contributing to health worldwide

The ecology and genetics of Chagas disease vectors in Ecuador, with emphasis on *Rhodnius ecuadoriensis* (Triatominae)

Fernando Abad-Franch

2002

Thesis submitted to the University of London in fulfilment of the requirements for the degree of Doctor of Philosophy (Ph.D.)

Supervised by Prof. Michael A. Miles

Pathogen Molecular Biology and Biochemistry Unit

Department of Infectious and Tropical Diseases

London School of Hygiene and Tropical Medicine

ABSTRACT

An estimate >11 million people are infected by *Trypanosoma cruzi* in Latin America. Chagas disease control programmes have been successful in the Southern Cone countries, and similar initiatives are underway in the Andean countries. In Ecuador prevalence is estimated as ~130000 people, with 2.5-3.8 million at risk; annual associated costs may reach >20 million US dollars. We studied ecological, genetic, and evolutionary aspects of *Rhodnius ecuadoriensis*, an important disease vector in western Ecuador and northwestern Peru. Sylvatic and synanthropic populations are sympatric in central Ecuador; only domestic-peridomestic colonies occur in the temperate Andean valleys of southern Ecuador and northern Peru. Both morphological-chromatic features and ecological-behavioural preferences seem to define a cline [sylvatic-large (north) → synanthropic-small (south)]. The ecology of domestic and sylvatic populations was studied using logistic regression. *Phytelephas aequatorialis* palms are the primary natural ecotope of *R. ecuadoriensis*; sylvatic bugs tend to favour male palms with large amounts of decomposing organic material and located in cropland/pasture fields. Poor households with mud walls, tiled roofs and large numbers of chickens were more likely to be infested. Isometry-free morphometric analysis consistently achieved >90% correct discrimination of sylvatic vs. synanthropic populations, supporting the use of metric variables in surveillance of reinfestations; size-free analyses revealed substantial divergence of Peruvian bugs from La Libertad. Mitochondrial DNA sequence polymorphisms (cytochrome *b* gene, 663 basepairs) were analysed; ~4% sequence divergence scored between Ecuadorian and Peruvian populations suggested they are independent phylogroups. Haplotype diversity and relationships indicate central coastal Ecuador as the centre of dispersal of this species, with isolated domestic populations in dry Andean valleys. The phylogeny of this species was explored using morphometric and molecular approaches. The monophyly of the 'Pacific *Rhodnius* lineage' (*pallescens*, *colombiensis* and *ecuadoriensis*) was confirmed, with the parapatric *pallescens* and *colombiensis* being very closely related; *R. pictipes* is the closest relative to this lineage among Amazonian species, with the *robustus* group forming a distinct, major clade.

Control of *R. ecuadoriensis* can contemplate local eradication in dry Andean valleys (southern Ecuador and northern Peru); special attention should be paid to peridomestic populations, including improvement of poultry management (burning-replacing nests every 15-30 days). Long-term interruption of disease transmission would benefit from educational interventions increasing awareness about Chagas disease and from housing improvements targeting mud walls and timber-and-tile roofs. In central-northern western Ecuador peridomestic palm trees may be the origin of reinfestations; environmental management (removing dead fronds and fibres from peridomestic palms), and continuous community-based surveillance are recommended. A comprehensive control programme over 15 years would probably result in interruption of disease transmission, and could bring savings of about 20 US$ per each dollar invested.

Acknowledgements

A few years ago I was told that a PhD was lonely business. Well this one wasn't. Many people helped carry out this project in different ways – and all their contributions brought improvement: the mistakes are my own.

Thanks first to my supervisor, Michael Miles. I have learnt many things from him, not only about bugs and trypanosomes; his solid support through these years was vital for the project.

Thanks also to Chris Schofield, who not only co-supervised this work – he in a way inspired it. He also provided essential support as the ECLAT Network general co-ordinator.

I especially wish to thank Fernando Monteiro (CDC-Fiocruz, Brazil) and JS Patterson (LSHTM) for their essential contribution to molecular and morphometric analyses, respectively, and to Ruth McNerney (LSHTM) for her great generosity. Most of the pictures of triatomine bugs are from JS Patterson's collection, and were used with his kind permission.

I am deeply grateful to all the colleagues and friends I worked with in Ecuador: Marcelo Aguilar, Pepe Racines (deceased in 1999), Aura Paucar, Charlie Carpio, Pancho Palomeque, Karyna Suárez, Mario Grijalva, Ángel Guevara, Luis Escalante, Giovanni Onore, the staff at both the Instituto Juan César García (Quito) and the Ministry of Public Health of Ecuador, and the students from the Central and Catholic Universities (Quito). The workers of the National Vector Control Service (SNEM) and the neighbours from fieldwork sites in various provinces made this study possible. Gloria, Ascen and Moli made my life in Ecuador easy and pleasant.

Many researchers within the ECLAT Network provided expert advice in the fields of ecology, vector biology and genetics, parasitology, statistics, or disease control. My sincere gratitude therefore to François Noireau (IRD-Fiocruz, Brazil), César Cuba Cuba (University of Brasília, Brazil), Jean-Pierre Dujardin (IRD, France), Ben Beard (CDC, USA), Nicolás Jaramillo (University of Antioquia, Colombia), Santiago Mas-Coma, Lola Bargues and Antonio Marcilla (University of Valencia, Spain), and José Jurberg (Fiocruz, Brazil). Dan Bausch (CDC) is partly responsible for my interest in triatomines.

At the LSHTM, Matthew Yeo, Sinead Fitzpatrick, Susi Solís, Isabel Mauricio, Michael Gaunt, Debbie Nolder, Diarmid Campbell-Lendrum, and Katrin Kuhn all helped me in different ways.

Various parts of this project were funded by the UNDP/World Bank/WHO TDR (grant 970195), the ECLAT Network, the LSHTM, the University General Hospital of Valencia (Spain), and the Cañada-Blanch Foundation (Spain).

I most definitely could not have made it without my family; their support came in any imaginable form... and always just at the right time. I see this thesis as an odd sort of collective 90th birthday present from all of us to my grandfather Paco.

This work is dedicated to José Luis Martínez (HRO and improving...).

CONTENTS ..... page

Aguilar VHM, **Abad-Franch F**, Racines VJ, Paucar CA (1999). Epidemiology of Chagas disease in Ecuador. A brief review. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 387-393.

Abad-Franch F, Noireau F, Paucar CA, Aguilar VHM, Carpio CC, Racines VJ (2000). The use of live-bait traps for the study of sylvatic *Rhodnius* populations (Hemiptera: Reduviidae) in palm trees. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **94**, 629-630.

Abad-Franch F, Aguilar VHM (2000). Control de la enfermedad de Chagas en el Ecuador. Datos y reflexiones para una política de Estado. *Revista del Instituto Juan César García*, **25**, 12-32.

Noireau F, Flores R, Gutiérrez T, **Abad-Franch F**, Flores E, Vargas F (2000). Natural ecotopes of *Triatoma infestans* dark morph and other sylvatic triatomines in the Bolivian Chaco. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **94**, 23-27.

Abad-Franch F, Paucar CA, Carpio CC, Cuba Cuba CA, Aguilar VHM, Miles MA (2001). Biogeography of Triatominae (Hemiptera: Reduviidae) in Ecuador: Implications for the design of control strategies. *Memórias do Instituto Oswaldo Cruz*, **96**, 611-620.

Marcilla A, Bargues MD, Ramsey JM, Magallón-Gastélum E, Salazar-Schettino PM, **Abad-Franch F**, Dujardin JP, Schofield CJ, Mas-Coma S (2001). The ITS-2 of the nuclear rDNA as a molecular marker for populations, species, and phylogenetic relationships in Triatominae (Hemiptera: Reduviidae), vectors of Chagas disease. *Molecular Phylogenetics and Evolution*, **18**, 136-142.

Abad-Franch F, Palomeque FS, Aguilar VHM (2001). *Control de la transmisión vectorial de la enfermedad de Chagas en el Ecuador*. FASBASE – Ministerio de Salud Pública del Ecuador, 63 pp.

Aguilar VHM, **Abad-Franch F**, Guevara AG, Racines VJ, Briones LA, Reyes LV (2001). *Guía operacional para el control de la enfermedad de Chagas en el Ecuador*. FASBASE – Ministerio de Salud Pública del Ecuador, 40 pp.

Noireau F, **Abad-Franch F**, Valente SAS, Dias-Lima A, Lopes CM, Cunha V, Valente VC, Palomeque FS, de Carvalho-Pinto CJ, Sherlock I, Aguilar M, Steindel M, Grisard EC, Jurberg J (2002). Trapping Triatominae (Hemiptera: Reduviidae) in silvatic habitats. *Memórias do Instituto Oswaldo Cruz*, **97**, 61-63.

Abad-Franch F, Aguilar VHM, Paucar CA, Lorosa ES, Noireau F (2002). Observations on the domestic ecology of *Rhodnius ecuadoriensis* (Triatominae). *Memórias do Instituto Oswaldo Cruz*, **97**, 199-202.

Cuba Cuba CA, **Abad-Franch F**, Roldán RJ, Vargas VF, Pollack VL, Miles MA (2002). The triatomines of northern Peru, with emphasis on the ecology and infection by trypanosomes of *Rhodnius ecuadoriensis* (Hemiptera: Reduviidae: Triatominae). *Memórias do Instituto Oswaldo Cruz*, **97**, 175-183.

Marcilla A, Bargues MD, **Abad-Franch F**, Panzera F, Carcavallo RU, Noireau F, Galvão C, Jurberg J, Miles MA, Dujardin JP, Mas-Coma S (2002). Nuclear rDNA ITS-2 sequences reveal polyphyly of *Panstrongylus* species (Hemiptera: Reduviidae: Triatominae), vectors of *Trypanosoma cruzi*. *Infection, Genetics and Evolution*, **1**, 225-235.

Abad-Franch F & Aguilar VHM (2002). Control of Chagas disease in the Andean countries, pp. 91-93 in (Guhl F & Schofield CJ eds.) *Proceedings of the IV Workshop on Population Genetics and Control of Triatominae, Cartagena de Indias, Colombia, August 2000.* Universidad de los Andes, Bogotá, Colombia.

Jaramillo ON, **Abad-Franch F**, Dujardin JP, Moreno J, Caro-Riaño H & Schofield CJ (2002). Clinal variation in the group *Rhodnius pallescens – R. colombiensis – R. ecuadoriensis*, pp. 95-104 in (Guhl F & Schofield CJ eds.) *Proceedings of the IV Workshop on Population Genetics and Control of Triatominae, Cartagena de Indias, Colombia, August 2000.* Universidad de los Andes, Bogotá, Colombia.

Noireau F, **Abad-Franch F** & Valente SAS (2002). Trapping Triatominae in silvatic habitats, pp. 183-185 in (Guhl F & Schofield CJ eds.) *Proceedings of the IV Workshop on Population Genetics and Control of Triatominae, Cartagena de Indias, Colombia, August 2000.* Universidad de los Andes, Bogotá, Colombia.

Tables.....**page**

Figures.....**page**

1. INTRODUCTION

1.1. Chagas disease

Chagas disease (American trypanosomiasis) was discovered in 1907 by the Brazilian physician Carlos Chagas (Chagas 1909, Prata 1999). A century later it is recognised as a major public health problem in virtually all Latin American countries (figure 1). In the early 90s, the World Health Organisation (WHO) estimated 18 million people were infected by *Trypanosoma cruzi*, the causative agent of the disease, with ~90 million people living under transmission risk conditions in the region. Mortality related to the disease reached 45000-50000 people each year, and the World Bank ranked Chagas disease as the most important parasitic disease in Latin America in terms of its impact on national economies and public health systems (producing a burden more than four times greater than all the rest of parasitic diseases plus leprosy considered together) (WHO 1991, 1997, World Bank 1993, Schofield & Dias 1996, Miles 1998, Dias & Schofield 1999, Moncayo 1999). However, co-ordinated control interventions throughout the Southern Cone countries have resulted in the elimination of disease transmission by *Triatoma infestans*, the most widespread domestic vector, in vast areas of Brazil, Argentina, Uruguay, Paraguay, Chile, and Bolivia; incidence among children and young adults has been reduced by an average of 72% in the area (WHO 1996, 1997, 1998, 2000, WHO/CTD 2002). Recent estimates indicate that about 11-12 million people are currently infected (Schmunis 1999a).

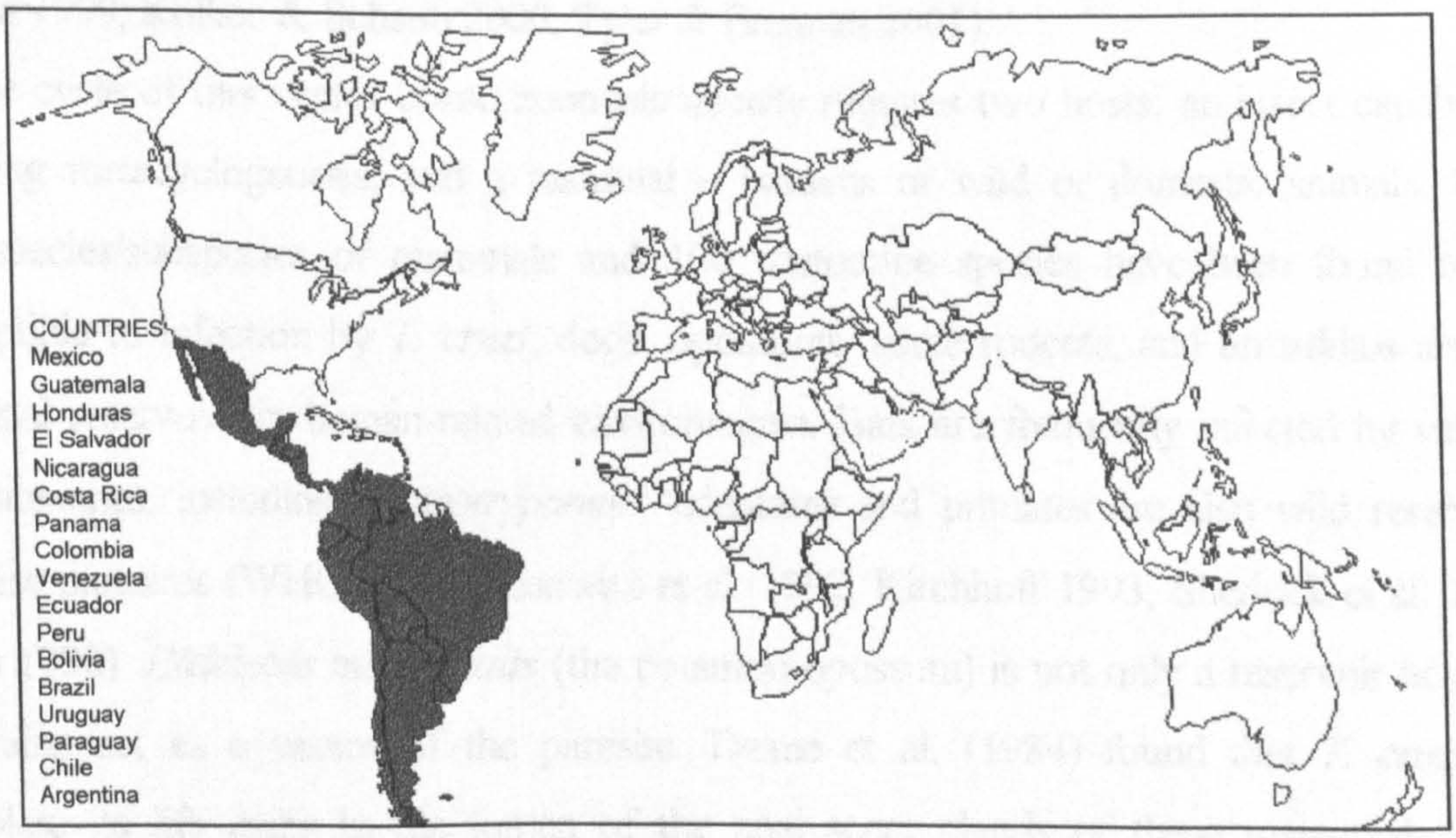


Figure 1. Geographic distribution of endemic Chagas disease (dark grey) and a list of endemic countries (from WHO/CTD)

1.1.1. THE PARASITE

Trypanosoma (Schizotrypanum) cruzi Chagas, 1909 (Kinetoplastida: Trypanosomatidae) is a flagellate blood protozoan transmitted mainly by contamination with the infected faeces of the insect vectors – haematophagous hemipterans belonging to the family Reduviidae, subfamily Triatominae (WHO 1991, Miles 1998).

1.1.1.1. Life cycle

The complex life cycle of *T. cruzi* involves four basic morphological forms:

i. Epimastigotes: replicative forms in the midgut of triatomine vectors;

ii. Metacyclic trypomastigotes: non-dividing forms in the vector hindgut; these are the infective forms present in insect faeces;

iii. Bloodstream trypomastigotes: extracellular, non-replicative forms present in the peripheral blood of mammalian hosts; and

iv. Amastigotes: intracellular replicative forms inside cells of various mammalian host tissues (forming the so-called pseudocysts when there are large numbers of parasites).

Bloodstream trypomastigotes constitute a pleomorphic population of 'broad' and 'slender' parasites; intermediate forms have been identified between and within the basic stages: sphaeromastigotes (between epi- and amastigote stages), intracellular intermediate forms (between amastigotes and slender trypomastigotes released from pseudocysts into the bloodstream), or the mid- and late-log growth phase epimastigotes. The relationships between these different forms and their biological significance are not clear in all cases (Souza 1999, Kollien & Schaub 2000, Tyler & Engman 2001).

The cycle of this vector-borne zoonosis usually requires two hosts: an insect capable of allowing metacyclogenesis, and a mammal – humans or wild or domestic animals. Over 200 species/subspecies of mammals and 100 triatomine species have been found to be susceptible to infection by *T. cruzi*; dogs, opossums, some rodents, and armadillos are the principal reservoirs in human-related environments. Bats are frequently infected by various trypanosomes, including *Schizotrypanum*; edentates and primates are also wild reservoirs of these parasites (WHO 1991, Tanowitz et al. 1992, Kirchhoff 1993, Sherlock et al. 1997, Miles 1998). *Didelphis marsupialis* (the common opossum) is not only a reservoir host but may also act as a vector of the parasite. Deane et al. (1984) found that *T. cruzi* can complete its life cycle in the lumen of the anal scent glands of these marsupials, from where metacyclic forms may directly infect other opossums; it has been proposed that this

could represent the ancestral route of transmission of the parasites (Schofield 2000a), but other authors consider that *T. cruzi* may have originated from insect kinetoplastids (Gaunt & Miles 2000). Birds, although refractory to *T. cruzi*, are important as hosts of triatomine bugs; some species and populations of triatomines exploit bird nest microhabitats, both in the wild and in peridomestic environments (Carcavallo et al. 1998a,b).

1.1.1.2. *Trypanosoma cruzi*: natural populations and diversity

Natural populations of *T. cruzi* show significant genetic and phenetic diversity. Various strains differ in their virulence, susceptibility to specific treatment, morphology, or antigenic structure. Techniques used for the characterisation of such diversity include analysis of multilocus enzyme electrophoresis (MLEE) profiles and other biochemical or immunological properties; total DNA or kinetoplast DNA minicircle amplification and hybridisation; riboprinting; randomly amplified polymorphic DNA (RAPD); and amplification/sequencing of selected genome fragments (see Momen 1999, Murta & Romanha 1999).

Some authors see a complex multiclonal origin as the most suitable explanation to this diversity (Tibayrenc et al. 1986, Tibayrenc & Ayala 1988), whilst others suggest that genetic exchange may contribute to the heterogeneity of sylvatic *T. cruzi* populations (Carrasco et al. 1996, Stothard et al. 1999, Machado & Ayala 2001, Miles et al. 2002). *T. cruzi* populations were divided into three main groups on the grounds of MLEE profiles (zymodemes Z1, Z2 and Z3) (Miles et al. 1977, 1978, 1980, 1981a, Barrett et al. 1980). Extensive isozymic characterisation led to the subdivision of these strains into a high number (up to 43) of individual zymodemes (Tibayrenc & Ayala 1988). Later on, molecular markers allowed for the distinction of two major evolutionary clades, defined as phylogenetic lineages 1 and 2 (Souto et al. 1996, Nunes et al. 1997, Fernandes et al. 1998, Oliveira et al. 1998, Stothard et al. 1998a, Zingales et al. 1998, 1999), and led to the definition of a coherent subspecific classification of *T. cruzi* populations into two major groups (*T. cruzi* I and II), with the latter encompassing Z2 and four further subgroups with consistent correspondences with the classical MLEE taxonomy (IIa=Z3, IIb=Z2, IIc=Z3/Z1 ASAT, IId=Bolivian Z2, and IIe=Paraguayan Z2) (Brisse et al. 2000, Miles et al. 2002). It is therefore recommended that *T. cruzi* strains should be grouped into two major lineages, *T. cruzi* I (Z1 or lineage 2) and *T. cruzi* II (Z2 or lineage 1) (anonymous 1999, Miles et al. 2002). Recently, Miles et al. (2002) put forward a comprehensive

proposal regarding parasite-host-vector-habitat associations (see also Gaunt & Miles 2000). It opens the possibility of establishing the epidemiological significance of the genetic diversity of *T. cruzi*, linking genotypes with different degrees of virulence, clinical outcomes, response to treatment, or biological behaviour of parasite strains within various hosts (Miles 1981a, Montamat et al. 1996). According to this proposal, *T. cruzi* I (which predominates in enzootic cycles in the Amazon basin and in domestic transmission cycles north of the Amazon) would be primarily associated with marsupial hosts (*Didelphis* spp.) and *Rhodnius* vectors; the association perhaps evolved (involving also *T. (Herpetosoma) rangeli*; cf. Stevens et al. 1999) in the abundant palm tree habitats of the Amazon basin, and may be as old as 90 million years (Miles et al. 2002, Gaunt & Miles 2002). *T. cruzi* II is the principal agent of human Chagas disease throughout the most heavily endemic areas of southern South America, where *T. infestans* acts as the main vector; Miles et al. (2002) proposed that this parasite lineage evolved with edentates (armadillos) in terrestrial ecotopes, shared also by rodents and several Triatomini. The parasites would have reached rodents secondarily, and possibly spread to human habitats when *T. infestans* (associated in the wild with rodents in rocky habitats) became domestic (Miles et al. 2002).

1.1.2. TRANSMISSION

Chagas disease can be considered as the result of complex ecological interactions between parasites, insect vectors, animal reservoir hosts, and human populations living under risk conditions. Human disease transmission seems favoured by various factors, both biological and social (Briceño-León 1990, WHO 1991, Miles 1998, Salvatella et al. 1998, Moreno & Carcavallo 1999).

1.1.2.1. Vector-borne transmission

Humans are considered as accidental hosts of *T. cruzi*. When an infected bug feeds on a person, metacyclic trypomastigotes may be deposited on the host body surface via vector excreta and reach the bloodstream through skin breaks or abrasions or through the mucosae – mainly the conjunctiva. This route of transmission accounts for about 80% of new human Chagas disease cases (WHO 1991, Tanowitz et al. 1992, Kirchhoff 1993, Schofield 1994, Miles 1998).

Over 100 species of Triatominae (out of the over 130 now recognised; see Section 1.2.) have been reported as naturally or experimentally infected by *T. cruzi*. Of these, only about 12 (those widely adapted to human environments) maintain **domestic transmission cycles**

and are considered of epidemiological importance; they all belong to the genera *Triatoma* [*T. infestans*, *T. dimidiata*, *T. brasiliensis*, *T. maculata*, *T. sordida*, *T. barberi*], *Rhodnius* [*R. prolixus*, *R. pallescens*, *R. ecuadoriensis*], and *Panstrongylus* [*P. megistus*, *P. herreri*, *P. rufotuberculatus*] (Carcavallo et al. 1997a, 1998a, 1999a, Sherlock et al. 1997, ECLAT 2002). In addition to their ability to colonise houses, other factors are involved in vectorial efficiency: feeding habits (anthropophyly, time lapse between feeding and defaecation, amount of blood ingested); infectivity and metacyclogenesis (amount of parasites in vector excreta and life cycle stage in which those parasites are excreted); longevity of insects; the relative distribution of populations of parasites, vectors, and mammal reservoir hosts; susceptibility of the latter to the infection, and levels and duration of parasitaemia (Lent & Wygodzinsky 1979, Gürtler et al. 1988, WHO 1991, Schofield 1994, Carcavallo et al. 1998b, Salvatella et al. 1998, Silveira 1999, Cohen & Gürtler 2001).

Enzootic transmission cycles occur in a great variety of ecotopes, where the parasite circulates among sylvatic triatomines and their mammalian hosts; only rarely become humans involved in these cycles, either when forest workers are attacked by triatomines (in the Brazilian Amazon *R. brethesi* may attack fibre gatherers working on *Leopoldinia piassaba* palms) or when adult bugs invade human habitats – a process perhaps favoured by artificial light sources. This latter mechanism is thought to be involved in most of disease transmission in the Amazon basin; adult bugs may transmit the infection either directly by feeding on the sleeping inhabitants or indirectly by contamination of presses used to prepare fruit juice (Lent & Wygodzinsky 1979, Coura et al. 1999, 2002, Moreno & Carcavallo 1999, Silveira 1999, Teixeira et al. 2001). Some bug species show a tendency, but only a limited capacity, to establish breeding colonies in human habitats; these are sometimes referred to as candidate domestic vectors (see Section 1.2.). In areas where the same vector species transmit *T. cruzi* in both enzootic and domestic environments, transmission cycles are said to be **continuous** or overlapping.

In some areas, the prevailing vector species in the domestic and sylvatic cycles are different; this is typically the case when synanthropic bugs spread out of their natural range associated with migrant people, and results in **separate** transmission cycles. Determining whether sylvatic and domestic cycles are overlapping or separate in a given geographical area has a profound bearing in the design of adequate strategies for disease control and surveillance; in the former case, the possibility of reinfestation of insecticide-treated

dwellings from sylvatic foci requires long-term entomological vigilance schemes, whereas in the latter eradication of synanthropic bug populations may be achieved through vertical spraying campaigns (Dujardin et al. 1991, Schofield 1994, Diotaiuti et al. 1995, Noireau et al. 1995, Dias & Diotaiuti 1998, Miles et al. 2002).

Several factors involved in household colonisation by triatomine bugs of various species have been identified; they often relate to low socio-economic status and poverty (Minter 1978, Mott et al. 1978, Lent & Wygodzinsky 1979, Zeledón & Vargas 1984, Briceño-León 1990, Barrett 1991, WHO 1991, Gürtler et al. 1992, Starr et al. 1992, Andrade et al. 1995a, Salvatella et al. 1998, Moreno & Carcavallo 1999, Oliveira-Lima et al. 2000, Cohen & Gürtler 2001; see also Chapter 5.):

i. Substandard housing: building materials, physical conditions of the dwellings, and management of the domestic environment. Mud walls (adobe, timber-and-clay, etc. – mainly if non-plastered), thatched or palm leaf roofs, earthen floors, storage of firewood and crops, or lack of proper domestic hygiene may favour infestation;

ii. In the peridomestic area, bugs may colonise chicken coops, dovecotes, corrals, pigsties, storerooms (for crops or firewood, where rodents frequently thrive), and even surrounding vegetation (e.g., bromeliads, hollow trees, or palm trees);

iii. The presence of domestic or synanthropic-opportunistic animals, involved in the parasite cycle (mammal reservoirs) and/or in the ecology of the vectors (mainly as blood sources), may also promote infestation;

iv. Environmental changes produced by man seem to play a role as well; sylvatic triatomines may invade human environments as a response to habitat destruction and reduction of natural host populations often associated with colonisation of forest areas where enzootic cycles occur;

v. Finally, the lack of knowledge about the disease and its modes of transmission among the population at risk can lead to tolerance towards infestation; this is often associated with the higher priority given by deprived householders to other, more immediate problems – including subsistence or acute diseases.

1.1.2.2. Blood transfusion

The second main transmission route is blood transfusion from infected donors (~16% of new Chagas disease cases). This is of greater importance in urban areas where triatomine vectors are absent (or only occasionally present) but to where many people migrate from endemic rural areas, being potential carriers of *T. cruzi*. Blood bank donation control through serological screening is therefore a necessary complement of any vector control programme (Dias & Brener 1984, Torres de Quinteros et al. 1990, WHO 1991, Moraes-Souza et al. 1994, Miles 1998, Moraes-Souza 1999, Schmunis 1999b).

1.1.2.3. Other routes of transmission

Other routes of *T. cruzi* transmission include (i) **congenital** transmission, most likely to occur in the last weeks of pregnancy or during delivery (about 2% of disease cases); (ii) **organ transplant** from infected donors (immunosuppressive therapy may result in severe acute disease forms); (iii) **oral** transmission, suspected in family outbreaks of acute disease reported from Brazil (likely related to the ingestion of infected fruit juices) and perhaps a risk for some hunting indigenous peoples of the Amazon (via ingestion of barely cooked game meat); oral transmission is probably also important among some *T. cruzi* reservoirs that may eat infected insects (triatomines or, in some cases, cimicid bugs); (iv) laboratory accidents; or, (v) in exceptional cases, perhaps breast feeding and sexual transmission (WHO 1991, Schofield 1994, Aguilar & Yépez 1996, Miles 1998, Sherlock 1999, Valente et al. 1999, Coura et al. 2002). As mentioned above, transmission from *D. marsupialis* can take place without the intervention of insect vectors through direct contamination by infected anal gland secretions. A recent paper points out the possibility that other arthropods, in this case sucking lice (Anoplura: Pediculidae) may also transmit the parasite among primates in some unusual circumstances (Argañaraz et al. 2001).

1.1.3. CLINICAL MANIFESTATIONS

A significant percentage of Chagas disease cases is asymptomatic and can only be detected by serological tests. Many others present only mild and nonspecific symptoms. Consequently, the proportion of cases that is diagnosed correctly (and in time to treat satisfactorily) in many areas of rural Latin America is probably very small. However, some general patterns may be outlined for each one of the phases and forms of the disease (for further information and details see Dias 1989, Espinosa et al. 1991, WHO 1991, Kirchhoff 1993, Miles 1998, Chapadeiro 1999, Corrêa-Oliveira et al. 1999, Dias

& Schofield 1999, Lopes 1999, Macêdo 1999, Manzullo & Chuit 1999, Ianni et al. 2001, Umezawa et al. 2001).

1.1.3.1. Incubation: from 7-10 days (vectorial transmission) to 7-40 days (transfusional transmission). Laboratory accidents may produce high initial parasitaemias and a reduced incubation period.

1.1.3.2. Acute phase: a proportion of these patients remain asymptomatic, but some symptoms and signs are usually present. It is more frequent in the first 20 years of life, with a higher incidence of severe cases in children under 5. Warning symptoms and signs include: (i) Romaña's sign: painless, unilateral, periophtalmic/palpebral oedema with satellite lymphadenopathy (produced by penetration of parasites through the conjunctiva). This lesion is the only pathognomonic sign of acute Chagas disease, but it is not frequent; it persists for 15-20 days. (ii) The cutaneous chagoma (an inflamed skin lesion at the site of parasite entry, with satellite lymphadenopathy): 20-25% of diagnosed acute cases; lasts 15-20 days. (iii) Fever (90-100% of cases): prolonged, irregular, does not respond to conventional treatment. (iv) Oedema (50-60% of cases): usually mild, extending from face to thorax; sometimes generalised (mainly in children). (v) Hepatosplenomegaly (30-50% of cases): usually mild; high frequency in congenital disease; splenomegaly (and lymphadenopathy) most frequent in transfusion-related transmission. (vi) Occasionally, generalised lymphadenopathy, rash, vomiting, diarrhoea, and anorexia. Other syndromes may also be diagnosed: acute diffuse myocarditis is frequently present but subclinical (rarely severe, but more dangerous for children under 5; ECG: sinus tachycardia, increased P-R interval, T-wave changes and low QRS voltage); acute meningoencephalitis is infrequent (except in immunodeficient patients and in congenital disease) and very serious, carrying a very poor prognosis.

Acute patients usually recover in 30-60 days, evolving towards the chronic indeterminate form (90% of cases). Less frequently (3%) they develop active myocarditis. Lethality reaches 2-7% (10-15% in some regions), mainly in children under 5 and immunodeficient patients.

1.1.3.3. Indeterminate chronic form: persistently positive serological tests in the absence of signs and symptoms (normal ECG and thorax/digestive X-Ray). About 50-60% of these cases remain stable, ~40% evolve symptomatic chronic forms (heart or digestive), and an undefined percentage of patients die suddenly.

1.1.3.4. Chronic chagasic cardiopathy is most frequent in male patients between 30-50 years. Chronic cardiac forms are characterised by the association of three syndromes (in different degrees): (i) *Arrhythmic syndrome* (most frequent): palpitations, bradycardia, syncope, Stokes-Adams (caused by a decreased brain blood flow); (ii) *Chronic cardiac insufficiency syndrome* (CCI): generally slow and progressive; causes mainly chest pain, dyspnoea, oedema of legs and hepatomegaly; and (iii) *Thrombo-embolic syndrome* is frequent (cardiac wall thrombi with embolism); cerebro-vascular accidents are common, and pulmonary and/or lower limb embolism also occur. Complementary tests reveal the following patterns: (a) Conventional ECG: ventricular tachycardia (frequently with ventricular extrasystoles [VE], which increases the risk of sudden death), right bundle branch block (RBBB, with ↑ QRS), atrium-ventricular block (AVB, with ↑ P-R interval and bradycardia), and alterations of re-polarisation (RT, with primary T wave changes). (b) Holter ECG: detection of transient, asymptomatic arrhythmias (especially ventricular extrasystoles, associated with an increased risk of sudden death). (c) Conventional X-Ray: increased cardiac volume. (d) Ecosonography: postero-inferior ventricular walls hypo- or akinetic, apical aneurysm, general ventricular enlargement with global reduced motility (this is common in other forms of myocardiopathy, and can cause alterations in cardiac valve function).

The following clinical classification of chronic chagasic cardiopathy was developed by WHO based on conventional clinical information and diagnostic test results.

Table 1. Chronic chagasic cardiopathy: WHO classification of clinical stages

STAGE	Serology	Clinical traits	X-Ray	ECG	RSD
I	+	–	–	–	–
II	+	+/–	–/+ (enlargement possible)	Mild alterations (RBBB, VE, RT)	+
III	+	+	Cardiomegaly +	Intermediate alterations (RBBB, AV hemiblock, hypokinetic areas)	+
IV	+	++ CCI	Cardiomegaly ++	Severe alterations (severe arrhythmias, large inactive areas, aneurysms)	+

CCI, RBBB, VE, RT, AV: see text; RSD: Risk of Sudden Death

The evolution of chronic chagasic heart patients can involve sudden death (usually by paroxystic tachycardia and ventricular fibrillation; in some cases related to mass pulmonary or brain embolisms); chronic cardiac insufficiency with asystolia; risk of death is associated with VE (higher risk), RBBB, RT and AVB (lower risk).

1.1.3.5. Digestive forms: megaoesophagus and megacolon are the principal digestive forms of chronic Chagas disease; rarely, other hollow organs can also be affected (digestive or urinary apparatus).

i. *Megaoesophagus*: most frequent in central and southeastern Brazil, mainly among males 20 to 40 years old; it is associated with cardiopathy in ~50% of cases. Clinical manifestations include: (a) Progressive dysphagia (100% of cases): patients need to drink in order to swallow solid food; (b) Regurgitation and risk of aspiration of oesophageal contents; and (c) Hypertrophy of parotid salivary glands ('feline face'). X-Ray examination with barium solution shows an increased oesophageal diameter, retention of contents (>1min), spasms, and a hypertonic distal third; assessment by dynamic X-Ray can be used in patient follow-up (revealing changes in the time required for oesophageal evacuation). These patients may develop malnutrition, potassium depletion, aspiration and lung disease, cancer, and oesophageal rupture.

Table 2. Chagasic oesophagopathy: WHO classification of clinical stages

STAGE	Serology	Clinical traits	X-Ray
I	+	Occasional dysphagia (solid food)	Normal or ↑ time of emptying
II	+	Constant dysphagia, pain, orthostatic regurgitation	↑ diameter, retention, hypertonic inferior third (spasms)
III	+	Mild-moderate dysphagia, regurgitation	↑↑ diameter, retention, hypotonic (spasms disappear)
IV	+	Dysphagia mild or absent, regurgitation	↑↑↑ diameter, retention ++, atonic

ii. *Megacolon* typically occurs in areas where megaoesophagus is also present; no gender differences have been reported, and it is most frequent in adults between 40 and 50 years. It produces progressive constipation (from days to weeks and even months) and accumulation of intestinal gas (frequent and early, causes abdominal distension), and may result in the formation of a faecaloma. X-Ray reveals the presence of gas; the use of radiological contrasts may increase the risk of perforation. The clinical evolution may include extremely severe conditions, including torsion, obstruction, toxic megacolon, perforation, and peritonitis.

The strong and persistent immune response to *T. cruzi* infection is considered to play an important role in the pathogenesis of both acute and chronic Chagas disease. However, the complex mechanisms involved are still poorly understood (Andrade 1999, Corrêa-Oliveira et al. 1999, León & Engman 2001).

1.1.4. DIAGNOSIS AND THERAPY

In the acute phase, direct identification of bloodstream trypomastigotes by **optic microscopy** is possible. The performance of this method can be improved by using appropriate concentration techniques (e.g., capillary tube centrifugation). Morphological characterisation by staining of blood samples (Giemsa) is especially recommended in areas where *T. cruzi* and *T. rangeli* are sympatric.

Xenodiagnosis is performed by allowing laboratory-reared insects to feed on suspect patients; after four weeks, insect faeces are examined in search of epimastigotes and metacyclic trypomastigotes. Only 20% to 50% of chronic patients have a positive xenodiagnosis; false-positives due to *T. rangeli* or *Blastocrithidia triatomae* may occur. **Culture** of blood samples on LIT (Liver Infusion-Tryptose) or BHI (Brain-Heart Infusion) media and inoculation of laboratory animals (irradiated mice) are also of limited utility in chronic patients. The use of **PCR**-based techniques for Chagas disease diagnosis and parasite detection/characterisation in patients, vectors and reservoirs has also been described (e.g. Ávila et al. 1991, Breniére et al. 1992, 1995, Guevara et al. 1996, 1997, Chiari 1999, Herwaldt et al. 2000, Britto et al. 2001).

Serological assays are widely used to detect anti-*T. cruzi* antibodies in endemic areas; they are the cornerstone of prevalence studies and therefore of crucial importance in control programmes. Techniques used are complement fixation reaction, indirect immuno-fluorescence, indirect and direct haemmaglutination or ELISA (Camargo 1999, Luquetti 1999, Umezawa & Silveira 1999). It is recommended that at least two different tests be performed in order to confirm the results (WHO 1991). Cross-reactions with antibodies against *Leishmania* and *T. rangeli* antigens may occur.

Specific treatment is only available for the acute phase of the disease. Nifurtimox (a nitrofuran) and Benznidazole (a nitroimidazole) are effective against bloodstream stages of the parasite. Some benefits may be expected in patients with indeterminate disease, but the indication has not yet been accurately established. Moreover, both drugs are toxic and cause severe side-effects. Other compounds are being tested against *T. cruzi*: Allopurinol failed to prove effective in a multicentric trial, and antifungal molecules as Ketoconazole or Itraconazole are showing moderate activity (Urbina 1999). Other triazole derivates, non-metabolisable pyrophosphate analogues, antiviral drugs, and others are also being tested in laboratory trials, but pharmaceutical corporations seem to show little interest in the

development of new treatments (Coura & de Castro 2002). The growing recognition that parasite persistence in the tissues of infected patients may play a central role in the pathogenesis of chronic Chagas disease (as opposed to the view that autoimmunity is the primary cause of chronic lesions) (Tarleton & Zang 1999, Tarleton 2001) has fostered debate about the indication of specific chemotherapy in chronic (indeterminate or symptomatic) patients (Urbina 1999). It is currently recommended that all seropositives under 12 years of age (and in general all those with less than 10 years of infection) should receive specific treatment. Although results from clinical trials are controversial, patients in the late chronic phase (over 10 years since infection) could also benefit from specific chemotherapy, mainly if they are in the indeterminate form or their lesions are not severe (Coura & de Castro 2002).

Benznidazole (RO-7-1051, 100mg pills) is the recommended specific treatment, and should be delivered as follows. Adults (except pregnant women): 5-7 mg/kg/day, 60 days. Children under 12 (both acute or just seropositive, including congenital transmission): 5-10 mg/kg/day, 60 days. Suspected accidental laboratory infection or infected blood transfusion: 7-10 mg/kg/day, 10 days.

Side effects are dose-dependent and include: malaise, dermatitis, anorexia and weight loss, abdominal pain, headache, loss of concentration, and peripheral polyneuropathies at the end of the treatment. Some cases of agranulocytosis and/or anaemia have been recorded, requiring interruption of treatment. Tolerance decreases with age, and side effects are less frequent in children.

Cardiopathy and digestive disease are treated symptomatically. Heart disease: general measures (repose, low-sodium diet), anti-arrhythmic drugs (amiodarone), anti-coagulants when there is embolism, and cardiac insufficiency treatment (except in stage IV, when there is no response) including cardiotonics, diuretics, vasodilators, etc. When there are complete RBBB or VE, a pacemaker must be implanted.

Treatment of oesophagopathy includes isosorbitol dinitrate, pneumatic dilation of the inferior oesophageal sphincter, and surgery in severe cases.

Megacolon is treated by reducing constipation (diet, mild laxatives, manual extraction of faecaloma), and several surgical techniques (see Meneghelli 1999, Silva 1999).

1.2. Triatominae (Hemiptera: Reduviidae)

1.2.1. BIOLOGY, SYSTEMATICS AND EVOLUTION

1.2.1.1. Biosystematics

·Systematics. The subfamily Triatominae Jeannel, 1919 groups all the obligate haematophagous reduviids. They belong to the order Hemiptera, suborder Heteroptera (true bugs), and share some fundamental characteristics; one of them (haematophagy) is used for the definition of the subfamily (in contrast with virtually all the rest of the Reduviidae, which are predatory bugs). The Triatominae are classified into five tribes, among which the most important (epidemiologically and in terms of number of species) are the Triatomini Jeannel, 1919 and the Rhodniini Pinto, 1926. All the *Triatoma* Laporte, 1832, *Panstrongylus* Berg, 1879, and *Rhodnius* Stål, 1859 species (i.e., all the epidemiologically significant triatomines) belong to these two tribes. Others (the Cavernicolini Usinger, 1944, Bolboderini Usinger, 1944, and Alberproseniini Martínez and Carcavallo, 1977) have never been incriminated in disease transmission (Lent & Wygodzinsky 1979, Schofield 1988, 1994, 1996, 2000b, Barrett 1991, Schofield & Dolling 1993, Salvatella et al. 1998, Carcavallo et al. 1999b, 2000, Silveira 1999). Seventeen genera and over 130 species of Triatominae have hitherto been recognised, although the validity of some species needs confirmation. Examples of taxonomic debate range from distinction of taxa within species complexes to the definition of genera and even tribes. The recent elevation of *Linshcosteus* to tribal status (the Linshcosteini), claims regarding the validity of *Meccus* Stål, 1859 (comprising species assigned to the *T. phyllosoma* complex), the controversial specific status of genetically distinct *R. robustus*-like populations in the Amazon-Orinoco basins, or the revalidation of *R. amazonicus* (previously synonymised with *R. pictipes*) are a few among those examples (Carcavallo et al. 2000, Monteiro et al. 2001, Bérenger & Pluot-Sigwalt 2002). More generally, the wealth of information on the biology of these blood-sucking bugs (with early allusions in documents dated in 1590) has not resulted in a reliable cladistic system that could help clarify the systematics of the subfamily or their historical biogeography (Lent & Wygodzinsky 1979). As discussed below, even the single/multiple evolutionary origins of the haematophagous habit, and therefore the mono- or polyphyletic character of the subfamily, are still unresolved.

Table 3. The Triatominae: tribes, genera and approximate number of species

Tribe	Genus	Number of species
Alberprosenìini	*Alberprosenia*	2
Bolboderini	*Belminus*	4
	Bolbodera	1
	Parabelminus	2
	Microtriatoma	2
Cavernicolini	*Cavernicola*	2
	Torrealbaia	1
Linshcosteini [a]	*Linshcosteus*	6
Triatomini	*Dipetalogaster*	1
	Eratyrus	2
	Hermanlentia	1
	Meccus [b]	—[c]
	Mepraia	1
	Panstrongylus	13 [d]
	Paratriatoma	1
	Triatoma	77 [e]
Rhodniini	*Psammolestes*	3
	Rhodnius	16

a-Generally considered as belonging in the Triatomini; *b*-Some authors query its validity; *c*-The ~7 species of *Meccus* were here included in *Triatoma*; *d*-*Panstrongylus turpiali*, synonymised with *P. chinai* by Lent (1997), is not included, although biogeographical considerations suggest it could be a valid taxon; *e*-Including *Meccus* spp.

The definition of the subfamily (plainly put as "Hematophagous Reduviidae" by Lent and Wygodzinsky [1979]; p. 175) emphasises a single trait as the most significant in the biology of the Triatominae, i.e. their obligate blood-feeding habit. It is not surprising therefore that modifications of their mouthparts and several other physiological adaptations to haematophagy are used as key taxonomic characters to distinguish them from other hemipterans (of which over 82000 species are known). The proboscis of the Triatominae has three segments, is straight and adpressed to the gula when in rest, and its distal extremity usually reaches the prosternum, where the stridulatory sulcus is located (*Linshcosteus* and *Cavernicola* are exceptions in that they lack stridulatory sulcus, present in most reduviids). Phytophagous bugs typically have a long and thin, four-segment proboscis, whereas the rostrum of predaceous Reduviidae is robust, heavily sclerotised, generally curved, and divided into three segments. In Triatominae, a membranous articulation allows the distal article of the rostrum to flex upwards when the insect is feeding (Lent & Wygodzinsky 1979, Schofield 1994, Carcavallo et al. 1999b, 2000).

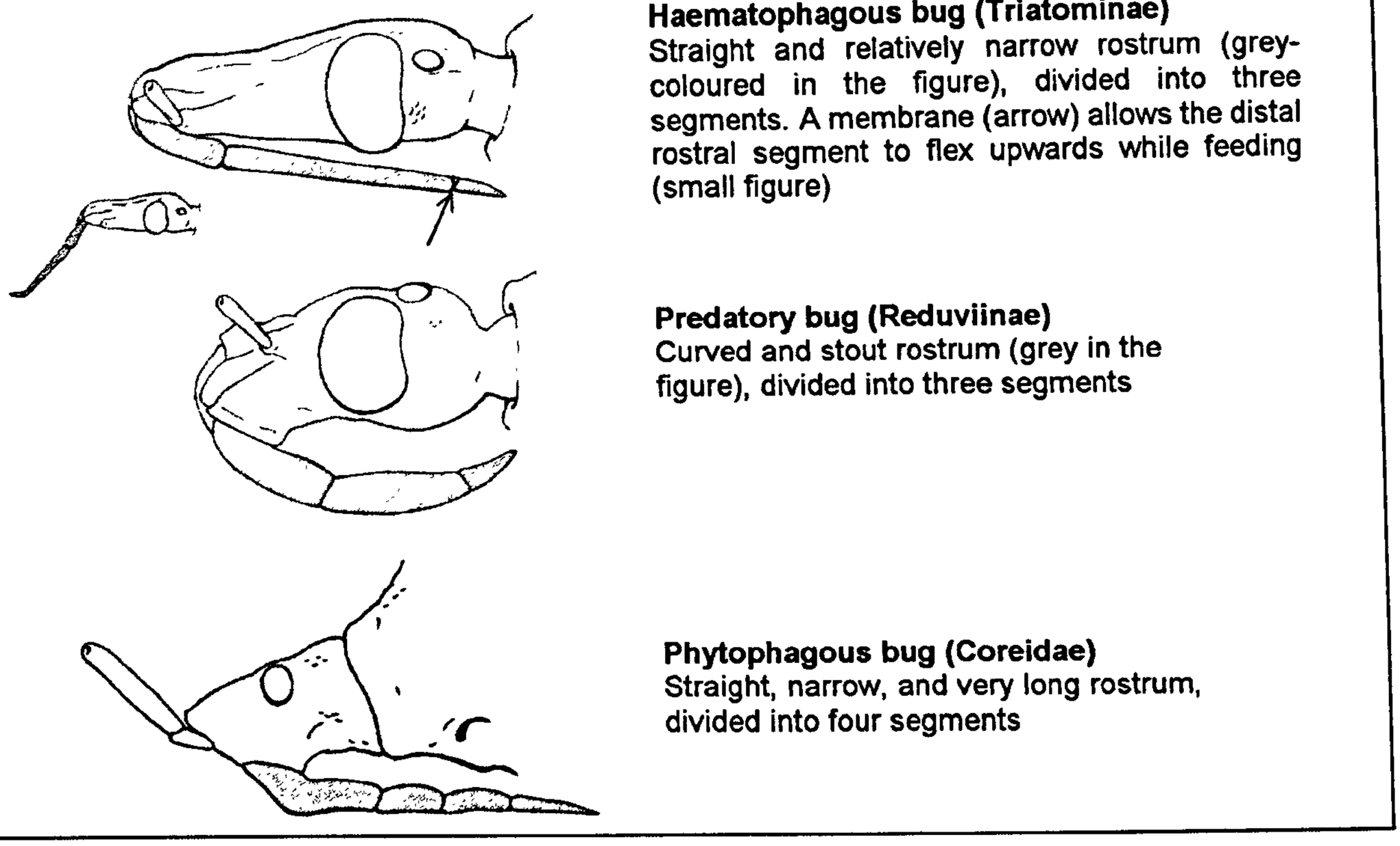


Figure 2. Mouthparts of haematophagous, predatory, and phytophagous hemipterans

Further characters used to separate the Triatominae from other reduviids are the lateral insertion of antennae, the absence of a well-developed interocular sulcus, and the absence of dorsal abdominal scent glands. However, the overall anatomical features of triatomines are notoriously similar to those of other reduviids (Lent & Wygodzinsky 1979, Schofield 2000a,b).

·Ecology·. Haematophagy is also central to many behavioural and ecological traits of the Triatominae, including of course their medical importance. Many species have adapted to live in particular microhabitats (both terrestrial and arboreal) where the availability of food (in terms of quantity and temporal stability) and protection from climate extremes seem to be the main favouring factors. The most important of these habitats include palm trees (the primary ecotope of virtually all *Rhodnius* species), hollow trees (a favoured ecotope for many Triatomini), rocky outcrops and stone piles (where several species of *Triatoma* may be found), bird nests and similar structures used by some arboreal mammals, bat caves, mammal subterranean burrows (e.g. armadillos), dead trees and the spaces under loose bark, and both epiphytic and terrestrial bromeliads. Human dwellings and peridomestic structures can also be colonised by several triatomine species; in such cases people, domestic animals, and opportunistic

mammals such as rodents and opossums provide the bugs with an abundant and very stable food source (Lent & Wygodzinsky 1979, Barrett 1991, Schofield 1994, 1996, Carcavallo et al 1998a,b, Salvatella et al. 1998, Gaunt & Miles 2000).

The vast majority of triatomine species are Neotropical, but several groups extend far beyond the tropics in the Americas, from the United States (roughly 40°N, *T. protracta*) to southern Argentina (about 46°S, *T. infestans*). Some *Triatoma* species occur in Asia, and are thought to have derived from the tropicopolitan *T. rubrofasciata*, a species associated with rodents that apparently dispersed to tropical harbours around the world as it adapted to live in rat-infested ships. The genus *Linshcosteus* is of uncertain origin and can only be found in India, apparently associated with reptiles and wild canids. Excluding *T. rubrofasciata*, the Triatominae are absent from both the Palaearctic and the Ethiopian regions (Lent & Wygodzinsky 1979, Carcavallo et al. 1997a, 1999c, Patterson et al. 2001, Galvão et al. 2002).

·Life cycle. Triatomines are hemimetabolous insects with five nymphal instars; to proceed from one stage to the next the bugs require at least one bloodmeal. Females usually lay several hundred eggs during their lifespan; numbers vary among species and depend on environmental factors such as food availability or climatic conditions. Egg laying begins about 15-30 days after copulation and may last for several months. Species that occupy terrestrial ecotopes generally lay loose eggs, whereas arboreal triatomines tend to fasten their eggs to the substrate; *Psammolestes arthuri* females are unique in that their eggs are laid in masses (Lent & Wygodzinsky 1979, Barrett 1991).

Embryonic development lasts between 10 and 30 days in most species, varying widely with temperature. First instar nymphs usually take their first bloodmeal 2-3 days after hatching. The complete life cycle of many species is typically of about one year, but it may be significantly faster under favourable environmental conditions. For instance, egg-to-adult development time in domestic populations of *T. infestans* varies from 6 months in constantly warm areas of central Brazil to 12 months in Argentina, where development and oviposition rates were significantly reduced during the cold winters (Schofield 1980, Gorla & Schofield 1989, Barrett 1991, Carcavallo 1999).

·Population dynamics. Triatomines are *k*-strategists; they usually exploit very stable habitats by developing finely balanced relationships with their hosts in the context of strong intersibling competition. Intrinsic population growth rates (R_0) are

typically very low, and tend to 1 when the population is at equilibrium. Several environmental factors may regulate the density of populations, but the availability of food seems to be the most relevant (Schofield 1980, 1994, 1997, Gorla & Schofield 1989, Schofield et al. 1999). In general, R_0 is >1 (i.e., the population is growing) when a particular habitat is occupied by a number of bugs below the carrying capacity of that habitat (in recently established colonies, when additional blood sources become available, or after heavy mortality – e.g. related to control interventions). Schofield et al. (1986a) found that defensive host responses increase with the number of bugs attempting to feed on that host, limiting the duration of bloodmeals. Therefore, the nutritional status of each individual bug deteriorates in denser colonies. Consequences include a longer development period (with reduced rate of recruitment of adults and increased probabilities of predation before reaching the reproductive stage), a decreased production of eggs per female, an increased mortality rate per stage, and an increased probability of adult bugs starting dispersive flights (Barrett 1991, Schofield 1997, Schofield et al. 1999). Thus, population growth rate declines (R_0=1 in stable populations, and R_0<1 when worse conditions place the population on its way to extinction) and density decreases to restore the balance between bug numbers and available food. Population dynamics provide an indication of the potential of different species to build up large colonies under certain environmental conditions, and about the capacity of population recovery after insecticide spraying.

Other relevant aspects involved in *T. cruzi* transmission, such as the time lapse between feeding and defaecation, may also be related to the density of bugs in a given environment (Kirk & Schofield 1987). The number of bloodmeals (and the amount of blood) required to complete development, the feeding time (from host skin piercing to repletion), the aggressivity of the bugs (time lapse between presentation of host and beginning of bloodmeal), etc., are also of interest and may vary with population density.

·Dispersal. Dispersive flight is one of the means by which density is regulated in natural populations. In some cases, it will lead to the invasion and possibly to the colonisation of human environments; many species enter human dwellings by flying from their natural habitats, and this appears to be a major route of disease transmission in the Amazon (Schofield & Matthews 1985, Naiff et al. 1998, Sherlock 1999, Coura et al. 1999, 2002). Triatomines are however relatively poor flyers, although this may vary

widely among species. Initiation of dispersive flights seems related to a poor nutritional status of the bugs; distance covered is usually in the range of 100 to 200m, but may be larger in many cases (Lehane & Schofield 1981, Schofield & Matthews 1985, Barrett 1991, Schofield et al. 1991a,b, Lehane et al. 1992, Noireau & Dujardin 2001). Passive dispersal with the host is important in some triatomines. Eggs and small nymphs of ornithophilic species (e.g. *T. sordida*, *Rhodnius*, *Psammolestes*) have been detected in the plumage of several birds, and synanthropic populations of *T. infestans*, *R. prolixus*, *T. rubrofasciata*, or *T. dimidiata* have followed their migrating human hosts over enormous distances (and by almost every possible transport means) to colonise territories far beyond their natural ranges (Barrett 1991, Schofield 1994).

1.2.1.2. Evolution

Whether haematophagy is a true autapomorphic character defining a natural clade (the Triatominae) or whether it arose more than once during the evolution of the reduviids remains a matter of controversy. In the most authoritative monograph on the systematics of the subfamily, the authors argue in favour of monophyly, affirming that

> "the obviously apomorphic traits of the triatomines, viz., the obligatory hematophagous condition and the upwardly flexible third rostral segment (...) are to our knowledge not found in any other reduviid. These characters are autapomorphic and thus of no value for determining relationships with other groups of Reduviidae, but they do establish the Triatominae as a monophyletic group" (Lent & Wygodzinsky [1979]; p. 177).

A competing view proposes that the subfamily is an artificial assemblage of different reduviid lineages that evolved haematophagy independently (Schofield 1988, 1994, 1996, 2000a,b). Circumstantial evidence to support this hypothesis comes from different sources, particularly from studies highlighting the differences between Triatomini and Rhodniini in terms of biochemical properties of salivary proteins (Pereira et al. 1996a, Ribeiro et al. 1998, Soares et al. 1998, Pereira 1999) and cuticular hydrocarbons (Juárez 1996, Juárez et al. 1999, 2000), egg exochorium architecture (Barata 1996, 1998), anatomical features of the male genitalia (Jurberg 1996) and antennal sensilla (Catalá & Schofield 1994, Catalá 1996, 1997, 1999), isoenzyme profiles (Dujardin et al. 1999a), DNA sequence polymorphisms (Stothard et al. 1998b, Lyman et al. 1999, Bargues et al. 2000, Marcilla et al. 2001, Gaunt & Miles 2002) and RAPD banding patterns (García et al. 1998), and from observations on blood-sucking behaviour (often facultative) and cleptohaematophagy in other hemipterans, including Lygaeidae (Cleradini), Reduviidae

(Emesinae, Harpactorinae, Peiratinae, Reduviinae, Apiomerinae), and Anthocoridae[Ø] (e.g. *Lyctocoris* spp.) (Schuh & Slater 1995, Schofield 1996, 2000a, Torres et al. 2000).

Preliminary observations suggesting a possible monophyletic origin of triatomine salivary anti-thrombin proteins, introduced by Gaunt and Miles (2000) as providing "compelling evidence that the evolution of haematophagous behaviour evolved from a single ancestor" (Gaunt & Miles [2000]; p. 564), have not been confirmed; rather, anti-thrombins of the same family have been discovered in some unrelated predatory reduviids, showing that the trait is not derived in blood-sucking bugs.

The issue is however likely to remain controversial until suitable outgroups are identified among the Reduviidae that may split the monophyly of the Triatominae (e.g., a sister group to the Rhodniini with respect to the Triatomini) in phylogenetic analyses ideally combining nuclear and cytoplasmic DNA sequence data; Schofield and Dujardin (1999) mentioned the suggestion by TV Barrett (cited as a personal communication) that the Rhodniini could derive from "an ancestral form similar to extant Stenopodinae".

There seems to be agreement in that haematophagy is a derivation of the predatory habits of the reduviid ancestor(s) of triatomines, but there is also debate about the evolutionary age of such an event (or events) (Gaunt & Miles 2000, Schofield 2000a, Stevens 2000). Some triatomine species (e.g., *Eratyrus mucronatus*, *T. rubrovaria*, *T. rubrofasciata*, *T. circummaculata*, and many Bolboderini) have been reported to feed on other insects and on spiders, suggesting haematophagy evolved recently. Morphological and habitat similarities between blood- and insect-feeding reduviids, the occasionally severe anaphylactic reactions of vertebrates to the bites of some triatomines (as for many predaceous reduviids), the use of cathepsins to digest bloodmeals, and the fact that intestinal symbiotic bacteria (required for the synthesis of vitamins that are scarce in vertebrate blood such as folates) are free in the gut lumen of triatomines (but enclosed in specialised organs or within intestinal cells in all other obligate blood-sucking insects), have all also been interpreted as showing imperfect adaptation of triatomines to blood-feeding, thus supporting the view of a recent evolution of haematophagy. An intermediate step of facultative haematophagy, perhaps aided by the nest-dwelling habits of many triatomines (in vertebrate nests and burrows detrivorous insects abound, attracting predatory bugs that will occasionally bite the vertebrates

---

[Ø]Cimicidae and Polyctenidae, probably offshoots of the Anthocoridae, are obligate blood-suckers

themselves), would have given rise to an obligate blood-sucking behaviour in most of the currently extant triatomines. On the other hand, the fine specialisations of several triatomines to exploit particular ecological niches (including hosts), together with the fact that marsupials and edentates were present (i.e. available as suitable hosts for blood-sucking arthropods) in South America at least 65 million years ago, have been interpreted as suggesting that haematophagy could be an ancient trait in Triatominae (Lent & Wygodzinsky 1979, Schofield 1988, 1994, 1996, 2000a,b, Carcavallo et al. 1999b, 2000, Schofield & Dujardin 1999, Gaunt & Miles 2000).

1.2.1.3. Synanthropic behaviour and epidemiological risk

In epidemiological terms it is however the current trends in triatomine behaviour that is genuinely relevant. When a particular triatomine species colonises human environments, it is likely that a domestic cycle of *T. cruzi* transmission will start. Some species have successfully adapted to human-related habitats; they eventually became major disease vectors – and the principal objectives of control interventions (Lent & Wygodzinsky 1979, Schofield 1994, Salvatella et al. 1998, Moreno & Carcavallo 1999, Silveira 1999). Among these species, the most significant is *T. infestans*. Its distribution includes most of southern South America, but international control activities (the Southern Cone Initiative) have contributed to the elimination of the species from large areas where it was artificially introduced by people in the recent past (Moncayo 1999, Schofield & Dias 1998, WHO/CTD 2002). *R. prolixus* is the main vector in northern South America and most of Central America. Other epidemiologically significant species are *T. dimidiata*, *T. brasiliensis*, and *P. megistus*. These are customarily considered as the primary vectors of human Chagas disease; they often maintain separate domestic transmission cycles in areas where sylvatic ecological niches are exploited by different species. Some species have adapted to human habitats in more restricted geographic areas, where they have become important disease vectors usually retaining sylvatic habits (resulting in overlapping transmission cycles); examples of these secondary vectors include *R. ecuadoriensis*, *T. maculata*, *T. sordida*, *R. pallescens*, *R. neglectus*, *R. nasutus*, *P. rufotuberculatus*, *P. herreri*, *T. barberi*, and some species of the *T. phyllosoma* complex. Finally, some species are sporadically reported to be invading-colonising households; they are considered as potentially dangerous, candidate domestic vectors. The following tables summarise the distribution

and epidemiologically relevant ecological features of triatomine species found to occur in human habitats. The records were organised in two main groups: Triatominae frequently found in human habitats [table 4] and sylvatic triatomine species occasionally found in human-related habitats [table 5]; several subdivisions were defined within each of these major groups, ranging from the very strongly synanthropic primary vectors to those species only occasionally found invading human dwellings. The main bibliographic reviews on triatomine bug ecology were consulted, and several original sources (not repeated when present in the reference lists of those reviews) were also revised (Lent & Wygodzinsky 1979, Carcavallo & Martínez 1985, Barrett 1991, Carcavallo et al. 1997a, 1998a, 1999c, Gonçalves et al. 1998, Salvatella et al. 1998, Angulo et al. 1999, Moreno & Carcavallo 1999, Schofield & Dujardin 1999, Silveira 1999, Jaramillo et al. 2000, Molina et al. 2000, Ramsey et al. 2000, Reyes-Lugo & Rodríguez-Acosta 2000, Vallejo et al. 2000, Abad-Franch et al. 2001a,b, 2002, ECLAT 2002, Cuba Cuba et al. 2002, Wolff & Castillo 2002).

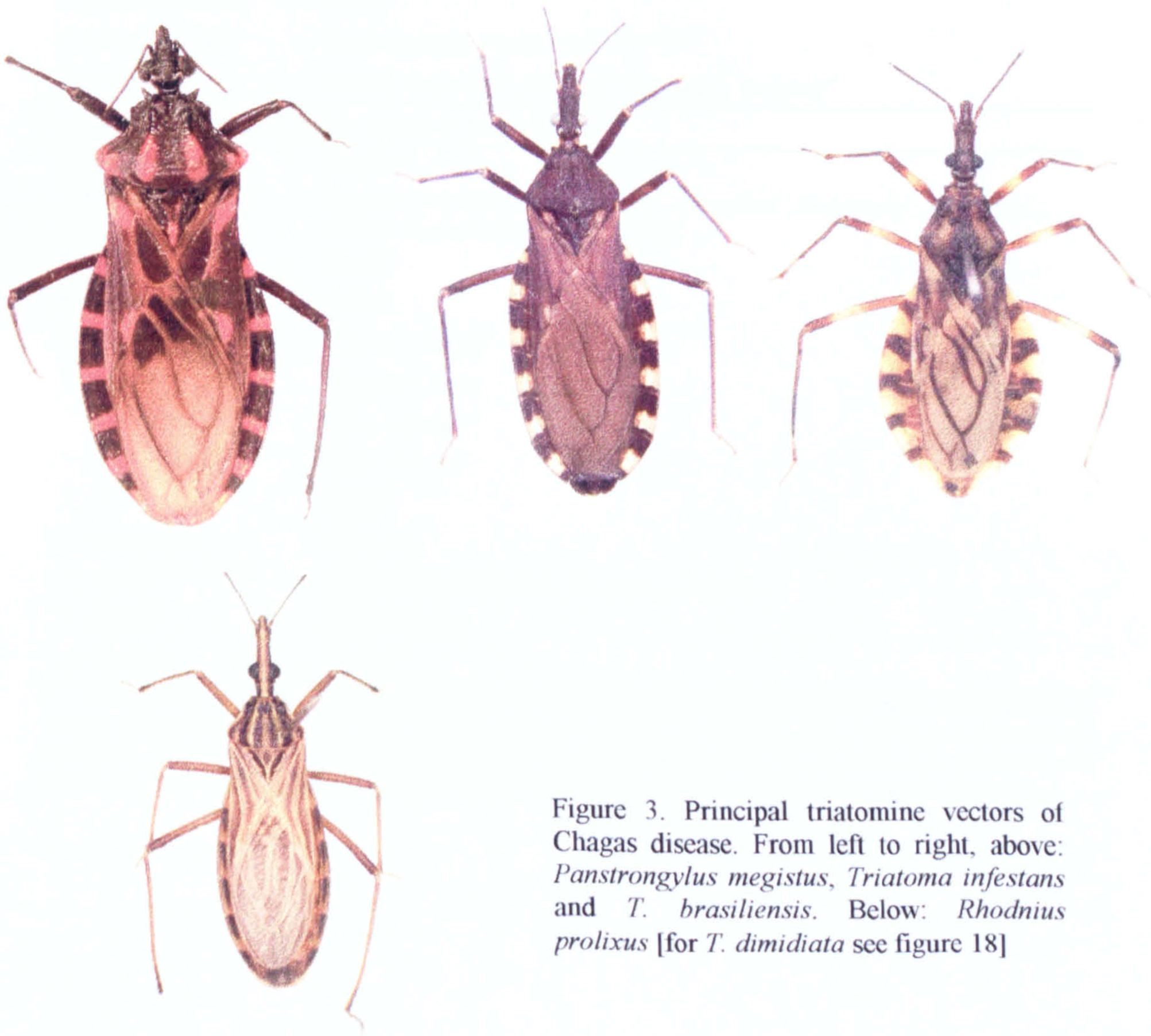


Figure 3. Principal triatomine vectors of Chagas disease. From left to right, above: *Panstrongylus megistus*, *Triatoma infestans* and *T. brasiliensis*. Below: *Rhodnius prolixus* [for *T. dimidiata* see figure 18]

Table 4. Distribution and degree of synanthropism of Triatominae frequently found in human habitats

Major disease vectors	
R. prolixus[1]	Central America-Southern Mexico[c, e], Venezuela[c], Colombia[c], Brazil, Suriname, Guyana, French Guiana[c], Trinidad
T. dimidiata[2]	Mexico[c], Central America[c], Colombia[c], Ecuador[c, e], Peru[c, e]
T. infestans[2]	Bolivia[c], Brazil[c, e], Argentina[c, e], Uruguay[c, e], Paraguay[c, e], Chile[c, e], Peru[c, e]
Species frequently colonising human environments	
P. megistus[2, e in some areas]	Brazil[c], Argentina[c], Uruguay[a], Paraguay[a], Bolivia[a]
P. herreri ⊗	Peru[c], Ecuador[b]
R. ecuadoriensis[1]	Ecuador[c], Peru[c]
R. pallescens[1]	Panama[c], Colombia[c], Belize[d], Costa Rica[d]
T. barberi	Mexico[c]
T. brasiliensis[2]	Brazil[c]
T. guasayana	Argentina[c], Bolivia[c], Paraguay[d]
T. maculata[2]	Colombia[c], Venezuela[c], Brazil[a], Guyana, French Guiana[d], Suriname[d], Aruba[d], Bonaire[d], Curaçao[d]
T. mazzottii	Mexico[c]
T. pallidipennis	Mexico[c]
T. phyllosoma	Mexico[c]
T. pseudomaculata	Brazil[c]
T. rubrofasciata[c]	Tropicopolitan – harbours[e in some areas]
T. sordida[2]	Brazil[c], Argentina[c], Bolivia[c], Paraguay[d], Uruguay[d]
Species showing synanthropic trends in some areas	
D. maxima	Mexico[a]
P. rufotuberculatus[2]	Bolivia[c], Ecuador[c], Peru[c], Brazil[b], Argentina[b], Venezuela[d], Colombia[c], Central America-Southern Mexico[d]
R. nasutus[1]	Brazil[c]
R. neglectus[1]	Brazil[c]
R. stali[1]	Bolivia[c], Brazil[d]
T. carrioni	Ecuador[c], Peru[c]
T. gerstaeckeri[c]	USA, Mexico
T. lecticularia[c*]	USA, Mexico
T. lenti	Brazil[c]
T. longipennis	Mexico[c]
T. nitida[occasionally c*]	Costa Rica, Honduras, Guatemala, Mexico
T. rubida[c*]	USA, Mexico
T. vitticeps	Brazil[c]

T = Triatoma; *R = Rhodnius*; *P = Panstrongylus*; *D = Dipetalogaster*; *a*-Found in human habitats (no further data); *b*-Adult specimens invading human habitats; *c*-Domestic or peridomestic colonies; *d*-No specific data; *e*-Artificially introduced; * indicates that only peridomestic colonies have been specifically reported; *1*-Primarily associated with palm trees; *2*-Occasionally reported from palm trees; ⊗*Panstrongylus herreri* and *P. lignarius* may be synonymous

Table 5. Sylvatic triatomine species occasionally found in human-related habitats in the Americas

Breeding colonies reported from human environments	
Mi. trinidadensis[2, b]	Brazil, Venezuela, Trinidad, Colombia, Peru, Bolivia[c*]
B. peruvianus	Peru[c]
E. cuspidatus[2]	Colombia[a], Ecuador[c*], Peru, Venezuela, Guatemala, Panama
E. mucronatus[2]	Bolivia[c], wide geographical range East of the Andes (Guatemala to Bolivia-Brazil)
M. gajardoi	Chile[c]
M. spinolai	Chile[c]
P. chinai	Ecuador[c], Peru[c]
P. diasi	Bolivia[d], Brazil[b, occasionally c*]
P. geniculatus[2]	Brazil[c*], most Central and South American countries[b, occasionally c]
P. guentheri[b, occasionally c*]	Argentina, Bolivia, Paraguay, Uruguay
P. howardi	Ecuador[b, occasionally c*]
P. lutzi	Brazil[c]
R. colombiensis[1]	Colombia[b, nymphs found in a few dwellings]
R. domesticus[2]	Brazil[c*]
R. pictipes[1]	Amazon basin[b, occasionally c, ‡] (Colombia, Venezuela, Ecuador, Peru, Brazil, Bolivia, Suriname, Guyana, French Guiana, Trinidad), Belize[d]
T. arthurneivai	Brazil[b, occasionally c*]
T. eratyrusiformis	Argentina[c*]
T. bolivari	Mexico[a*]
T. guazu[a*]	Paraguay, Brazil
T. hegneri	Mexico (islands: Cozumel, Quintana Roo)[c*]
T. melanosoma[■]	Argentina[c]
T. patagonica	Argentina[frequently c*, occasionally b]
T. picturata	Mexico[c*]
T. platensis	Argentina[c*], Bolivia[d], Paraguay[d], Uruguay[d]
T. protracta[b, occasionally c]	USA, Mexico
T. rubrovaria[c]	Argentina, Brazil, Uruguay
T. sanguisuga	USA[b, occasionally c]
T. venosa	Colombia[c], Ecuador, Costa Rica, Panama
T. williami	Brazil[a]

T = Triatoma; *R = Rhodnius*; *P = Panstrongylus*; *M = Mepraia*; *B = Belminus*; *Mi = Microtriatoma*; *a*-Found in human habitats (no further data); *b*-Adult specimens invading human habitats; *c*-Domestic or peridomestic colonies; *d*-No specific data; *indicates that only peridomestic colonies have been specifically reported; ‡-Probably the main Chagas disease vectors in the Amazon basin; *1*-Primarily associated with palm trees; *2*-Occasionally reported from palm trees; ■*Triatoma melanosoma* is probably a melanic form of *T. infestans*

Table 5 (continued). Sylvatic triatomines occasionally found in human habitats in the Americas

Adult specimens invading human habitats	
P. humeralis	Panama[b], Colombia
P. lignarius[2, b, ⊗]	Brazil, Guyana, Suriname, Venezuela, Ecuador
P. lutzi	Brazil[occasionally b]
P. tupynambai[a]	Brazil, Uruguay
Ps. coreodes[2, b]	Argentina, Paraguay, Bolivia, Brazil
R. brethesi[1]	Amazon[f] (Brazil, Venezuela)
R. neivai[1, b]	Venezuela, Colombia
R. robustus[1]	Amazon basin[b, f, ‡] (Colombia, Venezuela, Ecuador, Peru, Brazil, French Guiana)
T. bassolsae	Mexico[a]
T. bolivari	Mexico[a*]
T. breyeri	Argentina[b]
T. carcavalloi	Brazil[b]
T. circummaculata[a*]	Argentina, Brazil, Uruguay
T. costalimai	Brazil[b]
T. deanei	Brazil[b]
T. delpontei[b]	Argentina, Paraguay, Uruguay
T. dispar[b]	Costa Rica, Panama, Colombia, Ecuador
T. flavida	Cuba[b]
T. jurbergi[a]	Brazil
T. nigromaculata	Venezuela[b]
T. matogrossensis	Brazil[a]
T. melanocephala	Brazil[b]
T. obscura	Jamaica[b]
T. ryckmani[b]	Guatemala, Honduras, Nicaragua

T = Triatoma; *R = Rhodnius*; *P = Panstrongylus*; *Ps = Psammolestes*; *a*-Found in human habitats (no further data); *b*-Adult specimens invading human habitats; *f*-Wild bugs attack humans working on palm trees; *indicates that only peridomestic bugs have been specifically reported; ‡-Probably the main Chagas disease vectors in the Amazon basin; *1*-Primarily associated with palm trees; *2*-Occasionally reported from palm trees; ⊗*Panstrongylus herreri* and *lignarius* may be synonymous

1.2.2. TRIATOMINAE: METHODS OF STUDY

1.2.2.1. Systematics and evolution

·External morphological and chromatic characters. The taxonomy of triatomines is based on comparative analyses of morphological and chromatic characters; species concepts other than an exclusively phenetic one have seldom been explored (Usinger et al. 1966, Barrett 1996). Two major reviews (Lent & Wygodzinsky 1979, Carcavallo et al. 1997a, 1999a) summarise the knowledge on the morphological taxonomy of triatomines. In spite of extensive and detailed phenetic investigations, some species and populations still have controversial taxonomic status; external anatomical characters do not allow for clear distinction in all cases, and several taxa display a substantial degree of phenetic plasticity that may act either in the direction of

convergence or divergence, obscuring systematic considerations based on anatomical features (Lent & Wygodzinsky 1979, Dujardin et al. 1999b). Furthermore, the designation of ancestral (plesiomorphic) and derived (apomorphic) characters seems notoriously problematic in reduviids, preventing the use of Hennigian cladistics for the inference of phylogenetic relationships (Lent & Wygodzinsky 1979). Male genitalia anatomy has also been the subject of meticulous taxonomic studies; a comprehensive assessment of results obtained through the last 2-3 decades allowed for the exploration of phylogenetic relationships among different tribes (Lent & Jurberg 1985, 1987, Lent & Wygodzinsky 1979, Jurberg 1996, Jurberg et al. 1997, Pires et al. 1998). Other morphological techniques, such as the study of cuticular structures by scanning electron microscopy (e.g. Carcavallo et al. 1994, 1997b), the patterns of antennal sensilla (Catalá & Schofield 1994, Catalá 1996, 1997, 1999, Gracco & Catalá 2000), or eggshell structure (Barata 1996, 1998) have also been investigated.

Immature insects are wingless and lack ocelli; they are haematophagous and go through five developmental (nymphal) stages before moulting to adult (Lent & Wygodzinsky 1979, Galíndez Girón et al. 1998). All information related to morphological-chromatic characters in this work refers to adult insects; however, many of the molecular taxonomic tools discussed here are particularly helpful for the discrimination of nymphs, which are much more difficult to identify on morphological grounds than adult specimens.

·Morphometrics. In traditional morphometrics a series of standard measurements of the head capsule and hemelytra (sometimes also the thorax) of the bugs are taken and submitted to statistical analysis; various uni- and multivariate approaches are used for different purposes, but the general premise is that the patterns of variability and of similarity/difference revealed by metric analysis are a reflection of underlying genetic relationships. Several metric characters (linear measurements and ratios) are included in the classical descriptions of species, and recently further morphometric techniques have been developed to accurately separate species and even close geographic populations. Various types of multivariate discriminant analysis, particularly canonical variate analysis, are the methods of choice. These high-resolution techniques allow for the use of metric variables in the investigation of the structuring of conspecific bug populations. Applications include the surveillance of re-infestation of treated dwellings after control

interventions or the study of phenetic changes linked to the adaptation of bug populations to certain habitats (human domiciles, laboratories...). Traditional morphometric analyses may also be used to explore phylogenetic relationships among closely related entities (Dujardin & Casini 1996, Dujardin et al. 1997a,b, 1998a,b, 1999a-d, Galíndez Girón & Torres 1999, Soares et al. 1999, Patterson et al. 2001). More recently geometric techniques based on Cartesian coordinates and superimposition methods have been developed, but their application to the study of triatomine bugs is only incipient (Jaramillo 2000, Matías et al. 2001, Patterson 2002).

·Genetic approaches

i. *Cytogenetics.* Triatomines have holocentric chromosomes (i.e., lacking a discrete centromere), usually with a diploid complement of 2n=22 (20 autosomes plus XY in males and XX in females). Variations in the sex mechanism in *Triatoma* seem to follow a geographic pattern, with males of North and Central American species usually presenting X_1X_2Y, only found in *T. tibiamaculata* among the South American species so far surveyed (which typically present XY). Special cytogenetic techniques, such as C-banding and detailed analysis of heterochromatic regions, or the study of the meiotic behaviour of male chromosomes, have been used to complement morphological characterisation of Triatominae. Applications of these methods range from identification of similar species and detection of intraspecific variability to the investigation of evolutionary relationships at different levels of divergence (Panzera et al. 1992, 1995, 1997, 1998, 1999, Pérez et al. 1992, Panzera 1996).

ii. *Allozyme electrophoresis.* The analysis of the electrophoretic properties of allozymes [enzymes with identical function but distinct electrophoretic migration patterns (i.e., isoenzymes) encoded by different alleles of the same gene] has been extensively applied to the study of Triatominae. This approach has for decades represented the first choice to examine the molecular systematics and evolutionary relationships of many different organisms, including insects (Tibayrenc 1979, Thorpe & Solé-Cava 1994, Loxdale & Lushai 1998). It allows for the measurement of the degree of genetic variability within a species or population (as the proportion of heterozygotic loci), and interpopulation comparisons can be made regarding the relative frequencies at which polymorphic loci are scored; genetic distances and rates of gene flow (i.e. migration) between geographic populations can thus be calculated, and the structuring

of a metapopulation described in terms of *demes* or panmictic units (Thorpe & Solé-Cava 1994, Frías & Dujardin 1996, Loxdale & Lushai 1998). The genetic interpretation of isoenzyme electrophoretic patterns should however incorporate data from crossing experiments, which is not the case in many published studies; this may lead to overestimation of genetic distances and variability (Frías & Dujardin 1996).

In **taxonomy**, the main application of isoenzyme electrophoresis is probably the distinction of cryptic species (and the confirmation of the specific status of dubious populations); provided that enough individuals and loci are surveyed, genetic distances are usually higher between populations of closely related (but distinct) species than between conspecific populations, and reproductive isolation of sympatric populations may be inferred from allele frequencies and absence of putative hybrids (Tibayrenc 1979, Thorpe & Solé-Cava 1994). Cut-off values of genetic identity (Nei 1972) for species distinction have been proposed as $I \approx 0.85$ (Nei's genetic distance $D \approx 0.16$) based on comparisons among numerous different taxa, from mammals to plants (Thorpe & Solé-Cava 1994); in medically important insects (anophelines, sandflies and triatomines) D values above 0.1 are considered to indicate specific rank (Noireau et al. 1998). It has been noted however that for sympatric populations differences in gene frequencies are to be regarded as significant even if I values are high (Thorpe & Solé-Cava 1994). The finding of diagnostic loci allows for the assignation of isolated individuals to the populations they were drawn from; a locus is considered to be diagnostic when such a classification can be done with an error probability <0.05 (<0.01 in some cases) (Tibayrenc 1979).

Most triatomine species can be confidently distinguished by external morphological-chromatic characters; some groups are however more problematic, with a few of them being essentially isomorphic. The fact that descriptions of species based on a few type specimens can be vague and do not encompass intraspecific variation adds to the uncertainty in some cases. Some epidemiologically important taxa present such complications, and various researchers have turned to molecular systematics in an attempt to clarify their taxonomy. Examples are found both in the genus *Rhodnius* (mainly involving species of the *prolixus* group) and among *Triatoma* (*sordida-guasayana-patagonica-garciabesi*, *brasiliensis-petrochii*, or species belonging to the *oliveirai* and *phyllosoma* complexes).

In the case of *R. prolixus* (a major disease vector) and the closely related *R. robustus* (sylvatic and of comparatively minor medical importance), no fixed interspecific differences were found in several allozyme studies; the pairs *prolixus-robustus* and *nasutus-neglectus* could however be distinguished (Dujardin et al. 1991, Harry et al. 1992a,b, Harry 1993, Solano et al. 1996, Monteiro et al. 2002). Even when populations known to represent genetically distinct units (after mitochondrial DNA analysis) were compared, allozyme analysis revealed no differences between *prolixus* and *robustus*, and a single diagnostic locus was found between *nasutus* and *neglectus*; this was interpreted as a suggestion of recent divergence rather than conspecificity (Monteiro et al. 2001, 2002). Sylvatic populations of *R. prolixus* reported from the Magdalena valley (Tolima, Colombia) in 1995 were compared with domestic conspecifics found in sympatry using allozymes. Results were surprising in that they showed absence of gene flow between the two populations; several diagnostic loci and large genetic distances were scored (López & Moreno 1995). Subsequent studies confirmed the differences, suggesting that the sylvatic 'Tolima forms' were in fact a different, unnamed species related to the *pallescens-ecuadoriensis* group (Chávez et al. 1999, Dujardin et al. 1999a). In 1999 the new species was described as *R. colombiensis* (Moreno et al. 1999).

T. sordida, its former synonym *T. garciabesi*, *T. guasayana*, and *T. patagonica* are very similar morphologically but display distinct synanthropic trends; their overlapping ranges and high intraspecific anatomical variability make taxonomic determination complicated (Gorla et al. 1993). Allozyme studies confirmed *sordida*, *guasayana* and *patagonica* as valid taxa, and led to the revalidation of *T. garciabesi* and the discovery of two cryptic species within *T. sordida* (García et al. 1995a, Panzera et al. 1997, Noireau et al. 1998, Monteiro et al. 2001). The specific identity of *T. petrochii* was likewise confirmed using isoenzymes in a comparison with *T. brasiliensis* (Monteiro et al. 1998). Flores et al. (2001) found genetic distances not significantly different from zero between *T. longipennis*, *T. pallidipennis* and *T. picturata*, suggesting that species within the *phyllosoma* complex have diverged only recently (Flores et al. 2001), a view broadly supported by nuclear rDNA ITS-2 sequence data (Marcilla et al. 2001). Similarly, Noireau and colleagues failed to identify any isozymic differences between the closely related *T. williami* and *T. guazu* (members of the *oliveirai* complex from the Mato Grosso ecoregion), and questioned their validity (Noireau et al. 2002a).

The accurate taxonomic evaluation of field-collected specimens allows for detailed ecological investigations to be conducted; among the most interesting examples are the studies on Bolivian triatomines carried out by F Noireau and coworkers. After defining allozyme markers for the various species and populations (including *T. guasayana*, the two cryptic *T. sordida*, and a phenetically distinct sylvatic population of *T. infestans*), the ecotopes, behaviour, and associated epidemiological risk of those taxa were investigated in detail (Noireau et al. 1998, 1999a, 2000a,b, Noireau & Dujardin 2001).

Allozymes have also been used to explore **intraspecific variation** in triatomines (Dorea et al. 1982, Dujardin et al. 1987, 1998a,c, Dujardin 1990, Harry et al. 1992b, García et al. 1995b, Soares et al. 1999); results have shown that these markers are usually too conserved for taxonomic analysis of very closely related entities (Loxdale & Lushai 1998). When clear differences were scored the usual outcome was that more than one taxon was involved, either because of misidentification (such as in the case of *R. colombiensis*) or cryptic speciation (as with *T. sordida*) (Noireau et al. 1998, Moreno et al. 1999). The characterisation of phenetically diverse populations of *T. brasiliensis* may represent an exception (in that fixed allozyme differences were found between conspecific populations) or may result in new taxa being described (Costa et al. 1997, Costa 1999); Borges et al. (2000a) found no allozyme differences between three geographic populations of *T. brasiliensis brasiliensis*.

Relatively low levels of intraspecific variability represent a general feature in Triatominae. The proportion of variable loci ($P\approx0.33$ in *Triatoma*, 0.14 in *Rhodnius* and 0.23 in *Panstrongylus*) and associated gene diversity ($H\approx0.1$, 0.03, and 0.07 in the three main genera, respectively) are well in the lower range of the values usually scored for many insect groups[◊] (Schofield et al. 1995, 1999, Frías & Dujardin 1996). Mean heterozygosity values similar to those commonly found in triatomines have however been reported for other hemimetabolous insects (Harry 1992). In general it seems that habitat stability may be correlated with low levels of genetic diversity in many insects ($H\approx0.06$ for 117 species reviewed; Nevo 1978) and also in triatomines (Schofield et al. 1995); high overall values of genetic diversity reported for the Insecta could be the result of the inclusion of large amounts of data from drosophilid fruitflies (with $P\approx0.13$-

---

[◊]Unlike DNA sequence data, allozyme results are difficult to compare between laboratories; factors affecting the results include methods for the extraction and preparation of samples, electrophoresis conditions (buffer systems, electrical conditions, gels), or histochemical staining procedures

0.71 and $H \approx 0.025$-0.25) in the reviews (Harry 1992). From the standpoint of vector control-surveillance strategies, these low levels of genetic diversity in Triatominae have been regarded as an indication of the high vulnerability of many synanthropic populations to chemical control; these bug populations are in addition unlikely to develop insecticide resistance (Schofield et al. 1995, Guhl & Schofield 1996, Schofield & Dujardin 1997, Dujardin et al. 1998a, Monteiro et al. 2001).

Allozyme data have also been used to explore the **population genetics** of several triatomines. These studies are based on the comparison of allele frequencies between defined geographic or ecological populations; estimates of gene flow and migration rates can be calculated, and the structuring of the populations in *demes* (i.e., panmictic units where gene frequencies accord with expectations from random mating as described by the Hardy-Weinberg [HW] equilibrium) can be described (Thorpe & Solé-Cava 1994, Frías & Dujardin 1996). *T. infestans* populations for instance were panmictic within Andean villages in Bolivia and Peru, but strongly structured (and conforming to a model of isolation by distance) when different localities were compared. Analysis of allelic frequencies also allowed for the definition of the probable area of origin of *T. infestans* (Dujardin et al. 1998c). Further studies indicated that structuring within localities was also present, suggesting that the basic population unit would be represented by individual households (Breniére et al. 1998). Using a similar approach, Noireau et al. (1999b) showed that the panmictic unit of Bolivian populations of *T. sordida* (allozymic group 1) was larger than reported for *infestans*, with departures from HW equilibrium detected only between populations located over ~20km apart; this suggested a better dispersal capacity than previously thought for *T. sordida* (Noireau et al. 1999).

The **evolutionary interpretation** of allozymic results relies on the relatively straightforward relationships between zymograms and genes; each protein is a primary product of a gene (usually a single locus), which determines the structure of the peptide molecule with little or no environmental influence. In addition, those genes are (except for some linked loci) independent, and provide homologous characters easy to identify (Thorpe & Solé-Cava 1994, Dujardin et al. 1999d). Phylogenetic relationships may be explored by comparing measures of genetic identity/distance derived from allele frequencies (e.g., Nei 1972); an important assumption of distance-based approaches is

mutation rate homogeneity across taxa. Cladistic analyses rely on the ability to identify plesiomorphic and apomorphic character states in the dataset by comparing the ingroup with a suitable sister taxon; only shared-derived (synapomorphic) characters are used for phylogenetic inference purposes (Avise 1994, Thorpe & Solé-Cava 1994). Based on these premises, the phylogenetic relationships of several triatomine groups have been explored using allozyme techniques. These comparisons usually involved comparisons of genetic distances among a few, closely related species (e.g. García et al. 1995a, Pereira et al. 1996b, Solano et al. 1996, Panzera et al. 1997, Flores et al. 2001), but more complete investigations have been conducted with members of the tribe Rhodniini. Chávez et al. (1999) used genetic distances to construct an UPGMA dendrogram showing the relationships of nine species of *Rhodnius*. Three main clusters were identified: one basal clade [*pallescens* (*ecuadoriensis-colombiensis*•)] and two sister groups ([*prolixus* (*nasutus-neglectus*)] and [*stali* (*pictipes-brethesi*)]) (Chávez et al. 1999). Most of these relationships were confirmed by a cladistic analysis (using *T. infestans* as the outgroup), but *stali* and *pictipes* were sister taxa in their group ([*brethesi* (*pictipes-stali*)]), which appeared in a basalmost position in the cladogram; the position of *Psammolestes coreodes* within the tribe could not be satisfactorily resolved (Dujardin et al. 1999a). Recently FA Monteiro and collaborators addressed the phylogeny of the Rhodniini using 12 enzyme loci. Distance analysis produced an UPGMA dendrogram showing paraphyly of *Rhodnius*, with *Psammolestes tertius* as a sister group to the clade [(*prolixus-robustus*) (*nasutus-neglectus*)] within the cluster {*domesticus* (*Ps. tertius* [(*nasutus-neglectus*) (*prolixus-robustus*)])}. A second main clade was comprised of {*pictipes* (*brethesi* [*pallescens-ecuadoriensis*])} (Monteiro et al. 2002).

iii. *Molecular biological methods*. The analysis of DNA sequence polymorphisms has provided new insight on the taxonomy, population genetics, and evolutionary and phylogenetic relationships of many different taxa, including arthropods (Avise 1994, Simon et al. 1994, Friedrich & Tautz 1995, Collins & Paskewitz 1996, Crampton et al. 1996, Esseghir et al. 1997, Loxdale & Lushai 1998, Page & Holmes 1998, Donnelly et al. 2002, Gaunt & Miles 2002). Only a few among the many DNA-based techniques currently available have been hitherto applied to triatomines (Beard & Lyman 1999,

---

• *Rhodnius colombiensis*, undescribed at the time, was considered as a 'sylvatic form of *R. prolixus*' from Colombia until its affinities were discovered; it was then renamed as the 'Tolima form of *ecuadoriensis*'

Monteiro et al. 2001). These include randomly amplified polymorphic DNA (RAPD) (Carlier et al. 1996, Dujardin et al. 1998a, García et al. 1998, Noireau et al. 2000b, Borges et al. 2000, Jaramillo et al. 2001), species-specific length variation of rDNA amplicons (Jaramillo et al. 2001), single strand conformational polymorphism analysis (Stothard et al. 1998b), sequencing of selected nuclear (Lyman et al. 1999, Bargues et al. 2000, Marcilla et al. 2000, 2001, 2002) and mitochondrial gene fragments (García & Powell 1998, García 1999, Stothard et al. 1998b, Monteiro et al. 1999a,b, 2000, García et al. 2001, Gaunt & Miles 2002), and analysis of polymorphic microsatellite markers (Harry et al. 1998, Anderson et al. 2002).

·**RAPD**. Amplification of polymorphic DNA with random primers has been used to investigate genetic variability in several arthropod taxa, including medically important insects (e.g. Ballinger-Crabtree et al. 1992, Kambhampati et al. 1992, Adamson et al. 1993, Favia et al. 1994, Wilkerson et al. 1995). In triatomines, diversity among *T. infestans* and *T. sordida* populations was assessed in an early study by Carlier et al. (1996). The banding patterns clearly separated both species, and revealed higher intraspecific variability than observed with allozymes; within *T. infestans*, sylvatic and domestic populations were also well separated (Carlier et al. 1996). In a taxonomic study mainly involving closely related species of *Rhodnius*, García et al. (1998) found diagnostic profiles allowing for the separation of specimens belonging to the pairs *R. prolixus-robustus* and *R. neglectus-nasutus*. In an intraspecific study on Honduran and Colombian populations of *R. prolixus* (between which no allozyme differences were scored), RAPD patterns were population-diagnostic and showed reduced variability among Central American specimens, indicating recent dispersal of a genetically limited subset from a South American population and suggesting that local eradication of the vector could be contemplated in the former region (Dujardin et al. 1998a). Noireau et al. (2000b) characterised different Bolivian populations of *T. infestans* using various methods: while allozymes could not detect any significant variations, RAPD profiles separated two main clusters (bugs from the Andean highlands and those from the Chaco); within each clade, sylvatic and domestic populations could also be distinguished (Noireau et al. 2000b). Geographic populations belonging to the same phenetic sub-group of *T. brasiliensis* also showed distinct RAPD patterns (Borges et al. 2000a,b). Although dead specimens of all stages can be used for RAPD analysis, and

resolution is much higher than that of isoenzyme electrophoresis in detecting intraspecific variability, the use of random primers prompts the possibility of flawed results due to sample contamination; specific conditions used for the preparation of samples and PCR may also affect the results, reducing reliability and reproducibility. Finally, RAPD markers are dominant in heterozygous individuals, precluding the interpretation of results in genetic (Mendelian) terms (Loxdale & Lushai 1998, Beard & Lyman 1999).

·**Microsatellites** are series of short repetitive motifs [e.g., $(GT)_n$ or $(AT)_n$] within nuclear DNA; they are highly polymorphic, neutral, and exhibit Mendelian inheritance and codominance (Jarne & Lagoda 1996). Although their function and mode of evolution are not totally understood, the mentioned features result in microsatellites being considered as very promising tools for population genetic analysis when other markers (particularly allozymes) do not show an adequate degree of polymorphism at the subspecific level. Two preliminary works have been published reporting the identification and characterisation of microsatellite loci in triatomines. Harry et al. (1998) studied sylvatic populations of *R. pallescens* collected from *Attalea* palm trees in northern Colombia; six out of the 10 microsatellites evaluated were present in frequencies not different from those expected under HW equilibrium, suggesting panmixia among palm populations in the area; two to 20 alleles were observed per locus, and expected heterozygosity ranged from 0.32 to 0.94. Amplicons were also obtained using template DNA from *R. ecuadoriensis* (6 primer pairs) and *R. prolixus* (two primer pairs), but not for *T. infestans* (Harry et al. 1998). More recently, Anderson et al. (2002) identified and characterised eight microsatellite loci (6 to 27 alleles per locus) from populations of *T. dimidiata* from Mexico, Guatemala and Honduras; because of limited sample size no attempt was made to analyse results in terms of population genetics.

·**Sequencing** of selected genomic fragments allows for the direct assessment of DNA polymorphisms, providing researchers with the ultimate information for phylogenetic inference and evaluation of kinship among organisms and populations (from conspecific groups to extremely divergent taxa) (Avise 1994, Page & Holmes 1998). A large amount of information on the genetic constitution of medically important groups of insects has been accumulating over the last decade; two recent reviews present brief

summaries of molecular studies on the vectors of malaria (Donnelly et al. 2002) and Chagas disease (Monteiro et al. 2001). The use of DNA sequence data for both intra- and interspecific studies in Triatominae is more thoroughly discussed in Sections 6.3. and 7.3.; only a brief overview of published works is presented here.

The mitochondrial 16S ribosomal RNA gene was used to investigate the genetic variability and phylogenetic relationships of various triatomine species in an early study (Stothard et al. 1998b). A 400 basepair (bp) gene fragment was amplified by PCR and sequence variation assessed by single strand conformational polymorphism (SSCP) and direct sequencing. SSCP separated well the three genera, and also revealed intrageneric differences; a greater SSCP variation was detected within *Rhodnius* (represented by laboratory colonies of *prolixus*, *neglectus*, *nasutus*, *pictipes*, and *ecuadoriensis*) than in *Triatoma* (*infestans*, *protracta*, *platensis*, *delpontei*, and *nitida*; *Mepraia spinolai* was treated as *Triatoma* [see Lent et al. 1994]). Not surprisingly, *M. spinolai* (a different genus) and *T. protracta* (a North American species) were distinguished from *infestans*, *platensis*, and *delpontei* (three closely related species; see Pereira et al. 1996); the lack of differentiation between *T. nitida* (a Central American species with apparent affinities with *T. neotomae*; Lent & Wygodzinsky 1979) and the *infestans* group was on the contrary unexpected. Within *Rhodnius*, distinction among *prolixus*, *nasutus* and *neglectus* was problematic, but the group could be separated from the pair *pictipes-ecuadoriensis*. Phylogenetic relationships inferred from a 290bp alignment (8 species: *R. prolixus*, *R. pictipes*, *R. ecuadoriensis*, *T. infestans*, *T. platensis*, *T. delpontei*, *P. herreri*, and *P. megistus*) revealed two main clades (one comprising the Rhodniini and the other one the Triatomini). The separation (Kimura 2-parameter distances) between *pictipes-ecuadoriensis* and *prolixus* (0.19-0.29) was larger than that between *Panstrongylus* and *Triatoma* (0.12-0.16). Distances between *Rhodnius* and *Triatoma* ranged from 0.22 to 0.35, and those between *Rhodnius* and *Panstrongylus* from 0.25 to 0.43 (Stothard et al. 1998b).

The phylogeny of eleven species of the *T. infestans* complex (*T. infestans*, *T. guasayana*, *T. patagonica*, *T. sordida*, *T. platensis*, *T. delpontei*, *T. brasiliensis*, *T. rubrovaria*, *T. maculata*, *T. matogrossensis*, and *T. vitticeps*) plus *T. circummaculata*, *T. protracta*, *T. dimidiata*, and *T. mazzottii* (not belonging to that complex), has been explored by analysing the sequences of the 12S and 16S ribosomal RNA and the

cytochrome oxidase I (co*I*) genes; *R. prolixus*, *P. megistus* and *M. spinolai* were used as outgroups for different analyses (García & Powell 1998, García 1999, García et al. 2001). The phylogenies confirmed the close relatedness of *T. infestans* and *T. platensis*, and provided evidence suggesting mtDNA introgression between these interfertile species. *T. circummaculata* and *T. rubrovaria* appeared within the *infestans* complex; *T. vitticeps* was found to occupy a basalmost position to the South American *Triatoma* species, with *P. megistus* clustering (with low bootstrap support values) with North American species (*T. dimidiata*, *T. mazzottii* and *T. protracta*). *T. sordida*, unexpectedly close to *T. matogrossensis*, was very different from *T. guasayana* (García et al. 2001).

The mitochondrial large subunit rRNA (mtlsu rRNA, 383bp) and cytochrome *b* (cyt*b*, 399bp) genes have been used by Lyman et al. (1999) and Monteiro et al. (1999a,b, 2001). Separation of closely related species, including the almost sibling *R. prolixus–robustus–neglectus* or two species of the *phyllosoma* complex (*T. dimidiata* and *pallidipennis*) was achieved with both markers; phylogenetic trees (derived from distance matrices using neighbour-joining) were broadly in agreement and supported by relatively high bootstrap values. Notable exceptions were the uncertain placements of *P. megistus* and *Dipetalogaster maxima* (Lyman et al. 1999). In general, the Rhodniini were clearly separated from the Triatomini. In both the separate analyses of the mtDNA datasets (Lyman et al. 1999) and a combined analysis totalling 782bp (Monteiro et al. 2001), the clade comprising Central and North American species of *Triatoma* (*pallidipennis*, *dimidiata*, *sanguisuga*, *nitida*, *protracta*, and including *Dipetalogaster maxima*) was separated from the South American species group (*T. infestans*, *T. sordida* and *P. megistus* as indicated by the combined dataset), suggesting independent evolutionary histories; this basic split within the Triatomini was supported by bootstrap values of ~80% (mtlsu rRNA), ~75% (cyt*b*), and 61-81% (combined dataset) (Lyman et al. 1999, Monteiro et al. 2001). Furthermore, the fact that both *Dipetalogaster* and *Panstrongylus* were nested within *Triatoma* was indicative of the paraphyletic nature of this genus; similarly, *Psammolestes coreodes* appeared clearly within *Rhodnius*.

Monteiro et al. (1999b) evaluated the intra-specific variability of the cyt*b* gene and studied the relationships between species belonging to the *infestans* complex (*T. infestans*, *T. melanosoma*, *T. brasiliensis*, *T. sordida*, *T. garciabesi*, and *T. platensis*). *T. melanosoma* could not be separated from *T. infestans*, and some phenetically defined

populations of *T. brasiliensis* presented a higher degree of divergence than that recorded for closely related species. MtDNA sequences allowed the differentiation between *T. infestans* geographic populations from Bolivia, Brazil, and Argentina.

In a later survey, Monteiro et al. (2000) used mtDNA sequence diversity to assess relationships among several species of Rhodniini, including *Rhodnius* (*brethesi*, *pictipes*, *pallescens*, *colombiensis*, *domesticus*, *nasutus*, *neglectus*, *neivai*, and various populations of *prolixus* and *robustus*) and *Psammolestes* (*tertius* and *coreodes*). Both distance- and character state-based methods were used for phylogenetic reconstruction, using *T. infestans* as the outgroup; results confirmed the paraphyly of *Rhodnius*, with *Psammolestes* appearing closer to the '*prolixus* clade' than this was to the '*pictipes* clade'. Maximum parsimony analysis of 1429bp (including fragments of the mtlsu rRNA, mt cyt*b*, and the nuclear D2 variable region of the 28S rRNA gene) yielded two main clades: [(*brethesi-pictipes*) (*ecuadoriensis-pallescens*)] (72% bootstrap support) and {*Ps. tertius* [*neivai* (*domesticus* {*nasutus* [(*neglectus-prolixus*) (*robustus-prolixus*)]})]} (95% support). In general, the results suggested that the taxonomically problematic *R. prolixus* and *R. robustus* constitute valid entities, with synanthropic populations of the former presenting significantly lower genetic diversity than sylvatic specimens classified as *R. robustus* (Monteiro et al. 2000, 2001). In a cyt*b* (414bp), neighbour-joining tree, the '*prolixus* clade' {*domesticus* [*nasutus* (*robustus* {*neglectus-prolixus*})]} (support 67%), the '*pictipes* clade' {*pictipes* + *brethesi* [*pallescens* (*colombiensis-ecuadoriensis*)]} (51% bootstrap support), a '*Psammolestes* clade' (*tertius-coreodes*) (82%), and *R. neivai* formed a basal, four-branch polytomy (Monteiro et al. 2000).

These findings established the usefulness of mtDNA analysis for the study of triatomine molecular systematics and phylogeny (both at supra- and subspecific levels). The fact that the complete sequence of the mitochondrial genome of *T. dimidiata* has been obtained (Dotson & Beard 2001), together with the extensive knowledge about the biological properties (structural, functional and evolutionary) of metazoan mtDNA (see Section 6.3.), will facilitate the use of mtDNA in triatomine studies.

Nuclear genomic targets have also been used in molecular taxonomic and evolutionary studies on Triatominae. Apart from the combined analysis of mt gene fragments with the D2 variable region of the 28S rRNA gene mentioned above, the second internal transcribed spacer (ITS-2) of the nuclear rDNA has recently been tested

as a molecular marker for populations, species, and phylogenetic relationships in various Triatominae. These comparisons mainly involved Mesoamerican *Triatoma* species belonging to the *phyllosoma* complex, some other South American *Triatoma*, and several species of *Panstrongylus*; various populations of *T. infestans* and a few Rhodniini have also been analysed (Marcilla et al. 2000, 2001, 2002).

Phylogenetic analysis of ITS-2 sequence data revealed two major clades within the Triatomini; one was comprised of Central and North America species (including *T. dimidiata*, three species of the *phyllosoma* complex [*T. phyllosoma*, *T. mazzottii*, *T. longipennis*, *T. picturata*, and *T. pallidipennis*], *T. barberi*, and *D. maxima*) and a second one encompassing South American species (*T. infestans*, *T. sordida*, *T. brasiliensis*, and *P. megistus*) (Marcilla et al. 2001). This analysis was later extended to include several other species of *Panstrongylus* (*rufotuberculatus*, *geniculatus*, *chinai*, *lignarius*, and *herreri*); the topology of the inferred trees varied slightly, with the main difference referring to the tendency of all *Panstrongylus* species to cluster together with the Mesoamerican Triatomini, rather than with the South American species, even if with relatively low bootstrap and puzzle values (Marcilla et al. 2002). These studies provided evidence suggesting that some taxonomic rearrangements might be necessary with regard to the *phyllosoma* complex species (e.g., *picturata* and *longipennis* had identical sequences, and the degree of variability within *T. dimidiata* was larger than that recorded for the rest of *phyllosoma* species) and some *Panstrongylus* (notably, *lignarius* and *herreri* from various collection sites presented identical ITS-2 sequences). In addition, the unexpected clustering of *P. rufotuberculatus* as a sister taxon to the *dimidiata-phyllosoma* clade, with *T. barberi-D. maxima* occupying the immediate external branch, was interpreted as a strong indication of the non-monophyly of the genus *Panstrongylus* (Marcilla et al. 2002).

Further findings included the confirmation of the paraphyletic nature of the genus *Rhodnius*, with *Psammolestes tertius* clustering with *R. prolixus* and *R. stali* appearing in a basal position to that pair, and the 100% sequence identity between Ecuadorian and Honduran specimens of *T. dimidiata*, strongly suggesting that the former are recent derivatives of a Mesoamerican population probably introduced by people into western Ecuador in the recent past (Marcilla et al. 2001). Similar conclusions regarding human intervention in the passive dispersal of synanthropic bug populations were drawn from

the analysis of ITS-2 sequence variation among *T. infestans* geographic populations (Marcilla et al. 2000). Specimens collected from houses in seven localities of central Paraguay were found to have identical sequences, differing by only two transitional mutations and the elongation of one dinucleotide microsatellite from Bolivian bugs collected in the Andean highlands. Sequence homogeneity of Paraguayan populations suggested recent colonisation, probably originating from a synanthropic population dispersed by humans (Marcilla et al. 2000).

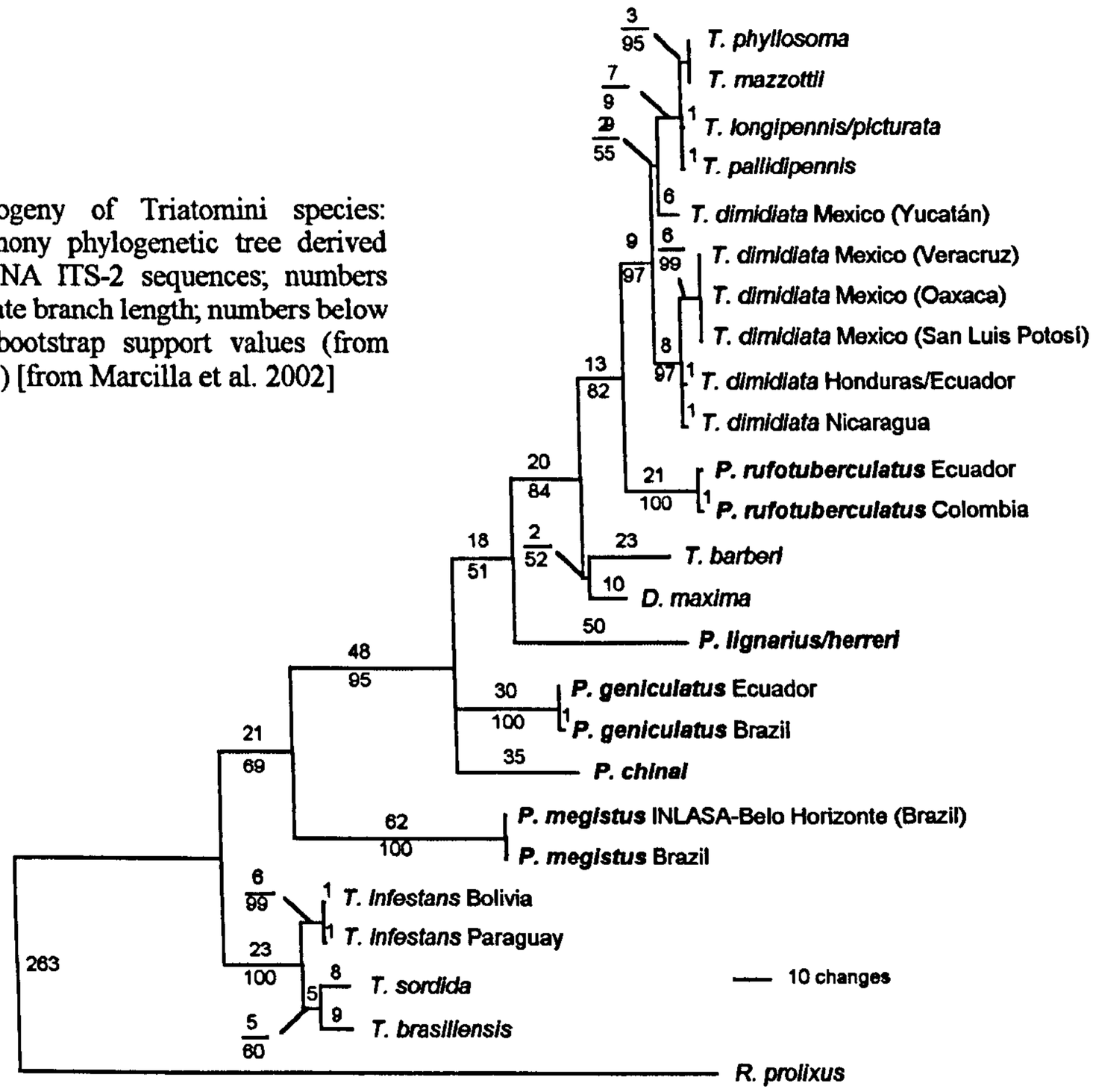


Figure 4. Phylogeny of Triatomini species: maximum parsimony phylogenetic tree derived from nuclear rDNA ITS-2 sequences; numbers above lines indicate branch length; numbers below lines represent bootstrap support values (from 1000 replications) [from Marcilla et al. 2002]

The 18S subunit of the nuclear rDNA is much more conserved than ITS-2 or mt protein-coding genes; in triatomines, the substitution rate seems to approach 1.8% sequence divergence per 100 million years, up to 55 times slower than ITS-2 (Bargues et al. 2000). These substitution rate estimates were used to calculate the time to common ancestry among various triatomine taxa; under the mentioned hypothesis of an 18S rDNA molecular clock, divergence between the ancestors of the Triatomini and Rhodniini would approach an estimate of ~48.9-64.4 million years ago (mya) (Palaeocene-Eocene), when Africa and South America were already separated (Bargues

et al. 2000). All estimates of divergence between Meso- and South American species of Triatomini (*T. infestans* vs. *phyllosoma* complex species or *D. maxima*) fell between 22.8 and 31.9 mya (18S) and between 19.5 to 34.1 mya (ITS-2), long before the Panama Isthmus linked North and South America in the middle Pliocene (~3 mya, although biogeographical evidence shows that some biotic interchange could have occurred from the late Miocene-early Pliocene, i.e. ~6-7 mya); these results were interpreted as strong evidence suggesting independent evolutionary origins of both groups, whose common ancestors would have diverged during the Oligocene-Miocene (Bargues et al. 2000; see also Cox & Moore 2000).

In a recent work, Gaunt and Miles (2002) used mt co*I* sequence and amino acid data to define a mitochondrial molecular clock hypothesis for various insect orders, including Hemiptera. Their results, strengthened by the observed congruence with the fossil record, with biogeographical evidence, and with independent estimates of divergence between lepidopterans, suggest that the evolutionary split of the ancestors of extant Triatomini (represented by 7 South American species of *Triatoma*, two *Panstrongylus*, and *Eratyrus mucronatus*) and Rhodniini (*R. prolixus*, *R. pictipes* and *R. neivai*) took place ~99.8-93.5 mya, roughly coinciding with the break-up of Gondwana during the Cretaceous (Gaunt & Miles 2002).

·Other approaches

Some alternative *biochemical approaches* have been applied to the study of triatomines. Electrophoresis of some salivary anti-haemostatic proteins of the Rhodniini or gas chromatography of cuticular hydrocarbons are techniques that can be used to solve some taxonomical problems, and may be also of help in defining phylogenetic relationships among Triatominae (Juárez 1996, Pereira 1999, Juárez et al. 1999, 2000).

Intercrossing experiments have provided valuable information about the systematics of several groups of triatomines (e.g. Usinger et al. 1966, Barrett 1996); the fact that the biological species concept does not prevail in the currently accepted taxonomic arrangement has however reduced the impact of such contributions. The almost purely typological approach favoured by H Lent and P Wygodzinsky in their monograph (Lent & Wygodzinsky 1979) represents still the mainstream view of triatomine systematics, and was indeed adopted by RU Carcavallo and coworkers in their recent revisions (Carcavallo et al. 1997a, 1999a, 2000). Thus, subspecies defined on the grounds of

intercrossing experiments (for instance among the *T. phyllosoma*, *T. protracta*, or *Paratriatoma hirsuta* groups) have been either elevated to specific rank or treated as variants within a species; in most of these cases it was argued that the information available from cross-mating experiments was inadequate. One exception was the recognition of *T. peninsularis* and *T. sinaloensis*, two sibling species that showed complete reproductive isolation in experimental (mutual and with *T. protracta*) crosses (Lent & Wygodzinsky 1979, Barrett 1996). New genetic data from allozymes (Flores et al. 2002), 18S rDNA (Bargues et al. 2000) and rDNA ITS-2 sequences (Marcilla et al. 2001) are providing evidence that several of the species of the *phyllosoma* complex should be better regarded as subspecies, as indicated by interfertility patterns (Mazzotti & Osorio 1942, Usinger 1944, Usinger et al. 1966). The results of extensive experimental crosses between colonies classified as either *R. prolixus* or *R. robustus* reported in 1996 by TV Barrett showed complex interfertility patterns between putative '*prolixus*' and material classified as '*robustus*' on morphological grounds (Barrett 1996). The author noted that the original description of *R. robustus* by Larrousse was hardly diagnostic and based on two females from different areas of the Amazon; the information on any character derived from new material, such as in the case of male genitalia, relies therefore on a dubious identification (Barrett 1996). These problems have deep epidemiological implications in areas where the existence of sylvatic populations of *R. prolixus* are suspected, and are beginning to be addressed using molecular techniques; overall, the results seem to suggest that several genetically distinct populations occur over the Amazon-Orinoco basins, and that a major problem with the results so far obtained is the accurate identification of the material used in the comparisons (Monteiro et al. 2001 and unpublished data).

Some specialists believe that reconsideration of the *species concept* is needed in triatomine systematics, particularly because it is becoming increasingly possible to incorporate phylogenetic information into the description of the biological discontinuity within the current subfamily (CJ Schofield, pers. comm.). Such evolutionary considerations were explicitly disregarded by Lent and Wygodzinsky (1979) on the grounds of the impossibility of working out a cladistic system with the morphological information available at the time; the only attempt these authors made (a cladogram of 13 species of *Panstrongylus* based on 21 characters) assumed monophyly of the genus

and showed patterns of relationships later disproved by rDNA ITS-2 sequence data – but some others, such as the tight kinship between *lignarius* and *herreri*, were confirmed (Lent & Wygodzinsky 1979, Marcilla et al. 2002).

A more realistic (i.e., '*natural*') systematic approach than the typological-nominalistic one currently favoured would most likely help improve our understanding of the biology and evolutionary trends of these medically important insects. Such a system would need not rely on *purely*, mutually exclusive typological, biological, or phylogenetic species concepts. The first one has provided the basic framework for the current systematic perception (which has been extremely useful for the definition, implementation and assessment of vector control interventions), but its limitations are becoming increasingly apparent – as reflected in the proliferation of new species described on the grounds of minor anatomical traits while genetically distinct taxa are overlooked because of their convergent phenotypes. The definition of biological species in Triatominae would still require extensive experimental work on interbreeding patterns; special attention must be drawn to the correct design of such experiments (including sound biogeographical and ecological considerations) to obtain meaningful results. Phylogenetic approaches are contributing to the (objective) definition of divergent triatomine lineages sharing a common pattern of ancestry, but the profound bearing of interfertility at the microevolutionary scale (best defined as reticulate genealogical or 'tokogenetic' relationships, and the domain of population genetics) should not be neglected; as Avise and Wollenberg observed, "the supposed sharp distinction between biological and phylogenetic species concepts is illusory. Historical descent and reproductive ties are related aspects of phylogeny and jointly illuminate biotic discontinuity" (Avise & Wollenberg 1997; abstract; see also Ayala & Fitch 1997). Avise and Johns (1999) have proposed the use of estimated divergence times to develop a standardised classification for all extant organisms. Their proposal does not include "species-level taxonomic assignments, where biological criteria including reproductive isolation should be applied" (Avise & Johns 1999, p. 7361).

1.2.2.2. Ecology

·Biogeography.[▽] The study of the spatial distribution of vector populations is crucial for the assessment of their relative epidemiological importance and for the development of control strategies. In this context, special attention is directed towards the definition of the natural ranges of species frequently reported as invading households from sylvatic ecotopes (Dujardin et al. 1991, WHO 1991, Schofield et al. 1995, 1996, Carcavallo et al. 1998a, Salvatella et al. 1998, Coura et al. 1999, 2002, Schofield & Ponce 1999, Silveira 1999). Biogeography studies the relationships between organisms and the geographic/climatic areas they occupy. When field data are incomplete (which is often the case for most triatomine species), this may help predict the actual distribution range of the vectors and their potential ability to disperse to suitable but as yet uncolonised areas (Cox & Moore 2000). Whenever clear associations between particular triatomine species and their habitats can be defined, such as in the case of several *Rhodnius* species and palm trees, the biogeography of those habitats may provide useful information about the range of the associated vector populations.

Biogeographical information may also help understand the evolutionary trends of a group of organisms. Thus, current theories about the evolution of members of the Rhodniini derived from the study of biogeographic traits of phenotypically defined species; this allowed for hypotheses to be put forward that could be tested by allozyme electrophoresis, comparative analysis of sensilla patterns, morphometrics, RAPD, and partial sequencing of selected genomic targets (Schofield & Dujardin 1999).

·Sylvatic populations. As a preliminary consideration, we might observe that 'sylvatic' and 'domestic' are anthropocentric concepts that hardly portray the complex reality of nature. 'Domestic' species of triatomines breed in a great variety of sylvatic ecotopes, and many 'sylvatic' triatomines show a patent (albeit geographically limited) capacity of colonising domestic habitats; other sylvatic species (such as some of the Amazonian *Rhodnius* and many *Panstrongylus*) are proving capable of exploiting food resources provided by humans, even without actually establishing breeding colonies in artificial habitats (e.g., Lent & Wygodzinsky 1979, Zeledón 1981, Barrett 1991, Schofield 1994, Noireau et al. 1994, 1995, 1997, 2000b, Carcavallo et al. 1998a,b, Dujardin et al. 1998b, Naiff et al. 1998, Pinho et al. 1998, Salvatella et al. 1998, Valente

---

[▽]See also Chapter 3

et al. 1998, Angulo et al. 1999, Moreno & Carcavallo 1999, Salomón et al. 1999, Silveira 1999, Reyes-Lugo & Rodríguez-Acosta 2000, Vallejo et al. 2000).

Studying sylvatic bug populations is logistically difficult, expensive, and time-consuming. Several techniques have been used for the detection and study of the natural ecotopes of different triatomines, including habitat inspection and dissection, tracking of mammals to their nests and burrows, and various types of baited traps (e.g. Miles 1976, Tonn et al. 1976, Miles et al. 1981b, 1983, Carcavallo 1985, Pizarro & Romaña 1998, Noireau et al. 1999c, 2000a, 2002, Abad-Franch et al. 2000). These basic methods, combined with a large number of circumstantial observations on the occurrence of various species in different environments and on host associations (including bloodmeal analysis; e.g. Zeledón et al. 1973, Carcavallo et al. 1998b, Costa et al. 1998, Canals et al. 2001), and with the results of light trapping and casual captures of adventitious bugs (e.g. Naiff et al. 1998, Zeledón et al. 2001a), have provided with abundant, yet fragmentary information about the general ecological features of different species of Triatominae. Several reviews have been published that summarise such information (Lent & Wygodzinsky 1979, Barrett 1991, Carcavallo et al. 1998a,b).

The Amazon basin provides an interesting example of the potential importance of sylvatic triatomines in Chagas disease transmission; it is furthermore pertinent to the present work in that several palm tree-living *Rhodnius* species are involved. The region has classically been considered as a non-endemic area, despite well-known enzootic cycles of *T. cruzi* involving mainly *Rhodnius* species (but also *Panstrongylus*, *Eratyrus*, *Cavernicola*, and others) (Miles et al. 1981b, 1983, Barrett 1991, Coura et al. 1999, 2002, Valente et al. 1999, Teixeira et al. 2001). Recent surveys have shown increasing rates of human *T. cruzi* infection in the region, giving rise to the thought that Chagas disease may become endemic in some areas of Amazonia (Chico et al. 1997, Coura 1990, Coura et al. 1994, 1999, 2002, Valente et al. 1999, Teixeira et al. 2001). Human-related ecological alterations (colonisation and new economic activities, deforestation, changes in traditional lifestyles etc.) are regarded as responsible for this new epidemiological pattern (see Coura 1990, Coura et al. 1994, 1999, 2002, Valente et al. 1999; for a general overview of the effects of deforestation on vector-borne diseases, see Walsh et al. 1993 and Molyneux 1997). These ecological changes visibly involve uncontrolled deforestation, frequently by means of slash-and-burn (see Cochrane et al.

1999, Laurance et al. 2002, Nepstad et al. 2002); it has been proposed that forest clearance could result in a reduced availability of blood and natural shelters for sylvatic triatomines. Simultaneously, permanent human settlements offer stable and abundant food sources to the bugs. A pattern of selective deforestation is often observed in which some palm trees are kept in strongly altered environments (Whitlaw & Chaniotis 1978, Miles et al. 1983, Barrett 1991). These palms may become the only suitable shelters for opportunistic rodents and marsupials, and also for triatomine bugs pre-adapted to palm trees (e.g., *R. pictipes*, *R. robustus*). Bug colonies can grow using opossum and rodents as hosts in palms located near human dwellings; when the system nears its carrying capacity, starved adult bugs will start dispersive flights more frequently, and some will end up in houses (a process perhaps favoured by the removal of physical obstacles caused by deforestation, and by the use of artificial light) and attempt to feed on the inhabitants. These sylvatic bugs ultimately become the most important vectors of Chagas disease in such areas (including the view that contamination of fruit presses by adventitious bugs flying from palms is a peculiar form of vector-borne transmission) (Miles et al. 1983, Barrett 1991, Sherlock 1999, Dias et al. 2001, Coura et al. 2002). There have been isolated reports of true domestic colonies of Amazonian triatomines. *P. geniculatus*, known to fly frequently into houses but thought to be unable to colonise human habitats, has successfully adapted to peridomestic pigsties in the Brazilian Amazon – feeding both on humans and pigs (Valente et al. 1998, 1999, Valente 1999).

The absence of domestic bug colonies, together with operational difficulties (small communities and farms scattered in vast jungle areas) and relatively low morbidity and mortality rates, means that traditional control approaches (household treatment with residual insecticides) are unlikely to be cost-effective in the Amazon. The development of alternative strategies critically depends on a better understanding of the ecology of disease transmission by non-domiciliated vectors. This in turn requires detailed studies of vector ecology at the population level, including the relationships between defined triatomine taxa and the risk of disease transmission in different ecological settings (Dias et al. 2001). Alternative control procedures (early case detection, selective focal spraying, physical barriers, intervention of sylvatic ecotopes near human settlements, integrated habitat management, etc.) will be required, but the definition of specific strategies still requires extensive operational research.

·Domestic vector populations. Chagas disease is primarily transmitted by domestic/peridomestic populations of triatomine bugs. The study of their biological, ecological and behavioural characteristics has contributed substantially to Chagas disease control throughout the Southern Cone countries (see WHO 1991, 1996, 1997, 1998, 2000, Dias & Schofield 1999, Moncayo 1999, Silveira 1999, Silveira & Vinhaes 1999, WHO/CTD 2002). The identification of ecological factors favouring colonisation of human habitats by triatomines has been addressed by many studies (e.g. Minter 1978, Mott et al. 1978, Lent & Wygodzinsky 1979, Zeledón & Vargas 1984, Briceño-León 1990, Starr et al. 1992, WHO 1991, Gürtler et al. 1992, Andrade et al. 1995a, Salvatella et al. 1998, Moreno & Carcavallo 1999, Oliveira-Lima et al. 2000, Cohen & Gürtler 2001, Espinoza-Gómez et al. 2002), but the full comprehension of the driving mechanisms of that process is still far from being achieved. Some species are commonly found in human-related environments (while others are rare), but virtually all triatomines (with the possible exception of those strongly associated with particular hosts/microhabitats, like *Cavernicola pilosa, T. delpontei,* or *Psammolestes* spp.) have the potential of invading artificial ecotopes (e.g., Dujardin et al. 1991, Noireau et al. 1994, 1995, Carcavallo et al. 1998a, Salvatella et al. 1998, Valente et al. 1998, 1999, Borges et al. 1999, Costa 1999, Silveira 1999, Soares et al. 1999, Valente 1999, Ramsey et al. 2000, Abad-Franch et al. 2001b, Wolff & Castillo 2002; see also tables 4 and 5). Synanthropic triatomine behaviour has been interpreted as a response of bug populations to ecological changes linked to human economic activities (see Carcavallo et al. 1998a, Salvatella et al. 1998, Curto de Casas et al. 1999, Moreno & Carcavallo 1999, Schofield et al. 1999). Once permanent breeding colonies are established in human environments (Barrett 1991, Schofield et al. 1999), the bugs may eventually spread to neighbouring homes and be passively carried by humans to different geographical areas (Schofield 1994, Dujardin et al. 1998a,c, Carcavallo et al. 1999c).

Schofield et al. (1999) have proposed the view that domestication "can be envisaged as an extension of the evolutionary route from predator to nest-dwelling bloodsucker" (p. 376). As observed by Barrett (1991), there is however "little evidence that colonies in houses are genetically different from wild populations of the same species" (Barrett 1991; p. 144). Most of the differences so far recorded may indeed be attributed to demographic processes that cannot be regarded as truly adaptive or evolutionary; thus,

the apparently reduced genetic variability of synanthropic bug colonies might reflect that only a limited subset of the original (sylvatic) gene pool is represented in domestic populations (Schofield et al. 1999), and changes in body size and sexual dimorphism may be readily associated to increases in survivorship and population density due to improved food availability, decreased predation, and enhanced microclimate stability in synanthropic environments (Dujardin et al. 1999c). Other changes, such as the correlation between the density of antennal sensilla and the number of habitats in which various species occur in nature, do not necessarily reflect synanthropism; they may conceivably be related to a long-term (evolutionary) process of adaptation to highly stable, nest-like habitats (Schofield et al. 1999), but these need not be man-made. The same authors acknowledged that, at least in some cases, 'synanthropism' probably is no more than a largely stochastic process – and also that a review of the information at hand "raises more questions than answers" (Schofield et al. 1999; p. 377).

1.3. Chagas disease in Ecuador

The public health dimension of Chagas disease seems to have been underestimated to a great extent in Ecuador. In this Section several sources of epidemiological information, including unpublished records and data from ongoing studies, are reviewed. Original epidemiological estimates relevant to the design of control schemes are derived from the critical revision and joint analysis of those data.

1.3.1. HISTORICAL OVERVIEW

Some pre-Columbian ceramics from the province of Manabí suggest that Romaña's sign was already known in those areas before the arrival of the Europeans (León 1949). During the Spanish conquest, some Pizarro soldiers suffered from a disease they described as 'eye sickness' acquired at the Portoviejo valley (Manabí) around 1530. According to Álvarez (1984), the descriptions resemble the characteristic lesions of Romaña's sign, and the author suggests that they might be attributed to Chagas disease. Stål and Whymper reported the presence of the principal vector, *Triatoma dimidiata*, Ecuador in the XIX century; the species was first described by Latreille in 1811, based on specimens collected by Humboldt and Bonpland in Ecuador (Lent & Wygodzinsky 1979, Aguilar et al. 1999). In 1917, Tamayo established the association between the insect bite and a clinical picture including local inflammation, oedema and fever, but it was not until 1930 that Arteaga confirmed the existence of American Trypanosomiasis in the zone of the Coastal Railroad (Guayaquil-Salinas). The following investigations certified that Chagas disease was endemic in urban Guayaquil, with *T. dimidiata* colonies breeding within the cane-and-wood houses. The Santa Ana and El Carmen hills, two deprived areas of the city, were the most strongly affected, and they seem to remain so nowadays (Arteaga 1930, Aguilar et al. 1999).

During the 40s and 50s new disease foci were reported from the provinces of Guayas, Manabí, Los Ríos, and in temperate areas of the Andean provinces of Loja, Azuay and Bolívar. It is today accepted that the main endemic areas are located in the provinces of Guayas, Manabí and El Oro, but new foci reported from the Amazon region and currently under investigation strongly suggest that the northern Ecuadorian Amazon is to be considered an endemic area as well. The lack of systematic studies in other provinces makes it complicated to assert that the disease is not endemic in areas (e.g., in the provinces of Los Ríos, Esmeraldas, Pastaza, Loja, Imbabura, Pichincha,

Azuay, etc.) where ecological and socio-economic traits are broadly comparable to those of well-known chagasic zones (Aguilar et al. 1999).

1.3.2. EPIDEMIOLOGY

WHO estimates indicate that some 30000 people (i.e., 0.25% of the population) are infected by *T. cruzi* in Ecuador (WHO 1991, Schofield 1994, Schofield & Dias 1996). This prevalence rate is noticeably smaller than accepted estimates for neighbouring countries with broadly comparable ecological and socio-economic characteristics: 0.25% in Ecuador, versus about 3.5% in Colombia and 3% in Peru (Schofield & Dias 1996, Guhl 1997, Guhl & Vallejo 1999).

Apparently, this estimate was calculated based primarily on prevalence rates reported for donations to blood banks in Quito, located in a non-endemic, triatomine-free area at ~2800m above sea level. Although rural immigrants from endemic zones surely arrive in Quito, migration involving potentially infected people has been mainly directed to the coastal city of Guayaquil, the largest urban, industrial, and commercial centre of the country, where prevalence rates are higher (see Aguilar & Yépez 1996, Aguilar et al. 1999, and below). This might be the origin of a relevant underestimation, and is probably one of the reasons why Chagas disease control has not been treated as a priority in the Ecuadorian public health agenda. In an attempt to investigate and clarify the situation, we reviewed the results of serological surveys carried out in the country, from the early investigations in the 1950s to those currently ongoing (table 6). These include studies in known endemic areas, various blood banks, and exploratory surveys in some zones considered as non-endemic. Although different techniques were used to detect anti-*T. cruzi* antibodies and diverse methods applied for the design of the studies, the general picture that emerged contrasts with the official figures, placing Ecuador in a situation comparable to that of other countries of the region. The fact however that a significant part of the Ecuadorian population live in Andean highlands where no domestic triatomines occur (with perhaps a few exceptions such as *T. carrioni* in some areas), and the absence of extremely efficient, synanthropic vector species (say, *T. infestans*, present in southern Peru, or *R. prolixus*, the main vector in northern Colombia), probably contribute to a lower general (country-wide) prevalence rate.

Table 6. Studies on seroprevalence of Chagas disease in Ecuador (modified from Aguilar et al. 1999)

Author, year, technique	Province	Locality	Positives		Observations
Montalván 1952	El Oro	Zaruma	29%		696 samples examined
Complement Fixation (CF)	El Oro	Machala	13.3%		
	Guayas	Gral. Vernaza	3 1%		
	Guayas	Salitre	11.8%		
	Manabí	**Portoviejo**	**3.8%**		
	Manabí	Chone	5 8%		
INH (1949-1957)	Coastal region	Various	13.9%		3333 samples examined
(reported by Rodríguez 1959)	(all provinces)				>80% ⊕ born in Coastal region
CF					>10% ⊕ born in Loja
Espinoza 1955	El Oro	Various	8.25%		Schoolchildren in rural areas and
CF	Guayas	Various	3.5%		in urban Guayaquil
	Guayas	Guayaquil (urban)	1.87%		
	Loja	Various	2 02%		
	Los Ríos	Various	1.47%		
Rodríguez 1959	Guayas	Various	8.8% (4 04% SC)		Guayas 320 samples; El Oro· 66
CF	El Oro	Various	22.7% (7.62% SC)		samples
	Manabí	**Portoviejo**	**4%**		
	Manabí	Bahía de Caráquez	3%		
	Loja	Various	2% (SC)		
	Esmeraldas	Various	4%		
	Los Ríos	Various	1 47% (SC)		
INH (1962-1967)	Coastal region	Various	2.96%		2160 samples (suspect patients)
(reported by Gómez 1968)	(all provinces)	Various	0.83%		3600 sera (random sampling)
CF					
Andrade A et al. (unpubl.)	Manabí	Picoazá	17%		521 samples
CF					
Mimori et al. 1985	Guayas	Pedro Carbo	4.3% (2.2% SC)		233 samples (446 SC)
Indirect Haemagglutination (IHA)	El Oro	Zaruma	15.5% (3.9% SC)		433 samples (305 SC)
SNEM-TDR 1986	El Oro	**Portovelo**	**17.1%**		Guayaquil (urban)· 2078 samples
(reported by Reyes 1992; further data:	El Oro	Piñas	14.6%		El Guavo: 43 samples
Ministry of Public Health, unpubl.)	El Oro	Zaruma	10.1%		Pasaje· 41 samples
Indirect Immunofluorescence (IFI)	El Oro	El Guavo	2.3%		
	El Oro	Pasaje	7.3%		
	Guayas	Guayaquil (urban)	2 6%		
Racines et al. 1994	El Oro	**Portovelo**, Piñas and	4 to 6	1.4%	Results by age groups
IFI + ELISA		Zaruma	6 to 8	1.13%	1514 samples examined
			8 to 10	1.52%	1.85% ⊕ for IgG
			10 to 12	2.2%	0.07% ⊕ for IgM
			12 to 14	1.88%	
			14 to 15	0.92%	
Guderian et al. 1994 (unpubl.)	El Oro	Marcabelí	7 2%		No further data available
ELISA/Recombinant antigen	El Oro	Pena	6%		
	El Oro	Balsas	11.4%		
Grijalva et al. 1995	Pichincha	Quito	9.25%		Quito Red Cross blood bank (335
ELISA + Western Blot					samples, 1992-93)
Chico et al. 1997	Napo/Orellana*	Various	6.03%		18 Quechua communities (1011
ELISA/Recombinant antigen					samples)
Grijalva et al. 1997	Pichincha	Quito	0.03%		Blood banks (62121 samples
ELISA	Various	Various	0.23%		total, 1994-96); mainly Andean
					region (also Manabí, Esmeraldas)
Romero et al. 1998	Guayas	Guayaquil	5.9%		Regional Hospital blood bank
MicroELISA					(2961 samples, 1996-97); ⊕ from
					Guayas, Manabí and El Oro
Guevara et al. 1999	Guayas	Guayaquil	1.1%		Blood banks (1423 [Guayaquil]
ELISA	El Oro	Machala	6.9%		and 203 [Machala] samples)
Córdova et al. 1999	El Oro	Piñas	11.1%		262 samples; all positives
IHA + microELISA (3 antigens)					confirmed with 4 tests
Racines & Grijalva 1999	Manabí	Paján (203 samples)	0.98%		Preliminary results; some of
INH/TDR/Ohio Universtity (unpubl.)	Manabí	**Portoviejo** (628 s.)	**1.91%**		them need to be confirmed (see
MicroELISA	Guayas	Balzar (178 s.)	0 56%		text also)
	Guayas	Guayaquil (2604 s.)	1.8%		
	Guayas	Pedro Carbo (94 s.)	1.06%		
	Sucumbíos	Lago Agrio (493 s)	2.23%		Amazon Region***
	Sucumbíos	Putumayo** (1232 s.)	1.3%		6365 samples; 0.8% ⊕
	Sucumbíos	Shushufindi (263 s.)	0%		Coastal Region
	Napo/Orellana*	Aguarico (1796 s)	0 39%		3718 samples; 1.7% ⊕
	Napo/Orellana	Coca (105 s.)	0%		Andean Region (Sierra)
	Napo/Orellana	El Chaco (311 s.)	0.32%		905 samples; 0.33% ⊕
	Napo/Orellana	J. Sachas (167 s)	0 6%		
	Napo/Orellana	Loreto (186 s.)	1.61%		Total
	Napo/Orellana	Orellana (495 s.)	1.61%		10988 samples
	Napo/Orellana	Quijos (40 s)	0%		1.06% ⊕
	Napo/Orellana	Tena (1050 s.)	0.2%		
	Pastaza	Various (227 s.)	0.44%		
	Cotopaxi	La Maná (501 s.)	0.4%		
	Cotopaxi	Bangua (404 s.)	0.2%		

*Napo/Orellana were separated in two provinces in 1998; **indigenous communities along the San Miguel-Putumayo river; ***confirmatory tests and wider sampling increased overall prevalence rate to 2.9% in the Amazon region (MJ Grijalva et al. unpublished); INH=National Institute of Hygiene; SNEM=National Vector Control Service; ⊕=seropositives; SC=school children; data in bold type correspond to areas studied in this project

An examination of recent surveys (field- and blood bank-based, most of them unpublished) helped us estimate the current epidemiological status of Chagas disease in the country. In an ongoing nation-wide study (National Institute of Hygiene, Ecuador, and Ohio University, USA), 10988 blood samples were analysed in 1999, including localities from the coastal, Andean, and Amazon regions. An overall figure of 1.06% of these samples was found to be positive by means of microELISA (Aguilar et al. 1999). Confirmatory tests subsequently carried out in ~7000 samples from the Amazon Region revealed an overall prevalence of 2.9% (MJ Grijalva, pers. comm.), with the majority of seropositives being native to the region and an epidemiological profile suggesting established endemicity and active transmission (figure 5).

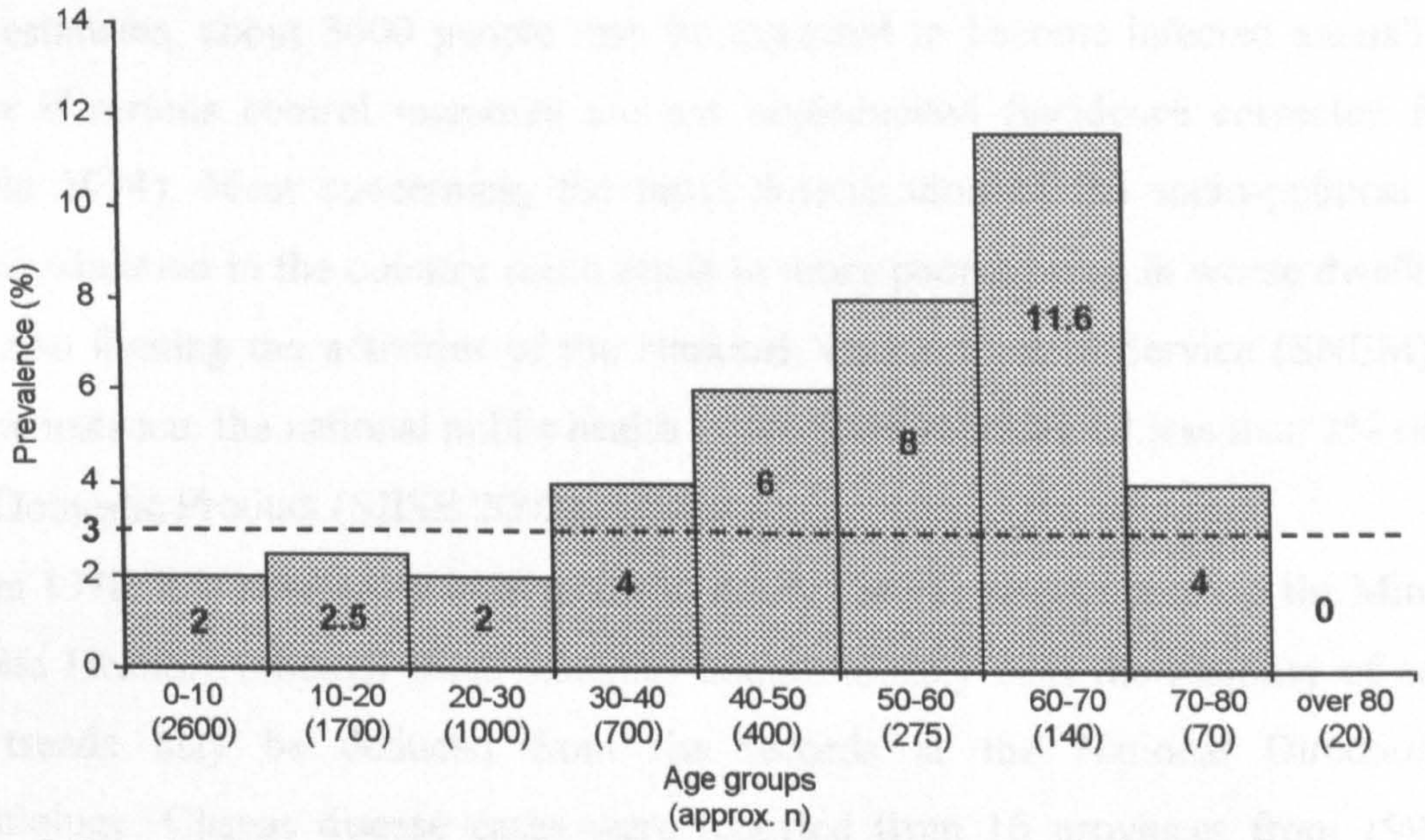


Figure 5. Prevalence of anti-*Trypanosoma cruzi* antibodies in the Ecuadorian Amazon region. The dashed line indicates overall mean prevalence (~3%). Data from MJ Grijalva (Ohio University, USA) and LE Escalante (National Institute of Hygiene, Ecuador) (with permission)

Guevara et al. (1999) reported prevalence rates of 1.1% in the Red Cross blood bank of Guayaquil and 6.9% in the Machala blood bank. In 2000, results from over 70000 blood samples (>90% of donations) analysed at the blood bank of Quito yielded an overall 0.1% seropositivity (HM Aguilar, pers. comm.). Taking into account only the available studies on blood banks from the main cities of Ecuador after 1995, the global prevalence rates are 0.1% in Quito (non-endemic area with low immigration from endemic zones) and 4.4% in Guayaquil (both endemic and recipient of immigration from rural endemic areas, and the largest urban centre of the country); results from smaller banks, including Machala, yield a global figure of 0.35% positives. Results of

field surveys conducted from 1990 to the present show a general prevalence rate of around 2.8% (>14200 samples and ~400 positives).

We consequently estimated that at least about 1% of the total Ecuadorian population (~125000-135000 people) may be infected by *T. cruzi*; no less than 2.2 to 3.5 million people (perhaps up to 5-6 million) live under risk conditions in areas where vector-borne transmission has been documented or is highly likely. This indicates that some 300 Ecuadorians probably die each year of Chagas disease; although official mortality records (available from 1979 to 1996, except for 1991-1992) do not reflect such a figure, 20% to 25% of deaths in rural Ecuador were classified as 'deaths with poorly defined diagnosis' (Ministry of Public Health, mortality records 1979-1996). According to our estimates, about 3000 people may be expected to become infected annually in Ecuador if serious control measures are not implemented (incidence corrected from Schofield 1994). Most concerning, the rapid deterioration of the socio-political and economic situation in the country could result in more people living in worse dwellings, and is also limiting the activities of the National Vector Control Service (SNEM). In 1998 for instance, the national public health expenditure represented less than 2% of the Gross Domestic Product (SIISE 2002).

From 1978, it is mandatory in Ecuador to notify Chagas disease cases to the Ministry of Public Health. Although these statistics unquestionably miss the majority of cases, some trends may be deduced from the records at the National Direction of Epidemiology. Chagas disease cases were reported from 16 provinces from 1990 to 2000; over 8.5 million people inhabit those provinces. Only four Andean provinces (Carchi, Cotopaxi, Tungurahua, and Chimborazo) did not report cases (table 7).

Table 7. Provinces of Ecuador with records of Chagas disease cases between 1990 and 2000

Coastal Region	Sierra (Andean Region)	Amazon Region
Esmeraldas	Imbabura	Sucumbíos
Manabí	Bolívar	Napo
Guayas	Cañar	Orellana
Los Ríos	Azuay	Pastaza
El Oro	Loja	Morona-Santiago
	Pichincha	Zamora-Chinchipe

Reported incidence from 1978 to 1999 shows a slight positive slope. Two high peaks recorded in 1984 and 1994 (figure 6) seem related to clinical-epidemiological surveys carried out by SNEM-TDR and Mimori and coworkers in the early 80s, and by various groups in the early 90s (see table 6 above).

Figure 6. Reported incidence of Chagas disease in Ecuador (cases/100000), 1978-1999
Data from the National Direction of Epidemiology, Ministry of Public Health of Ecuador

It is compulsory to test all blood donations for anti-*T. cruzi* antibodies in Ecuador, but the application of the directive still requires diagnostic kits, equipment and reagents, training of personnel, quality control schemes, and also a suitable information policy addressed to customers and those in charge of blood bank management (see for instance Grijalva et al. 1997, Abad-Franch & Aguilar 2002).

South America

Figure 7. Map of Ecuador

1.3.3. VECTORS

Vector-borne transmission is responsible for about 80% of new Chagas disease cases; in the absence of suitable treatments or vaccines, preventive action crucially depends on the control of synanthropic triatomine populations. In Ecuador, where a comprehensive control programme is as yet to be implemented (see below), the need for updated entomological information was identified as a critical issue. Particularly, no relevant information was available about *R. ecuadoriensis*, considered the second main disease vector in the country, and suitable control strategies were accordingly lacking.

The extraordinary biodiversity of Ecuador is also reflected in the subfamily Triatominae. We found reports indicating the presence of 18 species (over 13% of all those known in the world) in the relatively small Ecuadorian territory. These included old and doubtful reports of *T. infestans* and *R. prolixus*, and it also became later apparent that several records were probably due to misidentification of preserved specimens and/or labelling errors. There is general agreement in that *T. dimidiata* is the principal domestic vector in the country, but the situation with other species remains unclear. *R. ecuadoriensis*, *T. carrioni*, *P. rufotuberculatus*, and *P. chinai* have all been incriminated in disease transmission in the coastal/Andean foci, and *R. pictipes*, *R. robustus* and *P. geniculatus* are thought to be of importance in the Amazon region. The following table includes all triatomine species recorded in Ecuador (Aguilar et al. 1999, Abad-Franch et al. 2001a,b); a more detailed account of our findings and main conclusions is presented in Chapter 3.

Table 8. Triatomine species reported from Ecuador

Tribe	Genus	Species
Cavernicolini	*Cavernicola*	*Cavernicola pilosa*
Rhodniini	*Rhodnius*	*Rhodnius ecuadoriensis*
		Rhodnius pictipes
		Rhodnius robustus
		*Rhodnius prolixus**
Triatomini	*Triatoma*	*Triatoma dimidiata*
		Triatoma carrioni
		Triatoma venosa
		Triatoma dispar
		*Triatoma infestans**
	Panstrongylus	*Panstrongylus chinai*
		Panstrongylus rufotuberculatus
		Panstrongylus geniculatus
		Panstrongylus herreri
		Panstrongylus howardi
		*Panstrongylus lignarius**
	Eratyrus	*Eratyrus mucronatus*
		Eratyrus cuspidatus

*Dubious records

Vector control efforts. In the 1950s and 1960s, the National Institute of Hygiene and Tropical Medicine (INH) was officially in charge of Chagas disease vector control in Ecuador. A pilot campaign was carried out in 1963 in the cities of Guayaquil (province of Guayas) and Portoviejo (province of Manabí); although control interventions extended to other endemic areas, they were soon discontinued and coverage was incomplete.

In 1973, the malaria control service ('Servicio Nacional para la Erradicación de la Malaria y Control de Vectores', SNEM) was appointed by the Ministry of Public Health to carry out Chagas disease control activities. In 1978 a vertical spraying campaign (using the organophosphate malathion) was undertaken by the SNEM in Guayaquil and various localities of the Portoviejo river valley in Manabí. This was the last large-scale intervention specifically designed with the aim of reducing Chagas disease transmission in Ecuador; during the last 25 years, chemical control of domestic triatomine populations has depended upon the effects of mosquito control activities carried out by the SNEM (using mainly malathion, and also synthetic pyrethroids in some areas) (Aguilar et al. 1999, 2001, Abad-Franch & Aguilar 2002). Occasionally, the workers of the SNEM locally spray triatomine-infested houses and peridomiciles in response to requirements made by the community (often coinciding with epidemiological surveys related to research projects), but these interventions are not carried out within the framework of a national control programme; they as a result generally lack specific resources and continuity, and are almost never documented, hampering any attempts of evaluation.

From 1999 however, the Ministry of Public Health undertook a wide re-assessment of the epidemiological and control status of all vector-borne and tropical diseases in the country. As a result, Chagas disease control was officially put back in the list of priority public health issues, and a group of experts was commissioned to elaborate the technical guidelines for a national control programme. Various research groups were at the same time working on different clinical-parasitological, sero-epidemiological, and vectorial aspects of Chagas disease in Ecuador*. Two manuals were published by the Ministry of

---

*AG Guevara and coworkers (Vozandes Hospital, Quito); MJ Grijalva, J Racines (deceased), L Escalante, and colleagues (National Institute of Hygiene, Catholic University of Quito, and Ohio University, USA); and HM Aguilar and coworkers (Instituto 'Juan César García', Quito, and Ministry of Public Health); this project was developed in close collaboration with Dr HM Aguilar, and the rest of colleagues cited here generously provided help, assistance, and expert advice in numerous occasions

Public Health in 2001 with the conclusions and recommendations of the working groups on Chagas disease control (Abad-Franch et al. 2001a, Aguilar et al. 2001). However, financial constraints and political instability (e.g., four different Ministers of Public Health have headed the Ministry since 1998) have obstructed the implementation of such recommendations.

1.3.4. CLINICAL ASPECTS

Historical data show that, from the early 20s, it was not uncommon that clinical pictures compatible with Romaña's sign were diagnosed at hospitals in Guayaquil. Varas (1942) indicated that this form of periorbital oedema was extremely frequent in the city. Subsequent studies continued to show this trait (cf. Espinoza 1955, Rodríguez 1961 1963, Gómez 1968, Rassi 1979, Álvarez 1984). S Galindo registered 560 acute cases from the records of the National Institute of Hygiene and Tropical Medicine; the majority of these patients were from the provinces of Guayas, Manabí, El Oro, and Los Ríos, all in the coastal region (cf. Aguilar et al. 1999). Acute cases have also been recorded from the northern Amazon region; these usually corresponded to children presenting fever, generalised oedema, hepatosplenomegaly and signs of myocarditis (Amunárriz 1991, Amunárriz et al. 1991, Aguilar et al. 1999).

Chagasic cardiopathy has been identified as the predominant chronic form. Galindo (1958, 1959) found chagasic aetiology in 20% of 150 patients with heart disease in Guayaquil; 20.7% of chagasic patients were under 40 years old and presented severe cardiopathy, and over 50% of them died in the following 15 months. Gómez (1968) found electrocardiographic signs compatible with chagasic cardiopathy in 1.4% of randomly selected, apparently healthy people. Kawabata et al. (1987) reported that 40% of 154 seropositives from El Oro presented electrocardiographic abnormalities (vs. 8% of seronegatives), but the age profile of the patients (with ECG alterations in 64% of those over 60 years old) suggested relatively low rates of premature mortality; 22% of seropositives over 40 years old presented complete right bundle branch block. These authors found evidence suggesting lower prevalence of ECG abnormalities among seropositives from coastal localities of the province of Guayas than among patients from Andean communities of El Oro (Kawabata et al. 1987).

We have conservatively estimated that ~14000 patients (20% of the ~70000 seropositives over 30 years old) suffer different degrees of chronic cardiopathy in

Ecuador (Abad-Franch & Aguilar 2000). Digestive disease is also present in the country, with both megaoesophagus and severe cases of megacolon described in patients from different provinces (Guevara et al. 1997). It has been estimated that digestive forms may represent about 3% of all chronic chagasic patients, and AG Guevara (pers. comm.) suggested that megacolon may be more frequent than heart disease among chronic patients from El Oro (Aguilar et al. 1999, Abad-Franch & Aguilar 2000). However, most Chagas disease cases probably go undiagnosed by the weak primary health care network of rural Ecuador; even those cases with severe pathology may simply result in yet more 'deaths with poorly defined diagnosis' registered in official mortality records.

1.3.5. BURDEN

According to our prevalence estimates, some 120000-150000 people may carry *T. cruzi* infection in Ecuador, implying that 300 deaths and 3000 new cases would occur each year in the absence of systematic control interventions (modified from Hayes & Schofield 1990 and Schofield 1994). In Brazil, WHO estimations indicate that the 120000 deaths attributable to Chagas disease during 1989-1990 generated an economic loss of about 650 million US dollars (5400 US$ per death) in terms of disability-adjusted life years before the age of retirement (set at 62 years old) (WHO 1997). Thus, the 300 deaths/year in Ecuador (probably a conservative estimate) would generate equivalent annual losses of over 1.2 million US$ (4000 US$/death). Furthermore, the same WHO report indicates that 5000 US$ are spent annually in the medical care of each patient with serious heart or digestive chronic disease, including pacemakers and surgical procedures (WHO 1997). It can be assumed (again very conservatively) that ~14000 chagasic heart patients (20% of the ~70000 seropositives over 30 years old) live in Ecuador. According to local clinical patterns, approximately 10% of these patients would require specialised treatment; adjusting the cost to 3000 US$/patient/year, the overall investment required for the proper clinical management of these chronic heart patients would reach 4.5 million US$/year. Finally, Schofield (2000c) presented general estimates of costs associated with both acute and chronic chagasic patients (including medical expenses and productivity losses). According to these estimates, the ~3000 new (acute) cases expected each year in Ecuador would have associated costs approaching 1.2 US$ million/year (~400 US$/case); the costs associated with the (at least) 20000

chronic patients (~700 US$/case/year) would reach about 14 million US$/year (Abad-Franch & Aguilar 2000).

The overall economic burden of Chagas disease in Ecuador (calculated on the basis of conservative prevalence and morbi-mortality estimates) may therefore be of over 20 million US dollars each year. This is, for instance, approximately two-fold that associated with malaria in the country; malaria control is at the top of the official priority list among vector-borne diseases, followed by dengue. Only very recently the Ministry of Public Health included Chagas disease control in that list – as the third official priority (Abad-Franch & Aguilar 2000).

The cost of a comprehensive, long-term national programme for the control of Chagas disease transmission in Ecuador would not exceed 15 to 18 million dollars to be invested in a 15-year period; estimated savings would therefore reach some 20 US$ per US dollar invested in the programme, an estimate similar to that of 25 US$ advanced by WHO (Moncayo 1999). Taking into account the possibility of co-ordinated control activities covering Chagas disease and malaria (feasible in many coastal areas of the country), additional savings nearing 3 to 5 million US$/year could be anticipated (Abad-Franch & Aguilar 2000).

In 1997 a common, international initiative for the control of Chagas disease throughout the Andean countries (Venezuela, Colombia, Ecuador and northern Peru) was launched under the auspices of the WHO (UNDP/World Bank/WHO TDR 1997). The present project was developed, in co-ordination with the Ministry of Public Health of Ecuador, in the context of that initiative, and resulted in contributions to the development of the technical guidelines for a national control programme (Aguilar et al. 2001) and an additional, specific document on the control of vector-borne Chagas disease (Abad-Franch et al. 2001a). The principal results of the original research carried out in relation to that initiative constitute the main body of this thesis.

1.4. *Rhodnius ecuadoriensis*: general introduction

1.4.1. SYSTEMATICS

R. ecuadoriensis was first described in 1958 (Lent & León 1958). Type specimens were collected in synanthropic habitats in the Catamayo river valley, near the Peruvian border (La Toma, province of Loja, ~3°59'S, 79°21'W), and are deposited at the Fiocruz reference collections in Rio de Janeiro. *R. ecuadoriensis* is one of the 17 recognised species within the tribe Rhodniini. It is considered to belong to a group of closely related species including *R. pallescens* and *R. colombiensis*. These three species occur west of the Andes, while the rest of the genus is widespread on the eastern side of the cordillera – with the notable exception of domestic populations of *R. prolixus*. The Amazonian clade including *R. pictipes*, *R. brethesi* and *R. stali* (probably also *R. paraensis*) is usually considered as a sister group to *pallescens-colombiensis-ecuadoriensis* (Dujardin et al. 1999a, Lyman et al. 1999, Monteiro et al. 2000).

Males of *R. ecuadoriensis* are typically about 13mm long; females, as in all triatomines, are larger on average (about 14.5mm). Adult bugs are light brown-yellowish with a pattern of irregular dark markings dispersed on the body surface. The scutellum has 2+2 well-defined anterior carinae that merge into a single median carina on the posterior process. The median process of the male pygophore is triangular and has a rounded apex (Lent & León 1958, Lent & Wygodzinsky 1979). These morphological characters, mainly the colour and mottled pattern and the distinct carinae of the scutellum, place *R. ecuadoriensis* close to *R. pallescens* (and, to a lesser extent, *R. pictipes*). The close relationships with *R. colombiensis* (similar to *pallescens* but without the irregularly mottled pattern), not straightforward from anatomical characters, was demonstrated by isoenzyme studies (Chávez et al. 1999, Dujardin et al. 1999a).

1.4.2. EPIDEMIOLOGICAL SIGNIFICANCE

R. ecuadoriensis is an important local vector of Chagas disease in coastal areas and western Andean valleys of central-southern Ecuador and northern Peru (Aguilar et al. 1999, Abad-Franch et al. 2001b, Cuba Cuba et al. 2002). However, studies specifically addressing the epidemiological significance of the species are lacking. Results of limited serological surveys carried out in areas were *R. ecuadoriensis* and *T. dimidiata* (the main vector in the country) are the only (or largely prevailing) domiciliary species can be compared as an approximation to their relative epidemiological importance. *T. dimidiata* is

the only domestic vector in Guayaquil and other localities of the same province (Guayas), and in urban/semiurban areas of Manabí (Portoviejo, Manta, Chone), whereas *R. ecuadoriensis* prevails in the southern inter-Andean valleys of El Oro and Loja. Results from various studies (see table 6) are summarised in the following figure (Montalván 1952, Espinoza 1955, Rodríguez 1959, Mimori et al. 1985, Reyes 1992, Aguilar et al. 1999). While comparing results from different studies with broadly varying designs and methods is problematical, the trends of differences *within* surveys may help understand the vectorial role of each species.

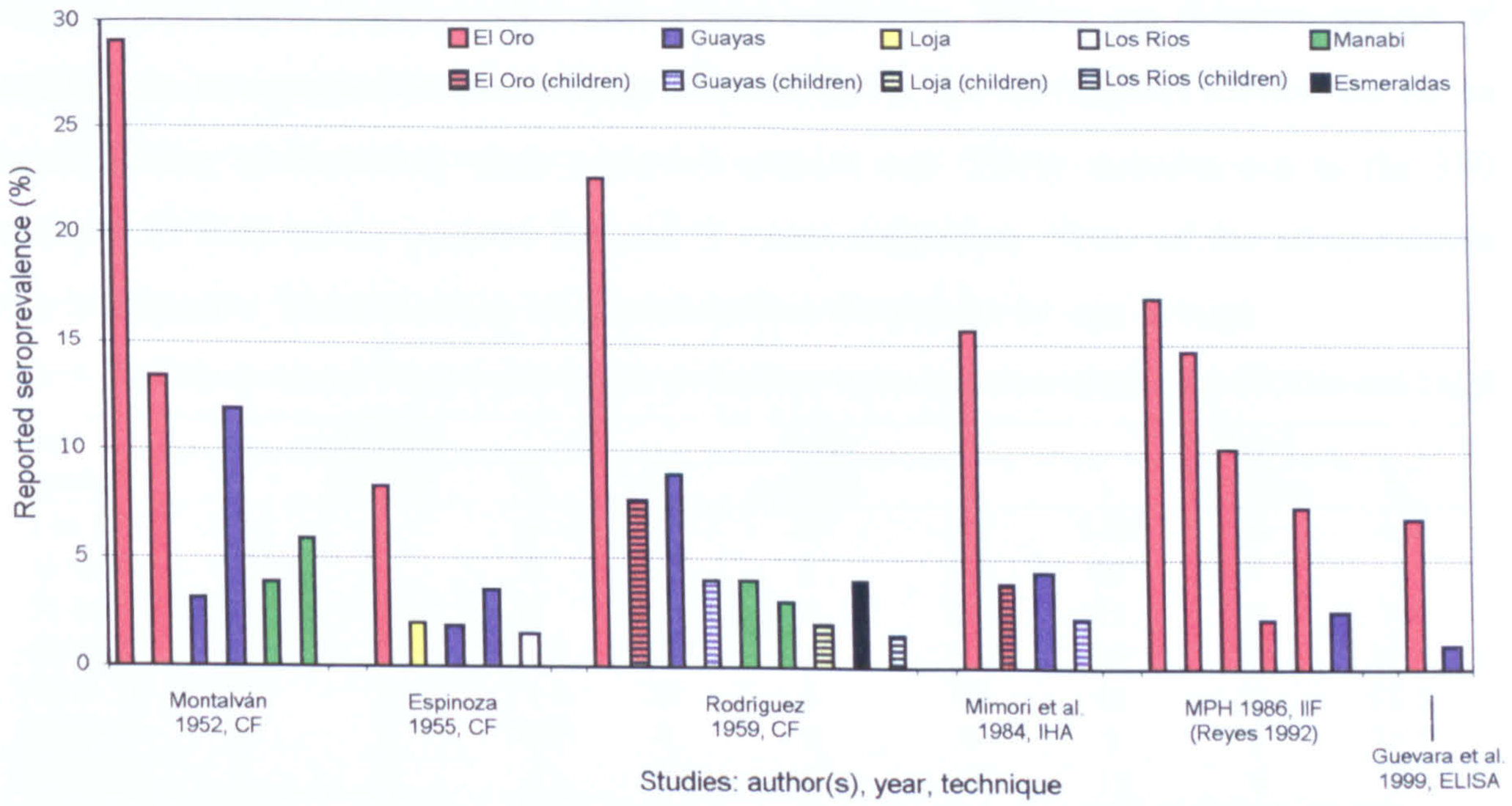


Figure 8. Comparison of reported seroprevalence in different localities of Ecuador. *Rhodnius ecuadoriensis* is the main domestic vector in El Oro and Loja, while *Triatoma dimidiata* prevails in Guayas and Manabí (and probably also in Los Ríos). See also table 6. CF=complement fixation; IHA=indirect haemagglutination; IIF=indirect immunofluorescence; ELISA=enzyme-linked immunosorbent assay; MPH=Ministry of Public Health

More recently, Garzón et al. (2002) reported 16.4% seroprevalence in El Oro (372 samples), 1.2% in Guayas (421 samples), and 0.4% in Manabí (680 samples) (microELISA with total parasite lysates and two synthetic peptides plus haemmaglutination). Other studies (ELISA with either crude epimastigote lysates or recombinant proteins used as antigens) did not include samples from both areas, making comparisons difficult. However, the trends suggested by the above results seem to be confirmed, with average prevalences of approximately 8% in El Oro and 1% in Guayas and urban Manabí (cf. Aguilar et al. 1999); in 1996, AG Guevara estimated overall prevalence rates of 13.8% in El Oro and 2.6% in Guayas (AG Guevara, unpublished report). Finally, a study on seroprevalence among children (4-15 years old; IIF plus

ELISA) in El Oro recently demonstrated active transmission, with ~2% of over 1500 samples positive (Racines et al. 1994), and Córdova et al. (1999) reported 11% seropositives (out of 262 samples) from the area of Piñas, El Oro (see also table 6).

Preliminary results of serological tests from two of the communities under study in the present project (El Lucero, Loja and Lourdes, El Oro) also show relatively high prevalence rates. The study was conducted in parallel to the entomological survey reported here and was under the co-ordination of the National Institute of Hygiene (INH, Ministry of Public Health). After informed consent, blood-spot samples were obtained onto filter paper, coded and stored until use. Below we present results of ELISA tests as reported to the officials responsible for the serological survey. As far as we are aware, confirmatory tests were not carried out. Thirty samples out of the 380 examined (7.9%) tested positive for anti-*T. cruzi* antibodies. None of the seropositives were immigrants. The following table summarises the results by age groups.

Table 9. Prevalence of anti-*Trypanosoma cruzi* antibodies in two localities of Ecuador (El Oro and Loja)

Age	El Oro			Loja			Total		
groups	n	positive	%	n	positive	%	n	positive	%
1 to 15	33	0	0	127	10	7.9	160	10	6.3
16 to 30	15	0	0	45	3	6.7	60	3	5
31 to 45	18	0	0	33	4	12.1	51	4	7.8
46 to 60	11	3	27.3	34	4	11.8	45	7	15.6
61 to 75	14	3	21.4	28	2	7.1	42	5	11.9
Over 75	1	1	100*	8	0	0	9	1	11.1
Missing	3	0	0	10	0	0	13	0	0
Total	95	7	**7.4**	285	23	**8.1**	380	30	**7.9**

*The only person over 75 years old in the sample was seropositive; this result was obviously due to chance

Positive tests were significantly more frequent among those reporting antecedents of bug bites (18 out of 123, 14.6% seropositivity) that among those not remembering having been bitten (8 out of 203, 3.9%) (Fisher's exact test p=0.0011; likelihood ratio test X^2=11.5, 1 df, p=0.0007; unadjusted odds ratio=4.18, 95% confidence interval 1.8-10.5; n=326). There was also a strong relationship between seropositivity and the presence of another seropositive in the same dwelling. The rates of seropositivity were 18.2% and 5.7% in houses with and without a seropositive person other than the one being investigated, respectively (Fisher's exact test p=0.0019; likelihood ratio test X^2=9.45, 1 df, p=0.002; unadjusted odds ratio=3.65, 95% confidence interval 1.6-7.96; n=380).

These results support the view that *R. ecuadoriensis* must be considered as the second main vector of Chagas disease in Ecuador. *T. dimidiata* is ranked first because its strongly synanthropic populations have a broader distribution that includes densely populated urban areas (Guayaquil, ~2 million people; Manta, ~180000 people; or Portoviejo, ~170000 people). However, it has been shown that *T. dimidiata* is not particularly efficient as a vector; in Honduras, a comparative study revealed prevalence rates of 15% (communities with only *T. dimidiata*; infestation=53%; 1234 samples) and 40% (communities with only *R. prolixus*; infestation=35%; 1337 samples) (Ponce 1996). Similar results were reported from Guatemala; 39% of 373 people tested seropositive in a community where *R. prolixus* was the main vector, vs. 9% out of 428 in another village where *T. dimidiata* prevailed (Paz-Bailey et al. 2002).

Natural *T. cruzi* infection rates are higher in *T. dimidiata* (>30% in Guayas-Manabí) than in *R. ecuadoriensis*: Loja 3.6% to 4%; Manabí 5% to 17.3%; El Oro 55.7% (Aguilar & Yépez 1996; AG Guevara, unpublished report). Lazo (1985) reviewed parasitological studies up to the mid 80s; 29.6% of 25436 *T. dimidiata*, and 3% of 5255 *R. ecuadoriensis* examined were reported as infected by *T. cruzi*-like trypanosomes.

1.4.3. ECOLOGY AND HABITATS

R. ecuadoriensis has adapted to human habitats in rural areas of the central-southern coastal region of Ecuador (mainly in Manabí and El Oro), the inter-Andean temperate valleys of the province of Loja, and in northern Peru (Defranc 1982, Aguilar et al. 1999, Carcavallo et al. 1999a, Abad-Franch et al. 2001a,b, Cuba Cuba et al. 2002). These synanthropic populations are related to domestic birds (mainly chickens and pigeons) and, in some cases, to guinea pigs, but only fragmentary studies are available. Some authors consider that the synanthropic behaviour of *R. ecuadoriensis* may be regarded as relatively recent, and that the species probably retain its sylvatic habits (Lent & Wygodzinsky 1979, Defranc 1982, Schofield 1994).

As with other aspects of the ecology of *R. ecuadoriensis*, little is known about the sylvatic habitats of this species. Like for all *Rhodnius* species, the primary natural habitat of *R. ecuadoriensis* is presumably arboreal. A few observations indicate that sylvatic populations occur in palm trees of the species *Phytelephas aequatorialis* Spruce (Romaña et al. 1994, Avilés et al. 1995a), and one record exists suggesting colonisation of the cultivated palm *Elaeis guineensis* (Carcavallo & Martínez 1985); the role of cacti

and hollow trees (Barrett 1991) is unclear. *Ph. aequatorialis* is endemic to western Ecuador; its biogeographic limits are related to excessive rainfall in the north (pluvial forests of the Chocó ecoregion in Colombia), excessive aridity in the south (northern Peru), and ~1500m altitude on the Andean foothills. The stipes of these palms reach ~10m in height in old (usually over a century) specimens. Separate sexes are discernible in adult palms (i.e., they are dioecious). Local residents make use of various products of these palms for different purposes. Leaves are used for thatching houses and corrals, and unripe fruits are edible (mainly used as livestock fodder). The most valuable product is the hardy endosperm of the seeds, known as vegetable ivory (the 'tagua'). There is a growing local industry related to tagua carving manufacture, and tagua nut disks are exported as raw material to manufacture buttons (Southgate 1997). Tagua exports were worth ~14 million US dollars in 1997. The economic importance of these palms for the locals implies that they are frequently maintained in peridomestic environments, mainly in the provinces of Manabí and Esmeraldas (Avilés et al. 1995a, Southgate 1997, Borchsenius et al. 1998).

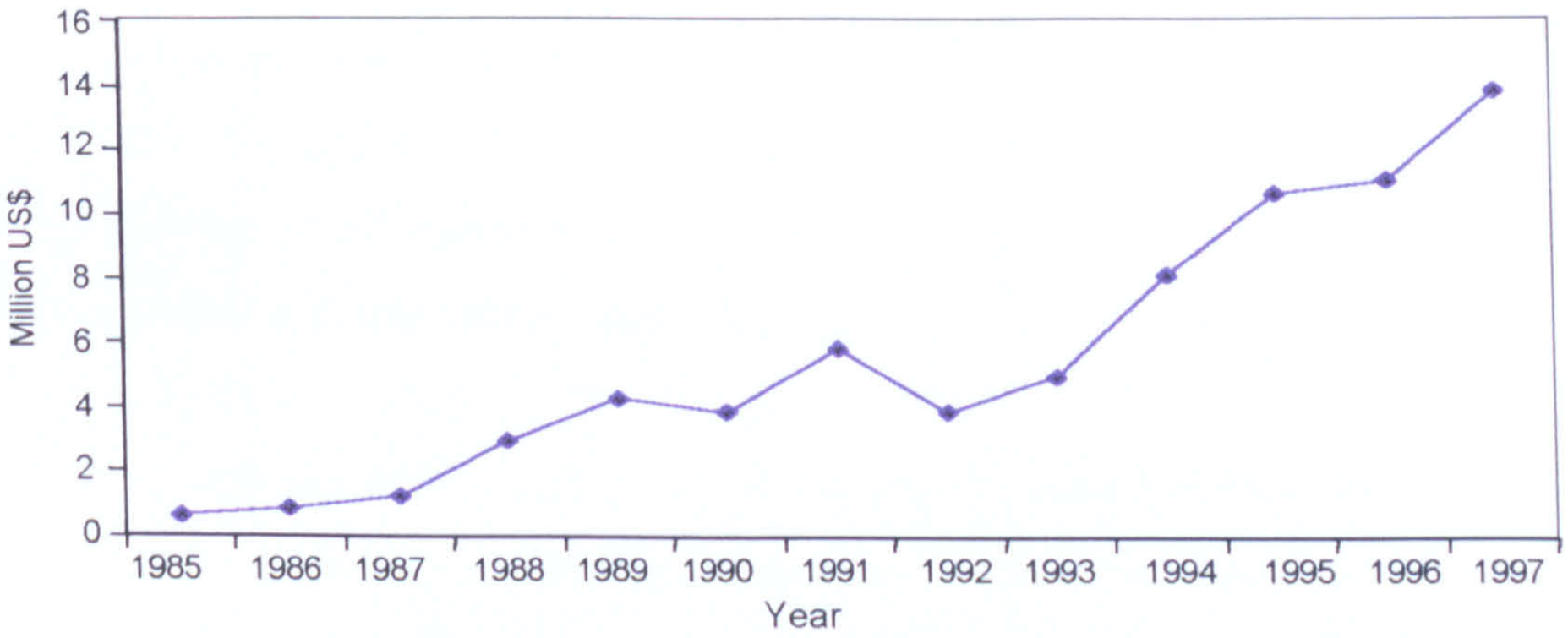


Figure 9. Tagua nut products export from Ecuador (1985-1997), in million US dollars
[Sources: www.ecuador.org/trdinv2.htm and www.bp.fin.ec/ext/grupo/docs/ecuadorexporta/ing/vegetabl.htm]

Not all palm tree habitats are however equally suitable for triatomines. Some of the characteristics of individual palms may result in favourable conditions for bugs – suitable microclimate, presence of hosts, specific architectural features (Whitlaw & Chaniotis 1978, Romaña et al. 1999). For a given palm species, the quantity of epiphytic plants, the amount of decomposing organic matter present around stems and on crowns, time-dependent features such as stipe height (representing palm age), or other ecological aspects such as the degree of human intervention on the environment in which palms are

located, could be related to a higher likelihood of palm infestation (Barrett 1991). These aspects were included in our field studies on *R. ecuadoriensis*.

The principal ecological features of *R. ecuadoriensis* remain poorly defined. Questions yet to be answered relate to the general biogeographic traits of the species (including the distribution of populations with different degrees of synanthropism), what factors may favour colonisation of human environments by these bugs, what is the potential epidemiological risk represented by sylvatic populations (and their main natural ecotopes), or what are the compositions and relationships of different populations over the geographical range of the species. In operational terms, the design of strategies for long-term control of Chagas disease transmission in western Ecuador and northern Peru will depend on the answers to these questions. This project was designed with the aim of clarifying the most relevant of these aspects by combining ecological and epidemiological approaches with new tools provided by molecular biology and morphometrics for the study of genetic and phenetic diversity.

2. AIMS

The general aim of all research activities within this project was to contribute to the design of suitable strategies for the control of vector-borne Chagas disease in Ecuador. We aimed at investigating the main ecological and genetic features of the most important disease vectors, with emphasis on the autochthonous species, *Rhodnius ecuadoriensis* Lent & León, 1958. Specifically, our objectives included:

1. To define the main ecological features of sylvatic and domestic populations of *R. ecuadoriensis* in western Ecuador.

2. To characterise the phenetic and genetic diversity of *R. ecuadoriensis* populations using digital morphometrics and mitochondrial DNA sequence data.
   2.1. To investigate the structuring and relationships of different *R. ecuadoriensis* populations.
   2.2. To explore the potential of molecular and phenetic markers for the distinction of epidemiologically diverse populations in the context of vector control and surveillance interventions.

3. To clarify the phylogenetic relationships of *R. ecuadoriensis* with *R. pallescens* and *R. colombiensis*, extending the analyses to other *Rhodnius* species.

4. To study selected aspects of the biology, ecology and genetics of other Ecuadorian triatomines.

5. To derive recommendations for sustainable vector control strategies in Ecuador and Peru.

3. BIOGEOGRAPHY AND GENERAL ECOLOGY OF *RHODNIUS ECUADORIENSIS* AND OTHER ECUADORIAN TRIATOMINES

3.1. General introduction

Biogeography is the study of organisms in space and time; this discipline seeks to understand the diversity of life forms, their current patterns of distribution and how they varied with time. These questions are addressed in the context of the intricate interplay between (present and past) geography, climate patterns, physical-chemical features of soils and water bodies, and different organisms (interacting in turn with each other and with their environments in communities and ecosystems – and thus changing under the logic of evolution). This knowledge is then integrated into general rules to build up a broader framework of understanding that may be used to accurately describe the system and to develop predictions about how its different parts might behave in the future under varying conditions (Cox & Moore 2000).

Ecologists and biogeographers have developed a classification of geographic areas with comparable basic characteristics – which allow for comparable communities of organisms to occur there. These areas, termed life zones, were demarcated on the basis of climate patterns (average maximum and minimum temperatures, annual rainfall, and potential evapotranspiration) and altitudinal ranges, which in turn define the type of vegetation that can naturally grow on each zone (Holdridge 1967). Generally speaking, each of these zones also bears typical animal communities; consequently, this system of habitat classification allows for the description of organismal distribution patterns. Many ecological studies have been based on this system of life zone classification, including several on Triatominae (e.g., Curto de Casas et al. 1999).

More recently, new techniques based on remote-sensing (RS) data derived from satellite images and geographic information systems (GIS) have been used for vector population and habitat mapping. This allows for the use of a large number of environmental variables to define descriptive/predictive models regarding distribution trends even when field data are incomplete (Kitron 2000, Bergquist 2001, Kuhn et al. 2002, Peterson et al. 2002, D Gorla pers. comm.).

Understanding of triatomine biogeography (current or historical) is still fragmentary. The majority of records correspond to domestic bug populations of the few species of utmost epidemiological significance. Many of these populations dispersed only recently,

associated with migrant humans and often carried to areas out of the natural range of the species. Data on the original sylvatic populations are comparatively scarce, and completely lacking for many species. The attempts to reconstruct the historical biogeography of triatomines have been hampered by the lack of a reliable cladistic system for classification based on morphological characters. However, molecular techniques provide suitable means for an improved understanding of the evolutionary relationships between the various taxa.

On a broad scale some **biogeographical trends** can however be outlined; they are relevant both in epidemiological terms and for phylogenetic interpretation. Firstly, the distribution of the subfamily is almost exclusively Neotropical-Nearctic. A few species occur in the Old World, including only two main groups: the tropicopolitan *T. rubrofasciata* and its close Asian relatives (seven species endemic to south China, Malaya, Indonesia, New Guinea, and northeast Australia), and the Indian genus *Linshcosteus*. It has been proposed that the species of the first group are recent derivatives from *T. rubrofasciata* specimens carried by man along sea trade routes linking the Americas with Asian harbours from the 16th century; *Linshcosteus* is on the contrary believed to have evolved independently in the Indian subcontinent (Schofield 1988, Gorla et al. 1997, Patterson et al. 2001, Galvão et al. 2002), and the tribe Linshcosteini was recently proposed (Carcavallo et al. 2000). These hypotheses need to be tested by means of molecular phylogenetic approaches (Patterson 2002); preliminary analyses of 16S rDNA sequence data show a close kinship between *T. rubrofasciata* and *Linshcosteus* sp. and favour the idea of a New World origin of all triatomines (Hypša et al. 2002).

The rest of the Triatominae occur in the Americas. Members of the tribe Triatomini are found in South-, Central-, North America, and in the Caribbean region, and include seven genera (*Triatoma*, *Panstrongylus*, *Dipetalogaster*, *Eratyrus*, *Mepraia*, *Hermanlentia*, and *Paratriatoma*; the genus *Meccus* would include the Mesoamerican species of the *phyllosoma* complex, but its validity needs to be confirmed) (Lent & Wygodzinsky 1979, Carcavallo et al. 2000). Evidence suggesting independent origins of the South- and Mesoamerican clades (the latter probably being a paraphyletic assemblage including *Panstrongylus* alongside *Dipetalogaster*, some *Triatoma*, the *phyllosoma-Meccus*, and perhaps *Paratriatoma*) has been obtained from morphometric

(Dujardin et al. 2000, Patterson et al. 2001) and genetic analyses (Bargues et al. 2000, García et al. 2001, Marcilla et al. 2001, 2002). Linking both regions from the Middle Pliocene is the isthmus of Panama, which re-closed definitively some two million years ago after a temporary interruption when sea levels rose in the Late Pliocene (see Cox & Moore 2000).

The Cavernicolini is composed of three species (*Cavernicola pilosa*, *C. lenti* and *Torrealbaia martinezi*) that occur mainly in the Amazon basin; *C. pilosa* has also been recorded from the Magdalena valley (Colombia) and Panama (Lent & Wygodzinsky 1979). Members of the Bolboderini also occur mainly in South America, but *Bolbodera scabrosa* is only known from Cuba, *Belminus costaricensis* from Costa Rica and Mexico, and *B. herreri* from Panama; *Microtriatoma trinidadensis* has been recorded from Panama to Bolivia (Lent & Wygodzinsky 1979). The Alberproseniini are only known to occur in Venezuela (*Alberprosenia goyovargasi*) and northeastern Brazil (*A. malheiroi*) (Carcavallo et al. 1999c).

The majority of species occur in South America, including the tribe Rhodniini. This is considered a monophyletic lineage comprising two genera and 19 species (the validity of a few being doubted by some specialists); they all share their preference for arboreal habitats, and the genus *Rhodnius* seems to be primarily adapted to palm trees, where the bugs feed mainly on mammals and birds – but also on reptiles and amphibians (Carcavallo et al. 1998a,b). *Psammolestes* species are associated with bird nests (Furnariidae and Psittacidae) and occur east of the Andes in open forests of Venezuela (*Ps. arthuri*), Brazil (*Ps. tertius*), Argentina, Bolivia, and Paraguay (*Ps. coreodes*).

Most *Rhodnius* species are found on the eastern side of the Andes. From north to south, these include *R. prolixus* (a major disease vector; domestic populations were probably introduced into north-western Colombia and Central America, but sylvatic forms are only known from palms in seasonally dry forests of the Venezuelan Llanos), *R. neivai* (adapted to dry environments in Venezuela and also reported from Colombia), *R. dalessandroi* (a rare Colombian species), *R. brethesi* (associated with *Leopoldinia piassaba* palms), *R. robustus* (populations of which, apparently subdivided into distinct geographic clades, are widely distributed over the Orinoco-Amazon system), *R. paraensis* (described from a few specimens from northeastern Brazilian Amazonia; a new finding in French Guiana was reported recently [Bérenger & Pluot-Sigwalt 2002]),

R. pictipes (widespread in the Amazon), *R. stali* (southwestern Amazonia-Mato Grosso in Bolivia and Brazil), *R. nasutus* (north-eastern Brazil), *R. domesticus* (Atlantic humid forests of Brazil), and *R. neglectus* (drier forests of central-southeastern Brazil). Recently a species allegedly similar to *R. dalessandroi* (but also resembling *neglectus*) was described from material collected in north-eastern Amazonian Brazil (Valente et al. 2001), and the validity of *R. amazonicus* (originally described by Almeida et al. [1973]) has been claimed by Bérenger and Pluot-Sigwalt (2002).

Rhodnius species are considered by some researchers to form two distinct clades separated by the Andes. East of the cordillera, the *R. pictipes* group comprises the virtually identical *R. stali* plus *R. brethesi*, *R. paraensis*, possibly *R. dalessandroi*, and now *R. amazonicus*. The rest of eastern *Rhodnius* species (the *robustus* group) are sometimes subdivided into two further groups – corresponding to regions north and south of the Amazon. The first is comprised of a putative 'northern form' of *R. robustus*, *R. prolixus*, and possibly *neivai*, and the second includes a 'southern form' of *robustus*, *nasutus*, *neglectus*, and *domesticus* (Schofield & Dujardin 1999).

A second major cluster of closely related *Rhodnius* species occurs west of the Andes, and is generally deemed to have closer relationships with the *R. pictipes* group than with any of the *robustus* lineages. It includes *R. ecuadoriensis* together with *R. pallescens* and the recently described *R. colombiensis* (Moreno et al.1999). These two latter species are found in northwestern Colombia, where they are sylvatic – associated with *Attalea butyracea* palm trees. *R. pallescens* may invade/colonise households in some areas, and is also found in Panama (where it is considered the primary vector of Chagas disease) and Costa Rica; a recent finding indicates its presence in southeast Nicaragua, where adult specimens were captured in houses (CJ Schofield, pers. comm.). *R. colombiensis* is only known from the central Magdalena valley in Colombia, and has only rarely been found in human environments (Molina et al. 2000, Vallejo et al. 2000). Phenetic similarities between *R. ecuadoriensis* and *R. pallescens* had been pointed out since the description of the former (Lent & León 1958, Lent & Wygodzinsky 1979), and results from isoenzyme and molecular studies largely support their close relatedness (see Section 7.3.). With regard to *R. colombiensis*, several studies have suggested that it may in fact be closer to *ecuadoriensis* than to the morphologically similar (and parapatric) *R. pallescens* (Dujardin et al. 1999a, Chávez et al. 1999, Monteiro et al. 2000).

3.2. Biogeography of *Rhodnius ecuadoriensis*

3.2.1. INTRODUCTION

The design of sound vector control strategies depends upon accurate knowledge on the current distribution and ecological preferences of insect populations involved (actually or potentially) in disease transmission. As field data are usually limited, we used a life zone-based approach to reconstruct the general biogeography of all known Ecuadorian triatomines. In the case of *R. ecuadoriensis*, a preliminary attempt to describe the potential distribution of the species using RS-GIS was also made in collaboration with David Gorla (CRILAR, Argentina) and Diarmid Campbell-Lendrum (LSHTM). The life zone biogeography of the species in Peru was analysed in collaboration with César A Cuba Cuba and his team. The association of sylvatic populations of *R. ecuadoriensis* with *Ph. aequatorialis* palm trees led us to include data on palm distribution and deforestation in biogeographical reconstruction and ecological analyses. Results were also interpreted in the context of interspecific relationships of *R. ecuadoriensis* with members of the same genus and with other Ecuadorian triatomines.

3.2.2. MATERIALS AND METHODS

For this part of the study, we reviewed all available published reports, unpublished records from the Vector Control Service (SNEM) and from Ecuadorian colleagues, and all triatomines deposited at the PUCE-QCAZ Invertebrates Museum (Catholic University of Ecuador, Quito), the Laboratório Nacional e Internacional de Referência em Taxonomia de Tratomíneos (Fiocruz, Rio de Janeiro, Brazil), and the Natural History Museum (London, UK). Our own fieldwork records were included in the analysis. For geographic information we used the guide by Miranda (1995), the 'Índice Toponímico' of the Army Geographic Institute (IGM 1978-82, 1982-96), and cartographic material provided by the IGM. Life zones were established following the ecological map of Ecuador proposed by Cañadas (1983) and the maps in Dodson & Gentry (1991), based on the life zones described by Holdridge (1967). Distribution maps were prepared by marking places of capture; infested life zones and life zones representing the potential distribution of each species (with no records but with ecological traits identical to areas where captures had been documented) were filled with different colours and numbered. The analysis included geographic location, life

zones, altitude, temperature, and annual rains recorded in each area. Only life zones under 2200m altitude were included in the analyses.

Additionally, the geographic coordinates of 52 Ecuadorian localities were *R. ecuadoriensis* had been collected were retrieved in the 'Índice Toponímico' (IGM 1978-82, 1982-96) and at the website www.calle.com/world. These approximate data were used as input for GIS mapping using four biophysical variables (air temperature [AT], land surface temperature [LST], medium infrared radiation [MIR], and vegetation index [NDVI]) recorded by meteorological satellites (NOAA series, National Oceanic and Atmospheric Administration) using AVHRR sensors (Advanced Very High Resolution Radiometer). The analysis was derived from a Fourier temporal analysis of monthly images from the period 1982-2000 including 14 statistical descriptors derived from each of the variables specified above (average, minimum, maximum, variability, amplitude and phase of AT, LST, MIR, and NDVI). Spatial resolution was 8x8km; images were obtained from the server ftp://daac.gsfc.nasa.gov/avhrr/global_8km/. The Idrisi V 2.0 software package was used for GIS analysis. Dr F Skov (Aarhus University, Denmark) kindly provided us with a GIS-based probability map showing the potential distribution of *Ph. aequatorialis* palms in western Ecuador.

For Peruvian populations, published records and the following sources of data were reviewed: the entomological collection, National Institute of Health, Lima, Peru; the reference collections at Fiocruz, Rio de Janeiro, Brazil; and records from the Ministry of Health, Lima, Peru – including unpublished reports by the Division of Epidemiology. Biogeographical information was obtained from Brack (1987) and Mostacero et al. (1996). Life zone classification was carried out after Curto de Casas et al. (1999).

3.2.3. RESULTS

3.2.3.1. Biogeography of *Rhodnius ecuadoriensis* in Ecuador

Records were found indicating the presence of *R. ecuadoriensis* in 82 localities of eight provinces of Ecuador; all records but a dubious one (an adult bug labelled as collected in the Amazon) correspond to southern latitudes. A few of these records (in Guayas, Tungurahua and Orellana) need to be confirmed (see below). A complete list of localities is provided in the Appendix. The presence of the species has been satisfactorily documented in the provinces of Pichincha, Manabí (where most of the records are from), Los Ríos (with a single record), El Oro, and Loja. The known

distribution of *R. ecuadoriensis* in western Ecuador comprises at least seven different life zones. Wide ranges of altitude (0-1800m in the life zones included in our study) and annual rainfall (from 125mm in the tropical desert scrub of coastal Manabí to 4000mm in the wet and moist forests of Pichincha/northern Manabí and Los Ríos) were recorded. The range of average minimum and maximum temperatures across life zones was 18°C (minimum, premontane forest areas) to 26°C (maximum, arid zones).

All reliable records correspond to the western side of the Andes (in central-northern Ecuador) and to southern inter-Andean valleys related to Pacific slope river systems. Sylvatic populations were only recorded from western Ecuador (territory below 900m altitude between the Andes and the Pacific coastline and comprised of plains extending westwards from the foothills of the Andes and a low range of coastal hills with maximum elevations of about 800m) (Dodson & Gentry 1991). The climate gets gradually more humid as one moves towards the Andes, as the zones nearest to the coast are affected by the cold Peruvian (Humboldt) current. Even in these dry areas night fogs provide substantial atmospheric humidity to the slopes and crests of coastal hills.

Table 10. Biogeography of *Rhodnius ecuadoriensis* in Ecuador

Life zones	AvT	Alt	Rain	Provinces	Other species
1 Tropical desert scrub	24-26	0-250	125-250	Manabí	*T. dimidiata*
2 Thick tropical bush	24-26	0-300	250-500	Manabí	*T. dimidiata* *P. geniculatus* *P. howardi*
3 Very dry tropical forest	24-26	0-300	500-1000	Manabí	*T. dimidiata* *P. rufotuberculatus* *P. chinai* *P. howardi*
4 Dry premontane forest	18-24	500-1800	500-1000	Manabí Loja	*T. dimidiata* *T. carrioni* *P. rufotuberculatus* *P. chinai* *E. cuspidatus*
5 Humid premontane forest	18-24	300-1800	1000-2000	El Oro	*T. carrioni* *T. venosa* *P. chinai*
6 Moist tropical forest	24-25	0-300	2000-4000	Pichincha/Manabí limit Los Ríos	*T. dimidiata* *T. dispar* *P. geniculatus* *E. cuspidatus*
7 Wet premontane forest	18-24	300-1800	2000-4000	Pichincha	*T. carrioni* *T. dispar* *T. venosa* *P. geniculatus* *P. rufotuberculatus*

AvT=mean minimal and maximal temperatures (in °C); Alt=altitude (in m); Rain=average annual rainfall (in mm); *T.=Triatoma*; *P.=Panstrongylus*; *E.=Eratyrus*; numbers in bold type correspond to life zones in the map below (figure 10)

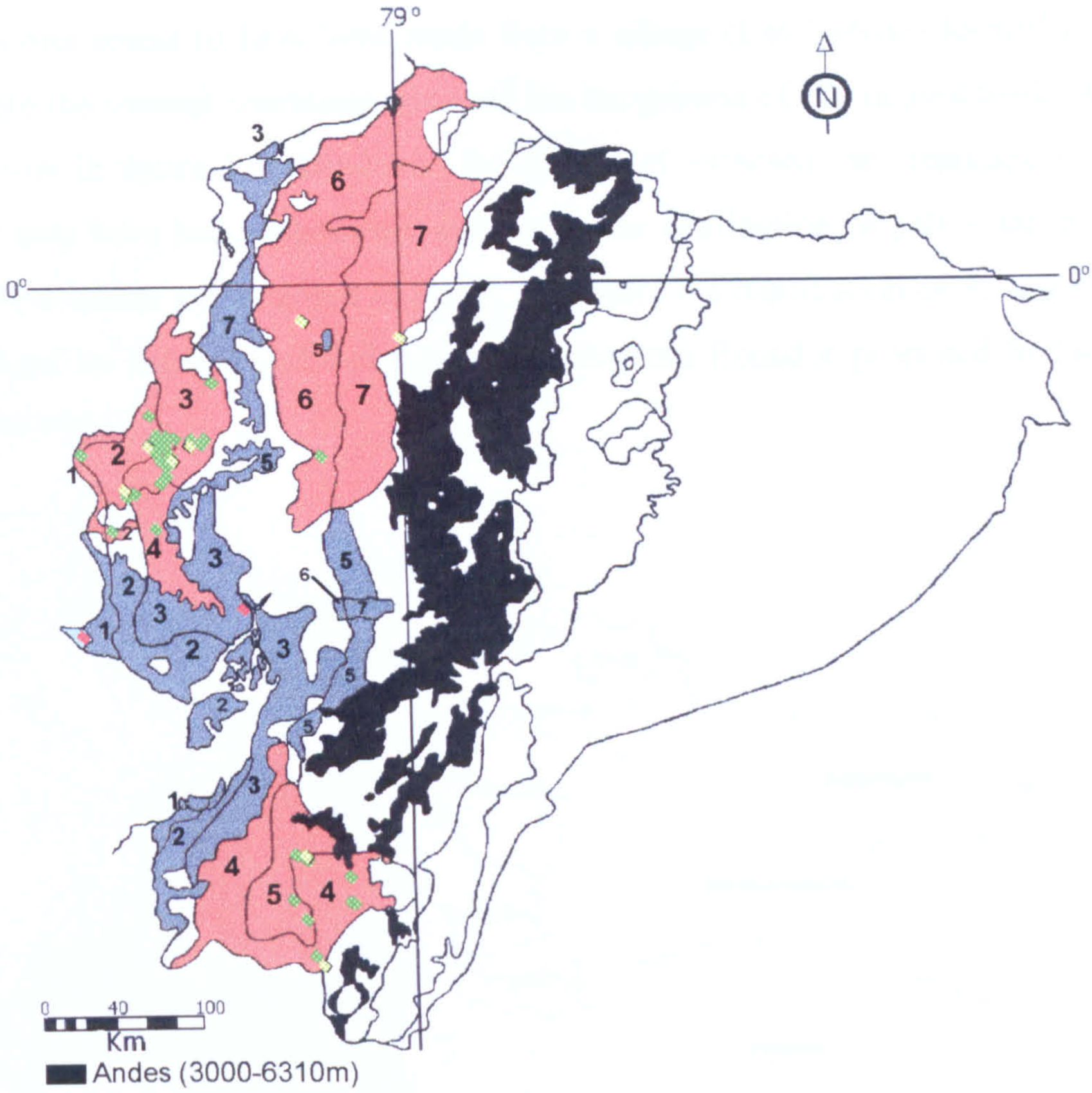


Figure 10. Biogeography of *Rhodnius ecuadoriensis* in Ecuador. Red areas correspond to life zones where the presence of this species has been documented (green marks indicate localities; our [positive] fieldwork sites are yellow); blue areas correspond to areas with the same ecological characteristics but from where no records of the species exist. Numbers correspond to life zones in table 10 (above). The red dots represent dubious records in Guayaquil and the Santa Elena peninsula; this latter life zone (tropical desert) is light blue, denoting that the presence of *R. ecuadoriensis* is very unlikely there

The relationship between the distribution of *R. ecuadoriensis* and that of the primary sylvatic ecotope of the species (*Ph. aequatorialis*) was also explored by plotting the localities from where the bugs had been reported on a map representing the maximum potential distribution of the palm trees. This habitat map was kindly produced by Dr F Skov (Aarhus University, Denmark) using 15 georeferenced observations of the occurrence of *Ph. aequatorialis* and data on altitude, temperature, rainfall, and soil type. An almost perfect match can be observed between both distributions, with the only exceptions of the dubious record of *R. ecuadoriensis* from Santa Elena (Guayas, tropical desert) and the collections of domestic bugs at high altitudes in the inter-Andean valleys

of Loja. Even in the tropical desert scrub life zone (Manabí), the record of *R. ecuadoriensis* seems to have been made from a village (Las Cañitas) located in a small area where the general conditions required for the growth of *Ph. aequatorialis* palms do exist (arrow in figure 11). However, the effects of extensive deforestation in western Ecuador may have had a significant impact on the distribution of palms and associated bugs. These trends are explored in figure 12, where the distribution of *R. ecuadoriensis* is presented on the maps of deforestation in western Ecuador proposed by Dodson & Gentry (1991).

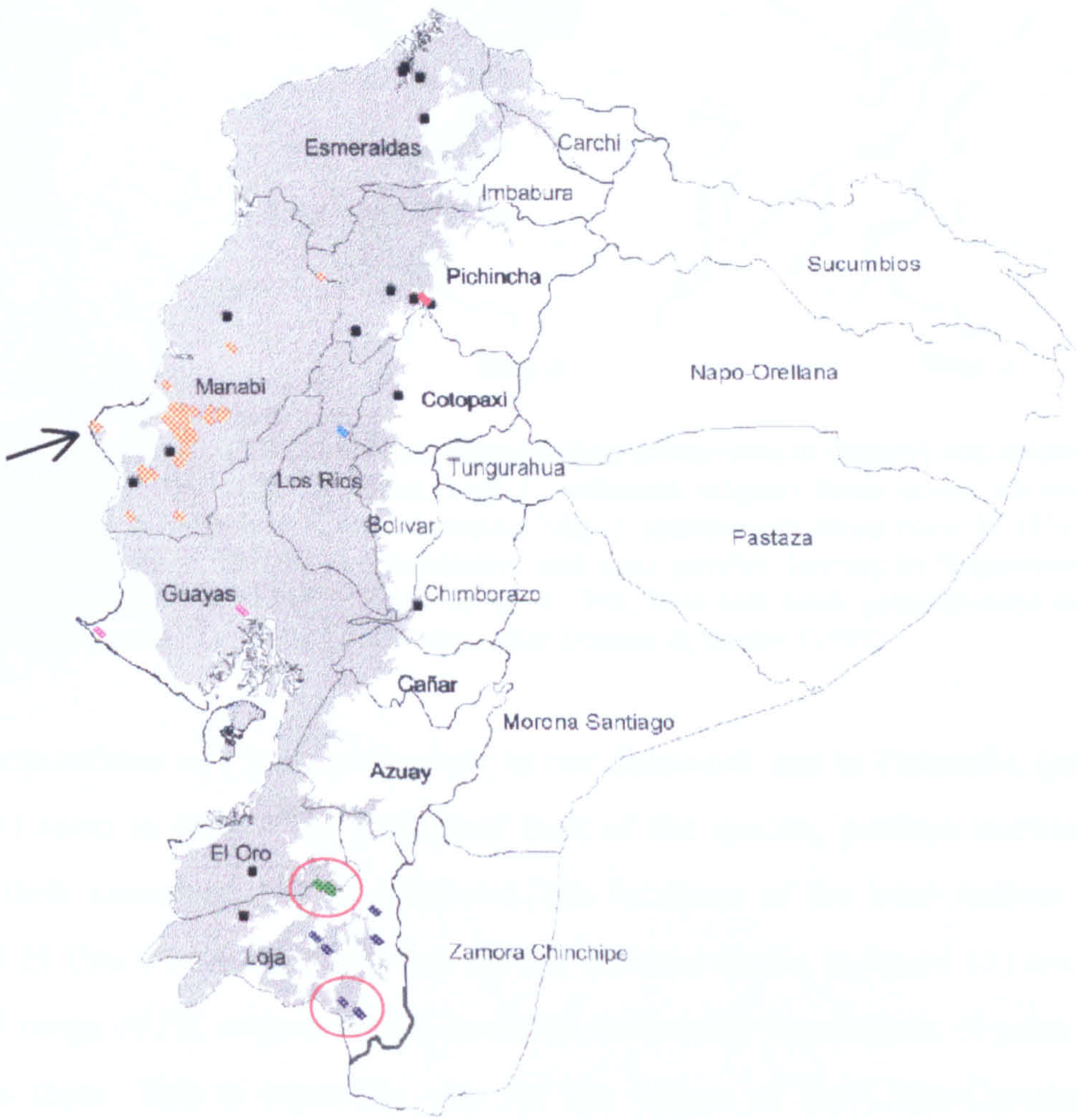


Figure 11. Biogeography of *Rhodnius ecuadoriensis* [orange, red, light blue, green, and dark blue marks=sites of capture in Manabí, Pichincha, Los Ríos, El Oro, and Loja (respectively)] and *Phytelephas aequatorialis* in Ecuador [black marks=georeferenced sites where the palms have been observed]. The grey area represents the (maximum) potential distribution of the palms. Red circles mark fieldwork areas in El Oro and Loja. The arrow indicates the locality of Las Cañitas, Manabí (see text). Pink dots represent dubious records of *R. ecuadoriensis* (Guayaquil and Santa Elena). *Ph. aequatorialis* data and map were kindly provided by Dr F Skov (Aarhus University, Denmark)

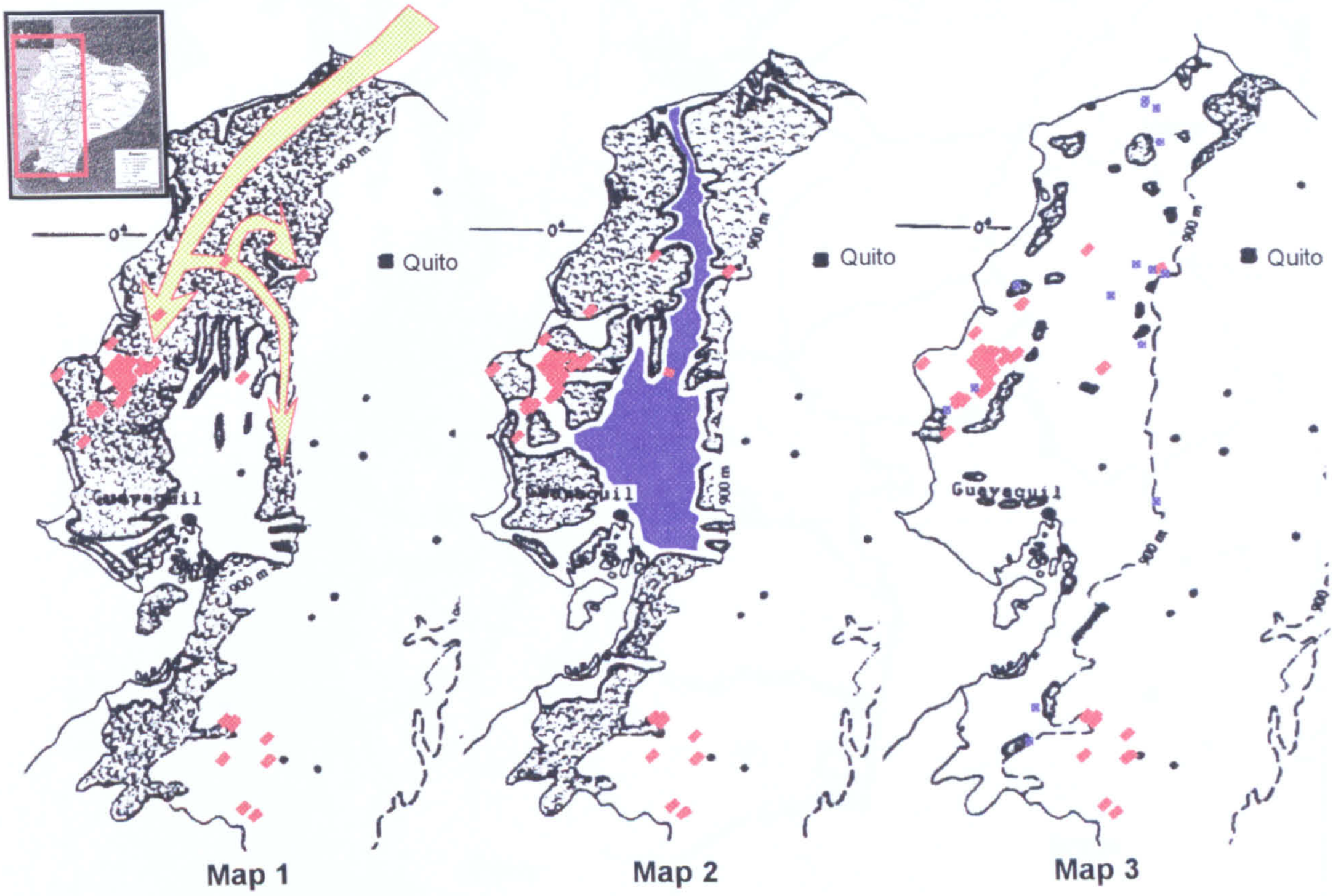


Figure 12. Biogeography of *Rhodnius ecuadoriensis* [red marks=sites of capture] and deforestation in western Ecuador (below 900m altitude). Map 1: estimated original forest cover; arrows indicate possible routes of dispersion of *R. ecuadoriensis*; Map 2: approximate forest cover in 1958; the blue area highlights the early effects of deforestation and their possible bearing on fragmentation of *R. ecuadoriensis* populations; Map 3: forest cover in 1988; blue dots mark georeferenced sites where *Phytelephas aequatorialis* palms occur. Maps after Dodson & Gentry (1991)

The populations of *Ph. aequatorialis* in our fieldwork site in Pichincha (red point in figure 11) seem to occupy the altitudinal limit of the species, perhaps marking also the limit of their associated bug populations. The localities of the inter-Andean valleys of Loja and El Oro where fieldwork was carried out (red circles in figure 11) are within the potential range of *Ph. aequatorialis*; however, no natural populations of palm trees were observed there. This is especially true for the valleys of the Chira-Catamayo system (Loja), where palms appear to be completely absent. Palm trees were seen in areas relatively close to our fieldwork site in El Oro (Lourdes), particularly along the road from Machala, but not in Lourdes itself or in neighbouring areas. Deforestation had obviously been intense and extensive in both our fieldwork areas in Loja and El Oro (both located above the altitude limit of the maps in figure 12).

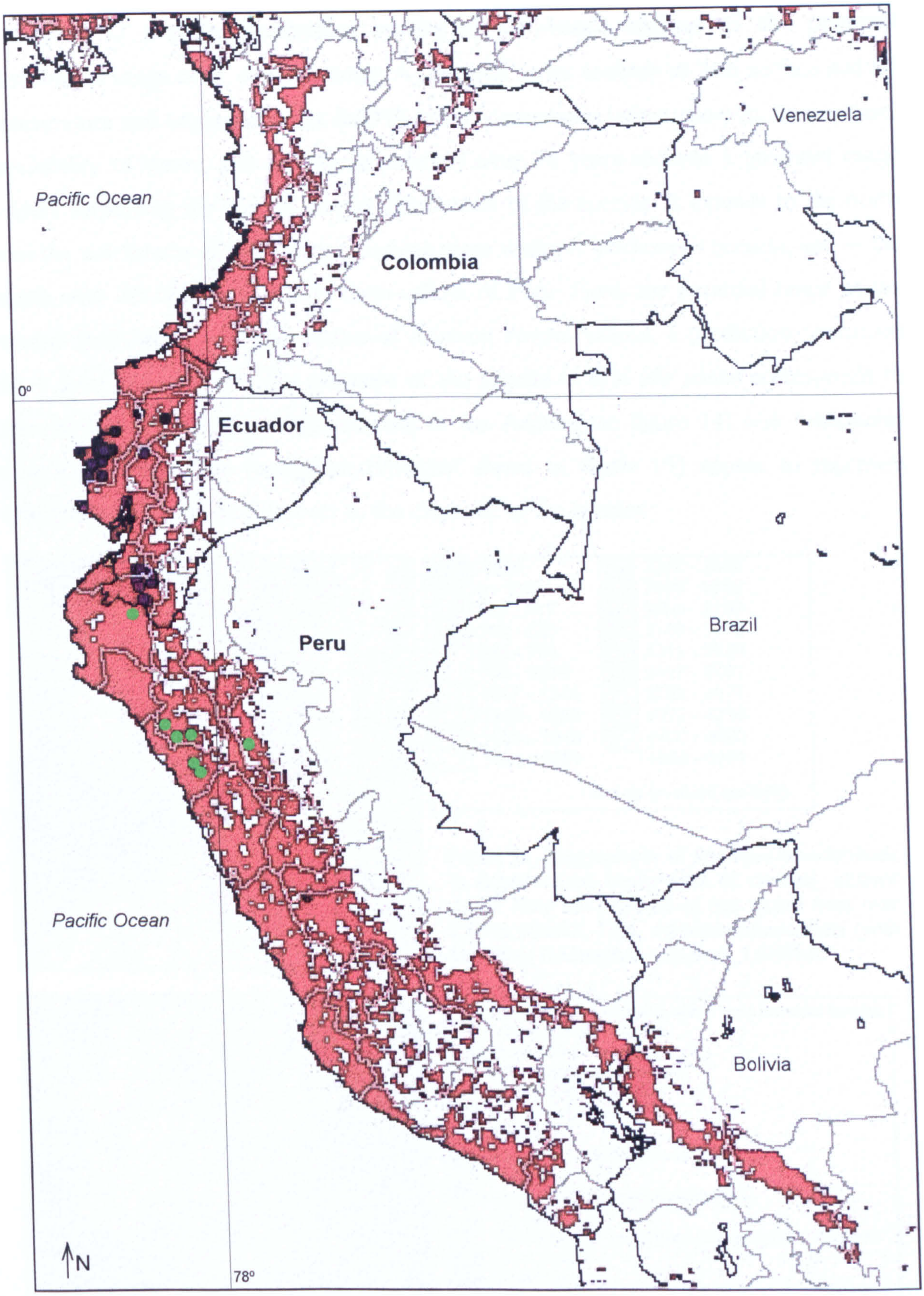


Figure 13. Biogeography of *Rhodnius ecuadoriensis* in Ecuador and Peru. Potential distribution (red area) map derived from 52 georeferenced capture sites in Ecuador (dark blue points) and biophysical data obtained from AVHRR-NOAA remote-sensing databases (see materials and methods). Green dots in Peru correspond to seven capture sites (data from F Vargas and CA Cuba Cuba) not included in the analysis; they were overlaid *a posteriori* on the map as a test for the predictions of the model. [In collaboration with D Gorla, CRILAR, Argentina]

Figure 13 presents preliminary results of GIS-based analysis of the potential distribution range of *R. ecuadoriensis*. A temporal series analysis of land surface and air temperature and vegetation data (NDVI index, comprising information on temperature, availability of water, and soil characteristics) over 18 years showed a potential range widely surpassing the current known distribution of the species. It extends to the north into the wet forests of the Chocó (reaching areas where *R. pallescens* occurs), and to the south over the arid coast and Andean valleys of Peru. Here, the potential range of the species includes the upper stretches of Amazon system valleys, a prediction confirmed by at least two records. The presence of the species in arid life zones corresponds to synanthropic populations. High altitudes in the Andes (see figure 14) and Amazonian environmental profiles (evergreen broadleaf forest in figure 15) appear to represent effective biogeographical barriers to the dispersal of the species.

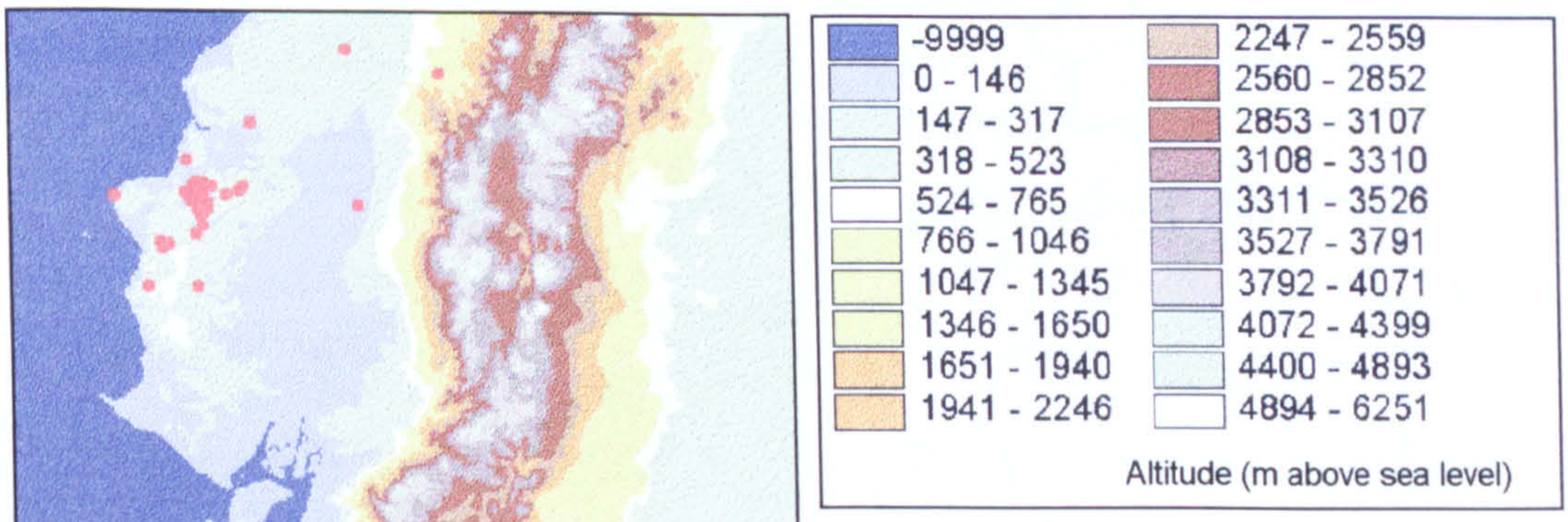


Figure 14. Biogeography of *Rhodnius ecuadoriensis* in Ecuador [red marks=sites of capture]: altitude range. Note the presence of one record from over 2500m (Gualal, Loja, domestic populations) [with help from D Campbell-Lendrum, LSHTM]

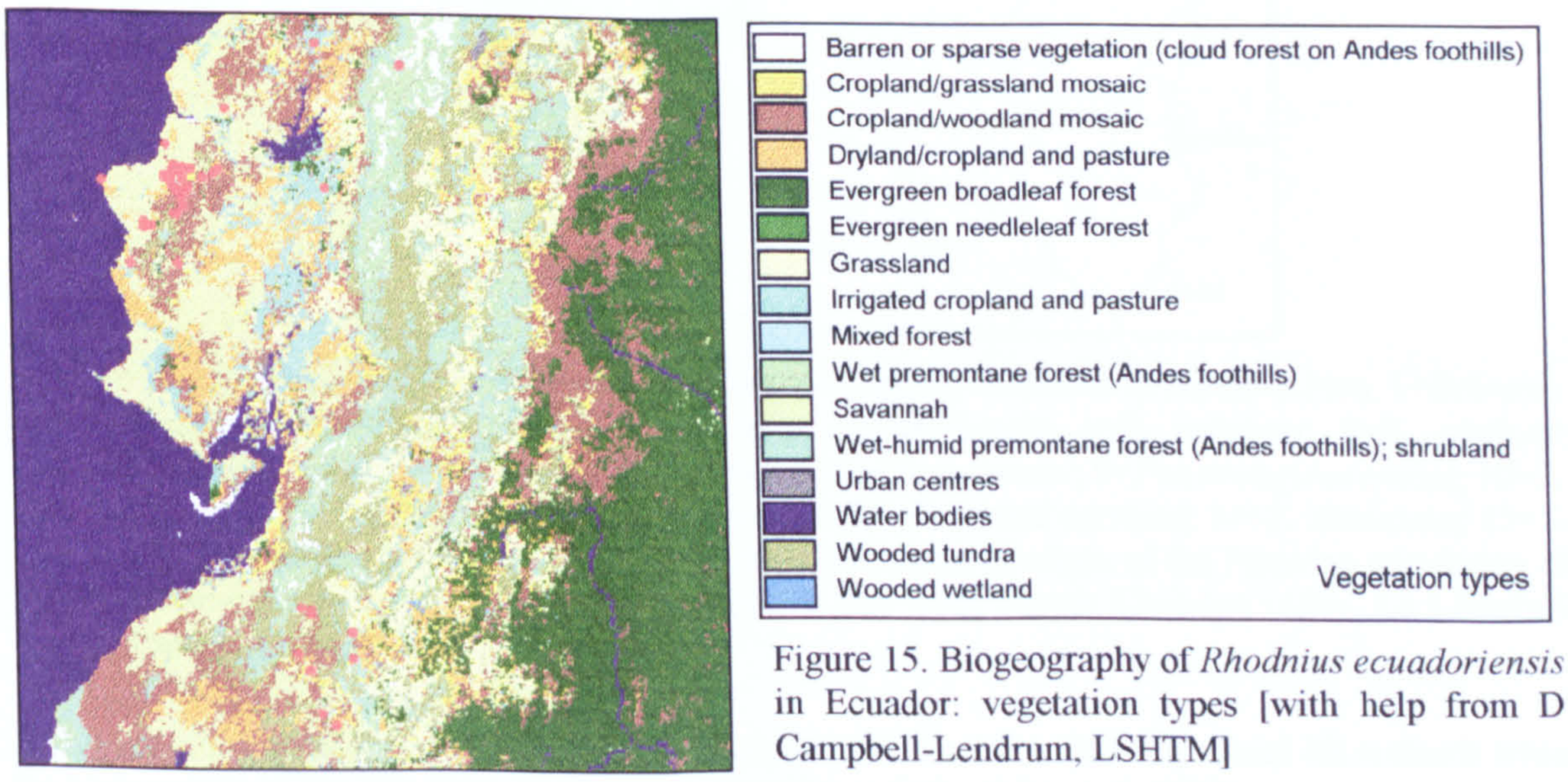


Figure 15. Biogeography of *Rhodnius ecuadoriensis* in Ecuador: vegetation types [with help from D Campbell-Lendrum, LSHTM]

3.2.3.2. Biogeography of *Rhodnius ecuadoriensis* in Peru*

In northern Peru, *R. ecuadoriensis* has been reported from five Departments and 19 provinces, comprising seven different life zones.

Table 11. Distribution of *Rhodnius ecuadoriensis* in northern Peru

Departments	Provinces	Valleys
Tumbes	Tumbes, Zarumilla	Tumbes, Zarumilla
Piura	Ayabaca, Huancabamba, Morropón, Piura	Huancabamba, Huarmaca, Piura
Lambayeque	Ferreñafe, Lambayeque	Zaña
Cajamarca	Jaén, Cutervo, Chota, San Miguel, Celendín, Cajamarca, Contumazá, San Benito	Cascas, Santa Ana
La Libertad	Trujillo, Otuzco, Cascas	Moche, Cascas, Alto Chicama, Huancay

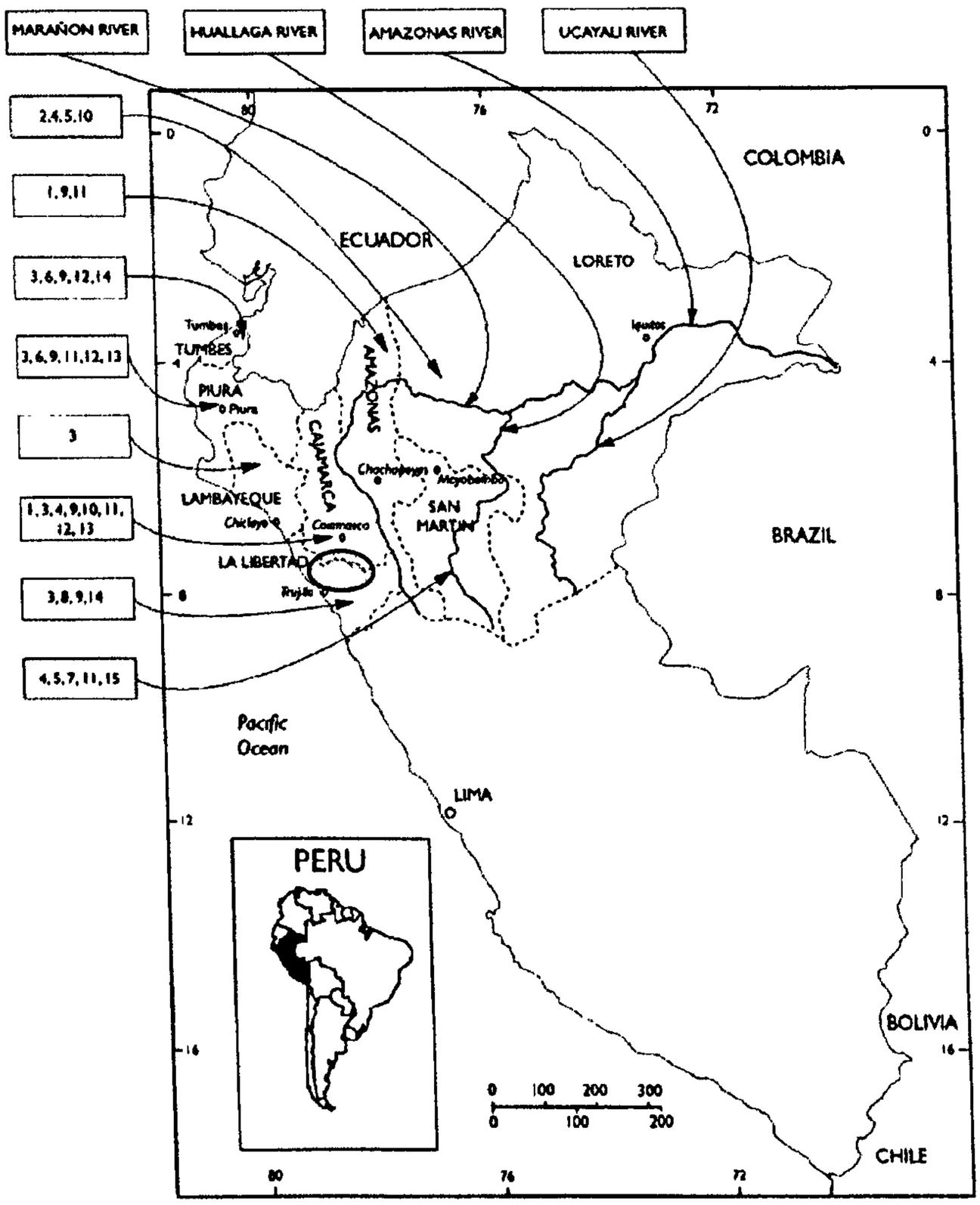


Figure 16. Distribution of *Rhodnius ecuadoriensis* and other triatomines in northern Peru. 1=*Belminus peruvianus*; 2=*Cavernicola pilosa*; **3=*Rhodnius ecuadoriensis***; 4=*R. robustus*; 5=*R. pictipes*; 6=*Eratyrus cuspidatus*; 7=*E. mucronatus*; 8=*Hermanlentia matsunoi*; 9=*Panstrongylus chinai*; 10=*P. geniculatus*; 11=*P. herreri*; 12=*P. rufotuberculatus*; 13=*Triatoma carrioni*; 14=*T. dimidiata*; 15=*T. nigromaculata* (dubious record). The circle indicates the area of origin of the Peruvian population of *R. ecuadoriensis* used for comparative purposes in this study (upper Chicama valley, La Libertad) [after Cuba Cuba et al. 2002]

*This part of the study was carried out in collaboration with Prof CA Cuba Cuba and his research team at the Universities of Trujillo, Peru and Brasília, Brazil (see Cuba Cuba et al. 2002)

Table 12. Biogeography of *Rhodnius ecuadoriensis* in northern Peru

Life zones	AvT	Rain	Alt	Other species
Tropical desert	29-30	0-125	0-125	*T. dimidiata* *P. chinai*
Tropical desert scrub	28.5-29	125-250	125-250	*T. dimidiata* *P. chinai* *P. geniculatus*
Premontane desert scrub	16.5-18	125-250	2000-2250	(none)
Thick tropical bush	28-28.5	250-500	250-375	*T. dimidiata* *P. chinai* *P. rufotuberculatus*
Very dry tropical forest	27-28	500-1000	375-500	*T. dimidiata* *E. mucronatus*
Dry tropical forest	26-27	1000-2000	500-625	*T. dimidiata* *P. rufotuberculatus* *P. geniculatus* *E. cuspidatus* *E. mucronatus*
Dry premontane forest	19.5-21	500-1000	1500-1750	*T. carrioni* *P. rufotuberculatus* *E. mucronatus*

AvT = average minimal and maximal temperatures (in °C); Alt = altitude (in m); Rain = average annual rainfall (in mm); *T.* = *Triatoma*; *P.* = *Panstrongylus*; *E.* = *Eratyrus*

In Peru, *R. ecuadoriensis* was always found in domestic-peridomestic environments in dry or very dry areas. *R. ecuadoriensis* appears capable of exploiting habitats with wide ranges of temperature (16.5-30°C) and altitude (0-2250m, with isolated records from ~2700m), and in areas with rainfall from virtually nil to 2000mm. The presence of *R. ecuadoriensis* in northern Peru was first recorded in 1955 (Herrer et al. 1972), and referred to domestic bugs collected in Tumbes (an arid coastal Department near the Ecuadorian border). Triatomine collections (over 3400 specimens in the period 1973-10981) from the same area included other known local species (mainly *P. chinai* and *T. dimidiata*); 3% of those specimens were *R. ecuadoriensis* – the only *Rhodnius* species known from the area. A recent field record (F Vargas, unpublished data) indicates that synanthropic populations of the species also occur in Andean areas of the Department of San Martín – close to the border with La Libertad.

3.2.4. DISCUSSION

R. ecuadoriensis occurs west of the Andes, between the equator and approximately 8°S (La Libertad, Peru). It has been reported from domestic habitats in virtually its whole range, but sylvatic populations seem geographically more restricted; they have only been found in central western Ecuador, with one isolated record from northern Peru (Abad-Franch et al. 2001b, Cuba Cuba et al. 2002). The pluvial forests of the Chocó ecoregion (mainly Colombian territory but extending into the Ecuadorian

province of Esmeraldas) seem to mark the northern limit to the species range; however, the presence of sylvatic populations in areas with *Phytelephas* palms cannot be ruled out. The general ecology of the Departments of La Libertad and Cajamarca (northern Peru) is very similar to that of other Peruvian Departments further south (Ancash, Lima). As suggested by a limited RS/GIS-based analysis, synanthropic bug populations could disperse to those areas. A few domestic populations of *R. ecuadoriensis* seem to have overcome the Andean barrier in northern Peru, with records from high (semiarid) valleys of the Huancabamba and Huallaga systems (both tributary of the Amazon) (Herrer et al. 1972, Cuba Cuba et al. 2002, F Vargas, unpubl.). Passive transport of synanthropic bugs by migrant people may be involved in this finding; it would seem unlikely that sylvatic populations occur east of the Andes.

In Ecuador, the rural population of the areas where *R. ecuadoriensis* has been collected (at the canton level) can be estimated at >500000 people (1990 official census); the total rural population of Manabí, El Oro and Loja (from where >90% of the records have been made) exceeds 1.2 million people (provisional data, 2001 census). Although the range of the species potentially includes about one third of Ecuador (~80000km^2 out of the total 273000km^2), the whole biogeographical range of *R. ecuadoriensis* may in fact be 'represented' in small coastal areas where low hills create an altitudinal gradient (from about 100 to over 600m) in which vegetation coverage gradually changes from tropical desert scrub-thick tropical bush-dry tropical forest (at the base and low altitudes) to dry premontane forest (on the slopes above 250-300m) to humid-moist-wet premontane forest (on the summit of the hills) (Dodson & Gentry 1991). Such areas are frequent in coastal Manabí, coinciding with the zone from where *R. ecuadoriensis* has been recorded at highest frequency – and the only one where both sylvatic and domestic populations of the species have been found in sympatry.

R. ecuadoriensis seems to occur only in dry life zones in Peru; the known natural ecotopes of the species (palm trees) are absent from such arid environments, raising doubts about the possibility that sylvatic populations exist there. The only finding of the species in an uninhabited area of Peru refers to a single nymph (stage V) collected from a hollow tree in an Andean valley of the Marañón system; the site (Hacienda Choloque) corresponds to the upper stretches (still a dry environment) of an eastern slope valley (the Huancabamba) (Herrer et al. 1972).

3.2.4.1. Relationships with other *Rhodnius* species

R. ecuadoriensis appears to be geographically isolated from its closest relatives within the genus *Rhodnius*. It is separated by the Andes from *R. pictipes* and *R. robustus* – both abundant in the Ecuadorian and Peruvian Amazonian territories. The pair *pallescens-colombiensis* and *R. ecuadoriensis* are also clearly allopatric; their respective known distributions (northwest Colombia and western Ecuador/northwest Peru) are separated by a large stretch of land including the Colombian Pacific coast (the Chocó ecoregion) and by the Central Massif of the Andes in southwest Colombia (figure 17). The closest known populations of *colombiensis* and *ecuadoriensis* were recorded in areas that are about 700-800km away and separated by mountain ranges consistently over 2000m above sea level. Even larger distances separate *R. ecuadoriensis* from the *pallescens* populations of northwest Colombia, but in this case the intermediate area is mainly comprised of lowland rainforest in the Darién-Chocó-Esmeraldas ecoregion (indicated in figure 17).

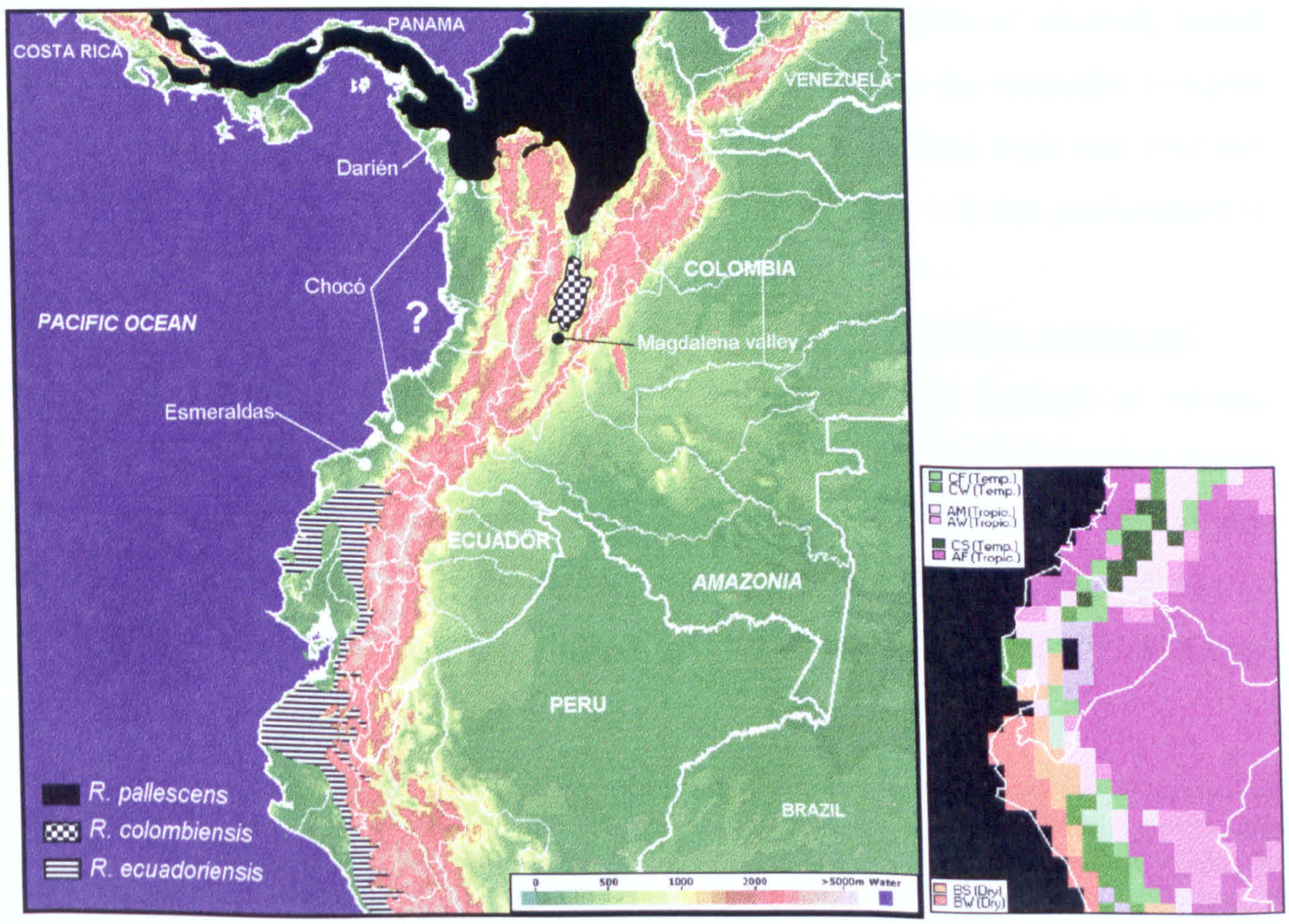


Figure 17. Biogeography of *Rhodnius ecuadoriensis* and related species. Approximate distribution areas were overlaid on an altitude map. The small map on the right shows a climate classification (Koppen system: A=Humid; B=Dry; C=Temperate). Note how the distribution of *R. ecuadoriensis* in Ecuador seems to follow the humid (pink) areas, but it extends to drier areas in northern Peru. The dark orange area in NW Peru largely corresponds to the Sechura desert

3.2.4.2. Endemic nature of *Rhodnius ecuadoriensis*

Apart from the questions about the possibility that intermediate populations occur in the Chocó (symbolised by a question mark in the figure above)[+], the biogeographical traits of *R. ecuadoriensis* suggest that this species is an endemism of the Ecuadorian coastal region. This view is supported by the fact that western Ecuador is considered as one of the areas with highest levels of endemism in the world. An estimated 20% of the flora of western Ecuador is for instance considered endemic, including the palms that constitute the natural habitat of *R. ecuadoriensis* or about 70 out of the 250 orchid species known to the area (Dodson & Gentry 1991, Borchsenius et al. 1998). Additional micro-endemisms have been found in botanic studies carried out at a finer scale within the region, many of them related to altitudinal gradients of humidity (and therefore of vegetation cover) on the hills of the coastal range.

These botanical observations suggest that natural habitat fragmentation in restricted life zones can promote speciation and increase biodiversity at the local level. In this context, *R. ecuadoriensis* is probably to be regarded as an endemic species that evolved to exploit a locally available habitat (*Ph. aequatorialis*) in the plots of relatively humid forests in western Ecuador. The map in figure 10 does not attain the resolution required to show these small humid areas present within the thick tropical bush and very dry tropical forest life zones (numbers 2 and 3 in figure 10 and table 10) that predominate in coastal Manabí.

3.2.4.3. Relationships with *Phytelephas* palms and other ecotopes

Ph. aequatorialis can be assumed to represent the primary ecotope of sylvatic populations of *R. ecuadoriensis*. However, adult bugs (including an inseminated female that laid viable eggs) have been collected in houses of otherwise non-infested communities in very dry areas of Manabí relatively distant from the nearest plot of humid forest with palms (~4-5 km). This suggests that the flight ability of these bugs

---

[+]According to the expert botanist, Dr Finn Borchsenius (pers. comm.), the relationships of the western Ecuadorian moist forest flora suggest a close affinity with Central American ecosystems – rather than with the neighbouring Chocó. This is also the case for numerous palm species belonging to diverse genera, including *Phytelephas*, *Astrocaryum*, *Bactris*, *Aiphanes*, and perhaps *Synecanthus*. Reflecting on these "puzzling" biogeographic findings, Dr Borchsenius thinks, "accepting that the 'Chocó gap' is not an artifact (which seems likely), maybe there have been periodic connections between seasonal forests of western Ecuador and those of the Magdalena-Cauca valleys due to climatic fluctuations" (pers. comm.). However, long-term political unrest and fighting in the Department of Cauca have hampered ecological studies in the area for decades, a trend that seems unlikely to change any time soon

allows them to extend their range from humid to drier areas – where they may establish new colonies if basic conditions are met, such as is often the case in human habitats. The records of the species in dry forests in Manabí and Loja always refer to synanthropic populations. Although the potential range of *Ph. aequatorialis* extends into the low parts of the Andean valleys of Loja, these palms appear to be absent from the area (where dry forest prevails). Palms were also absent from our fieldwork site in El Oro. Recent deforestation might have had a heavy impact on the populations of these palms in this province, where they can still be found in some small forest remnants (see figures 11 and 12). A rapid survey in one of these remnants yielded negative results, but further sampling might reveal sylvatic *R. ecuadoriensis* populations there. Finally, natural populations of palm trees are absent from northwestern Peru. Here, *R. ecuadoriensis* was recorded from arid the life zones (including tropical desert areas with virtually no rainfall) with a wide range of temperatures and altitude.

Together, these findings suggest that synanthropic bug populations have adapted to rather hostile (arid) conditions by exploiting the favourable microhabitats offered by human dwellings. Alternatively, the presence of the species in very dry life zones could indicate that sylvatic populations survive in arid climates by exploiting highly protected natural ecotopes (e.g., hollow trees). Such sylvatic-sylvatic transition (from palms in humid forests to hollow trees in arid areas) raises however some questions as to why a *Rhodnius* species (which probably evolved in humid forests with palm trees) should expand its range into tropical deserts, and how it survived competition from species already adapted to such environments (*P. chinai* and *T. carrioni* in southern Ecuador and northern Peru). Only *R. neivai* among the Rhodniini seems to have completed a comparable transition, occurring in semiarid and arid environments of Venezuela and Colombia associated with both palms and dead logs; *R. nasutus*, typical of the arid open forest of northeastern Brazil, is mainly found in palms and also in bird nests (Barrett 1991). Observations by Herrer et al. (1972) suggested not only that northern Peruvian populations of *R. ecuadoriensis* are well adapted to arid conditions, but also that their range might in fact be limited by excess humidity in, for instance, low areas of the Marañón valley. Surveys designed to inspect putative sylvatic ecotopes in arid-semiarid areas of southern Ecuador and northern Peru (specifically hollow trees, possibly using live-baited adhesive traps) would help clarify this question.

3.3. Ecology and biogeography of other triatomines in Ecuador

The main biogeographical features of other species of Triatominae recorded from Ecuador were also investigated during the course of this project. Five species, out of the 15 reported to occur in the country, have been implicated in the transmission of *T. cruzi* to people in different areas of Ecuador (*T. dimidiata* and *R. ecuadoriensis* in the coastal and western Andean foci and *R. pictipes*, *R. robustus* and *P. geniculatus* in the Amazon), but some others may also play a role in more restricted zones (*P. rufotuberculatus*, *P. howardi*, *T. carrioni*, *P. chinai*, and *P. herreri*). Other species recorded (*Eratyrus mucronatus*, *E. cuspidatus*, *Cavernicola pilosa*, *T. venosa*, and *T. dispar*) seem to be entirely sylvatic and therefore have little or no epidemiological relevance (Aguilar et al. 1999, Abad-Franch et al. 2001a,b). Published reports, unpublished data (from our fieldwork activities and from Ecuadorian colleagues), and material deposited at various entomological collections (Catholic University, Quito, Ecuador; Fiocruz, Rio de Janeiro, Brazil; and the Natural History Museum, London, UK) were reviewed and incorporated into the analyses. The main results are summarised in tables 13-14 and in biogeographical maps presented in figures 18 to 24. *P. lignarius* and *herreri* are considered here as a single species (Marcilla et al. 2002).

Seventeen out of 21 provinces of Ecuador have produced records of triatomines (excluding doubtful reports from two more provinces), corresponding to 11 life zones (Andean life zones above 2200m altitude not included); only *T. carrioni* has been reported from areas above 2200m. Annual rains in areas where triatomines occur ranged from 62.5-125mm/year in the coastal tropical desert and 2000-4000mm/year in the wet and moist forests (table 13). The range of average temperatures in these areas was from 12-18°C in low montane forest areas to 24-26°C in coastal dry tropical forests (table 13). Several species occur at sea level, and one record indicates the presence of *T. carrioni* at 2650m altitude. The maximum number of species recorded in a single life zone corresponded to the Amazon rainforest (8 species, excluding unconfirmed records of *T. dimidiata* and *R. ecuadoriensis*). These two latter species have the widest distribution ranges; *T. dimidiata* has been reported from 7 life zones, and *R. ecuadoriensis* from 5 (7 and 6 provinces, respectively). No records of triatomines from the Galápagos Islands were found.

Table 13. Life zone biogeography of Ecuadorian Triatominae

Life Zones	**T (°C)**	**Alt (m)**	**Rain (mm)**	**Species**[a]
Tropical desert	24	0 – 300	62.5 – 125	*Triatoma dimidiata* (W)
				Rhodnius ecuadoriensis (W)
Thick tropical bush	24 – 26	0 – 300	250 – 500	*T. dimidiata* (W)
				Panstrongylus geniculatus (W)
				P. howardi (W)
Very dry tropical forest	24 – 26	0 – 300	500 – 1000	*T. dimidiata* (W)
				R. ecuadoriensis (W)
				P. rufotuberculatus (W)
				P. chinai (W)
				P. howardi (W)
Dry premontane forest	18 – 24	300	500 – 1000	*T. dimidiata* (W)
				T. carrioni (W)
				R. ecuadoriensis (W)
				P. rufotuberculatus (W)
				P. chinai (W)
				Eratyrus cuspidatus (W)
Dry low montane forest	12 – 18	2000 – 2900	500 – 1000	*T. dimidiata* [b] (W)
				T. carrioni (Andes-W)
				P. chinai (W)
Dry tropical forest	24 – 25	0 – 300	1000 – 2000	*T. dimidiata* (W)
				P. geniculatus (W)
				P. rufotuberculatus (W)
Humid premontane forest	18 – 24	300 – 1800	1000 – 2000	*T. carrioni* (Andes-W)
				T. venosa (E-W)
				R. ecuadoriensis (W)
				P. chinai (W)
Humid low montane forest	12 – 18	2000 – 2900	1000 – 2000	*T. carrioni* (Andes-W)
Moist tropical forest	24 – 25	0-300 W	2000 – 4000	*T. dimidiata* (W)
		0-600 E		*T. dispar* (W)
				R. ecuadoriensis (W)
				R. pictipes (E)
				R. robustus (E)
				P. geniculatus (W-E)
				P. herreri/lignarius (E)
				P. rufotuberculatus (W)
				E. cuspidatus (W)
				E. mucronatus (E)
				Cavernicola pilosa (E)
Wet premontane forest	18 – 24	300-1800 W	2000 – 4000	*T. carrioni* (Andes-E)
		600-1800 E		*T. dispar* (W)
				T. venosa (E)
				R. ecuadoriensis (W)
				P. geniculatus (E-W)
				P. rufotuberculatus (W)
Wet low montane forest	18-24	1000 – 1800	2000 – 4000	*R. robustus* (E)

T = Temperature range; Alt = altitude; Rain = Annual rainfall; W = Western slope of the Andes (Pacific side); E = Eastern slope of the Andes (Amazon side). [a] Dubious records excluded; [b] This record corresponds to one specimen labelled as collected in the city of Loja, and has to be regarded with caution as no further nor previous reports from there have been made

Table 14. Distribution, ecology, and epidemiological significance of Ecuadorian Triatominae

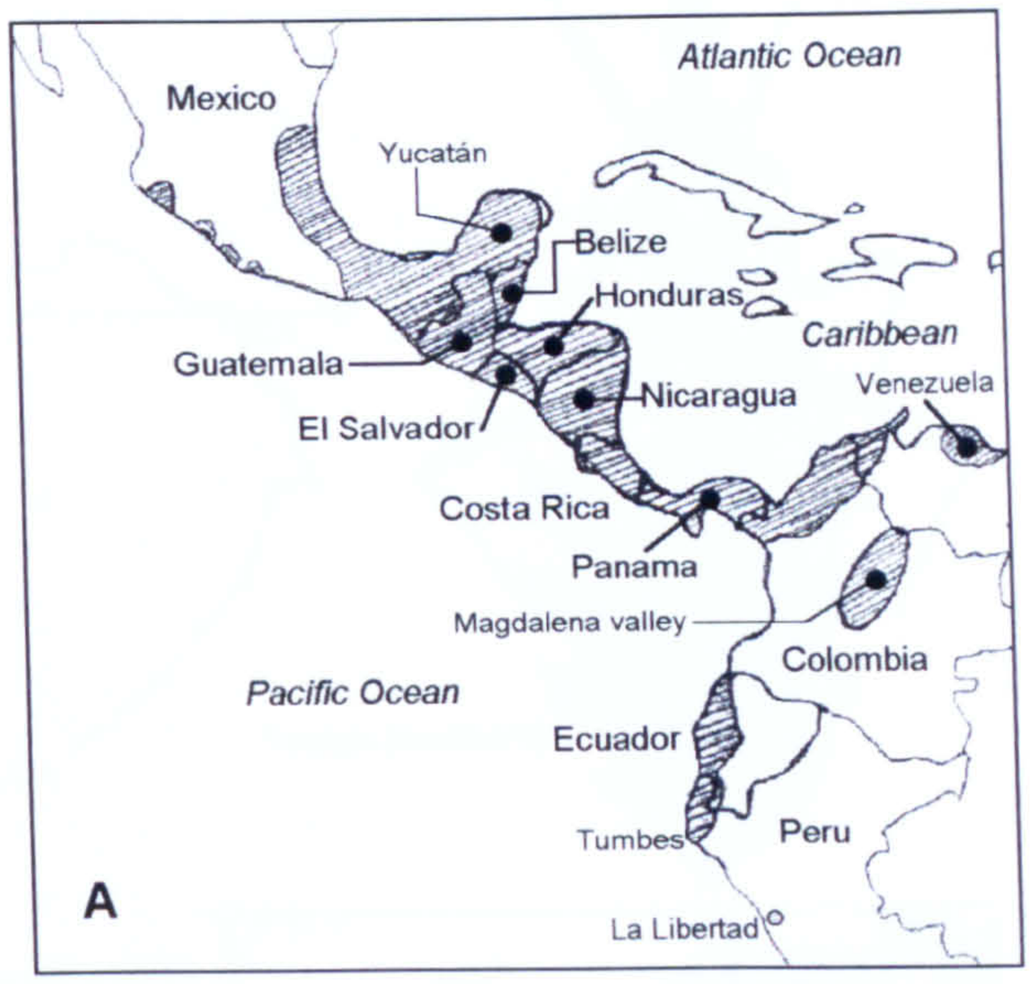
Species	Distribution (provinces) [a]	Epidemiological significance	Observations
Triatoma dimidiata (Latreille)	Manabí, Guayas, El Oro, Los Ríos, Loja, Esmeraldas, Pichincha; uncertain findings in the Amazon region (Napo and Sucumbíos)	The main vector of *T. cruzi* in Ecuador, responsible for the urban endemic focus of transmission in the city of Guayaquil and for continued transmission in other areas, including several coastal cities	Mainly dry life zones in central and southern coastal region. The apparent discontinuity in its distribution from Colombia to Peru merits further investigation. Sylvatic ecotopes in Ecuador unknown. Genetic and morphometric similarities with Mesoamerican specimens. Its presence in the Amazon needs confirmation
Triatoma carrioni Larrousse	Loja, Azuay, Cañar, El Oro, Pichincha, Cotopaxi, Zamora Chinchipe	Locally important in Andean valleys of the south, where it can be found in domestic environments. However, trends in records from Loja suggest it may have been replaced by *R. ecuadoriensis* in domestic habitats in some areas	Temperate valleys and highlands of the southern Andes, apparently extending to humid foothill forests on both slopes of the Andes; a V instar nymph apparently belonging to this species was captured in a bromeliad epiphyte in Cotopaxi (primary cloud forest >2000m altitude). The species has a vernacular name in Loja ('chinche de caballo'), suggesting frequent contact with humans
Triatoma venosa (Stål)	Azuay, Napo/Orellana	Sylvatic in Ecuador (domestic colonies reported from Colombia). Occasionally caught at light in or around houses. High altitudes recorded (Andean life zones)	Up to 2200 m altitude. Probably closely related to *T. carrioni*
Triatoma dispar Lent	Imbabura, Cotopaxi	Strictly sylvatic	Andean region; one record from Guayas (coastal lowlands) is probably erroneous. Closely related to *T. venosa* and *T. carrioni*
Eratyrus mucronatus Stål	Napo/Orellana	Sylvatic in Ecuador (reports of domestic colonies in Bolivia)	Amazonia (primary forest; two specimens captured by light trapping); one record from Esmeraldas is probably due to misidentification
Eratyrus cuspidatus Stål	Loja, Esmeraldas	Sylvatic; a few adults were collected from a peridomestic goat corral in Loja	Coastal region and temperate valleys of the western Andes
Cavernicola pilosa Barber	Napo/Orellana, Pastaza	Sylvatic	Amazon region (one specimen captured by light trapping)

a: reports on distribution: *T. dimidiata*: Lent & Wygodzinsky 1979, Lazo 1985, Defranc 1982, Abad-Franch et al. 2001a;*T. carrioni*: León 1949, Espinoza 1955, Lent & Wygodzinsky 1979, Defranc 1982, Reyes 1992, Abad-Franch et al. 2001a; *T. venosa*: Defranc 1982, Lent & Wygodzinsky 1979, Abad-Franch et al. 2001a; *T. dispar*: Lent & Wygodzinsky 1979, Abad-Franch et al. 2001a; *E. mucronatus*: Defranc 1982, Lent & Wygodzinsky 1979, Abad-Franch et al. 2001a; *E. cuspidatus*: Defranc 1982; *C. pilosa*: Lent & Wygodzinsky 1979, Abad-Franch et al. 2001a. Napo and Orellana were separated in 1998

Table 14. Distribution, ecology, and epidemiological significance of Ecuadorian Triatominae (continued)

Species	Distribution (provinces) [a]	Epidemiological significance	Observations
Rhodnius ecuadoriensis Lent & León	Manabí, Guayas, Los Ríos, El Oro, Loja, Pichincha	The second main vector of *T. cruzi* in the country. Can invade-colonise human environments and breed even within dwellings in good condition. Average human seroprevalence rates above 10% are typical in endemic areas where *R. ecuadoriensis* is the prevailing domestic vector	Central and southern coastal region; sylvatic populations reported from Manabí, Los Ríos and Pichincha. Associated with domestic birds (chicken, pigeons) and, in sylvatic environments in northern and central Ecuador, with *Phytelephas aequatorialis* palms; sylvatic populations not reported from southern Ecuador nor northern Peru
Rhodnius pictipes Stål	Sucumbíos, Napo/Orellana, Morona-Santiago	Probably involved in the transmission of *T. cruzi* in some areas. Adults frequently fly into houses, even in urban areas; reports indicating domestication deserve further investigation	Amazon region. Present in palm trees of five genera in Sucumbíos; extremely dense colonies in *Phytelephas tenuicaulis* palms located in pasture fields. *R. stali*, a species very closely related to *R. pictipes*, is absent from the Ecuadorian Amazon
Rhodnius robustus Larrousse	Sucumbíos, Napo/Orellana	Probably involved in the transmission of *T. cruzi* in some areas. Adults fly into houses; reports of domestic colonies need confirmation	Amazon region. Found in palm trees of five genera in Sucumbíos; dense colonies in *Attalea butyracea* palms. Populations visibly darker and smaller than typical forms were detected in the northern Ecuadorian Amazon. Alleged collections in coastal areas probably reflect labelling errors
Panstrongylus geniculatus (Latreille)	Imbabura, Manabí, Pichincha, Esmeraldas, Sucumbíos, Napo/Orellana	Possibly involved in the transmission of *T. cruzi* in some areas. Adults fly into houses; reports of domiciliation in Brazil, Venezuela and Colombia	Broad distribution (from Argentina to Mexico); it can be found on both slopes of the Andes (slight but noticeable chromatic differences recorded between both populations)
Panstrongylus rufotuberculatus (Champion)	Imbabura, Pichincha, Manabí, Loja, El Oro, Los Ríos, Guayas	Locally important in the southern Andes and the central lowlands, where it is truly domestic. One fatal case of acute Chagas disease (province of Pichincha) was recently attributed to a domestic colony of this species	Western slope of the Andes in Ecuador, but broad distribution in the Americas (from Argentina to Mexico)
Panstrongylus chinai (del Ponte)	Loja, El Oro	Unclear; reports of domestic colonies, mainly in timber-walled huts and chicken coops. An important local vector in Piura, northern Peru	Mainly sylvatic; arid-semiarid southwest region. This species has vernacular local names ('negrito', 'chupón') in some areas of Loja and El Oro, suggesting domiciliation may be frequent
Panstrongylus howardi (Neiva)	Manabí	Unclear; adults are occasionally found within human dwellings, and one peridomestic colony was reported	Endemic species apparently restricted to a small geographic area of arid climate; biology, habitats and hosts unknown
Panstrongylus herreri-lignarius Wygodzinsky-(Walker)	Sucumbíos, Napo/Orellana	Adults found in houses; it is the main vector of Chagas disease in northern Peru (Marañón valley)	*P. herreri-lignarius* are probably the same species; *lignarius* is widespread in Amazonia, and *herreri* seems limited to the areas close to the Andes in Peru-Ecuador

a: reports on distribution: *R. ecuadoriensis*: Lazo 1985, Defranc 1982, Carcavallo & Martínez 1985, Romaña et al. 1994, Abad-Franch et al. 2000, Abad-Franch et al. 2001a; *R. pictipes*: Espinoza 1955, Amunárriz 1991, Amunárriz et al. 1991, Chico et al. 1997, Abad-Franch et al. 2001a; *R. robustus*: Espinoza 1955, Amunárriz 1991, Amunárriz et al. 1991, Chico et al. 1997, Abad-Franch et al. 2001a; *P. geniculatus*: Espinoza 1955, Rodríguez 1959, Defranc 1982, Amunárriz 1991, Amunárriz et al. 1991, Chico et al. 1997, Abad-Franch et al. 2001a; *P. rufotuberculatus*: Lazo 1985, Defranc 1982, Reyes 1992, Abad-Franch et al. 2001a; *P. howardi*: Defranc 1982, Lent & Wygodzinsky 1979, Abad-Franch et al. 2001a; *P. chinai*: Defranc 1982, Lent & Wygodzinsky 1979, Reyes 1992; *P. herreri*: Lent & Wygodzinsky 1979, Aguilar et al. 1999, Abad-Franch et al. 2001a; *P. lignarius*: Rodríguez 1961, Defranc 1987, Lent & Wygodzinsky 1979, Abad-Franch et al. 2001a. Napo and Orellana were separated in 1998



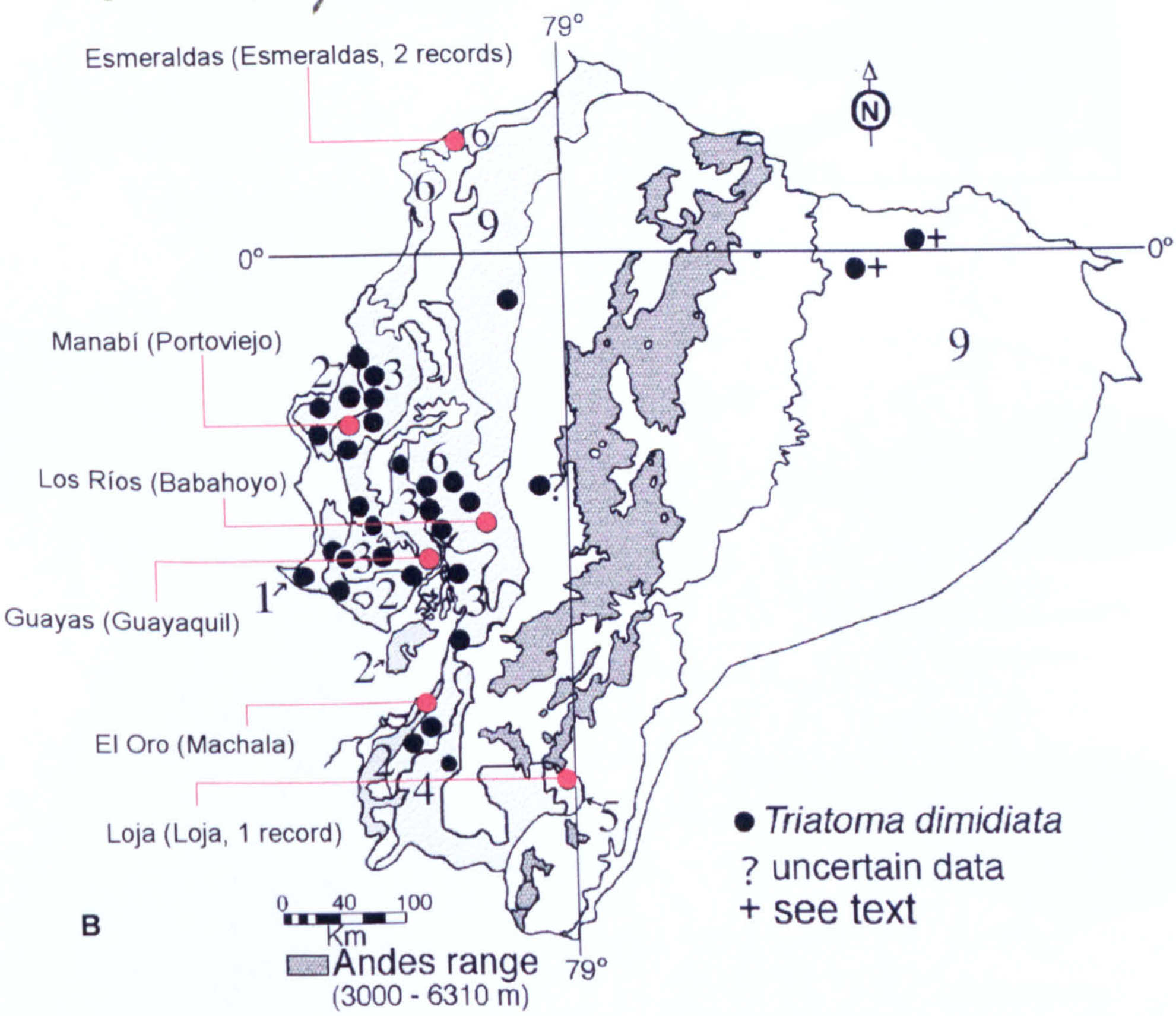


Figure 18. Biogeography of *Triatoma dimidiata*. **A**: Distribution; **B**: Life zone biogeography in Ecuador (life zones: 1-Tropical desert; 2-Thick tropical bush; 3-Very dry tropical forest; 4-Dry premontane forest; 5-Dry low montane forest; 6-Dry tropical forest; 9-Moist tropical forest). The presence of the species in urban areas is emphasised: *T. dimidiata* has been reported from the capital towns of all coastal (and one Andean) provinces in Ecuador (in parentheses)

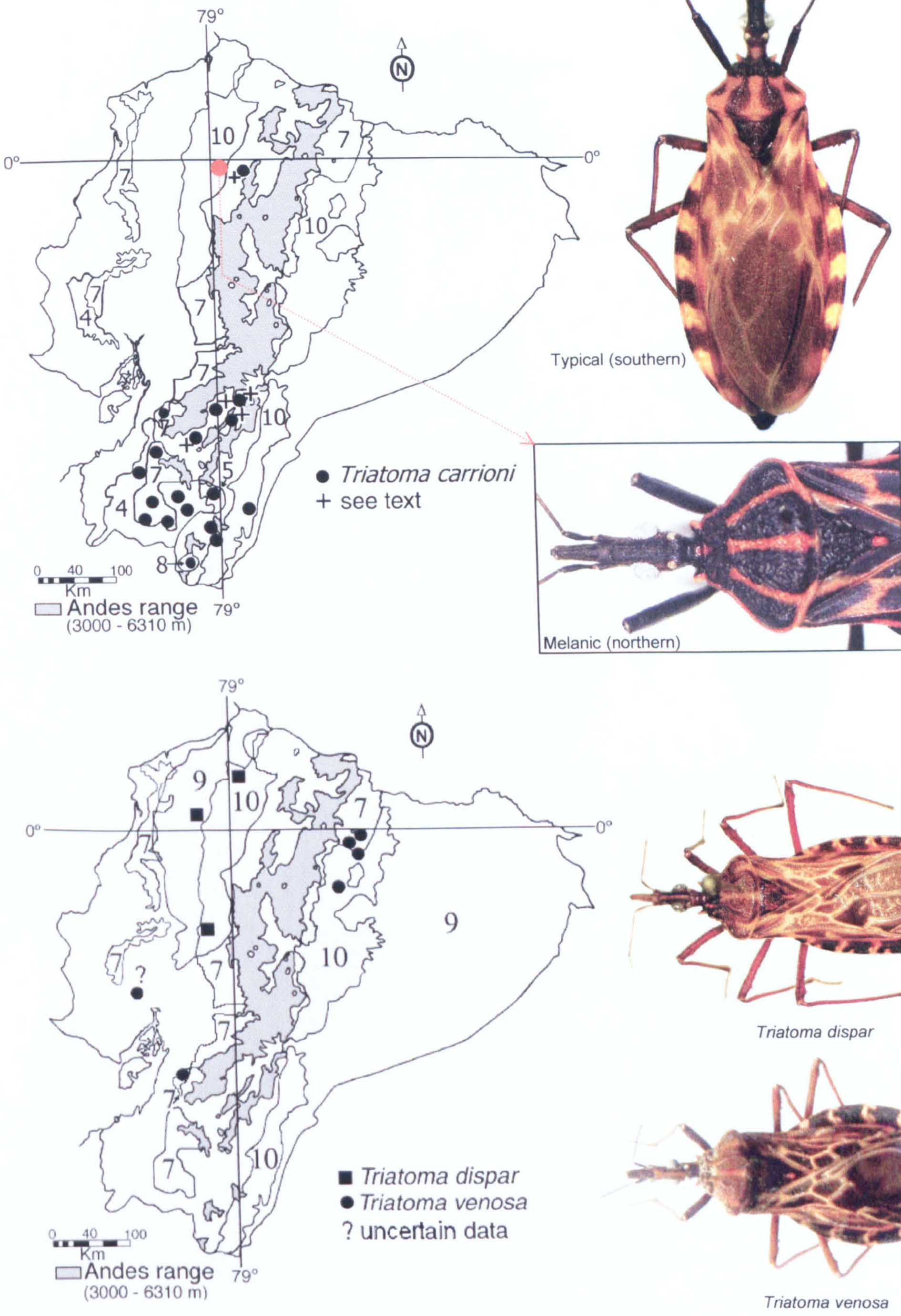
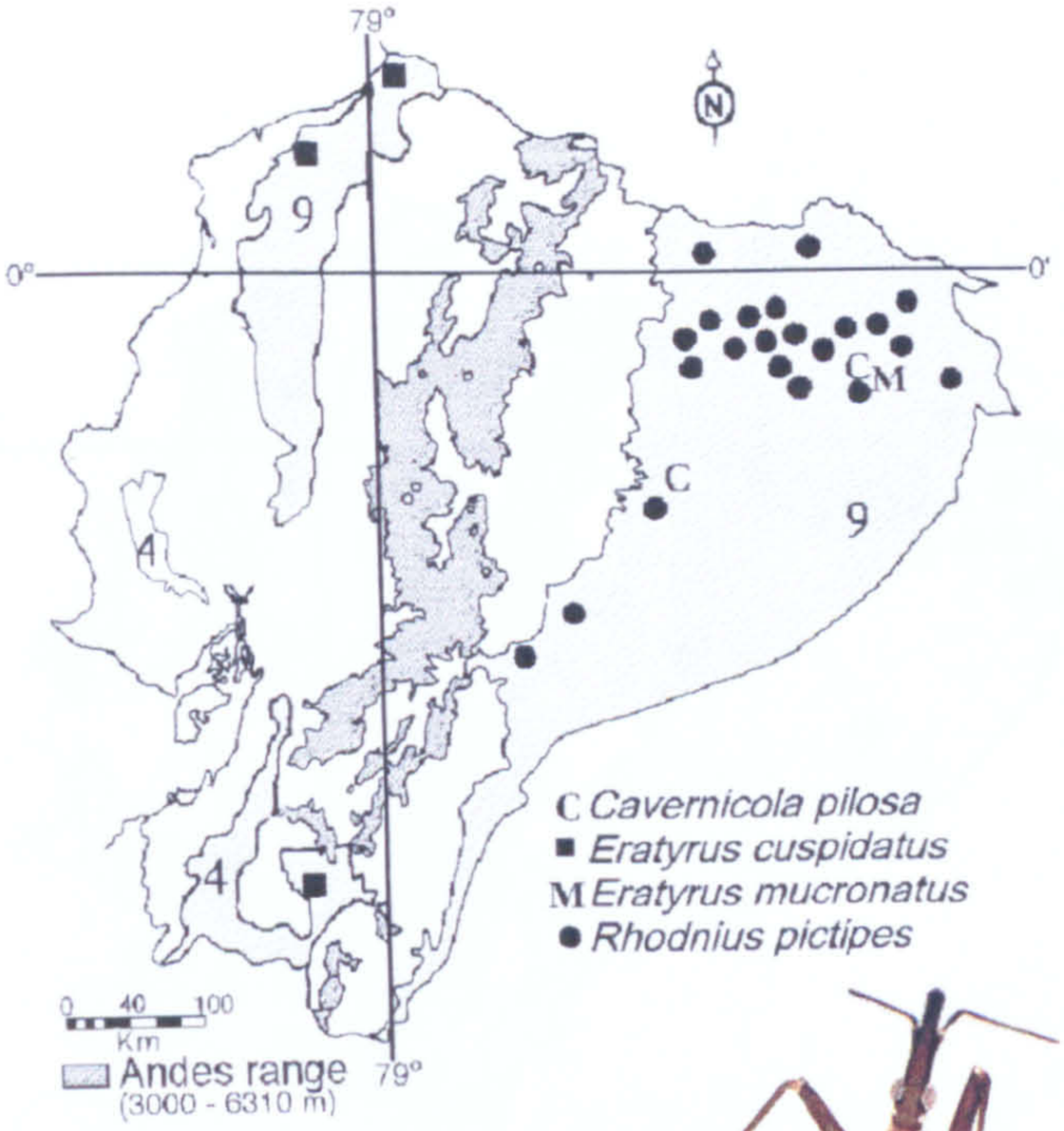


Figure 19. Biogeography of *Triatoma carrioni*, *T. dispar* and *T. venosa* in Ecuador (life zones: 4-Dry premontane forest; 5-Dry low montane forest; 7-Humid premontane forest; 8-Humid low montane forest; 9-Moist tropical forest; 10-Wet premontane forest)

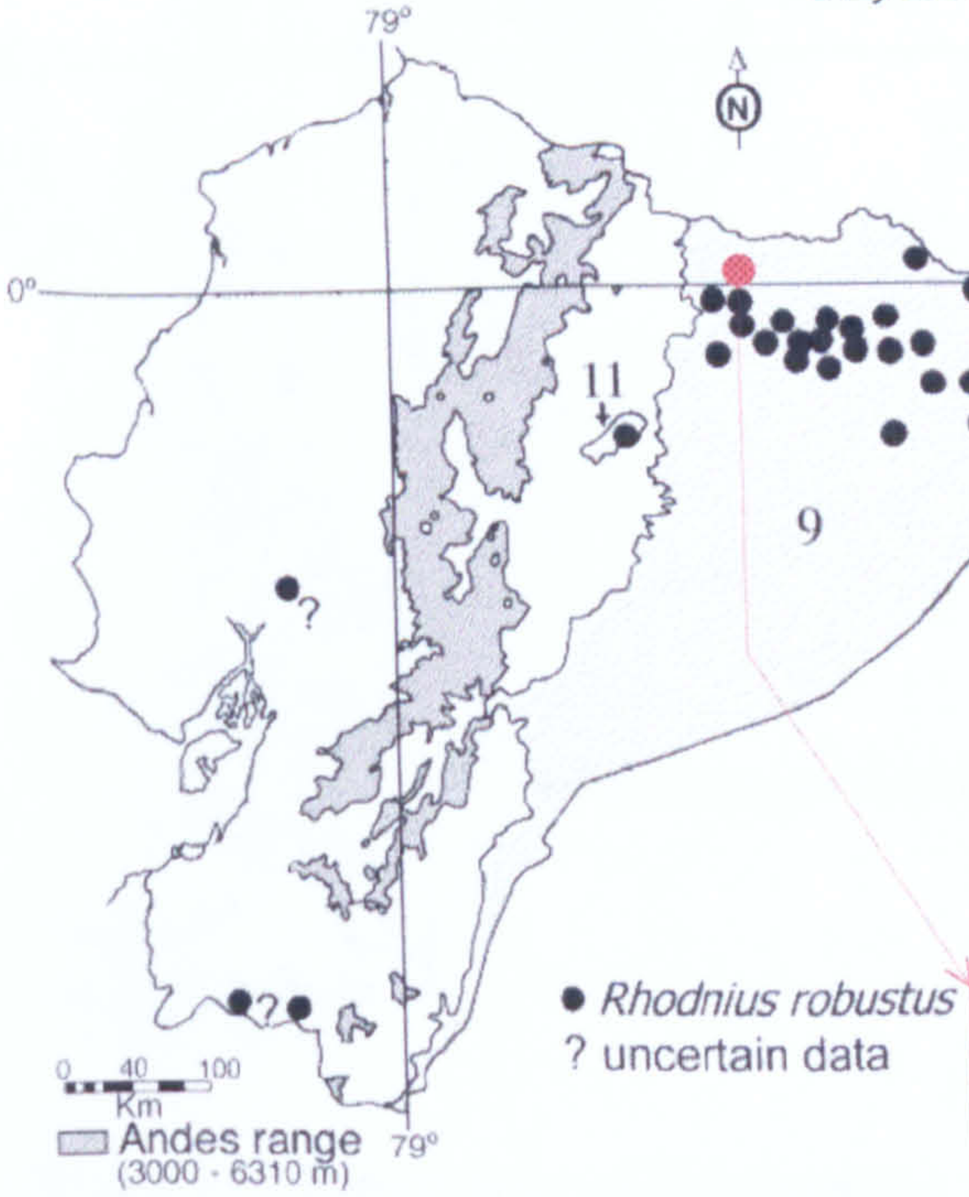


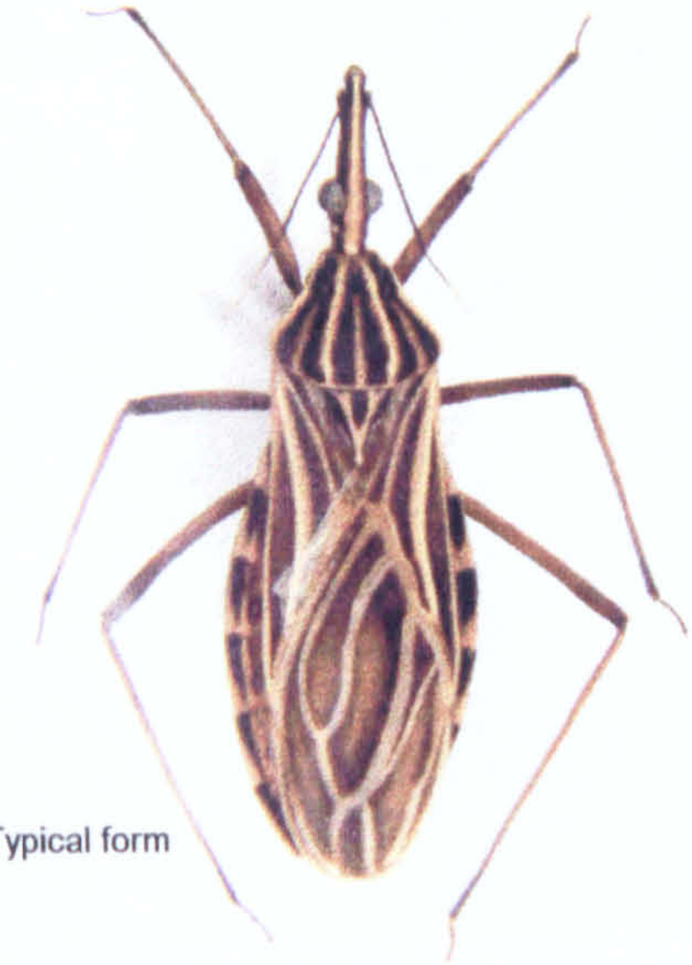
Rhodnius pictipes

Cavernicola pilosa

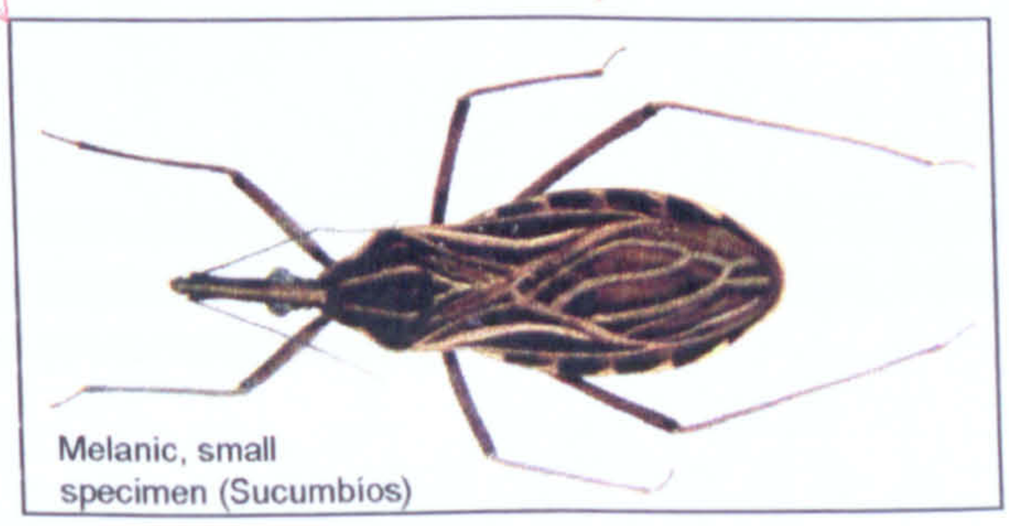
Eratyrus cuspidatus

Eratyrus mucronatus



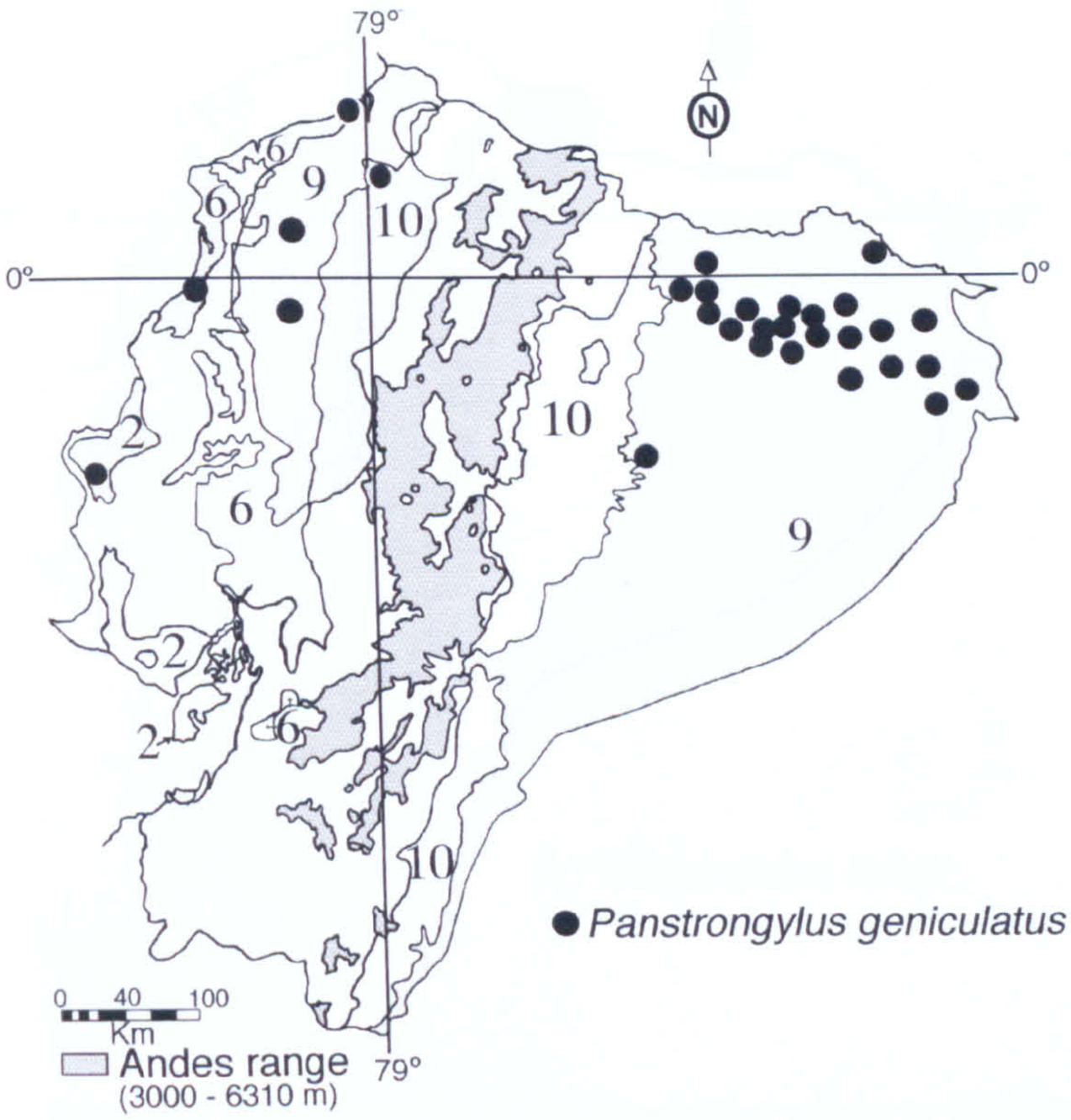


Typical form

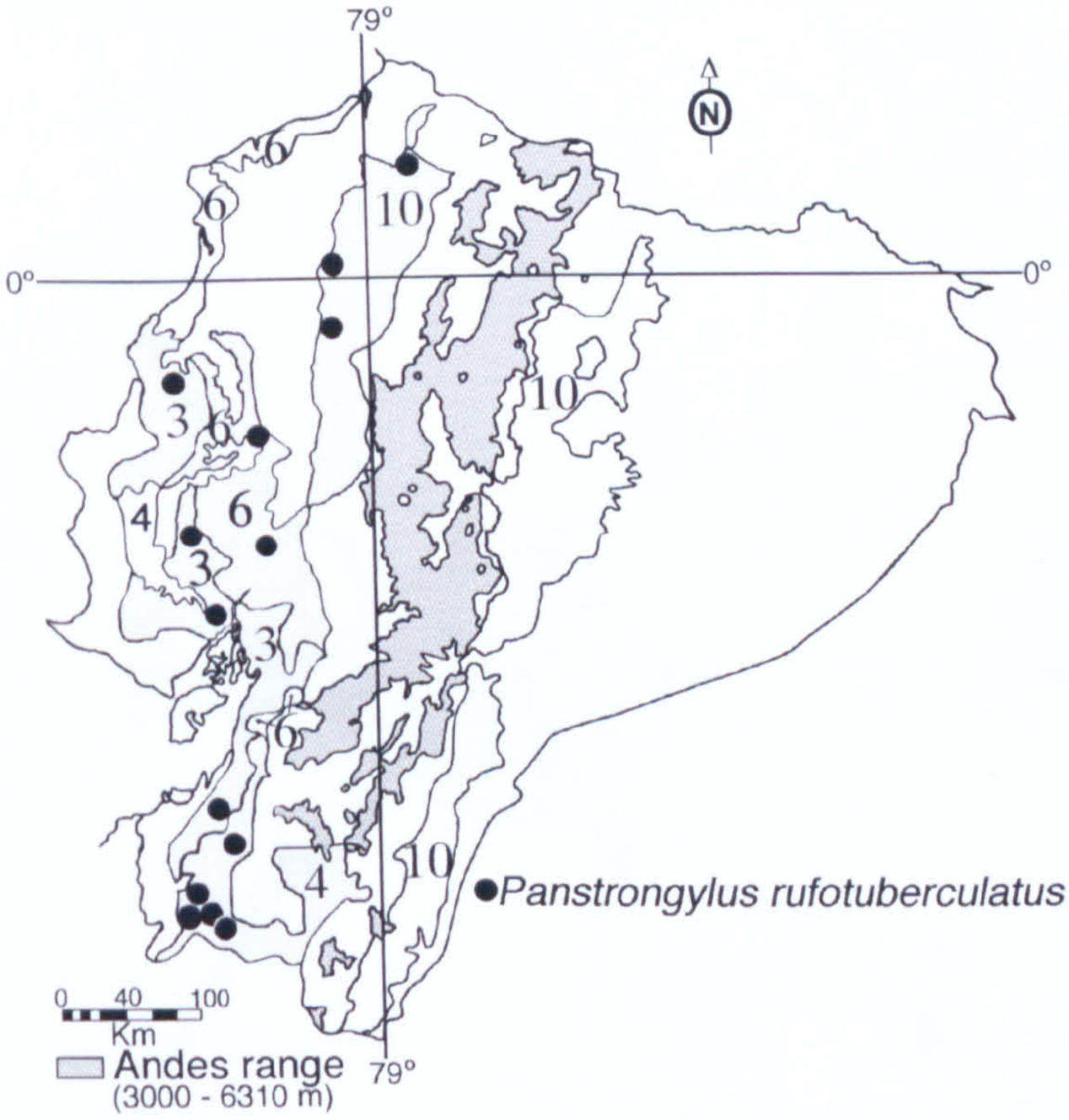


Melanic, small specimen (Sucumbios)

Figure 20. Biogeography of *Rhodnius pictipes*, *R. robustus*, *Cavernicola pilosa*, *Eratyrus cuspidatus*, and *E. mucronatus* in Ecuador (life zones: 4-Dry premontane forest; 9-Moist tropical forest; 11-Wet low montane forest)



● *Panstrongylus geniculatus*



●*Panstrongylus rufotuberculatus*

Figure 21. Biogeography of *Panstrongylus geniculatus* and *P. rufotuberculatus* in Ecuador (life zones: 2-Thick tropical bush; 3-Very dry tropical forest; 4-Dry premontane forest; 6-Dry tropical forest; 9-Moist tropical forest; 10-Wet premontane forest)

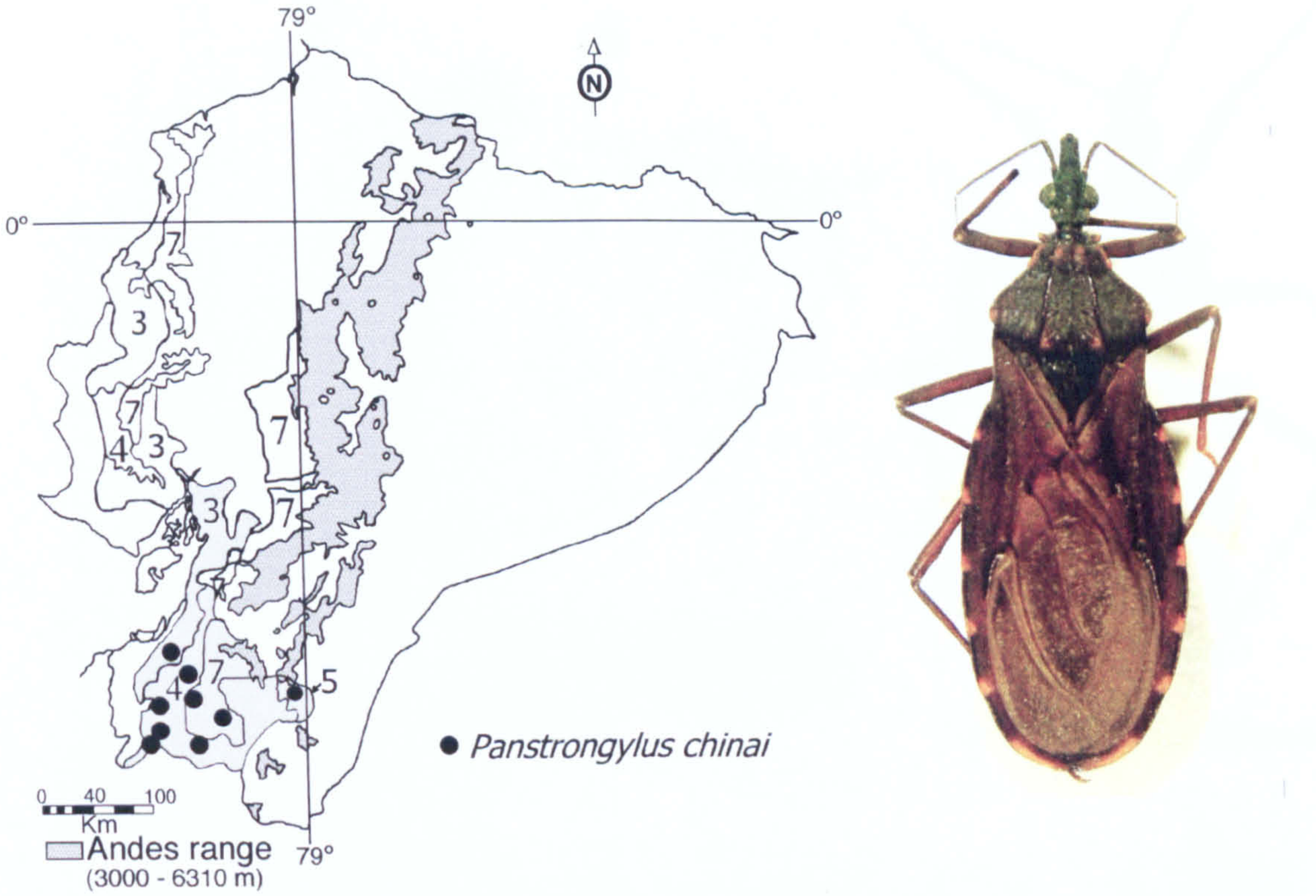


Figure 22. Biogeography of *Panstrongylus chinai* in Ecuador (life zones: 3-Very dry tropical forest; 4-Dry premontane forest; 5-Dry low montane forest; 7-Humid premontane forest)

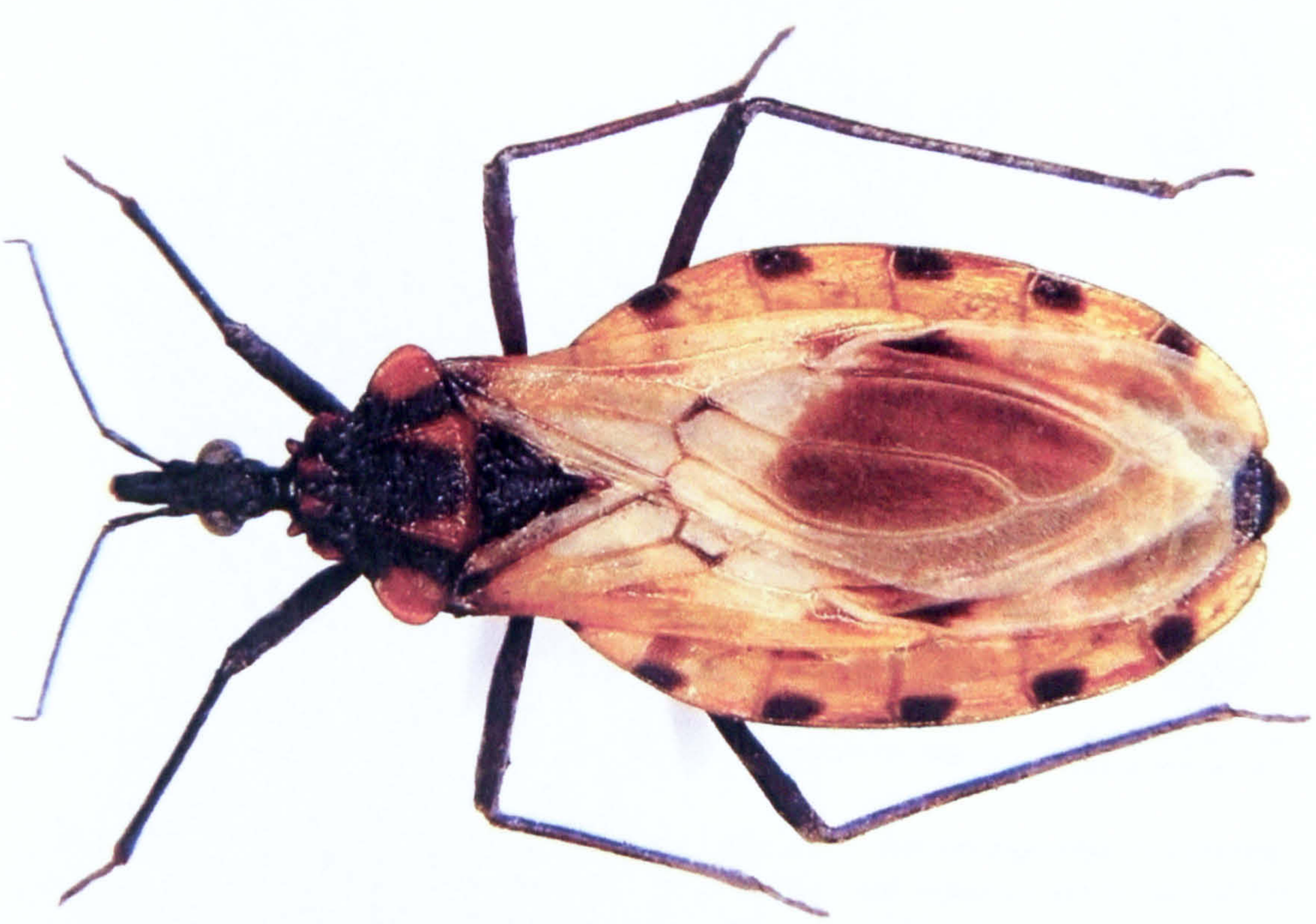
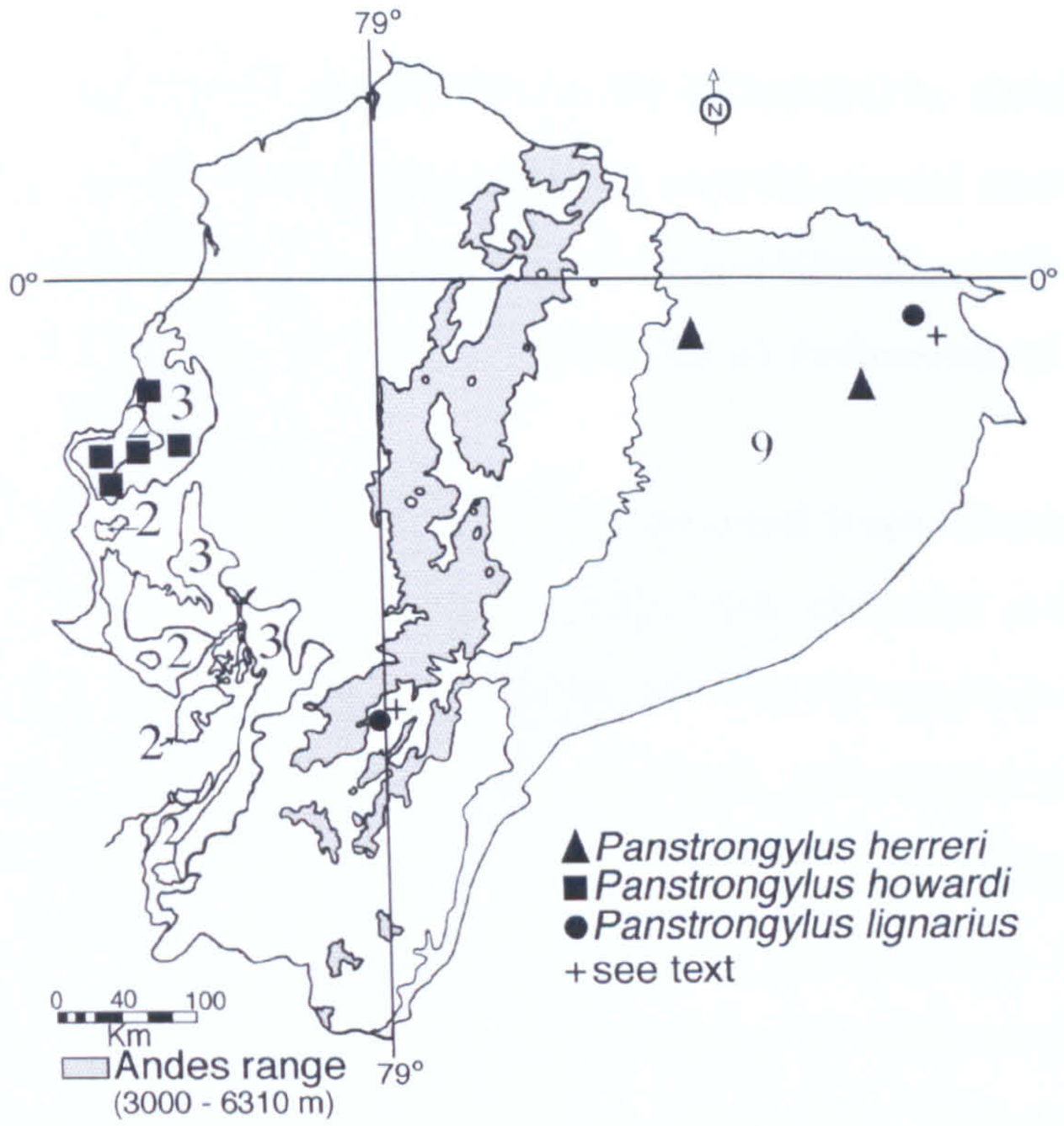
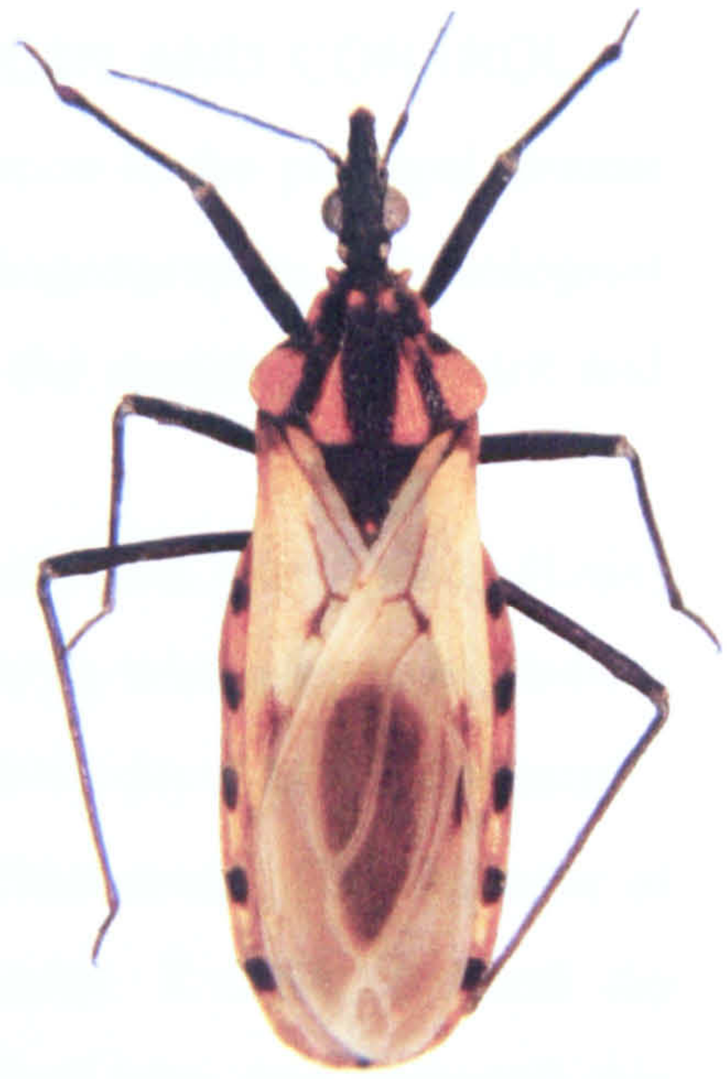


Figure 23. *Panstrongylus howardi*: female specimen collected in a house in Jipijapa (Manabí)



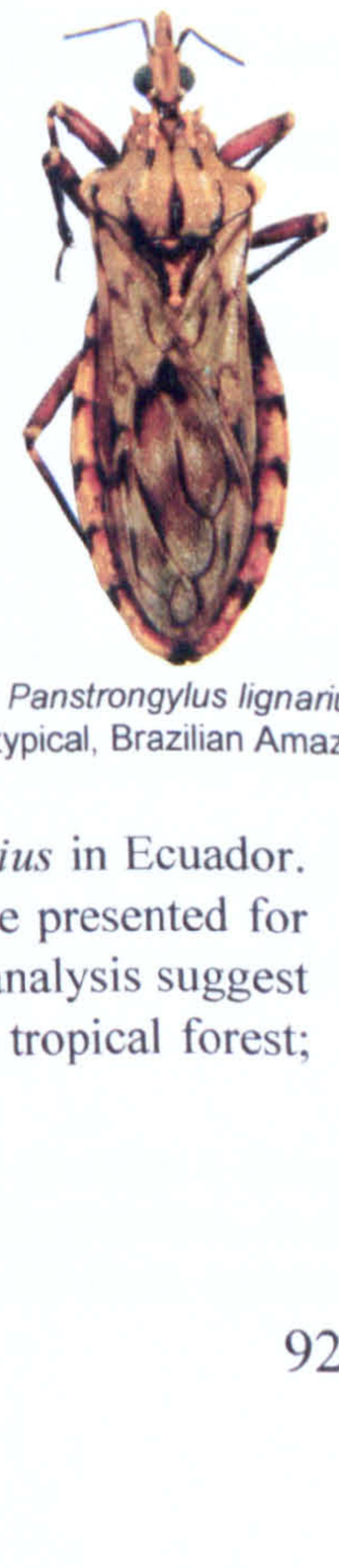


Panstrongylus howardi (male)

Panstrongylus herreri-like
(male, Ecuadorian Amazon)

Panstrongylus herreri-lignarius
(female, Ecuadorian Amazon)

Panstrongylus herreri
(typical, northern Peru)



Panstrongylus lignarius
(typical, Brazilian Amazon)

Figure 24. Biogeography of *Panstrongylus howardi* and *P. herreri-lignarius* in Ecuador. Ecuadorian material and typical specimens of *P. herreri* and *lignarius* are presented for comparison. Results from interbreeding experiments and DNA sequence analysis suggest they are a single species. (Life zones: 2-Thick tropical bush; 3-Very dry tropical forest; 9-Moist tropical forest)

3.3.1. *TRIATOMA DIMIDIATA* IN ECUADOR: ORIGIN AND CONTROL

In this general ecological evaluation we paid special attention to the principal disease vector in the country, *T. dimidiata*. Joint consideration of biogeographic and ecological data led to the hypothesis of an **artificial introduction** of the species to Ecuador and Peru.

In Ecuador, *T. dimidiata* has been reported from all coastal and one Andean (Loja) provinces (Abad-Franch et al. 2001a,b), and maintains a large urban focus of disease transmission in Guayaquil (Aguilar et al. 1999). It occurs in low, dry areas of the coast♦, including the tropical desert of Santa Elena and several urban areas. The presence of sylvatic populations has never been documented in Ecuador. R Zeledón cited the finding (in 1932) of adult specimens under the bark of a dead tree, and informed that adult specimens reportedly invaded dwellings in areas of new human settlements when electric light began to be installed (Zeledón 1981). These indirect records contrast with the fact that no further such observations have been produced over the last half-century. *P. howardi* is similar to *T. dimidiata* in both colour pattern and size (see figures 18, 23 and 24); in Manabí, it is often misidentified as *T. dimidiata* by Vector Control Service personnel. This complicates the assessment of unpublished reports and personal communications about adult *T. dimidiata* specimens found either in sylvatic habitats (dead logs are favoured ecotopes for many *Panstrongylus* species) or flying into houses (allegedly from sylvatic habitats) in the province. *T. dimidiata* has been found in large terrestrial bromeliads grown as fences around households in Manabí (Defranc 1982), and in periurban rubbish dumps of Portoviejo (capital of Manabí). Opossums and rodents are common in such fences and dumps, likely providing a stable and sufficient food source to the bugs. These should not, in our opinion, be regarded as true sylvatic ecotopes but just as peridomestic ones.

As a rule, introduced bug populations occupy only human-related ecotopes; the adaptation to highly stable human habitats is deemed irreversible, and only native species are expected to breed in truly sylvatic ecotopes within any given geographic area (Schofield et al. 1999). Systematic field research is required to verify (or reject) the

---

♦Some exceptions have been recorded: one adult specimen apparently collected in Loja (~2000 m altitude), two claimed captures in the Amazon, one record from the province of Bolívar (western Andean foothills), and one record from the humid lowlands of the province of Pichincha. All these specimens were collected in domestic-peridomestic environments

absence of sylvatic populations of *T. dimidiata* in the country, but available evidence and field observations indicate that they probably do not exist.

Some further data and observations would seem to support our idea of an artificial introduction of the species in Ecuador.

3.3.1.1. Biogeography

The distribution of *T. dimidiata* is largely discontinuous (figure 18) (Zeledón 1981, D'Alessandro & Barreto 1985, Carcavallo et al. 1999c), with its range clearly interrupted in the Chocó (Colombian Pacific coast). In northern Ecuador (province of Esmeraldas) the species was recorded only twice (in urban areas) (Rodríguez 1959, Defranc 1982, Lazo 1985, Abad-Franch et al. 2001b). The extension of the species to central-southern Colombia follows the Magdalena valley (Zeledón 1981, D'Alessandro & Barreto 1985, Carcavallo et al. 1999c). The tendency of *T. dimidiata* to occupy arid or semi-arid regions (rather than humid forests) in the South American Pacific coast suggests that the species may be absent from the Chocó. The Andean cordillera, which closes the Magdalena Valley in the south, and the humid forests of Nariño and Cauca (Colombia), and Esmeraldas (Ecuador) could have acted as biogeographic barriers that limited the dispersal of *T. dimidiata* to the south. The lack of systematic entomological surveys in the cited areas could also be a reason for this apparent discontinuity.

3.3.1.2. Historical data

The presence of important pre-Inca civilisations in what is now Ecuador is well documented (e.g., Meggers & Evans 1963). These populations occupied central-southern areas of the coastal region – in Manabí, Guayas and Los Ríos, with extensions towards the southern province of El Oro. This distribution is remarkably coincident with the current range of *T. dimidiata* in the country. Sea commerce with Mesomerican populations was a major economic activity; archaeological evidence suggests that these links were already established 1500-1200 years B.C. (Meggers 1963), with some authors dating the first contacts at ~5000 years B.C. (Ayala Mora 1993). These data suggest that domestic individuals of *T. dimidiata* might have been introduced to the Ecuadorian coastal region, perhaps in the pre-Columbian period (see also Álvarez 1984), then passively extended to their current range in Ecuador and Peru.

3.3.1.3. Phylogenetic relationships

It is generally accepted that *T. dimidiata* belongs to the *phyllosoma* complex, a group of closely related Mesoamerican-Caribbean species of *Triatoma* (Lent & Wygodzinsky 1979, Barrett 1991, CJ Schofield pers. comm.)]; this implies that *dimidiata* has Mesoamerican ancestry, and that its presence in South America can be regarded as a biogeographic anomaly. Contrary to this view, Carcavallo et al. (1999b, 2000) presented the hypothesis (based on 'plesiomorphic morphological conditions' [Carcavallo et al. 1999b; p. 939]) that *dimidiata* belongs to a different species complex of South American (Venezuela-Colombia) origin that extended southwest (Colombia-Ecuador-Peru), southeast (Colombia-Venezuela-Guyana), and northwards (Colombia-Central America-Mexico). Genetic evidence from both autosomal and mitochondrial loci (discussed below) clearly refutes this latter hypothesis, showing a close association of *dimidiata* with Mesoamerican species of the *phyllosoma* complex.

3.3.1.4. Clinal variation

Passive dispersal of domestic bugs associated with human activities may be expected to result in substantial similarity between populations occupying the 'origin' and 'destination' geographic areas. Thus, according to our hypothesis, Ecuadorian insects might be expected to resemble their Mesoamerican relatives rather than their Colombian conspecifics. Contrarily, a model of active dispersal through different ecological regions (as proposed by Carcavallo et al. [1999b, 2000]) gives rise to a cline in which any given population tends to resemble more the neighbouring ones than those located in distant areas. Lent and Wygodzinsky (1979) studied the ratio between the length of the head and its width across the eyes in 160 *T. dimidiata* from the entire range of the species, and found that it decreased (relatively longer head and smaller eyes) southwards from Mexico to Colombia. Ecuadorian and Peruvian specimens had however short heads and relatively large eyes, thus resembling their Mesoamerican relatives rather than those from neighbouring Colombia.

3.3.1.5. Morphometrics

We performed, in collaboration with S Solís-Mena and JS Patterson (LSHTM) a morphometric study including 100 specimens of *T. dimidiata* from Mexico, Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica, Panama, Colombia, and Ecuador. Results from both isometry- and allometry-free analyses of head capsule measurements

revealed a clear separation of Ecuadorian and Colombian populations. UPGMA dendrograms confirmed these trends and revealed a clinal tendency involving Central American and Colombian populations – but with Mexican and Ecuadorian populations appearing in independent branches.

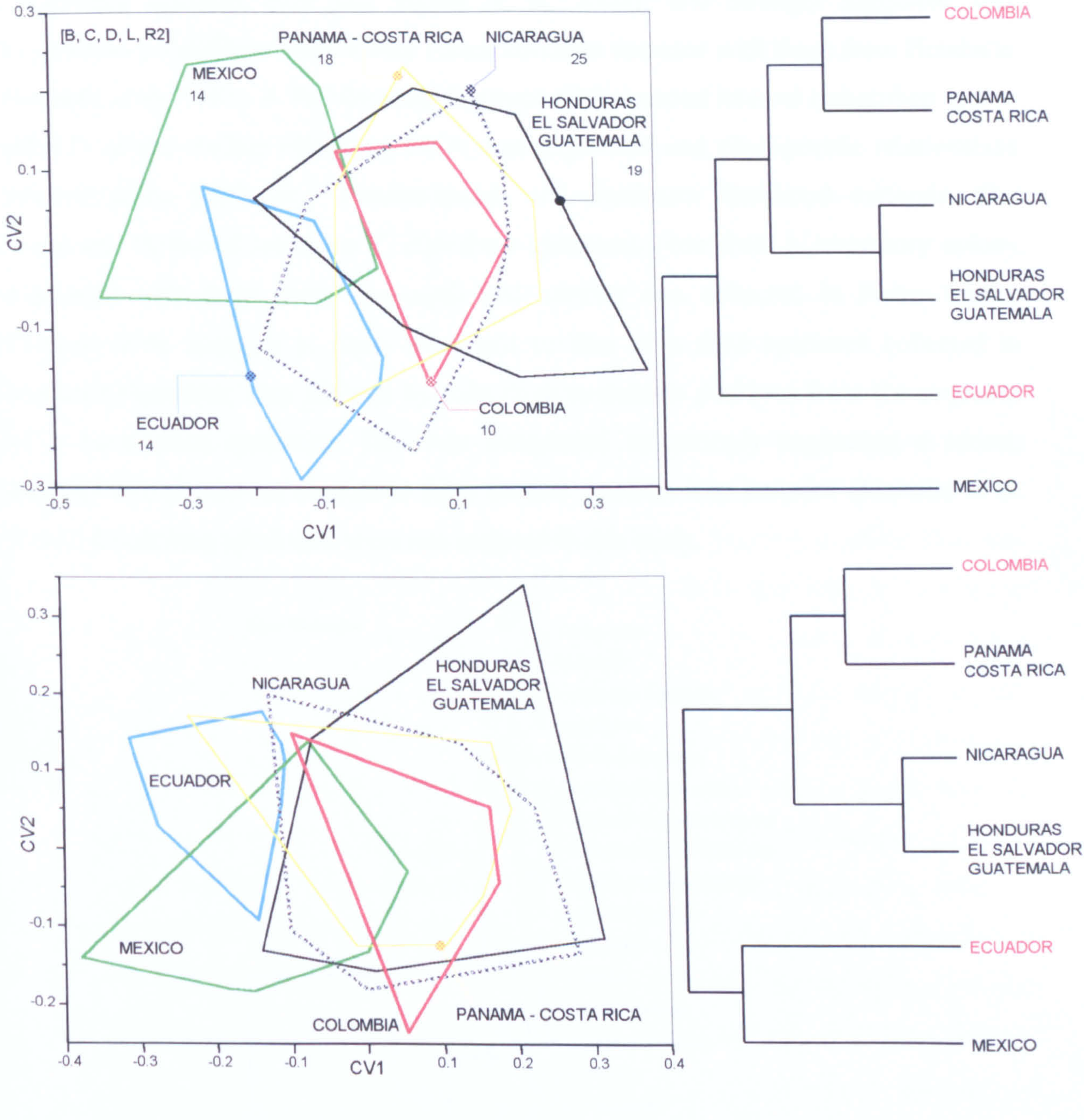


Figure 25. Morphometrics of *Triatoma dimidiata* populations: factorial maps and UPGMA dendrograms derived from Mahalanobis distances. Canonical variate analyses (upper: isometry-free; lower: allometry-free) show a clear separation of Ecuadorian and Colombian populations. The numbers of specimens in each group and the measurements used (in brackets) are indicated in the upper graph; details on methods used for measurement and analysis are described in detail in Section 6.2.2.

3.3.1.6. Molecular biology

Molecular phylogenetic analyses performed in collaboration with the Department of Parasitology of the University of Valencia, Spain (Dr A Marcilla, Dr MD Bargues and Prof. S Mas-Coma) and other groups confirmed the affiliation of *T. dimidiata* to the *phyllosoma* complex (see also Hypša et al. 2002), and strongly suggested that Ecuadorian populations share a very recent common ancestor with those from Honduras (Marcilla et al. 2001). A 581-basepair fragment of the second internal transcribed spacer (ITS-2) of the nuclear ribosomal DNA was sequenced and phylogenetic relationships inferred using parsimony, distance-based, and maximum likelihood methods. The sequences of two Ecuadorian *T. dimidiata* specimens (one from a laboratory colony originated with bugs from Guayaquil and another one collected in Pedro Carbo, Guayas) were identical to each other and to that of a third specimen collected in southern Honduras; they differed by only three nucleotide positions from the sequence of a Nicaraguan specimen. This was interpreted as strongly suggesting a recent, artificial introduction of *dimidiata* from Central America into Ecuador (Marcilla et al. 2001); Colombian specimens were not analysed in this study.

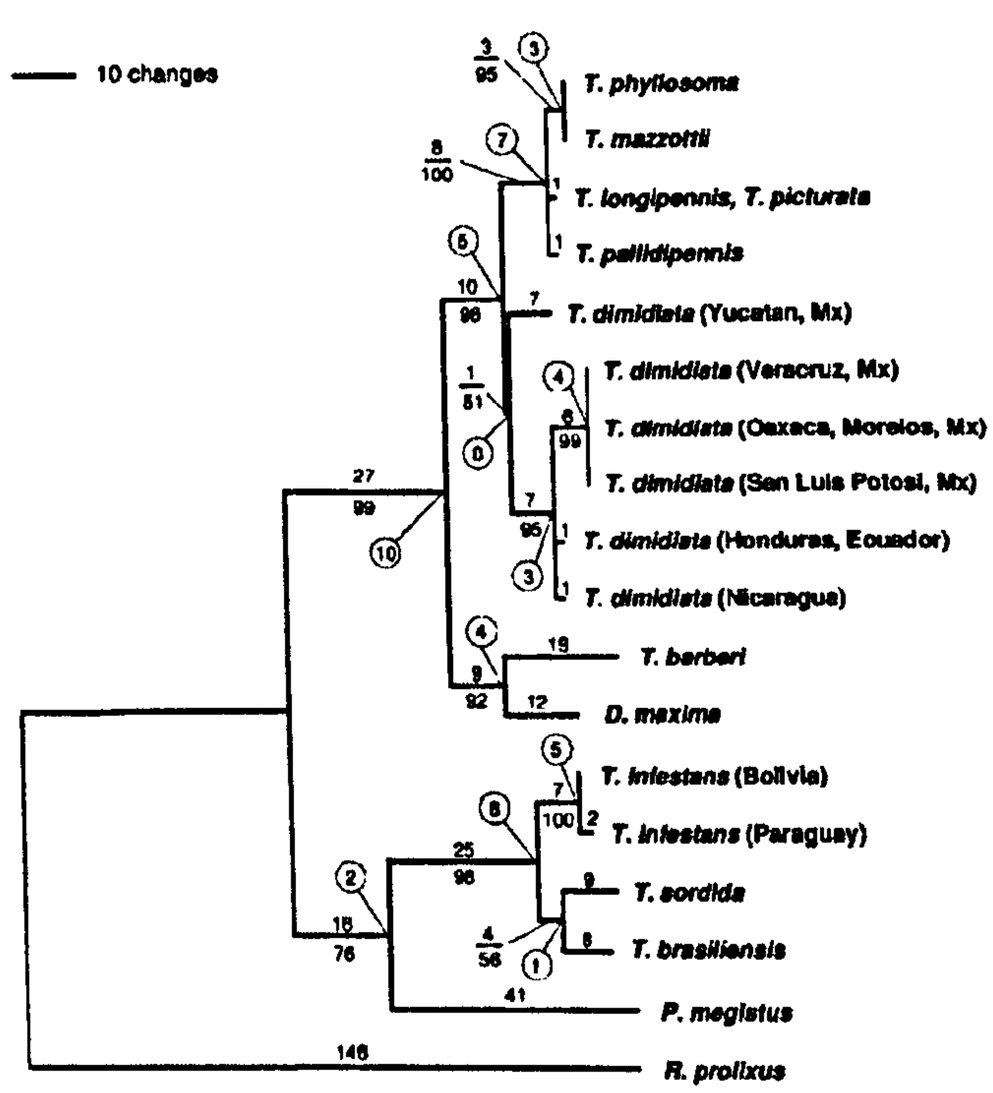


Figure 26. Phylogenetic relationships of *Triatoma dimidiata*. Maximum parsimony analysis of a 581bp fragment of the ITS-2 of the nuclear rDNA. Numbers above lines indicate branch lengths (tree steps), those below lines are bootstrap support values (from 1000 replicates), and numbers in circles indicate Bremer support values for the nodes. Note the well-supported monophyly of the *phyllosoma* + *dimidiata* clade, and the sequence identity between Ecuadorian and Honduran material [from Marcilla et al. 2001]

Further evidence was obtained from the analysis of mtDNA sequence data; based on a fragment of the mt ND4 gene, K Harris and coworkers demonstrated a very close relationship between Ecuadorian and Honduran specimens, and confirmed the similarities between these populations and those from Guatemala (CB Beard, pers. comm.). Similarly, preliminary results by S Solís-Mena and coworkers show that sequences (473bp of the mt ND5 gene) obtained from seven Ecuadorian specimens (field-collected in Guayas and Manabí) are identical. They differ by only one point mutation (a silent T/C transition, 0.2% sequence divergence) from those of Honduran specimens (from two localities) and from the sequences of three bugs collected in El Salvador – identical to the Honduran population. On the other hand, seven substitutions (~1.5% sequence divergence) were identified that separated Ecuadorian specimens from field-collected Colombian material (Solís-Mena et al., unpublished results). All these results show an unambiguously very close relationship between Ecuadorian and northern Central American populations of *T. dimidiata*, and are better explained by an artificial introduction of the species into Ecuador, firmly supporting our hypothesis.

Being *T. dimidiata* the main vector of Chagas disease in Ecuador, these findings may prove crucial for the design of suitable **control strategies** in the country. In Central America-southern Mexico, re-infestation of dwellings from sylvatic foci hampers control schemes; entomological vigilance is a key factor for the success of such control activities (e.g., Acevedo et al. 2000, Schofield 2000c). Control (but not eradication) of domestic bug populations is the objective of preventive programmes in areas where the species is also present in sylvatic environments. Artificially introduced, domestic triatomines are pre-adapted to human-related habitats; this favours disease transmission, but the absence of sylvatic foci limits the possibility of re-infestations. Control schemes may contemplate local eradication of such populations. Results presented here indicate that the strategy for the control of *T. dimidiata* in Ecuador and Peru should contemplate its eradication from domestic and peridomestic environments. An entomological surveillance system should subsequently be established with the aim of detecting any re-infestation from surviving bugs and the invasion of human-related environments by secondary, native triatomine species. The possibility that new introductions of *T. dimidiata* from Mesoamerica occur through sea trade probably persists, and will have to be considered in harbour cities of Ecuador and Peru.

3.3.2. OTHER TRIATOMINE SPECIES

3.3.2.1. *Triatoma carrioni*

This endemic species has adapted to human habitats in Andean valleys of southern Ecuador and northern Peru; it occupies a wide range of life zones (dry and humid, 1000-2650m altitude) and has been reported to feed on humans and horses (Lent & Wygodzinsky 1979). An adult specimen was captured by light trapping in the northern province of Pichincha (see figure 19). A nymph apparently belonging to this species was collected from an epiphytic bromeliad in the canopy of primary cloud forest in a neighbouring area. In the southern Andean provinces of Loja and Azuay, the species has been found in human-related habitats both in rural and urban areas (León 1949, Espinoza 1955, Defranc 1982). Some capture places (marked with + in the map in figure 19) are located in Andean life zones over 2200m; these findings correspond to human environments, but this does not preclude its presence in sylvatic habitats as well. Control of this species should contemplate the possibility of re-infestations from sylvatic foci; selective spraying of infested households should be complemented by a strong component of longitudinal surveillance.

3.3.2.2. *Rhodnius pictipes* and *Rhodnius robustus*

These sylvatic species are common in the Ecuadorian Amazon (where seropositivity ranges from 0.78% to 6.03%; cf. Aguilar et al. 1999 and Section 1.3.). Both species are only known to occur on the eastern side of the Andes (except for a single record of *R. pictipes* from Belize), and are considered as candidate vectors of human disease (Miles et al. 1981b). We have studied their presence in palm trees of the Ecuadorian Amazon region; 48.4% out of 64 palms surveyed using live-baited traps (see Section 4.2.3.) were positive, including five genera (*Attalea*, *Astrocaryum*, *Oenocarpus*, *Phytelephas*, and *Elaeis*) (Palomeque et al. 2000, Noireau et al. 2002b). Over 600 bugs were collected from those 31 infested palms. These observations suggest low levels of habitat specialisation in these species; only *Mauritia flexuosa* and the cultivated *Cocos nucifera* were negative. However, *Mauritia* palms seem to be a favoured ecotope for *R. neglectus* in central Brazil, and have also been found to harbour *R. prolixus* in Venezuela (cf. Pizarro & Romaña 1998), and *R. pallescens* and *R. prolixus* have both been collected from coconut palms (Carcavallo et al. 1998a, Romaña et al. 1999, Jaramillo et al. 2000). Another interesting finding is that both *R. pictipes* and *R. robustus* seem to have

successfully adapted to the artificially introduced African oil palm (*Elaeis guineensis*). This palm is extensively cultivated in the Ecuadorian Amazon, and very frequently found in peridomestic environments of the area. We found some 15 individuals of different nymphal stages (both species) in one out of two palms sampled by live bait trapping in Sucumbíos. Finally, while *R. robustus* prevailed in *Attalea* palms, *R. pictipes* was much more abundant in *Phytelephas tenuicaulis* (but small numbers of the non-prevailing species were captured from all infested palms). *Ph. tenuicaulis*, small palms with clonal stems, were heavily infested when located in pasture fields; we suspect that the very aggressive *R. pictipes* may feed on cattle resting beneath the palms, which are maintained in deforested areas to provide shadow to the livestock. In *Oenocarpus*, *Elaeis*, and *Astrocaryum* no remarkable differences in the relative abundance of each species were observed. Miles et al. (1981b) reported that *R. pictipes* prevails in *Acrocomia sclerocarpa* palm trees in the state of Pará, Brazil, and also colonises *Maximiliana regia*. *R. robustus* was found sharing these ecotopes, but in much smaller numbers. In *Attalea* palms, *R. robustus* hide in the angles between palm leaves and the trunk, while *R. pictipes* prefer the abundant rotting fibres and dead leaves that remain among the clonal trunks of *Ph. tenuicaulis* palms. In both cases, the colour of the insects melted with that of the substrates (dark brownish in *robustus* and their deep shelters in *Attalea*, and straw yellowish in *pictipes* and the dead fronds and fibres of *Phytelephas*).

The high apparent densities of the some of the colonies (overall crowding index=12.5±18.7) in palms located in secondary forest may be related to the frequent accounts of bugs found in domiciles in Amazonian Ecuador, because adult bugs are more likely to start a dispersive flight when starved. In fact, we manually captured many starved insects as they attacked us while working on or around the palms; some *R. pictipes* approached us by flying from heavily infested *Ph. tenuicaulis* palms. The possible epidemiological implications were supported by the results of a serological survey showing an overall prevalence rate of about 3% in the region (MJ Grijalva et al., unpublished; see Section 1.3.2.); natural infection with *T. cruzi*-like parasites of 35 *R. pictipes* analysed was ~31% (AG Guevara, pers. comm.).

Some of the putative *R. robustus* we captured were very dark and relatively small (figure 20). Their taxonomic status is being investigated, but external anatomical features broadly match the description of *R. robustus* by Lent & Wygodzinsky (1979).

In our revision of museum specimens, we found several insects labelled as *R. robustus* collected in areas of the Pacific slope of the Cordillera in Ecuador (Loja and Los Ríos provinces) during 1970-71 and deposited at the Herman Lent Collection (Fiocruz). This finding is most likely related to erroneous capture sites indicated on labels.

3.3.2.3. *Panstrongylus rufotuberculatus*

P. rufotuberculatus is truly domestic in some areas of southern Ecuador, where complete breeding colonies have been found inside dwellings (Barrett 1991, Avilés et al. 1995b, Abad-Franch et al. 2001a,b); in 1985, R Lazo considered this species as the second main vector of Chagas disease in Ecuador (Lazo 1985). Synanthropic behaviour has been previously reported in *P. rufotuberculatus* from Bolivia (Noireau et al. 1995, Dujardin et al. 1998), Peru (Lizaraso 1955, Calderón et al. 1985), and Colombia (Wolff & Castillo 2002). Adult bugs may also invade houses attracted to electric light (Lent & Wygodzinsky 1979, Salomón et al. 1999). The epidemiological significance of this species deserves further research in southern and central Ecuador. Recently, a fatal case of acute Chagas disease was reported from Santo Domingo de los Colorados (Province of Pichincha); an entomological investigation in the house of the patient revealed the presence of a small breeding colony of *P. rufotuberculatus* (with nymphs collected from beds), strongly suggesting transmission mediated by this species (Palomeque et al., unpublished). The possibility that this species colonises houses after domiciliated species are eliminated by control interventions has to be taken into account by control managers. *P. rufotuberculatus* occurs mainly in low, dry areas, but may also be found in zones of very humid premontane forest in northern Ecuador, where it is mainly sylvatic but may invade and occasionally colonise houses.

3.3.2.4. *Panstrongylus geniculatus*

P. geniculatus is broadly distributed throughout the continent (with records from Mexico to Argentina), and occurs on both slopes of the Andes. Specimens from the Ecuadorian Amazon and coastal regions display conspicuous, apparently constant chromatic differences. Bugs from the eastern side of the Andes have a lighter overall colour, and smaller dark markings on the pronotum; those collected on the Pacific side are darker and have larger dark markings, resulting in a more complex colour pattern on the pronotum. *P. geniculatus* has been mentioned as a potential vector in the

transmission foci described in the Ecuadorian Amazon (Chico et al. 1997, Aguilar et al. 1999). It seems to be readily attracted to electric light (Lent & Wygodzinsky 1979), and will also approach oil candles in the Ecuadorian Amazon (FS Palomeque, pers. comm.). Peridomestic colonies have been reported from the Brazilian Amazon (Valente et al. 1998, Valente 1999), and house infestation has been recorded in Venezuela (Reyes-Lugo & Rodríguez-Acosta 2000) and Colombia (Angulo et al. 1999); reports of true domiciliation in Ecuador (Amunárriz 1991, Chico et al. 1997) need confirmation. The species is to be considered as a potential secondary vector in its distribution areas.

3.3.2.5. *Panstrongylus howardi*

This little-known species, endemic to a dry area of Manabí, is rather commonly found entering human dwellings – usually adult specimens, except for one report of a breeding colony in a peridomicile (Defranc 1982). Its sylvatic habitats and hosts remain unknown. The possibility that *P. howardi* transmits *T. cruzi* to people by colonising or invading human-related structures points out the necessity for entomological studies of this species. After examining material collected in domiciles of Manabí, including as yet undescribed female specimens (figures 23-24), we concluded that the species is probably very closely related to *P. chinai*, as already suggested by Lent and Wygodzinsky (1979). Both occur in not very distant geographic areas (although they seem to be allopatric) and favour dry environments; moreover, detailed examination of chromatic patterns revealed that they share the basic design pattern, including the reddish markings on the pronotum. The fact that most *chinai* are strongly melanic (almost completely black in many cases) makes this correspondence hard to perceive, but we hypothesise that both species share a recent common ancestry; *P. chinai* may even represent simply a dark morph of *howardi*.

3.3.2.6. *Panstrongylus chinai*

P. chinai probably also plays a role in the sylvatic cycles of *T. cruzi* transmission in some areas of southern Ecuador and northern Peru, but only few data are available (cf. Barrett 1991, Abad-Franch et al. 2001b, Cuba Cuba et al. 2002). It has been found to breed in chicken coops and, occasionally, in human dwellings; in both cases, wooden structures seem to provide suitable habitats for this species, but stone walls of goat enclosures have also been found infested (Barrett 1991, Cuba Cuba et al. 2002). Adult specimens may fly into houses illuminated by artificial light (Lent & Wygodzinsky

1979, Cuba Cuba et al. 2002). The species may also behave as a secondary vector; it has been cited as the main domestic triatomine in the Department of Piura in northern Peru (Barrett 1991). Entomological surveillance is therefore a key for the success of control activities in the dry areas where this species occurs.

3.3.2.7. *Panstrongylus herreri*

This species was only known from Peru until we reported its presence in Ecuador (Aguilar et al. 1999, Abad-Franch et al. 2001b). It is domestic in areas of northern Peru related to the upper Marañón river system, where it is the main vector of Chagas disease (Lent & Wygodzinsky 1979, Calderón et al. 1985, Cuba Cuba et al. 2002). Regarding its occurrence in Ecuador, we first identified a male specimen collected in a light trap in a zone of primary Amazonian forest; later on, a female individual was captured in a dwelling of an indigenous village in the province of Napo, confirming our previous record. We also examined a *P. lignarius* female (captured in primary rainforest) that had some mixed characters of *P. lignarius* and *herreri* (figure 24). These closely related species showed reproductive compatibility under laboratory conditions (Barrett 1988), but no hybrids have been reported from nature (Lent & Wygodzinsky 1979). Recent results from rDNA ITS-2 sequence analysis have shown that material identified as either *P. herreri* or *P. lignarius* and collected in different parts of the Amazon drainage basin (*lignarius* from Brazil, *herreri* from Peru, and Ecuadorian material corresponding to the *herreri*-like male and the female with mixed traits described above) have identical sequences, strongly suggesting that the minor chromatic differences (*lignarius* presents a basal light annulus on the tibiae, which are uniformly dark in *herreri*) represent mere geographic variants (Marcilla et al. 2002; see also Section 1.2.2.). These results were interpreted as an indication that *herreri* should be synonymised with *lignarius*. The differences in geographic distributions and ecological preferences would perhaps be better represented by assigning subspecific status to the current species, with *P. l. lignarius* occupying sylvatic ecotopes in a large area of the central-eastern Amazon basin and *P. l. herreri* occurring in a more restricted area including the eastern slopes of the Andes in Ecuador-Peru and some inter-Andean valleys related to the Marañón system, where it has adapted to human habitats (Marcilla et al. 2002). The third species of the lignarius complex, *P. humeralis*, is phenetically distinct and is only known from Panama and Colombia (one record by Moreno & Jaramillo [1996]). The only previous

record of *P. lignarius* in Ecuador (in the Andean highlands city of Cuenca, province of Azuay, and therefore completely out of the natural range of the species) (Rodríguez 1959) is probably due to an erroneous capture site indicated on the label.

3.3.2.8. Other species

Other triatomine species (*Triatoma dispar*, *Triatoma venosa*, *Eratyrus mucronatus*, *Eratyrus cuspidatus*, and *Cavernicola pilosa*) seem to have little or no epidemiological significance in Ecuador; figures 19-20 and tables 13-14 show the main trends. Three of them (*T. venosa*, *E. cuspidatus*, and *E. mucronatus*) have however been reported to show some degree of synanthropism in different countries (Lent & Wygodzinsky 1979, D'Alessandro & Barreto 1985, Barrett 1991, Noireau et al. 1995), but seem to be mainly sylvatic in Ecuador.

3.4. Concluding remarks

The Ministry of Public Health is currently planning the strategy for the control of vector-borne Chagas disease in Ecuador. It will be based on updated information about the distribution and synanthropic behaviour of different triatomine species. The presence of a wide variety of them (~12% of all recognised species, and ~18% of South American species), in their majority present in wild environments, will be one of the main difficulties. Only one of these species, *T. dimidiata*, may be suspected of having been artificially introduced and therefore susceptible to eradication. The possibility that some southern domestic populations of *R. ecuadoriensis* are isolated from sylvatic foci seems plausible, opening the possibility of local elimination. Autochthonous species may however behave as secondary vectors, occupying empty niches when domestic triatomines are eliminated by insecticide spraying. A strong component of longitudinal surveillance with community involvement is recommended in such situations, complementing the use of residual pyrethroids (Dias 1991, Dias & Schofield 1999, Acevedo et al. 2000, Schofield 2000c).

4. SYLVATIC ECOLOGY OF *RHODNIUS ECUADORIENSIS*

4.1. Introduction

4.1.1. *RHODNIUS* AND PALM TREES

The Rhodniini are essentially arboreal triatomines. *Psammolestes* species are tightly associated with bird nests, and virtually all *Rhodnius* species breed in palm trees (Arecaceae). The few known exceptions are represented by (i) populations (of otherwise palm tree-living species) that colonise human environments, bird nests or (more rarely) mammal burrows; (ii) the apparent preference for epiphytic bromeliads of *R. domesticus* and some *R. pictipes*; (iii) a rare species, *R. paraensis*, found in tree holes inhabited by spiny rats; (iv) populations of *R. neivai* found in dead logs; and (v) occasional findings of *R. ecuadoriensis* in hollow trees (Lent & Wygodzinsky 1979, Barrett 1991, Carcavallo et al. 1998a, Abad-Franch et al. 2002). Palm tree-living *Rhodnius* species show varying patterns of habitat association, from apparently strict specialists infesting single palm species (e.g. *R. brethesi-Leopoldinia piassaba*) to generalist species known from several palm genera (e.g. *R. pictipes*, found in at least five genera of palms). The following table summarises the known palm tree habitats of different *Rhodnius* species.

Table 15. *Rhodnius* and palm trees

Species	Palm tree habitats*	Remarks
R. brethesi	*Leopoldinia piassaba*	Apparently specialised
R. colombiensis	*Attalea* (=*Maximiliana*) *butyracea* (=*macrocarpa, maracaibensis, marolepis, humboldtiana*)	Apparently specialised
R. dalessandroi	*Oenocarpus* (=*Jessenia*) *polycarpa*	Taxonomic status doubtful
R. domesticus	*Attalea* sp.	Preferentially in bromeliads and mammal nests
R. ecuadoriensis	*Phytelephas aequatorialis, Elaeis guineensis*	One record in *E. guineensis*
R. nasutus	*Copernicia cerifera* (=*prunifera*)	Arid life zones; also bird nests
R. neglectus	*Orbignya maritima, Or. oleifera, Or. martiana, Acrocomia macrocarpa, Ac. sclerocarpa* (=*aculeata*), *Mauritia vinifera, M. flexuosa, A. phalerata, A. speciosa, Arecastrum romanzoffianum, Syagrus oleracea*	Generalist; also bird nests
R. neivai	*Copernicia tectorum, Attalea* spp.	Arid environments
R. pallescens	*A. butyracea, Cocos nucifera, Elaeis oleifera, O. bataua, Co. tectorum*	Generalist
R. pictipes	*A. butyracea, A. maripa* (=*M. regia*), *A. speciosa, O. bataua, O. polycarpa, Astrocaryum urostachys, E. guineensis, Phytelephas tenuicaulis, Ac. sclerocarpa, Mauritia* sp., *Copernicia australis, Orbignya speciosa*	Generalist; also bromeliads; wide range across the Amazon
R. prolixus	*A. butyracea, elegans, Co. tectorum, Mauritia flexuosa, M. minor, Or. phalerata, Or. speciosa, Acrocomia sclerocarpa, Sabal mauritiiformis, C. nucifera, O. bataua, O. polycarpa, L. piassaba*	Generalist; also in trees, bird nests, bromeliads, and mammal shelters. Sylvatic in the Orinoco basin
R. robustus	*A. butyracea, A. maripa, A. speciosa, O. bataua, As. urostachys, E. guineensis, Ph. tenuicaulis, M. carana, Ac. sclerocarpa, Or. speciosa*	Perhaps encompassing several cryptic taxa; also in bromeliads and vertebrate nests

*At least 13 palm genera; the widespread use of synonyms in palm taxonomy complicates the assessment at the species level

The remarkable association between *Rhodnius* and palm trees is probably the result of an old ecological relationship. Together with adaptive features of *Rhodnius* likely related to their arboreal lifestyle (e.g. cryptic colouration, presence of climbing organs [spongy fossulae] on the tarsi, or the ability to fasten their eggs to the substrate), habitat associations have prompted hypotheses about the possibility that adaptation to the palm tree habitat was a key factor in the evolutionary origin of the Rhodniini. Based on mitochondrial cox*1* nucleotide and amino acid sequence data, Gaunt and Miles (2002) dated the split between Rhodniini (represented by *R. prolixus*, *R. pictipes* and *R. neivai*) and Triatomini (represented by seven South American species of *Triatoma*, *Eratyrus mucronatus*, and two *Panstrongylus* species) at 99.8-93.5 million years ago, and highlighted the fact that those estimates are relatively close to the age of the first known palm megafossils (84.5-71.3mya). The authors interpret these findings as 'opening up the possibility of either *Rhodnius*-palm co-evolution or the Rhodniini-Triatomini split resulting from adaptation of one genera to a specific habitat' (Gaunt & Miles 2002, p. 758). Co-evolution is to be considered as highly unlikely, because it implicitly requires that the organisms involved must exert some sort of *reciprocal* influence (such as in the case of sap-feeding hemipterans that act as vectors of palm pathogens), and it is difficult to envision what effect *Rhodnius* populations might have on the evolution of the palms they occupy. The second possibility seems more realistic in that it only implies that a lineage of hemipterans evolved in association with palms to yield the current tribe Rhodniini (perhaps even branching out in parallel in some instances), whereas other lineages preferentially exploited terrestrial ecotopes (but no co-evolution of *Triatoma*-saxicolous habitats can obviously be proposed). The results of molecular dating lend therefore circumstantial support to the idea of a close, old association between Rhodniini and palms, already well established from ecological studies (see reviews by Lent & Wygodzinsky 1979, Barrett 1991, Pizarro & Romaña 1998, Carcavallo et al. 1998a). A significant corollary is that the biogeographical range of most *Rhodnius* species coincides with that of the palm trees they occupy. In addition, it may be inferred that ancestral forms of *Rhodnius* probably inhabited humid forests (as do many of their extant descendant taxa, principally in the Amazon-Orinoco). The occupation of arid life zones is probably secondary for both palms (a typically tropical-subtropical family that probably first evolved during the warm late Cretaceous in the northern Gondwana floral

province; see Cox & Moore 2001) and *Rhodnius*, whereas it could well be the opposite for many *Triatoma* species groups, primarily associated with terrestrial ecotopes in dry regions and strikingly scarce in humid forest ecosystems (Barrett 1991).

Finally, it can also be noted that palm-living *Rhodnius* species do not necessarily develop sharp host associations; palm trees provide a suitable shelter to a great variety of small- and medium-sized vertebrates (birds, rodents, bats, carnivores, marsupials, reptiles, amphibians...), and blood-sucking bugs may thus exploit diverse food sources upon availability. Unlike some triatomines that have evolved fairly close associations with their hosts (e.g. *Cavernicola pilosa* with bats, *Panstrongylus geniculatus* with armadillos, species of the *T. protracta* complex with *Neotoma* woodrats, or *Psammolestes* with furnariid birds within the Rhodniini), many *Rhodnius* are rather generalist in their host preferences, and will feed on a variety of warm-blooded vertebrates and even on reptiles and amphibians (Carcavallo et al. 1998b). An association of Amazonian *Rhodnius* species with didelphid opossums, proposed after the observation that *T. cruzi* I predominates in both groups throughout the region (Gaunt & Miles 2000), probably reflects the relative abundance of these opportunistic marsupials in (or near) human settlements (i.e., where field studies are usually conducted) rather than a real predilection of the bugs for opossum blood; didelphids (probably ancient *T. cruzi* hosts) may in addition tend to use palms as refuges in modified forests (where palms tend to overgrow), suggesting that indications of a strong *Rhodnius-Didelphis* link may in fact be circumstantial, reflecting recent trends in human demography (with heavy immigration towards the Amazon) and their associated environmental alterations (see Nepstad et al. 2002).

4.1.2. *PHYTELEPHAS AEQUATORIALIS*: AN ECOTOPE FOR *RHODNIUS ECUADORIENSIS*

Because of the patent relationship between palms and *Rhodnius*, and because of the known biogeography of *R. ecuadoriensis*, our attempt to clarify the main ecological features of sylvatic populations of this species emphasised the study of palm tree ecotopes in western Ecuador. The fact that *R. ecuadoriensis* had previously been reported from the economically important (and therefore abundant near human settlements) *Ph. aequatorialis* (Romaña et al. 1994, Schofield 1994, Avilés et al. 1995a) led us to concentrate on that palm species, endemic to the western side of the Andes in

Ecuador (Henderson et al. 1995, Borchsenius et al. 1998). A single record from the African oil palm, *Elaeis guineensis* (extensively cultivated in several coastal and Amazonian Ecuadorian provinces, and often kept in peridomiciles as an ornamental species) was considered unlikely to indicate widespread adaptation of the bugs to those palms. The systematic use of pesticides in monoculture plantations probably prevents stable infestation of palms there. In their report of infested *E. guineensis* in the province of Los Ríos, Carcavallo and Martínez (1985) specify that, despite intensive searches, no nymphs were recovered from palms; eggs and adults were found among palm fronds where opossum beds were placed. The authors remark also that adult bugs commonly fly into houses in the area (Pichilingue, near the town of Quevedo), but they failed to find signs of colonisation in human environments (Carcavallo & Martínez 1985).

We aimed at detecting palm infestation to verify that *Ph. aequatorialis* are favoured ecotopes for sylvatic *R. ecuadoriensis*, but also explored possible associations between botanical traits and the presence/absence of bug colonies so that a general profile of 'high-risk palm ecotopes' could be outlined and environmental management-based strategies proposed for the control of peridomestic, palm-tree living vector populations. We tested a novel fieldwork method, live-baited adhesive traps (Noireau traps), used it for the first time to study *Rhodnius* populations inhabiting palm trees, and collected the specimens needed for phenotypic and molecular population studies.

4.2. Materials and methods

4.2.1. STUDY AREAS

We studied the presence of sylvatic populations of *R. ecuadoriensis* in *Ph. aequatorialis* palm trees of six localities of four ecological-geographic areas in western Ecuador (the region between 900m altitude on the Andes and the Pacific coast).

4.2.1.1. Andean foothills

The first study area, near the locality of Alluriquín (province of **Pichincha**), is located on the western slope of the Andes (~900m above sea level; approx. 79°00'W, 00°20'S), in an area comprised of wet premontane forest (Cañadas 1983). *Ph. aequatorialis* is abundant in the zone; other palm genera present in the area are *Iriartea*, *Attalea*, *Oenocarpus*, and *Bactris*. Here, we initially studied 11 *Ph. aequatorialis* palm trees by direct searches for bugs in their crowns. Later on, we investigated 56 *Ph. aequatorialis* palms using Noireau traps (see below); 88 traps were placed overnight on

the palms. We also undertook a manual capture of bugs in the organic matter and epiphytes present around the trunk of one palm tree. Part of these materials were cut down and examined on a white canvas (4 people searching during 3 hours). Finally, we dissected another palm by cutting it down and systematically inspecting it on a larger white canvas (4 people, 3 hours). These two palms were already known to be positive (triatomines were captured previously in live-bait traps).

4.2.1.2. Central lowlands

The lowlands of central coastal Ecuador (provinces of Pichincha and Manabí) are heavily deforested (see Dodson & Gentry 1991 and figures 12 and 37). Only dispersed palms remain on pasture/cultivated fields – excluding monoculture plantations of African oil palms. In Pichincha, we selected a rural area near Santo Domingo de los Colorados (**Comuna Chigüilpe**, approx. 79°13'W, 0°19'S and 640m altitude); here, indigenous people (the Tsáchilas) preserve a part of the original flora. However, only 4 palm trees (3 *Ph. aequatorialis* and one *Bactris* sp.) were surveyed because of the difficulty of finding them in this area. Several *Attalea colenda* (also endemic to western Ecuador) and *Iriartea deltoidea* (apparently the most abundant palm in this area), and a few *Oenocarpus* sp. palm trees were observed, but traps could not be placed on their crowns as their stems were more than 15m high. In this area, we used 6 trap-nights on those 4 palms.

Travelling westwards by the road Santo Domingo-Chone, the effects of deforestation are still very important. The predominant palm in these lowland pasture fields is *A. colenda* (replacing *Iriartea*), with stems usually over 15m in height (figure 37). We identified a remnant of old secondary forest in northeast Manabí (**Sitio La Roncadora**, canton El Carmen, close to the limit with Pichincha), and studied 16 palm trees in that forest and its fringes (approx. 79°32'W, 0°12'S and 400m altitude). Fifteen *Ph. aequatorialis* and one *A. colenda* were surveyed here with 43 trap-nights.

4.2.1.3. Coast

We applied the same methods in two localities of the canton Portoviejo, province of Manabí (one of them with previous records of domestic-peridomestic colonies of *R. ecuadoriensis*, and the other one without – but never studied before). Both localities are located in narrow valleys surrounded by low mountains not far from the coastline; these geological characteristics help determine the distinctive climatic traits of these areas,

with localised rainfall and dense mist allowing the growth of humid forests on the slopes and tips of the hills – and generating a somewhat humid local climate in the valleys. This permits the growth of *Ph. aequatorialis* palm trees, restricted to these mountainous zones in the coastal areas of Manabí – where the general climatic pattern is that of a dry tropical forest.

In **Pachinche Adentro** (approx. 80°20'W 1°07'S and 100m altitude) we studied 18 *Ph. aequatorialis* palms using 44 trap-nights. In **Chirijos** (approx. 80°15'W, 1°02'S and 100m), 18 *Ph. aequatorialis* were surveyed with 47 trap-nights. All these palms were located in agriculture fields near human dwellings. In Chirijos, many of the palms are taken care of by the inhabitants, who use their seeds and fronds for commercial purposes (seeds are sold for handicraft manufacture, and dry leaves are used for thatching). This sort of management is less frequent in Pachinche Adentro, where some of the locals merely collect seeds (and, occasionally, leaves) from semi-sylvatic *Ph. aequatorialis* that grow within their properties.

4.2.1.4. El Oro

Finally, ten *Ph. aequatorialis* palms were surveyed in the province of El Oro, where small humid forest remnants with palm trees have survived intense deforestation. We conducted a rapid survey in the surroundings of the locality of Balsas (approx. 3°47'S, 79°49'W, 600m altitude). Due to time and logistical constraints, only ten palms could be studied there: none of them were infested, and further data are not available for analysis.

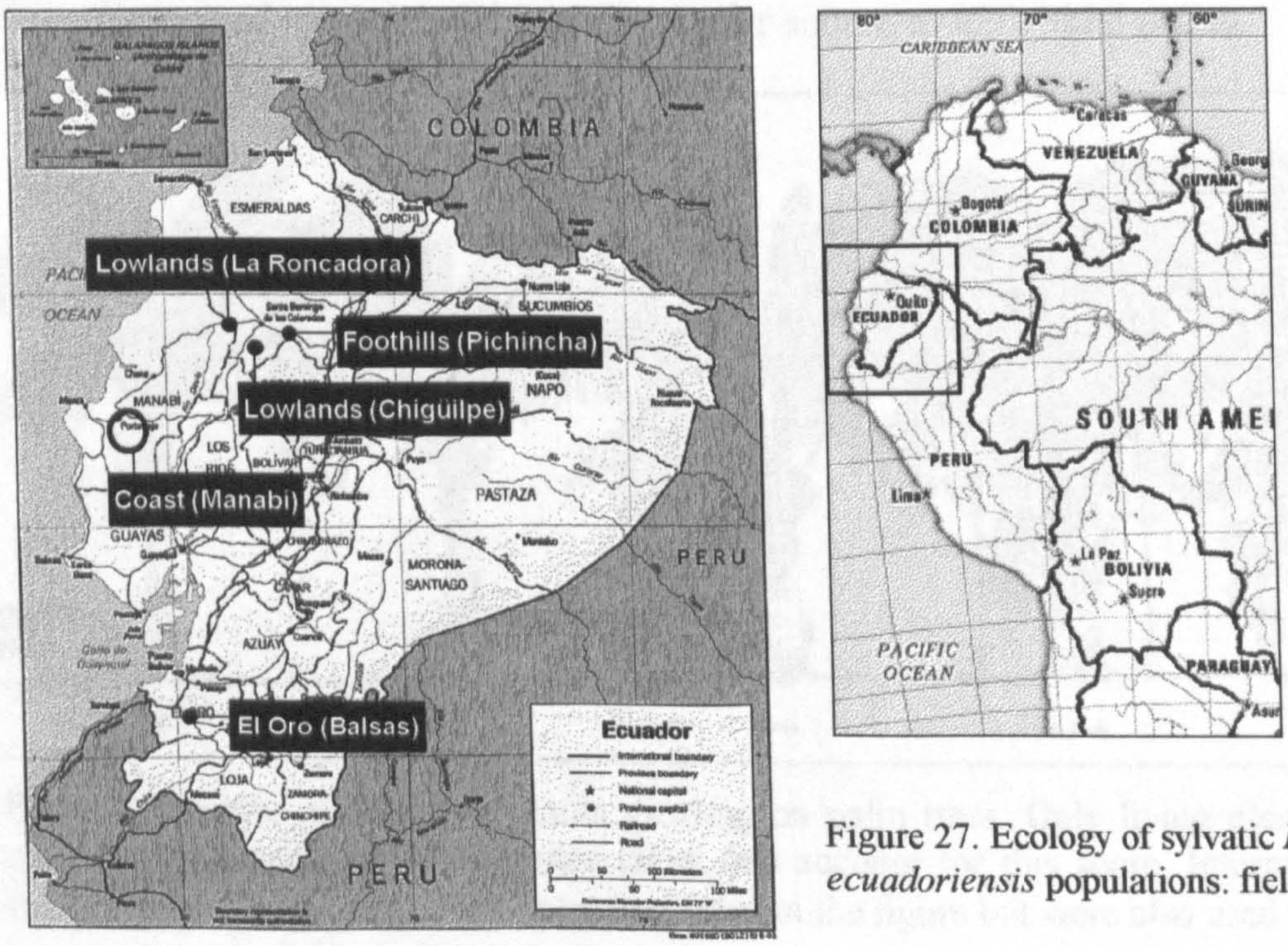


Figure 27. Ecology of sylvatic *Rhodnius ecuadoriensis* populations: fieldwork areas

4.2.2. PALM TREES

A total number of 110 *Ph. aequatorialis*, one *Attalea colenda*, and one *Bactris* sp. were studied (excluding 10 palms from Balsas, El Oro). No systematic, random sampling of palm trees in defined areas was performed; palms were selected on the basis of palm species (preferentially *Ph. aequatorialis*), location in accessible sites, and approximate agreement of the sample with the general aspect and variation of palm trees in each area. Preferential selection of some types of palms might have occurred, but results shown below, combined with field observations, suggest that surveyed palms were broadly comparable to the general populations of the species in each area. We recorded several botanical traits of the *Ph. aequatorialis* palm trees sampled, including locality, geographic-ecological area (Andean foothills, central lowlands, and coastal area; see figure 27), palm species, sex of palms (being *Ph. aequatorialis* dioecious), height of stipe, amount of epiphytic plants and of decomposing organic material (a semi-quantitative approach was used for these two latter parameters: a score between 0 and 4 with a 0.5 scale was given to each palm), and type of environment where each palm was located (primary forest, secondary forest, cropland-pasture, or urban-semiurban area). A combination of log-transformed scores of organic matter and epiphytes ('organic score') was devised for joint analysis of those variables:

'Organic score' = [log(0.5 + score epiphytes) + (2 x log[0.5 + score organic matter])] / 2

The following figures present schematic examples of the method used to assign different values of epiphyte and organic matter scores to individual palms.

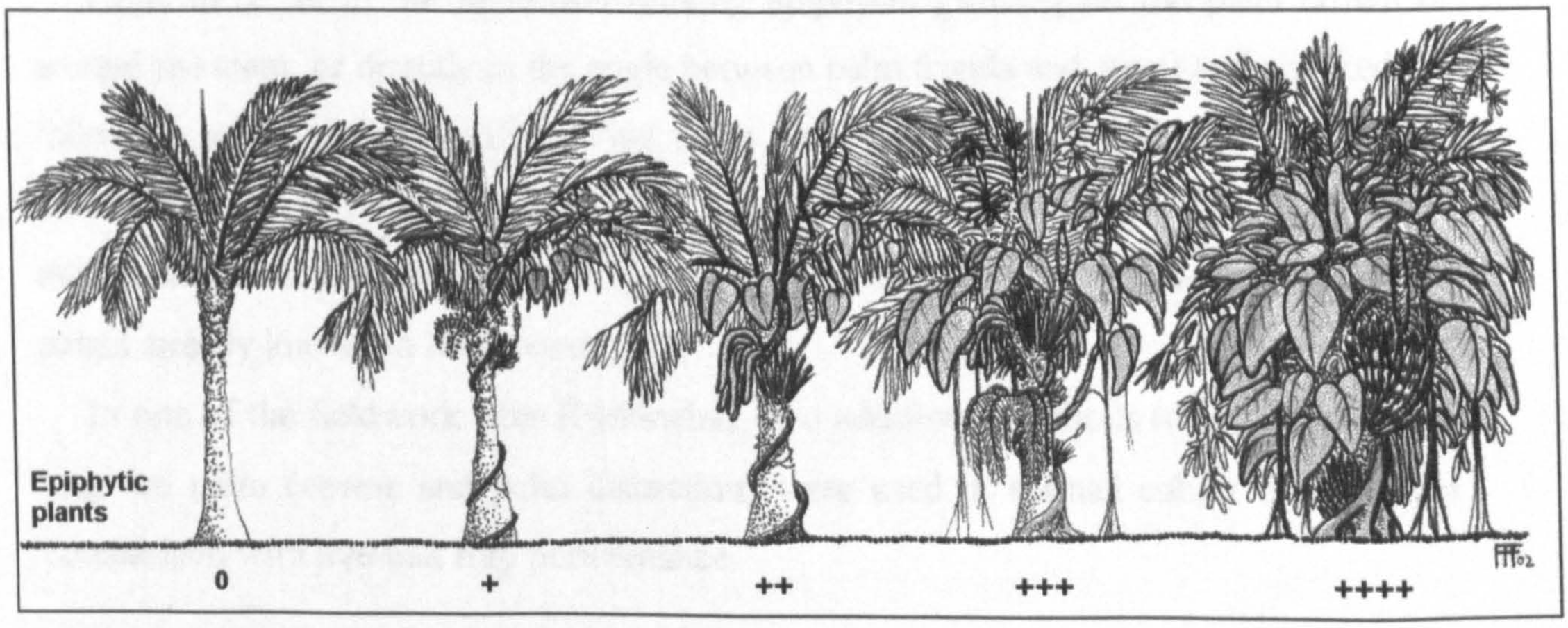


Figure 28. Score of epiphytic plants growing on palm trees. Only living plants (both true epiphytes and creeping plants) were taken into account for this score. Intermediate values (0½, +½, ++½, and +++½) are not represented in the figure but were also used in the study

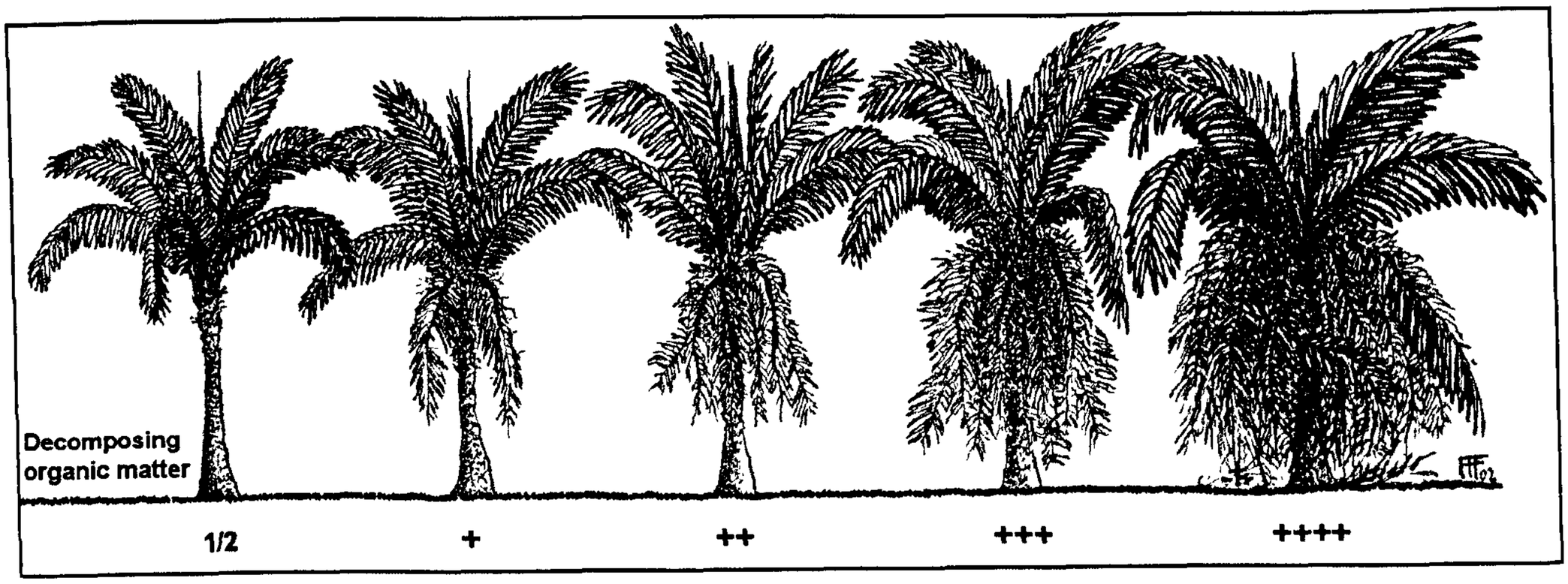


Figure 29. Score of decomposing organic matter on palm trees. Only dead organic material (dead leaves, fibres, dead epiphytes, and other vegetable debris) was taken into account for this score. Note that minimum values were always >0. Intermediate values (+½, ++½, and +++½), not represented in the figure, were also used in the study

4.2.3. LIVE-BAIT TRAPS

We modified the trapping method (live-baited adhesive traps) devised by Noireau et al. (1999a). Modified Noireau traps consisted of a plastic jar (15 x 7cm approximately) in which a white mouse was contained together with a small quantity of wood shavings and food (aiming to protect the animal from low night temperatures and starvation). Small drainage holes were made in the lower side of the containers, which were then closed with 1 mm-aperture wire mesh and wrapped around with double-coated adhesive tape (figure 30).

Traps were set in the afternoon (among epiphytes growing on the palm crown or around the stem, or directly in the angle between palm fronds and stem) and checked the following morning (after ~15h). Two traps were usually set in each palm; when the collection of a higher number of specimens was necessary (for population morphometric and molecular studies), a battery of four to five traps were set in single palms already known to be positive.

In one of the fieldwork sites (Pichincha), two additional methods (direct searches for bugs on palm crowns and palm dissection) were used in a small subset of palms for comparison with live-bait trap performance.

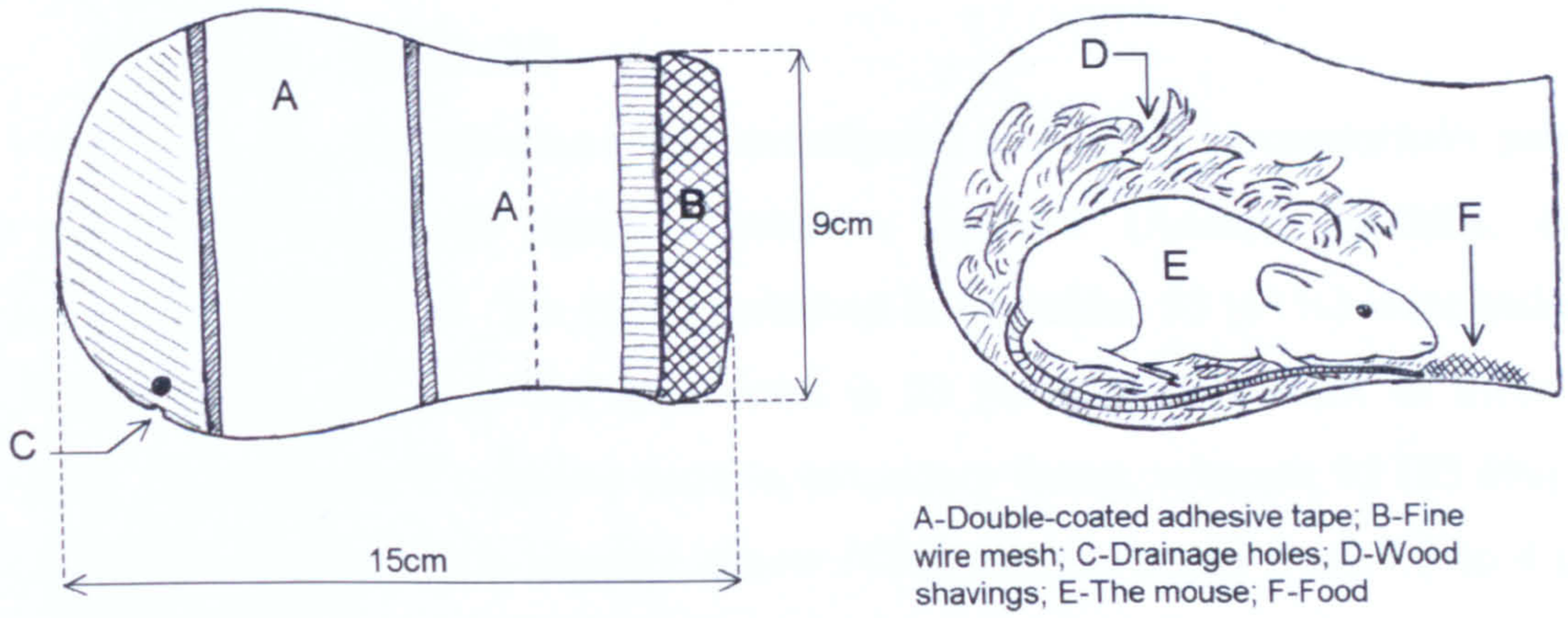


Figure 30. Live-baited traps for triatomines (Noireau traps): schematic design and mode of use

4.2.4. STATISTICS

Statistical approaches (descriptive and univariate) were similar to those used for the study of synanthropic bug populations, and are specified in detail in Chapter 5. 'Infestation' in this Section obviously refers to the presence of *R. ecuadoriensis* bugs in palm trees. Abbreviations used below are: KW: Kruskal-Wallis test; WT: Wilcoxon test; *t*: *t* statistic from Student's *t* test; df: degrees of freedom; p: significance probability; FET: Fisher's exact test; SD: standard deviation; uOR: unadjusted odds ratio (OR: odds ratio); CI: confidence interval.

4.3. Results

4.3.1. GENERAL RESULTS

Infestation by *R. ecuadoriensis* was investigated in 110 *Ph. aequatorialis* palms in three geographic-ecological areas in western Ecuador (Andean foothills, central lowlands, and coastal area). Sex was determined in 87 palms: 53 (61%) were males and 34 (39%) females. Sex was not determined in 23 palms (21%); most of these were subadults. Eighteen (16.4%) palms were in secondary forest, whereas 92 (83.6%) grew in cropland or pasture fields. Organic matter (OM) scores ranged from 0.5 to 4 (mean 1.8±0.8, median=1.5, quartiles 1-2), and those for epiphytic plants (EP) from 0 to 4 (mean 1.2±0.9, median=1, quartiles 0.5-1.5).

EP scores were non-significantly higher in ♀ palms than in ♂, whereas ♂ palms had significantly larger amounts of OM (t=-3.1, 85 df, p=0.0026) and were slightly taller than ♀. The 'organic score' (EP and OM) was similar in both sexes, with somewhat higher scores in ♂. Foothill palms had more EP than coastal ones (KW test X^2=82.8, 2 df, p<0.0001), with lowland specimens in an intermediate position – with no significant differences with either foothills or coast (Tukey-Kramer test). Palms from the three areas had comparable OM scores. The 'organic score' was significantly higher in the foothills than in the coast (Welch Anova F ratio=4.7, df=2, p=0.014), with lowland palms having intermediate scores (Tukey-Kramer test); a Bartlett test showed variances were unequal among groups. Palms located on cropland/pasture had more OM (WT X^2=6.3, 1 df, p=0.01) and EP (WT X^2=10.2, 1 df, p=0.0014) than those in secondary forest areas. Similarly, the 'organic score' was significantly higher in cropland/pasture palms (t=-3.5, 108 df, p=0.0006).

The height of the palm stipes ranged from 0.5m to 10m (mean 4.5±1.9, median=4.5, quartiles 3-6). Coastal palms had significantly taller stipes than those in the foothills and lowlands, which were comparable (Tukey-Kramer test). Heteroscedasticity led to the use of Welch Anova for the comparisons of means (F ratio=19.5, 2 df, p<0.0001).

Twenty-five out of 110 (22.7%) *Ph. aequatorialis* palm trees were infested by *R. ecuadoriensis*. The following table summarises the results obtained in three geographic-ecological areas [Andean foothills (Alluriquín), central lowlands (Chigüilpe + La Roncadora), and coastal Manabí (Pachinche Adentro + Chirijos)]. Most bugs were captured in live-bait traps, with a few collected manually from palm tree crowns.

Table 16. *Rhodnius ecuadoriensis* in *Phytelephas aequatorialis* palm trees in western Ecuador: entomological indices and characteristics of 110 palms surveyed

Variable	General	Foothills	Lowlands	Coastal Manabí
Palms sampled (infested)	110 (25)	56 (14)	18 (1)	36 (10)
Infestation index (% of palms with bugs)	22.7%	25%	5.3%	27.8%
Bugs captured	174	143	1	30
Density (bugs/palms sampled)	1.6	2.6	0.05	0.83
Crowding (bugs/infested palms) [SD, range]	7 [5.5, 1-22]	10.2 [4.9, 1-22]	1	3 [2.6, 1-9]
Colonisation index (palms with nymphs) [n]	92% [23]	93% [13]	100% [1]	90% [9]
Trap-nights used [average per palm sampled]	222 [2]	88 [1.6]	43 [2.4]	91 [2.5]
Trap-nights positive [%]	45 [20.3]	29 [33]	1 [2]	15 [16.5]
Palm stem height (average±SD, in m)	4.5±1.9	3.6±1.6	4.4±2.4	5.7±1.6
Palm sex ratio (M:F)	1:0.6	1:0.6	1:0.5	1:0.8
Organic matter (score mean±SD)	1.8±0.8	1.8±0.9	1.8±0.7	1.7±0.6
Epiphytic plants (score mean±SD)	1.2±0.9	1.6±1	1±0.5	0.7±0.5
'Organic score' (mean±SD)	0.9±0.5	1.1±0.6	1±0.4	0.8±0.3
Percentages of palms in cropland/forest	83.6/16.4	82/18	56/44	100/0

SD = standard deviation; M = male; F = female

4.3.2. BOTANICAL VARIABLES AND INFESTATION

4.3.2.1. Sex

Male palms were more frequently infested (34%, 18 out of 53) than females (17.7%, 6/34); this difference not statistically significant (FET p=0.14), and was larger in the coast (Pachinche Adentro + Chirijos) (40% ♂ vs. 12.5% ♀ infested) than in the foothills (Alluriquín: 42.9% ♂ vs. 33.3% ♀). Only one of 23 (4%) palm trees whose sex was not determined was infested. Considering sexes separately, stem height was not correlated with higher likelihood of infestation. Infested ♂ had larger amounts of OM than those non-infested (t=3, 51 df, p=0.004); the same was observed for the 'organic score' (t=2.5, 51 df, p=0.014), but not for the EP score (slightly higher in infested palms). In ♀, marginally non-significant differences between infested and non-infested palms were recorded for EP and OM (with higher scores in infested palms); the 'organic score' was significantly larger in infested trees (t=2.3, 32 df, p=0.03). Palms whose sex was not recorded were less likely to be infested than those with known sex; they also had significantly smaller stipes and comparable amounts of OM and EP.

4.3.2.2. Stem height

Stems were taller in infested (mean=5.3±1.7m) than in non-infested (4.3±1.9m) palms (t=2.46, 107 df, p=0.016). Palms were divided into two discrete groups according to stem height: stems under 3m high (n=22), and stems ≥3m (n=87). Infestation was more frequent in larger palms, but no significant relationship was found between stipe height and infestation in neither ♀ nor ♂ palms when considered separately, possibly

because sex was not determined in many juvenile trees. Palms with stems ≥3m were more likely to be infested (FET p=0.04); only one palm (♀) <3m (out of 22, 4.6%) was found to be positive, vs. 23 out of 87 larger palms (26.4%).

4.3.2.3. Decomposing organic matter

The most significant differences between infested and non-infested palms were those recorded in OM scores (t=3.13, 108 df, p=0.002 [a transformation of OM scores, namely $x_t = \log(0.5+x_i)$, was used for these analyses]).

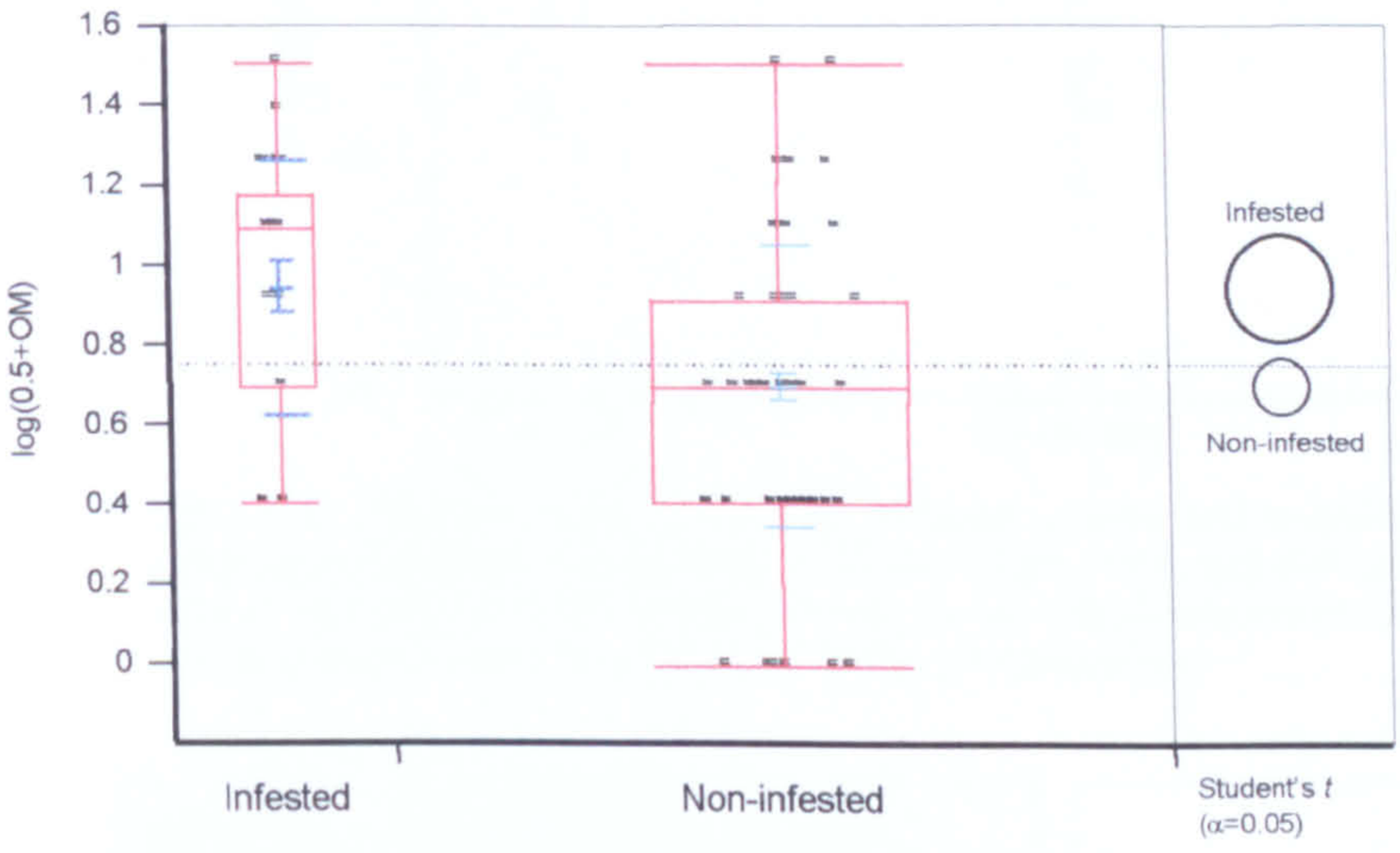


Figure 31. Organic matter score: differences between infested and non-infested *Phytelephas aequatorialis*. The overall average observed value is represented by the grey dotted line. Individual values: dots; means, standard errors, standard deviations: short blue lines; quantile boxplots: red (medians, 10%, 25%, 75%, and 90% quantiles)

Logistic regression illustrated this strong correlation [likelihood ratio test X^2=10.02 (1 df, p=0.0016); untransformed OM score: uOR=24.5, lower 95% CI limit >3].

Differences were highly significant between two groups of palms: those with a small (scores from 0 to 2, n=86) vs. those with a large (2.5 to 4, n=24) amount of OM; 54.2% of "dirty" palms vs. 14% of "clean" ones (FET p<0.0001) were infested. The difference was larger in ♂ (76.9% vs. 20%, FET p=0.0004, n=53) than in ♀ (60% vs. 10.3%, FET p=0.03, n=34). Palms in the group with higher OM scores (both ♂ and ♀) had significantly more epiphytes (t=-4.5, 108 df, p<0.0001) than 'cleaner' trees.

4.3.2.4. Epiphytes

EP scores were not correlated with the likelihood of infestation. Considering two discrete groups [small (scores 0 to 1.5, n=84) and large amount of EP (2 to 4, n=26)], significant differences (FET p=0.035) in infestation were recorded: 17.9% of palms with low EP scores, vs. 38.5% of those with more EP, were infested. In ♀ palms, infestation was more frequent among trees with higher EP scores (50% vs. 10.7%, FET p=0.05), but this was not verified in ♂ (50% vs. 29.3%, FET p=0.3).

4.3.2.5. 'Organic score'

The 'organic score', summarising data on both OM and EP scores in individual palms, was significantly higher among infested palms (t=2.9, 108 df, p=0.005). Similar results were obtained for both ♀ and ♂ palms, with a stronger association in ♂.

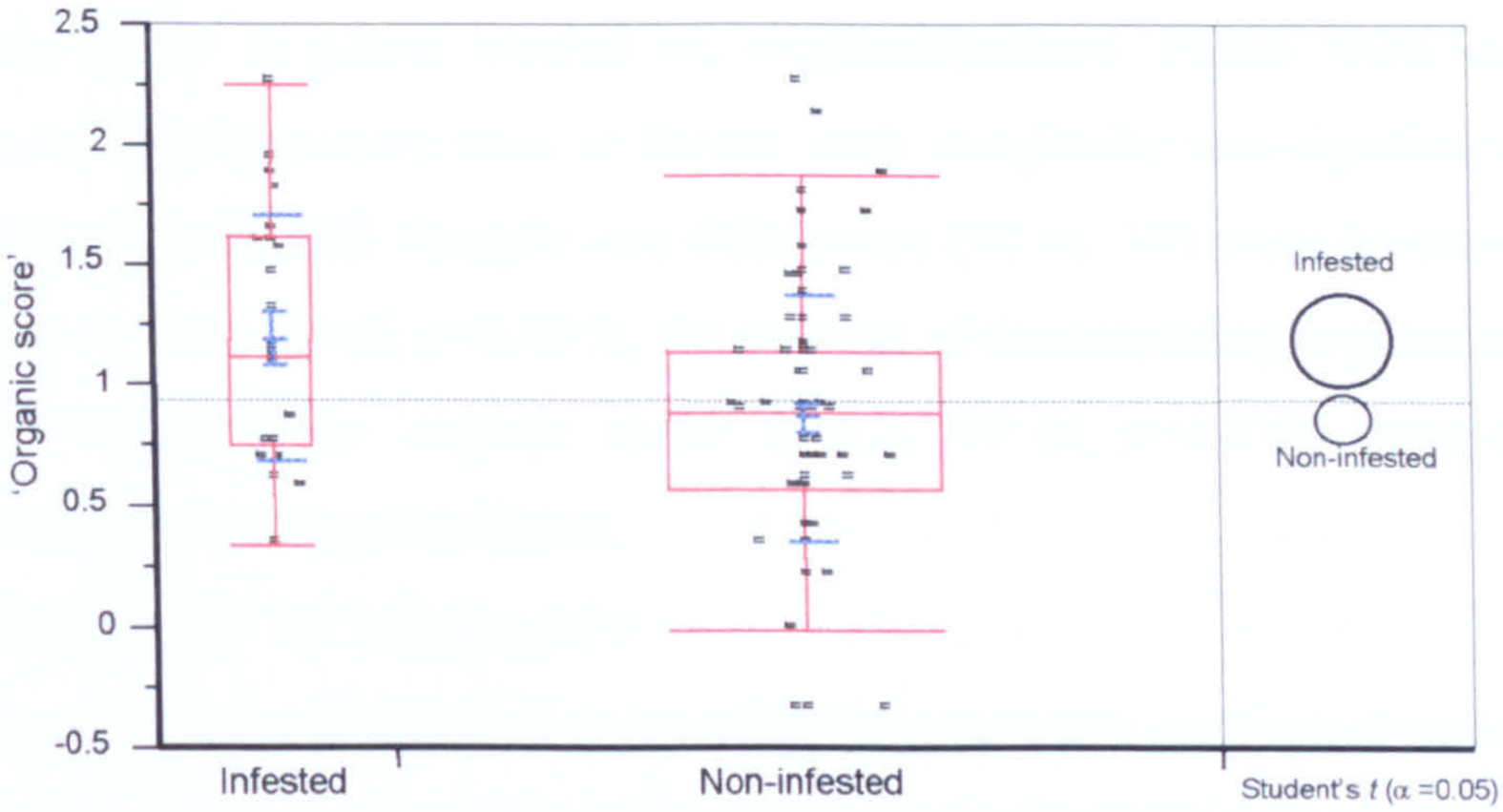


Figure 32. 'Organic score': differences between infested and non-infested *Phytelephas aequatorialis*. Quantile boxplots (red) indicate medians, 10%, 25%, 75%, and 90% quantiles; short blue lines indicate means, standard errors and standard deviations; individual values are represented by black dots; the overall average observed value is represented by the grey dotted line

Figure 33. *Phytelephas aequatorialis*: differences between palms from humid foothill forests (left) and from seasonally dry coastal valleys (right). Note the abundance of epiphytes on the foothill palm, whereas dead, dry leaves remain hanging around the trunk in coastal palms

4.3.2.6. Environment

All infested palms were located on land designated as cropland/pasture – and none of them in secondary forests. The amounts of OM (t=-2.6, 108 df, p=0.01) and EP (t=-3.2, 108 df, p=0.002), and therefore the 'organic score' (t=-3.5, 108 df, p=0.0006), were significantly larger in palms located on cropland/pasture. Palms were taller in these human-altered environments than in forest, with marginally non-significant differences (t=-1.9, 107 df, p=0.063). Sample size differences (92 vs. 18) were however important. Stem height (t=2.14, 89 df, p=0.035), the amount of decomposing organic matter (t=2.6, 90 df, p=0.01), and the 'organic score' (t=2.2, 90 df, p=0.032) were correlated to infestation in cropland/pasture palms.

4.3.3. RESULTS BY LOCALITY

Three groups were established (according to geographic-ecological traits) in order to analyse and compare results by locality: (1) **Andean foothills** (Alluriquín); (2) **Central lowlands** (Chigüilpe + La Roncadora); and (3) **Coast** (Pachinche Adentro + Chirijos). Differences in infestation were not statistically significant, although only one positive palm was detected in the central lowlands (see table 16, above). Details about each individual palm (by locality) can be found in the tables of the Appendix.

4.3.3.1. Andean foothills: Alluriquín, Pichincha

Direct searches in 11 palm crowns yielded negative results. By live-bait trapping, 25% of palms (14/56) were found to be infested; nymphs were captured in 13 palms (colonisation 93%). Out of 88 trap-nights utilised, 29 (33%) were found to be positive (with triatomine bugs adhered to the tape) when checked the following morning (4.9±3.4 bugs/positive trap-night; maximum=14 bugs in a single trap-night). We captured a total of 143 bugs. The mean number of bugs/positive palm (crowding) was 10.2±5 (from 3 to 22; median=9); density (bugs captured/palms examined) was 2.6±5.1. Of the total number, 140 bugs (98%) were nymphs of different stages (adults/nymphs index=0.02). Seven nymphs were captured in two positive palms by manual searches, with just one 5th instar nymph captured by complete dissection of one of these palms. No other triatomine species was found.

In Alluriquín, infested palms were significantly taller than those non-infested (t=2.6, 53 df, p=0.01) and had larger amounts of OM (t=2.4, 54 df, p=0.02) and EP (t=2.6, 54 df, p=0.01); the 'organic score' was consequently higher in infested palms (t=2.8, 54 df,

p=0.008). Significant differences (FET p=0.0014) were also recorded when palms were divided into two groups (small/large amounts of OM: ≤2/>2 in the OM score). Five out of 40 palms (12.5%) were infested in the former group, vs. nine out of 16 (56.3%) in the latter. The same was observed when two similar groups were defined in relation to the amount of EP (FET p=0.027); four infested palms (out of 32, 12.5%) were detected in the group with scores up to 1.5, vs. 10 out of 24 (41.7%) in the complementary group (scores from 2 to 4). Stems were significantly taller in the group with a large amount of organic material (t=-3.5, 53 df, p=0.001). There was in general a positive correlation between stem height and the amount of epiphytes and organic matter (and the 'organic score'), but linear regression showed low R^2 values (0.096, 0.1, and 0.12, respectively). No significant difference in the likelihood of infestation was recorded between male and female palms here; both sexes were comparable in height and had similar amounts of organic material and epiphytes. No infested palms were found in secondary forest (but only 10 palms were sampled in such an environment).

Figure 34. *Phytelephas aequatorialis* in the humid Andean foothills of Pichincha, Ecuador. Left: palms located in land designated as cropland/pasture; Right: secondary forest with palms

4.3.3.2. Lowlands: Chigüilpe, S. Domingo de los Colorados, Pichincha

No triatomines were captured in this area. All traps were negative, including those located in a *Ph. aequatorialis* palm occupied by a *Didelphis marsupialis*.

4.3.3.3. Lowlands: La Roncadora, Manabí

Just one nymph (stage II) was captured in the only trap found to be positive (2.3% of traps positive; 0.023 bugs/trap-night used, and 1 bug/positive trap-night). This trap was set in a *Ph. aequatorialis* palm located in a pasture field close to the fringe of old secondary forest; infestation index was 6.7% (one out of 15 *Ph. aequatorialis*). All the rest of palms (included those located in the forest, its fringes, and on pasture or agricultural fields) yielded negative results. Density was therefore 0.07 bugs/palm sampled, crowding one bug/positive palm, and colonisation index 100%. The fact that just one positive palm was detected prevents the possibility of further significant analysis. In this area, staff of the Vector Control Service had captured two adult *R. ecuadoriensis* inside houses, perhaps indicating a synanthropic trend that could not be observed in Alluriquín.

4.3.3.4. Coast: Pachinche Adentro, Manabí

Five out of 18 (27.8%) *Ph. aequatorialis* palms sampled in Pachinche Adentro (Manabí) were infested. Eleven bugs were captured from those palms (44 trap-nights, 8 positive [18%]). Density was 0.6 bugs/palm examined, crowding 2.2 bugs/positive palm, and colonisation index 100%. These were seemingly small colonies (2.2 bugs/infested palm), but invasion and colonisation of human environments (mainly peridomestic chicken coops and dovecotes) by these bugs had been reported in the locality. Only male palms (5 out of 12, 41.7%) were infested in Pachinche Adentro, with no bugs found in six female palms surveyed. All infested palms had stems over 3m tall; all palms in this locality had EP scores under 1.5. On the other hand, the only two palms with OM scores over 2 were infested, as were 3 out of 16 (18.8%) with lower scores; the trend was confirmed (on log[0.5+x]) by univariate logistic regression, with effect likelihood ratio test X^2=4.25 (1 df, p=0.039). All palms were in areas described as cropland/pasture. The rest of variables resulted in comparable indices of infestation. The sample of palms was however too small to draw conclusions from these results alone; a complementary study was conducted in a similar locality (Chirijos) of the same area.

4.3.3.5. Coast: Chirijos, Manabí

Five out of 18 *Ph. aequatorialis* were found to harbour *R. ecuadoriensis* (27.8%); one dead adult was found in a palm tree where traps yielded negative results. Overall density was 1.1 bugs/palm sampled, crowding 3.8 bugs per positive palm, and colonisation index 80%. From a total number of 47 trap-nights used, 15% were found to have triatomines adhered to the tape; 18 bugs were captured in these traps, excluding the dead adult found in one palm (0.4/trap-night utilised, and 2.6/positive trap-night). Here, locals often expressed that *Rhodnius* bugs enter houses and may bite people during the night; we learned from personnel of the Vector Control Service that a child from this community died of acute Chagas disease (with clinical and parasitological diagnosis) in 1999, and that other suspect cases had been reported in the past. Here, infestation was also more frequent in male palms (3 out of 8, 37.5%) than in females (2 out of 10, 20%); all infested palms had stems over 3m (but only one smaller palm was studied). All the palms surveyed in Chirijos had epiphyte scores up to 1.5; one out of 2 palms with organic matter scores over 2 was infested, as were 4 out of 16 (25%) with lower scores.

4.3.3.6. Coast: combined results

Statistical analyses were conducted considering the latter two coastal localities (Pachinche Adentro and Chirijos) together. Association of palm sex with higher likelihood of infestation was marginally non-significant (likelihood ratio test X^2=3.56, 1 df, p=0.06); two in 16 females (12.5%) and 8 in 20 male palms (40%) were infested. Male palms presented significantly higher amounts of decomposing organic material than females (Welch Anova F ratio=10.2, 1 df, p=0.004), whereas females had more epiphytes on average (WT X^2=8.97, 1 df, p=0.0027). Here, all palms were located in land designated as cropland/pasture and had epiphyte scores under 2. Infestation was more frequent in the group with OM scores >2 (75% [3 out of 4] vs. 21.9% [7 of 32]).

4.4. Discussion

We investigated 110 *Ph. aequatorialis* palms in western Ecuador (from the Andean foothills of Pichincha to the coastal valleys of Manabí) and found about 23% infested by *R. ecuadoriensis*. The comparison of this result with those from other surveys is limited by the fact that methods used for the selection of palms are not specified in most of the published reports; high infestation rates likely reflect preferential sampling of

palms suspected to harbour triatomines. Our selection criteria were also largely subjective; palms surveyed were located in accessible sites and were considered to approximately represent the general variation of palm tree populations in each area. The reasonably normal frequency distribution of palm stem heights (from 60cm to 10m) in our sample argues in favour of fair sampling (not biased towards larger trees, perhaps more likely to be infested).

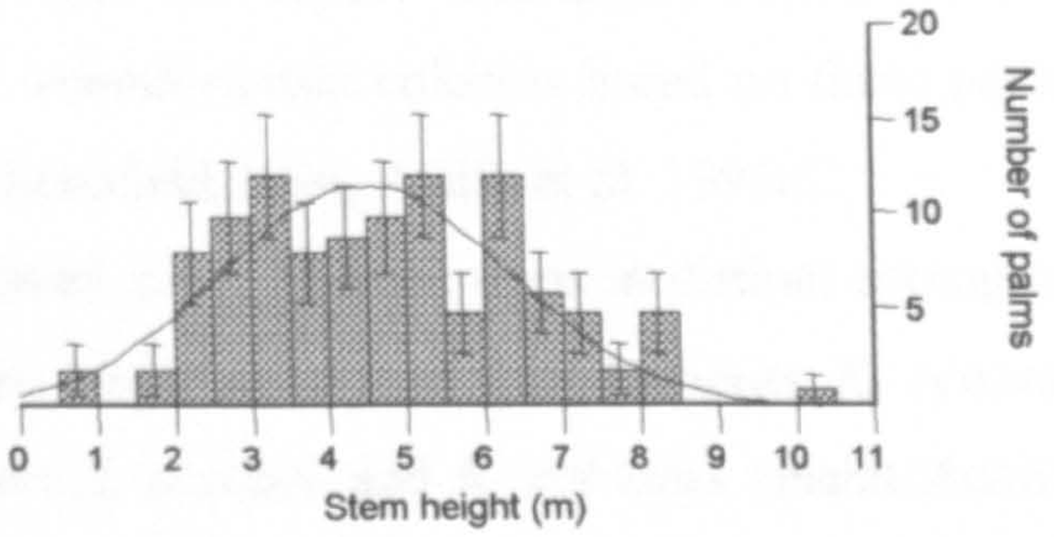


Figure 35. Stem height in 109 *Phytelephas aequatorialis* palm trees studied in western Ecuador: frequency distribution. Bars indicate standard errors; the curved line is a normal fit

The approximate amount of epiphytic plants (mean=1.2, median=1) showed a moderately skewed frequency distribution, while that of decomposing organic matter (mean=1.8, median=1.5) approached normality; this largely reflects the patterns observed during the survey (i.e., that the palms usually had relatively small amounts of epiphytes, whereas the quantities of organic matter seemed more evenly distributed), suggesting no systematic bias towards 'dirty' palms that could be suspected of harbouring bug colonies.

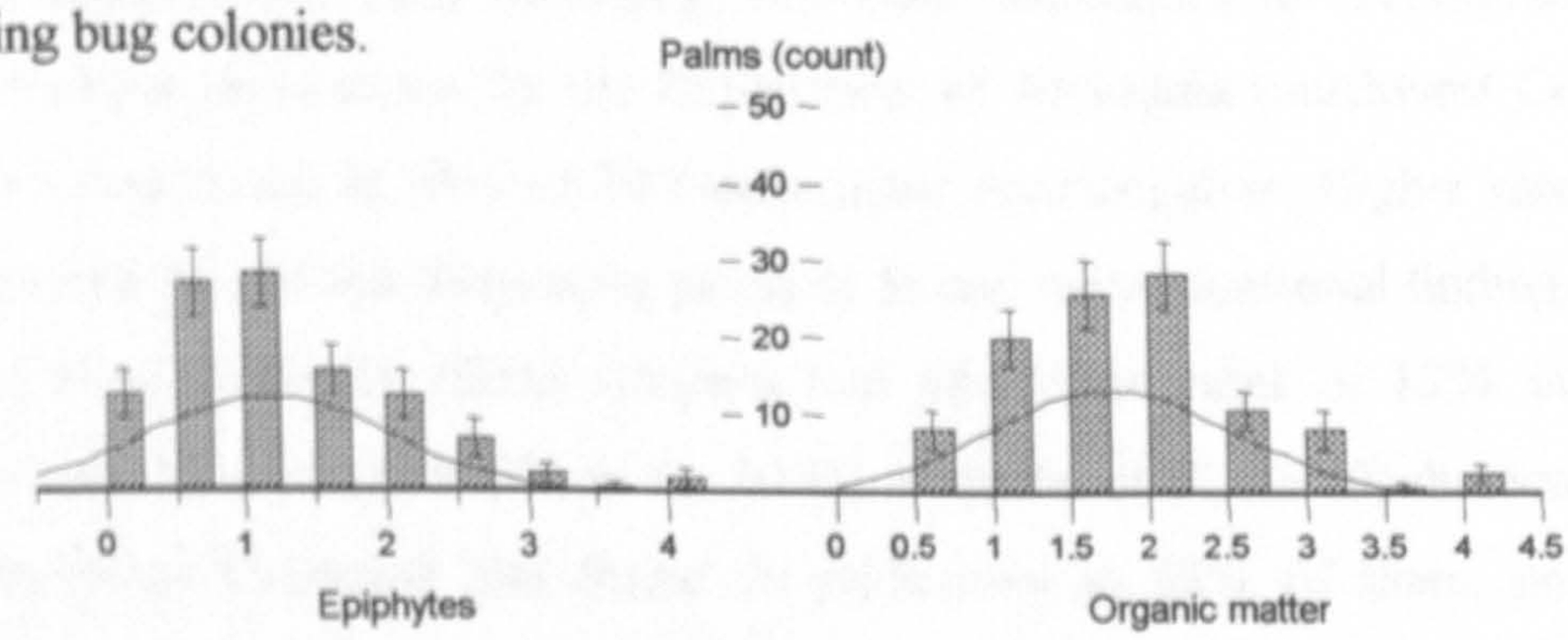


Figure 36. Approximate amounts of epiphytes (left) and decomposing organic matter (right) on 110 *Phytelephas aequatorialis* palm trees in western Ecuador: frequency distributions. Bars indicate standard errors; the curved lines are normal fits

There was a predominance of male palms in or sample, with 53 ♂ vs. 34 ♀ (and 23 palms for which sex was not determined). The fact that most of the palms studied were in cultivated (84%) rather than in forested (16%) areas reflects not only better accessibility but also the relative abundance of the palms in each type of environment and the epidemiological focus of the study (preferentially covering inhabited areas,

frequently deforested in varying degrees). Our study areas represented most of the altitudinal (0-1500m) and climatic (annual rainfall up to 4000mm) range of *Ph. aequatorialis*, but our sampling concentrated in central coastal Ecuador, omitting palm populations in the northern province of Esmeraldas and only including a few from the southern forest remnants in El Oro-Loja.

Previous studies in coastal Ecuador did not report infestation indices of *Ph. aequatorialis*, but merely the fact that *R. ecuadoriensis* colonies breed on these palms near rural dwellings (Romaña et al. 1994, Schofield 1994, Avilés et al. 1995a).

Recently two palm tree surveys performed using Noireau traps in distinct ecological areas of Brazil (the dry Caatinga, where *Copernicia prunifera* harbours *R. nasutus* populations, and the Amazon basin, where *R. pictipes* and *R. robustus* inhabit *Attalea* spp.) were reported showing infestation indices of 10% (Caatinga, 59 palms sampled) and 23% (Amazonia, 53 palms surveyed) (Noireau et al. 2002b). Results obtained by our group in the Ecuadorian Amazon, and reported in the same paper, show significantly higher infestation rates, with mixed *R. pictipes-R. robustus* colonies detected in almost half of 64 palms (including *Attalea*, *Oenocarpus*, *Astrocaryum*, and an Amazonian species of *Phytelephas*) (Noireau et al. 2002b).

Many authors have used traditional dissection techniques to investigate palm tree-living *Rhodnius* populations. In the Department of Antioquia (northwest Colombia), *R. pallescens* was found in 16% of 79 *Oenocarpus bataua* palms. Higher rates (82-91%) were reported for *Attalea butyracea* palms in Sucre, with occasional findings in coconut palms (*Cocos nucifera*); *Elaeis oleifera* had infestation rates of 82% in Colombian territory near Panama (Jaramillo et al. 2000). Romaña et al. (1999) dissected 51 palms in northwestern Colombia and found *R. pallescens* in 63% of them; positive palms included *A. butyracea* (25 of 26 infested, 555 bugs collected), *C. nucifera*, *E. oleifera*, and *Copernicia tectorum*. In an earlier survey in the Panama Canal Zone, Whitlaw and Chaniotis (1978) found 100% of 92 *A. butyracea* palms infested by mixed colonies of *R. pallescens* and *T. dimidiata*; 1114 bugs were collected (101 in one palm), of which 94.3% were *R. pallescens*. *R. colombiensis* is known from a restricted area within the Magdalena valley (Department of Tolima, Colombia); Moreno and Jaramillo (1996) mention that 13% of *A. butyracea* palms surveyed there were found to be infested.

We collected an average of 7 bugs per positive palm (1.6 bugs/palm surveyed) using live-baited adhesive traps (a few of those bugs were collected manually while setting and recovering the traps); in a review of 25 published works, Pizarro and Romaña (1998) report that habitat dissection yielded an average of 7.6 *Rhodnius* per palm studied. This difference most likely reflects the distinct methods utilised for both selection and investigation of palm trees. Systematic dissection of palms with suspected infestation will almost necessarily yield larger bug collections than virtually random Noireau trapping. Whitlaw and Chaniotis partially dissected 101 randomly selected palms in Panama and collected an average of 11 bugs/palm; because of the high infestation index (91%), crowding (12 bugs/infested palm) was similar to overall density (Whitlaw & Chaniotis 1978). Using Noireau traps, densities of 0.2 *R. nasutus* per surveyed *C. prunifera* have been reported from the arid Brazilian Caatinga (crowding=1.8), and 0.6 *Rhodnius* spp. (*pictipes* and *robustus*) were captured per palm studied (*Attalea* spp.) in Amazonian Brazil (crowding=2.5) (Noireau et al. 2002b). These results would suggest that sylvatic *R. ecuadoriensis* populations inhabiting *Ph. aequatorialis* reach higher densities than those other *Rhodnius* species do in their palm habitats, but better trap performance (e.g., because of differences in the quality of the adhesive tape used, climatic factors, aggressiveness of the bugs, etc.) could represent an alternative/complementary explanation. In this sense, we also investigated 64 palms of different genera in northeast (Amazonian) Ecuador, using the same traps as in the *ecuadoriensis* study, and collected (in combination with manual captures) an average of over 6 bugs/palm sampled (Noireau et al. 2002b). During this survey we found heavy infestations in some palms located in severely deforested areas (*R. pictipes* in *Ph. tenuicaulis* and *R. robustus* in *A. butyracea*), and the bugs displayed a strikingly aggressive behaviour, readily attacking us while we were setting the traps in open daylight and even probing the ladder we used to climb the palms, heated by the heavy sun, with their proboscides extended. These observations may, together with variations in trap performance, partially explain the differences with the mentioned report from the ecologically comparable Brazilian Amazon. On the other hand, ~1.4 trap/nights were used on average in each palm in this latter area (and just one in the Caatinga study), vs. ~1.7 trap/nights per palm tree in northeast Ecuador, and ~2 trap/nights per *Ph. aequatorialis* surveyed in western Ecuador.

4.4.1. BOTANICAL VARIABLES AND INFESTATION

4.4.1.1. General results

Thirty-four percent of male *Ph. aequatorialis* were infested in our sample, vs. only 18% of females. **Male and female palms** were different in some respects. Higher organic matter (OM) scores in males (WT X^2=9.02, 1 df, p=0.003) may be due to the fact that dead fronds and organic debris are often removed from female palms in areas were palm fruits and seeds are gathered. Locals usually pay less attention to male palm trees, which are considered of no use – except for leaves and natural pollination of females. This may explain the fact that sex-related differences in OM scores were larger in coastal areas (below). Also, the large male inflorescences and their husks contributed to higher OM scores – while female infrutescences are periodically removed in some areas. The overall pattern, including the fact that female palms tended to have more epiphytes than males did, suggested that sex-related differences in the likelihood of infestation could be related to the presence of larger amounts of decomposing OM in males; a straight link between male palm physiology and an increased suitability for bug colonisation seems unlikely. However, in some palms with large inflorescences, flower thermogenesis may influence microclimatic conditions to the advantage of various species of pollinating insects, perhaps attracting also more predators.

Increased infestation rates in taller palm trees (t=2.46, 107 df, p=0.016) likely reflect the fact that **stipe height** is age-related, and older palm trees have been available for the bugs (and for their vertebrate hosts) to establish colonies there for longer. Also, older palms tend to accumulate larger amounts of organic matter (but the relationship was weak in our sample, with linear regression $R^2 \approx 0.04$), possibly providing a more suitable microhabitat for the bugs.

High decomposing **organic matter** (OM) scores were apparently the most important single factor favouring palm infestation in our sample. OM comprised mainly dead leaves, husks, inflorescences-infrutescences, and fibres from the palm itself, but vegetable debris of other origins (largely dead epiphytic and creeping plants) was also taken into account for the assignation of the approximate scores used for analysis. These materials are present in different parts of the palm, including stems and crowns. In *Ph. aequatorialis*, the petioles of dead leaves usually fracture at 30-50cm from the base; broken fronds remain hanging around the trunk for some time, and leave a series of old

leaf bases when they finally fall down. Organic debris accumulates in the angles between these frond bases and the trunk, and in the axillae between leaves on the palm crown. In our experience, most OM accumulates at the transition between the trunk and the crown (where decaying fronds are still hanging and old leaf bases remain around the stem); most of the traps were set in that section of the palm.

Results from univariate logistic regression analysis suggested a very strong correlation between OM scores and the likelihood of palm infestation, with odds ratios over 24 and 29 and lower 99% confidence interval limits above 1.7 and 1.8 for crude and log-transformed scores, respectively. This was confirmed by comparing two discrete groups of palms defined after their OM scores. 'Dirty' *Ph. aequatorialis* palm trees (scores >2) had a significantly higher likelihood of being infested by *R. ecuadoriensis* than 'clean' ones (FET $p<0.0001$; relative risk[θ] [RR]=3.9). These results possibly reflect that the presence of vegetable debris helps create an adequate microenvironment (including buffered microclimate and abundant hiding places) for the bugs and, perhaps, also for their vertebrate hosts. Other authors had previously suggested that the presence of organic debris at the bases of palm fronds helps maintain relatively constant temperature and relative humidity values, even in areas where seasonal climate variation is important; vertebrate nests can often be found in those parts of the trees. These observations, together with considerations on the architectural complexity of some palm habitats, led to the suggestion that specific, and even individual, variation among palms might result in differential suitability for bug colonisation (Pizarro & Romaña 1998, Luz et al. 1999, Romaña et al. 1999, Jaramillo et al. 2000).

Although we also found 14% of 'clean' palms to be infested, our results largely support these conclusions, while suggesting that other factors might also be involved. For instance, the combination of male sex and a large amount of organic debris resulted in a high probability of the palm being infested; 80% of 'dirty' males were infested, while 80% of 'clean' males were not (FET $p=0.002$, RR=3.9). Under this arrangement, 60% of female palms with more than 2 in the organic matter score were infested, vs.

[θ]The relative risk is the ratio of the proportion of 'cases' to the total number of observations in two groups. In this case, the proportion of 'dirty' palms that were infested (13/24) divided by the proportion of 'clean' palms that were infested (12/86): 0.54/0.14=3.88. The odds ratio is the ratio of the probabilities of the 'event' (infestation) occurring and not: (13/11)/(12/74)=7.3 (see Bland & Altman 2000)

just 10% of those with OM scores up to 2 (FET p=0.03, RR=5.8). Scores of **epiphytic plants** (EP) were weakly, positively correlated with the amount of decomposing organic matter (linear fit R^2=0.27), probably because organic debris remains trapped among the creeping roots, branches and stems of these plants, whose growth is in turn favoured by the presence of decomposing organic matter; also, dead epiphytes contributed to higher OM scores. Overall, EP scores were not associated with increased likelihood of infestation. We devised a combination of log-transformed EP and OM scores for further comparison; the weight assigned to EP was half that of OM, reflecting the observation that while decomposing organic debris likely represents the main factor favouring the bugs, the presence of epiphytes may also have a bearing, especially in female palms (among which significantly higher infestation rates were recorded when EP scores were >2). This 'organic score' was also significantly higher among infested palms (t=2.9, 108 df, p=0.005).

Regarding the immediate **environment** of the palms, the fact that only 18 (16% of the total sample) were surveyed in forest areas indicates that further sampling could help discover infestation there. Forest bug colonies may be much smaller than those infesting palms located in human-altered environments, as it has been suggested for other *Rhodnius* species in the Ecuadorian Amazon (*R. pictipes* and *R. robustus*; Palomeque et al. 2000), the Magdalena valley (*R. colombiensis*; Vallejo et al. 2000), and northwestern Colombia (*R. pallescens*; Jaramillo et al. 2000). In this case, the failure to detect these colonies would be related to the sensitivity threshold of live-baited traps, but this aspect remains to be studied. Selective dissection of palms where Noireau traps captured different numbers of bugs would help clarify this aspect, and would provide a basis for estimating bug population densities after results from trapping.

4.4.1.2. Geographic-ecological heterogeneity

Infestation was more frequent in the **Andean foothills** near Alluriquín than in the two areas of the central lowlands (25% in Alluriquín vs. 5.3% in Chigüilpe-La Roncadora), and similar to that recorded from the coastal localities (28%). We found that one in four *Ph. aequatorialis* palms harboured *R. ecuadoriensis* colonies in that area of the foothills. The number of bugs captured (143, vs. 1 in the lowlands and 30 in the coast; density=2.5 vs. 0.05 and 0.83, respectively) was the most striking difference.

It probably reflects the fact that sylvatic populations of *R. ecuadoriensis* form larger palm tree-living colonies in the Andean foothills. A worse nutritional status (likely to occur in dense colonies) and a more aggressive behaviour of these bugs could be complementary or alternative explanations, as both could result in more bugs trying to attack the mice used as bait. A worse performance of traps in drier environments (where more dust and dry vegetable particles adhered to the tape) might also be involved. None of the several local villagers interviewed during our field trips knew the bugs, and we were repeatedly told that never these insects had been seen neither inside houses nor attacking people – even if infested palms were abundant near houses. Only once we were able to study a male specimen that was captured flying at night in a petrol station near Alluriquín. These bugs seem therefore to be strictly sylvatic in the area.

In the **coastal area** of Manabí our results suggest that it is not infrequent that peridomestic *Ph. aequatorialis* palms located in the narrow humid valleys (and possibly also those in forest remnants on the slopes of hills) of the province harbour small breeding colonies of *R. ecuadoriensis*. These bugs have demonstrated their ability to invade and colonise human environments (Defranc 1982, Carcavallo & Martínez 1985, Schofield 1994) in areas where disease transmission has been documented, and are therefore of epidemiological importance. We studied two localities with similar characteristics, distant some 15km from one another, and obtained comparable results. The likelihood of palm infestation was not affected by stem height or by the amounts of organic matter or epiphytic plants. Males were more likely to be infested; this is possibly related to the fact that they had significantly more decomposing organic material than females. In this area of the coast, tagua seeds are actively marketed for handicraft manufacture and for export. This means that female palms are usually taken care of by the locals, who remove dead leaves and other vegetable debris from the palms that produce infrutescences and seeds – but not from male palms. This results in male palms being more likely to accumulate organic matter, and this possibly favours infestation, as discussed above. When not managed in this way, the differences in the amount of organic matter and epiphytes recorded between male and female palms are not significant – as observed in Alluriquín. The different climatic patterns of foothills and coast could be also influencing the growth of epiphytes (in the coast all palms had EP scores below 2) and thus the accumulation of vegetable debris.

The low infestation rate found in the **central lowlands** (with no bugs detected in Chigüilpe, and just one specimen captured in La Roncadora) might reflect the effects of extensive deforestation on the populations of both *Ph. aequatorialis* palms and their associated *R. ecuadoriensis*. The first locality is located near Santo Domingo de los Colorados, the fourth largest town of Ecuador (~250000 inhabitants) and the area with the fastest growing population in the country, mainly because of high immigration rates (see Barros et al. 2001). These demographic trends are tightly linked to environmental alterations in the area; vast stretches of forest have been transformed into pasture and plantations (African oil palm, pineapple), mainly in the less mountainous zones – with a largest proportion of woodland respected on the Andean foothills east of the city (i.e., the area where Alluriquín is located).

Figure 37. Deforestation in the central lowlands of western Ecuador. Only a few palms (in this case *Attalea colenda*) remain in the pasture fields after forest clearing

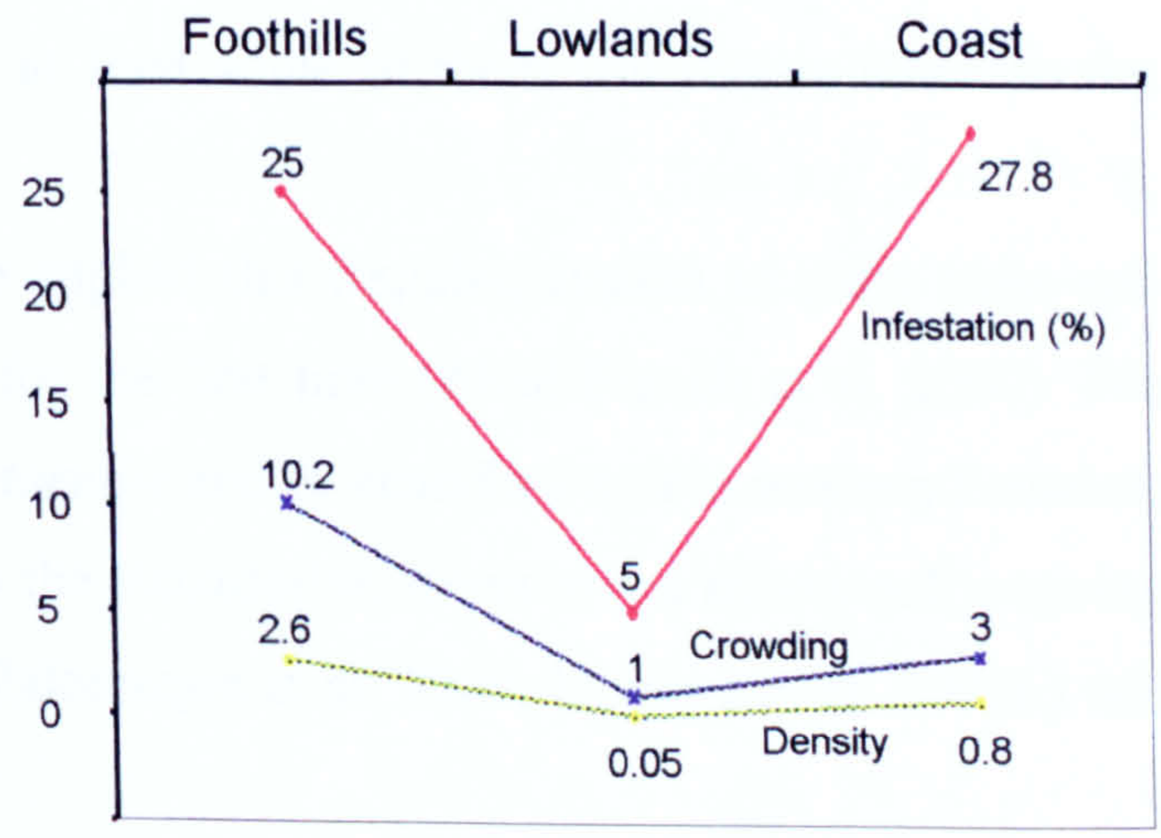
Only four palms were sampled in this area, in part because they were so scarce that we had to travel extensively to find a few of them in an indigenous community where part of the original vegetation had been preserved. This obviously precludes drawing any significant conclusion, but our perception was that extensive (and intense) deforestation might have had a severe impact on sylvatic *R. ecuadoriensis* populations near Santo Domingo, perhaps limiting them to small patches of modified forest where *Ph. aequatorialis* still grow. The absence of reports of *R. ecuadoriensis* in the area (where other triatomines have been captured regularly by workers of the Vector Control Service) might be envisaged as indirect evidence in support of this idea. We identified a patch of well-preserved, old secondary forest (~30ha) near the limit between the

provinces of Pichincha and Manabí (Sitio La Roncadora, ~35km west of Chigüilpe) and studied 15 *Ph. aequatorialis* and one *Attalea colenda* there. A single nymph (stage II) of *R. ecuadoriensis* was captured in a male palm located in a pasture field near the fringe of the forest. This finding suggests that *R. ecuadoriensis* may have survived deforestation in preserved areas where natural populations of *Ph. aequatorialis* still exist; one might speculate that these populations (of bugs and palms) could be fragmented, with small pockets perhaps isolated from each other, but no conclusions can as yet be proposed. The finding of two adult specimens of *R. ecuadoriensis* inside human dwellings in a nearby locality confirms that the species occurs in the central lowlands of western Ecuador, and indicates that at least in some localities the bugs display synanthropic behaviour and may interact with people. Interestingly, the overall morphology and chromatic pattern of these two bugs were distinct; one was similar to the foothill forms captured in Alluriquín, whereas the other one resembled the specimens collected in coastal Manabí.

Overall, our results suggest that two main clusters of sylvatic, palm tree-living *R. ecuadoriensis* populations occur in central-western Ecuador, one in the relatively well-preserved Andean humid forests and the other one in coastal areas of Manabí where *Ph. aequatorialis* are abundant. Furthermore, our findings are compatible with the existence of smaller bug populations in the intermediate zone (the central lowlands), where deforestation and habitat destruction-fragmentation has been extensive. The following table and figure summarise and compare the findings in the three geographic-ecological areas defined in this study.

Table 17 and Figure 38. *Rhodnius ecuadoriensis* and *Phytelephas aequatorialis* in western Ecuador: entomological indices

	Foothills	**Lowlands**	**Coast**
Infestation	25%	5.3%	27.8%
Density	2.6	0.05	0.8
Crowding	10.2	1	3
Colonisation	93%	100%	90%
Palms surveyed	56	18	36
Bugs captured	143	1	30



4.4.2. LIVE-BAITED ADHESIVE TRAPS (NOIREAU TRAPS)

The collection of sylvatic triatomines is a notoriously time-consuming, expensive, and logistically difficult undertaking. Results are usually scarce, even when suspect natural ecotopes are systematically dissected (Miles et al. 1981b, Pizarro & Romaña 1998). In addition, habitat dissection is destructive and there is usually no means for carrying out longitudinal studies that could yield valuable information on, for instance, the responses of bug populations to seasonal variations in climatic conditions, to changes in host species and/or numbers, or to control interventions in sylvatic ecotopes located near human dwellings (Noireau et al. 2002b). Finally, legal and ethical restrictions make it often impossible to dissect arboreal habitats in protected forests where the biology of sylvatic bug populations could be studied under truly natural conditions. Light trapping is also of limited value, because only starved adults of a few species fly readily to light traps and because the method provides no information on the natural ecotopes of the bugs (e.g., Whitlaw & Chaniotis 1978, Noireau et al. 2000a). Several authors have reported the use of animal-baited traps for triatomines (Rabinovich et al. 1976, Tonn et al. 1976, Carcavallo 1985), all as yet with poor results.

The use of small, live-baited adhesive traps for the capture of sylvatic triatomines was first reported by Noireau and coworkers in 1999; the system consists of small plastic pots (9 x 6cm) containing a live mouse (the bait) and covered with strong, double-sided adhesive tape (usually of the type used to fasten carpets). In the original work, the traps were suspended into hardwood tree holes of the Bolivian Chaco using a string, and were left there for periods between 8 and 15h before they were checked for triatomines (Noireau et al. 1999c). This simple trapping approach is based on the premise that starved triatomines will approach the bottle in an attempt to feed on the mouse (which they cannot reach), and at least some of them will remain stuck to the tape until the trap is recovered.

In collaboration with F Noireau we applied this trapping system to palm trees and their associated *Rhodnius* populations for the first time (Abad-Franch et al. 2000). We concentrated on the study of *Ph. aequatorialis* and *R. ecuadoriensis* in western Ecuador, but also extended our field activities to the Ecuadorian Amazon – a survey still ongoing from which some preliminary results (Palomeque et al. 2000, Noireau et al. 2002b) will also be presented in this discussion.

4.4.2.1. Results in Ecuador

We used 222 trap-nights in 110 *Ph. aequatorialis* in various geographic-ecological areas of western Ecuador (an average of 2 trap-nights per palm surveyed). Forty-five of those traps (20.3%) were found to have triatomines adhered to the tape when retrieved after ~15h. More widely in Ecuador, 333 trap-nights were used in palms of nine different genera (*Phytelephas*, *Attalea*, *Astrocaryum*, *Elaeis*, *Oenocarpus*, *Mauritia*, *Bactris*, *Iriartea*, and *Cocos*) on both sides of the Andes; 93 (27.9%) of those traps captured triatomines, and >600 bugs were collected during the surveys by combining traps and manual captures on palm tree crowns. With the Noireau traps, we were able to detect infestation in 32.6% out of 172 palms surveyed in Ecuador (and in all the genera above except *Mauritia*, *Bactris*, *Iriartea*, and *Cocos*). Additionally, it was possible to recover sufficient live specimens (adults and nymphs) to establish laboratory colonies (of *R. ecuadoriensis*, *R. pictipes* and *R. robustus*) and to enlarge reference collections for further studies and comparisons.

Live-bait trapping provided a quick and inexpensive system to readily detect infested palm trees. Live-baited adhesive traps allowed for the detection of seemingly small triatomine colonies in peridomestic palms, suggesting that the method could also be of value for disease transmission control purposes; infested palms could be detected and then monitored for reinfestation (or bug population recovery) after focal palm treatment with insecticide compounds. This would be particularly beneficial in areas where palm tree-living bug populations seem responsible for most of disease transmission and hamper long-term interruption of disease transmission. Potentially, the method could be of value in the vast Amazonian ecoregion and its fringes (Miles et al. 1981b, 1983, Barrett 1991, Coura et al. 1999, 2002, Noireau 1999, Teixeira et al. 2001), the Venezuelan Llanos and other parts of the Orinoco basin (Barrett 1991), the seasonally dry forests of northwestern Colombia (Pizarro & Romaña 1998, Romaña et al. 1999, Jaramillo et al. 2000) and several parts of southern Central America (Whitlaw & Chaniotis 1978), the Cerrado and Caatinga in Brazil (Barrett 1991, Dujardin et al. 1991, Diotaiuti 1999, Costa 1999, Soares et al. 1999), and in the central-northern coastal region of Ecuador (Abad-Franch et al. 2001). In all these zones different species of *Rhodnius* (*brethesi*, *pictipes*, *robustus*, *stali*, *prolixus*, *pallescens*, *neglectus*, *nasutus*, and *ecuadoriensis*) inhabit palm trees and pose a substantial health threat to the

inhabitants. Other potentially dangerous triatomines (e.g., *T. sordida*, *T. maculata*, or some populations of *T. dimidiata*) have also been recorded from palms (tables 4 and 5).

Other authors have reported the use of similar adhesive traps in different geographic areas and ecological conditions – and also involving distinct triatomine species. Our general results were comparable to those obtained by Noireau and coworkers in the Bolivian Chaco (where *T. infestans*, *T. sordida* and *T. guasayana* populations occur in hollow trees) in terms of percentage of positive traps: 27.5% (present study) vs. 27% and 22% (Noireau et al. 1999c and 2000a, respectively). Data from Noireau et al. (2002) show that 20% out of 72 traps captured *Rhodnius* (*pictipes* and *robustus*) in the Brazilian Amazon (up to 9 bugs/positive trap); in the Caatinga, 10% of 59 traps captured *R. nasutus* in *Copernicia prunifera* palms. Other habitats (hardwood trees, rockpiles, crags) have been studied with this approach, and various triatomine species have been captured (*T. sordida*, *T. guasayana*, and *T. infestans* in the Bolivian Chaco; *Panstrongylus megistus* in subtropical forests of Bolivia; *T. brasiliensis* and *T. pseudomaculata* in the Brazilian Caatinga; *T. klugi* in crags of the Serra Geral, Brazil; and bugs of the *T. phyllosoma* complex in Mexico) (Espinoza-Gómez et al. 2002, Noireau et al. 2002b). The use of mouse-baited traps in human environments has been less successful; this was attributed to the usually better nutritional status of synanthropic (vs. sylvatic) populations (Noireau et al. 2002b). It could be added that while the 'bait effect' of a small Swiss mouse may be significant in a palm tree where bugs must rely on (at best) one or two medium-sized, usually mobile vertebrates (opossums, birds...) for feeding, it is probably negligible in enclosed environments where a variety of mammals (humans, dogs, cats, rodents, sometimes goats, pigs, etc...) and birds (chickens, turkeys, pigeons, ducks...) are available to the bugs throughout the year. Limited data from Colima, Mexico show however that mouse-baited adhesive traps can capture triatomines indoors (Espinoza-Gómez et al. 2002).

4.4.2.2. Cost-effectiveness

Our general bug capture results in Ecuador (3.6 bugs captured per palm surveyed and ~12 bugs per positive palm [live-bait trapping plus direct searches in palm crowns]) question the necessity of indiscriminately cutting down and dissecting palms for these ecological studies, and demonstrate the efficacy of this approach. Based on data reviewed by Pizarro and Romaña (1998) (1390 palms cut down and 10564 *Rhodnius*

captured; i.e., 7.6 bugs/palm), the approximate cost-effectiveness of these two approaches (in terms of bug collection) can be compared as follows.

i. The complete dissection of one palm takes at least half a working day (for a team of four and using chainsaws); the cost can be estimated at no less than 80 US$/palm (salaries plus other expenses as calculated from our fieldwork activities, and without taking into account the cost of purchase and operation of chainsaws);

ii. The cost of our survey (172 palms overall) was ~3500 US$ (all fieldwork expenses included); the cost per palm was therefore ~20 US$;

iii. Palm dissection yielded ~7.5 bugs/palm surveyed (~10.7 US$/bug were required on average), and ~3.5 bugs were captured per palm surveyed in our study (~5.7 US$/bug).

Thus, not only was the ecological impact of research conducted with live bait traps negligible, but also the cost-effectiveness of this method in capturing bugs was roughly double that of the classical fell-and-dissection approach (5.7 US$/bug vs. 10.7 US$/bug). Although cutting down palms would probably have yielded significantly more bugs (perhaps about twice as many), our survey would have cost over 13700 US$ if based on that procedure, almost four-fold the 3500 US$ actually spent. Almost 700 palms could have been theoretically surveyed in Ecuador with 13700 US$ using Noireau traps.

4.4.2.3. Trap performance in western Ecuador

The performance of the traps was inferior (in terms of percentage of positive traps and number of bugs captured) in coastal Manabí (91 trap-nights used, 16.5% positive, 30 bugs collected) than in the Andean foothills near Alluriquín (88 traps-nights, 33% positive, 143 bugs captured). This difference was recorded despite the fact that more traps were set on average in each palm in Manabí (2.5 trap-nights/palm) than in the foothills (1.6 trap-nights/palm). The most likely explanation is therefore a lower density of bug colonies in the coastal localities, but other circumstances (less aggressive behaviour or better nutritional status of these populations) could also be involved. As mentioned above, a worse trap performance in the drier environment of Manabí could be related to dust and dry vegetable debris adhering to the tape; when this happened, some bugs and other insects were able to walk upon the adhesive surface without

getting trapped. Wet conditions (particularly rain) were however also observed to reduce the strength of the adhesive.

In the Amazon basin, our results (111 trap-nights used, 43% of them positive) are notably better than those obtained in western Ecuador and those reported by Noireau et al. (1999c, 2000a, 2002). Here, we found strikingly dense bug colonies in palms located in deforested areas; for instance, over 400 specimens were captured from palms in approximately two weeks of fieldwork in the outskirts of the town of Lago Agrio (Palomeque et al. 2000).

4.4.2.4. Final considerations (and limitations of the system)

This report presents the largest series of palms studied so far by means of live bait traps, and lends strong support to the suitability of this fieldwork tool for the study of sylvatic *Rhodnius* populations in palm trees. We have demonstrated the usefulness of live-bait traps in uncovering the presence of peridomestic, palm tree-living triatomine colonies. Adhesive traps detected small populations of bugs in palms of coastal Ecuador, suggesting that a similar approach could be used for the detection and monitoring of likewise small triatomine populations in peridomestic palm trees – and possibly in other ecotopes such as hollow trees (Noireau et al. 1999c, 2000a, 2002).

Apart from obvious limitations in sample size and incomplete coverage of the geographic range of *Ph. aequatorialis*, the use of ladders to climb palms prevented the study of very large palm trees (such as *A. colenda*, often with stipes more than 20m tall). Also, the lack of reference data on the possible infestation of palms found to be negative, or about the actual density and age structure of bug colonies in positive palms, limits the interpretation of our results; comparisons of live-bait trapping with palm dissection and bug population census could allow for a more complete analysis.

4.5. Conclusions and recommendations

Further studies on wider samples and other geographic-ecological regions are still necessary to improve our understanding of the ecology of sylvatic *Rhodnius* populations inhabiting palm trees in different parts of Latin America. The results reported here allowed us to outline some general trends in relation to *Ph. aequatorialis* palms and their associated *R. ecuadoriensis* populations in western Ecuador. Also, we added evidence in support of the usefulness of live-baited adhesive traps as a fieldwork tool; taking cost-effectiveness estimations and the advantages (ecological and scientific) of

preserving the studied palms into account, we may conclude that Noireau traps should represent the first choice for research addressing the ecology of palm tree-living triatomines.

·High-risk ecotopes. The definition of high-risk ecotopes (for instance, favoured habitats where sylvatic bug populations of species displaying synanthropic behaviour and frequently infected by *T. cruzi* may reach high densities) is of importance where contact between humans and sylvatic triatomines is likely to occur; in most cases, the risk increases when the suspect ecotope is located near a human settlement. As mentioned above, this seems to be the case in wide geographic areas (inhabited by several million people) across South America, where palm tree-living *Rhodnius* populations represent a serious public health concern. It has been noted that in some woodland areas (e.g. the southern fringe of the Amazon in Brazil, the northern Ecuadorian Amazon, or the canal zone in Panama) human activities, including deforestation, can increase the number of suitable ecotopes for palm tree-living triatomines near human habitations (Whitlaw & Chaniotis 1978, Barrett 1991, Palomeque et al. 2000). A pattern of selective deforestation in which palm trees are maintained in peridomestic environments is frequent in many parts of Latin America; in addition, some palms act as pioneer species of reforestation after slash-and-burn interventions. These palms (often peridomestic or periurban) are suitable shelters for opportunistic marsupials and rodents (*Didelphis marsupialis*, *Rattus* spp.) that proliferate in human-modified ecosystems. *Rhodnius* bugs, which are pre-adapted to palm trees and will feed on various vertebrates, may take advantage of this situation, forming large colonies in some palms. In the Ecuadorian Amazon for instance, peridomestic *Ph. tenuicaulis* palms are often heavily infested by *R. pictipes*, but when located in primary forest they harbour only small colonies (if infested at all); the same seems to occur with *R. robustus* and *A. butyracea* in the area (Palomeque et al. 2000), and similar trends have been noted for other *Rhodnius* species (Jaramillo et al. 2000, Vallejo et al. 2000). A reduced availability of food per bug when the system nears its carrying capacity results in adult starved specimens frequently flying into houses – probably the main source of epidemiological risk in the Amazon basin ecoregion (Teixeira et al. 2001, Coura et al. 2002).

In spite of these observations, the limited attempts to describe the links between palms, bugs, and epidemiological risk so far carried out have to our knowledge concentrated on a single botanical variable – palm species. A few authors had previously suggested that some of the characteristics of palm trees could favour infestation (see Barrett 1991, Romaña et al. 1999), but the associations largely remain to be studied.

·High-risk *Phytelephas aequatorialis* palms in western Ecuador. The combined analysis of the results obtained during our survey on sylvatic populations of *R. ecuadoriensis* helped depict a general 'outline' of infested *Ph. aequatorialis* palm trees. This approximation may be envisaged as a rough description of a high-risk palm ecotope. Infested *Ph. aequatorialis* were generally adult palms (i.e., specimens in which sex was discernible) with stems over 3m in height. Importantly, many of them could be classified as 'dirty' (with organic matter scores >2 over a maximum of 4), a trait more frequently found among male palms and the single most important factor favouring infestation. Infested palms also had relatively large amounts of epiphytic plants growing around their stipes and on their crowns (scores over 2, especially in the case of female palms). They were usually located in cropland or pasture fields rather than in forested areas – and consequently often near human dwellings. Although *R. ecuadoriensis* seems only able to form relatively small colonies on these palms, the epidemiological risk posed by the ability of these bugs to invade and colonise human habitats cannot be neglected.

In the Andean foothills of Alluriquín (Pichincha), the relative risk represented by infested palms seems to be less important than in the coast, as suggested by the fact that foothill bugs do not appear to display any synanthropic behaviour, even if the colonies reach higher apparent densities. In the coast, where Chagas disease can be transmitted by peridomestic, palm tree-living *R. ecuadoriensis* (which can in addition colonise artificial structures), the picture of the infested palm is less clear. Male palm trees located in altered environments were nonetheless more likely to harbour triatomine colonies in the area, possibly because they tended to have higher OM scores. Importantly, this latter fact seems related to the traditional management of female (seed-producing) palms in the area, suggesting that 'unmanaged' specimens are more likely to be infested. This notion may have considerable implications for disease control strategy

design, because it would imply that environmental management interventions aimed at reducing the amount of organic matter on palms could have a significant impact on peridomestic vector populations.

·Interactions between humans, palms and bugs. Several findings suggest that, at least in some areas, human activities could be affecting the dynamics of sylvatic populations of *R. ecuadoriensis* inhabiting *Ph. aequatorialis* palm trees.

Firstly, the fact that these palms are preserved in deforested areas for the economic value of their products results in the presence of suitable bug habitats (i.e., dense palm stands) in human-related environments, often near houses. On the other hand, deforestation in western Ecuador might have influenced the current geographic range of *R. ecuadoriensis*. Deforestation has been intense and extensive in the province of Pichincha, but massive environmental degradation did not take place on the mountain slopes. Sylvatic populations of *R. ecuadoriensis* might have disappeared from very heavily deforested areas like those surrounding Santo Domingo de los Colorados, where we failed to detect any bugs. The finding of one single nymph in La Roncadora (northeast Manabí) and of adult bugs inside human dwellings in a nearby locality make us believe that a few, small sylvatic colonies of the species might have survived in preserved forest patches with palm trees.

Finally, and perhaps more importantly, the fact that female palms are less likely to be infested in areas where they are managed by the locals (who remove organic debris and dead leaves, but do not use pesticides on palms) suggests that human intervention on individual palms could also be influencing infestation rates.

·Control approaches. Sylvatic populations of *R. ecuadoriensis* represent a potential risk for humans living in the same areas. These bugs occupy mainly palm trees, among which *Ph. aequatorialis* is probably the most favoured ecotope. Products obtained from these palms are an important part of the economy of some communities in the Ecuadorian coastal region (especially in Manabí and Esmeraldas), and palms are therefore often maintained near human dwellings. From these palms, adult bugs may reach dwellings and peridomestic structures (mainly chicken and pigeon houses); this process may lead to the stable colonisation of human environments by these triatomines, increasing the risk of *T. cruzi* transmission to the inhabitants. The situation in rural villages in El Oro and Loja, where domestic-peridomestic colonies of *R. ecuadoriensis*

are common, demonstrates that the species can adapt to human habitats; prevalence rates of *T. cruzi* infection are higher in these areas that in any other part of Ecuador.

Control strategies will have to contemplate different settings. In areas where the species is confined to human dwellings, local eradication by insecticide spraying could perhaps be contemplated, whereas the possibility of reinfestation from palm trees must be taken into account in other zones – mainly the coastal provinces of Manabí and Esmeraldas, and also some areas of Pichincha, Los Ríos, and Guayas. The presence of sylvatic *R. ecuadoriensis* in forest remnants with tagua palms in some small areas of El Oro and Loja should be investigated, even if no previous records suggest these bugs occur there.

An approach we might call '**integrated habitat management**' could be proposed for disease transmission control in areas where sylvatic *R. ecuadoriensis* populations have been detected in human settlements. It would integrate classical interventions in dwellings-peridomiciles (exclusion via insecticides) with domestic bird and palm tree management in the context of a joint, community-based control-surveillance system. The suggested approach would involve the following aspects:

i. Risk areas where *Ph. aequatorialis* stands grow near human dwellings could be traced down by following the trade routes of tagua seeds to the cultivation areas; remote sensing-GIS approaches could also be used to reconstruct the distribution of the palms (but they must be validated with field data on deforestation). Sero-entomological surveys would help describe transmission profiles, circumstances, and risk factors. Live-baited traps could be used to detect infestation in peridomestic palm trees;

ii. Spraying with residual pyrethroids should be carried out whenever an infested dwelling is discovered; surveys (and spraying if necessary) must include peridomestic chicken coops and dovecotes. Promoting better management of domestic birds would help maintain peridomiciles bug-free; periodically cleaning chicken coops in depth and burning and replacing nests in a 15 to 30-day period basis could be proposed;

iii. Palm tree management could complement control activities. Simple interventions could be endorsed in areas of tagua harvesting aiming to reduce palm infestation rates – at least in peridomiciles. For instance, removal of dead fronds and other organic debris (especially from male palms with stems >3m) could reduce palm infestation. In some cases large palm trees could be removed from a radius of ~200m from houses. Health

risks associated with tagua cultivation should be advertised to the locals and to the various organisations that foster this activity to improve awareness and to incorporate health safety concepts into tagua-related programmes (e.g. Southgate 1997);

iv. Community-based entomological surveillance is crucial whenever the risk of reinfestation of dwellings by triatomines in control areas is foreseeable (Dias 2000). Appropriate surveillance systems should be organised and maintained in areas where control of *R. ecuadoriensis* is undertaken and *Ph. aequatorialis* palms are present near human environments. The surveillance network should involve: (1) report of infestation by locals to designated personnel, (2) rapid assessment by control agents (including palm trees if deemed necessary), (3) design and implementation of control measures (as above), and (4) educational interventions (aiming either at establishing or reinforcing the collaborative network). Local health workers should also be prepared to detect, manage and report suspect disease cases (mainly acute forms), as the possibility of vector-borne transmission persists even if no bug colonies are detected during the surveys in or around houses.

·Further research

i. The apparent ability of live-baited traps to detect seemingly small sylvatic colonies of *R. ecuadoriensis* suggests that they might be used to survey and monitor the presence of such colonies in peridomestic palms. The use of alternative baits easier to handle (such as yeast or chemical attractants) could be explored. Some palms studied by means of live bait trapping could be subsequently cut down and dissected in order to work out the relationships between trapping results and the actual presence/absence, density, and composition (sex ratio, age structure, presence of other triatomines) of the colonies.

ii. Remote-sensing/GIS systems could be used to detect putative risk areas where tagua palms are abundant and close to human settlements. The combination of botanical data with current deforestation data could help define the actual range of this and other species of palm trees known as favoured habitats for *Rhodnius* bugs.

iii. The study of potential alternative habitats could be of interest in western Ecuador. The presence of another abundant endemic palm (*A. colenda*) in the area, and the fact that *Attalea* palms harbour various *Rhodnius* species (including *colombiensis*, *pallescens*, *pictipes*), make this species a good candidate for further studies. Carcavallo & Martínez (1985) reported the finding of *R. ecuadoriensis* in *E. guineensis* in the

province of Los Ríos. It would also be of interest to determine whether sylvatic populations of the species exist in southern Ecuador and in Peru; control interventions aiming to eradicate domestic-peridomestic populations from those areas could be organised if such populations were absent.

iv. The putative vertebrate hosts of natural populations of *R. ecuadoriensis* have not been studied. During our work on tagua palms, we observed various mammals (bats, opossums, small rodents, one spiny rat and one coati), birds (including medium-sized owls and others), and reptiles (mainly small lizards) taking shelter on palm crowns. The relationships between these and other vertebrates and the bugs could be investigated by means of bloodmeal identification.

v. Assessment of natural infection and characterisation of parasites circulating in bugs and mammals would help improve our knowledge on the sylvatic cycle of *T. cruzi* (and perhaps other trypanosomes) in Ecuador.

vi. The study of microclimatic conditions in palm trees would also be of interest. It has been suggested that relative humidity (RH) and temperature preferences of some bug species could play a key role in the process of invasion/colonisation of human habitats (CR Lazzari, pers. comm.). Thus, the adaptation of Amazonian *R. pictipes* and *R. robustus* to constant, extremely high RH in palm tree crowns (recorded averages over 95%, and minimum values ~80%) could be contributing to the observed fact that these bugs never colonise, although frequently invade, human dwellings – where average and minimum RH values are generally lower (FS Palomeque et al., unpublished data).

vii. The impact of alternative/complementary control approaches proposed here (the 'integrated habitat management') on bug behaviour and population dynamics, and on disease transmission, should be carefully assessed in the context of pilot interventions (including a thorough cost-effectiveness evaluation).

viii. Finally, the relationships between sylvatic and domestic *R. ecuadoriensis* populations were investigated in this project. Important phenetic, ecological, and behavioural differences were identified. Molecular and morphometric approaches were used to explore the relationships of *R. ecuadoriensis* populations. The phylogeny of the species in the context of the group *R. pallescens – R. colombiensis – R. ecuadoriensis* was also investigated.

5. DOMESTIC ECOLOGY OF *RHODNIUS ECUADORIENSIS*

5.1. Introduction

Domestic populations of Triatominae are the principal single source of human *T. cruzi* infection (Schofield 1994, Dias & Schofield 1999). The notion that these vectors may tend to favour households presenting some specific features was already present in the works by Carlos Chagas. *Panstrongylus megistus* (then identified as *Conorrhinus megistus*) was the main synanthropic species in Lassance (Minas Gerais, Brazil), where Chagas was working around 1907; he observed that the bugs thrived in the cracks and crevices of the mud-walled huts where the impoverished local population lived, and accordingly reached the conclusion that improvement of living standards would represent a major move towards the control of disease transmission (Chagas 1909, 1911, Dias & Schofield 1999, Lent 1999, Prata 1999). Later on, extensive investigations carried out at the 'Centro de Estudos e Profilaxia da Moléstia de Chagas' (a field research station of the Instituto Oswaldo Cruz in Minas Gerais) established the technological and operational foundation upon which current vector control programmes are built: the use of chemical insecticides in combination with community participation aided by comprehensive health education schemes – and with housing improvement playing an important role in lowering reinfestation rates and halting disease transmission in the long term (Briceño-León 1990, 1996, Ault 1994, Schofield 1994, Dias 1998a, 1999, Dias & Schofield 1999, Morel 1999, Rojas de Arias et al. 1999, Rojas de Arias 2001).

The Triatominae are however a very diverse assemblage of insects. Being largely a group of nest-dwelling, opportunistic blood-feeders, it is not surprising that many of the ecological differences among genera (and also between species groups, between species, and even between populations of single species) are related to their fine adaptations to particular microhabitats (Barrett 1991, Gaunt & Miles 2000, Schofield 2000b). The spatial distribution of some species may even tend to be discrete *within* human environments; for instance, in some parts of Brazil or Bolivia *T. sordida* prevails in the peridomicile while *T. infestans* is mainly found indoors (Barrett 1991, Schofield 1994, Noireau et al. 1999a, Pires et al. 1999). Considering peri- and intradomiciliary areas separately, *T. brasiliensis* is found more often in stone-walled animal pens and among piles of firewood or tiles than in fences or storerooms in northeast Brazil;

synanthropic populations of several *Rhodnius* species (e.g. *nasutus*, *neglectus*) preferentially infest peridomestic chicken coops, and *R. prolixus* is notorious for its ability to build up dense colonies in thatch roofs in Venezuela; *T. dimidiata* is in contrast rarely found above 1m from the floor in Costa Rica, although tile roofs were identified as a risk factor for infestation; finally, whilst in northwest Argentina *T. infestans* populations favour brushwood or grass-and-earth roofs, they are more commonly found in mud walls in Brazil (Lent & Wygodzinsky 1979, Barrett 1991, Starr et al. 1991, Gürtler et al. 1992, Schofield 1994, Salvatella et al. 1998, Oliveira-Lima et al. 2000).

As for other aspects of triatomine biology, most of the studies on the domestic ecology of the bugs refer to the primary disease vectors, and mainly to *T. infestans* (e.g. Cohen & Gürtler 2001; see below). It is well established that synanthropic *Rhodnius* species tend to infest houses with palm leaf or thatch roofs and chicken coops or dovecotes in the peridomicile, probably reflecting their association with arboreal habitats (mainly palms) and avian hosts in the wild; it has been proposed that eggs of *R. prolixus* (fastened to the substrate as in all Rhodniini) may be transported into houses when palm fronds are used to build roofs. Many species of Triatomini occupy terrestrial ecotopes in nature, and this too seems reflected in the preference for houses with mud walls and earthen floors of some populations of *Triatoma* (e.g., *infestans*, *dimidiata*) and *Panstrongylus* (e.g., *megistus*, *rufotuberculatus*) (Lent & Wygodzinsky 1979, Barrett 1991, Gaunt & Miles 2000).

Although some general patterns do apply to many of the principal vectors, H Lent and P Wygodzinsky observed that, when judged as a whole, synanthropic triatomines may be found "in all kinds of materials and structures" (Lent & Wygodzinsky 1979; p. 133), highlighting the need for specific studies if meaningful information (i.e., from which control measures can be derived) is to be obtained.

There is a conspicuous lack of studies on the domestic ecology of *R. ecuadoriensis*; this is in part a consequence (and perhaps in part a cause) of the relatively widespread perception that this species does not play a major role in disease transmission – in spite of epidemiological evidence on the contrary, applying at least to some geographic areas (see Section 1.4.2.).

5.2. Materials and methods

5.2.1. ENTOMOLOGICAL SURVEYS

A pre-selection of candidate study localities was performed after reviewing the relevant information extant on vector distribution and epidemiology of Chagas disease in Ecuador. Qualifying factors included existence of reports of domestic-peridomestic populations of *R. ecuadoriensis*, absence of evidence suggesting *T. dimidiata* was the main local vector, and feasibility of fieldwork. Preliminary visits to pre-selected localities allowed confirmation of their suitability, established collaborative links with local health authorities, and were used to make logistical arrangements.

Entomological surveys were carried out in three localities: El Lucero (canton Calvas, province of Loja), Lourdes (canton Portovelo, province of El Oro), and Pachinche Adentro (canton Portoviejo, province of Manabí). Demographic and sociological data were available from local health authorities.

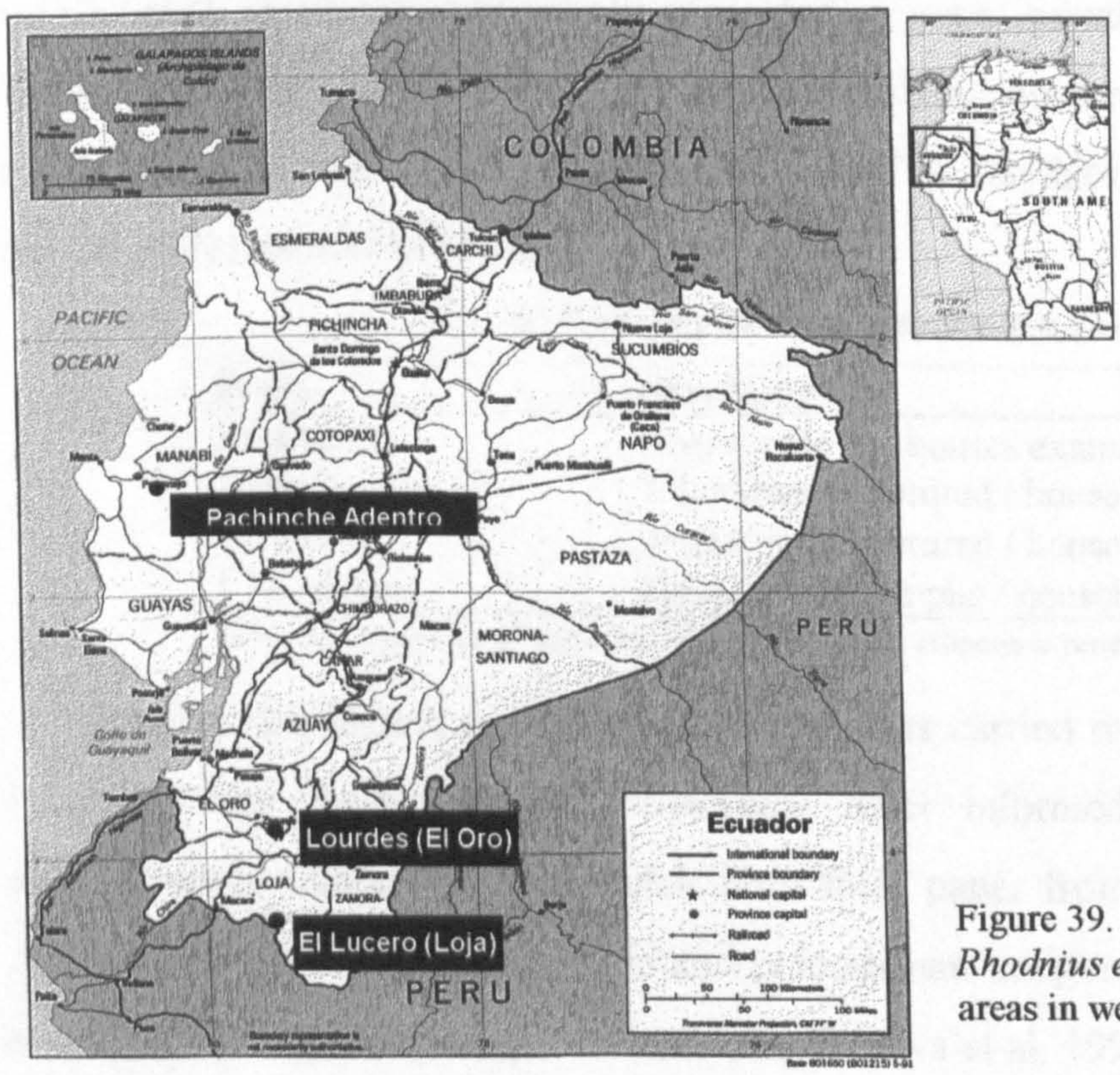


Figure 39. Domestic ecology of *Rhodnius ecuadoriensis*: fieldwork areas in western Ecuador

A representative sample of dwellings was randomly selected (using locality diagrams and house numbering established by the National Vector Control Service) for expected infestation rates below 2% (α=0.01 for confidence limits; calculations were made in EpiInfo 6.0). In each house, one dweller helped fill a detailed questionnaire, including

KAP (Knowledge, Attitudes and Practices) about the disease and its vectors. Triatomines were collected by hand, using forceps, gloves, torches, and labelled plastic flasks with filter paper. Dead specimens, exuviae, and eggs were also collected in some cases. Intradomiciliary inspection involved a two-people team (one worker of the National Vector Control Service plus one researcher) working in each dwelling for at least 30min; whenever triatomines or their traces (eggs, exuviae, dead bugs or faecal smears) were found, a thorough inspection was performed (two to four hours). Inspections began in the main bedroom, following clockwise; all surfaces and possible hiding places were inspected as thoroughly as possible (walls, roofs, floor; beds and other furniture; pictures and other objects hanging on walls; clothes; stored crops, building materials and firewood; and, if present indoors, the places where domestic animals were kept). No flushing-out or knock-down compounds were used.

A similar procedure was applied to the peridomiciliary area. All types of peridomestic structures were surveyed, including pens, corrals, domestic bird nests and coops, stored firewood, agriculture and/or building materials (stones, timber, tiles, adobe bricks...), hollow trees, palm trees, etc. The standardised entomological indices (WHO 1991) were calculated.

Table 18. WHO standardised entomological indices

Index	Description
Infestation	Households ⊕ / houses examined x 100
Density	Triatomines captured / households examined
Crowding	Triatomines captured / households ⊕
Colonisation	Houses with nymphs / households ⊕ x 100

⊕ = infested (presence of bugs of any developmental stage in houses or peridomiciles)

As mentioned above, a **serological survey** was carried out in Lourdes (El Oro) and Lucero (Loja) by Ecuadorian colleagues. After informed consent, capillary blood samples were obtained by fingerprick onto filter paper from inhabitants of the studied domiciles. ELISA tests using crude and recombinant antigens were performed on blood eluates following described procedures (see Grijalva et al. 1995, Guevara et al. 1999).

The **feeding profiles** of domestic/peridomestic *R. ecuadoriensis* from a highly infested dwelling in Loja were analysed by immunoprecipitation (Siqueira 1970); tests were carried out in a collaborating laboratory (Department of Entomology, Fiocruz, Rio de Janeiro, Brazil). Samples were obtained by pressing on the abdomen of recently collected, engorged specimens; drops of intestinal content were placed on filter paper,

labelled, and sent to the laboratory. Filter papers placed in the containers used for field collection, stained by insect faeces, were also analysed; these results, not individual-specific, were however indicative of the feeding preferences of the studied colonies (bugs from each collection site were placed in a separate container). A battery of 17 antisera [bird 1:10000, human 1:15000, dog 1:15000, cat 1:12000, goat 1:14000, horse 1:16000, pig 1:10000, opossum 1:15000, rodent 1:17000, tamandua 1:12000, capybara 1:14000, coati 1:13000, armadillo 1:15000, ox 1:15000, sheep 1:8000, toad 1:16000, and lizard 1:16000] was used.

5.2.2. STATISTICAL ANALYSES

Data analysis included descriptive statistics, univariate exploratory analyses, and multivariate logistic regression testing for the effects of covariates found to be related to the dependent variable (various forms of infestation, see below) in univariate analyses. The *variables and statistical tests* used in this study are defined below.

Current infestation: presence of *R. ecuadoriensis* bugs in artificial structures (domestic and/or peridomestic). The fact that only palm-tree living colonies of *R. ecuadoriensis* were detected in Manabí (with an additional adult bug captured within a house) led to the decision to limit the analysis of relationships between household variables and the likelihood of infestation to the communities of El Lucero (Loja; 84 DUs• studied) and Lourdes (El Oro; 36 DUs studied). Results obtained from *Ph. aequatorialis* palm trees in Pachinche Adentro (Manabí) are included in the analysis of the sylvatic ecology of *R. ecuadoriensis* presented in Chapter 4. **Intradomiciliary** and **peridomestic** current infestation were considered separately for some of the analyses.

Colonisation: presence of breeding colonies of *R. ecuadoriensis* (nymphs, eggs and/or exuviae) in domestic and/or peridomestic structures. The presence of adventitious adult bugs invading a domicile was considered to be most likely independent of any of the characteristics of households studied here – it could in any case be related to the infestation of neighbouring homes (or, in some parts of Ecuador, palm trees). Colonised dwellings corresponded exactly to those where peridomestic infestation was detected.

•DU = Domiciliary Unit (throughout the text hereafter); it comprises the house and the peridomestic area. 'Household' is sometimes used instead of DU, whereas 'dwelling' and 'house' refer exclusively to the buildings where people live

Reported infestation: report of the presence of *R. ecuadoriensis* bugs in the DU under study (i.e., the present family domicile), as recorded from dwellers during the interview. No distinction was made between the presence of breeding colonies and adult bugs invading the domicile. Reported infestation by the dwellers was in our sample strongly correlated to current infestation (see below); results from previous field studies suggest that longitudinal detection of infestation by householders outperforms active searches for bugs by investigators, which apparently yield false-negative results in many cases, even when pyrethrum compounds are used as flushing-out agents to dislodge the bugs from their hiding places (e.g. García-Zapata et al. 1992, 1993, Bryan et al. 1994, Andrade et al.1995a, Gürtler et al. 1995, Cuba Cuba et al. 2002). In our study areas, high levels of knowledge about *R. ecuadoriensis* were recorded (see below), supporting the view that most of the dwellers reporting infestation were likely to give true-positive answers. Modelling this variable can therefore be considered as a realistic approximation to the ecology of synanthropic populations of *R. ecuadoriensis*.

Reported recent infestation: a sub-group of the previous variable where positive DUs are those where dwellers reported having seen *R. ecuadoriensis* in the year before fieldwork.

Statistical approaches. Univariate statistics (Chi-square [X^2 hereafter], *t*-test, Fisher's exact test [FET hereafter], and non-parametric tests when the distribution assumptions of normality and equality of variances[+] were dubious [Wilcoxon test for two-level responses (WT), and Kruskal-Wallis test for multi-level responses (KW)]) were first used to explore relationships. Unadjusted odds ratios (uOR) and corresponding 95% CI were calculated from univariate logistic regression. However, performing repeated univariate comparisons could lead to erroneous estimates of significance probabilities (at α=0.05, an average of 5% p values will be <0.05 just by chance); in addition, univariate analyses do not allow for an assessment of the simultaneous effects of a range of covariates on the response variable. A multivariate logistic regression modelling approach was therefore used in which maximal models containing a binomial response variable (current infestation, reported infestation) and a set of potential explanatory

[+]The null hypothesis of homoscedasticity was tested using the Bartlett test in dubious cases; Welch Anova was used for comparisons whenever this hypothesis tested false

covariates (entomological, socio-economic, related to house quality and building materials, and related to domestic animals) were fit and then simplified using a step-wise process of deletion of non-significant terms as assessed from likelihood ratio tests (implying analysis of statistical significance in variations of deviance caused by each deletion by testing the null hypothesis that the parameter estimate for the term is zero [H_o: β_j=0]) (see Crawley 1993, Kleinbaum et al. 1998, SAS Institute 2000). The general expression of the logistic model describes the expected value of the outcome under study (i.e., $E(Y)$) in terms of the following formula:

$$E(Y) = 1 / 1 + \exp [- (\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_j X_j)]$$

The accuracy of each model was summarised as the area under the receiver operating characteristic (ROC) curve derived from the model (see Altman & Bland 1994, SAS Institute 2000). The model-predicted status (i.e., colonised/not, reportedly infested/not, etc.) of each DU was compared with the actual one by means of contingency analyses and FET. The covariates included in each maximal model corresponded to those showing highly significant relationships with the dependent variable in the univariate exploratory analyses. Following the general principle of parsimony, only minimal adequate models were kept. Predictions from these models were explored by simulation using different combinations of covariate values in the logit form of the model, namely

$$\text{logit}[\text{pr}(Y = 1)] = \beta_0 + \beta_1 (X_1) + \beta_2 (X_2) + \ldots + \beta_j (X_j) = \log[\text{odds}(Y = 1)] = \log[\text{pr}(Y = 1) / 1 - \text{pr}(Y = 1)]$$

where β_0 is the intercept and β_j the coefficients of the different covariates in the model. For dichotomous explanatory variables, X can take values of 1 (when the condition is present) and 0 (when the condition is absent). The probabilities associated with each particular case are therefore

$$\text{pr}(Y = 1) = 1 / 1 + \exp (\text{logit}[\text{pr}(Y = 1)]); \text{ and } \text{pr}(Y = 0) = 1 - \text{pr}(Y = 1)$$

Two- and three-dimensional plots were produced in Excel spreadsheets using these formulae, including different combinations of covariate values, to visualise the relationships between the covariates and their relative effects on the probabilities of the outcome.

5.3. Results

5.3.1. BASIC STATISTICS AND DIFFERENCES BETWEEN LOCALITIES

One hundred and eighty-eight houses were investigated in three communities of the provinces of **Manabí** (Pachinche Adentro [1°07'S 80°21'W, ~100m altitude], canton Portoviejo, 68 houses), **El Oro** (Lourdes, canton Portovelo [3°43'S 79°37'W, ~980m altitude], 36 houses), and **Loja** (El Lucero [4°24'S 79°28'W, ~1200m altitude], canton Calvas, 84 houses). These were small rural communities located in areas where the presence of *R. ecuadoriensis* had been previously recorded (Defranc 1982, Racines et al. 1994, Abad-Franch et al. 2001b). According to demographic data obtained from local health officials, ~1100 people lived in Pachinche Adentro (~175 households). Lourdes was a smaller village, with approximately 550 people (~140 households). The population of El Lucero was of ~800 inhabitants (~200 dwellings). In each locality, a representative sample of domiciliary units [DUs] was selected by systematic random sampling (confidence limits >95%) using house numbers and village diagrams by the Vector Control Service. Descriptive statistics include data from all three fieldwork localities and were derived using JMP® 4.0 (SAS Institute 2000).

In our sample, an average of 4.8±2.7 people (median=5, from 1 to 14) lived in each DU (significantly higher in Manabí [6.07±2.78] than in El Oro [3.92±2, up to 8] and Loja [4.08±2.45, up to 13]). The majority of families were originally from their areas of residence (100% in Manabí, 75% in El Oro, and 80% in Loja); immigrants came from the Amazon (one family in Loja) and Guayas (one family in Loja), with five families from Loja living in Lourdes (El Oro). Data on time of residence in their present localities were obtained from 168 families. They had been living in their communities for an average of 34.26±22.1 years (from 1 to 92, median=31.5). Families in Pachinche Adentro (Manabí) had lived there for a significantly (Kruskal-Wallis [KW] X^2=22.1, 2 df, p<0.0001) longer period (45.1±20.5 years, 3 to 75) than those in El Oro (23.03±20, 1 to 75) and Loja (31.8±21.1, 1 to 92).

Years	Families	%
1 to 9	21	12.5
10 to 19	32	19.1
20 to 39	50	29.8
40 to 59	32	19.1
60 or over	33	19.6
Total	168	100

Table 19. Entomological survey in western Ecuador: time of residence (in years) of 168 families at their present communities

5.3.1.1. Current infestation and infection

R. ecuadoriensis bugs were collected inside or around 15 out of the 188 houses surveyed (overall infestation index 8%). No other species of Triatominae were found. Infestation was lower (5.9%) in Manabí (one adult male collected in a house, and peridomestic palm tree colonies in three further DUs) than in El Oro (13.9%) or Loja (7.1%); no statistically significant difference was recorded between infestation rates (univariate logistic regression for infestation by locality yielded a whole model test X^2=1.96, 2 df, p=0.38). Bugs were captured inside 7 these 15 infested households (46.7%); two of them (13.3% of infested dwellings) had only intradomiciliary bugs – in both cases adult bugs invading the house, not breeding colonies. Peridomestic colonies were detected in 13 households (86.7% of infested DUs); these were found in chicken coops in El Oro and Loja (77%) and in peridomestic *Ph. aequatorialis* palm trees in Manabí (23%). Mixed peri- and intradomiciliary infestation was recorded in five dwellings (41.7% of infested households). Six hundred and ninety-one *R. ecuadoriensis* bugs were collected (excluding those from a palm tree located in an uninhabited smallholding in Manabí). The following table summarises these results.

Table 20. Domestic/peridomestic infestation by *Rhodnius ecuadoriensis* in three communities of western Ecuador: entomological indices

Entomological indices	General	Manabí	El Oro	Loja
DUs surveyed/infested (%)	188/15 (8)	68/4 (5.9)	36/5 (13.9)	84/6 (7.1)
DUs infested (breeding colonies)	15 (13)	4 (3*)	5 (4)**	6 (6)
Number of bugs captured	691	10	212	469
Density	3.67	0.15	5.89	5.58
Crowding	40.07	2.5	42.4	78.17
Colonisation index	86.7%	75%	80%	100%

*Breeding colonies were detected in peridomestic palm trees near these three houses; **adult bugs found invading one house, without evidence of colonisation

Results of serological tests for anti-*T. cruzi* antibodies were available from inhabitants of 116 houses in Lourdes and El Lucero (see Section 1.4.2.). Infected people lived in 22 of these houses (19%). The percentages were 15.2% in El Oro (5 out of 33 houses with data) and 20.5% in Loja (17 out of 83 households); the difference was not statistically significant.

5.3.1.2. Socio-economic data

The families were asked about their approximate monthly income; from 144 answers recorded, 65 families (44.1%) declared earning up to 50 US dollars a month, with 79 families (54.9%) earning 50 US$ or over. Overall mean was 54.25±67.6 (median=50

[quartiles 20-70, range 0-750]). Values in US dollars were calculated at the exchange rate at the time of fieldwork (when the Sucre was still the official Ecuadorian currency). The highest mean income was recorded in El Oro (72.2±29.7, median=70, n=23) and the lowest in Loja (37.5±33.3, median=32, n=60), with Manabí in an intermediate position (63.9±94.8, median=50, n=61) (KW X^2=22.86, 2 df, p<0.0001).

Analyses were repeated after excluding one outlier (reported monthly income 750 US$). Data were transformed ($\sqrt{x}$) to improve normality, but Bartlett test detected heteroscedasticity, preventing the use of parametric tests. Welch Anova confirmed the results (F ratio=11.57, 2 df, p<0.0001), and a Tukey-Kramer test showed that the mean income of families from Loja was significantly lower. After the exclusion of the mentioned outlier, a comparison of mean incomes ($\sqrt{x}$) of families (n=143) living in infested vs. non-infested dwellings was performed; variances were equal across groups. Mean income was significantly lower in households with infestation (current [t=-2.4, 141 df, p=0.016]; peridomestic [t=-2.7, 141 df, p=0.007]; reported in present home [t=-2.6, 141 df, p=0.01]; and reported in present or past home [t=-2.2, 141 df, p=0.03]).

The percentages of families earning over 50 US$ (82.6% in El Oro, 60% in Manabí, and just 38.3% in Loja) were also significantly different: likelihood ratio test [LR] X^2=15.4, 2 df, p=0.0005. Unadjusted odds ratios (for percentages of families in the 'under 50US$' group) were: Manabí=1 (reference), El Oro=0.1 (95% CI 0.02-0.49), and Loja=7.1 (95% CI 2.6-21.6). The following table summarises the findings.

Table 21. Entomological survey: monthly income of families in three communities of western Ecuador

Monthly family income (US$)	Families	%
0 to 20	39	27.1
20 to 40	23	16
40 to 60	42	29.2
60 to 80	22	15.3
80 to 100	10	6.9
100 to 150	6	4.2
Over 150	2	1.4
Total	144	100

The father and mother of each family were asked about their level of formal education (as the school years they had completed). We obtained 155 answers from fathers and 174 from mothers. Males from Manabí reported a significantly lower average of schooling than those from El Oro and Loja, as did women from Manabí in

relation to those from El Oro. Linear regression showed that levels of studies of mothers and fathers were positively correlated [studies ♀ = 2.3+0.5x(studies ♂); R^2=0.37].

Table 22. Entomological survey: formal education of family fathers (♂) and mothers (♀)in three communities of western Ecuador

School years completed	♂		♀	
	n	%	n	%
No school (0)	35	22.6	32	18.4
Under primary (1-5)	55	35.5	64	36.8
Primary (6)	51	32.9	64	36.8
Over primary (>6)	14	9	14	8.1
Total	155	100	174	100

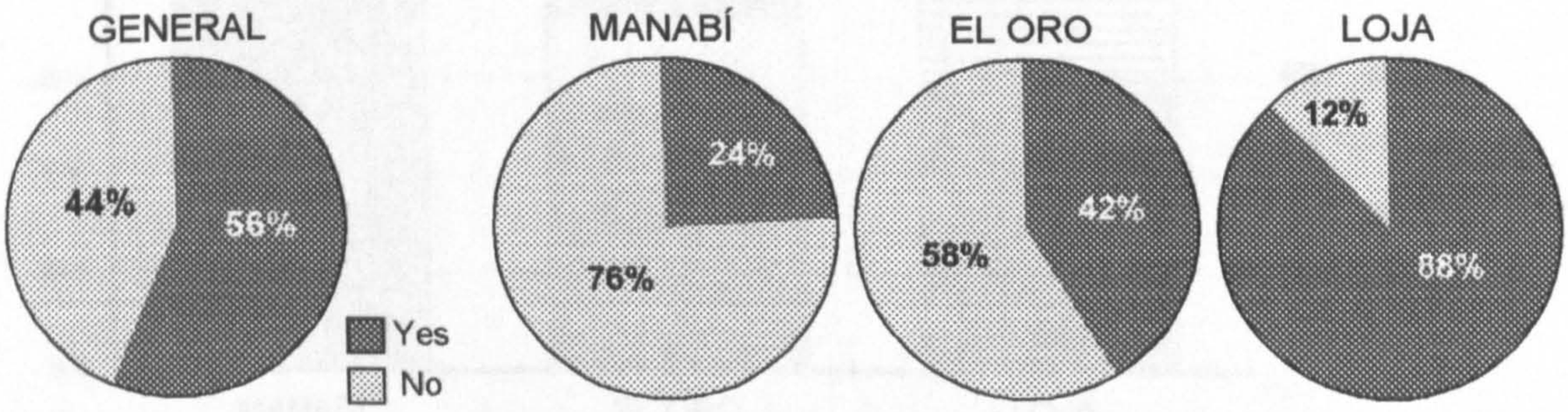
5.3.1.3. Vectors

We showed to the dwellers a collection of preserved triatomine specimens and colour, actual-size pictures of bugs of different species; percentages of people who recognised *R. ecuadoriensis* specimens and other species are presented below.

Table 23 and Figure 40. Entomological survey: people recognising *Rhodnius ecuadoriensis* specimens in three communities of western Ecuador

Recognise *R. ecuadoriensis*?	**General**		**Manabí**		**El Oro**		**Loja**	
	n	%	n	%	n	%	n	%
Yes	105	**55.9**	16	**23.5**	15	**41.7**	74	**88.1**
No	83	44.1	52	76.5	21	58.3	10	11.9
Total	188	100	68	100	36	100	84	100

Likelihood ratio test X^2=73.6, 2 df, p<0.0001; pairwise Fisher's exact test (FET) p: Manabí/El Oro=0.07; Loja/El Oro<0.0001; Loja/Manabí<0.0001



The percentage of people recognising other species was significantly higher in Manabí, where *T. dimidiata* was the only species seen (apart from *R. ecuadoriensis*), than in Loja.

Table 24. Entomological survey: people recognising vector species other than *Rhodnius ecuadoriensis* in three communities of western Ecuador

Recognise other vectors?	**General**		**Manabí**		**El Oro**		**Loja**	
	n	%	n	%	n	%	n	%
Yes	69	**38.5**	37	**54.4**	12	**33.3**	20	**26.7**
No	110	61.5	31	45.6	24	66.7	55	73.3
Total	179	100	68	100	36	100	75	100

Likelihood ratio test X^2=12.1, 2 df, p=0.002; pairwise FET p: Manabí/El Oro=0.06; Loja/El Oro=0.5; Loja/Manabí=0.001

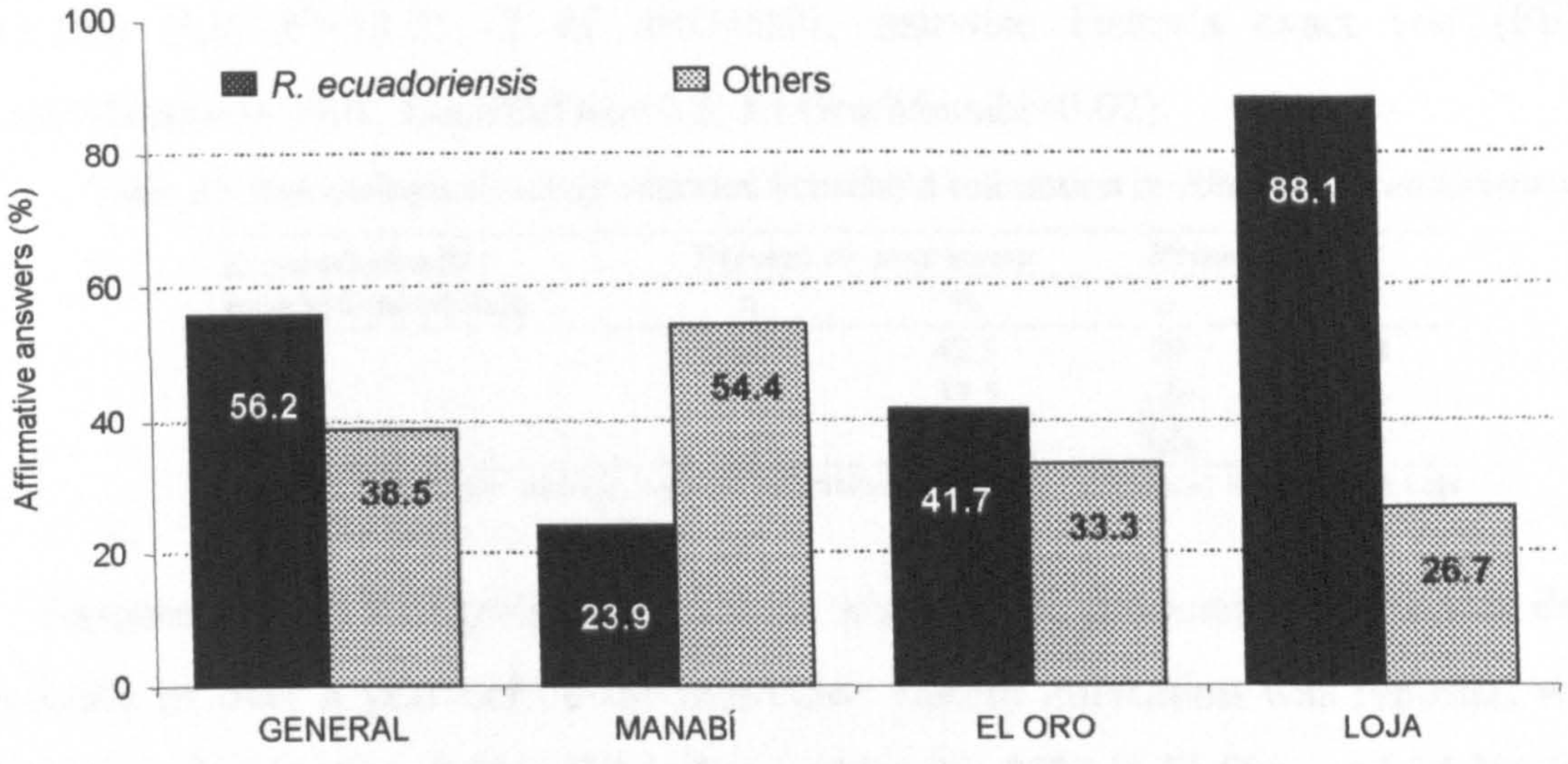


Figure 41. Entomological survey: percentages of affirmative answers indicating *Rhodnius ecuadoriensis* and other vector species

T. dimidiata was recognised by 85% of those giving affirmative answers; *T. carrioni*, *P. rufotuberculatus*, and '*Triatoma*' (without a clear species distinction) were all recorded in about 4% of answers. Finally, *P. chinai* was indicated in 2% of answers. Different patterns of answers were obtained in the three localities studied, as shown in the following figure.

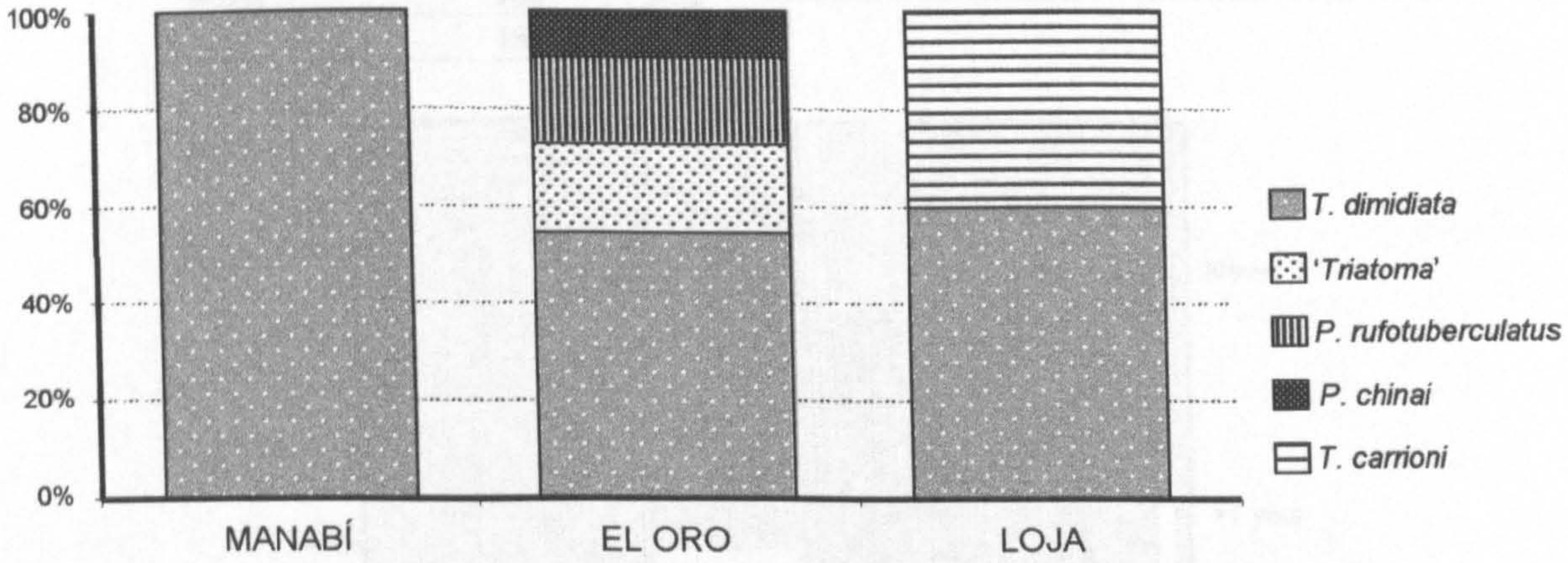


Figure 42. Entomological survey: percentages of answers indicating vector species other than *Rhodnius ecuadoriensis*

Regarding *R. ecuadoriensis*, dwellers were asked if they had seen these bugs in domestic or peridomestic environments (of either their present or past residences). Affirmative answers were recorded more frequently in Loja (65.5%) than in El Oro (36.1%) or Manabí (17.7%) (LR X^2=37.7, 2 df, p<0.0001). Fifty-nine people said they had seen the bugs in their present house (73.8% of all affirmative answers and 31.4% of the total); the percentage was higher in Loja (45.2%) than in El Oro (33.3%) or Manabí

(13.2%) (LR X^2=19.25, 2 df, p<0.0001; pairwise Fisher's exact test (FET) p: Loja/Manabí<0.0001, Loja/El Oro=0.3; El Oro/Manabí=0.02).

Table 25. Entomological survey: reported household infestation by *Rhodnius ecuadoriensis*

***R. ecuadoriensis*: reported infestation**	**Present or past house**		**Present house***	
	n	%	n	%
Yes	80	**42.5**	59	**31.4**
No	108	57.5	129	68.6
Total	188	100	188	100

*This was the variable used (as 'reported infestation') for further analyses on infestation in Loja and El Oro (below)

Responses were categorised to explore whether the presence of bugs was detected recently or over a year before the interview. Recent infestation was reported with the following frequencies: 8.8% of dwellers in Manabí, 25% in El Oro, and 14.3% in Loja; these differences were not statistically significant (LR X^2=4.5, 2 df, p=0.1; pairwise FET significance probabilities were above 0.05 in all comparisons).

Tables 26 and 27. Entomological survey: time since infestation by *Rhodnius ecuadoriensis* was last detected; general answers and results divided into two categories (< or > one year before fieldwork)

How long ago?	**n**	**%**
<6 months	20	10.6
One year	7	3.7
>1 year	53	28.2
Never	108	57.5
Total	188	100

Recent reported infestation	**n**	**%**
Yes (<1 year)	27	14.4
No (>1 year)	164	85.6
Total	188	100

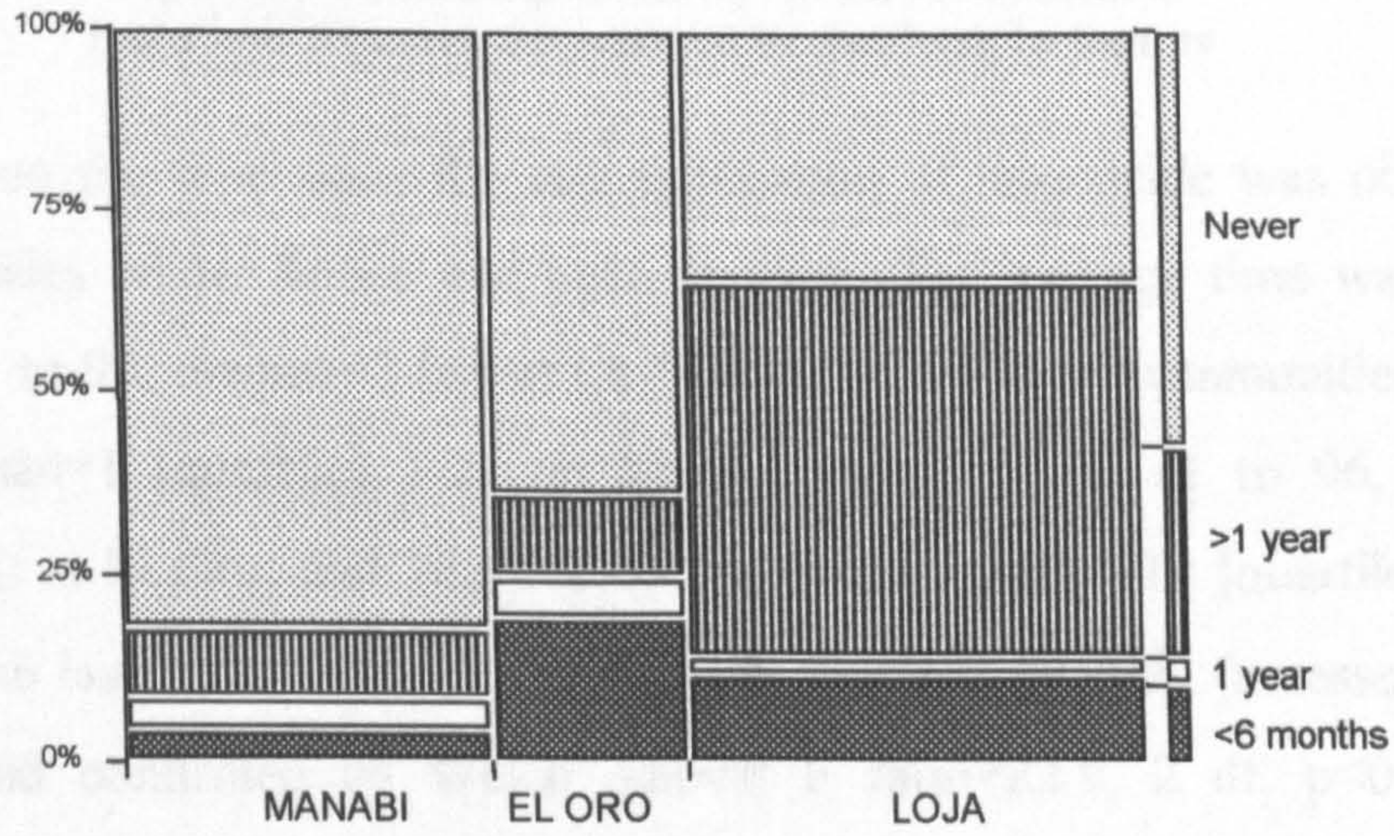


Figure 43. Entomological survey: time since reported infestation (present or past home) by *Rhodnius ecuadoriensis*; column width proportional to the number of answers from each locality; the general percentages by categories are represented in the column on the right

We recorded 83 valid answers indicating the places where *R. ecuadoriensis* bugs were seen. Intradomiciliary environments were mentioned in 55 of these answers (66.3% of the total), and peridomestic habitats in 17 (20.5%). Precise places included beds or bedrooms (mentioned in 24.1% of the answers), chicken coops (14.5%) or

house walls (16.9%). Mud walls were expressly cited in 12 answers (14.5%), whereas only four times was it mentioned that bugs are attracted to electric light (4.8%). Non-domestic environments were referred to in 13 occasions (15.7%).

5.3.1.4. History of household insecticide treatment

Inhabitants participating in the study were asked about the history of insecticide treatment of their dwellings (regardless of the technique, type of insecticide used, time since last treatment or whether the insecticide was applied by the vector control service or by the householder). Affirmative answers were obtained for 155 (83.8%) households (100% in Manabí, 84.2% in Loja, and 51.4% in El Oro; pairwise FET $p<0.001$ in all cases).

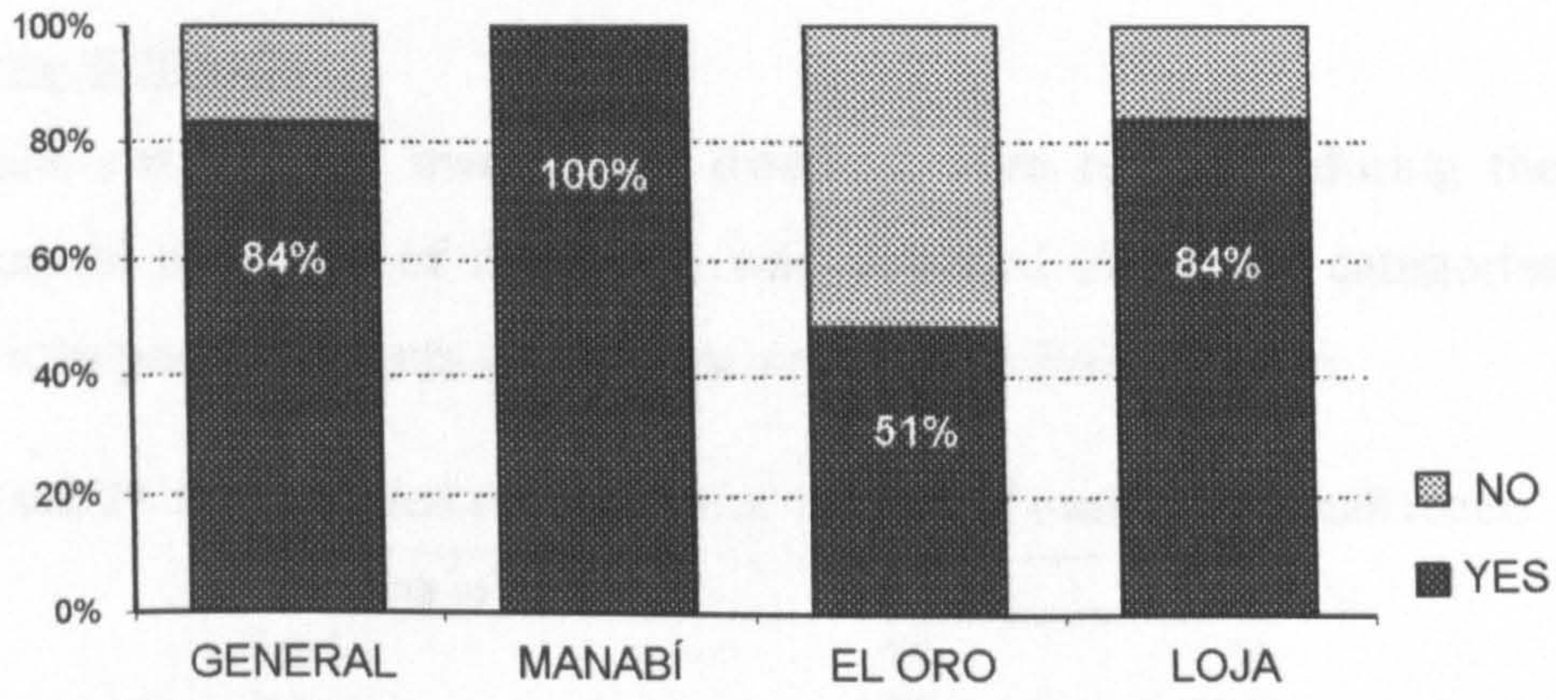


Figure 44. Entomological survey: history of household insecticide treatment (as reported by dwellers), by locality

Information on the time since the last application of insecticide was obtained from 153 of the families whose house had been sprayed. The average time was 9.95±18.2 months (from 1 to 96, median=2 [quartiles 1-12]) for the three communities; 1.28±1.36 (1 to 12, median=1 [quartiles 1-1]) in Manabí; 16.62±31.37 (1 to 96, median=4.5 [quartiles 1-12]) in El Oro; and 16.82±19.65 (1 to 96, median=12 [quartiles 12-12]) in Loja. Time since last treatment was significantly lower in Manabí (assessed by Tukey-Kramer test and confirmed by Welch Anova: F ratio=22.9, 2 df, $p<0.0001$). This variable was categorised into two groups (last spraying under or over 6 months) in order to calculate the percentage of recently treated houses.

Table 28. Entomological survey: percentage of houses recently treated with insecticides. General results

Time since treatment	Houses	%
< 6 months	84	54.9
≥ 6 months	69	45.1
Total	153	100

Houses in Loja (13% in the <6 months category) had been less recently treated than those in El Oro (50%); a significantly higher percentage (98.5%) of houses had been recently sprayed in Manabí (by the malaria control service, using deltamethrin in that area of the country) (LR X^2=124.6, 2 df, p<0.0001). Householders were also asked about the type of insecticide used in their homes; the most frequent from 100 valid answers were malathion (45%), deltamethrin (31%) (both used by the malaria control service in different areas of the country), and DDT (4%). Other responses included various commercial brands of both domestic and agricultural insecticides, diesel (sprayed on timber walls and floors in many parts of Ecuador for insect control) or, in one occasion, burning the aromatic wood of a local tree ('palo santo').

5.3.1.5. Households

Several features of each investigated dwelling were recorded during the surveys. First, the general condition of the house was classified into three categories ('good', 'poor' and 'very poor'). Results are summarised in the following table.

Table 29. Entomological survey: general condition of dwellings. Overall results

Condition of dwelling	n	%
Good	57	31
Poor	95	51.6
Very poor	32	17.4
Total	184	100

These results were further classified into two categories, 'good' and 'poor' (this latter including the two last groups of the former table, i.e. 69%), for analysis. Houses were significantly poorer (LR X^2=22.6, 2 df, p<0.0001) in El Oro, where only one dwelling (2.8%) was found to be in good general condition, while 26 were classified as 'poor' and 9 as 'very poor' (97.2%). In Manabí, 35.3% of houses were in good general condition, with 64.7% being poor. In Loja, these percentages were 40% and 60%, respectively.

Data on time since house construction were available from 159 families. The overall mean was 13.13±10.2 years (ranging from 1 to 60, median=11 [quartiles 5-19]). Dwellings in Manabí were built an average of 10.25±8.3 years ago (from 1 to 40, median=8 [quartiles 4-15]); this mean value was significantly lower (Welch Anova F ratio=5.4, 2 df, p=0.0065) than in El Oro (15.76±14.3, from 1 to 60, median=11 [quartiles 5.25-20]) or Loja (14.84±8.49, from 1 to 47, median=15 [quartiles 10.5-20]).

The sample was divided into two categories ('new' houses built up to 10 years ago, and 'old' houses built 10 years ago or over): 39.7% were classified as new and 60.3% were old. Under this arrangement, the houses in Loja were found to be significantly older than those in Manabí, with the dwellings of El Oro occupying an intermediate position (LR X^2=13.85, 2 df, p=0.001; pairwise FET: Manabí/Loja=0.0004; Manabí/El Oro=0.14; Loja/El Oro=0.15).

From 186 houses with valid data on wall building materials, 76 (40.9%) had walls made mainly of bricks (fired bricks or concrete blocks); 48 dwellings (25.8%) had mud walls (adobe blocks or bahareque – a mud-and-stick composite); 59 dwellings (31.7%) were mainly made of wood planks; and other materials were recorded from 3 dwellings (1.6%). Wall main building materials varied among localities, with timber prevailing in Manabí (77.6% of houses), bricks in El Oro (66.7%), and bricks (50.6%) and mud (49.4%) in Loja. These main materials were found in different combinations in some houses. A summary of results is presented in the following table.

Table 30. Entomological survey: wall building materials. General results

Material	Houses	%
Bricks	54	29
Adobe	33	17.7
Bahareque	9	4.8
Wood planks	48	25.8
Others	3	1.6
Bricks-adobe	5	2.7
Bricks-bahareque	2	1.1
Bricks-wood planks	7	3.8
Bricks-others	7	3.8
Adobe-bahareque	1	0.5
Adobe-others	1	0.5
Bahareque-wood planks	1	0.5
Bahareque-palm leaves	1	0.5
Bahareque-others	1	0.5
Wood planks-cane	2	1.1
Wood planks-others	8	4.3
Brick-wood-cane	1	0.5
Bahareque-cane-others	1	0.5
Wood-cane-others	1	0.5
Total	186	100

Walls made mainly of mud were thus present in 48 houses (25.8%), with a significantly higher proportion in Loja than in El Oro or Manabí. In some dwellings walls were only partially made with mud. The percentage of houses having mud in at

least some part of the walls was also significantly higher in Loja. In general, we found 72.3% of houses to have unplastered walls; this feature was less frequent in Loja. Unplastered mud walls were recorded from 33 houses (17.7%); this percentage was significantly higher in Loja than in Manabí or El Oro. Results are summarised in the following table.

Table 31. Entomological survey: wall building materials

Walls	General		Manabí		El Oro		Loja	
	n	%	n	%	n	%	n	%
Mainly mud	48	25.8	5	**7.5**	2	**5.6**	41	**49.4**
Others	138	74.2	62	92.5	34	94.4	42	50.6
Statistics	Likelihood ratio test X^2=46.35, 2 df, p<0.0001 (n=186)							
Partly mud	55	29.6	5	**7.5**	3	**8.3**	47	**56.6**
Others	131	70.4	62	92.5	33	91.7	36	43.4
Statistics	Likelihood ratio test X^2=56.05, 2 df, p<0.0001 (n=186)							
Unplastered	123	72.3	62	**94**	30	**85.7**	31	**44.9**
Plastered	47	27.7	4	6	5	14.3	38	55.1
Statistics	Likelihood ratio test X^2=46.6, 2 df, p<0.0001 (n=170)							
Unplastered mud	33	17.7	5	**7.5**	1	**2.8**	27	**32.5**
Others	153	82.3	62	92.5	35	97.2	56	67.5
Statistics	Likelihood ratio test X^2=24.5, 2 df, p<0.0001 (n=186)							
Wood planks	59	31.7	52	**77.6**	7	**19.4**	0	**0**
Others	127	68.3	15	22.4	29	80.6	83	100
Statistics	Likelihood ratio test X^2=125.7, 2 df, p<0.0001 (n=186)							

Tile roofs were the most frequently found (46.7% of 170 houses with data), followed by those made of planks of corrugated fibre cement, metal, or concrete (30%), and palm tree leaves (8.3%); other materials were recorded in 15% of dwellings. Eighty-five houses (47.2%) had roofs made at least partially with tiles (none of them in Manabí, where 62.7% of roofs were made of concrete, fibre cement or metal, with 21% made of palm leaves). Tile roofs were the most frequently found in Loja (80.8%) and El Oro (60%; 62.8% had at least a part made of tiles). The percentage of houses with (totally or partially) tiled roofs was marginally higher in Loja than in El Oro (FET p=0.06). Palm leaves were only used for roof thatching in Manabí, with the exception of one dwelling in Loja (with no information on the species of palm used).

Wood planks were used to make the floors of 47.1% of dwellings (85.3% of houses in Manabí, 66.7% of dwellings in El Oro, and only 7.2% of those in Loja); 25% of houses had earthen floors (43.4% of houses in Loja, 22.2% in El Oro, and 4.4% in Manabí); concrete floors (27.3% of the total) were more frequent in Loja (49.4%) than

in El Oro (11.1%) or Manabí (8.8%). Only one house (0.5% of 187 DUs with valid data) had a tiled floor (in Manabí).

The majority of households surveyed had two bedrooms (40.8%); 38.04% had just one bedroom, 15.8% had three, 4.9% four, and 0.5% (one house) had six bedrooms. Houses were in average larger in Manabí (2.18±1.02 bedrooms) than in Loja (1.69±0.82), with houses in El Oro in an intermediate position (1.83±0.71). Crowded conditions were considered to exist if three or more people slept in the same bedroom. This happened in 60.8% of our sample of dwellings, corresponding to 71.6% of families in Manabí, 63.9% in El Oro, and 50.6% in Loja. A significant difference was only recorded between Loja and Manabí (where crowding was more frequent, FET p=0.012).

5.3.1.6. Domestic animals

The presence of domestic animals (mammals and birds) in both intra- and peridomiciliary environments was thoroughly investigated during our surveys. Out of 188 families, 184 had at least one domestic animal (98%), with just four (2%) having no animals at all. No significant differences were recorded among localities.

Regarding birds, 90.9% of families had at least one (mainly chickens) in or around the house (about 10-12 birds/DU in average). Both in Manabí and in El Oro over 97% of families had birds; birds were present in 83% of DUs in Loja (LR X^2=11.4, 2 df, p=0.003). The average number of birds per DU was significantly lower in Loja (KW X^2=16.2, 2 df, p=0.0003; Anova [on $\sqrt{x}$] F ratio=7.6, 2 df, p=0.0007).

Table 32. Entomological survey: presence and abundance of domestic/peridomestic birds in domiciliary units

Birds	Presence*		Mean		StDev		Median		Quartiles		Range	
	Yes	No	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Manabí	66 (97.1)	2 (2.9)	13.6	14	9.8	9.6	10.5	11.5	6-20	6-20	0-50	1-50
El Oro	35 (97.2)	1 (2.8)	14.9	15.3	11.3	11.2	11	12	5-20.75	5-21	0-50	2-50
Loja	69 (83.1)	14 (16.9)	**9.4**	11.4	10.7	10.8	**6**	8	2-13	4-15	0-50	1-50
Total	170 (90.9)	17 (9.1)	12	13.2	10.7	10.5	10	10	4-18	5-20	0-50	1-50

*n (%). Numbers in bold indicate significantly less birds/DU in Loja (KW X^2=16.2, 2 df, p=0.0003); *a*=data from all DUs; *b*=data from DUs with birds (mode=10 for both groups)

Domestic mammals (mainly dogs, and also cats, pigs, guinea pigs, cattle, sheep, etc.) were present in/around 168 DUs (89.4% of 188), in an average quantity of 5.4±6.2 (median=3 [quartiles 2-7], ranging from 0 to 35). The results by locality were as follows (with no significant difference recorded). Manabí: 94% of DUs had mammals (mean=4.4±3.6, median=3 [quartiles 2-6.75], from 0 to 16); El Oro: mammals in 86%

of dwellings (mean=5.2±7.2, median=3 [1-6.75], 0 to 35); and Loja: 87% of DUs had mammals (mean=6.3±7.2, median=4 [2-9], 0 to 32).

Table 33. Entomological survey: presence and abundance of dogs in domiciliary units

Dogs	Presence*		Mean		StDev		Median		Quartiles		Range	
	Yes	No	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Manabí	39 (60)	26 (40)	1.1	1.8	1.2	1.01	1	1	0-2	1-2	0-5	1-5
El Oro	27 (75)	9 (25)	1.3	1.8	1.4	1.3	1	1	0.25-2	1-2	0-6	1-6
Loja	47 (56)	37 (44)	1.1	1.9	1.3	1.1	1	2	0-2	1-2	0-6	1-6
Total	113 (62.1)	72 (38.9)	1.1	1.8	1.2	1.1	1	1	0-2	1-2	0-6	1-6

*n (%). *a*=data from all DUs; *b*=data from DUs with dogs (mode was 1 for all groups)

Almost half (46%) of the families (data from 183 DUs) kept **animals inside domiciles**; the presence of these intradomiciliary animals was found in 34% of houses in Manabí, 36% in El Oro, and 60% in Loja (a significantly higher percentage: LR X^2=11.6, 2 df, p=0.003). The presence of domestic birds inside dwellings was recorded in 9.7% of 186 households with valid data (6% in Manabí, 11%in El Oro, and 12% in Loja). These were mainly chickens (present inside 8% of dwellings), ranging from a single hen in four houses to 29 birds kept in a particular domicile in Loja. A few families kept pigeons (one house), ducks (one house) and other birds (kept as pets, two houses).

Mammals were also present inside a high percentage of households (43.6%), with proportions varying among localities: 32.4% in Manabí, 36% in El Oro, and 56% in Loja (a significantly higher percentage; LR X^2=9.6, 2 df, p=0.008). Dogs were kept indoors in 15% of 185 households with valid data (one dog in 60.7% of them, and two dogs in 39.3%). All domiciles with intradomiciliary dogs were in El Oro (with 14% of houses keeping dogs inside) and Loja (27.4%). Cats were more frequently found indoors: 26.5% of 185 families providing valid answers were found to keep cats (one to five) inside the dwelling (29.2% of families in Manabí, 19.4% in El Oro, and 27.4% in Loja). Guinea pigs (an average of 10.16±7.5, ranging from two to 30 in positive DUs) were present inside 10.7% of dwellings surveyed (data from 185 families). All these houses were in Loja (16.7% of families kept guinea pigs indoors here; 8.07±6.1, from 2 to 20) and El Oro (14%; 16±8.6, from 8 to 30).

The presence of domestic **animals in peridomiciles** was also a frequent feature. Valid data were obtained from 188 households; 173 of these families (92%) kept at least one domestic bird or mammal around the house. These included 100% of DUs in

Manabí, 94.4% in El Oro, and 84.5% in Loja. Peridomestic birds were observed in 85.6% of DUs, including 97.1% in Manabí, 91.7% in El Oro, and 74% in Loja (significantly lower: LR X^2=19.4, 2 df, p<0.0001). One hundred and fifty-seven families (84% of 187 DUs with data) kept chickens around their domiciles, in numbers ranging from one to 50 birds (mean=12.86±10.07; median=10 [quartiles 5-20]). Keeping peridomestic chickens was significantly less frequent in Loja (71% of households) than in Manabí (97%) or El Oro (89%) (LR X^2=21.7, 2 df, p<0.0001). Families from Loja also had fewer chicken (average in positive DUs 11.3±10.7, from 1 to 50, median=8 [quartiles 5-15], n=59) than those from Manabí (13.2±9, 1 to 50, median=10.5 [quartiles 6-20], n=66) or El Oro (15.1±11, 4 to 50, median=12 [quartiles 5.25-22.25], n=32). Other peridomestic birds included pigeons (7% of houses, 5.4±3.6, from 1 to 14; Manabí: 13.2% of houses; El Oro: 8.3%; and Loja 1.3%), ducks (one house in El Oro), and turkeys (four houses in Loja); other birds were kept in five houses (two in Loja and three in El Oro).

Peridomestic mammals were observed in 74% of dwellings. The percentages by localities were 91.2% in Manabí (significantly higher, LR X^2=18.7, 2 df, p<0.0001), 66.7% in El Oro, and 63% in Loja. Peridomestic dogs were present in 47.3% of households, with a significantly lower (LR X^2=16.9, 2 df, p=0.0002) percentage recorded from Loja (31%; bold type in table 34) in comparison with Manabí (59%) or El Oro (64%). Owners of peridomestic dogs (n=89) had between one and six of those animals; a higher average (2.15±1.3, median=2; bold type in table 34) was recorded in Loja, but the differences with Manabí and El Oro were not statistically significant.

Table 34. Entomological survey: presence and abundance of peridomestic dogs in domiciliary units

Peridomestic dogs	Presence*		Mean		StDev		Median		Quartiles		Range	
	Yes	No	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Manabí	40 (58.8)	28 (41.2)	1.04	1.8	1.16	1	1	1	0-2	1-2	0-5	1-5
El Oro	23 (63.9)	13 (36.1)	1.17	1.8	1.4	1.4	1	1	0-1.75	1-2	0-6	1-6
Loja	26 (**31**)	58 (69)	0.7	**2.15**	1.2	1.3	0	**2**	0-1	1-3	0-6	1-6
Total	89 (47.3)	99 (52.7)	0.9	1.9	1.25	1.2	0	1	0-1	1-1.25	0-6	1-6

*n (%). *a*=data from all DUs; *b*=data from DUs with peridomestic dogs

Cats were present in the peridomestic areas of 22% of households, in numbers ranging between 1 and 4 (mean=1.8±1.03, median=1 [quartiles 1-3]). The percentages of families with peridomestic cats in the studied localities were comparable: 25% in Manabí (1.8±1.03, median=1), 30.6% in El Oro (1.64±1.03, median=1), and 20.3% in

Loja (2±1.06, median=2). Pigs were kept in the peridomiciles by 75 families (40% of houses). The percentage was significantly smaller (LR X^2=31.3, 2 df, p<0.0001) in El Oro (5.6%) than in Loja (40.5%) or Manabí (57.4%). The average number of pigs in those houses was also smaller in El Oro (2 houses with one pig each) than in Manabí (2.41±2.56, median=2 [quartiles 1-3], from 1 to 15) or Loja (4.73±3.32, median=3.5 [quartiles 2-8], 1 to 12); the general average was 3.43±3.13 (median=2 [quartiles 1-4]), ranging from 1 to 15. Guinea pigs were also present in some peridomiciles. Seven families (3.7%) had these animals around the house (average number 6.9±2.7, median=6 [5-8], from 4 to 12); peridomestic guinea pigs were kept by one family in Manabí (1.5%, five guinea pigs), two in El Oro (5.6%, 9±4.24, six in one house and 12 in the other), and four in Loja (4.8%, 6.25±2.06, from 4 to 8). Finally, the presence of other domestic mammals in peridomiciles (including for instance sheep, donkeys, horses, goats, cows, rabbits, etc.) was also investigated. They were present in 19.7% of houses (in an average number of 2.8±3.04, median=1 [quartiles 1-3], from 1 to 15), with percentages of 35.3% in Manabí (2.2±2.26, median=1, 1 to 9), 5.6% in El Oro (2±1.4, median=2, 1 to 3), and 13.1% in Loja (4.3±4.2, median=3, from 1 to 15).

5.3.1.7. Product storage

The investigation of households also involved gathering data about storage of agricultural products, firewood, building materials, etc. in both intra- and peridomestic environments. Out of 156 family questionnaires with valid information, 58 (37.2%) indicated the families stored these products indoors (31.7% in kitchens, 26.7% in storerooms, 16.7% in bedrooms, and 25% in other rooms). This percentage was lower (LR X^2=11.9, 2 df, p=0.003) in Manabí (19.2%) than in El Oro (41.2%) or Loja (48.6%). A higher proportion of families stored products around their houses (70.5% of 173 DUs with data), with a lower (LR X^2=16.7, 2 df, p=0.0002) percentage in Loja (53.5%) than in Manabí (81.8%) or El Oro (83.3%). Places for storage of these products included sheds or storerooms built on purpose in less that 20% of responses.

5.3.1.8. Knowledge, attitudes and practices (KAP) in relation to Chagas disease, its vectors, and putative conditions favouring triatomine infestation and disease transmission

A brief questionnaire was designed for this part of the study. As with the questions discussed so far, not all answers were available in all cases, either because the person being interviewed refused to answer some of the questions, because she or he could not give a clear answer, or occasionally because the answer was not accurately recorded.

The questionnaire was preferably answered by one adult in each of the 188 households surveyed. Family mothers were generally preferred (70% of the people interviewed were women), as they are largely in charge of the management of domestic and peridomestic environments in rural Ecuador. Data regarding age, formal education, and occupation of those responding were obtained at the beginning of the interview. Average age was 47.5±17.5 years, ranging from 15 to 92 years (174 answers). Results were comparable in Manabí (average 47±16.3 years; 68 answers), El Oro (44±18.2; 31 answers), and Loja (49.4±18.25; 75 answers). Regarding formal education, an average of 4.6±3.17 school years completed (from 0 to 13) was reported in 169 answers obtained. In Manabí, the reported average was significantly lower (KW X^2=13.9, 2 df, 0.001): Manabí, 3.56±2.7 school years, from 0 to 12; El Oro 5.97±3.5, 0 to 12; and Loja (5.01±3.18, 0 to 13). From 171 answers on occupation, ~56% of people interviewed were housewives, with agriculture/farming as the second main job.

Table 35. Occupation reported by dwellers in KAP questionnaires

Occupation	n	%
Housework	96	56.1
Agriculture/farming	45	26.3
None/jobless	19	11.1
Others	11	6.4
Total	171	100

·Knowledge. First, we asked whether people being interviewed had ever heard about a condition called 'Chagas disease'. Only 13.8% (26 out of 188 answers) gave an affirmative response. The percentages were 19.1% in Manabí (13 out of 68), 25% in El Oro (9 out of 36), and just 4.8% in Loja (4 out of 84, a significantly lower percentage: LR X^2=12.1, 2 df, p=0.0024).

Secondly, we asked about disease transmission. The percentages of positive answers were also low: 8.3% in the entire group, 14.9% in Manabí, 8.3% in El Oro, and 1.5% in

Loja (where 66 valid answers were obtained). This latter percentage was significantly smaller than that of Manabí (LR X^2=9.1, 2 df, p=0.01; FET Loja/Manabí: p=0.009); significant p values were not obtained in other comparisons.

Questions about triatomine vectors began by finding out whether the person being interviewed knew the bugs (both adult bugs and nymphal instars). Interviewers were allowed to 'help' with additional information – on the contrary, when the question was to find out whether people recognised the bugs (above) specimens and pictures were simply shown to the head of family. Results regarding knowledge of vectors (adults and nymphs) are summarised in the following tables and figures.

Table 36. KAP study: knowledge of adult triatomines

Adult vectors	General		Manabí		El Oro		Loja	
	n	%	n	%	n	%	n	%
Yes	136	**72.7**	46	**67.7**	16	**44.4**	74	**89.2**
No	51	27.3	22	32.3	20	55.6	9	10.8
Total	187	100	68	100	36	100	83	100

FET p values: 0.002 (Loja/Manabí), 0.02 (El Oro/Manabí) and <0.0001 (Loja/El Oro). LR X^2=27.1, 2 df, p<0.0001

Table 37. KAP study: knowledge of immature triatomines

Immature vectors	General		Manabí		El Oro		Loja	
	n	%	n	%	n	%	n	%
Yes	79	**42.7**	13	**19.7**	6	**16.7**	60	**72.3**
No	106	57.3	53	80.3	30	83.3	23	27.7
Total	185	100	66	100	36	100	83	100

Knowledge of nymphs was significantly better in Loja than in Manabí and El Oro (LR X^2=56.6, 2 df, p<0.0001), which were comparable. In all three localities, as well as in the general sample (FET p<0.0001), a significantly higher percentage of people knowing adult triatomine bugs did also know the nymphs.

Next we asked if these bugs may cause any nuisance to people, without mentioning disease at this point so that we could possibly get a more general idea bout what people think of and know about the bugs. Differences between localities in percentages of affirmative responses were statistically significant (LR X^2=21.2, p<0.0001): they were more frequent in Loja and only accounted for 26.5% of answers in El Oro, with Manabí in an intermediate position (pairwise FET: Loja/El Oro p<0.0001; Manabí/El Oro p=0.006; Loja/Manabí p=0.053).

Table 38. KAP study: "Do triatomines cause any nuisance?"

Do vectors cause any nuisance?	General		Manabí		El Oro		Loja	
	n	%	n	%	n	%	n	%
Yes	102	**57.6**	37	**56.1**	9	**26.5**	56	**72.7**
No	75	42.4	29	43.9	25	73.5	21	27.3
Total	177	100	66	100	34	100	77	100

After this, we specifically asked about the possibility of becoming infected or getting any disease from bug bites. A high percentage of affirmative responses was obtained (74.7%); the percentage was significantly smaller (LR X^2=7.55, 2 df, p=0.02) in Loja than in Manabí or El Oro (FET: Loja/Manabí p=0.04; Loja/El Oro p=0.03); results were comparable in these two latter localities (FET p=0.6). Examples of conditions thought to be caused by bug bites included skin problems (most frequent, including itch, ulcers, infection, inflammation, etc), anaemia, fever, and heart disease (percentages not recorded).

Table 39. KAP study: "Can bug bites transmit any disease?"

Can bug bites transmit diseases?	General		Manabí		El Oro		Loja	
	n	%	n	%	n	%	n	%
Yes	136	**74.7**	54	**80.6**	30	**85.7**	52	**65**
No	46	25.3	13	19.4	5	14.3	28	35
Total	182	100	67	100	35	100	80	100

Finally, the knowledge about vectors was further investigated by exploring what people knew about the habits of the bugs. Some additional questions were asked when necessary to confirm correct answers and reduce false-positive results. With regard to the ecotopes and hiding places of triatomines, answers considered correct included wall crevices, mud walls, palm trees, beds, spaces behind objects on walls (including wallpaper) or furniture, chicken coops, guinea pig pens, etc. The percentage of correct answers was significantly higher in Loja (LR X^2=14.9, 2 df, p=0.0006; pairwise FET: Loja/Manabí p=0.0004; Loja/El Oro p=0.013), with comparable results obtained in El Oro and Manabí (FET p=0.7).

Table 40. KAP study: "Do you know where the bugs live?"

Where the bugs live?	General		Manabi		El Oro		Loja	
	n	%	n	%	n	%	n	%
Correct	116	**64.1**	34	**50.7**	20	**55.6**	62	**79.5**
Wrong	65	35.9	33	49.3	16	44.4	16	20.5
Total	181	100	67	100	36	100	78	100

Regarding triatomine feeding habits, only responses stating bugs eat blood were considered to be correct. These were recorded more frequently in Loja, and represented <30% in Manabí. Differences were statistically significant.

Table 41. KAP study: "Do you know what bugs feed on?"

Triatomine feeding habits	General		Manabí		El Oro		Loja	
	n	%	n	%	n	%	n	%
Correct	99	**54.7**	18	**28.9**	18	**51.4**	63	**79.7**
Wrong	82	45.3	49	73.1	17	48.6	16	20.3
Total	181	100	67	100	35	100	79	100

Pairwise FET: Loja/Manabí p<0.0001; Loja/El Oro p=0.003; El Oro/Manabí p=0.02. LR X^2=43.2, 2 df, p<0.0001

A further comparison was performed taking into account four responses related to adults, nymphs, ecotopes and hiding places, and feeding habits of the bugs. Correct answers to different questions were assigned different weight in an attempt to reflect the accuracy of knowledge. Knowledge of adult bugs was assigned a value of 2; knowledge of nymphs was considered to reflect a considerably better level of information, so it was assigned a value of 3; a correct answer to the question of where bugs live was considered to have a lesser value (1) (other pest arthropods [cockroaches, bedbugs, chicken lice, mites, ticks] may share some ecotopes with triatomines); finally, a correct answer on blood-sucking was considered to reflect factual knowledge about bugs, and was assigned a value of 4. Wrong and missing (18 of 752 possible) answers were rated 0. Mean 'quality of knowledge' score was significantly (KW X^2=57.5, 2 df, p<0.0001) higher in Loja (7.64±3.06, median=9), with Manabí (3.46±2.72, median=3) and El Oro (3.94±3.43, median=4) having comparable results. The mean score value for whole sample was 5.4±3.61 (median=6).

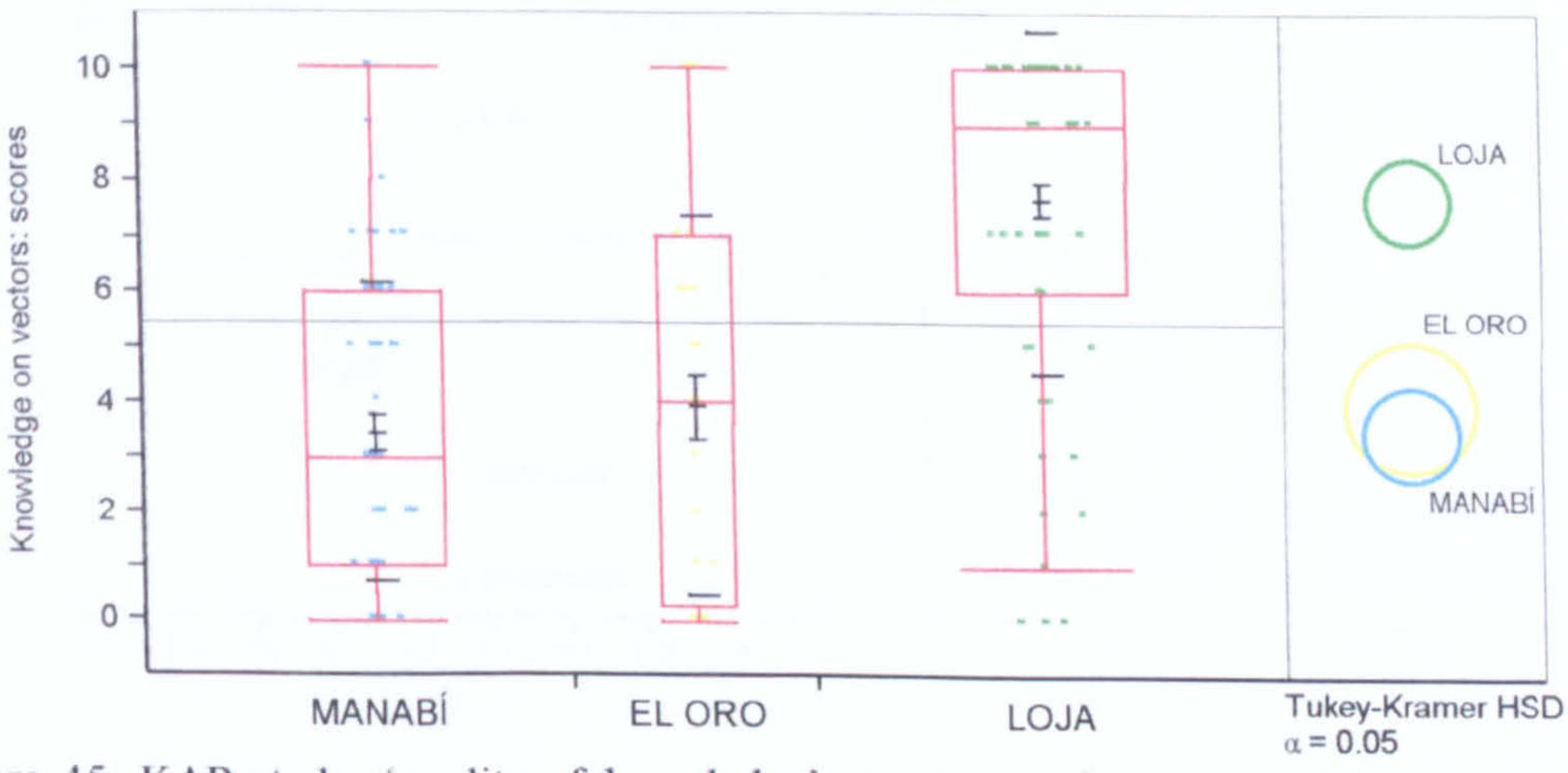


Figure 45. KAP study: 'quality of knowledge' on vectors. Scores combining answers to four questions (by locality); boxplots are quantile plots (showing median and 10%, 25%, 75% and 90% quantiles); short lines in each column indicate mean, standard errors and standard deviations

·Attitudes. The attitudes of inhabitants towards the presence of triatomine bugs in both intradomiciliary and peridomestic environments were also explored. Thus, the person being interviewed was asked how she or he felt (or would feel) about suffering intra- and peridomiciliary infestation by triatomine bugs. Responses were categorised into four groups (indifferent, minor nuisance, major nuisance, and intolerable); under this arrangement, results showed what appears to be a significantly higher degree of tolerance for intradomiciliary bugs (LR X^2=13.8, 2 df, p=0.03) in Loja, with comparable percentages in Manabí and El Oro. Tolerance towards peridomestic infestation was more important; it was lower in Manabí than in Loja and El Oro (LR X^2=35.7, 2 df, p<0.0001). Correspondence analysis plots were produced to explore these differences in tolerance.

Table 42. KAP study: attitudes towards intradomiciliary and peridomestic infestation

Attitudes: intradomiciliary vectors	**General**		**Manabí**		**El Oro**		**Loja**	
	n	%	n	%	n	%	n	%
Indifferent	15	8.2	4	5.9	1	2.8	10	12.8
Minor nuisance	39	21.4	13	19.1	7	19.4	19	24.4
Major nuisance	85	46.7	27	39.7	19	52.8	39	50
Intolerable	43	23.6	24	35.3	9	25	10	12.8
Total	182	100	68	100	36	100	78	100

Attitudes: peridomestic vectors	**General**		**Manabí**		**El Oro**		**Loja**	
	n	%	n	%	n	%	n	%
Indifferent	58	32.2	16	23.5	13	36.1	29	38.2
Minor nuisance	43	23.9	19	27.9	6	16.7	18	23.7
Major nuisance	51	28.3	10	14.7	15	41.6	26	34.2
Intolerable	28	15.6	23	33.8	2	15.6	3	3.9
Total	180	100	68	100	36	100	76	100

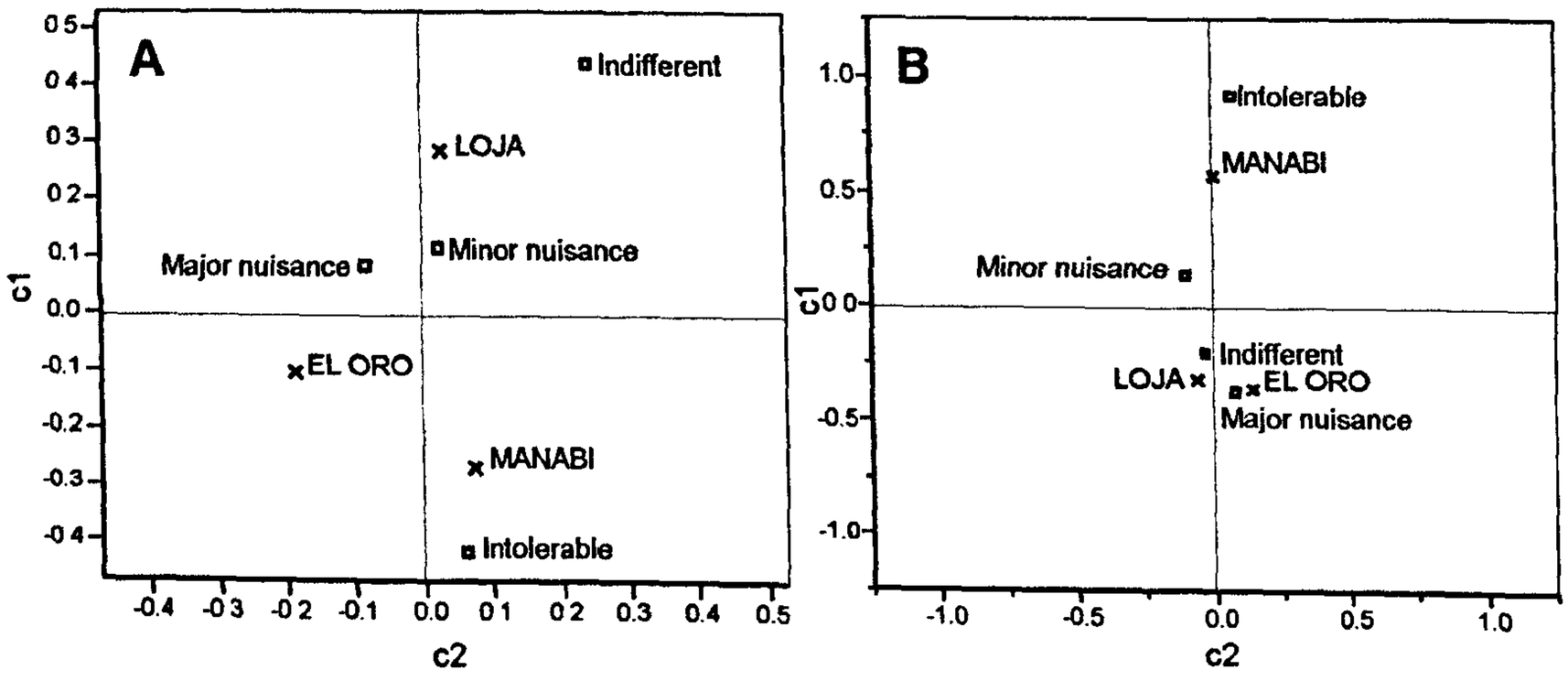


Figure 46. KAP study: attitudes towards intradomiciliary (**A**) and peridomestic (**B**) infestation by triatomines: correspondence analysis

·Practices. We started by asking whether the householder took any action *specifically* against triatomines, including insecticide spraying or any other possible intervention. We recorded 188 valid responses, 101 of which were affirmative (53.7%). This percentage did not vary significantly in the different localities (55.9% in Manabí, 50% in El Oro, and 53.6% in Loja). The following table summarises these results.

Table 43. KAP study: practices. Antivectorial action by householders

Action against vectors	General		Manabí		El Oro		Loja	
	n	%	n	%	n	%	n	%
Yes	101	**53.7**	38	**55.9**	18	**50**	45	**53.6**
No	87	46.3	30	44.1	18	50	39	46.4
Total	188	100	68	100	36	100	84	100

We then specifically asked about indoor insecticide spraying as an action taken against triatomine bugs *in particular*. Affirmative responses were obtained from 40 dwellers (21.3%); statistically significant differences between localities were recorded (LR X^2=7.03, p=0.03; FET: Loja/El Oro p=0.036, Loja/Manabí p=0.06, El Oro/Manabí p=0.06). Three people who affirmed they used insecticides lived in houses where data from the general interview indicated no history of insecticide spraying.

Table 44. KAP study: practices. Insecticide spraying against triatomines (two categories)

Insecticide against bugs	General		Manabí		El Oro		Loja	
	n	%	n	%	n	%	n	%
Yes	40	**21.3**	11	**16.2**	4	**11.1**	25	**29.8**
No	148	78.7	57	83.8	32	88.9	59	70.2
Total	188	100	68	100	36	100	84	100

Table 45. KAP study: practices. Use of insecticides against triatomines

Insecticide spraying	General		Manabí		El Oro		Loja	
	n	%	n	%	n	%	n	%
Regularly	18	9.6	11	16.2	1	2.8	6	7.2
Only if heavy infestation	22	11.7	0	0	3	8.3	19	22.6
Never	148	78.7	57	83.8	32	88.9	59	70.2
Total	188	100	68	100	36	100	84	100

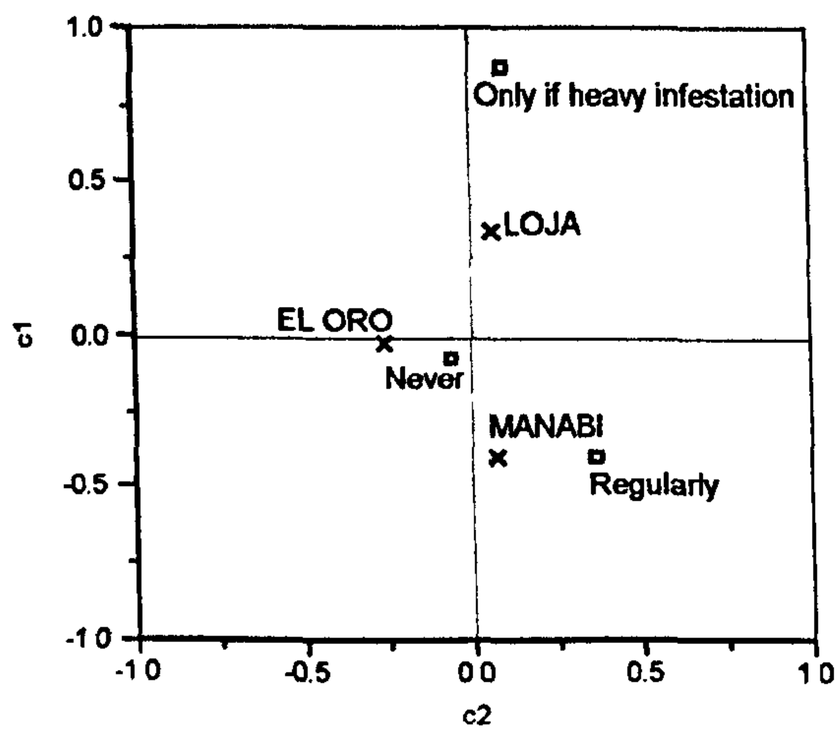


Figure 47. KAP study: practices. Use of insecticides against triatomines: correspondence analysis

Other initiatives were taken against triatomines in the studied localities. Thus, 15.4% of people indicated that they also kill individual bugs if they happen to see them in or around the house (22.1% in Manabí, 22.2% in El Oro and just 7.1% in Loja – a significantly smaller percentage). Special house cleaning activities (explicitly linked to the idea of avoiding the risk of infestation) were mentioned in 10% of the interviews (16.2% in Manabí, 8.3% in El Oro, and 6% in Loja). Other actions (spreading boiling water on walls, painting, removing wallpaper, etc.) were mentioned by 22% of the people being interviewed. Only 12.8% of those interviewed affirmed they use bednets. The percentage was higher in El Oro (22.2%), with 16.2% in Manabí and only 6% in Loja (a significantly smaller percentage compared to El Oro).

The dwellers were asked whether they let their domestic animals spend the night indoors. Affirmative responses were obtained from 68 people (36.2%). The percentage was higher in Loja (44.1%) than in Manabí (29.4%) or El Oro (30.6%); the differences were not significant. Only in 4.3% of households were domestic animals allowed to sleep inside the bedrooms (El Oro: 5.6%; Loja: 4.9%; Manabí: 2.9%). Asked about why they keep their domestic animals indoors, the dwellers mentioned rodent control in 36.4% of 66 valid answers. Habit was mentioned in a similar percentage of answers (34.8%), and was followed in frequency by the necessity of protecting the animals from predators and thieves (24.2%) and the use of dogs to guard the properties (7.6%).

The habits of domestic hygiene were also included in the survey. We asked the dwellers how often they performed in-depth cleaning of their houses; additionally, we posed the same question regarding the places where domestic animals are kept (including chicken houses, corrals, pigsties etc.). Responses obtained are summarised in the following tables.

Table 46. KAP study: practices. Domestic hygiene: frequency of in-depth house cleaning

Domestic hygiene: house cleaning	**General**		**Manabí**		**El Oro**		**Loja**	
	n	%	n	%	n	%	n	%
Weekly	70	44.9	40	59.7	6	17.7	24	43.6
Fortnightly	21	13.5	12	17.9	6	17.7	3	5.5
Monthly	29	18.6	8	11.9	10	29.4	11	20
Each two months	11	7.1	5	7.5	4	11.8	2	3.6
Each three months	9	5.8	1	1.55	2	5.9	6	10.9
Each six months	5	3.2	1	1.5	2	5.9	2	3.6
Once a year	11	7.1	0	0	4	11.8	7	12.7
Total	156	100	67	100	34	100	55	100

Table 47. KAP study: practices. Domestic hygiene: frequency of in-depth cleaning of domestic animal places

Domestic hygiene: cleaning of animal places	General		Manabí		El Oro		Loja	
	n	%	n	%	n	%	n	%
Each month	53	63.1	16	69.6	21	61.8	16	59.3
Each two months	6	7.1	2	8.7	3	8.8	1	3.7
Each three months	5	6	0	0	4	11.8	1	3.7
Each six months	2	2.4	0	0	1	2.9	1	3.7
Once a year	1	1.2	0	0	0	0	1	3.7
Never	17	20.2	5	21.7	5	14.7	7	25.9
Total	84	100	23	100	34	100	27	100

Finally, we included a series of questions about peridomestic palm trees. These could only be found in our study locality in Manabí, where *Ph. aequatorialis* are common near dwellings; thus, the analyses presented below refer only to Pachinche Adentro. First we asked about the reasons why palm trees are kept in the vicinity of houses. Eighteen answers were obtained, 60% of which referred to the use of palm leaves for roof thatching. Commerce of leaves and seeds was adduced by 10% of people responding, and one person (5%) said the palms were part of the fence surrounding his property. Finally, 15% of dwellers were not the owners of the plot, so the palms belonged to the landlord. Two households in El Oro had peridomestic coconut palms (*Cocos nucifera*) that were kept just because they were considered to be beautiful. In Manabí, 46 (67.7%) householders used palm tree leaves for some purpose. The uses given to the leaves are summarised in the following table.

Table 48. KAP study: practices. Use of palm tree leaves in Manabí

Use of palm leaves	Answers	%
Roof thatching	40	87
Roof and chicken coops	1	2.2
Roof and corrals	3	6.5
Roof and others	1	2.2
Roof, coops, corral, firewood	1	2.2
Total	46	100

Tagua nuts were said to be used by 17.7% of people interviewed in Manabí. The majority of answers about the uses of these seeds referred to food (the unripe tagua fruits have edible parts) and commerce (mainly in the vegetable ivory handicraft market). Both uses were combined in 41.7% of answers, with 33.3% referring only to food, and the rest (25%) only to commerce. No other palm products (stipes, fibre, etc.) were used by the householders participating in our study.

5.3.2. UNIVARIATE STATISTICS

As stated above, only results from the study localities in Loja (El Lucero, 84 DUs surveyed) and El Oro (Lourdes, 36 DUs) are included in these analyses. Palm tree-living colonies of *R. ecuadoriensis* detected in peridomiciles of Pachinche Adentro (Manabí) were certainly independent of any of the variables studied in this survey on the ecology of synanthropic bug populations. Data on infestation of *Phytelephas* palms of Pachinche Adentro were included in the study on the ecology of sylvatic populations.

5.3.2.1. Infestation

Only in one house (El Oro) with no peridomestic colonies were bugs found within the domicile; these were two adult bugs (with no evidence of a breeding colony), and the dwelling was located near other infested houses. On the other hand, 5 houses (50% of those infested) had only peridomestic colonies, and 5 more (50%) had both. Only one of the dwellers whose house presented infestation (in peridomestic chicken coops) reported not having seen the bugs in the past; thus, reported infestation (of the present domicile) was strongly correlated with current infestation (after FET and effect likelihood ratio test*) and peridomestic infestation; these latter figures were the same for DUs where bug breeding colonies were detected (i.e., all peridomestic colonies were *breeding* colonies, and no signs of true colonisation were detected in the household where only intradomiciliary bugs were found). All those living in dwellings with intradomiciliary bug colonies did report having seen the bugs (FET p=0.004).

Table 49. Entomological survey: univariate analyses.
Actual infestation by reported infestation (present home) by *Rhodnius ecuadoriensis*

Count Column % Row %		Current infestation		Total %	Peridomestic infestation*		Total %
		Yes	No		Yes	No	
Reported Infestation	Yes	10 **90.9** 20	40 **36.7** 80	50 41.7	9 **90** 18	41 **37.3** 82	50 41.7
	No	1 9.1 1.4	69 63.3 98.6	70 58.3	1 10 1.4	69 62.7 98.6	70 58.3
Total **%**		11 9.2	109 90.8	120 100	10 8.33	110 91.67	120 100
FET p / LR X^2 (p)		0.0007 / 13.01 (0.0003)			0.0016 / 11.2 (0.0008)		

Percentages of infested and non-infested households whose owners reported having seen the bugs in their homes are in bold type; *same figures as for colonisation (dwellings where breeding bug colonies were detected)

*In univariate logistic regression, the effect likelihood ratio test is equivalent to the whole model test, in which variations in deviance (differences in –log-likelihood) caused by the deletion of all covariates (just one in univariate analyses) except the intercept are assessed (i.e., the full and reduced models are compared)

In other words, report of infestation by dwellers allowed for the correct classification of 65.8% of DUs as infested/non-infested, with 10 true-positives, 69 true-negatives, 40 false-positives, and just one false-negative (i.e., ten out of 11 actually infested DUs [91%] were correctly classified). Report of recent (<1 year) infestation yielded 85.8% correctly classified DUs (FET p=0.0003), with 7 true-positives (reportedly and currently infested), 96 true-negatives, 13 false-positives (reported as infested but found negative after active searches) and four false-negatives (dwellers said not to have seen the bugs in these DUs in the year before fieldwork, but active searches yielded positive results).

5.3.2.2. Socio-economic data

The mean number of people living in currently infested and non-infested homes was comparable; the same was observed between houses with/without reported infestation. No significant correlation was found between current infestation and the origin of families or the number of years they had been living in their present communities. However, families in houses with reported infestation had in average been living longer (34.1±21 years, median=29 [quartiles 14.5-53.5], ranging from 5 to 72) than those in houses without reported infestation (26.1±20.9 years, median=20 [10-38.5], 1 to 92) in their present communities (t=2.3, 114 df, p=0.023 [t-test performed on $\sqrt{x}$ to improve normality]).

The average monthly income of families living in currently infested households was 20.75±22.5 US$ (median=14.5 [quartiles 3.875-37.5], from 0.5 to 60) (data from six families), whilst it was 49.2±35.86 US$ (median=50 [20-80], 0 to 150) in non-infested domiciles (n=77) [general mean=47.1±35.7, median=50 (quartiles 20-80)]. Differences between these values were marginally non-significant (Wilcoxon test [WT] X^2=3.2, 1 df, p=0.075), but univariate logistic regression showed a stronger negative correlation: uOR=0.01 (95% CI 0.00002-0.82) and LR X^2=4.26 (1 df, p=0.04) (figure 48 A). When comparing the incomes of families living in houses with (mean=37±31.8; median=30 [quartiles 5-60], 0 to 100) and without (mean=53.5±37, median=50 [quartiles 20-80], 0 to 150) reported infestation, WT similarly showed a weak negative correlation (X^2=3.7, 1 df, p=0.053). Univariate logistic regression yielded results (uOR=0.12 [95% CI 0.014-0.86]; LR test X^2=4.45 [1 df, p=0.035]) suggesting that family income may in fact be negatively correlated with the likelihood of reporting infestation (figure 48 B).

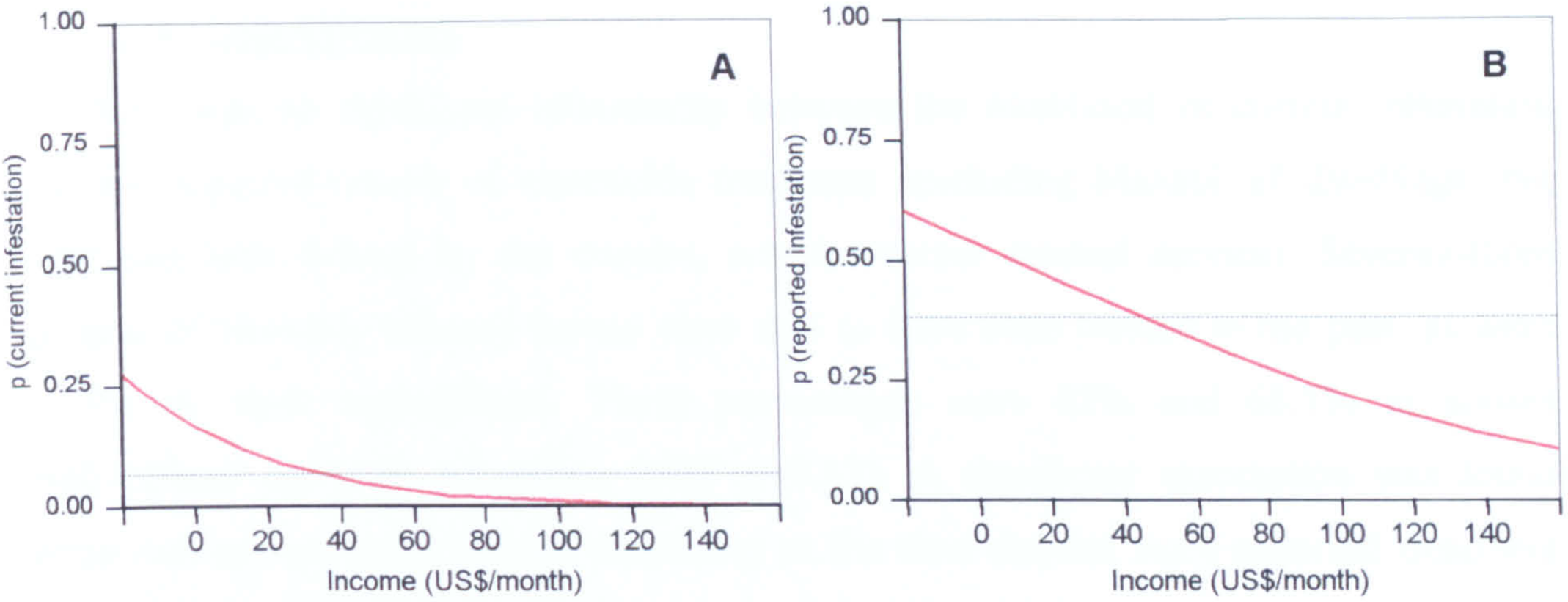


Figure 48. Entomological survey: univariate logistic regression. Probability of current (**A**) and reported (**B**) household infestation by *Rhodnius ecuadoriensis* in relation to the approximate (crude) monthly income of families (as reported by the head of each family); dotted grey lines represent the observed average probabilities; n=83

No significant differences in the levels of formal education of fathers (data from 94 men) and mothers (data from 108 women) in families living in currently infested or non-infested dwellings were detected. However, the likelihood of current DU infestation slightly decreased with increasing education levels of family mothers (but not fathers). Analysis of reported infestation also revealed a weak negative correlation – this time for both sexes. The education levels of both fathers and mothers were positively correlated with the monthly family income (F ratios 25.3 and 21.2, respectively [1 df, $p<0.0001$]).

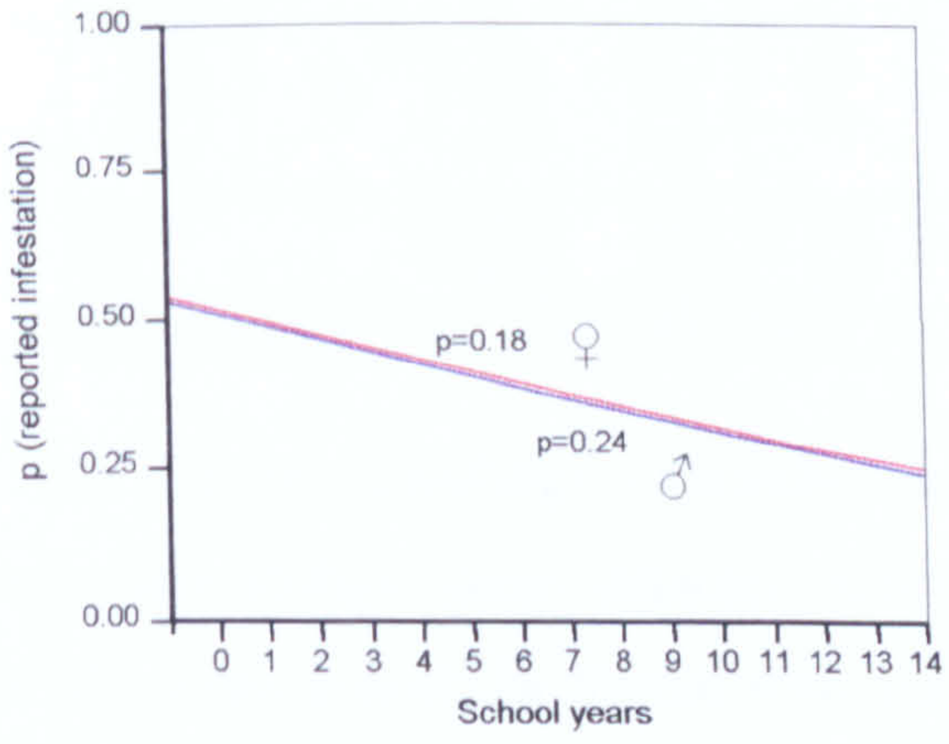


Figure 49. Entomological survey: univariate analyses. Probability of reported infestation as a function of levels of formal education of family mothers (♀) and fathers (♂). Significance probability values after likelihood ratio tests

5.3.2.3. Vectors

People living in households found to be infested during our survey were more likely to recognise *R. ecuadoriensis* specimens (91% recognised the bugs) than those living in non-infested homes (72.5%), but the difference was not significant (FET p=0.3). No significant difference was recorded when comparing households whose owners recognised other vector species (FET p=0.7).

5.3.2.4. Insecticides

There was no significant relationship between the likelihood of current infestation and the reported history of insecticide treatment (excluding Manabí, all dwellings [but one] had been treated by the owners, not the vector control service). Seventy-three percent of currently infested homes were said to have been treated in the past, as were 74.5% of those non-infested. These percentages were 82% and 68.7% in houses with/without reported infestation (FET p=0.13). A significant association was found when two groups were defined according to the time elapsed since reported treatment (under or over 6 months); 62.5% of currently infested dwellings were said to have been sprayed in the preceding 6 months, versus only 15.6% of non-infested domiciles (uOR=9.03 95% CI 1.96-49.02; n=85). All four houses with intradomiciliary infestation and data on recent insecticide treatment were said to have been treated by the owners in the six months before fieldwork, vs.16% of those without indoors bugs (FET p=0.0012).

Table 50. Entomological survey: univariate analyses.
Infestation by recent insecticide spraying (as reported by dwellers)

Count Column % Row %		Reported infestation		Total %	Current infestation		Total %	Recent reported infestation (<12 months)		Total %
		Yes	No		Yes	No		Yes	No	
Recently sprayed (<6 months)	Yes	8 **19.5** 47.1	9 **20.5** 52.9	17 20	5 **62.5** 29.4	12 **15.6** 70.6	17 20	5 **38.5** 62.5	3 **10.7** 37.5	8 19.5
	No	33 80.5 48.5	35 79.5 51.5	68 80	3 37.5 4.4	65 84.4 95.6	68 80	8 61.5 24.2	25 89.3 75.8	33 80.5
Total **%**		41 48.2	44 51.8	85 100	8 9.4	77 90.6	85 100	13 31.7	28 68.3	41 100
FET p / LR X^2 (p)		0.91 / 0.12 (0.9)			0.007 / 7.8 (0.005)			0.08 / 4.1 (0.04)		

The only two dwellings reportedly treated with deltamethrin were infested – one with an intradomiciliary colony detected, and the other with just adult bugs collected indoors. Of 43 houses reportedly treated with malathion, four (9.3%) were found to be infested: three were in Loja (two of them with bugs detected indoors and reportedly treated 2 and 3 months before the survey, respectively, and another one with just peridomestic infestation and treated a year before fieldwork), and one in El Oro (peridomestic infestation, treated one month before the survey). The only house where diesel was used as an insecticide (in El Oro) was also found to have peridomestic infestation. No infested houses were found where the dwellers used other insecticides.

5.3.2.5. Households

Current infestation was more frequent in houses in poor/very poor condition: 12% of these (10/83) were infested, vs. only 3% of houses in good condition (1/33); 91% of infested households were in poor/very poor condition, and just one currently infested DU was classified as good. These differences were not statistically significant. Similarly non-significant differences were recorded when analysing intradomiciliary and reportedly infested households, although the percentages of infested DUs were always higher in poorer households.

Time since house construction (general mean=15.2±11 years, median=15 [quartiles 8-20], ranging from 1 to 60) was also comparable in both infested and non-infested DUs, except when reported infestation was analysed: mean=18.1±12.3 for DUs with reported infestation (median=16 [11-20], 3 to 60) vs. 13±9.4 in DUs without (median=12.5 [3.25-19.75], 1 to 40); *t*-test [on $\sqrt{x}$] X^2=2.5 (89 df, p=0.014). Analysis of houses with/without recent reported infestation (up to 1 year, n=39) revealed no differences in time since construction (t=0.1, 37 df, p=0.9).

The analysis of wall building materials showed no significant differences between currently infested/non-infested DUs. Five out of 66 houses (7.6%) with brick walls were infested, as were 5 out of 43 (11.6%) with walls made mainly of mud. Adult bugs were collected in one out of 7 (14.3%) dwellings with timber walls. On the other hand, 33% of dwellers living in brick-walled houses reported past infestation, as did 60.5% of those living in mud houses, 14% of dwellers in houses with wooden walls, and 33% of those whose houses were made of other materials. The presence of intradomiciliary bug colonies was detected in two brick houses (both in Loja; one of them had some parts of the walls made of mud and was in poor condition, whereas the other one was a good, brick-walled house) and in three mud houses (two in Loja and one in El Oro). These results led to the idea of analysing infestation in dwellings with walls made totally or partially with mud (42% of 119 DUs with valid data) vs. all other dwellings (58%).

Six out of 50 (12%) houses with walls made totally or partially with mud were currently infested, vs. 5 of 69 (7.25%) in the complementary group (FET p=0.5). These percentages were 12% and 5.8% in houses with/without bug colonisation, and 8% and 2.9% in dwellings with/without indoors infestation. Reported infestation was more frequent in houses with walls built total or partially with mud: 58% of them were

reportedly infested, vs. 30.4% of houses with walls made of other materials (FET p=0.0045; uOR=3.16, 95% CI 1.5-6.8, n=119).

Both plastered and unplastered houses had comparable probabilities (if slightly higher for houses with unplastered walls) of being currently infested. Only the percentage of houses whose owners reported recent (up to 1 year) infestation (valid data available from 46 DUs) was significantly higher in the group with unplastered walls (55% vs. 12%; FET p=0.0047, uOR=9.2, 95% CI 2.1-65.7). Reported infestation was more frequent in houses with non-plastered mud walls (64.3% reportedly infested, vs. 35.2% of houses with different walls) (FET p=0.0085; uOR=3.3, 95% CI 1.4-8.3, n=119), but this type of walls was not associated with any other class of infestation (either current, intradomiciliary, with colonisation, or reported recent).

All currently infested houses had tiled roofs; five of them presented intradomiciliary infestation. FET p values were 0.06 (current infestation), 0.065 (colonisation), and 0.3 (intradomiciliary infestation). Dwellers reported infestation in 50.6% of houses with tiled roofs, vs. 14.3% in houses with other types of roofs (FET p=0.0008; uOR=6.14, 95% CI 2.15-22.25, n=113). The correlation between mud walls and tiled roofs was very strong (FET p<0.0001). Dwellers living in houses with walls made (totally or in part) of mud *and* a tiled roof were more likely to report past infestation (FET p=0.002, uOR=3.4, 95% CI 1.6-7.6, n=117), but differences in percentages were not significant for the rest of infestation categories. The combination of unplastered walls and roofs made mainly with tiles was also explored. Reported infestation was more frequent in this type of building (FET p=0.015, uOR=2.9, 95% CI 1.3-6.6); the same was observed with reported recent (<1 year) infestation (FET p=0.002, uOR=7.2, 95% CI 2.1-33.1).

The type of floor did not increase significantly the likelihood of current infestation; even if it was consistently higher in houses with earthen floors (for current infestation, colonisation, indoors infestation, and reported infestation), some of those with floors made with concrete or timber were also infested. Indoors breeding colonies were only found in one house with a concrete floor and in four with earthen floors.

Crowded living conditions (three or more people sharing a single bedroom), or the number of bedrooms in the house, did not affect the likelihood of infestation.

5.3.2.6. Domestic animals

All families living in currently infested domiciles had domestic animals, but this feature was so frequent (~97% of families had at least one animal) that no significant differences were recorded; the same occurred when an analysis of infestation in relation to the presence of peridomiciliary domestic animals (87.5% of families had them) was attempted. Infestation was less frequent in houses where animals were kept indoors, but differences were not statistically significant.

The total number of domestic animals (mammals plus birds) was found to be positively correlated to the likelihood of infestation. This relationship was statistically significant only for intradomiciliary (t=3.06, 118 df, p=0.003) and reported infestation (t=2.8, 118 df, p=0.005); it was slightly weaker when only the number of peridomestic animals was analysed (intradomiciliary infestation t=2.9, 116 df, p=0.005; reported infestation t=2.9, 116 df, p=0.004), and no correlation was found between the number of animals kept indoors and the various categories of infestation. The transformation $x_t=(0.5+x)^{1/3}$ resulted in an approximately normal frequency distribution, and was used for the t-tests (for indoor animals, crude data were analysed using non-parametric tests).

Separate analyses were conducted for chickens and dogs kept indoors or in the peridomicile. The number of dogs in the household was higher in infested houses (for both intradomiciliary [WT X^2=11.5, 1 df, p=0.0007] and current infestation [WT X^2=5.9, 1 df, p=0.015; uOR=36.8, 95% CI 3.4-489.4]). A significant difference was also recorded between reportedly infested/non-infested domiciles (WT X^2=5.7, 1 df, p=0.017; uOR=7.66, 95% CI 1.3-51.8) (figure 50 A), and a marginally non-significant one between households with/without bug colonisation and with/without peridomestic infestation (WT X^2=3.8, 1 df, p=0.051; uOR=16.9, 95% CI 1.4-207.7).

Reportedly infested dwellings also had more chickens* than those whose owners reported no infestation (t=2.7, 116 df, p=0.0089) (figure 50 B). No significant differences were recorded for current infestation, colonisation, or peridomestic infestation, but the presence of intradomiciliary bugs was associated with a significantly larger number of chickens in the DU (t=2.4, 116 df, p=0.017).

---

*For t-tests, data were transformed [$x_t=(0.5+x)^{1/4}$] to improve normality; the normal quantile plot showed a reasonably linear shape, and non-parametric tests (WT) were also carried out (results not shown) to confirm results from t-tests (for which homoscedasticity was checked using the Bartlett test)

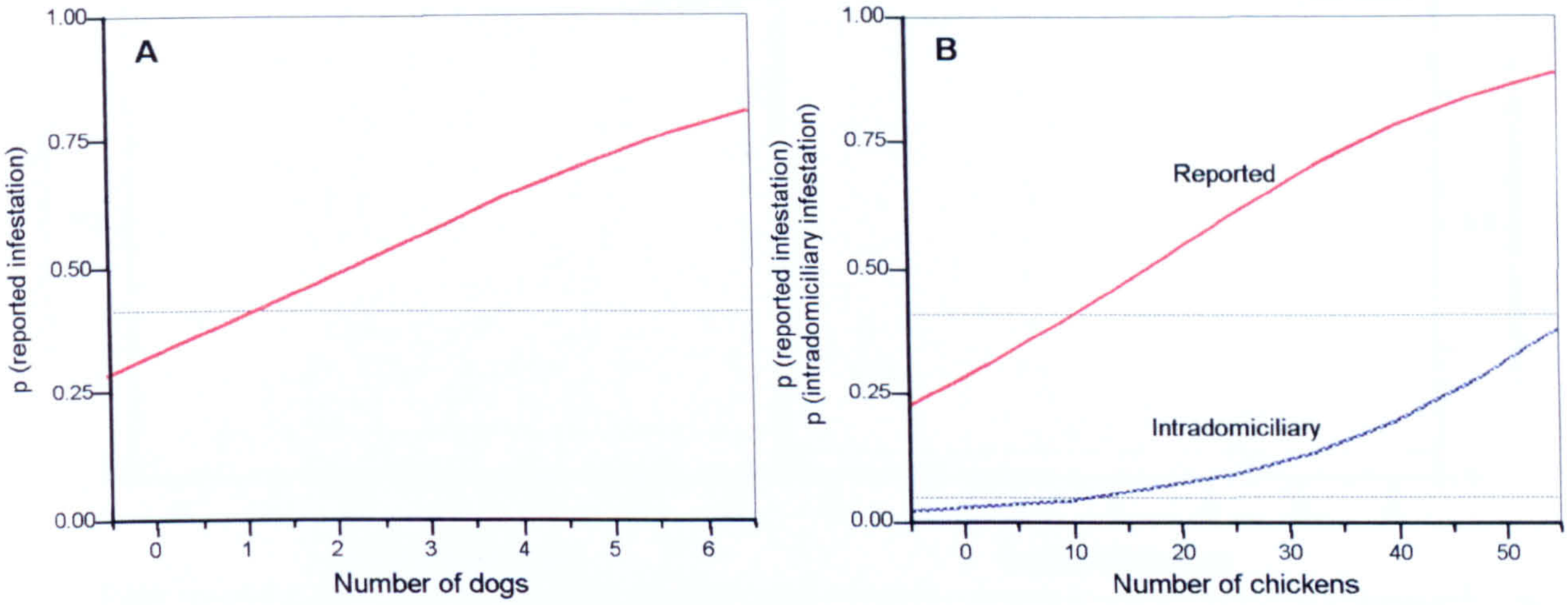


Figure 50. Entomological survey: univariate logistic fits. Probability of reported household infestation by *Rhodnius ecuadoriensis* in relation to the number of dogs (**A**) and chickens (**B**) kept in DUs. In **B**, the upper line corresponds to reported infestation, and the lower line to intradomiciliary infestation; statistical details were: (i)reported infestation: effect likelihood ratio test X^2=9.3, 1 df, p=0.002; uOR=16.2, 95% CI 2.6-128.6; and (ii)intradomiciliary infestation effect likelihood ratio test X^2=3.95, 1 df, p=0.047; uOR=20.3, 95% CI 1.05-357.3. Dotted grey lines indicate the observed average probabilities of dwellers reporting infestation (in **B**, the one on top corresponds to reported infestation)

The mere presence of chickens in the DU (assessed using a presence/absence variable) was not related to higher likelihood of any of the types of infestation studied here (FET p=1 for current infestation, colonisation, and peridomestic infestation, 0.6 for intradomiciliary infestation, and 0.3 for reported infestation). A similar lack of association was observed for the presence/absence of both peridomestic and intradomiciliary chickens.

Numbers of peridomestic dogs were found to be positively correlated with the likelihood of current infestation, intradomiciliary infestation, and reported infestation. As suspected, the inspection of frequency distribution histograms and normal quantile plots indicated non-normality, preventing the use of parametric statistics.

Table 51. Entomological survey: univariate analyses. Infestation and peridomestic dogs in households

Response variables	**Wilcoxon test**		**Univariate logistic regression**		
	X^2	p	Effect LR test X^2	Effect LR test p*	uOR (CI)
Current infestation	12.1	0.0005	12.35	0.0004	60.3 (6.26-787.8)
Colonisation**	9.1	0.0026	7.8	0.005	28.2 (2.8-319.2)
Intradomiciliary infestation	15.7	<0.0001	16.6	<0.0001	385 (20-19666)
Reported infestation	8.1	0.0043	7.7	0.0056	12.4 (2-97.6)
Recent reported infestation	8.13	0.004	9.3	0.002	20.7 (3-170)

WT=Wilcoxon test; WA=Welch Anova; LR=likelihood ratio; uOR=unadjusted odds ratio; CI=95% confidence interval; ns=not significant; *for one degree of freedom; **equivalent to peridomestic infestation

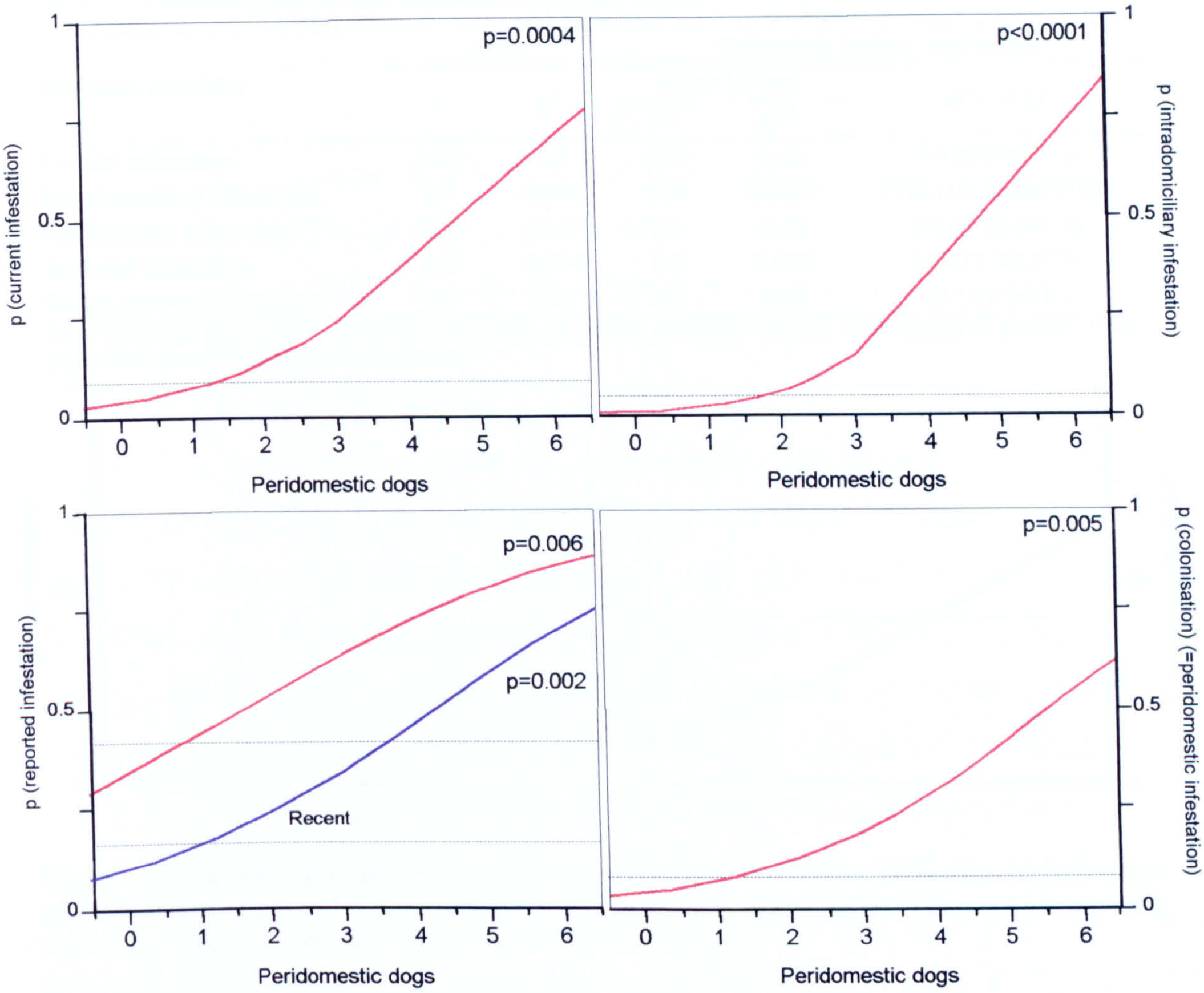


Figure 51. Entomological survey: univariate logistic fits. Probability of household infestation by *Rhodnius ecuadoriensis* in relation to the number of peridomestic dogs; X^2 p values (from effect likelihood ratio tests) are shown on each graph. The line with the "Recent" label corresponds to recent reported infestation. Dotted grey lines indicate the observed average probabilities (in the graph for reported infestation, the lower line corresponds to recent reported infestation). See also text and table 51

The numbers of dogs and chickens showed a weak positive correlation (as assessed from linear regression: R^2=0.07).

The number of guinea pigs inside dwellings had no effect on the likelihood of infestation; the same was observed for the number of peridomestic pigs. The total number of cats per DU was associated with a non-significant decrease of the probability of infestation.

Finally, an estimate of vertebrate biomass (B) was calculated for each household as

B = log[(number of human inhabitants)+(total domestic mammals)+½(total domestic birds)]

(the number of birds was halved because of their smaller average size) and its relationships with the likelihood of infestation by *R. ecuadoriensis* were explored.

Table 52. Entomological survey: univariate analyses. Infestation and 'biomass index' (*B*, see text)

Response variables	***t*-test**		**Univariate logistic regression**		
	t	p*	Effect LR test		uOR (CI)
			X^2	p**	
Current infestation	0.95	0.3	0.96	0.32	5.5 (0.2-237.3)
Intradomiciliary infestation	2.7	0.008	9.01	0.0027	6758 (15.3-22628118)
Peridomestic infestation***	0.68	0.5	0.5	0.48	3.5 (0.12-161.4)
Reported infestation	2.5	0.014	6.3	0.012	13.4 (1.7-127.7)
Recent reported infestation	2.1	0.04	4.7	0.03	23.1 (1.3-575)

LR = likelihood ratio; uOR = unadjusted odds ratio; CI = 95% confidence interval; *115 degrees of freedom; **one degree of freedom; ***equivalent to colonisation

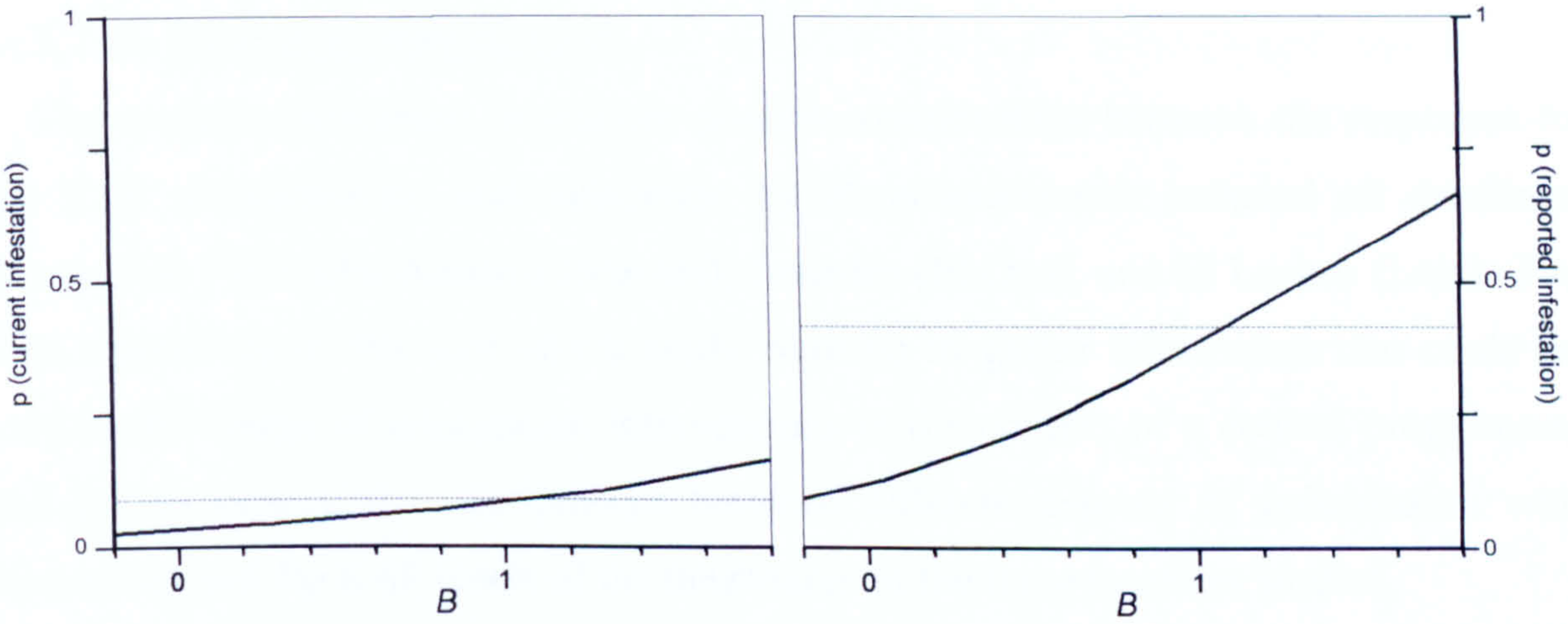


Figure 52. Entomological survey: univariate logistic fits. Probability of household infestation (current and reported) by *Rhodnius ecuadoriensis* in relation to an estimate of biomass (*B*, see text); dotted grey lines indicate the average observed probabilities

5.3.2.7. Product storage

Storage of products (mainly crops, firewood, and spare building materials) in the peridomicile increased the likelihood of current DU infestation; thus, 16% of dwellings with such products were infested, vs. none of the households without them (data from 107 DUs, FET p=0.007). Similar results were obtained for colonisation/peridomestic infestation (FET p=0.013), but no significant association was found for either reported (FET p=0.099) or intradomiciliary infestation (FET p=0.08).

A similar lack of association was found in the analysis of intradomiciliary product storage (n=104) – except for an increased percentage of dwellers reporting having seen the bugs in their homes recently (<1 year) (FET p=0.01).

5.3.2.8. Domestic hygiene

No infestation was detected in dwellings whose owners said they clean the house on a weekly basis, vs. 13.6% in households cleaned less often (FET p=0.048); the same trend was observed for colonisation (FET p=0.09) and intradomiciliary infestation (FET

p=0.16). Dwellers living in houses cleaned with different frequencies were equally likely to report infestation (FET p=1) (data from 89 DUs). Houses where chicken coops, corrals, pigsties, dovecotes etc. were cleaned at least once a month were less likely to be infested (8% vs. 16.7% infested DUs; FET p=0.4 [n=60]) or colonised (5.4% vs. 16.7%, FET p=0.2). The percentage of reportedly infested houses was also lower in those houses (29.7% vs. 54.2%; FET p=0.067). The difference was similar when recent (<1 year) reported infestation was analysed (36.4% vs. 77%; FET p=0.095, n=24).

5.3.2.9. KAP and infestation

The analyses performed to explore possible relationships between the responses to the KAP questionnaires and infestation by *R. ecuadoriensis* included all dwellings surveyed in Pachinche Adentro (Manabí), Lourdes (El Oro), and El Lucero (Loja). The main **objectives** of this part of the study were (i) to gather information that could be used in the design of educational interventions in the context of a control programme, and (ii) to explore the relationships between different degrees of cohabitation with triatomines and the KAP levels of the inhabitants of the three localities studied.

·General data. The ages of people answering the KAP questionnaires (data from 174 DUs; overall mean=47.5±17.5, median=45 [range: 15 to 92]) were comparable whether their domiciles were currently infested (44.3±16 years) or not (47.7±17.6). The same was observed when houses with/without reported infestation (50.17±15.9 and 46.3±18.1 years, respectively) were compared. No differences were recorded within any of the three communities when analysed separately, except for a lower mean age of dwellers living in infested DUs in Manabí (31.2 vs. 50 years); infestation here corresponds however to peridomestic palm trees (3 DUs) and one adult bug captured within a house. Finally, ages were also comparable between localities.

Formal education levels were also similar in infested and non-infested DUs, with similar percentages of people having no formal education, not having completed primary school, and having done so. However, no one having studied after primary school lived in a currently infested dwelling in our sample (n=169 domiciles). Again, the differences were even lower between reportedly infested/non-infested dwellings. Significant differences between communities were detected, with dwellers from Manabí having in average lower education levels.

·Knowledge. Of those living in currently infested DUs, 20% knew Chagas disease in our study group, vs. 13.3% of those living in non-infested houses (FET p=0.44, n=188). The percentages were also similar when comparing households with intra-, peridomestic, and reported infestation (FET p 1.0, 0.4 and 1.0, respectively). The same was found for knowledge about Chagas disease transmission; however, no one living in houses where intradomiciliary bugs were captured knew how the disease is transmitted. In general, the percentages of people knowing adult triatomines were comparable (with no statistically significant differences detected) in both infested and non-infested households, but they were somewhat higher among people living in infested houses (bold type in table 53). Differences were highly significant between the groups of households where past infestation had/had not been reported.

Table 53. Entomological/KAP survey: univariate analyses. Infestation by knowledge of adult vectors

Count Column % Row %		**Reported infestation**		**Total %**	**Current infestation**		**Total %**	**Intradomiciliary infestation**		**Total %**	**Peridomestic infestation**		**Total %**
		No	Yes		No	Yes		No	Yes		No	Yes	
Know adult bugs	No	48 38.1 94.1	3 4.9 5.9	51 27.3	49 28.5 96.1	2 13.3 3.92	51 27.3	50 27.8 98	1 14.3 2	51 27.3	50 28.7 98	1 7.7 2	51 27.2
	Yes	78 **61.9** 57.5	58 **95.1** 42.5	136 72.7	123 **71.5** 90.4	13 **86.7** 9.6	136 72.7	130 **72.2** 95.6	6 **85.7** 4.4	136 72.7	124 **71.3** 91.2	12 **92.3** 8.8	136 72.7
Total %		126 67.4	61 32.6	187 100	172 92	15 8	187 100	180 96.3	7 3.7	187 100	174 93	13 7	187 100
FET p		<0.0001			0.36			0.68			0.12		

The proportion of people knowing triatomine nymphs was significantly higher among those living in currently infested domiciles, as shown in the following table and figure. FET p values indicated strong correlation between these variables, especially when we compared households with and without peridomiciliary infestation (p=0.0023) and with/without reported infestation (FET p=0.0008).

Table 54. Entomological/KAP survey: univariate analyses. Infestation by knowledge of immature vectors

Count Column % Row %		**Reported infestation**		**Total %**	**Current infestation**		**Total %**	**Intradomiciliary infestation**		**Total %**	**Peridomestic infestation**		**Total %**
		No	Yes		No	Yes		No	Yes		No	Yes	
Know nymphs	No	82 66.1 77.4	24 39.3 22.6	106 57.3	103 60.6 97.2	3 20 2.8	106 57.3	105 59 99.1	1 14.3 0.9	106 57.3	104 60.5 98.1	2 15.4 1.9	106 57.3
	Yes	42 **33.9** 53.2	37 **60.7** 46.8	79 42.7	67 **39.4** 84.8	12 **80** 15.2	79 42.7	73 **41** 92.4	6 **85.7** 7.6	79 42.7	68 **39.5** 86.1	11 **84.6** 13.9	79 42.7
Total		124 67	61 33	185 100	170 91.9	15 8.1	185 100	178 96.2	7 3.8	185 100	172 93	13 7	185 100
FET p		0.0008			0.0047			0.043			0.0023		

The percentages of people knowing the habitats and feeding habits of bugs were similar in currently infested and non-infested DUs. Positive answers were significantly more frequent among those living in houses whose owners reported having seen the bugs in the past (FET $p<0.0001$ for habitats and 0.0004 for feeding habits; n=181).

Also, when the 'quality of knowledge' score (taking into account four responses related to the vectors, see above) was compared, we found that people living in infested (both currently and reported) households were likely to have a significantly better knowledge about the bugs than those living in vector-free environments. For these analyses, non-parametric tests (WT) and univariate logistic regression were employed.

Table 55. 'Quality of knowledge': comparison of mean/median scores obtained by people living in households with/without reported infestation and in infested/non-infested dwellings

Quality of knowledge	**Reported**		**Current**		**Intradomiciliary**		**Peridomestic**	
	Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median
Bugs present	7.23 (3.04)	7	7.53 (3.36)	9	8.57 (3.36)	10	7.85 (3)	9
Bugs absent	4.5 (3.5)	4.5	5.24 (3.6)	6	5.3 (3.57)	6	5.24 (3.6)	6
WT X^2 (p) [1 df]	24.2 (<0.0001)		5.24 (0.02)		5.75 (0.016)		5.76 (0.016)	

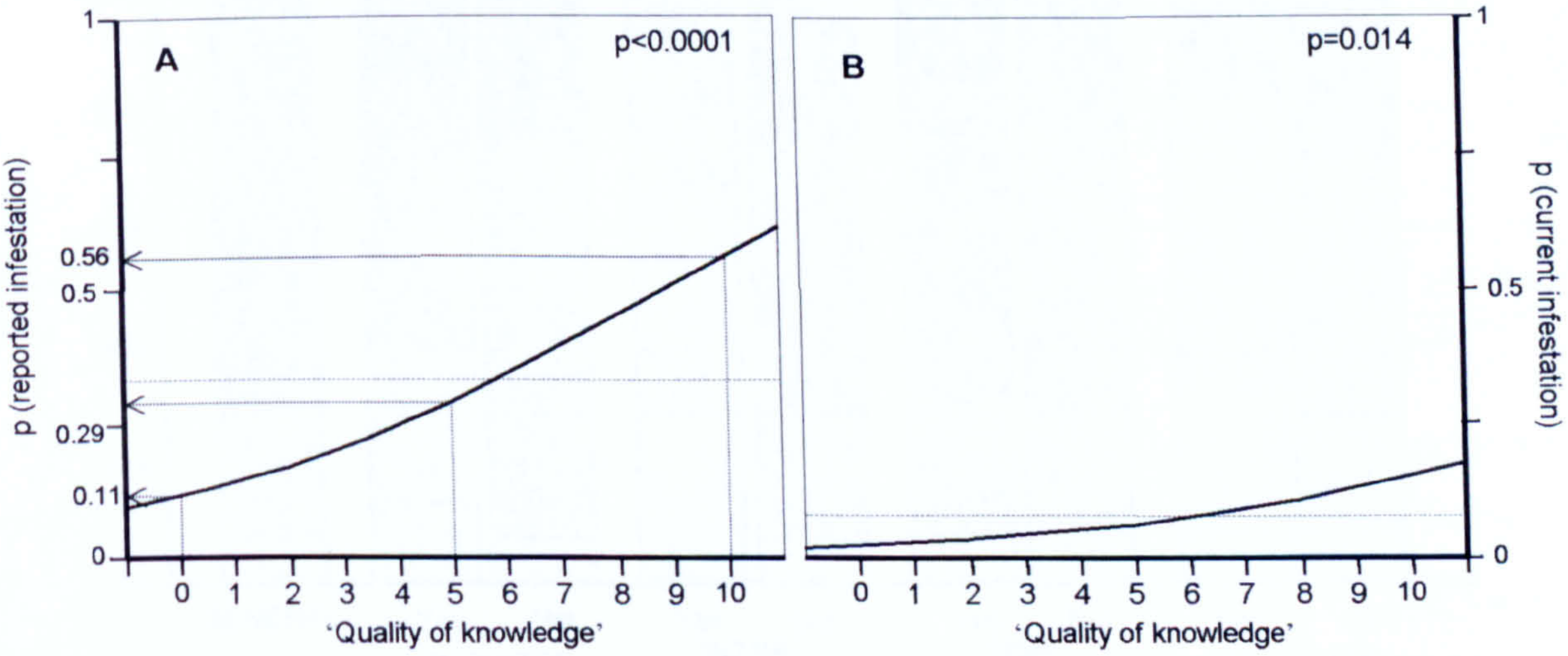


Figure 53. 'Quality of knowledge': logistic fit comparing knowledge scores and reported (**A**) and current (**B**) household infestation by *Rhodnius ecuadoriensis*. In **A**, *Y* values of 0.56, 0.29, and 0.11 are the probabilities of reporting infestation associated with scores of 10, 5 and 0, respectively, according to the logistic fit; the dotted line represents the average probability of dwellers reporting infestation. Significance probability values (p) obtained from effect likelihood ratio tests are presented on top right angle of each graph (X^2 values were 24.5 for **A** and 5.99 for **B** [1 df])

The percentages of people affirming that these bugs represent a nuisance did not differ significantly between currently infested and non-infested DUs, but affirmative answers were again significantly more frequent among people living (or who lived in the past) in households with reported infestation (75% vs. 48.7%, FET $p<0.0001$). In a separate analysis by locality, only in El Oro this difference remained significant, even if

affirmative answers were always more frequently recorded from reportedly infested DUs. No significant differences were detected when comparing the percentage of answers indicating that bug bites can transmit some diseases obtained in infested/non-infested households and in those with/without reported past infestation. On the contrary, answers stating that domestic animals can transmit diseases to people were more frequently obtained from people living in currently infested domiciles (FET p=0.04); the differences were however not statistically significant when houses with/without intradomiciliary, with/without peridomestic infestation, and with/without reported infestation were compared.

·Attitudes. Tolerance towards the presence of intradomiciliary bug colonies was comparable across the different groups, notwithstanding small differences recorded – such as a higher tolerance in houses actually presenting indoors infestation. The following figure shows the trends.

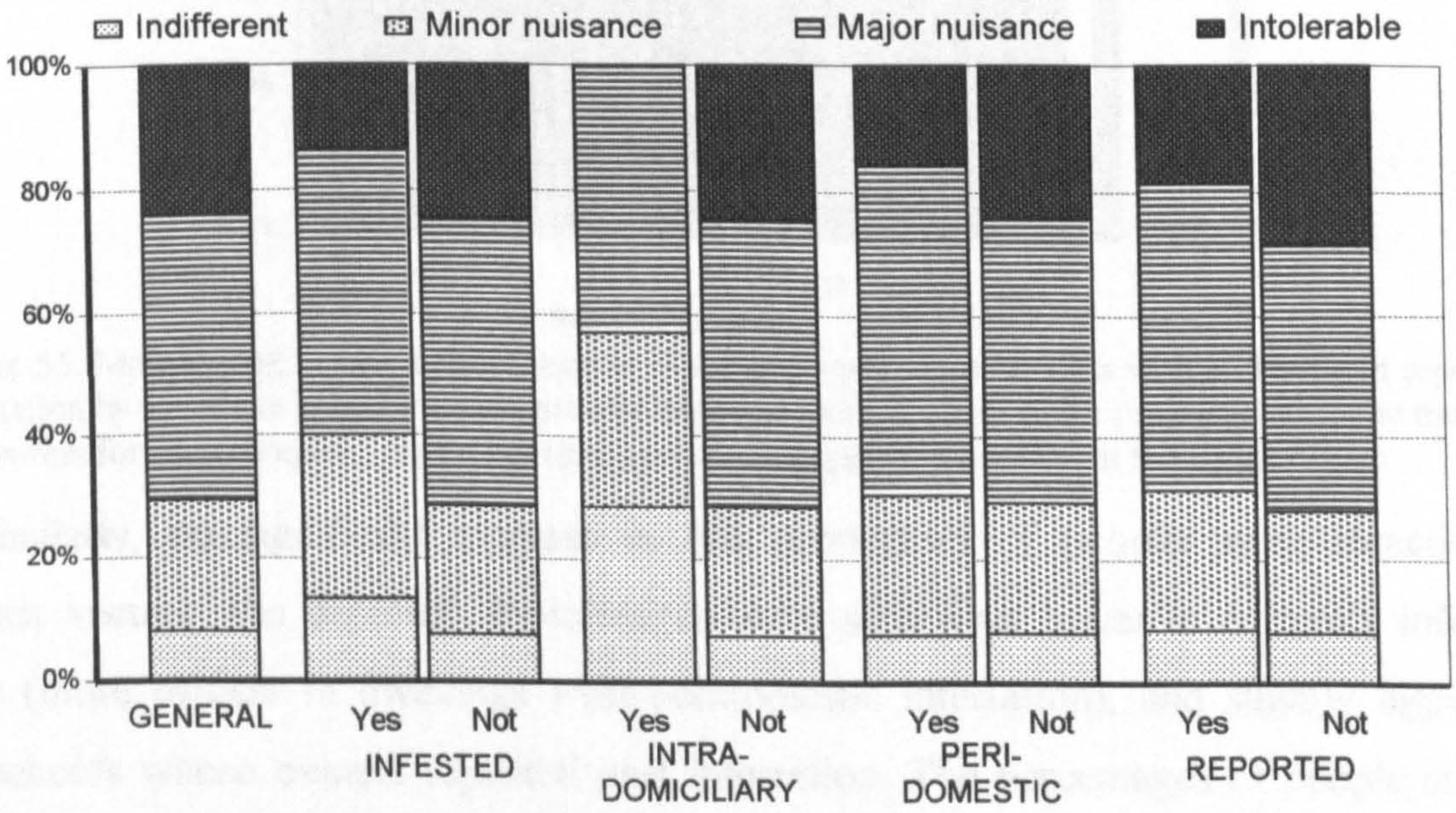


Figure 54. Entomological/KAP survey. Tolerance towards indoor *Rhodnius ecuadoriensis* colonies. General: results in the whole sample; Infested: currently infested/non-infested houses; Intradomiciliary: houses with/without intradomiciliary colonies; Peridomestic: houses with/without peridomestic colonies; Reported: dwellers reporting/not reporting past infestation

Similarly, no significant difference was recorded when analysing the tolerance towards peridomestic colonies of *R. ecuadoriensis*. However, contingency analysis showed that the number of people stating that the presence of these colonies is intolerable was less than half the expected value in houses where dwellers reported past infestation (4 vs. 9.5).

·Practices. The percentages of people taking some kind of action against the vectors were similar within the groups of currently infested/non-infested DUs, those with/without intradomiciliary bugs, and those with/without peridomestic colonies, yet they were always higher in houses with triatomines. The difference between percentages of people taking/not taking action against bugs was however statistically significant when comparing families living (or who lived in the past) in houses with/without reported infestation, with a higher proportion taking some sort of action in reportedly infested homes (FET p=0.003). The following figure presents a comparison of these percentages.

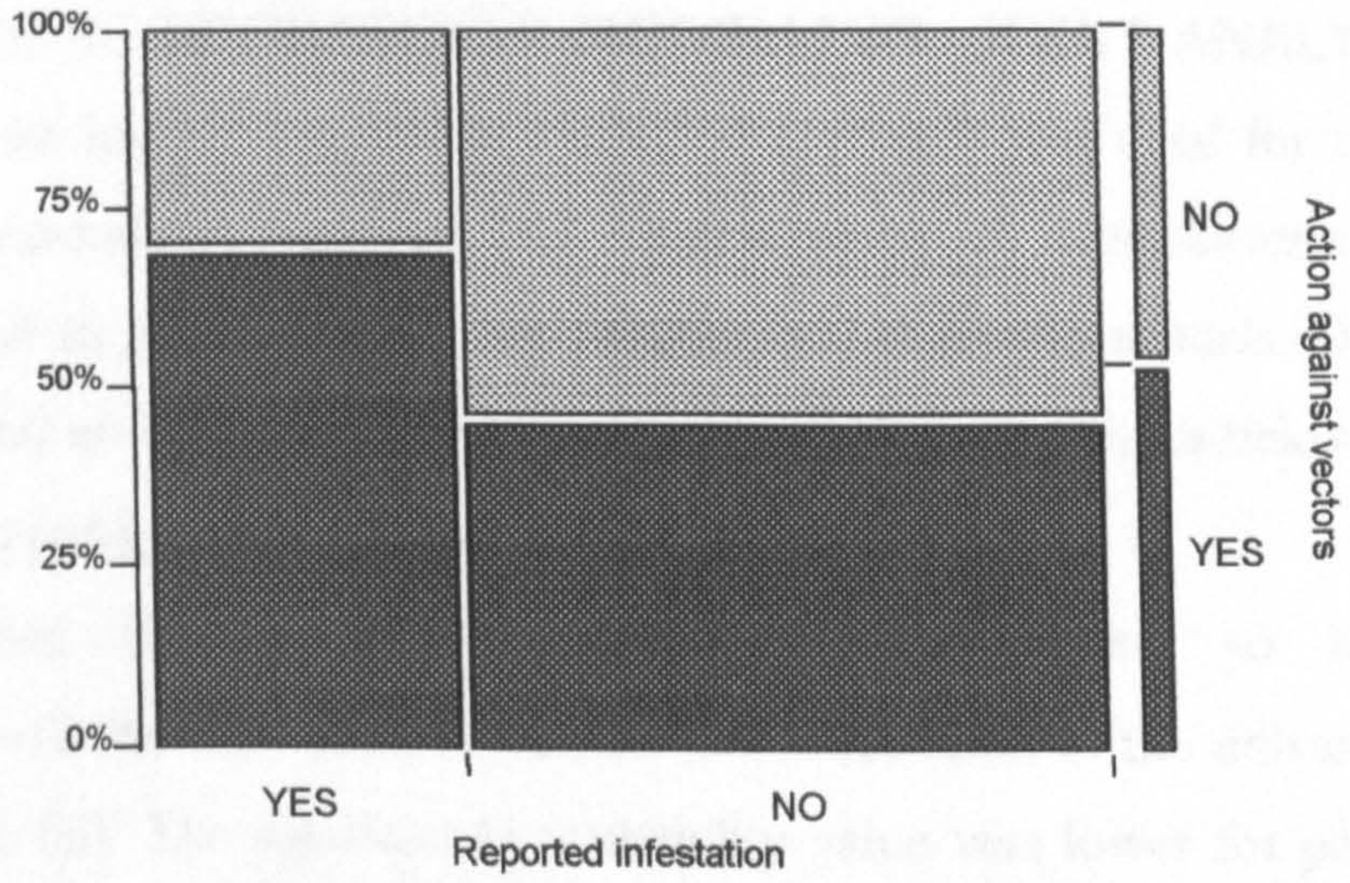


Figure 55. Mosaic plot: action against vectors (KAP answers) in households with and without reported infestation by *Rhodnius ecuadoriensis* (present or past home); column width proportional to the number of answers for each category; general percentages and categories presented in the right column

Similarly, no significant increase in the proportion of people using insecticides against vectors was detected. Percentages were somewhat lower in currently infested DUs (more evident in dwellings with peridomestic infestation), and slightly higher in households whose owners reported past infestation. The percentages of people stating that they kill single bugs as they detect them were higher in houses with current infestation (FET p=0.015), with peridomestic infestation (p=0.03), and in those whose owners reported past infestation (p=0.03); no significant difference was detected between houses with or without intradomiciliary colonies. We found no relationship between infestation and use of bednets; comparable percentages were also obtained for domestic hygiene, presence of domestic animals indoors, or frequency of cleaning the places where animals live. An exception was the fact that houses that were cleaned once a week or more often did not have intradomiciliary bug colonies (FET p=0.033); in

general, infestation rates were lower among these houses when compared to those cleaned less often (4.3% vs. 10.5%; FET p>0.2). Infestation was also less frequent in households where peridomestic chicken coops, corrals, dovecotes, pigsties, etc. were cleaned monthly or more often, but differences were not statistically significant (e.g. 28.3% vs. 45.2% reportedly infested, FET p>0.15).

The use of palm leaves in Manabí did not affect the proportion of infested households – as expected, taking into account that three out of four infested households had only palm tree-living peridomestic colonies, and only one adult bugs was found in the fourth one.

5.3.3. LOGISTIC REGRESSION MODELLING: JOINT ANALYSES

A multivariate logistic regression modelling approach was used for the investigation of possible associations between DU infestation by *R. ecuadoriensis* and different variables related to dwellings, their inhabitants, and domestic animals. Only results from El Lucero (Loja) and Lourdes (El Oro) are included in the analyses below.

5.3.3.1. Current infestation

The maximal (initial) model included variables found to have significant relationships with the likelihood of current DU infestation in the univariate exploratory analyses (table 56). The significance probability value was lower for peridomestic dogs than for total number of dogs; the former variable was therefore included in the model. The stepwise process of deletion of non-significant terms led to the exclusion of (in this order) recent insecticide treatment, family income, housekeeping (weekly or less often), and product storage in peridomiciles. The minimal adequate model (***MInf*-1**) was thus fitted (lack of fit test[▽] X^2=16.9, 10 df, p=0.08) including two covariates: report of infestation by the dwellers and number of peridomestic dogs in the DU (tables 56 and 57). A second model was fitted (***MInf*-2**) including current infestation as the response variable and two covariates related to those used in *MInf*-1: reported recent infestation (in the year before fieldwork) and total number of dogs in the DU (lack of fit test X^2=11.6, 10 df, p=0.3). Data from 120 households were available.

[▽]While the 'whole model' tests if the specified model is better than a reduced model (with only intercepts), the 'lack of fit' tests if a saturated model (with a parameter for each unique combination of *x* values) is better than the specified model; non-significant p values indicate that there is no need to add more complex (e.g. interaction) terms (SAS Institute 2000)

Table 56. Multivariate logistic regression. Covariates for DU infestation by *Rhodnius ecuadoriensis*

Current infestation	Univariate statistics				Multivariate modelling	
	Univariate tests		Logistic regression		LR X^2 (p)	OR (CI)
	Test statistic	p value	LR X^2 (p)	uOR (CI)		
Reported infestation	(FET)	0.0007	13 (0.0003)	17.2 (3-322)	9.1 (0.0025)[a]	13.7 (2.3-268.5)[a]
Dogs (peridomestic)	WT X^2=12.1	0.0005	12.4 (0.0004)	60 (6-788)	8.5 (0.0036)[a]	41 (3.4-664)[a]
Reported (recent)	(FET)	0.0003	14.04 (0.0002)	12.9 (3.4-55.4)	9.75 (0.0018)[b]	9.6 (2.4-43.1)[b]
Dogs (total)	WT X^2=5.86	0.015	8.55 (0.0035)	37 (3.4-489)	4.26 (0.039)[b]	16.2 (1.15-260)[b]
Recent spray (last 6 m)	(FET)	0.007	7.8 (0.005)	9 (2-49)	excluded	
Income	WT X^2=3.2	0.075	4.3 (0.04)	0.01 (0.00002-0.8)	excluded	
Housekeeping (>week)	(FET)	0.048	7 (0.008)	unstable	excluded	
Products (peridomicile)	(FET)	0.007	10.7 (0.001)	unstable	excluded	

a=Data from model *MInf*-1; *b*=Data from model *MInf*-2 (see text); LR = likelihood ratio test; uOR = unadjusted odds ratio; CI = 95% confidence interval; OR = adjusted odds ratio; FET = Fisher's exact test; WT = Wilcoxon test; 1 df (for all X^2 tests)

The parameter estimates of *MInf*-1 are shown in table 57. The whole model test showed a high significance (X^2=21.5, 2 df, p<0.0001). The area under the receiver operating characteristic [ROC] curve derived from the model was 0.86; for a specificity of 82%, the model achieved 83% sensitivity in the prediction on DU infestation (arrows in figure 56). Although *MInf*-1 correctly classified 90.8% of DUs as infested or not (FET p=0.04), nine out of 11 (81.8%) actually infested dwellings were classified as non-infested by the model (false-negatives). *MInf*-1 predictions included two true-positives, 107 true-negatives, two false-positives, and nine false-negatives (degree of agreement[x]: kappa=0.23, SE=0.15).

Table 57. Logistic regression model (*MInf*-1) for DU infestation by *Rhodnius ecuadoriensis*

Current infestation	PE (SE)	PE X^2 (p)	LR X^2 (p)	OR (CI)
Intercept	-3.65 (0.7)	27.4 (<0.0001)	---	---
Reported infestation	1.3 (0.55)	5.6 (0.018)	9.13 (0.0025)	13.7 (2.3-268.5)
Peridomestic dogs	0.62 (0.22)	7.9 (0.005)	8.47 (0.0036)	41 (3.4-664)

Whole model test p<0.0001; Lack of fit test p=0.08; PE = parameter estimates; SE = standard error; LR = effect likelihood ratio test; OR = odds ratio; CI = 95% confidence interval

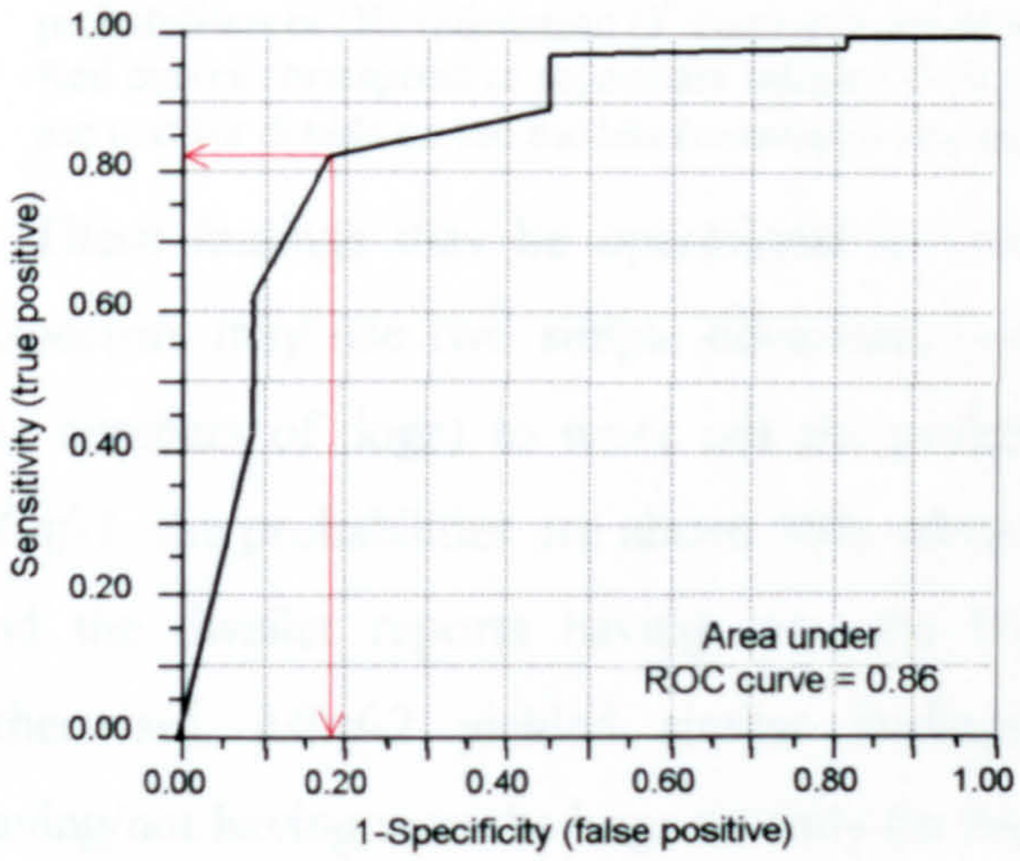


Figure 56. Receiver operating characteristic (ROC) curve derived from the logistic regression model *Minf*-1 for household infestation by *Rhodnius ecuadoriensis*

[x]Kappa values and degree of agreement (Landis & Koch 1977): 0-0.2=slight; 0.21-0.4=fair; 0.41-0.6=moderate; 0.61-0.8=substantial; 0.8-1=almost perfect to perfect

Statistical details of *MInf*-2 are presented in table 58. This model correctly classified 91.7% of dwellings as infested or not (FET p=0.02), with 108 true-negatives, two true-positives, one false-positive, and again nine false negatives (i.e., it failed to detect 81.8% of actually infested DUs). The area under the ROC curve derived from *MInf*-2 was 0.8; 82% and 87% sensitivities were associated with 73% and 64% specificities, respectively (kappa=0.26, SE=0.16).

Table 58. Logistic regression model (*MInf*-2) for DU infestation by *Rhodnius ecuadoriensis*

Current infestation	PE (SE)	PE X^2 (p)	LR X^2 (p)	OR (CI)
Intercept	-2.67 (0.6)	22.96 (<0.0001)	---	---
Reported (recent)	1.13 (0.36)	9.7 (0.0018)	9.75 (0.0018)	9.6 (2.4-43.1)
Dogs (total)	0.46 (0.23)	4.2 (0.04)	4.26 (0.039)	16.2 (1.15-260)

Whole model test p=0.0001; Lack of fit test p=0.3; PE = parameter estimates; SE = standard error; LR = effect likelihood ratio test; OR = odds ratio; CI = 95% confidence interval

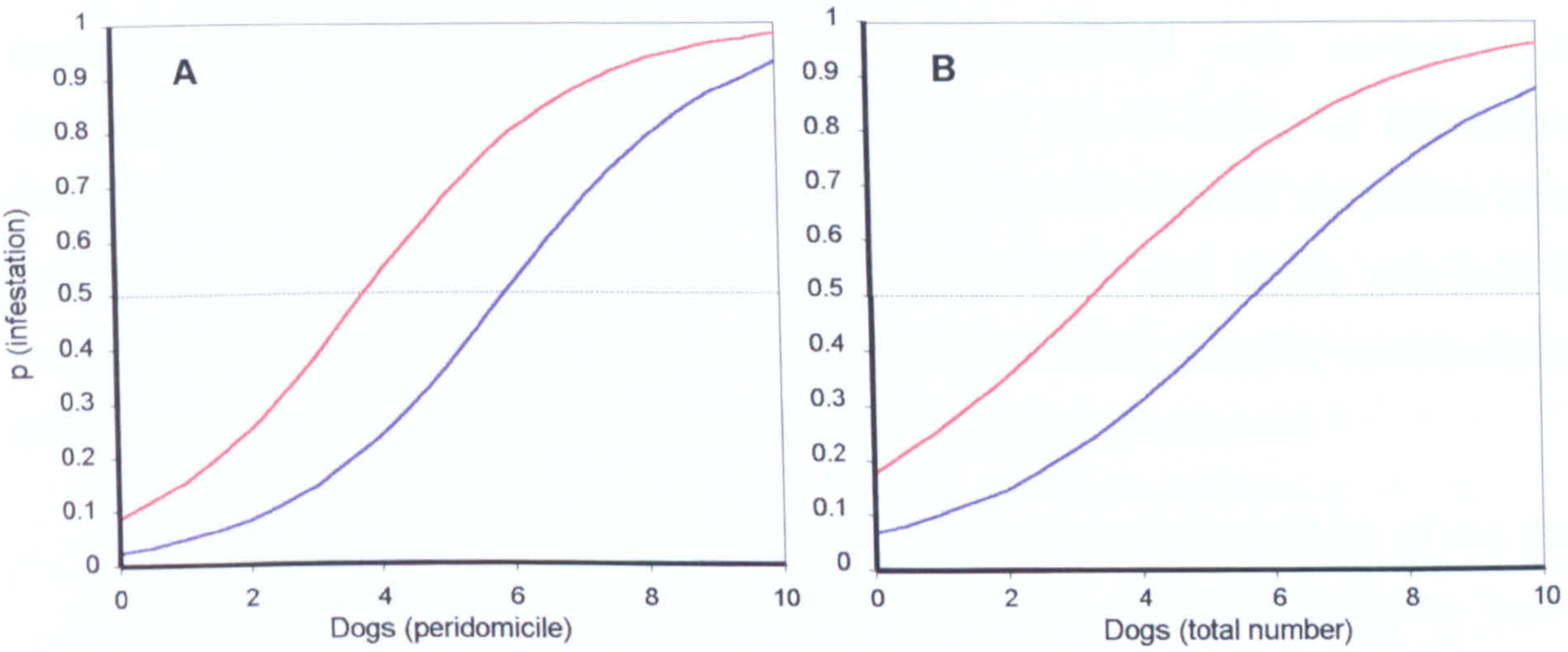


Figure 57. Predictions derived from the logistic regression models *MInf*-1 (**A**) and *MInf*-2 (**B**): probabilities of DU infestation (*Y* axes) as a function of reported infestation and numbers of dogs. Red curves correspond to reportedly infested DUs, and blue lines to reportedly non-infested DUs; see text for details on the models (covariates and associated statistics)

These findings may be operational in entomological surveys; thus, vector control inspectors may use two simple covariates (what dwellers report about infestation and the numbers of dogs) to work out the probabilities of each DU being infested. After *MInf*-1, the probabilities are above 50% when about 4 dogs are kept in the peridomicile and the dweller reports having seen the bugs in the household (and with 6 dogs otherwise); *MInf*-2 yielded similar findings for dwellings whose owners report having/not having seen the bugs recently (in the year before the survey takes place).

5.3.3.2. Reported household infestation

A larger set of possible covariates was available for the analysis of reported infestation. All those showing significant relationships (as assessed from results of univariate analyses) are presented in table 59. Reported infestation had the advantage of being much more frequent than the actual detection of bugs by our team during fieldwork (positive DUs accounted for 41.7% and 9.18%, respectively), allowing for richer analyses. The use of this variable is also supported by results of several field studies showing that detection of synanthropic bugs by householders is probably the most sensitive way to identify DU infestation (see above and discussion in Section 5.4.). An obvious caveat is that some false-positives may be included in the analyses (but active searches will yield some false-negatives); also, some details can be lost, such as precisely *when* (important for assessment of relationships with *current* DU characteristics) or *where* the bugs were seen, and the precise nature of infestation (breeding colonies or adult bugs). Both reported and *recent* reported infestation (the fraction of reportedly infested households where bugs were seen in the year before fieldwork) were available for analysis. Several models including different combinations of covariates were explored for both response variables with a double aim:

i. Determining 'diagnostic' characteristics of dwellings that could be of use for field surveys related to control interventions. This entails an attempt to define 'high-risk' households on the basis of simple traits easy to incorporate in routine activities of the vector control service. The models ***MRep*-1a** and **b** were devised with this aim.

ii. Ascertaining candidate targets for control interventions involving environmental management at the household level. This implies identifying key aspects of the ecology of synanthropic bug populations so that strategies to modify them (reducing the likelihood of infestation) may be put forward. Hence, candidate covariates were restricted to those amenable to intervention (not, for instance, the proportion of dwellers recognising the bugs, the family income, or the time each family had been living in their present community). ***MRep*-2** was defined following this rationale.

Table 59. Candidate covariates for reported domiciliary unit infestation by *Rhodnius ecuadoriensis*: univariate analyses and multivariate logistic regression modelling

Reported infestation	Univariate statistics				Multivariate modelling	
	Univariate tests		Logistic regression		LR X^2 (p)	OR (CI)
	Test statistic	p value	LR X^2 (p)	uOR (CI)		
Tiles in roof	(FET)	0.0008	12.65 (0.0004)	6.14 (2.15-22.25)	3.91 (0.048)*	3.3 (1.01-13.2)*
					6.4 (0.01)**	4.4 (1.4-17.4)**
Mud walls***	(FET)	0.0045	9.1 (0.0026)	3.16 (1.5-6.8)	4.46 (0.035)**	2.67 (1.1-6.91)**
Number of chickens	WT X^2=6.35	0.012	9.3 (0.0023)	16.2 (2.6-128.6)	5.65 (0.018)*	15.9 (1.6-246)*
					8.73 (0.003)**	22.7 (2.7-284)**
Income****	WT X^2=3.7	0.05	4.45 (0.035)	0.12 (0.014-0.86)	4.04 (0.045)*	0.11 (0.01-0.95)*
Time of residence	t=2.3	0.02[a]	5.3 (0.022)	6.4 (1.3-34.6)	excluded	
Age of house	t=2.5	0.014[b]	6.2 (0.013)	14.15 (1.7-148)	excluded	
Dogs (peridomestic)	WT X^2=8.14	0.004	7.7 (0.0056)	12.4 (2-97.6)	excluded	
Dogs (total)	WT X^2=5.7	0.0017	5.3 (0.022)	7.66 (1.3-51.8)	not included	
Biomass index	t=2.5	0.014[c]	6.3 (0.012)	13.4 (1.7-128)	not included	
Recognise vectors	(FET)	<0.0001	31.7 (<0.0001)	36.75 (7.4-668)	not included	

*Data from model *MRep*-1a; **data from *MRep*-1b (see text); ***excluded from *MRep*1-a; ****not included in *MRep*-1b; LR = likelihood ratio test; uOR = unadjusted odds ratio; CI = 95% confidence interval; OR = adjusted odds ratio; FET = Fisher's exact test; WT = Wilcoxon test; *a*=116 df; *b*=89 df; *c*=115 df; rest 1 df

A maximal model was fitted for reported infestation including the following covariates: time of residence of families in their present community, monthly family income, time elapsed since the house was built, houses with mud walls, houses with tiled roofs, number of chickens kept in the DU, and number of peridomestic dogs. Stepwise deletion of non-significant terms (using effect likelihood ratio tests) led to the exclusion of the following covariates (in this order): time of residence at the present community, mud walls, age of the house, and number of dogs. The resulting model (***MRep*-1a**) was considered to be the minimal adequate, and included data from 78 domiciles. The whole model test X^2 p was 0.0025 (X^2=14.36, 3 df), and the lack of fit test yielded a p=0.15 (X^2=40.6, 69 df). Parameter estimates and other statistical details are presented in table 60. The area under the ROC curve derived from model *MRep*-1a was 0.74, with 77% sensitivity for a specificity of 67% (arrows in figure 58, below); 67% sensitivity was associated with 77% specificity.

Table 60. Logistic regression model for reported infestation by *Rhodnius ecuadoriensis* (*MRep*-1a)

Reported infestation	PE (SE)	PE X^2 (p)	LR X^2 (p)	OR (CI)
Intercept	-0.73 (0.5)	1.92 (0.165)	—	—
Number of chickens	0.06 (0.025)	4.72 (0.03)	5.65 (0.018)	15.9 (1.6-246)
Tiled roof	0.6 (0.3)	3.49 (0.06)	3.91 (0.048)	3.3 (1.01-13.2)
Monthly income	-0.015 (0.008)	3.71 (0.054)	4.04 (0.045)	0.11 (0.01-0.95)

Whole model test p=0.0025; Lack of fit test p=0.15; PE = parameter estimates; SE = standard error; LR = likelihood ratio test; OR = odds ratio; CI = 95% confidence interval

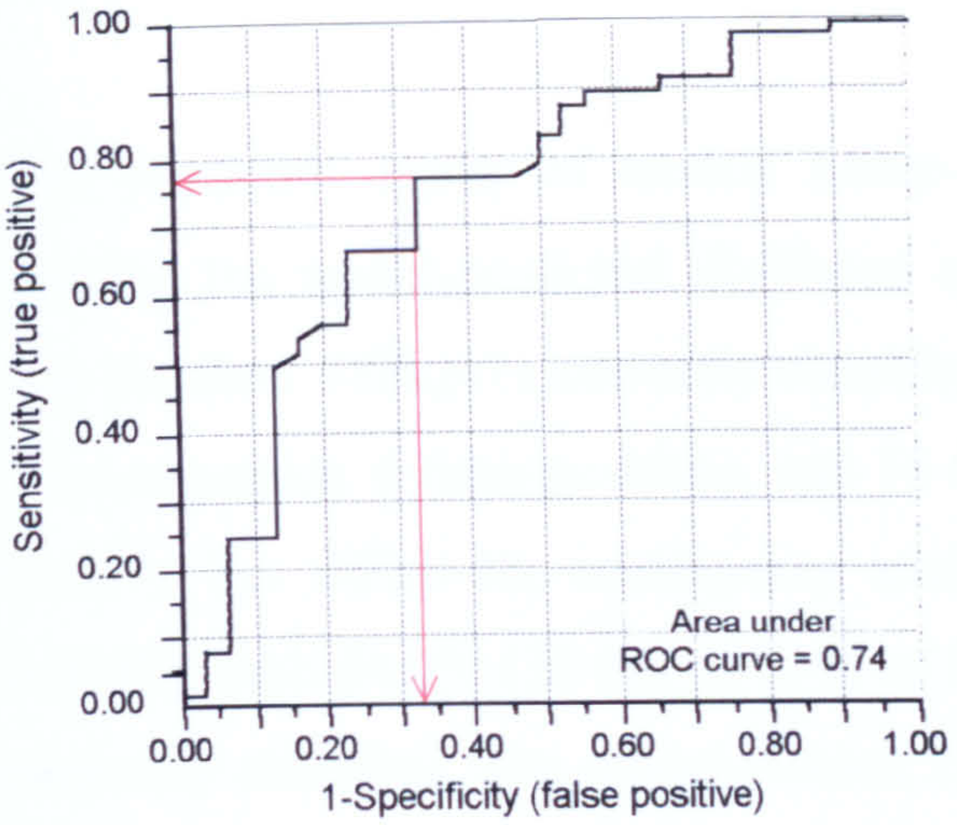


Figure 58. Receiver operating characteristic (ROC) curve derived from the logistic regression model *MRep*-1a for reported DU infestation

Considering the relatively small number of DUs for which family income was available (reflecting difficulties in obtaining this kind of information), a further model was fitted without including this covariate in the maximal initial model. In this case, both roofs with tiles and mud walls remained significantly correlated with the likelihood of infestation after likelihood ratio test-based deletion of covariates. The minimal adequate model (***MRep*-1b**) was derived from data from 110 DUs; whole model test X^2 was 25.33 (3 df, $p<0.0001$) and lack of fit test X^2 54.76 (45 df, $p=0.15$). The area under the ROC curve was 0.76 (71% sensitivity for 67% specificity, arrows in figure 59). Statistical details are presented in the following table.

Table 61. Logistic regression model for reported infestation by *Rhodnius ecuadoriensis* (*MRep*-1b)

Reported infestation	**PE (SE)**	**PE X^2 (p)**	**LR X^2 (p)**	**OR (CI)**
Intercept	-1.33 (0.4)	10.8 (0.001)	---	---
Number of chickens	0.062 (0.02)	6.97 (0.008)	8.73 (0.003)	22.7 (2.7-284)
Tiled roof	0.74 (0.3)	5.49 (0.019)	6.4 (0.01)	4.4 (1.4-17.4)
Mud walls	0.49 (0.2)	4.31 (0.038)	4.46 (0.035)	2.67 (1.1-6.91)

Whole model test $p<0.0001$; Lack of fit test $p=0.15$; PE = parameter estimates; SE = standard error; LR = likelihood ratio test; OR = odds ratio; CI = 95% confidence interval

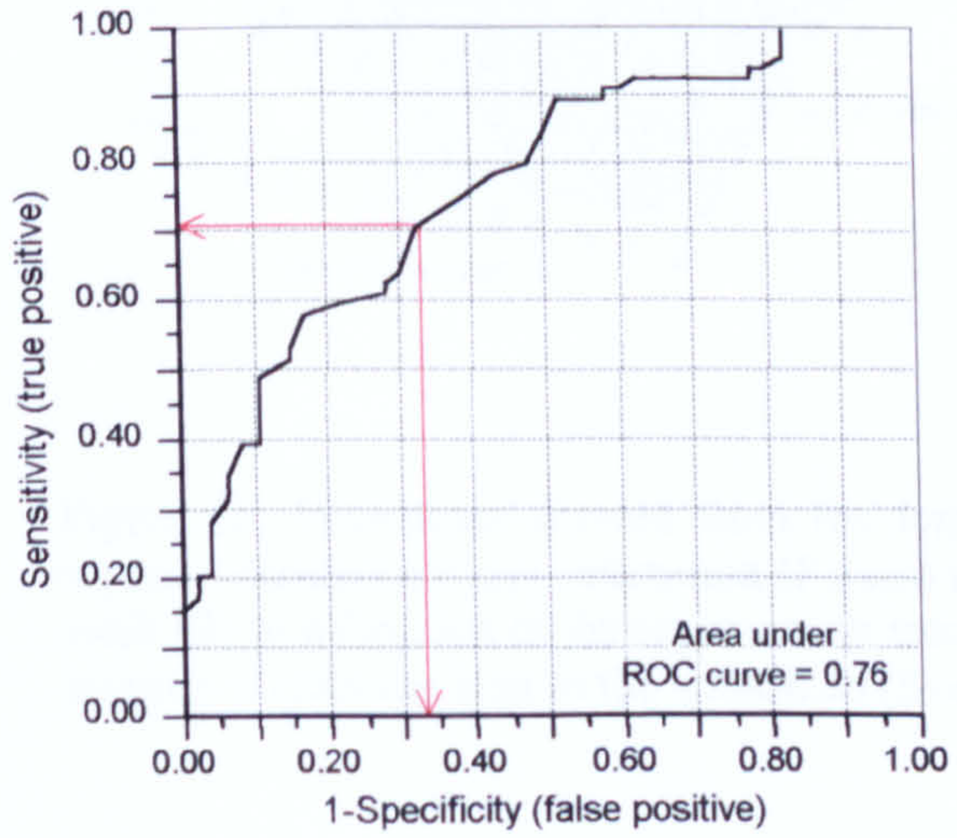


Figure 59. Receiver operating characteristic (ROC) curve derived from the logistic regression model *MRep*-1b for reported DU infestation

The predictive ability of models *MRep*-1a and *MRep*-1b was further checked by comparing the model-predicted likelihood of reported infestation and the actual report by the dwellers. *MRep*-1a correctly classified 56 of 78 (71.8%) DUs (14 true-positives, 42 true-negatives, 6 false-positives, and 16 false-negatives; FET p=0.0012; kappa=0.36, SE=0.01). For *MRep*-1b, contingency analysis showed a significant correlation (FET p=0.0037), with 64.6% (71 out of 110) of DUs correctly classified and 35.4% (39/110) erroneously classified. The model yielded 29 true-positives, 42 true-negatives, 22 false-positives and 17 false-negatives (kappa=0.28, SE=0.09). The predictions of both *MRep*-1a and *MRep*-1b were displayed graphically to show the relationships between the likelihood of reported infestation and the covariates in each model. Three- (*MRep*-1a, with one discrete and two continuous covariates) and a two-dimensional graphs (*MRep*-1b, with one continuous and two discrete covariates) were produced in Excel worksheets using the logit expressions of the models.

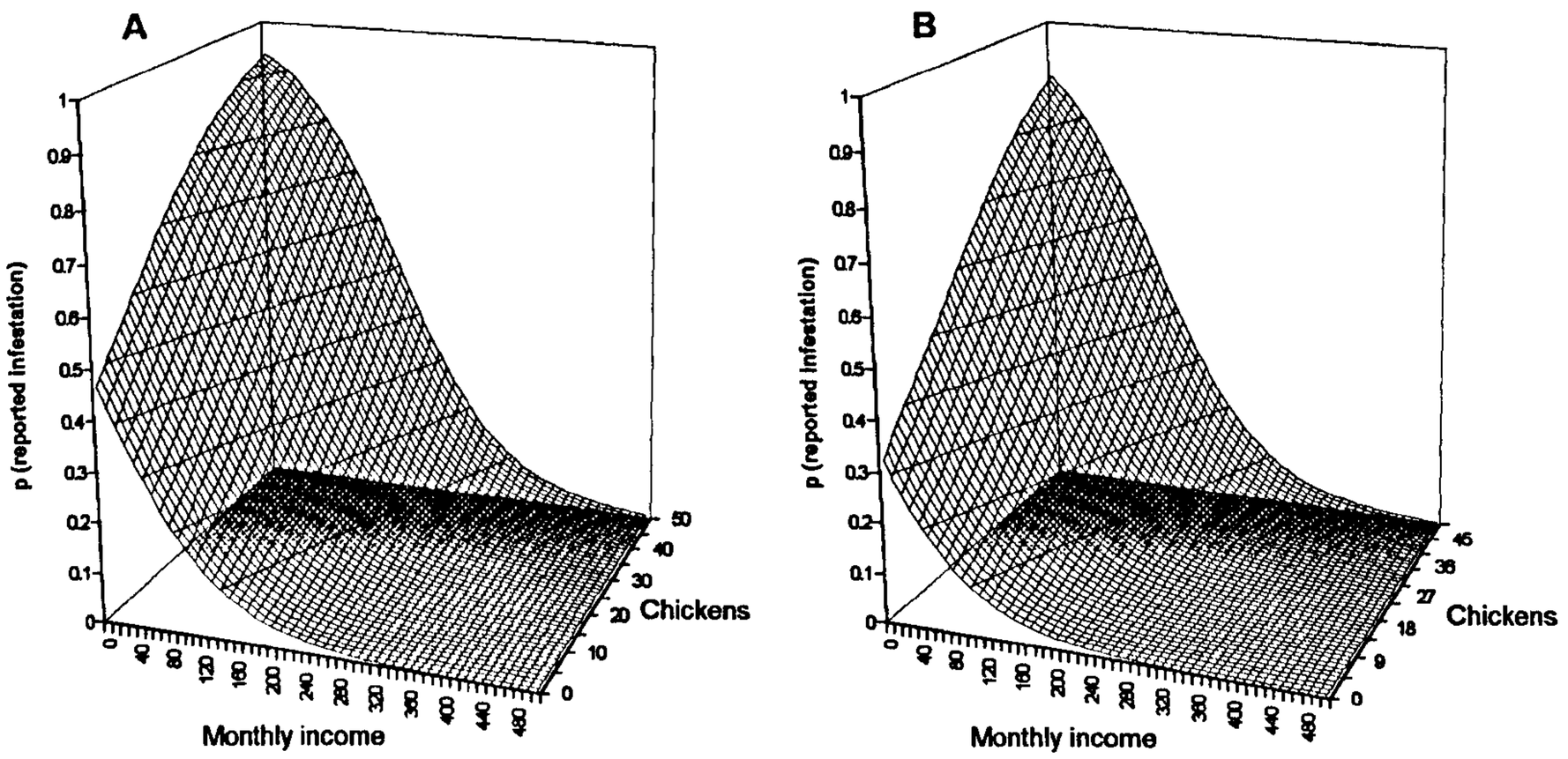


Figure 60. Predictions derived from the logistic regression model *MRep*-1a: probabilities of reported domiciliary unit infestation (*Y* axes) in households with tiled roofs (**A**) and other type of roofs (**B**) as a function of the approximate monthly income (from zero to 500 US dollars) and the number of chickens kept in the household (from none to 50)

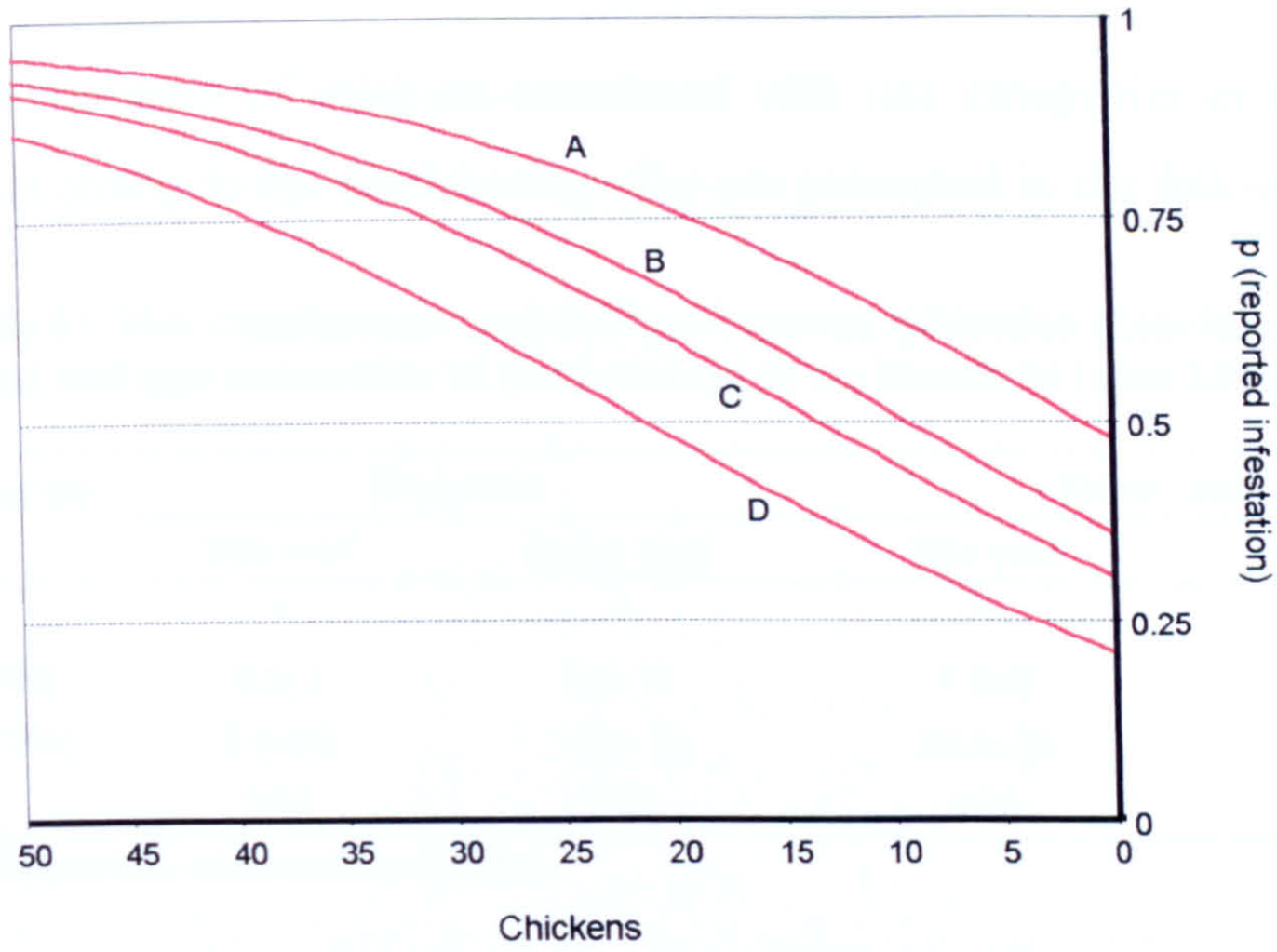


Figure 61. Predictions derived from the logistic regression model *MRep*-1b: probability of reported DU infestation (*Y* axis) for varying household conditions (A=house with tile roof and mud walls; B=tile roof and no mud walls; C=mud walls but no tiled roof; and D=house with no mud walls nor tiled roof) and decreasing numbers of chickens (from 50 to none). Dotted horizontal lines indicate 25%, 50%, and 75% probabilities of the dweller reporting infestation; these correspond to risk categories shown in table 63 below

According to results of *MRep*-1a, vector control field surveys could incorporate a system for ranking the risk of household infestation as a function of the type of roof (tiled vs. others), the reported family income, and the number of chickens kept in the DU. The following table presents a simplified proposal in this sense: four risk categories (low, medium, moderate, and high) and three income groups (up to 60US$/month [i.e., families below poverty line], between 60 and 150US$/month, and between 150 and 250) were arbitrarily defined for illustration; the table gives the number of chickens associated with each risk segment and income group in households with different types of roofing.

Table 62. Risk classification: probability of reported infestation according to roof type, approximate monthly income of the families, and number of chickens kept in the household (after *MRep*-1a)

Income*	**Tiled roof**			**Other roof**		
Risk	**up to 60**	**61 to 150**	**151 to 250**	**up to 60**	**61 to 150**	**151 to 250**
Low (0-25%)	**	0 to 17	0 to 49	0 to 9	0 to 33	>50***
Medium (25-50%)	0 to 18	18 to 22	50 or more	10 to 29	>33	**
Moderate (50-75%)	19 to 38	23 to 42	**	30 to 49	**	**
High (>75%)	>38	>42	**	>49	**	**

*Income in US$; **not applicable, independently of the numbers of chickens; ***maximum number used for simulations

Similarly, the numbers of chickens associated with risk categories in different types of household (according to the model *MRep*-1b) are presented in the following table.

Table 63. Risk classification: probability of reported infestation according to wall and roof type and number of chickens kept in the household (after *MRep*-1b)

Type of DU	Mud walls		Other walls	
Risk	**Tile roof**	**Other roof**	**Tile roof**	**Other roof**
Low (0-25%)	*	*	*	0 to 3
Medium (25-50%)	0 to 1	0 to 13	0 to 9	4 to 21
Moderate (50-75%)	2 to 16	14 to 30	10 to 26	22 to 38
High (>75%)	>16	>30	>26	>38

* not applicable, independently of the numbers of chickens

In order to deal with the second of the aforementioned objectives, a model was fitted for reported infestation (***MRep*-2**) only containing covariates amenable to intervention. This analysis was aimed at describing key ecological features of synanthropic bug populations, represented by various characteristics of each household. Past reported infestation was not considered as the most adequate response variable, since we were attempting to explore the relationships between the bugs and the *current* environmental conditions in each dwelling. This led to the decision of using *recent* reported infestation (dwellers reporting having seen the bugs in their households within the year before fieldwork) as the response variable for this model.

The maximal model for recent reported infestation included a selection of covariates drawn from a large number of candidate variables (table 64). Many of these variables were sub-groups or aggregates of others, as in the case of the various sub-divisions of domestic animals; only non-redundant covariates were included in the models. The initial model contained four covariates: houses with unplastered walls and a roof made mainly with tiles ('house conditions' for short), total number of chickens in the household, total number of dogs, and storage of products (firewood, crops etc.) inside the house. The analysis of variations in deviance caused by the deletion of each covariate led to the exclusion of product storage.

Table 64. Candidate covariates for recent reported domiciliary unit infestation by *Rhodnius ecuadoriensis*: univariate analyses and multivariate logistic regression modelling

Recent reported infestation (<1 year)	**Univariate statistics**				**Multivariate modelling***	
	Univariate tests		**Logistic regression**			
	Test statistic	p value	LR X^2 (p)	uOR (CI)	LR X^2 (p)	OR (CI)
'House conditions'	(FET)	0.002	10.8 (0.001)	7.2 (2-33)	4.5 (0.034)	4.45 (1.12-22.7)
Number of chickens	WT X^2=6.6	0.01	10.3 (0.0013)	28 (3.7-265)	4.3 (0.037)	11.5 (1.15-137)
Dogs total	WT X^2=4.7	0.03	6.4 (0.012)	13.6 (18-113)	5.3 (0.02)	18.2 (1.6-233)
Product storage indoors	(FET)	0.01	7.25 (0.007)	4.2 (1.5-14)	excluded	
Biomass	t=2.1[a]	0.039	4.7 (0.03)	23 (1.3-575)	not included	
Animals peridomicile	WT X^2=4.02	0.045	5.4 (0.02)	13.4 (1.5-146)	not included	
Mammals peridomicile	WT X^2=1.9	0.17	4.9 (0.03)	19 (1.4-294)	not included	
Birds peridomicile	WT X^2=2.97	0.08	7.86 (0.005)	16.3 (2.35-126)	not included	
Dogs peridomicile	WT X^2=8.1	0.0043	9.26 (0.0023)	20.7 (3-170)	not included	
Chickens peridomicile	WT X^2=3	0.08	7.4 (0.0064)	16 (2.2-131.5)	not included	
Animal places cleaning	(FET)	0.011	7.8 (0.0053)	6 (1.7-24.6)	not included	

*Data from model *MRep*-2 (see text); LR = likelihood ratio test; uOR = unadjusted odds ratio; CI = 95% confidence interval; OR = adjusted odds ratio; FET = Fisher's exact test; WT = Wilcoxon test; a=115 df; b=118 df; c=116 df; rest 1 df

The resulting model (*MRep*-2) included data from one hundred domiciles. The overall significance of the analysis was assessed by a whole model test; the associated significance probability was p<0.0001 (X^2=21.6, 3 df), and the lack of fit test yielded a p=0.9 (X^2=49.6, 64 df). Parameter estimates are shown in table 65. ROC curve analysis shows 85% sensitivity associated with a specificity of 67% (arrows in figure 62); 78% sensitivity was associated with 73% specificity.

Table 65. Logistic regression model for recent reported infestation (*MRep*-2)

Recent reported infestation	**PE (SE)**	**PE X^2 (p)**	**LR X^2 (p)**	**OR (CI)**
Intercept	-3.36 (0.6)	27.5 (<0.0001)	---	---
House conditions	0.75 (0.4)	4.03 (0.045)	4.5 (0.034)	4.45 (1.12-22.7)
Chickens	0.05 (0.02)	4.2 (0.04)	4.3 (0.037)	11.5 (1.15-137)
Dogs	0.5 (0.2)	5.4 (0.02)	5.3 (0.02)	18.2 (1.6-233)

Whole model test p<0.0001; Lack of fit test p=0.9; PE = parameter estimates; SE = standard error; LR = likelihood ratio test; OR = odds ratio; CI = 95% confidence interval

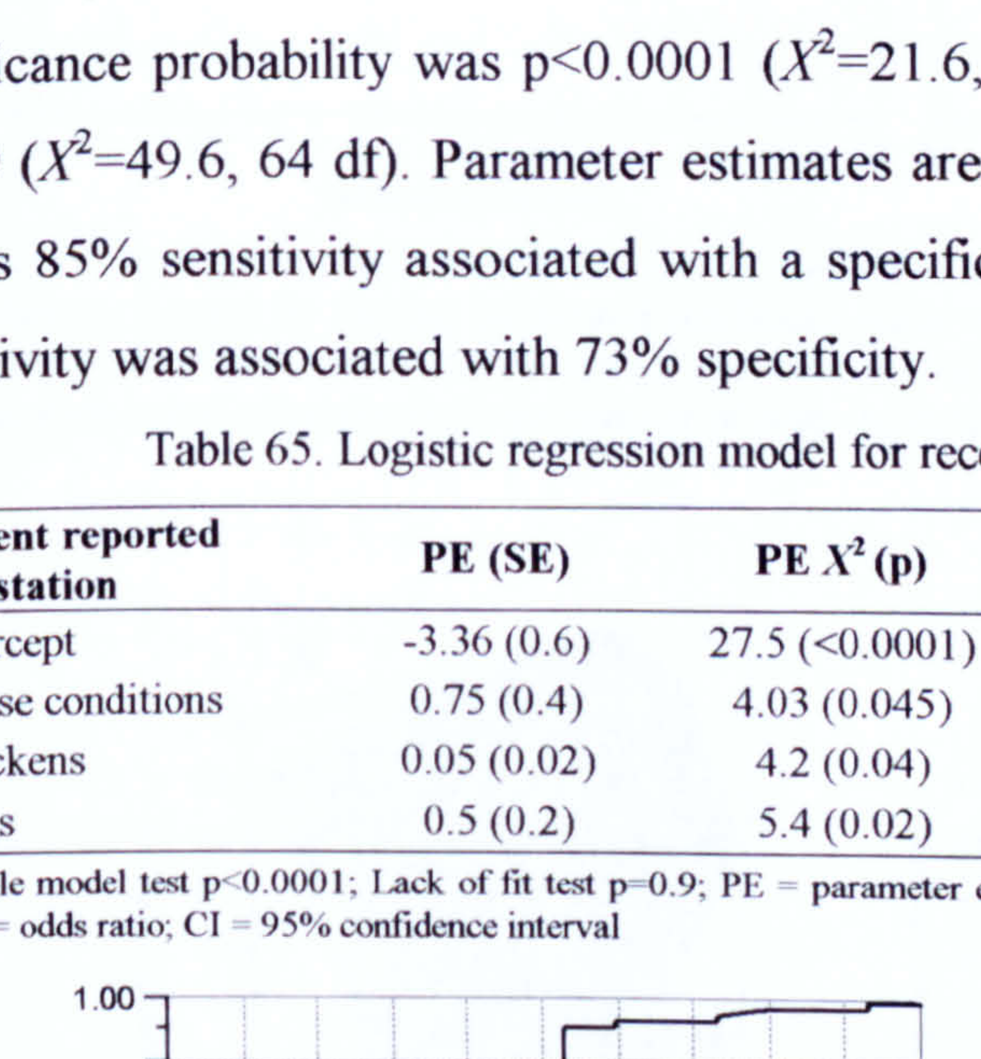


Figure 62. Receiver operating characteristic (ROC) curve derived from the logistic regression model *MRep*-2 for recent reported DU infestation

The model *MRep*-2 correctly classified 88% (88/100) of dwellings, with 6 true-positives, 82 true-negatives, 3 false-positives, and 9 false-negatives (FET p=0.0003; kappa=0.44, SE=0.13). In relation to current infestation (as found during the survey), *MRep*-2 correctly classified 87 out of 100 DUs (87%) (3 true-positives, 84 true-negatives, 6 false-positives, and 7 false-negatives [FET p=0.045]).

The predictions of *MRep*-2 for houses in different condition and with varying numbers of chickens and dogs are explored in the following figures.

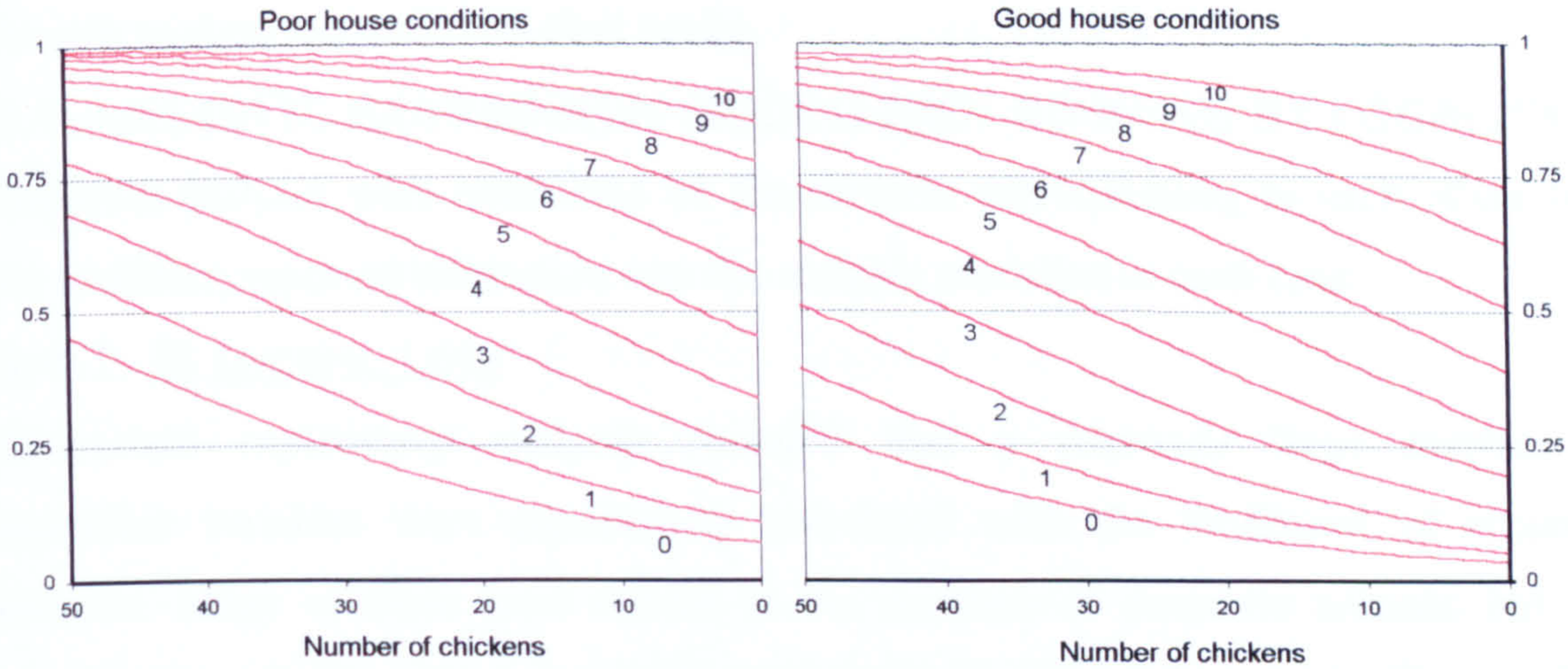


Figure 63. Predictions derived from the logistic regression model *MRep*-2 for probability of recent reported DU infestation (*Y* axes) for varying household conditions (poor = unplastered walls and tiled roofs; good = plastered walls and other type of roof) and decreasing numbers of chickens (from 50 to none; *X* axes) and dogs (from none to ten; each curve corresponds to the number of dogs indicated below the lines)

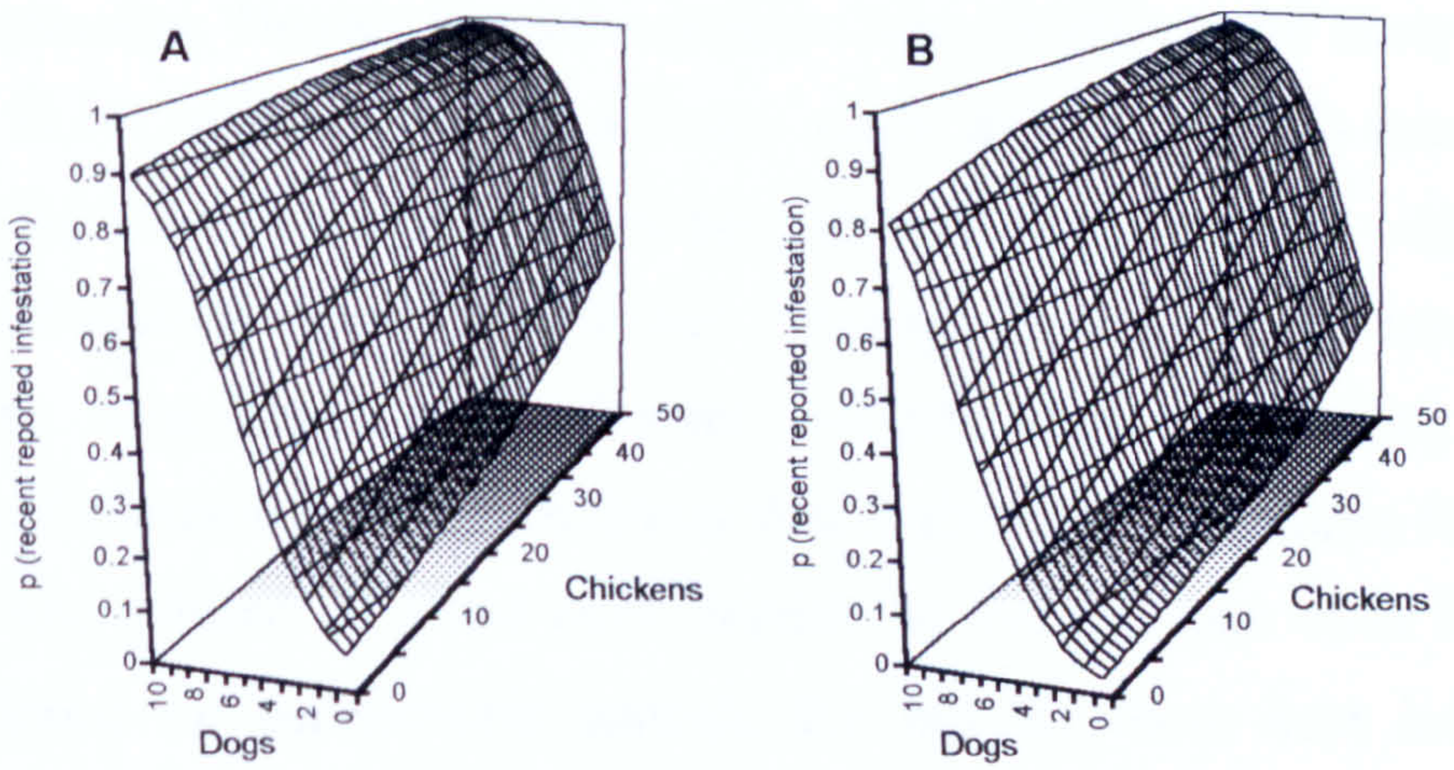


Figure 64. Predictions derived from *MRep*-2: recent reported DU infestation (*Y* axes) as a function of household conditions (**A**=poor, **B**=good, as defined above) and numbers of chickens (from none to 50) and dogs (from none to ten)

These predictions suggest that improvements on the roofing and plaster of dwellings might have a significant impact on the likelihood of those dwellings becoming infested. Houses with improved roofs and plastered walls can 'support' about seven chickens and six dogs (the median and maximum observed values, respectively) without reaching the 50% probability level. If, additionally, numbers of dogs are kept at up to three per household, the likelihood of recent reported infestation (valuable as indicator of actual infestation) does not reach 30% even in dwellings with as many as 35 chickens. Two dogs and seven chickens would be associated with a probability of about 11% (21% in DUs with unplastered walls and tiled roofs).

5.3.4. LOGISTIC REGRESSION MODELLING: RESULTS BY LOCALITY

Separate analyses were conducted on the datasets corresponding to each of the two study localities; reported infestation was the variable modelled in each case.

5.3.4.1. El Lucero, Loja

Univariate exploratory analyses revealed that a relatively large number of independent variables were significantly correlated with the likelihood of reported infestation. Many of these were related to the numbers of domestic animals, but the mere presence of either intra- or peridomestic animals did not have a significant effect in the outcome under investigation. Similarly non-significant increases in the likelihood of infestation (as reported by dwellers) were recorded for larger families, those with lower monthly incomes and for DUs where fathers and mothers had completed less years of schooling. Houses in poor condition were slightly more likely to be infested (54% vs. 34.4% of good houses), as were newer dwellings (54%) compared to older ones (45.5%). Dwellings made total or partially with mud and those with earthen floors were more frequently reported as infested. Although this was not the case for tile-roofed houses, the combination of mud walls and tile roofs significantly increased the likelihood of infestation. Further statistical details are presented in table 66.

Reportedly infested DUs had more animals on average than other households; this was confirmed for peridomestic animals, but not for those kept indoors. In more detailed analyses, the presence of peridomestic dogs had a significant effect on the likelihood of the dwellers reporting infestation (RR=2.01), but the total number of dogs did not. The presence of guinea pigs in the household was related to a significant increase in the likelihood of the dwellers reporting infestation (RR=2.05); such an

increase seemed to be independent of the number of guinea pigs kept in the DU. Overall, the average number of domestic mammals kept in reportedly infested households was higher than that in reportedly bug-free DUs, but univariate logistic regression showed the effect was marginally non-significant (LR X^2=3.8, 1 df, p=0.05, uOR=7.4, 95% CI 0.995-75.2).

On the contrary, while the presence/absence of chickens (either in general or in peridomiciles) did not have any significant effect, high numbers of these birds kept in DUs significantly increased the likelihood of reported infestation.

Other findings included the fact that storing crops or other agriculture products, firewood, or spare building materials in peridomiciles increased the frequency of reports indicating infestation; this was not the case for intradomiciliary product storage. Over 63.5% of households where peridomestic coops, pens and corrals were said to be cleaned less often than once a month were reportedly infested, vs. 31.3% of those with better hygiene practices (FET p=0.13); similarly, residents from houses reportedly cleaned on a weekly basis were slightly less likely to relate having seen the bugs in their homes. The following table presents a summary of these findings.

Table 66. Univariate analyses: reported infestation in El Lucero, Loja

Reported infestation	**Univariate tests**		**Univariate logistic regression**	
	Test statistic	p value	LR X^2 (p)	uOR (CI)
Mud in walls	(FET)	0.026	6.05 (0.014)	3.1 (1.3-7.9)
Unplastered mud walls	(FET)	0.036	4.8 (0.029)	2.8 (1.1-7.5)
Mud-and-tile house	(FET)	0.013	7.1 (0.0076)	3.4 (1.4-8.9)
Unplastered walls and tiled roof	(FET)	0.0495	4.2 (0.04)	2.8 (1.05-8.01)
Earthen floor	(FET)	0.0498	4.06 (0.044)	2.5 (1.02-6.1)
Biomass	t=2.5[a]	0.013	6.4 (0.01)	22.7 (1.96-363.5)
Total number of animals	WT X^2=6.8	0.009	9.3 (0.002)	48.5 (3.6-1151)
Number of peridomestic animals	WT X^2=6.85	0.009	10.6 (0.001)	131.5 (5.8-6415)
Total number of birds	WT X^2=6.7	0.01	10.5 (0.001)	56.5 (4.3-1423)
Number of peridomestic birds	WT X^2=5.4	0.02	8.9 (0.003)	39 (3.2-916)
Total number of chickens	WT X^2=6.9	0.009	10.5 (0.001)	63.3 (4.5-1713)
Number of peridomestic chickens	WT X^2=5.7	0.017	9.1 (0.003)	45 (3.4-1162)
Total number of mammals	WT X^2=4.2	0.04	3.8 (0.05)	7.4 (**0.995**-75.2)
Dogs peridomicile (yes/no)	(FET)	0.004	8.86 (0.003)	4.3 (1.63-12.1)
Number of peridomestic dogs	WT X^2=8.4	0.004	4.6 (0.033)	11.7 (1.2-170)
Guinea pigs (yes/no)	(FET)	0.005	8.6 (0.003)	5.5 (1.7-21.1)
Peridomestic product storage (yes/no)	(FET)	0.008	8.1 (0.0045)	4.1 (1.5-11.6)

LR = likelihood ratio test; uOR = unadjusted odds ratio; CI = 95% confidence interval; OR = adjusted odds ratio; FET = Fisher's exact test; WT = Wilcoxon test; a=79 df; t-tests were used after appropriate data transformation to verify significance probabilities obtained with non-parametric tests. In bold type: the uOR 95% CI included 1 for the total number of mammals in the household

The minimal adequate model for El Lucero (***MLoja***) contained data from 79 DUs and three covariates: dwellings with mud walls and tiled roofs ('house conditions' in the table below), the total number of chickens in the household, and the presence/absence of guinea pigs. The step-wise process of effect deletion after assessment of likelihood ratio tests led to the exclusion of (in this order) DUs with earthen floors and the number of dogs kept in peridomiciles. Whole model test X^2 was 20.7 (3 df, p=0.0001), and the lack of fit test X^2 was 48 (38 df, p=0.13). The area under the ROC curve derived from *MLoja* was >0.79, with 77% sensitivity associated with 70% specificity and 82% sensitivity with 64% specificity. *MLoja* correctly classified (as reportedly infested/not infested) 50 out of 79 DUs (63.3%); the pattern of reclassification included 33 true-positives, 17 true-negatives, 3 false-negatives, and 26 false-positives (FET p=0.0017; kappa=0.3, SE=0.09). About 91.7% (33/36) of reportedly infested DUs were correctly reclassified by the model. Regarding currently infested DUs, *MLoja* predicted six true-positives (i.e., it classified as infested 100% of actually infested DUs), 20 true-negatives, and 53 false-positives. Similarly, the model correctly reclassified 100% of DUs whose owners reported recent (<1 year) infestation, with 10 true-positives, 20 true-negatives, and 49 false-positives. All four dwellings with intradomiciliary bug colonies were classified as infested. The model and statistical details are presented in table 67.

Table 67. Logistic regression model for reported infestation in Loja (*MLoja*)

Reported infestation	PE (SE)	PE X^2 (p)	LR X^2 (p)	OR (CI)
Intercept	-0.5 (0.4)	1.29 (0.26)	—	—
House conditions	0.58 (0.26)	4.82 (0.028)	5.04 (0.025)	3.2 (1.16-9.3)
Guinea pigs (yes/no)	0.66 (0.34)	3.93 (0.047)	4.28 (0.039)	3.8 (1.07-15.7)
Chickens	0.07 (0.03)	5.4 (0.02)	7.26 (0.007)	38.3 (2.5-1207)

Whole model test p=0.0001; Lack of fit test p=0.13; PE = parameter estimates; SE = standard error; LR = likelihood ratio test; OR = odds ratio; CI = 95% confidence interval

Predictions of the model *MLoja* (the probabilities of the residents reporting infestation as a function of the number of chickens according to different house conditions and to the presence/absence of guinea pigs in the household) are presented in the following figure.

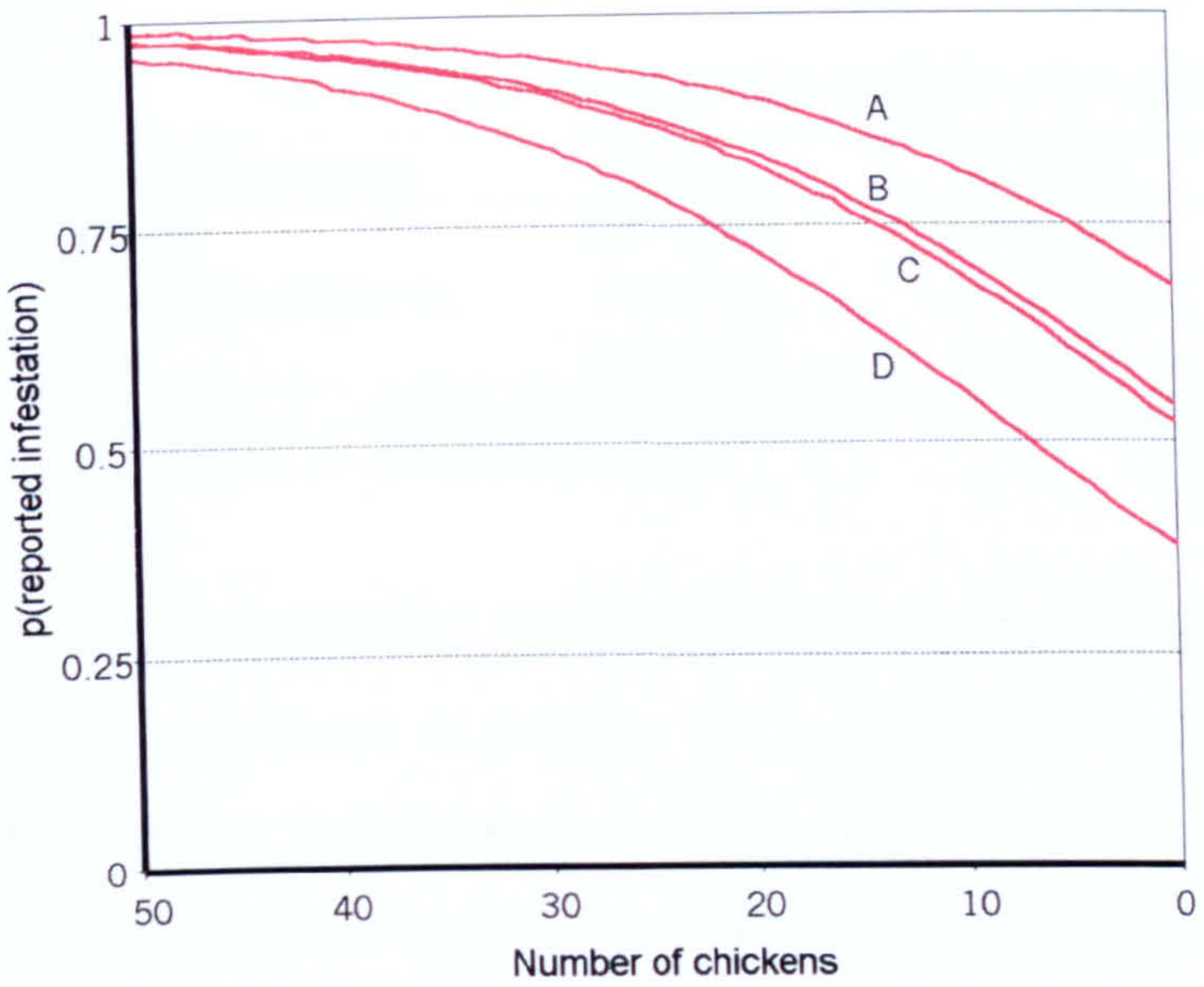


Figure 65. Predictions derived from the *MLoja* logistic regression model: probabilities of reported DU infestation (Y axis) for varying household conditions and decreasing number of chickens (X axis). **A** = mud-and-tile house with guinea pigs; **B** = other type of house with guinea pigs; **C** = mud-and-tile house without guinea pigs; **D** = other type of house without guinea pigs

5.3.4.2. Lourdes, El Oro

In El Oro (36 dwellings) only a few variables were associated with increased likelihood of reported infestation. These were used for multivariate logistic modelling. The resulting model (***MOro***) contained data from only 34 DUs and two covariates (after deletion of tiled roofs): age of the house (expressed as a categorical variable: DUs built under and over 10 years ago; older houses were more likely to be reported as infested), and number of dogs. Whole model test X^2 was 14.9 (2 df, p=0.0006), and lack of fit test X^2 6.77 (6 df, p=0.34). The area under the ROC curve was 0.85, with sensitivities of 69%, 80%, and 95% associated to specificities of 80%, 60%, and 42%, respectively. *MOro* correctly reclassified 70.6% of DUs (24 of 34): 5 true-positives and 19 true-negatives, with 3 false-positives and 7 false-negatives (i.e., it detected 41.7% of reportedly infested dwellings) (FET p=0.1; K=0.3, SE=0.17). Similarly, *MOro* performed poorly in detecting actual infestation, with 3 true-positive and two false-negative (i.e., it missed 40% of actually infested DUs); FET p was 0.07. The parameter estimates and other statistical details are presented in tables 68 and 69.

Table 68. Reported infestation by *Rhodnius ecuadoriensis* in El Oro: univariate analyses and multivariate logistic regression modelling

Reported infestation	**Univariate statistics**				**Multivariate modelling***	
	Univariate tests		**Logistic regression**		LR X^2 (p)	OR (CI)
	Test statistic	p value	LR X^2 (p)	uOR (CI)		
Age of house (new/old)	(FET)	0.01	8.03 (0.0046)	13.2 (2.03-263)	7.7 (0.0056)	25.4 (2.2-2100)
Dogs	WT X^2=4.55	0.033	7.03 (0.008)	119 (3.1-18901)	6.9 (0.0087)	155 (3.3-30258)
Tiled roof	(FET)	0.01	8.7 (0.003)	14.3 (2.2-284)	excluded	
Crowding	(FET)	0.01	3.8 (0.05)	0.24 (0.05-1.01)	not included	

*Data from model *MOro*; LR = likelihood ratio test; uOR = unadjusted odds ratio; CI = 95% confidence interval; OR = adjusted odds ratio; FET = Fisher's exact test; WT = Wilcoxon test; 34 df for *t*-test

Table 69. Logistic regression model for reported infestation in El Oro (*MOro*)

Reported infestation	PE (SE)	PE X^2 (p)	LR X^2 (p)	OR (CI)
Intercept	-2.67 (1.08)	6.16 (0.013)	—	—
Age of house (old/new)	1.62 (0.8)	4.06 (0.04)	7.7 (0.0056)	25.4 (2.2-2100)
Dogs	0.84 (0.37)	5.2 (0.02)	6.9 (0.0087)	155 (3.3-30258)

Whole model test p=0.0006; Lack of fit test p=0.34; PE = parameter estimates; SE = standard error; LR = likelihood ratio test; OR = odds ratio; CI = 95% confidence interval

5.3.5. LOGISTIC REGRESSION ANALYSIS: SEROPOSITIVITY

As mentioned in Section 1.4.2., results from 380 serological tests carried out by Ecuadorian collaborators in Loja and El Oro were available for joint consideration. Despite methodological reservations (e.g., positive results were not confirmed with an additional test, and the storage of some samples until processing seemed inadequate), we conducted some basic statistical analyses on these serological results (as reported to us by the laboratories where tests were carried out). These results must be regarded as purely tentative, but they nonetheless provide some insight (basically lacking in the literature) on the eco-epidemiology of *T. cruzi* human infection in areas where *R. ecuadoriensis* is the only synanthropic triatomine.

Logistic regression modelling was used to analyse the relative influence that different independent variables had on the likelihood of infection. A model was fitted including the covariates found to increase the risk of infection in univariate analyses: age, report of bug bites, and cohabitation (defined as the presence of *another* seropositive in the household where the case under consideration resided). No correlation with infestation or any other putative risk factor was detected. Likelihood ratio tests showed that removing age did not have a significant effect on the model; the minimal adequate model (***MSer***) was therefore fitted with the remaining two effects. The whole model test X^2 was 19.99 (2 df, $p<0.0001$), and the lack of fit test X^2 0.014 (1 df, $p>0.9$). Parameter estimates and other statistical details are presented in tables 70 and 71.

Table 70. Covariates for seropositivity: univariate analyses and logistic regression modelling

Seropositivity	Univariate statistics				Multivariate modelling*	
	Univariate tests		Logistic regression			
	Test statistic	p value	LR X^2 (p)	uOR (CI)	LR X^2 (p)	OR (CI)
Report of bug bites	(FET)	0.001	11.5 (0.0007)	4.2 (1.8-10.5)	10.5 (0.0012)	4.02 (1.7-10.2)
Cohabitation	(FET)	0.002	9.45 (0.002)	3.6 (1.6-7.9)	8.5 (0.0036)	3.97 (1.6-9.5)
Age ($[x]^{1/3}$)	t=2.3	0.024	4.9 (0.03)	5 (1.2-24)	excluded	

*Data from model *MSer*; LR=likelihood ratio test; uOR=unadjusted odds ratio; CI=95% confidence interval; OR=adjusted odds ratio; FET=Fisher's exact test; 1 df for all X^2 tests; 365 df for the t-test

Table 71. Logistic regression model (*MSer*) for seropositivity

Seropositivity	PE (SE)	PE X^2 (p)	LR X^2 (p)	OR (CI)
Intercept	-2.12 (0.2)	80.6 (<0.0001)	—	—
Report of bug bites	0.7 (0.2)	9.6 (0.0019)	10.5 (0.0012)	4.02 (1.7-10.2)
Cohabitation	0.7 (0.2)	9.3 (0.0022)	8.5 (0.0036)	3.97 (1.6-9.5)

Whole model test p<0.0001; Lack of fit test p>0.09; PE=parameter estimates; SE=standard error; LR=effect likelihood ratio test; OR=odds ratio; CI=95% confidence interval

The area under the ROC curve derived from *MSer* was 0.73, with poor relationships between sensitivity and specificity (e.g., sensitivities of 57% and 80% were associated with specificities of 80% and 47%, respectively). However, the predictive ability of the model was only of secondary interest, because no diagnostic approximation to seropositivity based on modelling results was to be used. When model predictions were explored, *MSer* classified all cases as seronegatives.

5.4. Discussion

5.4.1. GENERAL RESULTS: DESCRIPTIVE STATISTICS

5.4.1.1. Overall entomological indices

Comparing entomological indices as reported from triatomine field studies is not straightforward, despite the epithet of 'standardised' often applied to those indices after the WHO Technical Report on Chagas disease control (WHO 1991). The difficulty may be due to differences in bug species biology and population dynamics; it is however the methodological differences that really make comparisons complicated, even among studies on a single species. Apart from the selection of study areas (e.g., in relation to known levels of endemicity or prevalence of certain vector species) and the representativity of the samples, the use of flushing-out (pyrethroids) or knock-down agents (γ-BHC) to dislodge bugs may increase the sensitivity of manual searches, which in turn vary in effectiveness depending on factors such as the experience and motivation of the inspectors, the density of the bug population, the bug species involved, the structure of the houses, or the time spent examining each dwelling; longitudinal detection (e.g., with sensor boxes, paper sheets, or encouraging report of infestation by householders) performs even better. Infestation rates and apparent bug densities may thus vary widely, and discerning the effects of methodological variants may be problematic (Schofield 1978, Wood et al. 1993, Gürtler et al. 1993, 1995, Silva et al. 1999). For the general purposes of this discussion however, we have reviewed the results of some entomological surveys that may provide a suitable context for the

interpretation of our data; they were considered of interest mainly because some deal with the primary disease vectors and some refer to Ecuador and neighbouring countries (Colombia and Peru).

·Dispersion. Domestic-peridomestic infestation by *R. ecuadoriensis* seems relatively frequent in western Ecuador-northern Peru, but area-wide surveys are lacking. In Ecuador, synanthropic colonies of *R. ecuadoriensis* were detected in 25.5% of 145 localities in the province of Manabí, with 97% of bugs collected from peridomestic pigeon and chicken houses; in the same survey, *T. dimidiata* was found in 74.5% of villages (mainly indoors), and both species coexisted in 22% of localities (Defranc 1982, Lazo 1985, Ministry of Public Health, unpublished data). In northwestern Peru, Cuba Cuba et al. (2002) reported infestation (mainly intradomiciliary) in 47.6% of 21 localities surveyed in the Cascas district (Department of La Libertad). For comparison, in Venezuela (where *R. prolixus* is the main vector) reported dispersion indices were 25.1% in 1997 and 22.5% in 1996 (Guhl 1999); in Bahia, Brazil, dispersion of *T. infestans* fell from 36.9% (1979) to 2.7% (1994) because of control activities, but in Ceará, where the native *T. brasiliensis* prevails, the index remained high (97.3% in 1979 and 70.6% in 1993/94) in spite of control efforts (Silveira 1996).

·Infestation. We investigated 188 DUs in three communities, and found evidence of infestation in 15 of them (8%). This overall infestation index is substantially higher than the 0.7% reported for Manabí (canton Portoviejo; overall data from >12000 DUs surveyed in 20 localities) by Lazo (1982), but lower than the 14% (out of 259 DUs inspected) found in La Libertad, Peru, where preliminary results from longitudinal surveillance confirmed infestation rates of ~35% (Cuba Cuba et al. 2002). Herrer et al. (1972) investigated 121 households in northern Peru and found live *R. ecuadoriensis* specimens in seven (5.8%) of them. Average infestation rates (mainly by *R. prolixus*) in Venezuela were 1.8% in 1996 (data from over 40000 DUs) and 2.7% in 1997 (>39000 DUs inspected), with higher values recorded from some States: 10% in Guárico, 8% in Portuguesa (1997), or 13% in Lara (1996) (Guhl 1999); in Honduras, infestation by *R. prolixus* was detected in 35% of 375 DUs in 7 localities (Ponce 1996). Starr et al. (1991) reported 16% of ~500 DUs infested by *T. dimidiata* in Costa Rica, a value similar to the 12% found by Acevedo et al. (2000) in 49 DUs in Nicaragua. Low indices (1.5% to 3%) have been reported for *T. dimidiata* in Ecuador (Aguilar & Yépez 1996),

but bugs were found in over 40% of DUs in urban areas of Guayaquil (248 DUs inspected) and various localities of Manabí (>22700 DUs surveyed) (Defranc 1982, Lazo 1985); Ponce (1996) found 53% of 375 DUs infested in eight Honduran localities. *T. infestans* may infest over 90% of dwellings in some endemic areas of Argentina (Gürtler et al. 1992), over 42% in eastern Paraguay (Rojas de Arias et al. 1999), over 70% in southern Bolivia (Guillén et al. 1997), and 100% in hyperendemic areas of Cochabamba in the same country (Pless et al. 1992); a wide investigation in central Brazil (State of Goiás) found evidence of infestation by this species in 13% of 4232 DUs (Andrade et al. 1995a). In Bahia, Brazil, *T. infestans* was found in 66% of 2175 households in 1979, but control activities had reduced the rate to less than 1% (of 3620 DUs inspected) by 1994 (Silveira 1996). Regarding *T. brasiliensis*, infestation dropped from 57% (1979; 2500 DUs) to 8.6% (1994; 4500 DUs) in Ceará, Brazil, after the implementation of control measures (Silveira 1996), and pre-spray rates of about 24% (363 DUs) were reported from northeast Brazil (Oliveira Filho et al. 2000). Finally, *P. megistus* was found in 18% (out of 163), 22% (of 174), and 25% (of 183) of households in three surveys carried out in the same locality (in 1982, 1979, and 1974, respectively) in Bahia, Brazil (Piesman et al. 1985). Apart from the confirmation that *R. ecuadoriensis* may infest a significant proportion of dwellings (from 8% to 35%) in some areas within its relatively large geographic range (from central Ecuador to the Department of La Libertad in Peru), these infestation indices are well above the 5% proposed as a threshold value to indicate the need for extensive insecticide spraying of all houses in positive communities (Dias & Schofield 1999). The values are within the range of those reported from endemic areas where *R. prolixus* (e.g., 8% infestation in Portuguesa, Venezuela) and *T. infestans* (12.6% in Goiás, Brazil) are primary vectors. Considering that human infection rates are about three times higher in areas of Ecuador where *R. ecuadoriensis* prevails (7% to 10%) than in those where *T. dimidiata* is the main vector (2% to 4%) [a difference comparable to that between *dimidiata* and *R. prolixus* in Central America (see Section 1.4.2. and Ponce 1996, Paz-Bailey et al. 2002)], it seems obvious that the data fully endorse the need for a comprehensive control programme against *R. ecuadoriensis*.

·Crowding and density. Synanthropic colonies of *R. ecuadoriensis* appear to be small, with reported mean crowding (bugs/infested DU) of 19 (northern Peru; Herrer et

al. 1972) and eight [density=0.8 bugs/surveyed DU] (northern Peru; Cuba Cuba et al. 2002). However, in surveys conducted during the early 80s in Manabí over 5800 *R. ecuadoriensis* were collected from 79 DUs (mainly in peridomestic dovecotes and chicken coops; crowding=74, density=0.5) (Defranc 1982, Lazo 1985). We collected 691 specimens (excluding over 1000 eggs, most of them hatched) from peri- and intradomiciliary habitats of 15 households, yielding an average of 40 bugs/infested DU [density=3.7]. We thus confirmed that the potential of *R. ecuadoriensis* to build large synanthropic colonies may be substantial; specifically, we found that this applies also to southern, heavily synanthropic populations of Loja and El Oro (with average crowding of 62 bugs per infested DU, and 68 bugs/DU [density 5.7] excluding one domicile where only adult specimens were found). Over 220 bugs of all stages were collected from a single chicken nest in a household in Loja, where a detailed search yielded 279 live bugs (Abad-Franch et al. 2002).

Table 72. *Rhodnius ecuadoriensis* colonies in a heavily infested dwelling (house MP, El Lucero, Loja)

Site of capture	Eggs			Nymphs (instars)					Adults	Total
	Hatched	Not hatched		I	II	III	IV	V		
		alive	dead							
Beds	221	10	8	5	4	3	2	2	4	20 bugs/239 eggs
Chicken nests[a]	nr	nr	nr	35	72	77	7	6	27	224 bugs
Chicken nests[b]	nr	nr	nr	2	4	2	4	13	10	35 bugs
Total	221	10	8	42	80	82	13	21	41	279 bugs/239 eggs

nr = not recorded; *a*: two adjacent chicken nests dissected on white cardboard; *b*: bugs from two chicken nests and the hollow tree where these nests were located

Apart from large numbers of bugs collected in house demolition studies [e.g., >7900 *R. prolixus* in a single house in Venezuela (Rabinovich et al. 1979), and over 11400 bugs of the same species in Colombia (Sandoval et al. 2000a)], crowding averages of 39 [density 28.4] (*T. infestans*, Argentina; Gürtler et al. 1991), two [density 0.8] (*T. infestans*, Paraguay; Rojas de Arias et al. 1999), and 2.4 [density 1.4] (*T. brasiliensis*, Brazil; Silveira 1996) have been reported. Ramsey et al. (2000) found 10.1 *T. dimidiata* per infested DU in 13 localities of Oaxaca, Mexico. However, and as observed by Gürtler et al. (1992), the customary use of mean values as point estimates (and without parameters of dispersion) of bug density is probably unsound, because the frequency distribution of this kind of data is typically not normal; the practice is widespread mainly because a mean value was recommended by WHO in 1991; the "crowding index" was defined as the total number of bugs captured divided by the total number of

infested households [*multiplied by 100*, to further complicate things] (WHO 1991; see also Schofield 2001).

·Colonisation. Evidence of active breeding (eggs and nymphs) was found in all infested DUs except two, where only adult bugs were detected. These were most likely adventitious bugs flying into the houses from palms (Manabí) or neighbouring infested dwellings (El Oro); Carcavallo and Martínez mentioned the frequent finding of adult bugs of this species flying into houses "attracted to light" in central-western Ecuador, where eggs were found in palm trees (Carcavallo & Martínez 1985; pp. 156-157).

5.4.1.2. General socio-economic features of the study localities

·Family composition. The median family in our study sites was composed of 5 members (6 in Manabí and 4 in Loja or El Oro) living in a two-bedroom house; they had typically resided in their community for over 30 years and were not immigrants. They owned several chickens (about 10) and at least one dog.

·Income and poverty. Our study localities are small rural villages where the main economic activities are related to small-scale (often subsistence) agriculture and farming. Most of the residents are poor, with family monthly incomes not above the estimated average of 50-60 US$. Living standards were somewhat lower in Loja than in El Oro or Manabí, and this was confirmed by a significantly worse average income (about 35 US$/month) and an increased likelihood of earning less than 50 US$/month (OR≈7). It was estimated that 56% of the total Ecuadorian population lived below poverty line[+] in 1999 (46% in 1998); it is additionally well recognised that poverty is more widespread in rural areas (where poverty reached 77% in 1999, vs. 42% in urban areas), and that while the richest (largely urban) 10% of the population capitalises over 45% of the income, only 1% is available to the poorest 10%. In the rural areas of Loja where we conducted fieldwork indigence affects an estimate 42% of the people, with 95% of them living in poverty; the figures are slightly better in Manabí (20% and 80%, respectively) and the situation seems less dramatic in El Oro (9% and 53%, respectively) (Vos 2000, SIISE 2002).

In a general assessment of all families from which income data were obtained (n=143), we found that those living in infested households were significantly poorer. As

---

[+]Poverty line was estimated as 60 US$ (considered the threshold below which expenses related to the basic family needs cannot be met) for this calculation (cf. Vos 2000; p. 38)

mentioned above, the recognition of the relationship between poverty and Chagas disease is as old as the concept of the disease itself (Chagas 1909, 1911). This has important social, political, and philosophical implications. Perhaps the most salient is the acknowledgement that "social and political development could be sufficient to control Chagas disease" (Dias & Schofield 1999; p. 103); in other words, the realisation that the public health disaster (which probably reached its overwhelming dimensions only after Chagas first warnings) can largely be attributed to political neglect and lack of social responsibility – tightly linked in turn to the scarce political weight of the affected populations (Schofield & Dias 1996, Dias & Schofield 1999, Morel 1999, Abad-Franch & Aguilar 2000). Being largely a disease of those living in destitution, it has been noted that triatomine control may not be a top priority for the population at risk; they may tend to be more concerned with immediate survival issues, and the concept of a link between bugs and long-term health complications is often present only in the minds of the researchers (Barrett 1991, Briceño-León 1996, Dias 1998b).

·Formal education. Nearly half (43.5%) of 329 adults interviewed had completed their primary school education, but only 8.5% reported having studied further, and over 20% had no formal education. Although literacy indices are good in Ecuador (with reported average rates above 90%), the estimates for our study areas are somewhat lower (78% in Manabí, 87% in El Oro, and 84% in Loja); additionally, functional illiteracy reaches 53%, 35%, and 33% in the three zones (SIISE 1999). We accordingly found lower levels of formal education in Manabí.

5.4.1.3. Patterns of vector identification

Generally speaking, bugs that are frequently found infesting DUs in a particular area will be more likely to be identified by the dwellers among a collection of specimens and pictures. Our own collection included the most important local vectors (*T. dimidiata* and *R. ecuadoriensis*) and some secondary species that were known to occur in human habitats in the study areas (*P. rufotuberculatus*, *P. chinai*, and *T. carrioni*) (Abad-Franch et al. 2001b; see also Section 3.3.). The overall percentage of dwellers able to recognise *R. ecuadoriensis* (56%) was unevenly distributed: only 24% in Manabí, 42% in El Oro and a large 88% in Loja (LR $p<0.0001$). In the absence of any educational intervention that could have increased awareness in the southern localities, this could be attributed to a closer relationship between bugs and people in Loja (and to a lesser

extent in El Oro) than in Manabí; we therefore advanced the working hypothesis of a differential degree of synanthropism in the various populations of *R. ecuadoriensis* under investigation. Andrade and coworkers used a similar approach in central Brazil, and found that 90% of residents correctly identified *T. infestans* in a preparation with 8 hemipterans; the proportion was significantly higher among those living in infested households (94% vs. 83% of those living in vector-free DUs; Andrade et al. 1995b). In a survey conducted in a heavily endemic area of Cochabamba, Bolivia, Pless et al. (1992) found that all of the dwellers interviewed in Tabacal (a small rural community where all houses were infested) were able to correctly identify *T. infestans*.

We also detected interesting patterns of recognition of other triatomine species (179 answers). Thus, 35 people could recognise both *ecuadoriensis* and other species: 10 in Manabí, 8 in El Oro, and 17 in Loja; 61 people identified only *R. ecuadoriensis* specimens: 6, 7, and 48 in the three localities, respectively; 34 knew other species but not *ecuadoriensis* (27 of them in Manabí, 4 in El Oro and just 3 in Loja); and 49 people did not identify any species (25 in Manabí, 17 in El Oro and 7 in Loja). The following figure shows these trends.

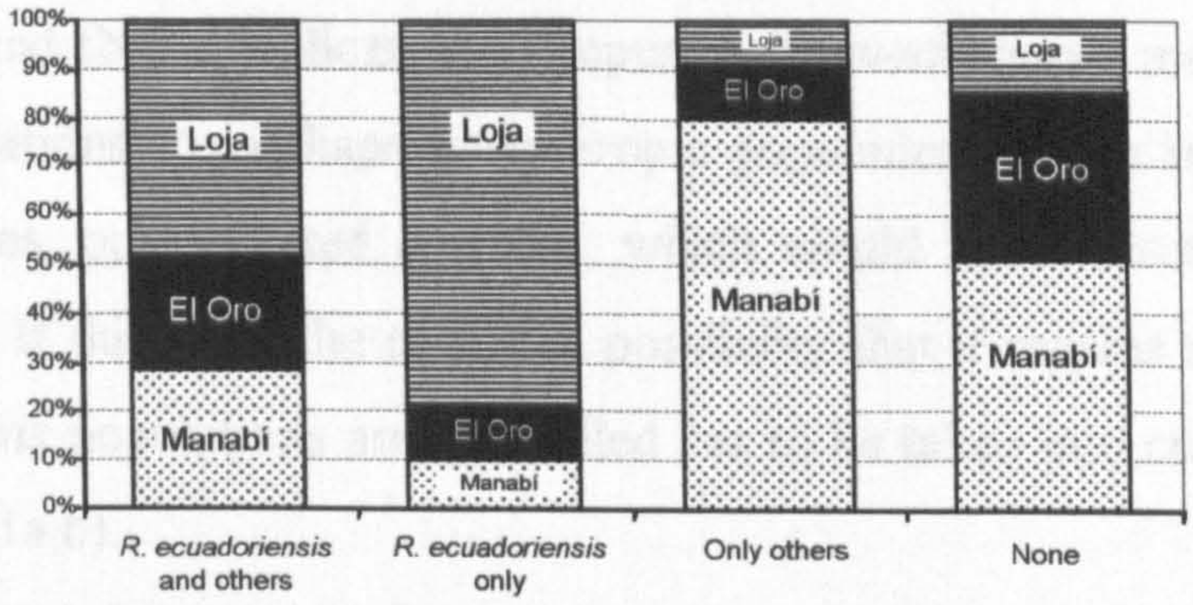


Figure 66. Entomological survey: patterns of vector identification by householders

It would seem therefore that *R. ecuadoriensis* is well recognised in Loja but not in Manabí. When analysing which particular species the dwellers recognised, it became apparent that *T. dimidiata* was the only species identified in Manabí, where in addition only 18% of householders had seen *R. ecuadoriensis* in or around their present or past domiciles. This corresponds well with the known occurrence of strongly synanthropic populations of *T. dimidiata* in the Portoviejo river basin, where the species was found in 32% of over 14200 DUs of 19 localities (including Pachinche Adentro, where both *dimidiata* and *ecuadoriensis* were collected) in a survey carried out in the early 1980s (Lazo 1985; Ministry of Public Health, unpublished data). In El Oro, *P. chinai* was

identified by one person, *P. rufotuberculatus* by two people, six identified *T. dimidiata* and two more thought they recognised *Triatoma* bugs without clearly distinguishing which species; it has been reported that in some localities of El Oro *P. rufotuberculatus* breeds within domiciles, and *P. chinai* may also display some degree of synanthropism in the area and in northern Peru (Barrett 1991, Avilés et al. 1995b, Abad-Franch et al. 2001a,b, Cuba Cuba et al. 2002). In Loja only five residents indicated a species other than *ecuadoriensis*: three indicated *T. dimidiata* and two *T. carrioni*; although only a single record of *dimidiata* exists from Loja (Abad-Franch et al. 2001a,b), it would not be surprising that some domestic specimens had passively spread to the temperate Andean valleys of the province carried by people. We however did not find evidence of the current presence of this species in any of our fieldwork localities. *T. carrioni* is an Andean triatomine whose existence in the valleys of Loja is well documented; it was considered (at least until about the 1960s) as an important domestic pest in southern Ecuador, where many records match the current distribution of synanthropic populations of *R. ecuadoriensis* (Defranc 1982, Lazo 1985, Abad-Franch et al. 2001b). The spatial and temporal coincidence between the apparent decline of *T. carrioni* in human habitats and the multiplication of reports of *ecuadoriensis* makes it tempting to hypothesise a relationship: perhaps synanthropic populations of the latter species, better adapted to houses, outcompeted *carrioni*, which would have retreated to its original sylvatic habitats. If this were the case, the possibility that it regains synanthropic habits after *ecuadoriensis* populations are controlled has to be taken into consideration (Abad-Franch et al. 2001a,b).

Reports of past infestation by *R. ecuadoriensis* (present homes) were more frequent in Loja (45%) and El Oro (33%) than in Manabí (13%), further suggesting that the vectors display a higher degree of synanthropism in the south. Five people in Manabí said they had seen the bugs inside their homes (on walls, flying around a light bulb, and inside beds in two occasions), and three referred to peridomestic environments (a chicken nest, firewood stored under the raised house, and a tagua palm). Together with results discussed above, this would seem to suggest that the bugs are only sporadic visitors in houses in our fieldwork community in Manabí. On the contrary, a closer association with human environments would account for the results obtained in El Oro (where 3 answers referred to beds, 3 more to chicken nests, one to bugs flying to lights,

one to house walls, and one to a peridomestic storeroom) and above all in Loja (where 14 answers mentioned beds or bedrooms, 23 walls or mud houses, 8 chicken coops, 2 other peridomestic structures, and 2 referred to the bugs being attracted to electric light).

All these results appear to indicate that a highly synanthropic bug population infests domiciles in Loja, living in close association with their human hosts and reaching high densities under favourable circumstances. In contrast, in Manabí the bugs are seldom found in houses; in fact, no colonies were detected in artificial habitats there: infestation of three (out of 4) DUs corresponded to peridomestic palm trees, and a single adult bug was collected in the remaining infested household. Note however that dense peridomestic colonies (in dovecotes and chicken coops) were documented in previous studies in Manabí (Defranc 1982, Lazo 1985), and that during our field activities in other localities of the province we found evidence of recent colonisation in one peridomicile (hatched eggs and exuviae, but no live bugs, were found in the underside of the wood plank floor of a raised house); finally, one female collected in the bed of a further domicile laid eggs that produced viable nymphs while kept isolated in captivity, showing that it had the potential of founding a breeding colony. The situation could be regarded as intermediate in El Oro, where the bugs are found mainly in peridomiciles but may also breed inside houses.

5.4.1.4. History of insecticide spraying

Two main aspects were identified that required consideration: (1) the fact that 84% of households had been reportedly treated (over half of them in the 6 months before fieldwork) and (2) the fact that in spite of such a high coverage 8% of dwellings were still infested. The patterns of insecticide use were also dissimilar among localities. Only half of the dwellers reported having had their houses treated in El Oro, whereas the percentage was over 80% in Loja and reached 100% in Manabí – where the spraying was carried out by the National Vector Control Service (SNEM), using deltamethrin, in the month before our survey. In Loja and El Oro the insecticides were in all cases but one applied by the residents themselves.

This may obviously be crucial for the interpretation of our results in Manabí: perhaps the unexpected zeal of the SNEM (suspectedly triggered by the fact that we were preparing our visit) had been so effective that no bugs were to be found in the locality. There are however some details worth considering. Firstly, infestation was detected in

28% of *Phytelephas* palms surveyed in the locality (see Section 4.3.); second, one adult male was found inside a domicile within a month since spraying; and finally, no evidence was found suggesting that bug colonies could have existed before the campaign (one would expect finding hatched or dead eggs, exuviae, faecal smears, or dead bugs, and also that some of the dwellers would have come across moribund bugs dislodged by the effects of the insecticide). Thus, it would seem that the risk of reinfestations from peridomestic palm tree colonies may still persist, indicating the need for longitudinal surveillance; the absence of substantial (above 5%) infestation of houses or artificial peridomestic structures means that (efficient) insecticide spraying should be focal and triggered by the report (and eventual confirmation) of bugs by the dwellers. The possibility of introducing control measures aimed at peridomestic, palm tree-living bug populations is discussed in Section 4.5.

In Loja and El Oro various types of insecticides (commercial malathion formulations in the majority of cases) were used by the dwellers; most DUs however had been treated >6 months before fieldwork or never treated (>85% of DUs overall). The relationship between infestation and use of insecticides in these localities is discussed below.

5.4.1.5. Housing conditions

In a general, qualitative assessment we found 70% of households to be in poor or very poor condition•. A median value of 11 years since construction was scored (8 in Manabí, 11 in El Oro, and 15 in Loja); 60% of houses were over 10 years old.

The type of construction varied among localities. As frequently observed in Ecuador, coastal houses tended to be wooden and raised ~2m above ground (78% of DUs in Manabí), traditionally with roofs made of sun-dried tagua fronds ('cade' or 'cady'; 21% of DUs in our sample in Manabí) that are progressively replaced with concrete, fibre-cement, metal sheets, etc. (63%). Over 85% of houses in Manabí had wooden floors. Mud-walled houses were most frequently found in Loja (>50% of them, including sun-dried adobe blocks and 'bahareque', a simple mud-and-stick composite), where roofs are usually built by overlaying loose tiles on a primitive but complex framework of bamboo canes (the 'caña guadúa', *Guadua angustifolia*) and/or timber (>80% of DUs),

---

•Note that many of the houses considered to be in good condition would have appeared poor by customary European standards. Generally speaking, brick-walled houses with concrete floors/roofs were classified as good, mainly if they were also in an acceptable state of hygiene. However, worse housing conditions were found in El Oro (just one dwelling classified as good) despite a higher proportion of brick houses (67%)

leaving a substantial gap (usually ~20-30cm) between the top of the walls and the roof eaves. Houses in these Andean valleys typically have earthen floors (>43% in our study locality), but many residents have covered them with concrete (~50%). Most dwellings had brick walls in El Oro (67%), often with tile roofs (>60%) and wooden (67%) or earthen (22%) floors – a somewhat intermediate profile between coastal and Andean houses. Wall materials also had a bearing on the percentage of houses having plastered walls; wood plank walls are never plastered (hence 94% unplastered houses in Manabí), brick walls only rarely (86% unplastered in El Oro), and mud walls often (45% unplastered in Loja, where 33% of houses had unplastered mud walls).

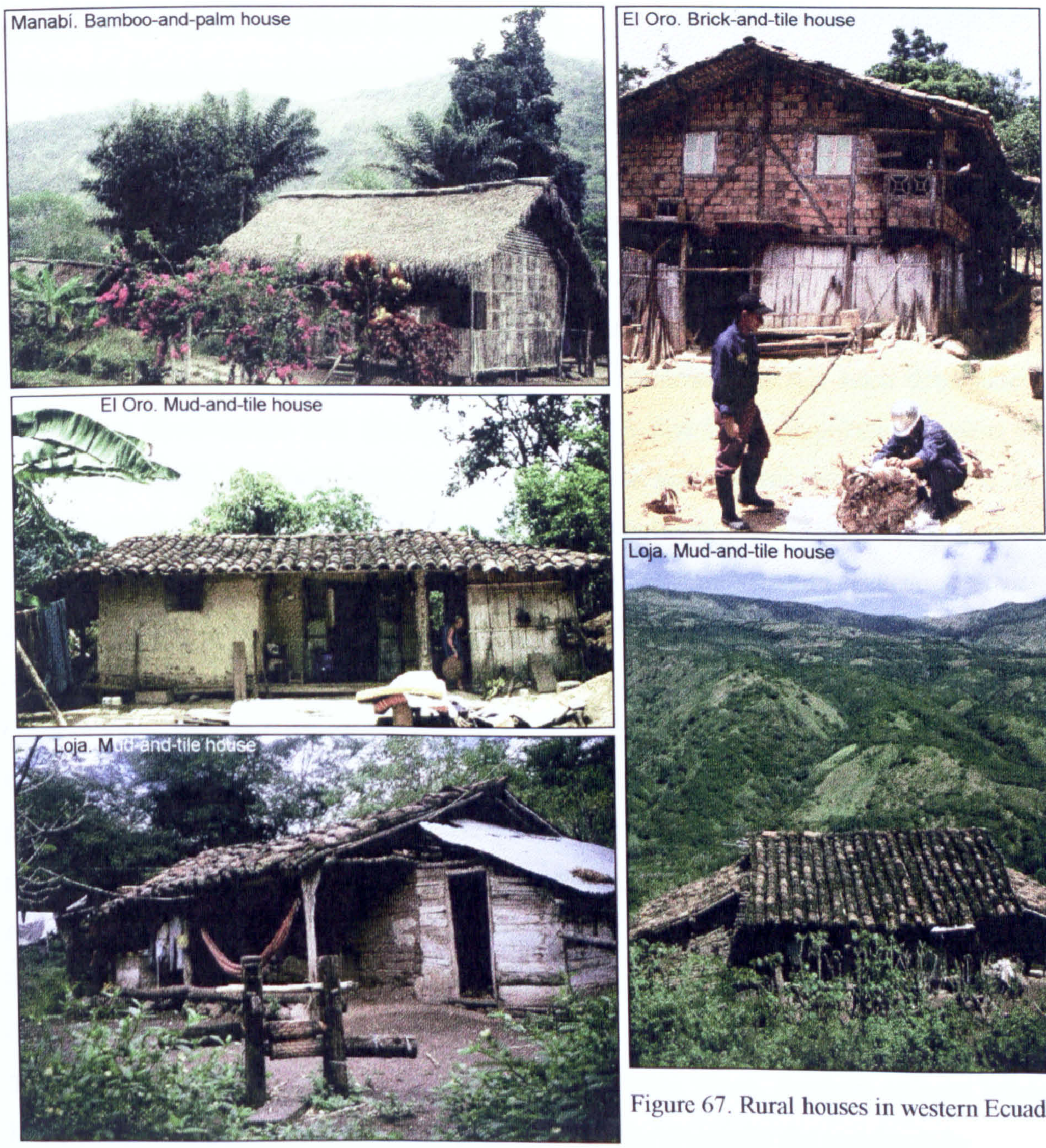


Figure 67. Rural houses in western Ecuador

5.4.1.6. Domestic animals

Synanthropic triatomines are, almost by definition, eclectic feeders. In the ecological transition from their original habitats to human environments they necessarily have to shift hosts. Domesticated animals may play a key role in infestation by providing the bugs with abundant and easily accessible food, usually throughout the year. While birds are only hosts (and may play a minor role as predators) of the bugs, mammals are also reservoirs of *T. cruzi*. It has been shown for instance that both poultry (indirectly by supporting large bug populations) and dogs (directly by being the main source of infective bloodmeals) play key roles in disease transmission by *T. infestans* within households in Argentina (Cohen & Gürtler 2001).

In rural Ecuador most peasant family own some animals; poultry and dogs are the most common. In agreement with this observation, 184 of the 188 (98%) families in our study had at least one animal: 113 of them had dogs (59 families had one dog, 30 had two, 15 three, and 9 had four or more dogs), and 168 had birds (most frequently about ten chickens). The importance of birds in the domestic ecology of *R. ecuadoriensis* had been suggested by observations of dense breeding colonies in dovecotes of Manabí and chicken coops in El Oro, but the possible role of mammals has not been determined (apart from observations that the bugs were found a few times in guinea pig pens in Peru and possibly associated with opossums in palms and hollow trees) (Herrer et al. 1972, Lent & Wygodzinsky 1979, Carcavallo & Martínez 1985, Lazo 1985, Barrett 1991). We studied for the first time the trophic relationships between *R. ecuadoriensis* and a variety of potential hosts in a heavily infested household in Loja. The versatility of these bugs, already remarked by Herrer et al. (1972) after a series of field observations, was confirmed by the finding of four types of blood (bird, rodent, opossum, and human) in a limited sample of 8 individuals collected from beds and peridomestic chicken nests. The finding of opossum and bird blood in a female specimen captured inside a bed (both vertebrates were absent from the intradomiciliary environment) suggested that some bugs may circulate from the peridomicile into the house, potentially introducing parasite strains from opossums into the domestic cycle. Human blood was only detected in bugs collected from the beds (Abad-Franch et al. 2002). However, no indication was found of dogs, pigs, sheep, donkeys or goat being hosts of the vectors in this household, even if they were present in the peridomicile.

Table 73. Feeding profiles of synanthropic *Rhodnius ecuadoriensis* in Loja (summary)

Blood source	No. of samples positive	%
Bird	17	65.4
Rodent	8	30.8
Human	4	15.4
Opossum	2	7.7

Table 74. Feeding profiles of *Rhodnius ecuadoriensis*, tested by immunoprecipitation

Sample	Place of capture	Results
01-individual (♀)	Bed	Human
02-individual (♀)	Bed	Opossum – bird
03-individual (nymph V)	Bed	Rodent
04-individual (♀)	Chicken nest	Bird – rodent
05-individual (♂)	Hollow tree with chicken nests	Bird
06-individual (nymph V)	Hollow tree with chicken nests	Bird
07-individual (nymph V)	Hollow tree with chicken nests	Opossum – bird
08-individual (nymph V)	Chicken nest	Bird
09 a-colony	Hollow tree with chicken nests	Bird – rodent
09 b-colony	Hollow tree with chicken nests	Bird
09 c-colony	Hollow tree with chicken nests	Rodent
10 a-colony	Chicken nest	Bird
10 b-colony	Chicken nest	Bird
10 c-colony	Chicken nest	Bird
11 a-colony	Bed	Human
11 b-colony	Bed	Bird
11 c-colony	Bed	Rodent
12 a-colony	Chicken nest	Bird
12 b-colony	Chicken nest	Bird
12 c-colony	Chicken nest	Bird
13 a-colony	Bed	Human
13 b-colony	Bed	Human – bird
13 c-colony	Bed	Rodent – bird
14 a-colony	Chicken nest	Bird
14 b-colony	Chicken nest	Rodent
14 c-colony	Chicken nest	Rodent

5.4.1.7. Product storage

Storing crops inside dwellings was identified as a risk factor favouring colonisation by *T. infestans* in central Brazil, where rats feeding on the stored crops probably provided in turn food to the bugs (Andrade et al. 1995). In addition, peridomestic colonies of several species of triatomines (e.g., *T. dimidiata*, *T. brasiliensis*, *T. phyllosoma*) have been found in piles of firewood, adobes, tiles, stones and other materials (e.g., Starr et al. 1991, Oliveira-Lima et al. 2000, Espinoza-Gómez et al. 2002). In the case of *T. dimidiata* in Costa Rica, the link between tile roofs and a higher (2.4-fold) risk of DU infestation (unexpected in a species rarely found over 1m above the ground) was identified as the heaps of spare tiles kept in peridomiciles, where

breeding colonies were detected (Starr et al. 1991). In some urban areas of Ecuador, piles of firewood and wood planks stored in patios serve as refuges for opossums, and colonies of *T. dimidiata* have been found associated with the marsupials (Lazo 1985, AG Guevara, pers. comm. and SNEM, unpubl. obs.). We investigated this aspect in our study localities; only 20% of families stored products indoors in Manabí, whereas about 40% did so in El Oro and almost 50% in Loja. These differences might reflect the diverse structures of the buildings; when they are raised over 2m above ground (almost all DUs in Manabí), most products are stored beneath the house, whereas in Loja this possibility did not exist because the houses are constructed directly on the ground (hence the lower proportion of families storing products in peridomiciles in Loja [50%] compared with Manabí or El Oro [80%]).

5.4.1.8. Knowledge, attitudes and practices (KAP)

·Sociological profile. People interviewed for the KAP study were of comparable ages (about 45-50 years old) in the three communities; most of them (~80%) were housewives or worked in agriculture/farming. Their levels of formal education were lower in Manabí, but given the type (and the subject) of questions, answers were most likely independent of the years of schooling.

·Knowledge

i. *The disease*. It was not unexpected that most people (over 86%) had never heard about Chagas disease ('mal de Chagas' or 'enfermedad de Chagas' in Spanish). The often mild and unspecific acute phase and a long latent period before the insidious signs of chronic illness appear (in only a fraction of those infected) may contribute to this lack of knowledge. In Ecuador, most acute cases probably go undiagnosed in rural areas, where basic medical care is dealt with by inexperienced and underpaid rural physicians (a year of rural general practice right after university is compulsory to obtain the full MD degree in Ecuador) with little (if any) technological and laboratory support; this adds to the fact that only heavily perceived, acute health problems (malaria, diarrhoeal diseases or dengue) are incorporated into health education interventions. The result is a generalised lack of awareness among the common people. That better results were obtained in Manabí (20% knew the disease) and El Oro (25%) than in Loja (5%) is probably related to previous entomological and epidemiological research carried out by different teams in the Portoviejo valley, including Pachinche Adentro (e.g., Defranc

1982, Lazo 1985, Avilés et al. 1995a), and in the area of Lourdes (e.g., Racines et al. 1994, Avilés et al. 1995b, Córdova et al. 1999), whereas no such surveys had apparently been conducted in El Lucero. Over 90% of answers indicated no knowledge about Chagas disease transmission routes; again, the proportion was higher in Loja (98.5%) than in Manabí (85%) or El Oro (92%).

ii. *The vectors (*Rhodnius ecuadoriensis*)*. Triatomine bugs were nonetheless well known by the residents, with 73% of them being able to identify adult *R. ecuadoriensis* specimens, mostly in Loja (~90%); lower percentages in Manabí (68%) and El Oro (just 44%) would again seem to reflect the stronger synanthropic behaviour of the Lojan bug population, and suggested that invasion of dwellings by adult bugs was not rare in Manabí. Indeed, only 20% of residents knew nymphal stages there, vs. over 72% in Loja – where breeding colonies were most often found indoors. The low percentage of affirmative answers in El Oro could be attributed to the bugs favouring peridomiciles in that area. The overall rate of knowledge of nymphs was of about 43%, suggesting that adult bugs (73%) are more frequently in contact with people – and perhaps also reflecting the fact that larger, flying bugs are more conspicuous than nymphs.

Just <60% of the respondents said that the bugs may be a cause of nuisance; this was again more frequent in Loja (73%), further suggesting a closer bug-human association. Most people in El Oro (73%) and >40% in Manabí did not seem to be bothered by the bugs. However, >85% of answers obtained in El Oro and >80% in Manabí indicated that bug bites may transmit diseases, with a significantly lower percentage in Loja (65%). This might indicate that although interaction bugs-people is relatively scarce in El Oro and Manabí, the educational effects of previous research activities persist in the population, at least to the extent needed to build an association between bug bites and the idea of a health threat. Pless et al. (1992) reported that only 50% of residents in a rural village of central-southern Bolivia (where infestation by *T. infestans* was detected in all households and >74% of people were seropositives) were aware of an association between bugs and disease.

A 'quality of knowledge' score was devised for joint assessment of answers to four questions regarding the basic biology of the vectors. The following table shows the scoring scheme used.

Table 75. The 'quality of knowledge' score

Question	Answer	
	Wrong	Right
Where do the bugs live?	0	1
Know adult bugs?	0	2
Know nymphs?	0	3
What do the bugs eat?	0	4

Those providing four correct answers had then a score of 10; this was the case of 46 out of 188 (24.5%) respondents: 2/68 in Manabí (3%), 4/36 in El Oro (11%), and 40/84 in Loja (48%). The median values were also significantly higher in Loja (median=9, vs. 3 in Manabí and 4 in El Oro), confirming that the residents of El Lucero had substantial knowledge about the bugs, despite their unawareness about the associated health risks. On the contrary, the poor general knowledge about the vectors in Manabí and El Oro was coupled with a better understanding of those risks. Also in relation to the possibility that domestic animals transmit diseases showed the inhabitants of Loja less concern (60% thought so) than those from Manabí (77%) and El Oro (80%).

·Attitudes. Describing and categorising the attitudes of people towards the presence of triatomine colonies was of course a subjective exercise; both the actual responses of people and what we observed during the interview were taken into account. For instance, that the presence of bugs was *intolerable* implied not only that we were told so by the respondent, but also that her or his attitude reflected 'zero tolerance'; this was not considered to be the case if, knowing that the house was infested, the dweller did not undertake resolute action against the bugs.

The results of this part of the study may be better interpreted in terms of tolerance towards infestation. TV Barrett described tolerance in the broader context of bug-host interaction, where defensive host behaviour is triggered by (mainly) the density of the bug colonies. He added that the "threshold of sensitivity of the host and effectiveness of the host reaction" are also implicated in the regulation of bug populations (Barrett 1991; p. 163). As mentioned above, social and economic deprivation may have an effect on tolerance, downgrading bug infestation in the scale of householder priorities. Other factors possibly involved may include the aggressiveness of the bugs, their size and other features making them more conspicuous (colour, audacity in reaching their human hosts in daytime), and the negative social consideration of infestation in some communities (Schofield 1994). In this sense, perhaps one would expect relatively high

tolerance towards an inconspicuous species such as *R. ecuadoriensis* (small, dull-coloured bugs), which in addition seems to favour avian hosts and usually builds comparatively small colonies within houses.

We found different patterns of tolerance in our surveys. Firstly, peridomestic colonies appeared to cause less concern than those established indoors; over 55% of people were indifferent to the former or considered them as a minor nuisance, vs. only about 30% with regard to intradomiciliary infestation. Some 24% deemed it intolerable that the bugs would infest their homes, but only ~16% thought so about peridomestic triatomines. Lower levels of tolerance towards indoor infestation were recorded in Manabí (75% of dwellers found these bugs intolerable or considered them a major nuisance) and El Oro (78%) than in Loja (63%); this likely reflects the lesser awareness of Lojan people with regard to health risks associated with triatomine infestation. Only one person (3%) in El Oro and 4 (6%) in Manabí declared themselves indifferent to indoors bug colonies, vs. 10 (13%) in Loja. Indifference was a more widespread attitude with regard to peridomestic infestation: 52% of people in Manabí, 53% in El Oro, and 62% in Loja paid little or no attention to that occurrence. In Manabí however ~34% deemed it intolerable, vs. 16% in El Oro and only 4% in Loja. Correspondence analysis graphically showed how Manabí residents tend to tolerate worse the presence of bugs, whereas those from Loja tend to be less cautious.

·Practices

i. *Action against vectors*. Tolerance trends were partially reflected in the patterns of action against triatomines. About half of the respondents affirmed taking some sort of antivectorial initiative, without significant differences between localities. Most dwellers (about 80%) never use insecticides *specifically* against triatomine infestation; correspondence analysis revealed some differences among villages, with Manabí residents being more likely to use insecticides regularly, those from El Oro to never using them, and householders from Loja showing a trend to spray only when the bugs build dense colonies in their homes. This perhaps also reflects the varying behaviour of the bugs: they recurrently invade houses in Manabí without establishing dense colonies (hence the need to spray on a regular basis), tend to remain in peridomiciles in El Oro (and their presence there tends to be well tolerated), and display a greater capacity to heavily infest dwellings in Loja.

ii. *Animals*. Domestic animals were preferentially kept outdoors in all three study localities; only in about 36% of households they were allowed inside during the night – and very rarely sharing people's bedrooms (~4% of DUs). This possibly helps keep the density of bug colonies low in some infested households, which in turn may reduce the rate of natural infection among the vectors; however, in the absence of room-mate animals, most of the bugs will probably feed on humans during the night (Cohen & Gürtler 2001). We observed that an engorged nymph (stage V) of *R. ecuadoriensis* collected from a bed in Loja (no domestic animals were allowed indoors in this dwelling) had actually fed on a rodent, indicating that the bugs may exploit alternative blood sources available in the intradomiciliary environment (Abad-Franch et al. 2002). It was in Loja were domestic animals were more frequently allowed indoors at night (in 44% of DUs).

iii. *Hygiene*. Most of the houses we visited were in a rather poor state of hygiene; however, most residents affirmed they perform in-depth cleaning every week. It seemed to us that this response was not reliable, partly because it was difficult to clearly define 'in-depth', and partly because many respondents visibly tended to overstate their keenness towards hygiene. A similar perception applies to the places where chickens and other domestic animals were kept around the houses.

iv. *Palms*. *Ph. aequatorialis* palm trees were only seen in our study locality in Manabí; they are a major feature of the landscape in Pachinche Adentro, and the locals use their fronds and seeds for various purposes. Of interest to our research was the use of palm leaves for roof thatching, known to favour household infestation by some *Rhodnius* species – most notably *prolixus* (Lent & Wygodzinsky 1979, Barrett 1991, Salvatella et al. 1998). Despite the fact that we found *R. ecuadoriensis* colonies in 28% of palms surveyed in the locality, and despite the reports that their eggs were found fastened to palm fronds in Los Ríos (Carcavallo & Martínez 1985), we found no evidence suggesting that any of the 14 dwellings with palm roofs could be infested, and none of the dwellers indicated the roofs as a source of infestation. The way in which palm fronds are processed for thatching in the area may help explain this finding. Instead of being used immediately, the leaves are sun-dried for a few weeks in large stacks; they are usually moved at intervals so that all of them receive direct sunlight for at least several days.

Triatomines have low resistance to sun exposure, and most will die after about 15min if they cannot find a suitable refuge (Schofield 2001); this probably implies that many of the bugs that could be present among the freshly collected fronds will not reach the dwellings alive. On the other hand, the incubation period of *R. ecuadoriensis* is of about one month at 28°C (see Section 5.5.); this period is shorter than the time fronds are normally left in the sun, meaning that most eggs will hatch before the leaves are used for thatching. It seems unlikely that small first instar nymphs will survive in the frond stacks and reach houses; in addition, high temperatures can accelerate embryonic development in triatomines, including *Rhodnius*. For instance, *R. neglectus* eggs hatched in 12-18 days at 28°C, but in only 9-16 at 33°C (Rocha et al. 2001a); similarly, incubation of *R. robustus* eggs was reduced from 14-21 days (28°C) to 10-17 days (33°C) (Rocha et al. 2001b). Furthermore, temperatures over 34°C-35°C seem to halt embryonic development in *R. prolixus* (Luz et al. 1999) and *R. robustus* (Rocha et al. 2001b). Thus, if temperatures in palm frond stacks rise to above 30°C, *R. ecuadoriensis* eggs could hatch before one month, probably increasing mortality among new-born nymphs before the leaves are used for thatching; if temperatures reach 35°C (which seems likely during sunny days in Manabí), some embryos will not complete their development. Together, these observations could explain the absence of infestation in palm frond roofs in Manabí.

Figure 68. Stacks of *Phytelephas aequatorialis* palm fronds ('cade') in Manabí

5.4.2. RISK FACTORS FOR HOUSEHOLD INFESTATION

5.4.2.1. Defining infestation

'Domiciliary unit infestation' is defined in different ways depending on the aims of the investigation. In the context of control interventions a household is considered as infested if any evidence of the presence of triatomines is found (live bugs of any stage, dead bugs or their remains, exuviae, eggs or eggshells, faecal streaks) inside or around the house; the peridomicile (whose definition is also variable) is comprised of all *artificial* structures associated with the house (chicken coops, storerooms, stables, pigsties, corrals etc.) (Schofield 2001). In entomological surveys aimed at describing baseline patterns of infestation the definitions may vary; although the one above is usually applied (e.g., Andrade et al. 1995a,b, Cuba Cuba et al. 2002), in some studies a house is classified as infested only if at least one live bug is collected (e.g., Gürtler et al. 1992), and both approaches may even be combined in single studies (Schofield 2001).

For the exploration of relationships between household characteristics and infestation, only the presence of *R. ecuadoriensis* bugs in artificial structures (domestic and/or peridomestic) was taken into account in our analyses. Results from Manabí, where only palm tree infestation was detected (with the single exception of an adult male captured in a house) were excluded; their inclusion in the overall assessment discussed above is justified by evidence suggesting limited interaction between palm tree-living bugs and humans, but such an interaction (involving invasion of houses by adult bugs) seemed obviously independent of the characteristics of DUs here studied.

On the other hand, it is generally recognised that active searches for bugs in human environments have a sensitivity threshold below which infestation goes undetected (Wisnivesky-Colli et al. 1987, Andrade et al. 1995b); an even larger proportion of actually infested dwellings may be missed when no flushing-out agents (e.g., pyrethrum or Deltamethrin at low dose) are employed (Schofield 1978, 2001, Gürtler et al. 1993, 1995). Infestation rates detected by active manual searches without the aid of a flushing-out agent are therefore likely to represent underestimates of the actual rates, with a number of false-negative DUs. It could be added that manual collections tend to be biased towards larger bugs (IV and V instar nymphs and adults; Gürtler et al. 1993) because they are more easily detected by the inspectors; adult *T. infestans* are between 21mm and 29mm long, whereas the largest synanthropic *R. ecuadoriensis* adult

specimens rarely reach 14.5mm, and the nymphs are obviously smaller. This fact, together with the cryptic colouration of *ecuadoriensis*, might have increased the number of false-negative DUs in our study; favouring detection was the fact that *R. ecuadoriensis* eggs are laid attached to the substrate (frequently wooden surfaces of coops and furniture), where the eggshells remain after hatching, whereas *Triatoma* eggs are laid loose, usually in non-accessible sites, making their detection difficult. However, Andrade et al. (1995b) reported that in a case-control study in central Brazil, live *T. infestans* were collected in 7% of 447 DUs; the infestation rate increased to 43% when the finding of exuviae, eggs, or bug faeces was taken into account.

Longitudinal detection of triatomines in households may provide more accurate estimates of actual infestation indices. Several 'passive' methods have been tested with good results, including sensor boxes (cardboard boxes fixed to bedroom walls for long periods and where bugs tend to take shelter) and papers (similarly used, and where bug faecal smears can be identified) (e.g., Schofield 1978, Schofield et al. 1986b, Wisnivesky-Colli et al. 1987, Gürtler et al. 1995, Guillén et al. 1997). Active longitudinal detection involves the collaboration of the dwellers in reporting the presence of bugs in their households (Dias 2000, Rojas de Arias 2001); several studies have concluded that this method may perform better than either passive detection or vertical active surveys, even when the involvement of large bug species (*T. infestans* or *P. megistus*) and the use of flushing-out chemicals improve the sensitivity of manual searches (García-Zapata & Marsden 1992, 1993, Andrade et al. 1995a, Gürtler et al. 1995, Rojas de Arias et al. 1999, Silva et al. 1999).

Following this rationale, and having to deal with the fact that bugs were actually found in only a relatively small proportion of dwellings (which decreased the statistical significance of the results, with some calculations yielding large standard errors), we based most of our analysis on the *report of the presence of bugs* by the householders. As mentioned, we found a strong correlation between reported and current infestation. The finding by Cuba Cuba et al. (2002) of a substantial increase in infestation rates (from 14% to 35%) by *R. ecuadoriensis* in northern Peru as a result of implementing longitudinal surveillance reinforces the idea that many DUs may be wrongly classified as non-infested after active manual searches. The use of dweller report of past infestation as an estimate of actual infestation introduces however the possibility of

using false-positives in the analyses, because an unknown fraction of dwellers may have misidentified the bugs or may have thought that reporting infestation could bring them some benefits (for instance, increasing the frequency of spraying by the SNEM). The good overall levels of knowledge about *R. ecuadoriensis* recorded in our survey moderates concern about inaccurate reports. Thus, a median of 7 (over a maximum of 10) was obtained in 'quality of knowledge' scores for Loja and El Oro considered together; the value was significantly higher in reportedly infested DUs (median=9) than in those where residents did not report having seen the bugs (median=6; Wilcoxon test X^2=9.02, 1 df, p=0.0027). We concluded that reported infestation may be regarded as a good estimate of the actual presence of synanthropic populations of *R. ecuadoriensis* in a given DU, and based most of our conclusions on the results obtained using that variable. Reported infestation will be referred to hereafter as **infestation**; DUs where we detected triatomines during our surveys will be referred to as **currently infested**.

5.4.2.2. Infestation and socio-economic data

Univariate analyses showed that the domiciles of families with lower monthly incomes were more likely to be infested. The unadjusted odds ratio indicated a consistent decrease in the likelihood of DU infestation with increasing incomes (uOR=0.12 or, equivalently, an 8.3 increase in the odds of infestation with decreasing incomes); the 95% CI did not include 1, supporting the validity of the finding. Multivariate logistic regression showed that the influence of income remained significant after adjusting for confounders (see model *MRep*-1a, tables 59 and 60).

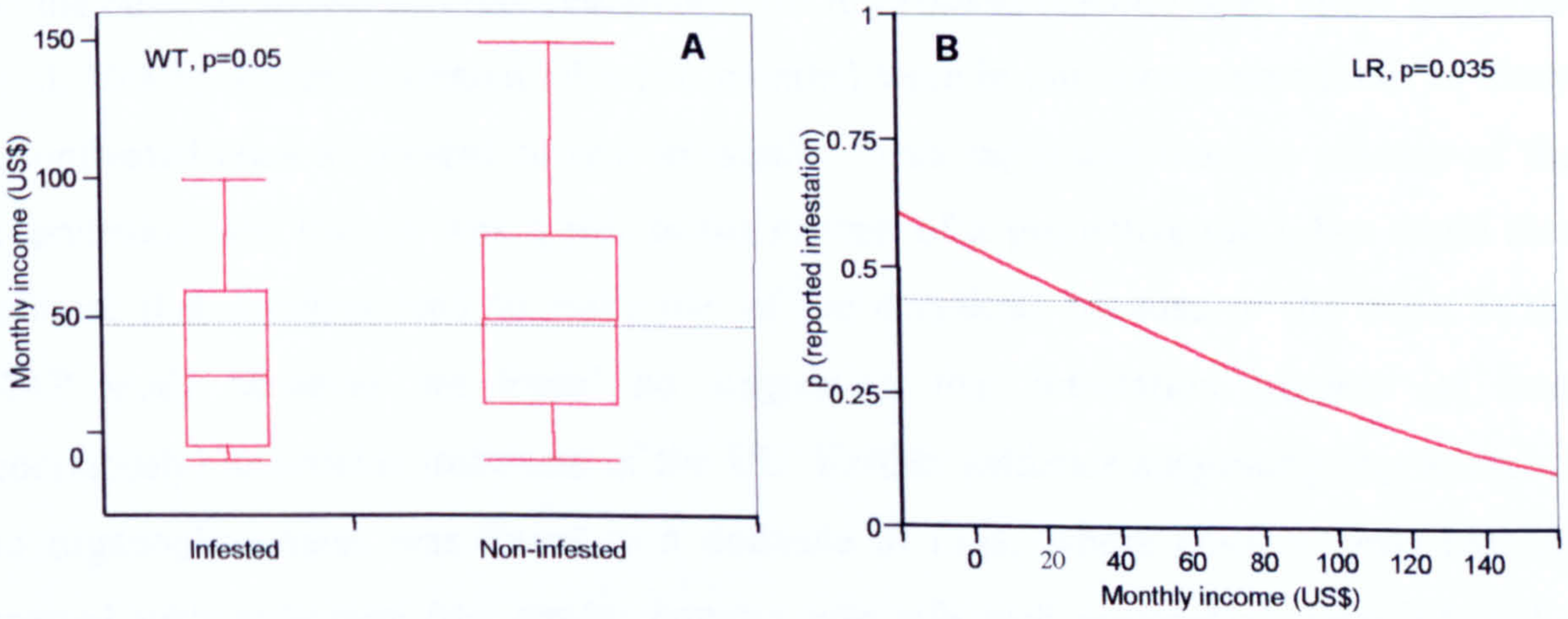


Figure 69. Univariate analyses: probability of household infestation as a function of the approximate monthly income of families. **A**: quantile plot showing the median income values and the 10%, 25%, 75%, and 90% quantiles (WT=Wilcoxon test); **B**: logistic fit (LR= likelihood ratio test); n=83 DUs

Lower levels of formal education were correlated with lower monthly incomes, but the trend towards higher likelihood of infestation in households whose owners had attended school for less years was not supported by statistically significant p values.

A large family size was found to be a potential risk factor for house infestation by *T. infestans* in Brazil (Marsden et al. 1982); this was not confirmed in our study, where the median number of people per DU was only slightly larger in infested (median=4) than in non-infested households (median=3.5).

5.4.2.3. Infestation and history of insecticide use in the household

The use of insecticides by householders may protect factor against infestation; it is also possible that the presence of bugs triggers such a practice, so that the relationship might be more complex than the intuitive equation 'insecticide = no infestation'. Gürtler and coworkers showed that although the regular domestic use of chemical insecticides reduced the density of *T. infestans* colonies in rural Argentina, the effects varied among houses with different types of roof, presumably because some structural features of grass-thatched roofs increased the residual availability of toxic particles; additionally, high infestation rates (above 80%) persisted in spite of the widespread use of (mainly) γ-BHC in the study locality (Gürtler et al. 1992). In our survey (Manabí excluded), the organophosphate malathion was the most commonly utilised by householders (as dry powder or as wettable powder applied with agriculture backpacks); the fact that 73% of currently infested households (and over 80% of those reportedly infested) had been treated with insecticides suggests that this compound may have incomplete lethal effects on the bugs, as shown by other researchers for *R. prolixus* (Picollo et al. 1990, Sandoval et al. 2000b). Recently treated DUs (<6 months) were in our survey nine times as likely as untreated ones to present current infestation. This suggested that the efficacy of the intervention was low (it was a risk factor instead of a protective one), but could also indicate that dwellers tend to make use of the chemicals because of the bugs; in the KAP study however we found no suggestion that infestation triggers (at least consciously) insecticide treatment of the DU. Further evidence suggesting bug tolerance to organophosphates was found in a domicile in Loja, where chicken nests recently treated with malathion (the smelly powder was still well noticeable) were infested – averaging ~65 per nest (Abad-Franch et al. 2002). The fact that two DUs reportedly sprayed with deltamethrin (residual pyrethroids are very effective against triatomines;

see Schofield 1994, 2001, Dias & Schofield 1999) were currently infested may indicate that incorrect application techniques (dosage of active ingredient, adequate coverage of surfaces, etc.) may also result in poor performance of chemical insecticides. Together, these findings illustrate the need for professional insecticide spraying in organised vector control campaigns; even if many rural householders may be familiar with the use of agricultural pesticides (some may even own a small backpack), only interventions carried out by trained personnel seem to produce suitable results.

5.4.2.4. Household features and infestation

·Age of the houses. Old houses may have increased chances of harbouring triatomine colonies (cf. Starr et al. 1991, Andrade et al. 1995a) for several reasons. For instance, they may be in a poorer state of conservation and hygiene than new homes, especially when low-quality materials and inadequate techniques are employed for building. With time, cracks and crevices appear in mud walls, which may also lose their plaster; the complex timber structures of primitive tiled or thatched roofs may also deteriorate, and are not easy to keep clean and rodent-free; earthen floors are difficult to clean, and even more so when (as frequently observed in poor rural communities) entire families sleep in small rooms, sometimes sharing them with their domestic animals and normally having to keep all their belongings in the same space (often in cardboard or wooden boxes stuck beneath beds, with clothes hanging from strings along the walls and rudimentary shelves where small goods and adornments accumulate). Thus, we may sense that in some cases households 'acquire' their risk factors over time.

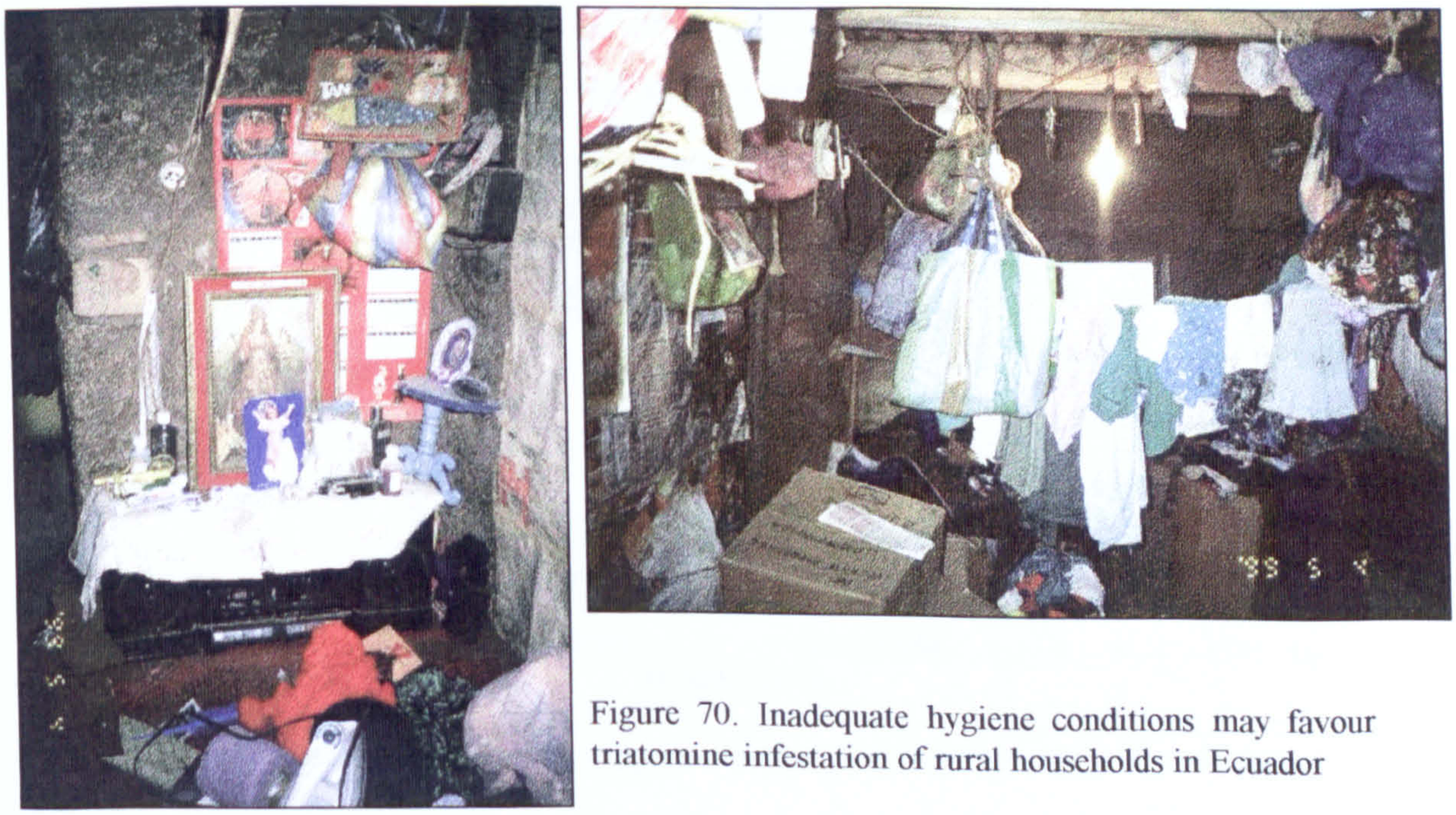


Figure 70. Inadequate hygiene conditions may favour triatomine infestation of rural households in Ecuador

However, in other circumstances it may well be the opposite. In areas of new settlements where impoverished immigrants arrive living conditions are generally appalling. Those newly arrived build precarious huts, often mud- or timber-walled and thatched, that may be rapidly colonised by triatomines (e.g., Albarracín-Veizaga et al. 1999); eventually however some of the immigrants will find a job and establish themselves in the area, and possibly will begin to improve their homes (covering walls with plaster and earthen floors with cement, replacing thatched roofs with corrugated metal sheets, and even having their homes sprayed). Herrer and coworkers mentioned that the densest *R. ecuadoriensis* colony they found in a survey (121 DUs) in rural areas of northern Peru corresponded to a "recently built household, where no insecticides had still been applied" (Herrer et al. 1972; p. 144).

Both mechanisms can act simultaneously at the level of single communities – some houses being improved over time while others deteriorate. A final average outcome of no apparent relationship between house age and infestation is therefore possible, and was suspected in our survey. We were left with the impression that those who escape desperate poverty readily embark on housing improvements, whereas those who remain in destitution see how their houses deteriorate without being able to prevent it; although the dataset showed no significant overall correlation between income and house quality, 37% (7/19) of those earning less than 15 US$/month lived in houses in very poor condition, vs. only 9.5% (2/21) of those earning over 75 US$/month. The time elapsed since the houses were built was comparable (WT X^2=0.3, 1 df, p=0.6) in both groups (median=16 and mean=16.6 years for the group of families earning ≤15 US$/month, vs. median=13 and mean=17.5 years in the group earning >75 US$/month).

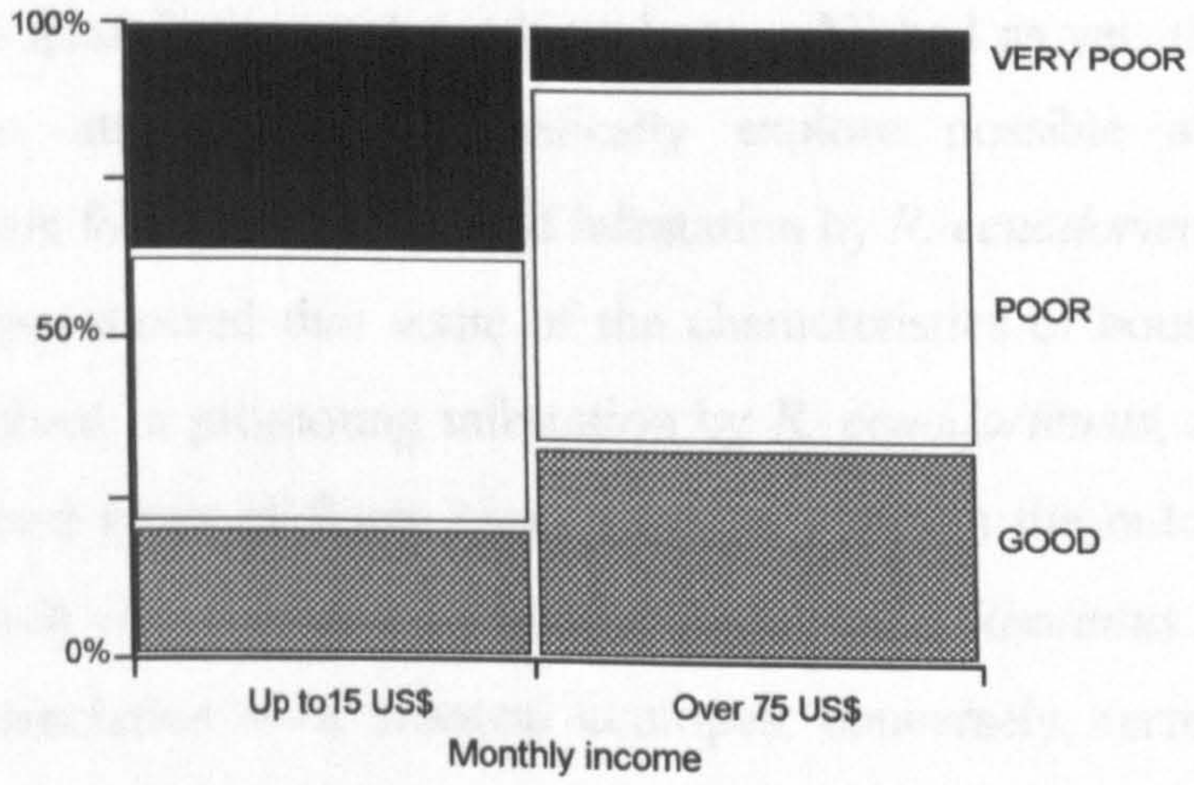


Figure 71. Entomological survey: approximate monthly income and quality of housing

Finally, older houses have simply been available as (more or less) fitting ecotopes for longer periods than newer ones. This means that reported infestation may not be suitable for exploring a possible increase in the risk of infestation with time, except perhaps because it may provide a rough confirmation that there is a time-cumulative increase in the probability that dwellers encounter bugs. Supporting this view, our results show that while reportedly infested households were older in average, no such difference was observed when assessing either current or recent (up to 1 year) reported infestation.

·Building materials. Unlike with other triatomine species (discussed above), we found no substantial information on the possible relationships between building materials and household infestation by *R. ecuadoriensis*. Based on limited field observations (data from seven infested households out of 121 surveyed in two localities of northern Peru), Herrer et al. (1972) indicated that mud-walled houses were preferentially infested; there, *R. ecuadoriensis* females laid their eggs either attached to wooden surfaces or in cracks and crevices of adobe walls of both houses and guinea pig pens. Thirty years later, CA Cuba Cuba and coworkers reported similar findings from a survey on 259 DUs (14% of which infested) in the Cascas district (La Libertad, Peru), where most of the bugs were found in houses "with non-plastered walls of adobe or 'quinchas' (mud/cane) and thatched or cane-and-clay roofs" (Cuba Cuba et al. 2002; p. 176). We were unable to find any specific information on building material preferences regarding Ecuadorian populations of *R. ecuadoriensis*, although the allusions to infestation of wooden dovecotes and chicken coops (occasionally with tagua palm thatching in Manabí) (Defranc 1982, Lazo 1985) could be regarded as an indication that some bugs may favour those materials. However, the significance of these observations is uncertain, and no quantitative analyses have been published as yet; the present work represents the first attempt to systematically explore possible associations and accordingly define risk factors for household infestation by *R. ecuadoriensis*.

Univariate analyses showed that some of the characteristics of household walls and roofs could be involved in promoting infestation by *R. ecuadoriensis*, and provided no evidence that different types of floors could have an effect in the outcome of interest. This would agree well with the basic biological features of a *Rhodnius* species, likely to have evolved in association with arboreal ecotopes; conversely, terrestrial species of *Triatoma* may prefer houses where earthen floors mimic their natural habitats,

particularly when nymphs cover themselves with dust particles to camouflage (Zeledón 1981, Barrett 1991, Starr et al. 1991).

The presence of **walls** made totally or partially with mud significantly increased the risk of DU infestation; assessment of contingency tables showed an associated relative risk of 1.9 (uOR=3.16, 95% CI 1.5-6.8, n=119 DUs). The uOR slightly increased when the absence of plaster in mud walls was taken into consideration, with unplastered mud walls presenting 3.3-fold (unadjusted) odds of being infested (95% CI 1.4-8.3; relative risk was 1.83). This was in agreement with observations mentioned above, and suggests that despite being an originally arboreal species, *R. ecuadoriensis* can take advantage of the micro-structural features of mud walls – where cracks, crevices, gaps and fissures provide the bugs with suitable hiding places (Abad-Franch et al. 2002) and, according to Herrer et al. (1972), perhaps also protected spaces to lay eggs. That mud-walled households were more likely to be infested was well recognised by many inhabitants of the study localities (mainly in Loja), as shown by the results of the KAP survey (above) and by detailed accounts obtained from numerous dwellers during the interviews. It must be noted however that mud-walled houses are typically found in Andean communities (both in Ecuador and Peru), but may be completely absent from many coastal areas where *R. ecuadoriensis* has been recorded – for instance in Manabí, see above. This means that these results are likely to have more impact in the control of the species in the temperate valleys where it seems to display a stronger synanthropic behaviour, and that other housing features not evaluated in our survey could be of importance in coastal communities where timber-walled houses are typical.

An unexpected result from exploratory univariate statistics was the strong correlation between tile **roofs** and a higher likelihood of infestation; contingency table analysis revealed a relative risk of 3.54 (uOR= 6.1, 95% CI 2.2-22.3) for tile-roofed houses. It was at first difficult to figure out how a tile roof could have a positive influence in the population biology of *R. ecuadoriensis* and encourage infestation. Analogous difficulties were encountered by Starr and coworkers when a similar finding arose from re-analysis of ecological data on synanthropic populations of *T. dimidiata* in Costa Rica (Starr et al. 1991). After examining original field records the authors concluded that it was not the roofs themselves that favoured bug colonies, but the presence of stacks of spare tiles (where immature bugs were collected) near the houses (Starr et al. 1991). In

the case of our survey, we favour a different interpretation. Firstly, we did not find any evidence of colonisation among piles of spare building materials (including timber, bricks, adobes, and tiles) in peridomestic areas. More importantly, we were able to examine over a hundred tile roofs during the survey, and were impressed by their architectural features. These roofs, usually built by the householders, consist of a rather primitive but very complex framework of trunks and/or large bamboo canes laid longitudinally (following the roof slope) and joint by smaller, transverse branches and/or reeds, tied with vegetable fibre strings or with metal wire; fired clay tiles are arranged loosely over this framework, without fastening them to each other or to the substrate. There is often a large gap between the top of the walls and the roof, important for ventilation in houses with normally small windows (if with any windows at all).

Figure 72. Typical tile roofs in Loja

We interpret the apparent preference of *R. ecuadoriensis* for houses with tile roofs in Loja-El Oro in the following terms:

i. *Structural suitability*. The very complex architecture of the roofs provides the bugs with numerous safe hiding places, generally inaccessible to any attempts householders may make to kill the bugs (perhaps including, at least to a certain extent, insecticide spraying). That structural-architectural features, rather than the building materials themselves, may favour domiciliary bug colonies was already observed in Argentina, where brushwood-earth roofs harboured more *T. infestans* than those made of compacted grass bundles (Gürtler et al. 1992).

ii. *Abundance of oviposition surfaces*. Although it has been mentioned that *R. ecuadoriensis* females may lay eggs in mud wall crevices, it is clear that these originally arboreal bugs do have a preference for vegetable surfaces – as also noted in the same report (Herrer et al. 1972). Cuba Cuba et al. (2002) specifically referred to the frequent finding of infested bamboo cane beds, often with numerous eggs fastened to the cane surface (CA Cuba Cuba, pers. comm.). We observed exactly the same thing during our surveys in both Loja and El Oro; in one household in Loja we dismantled two beds (one made of wood planks and the other with bamboo canes) and collected 239 *R. ecuadoriensis* eggs (Abad-Franch et al. 2002); we noticed that eggs were more frequently found on rough instead of smooth surfaces (for instance, on the inner rather than on the outer side of the canes, which are cut in half longitudinally and then flattened to construct the beds). The same preference for rough wooden surfaces was observed in the distribution of eggs in chicken coops; interestingly, parasitoidism by wasps (see Coscarón et al. 1999) was only detected among eggs collected outside houses (Abad-Franch et al. 2002).

Table 76. Eggs of *Rhodnius ecuadoriensis* collected in El Lucero (Loja)

Site of collection	**Hatched**	**Not hatched**				**Total (%)**
		Viable	Not viable		Dubious	
			P+	*P*–		
Intradomiciliary	434	14	0	13	3	464 (46.6)
Peridomestic	414	15	65	28	9	531 (53.4)
Total (%)	848 (85.2)	29 (2.9)	65 (6.5)	41 (4.1)	12 (1.2)	995

P+: eggs with signs of parasitoidism (larvae inside eggs or egg chorium presenting a circular orifice left by the emerging wasp); *P*–: eggs with no sign of parasitoidism; Dubious: apparently alive when collected, but producing no nymphs

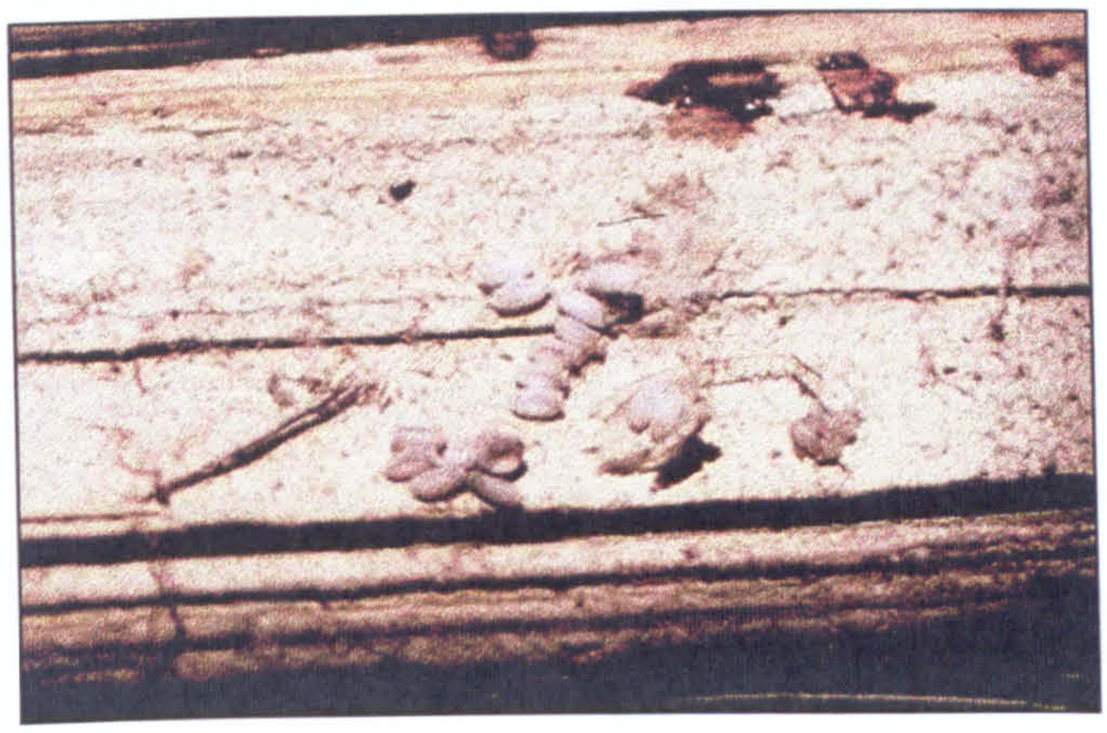


Figure 73. Eggs of *Rhodnius ecuadoriensis* on a bamboo cane bed (El Lucero, Loja)

We consequently believe that the timber roof structure may provide extremely abundant, suitable egg-laying surfaces to the female bugs inside these dwellings. It is however necessary to note that we did not observe this directly; demolition studies are usually required to conduct detailed observations on the ecology of roof-dwelling triatomine populations (e.g., Rabinovich et al. 1979, Gürtler et al. 1992).

iii. *Host availability*. The structurally complex roofs described here frequently provide refuge to synanthropic rodent populations, particularly when houses are not kept in a suitable state of hygiene and crops are stored without adequate protection inside or near the houses. Rats and mice are therefore extremely frequent in these households, and the residents in our study areas were well aware that rodents were breeding mainly in roofs and storerooms. In some cases, bird nests may also be found among the loose tiles, and even opossums will take refuge in the roofs occasionally. All these vertebrates may represent a suitable food supply for the bugs; our studies in a tile-roofed dwelling in Loja revealed that bugs collected indoors had fed on rodents (Abad-Franch et al. 2002), which were abundant in the roof. Andrade et al. (1995a) reported that the presence of rats significantly increased the likelihood of DU infestation by *T. infestans* in central Brazil.

iv. *Lack of barrier effect for peridomestic populations*. Most of the breeding colonies of *R. ecuadoriensis* detected in our survey were associated with peridomestic chicken coops, where they reached high densities (up to 221 bugs in a single chicken nest) under favourable conditions (Abad-Franch et al. 2002); as generally accepted for other secondary vectors that colonise peridomestic structures, the rate of migration of bugs towards the houses is likely to increase when the peridomestic system nears its

carrying capacity due to excess bug density in relation to a fixed number of hosts (Dias & Diotaiuti 1998). Obviously, physical barriers effectively separating both environments could prevent peridomestic bugs from getting inside the dwellings. We believe that tile roofs do not represent an effective barrier for *R. ecuadoriensis*. Firstly, a wide gap is usually present between walls and roof eaves; it was frequent in our study localities that chicken nests were placed against the house walls under the roof eaves, only a few inches below those gaps. A breeding bug colony was found within a brick house in good condition (in Loja) where infested chicken coops were thus positioned. In addition, the fact that tiles are laid loose over the timber structure means that any flying bugs landing on the large upper roof surface can easily work their way into the house by just walking through the spaces between the tiles. A fertilised female following this route would find herself in a structurally suitable, safe environment with plenty of adequate surfaces upon which to lay eggs and possibly with a large enough food supply to start a colony.

·Domestic animals. Almost 97% of families had domestic animals in our survey communities; the possible relationship between these potential hosts and triatomine infestation was therefore explored in terms of abundance (rather than presence/absence) of domestic animals in households. A general assessment disclosed infested DUs had on average a significantly larger amount of domestic animals (*t*-test p=0.005); as mentioned above, previous observations indicate chickens, pigeons, and guinea pigs may be hosts of *R. ecuadoriensis* in human habitats (Carcavallo et al. 1998b). However, detailed analyses of our dataset showed that DUs with chickens (85.6% of 118 households) only had a non-significant increase in the likelihood of infestation (FET p=0.3); although the unadjusted odds ratio was 1.8 (RR=1.5), the 95% CI included 1. A similar finding was reported regarding domestic *T. infestans* populations in central Brazil (uOR=1.5, 95% CI 0.9-2.3) (Andrade et al. 1995a). Similarly, no significant association was found suggesting houses with guinea pigs (20% out of 120 with data) were more likely to be infested (FET p=0.1; RR=1.6, uOR=2.3, 95% CI 0.94-5.9). Pigeons were present in only one infested DU (El Oro, 8 pigeons); although bugs were detected in a chicken coop located ~15m from the dwelling, there were no signs of infestation in the pigeon nests (wooden boxes attached to the brick wall under the roof eaves).

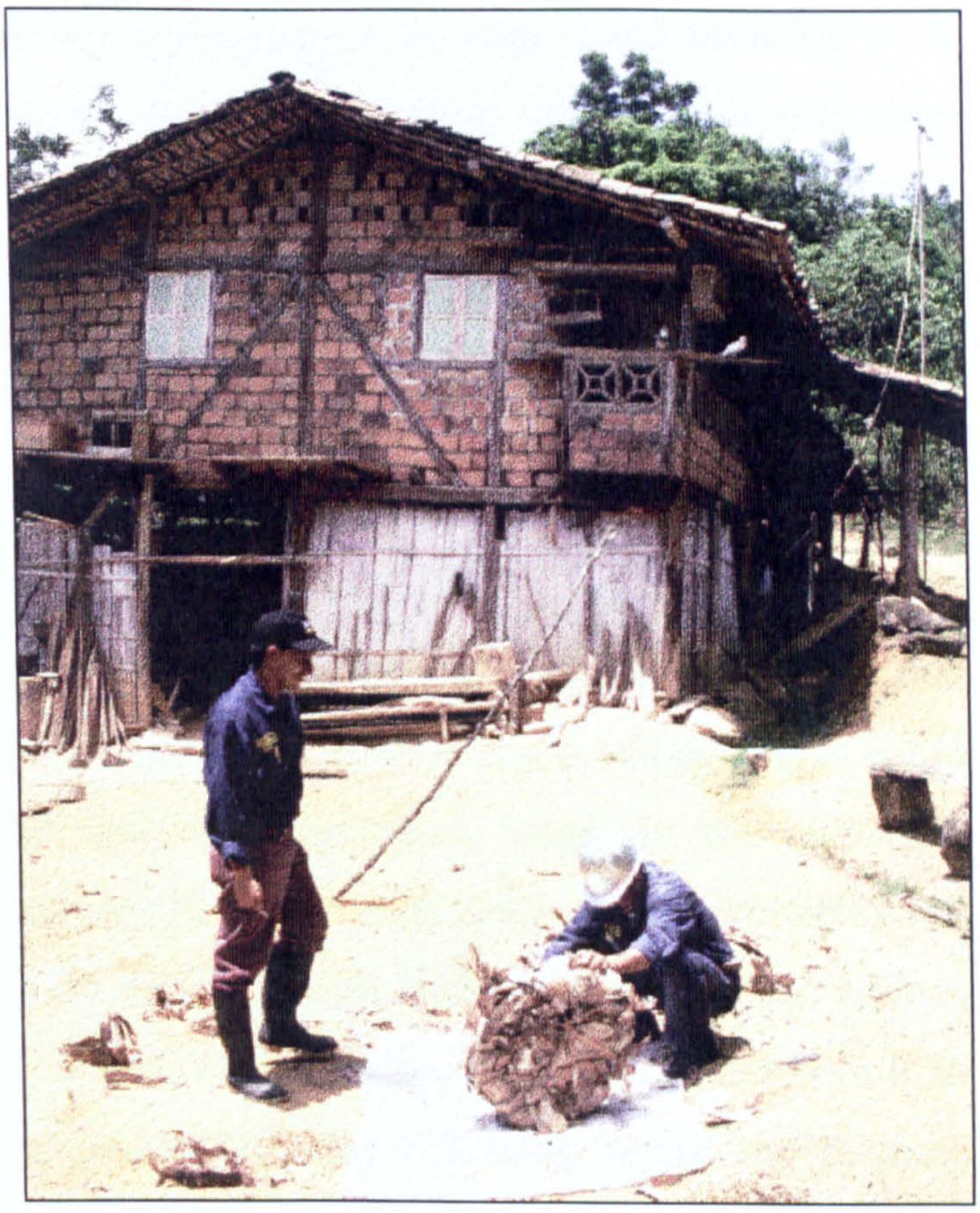


Figure 74. Infested household in El Oro. *Rhodnius ecuadoriensis* bugs (all stages) were recovered from a timber-and-tile-roofed chicken coop were 12 chickens were kept near the house; hen nests were made of banana tree leaves. No bugs were found in the house itself, or in pigeon nests located under the roof eaves. In the picture, workers of the National Vector Control Service (SNEM) examine a chicken nest to confirm infestation prior to spraying both the house and peridomestic structures with deltamethrin

While numbers of guinea pigs had no bearing on the probability of a DU being infested, the presence of bugs was more likely to be reported from households with larger numbers of **chickens** (uOR=16.2; the lower limit of the 99% CI was 1.5). Multivariate logistic regression confirmed this trend after adjusting for confounders, with OR=15.9 (*MRep*-1a), OR=22.7 (model *MRep*-1b), and OR=11.5 (*MRep*-2). This was entirely in agreement with our field observations; the bugs were clear and consistently associated with peridomestic chicken coops, where dense colonies may become established (Defranc 1982, Lazo 1985, Abad-Franch et al. 2002). We confirmed that although mainly ornithophilic, a significant fraction of feeding contacts of *R. ecuadoriensis* may involve mammals – even with availability of bird blood greatly exceeding that of mammal blood at the microhabitat level. Thus, eleven out of 17 (64.7%) intestinal content samples taken from bugs collected in chicken coops in a DU in Loja contained only bird blood, but in three of them (17.6%) only rodent blood was detected, two samples (11.8%) reacted with both bird and rodent antisera, and one bug (5.9%) had fed on both bird and opossum blood (Abad-Franch et al. 2002).

Although no previous data were available indicating that **dogs** could be hosts of *R. ecuadoriensis*, we found that DUs where dogs were present had a marginally non-significant higher likelihood of being infested (FET p=0.058, LR test p=0.047; RR=1.6); univariate logistic regression yielded an uOR=2.16, with 95% CI not including 1 (1.01-4.8), suggesting that an ecological relationship could in fact exist between dogs and *R. ecuadoriensis*. A quantitative analysis revealed that the number of dogs was correlated with a significant increase in the likelihood of household infestation (WT p=0.017, uOR=7.7); the relationship was stronger when only dogs kept in peridomiciles were considered (WT p=0.004, uOR=12.4), perhaps reflecting the fact that the majority of bugs were breeding outdoors in our study localities – and could therefore feed on peridomestic rather than intradomiciliary dogs. In the univariate models, the presence of two dogs in the DU was associated with a probability of infestation of 0.49, vs. 0.54 in DUs with 2 peridomestic dogs; similarly, 5 and 6 dogs were associated with probabilities of 0.8 and 0.86 (all dogs considered), and with 0.72 and 0.78 (considering only dogs kept outdoors). The following figure shows these trends for both reported and current household infestation.

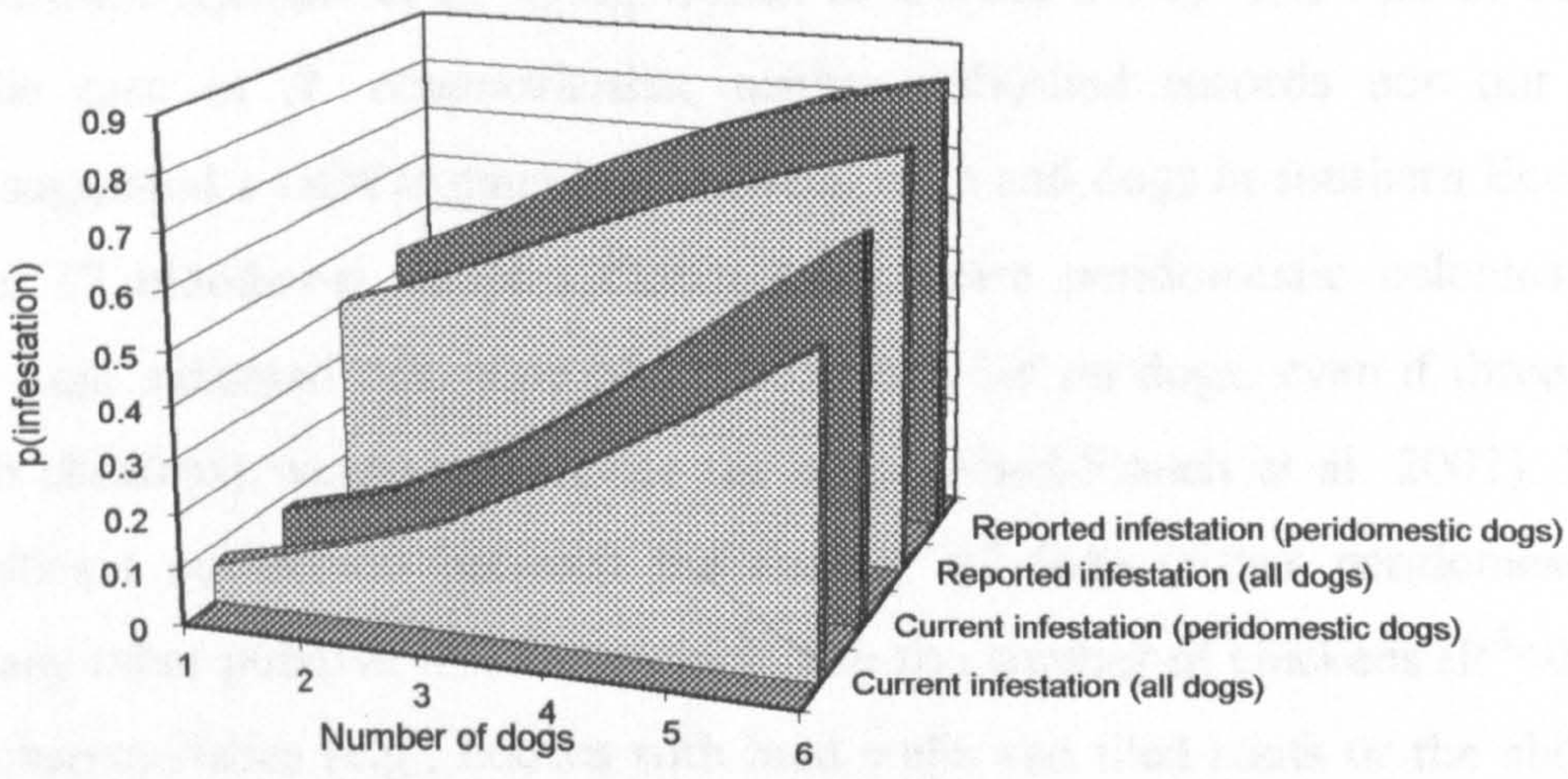


Figure 75. Dogs in households and infestation by *Rhodnius ecuadoriensis*: univariate logistic regression. Probabilities of infestation (current or reported) as a function of the number of peridomestic dogs (dark grey) and the total number of dogs (light grey) in the household

Taking these results into account, the likelihood of household infestation was modelled using the number of peridomestic dogs, rather than the total number, as a potential covariate. Multivariate logistic regression showed however that the correlation became non-significant after adjusting for confounders (LR test p=0.23), except for the separate analysis of the data from El Oro (only 34 DUs). Gürtler and coworkers have

shown that in Argentina domiciliary *T. infestans* are about three times more likely to take their bloodmeals from dogs or chickens than from humans, and that the number of intradomiciliary dogs is positively correlated with bug colony density (Gürtler et al. 1992, 1997); on the contrary, Andrade et al. (1995a) reported that the risk of DU infestation did not increase significantly in houses with dogs vs. those without them (uOR=1) in Brazil. Regarding disease transmission, the probability that a bug becomes infected after a bloodmeal from a seropositive dog was 12-fold that of getting the infection from a seropositive child, and 200 times higher than that from an infected adult (Gürtler et al. 1996), resulting in a positive correlation between the number of infected dogs and the rate of *T. cruzi* infection among the vectors (Gürtler et al. 1991); a similar increase in bug infection rates was observed in households with a single infected cat (but with no infected dogs) (Cohen & Gürtler 2001). Although chickens are not reservoirs of the parasite, and may divert a number of bugs from feeding on humans and other potentially infective vertebrates, their contribution to higher densities in bug populations probably has an influence on dispersal of bugs to neighbouring homes and increases the overall number of infected bugs within domiciles, and thus ultimately helps spread disease (Lehane et al. 1992, Cohen & Gürtler 2001). The role of dogs is unclear in the case of *R. ecuadoriensis*; neither published records nor our field observations suggested a tight trophic link between bugs and dogs in southern Ecuador. In this sense, 17 bloodmeal samples drawn from dense peridomestic colonies in a household in Loja indicated that none of the bugs had fed on dogs, even if three dogs (alongside 36 chickens) were available for the bugs (Abad-Franch et al. 2002). There was no significant correlation between the number of dogs (either peridomestic or overall) and any other putative risk factor, including the number of chickens ($R^2<0.1$) or several DU characteristics (e.g., houses with mud walls and tiled roofs or the ability of the householder to recognise *R. ecuadoriensis*). Although univariate results suggested that the bugs may feed on dogs in peridomestic areas, the available evidence is inconclusive. The only previous information regarding a possible trophic link between dogs and *R. ecuadoriensis* was reported by Herrer et al. (1972), who experimentally fed field-collected bugs on dogs, guinea pigs, chickens, rats, and mice. Further field research in southern Ecuador and northern Peru would need to pay attention to the ecological relationships between *R. ecuadoriensis* and dogs, both by performing

bloodmeal analyses and by ascertaining the prevalence of *T. cruzi* infection among dogs (Gürtler et al. 1991, Cohen & Gürtler 2002). It may in this sense be interesting to note that one of the main reasons why dogs were kept in peridomiciles in the rural areas where our surveys took place was the ability of these animals to "protect poultry from predators"; these are almost invariably *Didelphis marsupialis* opossums, called 'zorros' (literally 'foxes') in western Ecuador, which are abundant near human settlements and may prey on chicks, eggs, and chickens. The mix-bred, medium-sized dogs owned by many residents are extremely efficient in killing opossums, as we had the opportunity to see in Loja. When they hunt an opossum, the dogs kill it immediately by biting its neck and head, and although they do not eat it, obviously there is much contact between the blood of the marsupial and the oral mucosa of the dog, which probably also ingests some amount of blood. Taking into account the fact that *D. marsupialis* is probably the most important natural reservoir host of *T. cruzi* (Miles 1998), we propose that the hunting and killing of opossums by domestic dogs may contribute to build the links between sylvatic and domestic cycles of *T. cruzi* in many rural (and perhaps also suburban) areas of Latin America.

Figure 76. Killing of opossums (*Didelphis marsupialis* and others) by dogs may constitute a route of introduction of *Trypanosoma cruzi* strains from enzootic into peridomestic cycles

5.4.2.5. Results by locality

Some differences were recorded when results obtained in our two fieldwork localities were analysed separately. Thus, multivariate logistic regression showed that in **Loja** three covariates significantly increased the likelihood of DU infestation: houses with

mud walls and tiled roofs, the total number of chickens kept in the household, and the presence of guinea pigs. This was in agreement with the profile of infested DUs reported for Andean communities in northern Peru, including the possible importance of guinea pigs as hosts (Herrer et al. 1972, Cuba Cuba et al. 2002), and further questioned the (somewhat unexpected) finding that peridomestic dogs may play a key role in the domestic ecology of *R. ecuadoriensis* – at least in this Andean community. On the contrary, the number of dogs was associated (together with houses older than 10 years) with a higher risk of infestation in **El Oro**, where chickens seemed to have no effect in the outcome of interest. Clear differences in the building profiles of infested DUs between both localities were not unexpected, because houses are typically built with mud in the interior Andean valleys of Loja and with bricks and timber in El Oro. The only house where an intradomiciliary breeding bug colony was detected in El Oro had mud walls and a tile roof, which was atypical in the area; the owners were immigrants from Loja, and had accordingly reproduced the characteristic Lojan housing style, even if they had been living in El Oro for 65 years. Apart from bedroom infestation in this household (where chicken nests were also infested), all bug colonies were found in peridomestic chicken coops in Lourdes; this indicates that chickens are in fact important hosts, and (perhaps because of the limited sample size or because of unknown confounders) the analyses failed to detect a significant association.

5.4.2.6. Human infection

Notwithstanding the already mentioned reservations about the reliability of the results of serological tests, it may be worth noting that a clear association between seropositivity and both 'cohabitation' with another seropositive and reported antecedents of bug bites was disclosed by logistic regression analysis. The risk associated with the presence of a seropositive in the household (RR=3.17; OR=3.97, 95% CI 1.6-9.5) and with a history of bug bites (RR=3.7; OR=4.02, 95% CI 1.7-10.2) are suggestive of a good vectorial capacity of *R. ecuadoriensis*: when infected mammal reservoirs (here represented by the 'other' seropositive) are present in the house and people get bitten by the bugs, their risk of becoming seropositive increases significantly. This also argues in favour of studying seroprevalence among dogs in Ecuador and Peru.

5.5. Concluding remarks and recommendations

We have for the first time attempted to develop mathematical models aimed at describing the ecological interactions between *R. ecuadoriensis* and various elements of the domestic-peridomestic environment and predicting the effects of modifying those elements in the likelihood of a household becoming infested. Previously, only a few works used a similar approach (multivariate logistic regression modelling) to quantitatively define risk factors favouring the establishment of bug colonies in human habitats, but did not develop predictive models. Thus, Andrade et al. (1995a) modelled the likelihood of DU infestation by *T. infestans* as a function of several discrete covariates (complete/incomplete house building [largely equivalent to houses in good/poor condition in our study]; type of wall, floor and roof; crop storage; presence/absence of chickens, dogs, rodents, and pigs; and presence/absence of peridomestic animal shelters and palm trees), and found four of them to be associated with a significant increase in the likelihood of infestation after adjusting for confounders (house building, earthen floors, food storage and presence of rodents) (Andrade et al. 1995a). In this study, the presence of domestic animals (dogs, chickens, or pigs) had no significant effect on the risk of infestation, but, as observed in our survey, it could be that the number of them, rather than they mere presence, favours infestation – and increases the density of the colonies, promoting disease transmission (Piesman et al. 1985, Gürtler et al. 1992, Cohen & Gürtler 2001). Starr et al. (1991) studied the effects of various dwelling characteristics (good/poor general condition; type of floor, wall and roof; and indoor firewood storage) in the ecology of domestic *T. dimidiata* in Costa Rica. Previous results from observational studies and univariate analyses had shown that a poor sanitary condition of the house, earthen floors, and the presence of firewood could favour infestation (Zeledón et al. 1973, Zeledón & Vargas 1984); although multivariate analysis showed that 95% CI of adjusted OR for those covariates included 1, the authors considered that they should still be considered as potential risk factors (Starr et al. 1991). Host availability was not assessed by Starr and coworkers; it may be suspected from other studies that dogs, rodents, and chickens play a significant role in the domestic ecology of *T. dimidiata* (Zeledón 1981, Zeledón et al. 1973, 2001b).

Cohen and Gürtler observed that because of "the complexity of the natural system and the relative simplicity of any mathematical model, qualitative agreement is the most

that can be hoped for from the comparisons of model predictions and field observations" (Cohen & Gürtler 2001; p. 695); in addition, our analyses were constrained by the fact that infestation was actually detected in only a small number of households, probably (at least in part) because of the operational limitations of active searches (discussed in detail previously). However, the consistency (i.e., the 'qualitative agreement') of our findings (using reported infestation as the dependent variable for modelling) with field observations (e.g., importance of chickens as hosts and of structural house features in providing suitable refuges and breeding sites for the bugs) supports the satisfactoriness of our approach. Deriving flawless, definitive conclusions as to the ecology of synanthropic populations of *R. ecuadoriensis* was not among our objectives in this part of the study; we rather aimed at setting the basis for improved fieldwork methodologies that could be incorporated in large-scale, routine control activities (including the baseline studies of the preparatory phase). One major outcome of this research will therefore be the proposal of standardised fieldwork protocols with maximum efficiency as the optimality criterion.

Thus, according to the results here presented, **entomological surveys** should adopt slightly different tactics in areas with typical 'coastal' and 'Andean' house building patterns. Timber-walled coastal dwellings seem less propitious than mud-walled (mainly Andean, including the valleys of southern Ecuador and most of the species range in Peru) houses for the establishment of indoor bug colonies; however, special attention needs to be drawn to complex roof structures (mainly timber-and-tile), irrespective of wall building materials. A further major point would be the necessity for accurate inspection of chicken coops and dovecotes, with inspectors receiving specific training for the detection and identification of *Rhodnius* eggs, eggshells, and exuviae. The use of chemical flushing-out agents may represent and advantage for uncovering indoors infestation, but at least in Andean communities of southern Ecuador the accounts by the residents and their ability to recognise bug specimens seem to provide a highly reliable estimation of household infestation.

Intervention strategies may also be refined based on our results. In the short term, it seems particularly important to formally incorporate peridomestic structures used to keep poultry into control schemes. Not only they should be thoroughly inspected and sprayed; it would also be desirable that dwellers are vigorously encouraged to replace

hen nests on a monthly basis, burning old ones and at the same time checking that no signs of infestation are found in their coops. In this sense, educational interventions should emphasise the link between infestation and disease, and the fact that peridomiciliary bug colonies (not only those found indoors) also represent a serious health threat.

Operational design of chemical control interventions will have to consider the possible tolerance to malathion suggested by some of our observations. Synthetic pyrethroids should constitute the first choice, and bioassays should be performed to confirm the susceptibility of the bugs to the products utilised. Our studies with laboratory colonies founded with field-collected bugs from Loja show that *R. ecuadoriensis* incubation period is of about one month (details not shown); insecticides should therefore have lethal residual effects for over 30 days (both indoors and in peridomiciles). The same studies (bugs kept at 28±1°C and 75±5% relative humidity and fed fortnightly on restrained chickens) indicate the *R. ecuadoriensis* can only produce one generation per year [egg-to-adult development time was of ~431 days on average (median=389, minimum=126 days), with adult survival reaching over one year (mean=204±113 days, median=163, maximum=378)], suggesting a limited capacity for population recovery after control interventions.

Long-term control of disease transmission may benefit from housing improvements; according to our findings, changes aimed at reducing structural complexity of walls and roofs would have a maximal impact. This would entail walls with fewer crevices and cracks (properly plastered) and, importantly, a progressive replacement of traditional timber-and-tile roofs by others with a simpler structure and without timber frameworks (e.g., concrete or planks of fibre-cement – onto which tiles could perhaps be laid to improve insulation and increase acceptance by local residents). In general, we confirmed that poverty significantly increases the risk of infestation (probably reflecting poorer housing quality), supporting the view that social and economic development may have a substantial impact in disease transmission.

Overall, it would seem that after a classical attack phase based on pyrethroid spraying (paying special attention to peridomiciles), a relatively simple entomological surveillance system (aided by good levels of knowledge about the vectors among the residents) with emphasis on a better management of poultry and the report of infestation

by householders could reduce transmission very significantly in the large geographic range of strongly synanthropic *R. ecuadoriensis* populations; selective responses to reports by local residents would result in relative low investment requirements, improving cost-benefit ratios. In the areas where *Phytelephas* palms harbour sylvatic vector populations (mainly the central coastal region of Ecuador), the attack phase would have to be designed according primarily to household infestation rates by *T. dimidiata*. The possibility of subsequent occupation of the empty ecological niches by *R. ecuadoriensis* implies that community-based surveillance and selective interventions would be required to halt transmission in the long term; perhaps in this case, as suggested by results of our research on sylvatic *R. ecuadoriensis* populations, environmental management schemes aimed at high-risk palm tree ecotopes would help reduce the frequency of invasion-colonisation of artificial structures by vectors of sylvatic origin.

6. BIOLOGICAL DIVERSITY IN *RHODNIUS ECUADORIENSIS*

6.1. Morphological and chromatic variability

6.1.1. INTRODUCTION

R. ecuadoriensis was described in 1958 (Lent & León 1958) from material collected by LA León in 1955 in domiciles of La Toma (1270m above sea level, 3°59'S 79°21'W), a rural area of the temperate Andean valleys of the Catamayo-Chira river system (province of Loja, southern Ecuador). The type material was at the time of the original description found to be "of very small size and general aspect similar to that of *R. pallescens*"; the authors acknowledged that it was initially misidentified as *pallescens*, but biogeographical data (*pallescens* had only been reported from Panama), overall size (much larger in *pallescens*), some chromatic traits (with *pallescens* presenting better-defined connexival dorsal markings), and the shape of the median process of the pygophore, led to the conclusion that the Ecuadorian specimens belonged to a different taxon (Lent & León 1958; p. 181). Other mottled species (*R. pictipes* and the closely related *R. stali* and *R. amazonicus*) can be readily differentiated from *ecuadoriensis* (for instance, their tibiae are uniformly yellowish except for a transversal black band on the basal third); they are also much larger, and occur on the eastern (Amazonian) side of the Andes (Lent & Wygodzinsky 1979, Lent et al. 1993, Bérenger & Pluot-Sigwalt 2002).

The morphological and chromatic characteristics of *R. ecuadoriensis*, combined with its geographic distribution (allopatric to any other known species of the genus), allow therefore for unambiguous specific determination of field-collected material. Since the original description however, several geographic populations of the species have been recorded from a large area including Pacific coastal lowlands and temperate inter-Andean valleys of central and southern Ecuador and northern Peru (Abad-Franch et al. 2001b, Cuba Cuba et al. 2002). In addition, some of those populations seemed to display different ecological preferences, ranging from sylvatic colonies detected in palm trees of central-western Ecuador (Carcavallo & Martínez 1985, Avilés et al. 1995b) to synanthropic bugs both in Ecuador and Peru (Defranc 1982, Lazo 1985, Calderón et al. 1985, Abad-Franch et al. 2001b, Cuba Cuba et al. 2002). Despite the apparent biological diversity of these populations, and despite the possible epidemiological implications (because of suggestions that different populations display varying degrees of

synanthropism), no comparative inter-population analyses have been conducted in *R. ecuadoriensis*. Here we present a characterisation of several populations of the species, comprising most of its geographic range and ecological variants, based on external anatomical and chromatic features. The remarkable degree of intraspecific heterogeneity detected during this evaluation was further analysed by means of digital morphometrics and molecular biological methods (Sections 6.2. and 6.3.), and the main ecological traits of both sylvatic and synanthropic Ecuadorian populations were studied in a series of field surveys in western Ecuador (Chapters 4 and 5).

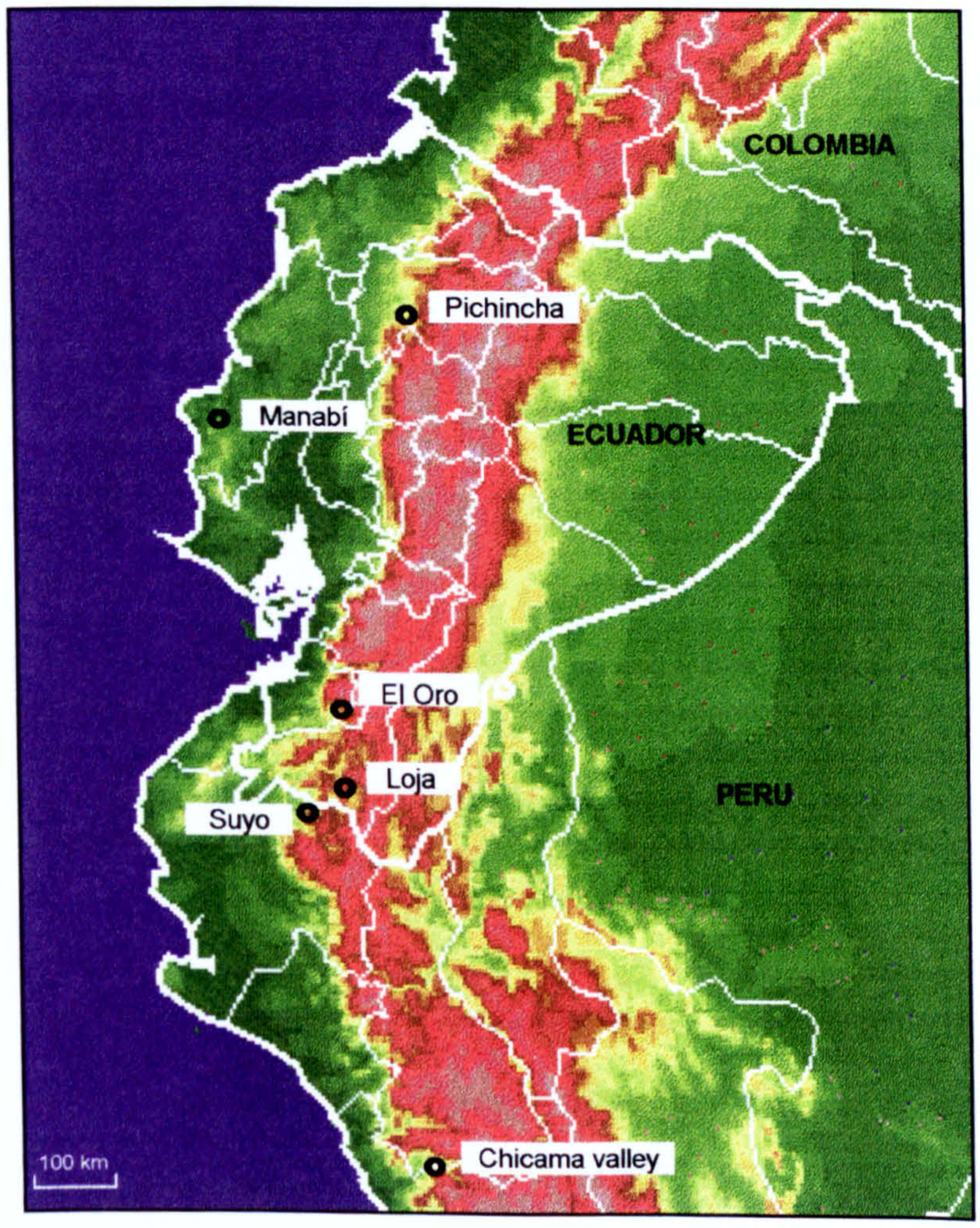


Figure 77. Population diversity in *Rhodnius ecuadoriensis*: origin of specimens in Ecuador (provinces of Pichincha, Manabí, El Oro, and Loja) and northern Peru (Suyo, Department of Piura, and upper Chicama valley, Department of La Libertad). All specimens were collected in areas of the western slope of the Andes; geographic locations are approximate

6.1.2. MATERIALS AND METHODS

We compared the type specimens (three males and two females deposited at the Herman Lent collection, Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos [LNIRTT], Departamento de Entomologia, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, Brazil) and the descriptions based on type material (Lent & León 1958, Lent & Wygodzinsky 1979) with bugs collected during our field surveys (natural populations from Loja, El Oro, Manabí, and Pichincha). Peruvian material collected in human environments in the Departments of Piura and La Libertad was made available by Prof. CA Cuba Cuba (University of Brasília, Brazil), and bugs from a colony founded with sylvatic specimens collected from *Phytelephas aequatorialis* palm trees in coastal Ecuador (Manabí) and kept at Fiocruz were kindly donated by Prof. J Jurberg (LNIRTT, Brazil). Our approach for this qualitative assessment combined the exhaustive review of morphological and chromatic traits (except for the complete dissection of male genitalia) with considerations on biogeographical records (Section 3.2.) and the known ecological preferences of the species (Sections 4.1. and 5.1.). For comparisons with closely related species, the descriptions and ecological remarks by Lent & León 1958, Lent & Wygodzinsky 1979, Carcavallo et al. 1997a, 1998a, and Moreno et al. 1999 were utilised; laboratory colony specimens of *R. pallescens* and *R. colombiensis* were made available by Dr NO Jaramillo (University of Antioquia, Medellín, Colombia) for comparative analyses.

6.1.3. RESULTS

Two major phenotypic groups were identified among the field-collected material examined that could be readily distinguished from each other. The first of these groups is comprised of bugs whose morphological and chromatic characteristics correspond well with descriptions based on type material. The phenotypes within this group were however heterogeneous, with size variants apparently related to the primary habitat of the bug populations (sylvatic vs. synanthropic) and with more subtle differences recorded between geographic populations of domestic-peridomestic vectors. The second major cluster was found to differ significantly from the type material, both in size and colouration; these bugs were only found in sylvatic environments in the province of Pichincha.

6.1.3.1. Typical forms of *Rhodnius ecuadoriensis*

·Synanthropic populations: Loja and El Oro. Specimens collected in the southern Ecuadorian provinces of Loja and El Oro were found to be virtually identical to the type material; these were synanthropic populations found in areas where the absence of palm trees suggested the possibility of isolation from sylvatic conspecifics (Section 3.2.4.). The main anatomical and chromatic features of these 'typical forms' corresponded therefore to the classical descriptions of the species summarised below.

Very small triatomines: total length 12.5-13.5mm (males) and 14.5mm (females). The overall colour of adult bugs is light brown-yellowish with dark brown markings (stripes and irregularly shaped dots) on the body and appendages; this pattern is especially conspicuous on the legs.

The head is granulose, relatively short and stout for the genus, approximately twice as long as wide across the eyes and very slightly longer than the pronotum. Eyes are medium-sized; in a lateral view, they slightly surpass the inferior (but do not attain the superior) limit of the head. The basal 3/5 of the second segment of the antennae is light brown, with the apical 2/5 much darker; the third segment is basally dark and apically lighter. The second rostral segment does not attain the neck, although it reaches the level of the ocelli and slightly surpasses it in some specimens. The neck is dark on the sides, with a light, broad medial band on the dorsal surface.

The anterior lobe of pronotum is granulose (with rounded and non-prominent anterolateral angles), and the posterior lobe rugose-granulose and light brown. Submedian carinae and lateral margins are yellowish, and the spaces between them present a mottled pattern with dark spots forming two darkish, poorly defined stripes in each of these spaces. The scutellum has 2+2 well-defined, yellowish anterior carinae that fuse into 1+1 on the centre of the scutellum and then into a single median carina on the posterior process (whose upper surface is therefore light coloured).

The hemelytra are straw-coloured, including veins; irregular dark stripes and spots with lighter dots cover the spaces between veins, giving an overall mottled aspect.

Legs are light yellowish brown with a conspicuous mottled pattern in which dark brown stripes and spots irregularly cover the surface of femora (where the pattern is

more conspicuous and dark markings can be seen to concentrate on the distal third) and tibiae (with darker distal extremes).

The ventral surface of abdomen is light brown-yellowish, irregularly mottled with dark brown. Dorsally, connexivum segments present a dark brown spot covering the anterior 1/3 to 1/2. The median process of the pygophore is shortly triangular and has a pointed apex.

·Synanthropic populations: northern Peru. Several Peruvian specimens of *R. ecuadoriensis* were generously made available for comparative analyses by CA Cuba Cuba (University of Brasília, Brazil). These were field-collected bugs found in human environments in the arid-semiarid Andean valleys of the Cascas district (upper Chicama valley, Department of La Libertad, ~500km south of the Ecuadorian border), except for one specimen collected in Suyo (Department of Piura, near the border with Loja).

The overall aspect of these bugs coincided with the type material described above; however, while the specimen from Suyo could not be distinguished from those collected in Loja or El Oro, Chicama bugs were noticeably lighter than the Ecuadorian material. This was more evident in the legs of the insects, where the dark mottled pattern was limited to small clusters of dots and stripes concentrated on the basal and distal thirds of femora (leaving a central, broad yellowish area with few or no black markings) and tibiae.

Peruvian bugs also seemed more slender than the typical specimens from Loja-El Oro; their legs for instance tended to be relatively longer and thinner. Width:length ratios for hind and medial femora were 1:8.82-9.2 and 1:7.2, respectively (Peruvian bugs from Chicama), vs. 1:8.81 and 1:5.7 (typical specimens from Loja). The pronotum also tended to be noticeably wider in the typical material than in the Peruvian population, with length:width ratios of 1:1.43 (Peru) vs. 1:1.68 (Loja). Heads of the Peruvian material were (on average) over 2.5mm long (2.4 to 2.68mm) and over 1.26mm (1.17-1.32mm) wide (ratio length:width 1:0.47-0.52), whereas they were about 2.34mm (2.1-2.6mm) long and 1.2mm (1.1-1.3mm) wide in southern Ecuadorian bugs (ratio length:width 1:0.49-0.56).

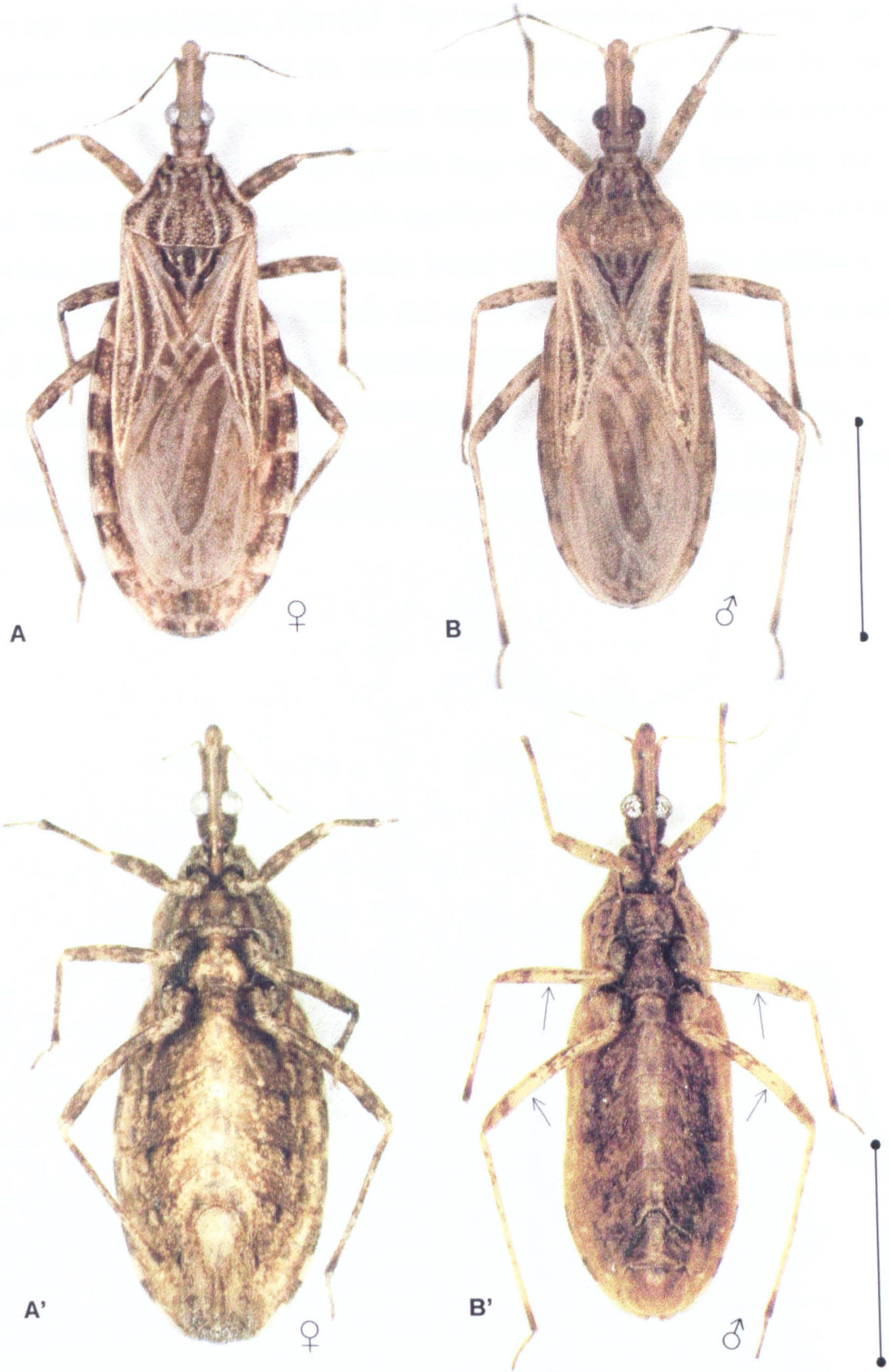


Figure 78. Phenotypic variation in *Rhodnius ecuadoriensis*: typical specimens from southern Ecuador (province of Loja, female [**A** = dorsal view, **A'** = ventral view]) and northern Peru (Department of La Libertad, male [**B** = dorsal view, **B'** = ventral view]). Note the lighter overall colouration and the more slender aspect (including longer legs) of the Peruvian material. The typical irregularly shaped dark markings are more abundant in the Ecuadorian specimen, particularly on the legs. These markings tend to appear in clusters on the basal and distal thirds of the femora, leaving a lighter band clearly perceptible in the Peruvian specimens (arrows). Scale bars: approx. 5mm

·Sylvatic populations: Manabí. *Phytelephas aequatorialis* palm trees harbour *R. ecuadoriensis* populations in the central coastal province of Manabí. The overall aspect and colouration of these specimens largely coincides with the descriptions of typical *ecuadoriensis* forms, but the sylvatic bugs are perceptibly larger than the type material. Thus, total length of females is usually of about 16mm, with males averaging ~14.5-15mm in length. The heads are also longer than in synanthropic specimens, with average values typically over 2.95mm (2.6 to 3.2mm); length:width ratio is usually 1:0.42-0.46. Legs are clearly longer and more slender than in typical specimens, with hind leg ratios as follows. Length femur:length tibia 1:1.4; width:length femur 1:11.5. Some light specimens are somewhat similar to Peruvian material in that the irregular dark markings tend to concentrate on the basal and distal thirds of the femora.

Figure 79. *Rhodnius ecuadoriensis* female specimen collected in central coastal Ecuador (province of Manabí). Note the non-uniform distribution of dark markings on the legs (mainly on hind femora, where the blackish dots concentrate on the basal and distal thirds, leaving an intermediate lighter area [arrow]). Scale bar: approx. 5mm

6.1.3.2. Pichincha forms

In the last months of 1998, a male specimen of *Rhodnius* was collected near Alluriquín, by the road Quito-Santo Domingo de los Colorados (province of Pichincha, western slope of the Andes, at approximately between 800 and 900m above sea level) and brought to us for identification. The site of capture was within the theoretical range of *R. ecuadoriensis*, which is not shared by any other known species of *Rhodnius*, but the overall morphology and colouration pattern of the specimen differed significantly from the descriptions of the type material. A more detailed examination evidenced the following:

(1) The specimen was noticeably larger than reported for *R. ecuadoriensis*, but smaller than other closely related species

Table 77. Size of typical *Rhodnius ecuadoriensis*, the Pichincha specimen, and related species

Measurements	*R. ecuadoriensis*	Pichincha	*R. colombiensis*	*R. pallescens*
Total length	12.5 – 13.5mm	17.2mm	17-18mm	21.5-23.5mm
Width of pronotum	2.5 – 3mm	3.9mm	4-5mm	4.5-5mm

(2) The Pichincha specimen was remarkably darker than reported for *ecuadoriensis*: the overall colour was dark brown, almost black, with lighter markings in head and pronotum (these marks being reddish); the mottled pattern, although present on all the body parts, was very inconspicuous (the dark background colour made the spots much less noticeable than they are in the yellowish typical *ecuadoriensis* and in *pallescens*);

(3) After removing one of the parameres, we could inspect the median process of pygophore of the genitalia of this male; this structure has been used in systematics of *Rhodnius* by several authors (see Lent 1958, Lent & Jurberg 1969, Lent & Wygodzinsky 1979, Jurberg 1996, Jurberg et al. 1997, Moreno et al. 1999); it is short, triangular, and with a pointed apex in *R. ecuadoriensis*. In the Pichincha male, this structure was triangular (as in all *Rhodnius* except *pictipes*, *stali*, *amazonicus*, and *paraensis*), but had a somewhat truncated apex;

(4) The head of this *Rhodnius* specimen (see figure 80) was much longer (3.5mm) than reported for *ecuadoriensis* (around 2.5mm), and shorter than in *pallescens* (around 4.5mm or more). Head length of the recently described *R. colombiensis* is of about 4mm.

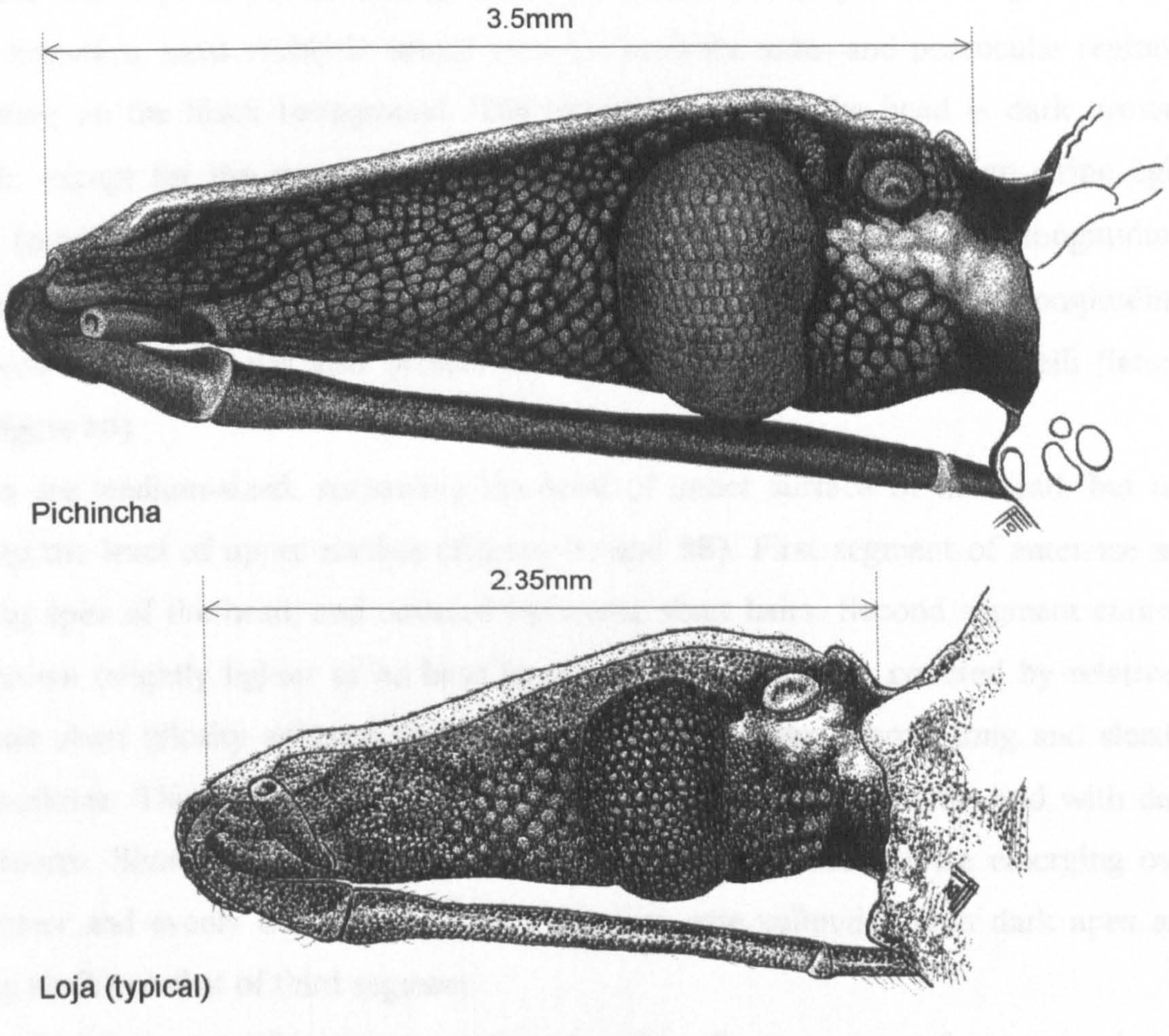


Figure 80. Heads of two *Rhodnius ecuadoriensis* male specimens (lateral view). Note the differences in size and shape between the sylvatic Pichincha forms (top figure) and synanthropic typical forms from southern Ecuador (bottom figure). The total length of head (neck→anteclypeus) is indicated in mm

Field surveys were carried out in the area of Andean foothill humid forests where this specimen was collected, and abundant material was recovered from *Ph. aequatorialis* palms using live-baited adhesive traps (Abad-Franch et al. 2000; see also Section 4.2.3.). The following **description** is based on that material.

Small triatomine (overall body length of males 17mm, of females 17.7mm). Overall colour dark brown, virtually blackish in many live specimens, with lighter brown markings (with an orange-reddish dye in many specimens) on various parts of the body and appendages (figures 81-85). Irregularly shaped dark spots are present on legs, but they are not very conspicuous as the brown-reddish background colour is only slightly lighter. A short golden pilosity is present on most of the integument.

Head granulose (more conspicuously on lateral view, especially in the post-ocular region); almost 2.5 times as long as wide across the eyes; and slightly longer than the

pronotum. Short golden setae emerge from the granules; many of these granules are orange-brownish, most visibly in lateral view (in both the ante- and postocular region), contrasting on the black background. The overall colour of the head is dark brown-blackish, except for the mentioned granules and a narrow dorsal, median stripe light brown (reddish in many live specimens), which completely covers the longitudinal median prominence – but does not surpass it (figures 80, 81, 88). 1+1 light longitudinal light brown-orange stripes also present in the postocular regions under ocelli (lateral view, figure 80).

Eyes are medium-sized, surpassing the level of under surface of the head, but not attaining the level of upper surface (figures 80 and 88). First segment of antennae not attaining apex of the head, and covered by scarce short hairs. Second segment entirely dark brown (slightly lighter at its base in some fair individuals), covered by relatively abundant short pilosity adhered to the surface and with very scarce, long and slender trichobothriae. Third segment light brown yellowish on its distal 3/4ths and with dark basal fourth. Short adherent hairs abundant, with longer trichobothriae emerging over the former and evenly distributed. Fourth segment pale yellowish with dark apex and pilosity similar to that of third segment.

The first segment of rostrum reaches the site of emergence of antennae in the antenniferous tubercle. The second segment is long, attaining the neck and clearly surpassing the level of ocelli. The third segment of the rostrum reaches the inferior groove of the prosternum in which the stridulatory sulcus is located, attaining the acetabuli of fore coxae (figure 80). Pilosity scarce on the rostrum: short setae on first and second segment, with longer and more slender, straight setae on the third segment.

Neck dark brown-blackish, with light brown-reddish longitudinal stripes (one wide band covering the median area of dorsal side of neck, and 1+1 narrower stripes below the level of ocelli – continuing the lateral stripes of postocular region; figure 80).

Pronotum divided into two lobes (anterior and posterior) by a relatively pronounced transversal groove that determines a quite conspicuous curvature of submedian carinae (figures 81-82). The anterior lobe is granulose, with a very dark brown-blackish background colour and a few small tubercles (with very short setae) in the spaces between submedian carinae and lateral margins. The space between carinae is black (an X-like shape is visible in some light specimens) and divided by a marked, median

longitudinal rut. Anterolateral angles not very prominent but slightly pointed; they are black with external margins and tips light brown-yellowish (same colour as the rest of the collar). Posterior lobe rugose-granulose, with background colour dark brown blackish and lighter brown tubercles (with very short, inconspicuous setae) regularly distributed in the spaces between carinae and between lateral margins and carinae. This arrangement results in an overall spotted pattern of the hind lobe of pronotum. Carinae and pronotum margins light brown (reddish in many specimens), well demarcated by the dark background colour. Posterolateral angles not very prominent (figures 81-82).

The scutellum is rugose, dark brown to black with 2+2 yellowish basal carinae that merge in 1+1 carinae at the body of scutellum and then into a single carina, with light colour extending to the process of the scutellum. The central furrow between submedian carinae presents marked, rough transversal rugosities (6 to 7). The posterior process is almost as long as the body of scutellum, yellowish on its upper side as a continuation of median carina. It is rugose on its base and has a rounded tip, lighter than the base.

Overall aspect of legs dark brown with short pilosity on all their surface; irregularly shaped, very dark markings overlay a slightly lighter, reddish brown background colour. Such mottled pattern is more evident on coxae and throchantera (where dark markings are very conspicuous and background colour is manifestly reddish in most specimens) and on femora (figure 84), but hardly noticeable on tibiae, whose distal third is almost black and covered by dense pilosity. Narrow, longitudinal black stripes are present on femora, and are particularly noticeable on the hind pair of legs. Tarsi are covered by longer hairs. Spongy fossulae are present in the fore and medium pairs of legs, both in males and females. Femora and tibiae are long and slender (ratio width:length of femora 1:6.9-9.6; ratio length of femora:length of tibiae 1:1.18-1.45).

Hemelytra brown, not attaining the hind extreme of abdomen. Membranes are slightly rugose and brown; round lighter, confluent spots are present on membrane cells. Venations light brown, thus being conspicuous onto the darker brown background. Corium well differentiated from membranes, with lighter venations and darker cells (the marginal one presenting a lighter central area) (figure 81).

Abdomen dark brown, covered by pale short setae. An overall pattern of light and dark spots is visible. Urosternites are blackish brown with a lighter spotted pattern. A triangular, lighter mark pointing towards the head is present on the median area of each

urosternite. Spiracles are visible as light round spots on the darker external part of urosternites, close to the margins (figures 83-84). The integument of the urosternites is finely granulose and transversally striate. Connexival segments dark brown-blackish. Yellowish markings (with reddish dye in many specimens) cover the posterior fourth of urotergites, including the suture and projecting forwards on the external half of segments (reaching the hind third of the marginal half of each tergite). The dark three-fourths of each segment present a longitudinal stripe of slightly lighter, small round dots (figure 85).

The median process of the pygophore is triangular. Its apex is not evidently pointed, but the tip is truncated in the majority of specimens examined (figure 86).

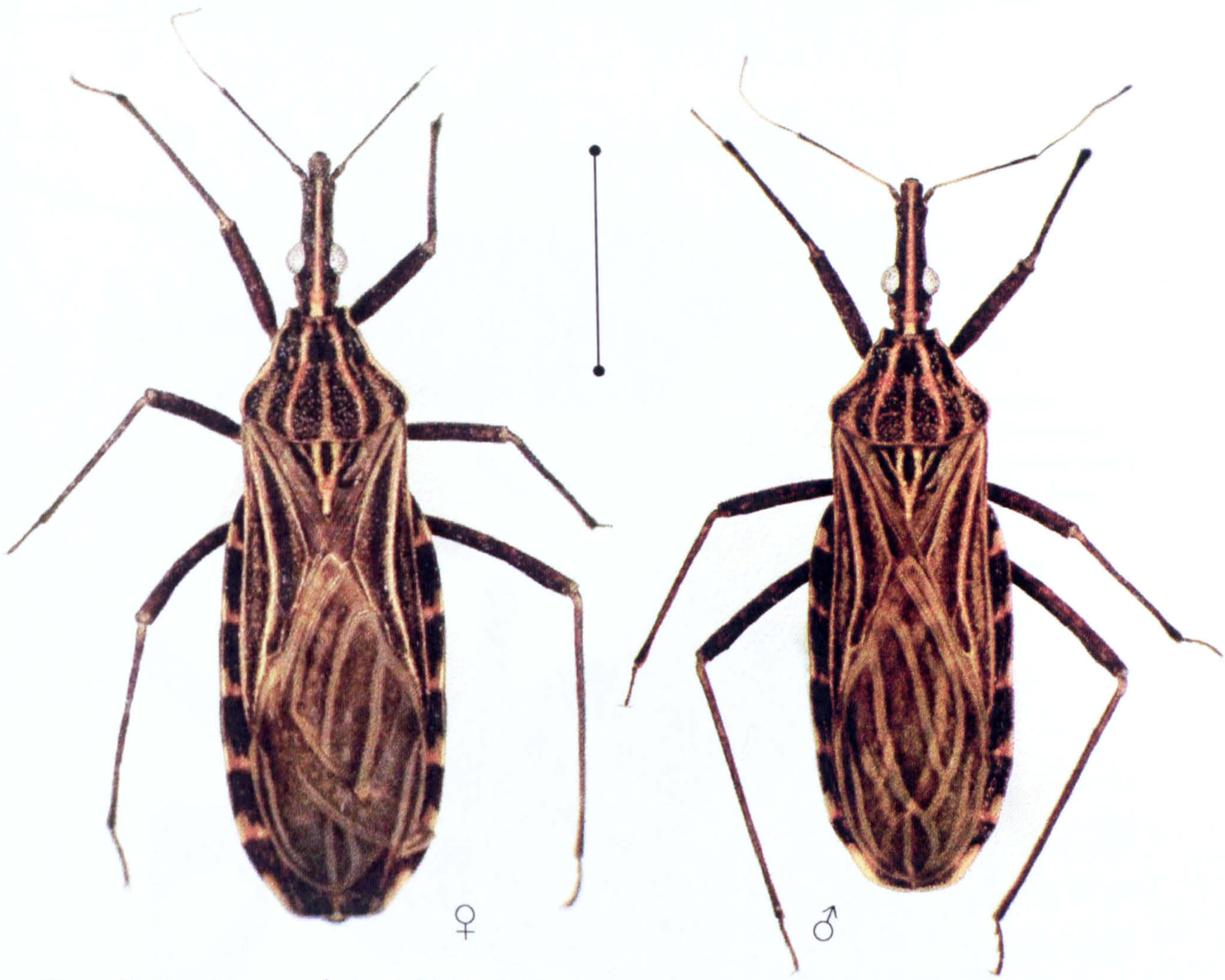


Figure 81. *Rhodnius ecuadoriensis* Pichincha forms (female and male, dorsal view). Note the overall dark colouration with reddish markings and the scarcely conspicuous mottled pattern of the legs. Scale bar: approx. 5mm

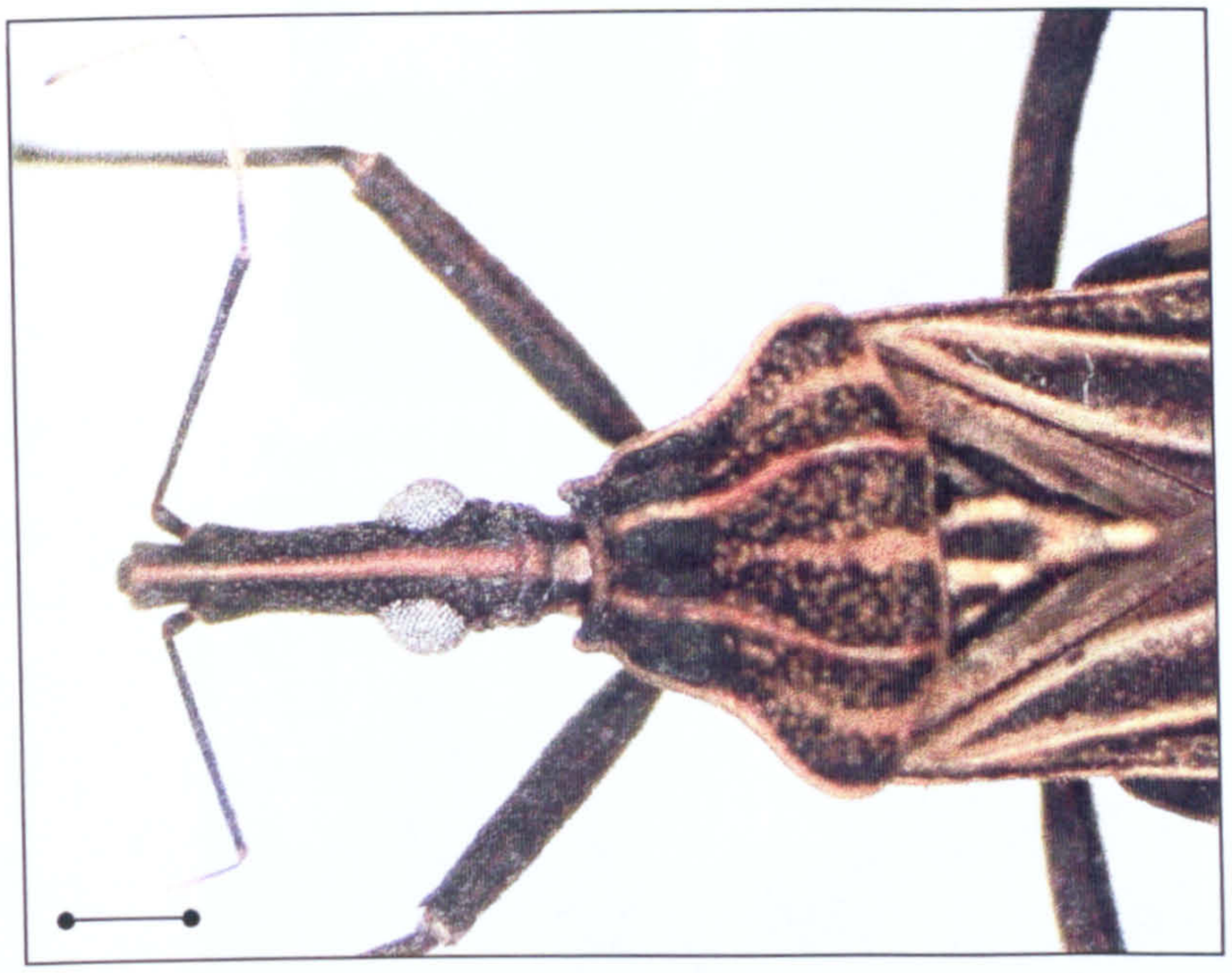


Figure 82. *Rhodnius ecuadoriensis* Pichincha forms. Head and pronotum in dorsal view. Note the reddish markings and the four antennal segments. Scale bar: approx 1mm

Figure 83. *Rhodnius ecuadoriensis* Pichincha form (female, ventral view). Scale bar: approx. 5mm

Figure 84. *Rhodnius ecuadoriensis* Pichincha forms. Detail of the chromatic pattern of thorax and abdomen in ventral view. Note the irregular dark markings overlaid on a reddish background on the trochantera and the arrow-shaped design of medial markings on urosternites

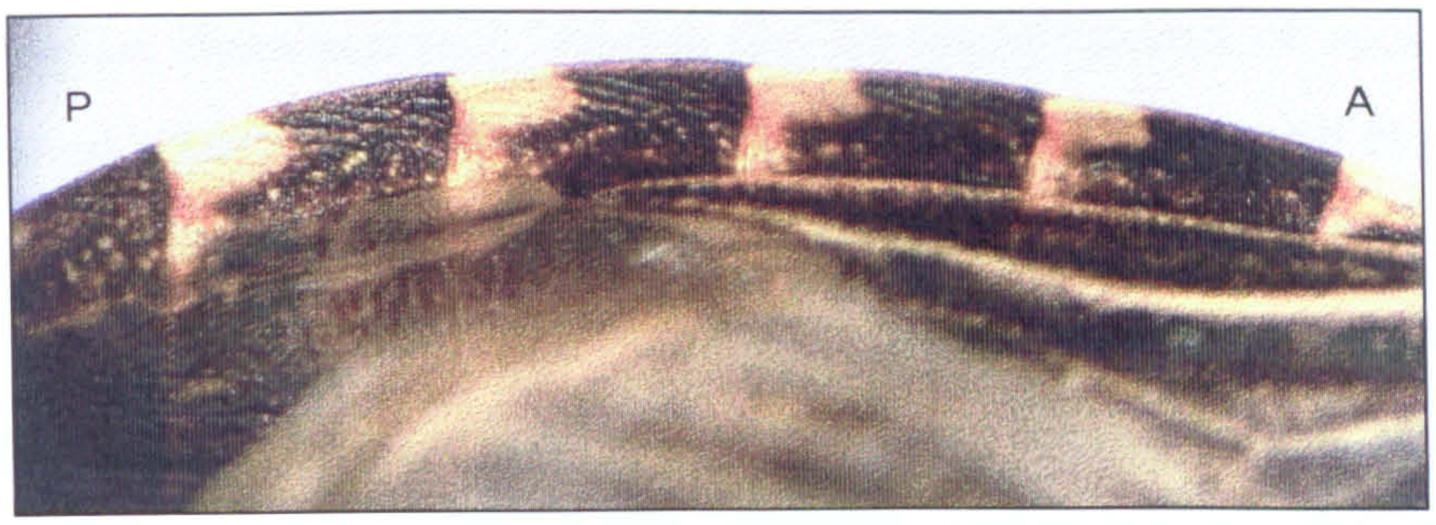


Figure 85. *Rhodnius ecuadoriensis* Pichincha forms. Detail of the chromatic pattern of the connexivum (dorsal view). A = anterior; P = posterior

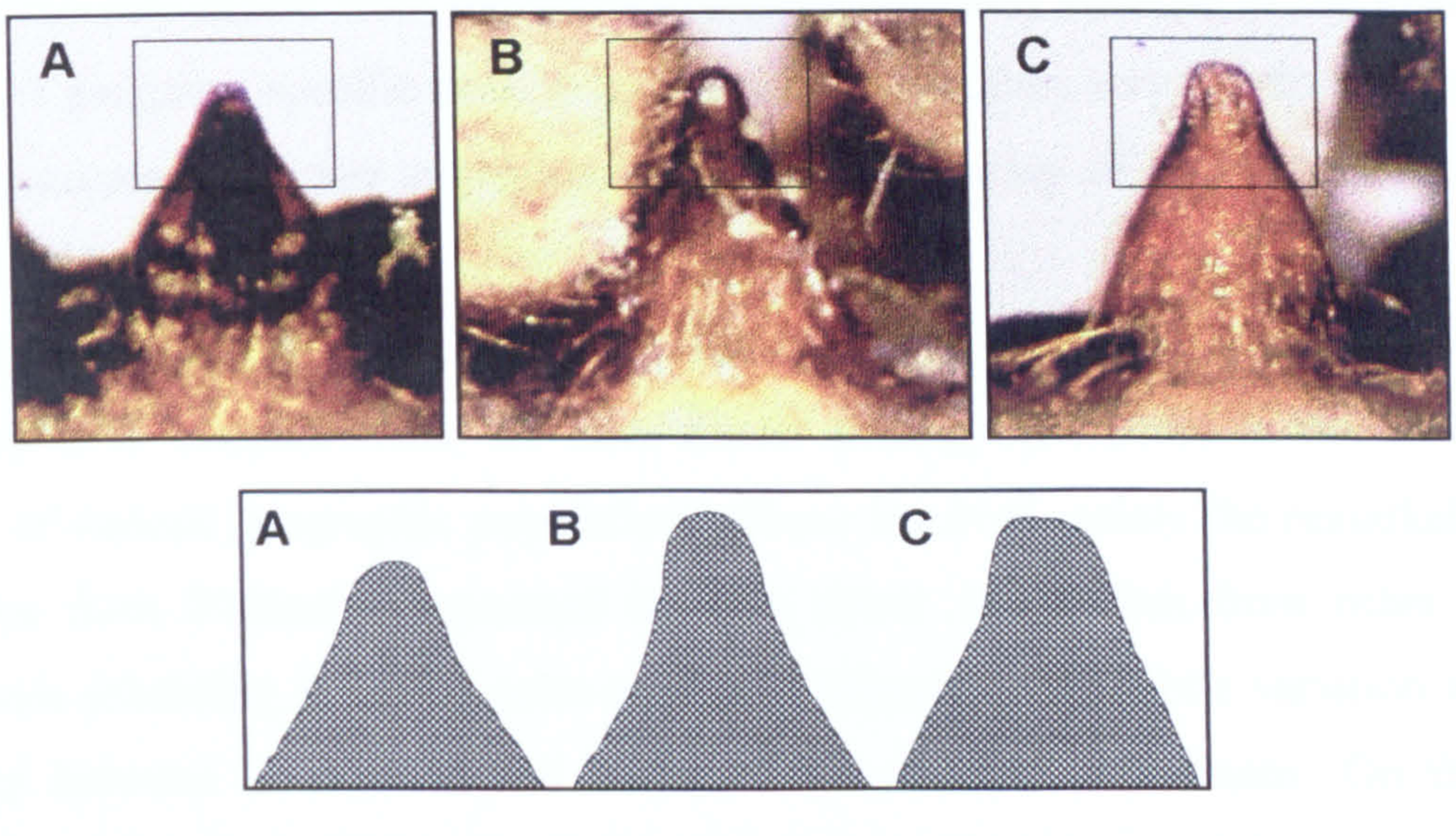


Figure 86. External male genitalia of *Rhodnius ecuadoriensis*: median process of the pygophore. Pictures (upper) and schematic outline of the apex of the process (lower). A = Loja; B = Manabí; C = Pichincha

6.1.4. DISCUSSION

6.1.4.1. Intraspecific variability in *Rhodnius ecuadoriensis*

Population phenotypic variability is a relatively common phenomenon in Triatominae; it has been proposed that ecological factors are the main forces underlying rapid morphological changes in triatomines (Dujardin et al. 1999b). Thus, adaptation of different populations to particular microhabitats may result in distinct phenotypes, and some geographic populations of species with large distribution ranges also exhibit consistent anatomical and chromatic differences. In some instances these phenotypic variants were considered to represent different species or subspecies (these latter not recognised currently), whereas in other cases they were treated simply as geographic forms (Lent & Wygodzinsky 1979). Triatomine phenotypic plasticity may be of particular significance, firstly because the entire taxonomic and systematic arrangement of the subfamily is based on anatomical characters and secondly because vector control

programmes rely on macroscopical features for the identification of specimens – and operational strategies are in turn defined according to the species present in each area.

Examples of intraspecific phenotypic plasticity in triatomines are abundant, and include more or less subtle changes in overall colour or marking design, size/shape character variants (often only detectable by morphometrics), and anatomical features ranging from slight variations in male genitalia structures to the striking alary polymorphism of *Mepraia spinolai*. It is worth noting however that *Rhodnius* species are conspicuously absent from the list of examples, probably because of the mainstream strategy of assigning specific rank to populations presenting very subtle differences in their phenotypes. The only exception would be the finding of two forms of *R. stali* ('pale' and 'dark') in Bolivia.

·Chromatic variation. Despite the absence of any previous allusions to chromatic variability in *R. ecuadoriensis*, we have found striking differences in the colouration patterns of various geographic populations. These involved mainly the remarkably dark specimens from Pichincha, separated by only about 180-200km from other sylvatic populations inhabiting the same palm species in Manabí; more subtle variation was also identified between Ecuadorian and Peruvian synanthropic populations. On the other hand, bugs from Manabí and those from southern Ecuador had basically identical chromatic patterns. The differences in colouration described here were constant in several hundred specimens examined, and primarily involved varying degrees of pigmentation intensity – rather than changes in marking design patterns.

Melanic forms of other triatomine species have been described; among the most notable examples was the finding of completely black specimens of *T. infestans* in northern Argentina, first described as a subspecies (*T. infestans melanosoma*), then elevated to species rank (as *T. melanosoma*), and recently shown to probably represent a population of *T. infestans* (Dujardin et al. 1999b, Monteiro et al. 1999b). Similarly, sylvatic dark morphs of *T. infestans* inhabit hardwood hollow trees in the Bolivian Chaco (Noireau et al. 2000a,b). Chromatic variants are also common in *T. brasiliensis*; three of them were treated as subspecies (*T. b. brasiliensis*, *T. b. melanica*, and *T. b. macromelasoma*) until they were synonymised, and a fourth, darker population (the Juazeiro population) was later described. In this case however, allozyme and mtDNA analyses indicated a correspondence between phenotypic and genetic variation (Costa

1999, Costa et al. 1997, Monteiro et al. 1999a). *T. rubrofasciata* is also a variable species, with virtually black specimens found in Hawaii, but no clear correspondences with morphometric variation were identified (Patterson et al. 2001). Further examples of intraspecific variation of overall pigmentation include *T. protracta* and *Paratriatoma hirsuta* in North America (Lent & Wygodzinsky 1979) or the mention of 'pale' and 'dark' varieties (with identical ITS-2 sequences) of *R. stali* (Marcilla et al. 2001).

In other cases the differences involve the design and patterns of colour markings. Thus, extensive variation has been described on the size, number, and distribution of dark spots on the ventral connexival segments of *P. geniculatus* (Lent & Wygodzinsky 1979), and we found apparently constant differences in the black markings of the pronotum between specimens collected on either the Pacific or Amazonian sides of the Ecuadorian Andes (Abad-Franch et al. 2001b). Similarly, the red areas of the pronotum of *T. rubrovaria* and *T. circummaculata* vary widely in size and shape (Lent & Wygodzinsky 1979).

In the interpretation of the patterns of chromatic variability observed in *R. ecuadoriensis* we consider the following aspects:

i. The main pattern of variation involves intensity of the pigmentation, rather than changes in the design of markings. Among specimens with typical colouration, more melanic forms may present a larger amount of irregular dark spots and markings, or these markings may have larger surfaces. Pichincha forms present large and abundant black markings and a reddish-brown, generally very dark background colour.

ii. The overall colouration of the two sylvatic populations considered here (Pichincha and Manabí) seem to correspond to differences in the microhabitat of the bugs. In the drier climate of Manabí, dead fronds and fibres of *Phytelephas aequatorialis* palms tend to dehydrate, resulting in a straw-like coloured habitat substrate; on the contrary, they decompose more quickly in the humid conditions of the Andean foothills of Pichincha, conferring a darker (actually dark brown-reddish) dye to the microhabitat. It seems therefore likely that the different colourations of specimens from both areas are related to camouflage.

iii. The fact that the 'typical' chromatic forms of *ecuadoriensis* are very similar in colour to the closely related *R. pallescens* (and, to a certain extent, to *R. pictipes*) is suggestive of this character state (light brown-yellowish background colour) being

plesiomorphic – with the dark brown-reddish background colour of the melanic Pichincha forms being derived.

iv. There appear to be some correspondences between the chromatic patterns and other characteristics of the different populations studied, including size/shape variation and ecological preferences; these correspondences are discussed below.

·Size and shape variation. Obvious differences in the average sizes of bugs from different populations were observed. As shown for other species, a general trend to reduced body size in synanthropic (vs. sylvatic) populations was revealed; this aspect was further explored by means of head capsule morphometrics, and is accordingly discussed in Section 6.2. A gradual reduction in overall size could be observed that broadly corresponded to a north-to-south axis, from the very large sylvatic forms of Pichincha to the small synanthropic populations of southern Ecuador and northern Peru – and with the bugs from Manabí occupying an intermediate position. Also in the same sense, the heads of the bugs appeared to be longer and relatively more slender in the northern sylvatic bugs. However, detailed examination of the Peruvian material showed that they tend to be slightly larger and have more elongated heads and legs, and less wide thoraces, than Ecuadorian synanthropic bugs from Loja and El Oro. These traits and colouration similarities observed between Peruvian bugs and some pale specimens from Manabí 'interrupt' what otherwise appears to be a tendency to clinal variation with clear geographic correspondences.

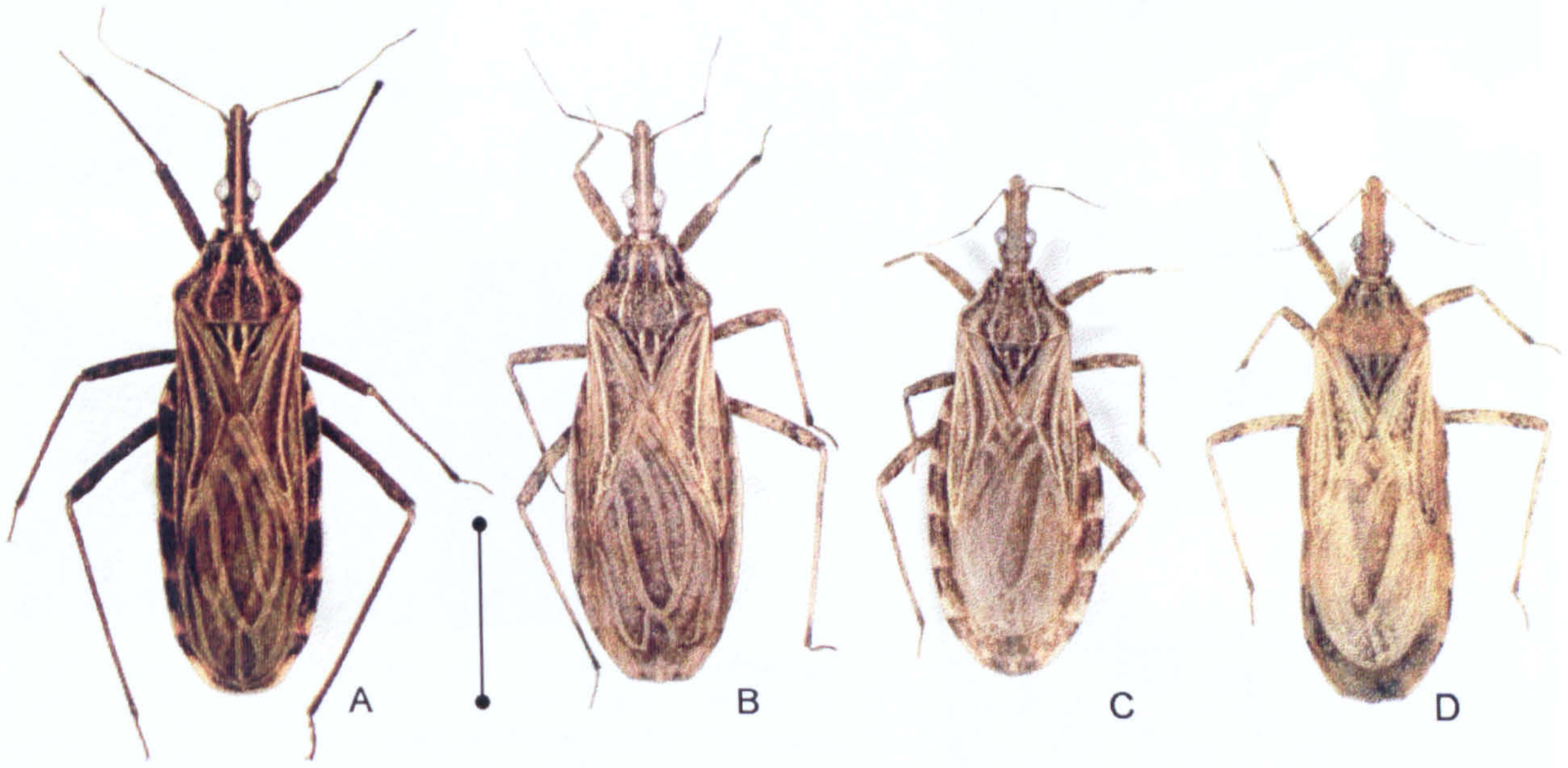
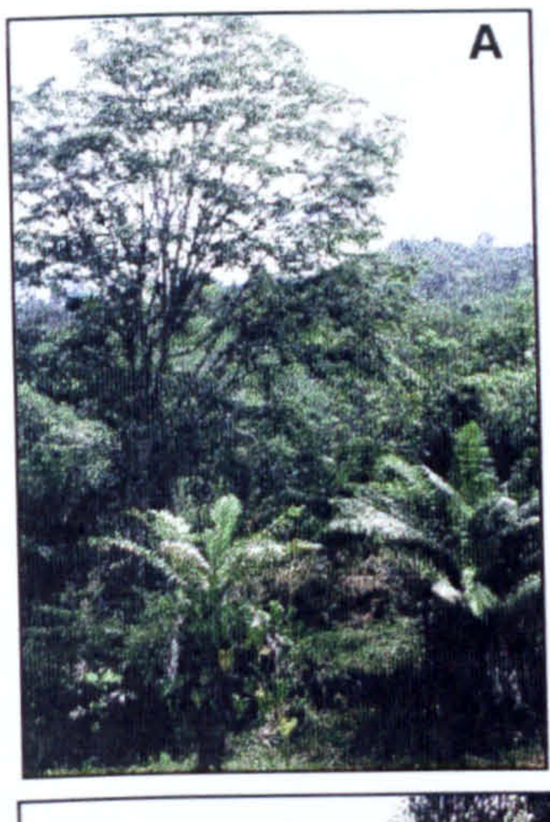
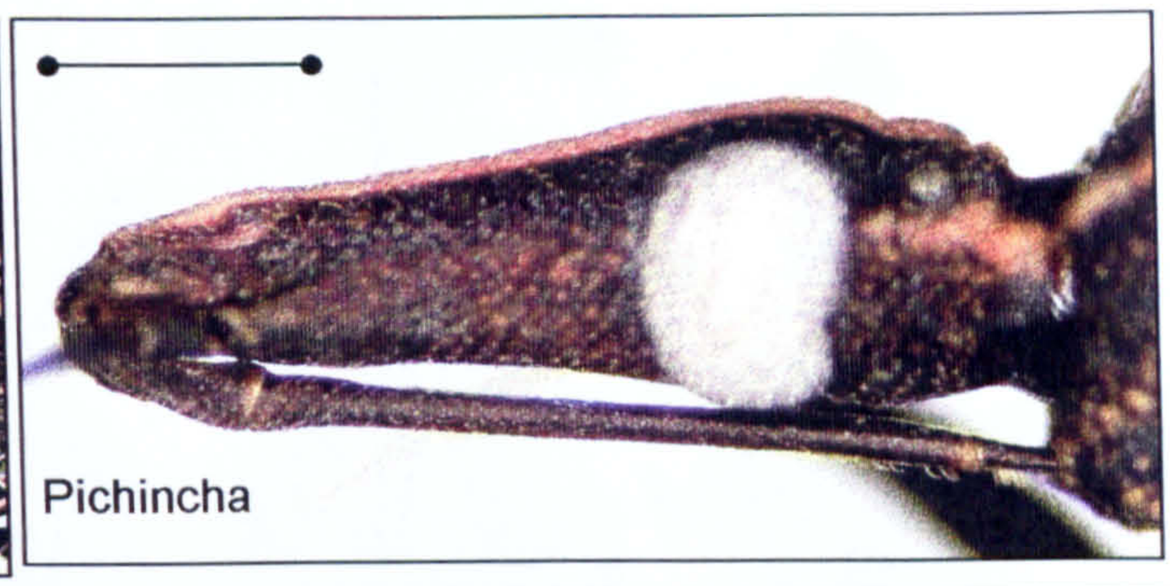
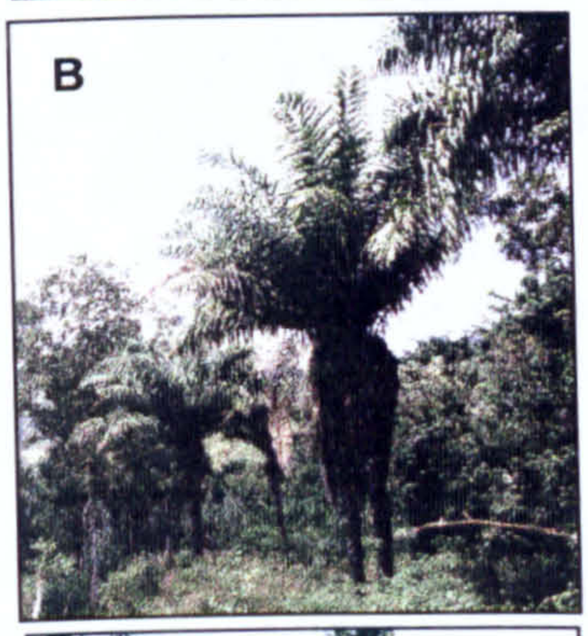
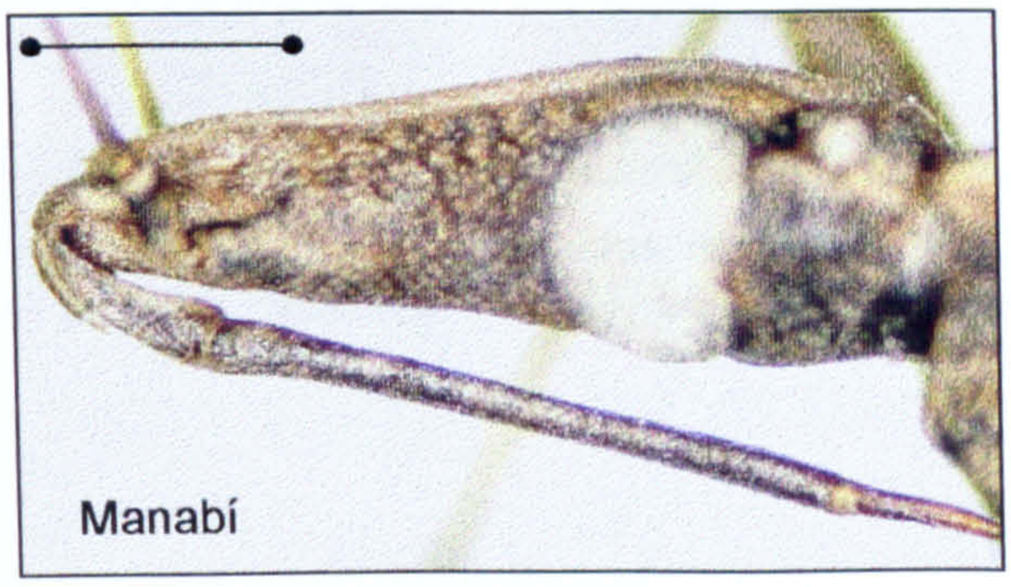


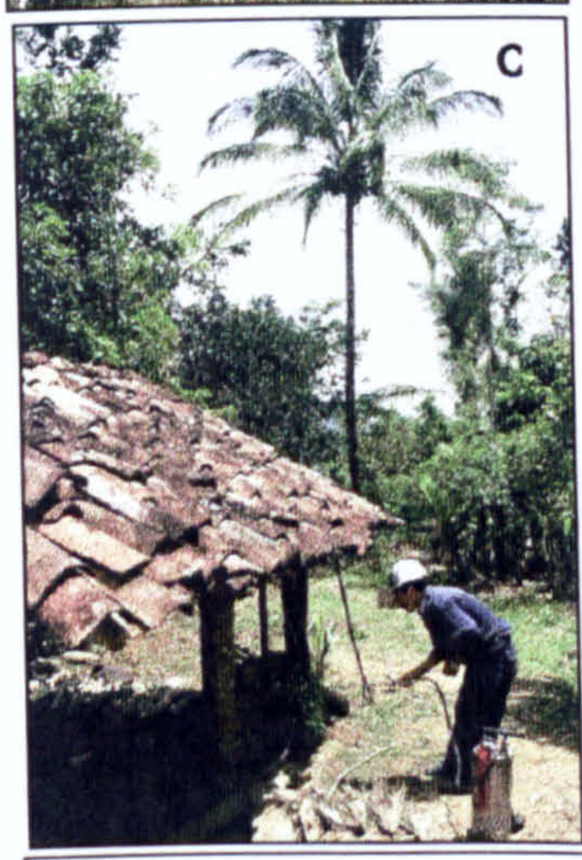
Figure 87. Phenotypic variation in *Rhodnius ecuadoriensis*. Overall aspect of adult specimens from different populations. A = Pichincha; B = Manabí; C = Loja; D = Peru. Scale bar: approx. 5mm

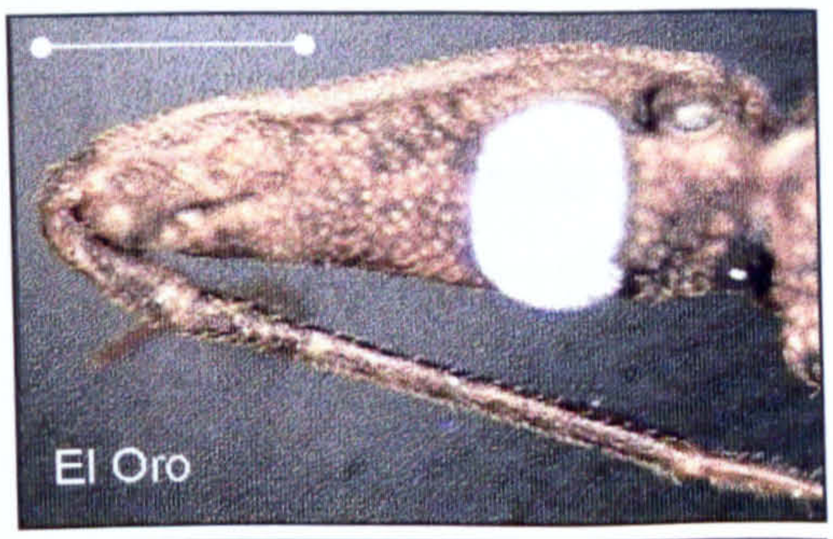


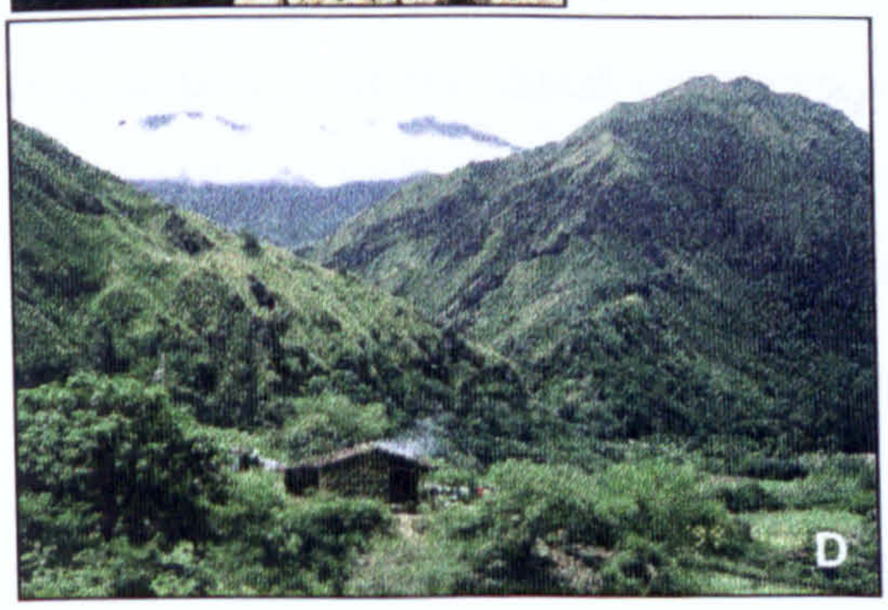


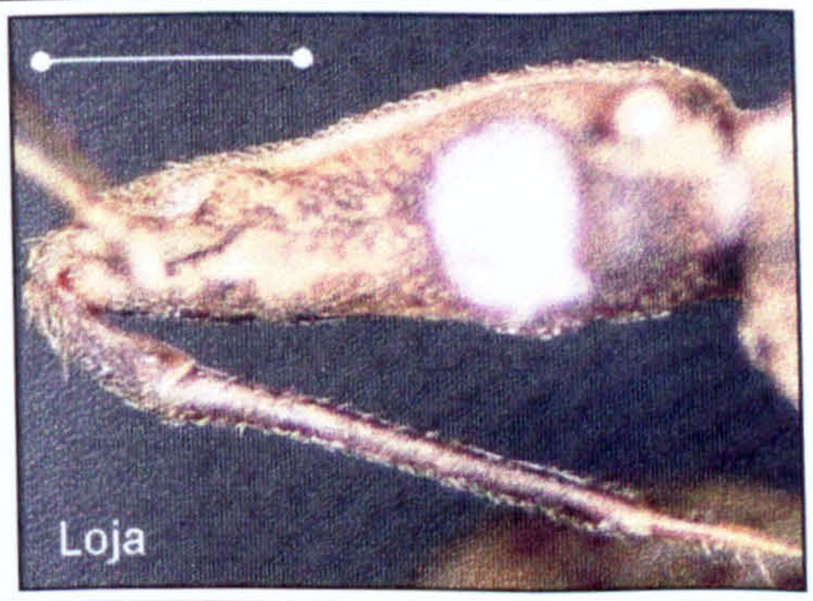












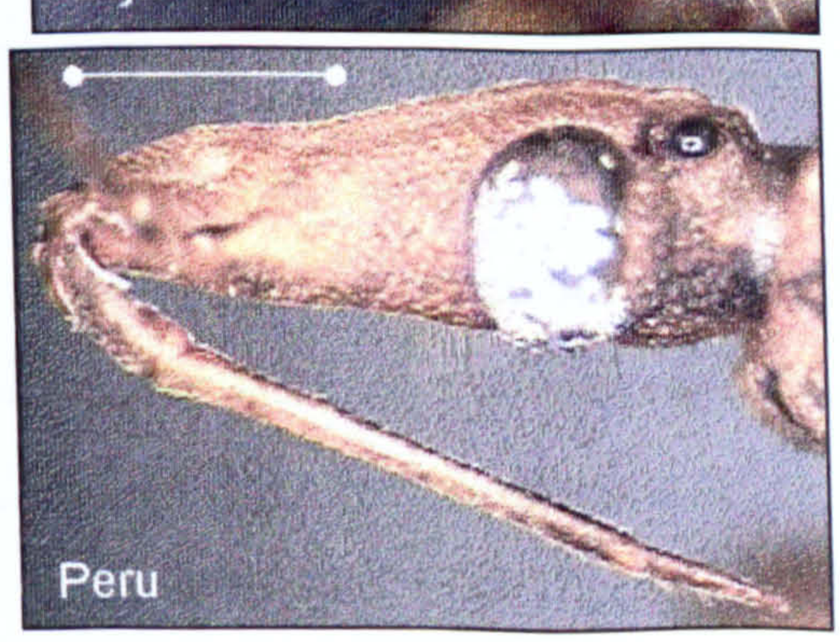


Figure 88. Phenotypic variation in *Rhodnius ecuadoriensis*: ecological correspondences. **A**=Andean foothill palms and microhabitat detail (Pichincha); **B**=Peridomestic *Phytelephas aequatorialis* in Manabí; **C**=Infested chicken coop in El Oro; **D**=Heavily infested domicile in the Andean valleys of Loja. [Pictures of heads were rescaled to approximate real (relative) differences among populations. Scale bars: approx. 1mm]

Figure 89. Phenotypic variation in *Rhodnius ecuadoriensis*. Head and thorax of adult specimens from different populations. From left to right: Pichincha, Manabí, Loja, and Peru. Scale bar: approx. 1mm

6.1.4.2. Interspecific comparisons

Although the overall chromatic pattern of *R. ecuadoriensis* is similar to that of the closely related *R. pallescens*, their mutual allopatry, clear differences in size (*pallescens* being about 21.5 to 23.5mm), and anatomical details of the head and male genitalia make specific determination unproblematic (Lent & Wygodzinsky 1979). *R. colombiensis*, the third species of the *pallescens* group, is similar to *pallescens* in several respects; its overall size is however somewhat smaller (about 17-20mm), and although it also has an overall yellowish colouration, *colombiensis* lacks the characteristic mottled pattern typical of both *pallescens* and *ecuadoriensis*. It is however worth noting that most *colombiensis* have irregular dark spots on their coxae and trochantera, and the distal extremes of their tibiae are very dark (Moreno et al. 1999). The heads of *pallescens* and *colombiensis* are similar not only in their general aspect, but also in their metric characters (Dujardin et al. 1999a; see also Section 7.2.). Both species differ significantly from typical *R. ecuadoriensis* in their head features, but comparisons with sylvatic populations of the latter species are lacking; our results indicate that both Pichincha and Manabí forms seem to occupy an intermediate position between *colombiensis* and typical *ecuadoriensis* specimens. The mean lengths of the heads of 109 specimens of these three species, including five different populations of *R. ecuadoriensis*, are presented in the following table.

Table 78. Length of the heads of *Rhodnius ecuadoriensis* and related species (in mm, mean±SD)

R. pallescens	***R. colombiensis***	***R. ecuadoriensis***				
		Pichincha	Manabí	Peru	El Oro	Loja
3.74±0.32	3.58±0.13	3.47±0.14	2.97±0.16	2.51±0.1	2.4±0.1	2.3±0.12

The relationships of these species (all of which occur on the western side of the Andes) with the somewhat similar *R. pictipes* (widespread in the Amazon basin) remain unclear. *R. pictipes* shares with the pallescens group the overall mottled aspect and some features of the pronotum (with a marked interlobe groove and conspicuously curved submedian carinae) and scutellum (2+2 carinae on the anterior section, merging into 1+1 and then into a single median carina on the posterior process). Typical of *pictipes* are the uniformly yellowish tibiae with a single, black transversal stripe on the basal third; mean head length in our sample of Ecuadorian *pictipes* was 4.09±0.16mm.

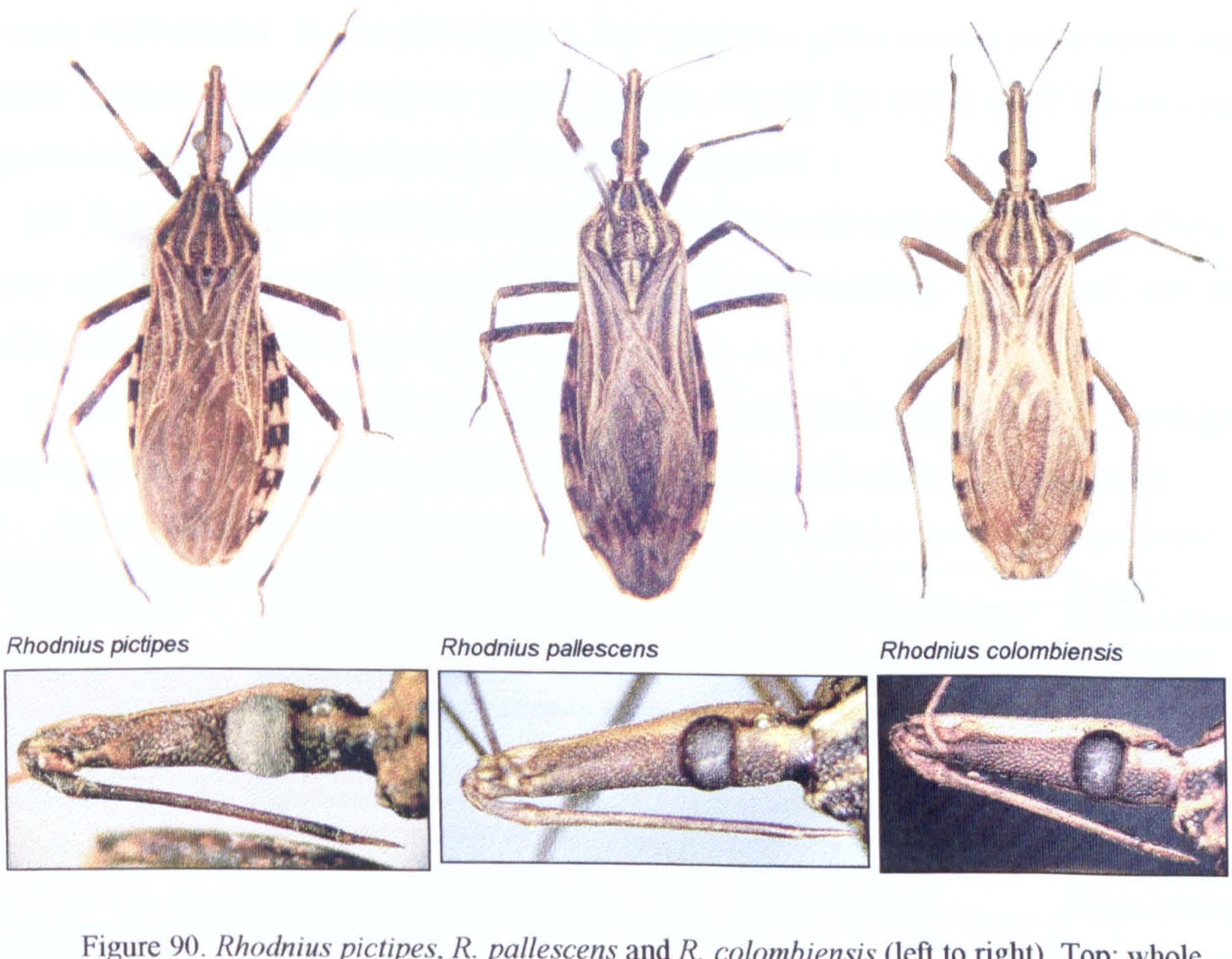


Figure 90. *Rhodnius pictipes*, *R. pallescens* and *R. colombiensis* (left to right). Top: whole insects; bottom: heads in lateral view. Note the elongated heads, mainly in *pictipes* and *pallescens*, and the similarity between *colombiensis* and *pallescens*

6.1.5. CONCLUSIONS

According to the results and considerations presented above, three different groups of *R. ecuadoriensis* may in our opinion be considered for comparative purposes:

(1) Strictly sylvatic bugs from *Pichincha* are large, very dark, with long heads, and a truncated median process of the pygophore (MPP). They inhabit *Ph. aequatorialis* palms in humid forests of northern foothills; here, the chromatic pattern of the insects provide an almost perfect camouflage as their dark brown-and-reddish colour is strikingly similar to that of the decaying palm fronds and fibres.

(2) In *Manabí* (~200km southwest from Pichincha), the insects have smaller average size, shorter heads, and an MPP with a pointed apex. They are somehow semi-sylvatic (they live in *P. aequatorialis* palms but frequently enter and can colonise human habitats). Their yellowish light colour mimics that of dead, dry palm leaves. Even if they belong to the same species, these palms are different from those found in the humid forests of Pichincha. In this drier region, few epiphytes grow around palm stems, and a lesser amount of rotting organic matter remains around the trunk; dead leaves usually remain hanging around the stem, and are straw-coloured.

(3) In *Loja*, *El Oro* and *Peru*, the bugs seem to be strictly synanthropic. They are very small, pale straw-like coloured with conspicuous mottling, have short and stout heads and an MPP with a pointed apex.

In the following table, the main morphological, chromatic, geographical, ecological, and behavioural traits of these different forms of *R. ecuadoriensis* are summarised.

Table 79. Morphological and chromatic variation among *Rhodnius ecuadoriensis* populations

Phenotypic forms	Morphological/chromatic traits	Distribution	Ecology
Pichincha forms	·Dark brown-black with reddish markings ·Mottled pattern inconspicuous due to dark background colour ·Remarkably larger than typical specimens ·Long and narrow heads ·MPP generally truncated	Northern-central foothills (Pichincha; perhaps also foothill forests of other western provinces)	Strictly sylvatic (*Phytelephas aequatorialis*)
Manabí forms	·Light brown-yellowish ·Mottled pattern conspicuous, ·Larger than typical specimens ·Heads longer than types ·MPP pointed	Central coastal region (Manabí, perhaps Los Ríos and some parts of Guayas and Esmeraldas)	Semi-sylvatic (*Phytelephas aequatorialis* and human habitats)
Southern forms	·Similar to species types ·Pale yellowish ·Mottled pattern conspicuous ·Small size ·Short and stout heads ·MPP pointed	Southern Ecuador (Loja, El Oro) and northern Peru	Domestic and peridomestic

MPP = median process of the pygophore

6.2. Morphometrics of *Rhodnius ecuadoriensis*

6.2.1. INTRODUCTION

Morphometric analyses have been widely applied in the context of numerical taxonomy. Their basic aim is to explore relationships among taxa (or operational taxonomic units, OTUs) by measuring continuous anatomical traits of individuals and characterising their variation among and within those OTUs. Phenetic variation is assumed to represent underlying genetic diversity, with metric characters governed generally by numerous alleles (polygenes), each one having a small effect on the phenotype. Continuous phenotypic variation among individuals results from the simultaneous expression of the linked polygenes governing each metric trait and from the concomitant effects of environmental factors. Metric variability may therefore be envisaged as a measure of population differentiation, but attention must be paid to the fact that different fractions of such variation may be related to genetic and environmental factors, respectively (Sorensen 1992, Sorensen & Foottit 1992, Dujardin et al. 1999a,b,d, 2000, Patterson et al. 2001, Patterson 2002). Several studies have suggested that environmental factors have a strong influence on the average size of triatomine bugs, with an overall reduction affecting laboratory-reared specimens when compared with their sylvatic conspecifics; this was also confirmed for field-collected specimens occupying sylvatic and synanthropic environments, with a significant decrease of size associated with adaptation to human-related environments (Harry 1992, 1994, Dujardin et al. 1997a,b, 1998a,b, 1999b,d, Galíndez Girón & Torres 1999). It may therefore be concluded that size-related traits should reflect the fraction of variation attributable to environmental factors, whereas size-independent shape variables may be more informative about the underlying genetic variability. This means that size-related variables can be more informative when intra-specific variation related to the adaptation of a vector population to synanthropic habitats is to be studied (for instance, to characterise potential reinfestation foci by comparing domestic and sylvatic populations). On the contrary, safer taxonomic and phylogenetic inferences can be made by concentrating on size-independent variables, more likely to reflect the genetic basis of phenotypic variation. Various approaches may be used to separate the effects of environmental and genetic factors on the metric traits of triatomine populations (Dujardin et al. 1999d). Some involve using bugs reared under identical conditions (the

'common garden' approach) or the comparison of sylvatic vs. laboratory-reared specimens. When genetic data are available, the correlation between genetic and metric distances among OTUs can be explored. Finally, several multivariate statistics approaches may be used to remove the influence of size from morphometric analyses.

Allometry has been defined as the effect that size variation has on the variability in shape, as some proportions may vary simply because of changes in body size. These allometric changes are regarded as less significant biologically than shape changes that are unrelated to size (Dujardin et al. 1999d). A particular case of allometry is isometry, in which individual measurements increase proportionally to increasing overall size. Thus, removing isometric (or, more generally, allometric) changes from morphometric analyses may help define biologically meaningful relationships among OTUs by focusing on the fraction of variation that is unrelated to environmental factors (Claridge & Gillham 1992, Patterson et al. 2001). Isometric size can be partially removed by centring the log-transformed measurements: for each specimen, the mean value of all variables is subtracted from each of the measurements (Patterson et al. 2001); these centred variables ('log-shape ratios'; Darroch & Mosiman 1985) are then used as the input for principal component and discriminant analyses. For a set of measurements, the OTUs under investigation can be tested for the existence of a common axis of allometric growth by using a common principal component approach (Klingenberg 1996, Patterson et al. 2001). If such a common axis is present in a given dataset, the first common principal component (CPC1) derived from the measurements accounts for most of the allometric growth, and the rest of CPCs can be used as allometry-free variables for further PCA and discriminant analyses (Dujardin & Le Pont 2000, Patterson et al. 2001). Finally, when results obtained using these procedures still show size-related variation, a more explicit approach may be used to remove size from the analysis. It is based on the premise that the first principal component (PC1) extracted from the covariance matrix of centred measurements usually represents a good estimate of overall body size of the organisms under study. Thus, the residuals of linear regressions of each variable versus PC1 can be envisaged as size-free, 'form' variables that may be used for PCA and discriminant analysis (Vitt et al. 1997, Dujardin et al. 1998b, 1999a,d). The effect of this procedure is equivalent to that of excluding PC1 from the discriminant analysis (Sorensen 1992).

Ordination methods allow for the representation of OTUs in a reduced character space defined by the major axes of variation (Pimentel 1992). Two main approaches, principal component analysis (CPA) and canonical variate analysis (CVA), are widely used in systematics (see Foottit & Sorensen 1992). PCA does not assume *a priori* divisions in the OTUs under study, concentrating on the relationships among variables and individuals within a single sample; it is frequently regarded as an exploratory procedure by which OTUs may be defined from a single conceptual group. Principal components are linear combinations of the variables; they are orthogonal (i.e., uncorrelated) and ranked in order of decreasing sample variance (i.e., the first PC explains the majority of the variation, and therefore maximally discriminates among individuals in the sample; PC2 accounts for the next larger portion of the total variance, but is constrained by the fact that it has to be uncorrelated to PC1). In discriminant analysis, the sample under study is divided *a priori* into OTUs; a set of linear (discriminant) functions is then calculated and used to evaluate the degrees of similarity/difference among OTUs, and the uniqueness of each group within the sample. These functions are those that best discriminate among the groups, and can be used to assign individuals to those OTUs. This approach therefore stresses the divergence between the groups by emphasising the effect of the variables that maximise among-group variance relative to within-group variance. When used to reduce dimensions of data (which is usually the case in systematics), discriminant analysis is termed canonical variate analysis (CVA). Canonical vectors (CVs) are discriminant functions, each representing a pattern of variation uncorrelated with other CVs; they contain coefficients calculated so that differences between groups are maximised (Foottit & Sorensen 1992, Pimentel 1992).

These methods were applied here to explore intraspecific relationships among *R. ecuadoriensis* populations. Two main hypotheses were tested. The first states that synanthropic populations can be distinguished from sylvatic ones (so that the origin of reinfestations of treated dwellings can be traced) using metric traits of the bugs involved; size-dependent variables are relevant to this part of the study, basically related to habitat adaptations. The second hypothesis is related to the evolutionary relationships of these populations, and involves the idea of a north-to-south axis of clinal variation

(the polarity of the changes being inferred from known relationships with northern Colombian *Rhodnius* species, namely *R. pallescens* and *R. colombiensis*).

6.2.2. MATERIALS AND METHODS

6.2.2.1. Bugs

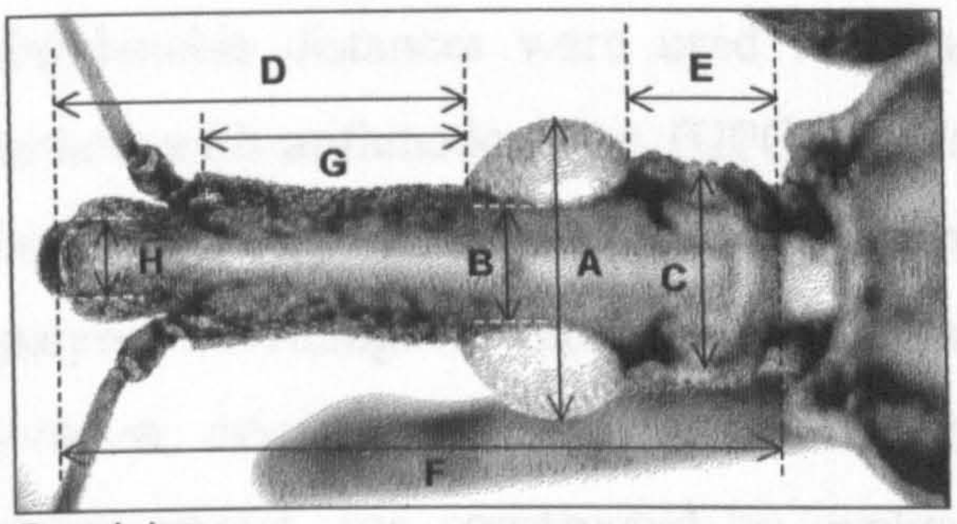
Seventy-nine adult *R. ecuadoriensis* specimens were used for this part of the study. They were mainly field-collected specimens, and comprised bugs from two sylvatic (Pichincha [n=15] and Manabí [n=17], although some of these latter were collected in human environments) and three synanthropic populations (El Oro [n=17], Loja [n=15], and northern Peru [n=15]). The details on each specimen are presented in the Appendix. *R. colombiensis* specimens (n=15) from a laboratory colony (University of Antioquia, Medellín, Colombia) were used as outgroup for some of the analyses.

6.2.2.2. Measurements

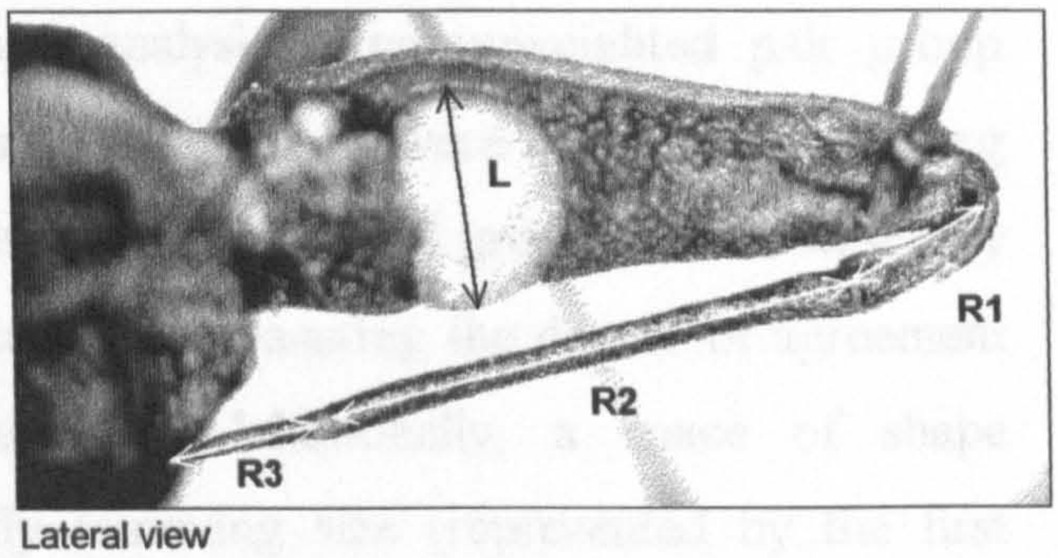
Bug head capsules are known to be useful for morphometric comparisons. They are rigid and easily preserved structures, their size and shape are fixed in each developmental stage, and traits related to their architectural complexity have been customarily used in taxonomic studies of Triatominae, including the reference to head measurement ratios in the descriptions of taxa (see Lent & Wygodzinsky 1979, Dujardin et al. 1999d). We used a semi-automatic method to compute twelve head measurements from the adult bugs under investigation (Patterson et al. 2001). A digital video camera (Euromoex Eurocam) was connected to a binocular microscope. Pinned, dry specimens were mounted on an entomological microscope stage (Bioquip, Gardena, CA, USA) that allowed for accurate orientation of heads, and illuminated using optic fibre. Images were captured into a computer using a Zipshot device (Zipshot, Fremont, CA, USA) and ArcSoft Photo Impression 2.5 software (ArcSoft, Fremont, CA, USA). Measurements were taken from stored image files (high resolution JPEG format) with Sigma ScanPro 5.0.0 image analysis software (Sigma Scan, SPS Inc.). A three-point calibration of image files was performed in Sigma ScanPro using the image of a 1-cm grid graticule with 100µm graduations captured at 25x magnification. Two calibrated images (lateral and dorsal view) from each specimen were analysed at 25x magnification by manually positioning the cursor at the start and end points of each measurement; values in mm were automatically recorded on an Excel spreadsheet. Measurements and their descriptions are presented in figure 91 and table 80.

Table 80. Morphometrics: head capsule measurements

Measurements		Description
Dorsal view	A	Outer (maximum) distance across eyes
	B	Synthlipsis: minimum width of inter-ocular space
	C	External (maximum) distance between ocelli
	D	Anteocular distance: anterior eye limit → base of anteclypeus
	E	Postocular distance: posterior eye limit → head/neck limit
	F	Total length of head: base of anteclypeus → head/neck limit
	G	Length of antenniferous tubercle: anterior eye limit → distal tip of tubercle
	H	Maximum width of anteclypeus
Lateral view	L	Maximum diameter of the eye
	R1	Length of first rostral segment
	R2	Length of second rostral segment
	R3	Length of third rostral segment



Dorsal view



Lateral view

Figure 91. Morphometrics: head capsule measurements

6.2.2.3. Statistical analysis

Descriptive statistics, including means, standard errors, standard deviations, medians, quartiles, and ranges of each variable were calculated from the original datasets for the whole sample and for groups defined by ecological (sylvatic vs. synanthropic populations) and geographic features (collection sites in different provinces and countries) (Appendix). These raw measurements were log-transformed and normality assessed by visual inspection of frequency histograms and normal quantile plots. Different subsets of measurements were selected for the various analyses performed.

Size variation among groups was studied by comparison of means using log-transformed measurement subsets; additional comparisons involved the mean values of those subsets. The 12-measurement dataset was used to explore sexual dimorphism (differences between males and females in size-related phenotypic traits).

·Isometry-free analysis. Prior to multivariate analysis, data were centred by row in order to remove isometric size. The resulting 'log-shape ratios' (Darroch &

Mosiman 1985, Patterson et al. 2001) were used as input for principal component analysis (PCA) on covariances. The amount of variation explained by each of the derived principal components ('shape variables') was assessed, and all of them but the last one (which did not contribute to the variability) were submitted to canonical variate analysis (CVA). The overall significance of multivariate CVA was assessed with the Wilk's lambda statistic. From this discriminant analysis, canonical vectors (CV) and Mahalanobis distances were calculated. The first two CVs (containing most of the variation) were used to plot the positions of each specimen on the 'shape discriminant space'; to improve clarity in the graphic output, polygons enclosing all points within each group ('convex hulls') were overlaid on the plots and individual dots removed. Mahalanobis distances were used for cluster analysis using unweighted pair group method with arithmetic mean (UPGMA), and dendrograms were constructed showing the relationships. Reclassification of specimens to their original groups was assessed by analysis of contingency tables and Kappa statistics (measuring the degree of agreement between original and CVA-derived groupings). Additionally, a space of shape measurements was constructed by explicitly removing size (represented by the first principal component, PC1) from the measured data, variable by variable. For this, residuals of linear regression of PC1 on each individual measurement were used as new variables for size-free discriminant analysis (Vitt et al. 1997, Dujardin et al. 1998b, 1999a,d). The derived CV1 and CV2 were plotted as above, and UPGMA dendrograms were constructed with Mahalanobis distances.

·Allometry-free analysis. Log-transformed measurements were tested for compatibility with a common allometric axis using X^2 statistics. Once a subset of variables fitting the model was obtained, they were submitted to a common principal component analysis (CPCA). Removing the first common principal component (CPC1), which represents growth effects, allows for the use of the remaining CPCs (allometry-free variables) as input for further PCA and CVA (Dujardin & Le Pont 2000, Patterson et al. 2001).

Different parts of the study were conducted using JMP 4.0.4 (SAS Institute 2000) and NTSYS 2.10y (Rohlf 2001) software.

6.2.3. RESULTS

6.2.3.1. Descriptive statistics

Basic descriptive statistics of raw measurements for the 79 *R. ecuadoriensis* bugs studied are presented in the Appendix.

6.2.3.2. Size variation

Significant differences among populations were found for all the measurements; Anova F ratios for each (log-transformed) measurement were: A=33.2, B=9.8, C=37.2, D=276, E=64.6, F=211, G=296, H=47.1, L=34.4, R1=54.8, R2=260, and R3=71.1 (4 df, $p<0.0001$ in all cases). Pairwise *t*-tests showed that bugs from Pichincha were significantly larger for all the variables (details not shown) except B (similar to Manabí). Regarding measurements A, D, E, G, L, R2, and R3 (selected for most of the analyses), the Manabí population was significantly smaller than that from Pichincha and larger that any of the synanthropic ones except for a non-significant difference with Peru in measurement L. Bugs from Peru and El Oro were similar for measurements A, L, R2, and R3. Finally, the population from Loja was the smallest; only L and R3 were similar to El Oro. To illustrate these trends, the mean value of all 12 log-transformed variables was analysed. Anova F ratio was highly significant (141, 4 df, $p<0.0001$), and pairwise *t*-test comparisons showed all groups were significantly different from each other. Size decreased from Pichincha into Manabí, Peru, El Oro, and Loja.

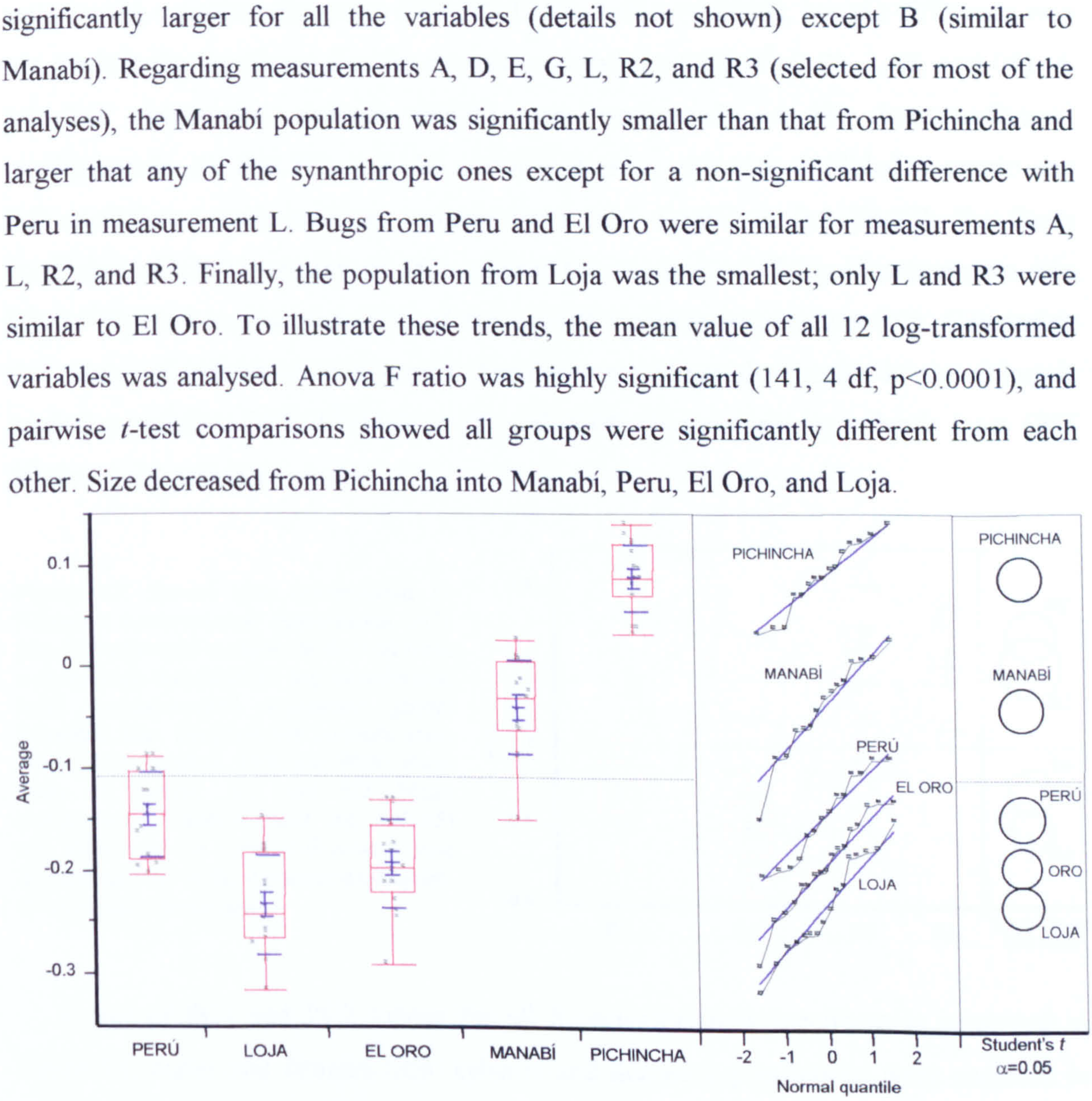


Figure 92. Size variation (using mean values of 12 log-transformed head measurements) among *Rhodnius ecuadoriensis* populations. Left: individual values (black dots), quantile boxes (median, quartiles, 10% and 90%; red), means, standard errors and standard deviations (short blue lines)

6.2.3.3. Sexual dimorphism

Sex ratio was (♀:♂) 1:0.86 for the whole sample, 1:1.04 for pooled synanthropic populations, and 1:0.63 for Manabí plus Pichincha. In general, females were significantly larger (details not shown) than males for all 12 log-transformed measurements except the length of the first rostral segment (R1; Wilcoxon test [WT] X^2=3.6, df=1, p=0.06). Females were also larger for the average of all log-measurements (WT X^2=9.5, 1 df, p=0.02). Synanthropic (El Oro, Loja and Peru) and sylvatic (Pichincha and Manabí) were pooled for analysis of sexual size dimorphism using the average of all 12 log-transformed measurements. Females were significantly larger than males in the synanthropic group (WT X^2=13.24, 1 df, p=0.0003), but the difference was not statistically significant for the sylvatic populations (WT X^2=3.01, 1 df, p=0.08). A principal component analysis (PCA) was performed on the log-transformed measurements; the first PC could be considered as an estimator of global isometric size (all coefficients were positive, ranging from 0.13 [logB] to 0.5 [logG]; the linear coefficient of the regression of PC1 against the average of all log-measurements was R^2=0.996), and represented 90% of the overall variance (eigenvalue=0.2). Comparing the means of individual PC1 scores, females were larger than males in the domestic pooled population (WT X^2=11.2, 1 df, p=0.0008), but not among sylvatic bugs (WT X^2=2.9, 1 df, p=0.09).

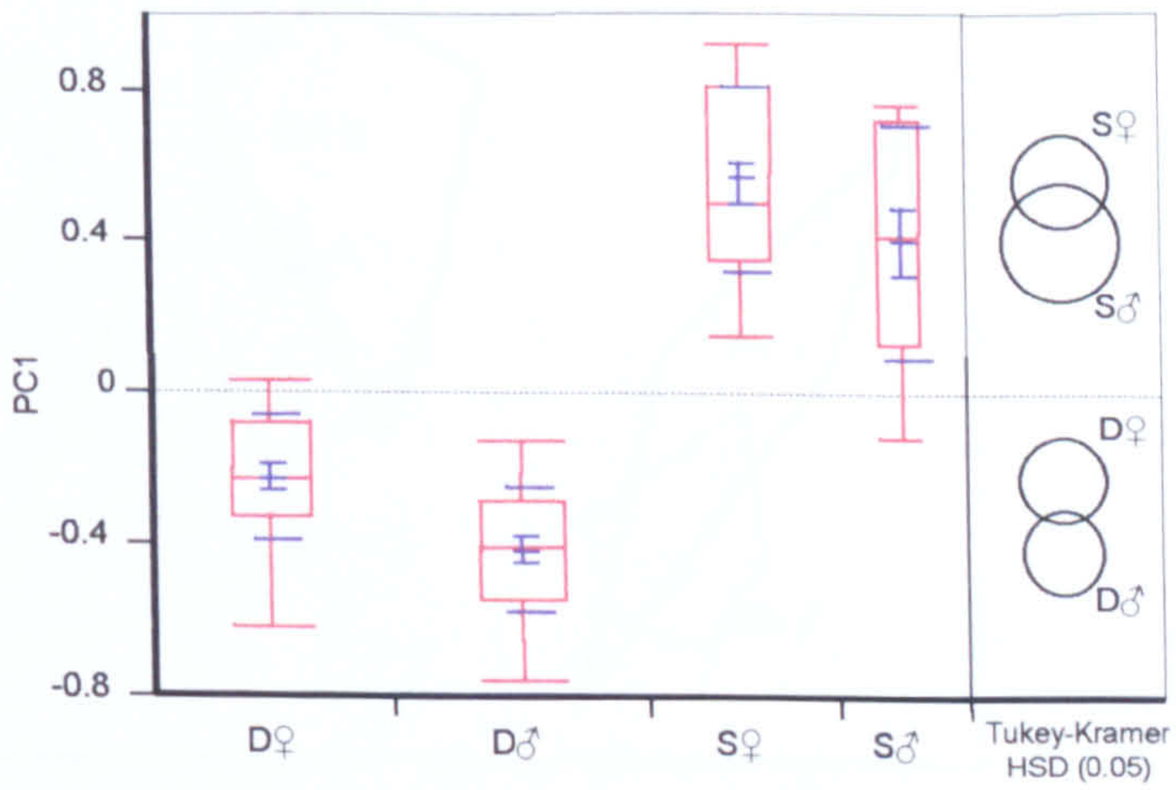


Figure 93. Sexual size dimorphism in *Rhodnius ecuadoriensis* populations. PC1 (the first principal component derived from a covariance matrix of 12 log-transformed head measurements) represents global isometric size. Boxplots are quantile plots (10th , 25th, median, 75th, and 90th), short lines are means, SE, and SD. Mean comparisons (Tukey-Kramer test, α=0.05) are shown on the right; differences were significant only in the domestic population. D=domestic; S=sylvatic

A plot of PC1 and PC2 values for all *R. ecuadoriensis* specimens is presented in figure 94; males and females from sylvatic and domestic populations were enclosed in convex polygons to show the relationships.

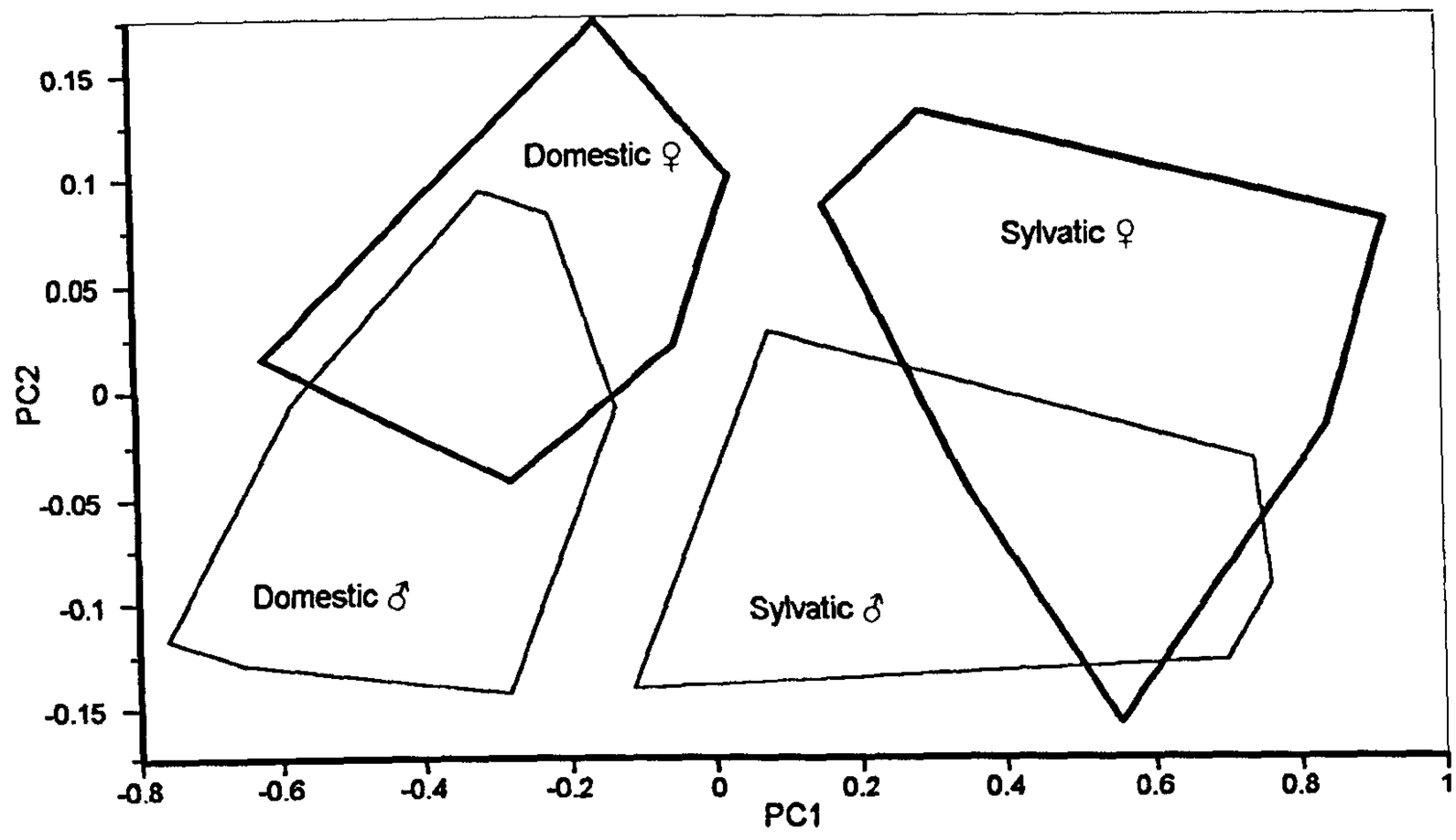


Figure 94. Sexual dimorphism in *Rhodnius ecuadoriensis* ecological populations. PC1 and PC2 are the first two principal components derived from a covariance matrix of 12 log-transformed head measurements; PC1 represents a vector of increasing global size

Separate analyses of PC1 scores by locality revealed however higher degrees of dimorphism in Manabí (9♀, 6♂; WT X^2=8, 1 df, p=0.005) than in Loja (8♀, 8♂; WT X^2=7.5, 1 df, p=0.006), Peru (7♀, 7♂; WT X^2=5.6, 1 df, p=0.006), or El Oro (8♀, 9♂; WT X^2=5.8, 1 df, p=0.016). Size differences between males and females from Pichincha were not statistically significant (9♀, 6♂; WT X^2=3.6, 1 df, p=0.06).

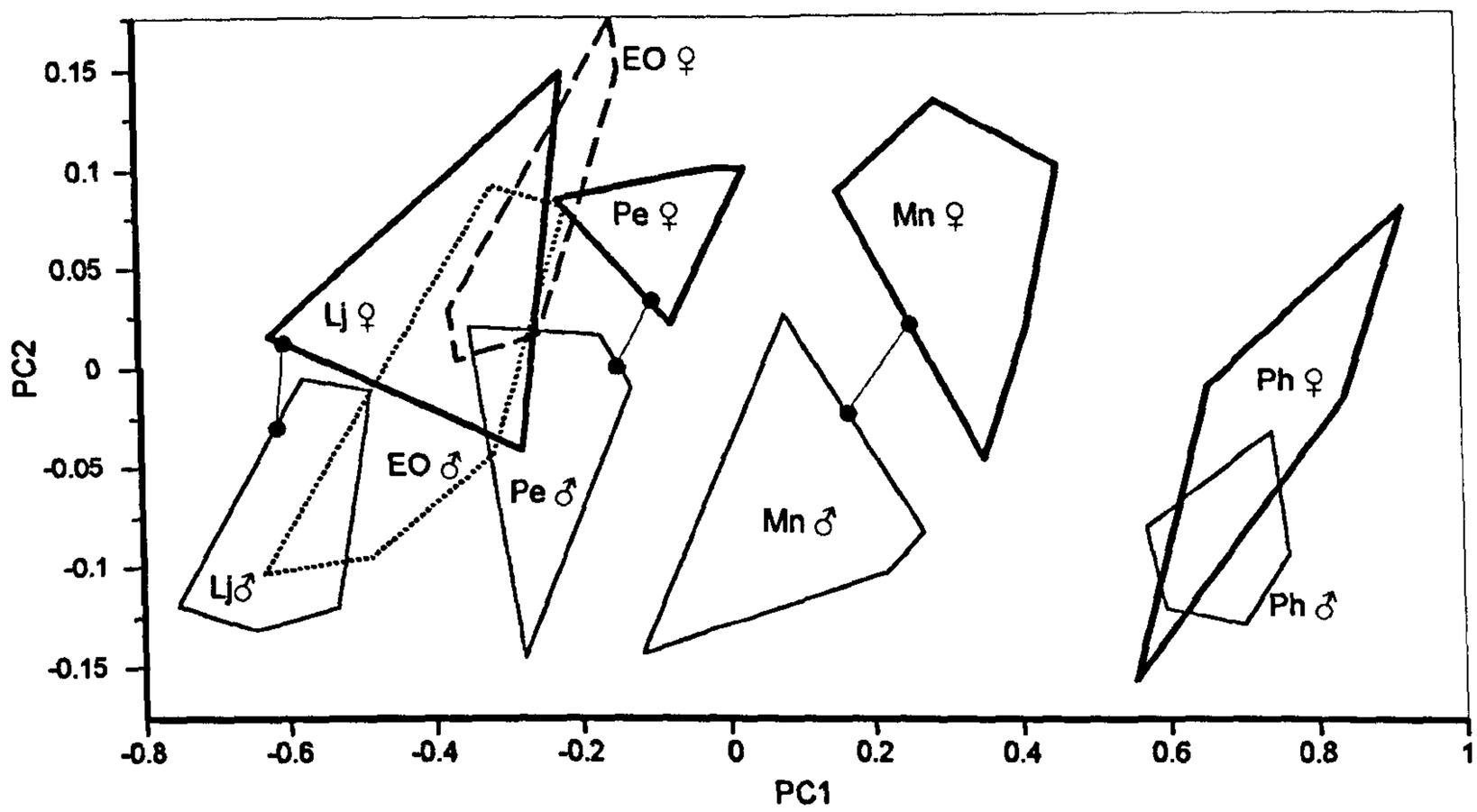


Figure 95. Sexual dimorphism in *Rhodnius ecuadoriensis* geographic populations. PC1 and PC2 are the first two principal components derived from a covariance matrix of 12 log-transformed head measurements; PC1 represents a vector of increasing global size. Lj=Loja, EO=El Oro, Pe=Peru, Mn=Manabí, and Ph=Pichincha

6.2.3.4. Isometry-free analyses

Seven measurements (A, D, E, G, L, R2, R3) were log-transformed and centred by row to obtain 'log-shape ratios'. Principal component analysis (PCA) on covariances showed the first six PCs accounted for 100% of the variability (with the first one explaining 74.3% of the observed variation). These six PCs were submitted to CVA; the multivariate significance test (Wilk's lambda) showed the analysis was highly significant (λ=0.024, 24 df, $p<0.0001$). Individual values for the first two canonical variables (CV) were plotted in the discriminant space and groups enclosed in convex hulls; results are shown in figure 96.

A complete discrimination between different ecological populations (sylvatic vs. synanthropic) was achieved (with 100% of bugs correctly classified in those categories and Kappa=0.9), mainly because of higher CV1 scores of synanthropic populations. Within these latter, some overlapping occurred between bugs from Loja and El Oro, and a single Peruvian specimen appeared in the middle of the Loja cluster (arrow in figure 96). When the origin of each bug was checked, it was discovered that this single bug had been collected in Suyo (Department of Piura), a locality only ~30km away from El Lucero (our fieldwork site in Loja). This bug was therefore reassigned to the Loja group and the analyses were repeated. Wilk's lambda was again highly significant (λ=0.0206, $p<0.0001$), and Kappa>0.9. Results are presented in figures 97-98.

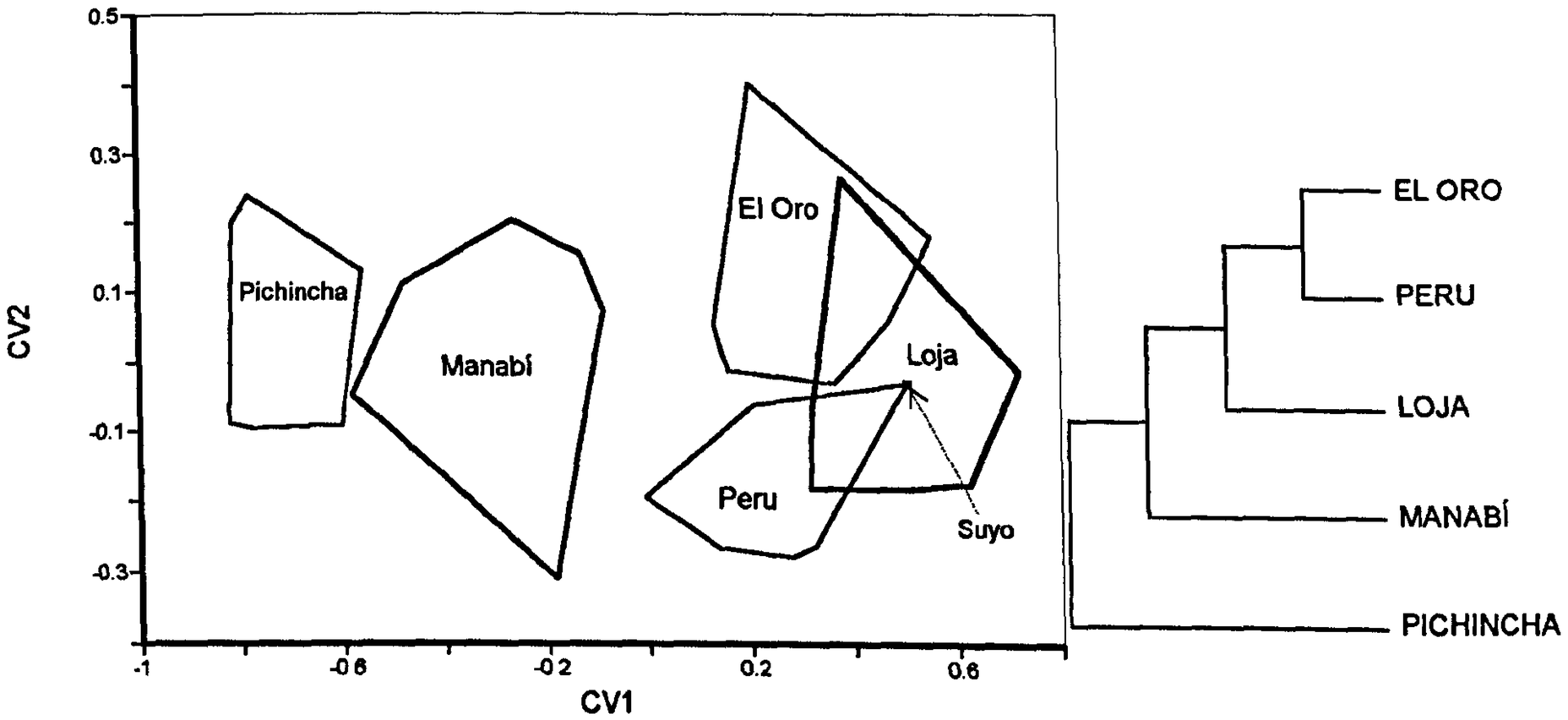


Figure 96. Canonical variate analysis of five *Rhodnius ecuadoriensis* populations: factorial map and UPGMA dendrogram based on Mahalanobis distances. A single bug collected in Suyo, Peru (arrow) was more closely related to the Loja group than to the Peruvian cluster. The localities were this specimen and those from Loja were collected are only ~30km away, whereas the rest of Peruvian bugs were captured in the upper Chicama valley (La Libertad, Peru), some 500km south of Loja. Note the complete discrimination between sylvatic (Pichincha and Manabí) and synanthropic (El Oro-Loja-Peru) populations on the first canonical vector (CV1)

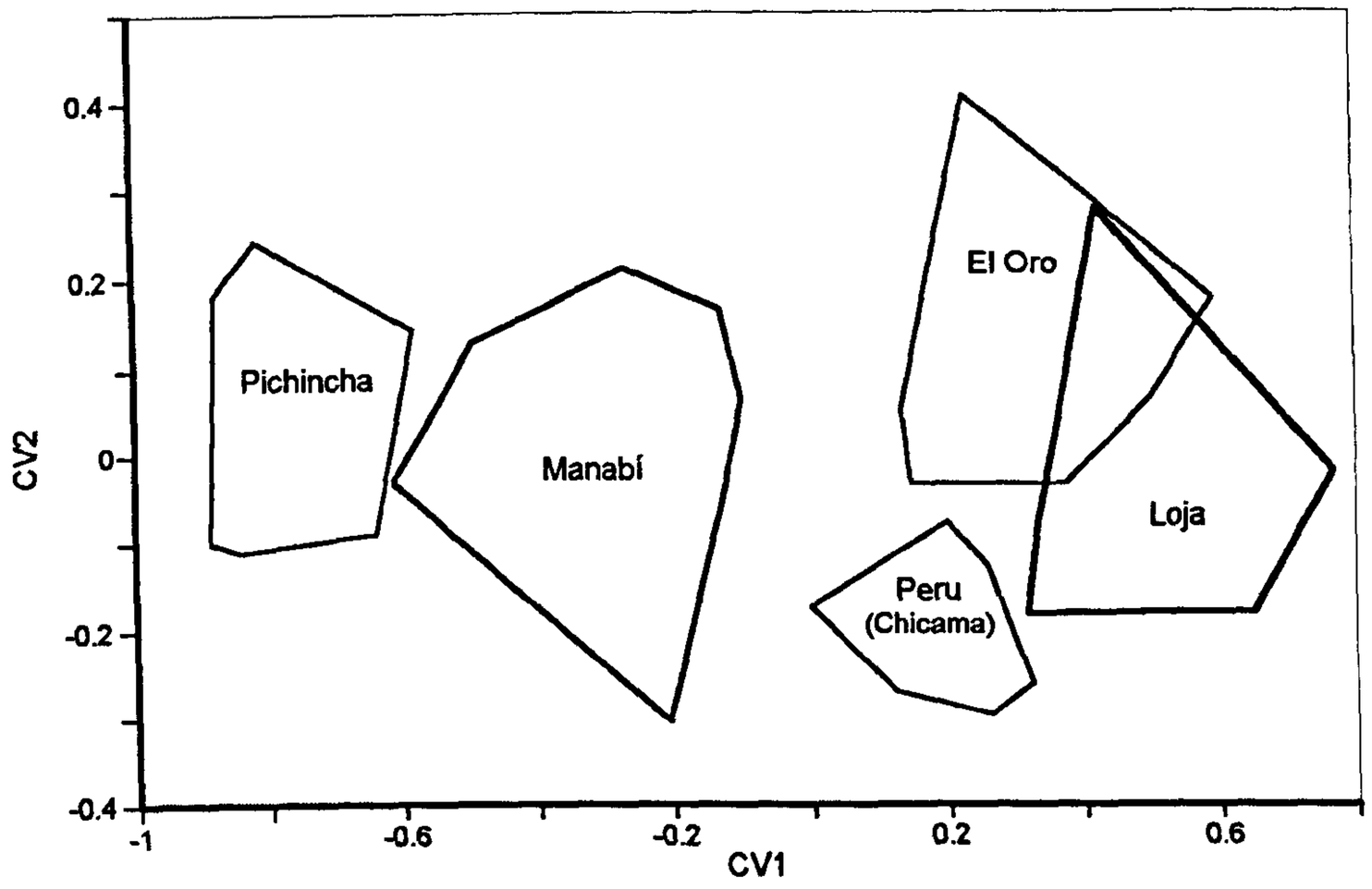


Figure 97. Canonical variate analysis of *Rhodnius ecuadoriensis* populations. One bug collected in Suyo, Peru was reassigned to the Loja group. Under this arrangement, Peruvian bugs from the Chicama valley (La Libertad) formed a completely distinct cluster, separated from Loja mainly on the first canonical vector (CV1) and from El Oro mainly on the second canonical vector (CV2)

Mahalanobis distances and canonical vectors derived from CVA were submitted to UPGMA cluster analysis. The resulting dendrograms are shown in the following figure.

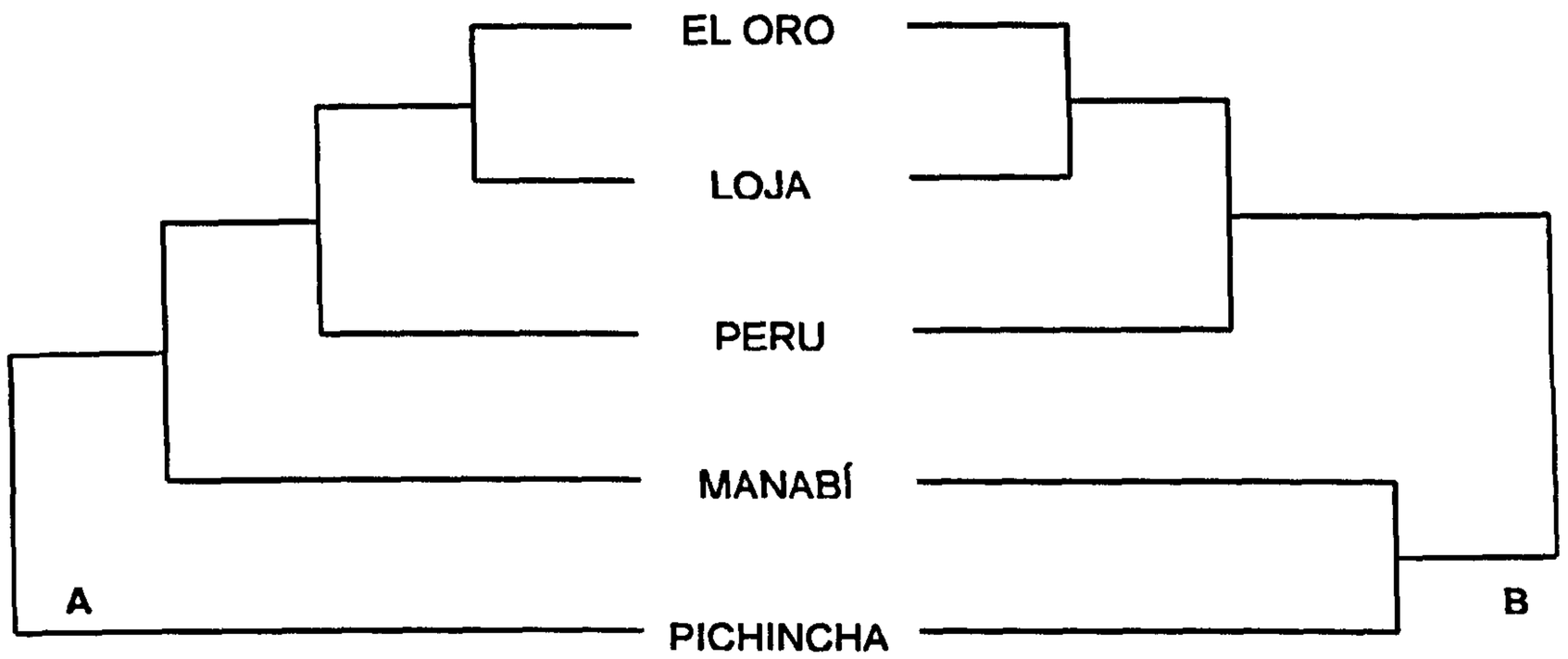


Figure 98. UPGMA dendrograms derived from Mahalanobis distances (**A**) and mean CV1 to CV4 values (**B**) after isometry-free canonical variate analysis of five *Rhodnius ecuadoriensis* populations. Note the definitiion of two main clusters (sylvatic – synanthropic) in **B**

Linear regression was used to verify possible relationships between CV1 and size (represented by the average value of all seven log-transformed measurements). The analysis demonstrated a strong negative correlation (y=0.025-0.27x; R^2=0.87), with larger bugs (Pichincha) having the lowest CV1 scores and the smallest specimens (from Loja) having CV1 values close to 1.

In order to minimise the influence of size, the residuals of linear regression of each variable on the first principal component were used as new variables for canonical variate analysis. All Ecuadorian populations were very similar to one another when the effect of size was removed, with bugs from Chicama (Peru) being the most distinct. This was confirmed by the dendrogram derived from Mahalanobis distances, with the Peruvian population occupying the most external branch as a sister group to all the Ecuadorian populations regardless of their ecological or geographic origin (figures 99 and 100). The lack of correlation between CV1 and size was confirmed by linear regression; R^2 was 0.000004.

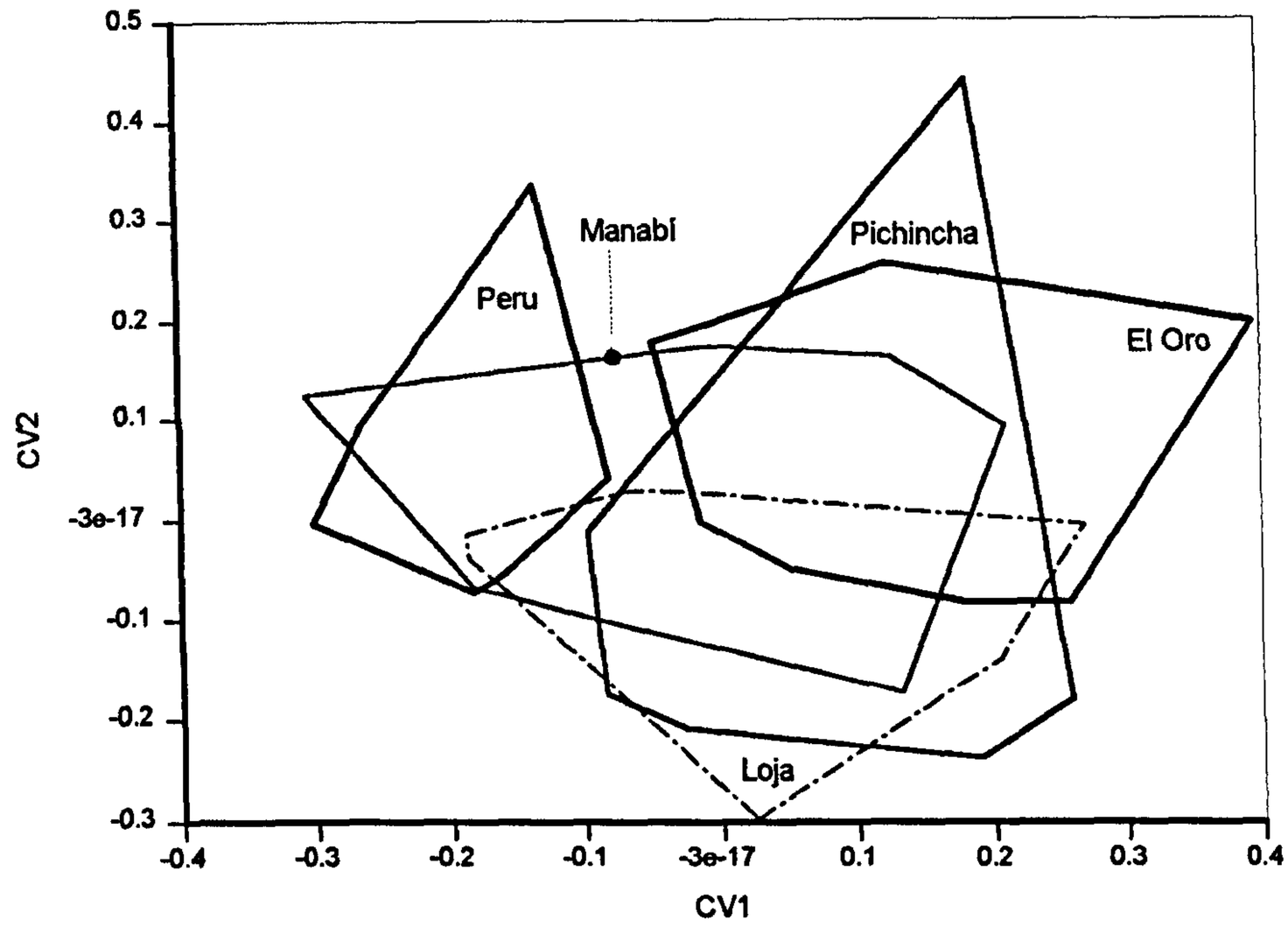


Figure 99. Canonical variate analysis of five *Rhodnius ecuadoriensis* populations: linear regression residuals (PC1 vs. measurements) were used as size-free (form) variables for discriminant analysis

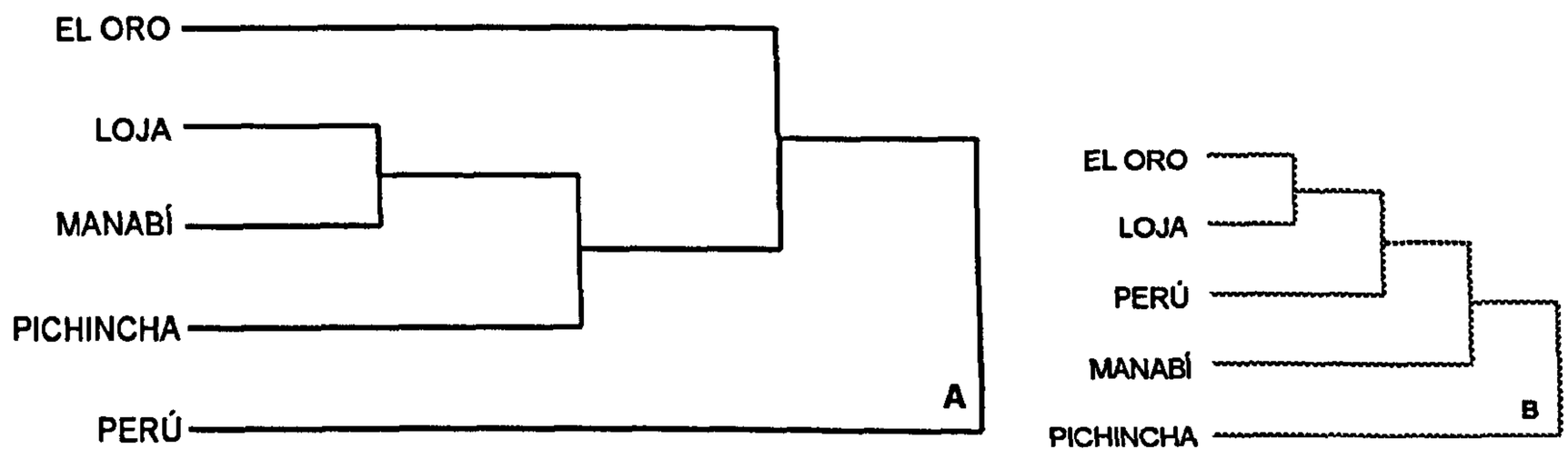


Figure 100. UPGMA dendrograms derived from Mahalanobis distances after size-free canonical variate analysis. Note the differences in tree topology between **A** (where size-free residuals were used) and **B** (CVA using log-transformed, centred measurements; see figure 98A above)

A more detailed discriminant analysis was conducted to explore possible structuring within each population. As precise sites of capture were available for most individual bugs from Manabí, El Oro, Loja, and Peru, these groups were submitted to CVA (using log-shape ratios as input for PCA) and the results plotted on the discriminant space as shown in figure 101.

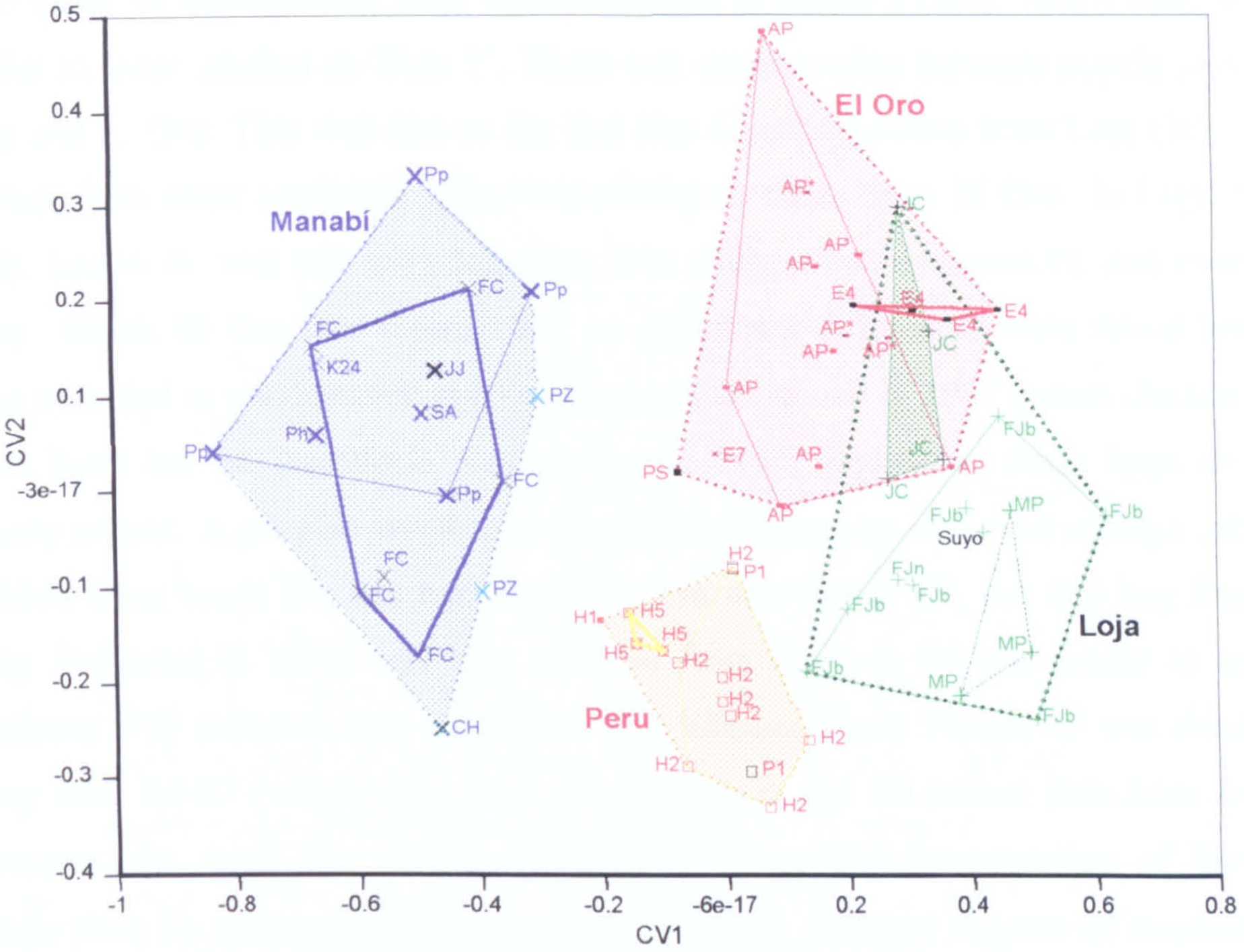


Figure 101. Canonical variate analysis of four *Rhodnius ecuadoriensis* populations. Within-population structuring was explored by labelling the positions of individual bugs on the discriminant space. Bugs from the same precise collection site were enclosed in a convex hull when ≥3 specimens were available.

- Manabí: Pp=Pachinche (palm); Ph=Pachinche (house); FC=Pachinche-Fiocruz (originally from palms); CH=Chirijos (palm); PZ=S. José de Picoazá (chicken nest); K24=km 24 (house); SA=Sta. Ana (house); JJ=Jipijapa (house)
- Peru: H1=House 1; H2=House 2; H5=House 5; P1=labelled as 'Peru-1'
- Loja: FJb=House FJ (bedroom); FJn=House FJ (chicken nest); MP=House MP (chicken nest); JC=House JC (chicken nest); Suyo=Suyo, Piura, Peru
- El Oro: AP=House AP (chicken nest; collected 1999); AP*=House AP (chicken nest; collected 1997); E4=House Entrada 4 (chicken nest); E7=House Entrada 7 (chicken nest); PS=House PS (bedroom)

These results show what appears to be a lack of structuring within the fundamentally sylvatic Manabí population. Thus, field-collected bugs from Pachinche Adentro were similar to those from a Fiocruz colony founded in 1992 with insects collected in palm trees of the same locality (and preserved pinned since 1999); size for instance was virtually identical in both subgroups (WT X^2=0.06, 1 df, p=0.81 [using the average of log-transformed measurements as the estimator of size]). Furthermore, bugs collected in

palm trees were similar to those found in human environments, with no suggestion of any trend towards differentiation. On the contrary, some within-population structuring was seen in bug collections from synanthropic habitats. The Peruvian population was completely separated from the Ecuadorian ones, and appeared to be more homogeneous, with all the specimens grouped in a small polygon. Within Peru, bugs from house 5 (H5) could be distinguished from those collected in house 2 (H2), which were in turn similar to those labelled as 'Peru 1'. There was some overlap between populations from Loja and El Oro. This was due to the fact that a subpopulation from Loja (JC), while distinct from other sympatric ones, was similar to those from El Oro. In Loja (figure 102), houses JC and MP were less than 1km apart, whereas house FJ was over 5km away. Within El Oro (see figure 103), no significant differences were found between bugs collected in peridomestic chicken coops of house AP in 1997 (when chicken nests were burnt but no insecticide was used) and 1999, suggesting these bugs are very closely related. A possible trend towards differentiation was observed in bugs collected in 1998 from house E4; this house was located near house E7, but one bug from this latter (collected in 1999) was very different from those in E4 and similar to another specimen (PS) collected from a bedroom in a different house. House AP was about 3km away from E4-E7 (which were close to each other) and PS (about 1km from E4-E7). However, the small size of these subgroups means that interpretation of fine-scale details must be approached with caution; in general, different degrees of structuring in sylvatic and synanthropic populations was the main feature revealed by these analyses.

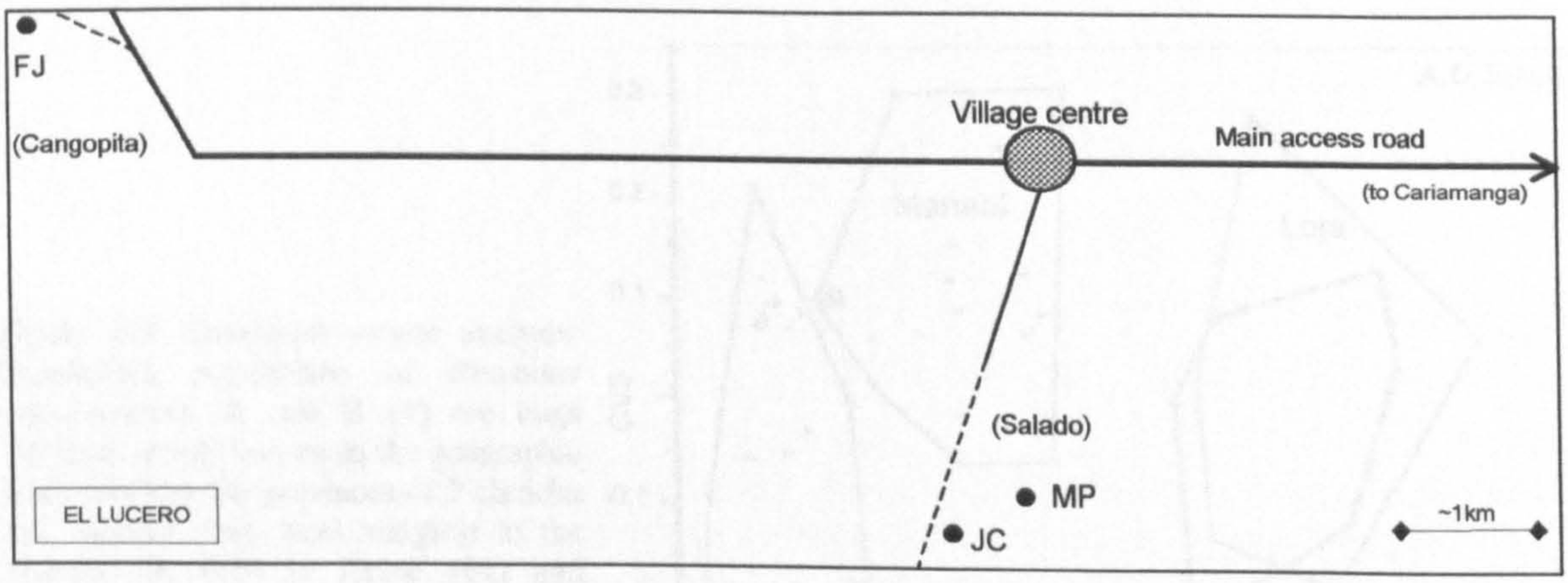


Figure 102. Diagram of El Lucero (Loja) showing the approximate relative positions of infested houses

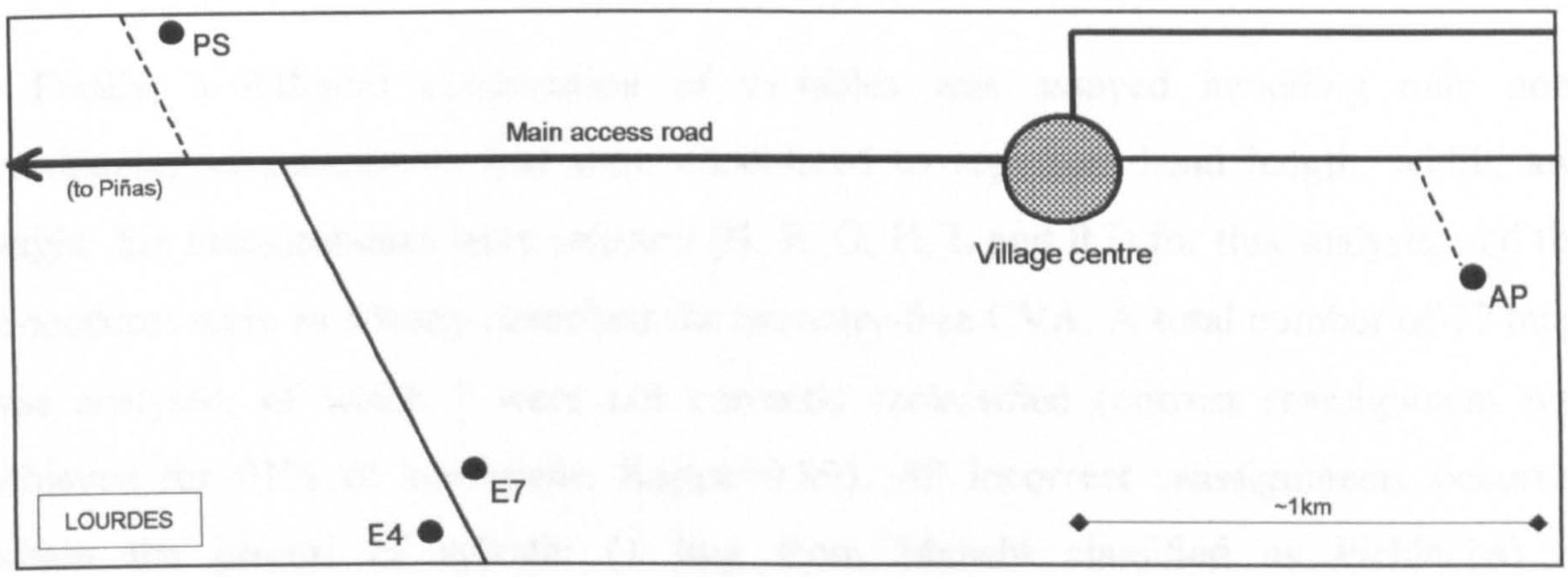


Figure 103. Diagram of Lourdes (El Oro) showing the approximate relative positions of infested houses

A different set of variables was used to investigate the finding of two specimens with distinct morphological-chromatic traits in the limit between the provinces of Manabí and Pichincha. Based on overall phenotype characteristics, one of these specimens was assigned to the Pichincha group and the other one to the Manabí group. Both were found in human environments; they were allegedly collected in a locality known as 'Kilómetro 24', on the road Santo Domingo de Los Colorados-Chone. Results of CVA using five log-shape ratios are shown in figure 104. The variables selected (A, D, E, H, and L) excluded those of the rostrum, because one of the specimens (Manabí-like, RecB) was deteriorated and lacked that structure. CVA analysis revealed a high degree of similarity, mainly on the second canonical vector (CV-2, *Y* axis). For the variables used, RecB had the smallest CV1 score in the Manabí group, with a value falling well within the Pichincha range.

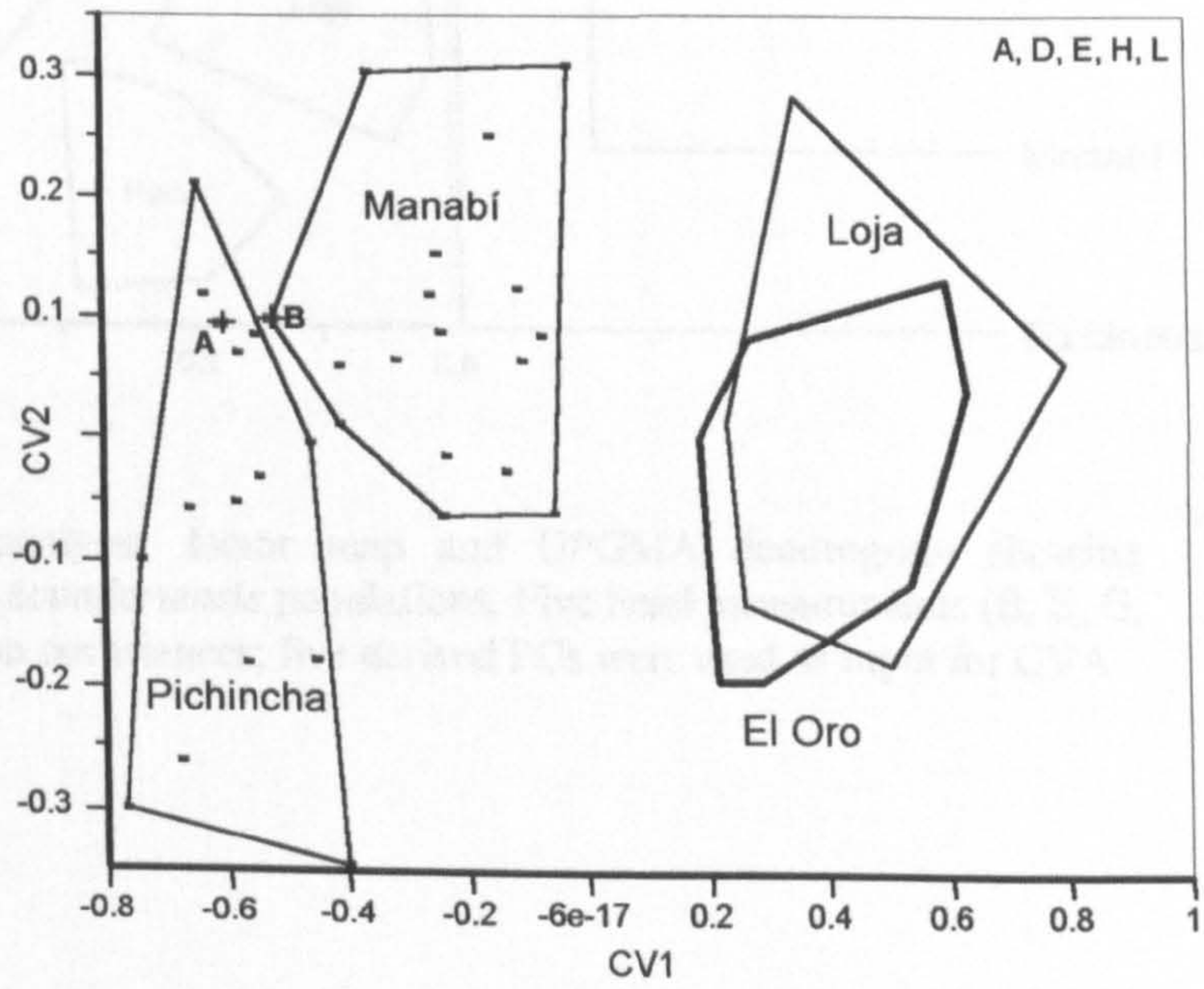


Figure 104. Canonical variate analysis: Ecuadorian populations of *Rhodnius ecuadoriensis*. **A** and **B** (+) are bugs collected inside houses in the geographic limit between the provinces of Pichincha and Manabí; they were assigned to the Manabí (**B**; K24 in figure 101) and Pichincha (**A**) groups morphological and chromatic characters. CVA revealed a high degree of similarity, mainly on the second canonical vector. Dots within polygons represent the positions of individual bugs

Finally, a different combination of variables was assayed including only non-overlapping measurements that were considered to represent head length, width, and height. Six measurements were selected (B, E, G, H, L and R2) for this analysis, and the procedures were as already described for isometry-free CVA. A total number of 77 bugs was analysed, of which 7 were not correctly reclassified (correct reassignment was achieved for 91% of specimens; Kappa=0.89). All incorrect reassignments occurred within the groups of sylvatic (1 bug from Manabí classified as Pichincha) or synanthropic (with some bugs from Loja and El Oro classified out of their original groups), and never across them. Wilk's lambda statistic was used to assess multivariate significance (λ=0.02, F≈25.3, 20 df, $p<0.0001$). The first PC accounted for 71.6% of variance, and was negatively correlated to size (as the average of the six log-measurements; y=-0.3-0.74x; R^2=0.74). Residuals of linear regression of PC1 vs. all variables were used as form variables for a size-free analysis (as above). Results were in agreement with those obtained earlier, and are summarised in the following figures.

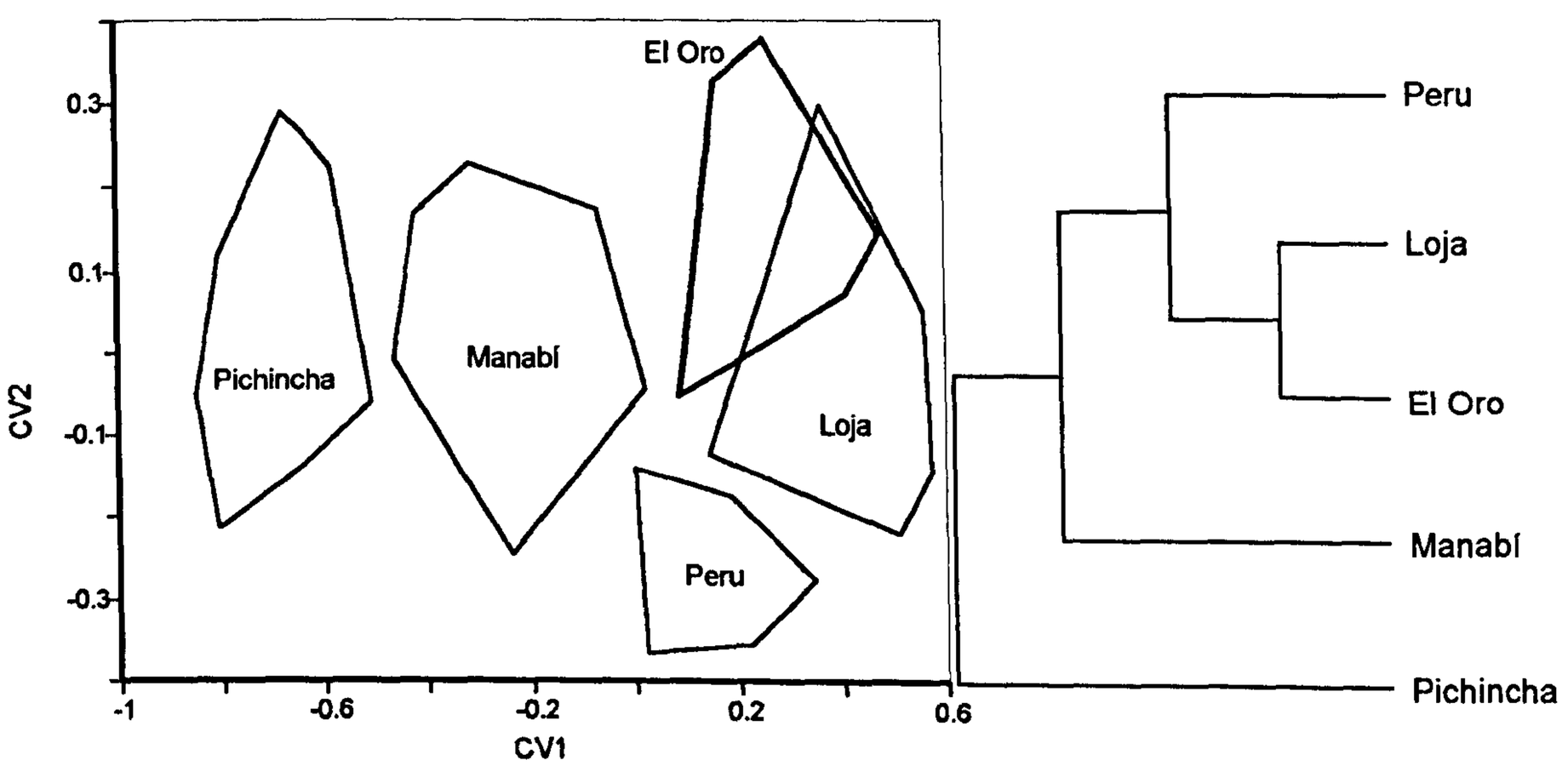


Figure 105. Canonical variate analysis: factor map and UPGMA dendrogram showing relationships among five *Rhodnius ecuadoriensis* populations. Five head measurements (B, E, G, H, L, and R2) were used for PCA on covariances; five derived PCs were used as input for CVA

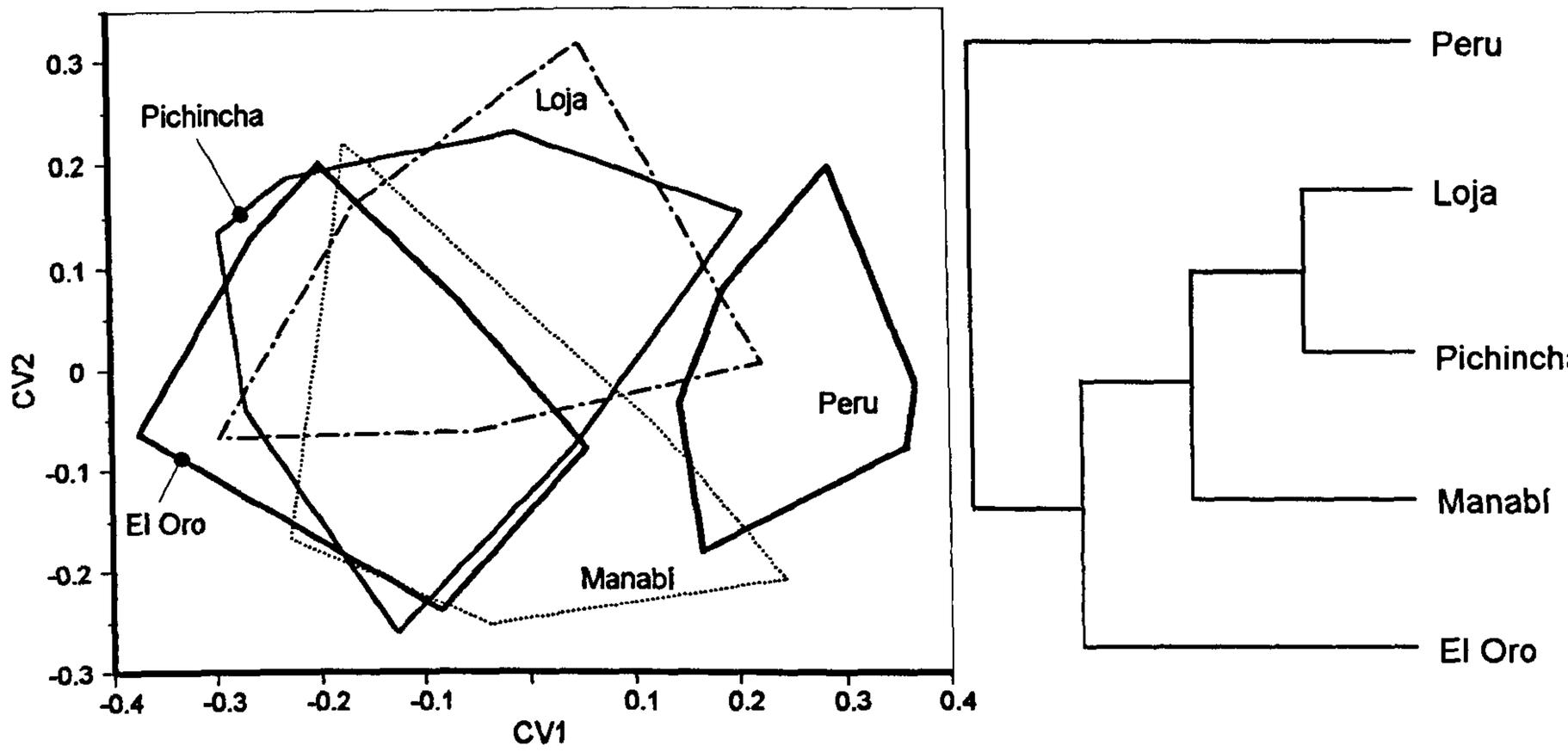


Figure 106. Canonical variate analysis of five *Rhodnius ecuadoriensis* populations: linear regression residuals (PC1 vs. measurements) were used as size-free (form) variables for discriminant analysis. Factor map (bivariate plot of CV1 vs. CV2) and UPGMA dendrogram derived from Mahalanobis distances. Note the changes in both factor map resolution and dendrogram branching order in relation to the previous figure (the same set of six measurements were used; see text for details)

6.2.3.5. Allometry-free analysis

A subset of five head measurements (A, C, D, G, and R2) was used for this part of the study; all five *R. ecuadoriensis* populations and 15 *R. colombiensis* (used as outgroup) were analysed (n=92) using a common principal component approach (CPC). Log-transformed variables were tested for compatibility with a common allometric axis. The fit with the CPC model was assessed using X^2 statistics with the NTSYS 2.10y software package; the data fitted well the model (X^2=62.3, 50 df, p=0.114), suggesting that a common growth axis is shared by all *R. ecuadoriensis* populations and also by *R. colombiensis*, at least for the variables considered here. All but the first CPCs (i.e., four 'form variables') were therefore used as input for PCA and discriminant analysis, and individual canonical scores were plotted on the discriminant space defined by the two first canonical vectors (CV1 and CV2). The multivariate analysis was highly significant (Wilk's λ=0.016, p<0.0001); results are shown in figures 107-108.

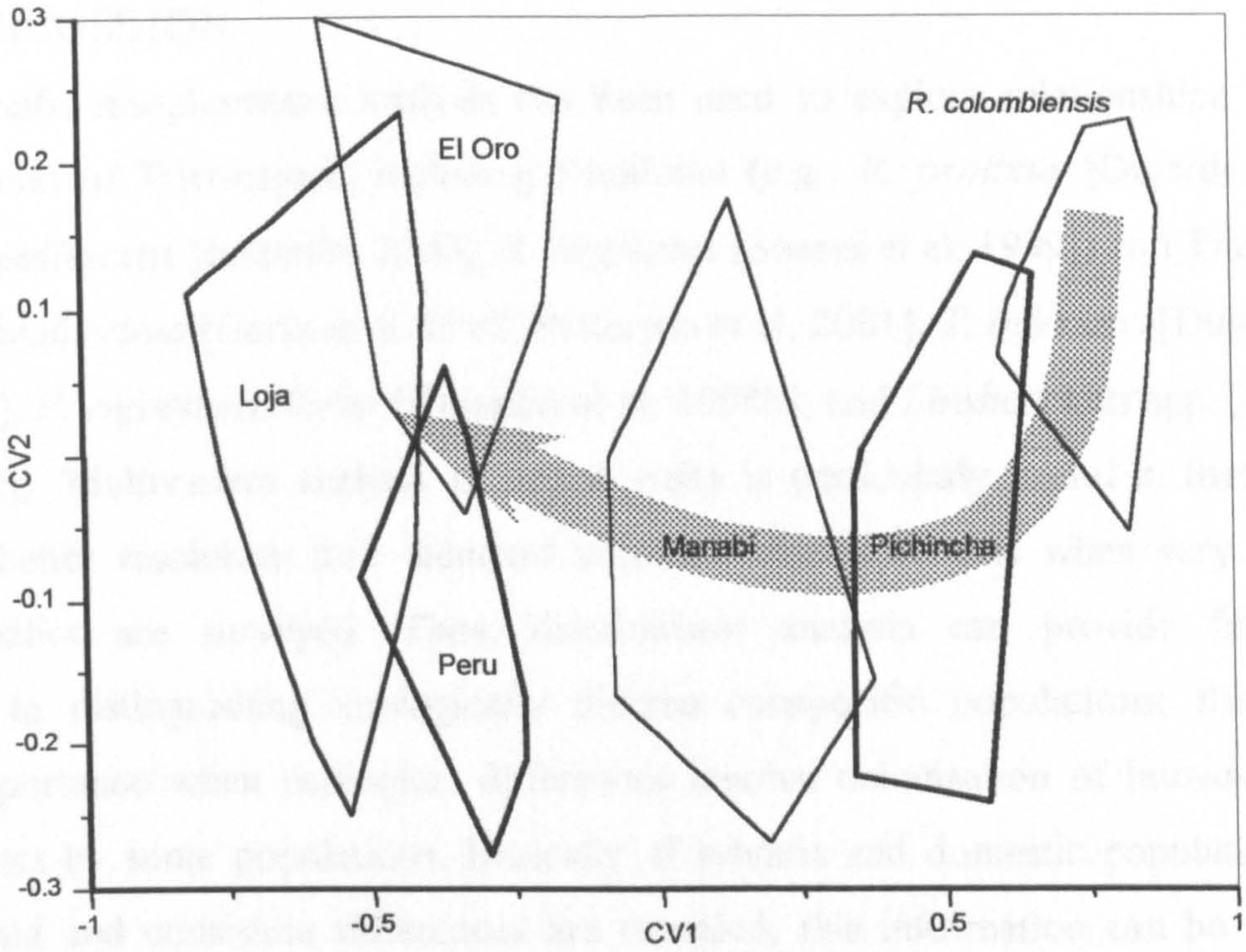


Figure 107. Morphometrics of *Rhodnius ecuadoriensis* populations: allometry-free analysis. Four common principal components (all but the first one) derived from five head measurements (A, C, D, G, R2) were submitted to CVA (see text for details). The grey arrow indicates a tentative north-to-south axis of progressive variation

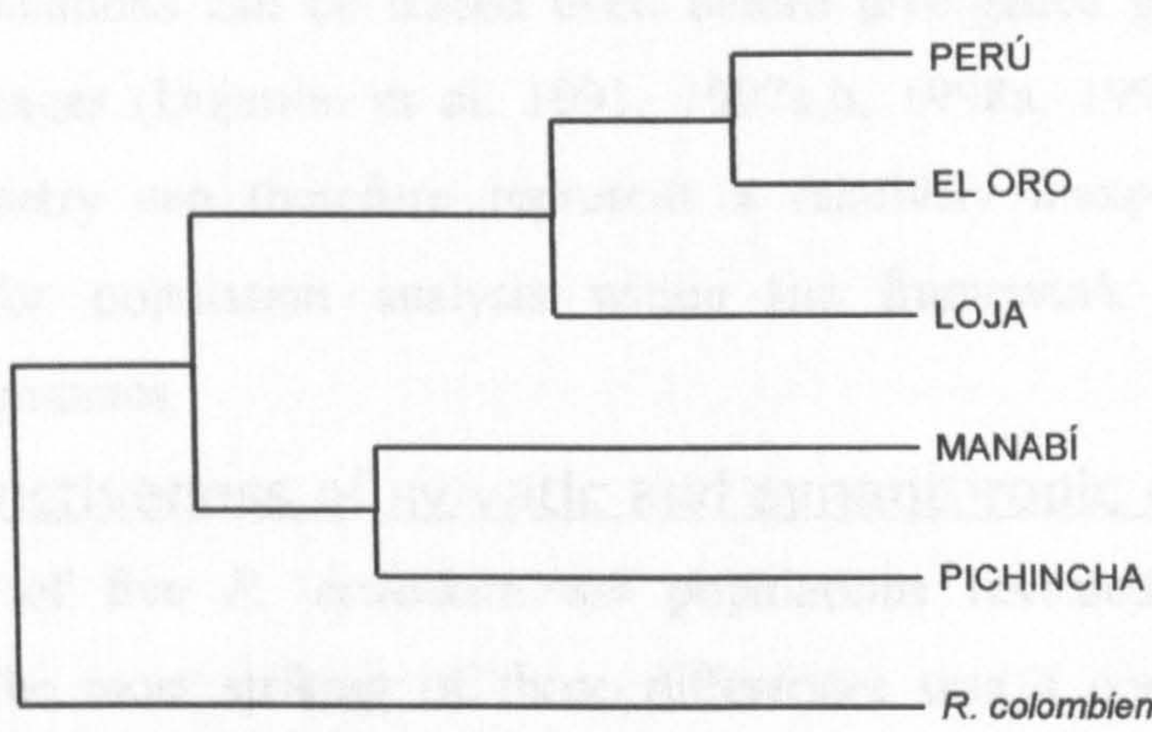


Figure 108. Morphometrics of *Rhodnius ecuadoriensis* populations: UPGMA dendrogram derived from Mahalanobis distances after allometry-free analysis

Results again show a clear distinction between populations exploiting different habitats – the primarily sylvatic Manabí-Pichincha cluster on the one hand and the synanthropic Loja-El Oro-Peru cluster on the other. However, the alignment of the various populations along CV1, similar to that of figure 97 (even if reversed), suggests that a size component is still influencing these results. Even if the first CPC was excluded from the analysis, it was still strongly correlated to CV1, with a high linear regression coefficient (R^2=0.885).

6.2.4. DISCUSSION

Intra-specific morphometric analysis has been used to explore relationships among several species of Triatominae, including Rhodniini (e.g., *R. prolixus* [Dujardin et al. 1998a], *R. pallescens* [Jaramillo 2000], *R. neglectus* [Soares et al. 1999]) and Triatomini (e.g., *T. rubrofasciata* [Gorla et al. 1997, Patterson et al. 2001], *T. infestans* [Dujardin et al. 1997a,b], *P. rufotuberculatus* [Dujardin et al. 1998b], and *Linshcosteus* spp. [Galvão et al. 2002]). Multivariate analysis of metric traits is particularly useful in that it can achieve a better resolution than standard allozyme electrophoresis when very closely related entities are surveyed. Thus, discriminant analysis can provide fine-scale resolution in distinguishing ecologically diverse conspecific populations; this is of greater importance when ecological differences involve colonisation of human-related environments by some populations. Basically, if sylvatic and domestic populations are characterised and consistent differences are revealed, this information can be used to investigate the origin of bugs found reinfesting dwellings after control interventions; similarly, the origin and routes of passive, man-mediated spread of strongly synanthropic populations can be traced even before divergence generates detectable, fixed genetic changes (Dujardin et al. 1991, 1997a,b, 1998a, 1999b, Patterson et al. 2001). Morphometry can therefore represent a relatively inexpensive, simple, and powerful tool for population analysis within the framework of vector control-surveillance programmes.

6.2.4.1. Distinctiveness of sylvatic and synanthropic populations

The analysis of five *R. ecuadoriensis* populations revealed a high degree of heterogeneity. The most striking of those differences was a complete separation of sylvatic and synanthropic populations, with 100% correct assignment of bugs to either group consistently achieved by CVA, and Kappa values close to 1 (corresponding to perfect agreement between original groups and reassignment by the model; Landis & Koch 1977). This indicates that a reference set of metric characters can be used for the surveillance of reinfestations within the vector control programmes in Ecuador and in northern Peru. The fact that a single specimen from Suyo was reclassified by the discriminant analysis as belonging to the Loja group (its natural geographic-ecological cluster) was encouraging in that sense, and further demonstrated the high sensitivity of CVA for resolving the origin of synanthropic bugs (Dujardin et al. 1998a). The apparent

trend towards higher intra-population morphometric structuring in synanthropic (vs. sylvatic) populations might reflect differences in the underlying genetic structure of both groups. Fixation of site-specific phenotypic traits at the micro-geographic level would be enhanced in domestic populations with little interbreeding, while panmictic sylvatic populations would tend to be phenetically homogeneous. Also, these latter may be assumed to be under stronger selective pressure, acting on the phenotypes via, for instance, predation or microclimatic constraints (both relatively relaxed in the protected synanthropic environments). This would in turn imply that 'pockets' of domestic bugs (inbreeding populations sharing micro-geographic sites) are isolated from each other, and that they lack the homogenising interconnections that would be provided by sylvatic populations occurring in sympatry. The finding of fixed, site-specific genetic differences (i.e., the detection of independent phylogroups [*sensu* Avise et al. 1987; see Avise and Walker 1998]) and relatively low levels of genetic diversity in synanthropic populations would be expected under the assumptions of this scenario.

Our results show that a very significant reduction in **size** is associated with adaptation of bugs to human environments; similar trends exist in other triatomines, but the magnitude of size decrease seems more important in *R. ecuadoriensis* than in *R. domesticus*, *T. infestans*, or *P. rufotuberculatus* (Dujardin et al. 1998b, 1999b,c). In general, size progressively decreased along a north-to-south axis (Pichincha → Manabí → El Oro → Loja), with the exception of Peruvian bugs, which were slightly (but significantly) larger on average than their Ecuadorian synanthropic counterparts.

We detected an overall increase in **size-related sexual dimorphism** in the transition from sylvatic (with no significant differences between males and females) to domestic (with significant differences) environments in *R. ecuadoriensis*. This finding apparently contradicts previous results from studies on *R. domesticus*, *T. infestans*, *R. prolixus* (Dujardin et al. 1999b), and *P. rufotuberculatus* (Dujardin et al. 1998b), where higher degrees of dimorphism were scored in sylvatic populations. This was interpreted in demographic terms (rather than simply reflecting different habitats), and the authors concluded that higer population density (and subsequent lower blood availability per bug) in domestic (or laboratory) environments may lead to a decrease in overall size, especially in females (with higher blood requirements); increased survivorship in those stable environments would result in more small individuals, mainly females, with the

consequent reduction in sexual dimorphism (Dujardin et al. 1999c). When the different populations studied here were analysed separately, it was revealed that the overall trend of higher dimorphism in synanthropic populations was due to the absence of significant differences between males and females from Pichincha; the Manabí population was indeed the most dimorphic for the traits surveyed, and various degrees of relative reduction in sexual dimorphism were scored in the three domestic-peridomestic populations. Thus, excluding Pichincha, the trends previously reported also seem to apply to *R. ecuadoriensis*. The low levels of dimorphism detected in Pichincha are difficult to interpret. Perhaps the large overall size of these bugs is a trait under selection, meaning that smaller males have limited chances of survival. This in turn could be related to the distinctive ecological and climatic characteristics of the habitats occupied by this population (humid and cool Andean foothill forests; see below).

6.2.4.2. Size-free analysis: divergence of the Peruvian population

Size-related features were the key of the powerful discrimination of populations obtained by CVA; when size was explicitly removed from each variable (by using the residuals of their linear regressions against PC1), a different picture emerged in which Ecuadorian populations appeared to be essentially similar to each other, whereas bugs from La Libertad (Peru) were the most distinct. Under the assumption that size-related variation is primarily related to environmental factors, this analysis of form should represent a more direct approximation to the genetic variation underlying the observed phenotypic diversity among populations; perfect multivariate partition of environmental factors is however to be regarded as operationally impossible (Sorensen 1992, Sorensen & Foottit 1992). Size-free CVA suggests that, despite extensive phenetic dissimilarities, all Ecuadorian populations share a common and very similar genetic background, whereas Peruvian bugs (except the Suyo specimen) appear to be well differentiated. Although these latter share many environmental characteristics with the southern Ecuadorian populations (Loja and El Oro), and are therefore similar in size, the area where they originated (the upper Chicama valley, Department of La Libertad) is separated from Ecuador by ~500km of very arid land, including the Sechura coastal desert. This suggests that long-term isolation of the Peruvian populations may have played a role in their distinctiveness, resulting in form traits even overwhelming the strong influence of a shared synanthropic environment on overall bug size. The fact that

no natural palm tree populations occur in northwestern Peru, discussed in Section 3.2., adds to the likelihood of such isolation.

6.2.4.3. Phenetic variability in sylvatic populations

The bugs from Manabí studied here had been collected mainly from palms, but the sample included adults found in synanthropic habitats. The lack of phenotypic structuring within this population suggests that both palm tree- and house-collected specimens are to be considered essentially sylvatic; the finding of adult specimens in human environments probably represents mere invasion of those habitats, without stable colonisation. The shallow micro-geographic structure likely reflects the combined effects of extensive interbreeding (implying good dispersal ability, as also suggested by field observations) and selective pressure (involving higher chances of survival of the observed phenotypes, perhaps because they help avoid predation and/or provide better fitness in relation to microclimatic conditions). Some specimens from a colony founded in 1992 with palm tree-collected bugs from one of our fieldwork localities were analysed and found to be indistinguishable from field-collected specimens. This shows that seven years of laboratory rearing (about seven generations) did not produce detectable changes in these specimens, and suggests that the evident differences between sylvatic and domestic bugs might reflect many generations of reciprocal isolation. Jaramillo et al. (2000) reported a similar finding in *R. pallescens*; size differences detected among field-collected populations were retained after the bugs were reared under identical laboratory conditions for several generations. On the other hand, Galíndez Girón and Torres (1999) compared metric traits of laboratory-reared bugs (colonies kept for 25 years) with the preserved, field-collected founders of the colonies. Significant, consistent differences were detected in *R. prolixus*, but *R. robustus* specimens from both groups were largely comparable (Galíndez Girón & Torres 1999).

Finally, sylvatic bugs from Pichincha and Manabí could also be readily distinguished from each other. The differences involved not only size-related metric characters (larger bugs in Pichincha, with reduced sexual dimorphism), but the bugs were also very different in colour (dark brown with reddish marks in Pichincha, light-yellowish with brown marks in Manabí; see Section 6.1.). This phenetic variability contrasts with the fact that both populations share the same primary habitat, *Ph. aequatorialis* palm trees. However, even if belonging to the same species, Pichincha and Manabí palms were

different in various respects, probably resulting in distinct microhabitat conditions. Microclimate differences, combined with strong positive selection related to avoidance of predation by camouflaging could explain the striking phenotypic variation in these sylvatic bug populations.

6.2.5. CONCLUSIONS

Combined, the findings presented here show that very important phenetic changes, mainly involving size-related characters, seem to take place when bugs adapt to human environments. These changes appear to require many generations to be detectable, suggesting that at least the most distinct populations probably have been isolated from each other for considerably long periods; the differential phenotypic structuring of sylvatic and synanthropic populations may be interpreted as further supporting the existence of such isolation.

However, phenetically distinct Ecuadorian populations seem to share their basic genetic background (as indirectly assessed by analysis of form), whereas Peruvian bugs from La Libertad appear to be the most strongly divergent.

Phenotypic plasticity is therefore probably acting both in the direction of divergence (separating genetically similar bugs that occupy different habitats) and convergence (making genetically distinct populations look similar when they occupy similar habitats).

Apart from considerations on the genetic correspondences of phenotypic diversity, it can be concluded that morphometric analysis may be extremely useful for monitoring reinfestations by *R. ecuadoriensis* in the context of operational control schemes; more generally, it may be possible to study behavioural and adaptive trends of morphometrically characterised populations of the species, perhaps directly using the information generated in this study (including characterised, preserved specimens and associated image files and measurements) as baseline databases. Such data and materials will be made available to Ecuadorian public health authorities.

The first of the hypotheses mentioned in the introduction to this Section (that synanthropic populations can be distinguished from sylvatic ones using metric traits) received therefore firm support from our results. The second hypothesis involves an assessment of evolutionary relationships among the populations under study. Morphometric data seem to favour the idea of a greater similarity of northern (sylvatic)

R. ecuadoriensis specimens with *R. colombiensis*, with an apparent clinal reduction in size along a north-to-south axis (see figure 107).

Morphometric data did not seem however to suffice for an accurate assessment of mutual evolutionary relationships at the subspecific level. The high sensitivity of the approach in detecting and characterising phenetic variation (mostly habitat-related) turns into a disadvantage when such phenetic plasticity involves both convergent (homoplastic) and divergent (apomorphic) change in the context of an apparently strong influence of environmental factors.

When isometric size was removed from the analysis a strong divergence of the Peruvian population was detected, perhaps signalling parallel genetic separation, but mutual relationships among Ecuadorian populations were poorly resolved. In this sense, clinal size reduction within Ecuadorian geographic-ecological groups might be interpreted as reflecting a phyletic pathway; it can for instance be assumed that anatomical traits of sylvatic bugs represent plesiomorphic phenotypic states, whereas the phenotypes of synanthropic bugs are derived. Also, polarised isometric change (sylvatic-to-domestic; i.e., large-to-small) may be envisaged as a more parsimonious (energy-efficient) transition than changes involving body shape, which require rearrangements in the simultaneous expression of polygenes (Sorensen & Foottit 1992). This rationale would add to the conclusion that the separation of Peruvian bugs (as shown by size-free comparisons) may reveal strong genetic divergence, whereas changes separating Ecuadorian ecological populations would represent relatively 'easy' transitions along a linear axis of size reduction. The evolutionary significance of these phenotypic relationships, if any, remains however uncertain. Further comparisons, including a direct assessment of genetic variability among different geographic-ecological populations are therefore required to clarify micro-evolutionary relationships and population structuring in *R. ecuadoriensis*.

6.3. Genetic diversity in *Rhodnius ecuadoriensis*

6.3.1. INTRODUCTION

Mitochondria are cytoplasmic organelles within eukaryotic cells; they are fundamentally involved in various metabolic processes and in apoptosis, disease, and aging (Boore 1999, Saccone et al. 2000). The most crucial of these functions relate to cell respiration: the oxidation of nutrients to produce adenosine 5'-triphosphate (ATP) takes place in these organelles. Mitochondria are believed to be of endosymbiotic origin, with phylogenetic affiliation to the α-subdivision of the Proteobacteria (Avise 1994, Page & Holmes 1998, Saccone et al. 2000, Turner et al. 2000). In Metazoa, each mitochondrion has a single DNA molecule, typically 15-20 kilobases in length and customarily containing about 37 genes coding for 2 rRNAs, 22 tRNAs, and 13 mRNAs responsible for the synthesis of proteins involved in electron transport and oxidative phosphorylation. Intergenic spacers are relatively rare and small, and there is a control region usually containing a series of tandem repeat units and involved in the initiation of transcription. Gene order is generally conserved; rearrangements have however been described – and used to explore relationships among strongly divergent taxa (Avise 1994, Simon et al. 1994, Boore et al. 1995, Boore 1999, Saccone et al. 1999). The sequence and organisation of the complete mitochondrial genomes of various animal taxa have been published, including several insects (e.g. Clary & Wolstenholme 1985, Beard et al. 1993, Crozier & Crozier 1993, Lewis et al. 1995, Spanos et al. 2000) and one triatomine species (*T. dimidiata*) (Dotson & Beard 2001). *T. dimidiata* mt genome is 17019bp long, and contains the 37 genes specified above; only seven intergenic spacers were found (just 1 to 5bp long, except for one comprised of 314bp and of unknown function). The control region is 2.1kb long, has 8 tandem repeat units (from 82 to 173bp long) and is organised in four regions; this arrangement is shared by *R. prolixus* (Dotson & Beard 2001).

The single, circular mtDNA molecule, present in multiple copies (~10^2-10^4 per cell, individuals being virtually homoplasmic), is maternally inherited through the egg cytoplasm (but 'paternal leakage' has been described in some taxa), replicates with essentially no recombination (although strict clonality may not be the case in a few exceptional circumstances), and presents significantly faster (up to ten-fold in mammals, and over three-fold in closely related *Drosophila* species) overall evolution

rates when compared to the nuclear genome (Tamura 1992a, Avise 1994, Saccone et al. 2000). These high substitution rates are in part due to defective repair mechanisms during replication, and are likely to affect preferentially some specific parts of the molecule, whereas functional and structural constraints tend to lower substitution rates in other sections (Avise 1994, Saccone et al. 2000). Examples of within-molecule rate heterogeneity include faster evolution of control and intergenic regions, third codon position nucleotides in protein-coding genes, or the bases of rRNA genes corresponding to unpaired loops (as opposed to helical stem sections) in the secondary structure of those genes; within these latter, conserved sections have been described in core helices, in unpaired regions between domains, and in relation to sites of ribosomal protein attachment, mRNA processing, and tRNA attachment (Simon et al. 1994, Rodríguez-Trelles et al. 2000, Saccone et al. 2000). Rate heterogeneity has also been described among different mt genes (for instance, rRNA and tRNA genes evolve more slowly on average than protein-coding ones) and between taxa (prompting controversies about the molecular clock hypothesis and contributing to the fundamental selectionist-neutralist debate on the modes of molecular evolution) (Avise 1994, Hafner et al. 1994, Ayala & Fitch 1997, Cavalli-Sforza 1997, 1998, Page & Holmes 1998, Gissi et al. 2000, Saccone et al. 2000, Nevo 2001). However, it has been noted that at low levels of divergence (at least when divergence times do not exceed roughly 3 million years, providing confidence that saturation has not occurred and that most substitutions are silent), and for various arthropod taxa, mtDNA synonymous substitutions seem to accumulate at a constant rate of about 1.1-1.2% per million years (my), with an overall 2.3% pairwise sequence divergence per my (Brower 1994). A global estimate for animal mtDNA is of about 2% divergence/my (Avise 1994), but limited taxon-specific rate heterogeneity has been identified (Gissi et al. 2000, Saccone et al. 2000).

In part because the structure, function, mode of inheritance, and evolutionary aspects of metazoan mtDNA are so well understood, and in part because many of these features make the molecule specially tractable as compared to nuclear DNA, mitochondrial genes are widely used in population genetics and in evolutionary studies at different levels of divergence. MtDNA gene order rearrangements have proven useful to resolve deep phylogenetic branches in metazoans, but did also show variation among some arthropod taxa (including insects), molluscs, and even between congeneric *Schistosoma*

blood flukes (Boore 1999, Boore et al. 1995, 1998, Black & Roehrdanz 1998, Saccone et al. 1999, Le et al. 2000a,b, Dotson & Beard 2001). On the other hand, nucleotide sequence polymorphisms of rapidly evolving mtDNA genes are more suitable for the assessment of relationships among recently diverged taxa (Avise 1994, Page & Holmes 1998, Saccone et al. 1999). This approach has been widely applied to investigate patterns of insect phylogeny within recognised lineages; not surprisingly, special attention has been directed towards disease vectors and crop pests, but *Drosophila* fruitflies, beetles, bees, butterflies, and many others have also been studied (e.g., Simon et al. 1994, Tang et al. 1996, Vogler & Pearson 1996, Conn et al. 1997, Esseghir et al. 1997, Gimeno et al. 1997, Glesson & Sarre 1997, Ready et al. 1997, O'Grady et al. 1998, Ishikawa et al. 1999, Rodríguez-Trelles et al. 2000, Walton et al. 2000, Litzenberger & Chapco 2001, Machado et al. 2001, Monteiro et al. 2001, Birungi & Munstermann 2002, Donnelly et al. 2002). Protein-coding genes are particularly useful for these purposes; although the codon triplet code places effective constraints on the rate of substitution at second codon positions, because of the degenerate nature of the genetic code (in mt genes a single tRNA species can decode four codons), many third and some first codon bases are relatively free to vary, especially when substitutions do not imply amino acid replacement (synonymous or silent substitutions) (Simon et al. 1994, Saccone et al. 1999). Furthermore, it has been found that silent sites evolve at similar rates in different mt protein-coding genes (which is not necessarily the case for the corresponding amino acid sequences) (see Clary & Wolstenholme 1985, Simon et al. 1994, Saccone et al. 1999). This feature has led to the conclusion that for any given sequence length, one mt protein-coding gene should be as informative as any other for phylogenetic inference purposes (Simon et al. 1994). Substitution rate heterogeneities have also been found in these genes, generally affecting distantly related taxa. For instance, cyt*b* genes seem to evolve six times slower in sharks than in mammals, and nonsilent substitutions in the cytochrome oxidase I and II genes were significantly more frequent in honeybees (*Apis mellifera*) than in *Drosophila* fruitflies (Simon et al. 1994).

The use of DNA approaches for the study of triatomine bugs has a short, albeit rather dynamic, history. Molecular studies have provided new insights into the systematics and evolutionary trends of the subfamily, with surveys ranging from inter-tribe to subspecific analyses, and from genotyping and species identification to elucidation of

inter-population relationships and inference of species phylogenies; both nuclear and mitochondrial gene sequence polymorphisms have been surveyed using different methodologies (from RAPD to single strand conformational polymorphism and direct sequencing). Beyond their intrinsic academic interest, many of these results have been used to refine and improve disease control strategies throughout Latin America, and will likely prove crucial for the study of bug species and populations displaying various degrees of synanthropic behaviour (see review by Monteiro et al. 2001, and García & Powell 1998, Stothard et al. 1998b, García et al. 1998, 2001, García 1999, Harry et al. 1998, Lyman et al. 1999, Monteiro et al. 1999, 2000, Bargues et al. 2000, Marcilla et al. 2000, 2001, 2002, Gaunt & Miles 2002).

We used mt cyt*b* sequence polymorphisms (based on a 663 basepair gene fragment) to investigate genetic diversity, relationships, and possible structuring in five populations of *R. ecuadoriensis* representing the entire known geographic range of the species. Specimens from the same populations were also subjected to qualitative (anatomical-chromatic) and quantitative (metric) phenetic analyses (Sections 6.1. and 6.2.); the vast majority of the material examined originated from field collections in well-characterised areas where intensive surveys were conducted to determine the main ecological and behavioural features of the bug populations (see Chapters 4-5). The analyses were further extended to encompass other closely related species of *Rhodnius* with the aim of clarifying the relationships of *R. ecuadoriensis* with its sister taxa. More generally, the phylogeny of the species of *Rhodnius* whose natural range includes the western side of the Andes (the 'Pacific *Rhodnius* lineage') was investigated (Chapter 7). The extent of these comparisons greatly benefited from the collaboration of several friends and colleagues who generously provided us with different materials (from bug specimens to sequence data; see below and Appendix), illuminating discussions, expert guidance, and constructive criticism. The framework provided by ECLAT was crucial to the establishment of these collaborative links.

Results of molecular analyses are discussed in terms of micro-evolutionary relationships among populations; the congruence of phenotypic and haplotypic patterns of diversity is addressed in that context, taking also into account biogeographic, ecological, and behavioural features of the bugs. Hypotheses regarding the possible relative contribution of different processes (e.g., demographic, phylogeographic,

ecological) to the observed population structuring of *R. ecuadoriensis* are derived from joint analysis of these results. Finally, suggestions for future work are outlined (including considerations on methodological improvement) and recommendations for operational design of vector control strategies in Ecuador and Peru are put forward.

6.3.2. MATERIALS AND METHODS

6.3.2.1. Bugs

A total number of 72 *R. ecuadoriensis* specimens representing five geographic populations were used for this part of the study. These included 20 specimens from Alluriquín (Pichincha), 14 from various collection sites in Manabí (three of them from a laboratory colony [Fiocruz, J Jurberg] founded in 1992 with bugs collected from palm trees in Pachinche Adentro – one of our fieldwork localities), nine bugs from Lourdes (El Oro), 15 from El Lucero (Loja), and 14 insects from Peru (13 field-collected in La Libertad [CA Cuba Cuba] and one from the reference *R. ecuadoriensis* laboratory colony at Fiocruz, Brazil). Overall, 34 *R. ecuadoriensis* bugs were essentially sylvatic (Pichincha and Manabí) and 38 synanthropic (El Oro, Loja and Peru). Additional material used for comparisons included *R. colombiensis*, *R. pallescens*, *R. pictipes*, and *R. robustus*. Further specimens were kindly made available by FA Monteiro (CDC-Fiocruz), S Fitzpatrick (LSHTM), JS Patterson (LSHTM), and CJ Schofield (ECLAT-LSHTM) (table 105). Further details about individual specimens can be found in the Appendix.

Table 81. Triatomine materials generously provided by friends and colleagues for genetic studies

Provider	Species	Material	Specifications*
FA Monteiro/CB Beard	*R. ecuadoriensis, R. Pictipes, R. pallescens, R. prolixus, R. robustus, R. nasutus, T. rubrovaria*	Sequences	PE (one), PIC-1, PAL-V, PRX-3 to 5, ROB-1 to 5, NAS
FA Monteiro/CJ Schofield	*R. pallescens*	Sequences	PAL-N1 to N3, PIC-2
CA Cuba Cuba	*R. ecuadoriensis*	Bugs	PE (all but one)
S Fitzpatrick/JS Patterson	*R. prolixus*	DNA	PRX-1 and 2
J Jurberg	*R. ecuadoriensis*	Bugs	Manabí-Fiocruz

R.=Rhodnius, T.=Triatoma; *see text for details on haplotypes and populations

6.3.2.2. DNA extraction and purification

Total genomic DNA was extracted from legs of dried or 70° ethanol-preserved triatomine specimens. In some cases (especially for nymphs) the whole insect was used. DNeasy extraction kits (Qiagen) were used, following the manufacturer's recommendations for processing animal tissues except for the preparation of tissue samples. Three to six legs (or whole bugs) were separated from individual bugs and ground to powder in an Eppendorf tube (1.5 ml); for this, the tube bottom was

submerged in liquid nitrogen until legs were frozen and a micro-pestle was used to crush the tissue. Tissue powder was mixed with 180µl of lysis buffer (ATL); 20µl of proteinase K were added, the mixture vortexed briefly and incubated overnight at 55°C. The mixture was then homogenised by vortexing for 15sec and 200µl of lysis buffer (AL) were added, mixed thoroughly by vortexing and left at 70°C for 10 minutes. 200µl of 100% ethanol were added, the mixture vortexed and the tubes centrifuged at 13000rpm for 3min. The liquid phase was recovered using disposable, sterile Pasteur pipettes, transferred to a DNeasy minicolumn sitting on a clean collection tube and left standing at room temperature for 30min. The columns were centrifuged at 8000rpm (1min) and the flow-through discarded. Washing buffer (AW1, 500µl) was pipetted into the column and left for 5min, then centrifuged at 8000rpm (1min) and the flow-through discarded. 500µl of a second washing buffer (AW2) were added, the columns left standing for 5min and then centrifuged at 13000rpm for 3min. The columns were placed in new, sterile 1.5ml Eppendorf tubes and 200µl of AE elution buffer (previously heated at 55°C for 10min) were pipetted directly onto the column membrane; the columns were left standing for 2 hours and then centrifuged at 8000rpm for 1min so that the eluted DNA was recovered in the Eppendorf tube; this first DNA elution was transferred to a sterile Eppendorf and stored at 4°C until use. The last step was repeated to obtain a second DNA elution.

6.3.2.3. PCR amplification

A ≈700 base pair (bp) fragment of the mitochondrial cytochrome *b* gene (cyt*b*) was amplified by PCR using primers generously provided by FA Monteiro and CB Beard:

Cyt*b*32F 5'-GGACG(AT)GG(AT)ATTTATTATGGATC (forward)

Cyt*b*33R 5'-GC(AT)CCAATTCA(AG)GTTA(AG)TAA (reverse)

These primers were designed by examination of conserved regions of the cyt*b* gene of *T. dimidiata* (Dotson & Beard 2001) and comparison with other insect cyt*b* sequences, and had been successfully tested in various *Rhodnius* species (FA Monteiro et al., unpublished). For PCR, 50µl reaction mixtures were prepared as follows: 5µl PCR buffer (with $MgCl_2$), 4µl dNTP mix, 2µl of each primer (10pmol/µl), 0.5µl *Taq* polymerase, 34.5µl H_2O, and 2µl template DNA. Alternatively, 4µl DNA (and 32.5µl H_2O) were used for some of the reactions.

PCR thermal cycles comprised a denaturation step (95°C for 5min) followed by 35 cycles of denaturation (94°C, 30sec), annealing (45, 47 or 48°C; 5sec), and extension (72°C, 1-2min); a final extension step (72°C, 7min) was followed by storage at 4°C indefinitely. Successful amplification was confirmed by 1% agarose gel electrophoresis of a 5μl aliquot of PCR product, using ethidium bromide staining. Reactions were considered positive when single bands of ≈700bp (assessed by comparison with appropriate DNA ladders) were detected using an ultraviolet transilluminator. One positive and one negative control were incorporated into each PCR run. Cyt*b* amplicons were purified using Microcon-PCR filter devices (Millipore) to remove primers and unincorporated dNTPs; the manufacturer's instructions were followed. Purified PCR products were eluted in 50μl TE buffer; products from specimens for which faint bands had been recorded in the agarose gels were eluted in 25μl TE buffer to increase the final DNA concentration. Purified products were checked by agarose gel electrophoresis as above and then stored at -20°C until use.

6.3.2.4. DNA sequencing

Direct sequencing was performed on both strands using fluorescent dye terminator chemistry and PCR primers. Sequencing reaction mixtures (20μl) were prepared as follows: 1μl template PCR product (3μl for specimens with faint bands after PCR), 6μl sequencing buffer, 2μl BigDye mix (Applied Biosystems), 1μl of either 32F or 32R primer, and 10μl H_2O (8μl for reactions with 3μl template). The following cycles (x25) were used: denaturation at 95°C (10sec), 50°C for 5sec (annealing), and extension for 4min (60°C); products were stored at 4°C until processing. Unincorporated dyes and primers were removed by means of Centri-sep columns (Princeton Separations), according to the manufacturer's recommendations. The samples were vacuum-dried and stored at -20°C until use. For analysis, sequencing reaction products were resuspended in 15μl of formamide and submitted to capillary automated sequencing in an ABI Prism 3100 apparatus (Applied Biosystems). The quality of the sequences was first assessed by inspection of each electropherogram. Poor quality sequences were discarded. Forward and reverse sequences were aligned to detect possible ambiguities; these were evaluated and corrected whenever possible so that a reliable consensus sequence could be computed for each specimen. Sequence Navigator 1.0.1 was used for this preliminary assessment. Consensus sequences were subsequently aligned manually.

6.3.2.5. Data analysis

Aligned sequences were analysed using MEGA 2.1 software (Kumar et al. 2001). **Basic statistics** included nucleotide composition and deduced amino acid sequences, patterns of base (transitions-transversions) and amino acid substitution (synonymous-nonsynonymous substitutions), numbers of variable sites and relative codon positions, number of parsimony informative sites, and patterns of codon usage. Nucleotide diversity was estimated for various groups of taxa.

Phylogenetic analyses included distance- and character state-based approaches. Matrices of pairwise genetic distances were calculated under various models of base substitution. As pointed out by Simon et al. (1994), all distance correction methods should produce comparable results when examining closely related taxa. Furthermore, when two distance correction measures give similar results, as was the case for the *R. ecuadoriensis* haplotype dataset under consideration, simple models (with smaller variance) should give better estimates of the correct tree provided that substitution rates do not vary among lineages (Kumar et al. 2001). For closely related taxa, therefore, distances based on the Jukes-Cantor (JC, Jukes & Cantor 1969) method (whose assumptions include equally probable base substitutions, absence of nucleotide bias, and equal probability of mutations across sites) or uncorrected distances (such as the proportion of variable sites, *p*) should work equally well as more complex models.

The Kimura two-parameter model (K2p, Kimura 1980) incorporates corrections for multiple hits at the same site taking into account transitional and transversional substitution rates. Base frequencies and substitution rates among sites are assumed to be equal. The Tamura three-parameter (T3p) model corrects for multiple hits taking into account the differences in transitional and transversional rates and the G+C-content bias, assuming equality of substitution rates among sites (Tamura 1992b). Finally, the Tamura-Nei model (TN) corrects for multiple hits taking into account substitution rate differences between nucleotides, inequality of nucleotide frequencies, and excess transitions; the rate of purine transitions is not assumed equal to the rate of pyrimidine transitions, but the assumption of equal substitution rates among sites does apply (Tamura & Nei 1993). These models can incorporate a gamma distribution in order to account for the variation in mutation rates among sites; the shape parameter of the gamma distribution is estimated as $\alpha \approx 0.44$ for the mt cyt*b* gene, indicating a large

proportion of sites with low substitution rates (Page & Holmes 1998). The various distance matrices were submitted to a neighbour-joining (NJ) algorithm (Saitou & Nei 1987) and phylogenetic trees were constructed. The statistical support for each clade in the derived topology was assessed by the bootstrap-resampling method (Felsenstein 1985) with at least 1000 replications.

Maximum parsimony (MP) analyses were conducted using a branch-and-bound algorithm for tree search. Consensus trees (strict and majority-rule) were computed whenever more than one most parsimonious tree was recovered. A bootstrap approach was used to assess the statistical support of the trees.

6.3.3. RESULTS

6.3.3.1. PCR amplification: size and identity of amplicons

PCR amplification of a ≈700bp fragment of the mitochondrial cytochrome *b* gene (mt cyt*b*) was successfully achieved for 73 *R. ecuadoriensis* specimens. These included 20 out of 22 from Pichincha (91% successful amplification), 15 out of 18 from Manabí (83.3%), 15 out of 16 from Loja (93.7%), 13 out of 13 from Peru (100%), and nine out of 17 from El Oro (53%). Good quality sequences were obtained from all but one specimen from Manabí, which was discarded. In order to avoid slightly lower quality sequence readings at the beginning and end of some sequences, 663bp (out of the 699bp available for most of the sequences) were used for all the analyses. The alignments contained no gaps. The identity of the cyt*b* sequence was confirmed by comparison with the corresponding gene (1132bp) of *T. dimidiata* (Dotson & Beard 2001; GenBank accession AF301594); the 663bp fragment corresponded to sites 340 to 1003 within the gene and contained no stop codons.

6.3.3.2. Basic statistics

Analysis of the 72 aligned *R. ecuadoriensis* cyt*b* sequences revealed 10 unique **haplotypes**, some of them recovered from different collection sites (see below and Appendix). The whole *ecuadoriensis* alignment yielded 42 variable sites (6.3%), seven of which were individual autapomorphs. When comparing only the 10-haplotype alignment, 13 of the 42 variable sites were parsimony-informative, whereas 29 were autapomorphs. Within the variable sites, 36 corresponded to third codon position substitutions (i.e., 16.3% out of 221 third codon position nucleotides were variable),

five to first codon position (2.6%), and only one (0.45%) to a second codon position (a T↔C transition in a codon whose first position had changed also).

The deduced **amino acid** sequence was very homogeneous, with only two of the 10 haplotypes corresponding to different peptides; these were both different from the rest and from each other by just one amino acid out of 221. One of them was found in a single Manabí bug (code 'Ind 14', a nymph V collected from a *Ph. aequatorialis* palm tree in Chirijos), and the substitution involved the first codon position: CTT↔ATT, i.e., Leucine↔Isoleucine. Two non-silent point mutations were detected in the unique haplotype found in all Peruvian bugs. These changes involved the first and second positions of a single codon (sites 361-362-363): while GTT was found in all the rest of haplotypes, Peruvian bugs had ACT (two transitional substitutions). The amino acid change involved was therefore Valine to Threonine; intermediate pathways would also have been nonsynonymous, and could be represented by either GCT (Alanine) or ATT (Isoleucine). No termination codons were identified. Leucine was the most frequent amino acid (16.74% on average), followed by Isoleucine (9.96%), Phenylalanine (8.6%), Glycine and Proline (both 6.8%), Valine (6.7%), Alanine and Asparagine (both 5.4%), Threonine (5.06%), Methionine and Serine (4.5%), Tryptophan (3.6%), Lysine (3.2%), Tyrosine (2.7%), Histidine and Glutamine (2.26%), Aspartic and Glutamic acids (1.8%), Arginine (1.36%), and Cysteine (0.45%).

With regard to **codon usage,** Leucine was preferentially coded by TTA (0.55 relative synonymous codon usage rate [*rscu*]), but the other five possible codons were also used (mainly CTA [*rscu*=0.25] and CTT [*rscu*=0.075]). Isoleucine was usually coded by ATT (*rscu*=0.88) rather than ATC (*rscu*=0.12), and Phenylalanine by TTT (*rscu*=0.74). GGA was used preferentially for Glycine (*rscu*=0.53); GGT (*rscu*=0.27) and the other two possible codons were also used. Proline codons were biased towards CCA (*rscu*=0.6) and against CCG (*rscu*=0), with the other two codons having the same *rscu* (0.2 each). Valine used mainly GTT (*rscu*=0.6); three Alanine codons had *rscu*≈0.3, with GCG being used less frequently. Other examples include 80% of Asparagines coded by AAT, Threonine only coded by ACT (*rscu*=0.55) and ACA (*rscu*=0.45), and all Methionines coded by ATA.

Average **nucleotide composition** for these haplotypes was T=37.7%; A=31.5%; C=18%; G=12.8%. A+T accounted in average for 69.2% of the 663 nucleotides of the fragment sequenced. Nucleotide composition (as the percentage of nucleotides containing each of the four bases) for each of the haplotypes found in *R. ecuadoriensis*, both for the entire mt cyt*b* gene fragment and for each codon position, is presented in the tables of the Appendix. The composition was similar across haplotypes, with A-T content ranging from 68.5% (LJ-3) to 69.9% (MN-6/PH).

6.3.3.3. Distribution of haplotypes in geographic populations

Six different haplotypes were detected in bugs collected in **Manabí** (haplotype codes MN-1 to MN-6). Three of these haplotypes (MN-1, MN-3 and MN-5) were only found in one specimen each, whereas MN-2 and MN-4 were shared by several bugs. MN-6 was found in one bug from Manabí and in the 20 specimens from Pichincha (PH). MN-4 was identical to the haplotype found in all the bugs from El Oro (EO). The precise sites of capture of each specimen were taken into consideration for haplotype coding. Thus, haplotype MN-2 was detected in bugs from two different collection sites (hence the codes MN-2a and MN-2b), and MN-4 in specimens from two distinct sites (MN-4a and MN-4b). The details about these haplotypes are presented in the following table.

Table 82. Cyt*b* haplotypes in *Rhodnius ecuadoriensis* from Manabí

Haplotype	Locality	Site of capture	Bugs	Code(s), sex / nymph stage (N)
MN-1	Pachinche Adentro	House*	1	Rec125 ♂
MN-2a	Pachinche Adentro	Palms [Fiocruz]	3	Rec79 ♀, Rec83 ♀, Rec86 ♂
MN-2b	Chirijos	Palm tree	1	Ind11 NV
MN-3	Pachinche Adentro	Palm tree	1	RecM1 ♂
MN-4a	Sta. Rosa – Jipijapa	House*	1	Rec128 ♀
MN-4b	Pachinche Adentro	Palm trees	5	RecM2 ♂, Ind22 NIII, Ind23 NIV, Ind24 NIV, Ind26 NIV
MN-5	Chirijos	Palm tree	1	Ind14 NV
MN-6	S. José de Picoazá	Chicken coop*	1	Rec130 ♀

*No evidence of colonisation (only adult specimens found)

Haplotype variability was also detected in the synanthropic specimens from **Loja**. Three unique haplotypes (LJ-1, LJ-2 and LJ-3) were found in the 15 bugs examined. Of these, LJ-1 was only found in a single specimen (Rec74, ♂) collected from a bed inside house 'FJ', located in the area of El Lucero known as Cangopita (see figure 102). Haplotype LJ-2 was shared by six specimens and LJ-3 by eight, as shown in table 83.

Table 83. Cyt*b* haplotypes in *Rhodnius ecuadoriensis* from Loja

Haplotype	Bug code	Area of collection	Site of capture	Sex
LJ-1	Rec74	Cangopita	Bed, house FJ	♂
LJ-2	Rec40	Salado	Chicken coop, house MP	♀
	Rec46	Cangopita	Bedroom, house FJ	♀
	Rec51	Salado	Chicken coop, house JC	♂
	Rec55	Salado	Chicken coop, house JC	♂
	Rec59	Salado	Chicken coop, house JC	♂
	Rec69	Cangopita	Bedroom, house FJ	♀
LJ-3	Rec32	Salado	Chicken coop, house MP	♂
	Rec33	Cangopita	Chicken coop, house FJ	♂
	Rec35	Salado	Chicken coop, house MP	♀
	Rec38	Salado	Chicken coop, house MP	♀
	Rec47	Salado	Chicken coop, house JC	♀
	Rec57	Cangopita	Bedroom, house FJ	♂
	Rec64	Cangopita	Bedroom, house FJ	♂
	Rec71	Cangopita	Bedroom, house FJ	♀

A single cyt*b* haplotype was recovered from the nine specimens from **El Oro**, including bugs collected in two different areas of the study locality: 'Entrada' (the village entry from the main road), and an area located past the village main square; they were some 3km from one another (see figure 103). The study also included specimens collected at different times (in 1997, 1998, and 1999). The haplotype found in all bugs (EO) was identical to MN-4.

Table 84. Cyt*b* haplotype (EO) in *Rhodnius ecuadoriensis* from El Oro

Haplotype	Bug code	Area of collection	Year, dwelling	Site of capture	Sex
EO	RecO2	Beyond village centre	1999, house AP	Chicken coop	♂
	RecO3	Beyond village centre	1999, house AP	Chicken coop	♀
	RecO4	Beyond village centre	1999, house AP	Chicken coop	♀
	RecO9	Entrada (left)	1999, house PS	Bedroom	♂
	Rec9	Beyond village centre	1997, house AP	Chicken coop	♂
	Rec17	Entrada (right)	1998, Entrada 4	Chicken coop	♂
	Rec22	Entrada (right)	1999, Entrada 7	Chicken coop	♀
	Rec26	Entrada (right)	1998, Entrada 4	Chicken coop	♂
	Rec27	Entrada (right)	1998, Entrada 4	Chicken coop	♀

The same lack of variability was observed in sylvatic bugs collected from *Ph. aequatorialis* palms in the province of **Pichincha**. Sequences from 20 specimens yielded the same haplotype (PH), which was identical to MN-6; both differed by only one point mutation (a third codon position transition C↔T at site 201) from MN-4 and EO. The precise palm tree in which specimens were collected was not recorded on their individual labels.

Finally, sequences from 13 specimens from La Libertad (**Peru**) also yielded a single haplotype (PEa). These bugs were collected by Peruvian colleagues [provided by CA Cuba Cuba] in the upper Chicama valley, and (according to the labels) they were found

in at least three different dwellings. One specimen from the Fiocruz reference colony for *R. ecuadoriensis*, founded in 1979 with bugs from the same geographic region (the Departments of northern Peru that lay south of the Sechura desert, i.e. La Libertad and Cajamarca), was also analysed; its sequence (haplotype PEb) was identical to that of all other Peruvian specimens. Interestingly, haplotype PE was the most distinct among all the *R. ecuadoriensis* populations studied; it was separated by 25 nucleotide substitutions from the most similar Ecuadorian haplotype (MN-1, see below).

The following figure presents the relative geographic location of fieldwork areas; the approximate geographic distances between localities and the haplotype codes corresponding to each population are indicated.

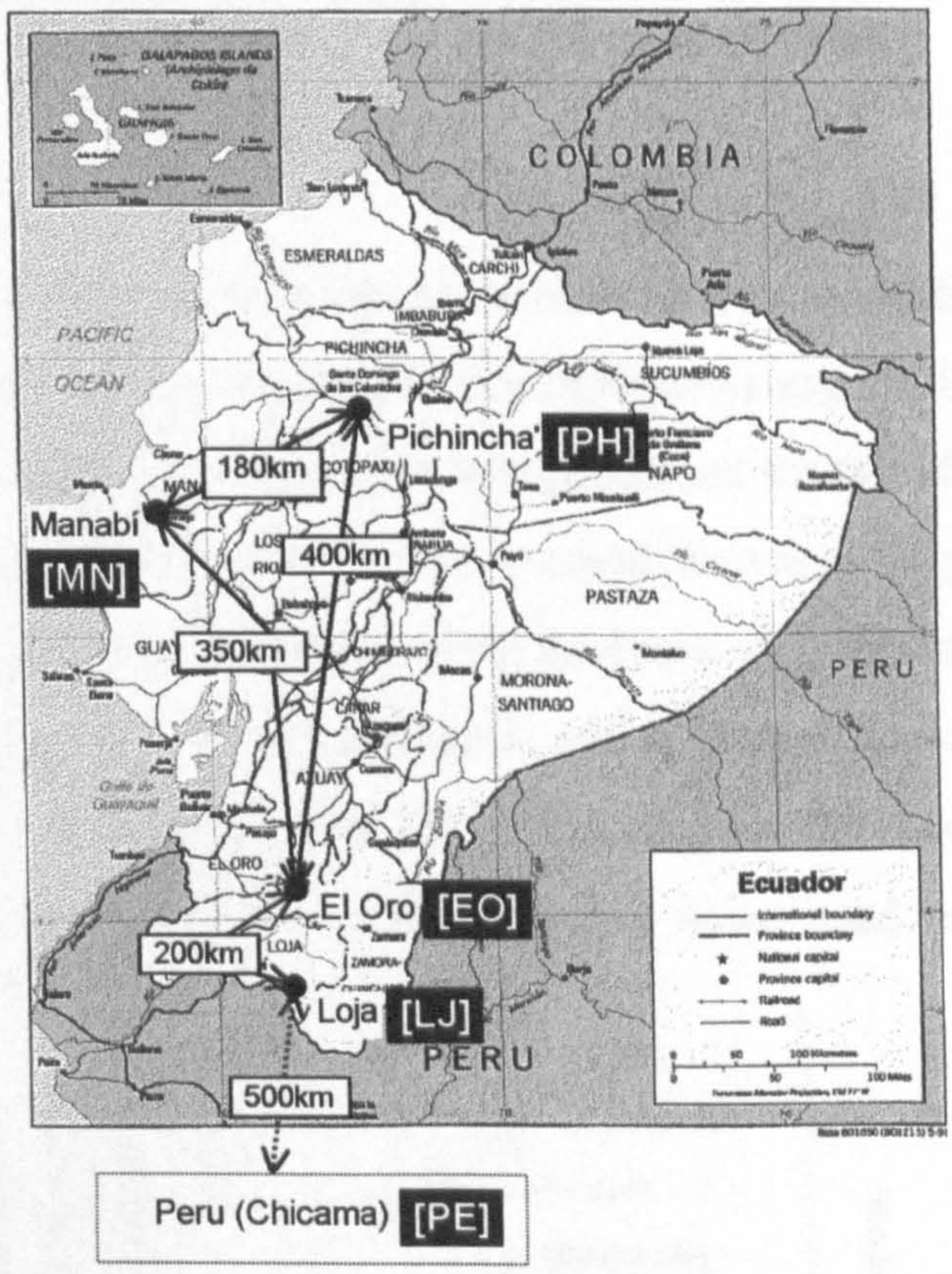


Figure 109. Geographic distribution of *Rhodnius ecuadoriensis* mt cyt*b* haplotypes. Sites of capture, haplotype codes and approximate geographic distances

6.3.3.4. Patterns of nucleotide sequence polymorphism

The overall average transition:transversion ratio was 8.3 (SE=4.02*), and increased to 10.6 (SE=5.85) when the entire dataset was analysed. Nucleotide diversity (average number of differences per site, π) was 0.0179 (SE=0.0029). Absolute nucleotide differences were scored between haplotypes and populations and distance matrices computed under different models of base substitution, from the simplest uncorrected *p*

* Standard errors (SE) were calculated by 500 bootstrap replications in MEGA 2.1 (Kumar et al. 2001)

distances to more complex distance-correction approaches such as the K2p model. The tables in the Appendix summarise the main results.

Overall mean distances within the 72-sequence alignment were 0.0179 (uncorrected *p* distance; SE=0.0029) and 0.0185 (K2p; SE=0.0031); the values for the 10-haplotype dataset were 0.0179 (SE=0.00295) and 0.0184 (SE=0.00299), respectively.

Mean distances and standard errors were also computed between geographic groups (including all sequences). The main results are presented in the tables of Appendix.

For the complete 72-sequence *ecuadoriensis* dataset, mean within-group distances (in the variable populations, i.e. Manabí and Loja) were as follows: Manabí 4.6 (SE=1.38) and Loja 1.3 (0.57) [absolute number of nucleotide differences]; Manabí 0.0069 (SE=0.002) and Loja 0.002 (SE=0.0009) [*p* distances]; these figures remained unchanged when more complex models (JC, K2p, Tamura 3-parameter, or Tamura-Nei) were used.

Mean within-group distances for the dataset containing only one example of each haplotype were: absolute nucleotide differences 5.4 (SE=1.48) in Manabí and 4.7 (SE=1.66) in Loja, and uncorrected distances Manabí=0.008 (0.002) and Loja=0.007 (0.003). Again, these latter figures did not vary under different models of base substitution, and correspond to the values of nucleotide diversity (π) for both populations. Mean between-group distances (and their SE) were also computed with the haplotype dataset (including PH and EO and their identical pairs in Manabí [MN-6 and MN-4]); the main results are summarised in the tables of the Appendix.

Saturation of transitions was not a concern for the dataset under consideration, because of the high TS/TV ratio (8.3 for the restricted haplotype dataset and 10.6 for the 72 sequences) and because all *p* distances were below the 0.09-0.1 saturation threshold for the cyt*b* gene (Meyer 1994, Griffiths 1997); maximum distances (involving pairwise comparisons between Ecuadorian and Peruvian haplotypes) were ~0.04. We compared pairwise frequencies of transitional and transversional substitutions along axes of total pairwise substitutions and postulated divergence time (after Brower 1994) between haplotypes. The plots show a steady increase in the frequency of transitional changes with genetic distance, without any evidence suggesting a trend towards saturation.

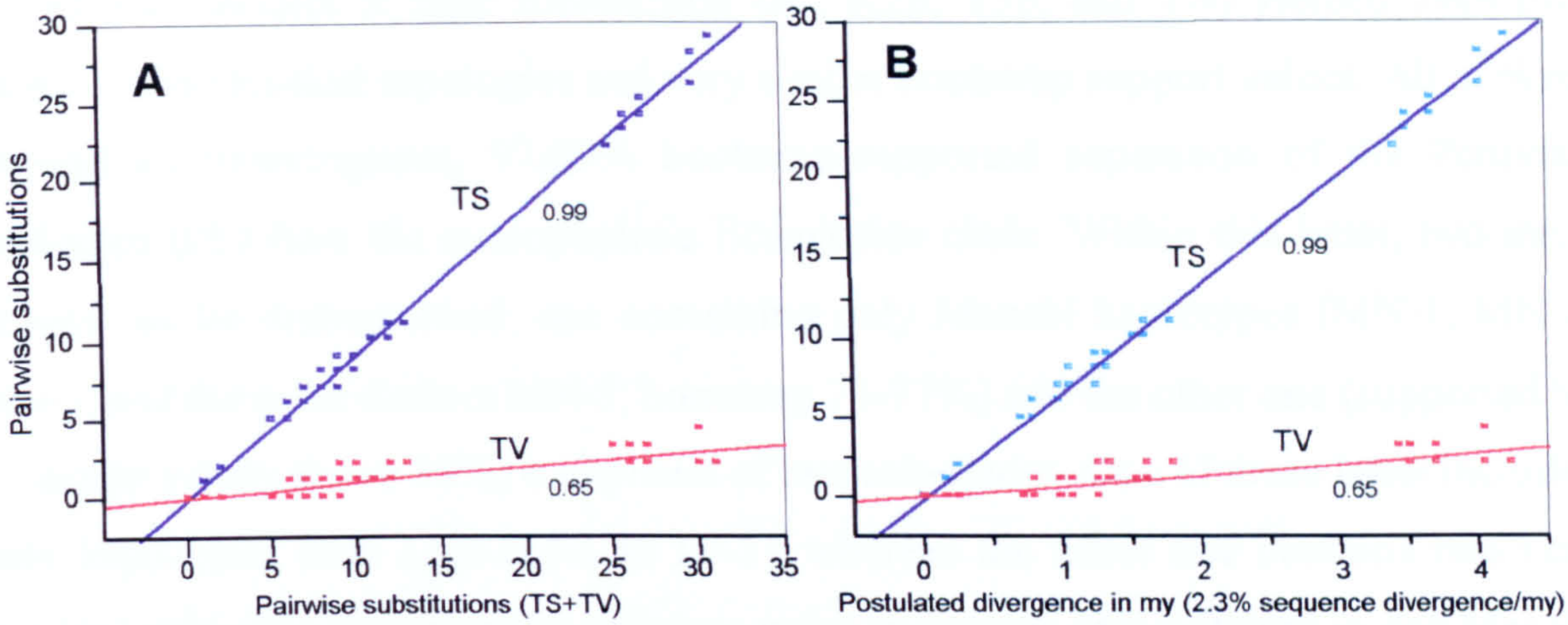
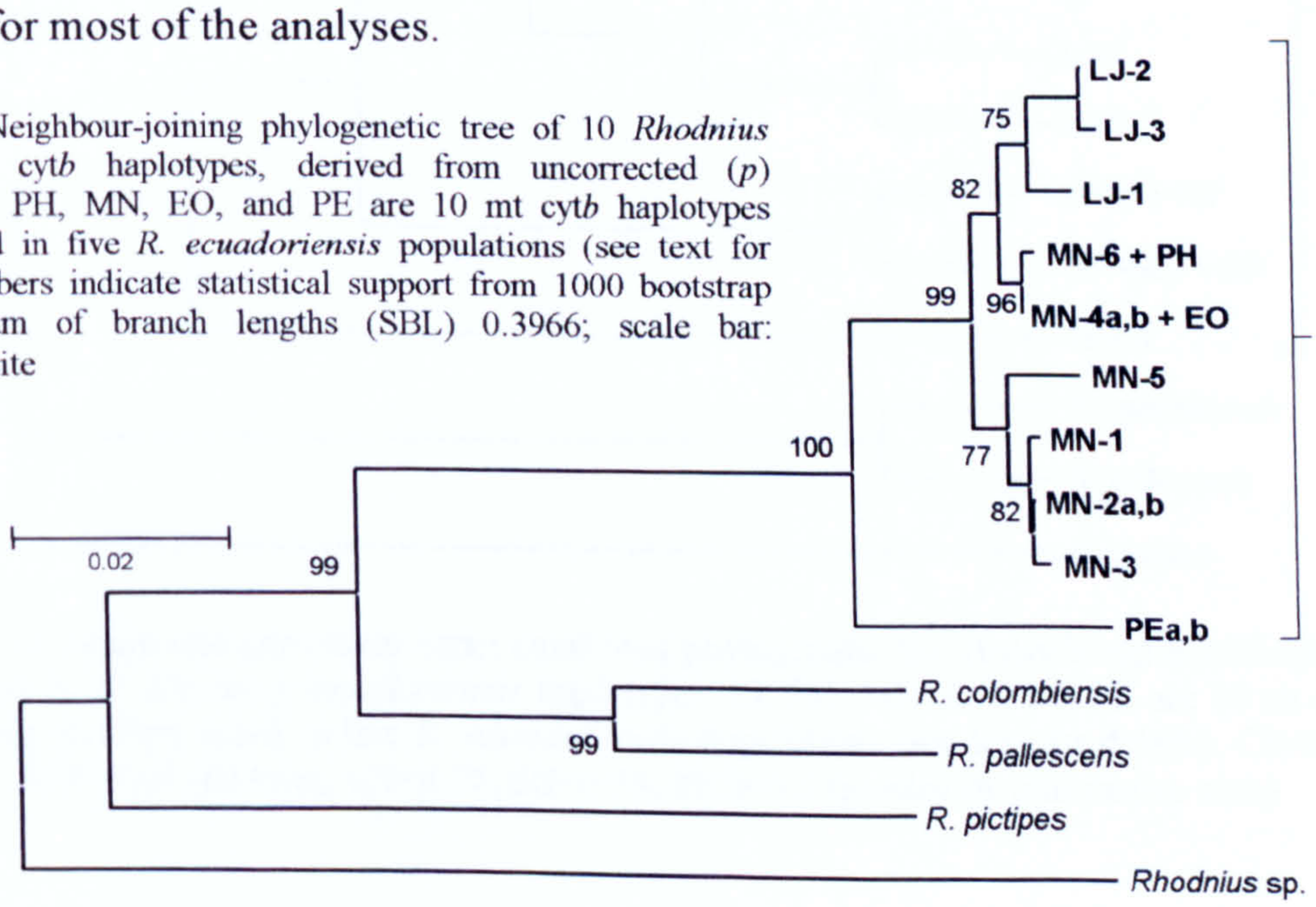


Figure 110. Pairwise transitional (TS) and transversional (TV) substitutions among *Rhodnius ecuadoriensis* haplotypes: linear regression versus (**A**) total number of substitutions (TS+TV) and (**B**) postulated time since divergence (based on an estimate of 2.3% pairwise sequence divergence per million year [my]; see Brower 1994). Linear coefficients of determination are given below each linear fit

6.3.3.5. Phylogenetic analyses

Neighbour-joining (NJ) tree topologies derived from various models were explored and their statistical support evaluated by the bootstrap-resampling technique, using 1000 replicates and a random seed number. NJ simplified trees were first produced for the 10 unique haplotypes found in the five *R. ecuadoriensis* populations studied. Subsequently the complete alignment (72 sequences) was submitted to the same analyses. Additional haplotypes from the closely related *R. pallescens*, *R. colombiensis*, and *R. pictipes*, and specimens identified as *R. robustus* and collected in palms of the Ecuadorian Amazon (pending specific determination, hence *Rhodnius* sp. in the figures), were used as outgroups for most of the analyses.

Figure 111. Neighbour-joining phylogenetic tree of 10 *Rhodnius ecuadoriensis* cyt*b* haplotypes, derived from uncorrected (*p*) distances. LJ, PH, MN, EO, and PE are 10 mt cyt*b* haplotypes (663bp) found in five *R. ecuadoriensis* populations (see text for details). Numbers indicate statistical support from 1000 bootstrap replicates; sum of branch lengths (SBL) 0.3966; scale bar: substitutions/site



Different models of base substitution (JC, K2p, T3p, and TN) yielded trees (not shown) with identical topologies and very similar bootstrap support values. All of them showed an unambiguous, 97-99% bootstrap-supported separation of the Peruvian haplotype (PE) from the monophyletic Ecuadorian clade. Within this latter, two main groups can be distinguished, one containing only Manabí haplotypes (MN-1, MN-2, MN-3, and the more distinct MN-5; bootstrap 74-77%) and the other one (supported by bootstrap values above 80%) comprised of two sub-clades. One of these latter includes only haplotypes from Loja (LJ-1 to LJ-3), whereas the other one contains two very similar haplotypes (separated by only one point mutation): MN-6 (found in one Manabí bug) and the identical PH (from sylvatic Pichincha bugs), and another haplotype found in specimens from Manabí (MN-4a and MN-4b) and El Oro (EO).

In order to further investigate the relationships between these haplotypes, additional trees were constructed using a **maximum parsimony** (MP) approach; a branch-and-bound algorithm was used to recover all MP trees, and statistical support was assessed with 1000 bootstrap replicates. Three equally parsimonious trees were recovered (tree length=216); strict consensus and a bootstrap (majority-rule) consensus trees are also presented.

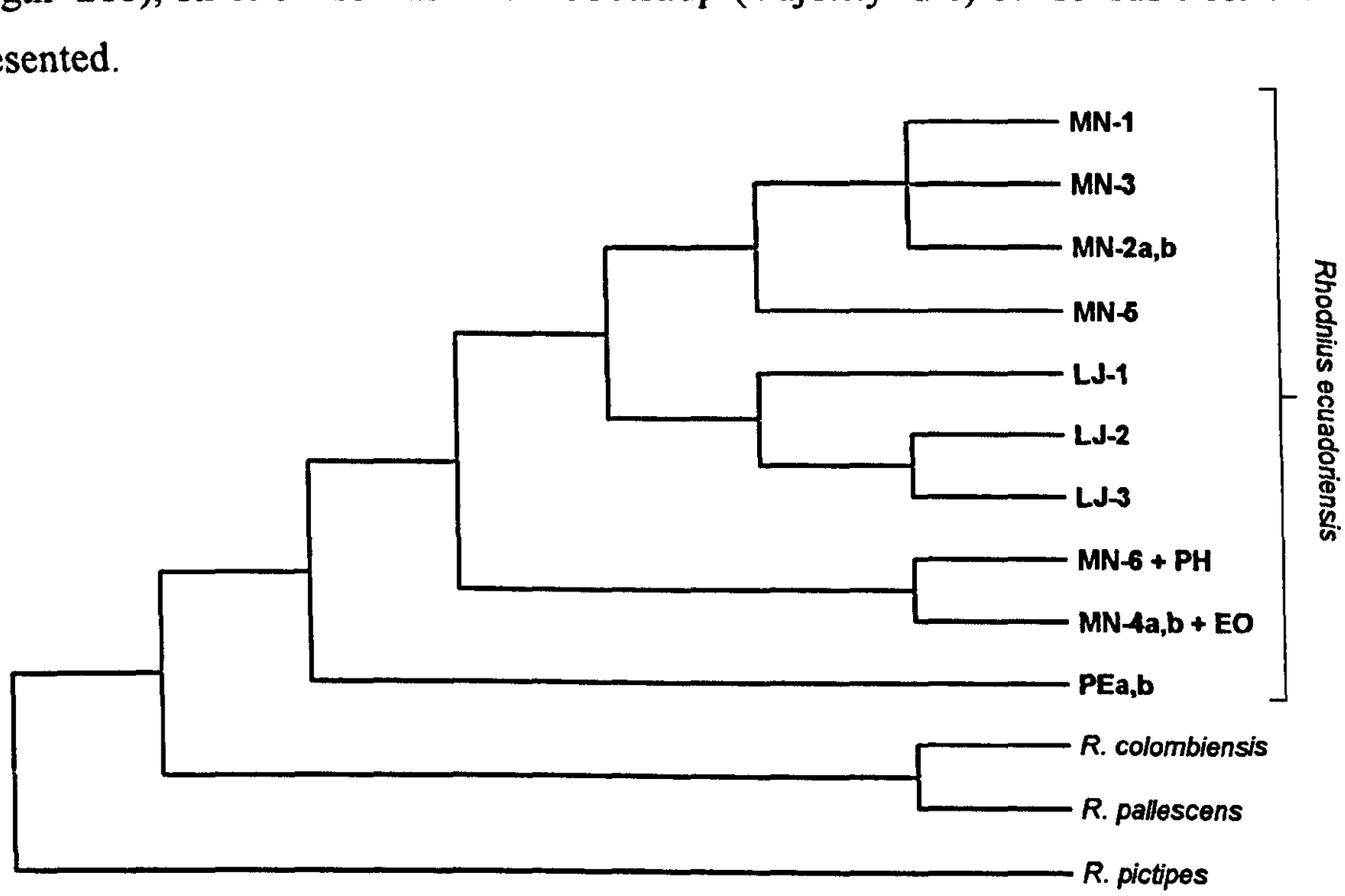


Figure 112. Maximum parsimony: strict consensus phylogenetic tree (branch-and-bound search algorithm) of 10 *Rhodnius ecuadoriensis* haplotypes. LJ, PH, MN, EO, and PE are 10 mt cyt*b* haplotypes (663bp) found in five *R. ecuadoriensis* populations (see text for details). CI=0.86, RI=0.79, RCI=0.68 (all sites); iCI=0.77, iRI=0.79, iRCI=0.6 (parsimony informative sites)

Figure 113. Maximum parsimony: bootstrap majority-rule consensus phylogenetic tree (branch-and-bound search algorithm) of 10 *Rhodnius ecuadoriensis* cyt*b* haplotypes. LJ, PH, MN, EO, and PE are 10 mt cyt*b* haplotypes (663bp) found in five *R. ecuadoriensis* populations. Numbers indicate statistical support from 1000 bootstrap replicates

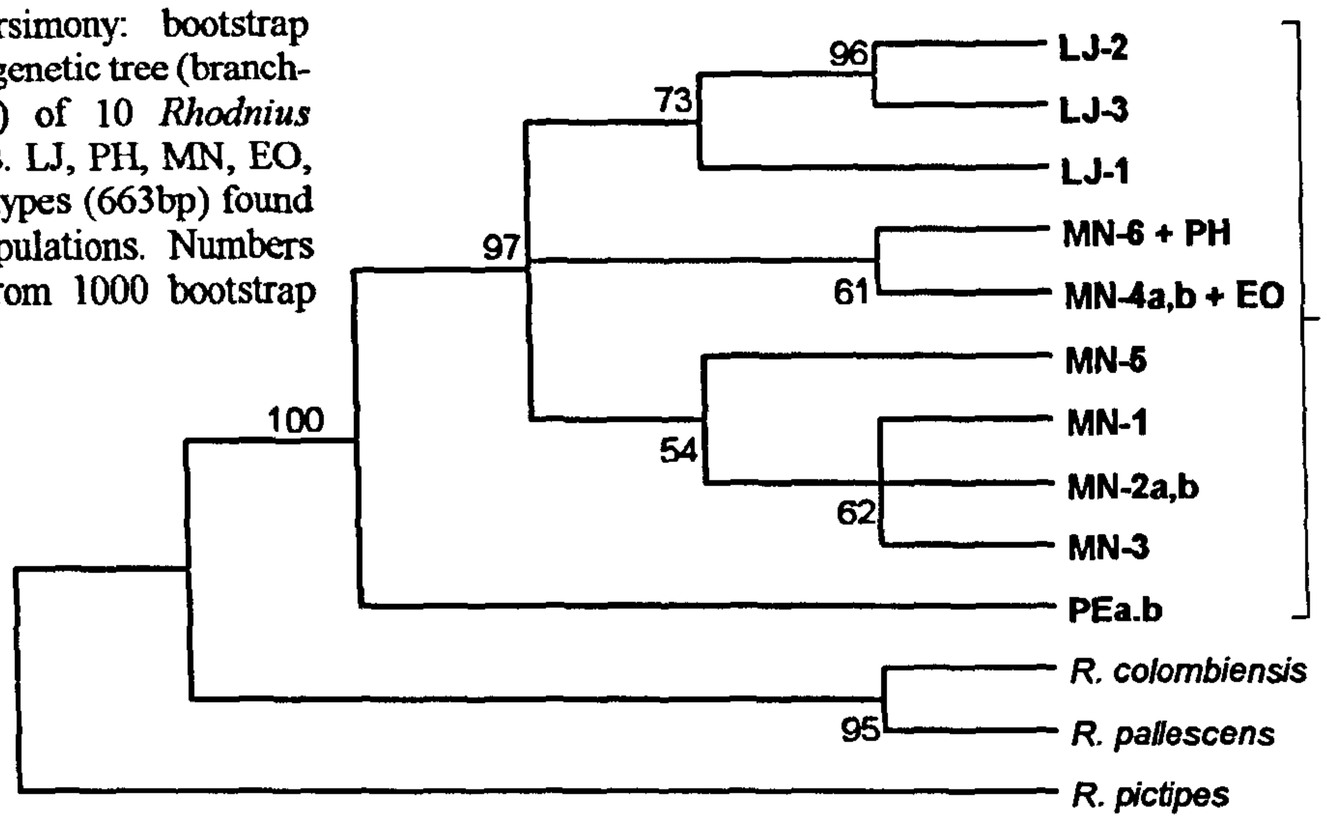
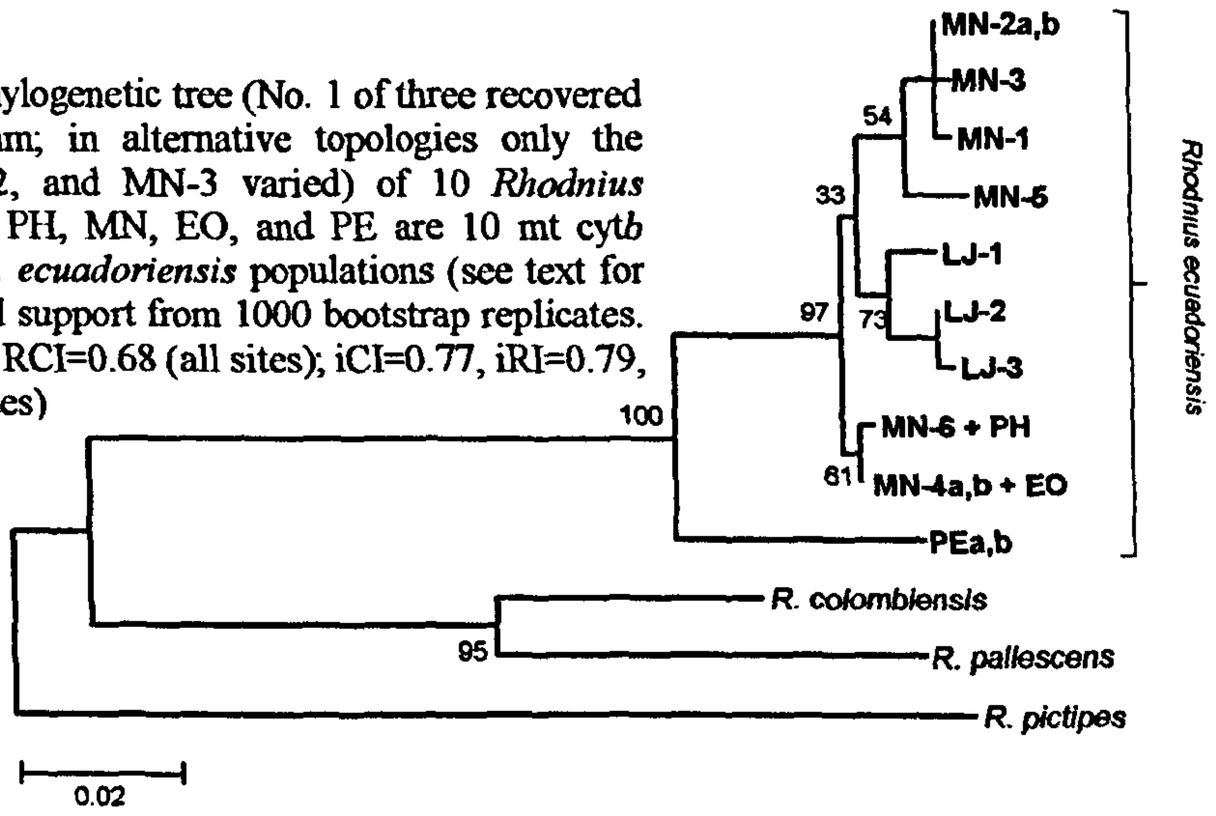


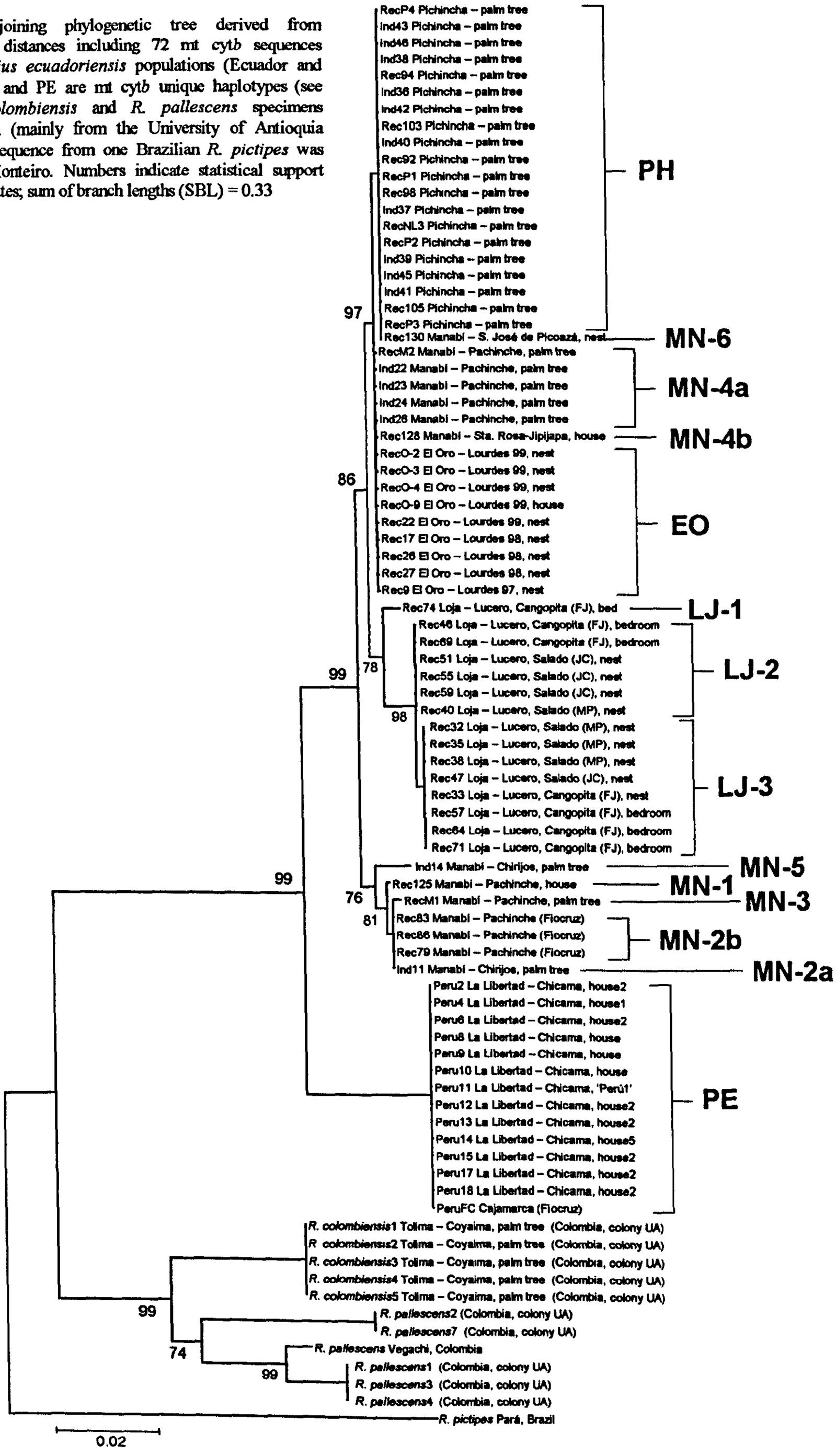
Figure 114. Maximum parsimony phylogenetic tree (No. 1 of three recovered using a branch-and-bound algorithm; in alternative topologies only the relative positions of MN-1, MN-2, and MN-3 varied) of 10 *Rhodnius ecuadoriensis* cyt*b* haplotypes. LJ, PH, MN, EO, and PE are 10 mt cyt*b* haplotypes (663bp) found in five *R. ecuadoriensis* populations (see text for details). Numbers indicate statistical support from 1000 bootstrap replicates. Tree length=216; CI=0.86, RI=0.79, RCI=0.68 (all sites); iCI=0.77, iRI=0.79, iRCI=0.6 (parsimony informative sites)



The relationships between *R. ecuadoriensis* haplotypes were relatively poorly defined in the MP analyses. The separation of the Peruvian lineage was nonetheless confirmed, with the monophyly of the Ecuadorian clade supported by bootstrap values >95%. The three main branches within Ecuador (Loja, Manabí, and a mixed Pichincha-Manabí-El Oro group) were also recognised, but they appeared forming a basal polytomy in the majority-rule bootstrap consensus tree. Support for internal nodes in these branches was low, with a highest value of 73% for the monophyly of Loja haplotypes.

A **complete NJ tree** (based on an uncorrected [*p*] pairwise distance matrix) was produced including seventy-two mitochondrial cyt*b* sequences from various *R. ecuadoriensis* populations and several examples of closely related *Rhodnius* species.

Figure 115. Neighbour-joining phylogenetic tree derived from uncorrected (*p*) pairwise distances including 72 mt cyt*b* sequences (663bp) from five *Rhodnius ecuadoriensis* populations (Ecuador and Peru). LJ, PH, MN, EO, and PE are mt cyt*b* unique haplotypes (see text for details). *R. colombiensis* and *R. pallescens* specimens originated from Colombia (mainly from the University of Antioquia [UA] laboratory), and a sequence from one Brazilian *R. pictipes* was made available by FA Monteiro. Numbers indicate statistical support from 1000 bootstrap replicates; sum of branch lengths (SBL) = 0.33



The topology of one additional NJ, K2p tree (not shown) was identical, and confirmed the results obtained in the simplified analyses conducted with only one representative of each haplotype. Bootstrap support values were in general somewhat higher in the tree based on uncorrected *p* distances. The lowest support value (76%) in the *R. ecuadoriensis* clade corresponds to the branch {[MN-1(MN-2 MN-3)] MN-5}.

The relationships between haplotypes were further explored by direct assessment of absolute nucleotide differences. A parsimonious **haplotype network** was manually constructed showing these relationships (figure 126); 39 intermediate haplotypes were inferred from the dataset that were not observed in the study. Single haplotypes were enclosed inside boxes whose margin thickness increased proportionally to the number of specimens presenting each haplotype: common haplotypes appear in thicker boxes and those found in single specimens in thinner boxes. These boxes were connected by lines showing the shortest mutation pathways between pairs; postulated (missing) haplotypes are shown as small circles along those lines, and the number and base change of each segregating site (see Appendix) are shown between each two haplotypes. Some homoplastic mutations were identified; the concern for phylogenetic analyses was moderated by the relatively high transition:transversion ratio, the apparent absence of saturation, and the low frequency of putative hypervariable sites within *ecuadoriensis*. The tentative connection of the *R. ecuadoriensis* network with the closest outgroup (*R. colombiensis*) places the postulated common ancestor of all *ecuadoriensis* haplotypes in a somewhat intermediate position between MN-4 and PE. The Peruvian haplotype (PE) is connected to the Ecuadorian clade near MN-4. Multiple substitutions were identified throughout the network: parallel mutations at sites 285 (MN-4 branch and LJ-1), 123 (PE and LJ-3), and 291 (Peru and the MN-1 to 5 branch); reversals (sites 462, 447, 309, 210, and 54); multiple hits (sites 543, 417, and 54 [where a reversal also occurred in MN-1]); and one coincidental substitution (site 531, PE and MN-1 to MN-5 branch). Sites 54, 531, 417, and 543 segregated at three bases (comparisons including *colombiensis*). The sequences of the 39 postulated haplotypes inferred from the network were reconstructed by post-order traversal, starting from observed sequences and proceeding towards the internal nodes (Page & Holmes 1998). A MP tree (not shown) was constructed using these haplotypes; it was 113 steps long, with CI=0.93, RI=0.98, RCI=0.9; iCI=0.83, iRI=0.98, and iRCI=0.82.

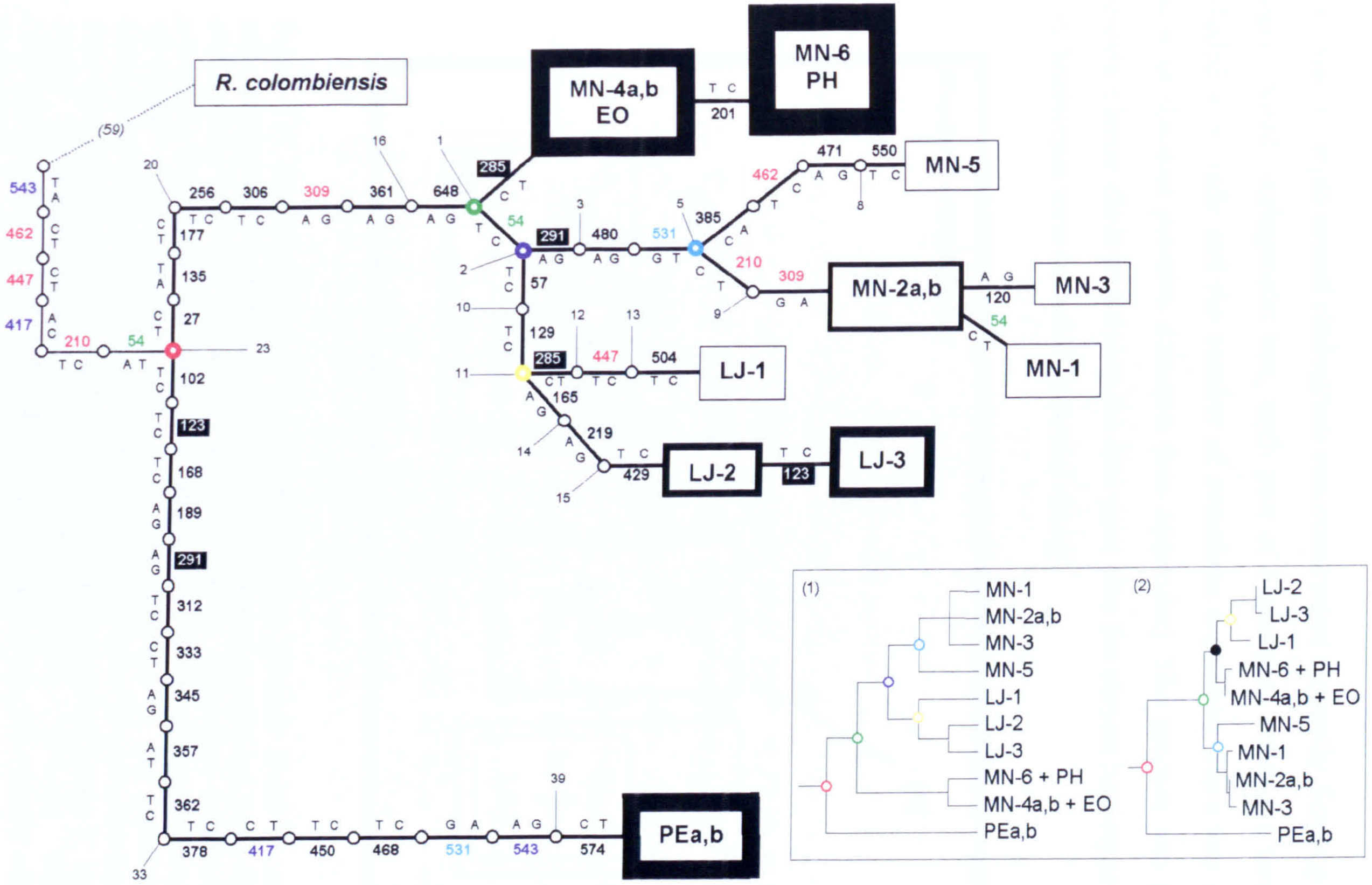


Figure 116. **Maximum parsimony haplotype network**: *Rhodnius ecuadoriensis* mt cyt*b*. Haplotypes were enclosed in boxes (margin thickness proportional to the number of specimens presenting each haplotype) connected by lines showing the shortest mutation pathways between pairs; small circles represent postulated haplotypes (Nos. 1 to 39 [only those within *ecuadoriensis* were numbered; some intermediate numbers are not shown but can be deduced]); numbers beside lines indicate sites in the partial cyt*b* sequence (base change is noted for each mutation). Coloured circles are the postulated common ancestors to various lineages (haplotypes 23, 1, 2, 5, 11). Coloured numbers are sites involved in multiple substitutions (red=reversals; blue=2 hits; green [site 54]=2 hits+reversal; white=parallel hits; light blue [site 531]=coincidental substitution). '*(59)*' refers to 59 synapomorphs (not shown) linking the *ecuadoriensis* network with *R. colombiensis*. Results of parsimony (1) and distance-based (2) phylogenetic analyses are shown for comparison (coloured circles=postulated common ancestors [as in the network]; note that in (2) the common ancestor to the MN1-3/LJ parsimony branch is lacking, hence the black dot instead of a blue circle). Nodes 23 (red) and 1 (green) had bootstrap >95%; node 11 (yellow circle) had ~75%. The network was 113 steps long

Finally, a simple **nested cladogram** was constructed manually. Starting at the tips of the distance-based phylogenetic tree, each pair of haplotypes sharing a terminal node was included in a clade and the number of mutations separating them was scored from the matrix of absolute pairwise distances (see Appendix). The process was then repeated to compute a larger clade containing the first pairs plus the closest haplotype, and so on until all 10 haplotypes were included in nested clades.

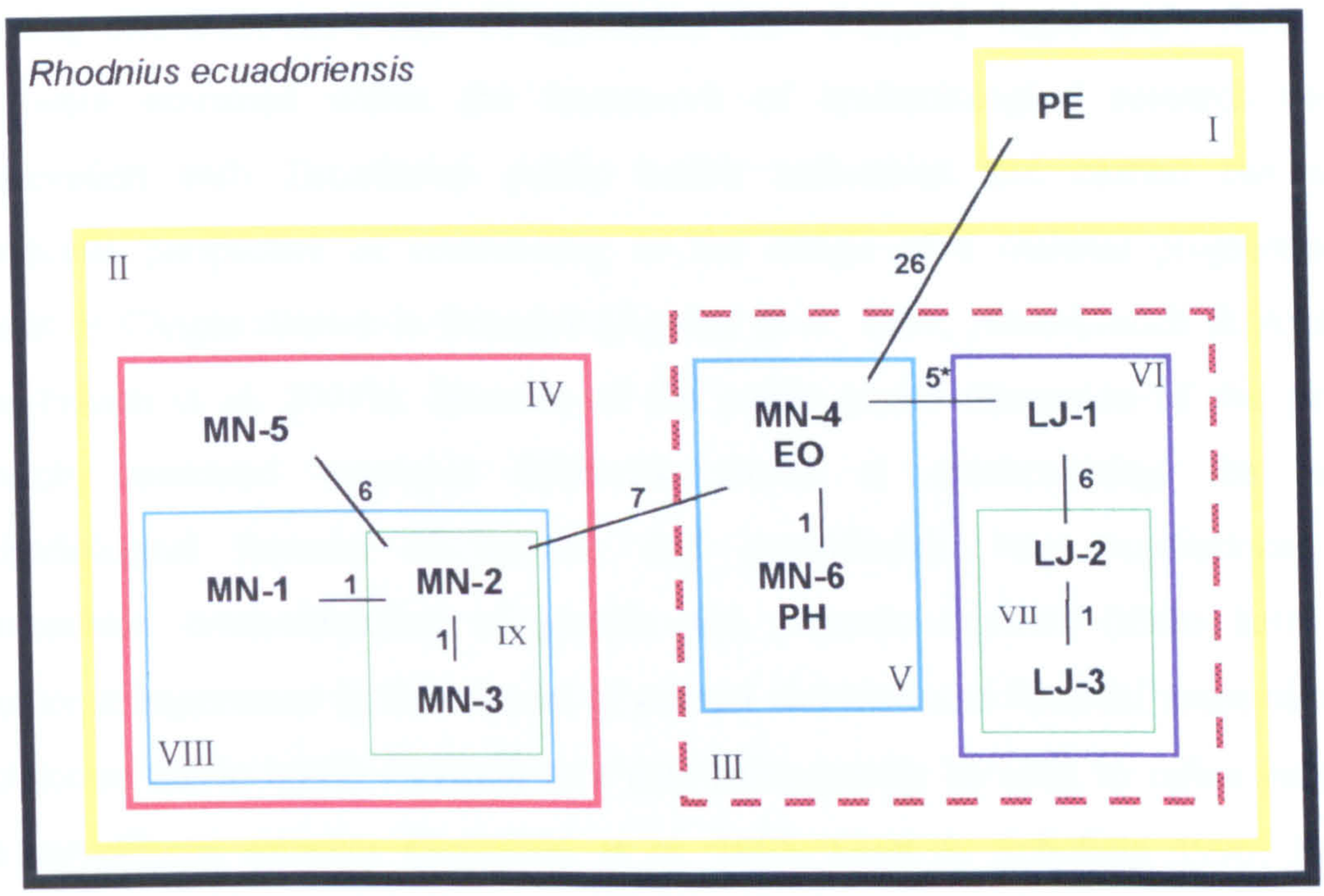


Figure 117. Nested cladogram showing relationships between *Rhodnius ecuadoriensis* mitochondrial cytochrome *b* haplotypes. The graph was constructed using distance-based phylogenetic trees and pairwise absolute nucleotide differences (numbers near lines connecting boxes) between haplotypes. Nine clades were identified: I=Peru; II=Ecuador; III=Manabí (partial)+Pichincha+Oro+Loja (the presence of one putative homoplasy between MN-4/EO and LJ-1 [site 285] increases the number of mutations separating them from 5 to 7 [see figure 126]; clade III is therefore only tentative [hence 5* and the dashed box]); IV=Manabí (partial); V=MN-4/EO+MN-6/PH; VI=Loja; VII=LJ-2+LJ-3; VIII=MN-1+MN-2+MN-3; IX=MN-2+MN-3 (see text for details on individual haplotypes)

6.3.4. DISCUSSION

This study represents, together with research on the phylogeography of Amazonian *Rhodnius* spp. carried out by FA Monteiro and colleagues (Monteiro et al., unpublished), the most comprehensive intraspecific molecular study so far carried out in Triatominae to our best knowledge. Field-collected specimens from five populations representing the entire geographic, ecological, and behavioural spectrum of *R. ecuadoriensis* (Abad-Franch et al. 2001b, Cuba Cuba et al. 2002) were submitted to mtDNA sequence analysis and compared with closely related taxa; overall, nucleotide polymorphisms of a 663bp cyt*b* gene fragment from more than 80 specimens were analysed. Importantly, these molecular data were examined within the framework of epidemiological research designed in collaboration with Ecuadorian public health authorities and carried out under the operational perspective of contributing to the design of a national programme for the control of Chagas disease in Ecuador (Aguilar et al. 1999, Abad-Franch & Aguilar 2000, Abad-Franch et al. 2001b). Because of the public health dimension of this project, our research combined extensive fieldwork aimed at characterising the main eco-epidemiological features of sylvatic and synanthropic bug populations with the simultaneous cross-validation of genetic and phenetic markers (these latter being of paramount importance in the context of current technical and financial constraints affecting Ecuadorian public health services) that may subsequently be used to refine vector control and surveillance schemes (Schofield et al. 1995, Guhl & Schofield 1996, Schofield & Dujardin 1997, Beard & Lyman 1999, Dujardin et al. 2000, Gaunt & Miles 2000, Noireau et al. 2000b, Monteiro et al. 2001).

As already mentioned in the introduction to this Section, mtDNA sequence data proved extremely useful for the examination of relationships among recently diverged taxa and populations of single species (Avise 1994, Esseghir et al. 1994, Simon et al. 1994, Tang et al. 1996, Gimeno et al. 1997, Page & Holmes 1998, Ishikawa et al. 1999, Walton et al. 2000, Litzsenberg & Chapco 2001, Machado et al. 2001, Monteiro et al. 2001, Birungi et al. 2002, Donnelly et al. 2002).

Our results demonstrated the potential utility of mitochondrial protein-coding genes in the investigation of population structuring and relationships in Triatominae, but also pointed out some of their limitations. Previous studies based on mitochondrial markers had

emphasised phylogenetic relationships at the supraspecific level, and are discussed in more detail in Section 7.3. (García & Powell 1998, Lyman et al. 1999, Monteiro et al. 2000, 2001, García et al. 2001). In 1999, Monteiro and coworkers reported the first survey of mt cyt*b* sequence polymorphisms among geographic and ecological populations of a triatomine vector species, *T. infestans* (Monteiro et al. 1999b). A 412bp fragment of the gene from 35 specimens of 10 populations (domestic and sylvatic, from Bolivia, Argentina and Brazil) was analysed. Additionally, morphometric data (CVA of wing measurements), allozyme profiles, cytogenetics, and cross-mating experiments were used to characterise the populations under study. Results revealed that two melanic forms (*T. melanosoma* from Uruguay and sylvatic dark morphs of *T. infestans* from the Bolivian Chaco) whose taxonomic status was considered unclear are probably phenotypic variants of *T. infestans*. Small but consistent differences were found between populations from Bolivia and those from Argentina and Brazil. In Bolivia, sylvatic and domestic Andean bugs had identical haplotypes, whereas the Chaco dark morphs presented three autapomorphs. Dark morphs differ from typical Andean *T. infestans* in several respects, including phenotypic traits (colouration, head and wing morphometry, antennal sensilla patterns) and geographic-ecological features (they occupy preferentially hollow hardwood trees in the Chaco, whereas sylvatic Andean populations occur in rocky habitats at very high altitudes), but allozyme, chromosome, interbreeding, and DNA studies (RAPDs and sequence polymorphisms) supported the idea of a single species (Noireau et al. 1997, 2000a,b, Monteiro et al. 1999b). Together, these results illustrated the suitability of mtDNA sequence analysis for population studies, and showed how the pictures provided by phenotype- and genotype-based assessments, apparently inconsistent, could be brought together by a comprehensive examination of different lines of evidence (Monteiro et al. 1999b, Noireau et al. 2000b). In addressing intraspecific diversity and population relationships in *R. ecuadoriensis* (with somewhat comparable patterns of variation), we followed a broadly similar rationale, emphasising the idea that combined consideration of data generated using various approaches and methods will likely produce better results.

More recently, Monteiro et al. (2000) used mtDNA sequence diversity in a survey of relationships among several species of Rhodniini. In this phylogenetic study, several geographic populations of *R. prolixus* (domestic from Venezuela, Colombia, Honduras, and Guatemala, and sylvatic from Brazil) were found to have virtually identical mt cyt*b*

haplotypes (414bp), suggesting recent dispersal of synanthropic forms mediated by people, a conclusion also supported by metric and RAPD analyses (Dujardin et al. 1998a). In addition, some putative *R. robustus* presented *prolixus* haplotypes, and the reverse case occurred with one Brazilian population labelled as *prolixus*. However, a neat overall separation between closely related taxa within the *prolixus* group (including the almost sibling *prolixus*, *robustus*, *nasutus*, and *neglectus*) was achieved, further showing that mtDNA sequence data can be used to address taxonomic problems that could not be fully resolved neither by conventional techniques (morphology, morphometrics, cross-mating experiments or isoenzymes) nor by single strand conformational polymorphisms (SSCP) of the mt 16S rRNA gene (Dujardin et al. 1991, Harry et al. 1992a, Harry 1993, Solano et al. 1993, Barrett 1996, Stothard et al. 1998b, Lyman et al. 1999, Schofield & Dujardin 1999). In this study, Monteiro et al. (2000) also analysed representatives of two distinct populations of *R. ecuadoriensis*. They were laboratory colony specimens reported as originally collected in Ecuador and Colombia, respectively, and mt cyt*b* polymorphisms were detected that could discriminate between them. These *ecuadoriensis* populations were firmly nested within a clade where *R. pallescens* appeared as a sister, basal taxon to a branch including *colombiensis* and *ecuadoriensis* (Monteiro et al. 2000). Later discussions and re-examination of the sequence dataset with FA Monteiro revealed however that *ecuadoriensis* and *colombiensis* specimens used in this study could have been mislabelled; in fact, the "Colombian" population of *ecuadoriensis* is probably derived from field collections carried out in Manabí in the early 1990s (sylvatic bugs from palm trees); the other *ecuadoriensis* specimen probably originated from domestic populations sampled in the mid 1990s from southern Ecuador (El Oro). Once these problems were solved, reanalysis of the cyt*b* dataset (two Ecuadorian and one Peruvian specimens) revealed apparently significant differences in nucleotide composition between Ecuadorian and Peruvian *ecuadoriensis* haplotypes (and smaller differences between sylvatic and domestic populations in Ecuador), suggesting that the gene could constitute a suitable, polymorphic molecular marker for intra-specific studies on *R. ecuadoriensis*.

6.3.4.1. Haplotype diversity in *Rhodnius ecuadoriensis*

Ten unique haplotypes were recovered from DNA extracts of 72 specimens from five populations (maximum geographic distance ~900km); a total number of 42 variable sites (6.3%) was scored from those haplotypes. Using 412bp of the cyt*b* gene, Monteiro et al. (1999b) isolated four unique haplotypes from 10 populations (35 bugs) of *T. infestans/T. melanosoma* (geographic distances often around 1000km, and up to >2000km); the percentage of variable sites was 1.94% (8 out of 412bp), and no intra-population variability was detected. Comparisons among *R. prolixus* populations from Honduras, Guatemala, Venezuela, and Colombia (36 specimens, 663bp of the mt cyt*b* gene) revealed just three haplotypes separated from each other by single point mutations (only 0.3% of sites were variable), but variation was much higher among populations identified as *R. robustus*; here, 17 unique haplotypes were found in 43 sylvatic bugs from various areas of the Amazon-Orinoco drainage basins, and 11.5% of sites were polymorphic (76/663) (Monteiro et al., unpublished). Finally, four cyt*b* haplotypes were found in 9 *R. pallescens* specimens from Colombia and Nicaragua (see Section 7.3.); 50 sites out of 663 were variable (7.54%). Comparing these results, it would appear that haplotype diversity and the proportion of variable sites per sequence length unit are significantly reduced in the most synanthropic triatomine species (*T. infestans* throughout the Southern Cone and *R. prolixus* in northern South America and Central America), whereas an entirely sylvatic entity (*R. robustus*) is highly variable; *R. pallescens* and *ecuadoriensis* (which retain sylvatic habitats but can colonise human environments) seem to present intermediate levels of variability. The low levels of polymorphism observed in *T. infestans* and *R. prolixus* may be attributed to recent, man-mediated dispersal of genetically restricted (mainly via founder effects and isolation from the original gene pool) domestic populations, combined with sampling bias towards synanthropic bugs even across wide geographic areas (Dujardin et al. 1998a, Schofield et al. 1999); on the other hand, phylogenetic analysis suggested *R. robustus*, as defined by its external anatomy, may encompass more than a single taxon (Monteiro et al., unpublished). Thus, assuming substitution rate homogeneity across the taxa under consideration, the observed genetic variability in *R. pallescens* and *R. ecuadoriensis* might represent more accurate estimates of interpopulation diversity within single triatomine species. However, as will be discussed later, the proportion of variable sites in *R. ecuadoriensis* haplotypes decreased to only 3.17% (21 out of 663) when the single,

strongly divergent Peruvian haplotype was excluded from the analysis. This latter value is similar to the 3.58% reported for the Old World *Leishmania* vector *Phlebotomus papatasi* (16 haplotypes found in 27 flies from 12 countries; the sequence contained 283bp of the mt cyt*b* gene plus 158bp from other segments, totalling 441bp) (Esseghir et al. 1997); Ishikawa et al. (1999) isolated 28 unique cyt*b* haplotypes (294bp) from the New World phlebotomine, *Lutzomyia whitmani* (31 specimens from 14 sites across Brazil), and found 26 variable sites (8.8%).

6.3.4.2. Distribution of haplotypes: geographic correspondences

The distribution of cyt*b* haplotypes found in *R. ecuadoriensis* only partially fitted expectations of strong population structuring based on geographic distance between collection sites and phenotypic heterogeneity revealed by morphological and metric comparisons (see Sections 6.1. and 6.2. and figure 109). Two populations with extremely distinct phenotypes, those from Pichincha (sylvatic) and El Oro (synanthropic), presented unique haplotypes (PH and EO, respectively) that diverged by a single point mutation (a T/C silent transition at site 201, a third codon position); some 400km separate both localities. Furthermore, haplotype PH was identical to MN-6, found in a single bug collected in San José de Picoazá (Manabí, ~200km from Pichincha) from a peridomestic chicken coop, and EO was in turn identical to another Manabí haplotype (MN-4; ~375 km from El Oro), found in bugs collected from palm trees in Pachinche Adentro and in a single female captured inside a house in Santa Rosa (Jipijapa). The highest levels of haplotype diversity were scored in Manabí. These fundamentally sylvatic bugs were collected from four rural localities near Portoviejo (the capital town of Manabí), and six haplotypes were isolated from 14 specimens. Intra-population diversity was high: nearly 2% of sites (13/663) were variable in the 6-haplotype alignment, with pairwise *p* distances ranging from 0.0015 to 0.015 (between MN-5 and MN-6, separated by 10 point mutations) and overall nucleotide diversity close to 0.01 (π=0.00895). No clear trend towards geographic population structuring was detected in Manabí, despite sampling of various sites (located on average about 30km from each other [~15 to 50km], but often separated by large areas of dry tropical forest with no palms) and ecotopes (with bugs collected directly from palms and found invading human habitats). Mean pairwise *p* distances between localities and other details about Manabí haplotypes are summarised in the following figure.

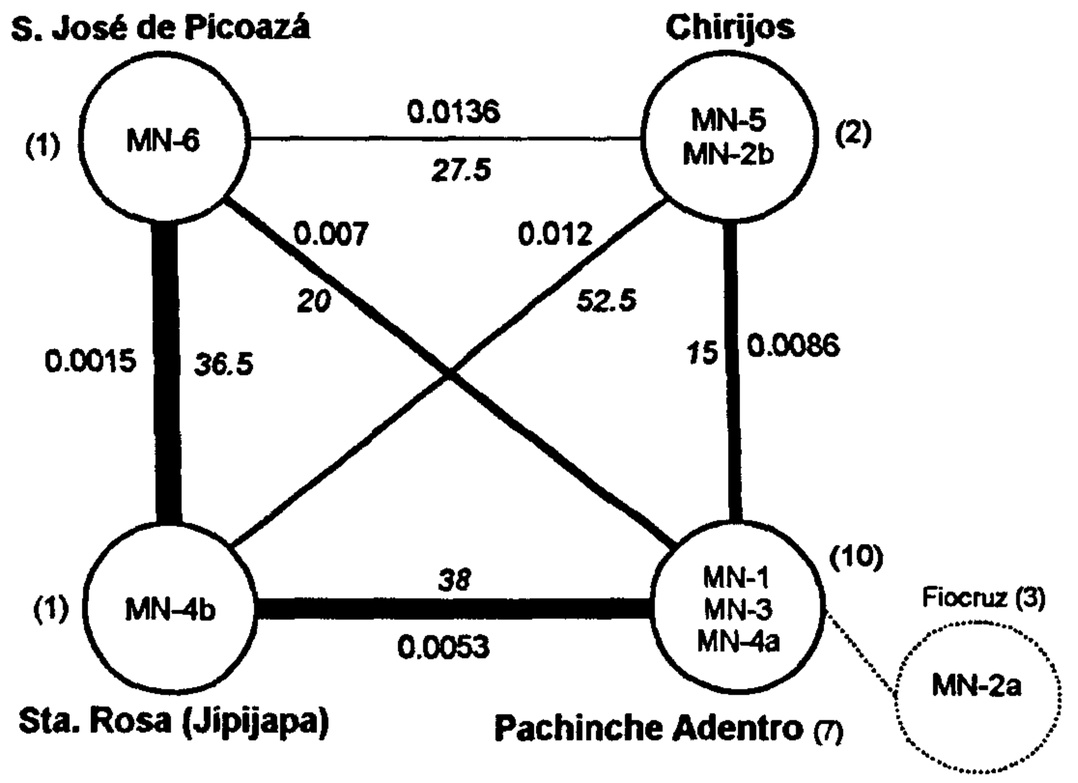


Figure 118. Mitochondrial cytb haplotypes in *Rhodnius ecuadoriensis* populations from Manabí. Each circle represents one locality (haplotype codes inside the circles). Numbers beside lines are mean pairwise *p* distances (thicker lines represent lower genetic distances) and approximate geographic distances (in km, italicised); in all cases, dry forest areas without palm trees separated the localities. Linear regression of genetic vs. geographic distances produced a coefficient of determination of 0.23

Similarly, no micro-geographic structuring was detected in the populations from Loja; here, three unique haplotypes were found in 15 specimens collected from two different areas of the locality (three houses). All three haplotypes were isolated from bugs collected in the same bedroom. These haplotypes were separated from MN-4/EO (the closest outside Loja) by seven point mutations; mean *p* distances between haplotypes from Loja and other populations were: Manabí=0.015 (geographic distance ~575km), El Oro=0.01 (~200km), Pichincha=0.01 (~580km), and Peru=0.046 (~500km). The uniqueness of the Loja haplotypes, and their evident monophyly, suggest that this population probably has a history of isolation from the others (even if the degree of divergence from other Ecuadorian populations falls within the range of haplotype diversity found in Manabí).

The strongly divergent Peruvian population was also the most geographically isolated. Not only was it separated from Ecuador by ~500km, but that distance is mainly comprised of very arid land. Mean genetic distance with the pooled Ecuadorian haplotypes was 0.0424 (K2p=0.044); using the same gene fragment analysed here, Monteiro et al. (unpublished) found smaller distances (3.3% sequence divergence) between sympatric populations of *R. prolixus* and *R. robustus* in Venezuela. Other comparisons between closely related species indicate overall cyt*b* sequence divergences ranging from 6% (*R. pallescens-colombiensis*) and 9% (*R. prolixus-nasutus*) to about 13% (*R. colombiensis-ecuadoriensis* or *T. infestans-T. brasiliensis*) (Monteiro et al. 1999b; see also Section 7.3.).

At the subspecific level, we have recorded differences (K2p) from 0.017 to 0.066 between populations of *R. pallescens*. JC Avise (2000) reported levels of intraspecific mtDNA sequence divergence reaching >2% (cited in Omland et al. 2000).

Overall, the pattern of haplotype distribution in *R. ecuadoriensis* seems to encompass two main units: (i) a broad area of shallow structuring and haplotype sharing corresponding to coastal western Ecuador and the humid foothills of the Andes (Manabí-Pichincha-El Oro), and (ii) restricted clusters of isolated populations in the drier, interior Andean valleys of southern Ecuador (Loja) and northern Peru (Chicama). A plot of approximate pairwise geographic distances between collection sites versus pairwise genetic (uncorrected) distances shows a positive correlation; however, geographic distances from Loja to both Chicama and Manabí (or Pichincha) are very similar (~500-600km), whereas genetic distances are remarkably different (>4.5% and ~1%, respectively), suggesting that other circumstances rather than simply linear distance are involved in the apparent isolation of the Peruvian population. The extremely dry environment of northwestern coastal Peru might help explain the finding, including the view that the Sechura desert might represent a true biogeographic barrier to the dispersal of the species (Cox & Moore 2000).

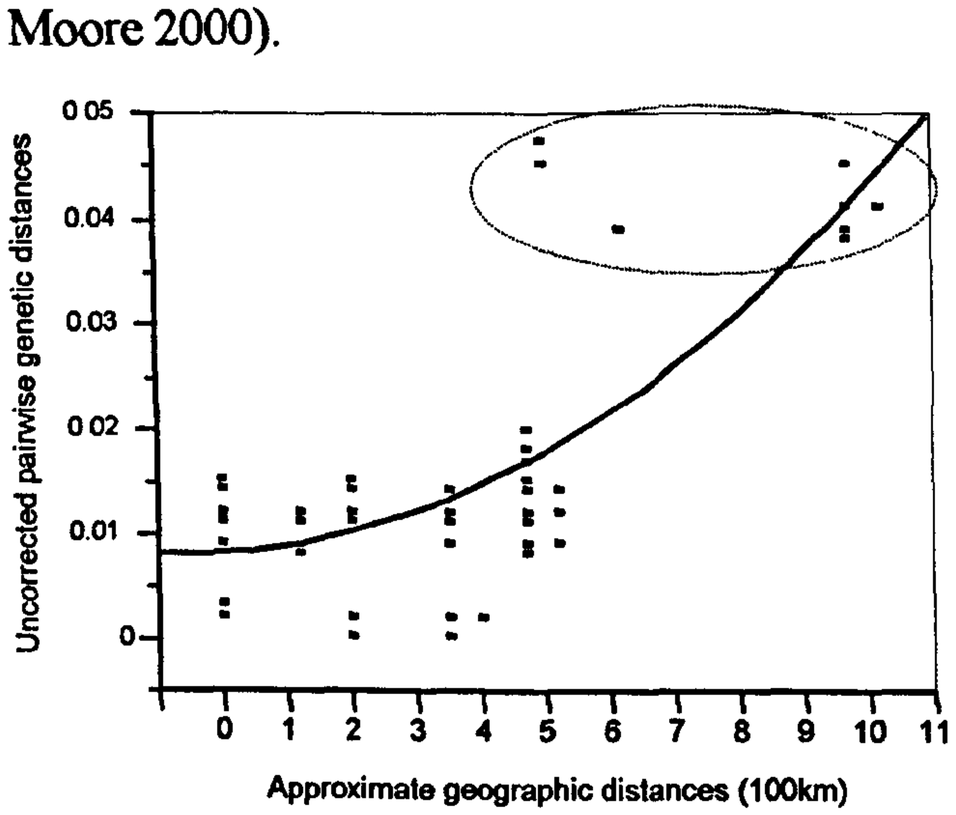


Figure 119. Genetic and geographic pairwise distances in *Rhodnius ecuadoriensis* populations; dots with genetic distance values ~4% correspond to the comparisons including Peruvian haplotypes; those involving Loja vs. Manabí or Pichincha are aligned over the (approx.) 500km mark, but have genetic distance values up to approx. 2%. The line is a second-order polynomial fit (R^2=0.58); pairwise comparisons involving Peruvian haplotypes are enclosed in an ellipse

The observed patterns of distribution and relationships of mt cyt*b* haplotypes in *R. ecuadoriensis* are summarised in the following figure; they were overlaid on a map showing the potential distribution of *Ph. aequatorialis*, the primary natural habitat of the

species; light green dots (barely visible) correspond to records of *R. ecuadoriensis* (see Section 3.2.). The approximate sites of capture of bugs presenting each haplotype are indicated with different colours for each population: Manabí=orange, Pichincha=dark red, El Oro=green, and Loja=blue. The red outlined area represents an hypothetical approximation to the distribution of sylvatic populations derived from known records and biogeographical data on the vectors and the palms they inhabit; although information regarding deforestation has been incorporated, the actual extent of habitat fragmentation is suspected to be in excess of what the map suggests (see Dodson & Gentry 1991, Borchsenius et al. 1998, and Section 3.2.3.1.). Possible routes of dispersal connecting the putative 'core' Ecuadorian populations (corresponding to the area of shallow genetic structuring mentioned above) with apparently isolated clusters (Loja and Peru) are suggested in the form of arrows extending from the red outline (with uncertainties regarding the connection with Peru indicated by a question mark). Similarly, the possibility that sylvatic forms, perhaps intermediate between *ecuadoriensis* and the Colombian species of the Pacific lineage (*pallescens* and *colombiensis*), occur in Esmeraldas and the Colombian Chocó is suggested (but the lack of data indicated by further question marks). The main differences and similarities between haplotypes found in different geographic areas are also indicated. The postulated routes of connection between MN-4 (Manabí) and EO (El Oro), and between MN-6 (Manabí) and PH (Pichincha) [i.e., identical pairs of haplotypes], are represented by narrow orange extensions departing from Manabí (as the likely centre of dispersal, see below) and reaching the other locations. The presence of a high mountain range separating El Oro and Loja is emphasised by a black boundary line between them. The complex blue outline in Loja reflects the fact that conditions potentially favourable for *Ph. aequatorialis* only exist in the lower parts of the dry inter-Andean valleys of the province (even if the palms themselves seem to be absent at present); the short blue arrow directed towards Peru indicates that domestic populations of *R. ecuadoriensis* have probably dispersed locally along the Chira river basin (see Section 6.2.3.). The true location of the Peruvian population from Chicama is not represented (hence the broken lines); the area of origin of those bugs is about 500km south of the Ecuadorian border (Department of La Libertad).

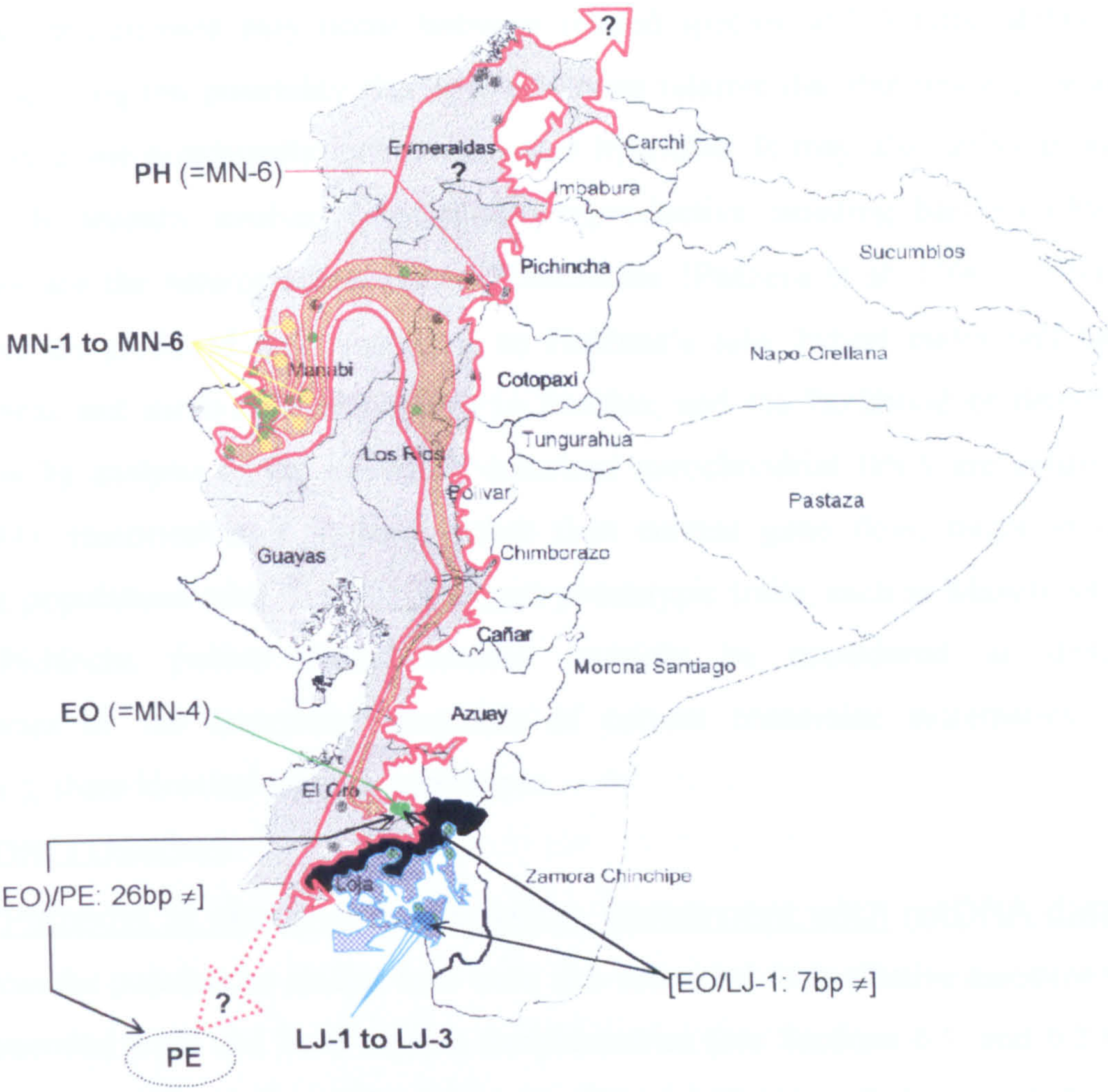


Figure 120. Geographic distribution and relationships of 10 mt cyt*b* haplotypes isolated from five populations of *Rhodnius ecuadoriensis*. Haplotype codes: MN=Manabí; PH=Pichincha; EO=El Oro; LJ=Loja; PE=Peru. bp=base pairs. The grey area of the map represents the (maximal) potential distribution of *Phytelephas aequatorialis* palms (provided by F Skov). See text and Sections 3.2. and 4.1. for further details

6.3.4.3. Patterns of similarity: gene flow and possible introgression

The pattern of haplotype sharing among populations suggested gene flow might exist (or have existed recently) between Manabí and El Oro (one haplotype [MN-4] was shared by two Manabí subgroups [MN4-a and b] and the population from El Oro [EO]), and between Manabí and Pichincha (MN-6, present in a single specimen from Manabí, and PH were identical). When comparing two populations with no shared haplotypes (such as Loja or Peru vs. all others), fixation indices such as *F*st will be 1 (indicating complete absence of gene flow) regardless of the actual kinship between the groups, hampering the accurate estimation of gene flow. On the other hand, shared mt haplotypes may be the result of historical introgression between otherwise divergent

phylogroups. Introgression may occur between related species with limited ability to interbreed, including the possibility that species whose relative distributions are usually allopatric may come accidentally into contact and hybridise. It may also affect groups that have only recently evolved (incomplete) reproductive isolating barriers (Avise 1994). Males are the heterogametic sex in Triatominae (Panzera et al. 1996, 1999); it can therefore be predicted that, according to Haldane's rule, hybrid males will have reduced fitness and survivorship compared to females, and the likelihood of detecting introgression by analysis of the maternally-inherited mitochondrial DNA are enhanced (Avise 1994). Historical introgression, rather than current gene flow, might in fact explain that populations with strongly divergent phenotypic traits, such as Manabí vs. El Oro or Pichincha (which would almost certainly be considered as distinct morphospecies by the customary standards of current triatomine systematics; see Section 6.1.), share identical mt cyt*b* haplotypes.

6.3.5. CONCLUSIONS

6.3.5.1. Patterns of phenotypic diversity: agreement with mtDNA data

Bugs from the populations studied here were also submitted to qualitative assessment of general anatomical traits and head capsule morphometrics (see Sections 6.1. and 6.2.). In general, striking interpopulation differences were detected; the overall degree of phenetic variation seemed above customary supraspecific rank for the tribe. Examples of triatomine species separated on the grounds of smaller phenetic differences would include *R. stali-R. pictipes* (Lent et al. 1993), the problematic *R. prolixus-robustus-nasutus-neglectus* group (Lent & Wygodzinsky 1979), *T. sordida-guasayana-patagonica* (Gorla et al. 1993), or some of the species of the *T. oliveirai* complex (Carcavallo et al. 2000). The principal patterns of phenotypic variation in *R. ecuadoriensis* involved (i) the discovery of the large, melanic, and strictly sylvatic Pichincha forms; (ii) a manifest size reduction of domestic-peridomestic populations; and (iii) a tendency to separation of the Peruvian population revealed by (size-free) analysis of form. The Manabí phenotypes presented intermediate characteristics (see Sections 6.1. and 6.2.). These findings were suggestive of a clinal series (with Pichincha phenotypes assumed plesiomorphic), and led to the speculation (also because of biogeographic end ecological considerations) that domestic and sylvatic populations (especially Pichincha vs. Loja-El Oro-Peru) could be isolated from each other and evolving independently, in part because of anthropogenic habitat destruction-

fragmentation and dispersal of bugs beyond their natural range (assumed to correspond to that of the palm trees they live in). Thus, we anticipated that molecular analyses might reveal (i) higher levels of polymorphism in Pichincha and Manabí, likely with shallow population structuring; and (ii) relative intrapopulation homogeneity of southern, synanthropic populations, including those from Peru, with perhaps a few, similar haplotypes fixed in each group (as a reflection of the effects of strict synanthropism). Similar patterns had been previously reported for other triatomine species known to have dispersed with their human hosts in recent historical time (Schofield 1994, Schofield et al. 1999). One exception was the significant differentiation of the Peruvian population revealed by size-free metric analyses, less influenced by environmental factors (which are known to produce mainly size-related variation) (Dujardin et al. 1998a, 1999a,b).

The picture that emerged from the analysis of cyt*b* haplotype polymorphisms agreed only partially with these expectations. Sylvatic populations were indeed the most variable, but this only applied to the bugs collected in Manabí. A single haplotype was isolated from 20 specimens from Pichincha, and it was also found in one bug from Manabí. Synanthropic bugs from El Oro had a single haplotype that, contrary to expectation as well, was also recovered from two different localities in Manabí. On the other hand, the genetic profile of the Loja population (unique, moderately divergent haplotypes) was more in accordance with our predictions; it suggested isolation from the sylvatic pool, lending support to the interpretations of ecological, biogeographic, and morphometric results. Finally, the divergence of the Peruvian population, suspected from metric analyses, was surprising in its magnitude, challenging the idea of recent, man-mediated passive dispersal.

6.3.5.2. Combined consideration of diverse lines of evidence

·Manabí. The populations from Manabí retain their sylvatic habitats in palm trees near dwellings; this resulted in the impossibility of detecting any consistent phenetic differences between bugs collected directly from palms and adult specimens found inside/around houses. In contrast, six cyt*b* haplotypes were found in 14 specimens (4 localities within the same area; 3 haplotypes in 7 field-collected specimens from Pachinche Adentro). The high degree of genetic diversity suggested that the coastal forests of Manabí (both on the humid hills and in the seasonally dry, narrow valleys among those hills) might be regarded as the

---

[+]Other *Phytelephas* species (closely related to *aequatorialis*) occur in the Pacific coast of Colombia: *Ph. tumacana* (endemic to the Department of Nariño) and *Ph. seamannii* (northwest Colombia [Antioquia, Chocó] and extending into Central America) (F Borchsenius, pers. comm.)

centre of dispersal of *R. ecuadoriensis* populations. The complex biogeographic structure of this area may help explain the high genetic heterogeneity of these bugs (Dodson & Gentry 1991). It is possible that some populations evolved different haplotypes while temporarily isolated in the 'islands' of humid forest represented by the hills and narrow valleys (separated from each other by a 'sea' of arid environments). Shared haplotypes detected in bugs from different localities (resulting in what appears to be a shallow micro-geographic structuring of populations) would be an indication of good dispersal capacity of some of the bugs, as suggested by findings of adult specimens in otherwise non-infested communities separated from the nearest humid forests by several km. Passive transport of nymphs with avian hosts could also link humid forest 'islands' in Manabí.

The overall geographic range of these sylvatic populations is probably related to that of its primary ecotope, *Ph. aequatorialis*. These palms are endemic to western Ecuador; their biogeographic boundaries are represented by the pluvial forests of the Colombian Chocó[+] in the north and by the very arid climate of northern Peru (with no native palms) in the south. In the southernmost extreme, these palms only occur on the plains and low foothills near the coastline in Loja and El Oro, but not in the interior, drier inter-Andean valleys. Genetic similarities (in the form of a shared cyt*b* haplotype) with synanthropic bugs from El Oro may be related to the existence (current or recent) of sylvatic populations of *R. ecuadoriensis* extending from Manabí into coastal areas of the southern provinces. However, marked phenetic differentiation suggests that contact between synanthropic and sylvatic bugs has not occurred in the south for many generations (see below).

·Pichincha. Sylvatic forms of *R. ecuadoriensis* were discovered in association with *Ph. aequatorialis* in the humid forests of the Andean foothills in northern-central Ecuador (province of Pichincha). Despite marked and consistent phenetic differences (morphological, chromatic and morphometric), these bugs appear to be genetically very similar to some of those from Manabí (and the population from El Oro), with cyt*b* haplotypes differing by only one nucleotide out of 663. One bug collected in a chicken coop in Manabí presented a cyt*b* haplotype identical to that found in the 20 Pichincha forms analysed.

Phenotypic plasticity related to the adaptation to specific microhabitats (known to be particularly rapid and intense in triatomines [Dujardin et al. 1999b], although other striking examples have been described [e.g. salamanders, see Parra-Olea & Wake 2001]), may help

explain these results. First, and on the grounds of the discussion presented above, we may suspect that the striking anatomical features (mainly size and colour) separating Pichincha forms from other *ecuadoriensis* are likely to be derived (and not plesiomorphs as initially thought – except for the observation of colour similarities between typical *ecuadoriensis* and *pallescens-colombiensis-pictipes*; see Section 6.1.). In this sense, explicitly removing size from metric analyses showed that most of the (powerful) discrimination achieved by previous CVAs was due to the influence of size, and that Pichincha bugs were hard to tell apart from other Ecuadorian populations because of their broadly comparable head forms. These considerations suggested that the Pichincha phenotypes could represent apomorphic states of a genetically similar, Manabí-like original population. A simple ecological observation could in fact account for the remarkable differences in both colour and overall size; because of climatic differences between the Andean foothills of Pichincha (colder and more humid) and the low coastal hills of Manabí (warmer and drier), the overall colour of the substrate where bugs live (i.e., mainly among epiphytes and dead organic matter on palm tree stems and crowns) is conspicuously different (dark brown-reddish in the former and straw-like yellowish in the latter). Natural populations of *R. ecuadoriensis* from each area seem to have evolved their divergent dyes to improve camouflage against the substrate (surely a trait under strong selective pressure). Regarding size, the finding of larger bugs in colder areas is simply in agreement with Bergmann's rule predictions (see Section 6.1.). It remains however unclear how long would it take for those chromatic and body size changes to become fixed, even in an isolated population under strong selection.

A further problem was encountered when the analysis of the mitochondrial gene fragment studied revealed a single haplotype for 20 Pichincha specimens. Several studies have shown that sylvatic populations of other triatomine species present higher genetic variability than their domestic conspecifics (e.g., Dujardin et al. 1998a,c, Borges et al. 1999, Schofield et al. 1999). The finding of a very homogeneous population may indicate that it recently went through a genetic bottleneck, perhaps related to the combination of strong positive selection and serial founder effects. A discrete catastrophic event with high mortality related to insecticide spraying is unlikely in the study area, although the use of pesticides in the small plantations were the palms were located was not investigated. On the other hand, the result could be related to sampling bias if the analysed bugs came from

a very small area (or even the same palm tree) and could therefore be suspected of having close kinship (i.e., being parents and offspring). This artifact cannot be ruled out completely because the exact palm from which each specimen was collected was not recorded on its individual label: all bugs collected in this locality were placed in a single container upon arrival to the insectary in Quito; those used in the analyses presented here were randomly withdrawn weeks later (~2 to 7 months). This introduces the possibility that we could have inadvertently used F1 offspring from perhaps a single female. However, this could only be true if the life cycle of these sylvatic forms were extremely shorter than that of their synanthropic conspecifics; we recorded egg-to-adult development times of ~14 months on average (bugs from Loja under favourable laboratory conditions). Thus, the possibility that adult specimens used in this study were born in the laboratory can be ruled out with confidence. Sampling methods and results do not support the plausibility of sampling bias either: 56 palms were searched (in two separate field trips, covering an area of approximately 5x2 km), of which 14 (25%) were infested; the average number of bugs found per infested palm was 10.2 (ranging from 1 to 22), making a total number of 143 (the majority of which were nymphs). Thus, even if most of the bugs were collected from a relatively small plot within the study area, the likelihood of 20 randomly selected bugs being close relatives seems very small (yet it remains a possibility).

Additional ecological considerations may help interpret these results. The area were fieldwork was carried out corresponds to the altitudinal biogeographic limit of *Ph. aequatorialis* (hence probably also to that of sylvatic populations of *R. ecuadoriensis*; see figures 11 and 14). Climate conditions in these boundary zones probably correspond to some of the extremes (lowest temperature, highest relative humidity values) both palms and bugs can endure. Thus, it is conceivable that under such conditions only a restricted subset of the bug population can reach the reproductive stage and pass genes to the next generation; this would be particularly noticeable if sampling took place short after an episode of high mortality (related, for instance, to bad weather conditions [for instance, El Niño southern oscillation 1997-1998 was the strongest in the century; see MSP 1999]), resulting in a strong selective sweep that removed a substantial proportion of previously extant polymorphisms. It has been recognised that the homogenising role of positive selection may be enhanced in peripheral subpopulations, which tend to be small and prone to suffer bottlenecks (Page & Holmes 1998). Peripheral isolation might in this case result

from extensive deforestation in the lowlands connecting the Andean foothills of Pichincha and the coastal area of Manabí where the most variable sylvatic populations of *R. ecuadoriensis* occur (Dodson & Gentry1991). This could have amplified the effects of selective pressure and contributed to low genetic variability and important phenetic differentiation in the Pichincha population, and might also be responsible for another 'domestic-like' trait of these bugs, namely the absence of significant sexual dimorphism (see Section 6.2.3.3.; Dujardin et al. 1999c). A further observation is also suggestive of high mortality among Pichincha populations. The nymphs:adults index we found in our collections was very low (0.02); we initially thought that adult bugs could escape from the adhesive tape of the traps, but a lower number of them in the natural colonies (compared to that of immature individuals) could also be a reason (Abad-Franch et al. 2000). Previous studies (conducted by palm dissection, and therefore probably biased towards larger bugs) reported an average index of 0.7, ranging from 0 to 2.6 (cf. Pizarro & Romaña 1998). In our surveys in the Amazon, *R. pictipes* and *R. robustus* adults were captured on many occasions in live-bait traps, suggesting that a poor performance of the tape could be ruled out; this would imply that mortality rates in the Pichincha population are very high, with only a few bugs completing their development to adults.

The fact that one specimen from Manabí shared its cyt*b* haplotype (MN-6) with the bugs from Pichincha would be explained if the substitution at site 201 (a T↔C transition that separates MN-4/EO from MN-6/PH) were homoplasious; alternatively, historical introgression of Manabí mtDNA into Pichincha bugs could be suspected (see below). Our attempts to find sylvatic *R. ecuadoriensis* (or records of their presence) in the lowlands of Pichincha yielded negative results, adding to the idea of peripheral isolation of the bugs from Pichincha; exceptions were the findings of a single nymph in an area of preserved forest and that of two adult bugs in dwellings of one locality near the border Manabí-Pichincha.

·El Oro. One of the synanthropic, southern Ecuadorian populations (El Oro) presented a single cyt*b* haplotype that was shared by some Manabí specimens, suggesting at first that sylvatic and domestic bugs might be in contact in the zone. The area were these domestic bugs were collected is in fact close to the southernmost known extreme of the natural distribution of *Ph. aequatorialis* palms, making it conceivable that sylvatic bug populations exist there (or did recently so) in association with forest remnants. However,

morphometric changes separating sylvatic and domestic specimens, and the fact that only one haplotype was detected in domestic bugs from various houses (separated by several km in some cases) and various collections (in three consecutive years), favoured the hypothesis of some degree of isolation. In fact, no palms were observed in the locality were fieldwork was carried out in El Oro; the nearest zone were *Phytelephas* palm trees occur is ~30km southwest, but rapid sampling of 10 *Ph. aequatorialis* palms in that area (using Noireau traps) yielded negative results. Another possibility was that the bugs from El Oro had retained an ancestral (Manabí) haplotype that became fixed by chance through a founder effect; the parsimonious haplotype network in figure 116 shows not only that EO/MN-4 is the closest haplotype to the postulated nearest common ancestor to all Ecuadorian populations, but also that the single point mutation separating both (site 285, a C↔T silent transition) has occurred at least twice (it is also present in LJ-1). Perhaps, therefore, the identity of EO and MN-4 is due to a combination of a relative recent (and fairly strong, as suggested by the absence of variability) founder effect and homoplasy. In the absence of extreme environmental conditions (invoked when considering the absence of haplotype diversity in Pichincha, but unlikely to occur in protected synanthropic habitats), perhaps repeated episodes of incomplete but high mortality (related for instance to insecticide spraying, a practice reported by roughly half of the families in El Oro) could help explain the finding of a single haplotype.

Observations of strong phenetic divergence in the presence of mtDNA similarities (identity in the case of the haplotype pairs MN-6/PH and MN-4/EO) can also be attributed to genetic introgression via hybridisation (Avise 1994). In this context, the remarkably diverse phenetic (and ecological-geographic) forms of *R. ecuadoriensis* would be regarded as distinct phylogroups (Pichincha, Manabí, and the synanthropic southern forms) with divergent evolutionary histories. On the other hand, as already discussed, the apparently allopatric distribution of these populations could be the result of historical processes (habitat destruction and fragmentation, dispersal of domestic bugs with migrant people) affecting the geographic range of both palms and bugs. It is at least conceivable that, in the recent past, *Phytelephas* palms extended through most of their potential range (figure 11), including southern Ecuador and extensively connecting the lowlands of Manabí with the Andean foothills of Pichincha. In that context, divergent phylogroups of *R. ecuadoriensis* could have interbred, giving rise to the observed pattern of haplotype sharing (Manabí-El

Oro and Manabí-Pichincha). In fact, specimens from Loja and Peru (virtually and effectively out of the range of *Ph. aequatorialis*, respectively) presented unique haplotypes, suggesting a longer history of reciprocal isolation (see below).

In the case of the Pichincha forms (the most strikingly distinct to the naked eye, and therefore the easiest to think of as a morphospecies), sympatric Manabí-like and Pichincha-like bugs were found (in the locality known as 'Kilómetro 24') that were morphometrically similar (see Section 6.2.3., figure 104), indicating the existence of a 'contact zone' where both forms could exchange genes. Molecular analyses were attempted on those two bugs, but we unfortunately could not obtain cyt*b* amplicons.

The problem of mtDNA introgression can be best approached by analysing nuclear gene polymorphisms in specimens already characterised using mtDNA, and then comparing the resulting nuclear and cytoplasmic gene genealogies (Avise 1994, Machado & Ayala 2001). Incongruent gene trees (nuclear divergence and mitochondrial similarity) would confirm historical introgression processes, whereas nuclear genealogies matching our mitochondrial results would suggest close relationships among the various populations – coupled with a remarkable degree of phenetic plasticity in *R. ecuadoriensis*. Suitable nuclear markers for specific determination and intra-specific analysis are currently available that have proven useful in Triatominae systematics and evolutionary genetics; these include allozymes (e.g. Dujardin et al. 1991, 1999a, Harry et al. 1992a,b, Harry 1993, Solano et al. 1996, Monteiro et al. 1998, 2002, Chávez et al. 1999) and selected nuclear gene fragments – for instance, the second internal transcribed spacer (ITS-2) of the ribosomal DNA has been successfully used to assess relationships among recently diverged taxa within both Triatomini and Rhodniini (Lyman et al. 1999, Monteiro et al. 2001, Bargues et al. 2000, Marcilla et al. 2000, 2001, 2002).

·Loja. In contrast with the findings described for Pichincha and El Oro, three unique haplotypes (not shared with any other population) were found in the strongly synanthropic bugs from Loja. These populations were collected in the valleys of the Chira-Catamayo river system, separated by a relatively high (>2000m) mountain range from the nearest fieldwork zone (El Oro, ~100km away in a straight line but ~200km away following routes below ~2000m altitude). These valleys (corresponding to dry premontane forest) are drier than those in El Oro (with humid premontane forest) and, although conditions allowing the growth of *Ph. aequatorialis* are theoretically present in some low areas, these (and any

other wild) palms are completely absent from the zone. *R. ecuadoriensis* are therefore probably isolated from sylvatic populations in the interior valleys of Loja, as reflected by both their phenetic traits (morphologically and morphometrically distinct from sylvatic bugs) and by the fact that they share no haplotype with any other population studied here. The fact that a single specimen from Suyo (a Peruvian locality near the Ecuadorian border) was found to be indistinguishable from those from Loja (both sites being ~30km away) by morphometric analyses favours the idea that these populations have extended locally in valleys with very similar ecological features, probably within the Chira river basin.

·Peru. The Peruvian population from the Chicama valley (Cascas District, Department of La Libertad) was found to be phenetically similar to (but distinguishable from) Ecuadorian synanthropic populations (mainly El Oro, ~600km away). Comparable size-related characters probably reflect the influence of a shared domestic habitat on the phenotypes. However, mtDNA analysis revealed very important genetic differences. The single, unique haplotype found in specimens from Chicama (collected in the same locality but from at least three different households) was 25bp different (~3.7%) from the most similar Ecuadorian one (in bugs from Manabí, ~975km away).

Both the absence of variability and (at least in part) the strong divergence could be explained by the isolation of a small subset of bugs in northwest Peru south of the Sechura desert. If the effective size of the original population was very small (implying a strong founder effect), important sequence divergence could accumulate in a relatively short period of isolation from the original gene pool, even in the absence of selective pressure. In the context of neutral allelic trees, one 'mainland' (original) and one 'island' (an isolated subset of the former) populations can evolve mutually exclusive mitochondrial lineages (i.e., reciprocal mtDNA monophyly since the time of isolation) in $4N_e$ generations (N_e being the effective population size of the 'island' population) (Avise & Wollenberg 1997, Donnelly et al. 2002). With, say, 10 founder *R. ecuadoriensis* females colonising a village in Peru and passing their mtDNA to their offspring (each female producing one reproductive daughter on average; see Schofield et al. 1999), 40 generations (for which the overall estimate of N_e will approximate the lowest N_e) would theoretically suffice to account for fixed differences in 'mainland' vs. 'island' mtDNA haplotypes. In addition, even relatively weak selection for local adaptation can dramatically reduce the waiting time to speciation (a measure of genetic differentiation) by orders of magnitude (Gavrilets

2000). Intersibling competition in the newly colonised ecotope and adaptation to the very arid climatic conditions in northern Peru could in fact be envisaged as selective forces likely to have enhanced differentiation of the Peruvian population. Pest control interventions may also contribute to reduced genetic variability in an isolated population confined to human habitats. Provided that a few bugs survive insecticide spraying, the subsequent genetic bottleneck will amplify the effects of genetic drift and substantially contribute to strong genetic structuring and reduced diversity (Donnelly et al. 2002).

The **intervention of humans** in the passive dispersal of a small founder population of domestic *R. ecuadoriensis* into Peru might therefore help explain our findings, including the fact that the known natural habitats of the species are absent from the arid northern Peru. However, when this mechanism has been invoked in other triatomines (notably *T. infestans* throughout the Southern Cone and *R. prolixus* in Central America, and probably also *T. dimidiata* in Ecuador and northern Peru), DNA analyses have shown that derived populations retain important similarities with the putative original ones (Dujardin et al. 1998a, Monteiro et al. 1999b, Marcilla et al. 2000, 2001). In general, while founder effects and inbreeding are expected to produce significant shifts in the frequencies of ancestral polymorphisms (and to considerably reduce genetic variability), the likelihood of strong divergence produced by new sequence polymorphisms remains low for considerable long periods, as *de novo* substitutions accumulate only at a (relatively) slow pace, especially in neutral alleles (Avise 1994). Tajima's relative rate tests showed no rate heterogeneity between Peruvian and Ecuadorian haplotypes (see Appendix), suggesting that the mt cyt*b* gene is evolving in a neutral fashion.

Alternatively, it is also possible that our Chicama population represents a subset of a more variable and widespread **Peruvian form of *R. ecuadoriensis*** (just as the monomorphic populations from Pichincha and El Oro are subsets of the Ecuadorian pool) that we simply failed to sample. In this scenario, the observed divergence patterns would probably have required a much longer period to become fixed, as the process would have initially involved a large (with a large N_e) population of *R. ecuadoriensis*. There is however no evidence that sylvatic populations currently exist in northern Peru, where only once it was claimed that a nymph of the species was found in an uninhabited area (Cuba Cuba et al. 2002). A potential explanation would involve the possibility that humid forests (and therefore palm trees) reached the Andean foothills of what today is northern Peru

during the warm and humid Pleistocene interglacial periods (Cox & Moore 2000). Sylvatic populations of (ancestral forms of) *R. ecuadoriensis* would thus have colonised those areas, and a subset of them would have remained isolated from its Ecuadorian relatives when forests again retreated northwards during the following glacial period. According to estimates of arthropod mtDNA sequence divergence per time unit (~2.3% per million year for recently diverged taxa; Brower 1994), the observed ~4% difference between Ecuadorian and Peruvian populations is compatible with ~1.7-1.8my of independent evolutionary histories; this estimate coincides with the beginning of the Donau glacial period*, which lasted for about 0.5my. It is not clear however how these sylvatic bugs would have survived the new conditions (cool and very arid climate in areas with no palm trees), but adaptation to highly protected arboreal microhabitats (hollow trees inhabited by vertebrates) provides an option; the above-mentioned record of sylvatic *R. ecuadoriensis* in Peru corresponds in fact to a *Schinus molle* hollow tree, indicating an obvious target for future field studies.

It must however be remembered that single-locus analysis of mtDNA provides only individual gene genealogies (the mt genome is, from the phylogenetic standpoint, a single nonrecombining locus with multiple alleles) that do not *necessarily* portray the overall organismal (or population) evolutionary history and may change from one (unlinked) locus to another (Avise 1994, Avise and Wollenberg 1997, Cavalli-Sforza 1998). In this sense, assessment of chromosomal polygene variation through (size-free) morphometric analyses represents a complementary source of multilocus genetic information; arguably, the degree of congruence between morphometric and molecular data might signal the amount of confidence we can have that a gene genealogy reasonably depicts the phylogeny of the organism carrying that gene. Critical to this view are the ability to identify the relative influence of environmental and genetic factors in metric analyses, and, more generally, the development of reliable methods to detect (and deal with) homoplasy.

In the case of Ecuadorian and Peruvian populations of *R. ecuadoriensis*, another source of genetic information is multilocus enzyme electrophoresis. Although comparisons explicitly designed to explore intra-specific relationships have not been published, Chávez et al. (1999) employed specimens from separate colonies originally founded with bugs

---

*Note however that some circularity of reasoning may be involved in this observation, because the rate of sequence divergence per time unit was calibrated using palaeoclimatological data (see Brower 1994)

from Ecuadorian (Manabí and El Oro) and Peruvian (Cajamarca, i.e. from the same colony as PEb) populations.

They investigated 17 loci (12 enzyme systems), in eight Rhodnius species, and reported that average enzyme polymorphism (0.15 for the whole sample) in *R. ecuadoriensis* (0.24) was only second to that found in *R. pictipes* (0.35), and higher than recorded for *pallescens* (0.12). Furthermore, different alleles (for which no heterozygotic forms were detected) of *Mdh* (malate dehydrogenase), *Pep3* and *Pep4* (aminopeptidase-B) were distributed 'according to the geographical origin of the specimens from Ecuador and Peru', suggesting a strong geographic structuring of these populations (Chávez et al. 1999; p. 303). Similar results were obtained by FA Monteiro and AM Solé-Cava (unpublished data), confirming the existence of diagnostic *Pep* alleles and showing that a further enzyme system also separated both groups (FA Monteiro, pers. comm.). On the other hand, Solano et al. (1996) studied Ecuadorian (originally from Manabí) and Peruvian specimens (from the same colony as PEb) using 14 enzyme systems (17 loci), including *Mdh* and *Pep*. *R. ecuadoriensis* presented single bands for all loci except *Mdh*, for which two unique alleles were species-diagnostic; the authors did not specify whether these alleles were distributed according to geography (Solano et al. 1996). Together, these findings can be suggestive of a long history of independent evolution of Ecuadorian and Peruvian populations of *ecuadoriensis*, perhaps involving incipient speciation (Noireau et al. 1998), but clearly support the need for complementary analyses based on nuclear markers.

6.3.5.3. Future work

i. In the short term, future molecular work on the different populations of *R. ecuadoriensis* characterised here will include a study of *nuclear rDNA ITS-2* sequence polymorphisms.

ii. An extension of phenotypic characterisation will include *traditional morphometrics of wings* and *geometric morphometric analyses of both head capsules and hemelytra*.

iii. *Cross-mating experiments* and *multilocus enzyme electrophoresis* are also being planned to clarify relationships between Ecuadorian and Peruvian populations and the role that historical introgression may have had in the observed mtDNA haplotype distribution.

iv. *Further sampling* would also be required; of special interest would be the study of intermediate geographic populations from northern Peru, the investigation of remnant *Phytelephas* forests in southern (El Oro-Loja) and western (along the Andes foothills and in Esmeraldas) Ecuador, and sampling of putative *Rhodnius* habitats (i.e., palm trees) in the Colombian Chocó.

Comparisons with biogeographic, ecological, molecular and morphometrics results presented here would help clarify the relative effects that various forces (such as phenetic plasticity, habitat associations, geographic isolation, migration patterns, and historical introgression) might have had in shaping the current patterns of diversity and distribution observed in the species. They would also provide further insight on the evolution of the various lineages within the genus *Rhodnius*, and will at the same time help define the usefulness of different molecular and phenetic approaches in deciphering those evolutionary pathways.

7. PHYLOGENY OF *RHODNIUS ECUADORIENSIS*

7.1. The 'Pacific *Rhodnius* lineage'

Within the Triatominae (obligate haematophagous reduviids), the tribe Rhodniini is characterised by the arboreal habitats of its members, many of which are primarily associated with palm trees. It contains two genera (*Rhodnius* and *Psammolestes*) and 17 species (14 *Rhodnius* and 3 *Psammolestes*) (table 85). The validity of *R. dalessandroi* (a rare, sylvatic species from Colombia) was doubted by Lent & Wygodzinsky (1979), but Martínez (1984) defended its taxonomic status. A new species (*R. milesi*) was described in 2001 based on material collected in the State of Pará (eastern Brazilian Amazon), and a recent paper claims validity for *R. amazonicus*, described in 1973 and later synonymised with *R. pictipes* (Almeida et al. 1973, Bérenger & Pluot-Sigwalt 2002).

Table 85. The tribe Rhodniini Pinto, 1926

Genus	Species	Authors
Rhodnius	*Rhodnius amazonicus**	Almeida, Santos & Sposina, 1973
Stål, 1859	*Rhodnius brethesi*	Matta, 1919
	Rhodnius colombiensis	Moreno, Galvão & Jurberg, 1999
	*Rhodnius dalessandroi**	Carcavallo & Barreto, 1976
	Rhodnius domesticus	Neiva & Pinto, 1923
	Rhodnius ecuadoriensis	Lent & León, 1958
	*Rhodnius milesi**	Valente et al., 2001
	Rhodnius nasutus	Stål, 1859
	Rhodnius neglectus	Lent, 1954
	Rhodnius neivai	Lent, 1953
	Rhodnius pallescens	Barber, 1932
	Rhodnius paraensis	Sherlock, Guitton & Miles, 1977
	Rhodnius pictipes	Stål, 1872
	Rhodnius prolixus	Stål, 1859
	Rhodnius robustus	Larrousse, 1927
	Rhodnius stali	Lent, Jurberg & Galvão, 1993
Psammolestes	*Psammolestes arthuri*	(Pinto, 1926)
Bergroth, 1911	*Psammolestes coreodes*	Bergroth, 1911
	Psammolestes tertius	Lent & Jurberg, 1965

*The validity of these species is doubted by some researchers

The Rhodniini are considered as a monophyletic group, in spite of substantial phenetic variation involving mainly the genus *Psammolestes* (Lent & Wygodzinsky 1979). The tribe is separated from other reduviids, including Triatominae, by the apical insertion of antennae, the elongated heads, and the presence of postocular callosities on the sides of the

head (Lent & Wygodzinsky 1979). Some characteristics of the male genitalia have also been used to define the tribe; the most salient of these is the absence of phallosome supports in all members of the tribe, with the exception of *R. pictipes* and the almost isomorphic *R. stali* (Jurberg 1996); the presence of this structure has been interpreted as a plesiomorphic state (it is shared by other triatomines and by predatory reduviids), with subsequent (apomorphic) loss in the Rhodniini, with the exception of *pictipes-stali* (which retain this 'ancestral' character) (Schofield & Dujardin 1999). Finally, the presence of nitrophorins in the salivary glands is typical of the tribe; nitrophorins are nitric oxide-carrying heme proteins with anticoagulant, vasodilatory and anti-histaminic activity, and are responsible for the characteristic reddish colouration of the salivary glands of the Rhodniini (Ribeiro et al. 1998, Sant'Anna et al. 2001).

The relationships among members of the tribe have been addressed by several studies. The most significant findings (in terms of taxonomy and systematics) refer to the mutual relationships of *Rhodnius* and *Psammolestes*. A species of this latter genus (*Ps. coreodes*) was found to be more closely related to the clade formed by *R. prolixus*, *R. robustus*, *R. nasutus*, *R. neglectus*, *R. domesticus*, and *R. neivai* than these were to *R. pallescens*, *R. colombiensis*, *R. ecuadoriensis*, *R. pictipes*, and *R. brethesi* (Monteiro et al. 2000, 2001, 2002). These studies, based on nucleotide sequences (from nuclear and mitochondrial DNA) and on isoenzyme electrophoresis, suggest that the genus *Rhodnius* is a paraphyletic assemblage including *Psammolestes*, and that the tribe Rhodniini would actually be monogeneric: the three *Psammolestes* species should consequently be renamed as *Rhodnius tertius*, *R. arthuri*, and *R. coreodes* – a view that also received partial support from ITS-2 rDNA sequence analysis (Marcilla et al. 2001). On the other hand, genetically distinct *Rhodnius* species prove difficult to discriminate on morphological grounds, and no discrete morphological characters can be used to distinguish *Rhodnius* species (except for the presence of phallosome supports in *R. pictipes* and *stali*). This has brought controversy about the taxonomic status and epidemiological importance of some species and populations, the example of *R. prolixus-R. robustus* being paradigmatic (e.g. Harry 1993, Schofield & Dujardin 1999, Monteiro et al. 2000, 2001).

Apart from the difficulties they pose to researchers, these facts indicate that at least for members of the tribe Rhodniini no simple, straight correlation between phenetic and genetic diversity is to be expected. Phenetically diverse species (even belonging to

different genera in the classical taxonomic arrangement) are genetically similar, while cryptic speciation is considered as a relatively common event by some researchers. For instance, *R. robustus* (as defined by morphological and chromatic characters) seems to comprise at least four to five genetically distinct lineages (Monteiro et al., unpubl.), and it has been suggested that at least some of them may represent different species.

The underlying causes of morphological plasticity in Triatominae (acting either in the sense of divergence or convergence) are poorly understood, but it has been proposed that environmental factors could play a major role (Dujardin et al. 1999b). Thus, the extreme phenetic divergence of *Psammolestes* (in relation to their sister *Rhodnius* species) could be related to their highly specialised adaptation to weaverbird nests (Furnariidae). On the other hand, isomorphic but genetically distinct entities within the *R. robustus* 'morphospecies' exploit virtually identical ecotopes (palm trees, preferentially *Attalea* spp.) throughout the Amazon-Orinoco drainage basins.

One interpretation of these findings suggests that here speciation could be related to isolation of different populations in Pleistocene forest refugia during dry, cold glacial periods (Haffer 1969), but environmental similarities would have promoted the retention of similar phenotypes (Monteiro et al., unpubl.). Other authors have proposed the existence of two main forms of *R. robustus*: a 'northern form' in the Orinoco-northern Amazon (forming a clade with *prolixus*, perhaps *neivai*, and *Psammolestes arthuri*) and a 'southern form' (related to *nasutus*, *neglectus*, *domesticus*, and *Psammolestes tertius-coreodes*) south of the Amazon; each of these 'forms' would be regarded as independent evolutionary units, with genetically distinct subgroups probably representing subspecies or geographic populations (Schofield & Dujardin 1999).

The evolutionary relationships of *R. ecuadoriensis* have been partially addressed in some general studies on the systematics and phylogeny of the Rhodniini; these analyses were based on allozymes (Solano et al. 1996, Dujardin et al. 1999a, Chávez et al. 1999, Monteiro et al. 2002), RAPD (García et al. 1998), and DNA sequence data (Stothard et al. 1998b, Lyman et al. 1999, Monteiro et al. 2000, 2001). All these results broadly agree in placing *R. ecuadoriensis* close to *R. pallescens*, as it had already been suggested by morphological and biogeographical observations (see Lent & Wygodzinsky 1979, Schofield 1994, Schofield & Dujardin 1999, Dujardin et al. 1999b). The recently described *R. colombiensis* (Moreno et al. 1999) was found to be more

closely related (as inferred from allozyme profiles using 12 enzyme systems and 17 loci) to *R. ecuadoriensis* than to *R. pallescens*, despite phenetic and biogeographical data suggesting a closer relationship with the latter; reported pairwise Nei standard genetic distances (*D*±SD) were: *pallescens-colombiensis* *D*=0.6±0.2; *pallescens-ecuadoriensis* *D*=0.7±0.2; and *colombiensis-ecuadoriensis* *D*=0.5±0.2 (Dujardin et al. 1999a). Monteiro et al. (2002) recently reported *D*=0.54 for the pair *pallescens-ecuadoriensis* after electrophoretic analysis of 11 enzyme systems (12 loci). Mahalanobis distances (Md) derived from a discriminant analysis on size-free metric variables were slightly smaller between *R. colombiensis* and *R. ecuadoriensis* (Md=26.6) than between *R. ecuadoriensis* and *R. pallescens* (Md=27.5), with the distance between *pallescens* and *colombiensis* being much smaller (Md=14.6) (Dujardin et al. 1999a). A comparison of a 414bp fragment of the mitochondrial cyt*b* gene placed *R. colombiensis* closer to *ecuadoriensis* than to *pallescens*, with 100% bootstrap support after 1000 replications in a phylogeny inferred from Kimura 2-paramater distances by neighbour-joining (Monteiro et al. 2000). These works also generally coincide in clustering *R. pallescens-colombiensis-ecuadoriensis* (sometimes referred to as the 'Pacific *Rhodnius* lineage', as all three species occur west of the Andes) together with the widespread Amazonian *R. pictipes*, in agreement with the classical taxonomic arrangement, but results are sometimes conflicting and as yet inconclusive in this regard (see Stothard et al. 1998b, Schofield & Dujardin 1999, Dujardin et al. 1999a, Lyman et al. 1999, Monteiro et al. 2000, 2001, 2002).

More recently, intercrossing experiments involving representatives of the three species produced infertile F1 hybrids from *R. pallescens* females x *R. colombiensis* males; all other combinations (including *pallescens* males x *colombiensis* females, *colombiensis* x *ecuadoriensis*, and *pallescens* x *ecuadoriensis*) did not produce F1 offspring (J Moreno, unpublished data). Up to date, cytogenetics studies have only been conducted in *R. pallescens* and *R. ecuadoriensis* (Panzera et al. 1998). As in all *Rhodnius*, these two species have a chromosomal complement equal to 20 autosomes plus XX in females and XY in males. *R. pallescens* presents 6 to 8 autosomic pairs with C heterochromatin – representing less than 10% of the total length of the autosomic complement. In contrast, the autosomes of the *R. ecuadoriensis* studied had no C-heterochromatic bands.

In an attempt to clarify the relationships within the 'Pacific *Rhodnius* lineage' and between these three species and other *Rhodnius* lineages, we analysed morphometric variation and mitochondrial cyt*b* sequence polymorphisms in seven species of *Rhodnius*. Representatives of the 'Pacific lineage' (*R. pallescens*, *R. colombiensis* and five populations of *R. ecuadoriensis*) and *R. pictipes* were examined by both methods, as were representatives of different populations of *R. prolixus* and *R. robustus* (including specimens collected in the Ecuadorian Amazon); *R. nasutus* was only subjected to molecular analysis.

7.2. Morphometrics of the Pacific *Rhodnius* lineage

7.2.1. INTRODUCTION

The use of morphometric traits for phylogenetic reconstruction relies on the assumption that variation of continuous phenotypic attributes is the expression of underlying genotype polymorphisms; the degree to which phenotypic traits differ between two groups is regarded as a reflection of their evolutionary distance. Because of several of its statistical properties, multivariate discriminant analysis has been widely used for the exploration of phylogenetic relationships among closely related, monophyletic taxa; the principal among those properties refer to the ability to generate orthogonal vectors (which can be used as uncorrelated variables) and the fact that it portrays relationships as divergence-based distances reflecting apomorphic information. It has been however emphasised that phylogenetic hypotheses derived from metric data should be corroborated with extrinsic information, better represented by DNA sequence polymorphisms (Pimentel 1992, Sorensen 1992, Sorensen & Foottit 1992). In the context of triatomine systematics, and particularly in the case of *Rhodnius* species, it has been noted that the analysis of metric characters represents the only non-molecular method that can be applied to the study of evolutionary relationships among closely related taxa, which cannot be readily distinguished on the basis of discrete anatomical features suitable for cladistic analysis (Dujardin et al. 1999a).

Discriminant function analysis (termed canonical variate analysis [CVA] when used to reduce the dimensions of data) emphasises unshared between-group variation (regarded as equivalent to apomorphic character information) while maximising intra-group cohesion (Pimentel 1992, Sorensen 1992, Foottit & Sorensen 1992, Dujardin et al. 1999a,e). In CVA, orthogonal factors are derived from the original dataset and used

as new, uncorrelated variables (canonical vectors) that separate the (*a priori*-defined) groups under investigation focusing on such unshared variation (Pimentel 1992). The effects of homoplasy are reduced (but not necessarily eliminated; it may persist if some phenotypes undergo reversal changes along a given vector) as each original metric character is split into orthogonal and non-redundant (uncorrelated 'by construction') canonical factors. Because of this latter attribute, orthogonal factors can be used as new characters that circumvent the problem of correlation affecting raw data (i.e., measurements taken directly from the specimens under investigation, which cannot be treated as independent for statistical analysis) (Sorensen & Foottit 1992, Dujardin et al. 1999e). Several statistical techniques have also been described that help concentrate on the genetic component of phenetic variation (as opposite to that attributable to environmental factors); in the case of triatomines, the focus is on partitioning of size-related features, known to be particularly sensitive to environmental stress. Such a problem is of greater importance when assessing relationships among conspecific populations; habitat correlations are crucial when the focus is evaluation of reinfestations.

7.2.2. MATERIALS AND METHODS

Details on the specimens used in morphometric analyses are provided in the Appendix. Briefly, 12 head measurements were taken from each of 153 *Rhodnius* specimens (79 *R. ecuadoriensis* representing five populations, 15 *R. colombiensis*, 15 *R. pallescens*, 15 *R. pictipes*, 15 *R. prolixus*, and 14 specimens classified as *R. robustus* after morphological characters); multivariate ordination methods (principal component analysis [PCA] and canonical variate analysis [CVA]) were used to assess relationships among species. Log-transformed measurements were centred to obtain 'log-shape ratios' that were submitted to PCA on covariances. Derived principal components (except for the last one, not contributing to the observed variation) were used as new variables for CVA; for some analyses, the residuals of linear regression of log-transformed measurements on the first PC were used as the input for CVA. Allometry-free analyses were conducted after identifying a subset of measurements from which a common growth axis could be derived; these log-transformed variables were submitted to common principal component (CPC) analysis. Further details on the methods used for morphometric comparisons are described in detail in Section 6.2.2.

7.2.3. RESULTS♦

7.2.3.1. Size variation

Size variation was analysed using seven log-transformed measurements (A, D, E, G, L, R2, and R3). For an initial analysis, eight groups were defined: '*R. ecuadoriensis*, Peru'; 'domestic *R. ecuadoriensis*, Ecuador'; 'sylvatic *R. ecuadoriensis*, Ecuador'; *R. colombiensis*; *R. pallescens*; *R. pictipes*; *R. prolixus*; and *R. robustus*. Group sample sizes were homogenised by randomly selecting subsets of *R. ecuadoriensis* specimens from Loja-El Oro ('domestic Ecuadorian *R. ecuadoriensis*', n=16) and Pichincha-Manabí ('sylvatic *ecuadoriensis*', n=16). Anova tests showed significant differences for all seven measurements; F ratios were: A=101.7, D=365.5*, E=88.2*, G=488*, L=67, R2=388.3*, and R3=104.3; for 7 degrees of freedom, significance probabilities were always <0.0001 [asterisks indicate heteroscedasticity for those measurements (details not shown); Welch Anova tests were therefore used for the comparisons].

A further comparison was performed with the mean value of the measurements. Unequal variances were also detected, and a Welch Anova test was therefore used to compare variation among groups (F ratio=270.1, 7 df, p<0.0001). Synanthropic *R. ecuadoriensis* bugs from Peru and Ecuador (Loja-El Oro) were significantly smaller, and size increased progressively in sylvatic *R. ecuadoriensis* (a very variable group), *R. prolixus* and *R. colombiensis* (which were virtually identical), *R. pallescens* and *R. pictipes* (*pallescens* being relatively very variable), and the largest and also variable *R. robustus*.

A gradual reduction in size was apparent within the 'Pacific clade'; the larger *R. pallescens* occurs in the northernmost extreme of the group range (northern Colombia), *R. colombiensis* in the central Magdalena valley, and *R. ecuadoriensis* in western Ecuador and northern Peru. Within these latter, a clinal north-to-south reduction in size involves all Ecuadorian populations, but Peruvian bugs from La Libertad are on average slightly larger than southern Ecuadorian populations (see Section 6.2.3.). *R. pictipes* specimens were larger on average than *R. pallescens* ones, but the difference was not statistically significant. These results are graphically summarised in the following figure.

♦Basic statistics of the twelve head measurements taken for morphometric comparisons are presented in the Appendix. For the description of these measurements see Section 6.2.2.

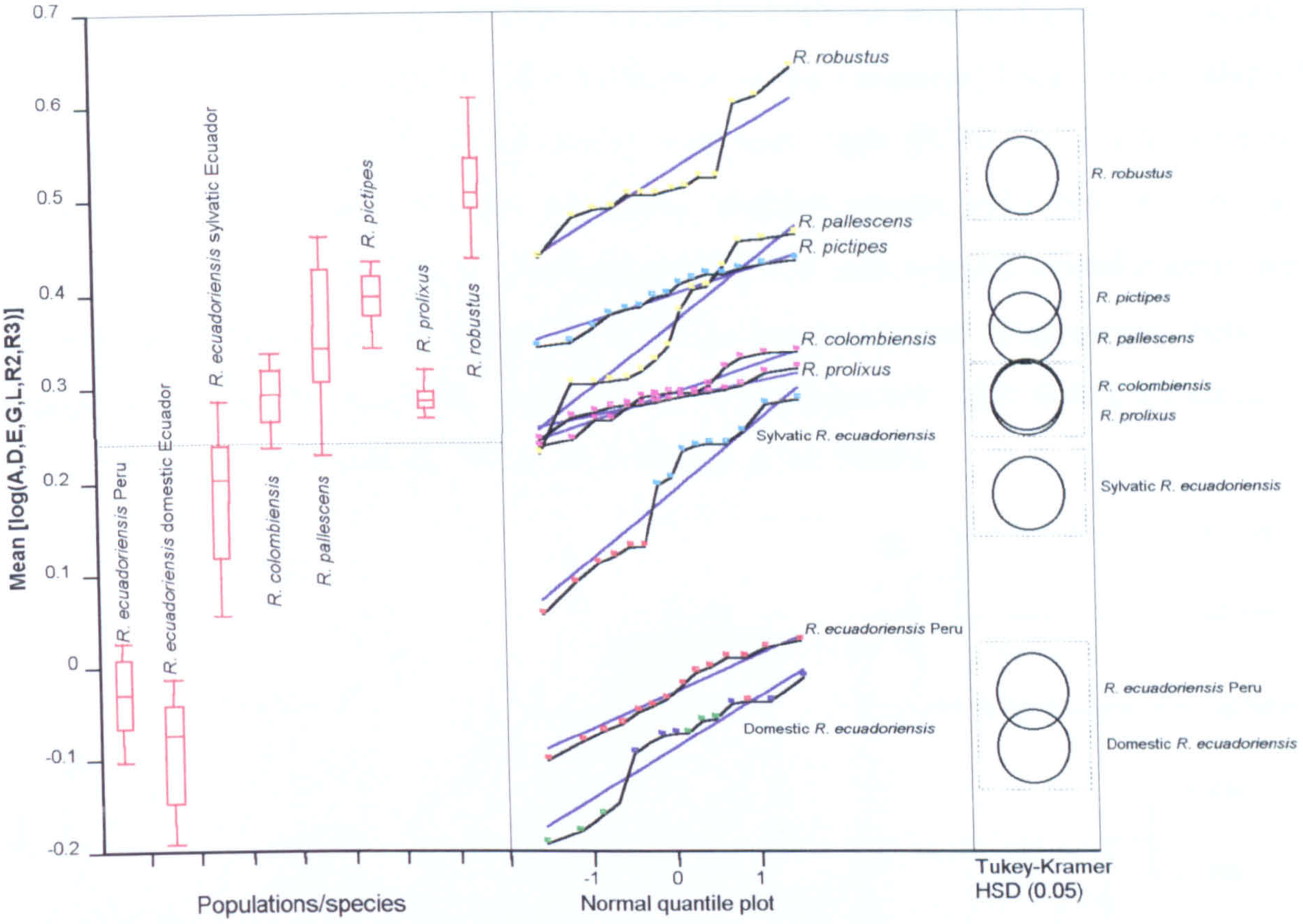


Figure 121. Size variation in six *Rhodnius* species. Boxplots show median values and 10%, 25%, 75% and 90% quantiles. Circles on the right show the relationships between groups (from a Tukey-Kramer test, α=0.05); dotted boxes enclose groups whose average measurements were not significantly different; note *R. prolixus* and *R. colombiensis* were virtually identical, with the former being very homogeneous; also note the similarity of synanthropic populations of *R. ecuadoriensis* (with Peruvian bugs being somewhat larger) and the very important difference between them and their sylvatic conspecifics

7.2.3.2. Isometry-free analysis

Isometry-free CVA was used to explore relationships among members of the Pacific lineage, *R. pictipes*, and two species of the *prolixus* group (*prolixus* and *robustus*). 'Log-shape ratios' were computed (from measurements A, D, E, G, L, R2, R3) and a PCA on covariances was performed. Several outliers identified by inspecting plots of Jackknife and Mahalanobis distances were excluded (n=116 bugs). The first six PCs were submitted to CVA. The multivariate significance of the discriminant analysis (as with all others below) was checked using the Wilk's lambda test (λ=0.0016, F≈34.2, 42 df, p<0.0001). Apart from the excellent inter-specific resolution, CVA shows a striking separation of sylvatic and synanthropic populations of *R. ecuadoriensis* (97.8% correct reassignation to their ecological groups); the degree of variability seems well above that separating some recognised species (e.g., *pallescens-colombiensis*). Sylvatic *R. ecuadoriensis* are intermediate between their domestic conspecifics and *colombiensis*-

pallescens. *R. pictipes* and *R. prolixus* had similar CV1 (*X* axis in figure 122) scores; this likely reflects size similarities (the coefficient of the [negative] linear relationship of CV1 on the average of all measurements was very high [R^2=0.9]). CVA correctly reassigned 97.4% of bugs to their respective original groups (113 out of 116; one *colombiensis* and one *pallescens* swapped group, and one sylvatic *ecuadoriensis* was classified as Peruvian); the Kappa statistic, measuring the degree of agreement between original and CVA-derived group assignations, was kappa=0.97 (SE=0.02), indicating a "perfect" agreement (Landis & Koch 1977, Matías et al. 2001).

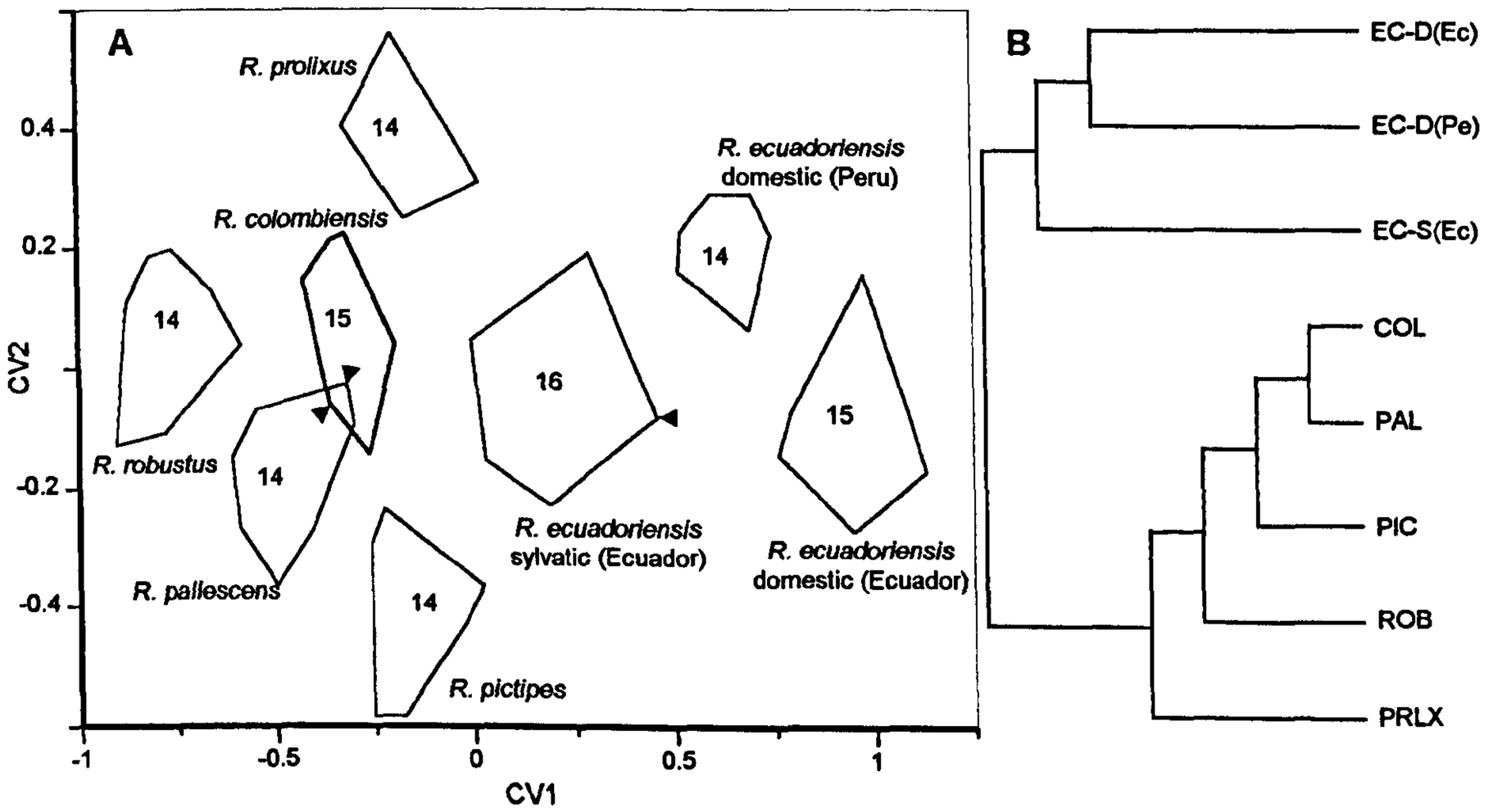


Figure 122. Isometry-free canonical variate analysis: relationships among *Rhodnius ecuadoriensis*, *R. colombiensis*, *R. pallescens*, *R. pictipes*, *R. prolixus*, and *R. robustus*. **A**: Factor map. Note the high phenetic variability of *R. ecuadoriensis* populations; sylvatic specimens were closer to *colombiensis* and *pallescens* than to some of their synanthropic conspecifics. Separation of groups on CV1 is influenced by their relative size. **B**: Single linkage hierarchical cluster analysis (individual mean scores on CV1, CV2, and CV3). EC=*R. ecuadoriensis* (D=domestic, S=sylvatic; Ec=Ecuador, Pe=Peru); COL=*R. colombiensis*; PAL=*R. pallescens*; PIC=*R. pictipes*; ROB=*R. robustus*; PRLX=*R. prolixus*. Numbers within polygons indicate group size (n_j); arrowheads point to CVA-misclassified specimens

A similar analysis including only *R. ecuadoriensis* and its closest relatives (*R. pictipes*, *R. pallescens* and *R. colombiensis*) plus an outgroup (*R. robustus*) confirmed the strong divergence of synanthropic forms of *R. ecuadoriensis*, with specimens from sylvatic habitats appearing closer to *pictipes-pallescens-colombiensis*. UPGMA cluster analysis placed *R. robustus* as a sister group to the clade {sylvatic *ecuadoriensis* [*pictipes* (*pallescens-colombiensis*)]}. Out of 102 specimens, 99 (97%) were correctly assigned to their original groups. Misclassification showed the same pattern as above. Kappa statistics showed again a perfect agreement: kappa=0.97, SE=0.02.

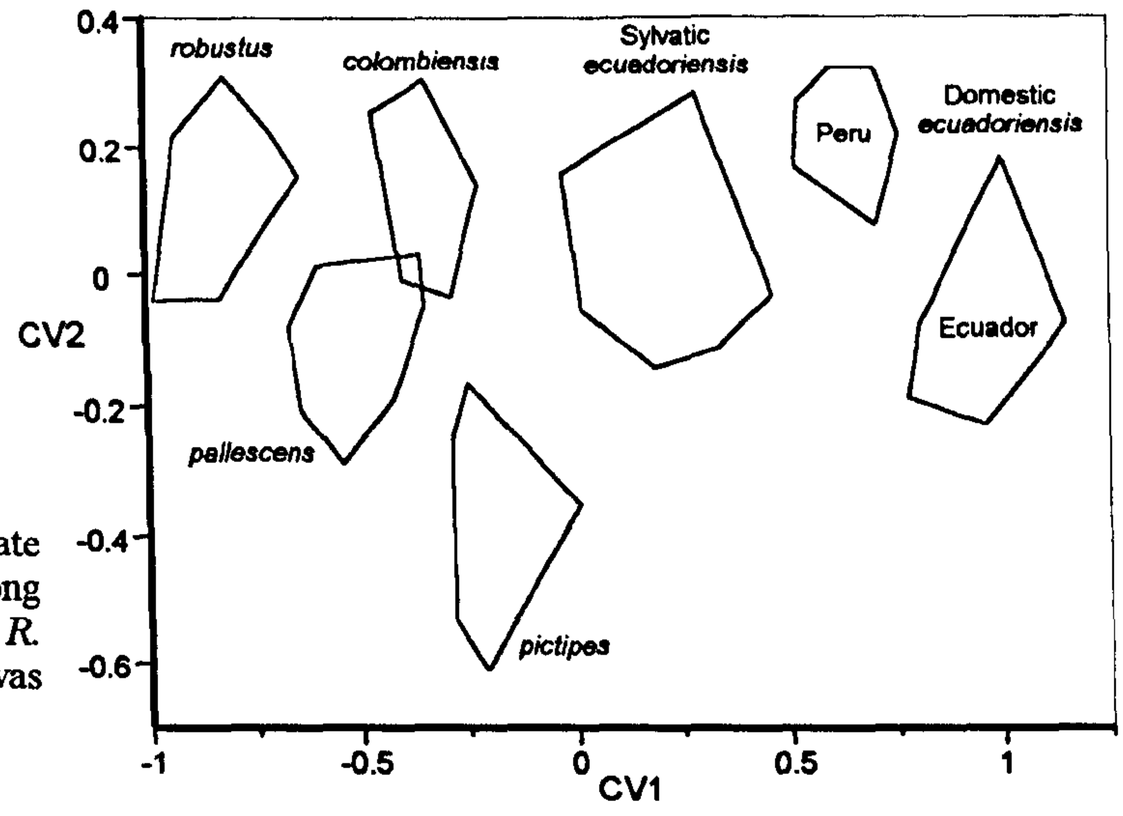


Figure 123. Isometry-free canonical variate analysis: inter-specific relationships among *Rhodnius ecuadoriensis*, *R. colombiensis*, *R. pallescens*, and *R. pictipes*; *R. robustus* was used as the outgroup

A further analysis was performed in an attempt to clarify the relationships of sylvatic *R. ecuadoriensis* with both synanthropic conspecifics and the closely related *pallescens* and *colombiensis*. For this, *R. pictipes* (with CV1 scores intermediate between sylvatic *ecuadoriensis* and the pair *pallescens-colombiensis*, probably reflecting intermediate size for the measurements used here) was excluded and CVA carried out on the remaining 89 specimens. Under these conditions, UPGMA cluster analysis recognised two major subdivisions, one corresponding to all the different *R. ecuadoriensis* populations and the second one encompassing *R. pallescens* and *R. colombiensis* (again found to be very closely related) plus *R. robustus*. CVA correctly classified 97.7% of bugs (86 out of 88); misclassification involved one *pallescens* and one *colombiensis* swapping groups (kappa=0.97, SE=0.02).

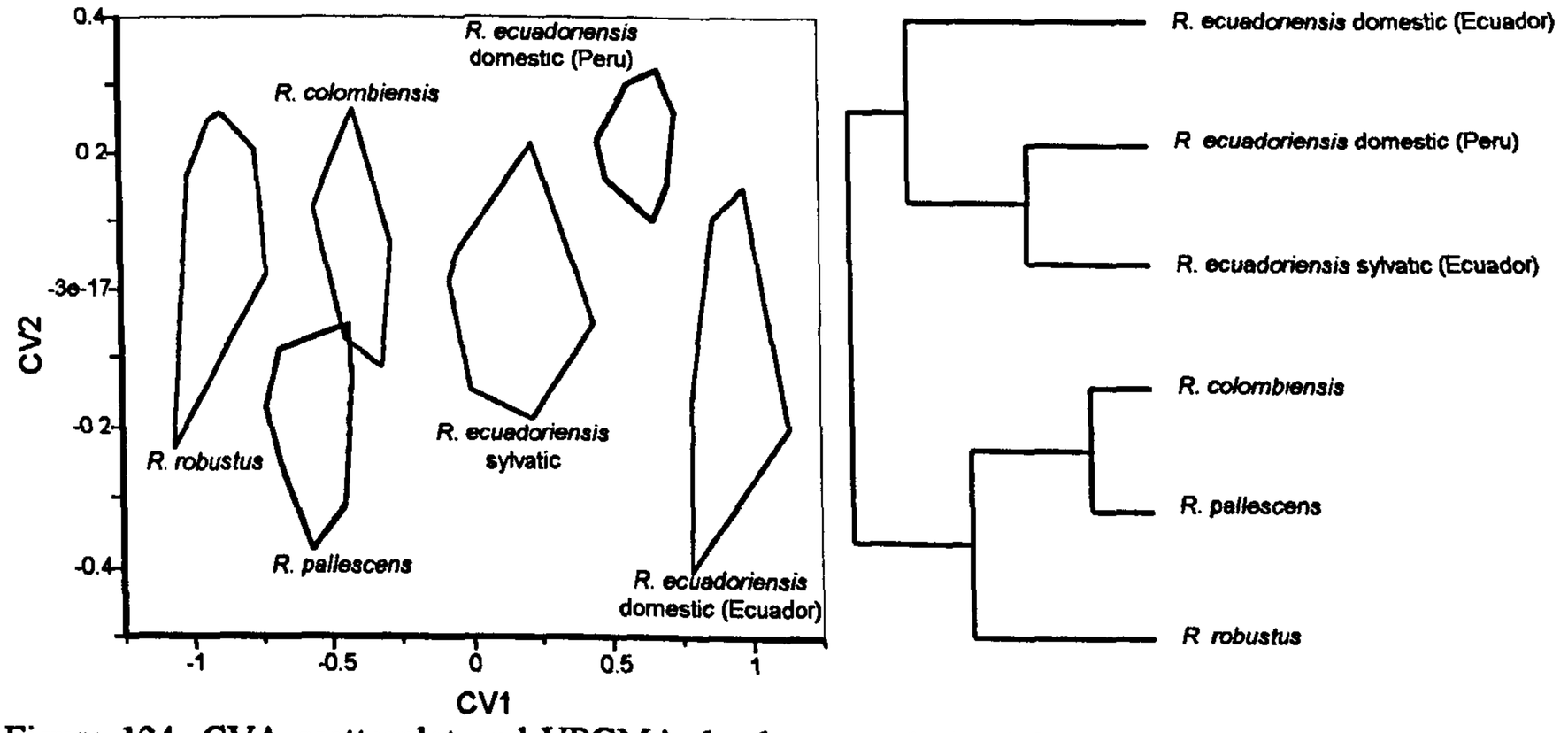


Figure 124. CVA scatterplot and UPGMA dendrogram derived from Mahalanobis distances: inter-specific relationships among *Rhodnius ecuadoriensis*, *R. colombiensis*, and *R. pallescens*, using 14 *R. robustus* specimens as the outgroup

These analyses were repeated including representatives of a different genus. Head capsules of 18 *Triatoma infestans* specimens were measured and the new dataset submitted to CVA. The discriminant scatterplots were distorted by the expansion of the discriminant space resulting from the inclusion of a distant outgroup, which had the effect of 'forcing' all *Rhodnius* together. UPGMA dendrograms derived from Mahalanobis distances confirmed that pooled Pichincha-Manabí *R. ecuadoriensis* specimens (the 'sylvatic' group) appear to be more similar to synanthropic *ecuadoriensis* populations than to the pair *pallescens-colombiensis*.

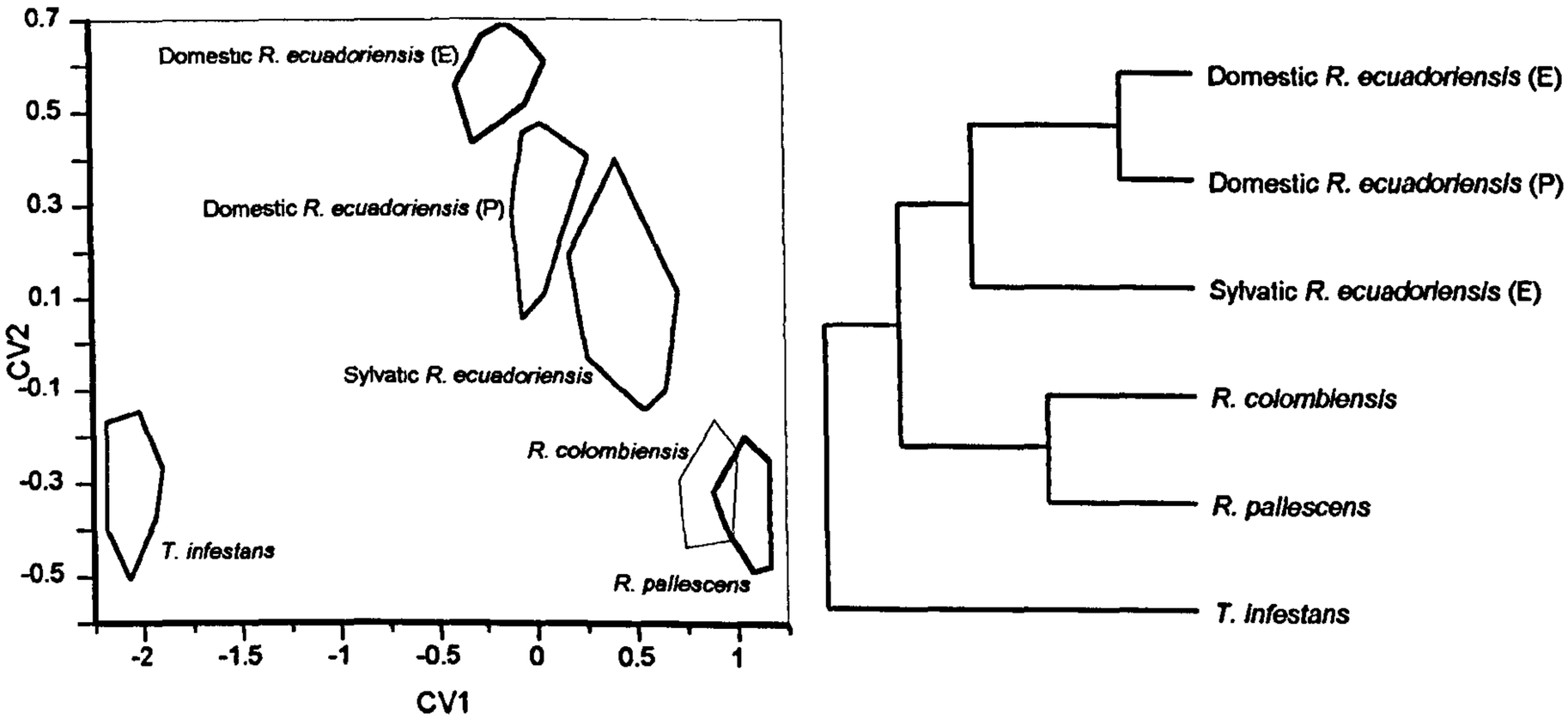


Figure 125. CVA scatterplot and UPGMA dendrogram derived from Mahalanobis distances: inter-specific relationships among *Rhodnius ecuadoriensis*, *R. colombiensis*, and *R. pallescens*, using 16 *Triatoma infestans* specimens as the outgroup. This CVA correctly classified 86 out of the 90 specimens studied (95.6%; kappa=0.95, SE=0.03). In the dendrogram, 'E' indicates Ecuador, and 'P' Peru

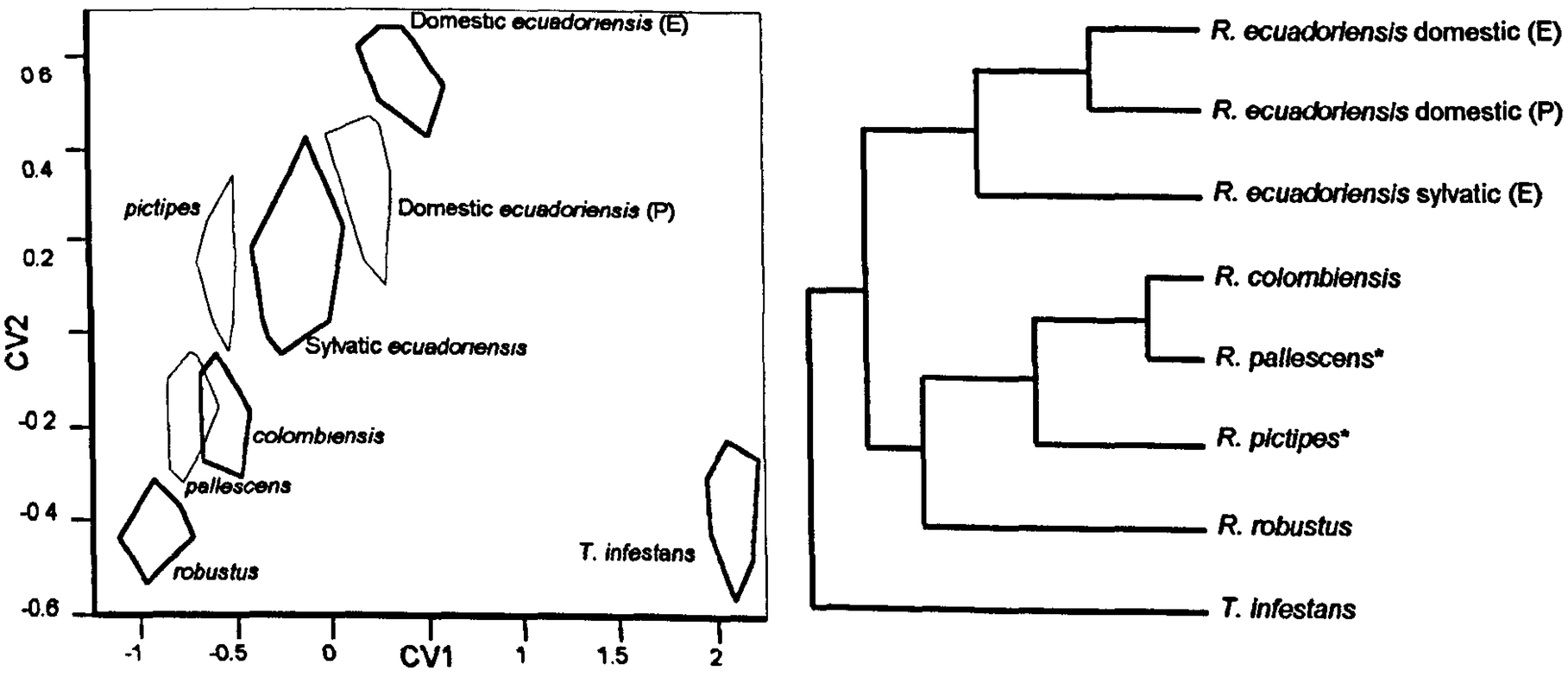


Figure 126. CVA scatterplot and UPGMA dendrogram derived from mean CV1 and CV2 scores: inter-specific relationships among *Rhodnius ecuadoriensis*, *R. colombiensis*, *R. pallescens*, *R. pictipes*, and *R. robustus* using *Triatoma infestans* as the outgroup. 97.5% of specimens (115 out of 118) were correctly classified (kappa=0.97, SE=0.02). 'E' indicates Ecuador, and 'P' Peru. **R. pictipes* and *R. pallescens* swapped positions in the dendrogram when the UPGMA algorithm was applied to Mahalanobis distances

Finally, the complete dataset (all *R. ecuadoriensis* populations and *T. infestans* as the outgroup) was submitted to CVA. The close affinity of sylvatic *R. ecuadoriensis* populations with *pallescens-colombiensis* and *R. pictipes* was only confirmed for Pichincha forms, with the Manabí population appearing as the basal branch of the cluster including the synanthropic populations of the species. This was confirmed by an analysis of the complete 'Pacific lineage' dataset (with *T. infestans* as the outgroup).

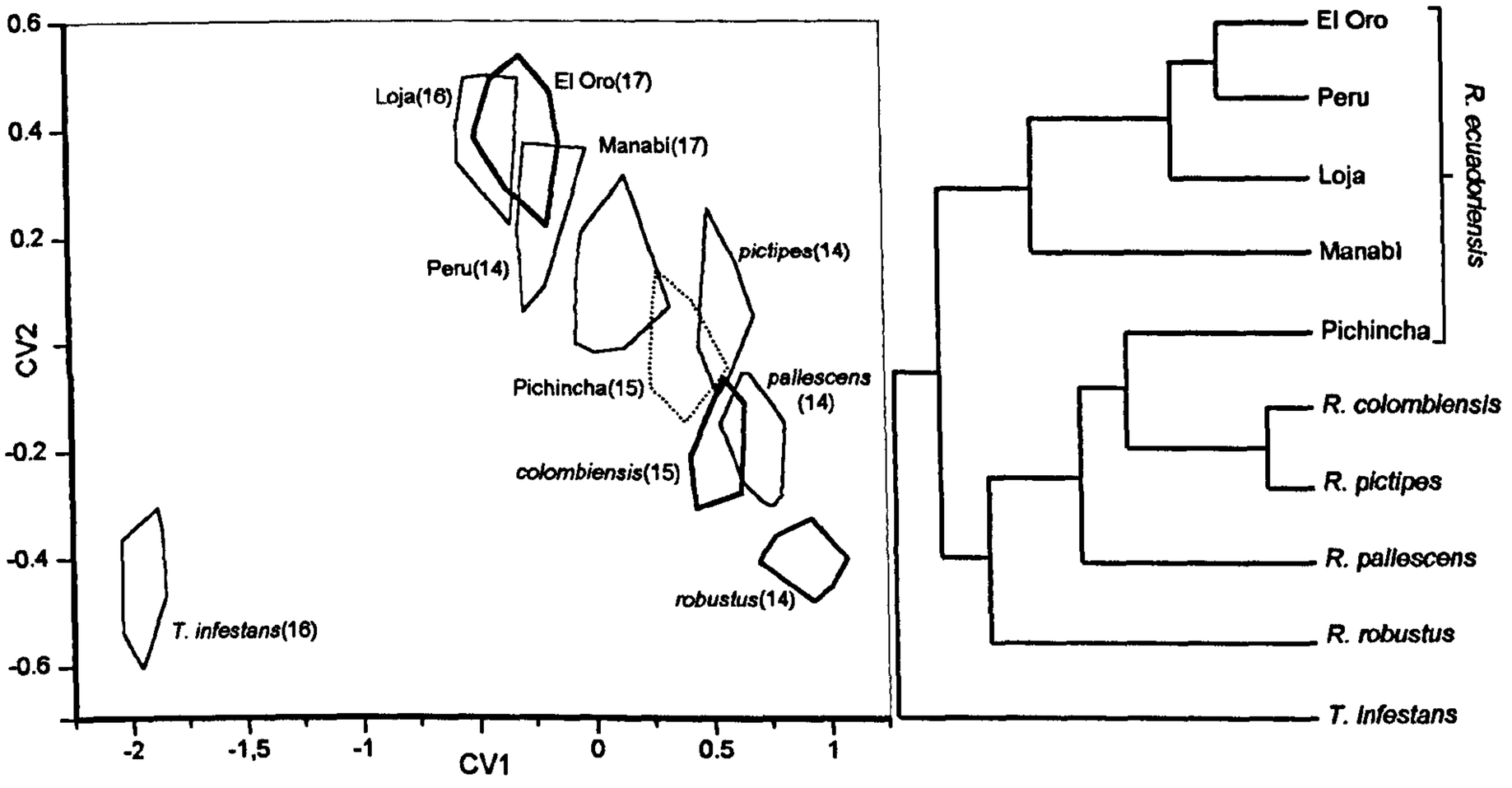


Figure 127. CVA factorial map and UPGMA dendrogram derived from Mahalanobis distances: inter-specific relationships among five populations of *Rhodnius ecuadoriensis*, *R. colombiensis*, *R. pallescens*, *R. pictipes*, and *R. robustus*, using *Triatoma infestans* as the outgroup. Note the clustering of *ecuadoriensis* Pichincha sylvatic forms with *colombiensis-pictipes*, and not with their conspecifics; note also the apparent convergence of *colombiensis* and *pictipes* on CV1, resulting in the pair being clustered together by the UPGMA algorithm. Out of 152 specimens, 14 (9.2%) were misclassified by CVA; 90.8% were reassigned to their original groups (kappa=0.9, SE=0.03)

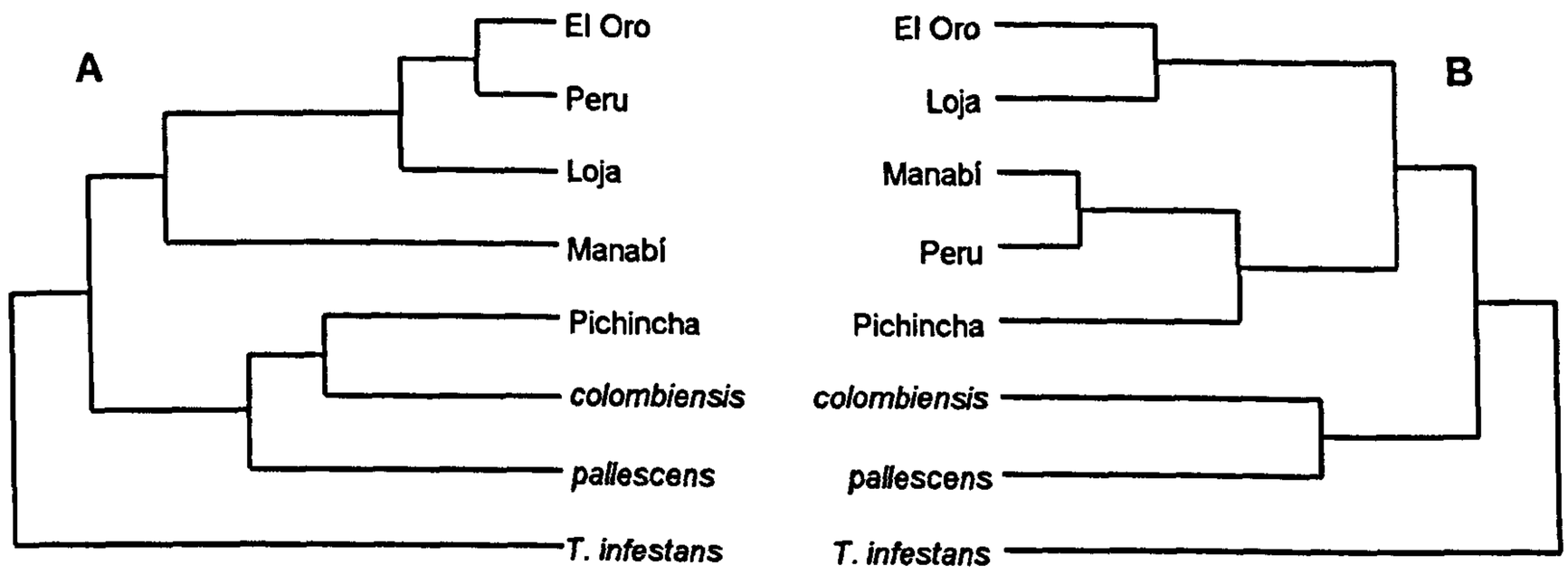


Figure 128. Interspecific relationships among members of the 'Pacific *Rhodnius* lineage'; *Triatoma infestans* was the outgroup. **A**: UPGMA dendrogram derived from Mahalanobis distances: sylvatic *R. ecuadoriensis* from Pichincha cluster with *pallescens-colombiensis*, and not with conspecific populations; **B**: UPGMA dendrogram derived from mean scores on CV1, CV2, and CV3; note the changes in branching order, with all *ecuadoriensis* populations forming a single clade and the Peruvian population appearing closer to sylvatic bugs from Manabí than to other synanthropic populations. n=124; CVA kappa=0.89, SE=0.03 (90.3% correct reassignation)

In an additional analysis, residuals of linear regression of each variable on PC1 were used as new variables to analyse these two latter datasets; further removing the influence of size helped clarify the patterns of relationship among the species under consideration. Cluster analysis placed the pairs *robustus-prolixus* and *colombiensis-pallescens* as sister groups; a second main cluster comprised the various *ecuadoriensis* populations (with a neat separation of Loja-El Oro from Peruvian and sylvatic specimens) and *R. pictipes*. The corresponding factorial map shows a good separation of *R. pictipes* on CV2.

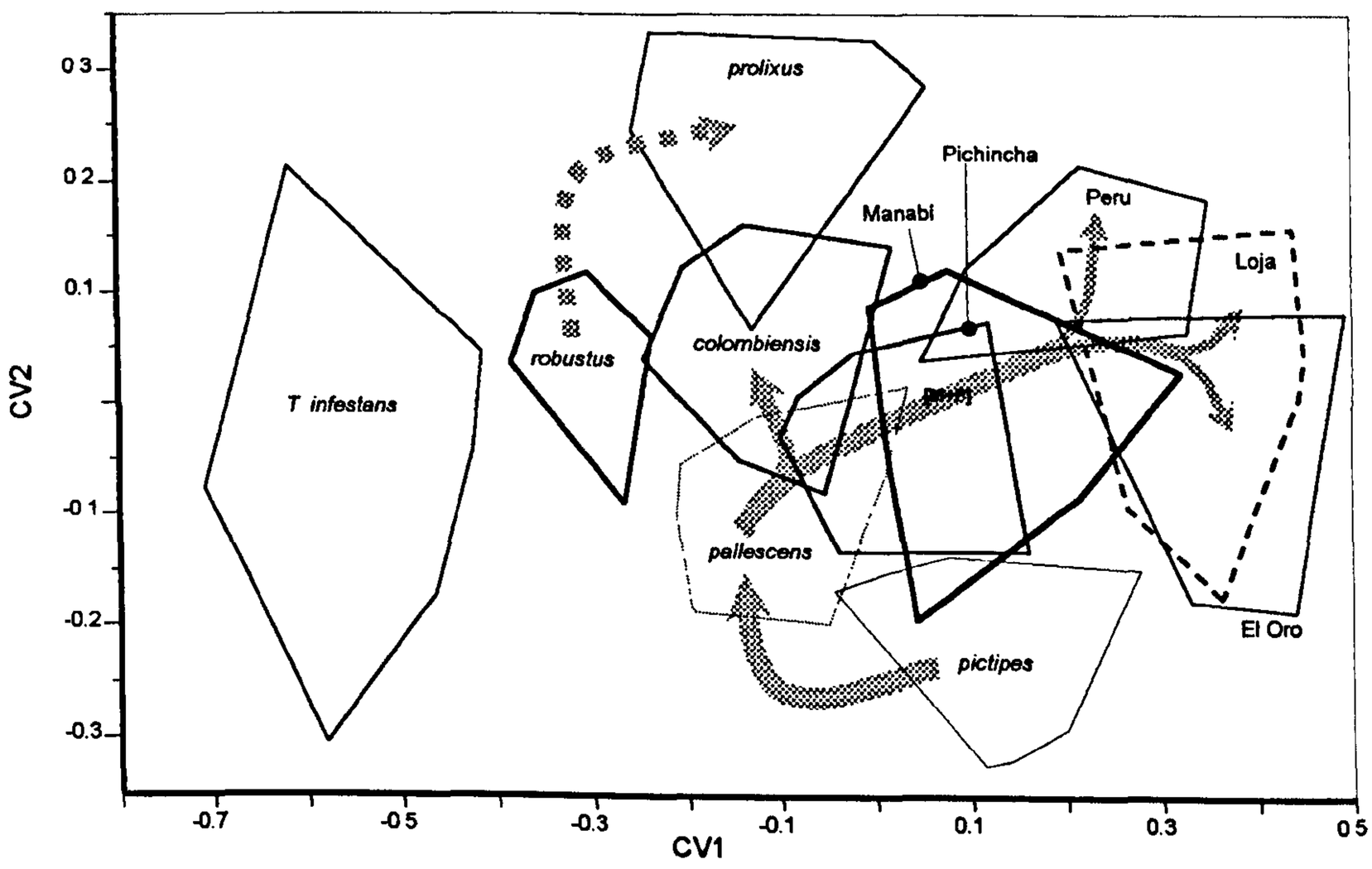


Figure 129. Canonical variate analysis of six *Rhodnius* species: linear regression residuals (PC1 vs. measurements) were used as size-free variables for discriminant analysis. Grey arrows represent tentative evolutionary routes; [M+P]=Manabí+Pichincha *R. ecuadoriensis* populations

An analysis restricted to the 'Pacific *Rhodnius* lineage' representatives suggests a close relationship between sylvatic *ecuadoriensis* populations and *pallescens-colombiensis*. Synanthropic Ecuadorian *R. ecuadoriensis* occupy a different branch, and Peruvian bugs cluster with sylvatic populations. The factorial map derived from CVA is suggestive of an axis of progressive north-to-south shape variation that might be interpreted as a putative evolutionary route starting from an ancestral form similar to *R. pallescens* [*pallescens* → *colombiensis* → Pichincha → Manabí → domestic *ecuadoriensis*].

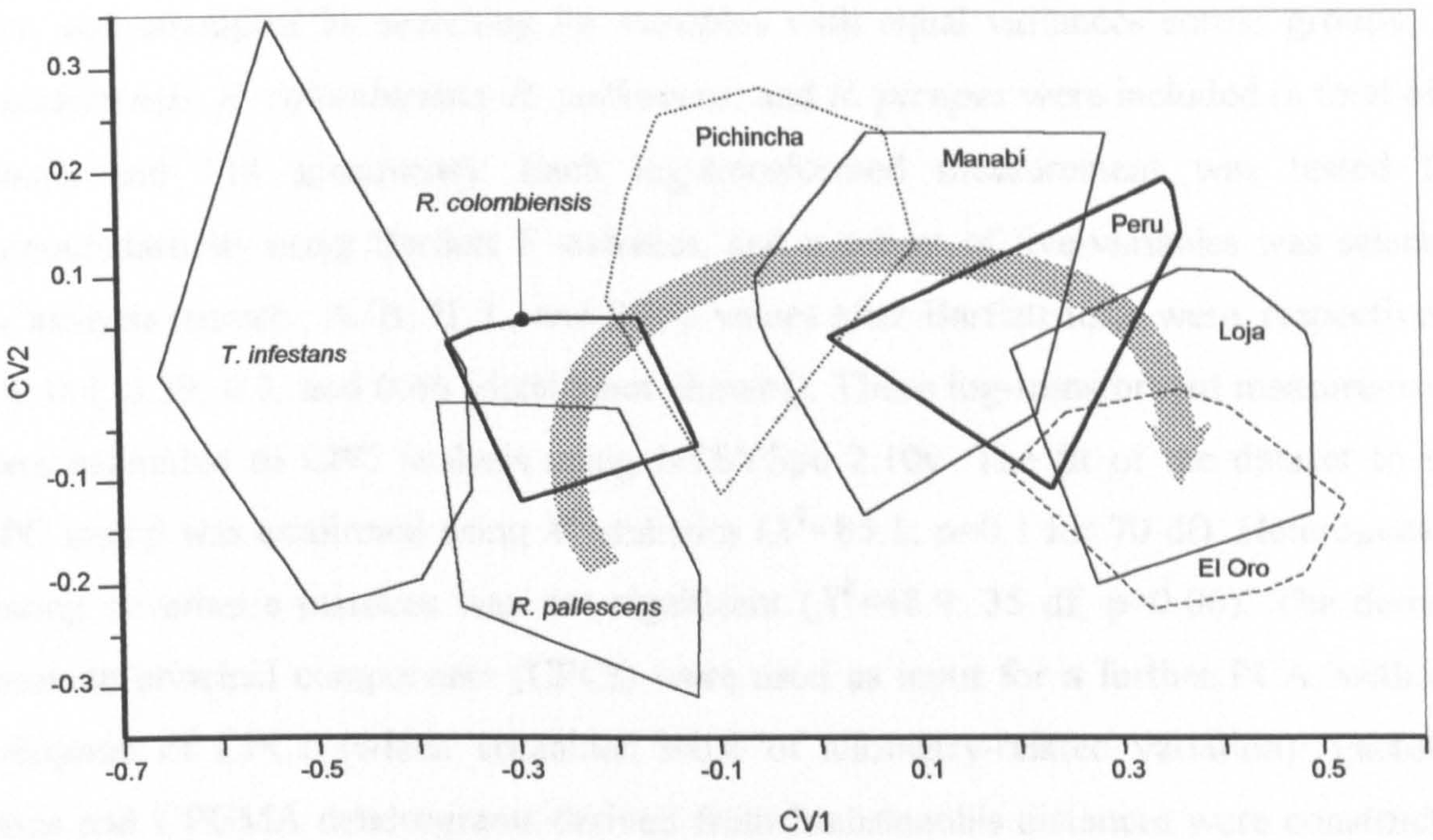


Figure 130. Canonical variate analysis of the *Rhodnius* species within the 'Pacific lineage': linear regression residuals were used as size-free variables for discriminant analysis. The grey arrow represents a tentative evolutionary route linking *R. pallescens* and *R. ecuadoriensis* through a north-to-south axis of progressive shape variation

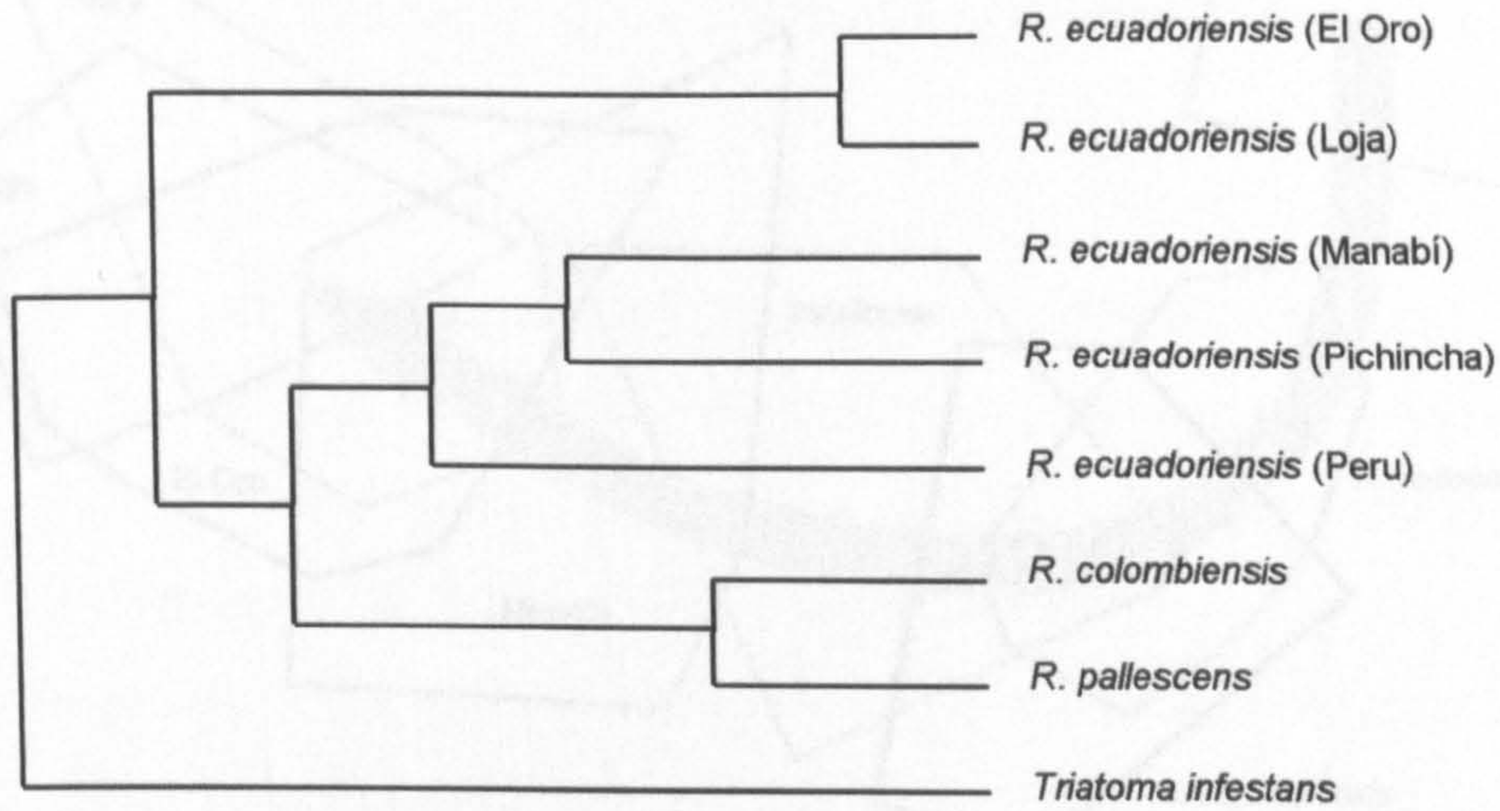


Figure 131. Relationships in the 'Pacific *Rhodnius* lineage': UPGMA dendrogram based on Mahalanobis distances (CVA on PC1 vs. log-shape ratios linear regression residuals)

7.2.3.3. Allometry-free analysis

Allometry-free analysis of closely related species (likely to share a common growth axis) was attempted by searching for variables with equal variances across groups; *R. ecuadoriensis*, *R. colombiensis*, *R. pallescens*, and *R. pictipes* were included (a total of 8 groups and 118 specimens). Each log-transformed measurement was tested for homoscedasticity using Bartlett F statistics, and a subset of five variables was selected for analysis (namely, A, B, H, L, and R3; p values after Bartlett tests were, respectively, 0.8, 0.4, 0.29, 0.3, and 0.46 [details not shown]). These log-transformed measurements were submitted to CPC analysis using NTSYSpc 2.10y. The fit of the dataset to the CPC model was confirmed using X^2 statistics (X^2=85.1; p=0.1 for 70 df). Heterogeneity among covariance matrices was not significant (X^2=48.9, 35 df, p=0.06). The derived common principal components (CPCs) were used as input for a further PCA, with the exception of CPC1 (which contained most of allometry-related variation). Factorial maps and UPGMA dendrograms derived from Mahalanobis distances were constructed after CVA.

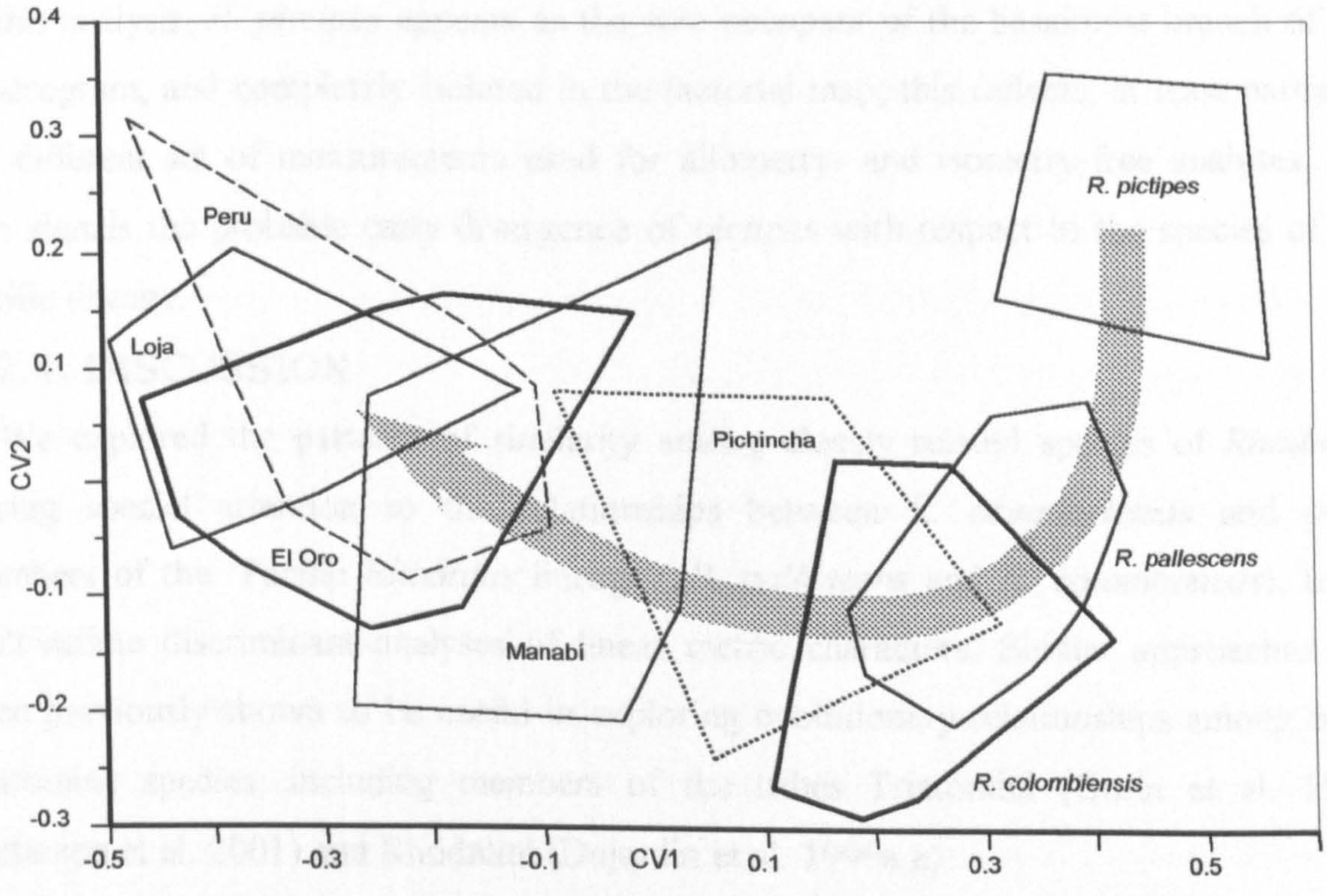


Figure 132. Allometry-free canonical variate analysis of four *Rhodnius* species: factorial map. Five log-transformed head measurements were used for common principal component (CPC) analysis; four derived CPCs (all except the first one, which represents allometric growth) were used as input for further discriminant analysis, and individual scores plotted on the discriminant space defined by the first two canonical vectors (CV1 and CV2). The grey arrow represents a tentative evolutionary route linking *R. pictipes* and *R. ecuadoriensis*

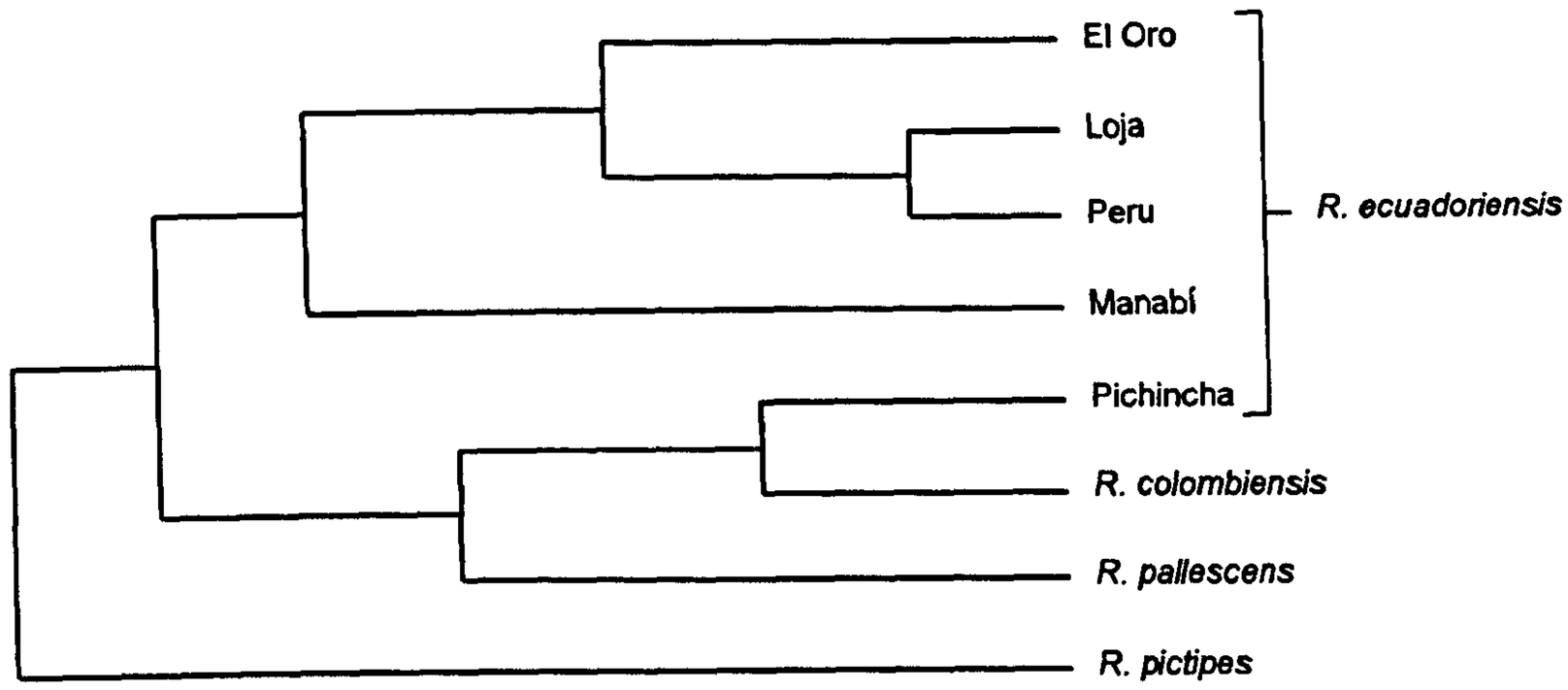


Figure 133. Allometry-free canonical variate analysis of four *Rhodnius* species: UPGMA dendrogram derived from Mahalanobis distances

These results confirmed that at least sylvatic bugs from Pichincha seem to share much of their head morphometric traits with *R. colombiensis*, suggesting a close relationship between both groups and leaving uncertainties about the true phylogenetic relationships between *ecuadoriensis* and *colombiensis*. The striking amount of phenetic variability found in *R. ecuadoriensis* was confirmed after removing allometric growth. In this analysis, *R. pictipes* appears as the sole occupant of the basalmost branch of the dendrogram, and completely isolated in the factorial map; this reflects, at least partially, the different set of measurements used for allometry- and isometry-free analyses, but also signals the probable early divergence of *pictipes* with respect to the species of the Pacific lineage.

7.2.4. DISCUSSION

We explored the patterns of similarity among closely related species of *Rhodnius*, paying special attention to the relationships between *R. ecuadoriensis* and other members of the 'Pacific *Rhodnius* lineage' (*R. pallescens* and *R. colombiensis*), using multivariate discriminant analyses of linear metric characters. Similar approaches had been previously shown to be useful in exploring evolutionary relationships among other triatomine species, including members of the tribes Triatomini (Gorla et al. 1997, Patterson et al. 2001) and Rhodniini (Dujardin et al. 1999a,e).

The first feature that became apparent in our study was a sharp **decline in size** involving the species of the 'Pacific lineage'; the largest of them (*R. pallescens*) occurs in the northernmost extreme of the geographic range of the group, and the smallest forms (synanthropic *R. ecuadoriensis*) in the southernmost areas. It has been proposed

that triatomine populations tend to be significantly smaller in the peripheral areas of their range, with larger bugs generally occurring near the putative centre of origin of the species; it was also suggested that a general decrease of genetic variability accompanies such clinal reduction of global size (Dujardin et al. 1998a, 1999d).

In our study, progressive size decline was associated with a likewise gradual **variation in head shape**, as revealed by both isometry- and allometry-free analyses; this was also confirmed by the results of CVA on 'form variables' derived from linear regression of log-transformed measurements on the first PC. Together, these results suggest the existence of a phylogenetic continuum connecting *R. pallescens* with the very closely related *R. colombiensis* and the various forms of *R. ecuadoriensis*. The link between these two latter seems to involve the northern sylvatic populations of *R. ecuadoriensis*, which in fact tended to cluster together with *colombiensis* and *pallescens* in UPGMA analyses – even if separation was clear on bidimensional factorial maps.

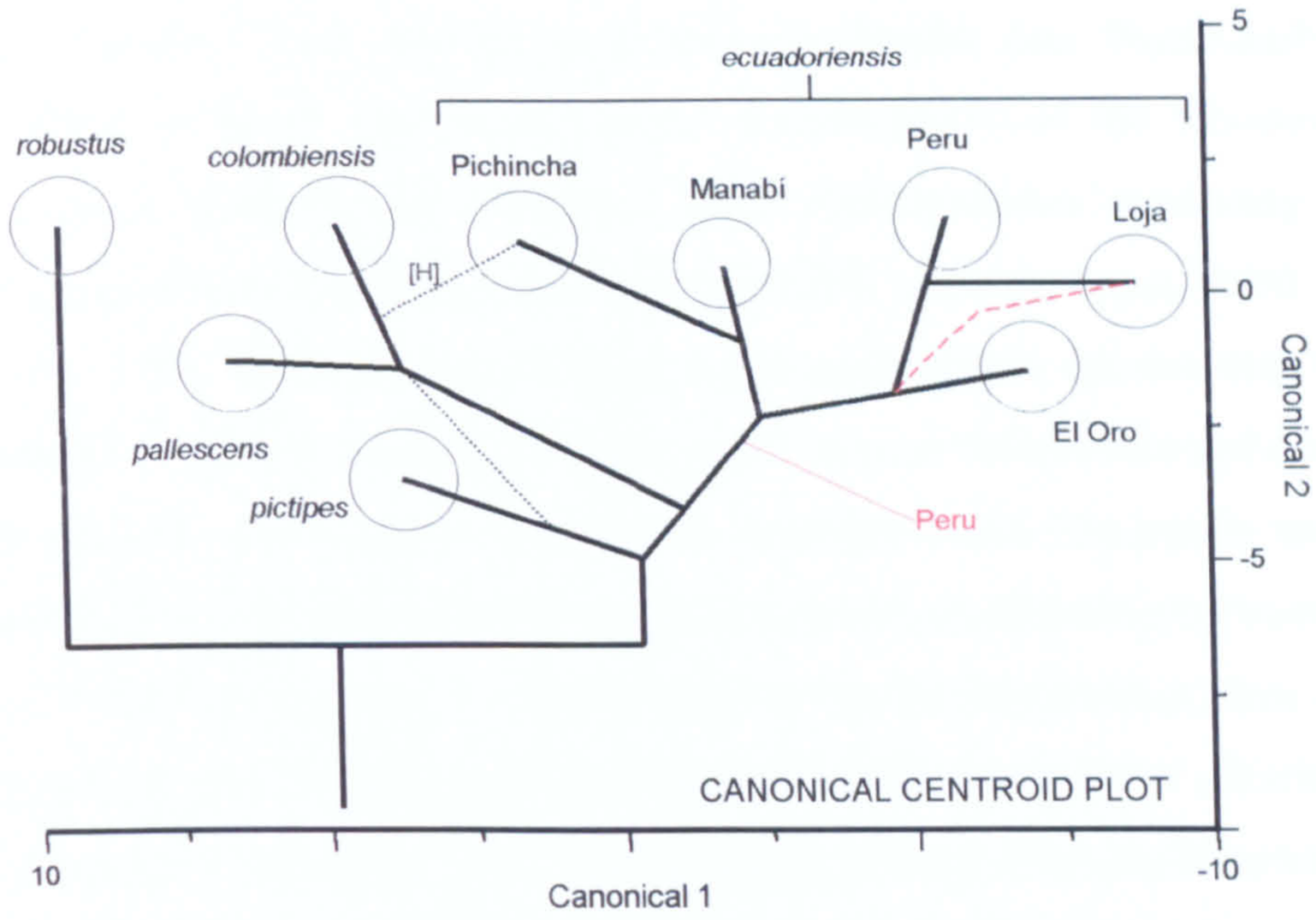
The fact that apparently spurious clusters (including species known to be distant relatives) persistently appeared in some UPGMA dendrograms is likely to reflect mere size similarities. Bidimensional discriminant plots (factorial maps) were useful in clarifying these relationships; they revealed for instance that the position of *R. pictipes* as a sister group to *R. colombiensis* (figure 126) was due to similar CV1 scores, and showed good separation of the taxa on CV2, much less influenced by size.

Overall, the picture that emerged from the metric analyses presented here places *R. pallescens* and *R. colombiensis* as very closely related entities; this is in agreement with both their relative geographic distributions (parapatric in north-western Colombia) and ecological preferences (with sylvatic populations strongly associated with *Attalea* palm trees). Northern sylvatic specimens of the highly variable *R. ecuadoriensis* are similar to the former pair of species, and represent the largest-sized population in a clinal series of gradual size reduction (and shape variation) within *ecuadoriensis*. This series involves intermediate forms in central coastal Ecuador (the essentially sylvatic Manabí population) and synanthropic populations in southern Ecuador and northern Peru. The possibility that Pichincha phenotypes are derived (Sections 6.1. and 6.3.) would however mean that similarities with *colombiensis* could be homoplasious, with plesiomorphic (Manabí) forms being readily distinguishable from the Colombian species.

The position of *R. pictipes* seems less clear; it shows however a consistent tendency to cluster with the members of the 'Pacific lineage' rather than with 'eastern' species such as the sympatric (Amazonian) *R. robustus*. The relationship between *pictipes* and the members of the 'Pacific clade' is not fully resolved. Phenetic similarities involving colouration patterns and overall size have been used to propose a close relationship with *pallescens*, *ecuadoriensis* and *colombiensis* (Lent & Wygodzinsky 1979, Schofield 1994). Isoenzyme analyses have shown a close association of *pictipes* with two Amazonian species, the almost isomorphic *R. stali* and the very distinct (both phenetically and ecologically) *R. brethesi* (Dujardin et al. 1999a,e, Chávez et al. 1999, Monteiro et al. 2002). Other studies (in which *brethesi* was not included) confirmed the close relationship between *pictipes* and *ecuadoriensis* by means of antennal sensilla patterns (Catalá & Schofield 1994, Catalá 1996) and RAPD profiles (García et al. 1998). Sequence analysis of the mitochondrial 16S ribosomal RNA gene produced similar results (Stothard et al. 1998b), and other comparisons (including fragments of mitochondrial and nuclear genes) also grouped *pictipes* with members of the 'Pacific lineage' – but with low bootstrap support values (Monteiro et al. 2000, 2001).

It is tempting to hypothesise that the gradual changes (in size, shape and form) observed here, involving all the species of the 'Pacific lineage' and possibly also *R. pictipes*, are a reflection of the evolutionary relationships among these taxa. This hypothesis, advanced by Schofield and Dujardin (1999), would entail a northern origin of the lineage, with an ancestral population perhaps related to *Attalea* palms (a favoured habitat of several *Rhodnius* species on both sides of the Andes) in the seasonally dry forests of northwest Colombia, with a subsequent southward dispersal and colonisation of new palm tree habitats. Adaptive radiation would have given rise to the different forms of the lineage, including the relatively widespread and eclectic *R. pallescens* (known from four countries and from various habitats, including palms of at least 5 genera and human-related environments), the geographically restricted *R. colombiensis* (only known from *A. butyracea* palms, with a few records from human habitats), and *R. ecuadoriensis* (associated with *Ph. aequatorialis* and human environments) (Jaramillo et al. 2000, Vallejo et al. 2000, Abad-Franch et al. 2000, 2001a, 2002, Cuba Cuba et al. 2002). The polarity of the connection between these species would be given by the wider range and ecological valence of the northern *R. pallescens*, which presents in

addition obvious morphological similarities with *R. pictipes* (the closest Amazonian relative of the group). Results of morphometric analyses presented here could be interpreted in the same sense, with *pallescens* consistently occupying the tip (as defined by similarity with *pictipes*) of the axis of progressive variation in size, shape, and form that seems to run from north to south across north-western South America.



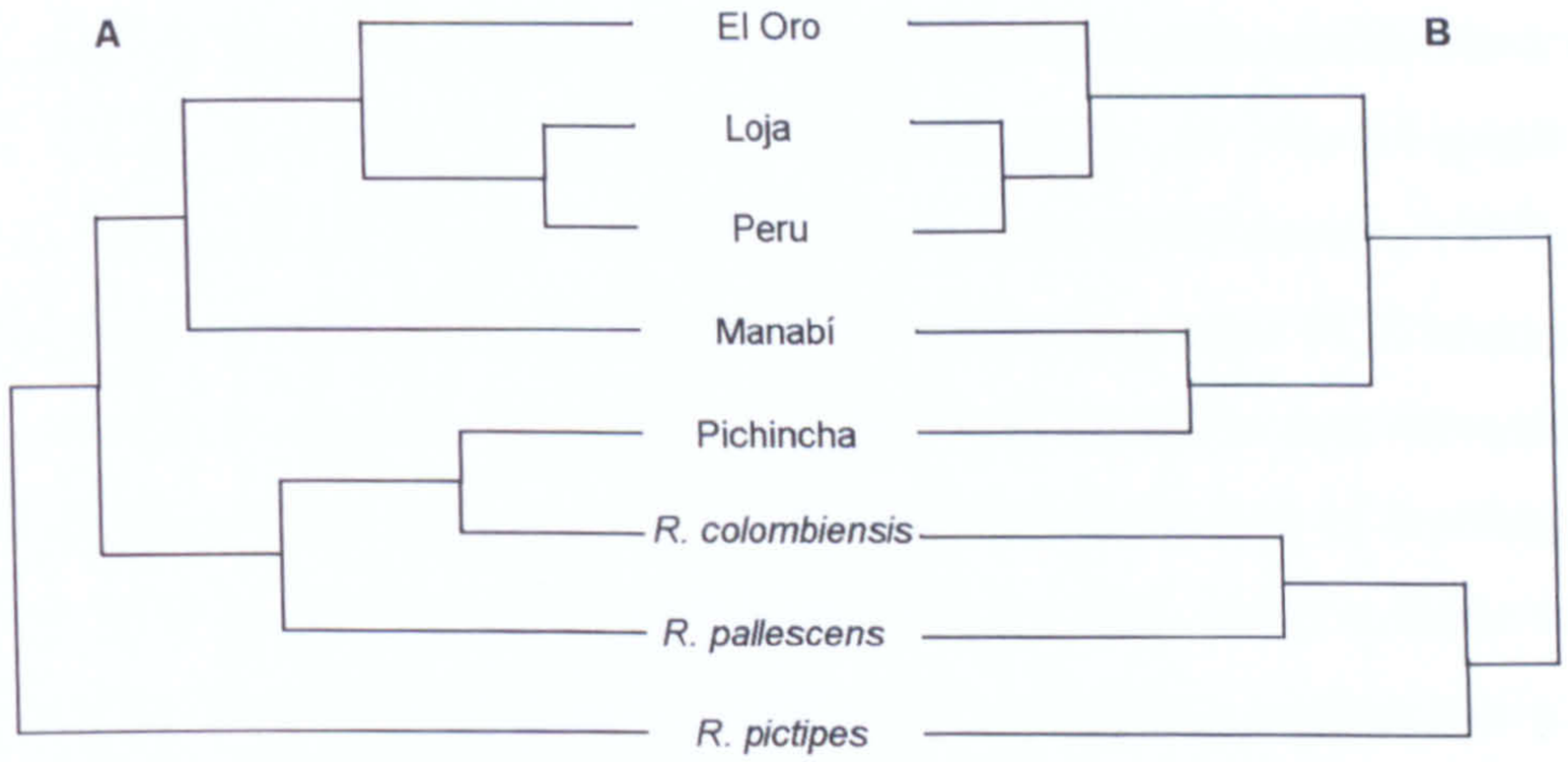


Figure 134. Phylogeny of the Pacific *Rhodnius* lineage: a morphometric approach. The canonical centroid plot (upper) was derived from CVA (7 log-transformed head measurements; see text for details); centroids represent mean group phenotypes (considered as the units of interest in the multivariate evolutionary model; Sorensen 1992). A phyletic network connecting centroids was overlaid on the plot; it was based on the dendrograms shown below and, partially, on prior knowledge on the relationships of the different species (essentially, the root of the network was placed between *robustus* and *pictipes*). The dendrograms were derived from different analyses [**A**: UPGMA tree derived from Mahalanobis distances after allometry-free CVA; **B**: single linkage hierarchical cluster analysis using mean values of CV1, CV2, and CV3 (distances between clusters are the minimum distances between observations in both clusters)]. Broken lines in the network represent alternative topologies (solid lines are the preferred alternatives). The alternative position of the Peruvian population of *ecuadoriensis* (in red) is derived from intraspecific size-free analyses (Section 6.2.), and was considered plausible after joint assessment of morphometric and biogeographic data; in this case, the Loja branch joins the network at the node from which El Oro diverges (red dotted line). [H] indicates probable homoplasy in the clustering of Pichincha with *colombiensis*

7.3. Molecular phylogenetics: evolution of the 'Pacific *Rhodnius* lineage'

7.3.1. INTRODUCTION

Several studies have been conducted to explore phylogenetic relationships among Triatominae (at various levels of divergence) using molecular data. Particularly relevant to the scope of this work are the confirmation of monophyly of the Rhodniini and the paraphyletic nature of the genus *Rhodnius* (with *Psammolestes* appearing as a highly specialised branch within *Rhodnius*) (Lyman et al. 1999, Monteiro et al. 2000, 2001, 2002, Marcilla et al. 2001). Especially significant is the problematic relationship between the species of *Rhodnius* found west of the Andes (*pallescens*, *colombiensis* and *ecuadoriensis*) with their congenerics in the Amazon-Orinoco drainage basins. On purely morphological grounds, Lent and Wygodzinsky (1979) suggested a close relationship between the species presenting a mottled pattern and well-developed 2+2 scutellar carinae, thus clustering *R. pictipes*, *pallescens* and *ecuadoriensis* as sister taxa; following these criteria, and taking also into account characteristics of the male genitalia (shape of the phallosome and median process of the pygophore, and presence of phallosome supports in some taxa), the group would also include *R. stali*, *R. amazonicus*, and perhaps *R. paraensis* (Sherlock et al. 1977, Lent et al. 1993, Schofield & Dujardin 1999, Bérenger & Pluot-Sigwalt 2002). *R. colombiensis*, identified at first as 'sylvatic *prolixus*' (López & Moreno 1995), was easily separated from the latter by allozymes, RAPD and other diagnostic PCR assays; this led to the reconsideration of its morphological traits, and the population was elevated to specific rank and placed as closely related to *R. pallescens* (despite the lack of mottling [except for the presence of irregular dark spots in the coxae and a dark distal extreme of tibiae], *R. colombiensis* shares with the parapatric *pallescens* and with *ecuadoriensis* a phallosome with an M-shaped upper margin) (López & Moreno 1995, Chávez et al. 1999, Dujardin et al. 1999a,e, Moreno et al. 1999, Jaramillo et al. 2001). A further, non-mottled Amazonian species of *Rhodnius* was found to belong to the *pictipes* cluster too; *R. brethesi* had allozyme (Dujardin et al. 1999a, Monteiro et al. 2002) and mtDNA sequence (Lyman et al. 1999, Monteiro et al. 2000, 2001) profiles similar to those of *pictipes* and *stali*, despite clear phenotypic dissimilarity. The possible inclusion of the disputed *R. dalessandroi* into this clade is based only on morphological considerations (Schofield & Dujardin 1999); it

has been compared to *R. brethesi* (Martínez 1984), but examination of the (deteriorated) type material (Fiocruz, Brazil) seems to favour a closer association with the *prolixus* group, as it was also noted by Barrett (1991; p. 147). The 'phenotypic *pictipes* group' is therefore comprised of 8 known species; 5 of them (*R. pictipes*, *stali*, *brethesi*, *paraensis*, and *amazonicus*) occur east of the Andes in the Amazon-Orinoco drainage system, whereas a clade of three closely related species is only found west of the Andes (*pallescens*, *colombiensis*, and *ecuadoriensis*)•. What are the precise relationships between these two geographic clades, and those between them and the rest of Rhodniini (comprising mainly the *prolixus* group and *Psammolestes*, all with natural ranges not reaching the western slope of the Andes)•, remains somewhat problematic. Some genetic studies did favour the clustering of *pictipes-stali-brethesi* with *pallescens-colombiensis-ecuadoriensis* (Schofield & Dujardin 1999, Monteiro et al. 2000, 2002), but others placed the *pallescens* clade as a basalmost lineage, with the *prolixus* and *pictipes* groups appearing as sister clades (Chávez et al. 1999, Dujardin et al. 1999a,e, Lyman et al. 1999, Monteiro et al. 2001). The first possibility is consistent with phenotypic traits, whereas the second fits better the respective biogeographical features of the groups under consideration. The difficulties in resolving the issue are further illustrated by the poor statistical support of the mutual relationships among the three clades in phylogenetic analyses of DNA sequence data; they either form a basal, unresolved polytomy or appear in clades with bootstrap support values below 75% (Lyman et al. 1999, Monteiro et al. 2000, 2001).

The following figures illustrate the current difficulties in establishing the mutual phylogenetic relationships among the main groups of species within the Rhodniini, with emphasis on the problematic placement of the *pictipes* group as a sister taxon of either the phenetically related members of the *pallescens* group or the biogeographically related species of the *prolixus* group. The trees were drawn manually taking into consideration the results of the various works cited above, and are rather speculative with regard to the

---

•Note however that there is a single record of *R. pictipes* from Belize (Lent & Wygodzinsky 1979), and that one species with uncertain affiliations, the almost totally black and xerophylic *R. neivai*, has been reported from both Venezuela and the Magdalena valley in Colombia (Barrett 1991). Finally, domestic populations of *R. ecuadoriensis* have been reported from the upper (arid) stretches of the Huancabamba river valley (a tributary of the Amazon) in northern Peru (Herrer et al. 1972, Cuba Cuba et al. 2002), and likewise domestic *R. prolixus* populations are common in northwestern Colombia and parts of Central America. These findings constitute however exceptions to the generally well-defined status of the Andes as a biogeographical barrier to the dispersal of natural (sylvatic) populations of extant *Rhodnius* species

position and relationships of some little-known species (e.g. *R. dalessandroi*, *R. amazonicus*, *R. paraensis*, or *R. milesi*) and the arrangement of some terminal branches (e.g. *nasutus-neglectus*).

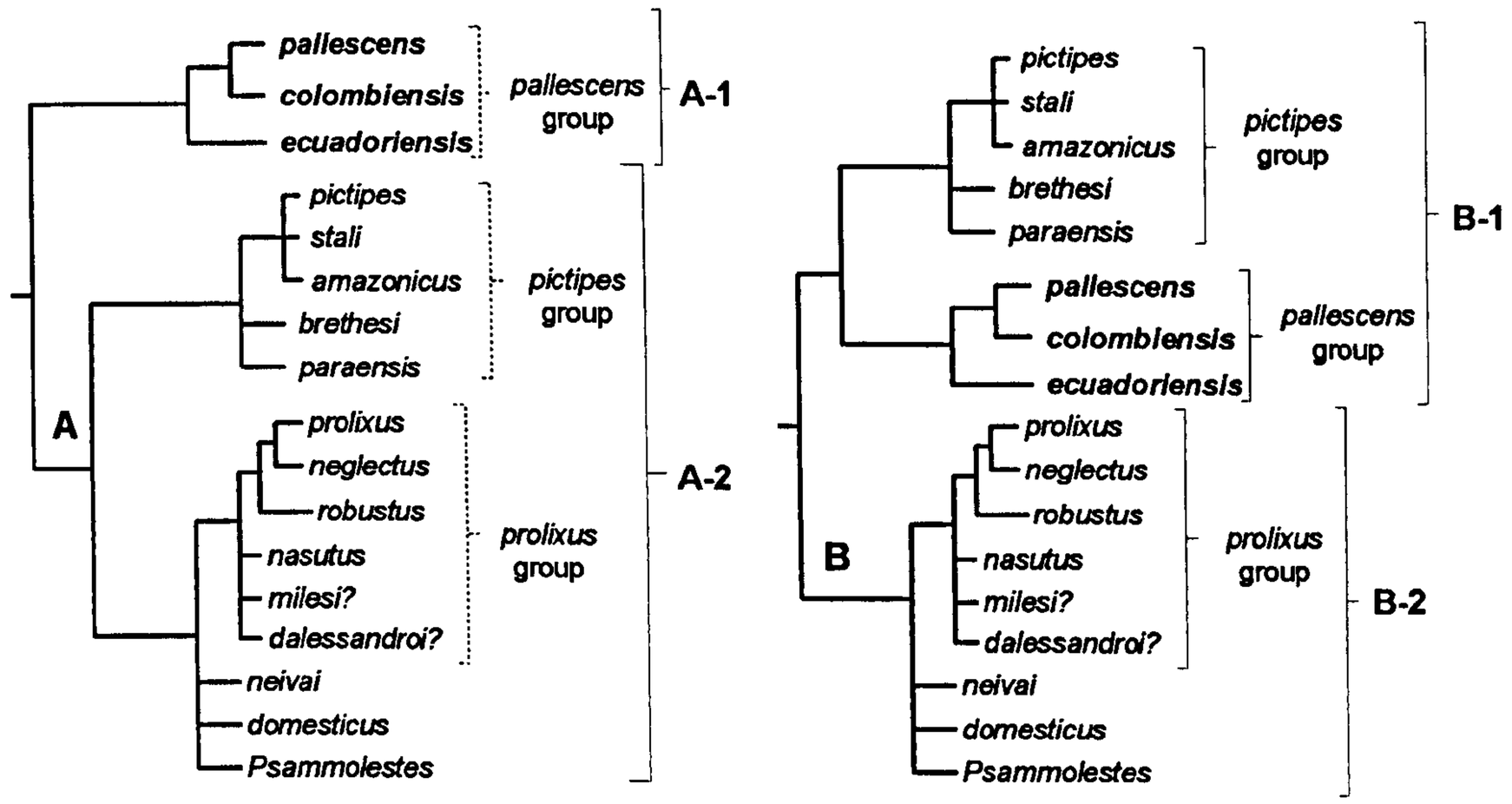


Figure 135. Phylogenetic relationships among the main species groups within the Rhodniini. **A**: The *pallescens* group appears in a basal position, and the *pictipes* and *prolixus* clades are sister groups; this arrangement agrees with biogeographical data, with the *pallescens* group restricted to the western slope of the Andes (Pacific clade, **A-1**) and separated from an Amazon-Orinoco clade (**A-2**) including the *prolixus* and *pictipes* groups. **B**: The basal bifurcation yields two clades, one including the *pallescens* and *pictipes* groups (**B-1**), and the other one comprised of the *prolixus* group species (**B-2**); this is in agreement with phenotypic traits. Unequivocal molecular results allowing for the rejection of either of these hypotheses (both of which provide intuitively appealing explanations) are not available

In the first part of our investigation, multivariate analysis of head measurements could not settle this phylogenetic question, partly because of the apparently homoplastic nature of some size-related characters and partly because of the noise introduced by extreme phenotypic variability within *R. ecuadoriensis*.

In order to further explore these phylogenetic hypotheses, independent evidence provided by molecular data (in the form of partial sequences of the mitochondrial cytochrome *b* [mt cyt*b*] gene) was analysed; essentially, these data involved the same species and populations studied by means of linear morphometrics, but the cyt*b* sequences of several further taxa, represented by specimens from various geographic and ecological origins, were kindly made available by FA Monteiro (CDC-Fiocruz).

7.3.2. MATERIALS AND METHODS

Partial sequences (663bp) of the mitochondrial cyt*b* gene were obtained from 102 specimens (72 *R. ecuadoriensis*, 5 *R. colombiensis*, 9 *R. pallescens*, 2 *R. pictipes*, 6 bugs classified as *R. robustus*, 5 *R. prolixus*, 1 *R. nasutus*, 1 *T. rubrovaria*, and 1 *T. dimidiata*). The sequences of *R. pictipes*, *R. nasutus*, four *R. pallescens*, *T. rubrovaria*, and various haplotypes of the *prolixus-robustus* group (except for one specimen preliminarily classified as *R. robustus* collected by us in Sucumbíos, Ecuador, and two Venezuelan *R. prolixus* specimens collected by S Fitzpatrick and JS Patterson) are part of a wider research on the phylogeny of the Rhodniini conducted by FA Monteiro and collaborators, and were used here with kind permission from Dr Monteiro. The *T. dimidiata* sequence was retrieved from GenBank (accession number AF301594; Dotson & Beard 2001). Phylogenetic analyses were based on both genetic distances and nucleotide character states. Further details on the methods used are presented in Section 6.3.2.

7.3.3. RESULTS

7.3.2.1. Basic statistics

Analyses were conducted first on an alignment of 88 sequences (662bp) corresponding to *R. ecuadoriensis* and its closest relatives within the 'Pacific *Rhodnius* lineage' (*R. pallescens* and *R. colombiensis*); the cyt*b* sequence from two *R. pictipes* were included as the outgroup for the analyses.

Table 86. Nucleotide composition (mt cyt*b*, 663bp) in four *Rhodnius* species (%)

Species	T	A	C	G	A+T
Rhodnius ecuadoriensis	37.8	31.6	17.9	12.7	69.4
Rhodnius colombiensis	37.4	32.1	17.8	12.7	69.5
Rhodnius pallescens	37.5	31.5	17.6	13.4	69
Rhodnius pictipes	36.3	32.1	18.6	13	68.4
Overall average	37.7	31.6	17.9	12.8	69.3

Out of the 663 nucleotide positions examined, 168 (25.34%) were variable, with four autapomorphs (unique to single individuals in the complete dataset) and 164 parsimony-informative substitutions. Considering the ingroup only, 132 sites were variable (19.9%); of these, 127 were parsimony-informative and five were autapomorphs: a C/A transversion (site 385, 1st codon position), an A/G transition (site 471, 3rd codon position), and a T/C change (site 550, 3rd codon position) in one *R. ecuadoriensis* haplotype (MN-5, found in a

single bug from Manabí); a T/C mutation at position 504 in the LJ-1 haplotype (a single *R. ecuadoriensis* specimen from Loja); and a C/T transition in a *R. pallescens* specimen from Vegachí, Antioquia, Colombia (site 618, 3rd codon position). Overall, 17 unique haplotypes were found in the 88-sequence dataset (15 excluding the outgroup). The deduced peptide sequence (221 amino acid long) presented 15 variable elements (6.8%) in the ingroup; 14 of these were parsimony-informative, with MN-5 presenting an autapomorphic change (Leucine to Isoleucine, codon 129, sites 385-387). The inclusion of the outgroup increased the figure to 20 variable amino acids (9.05%), 19 of them parsimony-informative.

Haplotype variability was higher in *R. ecuadoriensis*, with 10 unique haplotypes (72 bugs from five geographic areas). Intra-specific variability in other *Rhodnius* species was not a matter of investigation within this project, and sample sizes were therefore limited. A single, unique cyt*b* haplotype was detected in five laboratory-reared *R. colombiensis* specimens from a colony (University of Antioquia, Medellín, Colombia; bugs were kindly provided by NO Jaramillo) originally founded with 14 bugs collected in 1991 in *Attalea butyracea* palms in the central Magdalena valley (Coyaima, Tolima, Colombia). Colombian *R. pallescens* specimens were obtained from the same laboratory (undetermined origin and founding date), but here two different haplotypes were identified in the five specimens analysed; four additional *R. pallescens* cyt*b* sequences (one bug from Vegachí, Colombia, and 3 field-collected bugs from southern Nicaragua [CJ Schofield]) were made available by FA Monteiro (CDC, USA-Fiocruz, Brazil) for comparison. Finally, two *R. pictipes* haplotypes comprised our outgroup for this part of the study. These haplotypes were coded as shown in the following table (for details on *ecuadoriensis* haplotypes see Section 6.3.).

Table 87. Haplotype codes for phylogenetic analyses in the 'Pacific *Rhodnius* lineage'

Haplotype	Species	Group	Remarks
MN-1 to 6	*R. ecuadoriensis*	Manabí	Six haplotypes found in 14 bugs
EO	*R. ecuadoriensis*	El Oro	One haplotype in 8 bugs; identical to MN-4
PH	*R. ecuadoriensis*	Pichincha	Found in all 20 bugs of this group; identical to MN-6
LJ-1 to 3	*R. ecuadoriensis*	Loja	Three haplotypes found in 15 bugs
PE	*R. ecuadoriensis*	Peru	Unique to the group (14 bugs)
COL	*R. colombiensis*	Tolima	One haplotype in five bugs
PAL-1	*R. pallescens*	Laboratory UA	Shared by three bugs
PAL-2	*R. pallescens*	Laboratory UA	Shared by two bugs
PAL-V	*R. pallescens*	Vegachí (FA Monteiro)	One haplotype in one specimen
PAL-N	*R. pallescens*	Nicaragua (CJ Schofield)	One haplotype in three bugs
PIC-1	*R. pictipes*	Outgroup (FA Monteiro)	One haplotype in one specimen (Pará, Brazil)
PIC-2	*R. pictipes*	Outgroup (CJ Schofield)	One haplotype in one specimen (Amazonia)

Analyses were performed on an alignment containing only one example of each of these haplotypes (Appendix). Under this arrangement, 144 out of the 168 segregating sites were parsimony-informative, and 24 were autapomorphs. Transition:transversion ratio was 5.1 (SE=1.01) for the entire haplotype dataset and 6.09 (SE=1.29) for the ingroup. Separate analysis of pairwise transitional and transversional substitutions among the 17 unique haplotypes showed feeble indications that saturation of transitions might be occurring between the most distantly related pairs. Bivariate plots of TS and TV versus corrected genetic distances show that while transversions accumulate at a steadily increasing rate, the quadratic fit for transitions bends towards the *X* axis, even if very slightly; coefficients of determination of linear and second-order polynomial fits were largely equivalent. This trend seems more evident in inter-specific comparisons involving *R. pictipes*, whereas the TS plot is essentially linear for pairs including *pallescens*, *colombiensis*, and *ecuadoriensis*. Interestingly, pairwise comparisons between *pallescens* and *colombiensis* haplotypes seem to fall within the range of intra-specific variation (including *ecuadoriensis-ecuadoriensis*, *pallescens-pallescens*, and *pictipes-pictipes* haplotype comparisons).

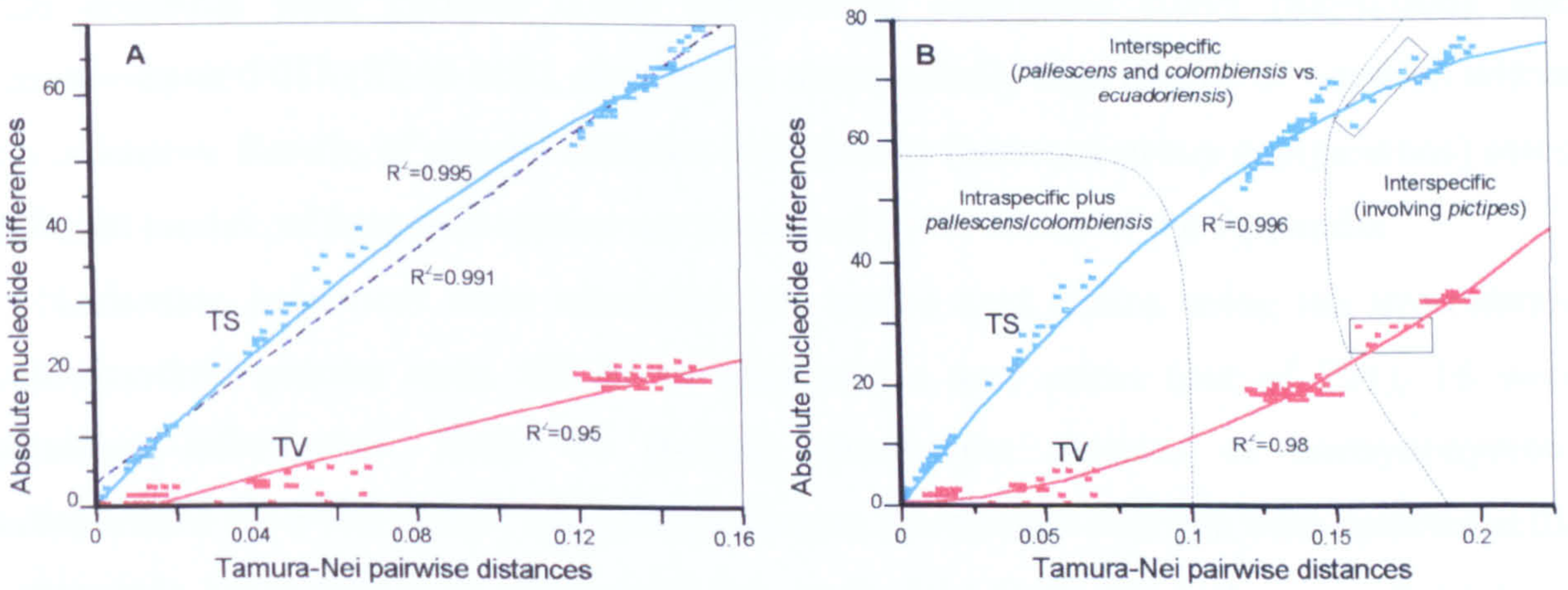


Figure 136. Transitional (**TS**) and transversional (**TV**) substitutions: pairwise comparisons among four *Rhodnius* species. Pairwise absolute differences were plotted against genetic distances corrected using the Tamura-Nei model. In **A**, comparisons involve haplotypes of *R. ecuadoriensis*, *R. pallescens* and *R. colombiensis*; transitional pairs (blue dots) seem to accumulate following a linear trend (R^2=0.991), although a quadratic fit (curved line) performed slightly better (R^2=0.995). In **B**, *R. pictipes* haplotypes (the outgroup sequences) were included; transitional substitutions (**TS**, blue dots) appear to be starting to saturate at the right end of the genetic distance axis (a linear fit produced a R^2 of 0.98, vs. 0.996 of the quadratic fit shown on the figure), whereas transversions (**TV**) accumulate at an increasing rate (linear regression R^2=0.95, vs. 0.98 of the quadratic fit shown). Dotted lines (in **B**) indicate the presence of three 'regions' in the plot (at low, intermediate, and high levels of divergence); the types of pairwise comparisons that fell within each of these regions are indicated. Small boxes enclose points corresponding to *pictipes* vs. *pallescens* and *colombiensis*

Variability was greater at third codon positions (as expected for a protein-coding gene), with 57% of sites (126 out of 221 third codon positions) presenting point mutations (i.e., 75% of variable sites were third codon positions); 15.84% of first positions were variable (35/221; 20.83% of variable sites were first codon positions), with second codon positions being highly conserved (3.17% [7/221] had changes; 4.17% of segregating sites were second codon positions). Overall nucleotide diversity (π) was 0.087 (SE=0.007) (0.069 [SE=0.006] when the *pictipes* haplotypes were excluded). Including *pictipes*, there were three segregating bases at 20 sites (51, 66, 72, 96, 99, 222, 234, 249, 276, 288, 289, 385, 447, 453, 456, 531, 537, 576, 590, and 633), and at sites 54, 291, and 543 all four bases were segregating; changes were silent for sites 54 and 543, but not for site 291 (a third position in a codon whose first nucleotide was also variable, resulting in 6 different triplets and 3 amino acids; see codon 97 in table 88). Nucleotide diversity among haplotypes was computed separately for *R. pictipes* (π=0.006, SE=0.003), *R. pallescens* (π=0.043, SE=0.005), and *R. ecuadoriensis* (π=0.018, SE=0.003). The associated within-group mean K2p distances were: *pictipes* 0.006 (SE=0.003), *pallescens* 0.045 (SE=0.006), and *ecuadoriensis* 0.018 (SE=0.003), showing an unexpectedly high degree of variation within *R. pallescens*. Results of genetic distance calculations (between-group comparisons) using different models of base substitution are presented in the tables of the Appendix.

Nucleotide sequences were translated into amino acid chains using the invertebrate mitochondrial genetic code. Of 20 variable amino acid states (out of 221), 16 were parsimony-informative. Table 88 (below) shows the patterns of nonsynonymous substitutions observed in the 17-haplotype dataset. Seven such changes were detected at the subspecific level: five in *R. pallescens*, two in *R. ecuadoriensis*, and one in *R. pictipes*. Amino acids found at highest frequencies were Leucine (averaging 16.4% in the 17 sequences), Isoleucine (10.25%), Phenylalanine (8.78%), Proline and Glycine (both with 6.79%), Valine (6.68%), and Alanine (5.51%). These frequencies were practically constant across haplotypes.

Table 88. Nonsynonymous substitutions in 17 mitochondrial cyt*b* haplotypes from four *Rhodnius* species

Codon (sites)	Position 1	2	3	Haplotype(s)*	Amino acid	Remarks
3 (7, 8, 9)	G	T	A	PIC	Valine	*pictipes*
	G	T	T	PAL, COL	Valine	*pallescens, colombiensis*
	A	T	T	MN, EO, PH, LJ, PE	Isoleucine	All *ecuadoriensis*
44 (130, 131, 132)	C	T	T	PIC	Leucine	*pictipes*
	T	T	A	PAL-V, PAL-2, PAL-N	Leucine	Some *pallescens*
	T	T	T	PAL-1, COL	Phenylalanine	Some *pallescens* and *colombiensis*
	C	T	A	MN, EO, PH, LJ, PE	Leucine	All *ecuadoriensis*
68 (202, 203, 204)	G	C	T	PIC, PAL-V, PAL-1, PAL-N, COL	Alanine	*pictipes*, most *pallescens* and *colombiensis*
	A	C	C	PAL-2	Threonine	Some *pallescens*
	G	C	C	MN, EO, PH, LJ, PE	Alanine	All *ecuadoriensis*
83 (247, 248, 249)	A	T	T	PIC	Isoleucine	*pictipes*
	G	T	G	PAL-1	Valine	Some *pallescens*
	G	T	A	All the rest	Valine	All but *pictipes* and PAL-1
94 (280, 281, 282)	A	A	T	All but PAL-2 and COL	Asparagine	*pictipes, ecuadoriensis* and some *pallescens*
	A	A	C	PAL-2	Asparagine	Only PAL-2
	A	G	T	COL	Serine	*colombiensis*
97 (289, 290, 291)	T	T	A	PIC	Leucine	*pictipes*
	A	T	C	PAL-V, PAL-1	Isoleucine	Two *pallescens* haplotypes
	A	T	T	PAL-2, PAL-N	Isoleucine	Two *pallescens* haplotypes
	A	T	A	COL	Methionine	Only *colombiensis*
	C	T	G	MN-1,2,3,5, PE	Leucine	Most Manabí and Peru
	C	T	A	MN-4(EO), MN-6(PH). LJ	Leucine	Manabí, El Oro, Pichincha, and Loja
102 (304, 305, 306)	G	A	T	All but *ecuadoriensis*	Aspartic acid	All but *ecuadoriensis*
	A	A	C	MN, EO, PH, LJ	Asparagine	All Ecuadorian *ecuadoriensis*
	A	A	T	PE	Asparagine	Peruvian *ecuadoriensis*
121 (361, 362, 363)	A	T	T	All but *ecuadoriensis*	Isoleucine	All but *ecuadoriensis*
	G	T	T	MN, EO, PH, LJ	Valine	All Ecuadorian *ecuadoriensis*
	A	C	T	PE	Threonine	Peruvian *ecuadoriensis*
129 (385, 386, 387)	T	T	A	PIC	Leucine	*pictipes*
	C	T	T	All but PIC and MN-5	Leucine	All but *pictipes* and one Manabí haplotype
	A	T	T	MN-5	Isoleucine	Only MN-5
136 (406, 407, 408)	C	A	A	PIC	Glutamine	*pictipes*
	C	G	A	All the rest	Arginine	All but *pictipes*
151 (451, 452, 453)	G	T	A	PIC	Valine	*pictipes*
	A	T	C	PAL-2	Isoleucine	Only PAL-2
	G	T	C	PAL-N	Valine	Only PAL-N
	G	T	T	All the rest	Valine	All but *pictipes* and PAL-2
184 (550, 551, 552)	C	T	T	PIC	Leucine	*pictipes*
	T	T	T	PAL, COL	Phenylalanine	*pallescens* and *colombiensis*
	T	T	A	All *ecuadoriensis* but MN-5	Leucine	All *ecuadoriensis* but MN-5
	C	T	A	MN-5	Leucine	Only MN-5
190 (568, 569, 570)	G	C	A	PIC	Alanine	*pictipes*
	A	T	T	PAL-2, COL	Isoleucine	Some *pallescens* and *colombiensis*
	G	T	T	All the rest	Valine	Some *pallescens* and *ecuadoriensis*
197 (589, 590, 591)	G	T	A	PIC	Valine	*pictipes*
	A	A	A	PAL	Lysine	All *pallescens*
	A	C	A	COL and all *ecuadoriensis*	Threonine	All *ecuadoriensis* and *colombiensis*
208 (622, 623, 624)	G	C	A	PIC	Alanine	*pictipes*
	G	T	A	PAL	Valine	All *pallescens*
	A	T	T	COL and all *ecuadoriensis*	Methionine	All *ecuadoriensis* and *colombiensis*
211 (631, 632, 633)	A	T	T	PIC	Isoleucine	*pictipes*
	G	T	A	PAL	Valine	All *pallescens*
	A	T	C	COL	Isoleucine	*colombiensis*
	G	T	T	All *ecuadoriensis*	Valine	All *ecuadoriensis*
215 (643, 644, 645)	A	C	A	PIC	Threonine	*pictipes*
	A	G	A	All the rest	Serine	All the rest
217 (649, 650, 651)	A	C	C	PIC-1	Threonine	One *pictipes*
	G	C	C	PIC-2	Alanine	One *pictipes*
	A	C	T	PAL-N	Threonine	One *pallescens* haplotype
	G	C	T	All the rest	Alanine	All the rest
218 (652, 653, 654)	G	C	C	PIC	Alanine	*pictipes*
	T	C	C	COL	Serine	*colombiensis*
	T	C	T	All the rest	Serine	*pallescens* and *ecuadoriensis*
219 (655, 656, 657)	A	T	A	PIC	Methionine	*pictipes*
	A	T	T	All the rest	Isoleucine	All the rest

*For haplotype codes see table 87 above; for details on *Rhodnius ecuadoriensis* haplotypes see also Section 6.3.

7.3.2.2. Phylogenetic analyses

Phylogenetic trees were reconstructed using different approaches; the analysis of nucleotide variation in the 663bp cyt*b* sequence among the **17-haplotype dataset** (including the members of the 'Pacific *Rhodnius* lineage' and *R. pictipes*) was first carried out with a distance-based approach. Neighbour-joining was used to construct the trees, and a bootstrap-resampling procedure to estimate statistical support.

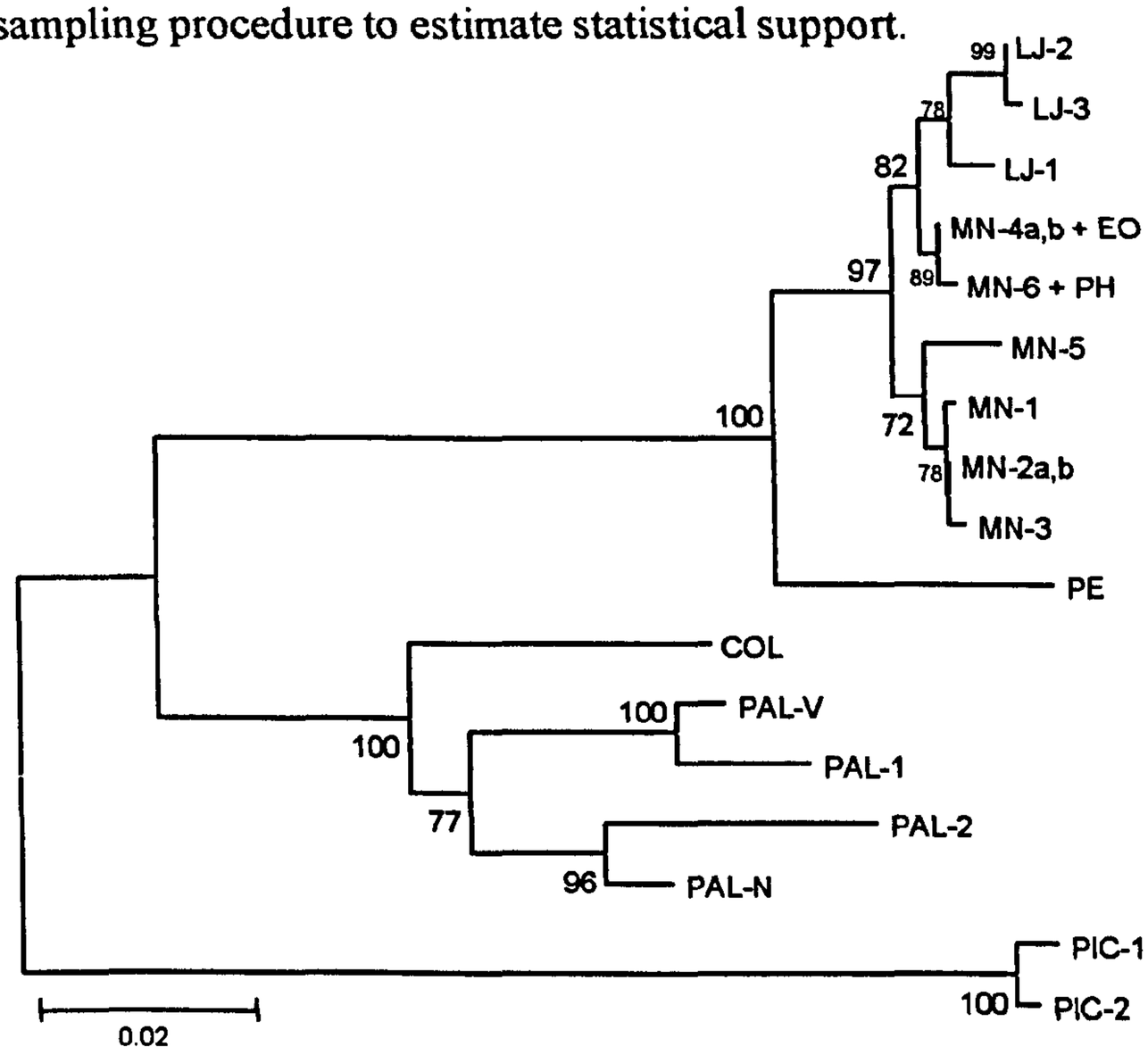


Figure 137. Phylogenetic relationships of members of the 'Pacific *Rhodnius* lineage'. Neighbour-joining tree derived from Kimura 2-parameter distances (SBL=0.37); 1000 bootstrap replicates. See text for haplotype codes

Distance-based phylogenies show the same topology whatever the model of substitution used to calculate pairwise distances (not shown: uncorrected *p* distances, T3p, and TN). The principal nodes are well supported, with bootstrap values above 95%; an exception is the *R. pallescens* branch, where bootstrap values are of about 75%. It is worth mentioning that in the context of this mitochondrial phylogeny the reciprocal relationships among taxa unequivocally show a closer kinship of *R. colombiensis* with *R. pallescens*, and not with *ecuadoriensis* as suggested by some studies (see below). In fact, the divergence between some *pallescens* haplotypes (PAL-1/PAL-2 *p* distance=0.062) is larger than that between some *pallescens* and *colombiensis* sequences (COL/PAL-V *p*=0.056; COL/PAL-1 *p*=0.06,

COL/PAL-N p=0.051). Distances between *colombiensis* and *ecuadoriensis* range from 0.112 (with MN-1,2, and 4) to 0.121 (with PE).

Character-state based phylogenies were reconstructed for the same taxa using standard maximum parsimony. A branch-and-bound search algorithm was used to recover the trees, and statistical support was assessed by the bootstrap-resampling method. Nine equally parsimonious trees (251 steps long) were recovered, and majority-rule and strict consensus trees were computed. Although the overall definition of the relationships was lower than in the neighbour-joining trees, the main subdivisions were well resolved. Some difficulties were apparent in ascertaining the topology of the *pallescens-colombiensis* clade and the position of haplotypes MN-4/EO and PH within the *ecuadoriensis* lineage. PAL-2 and PAL-N *pallescens* haplotypes tended to cluster with *R. colombiensis*, confirming unexpected results from distance-based analyses. The following figures show the topologies and statistics of the parsimony trees.

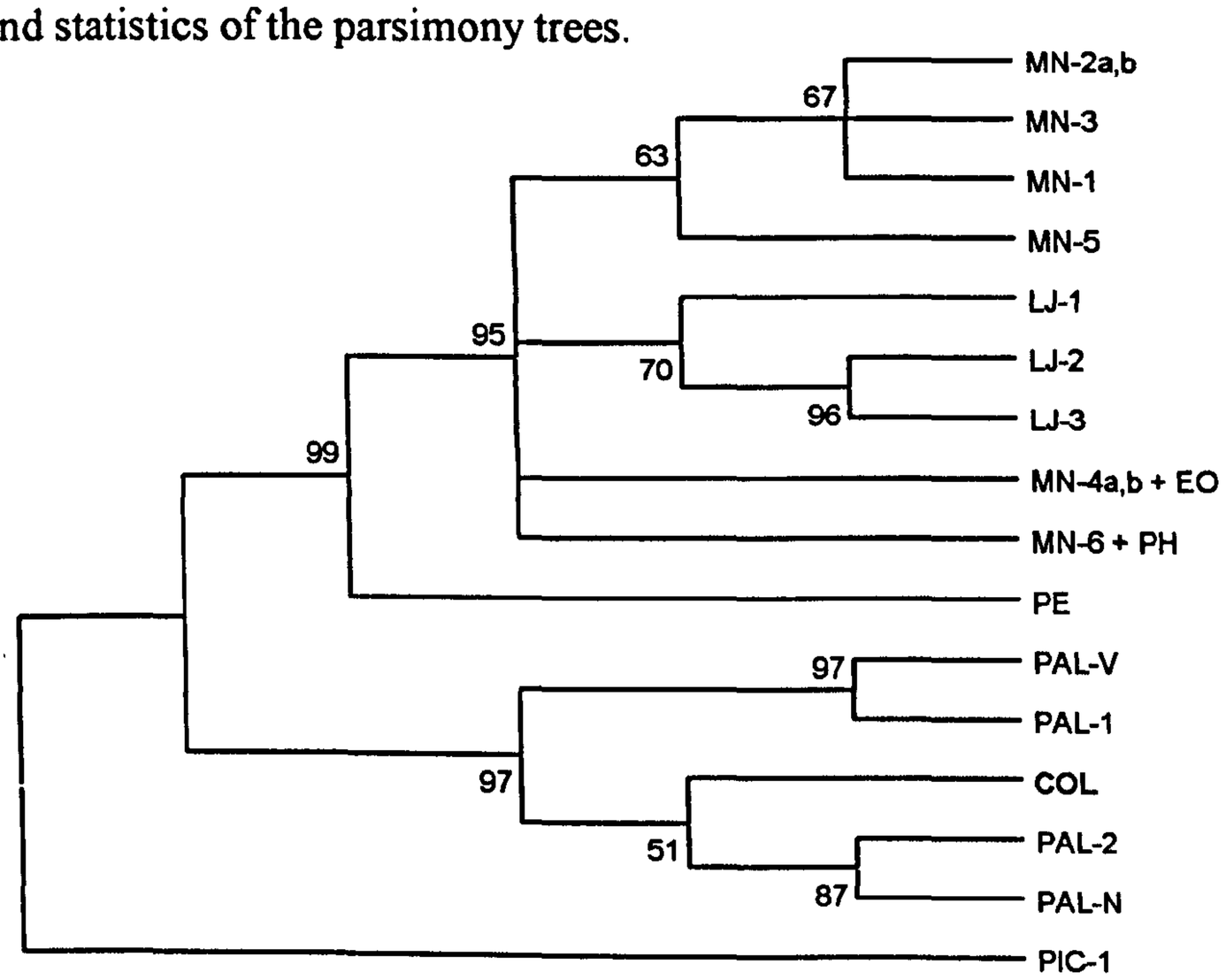


Figure 138. Phylogenetic relationships of members of the 'Pacific *Rhodnius* lineage'. Maximum parsimony (branch-and-bound search algorithm): majority-rule bootstrap consensus tree (1000 bootstrap replications). Tree length=251 (9 trees recovered); CI=0.77, RI=0.84, RCI=0.65 (all sites); iCI=0.69, iRI=0.84, iRCI=0.58 (parsimony-informative sites). Note the position of *R. colombiensis* (**COL**), nested within the *pallescens* clade

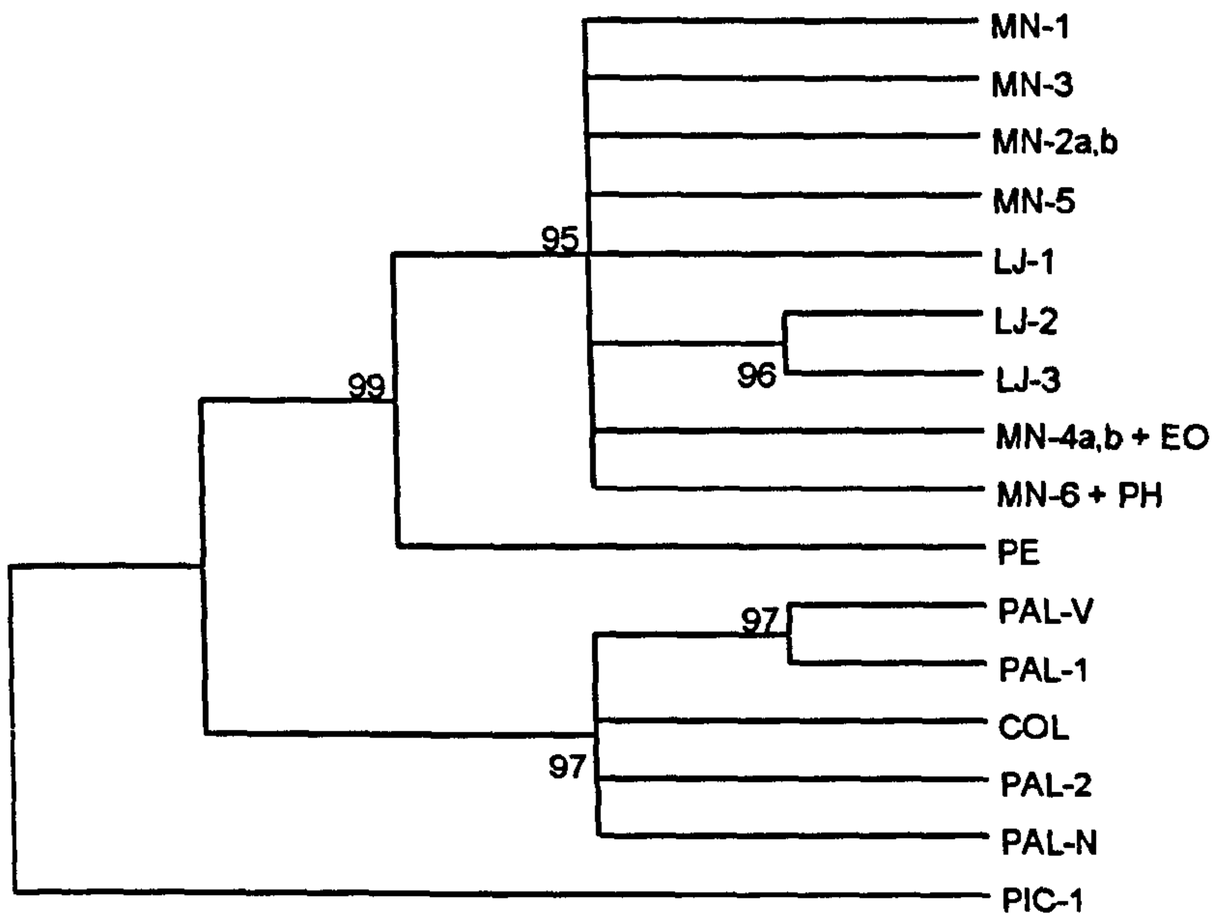


Figure 139. Phylogenetic relationships of members of the 'Pacific *Rhodnius* lineage'. Maximum parsimony (branch-and-bound search algorithm): semi-strict (95%) bootstrap consensus tree (1000 bootstrap replications). Tree length=251; CI=0.77, RI=0.84, RCI=0.65 (all sites); iCI=0.69, iRI=0.84, iRCI=0.58 (parsimony-informative sites)

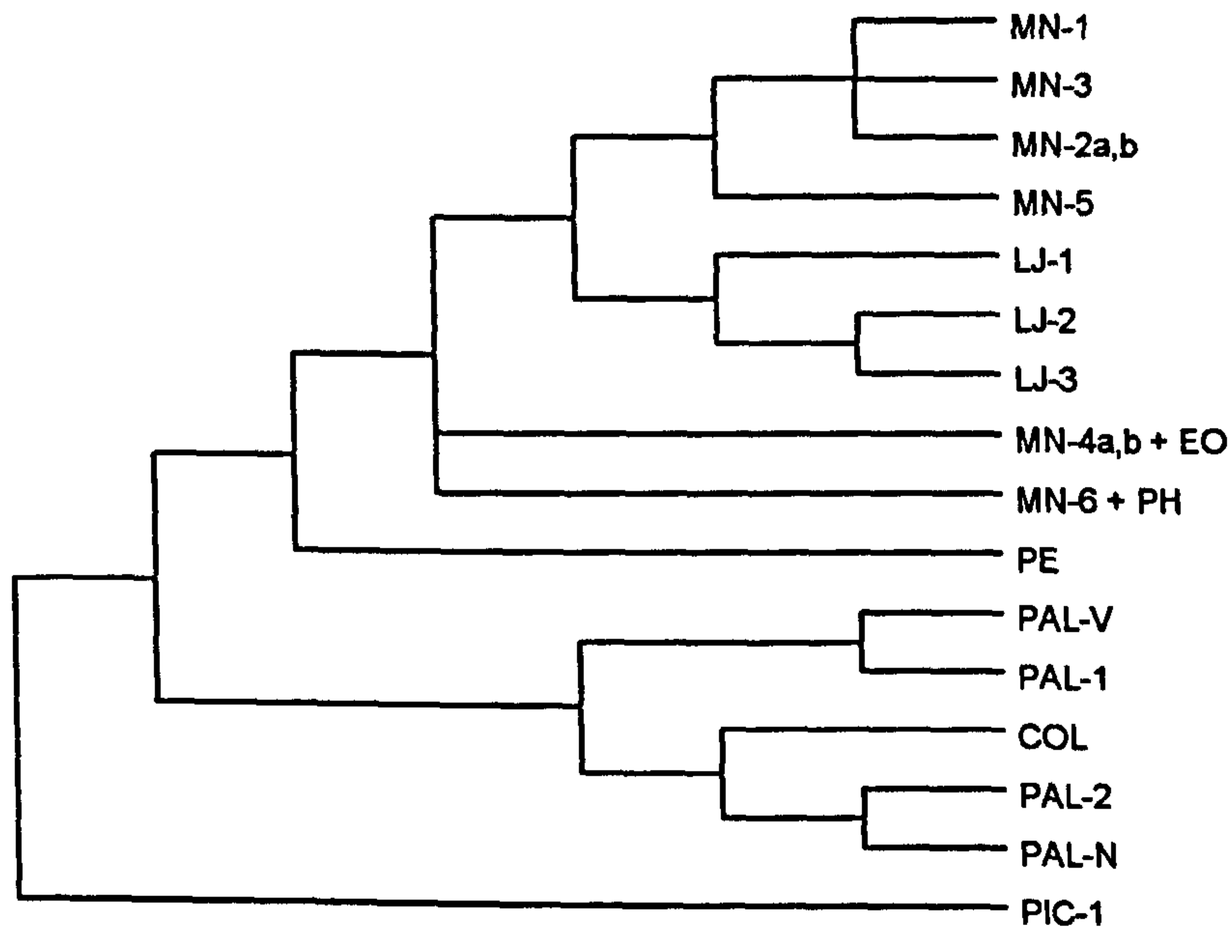


Figure 140. Phylogenetic relationships of members of the 'Pacific *Rhodnius* lineage'. Maximum parsimony (branch-and-bound search algorithm): strict (100%) topological consensus from 9 trees recovered. Tree length=251; CI=0.77, RI=0.84, RCI=0.65 (all sites); iCI=0.69, iRI=0.84, iRCI=0.58 (parsimony-informative sites)

Similar analyses were performed on a **100-haplotype dataset** including members of more distantly related *Rhodnius* species groups. Most of these sequences were kindly provided by FA Monteiro. Analyses included 5 *R. prolixus* (4 domestic and one sylvatic) from various origins: Francisco de Morazán, Honduras; Ortiz, Venezuela; Cojedes, Venezuela [sequences from FA Monteiro]; Lara, Venezuela; and Guárico (collected from a *Copernicia tectorum* palm tree), Venezuela [S Fitzpatrick and JS Patterson, LSHTM]. Five *R. robustus* sequences (Napo, Ecuador; Pará, Brazil; Cayenne, French Guiana; and two from Amazonas, Brazil) were obtained from FA Monteiro; one additional specimen, collected by us in Sucumbíos (northern Ecuadorian Amazon) and superficially similar to *R. robustus* (although somewhat smaller and darker than typical specimens, morphological identification using the keys by Lent & Wygodzinsky [1979] placed it closer to *robustus* than to any other taxon) was also sequenced. One *R. nasutus* (Piauí, Brazil, FA Monteiro) was also included. Finally, *T. rubrovaria* was used as the outgroup.

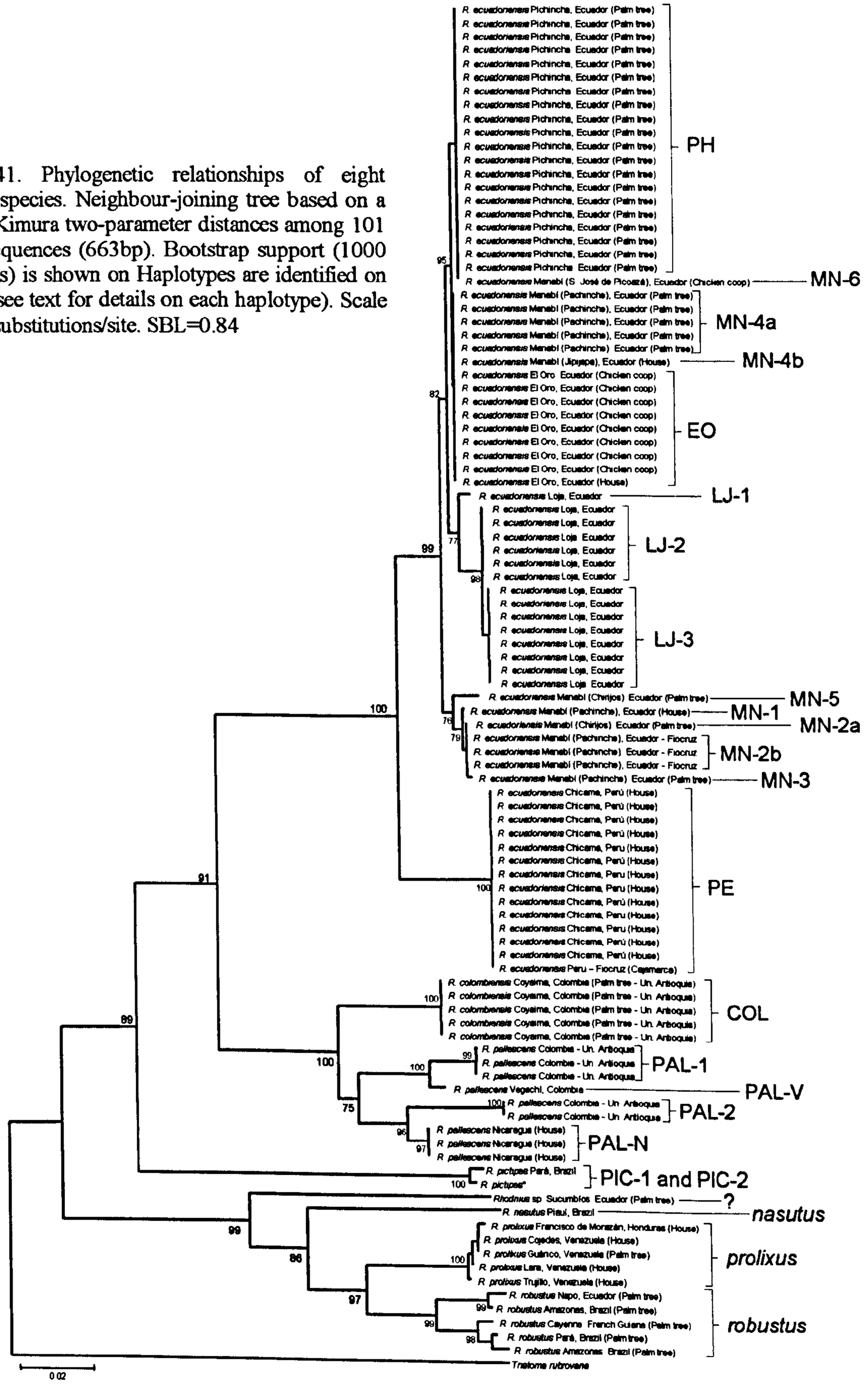
The alignment (excluding the outgroup) used for these analyses was comprised of 100 mt cyt*b* sequences. Out of 237 segregating sites, 213 were parsimony-informative (with 24 individual autapomorphs). Overall mean nucleotide diversity was 0.08 (SE=0.005); Jukes-Cantor (JC) and Kimura 2-parameter (K2p) overall mean distances were both 0.09, and more complex models yielded similar values (Tamura 3-parameter: T3p=0.09; Tamura-Nei: TN=0.095). These mean distances increased to >0.1 when a gamma distribution (shape parameter α=0.44; see Page & Holmes 1998, p.161) was incorporated: JC+Γ=0.12, K2p+Γ=0.13, TN+Γ=0.14. Within-group variation was scored in *R. ecuadoriensis* (π=0.0179, K2p=0.0185 [SE=0.003 for both]), *R. pallescens* (π=0.0365, K2p=0.0384 [SE=0.005]), *R. pictipes* (π=0.006, K2p=0.006 [SE=0.003]), *R. prolixus* (π=0.0018, K2p=0.0018 [SE=0.001]), and *R. robustus* (π=0.0226, K2p=0.0233 [SE=0.004]); five *R. colombiensis* specimens had the same haplotype, and *R. nasutus* and *Rhodnius* sp. (Sucumbíos, Ecuador) were represented by single specimens. Between-group mean uncorrected and K2-p distances were computed (see Appendix).

Average base composition was: T=37.2%, A=31.6%, C=18.3%, and G=12.9% (A+T accounted for about 69% of nucleotides). The inferred amino acid sequence (221 amino acids long) had 32 variable positions, of which 25 were parsimony-informative.

The resulting distance-based phylogenies have identical topologies (except for a few position swaps in terminal branches) and very similar bootstrap support values. They show two major, strongly supported monophyletic lineages within *Rhodnius* (bootstrap values ~90%). The first is comprised of *R. pictipes* and the 'Pacific lineage' species. *R. pictipes* appears in a basalmost position, with *pallescens-colombiensis* forming a 100%-supported clade displaying a sister relationship with the also strongly supported *R. ecuadoriensis* lineage. The difficulties in resolving the mutual relationships of *R. pallescens* and *colombiensis* mentioned above were confirmed, with the relative monophyly of *pallescens* supported by a weak bootstrap value (~75%); the specimens presenting PAL-1 and PAL-V haplotypes appear in an intermediate position between other *pallescens* and *colombiensis*. Within *R. ecuadoriensis*, the separation of the Peruvian population (PE) was confirmed, and the Ecuadorian populations formed two main branches already described above.

A second major clade corresponds to the species of the *R. nasutus-prolixus-robustus* lineage. One surprising result is the clear distinctiveness of the Sucumbíos '*R. robustus*' specimen. It is the sole occupant of the basalmost branch of the clade, with a 99%-100% bootstrap support. This is strong indication of this specimen belonging to a different, undescribed Amazonian species of *Rhodnius*. Further characterisation of these Ecuadorian Amazon *Rhodnius* populations was out of the scope of the present project, but will be undertaken in the future. It is interesting to note that other specimen identified as *R. robustus* and collected in the province of Napo (neighbouring Sucumbíos in the northern Ecuadorian Amazon) was found to be similar to other bugs of the same species collected in various parts of the Amazon basin, confirming that two genetically very distinct (but morphologically similar) taxa are sympatric in north-east Ecuador. *R. nasutus* appears in a position basal to the *prolixus-robustus* clade, where two main branches contain specimens of each of the species: all *R. prolixus* appear to be very similar, whereas a higher degree of genetic variation can be seen in the *robustus* clade. All these groupings were also supported by strong bootstrap values, usually above 90%. Virtually identical results were obtained using distances computed from uncorrected *p* distances and a Tamura three-parameter model (not shown).

Figure 141. Phylogenetic relationships of eight *Rhodnius* species. Neighbour-joining tree based on a matrix of Kimura two-parameter distances among 101 mt cyt*b* sequences (663bp). Bootstrap support (1000 replications) is shown on Haplotypes are identified on the right (see text for details on each haplotype). Scale bar: 0.02 substitutions/site. SBL=0.84



NJ phylogenetic trees were finally computed for a **27-haplotype dataset** including all *ecuadoriensis*, *pallescens*, *colombiensis*, and *pictipes* haplotypes plus the Sucumbíos *Rhodnius* sp., *R. nasutus*, three *prolixus*, and four *robustus* haplotypes (and *T. rubrovaria* as the outgroup). Several models of base substitution were assayed (*p* distances, Jukes-Cantor, Jukes-Cantor with gamma distribution [Γ, α=0.44], Kimura 2-parameter, K2p+Γ, Tamura 3-parameter, T3p+Γ, Tamura-Nei, and TN+Γ); the inferred trees showed complete topological stability. Results confirmed the observations described above, and are summarised in the following figures.

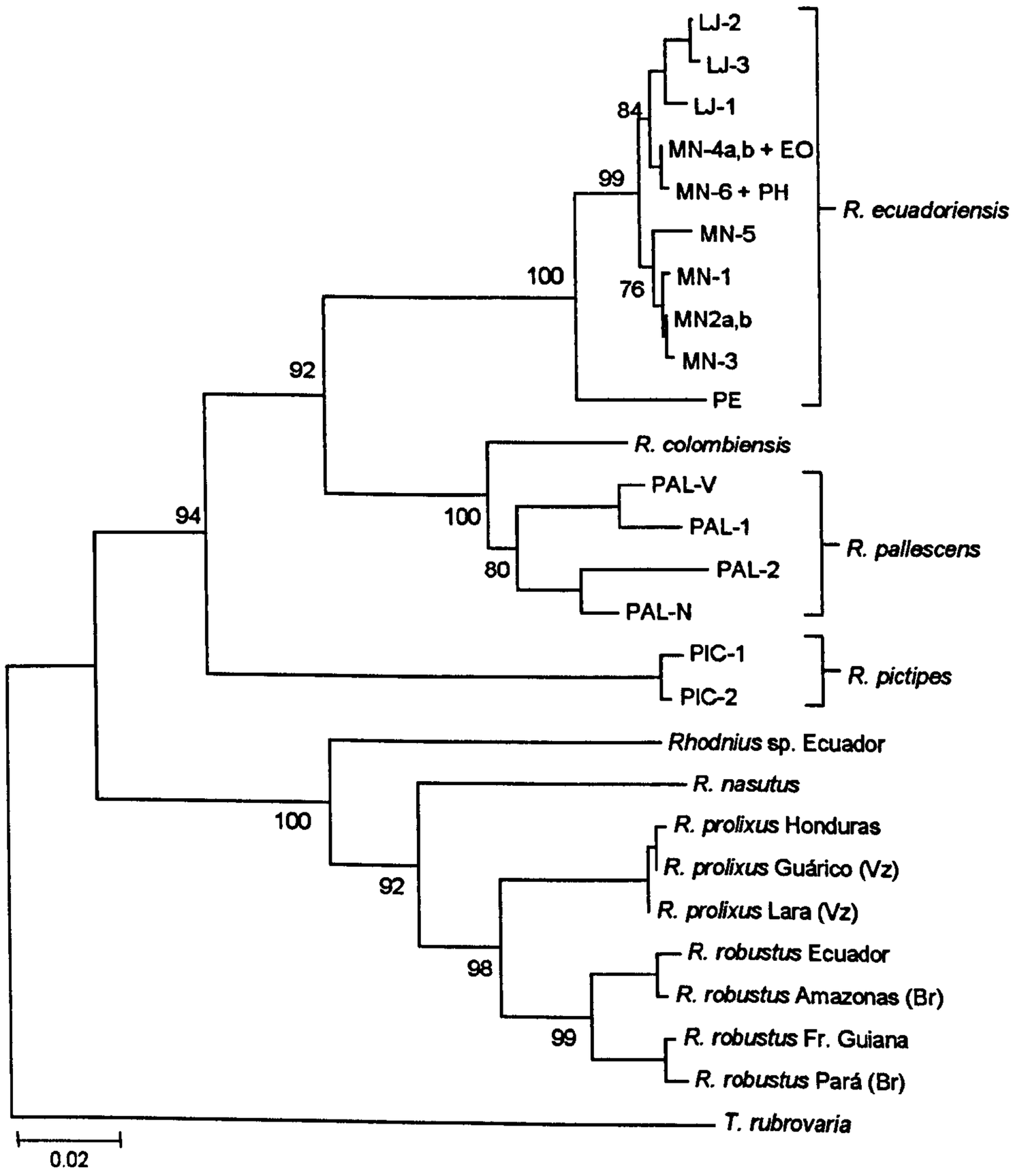


Figure 142. Phylogenetic relationships of *Rhodnius* species (Neighbour-Joining, 1000 bootstrap replicates) using Kimura 2-parameter distances; SBL=0.83

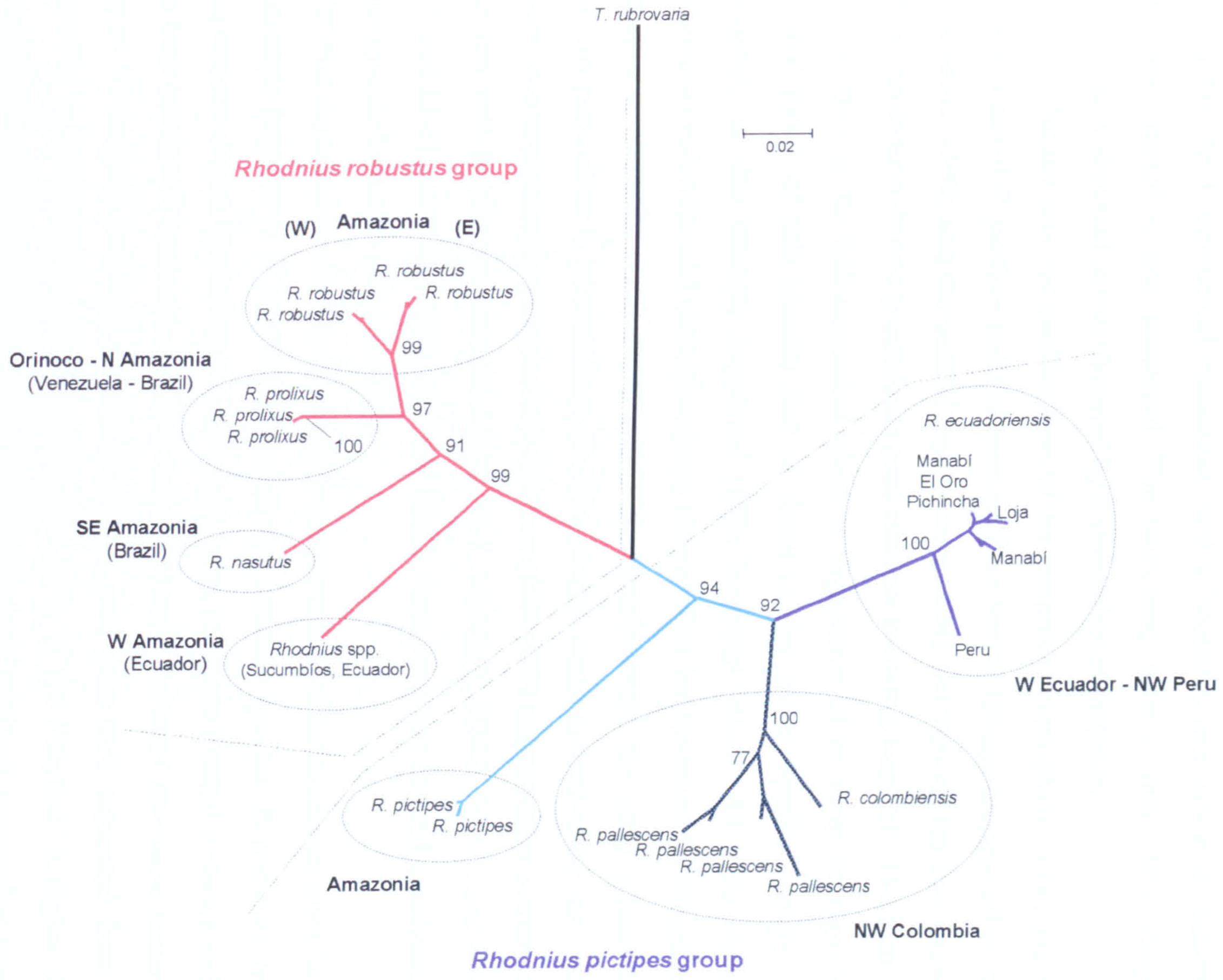


Figure 143. Phylogenetic relationships of *Rhodnius* species (NJ, 1000 bootstrap replicates); Tamura 3-parameter; SBL=0.84

Maximum parsimony analyses were performed on a restricted dataset of 26 haplotypes (plus *Triatoma rubrovaria* as the outgroup) to reduce computational requirements. A branch-and-bound search algorithm yielded 27 trees (573 steps long). A tree was computed showing the strict topological consensus, and a bootstrap majority-rule consensus tree (1000 replications) is also presented. The branching order in this MP phylogeny was fundamentally identical to that obtained by distance-based methods.

A major feature was the confirmation of the reciprocal monophyly of two main clades, one comprised of *R. pictipes* and the species of the Pacific lineage (supported by bootstrap values above 95%), and the second one encompassing the Sucumbíos specimen and the *nasutus-prolixus-robustus* group (with even stronger bootstrap support). The basal position of *R. pictipes* in its well-supported clade is suggestive of an early divergence of the ancestral forms of this species and of those in the extant Pacific lineage – whose monophyly is also strongly supported (98% bootstrap value). All trees recognised a [*pallescens+colombiensis*] clade (bootstrap above 95%), but the mutual relationships of the two species remained unresolved. Within *R. ecuadoriensis*, the early separation of the Peruvian population is unequivocal; the Ecuadorian clade is formed by two main sister groups, one containing haplotypes MN-4/EO and MN-6/PH and the other comprised of two branches with haplotypes from Manabí (MN-1, MN-2a,b, MN-3, MN-5) and Loja (LJ-1, LJ-2 and LJ-3) occupying each of them. These three branches formed a polytomy in the majority-rule bootstrap consensus tree.

Within the second main branch, the basalmost position of the specimen from Sucumbíos is strongly suggestive of its distinctiveness as a separate species. *R. prolixus* specimens from various origins (including two domestic bugs, one from Central America and another one from northern Venezuela, and third individual collected in a *Copernicia tectorum* palm tree in Venezuela) had very similar sequences, and were well separated from material identified as *R. robustus* collected in various parts of the Amazon. A higher degree of variability was detected among these latter specimens, with two geographic groups (one from the eastern and one from the central-western Amazon) recognised in different, well-supported branches. The reciprocal monophyly of *nasutus* vs. *prolixus-robustus* was not fully resolved, and received only 71% bootstrap support (the second lowest value, after the problematic *pallescens-colombiensis*, at the supraspecific level).

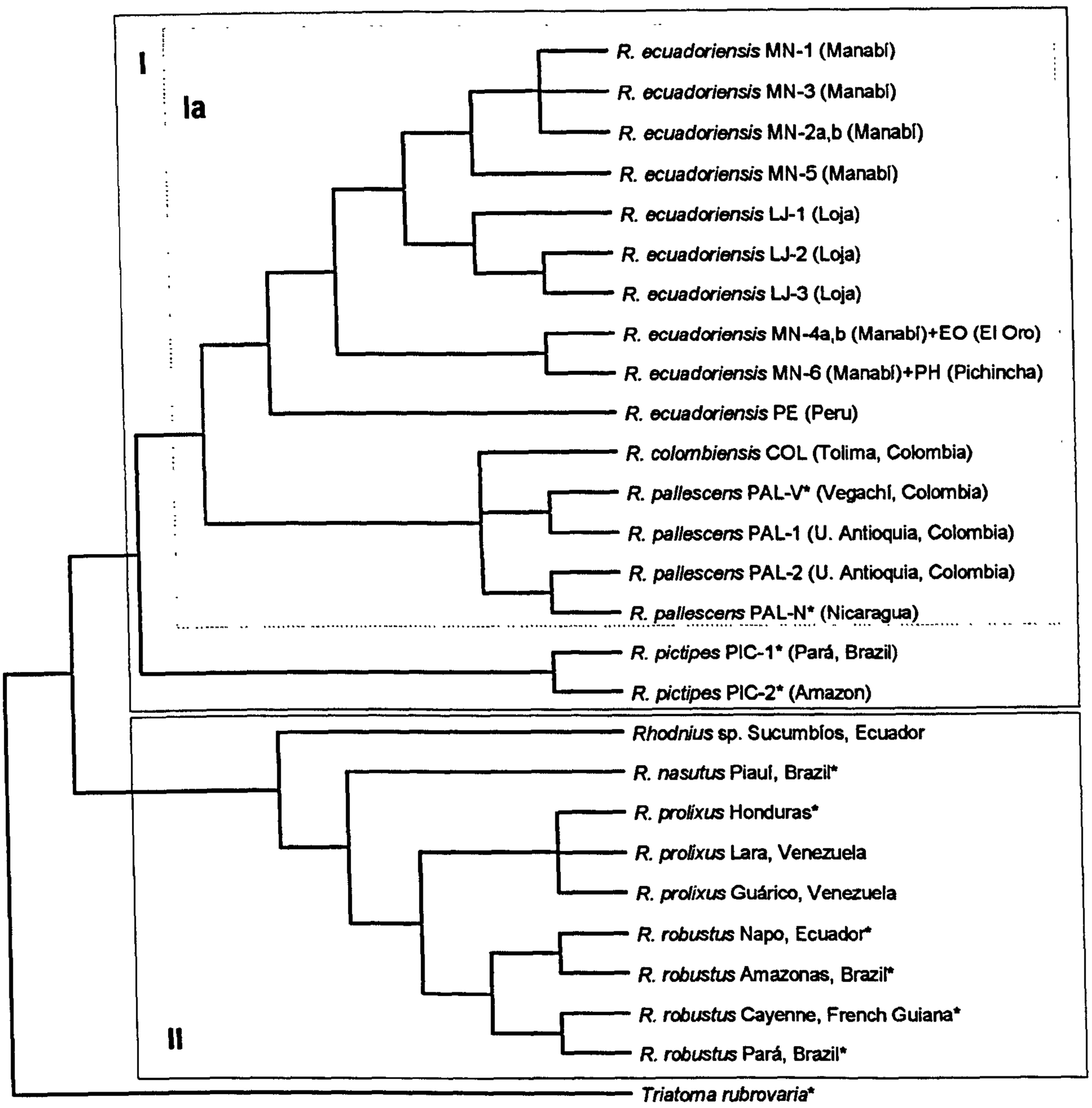


Figure 144. Phylogenetic relationships of *Rhodnius* species. Maximum parsimony (branch-and-bound search algorithm): strict consensus from 27 trees recovered. Major clades are identified in grey boxes: **I**=*R. pictipes* lineage, including the members of the 'Pacific lineage' (**Ia**, dotted box: *R. pallescens*, *R. colombiensis* and *R. ecuadoriensis*); **II**=*R. robustus* lineage, including *R. robustus* from different parts of the Amazon basin, *R. prolixus* from Central America and northern South America, *R. nasutus*, and the unidentified species found in palm trees of Sucumbíos, northeast Ecuador (*Rhodnius* sp.). Tree length=573; CI=0.62, RI=0.85, RCI=0.53 (all sites); iCI=0.58, iRI=0.85, iRCI=0.49 (parsimony-informative sites). Haplotypes with an asterisk (*) were kindly provided by FA Monteiro (CDC-Fiocruz) for comparison with new sequences obtained in this study; Venezuelan *R. prolixus* were collected by S Fitzpatrick and JS Patterson (LSHTM)

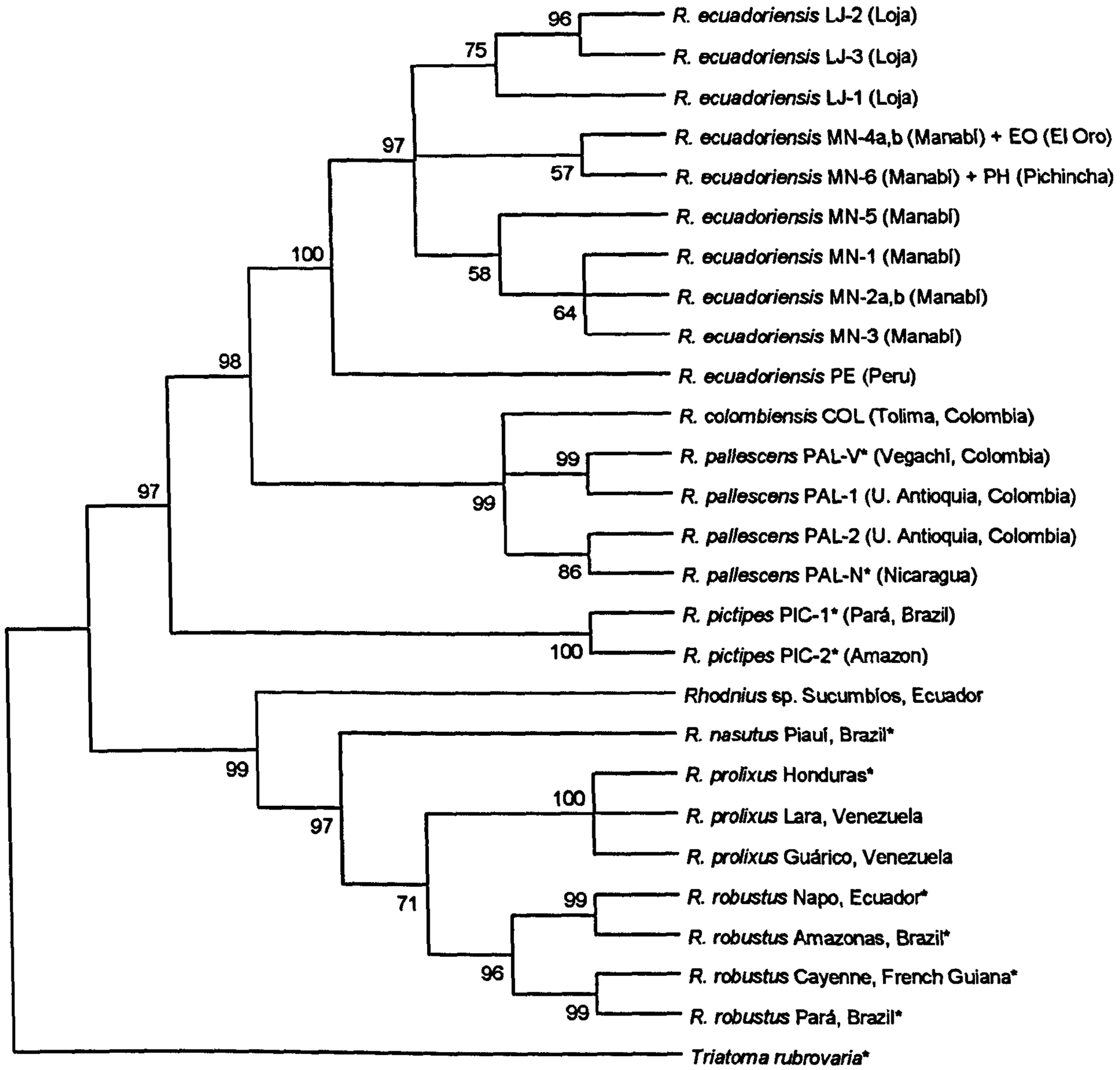


Figure 145. Phylogenetic relationships of *Rhodnius* species. Maximum parsimony (branch-and-bound search algorithm): majority-rule bootstrap consensus tree. Tree length=573; CI=0.62, RI=0.85, RCI=0.53 (all sites); iCI=0.58, iRI=0.85, iRCI=0.49 (parsimony-informative sites). Haplotypes with an asterisk (*) were kindly provided by FA Monteiro (CDC-Fiocruz) for comparison with new sequences obtained in this study; Venezuelan *R. prolixus* were collected by S Fitzpatrick and JS Patterson (LSHTM)

7.3.4. DISCUSSION

We have presented a complete mtDNA phylogeny of the Pacific *Rhodnius* lineage; the generosity of several friends, who allowed us to use their specimens (CJ Schofield, CA Cuba Cuba, JS Patterson, S Fitzpatrick) and unpublished sequence data (FA Monteiro), made it possible to extend the analyses to other groups of species.

The most relevant feature refers to the confirmation of the existence of two main, reciprocally monophyletic clades (the *pictipes* and *robustus* groups) within the genus *Rhodnius*. Based on results obtained by other researchers using diverse approaches, we have proposed the most likely affiliations of other *Rhodnius* species (and, to a lesser extent, of *Psammolestes*) to these main clades. Our results confirmed the monophyletic character of the Pacific *Rhodnius* lineage, clarified the mutual relationships of the species within that lineage, and favoured their affiliation to the *pictipes* main group.

Overall, the results of the mt cyt*b* phylogenies presented here lend strong support to the notion that the *pictipes* and *pallescens* groups share a common ancestry. With bootstrap support values consistently above 90% in both distance- and character state-based phylogenies, it is very unlikely that the branching order of the true tree involves a closer relationship of *R. pictipes* with the *robustus* group (even if only one representative of the *pictipes* group – *R. pictipes* itself – was available). This is in agreement with biogeographic-ecological considerations (see Schofield & Dujardin 1999) and with both quantitative (see Section 7.2.) and qualitative (Lent & Wygodzinsky 1979) phenetic analyses, but the issue was not unequivocally resolved by previous allozyme (Chávez et al. 1999, Dujardin et al. 1999a,e, Monteiro et al. 2002) or DNA studies (Stothard et al. 1998, Lyman et al. 1999, Monteiro et al. 2000, Hypša et al. 2002).

The relationships within the Pacific species clade remained largely unresolved. Allozyme data had shown that *colombiensis* might in fact be closer to *ecuadoriensis* than it is to *pallescens* (Chávez et al. 1999, Dujardin et al. 1999a,e), a view that received further support from mt cyt*b* DNA sequence analysis (Monteiro et al. 2000). Biogeographic and ecological considerations make these results counter-intuitive; *R. pallescens* and *R. colombiensis* are parapatric on the west side of the central Magdalena valley in Colombia, and both seem to favour *Attalea butyracea* palm trees as their primary natural ecotope (even if *pallescens* may colonise a variety of other habitats,

including human environments) (Jaramillo et al. 2000). In addition, both species have very similar phenotypes, not only in terms of overall aspect, size, and colouration (except for the mottling as discussed above), but also morphometrically as shown by results presented in Section 7.2. (Lent & Wygodzinsky 1979, Dujardin et al. 1999a,e, Moreno et al. 1999). Because of these similarities, we favoured the idea of a closer kinship between *pallescens* and *colombiensis* than between any of them and *ecuadoriensis* (allopatric, found preferentially in *Phytelephas* palm trees, and phenetically distinct), as shown in figure 135 (both **A** and **B**). The alternative hypothesis is roughly represented in the following figure, showing how *ecuadoriensis* is related to *colombiensis* and *pallescens* according to allozyme (Chávez et al. 1999, Dujardin et al. 1999a) and partial (414bp) mt cyt*b* sequence data (Monteiro et al. 2000).

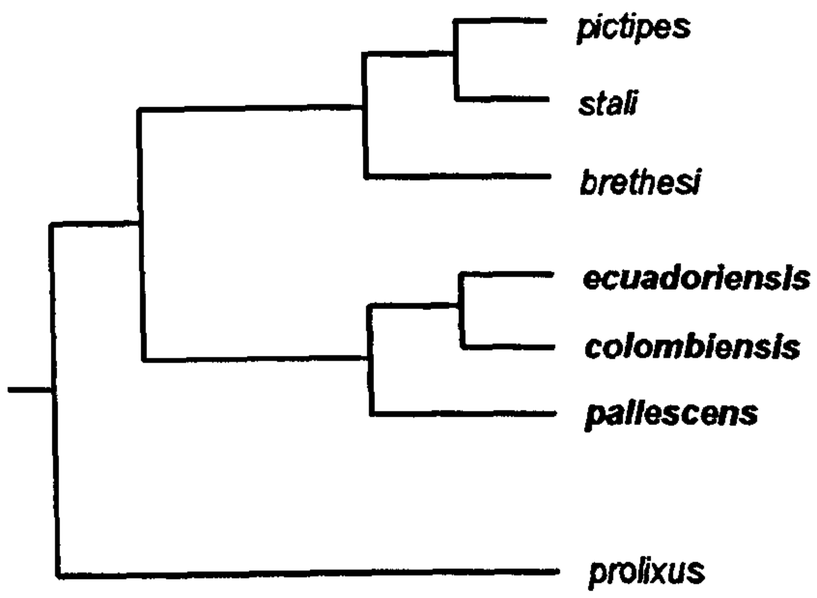


Figure 146. Phylogenetic relationships among species within the *Rhodnius pallescens* group. The tree was drawn by hand to present (roughly) the results obtained using allozymes and mtDNA sequence data. Compare the relative positions of *pallescens*, *colombiensis*, and *ecuadoriensis* with those in figure 135, showing a closer kinship between *pallescens* and *colombiensis*, as suggested by ecological and phenotypic similarities

Our results were in overt disagreement with the hypothesis of a sister-taxon relationship between *ecuadoriensis* and *colombiensis*. Firstly, obvious phenotypic similarities were revealed between *pallescens* and *colombiensis* by CVA of head measurements; a tendency of the sylvatic Pichincha forms of *ecuadoriensis* to cluster together with *colombiensis* was however detected in some UPGMA analyses (e.g. figures 127, 128**A**, 133), but not in others (e.g. figure 128**B**). Size-free analyses, in which the effects of environmental factors are reduced, showed a clear separation of both groups (figure 131).

This was convincingly substantiated by DNA sequence analyses. All mt cyt*b* phylogenies demonstrated the monophyletic character of the clade comprised by the Colombian species pair (*pallescens* and *colombiensis*); bootstrap values were always above 95%, and a 100% support was obtained in many of the analyses. Similarly, the

different populations of *R. ecuadoriensis* were confirmed as monophyletic with bootstrap values consistently reaching 99% to 100%. An intriguing feature was that, using exactly the same molecular marker (even if a shorter gene fragment), Monteiro et al. (2000) reported a 94% bootstrap-supported clade [*pallescens* (*colombiensis*, *ecuadoriensis*)] – and with an unequivocal 100% support for the monophyly of the two latter species. The contradiction was solved when, working on the new sequence data with Dr Monteiro, we realised that the '*colombiensis*' sequence presented in the paper (Monteiro et al. 2000) corresponded in fact to a Peruvian specimen of *R. ecuadoriensis*. The unexpectedly strong divergence between this and other *ecuadoriensis*, and the fact that allozyme data favoured the clustering of *ecuadoriensis* with *colombiensis* (Chávez et al. 1999, Dujardin et al. 1999a,e), resulted in the failure to notice the mislabelling.

Our results also showed that the separation of *colombiensis* from *pallescens* is in the range of that found among different populations of *pallescens*, which was much greater than expected after previous reports (based on allozyme and microsatellite data) indicated low genetic variability in *R. pallescens* (López & Moreno 1995, Harry et al. 1998, Jaramillo et al. 2000). Overall cyt*b* pairwise sequence divergence was ~6% for *pallescens* vs. *colombiensis* and ~12% for *colombiensis* vs. *ecuadoriensis*; differences from ~2% to >6% were recorded between different populations of *R. pallescens*, and nucleotide diversity was much higher in *pallescens* ($\pi \approx 0.037$) than in *ecuadoriensis* ($\pi \approx 0.018$), in spite of the wider sampling for the latter species. Together, these results may be interpreted as suggesting that the taxonomic status of *R. colombiensis* could fit the concept of subspecies (as a geographically and ecologically restricted subset of the main *pallescens* taxon presenting fixed, distinctive phenotypic features). Our results confirmed the occurrence of *R. pallescens* in Nicaragua, where some adult specimens examined here were collected in human dwellings (CJ Schofield, pers. comm.).

7.4. Conclusions

7.4.1. PHYLOGEOGRAPHY

In general, the hypothetical phylogenetic scenario proposed for the Pacific *Rhodnius* lineage on the grounds of metric analyses was broadly compatible with our mtDNA results; the geographic origin of the Pacific lineage remains unresolved, but the broader geographic and ecological valence of *R. pallescens*, together with obvious phenetic similarities with *pictipes* (a generalist, widely distributed species with male genitalia structures considered as plesiomorphic), may provide an indication of a northern origin and subsequent radiation towards the south. Genetic distances between *pictipes* and *pallescens* averaged ~0.14 (the same as for *colombiensis*), and were larger (~0.16) between *pictipes* and *ecuadoriensis*. *R. ecuadoriensis* appeared slightly closer to *colombiensis* ($p \approx 0.115$) than to *pallescens* ($p \approx 0.12$). Applying Brower's (1994) estimate of arthropod mt sequence divergence per million year (~2.3%) (see also Section 6.3.), *R. pictipes* and *R. pallescens-colombiensis* would share a common ancestor that lived in the late Miocene, about 6.1-6.3mya, just before the Andes increased their maximum altitude from ~2000 to over 4000m during the Pliocene (Cox & Moore 2000). Similarly, the estimated time since divergence of *ecuadoriensis* from its Colombian relatives (*pallescens* and *colombiensis*) may be estimated at about 5mya, roughly coinciding with the uplift of the Andes. A possible explanation to the current distribution of the members of the Pacific *Rhodnius* lineage would therefore combine the effects of adaptive radiation and vicariance. Thus, an initial population perhaps reached the western side of the (then low) Andes range during the Miocene by migrating from the eastern plains; subsequently, the rise of the Andes during the Pliocene split that population into two main clusters: the northern (now Colombian) cluster, comprised by the ancestral forms of *pallescens* (and of one phenetically distinct subset known as *colombiensis*), and an isolated pocket in the south (which adapted to new ecotopes and eventually gave rise to *R. ecuadoriensis*). Although there was evidence of a very slight trend towards saturation of transitions when comparing *R. pictipes* with the western species, the high coefficient of determination of the linear regression ($R^2=0.98$) suggests that the assumption of constant mutation rate is tenable for these closely related taxa. Prudence is however necessary in the interpretation of these results, which are only tentative; calibration of molecular clocks and the establishment of rate constancy across

taxa are notoriously problematic issues where a series of assumptions are involved (Avise 1994, Gaunt & Miles 2002); we have made some further assumptions as to the validity of applying Brower's estimate of sequence divergence per unit time (Brower 1994) to the organisms and gene fragments under consideration.

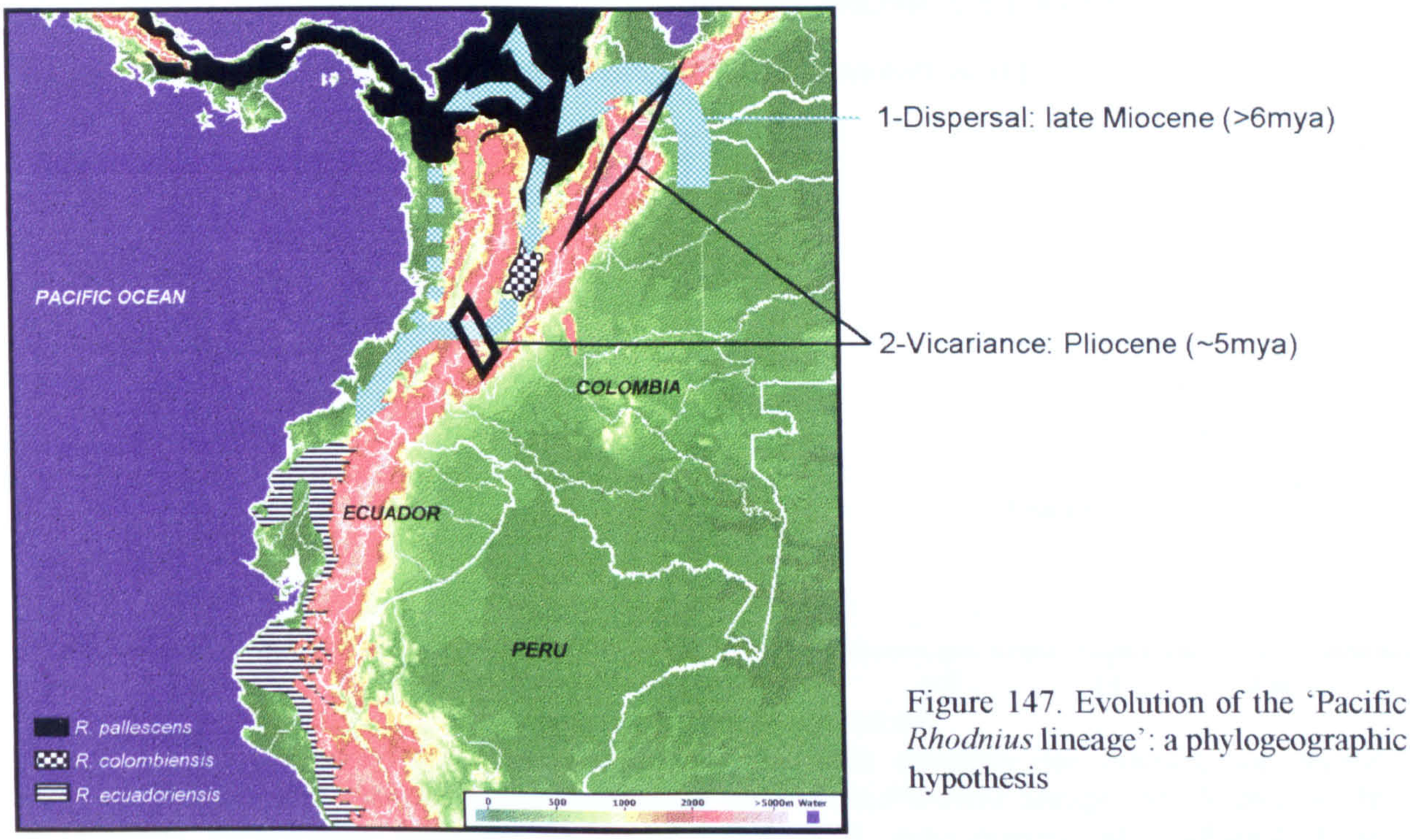


Figure 147. Evolution of the 'Pacific *Rhodnius* lineage': a phylogeographic hypothesis

7.4.2. PHENOTYPIC AND GENETIC DIVERSITY

The combined analysis of morphometric and molecular results illustrated a further example of phenetic plasticity among the Triatominae (Dujardin et al. 1999b), showing that genetically distinct populations (e.g. *colombiensis* vs. Pichincha forms of *ecuadoriensis*, *pictipes* vs. *colombiensis* or *prolixus* vs. *colombiensis*) may present convergent, size-related morphometric traits. Particularly striking was the finding of extensive morphometric variation within a single species (*R. ecuadoriensis*).

Overall, molecular data outperformed morphometrics for the definition of phylogenetic hypotheses regarding the phenetically distinct members of the two main groups of *Rhodnius* identified here. Discriminant analysis of metric characters was however confirmed as a powerful taxonomic tool, as substantiated by the patterns of reclassification of individual bugs to their respective, *a priori*-defined OTUs (with degrees of agreement always in the range of 'perfect' or 'almost perfect' [i.e., kappa>0.8]). The correlation between genetic and phenetic (Mahalanobis) distances is

explored in the following figure. The coefficient of determination of the quadratic fit increased from 0.6 to 0.75 when comparisons involving Pichincha forms of *R. ecuadoriensis* were removed from the analysis. In those comparisons we scored relatively low metric and large genetic distances (e.g., Pichincha forms vs. *R. colombiensis*) and *vice versa* (large metric and low genetic distances in comparisons of Pichincha forms vs. synanthropic populations of *R. ecuadoriensis*).

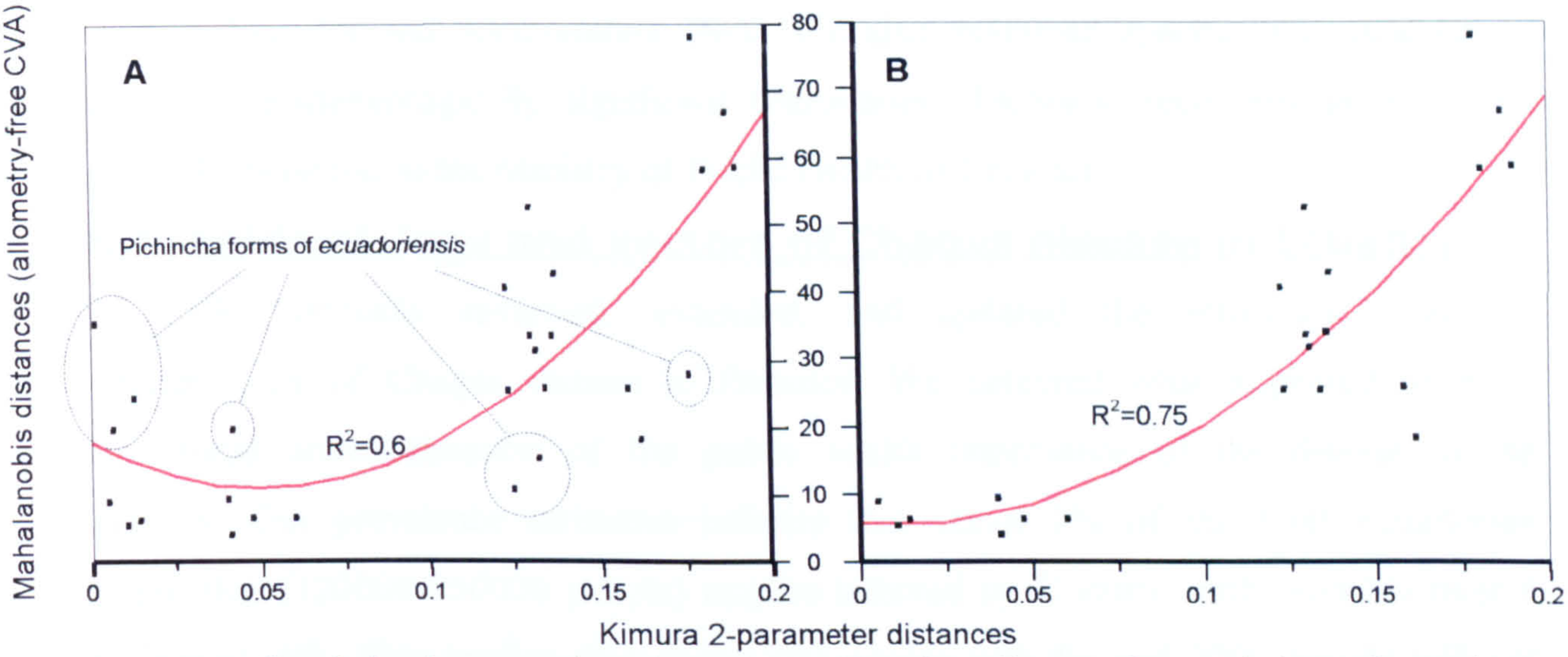


Figure 148. Correlation between phenetic (Mahalanobis, after allometry-free analysis) and genetic (Kimura 2-parameter) distances among members of the 'Pacific *Rhodnius* lineage' plus *R. pictipes*. In **A**, pairwise comparisons involving Pichincha forms of *R. ecuadoriensis* are indicated; those comparisons were removed from the analysis in **B**. Note how these forms are phenetically distant from other *ecuadoriensis* populations (genetic distances <0.05), but similar to genetically distant (K2p>0.1) bugs (*colombiensis*, *pallescens*, and even *pictipes* [K2p>0.15]). Coefficients of determination of quadratic fits are shown near the fit lines

7.4.3. A NEW *RHODNIUS* FROM THE AMAZON?

Finally, we have discovered what probably is a new species of *Rhodnius* among material collected from (mainly) *Attalea* palm trees in the northern Ecuadorian Amazon (province of Sucumbíos) (Palomeque et al. 2000, Noireau et al. 2002b). Interestingly, material originally collected in the same area and also identified as *R. robustus* presented a very different haplotype (9.7% pairwise sequence divergence); overall, the genetic distance between *R. robustus* and the undescribed bug was >10%. For the first time in triatomine systematics, mtDNA sequence data preceded phenetic considerations in the discovery of an undescribed taxon; a complete characterisation of these Amazonian populations will be carried out in the future.

8. CONCLUSIONS AND RECOMMENDATIONS

The primary aim of this project was to undertake research relevant to the design and implementation of the first national programme for the control of Chagas disease in Ecuador. After a general epidemiological assessment, we concentrated on ecological, genetic, and evolutionary aspects of a little-known triatomine species, *R. ecuadoriensis*, suspected of involvement in *T. cruzi* transmission to humans over a large area of western Ecuador and northwestern Peru. We also examined specific biological issues for other epidemiologically significant triatomines. Technical recommendations were officially delivered to the Ministry of Public Health of Ecuador.

8.1. Epidemiology and vectors of Chagas disease in Ecuador

1. We critically reviewed, extended, and updated the information on the epidemiology of Chagas disease in Ecuador. We detected what appeared to be a substantial underestimation of the public health importance of the disease in the country. Our prevalence estimates indicate that about 1% of the total Ecuadorian population (120000-150000 people) may be infected by *T. cruzi*, with probably over 4 million at risk. This implies that about 300 people will die and 3000 people will get infected each year in the absence of control measures. The economic burden of Chagas disease probably exceeds 20 million US dollars/year; savings of about 20 US$ per dollar invested in control activities can be anticipated if an adequate programme (costing a maximum of 1 million US$/year for 15 years) is implemented (Aguilar et al. 1999, Abad-Franch & Aguilar 2000).

2. We completed a baseline, general assessment of the biogeography and epidemiological significance of Ecuadorian triatomines; results from this ecological analysis were used to derive specific recommendations for the design of control strategies. The main vector of human disease in Ecuador is *T. dimidiata*; *R. ecuadoriensis* is important in some areas of central and southern western Ecuador, including temperate inter-Andean valleys where reported prevalence rates are the highest in the country. *P. rufotuberculatus* is a secondary vector with local importance, and *R. pictipes* and *R. robustus* are probably involved in foci of transmission detected in the Amazon region; *T. carrioni* and *P. chinai* are candidate vectors in the south (Abad-Franch et al. 2001b).

3. Our investigations (including biogeography, ecological features, morphometrics, and the combination of historical records) on the origin of Ecuadorian populations of *T. dimidiata* led us to put forward the hypothesis of an artificial introduction of this species to Ecuador and Peru. Results from morphometric and molecular biological data gave strong support to this hypothesis, showing a very close relationship with northern Mesoamerican populations. According to these data, local eradication of synanthropic populations of *T. dimidiata* may be attainable in Ecuador and Peru (Abad-Franch et al. 2001b, Marcilla et al. 2001, Solís-Mena et al. unpublished).

8.2. ***Rhodnius ecuadoriensis***

4. We defined the main biogeographical traits of *R. ecuadoriensis* in both Ecuador (Abad-Franch et al. 2001b) and Peru (Cuba Cuba et al. 2002). As with other *Rhodnius*, the range of sylvatic populations of the species is probably restricted to areas with palm trees; these are however absent from most of the dry life zones of southern Ecuador and northern Peru where strongly synanthropic bug populations are common. These patterns of distribution suggest that the species could have extended to arid life zones and Andean highlands associated with human migrations; however, the possibility that sylvatic populations exploit alternative habitats (e.g., hollow trees) in those areas cannot be ruled out with the data at hand and should be investigated.

5. The primary natural ecotope of *R. ecuadoriensis* is probably the endemic 'tagua' palm tree, *Ph. aequatorialis*. We studied the ecology of sylvatic populations of *R. ecuadoriensis* in over 100 tagua palms of western Ecuador, and found 23% of them infested. Higher apparent bug densities were recorded in the humid Andean foothills than in coastal communities, but evidence of synanthropic behaviour was only found in the coast; bug populations seem rare and small in the heavily deforested central lowlands. We defined high-risk palm trees based on simple biological features. Adult palms with stems over 3m and abundant decomposing organic matter (fibre, dead fronds) and epiphytes were more likely to be infested, mainly in human-altered environments (cropland/pasture). In the coast, traditional management of female palms seemed to lower infestation rates, suggesting the possibility that control measures aimed at palms could complement conventional interventions. An approach we termed 'integrated habitat management' was put forward combining chemical control,

environmental management (houses, peridomestic structures, poultry, and palms), and community-based longitudinal surveillance.

In this survey we used live-bait adhesive traps for the first time in palm trees (Abad-Franch et al. 2000); we demonstrated the value (including cost-effectiveness) of this approach for the study of sylvatic triatomine populations in their natural ecotopes, minimising both effort and ecological damage and allowing for longitudinal research (Abad-Franch et al. 2000, Noireau et al. 2000, 2002). Our results suggest that similar traps could be used for detecting and monitoring peridomestic bug infestations. The extensive application of this method to palm trees may be extremely helpful in the understanding of the dynamics of transmission in the Amazonian foci.

6. The main ecological features of synanthropic populations of *R. ecuadoriensis* were also investigated. Multivariate logistic regression was applied to field data from almost 200 domiciles and descriptive-predictive models were derived. The main findings relate to the link between lower family incomes and higher household infestation rates and the key role of poultry in the establishment of peri- and eventually intradomiciliary bug colonies. The risk of infestation was significantly higher in mud-walled houses; the epidemiological importance of timber-and-tile roofs evidenced by our results is probably related to structural features of these roofs. The possible role of dogs and guinea pigs in Andean communities, suggested by some analyses, should be more thoroughly studied. Different populations of *R. ecuadoriensis* display varying degrees of synanthropism, from sylvatic bugs invading and sometimes colonising human habitats (in the central coast) to mainly peridomestic populations (in El Oro) and both peri- and intradomiciliary colonies in the drier southern Andes (Loja and Peru) – where synanthropic populations may be dense and the bugs may feed on birds and mammals (rodents, opossums, and humans) (Abad-Franch et al. 2002).

Control strategies in southern Ecuador-northern Peru should combine extensive household spraying (with attention to peridomestic chicken coops, tiled roofs and mud walls) with health education aimed at improving poultry management, establishing a vigilance system, and increasing awareness with regard to the link between bugs and disease.

7. Extreme phenotypic diversity was detected among *R. ecuadoriensis* geographic-ecological populations; both qualitative and quantitative phenetic analyses showed

differences in the range of classical supraspecific groupings. The most striking differences relate to the discovery of melanic, very large, and strictly sylvatic forms of the species in palms of Andean foothill forests in central-northern Ecuador. They differ substantially from the type material (synanthropic bugs from southern Ecuador), but sylvatic bugs from the central coast of Ecuador seem to present intermediate characters. However, size-free morphometric analyses suggested a common genetic background of all Ecuadorian populations, whereas Peruvian material (superficially similar to southern Ecuadorian bugs) appeared to be well separated. Finally, isometry-free analyses consistently achieved ≈90% correct reclassification of specimens to their original geographic-ecological groups, showing the potential of this morphometric approach for entomological surveillance of reinfestations and suggesting that the bugs can undergo rapid and drastic morphological changes (mainly involving size, and acting either in the sense of divergence or convergence) as a result of ecological adaptations.

8. We used DNA sequence polymorphisms of the mitochondrial cytochrome *b* gene to analyse genetic diversity in *R. ecuadoriensis*. Two major clades were identified, corresponding to Ecuadorian and Peruvian populations – which probably constitute independent phylogroups. Average genetic distances of about 4% were scored, and are compatible with a Pleistocene common ancestor (~1.8-1.7mya). Ecuadorian populations were the most diverse; the seasonally humid forests of central northwest Ecuador may be regarded as the centre of dispersal of the species. Shared haplotypes were detected in the populations that occur within the natural range of *Ph. aequatorialis*, whereas bugs from interior, dry Andean valleys had unique haplotypes, including a small monophyletic cluster in Loja. This is suggestive of a certain degree of isolation of those Andean populations; the mechanism of dispersal to dry areas may have involved passive transportation with hosts (humans or other vertebrates) or active occupation of alternative (non-palm) habitats.

9. We combined morphometric and molecular approaches to explore the phylogenetic relationships of *R. ecuadoriensis* with the closely related *R. pallescens* and *R. colombiensis* (the 'Pacific *Rhodnius* lineage') and with other *Rhodnius* from the Amazon. We used mt cyt*b* sequence data to derive a robust phylogenetic hypothesis for eight species of *Rhodnius*, and established the monophyly of the Pacific lineage. Metric analyses suggested a progressive, north-to-south change in head shape (*pallescens* →

colombiensis → sylvatic *ecuadoriensis* → domestic *ecuadoriensis*). *R. pictipes* appeared in a basal position to the Pacific lineage in all mtDNA phylogenies; the 14% sequence divergence with *pallescens-colombiensis* is compatible with a late Miocene (6.3-6.1mya) common ancestor. This suggests that both dispersal and vicariance (related to the uplift of the Andes in the Pliocene) could have played major roles in the emergence and evolution of the Pacific lineage. The close relationship between *R. pallescens* and *R. colombiensis* was confirmed by both molecular and metric analyses; the ~11%-12% sequence divergence of that pair with regard to *ecuadoriensis* is compatible with Pliocene vicariance (~5mya).

8.3. Recommendations for control

10. Technical recommendations for the control of Chagas disease were delivered to the Ministry of Public Health of Ecuador in two documents (Abad-Franch et al. 2001a, Aguilar et al. 2001). The main aspects are listed below:

i. The diversity of actual (~5 species) and potential (~4-5 species) vectors highlights the need for flexible control strategies. The magnitude of the epidemiological risk and the operational design of control interventions are defined by the vector species and populations in each geographic area;

ii. *T. dimidiata* can probably be eliminated from western Ecuador-Peru: area-wide surveys will be required, and all houses in communities with infestation rates above 5% will have to be sprayed. Operational problems may be encountered in urban areas, where the bugs breed in the usually non-accessible spaces underneath timber house floors; perhaps fumigant canisters could be assayed in these cases;

iii. Control of strongly synanthropic populations of *R. ecuadoriensis* may follow a similar strategy in southern temperate Andean valleys with no palm trees. In areas of central-western Ecuador where sylvatic populations occur in palms interventions should be selective and focal; only houses where infestation is detected should be sprayed, and environmental management of potential peridomestic ecotopes (palms and bird coops) should be encouraged. Alternative sylvatic ecotopes (mainly hollow trees) should be investigated in southern Ecuador and northern Peru;

iv. Need for continuous and contiguous interventions. Elimination of *T. dimidiata* and southern populations of *R. ecuadoriensis* will require area-wide spraying; continuous surveillance and selective responses will be necessary to eliminate residual foci. The

incursion of secondary species (e.g., *P. rufotuberculatus*, *P. chinai*, *T. carrioni*) into domestic-peridomestic habitats is predictable; suitable monitoring with community participation will be necessary for long-term control;

v. Entomological-epidemiological surveillance will therefore play a key role in long-term interruption of transmission. Health education schemes can promote community participation; the link between bugs and disease should be emphasised, and the response of the control service to community requirements in the context of surveillance must be rapid and effective;

vi. In the Amazon foci, transmission is active and linked to non-domiciliated vectors; traditional control approaches will not be cost-effective in the area. Research is in progress aimed at defining the main ecological features of the vectors and helping devise innovative control-surveillance interventions (Palomeque et al. 2000, Noireau et al. 2002b).

vii. Adequate clinical management of patients is a crucial challenge for the public health system of Ecuador. Rural health workers must receive training and support to detect and manage acute cases; most chronic patients need continuous follow-up, and some require specialised care generally available in regional hospitals. Control of congenital transmission will require adequate follow-up of seropositive pregnant women;

viii. Screening of donations to blood banks, especially in coastal and Amazon towns, must be reinforced (in terms of equipment, reagents, and training) to ensure adequate coverage and quality standards;

ix. Control of Chagas disease can result in major savings for both the health and economic-productive systems of Ecuador. A relatively centralised and vertical decision-making scheme will probably be required for the initial attack phase (with area-wide surveys and insecticide spraying), but activities should become progressively more horizontal for an effective surveillance phase. The transfer of decision power to peripheral centres must be preceded by allocation of the necessary resources and by thorough training to ensure adequate temporal and spatial coverage and homogeneous quality standards. Transparency in the management of funds will be crucial for the success of the initiative; external audit will be needed in some instances, and suitable accountability guidelines must be clearly formulated.

9. REFERENCES

Abad-Franch F & Aguilar VHM (2000). Control de la enfermedad de Chagas en el Ecuador. Datos y reflexiones para una política de Estado. *Revista del Instituto Juan César García*, **25**, 12-32.

Abad-Franch F & Aguilar VHM (2002). Control of Chagas disease in the Andean countries, pp. 91-93 in (Guhl F & Schofield CJ eds.) *Proceedings of the fourth International Workshop on population genetics and control of Triatominae, Cartagena de Indias, Colombia, Aug. 2000.* Universidad de los Andes, Bogotá, Colombia.

Abad-Franch F, Aguilar VHM, Paucar CA, Lorosa ES & Noireau F (2002). Observations on the domestic ecology of *Rhodnius ecuadoriensis* (Triatominae). *Memórias do Instituto Oswaldo Cruz*, **97**, 199-202.

Abad-Franch F, Noireau F, Paucar CA, Aguilar VHM, Carpio CC & Racines VJ (2000). The use of live-bait traps for the study of sylvatic *Rhodnius* populations (Hemiptera: Reduviidae) in palm trees. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **94**, 629-630.

Abad-Franch F, Palomeque FS & Aguilar VHM (2001a). *Control de la transmisión vectorial de la enfermedad de Chagas en el Ecuador*. FASBASE – Ministerio de Salud Pública del Ecuador, Quito, Ecuador, 63 pp.

Abad-Franch F, Paucar CA, Carpio CC, Cuba Cuba CA, Aguilar VHM & Miles MA (2001b). Biogeography of Triatominae (Hemiptera: Reduviidae) in Ecuador: implications for the design of control strategies. *Memórias do Instituto Oswaldo Cruz*, **96**, 611-620.

Acevedo F, Godoy E & Schofield CJ (2000). Comparison of intervention strategies for control of *Triatoma dimidiata* in Nicaragua. *Memórias do Instituto Oswaldo Cruz*, **95**, 867-871.

Adamson RE, Ward RD, Feliciangeli MD & Maignon R (1993). The application of random amplified polymorphic DNA for sandfly species identification. *Medical and Veterinary Entomology*, 7, 203-207.

Aguilar VHM, Abad-Franch F, Guevara AG, Racines VJ, Briones LA & Reyes LV (2001). *Guía operacional para el control de la enfermedad de Chagas en el Ecuador*. FASBASE–Ministerio de Salud Pública del Ecuador, Quito, Ecuador, 40 pp.

Aguilar VHM, Abad-Franch F, Racines VJ & Paucar CA (1999). Epidemiology of Chagas disease in Ecuador. A brief review. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 387-393.

Aguilar VHM & Yépez R (1996). Evolución epidemiológica de la enfermedad de Chagas en el Ecuador, pp. 30-38 in (Schofield CJ, Dujardin JP & Jurberg J eds.) *Proceedings of the International Workshop on population genetics and control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995*. INDRE, Mexico City, Mexico.

Albarracín-Veizaga H, Carvalho ME, Nascimento EMM, Rodrigues VLCC, Casanova C & Barata JMS (1999). Chagas disease in an area of recent occupation in Cochabamba, Bolivia. *Revista de Saúde Pública*, **33**, 230-236.

Almeida FB, Santos EI & Sposina G (1973). Triatomíneos da Amazônia III. *Acta Amazónica*, **3**, 43-46.

Altman DG & Bland JM (1994). Statistics notes. Diagnostic tests 3: receiver operating characteristic plots. *British Medical Journal*, **309**, 188.

Álvarez CJ (1984). *Historia de la medicina tropical ecuatoriana III. Enfermedad de Chagas en el Ecuador*. Ed. Arquidiocesana Justicia y Paz, Guayaquil, Ecuador, 233 pp.

Amunárriz M (1991). Enfermedad de Chagas. Primer foco amazónico, pp. 27-37 in (Amunárriz M) *Estudios sobre patologías tropicales en la Amazonia ecuatoriana*. Ed. CICAME, Pompeya, Napo, Ecuador.

Amunárriz MU, Chico ME & Guderian RH (1991). Chagas disease in Ecuador: a sylvatic focus in the Amazon region. *Journal of Tropical Medicine and Hygiene*, **94**, 145-149.

Anderson JM, Lai JE, Dotson EM, Cordón-Rosales C, Ponce C, Norris DE & Beard CB (2002). Identification and characterization of microsatellite markers in the Chagas disease vector *Triatoma dimidiata*. *Infection, Genetics and Evolution*, **1**, 243-248.

Andrade ALSS, Zicker F, Oliveira RM, Silva IG, Silva SA, Andrade SS & Martelli CMT (1995a). Evaluation of risk factors for house infestation by *Triatoma infestans* in Brazil. *American Journal of Tropical Medicine and Hygiene*, **53**, 443-447.

Andrade ALSS, Zicker F, Silva IG, Souza JMP & Martelli CMT (1995b). Risk factors for *Trypanosoma cruzi* infection among children in central Brazil: a case-control study in vector control settings. *American Journal of Tropical Medicine and Hygiene*, **52**, 183-187

Andrade ZA (1999). Immunopathology of Chagas disease. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 71-80.

Angulo VM, Gutiérrez R, Rubio I, Joya M, Arismendi M, Esteban L & Sandoval CM (1999). Triatomíneos domiciliados y silvestres: impacto en la transmisión de la enfermedad de Chagas en Santander, pp. 72-76 in (Angulo VM ed.) *Memorias. Control y manejo de la trypanosomiasis americana. Curso-taller internacional.* Universidad Industrial de Santander–CINTROP, Bucaramanga, Colombia.

Anonymous (1999). Recommendations from a satellite meeting. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 429-432.

Argañaraz RE, Hubbard GB, Ramos LA, Ford AL, Nitz N, Leland MM, Vandeberg JL & Teixeira ARL (2001). Blood-sucking lice may disseminate *Trypanosoma cruzi* infection in baboons. *Revista do Instituto de Medicina Tropical de São Paulo*, **43**, 271-276.

Arteaga CL (1930). Investigaciones sobre la existencia de la enfermedad de Chagas en la zona del ferrocarril de la costa (Provincia del Guayas). *Revista de la Universidad de Guayaquil*, **1**, 89-101.

Ault SK (1994). Environmental management: a re-emerging vector control strategy. *American Journal of Tropical Medicine and Hygiene*, **50** (Suppl.), 35-49.

Ávila HA, Sigman DS, Cohen LM, Millikan RC & Simpson L (1991). Polymerase chain reaction amplification of *Trypanosoma cruzi* kinetoplast minicircle DNA isolated from whole blood lysates: diagnosis of chronic Chagas disease. *Molecular Biochemical Parasitology*, **48**, 221-222.

Avilés H, Cevallos P, Champaloux B, Goujon C, Lema F, Pajony R, Pays JF, Racines VJ & Romaña CA (1995a). *Rhodnius ecuadoriensis* en áreas endémicas de tripanosomiasis americana en Ecuador. *Parasitología al Día*, **19** (N° extraordinario), 231.

Avilés H, Cevallos P, Champaloux B, Goujon C, Lema F, Pajony R, Pays JF, Racines VJ & Romaña CA (1995b). *Panstrongylus rufotuberculatus* y características de la vivienda en la provincia de El Oro, Ecuador. *Parasitología al Día*, **19** (N° extraordinario), 230.

Avise JC (1994). *Molecular markers, natural history and evolution*. Chapman & Hall, New York, NY, USA, 511 pp.

Avise JC (2000). *Phylogeography: the history and formation of species*. Harvard University Press, Cambridge, MA, USA, 447 pp.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA & Saunders NC (1987). Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, **18**, 489-522.

Avise JC & Johns GC (1999). Proposal for a standardized temporal scheme of biological classification for extant species. *Proceedings of the National Academy of Sciences of the USA*, **96**, 7358-7363.

Avise JC & Walker D (1998). Pleistocene phylogeographic effects on avian populations and the speciation process. *Proceedings of the Royal Society London Series B*, **265**, 457-463.

Avise JC & Wollemberg K (1997). Phylogenetics and the origin of species. *Proceedings of the National Academy of Sciences of the USA*, **94**, 7748-7755.

Ayala FJ & Fitch WM (1997). Genetics an the origin of species: an introduction. *Proceedings of the National Academy of Sciences of the USA*, **94**, 7691-7697.

Ayala Mora E (ed.) (1993). *Nueva Historia del Ecuador, Vol. 14. Cronología comparada de la Historia ecuatoriana.* Corporación Editora Nacional/Editorial Grijalbo Ecuatoriana, Quito, Ecuador, 334 pp.

Ballinger-Crabtree ME, Black WC & Miller BB (1992). Use of genetic polymorphism detected by random polymerase chain reaction (RAPD-PCR) for differentiation and identification of *Aedes aegypti* subspecies and populations. *American Journal of Tropical Medicine and Hygiene*, **47**, 893-901.

Barata JMS (1996). Aspectos morfológicos de ovos de Triatominae, pp. 55-58 in (Schofield CJ, Dujardin JP & Jurberg J eds.) *Proceedings of the International Workshop on population genetics and control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995.* INDRE, Mexico City, Mexico.

Barata JMS (1998). Macroscopic and exochorial structures of Triatominae eggs, pp. 409-448 in (Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. II. Editora Fiocruz, Rio de Janeiro, Brazil.

Bargues MD, Marcilla JA, Ramsey J, Dujardin JP, Schofield CJ & Mas-Coma S (2000). Nuclear rDNA-based molecular clock of the evolution of Triatominae (Hemiptera: Reduviidae), vectors of Chagas disease. *Memórias do Instituto Oswaldo Cruz*, **95**, 567-573.

Barrett TV (1988). Current research on Amazonian Triatominae. *Memórias do Instituto Oswaldo Cruz*, **83** (Suppl. 1), 441-447.

Barrett TV (1991). Advances in triatomine bug ecology in relation to Chagas disease. *Advances in Disease Vector Research*, **8**, 143-176.

Barrett TV (1996). Species interfertility and crossing experiments in triatomine systematics, pp. 72-77 in (Schofield CJ, Dujardin JP & Jurberg J eds.) *Proceedings of the International Workshop on population genetics and control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995.* INDRE, Mexico City, Mexico.

Barrett TV, Hoff RH, Mott KE, Miles MA, Godfrey DG, Teixeira R & Souza AA (1980). Epidemiological aspects of three *Trypanosoma cruzi* zymodemes in Bahia State, Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **74**, 84-89.

Barros T, Barreto D, Pérez F, Santander R, Yépez E, Abad-Franch F & Aguilar VHM (2001). Un modelo de prevención primaria de las enfermedades de transmisión sexual y del VIH/sida en adolescentes. *Pan American Journal of Public Health*, **10**, 86-93.

Beard CB & Lyman DF (1999). DNA-based approaches for inter- and intraspecific analysis of Chagas disease vectors, pp. 74-77 in (Schofield CJ & Ponce C eds.) *Proceedings of the second International Workshop on population genetics and control of Triatominae, Tegucigalpa, Honduras, March 1998.* INDRE, Mexico City, Mexico.

Beard CB, Mills Hamm D & Collins FH (1993). The mitochondrial genome of the mosquito *Anopheles gambiae*: DNA sequence, genome organization, and comparisons with mitochondrial sequences of other insects. *Insect Molecular Biology*, **2**, 103-124.

Bérenger JM & Pluot-Sigwalt D (2002). *Rhodnius amazonicus* Almeida, Santos & Sposina, 1973, bona species, close to *R. pictipes* Stål, 1872 (Heteroptera, Reduviidae, Triatominae). *Memórias do Instituto Oswaldo Cruz*, **97**, 73-77.

Bergquist NR (2001). Vector-borne parasitic diseases: new trends in data collection and risk assessment. *Acta Tropica*, **79**, 13-20.

Birungi J & Munstermann LE (2002). Genetic structure of *Aedes albopictus* (Diptera: Culicidae) populations based on mitochondrial ND5 sequences: evidence for an independent invasion into Brazil and United States. *Annals of the Entomological Society of America*, **95**, 125-132.

Black WC & Roehrdanz RL (1998). Mitochondrial gene order is not conserved in arthropods: prostriate and metastriate tick mitochondrial genomes. *Molecular Biology and Evolution*, **15**, 1772-1785.

Bland JM & Altman DG (2000). Statistics notes. The odds ratio. *British Medical Journal*, **320**, 1468.

Boore JL (1999). Animal mitochondrial genomes. *Nucleic Acids Research*, **27**, 1767-1780.

Boore JL, Collins TM, Stanton D, Daehler LL & Brown WM (1995). Deducing the pattern of arthropod phylogeny from mitochondrial DNA rearrangements. *Nature*, **376**, 163-165.

Boore JL, Lavrov D & Brown WM (1998). Gene translocation links insects and crustaceans. *Nature*, **393**, 667-668.

Borchsenius F, Borgtoft Pedersen H & Balslev H (1998). *Manual to the palms of Ecuador*. AAU Reports **37**. Dept. of Systematic Botany, Aarhus University–Pontificia Universidad Católica del Ecuador, Quito. Aarhus, Denmark, 221 pp.

Borges EC, Dujardin JP, Schofield CJ, Romanha AJ & Diotaiuti L (2000). Genetic variability of *Triatoma brasiliensis* (Hemiptera: Reduviidae) populations. *Journal of Medical Entomology*, **37**, 872-877.

Borges EC, Pires HHR, Barbosa SE, Nunes CMS, Pereira MH, Romanha AJ & Diotaiuti L (1999). Genetic variability in Brazilian triatomines and the risk of domiciliation. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 371-373.

Borges EC, Romanha AJ & Diotaiuti L (2000). Uso do Random Amplified Polymorphic DNA (RAPD) no estudo populacional do *Triatoma brasiliensis* Neiva, 1911. *Cadernos de Saúde Pública*, **16** (Suppl. 2), 97-100.

Brack A (1987). Geografía de un país complejo, pp. 179-194 in (Dourojeanni MJ ed.) *Gran geografía del Perú: naturaleza y hombre*, Vol. II. Manfer – Juan Mejía Baca, Madrid, Spain.

Breniére SF, Bosseno MF, Revollo S, Rivera MT, Carlier Y & Tibayrenc M (1992). Direct identification of *Trypanosoma cruzi* natural clones in vectors and mammalian hosts by polymerase chain reaction amplification. *American Journal of Tropical Medicine and Hygiene*, **46**, 335-341.

Breniére SF, Bosseno MF, Telleria J, Carrasco R, Vargas F, Yaksic N & Noireau F (1995). Field application of polymerase chain reaction diagnosis and strain typing of *Trypanosoma cruzi* in Bolivia. *American Journal of Tropical Medicine and Hygiene*, **53**, 179-184.

Breniére SF, Bosseno MF, Vargas F, Yaksic N, Noireau F, Noel S, Dujardin JP & Tibayrenc M (1998). Smallness of the panmictic unit of *Triatoma infestans* (Hemiptera: Reduviidae). *Journal of Medical Entomology*, **35**, 911-917.

Briceño-León R (1990). *La casa enferma. Sociología de la enfermedad de Chagas*. Fondo Editorial Acta Científica Venezolana y Consorcio de Ediciones Capriles Caracas, Venezuela, 149 pp.

Briceño-León R (1996). El mejoramiento de la vivienda campesina: créditos y microcambio social, p. 86 in (Schofield CJ, Dujardin JP & Jurberg J eds.) *Proceedings of the International Workshop on population genetics and control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995*. INDRE, Mexico City, Mexico.

Brisse S, Barnabe C & Tibayrenc M (2000). Identification of six *Trypanosoma cruzi* phylogenetic lineages by random amplified polymorphic DNA and multilocus enzyme electrophoresis. *International Journal for Parasitology*, **30**, 35-44.

Britto C, Silveira C, Cardoso MA, Marques P, Luquetti A, Macêdo V & Fernandes O (2001). Parasite persistence in treated chagasic patients revealed by xenodiagnosis and polymerase chain reaction. *Memórias do Instituto Oswaldo Cruz*, **96**, 823-826.

Brower AVZ (1994). Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences of the USA*, **91**, 6491-6495.

Brugge VA, Schooley DA & Orchard I (2002). The biological activity of diuretic factors in *Rhodnius prolixus*. *Peptides*, **23**, 671-681.

Bryan RT, Balderrama F, Tonn RJ & Dias JCP (1994). Community participation in vector control: lessons from Chagas disease. *American Journal of Tropical Medicine and Hygiene*, **50** (6 Suppl.), 61-71.

Cabello DR & Galíndez Girón I (1998). Vital statistics of *Panstrongylus geniculatus* (Latreille 1811) (Hemiptera: Reduviidae) under experimental conditions. *Memórias do Instituto Oswaldo Cruz*, **93**, 257-262.

Calderón G, Cuzquén L, Figueroa Krap E, Náquira F, Carcavallo RU & Canale DM (1985). Perú, pp. 449-455 in (Carcavallo RU, Rabinovich JE & Tonn RJ eds.) *Factores biológicos y ecológicos en la enfermedad de Chagas*, Vol. II. Centro Panamericano de Ecología Humana y Salud OPS/OMS – Servicio Nacional de Chagas, Ministerio de Salud y Acción Social, Buenos Aires, Argentina.

Camargo ME (1999). Development in the etiologic diagnosis of Chagas disease. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 281-282.

Canals M, Cruzat L, Molina MC, Ferreira A & Cattan P (2001). Blood host sources of *Mepraia spinolai* (Heteroptera: Reduviidae), wild vector of Chagas disease in Chile. *Journal of Medical Entomology*, **38**, 303-307.

Cañadas L (1983). *Mapa bioclimático y ecológico del Ecuador*. MAG-PRONAREG, Quito, Ecuador, 210 pp.

Carcavallo RU (1985). Técnicas de estudio de triatominos en ambiente silvestre, pp. 49-52 in (Carcavallo RU, Rabinovich JE & Tonn RJ eds.) *Factores biológicos y ecológicos en la enfermedad de Chagas*, Vol. I. Centro Panamericano de Ecología Humana y Salud OPS/OMS–Servicio Nacional de Chagas, Ministerio de Salud y Acción Social, Buenos Aires, Argentina.

Carcavallo RU (1999). Climatic factors related to Chagas disease transmission. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 367-369.

Carcavallo RU & Barreto P (1976). Una nueva especie de *Rhodnius* Stål (Hemiptera, Reduviidae, Triatominae) de Colombia. *Boletín de la Dirección de Malariología y Saneamiento Ambiental*, **16**, 176-183.

Carcavallo RU, Curto de Casas SI, Sherlock IA, Galíndez Girón I, Jurberg J, Galvão C, Mena Segura CA & Noireau F (1999c). Geographical distribution and alti-latitudinal dispersion, pp. 747-792 in (Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. III. Editora Fiocruz, Rio de Janeiro, Brazil.

Carcavallo RU, Galíndez Girón I, Catalá S, Jurberg J, Lent H, Galvão C & Barata JMS (1997b). Some anatomic structures studied with Scanning Electron Microscopy (SEM), pp. 299-393 in (Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. I. Editora Fiocruz, Rio de Janeiro, Brazil.

Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H (eds.) (1997a). *Atlas of Chagas disease vectors in the Americas*, Vol. I. Editora Fiocruz, Rio de Janeiro, Brazil.

Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H (eds.) (1999a). *Atlas of Chagas disease vectors in the Americas*, Vol. III. Editora Fiocruz, Rio de Janeiro, Brazil.

Carcavallo RU, Jurberg J & Lent H (1999b). Phylogeny of the Triatominae. A-General approach, pp. 925-969 in (Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. III. Editora Fiocruz, Rio de Janeiro, Brazil.

Carcavallo RU, Jurberg J, Lent H, Noireau F & Galvão C (2000). Phylogeny of the Triatominae (Hemiptera: Reduviidae). Proposals for taxonomic arrangements. *Entomologia y Vectores*, **7** (Suppl. 1), 1-99.

Carcavallo RU, Jurberg J, Martínez A & Galíndez Girón I (1994). La región óculo-ocelar en el género *Rhodnius* Stål, 1895 (Hemiptera, Reduviidae, Triatominae). *Entomologia y Vectores*, **1**, 21-32.

Carcavallo RU & Martínez A (1985). Biología, ecología, distribución geográfica de los triatominos americanos, pp. 149-208 in (Carcavallo RU, Rabinovich JE & Tonn RJ eds.) *Factores biológicos y ecológicos en la enfermedad de Chagas*, Vol. II. Centro Panamericano de Ecología Humana y Salud OPS/OMS – Servicio Nacional de Chagas, Ministerio de Salud y Acción Social, Buenos Aires, Argentina.

Carcavallo RU, Rocha DS, Galíndez Girón I, Sherlock IA, Galvão C, Martínez A, Tonn RJ & Cortón E (1998b). Feeding sources and patterns, p. 537-560 in (Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. II. Editora Fiocruz, Rio de Janeiro, Brazil.

Carcavallo RU, Rodríguez MEF, Salvatella R, Curto de Casas SI, Sherlock IA, Galvão C, Rocha DS, Galíndez Girón I, Otero Arocha MA, Martínez A, Da Rosa JA, Canale DM, Farr TH & Barata JMS (1998a). Habitats and related fauna. A: General, p. 561-600 in (Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. II. Editora Fiocruz, Rio de Janeiro, Brazil.

Carlier L, Muñoz M & Dujardin JP (1996). A RAPD protocol for Triatominae, pp. 81-83 in (Schofield CJ, Dujardin JP & Jurberg J eds.) *Proceedings of the International Workshop on population genetics and control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995*. INDRE, Mexico City, Mexico.

Carrasco HJ, Frame IA, Valente SA & Miles MA (1996). Genetic exchange as a possible source of genomic diversity in sylvatic populations of *Trypanosoma cruzi*. *American Journal of Tropical Medicine and Hygiene*, **54**, 418-424.

Catalá S (1996). Los patrones de las sensilla, pp. 59-62 in (Schofield CJ, Dujardin JP & Jurberg J eds.) *Proceedings of the International Workshop on population genetics and control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995*. INDRE, Mexico City, Mexico.

Catalá S (1997). Antennal sensilla of Triatominae (Hemiptera: Reduviidae): a comparative study over five genera. *International Journal of Insect Morphology and Embryology*, **228**, 67-73.

Catalá S (1999). Patrones morfológicos de sensilla en las antenas de Triatominae: ¿reflejan la percepción del hábitat y los hospedadores?, pp. 49-52 in (Schofield CJ & Ponce C eds.) *Proceedings of the second International Workshop on population genetics and control of Triatominae, Tegucigalpa, Honduras, March 1998*. INDRE, Mexico City, Mexico.

Catalá S & Schofield CJ (1994). The antennal sensilla of *Rhodnius*. *Journal of Morphology*, **21**, 193-203.

Cavalli-Sforza L (1997). Genes, peoples, and languages. *Proceedings of the National Academy of Sciences of the USA*, **94**, 7719-7724.

Cavalli-Sforza L (1998). The DNA revolution in population genetics. *Trends in Genetics*, **14**, 60-65.

Chagas CRJ (1909). Nova Trypanozomiaze humana. Estudos sobre a morfologia e o ciclo evolutivo do *Schizotrypanum cruzi* n. gen., n. sp., ajente etiologico de nova entidade morbida no homem. *Memórias do Instituto Oswaldo Cruz*, **1**, 159-218.

Chagas CRJ (1911). Moléstia de Carlos Chagas. Conferência realizada em 7 de agosto de 1911, na Academia Nacional de Medicina. *Brazil Médico*, **25**, 340-375.

Chapadeiro E (1999). Clinical evolution and morbi-mortality in Chagas disease. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 309-310.

Chávez T, Moreno J & Dujardin JP (1999). Isoenzyme electrophoresis of *Rhodnius* species: a phenetic approach to relationships within the genus. *Annals of Tropical Medicine and Parasitology*, **93**, 229-307.

Chiari E (1999). Chagas disease diagnosis using polymerase chain reaction, hemoculture and serologic methods. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 299-300.

Chico HM, Sandoval C, Guevara EA, Calvopiña HM, Cooper PJ, Reed SG & Guderian RH (1997). Chagas disease in Ecuador: evidence for disease transmission in an indigenous population in the Amazon region. *Memórias do Instituto Oswaldo Cruz*, **92**, 317-320.

Claridge MF & Gillham (1992). Variation in populations of leafhoppers and planthoppers (Auchenorryncha): biotypes and biological species, pp. 241-259 in (Foottit RG & Sorensen JT eds.) *Ordination in the study of morphology, evolution and systematics of insects: applications and quantitative genetic rationales*. Elsevier, New York, NY, USA.

Clary DO & Wolstenholme DR (1985). The mitochondrial DNA molecule of *Drosophila yakuba*: nucleotide sequence, gene organization, and genetic code. *Journal of Molecular Evolution*, **22**, 252-271.

Cochrane MA, Alencar A, Schulze MD, Souza CMJr, Nepstad DC, Lefebvre P & Davidson EA (1999). Positive feedbacks in the fire dynamic of closed canopy tropical forests. *Science*, **284**, 1832-1835.

Cohen JE & Gürtler RE (2001). Modeling household transmission of American trypanosomiasis. *Science*, **293**, 694-698.

Collins FH & Paskewitz SM (1996). A review of the use of ribosomal DNA (rDNA) to differentiate among cryptic *Anopheles* species. *Insect Molecular Biology*, **5**, 1-9.

Conn JE, Mitchell SE & Cockburn AF (1997). Mitochondrial DNA variation within and between two species of Neotropical anopheline mosquitoes (Diptera: Culicidae). *Journal of Heredity*, **88**, 98-107.

Córdova MX, Bowen CA, Paredes WA, Gómez E, Garzón EA & Guevara AG (1999). Aislamiento de *Trypanosoma cruzi* a partir de pacientes chagásicos crónicos en áreas endémicas del Ecuador. *Boletín de Epidemiología (Ecuador)*, **3**, 14-17.

Corrêa-Oliveira R, Gomes JAS, Lemos EM, Cardoso GM, Reis DD, Adad S, Crema E, Martins-Filho OA, Costa MOR, Gazzinelli G & Bahia-Oliveira LMG (1999). The role of the immune response on the development of severe clinical forms of human Chagas disease. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 253-255.

Coscarón MC, Loiácono MS & De Santis L (1999). Predators and parasitoids, pp. 891-924 in (Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. III. Editora Fiocruz, Rio de Janeiro, Brazil.

Costa J (1999). The synanthropic process of Chagas disease vectors in Brazil, with special attention to *Triatoma brasiliensis* Neiva, 1911 (Hemiptera, Reduviidae, Triatominae). Population, genetical, ecological, and epidemiological aspects. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 239-241.

Costa J, Almeida JR, Britto C, Duarte R, Marchon-Silva V & Pacheco RS (1998). Ecotopes, natural infection and trophic resources of *Triatoma brasiliensis* (Hemiptera, Reduviidae, Triatominae). *Memórias do Instituto Oswaldo Cruz*, **93**, 7-13.

Costa J, Freitas-Sibajev MGR, Marchon-Silva V, Pires MQ & Pacheco RS (1997). Isoenzymes detect variation in populations of *Triatoma brasiliensis* (Hemiptera, Reduviidae, Triatominae). *Memórias do Instituto Oswaldo Cruz*, **92**, 459-464.

Coura JR (1990). The risk of endemic Chagas disease in the Brazilian Amazon. *Revista da Sociedade Brasileira de Medicina Tropical*, **23**, 67-70.

Coura JR & de Castro SL (2002). A critical review on Chagas disease chemotherapy. *Memórias do Instituto Oswaldo Cruz*, **97**, 3-24.

Coura JR, Junqueira ACV, Boia MN & Fernandes O (1999). Chagas disease: from bush to huts and houses. Is it the case of the Brazilian Amazon? *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 379-384.

Coura JR, Junqueira ACV, Fernandes O, Valente SAS & Miles MA (2002). Emerging Chagas disease in Amazonian Brazil. *Trends in Parasitology*, **18**, 171-176.

Coura JR, Junqueira ACV, Giordano CM & Funatsu IRK (1994). Chagas disease in the Brazilian Amazon. I – A short review. *Revista do Instituto de Medicina Tropical de Sâo Paulo*, **36**, 363-368.

Cox CB & Moore PD (2000). *Biogeography. An ecological and evolutionary approach*. Blackwell Science, Oxford, UK, 298 pp.

Crampton A, McKay I & Baker SC (1996). Phylogeny of ticks (Ixodida) inferred from nuclear ribosomal DNA. *International Journal for Parasitology*, **26**, 511-517.

Crawley MJ (1993). *GLIM for ecologists*. Blackwell Science, Oxford, UK, 379 pp.

Crozier RH & Crozier YC (1993). The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. *Genetics*, **133**, 97-117.

Cuba Cuba CA, Abad-Franch F, Roldán RJ, Vargas VF, Pollack VL & Miles MA (2002). The triatomines of northern Peru, with emphasis on the ecology and infection by trypanosomes of *Rhodnius ecuadoriensis* (Triatominae). *Memórias do Instituto Oswaldo Cruz*, **97**, 175-183.

Curto de Casas SI, Carcavallo RU, Galíndez Girón I & Burgos JJ (1999). Bioclimatic factors and zones of life, pp. 793-838 in (Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. III. Editora Fiocruz, Rio de Janeiro, Brazil.

D'Alessandro A & Barreto P (1985). Colombia, pp. 377-399 in (Carcavallo RU, Rabinovich JE & Tonn RJ eds.) *Factores biológicos y ecológicos de la enfermedad de Chagas*, Vol. II. Centro Panamericano de Ecología Humana y Salud OPS/OMS–Servicio Nacional de Chagas, Ministerio de Salud y Acción Social, Buenos Aires, Argentina.

Darroch JN & Mosiman JE (1985). Canonical and principal components of shape. *Biometrika*, **72**, 241-252.

Deane MP, Jansen AM & Lenzi HL (1984). *Trypanosoma cruzi*: vertebrate and invertebrate cycles in the same mammal host, the opossum *Didelphis marsupialis*. *Memórias do Instituto Oswaldo Cruz*, 79, 513-515.

Defranc MI (1982). *Enfermedad de Chagas*. Ed. Casa de la Cultura Ecuatoriana, Núcleo del Guayas, Guayaquil, Ecuador, 200 pp.

Dias JCP (1989). The indeterminate form of human chronic Chagas disease. A clinical epidemiological review. *Revista da Sociedade Brasileira de Medicina Tropical*, **22**, 147-156.

Dias JCP (1991). Chagas disease control in Brazil: which strategy after the attack phase?. *Annales de la Societé Belge de Médecine Tropicale*, **71** (Suppl. 1), 75-86.

Dias JCP (1998a). Problemas e possibilidades de participação comunitária no controle das grande endemias no Brasil. *Cadernos de Saúde Pública*, **14** (Suppl. 2), 19-37.

Dias JCP (1999). Recursos e estratégias da luta anti-chagásica nas Américas. Visão crítica, situação atual e perspectivas, pp. 13-24 in (Schofield CJ & Ponce C eds.) *Proceedings of the second International Workshop on population genetics and control of Triatominae, Tegucigalpa, Honduras, March 1998*. INDRE, Mexico City, Mexico.

Dias JCP (2000). Vigilância epidemiológica em doença de Chagas. *Cadernos de Saúde Pública*, **16** (Suppl. 2), 43-59.

Dias JCP & Brener S (1984). Chagas disease and blood transfusion. *Memórias do Instituto Oswaldo Cruz*, **79** (Suppl.), 139-147.

Dias JCP & Diotaiuti L (1998). Vectores secundarios de la enfermedad de Chagas en Brasil y perspectivas para su control, pp. 154-149 in (Guhl F & Jaramillo CA eds.) *Curso taller. Control de tripanosomosis americana y leishmaniosis: aspectos biológicos, genéticos y moleculares.* Universidad de Los Andes–CIMPAT, Santafé de Bogotá, Colombia.

Dias JCP & Schofield CJ (1999). The evolution of Chagas disease (American trypanosomiasis) control after 90 years since Carlos Chagas discovery. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 103-121.

Dias JCP, Vinhaes MC, Silveira AC, Schofield CJ, Cardoso B & Coura JR (2001). Pesquisas prioritárias sobre doença de Chagas na Amazónia: agenda de curto-médio prazo. *Revista da Sociedade Brasileira de Medicina Tropical*, **34**, 497-498.

Dias RB (1998b). "*Eu? Eu estou aí, compondo o mundo*". Uma experiência de controle de endemia, pesquisa e participação popular vivida em Cansanção, Minas Gerais, Brasil. *Cadernos de Saúde Pública*, **14** (Suppl. 2), 149-157.

Diotaiuti L (1999). Triatomíneos da Caatinga, pp. 96-100 in (Schofield CJ & Ponce C eds.) *Proceedings of the second International Workshop on population genetics and control of Triatominae, Tegucigalpa, Honduras, March 1998*. INDRE, Mexico City, Mexico.

Diotaiuti L, Pereira AS, Loiola CF, Fernandes AJ, Schofield CJ, Dujardin JP, Dias JCP & Chiari E (1995). Inter-relation of sylvatic and domestic transmission of *Trypanosoma cruzi* in areas with and without domestic vectorial transmission in Minas Gerais, Brazil. *Memórias do Instituto Oswaldo Cruz*, **90**, 443-448.

Dodson CH & Gentry AH (1991). Biological extinction in western Ecuador. *Annals of the Missouri Botanical Garden*, **78**, 273-295.

Donnelly MJ, Simard F & Lehman T (2002). Evolutionary studies of malaria vectors. *Trends in Parasitology*, **18**, 75-80.

Dorea RCC, Miles MA, Povoa MM & Souza A (1982). An electrophoretic approach to the taxonomy of Chagas disease vectors (Triatominae), pp. 643-647 in *Recent developments in the genetics of insect disease vectors.* Stipes Publishing, Champaign, IL, USA.

Dotson EM & Beard CB (2001). Sequence and organization of the mitochondrial genome of the Chagas disease vector, *Triatoma dimidiata*. *Insect Molecular Biology*, **10**, 205-225.

Dujardin JP (1990). *Intérét de la génetique des populations dans l'etude des vecteurs de la Trypanosomiase américaine*. PhD Thesis, Université de Liége, Belgium, 200 pp.

Dujardin JP, Bermúdez H, Casini C & Schofield CJ (1997a). The use of morphometrics in entomological surveillance of sylvatic foci of *Triatoma infestans* in Bolivia. *Acta Tropica*, **66**, 145-153.

Dujardin JP, Bermúdez H, Casini C, Schofield CJ & Tibayrenc M (1997b). Metric differences between sylvatic and domestic *Triatoma infestans* (Hemiptera: Reduviidae) in Bolivia. *Journal of Medical Entomology*, **34**, 544-551.

Dujardin JP & Casini C (1996). Morphometry, pp. 53-54 in (Schofield CJ, Dujardin JP & Jurberg J eds.) *Proceedings of the International Workshop on population genetics and control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995*. INDRE, Mexico City, Mexico.

Dujardin, JP, Chávez T, Hervas D & Machane M (1999e). Phylogenetic techniques applied to the Rhodniini, pp. 105-112 in (Schofield CJ & Ponce C eds.) *Proceedings of the second International Workshop on population genetics and control of Triatominae, Tegucigalpa, Honduras, March 1998*. INDRE, Mexico City, Mexico.

Dujardin JP, Chávez T, Machane M & Solís S (1999d). Size, shape and genetics. Sexual dimorphism and environment, pp. 53-62 in (Schofield CJ & Ponce C eds.) *Proceedings of the second International Workshop on population genetics and control of Triatominae, Tegucigalpa, Honduras, March 1998*. INDRE, Mexico City, Mexico.

Dujardin JP, Chávez T, Moreno JM, Machane M, Noireau F & Schofield CJ (1999a). Comparison of isoenzyme electrophoresis and morphometric analysis for phylogenetic reconstruction of the Rhodniini (Hemiptera: Reduviidae: Triatominae). *Journal of Medical Entomology*, **36**, 653-659.

Dujardin JP, García-Zapata MT, Jurberg J, Poelants P, Cardozo L, Panzera F, Dias JCP & Schofield CJ (1991). Which species of *Rhodnius* is invading houses in Brazil? *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **85**, 679-680.

Dujardin JP & Le Pont F (2000). Morphometrics of a Neotropical sandfly subspecies, *Lutzomyia carrerai thula*. *Comptes Redus de l'Academie des Sciences Séries III – Sciences de la Vie*, **323**, 273-279.

Dujardin JP, Muñoz M, Chavez T, Ponce C, Moreno J & Schofield CJ (1998a). The origin of *Rhodnius prolixus* in Central America. *Medical and Veterinary Entomology*, **12**, 113-115.

Dujardin JP, Panzera F & Schofield CJ (1999b). Triatominae as a model of morphological plasticity under ecological pressure. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 223-228.

Dujardin JP, Panzera F & Schofield CJ (2000). *Les vecteurs de la maladie de Chagas. Recherches taxonomiques, biologiques et génétiques*. Academie Royale des Sciences d'Outre Mer, Brussels, Belgium, 162 pp.

Dujardin JP, Schofield CJ & Tibayrenc M (1998c). Population structure of Andean *Triatoma infestans*: allozyme frequencies and their epidemiological relevance. *Medical and Veterinary Entomology*, **12**, 20-29.

Dujardin JP, Forgues G, Torrez M, Martínez E, Córdoba C & Gianella A (1998b). Morphometrics of domestic *Panstrongylus rufotuberculatus* in Bolivia. *Annals of Tropical Medicine and Parasitology*, **92**, 219-228.

Dujardin JP, Steindel M, Chávez T, Machane M & Schofield CJ (1999c). Changes in the sexual dimorphism of Triatominae in the transition from natural to artificial habitats. *Memórias do Instituto Oswaldo Cruz*, **94**, 565-569.

Dujardin JP, Tibayrenc M, Venegas E, Maldonado L, Desjeux P & Ayala FJ (1987). Isoenzyme evidence of lack of speciation between wild and domestic *Triatoma infestans* (Heteroptera: Reduviidae) in Bolivia. *Journal of Medical Entomology*, **24**, 40-45.

ECLAT – European Community-Latin American Network for Research on the Biology and Control of Triatominae (2002). *Species list.* http://www.eclat.fcien.edu.uy

Espinosa RA, Pericchi LR, Carrasco HA, Escalante A, Martínez O & González R (1991). Prognostic indicators of chronic chagasic cardiopathy. *International Journal of Cardiology*, **30**, 195-202.

Espinoza LA (1955). Epidemiología de la enfermedad de Chagas en la República del Ecuador. *Revista Ecuatoriana de Higiene y Medicina Tropical*, **12**, 25-105.

Espinoza-Gómez F, Maldonado-Rodríguez A, Coll-Cárdenas R, Hernández-Suárez CM & Fernández-Salas I (2002). Presence of Triatominae (Hemiptera, Reduviidae) and risk of transmission of Chagas disease in Colima, México. *Memórias do Instituto Oswaldo Cruz*, **97**, 25-30.

Esseghir S, Ready PD, Killick-Kendrick R & Ben-Ismail R (1997). Mitochondrial haplotypes and phylogeography of *Phlebotomus* vectors of *Leishmania major*. *Insect Molecular Biology*, **6**, 211-225.

Favia G, Dimopoulos G & Louis C (1994). Analysis of the *Anopheles gambiae* genome using RAPD markers. *Insect Molecular Biology*, **3**, 149-157.

Felsenstein J (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783-791.

Fernandes O, Mangia RH, Lisboa CV, Pinho AP, Morel CM, Zingales B, Campbell DA & Jansen AM (1998). The complexity of the sylvatic cycle of *Trypanosoma cruzi* in the Rio de Janeiro State (Brazil) revealed by the non-transcribed spacer of the mini-exon gene. *Parasitology*, **118**, 161-168.

Flores A, Magallón-Gastélum E, Bosseno MF, Ordóñez R, Lozano-Kasten F, Espinoza B, Ramsey J & Breniére SF (2001). Isoenzyme variability of five principal triatomine vector species of Chagas disease in Mexico. *Infection, Genetics and Evolution*, **1**, 21-28.

Foottit RG & Sorensen JT (1992). Ordination methods: their contrast to clustering and cladistic techniques, pp. 1-10 in (Foottit RG & Sorensen JT eds.) *Ordination in the study of morphology, evolution and systematics of insects: applications and quantitative genetic rationales*. Elsevier, New York, NY, USA.

Frías D & Dujardin JP (1996). Detección de la variación genética a nivel poblacional a través de electroforesis de isoenzimas, pp. 65-71 in (Schofield CJ, Dujardin JP & Jurberg J eds.) *Proceedings of the International Workshop on population genetics and control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995*. INDRE, Mexico City, Mexico.

Friedrich M & Tautz D (1995). Ribosomal DNA phylogeny of the major extant arthropod classes and the evolution of myriapods. *Nature*, **376**, 165-167.

Galíndez Girón I, Rocha DS, Lent H, Carcavallo RU, Jurberg J, Galvão C, Barbosa HS, Martínez A, Barata JMS & da Rosa JA (1998). Nymphal stages, pp. 449-514 in (Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. II. Editora Fiocruz, Rio de Janeiro, Brazil.

Galíndez Girón I & Torres E (1999). Morphometry in the systematics and ecology of the Triatominae, pp. 1115-1160 in (Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. III. Editora Fiocruz, Rio de Janeiro, Brazil.

Galindo SV (1958). Frecuencia chagásica en 150 cardiomegálicos. *Revista Ecuatoriana de Higiene y Medicina Tropical*, **15**, 205-216.

Galindo SV (1959). Cardiopatía chagásica crónica. Primeros casos en el Ecuador, demostrados parasitológicamente. *Revista Ecuatoriana de Higiene y Medicina Tropical*, **16**, 9-20.

Galvão C, Patterson JS, Rocha DS, Jurberg J, Carcavallo RU, Rajen K, Ambrose DP & Miles MA (2002). A new species of Triatominae from Tamil Nadu, India. *Medical and Veterinary Entomology*, **16**, 75-82.

García AL, Carrasco HJ, Schofield CJ, Valente SAS, Frame IA, Stothard JR & Miles MA (1998). Random amplification of polymorphic DNA as a tool for taxonomic studies of triatomine bugs (Hemiptera: Reduviidae). *Journal of Medical Entomology*, **35**, 38-45.

García BA (1999). Molecular phylogenetic relationships among species of the genus *Triatoma*, pp. 971-980 in (Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. III. Editora Fiocruz, Rio de Janeiro, Brazil.

García BA, Barata JMS & Blanco A (1995b). Enzyme polymorphism among *Triatoma infestans* (Hemiptera: Reduviidae) colonies. *Journal of Medical Entomology*, **32**, 126-133.

García BA, Canale DM & Blanco A (1995a). Genetic structure of four species of *Triatoma* (Hemiptera: Reduviidae) from Argentina. *Journal of Medical Entomology*, **32**, 134-137.

García BA & Powell JR (1998). Phylogeny of species of *Triatoma* (Hemiptera: Reduviidae) based on mitochondrial DNA sequences. *Journal of Medical Entomology*, **35**, 232-238.

García BA, Moriyama EN & Powell JR (2001). Mitochondrial DNA sequences of triatomines (Hemiptera: Reduviidae): phylogenetic relationships. *Journal of Medical Entomology*, **38**, 675-683.

García-Zapata MT & Marsden PD (1992). Control of the transmission of Chagas disease in Mambaí, Goiás, Brazil (1980-1989). *American Journal of Tropical Medicine and Hygiene*, **46**, 440-443.

García-Zapata MT & Marsden PD (1993). Chagas disease: control and surveillance through use of insecticides and community participation in Mambaí, Goiás, Brazil. *Bulletin of the Pan American Health Organization*, **27**, 265-279.

Garzón EA, Barnabé C, Córdova X, Bowen C, Paredes W, Gómez E, Ouaissi A, Tibayrenc M & Guevara AG (2002). *Trypanosoma cruzi* isoenzyme variability in Ecuador: first observation of zymodeme III genotypes in chronic chagasic patients. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **96**, 378-382.

Gaunt MW & Miles MA (2000). The ecotopes and evolution of triatomine bugs (Triatominae) and their associated trypanosomes. *Memórias do Instituto Oswaldo Cruz*, **95**, 557-565.

Gaunt MW & Miles MA (2002). An insect molecular clock dates the origin of the insects and accords with palaeontological and biogeographic landmarks. *Molecular Biology and Evolution*, **19**, 748-761.

Gavrilets S (2000). Waiting time to parapatric speciation. *Proceedings of the Royal Society London Series B*, **267**, 2483-2492.

Gimeno C, Belshaw R & Quicke DLJ (1997). Phylogenetic relationships of the Alysiinae/Opiinae (Hymenoptera: Braconidae) and the utility of cytochrome *b*, 16S and 28S D2 rRNA. *Insect Molecular Biology*, **6**, 273-284.

Gissi C, Reyes A, Pesole G & Saccone C (2000). Lineage-specific evolutionary rate in mammalian mtDNA. *Molecular Biology and Evolution*, **17**, 1022-1031.

Glesson DM & Sarre S (1997). Mitochondrial DNA variability and geographic origin of the sheep blowfly, *Lucilia cuprina* (Diptera: Calliphoridae), in New Zealand. *Bulletin of Entomological Research*, **87**, 265-272.

Gómez LLF (1968). El problema de la enfermedad de Chagas en Guayaquil. *Revista Ecuatoriana de Higiene y Medicina Tropical*, **25**, 3-10.

Gonçalves TCM, Oliveira E, Dias LS, Almeida MD, Nogueira WO & Pires FDA (1998). An investigation on the ecology of *Triatoma vitticeps* (Stål, 1859) and its possible role in the

transmission of *Trypanosoma cruzi*, in the locality of Triunfo, Santa Maria Magdalena Municipal District, State of Rio de Janeiro, Brazil. *Memórias do Instituto Oswaldo Cruz*, **93**, 711-171.

Gorla DE, Dujardin JP & Schofield CJ (1997). Biosystematics of Old World Triatominae. *Acta Tropica*, **63**, 127-140.

Gorla DE, Jurberg J, Catalá SS & Schofield CJ (1993). Systematics of *Triatoma sordida*, *T. guasayana* and *T. patagonica* (Hemiptera, Reduviidae). *Memórias do Instituto Oswaldo Cruz*, **88**, 379-385.

Gorla DE & Schofield CJ (1989). Population dynamics of *Triatoma infestans* under natural climatic conditions in the Argentine Chaco. *Medical and Veterinary Entomology*, **3**, 179-194.

Gracco M & Catalá S (2000). Inter-specific and developmental differences on the array of antennal chemoreceptors in four species of Triatominae (Hemiptera: Reduviidae). *Memórias do Instituto Oswaldo Cruz*, **95**, 67-74.

Griffiths CS (1997). Correlation of functional domains and rates of nucleotide substitution in cytochrome *b*. *Molecular Phylogenetics and Evolution*, **7**, 352-365.

Grijalva MJ, Chiriboga R, Racines VJ, Escalante L & Rowland EC (1997). Screening for *Trypanosoma cruzi* in the blood supply by the Red Cross Blood Bank in Quito, Ecuador. *American Journal of Tropical Medicine and Hygiene*, **57**, 740-741.

Grijalva MJ, Rowland EC, Powell MR, McCormick TS & Escalante L (1995). Blood donors in a vector-free zone of Ecuador potentially infected with *Trypanosoma cruzi*. *American Journal of Tropical Medicine and Hygiene*, **52**, 360-363.

Guevara AG, Eras JW, Recalde M, Vinueza L, Cooper PJ, Ouaissi A & Guderian RH (1997). Severe digestive pathology associated with chronic Chagas disease in Ecuador: report of two cases. *Revista da Sociedade Brasileira de Medicina Tropical*, **30**, 389-392.

Guevara AG, Ruiz CJC, Houghton RL, Reynolds L, Sleath P, Benson D, Ouaissi A & Guderian RH (1999). Evaluation of a recombinant protein (RTC24) and synthetic peptides in anti-*Trypanosoma cruzi* positive samples from blood bank donors in chagasic endemic areas of Ecuador. *Japanese Journal of Tropical Medicine and Hygiene*, **27**, 19-22.

Guevara AG, Taibi A, Billaut-Mullot O & Ouaissi A (1996). PCR-based detection of *Trypanosoma cruzi* useful for diagnosis of human Chagas disease. *Journal of Clinical Microbiology*, **34**, 485-486.

Guhl F (1997). Programa de control transfusional de *Trypanosoma cruzi* en Colombia, pp. 43-51, in (Guhl F ed.) *Memorias. Genética poblacional de triatomíneos aplicada al control vectorial de la enfermedad de Chagas. Curso postgrado.* Universidad de Los Andes/CIMPAT–Universidad de Antioquia, Santafé de Bogotá, Colombia.

Guhl F (1999). Programas en la eliminación de la transmisión de la enfermedad de Chagas en los países Andinos, pp. 38-43 in (Schofield CJ & Ponce C eds.) *Proceedings of the second International Workshop on population genetics and control of Triatominae, Tegucigalpa, Honduras, March 1998*. INDRE, Mexico City, Mexico.

Guhl F & Schofield CJ (1996). Population genetics and control of Triatominae. *Parasitology Today*, **12**, 169-170.

Guhl F & Vallejo GA (1999). Interruption of Chagas disease transmission in the Andean countries: Colombia. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl.1), 413-415.

Guillén G, Díaz R, Jemio A, Alfred Cassab J, Teixeira Pinto C & Schofield CJ (1997). Chagas disease vector control in Tupiza, southern Bolivia. *Memórias do Instituto Oswaldo Cruz*, **92**, 1-8.

Gürtler RE, Cécere MC, Rubel DN, Petersen RM, Schweigmann NJ, Lauricella MA, Bujas MA, Segura EL & Wisnivesky-Colli C (1991). Chagas disease in north-west Argentina: infected dogs as a risk

factor for the domestic transmission of *Trypanosoma cruzi*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **85**, 741-745.

Gürtler RE, Cécere MC, Rubel DN & Schweigmann NJ (1992). Determinants of the domiciliary density of *Triatoma infestans*, vector of Chagas disease. *Medical and Veterinary Entomology*, **6**, 75-83.

Gürtler RE, Cécere MC, Vázquez DP, Chuit R & Cohen JE (1996). Host-feeding patterns of domiciliary *Triatoma infestans* (Hemiptera: Reduviidae) in northwest Argentina: seasonal and instar variation. *Journal of Medical Entomology*, **33**, 15-26.

Gürtler RE, Chuit R, Cécere MC & Castañera MB (1995). Detecting domestic vectors of Chagas disease: a comparative trial of six methods in north-west Argentina. *Bulletin of the World Health Organization*, **73**, 487-494.

Gürtler RE, Cohen JE, Cécere MC & Chuit R (1997). Shifting host choices of the vector of Chagas disease, *Triatoma infestans*, in relation to the availability of hosts in houses in north-west Argentina. *Journal of Applied Ecology*, **34**, 699-715.

Gürtler RE, Schweigmann NJ, Cécere MC, Chuit R & Wisnivesky-Colli C (1993). Comparison of two sampling methods for domestic populations of *Triatoma infestans* in north-west Argentina. *Medical and Veterinary Entomology*, **7**, 238-242.

Gürtler RE, Wisnivesky-Colli C, Solarz ND, Lauricella M & Bujas M (1988). Dinámica de la transmisión de *Trypanosoma cruzi* en una zona rural de la Argentina: II. Relación entre la infección doméstica en niños y perros y la densidad de *Triatoma infestans* infectados. *Boletín de la Oficina Sanitaria Panamericana*, **104**, 130-143.

Haffer J (1969). Speciation in Amazonian forest birds. *Science*, **165**, 131-137.

Hafner MS, Sudman PD, Villablanca FX, Spradling TA, Demastes JW & Nadler SA (1994). Disparate rates of molecular evolution in cospeciating hosts and parasites. *Science*, **265**, 1087-1090.

Harry M (1992). *Variabilité génétique de populations vénézueliennes de* Rhodnius *spp., vectrices de* Trypanosoma cruzi, *parasite responsable de la maladie de Chagas*. PhD Thesis, Université Paris 6, Paris, France, 200 pp.

Harry M (1993). Isozymic data question the specific status of some blood-sucking bugs of the genus *Rhodnius*, vectors of Chagas disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87**, 492-493.

Harry M (1994). Morphometric variability in the Chagas disease vector *Rhodnius prolixus*. *Japanese Journal of Genetics*, **69**, 233-250.

Harry M, Galíndez Girón I & Cariou ML (1992a). Isozyme variability and differentiation between *Rhodnius prolixus, R. robustus* and *R. pictipes*, vectors of Chagas disease in Venezuela. *Medical and Veterinary Entomology*, **6**, 37-43.

Harry M, Moreno G & Goyffon M (1992b). Genetic variability in populations of *Rhodnius prolixus*, vector of Chagas disease in Venezuela. *Evolución Biológica*, **6**, 175-194.

Harry M, Poyet G, Romaña CA & Solignac M (1998). Identification and characterization of microsatellite markers in the bloodsucking bug *Rhodnius pallescens* (Heteroptera, Reduviidae). *Molecular Ecology*, **7**, 1784-1786.

Herrer A, Wygodzinsky P & Napan M (1972). Presencia de *Trypanosoma rangeli* Tejera, 1920, en el Perú. I. El insecto vector, *Rhodnius ecuadoriensis* Lent & León, 1958. *Revista de Biología Tropical*, **20**, 141-149.

Herwaldt B, Grijalva MJ, Newsome AL, McGhee CR, Powell MR, Nemec DG, Steurer FJ & Eberhard ML (2000). Use of polymerase chain reaction to diagnose the fifth reported US case of autochthonous transmission of *Trypanosoma cruzi*, in Tennessee, 1998. *Journal of Infectious Diseases*, **181**, 395-399.

Holdridge LR (1967). *Life zone ecology*. Tropical Science Centre, San José, Costa Rica, 206 pp.

Hypša V, Tietz DF, Zrzavý J, Rego ROM, Galvão C & Jurberg J (2002). Phylogeny and biogeography of Triatominae (Hemiptera: Reduviidae): molecular evidence of a New World origin of the Asiatic clade. *Molecular Phylogenetics and Evolution*, **23**, 447-457.

Ianni BM, Arteaga E, Frimm CC, Barretto ACP & Mady C (2001). Chagas heart disease: evolutive evaluation of electrocardiographic and ecocardiographic parameters in patients with the indeterminate form. *Arquivos Brasileiros de Cardiologia*, **77**, 59-62.

IGM (1978-82). *Índice Toponímico de la República del Ecuador (A-Q)*. Publicación del Instituto Geográfico Militar, Quito, Ecuador.

IGM (1982-96). *Índice Toponímico de la República del Ecuador (Q-Z)*. Publicación del Instituto Geográfico Militar, Quito, Ecuador.

Ishikawa EAY, Ready PD, Souza AA, Day CJ, Rangel EF, Davies CD & Shaw JJ (1999). A mitochondrial DNA phylogeny indicates close relationships between populations of *Lutzomyia whitmani* (Diptera, Psychodidae, Phlebotominae) from the rain-forest regions of Amazônia and northeast Brazil. *Memórias do Instituto Oswaldo Cruz*, **94**, 339-345.

Jaramillo C, Montaña MF, Castro LR, Vallejo GA & Guhl F (2001). Differentiation and genetic analysis of *Rhodnius prolixus* and *Rhodnius colombiensis* by rDNA and RAPD amplification. *Memórias do Instituto Oswaldo Cruz*, **96**, 1043-1048.

Jaramillo NO (2000). *Partición en tamaño y forma de los caracteres métricos y su interés en los estudios poblacionales aplicados a los Triatominae*. PhD Thesis, University of Antioquia, Medellín, Colombia, 200 pp.

Jaramillo NO, Schofield CJ, Gorla D, Caro-Riaño H, Moreno J, Mejía E & Dujardin JP (2000). The role of *Rhodnius pallescens* as a vector of Chagas disease in Colombia and Panama. *Research and Reviews in Parasitology*, **60**, 75-82.

Jarne P & Lagoda PJL (1996). Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution*, **11**, 424-429.

Juárez MP (1996). Hidrocarburos de triatominos: potencial como criterio taxonómico, pp. 63-64 in (Schofield CJ, Dujardin JP & Jurberg J eds.) *Proceedings of the International Workshop on Population Genetics and Control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995*. INDRE, Mexico City, Mexico.

Juárez MP, Fernández R & Dujardin JP (1999). Hidrocarburos cuticulares de Triatominae, pp. 63-67 in (Schofield CJ & Ponce C eds.) *Proceedings of the second International Workshop on Population Genetics and Control of Triatominae, Tegucigalpa, Honduras, March 1998*. INDRE, Mexico City, Mexico.

Juárez MP, Fernández R, Schofield CJ & Dujardin JP (2000). Intergeneric comparison of epicuticular hydrocarbons in Triatominae. *Research and Reviews in Parasitology*, **60**, 121-127.

Jukes T H & Cantor CR (1969). Evolution of protein molecules, pp. 21-132 in (Munro HN ed.) *Mammalian protein metabolism*. Academic Press, New York, NY, USA.

Jurberg J (1996). Uma abordagem filogenética entre os triatomineos baseada nas estruturas fálicas, pp. 51-52 in (Schofield CJ, Dujardin JP & Jurberg J eds.) *Proceedings of the International Workshop on population genetics and control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995*. INDRE, Mexico City, Mexico.

Jurberg J, Lent H & Galvão C (1997). The male genitalia and its importance in taxonomy, pp. 85-106 in (Carcavallo RU, Girón IG, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. I. Editora Fiocruz, Rio de Janeiro, Brazil.

Kambhampati S, Black WC & Rai KS (1992). Random amplified polymorphic DNA of mosquito species and populations (Diptera: Culicidae): techniques, statistical analysis, and applications. *Journal of Medical Entomology*, **29**, 939-945.

Kambhampati S & Smith PT (1995). PCR primers for the amplification of four insect mitochondrial gene fragments. *Insect Molecular Biology*, **4**, 233-236.

Kawabata M, Uchiyama T, Mimori T, Hashiguchi Y & de Coronel VV (1987). Association of electrocardiographic abnormalities with seropositivity to *Trypanosoma cruzi* in Ecuador. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **81**, 7-10.

Kimura M (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**, 111-120.

Kirchhoff LV (1993). Chagas disease. *Infectious Disease Clinics of North America*, **7**, 487-502.

Kirk ML & Schofield CJ (1987). Density-dependent timing of defecation by *Rhodnius prolixus*, and its implications for the transmission of *Trypanosoma cruzi*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **81**, 348-349.

Kitron U (2000). Risk maps: transmission and burden of vector-borne diseases. *Trends in Parasitology*, **94**, 324-325.

Kleinbaum DG, Kupper LL, Muller KE & Nizam A (1998). *Applied regression analysis and other multivariable methods*. Duxbury Press, Pacific Grove, CA, USA, 798 pp.

Klingenberg CP (1996). Multivariate allometry, pp. 23-49 in (Marcus LF, Corti M, Loy A, Naylor GJP & Slice D eds.) *Advances in morphometrics. Proceedings of the 1996 NATO-ASI on morphometrics*. Plenum Press, New York, NY, USA.

Kollien AH & Schaub GA (2000). The development of *Trypanosoma cruzi* in Triatominae. *Parasitology Today*, **16**, 381-387.

Kuhn KG, Campbell-Lendrum DH & Davies CR (2002). A continental risk map for malaria mosquito (Diptera: Culicidae) vectors in Europe. *Journal of Medical Entomology*, **39**, 621-630.

Kumar S, Tamura K, Jakobsen IB & Nei M (2001). MEGA2: Molecular Evolutionary Genetics Analysis software. Arizona State University, Tempe, AZ, USA.

Landis JR & Koch GG (1977). The measurement of observer agreement for categorical data. *Biometrics*, **33**, 159-174.

Laurance WF, Powell G & Hansen L (2002). A precarious future for Amazonia. *Trends in Ecology and Evolution*, **17**, 251-252.

Lazo R (1985). Ecuador, pp. 413-427 in (Carcavallo RU, Rabinovich JE & Tonn RJ eds.) *Factores biológicos y ecológicos en la enfermedad de Chagas*, Vol. II. Centro Panamericano de Ecología Humana y Salud OPS/OMS – Servicio Nacional de Chagas, Ministerio de Salud y Acción Social, Buenos Aires, Argentina.

Le TH, Blair D, Agatsuma T, Humair PF, Campbell NJH, Iwagami M, Littlewood DTJ, Peacock B, Johnston DA, Bartley J, Rollinson D, Herniou EA, Zarlenga DS & McManus DP (2000a). Phylogenies inferred from mitochondrial gene orders – a cautionary tale from the parasitic flatworms. *Molecular Biology and Evolution*, **17**, 1123-1125.

Le TH, Blair D & McManus DP (2000b). Mitochondrial genomes of human helminths and their use as markers in population genetics and phylogeny. *Acta Tropica*, **77**, 243-256.

Lehane MJ, McEwen PK, Whitaker CJ & Schofield CJ (1992). The role of temperature and nutritional status in flight initiation by *Triatoma infestans*. *Acta Tropica*, **52**, 27-38.

Lehane MJ & Schofield CJ (1981). Field experiments of dispersive flight by *Triatoma infestans*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **75**, 399-400.

Lent H (1958). Notes on an interesting structure of the pygophore of *Rhodnius* Stål, 1859 (Hemiptera, Reduviidae). *Revista Brasileira de Biologia*, **18**, 381-183.

Lent H (1997). Novos sinônimos de duas espécies de Triatominae da Venezuela (Hemiptera, Reduviidae). *Entomología y Vectores*, **4**, 67-70.

Lent H (1999). Evolução dos conhecimentos sobre vetores da doença de Chagas 90 anos após a sua descoberta. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 89-92.

Lent H & Jurberg J (1969). O gênero *Rhodnius* Stål, 1859, com um estudo sôbre a genitália das espécies (Hemiptera, Reduviidae, Triatominae). *Revista Brasileira de Biologia*, **29**, 487-560.

Lent H & Jurberg J (1985). Sobre a variaçao intra-específica em *Triatoma dimidiata* (Latreille) e *Triatoma infestans* (Klug) (Hemiptera: Reduviidae). *Memórias do Instituto Oswaldo Cruz*, **80**, 285-299.

Lent H & Jurberg J (1987). A genitália externa dos machos de sete espécies de *Triatoma* Laporte, 1832 da região neártica (Hemiptera, Reduviidae, Triatominae). *Memórias do Instituto Oswaldo Cruz*, **82**, 227-246.

Lent H, Jurberg J & Galvão C (1993). *Rhodnius stali* n. sp., afim de *Rhodnius pictipes* Stål, 1872 (Hemiptera, Reduviidae, Triatominae). *Memórias do Instituto Oswaldo Cruz*, **88**, 605-614.

Lent H, Jurberg J & Galvão C (1994). Revalidação do gênero *Mepraia* Mazza, Gajardo & Jorg, 1940 (Hemiptera, Reduviidae, Triatominae). *Memórias do Instituto Oswaldo Cruz*, **89**, 347-352.

Lent H & León LA (1958). Um novo *Rhodnius* Stål do Ecuador (Hemiptera, Reduviidae). *Revista Brasileira de Biologia*, **18**, 181-185.

Lent H & Wygodzinsky P (1979). Revision of the Triatominae (Hemiptera: Reduviidae), and their significance as vectors of Chagas disease. *Bulletin of the American Museum of Natural History*, **163**, 123-520.

León LA (1949). Información sobre el problema de la enfermedad de Chagas en el Ecuador. *Boletín de la Oficina Sanitaria Panamericana*, **28**, 569-585.

León JS & Engman DM (2001). Autoimmunity in Chagas heart disease. *International Journal for Parasitology*, **31**, 555-561.

Lewis DL, Farr CL & Kaguni LS (1995). *Drosophila melanogaster* mitochondrial DNA, completion of the nucleotide sequence and evolutionary comparisons. *Insect Molecular Biology*, **4**, 263-278.

Litzenberger G & Chapco W (2001). A molecular phylogeographic perspective on a fifty-year-old taxonomic issue in grasshopper systematics. *Heredity*, **86**, 54-59.

Lopes ER (1999). Sudden death in patients with Chagas disease. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 321-324.

López G & Moreno MJ (1995). Genetic variability and differentiation between populations of *Rhodnius prolixus* and *Rhodnius pallescens*, vectors of Chagas disease in Colombia. *Memórias do Instituto Oswaldo Cruz*, **90**, 353-357.

Loxdale HD & Lushai G (1998). Molecular markers in entomology. *Bulletin of Entomological Research*, **88**, 577-600.

Luquetti AO (1999). Evolution of knowledge on the etiological diagnosis of chagasic infection. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 283-284.

Luz C, Fargues J & Grunewald J (1999). Development of *Rhodnius prolixus* (Hemiptera: Reduviidae) under constant and cyclic conditions of temperature and humidity. *Memórias do Instituto Oswaldo Cruz*, **94**, 403-409.

Lyman DF, Monteiro FA, Escalante AA, Cordón-Rosales C, Wesson DM, Dujardin JP & Beard CB (1999). Mitochondrial DNA sequence variation among triatomine vectors of Chagas disease. *American Journal of Tropical Medicine and Hygiene*, **60**, 377-386.

Macêdo V (1999). Indeterminate form of Chagas disease. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 311-316.

Machado C & Ayala FJ (2001). Nucleotide sequences provide evidence of genetic exchange among distantly related lineages of *Trypanosoma cruzi*. *Proceedings of the National Academy of Sciences of the USA*, **98**, 7396-7401.

Machado CA, Jousselin E, Kjellberg F, Compton SG & Herre EA (2001). Phylogenetic relationships, historical biogeography and character evolution of fig-pollinating wasps. *Proceedings of the Royal Society London, Series B*, **268**, 685-694.

Manzullo EC & Chuit R (1999). Risk of death due to chronic chagasic cardiopathy. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 317-320.

Marcilla A, Bargues MD, Abad-Franch F, Panzera F, Carcavallo RU, Noireau F, Galvão C, Jurberg J, Miles MA, Dujardin JP & Mas-Coma S (2002). Nuclear rDNA ITS-2 sequences reveal polyphyly of *Panstrongylus* species (Hemiptera: Reduviidae: Triatominae), vectors of *Trypanosoma cruzi*. *Infection, Genetics and Evolution*, **1**, 225-235.

Marcilla A, Bargues MD, Ramsey JM, Magallón-Gastélum E, Salazar-Schettino PM, Abad-Franch F, Dujardin JP, Schofield CJ & Mas-Coma S (2001). The ITS-2 of the nuclear rDNA as a molecular marker for populations, species, and phylogenetic relationships in Triatominae (Hemiptera: Reduviidae), vectors of Chagas disease. *Molecular Phylogenetics and Evolution*, **18**, 136-142.

Marcilla A, Canese N, Acosta E, López A, Rojas de Arias A, Bargues MD & Mas-Coma S (2000). Populations of *Triatoma infestans* (Hemiptera: Reduviidae) from Paraguay: a molecular analysis based on the second internal transcribed spacer of the rDNA. *Research and Reviews in Parasitology*, **60**, 99-105.

Marsden PD, Virgens D, Magalhaes I, Tavares-Neto J, Ferreira R, Costa CH, Castro CN, Macêdo V & Prata A (1982). Ecologia doméstica do *Triatoma infestans* em Mambaí, Goiás, Brasil. *Revista do Instituto de Medicina Tropical de São Paulo*, **54**, 364-373.

Martínez A (1984). Caracterización taxionómica de *Rhodnius dalessandroi* Carcavallo & Barreto, 1976 (Hemiptera, Reduviidae, Triatominae). *Chagas*, **1**, 29-31.

Matías A, de la Riva JX, Torrez M & Dujardin JP (2001). *Rhodnius robustus* in Bolivia identified by its wings. *Memórias do Instituto Oswaldo Cruz*, **96**, 947-950.

Mazzotti L & Osorio MT (1942). Cruzamientos experimentales entre varias especies de triatomas. *Medicina (Mexico)*, **22**, 215-222.

Meggers BJ (1963). Cultural development in Latin America: an interpretative overview, pp. 131-145 in (Meggers BJ & Evans C eds.) *Aboriginal cultural development in Latin America: an interpretative review.* Smithsonian Miscellaneous Collections, Vol. **146**. The Smithsonian Institution, Washington, DC, USA.

Meggers BJ & Evans C (eds.) (1963). *Aboriginal cultural development in Latin America: an interpretative review.* Smithsonian Miscellaneous Collections, Vol. **146**. The Smithsonian Institution, Washington, DC, USA.

Meneghelli UG (1999). Clinical treatment of the digestive form of Chagas disease. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 341-342.

Meyer A (1994). Shortcomings of the cytochrome *b* gene as a molecular marker. *Trends in Ecology and Evolution*, **9**, 278-280.

Miles MA (1976). A simple method of tracking mammals and locating triatomine vectors of *Trypanosoma cruzi* in Amazonian forest. *American Journal of Tropical Medicine and Hygiene*, **25**, 671-674.

Miles MA (1998). New World Trypanosomiasis, pp. 238-302 in (Collier L, Balows A & Sussman M eds.) *Microbiology and microbial infections*, 9th Edition, Chapter 15, Vol. 5: Parasitology. Topley & Wilson's, London, UK.

Miles MA, Arias JR & Souza AA (1983). Chagas disease in the Amazon basin: V. Periurban palms as habitats of *Rhodnius robustus* and *Rhodnius pictipes* – triatomine vectors of Chagas disease. *Memórias do Instituto Oswaldo Cruz*, **78**, 391-398.

Miles MA, Lanham SM, Souza AA & Póvoa MM (1980). Further enzymic characters of *Trypanosoma cruzi* and their evaluation for strain identification. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **74**, 221-237.

Miles MA, Póvoa MM, Prata A, Cedillos RA, Souza AA & Macêdo V (1981a). Do radically dissimilar *Trypanosoma cruzi* strains (zymodemes) cause Venezuelan and Brazilian forms of Chagas disease? *The Lancet*, June 20 1981, 1338-1340.

Miles MA, Souza AA & Póvoa MM (1981b). Chagas disease in the Amazon basin: III. Ecotopes of ten triatomine bug species (Hemiptera: Reduviidae) from the vicinity of Belém, Pará State, Brazil. *Journal of Medical Entomology*, **18**, 266-278.

Miles MA, Souza AA, Póvoa MM, Shaw JJ, Lainson R & Toye PJ (1978). Isozymic heterogeneity of *Trypanosoma cruzi* in the first autochthonous patients with Chagas disease in Amazonian Brazil. *Nature*, **272**, 819-821.

Miles MA, Toye PJ, Oswald SC & Godfrey DG (1977). The identification by isoenzyme patterns of two distinct strain-groups of *Trypanosoma cruzi*, circulating independently in a rural area of Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **71**, 217-225.

Miles MA, Yeo M & Gaunt MW (2002). Genetic diversity of *Trypanosoma cruzi* and the epidemiology of Chagas disease, in (Kelly JM ed.) *Molecular mechanisms in the pathogenesis of Chagas disease*. Landes Bioscience, Austin, TX, USA, in press. [Available at www.eurekah.com].

Mimori T, Kawabata M, Gómez E, Vera de Coronel V, Aroca M, Flor T & Hasiguchi Y (1985). A seroepidemiological survey of Chagas Diseases and search for reservoir hosts in two endemic areas of Ecuador. *Japanese Journal of Tropical Medicine and Hygiene*, **13**, 245-250.

Minter DM (1978). Triatomine bugs and the household ecology of Chagas disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **72**, 85-93.

Miranda J (1995). *Elementos de cartografía básica*. Colección de Documentos Didácticos No. 1, Centro Panamericano de Estudios e Investigaciones Geográficas, Quito, Ecuador, 59 pp.

Molina JA, Gualdrón LE, Brochero HL, Olano VA, Barrios D & Guhl F (2000). Distribución actual e importancia epidemiológica de las especies de triatominos (Reduviidae: Triatominae) en Colombia. *Biomédica*, **20**, 344-360.

Molyneux DH (1997). Patterns of change in vector-borne diseases. *Annals of Tropical Medicine and Parasitology*, **91**, 827-839.

Momen H (1999). Taxonomy of *Trypanosoma cruzi*: a commentary on characterization and nomenclature. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 181-184.

Moncayo A (1999). Progress towards interruption of transmission of Chagas disease. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 401-404.

Montalván JA (1952). Ensayos de profilaxis de la enfermedad de Chagas en Guayaquil. *Revista Ecuatoriana de Higiene y Medicina Tropical*, **8-9**, 23-28.

Montamat EE, de Luca D'Oro GM, Gallerano RH, Sosa R & Blanco A (1996). Characterization of *Trypanosoma cruzi* populations by zymodemes: correlation with clinical picture. *American Journal of Tropical Medicine and Hygiene*, **55**, 625-628.

Monteiro F, Costa J & Beard CB (1999a). High levels of mitochondrial DNA sequence divergence among *Triatoma brasiliensis* Neiva, 1911 populations (Hemiptera, Reduviidae, Triatominae). *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 2), 244.

Monteiro F, Costa J & Solé-Cava AM (1998). Genetic confirmation of the specific status of *Triatoma petrochii* (Hemiptera: Reduviidae: Triatominae). *Annals of Tropical Medicine and Parasitology*, **92**, 897-900.

Monteiro FA, Escalante AA & Beard CB (2001). Molecular tools and triatomine systematics: a public health perspective. *Trends in Parasitology*, **17**, 344-347.

Monteiro FA, Lazoski C, Noireau F & Solé-Cava AM (2002). Allozyme relationships among ten species of Rhodniini, showing paraphyly of *Rhodnius* including *Psammolestes*. *Medical and Veterinary Entomology*, **16**, 83-90.

Monteiro F, Pérez R, Panzera F, Dujardin JP, Galvão C, Rocha D, Noireau F, Schofield CJ & Beard CB (1999b). Mitochondrial DNA variation of *Triatoma infestans* populations and its implication on the specific status of *T. melanosoma*. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 229-238.

Monteiro F, Wesson DM, Dotson EM, Schofield CJ & Beard CB (2000). Phylogeny and molecular taxonomy of the Rhodniini derived from mitochondrial and nuclear DNA sequences. *American Journal of Tropical Medicine and Hygiene*, **62**, 460-465.

Moore WS (1995). Inferring phylogenies from mtDNA variation: mitochondrial gene trees versus nuclear gene trees. *Evolution*, **49**, 718-726.

Moraes-Souza H (1999). Chagas infection transmission control: situation of transfusional transmission in Brazil and other countries of Latin America. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 419-423.

Moraes-Souza H, Wanderley DMV, Brener S, Nascimento RD, Figueiredo Antunes CM & Dias JCP (1994). Hemoterapia e doença de Chagas transfusional no Brasil. *Boletín de la Oficina Sanitaria Panamericana*, **116**, 406-418.

Morel CM (1999). Chagas disease, from discovery to control – and beyond: history, myths and lessons to take home. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 3-16.

Moreno AR & Carcavallo RU (1999). An ecological approach to Chagas disease epidemiology, pp. 981-1016 in (Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. III. Editora Fiocruz, Rio de Janeiro, Brazil.

Moreno MJ, Galvão C & Jurberg J (1999). *Rhodnius colombiensis* sp. n. da Colômbia, com quadros comparativos entre estruturas fálicas do gênero *Rhodnius* Stål, 1859 (Hemiptera, Reduviidae, Triatominae). *Entomología y Vectores*, **6**, 601-617.

Moreno MJ & Jaramillo NO (1996). Estudios epidemiológicos sobre la enfermedad de Chagas en los Departamentos de Antioquia, Sucre y Tolima, Colombia, p. 27 in (Schofield CJ, Dujardin JP & Jurberg J eds.) *Proceedings of the International Workshop on population genetics and control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995*. INDRE, Mexico City, Mexico.

Mostacero LJ, Mejía CF & Peláez PP (1996). *Fitogeografía del norte del Perú*. Concytec, Lima, Perú, 406 pp.

Mott KE, Muniz TM, Lehman JS, Hoff R, Morrow RH, Oliveira TS, Sherlock I & Draper CC (1978). House construction, triatomine distribution, and household distribution of seroreactivity to *Trypanosoma cruzi* in a rural community in northeast Brazil. *American Journal of Tropical Medicine and Hygiene*, **27**, 1116-1122.

MSP – Ministerio de Salud Pública del Ecuador (1999). *Memoria. Fenómeno de El Niño en el Ecuador 97-98*. Ministerio de Salud Pública del Ecuador, Quito, Ecuador, 93 pp.

Murta SMF & Romanha AJ (1999). Characterization of *Trypanosoma cruzi*. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 177-180.

Naiff MF, Naiff RD & Barrett TV (1998). Vetores selváticos de doença de Chagas na área urbana de Manaus (AM): atividade do vôo nas estações secas e chuvosas. *Revista da Sociedade Brasileira de Medicina Tropical*, **31**, 103-105.

Nei M (1972). Genetic distance between populations. *American Naturalist*, **106**, 283-292.

Nepstad D, McGrath D, Alencar A, Barros AC, Carvalho G, Santilli M & Vera Diaz MC (2002). Frontier governance in Amazonia. *Science*, **295**, 629-631.

Nevo E (1978). Genetic variation in natural populations: pattern and theory. *Theoretical Population Biology*, **13**, 121-177.

Nevo E (2001). Evolution of genome-phenome diversity under environmental stress. *Proceedings of the National Academy of Sciences of the USA*, **98**, 6233-6240.

Noireau F (1999). Triatominos candidatos vectores y realidad de los focos selváticos de *Triatoma infestans* en Bolivia, pp. 91-95 in (Schofield CJ & Ponce C eds.) *Proceedings of the second International Workshop on population genetics and control of Triatominae, Tegucigalpa, Honduras, March 1998*. INDRE, Mexico City, Mexico.

Noireau F, Abad-Franch F, Valente SAS, Dias-Lima A, Lopes CM, Cunha V, Valente VC, Palomeque FS, de Carvalho-Pinto CJ, Sherlock I, Aguilar M, Steindel M, Grisard EC & Jurberg J (2002b). Trapping Triatominae (Hemiptera: Reduviidae) in silvatic habitats. *Memórias do Instituto Oswaldo Cruz*, **97**, 61-63.

Noireau F, Bastrenta B, Catalá SS, Dujardin JP, Panzera F, Torres M, Pérez R, Jurberg J & Galvão C (2000b). Sylvatic population of *Triatoma infestans* from the Bolivian Chaco: from field collection to characterization. *Memórias do Instituto Oswaldo Cruz*, **95**, 119-122.

Noireau F, Bosseno MF, Carrasco R, Telleria J, Vargas F, Camacho C, Yaksic N & Brenière SF (1995). Sylvatic triatomines (Hemiptera: Reduviidae) in Bolivia: trends towards domesticity and possible infection with *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae). *Journal of Medical Entomology*, **32**, 594-598.

Noireau F, Bosseno MF, Vargas F & Brenière SF (1994). Apparent trend to domesticity observed in *Panstrongylus rufotuberculatus* Champion, 1899 (Hemiptera: Reduviidae) in Bolivia. *Research and Reviews in Parasitology*, **54**, 263-264.

Noireau F, dos Santos SM, Gumiel M, Dujardin JP, Soares MS, Carcavallo RU, Galvão C & Jurberg J (2002a). Phylogenetic relationships within the *oliveirai* complex (Hemiptera: Reduviidae: Triatominae). *Infection, Genetics and Evolution*, **2**, 11-17.

Noireau F & Dujardin JP (2001). Flight and nutritional status of sylvatic *Triatoma sordida* and *T. guasayana*. *Memórias do Instituto Oswaldo Cruz*, **96**, 385-389.

Noireau F, Flores R, Gutiérrez T, Abad-Franch F, Flores E & Vargas F (2000a). Natural ecotopes of *Triatoma infestans* dark morph and other sylvatic triatomines in the Bolivian Chaco. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **94**, 23-27.

Noireau F, Flores R, Gutiérrez T & Dujardin JP (1997). Detection of wild dark morphs of *Triatoma infestans* in the Bolivian Chaco. *Memórias do Instituto Oswaldo Cruz*, **92**, 583-584.

Noireau F, Flores R & Vargas F (1999c). Trapping sylvatic Triatominae (Reduviidae) in hollow trees. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **93**, 13-14.

Noireau F, Gutiérrez T, Flores R, Breniére SF, Bosseno MF & Wisnivesky-Colli C (1999a). Ecogenetics of *Triatoma sordida* and *Triatoma guasayana* (Hemiptera: Reduviidae) in the Bolivian Chaco. *Memórias do Instituto Oswaldo Cruz*, **94**, 451-457.

Noireau F, Gutiérrez T, Zegarra M, Flores R, Breniére SF, Cardozo L & Dujardin JP (1998). Cryptic speciation in *Triatoma sordida* (Hemiptera: Reduviidae) from the Bolivian Chaco. *Tropical Medicine and International Health*, **3**, 364-372.

Noireau F, Zegarra M, Ordóñez J, Gutiérrez T & Dujardin JP (1999b). Genetic structure of *Triatoma sordida* (Hemiptera: Reduviidae) domestic populations from Bolivia: application on control interventions. *Memórias do Instituto Oswaldo Cruz*, **94**, 347-351.

Nunes LR, de Carvalho MRC & Buck GA (1997). *Trypanosoma cruzi* strains partition in two groups based on the structure and function of the spliced leader RNA and rRNA gene promoters. *Molecular Biochemical Parasitology*, **86**, 211-224.

O'Grady PM, Clark JB & Kidwell MG (1998). Phylogeny of the *Drosophila saltans* species group based on combined analysis of nuclear and mitochondrial DNA sequences. *Molecular Biology and Evolution*, **15**, 656-664.

Oliveira RP, Broude NE, Macedo AM, Cantor CR, Smith CL & Pena SDJ (1998). Probing the genetic population structure of *Trypanosoma cruzi* with polymorphic microsatellites. *Proceedings of the National Academy of Sciences of the USA*, **95**, 3776-3780.

Oliveira Filho AM, Melo MTV, Santos CE, Faria Filho OF, Carneiro FCF, Oliveira-Lima JW, Vieira JBF, Gadelha FV & Ishihata J (2000). Tratamentos focais e totais com inseticidas de ação residual para o controle de *Triatoma brasiliensis* e *Triatoma pseudomaculata* no Nordeste brasileiro. *Cadernos de Saúde Pública*, **16** (Suppl. 2), 105-111.

Oliveira-Lima JW, Faria Filho OF, Vieira JBF, Gadelha FV & Oliveira Filho AM (2000). Alterações do peridomicílio e suas implicações para o controle de *Triatoma brasiliensis*. *Cadernos de Saúde Pública*, **16** (Suppl. 2), 75-81.

Omland KE, Tarr CL, Boarman WI, Marzluff JM & Fleischer RC (2000). Cryptic genetic variation and paraphyly in ravens. *Proceedings of the Royal Society London Series B*, **267**, 2475-2482.

Page RDM & Holmes E (1998). *Molecular evolution*. Blackwell Science, London, UK, 346 pp.

Palomeque FS, Abad-Franch F, Suárez K & Aguilar VHM (2000). Uso de trampas con cebo vivo para el estudio de poblaciones silvestres de *Rhodnius* (Reduviidae: Triatominae) en palmeras de la Amazonia ecuatoriana. Memorias, XXIV Jornadas Ecuatorianas de Biología, p. 108.

Panzera F (1996). Estudios cromosómicos en triatominos, pp. 78-80 in (Schofield CJ, Dujardin JP & Jurberg J eds.) *Proceedings of the International Workshop on population genetics and control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995*. INDRE, Mexico City, Mexico.

Panzera F, Álvarez F, Sánchez-Rufas J, Pérez R, Suja JA, Scvortzoff E, Estramil E, Dujardin JP & Salvatella R (1992). C-heterochromatin polymorphism in holocentric chromosomes of *Triatoma infestans* (Hemiptera – Reduviidae). *Genome*, **35**, 1068-1074.

Panzera F, Hornos S, Pereira J, Cestau R, Canale D, Diotaiuti L, Dujardin JP & Pérez R (1997). Genetic variability and geographic differentiation among three species of triatomine bugs (Hemiptera: Reduviidae). *American Journal of Tropical Medicine and Hygiene*, **57**, 732-739.

Panzera F, Pérez R, Hornos S & Nicolini P (1999). Citogenética y evolución cromosómica en triatominos, pp. 68-73 in (Schofield CJ & Ponce C eds.) *Proceedings of the second International Workshop on population genetics and control of Triatominae, Tegucigalpa, Honduras, March 1998*. INDRE, Mexico City, Mexico.

Panzera F, Pérez R, Hornos S, Panzera Y, Cestau R, Delgado V & Nicolini P (1996). Chromosome numbers in the Triatominae (Hemiptera-Reduviidae): a review. *Memórias do Instituto Oswaldo Cruz*, **91**, 515-518.

Panzera F, Pérez R, Panzera Y, Álvarez F, Scvortzoff E & Salvatella R (1995). Karyotype evolution in holocentric chromosomes of three related species of triatomines (Hemiptera – Reduviidae). *Chromosome Research*, **3**, 143-150.

Panzera F, Scvortzoff E, Pérez R, Panzera Y, Hornos S, Cestau R, Nicolini P, Delgado V, Álvarez F, Mazzella MC, Cossio G, Martínez M & Salvatella R (1998). Cytogenetics of triatomines, pp. 621-664 in (Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. II. Editora Fiocruz, Rio de Janeiro, Brazil.

Parra-Olea G & Wake DB (2001). Extreme morphological and ecological homoplasy in tropical salamanders. *Proceedings of the National Academy of Sciences of the USA*, **98**, 7888-7891.

Patterson JS (2002). *An evaluation of comparative morphometrics of triatomine bugs (Hemiptera: Reduviidae) as a taxonomic tool, and as an indicator of genetic diversity and evolutionary history*. MPhil-PhD Upgrading Report, LSHTM, London, UK, 100 pp.

Patterson JS, Schofield CJ, Dujardin JP & Miles MA (2001). Population morphometric analysis of the tropicopolitan bug *Triatoma rubrofasciata* and relationships with Old World species of *Triatoma*: evidence of New World ancestry. *Medical and Veterinary Entomology*, **15**, 443-451.

Paz-Bailey G, Monroy C, Rodas A, Rosales R, Tubaru R, Davies C, Lines J (2002). Incidence of *Trypanosoma cruzi* infection in two Guatemalan communities. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **96**, 48-52.

Pereira MH (1999). Saliva dos triatomíneos, pp. 78-80 in (Schofield CJ & Ponce C eds.) *Proceedings of the second International Workshop on population genetics and control of Triatominae, Tegucigalpa, Honduras, March 1998*. INDRE, Mexico City, Mexico.

Pereira MH, Souza ME, Vargas AP, Martins MS, Penido CM & Diotaiuti L (1996a). Anticoagulant activity of *Triatoma infestans* and *Panstrongylus megistus* saliva (Hemiptera: Triatominae). *Acta Tropica*, **61**, 255-261.

Pereira J, Dujardin JP, Salvatella R & Tibayrenc M (1996b). Enzymatic variability and phylogenetic relatedness among *Triatoma infestans, T. platensis, T. delpontei,* and *T. rubrovaria*. *Heredity*, **77**, 47-54.

Pérez R, Panzera Y, Scafiezzo S, Mazzella MC, Panzera F, Dujardin JP & Scvortzoof E (1992). Cytogenetics as a tool for triatomine species distinction (Hemiptera – Reduviidae). *Memórias do Instituto Oswaldo Cruz*, **87**, 353-361.

Peterson AT, Sánchez-Cordero V, Beard CB & Ramsey JM (2002). Ecologic niche modeling and potential reservoirs for Chagas disease, Mexico. *Emerging Infectious Diseases*, **8**, 662-667.

Picollo MI, Fontan E, Wood E & Zerba E (1990). The biochemical basis of tolerance to Malathion in *Rhodnius prolixus*. *Comparative Biochemistry and Physiology*, **96**, 361-365.

Piesman J, Sherlock IA, Mota E, Todd CW, Hoff R & Weller TH (1985). Association between household triatomine density and incidence of *Trypanosoma cruzi* infection during a nine-year study in Castro Alves, Bahia, Brazil. *American Journal of Tropical Medicine and Hygiene*, **34**, 866-869.

Pimentel RA (1992). An introduction to ordination, principal component analysis and discriminant analysis, pp. 11-28 in (Foottit RG & Sorensen JT eds.) *Ordination in the study of morphology, evolution and systematics of insects: applications and quantitative genetic rationales*. Elsevier, New York, NY, USA.

Pinho AP, Gonçalves TCM, Mangia RH, Russell NSH & Jansen AM (1998). The occurrence of *Rhodnius prolixus* Stål, 1859, naturally infected by *Trypanosoma cruzi* in the State of Rio de Janeiro, Brazil (Hemiptera, Reduviidae, Triatominae). *Memórias do Instituto Oswaldo Cruz*, **93**, 141-143.

Pires HHR, Barbosa SE, Margonari C, Jurberg J & Diotaiuti L (1998). Variations of the external male genitalia in three populations of *Triatoma infestans* Klug, 1834. *Memórias do Instituto Oswaldo Cruz*, **93**, 479-483.

Pires HHR, Borges EC, Andrade RE, Lorosa ES & Diotaiuti L (1999). Peridomiciliary infestation with *Triatoma sordida* Stål, 1859 in the County of Serra do Ramalho, Bahia, Brazil. *Memórias do Instituto Oswaldo Cruz*, **94**, 147-149.

Pizarro NJC & Romaña C (1998). Variación estacional de una población de *Rhodnius pallescens* Barber 1932 (Heteroptera: Triatominae) en la costa Caribe colombiana. *Bulletin de l'Institut Français d'Études Andines*, **27**, 309-325.

Pless M, Juranek D, Kozarsky P, Steurer F, Tapia G & Bermúdez H (1992). The epidemiology of Chagas disease in a hyperendemic area of Cochabamba, Bolivia: a clinical study including electrocardiography, seroreactivity to *Trypanosoma cruzi*, xenodiagnosis, and domiciliary triatomine distribution. *American Journal of Tropical Medicine and Hygiene*, **47**, 539-546.

Ponce C (1996). La enfermedad de Chagas en Centroamérica, pp. 41-42 in (Schofield CJ, Dujardin JP & Jurberg J eds.) *Proceedings of the International Workshop on population genetics and control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995*. INDRE, Mexico City, Mexico.

Prata A (1999). Evolution of the clinical and epidemiological knowledge about Chagas disease 90 years after its discovery. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 81-88.

Rabinovich JE, Carcavallo RU & Barreto MP (1976). Ecologic methods: marking, trapping and sampling for vector studies in the field, pp. 16-20 in *New approaches in American trypanosomiasis research*. Scientific Publication No. **318**, PAHO, Washington, DC, USA.

Rabinovich JE, Leal JA & Feliciangeli PD (1979). Domiciliary biting frequency and blood ingestion of the Chagas disease vector *Rhodnius prolixus* Stål (Hemiptera: Reduviidae), in Venezuela. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **73**, 272-283.

Racines VJ, Avilés H, Armijos R, Ortega M, Romaña C & Lema F (1994). Seroprevalencia de la infección a *Trypanosoma cruzi* en escolares de la Provincia del El Oro. *Microbiología*, **1**, 50.

Ramsey JM, Ordóñez R, Cruz-Celis A, Alvear AL, Chávez V, López R, Pintor JR, Gama F & Carrillo S (2000). Distribution of domestic Triatominae and stratification of Chagas disease transmission in Oaxaca, Mexico. *Medical and Veterinary Entomology*, **14**, 19-30.

Ready PD, Day JC, Souza AA, Rangel EF & Davies CR (1997). Mitochondrial DNA characterization of populations of *Lutzomyia whitmani* (Diptera: Psychodidae) incriminated in the peri-domestic and silvatic transmission of *Leishmania* species in Brazil. *Bulletin of Entomological Research*, **87**, 187-195.

Reyes LV (1992). Enfermedad de Chagas, pp. 134-137 in (Sempértegui R, Naranjo P & Padilla M eds.) *Panorama epidemiológico del Ecuador*. Ministerio de Salud Pública–UNICEF, Quito, Ecuador.

Reyes-Lugo M & Rodríguez-Acosta A (2000). Domiciliation of the sylvatic Chagas disease vector *Panstrongylus geniculatus* Latreille, 1811 (Triatominae: Reduviidae) in Venezuela. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **94**, 508.

Ribeiro JMC, Schneider M, Isaias T, Jurberg J, Galvão C & Guimarães JA (1998). Role of salivary antihemostatic components in blood feeding by triatomine bugs (Heteroptera). *Journal of Medical Entomology*, **35**, 599-610.

Rocha DS, Jurberg J, Carcavallo RU, Cunha V & Galvão C (2001a). Influência da temperatura e umidade na biologia de *Rhodnius neglectus* Lent, 1954 em laboratório (Hemiptera, Reduviidae, Triatominae). *Revista da Sociedade Brasileira de Medicina Tropical*, **34**, 357-363.

Rocha DS, Jurberg J, Carcavallo RU, Presgrave OAF, Cunha V & Galvão C (2001b). Influência da temperatura e umidade no desenvolvimento ninfal de *Rhodnius robustus*. *Revista de Saúde Pública*, **35**, 400-406.

Rodríguez JD (1959). Epidemiología de la enfermedad de Chagas en la República del Ecuador. *Revista Goiana de Medicina*, **5**, 411-438.

Rodríguez JD (1961). Nuevos datos sobre la enfermedad de Chagas en Guayaquil. *Revista Ecuatoriana de Higiene y Medicina Tropical*, **18**, 48-52.

Rodríguez JD (1963). Antecedentes y primeros resultados de la campaña antichagásica en un sector de la ciudad de Guayaquil. *Revista Ecuatoriana de Higiene y Medicina Tropical*, **20**, 13-15.

Rodríguez-Trelles F, Alarcón L & Fontdevilla A (2000). Molecular evolution and phylogeny of the *buzzatii* complex (*Drosophila repleta* group): a maximum-likelihood approach. *Molecular Biology and Evolution*, **17**, 1112-1122.

Rohlf FJ (2001). NTSYS. Numerical taxonomy and multivariate analysis system. Version 2.10y. Exeter Software, Setauket, New York, NY, USA.

Rojas de Arias A (2001). Chagas disease prevention through improved housing using an ecosystem approach to health. *Cadernos de Saúde Pública*, **17** (Suppl. 1), 89-97.

Rojas de Arias A, Ferro EA, Ferreira ME & Simancas LC (1999). Chagas disease vector control through different intervention modalities in endemic localities of Paraguay. *Bulletin of the World Health Organization*, **77**, 331-339.

Romaña CA, Pizarro JC, Rodas E & Guilbert E (1999). Palm trees as ecological indicators of risk areas for Chagas disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **93**, 594-595.

Romaña CA, Racines VJ, Avilés H & Lema F (1994). Observaciones de domiciliación de *Rhodnius ecuadoriensis* en focos endémicos de la enfermedad de Chagas en el Ecuador. *Microbiologia*, **1**, 59.

Romero CV, Macías CF, Suppo RJ, Macías CC & Tinoco ME (1998). Enfermedad de Chagas: incidencia en donantes de sangre, Hospital Teodoro Maldonado Carbo (Guayaquil), jun. 1996-jul. 1997. *Medicina (Quito)*, **4**, 132-134.

Saccone C, De Giorgi C, Gissi C, Pesole G & Reyes A (1999). Evolutionary genomics in Metazoa: the mitochondrial DNA as a model system. *Gene*, **238**, 195-209.

Saccone C, Gissi C, Lanave C, Larizza A, Pesole G & Reyes A (2000). Evolution of the mitochondrial genetic system: an overview. *Gene*, **261**, 153-159.

Saitou N & Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**, 406-425.

Salomón OD, Ripoll CM, Rivetti E & Carcavallo RU (1999). Presence of *Panstrongylus rufotuberculatus* (Champion, 1899) (Hemiptera: Reduviidae: Triatominae) in Argentina. *Memórias do Instituto Oswaldo Cruz*, **94**, 285-288.

Salvatella R, Rodríguez MEF, Curto de Casas SI, Barata JMS & Carcavallo RU (1998). Habitats and related fauna. B: Human environment: dwellings and peridomiciliary sites, pp. 601-619 in (Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. II. Editora Fiocruz, Rio de Janeiro, Brazil.

Sandoval CM, Amaya M, Luna SM & Angulo VM (2000b). Ensayos toxicológicos para el monitoreo de la susceptibilidad a los insecticidas en triatominos, pp. 113-117 in (Vallejo GA, Carranza MJC & Jaramillo JCJ eds.) *Memorias. Biología, epidemiología y control de la tripanosomosis americana y leishmaniosis. Curso-taller internacional*. Universidad del Tolima–Laboratorio de Investigaciones en Parasitología Tropical, Ibagué, Colombia.

Sandoval CM, Gutiérrez R, Luna SM, Amaya M, Esteban L, Ariza H & Angulo VM (2000a). High density of *Rhodnius prolixus* in a rural house in Colombia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **94**, 372-373.

Sant'Anna MRV, Diotaiuti L, Gontijo AF, Gontijo NF & Pereira MH (2001). Feeding behaviour of morphologically similar *Rhodnius* species: influence of mechanical characteristics and salivary function. *Journal of Insect Physiology*, **47**, 1459-1465.

SAS Institute (2000). JMP® version 4.0.4. Statistics and graphics guide. SAS Institute Inc., Cary, NC, USA.

Schmunis GA (1999a). Iniciativa del Cono Sur, pp. 26-31 in (Schofield CJ & Ponce C eds.) *Proceedings of the second International Workshop on Population Biology and Control of Triatominae, Tegucigalpa, Honduras, March 1998*. INDRE, Mexico City, Mexico.

Schmunis GA (1999b). Prevention of transfusional *Trypanosoma cruzi* infection in Latin America. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 93-101.

Schofield CJ (1978). A comparison of sampling techniques for domestic populations of Triatominae. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **72**, 449-455.

Schofield CJ (1980). Density regulation of domestic populations of *Triatoma infestans* in Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **74**, 761-769.

Schofield CJ (1985). Population dynamics and control of *Triatoma infestans*. *Annales de la Societé Belge de Médecine Tropicale*, **65** (Suppl. 1), 149-164.

Schofield CJ (1988). Biosystematics of the Triatominae, pp. 284-312 in (Service MW ed.) *Biosystematics of haematophagous insects*. Systematics Association Special Volume, Clarendon Press, Oxford, UK.

Schofield CJ (1994). *Triatominae: biologia y control*. Eurocommunica Publications, West Sussex, UK, 80 pp.

Schofield CJ (1996). Overview – Biosystematics of the Triatominae, pp. 45-50 in (Schofield CJ, Dujardin JP & Jurberg J eds.) *Proceedings of the International Workshop on population genetics and control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995*. INDRE, Mexico City, Mexico.

Schofield CJ (1997). Dinámica poblacional, pp. 65-68 in (Guhl F ed.) *Memorias. Curso postgrado. Genética poblacional de triatomíneos aplicada al control vectorial de la enfermedad de Chagas*. Universidad de Los Andes/CIMPAT–Universidad de Antioquia, Santafé de Bogotá, Colombia.

Schofield CJ (2000a). *Trypanosoma cruzi* – the vector-parasite paradox. *Memórias do Instituto Oswaldo Cruz*, **95**, 535-544.

Schofield CJ (2000b). Biosystematics and evolution of the Triatominae. *Cadernos de Saúde Pública*, **16** (Suppl. 2), 89-92.

Schofield CJ (2000c). *Challenges of Chagas disease vector control in Central America*. Position paper WHO/CDS/WHOPES/GCDPPH/2000.1. WHO, Communicable Diseases Control, Prevention and Eradication, WHO Pesticide Evaluation Scheme (WHOPES), Geneva, Switzerland, 36 pp.

Schofield CJ (2001). *Field testing and evaluation of insecticides for indoor residual spraying against domestic vectors of Chagas disease*. Document WHO/CSD/WHOPES/GCDPP/2001.1. WHO, Communicable Diseases Control, Prevention and Eradication, WHO Pesticide Evaluation Scheme (WHOPES), Geneva, Switzerland, 54 pp.

Schofield CJ & Dias JCP (1996). Introduction and historical overview, pp. 11-16 in (Schofield CJ, Dujardin JP & Jurberg J eds.) *Proceedings of the International Workshop on population genetics*

and control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995. INDRE, Mexico City, Mexico.

Schofield CJ & Dias JCP (1998). The Southern Cone Initiative against Chagas disease. *Advances in Parasitology*, **42**, 1-27.

Schofield CJ, Diotaiuti L & Dujardin JP (1999). The process of domestication in Triatominae. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 375-378.

Schofield CJ & Dolling WR (1993). Bedbugs and kissing-bugs, pp. 483-516 in (Lane CR & Crosskey RW eds.) *Medical insects and arachnids*. Chapman & Hall, London, UK.

Schofield CJ & Dujardin JP (1997). Chagas disease vector control in Central America. *Parasitology Today*, **13**, 141-144.

Schofield CJ & Dujardin JP (1999). Theories on the evolution of *Rhodnius*. *Actualidades Biológicas (Medellín)*, **21**, 183-197.

Schofield CJ, Dujardin JP & Jurberg J (1995). Population Genetics of Triatominae: support for vigilance and control interventions. *Memórias do Instituto Oswaldo Cruz*, **90** (Suppl 1), 57.

Schofield CJ, Dujardin JP & Jurberg J (eds.) (1996). *Proceedings of the International Workshop on population genetics and control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995*. INDRE, Mexico City, Mexico, 116 pp.

Schofield CJ, Lehane MJ, McEwen P, Catalá SS & Gorla DE (1991a). Dispersive flight by *Triatoma infestans*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **85**, 676-678.

Schofield CJ, Lehane MJ, McEwen P, Catalá SS & Gorla DE (1991b). Dispersive flight by *Triatoma infestans* under natural climatic conditions in Argentina. *Medical and Veterinary Entomology*, **6**, 51-56.

Schofield CJ & Matthews JN (1985). Theoretical approach to active dispersal and colonization of houses by *Triatoma infestans*. *Journal of Tropical Medicine and Hygiene*, **88**, 211-222.

Schofield CJ & Ponce C (eds.) (1999). *Proceedings of the second International Workshop on population genetics and control of Triatominae, Tegucigalpa, Honduras, March 1998*. INDRE, Mexico City, México, 131 pp.

Schofield CJ, Williams NG, Kirk ML, García-Zapata MT & Marsden PD (1986b). A key for identifying faecal smears to detect domestic infestations of triatomine bugs. *Revista da Sociedade Brasileira de Medicina Tropical*, **19**, 5-8.

Schofield CJ, Williams NG & Marshall TF (1986a). Density-dependent perception of triatomine bug bites. *Annals of Tropical Medicine and Parasitology*, **80**, 351-358.

Schuh RT & Slater JA (1995). *True bugs of the world (Hemiptera: Heteroptera). Classification and natural history*. Cornell University Press, New York, USA, 336 pp.

Service MW (1993). Community participation in vector-borne disease control. *Annals of Tropical Medicine and Parasitology*, **87**, 223-234.

Sherlock IA (1999). Epidemiology and dynamics of the vectorial transmission of Chagas disease. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 385-386.

Sherlock IA, Carcavallo RU & Galíndez Girón I (1997). List of natural and experimental flagellate infections in several Triatominae species, pp. 289-298 in (Carcavallo RU, Girón IG, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. I. Editora Fiocruz, Rio de Janeiro, Brazil.

Sherlock IA, Guitton N & Miles MA (1977). *Rhodnius paraensis* espécie nova do Estado do Pará, Brasil. *Acta Amazonica*, **7**, 71-74

SIISE–Servicio Integrado de Indicadores Sociales del Ecuador (1999). *Indicadores sociales en el Ecuador*. Compact disk edition, Quito, Ecuador.

SIISE–Servicio Integrado de Indicadores Sociales del Ecuador (2002). http://www.siise.gov.ec/

Silva AL (1999). Chagas disease surgery. *Memórias do Instituto Oswaldo Cruz*, 94 (Suppl. 1), 343-347.

Silva RA, Bonifácio PR & Wanderley DMV (1999). Doença de Chagas no Estado de São Paulo: comparação entre pesquisa ativa de triatomíneos em domicílios e notificação de sua presença pela população em área sob vigilância entomológica. *Revista da Sociedade Brasileira de Medicina Tropical*, **32**, 653-659.

Silveira AC (1996). O controle vetorial da doença de Chagas no Brasil. Resultados e perspectivas, pp. 89-94 in (Schofield CJ, Dujardin JP & Jurberg J eds.) *Proceedings of the International Workshop on population genetics and control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995*. INDRE, Mexico City, Mexico.

Silveira AC (1999). Current situation with the control of vector-borne Chagas disease transmission in the Americas, pp. 1161-1181 in (Carcavallo RU, Galíndez Girón I, Jurberg J & Lent H eds.) *Atlas of Chagas disease vectors in the Americas*, Vol. III. Editora Fiocruz, Rio de Janeiro, Brazil.

Silveira AC & Vinhaes MC (1999). Elimination of vector-borne transmission of Chagas disease. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 405-411.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H & Flook P (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved Polymerase Chain Reaction primers. *Annals of the Entomological Society of America*, **87**, 651-701.

Siqueira AF (1960). Estudos sobre a reação de precipitina aplicada a identificação de sangue ingerido por triatomíneos. *Revista do Instituto de Medicina Tropical de São Paulo*, **36**, 311-320.

Soares RPP, Barbosa SE, Dujardin JP, Schofield CJ, Siqueira AM & Diotaiuti L (1999). Characterizarion of *Rhodnius neglectus* from two regions of Brazil using isoenzymes, genitalia morphology and morphometry. *Memórias do Instituto Oswaldo Cruz*, **94**, 161-166.

Soares RPP, Gontijo NF, Romanha AJ, Diotaiuti L & Pereira MH (1998). Salivary heme proteins distinguish *Rhodnius prolixus* from *Rhodnius robustus* (Hemiptera: Reduviidae: Triatominae). *Acta Tropica*, **71**, 285-291.

Solano P, Dujardin JP, Schofield CJ, Romaña C & Tibayrenc M (1996). Isoenzymes as a tool for the identification of *Rhodnius* species. *Research and Reviews in Parasitology*, **56**, 41-47.

Solís-Mena S, Patterson JS & Abad-Franch F (2000). Studying the origin of Ecuadorian *Triatoma dimidiata* populations (Hemiptera: Reduviidae: Triatominae). Poster, The Trypanosome Evolution Workshop, University of Exeter–London School of Hygiene and Tropical Medicine, London, UK, February 2000.

Sorensen JT (1992). The use of discriminant function analysis for estimation of phylogeny: partitioning, perspective and problems, pp. 65-93 in (Foottit RG & Sorensen JT eds.) *Ordination in the study of morphology, evolution and systematics of insects: applications and quantitative genetic rationales*. Elsevier, New York, NY, USA.

Sorensen JT & Foottit RG (1992). The evolutionary quantitative genetic rationales for the use of ordination analyses in systematics: phylogenetic implications, pp. 29-53 in (Foottit RG & Sorensen JT eds.) *Ordination in the study of morphology, evolution and systematics of insects: applications and quantitative genetic rationales*. Elsevier, New York, NY, USA.

Southgate D (1997). *Alternatives for habitat protection and rural income generation*. Document ENV-107, The Inter-American Development Bank, Washington, DC, USA, 54 pp.

Souto R, Fernandes O, Macedo AM, Campbell D & Zingales B (1996). DNA markers define two major phylogenetic lineages of *Trypanosoma cruzi*. *Molecular Biochemical Parasitology*, **83**, 141-152.

Souza W (1999). A short review on the morphology of *Trypanosoma cruzi*: from 1909 to 1999. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 17-36.

Spanos L, Koutoumbas G, Kosyfakis M & Louis C (2000). The mitochondrial genome of the Mediterranean fruitfly, *Ceratitis capitata*. *Insect Molecular Biology*, **9**, 139-144.

Starr MD, Rojas JC, Zeledón R, Hird DW & Carpenter TE (1991). Chagas disease: risk factors for house infestation by *Triatoma dimidiata*, the major vector of *Trypanosoma cruzi* in Costa Rica. *American Journal of Epidemiology*, **133**, 740-747.

Stevens JR (2000). Blood-sucking: Old or new? Unravelling the evolution of *Trypanosoma cruzi*. *Parasitology Today*, **16**, 326.

Stevens JR, Teixeira MMG, Bingle LEH & Gibson WC (1999). The taxonomic position and evolutionary relationships of *Trypanosoma rangeli*. *International Journal for Parasitology*, **29**, 749-757.

Stothard JR, Frame IA, Carrasco HJ & Miles MA (1998a). On the molecular taxonomy of *Trypanosoma cruzi* using riboprinting. *Parasitology*, **117**, 243-247.

Stothard JR, Frame IA & Miles MA (1999). Genetic diversity and genetic exchange in *Trypanosoma cruzi*: dual drug-resistant "progeny" from episomal transformants. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl 1), 189-193.

Stothard JR, Yamamoto Y, Cherchi A, García AL, Valente SAS, Schofield CJ & Miles MA (1998b). A preliminary survey of mitochondrial sequence variation within triatomine bugs (Hemiptera: Reduviidae) using polymerase chain reaction-based single strand conformational polymorphism (SSCP) analysis and direct sequencing. *Bulletin of Entomological Research*, **88**, 553-560.

Tamura K (1992a). The rate and pattern of nucleotide substitution in *Drosophila* mitochondrial DNA. *Molecular Biology and Evolution*, **9**, 814-825.

Tamura K (1992b). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Molecular Biology and Evolution*, **9**, 678-687.

Tamura K & Nei M (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**, 512-526.

Tang J, Pruess K, Cupp EW & Unnasch TR (1996). Molecular phylogeny and typing of blackflies (Diptera: Simuliidae) that serve as vectors of human or bovine onchocerciasis. *Medical and Veterinary Entomology*, **10**, 228-234.

Tanowitz HB, Kirchhoff LV, Simon D, Morris SA, Weiss LM & Wittner M (1992). Chagas disease. *Clinical Microbiology Reviews*, **5**, 400-419.

Tarleton RL (2001). Parasite persistence in the aetiology of Chagas disease. *International Journal for Parasitology*, **31**, 550-554.

Tarleton RL & Zang (1999). Chagas disease aetiology: Autoimmunity or parasite persistence? *Parasitology Today*, **15**, 94-99.

Teixeira ARL, Monteiro PS, Rebelo JM, Argañaraz ER, Vieira D, Lauria-Pires L, Nascimento R, Vexenat CA, Silva AR, Ault SK & Costa JM (2001). Emerging Chagas disease: trophic network and cycle of transmission of *Trypanosoma cruzi* from palm trees in the Amazon. *Emerging Infectious Diseases*, **7**, 100-112.

Thorpe JP & Solé-Cava AM (1994). The use of allozyme electrophoresis in invertebrate systematics. *Zoologica Scripta*, **23**, 3-18.

Tibayrenc M (1979). Les isoenzymes et l'Entomologie médicale. *Cahiers ORSTOM, série Entomologie Médicale et Parasitologie*, **17**, 249-256.

Tibayrenc M & Ayala FJ (1988). Isozyme variability in *Trypanosoma cruzi*, the agent of Chagas disease: genetic, taxonomical, and epidemiological significance. *Evolution*, **42**, 277-292.

Tibayrenc M, Ward P, Moya A & Ayala FJ (1986). Natural populations of *Trypanosoma cruzi*, the agent of Chagas disease, have a complex multiclonal structure. *Proceedings of the National Academy of Sciences of the USA*, **83**, 115-119.

Tonn RJ, Carcavallo RU, Ortega R & Carrasquero B (1976). Métodos de estudio de triatominos en el medio silvestre. *Boletín de la Dirección de Malariología y Saneamiento Ambiental*, **16**, 146-152.

Torres M, Cárdenas E, Pérez S & Morales A (2000). Haematophagy and cleptohaematophagy of *Clerada apicicornis* (Hemiptera: Lygaeidae), a potential biological control agent of *Rhodnius prolixus* (Hemiptera: Reduviidae). *Memórias do Instituto Oswaldo Cruz*, **95**, 131-133.

Torres de Quinteros Z, Troncoso MC, Arnesi N, Boggio G & Sánchez S (1990). Comportamientos migratorios en donantes de sangre y su relación con la infección chagásica. *Cuadernos Médico Sociales*, **54**, 3-14.

Turner PC, McLennan AG, Bates AD & White MRH (2000). *Molecular biology*. BIOS Scientific Publishers, Oxford, UK, 346 pp.

Tyler KM & Engman DM (2001). The life cycle of *Trypanosoma cruzi* revisited. *International Journal for Parasitology*, **31**, 472-481.

Umezawa ES & Silveira JF (1999). Serological diagnosis of Chagas disease with purified and defined *Trypanosoma cruzi* antigens. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 285-288.

Umezawa ES, Stolf AMS, Corbett CEP & Shikanai-Yasuda MA (2001). Chagas disease. *The Lancet*, **357**, 797-799.

UNDP/World Bank/WHO TDR (1997). Andean countries initiative launched in Colombia. *TDR News*, **53**, 3.

Urbina JA (1999). Parasitological cure of Chagas disease: is it possible? Is it relevant? *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 349-355.

Usinger RL (1944). The Triatominae of North and Central America and the West Indies and their public health significance. *Public Health Bulletin*, **288**, 1-83.

Usinger RL, Wygodzinsky P & Ryckman RE (1966). The biosystematics of Triatominae. *Annual Review of Entomology*, **11**, 309-330.

Valente SAS, Valente VC & Fraiha Neto H (1999). Considerations on the epidemiology of Chagas disease in the Brazilian Amazon. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 395-398.

Valente VC (1999). Potential for domestication of *Panstrongylus geniculatus* (Latreille, 1811) (Liemiptera, Reduviidae, Triatominae) in the municipality of Muará, Marajó Island, State of Pará, Brazil. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 399-400.

Valente VC, Valente SAS, Carcavallo RU, Rocha SD, Galvão C & Jurberg J (2001). Considerações sobre uma nova espécie do gênero *Rhodnius* Stål, do Estado de Pará, Brasil (Hemiptera: Reduviidae: Triatominae). *Entomología y Vectores*, **8**, 65-80.

Valente VC, Valente SAS, Noireau F, Carrasco HJ & Miles MA (1998). Chagas disease in the Amazon basin: association of *Panstrongylus geniculatus* (Hemiptera: Reduviidae) with peridomestic pigs. *Journal of Medical Entomology*, **35**, 99-103.

Vallejo GA, Lozano LE, Carranza JC, Sánchez JL, Jaramillo JC, Guhl F, Jaramillo CA & Marinkelle CJ (2000). Ecología de los triatominos no domiciliados en Colombia con especial referencia a *Rhodnius colombiensis* en el Departamento del Tolima, pp. 22-28 in (Vallejo GA, Carranza MJC & Jaramillo JCJ eds.) *Memorias. Biología, epidemiología y control de la tripanosomosis americana y leishmaniosis. Curso-taller internacional*. Universidad del Tolima–Laboratorio de Investigaciones en Parasitología Tropical, Ibagué, Colombia.

Vitt LJ, Caldwell JP, Zani PA & Titus TA (1997). The role of habitat shift in the evolution of lizard morphology: evidence from tropical *Tropidurus*. *Proceedings of the National Academy of Sciences of the USA*, **94**, 3828-3832.

Vogler AP & Pearson DL (1996). A molecular phylogeny of the tiger beetles (Cicindelidae): congruence of mitochondrial and nuclear rDNA data sets. *Molecular Phylogenetics and Evolution*, **6**, 321-338.

Vos R (2000). *Liberalización económica, ajuste, distribución y pobreza en Ecuador, 1988-99*. Institute of Social Studies, The Hague, The Netherlands, 57 pp.

Walsh JF, Molyneux DH & Birley MH (1993). Deforestation: effects on vector-borne diseases. *Parasitology*, **106** (Suppl.), S55-S75.

Walton C, Handley JM, Tun-Lin W, Collins FH, Harbach RE, Baimai V & Butlin RK (2000). Population structure and population history of *Anopheles dirus* mosquitoes in Southeast Asia. *Molecular Biology and Evolution*, **17**, 962-974.

Whitlaw JT & Chaniotis BN (1978). Palm trees and Chagas disease in Panama. *American Journal of Tropical Medicine and Hygiene*, **27**, 873-881.

WHO (1991). Control of Chagas disease. *WHO Technical Report Series*, **811**, 95 pp.

WHO (1996). Chagas disease. Progress towards interruption of transmission. Argentina. *Weekly Epidemiological Record*, **2**, 12-15.

WHO (1997). Chagas disease. Interruption of transmission. Brazil. *Weekly Epidemiological Record*, **72**, 1-5.

WHO (1998). Chagas disease. Interruption of transmission. Uruguay. *Weekly Epidemiological Record*, **73**, 1-4.

WHO (2000). Chagas disease in Chile. Certification of interruption of transmission. *Weekly Epidemiological Record*, **75**, 9-16.

WHO/CTD (2002). *Chagas*. http://www.who.int/ctd/chagas/

Wilkerson RC, Parsons TJ, Klein TA, Caffigan TV, Bergo E & Consolim J (1995). Diagnosis by random amplified polymorphic DNA polymerase chain reaction of four cryptic species related to *Anopheles (Nysorhynchus) albitarsis* (Diptera: Culicidae) from Paraguay, Argentina, and Brazil. *Journal of Medical Entomology*, **32**, 697-704.

Wisnivesky-Colli C, Paulone I, Pérez A, Chuit R, Gualtieri J, Solarz N, Smith A & Segura EL (1987). A new tool for continuous detection of the presence of triatomine bugs, vectors of Chagas disease, in rural households. *Medicina (Buenos Aires)*, **47**, 45-50.

Wolff M & Castillo D (2002). Domiciliation trend of *Panstrongylus rufotuberculatus* in Colombia. *Memórias do Instituto Oswaldo Cruz*, **97**, 297-300.

Wood E, Licastro SA, Casabé N, Sivori J & Zerba E (1993). Evaluation of the flushing out activity of pyrethroids on *Triatoma infestans*. *Insect Science and its Application*, **14**, 651-655.

World Bank (1993). *World development report 1993. Investing in health*. Oxford University Press, New York, USA, 329 pp.

Zeledón R (1981). *El* Triatoma dimidiata *y su relación con la enfermedad de Chagas*. Ed. Universidad Estatal a Distancia, San José, Costa Rica, 146 pp.

Zeledón R, Montenegro VM & Zeledón O (2001b). Evidence of colonization of man-made ecotopes by *Triatoma dimidiata* (Latreille, 1811) in Costa Rica. *Memórias do Instituto Oswaldo Cruz*, **96**, 659-660.

Zeledón R, Solano G, Zúñiga A & Swartzwelder JC (1973). Biology and ethology of *Triatoma dimidiata* (Latreille, 1811) III. Habitat and blood sources. *Journal of Medical Entomology*, **10**, 363-370.

Zeledón R, Ugalde JA & Paniagua LA (2001a). Entomological and ecological aspects of six silvatic species of triatomines (Hemiptera, Reduviidae) from the collection of the National Biodiversity Institute of Costa Rica, Central America. *Memórias do Instituto Oswaldo Cruz*, **96**, 757-764.

Zeledón R & Vargas LG (1984). The role of dirt floors and of firewood in rural dwellings in the epidemiology of Chagas disease in Costa Rica. *American Journal of Tropical Medicine and Hygiene*, **33**, 232-235.

Zingales B, Souto RP, Mangia RH, Lisboa CV, Campbell DA, Coura JR, Jansen AM & Fernandes O (1998). Molecular epidemiology of American trypanosomiasis in Brazil based on dimorphisms of rRNA and mini-exon gene sequences. *International Journal of Parasitology*, **28**, 105-112.

Zingales B, Stolf BS, Souto RP, Fernandes O & Briones MRS (1999). Epidemiology, biochemistry and evolution of *Trypanosoma cruzi* lineages based on ribosomal RNA sequences. *Memórias do Instituto Oswaldo Cruz*, **94** (Suppl. 1), 159-164.

APPENDIX

APPENDIX TO MATERIALS
(a) Diversity in *Rhodnius ecuadoriensis*: bugs studied

Gruop	Label code	Locality	Collection	Site of capture	Sex	Morph	DNA sq	S/D
PERÚ	Peru 1	Chicama (La Libertad)	House 5	Synanthropic	M	+	-	D
PERÚ	Peru 2	Chicama (La Libertad)	House 2	Synanthropic	F	+	+	D
PERÚ	Peru 3	Suyo (Piura)	House 00S	Synanthropic	F	+	-	D
PERÚ	Peru 4	Chicama (La Libertad)	House 1	Synanthropic	M	+	+	D
PERÚ	Peru 5	Chicama (La Libertad)	Perú 1	Synanthropic	F	+	-	D
PERÚ	Peru 6	Chicama (La Libertad)	House 2	Synanthropic	F	+	+	D
PERÚ	Peru 7	Chicama (La Libertad)	House 2	Synanthropic	F	+	+	D
PERÚ	Peru 8	Chicama (La Libertad)	—	Synanthropic	M	-	+	D
PERÚ	Peru 9	Chicama (La Libertad)	—	Synanthropic	F	-	+	D
PERÚ	Peru 10	Chicama (La Libertad)	—	Synanthropic	M	-	+	D
PERÚ	Peru 11	Chicama (La Libertad)	Perú 1	Synanthropic	M	+	+	D
PERÚ	Peru 12	Chicama (La Libertad)	House 2	Synanthropic	M	+	+	D
PERÚ	Peru 13	Chicama (La Libertad)	House 2	Synanthropic	F	+	+	D
PERÚ	Peru 14	Chicama (La Libertad)	House 5	Synanthropic	F	+	+	D
PERÚ	Peru 15	Chicama (La Libertad)	House 2	Synanthropic	F	+	-	D
PERÚ	Peru 16	Chicama (La Libertad)	House 5	Synanthropic	M	+	-	D
PERÚ	Peru 17	Chicama (La Libertad)	House 2	Synanthropic	M	+	+	D
PERÚ	Peru 18	Chicama (La Libertad)	House 2	Synanthropic	M	+	+	D
PERÚ	Peru FC	Cajamarca	1979 (Fiocruz)	Synanthropic	—	-	+	D
LOJA	Rec 32	Salado, El Lucero (Loja)	1999, House MP	Peridomestic, tree with chicken nests	M	+	+	D
LOJA	Rec 33	Cangopita, El Lucero (Loja)	1999, House FJ	Peridomestic, chicken nest	M	+	+	D
LOJA	Rec 38	Salado, El Lucero (Loja)	1999, House MP	Peridomestic, tree with chicken nests	F	+	+	D
LOJA	Rec 40	Salado, El Lucero (Loja)	1999, House MP	Peridomestic, tree with chicken nests	F	+	+	D
LOJA	Rec 45	Cangopita, El Lucero (Loja)	1999, House FJ	Bedroom	M	+	-	D
LOJA	Rec 46	Cangopita, El Lucero (Loja)	1999, House FJ	Bedroom	F	+	+	D
LOJA	Rec 47	Salado, El Lucero (Loja)	1999, House JC	Peridomestic, chicken nest	F	-	+	D
LOJA	Rec 49	Salado, El Lucero (Loja)	1999, House JC	Peridomestic, chicken nest	F	+	-	D
LOJA	Rec 51	Salado, El Lucero (Loja)	1999, House JC	Peridomestic, chicken nest	M	+	+	D
LOJA	Rec 55	Salado, El Lucero (Loja)	1999, House JC	Peridomestic, chicken nest	M	+	+	D
LOJA	Rec 57	Cangopita, El Lucero (Loja)	1999, House FJ	Bedroom	M	+	+	D
LOJA	Rec 59	Salado, El Lucero (Loja)	1999, House JC	Peridomestic, chicken nest	M	+	+	D
LOJA	Rec 61	Cangopita, El Lucero (Loja)	1999, House FJ	Bedroom	F	+	-	D
LOJA	Rec 64	Cangopita, El Lucero (Loja)	1999, House FJ	Bedroom	M	+	+	D
LOJA	Rec 69	Cangopita, El Lucero (Loja)	1999, House FJ	Bedroom	M	-	+	D
LOJA	Rec 70	Cangopita, El Lucero (Loja)	1999, House FJ	Bedroom	F	+	-	D
LOJA	Rec 71	Cangopita, El Lucero (Loja)	1999, House FJ	Bedroom	F	+	+	D
LOJA	Rec 74	Cangopita, El Lucero (loja)	1999, House FJ	Bed	M	-	+	D
EL ORO	Rec O1	Lourdes (El Oro)	1999, House AP	Peridomestic, chicken nest	F	+	-	D
EL ORO	Rec O2	Lourdes (El Oro)	1999, House AP	Peridomestic, chicken nest	M	+	+	D
EL ORO	Rec O3	Lourdes (El Oro)	1999, House AP	Peridomestic, chicken nest	F	+	+	D
EL ORO	Rec O4	Lourdes (El Oro)	1999, House AP	Peridomestic, chicken nest	F	+	+	D
EL ORO	Rec O5	Lourdes (El Oro)	1999, House AP	Peridomestic, chicken nest	M	+	-	D
EL ORO	Rec O6	Lourdes (El Oro)	1999, House AP	Peridomestic, chicken nest	F	+	-	D
EL ORO	Rec O7	Lourdes (El Oro)	1999, House AP	Peridomestic, chicken nest	F	+	-	D
EL ORO	Rec O8	Lourdes (El Oro)	1999, House AP	Peridomestic, chicken nest	F	+	-	D
EL ORO	Rec O9	Lourdes (El Oro)	1999, House PS	Bedroom	M	+	+	D
EL ORO	Rec 9	Lourdes (El Oro)	1997, House AP	Peridomestic, chicken nest	M	+	+	D
EL ORO	Rec 10	Lourdes (El Oro)	1997, House AP	Peridomestic, chicken nest	M	+	-	D
EL ORO	Rec 13	Lourdes (El Oro)	1997, House AP	Peridomestic, chicken nest	M	+	-	D
EL ORO	Rec 17	Lourdes (El Oro)	1998, Entrada 4	Peridomestic, chicken nest	M	+	+	D
EL ORO	Rec 22	Lourdes (El Oro)	1999, Entrada 7	Peridomestic, chicken nest	F	+	+	D
EL ORO	Rec 26	Lourdes (El Oro)	1998, Entrada 4	Peridomestic, chicken nest	M	+	+	D
EL ORO	Rec 27	Lourdes (El Oro)	1998, Entrada 4	Peridomestic, chicken nest	F	+	+	D
EL ORO	Rec 28	Lourdes (El Oro)	1998, Entrada 4	Peridomestic, chicken nest	M	+	-	D
MANABÍ	Rec 79	Pachinche Adentro (Manabí)	1992 (Fiocruz)	*Phytelephas aequatorialis*	F	+	+	S
MANABÍ	Rec 81	Pachinche Adentro (Manabí)	1992 (Fiocruz)	*Phytelephas aequatorialis*	F	+	-	S
MANABÍ	Rec 83	Pachinche Adentro (Manabí)	1992 (Fiocruz)	*Phytelephas aequatorialis*	F	+	+	S
MANABÍ	Rec 84	Pachinche Adentro (Manabí)	1992 (Fiocruz)	*Phytelephas aequatorialis*	M	+	-	S
MANABÍ	Rec 86	Pachinche Adentro (Manabí)	1992 (Fiocruz)	*Phytelephas aequatorialis*	M	+	+	S
MANABÍ	Rec 88	Pachinche Adentro (Manabí)	1992 (Fiocruz)	*Phytelephas aequatorialis*	M	+	-	S
MANABÍ	Rec 89	Santa Ana (Manabí)	1987 (PUCE)	Synanthropic	F	+	-	S
MANABÍ	Rec 125	Pachinche Adentro (Manabí)	1999, House HM	Adult flying into house	M	+	+	S
MANABÍ	Rec 128	Sta. Rosa, Jipijapa (Manabí)	1999, House 00J	Bed	F	+	+	S
MANABÍ	Rec 129	S. José de Picoazá (Manabí)	2000, House 00P	Peridomestic, chicken nest	F	+	-	S
MANABÍ	Rec 130	S. José de Picoazá (Manabí)	2000, House 00P	Peridomestic, chicken nest	F	+	+	S
MANABÍ	Rec M1	Pachinche Adentro (Manabí)	1999, Palm	*Phytelephas aequatorialis*	M	+	+	S
MANABÍ	Rec M2	Pachinche Adentro (Manabí)	1999, Palm	*Phytelephas aequatorialis*	M	+	+	S
MANABÍ	Rec M3	Pachinche Adentro (Manabí)	1999, Palm	*Phytelephas aequatorialis*	F	+	-	S
MANABÍ	Rec M4	Pachinche Adentro (Manabí)	1999, Palm	*Phytelephas aequatorialis*	F	+	-	S
MANABÍ	Ind 2	Chirijos (Manabí)	2000, Palm	Found dead, *Phytelephas aequatorialis*	—	+	-	S
MANABÍ	Ind 11	Chirijos (Manabí)	2000, Palm	*Phytelephas aequatorialis*	NV	-	+	S
MANABÍ	Ind 14	Chirijos (Manabí)	2000, Palm	*Phytelephas aequatorialis*	NV	-	+	S
MANABÍ	Ind 22	Pachinche Adentro (Manabí)	1999, Palm	*Phytelephas aequatorialis*	NIII	-	+	S
MANABÍ	Ind 23	Pachinche Adentro (Manabí)	1999, Palm	*Phytelephas aequatorialis*	NIV	-	+	S
MANABÍ	Ind 24	Pachinche Adentro (Manabí)	1999, Palm	*Phytelephas aequatorialis*	NIV	-	+	S
MANABÍ	Ind 26	Pachinche Adentro (Manabí)	1999, Palm	*Phytelephas aequatorialis*	NIV	-	+	S
MANABÍ	Rec B	Km 24 (Manabí/Pichincha)	1999 (SNEM)	Adult found inside house	F	+	-	S

(a) Diversity in *Rhodnius ecuadoriensis*: bugs studied (continued)

Gruop	Label code	Locality	Collection	Site of capture	Sex	Morph	DNA sq	S/D
PICHINCHA	Rec 30	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	F	+	-	S
PICHINCHA	Rec 92	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	F	+	+	S
PICHINCHA	Rec 93	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	M	+	-	S
PICHINCHA	Rec 95	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	F	+	-	S
PICHINCHA	Rec 97	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	M	+	-	S
PICHINCHA	Rec 98	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	M	+	+	S
PICHINCHA	Rec 101	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	F	+	-	S
PICHINCHA	Rec 102	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	F	+	-	S
PICHINCHA	Rec 103	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	F	-	+	S
PICHINCHA	Rec 105	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	F	-	+	S
PICHINCHA	Rec 107	Coca (Orellana)	1987 (PUCE)	Palm, labelled as Coca (legit. Onore)	F	+	-	S
PICHINCHA	Rec P1	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	M	+	+	S
PICHINCHA	Rec P2	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	F	+	+	S
PICHINCHA	Rec P3	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	M	+	+	S
PICHINCHA	Rec P4	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	F	+	+	S
PICHINCHA	Ind 36	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	M	-	+	S
PICHINCHA	Ind 37	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	M	-	+	S
PICHINCHA	Ind 38	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	M	-	+	S
PICHINCHA	Ind 39	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	F	-	+	S
PICHINCHA	Ind 40	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	F	-	+	S
PICHINCHA	Ind 41	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	M	-	+	S
PICHINCHA	Ind 42	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	M	-	+	S
PICHINCHA	Ind 43	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	M	-	+	S
PICHINCHA	Ind 45	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	F	-	+	S
PICHINCHA	Ind 46	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	M	-	+	S
PICHINCHA	Rec A	Km 24 (Manabí/Pichincha)	1999 (SNEM)	Adult found inside house	M	+	-	S
PICHINCHA	Rec NL3	Alluriquín (Pichincha)	1999, Palm	*Phytelephas aequatorialis*	F	+	+	S

Morph=morphometrics (+=yes, -=no); DNA sq=DNA sequencing (+=yes, -=no); S/D=main habitat (D=domestic-peridomestic; S=sylvatic); SNEM= Ecuadorian National Vector Control Service, PUCE=Catholic University, Quito, Ecuador

(b) Phylogenetic relationships of *Rhodnius ecuadoriensis*: other species studied

Species	Code	Origin	Country	Original habitat	Sex	Morph	DNA sq	Remarks
R. colombiensis	Col1	Coyaima, Tolima	Colombia	*Attalea butyracea*	M	+	+	Lab UA
R. colombiensis	Col2	Coyaima, Tolima	Colombia	*Attalea butyracea*	F	+	+	Lab UA
R. colombiensis	Col3	Coyaima, Tolima	Colombia	*Attalea butyracea*	M	+	+	Lab UA
R. colombiensis	Col4	Coyaima, Tolima	Colombia	*Attalea butyracea*	F	+	+	Lab UA
R. colombiensis	Col5	Coyaima, Tolima	Colombia	*Attalea butyracea*	F	+	+	Lab UA
R. colombiensis	Col6	Coyaima, Tolima	Colombia	*Attalea butyracea*	F	+	-	Lab UA
R. colombiensis	Col7	Coyaima, Tolima	Colombia	*Attalea butyracea*	M	+	-	Lab UA
R. colombiensis	Col8	Coyaima, Tolima	Colombia	*Attalea butyracea*	M	+	-	Lab UA
R. colombiensis	Col9	Coyaima, Tolima	Colombia	*Attalea butyracea*	F	+	-	Lab UA
R. colombiensis	Col10	Coyaima, Tolima	Colombia	*Attalea butyracea*	M	+	-	Lab UA
R. colombiensis	Col11	Coyaima, Tolima	Colombia	*Attalea butyracea*	F	+	-	Lab UA
R. colombiensis	Col12	Coyaima, Tolima	Colombia	*Attalea butyracea*	F	+	-	Lab UA
R. colombiensis	Col13	Coyaima, Tolima	Colombia	*Attalea butyracea*	M	+	-	Lab UA
R. colombiensis	Col14	Coyaima, Tolima	Colombia	*Attalea butyracea*	M	+	-	Lab UA
R. colombiensis	Col15	Coyaima, Tolima	Colombia	*Attalea butyracea*	M	+	-	Lab UA
R. pallescens	Pal1	—	Colombia	—	M	+	+	Lab UA
R. pallescens	Pal2	—	Colombia	—	F	+	+	Lab UA
R. pallescens	Pal3	—	Colombia	—	M	+	+	Lab UA
R. pallescens	Pal4	—	Colombia	—	F	+	+	Lab UA
R. pallescens	Pal5	—	Colombia	—	F	+	+	Lab UA
R. pallescens	Pal6	—	Colombia	—	F	+	+	Lab UA
R. pallescens	Pal7	—	Colombia	—	M	+	+	Lab UA
R. pallescens	Pal8	—	Colombia	—	F	+	-	Lab UA
R. pallescens	Pal9	—	Colombia	—	F	+	+	Lab UA
R. pallescens	Pal10	—	Colombia	—	M	+	+	Lab UA
R. pallescens	Pal11	—	Colombia	—	F	+	+	Lab UA
R. pallescens	Pal12	—	Colombia	—	F	+	+	Lab UA
R. pallescens	Pal13	—	Colombia	—	M	+	+	Lab UA
R. pallescens	Pal14	—	Colombia	—	M	+	-	Lab UA
R. pallescens	Pal15	—	Colombia	—	M	+	+	Lab UA
R. pallescens	PalVe	Vegachí	Colombia	—	—	-	+	FA Monteiro
R. pallescens	PalN1	Nicaragua	Nicaragua	—	—	-	+	CJ Schofield/FA Monteiro
R. pallescens	PalN2	Nicaragua	Nicaragua	—	—	-	+	CJ Schofield/FA Monteiro
R. pictipes	Pic1	Yasuní	Ecuador			+	-	PUCE 1997
R. pictipes	Pic2	Yasuní	Ecuador			+	-	PUCE 1997
R. pictipes	Pic3	Lago Agrio	Ecuador	*Phytelepas tenuicaulis*		+	-	PUCE 1999
R. pictipes	Pic4	—	Ecuador	Light trap		+	-	PUCE
R. pictipes	Pic5	—	Ecuador	—		+	-	—
R. pictipes	Pic6	Loreto (km60)	Ecuador	House		+	-	SNEM
R. pictipes	Pic7	Jivino	Ecuador	Light trap		+	-	PUCE 1989
R. pictipes	Pic8	Yasuní	Ecuador	Light trap		+	-	PUCE 1996
R. pictipes	Pic9	Yasuní	Ecuador	Light trap		+	-	PUCE 1995
R. pictipes	Pic10	S. Pablo-Aguarico	Ecuador	Light trap		+	-	PUCE 1995
R. pictipes	Pic11	Zábalo	Ecuador	Light trap		+	-	PUCE 1996
R. pictipes	Pic12	Cuyabeno	Ecuador	Light trap	F	+	-	PUCE
R. pictipes	Pic13	Cuyabeno	Ecuador	Light trap	F	+	-	PUCE
R. pictipes	Pic14	Zábalo	Ecuador	Light trap	F	+	-	PUCE
R. pictipes	Pic15	Yasuní	Ecuador	Light trap	F	+	-	PUCE
R. pictipes	PicFM	Belém, Pará	Brazil	—	—	-	+	FA Monteiro
R. pictipes	PicCS	Amazonia		—	—	-	+	CJ Schofield/FA Monteiro
R. prolixus	Prx1	—	Colombia	Domestic	F	+	-	Lab CIMPAT
R. prolixus	Prx2	—	Colombia	Domestic	M	+	-	Lab CIMPAT
R. prolixus	Prx3	—	Colombia	Domestic	F	+	-	Lab CIMPAT
R. prolixus	Prx4	—	Colombia	Domestic	F	+	-	Lab CIMPAT
R. prolixus	Prx5	—	Colombia	Domestic	F	+	-	Lab CIMPAT
R. prolixus	Prx6	—	Colombia	Domestic	F	+	-	Lab CIMPAT
R. prolixus	Prx7	—	Colombia	Domestic	M	+	-	Lab CIMPAT
R. prolixus	Prx8	—	Colombia	Domestic	F	+	-	Lab CIMPAT
R. prolixus	Prx9	—	Colombia	Domestic	F	+	-	Lab CIMPAT
R. prolixus	Prx10	—	Colombia	Domestic	F	+	-	Lab CIMPAT
R. prolixus	Prx11	—	Colombia	Domestic	F	+	-	Lab CIMPAT
R. prolixus	Prx12	—	Colombia	Domestic	F	+	-	Lab CIMPAT
R. prolixus	Prx13	—	Colombia	Domestic	F	+	-	Lab CIMPAT
R. prolixus	Prx14	—	Colombia	Domestic	F	+	-	Lab CIMPAT
R. prolixus	Prx15	—	Colombia	Domestic	M	+	-	Lab CIMPAT
R. prolixus	PrxV1	Lara	Venezuela	Domestic	—	-	+	SFitzpatrick/JSPatterson
R. prolixus	PrxV2	Ortiz	Venezuela	Domestic	—	-	+	SFitzpatrick/JSPatterson
R. prolixus	PrxV3	Ortiz	Venezuela	Domestic	—	-	+	FA Monteiro
R. prolixus	PrxV4	Cojedes	Venezuela	Domestic	—	-	+	FA Monteiro
R. prolixus	PrxV5	Cojedes	Venezuela	Domestic	—	-	+	FA Monteiro
R. prolixus	PrxHn	F. Morazán	Honduras	Domestic	—	-	+	FA Monteiro
R. robustus	Rob1	Loreto	Ecuador	*Oenocarpus bataua*	M	+	-	Field col.
R. robustus	Rob2	Lago Agrio	Ecuador	*Attalea butyracea*	F	+	-	Field col.
R. robustus	Rob3	Loreto	Ecuador	*Oenocarpus bataua*	F	+	-	Field col.
R. robustus	Rob6	Sucumbíos	Ecuador	*Attalea butyracea*	M	+	-	Field col.
R. robustus	Rob31	Yasuní	Ecuador	Light trap	M	+	-	Field col.
R. robustus	Rob4	Yasuní	Ecuador	Light trap	M	+	-	Field col.

(b) Phylogenetic relationships of *Rhodnius ecuadoriensis*: other species studied (continued)

Species	Code	Origin	Country	Original habitat	Sex	Morph	DNA sq	Remarks
R. robustus	Rob7(B)	Lago Agrio	Ecuador	*Attalea butyracea*	F	+	-	Field col.
R. robustus	Rob8	Lago Agrio	Ecuador	---	---	+	-	Field col.
R. robustus	Rob9	J Sachas	Ecuador	*Attalea butyracea*	F	+	-	Field col.
R. robustus	Rob10	Yasuni	Ecuador	---	F	+	-	Field col.
R. robustus	Rob11	Lago Agrio	Ecuador	*Attalea butyracea*	M	+	-	Field col.
R. robustus	Rob12	Lago Agrio	Ecuador	*Attalea butyracea*	F	+	-	Field col.
R. robustus	Rob13	Lago Agrio	Ecuador	*Attalea butyracea*	M	+	-	Field col.
R. robustus	Rob14	Putumayo	Ecuador	House	F	+	-	Field col.
R. robustus	Rob15	Sucumbios	Ecuador	Palm	---	+	-	Field col.
R. robustus	Rob16	Sucumbios	Ecuador	Palm	---	-	+	Field col.
R. robustus	RobEc	Napo	Ecuador	Sylvatic, palm	---	-	+	FA Monteiro
R. robustus	RobB1	Balbina	Brazil	Sylvatic	---	-	+	FA Monteiro
R. prolixus	RobB2	Pará	Brazil	Sylvatic	---	-	+	FA Monteiro
R. robustus	RobFG	Cayenne	Fr. Guiana	Sylvatic	---	-	+	FA Monteiro
R. nasutus	Nas1	Piaui	Brazil	---	---	-	+	FA Monteiro
T. rubrovaria	Trbv	Sacramento	Brazil	---	---	-	+	FA Monteiro

Morph=morphometrics (+=yes, -=no), DNA sq=DNA sequencing (+=yes, -=no); UA=University of Antioquia, Medellín, Colombia; CIMPAT=University of Los Andes, Bogotá, Colombia; PUCE=Catholic University, Quito, Ecuador

APPENDIX TO SECTION 3.2.
Records of *Rhodnius ecuadoriensis* from Ecuador

Province	Canton	Municipality	Locality	Latitude	Longitude	Altitude (m)
Pichincha	Sto Domingo de los Colorados	Alluriquín	Alluriquín (*s*)	0.33	78.99	940
	—	—	Kilómetro 24	—	—	—
Manabí	El Carmen	—	La Roncadora (*s*)	0.2	79.53	400
	Portoviejo	Portoviejo	Portoviejo	1.05	80.45	37
		El Rodeo	El Rodeo	1.02	80.39	45
		Andrés de Vera	Mocora Afuera	1.01	80.18	160
			Guabito Afuera	1.075	80.45	174
			Limón Afuera	—	—	—
			Limón Adentro	—	—	—
			Naranjo Afuera	—	—	—
			Naranjo Adentro	—	—	—
			Mocora Adentro	—	—	—
	Colón	Colón	Colón	1.12	80.42	84
			El Cady	1.12	80.42	30
			Mapasingue	1.16	80.39	60
			Pachinche Afuera	1.15	80.39	30
			Pachinche en Medio	1.13	80.36	60
			Pachinche Adentro (*s*)	1.12	80.35	80
			Pachinche Entrada	—	—	—
			Quebrada de Maconta	1.09	80.36	300
			Estancia Vieja Afuera	1.14	80.4	15
			Estancia Vieja Adentro	1.13	80.39	35
	Picoazá	Picoazá	Picoazá	1.03	80.49	35
			Milagro	1.02	80.49	30
			Negrital	1.03	80.48	10
			Mejía	0.62	80.11	—
			Cruz del Guayabo	—	—	—
	Río Chico	Río Chico	Río Chico	1	80.43	169
			Zapote	0.99	80.16	420
			Quebrada Grande	0.97	80.38	100
			Pechiche	1.06	80.9	200
			Milagro	0.98	80.45	—
			San Gabriel	—	—	—
			Carretera S.Gabriel-Calderón	—	—	—
			Pinpiguasí (*s*)	—	—	—
			Tomatal	—	—	—
			Chacras Adentro	—	—	—
			La Balsita	—	—	—
			San Vicente	—	—	—
	Calderón	El Zapallo	El Zapallo	1.05	80.38	200
	Chirijos	Chirijos (*s*)	Chirijos (*s*)	1.03	80.25	—
	—	—	La Encantada	—	—	—
	—	—	Tigre	—	—	—
	—	—	Arreaga	—	—	—
Santa Ana	Santa Ana	Santa Ana	Santa Ana	1.22	80.38	150
			Sasay Adentro	—	—	—
			Sasay Afuera	—	—	—
	—	—	Tierras Negras	—	—	—
24 de Mayo	Sucre	Sucre	Sucre	1.27	80.43	154
			El Pueblito	1.26	80.42	120
			El Colorado	1.35	80.62	400
			Barro	—	—	—
			Bellavista	—	—	—

Records of *Rhodnius ecuadoriensis* from Ecuador (continued)

Province	Canton	Municipality	Locality	Latitude	Longitude	Altitude (m)
Manabí	Sucre	Charapotó	Charapotó	0.83	80.48	96
			Las Cañitas	1.6	80.7	600
			La Roncadora	—	—	—
	Jipijapa	Jipijapa	Jipijapa	1.33	80.58	316
			Santa Rosa	1.32	80.63	352
	Rocafuerte	Rocafuerte	Resbalón	0.96	80.43	110
			Pasaje	0.98	80.34	—
			Tierra Amarilla	—	—	—
			Valdez	—	—	—
		—	José A. Campos	—	—	—
	Portoviejo	—	San José de Picoazá	—	—	—
	Paján	Paján	Paján	1.57	80.42	151
El Oro	Portovelo	Portovelo	Portovelo	3.71	79.61	629
			Lourdes	3.72	79.62	984
	Piñas	Piñas	Piñas	3.68	79.68	1014
	Zaruma	Zaruma	Zaruma	3.68	79.62	1106
Loja	Catamayo	Catamayo	Catamayo	3.98	79.35	1531
		La Toma	La Toma	3.98	79.36	1266
	Calvas	El Lucero	El Lucero	4.4	79.47	1200
		Cariamanga	Cariamanga	4.33	79.55	2200
	Gonzanamá	Gonzanamá	Gonzanamá	3.97	79.7	1465
	Paltas	Paltas	Paltas	3.82	79.35	2720
			Catacocha	4.07	79.63	1886
Los Ríos	Quevedo	—	Pichilingue (*s*)	1.1	79.48	73
Guayas*	Guayaquil	Guayaquil	Guayaquil	2.15	79.88	5
	Sana Elena	Santa Elena	Santa Elena	2.23	80.85	26
Tungurahua*	Baños	Baños	Baños	1.39	78.42	1804
Orellana*	Coca	Coca	Coca			

(*s*) = sylvatic colonies confirmed; * = dubious records (not included in biogeographical analyses)

APPENDIX TO SECTION 4.3.

Table 1. *Rhodnius ecuadoriensis* in *Phytelephas aequatorialis* palms in Alluriquín, Pichincha

Palm code	Sex	Epiphytes	Organic matter	Stem height	Location	Result
A 1	♂	+++	+++	5	Cropland	Infested
A 2	♂	+ ½	+ ½	4	Cropland	Not infested
A 3	♀	++	+ ½	2.5	Cropland	Not infested
A 4	♂	++++	++++	6	Cropland	Infested
A 5	♂	++	++	5.5	Cropland	Not infested
A 6	♀	+ ½	+	4	Cropland	Infested
A 7	♀	++ ½	++	3	Cropland	Not infested
A 8	Nr	++++	++++	4.5	Cropland	Not infested
A 9	Nr	+++	++++	4.5	Cropland	Not infested
A 10	Nr	+++	+++	4.5	Cropland	Not infested
A 11	♀	0	½	3	Forest	Not infested
A 12	Nr	0	½	3	Forest	Not infested
A 13	Nr	0	½	4	Forest	Not infested
A 14 (C1)	♀	½	½	5	Cropland	Not infested
A 15 (C2)	♂	+	½	3.5	Cropland	Not infested
A 16 (C3)	♂	++	+ ½	3.5	Cropland	Not infested
A 17 (C4)	♀	+	½	4.5	Cropland	Not infested
A 18 (A)	♂	++	++	3	Cropland	Not infested
A 19 (B)	♂	+ ½	+	4.5	Cropland	Not infested
A 20 (C)	♂	++ ½	++	3	Cropland	Infested
A 21 (D)	♀	+	+	2.5	Cropland	Not infested
A 22 (E)	♀	++ ½	++ ½	2.5	Cropland	Infested
A 23 (F)	♂	++	+ ½	3	Cropland	Infested
A 24 (G)	♀	+ ½	+	3.5	Cropland	Not infested
A 25 (H)	Nr	++	+	Nr	Cropland	Infested
A 26 (I)	♂	++ ½	++ ½	5	Cropland	Infested
A 27 (J)	♂	+ ½	½	1.5	Cropland	Not infested
A 28 (K)	♂	++	++ ½	5.5	Cropland	Infested
A 29 (L)	♂	++	++ ½	4.5	Cropland	Not infested
A 30 (M)	♀	++ ½	++	4	Cropland	Not infested
A 31 (N)	♂	+ ½	+	3	Cropland	Infested
A 32 (Ñ)	♂	+	+ ½	6	Cropland	Not infested
A 33 (O)	♂	+	++ ½	5	Cropland	Infested
A 34 (P)	♀	++ ½	+++	4	Cropland	Infested
A 35 (1)	♂	½	++½	8	Cropland	Infested
A 36 (2)	♂	+	++	4	Cropland	Not infested
A 37 (3)	♀	++½	+++½	5	Cropland	Infested
A 38 (4)	Nr	++	+++	4	Cropland	Not infested
A 39 (5)	♂	+	++	3.5	Cropland	Not infested
A 40 (6)	♂	+½	++	6	Cropland	Not infested
A 41 (7)	Nr	++	+++	2.5	Cropland	Not infested
A 42 (8)	Nr	½	+½	3	Cropland	Not infested
A 43 (9)	Nr	+½	++	5	Cropland	Not infested
A 44 (10)	Nr	½	+½	2.5	Cropland	Not infested
A 45 (11)	Nr	+	+½	2	Cropland	Not infested
A 46 (12)	Nr	+	+	2.5	Cropland	Not infested
A 47 (13)	Nr	½	+	3	Forest	Not infested
A 48 (14)	Nr	++	+½	3.5	Forest	Not infested
A 49 (15)	Nr	+½	+	4	Forest	Not infested
A 50 (16)	Nr	½	++	3	Forest	Not infested
A 51 (17)	Nr	½	+½	2	Forest	Not infested
A 52 (18)	Nr	0	+½	0.6	Forest	Not infested
A 53 (19)	Nr	½	++	0.8	Forest	Not infested
A 54 (20)	Nr	½	+	3	Cropland	Not infested
A 55 (21)	Nr	+	+½	2.5	Cropland	Not infested
A 56 (22)	Nr	+++½	++½	3.5	Cropland	Not infested

Nr = not recorded; Location: Forest = secondary forest, Cropland = cropland/pasture

Table 2. Palms sampled in Chigüilpe, Pichincha

Palm code	Species (sex)	Epiphytes	Organic matter	Stem height	Location	Result
CL 1	*Ph. aequat.* (♀)	+	+½	3.5	Cropland	Not infested
CL 2	*Ph. aequat.* (♀)	+½	++½	2	Cropland	Not infested
CL 3	*Ph. aequat.* (♀)	+	++	2	Cropland	Not infested
CL 4*	*Bactris* sp.	+½	½	10	Cropland	Not infested

Ph. aequat.=Phytelephas aequatorialis; *Not included in the analyses

Table 3. *Rhodnius ecuadoriensis* in *Phytelephas aequatorialis* palms in La Roncadora, Manabí

Palm code	Species (sex)	Epiphytes	Organic matter	Stem height	Location	Result
RO 1	*Ph. aeq.* (♂)	+½	++½	10	Cropland	Not infested
RO 2	*Ph. aeq.* (♂)	½	+½	8	Forest	Not infested
RO 3	*Ph. aeq.* (♀)	½	+	6.5	Forest	Not infested
RO 4	*Ph. aeq.* (♂)	+	++	7	Forest	Not infested
RO 5	*Ph. aeq.* (♂)	++	+½	6	Forest	Not infested
RO 6	*Ph. aeq.* (♂)	+½	+++	4.5	Cropland	Infested
RO 7	*Ph. aeq.* (♂)	½	++	3	Forest	Not infested
RO 8	*Ph. aeq.* (♀)	½	+	2	Forest	Not infested
RO 9	*Ph. aeq.* (♂)	½	++	5.5	Forest	Not infested
RO 10	*Ph. aeq.* (♂)	½	+	2.5	Forest	Not infested
RO 11	*Ph. aeq.* (♂)	++	+++	4.5	Cropland	Not infested
RO 12	*Ph. aeq.* (♂)	½	++	2.5	Cropland	Not infested
RO 13	*Ph. aeq.* (♀)	+	+½	5	Cropland	Not infested
RO 14	*Ph. aeq.* (♂)	½	+	2	Cropland	Not infested
RO 15	*Ph. aeq.* (♂)	+	+	2	Cropland	Not infested
RO 16*	*Attalea colenda*	½	+	6	Forest	Not infested

Ph. aeq.=Phytelephas aequatorialis; *Not included in the analyses

Table 4. *Rhodnius ecuadoriensis* in *Phytelephas aequatorialis* palms in Pachinche Adentro, Manabí

Palm code	Sex	Epiphytes	Organic matter	Stem height	Location	Result
PA 1	♀	+	+ ½	5	Cropland	Not infested
PA 2	♂	½	++	4.5	Cropland	Not infested
PA 3	♂	0	+ ½	5	Cropland	Not infested
PA 4	♀	+	+	1.7	Cropland	Not infested
PA 5	♂	+	++	2	Cropland	Not infested
PA 6	♂	+ ½	+++	5	Cropland	Infested
PA 7	♀	+ ½	+ ½	5	Cropland	Not infested
PA 8	♂	+	+ ½	6.5	Cropland	Not infested
PA 9	♀	+ ½	+ ½	6	Cropland	Not infested
PA 10	♂	0	++ ½	6	Cropland	Infested
PA 11	♂	+	++	7	Cropland	Infested
PA 12	♂	0	++	8	Cropland	Not infested
PA 13	♂	0	++	6	Cropland	Not infested
PA 14	♂	+	++	5	Cropland	Infested
PA 15	♂	0	+ ½	7	Cropland	Infested
PA 16	♀	+	++	6.5	Cropland	Not infested
PA 17	♀	0	++	6.5	Cropland	Not infested
PA 18	♂	0	++	5.5	Cropland	Not infested

Table 5. *Rhodnius ecuadoriensis* in *Phytelephas aequatorialis* palms in Chirijos, Manabí

Palm code	Sex	Epiphytes	Organic matter	Stem height	Location	Result
CH 1	♀	+	+	7	Cropland	Not infested
CH 2	♀	+½	+	2.5	Cropland	Not infested
CH 3	♀	½	+½	7.5	Cropland	Infested
CH 4	♀	+	+	8	Cropland	Infested
CH 5	♀	+	+½	6.5	Cropland	Not infested
CH 6	♀	+	½	5.5	Cropland	Not infested
CH 7	♀	+	½	6	Cropland	Not infested
CH 8	♀	½	+	6	Cropland	Not infested
CH 9	♂	½	+	6	Cropland	Not infested
CH 10	♀	+	+½	6.5	Cropland	Not infested
CH 11	♂	0	++	7.5	Cropland	Infested
CH 12	♂	0	++½	7	Cropland	Infested
CH 13	♂	½	++	6	Cropland	Not infested
CH 14	♀	½	+++	8	Cropland	Not infested
CH 15	♂	½	+½	6	Cropland	Not infested
CH 16	♂	½	++	4	Cropland	Not infested
CH 17	♂	½	++	4.5	Cropland	Not infested
CH 18	♂	½	+½	3.5	Cropland	Infested

APPENDIX TO SECTION 6.2.

Table 1. Measurements taken for morphometrics (in mm): pooled *Rhodnius ecuadoriensis* populations

Measurements	Mean	SD	SE	Median	Quartiles	Range	n
A	1.28	0.1	0.01	1.28	1.2-1.36	1.1-1.52	79
B	0.49	0.05	0.01	0.49	0.46-0.53	0.37-0.61	79
C	0.85	0.09	0.01	0.84	0.78-0.91	0.66-1.04	79
D	1.54	0.3	0.03	1.42	1.3-1.75	1.1-2.2	79
E	0.56	0.08	0.01	0.53	0.5-0.616	0.42-0.75	79
F	2.73	0.45	0.05	2.55	2.38-3.07	2.1-3.72	79
G	1.06	0.26	0.03	0.98	0.85-1.25	0.7-1.68	79
H	0.32	0.03	0.004	0.32	0.29-0.34	0.27-0.4	79
L	0.85	0.09	0.01	0.84	0.79-0.9	0.7-1.06	79
R1*(see footnote)	0.69	0.09	0.01	0.66	0.63-0.75	0.52-0.88	77
R2	2.1	0.36	0.04	1.95	1.83-2.32	1.6-3	77
R3	0.62	0.07	0.01	0.6	0.57-0.66	0.49-0.77	77

SD = standard deviation; SE = standard error of the mean; n = number of specimens (in all tables); see figure 91 for measurements

Table 2. Measurements taken for morphometrics (in mm): pooled *Rhodnius ecuadoriensis* synanthropic populations (El Oro, Loja and Peru)

Measurements	Mean	SD	SE	Median	Quartiles	Range	n
A	1.23	0.06	0.01	1.22	1.18-1.28	1.11-1.33	47
B	0.47	0.04	0.01	0.48	0.44-0.5	0.37-0.56	47
C	0.79	0.05	0.01	0.79	0.75-0.83	0.66-0.89	47
D	1.33	0.1	0.01	1.33	1.25-1.39	1.1-1.49	47
E	0.5	0.04	0.01	0.51	0.48-0.52	0.42-0.56	47
F	2.41	0.15	0.02	2.43	2.3-2.5	2.09-2.69	47
G	0.88	0.08	0.01	0.88	0.82-0.94	0.71-1.02	47
H	0.3	0.02	0.003	0.29	0.28-0.31	0.27-0.34	47
L	0.8	0.05	0.01	0.81	0.77-0.84	0.7-0.9	47
R1	0.63	0.05	0.01	0.63	0.61-0.67	0.52-0.75	47
R2	1.86	0.11	0.02	1.85	1.79-1.94	1.62-2.04	47
R3	0.57	0.03	0.004	0.58	0.55-0.59	0.49-0.63	47

Table 3. Measurements taken for morphometrics (in mm): pooled *Rhodnius ecuadoriensis* sylvatic populations (Pichincha and Manabí)

Measurements	Mean	SD	SE	Median	Quartiles	Range	n
A	1.36	0.08	0.01	1.37	1.26-1.43	1.2-1.52	32
B	0.52	0.04	0.01	0.53	0.49-0.55	0.44-0.61	32
C	0.92	0.06	0.01	0.93	0.88-0.96	0.79-1.04	32
D	1.85	0.2	0.04	1.81	1.7-2.04	1.45-2.22	32
E	0.64	0.06	0.01	0.63	0.59-0.69	0.53-0.75	32
F	3.21	0.29	0.051	3.17	2.98-3.48	2.57-3.72	32
G	1.33	0.17	0.03	1.31	1.2-1.47	1.03-1.68	32
H	0.35	0.02	0.004	0.35	0.33-0.37	0.32-0.4	32
L	0.92	0.08	0.01	0.91	0.8-0.98	0.73-1.06	32
R1*(see footnote)	0.77	0.07	0.01	0.79	0.7-0.82	0.61-0.88	30
R2	2.49	0.27	0.05	2.48	2.19-2.69	1.95-2.99	30
R3	0.69	0.05	0.01	0.69	0.64-0.75	0.6-0.77	30

*Smaller sample sizes for rostrum measurements (R1, R2, R3) correspond to two deteriorated specimens (one found dead in a palm in Chirijos, Manabí, and the other collected by the Vector Control Service in a house in the limit between Manabí and Pichincha) that had lost their mouthparts; missing values were substituted by the mean of their geographic group (Manabí) for some of the analyses; in others, these bugs were excluded

Table 4. Measurements taken for morphometrics (in mm): Peru

Measurements	Mean	SD	SE	Median	Quartiles	Range	n
A	1.26	0.05	0.01	1.28	1.25-1.3	1.17-1.32	14
B	0.49	0.03	0.01	0.49	0.46-0.52	0.43-0.53	14
C	0.82	0.04	0.01	0.82	0.8-0.85	0.74-0.89	14
D	1.43	0.06	0.02	1.43	1.37-1.49	1.33-1.49	14
E	0.51	0.04	0.01	0.51	0.49-0.54	0.42-0.56	14
F	2.55	0.1	0.03	2.55	2.46-2.64	2.39-2.69	14
G	0.97	0.04	0.01	0.98	0.93-1.01	0.9-1.02	14
H	0.32	0.02	0.01	0.31	0.3-0.34	0.28-0.34	14
L	0.83	0.05	0.01	0.84	0.8-0.86	0.73-0.9	14
R1	0.68	0.04	0.01	0.68	0.66-0.68	0.62-0.75	14
R2	1.91	0.08	0.02	1.92	1.8-1.97	1.79-2.04	14
R3	0.58	0.04	0.01	0.58	8.57-0.6	0.49-0.63	14

Table 5. Measurements taken for morphometrics (in mm): Loja

Measurements	Mean	SD	SE	Median	Quartiles	Range	n
A	1.19	0.06	0.01	1.18	1.14-1.2	1.11-1.29	16
B	0.45	0.04	0.01	0.44	0.4-0.48	0.37-0.53	16
C	0.78	0.06	0.01	0.78	0.75-0.82	0.66-0.85	16
D	1.24	0.06	0.02	1.24	1.19-1.28	1.1-1.34	16
E	0.48	0.04	0.01	0.49	0.44-0.51	0.42-0.56	16
F	2.3	0.12	0.03	2.26	2.2-2.4	2.09-2.51	16
G	0.8	0.05	0.01	0.81	0.75-0.84	0.71-0.88	16
H	0.29	0.02	0.004	0.29	0.28-0.29	0.27-0.33	16
L	0.78	0.05	0.01	0.8	0.72-0.8	0.7-0.85	16
R1	0.62	0.03	0.01	0.63	0.59-0.64	0.54-0.66	16
R2	1.75	0.07	0.02	1.74	1.69-1.8	1.62-1.85	16
R3	0.57	0.02	0.01	0.58	0.55-0.59	0.53-0.62	16

Table 6. Measurements taken for morphometrics (in mm): El Oro

Measurements	Mean	SD	SE	Median	Quartiles	Range	n
A	1.24	0.06	0.02	1.26	1.15-1.29	1.11-1.33	17
B	0.49	0.04	0.01	0.48	0.46-0.53	0.42-0.56	17
C	0.78	0.05	0.01	0.78	0.74-0.82	0.68-0.87	17
D	1.32	0.06	0.01	1.3	1.28-1.38	1.2-1.39	17
E	0.51	0.03	0.01	0.5	0.49-0.52	0.47-0.55	17
F	2.4	0.1	0.02	2.4	2.3-2.48	2.18-2.55	17
G	0.87	0.05	0.01	0.88	0.8-0.9	0.77-0.94	17
H	0.29	0.02	0.004	0.29	0.28-0.3	0.27-0.32	17
L	0.8	0.04	0.01	0.8	0.77-0.83	0.72-0.86	17
R1	0.61	0.04	0.01	0.62	0.6-0.64	0.52-0.67	17
R2	1.91	0.08	0.02	1.91	1.85-1.95	1.76-2.04	17
R3	0.56	0.03	0.01	0.56	0.54-0.59	0.51-0.61	17

Table 7. Measurements taken for morphometrics (in mm): Manabí

Measurements	Mean	SD	SE	Median	Quartiles	Range	n
A	1.31	0.06	0.02	1.33	1.27-1.37	1.2-1.42	17
B	0.53	0.04	0.01	0.53	0.49-0.55	0.44-0.6	17
C	0.89	0.05	0.01	0.88	0.85-0.93	0.79-0.95	17
D	1.69	0.1	0.02	1.71	1.6-1.76	1.45-1.83	17
E	0.61	0.04	0.01	0.6	0.58-0.63	0.53-0.68	17
F	2.97	0.16	0.04	3.01	2.87-3.1	2.57-3.24	17
G	1.2	0.08	0.02	1.2	1.1-1.25	1.03-1.32	17
H	0.34	0.02	0.01	0.33	0.32-0.35	0.32-0.38	17
L	0.86	0.06	0.01	0.87	0.83-0.9	0.73-0.96	17
R1	0.72	0.07	0.02	0.7	0.65-0.78	0.61-0.82	15
R2	2.25	0.1	0.03	2.28	2.2-2.33	1.95-2.35	15
R3	0.65	0.03	0.01	0.65	0.63-0.66	0.6-0.71	15

Table 8. Measurements taken for morphometrics (in mm): Pichincha

Measurements	Mean	SD	SE	Median	Quartiles	Range	n
A	1.42	0.05	0.01	1.43	1.38-1.44	1.32-1.52	15
B	0.52	0.03	0.01	0.52	0.49-0.53	0.47-0.61	15
C	0.96	0.05	0.01	0.96	0.94-1	0.84-1.04	15
D	2.04	0.08	0.02	2.04	2-2.1	1.9-2.22	15
E	0.67	0.05	0.01	0.69	0.63-0.7	0.59-0.75	15
F	3.47	0.14	0.04	3.48	3.36-3.56	3.2-3.72	15
G	1.49	0.08	0.02	1.48	1.4-1.5	1.36-1.68	15
H	0.37	0.02	0.005	0.36	0.35-0.39	0.34-0.4	15
L	0.98	0.05	0.01	0.98	0.94-1.03	0.91-1.06	15
R1	0.82	0.04	0.01	0.82	0.8-0.85	0.75-0.88	15
R2	2.73	0.11	0.03	2.68	2.65-2.8	2.6-2.99	15
R3	0.73	0.04	0.01	0.75	0.7-0.76	0.64-0.77	15

Table 1. Mitochondrial cytochrome *b* haplotypes in *Rhodnius ecuadoriensis*: variable sites within a 663bp gene fragment

Haplotype* \ Site	27	54	57	102	120	123	129	135	165	168	177	189	201	210	219	256	285	291	306	309	312	333	345	357	361	362	378	385	417	429	447	450	462	468	471	480	504	531	543	550	574	648
MN-1♦ [Manabí] (Rec125)	C	T	T	T	A	T	T	T	A	T	C	A	C	T	A	C	C	G	C	A	T	C	A	A	G	T	T	C	C	T	T	T	T	T	A	G	T	T	A	T	C	G
MN-2a [Manabí] (Ind11)	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MN-2b [Manabí] (Rec86)	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MN-3♦ [Manabí] (RecM1)	-	C	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MN-4a [Manabí] (Ind22)	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	T	A	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	G	-	-	-	-
MN-4b [Manabí] (Rec128)	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	T	A	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	G	-	-	-	-
EO [El Oro] (Rec22)	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	T	A	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	G	-	-	-	-
MN-5♦ [Manabí] (Ind14)	-	C	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	G	-	-	-	-	-	-	-	A	-	-	-	-	C	-	G	-	-	-	-	C	-	-
MN-6 [Manabí] (Rec130)	-	-	-	-	-	-	-	-	-	-	-	-	-	T	C	-	-	T	A	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	G	-	-	-	-
PH [Pichincha] (RecP1)	-	-	-	-	-	-	-	-	-	-	-	-	-	T	C	-	-	T	A	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	G	-	-	-	-
LJ-1♦ [Loja] (Rec74)	-	C	C	-	-	-	C	-	-	-	-	-	-	C	-	-	T	A	-	G	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	A	C	G	-	-	-	-
LJ-2 [Loja] (Rec40)	-	C	C	-	-	-	C	-	G	-	-	-	-	C	G	-	-	A	-	G	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	A	-	G	-	-	-	
LJ-3 [Loja] (Rec32)	-	C	C	-	-	C	C	-	G	-	-	-	-	C	G	-	-	A	-	G	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	A	-	G	-	-	-	-
PEa [Peru] (Peru2)	T	-	-	C	-	C	-	A	-	C	T	G	-	C	-	T	-	-	T	-	C	T	G	T	**A**	**C**	**C**	-	T	-	-	C	-	C	-	A	-	A	G	-	T	A
PEb [Peru] (Peru Fiocruz)	T	-	-	C	-	C	-	A	-	C	T	G	-	C	-	T	-	-	T	-	C	T	G	T	**A**	**C**	**C**	-	T	-	-	C	-	C	-	A	-	A	G	-	T	A

*The area of origin of each haplotype is indicated in brackets; identical haplotypes found in different collection sites are presented (haplotype codes with a lower-case letter at the end, EO/MN4a-b, and PH/MN-6); codes in parentheses are examples of individual bugs presenting each cyt*b* haplotype; ♦indicates haplotypes that were unique to single specimens (see below for details); nucleotides in bold type and larger font size are those involved in nonsynonymous substitutions; sites 361 and 385 correspond to first codon positions, and site 362 is the only variable second codon position. There were three segregating bases at position 531 (column with dotted borders), whereas only two were found in the rest of variable sites

Table 2. Nucleotide composition (in %) of 10 *Rhodnius ecuadoriensis* cyt*b* haplotypes

Haplotype	T	A	C	G	Sites
MN-1 (Manabí)	38.2	31.5	17.6	12.7	663
MN-2a,b (Manabí)	38.0	31.5	17.8	12.7	663
MN-3 (Manabí)	38.0	31.4	17.8	12.8	663
MN-4a,b (Manabí) + **EO** (El Oro)	38.0	31.7	17.6	12.7	663
MN-5 (Manabí)	37.6	31.4	18.1	13.0	663
MN-6 (Manabí) + **PH** (Pichincha)	38.2	31.7	17.5	12.7	663
LJ-1 (Loja)	37.3	31.7	18.4	12.7	663
LJ-2 (Loja)	37.3	31.4	18.4	13.0	663
LJ-3 (Loja)	37.1	31.4	18.6	13.0	663
PEa,b (Peru)	37.7	31.7	17.9	12.7	663
Average	37.7	31.5	18.0	12.8	663

Table 3. Nucleotides of cyt*b* haplotypes in *Rhodnius ecuadoriensis*: bases at each codon position (%)

Haplotype	First codon position*				Second codon position*				Third codon position*			
	T	A	C	G	T	A	C	G	T	A	C	G
MN-1	29.0	29.0	19.5	22.6	46.6	19.5	20.8	13.1	38.9	46.2	12.7	2.3
MN-2a,b	29.0	29.0	19.5	22.6	46.6	19.5	20.8	13.1	38.5	46.2	13.1	2.3
MN-3	29.0	29.0	19.5	22.6	46.6	19.5	20.8	13.1	38.5	45.7	13.1	2.7
MN-4a,b + EO	29.0	29.0	19.5	22.6	46.6	19.5	20.8	13.1	38.5	46.6	12.7	2.3
MN-5	28.5	29.4	19.5	22.6	46.6	19.5	20.8	13.1	37.6	45.2	14.0	3.2
MN-6 + PH	29.0	29.0	19.5	22.6	46.6	19.5	20.8	13.1	38.9	46.6	12.2	2.3
LJ-1	29.0	29.0	19.5	22.6	46.6	19.5	20.8	13.1	36.2	46.6	14.9	2.3
LJ-2	29.0	29.0	19.5	22.6	46.6	19.5	20.8	13.1	36.2	45.7	14.9	3.2
LJ-3	29.0	29.0	19.5	22.6	46.6	19.5	20.8	13.1	35.7	45.7	15.4	3.2
PEa,b	29.9	29.4	18.6	22.2	46.2	19.5	21.3	13.1	37.1	46.2	14.0	2.7
Average	29.0	29.0	19.4	22.6	46.6	19.5	20.9	13.1	37.6	46.1	13.7	2.6

*The number of sites at each codon position was 221

Table 4. Absolute number of nucleotide differences (below diagonal) and uncorrected (*p*) distances (above diagonal) between *Rhodnius ecuadoriensis* cyt*b* haplotypes

Haplotype	MN-1	MN-2	MN-3	MN-4	MN-5	PH	LJ-1	LJ-2	LJ-3	PE
MN-1	-	0.00151	0.00302	0.00905	0.01056	0.01056	0.01659	0.01659	0.01810	0.03771
MN-2a,b	1	-	0.00151	0.01056	0.00905	0.01207	0.01508	0.01508	0.01659	0.03922
MN-3	2	1	-	0.01207	0.01056	0.01357	0.01659	0.01659	0.01810	0.04072
MN-4a.b + EO	6	7	8	-	0.01357	0.00151	0.00754	0.01056	0.01207	0.03922
MN-5	7	6	7	9	-	0.01508	0.01810	0.01810	0.01961	0.04525
MN-6 + PH	7	8	9	1	10	-	0.00905	0.01207	0.01357	0.04072
LJ-1	11	10	11	5	12	6	-	0.00905	0.01056	0.04676
LJ-2	11	10	11	7	12	8	6	-	0.00151	0.04676
LJ-3	12	11	12	8	13	9	7	1	-	0.04525
PEa,b	25	26	27	26	30	27	31	31	30	-

Table 5. Jukes-Cantor (below diagonal) and Kimura two-parameter (above diagonal) distances between *Rhodnius ecuadoriensis* cyt*b* haplotypes

Haplotype	MN-1	MN-2	MN-3	MN-4	MN-5	PH	LJ-1	LJ-2	LJ-3	PE
MN-1	-	0.00151	0.00303	0.00912	0.01066	0.01066	0.01685	0.01685	0.01841	0.03904
MN-2a,b	0.00151	-	0.00151	0.01066	0.00912	0.01220	0.01529	0.01529	0.01685	0.04066
MN-3	0.00302	0.00151	-	0.01220	0.01066	0.01374	0.01685	0.01685	0.01841	0.04229
MN-4a.b + EO	0.00910	0.01063	0.01216	-	0.01373	0.00151	0.00760	0.01067	0.01221	0.04072
MN-5	0.01063	0.00910	0.01063	0.01370	-	0.01528	0.01839	0.01839	0.01995	0.04716
MN-6 + PH	0.01063	0.01216	0.01370	0.00151	0.01524	-	0.00913	0.01221	0.01376	0.04235
LJ-1	0.01678	0.01524	0.01678	0.00758	0.01832	0.00910	-	0.00913	0.01067	0.04894
LJ-2	0.01678	0.01524	0.01678	0.01063	0.01832	0.01216	0.00910	-	0.00151	0.04894
LJ-3	0.01832	0.01678	0.01832	0.01216	0.01987	0.01370	0.01063	0.00151	-	0.04729
PEa,b	0.03869	0.04028	0.04187	0.04028	0.04667	0.04187	0.04828	0.04828	0.04667	-

Table 6. Mean between-population distances in *Rhodnius ecuadoriensis* haplotypes: absolute nucleotide differences (below diagonal) and uncorrected (*p*) distances (above diagonal)

Group	Manabí	Pichincha	El Oro	Loja	Peru
Manabí	-	0.007 (0.002)	0.0056 (0.0018)	0.014 (0.0036)	0.04 (0.0076)
Pichincha	4.8 (1.4)	-	0.0015 (0.0014)	0.013 (0.0038)	0.041 (0.0077)
El Oro	3.9 (1.2)	1 (0.9)	-	0.01 (0.0036)	0.039 (0.0078)
Loja	9.4 (2.46)	8.4 (2.6)	7.4 (2.4)	-	0.046 (0.008)
Peru	26.3 (4.78)	27 (4.99)	26 (5)	30.5 (5.35)	-

Numbers in parentheses are standard errors calculated by 500 bootstrap replications

Table 7. Mean between-population distances in *Rhodnius ecuadoriensis* haplotypes: Jukes-Cantor (below diagonal) and Kimura two-parameter distances (above diagonal)

Group	Manabí	Pichincha	El Oro	Loja	Peru
Manabí	-	0.007 (0.0022)	0.0057 (0.0018)	0.014 (0.0037)	0.041 (0.0078)
Pichincha	0.007 (0.002)	-	0.0015 (0.0015)	0.013 (0.004)	0.042 (0.0082)
El Oro	0.0056 (0.002)	0.0015 (0.0014)	-	0.01 (0.0037)	0.041 (0.0081)
Loja	0.014 (0.0037)	0.013 (0.0038)	0.01 (0.0036)	-	0.048 (0.0088)
Peru	0.04 (0.0076)	0.042 (0.0078)	0.04 (0.0079)	0.047 (0.0083)	-

Numbers in parentheses are standard errors calculated by 500 bootstrap replications

Table 8. Mean between-population distances in *Rhodnius ecuadoriensis* haplotypes: absolute nucleotide differences (below diagonal) and uncorrected (*p*) distances (above diagonal)

Group	Manabí	Pichincha	El Oro	Loja	Peru
Manabí	-	0.009 (0.003)	0.008 (0.002)	0.015 (0.003)	0.04 (0.008)
Pichincha	5.83 (1.59)	-	0.002 (0.001)	0.012 (0.003)	0.041 (0.008)
El Oro	5.17 (1.45)	1 (0.93)	-	0.01 (0.003)	0.039 (0.008)
Loja	9.94 (2.16)	7.67 (2.12)	6.67 (1.95)	-	0.046 (0.008)
Peru	26.83 (4.8)	27 (4.91)	26 (4.98)	30.67 (5.2)	-

Numbers in parentheses are standard errors calculated by 500 bootstrap replications

Table 9. Mean between-population distances in *Rhodnius ecuadoriensis* haplotypes: Jukes-Cantor (below diagonal) and Kimura two-parameter distances (above diagonal)

Group	Manabí	Pichincha	El Oro	Loja	Peru
Manabí	-	0.009 (0.003)	0.008 (0.002)	0.015 (0.004)	0.042 (0.008)
Pichincha	0.009 (0.003)	-	0.002 (0.001)	0.012 (0.004)	0.042 (0.008)
El Oro	0.008 (0.002)	0.002 (0.001)	-	0.01 (0.003)	0.041 (0.008)
Loja	0.015 (0.004)	0.012 (0.003)	0.01 (0.003)	-	0.048 (0.009)
Peru	0.042 (0.008)	0.042 (0.008)	0.04 (0.008)	0.048 (0.009)	-

Numbers in parentheses are standard errors calculated by 500 bootstrap replications

APPENDIX TO SECTION 7.2.

Table 1. Basic morphometric statistics: *Rhodnius colombiensis*, *R. pallescens* and *R. pictipes*

	Rhodnius colombiensis **(n=15)**					***Rhodnius pallescens*** **(n=15)**					***Rhodnius pictipes*** **(n=15)**				
	M	SD	MD	MX	MN	M	SD	MD	MX	MN	M	SD	MD	MX	MN
A	1.49	0.06	1.48	1.6	1.42	1.52	0.08	1.51	1.67	1.42	1.66	0.07	1.66	1.8	1.56
B	0.55	0.03	0.55	0.6	0.5	0.55	0.04	0.55	0.62	0.49	0.51	0.04	0.52	0.59	0.4
C	0.91	0.03	0.91	0.96	0.83	0.93	0.07	0.91	1.09	0.83	1	0.05	1	1.08	0.9
D	2.21	0.1	2.21	2.37	1.94	2.3	0.22	2.24	2.72	1.94	2.4	0.1	2.4	2.59	2.26
E	0.65	0.04	0.65	0.7	0.59	0.69	0.07	0.69	0.85	0.59	0.81	0.05	0.81	0.88	0.72
F	3.58	0.13	3.59	3.79	3.28	3.74	0.32	3.68	4.39	3.26	4.09	0.16	4.11	4.34	3.83
G	1.66	0.07	1.67	1.77	1.53	1.74	0.17	1.71	2.1	1.52	1.75	0.06	1.76	1.83	1.66
H	0.37	0.02	0.37	0.41	0.35	0.38	0.03	0.37	0.45	0.35	0.42	0.02	0.42	0.47	0.38
L	0.97	0.04	0.97	1.04	0.87	0.97	0.06	0.97	1.09	0.83	1.11	0.05	1.11	1.21	1.03
R1	0.8	0.04	0.79	0.87	0.75	0.85	0.09	0.83	1.07	0.75	0.97	0.05	0.97	1.05	0.88
R2	2.96	0.1	2.94	3.13	2.74	3.13	0.28	3.03	3.74	2.74	3.44	0.13	3.44	3.63	3.18
R3	0.77	0.03	0.76	0.84	0.72	0.77	0.04	0.76	0.84	0.67	0.76	0.03	0.75	0.82	0.72

M=mean; SD=standard deviation; MD=median; MX=maximum; MN=minimum (measurements: see figure 91)

Table 2. Basic morphometric statistics: *Rhodnius prolixus*, *R. robustus* and *Triatoma infestans*

	Rhodnius prolixus **(n=15)**					***Rhodnius robustus*** **(n=15)**					***Triatoma infestans*** **(n=18)**				
	M	SD	MD	MX	MN	M	SD	MD	MX	MN	M	SD	MD	MX	MN
A	1.63	0.06	1.65	1.72	1.53	1.77	0.12	1.74	2	1.54	2.49	0.1	2.51	2.63	2.27
B	0.57	0.05	0.59	0.65	0.48	0.58	0.05	0.57	0.66	0.48	1.06	0.05	1.06	1.17	0.96
C	0.98	0.03	0.97	1.05	0.93	1.05	0.08	1.04	1.21	0.94	1.29	0.08	1.31	1.39	1.15
D	2.16	0.06	2.15	2.28	2.02	3	0.19	2.95	3.45	2.76	2.61	0.13	2.62	2.85	2.41
E	0.62	0.02	0.62	0.65	0.6	0.77	0.05	0.76	0.87	0.7	0.65	0.06	0.65	0.76	0.57
F	3.59	0.07	3.59	3.73	3.46	4.7	0.31	4.58	5.41	4.32	4.31	0.2	4.28	4.67	4
G	1.62	0.05	1.61	1.7	1.52	2.36	0.17	2.31	2.74	2.17	1.22	0.06	1.21	1.31	1.11
H	0.42	0.02	0.42	0.46	0.38	0.46	0.02	0.46	0.5	0.42	0.47	0.03	0.47	0.52	0.41
L	1	0.05	0.98	1.12	0.95	1.16	0.08	1.16	1.3	1.04	1.4	0.07	1.39	1.52	1.29
R1	0.87	0.05	0.88	0.94	0.8	1.01	0.05	1.01	1.1	0.92	1.27	0.08	1.27	1.42	1.14
R2	2.79	0.08	2.79	2.92	2.63	3.92	0.21	3.87	4.35	3.64	2.56	0.14	2.58	2.74	2.17
R3	0.77	0.04	0.76	0.83	0.71	0.9	0.05	0.9	0.99	0.78	1.29	0.08	1.28	1.44	1.14

M=mean; SD=standard deviation; MD=median; MX=maximum; MN=minimum (measurements: see figure 91)

Note: see **Appendix to Section 6.2.** for measurements and basic statistics of *Rhodnius ecuadoriensis* populations

Table 1. Mitochondrial cytochrome *b* haplotypes in the 'Pacific *Rhodnius* lineage': variable sites and nucleotide substitutions

Ht/s	7	9	27	30	39	42	45	48	51	54	57	66	72	75	81	82	96	99	102	108	120	123	126	129	130	132	135	142	165	168	171	174	177	183	187	189	192	198	201	202	204	205	207	210	214	216	217	219	222	231	234	237	238	243	246	247
PIC-1	G	A	T	A	A	T	A	C	C	G	T	C	G	A	A	T	A	C	T	C	G	T	T	C	C	T	T	T	A	T	T	A	T	C	C	A	C	T	T	G	T	C	C	C	C	C	C	A	A	T	A	T	T	T	A	A
PIC-2	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PALV	-	T	-	-	-	A	C	-	A	A	-	G	-	-	-	-	T	T	-	T	A	-	-	T	T	A	A	C	G	-	-	-	-	-	-	-	-	C	C	-	-	T	A	T	T	A	T	G	C	C	C	C	-	C	G	G
PAL1	-	T	-	-	-	A	C	T	A	A	-	G	-	-	-	-	C	T	-	-	A	-	-	T	T	-	A	C	G	C	-	-	-	-	-	-	-	C	C	-	-	T	A	T	T	A	T	G	C	C	C	C	-	C	G	G
PAL2	-	T	-	G	G	A	C	-	A	A	-	G	A	G	G	C	T	T	-	-	-	-	-	T	T	A	A	C	-	-	-	-	-	T	-	-	-	C	C	A	C	T	A	T	T	A	T	G	C	C	C	C	-	C	-	G
PAL-N	-	T	-	-	G	A	C	-	A	A	-	A	A	-	G	C	T	T	-	T	-	-	-	T	T	A	A	C	-	-	-	-	-	T	-	-	-	C	C	-	-	T	A	T	T	A	T	-	C	C	C	C	-	C	-	G
COL	-	T	-	-	-	A	C	T	A	A	-	G	A	-	-	C	T	T	-	T	A	-	-	T	T	-	A	C	-	-	-	-	-	T	-	-	-	C	C	-	-	T	A	T	T	A	T	-	C	C	C	C	-	C	-	G
MN1	A	T	C	-	-	A	C	T	T	T	-	A	C	-	-	-	T	A	-	T	A	-	C	T	-	A	-	C	-	-	C	T	C	-	T	-	T	C	C	-	C	-	A	T	T	A	T	-	T	-	T	C	C	-	-	G
MN2	A	T	C	-	-	A	C	T	T	C	-	A	C	-	-	-	T	A	-	T	A	-	C	T	-	A	-	C	-	-	C	T	C	-	T	-	T	C	C	-	C	-	A	T	T	A	T	-	T	-	T	C	C	-	-	G
MN-3	A	T	C	-	-	A	C	T	T	C	-	A	C	-	-	-	T	A	-	T	-	-	C	T	-	A	-	C	-	-	C	T	C	-	T	-	T	C	C	-	C	-	A	T	T	A	T	-	T	-	T	C	C	-	-	G
MN4/EO	A	T	C	-	-	A	C	T	T	T	-	A	C	-	-	-	T	A	-	T	A	-	C	T	-	A	-	C	-	-	C	T	C	-	T	-	T	C	C	-	C	-	A	-	T	A	T	-	T	-	T	C	C	-	-	G
MN5	A	T	C	-	-	A	C	T	T	C	-	A	C	-	-	-	T	A	-	T	A	-	C	T	-	A	-	C	-	-	C	T	C	-	T	-	T	C	C	-	C	-	A	-	T	A	T	-	T	-	T	C	C	-	-	G
MN-6/PH	A	T	C	-	-	A	C	T	T	T	-	A	C	-	-	-	T	A	-	T	A	-	C	T	-	A	-	C	-	-	C	T	C	-	T	-	T	C	-	-	C	-	A	-	T	A	T	-	T	-	T	C	C	-	-	G
LJ1	A	T	C	-	-	A	C	T	T	C	C	A	C	-	-	-	T	A	-	T	A	-	C	-	-	A	-	C	-	-	C	T	C	-	T	-	T	C	C	-	C	-	A	-	T	A	T	-	T	-	T	C	C	-	-	G
LJ2	A	T	C	-	-	A	C	T	T	C	C	A	C	-	-	-	T	A	-	T	A	-	C	-	-	A	-	C	G	-	C	T	C	-	T	-	T	C	C	-	C	-	A	-	T	A	T	G	T	-	T	C	C	-	-	G
LJ3	A	T	C	-	-	A	C	T	T	C	C	A	C	-	-	-	T	A	-	T	A	C	C	-	-	A	-	C	G	-	C	T	C	-	T	-	T	C	C	-	C	-	A	-	T	A	T	G	T	-	T	C	C	-	-	G
PE	A	T	-	-	-	A	C	T	T	T	-	A	C	-	-	-	T	A	C	T	A	C	C	T	-	A	A	C	-	C	C	T	-	-	T	G	T	C	C	-	C	-	A	-	T	A	T	-	T	-	T	C	C	-	-	G

Table 1 (continued)

Ht/s	249	252	253	255	256	264	270	276	279	281	282	285	288	289	291	294	295	304	306	307	309	312	318	324	327	333	339	345	348	349	354	357	361	362	369	378	381	385	387	399	402	405	407	411	412	417	420	429	432	435	438	441
PIC-1	T	T	T	A	T	G	A	A	T	A	T	T	C	T	A	A	C	G	T	C	A	C	T	C	C	C	T	A	C	T	A	A	A	T	C	T	C	T	A	A	A	A	A	C	C	A	T	T	T	C	G	G
PIC-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PALV	A	C	C	-	-	A	-	-	C	-	-	-	T	A	C	G	T	-	-	T	-	T	C	-	T	-	-	-	T	C	-	-	-	-	T	-	T	C	T	G	G	C	G	T	-	-	C	-	C	T	A	A
PAL1	G	C	C	-	-	A	-	-	C	-	-	-	T	A	C	-	T	-	-	T	-	T	C	-	T	-	-	-	T	C	-	-	-	-	T	-	T	C	T	G	G	C	G	T	-	-	C	-	C	T	A	A
PAL2	A	C	C	-	-	A	-	G	-	-	C	C	T	A	T	G	T	-	-	T	-	T	C	-	-	-	-	-	-	C	G	-	-	-	T	-	T	C	T	G	G	C	G	-	-	-	-	-	-	-	A	A
PAL-N	A	C	C	-	-	A	-	G	-	-	-	-	T	A	T	G	T	-	-	T	-	T	C	-	T	-	-	-	-	C	-	-	-	-	T	-	T	C	T	G	-	C	G	-	-	-	-	-	-	T	A	A
COL	A	C	-	-	-	A	G	-	-	G	-	C	A	A	-	-	-	-	-	T	-	T	C	-	T	-	-	-	-	C	-	-	-	-	T	-	T	C	T	G	-	C	G	-	-	-	C	-	C	T	A	A
MN1	A	C	C	C	C	A	-	T	-	-	-	C	-	C	G	-	T	A	C	T	-	T	C	T	-	-	A	-	T	C	-	-	G	-	T	-	T	C	T	-	-	-	G	T	T	C	-	-	-	T	A	A
MN2	A	C	C	C	C	A	-	T	-	-	-	C	-	C	G	-	T	A	C	T	-	T	C	T	-	-	A	-	T	C	-	-	G	-	T	-	T	C	T	-	-	-	G	T	T	C	-	-	-	T	A	A
MN-3	A	C	C	C	C	A	-	T	-	-	-	C	-	C	G	-	T	A	C	T	-	T	C	T	-	-	A	-	T	C	-	-	G	-	T	-	T	C	T	-	-	-	G	T	T	C	-	-	-	T	A	A
MN4/EO	A	C	C	C	C	A	-	T	-	-	-	-	-	C	-	-	T	A	C	T	G	T	C	T	-	-	A	-	T	C	-	-	G	-	T	-	T	C	T	-	-	-	G	T	T	C	-	-	-	T	A	A
MN5	A	C	C	C	C	A	-	T	-	-	-	C	-	C	G	-	T	A	C	T	G	T	C	T	-	-	A	-	T	C	-	-	G	-	T	-	T	A	T	-	-	-	G	T	T	C	-	-	-	T	A	A
MN-6/PH	A	C	C	C	C	A	-	T	-	-	-	-	-	C	-	-	T	A	C	T	G	T	C	T	-	-	A	-	T	C	-	-	G	-	T	-	T	C	T	-	-	-	G	T	T	C	-	-	-	T	A	A
LJ1	A	C	C	C	C	A	-	T	-	-	-	-	-	C	-	-	T	A	C	T	G	T	C	T	-	-	A	-	T	C	-	-	G	-	T	-	T	C	T	-	-	-	G	T	T	C	-	-	-	T	A	A
LJ2	A	C	C	C	C	A	-	T	-	-	-	C	-	C	-	-	T	A	C	T	G	T	C	T	-	-	A	-	T	C	-	-	G	-	T	-	T	C	T	-	-	-	G	T	T	C	-	C	-	T	A	A
LJ3	A	C	C	C	C	A	-	T	-	-	-	C	-	C	-	-	T	A	C	T	G	T	C	T	-	-	A	-	T	C	-	-	G	-	T	-	T	C	T	-	-	-	G	T	T	C	-	C	-	T	A	A
PE	A	C	C	C	-	A	-	T	-	-	-	C	-	C	G	-	T	A	-	T	-	-	C	T	-	T	A	G	T	C	-	T	-	C	T	C	T	C	T	-	-	-	G	T	T	T	-	-	-	T	A	A

Table 1 (continued)

Ht/s	444	447	450	451	453	456	462	468	471	480	483	486	495	498	501	504	516	522	531	534	537	540	543	546	549	550	552	559	567	568	569	570	573	574	576	589	590	600	603	609	615	618	621	622	623	627	631	633	634	639	644	646	648	649	651	652	654	657	658	661
PIC-1	T	A	T	G	A	A	T	T	A	A	T	T	T	C	T	T	T	C	A	G	A	C	T	A	T	C	T	T	C	G	C	A	T	C	C	G	T	T	A	G	T	C	T	G	C	C	A	T	C	C	C	T	A	A	C	G	C	A	T	T
PIC-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-
PALV	-	C	-	-	T	T	C	-	-	-	-	-	C	-	-	-	-	T	-	A	T	T	C	-	A	T	-	C	T	-	T	T	-	-	G	A	A	-	-	A	-	T	-	-	T	T	G	A	T	T	G	C	-	G	T	T	T	T	-	C
PAL1	-	C	-	-	T	T	C	-	-	-	C	-	C	-	-	-	-	T	-	A	T	T	C	-	A	T	-	C	-	-	T	T	-	-	G	A	A	-	-	A	-	-	-	-	T	-	G	A	T	T	G	C	-	G	T	T	T	T	-	C
PAL2	C	C	-	A	C	T	C	-	-	-	-	-	C	-	-	-	-	T	-	A	T	T	C	G	A	T	-	C	T	A	T	T	C	-	A	A	A	C	-	A	-	-	-	-	T	-	G	A	T	T	G	C	-	G	T	T	T	T	-	-
PAL.N	C	C	-	-	C	T	C	-	-	-	-	-	C	-	-	-	-	T	-	A	T	T	C	-	A	T	-	-	T	-	T	T	-	T	A	A	A	C	-	A	-	-	-	-	T	-	G	A	T	T	G	C	-	-	T	T	T	T	-	-
COL	-	C	-	-	T	C	C	-	-	-	-	C	C	-	C	-	-	T	G	A	T	T	-	G	A	T	-	-	T	A	T	T	-	-	A	A	C	C	-	A	-	-	-	-	A	T	T	-	C	T	T	G	C	-	G	T	T	-	T	-	-
MN1	C	T	-	-	T	T	-	-	-	G	-	C	-	T	-	-	C	T	T	A	C	T	A	-	A	T	A	-	T	-	T	T	-	-	A	A	C	C	G	-	-	-	A	A	T	T	G	-	-	T	G	-	G	G	T	T	T	T	C	-
MN2	C	T	-	-	T	T	-	-	-	G	-	C	-	T	-	-	C	T	T	A	C	T	A	-	A	T	A	-	T	-	T	T	-	-	A	A	C	C	G	-	-	-	A	A	T	T	G	-	-	T	G	-	G	G	T	T	T	T	C	-
MN-3	C	T	-	-	T	T	-	-	-	G	-	C	-	T	-	-	C	T	T	A	C	T	A	-	A	T	A	-	T	-	T	T	-	-	A	A	C	C	G	-	-	-	A	A	T	T	G	-	-	T	G	-	G	G	T	T	T	T	C	-
MN4/EO	C	T	-	-	T	T	-	-	-	-	-	C	-	T	-	-	C	T	G	A	C	T	A	-	A	T	A	-	T	-	T	T	-	-	A	A	C	C	G	-	-	-	A	A	T	T	G	-	-	T	G	-	G	G	T	T	T	T	C	-
MN5	C	T	-	-	T	T	C	-	G	G	-	C	-	T	-	-	C	T	T	A	C	T	A	-	A	-	A	-	T	-	T	T	-	-	A	A	C	C	G	-	-	-	A	A	T	T	G	-	-	T	G	-	G	G	T	T	T	T	C	-
MN-6/PH	C	T	-	-	T	T	-	-	-	-	-	C	-	T	-	-	C	T	G	A	C	T	A	-	A	T	A	-	T	-	T	T	-	-	A	A	C	C	G	-	-	-	A	A	T	T	G	-	-	T	G	-	G	G	T	T	T	T	C	-
LJ1	C	C	-	-	T	T	-	-	-	-	-	C	-	T	-	C	C	T	G	A	C	T	A	-	A	T	A	-	T	-	T	T	-	-	A	A	C	C	G	-	-	-	A	A	T	T	G	-	-	T	G	-	G	G	T	T	T	T	C	-
LJ2	C	T	-	-	T	T	-	-	-	-	-	C	-	T	-	-	C	T	G	A	C	T	A	-	A	T	A	-	T	-	T	T	-	-	A	A	C	C	G	-	-	-	A	A	T	T	G	-	-	T	G	-	G	G	T	T	T	T	C	-
LJ3	C	T	-	-	T	T	-	-	-	-	-	C	-	T	-	-	C	T	G	A	C	T	A	-	A	T	A	-	T	-	T	T	-	-	A	A	C	C	G	-	-	-	A	A	T	T	G	-	-	T	G	-	G	G	T	T	T	T	C	-
PE	C	T	C	-	T	T	-	C	-	-	-	C	-	T	-	-	C	T	-	A	C	T	G	-	A	T	A	-	T	-	T	T	-	T	A	A	C	C	G	-	-	-	A	A	T	T	G	-	-	T	G	-	-	G	T	T	T	T	C	-

HT=haplotype (code, see text for details on each haplotype), s=sites in the 663bp cyt*b* fragment analysed

Between-group comparisons

Table 2. Absolute number of nucleotide differences (below diagonal) and uncorrected (*p*) distances (above diagonal): mean between-species values

Species	*R. pictipes*	*R. pallescens*	*R. colombiensis*	*R. ecuadoriensis*
R. pictipes	-	0.144 (0.012)	0.14 (0.013)	0.16 (0.013)
R. pallescens	95.75 (8)	-	0.058 (0.007)	0.12 (0.012)
R. colombiensis	93 (8.5)	38.25 (5.2)	-	0.115 (0.013)
R. ecuadoriensis	106.2 (9)	79.3 (7.9)	76.3 (8.4)	-

Values in parentheses are standard errors calculated by 500 bootstrap replications

Table 3. Jukes-Cantor (below) and Kimura 2-parameter distances (above): mean between-species values

Species	*R. pictipes*	*R. pallescens*	*R. colombiensis*	*R. ecuadoriensis*
R. pictipes	-	0.164 (0.016)	0.159 (0.017)	0.184 (0.018)
R. pallescens	0.16 (0.015)	-	0.061 (0.008)	0.133 (0.015)
R. colombiensis	0.115 (0.016)	0.06 (0.008)	-	0.128 (0.015)
R. ecuadoriensis	0.18 (0.017)	0.13 (0.014)	0.125 (0.015)	-

Values in parentheses are standard errors calculated by 500 bootstrap replications

Table 4. Tamura 3-parameter (below) and Tamura-Nei distances (above): mean between-species values

Species	*R. pictipes*	*R. pallescens*	*R. colombiensis*	*R. ecuadoriensis*
R. pictipes	-	0.168 (0.017)	0.163 (0.018)	0.191 (0.019)
R. pallescens	0.166 (0.016)	-	0.061 (0.008)	0.136 (0.016)
R. colombiensis	0.161 (0.017)	0.061 (0.008)	-	0.131 (0.017)
R. ecuadoriensis	0.187 (0.019)	0.135 (0.016)	0.13 (0.016)	-

Values in parentheses are standard errors calculated by 500 bootstrap replications

Table 5. Uncorrected (below) and Kimura 2-parameter distances (above): mean between-species values

Species	*R. ecu*	*R. col*	*R. pall*	*R. pict*	*Rh.* sp.	*R. nas*	*R. prlx*	*R. rob*
R. ecu	-	0.128 (0.016)	0.133 (0.015)	0.183 (0.018)	0.227 (0.019)	0.226 (0.018)	0.221 (0.018)	0.229 (0.019)
R. col	0.115 (0.012)	-	0.061 (0.008)	0.159 (0.017)	0.204 (0.019)	0.23 (0.02)	0.218 (0.019)	0.219 (0.019)
R. pall	0.12 (0.012)	0.057 (0.007)	-	0.162 (0.016)	0.225 (0.018)	0.239 (0.019)	0.226 (0.018)	0.219 (0.017)
R. pict	0.159 (0.014)	0.14 (0.013)	0.143 (0.012)	-	0.211 (0.019)	0.223 (0.021)	0.213 (0.02)	0.22 (0.02)
Rh. sp.	0.193 (0.013)	0.176 (0.014)	0.192 (0.014)	0.181 (0.014)	-	0.143 (0.016)	0.121 (0.014)	0.113 (0.013)
R. nas	0.193 (0.014)	0.196 (0.015)	0.202 (0.014)	0.19 (0.014)	0.127 (0.012)	-	0.098 (0.013)	0.1 (0.012)
R. prlx	0.189 (0.014)	0.187 (0.014)	0.193 (0.014)	0.182 (0.014)	0.109 (0.011)	0.09 (0.011)	-	0.064 (0.009)
R. rob	0.195 (0.014)	0.188 (0.014)	0.188 (0.013)	0.187 (0.014)	0.102 (0.01)	0.092 (0.01)	0.06 (0.008)	-

R. ecu=Rhodnius ecuadoriensis; *R. col=R. colombiensis*; *R. pall=R. pallescens*; *R. pict=R. pictipes*; *Rh.* sp.=*Rhodnius* sp., collected in Sucumbios, Ecuador; *R. nas=R. nasutus*, *R. prlx=R. prolixus*, *R. rob=R. robusuts*. Numbers in parentheses are standard errors calculated by 500 bootstrap replications

Tajima's relative rate tests

Tajima's relative rate tests were conducted to investigate rate heterogeneities between pairs of sequences within a 27-haplotype alignment. For each comparison, an adequate outgroup sequence was selected (for instance, one *R. pictipes* haplotype for comparisons among members of the Pacific lineage; one *R. prolixus* for *pictipes* vs. Pacific clade species; or *T. rubrovaria* for comparisons among members of the two main clades defined by phylogenetic analyses [the (*pictipes*-Pacific clade) vs. the (*robustus-prolixus-nasutus*) branches]). All significance probability values were well above 0.05, suggesting that, for the level of divergence of the *Rhodnius* species studied here, the mt cyt*b* gene evolves in a clocklike manner. One exception was detected in the comparisons of *R. pictipes* with *R. pallescens* and *R. colombiensis* when only transversional changes were used for the tests. In these cases, the Colombian species seemed to be evolving at a faster rate; the difference was statistically significant (p=0.02) for *pictipes* vs. *pallescens*, and marginally non-significant (p=0.06) for *pictipes* vs. *colombiensis*. These and other examples of pairwise comparisons (relevant to the phylogeny of *R. ecuadoriensis* and related species) are presented in the following table.

Table 6. Tajima's relative rate tests between *Rhodnius* species

Haplotype pair	Outgroup	AS: X^2 (p)***	TS: X^2 (p)***	TV: X^2 (p)***
PIC-1 vs. PAL-1	*Rhodnius prolixus*	0.49 (0.49)	0.29 (0.59)	5.56 (0.02)
PIC-1 vs. COL	*Rhodnius prolixus*	0.05 (0.8)	0.6 (0.44)	3.56 (0.06)
PIC-1 vs. MN-4	*Rhodnius prolixus*	0.29 (0.59)	0.02 (0.9)	1.64 (0.2)
PAL-1 vs. COL	*Rhodnius pictipes*	0.03 (0.8)	0 (1)	1 (0.32)
PAL-1 vs. MN-4	*Rhodnius pictipes*	1.5 (0.2)	0.47 (0.49)	1.9 (0.17)
COL vs. MN-4	*Rhodnius pictipes*	1.9 (0.17)	0.49 (0.49)	3 (0.08)
MN-4 vs. *Rhodnius* sp.*	*Triatoma rubrovaria*	0.04 (0.85)	0.24 (0.63)	0.11 (0.75)
MN-4 vs. *R. robustus* Napo**	*Triatoma rubrovaria*	1.13 (0.29)	1.2 (0.27)	0.1 (0.75)
PIC-1 vs. *R. robustus* Napo**	*Triatoma rubrovaria*	0.5 (0.48)	0.06 (0.8)	0.8 (0.37)
PAL-1 vs. *R. robustus* Napo**	*Triatoma rubrovaria*	0.24 (0.6)	0.58 (0.45)	0.02 (0.88)
COL vs. *R. robustus* Napo**	*Triatoma rubrovaria*	0.34 (0.56)	0.24 (0.6)	0.1 (0.75)

AS=all sites; TS=transitions; TV=transversions; PIC-1=*R. pictipes*; PAL-1=*R. pallescens*; COL=*R. colombiensis*; MN-4=*R. ecuadoriensis*; *collected in Sucumbíos, Ecuador (phenotypically similar to *R. robustus*); **provided by FA Monteiro (specimen originally collected by RU Carcavallo in a palm tree in Napo, Ecuador); ***p values for 1 df. See text for further details on haplotypes and specimens

PUBLICATIONS

Epidemiology of Chagas Disease in Ecuador. A Brief Review

H Marcelo Aguilar V//+, Fernando Abad-Franch/*, José Racines V**, Aura Paucar C**

Instituto 'Juan César García', Fundación Internacional de Ciencias Sociales y Salud, Casilla Postal 17-1106292 Quito, Ecuador *Pathogen Molecular Biology and Biochemistry Unit, Department of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK **Instituto Nacional de Higiene y Medicina Tropical 'Leopoldo Izquieta Pérez', Quito, Ecuador

Chagas disease is a complex public health problem that has been underestimated in Ecuador. Here we review the relevant published information, and present unpublished and new data that help to understand the current Chagas disease epidemiological situation and its evolution in the country. Three main characteristics have been identified: (i) persistence of Trypanosoma cruzi *transmission in already known foci; (ii) a marked endemicity in some urban areas of Guayaquil; and (iii) the transformation of new Amazon foci into truly endemic areas. The situation in other suspect areas remains uncertain. Five Triatominae species have been implicated in the transmission of* T. cruzi *to people in Ecuador* (Triatoma dimidiata, Rhodnius ecuadoriensis, R. pictipes, R. robustus *and* Panstrongylus geniculatus)*, but some others may also play a role in some areas* (P. rufotuberculatus, P. howardi, T. carrioni *and* P. chinai). *Other Triatominae reported seem to have little or no epidemiological relevance* (T. venosa, T. dispar, Eratyrus mucronatus, E. cuspidatus, P. lignarius *and* Cavernicola pilosa). *High frequency of acute cases and severe chronic disease has been observed. Although cardiomyopathy is more frequent, serious digestive disease is also present. It is estimated that around 120,000-200,000 people may be infected. 2.2 to 3.8 million people are estimated to live under transmission risk conditions.*

Key words: Chagas disease - Ecuador - epidemiology - Triatominae - prevalence - clinical traits - review

The current situation of Chagas disease in Ecuador is the subject of various ongoing epidemiological, entomological and clinical studies. With the aim of summarising the published information available and recent or unpublished data that may be remarkably helpful for researchers and control agents, we undertook a critical review about these crucial topics. Our purpose is to contribute to set the scientific basis necessary to the National Control Programme currently in preparation in Ecuador.

It is estimated that 2.24 to 3.8 million people in all, from a total population of around 11 million, are exposed to the risk of *Tripanosoma cruzi* transmission. These estimates indicate that 120,000-200,000 people would be infected, with chagasic cardiopathy as the dominant chronic form (Aguilar & Yépez 1996). Previous estimates suggested that only 30,000 people were infected (UNDP/World Bank/WHO TDR 1997).

HISTORICAL OVERVIEW

Some archaeological, pre-Columbian findings from the province of Manabí suggest that Romaña's sign was already known in those areas before the arrival of the Europeans to the coastal region of Ecuador (cf. Alvarez 1984). During the Spanish conquest, some of the Pizarro soldiers suffered from a disease they described as "eye sickness" acquired at the Portoviejo valley in Manabí around 1530. The descriptions resemble the characteristic lesions of Romaña's sign, and Álvarez (1984) attributes them to Chagas disease. This is also consistent with the high frequency of acute forms later recorded in the area.

Stal and Whymper (cf. Campos 1923) reported the presence of the principal vector, *Triatoma dimidiata*, in the Ecuadorian coastal region in the last century. In 1917, Tamayo established the association between the insect bite and a clinical picture including local inflammation, oedema and fever (Valenzuela 1939).

This work received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), grant no. 970195, and benefited from international support through the ECLAT Network. Dr Fernando Abad-Franch received funds from a Research Grant awarded by the Tropical Medicine Unit at the General University Hospital, University of Valencia, Spain.

+Corresponding author. Fax:+593-2-455797. E-mail: fijcg@uio.telconet.net

Received 9 June 1999
Accepted 9 August 1999

Arteaga (1930) studied the existence of American trypanosomiasis in the zone of the Coastal Railroad (Guayaquil-Salinas). In 1927 Arteaga verified the presence of human infection and triatomine bugs in the area of Santa Elena, and the following investigations certified that Chagas disease was endemic in the urban area of Guayaquil, with *T. dimidiata* colonies breeding within the cane and wood houses. The Santa Ana and El Carmen hills, two urban areas of Guayaquil, were the most strongly affected, and they seem to remain so nowadays.

During the 40s and 50s new disease foci were reported from the provinces of Guayas, Manabí, Los Ríos, and in temperate areas of the Andean provinces of Loja, Azuay and Bolívar. It is today accepted that the main endemic areas are located in the provinces of Guayas, Manabí and El Oro. But new foci reported from the Amazon region and currently under investigation (Amunárriz et al. 1991, Amunárriz 1991, Chico et al. 1997, Abad-Franch 1998, Abad-Franch et al. 1998a,b) strongly suggest that the northern Ecuadorian Amazon basin is to be considered an endemic area as well. The lack of systematic studies in other provinces makes it complicated to assert that the disease is not endemic in areas (e.g., in the provinces of Los Ríos, Esmeraldas, Pastaza, Loja, Imbabura, Pichincha, Azuay, etc.) where ecological and socioeconomic traits are quite similar to those of well-known chagasic zones.

TRIATOMINE VECTORS (HEMIPTERA: REDUVIIDAE)

The main vector species of Chagas disease in the Pacific slope of the Ecuadorian Andes are *T. dimidiata* and *Rhodnius ecuadoriensis*. *T. dimidiata* can be found in human dwellings in the provinces of Guayas, Manabí, Los Ríos, and El Oro (Lent & Wygodzinsky 1979, Defranc 1982, Lazo 1985). A recent observation includes the province of Loja (Abad-Franch et al., unpublished). *R. ecuadoriensis* has been reported from Manabí, Guayas, Loja and El Oro (Defranc 1982, Lazo 1985, Romaña et al. 1994), but our observations indicate that sylvatic forms of this species can be found in subtropical valleys of the province of Pichincha (Abad-Franch et al., unpublished), probably in relation to *Phytelephas* palm trees, as reported by Romaña et al. (1994) in other areas.

Three triatomine species seem to be involved in the Amazon basin foci: *R. pictipes*, *R. robustus* and *Panstrongylus geniculatus* (Espinoza 1955, Amunárriz 1991, Amunárriz et al. 1991, Chico et al. 1997, Abad-Franch et al. 1998a,b, Zabala unpublished). These five species may therefore be considered as the ones that actually transmit *T. cruzi* to people in the well-characterised endemic areas. The apparent trend to establish domestic and/or peridomestic colonies observed in the last three species in some areas of the Amazon basin (Barrett 1988, Amunárriz, 1991, Chico et al. 1997, Valente et al. 1998) is particularly worrying. However, adults have shown their capacity to fly into the houses to feed during the night from their breeding sites (palm trees, bromeliaceae, mammals burrows), without establishing permanent colonies within human-related structures (Lent & Wygodzinsky 1979, Miles et al. 1981, Barrett 1988, Schofield 1994). Some environmental changes introduced by man during the last 20 years seem to play a role in this process: colonisation of primary rainforest, deforestation, hunting, agriculture, breeding of domestic animals near the houses or the introduction of electric light may be factors involved (Abad-Franch 1998). *P. geniculatus* distribution in Ecuador is probably broader that reported (Manabí, Imbabura, Napo, Sucumbíos and Pichincha) (Rodríguez 1959, Defranc 1982, Amunárriz 1991, Amunárriz et al. 1991, Chico et al. 1997, Zabala unpublished); we studied several specimens belonging to this species from Quinindé (province of Esmeraldas), a zone where no previous reports indicate its presence (Abad-Franch et al., unpublished observations).

Other species seem to have some epidemiological significance in smaller areas. *T. carrioni* is a semidomestic species from southern Ecuador (León 1949, Espinoza 1955, Lent & Wygodzinsky 1979, Defranc 1982, Reyes 1992); nevertheless, recent observations indicate that its distribution may be significantly broader, reaching the subtropical valleys near Quito, in the province of Pichincha (Abad-Franch et al., unpublished observations).

P. rufotubercutalus can be found near or inside houses in the provinces of El Oro, Manabí and Loja (Defranc 1982, Lazo 1985, Reyes 1992, Zabala unpublished, Racines et al. unpublished). We have identified two adult specimens from the province of Pichincha; its presence in Guayas needs further investigation. This species seems to be adapting to human habitats in Bolivia, and it may be able to colonise dwellings after *T. infestans* eradication by spraying (Noireau et al. 1994).

P. howardi is considered to be of Ecuadorian origin (Lent & Wygodzinsky 1979, Defranc 1982). Its distribution seems to be very limited (in the province of Manabí), but few studies have been conducted in relation to this species. Our observations confirm that it is not uncommon to find adult specimens within human dwellings, and that misidentifications with *T. dimidiata* are not a rare event (even by trained personnel linked to the vector control service). These species, belonging to

different genera, share their general chromatic pattern and are of similar size. The actual vectorial role of this species at the local level needs to be established in Manabí.

Other species present in Ecuador and potentially involved in *T. cruzi* transmission are *T. venosa,* found to breed inside houses in some areas of Colombia (D'Alessandro & Barreto 1985), *Eraturys mucronatus*, a species able to invade and colonise human environments (Lent & Wygodzinsky 1979, D'Alessandro & Barreto 1985, Noireau et al. 1995) and *P. chinai* (Lent & Wygodzinsky 1979, Defranc 1982, Reyes 1992).

Cavernicola pilosa, *T. dispar* and *Panstrongylus lignarius*, apparently with no epidemiological importance, have been reported from the Amazon basin (Rodríguez 1961, Lent & Wygodzinsky 1979, Defranc 1987, Abad-Franch et al. 1998a, Zabala unpublished). We recently identified one adult male apparently belonging to the species *P. herreri* captured in the Amazon basin (Abad-Franch et al. in prep.). *E. cuspidatus* has also been reported from Ecuador (cf. Defranc 1982). Our studies indicate that *R. stali* (Lent et al. 1993) is not present in the Ecuadorian Amazon region. Reports indicating the presence of *R. prolixus* in El Oro and Loja, Manabí or Napo/Orellana are doubtful (Defranc 1982, Cueva & Romero 1987) and probably due to misidentification. Equally dubious is the record of *T. infestans* in Esmeraldas and Imbabura (cf. Defranc 1982).

HUMAN INFECTION

The *T. cruzi* infection prevalence rates in various areas of the country, as reported in different studies, are reviewed in Table, together with the authors, date and techniques used.

The interpretation of these data suggests that the main endemic areas, where the disease is still being actively transmitted, correspond to the provinces of El Oro in the southern coastal region, and Guayas and Manabí in central and northern Pacific coast. The northern Amazon region, including the provinces of Sucumbíos, Napo and Orellana, should be included in the list of endemic areas. This zone is characterised by intensive migratory pressure linked to petrol exploitation and subsequent colonisation. The possibility that the environmental changes introduced during the last 20 years might have favoured Chagas disease transmission in the area needs further investigation (Abad-Franch 1998). As mentioned, the situation in other provinces remains uncertain, but their common traits make us think that, at least in some zones, the disease may be present as well. Migration from endemic areas to the city of Guayaquil and the northern Amazon is very important from the early 70s; this trend may also play a role in the epidemiological pattern of these areas (Aguilar & Yépez 1996); the introduction of parasite strains from the coast needs further investigation, as all data indicate that vectors have not been passively transported to the area.

Grijalva et al. (1997, 1998) report a prevalence of 0.02% positives for *T. cruzi* infection among blood donors at the Red Cross Blood Bank in Quito, and 0.13% in samples from other provinces. In a previous report, Grijalva et al. (1995) reported prevalences of 12.1% and 6.1% positives in two collections from the Red Cross Blood Bank in Quito (345 samples in total, analysed by ELISA plus Western Blotting).

CLINICAL FEATURES

Acute clinical disease - Historical data show that from the early 20s it was not uncommon that clinical pictures compatible with the Romaña's sign were diagnosed at hospitals in Guayaquil. Varas (1942) indicate that this form of periorbital oedema was extremely frequent in the city. Subsequent studies continued to show this trait (Espinoza 1955, Rodríguez 1961, 1963, Gómez 1968, Rassi 1979, Álvarez 1984). In a recent series, Galindo (unpublished data) registered 560 acute cases from the records of the National Institute of Hygiene and Tropical Medicine. These cases are in the majority from the provinces of Guayas, Manabí, El Oro and Los Ríos, all in the coastal region (Galindo, pers. comm.). We studied a series of five acute cases from the northern Amazon in 1994 (unpublished data); all of them were children under nine with fever, generalised oedema, hepatosplenomegaly and signs of myocarditis.

Chronic chagas disease - Both heart and digestive forms of the disease have been reported from Ecuador. Galindo (1958, 1959) found chagasic etiology in 20% of 150 cardiac patients in Guayaquil. 20.68% of positives were under 40 years old and presented cardiopathy stage VI following the WHO/PAHO criteria (1974). More than 50% of those patients died in the next 15 months. Gómez (1968) found electrocardiographic signs compatible with chagasic cardiopathy in 1.4% of randomly selected, apparently healthy people. Kawabata et al. (1987) reported that 40% of 154 seropositives from El Oro and Guayas presented typical electrocardiographic abnormalities. In a series of 25 chagasic heart patients, we found that 53% met the WHO/PAHO criteria for cardiopathy stage I, and 47% for stage II and III (unpublished data).

Digestive forms are estimated to represent around 3% of chronic Chagas disease cases, and seem to be mainly from El Oro (Galindo, unpub-

TABLE

Epidemiological evolution of human *Trypanosoma cruzi* infection in Ecuador

Author, year, technique	Province	Locality	Positives	Observations
Montalván J 1950	El Oro	Zaruma	29%	696 samples examined
Complement fixation	El Oro	Machala	13.3%	
	Guayas	Gnal Vernaza	3.1%	
	Guayas	Salitre	11.8%	
	Manabí	Portoviejo	3.8%	
	Manabí	Chone	5.8%	
INH (1949-1957) (cf. Rodríguez JD 1959) Complement fixation	Coastal Region (all provinces)	Various	13.9%	3,333 samples examined Positives: >80% born in Coastal Region >10% born in the province of Loja
Espinoza L 1955	El Oro	Various	8.2%	Survey to schoolchildren in rural areas and in urban Guayaquil
Complement fixation	Guayas	Various	3.5%	
	Guayas	Guayaquil (urban)	1.9%	
	Loja	Various	2%	
	Los Ríos	Various	1.5%	
Rodríguez JD 1959	Guayas	Guayaquil	24% (GP)	GP = General Population; SC = School Children
Complement fixation	Guayas	Various	4% (SC)	
	El Oro	Various	7.6% (SC)	
	El Oro	Machala	7% (GP)	
	Manabí	Portoviejo	4% (GP)	
	Manabí	Bahía	3% (GP)	
	Loja	Various	2% (SC)	
	Esmeraldas	Various	4% (GP)	
	Los Ríos	Various	1.5% (SC)	
Gómez LLF 1968 INH (1962-1967) methods Complement fixation + Optic microscopy	Coastal region (all provinces)	Various	3% (CF) 2.8% (OM)	2,160 blood samples were examined by both
Andrade A et al. unpublished Complement Fixation	Manabí	Picoazá	17%	521 samples
Mimori T et al. 1985	Guayas	Pedro Carbo	4.3%	
IHA	El Oro	Zaruma	3.9%	
SNEM-TDR 1986	El Oro	Portovelo	17.1%	Guayaquil (urban): 2,078 samples
(cf. Reyes 1992; complementary data: Ministry of Public Health, unpublished report)	El Oro	Piñas	14.6%	
	El Oro	Zaruma	10.1%	El Guavo: 43 samples
	El Oro	El Guavo	2.3%	Pasaje: 41 samples
IFI	El Oro	Pasaje	7.3%	
	Guayas	Guayaquil (urban)	2.6%	
Racines VJ et al. 1994	El Oro	Portovelo, Piñas	4 to 6 1.4%	Results by age groups
IFI + ELISA		and Zaruma	6 to 8 1.3%	1,514 samples examined
			8 to 10 1.5%	1.8% + for IgG
			10 to 12 2.2%	0.1% + for IgM
			12 to 14 1.9%	
			14 to 15 0.9%	
Guderian R et al. 1994	El Oro	Marcabelc	7.2%	
(unpublished)	El Oro	Pena	6%	
Recombinant Antigen/ELISA	El Oro	Balzas	11.4%	

Author, year, technique	Province	Locality	Positives	Observations
Chico et al. 1997 RecombinantAntigen/ELISA	Napo and Orellana (see ([a]) below)	Various	6%	18 Quechua Communities (1,011 samples) surveyed
Racines & Grijalva 1999	Manabí	Paján (203 samples)	1%	Preliminary results; some of
INH/TDR/Ohio Universtity	Manabí	Portoviejo (628 sampl.)	1.9%	them need to be confirmed
(unpublished)	Guayas	Balzar (178 sampl.)	0.6%	([a])Napo/Orellana were
MicroELISA	Guayas	Guayaquil (2604 sampl.)	1.8%	separated in two provinces
	Guayas	Pedro Carbo (94 sampl.)	1.1%	in 1998
	Sucumbíos	Lago Agrio (493 sampl.)	2.3%	([b])Communities along the
	Sucumbíos	Putumayo(**) (1232 s.)	1.3%	San Miguel-Putumayo
	Sucumbíos	Shushufindi (263 s.)	0%	River
	Napo/Orellana([a])	Aguarico (1796 s.)	0.4%	
	Napo/Orellana	Coca (105 s.)	0%	Amazon region:
	Napo/Orellana	El Chaco (311 s.)	0.3%	6,365 samples; 0.8% +
	Napo/Orellana	J. Sachas (167 s.)	0.6%	Coastal region:
	Napo/Orellana	Loreto (186 s.)	1.6%	3,718 samples; 1.7% +
	Napo/Orellana	Orellana (495 s.)	1.6%	Andean sierra:
	Napo/Orellana	Quijos (40 sampl.)	0%	905 samples; 0.3% +
	Napo/Orellana	Tena (1050 s.)	0.2%	
	Pastaza	Various (227 sampl.)	0.4%	Total:
	Cotopaxi	La Maná (501 s.)	0.4%	10.988 samples
	Cotopaxi	Bangua (404 s.)	0.2%	1.1% positives

INH: Instituto Nacional de Higiene y Medicina Tropical 'Leopoldo Izquieta Pérez'; SNEM: Servicio Nacional para la Erradicación de la Malaria y Control de Vectores. Note: incomplete demographic data limit interpretation in terms of real prevalence in the general population; *a*: Napo/Orellana were separated in two provinces in 1998; *b*: communities along the San Miguel-Putumayo River.

lished). Guevara et al. (1997) reported two cases of severe digestive Chagas disease, confirmed by PCR, in patients from Loja (southwest) and Morona Santiago (south Amazon region) with megacolon. This digestive form seems to be more frequent than megaoesophagus, but further studies are required.

CONCLUSION

Chagas disease is a major public health problem classically underestimated in Ecuador. Prevalence estimates based upon infection rates reported from studied areas and demographic official data indicate that up to 200,000 people may be already infected, while data published by WHO report only 30,000 (UNDP/World Bank/WHO TDR 1997). The presence of a variety of actual or potential vector species, and recent data indicating that transmission actively persists, makes it imperative to accomplish a comprehensive and systematic control programme in the well-known endemic areas, and sero-entomological surveys in other coastal and Amazon provinces. Some of such studies are currently ongoing, but substantial efforts are still needed. A standardised methodology has to be established in order to enable comparisons between different studies. The dynamics of transmission in the Amazon region should be clarified as a research priority. Studies on vector biology and population genetics are also required (Schofield et al. 1995, 1996). In general, we understand that a serious and broad public health action, following the WHO recommendations (WHO 1991), and under the coordination of the Andean countries initiative to interrupt the transmission of Chagas disease (UNDP/World Bank/WHO TDR 1997), is essential to respond adequately to this important public health problem.

ACKNOWLEDGEMENTS

To Dr Chris J Schofield, Dr G Schmunis and Prof MA Miles for critical review of the manuscript.

REFERENCES

Abad-Franch F 1998. Impacto de las alteraciones medioambientales en la transmisión vectorial de *Trypanosoma cruzi*. Conference at the Round Table 'Alteraciones ambientales: impacto en el comportamiento de enfermedades emergentes y reemergentes'. V Congreso de la Sociedad Ecuatoriana de Microbiología/Asociación Latino-americana de Microbiología, Quito, Ecuador, July 1998 (Program and Abstracts in press).

Abad-Franch F, Onore G, Racines VJ, Aguilar VHM 1998a. Distribución de Triatominae (Hemiptera: Reduviidae), vectores de *Trypanosoma cruzi*, en la Amazonía ecuatoriana. Short Communication. V Congreso de la Sociedad Ecuatoriana de Microbiología/Asociación Latinoamericana de

Microbiología, Quito, Ecuador, July 1998 (Program and Abstracts in press).

Abad-Franch F, Racines VJ, Aguilar VHM, Grijalva M, Onore G 1998b. Extension of Chagas disease to the Ecuadorian Amazon basin: a new endemic area? Program and Abstracts of the 47th Annual Meeting of the American Society of Tropical Medicine and Hygiene, San Juan, Puerto Rico (meeting cancelled). *Am J Trop Med Hyg 59* (Suppl.): 329-330.

Aguilar VHM, Yépez R 1996. Evolución epidemiológica de la enfermedad de Chagas en Ecuador, p. 30-38. In CJ Schofield, JP Dujardin & J Jurberg (eds), *Proceedings of the International Workshop on Population Genetics and Control of Triatominae, Santo Domingo de los Colorados, Ecuador, 1995*. INDRE, Mexico City, 116 pp.

Álvarez CJ 1984. *Historia de la Medicina Tropical Ecuatoriana III. Enfermedad de Chagas en el Ecuador,* Arquidiocesana Justicia y Paz, Guayaquil, 233 pp.

Amunárriz M 1991. Enfermedad de Chagas. Primer foco amazónico, p. 27-37. In Amunárriz M *Estudios sobre Patologías Tropicales en la Amazonia Ecuatoriana*, CICAME, Pompeya, Napo, Ecuador.

Amunárriz MU, Chico ME, Guderian RH 1991. Chagas' disease in Ecuador: a sylvatic focus in the Amazon region. *Am J Trop Med Hyg 94:* 145-149.

Arteaga CL 1930. Investigaciones sobre la existencia de la enfermedad de Chagas en la zona del ferrocarril de la costa (Provincia del Guayas). *Rev Univ Guayaquil 1:* 89-101.

Barrett TV 1988. Current research on Amazonian Triatominae. *Mem Inst Oswaldo Cruz 83* (Suppl. I): 441-447.

Campos RF 1923. Notas bibliográficas sobre el *Triatoma dimidiata*. *Rev Col Vicente Rocafuerte 12-14:* 107-111.

Chico HM, Sandoval C, Guevara EA, Calvopiña HM, Cooper PJ, Reed SG, Guderian RH 1997. Chagas disease in Ecuador: evidence for disease transmission in an indigenous population in the Amazon region. *Mem Inst Oswaldo Cruz 92:* 317-320.

Cueva IO, Romero SR 1987. Estudio epidemiológico de la enfermedad de Chagas en la provincia de Loja. p. 151. *Los problemas de salud en el Ecuador. Memorias de los Simposios,* Dirección Nacional del Seguro Social Campesino, Quito, Ecuador.

D'Alessandro A, Barreto P 1985. Colombia, p. 377-399. In RU Carcavallo, JE Rabinovich & RJ Tonn (eds.) *Factores Biológicos y Ecológicos de la Enfermedad de Chagas*, vol. II: Centro Panamericano de Ecología Humana y Salud OPS/OMS Servicio Nacional de Chagas, Ministerio de Salud y Acción Social, República Argentina.

Defranc MI 1982. *Enfermedad de Chagas*. Casa de la Cultura Ecuatoriana, Núcleo del Guayas, Guayaquil, 275 pp.

Defranc MI 1987. Prevalencia de la enfermedad de Chagas en el Ecuador. Informe 1983-1986. *Rev Ecuat Hig Med Trop 37:* 13-47.

Espinoza LA 1955. Epidemiología de la enfermedad de Chagas en la República del Ecuador. *Rev Ecuat Hig Med Trop 12:* 25-105.

Galindo SV 1958. Frecuencia chagásica en 150 cardiomegálicos. *Rev Ecuat Hig Med Trop 15:* 205-216.

Galindo SV 1959. Cardiopatía chagásica crónica. Primeros casos en el Ecuador, demostrados parasitológicamente. *Rev Ecuat Hig Med Trop 16:* 9-20.

Gómez LLF 1968. El problema de la enfermedad de Chagas en Guayaquil. *Rev Ecuat Hig Med Trop 25:* 3-10.

Grijalva MJ, Chiriboga R, Racines VJ, Escalante L, Rowland EC 1997. Short Report: Screening for *Trypanosoma cruzi* in the blood supply by the Red Cross Blood Bank in Quito, Ecuador. *Am J Trop Med Hyg 57:* 740-741.

Grijalva MJ, Rowland EC, Powell MR, McCormick TS, Escalante L 1995. Blood donors in a vector-free zone of Ecuador potentially infected with *Trypanosoma cruzi*. *Am J Trop Med Hyg 52:* 360-363.

Grijalva MJ, Rowland EC, Escalante L, Costales J, Racines VJ 1998. Transmisión de la enfermedad de Chagas asociada a transfusiones sanguíneas en los bancos de sangre del Ecuador. Short Communication. V Congreso de la Sociedad Ecuatoriana de Microbiología/Asociación Latinoamericana de Microbiología, Quito, Ecuador, July 1998 (Program and Abstracts in press).

Guevara AG, Eras JW, Recalde M, Vinueza L, Cooper PJ, Ouassi A, Guderian RH 1997. Severe digestive pathology associated with chronic Chagas disease in Ecuador: report of two cases. *Rev Soc Bras Med Trop 30*: 389-392.

Kawabata M, Uchiyama T, Mimori T, Hashiguchi T, Vera de Coronel V 1987. Association of electrocardiographic abnormalities with seropositivity to *Trypanosoma cruzi* in Ecuador. *Trans R Soc Trop Med Hyg 81:* 7-10.

Lazo R 1985. Parásitos, reservorios, control. Situación regional de la enfermedad de Chagas en Ecuador, p. 413-427. In RU Carcavallo, JE Rabinovich & RJ Tonn (eds), *Factores Biológicos y Ecológicos de la Enfermedad de Chagas*, vol. II: Centro Panamericano de Ecología Humana y Salud OPS/OMS, Servicio Nacional de Chagas, Ministerio de Salud y Acción Social, República Argentina.

Lent H, Wygodzinsky P 1979. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. *Bull Am Mus Nat History 163:* 123-520.

Lent H, Jurberg J, Galvão C 1993. *Rhodnius stali* n. sp., afim de *Rhodnius pictipes* Stal, 1872 (Hemiptera: Reduviidae: Triatominae). *Mem Inst Oswaldo Cruz 88:* 605-614.

León LA 1949. Información sobre el problema de la enfermedad de Chagas en el Ecuador. *Bol Ofic Sanit Panam 28:* 569-585.

León LA, León B 1976. Paleopatología dermatológica ecuatoriana. *Medicina Rev Mex 1205.*

Miles MA, de Souza AA, Povoa M 1981. Chagas disease in the Amazon basin. III. Ecotopes of ten triatomine bug species (Hemiptera: Reduviidae) from the vicinity of Belém, Pará State, Brazil. *J Med Entomol 18:* 266-278.

Mimori T, Kawabata M, Gómez E, Vera de Coronel V, Aroca de M, Flor T, Hasiguchi Y 1985. A seroepidemiological survey of Chagas diseases and search for reservoir hosts in two endemic areas of Ecuador. *Japan J Trop Med Hyg 13:* 245-250.

Montalván JA 1950. Ensayos de profilaxis de la enfermedad de Chagas en Guayaquil. *Rev Ecuat Hig Med Trop 8-9* : 4.

Noireau F, Bosseno MF, Carrasco R, Telleria J, Vargas F, Camacho C, Yaksic N, Brenière SF 1995. Sylvatic triatomines (Hemiptera: Reduviidae) in Bolivia: trends toward domesticity and possible infection with *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae). *J Med Entomol 32:* 594-598.

Noireau F, Bosseno MF, Vargas F, Brenière SF 1994. Apparent trend to domesticity observed in *Panstrongylus rufotuberculatus* Champion, 1899 (Hemiptera: Reduviidae) in Bolivia. *Res Rev Parasitol 54:* 263-264.

Racines VJ, Avilés H, Armijos R, Ortega M, Romaña C, Lema F 1994. Seroprevalencia de la infección a *Trypanosoma cruzi* en escolares de la Provincia del El Oro. *Microbiología 1:* 50.

Rassi A 1979. Clínica: fase aguda, p. 249-264. In Z Brener & Z Andrade (eds) *Trypanosoma cruzi e Doença de Chagas*. Guanabara Koogan, Rio de Janeiro.

Reyes LV 1992. Enfermedad de Chagas, p. 134-137. In R Sempértegui, P Naranjo & M Padilla (eds) *Panorama Epidemiológico del Ecuador*. Ministerio de Salud Pública-Unicef. Quito, Ecuador, 252 pp.

Rodríguez JD 1959. Epidemiología de la enfermedad de Chagas en la República del Ecuador. *Rev Goiana Medica 5:* 411-438.

Rodríguez JD 1961. Nuevos datos sobre la enfermedad de Chagas en Guayaquil. *Rev Ecuat Hig Med Trop 18:* 48-52.

Rodríguez JD 1963. Antecedentes y primeros resultados de la campaña antichagásica en un sector de la ciudad de Guayaquil. *Rev Ecuat Hig Med Trop 20:* 13-15.

Romaña C, Racines VJ, Avilés H, Lema F 1994. Observaciones de domiciliación de *Rhodnius ecuadoriensis* en focos endémicos de la enfermedad de Chagas en el Ecuador. *Microbiología 1:* 59.

Schofield CJ 1994. *Triatominae. Biología y control.* Eurocommunica Publications, West Sussex, UK, 80 pp.

Schofield CJ, Dujardin JP & Jurberg J 1995. Population genetics of Triatominae: support for vigilance and control interventions. *Mem Inst Oswaldo Cruz 90* (Suppl. I): 57.

Schofield CJ, Dujardin JP, Jurberg J. 1996 *Proceedings of the International Workshop on Population Genetics and Control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995*. INDRE, Mexico City, 116 pp.

UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) 1997. Andean countries initiative launched in Colombia. *TDR News 53:* 3.

Valente VC, Valente AS, Noireau F, Carrasco HJ, Miles MA 1998. Chagas disease in the Amazon basin: association of *Panstrongylus geniculatus* (Hemiptera: Reduviidae) with domestic pigs. *J Med Entomol 35*: 99-103.

Valenzuela V 1939. Breve resumen sobre patología médica ecuatoriana y su distribución geográfica. *Rev Univ Guayaquil 10.*

Varas SJM 1942. Edema palpebral febril tripanosomiásico. *An Soc Med-Quir Guayas 22:* 979-998.

WHO-World Health Organization 1991. *Control of Chagas Disease*. WHO Technical Report Series 811, 95 pp.

WHO/PAHO 1974. Aspectos clínicos de la enfermedad de Chagas. Informe de una reunión conjunta OPS/OMS de investigadores. *Bol Ofic Sanit Panam.*

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (2000) 94, 629–630

Short Report

The use of live-bait traps for the study of sylvatic *Rhodnius* populations (Hemiptera: Reduviidae) in palm trees

F. Abad-Franch[1,3*], F. Noireau[2], A. Paucar C.[3+], H. M. Aguilar V.[3,4], C. Carpio C.[3] and J. Racines V.[4†]

[1]Pathogen Molecular Biology and Biochemistry Unit, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK; [2]Institut de Recherche pour le Développement (IRD) and Instituto Oswaldo Cruz, Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, avenida Brasil 4365, 21045-900 Rio de Janeiro, Brazil; [3]Unidad de Medicina Tropical, Instituto 'Juan Cesar García', CP 17-1106292 Quito, Ecuador; [4]Instituto Nacional de Higiene y Medicina Tropical 'Leopoldo Izquieta Pérez' Zona Norte, Iquique 2045 y Yaguachi, Quito, Ecuador

Keywords: *Rhodnius*, sylvatic populations, trapping, palm trees, methodology, Ecuador

Chagas disease is a major public health challenge for most Latin American countries. An initiative for the coordinated control of Chagas disease transmission throughout the Andean countries was launched in 1997. Since the early 1990s, control measures based on elimination of domestic/peridomestic triatomine colonies and screening of donor blood by serological testing have resulted in a reduction in incidence of ~70% in the Southern Cone countries (WHO, 1991; DIAS & SCHOFIELD, 1999; MONCAYO, 1999; WHO/CTD, 2000).

Various *Rhodnius* species are among the most important vectors of *Trypanosoma cruzi*, the causative agent of Chagas disease, in different countries of continental South and Central America. All of them have been reported to breed in arboreal habitats and many of them in palm trees of different genera (WHITLAW & CHANIOTIS, 1978; LENT & WYGODZINSKY, 1979; MILES *et al.*, 1983; PIZARRO & ROMAÑA, 1998). The study of the ecological traits and behavioural trends of sylvatic populations is a key to the understanding of the processes leading to the initial colonization of human dwellings by *Rhodnius* species, and recolonization following vector control interventions (DUJARDIN *et al.*, 1991; COSTA, 1999).

R. ecuadoriensis is a major vector of Chagas disease in Ecuador, and is able to colonize human-related habitats by migration from sylvatic ecotopes (LENT & WYGODZINSKY, 1979; ROMAÑA *et al.*, 1994; SCHOFIELD, 1994; AGUILAR *et al.*, 1999). Sylvatic populations of *R. ecuadoriensis* have been reported to occur in central and northern coastal regions, in palm trees of the genus *Phytelephas* (locally known as *palma de tagua*, used for handicraft manufacture and frequently maintained in the peridomicile) (AVILÉS *et al.*, 1995; BORCHSENIUS *et al.*, 1998). Here we report preliminary results of the use of live bait traps in the study of sylvatic *Rhodnius* populations in Ecuador.

The direct collection of sylvatic Triatominae is time consuming and logistically difficult. Results are usually scarce, even when dissection of natural ecotopes (e.g., palm trees) is undertaken (MILES *et al.*, 1981; PIZARRO & ROMAÑA, 1998). Light trapping is also of limited value, as only starved adults of few species fly readily to light traps. Several authors have reported the use of animal-baited traps for triatomines (RABINOVICH *et al.*, 1976; TONN *et al.*, 1976; CARCAVALLO, 1985), all as yet with poor results. Recently, NOIREAU *et al.* (1999, 2000) described a simple trapping system that produced excellent results in the study of sylvatic *Triatoma* (*T. sordida*, *T. guasayana*, and *T. infestans* dark morph) inhabiting hollow trees in the Bolivian Chaco. The system consists of a small plastic bottle containing a mouse as bait and covered with double-coated adhesive tape. We have applied this method, introducing several modifications aimed to improve its performance and the welfare of the mouse, to study sylvatic *Rhodnius* populations in palm trees in Ecuador.

The study area, nearby the locality of Alluriquín (province of Pichincha), is located on the western slope of the Andes (700–800 m above sea level; approximately 79°00′ W, 00°20′ S). The area comprises very humid subtropical forest (CAÑADAS, 1983). *Phytelephas aequatorialis* Spruce, a palm tree endemic to the Ecuadorian western slope of the Andes (BORCHSENIUS *et al.*, 1998), is abundant in the zone, and was suspected of being a favoured ecotope for *Rhodnius* insects. Our modified traps consisted of a plastic container, larger (~15 × 9 cm) than those used by NOIREAU *et al.* (1999), in which a mouse was contained together with a small quantity of wood shavings and food (aiming to protect the animal from low night temperatures and starvation). Small holes were made in the lower side of the bottles so that water from rain could not accumulate within. Containers were closed with 1 mm-aperture wire mesh and wrapped around with double-coated adhesive tape. They were located in different parts of the palm trees (among epiphytes growing around stems, or directly in the angle between palm fronds and the stem). Our objectives were: (i) to test the performance of modified live-bait traps in palm trees, and with *Rhodnius* species; (ii) to detect positive palm trees for further studies; and (iii) to capture some live specimens for laboratory studies. We initially studied 11 *P. aequatorialis* palm trees by direct searches for insects in their crowns. In a second attempt, we investigated 34 palm trees using the modified traps (placed overnight on the palms). We used a total of 56 trap-nights on these palms. We also undertook a manual capture of insects in the organic matter and epiphytes present around the trunk of 1 palm tree. Part of these materials were cut down and examined on a white sheet (4 people searching during 3 h). Finally, we dissected another palm by cutting it down and systematically inspecting it on a larger white sheet (4 people, 3 h). These 2 palms were already known to be positive (triatomines were captured previously in live-bait traps).

Direct searches in palm crowns yielded negative results. By live-bait trapping, 12 out of 34 palm trees were found to be positive (infestation index 35·3%) for the presence of *Rhodnius* breeding colonies (nymphs captured, colonisation index 100%) [insects were preliminarily identified as *R. ecuadoriensis* as described by LENT & WYGODZINSKY (1979); molecular taxonomy studies are ongoing]. Of 56 trap-nights, 27 (48%) were found to be positive (containing triatomine bugs adhered to the tape) when checked the following morning. The average number of insects per positive trap-night was 4·9, with a maximum of 14 individuals in a single trap-night. We captured a total of 141 *Rhodnius* insects. The crowding index (average number of insects per positive palm) was 11·75, and the density index (insects captured/palms examined) was 4·15. Of the total number, 139 bugs (99%) were nymphs of different stages, with just 2 adults (1 female and 1 male) (adults/nymphs index 0·014). Seven nymphs were captured in 2 positive palms by other means, with just 1 fifth-instar nymph captured

*Author for correspondence;
e-mail fernando.abad-franch@lshtm.ac.uk
+Current address: Systematics Research Collections, W-436 Nebraska Hall, University of Nebraska State Museum, Lincoln, NE 68588-0514, USA.
†Deceased in December 1999.

by complete dissection of 1 of these palms. No other triatomine species was found.

The performance of modified live-bait traps in this study is notably better than reported in 1999 and 2000 by NOIREAU *et al.* (48% positive against 27% and 21·9% positive, respectively); these differences may be related to the distinct triatomines and ecotopes studied (thus not necessarily to a superior trap design), but clearly reveal that this method yields excellent results in palms inhabited by *Rhodnius*. We recently captured 50 *Rhodnius* individuals (*R. pictipes* and *R. robustus*) in a single trap-night in a *P. tenuicaulis* palm in the Ecuadorian Amazon (F. S. Palomeque *et al.*, unpublished). Regarding palm dissection, in 25 published studies (cf. PIZARRO & ROMAÑA, 1998) 1390 palms were cut down and 10564 *Rhodnius* were captured (an average of 7·6 insects/palm); our results in Pichincha (4·15 insects/palm, this report), and more widely in Ecuador (6·3 insects/palm by live-bait trapping plus direct searches in palm crowns, F. Abad-Franch *et al.*, unpublished) question the necessity of indiscriminately cutting down and dissecting palms for this kind of study. The adults/nymphs index we found is very low (0·014); we speculate that adult insects may be able to escape from the adhesive tape, but a lower number of adults in sylvatic colonies—compared to that of immature individuals—may also be a reason. Previous studies (conducted by palm dissection, thus probably biased towards bigger insects) report an average adults/nymphs index of 0·7, range 0–2·6 (cf. PIZARRO & ROMAÑA, 1998). With the live-bait traps, no mouse mortality was recorded, and animals spending the night inside the modified traps were found to be in perfect condition (except for 1 mouse, which was attacked by small ants; avoidable by covering the wire mesh with finer cloth).

Our results strongly suggest that live-bait traps with adhesive tape could be extremely helpful in the study of sylvatic populations of different *Rhodnius* and other Triatominae species associated with palm trees in the wild. This method provides a quick and inexpensive way to readily detect positive palm trees, and could be also of value for disease transmission control purposes, as the presence of triatomines in peridomestic palms may be easily monitored using live-bait traps. The ecological impact of cutting down and dissecting palm trees to study wild triatomines could be reduced to the minimum (and, in many cases, avoided), as could the effort required for such studies. Additionally, it is possible to recover sufficient live specimens to establish laboratory colonies and enlarge reference collections for further studies and comparisons.

Acknowledgements

This study was supported by the United Nations Development Programme/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (Grant 970195), the ECLAT Network, and, partially, by the Tropical Medicine Unit (University General Hospital of Valencia, Spain), and the Cañada-Blanch Foundation (Valencia, Spain). Special thanks are due to Michael A. Miles and Christopher J. Schofield for guidance and review of the manuscript, and to Dr Ana Abad-Franch for her suggestions and help during fieldwork.

References

Aguilar, V. H. M., Abad-Franch, F., Racines, V. J. & Paucar, C. A. (1999). Epidemiology of Chagas disease in Ecuador. A brief review. *Memórias do Instituto Oswaldo Cruz*, **94** (supplement 1), 387–393.

Avilés, H., Cevallos, P., Champaloux, B., Goujon, C., Lema, F., Pajony, R., Pays, J. F., Racines, V. J. & Romaña, C. A. (1995). *Rhodnius ecuadoriensis* en áreas endémicas de tripanosomiasis americana en Ecuador. *Parasitología al Día*, **19** (no. extraordinario), 231.

Borchsenius, F., Borgtoft Pedersen, H. & Balslev, H. (1998). *Manual to the Palms of Ecuador*. Aarhus, Denmark: Department of Systematic Botany, Aarhus University–Pontificia Universidad Católica del Ecuador, Quito. AAU Reports 37.

Cañadas, L. (1983). *Mapa Bioclimático y Ecológico del Ecuador*. Quito, Ecuador: MAG-PRONAREG.

Carcavallo, R. U. (1985). Técnicas de estudio de triatominos en ambiente silvestre. In: *Factores Biológicos y Ecológicos en la Enfermedad de Chagas*, volume I, Carcavallo, R. U., Rabinovich, J. E. & Tonn, R. J. (editors). Buenos Aires: OPS-ECO/MSAS-SNCH, pp. 49–52.

Costa, J. (1999). The synanthropic process of Chagas disease vectors in Brazil, with special attention to *Triatoma brasiliensis* Neiva, 1911 (Hemiptera, Reduviidae, Triatominae). Population, genetic, ecological and epidemiological aspects. *Memórias do Instituto Oswaldo Cruz*, **94** (supplement 1), 239–241.

Dias, J. C. P. & Schofield, C. J. (1999). The evolution of Chagas disease (American trypanosomiasis) control after 90 years since Carlos Chagas discovery. *Memórias do Instituto Oswaldo Cruz*, **94** (supplement 1), 103–121.

Dujardin, J. P., García-Zapata, M. T., Jurberg, J., Poelants, P., Cardozo, L., Panzera, F., Dias, J. C. P. & Schofield, C. J. (1991). Which species of *Rhodnius* is invading houses in Brazil? *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **85**, 679–680.

Lent, H. & Wygodzinsky, P. (1979). Revision of the Triatominae (Hemiptera: Reduviidae), and their significance as vectors of Chagas disease. *Bulletin of the American Museum of Natural History*, **163**, 123–520.

Miles, M. A., de Souza, A. A. & Povoa, M. (1981). Chagas disease in the Amazon basin: III. Ecotopes of ten triatomine bugs species (Hemiptera: Reduviidae) from the vicinity of Belém, Pará State, Brazil. *Journal of Medical Entomology*, **18**, 266–278.

Miles, M. A., Arias, J. R. & de Souza, A. A. (1983). Chagas disease in the Amazon basin: V. Periurban palms as habitats of *Rhodnius robustus* and *Rhodnius pictipes*—triatomine vectors of Chagas disease. *Memórias do Instituto Oswaldo Cruz*, **78**, 391–398.

Moncayo, A. (1999). Progress towards interruption of transmission of Chagas disease. *Memórias do Instituto Oswaldo Cruz*, **94** (supplement 1), 401–404.

Noireau, F., Flores, R. & Vargas, F. (1999). Trapping sylvatic Triatominae (Reduviidae) in hollow trees. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **99**, 13–14.

Noireau, F., Flores, R., Gutiérrez, T., Abad-Franch, F., Flores, E. & Vargas, F. (2000). Natural ecotopes of *Triatoma infestans* dark morph and other sylvatic triatomines in the Bolivian Chaco. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **94**, 23–27.

Pizarro, J. C. N. & Romaña, C. (1998). Variación estacional de una población silvestre de *Rhodnius pallescens* Barber 1932 (Heteroptera: Triatominae) en la costa Caribe colombiana. *Bulletin de l'Institut Français d'Etudes Andines*, **27**, 309–325.

Rabinovich, J. E., Carcavallo, R. U. & Barretto, M. P. (1976). Ecologic methods: marking, trapping and sampling for vector studies in the field. In: *New Approaches in American Trypanosomiasis Research*, Washington: Pan American Health Organization, Scientific Publication no. 318, pp. 16–20.

Romaña, C. A., Racines, V. J., Avilés, H. & Lema, F. (1994). Observaciones de domiciliación de *Rhodnius ecuadoriensis* en focos endémicos de la enfermedad de Chagas en el Ecuador. *Microbiología*, **1**, 59.

Schofield, C. J. (1994). *Triatominae: Biología y Control*. West Sussex, UK: Eurocommunica Publications.

Tonn, R. J., Carcavallo, R. U., Ortega, R. & Carrasquero, B. (1976). Métodos de estudio de triatominos en el medio silvestre. *Boletín de la Dirección de Malariología y Saneamiento Ambiental*, **16**, 146–152.

Whitlaw, J. T. Jr & Chaniotis, B. N. (1978). Palm trees and Chagas disease in Panama. *American Journal of Tropical Medicine and Hygiene*, **27**, 873–881.

WHO (1991). *Control of Chagas Disease*. Geneva: World Health Organization, Technical Report Series, no. 811.

WHO/CTD (2000). *http://www.who.int/ctd/chagas*

Received 10 February 2000; revised 8 May 2000; accepted for publication 9 May 2000

REVISTA DEL INSTITUTO JUAN CESAR GARCIA

Quito - Ecuador

Sumario
Vol. 10, Nos 1-2
Enc - Dic, 2000

ISSN: 1390017X

Fundada en Enero de 1991 con el nombre de *Revista del Instituto Juan César García*

MENSAJE

ARTÍCULOS

TEMAS DE ACTUALIDAD

NOTAS

Control de la Enfermedad de Chagas en el Ecuador. Datos y Reflexiones para una Política de Estado *

Fernando Abad-Franch[1,2] y H. Marcelo Aguilar V.[1,3]

RESUMEN *La enfermedad de Chagas es uno de los graves problemas de salud de América Latina: más de 11 millones de personas están infectadas por Trypanosoma cruzi, el parásito que la produce. Felizmente, varios programas de control coordinados (Cono Sur, Área Andina, América Central, etc.) han resultado en la interrupción de la transmisión en grandes áreas del continente. Sólo Ecuador, entre los países andinos, carece de un programa estructurado de control. Se estima que ~120000 personas están infectadas en el país, lo que supone unas 300 muertes y unos 3000 nuevos casos por año. La carga financiera relacionada con la ausencia de medidas preventivas pueden calcularse en unos 30 millones de dólares anuales. Sin embargo, el costo de un programa de control no superaría los 1.6 millones de dólares/año durante los cinco primeros años y un millón/año durante los siguientes diez. La meta de eliminación de la transmisión, fijada para el año 2010 por la Comisión Intergubernamental de los países andinos, sólo podrá ser alcanzada emprendiendo sin retraso acciones sistemáticas para el control vectorial, el manejo adecuado de los pacientes y el tamizaje efectivo de las todas las donaciones realizadas en los bancos de sangre.*

PALABRAS CLAVE *Enfermedad de Chagas, clínica, epidemiología, aspectos económicos, control, políticas de salud pública, Ecuador*

En homenaje a la memoria del Dr. José Racines Vizuete

1 Unidad de Medicina Tropical, Instituto "Juan César García", Quito, Ecuador.
2 Pathogen Molecular Biology and Biochemistry Unit, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Londres, Reino Unido.
3 Instituto Nacional de Higiene y Medicina Tropical "Leopoldo Izquieta Pérez", Ministerio de Salud Pública del Ecuador, Quito, Ecuador.

* Las ideas para este trabajo surgieron del desarrollo de un proyecto de investigación sobre vectores de la enfermedad de Chagas ("Importancia epidemiológica de *Rhodnius ecuadoriensis* Lent y León, 1958 en Ecuador") ejecutado por el Instituto Juan César García en colaboración con la London School of Hygiene and Tropical Medicine y varios centros asociados a la Red Europea-Latinoamericana de Investigación sobre Biología y Control de Triatominae (ECLAT). Diversas partes del proyecto fueron finan-ciadas por OMS/TDR (Grant 970195), la Red ECLAT, la Unidad de Medicina Tropical del Hospital Gral. Universitario de Valencia (España) y la Fundación Cañada-Blanch (Valencia, España). Paralelamente, los autores, junto con otros investigadores y trabajadores de salud pública, participaron, asumiendo diferentes niveles de responsabilidad (desde lo estrictamente técnico a lo político), en el diseño del primer Programa Nacional para el control de la Enfermedad de Chagas en el Ecuador, en estrecha colaboración con las autoridades de la Subsecretaría Nacional de Medicina Tropical (Ministerio de Salud Pública del Ecuador).

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (2000) 94, 23–27

Natural ecotopes of *Triatoma infestans* dark morph and other sylvatic triatomines in the Bolivian Chaco

F. Noireau[1,2], R. Flores[3], T. Gutierrez[4], F. Abad-Franch[5], E. Flores[3] and F. Vargas[3] *[1]Institut de Recherche pour le Développement (IRD), France; [2]Instituto Oswaldo Cruz, Departamento de Entomologia, Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, CP 926, Rio de Janeiro, Brazil; [3]Instituto Boliviano de Biologia de Altura, CP 641, La Paz, Bolivia; [4]Universidad Autónoma Gabriel René Moreno, CP 2489, Santa Cruz, Bolivia; [5]Pathogen Molecular Biology and Biochemistry Unit, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK*

Abstract

A survey of natural ecotopes of *Triatoma infestans* dark morph and other triatomine sylvatic species was performed in an uninhabited area of the Bolivian Chaco. Among the 321 triatomines collected by light trapping, only 4 *T. infestans* dark morph specimens were identified. Predominant flying species were *T. guasayana* and *T. sordida* group 2 (51·7% and 37·1% of capture, respectively). The same species prevailed in terrestrial and epiphytic bromeliads where scarce *T. infestans* dark morph nymphal instars were also detected. In parrot nests *T. delpontei* prevailed broadly over other species (90·2% of the capture) and only 4 *T. infestans* dark morph adults were collected. In contrast, *T. infestans* dark morph was the predominant species captured in hollow trees (46·0% of the total collected). The abundance of immature forms (88·2% of the collection) shows that hollow trees constitute a favourable ecotope for this species. Of the 421 trees investigated, 33·7% were positive for triatomines. *T. infestans* dark morph, found inside 15·0% of them, also had higher apparent density than other species (average number of *T. infestans* in positive trees, 2·0 ± 1·6 vs 1·3 ± 0·6 for other species). Light trapping seems to be an efficient method to sample the *T. sordida–T. guasayana* complex in that it shows a similar distribution to that observed in natural ecotopes; however, this method is ineffective for the assessment of the local abundance of *T. infestans* dark morph.

Keywords: *Triatoma infestans*, *Triatoma sordida*, *Triatoma guasayana*, sylvatic populations, ecology, Bolivia

Introduction

Control of Chagas disease by the elimination of domestic populations of Triatominae has been successful over vast areas of the Southern Cone countries where *Triatoma infestans* is the main domestic vector (SCHOFIELD & DIAS, 1998). Nevertheless, in controlled areas, there are increasing reports of sylvatic triatomines invading human dwellings. Consequently, research activities must focus on originally sylvatic species adapting to peridomestic and domestic habitats. The entomological observations generated will assist in the adaptation of vector control strategies.

With regard to *T. infestans*, Bolivia is the only country where true sylvatic foci are documented. Sylvatic populations have been reported from valleys of Cochabamba and Sucre (TORRICO, 1946; DUJARDIN *et al.*, 1987; BERMUDEZ *et al.*, 1993). More recently, a sylvatic focus was also detected at Caracato, department of La Paz (F. Noireau, unpublished data). These sylvatic populations, which occur always amongst rock-piles, are most likely widespread through Andean valleys between 2400 and 2600 m altitude. Chromatic pattern, isozyme profiles and DNA sequence analysis are similar between domestic and sylvatic Andean specimens but random amplified polymorphic DNA (RAPD) and morphometrics allow them to be distinguished (DUJARDIN *et al.*, 1987, 1997; CARLIER *et al.*, 1996; MONTEIRO *et al.*, 1999).

Besides this Andean focus, another sylvatic *T. infestans* population was recently detected in the Bolivian Chaco (NOIREAU *et al.*, 1997b). The first reported specimens were adults captured in parrot nests and by light trapping. Because of chromatic differences with domestic *T. infestans* (overall darker coloration with small yellow markings on the connexivum), they were named dark morphs (DM). They present morphometric differences but isoenzymatic similarity with domestic *T. infestans*. This suggests that they represent a distinct population (NOIREAU *et al.*, 1997b), a hypothesis recently supported by DNA sequence analysis and cytogenetics (MONTEIRO *et al.*, 1999; F. Panzera, unpublished data).

The same region of the Bolivian Chaco is characterized by the sympatric occurrence of *T. guasayana* with 2 putative cryptic species of the *T. sordida* complex known as groups 1 and 2 (G1 and G2) (NOIREAU *et al.*, 1998, 1999b). Parrot nests in the region often contain ornithophilic triatomines characterized as *T. delpontei* by cytogenetics (F. Panzera, unpublished data). The current work improves the knowledge of these sylvatic species which are all (except *T. delpontei*) considered as candidate vectors because of their ability to colonize artificial structures.

Materials and Methods

Study area

The field work was carried out in the phytogeographical region of the Chaco, in the southern part of the Department of Santa Cruz, Bolivia. The main environmental characteristics of this semi-arid region are: (i) 300-m altitude, (ii) a mean temperature of 26°C, (iii) an average annual rainfall of about 600 mm, and (iv) a marked seasonality, with a dry season from March to October and a wet season the rest of the year. The surveyed area included La Choza (18° 34′ 516″ S; 62° 40′ 108″ W), an uninhabited site located on the way to Izozog, and the surrounding forest. The area is covered by a dense and thick vegetation (elevation 4–6 m) of hardwood trees dominated by *Ruprechtia triflora* with emerging trees up to 12 m high (including *Aspidosperma quebracho-blanco*, *Chorisia insignis*). In the lowest stratum thorn shrubs, bromeliads and cacti predominate.

Collection of triatomines

The current study was focused on *T. infestans* DM but also included other sylvatic triatomine species endemic to the area. Insects were collected by light-trap at the site of La Choza. The light system consisted of a vertical white cloth simultaneously illuminated by a 12-V fluorescent black light tube and a 150-V mercury vapour light. It was operated by portable generator from sunset to 22:30 for 3–5 successive nights in September 1995, 1996 and 1997, and for 18 nights in September 1998.

Various natural habitats such as bromeliads, parrot nests and hollow trees located near the site were searched

Address for correspondence: Dr F. Noireau, FIOCRUZ Instituto Oswaldo Cruz, Departamento de Entomologia – LNIRTT, Av. Brasil 4365, CP 926, 21045-900 Rio de Janeiro, RJ, Brazil; phone +55 21 598 4320, fax +55 21 290 9339, e-mail noireauf(a)ioc.fiocruz.br or noireauf(a)gene.dbbm.fiocruz.br

for the presence of triatomines during September and October 1998. Terrestrial (*Bromelia serra* and *B. hieronymi*) and epiphytic bromeliads (*Tillandsia* spp.) were cut off at the base and systematically dissected. Parrot nests inhabited by *Myiopsitta monacha cotorra* (colonial monk parrot) were carefully dismantled to allow each part to be examined. Mammal-baited traps as described by NOIREAU *et al.* (1999a) were used to capture insects living in hollow trees.

Processing of insects

The collected triatomines were placed in plastic bottles containing filter paper and transported to the laboratory. They were identified by morphology, according to LENT & WYGODZINSKY (1979). Isoenzyme analysis was performed to discriminate nymphal instars and adults of *T. sordida* G1, G2, and *T. guasayana*. All nymphal instars and adults pertaining to this complex were processed for 2 enzyme systems: malate dehydrogenase (MDH, EC 1.1.1.37) and isocitrate dehydrogenase (IDH, EC 1.1.1.42). Both enzyme systems provide a reliable identification of the 3 species (NOIREAU *et al.*, 1998). Although *T. sordida* G2 has not yet received formal nomenclatural recognition as a new species, it will be considered as a putative species in this work. Samples collected in hollow trees were examined for flagellates by direct microscope observation of faeces droplets at ×400 magnification.

Results

A total of 321 triatomines was captured by light-trapping (Table 1). According to their relative abundance, *T. guasayana* prevailed over *T. sordida* G2 and G1 (51·7%, 37·1% and 8·7% of capture, respectively). *T. infestans* DM and *T. delpontei* presented a low abundance (only 4 specimens of each species captured). The sex ratio for the whole collection was close to 1:1.

We searched for triatomines inside 197 terrestrial bromeliads (Table 2). *T. guasayana* and *T. sordida* G2 were the prevailing species (48·9% and 36·2%, respectively). Four nymphal instars of *T. infestans* DM and 3 *T. sordida* G1 specimens were also encountered. A similar distribution of species was observed inside 172 epiphytic bromeliads: *T. sordida* G2 and *T. guasayana* predominated over *T. infestans* DM (8 nymphal instars collected) and *T. sordida* G1 (Table 2). The infestation rate for both types of bromeliads was similar (17·3% for terrestrial bromeliads and 22·1% for epiphytes, respectively). According to the species, the triatomine burden of pooled terrestrial and epiphyte bromeliads is shown in Table 3. The average number of triatomines captured by positive plant was 1·5 ± 0·6. *T. infestans* DM and *T. sordida* G1 were scarcely found (2·2% and 1·9% of bromeliads, respectively). However, *T. sordida* G2 and *T. guasayana* were more frequently detected (in 9·5% of bromeliads for both species). A total of 78·6% of positive bromeliads contained only 1 species while 21·4% contained 2 species.

The dissection of 46 parrot nests showed that *T. delpontei* prevailed over *T. sordida* G1 (90·2% vs 6·3%, respectively). Only 4 *T. infestans* DM and 1 *T. sordida* G2 were also collected (Table 2). Except for *T. delpontei* which exhibited a great number of nymphal instars, only adult forms were detected for other species. *T. delpontei* was encountered inside 26 nests (56·6%) and *T. sordida* G1 inside 5 (10·9%). The 4 adult forms of *T. infestans* DM were captured inside 3 nests.

From a total of 732 bait-traps suspended in hollow trees, 160 (21·9%) collected live triatomines adhering to the tape. The distribution by species and age structure of the 276 captured triatomines is shown in Table 2. *T. infestans* DM was the predominant species (46·0% of the 276 triatomines captured), followed by *T. guasayana* (23·2%) and *T. sordida* G2 and G1 (17·0% and 13·8%, respectively). A great number of nymphal instars was collected, particularly of *T. infestans* DM and *T. sordida* G1 (88·2% and 71·1%, respectively). From a total of 421 trees investigated, 142 were positive for triatomines (33·7%). According to triatomine species, the apparent triatomine burden of infested trees is shown in Table 4. The average number of triatomines captured by positive tree was 1·9 ± 1·4. *T. infestans* DM, found inside 15·0% of trees, presented the highest apparent density: the average number of specimens captured by positive tree (2·0 ± 1·6) was greater than for any other species ($P < 0·05$) while the mean burdens for *T. sordida* G1, G2 and *T. guasayana* were similar. Most positive trees contained only 1 species (73·3%) while 23·2% had 2 species and 5 (3·5%) 3 species. Forty-five trees contained only *T. infestans* DM (31·7%) while 18 (12·7%) had *T. infestans* DM associated with another species.

The distribution of *T. sordida* G1, *T. sordida* G2 and *T. guasayana* adults did not differ significantly between captures performed by light-trapping, in bromeliads and hollow trees. In contrast, only 4 *T. infestans* DM were collected by light-trapping in spite of their abundance in hollow trees.

Faecal samples from 134 triatomines collected in hollow trees were examined by microscopy for flagellates. Only 2 (2·5%) *T. infestans* DM were positive. The infection rate of other species is summarized in Table 5.

Discussion

Ecological studies of Triatominae in areas where endemic species are hard to discriminate on morphological traits show the importance of using molecular tools for an accurate characterization. This is particularly so for the *T. sordida–T. guasayana* complex (NOIREAU *et al.*, 1998) and other Triatominae such as the *Rhodnius* genus (DUJARDIN *et al.*, 1991). In the Bolivian Chaco, multilocus enzyme electrophoresis allows reliable discrimination between 2 biological species pertaining to the *T. sordida* complex and named groups 1 and 2 (NOIREAU *et al.*, 1998). Later, this partition was confirmed by RAPD, morphometrics, cytogenetics and crossing experiments (J. P. Dujardin, B. Bastrenta, L. Garcia, F. Panzera and F. Noireau, unpublished data). Because these *T. sordida* groups could exhibit different behaviour, ecologicial studies should take the distinct population subdivision into consideration.

Light-trapping seems to be an efficient method to sample the *T. sordida–T. guasayana* complex as it shows

Table 1. Distribution of Triatominae captured by light-trapping in the Bolivian Chaco

Species	No. (%) of males	No. (%) of females	Total (%) of capture
T. sordida G1	9 (32·1)	19 (67·9)	28 (8·7)
T. sordida G2	68 (57·1)	51 (42·9)	119 (37·1)
T. guasayana	79 (47·6)	87 (52·4)	166 (51·7)
T. infestans DM	1 (25·0)	3 (75·0)	4 (1·2)
T. delpontei	3 (75·0)	1 (25·0)	4 (1·2)
Total	160 (49·8)	161 (50·2)	321 (100)

DM, dark morph. See the text for details of the methods used.

Table 2. Distribution of triatomines collected in natural ecotopes in the Bolivian Chaco

Ecotope	Species	No. of nymphs	No. of males	No. of females	No. of adults	Total (%) of capture
Terrestrial bromeliad	*T. sordida* G1	1	2	0	2	3 (6·4)
	T. sordida G2	0	9	8	17	17 (36·2)
	T. guasayana	0	16	7	23	23 (48·9)
	T. infestans DM	4	0	0	0	4 (8·5)
	Total	5	27	15	42	47 (100)
Epiphytic bromeliad	*T. sordida* G1	1	0	3	3	4 (7·3)
	T. sordida G2	0	9	13	22	22 (40·0)
	T. guasayana	1	9	11	20	21 (38·2)
	T. infestans DM	8	0	0	0	8 (14·5)
	Total	10	18	27	45	55 (100)
Parrot nest	*T. sordida* G1	0	2	7	9	9 (6·3)
	T. sordida G2	0	1	0	1	1 (0·7)
	T. guasayana	0	0	0	0	0
	T. infestans DM	0	3	1	4	4 (2·8)
	T. delpontei	69	33	27	60	129 (90·2)
	Total	69	39	35	74	143 (100)
Hollow tree	*T. sordida* G1	27	7	4	11	38 (13·8)
	T. sordida G2	9	21	17	38	47 (17·0)
	T. guasayana	3	30	31	61	64 (23·2)
	T. infestans DM	112	5	10	15	127 (46·0)
	Total	151	63	62	125	276 (100)
All ecotopes	*T. sordida* G1	29	11	14	25	54 (10·4)
	T. sordida G2	9	40	38	78	87 (16·7)
	T. guasayana	4	55	49	104	108 (20·7)
	T. infestans DM	124	8	11	19	143 (27·4)
	T. delpontei	69	33	27	60	129 (24·8)
	Total	235	147	139	286	521 (100)

DM, dark morph. See the text for details of the methods used.

Table 3. Triatomine burden in bromeliads in the Bolivian Chaco

Species	No. (%) of positive bromeliads	No. of triatomines	Mean no. (SD) of triatomines per positive plant	Range of density
T. sordida G1	7 (1·9)	7	1·0 (0·0)	1
T. sordida G2	35 (9·5)	39	1·1 (0·3)	1–2
T. guasayana	35 (9·5)	44	1·3 (0·5)	1–3
T. infestans DM	8 (2·2)	12	1·5 (0·8)	1–3
All species	70 (19·0)	102	1·5 (0·6)	1–3

The values given are combined results from 197 terrestrial bromeliads and 172 epiphytic bromeliads.
DM, dark morph.

Table 4. Apparent triatomine burden in trees in the Bolivian Chaco

Species	No. (%) of positive trees	No. of triatomines	Mean no. (SD) of triatomines by positive tree	Range of density
T. sordida G1	32 (7·6)	38	1·2 (0·5)	1–3
T. sordida G2	40 (9·5)	47	1·2 (0·4)	1–2
T. guasayana	50 (11·9)	64	1·3 (0·6)	1–4
T. infestans DM	63 (15·0)	127	2·0 (1·6)	1–11
All species	142 (33·7)	276	1·9 (1·4)	1–12

DM, dark morph.

a similar distribution to that observed in natural ecotopes such as hollow trees and bromeliads. Moreover, it confirms the dispersive flight ability of these species as reported by SCHOFIELD *et al.* (1991), WISNIVESKY-COLLI *et al.* (1993) and NOIREAU *et al.* (1999b). However, the light-trapping of *T. infestans* DM seems ineffective to assess the local abundance of this species. As suggested by studies on domestic populations, *T. infestans* has a lesser propensity to flight than *T. sordida* (SCHOFIELD *et al.*, 1992). WISNIVESKY-COLLI *et al.* (1993) reported that *T. guasayana* females attracted to light were slightly more numerous than males. In our

Table 5. Infection by flagellates of triatomines collected in hollow trees in the Bolivian Chaco

Species	No. examined	No. positive	% positive
T. sordida G1	22	2	9·1
T. sordida G2	16	0	0·0
T. guasayana	15	2	13·3
T. infestans DM	81	2	2·5
Total	134	6	4·5

DM, dark morph.

study, a similar result was observed in *T. guasayana* and *T. sordida* G1. On the contrary, the capture of males by light-trapping was greater in *T. sordida* G2. These collections performed during the end of the dry season discard the idea of a temporal sex ratio modification where males are more common early in the season and females at the end, as observed by EKKENS (1981) for *T. rubida* in Arizona.

In the Argentinean Chaco, sylvatic *T. guasayana* colonizes bromeliads, cacti and tree cavities (WISNIVESKY-COLLI *et al.*, 1997). Except for cacti (not considered in this study), the same ecotopes are infested in the Bolivian Chaco. In bromeliads and hollow trees, nymphs were collected in low numbers (1 and 3 specimens, respectively) whereas previous studies reported their greater abundance in such ecotopes (WISNIVESKY-COLLI *et al.*, 1997; NOIREAU *et al.*, 1999b). Nevertheless, the presence of nymphal instars allows particular ecotopes to be considered as breeding sites.

Both *T. sordida* groups were collected in bromeliads and hollow trees, although our observations suggest that bromeliads are not important breeding sites since we found no nymphal instars amongst these plants. By contrast, our finding of numerous nymphs in tree cavities suggests that these constitute a favourable ecotope for both species. In spite of the greater abundance of *T. sordida* G2 in this area, *T. sordida* G1 adults largely predominate over G2 in parrot nests but the absence of nymphal instars calls into question the role of these nests as breeding sites. The detection of *T. sordida* G1 in parrot nests opens the idea of passive transport of eggs of this species among the feathers of parrots, which could help to explain the wider distribution of this group. The capture of *T. delpontei* in nests occupied by parrots confirms previous reports of ABALOS & WYGODZINSKY (1951), CARCAVALLO & MARTÍNEZ (1985) and SALVATELLA *et al.* (1993). Until now, *T. delpontei* has not been reported from Bolivia, possibly owing to misidentification as *T. platensis*.

In hollow trees, 88·2% of collected *T. infestans* DM were nymphal instars. The abundance of immature forms shows that hollow trees certainly constitute a very favourable ecotope for this species. The vertebrate hosts living in such hollow trees were not investigated but we observed rodent faeces in various cavities suggesting a possible association between *T. infestans* DM and rodents. In Andean foci, sylvatic *T. infestans* were found associated with *Galea musteloides*, the wild guinea-pig (BERMUDEZ *et al.*, 1993). *T. melanosoma*, a species closely related to *T. infestans*, was captured under tree bark in its sylvatic habitat (A. Martínez, unpublished data) and the similarity of habitat between *T. infestans* DM and *T. melanosoma* suggests that both species share ecological traits regarding habitat and related fauna. The infrequent detection of young nymphal stages in bromeliads (7 stage 1, 3 stage 2 and 2 stage 3) makes us question the significance of these habitats as breeding sites, and the finding of only occasional adults of *T. infestans* DM in parrot nests suggests that these are also not the preferred breeding site of this species.

As reported by FORATTINI *et al.* (1973) and NOIREAU *et al.* (1997a), *T. sordida* does not form large colonies in artificial structures. A similar observation was reported by WISNIVESKY-COLLI *et al.* (1993) in relation to *T. guasayana* in Argentina. Their inability to build up significant populations may be due to the fact that both species rarely complete more than 1 generation per year (SCHOFIELD, 1994). Our data obtained in bromeliads and hollow trees, where only small colonies of these species were observed, support this biological trait. In contrast, *T. infestans* forms large populations in domestic/peridomestic structures where it generally completes 2 generations per year (SCHOFIELD, 1994). This reproductive ability was also reflected in sylvatic ecotopes such as hollow trees where *T. infestans* colonies are larger than those of *T. sordida* and *T. guasayana*. Moreover, laboratory studies demonstrate that *T. infestans* females lay more eggs than *T. sordida* and *T. guasayana* (CARCAVALLO & MARTÍNEZ, 1985). Differences of oviposition also may explain the size difference between populations of these species in artificial or sylvatic habitat.

T. sordida G1 and *T. guasayana* collected in hollow trees were found infected by flagellates. A previous analysis performed by polymerase chain reaction on faecal samples of *T. sordida* and *T. guasayana* from the same area indicates that these flagellates were probably *Trypanosoma cruzi* (NOIREAU *et al.*, 1999b). *Triatoma infestans* DM was also infected by flagellates but its infection rate (2·5%) was lower than observed in sylvatic Andean populations: 73% in Cochabamba according to BERMUDEZ *et al.* (1993) and 100% in Caracato according to F. Noireau (unpublished data).

Except for research on Andean populations of *T. infestans* in Bolivia (TORRICO, 1946; DUJARDIN *et al.*, 1987; BERMUDEZ *et al.*, 1993), very few studies have considered sylvatic foci. Former reports indicate that *T. infestans* was occasionally found in sylvatic conditions in Argentina (MAZZA, 1943; BEJARANO, 1967), Paraguay (VELASQUEZ & GONZALEZ, 1959) and Brazil (BARRETTO *et al.*, 1963). Despite these reports, it was considered that *T. infestans* did not maintain sylvatic foci in these areas because most specimens were found in ecotopes relatively close to human dwellings (USINGER *et al.*, 1966). In a review dedicated to such records, BEJARANO (1967) mentions eggs, nymphs and adults collected in a great variety of ecotopes such as rocks, trunks of fallen trees, hollow trees, shelters or burrows of marsupials and rodents and bird nests. The important investigation of *T. infestans* sylvatic populations must be again considered in non-Andean areas after the detection of a new focus in the Bolivian Chaco.

Isolation between sylvatic and domestic *T. infestans* populations from Andean valleys of Bolivia is strongly indicated by field experiments combined with morphometric studies as well as RAPD analysis (CARLIER *et al.*, 1996; DUJARDIN *et al.*, 1997). In the Chaco, continuous exchange of insects between sylvatic and domestic habitats also seems unlikely because of the marked chromatic and morphometric differences as well as differences in mitochondrial DNA and chromosome banding (MONTEIRO *et al.*, 1999; F. Noireau & J. P. Dujardin, unpublished data; F. Panzera, unpublished data). However, the capacity of *T. infestans* DM to invade domestic habitats is still unknown because the current study was performed in an uninhabited area. Further field studies carried out in an inhabited site would allow the determination of whether *T. infestans* DM presents a trend toward domesticity. At last, the detection of sylvatic *T. infestans* in the Bolivian Chaco leads to questions about the ancestral population of this species (CARCAVALLO, 1998), when it was classically considered that Andean populations represented the original sylvatic focus (SCHOFIELD, 1988).

Acknowledgements

This study received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (grant 970182) and benefited from interna-

tional collaboration through the ECLAT network. J. L. Botetano gave invaluable assistance in the field work. Special thanks to C. Schofield and R. Carcavallo for reviewing the manuscript.

References

Abalos, J. W. & Wygodzinsky, P. (1951). *Las Triatominae argentinas (Reduviidae, Hemiptera)*. Instituto de Medicina Regional, Monografia No. 2, Publicación 601, Tucumán.

Barretto, M. P., Siqueira, A. F. & Côrrea, F. M. A. (1963). Estudos sôbre reservatórios e vetores silvestres do *Trypanosoma cruzi*. I. Encontro do *Triatoma infestans* em ecótopos silvestres. *Revista do Instituto de Medicina Tropical de São Paulo*, 5, 289–293.

Bejarano, J. F. R. (1967). Estado selvático de *T. infestans* y otros aspectos a tener en cuenta para la eliminación de la enfermedad de Chagas. *2das Jornadas entomoepidemiológicas argentinas*, 3, 171–196.

Bermudez, H., Balderrama, F. & Torrico, F. (1993). Identification and characterization of wild foci of *Triatoma infestans* in Central Bolivia. *American Journal of Tropical Medicine and Hygiene*, 49 (supplement), 371.

Carcavallo, R. U. (1998). Phylogeny of Triatominae. The *Triatoma infestans* complex. *Memórias do Instituto Oswaldo Cruz*, 93 (supplement), 68–70.

Carcavallo, R. U. & Martínez, A. (1985). Biologia, ecologia y distribución geográfica de los triatominos americanos. In: *Factores biológicos y ecológicos en la enfermedad de Chagas*, Carcavallo, R. U., Rabinovich, J. E. & Tonn, R. J. (editors). Buenos Aires: OPS-ECO/MSAS-SNCH, Vol. 1, pp. 149–208.

Carlier, L., Muñoz, M. & Dujardin, J. P. (1996). RAPD. In: *Proceedings of the 1st International Workshop on Population Genetics and Control of Triatominae*, Schofield, Dujardin & Jurberg (editors). Mexico City: INDRE, pp. 81–83.

Dujardin, J. P., Tibayrenc, M., Venegas, E., Maldonado, P., Desjeux, P. & Ayala, F. J. (1987). Isozyme evidence of lack of speciation between wild and domestic *Triatoma infestans* (Heteroptera: Reduviidae) in Bolivia. *Journal of Medical Entomology*, 24, 40–45.

Dujardin, J. P., Garcia-Zapata, M. T., Jurberg, J., Roelants, P., Cardozo, L., Panzera, F., Dias, J. C. P. & Schofield, C. J. (1991). Which species of *Rhodnius* is invading houses in Brazil? *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 85, 679–680.

Dujardin, J. P., Bermudez, H., Casini, C., Schofield, C. J. & Tibayrenc, M. (1997). Metric differences between silvatic and domestic *Triatoma infestans* (Heteroptera: Reduviidae) in Bolivia. *Journal of Medical Entomology*, 34, 544–551.

Ekkens, D. B. (1981). Nocturnal flights of *Triatoma* (Hemiptera: Reduviidae) in Sabino Canyon, Arizona. *Journal of Medical Entomology*, 18, 211–227.

Forattini, O. P., Ferreira, O. A., Souza, J. M. P. de, Rabello, E. X., Rocha e Silva E. O. da & Rodrigues, F. W. (1973). Medida da infestação domiciliar por *Triatoma sordida*. *Revista de Saúde Pública, São Paulo*, 7, 241–250.

Lent, H. & Wygodzinsky, P. (1979). Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas disease. *Bulletin of the American Museum of Natural History*, 163, 127–520.

Mazza, S. (1943). Comprobaciones de *Triatoma platensis, Eutriatoma oswaldoi, Panstrongylus seai* y *Psammolestes coreodes* en la Provincia de Santiago del Estero, todos ellos sin infestación y de *Eutriatoma sordida* con infestación por *S. cruzi*. Otros datos sobre infestación esquizotripanósica natural silvestre de *Triatoma infestans*. *Prensa Medica Argentina*, 30, 1–23.

Monteiro, F. A., Perez, R., Panzera, F., Dujardin, J. P., Galvão, C., Rocha, D., Noireau, F., Schofield, C. & Beard, C. B. (1999). Mitochondrial DNA variation of *Triatoma infestans* populations and its implication on the specific status of *T. melanosoma*. *Memórias do Instituto Oswaldo Cruz*, 94 (supplement 1), 229–238.

Noireau, F., Brenière, F., Ordoñez, J., Cardozo, L., Morochi, W., Gutierrez, T., Bosseno, M. F., Garcia, S., Vargas, F., Yaksic, N., Dujardin, J. P., Peredo, C. & Wisnivesky-Colli, C. (1997a). Low probability of transmission of *Trypanosoma cruzi* to humans by domiciliary *Triatoma sordida* in Bolivia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 91, 653–656.

Noireau, F., Flores, R., Gutierrez, T. & Dujardin, J. P. (1997b). Detection of wild dark morphs of *Triatoma infestans* in the Bolivian Chaco. *Memórias do Instituto Oswaldo Cruz*, 92, 583–584.

Noireau, F., Gutierrez, T., Zegarra, M., Flores, R., Brenière, F., Cardozo, L. & Dujardin, J.-P. (1998). Cryptic speciation in *Triatoma sordida* (Hemiptera: Reduviidae) from the Bolivian Chaco. *Tropical Medicine and International Health*, 3, 364–372.

Noireau, F., Flores, R. & Vargas, F. (1999a). Trapping sylvatic Triatominae (Reduviidae) in hollow trees. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 93, 13–14.

Noireau, F., Gutierrez, T., Flores, R., Brenière, F., Bosseno, M. F. & Wisnivesky-Colli, C. (1999b). Ecogenetics of *Triatoma sordida* and *T. guasayana* (Hemiptera: Reduviidae) in the Bolivian Chaco. *Memórias do Instituto Oswaldo Cruz*, 94, 451–457.

Salvatella, R., Basmadjian, Y., Rosa, R. & Puime, A. (1993). *Triatoma delpontei* Romaña & Abalos, 1947 (Hemiptera, Triatominae) en el Estado Brasileño de "Rio Grande do Sul". *Revista do Instituto de Medicina Tropical de São Paulo*, 35, 73–76.

Schofield, C. J. (1988). Biosystematics of the Triatominae. In: *Biosystematics of Haematophagous Insects*, Service, M. W. (editor). Oxford: Clarendon Press, Systematics Association Special Volume 37, 284–312.

Schofield, C. J. (1994). *Triatominae: Biology and Control*. West Sussex: Eurocommunica Publications Ed.

Schofield, C. J. & Dias, J. C. P. (1998). The Southern Cone Initiative against Chagas disease. *Advances in Parasitology*, 42, 1–27.

Schofield, C. J., Lehane, M. J., McEwan, P., Catalá, S. S. & Gorla, D. E. (1991). Dispersive flight by *Triatoma sordida*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 85, 676–678.

Schofield, C. J., Lehane, M. J., McEwan, P., Catalá, S. S. & Gorla, D. E. (1992). Dispersive flight by *Triatoma infestans* under natural climatic conditions in Argentina. *Medical and Veterinary Entomology*, 6, 313–317.

Torrico, R. A. (1946). Hallazgo de *Eratyrus mucronatus*, infestación natural de "vinchucas" de cerro y *Eutriatoma sordida* en Cochabamba. *Anales del Laboratorio Central, Cochabamba*, 1, 19–23.

Usinger, R. L., Wygodzinsky, P. & Ryckman, R. E. (1966). The biosystematics of Triatominae. *Annual Review of Entomology*, 11, 309–330.

Velasquez, C. J. & González, G. (1959). Aspectos de la enfermedad de Chagas en Paraguay. *Revista Goiana de Medicina*, 5, 357–373.

Wisnivesky-Colli, C., Gürtler, R. E., Solarz, N. D., Schweigmann, N. J., Pietrokovsky, S. M., Alberti, A. & Flo, J. (1993). Dispersive flight and house invasion by *Triatoma guasayana* and *Triatoma sordida* in Argentina. *Memórias do Instituto Oswaldo Cruz*, 88, 27–32.

Wisnivesky-Colli, C., Schweigmann, N. J., Pietrokovsky, S., Bottazi, V. & Rabinovich, J. E. (1997). Spatial distribution of *Triatoma guasayana* (Hemiptera: Reduviidae) in hardwood forest biotopes in Santiago de Estero, Argentina. *Journal of Medical Entomology*, 34, 102–109.

Received 27 July 1999; accepted for publication 1 September 1999

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (2000) 94, 28–32

Transmission ecology of the fly *Musca sorbens*, a putative vector of trachoma

Paul M. Emerson[1,2,3], Robin L. Bailey[1,2], Olaimatu S. Mahdi[1], Gijs E. L. Walraven[1] and Steve W. Lindsay[1,3] *[1]Medical Research Council Laboratories, P.O. Box 273, Banjul, The Gambia; [2]Clinical Research Unit, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 5HT, UK; [3]Department of Biological Sciences, Durham University, South Road, Durham DH1 3LE, UK*

Abstract

Recent evidence suggests that eye-seeking flies are important trachoma vectors. We conducted a series of investigations to identify which species of synanthropic flies are potential vector(s) of this blinding disease in The Gambia. Several species of fly were caught in fish-baited attractant traps placed in villages throughout the year (1997/98) but only 2 species, *Musca sorbens* and *M. domestica*, were caught from the eyes of children. *M. sorbens* comprised <10% of the total number of flies caught with attractant traps but was responsible for >90% of fly–eye contacts, the remainder were made by *M. domestica*. All fly species were more numerous in the wet season than the dry season. Eyes of young children are considered to be the main reservoir of *Chlamydia trachomatis*, the causative agent of trachoma. Collections of eye-seeking flies from children showed frequent fly–eye contacts (median [interquartile range], 3 [1·5–7] every 15 min). Children with potentially infective ocular or nasal discharge had twice as many fly–eye contacts than children with no discharge ($P < 0{\cdot}001$). There was no difference in exposure to fly–eye contacts if a child sat inside or outside a house ($P = 0{\cdot}273$). Female flies were more commonly caught from eyes than male ($P < 0{\cdot}001$). The presence of *Chlamydia* DNA was demonstrated by PCR on 2 of 395 flies caught from the eyes of children with a current active trachoma infection. Both positive flies were *M. sorbens*, one male and the other female. Further elucidation of *M. sorbens* behavioural ecology and the development of sustainable strategies to control these flies should be a priority. It is likely that *M. sorbens* is the principal insect vector of trachoma in The Gambia.

Keywords: *Musca domestica*, *Musca sorbens*, trachoma, *Chlamydia trachomatis*, transmission, vector behaviour, The Gambia

Introduction

Trachoma is an infectious blinding disease caused by the bacterium *Chlamydia trachomatis*. Worldwide, between 300 and 500 million people are believed to be affected, of whom an estimated 5·8 million are blind, making trachoma second only to cataract as a cause of blindness and the most common form of infectious blindness (THYLEFORS *et al.*, 1995).

The challenge of controlling trachoma has led to the Global Elimination of Trachoma by the Year 2020 Initiative (GET 2020), a WHO alliance whose aim is to eliminate trachoma as a blinding disease by 2020. It is understood that blindness from trachoma is a result of frequent infections over many years (MABEY *et al.*, 1992), but the routes of infection are incompletely understood. Several routes, with varying epidemiological significance, may exist. The most likely are fingers, fomites (infective discharges on clothes, bed sheets, towels, etc.) and flies (MACCALLAN, 1931; MUÑOZ & WEST, 1997). The eyes of children with active trachoma aré believed to be the principal reservoir of disease in endemic areas (MABEY *et al.*, 1992). Studies in The Gambia (BAILEY *et al.*, 1989) and Tanzania (WEST *et al.*, 1991) have found that cases of active trachoma cluster by household, supporting the idea that transmission occurs between subjects in close proximity.

Until recently the evidence implicating synanthropic flies as vectors of trachoma was largely anecdotal or circumstantial. Flies had been shown to be capable of transferring fluorescein between children's eyes (JONES, 1975), *Chl. trachomatis* had been cultured from flies after they were fed on heavily infected laboratory cultures (FORSEY & DAROUGAR, 1981) and high fly densities had been associated with outbreaks of trachoma in Morocco (REINHARDS *et al.*, 1968) and Egypt (MAXWELL LYONS & ABDINE, 1952; HAFEZ & ATTIA, 1958a).

A recent study from The Gambia (EMERSON *et al.*, 1999) provides the best evidence to date that flies are important in trachoma transmission. The study was conducted in 2 pairs of villages; one village from each pair received fly control using insecticide for 3 months, the other acted as a control. Fly control decreased the numbers of Muscid flies by around 75% and reduced fly–eye contact by >95% compared to controls. Cross-sectional trachoma surveys conducted at baseline and after 3 months showed that in the absence of flies there were 75% (95% CI 36–91) fewer new prevalent cases of trachoma in intervention villages compared to controls. On the basis of this result, fly control is likely to be the focus of interventions to interrupt trachoma transmission. This study was designed to identify the most likely vector(s) and to characterize exposure to the flies.

Address for correspondence: P. Emerson, MRC, P.O. Box 273, Banjul, The Gambia; fax +220 735 512,
e-mail pemerson@mrc.gm

Materials and Methods

Study site

The study was conducted in the Sanjal region of The Gambia between May 1997 and May 1998. Flies were collected from Wollof hamlets (300–700 inhabitants) which consisted of 12 to 20 family compounds. Houses in the hamlets were typically (>90%) single roomed and constructed from mud blocks. Most houses were roofed with grass thatch, the remainder with corrugated iron. Goats, sheep, dogs and poultry roamed freely between the houses during the day but were shut in or penned at night. Horses, donkeys and cattle were enclosed in pens or tethered adjacent to the owner's house. Houses and compounds were swept daily, with the refuse being piled outside each compound and occasionally burnt. There were few latrines and most adults defaecated away from the settlement in the bush. Young children defaecated in the compounds and carers cleaned up after them, throwing the faeces on to the refuse piles.

Background trachoma prevalence

Prior to the collection of entomological data all people aged ⩾ 3 months and resident in the hamlets were screened for trachoma by a community ophthalmic nurse from the Gambian National Eye Care Programme. Screening was conducted by everting the upper eyelid and visually examining the tarsal plate with a torch and a ×2·5 binocular loup. Eyes were graded according to the WHO simplified scale (THYLEFORS *et al.*, 1987), which classifies active trachoma as the presence of follicular trachoma (5 or more follicles >0·5 mm visible) or intense trachoma (50% of tarsal plate obscured by inflammation). People with symptomatic trachoma were offered

Biogeography of Triatominae (Hemiptera: Reduviidae) in Ecuador: Implications for the Design of Control Strategies

Fernando Abad-Franch/*, Aura Paucar C*/++, Carlos Carpio C*, César A Cuba Cuba, H Marcelo Aguilar V*/***, Michael A Miles/+**

Pathogen Molecular Biology and Biochemistry Unit, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel St., London WC1E 7HT, UK *Unidad de Medicina Tropical, Instituto 'Juan César García', Quito, Ecuador **Unidade de Parasitologia Médica-Patologia, Faculdade de Medicina, Universidade de Brasília, DF, Brasil ***Instituto Nacional de Higiene y Medicina Tropical 'Leopoldo Izquieta Pérez', Quito, Ecuador

Chagas disease control strategies strongly depend on the triatomine vector species involved in Trypanosoma cruzi *transmission within each area. Here we report the results of the identification of specimens belonging to various species of Triatominae captured in Ecuador (15 species from 17 provinces) and deposited in the entomological collections of the Catholic University of Ecuador (Quito), Instituto Oswaldo Cruz (Brazil), the Natural History Museum London (UK), the London School of Hygiene and Tropical Medicine (UK), the National Institute of Hygiene (Quito), and the Vozandes Hospital (Quito). A critical review of published information and new field records are presented. We analysed these data in relation to the life zones where triatomines occur (11 life zones, excluding those over 2,200 m altitude), and provide biogeographical maps for each species. These records are discussed in terms of epidemiological significance and design of control strategies. Findings relevant to the control of the main vector species are emphasised. Different lines of evidence suggest that* Triatoma dimidiata *is not native to Ecuador-Peru, and that synanthropic populations of* Rhodnius ecuadoriensis *in southern Ecuador-northern Peru might be isolated from their sylvatic conspecifics. Local eradication of* T. dimidiata *and these* R. ecuadoriensis *populations might therefore be attainable. However, the presence of a wide variety of native species indicates the necessity for a strong longitudinal surveillance system.*

Key words: Triatominae - biogeography - Chagas disease - control - *Triatoma dimidiata* - *Rhodnius ecuadoriensis* - Ecuador

Around 3 million people live under risk conditions for *Trypanosoma cruzi* transmission in Ecuador; prevalence estimates indicate that ~150,000 people are already infected (Aguilar et al. 1999). Sixteen triatomine species have been reported from the country (excluding doubtful records of *Triatoma infestans* and *Rhodnius prolixus*) (Aguilar et al. 1999). At least 13 of these species (see Tables I-III) are actual or potential vectors of Chagas disease (Lent & Wygodzinsky 1979, WHO 1991, Abad-Franch 2000). This complex situation is currently being investigated in the context of the National Programme for Chagas Disease Control in Ecuador. Our aim is to contribute to these efforts by analysing and completing the relevant information about Chagas disease triatomine vectors in Ecuador.

From 1998 to 2000, and as part of our research on the epidemiological significance of *Rhodnius* species in Ecuador and Peru, we undertook a revision of the Triatominae kept at various important entomological collections. During that revision, new data on distribution of some species in Ecuador became apparent. We added entomological records from our own fieldwork and from research being carried out by Ecuadorian colleagues. Here we report those new records, together with previously published data, and analyse them in relation to the main ecological and epidemiological traits of the different species. Finally, recommendations on vector control strategies are put forward.

Supported by the UNDP/World Bank/WHO TDR (Grant 970195), the ECLAT Network, the Tropical Medicine Unit at the University General Hospital of Valencia (Spain), the Cañada-Blanch Foundation (Spain), and Capes (Ministério de Educação e Cultura, Brasil).

+Corresponding author. Fax: +44-207-636.8739. E-mail: michael.miles@lshtm.ac.uk

++Current address: Systematics Research Collections, W-436 Nebraska Hall, University of Nebraska State Museum Lincoln, NE 68588-0514 USA

Received 31 July 2000
Accepted 21 November 2000

MATERIALS AND METHODS

For this study, we reviewed available published reports, and the specimens belonging to the subfamily Triatominae deposited at the Invertebrate Museum of the Catholic University of Ecuador, Quito, the Herman Lent and Rodolfo Carcavallo Collections (Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Departamento de Entomologia, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil), the Natural History Museum, London (UK), the London School of Hygiene and Tropical Medicine (UK), the Vozandes Hospital (Quito, Ecuador), and the National Institute of Hygiene and Tropical Medicine (Quito, Ecuador). Fieldwork and unpublished records were also included in our analysis.

Notes on epidemiological significance of each species were prepared on the basis of published information, unpublished reports, and field observations. For geographical information, we used the guide by Miranda (1995), the "Índice Toponímico" of the Instituto Geográfico Militar (IGM 1978-82, 1982-96), and cartographic material provided by the IGM. Life zones were established following the ecological maps proposed by Cañadas (1983) and Cañadas and Estrada (1978, cf. Dodson & Gentry 1991), based on the life zones described by Holdridge (1967). We included life zones under 2,200 m altitude in the analysis. In the distribution maps (Figure), marks indicate places of capture. Life zones where each species occur are light grey, and we added the outline and number of the life zones representing the potential distribution of each species (areas from where no records exist, but where ecological features are identical to other zones where captures have been made).

RESULTS*

Results on geographical and ecological distribution of species are shown in the Figure and in Tables I-III; 17 out of 21 provinces have produced records of triatomines (excluding doubtful reports from two more provinces). The studied species occupy 11 life zones in Ecuador. Life zones in the Andes range (2,200-6,310 m altitude) were not included in the analysis; apparently, only *T. carrioni* occurs there. Annual rains in areas where triatomines occur range between 62.5-125 mm/year in the tropical desert and 2,000-4,000 mm/year in the wet and moist forests (Table IV). Average temperatures ranged from 12-18°C in the low montane forest areas to 24-26°C in the coastal dry tropical forests (Table IV). Altitude range was 0-2,650 m (Tables I, IV). The maximum number of species recorded in a single life zone corresponds to the Amazon rainforest (seven species, excluding unconfirmed records of *T. dimidiata* and *R. ecuadoriensis*). These two latter species, deemed the main Chagas disease vectors in the country, have the widest distribution range; *T. dimidiata* has been reported from six different life zones in the country and *R. ecuadoriensis* from five (see Tables I, II, IV). The distributions of these and all the other species in relation to life zones are also summarised in Table IV.

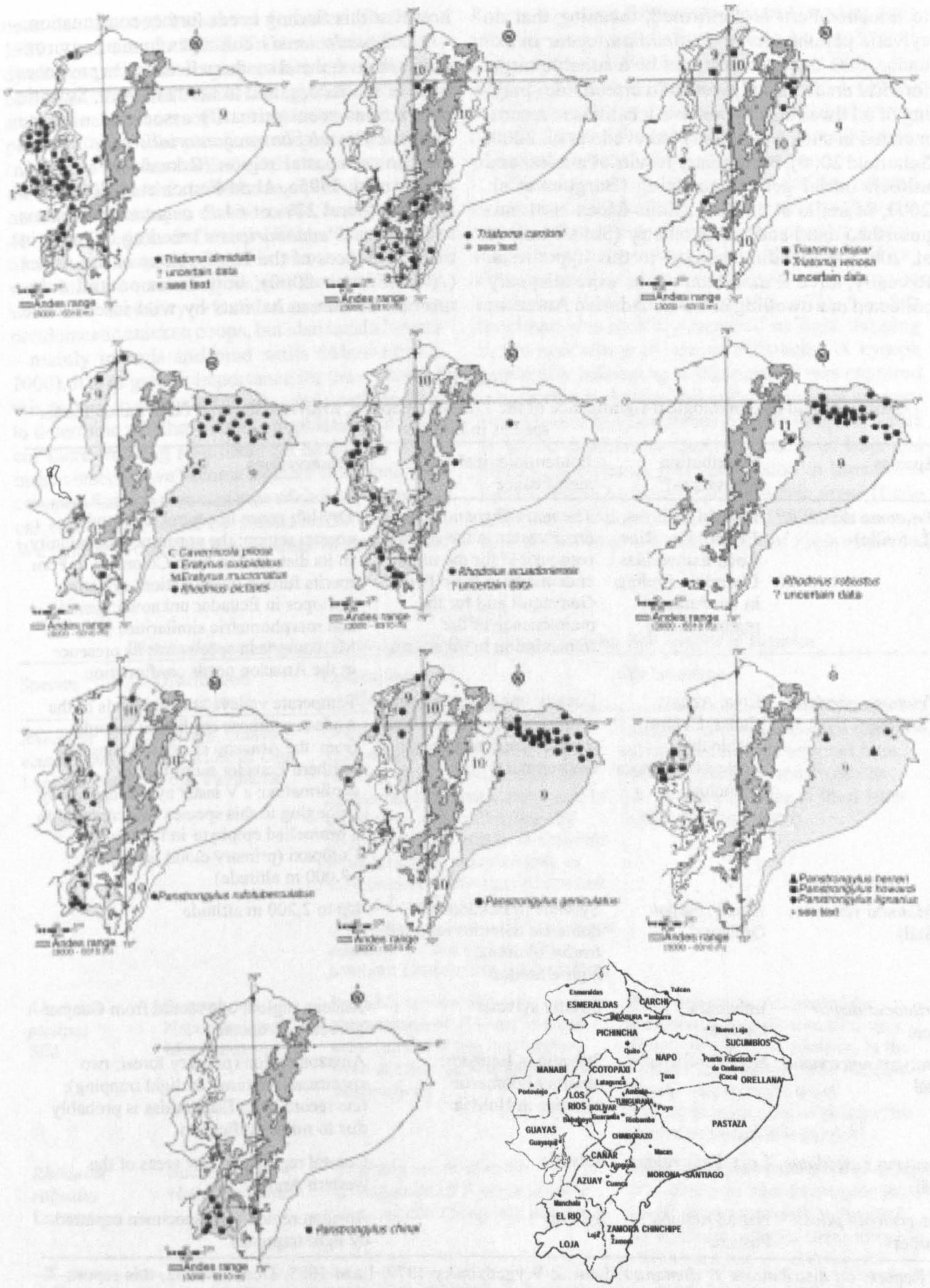
No records from the Galápagos Islands were found.

DISCUSSION

Complementary sampling and further analysis are indispensable to achieve an accurate idea of how triatomines are distributed in Ecuador, the way they are related to different ecological zones, their synanthropic behaviour, and their inter- and intraspecific relations. Records from some provinces are limited or do not exist. The ecological complexity of Ecuador is illustrated in the diversity of life zones (from desertic areas to pluvial forests). The high number of triatomine species also reflects such diversity.

T. dimidiata occurs only in low, dry areas of the coast, including the tropical desert in the Santa Elena peninsula (Guayas), and seems to be always in a domestic habitat. Only one report of adult specimens found in an allegedly sylvatic environment (under the bark of a dead tree) (cf. Zeledón 1981) was found. However, the actual presence of sylvatic colonies of this species in Ecuador has never been documented; specimens found in peridomestic terrestrial bromeliads and periurban rubbish dumps in Manabí (Vector Control Service, unpublished observations) cannot be considered as truly sylvatic. This fact, together with the apparent discontinuity in the distribution of the species from Mexico to Ecuador (absent from southern Colombia, except in some localities of the upper Magdalena valley) (Zeledón 1981, D'Alessandro & Barreto 1985, Carcavallo et al. 1999), makes us contemplate the hypothesis that it was introduced into coastal areas of Ecuador and Peru. Sea trade is known to have linked Mesoamerican and Ecuadorian-Peruvian cultures from ~1500 BC (Meggers & Evans 1963). As pointed out by Schofield et al. (1999), genetic and phenetic simplification generally impedes the re-adaptation of strictly domestic triatomines to new, unstable sylvatic habitats. If the introduction of such *T dimidiata* populations

*New field records were obtained after the preparation of the manuscript (province of Pichincha, life zone 9): *Triatoma dimidiata* (domestic colony), *Rhodnius ecuadoriensis* (adults in house), and *Panstrongylus rufotuberculatus* (adults in houses).



Biogeography of Ecuadorian Triatominae. Life zones - 1: tropical desert; 2: thick tropical bush; 3: very dry tropical forest; 4: dry premontane forest; 5: dry low montane forest; 6: dry tropical forest; 7: humid premontane forest; 8: humid low montane forest; 9: moist tropical forest; 10: wet premontane forest; 11: wet low montane forest (Ecuador Provinces); actual distribution: light grey areas; potential distribution: outlined white areas with number of life zone.

to Ecuador-Peru is confirmed, meaning that no sylvatic populations of *T. dimidiata* occur in Ecuador, then the species might be a suitable target for local eradication (pyrethroid insecticide spraying of all dwellings in positive localities is recommended in such a situation) (Acevedo et al. 2000, Schofield 2000). Preliminary results of nuclear and mitochondrial gene sequencing (Bargues et al. 2000, Marcilla et al. 2001, Solís-Mena et al. unpublished data) and morphometry (Solís-Mena et al. 2000) are lending support to this hypothesis. Recently, three *T. dimidiata* adults were allegedly collected in a dwelling in the Ecuadorian Amazon; however this finding needs further confirmation.

R. ecuadoriensis colonises human environments in central and southern Ecuadorian provinces west of the Andes, and in northern Peru. Sylvatic populations seem primarily associated with the endemic *Phytelephas aequatorialis* palm trees in the central coastal region (Romaña et al. 1994, Avilés et al. 1995a, Abad-Franch et al. 2000). We recently found 27% of 64 *P. aequatorialis* palms to harbour *R. ecuadoriensis* breeding colonies in three provinces of the Pacific slope of the Andes (Abad-Franch 2000); both invasion and colonisation of human habitats by wild insects have

TABLE I

Distribution and epidemiological significance of the *Triatoma* Laporte, *Eratyrus* Stal, and *Cavernicola* Barber species in Ecuador

Species	Distribution (provinces)[a]	Epidemiological significance	Observations
Triatoma dimidiata (Latreille)	Manabí, Guayas, El Oro, Los Ríos, Loja, Esmeraldas Uncertain finding in the Amazon region (Napo)	The main *Trypanosoma cruzi* vector in the country; responsible for the urban endemicity in the city of Guayaquil and for the maintenance of the transmission in other areas	Dry life zones in central and southern coastal region; the apparent discontinuity in its distribution from Colombia to Peru merits further investigation; sylvatic ecotopes in Ecuador unknown; genetic and morphometric similarities with Mesoamerican specimens; its presence in the Amazon needs confirmation
Triatoma carrioni Larrousse	Loja, Azuay, Cañar, El Oro, Pichincha, Cotopaxi, Zamora Chinchipe	Locally important in areas of the south, where colonies can be found in domestic environments	Temperate valleys and highlands of the Andean southern cordillera; records from the Amazon slope of Andes and northern Ecuador merit further confirmation; a V instar nymph apparently belonging to this species was captured in a bromeliad epiphyte in La Otonga, Cotopaxi (primary cloud forest, >2,000 m altitude)
Triatoma venosa (Stål)	Azuay, Napo/ Orellana[b]	Sylvatic in Ecuador – domestic colonies reported from Colombia; high altitudes	Up to 2,200 m altitude
Triatoma dispar Lent	Imbabura, Cotopaxi	Strictly sylvatic	Andean region; one record from Guayas probably erroneous
Eratyrus mucronatus Stål	Napo/Orellana[b]	Sylvatic in Ecuador; reports of domestic colonies in Bolivia	Amazon region (primary forest; two specimens captured by light trapping); one record from Esmeraldas is probably due to misidentification
Eratyrus cuspidatus Stål	Loja, Esmeraldas	Sylvatic	Coastal region and low areas of the western Andes
Cavernicola pilosa Barber	Napo/Orellana[b], Pastaza	Sylvatic	Amazon region (one specimen captured by light trapping)

a: Reports on distribution: *T. dimidiata*: Lent & Wygodzinsky 1979, Lazo 1985, Defranc 1982, this report; *T. carrioni*: Leon 1949, Espinoza 1955, Lent & Wygodzinsky 1979, Defranc 1982, Reyes 1982, this report; *T. venosa*: Defranc 1982, Lent & Wygodzinsky 1979, this report; *T. dispar*: Lent & Wygodzinsky 1979, this report; *E. mucronatus*: Defranc 1982, Lent & Wygodzinsky 1979, this report; *E. cuspidatus*: Defranc 1982; *C. pilosa*: Lent & Wygodzinsky 1979, this report; *b:* Napo and Orellana were separated into two provinces in 1998.

been documented in coastal Ecuador (Defranc 1987, Abad-Franch 2000). Treatment of infested dwellings and monitoring of re-infestations should be the recommended control strategy there. We also studied the presence of *R. ecuadoriensis* in temperate valleys of the provinces of Loja and El Oro (near the Peruvian border). Palm trees are completely absent in the studied areas, probably due to massive deforestation that occurred in the last 30-40 years (Dodson & Gentry 1991). Nine percent of 118 dwellings surveyed in two communities were infested (crowding indices from 42 to 78), with the majority of colonies breeding in peridomestic chicken coops, but also inside houses – mainly in beds and mud walls (Abad-Franch 2000). It is of greater importance for the control of this species in southern Ecuador and northern Peru to determine whether sylvatic populations still occur there (making re-infestation likely), or if domestic insects have become isolated from their wild conspecifics as a consequence of deforestation (local eradication could be attainable in this case). We have also detected the presence of strictly wild *R. ecuadoriensis* forms in northern Ecuador; their specific status needs however to be confirmed (Abad-Franch 2000, Abad-Franch et al. 2000). Finally, we found two specimens labelled as collected on the eastern slope of the Andes (marked as *uncertain data* on the map, as we suspect erroneous places of capture indicated on labels).

T. carrioni, an endemic species that has adapted to human habitats in the Andean valleys of southern Ecuador and northern Peru, occupies a wide range of life zones (dry and humid, 1,000-2,650 m altitude) and has been reported to feed on humans and horses (Lent & Wygodzinsky 1979). An adult specimen was recently captured by light trapping in the northern province of Pichincha. A nymph apparently belonging to this species was captured in an epiphytic bromeliad in the canopy of primary cloud forest in a neighbouring area (Figure, Table I). In the southern Andean provinces of Loja and Azuay, the species has been found in human-related habitats both in rural and urban areas (León 1949, Espinoza 1955, Defranc 1982). Some capture places (marked with + in the Figure) are lo-

TABLE II

Distribution and epidemiological significance of the *Rhodnius* Stål species in Ecuador

Species	Distribution (provinces)[a]	Epidemiological significance	Observations
Rhodnius ecuadoriensis Lent & León	Manabí, Guayas, Los Ríos, El Oro, Loja, Pichincha	Considered as the second main *Trypanosoma cruzi* vector in the country; able to invade-colonise human environments, and found to breed even within dwellings in good condition; related to domestic birds (chicken, pigeons) and, in wild environments in northern and central Ecuador, to palm trees (*Phytelephas aequatorialis*); sylvatic populations not reported from southern Ecuador nor northern Peru	Central and southern coastal region; sylvatic populations reported from Manabí, Los Ríos, and Pichincha (the taxonomic status of these latter is under investigation)
Rhodnius pictipes Stål	Sucumbíos, Napo/Orellana[b], Morona-Santiago	Probably involved in the transmission of *T. cruzi* in some areas; adults flying into houses, even in urban areas (unpublished observation)	Amazon region; we studied the presence of *R. stali*, a species very closely related to *R. pictipes*, in the Ecuadorian Amazon, with negative results (unpublished data); present in palm trees of at least five genera in Sucumbíos (unpubl.)
Rhodnius robustus Larrousse	Sucumbíos, Napo/Orellana[b]	Probably involved in the transmission of *T. cruzi* in some areas; adults flying into houses	Amazon region; palm trees of at least five genera in Sucumbíos (unpubl.); labelling errors probably involved in the finding of specimens allegedly collected in the Coastal region

a: Reports on distribution: *R. ecuadoriensis*: Lazo 1985, Defranc 1982, Carcavallo & Martínez 1985, Romaña et al. 1994, Abad-Franch et al. 2000, this report; *R. pictipes*: Espinoza 1955, Amunárriz 1991, Amunárriz et al. 1991, Chico et al. 1997, this report; *R. robustus*: Espinoza 1955, Amunárriz 1991, Amunárriz et al. 1991, Chico et al. 1997, this report; *b*: Napo and Orellana were separated into two provinces in 1998.

cated in Andean life zones over 2,200 m; data available indicate that the species has only been found there in human environments, but this does not preclude its presence in wild habitats as well. Control of this species should contemplate the possibility of re-infestations from wild populations; treating of infested homes and a strong surveillance component must therefore be recommended.

R. pictipes and *R. robustus* are recorded from the Ecuadorian Amazon – where seropositivity ranges from 0.8% to 6% (cf. Aguilar et al. 1999). We recently studied their presence in palm trees in the Ecuadorian Amazon region. Forty-six percent out of 56 palms surveyed were positive, including five genera (*Attalea, Astrocaryum, Oenocarpus, Phytelephas,* and *Elaeis*) (Palomeque et al. 2000). Several specimens labelled as *R. robustus* collected in western Ecuador (Loja and Los Ríos), are deposited at the H. Lent Collection (Instituto Oswaldo Cruz). This finding is most likely related to erroneous capture sites indicated on labels. Our investigations confirm that *R. stali*, a synanthropic species close to *R. pictipes* (Lent et al. 1993), is not known in the Ecuadorian Amazon.

P. rufotuberculatus is truly domestic in some areas of southern Ecuador, where breeding colonies have been found inside dwellings (Avilés et al. 1995b, Abad-Franch 2000). Similar behaviour has also been reported from Bolivia (Noireau et al. 1995, Dujardin et al. 1998) and Peru (Lizaraso 1955, Calderón et al. 1985). Adult insects may also invade houses attracted to electric light (Lent & Wygodzinsky 1979, Salomón et al. 1999). The

TABLE III

Distribution and epidemiological significance of the *Panstrongylus* Berg species in Ecuador

Species	Distribution (provinces)[a]	Epidemiological significance	Observations
Panstrongylus geniculatus (Latreille)	Imbabura, Manabí, Pichincha, Esmeraldas, Sucumbíos, Napo/Orellana[b]	Possibly involved in the transmission of *Trypanosoma cruzi* in some areas; adults flying into houses; reports of a trend towards domiciliation in Brazil	Broad distribution (from Argentina to Mexico); it can be found on both slopes of the Andes (slight but noticeable chromatic differences recorded between both populations)
Panstrongylus rufotuberculatus (Champion)	Imbabura, Pichincha, Manabí, Loja, El Oro, Los Ríos, Guayas	Locally important in areas of the south, where it is truly domestic	Western slope of the Andes in Ecuador, but broad distribution in the Americas (from Argentina to Mexico)
Panstrongylus howardi (Neiva)	Manabí	Unclear; adults found within human dwellings	Endemic species apparently restricted to a small geographical area; biology, sylvatic habitats and hosts unknown
Panstrongylus chinai (del Ponte)	Loja, El Oro	Unclear; reports on domestic colonies	Mainly sylvatic; southwestern region
Panstrongylus herreri Wygodzinsky	Napo/Orellana[b]	Sylvatic; one female captured inside a house; domestic in northern Peru, where it is the main vector of Chagas disease	Two records by our group in the Amazon basin; its presence in Ecuador could explain the record of *P. lignarius* from Azuay (see text and below)
Panstrongylus lignarius (Walker)	Sucumbíos	Sylvatic; recorded only from rainforests, with the exception of Cuenca (Azuay) in Ecuador (thus probably erroneous)	Only two records (western slope of Andes); its relation with *P. herreri* needs further investigation (a sylvatic specimen with mixed characters was collected in northern Ecuador Amazon); the record of the species in Azuay is probably due to misidentification or a labelling error

a: reports on distribution: *P. geniculatus*: Espinoza 1955, Rodríguez 1959, Defranc 1982, Amunárriz 1991, Amunárriz et al. 1991, Chico et al. 1997, this report; *P. rufotubercutalus*: Lazo 1985, Defranc 1982, Reyes 1992, this report; *P. howardi*: Defranc 1982, Lent & Wygodzinsky 1979, this report; *P. chinai*: Defranc 1982, Lent & Wygodzinsky 1979, Reyes 1992; *P. herreri*: Lent & Wygodzinsky 1979, Aguilar et al. 1999, this report; *P. lignarius*: Rodríguez 1961, Defranc 1987, Lent & Wygodzinsky 1979, this report; *b:* Napo and Orellana were separated into two provinces in 1998.

epidemiological significance of this species deserves further research in southern Ecuador. The possibility that this species colonises houses after domiciliated species are eliminated by control interventions has to be taken into account by control managers. The species occurs mainly in low, dry areas, but may also be found in zones of very humid premontane forest in northern Ecuador, where it is sylvatic but has been captured in houses when flying attracted to light.

TABLE IV

Main biogeographical traits of Ecuadorian Triatominae

Life zones	Temperature (°C)	Altitude (m)	Annual rains (mm)	Species[a]
Tropical desert	24	0-300	62.5-125	*Triatoma dimidiata* *Rhodnius ecuadoriensis*
Thick tropical bush	24-26	0-300	250-500	*T. dimidiata* *Panstrongylus geniculatus* *P. howardi*
Very dry tropical forest	24-26	0-300	500-1,000	*T. dimidiata* *R. ecuadoriensis* *P. rufotuberculatus* *P. chinai* *P. howardi*
Dry premontane forest	18-24	300	500-1,000	*T. dimidiata* *T. carrioni* *R. ecuadoriensis* *P. rufotuberculatus* *P. chinai* *Eratyrus cuspidatus*
Dry low montane forest	12-18	2,000-2,900	500-1,000	*T. carrioni* *T. dimidiata*[b] *P. chinai*
Dry tropical forest	24-25	0-300	1,000-2,000	*T. dimidiata* *P. geniculatus* *P. rufotuberculatus*
Humid premontane forest	18-24	300-1800	1,000-2,000	*T. carrioni* *T. venosa* *R. ecuadoriensis* *P. chinai*
Humid low montane forest	12-18	2,000-2,900	1,000-2,000	*T. carrioni*
Moist tropical forest[c]	24-25	0-300 W up to 600 E	2,000-4,000	*T. dispar* *R. pictipes* *R. robustus* *P. geniculatus* *P. herreri/lignarius* *E. cuspidatus* (W) *E. mucronatus* (E) *Cavernicola pilosa*
Wet premontane forest	18-24	300-1,800 W 600-1,800 E	2,000-4,000	*T. carrioni* *T. dispar* *T. venosa* *R. ecuadoriensis* *P. geniculatus* *P. rufotuberculatus*
Wet low montane forest	18-24	1,000-1,800	2,000-4,000	*R. robustus*

W: western slope of the Andes (Pacific side); E: eastern slope of the Andes (Amazon side), *a*: dubious records excluded; *b*: this record corresponds to one specimen labelled as collected in the city of Loja, and has to be regarded with caution as no further nor previous reports have been made; *c*: see footnote in Results.

P. geniculatus is broadly distributed throughout the continent, and occurs on both slopes of the Andes. Specimens from the Ecuadorian Amazon and coastal regions display conspicuous, apparently constant chromatic differences. The species has been involved in disease transmission in the Ecuadorian Amazon foci (cf. Aguilar et al. 1999). It seems to be readily attracted to electric light (Lent & Wygodzinsky 1979), and will also approach oil candles in the Ecuadorian Amazon (FS Palomeque, pers. commun.). Peridomestic colonies have been reported from the Brazilian Amazon (Valente et al. 1998, Valente 1999), but reports of domiciliation in Ecuador (Amunárriz 1991, Chico et al. 1997) need confirmation. The species is to be considered as a potential secondary vector in its distribution areas.

P. howardi, a little known species endemic to a dry area of Manabí, is quite commonly found entering human dwellings – as adult specimens, except for one report of a breeding colony in a peridomicile (Defranc 1982). Its sylvatic habitats and hosts remain unknown. The possibility that *P. howardi* transmits *T. cruzi* to people by colonising or invading human-related structures points to the necessity for entomological studies of this species.

P. chinai probably also plays a role in the wild cycle of *T. cruzi* transmission in some areas of southern Ecuador and northern Peru, but only few data are available. It has been found to breed in chicken coops and, occasionally, in human dwellings. Adults may be attracted to artificial light (Lent & Wygodzinsky 1979). The species may also behave as a secondary vector, thus entomological surveillance is a key for the success of control activities.

P. herreri has only been reported once from Ecuador (Aguilar et al. 1999). It is domestic in northern Peru, where it is the main vector of Chagas disease (Herrer 1956, 1977, Lent & Wygodzinsky 1979, Calderón et al. 1985). We recently identified a female *P. herreri* captured in a dwelling of an indigenous village in the province of Napo, confirming our previous record. We also examined a *P. lignarius* female (captured in primary rainforest) which has some mixed characters of *P. lignarius* and *herreri*. These closely related species showed reproductive compatibility under laboratory conditions (Barrett 1988), but no hybrids have been reported from nature (Lent & Wygodzinsky 1979). Their taxonomic status needs to be clarified as it has epidemiological implications. The only previous record of *P. lignarius* in Ecuador (in the Andean highlands city of Cuenca, province of Azuay) (Rodríguez 1959) may be due to misidentification with *P. herreri*, or to an erroneous capture site indicated on the label.

Other Triatominae species (*T. dispar, T. venosa, Eratyrus mucronatus, E. cuspidatus* and *Cavernicola pilosa*) seem to have little or no epidemiological significance in Ecuador; Figure and Table I show the main trends. Three of them (*T. venosa, E. cuspidatus,* and *E. mucronatus*) have however been reported to show some degree of synanthropism in different countries (Lent & Wygodzinsky 1979, D'Alessandro & Barreto 1985, Noireau et al. 1994), but seem to be mainly sylvatic in Ecuador.

The Ministry of Public Health is currently developing the strategy for the control of vector-borne Chagas disease in Ecuador. It will be based on updated information about the distribution and synanthropic behaviour of different triatomine species. The presence of a wide variety of them (~12% of all recognised species, and ~18% of South American species), in their majority present in wild environments, will be one of the main difficulties. Only one of these species, *T. dimidiata*, may be suspected of having been artificially introduced and therefore susceptible to eradication. The possibility that some southern domestic populations of *R. ecuadoriensis* are isolated from wild foci must be ascertained; if so, local elimination might also be attainable. Autochthonous species may behave as secondary vectors, occupying empty niches when domestic triatomines are eliminated by insecticide spraying. Various species are in their way to true domestication, while others show ability to invade houses without establishing breeding colonies there. A strong component of longitudinal vigilance with community involvement is recommended in such situations, complementing the use of residual pyrethroids (Dias 1991, Dias & Schofield 1999, Acevedo et al. 2000, Schofield 2000). This work aims to be a contribution to the development of the technical basis for the National Control Programme currently in preparation (including definition of risk areas, sero-entomological surveys, spraying interventions, and the establishment of longitudinal surveillance systems), and to provide researchers and public health authorities with baseline knowledge indispensable for the design of adequate strategies.

ACKNOWLEDGEMENTS

To CJ Schofield and J Jurberg for their help during the preparation of this work. To AG Guevara (Vozandes Hospital, Quito) and J Racines V (National Institute of Hygiene, Quito) for providing us with entomological records from their fieldwork, and to G Onore and M Webb for permitting us to review the material deposited at the Invertebrate Museum of the Catholic University of Quito and the Natural History Museum (London), respectively.

REFERENCES

Abad-Franch F 2000. *Ecology and Genetics of Chagas Disease Vectors in Ecuador. Implications for the Design of Control Strategies*, MPhil to PhD Upgrading Report, London School of Hygiene and Tropical Medicine, University of London, UK, 150 pp.

Abad-Franch F, Noireau F, Paucar CA, Aguilar VHM, Carpio CC, Racines VJ 2000. The use of live bait traps for the study of sylvatic *Rhodnius* populations (Hemiptera: Reduviidae) in palm trees. *Trans R Soc Trop Med Hyg 94*: 629-630.

Acevedo F, Godoy E, Schofield CJ 2000. Comparison of intervention strategies for control of *Triatoma dimidiata* in Nicaragua. *Mem Inst Oswaldo Cruz 95*: 867-871.

Aguilar VHM, Abad-Franch F, Paucar CA, Racines VJ 1999. Epidemiology of Chagas disease in Ecuador. A brief review. *Mem Inst Oswaldo Cruz 94* (Suppl. I): 387-393.

Amunárriz MU 1991. Enfermedad de Chagas. Primer foco amazónico. In MU Amunárriz, *Estudios sobre Patologías Tropicales en la Amazonia Ecuatoriana*, Ed. CICAME, Pompeya, Napo, Ecuador, p. 27-37.

Amunárriz MU, Chico ME, Guderian RH 1991. Chagas disease in Ecuador: a sylvatic focus in the Amazon region. *J Trop Med Hyg 94*: 145-149.

Avilés H, Cevallos P, Champaloux B, Goujon C, Lema F, Pajony R, Pays JF, Racines VJ, Romaña CA 1995a. *Rhodnius ecuadoriensis* en áreas endémicas de tripanosomiasis americana en Ecuador. *Parasitol al Día 19* (No. extraordinario): 231.

Avilés H, Cevallos P, Champaloux B, Goujon C, Lema F, Pajony R, Pays JF, Racines VJ, Romaña CA 1995b. *Panstrongylus rufotuberculatus* y características de la vivienda en la provincia de El Oro, Ecuador. *Parasitol al Día 19* (No. extraordinario): 230.

Bargues MD, Marcilla A, Ramsey JM, Dujardin JP, Schofield CJ, Mas-Coma S 2000. Nuclear rDNA-based molecular clock of the evolution of Triatominae (Hemiptera: Reduviidae), vectors of Chagas disease. *Mem Inst Oswaldo Cruz 95*: 567-573.

Barrett TV 1988. Current research on Amazonian Triatominae. *Mem Inst Oswaldo Cruz 83* (Suppl I): 441-447.

Calderón FGE, Figueroa K, Náquira F, Carcavallo RU, Canale DM 1985. Perú. In RU Carcavallo, JE Rabinovich, RJ Tonn (eds), *Factores Biológicos y Ecológicos de la Enfermedad de Chagas*, vol. II, CPEHS OPS/OMS, SNCh, Ministerio de Salud y Acción Social, República Argentina, p. 449-456.

Cañadas L 1983. *Mapa Bioclimático y Ecológico del Ecuador*, MAG-PRONAREG, Quito, Ecuador.

Cañadas L, Estrada W 1978. *Ecuador Mapa Ecológico*, MAG-PRONAREG, Quito, Ecuador.

Carcavallo RU, Martínez A 1985. Biología, ecología y distribución geográfica de los triatominos americanos. In RU Carcavallo, JE Rabinovich, RJ Tonn (eds), *Factores Biológicos y Ecológicos en la Enfermedad de Chagas*, Vol. I. CPEHS OPS/OMS, SNCh, Ministerio de Salud y Acción Social, República Argentina.

Carcavallo RU, Curto de Casas SI, Sherlock IA, Galíndez Girón I, Jurberg J, Galvão C, Mena Segura CA, Noireau F 1999 Geographical distribution and alti-latitudinal dispersion. In RU Carcavallo, I Galíndez Girón, J Jurberg, H Lent (eds), *Atlas of Chagas Disease Vectors in the Americas*, vol. III, Editora Fiocruz, Rio de Janeiro, p. 747-792.

Chico HM, Sandoval C, Guevara EA, Calvopiña HM, Cooper PJ, Reed SG, Guderian RH 1997. Chagas disease in Ecuador: evidence for disease transmission in an indigenous population in the Amazon region. *Mem Inst Oswaldo Cruz 92*: 317-320.

D'Alessandro A, Barreto P 1985. Colombia. In RU Carcavallo, JE Rabinovich, RJ Tonn (eds), *Factores Biológicos y Ecológicos de la Enfermedad de Chagas*, vol. II, CPEHS OPS/OMS, SNCh, Ministerio de Salud y Acción Social, República Argentina, p. 377-399.

Defranc MI 1982. *Enfermedad de Chagas*, Ed. Casa de la Cultura Ecuatoriana, Núcleo del Guayas, Guayaquil.

Defranc MI 1987. Prevalencia de la enfermedad de Chagas en el Ecuador. Informe 1983-1986. *Rev Ecuat Hig Med Trop 37*: 13-47.

Dias JCP 1991. Chagas disease control in Brazil: which strategy after the attack phase? *Ann Soc Belge Med Trop 71* (Suppl. I): 75-86.

Dias JCP, Schofield CJ 1999. The evolution of Chagas disease (American Trypanosomiasis) control after 90 years since Carlos Chagas discovery. *Mem Inst Oswaldo Cruz 94* (Suppl. 1): 103-121.

Dodson CH, Gentry AH 1991. Biological extinction in western Ecuador. *Ann Missouri Botanical Garden 78*: 273-295.

Dujardin JP, Forgues G, Torrez M, Martínez E, Córdoba C, Gianella A 1998. Morphometrics of domestic *Panstrongylus rufotuberculatus* in Bolivia. *Ann Trop Med Parasitol 92*: 219-228.

Espinoza LA 1955. Epidemiología de la enfermedad de Chagas en la República del Ecuador. *Rev Ecuat Hig Med Trop 12*: 25-105.

Herrer A 1956. Observaciones sobre la enfermedad de Chagas en la Provincia de Moyobamba (Depto. de San Martín). *Rev Medicina Experimental Lima 10*: 59-74.

Herrer A 1977. Reseña de la Entomología Médica en el Perú. I. Principales aspectos entomológicos en la bartonellosis, la trypanosomiasis y la leishmaniasis. *Rev Per Entomol 20*: 19-24.

Holdridge LR 1967. *Life Zone Ecology*, Tropical Science Center, San José, Costa Rica.

IGM-Instituto Geográfico Militar 1978-82. *Índice Toponímico de la República del Ecuador*, Publicación del Instituto Geográfico Militar (A-Q), Quito, Ecuador.

IGM-Instituto Geográfico Militar 1982-96. *Índice Toponímico de la República del Ecuador*, Publicación del Instituto Geográfico Militar (Q-Z), Quito, Ecuador.

Lazo R 1985. Ecuador. In RU Carcavallo, JE Rabinovich, RJ Tonn (eds), *Factores Biológicos y Ecológicos de*

la Enfermedad de Chagas, vol. II, CPEHS OPS/OMS, SNCh, Ministerio de Salud y Acción Social, República Argentina, p. 413-427.

Lent H, Wygodzinsky P 1979. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas disease. *Bull Am Mus Nat History 163*: 123-520.

Lent H, Jurberg J, Galvão C 1993. *Rhodnius stali* n. sp., afim de *Rhodnius pictipes* Stal, 1872 (Hemiptera: Reduviidae: Triatominae). *Mem Inst Oswaldo Cruz 88*: 605-614.

León LA 1949. Información sobre el problema de la enfermedad de Chagas en el Ecuador. *Bol Ofic Sanit Panam 28*: 569-585.

Lizaraso Y 1955. Nota sobre el hallazgo de *Triatoma dimidiata* y *Panstrongylus rufotuberculatus* en el Perú. *Rev Med Experimental Lima 9*: 119-121.

Marcilla JA, Bargues MD, Ramsey JM, Magallón-Gastélum E, Salazar-Schettino PM, Abad-Franch F, Dujardin JP, Schofield CJ, Mas-Coma S 2001. The ITS-2 of the nuclear rDNA as a molecular marker for populations, species, and phylogenetic relationships in Triatominae (Hemiptera: Reduviidae), vectors of Chagas disease. *Mol Phylogenet Evol 18*: 136-142.

Meggers BJ, Evans C 1963. *Aboriginal Cultural Development in Latin America: an Interpretative Review*, Smithsonian Miscellaneous Collections, vol. 146, no.1, The Smithsonian Institution, Washington DC, USA.

Miranda J 1995. *Elementos de Cartografía Básica*, Colección de Documentos Didácticos no.1, Centro Panamericano de Estudios e Investigaciones Geográficas, Quito, Ecuador.

Noireau F, Bosseno MF, Carrasco R, Tellería J, Vargas F, Camacho C, Yaksic N, Brenière SF 1995. Sylvatic triatomines (Hemiptera: Reduviidae) in Bolivia: trends towards domesticity and possible infection with *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae). *J Med Entomol 32*: 594-598.

Palomeque FS, Abad-Franch F, Suárez K, Aguilar VHM 2000. Uso de trampas de cebo vivo para el estudio de poblaciones silvestres de *Rhodnius* (Reduviidae: Triatominae) en palmeras de la Amazonia Ecuatoriana. Memorias, XIV Jornadas Equatorianas de Biología, p. 108.

Romaña C, Racines VJ, Avilés H, Lema F 1994. Observaciones de domiciliación de *Rhodnius ecuadoriensis* en focos endémicos de la enfermedad de Chagas en el Ecuador. *Microbiología 1*: 59.

Reyes LV 1992. Enfermedad de Chagas. In R Sempértegui, P Naranjo, M Padilla (eds), *Panorama Epidemiológico del Ecuador*, Ministerio de Salud Pública, UNICEF, Quito, Ecuador, p. 134-137.

Rodríguez JD 1959. Epidemiología de la enfermedad de Chagas en la República del Ecuador. *Rev Goiana Med 5*: 411-438.

Rodríguez JD 1961. Nuevos datos sobre la enfermedad de Chagas en Guayaquil. *Rev Ecuat Hig Med Trop 18*: 48-52.

Salomón OD, Ripoll CM, Rivetti E, Carcavallo RU 1999. Presence of *Panstrongylus rufotuberculatus* (Champion, 1899) (Hemiptera: Reduviidae: Triatominae) in Argentina. *Mem Inst Oswaldo Cruz 94*: 285-288.

Schofield CJ 2000. Challenges of Chagas disease vector control in Central America. Position paper WHO/CDS/WHOPES/GCDPPH/2000.1. WHO, Communicable Diseases Control, Prevention and Eradication, WHO Pesticide Evaluation Scheme (WHOPES), Geneva, Switzerland.

Schofield CJ, Diotaiuti L, Dujardin JP 1999. The process of domestication in Triatominae. *Mem Inst Oswaldo Cruz 94* (Suppl. I): 375-378

Solís-Mena S, Patterson JS, Abad-Franch F 2000. Studying the origin of Ecuadorian *Triatoma dimidiata* populations (Hemiptera: Reduviidae: Triatominae). Poster, The Trypanosome Evolution Workshop, University of Exeter/London School of Hygiene and Tropical Medicine, London, UK.

Valente VC 1999. Potential for domestication of *Panstrongylus geniculatus* (Latreille, 1811) (Liemiptera, Reduviidae, Triatominae) in the municipality of Muará, Marajó Island, State of Pará, Brazil. *Mem Inst Oswaldo Cruz 94* (Suppl. I): 399-400.

Valente VC, Valente SAS, Noireau F, Carrasco HJ, Miles MA 1998. Chagas disease in the Amazon basin: association of *Panstrongylus geniculatus* (Hemiptera: Reduviidae) with peridomestic pigs. *J Med Entomol 35*: 99-103.

WHO-World Health Organization 1991. Control of Chagas disease. WHO Technical Report Series 811.

Zeledón R 1981. *El Triatoma dimidiata y su Relación con la Enfermedad de Chagas*, Ed. Universidad Estatal a Distancia, San José, Costa Rica, 146 pp.

Molecular Phylogenetics and Evolution
Vol. 18, No. 1, January, pp. 136–142, 2001
doi:10.1006/mpev.2000.0864, available online at http://www.idealibrary.com on IDEAL®

The ITS-2 of the Nuclear rDNA as a Molecular Marker for Populations, Species, and Phylogenetic Relationships in Triatominae (Hemiptera: Reduviidae), Vectors of Chagas Disease

Antonio Marcilla,* Maria Dolores Bargues,* Janine M. Ramsey,†
Ezequiel Magallon-Gastelum,‡ Paz María Salazar-Schettino,§ Fernando Abad-Franch,¶,‖
Jean-Pierre Dujardin,** Christopher John Schofield,‖ and Santiago Mas-Coma*

*Departamento de Parasitologia, Facultad de Farmacia, Universidad de Valencia, Av. Vicente Andres Estelles s/n, 46100 Burjassot, Valencia, Spain; †Centro de Investigaciones sobre Enfermedades Infecciosas, Instituto Nacional de Salud Publica, Av. Universidad 655, Col. Santa Maria Ahuacatitlan, 62508 Cuernavaca, Morelos, Mexico; ‡Departamento de Salud Publica, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Aptdo. Postal 4-119, Guadalajara, Jalisco, Mexico; §Laboratorio Biologia de Parasitos, Departamento de Microbiologia y Parasitologia, Facultad de Medicina, U.N.A.M., Mexico, D.F., Mexico; ¶Instituto "Juan Cesar Garcia", Fundacion Internacional de Ciencias Sociales y Salud, Casilla Postal 17-1106292 Quito, Ecuador; **UMR IRD/ORSTOM-CNRS 9926, Institut de Recherche pour le Developpement (IRD), Av. Agropolis 911, 34032 Montpellier Cedex 1, France; and ‖Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1 E7HT, United Kingdom

Received April 5, 2000

The nucleotide sequences of the rDNA second internal transcribed spacer (ITS-2) of 31 populations of 12 and 3 species of the two main Triatominae tribes Triatomini and Rhodniini, including the most important Chagas disease vectors, were obtained. Sequence comparisons and parsimony, distance, and maximum-likelihood analyses indicate that ITS-2 is a useful marker for resolving supraspecific, specific, subspecific, and even sometimes population-level relationships in Triatominae. Results were markedly different between species of Triatomini and Rhodniini, suggesting polyphyly. Phylogenetic trees support an old divergence between South American and North–Central American Triatomini and query the validity of some genera (*Dipetalogaster, Psammolestes*). The very low sequence variation between species of the *phyllosoma* complex suggests that subspecific ranking would be more appropriate. *Triatoma dimidiata* proves to be a clearly differentiated species, with several populations evidencing a clinal variation along a north–south axis and a population from Yucatan showing differences consistent with specific status. © 2001 Academic Press

Key Words: **ribosomal DNA; ITS-2; Triatominae; Hemiptera; nucleotide sequence; phylogenetic analyses.**

INTRODUCTION

The reduviid subfamily Triatominae (Hemiptera: Prosorrhyncha) includes 129 blood-sucking bug species (Dujardin *et al.*, 2000) of medical interest because of acting as vectors of *Trypanosoma cruzi*, which causes Chagas disease. The most important are *Triatoma infestans*, of wide distribution throughout the southern and northeastern parts of South America, *T. brasiliensis* in northeastern Brazil, *T. dimidiata* in Mexico, Central America, Colombia, Ecuador and Peru, *Rhodnius prolixus* in parts of Central America, Venezuela, and Colombia, *Panstrongylus megistus* in Brazil and Paraguay, and *T. sordida* in Brazil, Paraguay, Argentina, and Uruguay.

In Mexico, up to 18 species have been reported with natural infection by *T. cruzi* (Lent and Wygodzinsky, 1979; Zarate and Zarate, 1985), among which are the species belonging to the *T. phyllosoma* complex (Schofield, 1994). The taxonomic problem posed by this complex concerns the species/subspecies rank of its members (Usinger, 1944; Lent and Wygodzinsky, 1979), several of which are capable of cross-species mating in the laboratory, with production of viable F1 offspring (Mazzotti and Osorio, 1942). Further studies using molecular tools are urgently needed to clarify the validity of species/subspecies designations and for eco-epidemiological studies of the importance of the different complex members in the transmission of Chagas disease in Mexico.

Different regions of the nuclear ribosomal DNA (rDNA) have been shown to be valuable targets for defining markers for systematic and phylogenetic studies. The two internal transcribed spacers (ITS-1 and ITS-2) are useful for resolving affiliations of closely related taxa that have diverged relatively recently (<50 million years ago) and excellent markers for spe-

1055-7903/01 $35.00

cies distinction and hibridization experiments. Moreover, ITSs usually present tandemly repeated sequences or microsatellites (Almeyda-Artigas *et al.*, 2000), whose unit of repetition is between 1 and 5 bp and which are very good polymorphic molecular markers for the differentiation of populations within a given species (Jarne and Lagoda, 1996). The ITS sequences use to have the same length in different species of the same genus; however, pronounced differences in ITS length related to the presence of microsatellite sequences have been sometimes found (Almeyda-Artigas *et al.*, 2000). The sequencing of only one of the two spacers is sufficient, due to the structural conservatism and apparent coevolution of ITS regions.

This paper aims to analyze the usefulness of the ITS-2 in Triatominae. Especially selected species, the most important Chagas disease vectors included, were used to verify the information that this spacer can furnish about their phylogeny, as well as to analyze relationships among tribes, genera, closely related species, and different populations within a given species. This paper demonstrates the ITS-2 suitability for future molecular systematic studies of this important group of vectors.

MATERIALS AND METHODS

Triatominae Materials and Molecular Techniques

A total of 35 adult specimens from 31 populations of 12 species of Triatomini and 3 species of Rhodniini were studied (Table 1). The DNA was extracted from more than 1 specimen of a given population and from more than 1 population of a given species when necessary for sequence conservation verification studies. Complete dry triatomine bugs and legs were used for DNA extraction according to the standard phenol–chloroform technique (Sambrook *et al.*, 1989). DNA extraction was performed according to Bargues and Mas-Coma (1997). The fragment corresponding to a 127-bp sequence of the 5.8S rRNA gene and the ITS-2 were amplified by PCR following Almeyda-Artigas *et al.* (2000). The first PCR amplification was performed using forward (5′-CTAAGCGGTGGATCACTCGG) (5,8T) and reverse (5′-GCACTATCAAGCAACACGACTC) (28T) primers. The PCR fragment obtained was subcloned in the pGEM-T vector system (Promega, Madison, WI) and sequenced using vector primers (T7 and SP6). Internal primers were designed for ITS-2 amplification. Sequencing was performed on both strands by the dideoxy chain-termination method and with the *Taq* dye-terminator chemistry kit for ABI 373A (Perkin–Elmer, Foster City, CA), using PCR primers.

Sequence Alignment and Phylogenetic Analysis

Sequences were aligned using CLUSTAL W version 1.8 (Thompson *et al.*, 1994). Aligned sequences are obtainable at the EMBL database (ftp://ftp.ebi.ac.uk/pub/databases/embl/align/) or on request from the senior author. Distance, maximum-parsimony (MP), and maximum-likelihood (ML) methods were used in phylogeny reconstruction. For distance analysis, neighbor-joining (NJ) trees were generated from a Kimura two-parameter (Kimura, 1980) and a Tamura–Nei (Tamura and Nei, 1993) distance matrices, using PAUP v4.0b 3a (Swofford, 1999) and the TREECON v1.3b (Van De Peer and De Wachter, 1994) programs. Statistical support of each NJ tree was assessed with bootstrap-resampling technique (Felsenstein, 1985) over 1000 replications. MP analyses were performed using heuristic and branch-and-bound algorithms provided in PAUP, relative support for branches being assessed by Bremer values (Bremer, 1988, 1994) using the TreeRot.v2 program (Sorenson, 1999) and the bootstrap (1000 replicates, each with simple addition sequences and TBR branch swapping). ML trees were constructed with PAUP using the HKY85 model (Hasegawa *et al.*, 1985), a quartet puzzling analysis (with 1000 puzzling steps) being employed to assess tree precision.

RESULTS

Sequence Analyses

A total of 22 Triatominae ITS-2 sequences have been deposited in the GenBank–EMBL (Table 1). Rhodniini species show an ITS-2 markedly longer than Triatomini species (Table 1). Of the 884 positions including gaps required to align all the 20 different sequences of the 12 Triatomini and 3 Rhodniini species studied (*T. longipennis* and *T. picturata* presented identical sequence, as did the two *R. stali* varieties), 706 nucleotide positions (79.8%) were variable. Base composition was strongly A + T biased: 76.7% in Triatomini; 76.2% in Rhodniini. Because of the high variation detected in the alignment of all Triatominae studied, we decided to consider both tribes separately.

Triatomini. *T. dimidiata* sequences from Honduras and Ecuador were identical and differed in only 3 positions from that of Nicaragua. Mexican *T. dimidiata* sequences (Yucatan populations excluded) were identical, except for one deletion detected in San Luis Potosi populations and the interrupted microsatellite $(AT)_5$ TTT $(AT)_6$ in Veracruz, whereas all other presented $(AT)_5$ TTT $(AT)_7$. They differ in 7–10 nucleotide positions from those of Honduras, Ecuador, and Nicaragua. *T. dimidiata* (Yucatan populations excluded) proved to be a species clearly differentiated from the other taxa of the *T. phyllosoma* complex, owing to the high number (25–34) of nucleotide differences between the *T. dimidiata* populations and the phyllosoma complex members.

Sequence divergence between the five Mexican *T. phyllosoma* complex species (*T. dimidiata* populations

TABLE 1

Species of Triatominae Chosen for Sequencing of rDNA ITS-2

Taxon	Source[a]	GenBank Accession No.	Length (bp)
Triatomini			
Triatoma infestans Klug, 1834	La Paz, Bolivia	AJ286874	454
	1° de Marzo, Cordillera, Paraguay	AJ289876	458
Triatoma sordida (Stal, 1859)	Izozog, Sta. Cruz, Bolivia	AJ293589	446
Triatoma brasiliensis Neiva, 1911	Ceara, Brazil	AJ293591	465
Triatoma barberi Usinger, 1939	Rojas de Cuauhtemoc, Oaxaca, Mexico	AJ293590	470
Triatoma dimidiata Latreille, 1811	Oaxaca, Mexico	AJ286878	477
	Cajones, Morelos, Mexico	AJ286878	477
	Emiliano Zapata, Veracruz, Mexico	AJ286877	475
	Tanchahuil, San Luis Potosi, Mexico	AJ286879	476
	Barrio Tzitzi, San Luis Potosi, Mexico	AJ286879	476
	Izamal, Yucatan, Mexico	AJ286880	473
	Yaxkukul, Yucatan, Mexico	AJ286880	473
	San Jose, southern Honduras	AJ286875	476
	Pedro Carbo, Guayaquil, Ecuador	AJ286875	476
	laboratory raised, Guayaquil, Ecuador	AJ286875	476
	Madriz, Nicaragua	AJ286876	477
Triatoma phyllosoma Burmeister, 1835 (= *Meccus phyllosoma* Stal, 1859)	San Pedro Totolapan, Oaxaca, Mexico	AJ286881	470
	El Camaron, Oaxaca, Mexico	AJ286881	470
	Chalcatzingo, Morelos, Mexico	AJ286882	470
Triatoma pallidipennis (Stal, 1872) (= *Meccus pallidipennis* Stal, 1872; = *T. phyllosoma pallidipennis* Usinger, 1944; = *T. p. usingeri* Mazzotti, 1943 *pro parte*)			
Triatoma longipennis Usinger, 1939 (= *T. phyllosoma intermedia* Usinger, 1944; = *T. p. longipennis* Usinger, 1944; = *T. p. usingeri* Mazzotti, 1943 *pro parte*)	San Martin de Hidalgo, Jalisco, Mexico (2)	AJ286883	470
	Cuxpala, Zacatecas, Mexico (2)	AJ286883	470
	Nogueras, Colima, Mexico	AJ286883	470
	Colorado de la Mora, Nayarit, Mexico	AJ286883	470
	San Martin de Hidalgo, Jalisco, Mexico (2)	AJ286884	470
Triatoma picturata Usinger, 1939 (= *T. phyllosoma picturata* Usinger, 1944)	Oaxaca, Mexico	AJ286885	468
Triatoma mazzottii Usinger, 1941 (= *Meccus phyllosoma* Champion, 1899 *nec* Burmeister, 1835; = *T. phyllosoma mazzottii* Usinger, 1944)	Lab strain INLASA, La Paz, Bolivia orig. from Fiocruz, Belo Horizonte, Brazil	AJ286886	559
Panstrongylus megistus (Burmeister, 1835)	La Roma, Baja California, Mexico	AJ286887	475
Dipetalogaster maxima (Uhler, 1894)	Reference strain, Institute Oswaldo Cruz, Rio de Janeiro, Brazil	AJ286888	694
Rhodniini			
Rhodnius prolixus Stal, 1859	Alto Beni, Bolivia	AJ286889	684
Rhodnius stali Lent, Jurberg et Galvão, 1993	Alto Beni, Bolivia (2)	AJ286890	684
pale variety	Realeza, Ceara, Brazil	AJ286891	696
dark variety			
Psammolestes tertius Lent et Jurberg, 1965			

[a] Number of specimens sequenced in parentheses (noted only when more than one specimen were sequenced).

excluded) was even lower (1.5%) than that between *T. dimidiata* populations, pairwise comparisons showing only two to six nucleotide differences. All species presented the microsatellite $(AT)_4$ TTT $(AT)_6$, except *T. mazzottii*, which presented $(AT)_4$ TTT $(AT)_5$. Concerning *T. phyllosoma*, *T. picturata*, and *T. longipennis*, no differences were found between populations or specimens studied within each species. Moreover, *T. longipennis* and *T. picturata* were identical.

Both *T. dimidiata* populations from Yucatan were identical, showing a high number of nucleotide differences compared to the other *T. dimidiata* populations of Mexico (24–27), Honduras–Ecuador (21), and Nicaragua (23) and to the phyllosoma complex members (20–23).

T. infestans populations from Bolivia and Paraguay presented two transversions and a different length due to a different number of a microsatellite repeat (AT_6 in Bolivia and AT_8 in Paraguay).

The alignment of the 15 *Triatoma* species was 505 positions long, showing a 35.8% sequence divergence. Interestingly, ITS-2 lengths of South American species were always shorter than those of North and Central American species. *T. sordida* presented the shortest length, mainly related to the supression of a 14-bp zone compared to the other South American *Triatoma* spe-

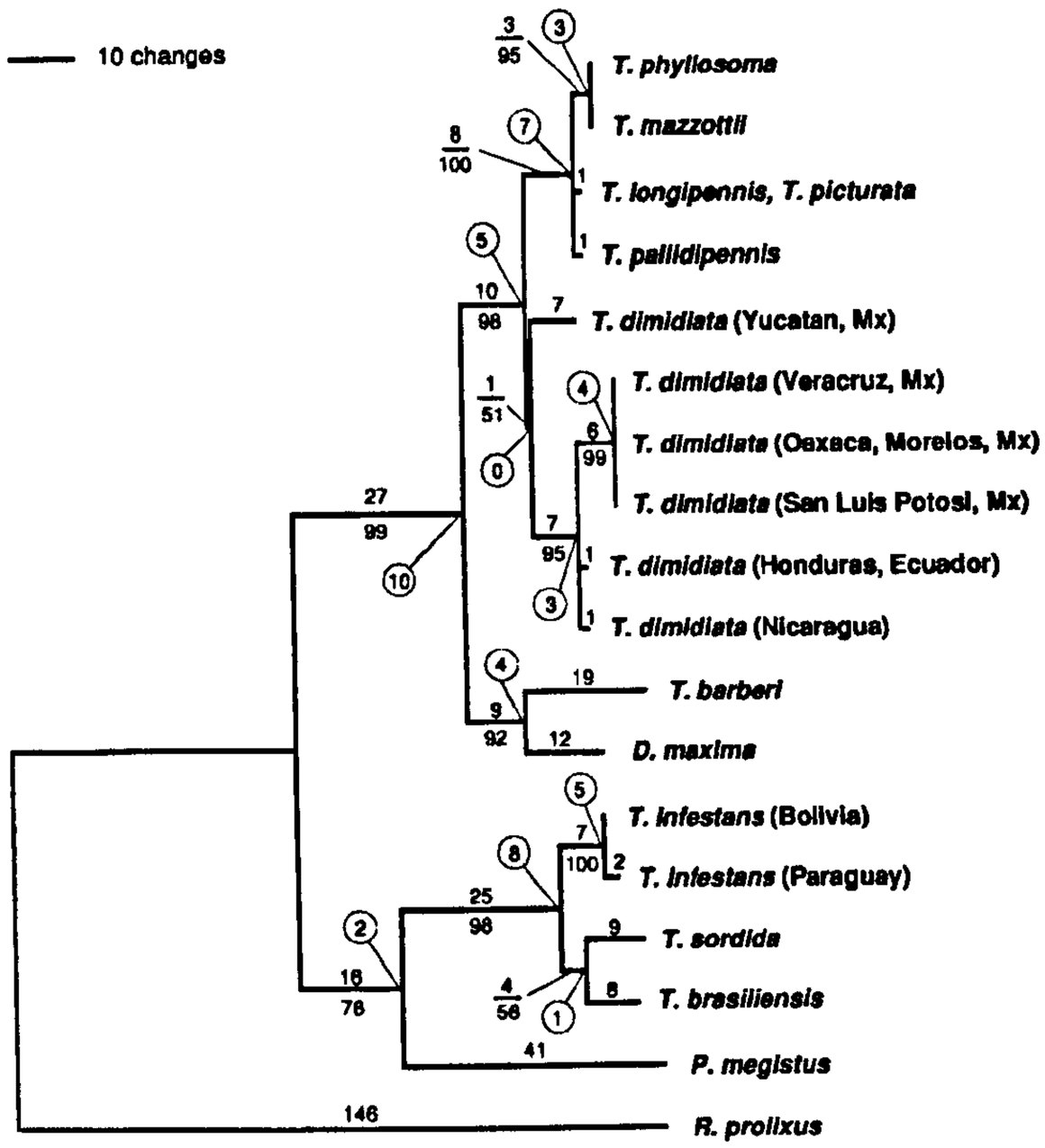


FIG. 1. Phylogenetic tree of the Triatomini species studied, based on maximum-parsimony analysis using the heuristic option. Numbers above the line indicate branch lengths (steps); numbers below the line represent the percentage of 1000 bootstrap replicates. Encircled numbers indicate Bremer support values for nodes.

cies. The complete alignment of the ITS-2 sequences of the 18 Triatomini species analyzed showed a length of 581 positions. Of these, 294 sites were constant and 97 sites were phylogenetically informative.

Rhodniini. The alignment of the ITS-2 sequences obtained from Rhodniini species consisted of 815 characters. Only a low percentage of similarity of 52.8% was detected, each species studied presenting a markedly different ITS-2 sequence. The ITS-2 of the *R. stali* pale and dark varieties were identical.

Phylogenetic Analyses

Due to the substantial variation in the alignment of all Triatominae ITS-2 sequences, we constructed phylogenetic trees for Triatomini and for Rhodniini species separately.

Parsimony analysis, using the heuristic option, of the aligned sequences of the Triatomini species, with *R. prolixus* as outgroup, yielded a single most-parsimonious tree (Fig. 1). The tree obtained was 371 steps long. The consistency index (CI) and the homoplasy index (HI) were 0.870 and 0.129, respectively. CI and HI excluding uninformative characters were 0.755 and 0.245, respectively. The retention index (RI) and the rescaled consistency index (RC) were 0.861 and 0.750, respectively. Two different clades separating North and Central American Triatomini from South American members of the same tribe appeared. A clear divergence of five *T. dimidiata* populations from the other species of the *T. phyllosoma* complex appeared, with very high bootstrap values supporting the close relationships between the *T. dimidiata* populations (95%) and between the other *T. phyllosoma* complex species (100%). *T. dimidiata* from Yucatan was included in the cluster of the other *T. dimidiata* populations but with only a low bootstrap value (51%). *Dipetalogaster maxima* and *T. barberi* clustered together with a high bootstrap value (92%) and represent a sister group of the *T. phyllosoma–T. dimidiata* lineages. *Panstrongylus megistus* appeared basal to the South American species belonging to the *Triatoma* genus. Bremer values supporting nodes were high (decay index ≥4) for 7 of the 12 test clades, the total support index *ti* being 0.135.

The topologies of the Triatomini phylogenetic trees derived from Kimura two-parameter and Tamura–Nei distance matrices using the NJ method (Fig. 2) were similar to each other and similar to the MP tree. The two groupings of North–Central and South American species again appear. NJ trees confirm the divergence between *T. dimidiata* and the other species of the *T. phyllosoma* complex. These two branches are supported with a 100% bootstrap value each. *T. dimidiata* from Yucatan is linked to the other *T. dimidiata* populations with only a 61% bootstrap support. Low bootstrap values only appear in the groupings of *T. dimidiata* populations from Veracuz and Oaxaca, those of Honduras–Ecuador and Nicaragua, and those of *T. brasiliensis* and *T. sordida*.

The topology (tree not shown) which resulted from the ML search (likelihood = −2566.05245) and the respective supports for the branches were similar to those obtained with the MP and NJ methods.

The Rhodniini species were analyzed using *T. infestans* as outgroup. With MP analysis, using the branch-and-bound and heuristic algorithms and with NJ analysis using the distance data measured with the Kimura two-parameter, the topology and the bootstrap values obtained in both analyses were identical (Fig. 3). *R. stali* appears basal to the *R. prolixus–P. tertius* clade with a 100% bootstrap value.

DISCUSSION

Despite the small and limited sample size, the results of sequence comparisons, and MP, NJ, and ML analyses, indicate that the ITS-2 spacer is a useful marker for resolving supraspecific, specific, and subspecific relationships in Triatominae. Although phylogenetic analyses appear sometimes to be unable to resolve population-level relationships, the detection of some interpopulational punctual nucleotide variations and mainly the presence of different number of micro-

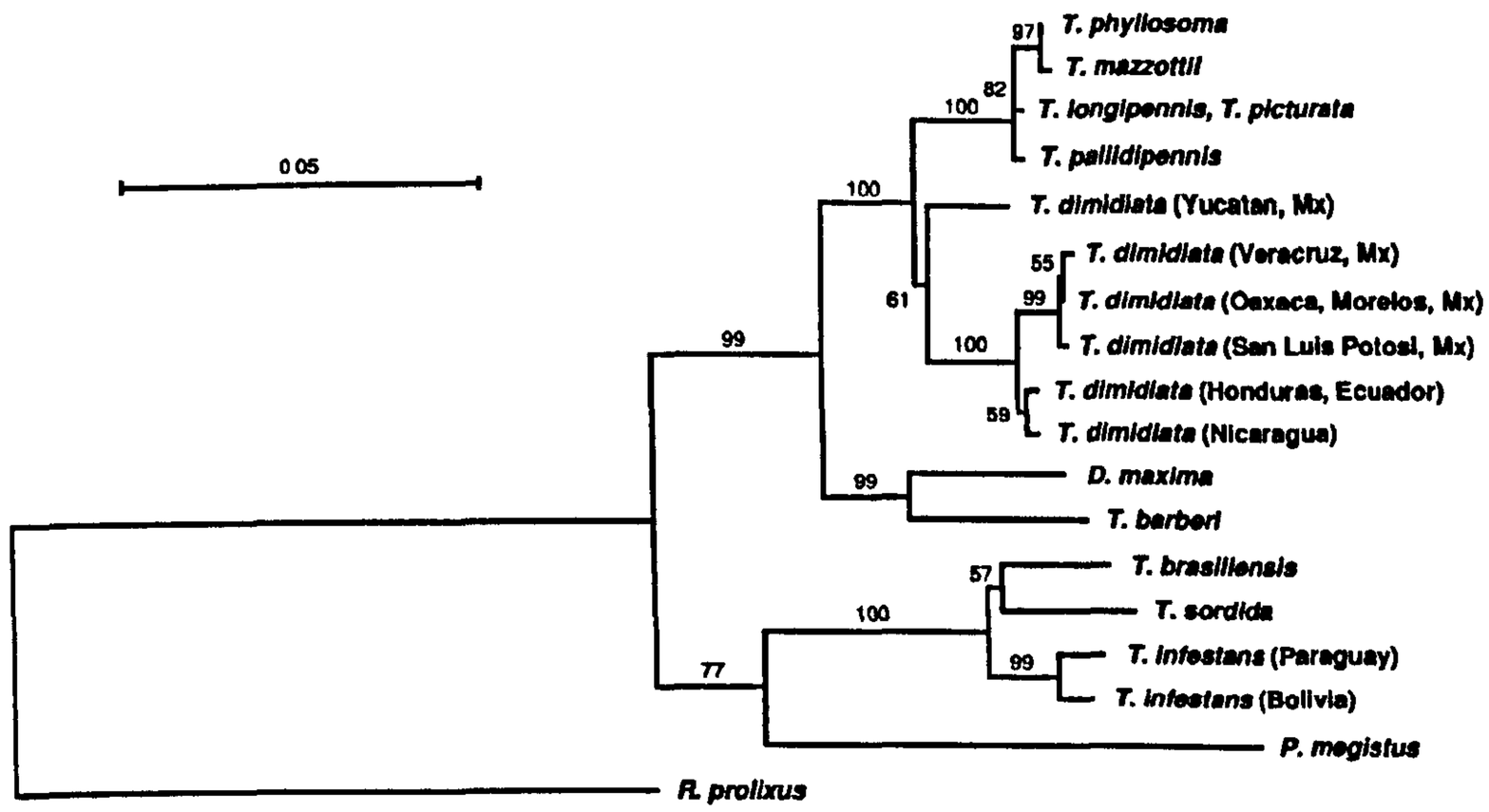


FIG. 2. Phylogenetic tree of the Triatomini species studied, derived from the distance data (Kimura two-parameter) using the neighbor-joining method. Numbers represent the percentage of 1000 bootstrap replicates.

satellite repeats show that ITS-2 may also be useful for population analyses within given species. ITS-2 proves to be a useful marker despite the marked A + T bias. Although the G + C composition of ITSs tends to be around 50% in many organisms (e.g., Remigio and Blair, 1997), nucleotide compositions biased toward G + C or A + T have often been found. Examples of bias toward A + T include 80% in ITS-2 of *Drosophila melanogaster* (Tautz *et al.*, 1988) and 64–71% in Nematoda (e.g., Chilton *et al.*, 1997). In general, however, balanced DNA regions seem better for taxonomic and phylogenetic studies since substitutions become more easily detectable when bases occur with approximately similar frequency.

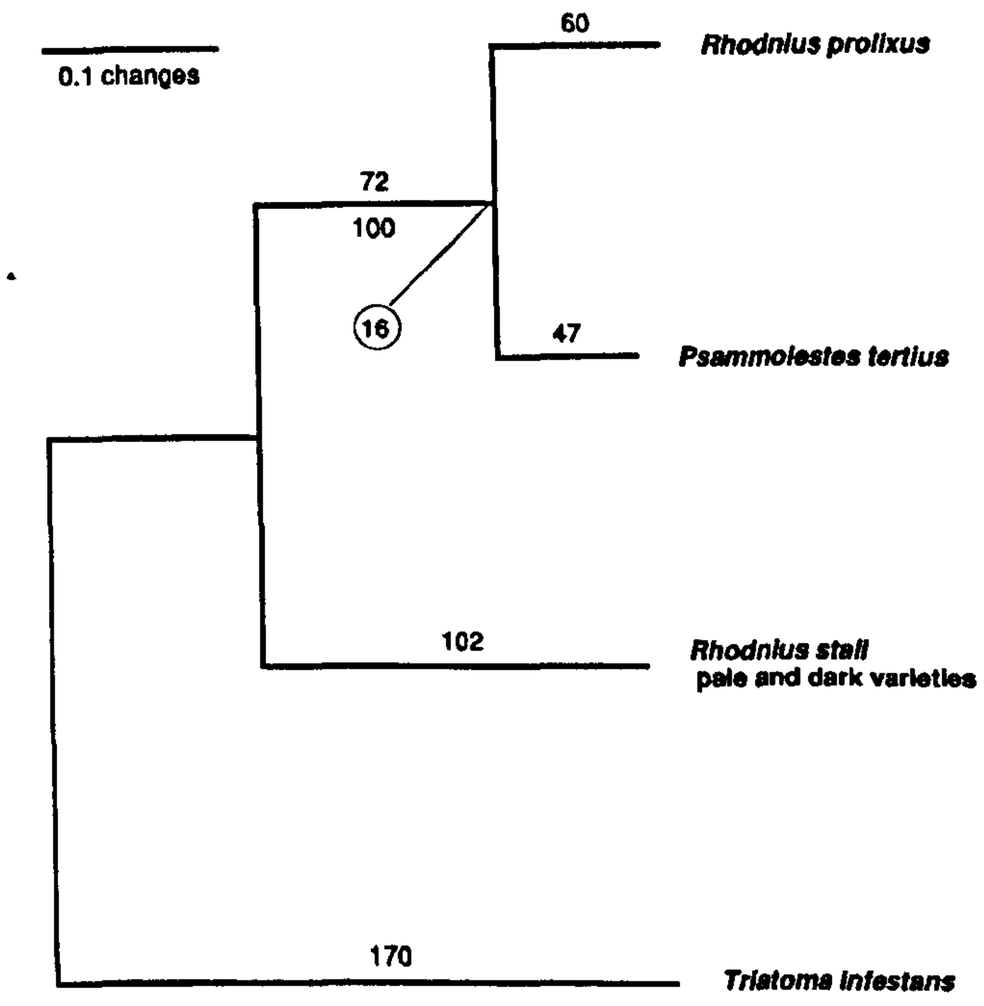


FIG. 3. Phylogenetic tree of the Rhodniini species studied, obtained with both parsimony analysis using the branch-and-bound algorithm (CI = 0.978; RI = 0.697) and neighbor-joining analysis using the distance data measured with the Kimura two-parameter. Numbers above the line indicate branch lengths (steps) obtained with parsimony analysis; numbers below the line represent the percentage of 1000 bootstrap replicates obtained with both methods. Encircled number indicates Bremer support value for node.

The ITS-2 sequences were markedly different between species of Triatomini and Rhodniini. These differences support current theory that envisages the Triatominae as a polyphyletic assemblage derived from several different reduviid lineages, which have converged in response to parallel demands of the blood-sucking habit. Initial arguments for polyphyly were advanced by Schofield (1988) based largely on biogeographic and ecological characteristics. Since then, comparative studies of the Triatomini and Rhodniini provide further evidence, based on isoenzyme comparisons (Dujardin *et al.*, 1999), RAPD patterns (Garcia *et al.*, 1998), and preliminary mitochondrial DNA sequence studies (Stothard *et al.*, 1998; Lyman *et al.*, 1999). All these studies show major divergence between the Triatomini and the Rhodniini consistent with a polyphyletic origin. Detailed comparison of antennal sensilla patterns (Catala, 1997) and genital structure (Jurberg, 1996) also suggests that these two tribes have evolved from different ancestral forms, as does the absence of salivary nitrophorins in the Triatomini, compared to their characteristic presence in the Rhodniini (Soares *et al.*, 1998).

Within the Triatomini, our results support the idea of an old divergence between South American and North–Central American forms, shown by the primary dichotomy between *P. megistus* plus *T. infestans*, *T.*

brasiliensis, and *T. sordida* from South America and *D. maxima* and *T. barberi* plus *T. dimidiata* and species of the phyllosoma complex from North–Central America (Figs. 1 and 2). Within the Rhodniini, *Psammolestes* clearly clustered with *R. prolixus* rather than with *R. stali* (Fig. 3) in accordance with the idea that *Psammolestes* was derived from the *prolixus/robustus* clade (Schofield and Dujardin, 1999). Phylogenetic trees query the validity of the genera *Psammolestes* and *Dipetalogaster*.

In our analysis of the Central American and Mexican species of *Triatoma*, the *dimidiata* from Yucatan appears well separated from other populations of *dimidiata* and from populations of the *phyllosoma* complex. The classification of these species has led to some confusion. The *phyllosoma* species (represented here by *phyllosoma, mazzottii, longipennis, picturata,* and *pallidipennis*) were considered subspecies of *T. phyllosoma* by Usinger (1944) but were raised to specific rank by Lent and Wygodzinsky (1979) within the so-called *phyllosoma* complex. The concept of this *phyllosoma* complex was then extended by Schofield (1988) to include *T. dimidiata*. There is little doubt that these species are closely related, but it is worth emphasizing that there are very few ITS-2 sequence differences between the original *phyllosoma* species (even less than those between the *T. dimidiata* populations from Mexico, Honduras, and Nicaragua). These differences involve only two to four nucleotide differences and dinucleotide microsatellite repeats, or even no differences at all in the case of *longipennis* and *picturata*. Such a low number of nucleotide differences are generally considered to reflect organisms capable of hybridization (Remigio and Blair, 1997) and available studies indeed indicate the production of fertile hybrids, for example in crosses between *pallidipennis* and *picturata* (Mazzotti and Osorio, 1942). Subspecific ranking of these entities may therefore be more appropriate, as originally proposed by Usinger (1944), reflecting populations that can usually be morphologically distinguished and which generally show different geographical distributions with little overlap.

T. dimidiata proves to be a species clearly differentiated from the *T. phyllosoma* complex. It includes, moreover, several populations already following different evolutionary divergences in which geographical isolation appears to develop an important influence. Classification of *T. dimidiata* has also involved subspecific divisions, with the darker northern forms denoted *T. d. maculipennis*, the intermediate Central American forms *T. d. dimidiata*, and the southern forms *T. d. capitata* (Usinger, 1944), all of which were synonymized by Lent and Wygodzinsky (1979). Our results indicate little differences between *dimidiata* populations from southern Mexico, Honduras, Nicaragua, and Ecuador, although that from Yucatan shows differences consistent with specific status. Southern Mexico *T. dimidiata* populations (Oaxaca, Morelos, Veracruz, and San Luis Potosi) were almost indistinguishable, but differences increase when compared to the populations of Honduras and Nicaragua. This could be interpreted as evidence of clinal variation along a north–south axis. Finally, the total absence of differences between the population of Honduras and those of Ecuador may indicate that the Ecuadorian specimens derive from introduced specimens, recently transported by Man.

ACKNOWLEDGMENTS

This work benefited from international collaboration through the ECLAT network. Funding was obtained from a Project (Contract No. IC18-CT98-0366) of the INCO-DC Programme, European Commission (DG XII), Brussels, EU, the AVINA Foundation, Switzerland, and the Research Promotion Programme, University of Valencia, Spain. Specimen donation by Dr. J. C. P. Dias (Brazil), Dr. A. Rojas de Arias (Paraguay), and G. Rojas W. (Mexico) is acknowledged. Dr. David Swofford generously provided the beta test version of PAUP 4.0b 3a.

REFERENCES

Almeyda-Artigas, R. J., Bargues, M. D., and Mas-Coma, S. (2000). ITS-2 rDNA sequencing of *Gnathostoma* species (Nematoda) and elucidation of the species causing human gnathostomiasis in the Americas. *J. Parasitol.* **86:** 537–544.

Bargues, M. D., and Mas-Coma, S. (1997). Phylogenetic analysis of lymnaeid snails based on 18S rDNA sequences. *Mol. Biol. Evol.* **14:** 569–577.

Bremer, K. (1988). The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42:** 795–803.

Bremer, K. (1994). Branch support and tree stability. *Cladistics* **10:** 295–304.

Catala, S. (1997). Antennal sensilla of Triatominae (Hemiptera, Reduviidae): A comparative study over five genera. *Int. J. Insect Morphol. Embryol.* **26:** 67–73.

Chilton, N. B., Gasser, R. B., and Beveridge, I. (1997). Phylogenetic relationships of Australian strongyloid nematodes inferred from ribosomal DNA sequence data. *Int. J. Parasitol.* **27:** 1481–1494.

Dujardin, J. P., Chavez, T., Moreno, J. M., Machane, M., Noireau, F., and Schofield, C. J. (1999). Comparison of isoenzyme electrophoresis and morphometric analysis for phylogenetic reconstruction of the Rhodniini (Hemiptera: Reduviidae: Triatominae). *J. Med. Entomol.* **36:** 653–659.

Dujardin, J. P., Schofield, C. J., and Panzera, F. (2000). "Les Vecteurs de la Maladie de Chagas: Recherches Taxinomiques, Biologiques et Génétiques," Académie Royale des Sciences d'Outre Mer, Classe des Sciences Naturelles et Médicales, N.S., **24.**

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39:** 783–791.

Garcia, A. L., Carrasco, H. J., Schofield, C. J., Valente, S. A., Frame, I. A., Stothard, R., and Miles, M. A. (1998). Random amplification of polymorphic DNA as a tool for taxonomic studies of Triatomine bugs (Hemiptera: Reduviidae). *J. Med. Entomol.* **35:** 38–45.

Hasegawa, M., Kishino, H., and Yano, T. (1985). Dating of the human–ape splitting by molecular clock of mitochondrial DNA. *J. Mol. Evol.* **21:** 160–174.

Jarne, P., and Lagoda, P. J. L. (1996). Microsatellites, from molecules to populations and back. *Trends Ecol. Evol.* **11:** 424–429.

Jurberg, J. (1996). Uma abordagem filogenetica entre os Triatomineos baseada nas estruturas falicas. *In* "Proceedings of the International Workshop on Population Genetics and Control of Triatominae, Santo Domingo de los Colorados, Ecuador" (C. J. Schofield, J. P. Dujardin, and J. Jurberg, Eds.), pp. 51–52. INDRE, Mexico City.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16:** 111–120.

Lent, H., and Wygodzinsky, P. (1979). Revision of the Triatominae (Hemiptera, Reduviidae) and their significance as vectors of Chagas' disease. *Bull. Am. Mus. Nat. Hist.* **163:** 123–520.

Lyman, D. F., Monteiro, F. A., Escalante, A. A., Cordon-Rosales, C., Wesson, D. M., Dujardin, J. P., and Beard, C. B. (1999). Mitochondrial DNA sequence variation among triatomine vectors of Chagas disease. *Am. J. Trop. Med. Hyg.* **60:** 377–386.

Mazzotti, L., and Osorio, M. T. (1942). Cruzamientos experimentales entre varias especies de Triatomas. *Rev. Mex. Med.* **22:** 215–222.

Remigio, E. A., and Blair, D. (1997). Relationships among problematic North American stagnicoline snails (Pulmonata: Lymnaeidae) reinvestigated using nuclear ribosomal DNA internal transcribed spacer sequences. *Can. J. Zool.* **75:** 1540–1545.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular Cloning: A Laboratory Manual," 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schofield, C. J. (1988). The biosystematics of Triatominae. In "Biosystematics of Haematophagous Insects" (M. W. Service, Ed.), pp. 284–312. Systematics Association Special Volume 37, Clarenden, Oxford.

Schofield, C. J. (1994). "Triatominae. Biology and Control," Eurocommunica Publications, West Sussex, UK.

Schofield, C. J., and Dujardin, J. P. (1999). Theories on the evolution of *Rhodnius*. *Actual. Biol.* **21:** 183–197.

Soares, R. P. P., Gontijo, N. F., Romanha, A. J., Diotaiuti, L., and Pereira, M. H. (1998). Salivary heme proteins distinguish *Rhodnius prolixus* from *Rhodnius robustus* (Hemiptera: Reduviidae: Triatominae). *Acta Trop.* **71:** 285–291.

Sorenson, M.D. (1999). TreeRot, version 2. Boston Univ., Boston, MA.

Stothard, J. R., Yamamoto, Y., Cherchi, A., Garcia, A. L., Valente, S. A. S., Schofield, C. J., and Miles, M. A. (1998). A preliminary survey of mitochondrial sequence variation within triatomine bugs (Hemiptera: Reduviidae) using polymerase chain reaction-based single strand conformational polymorphism (SSCP) analysis and direct sequencing. *Bull. Entomol. Res.* **88:** 553–560.

Swofford, D. L. (1999). PAUP: Phylogenetic analysis using parsimony, Version 4.0b 3a. Computer program distributed by the Smithsonian Institution, Sinauer, Sunderland, MA.

Tamura, K., and Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* **10:** 512–526.

Tautz, D., Hancock, J. M., Webb, D. A., Tautz, C., and Dover, G. A. (1988). Complete sequences of the rRNA genes of *Drosophila melanogaster*. *Mol. Biol. Evol.* **5:** 366–376.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity and progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22:** 4673–4680.

Usinger, R. (1944). The Triatominae of North and Central America and the West Indies and their public health significance. *Publ. Health Bull.* **288:** 1–83.

Van De Peer, Y., and De Wachter, R. (1994). TREECON for Windows: A software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput. Appl. Biosci.* **10:** 569–570.

Zarate, L. G., and Zarate, R. J. (1985). A checklist of the Triatominae (Hemiptera: Reduviidae) of Mexico. *Int. J. Entomol.* **27:** 102–127.

FASBASE-MSP

CONTROL DE LA TRANSMISIÓN VECTORIAL DE LA ENFERMEDAD DE CHAGAS EN EL ECUADOR

Un documento de trabajo para el personal de entomología del Servicio Nacional para la Erradicación de la Malaria y Control de Vectores (SNEM).

Fernando Abad-Franch

Unidad de Biología Molecular y Bioquímica
de Patógenos, Departamento de Enfermedades Infecciosas y Tropicales,
London School of Hygiene and Tropical Medicine, Londres, Reino Unido.

Unidad de Medicina Tropical, Instituto "Juan César García",
Quito, Ecuador.

Francisco S. Palomeque

Pontificia Universidad Católica del Ecuador, Departamento de Ciencias Biológicas,

Unidad de Medicina Tropical, Inst. "Juan César García"
Quito - Ecuador

H. Marcelo Aguilar V.

Instituto Nacional de Higiene Leopoldo Izquieta Pérez, Zona Norte

Consultor en Enfermedades Tropicales del Proyecto
FASBASE 3510 - 2 EC

Quito, Ecuador, Marzo de 2001

FASBASE - MSP

MINISTERIO DE SALUD PUBLICA

SUBSECRETARÍA NACIONAL DE MEDICINA TROPICAL

GUÍA OPERACIONAL PARA EL CONTROL DE LA ENFERMEDAD DE CHAGAS EN EL ECUADOR

Dr. H. Marcelo Aguilar Velasco1,2,
Dr. Fernando Abad-Franch2,3,
Dr. Ángel G. Guevara4, Dr. José Racines Vizuete1,
Dra. Alba Briones Lavayen5 y Dr. Víctor Reyes Lituma6

FASBASE PROYECTO DE FORTALECIMIENTO Y AMPLIACION DE LOS SERVICIOS BASICOS DE SALUD EN EL ECUADOR

Trapping Triatominae in Silvatic Habitats

François Noireau/*/+, Fernando Abad-Franch, Sebastião AS Valente***, Artur Dias-Lima****, Catarina M Lopes, Vanda Cunha, Vera C Valente***, Francisco S Palomeque*****, Carlos José de Carvalho-Pinto******, Ítalo Sherlock****, Marcelo Aguilar*******, Mario Steindel******, Edmundo C Grisard******, José Jurberg**

Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Departamento de Entomologia, Instituto Oswaldo Cruz-Fiocruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil *Institut de Recherche pour le Développement UR016, Paris, France **Pathogen Molecular Biology and Biochemistry Unit, Department of Infectious and Tropical Diseases, LSHTM, London, UK ***Instituto Evandro Chagas, Programa de Doença de Chagas, Ananindeua, PA, Brasil ****Centro de Pesquisa Gonçalo Moniz-Fiocruz, Salvador, BA, Brasil *****Departamento de Biología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador ******Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Catarina, Florianópolis, SC, Brasil *******Unidad de Medicina Tropical, Instituto Juan César García, Quito, Ecuador

Large-scale trials of a trapping system designed to collect silvatic Triatominae are reported. Live-baited adhesive traps were tested in various ecosystems and different triatomine habitats (arboreal and terrestrial). The trials were always successful, with a rate of positive habitats generally over 20% and reaching 48.4% for palm trees of the Amazon basin. Eleven species of Triatominae belonging to the three genera of public health importance (Triatoma, Rhodnius *and* Panstrongylus) *were captured. This trapping system provides an effective way to detect the presence of triatomines in terrestrial and arboreal silvatic habitats and represents a promising tool for ecological studies. Various lines of research are contemplated to improve the performance of this trapping system.*

Key words: Triatominae - trapping system - silvatic environment - terrestrial ecotopes - arboreal ecotopes

The control of domestic vector species of Triatominae is being successfully pursued in most of the Southern Cone countries and is being developed in the Andean countries and Central America. In areas with successful control programmes, report of silvatic species invading human dwellings leads research to be focused on their original habitats. Observations on the ecology and behaviour of these silvatic triatomines will assist in devising strategies for control-surveillance in areas where they invade or colonize synanthropic habitats (Schofield et al. 1999).

Understanding of the ecology and biology of Triatominae in their natural habitats is fragmentary, principally because collection of specimens is laborious and time-consuming. Light trapping may be effective in open vegetation, but only small numbers of starved adults of those species that are light-attracted can be captured. An alternative is meticulous "habitat dissection" of the great variety of potential ecotopes where triatomines breed, including hollow trees, palm tree crowns, bromeliads, rock piles, burrows, and bird-nests. The few animal-baited trapping devices previously designed to sample silvatic triatomines have yielded poor results (Rabinovich et al. 1976, Carcavallo 1985).

Recently, the use of a simple trapping system to collect silvatic triatomines was reported (Noireau et al. 1999, 2000). Here we present results of the first trials of this trapping system in various ecosystems and different triatomine habitats.

MATERIALS AND METHODS

The traps consisted of small plastic containers (250 or 500 cm^3) closed with wire mesh and partially covered with double-sided adhesive tape (Figure). They contained a mouse as bait together with a small quantity of wood shavings and food. Initially designed for collecting silvatic *Triatoma* in hollow trees, the system was later applied to the capture and study of *Rhodnius* species in palm tree crowns (Abad-Franch et al. 2000, Palomeque et al. 2000, Valente et al. 2000).

This trapping system has now been tested in various ecosystems (Chaco, Caatinga, Amazon basin, and subtropical humid forests) and in four types of triatomine habitats (hardwood trees, palm trees, rock piles and crags). Traps were suspended in hollows located in trunks and limbs of hardwood trees, or were placed in the crown of palm trees, among rocks or inside crevices. One to four

The trapping system

Research supported by the CNPq, IRD, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (grants 970195 and A00206), Fiocruz, Funasa, ECLAT network, the Cañada-Blanch Foundation and the University General Hospital (Valencia, Spain).

+Corresponding author. Fax: +55-21-2573-4468. E-mail: noireauf@ioc.fiocruz.br

Received 8 May 2001
Accepted 16 August 2001

traps were generally used for each site, depending on its dimensions and the number of accessible triatomine shelters (hollows in the case of trees). Four traps were usually set in each palm crown. Traps were commonly set in the afternoon and inspected the next morning, approximately 15 h later, in order to avoid the midday heat.

RESULTS

With the live-bait traps, some mouse mortality was recorded (< 2%). It was generally due to the attack by ants and is avoidable by covering the wire mesh with finer cloth. Results of the different capture series according to the ecosystem and triatomine habitat are shown in Table I. Trials performed in favourable habitats known to harbour triatomines were always successful, with a positivity rate higher than 20% and reaching 33.7% in hardwood trees in the Bolivian Chaco, 38.5% in rock piles of the Caatinga, and 48.4% in palm trees of the Ecuadorian Amazon basin. The apparent density of insects per positive habitat was highest for palm trees in the Amazon basin and West Andean foothills in Ecuador (12.5 and 10.2, respectively), and reached 6.8 in rock piles. Eleven species of Triatominae (six *Triatoma*, four *Rhodnius*, and one *Panstrongylus* species) have been captured with this method (Table I).

Results on the efficacy of the trapping system are shown in Table II. The positivity rate of traps was higher in palm crowns and rock crevices than in hollow trees. The number of insects adhered to the tape was usually low (one or two insects) but could reach high densities (> 10, and up to 51) in some palm tree crowns.

DISCUSSION

The increasing reports of silvatic triatomine species invading (and sometimes colonizing) peridomestic and domestic habitats endorse the need for research on their original wild populations and habitats. The study of these habitats by means of traditional approaches (random manual searches and dissection) is laborious and destructive, hampering the development of more detailed studies. Hence, many of the characteristics of triatomine habitats are often obscure and remain to be more thoroughly investigated (Romaña et al. 1999, Gaunt & Miles 2000). Live-baited adhesive traps could help overcome some of these problems.

TABLE I

Positive habitats and species of Triatominae collected

Study area	Habitat	No. of habitats surveyed (% positive)	Mean no. (SD) of insects by positive habitat	Range of density	Species of Triatominae collected
Chaco (Bol)	Hardwood trees [a]	421 (33.7)	1.9 (1.4)	1-12	*Triatoma sordida* *T. guasayana* *T. infestans* (dark morph)
Caatinga (Bra)	Hardwood trees [b]	67 (17.9)	1.9 (1.2)	1-6	*T. pseudomaculata*
East Andean foothills (Bol)	Hardwood trees	14 (21.4)	2.0 (0.7)	1-3	*Panstrongylus megistus*
Caatinga (Bra)	Rock piles	13 (38.5)	6.8 (3.8)	3-13	*T. brasiliensis*
Serra Geral (Bra)	Crags	10 (20)	1.5 (0.8)	1-4	*T. klugi*
Caatinga (Bra)	Palm trees [c]	59 (10.2)	1.8 (1.4)	1-6	*Rhodnius nasutus*
Amazon basin (Bra)	Palm trees [d]	53 (22.6)	2.5 (2.1)	1-14	*R. pictipes* *R. robustus*
Amazon basin (Ecu)	Palm trees [e]	64 (48.4)	12.5 (18.7) [g]	1-98 [g]	*R. pictipes* *R. robustus*
West Andean foothills (Ecu)	Palm trees [f]	56 (25)	10.2 (4.9)	3-22	*R. ecuadoriensis*
Central coast lowlands (Ecu)	Palm trees [f]	16 (6.2)	Only 1 bug captured	-	*R. ecuadoriensis*
Coastal area (Ecu)	Palm trees [f]	36 (27.8)	3.0 (2.6)	1-9	*R. ecuadoriensis*

a: dominated by *Ruprechtia triflora*; *b*: *Caesalpinia pyramidalis*, *Spondias tuberosa*, *Bumelia sartorum*, *Anadenanthera colubrina* and *Astronium urundeuva* resulted positive; *c*: *Copernicia prunifera*; *d*: dominated by *Attalea regia* and *A. speciosa*; *e*: *Attalea*, *Phytelephas*, *Astrocaryum*, *Oenocarpus* and *Elaeis* resulted positive; *f*: dominated by *P. aequatorialis* (palma de tágua); *g*: combining traps and manual capture on palm crowns; Bol: Bolivia; Ecu: Ecuador; Bra: Brazil

TABLE II

Efficacy of the trapping system

Study area	Ecotope	Traps placed (% positive)	Mean no. (SD) of insects by positive traps	Range of density
Chaco (Bol)	Hollow trees	732 (21.9)	1.7 (1.0)	1-8
Caatinga (Bra)	Hollow trees	78 (17.9)	1.5 (0.7)	1-3
East Andean foothills (Bol)	Hollow trees	27 (18.5)	1.2 (0.3)	1-2
Caatinga (Bra)	Rock crevices	29 (27.6)	3.4 (1.6)	1-7
Serra Geral (Bra)	Crag crevices	60 (21.6)	1.5 (0.8)	1-4
West Andean foothills (Ecu)	Palm trees	88 (32.9)	4.9 (3.4)	1-14
Central coast lowlands (Ecu)	Palm trees	43 (2.3)	*a*	-
Coastal area (Ecu)	Palm trees	91 (16.5)	1.9 (1.3)	1-6
Amazon basin (Ecu)	Palm trees	111 (43.2)	5.2 (9.1)	1-51
Caatinga (Bra)	Palm trees	59 (10.2)	1.8 (1.4)	1-6
Amazon basin (Bra)	Palm trees	72 (19.4)	1.7 (1.2)	1-9

Bol: Bolivia; Ecu: Ecuador; Bra: Brazil; *a*: only 1 bug captured.

Results of several independent studies presented here suggest live-baited trapping provides a quick, simple and inexpensive way to detect the presence of triatomine populations in silvatic habitats. Four important triatomine ecotopes were successfully searched by this method. Other terrestrial (burrows, bromeliads, tree root cavities) and arboreal habitats (bromeliad epiphytes) remain to be investigated. Eleven species of Triatominae were captured in various environments, suggesting that the system may be applied to other species as well. Repeated series of captures in positive habitats increased the number of bugs captured, allowing for a more accurate assessment of the density of colonies. Starved bugs were more likely to be attracted by the bait; thus, the proportion of bugs captured in each ecotope would be inversely correlated to the nutritional status of the population. This study confirms that the nutritional status of silvatic bugs is generally very poor and may explain the unsuccessful results obtained by live-baited trapping in domestic habitat where triatomines are commonly fed (Tonn et al. 1976, Noireau & Dujardin 2001). Using this trapping system, the ecological (and economical) damage caused by felling and dissecting trees to study associated triatomines may be avoided. With regard to the capture of *T. klugi* in crag crevices, previous attempts using light trapping and active searching in crevices were unsuccessful.

Live-baited adhesive traps represent a promising tool to identify triatomine habitats that may thereafter be studied longitudinally (including for instance the response of bug populations to variations in microclimatic conditions or hosts, or to biological control interventions). By allowing the capture of silvatic specimens, several entomological factors can be investigated (e.g. geographic range, density and structuring of bug populations or species, natural infection by trypanosomes etc.). Furthermore, our knowledge on the ecology of these populations (association to specific habitats, behavioural differences in relation to vertebrate hosts etc.) may benefit from this sampling technique. Live-baited traps may help define "high-risk" ecotopes (e.g., containing dense bug colonies of species displaying synanthropic behaviour and frequently infected by *Trypanosoma cruzi*) nearby human dwellings, an important issue in areas where invasion and re-infestation of houses by silvatic vectors hinder long-term interruption of vector-borne transmission.

Various lines of research could be explored to improve the performance of this trapping system. Replacement of the live bait by a chemical attractant would make traps easier to handle in the field, and could help reduce their size so that smaller ecotopes (hollow trees and rock crevices) might be studied. Finally, the design of a trapping system for intra- and peridomestic habitats could be of use for entomological vigilance after control interventions.

ACKNOWLEDGEMENTS

To Michael A Miles for the revision of an earlier version of the manuscript. To Teresa Gutierrez, Rosmary Flores, José Luis Botetano, Carlos Carpio, Karyna Suárez, José Elson Abud de Araújo, Carlos Alberto Rodrigues, Gilberto César Rodrigues and Nélio do Nascimento Farias for their invaluable assistance in the field work. To Laure Emperaire for reviewing the identification of trees and CJ Schofield for the useful comments on the manuscript.

REFERENCES

Abad-Franch F, Noireau F, Paucar A, Aguilar M, Carpio C, Racines J 2000. The use of live bait traps for the study of sylvatic *Rhodnius* populations (Hemiptera: Reduviidae) in palm trees. *Trans R Soc Trop Med Hyg 94*: 629-630.

Carcavallo RU 1985. Técnicas de estudio de triatominos en ambiente silvestre. In RU Carcavallo, JE Rabinovich, RJ Tonn (eds), *Factores Biológicos y Ecológicos en la Enfermedad de Chagas*, vol. 1, OPS-ECO/MSAS-SNCH, Buenos Aires, p. 49-52.

Gaunt M, Miles M 2000. The ecotopes and evolution of triatomine bugs (Triatominae) and their associated trypanosomes. *Mem Inst Oswaldo Cruz 95*: 557-565.

Noireau F, Dujardin JP 2001. Flight and nutritionnal status of sylvatic *Triatoma sordida* and *T. guasayana*. *Mem Inst Oswaldo Cruz 96*: 385-389.

Noireau F, Flores R, Vargas F 1999. Trapping sylvatic Triatominae (Reduviidae) in hollow trees. *Trans R Soc Trop Med Hyg 93*: 13-14.

Noireau F, Flores R, Gutierrez T, Abad-Franch F, Flores E, Vargas F 2000. Natural ecotopes of *Triatoma infestans* dark morph and other sylvatic triatomines in the Bolivian Chaco. *Trans R Soc Trop Med Hyg 94*: 23-27.

Palomeque FS, Abad-Franch F, Suárez K, Aguilar VHM 2000. Uso de trampas con cebo vivo para el estudio de poblaciones silvestres de *Rhodnius* (Reduviidae: Triatominae) en palmeras de la Amazonia ecuatoriana. Memorias, XXIV Jornadas Ecuatorianas de Biología, p. 108.

Rabinovich JE, Carcavallo RU, Barretto MP 1976. Ecologic methods: marking, trapping, and sampling for vector studies in the field. In *New Approaches in American Trypanosomiasis Research*, PAHO scientific publication 318, Washington, p. 16-20.

Romaña C, Pizzaro NJC, Rodas E, Guilbert E 1999. Palm trees as ecological indicators of risk areas for Chagas disease. *Trans R Soc Trop Med Hyg 93*: 594-595.

Schofield CJ, Diotaiuti L, Dujardin JP 1999. The process of domestication in Triatominae. *Mem Inst Oswaldo Cruz 94* (Suppl. 1): 375-378.

Tonn RJ, Otero MA, Jiménez J 1976. Comparación del método hora-hombre con la trampa Gómez-Núñez en la búsqueda de *Rhodnius prolixus*. *Bol Dir Malar Saneam Ambiental 16*: 269-275.

Valente VC, Valente SAS, Rodrigues CA, Souza GCR, Noireau F, Lopes CM, Cunha V 2000. Estudo preliminar da eficiência de captura de triatomíneos silvestres utilizando armadilhas com fita adesiva em Bragança, Pará. *Rev Soc Bras Med Trop 33* (Suppl. 2): 94-95.

SHORT COMMUNICATION

Observations on the Domestic Ecology of *Rhodnius ecuadoriensis* (Triatominae)

F Abad-Franch/*/+, HM Aguilar V*/, A Paucar C*, ES Lorosa***, F Noireau***/****

Pathogen Molecular Biology and Biochemistry Unit, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel St., London WC1E 7HT, UK *Unidad de Medicina Tropical, Instituto 'Juan César García', Quito, Ecuador **Instituto Nacional de Higiene y Medicina Tropical 'Leopoldo Izquieta Pérez', Quito, Ecuador ***Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Departamento de Entomologia, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, RJ, Brazil ****Institut de Recherche pour le Développement, UR016, France

Rhodnius ecuadoriensis *infests peridomiciles and colonises houses in rural southern Ecuador. Six out of 84 dwellings (7%) surveyed in a rural village were infested (78 bugs/infested domicile; 279 bugs were collected in a single dwelling). Precipitin tests revealed* R. ecuadoriensis *fed on birds (65%), rodents (31%), marsupials (8%), and humans (15%) – mixed bloodmeals detected in 37.5% of individual samples.* Trypanosoma cruzi *from opossums and rodents may thus be introduced into the domestic cycle. Wasp parasitoidism was detected in 6.5% of 995* R. ecuadoriensis *eggs (only in peridomestic habitats). Control strategies should integrate insecticide spraying (indoors and peridomestic), better management of poultry, and housing improvements. A possible inefficacy of Malathion is reported.*

Key words: *Rhodnius ecuadoriensis* - ecology - feeding - Chagas disease - control - Ecuador

Chagas disease, caused by *Trypanosoma cruzi*, is a major public health challenge in Latin America. Since the early 90s (when ~18 million people were estimated to be infected), control interventions (elimination of domestic vectors plus blood bank control) have reduced incidence by ~70% in the Southern Cone countries (WHO 1991, Dias & Schofield 1999, Moncayo 1999, WHO/CTD 2001). Aguilar et al. (1999) estimated some 150,000 people may be infected by *T. cruzi* in Ecuador, with ~3 million living at risk. Epidemiological evidence indicates that *Rhodnius ecuadoriensis* is a significant disease vector in southern Ecuador (with reported prevalence rates up to 17%, including 9% at the blood bank of Machala) and northern Peru, where control interventions are being designed; however, very few studies on the ecology and behaviour of the species have been published (see Aguilar et al. 1999, Guevara et al. 1999, Abad-Franch et al. 2001, Cuba Cuba et al. this vol., p. 175-183). It colonises human habitats, and sylvatic populations breed in *Phytelephas aequatorialis* palms in western Ecuador (Lent & Wygodzinsky 1979, Lazo 1985, Barrett 1991, Schofield 1994, Abad-Franch et al. 2000, 2001). Aiming to understand better the domestic-peridomestic ecology of this species, we studied the situation in a rural area of southern Ecuador, and present here some preliminary results.

The locality of El Lucero (~1,400 m altitude, 79°30'W 4°10'S) belongs to the province of Loja, where Chagas disease is endemic (cf. Aguilar et al. 1999). The original dry premontane forest has been widely replaced by agriculture and livestock farms. No wild palm trees were observed in the area. We inspected 84 dwellings (sample representative for 2% expected infestation) and found 7.1% to be infested. Bugs were breeding mainly in peridomestic chicken coops and (in smaller numbers) inside bedrooms; 469 bugs of all stages (an average of 78.2 bugs/infested house) and 995 eggs were collected in the locality (Abad-Franch 2000). Eggs were inspected to detect parasitoidism by Hymenoptera (see Coscarón et al. 1999).

We studied in depth one house presenting heavy infestation, where two adults and four children lived by subsistence farming. The general condition of the dwelling was very poor (mud-stick, non-plastered walls; roof made of tiles, bamboo, and trunks; and earthen floor). All six people shared two beds in a 6.3m^2-room; 36 chickens, 3 dogs, 6 pigs, 4 sheep, a donkey, and a goat were kept in the peridomicile. The house was surrounded by cropland and patches of modified dry premontane forest. Both adults answered a brief questionnaire on Chagas disease and its vectors. A four hour-search for bugs was conducted in and around the domicile (4 inspectors); beds and two chicken nests were systematically dismantled. Precipitin bloodmeal tests were performed on 26 bug faecal samples collected on filter paper. Eight individual bugs and the papers placed in containers used for bug collection, stained by bug faeces, were analysed (the latter results, although not individual-specific, reveal the feeding preferences of the colonies). A 17-antiserum battery was used (human 1:15,000, bird 1:10,000, opossum 1:15,000,

Research supported by WHO/TDR (Grant 970195), the ECLAT Network, the Cañada-Blanch Foundation (Spain), and the University General Hospital of Valencia (Spain).
+Corresponding author. Fax: +44-207-636.8739. E-mail: Fernando.Abad-Franch@lshtm.ac.uk
Received 27 July 2001
Accepted 13 August 2001

rodent 1:17,000, dog 1:15,000, cat 1:12,000, sheep 1:8,000, goat 1:14,000, pig 1:10,000, horse 1:16,000, ox 1:15,000, coati 1:13,000, tamandua 1:12,000, capybara 1:14,000, armadillo 1:15,000, toad 1:16,000, lizard 1:16,000).

Adults and nymphs of *R. ecuadoriensis* (but no other species) were readily recognised by the inhabitants, who said that bugs were common during all seasons. They had never heard before about Chagas disease, but other conditions were thought to be caused by bug bites ("allergy, itch, anaemia"). They knew that bugs live in mud wall crevices, beds, and chicken nests, and that they "eat blood" from people and poultry. The presence of triatomines inside the house was described as a daily major nuisance. Peridomestic colonies were also considered an important pest, as they suck blood from hens, reducing the production of eggs – hence contributing to malnutrition of children. Bednets were said to be ineffective against triatomines, as bugs "hide *within* the beds". The householders avoided using chemical insecticides inside the house, as they were deemed harmful for the children. A Malathion product used in chicken nests proved quite ineffective – two nests recently treated were infested. Bugs hiding in wall cracks within the house were regularly killed with needles, and boiling water was spread sporadically on infested walls and chicken nests – which were made of dry branches of *Dodonaea viscosa* ("chamana") and grass.

Forty-one *R. ecuadoriensis* adults and over 230 nymphs were captured in this dwelling. Beds, bedroom walls, chicken nests, and a peridomestic hollow tree (where two hens were nesting) were infested. Twenty bugs (all stages) were collected from the two beds; they took shelter in clefts between wood planks or among vegetal fibres used to tie up different parts of beds. Eggs were systematically collected from beds (239 eggs found). A few adult bugs were observed to hide in wall crevices beside the beds. Exuviae and eggs were found in nests, beds, and behind objects hanging on walls. 221 bugs of all stages were found in a nest occupied by a hen, but only three in an adjacent empty nest. Finally, 35 bugs were found in two chicken nests (recently treated with Malathion) located upon a hollow tree near the house; adults and nymphs were also found in the tree itself (Table I).

These triatomines proved rather eclectic in relation to their feeding habits (Table II). They appear to be mainly ornithophilic (65.4% of samples contained bird blood), but rodents (30.8%), humans (15.4%), and opossums (7.7%) are also hosts; 37.5% of individual samples were positive for more than one type of blood. Bugs fed on birds, opossums, and rodents were collected from beds, indicating that bugs circulate from the peridomestic environment to the house (where no birds or opossums were present) and pointing out the risk of introduction of parasites from opossums and rodents into the domestic cycle.

Only 6.5% out of 995 *R. ecuadoriensis* eggs collected in the study locality presented evidence of parasitoidism by Hymenoptera (probably Scelionidae) (Table III). All of them were found in peridomiciles, suggesting that parasitoid wasps do not reach triatomine eggs inside dwellings; the impact of parasitoidism in the demography of *R. ecuadoriensis* in the area is probably insignificant.

This example describes some little-known aspects of the ecology of *R. ecuadoriensis* in human environments (trophic resources, population age structure, preferred microhabitat, impact of parasitoidism), providing valuable data for improved control strategies – currently being planned in the context of the Andean Countries initiative (WHO/TDR 1997). The strong synanthropic behaviour of the species and the virtual absence of palm trees in southern Ecuador and northern Peru suggest that *R. ecuadoriensis* might have spread there in association with humans; if this is confirmed, local eradication might be attainable (Abad-Franch et al. 2001). The combination of an adequate substrate (dwelling in poor condition, abundant domestic birds around houses) with the lack of effective control measures makes human environments likely to become infested by *R. ecuadoriensis*. This species can establish large colonies associated with poultry and has proven able to migrate from peridomiciles into houses, feeding both on mammals and birds. Combined, these features result in a good capacity for house infestation from peridomestic colonies; *T. cruzi* strains from wild and peridomestic mammals may thus be introduced into the domestic cycle. Knowledge, beliefs and customs of inhabitants regarding triatomines are central to health education in community-based surveillance systems; although we found some to be wrong, most of them may be beneficial if slightly modified. Control of the species in the area should integrate residual insecticide spraying (both indoors and peridomestic) with an improved man-

TABLE I

Rhodnius ecuadoriensis domestic and peridomestic colonies in a heavily infested dwelling (El Lucero, Loja, Ecuador)

	Eggs			Nymphs (instars)						
	Hatched	Not hatched								
Site of capture		Alive	Dead	I	II	III	IV	V	Adults	Total
Beds	221	10	8	5	4	3	2	2	4	20 bugs/239 eggs
Chicken nests 1[a]	nr	nr	nr	35	72	77	7	6	27	224 bugs
Chicken nests 2 [b]	nr	nr	nr	2	4	2	4	13	10	35 bugs
Total	221	10	8	42	80	82	13	21	41	279 bugs/239 eggs

nr: not recorded; *a*: two adjacent chicken nests dissected on white cardboard; *b*: bugs from two chicken nests and the hollow tree where these nests were located.

TABLE II

Feeding profiles of *Rhodnius ecuadoriensis* collected in a heavily infested dwelling (El Lucero, Loja, Ecuador), tested by immunoprecipitation

Sample	Place of capture	Results
01-individual (G)	Hollow tree with chicken nests	Bird
02-individual (nymph V)	Hollow tree with chicken nests	Bird
03-individual (nymph V)	Hollow tree with chicken nests	Opossum – Bird
04-individual (E)	Chicken nest	Bird – Rodent
05-individual (nymph V)	Chicken nest	Bird
06-individual (E)	Bed	Human
07-individual (E)	Bed	Opossum – Bird
08-individual (nymph V)	Bed	Rodent
09 to 11-colony 1[a]	Hollow tree with chicken nests	Bird – Rodent
12 to 20-colony 2[b]	Chicken nest	Bird – Rodent
21 to 26-colony 3[c]	Bed	Human – Bird – Rodent

a: colony 1: 35 bugs collected in two chicken nests and the hollow tree where these nests were located; *b*: colony 2: 224 bugs collected in two chicken nests dissected; *c*: colony 3: 20 bugs collected in two beds.

TABLE III

Eggs of *Rhodnius ecuadoriensis* collected in El Lucero (Loja, Ecuador)

Site of collection	Hatched	Not hatched				Total (%)
		Viable	Not viable		Dubious [a]	
			P+	*P*–		
Intra-domiciliary	434	14	0	13	3	464 (46.6)
Peridomestic	414	15	65	28	9	531 (53.4)
Total (%)	848 (85.2)	29 (2.9)	65 (6.5)	41 (4.1)	12 (1.2)	995

P+: eggs with evident signs of parasitoidism by Hymenoptera (larvae inside eggs or egg corium presenting a circular orifice left by the emerging wasp); *P*–: eggs with no evident sign of parasitoidism; *a*: apparently alive when collected, but producing no nymphs.

agement of domestic and peridomestic environments (burning/replacement of hen nests each 15-30 days, and better domestic hygiene), and community-based surveillance. The possible resistance of *R. ecuadoriensis* to Malathion preparations should be investigated, as this insecticide has been extensively used for malaria control in Ecuador.

ACKNOWLEDGEMENTS

To the people of El Lucero. To MA Miles and CJ Schofield for guidance and critical review of the manuscript, and to the anonymous reviewers whose comments improved the original text.

REFERENCES

Abad-Franch F 2000. *Ecology and Genetics of Chagas Disease Vectors in Ecuador. Implications for the Design of Control Strategies*, MPhil-PhD Upgrading Report, LSHTM, London, 150 pp.

Abad-Franch F, Noireau F, Paucar CA, Aguilar VHM, Carpio CC, Racines VJ 2000. The use of live-bait traps for the study of sylvatic *Rhodnius* populations (Hemiptera: Reduviidae) in palm trees. *Trans R Soc Trop Med Hyg 94*: 629-630.

Abad-Franch F, Paucar CA, Carpio CC, Cuba Cuba CA, Aguilar VHM, Miles MA 2001. Biogeography of Triatominae (Hemiptera: Reduviidae) in Ecuador: implications for the design of control strategies. *Mem Inst Oswaldo Cruz 96*: 611-620.

Aguilar VHM, Abad-Franch F, Racines VJ, Paucar CA 1999. Epidemiology of Chagas disease in Ecuador. A brief review. *Mem Inst Oswaldo Cruz 94* (Suppl. I): 387-393.

Barrett TV 1991. Advances in triatomine bug ecology in relation to Chagas disease. *Adv Dis Vector Res 8*: 143-176.

Coscarón MC, Loiácono MS, De Santis L 1999. Predators and parasitoids. In RU Carcavallo, I Galíndez Girón, J Jurberg, H Lent (eds), *Atlas of Chagas Disease Vectors in the Americas*, vol. III, Fiocruz, Rio de Janeiro, p. 891-924.

Dias JCP, Schofield CJ 1999. The evolution of Chagas disease (American trypanosomiasis) control after 90 years since Carlos Chagas discovery. *Mem Inst Oswaldo Cruz 94* (Suppl. I): 103-121.

Guevara AG, Ruiz CJC, Houghton RL, Reynolds L, Sleath P, Benson D, Ouaissi A, Guderian RH 1999. Evaluation of a recombinant protein (RTC24) and synthetic peptides in anti-*Trypanosoma cruzi* positive samples from blood bank donors in chagasic endemic areas of Ecuador. *Japan J Trop Med Hyg 27*: 19-22.

Lazo R 1985. Ecuador. In RU Carcavallo, JE Rabinovich, RJ Tonn (eds), *Factores Biológicos y Ecológicos de la Enfermedad de Chagas*, vol. II, CPEHS OPS/OMS, SNCh, Ministerio de Salud y Acción Social, Argentina, p. 413-427.

Lent H, Wygodzinsky P 1979. Revision of the Triatominae (Hemiptera: Reduviidae), and their significance as vectors of Chagas disease. *Bull Am Mus Nat History 163*: 123-520.

Moncayo A 1999. Progress towards interruption of transmis-

sion of Chagas disease. *Mem Inst Oswaldo Cruz 94* (Suppl. I): 401-404.

Schofield CJ 1994. *Triatominae: Biología y Control*, Eurocommunica Publications, West Sussex, 80 pp.

WHO/TDR-World Health Organization 1997. Andean countries initiative launched in Colombia. *TDR News 53*: 3.

WHO-World Health Organization 1991. Control of Chagas disease, WHO Technical Report Series 811, 95 pp.

WHO/CTD-World Health Organization 2001. *Chagas*. http://www.who.int/ctd/chagas

The Triatomines of Northern Peru, with Emphasis on the Ecology and Infection by Trypanosomes of *Rhodnius ecuadoriensis* (Triatominae)

César A Cuba Cuba/*, Fernando Abad-Franch*/, Judith Roldán Rodríguez***, Franklin Vargas Vásquez***, Luis Pollack Velásquez***, Michael A Miles*/+**

Unidade de Parasitologia Médica-Patologia, Faculdade de Medicina, Universidade de Brasília, Brasília, DF, Brasil *Pathogen Molecular Biology and Biochemistry Unit, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel St., London WC1E 7HT, UK **Unidad de Medicina Tropical, Instituto 'Juan César García', Quito, Ecuador ***Departamento de Microbiología y Parasitología, Universidad Nacional de Trujillo, Trujillo, Perú

Information on the distribution and synanthropic behaviour of triatomines is essential for Chagas disease vector control. This work summarises such information from northern Peru, and presents new data on Rhodnius ecuadoriensis *– an important local vector infesting 10-35% of dwellings in some zones. Three species are strongly synanthropic and may be suitable targets for chemical control of domestic/peridomestic bug populations.* Panstrongylus herreri, *the main domestic vector in the area, is probably present in sylvatic ecotopes in the Marañón river system.* R. ecuadoriensis *and* Triatoma dimidiata *seem exclusively domestic; biogeographical and ecological data suggest they might have spread in association with humans in northern Peru. Confirmation of this hypothesis would result in a local eradication strategy being recommended. Presence of trypanosome natural infection was assessed in 257* R. ecuadoriensis; Trypanosoma rangeli *was detected in 4% of bugs. Six further triatomine species are potential disease vectors in the region* (T. carrioni, P. chinai, P. rufotuberculatus, P. geniculatus, R. pictipes, *and* R. robustus), *whilst* Eratyrus mucronatus, E. cuspidatus, Cavernicola pilosa, Hermanlentia matsunoi, *and* Belminus peruvianus *have little or no epidemiological significance. A strong community-based entomological surveillance system and collaboration with Ecuadorian public health authorities and researchers are recommended.*

Key words: Triatominae - ecology - Chagas disease - *Rhodnius ecuadoriensis* - *Trypanosoma rangeli* - *Trypanosoma cruzi* - Peru

Triatomine bugs transmit *Trypanosoma cruzi*, the causative agent of Chagas disease (Miles 1998). It is estimated that around 650,000-680,000 people might be infected by *T. cruzi* in Peru, with 5 to 6.8 million people living at risk. These epidemiological data largely refer to southern Peru, where *Triatoma infestans* is the primary vector (WHO 1991, Barreda 1996, Dias & Schofield 1999, Guhl 1999). Control activities do not incorporate the northern provinces (where *T. infestans* is absent), partially because of lacking updated epidemiological and entomological information. Although no representative serological data are available, prevalence may be estimated as 1% to 2% (67,000-134,000 people), with about 20% of the population living at risk (~1.34 million people), based on estimations for the whole country presented by Guhl (1999) (prevalence 2.5%, and 25% of the population at risk). Sixteen triatomine species have been reported from the area, 13 of which can be naturally infected by *T. cruzi* (Lent & Wygodzinsky 1979, Calderón et al. 1985, Guillén et al. 1989, Carcavallo et al. 1999a, and this report). *T. dimidiata*, *Panstrongylus herreri*, and *Rhodnius ecuadoriensis* are well adapted to indoors breeding, and are considered significant disease vectors. *T. carrioni*, *P. chinai*, and *P. rufotuberculatus* also breed inside houses in particular areas of Ecuador, Peru and Bolivia. *P. geniculatus* can colonise peridomestic pigsties in the Brazilian Amazon and has been found in houses in Colombia and Venezuela. *R. robustus* and *R. pictipes* are sporadic vectors of human disease in the Amazon, where adult bugs frequently invade homes; only very seldom have domestic colonies of *R. pictipes* been reported. *Eratyrus mucronatus*, *E. cuspidatus*, *Cavernicola pilosa*, and *T. nigromaculata* have little or no epidemiological significance (Lent & Wygodzinsky 1979, Miles et al. 1981, 1983, Barrett 1991, Noireau et al. 1994, 1995, Sherlock et al. 1997, Carcavallo et al. 1998b, Valente et al. 1998, 1999, Angulo et al. 1999, Reyes-Lugo & Rodríguez-Acosta 2000, Abad-Franch et al. 2001, Teixeira et al. 2001). Additionally, *Rhodnius* species act as vectors of *T. rangeli* (Sherlock et al. 1997, Cuba Cuba 1998, Miles 1998). Here we review the main biogeographical traits of these triatomines in northern Peru, and present new data from field research on *R. ecuadoriensis*. The role of *P. herreri*, *T. dimidiata*, and *P. chinai* as actual or potential disease vectors is also emphasised.

MATERIALS AND METHODS

Study area - Peru is divided into three main physiographical areas: the coastal region, the mountain-

Supported by Capes (Ministério de Educação e Cultura, Brasil), the Oficina Nacional de Epidemiología and the Instituto Nacional de Salud (Ministerio de Salud Pública, Perú). Additional funding was received from TDR (grant 970195), the ECLAT Network and the Cañada-Blanch Foundation (Spain).
+Corresponding author. Fax: +44-207-636.8739. E-mail: michael.miles@lshtm.ac.uk
Received 21 May 2001
Accepted 16 August 2001

ous Andean region, and the Amazonian region. In northern Peru, the Andes split into three branches (eastern, central, and western; this latter separates the Pacific and Amazon slopes). There is a complex pattern of temperate valleys with rivers flowing towards either the Pacific or Amazon slopes. The northern coastal region (0-800 m altitude) presents a dry climate with xerophytic areas and valleys where permanent rivers allow the growth of thick, evergreen vegetation, and various crops. The northern Andean region (800-4,947 m altitude) includes the highlands, the valleys of rivers flowing into the Pacific Ocean, and the upper stretches of some tributaries of the Amazon. The climate is arid-semiarid, with various cactus species and without palm trees. The Amazon region includes plains covered by dense rain forests, the eastern Andean humid foothills and valleys, and the eastern branch of the Andes ("selva alta"). Eight political constituencies ("Departamentos") comprise the area of interest of the present study: Tumbes, Piura, Lambayeque, La Libertad, Cajamarca (western slope, although some rivers flow into the Amazon), Amazonas, San Martín, and Loreto (eastern slope and Amazon basin). Some 6.7 million people (~25% of Peruvian population) live in the area (558,000 km^2, 43% of Peru).

Collection of data - Available published reports and the following sources of data were reviewed: (i) the entomological collection, National Institute of Health, Lima, Peru; (ii) the reference collections at Fiocruz, Rio de Janeiro, Brazil; and (iii) records from the Ministry of Health, Lima, Peru – including unpublished reports by the Division of Epidemiology. Our fieldwork records and observations complemented these data. Biogeographical information was obtained from Brack (1987) and Mostacero et al. (1996). Life zone classification was carried out after Curto de Casas et al. (1999).

Entomological surveys - Surveys were carried out in 21 rural localities of Cascas district, La Libertad. A representative sample of dwellings was randomly selected (expected infestation 2.5%, confidence level 95%), and 259 domiciliary units (DUs: domiciles+peridomiciles) were inspected (man-hour method). Live or dead triatomines, exuviae, eggs, or faeces, were considered to indicate infestation. A longitudinal, 2-year entomological survey is also being carried out in other rural areas of La Libertad and Cajamarca, using both active and passive methods to detect DU infestation (detailed results will be presented elsewhere). Natural infection of *R. ecuadoriensis* was assessed in haemolymph, salivary glands, and intestinal contents of bugs; parasites were isolated and identified after Cuba Cuba (1998).

RESULTS

The records of triatomine species from northern Peru are summarised in Tables I and II and in the Figure. Sixteen species were recorded (including a few dubious records discussed below); they occupy 15 different life zones in the region (Table III).

In the Cascas valley (La Libertad), 10 out of 21 (47.6%) localities surveyed were positive for the presence of *R. ecuadoriensis*; 10% of dwellings were infested (adults, nymphs, eggs, and exuviae found indoors; no peridomestic colonies were detected after systematic searches in chicken coops and corrals). There were 204 insects collected (0.8/house surveyed, 7.8/infested house), mainly from houses with non-plastered walls of adobe or "quinchas" (mud/cane) and thatched or cane-and-clay roofs. Beds made of cane were frequently infested. When active and passive methods to detect infestation were combined, overall infestation rate increased to ~35%, and both intra- and peridomestic *R. ecuadoriensis* colonies were detected (preliminary results from the ongoing, 2-year longitudinal survey; authors, unpublished data). *R. ecuadoriensis* was only found in arid environments, and at altitudes up to 2,700 m – the highest value for the species. Bugs infected by *T. rangeli* were collected in two domiciles only, where 19% (10/53) insects were infected; salivary gland infection was confirmed in four bugs (7.5%). Overall *T. rangeli* infection index was 4% (10/257).

P. chinai is predominantly sylvatic in the study area. Peridomestic colonies were detected in stone wall goat enclosures and among clay blocks; nymphs camouflage by covering themselves with dust. Adult males invade synanthropic habitats; they were frequently captured in the main square of the town of Cascas, apparently attracted to artificial light. Domestic colonies were detected in Piura, nearby the Ecuadorian border.

Although updated information is scarce, the main animal reservoirs of *T. cruzi* in the area seem to be marsupials (*Didelphis* spp.), rodents (*Rattus* spp., *Cavia porcellus*), and, in the Amazon region, primates and bats (cf. Calderón et al. 1985, Jara et al. 1998).

TABLE I

Triatominae reported from northern Peru

Tribe	Species [a]
Bolboderini	1 *Belminus peruvianus* Herrer, Lent & Wygodzinsky, 1954
Cavernicolini	2 *Cavernicola pilosa* Barber, 1937
Rhodniini	3 *Rhodnius ecuadoriensis* Lent & León, 1958 4 *Rhodnius robustus* Larrousse, 1927 5 *Rhodnius pictipes* Stål, 1872
Triatomini	6 *Eratyrus cuspidatus* Stål, 1859 7 *Eratyrus mucronatus* Stål, 1859 8 *Hermanlentia matsunoi* (Fernández-Loayza, 1989) 9 *Panstrongylus chinai* (Del Ponte, 1929) 10 *Panstrongylus geniculatus* (Latreille, 1811) 11 *Panstrongylus herreri* Wygodzinsky, 1984 12 *Panstrongylus rufotuberculatus* (Champion, 1899) 13 *Triatoma carrioni* Larrousse, 1926 14 *Triatoma dimidiata* (Latreille, 1811) 15 *Triatoma nigromaculata* (Stål, 1872) (see text)

a: numbers used in the map for species distribution; *Panstrongylus lignarius* not included (see text).

TABLE II

Biogeography of triatomine species reported from northern Peru

Species	Geography [c]	Valleys	Biology, natural infection
Triatoma dimidiata [a]	Tumbes: Tumbes, Zarumilla	Tumbes, Zarumilla	Domestic-peridomestic
	La Libertad: Pacasmayo	Chamán	Domestic-peridomestic
Triatoma carrioni [a, b]	Piura: Huancabamba (E), Ayabaca (W)	Huancabamba, Quiroz	Domestic-peridomestic
	Cajamarca: Jaén, Santa Cruz, San Miguel, Cutervo, Chota	Nd	Domestic-peridomestic; *Tc* (Jaén)
Triatoma nigromaculata [b]	San Martín: Lamas (see text)	Mayo	Domestic
Hermanlentia matsunoi [b]	La Libertad: Pataz	Upper Marañón	Sylvatic (caves)
Cavernicola pilosa [b]	Loreto: Iquitos, Francisco de Orellana	Amazon basin	Sylvatic, hollow trees with bats
Eratyrus mucronatus [b]	San Martín: Huallaga	Huallaga	Sylvatic
	Loreto: Coronel Portillo	Marañón system	Sylvatic
Eratyrus cuspidatus [a]	Tumbes: Zarumilla	Tumbes, Zarumilla	Sylvatic
	Piura: Ayabaca	Nd	Nd
Belminus peruvianus [b]	Cajamarca: Jaén	Upper Marañón	Peridomestic colonies reported
	Amazonas	Marañón	Sylvatic
Rhodnius ecuadoriensis [a,b]	Tumbes: Tumbes, Zarumilla	Tumbes, Zarumilla	Domestic; *Tc*
	Piura: Ayabaca, Huancabamba, Morropón, Piura	Huancabamba, Huarmaca, Piura	Domestic-peridomestic (*Schinus molle* tree holes); *Tc, Tr*
	Lambayeque: Ferreñafe, Lambayeque	Zaña	Peridomestic; *Tc*
	Cajamarca: Jaén, Cutervo, Chota, San Miguel, Celendín, Cajamarca, Contumazá, San Benito	Cascas, Santa Ana	Domestic-peridomestic; *Tr*
	La Libertad: Trujillo, Otuzco, Cascas	Moche, Cascas, Alto Chicama, Huancay	Domestic, peridomestic (guinea pig corrals); *Tr*
Rhodnius robustus [b]	San Martín	Huallaga	Sylvatic (palms); *Tc*
	Loreto: Coronel Portillo	Yarinacocha, Ucayali	Sylvatic (palms)
Rhodnius pictipes [b]	Loreto: Coronel Portillo	Yurimaguas, Callerías, Yarinacocha	Sylvatic (palms); *Tc*
	San Martín	Huallaga valley (Huallobamba)	Domestic colonies claimed
Panstrongylus herreri [b, (a?)]	San Martín: Moyobamba, Rioja	Marañón, Huallaga	Domestic; *Tc*
	Amazonas: Bagua, Rodriguez de Mendoza	Marañón	Domestic
	Cajamarca: Jaén, Cutervo, San Ignacio, Santa Cruz	Marañón, Huallaga	Domestic; *Tc*
	Piura: Ayabaca	Nd	Peridomestic
Panstrongylus chinai [a, b]	Piura: Huancabamba, Ayabaca, Morropón, Paita, Piura, Sullana, Talara	Huancabamba, Chira	Peridomestic; domestic; *Tc*
	Tumbes: Zarumilla, Tumbes, Comandante Villar	Zarumilla, Tumbes	Peridomestic; *Tc*
	Lambayeque: Lambayeque	Zaña	Peridomestic; *Tc*
	La Libertad: Trujillo, Huamacucho, Otuzco, Bolívar, Chepén	Chamán	Peridomestic; *Tc*
	Cajamarca: Cajamarca, Contumazá, Celendín, Santa Cruz	Inter-Andean and transversal valleys	Peridomestic; sylvatic (attracted to light); *Tc*
	Amazonas: Bagua, Rodríguez de Mendoza, Chachapoyas	Amazon basin	Peridomestic; sylvatic
Panstrongylus geniculatus [b]	Cajamarca: Jaén, San Ignacio, Cutervo	Nd	Sylvatic; *Tc*
	Loreto: Coronel Portillo	Amazon basin	Sylvatic; *Tc*
Panstrongylus rufotuberculatus [a]	Tumbes: Comandante Villar	Tumbes	Sylvatic; peridomestic
	Piura: Ayabaca	Nd	Nd
Panstrongylus lignarius [b]	San Martín (see text)	Amazon basin	Sylvatic

a: reported from the Pacific slope; *b*: reported from the Amazon slope; *c*: Departments: provinces; *Tc*: *Trypanosoma cruzi*; *Tr*: *Trypanosoma rangeli*; Nd: no specific data. Additional information was obtained from Herrer and Wygodzinsky (1954), Herrer (1956, 1959, 1977), and Fernández-Loayza (1989).

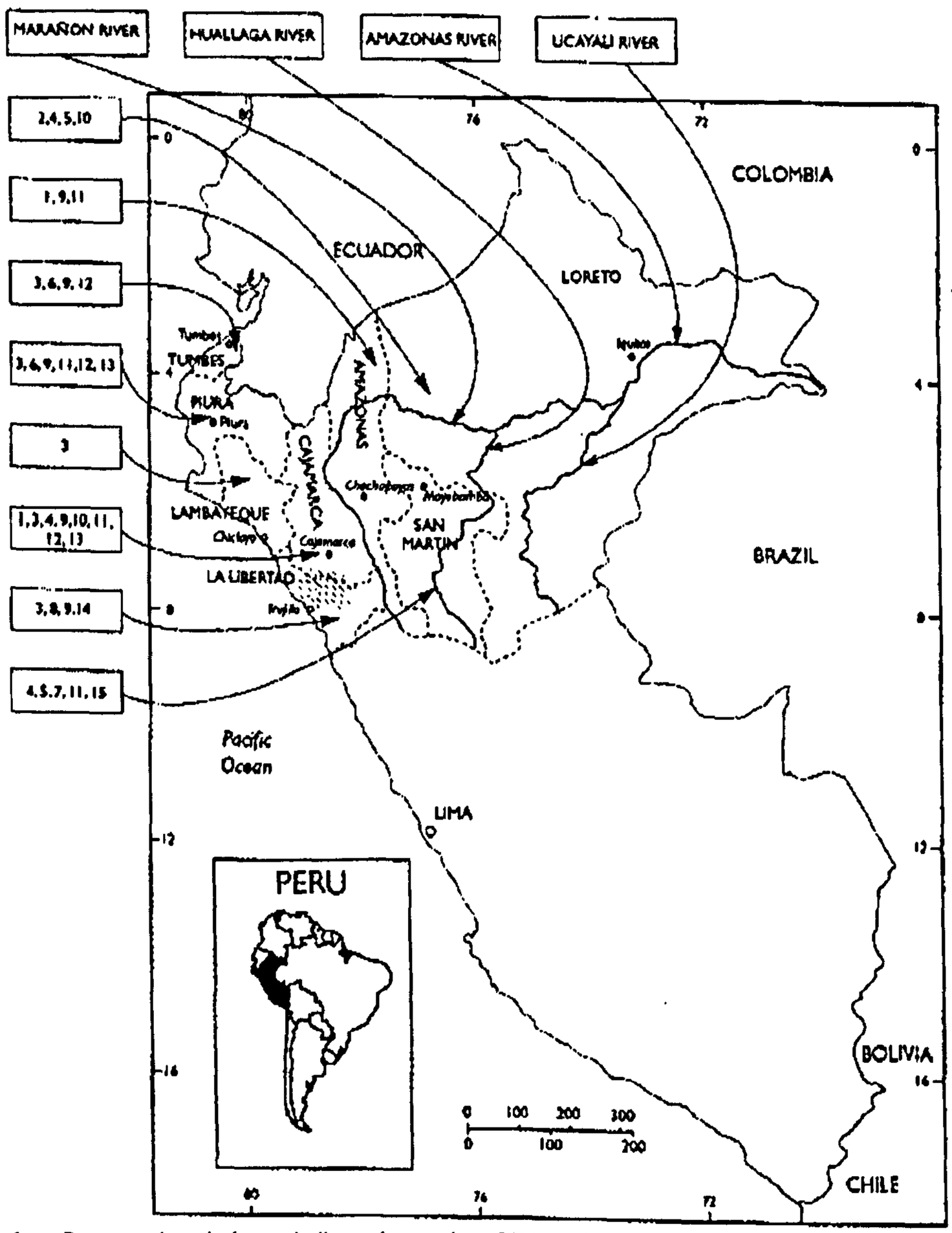
DISCUSSION

T. dimidiata, P. herreri, and *R. ecuadoriensis* are strongly synanthropic in northern Peru, and six more species can invade and sometimes colonise houses or peridomestic structures. Three of them may act as secondary disease vectors (*T. carrioni*, *P. chinai*, and *P. rufotuberculatus*), and *R. pictipes*, *R. robustus* and *P. geniculatus* may also be of some importance. Our study area comprised 15 life zones (including desert coastal lowlands, temperate Andean valleys, and humid forests). Some triatomines may have spread following these valleys, and have reached areas that appear out of their usual range.

Triatomine species

P. herreri is considered the principal vector of Chagas disease in northern Peru. Its distribution includes 5 Departments and 11 provinces, and it may be found at altitudes up to 1,500 m. It preferentially occupies humid life zones, and it is domestic-peridomestic in our study area (Herrer 1955, Lent & Wygodzinsky 1979, Calderón et al. 1985, Carcavallo et al. 1998b, 1999a, Curto de Casas et al. 1999). The species was recently reported from Ecuador (Aguilar et al. 1999). *P. herreri* shares most of its external characters with *P. lignarius*, a sylvatic Amazonian triatomine (see Lent & Wygodzinsky 1979). The only record of *P. lignarius* from Peru (cf. Calderón et al. 1985) may be due to misidentification; however, the taxonomic status of these two species remains unclear (Carcavallo et al. 1999b).

T. dimidiata is strongly synanthropic in northern Peru and in coastal Ecuador, where it is an important Chagas disease vector (Lent & Wygodzinsky 1979, Lazo 1985, Schofield 1994, Aguilar et al. 1999). In Peru, the species seems restricted to low, dry areas of Tumbes and La Libertad (its distribution being discrete rather than con-



Departments of northern Peru; numbers in boxes indicate the species of Triatominae reported from each Department (numbers as in Table I). The area where field studies on *Rhodnius ecuadoriensis* were conducted is marked with diagonal lines.

TABLE III

Triatomines from northern Peru: life zone ecology

Life zones [a]	Annual rain (mm)	Temperature (°C)	Altitude (m)	Species
Tropical desert	0-125	29-30	0-125	*T. dimidiata* *R. ecuadoriensis* *P. chinai*
Premontane desert	0-125	15-16.5	2250-2500	*P. chinai*
Tropical desert scrub	125-250	28.5-29	125-250	*T. dimidiata* *R. ecuadoriensis* *P. chinai* *P. geniculatus*
Premontane desert scrub	125-250	16.5-18	2000-2250	*R. ecuadoriensis*
Tropical thorn scrub	250-500	28-28.5	250-375	*T. dimidiata* *R. ecuadoriensis* *P. chinai* *P. rufotuberculatus*
Premontane thorn scrub	250-500	18-19.5	1750-2000	*P. chinai* *E. mucronatus*
Very dry tropical forest	500-1000	27-28	375-500	*T. dimidiata* *R. ecuadoriensis* *E. mucronatus*
Dry tropical forest	1000-2000	26-27	500-625	*T. dimidiata* *R. ecuadoriensis* *P. rufotuberculatus* *P. geniculatus* *E. cuspidatus* *E. mucronatus*
Dry premontane forest	500-1000	19.5-21	1500-1750	*T. carrioni* *R. ecuadoriensis* *P. rufotuberculatus* *E. mucronatus*
Tropical wet forest	2000-4000	25.5-26	625-750	*R. pictipes* *T. nigromaculata (?)* *R. robustus* *P. herreri* *P. geniculatus* *E. cuspidatus* *E. mucronatus* *C. pilosa*
Premontane wet forest	1000-2000	21-22.5	1250-1500	*T. carrioni* *P. herreri* *P. rufotuberculatus* *E. cuspidatus* *E. mucronatus* *H. matsunoi*
Tropical moist forest	4000-8000	25-25.5	750-875	*P. rufotuberculatus*
Premontane moist forest	2000-4000	22.5-24	1000-1250	*T. carrioni* *P. rufotuberculatus* *P. geniculatus* *B. peruvianus*
Tropical pluvial forest	> 8000	24-25	875-1000	*R. robustus* *B. peruvianus*
Low montane desert scrub	250-500	13.5-15	2500-2750	*T. carrioni*

a: life zones based on Curto de Casas et al. (1999); *T.: Triatoma; R.: Rhodnius; P.: Panstrongylus; E.: Eratyrus; B.: Belminus; C.: Cavernicola; H.: Hermanlentia*

tinuous) (Lizaraso 1955, Hidalgo 1957, Jara et al. 1998). Sylvatic populations have not been documented in Peru or Ecuador (cf. Abad-Franch et al. 2001). These features, and the discontinuity of the distribution of *T. dimidiata* in southern Colombia (only reported from the upper Magdalena valley) (Zeledón 1981, D'Alessandro & Barreto 1985), could be explained by an artificial introduction of the species to Ecuador and Peru. Preliminary results of morphometric (Abad-Franch 2000) and molecular studies (Marcilla et al. 2001) are lending support to this hypothesis. If confirmed, this would imply that eradication of the species from the region might be attainable (see Schofield 2000).

R. ecuadoriensis is a significant disease vector in southern Ecuador and northern Peru (Lent & Wygodzinsky 1979, Schofield 1994, Aguilar et al. 1999); even so, the studies on its ecology, behaviour, or vectorial role, are scarce and limited. It was first reported from Peru in 1955 (Llanos 1961, Herrer et al. 1972). Cuba Cuba et al. (1972) described domestic colonies in Cajamarca (7% infected by *T. rangeli*). F Vargas V (unpubl.) found 25% of 463 specimens infected by *T. rangeli* in La Libertad, and Castillo (1995) reported peridomestic colonies of the species from the Zaña valley, Lambayeque. Calderón (1996) reported that 3% of 3,450 triatomines captured in Tumbes and Piura (1973-1981) were *R. ecuadoriensis*. Sylvatic populations of this species occupy *Phytelephas aequatorialis* palm trees (endemic to humid areas of western Ecuador) (Borchsenius et al. 1998, Abad-Franch et al. 2000, 2001). Other ecotopes recorded are *Elaeis guineensis*, an artificially introduced, cultivated African palm (infested in Los Ríos, Ecuador, although only eggs and adults were found – the absence of nymphs perhaps indicating failure to colonise the ecotope; see Carcavallo & Martínez 1985), and a single record of the species in a hollow tree in an allegedly uninhabited area (Herrer et al. 1972); its presence in cacti has been cited (cf. Barrett 1991) but probably represents temporary occupation of the nest of a vertebrate near to an infested dwelling. Palm trees, the true primary habitat of the species, are absent from the arid-semiarid areas where *R. ecuadoriensis* is strongly synanthropic. The only documented finding of the species in an uninhabited area of Peru refers to a single nymph collected from a *Schinus molle* hollow tree. These observations suggest that *R. ecuadoriensis* probably has no truly sylvatic ecotope in northern Peru, and that its presence in the region is probably related to passive transportation in association with humans (perhaps about the early 1950s, as it was never reported before from Peru). The occurrence of the species within houses at high altitudes and in very arid zones also suggests a close bug-human association. Such a hypothesis would be rejected if truly sylvatic colonies were satisfactorily documented, and is currently being tested by means of morphometric and molecular analyses using different bug populations. If the hypothesis were not rejected, this would mean that a local eradication strategy could be implemented in northern Peru and some areas of Ecuador with good chances of success.

Our results show a moderately high house infestation rate (10%), with small breeding colonies inside houses rather than in peridomiciles. However, an overall infestation rate of 35% was revealed by a 2-year longitudinal survey in La Libertad (authors, unpubl.). The studied localities are located in arid valleys of the western slope of the Andes. Available data agree in that domestic-peridomestic populations of *R. ecuadoriensis* can be found in similar valleys throughout north-western Peru, suggesting that they constitute a favourable biotope for the species; however, further studies are required to accurately define the biogeography of the species in the country.

Natural infection by *T. cruzi* was absent in 257 *R. ecuadoriensis* examined, whereas *T. rangeli* was detected in 4% of bugs, suggesting that diagnostic tests for *T. cruzi* might yield false-positive results in some cases (Cuba Cuba 1998). This finding does not preclude however that *T. cruzi* may be transmitted by this species (e.g. Llanos 1961, Herrer et al. 1972, Lazo 1985, Castillo 1995).

P. chinai occurs on both slopes of the Andes in northern Peru and southern Ecuador. Although mainly sylvatic, its ability to invade and occasionally colonise DUs has been documented (Lent & Wygodzinsky 1979, Calderón et al. 1985, Lazo 1985). The species transmits *T. cruzi* among rats (*Rattus norvegicus*) and marsupials (*Didelphis* spp.) in Peru, and infected *P. chinai* nymphs were found in peridomestic ecotopes in La Libertad (Jara et al. 1998). The species is to be considered as a potential secondary vector in its distribution areas. Despite the claims that the species can be found in Venezuela (cf. Carcavallo et al. 1998a), we deem that it is most likely endemic to arid areas of northern Peru-southern Ecuador; those reports may be due to erroneous labelling of specimens or misidentification, and have led to concern that the species might be widespread in the Amazon and present in Colombia (Molina et al. 2000).

T. carrioni occasionally colonises human habitats in southern Ecuador and northern Peru. It occurs in both dry and humid life zones up to 2,650 m altitude (Lumbreras et al. 1955, Lent & Wygodzinsky 1979, Defranc 1982, Lazo 1985). In southern Ecuador, *T. carrioni* was considered an important pest until it was apparently replaced by *R. ecuadoriensis* (already present in the area in 1958, when the species was described from domestic bugs from La Toma, Loja) (Lent & León 1958, Defranc 1982, Lazo 1985). The presence of wild populations indicates that the possibility of re-infestation of treated dwellings persists.

P. rufotuberculatus is domestic in some areas of Peru, Ecuador, and Bolivia (Lizaraso 1955, Calderón et al. 1985, Lazo 1985, Noireau et al. 1994, Dujardin et al. 1998, Abad-Franch et al. 2001). Its epidemiological role deserves further research, at least in the Andean valleys and foothills where it seems adapted to human habitats. The species occurs mainly in low, dry areas, but may also be found in humid forests (and in higher valleys in southern Peru).

P. geniculatus occurs mainly in the Amazonian rainforests of Loreto, but has also been collected in Cajamarca (Lumbreras 1972). This is largely a sylvatic species, but seems to be readily attracted to electric light and occasionally colonises peridomestic pigsties and domiciles (Valente et al. 1998, Angulo et al. 1999, Reyes-Lugo & Rodríguez-Acosta 2000).

R. pictipes and *R. robustus* are reported from a wide geographical range in the Amazon. Records from

Cajamarca (Lumbreras 1972) and Trujillo (Fiocruz collections) are probably due to labelling errors. The biological characteristics of these sylvatic, palm tree-living Amazonian triatomines make us believe that adaptation to dry highlands with no palms, and thus their presence on the western slope of the Andes, is unlikely.

Other species (*E. mucronatus, E. cuspidatus, Belminus peruvianus, Hermanlentia matsunoi,* and *C. pilosa*) have little or no epidemiological significance in Peru. *E. cuspidatus* and *E. mucronatus* have been reported to show some degree of synanthropism in different countries (Lent & Wygodzinsky 1979, Noireau et al. 1995) but are still mainly sylvatic.

The presence of *T. nigromaculata* in Peru has been reported once (Calderón & Monzón 1995). Previously, the species was only known from Venezuela, where it is sylvatic (Lent & Wygodzinsky 1979). The probable affiliation of *T. nigromaculata* to the *dispar* complex (including *T. dispar, T. venosa,* and *T. carrioni*) may help explain this record. Although the species presents a quite characteristic chromatic pattern, it is conceivable that a pale variety of *T. carrioni* could have been misclassified as *T. nigromaculata*. The record deserves however further investigation.

Finally, it is worth mentioning that the main vector in the south of Peru, *T. infestans*, has never been reported from the area of interest of the present study. From its Bolivian origin, the species reached suburban areas of Lima, probably in association with rural immigrants. However, the migratory movements from the south rarely reach the northern Departments, as there are no higher standards of living in the north. Thus, the likelihood of *T. infestans* being introduced seems low.

The presence of a wide variety of triatomines in northern Peru (~12% of all recognised species), many of them occupying wild environments, could represent an important difficulty for disease control in the zone. *T. dimidiata, P. herreri*, and *R. ecuadoriensis* may constitute suitable targets for interventions against synanthropic bug populations, but the presence of other potential vector species (mainly *T. carrioni*, *P. rufotuberculatus* and *P. chinai*, and possibly also *P. geniculatus*, *R. pictipes*, and *R. robustus* – whose role as vectors needs to be clarified) must be taken into account. Some of these autochthonous species may behave as secondary vectors, occupying empty niches after domestic insects are eliminated by insecticide spraying. A strong longitudinal vigilance system with community involvement should be established to complement extensive residual insecticide spraying (Dias & Schofield 1999, Dias 2000). Finally, the control of vector-borne Chagas disease in the region must be developed on both sides of the Peruvian-Ecuadorian border. Strong collaborative links between public health authorities and research groups from both countries must be actively promoted and encouraged.

REFERENCES

Abad-Franch F 2000. *Ecology and Genetics of Chagas Disease Vectors in Ecuador. Implications for the Design of Control Strategies*, MPhil-PhD Upgrading Report, LSHTM, London, 150 pp.

Abad-Franch F, Noireau F, Paucar CA, Aguilar VHM, Carpio CC, Racines VJ 2000. The use of live-bait traps for the study of sylvatic *Rhodnius* populations (Hemiptera: Reduviidae) in palm trees. *Trans R Soc Trop Med Hyg 94*: 629-630.

Abad-Franch F, Paucar CA, Carpio CC, Cuba Cuba CA, Aguilar VHM, Miles MA 2001. Biogeography of Triatominae (Hemiptera: Reduviidae) in Ecuador: implications for the design of control strategies. *Mem Inst Oswaldo Cruz 96*: 611-620.

Aguilar VHM, Abad-Franch F, Racines VJ, Paucar CA 1999. Epidemiology of Chagas disease in Ecuador. A brief review. *Mem Inst Oswaldo Cruz 94* (Suppl. I): 387-393.

Angulo VM, Gutiérrez R, Rubio I, Joya M, Arismendi M, Esteban L, Sandoval CM 1999. Triatomíneos domiciliados y silvestres: impacto en la transmisión de la enfermedad de Chagas en Santander. In VM Angulo, *Control y Manejo de la Trypanosomiasis Americana. Curso-taller Internacional*, Universidad Industrial de Santander-CINTROP, Bucaramanga, Colombia, p. 72-76.

Barreda G 1996. Enfermedad de Chagas en el Perú. In CJ Schofield, JP Dujardin, J Jurberg (eds), *Proceedings of the International Workshop on Population Genetics and Control of Triatominae, Santo Domingo de los Colorados, Ecuador, Sept. 1995*, INDRE, Mexico City, Mexico, p. 39.

Barrett TV 1991. Advances in triatomine bug ecology in relation to Chagas disease. *Adv Dis Vector Res 8*: 143-176.

Borchsenius F, Borgtoft Pedersen H, Balslev H 1998. *Manual to the Palms of Ecuador*, AAU Reports 37, Aarhus University, Universidad Católica del Ecuador, Aarhus, Denmark, 221 pp.

Brack A 1987. Ecología de un país complejo. In MJ Dourojeanni, *Gran Geografía del Perú: Naturaleza y Hombre*, vol. II, Manfer Juan Mejía Baca, Spain, p. 179-194.

Calderón FGE 1996. Chinches triatominos (Hemiptera: Reduviidae) de la Región Grau, Perú. *Rev Peruana Entomol 38*: 19-22.

Calderón FGE, Monzón L 1995. Nota científica: primer hallazgo de *Triatoma nigromaculata* (Stål, 1872) en el Perú. *Rev Peruana Entomol 37*: 124.

Calderón FGE, Figueroa K, Náquira F, Carcavallo RU, Canale DM 1985. Perú. In RU Carcavallo, JE Rabinovich, RJ Tonn (eds), *Factores Biológicos y Ecológicos de la Enfermedad de Chagas*, vol. II, CPEHS OPS/OMS, SNCh, Ministerio de Salud y Acción Social, Argentina, p. 449-456.

Carcavallo RU, Martínez A 1985. Biología, ecología, distribución geográfica de los triatominos americanos. In RU Carcavallo, JE Rabinovich, RJ Tonn (eds), *Factores Biológicos y Ecológicos en la Enfermedad de Chagas*, vol. I, CPEHS OPS/OMS, SNCh, Ministerio de Salud y Acción Social, Argentina, p. 149-208.

Carcavallo RU, Curto de Casas SI, Sherlock IA, Galíndez Girón I, Jurberg J, Galvão C, Mena Segura CA, Noireau F 1999a. Geographical distribution and alti-latitudinal dispersion. In RU Carcavallo, I Galíndez Girón, J Jurberg, H Lent (eds), *Atlas of Chagas Disease Vectors in the Americas*, vol. III, Fiocruz, Rio de Janeiro, p. 747-792.

Carcavallo RU, Galvão C, Lent H 1998a. *Triatoma jurbergi* sp. n. do norte do Estado do Mato Grosso, Brasil (Hemiptera, Reduviidae, Triatominae), com uma atualização das sinonímias e outros táxons. *Mem Inst Oswaldo Cruz 93*: 459-464.

Carcavallo RU, Jurberg J, Lent H 1999b. Phylogeny of the Triatominae. A - General approach. In RU Carcavallo, I Galíndez Girón, J Jurberg, H Lent (eds), *Atlas of Chagas Disease Vectors in the Americas*, vol. III, Fiocruz, Rio de Janeiro, p. 925-969.

Carcavallo RU, Rodríguez MEF, Salvatella R, Curto de Casas SI, Sherlock IA, Galvão C, Rocha DS, Galíndez Girón I, Otero Arocha MA, Martínez A, Da Rosa JA, Canale DM, Farr TH, Barata JMS 1998b. Habitats and related fauna. A: General. In RU Carcavallo, I Galíndez Girón, J Jurberg, H Lent (eds), *Atlas of Chagas Disease Vectors in the Americas*, vol. II, Fiocruz, Rio de Janeiro, p. 561-600.

Castillo RO 1995. *Rhodnius ecuadoriensis* naturalmente infectado por *Trypanosoma cruzi* en el valle de Zaña, Lambayeque. II Congreso Peruano de Parasitología, Trujillo, Perú, p. 38.

Cuba Cuba CA 1998. Revisión de los aspectos biológicos y diagnósticos del *Trypanosoma (Herpetosoma) rangeli. Rev Soc Bras Med Trop 31*: 207-220.

Cuba Cuba CA, Morales N, Fernández E, Fernández W 1972. Hallazgo de *Rhodnius ecuadoriensis* Lent & León, 1958 infectado naturalmente por trypanosomas semejantes a *Trypanosoma rangeli* Tejera, 1920 en caseríos de la Provincia de Cascas, Contumazá, Perú. *Rev Inst Med Trop São Paulo 14*: 191-202.

Curto de Casas SI, Carcavallo RU, Galíndez Girón I, Burgos JJ 1999. Bioclimatic factors and zones of life. In RU Carcavallo, I Galíndez Girón, J Jurberg, H Lent (eds), *Atlas of Chagas Disease Vectors in the Americas*, vol. III, Fiocruz, Rio de Janeiro, p. 793-838.

D'Alessandro A, Barreto P 1985. Colombia. In RU Carcavallo, JE Rabinovich, RJ Tonn (eds), *Factores Biológicos y Ecológicos de la Enfermedad de Chagas,* vol. II, CPEHS OPS/OMS, SNCh, Ministerio de Salud y Acción Social, Argentina, p. 377-399.

Defranc MI 1982. *Enfermedad de Chagas*, Casa de la Cultura Ecuatoriana, Núcleo del Guayas, Guayaquil, Ecuador, 200 pp.

Dias JCP 2000. Vigilância epidemiológica em doença de Chagas. *Cad Saúde Púb 16* (Suppl. 2): 43-59.

Dias JCP, Schofield CJ 1999. The evolution of Chagas disease (American trypanosomiasis) control after 90 years since Carlos Chagas discovery. *Mem Inst Oswaldo Cruz 94* (Suppl. I): 103-121.

Dujardin JP, Forgues G, Torrez M, Martínez E, Córdoba C, Gianella A 1998. Morphometrics of domestic *Panstrongylus rufotuberculatus* in Bolivia. *Ann Trop Med Parasitol 92*: 219-228.

Fernández-Loayza R 1989. *Triatoma matsunoi*, nueva especie del norte peruano (Hemiptera, Reduviidae, Triatominae). *Rev Peruana Entomol 31*: 21-24.

Guhl F 1999. Programas en la eliminación de la transmisión de la enfermedad de Chagas en los países andinos. In CJ Schofield, C Ponce (eds), *Proceedings of the Second International Workshop on Population Genetics and Control of Triatominae, Tegucigalpa, Honduras, March 1998*, INDRE, Mexico City, p. 38-43.

Guillén Z, Cáceres I, Elliot A, Ramírez J 1989. Triatominos del norte peruano y su importancia como vectores de *Trypanosoma* spp. *Rev Peruana Entomol 31*: 25-30.

Herrer A 1955. Trypanosomiasis Americana en el Perú V. Triatominos del valle interandino del Marañón. *Rev Med Exp Lima 9*: 69-81.

Herrer A 1956. Observaciones sobre la enfermedad de Chagas en la Provincia de Moyobamba (Departamento de San Martín). *Rev Med Exp Lima 10*: 59-74.

Herrer A 1959. La enfermedad de Chagas en el Perú: breve revisión de los conocimientos adquiridos hasta 1958. *Rev Goiana Med 5*: 389-409.

Herrer A 1977. Reseña de la Entomología Médica en el Perú. I. Principales aspectos entomológicos en la bartonelosis, la trypanosomiasis y la leishmaniasis. *Rev Peruana Entomol 20*: 19-24.

Herrer A, Wygodzinsky P 1954. Contribución al conocimiento del género *Belminus* Stål, 1859 (Triatominae, Reduviidae, Hemiptera). *An Inst Med Reg Tucumán 4*: 85-105.

Herrer A, Wygodzinsky P, Napan M 1972. Presencia de *Trypanosoma rangeli* Tejera, 1920, en el Perú. I. El insecto vector, *Rhodnius ecuadoriensis* Lent & León, 1958. *Rev Biol Trop 20*: 141-149.

Hidalgo R 1957. Trypanosomiasis Americana en el Perú. Observaciones entomológicas en el Departamento de Tumbes. *Rev Med Exp Lima 11*: 71-85.

Jara CA, Escalante H, Roldán J, Díaz Limai E 1998. Distribución y frecuencia de infección por *Trypanosoma cruzi* de triatominos y *Ovis aries* en el valle de Chamán, La Libertad, Perú. *Sciendo 1*: 23-31.

Lazo R 1985. Ecuador. In RU Carcavallo, JE Rabinovich, RJ Tonn (eds), *Factores Biológicos y Ecológicos de la Enfermedad de Chagas*, vol. II, CPEHS OPS/OMS, SNCh, Ministerio de Salud y Acción Social, Argentina, p. 413-427.

Lent H, León LA 1958. Um novo *Rhodnius* Stål do Ecuador (Hemiptera, Reduviidae). *Rev Bras Biol 18*: 181-185.

Lent H, Wygodzinsky P 1979. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas disease. *Bull Am Mus Nat History 163*: 123-520.

Lizaraso Y 1955. Nota sobre el hallazgo de *Triatoma dimidiata* y *Panstrongylus rufotuberculatus* en el Perú. *Rev Med Exp Lima 9*: 119-121.

Llanos B 1961. Hallazgo en el Perú del *Rhodnius ecuadoriensis* Lent & León, 1958 naturalmente infectado por el *Trypanosoma cruzi. Arch Per Patol Clín Lima 15*: 133-140.

Lumbreras H 1972. El problema de la enfermedad de Chagas en los Departamentos del Perú. *Rev Viernes Méd Lima 23*: 43-77.

Lumbreras H, Arrarte JA, Tejada A 1955. Nota acerca de la presencia del *Panstrongylus herreri* y *Triatoma carrioni* en el distrito de Querocotillo, Provincia de Cutervo del Departamento de Cajamarca. *Rev Méd Peruana 26*: 444-446.

Marcilla A, Bargues MD, Ramsey JM, Magallón-Gastélum E, Salazar-Schettino PM, Abad-Franch F, Dujardin JP, Schofield CJ, Mas-Coma S 2001. The ITS-2 of the nuclear rDNA as a molecular marker for populations, species, and phylogenetic relationships in Triatominae (Hemiptera: Reduviidae), vectors of Chagas disease. *Mol Phylogenet Evol 18*: 136-142.

Miles MA 1998. New World Trypanosomiasis. In L Collier, A Balows, M Sussman (eds), *Microbiology and Microbial Infections, vol. 5: Parasitology*, Topley & Wilson's, UK, p. 238-302.

Miles MA, Arias RJ, Souza AA 1983. Chagas disease in the Amazon basin: V. Periurban palms as habitats of *Rhodnius robustus* and *Rhodnius pictipes* – triatomine vectors of Chagas disease. *Mem Inst Oswaldo Cruz 78*: 391-398.

Miles MA, Souza AA, Povoa M 1981. Chagas disease in the Amazon basin. III. Ecotopes of ten triatomine bugs species (Hemiptera: Reduviidae) from the vicinity of Belém, Pará State, Brazil. *J Med Entomol 18*: 266-278.

Molina JA, Gualdrón LE, Brochero HL, Olano VA, Barrios D, Guhl F 2000. Distribución actual e importancia epidemiológica de las especies de triatominos (Reduviidae: Triatominae) en Colombia. *Biomedica 20*: 344-360.

Mostacero LJ, Mejía F, Peláez FP 1996. *Fitogeografía del Norte del Perú*, Concytec, Lima, Peru.

Noireau F, Bosseno MF, Carrasco R, Telleria J, Vargas F, Camacho C, Yaksic N, Brenière SF 1995. Sylvatic triatomines (Hemiptera: Reduviidae) in Bolivia: trends towards domesticity and possible infection with *Trypanosoma cruzi*

(Kineto-plastida: Trypanosomatidae). *J Med Entomol 32*: 594-598.

Noireau F, Bosseno MF, Vargas F, Brenière SF 1994. Apparent trend to domesticity observed in *Panstrongylus rufotuberculatus* Champion, 1899 (Hemiptera: Reduviidae) in Bolivia. *Res Rev Parasitol 54*: 263-264.

Reyes-Lugo M, Rodríguez-Acosta A 2000. Domiciliation of the sylvatic Chagas disease vector *Panstrongylus geniculatus* Latreille, 1811 (Triatominae: Reduviidae) in Venezuela. *Trans R Soc Trop Med Hyg 94*: 508.

Schofield CJ 1994. *Triatominae. Biologia y Control*, Eurocommunica Publications, West Sussex, 80 pp.

Schofield CJ 2000. *Challenges of Chagas Disease Vector Control in Central America*, Position paper WHO/CDS/WHOPES/GCDPPH/2000.1, WHO, Geneva, 36 pp.

Sherlock IA, Carcavallo RU, Galíndez Girón I 1997. List of natural and experimental flagellate infections in several Triatominae species. In RU Carcavallo, I Galíndez Girón, J Jurberg, H Lent (eds), *Atlas of Chagas Disease Vectors in the Americas*, vol. I, Fiocruz, Rio de Janeiro, p. 289-298.

Teixeira ARL, Monteiro PS, Rebelo JM, Argañaraz ER, Vieira D, Lauria-Pires L, Nascimento R, Vexenat CA, Silva AR, Ault SK, Costa JM 2001. Emerging Chagas disease: trophic network and cycle of transmission of *Trypanosoma cruzi* from palm trees in the Amazon. *Emerg Infect Dis 7*: 100-112.

Valente SAS, Valente VC, Fraiha Neto H 1999. Considerations on the epidemiology of Chagas disease in the Brazilian Amazon. *Mem Inst Oswaldo Cruz 94* (Suppl. I): 395-398.

Valente VC, Valente SAS, Noireau F, Carrasco HJ, Miles MA 1998. Chagas disease in the Amazon basin: association of *Panstrongylus geniculatus* (Hemiptera: Reduviidae) with domestic pigs. *J Med Entomol 35*: 99-103.

WHO-World Health Organization 1991. *Control of Chagas disease*, WHO Technical Report, Series 811.

Zeledón R 1981. *El Triatoma dimidiata y su Relación con la Enfermedad de Chagas*, Universidad Estatal a Distancia, San José, Costa Rica, 146 pp.

ELSEVIER

Infection, Genetics and Evolution 1 (2002) 225–235

Infection, Genetics and Evolution

www.elsevier.com/locate/meegid

Nuclear rDNA ITS-2 sequences reveal polyphyly of *Panstrongylus* species (Hemiptera: Reduviidae: Triatominae), vectors of *Trypanosoma cruzi*☆

A. Marcilla[a], M.D. Bargues[a,*], F. Abad-Franch[b,c], F. Panzera[d], R.U. Carcavallo[e], F. Noireau[e,f], C. Galvão[e], J. Jurberg[e], M.A. Miles[b], J.P. Dujardin[g], S. Mas-Coma[a]

[a] *Departamento de Parasitología, Facultad de Farmacia, Universidad de Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot-Valencia, Valencia, Spain*
[b] *Pathogen Molecular Biology and Biochemistry Unit, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1 E7HT, UK*
[c] *Instituto "Juan César García", Fundación Internacional de Ciencias Sociales y Salud, Casilla Postal 17-1106292 Quito, Ecuador*
[d] *Sección de Genética Evolutiva, Instituto de Biología, Facultad de Ciencias, Universidad de la República, Calle Igua 4225, 11400 Montevideo, Uruguay*
[e] *Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Departamento de Entomologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brazil*
[f] *URO16, Institut de Recherche pour le Développement (IRD), Av. Agropolis 911, 34032 Montpellier Cedex 1, France*
[g] *UMR IRD/ORSTOM-CNRS 9926, URO62, Institut de Recherche pour le Développement (IRD), Av. Agropolis 911, 34032 Montpellier Cedex 1, France*

Received 24 July 2001; received in revised form 12 January 2002; accepted 25 January 2002

Abstract

Panstrongylus species are widely distributed throughout the Americas, where they act as vectors of *Trypanosoma cruzi*, agent of Chagas disease. Their intraspecific relationships, taxonomic position and phylogeny in relation to other Triatomini were explored using ribosomal DNA (rDNA) internal transcribed spacer 2 (ITS-2) sequence polymorphisms and maximum parsimony, distance and maximum likelihood analyses of 10 populations representing six species of the genus (*P. megistus*, *P. geniculatus*, *P. rufotuberculatus*, *P. lignarius*, *P. herreri* and *P. chinai*). At the subspecific level, *P. megistus* appeared more homogeneous than *P. rufotuberculatus* and *P. geniculatus* (both with broader distribution). Several dinucleotide microsatellites were detected in the sequences of given species. Many of these microsatellites (GC, TA, GT and AT) showed different number of repeats in different populations and thus, may be very useful for population differentiation and dynamics analyses in future studies. The sequences of *P. lignarius* (considered sylvatic) and *P. herreri* (a major disease vector in Peru) were identical, suggesting that these species should be synonymised. Intrageneric analysis showed a clear separation of *P. rufotuberculatus*, with closest relationships between *P. geniculatus* and *P. chinai*, and *P. megistus* occupying a separate branch. Genetic distances between *Panstrongylus* species (0.11585–0.22131) were higher than those between *Panstrongylus* and other Triatomini (16 species from central and North America and South America) (0.08617–0.11039). The distance between *P. megistus* and *P. lignarius/herreri* (0.22131) was the largest so far recorded in the tribe. The pronounced differences in length and nucleotide composition suggest a relatively old divergence of *Panstrongylus* species. *P. rufotuberculatus* was closer to Mesoamerican *Triatoma*, *Meccus* and *Dipetalogaster* species than to other *Panstrongylus*. All *Panstrongylus* clustered with the Mesoamerican clade; *P. rufotuberculatus* clustered with the *phyllosoma* complex and *T. dimidiata*, with *D. maxima* and *T. barberi* in a basal position. The rest of *Panstrongylus* appeared paraphyletically in the tree. This is evidence suggesting polyphyly within the genus *Panstrongylus*, whose species may be related to the ancestors giving rise to central and North American Triatomini.

Keywords: *Panstrongylus* species; Triatominae; Chagas disease vectors; rDNA ITS-2 sequences; Taxonomy; Phylogeny

1. Introduction

The Triatominae (Hemiptera: Reduviidae) are notorious as the vectors of *Trypanosoma cruzi*, which infects a great variety of sylvatic and domestic mammals and causes American trypanosomiasis (Chagas disease) in humans throughout Latin America. Over 12 million people are infected by this parasite, with about 90 million considered at risk in endemic areas. No vaccine is available and except in the very early stage of the infection, there is no effective chemotherapy (WHO, 1991). A total of 133 species of Triatominae are currently recognised, grouped into 18 genera forming five tribes (Dujardin et al., 2000; Carcavallo et al., 2000). Over half of these species have been naturally or experimentally infected

☆ New nucleotide sequence data reported in this paper are available in the GenBank™, EMBL and DDBJ databases under the accession numbers listed in Table 1.

* Corresponding author. Tel.: +34-96-386-4298; fax: +34-96-386-4769.
E-mail address. M.D.Bargues@uv.es (M.D. Bargues).

1567-1348/02/$ – see front matter
PII: S1567-1348(02)00029-1

with *T. cruzi* and according to their similar behaviour and physiology, all species must be regarded as potential vectors.

Species of greatest epidemiological significance are those that have adapted to live in close association with humans, mainly infesting rural dwellings in poor condition. However, an increasing number of species seems to be following a similar adaptive route from sylvatic to domestic habitats (Schofield et al., 1999) and understanding of this evolutionary transition is of considerable importance in relation to epidemiological surveillance and control of Chagas disease vectors (WHO, 1991).

The genus *Panstrongylus* Berg, 1879 is, together with *Triatoma* Laporte, 1832 (Triatomini) and *Rhodnius* Stal, 1859 (Rhodniini), one of the genera of foremost epidemiological importance. It comprises 14 species, four of which may develop domestic colonies in some geographic areas, six are sylvatic species occasionally recorded in the domestic environment and two are exclusively sylvatic species (Dujardin et al., 2000). A rare Brazilian species, *P. lenti* Galvão et Palma, 1968 is known from only two individuals and *P. sherlocki* Jurberg, Carcavallo et Lent, 2001 from only one specimen (Jurberg et al., 2001).

Four species can colonise human habitats. *P. megistus* (Burmeister, 1835) has great epidemiological importance (it was in fact the first Chagas disease vector to be described); it has been reported from Brazil, Argentina, Paraguay and Uruguay (Dujardin et al., 2000) and recently also southeastern Bolivia (Noireau et al., 1999); *P. rufotuberculatus* (Champion, 1899) is broadly distributed in South America, central America and Mexico, often infected by *T. cruzi*, and is domiciliated in some parts of Bolivia (Noireau et al., 1994; Dujardin et al., 1998), Peru and Ecuador (Abad-Franch et al., 2001); *P. chinai* (Del Ponte, 1929) and *P. herreri* Wygodzinsky, 1948, both known from Peru and Ecuador (Aguilar et al., 1999; Carcavallo et al., 1999a; Abad-Franch et al., 2001), have been reported from domestic environments and infected by *T. cruzi* (Dujardin et al., 2000).

Several sylvatic species have occasionally been recorded in the domestic environment or attracted by electric light into houses. *P. geniculatus* (Latreille, 1811) is very broadly distributed through South America, central America and Mexico and colonises peridomestic pigsties in Brazil (Valente et al., 1998); *P. lutzi* (Neiva et Pinto, 1923) is limited to Brazil; *P. howardi* (Neiva, 1911) only occurs in Ecuador; *P. guentheri* Berg, 1879 is found in Argentina, Uruguay, Paraguay and in southern Bolivia; *P. humeralis* (Usinger, 1939) is only known from Panama; and *P. diasi* Pinto et Lent, 1946 is widely distributed in Brazil and also recorded in Bolivia (Carcavallo et al., 1999a). All these species, except *P. lutzi* and *P. diasi*, have been found infected by *T. cruzi* (Dujardin et al., 2000).

Finally, two species appear to be exclusively sylvatic, but naturally infected by *T. cruzi*: *P. lignarius* (Walker, 1873) known from Brazil, Guyana, Suriname and Venezuela, the records from Ecuador pending confirmation (Abad-Franch et al., 2001) and *P. tupynambai* Lent, 1942, which is found under stones in Brazil and Uruguay (Carcavallo et al., 1999a; Dujardin et al., 2000).

Although some *Panstrongylus* species, such as *P. megistus*, can be found in palm crowns, all the species in the genus are predominantly associated with terrestrial burrows, tree root cavities and/or arboreal tree holes. A sylvatic habitat of the highly domiciliated species *P. megistus* is hollow trees with *Didelphis* (Gaunt and Miles, 2000).

The recent reports about the increasing frequency of *Panstrongylus* species displaying ability to invade and colonise human habitats are focusing the interest of medical entomologists and Chagas disease control managers throughout Latin America (Noireau et al., 1994, 1995; Chico et al., 1997; Dujardin et al., 1998; Valente et al., 1998; Aguilar et al., 1999; Borges et al., 1999; Abad-Franch et al., 2001). A more accurate knowledge of these triatomine species, including distributions, adaptive trends towards domesticity, population dynamics, vectorial capacity and susceptibility to insecticides, would be important within the Chagas control programmes and essential in localities where they are presently colonising human structures (Noireau et al., 1994). An improved knowledge on the interspecific relationships within this genus may significantly help understand the dynamics of the synanthropic behaviour of some species. It will additionally strengthen the ability of researchers and control managers to make some predictions in regard of the potential epidemiological role of each species in their respective areas, allowing for anticipatory decision-making.

In eukaryotes, ribosomal DNA (rDNA) consists of multiple copies of tandemly repeated transcriptional units. Each transcriptional unit consists of regions that code for three ribosomal subunits (18S, 5.8S and 28S) separated by two spacers, internal transcribed spacers 1 and 2 (ITS-1 and ITS-2) (Hillis and Dixon, 1991). Ribosomal DNA has been used in phylogenetic studies at several taxonomic levels, ranging from major phyla to populations (Brower and DeSalle, 1994; Bargues and Mas-Coma, 1997). This broad utility of rDNA is because the multiple copies per genome are usually tandemly repeated and the non-coding spacers evolve faster than the coding regions (Hillis and Dixon, 1991). Like other multigene families, individual rDNA copies are not believed to accumulate mutations independently, thus resulting in little intragenomic or intraspecific variation but substantial interspecific differentiation. Concerted evolution of rDNA within species has resulted in the use of the faster evolving spacers, not only for the reconstruction of phylogenies, but as diagnostic markers for differentiating species, including proximal and cryptic species (Bargues et al., 2001).

Nuclear rDNA sequences have recently shown their usefulness in triatomine bugs for the above-mentioned purposes (Bargues et al., 2000, 2002). The ITS-2 of the rDNA has proved to be a good molecular marker for populations, species and phylogenetic relationships in Triatominae (Marcilla et al., 2000, 2001), because no intragenomic polymorphism at this locus, as reported in mosquitoes (Onyabe

and Conn, 1999), has been found in triatomine bugs up to the present. The aim of the present paper is to characterise the rDNA ITS-2 sequences of the species of the genus *Panstrongylus* with wider geographic range and higher epidemiological significance, analyse their intra- and inter-specific relationships and compare them with other species of the same tribe, mainly belonging to the closely related genus *Triatoma*.

2. Materials and methods

2.1. Triatominae materials

Specimens from 10 populations of six species of the genus *Panstrongylus* were studied (Table 1). Genomic DNA was extracted from more than one specimen of a given population and from more than one population of a given species when necessary for sequence conservation verification studies, mainly in cases of microsatellite detection or when unexpected results were obtained.

2.2. Molecular techniques

2.2.1. DNA extraction

Triatomine legs fixed in 70% cold ethanol were used for DNA extraction according to the standard phenol/chloroform technique (Sambrook et al., 1989). Each bug was examined individually and was processed essentially as previously described (Marcilla et al., 2001). One or two legs were disrupted with flame-sterilised scissors, placed in 1.5 ml microcentrifuge tubes together with an homogeniser and suspended in 400 μl of lysis buffer (10 mM Tris–HCl, pH 8.0, 100 mM EDTA, 100 mM NaCl, 1% sodium dodecyl sulphate) containing 500 μg/ml proteinase K (Promega, Madison, WI). The following steps were performed according to methods outlined previously (Bargues and Mas-Coma, 1997). The lysed preparation was gently mixed and then incubated for 4 h at 55 °C with alternate shaking each 15 min. For the extraction of total DNA, three steps were followed. In the first, there was an equal volume of phenol; in the second, 200 μl of phenol and 200 μl of chloroform/isoamyl alcohol (24/1) were used; in the third, 400 μl of chloroform/isoamyl alcohol (24/1) were employed. After each extraction step, phases were separated at 12,000 × *g* for 3 min. The aqueous phase was precipitated with 1/10 volume of 4 M ammonium acetate and 2.5 volumes of 100% ethanol and refrigerated at −20 °C. The spooled DNA or pellet obtained was washed in 70% ethanol, centrifuged at 12,000–13,000 × *g* for 5–10 min at 4 °C and briefly air-dried. The precipitated DNA was redissolved in a small volume (20–100 μl) of sterile TE buffer (10 mM Tris–HCl, pH 7.6, 1 mM EDTA) and stored at −20 °C until use.

2.2.2. rDNA sequence amplification

The fragment corresponding to a 127 bp sequence of the 5.8S rRNA gene and the ITS-2 of each triatomine bug was amplified by the polymerase chain reaction (PCR) using specific primers as previously described (Marcilla et al., 2001). Double or multiple bands in PCR products were never observed.

2.2.3. Purification and quantification of PCR products

Primers and nucleotides were removed from PCR products by purification on Wizard® PCR Preps DNA purification system (Promega, Madison, WI) according to the manufacturer's protocol and resuspended in 50 μl of 10 mM TE buffer (pH 7.6). The final DNA concentration was determined by measuring the absorbance at 260 and 280 nm.

2.2.4. DNA sequencing

Sequencing of the ITS-2 of the rDNA was performed on both strands by the dideoxy chain-termination method (Sanger et al., 1977) and was carried out with the *Taq* dye-terminator chemistry kit for ABI 373A (Perkin-Elmer,

Table 1
Panstrongylus species and populations studied, including geographic origins, nucleotide length and composition of the ITS-2 sequences obtained and corresponding GenBank™ accession numbers

Species of *Panstrongylus*	Populations studied (geographic origin)	ITS-2 length (bp)	AT content (%)	Accesion number
P. megistus (Burmeister, 1835)	Pampulha, Minas Gerais, Brazil	600	75.2	AJ306542
P. geniculatus (Latreille, 1811)	Yasuní, Orellana, Ecuador	506	76.8	AJ306543
	Belén, Pará, Brazil	510	76.6	AJ306544
P. rufotuberculatus (Champion, 1899)	Guayacón, El Oro, Ecuador	470	76.7	AJ306545
	El Carmen, Santander, Colombia	472	76.8	AJ306546
P. chinai (Del Ponte, 1929)	Laboratory strain, INHMT[a], Quito, Ecuador	503	76.7	AJ306547
P. lignarius (Walker, 1873)	San Pablo, Sucumbios, Ecuador	492	78.6	AJ306548
	Santa Bárbara, Pará, Brazil	492	78.6	AJ306549
P. herreri (Wigodzinsky, 1948)	Yasuní, Orellana, Ecuador	492	78.6	AJ306550
	Laboratory strain Fiocruz, origin from Cajamarca, Perú	492	78.6	AJ306551

[a] INHMT: Instituto Nacional de Higiene y Medicina Tropical.

Foster City, CA), using PCR primers. Poor quality sequences were never obtained.

2.3. Software programmes used

2.3.1. For sequence alignment

For all data sets, to ensure that sequences of ITS-2 would begin at the same position, a 127 bp long fragment of the 5.8S rRNA gene was also sequenced. Sequences were aligned using CLUSTAL-W version 1.8 (Thompson et al., 1994) and introducing sequences in different orders at random to reduce biases (Lake, 1991). The alignments were made including the *Panstrongylus* species studied together with other known triatomine bug sequences. Several rDNA ITS-2 sequences of species of Triatomini present in GenBank™ and EMBL were used: *Triatoma infestans* from Bolivia (AJ286874) and Paraguay (AJ289876); *T. sordida* from Bolivia (AJ293589); *T. brasiliensis* from Brazil (AJ293591); *T. dimidiata* from Mexico (different origins: AJ286877, AJ286878, AJ286879 and AJ286880), Honduras and Ecuador (AJ286875) and Nicaragua (AJ286876); *T. phyllosoma* (AJ286881), *T. pallidipennis* (AJ286882), *T. longipennis* (AJ286883), *T. picturata* (AJ286884), *T. mazzottii* (AJ286885), *T. barberi* (AJ293590) and *Dipetalogaster maxima* (AJ286887), all from Mexico (Marcilla et al., 2001). The five species of the *phyllosoma* complex have been very recently included in the genus *Meccus* Stal, 1859 by Carcavallo et al. (2000). *Panstrongylus megistus* from the laboratory strain of INLASA, La Paz, Bolivia, derived from Fiocruz, Belo Horizonte, Brazil (AJ286886), the only ITS-2 sequence of *Panstrongylus* presently available in GenBank™, was used for comparison; previously described as composed of 559 bp (Marcilla et al., 2001), its full length of 598 bp is used in the present paper. *Rhodnius prolixus* (Rhodniini) (AJ286888) (Marcilla et al., 2001) was also used as outgroup in different phylogenetic analyses.

2.3.2. For phylogenetic analysis

Maximum parsimony (MP), distance and maximum likelihood (ML) methods were used in phylogeny reconstruction. All these analyses were performed using algorithms provided in PAUP v.4.0b 6 for Macintosh (Swofford, 2001) and TREECON v1.3b for Windows (Van De Peer and De Wachter, 1997).

MP analysis was performed using the heuristic algorithm. To assess the relative support for internal nodes, a bootstrap-resampling approach (with 1000 replicates) was used. Alignment gaps were treated as missing character-states for the analyses. Only minimal length trees were kept. Polytomies were permitted. Accelerated transformation was used for character-state optimisation.

For distance analysis, neighbour-joining (NJ) trees (Saitou and Nei, 1987) were generated from four different models because of the A + T bias found: Tamura-Nei, Kimura two-parameter, Kimura two-parameter using γ-corrected distances and Kimura three-parameter. Support of each NJ tree was assessed with bootstrap-resampling technique (Felsenstein, 1985) over 1000 replications.

ML trees were constructed utilising the HKY85 model of DNA substitution assuming that all sites evolve at the same rate and the transition/transversion rate = 2 (κ = 5.630). Because of the A + T bias detected, transition/transversion rates of 4, 6 and 8 were also tested. To provide an assessment of the precision of the trees, a quartet puzzling analysis was employed (with 1000 puzzling steps).

3. Results

3.1. Sequence analysis

A total of 10 ITS-2 sequences of species of the genus *Panstrongylus* have been deposited in the GenBank™ and EMBL (see accesion numbers in Table 1). The length of the spacer ranged from 470 (*P. rufotuberculatus* from Ecuador) to 600 bp (*P. megistus* from Pampulha, Minas Gerais, Brazil) (Table 1). Base composition was clearly biased to A + T content (mean 76.5% when including *P. megistus* from the laboratory strain of La Paz, according to Marcilla et al., 2001) (Table 1).

When comparing populations of a given species, *P. megistus* from Pampulha, Minas Gerais, Brazil, differed in only one microsatellite from the *P. megistus* laboratory strain of La Paz (Marcilla et al., 2001), giving rise to a different length: $(GC)_3$ in the 600 bp long sequence from Pampulha, whereas, $(GC)_2$ in the 598 bp long sequence from La Paz. Five nucleotide differences were detected between the populations of *P. geniculatus* from Ecuador and Brazil: one transition A/C and two microsatellite extensions giving rise to the 4 bp longer sequence in the Brazilian population: $(TA)_4$ and $(GT)_1$ in Ecuador and $(TA)_5$ and $(GT)_2$ in Brazil. Four nucleotides distinguished the ITS-2 of the two populations of *P. rufotuberculatus* from Ecuador and Colombia: two mutations (one transition A/G and one transversion T/A) and one microsatellite explaining the 2 bp difference in their length [$(AT)_5$ in the Ecuadorian population and $(AT)_6$ in the Colombian one)]. The two populations of *P. lignarius* from Ecuador and Brazil were identical, as were those of *P. herreri* from Ecuador and Peru.

Interspecific analysis revealed that sequences from *P. lignarius* and *P. herreri* were identical. Absolute nucleotide differences studied in pairwise comparisons and total character differences obtained in the K-2 distance matrix including only the *Panstrongylus* species in the alignment according to PAUP (table not shown), respectively, between the ITS-2 sequences of all other *Panstrongylus* species appear to be very high: 166–168 and 73–77 between *P. megistus* and *P. geniculatus*; 209–213 and 79 between *P. megistus* and *P. rufotuberculatus*; 165–167 and 76 between *P. megistus* and *P. chinai*; 199–201 and 106 between *P. megistus* and *P. lignarius/herreri*; 106–113 and 64 between

Table 2
Genetic distances (mean character differences) between species of *Panstrongylus* and species of other genera of Triatomini (*Triatoma*, *Meccus* and *Dipetalogaster*)[a]

	P. megistus	*P. geniculatus*	*P. rufotuberculatus*	*P. chinai*	*Triatoma*, *Meccus* and *Dipetalogaster* species	
					Central and North America	South America
P. megistus					0.14437–0.17697	0.19426–0.21336
P. geniculatus	0.17624–0.18271				0.08817–0.11015	0.17873–0.18142
P. rufotuberculatus	0.18298–0.18220	0.12826–0.12771			0.08617–0.10181	0.17209–0.17967
P. chinai	0.17097	0.11585–0.11717	0.12009–0.11957		0.09649–0.11039	0.17460–0.18014
P. lignarius/herreri	0.22131	0.16279–0.16842	0.17111–0.17035	0.15254	0.16071–0.18667	0.20465–0.21615
Panstrongylus species	–	–	–	–	0.08617–0.18667	0.17209–0.21615

[a] Data summarised from the K-2 distance matrix including all the 24 different ITS-2 sequences available from Triatomini species in the alignment, according to PAUP.

P. geniculatus and *P. rufotuberculatus*; 68 and 47–49 between *P. geniculatus* and *P. chinai*; 114–115 and 79–81 between *P. geniculatus* and *P. lignarius/herreri*; 101–103 and 54 between *P. rufotuberculatus* and *P. chinai*; 120–123 and 73 between *P. rufotuberculatus* and *P. lignarius/herreri*.

When comparing species of *Panstrongylus* with species of other genera of Triatomini (*Triatoma*, *Meccus* and *Dipetalogaster* Usinger, 1939), genetic distances, obtained in the K-2 distance matrix including all the 24 different ITS-2 sequences available from Triatomini species in the alignment according to PAUP (see summarised Table 2), were surprising. Thus, genetic distances between the different *Panstrongylus* species (0.11585–0.22131) are larger than those of central and North American *Triatoma*, *Meccus* and *Dipetalogaster* species versus *P. rufotuberculatus* (0.08617–0.10181), versus *P. geniculatus* (0.08817–0.11015) and versus *P. chinai* (0.09649–0.11039). On the contrary, *Panstrongylus* species in general appear to be very far away from South American *Triatoma* species (0.17209–0.21615), similarly as central and North American *Triatoma*, *Meccus* and *Dipetalogaster* species are from South American *Triatoma* species (0.16629–0.18970). Interestingly, the genetic distance between *P. megistus* and *P. lignarius/herreri* (0.22131) is the highest so far recorded between two Triatomini species, even larger than that between the two most separated species belonging to different genera, *T. sordida* and *P. lignarius/herreri* (0.21615).

3.2. Phylogenetic analyses

For phylogenetic reconstruction, two kinds of analyses were carried out, one only with *Panstrongylus* species and another including all Triatomini species.

Phylogenetic trees only including the eight different ITS-2 sequences representing the populations of the six *Panstrongylus* species studied, were constructed using different outgroups. The most consistent results were obtained when *T. infestans* (Paraguay population) was used as outgroup. The convenience of using this outgroup lies in that South American *Triatoma* species appear clustered and in a clade different from that of central and North American Triatomini species in phylogenetic trees inferred from rDNA ITS-2 sequences (Marcilla et al., 2001). A 605 position long alignment was obtained. Of these, 384 sites were constant and 131 were parsimony-informative. Gaps, indicating insertions and deletions, were present throughout the sequences.

Parsimony analysis, using the heuristic option, of the aligned sequences yielded a single most-parsimonious tree (Fig. 1A). The tree obtained was 281 steps long. The consistency index (CI) and the homoplasy index (HI) were 0.900 and 0.099, respectively. CI and HI excluding uninformative characters were 0.850 and 0.150, respectively. The retention index (RI), the rescaled consistency index (RC) and the Goloboff-fits (G-fits) were 0.846, 0.762 and −124.100, respectively. Two different clades were obtained, one including only *P. rufotuberculatus* and clearly separated from another clade including *P. geniculatus*, *P. chinai*, *P. lignarius/herreri* and *P. megistus*. In this second clade, supported only by a 65% of bootstrap value, three paraphyletic branches appear: one for *P. geniculatus* and *P. chinai* with a 72% of bootstrap support, another for *P. lignarius/herreri* and the last one for *P. megistus*.

The topology of the trees derived from the distance data and bootstrap values using the NJ method according to the four models applied (trees not shown) did not solve the phylogeny, only showing a paraphyly of the four branches of *P. rufotuberculatus*, *P. geniculatus/chinai*, *P. lignarius/herreri* and *P. megistus*.

ML analysis using the transition/transversion rate of 2 generated a tree (likelihood = −1975.21255), the number of quartets examined being 126 using least-squares method with ML distances. The topology was similar to that obtained from parsimony analysis, but without the paraphyly shown by the latter. In the ML tree, *P. lignarius/herreri* appeared in a position basal to the clade including a branch with *P. geniculatus* and *P. chinai* and another branch with *P. megistus* (Fig. 1B). ML analyses using the different transition/transversion rates of 4, 6 and 8 furnished trees showing identical topology and increasing puzzle values.

Phylogenetic analyses including 24 Triatomini ITS-2 sequences (eight for *Panstrongylus* species and 16 of other Triatomini species) were performed using *R. prolixus* as

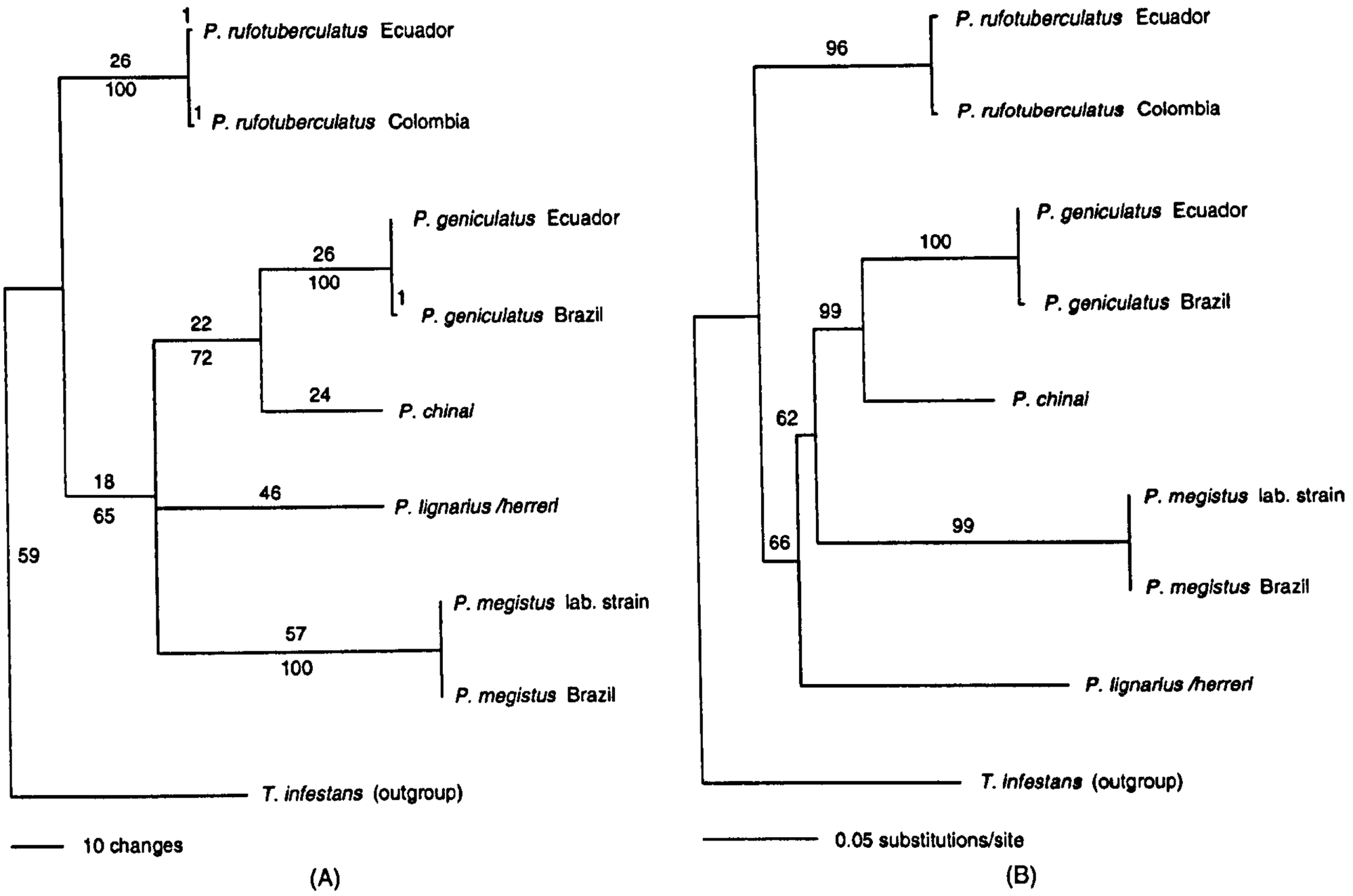


Fig. 1. Phylogenetic trees of the *Panstrongylus* species studied, using *Triatoma infestans* as outgroup: (A) based on MP analysis using the heuristic option; numbers above the line indicate branch lengths (steps); numbers below the line represent the percentage of 1000 bootstrap replicates; (B) derived from the ML model; scale bar indicate the number of substitutions per sequence position; numbers represent the percentage of 1000 puzzling replicates.

outgroup. A 730 position long alignment was obtained. Of these, 315 sites were constant and 217 were parsimony-informative. All MP, NJ and ML analyses yielded similar trees where the *Panstrongylus* species did not clade together.

Parsimony analysis, using the heuristic option, of the aligned sequences yielded a single most-parsimonious tree (Fig. 2A). The tree obtained was 717 steps long. The CI and the HI were 0.792 and 0.208, respectively. CI and HI excluding uninformative characters were 0.699 and 0.300, respectively. The RI and the RC were 0.808 and 0.640, respectively. In this MP tree, the *Panstrongylus* species appeared in the clade which also includes the central and North American *Triatoma*, *Meccus* and *Dipetalogaster* species, with a 69% bootstrap support. In this clade, *P. megistus* had a position basal to the remaining species. It is worth mentioning that *P. rufotuberculatus* appeared clustering with the species of the *phyllosoma* complex with a high bootstrap value of 82%, *T. dimidiata* representing a sister group. The position of the *T. barberi—D. maxima* branch basal to the *P. rufotuberculatus—M. phyllosoma/T. dimidiata* clade, with a 84% support value, represents a polyphyly for the *Panstrongylus* species, among which *P. chinai*, *P. geniculatus* and *P. lignarius/herreri* appear paraphyletically in the tree.

The phylogenetic trees derived from the Tamura-Nei, Kimura two-parameter, Kimura two-parameter using γ-corrected distances and Kimura three-parameter models showed similar topologies, although that obtained with Kimura two-parameter distance data (Table 3) presented the highest bootstrap supports. The topology of this NJ tree (tree not shown) was similar to that of the MP tree (Fig. 2A), although bootstrap values using the NJ method were somewhat lower. The clustering of *P. rufotuberculatus* with *M. phyllosoma/T. dimidiata* was supported by a 69% bootstrap value, with the *T. barberi—D. maxima* branch appearing basal to the *P. rufotuberculatus—M. phyllosoma/T. dimidiata* clade. *P. geniculatus*, *P. chinai* and *P. lignarius/herreri* appeared paraphyletically linked to the above-mentioned central and North American Triatomini species with a 76% support.

ML analysis using the transition/transversion rate of 2 generated a tree (likelihood = −3737.67876), the number of quartets examined being 12,650 using least-squares method with ML distances (Fig. 2B). In this ML tree, the presence of puzzle values in all the nodes, despite the high number of sequences included, is worth mentioning. *P. rufotuberculatus* also clustered with *M. phyllosoma/T. dimidiata*, with a 60% puzzle value, the *T. barberi—D. maxima* branch appearing basal to the latter grouping. *P. megistus* appeared basal to the 76% supported grouping of the other *Panstrongylus* species with the central and North American Triatomini, among which there was a clade including *P. geniculatus*, *P. chinai*

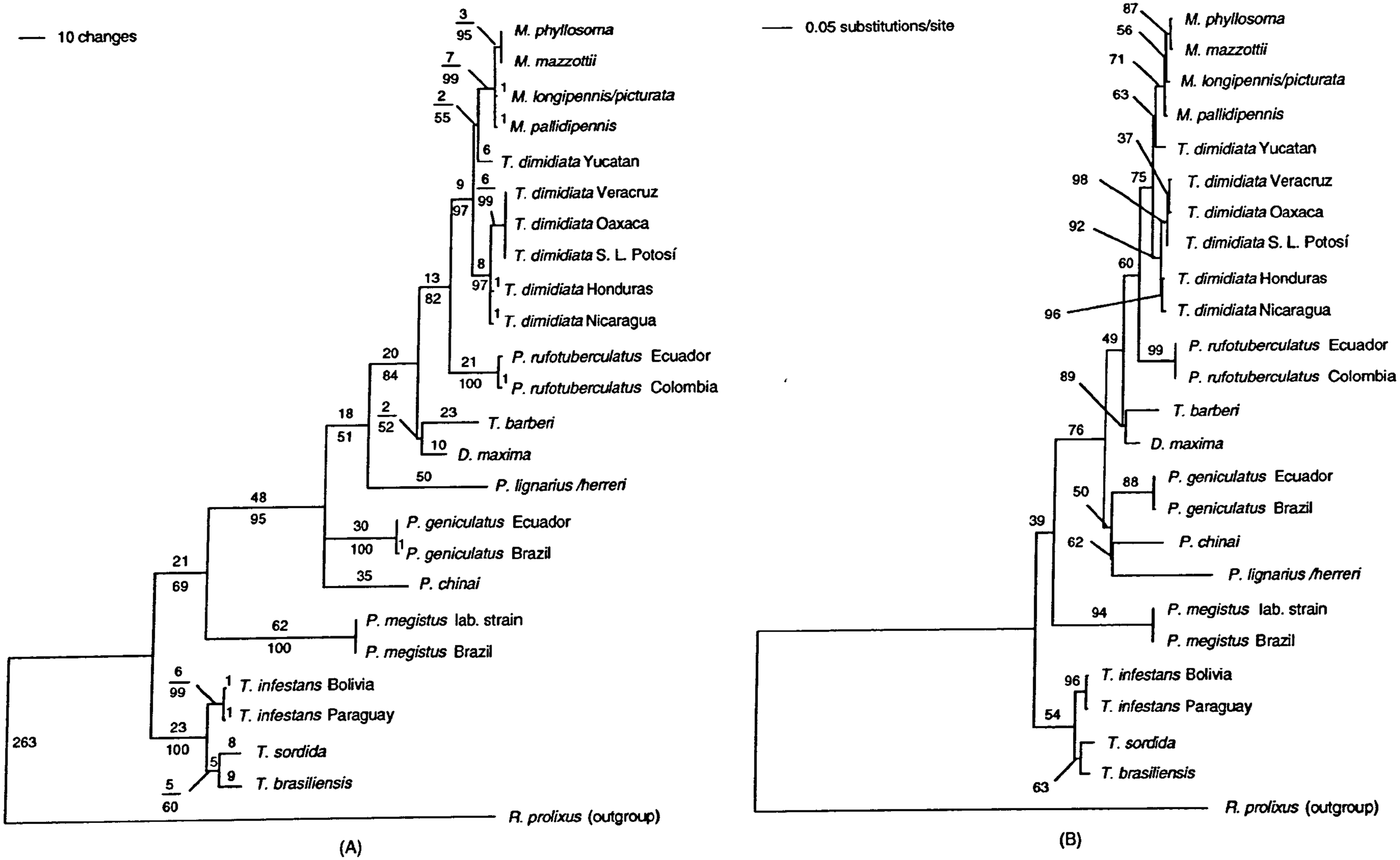


Fig. 2. Phylogenetic trees of Triatomini species, using *Rhodnius prolixus* as outgroup: (A) based on MP analysis using the heuristic option; numbers above the line indicate branch lengths (steps); numbers below the line represent the percentage of 1000 bootstrap replicates; (B) derived from the ML model; scale bar indicate the number of substitutions per sequence position; numbers represent the percentage of 1000 puzzling replicates.

Table 3
Pairwise comparisons of nucleotide divergences according to Kimura two-parameter model for the whole set of 25 ITS-2 sequences from the triatomine populations analysed[a]

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	*M. phyllosoma*	–	0.00000	0.00851	0.00851	0.03419	0 04904	0.04904	0.04915	0.04060	0.04478	0.10606	0.08061	0.18014
2	*M. mazzottii*	0	–	0.00855	0.00855	0.03433	0.04925	0.04925	0.04936	0.04077	0.04497	0.10652	0.08096	0.18097
3	*M. longipennis/picturata*	4	4	–	0.00426	0.02991	0 04478	0.04478	0.04487	0.03632	0.04051	0.10173	0.07843	0.18014
4	*M. pallidipennis*	4	4	2	–	0.02991	0.04478	0.04478	0.04487	0.03632	0.04051	0.09740	0.07407	0.17783
5	*T. dimidiata* Yucatan (Mexico)	16	16	14	14	–	0.04043	0.04025	0.04025	0.03185	0.03602	0.10390	0.08061	0.18307
6	*T. dimidiata* Veracruz (Mexico)	23	23	21	21	19	–	0.00000	0.00000	0.01477	0.01474	0.10151	0.08061	0.18851
7	*T. dimidiata* Oaxaca (Mexico)	23	23	21	21	19	0	–	0.00000	0.01471	0.01468	0.10151	0.08061	0.18764
8	*T. dimidiata* S.L. Potosi (Mexico)	23	23	21	21	19	0	0	–	0.01474	0.01471	0.10151	0.08061	0.18764
9	*T. dimidiata* (Honduras)	19	19	17	17	15	7	7	7	–	0.00420	0.09957	0.07642	0.18578
10	*T. dimidiata* (Nicaragua)	21	21	19	19	17	7	7	7	2	–	0.10151	0.08061	0.18764
11	*T. barberi*	49	49	47	45	48	47	47	47	46	47	–	0.07143	0.18750
12	*D. maxima*	37	37	36	34	37	37	37	37	35	37	33	–	0.17169
13	*T. infestans* (Bolivia)	78	78	78	77	80	82	82	82	81	82	81	74	–
14	*T. infestans* (Paraguay)	78	78	78	77	80	82	82	82	81	82	81	74	2
15	*T. sordida*	75	75	75	74	77	77	77	77	78	80	82	69	17
16	*T. brasiliensis*	82	82	82	81	82	85	85	85	85	86	82	72	16
17	*P. rufotuberculatus* (Ecuador)	36	36	34	35	38	35	35	35	36	37	45	35	78
18	*P. rufotuberculatus* (Colombia)	36	36	34	35	38	35	35	35	36	37	47	37	78
19	*P. geniculatus* (Ecuador)	55	55	53	51	56	56	56	55	53	54	59	54	75
20	*P. geniculatus* (Brazil)	55	55	53	51	56	56	56	55	53	54	60	55	75
21	*P. chinai* (Ecuador)	56	56	56	54	58	56	56	56	55	56	60	54	86
22	*P. lignarius/herreri*	82	82	78	78	81	80	80	79	78	79	83	70	103
23	*P. megistus* (laboratory strain INLASA)	89	89	87	87	87	97	97	97	92	93	97	91	81
24	*P megistus* (Brazil)	90	90	88	88	88	98	98	98	93	94	98	92	81
25	*R. prolixus*	224	222	224	222	224	230	231	230	227	228	231	226	213

		14	15	16	17	18	19	20	21	22	23	24	25
1	*M. phyllosoma*	0.18014	0.17730	0.18721	0.08200	0.08163	0.11879	0.11853	0.12148	0.18263	0.19099	0.19272	0.47966
2	*M. mazzottii*	0.18097	0.17815	0.18807	0.08200	0.08200	0.11931	0.11905	0.12200	0.18345	0.19181	0.19355	0.47742
3	*M. longipennis/picturata*	0.18014	0.17730	0.18721	0.07745	0.07710	0.11447	0.11422	0.12148	0.17372	0.18670	0.18844	0.47966
4	*M. pallidipennis*	0.17783	0 17494	0.18493	0.07973	0.07937	0.11015	0.10991	0.11714	0.17372	0.18670	0.18844	0.47537
5	*T. dimidiata* Yucatan (Mexico)	0.18307	0 18160	0.18552	0.08676	0.08636	0.12043	0.12017	0.12500	0.18080	0.18511	0.18684	0.47660
6	*T. dimidiata* Veracruz (Mexico)	0.18851	0.18203	0.19318	0.07973	0.07937	0.12043	0.12017	0.12095	0.17817	0.20726	0.20896	0.48729
7	*T. dimidiata* Oaxaca (Mexico)	0.18764	0.18203	0.19231	0.07973	0.07937	0.11991	0.11966	0.12043	0.17817	0.20638	0.20807	0.48734
8	*T. dimidiata* S.L. Potosi (Mexico)	0.18764	0 18203	0.19231	0.07991	0.07955	0.11803	0.11777	0.12043	0.17634	0.20638	0.20807	0.48626
9	*T. dimidiata* (Honduras)	0.18578	0.18483	0.19274	0.08219	0.08182	0.11373	0.11349	0.11853	0.17411	0.19616	0.19787	0.47992
10	*T. dimidiata* (Nicaragua)	0.18764	0 18913	0.19457	0.08428	0.08390	0.11563	0.11538	0.12043	0.17595	0.19787	0.19958	0.48101
11	*T. barberi*	0.18750	0.19431	0.18764	0.10204	0.10609	0.12798	0.12987	0.12987	0.18652	0.20771	0.20940	0.49465
12	*D. maxima*	0.17169	0.16390	0.16552	0.08009	0.08428	0.11765	0.11957	0.11739	0.15695	0.19570	0.19742	0.48394
13	*T. infestans* (Bolivia)	0.00441	0.03908	0.03532	0.18440	0.18353	0.17007	0.17007	0.19413	0.24122	0.17881	0.17841	0.47228
14	*T. infestans* (Paraguay)	–	0 04368	0.03532	0.18203	0.18118	0.17007	0.17007	0.19413	0.23888	0.18102	0.18062	0.47692
15	*T. sordida*	19	–	0.03837	0.18735	0.18644	0.17442	0.17442	0.19400	0.25000	0.18427	0.18386	0.45946
16	*T. brasiliensis*	16	17	–	0.18545	0.18458	0.18708	0.18708	0.19868	0.23733	0.19654	0.19612	0.48485
17	*P. rufotuberculatus* (Ecuador)	77	77	79	–	0.00426	0.13913	0.13913	0.15066	0.17411	0.23126	0.23291	0.50325
18	*P rufotuberculatus* (Colombia)	77	77	79	2	–	0.13853	0.13853	0.15000	0.17333	0.23028	0.23191	0.50324
19	*P. geniculatus* (Ecuador)	75	75	84	64	64	–	0.00198	0.12220	0.17722	0.22156	0.22311	0.47887
20	*P geniculatus* (Brazil)	75	75	84	64	64	1	–	0.12348	0.18067	0.22266	0.22421	0.48104
21	*P. chinai* (Ecuador)	86	84	90	69	69	60	61	–	0.17660	0.22645	0.22800	0.48889
22	*P lignarius/herreri*	102	105	103	78	78	84	86	83	–	0.28247	0.28395	0.50932
23	*P. megistus* (laboratory strain INLASA)	82	82	91	108	108	111	112	113	137	–	0.00000	0.51779
24	*P. megistus* (Brazil)	82	82	91	109	109	112	113	114	138	0	–	0.51950
25	*R. prolixus*	217	204	224	232	233	238	241	242	246	291	293	–

[a] Below diagonal: total character differences; above diagonal: mean character differences.

and *P. lignarius/herreri*, the two latter grouped in one branch with a 62% value. ML analyses using the different transition/transversion rates of 4, 6 and 8 furnished trees showing similar topology with somewhat lower puzzle values; the only worth-mentioning difference was the appearance of the two *P. megistus* populations whether in the same branch of the South American *Triatoma* species studied (*T. infestans*, *T. sordida* and *T. brasiliensis*) with 43 and 35 puzzle values when applying the ratios of 4 and 6, respectively, or independently in a clade basal to all other Triatomini species included with a puzzle value of 68 when the ratio of 8.

4. Discussion

The results obtained in rDNA ITS-2 sequencing of *Panstrongylus* species offer further evidences in support of the usefulness of this spacer as a good marker for resolving supraspecific, specific and subspecific relationships in Triatominae, as already suggested by Marcilla et al. (2001). ITS-2 base composition biased to A + T content in *Panstrongylus* species is in agreement with the values (76.7%) previously found in other triatomines (Marcilla et al., 2001).

ITS-2 length range found in *Panstrongylus* species agrees with that found in Triatomini and is shorter than in Rhodniini (Marcilla et al., 2001). ITS-2 length variation between different populations of given species slightly differ because of a different number of repeats of several dinucleotide microsatellites. Microsatellites have already been detected in the rDNA ITS-2 of other organisms (see review in Almeyda-Artigas et al., 2000), as well as in other triatomines (Marcilla et al., 2001). Neither the origin of microsatellites, nor their mutation model evolution and function, if any, are fully understood (Remigio and Blair, 1997; Jarne et al., 1998), but a recent, extensive bibliography proves that microsatellite alleles exhibit an extreme intraspecific variability, neutrality, Mendelian inheritance, codominance and high mutation rates. They are, therefore, very good polymorphic molecular markers for the differentiation of populations within a given species (see review by Jarne and Lagoda, 1996). Hence, the results of this paper suggest that many of the microsatellites detected in the ITS-2 may be very useful for population differentiation and dynamics analyses within *Panstrongylus* species in future studies.

ITS-2 length variation not related to microsatellite repeats was unexpectedly high between different *Panstrongylus* species. With very few exceptions, the rDNA ITS-2 sequences have the same or very similar length in different species of the same genus in different groups of organisms (see reviews in Mas-Coma, 1999 and Almeyda-Artigas et al., 2000). Previous studies on the ITS-2 of triatomine bugs suggested that this spacer followed this length rule both in Triatomini and Rhodniini (Marcilla et al., 2001). The pronounced differences in length detected in *Panstrongylus* species may perhaps reflect a relatively old origin of this genus. The great differences in nucleotide composition between *Panstrongylus* species also support a relatively old separation of these species, according to the nuclear rDNA-based molecular clock pattern followed by Triatominae in their evolution (Bargues et al., 2000).

The absence of nucleotide differences between the population of Yasuní, Orellana and that from the laboratory strain of Fiocruz (originally from Cajamarca, Peru), verifies the correct classification of *P. herreri* made by Aguilar et al. (1999) and Abad-Franch et al. (2001) and expands the geographical distribution of this species to Ecuador. Similarly, the ITS-2 sequences of the bug populations from San Pablo, Sucumbíos, Ecuador and Santa Bárbara, Pará, Brazil being identical confirm the classification of the material from Ecuador as belonging to *P. lignarius* made by Abad-Franch et al. (2001).

Interestingly, not a single nucleotide difference was detected between the sequences of the species *P. lignarius* and *P. herreri*. According to the characteristics of the ITS-2 as a species marker (Mas-Coma, 1999), this indicates that in fact there is only one species, meaning that *herreri* would enter as a synonym of *lignarius*. As already mentioned by Carcavallo et al. (1999b), these two species are so similar that they are often difficult to distinguish, the differentiation being mainly based on their allopatric geographical distributions and ecological aspects, *P. herreri* having adapted to other habitats (including human dwellings) through its trophic link to guinea pigs (Herrer, 1960). Although Lent and Wygodzinsky (1979) reported that no intermediate forms have been found, Barrett (1988) already proved that both species cross-fertilise giving rise to hybrids. If there would be an applied interest to differentiate them and as long as valid distinctive morphological characters exist, subspecific status would perfectly fit the present knowledge: *P. lignarius lignarius* occupying a large area of the central-eastern Amazon basin and *P. lignarius/herreri* in a more restricted area including the eastern slopes of the Andes in Ecuador and Peru, and some inter-Andean valleys related to the Marañón river system. This case appears to be similar to that of the species of the *phyllosoma* complex in Mexico (Marcilla et al., 2001) and has serious epidemiological implications. In fact, *P. herreri* is the main domestic vector of Chagas disease in northern Peru (Calderón et al., 1985), whereas, *P. lignarius* is considered as exclusively sylvatic. Our results suggest that the species (*lignarius/herreri*) has a potential for domiciliation higher than previously thought, as demonstrated by the strong synanthropism of one population (known as *P. herreri*), whose biogeographic range is in addition broader than reported to date, including primary Amazonian forests of Ecuador (see Carcavallo et al., 1999a; Abad-Franch et al., 2001).

In all of the different phylogenetic trees obtained, all *Panstrongylus* species appear clustering with the central and North American species of other Triatomini and consequently in a clade different from that of the South American Triatomini species. The results obtained by Marcilla et al.

(2001) were adding support for the idea of an old divergence between South American and central and North American forms. The present paper suggests that *Panstrongylus* species may be related to the ancestors giving rise to central and North American Triatomini. The broad geographic distribution of some species of *Panstrongylus* in the northern part of the Neotropical region, such as *P. geniculatus*, *P. lignarius/herreri* (with *P. humeralis* in central America) and above all *P. rufotuberculatus*, whose wide area of distribution also expands into central America (Carcavallo et al., 1999a) and which occupies very large climatic and altitude ranges, from lowland rainforests to arid highlands and subtropical forest of intermediate altitude (Noireau et al., 1994), fit in such an hypothesis.

The phylogenies inferred from ITS-2 sequence analyses markedly differ from the cladogram of hypothesised phylogenetic relationships of the genus *Panstrongylus* based on plesiomorphic and apomorphic traits constructed by Lent and Wygodzinsky (1979) (see also Carcavallo et al., 1999b, Fig. 21.10 and Table 2), except for the similitude of *P. lignarius* and *P. herreri*. However, when dealing with evolutionary units within Triatominae, Dujardin et al. (1999) already noted that no good correlation between morphological and genetic relationships was to be expected.

Moreover, the phylogenetic trees here obtained also suggest that the genus *Panstrongylus* is polyphyletic, with *P. rufotuberculatus* separated from all other species of the genus. This hypothesis is also supported by the ITS-2 length variation and the large genetic distances found between the species studied. Although a larger sample including more species (such as those of lesser medical importance) and populations of this genus needs to be analysed to definitively address the question, the rDNA results already suggest the convenience of introducing supraspecific or perhaps better generic differentiation within the present *Panstrongylus* taxon. Unfortunately, no information on DNA sequences of the *Panstrongylus* species analysed in this paper is available at present, neither from mitochondrial genes nor from other nuclear ribosomal genes or spacers, as to corroborate the above mentioned hypothesis of polyphyly.

The phylogenetic trees obtained in this study also suggest a polyphyly of South American and central and North American *Triatoma* species, as already observed by Marcilla et al. (2001). Although this may support the validity of the genus *Meccus* for the central and North American species, neither ITS-2 nor 18S rDNA (Bargues et al., 2000) sequence results agree with the exclusion of *T. dimidiata* from this genus.

Acknowledgements

This work was supported by the Project no. 3042/2000 of the Dirección General de Cooperación para el Desarrollo, Presidencia de Gobierno de la Generalitat Valenciana, Valencia, Spain. Moreover, it benefitted from international collaboration through the ECLAT network. Additional financial support was obtained from a Project (Contract no. IC18-CT98-0366) of the INCO-DC Programme of the Commission of the European Communities (DG XII: Science, Research and Development), Brussels, EU, the AVINA Foundation, Switzerland, the Research Promotion Programme of the University of Valencia, Valencia, Spain, the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Fundação Nacional de Saúde, Brazil. Fieldwork in Ecuador was supported by Grant no. 790195 of the UNDP/World Bank/WHO TDR Programme. F. Panzera benefited from a funding by the Conselleria de Cultura i Educació of the Valencia Government, Spain. Dr. David Swofford generously provided the beta test version of PAUP v.4.0b 6 to M. D. Bargues. The PUCE-QCAZ Invertebrates Museum (Pontificia Universidad Católica del Ecuador, Quito, Ecuador) kindly provided specimens from its entomological collections.

References

Abad-Franch, F., Paucar, C.A., Carpio, C.C., Cuba Cuba, C.A., Aguilar, V.H.M., Miles, M.A., 2001. Biogeography of Triatominae (Hemiptera: Reduviidae) in Ecuador: implications for the design of control strategies. Mem. Inst. Oswaldo Cruz 96, 611–620.

Aguilar, V.H.M., Abad-Franch, F., Racines, V.J., Paucar, C.A., 1999. Epidemiology of Chagas disease in Ecuador. A brief review. Mem. Inst. Oswaldo Cruz 94 (Suppl. 1), 387–393.

Almeyda-Artigas, R.J., Bargues, M.D., Mas-Coma, S., 2000. rDNA of *Gnathostoma* species (Nematoda): ITS-2 microsatellites and 5.8S gene secondary structure. Res. Rev. Parasitol. 60, 51–56.

Bargues, M.D., Mas-Coma, S., 1997. Phylogenetic analysis of lymnaeid snails based on 18S rDNA sequences. Mol. Biol. Evol. 14, 569–577.

Bargues, M.D., Marcilla, A., Ramsey, J., Dujardin, J.P., Schofield, C.J., Mas-Coma, S., 2000. Nuclear rDNA-based molecular clock of the evolution of Triatominae (Hemiptera: Reduviidae), vectors of Chagas disease. Mem. Inst. Oswaldo Cruz 95, 567–573.

Bargues, M.D., Vigo, M., Horak, P., Dvorak, J., Patzner, R.A., Pointier, J.P., Jackiewicz, M., Meier-Brook, C., Mas-Coma, S., 2001. European Lymnaeidae (Mollusca: Gastropoda), intermediate hosts of trematodiases, based on nuclear ribosomal DNA ITS-2 sequences. Infect. Genet. Evol. 16, 1–23.

Bargues, M.D., Marcilla, A., Dujardin, J.P., Mas-Coma, S., 2002. Triatominae vectors of Chagas disease: a molecular perspective based on nuclear ribosomal DNA markers. In: Baker, J. (Ed.), Douglas Baker Supplement, Transactions of the Royal Society of Tropical Medicine and Hygiene, in press.

Barrett, T.V., 1988. Current research on Amazonian Triatominae. Mem. Inst. Oswaldo Cruz 83 (Suppl. 1), 441–447.

Borges, E.C., Pires, H.H.R., Barbosa, S.E., Nunes, C.M.S., Pereira, M.H., Romanha, A.J., Diotaiuti, L., 1999. Genetic variability in Brazilian triatomines and the risk of domiciliation. Mem. Inst. Oswaldo Cruz 94 (Suppl. 1), 371–373.

Brower, A.V.Z., DeSalle, R., 1994. Practical and theoretical considerations for choice of a DNA sequence region in insect molecular systematics, with a short review of published studies using nuclear gene regions. Ann. Entomol. Soc. Am. 87, 702–716.

Calderón, F.G.E., Figueroa, K., Naquira, F., Carcavallo, R.U., Canale, D.M., 1985. Perú. In: Carcavallo, R.U., Rabinovich, J.E., Tonn, R.J. (Eds.), Factores biológicos y ecológicos de la enfermedad de Chagas, Vol. 2. OPS-ECO/MSAS-SNCH, Buenos Aires, pp. 449–456.

Carcavallo, R.U., Curto de Casas, S.I., Sherlock, I.A., Girón, I.G., Jurberg, J., Galvão, C., Mena Segura, C.A., Noireau, F., 1999a. Geographical distribution and alti-latitudinal dispersion. In: Carcavallo, R.U., Girón, I.G., Jurberg, J., Lent, H. (Eds.), Atlas of Chagas Disease Vectors in the Americas, Vol. 3. Editora Fiocruz, Rio de Janeiro, pp. 747–792.

Carcavallo, R.U., Jurberg, J., Lent, H., 1999b. Phylogeny of the Triatominae. Part A. General approach. In: Carcavallo, R.U., Girón, I.G., Jurberg, J., Lent, H. (Eds.), Atlas of Chagas Disease Vectors in the Americas, Vol. 3. Editora Fiocruz, Rio de Janeiro, pp. 925–980.

Carcavallo, R.U., Jurberg, J., Lent, H., Noireau, F., Galvão, C., 2000. Phylogeny of the Triatominae (Hemiptera, Reduviidae). Proposals for taxonomic arrangements. Entomol. Vect. 7 (Suppl. 1), 1–99.

Chico, H.M., Sandoval, C., Guevara, E.A., Calvopiña, H.M., Cooper, P.J., Reed, S.G., Guderian, R.H., 1997. Chagas disease in Ecuador: evidence for disease transmission in an indigenous population in the Amazon region. Mem. Inst. Oswaldo Cruz 92, 317–320.

Dujardin, J.P., Forgues, G., Torrez, M., Martínez, E., Córdoba, C., Gianella, A., 1998. Morphometrics of domestic *Panstrongylus rufotuberculatus* in Bolivia. Ann. Trop. Med. Parasitol. 92, 219–228.

Dujardin, J.P., Panzera, F., Schofield, C.J., 1999. Triatominae as a model of morphological plasticity under ecological pressure. Mem. Inst. Oswaldo Cruz 94 (Suppl. 1), 223–228.

Dujardin, J.P., Schofield, C.J., Panzera, F., 2000. Les Vecteurs de la Maladie de Chagas. Recherches taxonomiques, biologiques et génétiques, Vol. 24, no. 5. Académie Royale des Sciences d'Outre Mer, Classe des Sciences Naturelles et Médicales, N.S., 162 pp.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Gaunt, M., Miles, M.A., 2000. The ecotopes and evolution of triatomine bugs (Triatominae) and their associated trypanosomes. Mem. Inst. Oswaldo Cruz 95, 557–565.

Herrer, A., 1960. Distribución geográfica de la enfermedad de Chagas y de sus vectores en el Perú. Bol. Of. San. Pan. 49, 572–581.

Hillis, D.M., Dixon, M.T., 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Q. Rev. Biol. 66, 411–453.

Jarne, P., Lagoda, P.J.L., 1996. Microsatellites, from molecules to populations and back. Trends Ecol. Evol. 11, 424–429.

Jarne, P., David, P., Viard, F., 1998. Microsatellites, transposable elements and the X chromosome. Mol. Biol. Evol. 15, 28–34.

Jurberg, J., Carcavallo, R.U., Lent, H., 2001. *Panstrongylus sherlocki* sp. n. do estado da Bahia (Hemiptera, Reduviidae, Triatominae). Entomol. Vect. 8, 261–274.

Lake, J.A., 1991. The order of sequence alignment can bias the selection of tree topology. Mol. Biol. Evol. 8, 378–385.

Lent, H., Wygodzinsky, P., 1979. Revision of the Triatominae (Hemiptera, Reduviidae) and their significance as vectors of Chagas disease. Bull. Am. Mus. Nat. Hist. 163, 123–520.

Mas-Coma, S., 1999. Los espaciadores transcritos internos (ITSs) del ADN ribosomal como marcadores en sistemática, ecología, evolución y filogenia de parásitos y vectores. In: Proceedings of the 14th Congress of the Latinoamerican Federation Parasitology (FLAP), Acapulco, pp. 5–6.

Marcilla, A., Canese, A., Acosta, N., López, E., Rojas de Arias, A., Bargues, M.D., Mas-Coma, S., 2000. Populations of *Triatoma infestans* (Hemiptera: Reduviidae) from Paraguay: a molecular analysis based on the second internal transcribed spacer of the rDNA. Res. Rev. Parasitol. 60, 99–105.

Marcilla, A., Bargues, M.D., Ramsey, J., Magallón-Gastelum, E., Salazar-Schettino, P.M., Abad-Franch, F., Dujardin, J.P., Schofield, C.J., Mas-Coma, S., 2001. The ITS-2 of the nuclear rDNA as a molecular marker for populations, species and phylogenetic relationships in Triatominae (Hemiptera: Reduviidae), vectors of Chagas disease. Mol. Phyl. Evol. 18, 136–142.

Noireau, F., Bosseno, M.F., Vargas, F., Brenière, S.F., 1994. Apparent trend to domesticity observed in *Panstrongylus rufotuberculatus* Champion, 1899 (Hemiptera: Reduviidae) in Bolivia. Res. Rev. Parasitol. 54, 263–264.

Noireau, F., Bosseno, M.F., Carrasco, R., Tellería, J., Vargas, F., Camacho, C., Yaksic, N., Brenière, S.F., 1995. Sylvatic triatomines (Hemiptera: Reduviidae) in Bolivia: trends towards domesticity and possible infection with *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae). J. Med. Entomol. 32, 594–598.

Noireau, F., Flores, R., Gutierrez, T., Vargas, F., Brenière, S.F., Dujardin, J.P., 1999. Triatominae en Bolivia: importancia actual de los candidatos vectores. In: Alfred Cassab, J., Noireau, F., Guillén, G. (Eds.), La Enfermedad de Chagas en Bolivia—Conocimientos científicos al inicio del Programa de Control (1998–2002), Ministerio de Salud y Previsión Social, OMS/OPS, IRD & IBBA, La Paz, pp. 127–133.

Onyabe, D.Y., Conn, J.E., 1999. Intragenomic heterogeneity of a ribosomal DNA spacer (ITS2) varies regionally in the Neotropical malaria vector *Anopheles nuneztovari* (Diptera: Culicidae). Insect Mol. Biol. 8, 435–442.

Remigio, E.A., Blair, D., 1997. Relationships among problematic North American stagnicoline snails (Pulmonata: Lymnaeidae) reinvestigated using nuclear ribosomal DNA internal transcribed spacer sequences. Can. J. Zool. 75, 1540–1545.

Saitou, N., Nei, M., 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning. A Laboratory Manual. 2nd Edition, Vols. 1–3. Cold Spring Harbor Laboratory Press, New York, USA.

Sanger, F., Nicklen, S., Coulson, A.R., 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A. 74, 5463–5467.

Schofield, C.J., Diotaiuti, L., Dujardin, J.P., 1999. The process of domestication in Triatominae. Mem. Inst. Oswaldo Cruz 94 (Suppl. 1), 375–378.

Swofford, D.L., 2001. PAUP*: phylogenetic analysis using parsimony (* and other methods). Version 4. Computer program distributed by the Smithsonian Institution, Sinauer Associates Inc. Publishers, Sunderland, MA.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL-W: improving the sensitivity and progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Valente, V.C., Valente, S.A.S., Noireau, F., Carrasco, H.J., Miles, M.A., 1998. Chagas disease in the Amazon basin: association of *Panstrongylus geniculatus* (Hemiptera: Reduviidae) with domestic pigs. J. Med. Entomol. 35, 99–103.

Van De Peer, Y., De Wachter, R., 1997. Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. Comput. Appl. Biosci. 13, 227–230.

WHO, 1991. Control of Chagas disease. Report of a WHO Expert Committee, World Health Organisation, Geneva, WHO Technical Report Series 811, pp. 1–95.

Proceedings of the

FOURTH INTERNATIONAL WORKSHOP ON POPULATION GENETICS AND CONTROL OF TRIATOMINAE

Cuarto Taller Internacional sobre Genética Poblacional y Control de Triatomineos

(ECLAT 4)

Punta Iguana, Cartagena, Colombia
16-18 August 2000

Editores:
Felipe Guhl
C.J. Schofield

CONTROL OF CHAGAS DISEASE IN THE ANDEAN COUNTRIES

Fernando Abad-Franch [1] ***& H. Marcelo Aguilar V.*** [2]

[1]Pathogen Molecular Biology and Biochemistry Unit, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel St., London WC1E 7HT, UK.
[2]Instituto Nacional de Higiene y Medicina Tropical 'Leopoldo Izquieta Pérez', Ministerio de Salud Pública del Ecuador, Iquique 2045 y Yaguachi, Quito, Ecuador.

Control of Chagas disease has become a priority for almost all Latin American countries. The elimination of *Trypanosoma cruzi* transmission by *Triatoma infestans* in vast areas of the Southern Cone countries has encouraged the implementation of similar endeavours in Central America and the Andean countries (WHO 1997, 1998). In the context of the Andean countries Initiative, the 'Third Sub-regional Meeting for the Control of Transfusional and Vectorial Transmission of Chagas Disease' was held in Guayaquil, Ecuador, in June 2000. The meeting was organised by the National Sub-secretary for Tropical Medicine (Ministry of Public Health of Ecuador) under the auspices of the WHO/PAHO. Representatives of Venezuela, Colombia, Ecuador, the WHO/PAHO, and of various research centres developing activities linked to the control of Chagas disease in Ecuador participated at the meeting. Several technical presentations, two round tables (on transfusional and vectorial transmission, respectively), and four working groups were organised.

In Venezuela, six million people are estimated to be at risk of acquiring *T. cruzi*; *Rhodnius prolixus* and *T. maculata* are the main triatomine vectors. The Control Programme has 40 years of history; it includes housing improvement alongside residual insecticide spraying. Seroprevalence has decreased as a result of these activities (<1% in children under 10); recent estimates indicate that general prevalence is ~9%. House infestation by triatomines occurs in less than 20% of localities. Serological screening of blood donations is compulsory and reaches 100% of donors.

The following challenges for the future of the Programme have been identified: integration of local health services; guarantee of adequate treatment and follow-up of those infected; need for serological surveys on schoolchildren and in suspect areas; standarisation of serological diagnosis; maintenance of serological screening of all blood donors; and the incorporation of health education/community participation to the prevention/control activities.

In Colombia, the national control Programme began in 1996, and integrates vectorial, transfusional, and congenital transmission control with patient management, housing improvements, and epidemiological/entomological surveillance. Some 2.8 million people live under risk conditions in the country; *R. prolixus* and *T. dimidiata* are considered as the main vectors. The exploratory phase of the Programme took place in 1999-2000; high-risk areas were identified, and trial interventions (residual spraying, housing improvements, and health education) are currently

Clinal variation in the group *RHODNIUS PALLESCENS - R. COLOMBIENSIS - R. ECUADORIENSIS.*

Nicolás Jaramillo O.[1], Fernando Abad-Franch[2,3], Jean Pierre Dujardin[4], Jaime Moreno[1], Harling Caro-Riaño[1], Christopher John Schofield[3]

[1] Address for correspondence: Instituto de Biología, Universidad de Antioquia, AA 1226, Medellín, Colombia. E-mail: njaram@epm.net.co

[2] Unidad de Medicina Tropical, Instituto Juan César García, CP 17-1106292, Quito, Ecuador. E-mail: Fernando.Abad-Franch@lshtm.ac.uk

[3] Pathogen Molecular Biology and Biochemistry Unit, Dept. of Infectious and Tropical Diseases, LSHTM, London WC1E 7HT, UK. E-mail: CJ.Schofield@wanadoo.fr

[4] 4770 Buford Highway, bldg 102, Room 2116,Chamblee Georgia 30341, USA. Fax : + 1 770 488 4258. Phone : + 1 770 488 3607. E-mail: fzd7@cdc.gov

INTRODUCTION

The dynamics of migration and geographical dispersion of triatomines probably involve a process of genetic and phenetic simplification linked to a series of founder effects followed by genetic drift and to the various determinants of selective pressure that occur in each new hábitat. As a result, morphological and genetic clines often appear (Dujardin et al. 1998).

Various lines of evidence currently suggest that the bottleneck constituted by the Sierra Nevada and the northernmost end of the Andes in Colombia could have resulted in a founder effect that gave rise to *Rhodnius pallescens* from an Amazonian *pictipes*-like ancestor (Schofield & Dujardin 1999). It is likely that *R. pallescens* then spread associated with palm trees, which are the natural primary hábitat of the species, to its current range (towards Panamá and Costa Rica, and southwards following the Magdalena river valley in Colombia). In the central-southern Magdalena valley the species would have given rise to *R. colombiensis* (recently described from specimens collected from palm trees in Coyaima, Tolima) (Moreno et al. 1999); finally, these bugs would have reached western Ecuador and diverged to originate the third species of the clade, *R. ecuadoriensis*, which arrived northern Peru in more recent dates (Schofield & Dujardin 1999).

Here we present an overview of the evidence regarding both the genetic relatedness of *R. pallescens*, *R. colombiensis*, and *R. ecuadoriensis*, and the progressive variation of their morphological, ecological-behavioural, and genetic characteristics.

TRAPPING TRIATOMINAE IN SILVATIC HABITATS

François Noireau, Fernando Abad-Franch, Aldo Valente

The control of domestic vector species of Triatominae has been successful in most of the Southern Cone countries and is being developed in Andean countries and Central America. In controlled areas, the report of silvatic species invading human dwellings leads research activities to be focused on their original populations and observations generated will assist in the adaptation of vector control strategies.

The collection of triatomines in their natural environment is very laborious and time-consuming. The light trapping has the disadvantage of capturing only starved adults of attracted species. Other methods include the inspection of a great variety of potential ecotopes such as hollow trees, bark of trees, palm crown, bromeliads, rocks, burrows of marsupials or rodents and bird-nests. The few bait-traps previously designed to sample silvatic triatomines yield poor results (Rabinovich *et al.*, 1976; Carcavallo, 1985) .

Recently, Noireau *et al.* (1999 & 2000) reported the trial of a simple trapping system to collect triatomines in silvatic ambient. The system was a small plastic container (30 or 60 cm^3) closed with wire mesh and covered in the superior part with double-coated adhesive tape. It contained a mouse as bait. Initially designed for collecting silvatic *Triatoma* in hollow trees, it was later fitted to capture *Rhodnius* species in palm crown (Abad-Franch *et al.*, 2000).

Up to now the trapping system was particularly tested in three different ecosystems (Chaco, subtropical and tropical forest) and two sorts of ecotopes (hollow tree and palm crown). Results are showed in Table 1. In the semi-arid region of the Chaco, the traps were commonly suspended for 15 h approximately by night in hollows situated in trunks and limbs from the lowest stratum as well as emerging trees. Placed for only 8 h in the daytime, they showed also a good efficiency compared with night collection (results not showed). One to three units were placed inside each tree. Finally, from a total of 421 trees investigated, 34% contained triatomines inside one hollow at least. Trapping in palm tree was easier for the selection of investigated ecotope. Larger and more númerous traps (up to 6 units) were used inside each palm crown. The percentage of positive palm trees was similar to hardwood trees from the Chaco.