The analysis of the relationship between silvatic and domestic populations of *Rhodnius prolixus/robustus* (Hemiptera: Reduviidae) in Venezuela by morphometric and molecular methods.

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Abstract

Despite four decades of control of Chagas disease in Venezuela, domestic infestations still persist and transmission of *Trypanosoma cruzi* may be increasing. This is in contrast to the Southern cone region where control has successfully eliminated domestic populations of the main vector *Triatoma infestans* over large areas. However unlike *T. infestans,* the main vector in Venezuela, *Rhodnius prolixus*, has a widespread silvatic distribution occurring primarily in palm trees, which are a ubiquitous feature of the Venezuelan landscape. The palm tree is an important part of campensino life and is maintained for fruit, shade and for use in house construction. Control failures may be due to reinvasion of houses by these prevalent silvatic populations. However, debate exists as to whether the silvatic populations are in fact *Rhodnius robustus,* a related species of minor epidemiological importance, and therefore no threat to control. With an estimated 800,000 people infected with *T. cruzi* in Venezuela and a further 3 million at risk of infection, an effective control programme is required, which necessitates that this relationship between silvatic and domestic populations is resolved.

This study was undertaken in order to (1) confirm the identity of silvatic populations of *Rhodnius* in Venezuela and (2) determine if domestic and silvatic populations are isolated, thus to clarify the role of silvatic populations in maintaining house infestations. To achieve these aims field collected silvatic and domestic populations of *Rhodnius* from 5 States were analysed by genetic methods, direct sequencing (cytochrome b, D2) and microsatellites, and by geometric morphometric analysis.

A total of 551 specimens from 31 localities in six Venezuelan states were analysed by direct sequencing of cytochrome b (*cytb*). Results confirmed the presence of *R. prolixus* in both silvatic and domestic ecotopes, dispelling the belief that all silvatic populations are *R. robustus*. *Rhodnius robustus* does however occur and was found in this study in the Andean State Trujillo. Here it was limited to the silvatic environment. This project found that silvatic and domestic populations of *R. prolixus* are not isolated, sharing 6

haplotypes, including four silvatic haplotypes also detected in domestic nymphs, indicating that silvatic specimens are capable of domestic colonisation. This was also confirmed from the analysis of adjacent domestic and silvatic populations in Portuguesa and Barinas State where population homogeneity was detected. Additionally *cytb* analysis identified an introgression event between Amazonian *R. robustus* and *R. prolixus,* confirmed by incongruence of *cytb* and D2 nuclear characterisation. Phylogenetic analysis of specimens was also undertaken.

To investigate further fine scale population heterogeneity a panel of microsatellite markers, hitherto unavailable, was developed for *R. prolixus*, using an enrichment technique. A panel of 10 loci was available for analysis following PCR screening and linkage analysis. A total of 555 specimens were analysed from 33 populations. Microsatellite analysis also detected population homogeneity between ecotopes, including adjacent populations, indicating that silvatic populations are not isolated. Population heterogeneity was greater among localities in Portuguesa than Barinas, may be due to landscape variation. Differences between control programmes may also play a role.

Geometric morphometrics identified shape similarity between populations across all States. However, shape convergence by ecotope was detected and results indicated that morphometrics might be of limited use for the analysis of populations of *R. prolixus,* with the exception of post-control reinvasion\recrudescent studies.

The three methods did not always concur precisely. However comparison was difficult due to detected introgression and shape convergence distorting, respectively, mitochondrial and morphometric analysis. Results indicated that a combined use of microsatellites and morphometrics would be beneficial in the analysis of adjacent domestic and silvatic populations. A similar pattern of a lack of isolation between silvatic and domestic ecotopes was detected by both genetic methods, with limited morphometrics overlap detected; broadly all three methods showed that populations

from differing ecotopes are not isolated. This is also supported by a parallel project on risk-factor analysis.

From this study it is clear that silvatic populations of *R. prolixus* present an unquestionable threat to the successful control of Chagas disease in some endemic regions of Venezuela and, unlike the Southern cone, elimination of domestic infestations may not be possible in areas where silvatic *R. prolixus* occur. A restructuring of the control programme in Venezuela is required to deal with this silvatic threat. This may include increased vigilance and methodical respraying and the incorporation of novel approaches to deal with silvatic invasion such as use of insecticide treated curtains; ultimately more investment in the improvement of the rural rancho is required.

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Contents

List of Figures

List of Tables

List of Abbreviations

18S	18 subunit RNA
28S	28 subunit RNA
AIDS	acquired immunodeficiency syndrome.
ALA	alanine
BDH	PCR-grade water
BHC	benzene hexachloride (insecticide).
bp	basepair
CDCP	Chagas disease control program
CPCA	common principal component analysis
CV1	canonical variable 1
CV2	canonical variable 2
CV3	canonical variable 3
CVA	canonical variate analysis
cytb	cytochrome b
D2	28S ribosomal RNA
DDT	4,4'-(2,2,2-trichloroethane-1,1-diyl)bis(chlorobenzene) (insecticide)
DNA	deoxyribonucleic acid
DNTP	deoxynucleoitide triphosphate
F1	first filial generation
FIS FST FIT	Wright's F statistics
FOI	force of infection
GDEHSC	General Direction of Environmental Health and Sanitary Control
GIS	geographical information system
GPA	generalised procrustes superimposition algorithm
HCH	hexachlorocyclohexane (insecticide).
HWE	hardy weinberg equilibrium
IAM	infinate alleles model
IBD	isolation by distance
IFAT	immunofluorescence antibody test
IIS	identical in state
IPTG	isopropyl b-D-thiogalactopyranoside
ITS-1	internal transcribed spacer 1 (ribosomal RNA gene).
ITS-2	internal transcribed spacer 2 (ribosomal RNA gene)
KAM	k-allele model
KCL	potassium chloride
LB	luria-bertani
MgCl2	magnesium chloride
ML	maximum likelihood
MP	maximum parsimony
mRNA	messenger RNA
mtcytb	mitochondrial cytochrome b
mtDNA	mitochondrial DNA

mtlsurRNA	mitochondrial large subunit ribosomal RNA
Mya	million years ago
NaCl	sodium chloride
NaOAc	sodium acetate
Ne	effective population size
NJ	neighbour joining
Nm	indice of gene flow
NS	non-significant
Oligo	oligonucleotide
PAHO	Pan American Health Organisation
PCA	principle components analysis
PCR	polymerase chain reaction
RAPDs	randomly amplified polymorphic DNA
REV	reversible rates
RNA	ribonucleic acid
rRNA	ribosomal RNA
RST	Slatkin 1995 analogue of FST
SMM	stepwise mutation model
SOC	a liquid growth medium
SSC	sodium citrate buffer
SSCP	single strand conformational polymorphisms
SSR	simple sequence repeat
Taq	*Thermus aquaticus*
TE	tris-EDTA
THR	threonine
TPM	two phase mutation model
TPS	thin plate spline
TRIS-HCL	thris hydrochloride
tRNA	transfer RNA
TS\TV	transition\transversion ratio
UPGMA	unweighted pair group method with arithmetic mean
UV	ultraviolet
W/B	washing/binding buffer
WHO	World Health Organization
X-GAL	5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside

1 Chagas disease

First described by Carlos Chagas in Brazil in 1909, human Chagas disease is a chronic parasitic infection that can result in severe debilitation through impairment of essential organs (Dias *et al.*, 2002, Schofield 1994). It is a major source of morbidity and mortality in endemic areas in Latin America surpassing the combined effects of malaria, leishmaniasis and schistosomiasis (Dias & Schofield 1999, World Bank 1993).

Ten years ago serological data estimated that 16-18 million people were infected with the disease and a further 100 million lived at risk from transmission (WHO 1991), resulting in a suggested 45,000 deaths annually (Rozendaal 1997). In addition to being a major public health problem Chagas disease has also been a considerable economic burden to these endemic countries with losses said to equate to over $6.5 billion a year, a factor that has contributed to political will to control this disease (Schofield & Dias 1999, Dias & Schofield 1999).

Following large-scale control initiatives, at both national and international levels, the number infected is now estimated at over 12 million (Morel & Lazdins 2003, Dias *et al.*, 2002, Schmunis 1999). However, there is continued transmission and risk of infection in rural areas, with 200,000 new cases a year in 15 countries predicted in the absence of control, a prospect facilitated by poverty, social deprivation and low political priority (Morel & Lazdins 2003, Dias & Schofield 1999, WHO 1991, Hayes & Schofield 1991, Dias 1987). Success has been achieved but continued commitment to control is required, with risk of infection existing where programmes are not fully executed e.g. Mexico, or where new areas are being colonised e.g. the Amazon, and importantly continued vigilance is required to prevent recrudescence of transmission in those areas where control has been successful and disease cases reduced (Dias *et al.*, 2002).

1.1 Life cycle of *Trypanosoma cruzi*

The causative agent of Chagas disease is a haemoflagellate protozoan, *Trypanosoma cruzi* (family Trypanosomatidae, order Kinetoplastida), with a complex life cycle

involving various developmental stages (see Figure 1 belowand Figure 2 on page 28) and passage through a haematophagous invertebrate vector (family Reduviidae, subfamily Triatominae) and one of numerous vertebrate hosts (Schofield 1994, Rodriques Coura & de Castro 2002, WHO 1991).

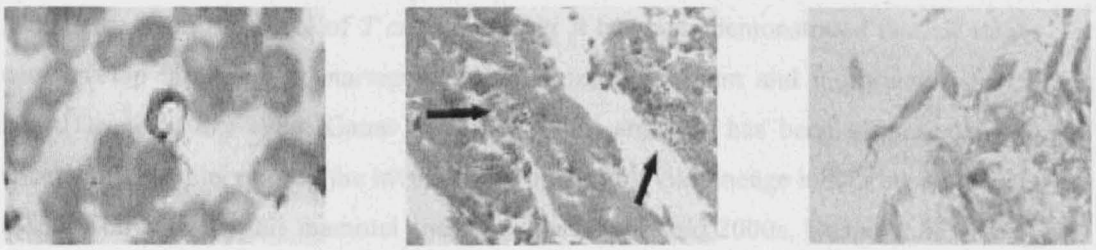

Figure 1. The main developmental stages of *Trypanosoma cruzi*.
A. Mammalian blood form trypomastigotes **B.** Intracellular amastigotes, **C.** epimastigote forms in triatomine bug faeces (courtesy Dr Matthew Yeo).

T. cruzi life stages vary morphologically, in terms of size, shape and the position of the kinetoplast in relation to the nucleus and flagellum (Schofield 1994). The three main stages of the life cycle are as follows (see Figure 1and Figure 2): non-replicating flagellated **trypomastigotes (A)** circulate in the blood of infected vertebrates where they invade non-phagocytic and phagocytic cells in various tissues, particularly the heart, smooth and skeletal muscle. These transform to small non-flagellated forms, **amastigotes (B)**, which multiple within the cell forming **pseudocysts.** Following differentiation into trypomastigotes and cell lysis these forms are released into the bloodstream where they circulate or reinvade cells.

When a triatomine bug feeds on an infected host, blood trypomastigotes may be ingested and development of infective stages occurs over a period of 3-4 weeks. In the vector, trypomastigotes develop into amastigotes in the midgut, and then differentiate into non-infective **epimastigotes (c)**, which replicate by binary fission in the hindgut of the vector. Passing to the rectum they attach to the wall and divide, yielding infective **metacyclic trypomastigotes**. The life cycle is complete upon transmission to a vertebrate, via a contaminative route as opposed to inoculation. During or shortly after

feeding, depending on the bug species, defecation occurs and transmission ensues when infective metacylic stages in the faeces enter the bloodstream through an abrasion in the skin or through the mucosa (Schofield 1994, Macedo *et al.,* 2002). Once infected with *T. cruzi* the bug is a vector for life but transovarian transmission does not occur.

This is the main life cycle of *T cruzi*, however it has been demonstrated that all stages can develop in *Didelphis marsupialis*, the common opossum and important reservoir host (Deane *et al.,* 1984, Gaunt & Miles 2000), and this has been suggested as an ancestral life cycle, prior to the involvement of the reduviid lineage indicating an ancient association between this mammal and protozoan (Schofield 2000a, Deane *et al.,* 1984). Direct host infection is also possible through oral ingestion, blood transfusion (Schmunis, 1999), organ transplant, (Riarte *et al.,* 1999), laboratory accidents and congenital transmission (Nisida *et al.,* 1999). In areas of South America free from vectorial transmission, congenital transmission is responsible for new cases of Chagas disease.

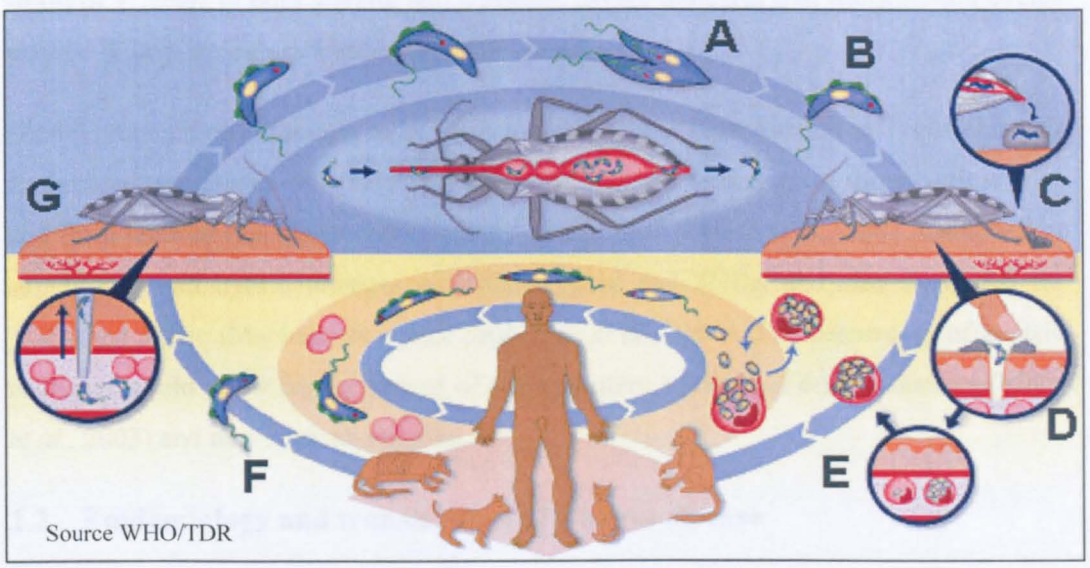

Figure 2. Summary of the life cycle of *Trypanosoma cruzi.*
A. Dividing epimastigotes, **B.** Infective metacyclics, **C.** Excreted infective metacyclics, **D.** Host cell infection, **E.** Pseudocysts containing dividing amastigotes, **F.** Circulating trypomastigotes, **G.** Ingestion of trypomastigotes by insect vector.

1.1.1 The genetic diversity of *T. cruzi*

The characterisation of *T. cruzi* is essential to the understanding of the epidemiology of Chagas disease. Genomic heterogeneity was detected in a number of studies employing a variety of analytical methods including isoenzymes (giving the original subgrouping Z1, Z2 Z3) (Miles *et al.*, 1977, 1978), mini-exon DNA (Souto *et al.*, 1996), microsatellites (Oliveira *et al.*, 1998) and RAPDs (Carrasco *et al.*, 1996, Steindel *et al.*, 1993) and has long been suspected to be due to differing phenotypic forms, clinical manifestations and treatment outcomes (Miles *et al.*, 2003, Momen 1999, Campbell *et al.*, 2004). Two principle subdivisions have now been designated, *T. cruzi* I (Z1) and *T. cruzi* II (Z2, Z3) (Miles *et al.*, 2003, Anon 1999b), with five sublineages (II*a*-e) known within *T. cruzi* II. *T. cruzi* I is dominant in domestic cycles north of the Amazon and zoonotic cycles in the Amazon basin and is commonly associated with opossums such as *D. marsupialis*, whilst *T. cruzi* II is involved in transmission in the southern cone countries associated with armadillos (*Dasypus novemcinctus*). The presence of the same strain of *T. cruzi* in both silvatic and domestic cycles indicates that transmission cycles may be linked, as seen in Venezuela (Miles *et al.*, 1981).

Clonal propagation is thought to be the principal form of reproduction in *T. cuzi*, leading to predominant clones but there is evidence of genetic exchange in the silvatic cycles and from laboratory experiments (Carrasco *et al.*, 1996, Gaunt *et al.*, 2003, Campbell *et al.*, 2004), with hybrid lineages identified, TCIId and TCIIe, and also suggested for TCIIa and TCIIc (Machado & Ayala 2001, Yeo *et al.*, 2005). The occurrence of genetic exchange could allow for the spread of more virulent strains and drug resistance (Miles *et al.*, 2003) and new host adaptations.

1.2 Epidemiology and transmission of Chagas disease

Trypanosoma cruzi is endemic in the Americas as a zoonosis of small nest dwelling mammals, with over 100 indigenous species found naturally infected (Schofield 1994) including opossums, armadillos and wild rodents. Silvatic species of Triatominae maintain this zoonosis, often inhabiting the same ecotopes as these mammals due to

their obligate haematophagy e.g. in nests, opossum lodges, palm trees and rock piles (Schofield *et al.*, 1999). The destruction of silvatic ecotopes by humans has allowed for both vectors and reservoirs to invade and colonise houses (De Andrade *et al.*, 1995).

While both disease reservoirs and vectors have a wide distribution in the Americas, including the southern states of the USA, endemic disease in humans is restricted to Central and South America (Schofield 2000a, Beard *et al.*, 2003). This clearly reflects the role of poverty in the transmission of Chagas disease, with only five cases of human transmission noted in the USA (Beard *et al.*, 2003). Social deprivation such as poor housing conditions and hygiene is essential and unfortunately this is prevalent in Central and South America. Even within a single village in South America different housing conditions make a difference, it is strange to visit neighbouring houses where infestation, and therefore disease risk, varies due to differences in construction and hygiene levels. It is clear that tackling basic issues related to poverty would ease greatly the burden of this disease. Important factors relating to vector competence include a tendency to enter houses, feeding preferences and defecation patterns. (Gurtler *et al.*, 1992, De Andrade *et al.*, 1995, Starr *et al.*, 1991, Schofield 1994). In humans Chagas disease transmission is primarily vector based (80%) and while all species of Triatominae are considered potential vectors, only those that have come to live in close association with man are of epidemiological significance (Schofield 1994).

Two main cycles of vector transmission exist, silvatic and domestic, which may overlap (see Figure 3 on page 31and Figure 4 on page 34). The **silvatic/enzoonotic cycle** is the predominant natural cycle between reservoirs and their associated vectors and the particulars of this cycle are largely unknown (Jansen *et al.*, 1999, Gaunt & Miles 2000), with transmission probably occurring through ingestion of infected bugs in addition to contact with infected faeces/urine (Diotaiuti *et al.*, 1995). Human infection is sporadic, limited to occasional cases where adult bugs enter houses or peridomestic areas to feed, perhaps attracted by light, but without colonisation e.g. *Rhodnius* species in the Amazon, (Coura *et al.*, 2002). Other examples include attacks on forest workers by hungry silvatic triatomines or outbreaks following oral transmission in particular from palm juice

presses (Coura *et al.*, 1994, 2002, Valente *et al.*, 2000, Miles *et al.*, 2003) (see Figure 3 below). Although human involvement is limited, this cycle is important in terms of reservoir maintenance, and allows for infected synanthropic mammals e.g. rats and opossums to enter the domestic/peridomestic environment introducing new strains of the parasite and increasing the risk of Chagas disease (De Andrade *et al.*, 1995, Macedo *et al.*, 2002). In addition while many silvatic Triatominae are highly adapted to their vertebrate hosts and specific ecotopes others are more eclectic with anthropophilic tendencies. As man disturbs this cycle, through encroachment, or removes the main domestic vector populations through control, new niches are created which are open to occupation by these opportunistic species (Schofield 2000b).

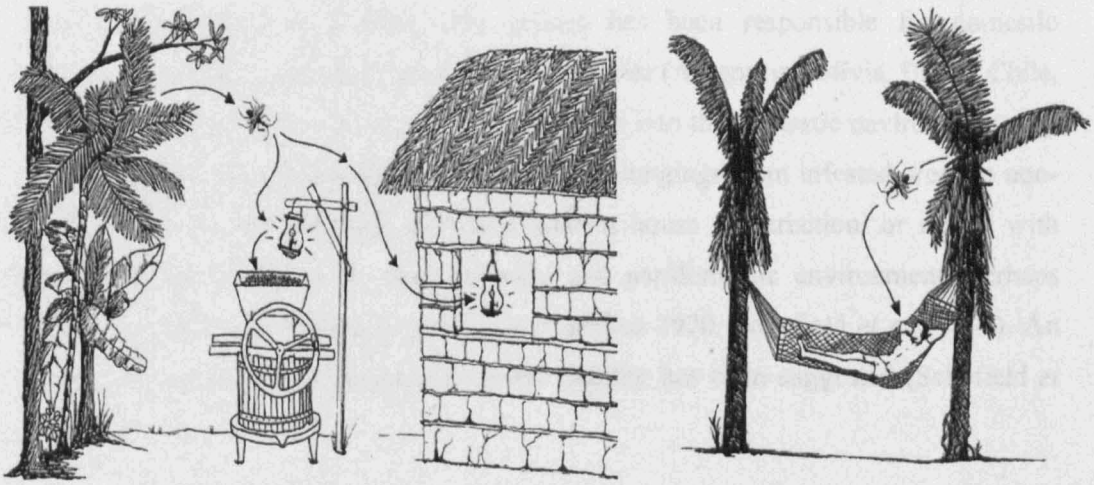

Figure 3. Role of silvatic specimens of Triatominae in sporadic cases of human Chagas disease. (Courtesy of James S. Patterson).

The most significant cycle in terms of human disease burden and public health control is the **domestic transmission cycle** (see Figure 4 on page 34). In the rural communities of Latin America substandard housing, consisting of traditional wattle and daub structures, often with palm or thatched roofs, have created artificial ecotopes that allow for the colonisation of the domestic environment by silvatic species of Triatominae. This new territory has proved advantageous with its constant blood supply and superior shelter from predators in the many cracks and crevices of the mud walls, thus allowing for large

populations of bugs to develop. The rate of disease transmission in this environment is dependent on several factors including density of vector populations, vector anthropophily, longevity, susceptibility to infection, feeding and defecation patterns; the most effective vectors are those that defecate during or shortly after blood uptake (Guhl & Vallejo 1999). Important host factors include parasitaemia levels and reaction to vector biting, with irritability resulting in short feeding times and insufficient bloodmeal uptake (Schofield 1994). High numbers of people per household, low levels of hygiene and indoor crop storage are also important factors (Gurtler *et al.*, 1992, Starr *et al.*, 1991, De Andrade *et al.*, 1995). The main reservoirs in this cycle are humans, also dogs which are more tolerant of biting and important in the maintenance of disease (Gurtler *et al.*, 1992). *Triatoma infestans* has thrived in the domestic environment, isolated from its original silvatic foci in Bolivia, this species has been responsible for domestic transmission of Chagas in six South America countries (Argentina Bolivia, Brazil, Chile, Paraguay, Uruguay) (Miles *et al.*, 2003). Movement into the domestic environment may be **passive**, with bugs transported by man in his belongings from infested areas to non-infected areas, or with infested materials used in house construction, or **active** with silvatic specimens flying into the domestic and peridomestic environment, perhaps attracted by light and establishing colonies (Gamboa 1970, Schofield *et al.*, 1999). An evolutionary trend towards increased habitat stability has been suggested (Schofield *et al.*, 1999).

The domestic cycle may be separate from an adjacent silvatic cycle or they may overlap (Figure 4 on page 34). In areas of Bahia State, Brazil houses are infested by the triatomine bug *Panstrongylus megistus*, while adjacent silvatic cycles are maintained by the species *Triatoma tibiamaculata*, colonising bromeliad epiphytes in association with the opossum *Didelphis albiventris* (A). Separate cycles of *T. cruzi* are also maintained with *T cruzi* II found in the domestic cycle while *T cruzi* I is in the silvatic cycle (Miles *et al.*, 2003). Examples of overlapping cycles can be seen in north-eastern Brazil where *Triatoma brasiliensis* can invade houses from adjacent silvatic populations. In Venezuela where the main vector species, *Rhodnius prolixus*, has both silvatic (palm

dwelling) and domestic populations, overlapping transmission cycles have also been proposed (B).

In addition to vector transmission a major source of infection is through infected **blood transfusion** (16%), overcoming the low socio-economic conditions traditionally characterising Chagas epidemiology and introducing the disease as an urban threat. This became a major source of transmission following rural to urban migrations in the 1970s and 80s (Moncayo 1999); it has been estimated that 300,000 infected people are living in São Paulo (Schofield 1994). Prior to control of *T. cruzi*, infection in blood banks varied from 3 to 53%, a prevalence greater than those of HIV or hepatitis (Anon 1999a). This has also highlighted the threat of Chagas disease on an international level with an estimated 25,000-100,000 infected Latin American immigrants in the USA and the need for precautionary measures taken in blood screening even in England. Other non-vector sources of infection including congenital transmission, in up to 4% of births from infected mothers (Dias & Schofield 1999) and organ transplant, in particular renal transplants, are also a threat outside the endemic area (Zayas *et al.*, 2002).

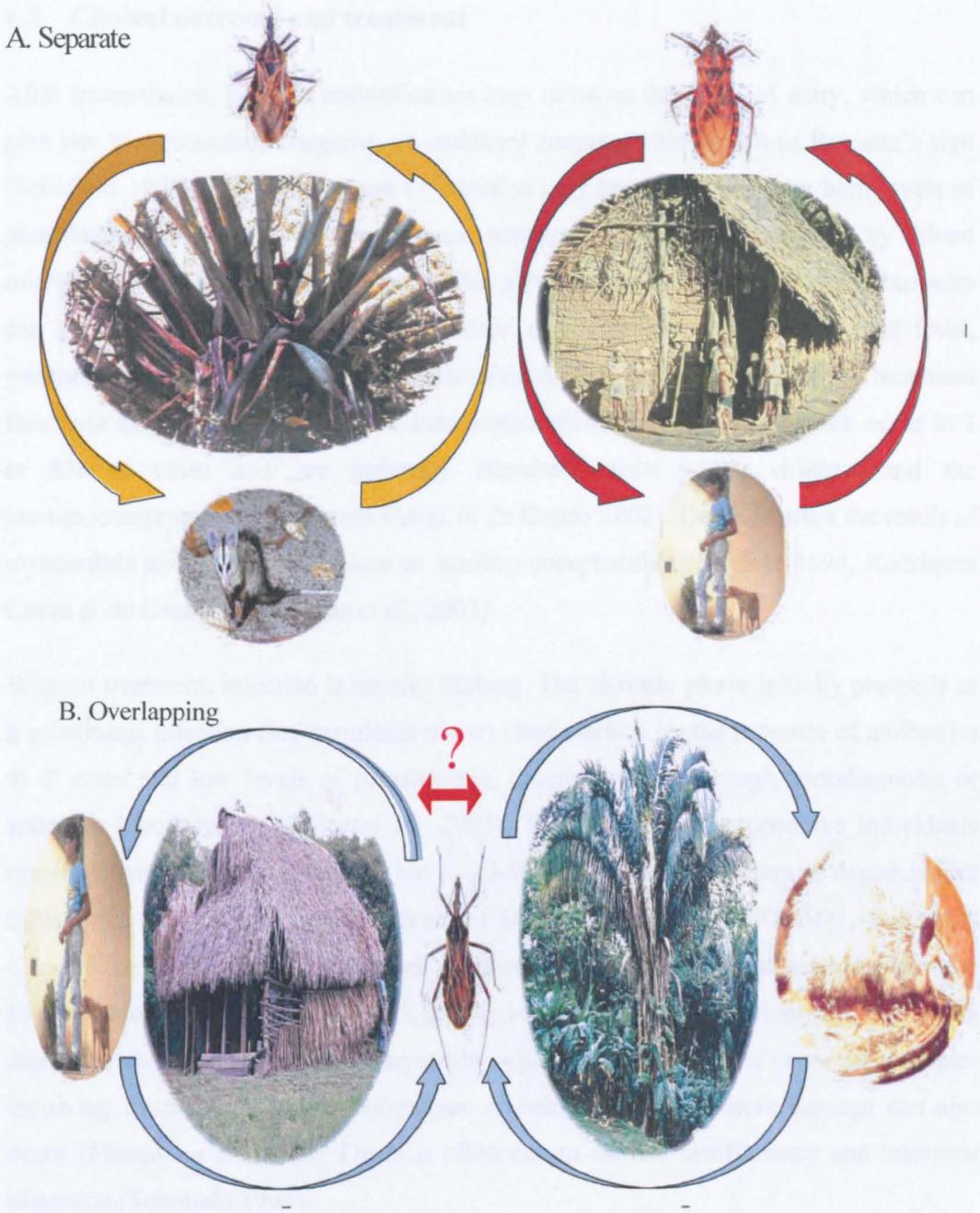

Figure 4. Domestic and silvatic transmission cycles
A Separate: silvatic cycles are maintained by the species *Triatoma tibiamaculata* in association with bromeliad epiphytes and the opossum *Didelphis albiventris,* with domestic colonies of *Panstrongylus. megistus.* **B** Overlapping: *Rhodnius prolixus* in Venezuela found in houses and palms close to houses in association with various birds and mammals in particular *Didelphis marsupialis.*

1.3 Clinical outcome and treatment

After transmission, parasite multiplication may occur at the point of entry, which can give rise to a cutaneous chagoma, or unilateral conjunctivitis known as Romana's sign (Schofield 1994). The **acute phase** of infection may be characterised by high levels of parasitaemia, the result of continuous parasite replication, detectable by blood microscopy and concentration methods. This phase is generally subclinical and exposure can go unnoticed. Symptoms, if they occur, can be non-specific and include fever, generalised lymphadenopathy, mild heptosplenomegaly and in the absence of treatment these can last for up to two months. Fatal outcomes at this stage of infection occur in 2 to 8% of cases and are generally associated with young children and the immunocompromised (Rodriques Coura & de Castro 2002). Death is often the result of myocarditis and congestive failure or meningoencephalitis (Schofield 1994, Rodriques Coura & de Castro 2002, Zayas *et al.*, 2002).

Without treatment, infection is usually lifelong. The **chronic phase** initially proceeds as a subclinical infection (indeterminate phase) characterised by the presence of antibodies to *T. cruzi* and low levels of parasitaemia, detectable only through xenodiagnosis or intensive blood culture (Miles *et al.*, 2003). The majority of seropositive individuals remain asymptomatic indefinitely, but in 20-50% of cases, often years or decades after initial infection, chronic manifestations of infection develop (WHO 1991, Rodriques Coura & de Castro 2002). There are no laboratory or clinical indicators to predict the likelihood of disease (Rodriques Coura & de Castro 2002). Development of chronic disease is characterised by cardiomyopathy with conduction defects or megasyndromes involving dysfunction of the oesophagus or colon, peripheral nerve damage can also occur (Macedo *et al.*, 2002). Death is often due to cardiac insufficiency and intestinal distortion (Schofield 1994).

Clinical outcomes vary in different geographical areas. In northern South America and Central America, megasyndromes are rare, cardiomyopathy is variable in distribution and infection is more benign, whereas in the southern cone countries cardiomyopathy

and megasyndromes are common (Marsden 1996, Dias & Schofield 1999). It is thought these varied clinical outcomes could be linked to *T. cruzi* diversity, with certain strains differing in virulence from others and could also be linked with variable patient response (Rodriques Coura & de Castro 2002). With the *T. cruzi* genome project complete genes associated with pathogenicity and other features of this protozoan will hopefully be identified (El-Sayed *et al.*, 2005).

No vaccine is available, and two drugs are currently employed for Chagas treatment: nifurtimox and benznidazole. Treatment success varies with phase of infection, age of patient, dosage and geography linked to parasite strain (Macedo *et al.*, 2002). Clearance of parasitaemia is possible when drugs are administered early in acute cases (30-70% of cases). Treatment is recommended in all acute cases, congenital cases and accidental transmission (Rodriques Coura & de Castro 2002, Schofield 1994, Dias & Schofield 1999). Drugs can also be administered in early and late chronic phases to attempt to prevent disease progression or lessen severity, but also to reduce human *T. cruzi* reservoirs (Dias & Schofield 1999). Whilst these drugs are available there are problems related to their use in large scale interventions including **delivery:** in rural areas where needs are greatest but medical contact limited, **clinical:** recognition of the acute phase when drugs are most effective is difficult and infections often go undiagnosed, **side effects:** can be severe and are a common result of treatment. Treatment of symptoms are more common for the chronic phase of infection such as anti-arrhythmic drugs and surgical correction for megasyndromes (Schofield 1994, Dias & Schofield 1999). More effective chemotherapy is required including less toxic drugs in shorter courses for effective treatment of acute cases, and the existing infected population. However, very few drug companies are interested in this area, but hope may lie in the screening of available developed drugs for activity against *T. cruzi* (Rodriques Coura & de Castro 2002, Miles *et al.*, 2003). The genome of *T. cruzi* may also reveal new drug targets or possible vaccine candidates.

1.4 Interventions and control programmes

Interventions for the control of Chagas disease are based on (1) the prevention of transmission of *T. cruzi* through the elimination of domestic vector populations, (2) the prevention of infected blood transfusion and congenital transmission and (3) through health education in rural endemic areas. Control aims to prevent new cases in uninfected children, and adults and reduce morbidity and mortality in those already infected. Chagas disease has a high economic impact in Latin America with economic losses of US$ 8156 million calculated due to disability alone, while medical costs of US$ 53 million have been suggested for the lifetime treatment of chronic patients in Brazil (Dias & Schofield 1999). This has led to interest in control of Chagas among Latin American governments (Dias & Schofield 1999, Schofield 1994).

1.4.1 Vector control

Vector control is seen as essential for prevention of infection since Carlos Chagas first discovered the role of the triatomine bug in transmission. The effectiveness of control efforts was demonstrated initially by successful programmes in Brazil and Venezuela and recently by the multinational southern cone initiative.

The mainstay of vector control is the spraying of synthetic insecticides on rural housing (internal walls and roof surfaces and external walls) and on peridomestic animal enclosures (Dias & Schofield 1999). Synthetic pryrethroids e.g. deltamethrin and cyfluthrin are currently used and, although these are more expensive than previously used insecticides e.g. BHC, they are overall more cost effective, with higher efficiencies at lower dosages and longer residual effects resulting in less frequent reapplications. These insecticides are also relatively non-toxic and are more acceptable to householders, being odourless and non-staining, overcoming problems previously encountered (Schofield 1994, Dias & Schofield 1999).

A systematic approach to control has been developed based on successes in Brazil with programmes divided into three phases: **preparatory, attack** and **vigilance** (Dias 1987).

The initial **preparatory phase** involves identifying and mapping areas to be treated, manual sampling of bugs from a selection of houses in each area and peridomestic habitats. Collection of any baseline data available and training of staff also occurs. Costs and details of the control strategy are devised. In the **attack phase** all houses in target areas are sprayed, regardless of infestation status. This is followed by surveillance for reinfestations and respraying when necessary in the **vigilance phase**. Surveillance takes the form of active postspray house inspections, carried out to check for bugs, but passive surveillance can also be employed using Gomez-Nunez or Maria sensor boxes. These boxes are placed within houses to create artificial refuges for bugs; continued presence of bugs is visible from eggs and faecal stains left on the pleated paper placed within, but these methods are not as precise as active searches (Schofield 1994, Dias & Schofield 1999). Community participation has been vital in the Southern Cone, whereby householders collect and report any bug infestations to a local information point; once infestations are confirmed houses within 20m of the area are resprayed (Dias & Schofield 1999, Dias 1987). This vigilance phase also depends on serological surveys in children born after the launch of the programme in order to identify new infections and areas of continued transmission (Feliciangeli *et al.*, 2003) excluding congenital cases and passive antibodies in infants less than 9 months of age.

House improvement to prevent colonisation of the domestic environment is also important. This is achieved through plastering of wall cracks and crevices, replacing palm and thatch roofs with corrugated iron or tiles, cementing floors and removing animal enclosures from the immediate housing area (Schofield 1994, Dias & Schofield 1999). This is a long-term approach to control and is expensive, however it is beneficial for the control not only of Chagas disease but also other social diseases. High costs and non-co-operation of landowners reduce the feasibility of this approach.

1.4.2 Transfusion control

Although a proven risk for transmission since 1962 the screening of blood on a wide scale only began with the emergence of AIDS (Dias *et al.*, 2002). Interruption of

transfusion transmission is achieved by screening blood samples from donors using a variety of serological techniques such as the indirect haemaglutination or the indirect immunofluorescence test (IFAT) (Dias & Schofield 1999). National policy and legislation is essential for mandatory screening and full coverage. Treatment of infected human reservoirs is also essential if risk is to be reduced (Rodriques Coura & de Castro 2002). Blood screening is established in all major control programmes.

1.4.3 Control programmes

1.4.3.1 Southern cone initiative

This is a multinational initiative established in 1991 for the control of the transmission of Chagas disease in participating countries (Argentina, Bolivia, Chile, Paraguay, Uruguay and Peru) through the eradication of domestic and peridomestic populations of *T. infestans*, and other locally important species, and the prevention of infected blood transfusions through implementation and improvement of blood screening procedures (Dias & Schofield 1999, Schofield & Dias 1999).

Eradication was deemed possible, as *T. infestans* was considered exclusively domestic throughout the region with the exception of genetically isolated silvatic Andean and Chaco populations in Bolivia, therefore reinvasion from silvatic populations would not jeopardise control. However recent research indicates that silvatic foci of *T. infestans* are more widespread than previously believed within Bolivia, including reports at the border with Paraguay and within Argentina (Noireau *et al.*, 2005). Further studies are needed to assess how widespread these foci are and evaluate the risk they pose to effective control. Control on an international level may reduce the chance of reinvasion from untreated foci by movement of people across borders, which may have been responsible for the present day distribution of *T. infestans*, presumably from original silvatic foci in Bolivia (Schofield & Dias 1999).

This initiative has had major successes with the distribution of *T. infestans* greatly reduced with large formerly endemic areas in Brazil (6 States by 2000) and Argentina

(10 provinces by 2002) now free from transmission, and Uruguay and Chile certified free of transmission in 1997 and 1999 respectively. Disease incidence has dropped by an average of 94% in the area. From an economic point of view returns of $7.16 per dollar have been calculated in Brazil for $420 million invested in control from 1975-1995. Economic benefits arise from reduced morbidity and decreased medical costs (saving US$53 million) (Dias *et al.*, 2002, WHO 2002).

1.4.3.2 Andean Pact and Central American initiative

The success of the southern cone programme has inspired similar control collaborations: the Andean Pact control programme in 1997 (Venezuela, Colombia, Peru, and Ecuador) the Central American initiative in 1997 (Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua and Panama) (Schofield 2000b). The main vectors in these countries are *R. prolixus* and *Triatoma dimidiata*, with contributions from *Rhodnius pallescens* in Panama and *Rhodnius ecuadoriensis* in northern Peru (Dias *et al.*, 2002). There are an estimated 5-6 million people infected and 25 million at risk from infection (Moncayo 1999). Established vector control strategies must be adapted as the main vector species in these countries have silvatic foci, adaptations may include continued surveillance, more frequent selective respraying, or development of new methodologies aimed at the control of silvatic foci (Feliciangeli *et al.*, 2003, Dias & Schofield 1999). The main emphases of these programmes are again vector control and transfusion control.

While control is feasible, challenges in addition to reinvasion include maintenance of political interest in control when disease numbers fall (Feliciangeli *et al.*, 2003, Dias *et al.*, 2002).

1.5 Taxonomy of Triatominae

The Triatominae (order Hemiptera, suborder Heteroptera), a subfamily of the Reduviidae, are characterised by their obligate haematophagy, other family members

being predaceous (Schofield 1994, Lent & Wygodzinsky 1979). All life stages require blood for development and reproduction (Schofield 1994).

The subfamily comprises at present approximately 137 species, classified into six tribes and 17 genera (see Table 1 on page 42). The majority are silvatic and are of little epidemiological importance; only twelve species of two tribes are currently considered significant vectors due to their ability to invade and/or colonise houses and the peridomestic area (see Table 1). The five most important species in terms of domestic transmission and public health control, with wide distributions in different geographical areas are *T. infestans, R. prolixus, P. megistus, T. dimidiata* and *T. brasiliensis* (see Figure 5 below).

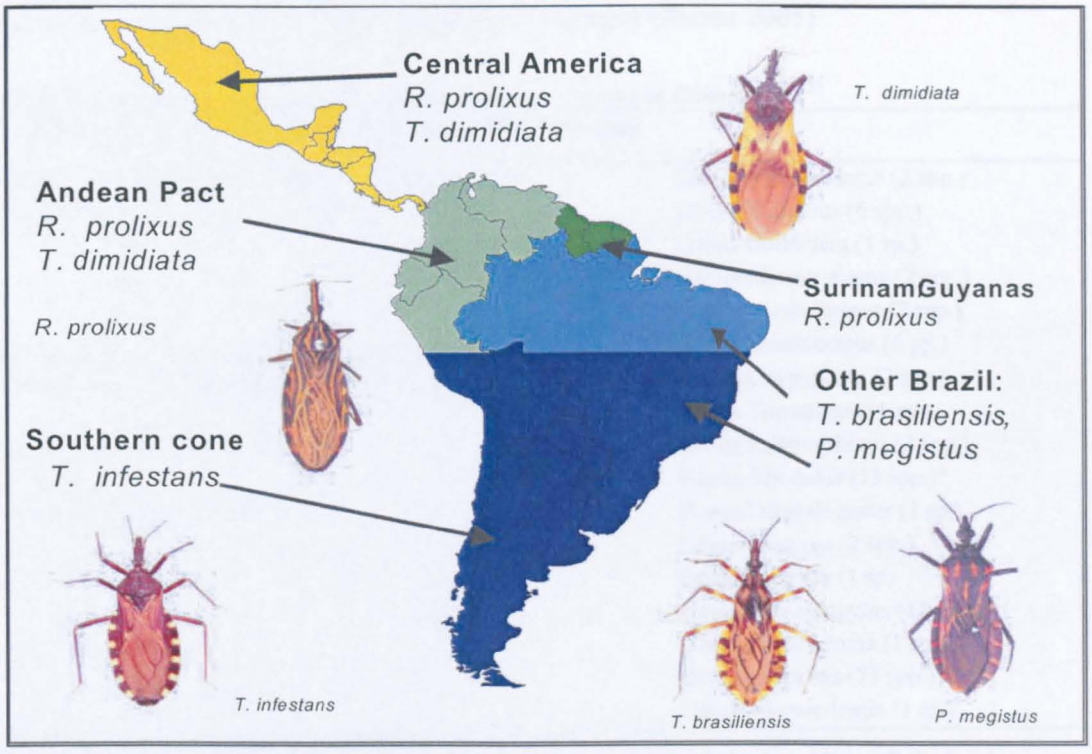

Figure 5. Main vector species of Triatominae and their geographical distribution
Triatoma dimidiata, Triatoma infestans, Panstrongylus megistus, Rhodnius prolixus, T. brasiliensis.

The Triatominae are primarily neotropical with the majority of species found in the New World. However, species are also found in India, China, Malaysia, Indonesia and Australia (Poinar 2005, Patterson *et al.*, 2001). There is evidence that Old World species have New World origins (Patterson *et al.*, 2001, Poinar 2005), although the placement of the genus *Linshcosteus* is debated (Hypsa *et al.*, 2002). Triatominae may have arisen in the mid-Cretaceous period and spread through North America via the Proto-Antillean landmass; its presence is noted in Dominican amber (*Triatoma dominicana*) together with ancient trypanosomes possibly of bat origin (Poinar 2005). From North America the subfamily may have passed into Asia via the Beringia land bridge (Late Cretaceous) and spread south and northward to Australia. The present fragmentary species distribution may have been due to climate cooling in the mid-Eocene-Oligocene eliminating species or causing a southward migration (Poinar 2005).

Table 1. Triatomine systematics the principal vectors are found **in three genera^.**

Order Hemiptera, **Family** Reduviidae, **Subfamily** Triatominae	
Tribe Alberprosenìini Martinez & Carcavallo, 1977	Genus Alberprosenia (2 spp.)
Tribe Bolboderini Usinger, 1944	Genus Belminus (6 spp.)
	Genus Bolbodera (1 sp.)
	Genus Microtriatoma (2 spp.)
	Genus Parabelminus (2 spp.)
Tribe Linshcosteini Carcavallo et al., (2000)[a]	Genus Linshcosteus (6 pp.)
Tribe Cavernicolini Usinger, 1944	Genus Cavernicola (2 spp.)
	Genus Torrealbaia (1 sp.)
Tribe Rhodniini Pinto, 1926	Genus Psammolestes (3 spp.)
	Genus Rhodnius (13 spp.)^
Tribe Triatomini Jeannel, 1919	Genus Dipetalogaster (1 sp.)
	Genus Eratyrus (2 spp.)
	Genus Mepraia (1 sp)
	Genus Panstrongylus (13 spp.)^
	Genus Paratriatoma (1 sp.)
	Genus Triatoma (77 spp.)^
	Genus Hermanlentia (1 sp.)

Schofield (2000c), Galvao *et al.*, (2003). [a] recent elevation, previously included in the Tribe Triatomini.

The genus *Linshcosteus* is limited to the Indian subcontinent and members are identified from an abbreviated rostrum and lack a stridulary suculus (Galvao *et al.*, 2002). An independent origin has been suggesting for the genus (Gorla *et al.*, 1997, Schofield

2000a). However in a recent phylogenetic analysis of 57 species of Triatominae from 9 genera, *Linshcosteus* clustered within the Triatomini, thus casting doubt on this theory (Hypsa *et al.*, 2002).

The Triatominae are believed to have evolved from nest dwelling predaceous Reduviidae (Schofield 1988, 2000a, 2000c, Schaffer 2003). Opinions differ, however, as to whether this occurred once and all species share a single common ancestor (Lent & Wygodzinsky 1979, Gaunt & Miles 2000, Hypsa *et al.*, 2002, Schuh & Slater 1995), or originate from different predator species resulting in a polyphyletic assemblage (Schofield 1988, 2000a, 2000c, Dujardin *et al.*, 1999a, Stothard *et al.*, 1998, Lyman *et al.*, 1999, Marcilla *et al.*, 2001, Bargues *et al.*, 2000, Schaffer 2003, de Paula *et al.*, 2005).

It has been argued that monophyly is inconsistent with species distributions and specialisations (Schofield 1988) and incompatible with genetic characterisation, which has been increasingly adopted in the analysis of this subfamily (RAPDs, DNA sequence polymorphisms) which has established deep clade separation between the Rhodniini and Triatomini (Monteiro *et al.*, 2000, Stothard *et al.*, 1998, Lyman *et al.*, 1999, Marcilla *et al.*, 2001, Bargues *et al.*, 2000, Garcia & Powell 1998). This division is also supported by evidence from saliva and sensillae (Ribeiro *et al.*, 1998, Soares *et al.*, 1998, Catala 1997), egg exochorium architecture (Barata 1996, 1998) and isoenzymes (Dujardin *et al.*, 1999b).

Shared morphology, from the polyphyletic viewpoint, is the result of convergence due to a haematophagic existence and predatory ancestry as opposed to genetic relatedness (Schofield 2000c). The inclusion of reduviids from other subfamilies and other triatomine species from other tribes in DNA studies could help to solve this question (Bargues *et al.*, 2002). A recent study examined this using 16S mitochondrial sequences from Genbank for 43 species of Triatomini and 14 species of Rhodniini together with 15 species of predatory Reduviidae from five subfamilies as outgroups (de Paula *et al.*, 2005). Parsimony and maximum likelihood trees supported the polyphyly of the

Triatomini and Rhodniini tribes but also identified sister groups of predatory reduviids for each tribe (the Reduviinae to Triatomini and Stenopodinae, Salyvatinae or Vesciinae with the Rhodniini). This result was in contrast to those of Hypsa *et al.*, (2002) where a phylogeny based on 12S and 16S RNA sequences of 57 species of the Triatominae including members of the tribes Rhodniini, Linshcosteini, and Triatomini suggested a monophyletic origin of the subfamily. This study used fewer Reduviid subfamilies and included more distantly related species of the order Hemiptera and Homoptera, which may have prevented the detection of polyphyly (de Paula *et al.*, 2005).

Triatominae are classified by morphological and chromatic characteristics of the exoskeleton to define species and genera (see Lent & Wygodzinsky 1979) including the structure of cuticular surfaces, pigmentation patterns and coloration of legs, pronotum and connexivum, length ratios of antennal and rostral segments and the anteocular and postocular regions. The medically important genera are distinguished by the point of insertion of the antennae in relation to the eyes or the apex of the head. While this approach allows for the identification of the majority of species, some species show variations e.g. colour morphs such as *T. infestans* dark morph and similarity between different species e.g. *R. prolixus* and *Rhodnius robustus, Triatoma platensis and Triatoma delpontei*, which have led to difficulties in correct taxonomic classification and confusion over species status (Dujardin *et al.*, 1999a, Harry 1993a). Male genital structure has been employed in the characterisation and separation of similar species, but has not always been reliable, e.g. *R. neglectus* and *R. nasutus* (Lent & Wygodzinsky 1979, Jurberg 1996, Harry 1993a). Patterns of sensillary receptors on antennae and eggshell structure have also been used (Catala & Schofield 1994, Catala 1997, Gracco & Catala 2000, Barata 1996, 1998).

Researchers are increasingly applying new methodologies to Triatomine classification to solve these problems (Monteiro *et al.*, 2001), including morphometrics and genetic studies using isoenzymes and molecular methods such as direct sequencing (see excellent review of methods and studies applied to Triatominae by Abad-Franch &

Monteiro 2005). Correct species identification is essential for accurate vector incrimination and control.

1.5.1 Morphometrics

Descriptions of morphological form have always been used in the classification of organisms (Adams *et al.*, 2004). Early morphometrics was based on simple comparisons of mean values of one or more measurable traits. Modern morphometrics developed with advances in statistical analysis that allowed for the description of shape variation within and among groups (Adams *et al.*, 2004).

"Morphometry is the measurement and analysis of form, and the main premise of morphometrics is that a statistical analysis of genetic variability expressed by morphological characters is a measure of population differentiation and ultimately speciation" (Patterson 2002). Morphometrics is currently classified into two domains; traditional and geometric morphometrics.

1.5.1.1 Traditional morphometrics

Based on the multivariant analysis of a series of standard measurements (linear) for example of the head, wings (hymelytra) or thorax of insects. Two main orthogonal factors, size and shape, govern morphology (Brookstein *et al.*, 1985). Temporal, environmental or ontogenetic factors can effect variation in size and therefore can have a confounding effect on analysis (Hutcheson *et al.*, 1995, Patterson *et al.*, 2001). Linear measurements are normally strongly correlated with size (Adams *et al.*, 2004).

Analytical methods employed in traditional morphometrics may depend on the focus of the study, if evolutionary differences between species or geographic populations is of interest size correction is required to eliminate isometric size in order to focus on evolutionary (genetic) differences as to opposed environmental factors. The removal of isometric size is achieved by log transforming the data to equal variance among groups and variables, and then subtracting the mean for each row (Patterson *et al.*, 2001,

Hutcheson *et al.*, 1995). These values are then used for univariate and multivariate analysis. Multivariate analysis requires a greater number of individuals than the variables tested (Bustamente *et al.*, 2004, Patterson *et al.*, 2001).

Multivariate techniques employed include principal component analyses (PCA) and canonical variate analysis (CVA). These are ordination procedures which reduce dimensions within the data by calculating linear combinations of the original variables to produce one or two components, which represent the majority of the shape variability within and between specimens (Hutchinson *et al.*, 1995). Principal component analyses (PCA) summarises total variation in all samples as a single group. The new linear variables produced are termed principal components (PC) and they are orthogonal (Patterson *et al.*, 2001, Hutcheson *et al.*, 1995). This is followed by a discriminant analysis, canonical variate analysis (CVA), which explores between-group variation in relation to the collective within-group variation (Patterson *et al.*, 2001, Hutcheson *et al.*, 1995, Abad-Franch 2003). The discriminant variables produced are termed canonical variates (CV) and n-1 are produced, where n is the number of groups analysed.

If investigating the relationship between similar groups, e.g. closely related species or intraspecific comparisons, it is necessary to also account for allometric effects (changes in shape with size). A common principal component analysis (CPCA) is used to test for the existence of a common axis of allometric growth. If a common axis is detected, the first CPC value is disregarded as this represents growth effects and the remaining values can be used for PCA and CVA analysis (Dujardin & Le Pont 2000, Patterson *et al.*, 2001).

1.5.1.2 Geometric morphometrics

This is a more recent morphometric technique based on the use of Cartesian coordinates (2d or 3d) and superimposition methods to analyse shape variation between specimens (Adams *et al.*, 2004). Landmarks cannot be analysed directly as they are affected by variation in digitising position, orientation and scale, and these non-shape effects must

be removed (Adams *et al.*, 2004). To achieve this, the landmarks of the compared specimens are overlaid using a generalised procrustes superimposition algorithm (GPA); this is an iterative least squares estimate, which scales, translates and rotates the landmark configurations to minimise the square differences between them and to produce a mean shape configuration. Each iteration updates the mean configuration (Brookstein 1991). The superimposition translates the centroid of each landmark to the origin, then scales the configurations to a common size and lastly rotates the configurations to minimise the square difference between the landmarks. Centroid size is removed by ratios (Adams *et al.*, 2004, Patterson 2002). Centroid Size is the square root of the sum of squared distances of a set of landmarks from their centroid.

Shape differences between the compared specimens are measured as the difference between the positions of specimen landmarks to the corresponding landmark of the consensus; these differences are termed procrustes residuals. These residuals are free of scale, rotation and position; however they are non-Euclidean as after superimposition (GPA) they lie on a multidimensional curved shape surface called Kendall's shape space (Patterson 2002, see Figure 6 on page 48). These residuals are subjected to Thin Plate Spline (TPS) analysis (TPSrelw software v1.29 Rohlf 2003) which projects them into a linear orthogonal plane tangent to Kendall shape space with Euclidean geometry and also allows for the visualisation of shape variation as displacement vectors on transformation grids (TPS visualisation\grids, see Figure 7 on page 49) (Patterson 2002, Adams *et al.*, 2004). Shape differences termed partial warps consist of 'affine' (global stretching on grids) and 'non-affine' (non linear localised distortions) components of shape change and these are visible distortions on the grids (Patterson 2002). These partial warps are the variables subjected to multivariate analysis (PCA and CVA on page 45).

Each CV produced from CVA analysis represents a unique pattern of shape variation and can be used to plot individuals in 'shape discriminant space' (Abad-Franch 2003, Patterson 2002). Here a single point represents the shape of a specimen and individuals with similar shape variation will group together. Typically the first two CVs are used as

they represent the majority of variability detected (Patterson 2002). Shape variation represented by the CVs is used to reclassify individuals to groups using a contingency table.

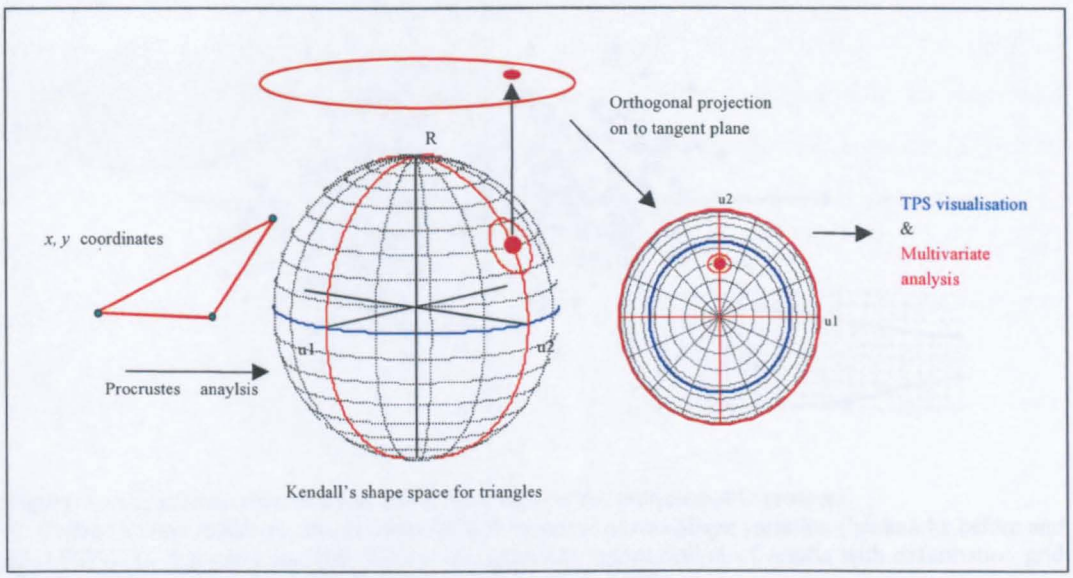

Figure 6. An illustration of the orthogonal projection from Kendal's shape space (from Patterson 2002).

Geometric morphometrics has several advantages; it allows the recovery of the geometry of the original form by preserving the information on spatial arrangements of the organism, linear measurements are usually inadequate (Adams *et al.,* 2004, see Figure 7 on page 49), it also reduces the effects of differential growth based on environmental causes (Rohlf & Marcus 1993). This removal of growth effects indicates that differences detected are due to genetic as opposed to environmental differences. Although traditional morphometrics does allow for size correction, distances are highly correlated with size.

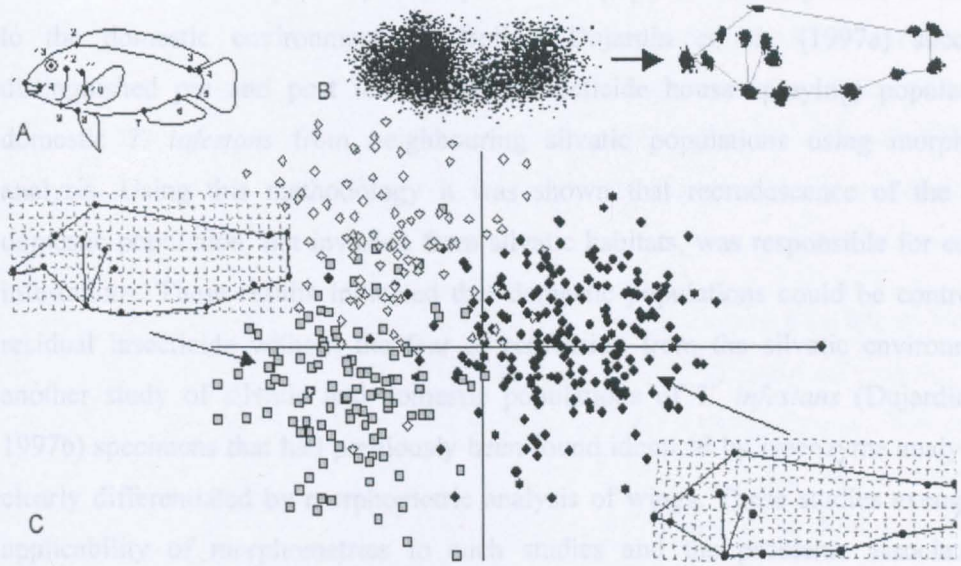

Figure 7. A graphical representation of the main steps of the morphometric protocol.
A. Capture of raw landmark data (cichlid fish) B Removal of non-shape variation (landmarks before and after GPA). C. Statistical analysis (CVA) and graphical representation of results with deformation grid showing different shape representations (from Adams *et al.*, 2004)

1.5.1.3 Previous studies

Prior to the nineties, morphometrics was applied to Triatominae in terms of classical metric measurements (linear measurements or ratios) used solely for species classification. New approaches to morphometrics were first applied to the interspecific analysis of *Triatoma sordida*, *Triatoma guasayana*, and *Triatoma patagonica* (Gorla *et al.*, 1993), where multivariate analysis clearly distinguished *T. sordida* from *T. guasayana* and *T. patagonica* but *T. guasayana* and *T. patagonica* exhibited similarity with 2.44% classification error.

Studies to date have primarily used traditional morphometrics to resolve geographic and ecological variations of species (Monroy *et al.*, 2003, Bustamante *et al.*, 2004, Borges *et al.*, 2005, 2000, Fernandez *et al.*, 2005) or to solve systematic and population questions (Patterson *et al.*, 2001, Soares *et al.*, 1999, Dujardin *et al.*, 1997a, 1998a, 1998b, 1999b, Gorla *et al.*, 1993, Harry 1994). Important applications in relation to disease control

have included the analysis of postspray domestic populations and process of adaptation to the domestic environment. In Bolivia Dujardin *et al.*, (1997a) successfully distinguished pre and post intervention (insecticide house spraying) populations of domestic *T. infestans* from neighbouring silvatic populations using morphometric analysis. Using this methodology it was shown that recrudescence of the original domestic population, not invasion from silvatic habitats, was responsible for continued infestations. These results indicated that domestic populations could be controlled by residual insecticide without the fear of reinvasion from the silvatic environment. In another study of silvatic and domestic populations of *T. infestans* (Dujardin *et al.*, 1997b) specimens that had previously been found identical by isoenzyme analysis were clearly differentiated by morphometric analysis of wings. These studies exemplify the applicability of morphometrics to such studies and the problems associated with isoenzymes in the differentiation of recently diverged populations.

Trends in the adaptation to the domestic environment have also been investigated using morphometrics. Dujardin *et al.*, 1998a investigated the increasing tendency of the predominantly silvatic *Panstrongylus rufotuberculatus* to enter houses in Bolivia and colonise with nymphal stages detected (Dujardin *et al.*, 1998a). Morphometric analysis successfully distinguished domestic *P. rufotuberculatus,* collected from houses in adjacent localities in La Paz, from silvatic specimens from museum collections. An important result from this study was the detection of sexual dimorphism in domestic specimens. Dimorphism between sexes is reduced in laboratory colonies with increasing generations, therefore its detection in domestic colonies in La Paz may indicate that domestication was a recent event and its presence could be used as a future indicator of domestic adaptation (Dujardin *et al.*, 1998a). *T. dimidiata* has a wide geographical distribution from Mexico to Ecuador and exhibits variability in synanthrophic behaviour, coloration and genital structure. Traditional morphometrics of the heads of eight populations from its range showed significant shape differences in relation to geographical distribution and suggested that populations were diverging, as detected with ITS-2 sequencing (a segment of ribosomal RNA, the internal transcribed spacer,

Bustamante *et al.*, 2004, Marcilla *et al.*, 2001). Two populations exhibited shape similarity unrelated to the cline, possibly due to similar ecotope. Morphometric analysis was also used to investigate the assignment of *T. dimidiata* to the *phyllosoma* complex, and its relationship to the *protacta* complex. The complexes were clearly distinguishable and *T. dimidiata* was placed within the *phyllosoma* complex, in agreement with ITS-2 analysis.

Dujardin *et al.*, (1999b) compared phylogenetic structure of the tribe Rhodniini, derived from isoenzyme analysis, with relation to Mahalanobis distances (see page 132) derived from morphometric comparisons of the head and wings of specimens. Both trees were similar, identifying three main groupings and differing only with respect to the position of *R. prolixus*, which was grouped with *R. nasutus* and *R. neglectus* in isoenzyme analysis but with *R. pictipes* in morphometric analysis. Neis genetic distances and Mahalanobis distances were also significantly correlated.

Geometric morphometrics is now increasingly applied to Triatominae, initially for taxonomic analysis (Matias *et al.*, 2001, Villegas *et al.*, 2002) but now also for intraspecific studies (Jaramillo *et al.*, 2002, Schachter-Broide *et al.*, 2004a, 2004b, Acosta *et al.*, 2004, Cuartas *et al.*, 2004). On a taxonomic level wing shape variation clearly distinguished *R. prolixus* from *R. robustus* supporting their separate species status (Villegas *et al.*, 2002). Population heterogeneity of *T. infestans* from three Argentinean villages, within $10km^2$, was investigated using wing geometric morphometry. Wing shape heterogeneity was detected within villages but also between ecotopes and individual collection sites (Schachter-Broide *et al.*, 2004a). These results were in agreement with previous isoenzyme analysis of *T. infestans* where individual collection sites e.g. a single house was found to represent a single population deme (Breniere *et al.*, 1998). Reclassification data based on size and shape variation was highest for individual sites. The same authors also investigated seasonal variations in spatial structuring for five sites (four goat and 1 pig coral) within one of these villages. Populations of *T. infestans* were collected in October 2002 and March 2003. Greater shape heterogeneity was found to exist between populations collected in October, than

populations collected in March. Eighty-three specimens collected in March were then compared for shape similarity with October populations to identify a possible source population. These specimens were found to share greater shape similarity with the pig coral population, suggesting this site was the source of dispersion (Schachter-Broide *et al.*, 2004b).

Morphometrics has been used in the analysis of a number of important vector species including sandflies (Dujardin & Le Pont 2000, Dujardin *et al.*, 1997c, 2004, De La Riva *et al.*, 2001), ticks (Hutcheson *et al.*, 1995), blackflies (Kruger & Garms 1999) and tsetse flies (Patterson & Schofield 2005). Morphometrics offers the advantages for species analysis of being cheap and robust.

1.5.2 Genetic methods

1.5.2.1 Basic concepts in population genetics

1.5.2.1.1 Hardy-Weinberg equilibrium (HWE)

The Hardy-Weinberg law illustrates the influence of random mating on allele and genotype frequencies in diploid populations. Under HWE allele frequencies remain in equilibrium over time in large randomly mating populations, without immigration or selection, allowing for the maintenance of genetic variation and rare alleles between generations (Hartl & Clarke 1997).

The relationship between allele frequencies and genotype frequencies is computed as follows; for biallelic loci, $p^2 + 2pq + q^2=1$ where p^2 and q^2 represent the expected frequencies of homozygote genotypes, AA and aa, in zygotes of any generation, and 2pq the heterozygote frequency Aa, p and q are the allele frequencies of A and a in gametes of the previous generation, $p + q = 1$ (Hartl & Clarke 1997). Heterozygosity (H) can also be calculated as H=1-homozygosity, $H= 1- (p^2+ q^2)$. Hardy Weinberg can be applied to genes with 3 or more alleles by extending the binomial expansion e.g. for a 3 allele

system with allele frequencies p, q and r, $(p + q + r)^2 = p^2 + r^2 + q^2 + 2pq + 2pr + 2qr$, p + q + r = 1.

A population is in Hardy Weinberg equilibrium when the observed allele frequencies do not vary significantly from expected. However this concept is based on a number of assumptions including large population size, random mating, no significant mutation or immigration or selection, all of which are violated in nature for example through inbreeding or genetic drift (Hartl & Clarke 1997). Hardy Weinberg law functions as a null hypothesis for the investigation of various factors driving population differentiation.

1.5.2.1.2 Linkage equilibrium

In a randomly mating population alleles at different loci are in random association (linkage equilibrium) and the frequency of a gamete carrying a given combination of alleles is equal to the product of the frequency of those alleles. Linkage disequilibrium occurs when alleles at two loci are found together more frequently than expected by chance. If populations are randomly mating, linkage equilibrium is expected to eventually to be achieved. Significant linkage disequilibrium can be detected when two or more subpopulations that differ in allele frequencies are combined as one population or when a particular combination of alleles occurs more frequently than expected due to natural selection (Hartl & Clarke 1997, Page & Homes 2000).

1.5.2.1.3 Population heterogeneity

Population subdivision (different allele frequencies) can result in departures from HWE and the rejection of this null hypothesis (Page & Homes 2000, Hartl & Clarke 1997). Three fixation indices F_{IS}, F_{ST} and F_{IT} were developed by Wright to evaluate subdivision and inbreeding among three levels of a population; the individuals in relation to their own subpopulation (I), the subpopulation (S) and the total population (T) (Pages & Homes 2000). These indices measure the reduction in heterozygosity between the hierarchical levels compared to levels expected under random mating (Hartl & Clarke 1997). When populations are subdivided heterozygosity is reduced due to smaller

population size and the effects of inbreeding or genetic drift, both of which result in allele fixation.

F_{IS}, termed the inbreeding coefficient, measures the reduction in heterozygosity of an individual in relation to their own subpopulation as a result of inbreeding (Page & Homes 2000). Values range from −1 to +1. Negative values are associated with heterozygote excess, which is indicative of outbreeding while positive values indicate homozygous excess due to inbreeding.

F_{ST} indices summarise the division of genetic diversity within and between populations as a measure of heterozygosity. Guidelines indicate that at F_{ST}=0.0-0.05 population heterogeneity is minimal, at F_{ST}=0.05-0.15 moderate, at F_{ST}=0.15-0.25 great, and at higher values very great (Hartl & Clarke 1997). When F_{ST}=0 total panmixia is implied while when F_{ST}=1 populations are completely isolated. Unlike F_{IS} values are always positive and negative values are noted as zero. F_{ST} analysis is based on an infinite alleles model (IAM) and assumes new alleles in populations are primarily due to migration not mutation.

F_{IT} measures total heterozygote deficiency for individuals relative to the total population thereby measuring both inbreeding and population subdivision effects (Page & Homes 2000).

R_{ST} is an analogue of F_{ST} developed for the analysis of microsatellite data. Based on the stepwise mutation model (SMM) R_{ST} calculations incorporate the mutational differences between alleles (Slatkin 1995). R_{ST} may be more appropriate when populations or species are genetically distant as mutation rates between alleles would be larger and more informative, and the effect of mutation greater than migration (Chambers & MacAvoy 2000). However for a limited number of loci and individuals F_{ST} may be more appropriate than R_{ST} (Gaggiotti *et al.*, 1999, Fredsted *et al.*, 2005). High variance of R_{ST} can occur under SMM (Gaggiotti *et al.*, 1999). Examples of marked differences between F_{ST} and R_{ST} have been quoted for the same data sets (Lugon-Moulin *et al.*, 1999).

1.5.2.1.4 Genetic drift

Genetic drift causes deviation from HWE because random sampling of the gametes, which succeed in fertilisation, leads to fluctuations of allele frequencies between generations. As time passes certain alleles will be fixed by chance alone; such effects are much greater in small populations (Hartl & Clarke 1997, Page & Homes 2000). When populations are subdivided genetic drift can lead to fixation of different alleles in each population. Genetic drift is therefore responsible for the reduction of genetic variation within a population while increasing genetic difference between subpopulations, as with inbreeding (Page & Homes 2000).

Other population phenomena can affect heterozygosity levels and HWE such as population bottlenecks and founder effects. Population bottlenecks occur following a severe reduction in population number. This results in a reduction of genetic variation, as the majority of alleles, especially rare alleles are lost. A smaller population will also lead to increased inbreeding and increased levels of homozygosity (Page & Homes 2000). Founder affects also produce similar population changes whereby heterozygosity is decreased as fewer alleles are represented in the newly founded population due to limited number of colonizers. Genetic drift and selection following foundation can result in different alleles fixed in the newly established population in comparison to the founding population (Pages & Homes 2000).

1.5.2.1.5 Geneflow

While population subdivision and genetic drift cause population differentiation, geneflow can homogenize populations. Geneflow introduces new genetic variation into populations, in addition to mutation but at a greater rate (Page & Homes 2000, Hartl & Clarke 1997). Geneflow occurs when an individual migrates from one subpopulation to another and successfully interbreeds. Through geneflow the genetic variation between subdivided populations is therefore reduced, by the homogenisation of allele frequencies while intrapopulation heterozygosity is increased (Page & Homes 2000).

Two main models governing geneflow are the island model and the stepping stone model. Under the island model geneflow occurs with equal probability between a series of small subpopulations within a larger population. Under the stepping stone model, geneflow is limited to adjacent populations, resulting in isolation by distance and a cline in allele frequencies. Limited levels of migration can be sufficient to prevent population divergence and one migrant per generation is sufficient, regardless of population size (Hartl & Clarke 1997, Page & Homes 2000).

F_{ST} has previously been used to estimate measures of geneflow via the equation $F_{ST}=1/(4Nm+1)$ (see Glossary on page 310) based on an island population model by Wright, however such measures may be incorrect (Whitlock & McCauley 1999, Gaggiotti *et al.*, 1999) because they rely on assumptions of a low mutation rate relative to the migration rate, an absence of selection and drift-migration equilibrium. $F_{ST}=1/(4Nm+1)$ is unable to distinguish between past and current geneflow, and recently separated populations may exhibit low F_{ST} values, due to retained polymorphism. This would result in overestimation of geneflow or Nm, the number of migrants per population per generation.

1.5.2.2 Isoenzyme analysis

Alloenzymes of a given enzyme are the product of different alleles at a specific locus, which have differing electrophoretic charges and therefore migration rates in gel electrophoresis. Genetic variability within or between populations or species is seen as the degree of heterozygosity at different polymorphic loci and their relative frequencies in different populations, genetic distances and rates of geneflow can be calculated. Alloenzymes have been the mainstay of molecular analysis in Triatominae, accounting for 65% of published work (Monteiro *et al.*, 2001) addressing such issues as genetic variability between populations of the same species (Dujardin *et al.*, 1987, 1996, 1998c, Soares *et al.*, 1999, Costa *et al.*, 1997, Borges *et al.*, 2000, Breniere *et al.*, 1998, Noireau *et al.*, 1998), between species (Harry *et al.*, 1992, Harry 1993b, Lopez & Moreno 1995, Solano *et al.*, 1996, Pereira *et al.*, 1996) and investigation of phylogenetic relationships

(Chavez *et al.*, 1999, Dujardin *et al.*, 1999b, Monteiro *et al.*, 2002, see Abad-Franch & Monteiro 2005).

Using isoenzymes it was demonstrated that *T. infestans* specimens collected 6 months after insecticide spraying in 3 houses in Bolivia were surviving members of the original population and not migrants from neighbouring untreated villages (Dujardin *et al.*, 1996). Isoenzymes used to compare populations of silvatic and domestic *T. infestans* from Bolivia failed to detect population differentiation at 19 loci (Dujardin *et al.*, 1987). This, however, is in contrast to later studies using morphometrics and RAPD analysis (Dujardin *et al.*, 1997b, Carlier *et al.*, 1996). Isoenzymes have been used to investigate *T. infestans* population heterogeneity, and it was found, while panmixia can occur at village level (Dujardin *et al.*, 1998c), population isolation is also possible within villages (Breniere *et al.*, 1998). Populations between differing villages have also been shown to be isolated (Dujardin *et al.*, 1998c), as seen in Dujardin *et al.*, (1996).

For studies on a taxonomic level allozymes have often proved too conserved to distinguish closely related species e.g. the analysis of allozyme loci in *R. prolixus* and *R. robustus* failed to detect fixed differences in several studies and led to questions on their species specific status (Dujardin *et al.*, 1991, Harry *et al.*, 1992, Harry 1993b), although the lack of differentiation was later suggested to be a sign of recent divergence (Monteiro *et al.*, 2002). However isoenzyme analysis has successfully detected cryptic speciation within *T. sordida* populations in Bolivia, identifying one primarily silvatic species (Noireau *et al.*, 1998). The species status of *T. petrochii*, (from *T. brasiliensis*) was also validated by isoenzyme analysis (Monteiro *et al.*, 1998).

A problem associated with isoenzymes is that homozygosity can be over-estimated due to degeneracy, that is changes in the genetic sequence can still code for the same amino acid. Hence genetic divergence can be underestimated (positional homology). Isoenzymes can be too conserved and can result in the misinterpretation of species relationships, particularly with recent speciation events (Monteiro *et al.*, 2002). In Triatominae low levels of genetic diversity are found and thus isoenzymes may be too

conserved to provide useful characteristics (Schofield 1994). Living or freshly frozen materials are also required (Stothard *et al.*, 1998, Abad-Franch & Monteiro 2005).

1.5.2.3 Molecular biological methods

The use of DNA based methods in the investigation of triatomine systematics and evolution has been an increasing trend (Monteiro *et al.*, 2001). Methods used include randomly amplified DNA (RAPDs) (Carlier *et al.*, 1996, Garcia *et al.*, 1998, Borges *et al.*, 2000, 2005, Jaramillo *et al.*, 2001) and single strand conformational polymorphisms (SSCP) (Stothard *et al.*, 1998), examining intraspecific and interspecific, relationships within and between the genera *Triatoma*, *Panstrongylus* and *Rhodnius*. Direct sequencing of polymorphic fragments of mitochondrial (Garcia & Powell 1998, Stothard *et al.*, 1998, Lyman *et al.*, 1999, Monteiro *et al.*, 1999a 1999b 2000, 2003, 2004, Garcia *et al.*, 2001, 2003), and nuclear genes (Monteiro *et al.*, 2000, Bargues *et al.*, 2000, 2002, Marcilla *et al.*, 2000, 2001, 2002). These methods have played a new role in interpopulation analysis and determining interspecific relationships at genus, tribe and family level. Microsatellite markers have also recently been published in *R. pallescens* (Harry *et al.*, 1998), *T. dimidiata* (Anderson *et al.*, 2002) and *T. infestans* (Garcia *et al.*, 2004).

1.5.2.3.1 RAPDs

In RAPD analysis banding patterns are generated through the amplification of polymorphic DNA using a series of random primers. DNA product is visualised by gel electrophoresis for the presence of distinct/shared banding patterns. By sampling the genome this technique generally identifies a greater degree of genetic variability than isoenzyme analysis (Dorn *et al.*, 2003, Borges *et al.*, 2000, Garcia *et al.*, 1998). It can also be used on dead material and on all life stages, and multiple samples can be visualised (Dorn *et al.*, 2003, Abad-Franch & Monteiro 2005). This technique has been widely applied to triatomine studies (Carlier *et al.*, 1996, Garcia *et al.*, 1998, Borges *et al.*, 2000, 2005, Jaramillo *et al.*, 2001, Feliciangeli *et al.*, 2002, Pacheco *et al.*, 2003). In

intraspecific comparison of three domestic *T. brasiliensis* populations RAPDs allowed for their clear discrimination, showing isolation by genetic distance where isoenzyme profiles were identical. Later the same author distinguished silvatic and domestic populations of *T. brasiliensis*, with peridomestic populations showing intermediate profiles. (Borges *et al.*, 2000, 2005). Importantly, an interspecific study on *Rhodnius* species using RAPDs identified genetic differences between the closely related species pairs of *R. prolixus-R. robustus* and *R. neglectus-R. nasutus*, for which isoenzyme analysis had not detected differences (Garcia *et al.*, 1998, Feliciangeli *et al.*, 2002).

RAPDS have been successful in the separation of *T. sordida* from *T. infestans* (Carlier *et al.*, 1996). RAPDS allowed for the differentiation of silvatic and domestic populations of *T. infestans*, with silvatic populations showing greater genetic variation, in another study isoenzymes did not detect differences at 19 loci (Carlier *et al.*, 1996, Dujardin *et al.*, 1987). RAPDs suggested geneflow between domestic, peridomestic and silvatic populations of *T. dimidiata* in Colombia (Ramierez *et al.*, 2005) indicating that silvatic and peridomestic populations represent a threat to domestic control in that area.

The benefits of using RAPD analysis are that the system is cheap and requires few items of equipment and reagents. Problems with RAPD analysis include vulnerability to contamination and profile variability can arise due to differing PCR conditions as opposed to genetic polymorphisms. The markers are not co-dominant therefore heterozygosity cannot be determined for population genetics studies (Abad-Franch & Monteiro 2005).

1.5.2.3.2 Mitochondrial and nuclear sequencing studies

Preliminary studies using DNA sequencing examined genetic variability and evolutionary relationships (Stothard *et al.*, 1998, Garcia & Powell 1998, Lyman *et al.*, 1999, Monteiro *et al.*, 2000). The identification of subspecies and closely related species has also been investigated, together with analysis of population variability (Lyman *et al.*, 1999, Monteiro *et al.*, 1999a, 1999b, 2000, 2003).

Mitochondria have initially been the target of choice. The mitochondrial genome has been sequenced for one triatomine species, *T. dimidiata*, and was found to be 17019 bp in length and contain genes coding for proteins required for cell respiration (2 rRNAs, 22 tRNAs and 13 mRNAs) (Dotson & Beard 2001, Abad-Franch & Monteiro 2005). Mitochondria are widely used as they are easy to manipulate, are clonally inherited along the maternal line, they are single copy, non-recombinant and are abundant in cells ($\sim 10^2$-10^4) (Simon *et al.*, 1994, Abad-Franch & Monteiro 2005). They also have faster evolutionary rates than in the nuclear genome, and are therefore useful in the study of recently diverged taxa (Avise 1994, Loxdale & Lushai 1998, Simon *et al.*, 1994). While these faster evolving sites allow for rapid evolving genetic markers, it also reduces their usefulness in the examination of wider phylogenies, with saturation increasingly likely as divergence increases between taxa (Simon *et al.*, 1994). As mitochondria are maternally inherited, mitochondrial DNA has a lower effective population size (Ne), variants increase at a greater rate than for nuclear DNA (Sunnucks 2000) and F_{ST} values for mitochondrial DNA are approximately 4 times larger than nuclear DNA (Krafsur *et al.*, 2001, Avise 1994).

Studies using mitochondrial DNA sequencing have included investigations on species identification and evolution within the subfamily Triatominae (Stothard *et al.*, 1998, Lyman *et al.*, 1999, Hypsa *et al.*, 2002, Sainz *et al.*, 2004), at tribe level in the Rhodniini (Monteiro *et al.*, 2003) and at species level examining populations and species complexes (Garcia & Powell 1998, Garcia *et al.*, 2003, Monteiro *et al.*, 1999a 1999b, 2003, 2004). Mitochondrial genes used include fragments of the protein coding gene cytochrome b (mt*cytb*) and cytochrome oxidase 1 and the large subunit ribosomal RNA (mtlsurRNA), 12S and 16S ribosomal RNA. Studies have confirmed the deep clade separation of the Triatomini and Rhodniini and paraphyly within the Triatomini and the separation of morphologically similar species *R. prolixus, R. robustus, R. nasutus and R. neglectus*, and confirmation of same species status for phenotypic variants e.g. *T. infestans* and *T. melanosoma*, but also detected variation in established species, thereby of value for solving classification problems (see Abad-Franch & Monteiro 2005 for

detailed review). Interestingly, mitochondrial DNA was also capable of distinguishing populations of *T. infestans* from differing geographical areas in Argentina, with 4 of 5 populations exhibiting 2 to 7 haplotypes (Garcia *et al.*, 2003). The number of private haplotypes detected in populations indicated limited gene flow occurring among populations and significant levels of genetic variation were detected.

Problems with analysing closely related species using mitochondrial targets are current or past introgression events, whereby horizontal gene transfer occurs between two species following a hybridisation event, with subsequent backcrossing. Although specimens may be morphologically distinct or distinguishable by other genetic means e.g. isoenzymes, they appear as conspecific species by mitochondrial analysis, e.g. *T. platensis* and *T. infestans* (Garcia & Powell 1998). To check for introgression, incongruence between mitochondrial and nuclear trees can be examined. Monteiro *et al.*, (2000, 2003) sequenced 28S rDNA (D2) for a subset of specimens, in addition to mitochondrial target *cytb*, to check for introgression. Incomplete mtDNA lineage sorting may also be a problem where two different species retain sequences that were found in their common ancestor (Abad-Franch & Monteiro 2005, Monteiro *et al.*, 2004).

Nuclear targets have also been employed including ribosomal targets such as quickly evolving second internal transcribed spacer (ITS-2) (Bargues *et al.*, 2000, 2002, Marcilla *et al.*, 2000, 2001, 2002). Ribosomal DNA sequences are often used in molecular systematics as they contain highly conserved (18S) and variable sequences (ITS-1, ITS-2). These can be used to construct phylogenetic relationships between both distantly related and closely related species. Differences in the nucleotide sequence composition and length of the two internal transcribed spaces (ITS-1, ITS-2) are useful for resolving genetic relationships between closely related taxa that have diverged relatively recently (<50 million years ago) (Marcilla *et al.*, 2000, 2001). The sequencing of ITS-2 in Triatominae has proved a useful marker for species and subspecies differentiation and was capable of population differentiation (Marcilla *et al.*, 2000, 2002). The analysis of ITS-2 sequences from 31 populations of 15 species of the two main tribes of Triatominae, Rhodniini and Triatomini, supported the polyphyletic view of the

subfamily with marked differences between the sequences in both length and composition, therefore requiring separate phylogenetic analysis of the tribes (Marcilla *et al.*, 2001). The monophyly of the *Rhodniini* tribe was also confirmed with *R. prolixus* and *Psammolestes tertius* clustering together (Marcilla *et al.*, 2001). Polyphyly within the genus *Panstrongylus* was also detected with ITS-2 analysis with *P. rufotuberculatus* clustering with the *T. dimidiata-phyllosoma* clade (Marcilla *et al.*, 2002).

The more slowly evolving 18S rDNA which codes for the small subunit rRNAs is useful for examining wider phylogenies, evolving 23 to 55 times slower than ITS-2, with substitution rates close to 1.8% per 100 million years (Bargues *et al.*, 2000). Bargues *et al.*, 2000 developed a molecular clock based on 18S rDNA and ITS-2 sequences. This was used to estimate divergence times between the tribes Rhodniini and Triatomini (48.9-64.4 million years ago mya). Phylogenetic trees derived from 18S sequences also support deep divergence between the Triatomini and Rhodniini and the separation of North and Central American species of Triatominae from South American (Bargues *et al.*, 2002). However this gene fragment is highly conserved and was unable to distinguish the genera *Panstrongylus* and *Dipetalogaster* from the genus *Triatoma* and between closely related species such as the *Triatoma phyllosoma* complex (Bargues *et al.*, 2002).

1.5.2.3.3 Microsatellites

Microsatellites or simple sequence repeat (SSR) are short regions of tandemly repeated DNA sequence 2-6 bp in length, e.g. (AC)n or (GATA)n, although this definition can vary from 1-8bp (Chambers & MacAvoy 2000). They are neutral, codominant and inherited in a mendelian fashion. These features make microsatellites ideal markers for population analysis and account for their increasing use in this field (Jarne & Lagoda 1996, Zhang *et al.*, 2003, Page & Holmes 2000, Schlötterer 2000). In addition microsatellites often exhibit high variation in the numbers of repeats in individuals from different populations and are therefore useful in detecting levels of genetic variability within and between populations even at fine scale resolution (Loxdale & Lushai 1998,

Tautz & Schlotterer 1994, Jarne & Lagoda 1996, Page & Holmes 2000). Species that exhibit low levels of alloenzyme and mitochondrial diversity have demonstrated variability at microsatellite loci (Estoup *et al.*, 1996, 1998, Paetkau & Strobeck 1994, Murray 1996).

Dinucleotide repeats e.g. (AC)n are the most common form of microsatellite repeat (Ashley & Dow 1994, Jarne & Lagoda 1996). Microsatellites have a wide distribution in eukaryotic genomes, primarily in non-coding DNA, and occur in low numbers in prokaryotes (Ellegren 2004, Ashley & Dow 1994). Microsatellites are more frequent and greater in length in vertebrates than invertebrates (Chambers & MacAvoy 2000, Schlötterer 2000). Mutation rates calculated for microsatellite are high (of the order of 10^{-2} to 10^{-6} Jarne & Lagoda 1996, Handcock 1999).

Replication slippage is thought to be the primary mechanism governing changes in base length repeats in microsatellites (Chambers & MacAvoy 2000, Schlötterer 2000). During replication the nascent strand may disassociate from the template strand and, upon re-annealing the repetitive nature of the microsatellite may cause the nascent strand to align out of sync. Continued replication without repair would result in the insertion or deletion of repeat units depending on occurrence of a loop structure in the template or the nascent strand (Handcock 1999, Ellegren 2004). Evidence for the role of unequal crossing over or gene conversion in the mutation of microsatellites has yet to be fully substantiated (Ellegren 2004, Handcock 1999, Schlötterer 2000, Ashley & Dow 1994).

The use of microsatellites in population and phylogenetic analysis requires an evolutionary model to explain the allelic variation detected and their genetic relationship. Two main theoretical models have been suggested to explain microsatellite evolution. The "infinite alleles model" (IAM Kimura & Crow 1964) whereby each new mutation, at rate μ, can involve the loss and gain of an unspecified number of repeats, with each mutation resulting in a new allele. The "stepwise mutation model" (SMM Kimura & Ohta 1978), in agreement with slippage strand mutation mechanism, states that new alleles are derived from the loss or gain of single repeat units with an equal

probability μ. Under this mechanism alleles of similar length are more related and alleles may mutate into an existing allele (Jarne & Lagoda 1996, Estoup & Cornuet 1999). The "two-phase model" (TPM, Di Rienzo *et al.*, 1994) is a modification of the SMM model that also allows for the gain/loss of repeat units by several orders of magnitude. Under the "*K*-allele model" (KAM) (Crow and Kimura 1970) the number of *K* alleles are limited, and an allele can mutate to an existing allele $K - 1$ with a probability of $\mu/(K - 1)$ (Estoup & Cornuet 1999, Jarne & Lagoda 1996). Under IAM and KAM mutational steps between alleles does not provide information on their relationship (Jarne & Lagoda 1996, Estoup & Cornuet 1999). A number of studies have been undertaken to investigate which models fit microsatellite data using both observed and theoretical analysis (Jarne & Lagoda 1996, Chambers & MacAvoy 2000, Estoup & Cornuet 1999), however, results have been conflicting and are subject to debate. These mutational models may be too simplistic and additional factors may affect rates of mutation. Studies have indicated that mutation rate and direction can vary with size of repeat unit, repeat length with longer repeats showing greater polymorphism (Chambers & MacAvoy 2000, Jarne & Lagoda 1996, Schlötterer 2000, Estoup & Cornuet 1999). Mutation may also vary with the purity of the repeat array, with uninterrupted repeats displaying greater polymorphism (Estoup & Cornuet 1999). There also appears to be an upper size limit on the mutational process (Chambers & MacAvoy 2000). Models have been developed incorporating some of these complications (Ellegren 2004, Jarne & Lagoda 1996).

Alleles in populations are subjected to mutation, selection, and genetic drift to produce population allele specific patterns (Chambers & MacAvoy 2000). In microsatellite analysis alleles are primarily scored by size variation following amplification by PCR, although direct sequence comparisons can also be employed (Jarne & Lagoda 1996). Direct sequencing of alleles at loci have suggested that indels (see Glossary on page 310) in microsatellite flanking regions can generate length variation (Anderson *et al.*, 2000, Estoup & Cornuet 1999), and can result in problems scoring alleles by size due to homoplasy (see Glossary on page 310).

Microsatellite markers have a number of problems associated with their use and analysis. Loci can be subject to null alleles whereby alleles fail to amplify due to a mutation in the primer binding site. This is noticeable in homozygotes where the PCR product is visually absent, however nulls may go unnoticed in heterozygotes when only one allele fails to amplify. The presence of null alleles increases or decreases estimates of population heterogeneity. Null alleles may be detected in data sets by testing for heterozygote deficiencies, which result in departures from Hardy Weinberg equilibrium, but this may also be due to natural population phenomena such as inbreeding (Jarne & Lagoda 1996). *In vitro* PCR slippage can also be a problem for accurate genotyping by the production of stutter bands, which differ from the true allele by multiples of the repeat unit and are most common in dinucleotide repeats (Ellegren 2004). Microsatellites can also suffer from size homoplasy, whereby two alleles can be the same size (Identical In State IIS) without originating from a common ancestral allele (Identical By Descent) (Jarne & Lagoda 1996, Chambers & MacAvoy 2000). Over large population divergence times, with high mutation rates, homoplasy can be a problem in the analysis of populations using the SMM or TPM mutation models (Estoup & Cornuet 1999). Sequencing of same sized alleles may reveal homoplasy, however alleles with identical sequence may have been derived from different mutational steps (Jarne & Lagoda 1996), variation in flanking sequence may be informative (Anderson *et al.*, 2000), or studying interrupted sequence as opposed to pure repeats (Estoup *et al.*, 1995).

The identification of microsatellite markers can also be time consuming. For the majority of species the genome sequence is not available and library construction is often required for the identification of repeat regions for primer design. However PCR screening of available microsatellite primers for related taxa may allow for successful amplification, if primers have been designed from conserved flanking regions (Harry *et al.*, 1998, Garcia *et al.*, 2004).

Microsatellite analysis has been applied extensively to population genetic analysis in social insects (Jaquiery *et al.*, 2005, Dronnet *et al.*, 2005) and *Drosophila* (Noor *et al.*, 2000). Microsatellite markers have also been isolated from, and used in, the analysis of a

number of important vector species such as tsetse flies (Solano *et al.*, 1998, 2000, Krafsur *et al.*, 2001, Krafsur 2002), sandflies (Maingon *et al.*, 2003, Aransay *et al.*, 2003), blackflies (Dumas *et al.*, 1998) and various species of mosquitoes (Braginets *et al.*, 2003, Huber *et al.*, 2004, Lehmann *et al.*, 2003, Rongnoparut *et al.*, 1999, Donnelly & Townson 2000, Norris *et al.*, 2001, Huber *et al.*, 2002). Understanding population heterogeneity in vector species is essential for understanding disease epidemiology and in devising successful strategies for disease control such as sterile male release for mosquitoes or tsetse flies.

Krafsur & Endsley (2002) investigated genetic heterogeneity among seven populations of *Glossina morsitans submorsitans* from the Gambia and Ethiopia and 6 populations of *G. m. morsitans* from Zimbabwe. Mark release recapture studies suggested active dispersal, however, mitochondrial analysis indicated high levels of heterogeneity. Microsatellite analysis supported population heterogeneity (F_{ST}= 0.18, F_{ST}=0.17). Six populations of *Glossina morsitans centralis* were also investigated using mitochondrial and microsatellite analysis (Krafsur *et al.*, 2001). Both markers showed evidence of population heterogeneity and restricted geneflow correlated with geographical distances, however the F_{ST} estimate from mitochondrial data was greater (F_{ST} = 0.866, F_{ST} =0.186). This discrepancy was suggested to be the result of the sensitivity of mitochondria to genetic drift as, due to their maternal inheritance, mitochondria have approximately one quarter of the effective population size of nuclear genes. Homoplasy due to high mutation rates of microsatellites was also suggested as a possible influence. Population substructure in both studies was suggested to have originated with the rindepest epizootic, which resulted in population reduction and isolation.

Among insect vector species, mosquitoes have been the most extensively studied by microsatellite analysis, in particular species of the *Anopheles gambiae* complex. Lehmann *et al.*, 1997 studied populations of *An. gambiae* using both microsatellite (five loci) and mitochondrial analysis (ND5 gene). Specimens were collected from houses distributed over a 50km radius. Population heterogeneity was not detected by either marker, suggesting the panmictic unit for *An. gambiae* is greater than 50km. A study

comparing *An. gambiae* populations from East and West Africa over the distribution extremes (over 6000km) also detected geneflow (mean F_{ST}= 0.016, Nm 7.7). R_{ST} values detected were higher and unusually were similar to the results obtained for the more conserved alloenzymes (mean R_{ST} = 0.036 Nm=3.4) (Lehmann *et al.*, 1996). The authors suggested that factors inherent in microsatellite evolution unaccounted for in analysis might be reflected in these results (Lehmann *et al.*, 1996). Recent population range expansion was also suggested to explain lack of population divergence, rather than geneflow between populations of *An. gambiae* and *An. arabiensis* over 6000km (Besansky *et al.*, 1997).

Anopheles funestis in Kenya is an important vector during the dry season. An uneven dispersal related to habitat distribution was suggestive of population isolation. However, results from cytogenetic and mitochondrial data were contradictory, with cytogenic data suggesting panmixia. Braginets *et al.*, (2003) studied 4 populations from western and coastal Kenya at 7 microsatellite loci. Populations within each area were panmictic, however, regions were isolated (F_{ST} = 0.208, R_{ST} 0.158), possibly due to a geographic barrier. Results indicated that the smallest panmictic unit for *An. funestis* is greater than 50km radius. Results obtained were lower than those values generated from cytogenetic data, again size homoplasy and high mutation rates governing microsatellite evolution were suggested as a possible explanation.

Limited genetic variation has been detected among populations of *Anopheles maculatus* in Thailand using direct sequencing of ribosomal and RAPD analysis. Rongnoparut *et al.*, (1999) investigated population subdivision at 7 microsatellite loci among 8 populations. All populations exhibited geneflow (Nm>4) over an 1100km radius. Within regions panmixia was greater among northern populations (Rst=0.019, Nm=12.91), over distances of 550km, while Southern populations were more heterogeneous (Rst=0.031, Nm 7.81). Microsatellite data therefore supported low levels of population differentiation detected in previous studies.

Work on microsatellites in Triatominae has been limited to three published studies. Triatominae have holocentric chromosomes, normally with a diploid complement (*R. prolixus* 2n= 20A + XX\XY). Harry *et al.*, (1998) described microsatellite loci in *R. pallescens* from 36 palm trees in Colombia. Ten microsatellite primers were developed and the number of alleles per locus ranged from 2 to 20, with expected heterozygosity ranging from 0.32-0.94. All but four of the microsatellite loci were in frequencies expected under Hardy Weinberg equilibrium. These primers were also tested for successful amplification in the related species *R. prolixus* (two loci amplified) and *R. ecuadoriensis* (six loci amplified). All loci failed to amplify in *T. infestans*. Anderson *et al.*, (2002) developed eight microsatellite loci for *T. dimidiata* populations from Guatemala, Honduras and Mexico. Six to 27 alleles per locus were identified, but further population analysis was not carried out due to the small sample size used. Garcia *et al.*, (2004) isolated 30 microsatellite loci for *T. infestans* from a partial genomic library. Ten loci were tested, on the basis of clear peak resolution, in the analysis of 34 domestic/peridomestic specimens of *T. infestans* from Argentina. Alleles detected per locus varied in populations from 5 to 19, with observed heterozygosity from 0.242 to 0.938. Four of the ten loci analysed exhibited heterozygote deficiency ($P<0.01$-0.001). This may be evidence of population subdivision or due to the failed amplification of heterozygotes (null alleles). Amplification in the related species *T. guasayana* and *T. sordida* was successful at five and two loci respectively.

2 Chagas disease in Venezuela

Since it was first described in Venezuela, in 1919, Chagas disease has been increasingly recognised as a major cause of morbidity and mortality. It ranked within the first five major causes of death in the country for three decades (Ache & Matos 2001). Vector control was executed early in Venezuela, with funds available from the rich petroleum based economy and expertise from anti-malarial campaigns, reducing the impact of the disease, such that by 1980 Chagas disease fluctuated between 13th and 18th position in annual death rates. However, despite four decades of control active transmission still occurs in persistent endemic areas. Given the relative rapidity with which transmission has been interrupted in the Southern Cone it has been suggested that localised control failures or reinvasion of silvatic species of the main domestic vector, *R. prolixus*, could be maintaining transmission (Feliciangeli *et al.*, 2003).

2.1 Geographical distribution

The Chagas endemic area in Venezuela is limited to the piedmonts of the Andean Cordillera and the northern and central mountain ranges 1-500m above sea level, with limited foci also occurring up to 2500m above sea level. This region covers 101,488 km^2 (11.1% of the national territory) and encompasses areas of 12 States: Barinas, Portuguesa, Lara, Cojedes, Yaracuy, Falcon, Carabobo, Guarico, the Federal District, Anzoategui and Monagas (Ache & Matos 2001) (see Figure 8 on page 70). Low levels of transmission also occur in the llanos and coastal plains consisting of approx. 263,512km^2 (Ache & Matos 2001, Feliciangeli *et al.*, 2003). Vector transmission has not been reported in the States of Amazonas, Bolivar or Delta Amacuro, although the main vectors have been recorded, (Ache & Matos 2001, Ramirez-Perez 1987).

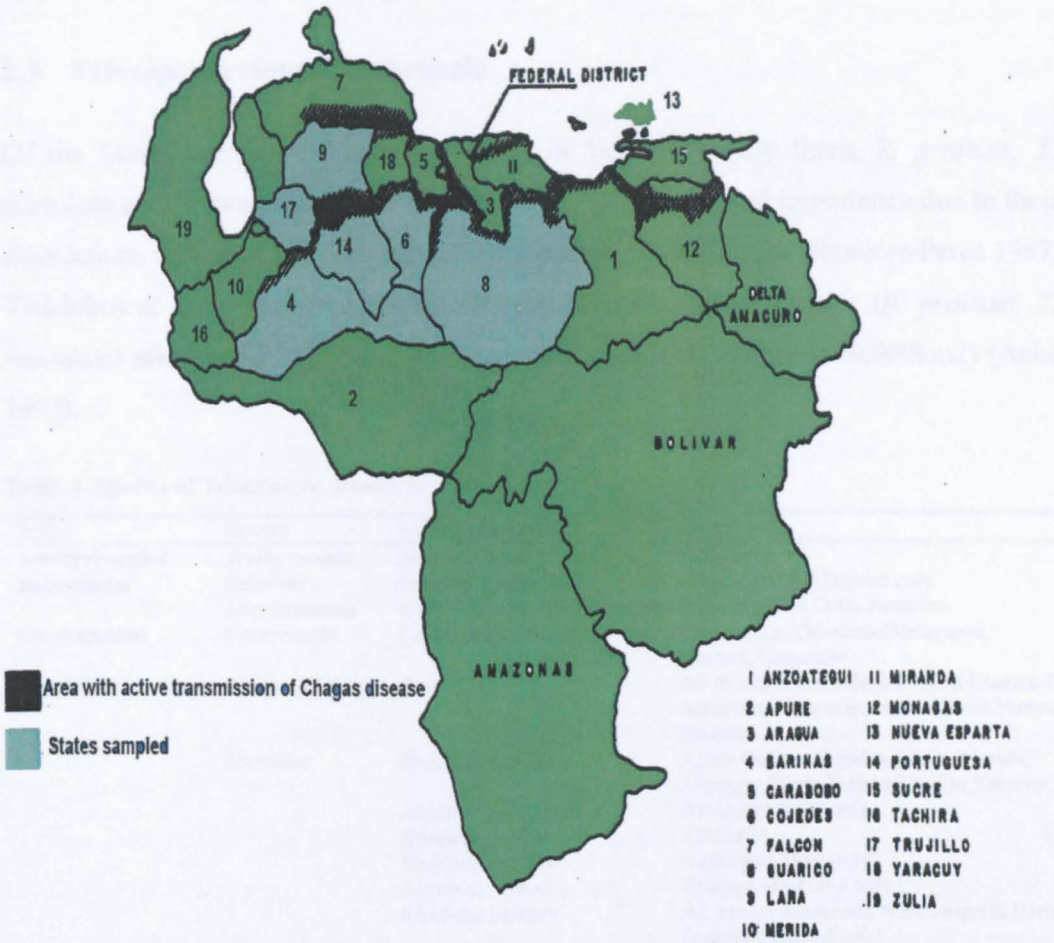

Figure 8. Map of Chagas endemic area in Venezuela, adapted from Ache & Matos (2001).
States: Barinas (4), Cojedes (6), Guarico (8), Lara (9), Portuguesa (14) and Trujillo (17) have been sampled for this study.

2.2 Epidemiology

In Venezuela it is estimated that 6 million people are at risk from Chagas disease (WHO 2002b). Transmission is primarily rural and vector based with screening of all blood donors mandatory in private and public blood banks since 1977 (Ache & Matos 2001, Feliciangeli *et al.,* 2003). Transmission often occurs where poor people move into

endemic foci in these mountainous regions often to sustain a living through the cultivation of coffee and other agricultural goods.

2.3 Principal vectors in Venezuela

Of the twenty species currently described in Venezuela only three, *R. prolixus, T. maculata* and *P. geniculatus*, are considered of epidemiological importance due to their associations with man and their role in the transmission of *T. cruzi* (Ramirez-Perez 1987, Villalobos *et al.*, 1994) (see Table 2 below). The two major species (*R. prolixus, T. maculata*) are found in 59.9% of the total territory of the country (549,000km2) (Ache 1993).

Table 2. Species of Triatominae present in Venezuela.

Tribe	Genera	Species	States
Alberproseniini	*Alberprosenia*	*Alberprosenia goyovargasi*	Zuila only
Bolboderini	*Belminus*	*Belminus rugulosus*	Aragua Federal District only
	Microtriatoma	*Microtriatoma trinidadensis*	Sucre, Federal Delta Amacuro
Cavernicolini	*Cavernicola*	*Cavernicola pilosus*	Cojedes,Lara,Miranda,Portuguesa, Tachira,Amazonas
Rhodniini	*Psammolestes*	*Psammolestes arthuri*	All except Zulia,Falcon,Federal District, Delta Amacuro,Amazonas,Bolivar,Sucre,Nueva Esparta
	Rhodnius	*Rhodnius robustus*	Apure,Barinas,Cojedes,Falcon,Merida, Monagas,Sucre,Tachira,Trujillo,Yaracuy
		Rhodnius pallescens	Amazonas State only
		Rhodnius prolixus^	All States
		Rhodnius brethesi	Amazonas State only
		Rhodnius neivai	Falcon Lara Zuilia only
		Rhodnius pictipes	All except Amazonas Nueva esparta Barinas, Guarico Fedral district
Triatomini	*Eratyrus*	*Eratyrus cuspidatus*	Anzoategui,Aragua,Falcon,Sucure, Trachira,,Zulia Trujillo,
		Eratyrus mucronatus	All except Apure,Amazonas,Delta Amacuro, Anzoategui,Miranda,Federal district,Nueva Esparta
	Panstrongylus	***Panstrongylus geniculatus^***	All except Barinas,Apure, Nueva Esparta
		Panstrongylus lignarius	Portuguesa only
		Panstrongylus rufotuberculatus	All except Merida,Miranda,Bolivar, Apure, Barinas Anzoategui ,Nueva Esparta
	Triatoma	*Triatoma dimidiata*	Bolivar,Carabobo,Cojedes,Falcon,Yaracuy, Federal district, Federal Delta Amacuro
		Triatoma nigromaculata	All except Zuila,Falcon,Apure,Guarico,Nueva Esparta,Amazonas,Miranda,Carabobo
		Triatoma maculata^	All States
		Triatoma rubrofasciata	Anzoategui, Aragua only

Important vector **species^** (Ramirez Perez 1987)

2.3.1 *Rhodnius prolixus* Stal 1859

Rhodnius prolixus is considered the main vector of Chagas disease in Venezuela, due to its wide distribution (all States), its considerable ability to invade and colonise rural houses and, importantly, due to its vectorial efficiency. In Venezuela *R. prolixus* has been found from above sea level to 2000m, in a variety of biomes including tropical woodlands and mountain humid forests (Carcavallo *et al.*, 1978, Ramirez-Perez 1987). It is reported in 79.1% of municipalities (Ache 1993).

Prior to 1961 this species was believed to be primarily domestic, and therefore amenable to control. However Gamboa (1961) demonstrated its presence in a variety of silvatic ecotopes including dry trees, nests of the bird *Mycteria americana* (wood stork) and also in a diversity of palm species. Fifty-two of 101 palm trees dissected being positive and yielding 331 specimens of *R. prolixus.* Gamboa emphasised the importance of the silvatic cycle of this species.

These findings stimulated extensive research on these silvatic populations throughout all endemic States on the basis that effective vector control required an understanding of their ecology and relationship to domestic populations. Important aspects investigated included variety of habitats infested, food sources and levels of *T. cruzi* infectivity of these silvatic specimens. Migration between the silvatic and domestic ecotopes was also studied. These investigations were carried out in numerous States including Portuguesa, Barinas, Apure, Anzoategui, Carabobo, Cojedes, Falcon, Guarico, Lara, Monagas, Sucre, Trachira, Yaracuy, Zulia and a range of biomes such as dry tropical forest, humid tropical forest and savannah (Gamboa 1970, Carcavallo *et al.*, 1978, Tonn *et al.*, 1976a, Pifano 1973, Gomez-Nunez 1963, 1969). The methods used included palm dissection, baited traps, light traps and mark, release-recapture studies. These studies confirmed the existence of silvatic populations and it was soon realised that *R. prolixus* occurred in two very different ecotopes. Some researchers argued that these extensive populations would make control of Chagas disease extremely difficult due to possible reinvasion from silvatic ecotopes after insecticide control (Carcavallo *et al.*, 1978, Pifano 1973).

In these studies the palm tree was revealed as a very important habitat, suggested as the primary ecotope from where the species moved to other secondary habitats such as birds nests and rural houses (Gamboa 1970, Pifano 1973). The palm is an important ecotope for most species of the genus *Rhodnius* with a few exceptions e.g. *Rhodnius domesticus,* and for which they are highly adapted e.g. climbing organs and coloration (Gaunt & Miles 2000). Of the 102 palm species present in Venezuela (Fred Stauffer, pers. communication) 24 were examined, primarily though laborious dissection, and 8 species were found to harbour colonies of *R. prolixus* and other silvatic triatomine species (see Table 3 below).

Table 3. Palm species found infested with *R. prolixus* in Venezuela

Species Name	Common Name
Acrocomia aculeata	palma corozo
Attalea butyracea	palma yagua
Copernitia tectorum	palma llanera
Leopoldinia piassaba	palma chiquechique
Mauritia flexuosa	palma moriche
Oenocarpus bataua	palma seje
Sabal mauritiiformis	palma redonda
Syagrus orinocensis	palma coroba

(Gamboa 1970, Tonn *et al.*, 1976a)

Associations between species type and infestation varied with areas sampled and also numbers of palms available for dissection, so while Gamboa (1961) found a greater number of *Attalea butyracea* infested, studies carried out by Carcavallo *et al.*, 1978 found *Acrocomia aculeata* on average more infested, followed by *Mauritia flexuosa, Sabal mauritiiformis, Copernitia tectorum* and lastly by *A. butyracea.* However, certain species, due to their wide distribution and common infestation were regarded as important ecotopes in particular *A. butyracea* (palma yagua), also commonly used in house construction (Gamboa 1970).

Bug density levels also varied between palm species, this led to the suggestion that the structure of certain palm species influenced these levels by providing greater protection from predators and more suitable refuges for mammals (Gamboa 1970). However, bug levels were also found to demonstrate seasonal bias, with larger numbers occurring in

May at the start of the rainy season when egg exclusion is greatest and when increased number of nesting mammals are found due to ground habitat flooding (Pinero & Torrealba 1977). Overall, relatively low populations numbers, 3 to 18.7 bugs, have been detected in the silvatic environment, due to long feeding cycles and high predatory pressures (Gamboa 1970, Carcavallo *et al.*, 1978). Population distribution within the palms was vertical and age structured, with eggs and nymphs, which are more abundant and require higher humidity, occurring from the superior third of the palm between the bases of the leaves and the trunk where water retention is higher, while adults were found in the crown (Pifano 1973, Gamboa 1970, Gomez-Nunez 1963). Eggs were found individually or in small groups glued to the barbs of the leaves, suggesting that domestic colonisation followed the use of these egg infested palm leaves in roof construction (Gamboa 1970, Gomez-Nunez 1963).

Infection rates of palm dwelling populations with *T. cruzi* in several States ranged from 12.5% to 26.6% of specimens examined, although high levels of 47.5% and 77% were also noted (Carcavallo *et al.*, 1978, Pifano 1973). Precipitin tests used to investigate the major food sources of 278 palm captured *R. prolixus*, identified the blood of a number of mammals, in particular *Caluromys philander* (24.5%) (the bare-tailed woolly opossum), *D. marsupialis* (23.7%) (the common opossum) *Rattus rattus* (4.3%) (the common rat) *Oecomys concolor* (12.2%) (arboreal rice rat), birds (5.8%) and lizards (7.9%), mixed bloodmeals were also identified (Pifano 1973, Carcavallo & Tonn 1985).

Mobility studies were carried out by Gomez-Nunez (1969) using radioactive isotopes. Migration was detected between palms and houses but not vice versa. It was suggested that due to high predatory pressures direct infestations of houses by actively migrating silvatic specimens was a threat only when highly infested palms were in close proximity. It was concluded that infestation was primarily passive following the use of infested palm leaves for roofing (Gomez-Nunez 1969). However flight studies on *T. infestans* have indicated that bugs can actively fly over larger distances than detected by Gomez-Nunez (1969), and it has been suggested that the weight of the marker may have impaired the flight ability of marked bugs (Schofield *et al.*, 1992). Studies have found

that active flight in Triatominae is associated with nutritional status, as well as wind and ambient temperature (Schofield *et al.*, 1992, Lehane *et al.*, 1992). In a 12 month study in Venezuela using light traps *R. prolixus* was the most frequent species collected and numbers collected increased during the wet season (Tonn *et al.*, 1978a), so flight patterns may be seasonal.

A recent study was undertaken on risk factors for house infestations in Barinas State in Venezuela based on a survey of 552 houses and 1068 peridomestic outbuildings (chicken huts, cow sheds, pigsties) in 18 villages (Sanchez-Martin *et al.*, 2004, 2005). This study determined that the risk of a house been infested was just significantly higher when within 100m of 10 or more palm trees (p=0.05, p=0.07 for colonisation indicating nymphs present), while infestation and colonisation of outbuildings was highly positively associated when within 100m of 5 or more palm trees (p=0.008, p=0.006), in particular the palm species *A. butyracea* (p-value=0.006). House infestation and colonisation was also significantly associated with the density of bugs in the peridomestic area (p-value=0.05, 0.005). The study found that 75% of houses were located within 100m of a palm tree (92% of which were *A. butyracea*), and 49% had greater than 10 palms within that distance. The average minimum distance from a house to a palm in the study was 27m. In this study *R. prolixus* were collected in 89 of 119 palms surveyed (75%) (Sanchez-Martin *et al.*, 2005). These data suggest that palm dwelling *R. prolixus* within 100m of houses may be invading and colonising the domestic environment, as may peridomestic specimens.

Controversy arose as to whether the palm tree really is a silvatic ecotope for *R. prolixus* when Lent and Valderrama (1973) recorded the presence of the morphologically similar species *R. robustus* in palms in an area between Merida and Zulia States. Its wide distribution was later described by Tonn *et al.*, (1976b) and this led to the debate, which has continued to today, as to whether all palm dwelling *R. prolixus* are in fact *R. robustus*, and therefore of no threat to control, although the *R. robustus* is attracted to light and may be responsible for sporadic cases of Chagas at least in western Venezuela (Feliciangeli *et al.*, 2002, Herber & Kroeger 2003).

Other silvatic ecotopes included the nests of numerous bird species in particular *Jabiru mycteria* (the Jabiru stork), also *Cercibis oxycerca* (the sharp-tailed ibis) (Gamboa 1970).

In houses *R. prolixus* populations encounter conditions similar to the silvatic ecotope but more favourable, with constant food supplies and few natural predators, thereby allowing large populations to be attained. In this environment specimens are frequently found in beds, clothes, boxes, resting in cracks of walls and in the palms of the roof (Carcavallo & Tonn 1985). Studies showed that houses with bahareque walls (mud and wattle), earth floors and zinc roofs or palm roofs are more likely to be infested, with palm roofed houses maintaining much larger populations (Gamboa & Perez-Rios 1965, Tonn *et al.*, 1978b). When palm roofs were replaced by zinc, a reduction in the number of houses infested was noted (Gamboa 1973). These studies indicate that the presence of a palm roof plays an important role in domestic infestations. However, Sanchez-Martin *et al.*, (2004, 2005) found the presence of a palm roof as opposed to a metal roof was positively associated with house infestation only when the palm roof was under 1 year old (p value=0.01). This indicates that bugs may be moved passively into the domestic environment via eggs glued to palm leaves as previously believed (Gomez-Nunez 1969). Inhabitants in this study (Sanchez-Martin *et al.*, 2005) also noted that *R. prolixus* bugs would appear in the house after the roof was thatched with palm leaves. Houses in this study region were more commonly infested with *R. prolixus* (15.6% in houses, with 5.5% showing signs of colonisation) with a geometric mean of 2.3 bugs inside houses, while *T. maculata* was collected more frequently in the peridomestic environment (4.3% in houses, with 2.5% colonisation) (Sanchez-Martin *et al.*, 2004, 2005).

2.3.2 *Triatoma maculata* Erichson 1848

This species in Venezuela is considered secondary in importance to *R. prolixus* in terms of transmission of *T. cruzi* (Tonn *et al.*, 1978c). It has a wide distribution, is found in all States, primarily in the llanos and coastal areas occurring up to 500m above sea level, but is uncommon in mountainous areas (Tonn *et al.*, 1978c). This species has an

overlapping distribution with *R. prolixus* and studies have found them co-inhabiting both domestic and silvatic ecotopes (Gamboa & Perez Rios 1965, Pinero & Torrealba 1977, Gamboa 1970 Sanchez-Martin *et al.*, 2004, 2005). However *T. maculata* has been considered predominantly an ornithophilic species, found infesting the peridomestic area (Tonn *et al.*, 1978c).

In the silvatic environment the species is most frequently found under the bark of dried trees. Other ecotopes include several species of palm in particular of the genera *Acrocomia, Copernitia, Sabal* and *Scheelea,* where they are often found with *R. prolixus* and *R. robustus,* in bird nests including *M. americana* and in animal burrows of rodents (Tonn *et al.*, 1978c, Gamboa 1970). Levels of *T. cruzi* infection in silvatic specimens have been found to range from 1.4% to 13.15% and bloodmeal analysis has indicated a tendency to feed on birds (Pifano 1973).

Its importance as a domestic vector varies. Many studies have reported a limited occurrence of *T. maculata* within houses, with only 2 houses in 7,630 investigated in Merida infested with the species, others have reported 4.5% of houses investigated infested in the same State (Gamboa 1963, Gamboa & Perez Rios 1964). In Guarico, in one study 9.2% of houses were found to harbour *T. maculata* but infestations in 29.6% of investigated houses have also been described, however, in this study a greater number of specimens were found in the peridomestic area (Gamboa & Perez Rios 1964, 1965, Tonn *et al.*, 1978c). In the domestic environment *T. maculata* specimens have been found in the roof and walls; other important refuges include clothes, boxes and beds (Tonn *et al.*, 1978c, Gamboa & Perez-Rios 1965). In general, *R. prolixus* appears to be a more efficient coloniser and, in domestic infestations with both *T. maculata* and *R. prolixus, T. maculata* are often found resting in different areas of the house to *R. prolixus,* indicating a degree of competition (Gamboa & Perez-Rios 1965). However, it has been found that the type of roof, palm or zinc, plays a role in this (Gamboa 1970, Gamboa & Perez-Rios 1965). Even when specimens of *T. maculata* have been found, the level of infection with *T. cruzi* has been low, with 0.98% to 3.3% of specimens infected in different studies, and bloodmeal analysis has shown a limited tendency to

feed on man, with birds and other mammals appearing as more common food sources (Tonn *et al.*, 1978c). However, infestations in the peridomestic area are common and extensive, most frequently in association with the nests of chickens and pigeons but also in firewood and posts (Tonn *et al.*, 1978a, Gamboa 1970). Surveys have shown, through bloodmeal analysis, that specimens in this environment also feed primarily on birds (Tonn *et al.*, 1978c), resulting in the low level of *T. cruzi* detected. Sanchez-Martin *et al.*, 2004, 2005) found infestation and colonisation of the domestic environment by *T. maculata* in Barinas State to be significantly associated with the presence of nests inside houses (p-value=0.002, 0.001), this was also true of the peridomestic area with infestations occurring in only 3/524 outbuildings surveyed without nests.

It appears from studies that *T. maculata* is a silvatic species with a tendency to colonise domestic and peridomestic habitats; it has been suggested that it is in the process of adapting to the human environment (Gamboa 1970, Tonn *et al.*, 1978c), and has importantly been reported to replace *R. prolixus* populations that have been eliminated from the domestic environment through insecticide control (Gamboa 1963, 1970, Gomez-Nunez 1963). Therefore its presence in large numbers in the peridomestic environment is a threat to Chagas disease control.

2.3.3 *Panstrongylus geniculatus* Latreille 1811

This primarily silvatic species has a wide geographical distribution, known from the Federal districts and all States in Venezuela except Apure and Barinas (Ramirez-Pirez 1987). Silvatic ecotopes include burrows of the armadillo *D. novemcinctus*, with which it is often associated (Pifano 1969, Lent & Wygodzinsky 1979), the burrows of other small animals such *as R. rattus* and under the bark of dried trees (Gamboa 1970, Tonn *et al.*, 1978c). However, this species is light-attracted and starved adults are found to invade the peridomestic and domestic environments to feed on man or his domestic animals (Gomez-Nunez 1963, Gamboa 1970, pers. observation), introducing *T. cruzi* from the silvatic environment in to the domestic cycle (Pifano 1969, Lent &

Wygodzinsky 1979). Pifano (1969) reported accounts of *P. geniculatus* feeding on children.

A recent study in Venezuela indicates that *P. geniculatus* not only repeatedly enters the domestic environment but successfully feeds on inhabitants and could be in the process of domestication (Carrasco *et al.*, 2005, Feliciangeli *et al.*, 2004). Eighty-eight *P. geniculatus* adults were collected at night in houses in the Metropolitan district, including the capital Caracas, and Miranda and Vargas States. Residents reported that they were bitten. More than 70% of these bugs were found to be infected with *T. cruzi I* and 60.2% of the bugs gave positive reaction to the human antiserum (40.9% of which were positive to *T. cruzi*). Importantly one householder from the capital Caracas collected, in addition to adults, a batch of eggs and nymphs. Two fatalities from acute Chagas disease have been recorded in Caracas recently (Carrasco *et al.*, 2005)

Troublingly, there have been increased reports of the presence of peridomestic and domestic colonies of *P. geniculatus*, in pigsties in Brazil (Valente *et al.*, 1998) and in houses in Venezuela (Reyes-Lugo & Rodriguez-Acosta 2000, Feliciangeli *et al.*, 2004, Carrasco *et al.*, 2005). In Venezuela, a domestic colony was reported in Miranda State (3 adults and 17 nymphs) found in a cavity within the house in association with *R. rattus*. Bugs were also reported feeding on children and the examination of faeces indicated the presence of *T. cruzi* (Reyes-Lugo & Rodriguez-Acosta 2000). In three houses in Lara State 1 adult and 10 nymphs were found coinfesting with *R. prolixus*. One *P. geniculatus* nymph positive to human antisera was found infected with *T. cruzi* I (Feliciangeli *et al.*, 2004).

From these studies it is obvious that *P. geniculatus* may pose a serious threat to disease control in Venezuela in the future. Studies are required to understand the stimulus for domestic invasion, Carrasco *et al.*, (2005) found more females than males and concluded that starvation and the need for blood for oogenesis may be the stimulus.

2.4 Transmission cycles

The **silvatic cycle** of *T. cruzi* in Venezuela is maintained by numerous silvatic species, including populations of the three main vectors, living in close association with mammalian reservoirs in a variety of ecotopes such as *P. arthuri,* which is associated with furnariid birds and *R. pictipes* which is found in palms of the genus *Attalea* in association with marsupials and rodents (Carcavallo *et al.,* 1978, Feliciangeli *et al.,* 2002). Important reservoirs in Venezuela identified by Pifano (1973) included *Didelphis, Caluromys, R. rattus,* with high percentage of infection found in *Didelphis* (86.53% infected) and *Caluromys* (43.23% infected). In this study Pifano concluded that *Didelphis* played an important role in the introduction of infection into the human cycle due to its abundance, its synanthropic behaviour, often foraging and nesting in human domestic areas and due to low but consistent levels of infection. Fifty-nine species trapped in Venezuela were examined for trypanosomes by xenodiagnosis. Tonn *et al.,* (1982) identified 15 species as hosts for *T. cruzi* in Venezuela *C. philander* (bare tailed woolly opossum), *C. lanatus* (western woolly opossum), *Monodelphis brevicaudata* (short tailed opossum), *Marmosa robinsoni* (pale-bellied mouse opossum), *D. marsupialis* (common opossum), *Tamandua tetradactyla* (lesser anteater), *D. novemcinctus* (nine banded armadillo), *Procyon cancrivorus* (crab eating racoon), *Sciurus granatensis* (red tailed squirrel), *Heteromys anomalus* (pocket mouse), *Proechimys semispinosus* (spiny rat), *Echimys semivillosus* (speckled tree rat), *Dasyprocta agouti* (agouti), *Coendu prehensilis* (porcupine) and *Cebus* (capuchin monkeys 2 spp). In this study *D. marsupialis* was the most important reservoir host for *T. cruzi* with 44.5% of specimens examined infected (Tonn *et al.,* 1982), as noted previously (Telford *et al.,* 1981). Other species harbouring and introducing infection into the domestic environment include *R. rattus,* (9.8% of specimens investigated) and *Mus musculus,* (1.6%) in various States in Venezuela (Tonn *et al.,* 1983).

The **domestic transmission cycle** is the most important cycle in relation to Chagas disease transmission. Housing with unhygienic conditions and constructed from natural materials, creates a niche similar to the natural ecotope and allows for the colonisation,

feeding and high procreation of infesting *R. prolixus* and *T. maculata* populations. Here infection cycles are mainly between humans and vectors, but dogs have also been demonstrated as an important influence in maintaining infection (Anon 1999a). Tonn *et al.,* (1983) studied *T. cruzi* reservoirs in the domestic environment in various States and found 7.4% of dogs infected with *T. cruzi* and 7.0% of cats; in other domestic animals *T. cruzi* was low or absent, 1 of 269 pigs examined was found to be infected, no goats examined were infected.

Interesting studies have been carried out in Venezuela on the association of house type and triatomine infestation. The traditional 'ranchos' consisting of earth floors, baharque walls (mud and wattle) and either palm or metal roofs were found to be the most likely to be infested. When these ranchos were further grouped in one study by roof type, (group 1 metal roof, group 2 palm roof), it was found that group 1 houses were more commonly infested, although a bias existed in this study as these represented 63% of all the houses investigated. However, greater densities of *R. prolixus* were found in group 2 (Gamboa & Perez-Rios 1965). It has been demonstrated that the replacement of palm roof with metal greatly reduces the numbers of *R. prolixus* infesting, however, *T. maculata* levels seem unaffected by this control measure, and for this species the levels infesting the peridomestic area are a greater influence on house infestations (Gamboa 1970, Tonn *et al.,* 1978c).

2.5 Control programme in Venezuela

The national Chagas disease control programme (CDCP) was officially established in 1966 with the aim of interrupting intradomestic transmission through the control of the main vector species *R. prolixus* and *T. maculata.* At this time national prevalence levels indicated that 500,000 people were infected, with prevalence rates up to 45% in rural endemic areas (Feliciangeli *et al.,* 2003). In 1965 entomological surveys indicated that infestations were present in 14,209 villages in an area covering 750,000 km2. Control was to be achieved through residual insecticide spraying, the improvement of rural housing and health education (Ache & Matos 2001, Feliciangeli *et al.,* 2003). Prevention

of transmission through infected blood transfusion began in 1977 when mandatory screening of all donated blood was established (Ache & Matos 2001); coverage is now 100% (Anon 1999a).

While the programme was established in 1966 efforts to control vectors began two decades earlier, in 1945, incorporated into the malarial control campaign as an indirect benefit to indoor spraying against malaria with DDT, but this insecticide proved ineffective against triatomines. Vector control activities increased when dieldrin and HCH were found to reduce substantially domestic and peridomestic populations of triatomine bugs and impact on Chagas disease transmission. The National Rural Housing programme was initiated to complement chemical control by providing loans to rural householders to replace the traditional 'rancho' with cement block, zinc roofed houses. A total of 443,522 of these houses have been built (Ache & Matos 2001, Feliciangeli *et al.*, 2003). However the program now focuses on improvement of existing houses (Feliciangeli *et al.*, 2003).

A national control programme for Chagas was established due to feasibility of control seen in these early years and due to impact made. The General Direction of Environmental Health and Sanitary Control (GDEHSC) runs the programme, which sets national objectives and allocates funding to States, the amount dependent on serological and entomological data submitted for the previous year (Sanchez-Martin 2002).

The national objectives for the control programme are as follows **1** the reduction of seroprevalence in the under 10 age class to less than 0.5%, **2** the decrease in *R. prolixus* infestation indices to less than 2% of sampled houses (with less than 20% of sampled localities having any infested houses) **3** to decrease *T. cruzi* prevalence in domestic *R. prolixus* to less than 0.5% (Feliciangeli *et al.*, 2003).

Activities of the control program are as follows;

1. Entomological surveys: The identification of infested houses via manual searches of the houses, contents and the peridomestic area. The triatomines collected are kept for

parasitological investigation for *T. cruzi* (Sanchez-Martin 2002, Feliciangeli *et al.*, 2003). The number of houses visited per year depends on the budget and villages inspected may include houses not previously visited, visited previously but not in the last 8 years, or where householders have brought samples of triatomines to the State office of GDEHSC and they are infected with *T. cruzi* (Sanchez-Martin 2002). In malaria endemic areas indoor spraying against malarial vectors is assumed to also control triatomine populations (Sanchez-Martin 2002).

2. Serological surveys: Blood samples are taken from children under 10 years in order to monitor trends in infection, and identify areas of transmission. Entomological and serological surveys do not always occur in the same area (Feliciangeli *et al.*, 2003).

3. House spraying: Positive houses (at least one bug) and all other surrounding houses and peridomestic sites in the area are sprayed with fenitrothion (Feliciangeli *et al.*, 2003, Sanchez-Martin 2002). Areas are not routinely revisited within 6 months to check control efficacy (Feliciangeli *et al.*, 2003).

4. House improvements: An established programme exists as discussed but since 1998 no improvements have taken place due to lack of funding (Sanchez-Martin 2002)

HCH was replaced with the organophosphate fenitrothion, following low levels of resistance detected (Nocerino 1972). A reduction in resources for control has resulted in a decrease in surveys and spraying activities, the number of municipalities surveyed has dropped from 110-150 per State per year in 1950-1980 to 15-18 per year 1990-1998 (Ache & Matos 2001). The priority of Chagas disease has dropped due to lower infection and infestation rates, but also with the re-emergence of malaria and outbreaks of haemorrhagic dengue (Feliciangeli *et al.*, 2003). However Venezuela joined the Andean pact in 1997 thereby committing itself to Chagas disease control with the aim of cessation of vectorial transmission by 2010 (WHO 1997, Anon 1999a).

2.5.1 Control outcome

Published data from the CDCP has looked promising including the reduction of the original endemic areas from 750,000 km2 to 365,000km2 and a reduction in the housing infestation index from 60-80% in 1958-1968, with approx. 30-50 bugs per house, to 1.6%-4% and approx. 3-4 bugs per house in 1990-1998 (Ache & Matos 2001). The overall national rural seroprevalence has declined from 44.5% in 1958-1968 to 9.2% in 1990-1998 (Ache & Matos 2001). In children under 10yrs the figures were 20.5% and 0.5% respectively (Ache & Matos 2001). However, recent reanalysis of CDCP data on a national and State level indicates that although a substantial reduction has occurred, transmission during the last 10 years could be increasing, with a significant increase in the seroprevalence of children under the age of 15yrs (Feliciangeli *et al.*, 2003) and while house infestation indexes have decreased the overall distribution of triatomines has not, with *R. prolixus* still present in 79.1% of municipalities (Ache 1993).

Differing results may be due to variation in sampling rate and endemic status of areas sampled and variation in the diagnostic methods used to confirm seropositivity over the decade. As Chagas confers lifelong seropositivity, prevalence data will include old and new cases, therefore the use of serological data is problematic (Feliciangeli *et al.*, 2003). Feliciangeli *et al.*, (2003) used recent age stratified seroprevalence data to overcome this bias and calculated retrospectively the average force of infection (FOI) for each year between 1945 and 1999. Interestingly this analysis showed that although the force of *T. cruzi* infection has not decreased in the last 20yrs, and included a slight increase in the 1990s. Age prevalence data indicated that infection rates in children under 10 between 1996-1999 exceeded the national target of 0.5% in certain States such as Portuguesa (1.3%), Barinas (0.9%) and Yaracuy (0.8%).

Trends in infestation prevalence depend on the sampling effort and its distribution in more or less endemic areas, which varies from year to year according to resources available (Feliciangeli *et al.*, 2003). Analysis by Feliciangeli *et al.*, (2003) of entomological indices of >250,000 houses between 1990-1999 using binomial

regression with logistic errors, including variables to account for these sampling biases found infestation rates have not decreased in the last ten years (Feliciangeli *et al.*, 2003). A significant proportion of previously sprayed villages were also found infested.

2.5.2 Problems facing effective control

While great progress has been made in control of Chagas disease, Venezuela has yet to achieve the same standards attained in the southern cone countries (established 1991). Thirty-six years after the start of the control program transmission of Chagas disease in Venezuela still persists. There are several possible explanations for the persistence of transmission, such as the lack of a vigilance phase, integral to the Southern Cone programme, so that residual populations in Venezuela go unnoticed. However, one area that strongly merits attention is the role of silvatic populations of the main vector species in Venezuela, *R. prolixus,* in maintaining disease transmission after control by invasion and colonisation of the domestic environment. This is in marked contrast with the southern cone vector, *T. infestans*, which is primarily domestic throughout most of its range, although the limited range of silvatic foci has recently been questioned (Noireau *et al.*, 2005). Therefore, to gauge correctly the control strategy required to eliminate domestic populations and transmission, it is necessary to study the degree of interaction between silvatic and domestic vector populations (Guhl & Vallejo 1999).

3 The *Rhodnius prolixus* and *Rhodnius robustus* enigma

This controversy relates to the species relationship between the highly synanthropic *R. prolixus*, the primary vector of Chagas disease in Venezuela, Colombia and parts of Central America, which is also said to have silvatic populations in palms in Venezuela and Colombia, and the morphologically similar *R. robustus*, a silvatic species of little medical importance found only in palm tree crowns from northern Brazil, the Guyana's and Venezuela to Colombia, and occurring sympatrically with *R. prolixus* over part of its range. The debate has been fuelled by conflicting outcomes of various studies investigating their possible conspecific status (Harry *et al.*, 1992, Harry 1993a, 1993b, 1994, Monteiro *et al.*, 2000, 2002, 2003). It has been suggested that *R. prolixus* is solely a domestic vector and all silvatic populations are *R. robustus*, from which it has been derived after colonisation in Venezuela in a manner similar to the domestication of *T. infestans* from silvatic populations in Bolivia (Schofield & Dujardin 1999). This implies that the control of *R. prolixus* throughout its range should be viable.

3.1 The genus *Rhodnius*

The tribe Rhodniini Pinto 1926 consists of two genera, *Rhodnius* Stal 1859 and *Psammolestes* Bergith 1911. The tribe is believed to be monophyletic, defined by synapomorphies including apical insertion of the antennae, the presence of distinct postocular callosities, modified basal struts of the male genitalia, presence of nitrophorins, which give the salivary glands a red hue (Lent & Wygodzinsky 1979, Ribeiro *et al.*, 1998, Soares *et al.*, 2000) and recently from mitochondrial and nuclear studies (Lyman *et al.*, 1999, Monteiro *et al.*, 2000, Marcilla *et al.*, 2001). The genus *Psammolestes* comprises three species associated with funariid birds and they are of little epidemiological importance (Schofield &Dujardin 1999).

The genus *Rhodnius* contains 14 species principally arboreal in habitat, often found infesting palms, hollow trees and epiphytic bromeliads and feeding on a variety of hosts including birds, marsupials and rodents (Monteiro *et al.*, 2003). The genus, which is

widely distributed in South and central America, also includes several synanthropic species, in addition to *R. prolixus,* which have adapted to the domestic and peridomestic environment and are important vectors of Chagas disease, such as *R. pallescens,* the primary vector in Panama, *R. ecuadoriensis,* of local importance in Ecuador and northern Peru (Monteiro *et al.,* 2000) and peridomestic species including *R. stali* in Bolivia, *R. neglectus* and *R. nasutus* in Brazil, which can also be of local importance and increasingly found where *T. infestans* has been removed through control (Dujardin *et al.,* 1999a).

Results from morphometric and isoenzyme studies have generally revealed consistent groupings in the genus *Rhodnius,* which exhibit a strong geographical structure separating the genus east and west of the Andes (Dujardin *et al.,* 1999b, Chavez *et al.,* 1999, Schofield & Dujardin 1999, Monteiro *et al.,* 2000). Recent studies using mitochondrial and nuclear analysis to determine species identity, taxonomic relationships and phylogeny of the genus support the monophyly of the tribe, while indicating a paraphyletic structure. Two main clades have been revealed by this method, a '*prolixus* group' clade containing (*R. prolixus, R. robustus, R. neglectus, R. nasutus*) together with *R. domesticus* and *R. neivai.* The second clade includes *R. pictipes, R. brethesi, R. ecuadoriensis* and *R. pallescens. Psammolestes* species clustered together but were seen to be close to the '*prolixus*' clade (Lyman *et al.,* 1999, Monteiro *et al.,* 2000, Stothard *et al.,* 1998, Schofield & Dujardin 1999). These genetic clusters are largely consistent with the hypothesis about evolution of the genus put forward by Schofield & Dujardin 1999, and broadly corresponding to prior isoenzyme and morphometric determinations, whereby the ancestral form of the genus, perhaps represented today by the widespread generalist *R. pictipes,* inhabiting the Amazonian – Orinoco basin followed three main adaptive radiations one northwest through the Andean cordillera into Colombia and southern Central America to give the *R. pallescens* and a southward cline into northern Peru to give *R. colombiensis* and *R. ecuadoriensis.* The second radiation northeastwards into the Llanos of Venezuela to give *R. robustus* and *R. prolixus* and southwards to the savannah areas in Brazil giving rise to *R.*

neglectus/R. nasutus/R. domesticus and a third radiation within the forests themselves to give rise to *R. brethesi* and *R. neivai.* The present geographical distribution thereby reflects their phylogenetic relationships and the hypothesis about the evolution of the genus (Schofield & Dujardin 1999).

3.2 Methods of identification and previous studies

The species of the genus *Rhodnius,* while similar in appearance, are generally distinguishable by the morphology of the male genitalia based on the shape and basal width of the median process of the pygophore (Lent & Jurberg 1969, Lent & Wygodzinsky 1979). However it has been found that for certain species of the genus this is not a discrete attribute with large series showing morphological overlap (Harry 1993a, Dujardin *et al.,* 1999b, Chavez *et al.,* 1999). Increasingly important as secondary vectors, *R. neglectus* and *R. nasutus,* although indistinguishable by isoenzymes (Solano *et al.,* 1996, Dujardin *et al.,* 1999b) can be separated by morphometrics, (Harry 1994, Dujardin *et al.,* 1999b), which combined with discrete distributions, allows for easier identification (Schofield 1994).

However, the separation of *R. prolixus* and *R. robustus,* has been problematic with several studies using both morphometric and isoenzyme techniques failing to detect intraspecific differences, resulting in questions on the validity of *R. robustus* as a taxonomic entity (Harry *et al.,* 1992, Harry 1993a, 1993b, 1994, Monteiro *et al.,* 2002). The basis for the establishment of the species was questionable with the initial description based on two specimens larger and darker than the rest of a series; although later reaffirmed by genital structures, the shape of the basal plate struts of the aedeagus differ, this too has proven an unsatisfactory means of division (Monteiro *et al.,* 2003, Harry 1993a). In *R. robustus* the anteocular region is reportedly 4 times the post ocular region, while the distance between the eyes dorsally is larger than the with of the eyes in the dorsal view, this has not proved reliable. Others, however, have viewed apparent conspecific status as an indication of recent speciation, with isoenzymes, due to their conserved nature, unable to detect this genetic divergence (Solano *et al.,* 1996, Monteiro

et al., 2003). Studies using interspecies crossing experiments have added to the confusion, with reproductive compatibility demonstrated between populations indicating a single species (Barrett 1996, Solano *et al.*, 1996, Schofield & Dujardin 1999) but decreased fecundity has also been noted, signifying a degree of reproductive isolation (Galindez-Giron *et al.*, 1994). The use of misidentified samples has also been suggested as an explanation, with the lack of intraspecific differences in studies due to same species comparisons using laboratory colonies of uncertain identity and origin. (Garcia *et al.*, 1998, Stothard *et al.*, 1998, Schofield & Dujardin 1999, Monteiro *et al.*, 2003). A study demonstrating isoenzyme differences between silvatic and domestic populations in Colombia added to the uncertainty but it was later shown that silvatic specimens used in comparisons with domestic *R. prolixus* populations represented a new species of *Rhodnius*, later named *R. colombiensis*, and found to be closely related to the *R. ecuadoriensis – R. pallescens* grouping (Lopez & Moreno 1995, Chavez *et al.*, 1999, Dujardin *et al.*, 1999b, Moreno *et al.*, 1999).

DNA based methods, for example RAPDs and DNA sequencing, have been increasing utilized in triatomine systematics and have played a new role in assisting species determination (Monteiro *et al.*, 2001). Such techniques have been applied to the *R. prolixus/robustus* question, with results not only supporting the validity of *R. robustus* as a taxonomic entity but also indicating strong genetic variation within this species, suggesting that *R. robustus* might consist of more than one cryptic species (Lyman *et al.*, 1999, Monteiro *et al.*, 2000, 2001, 2003). Genetic differences between the species were initially identified from the inspection of RAPD profiles (Garcia *et al.*, 1998). Genetic divergences akin to species comparisons with *R. neglectus* were demonstrated (6.8%, 27 of 399bp in mt*cytb*) in a preliminary study based on the sequence analysis of two mitochondrial genes (mtlsurRNA 383bp and mt*cytb* 399bp), thereby supporting the validity of the *R. robustus* taxon (Lyman *et al.*, 1999). The results of this study were later confirmed, as part of a wider phylogenetic study of the tribe Rhodniini, using larger sample sizes and including a fragment of a nuclear gene (D2 28S RNA gene 632bp) to compare phylogenies (Monteiro *et al.*, 2000). Interestingly the *R. prolixus* samples

analysed, although from several countries, were found to be very homogenous, while *R. robustus* samples primarily from Brazil were heterogeneous, exhibiting greater variability with a higher degree of genetic structuring (Monteiro *et al.*, 2000). This study also identified silvatic *R. prolixus* from Brazil, contradicting the view that all silvatic specimens are *R. robustus*. The populations of this study were later re-analysed using alloenzymes at 12 loci; no intraspecific differences were identified, indicating the limited applicability of this technique (Monteiro *et al.*, 2002).

An in-depth study of these taxa was implemented by the same author to investigate further the phylogeographic structure and the heterogeneous nature of *R. robustus*. Sequence analyses (663bp of mt*cytb* gene, 630bp D2) of 26 populations from 7 countries yielded 21 unique haplotypes and indicated that *R. prolixus* and *R. robustus* are closely related but separate species (Monteiro *et al.*, 2003). This study confirmed the homogeneity of *R. prolixus*, three haplotypes were recovered but low levels of nucleotide diversity were detected (π =0 within regions π = 0.0008 between), while the *R. robustus* taxon was found to be a paraphyletic assemblage of several haplotypes with deep clades indicating long divergence times and the possible existence of a species complex.

The phylogenetic tree produced consisted of five geographically structured monophyletic clades, one clade composed of *R. prolixus* samples and four clades (I – IV) *R. robustus*, with clade I representative of Venezuelan samples and clades II-IV Amazonian. The paraphyly of the *R. robustus* taxon was evident by the more proximate clustering of clade I with *R. prolixus* samples (3.3% mean sequence divergence), than to the other Amazonian clades (7.4% mean sequence divergence). Divergence levels between the Amazonian clades ranged from 2.3% (II and IV) to 4% (II and III). These divergence levels within the *R. robustus* samples studied indicated possible cryptic species and the need for a re-evaluation the *R. robustus* taxon. This study also confirmed the existence of silvatic specimens of *R. prolixus* in Venezuela, the validity of which was questioned following the discovery of silvatic *R. robustus* in the country (Gamboa 1963, 1970, Gomez-Nunez 1963, Lent & Valderrama 1973). Genetic differences

between the two species have also been demonstrated by the electrophoresis of salivary heme proteins, which also found *R. prolixus* to be less genetically variable (Soares *et al.*, 1998, 2000).

3.3 Implications for control

Distinction between *R. prolixus/robustus* has serious implications for control. While *R. prolixus* is an important vector and its eradication is the main aim of the Andean Pact and the Central American control programmes, *R. robustus* is thought to be of minor epidemiological importance, seemingly unable to colonise houses, e.g. in the Amazon, and it is responsible for only sporadic cases of disease. Therefore the existence of two morphologically identical species, with overlapping distributions is confusing for correct vector incrimination. Further complicating the issue is the existence of silvatic *R. prolixus* populations. While genetically distinct *R. robustus* populations may not represent a threat to control, reinvasion from these silvatic *R. prolixus* populations may require modification of control strategies. If these populations are, as the molecular data indicates, *R. prolixus,* controversy remains as to whether (a) these species continually invade substandard rural housing after control efforts and thereby represent a threat to effective control, or (b) have done so only infrequently in their evolutionary history, giving rise to a primarily domestic species. If reinvasion from silvatic populations commonly occurs then sustained control in Venezuela will be problematic. Control in areas where silvatic populations are nonexistent, e.g. Central America, is seen as increasingly possible with genetic studies showing limited genetic variability in populations of *R. prolixus* in this area.

4 Aims

To investigate, in the context of vector control strategies, the relationship between domestic and silvatic populations of *Rhodnius* in Venezuela using both geometric morphometrics and molecular analysis (direct sequencing and microsatellite analysis).

4.1 Objectives

4.1.1 Sample collection

- To undertake fieldwork for the collection of specimens of *Rhodnius* from silvatic and domestic ecotopes in Venezuela, in collaboration with the Ministry of Health, and through the use of live bait traps, palm dissection and active house searches.

4.1.2 Cytochrome b

- To clarify the identity of field collected silvatic populations of *Rhodnius* from palm trees in Venezuela and domestic populations via direct sequencing of mitochondrial cytochrome b (*cytb*).
- To identify common shared haplotypes between silvatic and domestic ecotopes and investigate the relationship between silvatic and domestic populations using F_{ST} indices of population division.
- To compare *cytb* and D2 sequence results to check for possible introgression.
- To analyse the genetic relationship of all determined haplotypes.

4.1.3 Microsatellite analysis

- To develop a panel of polymorphic microsatellite markers for *R. prolixus,* which have not hitherto been available.
- To employ these microsatellite loci in the analysis of heterogeneity between silvatic and domestic populations of *Rhodnius*.
- To compare microsatellite analysis with genetic analysis by cytochrome b.

4.1.4 Geometric morphometrics

- To investigate shape variation between silvatic and domestic populations of *Rhodnius* in Venezuela.
- To compare results with cytb and microsatellite analysis.

This project aimed to conclude whether there is continuity between silvatic and domestic populations and obtain a wider understanding of the relationship between these populations.

It was intended to assess the significance of these results for the control of Chagas disease in Venezuela, and to provide information that might allow for the design of more suitable control strategies.

5 Materials and Methods

5.1 Study area

Venezuela is in the north of South America and borders both the Caribbean Sea and the Atlantic Ocean. Land borders include Brazil to the South, Guyana to the East and Colombia to the West.

For the purpose of this study field work was carried in July 2001, October-November 2003 and September-December 2004, in the States of Lara, Portuguesa, Guarico Cojedes, Barinas, and Trujillo (see Figure 8 on page 70). Fieldwork involved the survey of silvatic, peridomestic and domestic ecotopes in localities of these States in collaboration with the GDEHSC State chiefs and field inspectors (see abbreviations page 25). Field sites were chosen with the field inspectors, based on recent inspection data and on the basis of recent spray records. The arrival of a community representative with bug samples and information on local infestations also played a role. Specimens from Barinas and Portuguesa were kindly provided by collaborators, Mrs M. Sanchez-Martin (LSHTM) and Prof Feliciangeli (BIOMED, Venezuela), and were also collected in the field.

Lara State is situated in north-western Venezuela. In Lara in 2000 two houses were found infested in mountainous localities (Guamarito and Salvador) but palm searches were negative. Interestingly, searches for palm dwelling *R. prolixus* in Lara State by collaborators have also proved fruitless (Prof. Feliciangeli pers. communication). During the extensive searches for silvatic *R. prolixus* in the 1970s silvatic infestations in palms were also not encountered in Lara State. A total of 24 specimens from this State were examined by direct sequencing, 17 by microsatellite analysis, and 19 by morphometrics.

In **Guarico** State collection sites were in 4 localities (El Sombero, El Manguito, Bravero, Ortiz) situated in the central flat plains of Venezuela (Los Llanos), dry lowland savannah. Here the predominant palm species was *C. tectoreum* (palma llanera). The houses inspected in Guarico were of higher quality with the traditional rancho replaced

in these areas by cement block structures as part of the National Programme for housing improvement in the 1960s. All houses inspected were negative and samples were isolated from palms only. A total of 21 specimens were analysed by direct sequencing, while 20 were analysed by morphometrics. Specimens were not analysed at microsatellite loci.

Portuguesa State is situated northwest in the central plains of Venezuela. In this State collections were made in foothill humid forest, interrupted by the small scale cultivation of maize, coffee and bananas in the localities Terronal, Palo Gacho, Laurianito, Casa Rena, Peña Negra, Palmarito (Municipality Araure) and Los Rastrojos (Municipality Sucre). In these sites the predominant palm species was *A. butyracea* (palma yagua). The houses primarily consisted of the traditional 'rancho', constructed of wattle and daub with palm and corrugated iron roofs. Specimens from San Bartolo, Santa Lucia (Municipo Ospino), El Mosquito, Qdra Negra (Municipality Sucre) and Morichal (Municipality Araure) were provided by a collaborator (Prof. D. Feliciangeli BIOMED). In Santa Lucia and San Bartolo there was much deforestation and land clearance for coffee growing, and palms were noticeable less abundant. Specimens originated in silvatic, peridomestic and domestic ecotopes. From Portuguesa a total of 287 specimens were analysed by direct sequencing, 21 by morphometrics and 243 by microsatellite analysis.

Cojedes lies in midwestern Venezuela. In this State three Municipalities were inspected (Falcon, Juan A. Bravo and Pao) however only a single house infestation was detected in the locality Las Queseras (Municipality Falcon). A single palm (*C. tectorum,* palm Ilanera) was dissected adjacent to the infested house and was found positive. A total of 46 specimens were analysed by direct sequencing, 21 by morphometrics and 48 by microsatellites.

In the Andean State of **Trujillo** in western Venezuela, domestic infestations are lower as houses are generally of higher quality, replaced in the national programme, but also due to lower nighttime temperatures and higher standards of living. One house was found

infested in Trujillo (Loma de Amarillo) much to the surprise of the GDEHSC personnel. It was a localised infestation in a bedroom attached to the house, consisting of palm roof and walls. Palms surrounding the house were negative with the exception of two 1st instar nymphs collected in a nest. Interestingly in this State many householders reported adults, arriving at night when the light was used, often during the rainy season, but not colonising houses. A householder in the locality Palma Real gave a single adult that had entered the house at night (the house was negative). A single palm was dissected in La Juventud and was found positive. In this area adult *Rhodnius* specimens, thought to be *R. robustus*, are known to enter houses at night to feed but not colonise (Herber & Kroeger 2003). A total of 27 specimens were analysed from this State by direct sequencing, including 3 specimens taken from the insectary at the Universidad de Los Andes, with 31 analysed by morphometrics (14 from the insectary). Twenty-six domestic specimens were analysed with microsatellites.

Barinas is situated in south-western Venezuela and all sampled localities were situated in Los Llanos where the predominant palm species was *A. butyracea* (palma yagua). Collaborators provided all but 11 specimens analysed from this State. Specimens from localities Cascabel, Guaranda, Laguna Hermosa, G. Paraguey, Parcelamiento, 19 Abril, and Rio Bravo II were provided by a collaborator (Mrs Sanchez-Martin). In these localities land usage was primarily for cattle farming and the cultivation of maize, yucca and bananas. Houses had walls of wood (most frequent) or cement blocks, with metal or palm roofs (Sanchez-Martin *et al.*, 2005). A total of 17 specimens from the localities Obispos, Santa Elena de Caramuca, San Isidero and Carreteron were collected in 2001-2003 by a collaborator (Prof. Feliciangeli). While an additional 11 specimens, 8 from the locality Carreteron and 3 from Acequita were collected on a field trip to Barinas in 2003. Housing and land usage was similar across all localities. A total of 146 specimens from domestic, silvatic and peridomestic ecotopes in this State were analysed by direct sequencing and 221 by microsatellite analysis. Only 18 adults were analysed by morphometrics, with morphometrics of donated specimens been undertaken in a separate project.

5.2 Sampling methods used

Silvatic collections were made with live bait traps, as used by Abad-Franch *et al.*, (2000), consisting of a small plastic container into which was placed a live mouse with bedding and food. The trap was sealed with a perforated screw-on lid and the outer surface was covered with double sided adhesive carpet tape. An overnight trapping system was used, with 4 to 6 palms typically sampled each night. Using an adjustable ladder the baited traps (4-5 per palm) were placed in the palm crown, close to the fronds. The next day traps were retrieved and any bugs attached to the tape were carefully removed with a forceps and placed in collection tubes noting date and place of collection, GIS coordinate data were also taken for some collection sites (see Figure 9 on page 98). Palm dissections, with consent of landowner, were also carried out to collect silvatic specimens, this methodology was used when a ladder was not available or palms were too high. The palm was cut at the base and cleared from the base up to the crown using a machete, removing and inspecting each layer (see Figure 9). All bugs collected were placed in collection tubes, noting date and place of collection.

Figure 9. Methods employed in the collection of silvatic specimens.
A-live bait trap, B-placement of trap in palm, C- palm dissection

Domestic collections were made by traditional search and capture method, without the use of irritant spray, using forceps and flashlights and without setting a time limit (see Figure 10 on page 99). Permission to search was obtained by oral consent and householders were usually willing to help. The householder would generally know if the house was infested. Walls, beds and roofs (when possible) were inspected, together with any boxes or sacks present in the house. Peridomestic searches of chicken huts and other shelters for domestic animals were also carried out. All bugs collected were placed in collection tubes, noting date and place of collection. All specimens captured were identified using the keys of Lent and Wygodzinsky (1979).

Figure 10. Typical housing conditions and method employed in the collection of domestic specimens. Note proximity of palm to house.

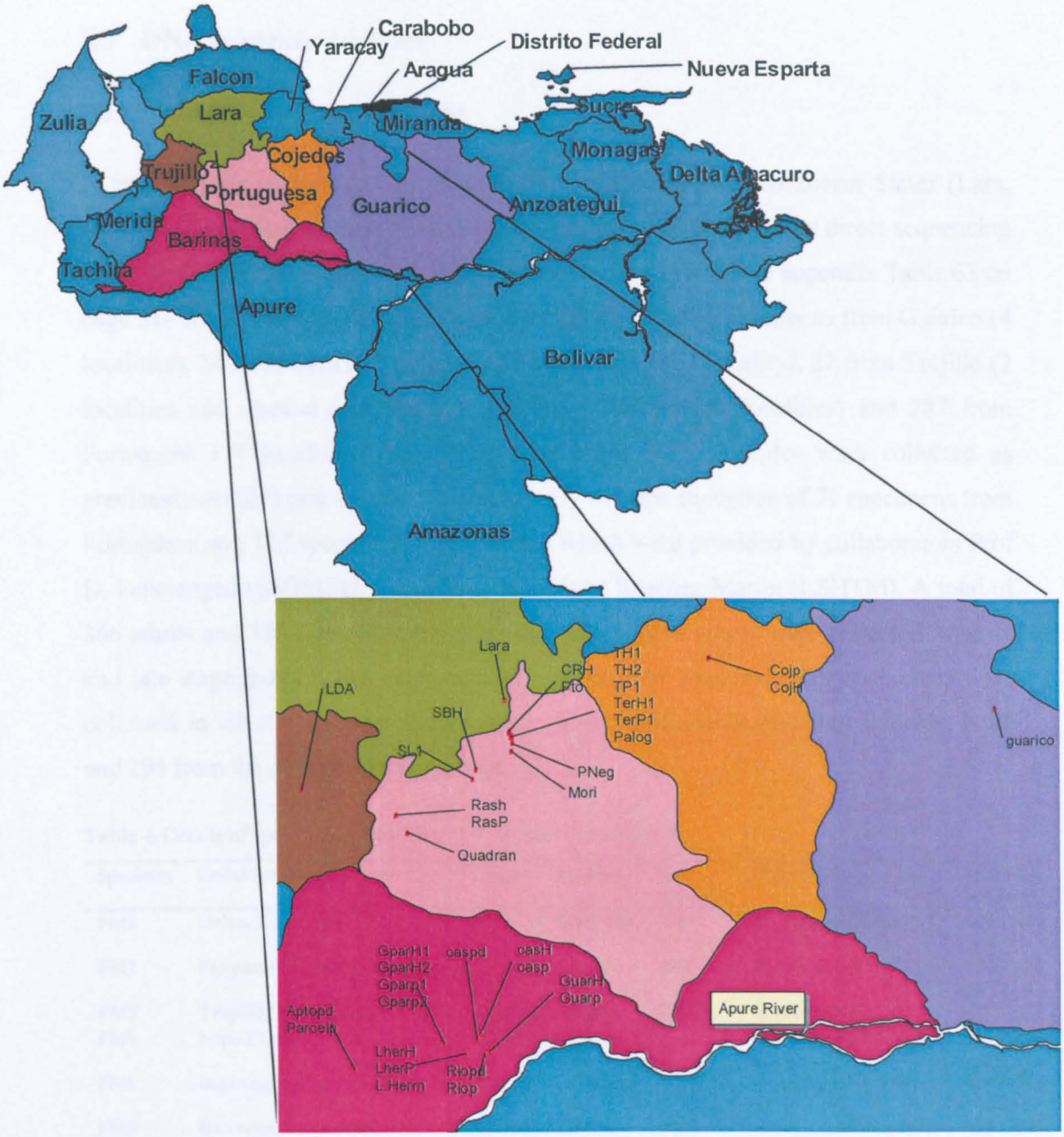

Figure 11. Map illustrating sample sites with available coordinates (courtesy of Martin Llewellyn).
Apto=19 Abril pd, Parcelp=Parcelamiento p, LherH= Laguna Hermosa h, LherP= Laguna Hermosa p, L.Herm=Laguna Hermosa pd, Caspd=Cascabel pd, cas H=Cascabel H, casp=Cascabel p, GuarH=Guaranda h, Guarp=Guarp, Riopd=Rio Bravo II pd, Riop=Rio p, Quadran= Qdra. Negra h, Rash=Los Rastrojos h, RasP=Los Rastrojos p, SL=Santa Lucia h, SBH=San Bartolo h, LDA=Trujillo, CRH=Casa Rena h, Pto= Palmarito, PNeg=Peña Negra, Mori=Morichal, Cojp=Cojedes p, Cojh=Cojedes h, TH1=Terronal h1 01, TH2=Terronal h2 01, TP1=Terronal h2 palm 01, TerH1=Terronal h1 03, TerP1=Terronal h2 palm 03.

5.3 DNA sequence analyses

5.3.1 Samples and fragment used

A total number of 551 specimens from 31 localities in six Venezuelan States (Lara, Guarico, Portuguesa Barinas, Cojedes and Trujillo) were analysed by direct sequencing of a fragment of the mitochondrial gene cytochrome *b* (*cytb*) (see appendix Table 63 on page 314 for full sample details). These samples included 21 specimens from Guarico (4 localities), 24 from Lara (2 localities), 46 from Cojedes (1 locality), 27 from Trujillo (2 localities and insectary specimens), 146 from Barinas (12 localities) and 287 from Portuguesa (11 localities) (see Table 5 on page 102). Samples were collected as previously detailed (see section 5.2 on page 97) with the exception of 71 specimens from Portuguesa and 137 specimens from Barinas which were provided by collaborators Prof D. Feliciangeli (BIOMED, Venezuela) and Maria Sanchez-Martin (LSHTM). A total of 366 adults and 185 nymphs were examined, 75 of these adults were collected as early and late stage nymphs that later moulted. Of the total examined 219 specimens were collected in silvatic ecotopes (palm trees), 38 in peridomestic ecotopes (chicken huts) and 294 from the domestic environment.

Table 4. Details of specimens provided by Dr. Monteiro (FIOCRUZ).

Specimen	Collection site	Origin	Ecotope	Date collected	Haplotype	Accession numbers*
FM1	**Orica, Honduras**	**Field**	**Domestic**	**1999**	***R. prolixus* Clade I^**	**AF421339 (prHO)**
FM2	**Panpanito,Trujillo, Venezuela**	**Colony**	**Silvatic**	**1997**	***R. robustus* Clade I^**	AF421340 (roVE1)
FM3	**Trujillo, Venezuela**	**Colony**	**Silvatic**	**1988**	***R. robustus* Clade 1^**	-
FM4	Napo Ecuador	Colony	Silvatic	-	*R. robustus* Clade II	AF421341 (roEC)
FM5	Itupirangan, Para, Brazil	Colony	Silvatic	1984	*R. robustus* Clade III	AF421342 (roBR4)
FM6	Barcarena, Para, Brazil	Colony	Silvatic	1996	*R. robustus* Clade IV	AF421343 (roBR7)
FM7	**Brazil**	**Colony**	**Silvatic**	-	***R. robustus* Clade IV^**	-
FM8	**Trujillo, Venezuela**	**Colony**	**Silvatic**	**1997**	***R. robustus* Clade I^**	-

Haplotypes^ found in our study. * labels in parenthesis are actual labels used in Monteiro *et al.*, (2003), haplotype description as used in Monteiro *et al.*, (2003).

Table 5. Summary of the Venezuelan samples used for *cytb* analysis

State	Locality	N[a]	Nymph[c]	Adult[c]	Habitat[b]	Year
Lara	Guamarito	22	9	8♀ 4♂ 1NI	H	2001
	Salvador	2	-	2♂	H	2001
Guarico	El Sombero	6	3	1♀ 2 NI	P	2001
	El Manguito	8	8	-	P	2001
	Bravero	3	3	-	P	2001
	Ortiz	4	-	1♀ 1♂ 2 NI	P	2001
Portuguesa	Terronal	142	68	41♀ 21♂ 12 NI	H/P	2001/2003
	Peña Negra	10	6	3♀ 1♂	H	2001
	Palmarito	5	5	-	H/P	2001
	San Bartolo	29	17	4♀ 6♂ 2 NI	H	2002
	Santa Lucia	17	7	7♀ 1♂ 2 NI	H	2002
	Casa Rena	17	5	7♀ 3♂ 2 NI	H/P/PD	2003/2001
	Qdra. Negra	12	-	3♀ 8♂ 1 NI	H	2002
	Palo Gacho	10	10	-	P	2001
	Morichal	5	-	5 NI	H	2002
	El Mosquito	8	1	1♂ 6 NI	H	2002
	Los Rastrojos	32	31	1♂	H/P	2004
Trujillo	Loma de Amarillo	21	8	5♀ 8♂	H	2003
	La Juventud	3	-	1♀ 1♂ 1 NI	P	2003
	Insectary	3	-	3♀	P	-
Barinas	S. E. Caramuca	2	-	2♂	P	2002
	Obispos	1	-	1♀	H	2001
	San Isidero	1	-	1 NI	H	2003
	Acequita	3	-	3 NI	PD	2003
	Carreteron	20	5	8♀ 3♂ 4 NI	H/P	2003
	Cascabel	32	12	12♀ 8♂	H/P/PD	2003
	Guaranda	17	10	3♀ 4♂	H/P	2003
	Laguna Hermosa	27	9	8♀ 10♂	H/P/PD	2003
	G. Paraguey	8	-	8♀	H	2003
	Parcelamiento	10	6	3♀ 1♂	H	2003
	Apto. 19 Abril	10	4	5♀ 1♂	PD	2003
	Rio Bravo II	15	7	5♀ 3♂	PD/P	2003
Cojedes	Las Queseras	46	27	6♀ 13♂	H/P	2004
Total	-	551	258	293	-	-

a No. of specimens analysed. b H= house, PD= peridomestic, P= palm C=Adult and nymph life stage as at point of analysis unless otherwise noted in appendix Table 63 on page 314, NI=adult sex unknown ♀ =female ♂ =male. See appendix Table 63 on page 314 for further details.

In addition 8 *cytb* nucleotide sequences (FM1-FM8) were provided by Dr F. Monteiro (FIOCRUZ, Brazil) (see Table 4 on page 101). Published sequence data for three outgroups, *R. pallescens*, *R. neglectus* and *T. infestans* used were taken from Genbank, accession numbers, AF045720, AF045716 and AF045721 respectively. A subset of 9 specimens characterised by *cytb* were also analysed by D2 to check for introgression between *R. prolixus* and *R. robustus* lineages.

5.3.2 Isolation and purification of genomic DNA

Total genomic DNA was isolated from 4-6 legs of dried or ethanol preserved specimens. For nymphs the whole insect was used, minus the abdomen in larger stages. Qiagen Dneasy^{Tm} extraction kits were used for extraction of high quality DNA following the manufacturer's protocol for the isolation of DNA from animal tissues. The legs were placed in a 1.5ml eppendorf and ground prior to extraction using a plastic micro-pestle and liquid nitrogen. DNA isolated was carefully labelled and stored at –20°C until further use.

5.3.3 PCR amplification

Standard PCR techniques were used to amplify an approx. 700bp fragment of the mitochondrial *cytb* gene using primers previously shown to amplify product in various *Rhodnius* species (Monteiro *et al.*, 2003). These primers were designed by comparisons of the conserved regions of the *cytb* gene of *T. dimidiata* (Dolston & Beard 2001) and other published insect *cytb* sequences (Monteiro *et al.*, 2003).

Forward primer *Cytb*7432F 5`-GGACG(AT)GG(AT)ATTTATTATGGATC

Reverse primer *Cytb*7433R 5`-GC(AT)CCAATTCA(AG)GTTA(AG)TAA

PCR amplification was preformed in a 50ul reaction mix containing 3.0µl of extracted DNA, 125pmol of each primer, 1 unit of *Taq* DNA polymerase (Bioline) 2mM of each dNTP, 1.5mM $MgCl_2$, 10mM TRIS-HCL pH 9.0, 50 mM KCL, 0.01% gelatin, 0.1% Triton X-100. Reaction conditions were as follows: an initial denaturation step at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 45 s, and extension at 72°C for 45 s, this was followed by a final extension step at 72°C for 5 min. Cycle amplification was performed on either a Primus 96 plus (MWG AG Biotech) or a PTC-100R (MJ research) thermal cycle sequencer.

Successful amplification was confirmed by running 5µl of PCR product in tandem with 5µl of HyperLadder IV (Bioline) on a 1% agarose gel (90V/30 mins) stained with

ethidium bromide and visualized under ultraviolet light. Amplified products were then purified using either a spin column format with QIAquick™ kit (Qiagen) or directly using Quick-clean™ (Bioline), using methods specified by the manufacturers. Purified products were rechecked by agarose gel electrophoresis.

When weak bands were detected reaction conditions were changed as follows: in the reaction mix DNA volume was increased (up to 8µl) and 1 unit of *Taq* Extender™ (Stratagene) was used with replacement 10X *Taq* Extender buffer. *Taq* Extender™ is a PCR *Taq* additive that improves reliability and yield of standard *Taq* based amplifications. The PCR cycling conditions were also changed; the annealing temperature was dropped from 50°C to 45°C.

In order to test the possibility of mtDNA introgression between the *R. prolixus* and *R. robustus* lineages, a selection of 9 specimens (previously characterised by *cytb* as *R. robustus* and *R. prolixus*) were also sequenced for a fragment of the D2 variable region of 28S RNA (Porter & Collins 1996, Monteiro *et al.*, 2003). An additional five D2 sequences were available in Genbank for comparison (Monteiro *et al.*, 2003, Lyman *et al.*, 1999, see Table 6 below for details). *Rhodnius neglectus* was used as an outgroup (AF435856). Haplotype prVE5 (AF435862) was amplified from 1 of 4 silvatic specimens from our study from the locality Ortiz in Guarico, (62-65 ortiz, see appendix Table 63 on page 314).

Table 6. Details of specimens from Genbank used in D_2 analysis

Specimen	Collection site	Origin	Ecotope	Date collected	*Cytb* Haplotype *	Accession numbers
prVE5	Guarico, Venezuela	Field	Silvatic	2001	*R. prolixus*^	AF435862 (62-65 ortiz) **
prCO1	Coyaima, Colombia	Colony	Domestic	1997	*R. prolixus*	AF435860
roVE2	Trujillo, Venezuela	Colony	Silvatic	1988	*R. robustus* Clade I	AF435861
roBR4	Para, Brazil	Colony	Silvatic	1984	*R. robustus* Clade III	AF435857 (FM5)
roBR8	Amazonas, Brazil	Colony	Silvatic	1983	*R. robustus* Clade IV	AF435859

* *cytb* haplotype and clade from maximum parsimony tree in Monteiro *et al.*, (2003) paper. ^ represents specimens from current study (62-65 ortiz). ** in parenthesis label in current study. (see Monteiro *et al.*, 2003, Lyman *et al.*, 1999 for further details).

Standard PCR techniques were used to amplify an approx. 650 bp fragment of the gene using primers previously shown to amplify product in *Rhodnius* species (Monteiro *et al.*, 2003) (see Table 7 below). The following cycling conditions were employed; 25 cycles of denaturation at 94°C for 1min, annealing at 50°C for 2 mins, and extension at 72°C for 2 mins. Cycle amplification was preformed on either a Primus 96 plus (MWG AG Biotech). Primers as follows:

Forward primer *D2*F 5`-GCGAGTCGTGTTGCTTGATAGTGCAG

Reverse primer *D2*R 5`-TTGGTCCGTGTTTCAAGACGGG

Table 7. Specimens from current study used for D2 direct sequencing

Specimen	Origin	Ecotope	Life stage	*Cytb* Haplotype
Coj7p	Las Queseras, Cojedes	palm	Adult male	*R. prolixus* haplotype 1
Coj8p	Las Queseras, Cojedes	palm	Adult female	*R. robustus* haplotype 3
Coj20p	Las Queseras, Cojedes	palm	Adult male	*R. robustus* haplotype 3
24 terr	Terronal, Portuguesa	house 1	Adult female	*R. robustus* haplotype 3
57 terr	Terronal, Portuguesa	house 1	nymph	*R. prolixus* haplotype 1
20 terr	Terronal, Portuguesa	house 1	Adult male	*R. robustus* haplotype 3
30 terr	Terronal, Portuguesa	house 1	Adult female	*R. prolixus* haplotype 1
Ldat2	Loma de Amarillo, Trujillo	house	nymph	*R. prolixus* haplotype 5
CP7	Casa Rena, Portuguesa	house	nymph	*R. robustus* haplotype 3

5.3.4 DNA sequencing

Purified PCR products were sequenced by fluorescent dye terminator chemistry using ABI Prism® Bigdye™ ready reaction kits V2.0 and V3.1 (Applied Biosystematics). Sequencing amplifications were performed in 10µl reactions, halving the recommended reaction protocol. The reaction mix was as follows: 5-20ng of purified PCR product, 10pm forward or reverse primer, 1µl ready reaction premix, 1.5µl BigDye sequencing buffer. Purified PCR product concentration was approximated on band intensity from gel electrophoresis in comparison with the size standard Hyperladder IV. Sequencing reaction conditions were as follows: rapid thermal ramp to 96°C (ramp at 1°C/sec), 96°C for 30 s, rapid thermal ramp to 50°C (ramp at 1°C/sec), 50°C for 20s, rapid thermal ramp to 60°C (ramp at 1°C/sec), 60°C for 4 mins. The cycle was repeated 25 times.

Sequencing samples were purified to remove unincorporated dyes using two methods, depending on sample numbers. For sample sizes less than 48 isopropanol/ethanol precipitation in 1.5µl microcentrifuge tubes was used following the ABI Prism® protocol for BigDye™ V2.1. When 48 to 96 samples were sequenced products were cleaned in plates using a modification of the BigDye™ 3.1V protocol for sodium acetate/ethanol precipitation. To each sample well 3µl of 3M NaOAc pH 4.6, 65.8µl of 95% ethanol and 21.2µl of H_2O were added. The plate was covered with sticky foil, vortexed quickly and left on ice for 20 mins. The plate was then spun at 3000g for 30 mins at 4°C in a plate centrifuge. The foil was removed and liquid was allowed to drain by inverting the plate on blue lab roll. The plate was then spun inverted on paper for 15 sec at 50g to remove excess liquid. Ice cold 70% ethanol was then added to each well, the plate was inverted five times and then spun for 10 mins at 3000g/4°C. Excess liquid was removed as previously detailed. After clean up tubes and plates were carefully labelled and kept at -20°C. Prior to electrophoresis 10µl HiDi formamide (ABI) was added to each sample and samples were then denatured at 95°C for 2-4mins.

The samples were analysed on an ABI Prism 377 automated DNA sequencer (PE Applied Biosystematics) or on a 48 capillary ABI 3730 DNA analyser. Forward and reverse sequences were imported for editing and alignment into the auto-assembler programmes Sequence Navigator V1.01 (Perkin-Elmer) and BioEdit V7.0.4.1 (Hall 1999). Here ambiguities were resolved, and sections of poor sequence resolution removed, forward and reverse sequences were used to produce a consensus sequence.

5.3.5 Data analysis

5.3.5.1 Statistical analysis

Sequence identity was confirmed by comparison with data in Genbank. Statistical analysis of the data included the investigation of nucleotide and amino acid composition and substitution such as transition\transversions and synonymous/nonsynonymous substitutions using Mega v 2.1 software (Kumar *et al.*, 2001) (see Glossary on page

310). The number of variable sites and their codon position together with the number of parsimonious informative sites (see Glossary on page 310) and codon usage were also determined.

5.3.5.2 Population heterogeneity

Intrapopulation comparisons were based on the indices of population heterogeneity F_{ST} (Weir & Cockerhams 1984 unbiased estimator) generated using Arlequin v2.000. F_{ST} values were used to create UPMGA trees using Mega V3 (Kumar *et al.*, 2004) (see Glossary on page 310). The index F_{ST} measures the amount of observed variation attributable to grouping samples into subpopulations. The significance of the estimates from 0 was determined by non parametric permutation, whereby genotypes are shuffled among the groups a great number of times (10,000 permutations) and from each new data set the F_{ST} values are re-estimated. The proportion of F_{ST} values larger than or equal to the estimates from the original data set gives the p-value. The null hypothesis of the test is no difference between the populations. F_{ST} investigates nonrandom mating among subpopulations relative to the entire population and is calculated using allele frequencies. Analysis is based on an infinite alleles model (IAM).

The nominal significance level was adjusted for multiple comparisons using the sequential Bonferroni procedure (Rice 1989). The adjusted procedure is carried out as multiple statistical tests increase the overall probability of committing a type-1 error, i.e. incorrectly rejecting a null hypothesis. The adjustment consists of setting a lower threshold for the nominal significance level (see Rice 1989). The p-values are grouped in ascending order, and k, the number of tests is calculated. The smallest p-value is then compared to α / k (nominal level/ the number of statistical tests). If the smallest p-value is smaller and therefore significant, then the second smallest p-value is compared α / (k-1) and if significant the third α / (k-2), until a p-value is larger and therefore it and all higher p-values are not significant.

5.3.5.3 Phylogenetic analysis

Phylogenetic analysis of aligned sequences was performed using both distance and discrete based methods. Neighbour-joining trees were constructed using Mega v 2.1 software (Kumar *et al.*, 2001) applying various models of sequence evolution to convert aligned sequences into matrices of pairwise genetic distances. The models used to measure the evolutionary divergence between aligned sequences included Kimura-2 parameter, Jukes Cantor distance, Tajima-Nei distance, Tamura-Nei parameter, see Glossary on page 310). Statistical support for clades was assessed by the bootstrap method (Felsenstein, 1985) with 1000 replications (see Glossary on page 310). Maximum likelihood trees were produced in PAUP V4.0 (Swofford, 2002) incorporating a 4-category discrete gamma distribution to model mutation rate heterogeneity across the gene (see Glossary on page 310). A reversible rates (REV) mutation matrix was employed and is a variation of the transition/transversion ratio (see Glossary on page 310). Parameters were estimated via maximum likelihood using a reiterative (repeated) heuristic search until default conditions determined the maximum likelihood had been reached (see Glossary on page 310).

5.3.5.4 Spanning haplotype networks.

Minimum haplotype networks were constructed using the programme TCS V1.20 (Clement *et al.*, 2000) for the 18 haplotypes detected together with FM4-FM6 (FM1-FM2, FM7-FM8 were not included) and based on the parsimony (see Glossary on page 310) using the algorithm of Templeton *et al.*, 1992. The TCS v1.18 programme calculates the frequencies of haplotypes and determines outgroup probabilities, which are taken as estimates of haplotype age. The haplotype with the highest outgroup probability is determined as the ancestor (displayed in a square). Distances between taxa are determined from pairwise comparisons and the probability of parsimony is then calculated for these differences until a 95% cut off (Templeton *et al.*, 1992). The maximum number of mutational connections between haplotypes is based on these

differences and networks are created using the parsimony criterion (see Clement *et al.*, 2000).

The distances between haplotypes 3, FM4-FM6, 17, 16 and 18 exceeded the limitation of mutational steps that can be inferred using TCS1.20 programme, and were therefore analysed in separate groups: haplotypes 16-18, haplotype 3, FM4-FM6.

5.4 Isolation and analysis of microsatellite loci.

Microsatellite loci were isolated from a series of enriched libraries constructed using a protocol developed by Bloor *et al.*, (2001). This method is based on the use of magnetic beads (dynabeads) and biotinylated probes.

Total DNA was digested with a blunt end restriction enzyme, fragments were then size selected, purified and ligated to the required oligonucleotides. These ligated fragments termed adapters were then amplified by PCR, using free adaptors as primers. Biotinylated probes containing sequences complementary to the required microsatellite e.g. CAA were linked to magnetic beads and used to search for microsatellite sequences in the digested DNA. These beads were collected and washed repeatedly and the enriched DNA used as a template for PCR. The PCR product was subsequently ligated to a plasmid and bacteria were transformed. The libraries created were screened by PCR\streak plate and colonies found to contain inserts were purified and sequenced. Forward and reverse primer pairs, flanking suitable microsatellite regions, were then designed using PRIMER2 (Kemp 1993).

Four separate partial genomic libraries were constructed, enriched for (i) CA & CAA, (ii) GATA and a mixture of (iii) GAA & AAAG and (iv) GT& GTG repeat motifs.

5.4.1 Adaptor preparation

Two oligonucleotides with complementary sequences were annealed and used as an adaptor for total DNA digestion. Sticky end adapters were prepared for digestion with *Sau*3A1.

1. 10µl oligonucleotide A and 10µl oligonucleotide B (200 pmol/µl) were mixed by gentle pipetting in a 0.5 ml microcentrifuge tube and briefly spun:

Sticky end adaptor (*Sau*3A1-specific).

Oligo A 5' GGC CAG AGA CCC CAA GCT TCG 3'[21-mer]

Oligo B 5' PO_4 - GAT CCG AAG CTT GGG GTC TCT GGC C 3'[25-mer]

Adaptor GGC CAG AGA CCC CAA GCT TCG

CCG GTC TCT GGG GTT CGA AGC CTA G - PO_4

[*Sau*3A1 restriction site]

2. The mixture was denatured at 80°C for 2-5 mins on a thermocycler, and annealed by cooling at room temperature for 1hr.

3. 60µl of PCR-grade water (BDH) was added [i.e. to a total of 80µl] and the mixture was aliquoted and stored at -20°C.

5.4.2 Digestion of genomic DNA and adapter ligation

Genomic DNA for library construction was isolated from the legs of 11 field caught *R. prolixus* specimens collected in 2001 in Lara State Venezuela. Total DNA was extracted using a standard phenol chloroform protocol. DNA concentration was measured using a spectrophotometer and 9-20μg of total genomic DNA was used to construct each library. High quality genomic DNA is essential for library construction in order to avoid poor downstream sequence results. *Sau*3A1 restriction enzyme was chosen because in a digestion test of 1μg of DNA the enzyme produced a suitable size distribution of fragments.

5.4.2.1 Total DNA digestion

The restriction enzyme was added last and returned immediately to –20°C.

1. The following components were mixed in a 0.5ml microcentrifuge tube:

11.4µl	Genomic DNA (20μg)[1]
9.0µl	10X enzyme buffer (Final concentration 1X)
10.0µl	*Sau*3A1 enzyme (24U)
59.64µl	PCR H_2O
90.0µl	Total volume

2. The mix was incubated at 37°C/1 hr and deactivated at 65°C for 5-10 mins.

5.4.2.2 Adaptor ligation

1. The following were added to the DNA mixture treated with a restriction enzyme.

100.0µl	2X T_4 DNA ligase buffer (Final concentration 1X)
60.0µl	PCR grade H_2O
20.0µl	Adaptor preparation (25 pmol/µl)
20.0µl	T_4 DNA ligase (40U)
200.0µl	Total volume

2. The mixture was mixed gently by pipetting and incubated at room temperature for 1hr or overnight at 4°C.

3. After incubation the enzyme was deactivated at 65°C for 5-10 mins.

4. When several samples were treated with a restriction enzyme, the digested DNA was pooled and concentrated using a micron YM-50 spin column and diluted in 50µl PCR grade water.

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[1] As the initial digested size fragments were small, three reactions were carried out at different concentrations of DNA and restriction enzyme (parenthesis)- 9μg (20U), 20μg (24U) and 25μg (30U). A shorter incubation time was also used (protocol recommends 1.5-2 hrs). All digests were later gel purified, pooled and concentrated using a micron YM-50 spin column (Millipore).

5.4.3 Size selection and PCR of adaptor-ligated DNA

5.4.3.1 Size selection

1. The total adaptor ligated DNA was run in a 1.8% NuSieve agarose gel with ethidium bromide (0.5μg/ml) for 1hr at 60 V. A 100bp PCR ladder (Promega) was run concurrently for differentiation of fragment sizes.

2. The gel was visualised by a UV transilluminator and bands of sizes 400-1000bp[2] were quickly removed using a sterile scalpel blade.

3. The excised gel was then purified using a QIAquick™ gel extraction kit.

5.4.3.2 PCR of adaptor-ligated DNA

1. A PCR was performed to test the success of the ligation as follows:

Step 1	95°C for 5mins	1.25µl Oligonucleotide A (200 pmol/µl)
Step 2	95°C for 50sec	10.25µl PCR grade water (BHD)
Step 3	56°C for 1min	1.00µl size selected DNA
Step 4	72°C for 2mins	12.50µl Ready Mix (ABgene)
Step 5	Go to step 2 thirty times	25.00µl Total volume
Step 6	72°C for 10mins	
Step 7	6°C hold	

2. 5µl of PCR product was run on a 2% agarose gel with a 100bp ladder (1hr/60V). The presence of a smear between 400-1000bp demonstrated successful adaptor ligation.

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[2] 400-1000bp fragments are isolated as fragments over 1,000bp are difficult to sequence completely and sequences smaller than 400bp limit primer sites.

5.4.4 Capture of microsatellite containing DNA fragments

1. 100µl of streptavadin-coated magnetic beads (10mg/ml) (M-280 Dynabeads, Dynal) were added to a 1.5ml microcentrifuge tube and washed in 100µl of 1 X Washing/Binding (W/B) buffer.

2. The supernatant was removed by placing the tube by a magnetic stand, causing the beads to migrate to the side. The wash in step (1) was repeated and supernatant removed.

3. The beads were resuspended in 200µl of 2 X W/B buffer and 3µl of 3'-biotinylated oligonucleotide probe was added (1.5µl of each probe if combined)[3]. The total volume was then increased to 400µl with PCR-grade water (BDH).

4. The bead mixture was incubated at room temperature for 30 mins, with gentle agitation every 10 mins.

5. The mixture was washed once in 400µl of 1 X W/B and twice in 400µl of 6 X SSC, the supernatant removed as previously detailed.

6. The beads were re-suspended in 50µl 6 X SSC and then incubated at the probe-specific hybridisation temperature[3].

7. The following was placed in a tube, mixed gently by pipetting and briefly spun:

10µl	digested/adaptor-ligated/size-selected DNA
5µl	oligonucleotide A (20 pmol)
15µl	20 X SSC [final concentration 6 X]
20µl	PCR-grade water (BDH)
50 µl	Total volume

[3] Four libraries were created using the probes CA/CAA (60°C), GATA (48°C), GAA/AAAG (48°C), GT/GTG (60°C). Probes can be combined as long as they do not complement and have similar annealing/hybridisation temperatures (in parenthesis).

8. A thermal cycler was set as follows:

Step 1	95°C	10 mins	
Step 2	X°C	30 mins	[the probe-specific hybridisation temperature][3]
Step 3	70°C	2 hr	

9. The DNA mixture (7) was incubated in a thermal cycler at 95°C for 10 min.

10. The re-suspended beads (6) were added as the temperature ramped to X°C. The sample was incubated at X°C for 30 min with gentle agitation every 5 min.

11. The supernatant was removed and the beads re-suspended in 100µl of 2 X SSC.

12. The mixture was then transferred to a 1.5ml microcentrifuge tube and washed four times in 1ml of 2 X SSC with a 5 min incubation at room temperature for each wash. It is critical that beads do not dry out between washes.

13. Next step12 was repeated using 1 ml of 1 X SSC.

14. The beads were resuspended in 100 µl of 1 X SSC and aliquoted into four 25µl samples, 250µl of 1 X SSC was then added to each aliquot. The aliquots were incubated at the probe-specific temperature for 10 min.

15. The supernatant was quickly removed and the beads rinsed for 30s at room temperature in 400µl 1 X TE.

16. Next step 15 was repeated with 400µl 50 mM NaCl.

17. Finally each aliquot was re-suspend in 50 µl PCR-grade water (BDH).

To create large volumes of double stranded enriched DNA a PCR was carried out:

Step 1	95°C 3 min	25 µl	2 X Reddy-Mix (ABgene)
Step 2	95°C 30 s	3 µl	oligonucleotide A (10 pmol/µl)
Step 3	X°C 30 s [4]*	8 µl	bead suspension (40µg)
Step 4	72°C 45 s	14 µl	PCR-grade water (BDH)
Step 5	go to step 2 five times	50 µl	Total volume
Step 6	92°C 30 s		
Step 7	X°C 30 s [4]*		
Step 8	72°C 55 s		
Step 9	go to step 6 thirty times		
Step 10	72°C 30 min		
Step 11	4°C hold		

A smear between 400-1000 bp indicated the procedure was successful (2% gel at 100 V for 15-20 min 5µl PCR product). The PCR product was cleaned (Qiagen Quick Clean™) and ligated into pGEM®-T vector within 24hrs.

5.4.5 Construction of enriched microsatellite library

5.4.5.1 Ligation of microsatellite DNA into pGEM®-T vector.

The following reaction was set up in 0.5 ml centrifuge tubes and left overnight.

5 µl	2 X Rapid Ligation buffer (Promega)
1 µl	pGEM®-T vector (50 ng) (Promega)
X µl	PCR product[5]
1 µl	T_4 DNA ligase (3 Weiss units/µl)
X µl	PCR-grade water (BDH)
10 µl	Total volume

[4] probe-specific annealing temperature

[5] Three ratios of vector:insert were tried 3:1, 1:1, 1:3 to maximise transformation, requiring 25ng, 8.33ng, 2.77ng DNA respectively. PCR product was quantified using a spectrophotometer and volumes added ranged from 3µl to 0.4µl

5.4.5.2 Construction of enriched microsatellite library

The libraries were constructed using a Promega pGem-T® vector cloning kit following the manufacturer's recommended protocol.

1. 2µl of each ligation reaction, together with 50µl JM109 High Efficiency Competent Cells were added to a 1.5 ml centrifuge tube and left on ice for 20 mins.

2. The cells were then heat-shocked for 45 sec in a water bath at 42°C and returned to ice for 2 min.

3. 950µl of room temperature SOC medium was then added, and the tubes incubated at 37°C with shaking at 150 rpm for 1.5 hr.

4. 100µl of each transformation was plated on LB/ampicillin/IPTG/X-gal plates and incubated overnight at 37°C.

With Promega kits white colonies generally contain inserts, due to interruption of the coding sequence of β-galactosidase, allowing for recombinants to be identified by colour screening.

5.4.5.3 Working library construction

1. Identified white colonies were transferred, using 200ul pipette tips, to 96 well plates containing 100µl of LB/ampicillin (100 µg/ml), one colony per well.

2. The 96 well-plates were incubated at 37°C for 3-4 hours and then were stored for screening at 4°C for up to 3 weeks.

For long term storage of constructed libraries, 10µl from the well of each working library was transferred to the corresponding well in a second 96 well plate and following an incubation period, (37°C for 3-4 hrs), 100 mls of a sterile 30% glycerol stock was added to each well (30mls glycerol/70mls LB). This allows plates to be stored

indefinitely. Stabs (LB/Amp 10ug/ml) were also constructed for colonies from positive streak plates (as detailed below).

5.4.6 Screening working libraries for inserts

Parallel to working library construction, the colonies were screened, by PCR, for the presence of inserts.

1. 20µl of the PCR master mix below was placed in each well of a 96-well PCR plate.

5.0µl	Oligo A (10 pmol/µl)
2.5µl	probe oligonucleotide (10 pmol/µl)
2.5µl	PCR-grade water (BDH)
10µl	2 X Reddy Mix (ABgene)
20µl	Total volume

2. Each pipette tip used to transfer a colony to the working library plate was additionally swirled in a corresponding well of a PCR plate. PCR conditions used were as previously stated (see section 5.4.4 on page 114).

3. The presence of a microsatellite insert in the vector was indicated by a double-banded PCR product (2% gel at 100 V for 15-20 min).

4. Streak plates (LB/amp 100ug/ml) were created from library wells where microsatellites inserts were detected (doubled banded PCR). Individual colonies from each streak plate were tested for the presence of double banded inserts by PCR (as stated above). Streaking was carried out to ensure that the presence of a double band was not due to the accidental transfer of two colonies to the library plate.

5. Individual colonies from each streak plate were grown overnight in 4-5mls LB/amp broth. Plasmids were extracted from those cultures that tested positive by both PCRs (library and streak plate) using a Qiagen miniprep® kit.

5.4.7 Sequencing of positive inserts

Plasmids from positive clones were subsequently sequenced using Big Dye™ chemistry V3.1 (Applied Biosystems) and electrophoresis on an ABI 377. A 10µl final reaction mix was used as follows: 200-300ng of purified PCR product, 3.2pm M13 forward or reverse primer, 2µl ready reaction premix and 1.0µl BigDye sequencing buffer. Sequencing cycling conditions were as previously detailed (see section 5.3.4 on page 105). Samples for sequencing were purified to remove unincorporated dyes using the BigDye™ 3.1V protocol for sodium acetate ethanol precipitation (see section 5.3.4).

5.4.8 Primer design

Primers were designed using PRIMER2 (Kemp 1993) from the flanking regions of suitable sequences found to contain repeat motifs. Some sequences were unsuitable for designing primers because the sequence at one (or both) flanking regions was repeated across different microsatellite loci, BioEdit V7.0.4 (Hall 1999) was used to check for repeat sequences, or primer sites were too close to the microsatellite repeat or problems such as hairpin structures prevented the design of suitable primer sites.

5.4.9 Microsatellite amplification

Primer pairs were tested for successful amplification in a 10µl reaction mix containing 50ng/µl DNA, 10pmol of each primer, and 5µl ReddyMix (ABgene). Cycle conditions were as previously detailed (see section 5.4.4 on page 114). When primers failed to amplify or when multibands were detected annealing temperatures were increased or decreased accordingly, and $MgCl_2$ levels were adjusted.

5.4.10 Genescan analysis

A subset of primers that amplified successfully was selected for fluorescent labelling for genescan analysis. Forward primers were labelled using a 5' fluorescent dye (6-FAM, PET, NED, VIC). The dyes were assigned according to PCR product size, with loci of

similar size labelled differently when possible and vice versa. This allowed for multiple samples to be pooled prior to gel electrophoresis and run in the same lane.

Products were amplified in a 10µl reaction consisting of 1-3µl DNA, 1µl dNTP, 1µl buffer, 0.2µl $MgCl_2$, 0.2µl *Taq* polymerase and 5pm of each forward/reverse primer. When loci failed to multiply in samples, the annealing temperature was lowered and $MgCl_2$ concentration adjusted (maximum 0.4µl). *Taq* extender (Stratagene) was also employed.

For genescan a final reaction mix of 10µl-11.5µl was used consisting of 9.25µl Hidi-formamide, 0.25µl of the size standard Liz 500 (ABI), and 0.5µl of each labelled PCR product was added for up to four different sized or labelled loci. This mixture was then denatured for 2-5 mins at 95°C and then run on an ABI 3730 48 capillary DNA analyser (Applied Biosystems, Warrington, Cheshire, United Kingdom). Allele data was generated using Genemapper software V3 (ABI). This allowed for configurable, automated allele calling by measurement of allele length and quantification of allele peaks in relation to the size standard provided (Liz 500).

5.4.10.1 Specimens used for microsatellite analysis.

A total of 555 *R. prolixus* specimens were used for microsatellite amplification, from 5 States grouped into 33 populations, including 4 adjacent populations (house and palm, house and peridomestic) (see appendix Table 64 on page 335). Populations were analysed by a total of 10 loci (33 populations), an additional two loci were amplified for a subset of 20 populations. Following amplification two loci were excluded from analysis List14-041 and List14-076 (see section 7.1.2 on page 179). This left two sets of 9 and 10 loci. These two loci sets shall be referred to as **Set 1**; List14-056, List14-17, List14-042, List14-010, List14-064, List14-013, List14-21, List14-025, List14-037 (9 loci) and **Set 2**: Set 1 plus List14-079 (10 loci). Comparisons across all populations (33) were made using data from 9 loci, while comparisons within populations were made according to the number of loci amplified (set 1 or set 2).

5.4.11 Microsatellite data analysis

The nominal significance level for multiple comparisons was adjusted using the sequential Bonferroni procedure (Rice 1989, on page 107).

5.4.11.1 Hardy-Weinberg Equilibrium

Arlequin V2.000 (Schneider *et al.*, 2000) was used to calculate observed and expected heterozygosity and to test for significant deviations from expected Hardy-Weinberg conditions (HWE) at each locus in the total pooled population (555 specimens) and within each individual population. The null hypothesis is the random union of gametes. This analysis uses a contingency table and p-values are generated by resampling of the data using the Guo & Thompson's (1992) Markov-chain random walk algorithm (10,000 steps). Using this algorithm the various replicate contingency tables are explored and the p-values are based on the proportion of the replicate tables with a probability distribution smaller or equal to the original data. GENEPOP V3.4 (Raymond & Rousset, 1995) was employed to investigate deviations from HWE by testing for heterozygosity deficiency and heterozygosity excess at each locus and in each individual population. Wright's (1951) inbreeding coefficient (F_{IS}) was also calculated in GENEPOP V3.4 using the estimator of Weir & Cockerham (1984).

Summary statistics of mean allele number, allele and genotype frequencies at each locus were generated in Powermarker V3.23 (Liu & Muse 2005) and Fstat V2.932 (Goudet 1995). Measures of allele richness were calculated for each locus in each population together with minimum and maximum allele number per locus per population using Fstat V2.932 (Goudet 1995). Allelic richness is a measure of allele number but is independent of sample size and based on the rarefaction index of Hurlbert (1971), and therefore allows different sample sizes to be compared. Values of F_{IS} at population level (over all loci) were also calculated using Fstat V2.932 (Goudet 1995).

5.4.11.2 Linkage Disequilibrium

Linkage disequilibrium among all locus pair combinations was also examined using the procedure implemented by GENEPOP V3.4 (Raymond & Rousset 1995), using default settings of 1000 iterations per 100 batches. This uses a contingency table to test for deviations from the null hypothesis of independence of genotypes at one locus from other loci. The significance is tested via an exact test using a Markov chain algorithm to explore replicate contingency tables (see 5.4.11.1 on page 121).

5.4.11.3 Intrapopulation comparisons

Intrapopulation comparisons were based on the indices of population heterogeneity F_{ST} (Weir & Cockerhams 1984 unbiased estimator) and R_{ST} (Slatkin 1995) generated using Arlequin v2.000. The indices F_{ST} and R_{ST} measure the amount of observed variation attributable to grouping samples into subpopulations (see section 1.5.2.1.3 on page 53). The significance of the estimates (F_{ST} and R_{ST}) from 0 was determined by 10,000 permutations of genotypes among samples (null hypothesis of no difference between the populations, see on page 107). F_{ST} investigates non-random mating among subpopulations relative to the entire population and is based on variance in allele frequencies. Analysis is based on an infinite alleles model (IAM) and assumes new alleles in populations are due to migration not mutation. R_{ST} is an analogue of F_{ST} developed for the analysis of microsatellite data. R_{ST} calculations incorporate the mutational differences between alleles and are modelled on stepwise mutation (SMM) with analysis based on variance in repeat number (Slatkin 1995). With limited numbers of loci (<20) and/or individuals (n<10) F_{ST} may be more suitable than R_{ST} (Fredsted *et al.*, 2005, Gaggiotti *et al.*, 1999). High variance can occur in R_{ST} analysis under SMM (Gaggiotti *et al.*, 1999).

Population genetic diversity and heterogeneity were investigated at a number of hierarchical levels (1) population level (33 groups) (2) by State (5 groups) (3) State partitioned by ecotope (10 groups) (4) division of all specimens by ecotope (3 groups).

UPGMA trees (see Glossary on page 310) were also produced using Mega V3 from F_{ST} values generated at State level within Portuguesa and Barinas and for all 33 localities analysed.

5.4.11.4 Isolation by distance (IBD)

The relationship between geographical and genetic distance (IBD) over the study area was assessed by testing the correlation between $F_{ST}/(1-F_{ST})$ against log transformed (ln) geographic distances. This was undertaken to investigate if genetic differences between populations increased with the geographic distances between them i.e. isolation by distance. Rousset (1997) showed that a linear relationship occurs between natural log of geographical distance and $F_{ST}/(1-F_{ST})$ in two dimensional habitats. Geographic distances (miles) were generated between all collection sites from coordinate data using ArcView GIS V3.3. The significance of the correlation between the matrices of distances was examined by a Mantel test using a permutation procedure (9999 permutations) in GenAlex (Peakall & Smouse 2005).

5.4.11.5 Genetic distance measures

To measure genetic relatedness between populations four genetic distance were calculated (1) Nei's unbiased genetic distance measure D_S (Nei 1972) based on allele frequencies and the IAM mutation model, (2) D_{PS} based on the number of shared alleles per locus, (Bowcock *et al.*, 1994), (3) D_{SW} (Shriver *et al.*, 1995) a genetic distance based on Nei's minimum genetic distance and the SMM mutation model and (4) delta mu squared, D_{MU}, also incorporating SMM (Goldstein *et al.*, 1995). All populations were analysed (33 groups) at 9 loci (set 1). All distances were calculated using MICROSAT (with 500 bootstrap replicates for D_S, see Glossary on page 310). Details of distances measures are available in Paetkau *et al.*, (1997) and MICROSAT distance manual. A neighbour joining tree (see Glossary on page 310) was produced for D_S distances using PHYLIP (Felsenstein 1993). This distance was chosen as it has been shown to work well in the analysis of closely related populations in Paetkau *et al.*, (1997). For each genetic

distance measure pairwise comparisons were made with geographic distances using a Mantel test in GenAlEx (9999 permutations, Peakall & Smouse 2005). This was undertaken to investigate if genetic distances between populations increased with the geographic distances between them. The genetic distance D_S (500 bootstrap replicates) was also calculated between groups within Portuguesa (13 groups, set 1) and Barinas (16 groups, set 2), with trees produced as detailed above.

5.4.11.6 Assignment test

Assignment test were carried out using Geneclass2 (Piry *et al.*, 2004). Both frequency (Paetkau *et al.*, 2004) and Bayesian (Rannala & Mountain 1997) assignment methods were used. These methods assign individuals to populations from which they are most likely to have originated on the basis of their microsatellite genotype (their genotype likelihood distribution). Our analysis is based on the analysis of 9 microsatellite loci. Likelihood is the probability of the data for a given set of parameters. Both assignment methods calculate the genotype likelihood distributions of the reference populations and then compare the likelihood distribution of the individual to each population. The specimen is then assigned to the population with the highest log likelihood (i.e. the population with the least negative log-likelihood value). The leave one out option was employed, which removes the individual under consideration when calculating the allele frequencies for each population. Bayesian analysis differs from frequency analysis in that it uses known information to help in the assignment of an individual, termed the prior distribution, here the allele frequencies in the reference population.

Populations are defined *a priori* with 555 individuals at 9 loci divided by (1) site of collection; 33 groups, (2) locality level; 17 groups (3) State level; 5 groups (4) State by ecotope; 10 groups (5) ecotope; 3 groups. The program assumes both Hardy Weinberg equilibrium and linkage equilibrium within populations.

5.4.11.7 Microsatellites and mitochondrial data

Table 8. Specimens characterised by mt*cytb* and microsatellite loci (set 1).

Population	Population size	Cytb haplotypes*
Terronal h1 01	19	Haplotype 1 (7), Haplotype 3 (12)
Terronal h2 01	15	Haplotype 1 (9), Haplotype 3 (6)
Terronal h2 p01	23	Haplotype 1 (18), Haplotype 3 (5)
Terronal h1 03	10	Haplotype 1 (3), Haplotype 3 (7)
Terronal h2 p03	34	Haplotype 1 (27), Haplotype 3 (7)
San Bartolo h1	14	Haplotype 1
San Bartolo h2	8	Haplotype 1
Santa Lucia h	13	Haplotype 9 (11), Haplotype 1 (2)
Casa Rena h	10	Haplotype 3
Palo Gacho p	7	Haplotype 1 (4), Haplotype 3 (3)
Los Rastrojos h	22	Haplotype 1 (20), Haplotype 2 (2)
Los Rastrojos p	10	Haplotype 1
Lara h	15	Haplotype 1
Cojedes p	24	Haplotype 1 (22), Haplotype 3 (2)
Cojedes h	22	Haplotype 1
Trujillo h	21	Haplotype 16 (1) Haplotype 5 (20)
Cascabel pd	9	Haplotype 1 (5), Haplotype 2 (3), Haplotype 4 (1)
Cascabel h	8	Haplotype 1 (6), Haplotype 2 (1), Haplotype 5 (1)
Cascabel p	15	Haplotype 1 (8), Haplotype 2 (6), Haplotype 5 (1)
Guaranda h	5	Haplotype 1
Guaranda p	11	Haplotype 1 (10), Haplotype 10 (11)
Laguna Hermosa h	10	Haplotype 1 (9), Haplotype 5 (1)
Laguna Hermosa p	9	Haplotype 1 (6), Haplotype 5 (1), Haplotype 2 (1), Haplotype 11 (1)
Laguna Hermosa pd	7	Haplotype 1 (5), Haplotype 12 (1), Haplotype 5 (1)
19 Abril pd	10	Haplotype 14
Parcelamiento p	7	Haplotype 1 (5), Haplotype 14 (2)
Rio Bravo II pd	5	Haplotype 1 (4), Haplotype 2 (1)
Rio Bravo II p	6	Haplotype 1 (4), Haplotype 2 (2)

p=palm h=house pd= peridomestic, numbers in parenthesis represent no of specimens for each haplotype

A total of 369 specimens from 28 populations were characterised by both *cytb* direct sequencing and microsatellite characterisation (9 loci; set 1). Degrees of population heterogeneity detected by both markers was analysed by comparing F_{ST} values generated between populations characterized by both methods. UPGMA trees were produced for F_{ST} values generated using MEGA V3 (Kumar *et al.*, 2004). Comparisons of pairwise indices for both markers were made using a Mantel test in GenAlEx (9999 permutations, Peakall & Smouse 2005).

5.5 Geometric morphometric analysis

The aim of this section of the study was to apply novel geometric morphometric analysis to assess wing shape variation between domestic, peridomestic and silvatic populations of *R. prolixus*. Morphometric analysis was compared with genetic characterisation (*cytb* and microsatellites) of subsets of specimens analysed by both means.

The left wings of adult specimens were subjected to geometric morphometrics analysis investigating shape variation at various hierarchical levels. Wings (hymelytra) were used in analysis, as they are rigid and easily preserved structures, which makes them suitable for morphometric analysis (Dujardin *et al.*, 1997b), and they are flat and 2 dimensional, which makes them easier to photograph. Wings have previously been employed successfully for geometric morphometric analysis of Triatominae, including *R. prolixus*, although at a taxonomic level (Villegas *et al.*, 2001, Feliciangeli *et al.*, 2002). Wings were capable of distinguishing *R. robustus* and *R. prolixus* (Villegas *et al.*, 2002, Matias *et al.*, 2001), while head data had proved inconclusive (Harry 1994).

Unfortunately small numbers of adults limited population comparisons. Specimens were grouped at the lowest hierarchical level possible (see Table 9 on page 127), including 11 populations, 6 groupings by locality and 3 groups by State (encompassing various localities). Adjacent house and palm comparisons were limited to Cojedes (a single house and palm in Las Queseras) and Terronal (a single house and 2 palms).

Shape variation was explored in five ways (1) within a single locality, Terronal in Portuguesa State, comparing 5 populations (3 domestic and 2 silvatic) to investigate variation across a small scale (2) within Portuguesa State (12 groups) (3) across States (20 groups) and (4) globally by ecotope. (5) Comparisons were also made between subsets of specimens analysed by both morphometric and genetic analysis (*cytb* and microsatellite) (see appendix Table 70 on page 367, Table 71 on page 378, Table 72 on page 386, Table 73 on page 394).

5.5.1 Within Terronal

The locality Terronal within Portuguesa State was analysed separately to investigate shape variation among the 5 different populations sampled including three domestic populations, with house 1 sampled in 2001 and again in 2003 and two silvatic populations from separate years adjacent to house 2 (see Table 9 below).

5.5.2 Within Portuguesa

Due to the large numbers available, including Terronal populations (212 specimens), Portuguesa State was initially analysed separately. Specimens were divided by locality and ecotope with Terronal specimens separated by year as previous (12 group comparisons; 8 domestic, 1 peridomestic and 3 silvatic) (see Table 9).

5.5.3 Across State groups

Table 9. Summary of specimens used in analysis of shape by State and ecotope.

State	Locality	Ecotope	Neco	State total ^
Portuguesa	Casa Rena*	Domestic	8	8
	Terronal h1 01*	Domestic	26	81
	Terronal h2 01**	Silvatic	40	-
	Terronal h2 01*	Domestic	15	-
	Terronal h2 03*	Silvatic	27	35
	Terronal h1 03*	Domestic	8	-
	Palo Gacho	Silvatic	20	20
	Qdra Negra	Domestic	14	14
	San Bartolo	Domestic	15	15
	Laurianito*	Peridomestic	20	20
	Morichal	Domestic	9	9
	El Mosquito	Domestic	10	10
Trujillo	Varios	Silvatic	14	31
	Loma de Amarillo\ Palma Real **	Domestic	17	-
Guarico	Various	Silvatic	20	20
Lara	Guamarito\Salvador**	Domestic	19	19
Cojedes	Las Queseras*	Silvatic	14	21
	Las Queseras*	Domestic	7	-
Barinas	Carreteron	Domestic	18	18
Merida	Various	Silvatic	5	5
Total	-	-	-	**326**

Neco = number of specimens per ecotope. ^ =total per locality in Portuguesa State (per year Terronal). See appendix Table 70 on page 367 for further details. * = specimens in locality from single population, ** primarily single population with the exception of 1 specimens in Trujillo, 2 in Lara and 7 specimens in Terronal. p=palm h=house pd= peridomestic.

A total of 326 adults were divided by State-locality and ecotope (7 States; Portuguesa, Trujillo, Guarico, Lara, Cojedes, Barinas and Merida). Portuguesa specimens, due to large numbers, were subdivided as previously, giving a total of 20 group comparisons (see Table 9 on page 127). Domestic groups dominated (12) followed by silvatic (7), with a single peridomestic population analysed. Cojedes, Lara and Trujillo samples came primarily from single populations, and Barinas samples from a single locality (Carreteron).

5.5.4 Analysis by ecotope

A total of 306 specimens from the study States, including the 5 silvatic specimens from Merida, were grouped globally by ecotope (domestic 166 and silvatic 140) to see if an overall shape difference could be seen between ecotopes. Peridomestic samples were excluded due to low numbers (20 specimens).

5.5.5 Variation in shape in relation to genetic variation

5.5.5.1 Cytochrome b

A subset of specimens characterised by *cytb* were grouped by haplotype and analysed for the existence of shape variation associated with different haplotypes. Due to limited adult numbers and wings available for each haplotype only four groups could be compared (haplotype 1, 2, 3 and 5), totalling 237 specimens (see Table 10 below).

Table 10. Summary of specimens, grouped by *cytb* haplotype and ecotope, used in analysis of shape.

Haplotype group	Silvatic specimens	Domestic specimens	Total
Haplotype 1	75	87	162
Haplotype 2	12	-	12
Haplotype 3	14	34	48
Haplotype 5	-	15	15
Total	101	136	237

5.5.5.1.1 Populations and cytb variation

A total of 18 population groups characterised by both *cytb* and morphometrics were compared to investigate variation detected by both methodologies, totalling 233 specimens (see Table 11 below). Peridomestic specimens were excluded due to small numbers. A Mantel test was also used to compare shape differences in the form of Mahalanobis distances with genetic differences (F_{ST}) generated between specimens characterised by *cytb*.

Table 11. Summary of populations analysed by both *cytb* and morphometrics.

Locality	N	Haplotype
Barinas h	14	Haplotype 1 (9), **Haplotype 3 (1)**, Haplotype 4 (2), Haplotype 5 (1), Haplotype 7 (1)
Casa Rena h*	7	**Haplotype 3 (7)**
Cojedes h*	6	Haplotype 1 (6)
Cojedes p*	14	Haplotype 1 (12), **Haplotype 3 (2)**
Guarico p	16	Haplotype 1 (5), Haplotype 2 (11)
Lara h**	19	Haplotype 1
Morichal h	5	Haplotype 1 (4), **Haplotype 3 (1)**
Palo Gacho p	10	Haplotype 1 (6), **Haplotype 3 (4)**
Qdra Negra h	12	Haplotype 1 (8), Haplotype 2 (1), Haplotype 5 (2), Haplotype 8 (1)
San Bartolo h	12	Haplotype 1 (12)
Terronal h1 03*	7	**Haplotype 3 (6)**, Haplotype 1 (1)
Terronal h1 01*	18	Haplotype 1 (7), **Haplotype 3 (11)**
Terronal h2 01*	14	Haplotype 1 (8), **Haplotype 3 (6)**
Terronal h2 p 01**	30	Haplotype 1 (24), Haplotype 2 (1), **Haplotype 3 (5)**
Terronal h2 p 03*	24	Haplotype 1 (22), **Haplotype 3 (2)**
Trujillo h*	13	Haplotype 5 (12), Haplotype 16 (1)
Trujillo p	6	Haplotype 16 (3), Haplotype 17 (2), Haplotype 18 (1)
El Mosquito h	6	Haplotype 1 (5), Haplotype 2 (1)
Total	**233**	

h=house p=palm 01=2001 03=2003. In parenthesis numbers of each haplotype per population. * = specimens in locality from single population, ** primarily single population with the exception of 2 specimens in Lara and 6 specimens in Terronal (see appendix Table 72 on page 386). In bold the introgressed haplotype 3. N= population size.

5.5.5.2 Microsatellites

A total of 190 specimens were analysed by both microsatellite and geometric morphometric analysis from 4 States representing all ecotopes (see Table 12 on page 130). A Mantel test was also used to compare shape differences in the form of

Mahalanobis distances with genetic differences (F_{ST}) generated between compared populations.

Table 12. Summary of specimens used in analysis of shape by microsatellite

State	Localities	Number of specimens	Ecotope
Lara	Guanarito/Salvador**	13	Domestic
Cojedes	Las Queseras*	7	Domestic
		14	Silvatic
Trujillo	Loma de Amarillo *	16	Domestic
Portuguesa	Casa Rena *	8	Domestic
	Laurianito *	16	Peridomestic
	Palo Gacho	13	Silvatic
	San Bartolo	12	Domestic
	Terronal h1 01*	23	Domestic
	Terronal h1 03*	7	Domestic
	Terronal h2 01*	12	Domestic
	Terronal h2 p 01*	24	Silvatic
	Terronal h2 p03*	25	Silvatic

* = specimens in locality from single population, ** primarily single population with the exception of 2 specimens in Lara (see appendix Table 73 on page 394). p=palm h=house pd= peridomestic, 01=2001, 03=2003.

5.5.6 Morphometrics protocol

The geometric morphometrics procedure involved the measurement of landmarks (two dimensional Cartesian coordinates (xy) for homologous points on the dorsal surface of the left wing of each specimen (see Figure 12 on page 131) and superimposition procedures for the analysis of shape (see section 1.5.1.2 on page 46, Figure 6 on page 48, Figure 7 on page 49).

5.5.6.1 Image collection

A protocol of video photography and computerised image analysis of morphometric data was used as in Patterson *et al.*, (2001). A microscope video camera (Euromoex Eurocam) was attached to the right eyepiece of a binocular dissecting microscope. Still pictures of the specimen wing were recorded by an image-capturing device (Zipshot). Wings were carefully removed from specimens prior to image collection and mounted between two glass slides, which were sealed and carefully labelled. Upon capture still

images were carefully labelled and immediately transferred and stored in a computer via Arc Sort Photo Impression (Arc Soft v2.5) software for later analysis.

5.5.6.2 Data collection

A series of nine landmarks were taken from the stored image of the left wing of each insect using TPSdig software (TPSdig version 1.28 Rohlf 2000). All landmarks were taken, in strict rotation and by the same operator (starting 1 through to 9). Of the nine landmarks taken seven were 'type I' (2,3,5,6,7,8,9-tissue intersections) and two were of 'type II' (1,4) (Brookstein 1991).

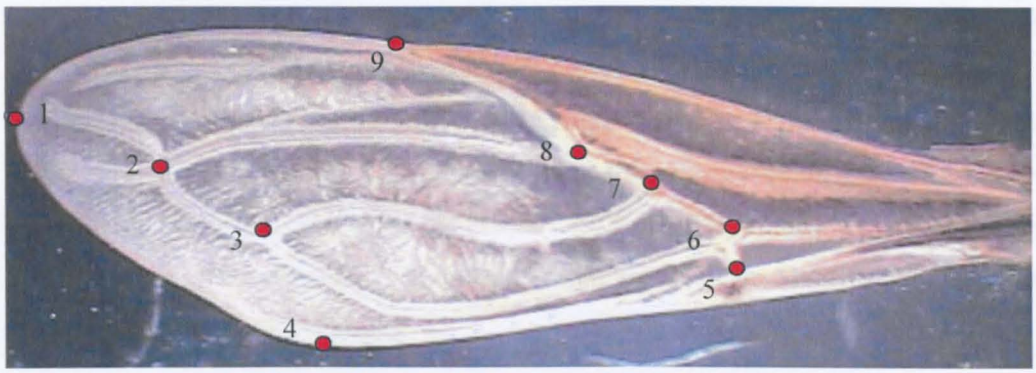

Figure 12. Landmarks of left wing (1-9) used in the analysis of shape variation.

5.5.6.3 Shape analysis

Following generalised procrustes superimposition (GPS) and thin plate spline analysis (TPS) the shape variables produced, 'partial warp values,' were subjected to principal components analysis (PCA, see section 1.5.1.2 on page 46, 1.5.1.1 on page 45) using JMP V4.02 (SAS Institute Inc.). The resulting shape variables were analysed by discriminant canonical variate analysis (CVA, see section 1.5.1.1 on page 45) with data grouped *a priori,* inorder to investigate shape discrimination within and between these groups. Values from the first two canonical variables (CV1 and CV2) were used to plot the position of each specimen in the 'shape discriminant space' (see page 48). Distance trees were produced by cluster analysis using Mahalanobis distances generated from

CVA (JMP V4.02, PAD Dujardin 2005, PHYLIP Felsenstein 1993). Mahalanobis distance is calculated between two points in the space defined by two or more correlated variables and differ from Euclidean distance by considering the correlations of the data set. A oneway ANOVA was used to analyse the pattern of shape distribution for each canonical variable and significant differences between groups means was tested using the Tukey-Kramer test (JMP V4.02). An ANOVA can inform means are significantly different but do not inform which exact group means differ. The Tukey-Kramer test gives information on the differences between group means while allowing for multiple comparisons.

Reclassification of specimens to their correct groups was investigated after CVA. The test statistic Kappa indicated the strength of correct reclassification on a scale from 0 to 1 between the compared groups (JMP V4.02). Mantel tests were carried out in GenAlEx (9999 permutations, Peakall & Smouse 2005). Multivariate significance of CVA was tested by the Wilk's Lambda statistic (JMP V4.02). Wilk's lambda test statistic ranges from 1 to 0, with values close to 0 indicating that group means are different and values close to 1 indicating no difference.

5.5.6.4 Allometric analysis

It is necessary to also account for allometric effects (change of shape with size) in the analysis. Estimates of global size was generated using the isometric size estimator 'centroid size' generated from landmark data by TPSregr v1.22 (Rohlf 2000). Centroid size is the square root of the sum of the square distances between the centre of the object to its landmarks. To explore shape variation with variation in size (allometry), centroid size was plotted against CV1, for each data set analysed.

6 DNA Sequence analyses

The aim of this section of the study was to characterise silvatic and domestic populations of *Rhodnius* by direct sequencing of a fragment of the mitochondrial cytochrome b gene (*cytb*), to confirm the identity of silvatic bugs of the genus *Rhodnius* and to determine if silvatic and domestic populations are isolated. Additionally to check for introgression between *R. prolixus* and *R. robustus*.

6.1 Results

6.1.1 Sequences produced

PCR amplification of a ~700bp of the mitochondrial *cytb* gene was achieved for 551 specimens. A 415 bp consensus sequence was produced for the majority of the specimens analysed with 10 alignments producing a shorter consensus ranging from 392bp to 408bp (see appendix Table 63 on page 314).

Table 13. Results of Genbank comparisons for the 18 *Rhodnius* haplotypes found in this study.

Haplotype	Specimen match	Score	Identity	Genbank Accession No.
Haplotype 1	*R. prolixus* 663 bp	823 (e =0.0)	415/415 (100%)	AF421339^
Haplotype 2	*R. prolixus* 663 bp	815 (e= 0.0)	414/415 (99%)	AF421339^
Haplotype 3	*R. robustus* 663 bp	775 (e = 0.0)	409/415 (98%)	AF421343^
Haplotype 4	*R. prolixus* 663 bp	807 (e=0.0)	413/415 (99%)	AF421339^
Haplotype 5	*R. prolixus* 663 bp	815 (e= 0.0)	414/415 (99%)	AF421339^
Haplotype 6	*R. prolixus* 663 bp	807 (e=0.0)	413/415 (99%)	AF421339^
Haplotype 7	*R. prolixus* 663 bp	799 (e=0.0)	412/415 (99%)	AF421339^
Haplotype 8	*R. prolixus* 663 bp	791 (e=0.0)	411/415 (99%)	AF421339^
Haplotype 9	*R. prolixus* 663 bp	815 (e= 0.0)	414/415 (99%)	AF421339^
Haplotype 10	*R. prolixus* 663 bp	807 (e=0.0)	413/415 (99%)	AF421339^
Haplotype 11	*R. prolixus* 663 bp	799 (e=0.0)	412/415 (99%)	AF421339^
Haplotype 12	*R. prolixus* 663 bp	807 (e=0.0)	413/415 (99%)	AF421339^
Haplotype 13	*R. prolixus* 663 bp	815 (e= 0.0)	414/415 (99%)	AF421339^
Haplotype 14	*R. prolixus* 663 bp	815 (e= 0.0)	414/415 (99%)	AF421339^
Haplotype 15	*R. prolixus* 663 bp	815 (e= 0.0)	414/415 (99%)	AF421339^
Haplotype 16	*R. robustus* 663 bp	783 (e = 0.0)	398/399 (99%)	AF421340^
Haplotype 17	*R. robustus* 663 bp	791 (e=0.0)	399/399 (100%)	AF421340^
Haplotype 18	*R. robustus* 663 bp	775 (e=0.0)	397/399 (99%)	AF421340^

Note ^ specimens **among** the eight sequences (FM1-FM8) donated by collaborator (details in Monteiro *et al.*, 2003).

The absence of insertions or deletions allowed for an unambiguous sequence alignment. Sequence identity was confirmed by comparison with data in Genbank and sequence haplotypes were found to have the highest identity with specimens listed in Table 13 on page 133. This table indicates that haplotypes 1 to 15 are forms of *R. prolixus cytb* with the exception of haplotype 3, which is *R. robustus*. Haplotypes 16 to 18 also show greatest similarity with *R. robustus*

6.1.2 Basic statistical analysis

The analysis of the 551 sequences (excluding the outgroups and donated FM sequences) revealed 18 haplotypes, 14 of which were unique to single States (private haplotypes) and 8 of these unique haplotypes were found only once in the study (singletons) (see Figure 13 on page 136, Table 14 on page 135and appendix Table 63 on page 314).

Haplotypes occurred among the specimens in different frequencies. Haplotype 1 accounted for more than half the sequences analysed (67.7%) and had the widest distribution, occurring in all States with the exception of Trujillo and in all ecotopes. Haplotype 3 (putative *R. robustus*) was the next most frequently encountered haplotype (12.5%), however, this haplotype occurred primarily in Portuguesa, with a total of three specimens detected in other States (see Table 14, appendix Table 63). This haplotype was also found in all ecotopes, with more than 50% found in houses. Haplotype frequencies also varied in different States; Lara was homogenous for haplotype 1, while in Guarico haplotype 1 (35.3%) and haplotype 2 (64.7%) occurred. In Portuguesa haplotype 1 (67.3%) and haplotype 3 (32.7%) were dominant among the six haplotype detected, while in Trujillo haplotype 5 dominated (74.1%). Among the 11 haplotypes detected in Barinas haplotype 1 (33.3%) and haplotype 14 (66.7%) occurred most frequently. In Cojedes haplotype 1 dominated (96%).

The alignment of the 18 haplotypes yielded 46 variable sites (11.1% polymorphism), 16 of which were parsimony informative (3.9%), while 30 were autapomorphic (see Figure 13 on page 136), (see Glossary on page 310) When the eight extra sequences (FM1-

FM8) from Monteiro were added polymorphism increased to 12.8%, with 39 of the 53 variable sites parsimony-informative (9.4%). In the alignment of all 551 specimens sequenced, 40 sites were parsimonious informative (9.6%). As expected for a protein coding gene, third base codons were most variable. The estimated nucleotide frequencies were T=31.6%, C=23.8%, A=31.6% and G=13.1%, corresponding to a high A:T rich content (63.2%) as expected for insect mitochondrial DNA. Codon frequency analysis for of all haplotypes demonstrated a preference for base T in the second codon position (42.8%) and base A at the third codon position (43.8%).

Table 14. The distribution of haplotypes detected in the study per State.

Haplotype	Lara	Portuguesa	Guarico	Barinas	Cojedes	Trujillo	Total
Haplotype 1	24 (9N)	200 (58N)	7 (4N)	98 (2N)	44 (15N)	-	373
Haplotype 2	-	6 (2N)	12 (11N)	16	-	-	34
Haplotype 3	-	66 (14N)	-	1	2	-	69
Haplotype 4	-	-	-	6	-	-	6
Haplotype 5	-	2	-	7	-	20 (8N)	29
Haplotype 6	-	-	1	-	-	-	1
Haplotype 7	-	-	-	1	-	-	1
Haplotype 8	-	1	-	-	-	-	1
Haplotype 9	-	12 (6N)	-	-	-	-	12
Haplotype 10	-	-	-	1	-	-	1
Haplotype 11	-	-	-	1	-	-	1
Haplotype 12	-	-	-	2	-	-	2
Haplotype 13	-	-	1	-	-	-	1
Haplotype 14	-	-	-	12	-	-	12
Haplotype 15	-	-	-	1	-	-	1
Haplotype 16	-	-	-	-	-	4	4
Haplotype 17	-	-	-	-	-	2	2
Haplotype 18	-	-	-	-	-	1	1
State total	24	287	21	146	46	27	551

In parenthesis number of domestic nymphs analysed per haplotype in each State indicating colonisation of that haplotype.

The deduced amino acid sequence among the haplotypes examined was very homogenous with only 1 of the 138 amino acids encoded involving nonsynonymous peptide changes which were parsimonious informative (resulting in alanine (ALA) instead of threonine (THR) in 10 haplotypes). Of the twenty amino acids encoded

leucine was the most frequent (17.4%), followed by isoleucine (9.4%), phenylalanine (8.7%), proline (8.0%) and glycine (6.5%). No stop codons were recorded.

All 46 variable sites between the haplotype sequences involved base changes (point mutations), no insertions or deletions occurred. Of these, 8 base changes consisted of transitional changes and 1 transversional, with a transition/transversion ratio of 12.4 for the gene fragment, the transitional change most common was T↔C and this occurred with the most frequency at the third base.

```
                11 1111111111 1222222222 3333333333 333344
          3446678900 0124455668 9234778999 0011233345 688900
          3253934336 9401309053 8273032147 5658706924 917325
             R   R  R  R                  R
Hap1      ACTCCGCCAG TTCACTCCAC CATAAGTCCA TCCGCCTCGT CACTAA (373)
Hap2      ........G. .......... .......... .......... ...... (34)
Hap3      .AC.T...GA .CT.TCTTGT TGC.GACTA. .T.AT..TAC .G.C.. (69)
Hap4^     ........G. .......... ........T. .......... ...... (6)
Hap5      .......... .......... ........T. .......... ...... (29)
Hap6*     .......AG. .......... .......... .......... ......
Hap7*     ........G. C......... ........T. .......... ......
Hap8*     ......T... .......... ........T. ..T....... T.....
Hap9^     .......... .....C.... .......... .......... ...... (12)
Hap10*    ........G. ...G...... .......... .......... ......
Hap11*    ........G. .......... ........T. C......... ......
Hap12^    .......... .......... ........T. .......... T..... (2)
Hap13*    ...T...A.. .......... .......... .......... ......
Hap14^    .....A.... .......... .......... .......... ...... (12)
Hap15*    .......... .......... .......... .......... ..T...
Hap16^    G......AG. ...G..TT.. ...G.....G ......C.A. ....GG (4)
Hap17^    G......AG. ...G..TT.. ...G.....G .....TC.A. ....GG (2)
Hap18*    G......AG. ...G..TT.. ...G.....G ...A..C.A. ....GG
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Figure 13. Alignment of the polymorphic sections of the 18 haplotypes detected in study. Hap=haplotype. *Indicates haplotypes that were unique to a single species, ^ indicates haplotypes unique to a single State. R indicates the positions in which a bp substitution would result in an amino acid replacement (total alignment 551 sequences). Numbers in parenthesis represent the frequency of that haplotype in the study.

6.1.3 Distribution of haplotypes

The distribution of haplotypes is examined on a locality, ecotope and State level (see Table 14 on page 135, appendix Table 63 on page 314). Specimens are pooled by ecotope within a locality when collected from multiple sources in low numbers.

Population diversity and heterogeneity was investigated at a number of hierarchical levels (1) collection sites within States (34 groups) (2) division of all specimens by ecotope (3 groups) (3) by State (5 groups) and (4) State partitioned by ecotope (11 groups). Indices of population subdivision (F_{ST}) were derived from haplotype frequencies using Arlequin V2.000.

6.1.3.1 Genetic diversity and heterogeneity at locality level

In **Lara** two localities were sampled (Guamarito and Salvador) and specimens were recovered from the domestic environment only. A total of 24 specimens were analysed by direct sequencing revealing a very homogenous group sharing a single haplotype (haplotype 1). Both adults and nymphs (9 specimens) were collected in Guamarito indicating the presence of a domestic colony. At a State level Lara was the least heterogeneous with all other States presenting 2 to 11 haplotypes.

In **Guarico** a total of 21 specimens were analysed from silvatic ecotopes only in four localities (El Sombero, El Manguito, Bravero, Ortiz). Four haplotypes were detected including two unique to this State (haplotypes 1, 2, 6, 13). Haplotypes were very similar differing at only 3 sites. Both adults and nymphs were analysed. Haplotype 6 and 13 were represented by single adults only. Nucleotide diversity (π) for Guarico was low (0.002 ± 0.002) as haplotypes detected were very similar differing at only 3 sites.

In **Cojedes** 46 specimens were collected from a single house and adjacent palm (24) in the locality of Las Queseras, both ecotopes included both adults and nymphs. Domestic specimens represented a very homogenous group revealing a single haplotype (Haplotype 1), which was shared with the adjacent palm population. An additional haplotype (haplotype 3) was detected in two adult specimens from the palm. This haplotype is shared with silvatic and domestic adult and nymph specimens from Portuguesa and a single domestic adult from Barinas. Nucleotide diversity (π) for Cojedes was higher ($0.01 \pm$ s.e. 0.004) as although only 2 haplotypes were detected

these differed at 29 polymorphic sites. F_{ST} between the ecotopes was low and non-significant (F_{ST}=0.04, p-value=0.494).

In **Trujillo** a total of 27 specimens were analysed and 4 haplotypes were detected. Twenty- one specimens were collected from a single house. The presence of nymphs (8) indicated a domestic colony. The population was quite homogenous with 20 specimens sharing a single haplotype (haplotype 5), also detected in Portuguesa and Barinas, including silvatic ecotopes. A single domestic adult female was determined as haplotype 16 (Venezuelan *R. robustus*), unique to this State. Three of the 6 silvatic specimens analysed were collected in the field (La Juventud) and three originated from named *R. robustus* colonies in the University of Los Andes. These six specimens revealed 3 haplotypes unique to this State (haplotypes 16,17, 18). In contrast to the other States in the study haplotype 1 was not detected in Trujillo. Nucleotide diversity (π) for Trujillo was high (0.01 ± s.e. 0.007) with the 4 haplotypes detected differing by 15 polymorphic sites. F_{ST} between silvatic and domestic ecotopes was very high and significant (F_{ST}=0.75, p-value<0.0001).

Table 15. Summary of the haplotypes detected in localities in Portuguesa State.

Locality	Specimen no.	Ecotope	Haplotypes detected
Terronal	142	Domestic, Silvatic, Peridomestic	Haplotype 1,2,3
Peña Negra	10	Domestic	Haplotype 1
Palmarito	5	Domestic, Silvatic	Haplotype 1,3
San Bartolo	29	Domestic	Haplotype 1
Santa Lucia	17	Domestic	Haplotype 1, 9*
Casa Rena	17	Domestic, Silvatic, Peridomestic	Haplotype 1,3
Qdra Negra	12	Domestic	Haplotype 1,2,5,8*
Palo Gacho	10	Silvatic	Haplotype 1,3
Morichal	5	Domestic	Haplotype 1,3
El Mosquito	8	Domestic	Haplotype 1,2
Los Rastrojos	32	Domestic, Silvatic	Haplotype 1,2

* indicates unique to State

In **Portuguesa** State 11 localities were sampled (Terronal, Peña Negra, Palmarito, San Bartolo, Santa Lucia, Casa Rena, Qdra. Negra, Palo Gacho, Morichal, El Mosquito and Los Rastrojos) (see Table 15 above, appendix Table 63 on page 314). A total of 287

specimens were analysed including 180 domestic, 3 peridomestic and 104 silvatic. Analysis identified 6 haplotypes two of which were dominant, haplotype 1 (69.7%) and haplotype 3 (22.9%). Haplotype 1 occurred in all 10 localities, in domestic, silvatic and peridomestic ecotopes and in both adults and nymphs. Haplotype 3 was detected in 5 of the 10 localities (Terronal, Palo Gacho, Palmarito, Casa Rena and Morichal), in all ecotopes, and in nymphs and adults in both silvatic and domestic ecotopes. Two haplotypes unique to this State were also detected (Haplotype 8 and 9). Nucleotide diversity (π) was highest in Portuguesa (0.03 ± s.e. 0.013) with the 6 haplotypes detected differing in 32 polymorphic sites.

Domestic specimens in **Terronal** were collected from two houses in 2001, which were resampled in 2003. Silvatic specimens were collected from different sites in both years. A total of 32 specimens were analysed from house 1 over both years, nymphs present in both years indicated colonisation (7 nymphs analysed from 2001, 6 nymphs 2003). Two haplotypes were detected and in similar proportions each year, haplotype 3 (70%) and haplotype 1. Six silvatic specimens collected from a nearby palm were homogenous for haplotype 1.

Table 16. F_{ST} values (p-values above diagonal) from pairwise comparisons of populations in Terronal.

	House 1 01	House 2 01	Palm house 2 01	House 1 03	Palm house 1 03	Palm house 2 03
House 1 01	-	0.020	**<0.001**	0.065	0.005	**0.001**
House 2 01	0.18	-	0.184	0.698	0.035	0.261
Palm house 2 01	0.40	0.03	-	0.437	0.250	0.767
House 1 03	0.20	-0.07	-0.02	-	0.252	0.659
Palm house 1 03	0.54	0.16	0.01	0.14	-	0.052
Palm house 2 03	0.37	0.01	-0.03	-0.04	0.04	-

Values in bold significant after Bonferroni correction k=15, p1=0.05/15, p≤0.004.

A total of 104 specimens were analysed from Terronal house 2 from both years, including 31 domestic (including 13 nymphs analysed from 2001), 1 peridomestic and 72 silvatic specimens from various palms. Haplotype 1 dominated silvatic ecotopes each year (81%) followed by haplotype 3. Haplotype 2 was detected in a single silvatic

specimen in 2001. Haplotype 1 was also dominant in houses (67% in 2001), found together with haplotype 3; only four domestic specimens were collected in 2003.

Population heterogeneity was examined between samples divided by ecotope and year of collection. Specimens from house 2 collected in 2003 were omitted due to small numbers (appendix Table 63 on page 314). F_{ST} values ranged from 0 to 0.54. Two pairwise comparisons remained significant after sequential Bonferroni correction (see page 107), between house 1 01 and palm populations at house 2 (F_{ST}=0.54, 0.37, p-value $\leq$ 0.001). Population subdivision was low and non-significant between all ecotopes, between house 2 domestic and silvatic ecotopes (F_{ST}=0.03, 0.01, p-value=0.18, 0.26), between silvatic ecotopes (F_{ST}= -0.03 to 0,04) (see Table 16 on page 139).

The 10 specimens analysed from a single house in the locality **Peña Negra** were homogenous for haplotype 1 (adults and nymphs). Five nymph specimens analysed from silvatic and domestic ecotopes in the locality **Palmarito** were heterogeneous for haplotype 1 and 3. In **San Bartolo** 29 specimens (adults and nymphs) from three houses were homogenous for haplotype 1. The analysis of 17 domestic specimens (adults and nymphs) from 4 houses in the locality **Santa Lucia** revealed a unique and dominant haplotype (haplotype 9) in addition to haplotype 1.

In **Casa Rena** haplotype 3 dominated among the 10 domestic (including 3 nymphs), 2 peridomestic and three silvatic specimens analysed (adults and nymphs) with haplotype 1 detected in only two silvatic specimens. In **Qdra. Negra** 12 domestic adult specimens (from various sources) were analysed, revealing three haplotypes (haplotype 1, 2, 5) and a unique haplotype 8 in a single adult specimen. In **Palo Gacho** the analysis of 10 silvatic nymph specimens revealed a dominant haplotype 3 occurring with haplotype 1. Two haplotypes (haplotype 1 and 3) were detected among the 5 domestic adult specimens from **Morichal**, while haplotypes 1 and 2 were detected among 8 domestic specimens (1 nymph) from the locality **El Mosquito.**

Twenty-two specimens (1 adult male and 21 nymphs) were analysed from the locality **Los Rastrojos** from a single house and 10 specimens from an adjacent palm (all nymphs). Both ecotopes shared haplotype 1, while 2 domestic specimens were identified as haplotype 2. Population heterogeneity was not detected between silvatic and domestic specimens (F_{ST}= -0.01, p-value=0.084) (see Table 17 below)

Population heterogeneity was also investigated between localities within Portuguesa. Samples were divided by ecotope. Localities Palmarito, Morichal and El Mosquito were excluded due to small numbers. Casa Rena silvatic/peridomestic specimens and Terronal house 2003 were also excluded (Table 17 below).

Table 17. F_{ST} values (p-values above diagonal) from pairwise comparisons of populations in Portuguesa.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Terronal h1 01	-	0.022	**<0.0001**	0.724	**0.0001**	**<0.0001**	0.0004	0.139	**<0.0001**	**<0.0001**	0.001	0.0002	0.018
2 Terronal h2 01	0.18	-	0.189	0.185	0.256	0.010	0.078	1.000	0.001	**<0.0001**	0.080	0.082	0.0002
3 Terronal h2 p01	0.40	0.03	-	0.007	0.771	0.164	0.267	0.185	0.019	**<0.0001**	0.114	0.265	**<0.0001**
4 Terronal h1 03	0.00	0.06	0.27	-	0.017	0.0003	0.006	0.217	**<0.0001**	**<0.0001**	0.014	0.006	0.024
5 Terronal h2 p03	0.37	0.01	0.00	0.24	-	0.071	0.184	0.224	0.013	**<0.0001**	0.074	0.185	**<0.0001**
6 Los Rastrojos h	0.56	0.18	0.04	0.46	0.07	-	0.084	0.006	0.186	**<0.0001**	0.126	0.093	**<0.0001**
7 Los Rastrojos p	0.58	0.20	0.05	0.49	0.08	0.00	-	0.085	1.000	0.001	0.097	1.000	**<0.0001**
8 Palo Gacho p	0.09	0.00	0.07	0.00	0.04	0.28	0.33	-	0.003	0.001	0.113	0.088	0.013
9 San Bartolo h	0.70	0.32	0.12	0.66	0.16	0.07	0.00	0.54	-	**<0.0001**	0.005	1.000	**<0.0001**
10 Santa Lucia h	0.51	0.44	0.51	0.45	0.51	0.60	0.63	0.42	0.75	-	0.001	0.001	**<0.0001**
11 Qdra Negra h	0.37	0.09	0.05	0.24	0.07	0.08	0.14	0.09	0.30	0.38	-	0.101	**<0.0001**
12 Peña Negra h	0.58	0.20	0.05	0.49	0.08	0.00	0.00	0.33	0.00	0.63	0.14	-	**<0.0001**
13 Casa Rena h	0.20	0.55	0.72	0.33	0.71	0.88	1.00	0.56	1.00	0.73	0.70	1 00	-

Values in bold remain significant following Bonferroni correction k=561, p1=0.05/561 , p≤0.0001. (Table is subsection from all State locality comparisons see Table 20 on page 148).

From the UPGMA tree produced (see Figure 14 on page 142) population homogeneity detected between ecotopes at locality level is also seen to occur at the State level. Isolation of Santa Lucia from the majority of other populations is also visible with F_{ST} ranging from 0.38 to 0.75 and all but 4 pairwise comparisons were significant, with exceptions including Los Rastrojos and Palo Gacho palm (see Table 17 above). Casa Rena was also significantly different from the majority of populations, with exception of Terronal h1 01/03, h2 01 and Palo Gacho (F_{ST}= 0.20-1.0) (see Table 17). From 78

pairwise comparisons within Portuguesa State 20 remained significant after Bonferroni correction (27%), indicating insignificant population heterogeneity is found within the State (see Table 17 on page 141).

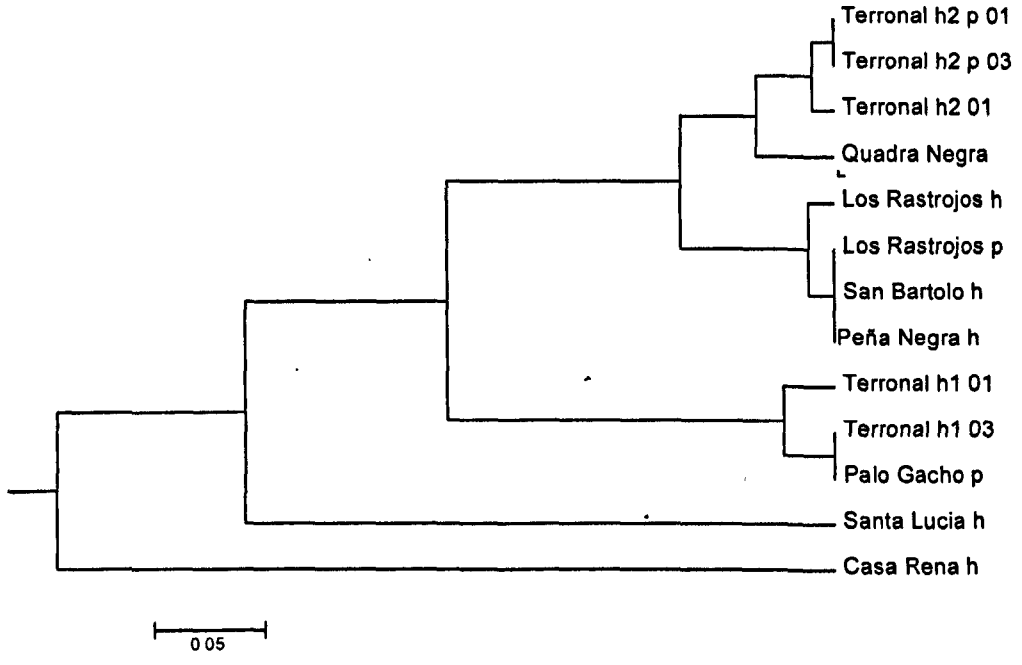

Figure 14. UPGMA tree of pairwise F_{ST} values between localities in Portuguesa. h=house, p=palm.

In **Barinas** State 12 localities were sampled (S. Elena de Caramuca, Obispos, San Isidero Acequita, Carreteron, Cascabel, Guaranda, Laguna Hermosa, G. Paraguey, Parcelamiento, 19 Abril, Rio Bravo II) in both silvatic, domestic and peridomestic ecotopes. Rio Bravo II was sampled at adjacent peridomestic and silvatic ecotopes. A total of 146 specimens were analysed, 47 of which were collected in the domestic environment, 35 in the peridomestic, and 64 from palms. At State level Barinas was the most heterogeneous in the study in terms of haplotype number. A total of 11 haplotypes were identified, seven of which were unique to this State and four of which occurred in single specimens. Nucleotide diversity (π) was determined as 0.003 ± s.e. 0.002, values were low as 10 haplotypes were very similar with only 8 variable sites. When haplotype

3 was included variability increased to 35 sites but this haplotype was detected in a single specimen.

Table 18. Summary of haplotypes detected in localities in Barinas State

Locality	No.	Ecotopes	Haplotypes detected
Santa Elena de Caramuca	2	Silvatic	Haplotype 4*
Obispos	1	Domestic	Haplotype 1
San Isidero	1	Domestic	Haplotype 1
Acequita	3	Peridomestic	Haplotype 1
Carreteron	20	Domestic, Silvatic	Haplotype 1, 3,4*,5, 7 *
Cascabel	32	Domestic, Silvatic, Peridomestic	Haplotype 1,2 4*,5, 12*
Guaranda	17	Domestic, Silvatic	Haplotype 1,2,10*
Laguna Hermosa	27	Domestic, Peridomestic, Silvatic	Haplotype 1,2,5,11*,12*
G. Paraguey	8	Domestic	Haplotype 1,2,5
Parcelamiento	10	Silvatic	Haplotype 1,14*,15*
19 Abril	10	Peridomestic	Haplotype 14*
Rio Bravo II	15	Peridomestic (6), Silvatic (9)	Haplotype 1,2

* indicates haplotypes unique to this State. No.=number of specimens amplified

Localities **Obispos, San Isidero, S. Elena** and **Acequita** were represented by a total of 7 specimens from all ecotopes. Two haplotypes were detected: haplotype 1 and haplotype 4 (unique to Barinas). These localities were excluded from analysis by F_{ST} due to small numbers.

A total of 20 specimens were analysed from the locality **Carreteron** including specimens from domestic and silvatic ecotopes. Domestic specimens (multiple sources) were more heterogeneous, represented by five haplotypes, four of which were shared with other localities in the study (haplotype 1, 3, 4 and 5) and one unique haplotype detected in a single adult (haplotype 7). Silvatic specimens were homogenous for haplotype 1. F_{ST} values were high but non-significant (F_{ST}= 0.17, p-value=0.021) (see Table 19 on page 145).

A total of 32 specimens were analysed from the locality **Cascabel** including 8 adult domestic specimens, 9 peridomestic (including 3 nymphs) and 15 silvatic specimens (populations not adjacent). The silvatic specimens were the most heterogeneous presenting 4 haplotypes, including haplotype 12, unique to this State and shared with a

single peridomestic specimen from Laguna Hermosa. All ecotopes shared haplotypes 1 and 2, while haplotype 4 was detected in a single peridomestic nymph and haplotype 5 in single silvatic and domestic specimens. F_{ST} values were highest between silvatic and domestic ecotopes (F_{ST}= 0.04), however all pairwise comparisons were non-significant (p-values= 0.28-0.93), indicating a lack of population heterogeneity (see Table 19 on page 145).

In **Guaranda** five domestic (including 2 nymphs) and 12 silvatic specimens were analysed (populations not adjacent). The silvatic specimens were more heterogeneous displaying three haplotypes including haplotype 10, a singleton, and haplotype 1 and 2. Domestic specimens were homogenous for haplotype 1. F_{ST} values indicated lack of population heterogeneity (F_{ST}= -0.06, p-value=0.51) (see Table 19).

In the locality **Laguna Hermosa** a total of 27 specimens were analysed, 11 domestic (all adults), 9 silvatic specimens and 7 peridomestic (including 3 nymphs). All three ecotopes shared common haplotypes (1 and 5). Silvatic specimens were more heterogeneous with the detection of two additional haplotypes in two specimens (haplotype 2 and the singleton haplotype 11). Haplotype 12 also occurred in a single peridomestic specimen. F_{ST} values were greatest between silvatic and domestic populations (F_{ST}= 0.02), but all pairwise comparisons were non-significant, and no heterogeneity was detected between ecotopes (see Table 19 on page 145)

A total of 8 adult specimens were analysed from two houses in **G. Paraguey** where 3 haplotypes were detected (haplotypes 1, 2 and 5) (populations not adjacent). Ten silvatic specimens from **Parcelamiento** presented two haplotypes in addition to haplotype 1; haplotype 15 (a singleton) and haplotype 14, unique to this State and shared with the 10 peridomestic specimens (including 4 nymphs) analysed from the locality **19 Abril**. Apto specimens were found to be significantly different from all other populations (F_{ST}= 0.62-1.0, p-value<0.001), with the exception of Guaranda house and Parcelamiento palm, although F_{ST} values were very high (F_{ST}=0.64, 1.00).

Table 19. F_{ST} values (p-values above diagonal) from pairwise comparisons of localities in Barinas State.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 Carreteron p	-	0.021	0.054	0.198	0.044	1.000	0.511	1.000	**<0.0001**	0.063	0.193	0.051	**<0.0001**	**<0.0001**	0.178
2 Carreteron h	0.17	-	0.298	0.332	0.052	0.109	0.054	0.049	0.369	0.405	0.339	0.218	**<0.0001**	0.139	0.148
3 Cascabel pd	0.24	0.01	-	0.269	0.926	0.227	0.099	0.070	0.287	0.666	0.272	0.168	**<0.0001**	0.230	0.612
4 Cascabel h	0.05	0.00	0.00	-	0.280	0.484	1.000	0.688	1.000	0.842	1.000	0.473	**<0.0001**	0.676	1.000
5 Cascabel p	0.28	0.09	0.00	0.04	-	0.092	0.055	0.018	0.156	0.293	0.278	0.053	**<0.0001**	0.234	0.265
6 Guaranda h	0.00	0.12	0.18	0.00	0.24	-	0.512	1.000	0.155	0.506	0.485	0.498	0.0004	1.000	0.115
7 Guaranda p	0.00	0.08	0.07	0.00	0.15	0.00	-	1.000	0.609	0.770	1.000	0.247	**<0.0001**	1.000	0.801
8 Laguna Hermosa h	0.00	0.12	0.19	0.00	0.24	0.00	0.00	-	0.534	0.289	0.695	0.091	**<0.0001**	0.511	0.334
9 Laguna Hermosa pd	0.08	0.00	0.03	0.00	0.08	0.02	0.00	0.00	-	1.000	1.000	0.630	**<0.0001**	1.000	0.487
10 Laguna Hermosa pd	0.09	0.00	0.00	0.00	0.02	0.03	0.00	0.02	0.00	-	0.855	0.629	**<0.0001**	0.558	1.000
11 G. Paraguey h	0.05	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	-	0.475	**<0.0001**	0.696	1.000
12 Parcelamiento p	0.10	0.03	0.06	0.00	0.13	0.05	0.01	0.05	0.00	0.00	0.00	-	0.0007	0.559	0.435
13 Apt Abril 19 pd	1.00	0.62	0.69	0.79	0.62	1.00	0.83	0.90	0.78	0.72	0.79	0.68	-	**<0.0001**	**<0.0001**
14 Rio Bravo II pd	0.03	0.04	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.88	-	1.000
15 Rio Bravo II p	0.09	0.06	0.00	0.00	0.03	0.03	0.00	0.03	0.00	0.00	0.00	0.01	0.82	0.00	-

Values in bold remain significant following Bonferroni correction k=561, p1=0.05/561 , p≤0.0001. h=house, p=palm, pd=peridomestic. (Table is subsection from all State locality comparisons see Table 20 on page 148).

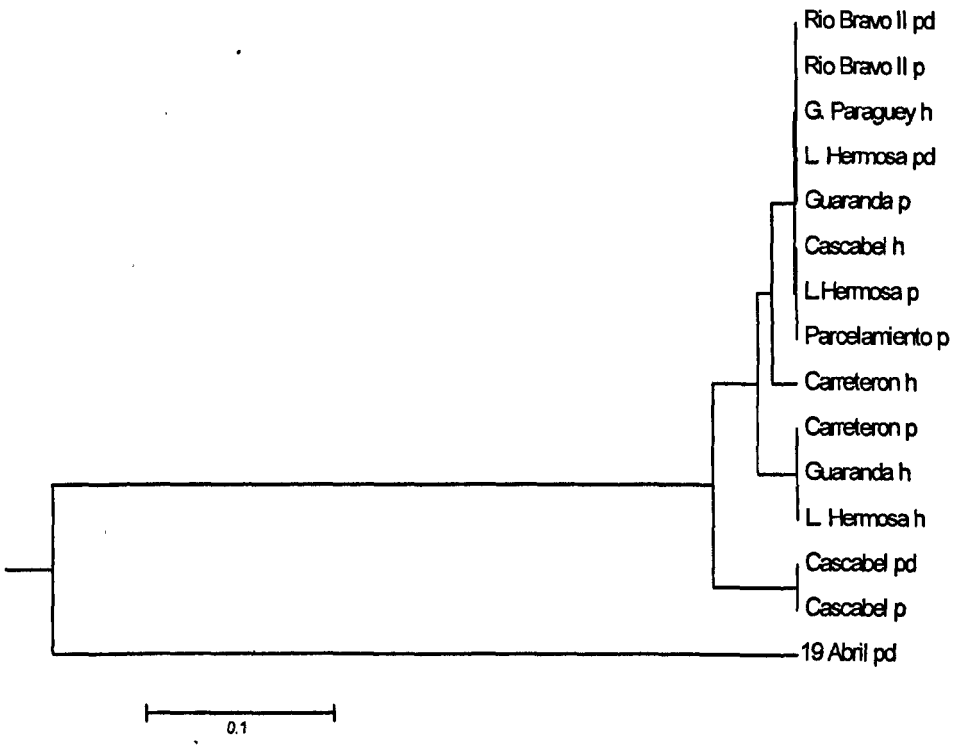

Figure 15. An UPGMA tree for pairwise F_{ST} values between localities in Barinas. h=house, p=palm, pd=peridomestic

In **Rio Bravo II** the peridomestic population, including 4 nymphs, and adjacent silvatic population presented a mixture of haplotype 2 and haplotype 1 specimens. Low values of F_{ST} indicated a lack of population division (F_{ST} = -0.15 p-value=1.0)

From the UPGMA tree produced (see Figure 15 above) homogeneity detected between different ecotopes at locality level is also found at State level. Isolation of Apto Abril 19 from other populations is also visible with F_{ST} ranging from 0.62 to 1.0 (see Table 19 on page 145). From 105 pairwise comparisons within Barinas State only those between 19 Abril pd remained significant after Bonferroni correction (13%), indicating weak population heterogeneity (see Table 19). No significant population heterogeneity was detected between Cascabel peridomestic and palm, Rio Bravo II peridomestic and palm.

6.1.3.2 Comparisons across all localities

Across all localities 561 pairwise comparisons were made, 144 of which remained significant following Bonferroni correction (26%) (see Table 20 on page 148). A total of 376 pairwise comparisons were made between populations from differing States, 109 remained significant following Bonferroni correction (29%), indicating that heterogeneity also occurs at State level. From the UPGMA tree produced (see Figure 16 on page 149) population homogeneity between different ecotopes at locality level also occurs across all States. However, populations of 19 Abril, Casa Rena, Santa Lucia and Trujillo remained significantly differentiated from the majority of other populations (see Table 20).

Heterogeneity detected at individual State level (see Figure 14 on page 142, Figure 15 on page 146) is largely maintained e.g. the cluster Rio Bravo to Parcelamiento is also found in the Barinas state F_{ST} tree, with the addition of locality Qdra Negra from Portuguesa (see Figure 16). The cluster of populations from Terronal house 2 is also maintained in the comparison of all States, as with Terronal h1 01. Some locality relationships have been rearranged with Guaranda domestic specimens clustering with San Bartolo, and Cascabel palm with Guarico palm.

Table 20. F_{ST} values (p-values above diagonal) from pairwise comparisons of localities across all States.

Pop	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1		0.02	**<0.01**	0.72	**<0.01**	**<0.01**	<0.01	0.14	**<0.01**	**<0.01**	<0.01	<0.01	0.018	**<0.01**	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	**<0.01**	0.01	<0.01	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	<0.01
2	0.18		0.19	0.19	0.26	0.01	0.08	1.00	0.001	**<0.01**	0.00	0.08	**<0.01**	0.15	0.08	0.03	0.17	**<0.01**	0.28	0.01	0.05	0.22	0.13	0.16	0.09	**<0.01**	0.14	0.11	**<0.01**	0.04	**<0.01**	**<0.01**	**<0.01**	**<0.01**
3	0.40	0.03		0.01	0.77	0.16	0.27	0.19	0.02	**<0.01**	0.11	0.27	**<0.01**	0.34	0.03	0.03	0.33	**<0.01**	0.24	0.38	0.23	0.34	0.21	0.33	0.15	**<0.01**	0.57	0.28	0.05	0.24	0.014	**<0.01**	**<0.01**	**<0.01**
4	-0.03	0.06	0.27		0.02	**<0.01**	0.01	0.22	**<0.01**	**<0.01**	0.01	0.02	0.024	0.02	0.03	0.02	0.018	**<0.01**	0.04	0.01	0.01	0.04	0.02	0.02	0.02	**<0.01**	0.04	0.01	**<0.01**	**<0.01**	**<0.001**	**<0.01**	**<0.01**	**<0.01**
5	0.37	0.01	-0.02	0.24		0.07	0.18	0.22	0.01	**<0.01**	0.07	0.19	**<0.01**	0.08	0.04	0.02	0.26	0.001	0.56	0.23	0.20	0.31	0.18	0.26	0.11	**<0.01**	0.32	0.17	0.01	0.14	0.03	**<0.01**	**<0.01**	**<0.01**
6	0.56	0.18	0.04	0.46	0.07		0.08	0.01	0.19	**<0.01**	0.13	0.09	**<0.01**	1.00	<0.01	0.02	0.55	0.01	1.00	0.70	0.69	0.10	0.13	0.55	0.02	**<0.01**	1.00	0.56	0.50	0.47	0.22	<0.01	**<0.01**	**<0.01**
7	0.58	0.20	0.05	0.49	0.08	0.00		0.09	1.00	<0.01	0.10	1.00	**<0.01**	1.00	0.02	0.03	0.18	<0.01	1.00	0.49	0.48	0.15	0.08	0.19	0.21	**<0.01**	0.39	0.21	1.00	0.57	1.00	<0.01	**<0.01**	**<0.01**
8	0.09	-0.06	0.07	-0.03	0.04	0.28	0.33		<0.01	<0.01	0.11	0.09	0.013	0.014	0.18	0.12	0.12	0.03	0.23	0.10	0.04	0.16	0.18	0.11	0.12	**<0.01**	0.24	0.12	0.01	0.01	<0 01	<0.01	**<0.01**	<0.01
9	0.70	0.32	0.12	0 66	0.16	0.07	0.00	0.54		**<0.01**	0.01	1.00	**<0.01**	1.00	<0.01	**<0.01**	0.04	**<0.01**	1.00	0.08	0.28	**<0.01**	0.01	0.04	0.02	**<0.01**	0.16	**<0.01**	1.00	0.20	1.00	<0.01	**<0.01**	**<0.01**
10	0.51	0 44	0.51	0.45	0.51	0.60	0 63	0.42	0.75		<0.01	<0.01	**<0.01**	**<0.01**	<0.01	<0.01	<0.01	**<0.01**	**<0.01**	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	**<0.01**	<0.01	<0.01	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
11	0.37	0.09	0.05	0.24	0.07	0.08	0 14	0.09	0.30	0.38		0.10	**<0.01**	0.24	0.33	0.32	0.86	0.18	0.14	0.33	0.38	1.00	1.00	0.86	0.39	**<0.01**	0.84	0.60	0.01	0.02	0.01	0.011	**<0.01**	0.001
12	0.58	0.20	0.05	0.49	0.08	0.00	0 00	0.33	0.00	0.63	0.14		**<0.01**	1.00	0.02	0.03	0.18	0.01	1.00	0.48	0.49	0.15	0.09	0.18	0.21	**<0.01**	0.37	0.20	1.00	0.56	1.00	<0.01	**<0.01**	<0 001
13	0.20	0.55	0.72	0.33	0.71	0.88	1.00	0.56	1.00	0.73	0.70	1.00		**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	<0.01	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	<0.01
14	0.55	0.17	0.03	0.44	0.05	-0.03	0.00	0.27	0.00	0.59	0.09	0.00	1.00		0.02	0.02	0.20	0.04	1.00	0.51	1.00	**<0.01**	0.06	0.19	0.05	**<0.01**	**<0.01**	0.18	1.00	0.41	1.00	0.006	**<0.01**	<0.01
15	0.28	0.07	0.11	0.16	0.11	0.19	0.21	0.05	0.39	0.34	0.00	0.21	0.59	0.17		0.30	0.33	0.05	0.12	0.05	0.05	0.37	0.41	0.34	0.22	**<0.01**	0.14	0.15	**<0.01**	0.01	**<0.01**	0.003	**<0.01**	<0.01
16	0.36	0.15	0.16	0.24	0.18	0 20	0.30	0.12	0 51	0.37	0.01	0 30	0.69	0.24	0.01		0.27	0.93	0.23	0.10	0.07	0.29	0.67	0.27	0.17	**<0.01**	0.23	0.61	**<0.01**	0.01	<0.01	0.301	**<0.01**	<0.01
17	0.40	0.07	0.00	0.26	0.03	0.00	0.11	0.09	0.30	0.42	-0 10	0.11	0.79	0.05	0.00	-0.02		0.28	0.48	1.00	0.69	1.00	0.84	1.00	0.47	**<0.01**	0.68	1.00	0.06	0.07	0.06	0.049	**<0.01**	<0.01
18	0.36	0.20	0.23	0.26	0.25	0.26	0.33	0.17	0.50	0.37	0.05	0 33	0.62	0.28	0.09	-0.07	0.04		0.09	0.05	0.02	0.16	0.29	0.28	0.05	**<0.01**	0.23	0.27	**<0.01**	**<0.01**	**<0.01**	0.416	**<0.01**	<0.01
19	0.52	0.14	-0.01	0.40	0.02	-0.07	0.00	0.22	0.00	0.56	0 04	0 00	1.00	0.00	0.12	0.18	0.00	0.24		0.51	1.00	0.16	0.51	0.49	0.50	<0.01	1.00	0.12	1.00	1.00	1.00	0.025	**<0.01**	<0.01
20	0.46	0.11	0.00	0.34	0.03	-0.04	0.03	0.16	0.15	0.49	0.00	0.03	0 83	-0.01	0.08	0.07	-0.07	0.15	-0.06		1.00	0.61	0.77	1.00	0.25	**<0.01**	1.00	0.80	**<0.01**	0.32	0.11	0.004	**<0.01**	<0.001
21	0.51	0.14	0.02	0.40	0.04	-0.02	-0.01	0.22	0.10	0.55	0.02	-0 01	0.90	-0.05	0.12	0.19	-0.04	0.24	-0.09	-0.03		0.53	0.29	0.70	0.09	**<0.01**	0.51	0.34	0.33	0.69	0.31	0.002	**<0.01**	<0.001
22	0.38	0.07	0.02	0.24	0.04	0.07	0.15	0.07	0.36	0.40	-0 09	0.15	0.78	0.08	-0.02	0.03	-0.11	0.08	0.02	-0.03	-0.02		1.00	1.00	0.63	**<0.01**	1.00	0.49	0.05	0.09	**<0.01**	0.015	**<0.01**	<0.01
23	0.36	0.07	0.04	0.23	0.05	0.06	0.14	0.07	0.33	0.38	-0.08	0.14	0.72	0.09	-0.01	-0.03	-0.11	0.02	0.03	-0.03	0.02	-0.09		0.86	0.63	**<0.01**	0.56	1.00	**<0.01**	0.05	0.02	0.036	**<0.01**	<0.01
24	0.40	0.07	0.00	0.26	0.03	0.00	0.11	0.09	0.30	0.42	-0.10	0.11	0.79	0.05	0.00	-0.02	-0.14	0.04	0.00	-0.07	-0.04	-0.11	-0.11		0.48	**<0.01**	0.70	1.00	0.06	0.08	0.06	0.047	**<0.01**	<0.01
25	0.39	0.09	0.06	0.26	0.07	0.10	0.15	0.10	0.33	0.41	-0.01	0.15	0.74	0.10	0.03	0.06	-0.03	0.13	0.05	0.01	0.05	-0.03	-0.03	-0.03		<0.01	0.56	0.44	0.03	0.04	0.02	0.004	**<0.01**	<0.01
26	0.70	0.69	0.76	0.70	0.76	0.88	1 00	0.73	1.00	0.73	0.70	1.00	1.00	1.00	0.62	0.69	0.79	0.62	1.00	0.83	0.90	0.78	0.72	0.79	0.68		**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	<0.01
27	0.44	0.08	-0 03	0.30	0.01	-0.08	0.09	0.11	0.31	0.47	-0.06	0.09	0.88	0.03	0.04	-0.03	-0.13	0.05	-0.03	-0 12	-0.04	-0.06	-0.09	-0.13	-0.03	0.88		1.00	0.22	0.23	0.20	0.061	**<0.01**	<0.01
28	0.43	0.11	0.03	0.30	0.06	0.00	0.14	0.13	0.33	0.46	-0.03	0.14	0.82	0.09	0.06	-0.04	-0.10	0.03	0.03	-0.06	0.03	-0.02	-0.06	-0 10	0.01	0.82	-0.15		0.08	0.14	0.07	0.070	**<0.01**	<0.01
29	0.67	0.28	0.10	0.61	0.14	0.05	0.00	0.48	0.00	0.72	0.25	0.00	1.00	0.00	0.33	0.45	0.24	0.45	0.00	0.11	0.07	0.30	0.28	0.24	0.28	1.00	0.25	0.28		0.49	1.00	**<0.01**	**<0.01**	**<0.01**
30	0.53	0.14	0.00	0.43	0.02	0.00	-0.01	0.24	0.06	0.61	0.11	-0.01	0.88	-0.04	0.20	0.28	0.06	0.33	-0.07	0.00	-0.02	0.09	0.11	0.06	0.12	0 89	0.01	0.09	0.04		**<0.01**	**<0.01**	**<0.01**	**<0.01**
31	0.68	0.29	0.11	0.62	0.14	0.05	0.00	0.50	0.00	0.73	0.26	0.00	1.00	0.00	0.35	0.47	0.26	0.46	0.00	0.12	0.08	0.32	0.29	0.26	0.29	1.00	0.27	0.29	0.00	0.04		**<0.01**	**<0.01**	**<0.01**
32	0.42	0.33	0.39	0.35	0.40	0.42	0.47	0.30	0.61	0.43	0.21	0.47	0.63	0.43	0.23	0.02	0.21	-0 01	0.40	0.31	0.40	0.26	0.18	0.21	0.28	0.63	0 22	0.18	0.57	0.47	0.58		**<0.01**	<0.01
33	0.72	0.70	0.76	0.72	0.76	0.86	0.94	0.75	0.96	0.75	0.67	0.94	0.94	0.93	0 63	0.72	0.76	0.63	0.92	0 82	0.86	0.75	0.71	0.76	0.76	0.94	0.85	0.81	0.95	0.87	0 96	0.66		**<0.01**
34	0 45	0 45	0 57	0.39	0 57	0.67	0.72	0.38	0 86	0.46	0 37	0.72	0 72	0.66	0 29	0.32	0.41	0.31	0.60	0 52	0.61	0 38	0.35	0.41	0.40	0 72	0 47	0.46	0 83	0.69	0.84	0.36	0 73	

Values in bold remain significant following Bonferroni correction k=561, p1=0.05/561 , p≤0.0001. h=house, p=palm, pd=peridomestic

Pops 1 Terronal h1 01, **2** Terronal h2 01, **3** Terronal h2 p01, **4** Terronal h1 03, **5** Terronal h2 p03, **6** Los Rastrojos h, **7** Los Rastrojos p, **8** Palo Gacho p, **9** San Bartolo h, **10** Santa Lucia h **11** Qdra Negra h, **12** Peña Negra h, **13** Casa Rena h, **14** Carreteron p, **15** Carreteron h, **16** Cascabel pd, **17** Cascabel h, **18** Cascabel p, **19** Guaranda h, **20** Guaranda p, **21** L. Hermosa h , **22** L. Hermosa pd, **23** L. Hermosa p, **24** G. Paraguey h, **25** Parcelamiento p, **26** Apto. 19 Abril pd, **27** Rio Bravo II pd, **28** Rio Bravo II p, **29** Cojedes h, **30** Cojedes p, **31** Lara h , **32** Guarico p, **33** Trujillo h, **34** Trujillo p

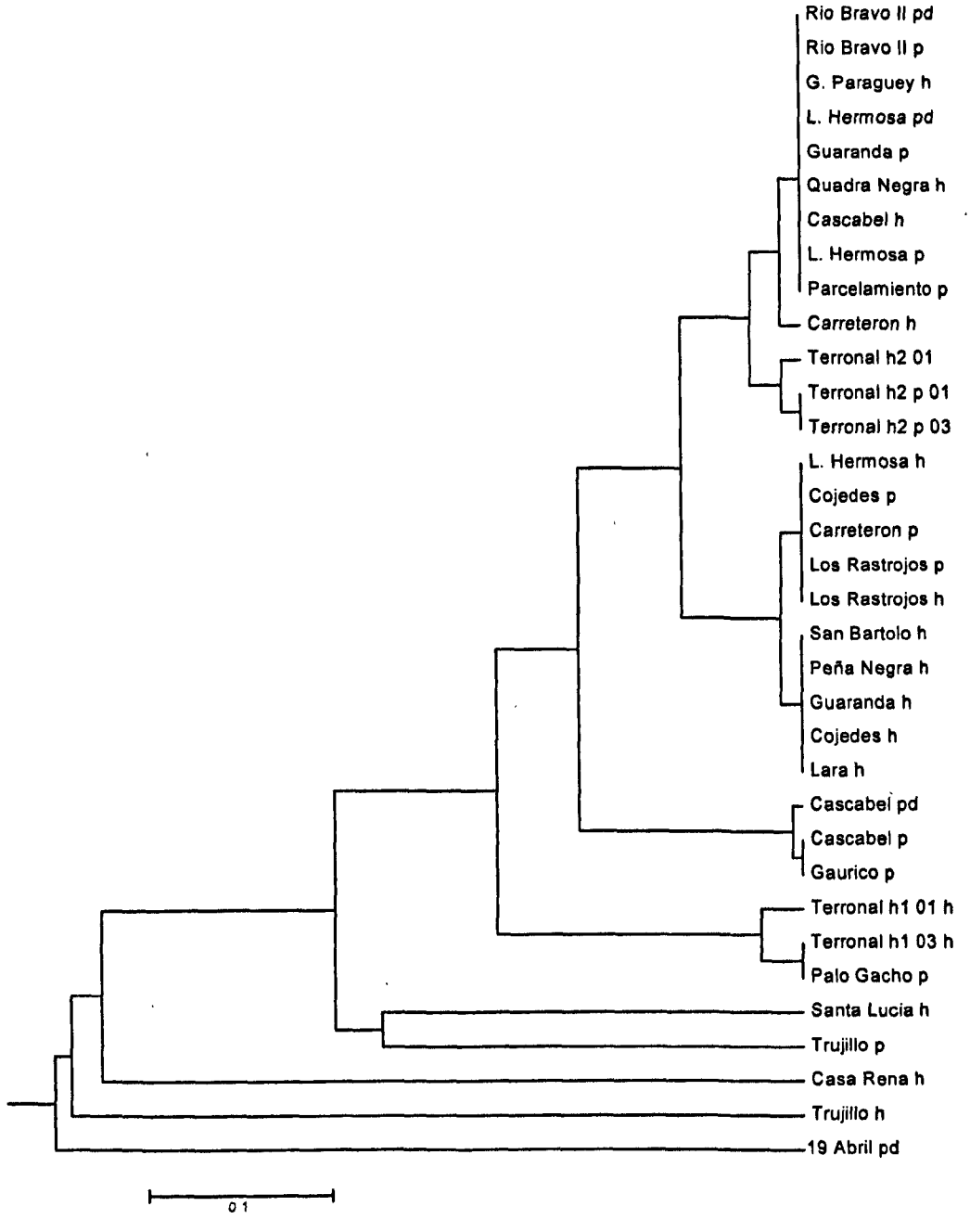

Figure 16. An UPGMA tree for pairwise F_{ST} values between localities from all States. h=house, p=palm, pd=peridomestic.

6.1.3.3 Specimens grouped by State

Population heterogeneity was also investigated between specimens grouped by State of collection (6 groups), and additionally State groups were further divided by ecotope (11 groups). The number of haplotypes per State varied from 1 to 11, with highest numbers in Barinas. Barinas also gave the highest number of private haplotypes (7). For comparisons of states, F_{ST} values were high and significantly different from zero (after Bonferroni correction), with the exception of Lara and Cojedes State (F_{ST}= 0.002 p-value=0.55) (see Table 21 below). Other pairwise comparisons ranged from F_{ST}=0.06 (p-value<0.001) between Barinas and Portuguesa and F_{ST}=0.77 (p-value<0.001) between Trujillo and Cojedes/Lara.

Table 21. F_{ST} values (p-values above diagonal) for pairwise comparisons for specimens grouped by State.

State	N	1	2	3	4	5	6
1 Portuguesa	287	-	**<0.001**	**<0.001**	**0.003**	**<0.001**	**<0.001**
2 Barinas	146	0.06	-	**<0.001**	**0.002**	**<0.001**	**<0.001**
3 Guarico	21	0.33	0.23	-	**<0.001**	**<0.001**	**<0.001**
4 Lara	24	0.13	0.11	0.58	-	**<0.001**	0.547
5 Trujillo	27	0.54	0.48	0.49	0.77	-	**<0.001**
6 Cojedes	46	0.10	0.1	0.59	0.002	0.77	-

Values in bold remain significant after Bonferroni correction (k=15, p1=0.05/15, p≤0.005). N=number of specimen analysed.

State samples were further subdivided by ecotope (11 groups) to investigate heterogeneity. Of the 55 pairwise comparisons 37 remained significant following Bonferroni correction (see Table 22 on page 151). F_{ST} values ranged from 0.01 to 0.95. Within States comparisons between ecotopes were non-significant in Portuguesa (F_{ST}=0.03, p-value=0.021), Cojedes (F_{ST}=0.04, p-value=0.492). In Barinas State significant heterogeneity was detected between peridomestic and domestic specimens (F_{ST}=0.11, p-value=0.001). In Trujillo silvatic and domestic specimens was significantly different from each other and to all other populations. Guarico was also significantly different from all other populations (F_{ST}=0.18-0.66, p-value ≤0.001).

Table 22. F_{ST} values (p-values above diagonal) from pairwise comparisons of specimens grouped by State and ecotope.

State ecotope	1	2	3	4	5	6	7	8	9	10	11
1 Portuguesa h	-	0.021	0.004	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.002	0.017	**<0.001**	**0.001**
2 Portuguesa p	0.03	-	0.026	**0.001**	**<0.001**	**<0.001**	**<0.001**	0.023	0.149	**<0.001**	0.020
3 Barinas h	0.06	0.04	-	0.185	**0.001**	**<0.001**	**<0.001**	0.027	0.089	**<0.001**	0.015
4 Barinas p	0.07	0.08	0.01	-	0.012	**<0.001**	**<0.001**	0.005	0.016	**<0.001**	0.004
5 Barinas pd	0.12	0.19	0.11	0.06	-	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.001**	**<0.001**
6 Trujillo h	0.58	0.73	0.68	0.62	0.57	-	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
7 Trujillo p	0.42	0.59	0.51	0.43	0.32	0.73	-	**<0.001**	**<0.001**	**0.001**	**<0.001**
8 Cojedes h	0.15	0.10	0.08	0.12	0.27	0.95	0.83	-	0.492	**<0.001**	1.000
9 Cojedes p	0.08	0.02	0.03	0.07	0.20	0.87	0.69	0.04	-	**<0.001**	**<0.001**
10 Guarico p	0.29	0.43	0.33	0.21	0.18	0.66	0.36	0.57	0.47	-	**<0.001**
11 Lara h	0.15	0.11	0.09	0.12	0.28	0.96	0.84	0.00	0.04	0.58	-

Values in bold remain significant after Bonferroni correction k=55, p1=0.05/55 (p≤0.001). h=house, p=palm, pd=peridomestic

6.1.3.4 Specimens grouped by ecotope

Table 23. Summary of haplotype distribution by ecotope among the 551 samples sequenced.

Ecotope	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	Total
Silvatic Palm	156	23	22	2	2	1*	-	-	-	1*	1*	1	1*	2	1*	3	2*	1*	219
Domestic House	198	7	45	3	26	-	1*	1*	12*	-	-	-	-	10	-	1	-	-	304
Peridom	19	4	2	1	1	-	-	-	-	-	-	1	-	-	-	-	-	-	28
Total	373	34	69	6	29	1	1	1	12	1	1	2	1	12	1	4	2	1	551

H= haplotype, Peridom=peridomestic

Of the 551 specimens analysed 294 were collected in domestic ecotopes, 219 in silvatic and 38 in peridomestic (see Table 23 above). Haplotype 1 was the dominant haplotype in all ecotopes. Silvatic ecotopes showed the greatest heterogeneity, with a total of 15 haplotypes detected, 7 of which were found exclusively in silvatic specimens. Domestic ecotopes also showed heterogeneity with 10 haplotypes detected including 3 unique. Six haplotypes were found among the peridomestic specimens analysed, all of which were shared with domestic and peridomestic specimens. Significant heterogeneity was detected between specimens grouped by silvatic and domestic ecotopes (F_{ST}=0.02, p-

value=0.004). All other comparisons were non-significant (F_{ST}=0.01, F_{ST}= -0.01, p-value= 0.24, 0.85).

6.1.4 Distance analysis

Nucleotide differences were calculated between the 18 haplotypes detected and donated sequences FM1-8. Distance matrices were constructed using different models of base substitution (Kimura 2-parameter, Jukes Cantor, Tamura 3-parameter, Tajima-Nei, see the Glossary on page 310). As the use of different models did not vary results greatly only Kimura 2-parameter and Jukes Cantor are presented (see Table 24 on page 155). Uncorrected sequence divergence between the 18 haplotypes was calculated (=100x (number of nucleotides different/number of nucleotide sites) and ranged from 0.2% to 8.1%. From the alignment of the polymorphic sections of the 18 haplotypes (see Figure 13 on page 136) it is clear that haplotype 3 and haplotypes 16-18 are the most divergent. Sequence similarity was also investigated between haplotypes and the donated *R. robustus* and *R. prolixus* samples from Monteiro (FM1-FM8). The overall mean genetic distances within the 551 sequence alignment was 0.018 (s.e. 0.003, uncorrected p-distance) and 0.019 (s.e. 0.003, Kimura 2-parameter, Jukes Cantor). For the entire data set (551 sequences including FM1-8 but excluding the outgroups) the overall mean genetic distance increased marginally to 0.020 (s.e. 0.004, Kimura 2-parameter, Jukes Cantor). Saturation of transitions was not considered a problem because of the high transition\transversion ratio (the Glossary on page 310). The exclusion of third codons in phylogenetic analysis caused a decrease in bootstrap values indicating that such sites unlikely to be saturated.

6.1.5 Phylogenetic analysis

The sequence produced was subjected to distance and discrete analysis, together with the sequences (FM1-8) and three outgroups from Genbank (*R. pallescens, R. neglectus* and *T. infestans*). A neighbour joining tree was also produced for a subset of 9 specimens characterised by D2 (28S ribosomal RNA), together with 5 sequences taken from Genbank, including the outgroup *R. nasutus.*

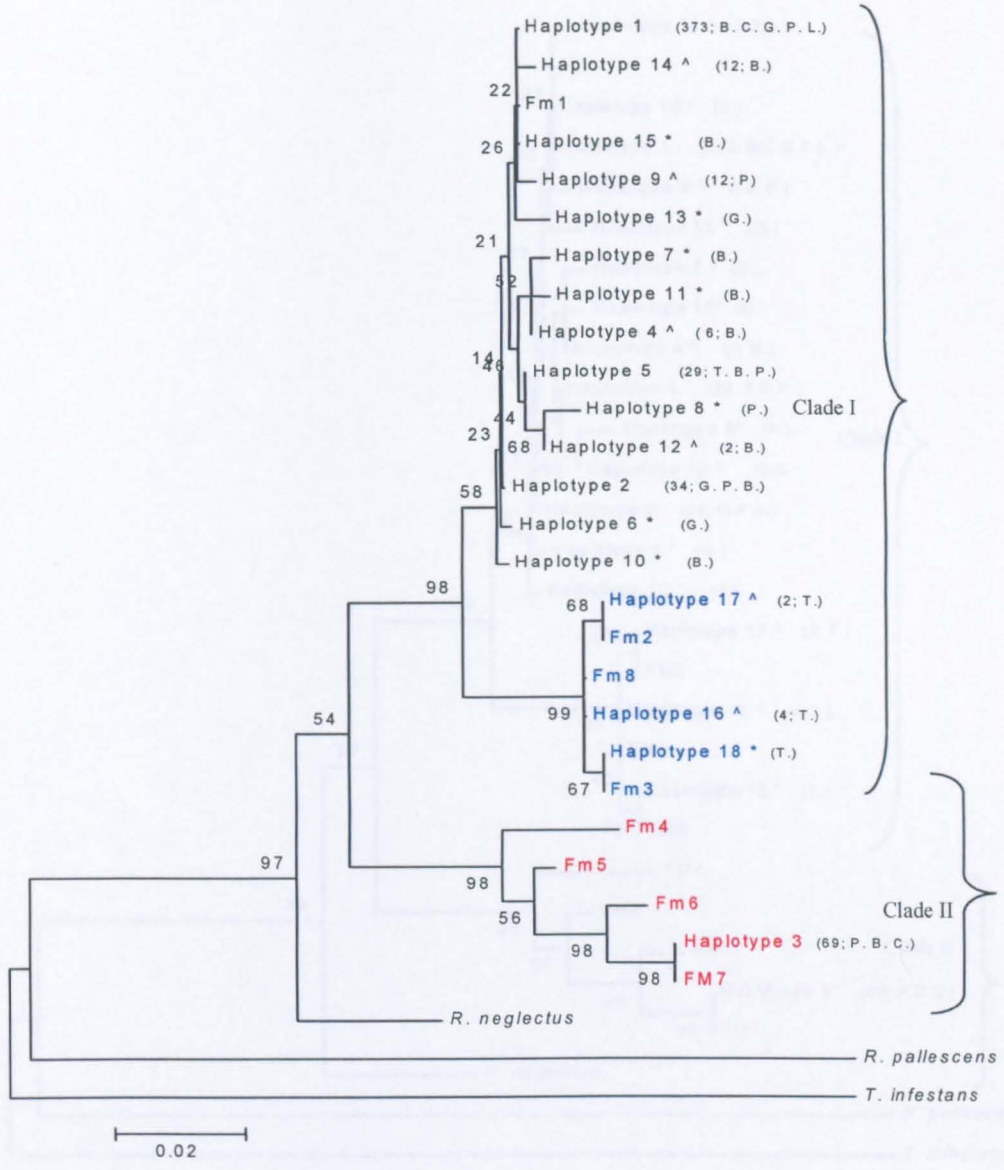

Figure 17. Neighbour-joining phylogenetic tree derived from Kimura-2 parameter distances. Statistical support from 1000 bootstrap replicates. Haplotypes 1-18 were found among the 551 *Rhodnius* specimens studied. FM1-8 were donated sequences (see Table 4 on page 101). Haplotypes in **blue** represent Venezuelan *Rhodnius robustus*, with those in **red** Amazonian *Rhodnius robustus,* in **black** *Rhodnius prolixus.* * Indicates haplotypes unique to single specimens. ^ Indicates haplotypes unique to single States/localities. Numbers in parenthesis are the frequency of occurrence of the haplotypes among the 551 specimens. Letters in parenthesis indicate States in which the haplotypes occurred; P. – Portuguesa, B. – Barinas, C. – Cojedes, T. – Trujillo, L. – Lara, G. – Guarico.

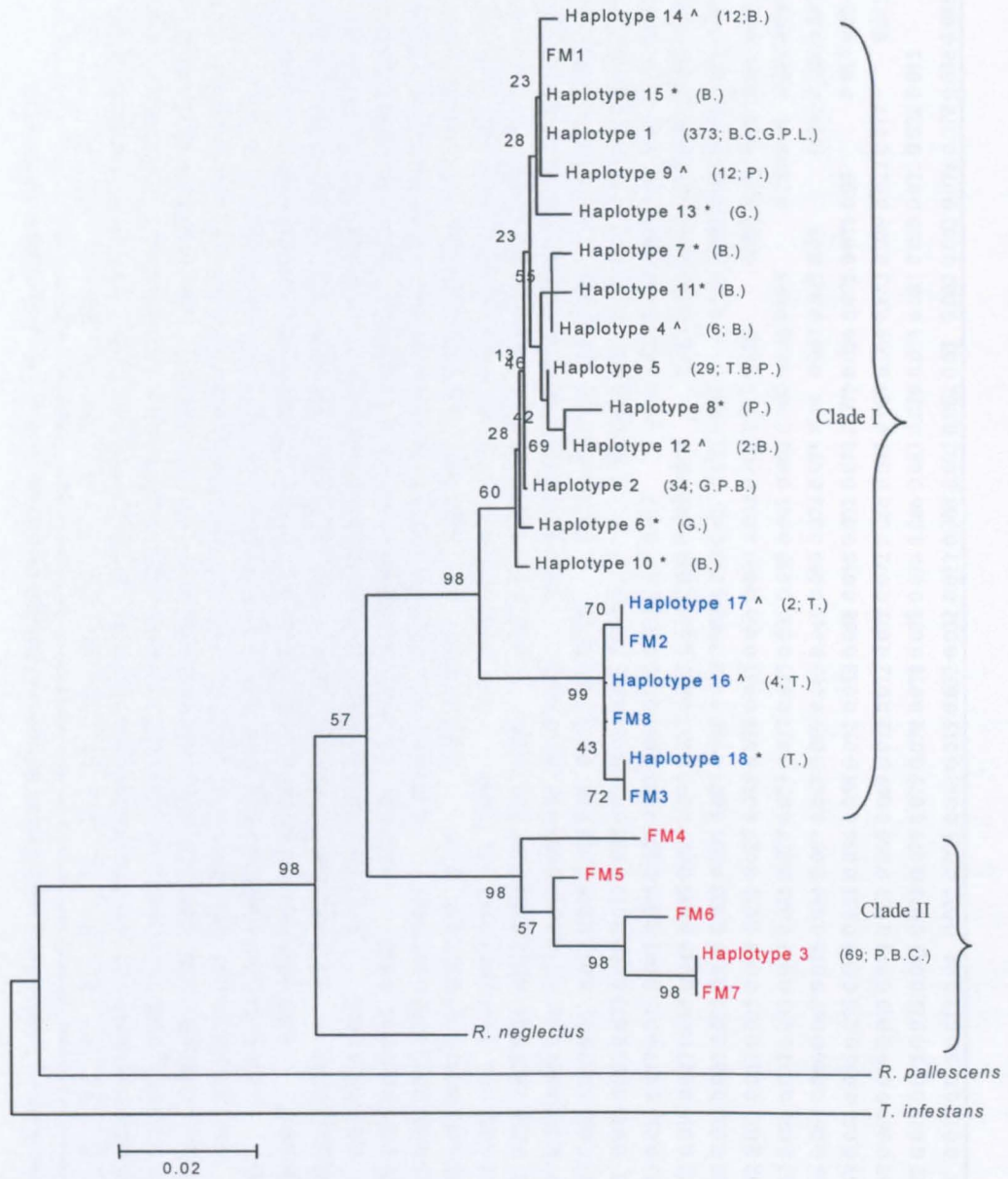

Figure 18. Neighbour-joining phylogenetic tree derived from Jukes-Cantor pairwise distances. Statistical support from 1000 bootstrap replicates. Haplotypes 1-18 were found among the 551 *Rhodnius* specimens studied. FM1-8 were donated sequences (see Table 4 on page 101). * Indicates haplotypes unique to single specimens. ^ Indicates haplotypes unique to single States/localities. Numbers in parenthesis are the frequency of occurrence of the haplotypes among the 551 specimens. Haplotypes in blue represent Venezuelan *R. robustus*, with those in **red** Amazonian *R. robustus*. Letters in parenthesis indicate the States in which the haplotypes occurred; P. – Portuguesa, B. – Barinas, C. – Cojedes, T. – Trujillo, L. – Lara, G. – Guarico.

Table 24. Pairwise genetic distances, Kimura-2 (below) and Jukes Cantor, between the 18 haplotypes from study and donated sequences FM1-FM8.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Haplotype 1		0.002	0.08	0.005	0.002	0.005	0.007	0.01	0.002	0.005	0.007	0.005	0.005	0.002	0.002	0.031	0.033	0.033	0.000	0.033	0.033	0.071	0.059	0.074	0.08	0.031
Haplotype 2	0.002		0.077	0.002	0.005	0.002	0.005	0.012	0.005	0.002	0.005	0.007	0.007	0.005	0.005	0.028	0.031	0.031	0.002	0.031	0.031	0.068	0.056	0.071	0.077	0.028
Haplotype 3	0.075	0.072		0.077	0.08	0.073	0.08	0.09	0.077	0.08	0.08	0.084	0.087	0.084	0.084	0.093	0.09	0.097	0.08	0.09	0.097	0.033	0.023	0.015	0.000	0.093
Haplotype 4	0.005	0.002	0.072		0.002	0.005	0.002	0.01	0.007	0.005	0.002	0.005	0.01	0.007	0.007	0.031	0.033	0.033	0.005	0.033	0.033	0.068	0.056	0.071	0.077	0.031
Haplotype 5	0.002	0.005	0.075	0.002		0.007	0.005	0.007	0.005	0.007	0.005	0.002	0.007	0.005	0.005	0.033	0.036	0.036	0.002	0.036	0.036	0.071	0.059	0.074	0.08	0.033
Haplotype 6	0.005	0.002	0.075	0.005	0.007		0.007	0.015	0.007	0.005	0.007	0.01	0.007	0.007	0.007	0.024	0.027	0.027	0.005	0.027	0.027	0.065	0.055	0.068	0.073	0.024
Haplotype 7	0.007	0.005	0.075	0.002	0.005	0.007		0.012	0.01	0.007	0.005	0.007	0.012	0.01	0.01	0.033	0.036	0 036	0.007	0.036	0.036	0.071	0.059	0.074	0.08	0.033
Haplotype 8	0.01	0.012	0.083	0.01	0.007	0.015	0.012		0.012	0.015	0.012	0.005	0.015	0.012	0.012	0.042	0.044	0.044	0.01	0.044	0.044	0.08	0.068	0.084	0.09	0.042
Haplotype 9	0.002	0.005	0.072	0.007	0.005	0.007	0.01	0.012		0.007	0.01	0.007	0.007	0.005	0.005	0.033	0.036	0.036	0.002	0.036	0.036	0.068	0.056	0.071	0.077	0.033
Haplotype 10	0.005	0.002	0.075	0.005	0.007	0.005	0.007	0.015	0.007		0.007	0.01	0.01	0.007	0.007	0.025	0.028	0.028	0.005	0.028	0.028	0.071	0.059	0.074	0.08	0.025
Haplotype 11	0.007	0.005	0.075	0.002	0.005	0.007	0.005	0.012	0.01	0.007		0.007	0.012	0.01	0.01	0.033	0.036	0.036	0.007	0.036	0.036	0.071	0.059	0.074	0.08	0.033
Haplotype 12	0.005	0.007	0.078	0.005	0.002	0.01	0.007	0.005	0.007	0.01	0.007		0.01	0.007	0.007	0.036	0.039	0.039	0.005	0.039	0.039	0.074	0.062	0.077	0.084	0.036
Haplotype 13	0.002	0.005	0.078	0.007	0.005	0.007	0.01	0.012	0.005	0.007	0.01	0.007		0.007	0.007	0.031	0.033	0.033	0.005	0.033	0.033	0.077	0.064	0.08	0.087	0.031
Haplotype 14	0.002	0.005	0.078	0.007	0.005	0.007	0.01	0.012	0.005	0.007	0.01	0.007	0.005		0.005	0.033	0.036	0.036	0.002	0.036	0.036	0.074	0.062	0.077	0.084	0.033
Haplotype 15	0.002	0.005	0.078	0.007	0.005	0.007	0 01	0.012	0.005	0.007	0.01	0.007	0.005	0.005		0.033	0.036	0.036	0.002	0.036	0.036	0.074	0.062	0.077	0.084	0.033
Haplotype 16	0.030	0.027	0.086	0.03	0.032	0.025	0.032	0.040	0.032	0.025	0.032	0.035	0.032	0.032	0.032		0.002	0.002	0.031	0.002	0.002	0.083	0.077	0.093	0.093	0.000
Haplotype 17	0.032	0.03	0.083	0.032	0.035	0.027	0.035	0.043	0.035	0.027	0.035	0.037	0.035	0.035	0.035	0.002		0.005	0.033	0.000	0.005	0.08	0.08	0.09	0.09	0.002
Haplotype 18	0.032	0.03	0.089	0.032	0.035	0.027	0.035	0.043	0.035	0.027	0.035	0.037	0.035	0.035	0.035	0.002	0.005		0.033	0.005	0.000	0.087	0.08	0.097	0.097	0.002
FM 1	0.000	0.002	0.075	0.005	0.002	0.005	0.007	0.010	0.002	0.005	0.007	0.005	0.002	0.002	0.002	0.03	0.032	0.032		0.033	0.033	0.071	0.059	0.074	0.08	0.031
FM 2	0.032	0.03	0.083	0.032	0.035	0.027	0.035	0.043	0.035	0.027	0.035	0.037	0.035	0.035	0.035	0.002	0.000	0.005	0.032		0.005	0.08	0.08	0.09	0.09	0.002
FM 3	0.032	0.03	0.089	0.032	0.035	0.027	0.035	0.043	0.035	0.027	0.035	0.037	0.035	0.035	0.035	0.002	0.005	0.000	0.032	0.005		0.087	0.08	0.097	0.097	0.002
FM 4	0.067	0.064	0.032	0.064	0.067	0.066	0.067	0.075	0.064	0.067	0.067	0.069	0.069	0.069	0.069	0.078	0.075	0.08	0.067	0.075	0.08		0.031	0.028	0.033	0.083
FM 5	0.056	0.053	0.022	0.053	0.056	0.056	0.056	0.064	0.053	0.056	0.056	0.058	0.058	0.058	0.058	0.072	0.075	0.075	0.056	0.075	0.075	0.03		0.017	0.023	0.077
FM6	0.069	0.067	0.015	0.067	0.069	0.069	0.069	0.078	0.067	0.069	0.069	0.072	0.072	0.072	0.072	0.086	0.083	0.089	0.069	0.083	0.089	0.027	0.017		0.015	0.093
FM7	0.075	0.072	0.000	0.072	0.075	0.075	0.075	0.083	0.072	0.075	0.075	0.078	0.078	0.078	0.078	0.086	0.083	0.089	0.075	0.083	0.089	0.032	0.022	0.015		0.093
FM8	0.03	0.027	0.086	0 03	0 032	0.025	0 032	0.04	0.032	0.025	0.032	0.035	0.032	0.032	0.032	0 000	0.002	0.002	0.03	0.002	0.002	0.078	0.072	0.086	0.086	

6.1.5.1 Neighbour-Joining (NJ)

Various distance models were used to produce Neighbour-joining (NJ) tree topologies including Kimura 2-parameter, Jukes Cantor parameter and Tamura-Tajima parameter (see Glossary on page 310). Statistical support for trees produced was determined by bootstrap technique using 1000 replicates (see Glossary on page 310). The analysis was also carried out with the exclusion of third codon positions in order to detect any indication of saturation at these sites. All sites were equally weighted. Data for Kimura-2 and Jukes Cantor are presented (see Table 24 on page 155, Figure 17 on page 153, Figure 18 on page 154).

The trees produced by the various base substitution methods had broadly identical topologies, with slight rearrangements of haplotype 1, 14 and 15 and with comparable high bootstrap values for all major clades (see Figure 17, Figure 18). The 18 haplotypes detected in this study divided into two major polyphyletic clades (clades I and II, with 97-99% bootstrap support) within these trees regardless of the distance method applied. Haplotypes 1, 2 and haplotypes 4-18 formed one clade, together with samples FM1-3 and FM8 from Monteiro (clade I), and separate from haplotype 3, which occurred in clade II, together with the remaining samples from Monteiro (FM4-7). Within clade I two main groups could be distinguished with haplotypes 1 and 2 and haplotypes 4-15 grouped together with sample FM 1 (56-65% bootstrap support), all *R. prolixus cytb* haplotypes from Genbank comparisons. Within this clade some haplotypes were more distantly related, with haplotypes 1, 2, 15 and FM1 the most closely related. Haplotypes 16-18 formed a second separate group within clade 1 with FM2, FM3 and FM8 (99% bootstrap support), with haplotype 17 grouping with FM2 (68-70% bootstrap support), haplotype 18 with FM3 (67-72% bootstrap support) and haplotype 16 with FM8 (all Venezuelan *R. robustus cytb* haplotypes from Genbank comparisons).

Within clade II samples FM4-FM6 were heterogeneous and occurred on different branches within the clade, however, sample FM7 and haplotype 3 were homogeneous (98% bootstrap support) and clustered together.

The exclusion of third codon positions from the analysis caused an overall decrease in bootstrap values indicating that such sites contribute useful phylogenetic information and as such are unlikely to be saturated (Monteiro *et al.*, 2003).

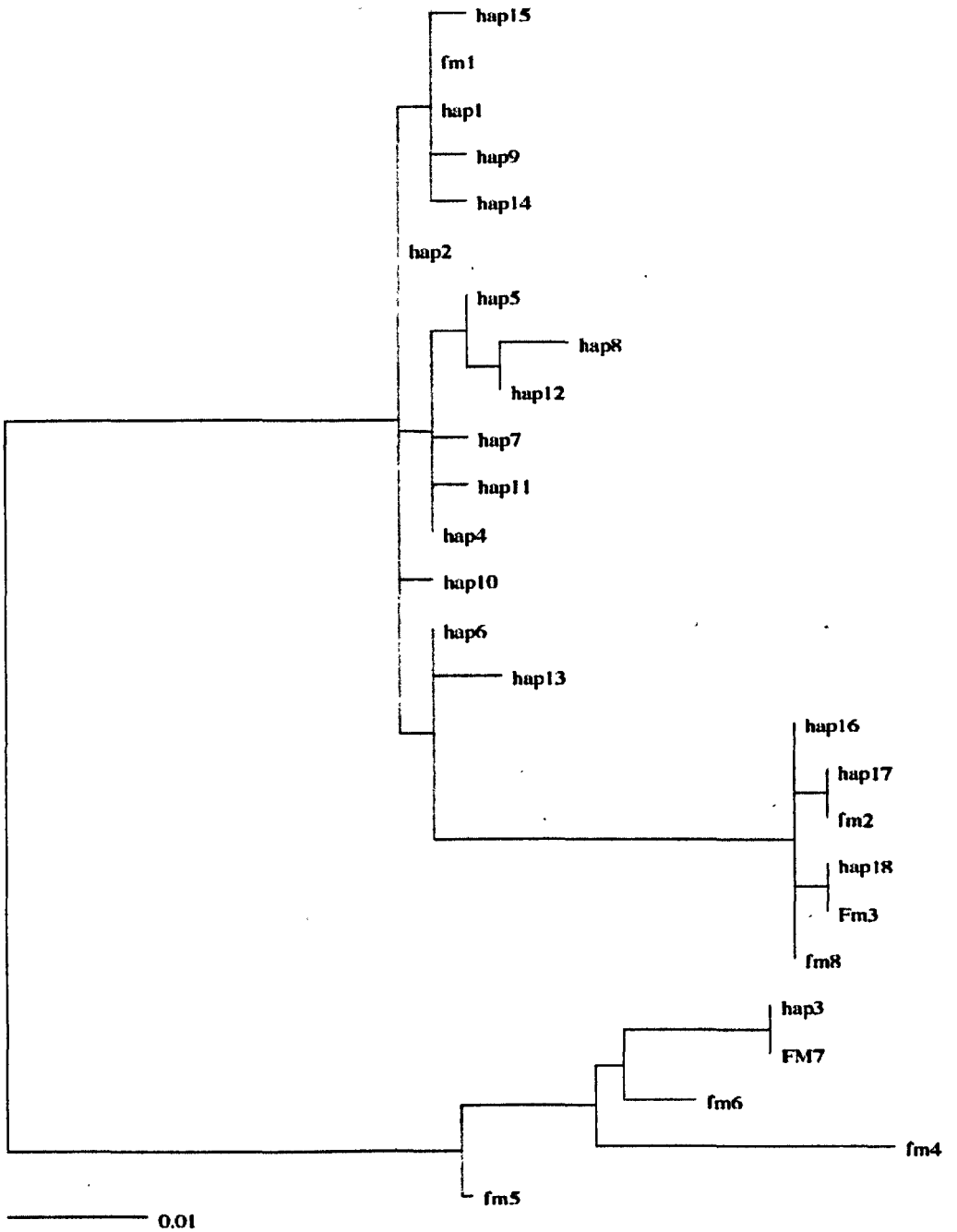

Figure 19. Maximum Likelihood tree of detected *Rhodnius* haplotypes 1-18 and donated sequences. hap=haplotype. hap = haplotype. fm = FM (see Table 4 on page 101).

6.1.5.2 Maximum Likelihood analysis

Maximum likelihood trees were produced in PAUP V4.0 based on a model incorporating a 4-category discrete gamma distribution to model mutation rate heterogeneity across the gene (see Glossary on page 310). A reversible rates (REV) mutation matrix was employed and is a variation on the transition\transversion ratio (see Glossary on page 310). Parameters were estimated via maximum likelihood using a reiterative heuristic search until default conditions determined the maximum likelihood had been reached (see Glossary on page 310).

Using this method the two main clades are maintained, however relationships within the clades have changed (see Figure 19 on page 158). Interestingly a closer relationship between the Venezuelan *R. robustus* haplotype group (haplotype 16 to FM8 in the tree) and the *R. prolixus* haplotype group (haplotype 15 to 13 in the tree) is found. Within the *R. prolixus* haplotype group haplotype 2 appears as the basal haplotype suggesting that this may be an ancestor to the other *R. prolixus cytb* haplotypes in the tree. This tree confirms a deep split between clades I and II. Within clade II a distant genetic relationships between haplotypes is seen, with the exception of FM7 and haplotype 3.

6.1.5.3 Haplotype networks

Three haplotype networks were produced using the program TCS1.8 for haplotypes detected in the study (see Figure 20 on page 160, Figure 21 on page 160, Figure 22 on page 161). The numbers on branches represent the site in the sequence that is changed at that particular step.

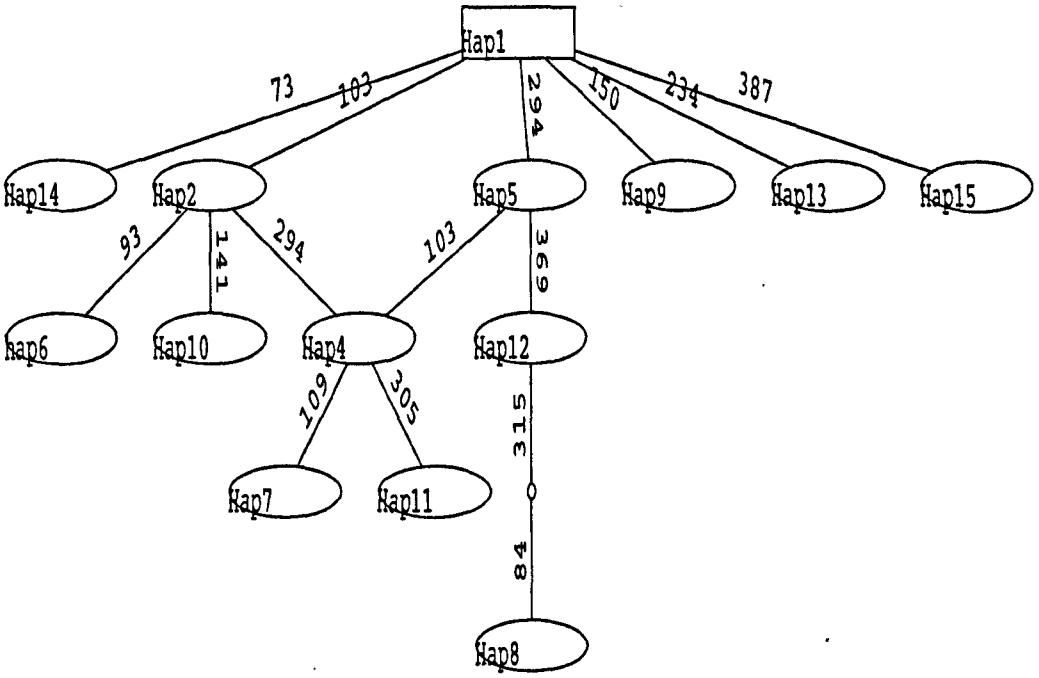

Figure 20. Haplotype network for *Rhodnius prolixus* haplotypes 1-2, 4-15, detected in the study. Hap=haplotype (FM1 not included as identical to haplotype 1).

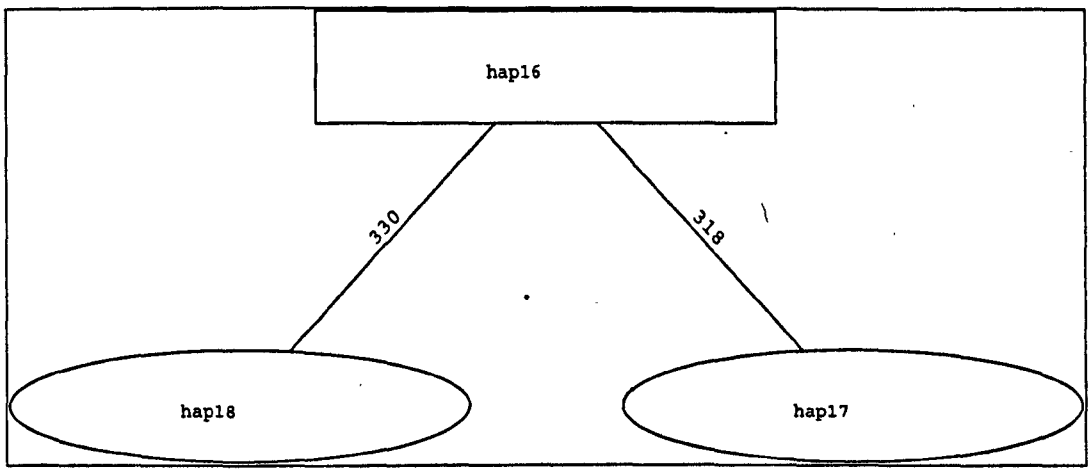

Figure 21. Haplotype network for Venezuelan *Rhodnius robustus* haplotypes 16-18 detected. hap=haplotype. (FM2, FM3, FM8 not included as identical to haplotypes analysed).

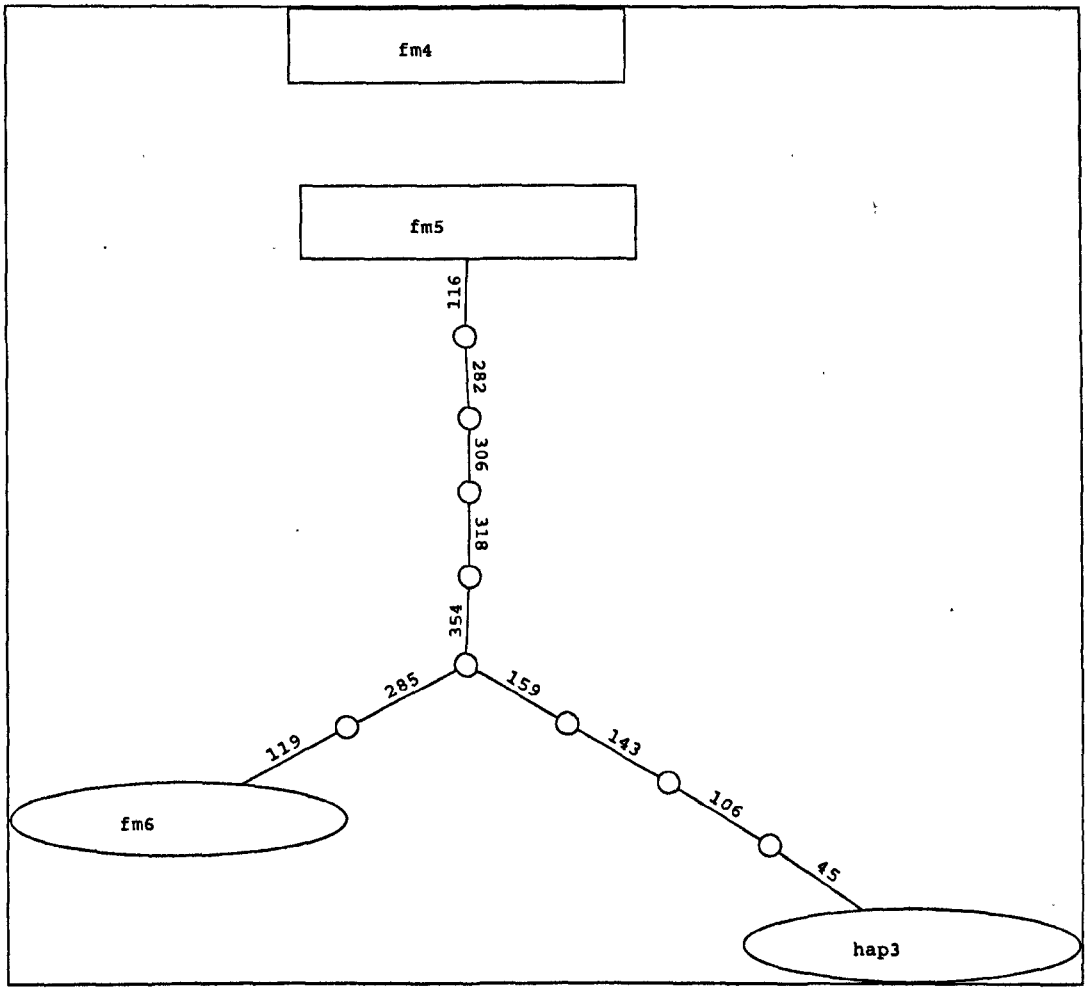

Figure 22. Haplotype network for Amazonian *Rhodnius robustus* haplotype 3 and FM4-6. hap=haplotype, fm=FM.

From the *R. prolixus* haplotype network haplotype 1 is taken as the most likely common ancestor to all other haplotypes present (see Figure 20 on page 160). From haplotype 1 a direct origin for haplotypes 2, 5, 9, 13, 14 and 15 is suggested. Haplotype 2 is the suggested ancestor to haplotypes 6 and 10. Haplotype 2 and 5 are equally likely as ancestors for haplotype 4. Haplotype 4 gave rise to haplotypes 7 and 11. Haplotype 5 is the suggested ancestor to haplotype 12, which consequently gave rise to the unknown ancestor of haplotype 8. From this network haplotype 8 is seen to be the most distant

from the ancestral haplotype 1, requiring 4 mutational steps, followed by haplotypes 7 and 11 at 3 steps.

Separate networks were generated for haplotypes 16-18 (see Figure 21 on page 160). Haplotype 16 is the suggested common ancestor to both haplotypes 17 and 18, also seen in the ML tree (see Figure 19 on page 158). Within the Amazonian *R. robustus* clade FM5 was taken as the ancestral haplotype from which FM6 and haplotype 3 eventually arose, passing through 5 common undetected intermediate sequences, with one additional single ancestor for FM 6, and 3 to haplotype 3 (see Figure 22 on page 161). Analysis could not place the more distantly related FM4 (11-13bp different).

No ancestral path could be suggested to link clusters due to the large number of base pair differences, with haplotype 3 differing by 29 sites from haplotype 1 and 32-34 bp from haplotypes 16-18. Haplotypes 16-18 differed from haplotype 1 by 12-13 bp.

6.1.5.4 D2 Sequence analysis and introgression

In order to check for the possibility of mtDNA introgression between the *R. prolixus* and *R. robustus* lineages a subset of 9 specimens, previously identified as *R. prolixus* and *R. robustus* from *cytb* analysis, were sequenced for a fragment of the D2 variable region of the 28S RNA gene (Monteiro *et al.*, 2000) (see Table 7 on page 105). An additional 5 sequences were taken from Genbank for specimens of known *cytb* characterisation (Monteiro *et al.*, 2003) (see Table 6 on page 104). The 519bp alignment (excluding the outgroup *R. neglectus*) revealed 4 variable sites, 1 site was parsimony-informative. Specimens from this study characterised as *cytb* haplotype 1 (putative *R. prolixus*) and haplotype 3 (putative Amazonian *R. robustus*) presented identical D2 haplotypes (D2-a), also shared with roVE2 (Venezuelan *R. robustus* from Genbank) (see Figure 23 on page 163). Specimens from Genbank, roBR4 and roBR8, characterised as *cytb* Amazonian *R. robustus* (Monteiro *et al.*, 2003) presented variant D2 haplotypes (D2-b, D2-c) differing from D2-a by 2 (T↔C) and 3 sites (2 x A↔G, T↔C) respectively, and from each other at 3 sites (2 x A↔G, T↔C). From *cytb* characterisation, haplotype 3 from our study

(putative Amazonian *R. robustus*) would be expected to show a similar variant D2 haplotype as that found in roBR8, as roBR8 occurs in the same cluster (IV) as this haplotype (FM7) in the maximum parsimony tree in Monteiro *et al.,* 2003. This incongruence between cytochrome and D2 characterisation suggests a past introgression event between *R. prolixus* and *R. robustus* lineages represented by haplotype 1 and 3.

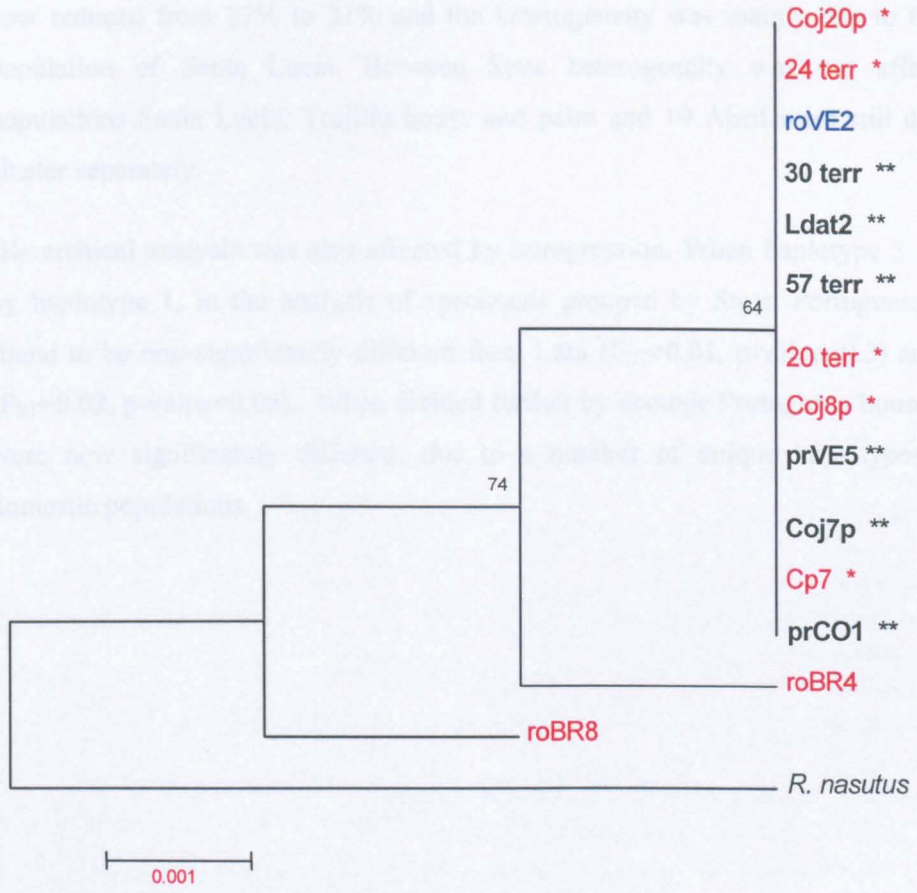

Figure 23. Neighbour joining tree for D2 sequences.
Labels in red=Amazonian *Rhodnius robustus cytb* with ***= Haplotype 3. Labels in Bold**** *Rhodnius prolixus* haplotypes. roBR8, roBR4 = Amazonian *Rhodnius robustus* (Genbank), roVE2= Venezuelan *Rhodnius robustus* (Genbank), prVE5= *Rhodnius prolixus* Venezuela (from our study samples collected in Ortiz, Guarico) (Genbank), prCO1= *Rhodnius prolixus* Colombia (Genbank).

This introgression event had an effect on the F_{ST} calculated between localities in Portuguesa, with haplotype 3 largely restricted to this State. If haplotype 3 is regarded as haplotype 1, the most common *R. prolixus* haplotype, population heterogeneity is reduced. Significant heterogeneity was reduced between Terronal populations and Casa Rena house population was no longer distinct (see Figure 16 on page 149, Figure 24 on page 165). The number of significantly different comparisons within Portuguesa was now reduced from 27% to 21% and the heterogeneity was mainly due to the distinct population of Santa Lucia. Between State heterogeneity was not affected. The populations Santa Lucia, Trujillo house and palm and 19 Abril were still distinct and cluster separately.

Hierarchical analysis was also affected by introgression. When haplotype 3 is replaced by haplotype 1, in the analysis of specimens grouped by State, Portuguesa was now found to be non-significantly different from Lara (F_{ST}=0.01, p-value=0.3) and Cojedes (F_{ST}=0.02, p-value=0.06). When divided further by ecotope Portuguesa house and palm were now significantly different, due to a number of unique haplotypes found in domestic populations.

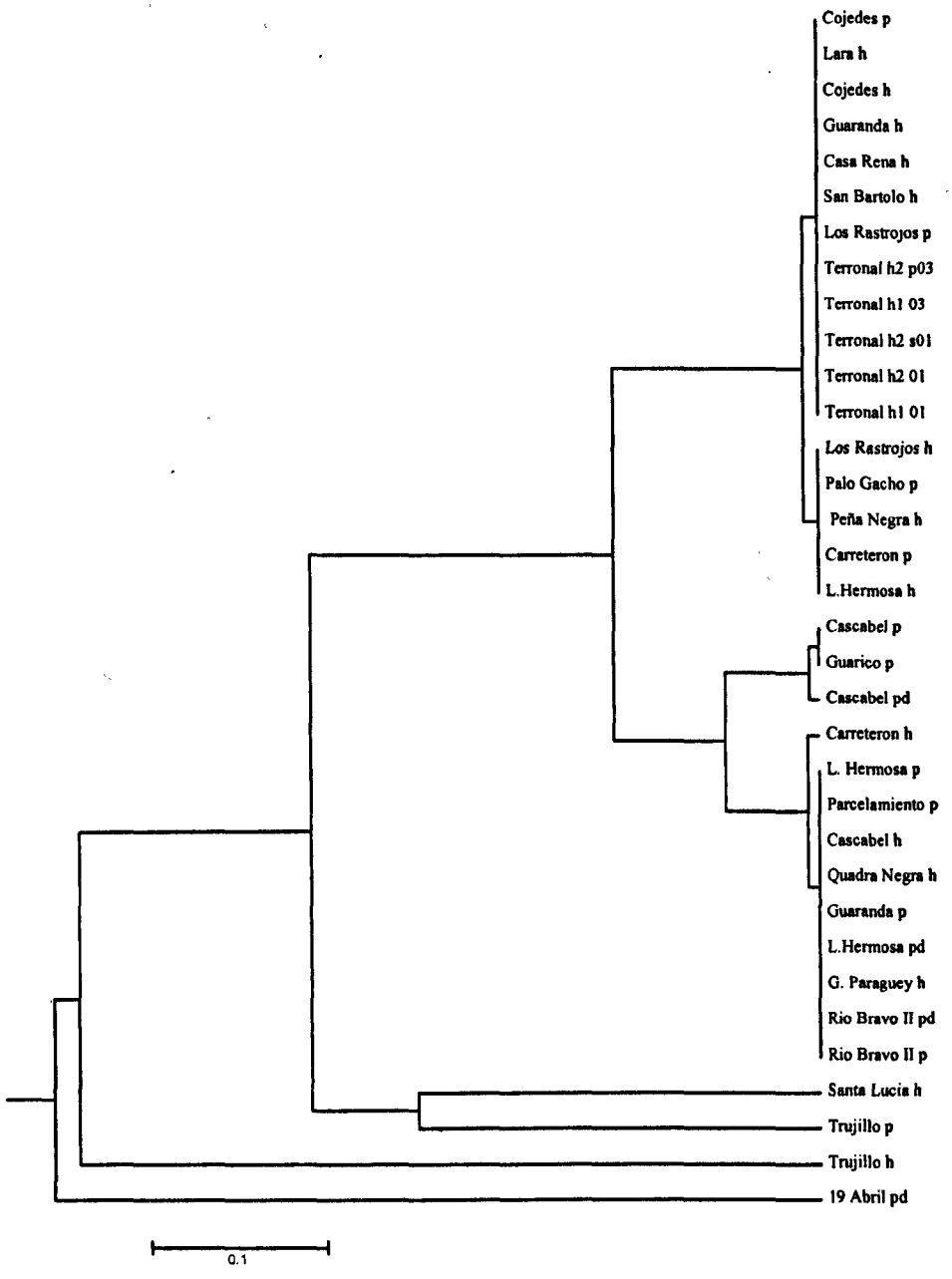

Figure 24. UPGMA tree of F_{ST} values between localities across all States; accounting for introgression. h=house, p=palm, pd=peridomestic.

6.2 Discussion

Mitochondrial *cytb* sequence analysis was used to investigate the genetic identity and diversity between silvatic and domestic field collected specimens of *R. prolixus* from 32 different sites within 6 States in Venezuela. Sequence polymorphism was analysed among haplotypes detected. Comparisons were also made with *R. prolixus* from Honduras and seven specimens of the closely related species *R. robustus* from three countries, Venezuela, Ecuador and Brazil (FM1-FM8) (see Table 4 on page 101). Phylogenetic trees were constructed using sister taxa *R. pallescens, R. neglectus* and distantly related *T. infestans* as outgroups. Mitochondrial DNA analysis has been used widely in triatomine studies and as previously described has also been used to question intra and interspecific relationships within the tribe Rhodniini (Lyman *et al.*, 1999, Monteiro *et al.*, 2000, 2003). A subset of 9 specimens were analysed using a segment of the D2 region of 28S rRNA to check for introgression between *R. prolixus* and *R. robustus* lineages. Population homogeneity was investigated at several hierarchical levels using pairwise estimates of F_{ST}.

6.2.1 Sequence polymorphisms and haplotype diversity

This study revealed 18 haplotypes from the 551 specimens analysed, 46 variable sites were determined (11.1%) (see Figure 13 on page 136). This is similar to previous *cytb* sequence comparisons within the '*prolixus* species' group, 6.7% of a 389 bp *cytb* fragment was found to be polymorphic (Lyman *et al.*, 1999) and 16.7% of a 663 bp fragment (Monteiro *et al.*, 2003). The nucleotide frequencies and rich A:T content were in keeping with previous analysis of this gene fragment in Triatominae (Lyman *et al.*, 1999, Stothard *et al.*, 1998, Monteiro *et al.*, 2004).

The sequences were analysed for homology to published sequences in Genbank and were found to have the highest similarity with *R. prolixus* and *R. robustus cytb* sequences (99-100%) (see Table 13 on page 133). Identity scores for haplotypes 1 and 2 and haplotypes 4 to 15 showed greatest similarities to *R. prolixus* haplotypes (99-100%),

whilst haplotype 3 and haplotypes 16 to 18 corresponded with *R. robustus cytb* fragments (98-100%). This was in agreement with the phylogenetic trees produced in this study (see Figure 17 on page 153, Figure 18 on page 154).

Haplotype 1, *R. prolixus* from Genbank comparisons, occurred in 67.7% of all specimens examined and was found in all States with the exception of Trujillo, occurring in both silvatic and domestic ecotopes in adults and nymphs. Maximum parsimony (MP) analysis using the algorithm of Templeton *et al.*, (1992) identified this widespread haplotype as ancestral to the other *R. prolixus* haplotypes detected (see Figure 20 on page 160). This haplotype was identical to FM1, donated *R. prolixus* sequence from Honduras (0.0 Kimura-2 genetic distance). Haplotypes 2 and haplotypes 4 to 15 were very similar genetically to haplotype 1, separated by only 1 to 4 base pair differences with genetic distances of 0.002-0.01 (Kimura-2 parameter) (see Figure 13 on page 136, Table 24 on page 155). Of the *R. prolixus* variant haplotypes, haplotype 8 had the largest number of base pair differences with four C↔T transitions, two of which were unique to this haplotype (0.01 Kimura-2 parameter). Haplotype 2 was separated from haplotype 1 by a single A↔G transition (0.002 Kimura-2 parameter) (see Figure 13, Table 24). This transition was not unique and was shared by nine other haplotypes in the study (see Figure 13). Maximum likelihood (ML) analysis placed this haplotype basal to all *R. prolixus* variant haplotypes (see Figure 19 on page 158). Thus maximum likelihood analysis proved more powerful than MP, also resolving the relationship between *R. prolixus* and *R. robustus* clades (see Figure 19).

An alignment of these 14 *R. prolixus* type haplotypes from our study and donated sequence FM1 (*R. prolixus* Honduras) detected 13 variable sites, 4 of which were parsimony-informative (see Figure 13). These sites were as follows; a C↔T transition shared by haplotypes 6 and 13, an A↔G transition shared by haplotypes 2,4,6,7,10,11, a C↔T transition shared by haplotype 4, 5,7,8, 11 and 12 and finally a T↔C transition shared by haplotypes 8 and 12. These sites are reflected in the topology of the trees produced by neighbour joining methods for example within clade 1 we can see a clear

grouping of haplotype 8 and 12, influenced by 2 shared T↔C transitions (68% bootstrap support). Haplotype 5 is associated with this group due to a shared T↔C transition (46% bootstrap support). This is also reflected in the lower genetic distances between haplotype 8 and haplotypes 12 and 5 (0.005 and 0.007 Kimura-2 parameter respectively) in comparisons with haplotype 8 and the other *R. prolixus* haplotypes (0.010-0.015). Haplotypes 7, 11 and 4 group together due to two shared transitions (52% bootstrap support) (see Figure 13 on page 136, Table 24 on page 155, Figure 17 on page 153, Figure 18 on page 154).

Haplotypes 16, 17 and 18 grouped together within clade I, differing at only 2 sites (A↔G, C↔T) (see Figure 13). These haplotypes shared a number of unique basepair changes (5 x A↔G, T↔C). These putative Venezuelan *R. robustus* shared a greater number of basepairs with haplotype 1 (97%) than putative Amazonian *R. robustus,* (haplotype 3 92%), as seen from the phylogenetic trees produced (see Figure 13, Figure 17, Figure 18).

The second clade consisted of *R. robustus* specimens from the Amazon in Brazil and Ecuador together with haplotype 3. Haplotype 3, identified as *R. robustus* from Genbank comparisons, differed greatly from the other haplotypes detected in the study. There were 29 base pair differences from haplotype 1 (18 T↔C, 9 A↔G and C↔A), and 34 base pairs from haplotype 18 (18 T↔C, 13 A↔G and 3 C↔A), while haplotype 18 and haplotype 1 differed at only 13 sites (see Figure 13, Figure 17, Figure 18).

If haplotypes 1, 2 and haplotypes 4 to 15 are *R. prolixus,* as Genbank comparisons imply, the low variability detected between these haplotypes from 6 States within Venezuela and in comparison to the Honduran specimen FM1, is in agreement with previous studies (0.002-0.01 Kimura-2 genetic distance; π 0.007 s.e. 0.002 Kimura-2 Parameter) (see Table 24). Low levels of sequence variability were detected by *cytb* sequence analysis of *R. prolixus* from several countries (Brazil, Venezuela, Colombia, Honduras and Guatemala) where these genetically similar samples formed a single cluster in both maximum parsimony and neighbour-joining analysis (Monteiro *et al.*,

2000). In another study by the same author three haplotypes of *R. prolixus* were recovered from populations from four different countries (Venezuela, Honduras, Guatemala, Colombia) but they differed by single point mutations and sequence diversity between the different areas was very low (π =0.0008, Monteiro *et al.*, 2003). The low level of genetic diversity was attributed to a recent bottleneck and subsequent dispersal by man (Monteiro *et al.*, 2003). While sequence diversity was low among our *R. prolixus* samples, it showed a greater degree of variation, with five haplotypes differing by two point mutations, two by three point mutations and one haplotype (haplotype 8) differing by 4-point mutations, in comparison to Monteiro *et al.*, (2003). This may reflect the variation that exists naturally in field as opposed to colony specimens.

Interestingly, our data showed that the *R. prolixus* haplotypes 1, 2, 4 to 6 and 10-15 were detected in palm specimens (haplotypes 6, 10, 11 & 13 in palms only) (see Table 23 on page 151, appendix Table 63 on page 314), indicating the existence of silvatic *R. prolixus* in Venezuela in all States sampled, with the exception of Trujillo and Lara. Silvatic *R. prolixus* was said to occur in Venezuela but its validity was questioned on the basis of the identification of *R. robustus* in palms in 1973 (see Chapter 3 on page 86). The findings here are in keeping with genetic studies, which also identified silvatic *prolixus* in the State of Guarico (Monteiro *et al.*, 2000, 2003) and in Brazil (Monteiro *et al.*, 2000). The identification of field caught specimens as silvatic *R. prolixus,* as opposed to the use of colony specimens, possibly of uncertain origin, makes these observations unequivocal. The presence of *R. prolixus* in palms in these areas may represent a threat to control with *R. prolixus* haplotypes 1, 2 4,5,7, 8, and 9 specimens present in houses, with haplotypes 1, 2, 3, 5, and 9 capable of colonization (nymphal stages recorded). This was noted in all States except Guarico where domestic infestations were not detected (see Table 23, appendix Table 63 on page 314). Domestic infestations were present in that area of Guarico before housing improvements occurred as part of the National control programme. If poor quality housing, such as traditional palm roofed, wattle and daub structures known to be suitable ecotopes for *R. prolixus,*

were re-established in this area domestic infestations may occur once again. The apparent absence of silvatic populations of haplotype 1 in Lara is beneficial for control in that the reinvasion of the domestic environment by silvatic species, following insecticide spraying, might not occur.

In Trujillo silvatic specimens analysed were identified as Venezuelan *R. robustus* from Genbank comparisons (99-100%), also confirming its presence in Venezuela. Within Clade 1, of all distance trees produced, a second group was formed (99% boot strap support) by haplotypes detected in seven specimens from Trujillo together with samples FM2, FM8 and FM3, donated *R. robustus* sequences also from Trujillo, Venezuela (see Figure 17 on page 153, Figure 18 on page 154). Six of these Trujillo specimens were silvatic in origin. A single female from a house in Loma de Amarillo was also determined as *R. robustus*, all other specimens, adults and nymphs, in the same house were *R. prolixus* haplotype 5, indicating that the adult female may have entered the house from surrounding palms to feed. This heavily infested house in Loma de Amarillo was very unusual for the area (Field inspector from Trujillo, pers. communication). The infestation was localized to a single bedroom annexed to the house, which had a palm roof and walls as opposed to the main house, which had a zinc roof and baharque walls. It was not possible to identify the source of the infestation, but two *Rhodnius* 1st instars were taken from a nest in surrounding palms. Monteiro *et al.*, 2003 also identified *R. prolixus* in Trujillo from insectary colonies that were established with specimens collected from houses (Panpan and Panpanito). These six specimens differed from Honduran *R. prolixus* (FM1 sequence in our analysis) by a single A↔G transition, while haplotype 5, identified in our study, differed by a single C↔T transition. These colony samples therefore represent an additional *R. prolixus* haplotype from Trujillo. During house searches in Trujillo most householders reported that bugs arrived at night, attracted by the light to feed but did not stay, active searches did not find any signs of colonization outside of Loma de Amarillo. In the locality La Juventud Trujillo, where three of our silvatic specimens originated, the presence of bugs in houses in the area appears to be primarily linked to invading silvatic *R. robustus* adults which arrive at

night to feed but do not colonise (Herber & Kroeger 2003). In a pilot study in this area on the use of insecticide impregnated curtains for the control of invading silvatic bugs 38 adult bugs identified as *R. robustus* were collected in a 4-week period. Thirty-two percent of householders said silvatic bugs entered the house at night. *R. robustus* is thought as a silvatic species of limited medical importance whose role in Chagas transmission is limited to sporadic cases (Feliciangeli *et al.*, 2002, 2003, Miles *et al.*, 1983). Invading silvatic *R. robustus* has been implicated in transmission of *T. cruzi* in western Venezuela (Feliciangeli *et al.*, 2002). However, domestic colonies of *R. robustus* have never been detected in Venezuela even in areas of where *R. robustus* has been implicated in cases of Chagas such as Merida (Feliciangeli *et al.*, 2002). An entomological survey of 18 houses in Merida, including an area where a fatal case of acute transmission had been recorded did not find signs of colonization but 137 *R. robustus* were collected from palms in the area, 10 of the 54 specimens examined for *T. cruzi* were positive (Feliciangeli *et al.*, 2002).

Haplotype 3 was the most divergent haplotype detected among our specimens and by Genbank comparisons. This haplotype also shared highest identity with *R. robustus*, but samples of Amazonian origin. This haplotype was the second most frequent haplotype in the study (12.5%) but it had limited distribution, occurring primarily in Portuguesa, with a total of three specimens found in other States (Cojedes and Barinas) (see Table 14 on page 135). Genetic distances calculated between this haplotype and the others identified ranged from 0.072-0.089 (Kimura 2-parameter) (see Table 24 on page 155). Haplotype 3 was found to be identical to FM7, a *R. robustus* sample from the Amazon. These samples formed the second main clade (clade II) in all phylogenetic trees produced, together with *R. robustus* samples FM4, FM5 and FM6 from Brazil and Ecuador (see Figure 17 on page 153, Figure 18 on page 154, Figure 19 on page 158). Interestingly, this *cytb R. robustus* haplotype was closer genetically to the *R. robustus* specimens from the Amazon than to identified *R. robustus* specimens from Venezuela (haplotypes 16-18), with genetic distances between haplotype 3 and FM4-6 ranging from 0.015-0.03, with 0.083-0.089 calculated to haplotypes 16-18 (Kimura 2-parameter) (see Table 24).

Uncorrected sequence divergences were calculated as 7.7-8.2% between haplotype 3 and haplotypes 16-18. Using a sequence divergence rate of 2.3% per million years for arthropod mtDNA sequence divergence (Brower 1994, Monteiro *et al.*, 2002) haplotype 3 and haplotypes 16-17 specimens have an estimated separation time from their last common ancestor in the region of 3.4 million years ago (mya), i.e. in the Pleistocene era. Uncorrected sequence divergence rates between haplotype 3 and the other Amazonian *R. robustus* haplotypes FM4-6 ranged from 0.6 (FM6) to 1.3 million years ago (FM4). Sequence diversity between haplotype 3 and the other Amazonian *R. robustus* haplotypes was calculated as 0.009 (s.e. 0.003) with genetic distances (Kimura 2-parameter) of 0.032 (s.e. 0.009 FM4), 0.022 (s.e. 0.007 FM5) and 0.015 (s.e. 0.006 FM6), indicating a closer genetic relationship (see Table 24 on page 155). The level of divergence between the Amazonian and Venezuelan *R. robustus* is similar to the value (7.2%) determined by Monteiro *et al.*, 2003 using a 663 bp *cytb* fragment. However, an important difference in relation to this study was the identification of Amazonian *R. robustus* among our Venezuelan specimens, in addition to Venezuelan *R. robustus* haplotypes. This disagrees with the discrete geographical distributions and structuring of *R. robustus* haplotypes determined by Monteiro *et al.*, 2003 (see section 3.2 on page 88).

Another important finding was the identification of haplotype 3, putative *R. robustus*, in both domestic (45 specimens) and peridomestic ecotopes (2 specimens) in addition to silvatic ecotopes (20 specimens). Domestic specimens from three houses in Portuguesa included 14 early and late stage nymphs in addition to adults (localities Terronal, Casa Rena), indicating the presence of domestic colonies in Venezuela of this putative species (see appendix Table 63 on page 314). This strongly contrasts with the belief that *R. robustus* is primarily a silvatic species as previously discussed. It is surprising that these morphologically indistinguishable specimens, identified as *R. prolixus* (Lent & Wygodzinsky 1979), should present such differing genetic characterizations. While this could be explained by convergence, the presence of active domestic colonies of Amazonian *R. robustus* is uncharacteristic as it is thought that high humidity requirements restrict this species to the more humid environment of Amazonian palms.

Additionally Amazonian *R. robustus,* unlike Venezuelan, has not been identified as light attracted or recorded entering houses (Feliciangeli *et al.,* 2002). Nevertheless, perhaps as this species spread from the Amazon-Orinoco forest northwards into the llanos of Venezuela, (Schofield & Dujardin 1999), it became more adapted to the drier conditions of this region, allowing a greater potential for domestication. However, a more plausible explanation for these interesting results is introgression, whereby through a past hybridisation event and horizontal gene transfer, the mitochondria of these specimens share similarity to Amazonian *R. robustus.* Since mitochondrial DNA does not recombine or segregate, some descendants of the hybrids may contain the mitochondrial genome of *R. robustus,* even though backcrossing has made their nuclear genome appear like that of *R. prolixus.* Introgression was suggested as an explanation for the molecular similarity, determined by mitochondrial sequence analysis, between the morphologically divergent *T. platensis* and *T. infestans* (Garcia & Powell 1998) and has been detected among other insects e.g. between sandfly vector populations in Colombia, *Lutzomyia townsendi* and *L. youngi,* which were separated by nuclear data but showed introgression by *cytb* analysis (Testa *et al.,* 2002) and *Anopheles bwambae* and other species of the *A. gambiae* complex in Uganda (Thelwell *et al.,* 2000). Given that *R. prolixus* and *R. robustus* are capable of hybridisation, an introgression event is possible (Barrett 1996, Solano *et al.,* 1996). To check that *cytb* results are not skewed due to introgression, comparisons can be made to a nuclear marker, in the case of this study D2. Results produced can be checked for incongruence, where nuclear similarity would be demonstrated for mitochondrial divergent specimens (Avise 1994). In this study a subset of 9 bugs were analysed for a 586bp segment of D2. Haplotype 1 and haplotype 3 specimens presented the same D2 sequence indicating that introgression has indeed taken place. Therefore these domestic "*R. robustus*" are *R. prolixus* with introgressed mitochondrial DNA, in accord with their colonisation behaviour. From our data from six States it appears that true *R. robustus* does, however, exists in Venezuela, in the Andean region in the State of Trujillo and published data also indicate its presence in Merida (Feliciangeli *et al.,* 2002).

6.2.2 Evolutionary relationships of haplotypes

Maximum parsimony networks (TCS v1.20) suggested haplotype 1 as the ancestor from which all the *cytb R. prolixus* haplotypes evolved, with haplotype 8 showing greatest level of divergence. Within the Venezuelan *R. robustus* cluster, haplotype 16 was ancestral, while FM5 was suggested as the common ancestor to the Amazonian *R. robustus*. However, maximum parsimony was not capable of inferring a relationship between the *R. prolixus* haplotypes and haplotypes 16-18 (Venezuelan *R. robustus*) and haplotype 3, FM4-6 (Amazonian *R. robustus*), or FM4 within the Amazonian *R. robustus* cluster (see Figure 20 on page 160, Figure 21 on page 160, Figure 22 on page 161). Under maximum likelihood (see Figure 19 on page 158) haplotype 2 was suggested as the ancestral haplotype to the *R. prolixus* group while it appears from the tree that Venezuelan *R. robustus* may have evolved from *R. prolixus*, showing closest association to the unique haplotypes 6 and 13 found in two silvatic specimens from Guarico. A maximum likelihood tree showed haplotypes within the cluster of Amazonian *R. robustus* (FM5-FM7) to be distantly related. This, coupled with the closer genetic relationship of Venezuelan *R. robustus* to *R. prolixus* supports the suggestion that *R. robustus* may in fact be several cryptic species with a paraphyletic origin as suggested by Monteiro *et al.*, 2003.

6.2.3 Population heterogeneity

From the study of 551 specimens it is clear that common haplotypes are occurring across all ecotopes with haplotype 1-5 occurring in all States (see Table 14 on page 135). This is suggestive of a lack of population differentiation. Population homogeneity was investigated at various hierarchical levels (1) collection area, (2) by State of collection, State divided by ecotope and (3) by ecotope using the F_{ST} index of Weir & Cockerhams (984).

Homogeneity was detected at all levels of analysis. At the lowest hierarchical level, the collection site, a lack of heterogeneity was detected between adjacent ecotopes in the

Terronal, Los Rastrojos, Cojedes and Rio Bravo II. Results indicate populations are not isolated and movement must be occurring across ecotopes. Pairwise comparisons were non-significant between house and palm and peridomestic sites. A lack of population division was also detected across locality level.

Within the locality Terronal, low and non-significant heterogeneity was detected between Terronal h2 and silvatic palms near that house (F_{ST}=0.03, 0.01 p-value=0.18, 0.26), also between the silvatic palms (F_{ST}= -0.03). F_{ST} values from pairwise comparisons with domestic specimens from 2003 were fairly high but non-significant (see Table 16 on page 139). Higher but non-significant F_{ST} indices were detected between Terronal h1 03 and palm specimens collected near that house (F_{ST}=0.14, p-value=0.25). When the introgressed haplotype 3 is taken into account, by regarding it as haplotype 1, populations in Terronal are homogeneous. Similarly within the locality Los Rastrojos the comparison of a single house and adjacent palm population showed no significant population differentiation (F_{ST}=-0.01 p-value=0.08) (see Table 17 on page 141). However, comparisons across Portuguesa State (see Table 17, Figure 14 on page 142) indicate that population isolation can occur with domestic samples from Santa Lucia and Casa Rena significantly different from the majority of other groups. Casa Rena is distinct being composed solely of haplotype 3, therefore its isolation is distorted by introgression.

A similar picture is revealed in Barinas State where 12 localities were sampled. Within the locality Cascabel, pairwise comparisons between domestic, peridomestic and palm populations gave non-significant differences, similarly in Guaranda (silvatic and domestic) and between adjacent populations in Rio Bravo (peridomestic and silvatic) (see Table 19 on page 145). From Figure 15 (on page 146) across the State population homogeneity is visible between different ecotopes, however, population isolation can also occur, with 19 Abril significantly different from most other populations.

When all States are compared, high population differentiation is maintained for some populations from varying ecotopes (Trujillo, Casa Rena, Santa Lucia, Apto. 19 Abril),

while low and non-significant for others (see Figure 16 on page 149), with Casa Rena differentiated due to introgression.

At State level analysis detected some homogeneity between Cojedes and Lara populations, while all other comparisons were significantly different. Even at this gross level homogeneity can be detected (see Table 21 on page 150). Differentiation decreased when introgression was taken into account, with Portuguesa also non-significantly different from Lara. When the State groups were subdivided further by ecotope, population differentiation decreased (see Table 22 on page 151). Within States population homogeneity was detected between different ecotopes, such as palm and domestic and peridomestic ecotopes in Barinas, within Cojedes pairwise comparisons were higher but non-significant (F_{ST} 0.04, p=0.5). Palm and domestic ecotopes in Portuguesa were significantly different after accounting for haplotype 3, due to a number of unique haplotypes found in the domestic environment.

6.2.4 Conclusions

Important conclusions from this analysis are:

1. The confirmation of the presence of silvatic *R. prolixus* in Venezuela (previously disputed see Chapter 3 on page 86), in addition to domestic colonies of the same species.

2. Identification of introgression between *R. prolixus* and Amazonian *R. robustus*.

3. The identification of true *R. robustus* in the Andean region.

4. Importantly, evidence indicates that *R. robustus* is limited to the silvatic, environment in this area with house colonies identified as *R. prolixus*, although adults can enter to feed.

5. Most importantly for control of Chagas disease, data analysis indicates that silvatic and domestic populations of *Rhodnius prolixus* are not always genetically differentiated, including 5 adjacent populations. Thus silvatic populations can play a role in house infestation.

These data support the data of Sanchez-Martin *et al.*, (2005) where infested palms within 100m of houses were identified as risk factors for infestation, as was the presence of a palm roof less than 1 year old (passive transfer between ecotopes).

Further investigation of population homogeneity was undertaken using polymorphic microsatellites markers.

7 Microsatellite analysis

The aim of this section of the study was to apply novel microsatellite markers generated for *R. prolixus* to the analysis of silvatic, peridomestic and domestic populations in order to estimate population heterogeneity between these differing ecotopes and to determine if silvatic and domestic populations are isolated. The results for a subset of specimens analysed by both microsatellites and *cytb* were also compared.

7.1 Results

7.1.1 Library

A total of four microsatellite libraries were created using the magnetic bead and biotinylated probe enrichment technique for the following repeat motifs (1) CA/CAA (2) GATA (3) GAA/AAAG and (4) GT/GTG. Three libraries were screened for the presence of microsatellite repeats, totalling 576 clones, with library 4 unscreened to date (see Table 25 below).

Table 25. Summary of *Rhodnius prolixus* microsatellite enriched libraries.

Library	Repeat	No. of 96 well plates screened	No. of positive clones	No. of clones sequenced	No. of primers designed* (primers ordered)
1	CA/CAA	4	72	68	78 (44)
2	GATA	1	2	2	1 (1)
3	GAA/AAAG	1	20	20	9 (7)
4	GT/GTG	unscreened	-	-	-
Total			94	90	88 (52)

*Multiple primers were produced from some individual clones, in this situation a single primer pair was ordered based on higher annealing temperature and greater fragment length (Primer2, Kemp 1993).

From these libraries 94 positive clones were identified (by PCR, streak plate), 90 of which were sequenced. Thirty-eight of the sequenced clones were not used for primer design because 9 had identical flanking regions (BioEdit V7.0.4.1), 1 sequence was a false positive lacking a repeat motif and in 6 sequences primers could not be designed from one flanking region. Additionally 22 of the 38 clones required re-sequencing, the presence of a repeat motif could not be confirmed in 5 of these sequences. A total of 84

positive clones identified by PCR and streak plates were shown to contain repeat satellite motifs (90 minus 1 false positive and 5 unconfirmed clones that required resequencing).

7.1.2 Primers

A total of 52 primer pairs were designed and ordered (MWG Biotech) (see appendix Table 65 on page 356). These were tested for amplification by PCR. Extra primers were produced for each individual clone (see appendix Table 66 on page 360).

Of the 52 primers ordered 12 pairs did not amplify or produce consistent amplification. However, additional primers are available for these loci and these may allow for successful amplification when tested. Two primer pairs (List14-069/List-14072) were later found to flank the same region and List14-069 was therefore discarded. Of the 39 remaining primers, 36 flanked dinucleotide repeats and 3 flanked trinucleotide repeats. A total of 21 forward primers were fluorescent labelled, each flanking dinucleotide repeats (see Table 26 on page 180, see appendix Table 67 on page 364). Seven fluorescent primer pairs were subsequently dropped due to inconsistent amplification (List14-039, List14-061, List14-055, List14-019, List14-067, List14-050 and List14-007) and require further optimisation and testing with the additional designed primers (see appendix Table 66 on page 360). Two loci amplified but stuttering patterns made them difficult to score and so they were not used further (List14-032, List14-014). This left a total of 12 working primer pairs (see Table 26). List14-041, while amplified, was not used in analysis as large alleles detected were of uncertain origin and may have been the result of cross locus amplification, therefore this locus requires sequencing for confirmation. List14-076, while amplified successfully for specimens, was later found to exhibit significant linkage with List14-056 (GENEPOP V3.4 Raymond & Rousset 1995) in 13 of 20 populations (p-values <0.0001, 0.0004) and was therefore excluded from analysis (see Table 28 on page 184).

Table 26. Fluorescent primers used in specimen amplification.

Locus	Primer sequence (5'-3')	F-Dye	Repeat motif	Annel. Temp °C	Cloned allele size
LIST14-013F	CATACTACACGCACACAAGACC	PET	$[AC]_{10}$	55	341bp
LIST14-013R	ATACTCGCATCAAGCCATTTGG				
LIST14-021F	AACCTCTGAACACATCAAATGG	NED	$[TG]_{10}$	55	297bp
LIST14-021R	AGCTACCTCTTGCCTCTACG				
LIST14-037F	GGCGACACCCCATAGAAACC	PET	$[GT]_8$	55	239bp
LIST14-037R	ATTAAAGAACGGAAACCCCACC				
LIST14-041F+	CCAATACAACACATACACTCG	VIC	$[CT]_{18}$	50	160bp
LIST14-041R+	ATCTGACACGACGTGATTCC				
LIST14-025F	CCGCTCTATCAACTACTCC	NED	$[TC]_9[AC]_7N_{13}[AC]_7$	50	180bp
LIST14-025R	GATCCCTTATGTTTCTCAGC				
LIST14-056F	TTTCCATTTGGCTCGTTTTGC	PET	$[CA]_{16}\ N_{14}\ [CT]_6$	57	167bp
LIST14-056R	GATAGTGCGATACATTTTGC				
LIST14-042F	TACTTCCGACTGACAACCG	FAM	$[GT]_9$	50	170bp
LIST14-042R	GGTTTTAGTTCACCAATAGC				
LIST14-064F	AGAAAATGAGCAAAACGGCC	FAM	$[GT]_{10}$	57	242bp
LIST14-064R	ACAGGCAAACAACTATGACG				
LIST14-017F	ATTGAAGGTTACTACTTGCTGC	FAM	$[TG]_{12}$	57	161bp
LIST14-017R	ACGCTGCTTCATTTTTTAGTGG				
LIST14-010F	AATGATGACTGTATTGATGGGC	FAM	$[CA]_9$	52	322bp
LIST14-010R	TTCGACCAACAACAACTTCCC				
LIST14-076F +	AGATAGTGCGATACATTTTGCG	FAM	$[AG]_6\ N_{14}\ [TG]_{17}$	52	218bp
LIST14-076R +	GTTAGAGTTGTCCTCAAGAAGC				
LIST14-079F	TAGAGTTTTTGCTCCTGTTAGC	FAM	$[CA]_9\ N_2\ [CA]_{10}$	52	314bp
LIST14-079R	TCCTATCTTTCGGTAAGTCCG				

+ Loci List14-041, List14-076 were not used in analysis; F-Dye fluorescent dye used on forward primer only. PET=Red, NED=Yellow, VIC=Green, FAM=Blue. Annel= Annealing. F=forward primer R=reverse primer.

Locus List14-017 showed significant heterozygosity deficiency in 11 populations. A Mantel test was carried out to investigate if this excess heterozygosity influenced heterogeneity among the 33 populations analysed. The comparison of F_{ST} values generated with and without List14-017 were highly significantly correlated (R^2=0.9 p-value=0.0001). As population heterogeneity was not affected by the excess homozygosity this locus was included. R^2 values range from 0 to 1. Its is the fraction of shared variance in the two compared variables i.e. R^2=0.06, therefore 6% of the variance in X can be explained by variation in Y at nominal significance level $p<0.05$.

7.1.3 Summary of loci

7.1.3.1 Null alleles

Null alleles can be problematical in microsatellite analysis (Rongnoparut *et al.*, 1996, Donnelly *et al.*, 1999), and can result in departures from HWE. Heterozygotes with a single null allele can be mistakenly identified as homozygous for the amplifying allele. Nulls at a locus are only obvious in homozygotes where there is failure to amplify. In this study 54 specimens consistently failed to amplify at a single locus and 2 specimens failed at two loci when all other loci for these specimens amplified.

Table 27. Summary of data at amplified loci

Locus	N	A_N	N_A	Common alleles*	Allele size range	P_A	N_G	Common genotypes*
List14-056	555	4	11	170bp (0.6) 172bp (0.2)	158-184bp	164bp	30 (7)	170/170bp (0.3) 170/172bp (0.2)
List14-017	555	5	7	158bp (0.4) 160bp (0.3)	152-166bp	-	15 (0)	158/158bp (0.3) 160/160bp (0.3)
List14-042	555	3	3	170bp (0.5) 172bp (0.5)	170-174bp	-	5 (0)	170/172bp (0.4) 170/170bp (0.3)
List14-010	555	11	12	323bp (0.8) 325bp (0.1)	311-339bp	-	31 (16)	323/323bp (0.7) 323/325bp (0.1)
List14-064	555	5	6	243bp (0.7) 241bp (0.2)	237-247bp	239bp	13 (3)	243/243bp (0.6) 241/243bp (0.2)
List14-013	555	10	6	343bp (0.5) 341bp (0.4)	335-345bp	-	12 (3)	341/342bp (0.4) 343/343bp (0.3)
List13-021	555	1	4	291bp (0.8) 295bp (0.1)	291-299bp	-	10 (0)	291/291bp (0.7) 291/295bp (0.1)
List14-025	555	6	7	179bp (0.7) 177bp (0.2)	163-181bp	163bp 173bp	10 (2)	179/179bp (0.5) 177/179bp (0.3)
List14-037	555	10	9	241bp (0.6) 243bp (0.4)	231-253bp	-	15 (3)	241/243bp (0.4) 241/241bp (0.3)
List14-041+	555	3	6	140bp (0.8) 160bp (0.1)	140-160bp	152bp	9 (1)	140/140bp (0.7) 140/160bp (0.2)
List14-076+	305	3	12	219bp (0.5) 221bp (0.3)	203-235bp	213bp	25 (8)	219/221bp (0.3) 219/219bp (0.2)
List14-079	305	1	10	309bp (0.6) 313bp (0.3)	293-329bp	293bp 319bp 329bp	9 (6)	309/309bp (0.4) 309/313bp (0.2)

+Loci List14-041, List14-076 were not used in analysis. N= no. of specimens amplified, A_N =null alleles, N_A = number of alleles per locus. P_A =private alleles, N_G= number of genotypes detected per locus, in parenthesis no. unique. * Frequency in parenthesis.

All 10 loci experienced such nulls with the greatest number occurring at loci List14-010, List14-037, List14-013 (11 and 10 specimens respectively) and the least at List14-079 and List14-021 (see Table 27 on page 181). Non-amplifying alleles (heterozygote nulls) may also be present and may explain the heterozygote deficiency prevalent at locus List14-017. The greatest number of nulls per population occurred in Terronal h2 palm 03, followed by Parcelamiento palm and Laguna Hermosa peridomestic populations (see Table 32 on page 190, Table 34 on page 195, Table 36 on page 200).

The least polymorphic locus in the study was List14-042 with only three alleles detected, while List14-010 was the most with 12 alleles, however the frequency of the most common allele (323bp) was 0.8 (80%) as most alleles were rare, also resulting in the largest number of unique genotypes. List14-076 also scored 12 alleles but as discussed was not used in analysis. List14-056 followed closely at 11 alleles (60% of alleles were 170bp). The greatest number of private alleles (3) occurred at locus List14-079, even with fewer overall specimens analysed (305 in total). The frequency of the most common alleles varied at loci from 0.4-0.8, with major genotype frequencies varying from 0.3-0.7. Allele size ranges varied over loci with the smallest number of base pair differences between alleles detected at List14-042 (4) and the largest at List14-079 (36).

7.1.4 Tests for linkage disequilibrium

Linkage equilibrium between all pairs of loci in each population was examined using a contingency table generated in GENEPOP V3.4 (Raymond & Rousset 1995) and based on the null hypothesis 'genotypes at one locus are independent from genotypes at the other locus'. The significance of the test was determined via an exact test employing a Markov chain.

Significant linkage disequilibrium was detected in 96 locus pairs across 30 populations, however only 17 pairs remained significant after sequential Bonferroni correction; List14-017 with List14-042 in San Bartolo h1 (p-value=0.001), List14-010 with List14-

013 in Guaranda palm (p-value0.0004), List14-010 and List14-025 in Cojedes palm (p-value=0.0006) and List14-056 with List14-076 (p-value<0.0001, 0.0005 in 14 populations) (see Table 28 on page 184). Due to the consistent linkage pattern exhibited by List14-056 with List14-076, locus List14-076 was excluded from analysis. All other loci were determined to be in linkage equilibrium as they did not exhibit a consistent pattern of significant linkage. Fisher's exact test across each pair of loci in all populations detected significant linkage at two locus pairs, List14-056 with List14-013 (p-value=0.001) and List14-056 with List14-076 (p-value<0.0001), both remained significant after Bonferroni correction (k=9, k=10).

Table 28. Exact p-values for linkage disequilibrium between all pairs of loci in each population.

Population	Pairs of loci"		P value^	Population	Pairs of loci"		P value^
Terronal h1 01	17	25	0.04	Cascabel house	21	76	0.03
Terronal h1 01	42	25	0.04	Cascabel house	56	37	0.04
Terronal h2 palm 01	10	25	0.04	Cascabel house	64	76	0.04
Terronal h2 palm 01	42	64	0.02	Cascabel palm	17	21	0 01
Terronal h2 palm 01	56	37	0.02	Cascabel palm	42	79	0.01
Terronal h2 palm 01	56	64	0.02	Cascabel palm	56	13	0.01
Terronal h2 palm 01	64	13	0.02	Cascabel palm	56	25	0.01
Terronal h1 03	64	37	0.04	Cascabel palm	25	76	0.01
Terronal h2 palm 03	17	13	0.002	Cascabel palm	**56**	**76**	**<0.0001**
Terronal h2 palm 03	25	37	0.002	Guaranda house	25	37	0.04
Terronal h2 palm 03	56	25	0.002	Guaranda house	42	64	0.03
Terronal h2 palm 03	56	37	0.02	Guaranda house	56	76	0 02
Terronal h2 palm 03	56	17	0.04	Guaranda palm	**10**	**13**	**0.0004**
Terronal h2 palm 03	64	13	0.01	Guaranda palm	**56**	**76**	**<0.0001**
Terronal h2 palm 03	64	37	0.02	Guaranda palm	17	76	0.04
San Bartolo h1	13	21	0.04	Laguna Hermosa house	42	13	0.04
San Bartolo h1	**17**	**42**	**0.001**	Laguna Hermosa house	56	21	0 01
San Bartolo h2	56	37	0.02	Laguna Hermosa house	21	76	0.02
Laurianito pd	17	64	0.02	Laguna Hermosa house	**56**	**76**	**<0.0001**
Laurianito pd	17	25	0.04	Laguna Hermosa palm	13	76	0.01
Laurianito pd	56	13	0.01	Laguna Hermosa palm	10	21	0.03
Laurianito pd	64	25	0.04	Laguna Hermosa palm	17	10	0.03
Palo Gacho palm	42	25	0.01	Laguna Hermosa palm	56	13	0 02
Los Rastrojos house	**56**	**76**	**<0.0001**	Laguna Hermosa palm	64	79	0.003
Los Rastrojos house	13	76	0.002	Laguna Hermosa palm	**56**	**76**	**<0.0001**
Los Rastrojos house	56	13	0.002	Laguna Hermosa pd	**56**	**76**	**<0.0001**
Los Rastrojos house	21	37	0.01	Laguna Hermosa pd	10	79	0.01
Los Rastrojos house	42	21	0.012	Laguna Hermosa pd	10	76	0.04
Los Rastrojos house	56	13	0.002	G. Paraguey h1	13	25	0.03
Los Rastrojos house	10	79	0.02	G. Paraguey h1	56	76	0.04
Los Rastrojos palm	**56**	**76**	**0.0005**	G. Paraguey h2	**56**	**76**	**<0.0001**
Lara house	42	10	0.01	G. Paraguey h2	42	64	0 01
Cojedes palm	**56**	**76**	**<0.0001**	G. Paraguey h2	64	79	0.04
Cojedes palm	**10**	**25**	**0.0006**	G. Paraguey p1	**56**	**76**	**<0.0001**
Cojedes palm	21	37	0.04	G. Paraguey p2	**56**	**76**	**<0.0001**
Cojedes house	**56**	**76**	**<0.0001**	19 Abril pd	13	79	0.03
Cojedes house	64	37	0.03	19 Abril pd	**56**	**76**	**<0.0001**
Cojedes house	10	64	0.03	Parcelamiento palm	64	21	0.04
Cojedes house	56	13	0.03	Parcelamiento palm	56	76	0.004
Trujillo h	17	21	0.01	Rio Bravo II pd	**56**	**76**	**<0.0001**
Trujillo h	17	10	0.04	Rio Bravo II pd	10	13	0.02
Trujillo h	56	21	0 01	Rio Bravo II pd	37	76	0.02

Population	Pairs of loci"		P value^	Population	Pairs of loci"		P value^
Trujillo h	56	10	0.03	Rio Bravo II pd	42	37	0.01
Cascabel pd	17	64	0.02	Rio Bravo II pd	56	37	0.03
Cascabel pd	21	37	0.004	Rio Bravo II pd	56	21	0.04
Cascabel pd	56	10	0.02	Rio Bravo II pd	21	76	0.03
				Rio Bravo II palm	42	10	0 01

"loci missing List14- prefix before numbers. ^Exact p value. **Values in Bold** significant after sequential Bonferroni correction (Rice 1989) k=36 p1=0.05/36 (9 loci) k=55 p1=0.05/55 (11 loci). In analysis k also varied within populations according to the number of loci comparisons possible with some noted by Genepop as "Not possible" and "No information" see Genepop manual. p=palm h=house pd=peridomestic

7.1.5 Tests for Hardy Weinberg Equilibrium (HWE)

A test for departure from HWE, using the exact test in Arlequin V2.000 (Schneider *et al.*, 2000), was conducted across all loci for the pooled data set of 555 specimens (set 1) and 305 specimens (set 2). In the pooled data set 7 of 10 microsatellite loci significantly fall out of expected HWE after sequential Bonferroni correction, the exceptions being List14-013, List14-025 and List14-037. Significant heterozygosity deficiency was calculated at all loci except List14-013, List14-025, List14-042 and List14-010 (see Table 29 on page 186) (GENEPOP V3.4 Raymond & Rousset 1995). Significant heterozygosity excess was not detected (GENEPOP V3.4 Raymond & Rousset 1995). F_{IS} (Weir & Cockerham 1984) also detected deviations from random mating and ranged from 0.01 (List14-037) to 0.54 (List14-017), with positive values indicating heterozygote deficiency at all loci, although not always significant (see Table 29).

Loci in each population were also tested for significant departure from HWE. A number of loci were monomorphic in populations and were therefore not tested as seen in Santa Lucia, San Bartolo and Casa Rena populations in Portuguesa. Six loci in 17 populations departed from equilibrium following sequential Bonferroni correction see Table 30 on page 187, Table 31 on page 188). Most departures were related to List14-017 (11 populations), with significant excess homozygosity detected in all with the exception of San Bartolo h2 and Los Rastrojos h (GENEPOP). Significant excess homozygosity at this locus was also detected in Cascabel palm, Guaranda palm, Parcelamiento palm and Rio

Bravo II palm but loci did not depart significantly from HWE after Bonferroni correction.

Departures from HWE occurred most frequently in the population Rio Bravo II pd with 3 of 10 loci out of HWE (List14-056 F_{IS}= -0.23, List14-010 F_{IS}= +0.24, List14-013 F_{IS}= -0.78). In Terronal palm 01 two loci were out of HWE, both loci exhibited significant homozygosity excess (List14-064 F_{IS}= +0.92, List14-017 F_{IS}= +0.62). In San Bartolo h2 two loci departed from HWE (List14-021 F_{IS}= +0.71, List14-017 F_{IS}= +0.5), significant homozygosity excess was detected at List14-021 (see Table 30 on page 187, Table 31 on page 188).

Other loci affected by HWE were List14-056 in Santa Lucia (F_{IS}=-0.35, not significant), List14-010 in G. Paraguey p2 (F_{IS}=+0.74, significant), List14-064 in Terronal h2 01 (F_{IS}=+0.78, significant) and G. Paraguey h1 (F_{IS}=+0.69, not significant), and List14-021 in Cascabel house (F_{IS}=+0.6, not significant).

Table 29. Summary of data at the 10 polymorphic loci used in analysis.

Locus	Sample No.	No. of non missing alleles	Allele No.	Major allele Frequency	H_O	H_E	P-value	F_{IS} (W&C)
List14-056	555	551	11	0.56	0.55	0.62	**<0.0001**	+0.113
List14-017	555	550	7	0.43	0.31	0.66	**<0.0001**	+0.528
List14-042	555	552	3	0.53	0.40	0.50	**0.0003**	+0.197
List14-010	555	544	12	0.83	0.26	0.32	**<0.0001**	+0.180
List14-064	555	550	6	0.74	0.27	0.42	**<0.0001**	+0.355
List14-013	555	545	6	0.54	0.50	0.53	0.062	+0.061
List14-021	555	554	4	0.78	0.27	0.37	**<0.0001**	+0.274
List14-025	555	549	7	0.71	0.43	0.44	0.010	+0.029
List14-037	555	545	9	0.59	0.51	0.51	0.095	+0.005
List14-079	305	304	10	0.62	0.41	0.55	**<0.0001**	+0.254

H_O, H_E=Observed and Expected heterozygosity, P-value- exact probability for expected Hardy Weinberg equilibrium conditions for each locus/population combination (Arlequin v2.1). F_{IS}= Weir & Cockerham (1984) (GENEPOP V3.4). **Values in bold** significant after sequential Bonferroni correction k=9, p1=0.05/9.

Table 30. Summary of data per population per locus (List14-056, List14-017, List14-042, List14-010, List14-064)

	List14-056						List14-017					List14-042					List14-010					Liat14-064				
Pop	N	N_A	H_O	H_E	P	F_{IS}	N_A	H_O	H_E	P	F_{IS}	N_A	H_O	H_E	P	F_{IS}	N_A	H_O	H_E	P	F_{IS}	N_A	H_O	H_E	P	F_{IS}
1	26	5	0.39	0.47	0.31	+0.17	4	**0.35**	**0.67**	**0.004**	+0.47	2	0.27	0.34	0.29	+0.21	2	0.08	0.19	0.12	+0 47	2	0.19	0.27	0 37	+0.19
2	18	6	0.50	0.61	0.41	+0.12	3	**0.33**	**0.70**	**0.02**	+0.51	2	0 56	0.51	1	-0.08	3	0.22	0.35	0.20	+0.26	2	**0.06**	**0.30**	**0.01**	+0.78
3	26	4	0.60	0.54	0.76	-0.11	3	0.16	0.4	**0.001**	+0.62	2	0 08	0.11	1	-0.02	2	0.04	0.08	1	-	2	**0.04**	**0.49**	**<0.0001**	+0 92
4	10	3	0.50	0.50	1	-0.18	3	0.40	0.79	0.20	+0.44	2	0 10	0.35	0.16	+0.64	4	0.40	0.36	1	-0.11	2	0.63	0.53	1	-0.21
5	39	7	0.68	0.68	0.59	-0.00	4	**0.26**	**0.67**	**<0.0001**	+0.61	2	0 56	0.54	0.54	-0.12	2	0.16	0.20	1	-0.07	3	0.31	0.43	0.31	+0.22
6	14	3	0.14	0.27	0.08	+0.47	4	**0.29**	**0.74**	**0.001**	+0.60	2	0.5	0.50	1	-0 01	1	M	M	M	M	1	M	M	M	M
7	14	3	0.21	0.37	0.37	+0.33	4	**0.36**	**0.71**	**0.006**	+0.50	2	0.21	0.52	0.07	+0.54	1	M	M	M	M	1	M	M	M	M
8	13	3	**0.77**	**0.62**	**0.004**	-0.35	2	0.08	0.15	1	-	1	M	M	M	M	1	M	M	M	M	1	M	M	M	M
9	11	3	0.64	0.54	0.63	-0.35	3	0.82	0.66	0.85	-0.25	2	0.36	0.52	0.54	+0.26	1	M	M	M	M	2	0.18	0 26	1	-0.05
10	21	6	0.52	0.57	0.69	+0.04	3	**0.19**	**0.65**	**<0.0001**	+0.69	2	0.43	0.51	0.66	+0.16	4	0.33	0.34	1	-0.11	2	0.38	0.45	1	+0.09
11	15	6	0.53	0.55	0.50	+0.03	3	0.33	0.60	0.17	+0.39	2	0.27	0.56	0.11	+0.47	2	0.13	0.19	1	-0 04	2	0.20	0.35	0.33	+0.31
12	24	2	0.42	0.38	1	-0 09	3	**0.17**	**0.30**	**0.006**	+0.38	2	0.50	0.50	1	-0.05	5	0.63	0.66	0.75	+0.01	1	M	M	M	M
13	12	5	0.67	0.67	1	-0.05	3	0.17	0.53	0.01	+0.64	2	0.75	0.52	0.24	-0.48	4	0.50	0.51	0.12	+0.02	2	0.00	0.26	0.05	+1
14	17	2	0.42	0.44	1	-0.03	3	0.41	0.52	0.05	+0.15	2	0.18	0.49	0.02	+0.62	3	0.19	0.24	1	-0.05	2	0.18	0.46	0.04	+0.57
15	24	4	0.35	0.44	0 27	+0.20	4	**0.29**	**0.77**	**<0.0001**	+0.62	2	0.67	0.50	0.11	-0.35	4	0.58	0.57	0.28	-0.07	2	0 50	0.48	1	-0.05
16	24	2	0.25	0.37	0.23	+0.26	3	**0.33**	**0.65**	**0.0007**	+0.46	2	0.33	0.45	0.34	+0.21	2	0.08	0.12	1	-0.02	2	0.33	0.34	1	+0.01
17	26	3	0.27	0.32	0.10	+0.17	3	**0.31**	**0.53**	**0.006**	+0.40	2	0.15	0.18	1	-0.06	4	0.35	0 45	0.02	+0.18	3	0.04	0.15	0.02	+0.66
18	11	3	0.36	0.39	1	-0.13	3	0.27	0.53	0.14	+0.41	2	0.36	0.54	0.53	+0.26	2	0.20	0.28	1	-0.06	3	0.46	0.46	0.60	+0.00
19	10	4	0 80	0.64	0.55	-0.26	3	0.50	0 64	0.09	+0.22	2	0.60	0.51	1	-0.20	4	0.22	0.41	0.12	+0.46	4	0.40	0.62	0.38	+0.29
20	24	8	0 88	0.79	0 85	-0.11	5	0.38	0.58	0.02	+0.35	2	0.42	0.52	0.67	+0.16	7	0.38	0.46	0.04	+0.14	4	0.42	0.54	0.06	+0.23
21	11	5	0.64	0.72	0.29	+0.05	4	0.45	0.71	0.33	+0.30	2	0.45	0.54	1	+0.00	2	0.10	0.19	1	-	4	0.27	0.54	0.013	+0 49
22	20	8	0.85	0.75	0.21	-0.14	4	0.35	0.67	0.01	+0.47	3	0.55	0.55	1	-0.06	4	0.25	0.36	0 04	+0.21	4	0.65	0.64	0.66	-0.02
23	16	3	0 81	0.69	0.20	-0.19	3	0.31	0.59	0.03	+0.45	2	0.63	0.51	0.61	-0.24	6	0.50	0.56	0 21	+0.04	3	0.38	0.50	0.02	+0.19
24	17	5	0.77	0.72	0 80	-0.07	4	0.41	0.64	0.10	+0.30	2	0.69	0.50	0.16	-0.40	6	0.44	0.48	0.33	-0.01	4	0.31	0.52	0.08	+0.34
25	13	4	0.62	0.76	0.48	+0 13	3	0.27	0.58	0.04	+0.55	2	0.62	0.49	0.57	-0.26	4	0.42	0.44	1	-0.13	3	0.39	0.40	1	-0.13
26	11	3	0.36	0.65	0.08	+0 39	4	0.46	0.72	0.20	+0.34	3	0.36	0.50	0.60	+0.18	2	0.09	0.18	1	-	3	**0.18**	**0.65**	**0.003**	**+0.69**
27	12	3	0.58	0.57	1	-0 03	4	0.33	0.63	0.03	+0.48	2	0.58	0.52	1	-0.13	4	0.33	0.50	0 56	+0.22	5	0.50	0.44	1	-0.16
28	12	4	0 42	0 66	0.19	+0 33	3	**0.18**	**0.66**	**0.003**	+0.70	3	0.42	0.55	0.55	+0.26	4	0.42	0.42	0.39	+0.02	3	0.33	0.37	1	-0.11
29	11	5	0.73	0.74	0.32	+0.02	3	0.46	0.57	0.07	+0.21	2	0.45	0.46	1	+0.00	4	**0.09**	**0.41**	**0.002**	+0.74	3	0 36	0.62	0.02	+0.36
30	13	4	0.62	0.69	0.67	+0 11	3	0.08	0.29	0 04	+0.66	2	0.23	0.58	0.08	+0.56	2	0.08	0.15	1	-	2	0.54	0.55	1	-0.06
31	13	5	0.52	0.59	0 48	+0.02	4	0.31	0.66	0 01	+0.52	2	0.42	0.60	0.59	+0.20	2	0.25	0.41	0.41	+0.28	4	0.46	0.58	0.64	+0.21
32	17	5	**0.94**	**0.77**	**0.006**	-0.23	2	0.31	0.47	0.27	+0.34	2	0.24	0.47	0.09	+0 46	4	**0.50**	**0.68**	**0.0003**	+0.24	2	0.13	0.18	1	-0.03
33	10	6	0.70	0.85	1	+0 13	4	0 30	0 73	0 02	+0.59	3	0.44	0 67	0 72	+0.30	4	0.33	0.56	0.14	+0 31	5	0 60	0.63	0 80	-0 06

N= number of specimens amplified, N_A= number of alleles, H_O, H_E=Observed and Expected heterozygosity, P=exact probability for expected Hardy Weinberg equilibrium conditions for each locus/population combination (Arlequin v2.1), M= monomorphic. F_{IS}= Weir & Cockerham (1984) (GENEPOP V3.4). **Values in bold** departures from HWE significant after Bonferroni correction, populations analysed at 9 loci k=9, p1=0.05/9, at 10 loci k=10, p1=0.05/10. Population numbering as in Table 33 on page 191.

Table 31. Summary of data per population per locus (List14-013, List14-021, List14-025, List14-037, List14-079).

	List14-013					List14-021					List14-025					List14-037					List14-079 ^				
Pop	N_A	H_O	H_E	P	F_{IS}	N_A	H_O	H_E	P	F_{IS}	N_A	H_O	H_E	P	F_{IS}	N_A	H_O	H_E	P	F_{IS}	N_A	H_O	H_E	P	F_{IS}
1	2	0.48	0.53	1.00	+0.06	2	0.15	0.35	0.03	+0.52	2	0.42	0.38	1	-0.11	2	0.48	0.50	1	-0.02	NA	-	-	-	-
2	2	0 65	0.54	0.36	-0.27	2	0 06	0.11	1	-	2	0.39	0.37	1	-0.21	2	0.44	0.40	0.53	-0.26	NA	-	-	-	-
3	2	0.38	0.36	1	-0.06	2	0.12	0.15	1	-0.04	2	0.19	0.24	0.37	+0.19	2	0.38	0.50	0.42	+0.23	NA	-	-	-	-
4	2	0.50	0.48	1	-0.05	3	0.10	0.37	0.06	+0.65	2	0.50	0.48	1	-0.05	2	0.70	0.56	0.52	-0.37	NA	-	-	-	-
5	2	0 54	0.53	0.74	-0.09	2	0.08	0.12	1	-0.03	3	0.56	0.51	0.88	-0.10	2	0.45	0.41	0.69	-0.11	NA	-	-	-	-
6	2	0.71	0.55	0.27	-0.43	4	0.64	0.70	0.59	+0 07	3	0.43	0.36	1	-0.19	2	0.46	0.49	1	+0.07	NA	-	-	-	-
7	2	0.36	0.52	0.32	+0.32	4	**0.21**	**0.77**	**0.0004**	+0.71	3	0.42	0.42	1	-0.17	2	0.69	0.51	0.28	-0.39	NA	-	-	-	-
8	2	0.23	0.48	0.16	+0.45	2	0.38	0 52	0.58	+0.19	2	0.23	0.21	1	-0.09	2	0.38	0.58	0.57	+0.25	NA	-	-	-	-
9	2	0 46	0.54	1	+0.11	1	M	M	M	M	3	0.27	0.56	0.02	+0.46	2	0.27	0.33	1	-0.11	NA	-	-	-	-
10	2	0.50	0 43	0 62	-0.17	4	0.24	0 26	0.35	+0.10	3	0 45	0.53	0.65	+0.08	2	0.43	0.35	0.53	-0.25	NA	-	-	-	-
11	2	0.33	0.50	0.54	+0.24	1	M	M	M	M	3	0.27	0.41	0.45	+0.23	2	0.53	0.50	1	-0.17	NA	-	-	-	-
12	2	0.50	0.51	1	+0.02	3	0.13	0.17	1	-0.03	2	0.13	0.16	1	-0.05	3	0.38	0.52	0 33	+0.24	3	0.37	0.32	1	-0.18
13	2	0.67	0.51	0.55	-0.33	3	0.50	0.51	0.02	+0.02	2	0 17	0.24	1	-0.05	4	0.42	0.37	1	-0.12	2	0.17	0.24	1	-0.05
14	2	0.29	0.34	0.54	+0.13	2	0.06	0.22	0.09	+0.65	2	0.06	0.12	1	-	2	0.53	0.40	0.28	-0.33	NA	-	-	-	-
15	3	0.44	0.52	0.78	+0.14	2	0.04	0.08	1	-	3	0 46	0.46	1	+0.01	4	0.48	0.48	1	-0.06	2	0.29	0.25	1	-0.15
16	2	0.52	0.46	0.66	-0.13	2	0.00	0.12	0.02	+1	3	0.50	0.51	0.15	+0.03	3	0.38	0.51	0.26	+0.22	1	M	M	M	M
17	2	0.13	0.23	0.21	+0.35	2	0.12	0.22	0.19	+0.35	4	0.08	0.15	0.05	+0.32	4	0.46	0.52	0.06	+0.12	NA	-	-	-	-
18	4	0.82	0 71	0.33	-0.15	3	0.55	0.56	1	+0.02	2	0.45	0.37	1	-0.25	3	0.45	0.65	0.75	+0.26	3	0.46	0.71	0.28	+0.29
19	3	0.50	0.55	1	+0.06	3	**0.20**	**0.55**	**0.003**	+0 60	2	0.40	0.44	1	+0.10	3	0.70	0.56	0.74	-0.26	3	0.5	0.68	0.08	+0.17
20	5	0.46	0.64	0.05	+0.25	3	0.58	0.63	0.41	+0.07	3	0.52	0.62	0.61	+0.11	3	0.67	0.55	0.20	-0.27	6	0.5	0.49	0.90	-0.09
21	3	0.46	0.60	0.28	+0.25	3	0.09	0.40	0 01	+0.77	3	0.55	0.45	1	-0.22	4	0.73	0.68	1	-0.08	3	0.64	0.48	0.64	-0.35
22	4	0.50	0.60	0.47	+0 18	3	0.40	0.38	1	-0.19	2	0.58	0.54	0 66	-0.15	4	0.67	0.57	0.7	-0.18	4	0.4	0.43	0.32	-0.03
23	4	0.69	0.56	0.16	-0.23	3	0.50	0.59	0.15	+0.15	3	0.75	0.59	0.20	-0.36	4	0.56	0.54	0.05	-0.07	5	0.75	0.61	0.72	-0.23
24	4	0.59	0.68	0.06	+0 07	3	0.47	0.53	0.36	+0.10	4	0.65	0.59	0.90	-0 09	4	0.53	0.57	0.60	+0.07	7	0.59	0 66	0.62	+0.11
25	2	0.46	0.44	1	-0.04	2	0.08	0.15	-	-	2	0.69	0.47	0.21	-0.50	2	0.46	0.49	1	+0.07	3	0.33	0.37	1	-0.11
26	2	0.55	0.52	1	-0 05	3	0.36	0.56	0.25	+0.28	3	0.73	0.58	0.78	-0.26	3	0.64	0.57	1	-0.13	5	0.36	0.62	0.07	+0.37
27	3	0.46	0.59	0.75	+0.16	3	0.17	0.51	0.01	+0.67	4	0.58	0.66	0.77	+0.12	3	0.58	0.56	1	-0.04	3	0.33	0.51	0.20	+0.35
28	5	0.58	0.70	0 85	+0.09	3	0.75	0.56	0.41	-0.36	3	0 67	0.50	0.64	-0.39	2	0 50	0.55	1	+0.02	4	0.42	0.66	0.28	+0.30
29	3	0.55	0.50	1	-0.26	3	0.73	0.54	0.35	-0 38	3	0.46	0.46	1	-0.16	3	0.89	0.58	0.08	-0.58	5	0.45	0 67	0.12	+0.27
30	2	0.46	0 37	1	-0.26	3	0.69	0.66	0 89	-0 06	4	0.39	0.44	0.68	+0.14	2	0.38	0.39	1	-0.20	3	0.54	0.66	0.57	+0.19
31	3	0.54	0.54	1	+0.01	3	0.39	0.45	0.52	+0.02	4	0.75	0.58	0.88	-0.30	4	0 67	0.62	0 84	-0.14	4	0.69	0.71	0.66	-0.03
32	2	**0.88**	**0.51**	**0.003**	-0.78	3	0.47	0.59	0.77	+0.12	3	0.47	0.52	1	+0.10	3	0.59	0.58	0.52	-0.01	4	0.35	0.36	1	-0.12
33	4	0.70	0.54	0 74	-0.33	2	0.60	0 56	1	-0 20	3	0 60	0 65	0.73	-0.07	2	0 60	0.57	1	-0 15	3	0.30	0.42	0.48	+0.29

N= number of specimens amplified, N_A= number of alleles, H_O, H_E=Observed and Expected heterozygosity, P=exact probability for expected Hardy Weinberg equilibrium conditions for each locus/population combination (Arlequin v2.1). F_{IS}= Weir & Cockerham (1984) (GENEPOP V3.4). **Values in bold** departures from HWE significant after Bonferroni correction, populations analysed at 9 loci k=9, p1=0.05/9, at 10 loci k=10, p1=0.05/10. ^ List14-079 amplified in subset of populations. M= monomorphic, NA= not amplified. Population numbering as in Table 33 on page 191.

7.1.6 Population genetic diversity and heterogeneity

Population genetic diversity and heterogeneity was investigated at a number of hierarchical levels (1) population level (33 groups) (2) by State (5 groups) (3) State partitioned by ecotope (10 groups) (4) division of all specimens by ecotope (3 groups)

Intrapopulation analysis was investigated using pairwise estimates of F_{ST} generated in Arlequin V2.000 with the probability of differentiation estimated over 10,000 randomisations (see Table 33 on page 191). F_{ST} and R_{ST} estimates can vary for the same data set (Lugon-Moulin *et al.,* 1999). In this study a significant but weak correlation was detected between F_{ST} and R_{ST} values for population comparisons (R^2 =0.4, p=0.0001 Mantel test 9999 permutations GenAlEx). As F_{ST} performs well in data sets with limited loci and sample numbers it was decided to use this index. The number of significant differences detected by R_{ST} was lower in both Portuguesa State and Barinas, however the same discrete populations were detected by both methods, although to a lesser degree by R_{ST}. F_{ST} will show patterns of divergence caused by drift, while R_{ST} reflects mutational differences. Both indices detected homogeneity between ecotopes however some discrepancies were noted. For example in the locality Cascabel R_{ST} indicated population homogeneity between palm and peridomestic ecotopes (R_{ST}=0), while F_{ST} indicated a higher but non-significant difference (F_{ST}=0.06).

7.1.6.1 Populations

The number of polymorphic loci in populations ranged from 6-10, with 85% of all populations polymorphic at all loci (see Table 32 on page 190, Table 34 on page 195, Table 36 on page 200). The mean number of alleles per locus in a population varied from 1.9 (Santa Lucia domestic) to 4.7 (Cascabel palm), with allele richness (see page 121) also lowest in Santa Lucia (1.7) and highest in Cascabel and Laguna Hermosa palm populations (3.58 and 3.49 respectively). Specimens in Santa Lucia were monomorphic at three loci and in San Bartolo and Casa Rena populations at two loci. The number of private alleles detected in the study was low, nine in total, four of which occurred in a

single domestic population in Loma de Amarillo, Trujillo State (see Table 32, Table 34, Table 36). Mean observed heterozygosity ranged from 0.2-0.6 and expected heterozygosity between 0.3-0.6. Heterozygosity and the mean number of alleles varied between samples. Pairwise F_{ST} estimates are shown in Table 33 on page 191.

7.1.6.1.1 Portuguesa State

Table 32. Summary of population diversity in Portuguesa State

Population	Ecotope	N	Nloci	Npol	A	A_M	A_N	P_A	H_O	H_E	F_{IS}
Terronal h1 01	House	26	9	9	2.3	2.6	3	-	0.31	0.41	0.20
Terronal h2 01	House	18	9	9	2.4	2.7	1	-	0.38	0.45	0.10
Terronal h2 p01	Palm	26	9	9	2.0	2.4	2	-	0.22	0.32	0.28
Terronal h1 03	House	10	9	9	2.5	2.5	2	-	0.43	0.49	0.06
Terronal h2 p03	Palm	39	9	9	2.4	3.0	5	-	0.40	0.45	0.07
San Bartolo h1	House	14	9	7	2.4	2.4	1	-	0.35	0.40	0.10
San Bartolo h2	House	14	9	7	2.3	2.3	3	-	0.27	0.42	0.32
Santa Lucia	House	13	9	6	1.7	1.9	-	-	0.23	0.28	0.08
Casa Rena	House	11	9	7	2.2	2.2	-	-	0.33	0.38	0.02
Laurianito	Peridomestic	21	9	9	2.7	3.1	2	-	0.39	0.46	0.11
Palo Gacho	Palm	15	9	8	2.3	2.6	-	-	0.29	0.41	0.20
Los Rastrojos*	House	24	10	9	2.3	2.6	1	1	0.32	0.35	0.03
Los Rastrojos*	Palm	12	10	10	2.7	2.9	1	1	0.40	0.44	0.00

N= no of specimens, Nloci= No of Loci, Npol= No of polymorphic loci . A= allele richness averaged over all loci analysed in pop. A_M= mean no of alleles, A_N= null alleles. P_A=private alleles, H_O H_E = Observed and expected heterozygosity averaged values over all loci. * for 9 loci A_M = 2.6, 3.0 He= 0.33, 0.43. A=2.2, 2.7. F_{IS}= inbreeding coefficient averaged over all loci (Fstat).

A total of 243 specimens consisting of 13 populations from 7 localities in Portuguesa were analysed in this study. These included 130 domestic, 92 silvatic and 21 peridomestic specimens. One house was sampled twice in different years (Terronal h1 in 2001 and 2003). Two localities were sampled at adjacent houses and palms (Terronal and Los Rastrojos).

Table 33. F_{ST} values (p-values above diagonal) for pairwise comparisons of all specimens grouped by State locality and ecotope.

Pop	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Pop 1		0.02	**<0.01**	0.03	<0.01	<0.01	0.03	**<0.01**	0.01	0.03	0.02	**<0.01**	<0.01	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.10	0.01	**<0.01**	0.01	**<0.01**	**<0.01**	**<0.01**	<0.01	0.11	<0.01	0.05	**<0.01**	**<0.01**	<0.01	**<0.01**	**<0.01**
Pop 2	0.03		**<0.01**	<0.01	0 42	**<0.01**	**<0.01**	**<0.01**	0.58	0.08	0.50	**<0.01**	<0.01	**<0.01**	**<0.01**	**<0.01**	**<0.01**	<0.01	0.08	**<0.01**	0.05	<0.01	**<0.01**	**<0.01**	<0.01	**<0.01**	<0.01	0.02	**<0.01**	**<0.01**	0.01	**<0.01**	**<0.01**
Pop 3	0.13	0.20		0.02	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Pop 4	0.05	0.09	0.06		0.002	**<0.01**	<0.01	**<0.01**	<0.01	0.03	0.02	**<0.01**	**<0.01**	<0.01	**<0.01**	**<0.01**	**<0.01**	0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0 42	<0.01	0.01	<0.01	**<0.01**	0.06	<0 01	0.01
Pop 5	0.04	0.00	0.17	0.07		**<0.01**	**<0.01**	**<0.01**	0.15	0.01	0.03	**<0.01**	**0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.04	**0.01**	0.06	0.01	**0.01**	<0 01	0.01	0 01	<0 01	<0.01	<0.01	**<0.01**	0.01	**<0.01**	<0 01
Pop 6	0.06	0.10	0.26	0.10	0.12		0.10	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	<0.01	**<0.01**	<0.01	**<0.01**	**<0.01**	**<0.01**	0.01	<0.01	**<0.01**	0.01	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Pop 7	0.04	0.10	0.24	0.10	0.11	-0.03		**<0.01**	**<0.01**	<0.01	<0.01	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.07	<0.01	**<0.01**	0.01	**<0.01**	**<0.01**	**<0.01**	0.01	0.05	**<0.01**	0.09	<0.01	**<0.01**	**<0.01**	**<0.01**	<0.01
Pop 8	0.17	0.19	0.29	0.22	0.20	0.22	0.20		**<0.01**	**<0.01**	**<0.01**	**<0.01**	<0.01	**0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Pop 9	0.06	0.00	0.23	0.14	0.02	0.13	0.13	0.24		0.12	0.20	**<0.01**	0.01	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.08	**<0.01**	0.10	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	0.06	**<0.01**	**<0.01**	0.03	**<0.01**	**<0.01**
Pop 10	0.03	0.02	0.13	0.04	0.03	0.09	0.08	0.23	0.02		0.04	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.08	<0.01	0.01	<0.01	**<0.01**	**<0.01**	<0.01	<0.01	0.01	<0.01	0.18	**<0.01**	**<0.01**	0.08	**<0.01**	**<0.01**
Pop 11	0.04	0.00	0.16	0.07	0.03	0.12	0.11	0.14	0.02	0.04		0.0004	<0.01	0.01	**<0.01**	**<0.01**	**<0.01**	<0.01	0.22	<0.01	0.04	<0.01	**<0.01**	**0.01**	0.07	0.06	<0.01	0.01	0.02	**<0.01**	0.08	**<0.01**	<0.01
Pop 12	0.12	0.07	0.30	0.19	0.12	0.18	0.18	0.13	0.11	0.16	0.07		0.01	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Pop 13	0.10	0.06	0.26	0.17	0.08	0.16	0.15	0.13	0.07	0.12	0.08	0.04		**<0.01**	**<0.01**	**<0.01**	**<0.01**	<0.01	0.01	**0.01**	0 01	**0.01**	**<0.01**	**<0.01**	**0.01**	0.01	<0.01	<0 01	<0.01	**<0.01**	<0.01	**<0.01**	**<0.01**
Pop 14	0.14	0.13	0.23	0.10	0.16	0.12	0.15	0.16	0.19	0.16	0.07	0.14	0.19		**<0.01**	**<0.01**	**<0.01**	**<0.01**	<0.01	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	<0.01	<0.01	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Pop 15	0.14	0.10	0.26	0.14	0.10	0.18	0.18	0.29	0.11	0.11	0.14	0.17	0.11	0.22		**<0.01**	**<0.01**	**0.01**	**<0.01**	**<0.01**	**0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Pop 16	0.16	0.14	0.29	0.24	0.11	0.22	0.21	0.35	0.11	0.14	0.20	0.26	0.16	0.33	0.15		**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Pop 17	0.20	0.14	0.40	0.31	0.14	0.25	0.24	0.42	0.14	0.20	0.21	0.22	0.17	0.33	0.19	0.20		**<0.01**	**<0.01**	**<0.01**	<0.01	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Pop 18	0.03	0.09	0.16	0.07	0.09	0.07	0.04	0.24	0.11	0.06	0.10	0.18	0.12	0.18	0.13	0.18	0.18		0.08	0.02	0.10	<0.01	0.01	0.04	<0.01	0.08	0.01	0.44	0.01	<0.01	0.03	0.01	0.02
Pop 19	0.06	0.03	0.17	0.05	0.03	0.08	0.08	0.18	0.04	0.03	0.02	0.10	0.06	0.08	0.09	0.15	0.17	0.04		0.64	0.73	0.77	0.21	0.46	0 26	0.31	0 63	0.86	0.97	<0.01	0.80	0.01	0.66
Pop 20	0.08	0.08	0.16	0.08	0.06	0.11	0.09	0.18	0.08	0.06	0.08	0.16	0.08	0.15	0.13	0.12	0.18	0.04	0.00		0.23	0.21	0.26	0.99	0 01	0.06	0.74	0.64	0.19	<0.01	0.22	<0.01	0.64
Pop 21	0.06	0.03	0.19	0.09	0.03	0.08	0.08	0.21	0.03	0.05	0.05	0.12	0.06	0.13	0.08	0.10	0.09	0.03	-0.01	0.01		0 81	0.01	0.34	0.04	0.13	0.13	0.70	0.46	<0.01	0.59	<0.01	0.06
Pop 22	0.07	0.06	0.16	0.06	0.03	0.11	0.10	0.19	0.06	0.06	0.06	0.15	0.09	0.13	0.09	0.10	0.16	0.06	-0.01	0.01	-0.01		0.02	0.50	0 10	0.10	0.32	0.26	0.06	**<0.01**	0.28	<0 01	0.22
Pop 23	0.10	0.11	0.21	0.12	0.08	0.14	0.12	0.24	0.09	0.08	0.11	0.20	0.11	0.21	0.15	0.13	0.22	0.06	0.01	0.01	0.05	0.02		0.31	<0.01	<0.01	0.38	0.14	<0.01	**<0.01**	0.01	0.01	0 85
Pop 24	0.07	0.08	0.17	0.08	0.05	0.10	0.08	0.22	0.07	0.06	0.09	0.17	0.10	0.17	0.12	0.10	0.17	0.03	0.00	-0.01	0.01	0.00	0.00		0.02	0.05	0.82	0.89	0.07	**<0.01**	0.11	0.016	0.80
Pop 25	0.06	0.06	0.17	0.05	0.05	0.11	0.10	0.14	0.08	0.08	0.03	0.10	0.10	0.07	0.15	0.15	0.24	0.10	0.01	0.04	0.04	0.02	0.07	0.03		0 17	0.34	0.03	0.03	**<0.01**	0.03	<0.01	0.02
Pop 26	0.03	0.06	0.11	0.01	0.05	0.07	0.05	0.11	0.08	0.06	0.04	0.12	0.08	0.08	0.11	0.17	0.24	0.04	0.01	0.03	0.03	0.02	0.06	0.03	0.02		0.19	0.11	0.06	**<0.01**	0.42	<0.01	0.06
Pop 27	0.09	0.08	0.20	0.10	0.06	0.12	0.10	0.17	0.07	0.08	0.08	0.14	0.08	0.16	0.14	0.10	0.22	0.07	0.00	-0.01	0.03	0.01	0.00	-0.01	0.01	0.02		0.42	0.22	**<0.01**	0.10	<0.01	0.70
Pop 28	0.03	0.04	0.17	0.07	0.04	0.05	0.03	0.20	0.03	0.02	0.06	0.13	0.07	0.14	0.11	0.10	0.15	0.00	-0.02	0.00	-0.01	0.01	0.01	-0.01	0.04	0.03	0.01		0.18	0.01	0.36	0.02	0.46
Pop 29	0.10	0.07	0.21	0.09	0.07	0.10	0.10	0.18	0.07	0.07	0.05	0.12	0.08	0.08	0.13	0.18	0.19	0.07	-0.03	0.01	0.00	0.02	0.05	0.02	0.03	0.04	0.02	0.02		<0.01	0.26	<0.01	0.17
Pop 30	0.15	0.17	0.18	0.18	0.15	0.20	0.18	0.33	0.15	0.09	0.17	0.30	0.18	0.27	0.22	0.19	0.28	0.10	0.08	0.07	0.10	0.11	0.07	0.09	0.18	0.13	0.12	0.06	0.09		<0.01	**<0.01**	<0.01
Pop 31	0.06	0.04	0.12	0.04	0.04	0.11	0.10	0.19	0.05	0.02	0.03	0.12	0.07	0.11	0.09	0.15	0.17	0.05	-0.01	0.01	0.00	0.01	0.04	0.02	0.03	0.01	0.03	0.01	0.01	0.08		**<0.01**	0.04
Pop 32	0.08	0.12	0.22	0.13	0.10	0.14	0.11	0.22	0.14	0.11	0.12	0.17	0.11	0.21	0.17	0.19	0.23	0.06	0.06	0.05	0.08	0.08	0.04	0.03	0.09	0.06	0.06	0.05	0.09	0.13	0.09		0.05
Pop 33	0.09	0.10	0.22	0.08	0.07	0.11	0.10	0.24	0.10	0.07	0 10	0.19	0.13	0.17	0.14	0.15	0.24	0.06	-0.01	0.00	0.04	0.01	-0.01	-0.01	0.04	0.04	-0.01	0.00	0.02	0.10	0.04	0.04	*

Values in bold **non-significant** after Bonferroni correction k=528, p1=0.05/528 p≤0.0001 (microsatellite set 1).

Pop: 1 Terronal h1 01, **2** Terronal h2 01, **3** Terronal h2 p01,**4** Terronal h1 03, **5** Terronal h2 p03, **6** San Bartolo h1 ,**7** San Bartolo h2, **8** Santa Lucia h , **9** Casa Rena h, **10** Laurianito pd, **11** Palo Gacho, **12** Los Rastrojos h, **13** Los Rastrojos p, **14** Lara h, **15** Cojedes p, **16** Cojedes h, **17** Trujillo h, **18** Cascabel pd, **19** Cascabel h, **20** Cascabel p, **21** Guaranda h, **22** Guaranda p, **23** L. Hermosa h, **24** L. Hermosa p, **25** L. Hermosa pd, **26** G. Paraguey h1, **27** G. Paraguey h2, **28** G. Paraguey p1, **29** G. Paraguey p2, **30** 19 Abril pd, **31** Parcelamiento p, **32** Rio Bravo II pd, **33** Rio Bravo II p.

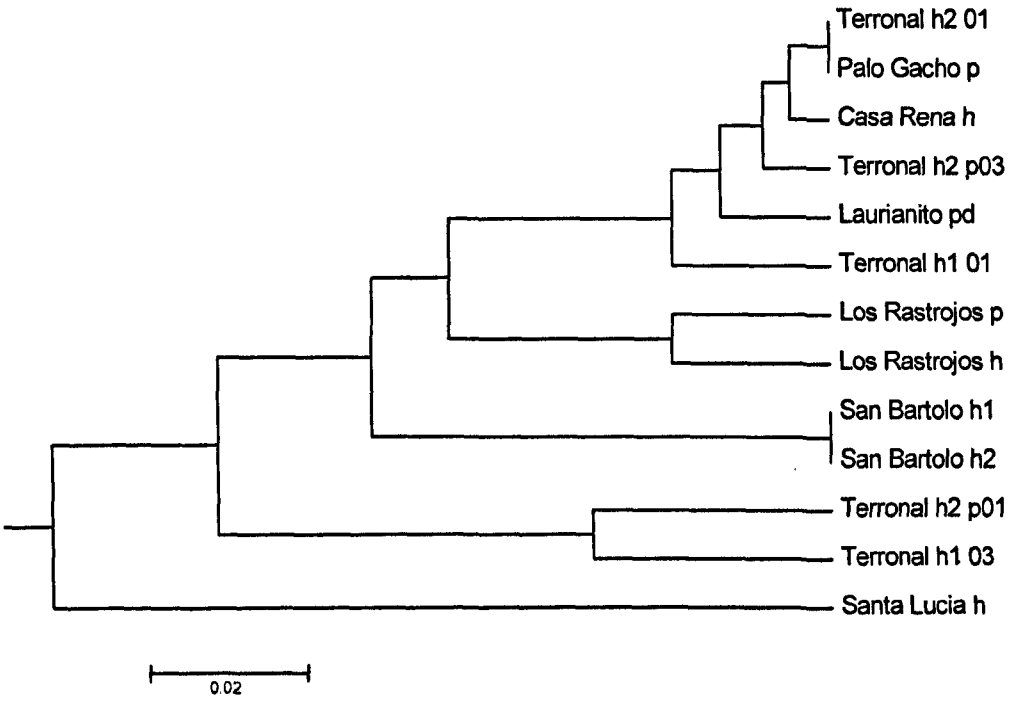

Figure 25. An UPGMA tree for pairwise F_{ST} values between localities in Portuguesa State. h=house, p=palm, pd=peridomestic, 01=2001, 03=2003.

The comparison of populations within Portuguesa State revealed varying degrees of heterogeneity (see Table 33 on page 191). Of the 78 pairwise F_{ST} comparisons within Portuguesa 43 (55%) were significantly different from 0 after Bonferroni correction (F_{ST} range=0-0.3).

Homogeneity was detected between the two adjacent house and palm populations in Portuguesa: in Terronal between house 2 and palm 03 (F_{ST}=0 p-values=0.42) and in Los Rastrojos, (F_{ST}=0.04 p-value=0.006 set 1 and 2). Los Rastrojos house and palm were significantly different from the majority of other populations in Portuguesa (see Table 33 on page 191) and cluster separately (see Figure 25 above).

A lack of significant population heterogeneity was also detected between domestic ecotopes within localities in Portuguesa. San Bartolo house 1 and house 2 were not significantly different (F_{ST}=0 p-value=0.997). Low and non-significant values were

detected among domestic ecotopes in Terronal, house 1 and house 2 2001 (F_{ST}= 0.03, p-value=0.02) and house 1 from each sampling year (F_{ST}= 0.05, p-value=0.025).

The UPGMA tree (see Figure 25 on page 192) illustrates the structural relationship among localities within Portuguesa based on the F_{ST} indices calculated. Structural similarity is also visible between populations from different ecotopes for example between Terronal domestic (h2) and Palo Gacho (palm) (F_{ST}=0 p-value= 0.495) and within ecotopes San Bartolo h1 and h2. Localities Casa Rena and Laurianito also exhibited low and insignificant pairwise differences with populations in Terronal (see Table 33 on page 191, Figure 25).

The isolation of the domestic population Santa Lucia is visible. Santa Lucia was significantly different from all populations in Portuguesa (F_{ST}=0.1-0.2), with the exception of Los Rastrojos palm, where high but non-significant F_{ST} values were detected. Santa Lucia was also significantly different from the majority of populations in the study (F_{STmax}=0.42). Three loci were monomorphic in this population, List14-042, List14-010 and List14-064 fixed for alleles 170bp, 323bp and 243bp respectively. The mean number of alleles and allele richness detected in this population was the lowest in the study (1.9 and 1.7), indicating possible drift. San Bartolo h1 and h2 were also significantly different from the majority of other populations in Portuguesa, and cluster together (see Table 33, Figure 25). Both populations were monomorphic at the two loci (List14-010 and List14-064) and were fixed for the same alleles (323bp and 243bp).

Significant population differentiation was detected between Terronal palm populations (F_{ST}=0.2, p-value<0.0001), and between Terronal palm and domestic populations (house 2 2001) from the same year (F_{ST}=0.1, 0.2 p-value<0.0001), which cluster separately on the tree (see Table 33, Figure 25). Terronal palm 1 01 was significantly different from all other populations in Portuguesa (F_{ST}=0.13 - 0.3), with the exception of Terronal h1 03. In the population Terronal palm 1 01, loci List14-010, List14-042 were frequently fixed for alleles 323bp and 170bp and observed heterozygosity and allele richness was

low. Terronal palm 1 01 was also significantly different from the majority of populations in the study (F_{STmax}=0.3).

While significant heterogeneity was detected in the State, due to what appears to be population isolation and drift, population homogeneity was also detected, importantly across house and palm ecotopes, including adjacent populations, which indicates that populations in these differing ecotopes are not always isolated.

7.1.6.1.2 Barinas State

A total of 221 specimens were analysed from Barinas State, divided into 16 populations from 7 localities, all populations were analysed at 10 loci. In 5 of the 7 localities two or more ecotopes were sampled, and population homogeneity was detected, including between adjacent populations in Rio Bravo II. These specimens included 60 domestic specimens, 54 peridomestic and 107 silvatic specimens.

Average allele richness was greater in Barinas State (3.1 with both loci sets) than Portuguesa (2.3). Expected heterozygosity was higher and ranged from 0.5 to 0.6 (see Table 34 on page 195, for population data per locus see Table 30 on page 187, Table 31 on page 188). Pairwise comparisons between localities within Barinas (10 loci) detected population homogeneity, with only 17 of 120 pairwise F_{ST} comparisons (14%) significantly different from zero (F_{ST}=0 to 0.2) (see Table 35 on page 196).

Table 34. Summary of population diversity in Barinas State

Locality	Ecotope	N	Nloci	Npol	A*	A_M*	A_N	P_A	H_O	H_E*	F_{IS}
Cascabel	Peridomestic	11	10	10	2.7	2.8	1	-	0.44	0.52	0.09
Cascabel	House	10	10	10	3.1	3.1	1	-	0.48	0.56	0.10
Cascabel	Palm	24	10	10	3.6	4.6	2	1	0.52	0.58	0.08
Guaranda	House	11	10	10	3.2	3.3	1	-	0.44	0.53	0.13
Guaranda	Palm	20	10	10	3.3	4.0	3	-	0.52	0.55	0.02
Laguna Hermosa	House	16	10	10	3.2	3.6	-	1	0.59	0.57	-0.07
Laguna Hermosa	Palm	17	10	10	3.6	4.3	3	1	0.55	0.59	0.04
Laguna Hermosa	Peridomestic	13	10	10	2.6	2.7	4	-	0.43	0.46	-0.01
G. Paraguey h1	House	11	10	10	3.0	3.1	-	-	0.41	0.56	0.20
G. Paraguey h2	House	12	10	10	3.2	3.4	1	-	0.44	0.55	0.17
G. Paraguey p1	Palm	12	10	10	3.2	3.4	1	-	0.47	0.56	0.12
G. Paraguey p2	Palm	11	10	10	3.3	3.4	2	-	0.52	0.56	0.01
19 Abril	Peridomestic	13	10	10	2.5	2.7	-	-	0.40	0.48	0.11
Parcelamiento	Palm	13	10	10	3.2	3.5	4	-	0.50	0.57	0.08
Rio Bravo II	Peridomestic	17	10	10	2.7	3.0	3	-	0.49	0.51	0.01
Rio Bravo II	Palm	10	10	10	3.5	3.6	2	-	0.52	0.62	0.10

N= no of specimens, Nloci= No of Loci, Npol= No of polymorphic loci . A= allele richness averaged over all loci analysed in pop. A_M= mean no of alleles, A_N = Null alleles, H_O, H_E = Observed and expected heterozygosity averaged values over all loci. * for 9 loci average A= 3.1, average $A_{M=3.3}$. HE range= 0.42-0.59, He average 0.52.

Within the locality **Laguna Hermosa** homogeneity was detected between house and palm populations (F_{ST}=0, p-value=0.38), while pairwise comparison between house and peridomestic specimens gave F_{ST} values that were higher but non significant (F_{ST}=0.06 p-value=0.0004). All comparisons were non-significant (see Table 35 on page 196).

Three populations from different ecotopes within the locality **Cascabel** revealed varying degrees of population heterogeneity. Population difference was lowest between the palm and domestic populations (F_{ST}=0, p-value=0.397) and highest between palm and peridomestic specimens (F_{ST}=0.05, p-value=0.005). All comparisons were non-significant. In **Guaranda** between a single house and palm (F_{ST}=0, p-value=0.80).

From estimates of F_{ST} population heterogeneity within the locality **G. Paraguey** was low (F_{ST} 0.01-0.03), with all population comparisons non-significant. Analyses of heterogeneity between adjacent **Rio Bravo II** populations was non-significant (F_{ST}=0.04 p=0.045).

Table 35. F_{ST} values (p-values above diagonal) for pairwise comparisons of all specimens groups analysed at 10 loci.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. Los Rastrojos h		0.006	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
2. Los Rastrojos p	0.04		**<0.0001**	**<0.0001**	**<0.0001**	**0.0002**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0001**
3. Cojedes p	0.24	0.20		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
4. Cojedes h	0.35	0.30	0.15		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
5. Cascabel pd	0.19	0.15	0.14	0.21		0.105	0.005	0.102	**0.0001**	0.007	0.060	**0.0003**	0.116	0.008	0.543	0.005	**0.0002**	0.009	0.002	0.013
6. Cascabel h	0.13	0.10	0.10	0.17	0.03		0.397	0.741	0 472	0.224	0.596	0.167	0.376	0 697	0.926	0.899	0.0016	0.350	0.006	0.437
7. Cascabel *p*	0.21	0.16	0.12	0.12	0.05	0.00		0.202	0.263	0.213	0.853	0.009	0.064	0.684	0.325	0.182	**<0.0001**	0.012	0.004	0.663
8. Guaranda h	0.16	0.13	0.08	0.11	0.03	-0.01	0.01		0.745	0.010	0.375	0.037	0.170	0.185	0.716	0.389	**0.0001**	0.118	0.003	0.042
9. Guaranda p	0.21	0.18	0 09	0.10	0.06	0.00	0.01	-0.01		0.014	0.247	0.136	0.101	0.276	0.096	0.051	**<0.0001**	0.007	**0.0003**	0.270
10. L. Hermosa h	0.23	0.17	0.14	0.14	0.05	0.01	0.01	0.04	0.02		0.382	0.0004	0.007	0.442	0.132	0.006	**<0.0001**	0.001	0.004	0.780
11. L. Hermosa p	0.19	0.14	0.12	0.12	0.03	0.00	-0.01	0.01	0.01	0.00		0.012	0.081	0.844	0.934	0.069	**<0.0001**	0.024	0.008	0.546
12. L. Hermosa pd	0.18	0 19	0.14	0.15	0.10	0.02	0.04	0.04	0.01	0.06	0.03		0.188	0.363	0.016	0.034	**<0.0001**	0.001	0.001	0.030
13. G. Paraguey h1	0.16	0.14	0.11	0 18	0.03	0.01	0 03	0.03	0.02	0.05	0.02	0.02		0.269	0.142	0.077	**0.0001**	0.163	0.009	0.065
14 G. Paraguey h2	0.18	0.14	0.13	0.12	0.06	-0.01	0.00	0.02	0.01	0.00	-0.01	0.01	0.02		0.474	0.198	**0.0001**	0.012	0.007	0.612
15. G. Paraguey p1	0.15	0.11	0.11	0.13	0.00	-0.02	0.01	-0.01	0.02	0.01	-0.01	0.04	0.03	0.01		0.128	0.0016	0.078	0.006	0.218
16. G. Paraguey p2	0.18	0.15	0.13	0.19	0.06	-0.02	0.01	0.01	0.02	0.04	0.02	0.03	0.03	0.02	0.02		**0.0001**	0.030	**0.0003**	0.232
17. 19 Abril pd	0.31	0.23	0.22	0.23	0.10	0.08	0.08	0.10	0.12	0.07	0.08	0.19	0.13	0.12	0.07	0.10		0.002	**<0.0001**	**<0.0001**
18. Parcelamiento p	0.17	0.14	0.12	0.19	0.06	0.01	0.03	0.02	0.04	0.05	0.03	0.06	0.02	0.04	0.03	0.03	0.07		**0.0001**	0.002
19. Rio Bravo II pd	0.23	0.20	0.16	0 19	0.07	0.06	0.04	0.07	0.07	0.04	0.04	0.08	0.06	0.06	0.05	0.08	0.14	0.11		0.045
20. Rio Bravo II pd	0 25	0.21	0.13	0 15	0.06	0 01	0 00	0 04	0.01	-0.01	0.00	0 03	0 04	0.00	0 02	0 01	0.11	0.06	0 04	

Values in Bold significant after sequential Bonferroni correction k=190, p1=0.05/190 p≤0.0003

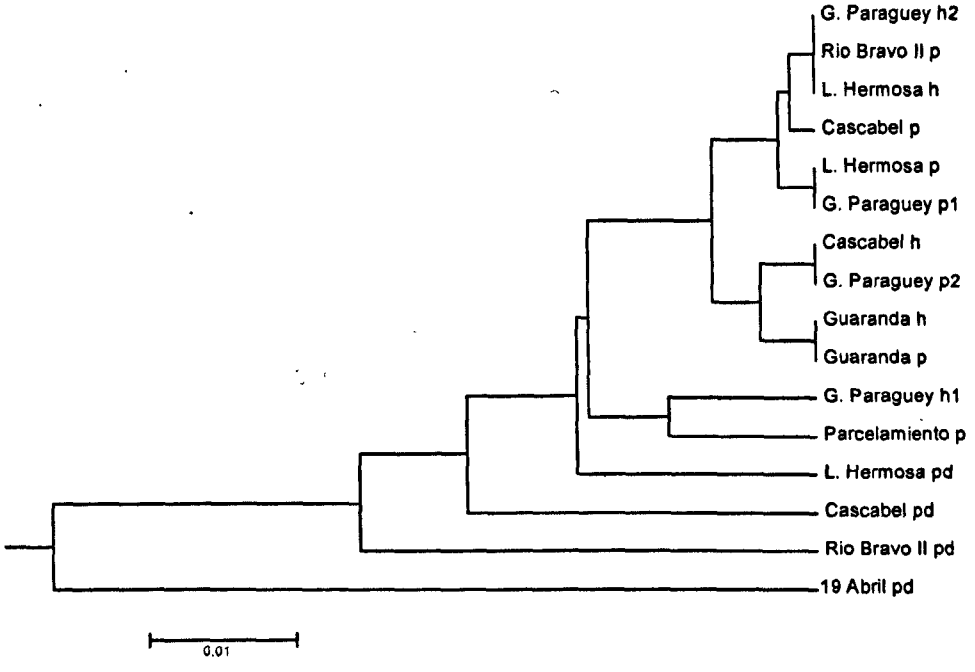

Figure 26. An UPGMA tree for pairwise F_{ST} values between localities within Barinas (10 loci). h=house, p=palm, pd=peridomestic

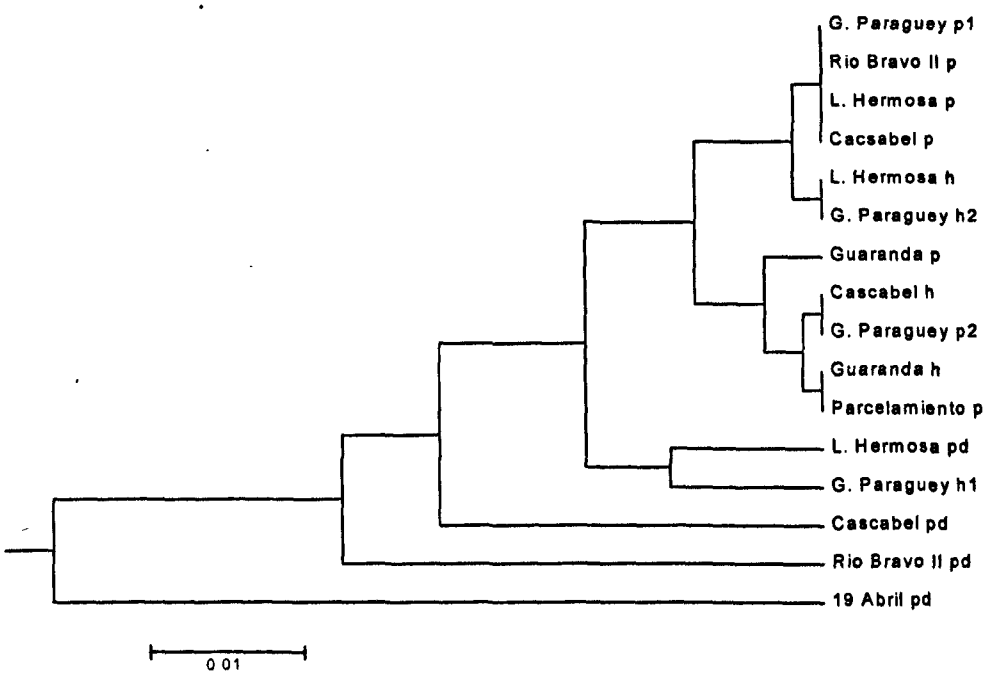

Figure 27. An UPGMA tree for pairwise F_{ST} values between localities within Barinas (9 loci). h=house, p=palm, pd=peridomestic.

From Figure 26 on page 197 homogeneity is visible between silvatic and domestic populations across the State of Barinas (10 loci), including across palm populations, (Laguna Hermosa and G. Paraguey palms), across domestic populations (Laguna Hermosa house, G. Paraguey house 2), and between ecotopes (Guaranda house and palm).

Detected heterogeneity within Barinas was related to the population 19 Abril pd. This population exhibited high values of F_{ST} for all pairwise comparisons (F_{ST} 0.07-0.13) and was significantly different from the majority of populations (see Table 35 on page 196). The population isolation is also visible from Figure 27 and Figure 26. Rio bravo II pd also exhibited high values of F_{ST} in the majority of pairwise comparisons and also clustered distantly on the tree.

With nine loci (see Figure 27 above) the clustering was broadly the same with changes including rearrangement of the relationship between domestic populations G. Paraguey

h2 and Laguna Hermosa with palm populations from the same localities. Guaranda house and palm cluster is disrupted, with Guaranda house now grouping with the Parcelamiento palm population.

7.1.6.1.3 Lara State

A single domestic population was analysed in this State. Mean allele number and richness were low (2.2, 2.0) (see Table 36 on page 200). This population was significantly different by pairwise comparisons from the majority of populations analysed, (F_{ST} 0.07-0.33). High but non-significant values were detected with Palo Gacho, Terronal h1 03, Cascabel domestic and G. Paraguey palm 1 populations (see Table 33 on page 191).

7.1.6.1.4 Cojedes State

In the locality Las Quebralitas in Cojedes intrapopulation analysis by F_{ST} indicated significant heterogeneity between a single silvatic and adjacent domestic population (F_{ST}= 0.15 p-value<0.0001, see Table 35 on page 196). These populations were also significantly different from the majority other populations (see Table 33).

7.1.6.1.5 Trujillo State

A single domestic population was analysed in Trujillo from the locality Loma de Amarillo and was found significantly different from all other populations from F_{ST} comparisons (range 0.14-0.4) with the exception of Guaranda house (see Table 36). Four private alleles were detected in this population, at List14-017 allele 154, at List14-064 allele 239 and at List14-025 alleles 163 and 173. All private alleles were detected in the single female adult identified as *R. robustus* by *cytb* analysis.

Table 36. Summary of population diversity in the States of Lara, Cojedes and Trujillo.

State	Ecotope	N	Nloci	Npol	A	A_M	N_U	P alleles	H_O	H_E	F_{IS}
Lara	House	17	9	9	2.03	2.2	1	-	0.257	0.359	0.204
Cojedes	Palm	24	10	10	2.63	3.0	3	1	0.41	0.455	0.078
Cojedes	House	24	10	10	2.09	2.2	1	-	0.272	0.353	0.182
Trujillo	House	26	9	9	2.17	3.0	2	3	0.212	0.305	0.250

N= no of specimens, Nloci= No of Loci, Npol= No of polymorphic loci. A= allele richness. A_M= mean no of alleles, H_O, H_E = Observed and expected heterozygosity averaged values over all loci. * for 9 loci average A= 3.1, average A_M=3.3 H_E range= 0.42-0.59, H_E average 0.52.

Over all 33 populations (528 F_{ST} pairwise comparisons, 9 loci) 267 significant differences were calculated (F_{ST} ranged from 0.0-0.42). Of the 329 pairwise comparisons among populations from different States 214 were significantly different following Bonferroni correction (65%), values of F_{ST} ranged from F_{ST}=0.01 to 0.42.

From Figure 28 on page 201 the pattern of homogeneity seen between ecotopes within States can be seen to occur across all States such as Terronal h2 01 and Palo Gacho palm, Guaranda house and Parcelamiento palm, Cascabel and G. Paraguey p2. Population homogeneity between silvatic ecotopes is also visible G. Paraguey p1, Rio Bravo II p, Laguna Hermosa p and Cascabel p, also between domestic ecotopes, Laguna Hermosa and G. Paraguey h2.

From Figure 28, 7 populations appear distinct, and do not form clusters with other populations (Cojedes house and palm, Lara, Santa Lucia, Terronal h2 palm 01, 19 Abril and Trujillo). Domestic specimens from San Bartolo clustered together and were significantly differentiated from other populations, as with Los Rastrojos house and palm populations. Clusters within States (see Figure 25 on page 192, Figure 27 on page 198) are broadly maintained, with State localities remaining separate, with the exception of localities Cascabel pd, G. Paraguey h1 and Rio Bravo p with localities in Portuguesa.

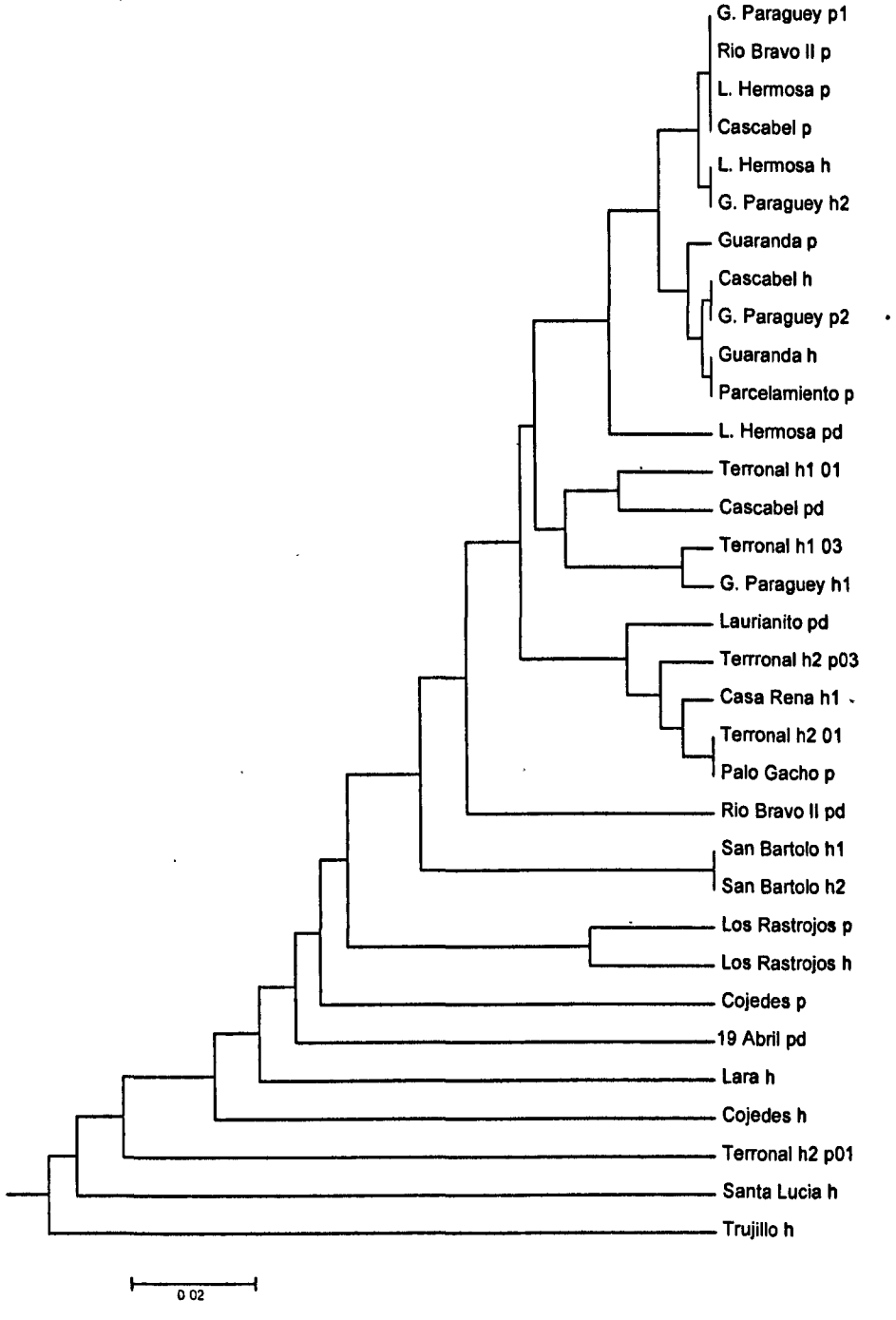

Figure 28. An UPGMA tree for pairwise F_{ST} values between all State localities (9 loci). h=house, p=palm, pd=peridomestic. 01=2001, 03=2003.

7.1.6.2 State comparisons

Population heterogeneity and diversity were also investigated between specimens grouped by State, and State groups divided by ecotope (set 1). All State comparison values of F_{ST} were high and significantly different from zero (after Bonferroni correction) ranging from F_{ST}=0.33 (p-value<0.0001) between Lara and Trujillo and F_{ST}=0.04 (p-value<0.0001) between Barinas and Portuguesa.

When State samples were further subdivided by ecotope (10 groups) to investigate population homogeneity. F_{ST} comparisons were still highly significant between States but within States population homogeneity was detected between ecotopes. Barinas house and Barinas palm and peridomestic were not significantly different. In Portuguesa palm and peridomestic comparison was also non-significant (F_{ST}=0.02). F_{ST} values were highest for comparisons between the States of Trujillo, Lara and Cojedes with all other States (range F_{ST}=0.1-0.3).

Table 37. Summary of diversity for State groupings divided by ecotope

State	Ecotopes	N	No. loci	Unbiased Hz	Mean Allele No.	Mean allele richness
Portuguesa	H PD P	243	9	0.42	4.44	2.98
Lara	H	17	9	0.32	2.22	2.22
Cojedes	H P	48	9	0.45	3.11	2.76
Trujillo	H	26	9	0.28	3.00	2.61
Barinas	H PD P	221	9	0.54	6.44	3.87

Unbiased Hz =Gene diversity (unbiased Nei 1987), H=House, PD= peridomestic, P=palm

7.1.6.3 Ecotope comparisons

The 555 specimens were divided by ecotope to examine diversity and population heterogeneity at nine loci (set 1). This included 257 domestic specimens, 223 silvatic and 75 peridomestic. F_{ST} values were low but significantly different from zero for all group comparisons after Bonferroni correction (F_{ST}=0.01-0.02, p-value<0.0001, 0.0002). While the mean number of alleles per locus was greatest in the domestic environment (6.44), the mean allele richness was greatest in palms (5.1). Gene diversity (unbiased Nei 1987) was highest in palms (0.51, followed by the peridomestic 0.49).

Table 38. Summary of diversity for specimens divided by ecotope

Ecotope	Sample size	Loci no	Unbiased Hz	Mean allele no	Mean allele richness
House	257	9	0.4522	6.44	0.452
Palm	223	9	0.5100	6.33	0.510
Peridomestic	75	9	0.4935	4.44	0.494

Unbiased Hz =Gene diversity (unbiased Nei 1987), the mean expected heterozygosity per locus in a population.

7.1.6.4 Isolation by distance (IBD)

Tests for IBD ($F_{ST}/(1-F_{ST})$ against log transformed (ln) distances were conducted at various hierarchical levels (1) across State level (2) locality level (3) population level (4) within Portuguesa and Barinas State. Patterns were weakly correlated but significant at population level (33 groups; R^2=0.06 p-value=0.0001), locality level (17 groups; R^2=0.06 p-value=0.0001) and non-significant at State level (5 groups; R^2=0.01 p-value=0.64). Patterns were weakly correlated but significant within Portuguesa State (13 groups; R^2=0.07 p-value=0.01), within Barinas (16 groups; R^2=0.02 p-value=0.01). R^2 values range from 0 to 1. Its value represents the fraction of shared variance in the two compared variables i.e. R^2=0.06, therefore 6% of the variance in X can be explained by variation in Y at nominal significance level $p<0.05$.

7.1.6.5 Genetic distances

For matrices of genetic distances see Table 39 on page 204 and appendix Table 69 on page 366). Figure 30 on page 208 is a neighbour joining tree for all populations compared produced in PHYLIP Neighbour.exe (Felsenstein 1993) and visualised in MEGA V3 (Kumar *et al.*, 2004) using Nei's standard genetic distances D_S (MICROSAT, 500 bootstrap replicates). Bootstrap values were weak and were therefore not included. Genetic distances ranged from D_S=0-0.3 for all 33 populations, with D_S=0-0.2 within Portuguesa and D_S=0-0.1 within Barinas. When groups were analysed separately bootstrapping values increased and were highest within Portuguesa State (see Figure 29 on page 206), but were still predominantly low in Barinas (data not shown).

Table 39. Genetic distances between populations **DS** (below diagonal NEI 1972) and **Dps** (above diagonal Bowcock *et al.*, 1994).

Populations	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1 Terronal h1 01		0.13	0.20	0.17	0.15	0.20	0.16	0.25	0.16	0.15	0.15	0.21	0.26	0.23	0.26	0.24	0.25	0.17	0.21	0.26	0.19	0.23	0.27	0.23	0.21	0.17	0.26	0.17	0.26	0.29	0.21	0.22	0.27
2 Terronal h2 01	0.02		0.27	0.21	0.09	0.23	0.22	0.29	0.12	0.13	0.11	0.18	0.21	0.24	0.21	0.24	0.25	0.24	0.20	0.27	0.20	0.22	0.30	0.26	0.19	0.23	0.25	0.20	0.23	0.31	0.21	0.28	0.28
3 Terronal h2 p01	0.07	0.13		0.19	0.27	0.32	0.29	0.23	0.27	0.23	0.22	0.32	0.36	0.27	0.35	0.34	0.35	0.25	0.28	0.32	0.29	0.30	0.34	0.31	0.26	0.24	0.32	0.27	0.30	0.27	0.24	0.35	0.35
4 Terronal h1 03	0.03	0.07	0.03		0.21	0.24	0.22	0.32	0.25	0.17	0 21	0.30	0.37	0.23	0.30	0.35	0.37	0.25	0.24	0.29	0.28	0.24	0.34	0.28	0.20	0.20	0.28	0.26	0.27	0.35	0.22	0.32	0.29
5 Terronal h2 p03	0.03	0.00	0.12	0.06		0.28	0.26	0.32	0.13	0.14	0.14	0.24	0.24	0.26	0.22	0.20	0.27	0.24	0.19	0.24	0.18	0.18	0.26	0.23	0.19	0.20	0.22	0.21	0.25	0.29	0.20	0.26	0.25
6 San Bartolo h1	0.04	0.07	0.18	0.08	0.10		0.05	0.28	0.24	0.24	0.23	0.27	0.30	0.24	0.32	0.32	0.34	0.20	0.27	0.30	0.26	0.30	0.33	0.29	0.25	0.22	0.30	0.23	0.27	0.33	0.28	0.31	0.31
7 San Bartolo h2	0.03	0.07	0.15	0.08	0.09	-0.02		0.26	0.24	0.23	0.23	0.26	0.29	0.25	0.32	0.31	0.32	0.18	0.26	0.29	0.25	0.28	0.32	0.27	0.26	0.19	0.29	0.21	0.26	0.31	0.26	0.28	0.30
8 Santa Lucia h1	0.10	0.11	0.16	0.13	0.14	0.13	0.11		0.27	0.32	0.23	0.21	0.23	0.25	0.41	0.39	0.37	0.33	0.29	0.33	0.31	0.34	0.37	0.35	0.26	0.25	0.30	0.30	0.29	0.36	0.30	0.36	0.39
9 Casa Rena h	0.04	0.00	0.14	0.10	0.01	0.09	0.09	0.13		0.16	0.14	0.22	0.23	0.28	0.22	0.20	0.24	0.25	0.20	0.25	0.18	0.21	0.27	0.24	0.20	0.22	0.23	0.19	0.24	0.26	0.19	0.31	0.28
10 Laurianito pd	0.02	0.01	0.08	0.04	0.02	0.07	0.07	0.15	0.01		0.14	0.26	0.28	0.24	0.26	0.25	0.30	0.23	0.21	0.24	0.22	0.24	0.27	0.24	0.21	0.24	0.25	0.18	0.24	0.24	0.18	0.29	0.26
11 Palo Gacho p	0.03	0.00	0.09	0.05	0.02	0.08	0.08	0.07	0.01	0.02		0.18	0.24	0.17	0.27	0.29	0.28	0.23	0.19	0.27	0.21	0.25	0.29	0.28	0.14	0.21	0.23	0.22	0.19	0.29	0.19	0.29	0.28
12 Los Rastrojos h	0.08	0.04	0.20	0.13	0.09	0.12	0.12	0.06	0.07	0.11	0.04		0.18	0.23	0.27	0.33	0.26	0.29	0.28	0.34	0.28	0.31	0.36	0.32	0.24	0.27	0.29	0.27	0.29	0.39	0.28	0.30	0.37
13 Los Rastrojos p	0.07	0.04	0.19	0.17	0.07	0.13	0.11	0.07	0.05	0.10	0.06	0.02		0.32	0.26	0.29	0.29	0.30	0.27	0.29	0.26	0.28	0.31	0.29	0.26	0.27	0.26	0.26	0.27	0.32	0.27	0.31	0.35
14 Lara h	0.09	0.08	0.13	0.06	0.11	0.07	0.09	0.08	0.12	0.11	0.04	0.08	0.14		0.36	0.39	0.35	0.29	0.24	0.35	0.29	0.30	0.37	0.34	0.20	0.24	0.31	0.30	0.23	0.33	0.27	0.37	0.33
15 Cojedes p	0.12	0.08	0.21	0.15	0.09	0.16	0.17	0.24	0.08	0.10	0.11	0.13	0.11	0.18		0.23	0.31	0.30	0.27	0.33	0.27	0.28	0.34	0.32	0.32	0.29	0.34	0.28	0.32	0.36	0.27	0.37	0.34
16 Cojedes h	0.11	0.10	0.20	0.21	0.08	0.17	0.16	0.26	0.07	0.11	0.15	0.19	0.12	0.26	0.12		0.28	0.31	0.30	0.31	0.24	0.27	0.30	0.28	0.28	0.30	0.27	0.25	0.33	0.31	0.30	0.34	0.33
17 Trujillo h	0.12	0.08	0.27	0.23	0.09	0.16	0.15	0.27	0.07	0.13	0.12	0.13	0.10	0.21	0.13	0.12		0.30	0.30	0.34	0.26	0.33	0.34	0.32	0.33	0.36	0.35	0.28	0.33	0.38	0.28	0.33	0.39
18 Cascabel pd	0.02	0.07	0.09	0.07	0.08	0.05	0.03	0.16	0.08	0.05	0.07	0.13	0.12	0.12	0.14	0.15	0.11		0.22	0.23	0.20	0.23	0.24	0.20	0.26	0.20	0.24	0.17	0.23	0.27	0.25	0.23	0.25
19 Cascabel h	0.04	0.01	0.10	0.05	0.02	0.06	0.06	0.10	0.01	0.02	0.00	0.06	0.06	0.04	0.10	0.11	0.10	0.04		0.14	0.17	0.14	0.19	0.17	0.18	0.18	0.14	0.13	0.13	0.23	0.15	0.27	0.16
20 Cascabel p	0.07	0.07	0.12	0.10	0.06	0.10	0.09	0.14	0.06	0.06	0.06	0.13	0.09	0.13	0.16	0.10	0.13	0.05	-0.01		0.19	0.15	0.16	0.10	0.23	0.20	0.13	0.14	0.19	0.24	0.17	0.23	0.16
21 Guaranda h	0.04	0.02	0.13	0.09	0.02	0.07	0.06	0.14	0.02	0.04	0.03	0.09	0.06	0.09	0.09	0.07	0.04	0.03	-0.01	0.02		0.15	0.25	0.19	0.23	0.21	0.23	0.18	0.18	0.27	0.17	0.27	0.25
22 Guaranda p	0.06	0.04	0.11	0.06	0.02	0.10	0.09	0.15	0.04	0.05	0.04	0.11	0.10	0.10	0.10	0.08	0.11	0.06	-0.01	0.01	-0.01		0.20	0.14	0.19	0.19	0.17	0.17	0.20	0.28	0.17	0.28	0.18
23 L. Hermosa h	0.08	0.10	0.16	0.14	0.07	0.14	0.12	0.19	0.07	0.08	0.10	0.18	0.12	0.19	0.17	0.10	0.16	0.07	0.02	0.01	0.06	0.03		0.16	0.25	0.26	0.17	0.19	0.25	0.24	0.22	0.24	0.16
24 L. Hermosa p	0.05	0.06	0.12	0.09	0.04	0.09	0.07	0.17	0.05	0.05	0.07	0.13	0.10	0.14	0.15	0.07	0.11	0.03	0.00	-0.02	0.01	0.00	0.01		0.20	0.21	0.12	0.13	0.19	0.27	0.21	0.22	0.17
25 L. Hermosa pd	0.05	0.04	0.11	0.05	0.04	0.09	0.09	0.07	0.05	0.07	0.02	0.06	0.08	0.04	0.15	0.12	0.16	0.09	0.01	0.04	0.04	0.01	0.07	0.03		0.20	0.16	0.21	0.20	0.33	0.22	0.27	0.23
26 G. Paraguey h1	0.01	0.05	0.06	0.01	0.04	0.05	0.04	0.05	0.06	0.05	0.02	0.08	0.08	0.05	0.12	0.14	0.17	0.03	0.01	0.03	0.03	0.03	0.07	0.03	0.02		0.20	0.21	0.20	0.29	0.17	0.26	0.25
27 G. Paraguey h2	0.07	0.07	0.13	0.10	0.05	0.11	0.10	0.11	0.04	0.07	0.06	0.10	0.07	0.11	0.16	0.08	0.15	0.08	0.00	-0.01	0.04	0.01	0.01	-0.01	0.00	0.03		0.16	0.18	0.28	0.20	0.25	0.16
28 G. Paraguey p1	0.02	0.03	0.11	0.07	0.03	0.04	0.03	0.14	0.02	0.01	0.04	0.09	0.07	0.11	0.12	0.08	0.08	0.00	-0.02	-0.01	0.00	0.01	0.02	-0.02	0.04	0.04	0.01		0 19	0.23	0.18	0.24	0.19
29 G. Paraguey p2	0.08	0.05	0.14	0.10	0.06	0.08	0.08	0.11	0.05	0.06	0.03	0.08	0.07	0.04	0.14	0.15	0.12	0.07	-0.03	0.01	0.01	0.02	0.06	0.02	0.02	0.04	0.02	0.02		0.23	0.20	0.28	0.19
30 19 Abril pd	0.12	0.14	0.11	0.16	0.13	0.17	0.15	0.25	0.11	0.07	0.13	0.25	0.17	0.21	0.22	0.15	0.20	0.09	0.07	0.06	0.09	0.11	0.07	0.08	0.17	0.13	0.11	0.05	0.08		0.23	0.33	0.28
31 Parcelamiento p	0.04	0.03	0.06	0.03	0.03	0.08	0.08	0.11	0.02	0.02	0.01	0.08	0.07	0.06	0.10	0.12	0.10	0.04	-0.02	0.01	0.00	0.01	0.05	0.02	0.03	0.01	0.03	0.01	0.01	0.07		0.30	0.23
32 Rio Bravo II pd	0.06	0.10	0.17	0.14	0.10	0.13	0.11	0.16	0.12	0.10	0.10	0.14	0.11	0.18	0.20	0.17	0.16	0.06	0.07	0.06	0.09	0.09	0.05	0.04	0.09	0.07	0.07	0.05	0.10	0.13	0.10		0.25
33 Rio Bravo II p	0.06	0 07	0.15	0.09	0.05	0 09	0.08	0.17	0 06	0.05	0.06	0.15	0.14	0.12	0.16	0.11	0.17	0.06	-0.01	-0.01	0.04	0.01	-0.02	-0.02	0.03	0.04	-0.01	0.00	0 01	0.08	0.04	0.05	

p-palm, pd-peridomestic, h=house, 01-2001, 03=2003

Pairwise comparisons using Nei's genetic distance (D_S) also detected a close genetic relationship between adjacent populations, house and palm, peridomestic and palm populations in both Portuguesa and Barinas, also reflected in F_{ST} values;

- Los Rastrojos house and palm populations (D_S=0.02), (F_{ST}=0.04, p-value=0.006).
- Terronal h2 01 and palm 03 (D_S=0), (F_{ST}=0, p-value=0.424).
- Rio Bravo II populations (D_S=0.05), (F_{ST}=0.04, p-value=0.046).

Between house, palm and peridomestic populations at locality level;

- Guaranda house and palm (D_S=0), (F_{ST}=0, p-value=0.811).
- Cascabel house and palm (D_S=0), (F_{ST}=0, p-value=0.642), Cascabel pd with house and palm (D_S=0.04-0.05), (F_{ST}=0.04, p-value=0.080, 0.016).
- G. Paraguey house and palms (D_S=0.01-0.04), (F_{ST}=0.01-0.04, p-value=0.06-0.42).
- Laguna Hermosa house and palm (D_S=0.01), (F_{ST}=0, p-value=0.309), palm and peridomestic (D_S=0.03), (F_{ST}=0.03, p-value=0.019).

Close genetic relationships were also detected within ecotopes;

- Between domestic populations in San Bartolo (D_S=0), (F_{ST}=0, p-value=0.997).
- Between domestic populations in Terronal (D_S=0.02-0.03), (F_{ST}=0.03, 0.05, p-value=0.020, 0.025).
- Between silvatic ecotopes Palo Gacho palm and Terronal h2 p03 (D_S=0.02), (F_{ST}=0.03, p-value=0.029).
- Between G. Paraguey p1 and p2 (D_S=0.02), (F_{ST}=0.02, p-value=0.181).

However, adjacent Cojedes house and palm were distantly related (D_S=0.12), (F_{ST}=0.15 p-value<0.0001).

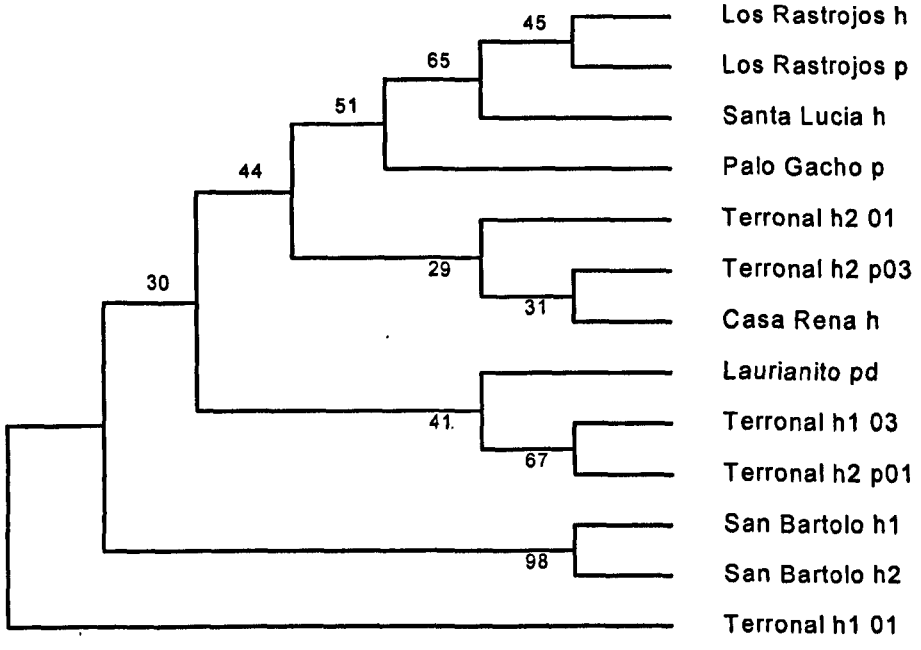

Figure 29. Majority rules consensus tree (D_S 500 bootstrap replicates) for localities in Portuguesa.

Within Portuguesa high bootstrap values were detected between San Bartolo h1 and h2 (see Figure 29 above). Weak clustering was detected between Terronal h2 p03 and Casa Rena h (D_S=0.01, 31%), together with Terronal h2 01 (29%, D_S=0 to Casa Rena, D_S=0 to h2 p03). These groups also clustered in the F_{ST} tree (see Figure 25 on page 192), as did Los Rastrojos house and palm. Santa Lucia, although distantly related, clustered with Los Rastrojos in 65% of trees. These populations were different from the majority of other populations within Portuguesa, also detected with F_{ST}. Terronal h2 p01 and Terronal h1 01, although more distantly related, cluster. Laurianito was also associated. All other clusters within Portuguesa were weak.

Within Barinas State bootstrapping values were low with only two groups associated in 50% of trees, Parcelamiento and 19 Abril (DS=0.07) and Laguna Hermosa h and Rio

Bravo II palm populations (D_S=0). Guaranda house and palm clustered in 32% of trees, as did G. Paraguey p2 and Cascabel h (37%).

In the analysis of all 33 populations bootstraps were very low and therefore not employed (see Figure 30 on page 208). As bootstrapping was not reliable it is difficult to assess the strength of branching patterns detected. Only Santa Bartolo h1 and h2 (92%), Terronal h1 03 and h2 01 (59%) and Rio Bravo p II and Laguna Hermosa h (58%) gave some high clustering values. Some grouping by State of origin is evident for populations from Portuguesa (Santa Lucia to Terronal palm 03) and Barinas (Parcelamiento to Cascabel house).

Small genetic distances were detected within and between ecotopes as seen from the grouping of Casa Rena h, Laurianito pd, Terronal h2 01, and Terronal h2 p03 (D_S=0 to 0.03). Small genetic distances were also detected between Cascabel h and G. Paraguey palm (D_S=0), Cascabel p and Laguna Hermosa palm (D_S=0), Laguna Hermosa h and Rio Bravo II palm (D_S=0). The clustering of San Bartolo, Los Rastrojos and Santa Lucia with Palo Gacho, Terronal h2 p01 and house 01 03 in the genetic distance tree was also reflected in the Portuguesa bootstrapped tree (see Figure 29 on page 206). Distant relationships between populations detected by F_{ST} are also seen from genetic distances calculated for San Bartolo, Lara, Cojedes, Trujillo, 19 Abril, Rio Bravo II pd, as noted by the presence of long branches (see Figure 30 on page 208).

All genetic distances were correlated with geographic distances (km) and were found to be weakly correlated but significant; D_{MU} (R^2=0.08 p-value=0.0004), D_S (R^2=0.2 p-value=0.0001), D_{PS} (R^2=0.3 p-value=0.0001) and D_{SW} (R^2=0.2 p-value=0.01). R^2 values range from 0 to 1. Its value represents the fraction of shared variance in the two compared variables i.e. R^2=0.08, therefore 8% of the variance in X can be explained by variation in Y at nominal significance level $p<0.05$.

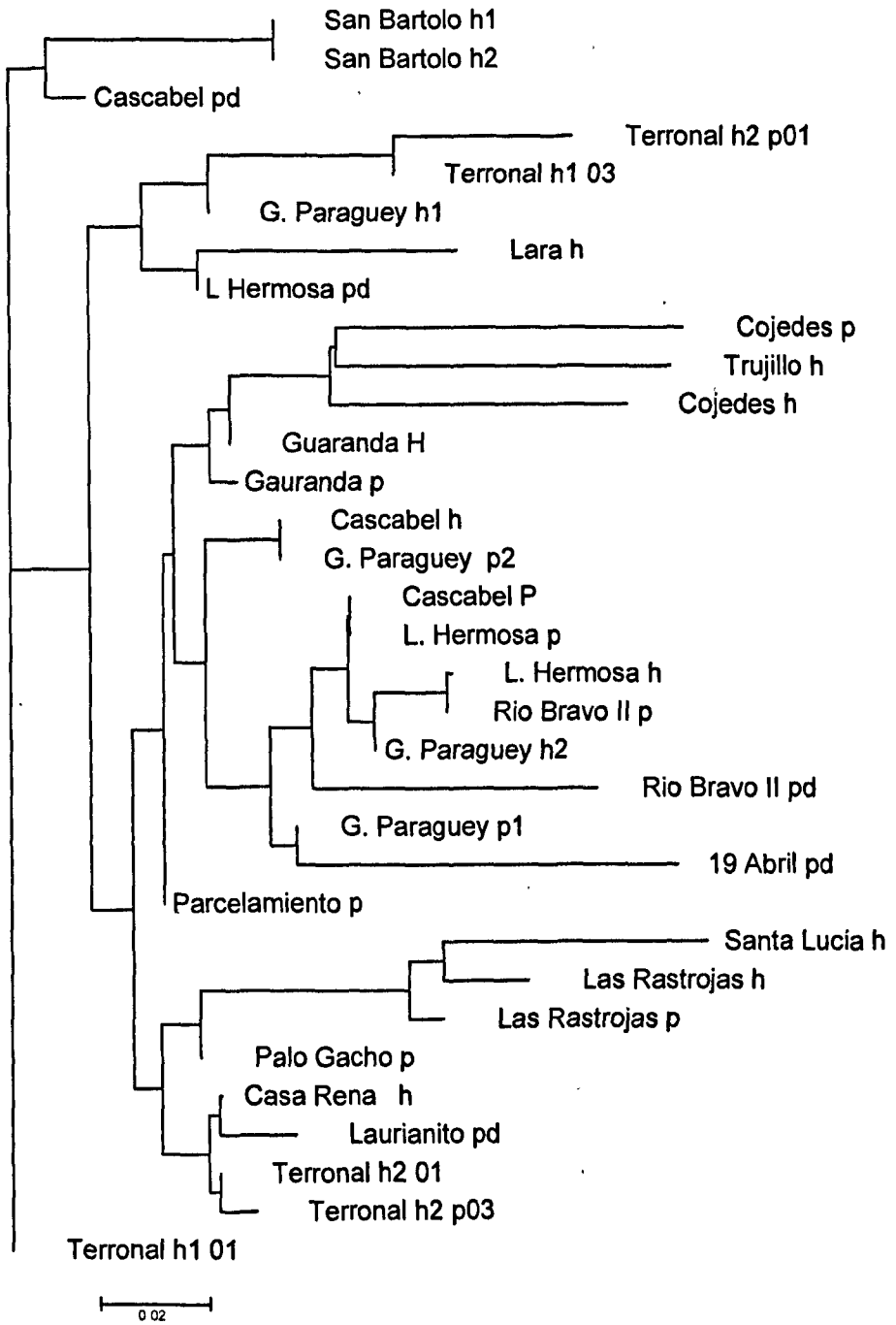

Figure 30. Neighbour Joining tree of DS genetic distance (Nei 1972) from Microsat. p=palm, h=house, pd=peridomestic 01=2001, 03=2003. Bootstrap values not used as too low.

Table 40. F_{ST} values for pairwise comparisons of 28 populations analysed by both microsatellite (below diagonal) and *cytb* direct sequencing (above).

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 Terronal h1 01		0.00	0.27	0.00	0.31	**0.57**	**0.51**	**0.58**	0.25	0.00	**0.51**	**0.53**	0.58	**0.48**	**0.63**	**0.71**	0.30	0.34	0.31	**0.46**	0.46	0.44	0.30	0.32	**0.69**	0.34	0.35	0.33
2 Terronal h2 01	0.03		0.31	0.00	0.35	**0.63**	0.57	**0.60**	0.23	0.01	**0.56**	**0.59**	0.64	**0.54**	**0.69**	**0.74**	0.33	0.37	0.33	0.52	0.51	0.49	0.33	0.35	**0.72**	0.38	0.39	0.36
3 Terronal h2 p01	**0.15**	**0.19**		0.35	0.00	0.13	0.08	**0.63**	**0.70**	0.02	0.08	0.10	0.14	0.03	0.18	**0.77**	0.15	0.02	0.22	0.03	0.05	0.04	0.04	0.02	**0.76**	0.08	0.00	0.10
4 Terronal h1 03	0.05	0.07	0.05		0.39	**0.71**	0.64	**0.62**	0.22	0.02	**0.61**	0.67	**0.72**	**0.60**	**0.77**	**0.78**	0.34	0.40	0.34	0.58	0.56	0.54	0.35	0.37	**0.77**	0.40	0.42	0.38
5 Terronal h2 p03	0.06	0.00	**0.18**	0.08		0.11	0.07	**0.64**	**0.71**	0.05	0.07	0.08	0.11	0.02	0.14	**0.76**	0.18	0.03	0.25	0.02	0.04	0.03	0.05	0.04	**0.76**	0.09	0.00	0.12
6 San Bartolo h1	0.07	**0.10**	**0.27**	**0.10**	**0.13**		0.00	**0.84**	**1.00**	0.48	0.02	0.00	0.00	0.01	0.00	**0.94**	0.36	0.16	0.37	0.00	0.02	**0.04**	0.19	0.21	**1.00**	0.30	0.22	0.39
7 San Bartolo h2	0.06	0.11	**0.23**	0.09	**0.12**	0.00		0.80	**1.00**	0.36	0.00	0.00	0.00	0.00	0.00	**0.93**	0.26	0.07	0.30	0.00	0.00	0.00	0.11	**0.11**	**1.00**	**0.19**	0.10	0.26
8 Santa Lucia h	**0.14**	**0.18**	**0.30**	**0.22**	**0.21**	**0.22**	**0.20**		**0.84**	0.57	**0.75**	**0.81**	**0.84**	**0.76**	**0.87**	**0.83**	0.52	**0.60**	**0.49**	0.76	**0.73**	0.72	**0.54**	0.58	**0.84**	0.60	0.64	**0.58**
9 Casa Rena h	0.08	0.00	**0.27**	**0.16**	0.01	**0.14**	**0.15**	**0.25**		0.57	**0.88**	**1.00**	**1.00**	**0.88**	**1.00**	**0.94**	**0.69**	**0.79**	**0.62**	1.00	**0.90**	**0.90**	**0.72**	**0.78**	**1.00**	**0.80**	0.87	**0.80**
10 Palo Gacho p	0.05	0.00	0.15	0.04	0.01	0.10	0.09	**0.17**	0.02		0.33	**0.41**	0.49	0.28	0.57	**0.77**	0.11	0.10	0.15	0.27	0.26	0.24	0.07	0.07	**0.76**	0.11	0.09	0.11
11 Los Rastrojos h	**0.10**	0.06	**0.31**	**0.19**	**0.12**	**0.17**	**0.19**	**0.12**	**0.11**	0.09		0.00	0.02	0.00	0.05	**0.86**	0.20	0.00	0.26	0.00	0.00	0.00	0.06	0.07	**0.88**	0.15	0.00	0.12
12 Los Rastrojos p	0.08	0.03	**0.25**	**0.15**	0.06	**0.14**	0.13	**0.13**	0.04	0.06	0.05		0.00	0.00	0.00	**0.94**	0.30	0.11	0.33	0.00	0.00	0.00	0.14	0.15	**1.00**	0.23	**0.15**	0.31
13 Lara h	**0.15**	**0.13**	0.22	0.09	**0.17**	**0.12**	0.13	**0.19**	**0.21**	0.06	**0.15**	**0.18**		0.02	0.00	**0.94**	0.38	0.17	0.38	0.00	**0.03**	**0.04**	0.21	**0.22**	**1.00**	0.32	0.24	0.40
14 Cojedes p	**0.15**	**0.09**	**0.26**	**0.14**	**0.10**	**0.18**	**0.20**	**0.29**	**0.11**	**0.12**	**0.17**	**0.09**	**0.21**		0.04	**0.87**	0.28	0.06	0.33	0.00	0.00	0.00	0.11	0.09	**0.89**	0.16	0.03	0.23
15 Cojedes h	**0.18**	**0.13**	**0.29**	**0.23**	**0.10**	**0.21**	**0.18**	**0.34**	**0.09**	**0.15**	**0.26**	**0.11**	**0.31**	**0.14**		**0.95**	**0.45**	0.24	0.45	0.00	0.07	0.09	**0.28**	0.30	**1.00**	0.40	0.33	0.49
16 Trujillo h	**0.25**	**0.14**	**0.41**	**0.32**	**0.14**	**0.26**	**0.28**	**0.42**	**0.14**	**0.21**	**0.21**	**0.15**	**0.33**	**0.18**	**0.19**		**0.72**	**0.76**	**0.63**	**0.92**	**0.87**	**0.86**	**0.71**	**0.75**	**0.94**	**0.80**	**0.84**	**0.80**
17 Cascabel pd	0.05	0.08	0.16	0.06	0.09	0.08	0.03	**0.25**	0.14	0.08	**0.18**	0.11	**0.17**	**0.13**	**0.18**	**0.20**		0.00	0.00	0.18	0.19	0.17	0.00	0.03	**0.69**	0.07	0.00	0.00
18 Cascabel h	0.04	0.00	0.15	0.04	0.03	0.07	0.05	**0.14**	0.04	0.00	0.06	0.02	0.05	0.09	**0.13**	**0.18**	0.00		0.04	0.00	0.00	0.00	0.00	0.00	**0.79**	0.00	0.00	0.00
19 Cascabel p	**0.11**	**0.08**	**0.22**	**0.11**	0.08	**0.12**	0.08	**0.20**	**0.09**	0.05	**0.17**	0.06	**0.17**	**0.14**	**0.13**	**0.19**	0.00	0.01		0.24	0.25	0.22	0.02	0.08	**0.62**	0.13	0.00	0.00
20 Guaranda h	0.09	0.00	0.24	0.08	0.01	0.12	0.12	0.27	0.04	0.00	0.09	0.05	0.13	0.05	0.13	0.09	0.18	0.00	0.03		0.00	0.00	0.03	0.02	**1.00**	0.10	0.00	0.16
21 Guaranda p	0.09	0.05	0.14	0.04	0.05	**0.11**	0.08	**0.15**	0.08	0.00	**0.12**	0.05	0.08	**0.10**	**0.13**	**0.21**	0.19	0.00	0.00	0.00		0.00	0.04	0.02	**0.90**	0.09	0.00	0.14
22 L. Hermosa h	0.08	0.05	**0.18**	0.09	0.05	**0.13**	0.09	**0.22**	0.06	0.02	**0.16**	0.06	**0.17**	**0.11**	**0.11**	**0.20**	0.17	0.00	0.01	0.01	0.00		0.00	0.00	**0.90**	0.08	0.00	0.12
23 L. Hermosa p	**0.15**	0.12	**0.26**	0.15	**0.10**	**0.15**	0.08	**0.30**	0.12	0.09	**0.24**	0.12	**0.24**	**0.17**	0.09	**0.20**	0.00	0.05	0.00	0.04	0.03	0.02		0.00	**0.72**	0.00	0.00	0.00
24 L. Hermosa pd	0.08	0.05	0.20	0.07	0.06	0.12	0.09	**0.14**	0.09	0.00	0.11	0.07	0.07	**0.15**	**0.14**	**0.28**	0.03	0.00	0.06	0.06	0.01	0.04	0.10		**0.78**	0.00	0.00	0.00
25 19 Abril pd	**0.15**	**0.15**	**0.19**	0.16	**0.15**	**0.19**	0.13	**0.32**	**0.17**	0.13	**0.29**	**0.14**	**0.25**	**0.21**	**0.18**	**0.28**	0.69	0.08	0.09	0.15	0.10	0.04	0.10	0.17		**0.72**	**0.87**	**0.80**
26 Parcelamiento p	0.15	0.08	0.18	0.08	0.09	**0.16**	0.13	**0.24**	0.12	0.04	**0.17**	0.07	0.13	**0.12**	**0.18**	**0.20**	0.07	0.00	0.00	0.02	0.00	0.03	0.05	0.07	0.10		0.00	0.04
27 Rio Bravo II pd	0.07	0.07	0.20	0.06	0.04	0.11	0.06	0.23	0.12	0.01	**0.18**	0.11	0.13	**0.14**	0.14	**0.25**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.03		0.00
28 Rio bravo II p	0.04	0.06	0.20	0.05	0.07	0.08	0.05	**0.23**	0.11	0.04	**0.18**	0.09	**0.15**	0.11	**0.16**	**0.27**	0.00	0.00	0.01	0.04	0 02	0.00	0.02	0.04	0 08	0.06	0.00	

Values in bold remain significant following Bonferroni correction, k=378, p1=0.05/378 p≤0.0001. h=house, p=palm, pd=peridomestic. 01=2001, 03=2003.

7.1.6.6 Microsatellite and cytochrome b analysis.

A total of 369 specimens from 28 populations were characterised by both *cytb* direct sequencing and microsatellites (9 loci; set 1) (see Table 8 on page 125). Levels of population heterogeneity detected by both markers was analysed by comparing F_{ST} values between populations characterised by both methods (28 groups excluding G. Paraguey domestic specimens due to low numbers) (see Table 40 on page 209). As seen from Table 40 microsatellite data detected more heterogeneity than mitochondrial analysis.

7.1.6.6.1 Adjacent populations

In the comparison of adjacent populations similar results were obtained for both methods. Some population heterogeneity detected by sequence data is related to the presence of the introgressed haplotype 3 (Amazonian *R. robustus*). If this haplotype is excluded or considered as haplotype 1 (the most common *R. prolixus* haplotype occurring in the same ecotope) populations are more homogeneous.

- In Rio Bravo II between palm and peridomestic populations (F_{STseq}=0, $F_{STmicro}$=0).
- In Los Rastrojos, adjacent palm and domestic populations were homogeneous (F_{STseq}=0 $F_{STmicro}$=0.05).
- In the locality Terronal population homogeneity was detected between adjacent Terronal h2 01 and palm 03 by microsatellite analysis ($F_{STmicro}$=0). Higher but non-significant heterogeneity was detected by sequence analysis (F_{STseq}=0.35), however when introgressed haplotype 3 is taken into account (F_{STseq}=0).

7.1.6.6.2 At locality level

- In San Bartolo population homogeneity was evident between house 1 or 2 by both markers (F_{STseq}=0, $F_{STmicro}$=0).

- In Guaranda house and palm populations were also homogeneous (F_{STseq}=0, $F_{STmicro}$=0).

- In Laguna Hermosa domestic, peridomestic and silvatic populations were homogeneous. $F_{STmicro}$ values were higher between house and peridomestic populations but were not significant ($F_{STmicro}$=0.02-0.1, F_{STseq}=0).

- In the locality Cascabel population comparisons (domestic, silvatic and peridomestic) were non-significant by both markers but higher values of F_{STseq} were detected between house and palm populations ($F_{STmicro}$=0.01, F_{STseq}=0.04).

- Homogeneity was also detected between domestic populations in Terronal, house 1 from both sampling years (01, 03) and house 1 01 and house 2 01. $F_{STmicro}$ values were higher but not significant ($F_{STmicro}$=0.03-0.05, F_{STseq}=0).

- Higher but non-significant levels of heterogeneity was also detected between Terronal house 1 01 and the palm population from the same year (palm 01) ($F_{STmicro}$=0.05, F_{STseq}=0).

Both markers clearly detect a lack of population heterogeneity between silvatic, domestic and peridomestic populations, which indicates that these populations are not isolated.

Nevertheless, both markers also detected contrasting patterns of population heterogeneity.

- In Cojedes heterogeneity detected between adjacent domestic and silvatic populations was high and significant by microsatellite analysis but low by sequence analysis ($F_{STmicro}$=0.14, F_{STseq}=0.04). When the introgressed haplotype is considered F_{STseq}=0.

- Within the locality Terronal significant heterogeneity was detected by microsatellite analysis in three population comparisons, all in relation to house 2 palm 01, while all comparisons were non significant by cytb analysis.

7.1.6.6.3 All populations

For heterogeneity detected across all populations see Table 40 on page 209, Figure 31 on page 214 and Figure 32 on page 215.

- Within Barinas State population heterogeneity was low for both markers across populations.

- Detected heterogeneity was related to the population 19 Abril pd. This population was distinct from all populations by sequence data (F_{STseq}=0.68-1.0) with the exception of Parcelamiento palm, however while $F_{STmicro}$ indices were high all pairwise comparisons were non-significant within Barinas after Bonferroni correction. 19 Abril pd clusters separately on both trees.

- Greater population heterogeneity was detected within Portuguesa by microsatellite analysis. Santa Lucia was significantly different from the majority of populations in the study by both markers, and was distinct in both trees. This population clusters with Los Rastrojos house in the microsatellite tree, which was also distinct by microsatellite data but not distinct by sequence data.

- Within Portuguesa Terronal house 2 palm 01, Los Rastrojos palm, San Bartolo populations were also distinct by microsatellite analysis.

- The domestic population from Trujillo was also distinct from the majority of populations by both markers (F_{STseq}=0.71-0.94, $F_{STmicro}$=0.14-42). This population also clusters separately on both trees.

- Populations from the Lara and Cojedes States were also distinct by microsatellite analysis.

- Across all populations significant differences between populations from different States was detected by microsatellite analysis and some clustering by State is visible from microsatellite data (see Figure 31 on page 214), some clustering by sequence data for Barinas localities is also detected (Cascabel to Rio Bravo II).

Population heterogeneity detected by microsatellite loci was generally higher over all populations, however a similar picture by both markers was detected at fine population levels.

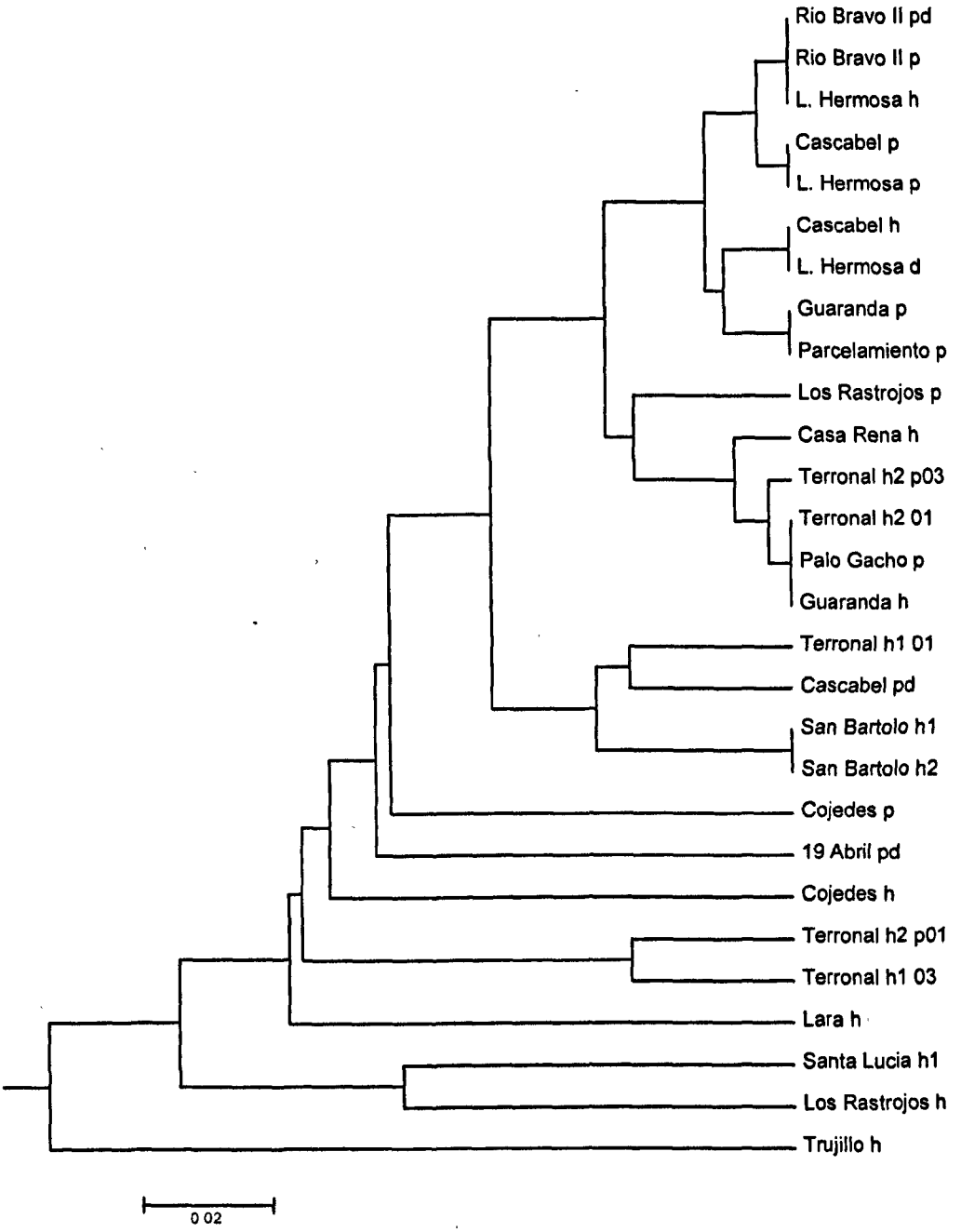

Figure 31. An UPGMA tree for pairwise F_{ST} values (microsatellite data) between all localities characterised by both microsatellite and *cytb*.
h=house, p=palm, pd=peridomestic. 01=2001, 03=2003. L. Hermosa= Laguna Hermosa.

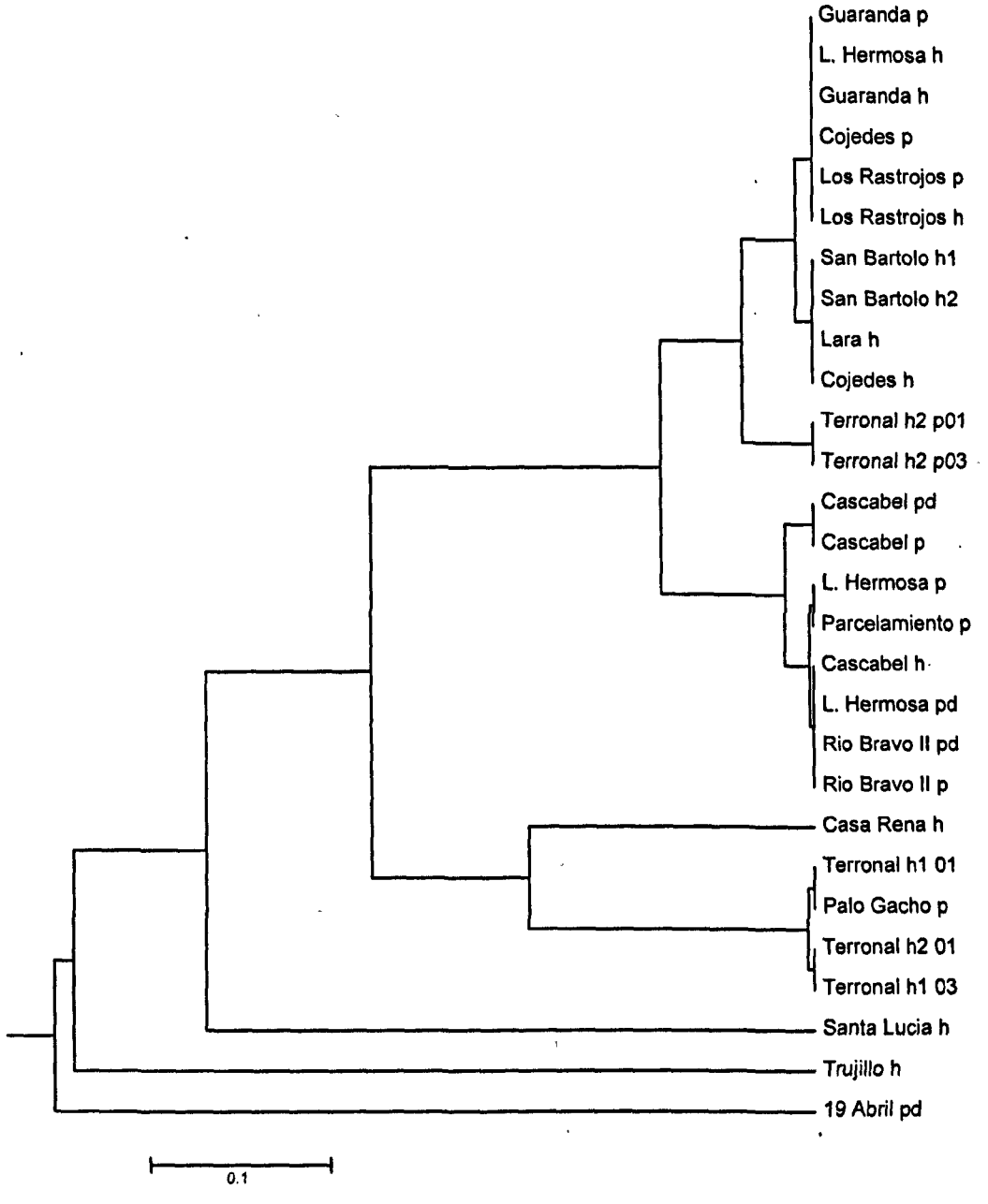

Figure 32. An UPGMA tree for pairwise F_{ST} values (*cytb* data) between all localities characterised by both microsatellite and *cytb*.
h=house, p=palm, pd=peridomestic. 01=2001, 03=2003

7.1.6.7 Assignment tests

Individual assignment test was carried out using Geneclass2 (Piry *et al.*, 2004) applying 1) Bayesian (Rannala & Mountain 1997) and 2) frequency based methods (Paetkau *et al.*, 1995) to assign individuals to their most likely population (see section 5.4.11.6 on page 124). Population groups were defined *a priori* with 555 individuals at 9 loci divided by (1) site of collection; 33 groups, (2) locality level; 17 groups (3) State level; 5 groups (4) State by ecotope; 10 groups (5) ecotope; 3 groups.

7.1.6.7.1 Population level

Results for assignment tests at population level were poor with few individuals reassigned correctly (see Table 41 on page 217). This is not surprising given the low levels of heterogeneity detected among populations (F_{ST}). The quality index, based on the mean reassignment scores for individuals placed in the correct population, was low for both assignment methods used (27.0-28.3-%). The numbers of individuals correctly assigned was less than 35% (178, 186 specimens).

Terronal house 2 specimens were more frequently assigned to Terronal palm and Casa Rena domestic populations than correctly assigned, this pattern was also detected in other populations. San Bartolo h1 and h2 specimens were equally as likely to be from either population. In three Barinas populations no correct assignments occurred (Guaranda house, Laguna Hermosa palm, Cascabel house).

Highest numbers of correctly assigned individuals occurred in populations Terronal h2 palm 01 (69%), Santa Lucia (85%), Los Rastrojos house (63%), Cojedes house and palm (71%), Rio Bravo pd (65%), Loma de Amarillo (Trujillo) (85%) and Apto. pd (62%).

Table 41. The assignment of specimens using Bayesian analysis among the 33 populations sampled in this study (Rannala & Mountain 1997).

Population	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1 Terronal h1 01	25	**5**	2	4	-	2	1	-	-	1	-	1	-	-	1	-	-	1	3	-	-	-	-	-	-	1	1	-	-	-	-	-	1	1
2 Terronal h2 01	18	2	**2**	-	1	3	1	-	1	3	1	2	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-
3 Terronal h2 p01	26	4	-	**18**	-	1	-	-	-	-	1	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4 Terronal h1 03	10	-	1	2	**3**	1	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
5 Terronal h2 p03	39	2	5	2	1	7	-	-	2	1	3	8	-	-	-	1	3	2	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-
6 San Bartolo h1	14	2	-	-	-	-	**2**	8	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
7 San Bartolo h2	14	2	-	-	-	-	7	2	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
8 Santa Lucia h1	13	-	-	-	-	-	-	-	**11**	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9 Casa Rena h1 1	11	1	-	1	-	2	-	-	-	**3**	1	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
10 Laurianito pd	21	1	4	3	1	1	1	1	-	4	1	2	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-
11 Palo Gacho p	15	1	-	2	-	4	-	-	1	2	-	1	1	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
12 Los Rastrojos h	24	2	-	-	-	-	-	-	1	-	-	1	**15**	1	2	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-
13 Los Rastrojos p	12	-	-	-	-	-	-	-	1	-	-	-	2	**4**	-	1	2	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
14 Lara h	17	-	-	-	-	-	2	-	3	-	-	1	-	-	**8**	-	-	-	-	-	-	-	-	-	-	1	-	-	-	2	-	-	-	-
15 Cojedes p	24	-	-	-	-	-	-	-	1	-	-	-	-	1	-	**17**	**4**	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16 Cojedes h	24	1	-	-	-	-	-	-	-	3	-	-	-	-	-	2	**17**	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
17 Trujillo h	26	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	**22**	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-
18 Cascabel pd	11	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**6**	-	-	1	-	-	-	-	-	-	1	-	-	-	1	-
19 Cascabel h	10	1	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	**0**	-	-	1	2	-	1	-	1	-	-	1	1	-	-
20 Cascabel p	24	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	1	-	-	3	**3**	-	2	1	4	-	2	1	1	1	2	1	-	-
21 Guaranda h	11	-	-	-	-	-	-	1	-	1	-	-	-	-	1	1	-	-	-	-	-	1	2		-	1	-	-	-	1	1	-	1	-
22 Guaranda p	20	-	1	1	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	2	1	2	2	1	-	-	-	3	3	1	1	-	-	-
23 L. Hermosa h	16	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-		-	7	2	1	1	-	1	-	1	-	-	1
24 L. Hermosa p	17	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	2	3	1	-	-	**0**	2	-	4	-	-	-	1	2	-
25 L. Hermosa pd	13	-	-	-	-	2	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-		-	-	1	**3**	1	3	-	1	-	-	-	-
26 G. Paraguey h1	11	-	-	2	-	-	-	1	1	-	-	-	1	1	-	-	-	-	-	-	1		-	-	-	-	**0**	1	1	-	-	2	-	-
27 G. Paraguey h2	12	-	-	-	-	-	-	-	1	1	-	-	-	-	1	-	1	-	-	-	-		1	-	2	-	-	1	1	-	1	-	-	2
28 G. Paraguey p1	12	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	2	2	-		2	-	-	1	-	-	1	1	1	-	-	-
29 G. Paraguey p2	11	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	1	1	1		-	-	-	-	-	1	1	1	1	-	1	1
30 19 Abril pd	13	-	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-		-	2	-	-	-	-	-	-	**8**	-	-	-
31 Parcelamiento p	13	-	-	-	-	-	-	-	-	-	1	1	-	1	-	-	-	-	1	-	-	1	-	-	-	2	2	-	-	-	1	**3**	-	-
32 Rio Bravo II pd	17	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	**11**	-
33 Rio Bravo II p	10	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-		-	-	2	-	2	1	-	1	1	-	-	1

N=number of specimens\individuals per population. h=house, p=palm, pd=peridomestic. Values in **bold** = the number of individuals assign correctly to their population.

7.1.6.7.2 State level

The quality index was higher for specimens analysed by State of collection (63%, 70.3%). Higher correct assignment rates were also detected with 70% specimens reassigned correctly (387, 426). Broadly similar results were obtained for both assignment methods with Rannala & Mountain (1997) performing better for the correct assignment of Portuguesa and Barinas specimens. The assignment scores for correctly assigned individuals were higher and greater differences were detected between the log likelihoods used to allocate individuals to groups (see Table 43 below). Reassignment was highest for the States Lara, Trujillo and Cojedes, with Trujillo the most distinct (see Table 42 below). Barinas specimens were most frequently misassigned to Portuguesa State.

Table 42. Assignment of individuals at State level (Rannala & Mountain 1997)

State	N	Portuguesa	Lara	Cojedes	Trujillo	Barinas
Portuguesa	243	**186** (77)	19	15	5	18
Lara	17	1	**16** (94)	-	-	-
Cojedes	48	1	1	**42** (88)	1	3
Trujillo	26	1	2	1	**22** (85)	-
Barinas	221	36	12	9	4	**160** (72)

N= number of specimens\individuals. Values in bold = number of individuals correctly assigned, in parenthesis % correctly assigned.

Table 43. Mean assignment scores and Log likelihood scores of individuals at State level (Rannala & Mountain 1997)

State	Mean assignment Score (%)*	Mean Log (L)1	Mean Log (L)2	Mean Log (L)3	Mean Log (L)4	Mean Log (L)5
1Portuguesa	78	**-4.8**	-7.8	-7.3	-8.2	-5.9
2 Lara	84	-5.2	**-3.7**	-8.7	-8.8	-5.6
3Cojedes	90	-7.9	-11.9	**-5.1**	-9.9	-7.5
4 Trujillo	95	-5.4	-7.9	-5.6	**-2.8**	-5.3
5 Barinas	89	-9.0	-12.0	-10.4	-11.1	**-6.8**

* the average of all individual assignment scores for those specimens assigned to correct State. ^ the average of all Log (L) scores derived from likelihood values of each individual from each State placed into each State group.

7.1.6.7.3 State divided by ecotope

When specimens were divided by ecotope within their State of collection, assignment accuracy decreased further (quality indexes of 36-40%) and the numbers of individuals correctly assigned dropped to 230 and 253 (41-46%). Broadly similar results were obtained for both assignment methods with Rannala & Mountain (1997) performing better for the correct assignment of Portuguesa house/palm and Barinas house/palm specimens. As seen from F_{ST} comparisons, assignment of individuals was greatest among ecotopes within States. In Barinas house population specimens were more frequently assigned to the palm and peridomestic groups than correctly assigned. In Portuguesa domestic specimens were most frequently assigned to peridomestic populations, with palm specimens most frequently assigned house populations. Peridomestic sites were fewer in number and were more frequently assigned to the palm group. States of Trujillo Lara and Cojedes were less heterogeneous than the other States with higher rates of correct assignment (79-91%), again few specimens were assigned to Trujillo (see Table 44 below, Table 45 on page 220).

Table 44. Assignment of individuals at State ecotope level (Rannala & Mountain 1997).

State ecotope	N	1	2	3	4	5	6	7	8	9	10
1 Barinas h	60	**11** (18)	14	12	3	4	5	6	4	-	1
2 Barinas p	107	27	**36** (34)	20	3	1	3	5	5	6	1
3 Barinas pd	54	8	7	**21** (39)	2	-	2	5	5	3	1
4 Cojedes h	23	1	-	-	**19** (83)	2	-	-	-	-	1
5 Cojedes p	24	1	-	-	4	**18** (76)	1	-	-	-	-
6 Lara h	17	-	-	-	-	-	**13** (76)	4	-	-	-
7 Portuguesa h	131	5	-	6	3	2	8	**64** (49)	17	23	3
8 Portuguesa p	91	-	1	7	6	2	6	17	**41** (45)	9	2
9 Portuguesa pd	21	-	-	3	1	-	-	3	6	**8** (38)	-
10 Trujillo h	26	-	-	-	1	-	1	1	-	1	**22** (85)

N= number of specimens\individuals. Values in bold = number of individuals correctly assigned, in parenthesis % correctly assigned. p=palm, h=house, pd=peridomestic.

Table 45. Mean assignment score and Log likelihood scores of individuals at State ecotope level (Rannala & Mountain 1997)

State by ecotope	M assign. (%)*	^MLog (L)1	Mlog (L)2	MLog (L)3	MLog (L)4	MLog (L)5	Mlog (L)6	Mlog (L)7	Mlog (L)8	Mlog (L)9	MLog (L)10
1 Barinas h	58.6	-6.3	-6.7	-7.5	-10.9	-10.1	-12.3	-9.7	-8.9	-9.4	-11.1
2 Barinas p	62.9	-7.7	-7.1	-8.3	-11.9	-10.6	-12.3	-10.4	-9.8	-9.4	-11.7
3 Barinas pd	77.2	-7.3	-7.5	-5.7	-11.3	-10.8	-11.8	-10.6	-8.7	-8.4	-10.9
4 Cojedes h	91.0	-5.8	-6.2	-6.5	-3.7	-5.8	-12.3	-6.9	-5.9	-7.0	-7.7
5 Cojedes p	91.0	-9.1	-8.9	-10.3	-8.0	-5.2	-11.5	-11.0	-9.3	-10.9	-12.4
6 Lara h	79.1	-5.8	-5.7	-5.8	-11.3	-8.7	-3.7	-6.5	-5.4	-6.0	-9.2
7 Portuguesa h	55.4	-5.8	-5.8	-5.9	-8.1	-6.5	-6.8	-4.4	-5.6	-5.9	-7.7
8 Portuguesa p	61.7	-6.7	-6.2	-6.1	-9.2	-8.4	-8.8	-5.6	-4.5	-5.2	-8.7
9 Portuguesa pd	63.2	-7.3	-6.4	-6.9	-7.5	-7.7	-8.5	-5.7	-6.0	-4.9	-8.9
10 Trujillo h	90.2	-5.2	-5.4	-5.2	-6.3	-5.9	-7.9	-5.7	-5.2	-5.8	-2.8

* Massign.=mean assignment score - the average of all individual assignment scores for those specimens assigned to correct State ecotope. ^ the average of all Log (L) scores derived from likelihood values of each individual from each State ecotope placed into each State ecotope group. p=palm, h=house, pd=peridomestic

7.1.6.7.4 Ecotope

The quality indexes for both methods were low (1; 44.2%, 2; 46.4%). Correct assignment rates were also low with only half of specimens reassigned correctly by both methods (1; 278, 2; 294). Broadly similar results were obtained for both methods, with Rannala & Mountain (1997) performing better for the correct assignment of domestic and silvatic specimens. However, even when individuals were correctly assigned, the assignment scores were often low and minimal differences were detected between the log likelihoods values used for assignment of individuals to groups (based on minimum negative Log (L) value) (see Table 47 on page 221). Numbers of correctly assigned individuals was highest for domestic specimens and lowest for silvatic specimens (see Table 46 on page 221). Domestic specimens were more frequently reassigned to the silvatic group, while silvatic specimens were most frequently assigned to peridomestic. Peridomestic specimens were most frequently assigned to domestic.

Table 46. Assignment of individuals at ecotope level (Rannala & Mountain 1997)

Ecotope	N	Domestic	Silvatic	Peridomestic
Domestic house	257	**163** (63)	52	42
Silvatic palm	223	59	**94** (42)	70
Peridomestic	75	21	17	**37** (49)

N= number of specimens\individuals. Values in bold = number of individuals correctly assigned, in parenthesis % correctly assigned.

Table 47. Mean assignment scores and log likelihood scores Log (L) of individuals at ecotope level (Rannala & Mountain 1997)

Ecotope	Mean assignment Score (%)*	Mean Log (L) 1^	Mean Log (L) 2	Mean Log (L) 3
1 Domestic	60.0	**-4.7**	**-5.2**	**-5.4**
2 Silvatic	71.1	**-7.8**	**-7.0**	**-8.5**
3 Peridomestic	75.7	**-7.2**	**-7.1**	**-5.8**

*=the average of all individual assignment scores for those specimens assigned to correct ecotope. ^ the average of all Log (L) scores derived from likelihood values of each individual from each ecotope placed into each ecotope group.

7.2 Discussion

To investigate the relationship between silvatic and domestic populations of *R. prolixus* in Venezuela a panel of microsatellite markers was developed. These genetic markers, as discussed (see section 1.5.2.3.3 on page 62), have attributes suitable for population genetics and have also proven highly polymorphic in species where low levels of isoenzyme polymorphism have been detected (Hughes & Queller 1993, Estoup *et al.*, 1998), as noted for Triatominae (Abad-Franch & Monteiro 2005) including *R. prolixus* (Harry *et al.*, 1992, 1993b). This approach was undertaken because knowledge of population heterogeneity may allow for the development of more effective strategies for the control of Chagas disease.

The magnetic bead and biotinylated probe enrichment technique proved successful with 93 positive clones identified (1 false positive), 84 of which were shown to contain microsatellite repeats (15% of total clones screened). Harry *et al.*, (1998) using a partial genomic library for *R. pallescens* found 2.3% of clones to contain repeat motifs, while Garcia *et al.*, (2004) found 210 positive clones (1.31% of clones screened).

From the total of 52 primer pairs initially designed a working panel of 11 fluorescent loci was developed, all were considered unlinked and neutral. Polymorphism varied from 3 alleles at List14-042 to 12 alleles at List14-010. Heterozygosity was moderate: 0.3 to 0.6 The analysis of 36 field caught specimens of the related species *R. pallescens* detected allele ranges of 2-20 per locus from a panel of 10 microsatellite loci (Harry *et al.*, 1998), so allele numbers in the present study were more conservative.

Null alleles were observed with a total of 56 specimens consistently failing to amplify at 1 or 2 loci. Nulls can be a common feature in microsatellite amplification in insects including vector species such as sandflies (Maingon *et al.*, 2003), tsetse flies (Krafsur & Endsley 2002) mosquitoes (Donnelly *et al.*, 1999, Rongnoparut *et al.*, 1996, Lehmann *et al.*, 1996b, Kamau *et al.*, 1999), although they were not reported in studies of other Triatominae (Harry *et al.*, 1998, Garcia *et al.*, 2004, Anderson *et al.*, 2002), however

this may have been a factor of the small numbers of samples amplified. Null alleles can result in departures from Hardy Weinberg equilibrium (HWE) due to heterozygote deficiency, but departures may also occur naturally in populations for example due to inbreeding or the Wahlund effect (see Glossary page 310). Significant departures from HWE were detected at 6 loci in 17 populations after Bonferroni correction. Departures generally consisted of heterozygote deficiency particularly at locus List14-017, which may be due to hidden nulls. Similar values of F_{ST} were generated with and without this locus List14-017 so population heterogeneity was not greatly affected. All other populations in the study were in HWE.

7.2.1 Population heterogeneity

Population heterogeneity was investigated both within and between silvatic, peridomestic and domestic populations including adjacent populations, also by State and by ecotope.

7.2.1.1 Adjacent populations

Four localities analysed by microsatellites were sampled in adjacent populations. When these populations were analysed for heterogeneity 3 exhibited non-significant differences between populations including house and palm and peridomestic and palm ecotopes (see Table 33 on page 191). These were (1) Terronal h2 and palm 03, (2) Los Rastrojos house and palm, (1) between Rio Bravo II pd and palm. Similarity of these populations was also confirmed by genetic analysis (D_S) (see section 7.1.6.5 on page 203). Homogeneity was also confirmed by *cytb* direct sequencing for a subset of specimens analysed by both methods.

Significant heterogeneity was also detected between some adjacent populations. Isolation between adjacent house and palm populations was identified in Cojedes by microsatellite analysis. The domestic infestation was localised to a bed in a house occupied by a single youth. The silvatic population was taken from a palm that supported several types of wild life including parrots and bats. This availability of blood

supply may have resulted in limited domestic colonisation of specimens from this palm, followed by genetic drifting of the two populations. Two other palms were equally as close to the house and may have been the source of the domestic infestation. In the locality Terronal, house 2 and adjacent palm 01 were also significantly different, but Terronal house 2 and palm 03 was not significantly different.

7.2.1.2 Populations within localities

Homogeneity was also detected across populations within localities including between and within ecotopes. Between house and palm ecotopes (1) Laguna Hermosa house and palm, (2) Cascabel house and palm, (3) Guaranda house and palm (4) G. Paraguey house and palms populations. Homogeneity for each of these populations was also confirmed by *cytb* direct sequencing for a subset of specimens analysed by both methods. Homogeneity between peridomestic and silvatic\domestic ecotopes was also detected including Laguna Hermosa palm and pd and Cascabel palm and pd. Greater, but non-significant, heterogeneity was detected between Laguna Hermosa house and peridomestic by microsatellite analysis. These results were also confirmed by *cytb* direct sequencing for a subset of specimens analysed by both methods (see section 7.1.6.6 on page 210).

These results indicate that specimens of *R. prolixus* from silvatic, domestic and peridomestic ecotopes in Venezuela are not always isolated. This agrees with the results of risk factor analysis (Sanchez-Martin *et al.*, 2005). Homogeneity was also detected within domestic ecotopes and within silvatic ecotopes including San Bartolo house 1 and 2 and Terronal domestic populations (h1 01 and 03, h1 01 and h2 01). This was also confirmed by sequence data.

7.2.2 States

Within States results varied with high levels of population heterogeneity detected among populations in Portuguesa (55% of pairwise comparisons), and low levels of

heterogeneity was detected within Barinas State (14%). Population heterogeneity between States was also high (see Table 33 on page 191, Figure 28 on page 201).

Lack of population differences was detected across localities in Portuguesa, including between silvatic and domestic ecotopes. For example peridomestic specimens from Laurianito were not significantly different from domestic populations in Terronal or palm 03. Palo Gacho was not significantly different from Terronal h2 01 (see Figure 25 on page 192).

Within Portuguesa significant population isolation was also detected for Santa Lucia, San Bartolo, Los Rastrojos, Terronal h2 p01 (see Figure 25). Distinct populations may be the results of genetic drift with populations showing allele fixation at two or three loci and private alleles detected in both Los Rastrojos house and palm (see Table 30 on page 187, Table 31 on page 188).

Populations from Santa Lucia and San Bartolo (municipality Ospino) were sampled in a different mountain area to Terronal, Casa Rena, Laurianito and Palo Gacho (all from the municipality Araure). In Ospino land clearance for coffee growing had noticeably changed the landscape and few palms were visible. This may explain the distinction of these populations. Los Rastrojos (municipality Sucre) was also distinct from other populations in Portuguesa; this locality was also distant from Araure and was situated close to the borders of Trujillo State. However isolation by distance was not detected among localities and Terronal h2 p01 were also distinct even within the municipality Araure, so population isolation does not necessarily require great distances.

Important outcome of the analysis is that within Portuguesa results indicated that a lack of population division may be found across all ecotopes and therefore populations in these differing ecotopes are not isolated. It indicates that movement of bugs is occurring between and within different ecotopes. Analysis of all specimens from Portuguesa State divided by ecotope also detected a lack of heterogeneity at this macro scale between palm and peridomestic populations (see section 7.2.4 on page 228).

Within the State of Barinas a different picture emerged, with less than 20% of all pairwise population comparisons significant. Again within the State non-significant population division was detected between ecotopes including domestic and silvatic populations (Cascabel, Guaranda), domestic, silvatic and peridomestic populations (Cascabel), and within ecotopes (G. Paraguey) (see section 7.1.6.1.2 on page 194). Across localities in Barinas homogeneity was detected within and between differing ecotopes (see Figure 26 on page 197). In Barinas population heterogeneity was not significant between ecotopes (see section 7.1.6.2 on page 202).

High levels of heterogeneity (F_{ST} values) and significant population differences within Barinas were related to a single peridomestic population 19 Abril. Unlike Portuguesa where all populations came from various mountainous regions, Barinas populations came from the Llanos, the flat plains of Venezuela, which may explain the greater extent of population homogeneity. Infestations in the foothills in Barinas occur to a lesser extent (M. Sanchez-Martin pers. communication). Apto. 19 Abril pd came from the extreme distribution of sampled houses and from an area where *T. maculata* infestations were more common, a factor that could add to population isolation (M Sanchez-Martin pers. communication). In areas where *T. maculata* and *R. prolixus* occur *T. maculata* is normally found in the peridomestic environment with *R. prolixus* in the domestic environment, indicating a degree of competition between the species, and when they occur in the same house they are often found in different parts with *T. maculata* most commonly found in lower walls where chickens often rest (see section 2.3.2 on page 76). However, the silvatic Parcelamiento population also came from this region and was not distinct.

Between States significant heterogeneity was detected (see Table 33 on page 191, Figure 28 on page 201), with the majority of population pairwise comparisons showing significant differences, also detected when populations were merged to state level and compared (see section 7.1.6.2 on page 202).

Trujillo domestic specimens were significantly different from almost all other populations. This is not surprising given they are separated by the Andes mountain range. Even within Trujillo few domestic infestations are detected (Dr A. Rojas pers. communication) and predominance of silvatic *R. robustus* could lead to further population isolation. This population was also significantly different by *cytb* analysis (see Table 40 on page 209). Interestingly, although a single female *R. robustus* (*cytb* characterisation) was detected in this domestic population, this specimen showed unique alleles in microsatellite analysis, indicating that this female, while found in the house, appears not to be a member of the colony.

Specimens from Lara State were also distinct. As discussed silvatic specimens in Lara have never been successfully detected (see section 5.1 on page 94), and this may mean domestic populations are prone to isolation and genetic drift. Significant population differences were not detected by *cytb* analysis.

Our results indicate that *R. prolixus* has low genetic variability and can have a low levels of population heterogeneity up to State level, with comparison across States more likely to give significant differences. As discussed earlier Sanchez-Martin *et al.*, (2005) found the risk of a house been infested was just significantly higher when within 100m of 10 or more palm trees (p=0.05, p=0.07 for colonisation), while infestation and colonisation of outbuildings (chicken huts, cow sheds, pigsties) were highly positively associated when within 100m of 5 or more palm trees (p=0.008, p=0.006), in particular the palm species, *A. butyracea,* (p value=0.006). House infestation and colonisation was also significantly associated with the density of bugs in the peridomestic area (p-value=0.05, 0.005). The presence of a palm roof was positively associated with house infestation when the roof was less than 1 year old (p value=0.01). This indicates that bugs may also be moved passively into the domestic environment via eggs glued to palm leaves as previously believed (see section 2.3.1 on page 72). This risk factor data also support the idea of movement of bugs between ecotopes. The presence of silvatic populations of *R. prolixus* in palms therefore represents a momentous challenge to Chagas disease control.

7.2.3 State

Specimens were clustered and analysed by State of origin were distinct. This confirms earlier significant differences detected between States. Interestingly when State groups were further subdivided by ecotope, homogeneity was detected in Barinas across domestic and silvatic/peridomestic groups. The same was true between palm and peridomestic ecotopes in Portuguesa, indicating much homogeneity between these ecotopes within states. Isolation between States was not correlated with geographic distances.

7.2.4 Ecotope

When specimens were divided by ecotope, silvatic ecotopes proved to be the most heterogeneous environment, with higher levels of allele richness and gene diversity detected, with peridomestic environment being intermediate. Higher diversity would be expected in silvatic ecotopes as it is the natural environment for *R. prolixus.* All pairwise comparisons between the three ecotope groups were significant, which would be expected given the earlier detection of distinct populations within ecotopes.

7.2.5 Microsatellite and *cytb* analysis.

From the comparison of both markers it is clear that a higher degree of population heterogeneity was generally detected by microsatellite analysis. Differences in population heterogeneity detected by both markers may be due to differing selective pressures and mutational rates associated with the different genes. Microsatellites are neutral noncoding fragments of DNA, with variation linked to high rates of slippage (see section 1.5.2.3.3 on page 62) while *cytb*-genes that encode proteins involved in metabolic processes may be less polymorphic due to selective constraints (Maingon *et al.*, 2003). Mitochondrial DNA, however, is sensitive to genetic drift as, due to their maternal inheritance, mitochondria have approximately one quarter of the effective population size of nuclear genes (Krafsur *et al.*, 2001).

Importantly, as described in 7.1.6.6 on page 210, in several localities including Terronal, San Bartolo, Los Rastrojos, Guaranda, Rio Bravo II, Laguna Hermosa and Cascabel, populations from differing ecotopes (house palm and peridomestic) were analysed by both microsatellite and *cytb* analysis and homogeneity was detected by both methods, including adjacent populations. Distinct populations were also detected by both methods (Trujillo, Santa Lucia and 19 Abril).

However, as pointed out in section 7.1.6.6 on page 210 the methods sometimes gave conflicting results, for example, in Cojedes but also in Portuguesa where a number of populations found distinct by microsatellite analysis were not distinct by *cytb* analysis. With high rates of variation associated with microsatellites, and selective neutrality, it is to be expected that microsatellites are more sensitive to population heterogeneity (Anderson *et al.*, 2002).

7.2.6 Assignment

Assignment test (see 5.4.11.6 on page 124) results were similar to those obtained from F_{ST} with poor assignment at population level, due to limited population heterogeneity with only individuals from highly differentiated populations assigned correctly in the majority of cases. These results are not surprising as accuracy of assignment decreases when population differences are low while those populations separated by large genetic distances and high F_{ST} indices are assigned with greater accuracy (Maingon *et al.*, 2003, Waser & Strobeck 1998, Burns *et al.*, 2004).

Highest scores for assignment were detected when data were grouped by State, indicating that at State level significant population differentiation is visible (as seen from F_{ST} indices). Barinas and Portuguesa specimens shared some similarity, with Portuguesa also showing similarity to Lara and Cojedes States. Accuracy of assignment dropped when State groups were separated by ecotope, specimens were reassigned with greatest frequency within differing State ecotopes, as observed by F_{ST} analysis.

Assignment scores by ecotope were moderate, with highest reassignment detected for domestic populations. Domestic populations were most frequently assigned to silvatic ecotopes, indicating much shared similarity between these ecotopes, however silvatic populations were more frequently assigned to peridomestic ones, indicating that this ecotope may be an important intermediary between silvatic and domestic ecotopes, with peridomestic specimens more frequently assigned to domestic populations.

No published studies are available on Triatomine population heterogeneity using microsatellites for comparison. Population studies using more conserved isoenzyme analysis have indicated that silvatic and domestic populations of *T. infestans* were panmictic (Dujardin *et al.*, 1987). However this was in contrast to later studies using morphometrics and RAPD analysis (Dujardin *et al.*, 1997b, Carlier *et al.*, 1996). Other studies using isoenzyme analysis have detected panmixia for *T. infestans* populations between houses at village level (Dujardin *et al.*, 1998c), with isolation by distance detected between villages, however a population deme within a single house has also been detected (Breniere *et al.*, 1998). Isolation of silvatic and domestic populations of *R. prolixus* in Colombia detected by isoenzyme analysis was later revised with the identification of silvatic populations as a new species, *R. colombiensis* (Lopez & Moreno 1995). RAPD and morphometric analysis have been used in the analysis of *T. brasiliensis* from silvatic, peridomestic and domestic ecotopes and indicated that populations were not isolated (Borges *et al.*, 2005). These studies indicate populations of triatomines in differing ecotopes are not always isolated. The most similar study is the analysis of 36 silvatic specimens of *R. pallescens* by Harry *et al.*, (1998) but as numbers were small, true population heterogeneity was difficult to assess. All but four of the microsatellite loci were at frequencies expected with Hardy Weinberg equilibrium. Studies on the population of mosquitoes have detected limited population heterogeneity over large distances for example in the studies of Rongnoparut *et al.*, (1999) on *An. maculatus* populations over 1100km and Lehmann *et al.*, (1996) for *An. gambiae* populations from East and West Africa separated by distances up to 6000km.

Nine or ten loci were used and additional loci would be advantageous. Polymorphism was low to moderate for the majority of loci and excess homozygosity at one locus may indicate nulls (List14-017). This might have resulted in under estimation of divergence between populations. It is also possible that other loci exhibit hidden nulls with 54 specimens failing to amplify at one or more loci. However, mitochondrial and risk factor analysis also support a similar picture of limited population differentiation between silvatic and domestic ecotopes.

7.2.7 Conclusions

Important conclusions from this microsatellite analysis are:

1. Most importantly for the control of Chagas disease, data analysis indicates that silvatic, peridomestic and domestic populations of *R. prolixus* are not always genetically differentiated, including 3 adjacent populations. Thus silvatic and peridomestic specimens can play a role in house infestation.

2. Analysis broadly agrees with *cytb* and risk factor analysis (Sanchez-Martin *et al.*, 2005).

3. Future effective control of Chagas disease in Venezuela requires a restructuring of the control program to deal with this credible silvatic threat (see chapter 9 on page 268).

8 Geometric morphometric analysis

The aim of this section of the study is to assess wing shape variation between silvatic and domestic populations of *R. prolixus* using novel geometric morphometrics. Results were also compared with genetic characterisation, *cytb* and microsatellites, for subsets of specimens analysed by these methods.

The locality Terronal within Portuguesa was first analysed separately to investigate shape variation in a limited area between silvatic and domestic populations. Due to large numbers, specimens within Portuguesa State were analysed next, consisting of silvatic or domestic groups at population\locality level (13 groups). State comparisons were then made, with specimens divided by locality\State of collection and ecotope (326 specimens in 20 groups). Specimens were also grouped by ecotope and analysed (306 specimens in 2 groups).

Comparisons with genetic characterisation were as follows: (1) For *cytb* characterisation by (i) haplotype (4 groups; haplotype 1, haplotype 2, haplotype 3 and haplotype 5, 237 specimens in total) (ii) for a subset of populations characterised by both morphometrics and *cytb* (18 groups 233 specimens in total) (2) For microsatellite characterisation; a subset of populations characterised by both morphometrics and microsatellites were analysed (12 groups, 190 specimens in total). As the majority of specimens were collected in Portuguesa, this State was further subdivided by locality and year of collection (where appropriate).

8.1 Results

8.1.1 Analysis of specimens in Terronal

In Terronal five populations were compared consisting of 116 specimens divided into 3 domestic and 2 silvatic groups. Domestic populations came from two houses, with house 1 represented in two different years (2001, 2003); specimens from palm 01 were from multiple palms (see Table 9 on page 127).

CVA analysis (see page 46) detected significant differences between groups, ($p<0.0001$), however Wilk's lambda value was moderate (Wilk's lambda=0.2, see page 132). Moderate values here indicate that while populations exhibit significant shape differences, these differences are not pronounced.

From the plot of CV1 against CV2 (see Figure 33 and see page 47) significant shape overlap can be seen between domestic populations, with house 1 03 more distinct. The pairwise comparison of means by the Tukey-Kramer test (see page 132) detected shape similarity between all domestic populations, with Terronal h1 03 differing by CV3 only (accounting for 21% shape variation) (see Table 49 on page 235). Palm populations show no shape overlap, by CV1, but were similar in shape by CV2. Significant shape similarity was detected between silvatic populations by Tukey-Kramer (CV2 and CV3, see Table 49). Limited overlap occurred between silvatic and domestic populations (see Figure 33 on page 234), with CV2 dividing populations by ecotope.

Reclassification data (see page 48) found bugs from house 1 01 to be heterogeneous in shape with specimens placed in each of the compared populations, with highest numbers reclassified as Terronal h2 01 and Terronal h2 p01. House 2 01 was also heterogeneous in shape with only 47% of specimens correctly re-classified, with the largest number of incorrect classified specimens placed in house 1 03 (27%). House 1 03 exhibited the highest correct reclassification, and population distinction was also visible from Table 33 on page 191 and Figure 34 on page 235. However with fewer specimens analysed in this population, total shape heterogeneity may not have been captured. Each palm population also showed high levels of correct reclassification (85% and 73%, see Table 48 on page 234).

From the Mahalanobis distances, (see page 132), house 1 01 and house 2 01 are the most similar in shape, with palm 01 closer in shape to house 01 and 02 than house 1 03. House 1 03 and palm 03 clustered separately (see Figure 34 on page 235). Reclassification data also indicated some shape similarity between palm 01 and house 1

01 (10% and 15.4% misclassified) and between house 2 and palm 01 (13.3% misclassified).

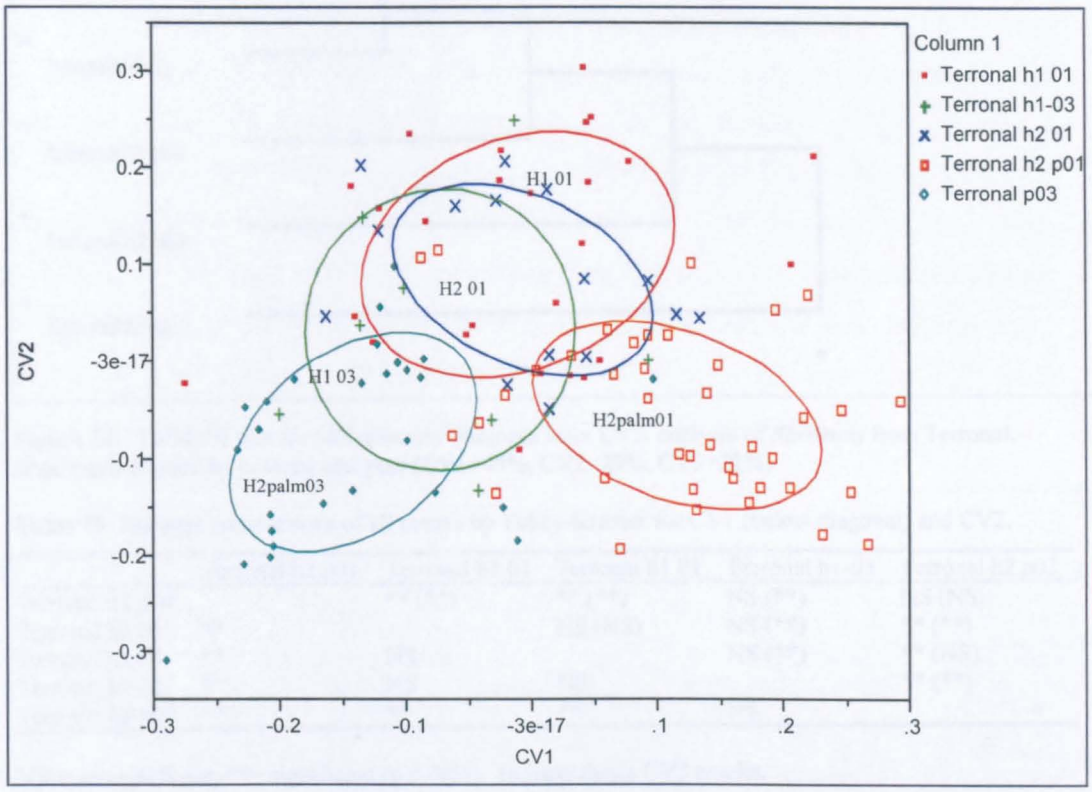

Figure 33. CVA analysis after PCA of Terronal specimens grouped by ecotope and year. Ellipses enclose **50%** distribution of specimens in the shape discriminate space defined by CV1 (accounting 44% of total variation among groups) and CV2 (29% of total variation). (Wilk's lambda=0.2, CVA p-value ≤ 0.0001). (CV3=21%).

Table 48. Reclassification scores after CVA analysis of Terronal specimens divided by ecotope and year.

	Terronal h1 01	Terronal h1 03	Terronal h2 01	Terronal h2 p01	Terronal h2 p03	Total
Terronal h1 01	**15 (57.7)**	1 (3.9)	4 (15.4)	4 (15.4)	2 (7.7)	26
Terronal h1 03	-	**7 (87.5)**	-	1 (12.5)	-	8
Terronal h2 01	2 (13.3)	4 (26.7)	**7 (46.7)**	2 (13.3)	-	15
Terronal h2 p01	4 (10.0)	2 (5.0)	2 (5.0)	**29 (72.5)**	3 (7.5)	40
Terronal h2 p03	1 (3.7)	-	2 (7.4)	1 (3.7)	**23 (85.2)**	27
Total	22	14	15	37	28	116

Parenthesis = percentage reclassified. **Bold values** numbers and percentages for groups reclassified correctly. (Kappa value=0.6, Kappa measures the degree of agreement between compared variable on a scale from 0 to 1).

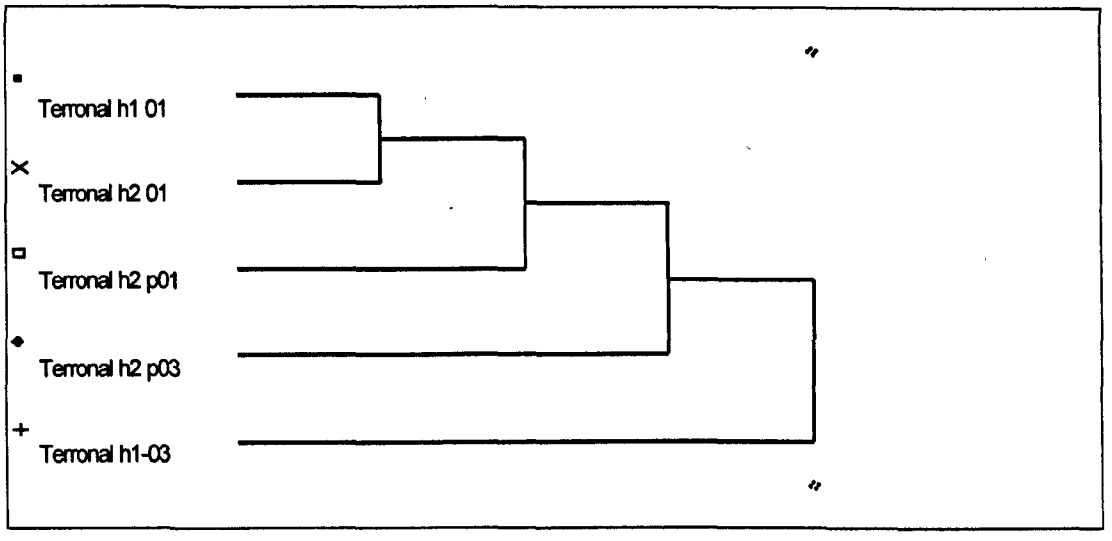

Figure 34. UPMGA tree for Mahalanobis distances from CVA analysis of *Rhodnius* from Terronal. Specimens divided by ecotope and year (CV1=44%, CV2=29%, CV3=22%).

Table 49. Pairwise comparisons of all means by Tukey-Kramer for CV1 (below diagonal) and CV2.

	Terronal h2 p01	Terronal h2 01	Terronal h1 01	Terronal h1-03	Terronal h2 p03
Terronal h2 p01		** (**)	** (**)	NS (**)	NS (NS)
Terronal h2 01	**		NS (NS)	NS (**)	** (**)
Terronal h1 01	**	NS		NS (**)	** (NS)
Terronal h1 03	**	NS	NS		** (**)
Terronal h2 p03	**	**	**	NS	

NS=non-significant, **= significant ($p < 0.05$). In parenthesis CV3 results.

In summary, for this locality

- Domestic populations are similar in shape, although house 1 03 is more distinct.
- Domestic and silvatic populations, while sharing some limited shape similarity, are distinguishable.
- Silvatic populations, while sharing some shape similarity, are also distinguishable by CV1.
- Shape differences while present are not extremely pronounced.
- No group was 100% correctly reclassified (Kappa 0.6).

8.1.2 Analysis of localities within Portuguesa

A total of 212 specimens from Portuguesa State from 8 localities were analysed by ecotope (8 domestic groups, 1 peridomestic and 3 silvatic groups) (see Table 9 on page 127). Specimens from the locality Terronal were divided by year of collection. Groups were as follows; 20 Laurianito (peridomestic), 15 San Bartolo, 14 Qdra Negra, 9 Morichal, 10 El Mosquito, 8 Casa Rena (all domestic) and 20 Palo Gacho (silvatic), 81 specimens from Terronal in 2001 and 35 specimens from Terronal in 2003 (both domestic and silvatic). CVA analysis detected significant differences between groups, ($p<0.0001$), however Wilk's lambda value was moderate (Wilk's lambda=0.14), indicating shape differences were not very pronounced. No significant allometric trend was detected, so shape differences are unrelated to size (data not shown).

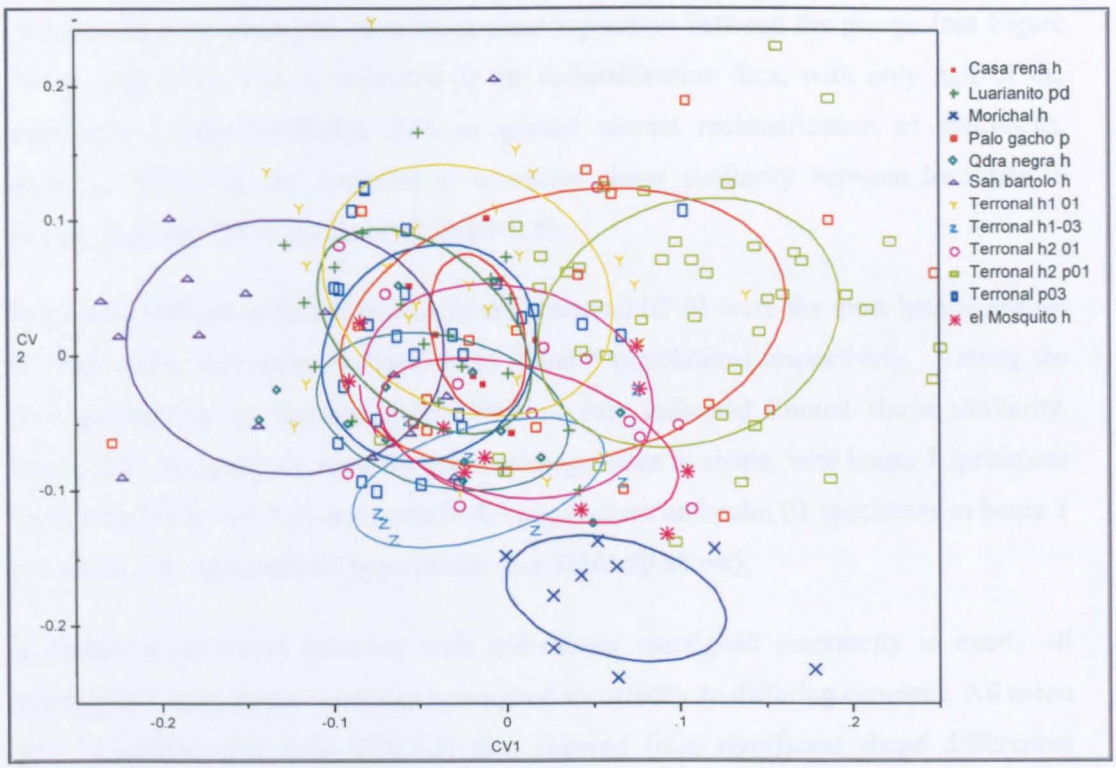

Figure 35. CVA analysis after PCA of specimens from Portuguesa grouped by locality and ecotope. Terronal specimens were divided by year of collection. Ellipses enclose **50%** distribution of specimens in the shape discriminate space defined by CV1 (accounting 33% of total variation among groups) and CV2 (22%). (CV3=18%).

Table 50. Reclassification scores after CVA analysis of specimens from Portuguesa State.

Locality	1	2	3	4	5	6	7	8	9	10	11	12	Total ^
1 Casa Rena h *	**4**	1	-	-	-	-	2	-	1	-	-	-	8 (50)
2 Laurianito pd	3	**7**	-	-	1	-	3	-	2	-	4	-	20 (35)
3 Morichal h *	-	-	**8**	-	-	-	-	1	-	-	-	-	9 (89)
4 Palo Gacho p	2	1	1	**7**	1	1	2	2	1	2	-	-	20 (35)
5 Qdra Negra h	1	-	-	-	**6**	-	2	2	1	-	2	-	14 (43)
6 San Bartolo h *	-	2	-	-	1	**9**	1	-	2	-	-	-	15 (60)
7 Terronal h1 01	4	-	-	1	1	3	**10**	-	2	3	2	-	26 (39)
8 Terronal h1 03 *	-	-	-	1	1	-	-	**6**	-	-	-	-	8 (75)
9 Terronal h2 01	2	1	1	2	1	1	1	1	**5**	-	-	-	15 (33)
10 Terronal h2 p01 *	1	-	4	4	1	-	-	1	1	**26**	2	-	40 (65)
11 Terronal h2 p03 *	-	2	-	3	3	2	-	1	-	1	**15**	-	27 (56)
12 El Mosquito h	-	-	2	-	2	1	-	2	-	2	-	**1**	10 (10)
Total	17	14	16	18	18	17	21	16	15	34	25	1	212

* ≥ 50% correct reclassification. ^ Number in parenthesis represents % of group total correctly reclassified. h=house, p=palm, pd=peridomestic. 01=2001, 03=2003. Kappa=0.4.

The plot of CV1 and CV2, accounting for 55% of total variation, shows the majority of populations to be homogenous without clear separation between the groups (see Figure 35 on page 236). This is reflected in the reclassification data, with only half of the population groups exhibiting 50% or greater correct reclassification of specimens, therefore indicating the presence of extensive shape similarity between localities in Portuguesa (see Table 50 above) (Kappa=0.4).

From reclassification data Palo Gacho and Terronal h2 01 were the most heterogeneous in shape, with specimens reclassified in 9 and 8 populations respectively. Among the five populations in Terronal reclassification data indicated limited shape similarity. House 1 01 and palm 01 were the most heterogeneous in shape, with house 1 specimens reclassified in house 2 01 and palm 01/03 populations and palm 01 specimens in house 1 03, house 2 01 and palm 03 populations (see Table 50 above).

Reclassification errors occurred with individuals reassigned incorrectly to nearly all populations. Specimens were also reassigned incorrectly to differing ecotopes. All mean pairs Tukey-Kramer tests (CV1-3) also showed little significant shape differences between localities (see Table 51 on page 239), with Terronal h2 p01, Morichal, and San Bartolo most divergent in shape (see Figure 36 on page 238). Terronal palm 03 showed limited difference by CV3, as also seen from reclassification data.

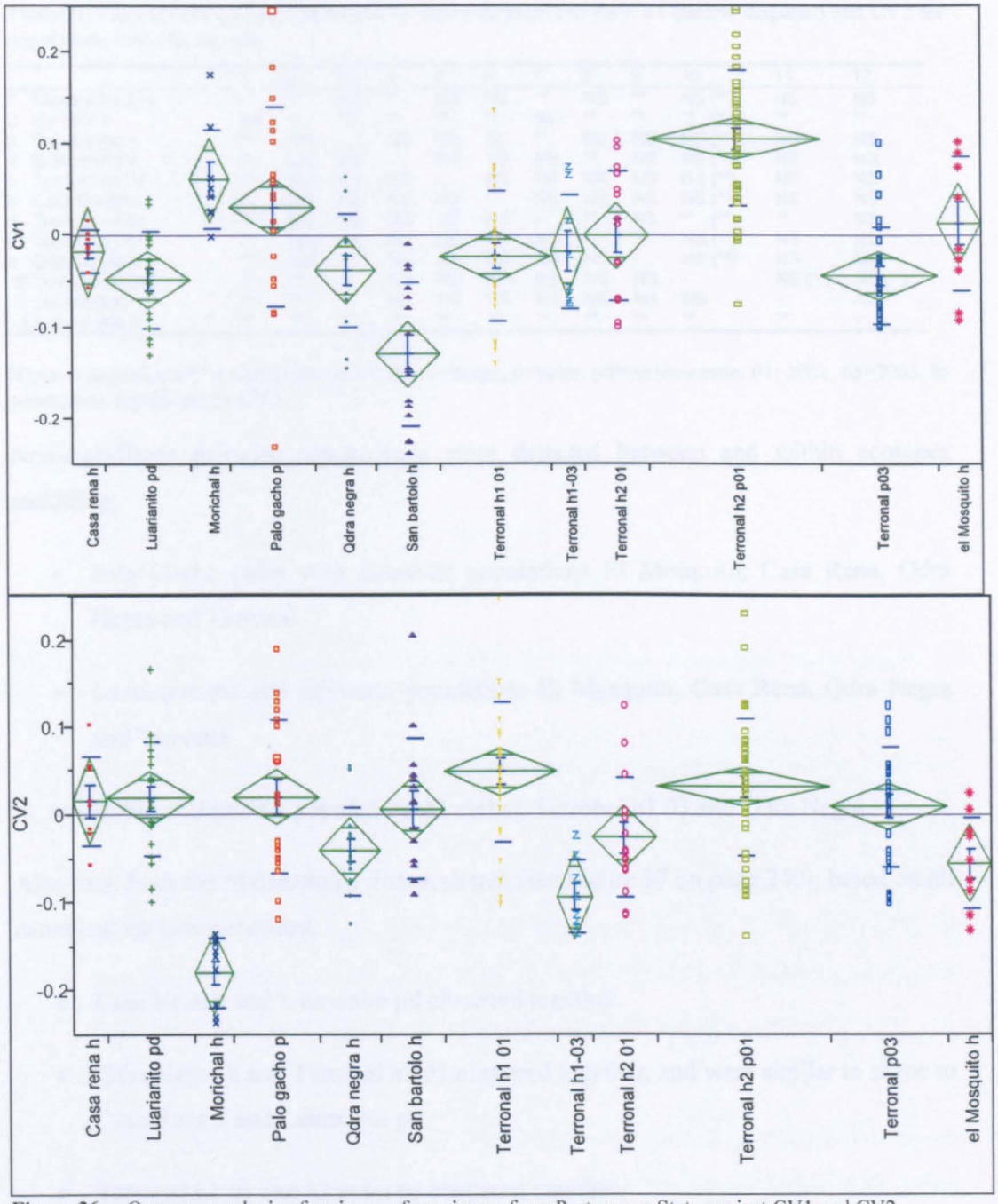

Figure 36. One-way analysis of variance of specimens from Portuguesa State against CV1 and CV2. CV1 (upper plot) accounts for 33% and CV2 (lower plot) 22% of the total variance for wing shape between haplotype groups. h=house, p=palm, pd=peridomestic. 01=2001, 03=2003.

Table 51. Pairwise comparisons of means by Tukey-Kramer test for CV1 (below diagonal) and CV2 for populations from Portuguesa.

	1	2	3	4	5	6	7	8	9	10	11	12
1 Terronal h2 p01	-	**	NS	**	NS	NS	**	NS	**	NS (**)	NS	NS
2 Morichal h	NS	-	**	**	**	**	NS	**	**	** (**)	**	**
3 Palo Gacho p	**	NS	-	NS	NS	NS	**	NS	NS	NS (**)	NS	NS
4 El Mosquito h	**	NS	NS	-	NS	NS	NS	**	NS	NS (**)	NS	NS
5 Terronal h2 01	**	NS	NS	NS	-	NS	NS	NS	NS	NS (**)	NS	NS
6 Casa Rena h	**	NS	NS	NS	NS	-	NS	NS	NS	NS (**)	NS	NS
7 Terronal h1 03	**	NS	NS	NS	NS	NS	-	**	NS	** (**)	**	NS
8 Terronal h1 01	**	NS	NS	NS	NS	NS	NS	-	**	NS (**)	NS	NS
9 Qdra Negra h	**	NS	NS	NS	NS	NS	NS	NS	-	NS (**)	NS	NS
10 Terronal h2 p03	**	**	**	NS	NS	NS	NS	NS	NS	-	NS (**)	NS (**)
11 Laurianito pd	**	**	**	NS	NS	NS	NS	NS	NS	NS	-	NS
12 San Bartolo h	**	**	**	**	**	**	**	**	**	**	**	-

NS=non-significant **= significant ($p < 0.05$). h=house, p=palm, pd=peridomestic. 01=2001, 03=2003. In parenthesis significant by CV3.

Non-significant pairwise comparisons were detected between and within ecotopes including;

- Palo Gacho palm with domestic populations El Mosquito, Casa Rena, Qdra Negra and Terronal.
- Laurianito pd and domestic populations El Mosquito, Casa Rena, Qdra Negra and Terronal
- Between domestic populations Morichal, Terronal h1 03 and Qdra Negra.

Also seen from the Mahalanobis distances tree (see Figure 37 on page 240), based on all canonical variants produced,

- Casa Rena h and Laurianito pd clustered together.
- Qdra Negra h and Terronal h2 01 clustered together, and were similar in shape to Casa Rena h and Laurianito pd.
- Terronal h1 01 and h2 palm 03 clustered together.
- Palo Gacho p and Terronal h2 p01 clustered together.
- Adjacent house and palm Terronal h2 01, palm 01\03 clustered separately.

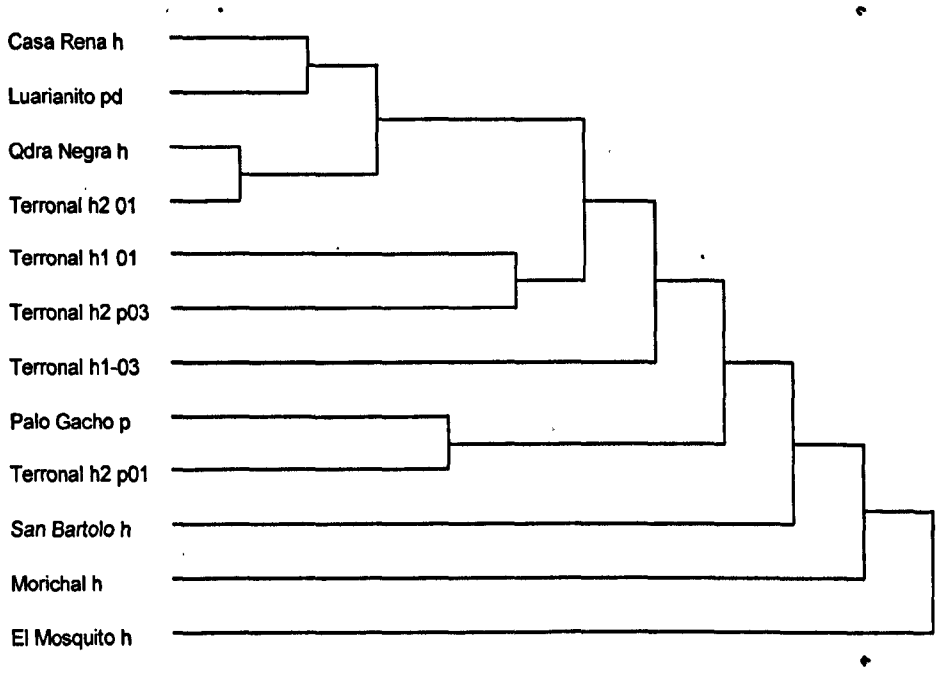

Figure 37. UPMGA tree for Mahalanobis distances from CVA analysis of specimens from Portuguesa. Specimens were grouped by locality and ecotope, with Terronal specimens divided by year. h=house, p=palm, pd=peridomestic. 01=2001, 03=2003

From Figure 35 on page 236 and Figure 37 domestic populations Morichal and San Bartolo appear largely distinct from other populations.

- In Morichal 89% of specimens were correctly reclassified. Few specimens were analysed so total shape heterogeneity may not have been captured. While distinct, Morichal, was similar in shape by CV1 and CV3 with other populations within Portuguesa including El Mosquito, Qdra Negra and Terronal h1 01 (all CVs) (see Table 51 on page 239).

- In San Bartolo 60% of domestic specimens were correctly reclassified. Shape difference occurred by CV1, while shape similarity was detected with other populations by CV2 and CV3 (see Table 51).

- In Terronal h2 p01 65% of specimens were correctly reclassified, 35% incorrect reclassification indicated some shape overlap and specimens were most frequently reclassified in Palo Gacho and Morichal. While largely distinct by CV1, Terronal palm 01 shared shape similarity with other populations by CV2 and CV3 (see Table 51 on page 239).

- Terronal h1 03 also showed a high rate of correct reclassification, as seen in previous analysis (see section 8.1.1 on page 232).

8.1.3 Analysis of specimens across State level.

In order to investigate shape variability among specimens across States and ecotopes 326 specimens were grouped by State of collection and analysed (see Table 9 on page 127). This included 19 specimens from Lara (domestic only), 20 from Guarico (silvatic only), 31 from Trujillo (domestic and silvatic), 21 from Cojedes (domestic and silvatic), and 18 from Barinas (Carreteron, domestic only). Specimens from Cojedes, Lara and domestic Trujillo were primarily from single populations and Barinas from a single locality. An additional 5 silvatic specimens from the State of Merida were also analysed. Due to their large number the 212 specimens from Portuguesa were divided as in preceding analysis (see section 8.1.2 on page 236). The 326 specimens consisted of 166 collected in domestic ecotopes, 20 from peridomestic and 140 from silvatic, in a total of 20 groups. Eleven of the 14 silvatic Trujillo specimens were taken from insect colonies in the Universidad de Los Andes and therefore may not represent true silvatic shape variation for the State.

CVA analysis was significant (p-value<0.0001) and Wilk's lambda statistic was 0.06, i.e. lower than previously detected and indicating that shape differences detected at State level were more pronounced, although still subtle. No significant allometric trend was detected, so shape differences are unrelated to size (data not shown).

As detected in Portuguesa State resolution across all State groups was also poor by wing shape data (plot CV1 against CV2 not shown). Reclassification data for State groups

indicate that there is much shared shape variation in different States with only half of the populations showing 50% or greater correct reclassification (max 86%). For the remaining ten groups classification varied from 20-43% (see Table 52 below). All groupings were assigned incorrect individuals, ranging from 3 to Morichal and 10 to El Mosquito. Specimens were also reassigned to different ecotopes and different States. Shape similarity between groups is also visible from one-way analysis of variance (see Figure 38 on page 244), where CV1 accounts for 27% of total variation between the groups (CV2 22%). The all pairs mean Tukey-Kramer test also indicated significant shape similarity across CV1, CV2 (see Table 53 on page 243).

Table 52. Reclassification scores after CVA analysis of specimens grouped by State and ecotope.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total ^
1 Cojedes p	5	-	-	1	2	-	-	1	1	-	-	-	-	-	2	-	-	-	-	2	14 (36)
2 Guarico p *	-	11	-	1		-	-	-	-	2	1	1	-	1	-	1	-	1	1	-	20 (55)
3 Carreteron h^	-	-	6	-	1	1	-	1	1	1	-	1	1	-	-	-	-	-	1	4	18 (33)
4 Casa Rena h	-	-	-	3	-	-	-	-	-	-	1	2	-	1	-	-	-	-	-	1	8 (38)
5 Cojedes h	-	-	-	1	3	-	-	1	1	-	-	-	-	-	-	-	1	-	-	-	7 (43)
6 Lara h *	-	-	2	1	-	13	-	-	-	-	-	1	-	-	-	-	2	-	-	-	19 (68)
7 Laurianito pd	-	-	3	1	-	-	4	-	1	1	2	1	-	3	4	-	-	-	-	-	20 (20)
8 Morichal h *	-	-	-	-	1	-	-	7	-	-	-	-	1	-	-	-	-	-	-	-	9 (78)
9 El Mosquito h	3	-	-	-	-	1	1	-	4	-	-	-	1	-	-	-	-	-	-	-	10 (40)
10 Palo Gacho p	1	3	-	1	-	1	-	-	-	6	2	1	1	1	-	2	-	1	-	-	20 (30)
11 San Bartolo h *	-	1	1	-	-	-	2	-	-	1	8	-	-	-	-	-	1	-	-	1	15 (53)
12 Terronal h1 01	-	2	-	1	2	2	-	-	1	1	1	9	-	1	2	4	-	-	-	-	26 (35)
13 Terronal h1 03*	-	1	-	-	1	-	-	-	-	-	-	-	4	-	1	-	-	1	-	-	8 (50)
14 Terronal h2 01	-	-	1	-	-	-	1	-	-	2	-	2	4	4	-	-	-	-	1	-	15 (27)
15 Terronal h2p03*	-	-	-	1	-	-	1	-	1	2	1	1	-	-	17	1	-	-	-	2	27 (63)
16 Terronal h2 p01*	3	5	-	-	1	-	-	-	-	5	-	1	-	1	-	20	2	-	1	1	40 (50)
17 Trujillo h *	-	-	-	-	-	2	-	-	1	1	-	-	1	-	-	-	9	1	-	2	17 (53)
18 Trujillo p *	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	12	-	-	14 (86)
19 Merida p *	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	4	-	5 (80)
20 Qdra Negra h	-	-	1	1	2	2	-	-	1	-	-	-	-	-	1	-	-	-	-	6	14 (43)
Total	12	23	14	12	14	22	9	10	12	22	16	20	14	13	27	28	15	16	8	19	326

*≥50% correct reclassification, ^ %correctly reclassified in parenthesis. h=house p=palm, pd=peridomestic, 01=2001, 03=2003. ^Carreteron = Barinas.

Palo Gacho palm and Terronal house 01 groups were the most heterogeneous, with specimens misclassified in 10 other groups. Laurianito was also heterogeneous in shape, with specimens designated to 8 other groups, including an equal and greater number of

specimens misclassified than correctly classified. Specimens from Carreteron (Barinas State) also proved heterogeneous in shape (see Table 52 on page 242).

Table 53. Pairwise comparisons of means by Tukey-Kramer test for CV1 (below diagonal) and CV2 for specimens grouped by State and ecotope.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1 Terronal h2 p 01	-	NS	NS	**	**	**	**	**	NS	NS	NS	NS	**	**	NS	NS	**	NS	**	NS
2 Guarico p	NS	-	NS	**	NS	**	NS	**	NS	NS	NS	NS	**	**	NS	NS	**	NS	NS	NS
3 Palo Gacho p	NS	NS	-	NS	NS	**	NS	**	NS	NS	NS	NS	**	**	NS	NS	**	NS	NS	NS
4 Terronal h1 01	NS	NS	NS	-	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	**	NS	NS	**
5 Casa Rena h	NS	NS	NS	NS	-	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	**	NS	NS	**
6 Terronal h2 p03	**	NS	NS	NS	NS	-	NS	NS	**	NS	NS	NS	NS	**	NS	NS	**	NS	NS	**
7 Laurianito pd	**	NS	NS	NS	NS	NS	-	NS	NS	NS	NS	NS	NS	**	NS	NS	**	NS	NS	**
8 Qdra Negra h	**	**	NS	NS	NS	NS	NS	-	**	NS	NS	NS	NS	**	NS	NS	**	**	NS	**
9 Terronal h2 01	**	**	**	NS	NS	NS	NS	NS	-	NS	NS	NS	**	**	NS	NS	**	NS	NS	NS
10 Cojedes h	**	NS	NS	NS	NS	NS	NS	NS	NS	-	NS	NS	NS	**	NS	NS	**	NS	NS	NS
11 Cojedes p	**	**	**	NS	NS	NS	NS	NS	NS	NS	-	NS	**	**	NS	NS	**	NS	NS	NS
12 El Mosquito h	**	**	**	NS	NS	NS	NS	NS	NS	NS	NS	-	NS	**	NS	NS	**	NS	NS	NS
13 San Bartolo h	**	**	**	**	NS	**	NS	NS	NS	NS	NS	NS	-	**	**	NS	**	**	NS	**
14 Merida p	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	**	**	NS	**	**	**
15 Lara h	**	**	**	**	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	-	NS	**	NS	NS	NS
16 Barinas h	**	**	**	**	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	**	NS	NS	NS
17 Trujillo p	**	**	**	**	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	**	**	NS
18 Terronal h1 03	**	**	**	**	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	NS	NS
19 Trujillo h	**	**	**	**	**	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	**
20 Morichal h	**	**	**	**	**	**	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-

** =significant ($p<0.05$). NS=not significant h=house p=palm, pd=peridomestic 01=2001, 03=2003. Lara, Guarico and Terronal palm 03 showed shape distinction by **CV3.**

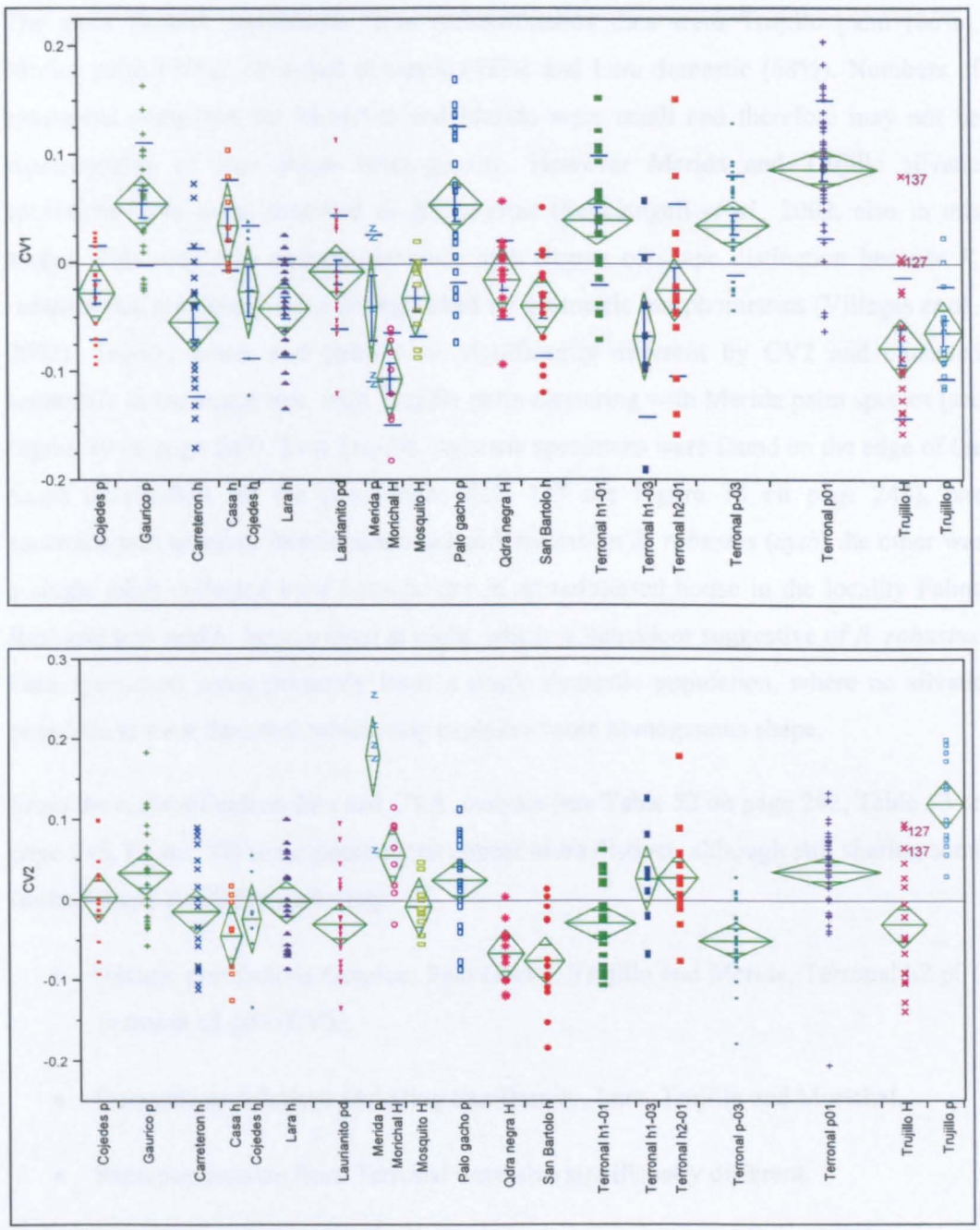

Figure 38. One-way analysis of variance of specimens grouped by State against CV1 and CV2. CV1 (upper plot) accounts for 27% of the total variation between groups and CV2 (lower) 22%. h=house, p=palm, pd=peridomestic 01=2001, 03=2003.

The most distinct populations from reclassification data were Trujillo palm (86%), Merida palm (80%), Morichal domestic (78%) and Lara domestic (68%). Numbers of specimens compared for Morichal and Merida were small and therefore may not be representative of true shape heterogeneity. However Merida and Trujillo silvatic specimens have been identified as *R. robustus* (Feliciangeli *et al.*, 2002, also in this study). This may also account for their high degree of shape distinction because *R. robustus* has previously been distinguished by geometric morphometrics (Villegas *et al.*, 2002). Trujillo house and palm were significantly different by CV2 and clustered separately in the shape tree, with Trujillo palm clustering with Merida palm species (see Figure 39 on page 247). Two Trujillo domestic specimens were found on the edge of the shape distribution for the population (127, 137 see Figure 38 on page 244), one specimen was an adult female identified as Venezuelan *R. robustus* (*cytb*) the other was a single adult collected by a householder in an uninfested house in the locality Palma Real and was said to have arrived at night, which is behaviour suggestive of *R. robustus*. Lara specimens came primarily from a single domestic population, where no silvatic populations were detected, which may explain a more homogenous shape.

From the reclassification data and CVA analysis (see Table 52 on page 242, Table 53 on page 243, Figure 38) some populations appear more distinct, although still sharing some limited shape similarity including;

- Silvatic populations Guarico, Palo Gacho, Trujillo and Merida, Terronal h2 p01, Terronal h2 p03 (CV3).
- Domestic populations including San Bartolo, Lara, Trujillo and Morichal.
- Palm populations from Terronal were also significantly different.

From the All pairs mean Tukey-Kramer test some shape similarity is detected within and between ecotopes including;

- Palo Gacho palm and domestic populations Terronal h1 01, Casa Rena and Cojedes.
- Terronal h2 p01 with Guarico and Palo Gacho palms
- Terronal h1 01\03 and house 02, Cojedes, El Mosquito, Lara and Casa Rena.

From the Mahalanobis distances tree, based on all CVA analysis (see Figure 39 on page 247), population similarity within and between ecotopes is visible between;

- adjacent population Cojedes house and palm
- domestic Casa Rena and Terronal h2 01, with Laurianito pd.
- Barinas and Trujillo domestic populations.
- San Bartolo h and Terronal h2 p03.

Adjacent house and palm populations from Terronal cluster separately. The distinction of Trujillo and Merida palm populations is also visible, as is domestic population Morichal and Lara. Interestingly clustering by ecotope occurs and a general shape similarity determined by ecotope exists, with palm populations more similar in shape and clustering together (Guarico palm to Terronal palm 03) (see Figure 39).

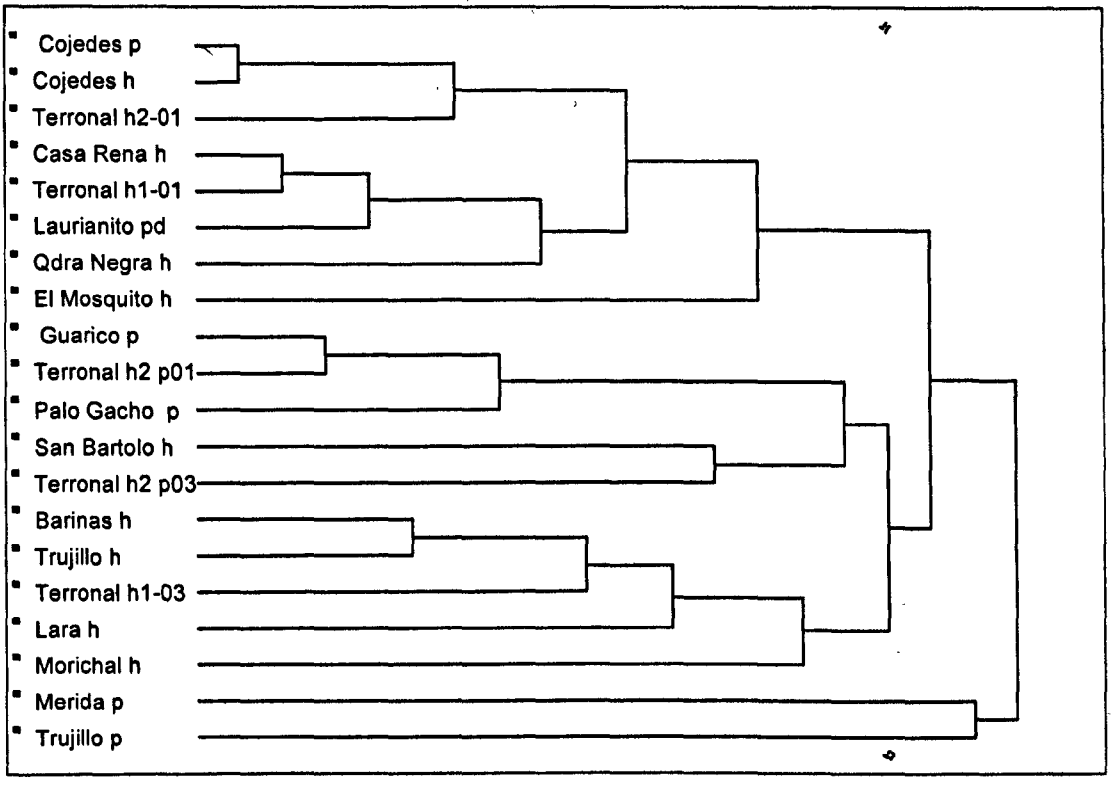

Figure 39. UPMGA tree for Mahalanobis distances from CVA of specimens by State and ecotope. Portuguesa specimens further subdivided by locality and year of collection.

8.1.4 Analysis of specimens by ecotope

For analysis of shape differences between silvatic and domestic ecotopes 306 specimens from the six study States were used (see appendix Table 70 on page 367) (166 domestic and 145 silvatic specimens). Peridomestic specimens were excluded due to limited numbers (20).

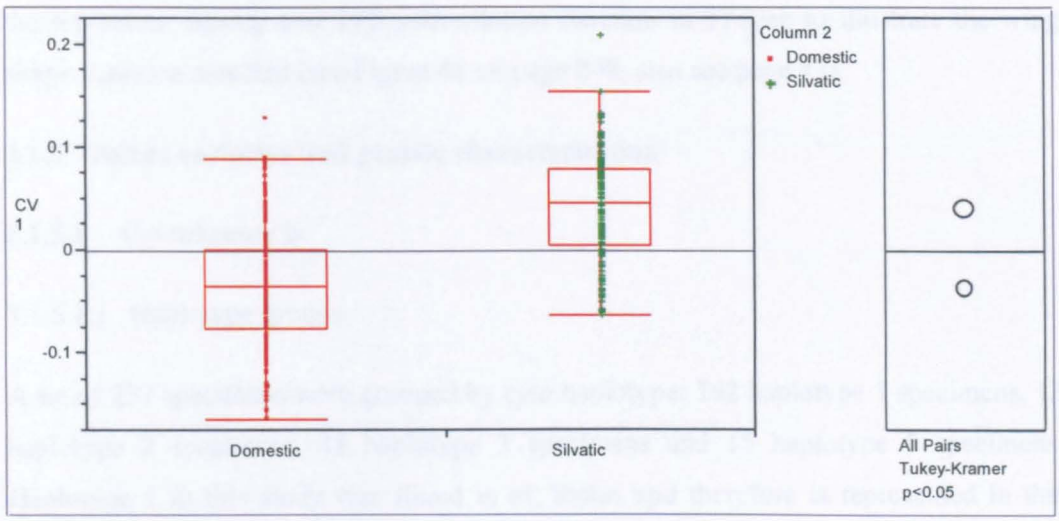

Figure 40. One-way analysis of variance of specimens divided by ecotope.
CV1 accounts for 100% of the total variation between groups. All pairs Tukey-Kramer test of significance $p<0.05$.

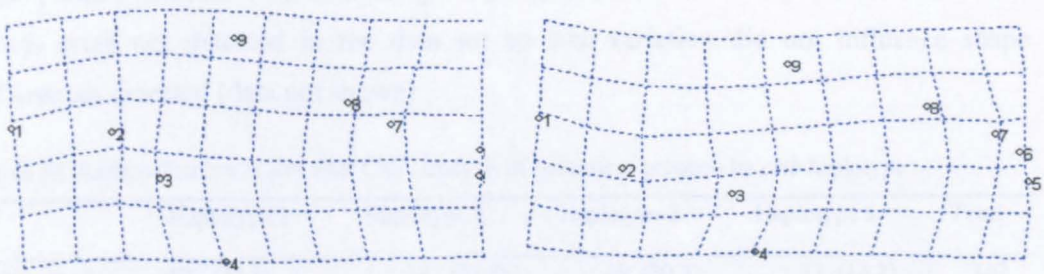

Figure 41. Thin plate spline grids of wing shape as deformations of average shape.
Grids show the deformation exaggerated by a factor of 3. Left grid extreme domestic shape, right grid extreme silvatic shape.

The all pairs Tukey-Kramer test was significant. Reclassification of specimens after CVA analysis was high (75% for both ecotopes). From the results a clear shape difference can be detected by wing morphometric analysis between specimens collected in domestic environments as compared to specimens collected in silvatic environments. Significant allometric trends were not detected in the data set, i.e. shape differences were not influenced by variation in size of specimens (data not shown). Shape changes associated with CV1 were visualised as Thin plate spline grids (TPS) generated using

the regression facility and TPS interpolation function in TPSreg to illustrate the wing shape variation detected (see Figure 41 on page 248, also see page 47).

8.1.5 Shape variation and genetic characterisation

8.1.5.1 Cytochrome b

8.1.5.1.1 Haplotype groups

A set of 237 specimens were grouped by *cytb* haplotype; 162 haplotype 1 specimens, 12 haplotype 2 specimens, 48 haplotype 3 specimens and 15 haplotype 5 specimens. Haplotype 1 in this study was found in all States and therefore is represented in this analysis by specimens across this geographic distribution. CVA analysis detected significant differences between groups, ($p<0.0001$), however Wilk's lambda value was high (Wilk's lambda=0.7), indicating shape differences were very subtle. Allometric trends were not detected in the data set so size variation did not influence shape differences detected (data not shown).

Table 54. Reclassification scores after CVA analysis of specimens grouped by *cytb* haplotype

	Haplotype 1	Haplotype 2	Haplotype 3	Haplotype 5	Total
Haplotype 1	**52 (32.1)**	38 (23.5)	49 (30.3)	23 (14.2)	162
Haplotype 2	1 (8.3)	**8 (66.7)**	3 (25.0)	-	12
Haplotype 3	12 (25.0)	10 (20.8)	**22 (45.8)**	4 (8.3)	48
Haplotype 5	-	-	1 (6.7)	**14 (93.3)**	15
Total	65	56	75	41	237

Kappa=0.2

From CVA analysis of wing shape it is clear that haplotype 1 and 3 showed significant overlap, a lack of difference was also detected by all pairs Tukey-Kramer test and reclassification data (see Figure 42 on page 250 and Table 54 above). Haplotype 5 forms a distinct group, differentiated from haplotype 1, 2 and 3 by CV1 (see Figure 42) and reflected in the high levels of correct reclassification (93%) for this haplotype (see Table 54 above). Haplotype 2 showed some shape difference, this is also reflected in reclassification data. The all pairs Tukey-Kramer means test from one-way analysis of

variance indicated that haplotype 5 was significantly different ($p<0.05$) from all other haplotype groups, while all other comparisons were not significant.

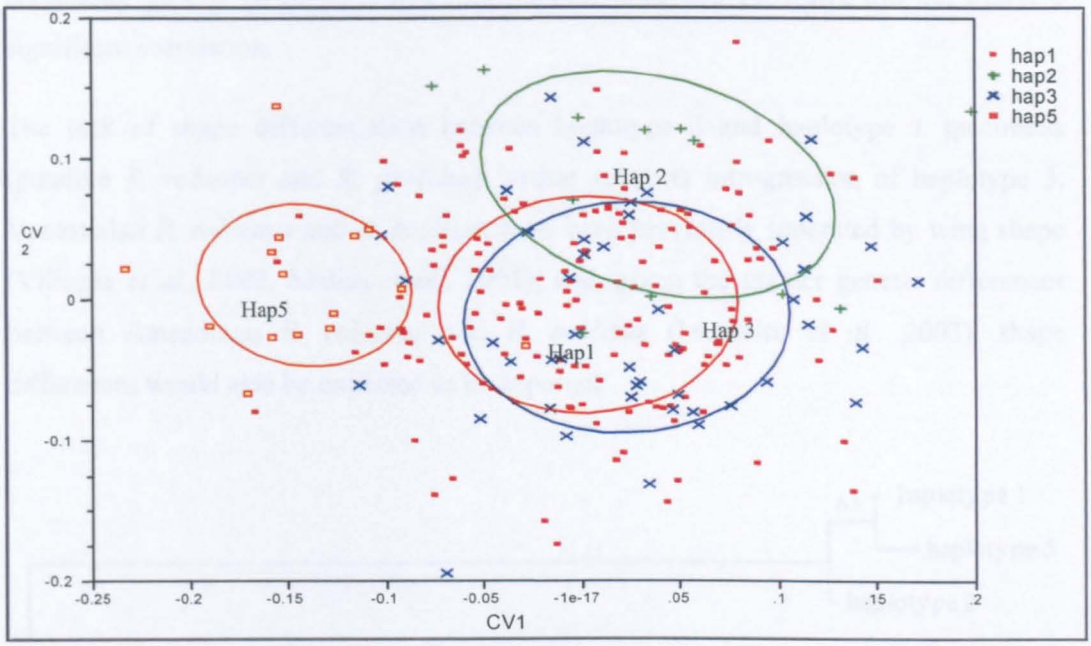

Figure 42. CVA analysis after PCA for specimens grouped by *cytb* haplotype.
Ellipses enclose 50% distribution of specimens in the shape discriminant space. CV1 accounts for 73% and CV2 for 19% of the total variance between haplotypes. hap=haplotype

A neighbour-joining tree for genetic distances (Kimura-2 1000 bootstraps MEGA v2.1) between haplotypes was compared with a neighbour-joining tree produced from Mahalanobis distances determined from shape variability (PAD Dujardin 2005, PHYLIP Felsenstein 1993) (see Figure 43 on page 251). As seen from comparisons with phylogenetic tree the shape relationships between haplotypes are incongruent with results from genetic analysis. In *cytb* genetic analysis haplotypes 1 and 3 were found to be the least similar, differing by 29 point mutations and with calculated genetic distances of 0.08 (Kimura 2-parameter) (see Table 24 on page 155), whilst haplotype 1, 2 and 5 were close genetically, separated by a single point mutation and a minimal calculated genetic distance of 0.002 (Kimura 2-parameter, Jukes Cantor). From the shape tree haplotype 1, 2 and 3 exhibit similar shape, while haplotype 5 is distinct. Permutations of Mahalanobis distances (500 in PAD) found haplotype 5 to be significantly different

from all other haplotypes, while all other comparisons were not significant, as with the Tukey-Kramer significance test. A Mantel test (9999 permutations, GenAlEx) comparing genetic (Kimura-2) and Mahalanobis distances generated did not detect a significant correlation.

The lack of shape differentiation between haplotype 3 and haplotype 1 specimens (putative *R. robustus* and *R. prolixus*) further supports introgression of haplotype 3. Venezuelan *R. robustus* and *R. prolixus* have been previously separated by wing shape (Villegas *et al.*, 2002, Matias *et al.*, 2001), and, given the greater genetic differences between Amazonian *R. robustus* and *R. prolixus* (Monteiro *et al.*, 2003), shape differences would also be expected to be apparent.

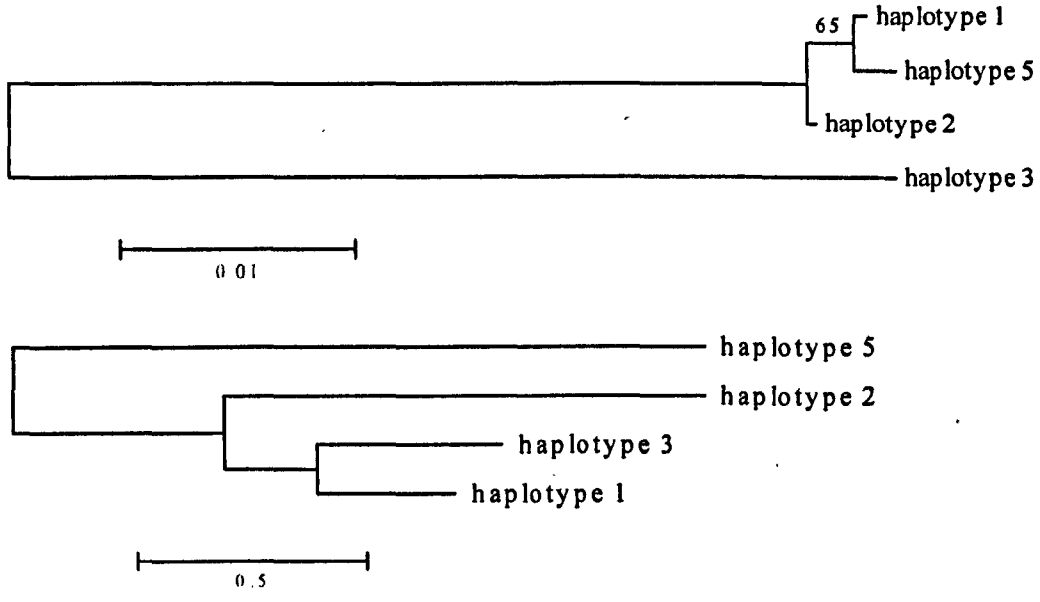

Figure 43. Comparison of *cytb* genetic tree (upper) and shape tree (lower) for 4 haplotype groups. *Cytb* produced by neighbour-joining (Kimura 2-parameter) and shape tree by neighbour-joining (PAD) using Mahalanobis distances from CVA analysis for specimens grouped by *cytb* haplotype.

Twelve of the haplotype 5 specimens (80%) originated from a single domestic population in Loma de Amarillo Trujillo. Domestic specimens from this State were also shown to be relatively distinct from other populations (53% correct reclassification) and

significantly different from 7 other State groups (divided by ecotope) by one-way analysis of variance (see Table 52 on page 242, Table 53 on page 243).

8.1.5.2 *Cytb* and morphometric analysis at population level

Eighteen population groups from 6 States were analysed by both morphometric and *cytb* direct sequencing (233 specimens in total) (see Table 11 on page 129). CVA analysis detected significant differences between groups, ($p<0.0001$) with Wilk's lambda similar to previous analyses (Wilk's lambda 0.05). A significant allometric trend was not detected in the data (data not shown).

Table 55. Reclassification scores after CVA analysis of groups characterised by *cytb* and morphometrics.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total^
1 Barinas h	3	-	2	-	-	-	2	1	2	-	-	-	-	-	1	1	-	2	14 (21)
2 Casa Rena h *	-	4		-	-	-	-	-	1	-	-	1	1	-	-	-	-	-	7 (57)
3 Cojedes h	-	1	2	1	-	-	-	-		-	-	1	-	-	-	1	-	-	6 (33)
4 Cojedes p	-	1	1	6	-	-	1	-	2	-	-	-	-	-	2	-	-	1	14 (43)
5 Guarico p *	-	2	-	-	9	-	-	1	-	-	-	-	1	2	-	-	1	-	16 (56)
6 Lara h *	1	1	-	-	-	12	-	1	-	-	-	2	-	-	-	2	-	-	19 (63)
7 Morichal h *	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	5 (100)
8 Palo Gacho p *	-	-	-	-	-	-	-	5	-	1	-	-	1	3	-	-	-	-	10 (50)
9 Qdra Negra h *	-	1	1	-	-	2	-	-	6	-	-	-	-	-	1	-	-	1	12 (50)
10 San Bartolo h *	-	1	-	-	-	-	-	-	1	8	-	-	-	-	-	1	-	1	12 (67)
11 Terronal h1 03 *	-	-	-	-	1	-	-	-	-	-	6	-	-	-	-	-	-	-	7 (86)
12 Terronal h1 01	-	1	2	-	2	1	-	1	-	1	0	7	1	-	1	-	-	1	18 (39)
13 Terronal h2 01	1	-	-	-	-	-	-	-	-	-	4	2	5	1	-	-	-	1	14 (36)
14 Terronal h2 p01 *	-	-	-	3	1	-	-	3	1	-	-	2	-	18	1	-	-	1	30 (60)
15 Terronal h2 p03 *	0	1	-	-	-	-	-	2	2	-	-	1	-	-	16	-	-	2	24 (67)
16 Trujillo H *	-	-	-	1	-	2	-	-	-	-	-	-	-	-	-	10	-	-	13 (77)
17 Trujillo p *	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	6	-	6 (100)
18 El Mosquito h *	-	-	-	1	-	1	-	1	-	-	-	-	-	-	-	-	-	3	6 (50)
Total	5	13	8	12	13	18	8	15	15	10	10	16	9	24	22	15	7	13	233

*≥50% correct reclassification, ^ %correctly reclassified in parenthesis. h=house p=palm, 01=2001, 03=2003.

The removal of two populations (Laurianito and Merida) and a reduction in population sizes led to an increase in population distinction with a higher number of populations (13) showing 50% or greater correct reclassification, with Palo Gacho, Casa Rena and Qdra Negra now more distinct (see Table 55 above). However, significant population shape overlap still occurred (plot of CV1 and CV2 not shown).

Table 56. Pairwise comparisons of means by Tukey-Kramer test for CV1 (below diagonal) and CV2 for groups characterised by both *cytb* and morphometrics.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 Terronal h2 p01	-	NS	**	NS	**	NS	NS	NS	NS	**	NS	NS	NS	NS	**	NS	**	NS
2 Palo Gacho p	NS	-	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS
3 Terronal h2 p03	NS	NS	-	**	NS	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS
4 Guarico p	NS	NS	NS	-	**	NS	NS	**	**	**	**	**	NS	**	**	**	NS	**
5 Trujillo p	NS	NS	NS	NS	-	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	**
6 Terronal h1 01	**	NS	NS	NS	NS	-	NS	**	NS	**	NS	NS	NS	NS	**	NS	NS	NS
7 Casa Rena h	**	NS	NS	NS	NS	NS	-	NS	NS	**	NS	NS	NS	NS	**	NS	NS	NS
8 Cojedes p	**	**	**	NS	NS	NS	NS	-	NS	NS	NS	NS	NS	NS	NS	NS	**	NS
9 Terronal h2 01	**	**	**	NS	NS	NS	NS	NS	-	NS	NS	NS	NS	NS	NS	NS	**	NS
10 Terronal h1 03	**	NS	**	NS	NS	NS	NS	NS	NS	-	NS	NS	NS	NS	NS	NS	**	NS
11 Cojedes h	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	NS	NS	NS	NS	NS	**	NS
12 Qdra Negra h	**	**	**	NS	NS	NS	NS	NS	NS	NS	NS	-	NS	NS	NS	NS	**	NS
13 El Mosquito h	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	NS	NS	NS	**	NS
14 Barinas h	**	**	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	NS	NS	**	NS
15 Morichal h	**	**	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	NS	**	NS
16 San Bartolo h	**	**	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	**	NS
17 Lara h	**	**	**	**	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	**
18 Trujillo h	**	**	**	**	**	**	**	**	**	NS	NS	**	NS	NS	NS	NS	NS	-

**= significant p<0.05 NS=non-significant, h=house p=palm, 01=2001, 03=2003.

The majority of pairwise comparisons were not significant by both methods, suggesting limited population heterogeneity was detected by both means (see Table 56 above, Table 57 on page 254). However a Mantel test between shape Mahalanobis distances and F_{ST} values showed that these were not significantly correlated (R^2=0.02, p-value=0.12).

The results of morphometrics and *cytb* analysis of populations were compared between population pairs (see Table 58 on page 255). Results indicate that both methods can detect the same pattern of heterogeneity between populations for example;

- Cojedes house and palm.
- Trujillo house and palm.
- Terronal h1 01 and house 2 01.
- Terronal house 1 03 and house 2 01.

Table 57. F_{ST} values (p-values above) from pairwise comparisons of populations characterised by both *cytb* and morphometrics.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 Barinas h	-	**0.0003**	0.074	0.220	0.002	0.007	0.898	0.274	0.700	0.039	0.001	0.014	0.137	0.180	0.028	**<0.0001**	0.001	0.376
2 Casa Rena h	0.58	-	0.001	0.0008	**<0.0001**	**<0.0001**	**<0.0001**	0.035	**<0.0001**	**<0.0001**	**<0.0001**	0.133	0.016	**0.0002**	**<0.0001**	**<0.0001**	0.0004	**0.0003**
3 Cojedes h	0.08	1.00	-	0.556	0.010	1.000	0.459	0.236	0.301	1.000	0.052	0.004	0.116	0.562	1.000	**<0.0001**	**<0.001**	0.467
4 Cojedes p	0 04	0.80	0.00	-	**0.0001**	0.174	1.000	0.049	0.177	0.474	0.004	0 014	0.034	0.774	0.610	**<0.0001**	**<0.001**	0.677
5 Guarico p	0.33	0.70	0.56	0.50	-	**<0.0001**	0.013	0.001	0.005	0.0004	**<0.0001**	**<0.0001**	0.0004	**<0.0001**	**<0.0001**	**<0.0001**	**0.0002**	0.03
6 Lara h	0.24	1.00	0.00	0.11	0.69	-	0 197	**<0.0001**	0.016	1.000	**<0.0001**	**<0.0001**	0.002	0.068	0.500	**<0.0001**	**<0.001**	0.26
7 Morichal h	0.00	0.79	0.04	0.00	0.42	0.30	-	0.251	0.384	**<0.0001**	0.027	0.038	0.610	1.000	1.000	**0.0001**	0.003	0.460
8 Palo Gacho p	0.03	0.50	0.25	0 08	0.40	0.45	0 00	-	0.113	0.032	0.137	0.255	0.682	0.097	0.005	**<0.0001**	0.0006	0.102
9 Qdra Negra h	0.00	0.66	0.07	0.04	0.31	0.23	0.00	0.09	-	0.092	**0.0002**	0.005	0.067	0.174	0.024	**0.0001**	**0.0002**	0.686
10 San Bartolo h	0.17	1.00	0.00	0.06	0.63	0.00	0.19	0.37	0.16	-	**<0.0001**	0.006	0.0131	0.164	0.097	**<0.0001**	**<0.001**	0.371
11 Terronal h1 03	0.42	0.00	0.82	0.64	0.58	0.91	0.53	0.26	0.50	0.88	-	0.363	0.162	0.002	0.0004	**<0.0001**	0 001	0 063
12 Terronal h1 01	0.21	0.24	0.46	0.33	0.45	0.60	0.18	0.01	0.29	0.54	0.04	-	0.312	0.004	**0.0003**	**<0.0001**	0 001	0.019
13 Terronal h2 01	0.06	0.44	0.27	0.12	0.40	0.43	0.00	0.00	0.12	0.36	0.23	0.00	-	0.104	0.017	**<0.0001**	0.002	0.122
14 Terronal h2 p01	0.04	0.69	0.02	0.00	0.46	0.11	0.00	0.05	0.04	0.07	0.56	0.29	0.09	-	0.220	**<0.0001**	**<0.0001**	0.460
15 Terronal h2 p03	0.12	0 87	0.00	0.00	0.60	0.03	0.00	0.24	0.11	0.00	0.76	0.46	0.26	0.00	-	**<0.0001**	**<0.0001**	0.120
16 Trujillo h	0 58	0.90	0.89	0.79	0 68	0.94	0.77	0.68	0.58	0.92	0 80	0.65	0.65	0.72	0.84	-	**<0.0001**	**<0.0001**
17 Trujillo p	0.33	0.66	0.63	0.57	0.44	0.81	0.42	0.38	0 37	0.74	0.50	0.42	0.39	0.55	0.69	0.63	-	0.001
18 El Mosquito h	0.01	0 86	0 00	0 00	0 38	0.16	0.00	0.14	0 00	0 08	0 67	0.37	0.17	0 00	0 00	0.80	0 50	-

Values in bold significant after Bonferroni correction k=153, p1=0.05/153, p≤0.0003. h=house, p=palm, 01=2001, 03=2003.

However, this is not always the case and some conflicting patterns were detected; Terronal h2 p03 and p01 were significantly different by shape analysis (CV2) but not by *cytb* analysis. There were more differing outcomes between both methods when the introgressed haplotype 3 was taken into account because this is responsible for significant differences by *cytb* analysis among populations in Terronal and other populations in which it is present (see Table 11 on page 129). When haplotype 3 is interchanged for haplotype 1, heterogeneity among populations in Terronal is non-significant. Population differences in Terronal by morphometrics relate to house/palm comparisons, with the exception of Terronal h1 01 and h 03 and Terronal palm 01 and palm 03.

Table 58. Results for cytochrome b and morphometric analysis of palm and house population pairs

Compared populations	Cytb results	Cytb results (h3=h1)	Morphometric
Cojedes house\palm	NS	NS	NS
Terronal house 2 01\house 2 palm 01	NS	NS	** (CV1)
Terronal house 2 01\house 2 palm 03	NS	NS	** (CV1)
Terronal house 1 01\house 1 03	NS	NS	** (CV2)
Terronal house 1 01\ house 2 palm 01	NS	NS	** (CV1)
Terronal house 1 01\ house 2 palm 03	**	NS	** (CV2)
Terronal house 1 03\ house 2 palm 01	NS	NS	**
Terronal house 1 03\ house 2 palm 03	NS	NS	** (CV1)
Terronal house 1 01\ house 2 01	NS	NS	NS
Terronal house 1 03\ house 2 01	NS	NS	NS
Terronal house 2 p01\ house 2 p03	NS	NS	** (CV2)
Trujillo house \palm	**	-	**

NS=not significantly different, **=significantly different (see Table 56 on page 253, Table 57 on page 254). In parenthesis significant CV variable if shape is significant by one CV only.

Discrete populations detected by each method can also be compared for congruence. From CVA analysis (see Table 55 on page 252, Table 56 on page 253) populations Terronal h2 p01 and p03, Palo Gacho palm, Guarico palm, Trujillo house and Lara house were the most distinct, differing significantly from a number of populations by either CV1 or CV2 shape variables. Trujillo palm, while distinct from reclassification data, did not show significant shape difference by Tukey-Kramer, however in earlier analysis with greater numbers shape differences were more pronounced (see Figure 38 on page 244). From pairwise F_{ST} comparisons Trujillo house and palm, Guarico and

Lara were also distinct, in addition to Casa Rena and Terronal h1 03. However, when haplotype 3 is taken into account, only Trujillo house, palm and Guarico remain distinct (see Figure 46 on page 258).

The relationship between all groups by both methods is visualised in tree format (see Figure 44 below, Figure 45 on page 257). In both trees Trujillo palm is distantly related, with house specimens also isolated in *cytb* analysis. From the different clustering patterns in these trees it seems that ecotope has an important influence on shape variation, with clusters of domestic and silvatic populations visible. Some shape overlap occurs between ecotopes for example between with Cojedes house and palm and Terronal house 2 01. The clear division does not exist for sequence data, with homogeneity detected between ecotopes. This shape clustering due to ecotope, together with introgression of *cytb* data, would make comparisons between both analysis methods difficult.

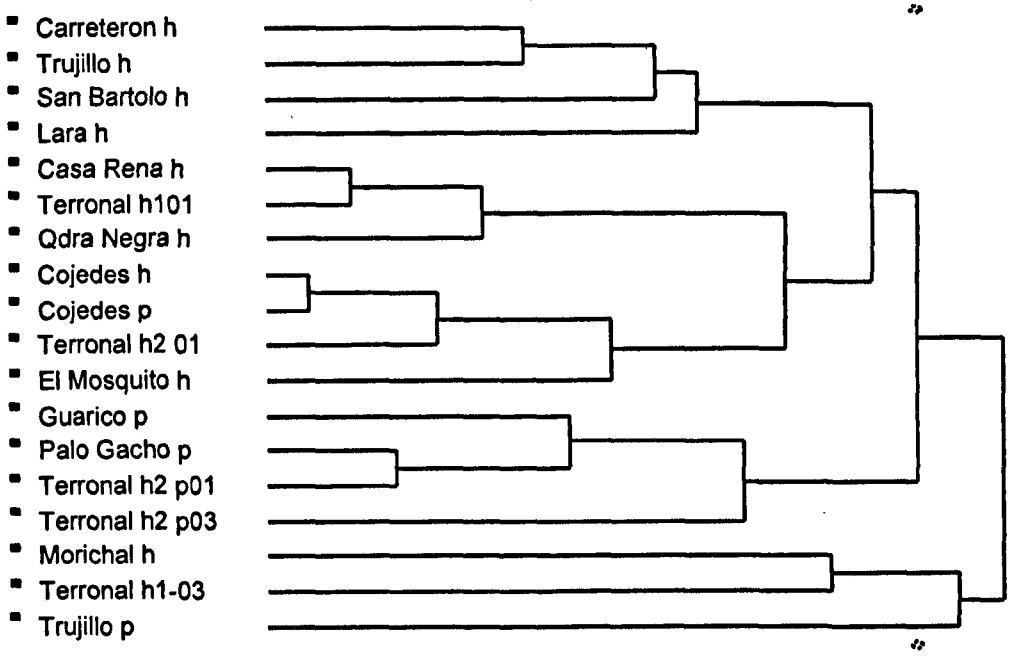

Figure 44. UPGMA tree of Mahalanobis distances after CVA of populations characterised by *cytb*. h=house, p=palm.

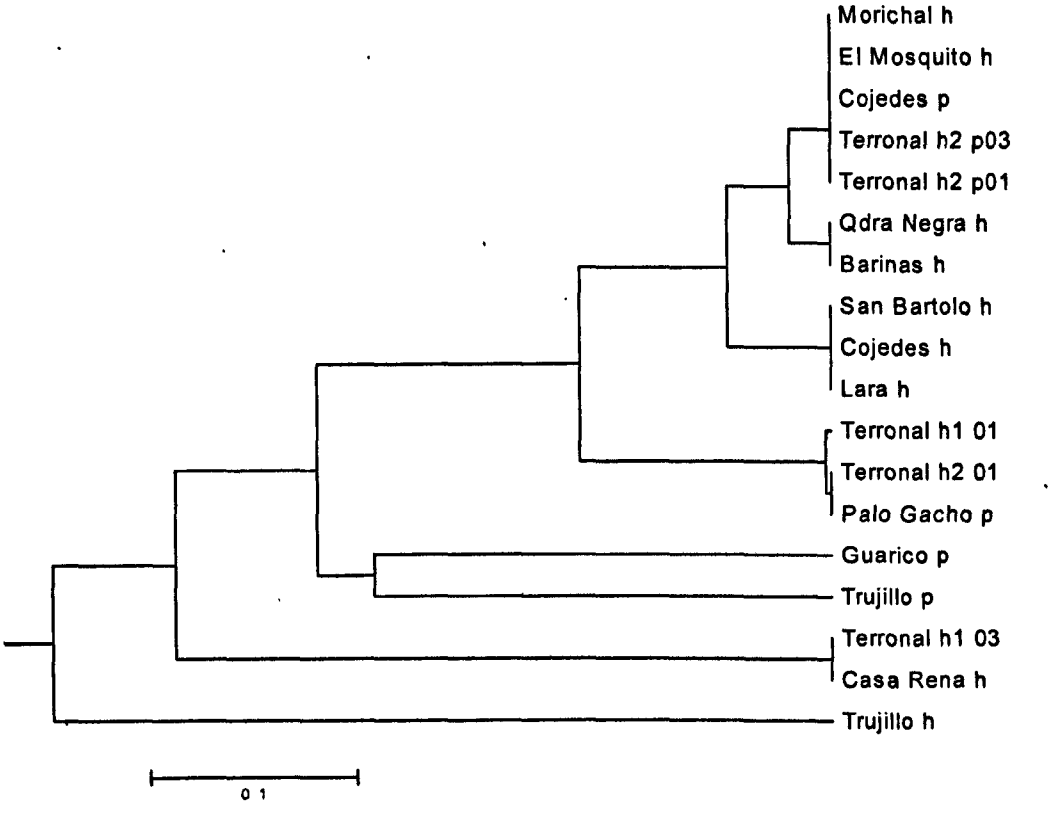

Figure 45. UPGMA tree of F_{ST} values from sequence data of populations used in morphometric analysis. h=house, p=palm. 01=2001, 03=2003.

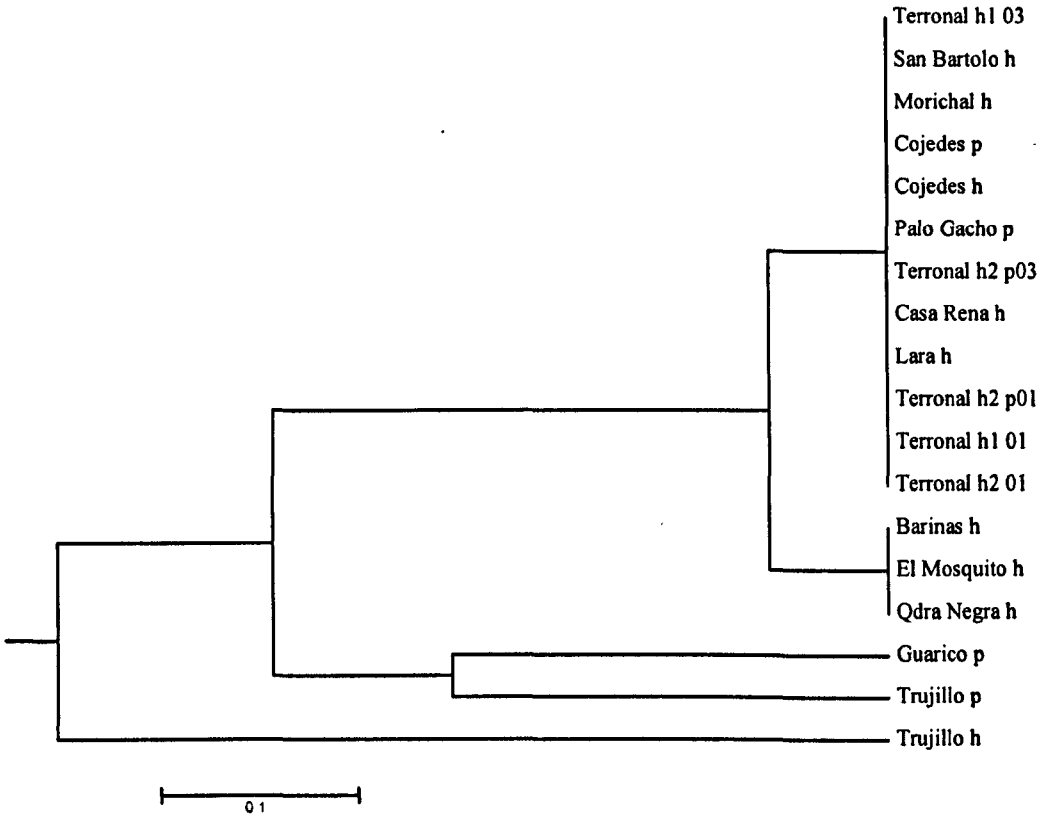

Figure 46. UPGMA tree of F_{ST} values for sequence data when introgression is taken into account. h=house, p=palm. 01=2001, 03=2003.

8.1.5.3 Shape and microsatellite variation

A total of 190 specimens were analysed by both microsatellite and geometric morphometric analysis originating from 4 States and all ecotopes (see Table 12 on page 130). CVA analysis was significant (p-value<0.0001) and Wilk's lambda value for analysis was similar to previous analyses (Wilk's lambda=0.06). A significant allometric trend was not detected in the data (data not shown).

A reduction in population size and number of populations compared resulted in greater distinction among populations, with 10 of 13 populations showing 50% or greater correct reclassification (see Table 59 on page 259). Significant shape overlap was still

detected among groups, (plot CV1 and CV2 not shown) (see Table 61 on page 260). Population heterogeneity detected by microsatellites was high, with 48 of 78 pairwise comparisons significantly different. Mantel tests between shape Mahalanobis distances and F_{ST} were not significantly correlated (R^2=0.003 p=0.6).

Table 59. Reclassification of specimens in shape discriminate space from CVA analysis of specimens analysed by microsatellites and morphometrics.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
1 Cojedes h *	4	1	-	-	-	-	-	-	-	-	-	1	1	7 (57)
2 Cojedes p	3	6	-	1	-	-	1	-	-	-	2	1	-	14 (42)
3 Lara d *	-	-	10	-	-	-	-	1	-	-	-	2	-	13 (77)
4 Laurianito pd	1	-	-	3	2	2	3	1	-	-	2	2	-	16 (19)
5 Palo Gacho p *		1	-	1	7	-	1	-	-	3	-	-	-	13 (54)
6 San Bartolo h *	-	-	-	4	1	7	-	-	-	-	-	-	-	12 (58)
7 Terronal h2 01 *	-	-	-	-	2	-	6	1	3	-	-	-	-	12 (50)
8 Terronal h1 02	1	-	2	-	1	-	3	10	-	2	1	-	3	23 (44)
9 Terronal h1 03 *	-	-	-	-	-	-	-	1	4	-	1	1	-	7 (57)
10 Terronal h2 p01 *	-	1	-	-	2	-	-	2	-	18	1	-	-	24 (75)
11 Terronal h2 p03 *	2	-	-	2	1	-	-	-	-	1	17	-	2	25 (68)
12 Trujillo h *	-	1	2	-	-	-	-	-	2	-	-	11	-	16 (69)
13 Casa Rena h *	-	-	-	-	-	1	-	1	-	-	-	-	6	8 (75)
Total	11	10	14	11	16	10	14	17	9	24	24	18	12	190

*≥50% correct reclassification, ^ % correctly reclassified in parenthesis. h=house, p=palm, pd=peridomestic, 01=2001, 03=2003.

Table 60. F_{ST} values generated in Arlequin V2.0 for specimen groups characterized by both morphometrics and microsatellites.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Cojedes h		**<0.0001**	0.003	0.034	0.002	0.017	0.096	**<0.0001**	0.001	**0.0003**	0.217	**<0.0001**	0.058
2 Cojedes p	0.20		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.045	**0.0002**	**<0.0001**	0.002	0.001	**<0.0001**	**<0.0001**
3 Lara h	0.08	0.21		0.010	**<0.0001**	**0.0002**	**0.0003**	**<0.0001**	**0.0001**	**0.0001**	0.003	**<0.0001**	**0.0006**
4 Laurianito pd	0 07	0.32	0.09		**0.0004**	**<0.0001**	**0.0001**	**<0.0001**	**<0.0001**	0.0008	**<0.0001**	**<0.0001**	**<0.0001**
5 Palo Gacho h	0.10	0.29	0.13	0.15		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
6 San Bartolo h	0.06	0.15	0.09	0.16	0 19		0.059	0.003	0.262	0.025	0.165	**<0.0001**	0.014
7 Terronal h2 01	0.04	0.05	0.11	0.21	0.20	0.04		**<0.0001**	0 021	0.005	0.781	**<0.0001**	0.004
8 Terronal h1 01	0.17	0.15	0.17	0.26	0.26	0.08	0.12		0.033	**<0.0001**	**0.0001**	**<0.0001**	**<0.0001**
9 Terronal h1 03	0.10	0.13	0.11	0.20	0.19	0.01	0.05	0.04		0.016	0.184	**<0.0001**	0.015
10 Terronal h2 p01	0.22	0.12	0.16	0.28	0.32	0.06	0.11	0.13	0.07		**0.0006**	0.037	**<0.0001**
11 Terronal h2 p03	0.01	0.11	0.06	0.16	0.12	0.02	-0.01	0.10	0.02	0.12		**<0.0001**	0.159
12 Trujillo h	0.29	0.26	0.27	0.35	0.41	0.12	0.20	0.29	0.15	0.07	0.22		**<0.0001**
13 Casa Rena h	0 03	0.18	0 07	0.13	0.13	0.03	0 06	0.11	0 03	0 13	0 01	0 22	

Figures in bold p-values significantly different after Bonferroni correction k=78, p1=0.05/78 p≤0.0006 h=house, p=palm, pd=peridomestic, 01=2001, 03=2003.

Table 61. Pairwise comparisons of means by Tukey-Kramer test for CV1 (below diagonal) and CV2 for groups analysed by both microsatellites and morphometrics.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Terronal h2 p01		**	NS	NS	NS	**	NS	NS	NS	NS	NS	**	NS
2 Terronal h2 p03	**		**	**	**	**	**	**	NS	NS	**	NS	**
3 Palo Gacho h	**	NS		NS	NS	NS	NS	NS	NS	NS	**	NS	NS
4 Casa Rena h	**	NS	NS		NS	NS	NS	NS	NS	NS	**	NS	NS
5 Terronal h1-02	**	NS	NS	NS		NS	NS	NS	NS	NS	**	NS	NS
6 Laurianito pd	**	**	NS	NS	NS		NS	NS	NS	NS	**	NS	NS
7 Terronal h2 01	**	**	NS	NS	NS	NS		NS	NS	NS	**	NS	NS
8 Cojedes p	**	**	NS	NS	NS	NS	NS		NS	NS	**	NS	NS
9 Cojedes h	**	NS	NS	NS	NS	NS	NS	NS		NS	**	NS	NS
10 Terronal h1 03	**	**	NS	NS	NS	NS	NS	NS	NS		**	NS	NS
11 Lara h	**	**	**	NS	**	NS	NS	NS	NS	NS		**	**
12 San Bartolo h	**	**	**	NS	**	NS	NS	NS	NS	NS	NS		NS
13 Trujillo h	**	**	**	**	**	**	**	**	**	NS	NS	NS	

**= significant p<0.05, NS=non-significant, h=house, p=palm, pd=peridomestic, 01=2001, 03=2003.

While results differ considerably, population heterogeneity detected by both methods for paired populations was compared at a fine scale within Cojedes and across the locality Terronal (see Table 62 below). Population homogeneity was detected by both methods between domestic ecotopes in Terronal, house 1 03 and house 1 01, house 2 01. Significant heterogeneity was also detected between palm populations in Terronal by both methods, as was also the case between house 1 01 and palm 01, palm 03.

Table 62. Results for microsatellite and morphometric analysis of palm and house population pairs

Compared populations	Morphometric results	Microsatellite results
Cojedes house\palm	NS	**
Terronal house 2 01\house 2 palm 01	** (CV1)	NS
Terronal house 2 01\house 2 palm 03	**	NS
Terronal house 1 01\ house 2 palm 01	** (CV1)	**
Terronal house 1 01\ house 2 palm 03	** (CV2)	**
Terronal house 1 03\ house 2 palm 01	** (CV1)	NS
Terronal house 1 03\ house 2 palm 03	** (CV1)	NS
Terronal house 1 01\ house 2 01	NS	**
Terronal house 1 03\ house 2 01	NS	NS
Terronal house 1 01\house 1 03	NS	NS
Terronal house 2 palm 01\ house 2 palm 03	**	**

Morphometric data NS=not significantly different, **=significantly different (p=0.05) (see Table 61 above) Microsatellite data see Table 60 on page 259. In parenthesis significant CV variable if shape is significant by one CV only.

However microsatellites and morphometrics did not always present the same picture. In Cojedes shape homogeneity was detected between the house and palm populations by morphometrics but population heterogeneity was detected by microsatellite analysis. However, morphometric data indicated significant shape difference between Terronal h2 01 and palm 01 and palm 03, while microsatellites did not detect population differences.

While the majority of F_{ST} pairwise values were high, Palo Gacho p, San Bartolo, Trujillo h and Lara appeared the most distinct (see Table 60 on page 259). In shape data Trujillo h and Lara showed significant shape differences by CVA analysis, together with Terronal h2 p01, p03. San Bartolo showed some significant differences, although more pronounced in earlier analysis, as did Palo Gacho (see Table 61 on page 260, Table 53 on page 243). Relationship between populations by both methods is visualised in tree format (see Figure 47 below, Figure 48 on page 262). Shape data clustering is again influenced by ecotope. The isolation of Lara San Bartolo and Trujillo is visible in both trees.

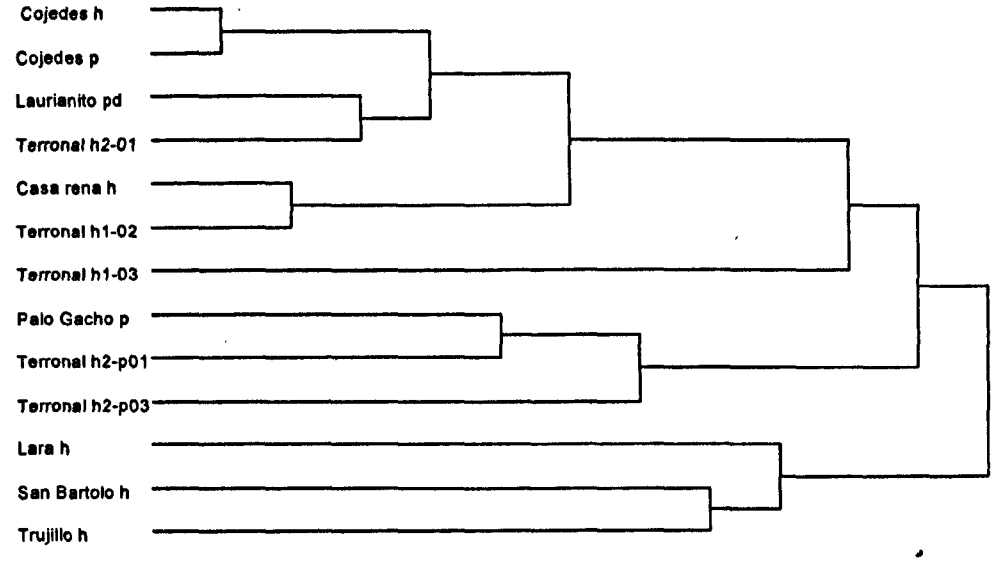

Figure 47. UPMGA tree of Mahalanobis distances after CVA of specimens analysed by morphometrics and microsatellites.
h=house, p= palm, pd=peridomestic, 01=2001, 03=2003.

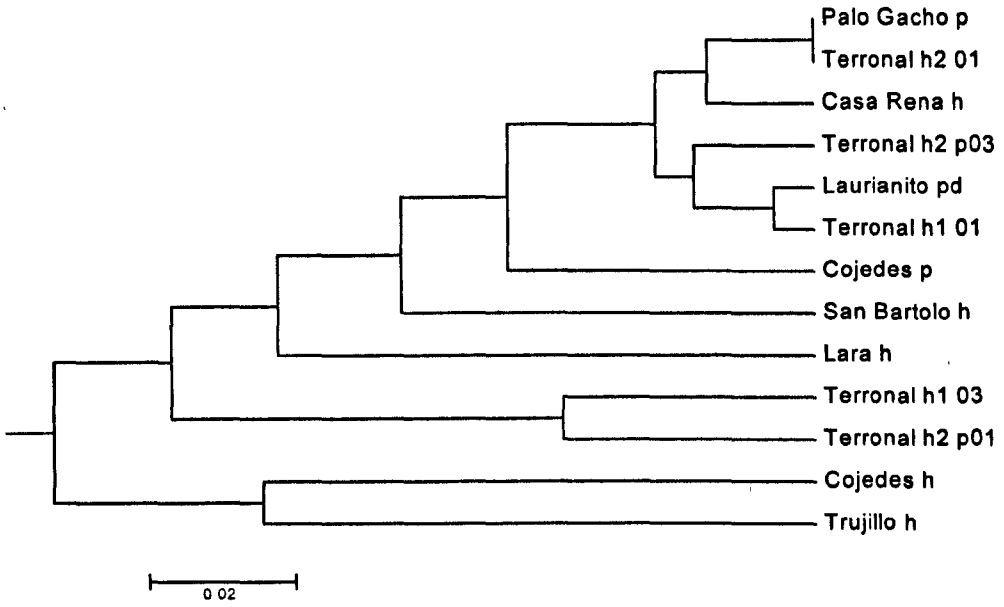

Figure 48. UPMGA tree of F_{ST} values of specimens analysed by morphometrics and microsatellites. h=house, p=palm, pd=peridomestic, 01=2001, 03=2003.

8.2 Discussion

"Morphometry is the measurement and analysis of form, and the main premise of morphometrics is that a statistical analysis of genetic variability expressed by morphological characters is a measure of population differentiation and ultimately speciation" (Patterson 2002). Geometric morphometrics was used in this study to analyse the relationship between domestic and silvatic populations of *R. prolixus* specimens. Comparisons with genetic characterisation (*cytb* and microsatellite) of subsets of the same specimens were also made.

Morphometric analysis has been used previously in the analysis of Triatominae, as discussed in section 1.5.1 on page 45. Traditional morphometrics was used to distinguish domestic populations of *T. infestans* in Bolivia from neighbouring silvatic populations, including specimens previously found identical by isoenzyme analysis (Dujardin *et al.*, 1997a 1997b) and geographical populations of *T. dimidiata* (Bustamante *et al.*, 2004). Silvatic and domestic specimens of *P. rufotuberculatus* in Bolivia were distinguished by traditional morphometrics (Dujardin *et al.*, 1998a). Populations of *T. infestans* have been analysed using geometric morphometrics (Schachter-Broide *et al.*, 2004) and populations showed shape differentiation in comparisons between single collection sites and across villages, agreeing with isoenzyme analysis that a single house can represent a population deme (Breniere *et al.*, 1998). The results from morphometric analysis of wild caught silvatic and domestic populations of *Rhodnius* in Venezuela provided some interesting data, and shed light on the applicability of this tool for the analysis of such populations. Geometric morphometrics was used to compare populations at various hierarchical levels; within a locality, including adjacent house and palm populations (Terronal), within Portuguesa State and across six States.

At a fine scale between house and palm populations, population differentiation was visible, although subtle and with limited overlap, for example within Terronal, with house populations more similar in shape. Adjacent domestic and silvatic populations,

Terronal house 2 and palms 01 and 03, were also different, with palm 01 showing limited shape similarity. However with increasing numbers of populations analysed, over greater geographical areas e.g. State level, the differentiation became increasingly difficult, with significant population shape overlap detected. In general these results showed, that while some distinct populations were detected, geometric morphometrics has reduced utility in the comparison of populations over greater geographical areas.

While some shape similarity was detected between domestic and silvatic ecotopes, e.g. Palo Gacho and Casa Rena, El Mosquito, Qdra Negra, the overall shape pattern as exemplified in the Mahalanobis trees indicated that some environmental influence or selection might be affecting the wing shape detected, with clusters of palms noticeable (see Figure 39 on page 247). This was also shown in the group analysis of all domestic and silvatic populations (see Figure 40 on page 248). Wing shapes may differ between ecotopes due to developmental differences linked to a differing environment such as temperature, relative humidity or environmental stress due to differences in feeding cycles or egg development. However shape differences could also be liked to genetic selection for example palm bugs may have differing wing shape linked to a greater need for dispersal, perhaps due to longer periods of starvation, which might be less common in domestic populations. These results indicate that the applicability of morphometrics alone in the comparison of wild caught silvatic and domestic populations of *Rhodnius* may be limited, as it may not be a true reflection of underling genetic similarity.

Morphometrics and *cytb* results were mostly incongruent but with introgression, the influence of selection\environment on shape and the independent evolution of the mitochondrial genome from the nuclear genome this incongruence may be expected. Morphometrics proved, however, very useful in the confirmation of introgression between haplotype 3 and haplotype 1. If haplotype 3 was truly *R. robustus*, shape differentiation would be expected between these different species, as detected between *R. prolix*us and Venezuelan *R. robustus* (Villages *et al.*, 2002), and as detected in this study with the separate clustering of Merida and Trujillo silvatic populations, thought to be *R. robustus*. While convergence through shared ecotope could be suggested, this was

ruled out by the identical D2 haplotypes shared by both Venezuelan *R. robustus* and *R. prolixus* (see section 6.1.5.4 on page 162), and additionally in this study Trujillo silvatic specimens, although primarily from established laboratory colonies (similar ecotope to the domestic environment), were still distinguishable from domestic specimens.

Results from morphometrics and microsatellites, while showing some incongruence appeared more compatible. An interesting pattern emerged in the data that indicates that combined use of both methodologies would be beneficial. If selection is acting on wing shape according to ecotope, shape differences may develop faster than neutral drift in microsatellites. From Table 62 on page 260 it can be proposed that:

1. As Terronal h1 01 and h2 palm 01, palm 03 are significantly different by both methods, which implies that these populations may have always been isolated or isolated for a sufficient period for 'domestic' shape selection and microsatellite drift to concur.

2. In Terronal h2 01 and palm 01, palm 03 are not significantly different by microsatellite analysis, however selection for 'domestic shape' may have occurred at a faster rate and from morphometric analysis these populations are differentiated. If morphometrics alone had been used to compare these populations, a conclusion of a lack of exchange between populations from these differing ecotopes would have been concluded.

3. Adjacent domestic and silvatic populations in Cojedes were found significantly different by microsatellite analysis but similar in shape. These domestic and silvatic populations would be similar in shape if this was a recent invasion from another palm, due to a shared common ecotope before selection had time to act, but different genetically as each palm population was isolated. This domestic infestation was limited to a single bed, few adults were encountered and the population was primarily early stage nymphs, suggesting that it may have been a recent invasion. As previously discussed, (see page 225), several palms

surrounded this house, and each was equally as likely to establish this domestic population.

4. These results may also suggest that where silvatic and domestic populations show similar shape, similar microclimates may occur in houses and palms in that area, and this may be reflected in shape.

These results indicate that the use of morphometrics alone could be misleading for population analysis, but may be useful for distinguishing recent invasion from silvatic ecotopes from recrudescence of original domestic populations after control efforts, if populations were collected and analysed before 'ecotope shape' selection had taken place. Recrudescent populations would have a 'domestic shape' while recent invading silvatic populations would have a 'palm shape'. The comparison, by morphometrics, of domestic populations of *T. infestans* before and after spraying and adjacent silvatic specimens showed that domestic populations had survived the control effort, indicated by the same shape detected across domestic populations (Dujardin *et al.*, 1997b).

Results from this study indicate that morphometrics and microsatellites, when used in combination, could provide an interesting complementary analysis. Microsatellite technology is costly and not widely available, as opposed to the application of morphometrics, which is a cheap system to establish, with costs only related to the initial price of a computer and camera and no additional reagents or running costs required. Morphometrics for the population analysis of specimens from differing ecotopes, apart from detecting recent invasion\recrudescence would benefit from the establishment of separate laboratory colonies of initial wild caught specimens and raised to F1 generations under the same conditions, could then be compared. Thus results would be based on genetic differences, free from environmental influences.

8.2.1 Conclusions

Important conclusions from this analysis are:

- Importantly morphometrics detected similar shape between populations across States, indicating that populations are not isolated.
- Importantly convergence in shape due to a common ecotope was detected, indicating that morphometrics might be of limited use for general population comparisons.
- Analysis indicated that this limitation might be overcome by using morphometrics in conjunction with microsatellite markers.
- Analysis indicated that morphometrics would be applicable to post control reinvasion\recrudescent studies.

9 Conclusions

The national program for control of Chagas disease in Venezuela has been established since 1966. Although disease incidence was initially greatly reduced, recent data suggests transmission may in fact now be increasing (Feliciangeli *et al.*, 2003). A possible explanation for this continued transmission is the abundant silvatic foci of *R. prolixus* in palms, identified in extensive silvatic investigations in endemic areas of Venezuela in the 1970s (Gamboa 1970, Tonn *et al.*, 1976a, Carcavallo *et al.*, 1978). These populations may continually invade domestic ecotopes following control. It is essential to determine the degree of interaction between specimens from silvatic and domestic ecotopes, as with limited resources available for control, strategies must be effective.

This project aimed to determine the relationship between these populations and also to resolve the debate regarding the identity of silvatic populations of *Rhodnius*. To address the project objectives we employed genetic and geometric morphometric methods. Valuable results were produced by each method, giving different degrees of resolution and complementary insight. A broadly similar picture of shared *cytb* haplotypes and population homogeneity detected from microsatellite analysis indicated that silvatic and domestic populations are not isolated, and that geneflow is occurring. Morphometric data were more complex to interpret, partially due to the occurrence of shape convergence by ecotope.

From the results of this study any doubt of the existence of silvatic *R. prolixus* can be unequivocally laid to rest. Sequence data identified 18 haplotypes, 14 of which were variant forms of *R. prolixus* and 11 of which were detected in palms. Importantly, 6 of the shared *R. prolixus* haplotypes, including the introgressed haplotype 3, were detected in both silvatic and domestic ecotopes, indicating that populations in these ecotopes are not isolated. Furthermore, 4 of these haplotypes were also detected in nymphs, confirming that silvatic haplotypes occur within the domestic colonies. Silvatic *R. prolixus* was identified in all States sampled, with the exception of Trujillo and Lara. A

lack of significant heterogeneity between populations, adjacent and within localities, from palm, peridomestic and domestic ecotopes in both Portuguesa and Barinas State, also confirmed that populations from differing ecotopes are not isolated. Our study also detected for the first time an introgression event between *R. robustus* and *R. prolixus*. True Venezuelan *R. robustus* was identified only in Trujillo State, where it was not found to colonise houses.

Population heterogeneity was investigated further using polymorphic microsatellite markers. Homogeneity was detected between populations, adjacent and within localities, from domestic, silvatic and peridomestic ecotopes in both Portuguesa and Barinas. Within States, population heterogeneity was highest in Portuguesa, and may have been related to the fact that samples came from localities in mountainous areas. This was in contrast to Barinas where detected heterogeneity was related to a single population from the edge of the distribution of the sampled populations. In Barinas populations came from the flat lands, the Llanos, which could allow for easier mixing of populations. A domestic population from the Andean region also proved to be significantly different from all populations, suggesting that the Andes mountain range may be a significant barrier to geneflow. In comparison to *cytb*, a higher degree of population heterogeneity was detected by microsatellite analysis. However, geneflow between populations was confirmed by both methods, and both methods proved sensitive to drift. The Chagas disease control programmes in Barinas and Portuguesa also differ. While both States officially have a programme for Chagas control, in Barinas house spraying is focused in malaria endemic areas (M Sanchez-Martin pers. communication). However, no difference seems to exist between time required for reinfestation to occur or reinfestation rates following spraying in both States, but with fewer villages reinspected in Barinas State following spraying, true variation in effectiveness of the programmes is difficult to determine (M Sanchez-Martin pers. communication). In Portuguesa 20% of villages identified as positive are sprayed within a year, with only 5% in Barinas (M Sanchez-Martin pers. communication). Higher spraying coverage in Portuguesa may result in a greater degree of population isolation.

Morphometrics detected shape similarity within and between populations across States. However, some convergence in shape due to common ecotope was detected. Analysis indicated that morphometrics, while applicable to detecting postcontrol reinvasion\recrudescent, might be limited for general population comparisons unless used in conjunction with microsatellite markers or if populations are previously bred under identical laboratory conditions to investigate the environmental component of shape variation.

Movement of bugs between ecotopes probably occurs both actively and passively. In triatomine bugs, including *R. prolixus,* flight is often associated with starvation (Schofield *et al.*, 1992, Lehane *et al.*, 1992) and is thought to be linked to population density (Schofield 1994). Studies have shown correlations between starvation and flight initiation, and distance travelled can be affected by ambient temperature and wind speeds (Lehane *et al.*, 1992, Schofield *et al.*, 1992). Gomez Nunez (1969) in a mark release recapture study, using isotope markers, detected movement from palms to houses but not vice versa. Results indicated that house infestation could occur from heavily infested palms close to houses, but due to small distances travelled and high predatory pressure, it was concluded that infestation was primarily passive following the use of infested palm leaves in roof construction. Risk factor analysis study by Sanchez-Martin *et al.*, (2005) also detected an association between palm roofs and infestation. Restriction or elimination of the use of palm roofs on dwellings must therefore be an important element of control strategies, although it is important that a substitute roofing material is readily available to the inhabitants. Movement may also be passive in relation to human dispersal, with eggs and nymphal stages in clothing and or boxes. *R. prolixus* eggs are commonly glued to clothes (pers. observation). *T. infestans* is proposed to have been spread passively over much of its range (Schofield 1994). Birds and small mammals may also play a role with eggs found glued to the plumage of birds (Schofield 1994).

From these data it is clear that silvatic populations of *R. prolixus* in Venezuela represent a unmistakable threat to successful control of Chagas disease, as also indicated by risk

factor analysis data (Sanchez-Martin *et al.*, 2005) and long suspected since populations of *R. prolixus* were first identified in palm trees (Gamboa 1961).

Results indicate that the current programme of house spraying in Venezuela will never achieve the levels of success seen in the Southern cone, where *T. infestans* has been eradicated over large areas. At least in some endemic regions the control programme will have to be modified to deal with this continual threat of reinvasion in order to maintain house infestations/colonisations at a low level.

Modification may include more frequent spraying of houses, combined with vigilance for reinfestations as an integral part of the control programme, combined with alternative control methods such as insecticide treated curtains, which have been shown to be effective in Merida against invading silvatic adults (Herber & Kroeger 2003) or bednets (Kroeger *et al.*, 2003). Clearance of palms or treatment of palms near houses with insecticide may also be an option. Further trials of agricultural spraying procedures for control of *Rhodnius* populations in palms would be valuable. Increased levels of housing improvements, although expensive, seems increasingly important for long term control by creating a domestic environment unsuitable to colonisation by silvatic bugs.

This project has therefore made a considerable contribution to understanding of *Rhodnius* populations in the context of disease epidemiology and vector control in Venezuela. Additional genetic studies would clarify further the taxonomy of *Rhodnius* species and experimental studies may give insight into morphometric plasticity. Parallel comparative studies of silvatic and domestic strains of *T. cruzi* are in progress, which will also give information as to ecotope interaction from the similarity of strains circulating. An important follow up to this project may be to define population interaction more extensively in the Andean region, for example in regions of Colombia, where silvatic and domestic *Rhodnius* populations also occur, to determine if reinvasion is also a problem. This would allow more detailed prioritisation of control interventions and tailoring of control to regional circumstances. Modified control strategies to counteract the threat of reinvasion could be tested further, such as widespread provision

of alternative, acceptable low cost roofing, the removal or treatment of palms and improved spraying and surveillance, in order to reduce the burden of Chagas disease in rural areas.

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11 Glossary of Terms

Autapomorphic: a derived trait, i.e. an altered ancestral state, unique to a taxon. In this case a unique nucleotide.

Bootstrap method: is used to assess the reliability of a dataset or statistical estimate, in phylogenetics, to assess clusters. Felsenstein's bootstrap for phylogenetic trees creates pseudoreplicates of the original dataset by random sampling with replacement. In each replicate a new data set is formed and analysed. The bootstrap value produced for each clade in the final tree is based on the frequency with which a given clade is found in the pseudoreplicates.

$F_{ST} = 1/(4Nm+1)$ can be used to estimate gene flow; N is the effective population size, m is the effective proportion of immigrants, and F_{ST} is the fixation index.

Gamma distribution: this parameter allows for variation in the rate of nucleotide substitution changes from site to site e.g. depending on the gene target certain areas of gene maybe more likely to undergo mutation than other sites.

Homoplasy: alleles the same size, but not derived from a common allele ancestor i.e. they are identical in state (IIS) but not identical by decent (IBD).

Heuristic method: Finding the optimal tree in phylogenetics often requires extensive analysis, to overcome this heuristics is often used. This is a method of searching data for the most parsimonious tree, thereby increasing the speed of analysis, but possibly giving less than accurate results.

Indels: insertion\deletion; the alignment of multiple sequences often needs the introduction of gaps as some sequences may have insertions or deletions, these alignment gaps are called indels.

Jukes-Cantor: when calculating the distance between sequences this model assumes equal frequencies each of the 4 bases, A, T, C, and G, and an equal rate of nucleotide substitution.

Kimura 2-parameter: when calculating the distance between sequences this model assumes equal frequencies each of the 4 bases, A, T, C, and G, and differential transitional and transversional substitutions rates (a transitional\transversional bias).

Maximum likelihood: is a discrete method of tree building, which uses an evolutionary model set by the user to score and rank trees, in terms of their likelihood. It analyses data in its raw form, not derived distances and as such is often thought to be more powerful. The correct evolutionary tree is that with the highest likelihood score under that model.

Maximum parsimony\ Parsimony: aims to recreate the ancestral sequences of the data on the basis of a minimum number of evolutionary changes, giving the optimal tree or network.

Neighbour-joining: is a tree building method based on a clustering algorithm and the minimum evolutionary principle. Pairwise genetic distances are calculated between the sequences according to an evolutionary model e.g. the Kimura-2 parameter. A phylogenetic tree is then constructed by linking the least distant pairs of sequences, the tree with the least total branch length is preferred at each step. The tree produced is unrooted.

Parsimony-informative: a site is parsimony-informative if it exhibits at least two variant nucleotides or amino acids, with at least two of the variants occurring twice or more.

Purines: the nucleotides Adenine (A) and Guanine (G) are purines.

Pyrimidines: the nucleotides Cytosine (C) and Thymine (T) are pyrimidines.

Rev rates mutation model: when calculating the distance between sequences this model allows for unequal base frequencies (A, T, G, C), and allows for different rates of base substitution e.g. in AT rich insect mitochondria A and T are more frequent and more likely to undergo base substitution. This model also allows for back mutations.

Synonymous change: a nucleotide substitution that does not change the encoded amino acid (antonym nonsynonymous).

Tajima-Nei: when calculating the distance between sequences this model allows variation in the frequency of the four nucleotides bases, A, T, C, and G, but the rate of nucleotide substitution is equal for all.

Tamura-Nei: when calculating the distance between sequences this model allows for variation in the frequency of the four nucleotides bases, A, T, C, and G, and differential transitional and transversional substitutions rates (a transitional\transversional bias).

Transition: a transition is the substitution of purine by a purine, or a pyrimidine by a pyrimidine at a nucleotide site.

Transversion: a transversion is a change from a purine to a pyrimidine at a nucleotide site, or vice versa.

Transition/Transversion Ratio: the ratio of the number of transitions to the number of transversions in a data set. In sequence data transitions occur more frequently than transversions, and can reach saturation quickly. As the rate of transitional changes differs from the rate of transversions this could bias distance analysis.

UPGMA: Unweighted Pair Group Method with Arithmetic Mean is a clustering method for creating phylogenetic trees that assumes a constant evolution rate and produces a rooted tree. Initial clusters are formed based on minimum distance between pairs, the average distance between paired clusters is then calculated and those separated by the minimum distance grouped into a higher-level cluster, the average distance is recalculated until the last two clusters are joined.

Wahlund effect: is a reduction in heterozygosity, greater than expected, in populations due to the presence of population substructure. When a series of small subpopulations in HWE and with differing allele frequencies are pooled as a single population the Wahlund effect occurs.

This **Glossary** has been produced from a mixture of definitions from the following sources:

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12 Appendix

Table 63. Details of specimens used in direct sequencing by cytochrome b and D2.

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
Lara									
69 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Adult***	*R. prolixus* haplotype 1	415 bp
74 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
75 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
78 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
81 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
84 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20 757'	01/08/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
90 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
94 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20 757'	01/08/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
76 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20 757'	01/08/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
79 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Adult***male	*R. prolixus* haplotype 1	415 bp
95 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Adult***male	*R. prolixus* haplotype 1	415 bp
98 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Adult***male	*R. prolixus* haplotype 1	415 bp
96 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Adult***male	*R. prolixus* haplotype 1	415 bp
107 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Nymph* female	*R. prolixus* haplotype 1	415 bp
122 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Nymph* female	*R. prolixus* haplotype 1	415 bp
131 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Nymph* female	*R. prolixus* haplotype 1	415 bp
123 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Nymph* female	*R. prolixus* haplotype 1	415 bp
130 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Nymph* female	*R. prolixus* haplotype 1	415 bp
125 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Nymph* male	*R. prolixus* haplotype 1	415 bp
126 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20 757'	01/08/2001	Nymph* male	*R. prolixus* haplotype 1	415 bp
129 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Nymph* male	*R. prolixus* haplotype 1	415 bp
110 lara	Lara	Guamito	Domestic	House 1	N09°45.858' W069°20.757'	01/08/2001	Nymph* male	*R. prolixus* haplotype 1	415 bp
102 lara	Lara	Salvador	Domestic	House 2	N09°45.858' W069°20.757'	02/08/2001	Adult***male	*R. prolixus* haplotype 1	415 bp
103 lara	Lara	Salvador	Domestic	House 2	N09°45.858' W069°20.757'	02/08/2002	Adult***male	*R. prolixus* haplotype 1	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
Guarico									
172 gua	Guarico	Bravero	Silvatic Palm	Not near house	N09°25.670' W067°31.634'	26/07/2001	Nymph* female	*R. prolixus* haplotype 1	415 bp
159 gua	Guarico	Bravero	Silvatic Palm	Not near house	N09°25.670' W067°31.634'	26/07/2001	Nymph* female	*R. prolixus* haplotype 1	415 bp
171 gua	Guarico	Bravero	Silvatic Palm	Not near house	N09°25.670' W067°31.634'	26/07/2001	Nymph* male	*R. prolixus* haplotype 1	415 bp
160 gua	Guarico	El Manguito	Silvatic Palm	Not near house	N09°25.670' W067°31.634'	25/07/2001	Nymph* female	*R. prolixus* haplotype 2	415 bp
112 gua	Guarico	El Manguito	Silvatic Palm	Not near house	N09°25.670' W067°31.634'	25/07/2001	Nymph* female	*R. prolixus* haplotype 2	415 bp
113 gua	Guarico	El Manguito	Silvatic Palm	Not near house	N09°25.670' W067°31.634'	25/07/2001	Nymph*	*R. prolixus* haplotype 2	415 bp
118 gua	Guarico	El Manguito	Silvatic Palm	Not near house	N09°25.670' W067°31.634'	25/07/2001	Nymph* male	*R. prolixus* haplotype 2	415 bp
119 gua	Guarico	El Manguito	Silvatic Palm	Not near house	N09°25.670' W067°31.634'	25/07/2001	Nymph* female	*R. prolixus* haplotype 2	415 bp
120 gua	Guarico	El Manguito	Silvatic Palm	Not near house	N09°25.670' W067°31.634'	25/07/2001	Nymph* female	*R. prolixus* haplotype 2	415 bp
182 gua	Guarico	El Manguito	Silvatic Palm	Not near house	N09°25.670' W067°31.634'	25/07/2001	Nymph* female	*R. prolixus* haplotype 2	415 bp
184 gua	Guarico	El Manguito	Silvatic Palm	Not near house	N09°25.670' W067°31.634'	26/07/2001	Nymph* male	*R. prolixus* haplotype 2	415 bp
61 pericoco	Guarico	El Sombero	Silvatic Palm	Not near house	N09°25.670' W067°31.634'	25/07/2001	Adult	*R. prolixus* haplotype 13	415 bp
66 gua	Guarico	El Sombero	Silvatic Palm	Not near house	N09°25.670' W067°31.634'	25/07/2001	Adult***female	*R. prolixus* haplotype 2	415 bp
106 gua	Guarico	El Sombero	Silvatic Palm	Not near house	N09°25.670' W067°31.634'	25/07/2001	Nymph* male	*R. prolixus* haplotype 2	415 bp
gua	Guarico	El Sombero	Silvatic Palm	Not near house	N09°25.670' W067°31 634'	25/07/2001	Nymph* female	*R. prolixus* haplotype 2	415 bp
111 gua	Guarico	El Sombero	Silvatic Palm	Not near house	N09°25.670' W067°31 634'	25/07/2001	Nymph* female	*R. prolixus* haplotype 2	415 bp
61 psaber	Guarico	El Sombero	Silvatic palm3	Not near house	N09°25.670' W067°31.634'	25/07/2001	Adult	*R. prolixus* haplotype 6	415 bp
62 ortiz	Guarico	Ortiz	Silvatic Palm 1	Not near house	N09°25.670' W067°31.634'	27/07/2001	Adult female	*R. prolixus* haplotype 1	415 bp
63 ortiz	Guarico	Ortiz	Silvatic Palm 1	Not near house	N09°25.670' W067°31 634'	27/07/2001	Adult	*R. prolixus* haplotype 1	415 bp
64 ortiz	Guarico	Ortiz	Silvatic Palm 1	Not near house	N09°25.670' W067°31.634'	27/07/2001	Adult	*R. prolixus* haplotype 1	415 bp
65 ortiz	Guarico	Ortiz	Silvatic Palm 2	Not near house	N09°25.670' W067°31 634'	26/07/2001	Adult male	*R. prolixus* haplotype 1	415 bp
Cojedes									
COJ1H	Cojedes	Las Queseras	Domestic	J. P. Arraez	N09°47.301 W068°19.892	16/09/2004	Adult male	*R. prolixus* haplotype 1	415 bp
COJ3H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Adult male	*R. prolixus* haplotype 1	415 bp
COJ4H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ5H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph** female	*R. prolixus* haplotype 1	415 bp
COJ6H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph** male	*R. prolixus* haplotype 1	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
C0J7H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph** male	*R. prolixus* haplotype 1	415 bp
C0J8H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ9H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ10H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ11H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Adult male	*R. prolixus* haplotype 1	415 bp
COJ12H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Adult female	*R. prolixus* haplotype 1	415 bp
COJ13H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ14H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ15H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ17H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ18H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
C0J19H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
C0J20H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ21H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19 892	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ22H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ23H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19 892	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ24H	Cojedes	Las Queseras	Domestic	J.P. Arraez	N09°47.301 W068°19 892	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ1P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19 916	16/09/2004	Adult female	*R. prolixus* haplotype 1	415 bp
COJ2P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ3P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Adult male	*R. prolixus* haplotype 1	415 bp
COJ4P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47 304 W068°19 916	16/09/2004	Adult male	*R. prolixus* haplotype 1	415 bp
COJ5P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Adult female	*R. prolixus* haplotype 1	415 bp
COJ6P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19 916	16/09/2004	Adult male	*R. prolixus* haplotype 1	415 bp
C0J7P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19 916	16/09/2004	Adult male	*R. prolixus* haplotype 1	415 bp
C0J8P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Adult female	*R. robustus* haplotype 3	415 bp
COJ9P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Adult female	*R. prolixus* haplotype 1	415 bp
COJ10P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Adult male	*R. prolixus* haplotype 1	415 bp
COJ11P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Adult male	*R. prolixus* haplotype 1	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
COJ12P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ13P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ14P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ15P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Adult female	*R. prolixus* haplotype 1	415 bp
COJ16P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Adult male	*R. prolixus* haplotype 1	415 bp
COJ17P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Adult male	*R. prolixus* haplotype 1	415 bp
COJ18P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
C0J19P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19 916	16/09/2004	Adult male	*R. prolixus* haplotype 1	415 bp
C0J20P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Adult male	*R. robustus* haplotype 3	415 bp
COJ21P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ22P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ23P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
COJ24P	Cojedes	Las Queseras	Silvatic Palm	J.P. Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
Trujillo									
LDAT14	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult*** female	*R. prolixus* haplotype 5	415 bp
LDAT19	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***female	*R. prolixus* haplotype 5	415 bp
LDAT20	Trujillo	Loma de Amarillo	Domestic	House 1	N 09 5 W -70.417	10/11/2003	Adult***male	*R. prolixus* haplotype 5	415 bp
LDAT1	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult female	*R. robustus* haplotype 16	415 bp
LDAT13	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult ***male	*R. prolixus* haplotype 5	415 bp
LDAT24	Trujillo	Loma de Amarillo	Domestic	House 1	N 09 5 W -70.417	10/11/2003	Adult***female	*R. prolixus* haplotype 5	415 bp
LDAT10	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***male	*R. prolixus* haplotype 5	415 bp
LDAT12	Trujillo	Loma de Amarillo	Domestic	House 1	N 09 5 W -70.417	10/11/2003	Adult***male	*R. prolixus* haplotype 5	415 bp
LDAT21	Trujillo	Loma de Amarillo	Domestic	House 1	N 09 5 W -70.417	10/11/2003	Adult***male	*R. prolixus* haplotype 5	415 bp
LDAT22	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***male	*R prolixus* haplotype 5	415 bp
LDAT23	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70 417	10/11/2003	Adult***male	*R. prolixus* haplotype 5	415 bp
LDAT25	Trujillo	Loma de Amarillo	Domestic	House 1	N 09 5 W -70.417	10/11/2003	Adult***male	*R. prolixus* haplotype 5	406 bp
LDAT26	Trujillo	Loma de Amarillo	Domestic	House 1	N 09 5 W -70.417	10/11/2003	Adult***male	*R. prolixus* haplotype 5	415 bp
LDAT17	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	*R. prolixus* haplotype 5	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
LDAT18	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	*R. prolixus* haplotype 5	415 bp
LDAT2	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	*R. prolixus* haplotype 5	415 bp
LDAT3	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	*R. prolixus* haplotype 5	406 bp
LDAT4	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	*R. prolixus* haplotype 5	415 bp
LDAT6	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	*R. prolixus* haplotype 5	415 bp
LDAT7	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	*R. prolixus* haplotype 5	415 bp
LDAT9	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	*R. prolixus* haplotype 5	415 bp
LJT2	Trujillo	LA Juventud	Silvatic Palm	House 5	N 09.5 W -70.417	10/11/2003	Adult male	*R. robustus* haplotype 17	415 bp
LJT3	Trujillo	LA Juventud	Silvatic Palm	House 5	N 09.5 W -70.417	10/11/2003	Adult female	*R. robustus* haplotype 16	415 bp
LJT4	Trujillo	LA Juventud	Silvatic Palm	House 5	N 09.5 W -70 417	10/11/2003	Adult	*R. robustus* haplotype 17	415 bp
INS1	Trujillo	Insectary	Silvatic Palm	La Viscosa	N 09.5 W -70.417	04/05/1995	Adult female	*R. robustus* haplotype 16	415 bp
INS2	Trujillo	Insectary	Silvatic Palm	Vitro	N 09.5 W -70.417	18/10/1995	Adult female	*R. robustus* haplotype 16	415 bp
INS3	Trujillo	Insectary	Silvatic Palm	Trados de la Villa	N 09.5 W -70.417	19/07/1995	Adult female	*R. robustus* haplotype 18	415 bp
Portuguesa									
1 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
2 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
7 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***	*R. prolixus* haplotype 1	415 bp
9 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
10 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***male	*R. prolixus* haplotype 1	415 bp
13 TERR	Portuguesa	Terronal	Peridomestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
16 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***male	*R. prolixus* haplotype 1	415 bp
5 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
52 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph** male	*R. prolixus* haplotype 1	415 bp
53 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph** female	*R. prolixus* haplotype 1	415 bp
TERR2E	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
Terr2G	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
TERR2H	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
Terr2I	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
Terr2J	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21 179'	09/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
Terr2K	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
TER2A	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***male	*R. prolixus* haplotype 1	415 bp
Ter2B	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
TERR2 2HA	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
6 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult	*R. robustus* haplotype 3	415 bp
11 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***female	*R. robustus* haplotype 3	415 bp
15 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***female	*R. robustus* haplotype 3	415 bp
17 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34 689' W069°21.179'	09/07/2001	Adult***male	*R. robustus* haplotype 3	415 bp
8 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***female	*R. robustus* haplotype 3	415 bp
54 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph** male	*R. robustus* haplotype 3	415 bp
TERR2 2hb	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph***	*R. robustus* haplotype 3	415 bp
TERR2B	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph***	*R. robustus* haplotype 3	415 bp
Terr2F	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph***	*R. robustus* haplotype 3	415 bp
43 TERFP	Portuguesa	Terronal	Silvatic Palm 2	House 2 01	N09°34.659' W069°21.355'	13/07/2001	Adult female	*R. prolixus* haplotype 1	415 bp
45 TERR	Portuguesa	Terronal	Silvatic Palm 2	House 2 01	N09°34.659' W069°21.355'	13/07/2001	Adult male	*R. prolixus* haplotype 1	415 bp
TER2P3A	Portuguesa	Terronal	Silvatic Palm 3	House 2 01	N09°34.688' W069°21.205'	09/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
TER2P3B	Portuguesa	Terronal	Silvatic Palm 3	House 2 01	N09°34.688' W069°21.205'	09/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
Ter2P1A	Portuguesa	Terronal	Silvatic Palm 4	House 2 01	N09°34.709' W069°21.174'	09/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
108TERFP	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* male	*R. prolixus* haplotype 1	415 bp
116 TERFP	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34 659' W069°21.355'	July 01	Nymph* female	*R. prolixus* haplotype 1	415 bp
138 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph*	*R. prolixus* haplotype 1	415 bp
140 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34 659' W069°21.355'	July 01	Nymph* female	*R. prolixus* haplotype 1	415 bp
143 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34 659' W069°21.355'	July 01	Nymph* male	*R. prolixus* haplotype 1	415 bp
144 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34 659' W069°21.355'	July 01	Nymph* male	*R. prolixus* haplotype 1	415 bp
146 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* female	*R. prolixus* haplotype 1	415 bp
151 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* male	*R. prolixus* haplotype 1	415 bp
152 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* male	*R. prolixus* haplotype 1	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
154 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* male	*R. prolixus* haplotype 1	415 bp
155 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* male	*R. prolixus* haplotype 1	415 bp
156 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34 659' W069°21.355'	July 01	Nymph* female	*R. prolixus* haplotype 1	415 bp
158 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* female	*R. prolixus* haplotype 1	415 bp
162 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* male	*R. prolixus* haplotype 1	415 bp
163 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* male	*R. prolixus* haplotype 1	415 bp
164 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* male	*R. prolixus* haplotype 1	415 bp
168 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* male	*R. prolixus* haplotype 1	415 bp
170 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* female	*R. prolixus* haplotype 1	415 bp
174 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34 659' W069°21.355'	July 01	Nymph* female	*R. prolixus* haplotype 1	415 bp
176 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34 659' W069°21.355'	July 01	Nymph* male	*R. prolixus* haplotype 1	415 bp
179 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* female	*R. prolixus* haplotype 1	415 bp
183 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34 659' W069°21.355'	July 01	Nymph* female	*R. prolixus* haplotype 1	415 bp
186 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* female	*R. prolixus* haplotype 1	406 bp
187 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* male	*R. prolixus* haplotype 1	415 bp
188 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* female	*R. prolixus* haplotype 1	415 bp
44 TERFP	Portuguesa	Terronal	Silvatic Palm 2	House 2 01	N09°34.659' W069°21.355'	13/07/2001	Adult female	*R. prolixus* haplotype 2	415 bp
38 TERFP	Portuguesa	Terronal	Silvatic Palm 1	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult female	*R. robustus* haplotype 3	415 bp
134 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* male	*R. robustus* haplotype 3	415 bp
136 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* male	*R. robustus* haplotype 3	415 bp
145 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* female	*R. robustus* haplotype 3	415 bp
157 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34 659' W069°21.355'	July 01	Nymph* female	*R. robustus* haplotype 3	415 bp
173 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	July 01	Nymph* male	*R. robustus* haplotype 3	415 bp
21 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
23 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34 469' W069°20.595'	09/07/2001	Adult***male	*R. prolixus* haplotype 1	415 bp
25 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
30 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***female	*R. prolixus* haplotype 1	394 bp
57 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Nymph**male	*R. prolixus* haplotype 1	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
33 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***male	*R. prolixus* haplotype 1	415 bp
48 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Nymph**	*R. prolixus* haplotype 1	415 bp
133 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Nymph* male	*R. robustus* haplotype 3	415 bp
55 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Nymph** female	*R. robustus* haplotype 3	415 bp
37 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***female	*R. robustus* haplotype 3	415 bp
18 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***male	*R. robustus* haplotype 3	415 bp
19 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***female	*R. robustus* haplotype 3	415 bp
20 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34 469' W069°20.595'	09/07/2001	Adult***male	*R. robustus* haplotype 3	415 bp
22 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***female	*R. robustus* haplotype 3	415 bp
24 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***female	*R. robustus* haplotype 3	415 bp
27 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***female	*R. robustus* haplotype 3	415 bp
28 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***male	*R. robustus* haplotype 3	415 bp
29 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***male	*R. robustus* haplotype 3	415 bp
31 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20 595'	09/07/2001	Adult***male	*R. robustus* haplotype 3	415 bp
49 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Nymph** male	*R. robustus* haplotype 3	415 bp
46 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Nymph** female	*R. robustus* haplotype 3	415 bp
50 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Nymph** female	*R. robustus* haplotype 3	415 bp
T1P11	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T1P14	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T1P15	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T1P12	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	November 03	Adult***male	*R. robustus* haplotype 3	415 bp
T1P13	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	November 03	Adult***	*R. robustus* haplotype 3	415 bp
T1P16	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	November 03	Adult***female	*R. robustus* haplotype 3	415 bp
T1P4	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	November 03	Nymph***	*R. robustus* haplotype 3	415 bp
T1P5	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	November 03	Adult***	*R. robustus* haplotype 3	415 bp
T1P6	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	November 03	Adult***male	*R. robustus* haplotype 3	415 bp
T1P7	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	November 03	Adult***female	*R. robustus* haplotype 3	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
T1P1	Portuguesa	Terronal	Silvatic palm	House 1 03	N09°34.469' W069°20.595'	November 03	Adult***male	*R. prolixus* haplotype 1	415 bp
T1P10	Portuguesa	Terronal	Silvatic palm	House 1 03	N09°34.469' W069°20.595'	November 03	Nymph***	*R. prolixus* haplotype 1	415 bp
T1P2	Portuguesa	Terronal	Silvatic palm	House 1 03	N09°34.469' W069°20.595'	November 03	Nymph***	*R. prolixus* haplotype 1	415 bp
T1P3	Portuguesa	Terronal	Silvatic palm	House 1 03	N09°34.469' W069°20.595'	November 03	Nymph***	*R. prolixus* haplotype 1	415 bp
T1P8	Portuguesa	Terronal	Silvatic palm	House 1 03	N09°34.469' W069°20.595'	November 03	Nymph***	*R. prolixus* haplotype 1	415 bp
T1P9	Portuguesa	Terronal	Silvatic palm	House 1 03	N09°34.469' W069°20.595'	November 03	Nymph***	*R. prolixus* haplotype 1	415 bp
T2P3	Portuguesa	Terronal	Domestic	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T2P33	Portuguesa	Terronal	Domestic	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***	*R. prolixus* haplotype 1	415 bp
T2P34	Portuguesa	Terronal	Domestic	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***male	*R. prolixus* haplotype 1	415 bp
T2P16	Portuguesa	Terronal	Domestic	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***female	*R. robustus* haplotype 3	415 bp
T2P1	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***male	*R. prolixus* haplotype 1	415 bp
T2P13	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Nymph***	*R. prolixus* haplotype 1	415 bp
T2P15	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T2P18	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***	*R. prolixus* haplotype 1	415 bp
T2P2	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T2P20	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***	*R. prolixus* haplotype 1	415 bp
T2P21	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T2P23	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T2P25	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***male	*R. prolixus* haplotype 1	415 bp
T2P26	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T2P27	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T2P28	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Nymph***	*R. prolixus* haplotype 1	415 bp
T2P29	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T2P30	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34 689' W069°21.179'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T2P31	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T2P32	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T2P35	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***male	*R. prolixus* haplotype 1	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
T2P36	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***male	*R. prolixus* haplotype 1	415 bp
T2P37	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T2P38	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34 689' W069°21.179'	November 03	Adult***	*R. prolixus* haplotype 1	415 bp
T2P39	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T2P40	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***	*R. prolixus* haplotype 1	415 bp
T2P41	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***	*R. prolixus* haplotype 1	415 bp
T2P43	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***female	*R. prolixus* haplotype 1	415 bp
T2P44	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***male	*R. prolixus* haplotype 1	415 bp
T2P7	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Nymph***	*R. prolixus* haplotype 1	415 bp
T2P8	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34 689' W069°21.179'	November 03	Nymph***	*R. prolixus* haplotype 1	415 bp
T2P9	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Nymph***	*R. prolixus* haplotype 1	415 bp
T2P12	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Nymph***	*R. robustus* haplotype 3	415 bp
T2P14	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Nymph***	*R. robustus* haplotype 3	415 bp
T2P17	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***female	*R. robustus* haplotype 3	415 bp
T2P19	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***	*R. robustus* haplotype 3	415 bp
T2P4	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Nymph***	*R. robustus* haplotype 3	415 bp
T2P5	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Nymph***	*R. robustus* haplotype 3	415 bp
T2P6	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	November 03	Adult***	*R. robustus* haplotype 3	415 bp
39 Pen	Portuguesa	Peña Negra	Domestic	House 1 Peña Negra	N09°33.990' W069°21.038'	09/07/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
40 Pen	Portuguesa	Peña Negra	Domestic	House 1 Peña Negra	N09°33.990' W069°21.038'	09/07/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
41 pen	Portuguesa	Peña Negra	Domestic	House 1	N09°33.990' W069°21.038'	09/07/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
42 pen	Portuguesa	Peña Negra	Domestic	House 1	N09°33.990' W069°21.038'	09/07/2001	Adult***male	*R. prolixus* haplotype 1	415 bp
PenNA	Portuguesa	Peña Negra	Domestic	House 1	N09°33 990' W069°21.038'	09/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
PenNB	Portuguesa	Peña Negra	Domestic	House 1	N09°33.990' W069°21.038'	09/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
PenNC	Portuguesa	Peña Negra	Domestic	House 1	N09°33 990' W069°21.038'	09/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
PenNE	Portuguesa	Peña Negra	Domestic	House 1	N09°33.990' W069°21.038'	09/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
PenNF	Portuguesa	Peña Negra	Domestic	House 1	N09°33.990' W069°21.038'	09/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
PenNG	Portuguesa	Peña Negra	Domestic	House 1	N09°33.990' W069°21.038'	09/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
P-rito A	Portuguesa	Palmarito	Domestic	House 1	N09°36.805' W069°21.829'	10/07/2001	Nymph***	*R. robustus* haplotype 3	415 bp
P-rito C	Portuguesa	Palmarito	Domestic	House 1	N09°36.805' W069°21.829'	10/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
P-rito D	Portuguesa	Palmarito	Domestic	House 1	N09°36.805' W069°21.829'	10/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
P-rito E	Portuguesa	Palmarito	Domestic	House 1	N09°36.805' W069°21.829'	10/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
Palmito C	Portuguesa	Palmarito	Silvatic palm 1	House 1	N09°36.846' W069°21.756'	10/07/2001	Nymph***	*R. prolixus* haplotype 1	415 bp
POSB19A	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Adult***male	*R. prolixus* haplotype 1	415 bp
POSB19B	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Adult***male	*R. prolixus* haplotype 1	415 bp
POSB19C	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Adult***male	*R. prolixus* haplotype 1	415 bp
POSB19D	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSB19E	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Adult***	*R. prolixus* haplotype 1	415 bp
POSB19F	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Adult***female	*R. prolixus* haplotype 1	415 bp
POSB19G	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Adult*** male	*R. prolixus* haplotype 1	415 bp
POSB19H	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSB19I	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSB19J	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSB19K	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Adult***female	*R. prolixus* haplotype 1	415 bp
POSB19L	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSB19M	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSB19N	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSB25a	Portuguesa	San Bartolo	Domestic	House 25	N09°27.218' W069°32.745'	03/07/2002	Adult***female	*R. prolixus* haplotype 1	415 bp
POSB25b	Portuguesa	San Bartolo	Domestic	House 25	N09°27.218' W069°32.745'	03/07/2002	Adult***	*R. prolixus* haplotype 1	415 bp
POSBMAR11	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSBMAR12	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSBMAR15	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Adult***male	*R. prolixus* haplotype 1	415 bp
POSBMAR17	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Adult***male	*R. prolixus* haplotype 1	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
POSBMAR19	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Adult***female	*R. prolixus* haplotype 1	415 bp
POSBMAR2	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSBMAR3	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSBMAR4	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSBMAR5	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSBMAR6	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSBMAR8	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSBMARP1	Portuguesa	San Bartolo	Peridomestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSBMARP2	Portuguesa	San Bartolo	Peridomestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSL89A	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Nymph***	*R. prolixus* haplotype 9	415 bp
POSL89B	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Nymph***	*R. prolixus* haplotype 9	415 bp
POSL89C	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Adult***	*R. prolixus* haplotype 9	415 bp
POSL89D	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Adult***female	*R. prolixus* haplotype 9	415 bp
POSL89E	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Adult***female	*R. prolixus* haplotype 9	415 bp
POSL89F	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Nymph***	*R. prolixus* haplotype 9	415 bp
POSL89G	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Nymph***	*R. prolixus* haplotype 9	415 bp
POSL89H	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Nymph***	*R. prolixus* haplotype 9	415 bp
POSL89I	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Adult***	*R. prolixus* haplotype 1	415 bp
POSL89J	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Nymph***	*R. prolixus* haplotype 9	415 bp
POSL89K	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Adult***female	*R. prolixus* haplotype 9	415 bp
POSL89L	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Adult***female	*R. prolixus* haplotype 9	415 bp
POSL89M	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Nymph***	*R. prolixus* haplotype 1	415 bp
POSL89N	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Adult***female	*R. prolixus* haplotype 1	415 bp
POSL94A	Portuguesa	Santa Lucia	Domestic	House 94	N 9.4102 W -69.575	01/07/2002	Adult***male	*R prolixus* haplotype 9	415 bp
POSL49A	Portuguesa	Santa Lucia	Domestic	House 49	N 9.4102 W -69.575	01/07/2002	Adult***female	*R prolixus* haplotype 1	415 bp
POSL50A	Portuguesa	Santa Lucia	Domestic	House 50	N 9.4102 W -69.575	01/07/2002	Adult ***female	*R prolixus* haplotype 1	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
CP7	Portuguesa	Casa Rena	Domestic	M. Barreto	N09°36.189' W069°21.554'	December 03	Nymph***	*R. robustus* haplotype 3	415 bp
CP8	Portuguesa	Casa Rena	Domestic	M. Barreto	N09°36.189' W069°21.554'	December 03	Nymph***	*R. robustus* haplotype 3	415 bp
CP9	Portuguesa	Casa Rena	Domestic	M. Barreto	N09°36.189' W069°21.554'	December 03	Nymph***	*R. robustus* haplotype 3	415 bp
CP10	Portuguesa	Casa Rena	Domestic	M. Barreto	N09°36.189' W069°21.554'	December 03	Adult***female	*R. robustus* haplotype 3	415 bp
CP11	Portuguesa	Casa Rena	Domestic	M. Barreto	N09°36.189' W069°21 554'	December 03	Adult***female	*R. robustus* haplotype 3	415 bp
CP13	Portuguesa	Casa Rena	Domestic	M. Barreto	N09°36.189' W069°21 554'	December 03	Adult***	*R. robustus* haplotype 3	415 bp
CP14	Portuguesa	Casa Rena	Domestic	M. Barreto	N09°36.189' W069°21.554'	December 03	Adult***female	*R. robustus* haplotype 3	415 bp
CP15	Portuguesa	Casa Rena	Domestic	M. Barreto	N09°36.189' W069°21.554'	December 03	Adult***female	*R. robustus* haplotype 3	415 bp
CP16	Portuguesa	Casa Rena	Domestic	M. Barreto	N09°36.189' W069°21.554'	December 03	Adult***female	*R. robustus* haplotype 3	415 bp
CP17	Portuguesa	Casa Rena	Domestic	M. Barreto	N09°36.189' W069°21.554'	December 03	Adult***female	*R. robustus* haplotype 3	415 bp
CP3	Portuguesa	Casa Rena	Peridomestic	M. Barreto	N09°36.189' W069°21.554'	December 03	Adult***female	*R. robustus* haplotype 3	415 bp
CP5	Portuguesa	Casa Rena	Peridomestic	M. Barreto	N09°36.189' W069°21.554'	December 03	Adult***	*R. robustus* haplotype 3	415 bp
CP2	Portuguesa	Casa Rena	Silvatic palm	M. Barreto	N09°36.189' W069°21.554'	December 03	Adult***male	*R. prolixus* haplotype 1	415 bp
CP6	Portuguesa	Casa Rena	Silvatic palm	M. Barreto	N09°36.189' W069°21.554'	December 03	Adult***male	*R. prolixus* haplotype 1	415 bp
CP1	Portuguesa	Casa Rena	Silvatic palm	M. Barreto	N09°36.189' W069°21.554'	December 03	Adult***male	*R. robustus* haplotype 3	415 bp
Casa	Portuguesa	Casa Rena	Silvatic palm	Not near house	N09°36.189' W069°21.554'	11/07/2001	Nymph***	*R. robustus* haplotype 3	415 bp
CasarPA	Portuguesa	Casa Rena	Silvatic palm	Not near house	N09°36.050' W069°20.836'	11/07/2001	Nymph***	*R. robustus* haplotype 3	415 bp
POQN20B	Portuguesa	Quadra Negras	Domestic	House 20	N09°12.072' W069°56.257'	2001	Adult***female	*R. prolixus* haplotype 1	415 bp
POQN20C	Portuguesa	Quadra Negras	Domestic	House 20	N09°12.072' W069°56.257'	2001	Adult***male	*R. prolixus* haplotype 1	415 bp
POQN22.1bl	Portuguesa	Quadra Negras	Domestic	House 22.1	N09°12.072' W069°56.257'	2001	Adult***male	*R. prolixus* haplotype 1	415 bp
POQN26A	Portuguesa	Quadra Negras	Domestic	House 26	N09°12.072' W069°56.257'	2001	Adult***male	*R. prolixus* haplotype 1	415 bp
POQN27A	Portuguesa	Quadra Negras	Domestic	House 27	N09°12.072' W069°56.257'	2001	Adult***female	*R. prolixus* haplotype 1	415 bp
POQN27B	Portuguesa	Quadra Negras	Domestic	House 27	N09°12.072' W069°56.257'	2001	Adult***female	*R. prolixus* haplotype 1	415 bp
POQN30.1A	Portuguesa	Quadra Negras	Domestic	House 30.1	N09°12 072' W069°56.257'	2001	Adult***male	*R. prolixus* haplotype 1	415 bp
POQU114A	Portuguesa	Quadra Negras	Domestic	House 114	N09°12.072' W069°56.257'	2001	Adult***male	*R. prolixus* haplotype 1	415 bp
POQN20F	Portuguesa	Quadra Negras	Domestic	House 20	N09°12 072' W069°56.257'	2001	Adult***male	*R. prolixus* haplotype 2	415 bp
POQN20D	Portuguesa	Quadra Negras	Domestic	House 20	N09°12.072' W069°56.257'	2001	Adult***male	*R. prolixus* haplotype 5	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
POQN20E	Portuguesa	Quadra Negras	Domestic	House 20	N09°12.072' W069°56.257'	2001	Adult***male	*R. prolixus* haplotype 5	415 bp
POQN22.1A	Portuguesa	Quadra Negras	Domestic	House 22.1	N09°12.072' W069°56.257'	2001	Adult***	*R. prolixus* haplotype 8	415 bp
110 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph* female	*R. prolixus* haplotype 1	415 bp
121 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph*female	*R. robustus* haplotype 3	415 bp
147 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph*female	*R. robustus* haplotype 3	415 bp
149 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph* male	*R. prolixus* haplotype 1	415 bp
15 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20 737'	17/07/2001	Nymph*female	*R. prolixus* haplotype 1	415 bp
153 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph*female	*R. prolixus* haplotype 1	415 bp
161 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph* male	*R. prolixus* haplotype 1	415 bp
185 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph*female	*R. robustus* haplotype 3	415 bp
139 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph* male	*R. prolixus* haplotype 1	415 bp
137 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph*female	*R. robustus* haplotype 3	415 bp
POMO10.1B	Portuguesa	Morichal	Domestic	House 10.1	N09°31.976' W069°22.426'	2001	Adult***	*R. prolixus* haplotype 1	415 bp
POMO10.1C	Portuguesa	Morichal	Domestic	House 10.1	N09°31.976' W069°22.426'	2001	Adult***	*R. prolixus* haplotype 1	415 bp
POMO10.1F	Portuguesa	Morichal	Domestic	House 10.1	N09°31.976' W069°22.426'	2001	Adult***	*R. prolixus* haplotype 1	415 bp
POMO10.1G	Portuguesa	Morichal	Domestic	House 10.1	N09°31.976' W069°22.426'	2001	Adult***	*R. prolixus* haplotype 1	415 bp
POMO10A	Portuguesa	Morichal	Domestic	House 10	N09°31.976' W069°22.426'	2001	Adult***	*R. robustus* haplotype 3	415 bp
POMO21A	Portuguesa	El Mosquito	Domestic	House 21	MC*	2001	Adult***	*R. prolixus* haplotype 1	415 bp
POMO21C	Portuguesa	El Mosquito	Domestic	House 21	MC*	2001	Adult***male	*R. prolixus* haplotype 1	415 bp
POMO21B	Portuguesa	El Mosquito	Domestic	House 21	MC*	2001	Adult***	*R. prolixus* haplotype 2	415 bp
POMO40A	Portuguesa	El Mosquito	Domestic	House 40	MC*	2001	Adult***	*R. prolixus* haplotype 1	415 bp
POMO48A	Portuguesa	El Mosquito	Domestic	House 48	MC*	2001	Adult***	*R. prolixus* haplotype 1	415 bp
POMO8A	Portuguesa	El Mosquito	Domestic	House 8	MC*	2001	Adult***	*R. prolixus* haplotype 1	415 bp
POMO8D	Portuguesa	El Mosquito	Domestic	House 8	MC*	2001	Adult***	*R. prolixus* haplotype 2	415 bp
POMOINTRAB	Portuguesa	El Mosquito	Domestic	-	MC*	2001	Nymph***	*R. prolixus* haplotype 1	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
RASH1	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Adult***male	*R. prolixus* haplotype 1	415 bp
RASH2	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958 615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH3	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH4	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH5	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH6	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH7	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 2	415 bp
RASH8	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH9	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH10	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH11	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH12	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH14	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH16	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH17	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH18	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH19	Portuguesa	Los Rastrojos	Domestic	J.P Delgado	N0917.636 W06958 615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH20	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 2	415 bp
RASH21	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH22	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH23	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASH24	Portuguesa	Los Rastrojos	Domestic	J.P. Delgado	N0917.636 W06958 615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASP1	Portuguesa	Los Rastrojos	Silvatic Palm	J.P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASP2	Portuguesa	Los Rastrojos	Silvatic Palm	J.P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASP3	Portuguesa	Los Rastrojos	Silvatic Palm	J.P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASP4	Portuguesa	Los Rastrojos	Silvatic Palm	J.P Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASP5	Portuguesa	Los Rastrojos	Silvatic Palm	J.P. Delgado	N09 17.627 W069 58 615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
RASP6	Portuguesa	Los Rastrojos	Silvatic Palm	J.P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASP7	Portuguesa	Los Rastrojos	Silvatic Palm	J.P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASP8	Portuguesa	Los Rastrojos	Silvatic Palm	J.P. Delgado	N09 17.627 W069 58 615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASP10	Portuguesa	Los Rastrojos	Silvatic Palm	J.P. Delgado	N09 17.627 W069 58 615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
RASP11	Portuguesa	Los Rastrojos	Silvatic Palm	J.P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1	415 bp
Barinas									
bar1	Barinas	Santha Elena de Caramuca	Silvatic palm	-	MC*	21/09/2002	Adult***male	*R. prolixus* haplotype 4	415 bp
bar2	Barinas	Santha Elena de Caramuca	Silvatic palm	-	MC*	21/09/2002	Adult***male	*R. prolixus* haplotype 4	415 bp
barh	Barinas	Obispos	Domestic	-	MC*	23/09/2001	Adult***female	*R. prolixus* haplotype 1	415 bp
A1B1	Barinas	Acequita	Peridomestic	Peridomestic	MC*	28/10/2003	Adult***	*R. prolixus* haplotype 1	415 bp
A1B3	Barinas	Acequita	Peridomestic	Peridomestic	MC*	28/10/2003	Adult***	*R. prolixus* haplotype 1	415 bp
A1B4	Barinas	Acequita	Peridomestic	Peridomestic	MC*	28/10/2003	Adult***	*R. prolixus* haplotype 1	415 bp
CAR1B1	Barinas	Carreteron	Silvatic Palm	Palm 2House 1	MC*	01/11/2003	Adult***	*R. prolixus* haplotype 1	415 bp
CAR1B10	Barinas	Carreteron	Silvatic Palm	Palm 1House 1	MC*	01/11/2003	Adult***	*R. prolixus* haplotype 1	415 bp
CAR1B2	Barinas	Carreteron	Silvatic Palm	Palm 1House 1	MC*	01/11/2003	Nymph***	*R. prolixus* haplotype 1	415 bp
CAR1B4	Barinas	Carreteron	Silvatic Palm	Palm 1House 1	MC*	01/11/2003	Nymph***	*R. prolixus* haplotype 1	415 bp
CAR1B5	Barinas	Carreteron	Silvatic Palm	Palm 1House 1	MC*	01/11/2003	Nymph***	*R. prolixus* haplotype 1	415 bp
CAR1B6	Barinas	Carreteron	Silvatic Palm	Palm 1House 1	MC*	01/11/2003	Nymph***	*R. prolixus* haplotype 1	415 bp
CAR1B8	Barinas	Carreteron	Silvatic Palm	Palm 1House 1	MC*	01/11/2003	Nymph***	*R. prolixus* haplotype 1	415 bp
CAR1B11	Barinas	Carreteron	Domestic	House 1	MC*	01/11/2003	Adult***	*R. prolixus* haplotype 4	415 bp
CAR1B9	Barinas	Carreteron	Domestic	House 1	MC*	27/06/2003	Adult***male	*R. prolixus* haplotype 1	415 bp
CAR2B2	Barinas	Carreteron	Domestic	House 2	MC*	25/06/2003	Adult***female	*R. prolixus* haplotype 4	415 bp
CAR2B3	Barinas	Carreteron	Domestic	House 2	MC*	25/06/2003	Adult***female	*R. prolixus* haplotype 1	415 bp
CAR2B4	Barinas	Carreteron	Domestic	House 2	MC*	25/06/2003	Adult***female	*R. prolixus* haplotype 1	415 bp
CAR2B5	Barinas	Carreteron	Domestic	House 2	MC*	25/06/2003	Adult***female	*R. prolixus* haplotype 5	415 bp
CAR2B6	Barinas	Carreteron	Domestic	House 2	MC*	25/06/2003	Adult***female	*R. prolixus* haplotype 4	415 bp
CAR2B7	Barinas	Carreteron	Domestic	House 2	MC*	25/06/2003	Adult ***female	*R. prolixus* haplotype 1	415 bp
CAR2B8	Barinas	Carreteron	Domestic	House 2	MC*	25/06/2003	Adult***male	*R. robustus* haplotype 3	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
CAR3B2	Barinas	Carreteron	Domestic	House 3	MC*	25/06/2003	Adult***	*R. prolixus* haplotype 1	415 bp
CAR4B3	Barinas	Carreteron	Domestic	House 4	MC*	25/06/2003	Adult***female	*R. prolixus* haplotype 1	415 bp
CAR4B5	Barinas	Carreteron	Domestic	House 4	MC*	25/06/2003	Adult***female	*R. prolixus* haplotype 1	415 bp
CAR5B1	Barinas	Carreteron	Domestic	House 5	MC*	25/06/2003	Adult***male	*R. prolixus* haplotype 7	415 bp
CAR6B1	Barinas	San Isidero	Domestic	House 6	MC*	28/06/2003	Adult***	*R. prolixus* haplotype 1	415 bp
01071fpd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Adult Female	*R. prolixus* haplotype 1	415 bp
01072fpd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Adult female	*R. prolixus* haplotype 1	415 bp
01073fpd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Adult female	*R. prolixus* haplotype 1	415 bp
01075fpd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Adult female	*R. prolixus* haplotype 1	415 bp
01076fpd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Adult female	*R. prolixus* haplotype 2	415 bp
01077fpd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Adult female	*R. prolixus* haplotype 1	415 bp
01075npd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Nymph***	*R. prolixus* haplotype 2	415 bp
01072npd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Nymph***	*R. prolixus* haplotype 2	415 bp
01074npd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Nymph***	*R. prolixus* haplotype 4	415 bp
01231fh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult female	*R. prolixus* haplotype 1	392 bp
01232fh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult female	*R. prolixus* haplotype 1	415 bp
01233fh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult female	*R. prolixus* haplotype 1	415 bp
t	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult female	*R. prolixus* haplotype 5	415 bp
01236mh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult male	*R. prolixus* haplotype 1	415 bp
01237mh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult male	*R. prolixus* haplotype 1	415 bp
01238mh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult male	*R. prolixus* haplotype 2	415 bp
01239mh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult male	*R. prolixus* haplotype 1	415 bp
01p1f2	Barinas	Cascabel	Silvatic Palm	House 2	N 8.174181 W -69.74433	8-10-03	Adult female	*R. prolixus* haplotype 1	415 bp
01p1f4	Barinas	Cascabel	Silvatic Palm	House 2	N 8.174181 W -69.74433	8-10-03	Adult female	*R. prolixus* haplotype 2	415 bp
01p1t1m1	Barinas	Cascabel	Silvatic Palm	House 2	N 8.174181 W -69.74433	8-10-03	Adult male	*R. prolixus* haplotype 1	415 bp
01p1m5	Barinas	Cascabel	Silvatic Palm	House 2	N 8.174181 W -69.74433	8-10-03	Adult male	*R. prolixus* haplotype 1	415 bp
01p1m6	Barinas	Cascabel	Silvatic Palm	House 2	N 8.174181 W -69.74433	8-10-03	Adult male	*R. prolixus* haplotype 2	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
01p1m7	Barinas	Cascabel	Silvatic Palm	House 2	N 8.174181 W -69.74433	8-10-03	Adult male	*R. prolixus* haplotype 1	415 bp
01p1n5	Barinas	Cascabel	Silvatic Palm	House 2	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 1	415 bp
01p1n3	Barinas	Cascabel	Silvatic Palm	House 2	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 1	415 bp
01p1n25	Barinas	Cascabel	Silvatic Palm	House 2	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 2	415 bp
01p1n8	Barinas	Cascabel	Silvatic Palm	House 2	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 1	415 bp
01p1n6	Barinas	Cascabel	Silvatic Palm	House 2	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 2	415 bp
01p1n7	Barinas	Cascabel	Silvatic Palm	House 2	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 5	415 bp
01p1n4	Barinas	Cascabel	Silvatic Palm	House 2	N 8.174181 W -69.74433	8-10-03	Nymph***	R. prolixus haplotype 12	415 bp
01p1n9	Barinas	Cascabel	Silvatic Palm	House 2	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 2	415 bp
01p1n11	Barinas	Cascabel	Silvatic Palm	House 2	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 2	415 bp
06095fh	Barinas	Guaranda	Domestic	House 9	N8.084781 W-69.7035	-	Adult female	*R. prolixus* haplotype 1	415 bp
06097fh	Barinas	Guaranda	Domestic	House 9	N8.084781 W-69.7035	-	Adult female	*R. prolixus* haplotype 1	415 bp
06098fh	Barinas	Guaranda	Domestic	House 9	N8.084781 W-69.7035	-	Adult female	*R. prolixus* haplotype 1	415 bp
06091nh	Barinas	Guaranda	Domestic	House 9	N8.084781 W-69.7035	-	Nymph***	*R. prolixus* haplotype 1	415 bp
06092nh	Barinas	Guaranda	Domestic	House 9	N8.084781 W-69.7035	-	Nymph***	*R. prolixus* haplotype 1	415 bp
06p2m2	Barinas	Guaranda	Silvatic Palm	House E. Quintero	N8.084781 W-69.7035	24-10-03	Adult male	*R. prolixus* haplotype 1	415 bp
06p2m3	Barinas	Guaranda	Silvatic Palm	House E. Quintero	N8.084781 W-69.7035	24-10-03	Adult male	*R. prolixus* haplotype 1	415 bp
06p2m4	Barinas	Guaranda	Silvatic Palm	House E. Quintero	N8.084781 W-69.7035	24-10-03	Adult male	*R. prolixus* haplotype 1	415 bp
06p2n1	Barinas	Guaranda	Silvatic Palm	House E. Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	*R. prolixus* haplotype 1	415 bp
06p2n4	Barinas	Guaranda	Silvatic Palm	House E. Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	*R. prolixus* haplotype 1	415 bp
06p2n7	Barinas	Guaranda	Silvatic Palm	House E. Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	*R. prolixus* haplotype 1	415 bp
06p2n9	Barinas	Guaranda	Silvatic Palm	House E. Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	*R. prolixus* haplotype 1	415 bp
06p2m5	Barinas	Guaranda	Silvatic Palm	House E. Quintero	N8.084781 W-69.7035	24-10-03	Adult male	*R. prolixus* haplotype 10	415 bp
06p2n3	Barinas	Guaranda	Silvatic Palm	House E. Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	*R. prolixus* haplotype 2	415 bp
06p2n5	Barinas	Guaranda	Silvatic Palm	House E. Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	*R. prolixus* haplotype 1	415 bp
06p2n6	Barinas	Guaranda	Silvatic Palm	House E. Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	*R. prolixus* haplotype 1	415 bp
06p2n8	Barinas	Guaranda	Silvatic Palm	House E. Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	*R. prolixus* haplotype 1	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
07032MH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult male	*R. prolixus* haplotype 1	415 bp
07033MH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult male	*R. prolixus* haplotype 1	415 bp
07036MH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult male	*R. prolixus* haplotype 1	415 bp
07038FH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult female	*R. prolixus* haplotype 1	415 bp
070311FH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult female	*R. prolixus* haplotype 1	415 bp
070312FH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult female	*R. prolixus* haplotype 1	415 bp
070313MH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult male	*R. prolixus* haplotype 1	415 bp
070314MH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult male	*R. prolixus* haplotype 1	415 bp
070315FH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult female	*R. prolixus* haplotype 1	415 bp
07037MH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult male	*R. prolixus* haplotype 1	401 bp
07034MH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult male	*R. prolixus* haplotype 5	408 bp
07P2F1	Barinas	Laguna Hermosa	Silvatic palm	House P. Monagas	N 8.089 W-69.8314	29-10-03	Adult female	*R. prolixus* haplotype 1	415 bp
07P2F3	Barinas	Laguna Hermosa	Silvatic palm	House P. Monagas	N 8.089 W-69.8314	29-10-03	Adult female	*R. prolixus* haplotype 1	415 bp
07P2N3	Barinas	Laguna Hermosa	Silvatic palm	House P. Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	*R. prolixus* haplotype 1	415 bp
07P2N4	Barinas	Laguna Hermosa	Silvatic palm	House P. Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	*R. prolixus* haplotype 1	415 bp
07P2N5	Barinas	Laguna Hermosa	Silvatic palm	House P. Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	*R. prolixus* haplotype 1	415 bp
07P2N8	Barinas	Laguna Hermosa	Silvatic palm	House P. Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	*R. prolixus* haplotype 1	415 bp
07P2F2	Barinas	Laguna Hermosa	Silvatic palm	House P. Monagas	N 8.089 W-69.8314	29-10-03	Adult female	*R. prolixus* haplotype 5	415 bp
07P2N2	Barinas	Laguna Hermosa	Silvatic palm	House P. Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	*R. prolixus* haplotype 2	415 bp
07P2N6	Barinas	Laguna Hermosa	Silvatic palm	House P. Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	*R. prolixus* haplotype 11	415 bp
07293FPD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Adult female	*R. prolixus* haplotype 1	415 bp
0729N1PD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Nymph***	*R. prolixus* haplotype 1	415 bp
0729N2PD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Nymph***	*R. prolixus* haplotype 1	415 bp
0729N3PD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Nymph***	*R. prolixus* haplotype 1	415 bp
07295MPD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Adult male	*R. prolixus* haplotype 1	407 bp
07297MPD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Adult male	*R. prolixus* haplotype 5	415 bp
07296MPD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Adult male	*R. prolixus* haplotype 12	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
12112FH	Barinas	G. Paraguey	Domestic	House 11	N8.1531 W-69.919	-	Adult female	*R. prolixus* haplotype 1	415 bp
12114FH	Barinas	G. Paraguey	Domestic	House 11	N8.1531 W-69.919	-	Adult female	*R. prolixus* haplotype 1	415 bp
12116FH	Barinas	G. Paraguey	Domestic	House 11	N8.1531 W-69.919	-	Adult female	*R. prolixus* haplotype 1	415 bp
12131FH	Barinas	G. Paraguey	Domestic	House 13	N8.1531 W-69.919	-	Adult female	*R. prolixus* haplotype 2	415 bp
12132FH	Barinas	G. Paraguey	Domestic	House 13	N8.1531 W-69.919	-	Adult female	*R. prolixus* haplotype 1	415 bp
12134FH	Barinas	G. Paraguey	Domestic	House 13	N8.1531 W-69.919	-	Adult female	*R. prolixus* haplotype 1	415 bp
12135FH	Barinas	G. Paraguey	Domestic	House 13	N8.1531 W-69.919	-	Adult female	*R. prolixus* haplotype 5	415 bp
12136FH	Barinas	G. Paraguey	Domestic	House 13	N8.1531 W-69.919	-	Adult female	*R. prolixus* haplotype 1	415 bp
13P1F1	Barinas	Parcelamiento	Silvatic palm	House Peralta	N8.09622 W-70.3829	20-1-03	Adult female	*R. prolixus* haplotype 1	415 bp
13P2F2	Barinas	Parcelamiento	Silvatic palm	House Belandria	N8.09622 W-70.3829	20-1-03	Adult female	*R. prolixus* haplotype 1	415 bp
13P2N1	Barinas	Parcelamiento	Silvatic palm	House Belandria	N8.09622 W-70.3829	20-1-03	Nymph***	*R. prolixus* haplotype 1	415 bp
13P2N2	Barinas	Parcelamiento	Silvatic palm	House Belandria	N8.09622 W-70.3829	20-1-03	Nymph***	*R. prolixus* haplotype 1	415 bp
13P2N4	Barinas	Parcelamiento	Silvatic palm	House Belandria	N8.09622 W-70.3829	20-1-03	Nymph***	*R. prolixus* haplotype 1	415 bp
13P2N6	Barinas	Parcelamiento	Silvatic palm	House Belandria	N8.09622 W-70.3829	20-1-03	Nymph***	*R. prolixus* haplotype 1	415 bp
13P2N7	Barinas	Parcelamiento	Silvatic palm	House Belandria	N8.09622 W-70.3829	20-1-03	Nymph***	*R. prolixus* haplotype 1	415 bp
13P2F1	Barinas	Parcelamiento	Silvatic palm	House Belandria	N8.09622 W-70.3829	20-1-03	Adult female	*R. prolixus* haplotype 14	415 bp
13P2N3	Barinas	Parcelamiento	Silvatic palm	House Belandria	N8.09622 W-70.3829	20-1-03	Nymph***	*R. prolixus* haplotype 14	415 bp
13P1TM1	Barinas	Parcelamiento	Silvatic palm	House Castillo	N8.09622 W-70.3829	20-1-03	Adult male	*R. prolixus* haplotype 15	415 bp
13123fpd	Barinas	19 Abril	Peridomestic	House 12	N8.09622 W-70.3829	-	Adult female	*R. prolixus* haplotype 14	415 bp
13124fpd	Barinas	19 Abril	Peridomestic	House 12	N8.09622 W-70.3829	-	Adult female	*R. prolixus* haplotype 14	415 bp
13125fpd	Barinas	19 Abril	Peridomestic	House 12	N8.09622 W-70.3829	-	Adult female	*R. prolixus* haplotype 14	415 bp
13126mpd	Barinas	19 Abril	Peridomestic	House 12	N8.09622 W-70.3829	-	Adult male	*R. prolixus* haplotype 14	415 bp
1312N1PD	Barinas	19 Abril	Peridomestic	House 12	N8.09622 W-70.3829	-	Nymph***	*R. prolixus* haplotype 14	415 bp
1312N2PD	Barinas	19 Abril	Peridomestic	House 12	N8.09622 W-70.3829	-	Nymph***	*R. prolixus* haplotype 14	415 bp
1312N7PD	Barinas	19 Abril	Peridomestic	House 12	N8.09622 W-70.3829	-	Nymph***	*R. prolixus* haplotype 14	415 bp
1312N8PD	Barinas	19 Abril	Peridomestic	House 12	N8.09622 W-70.3829	-	Nymph***	*R. prolixus* haplotype 14	415 bp

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage++	*Cytb* haplotype	Fragment size
13122fpd	Barinas	19 Abril	Peridomestic	House 12	N8.09622 W-70.3829	-	Adult female	*R. prolixus* haplotype 14	406 bp
13121fpd	Barinas	19 Abril	Peridomestic	House 12	N8.09622 W-70.3829	-	Adult female	*R. prolixus* haplotype 14	415 bp
18071FPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	-	Adult female	*R. prolixus* haplotype 1	415 bp
18078FPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	-	Adult female	*R. prolixus* haplotype 1	415 bp
18072NPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	-	Nymph***	*R. prolixus* haplotype 1	415 bp
18073NPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	-	Nymph***	*R. prolixus* haplotype 1	415 bp
18074NPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	-	Nymph***	*R. prolixus* haplotype 1	415 bp
18071NPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	-	Nymph***	*R. prolixus* haplotype 2	415 bp
18P1F3	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	-	Adult female	*R. prolixus* haplotype 1	415 bp
18P1F4	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	-	Adult female	*R. prolixus* haplotype 1	415 bp
18P1M6	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	-	Adult male	*R. prolixus* haplotype 1	415 bp
18P1M8	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	-	Adult male	*R. prolixus* haplotype 1	415 bp
18P1F2	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	-	Adult female	*R. prolixus* haplotype 2	415 bp
18P1M7	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	-	Adult male	*R. prolixus* haplotype 2	415 bp
18P2N3	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	-	Nymph***	*R. prolixus* haplotype 1	415 bp
18P2N2	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	-	Nymph***	*R. prolixus* haplotype 1	415 bp
18P2N1	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	-	Nymph***	*R. prolixus* haplotype 1	406 bp

^ Coordinates not always exact point of collection but within locality, some coordinates in decimal degrees. MC =no coordinate data available, ++ life stage at point of analysis not always known some adults may have been collected as nymphs, some adults sex not known. Nymph*= matured in lab to Adult prior to analysis, Nymph**= mature Nymphs on collection emerged as adults shortly after collection, Nymph***=analysed as Nymphs, Adult*** adults may have emerged from late stage nymphs, as adults were not kept separate from nymphs.

Table 64. Details of specimens used in microsatellite analysis.

Label	State	Locality	Ecotope	Location	GIS coordinates ^**	Collection date	Life Stage	Cytb haplotype
Portuguesa								
18 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***male	*R. robustus* haplotype 3
19 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***female	*R. robustus* haplotype 3
20 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***male	*R. robustus* haplotype 3
21 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***female	*R. prolixus* haplotype 1
22 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***female	*R. robustus* haplotype 3
23 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***male	*R. prolixus* haplotype 1
24 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***female	*R. robustus* haplotype 3
25 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***female	*R. prolixus* haplotype 1
26 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***	-
27 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***female	*R. robustus* haplotype 3
28 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***male	*R. robustus* haplotype 3
29 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***male	*R. robustus* haplotype 3
30 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***female	*R. prolixus* haplotype 1
31 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***male	*R. robustus* haplotype 3
32 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***	-
33 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***male	*R. prolixus* haplotype 1
34 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***	-
35 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***	-
36 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***	-
37 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***female	*R. robustus* haplotype 3
47 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***	-
48 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Nymph**	*R. prolixus* haplotype 1
49 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Nymph** male	*R. robustus* haplotype 3
55 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Nymph** female	*R. robustus* haplotype 3
57 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Nymph** male	*R. prolixus* haplotype 1
58 TERR	Portuguesa	Terronal	Domestic	House 1 01	N09°34.469' W069°20.595'	09/07/2001	Adult***	-

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage	Cytb haplotype
10 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***male	*R. prolixus* haplotype 1
11 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***female	*R. robustus* haplotype 3
14TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***	-
15 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***female	*R. robustus* haplotype 3
17 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***	*R. robustus* haplotype 3
1TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***female	*R. prolixus* haplotype 1
2 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***female	*R. prolixus* haplotype 1
4 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***	-
5 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***female	*R. prolixus* haplotype 1
52 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph** male	*R. prolixus* haplotype 1
53 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph** female	*R. prolixus* haplotype 1
6 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***	*R. robustus* haplotype 3
7TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***	*R. prolixus* haplotype 1
8 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***female	*R. robustus* haplotype 3
9 TERR	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Adult***female	*R. prolixus* haplotype 1
TERR2A	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph***	*R. prolixus* haplotype 1
TERR2B	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph***	*R. robustus* haplotype 3
TERR2E	Portuguesa	Terronal	Domestic	House 2 01	N09°34.689' W069°21.179'	09/07/2001	Nymph***	*R. prolixus* haplotype 1
134 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* male	*R. robustus* haplotype 3
136 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* male	*R. robustus* haplotype 3
138 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph*	*R. prolixus* haplotype 1
141 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph*	-
144 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* male	*R. prolixus* haplotype 1
145 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* female	*R. robustus* haplotype 3
148 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph*	-
150 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph*	-
152 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* male	*R. prolixus* haplotype 1

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage	Cytb haplotype
154 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* male	*R. prolixus* haplotype 1
155 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* male	*R. prolixus* haplotype 1
156 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* female	*R. prolixus* haplotype 1
157 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* female	*R. robustus* haplotype 3
158 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* female	*R. prolixus* haplotype 1
162 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* male	*R. prolixus* haplotype 1
163 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* male	*R. prolixus* haplotype 1
164 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* male	*R. prolixus* haplotype 1
168 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* male	*R. prolixus* haplotype 1
170 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* female	*R. prolixus* haplotype 1
173 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* male	*R. robustus* haplotype 3
174 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* female	*R. prolixus* haplotype 1
176 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* male	*R. prolixus* haplotype 1
179 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* female	*R. prolixus* haplotype 1
183 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* female	*R. prolixus* haplotype 1
187 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* male	*R. prolixus* haplotype 1
188 TERR	Portuguesa	Terronal	Silvatic Palm	House 2 01	N09°34.659' W069°21.355'	Jul-01	Nymph* female	*R. prolixus* haplotype 1
T1P11	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	Nov-03	Adult***female	*R. prolixus* haplotype 1
T1P12	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	Nov-03	Adult***male	*R. robustus* haplotype 3
T1P13	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	Nov-03	Adult***	*R. robustus* haplotype 3
T1P14	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	Nov-03	Adult***female	*R. prolixus* haplotype 1
T1P15	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	Nov-03	Adult***female	*R. prolixus* haplotype 1
T1P16	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	Nov-03	Adult***female	*R. robustus* haplotype 3
T1P4	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	Nov-03	Nymph***	*R. robustus* haplotype 3
T1P5	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	Nov-03	Adult***	*R. robustus* haplotype 3
T1P6	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	Nov-03	Adult***male	*R. robustus* haplotype 3
T1P7	Portuguesa	Terronal	Domestic	House 1 03	N09°34.469' W069°20.595'	Nov-03	Adult***female	*R. robustus* haplotype 3

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage	Cytb haplotype
T2P1	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***male	*R. prolixus* haplotype 1
T2P10	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Nymph***	-
T2P11	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Nymph***	-
T2P12	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Nymph***	*R. robustus* haplotype 3
T2P13	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Nymph***	*R. prolixus* haplotype 1
T2P14	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Nymph***	*R. robustus* haplotype 3
T2P15	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***female	*R. prolixus* haplotype 1
T2P17	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***female	*R. robustus* haplotype 3
T2P18	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***	*R. prolixus* haplotype 1
T2P19	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***	*R. robustus* haplotype 3
T2P2	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***female	*R. prolixus* haplotype 1
T2P20	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***	*R. prolixus* haplotype 1
T2P21	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***female	*R. prolixus* haplotype 1
T2P22	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***	-
T2P23	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***female	*R. prolixus* haplotype 1
T2P24	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***	-
T2P25	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***male	*R. prolixus* haplotype 1
T2P26	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***female	*R. prolixus* haplotype 1
T2P27	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***female	*R. prolixus* haplotype 1
T2P28	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Nymph***	*R. prolixus* haplotype 1
T2P29	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***female	*R. prolixus* haplotype 1
T2P30	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***female	*R. prolixus* haplotype 1
T2P31	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***female	*R. prolixus* haplotype 1
T2P32	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***female	*R. prolixus* haplotype 1
T2P35	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***male	*R. prolixus* haplotype 1
T2P36	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***male	*R. prolixus* haplotype 1
T2P37	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***female	*R. prolixus* haplotype 1
T2P38	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***	*R. prolixus* haplotype 1
T2P39	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***female	*R. prolixus* haplotype 1

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage	Cytb haplotype
T2P4	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Nymph***	*R. robustus* haplotype 3
T2P41	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***	*R. prolixus* haplotype 1
T2P42	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***	-
T2P43	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***female	*R. prolixus* haplotype 1
T2P44	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***male	*R. prolixus* haplotype 1
T2P5	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Nymph***	*R. robustus* haplotype 3
T2P6	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Adult***	*R. robustus* haplotype 3
T2P7	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Nymph***	*R. prolixus* haplotype 1
T2P8	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Nymph***	*R. prolixus* haplotype 1
T2P9	Portuguesa	Terronal	Silvatic palm	House 2 03	N09°34.689' W069°21.179'	Nov-03	Nymph***	*R. prolixus* haplotype 1
POSB19A	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Adult***male	*R. prolixus* haplotype 1
POSB19B	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Adult***male	*R. prolixus* haplotype 1
POSB19C	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Adult***male	*R. prolixus* haplotype 1
POSB19D	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1
POSB19E	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Adult***	*R. prolixus* haplotype 1
POSB19F	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Adult***female	*R. prolixus* haplotype 1
POSB19G	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Adult***male	*R. prolixus* haplotype 1
POSB19H	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1
POSB19I	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1
POSB19J	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1
POSB19K	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Adult***female	*R. prolixus* haplotype 1
POSB19L	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1
POSB19M	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1
POSB19N	Portuguesa	San Bartolo	Domestic	House 1	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1
POSBMAR10	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	-
POSBMAR11	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1
POSBMAR12	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1
POSBMAR14	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Adult***	-

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage	Cytb haplotype
POSBMAR15	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Adult***	*R. prolixus* haplotype 1
POSBMAR16	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Adult***	-
POSBMAR17	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Adult***male	*R. prolixus* haplotype 1
POSBMAR18	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Adult***	-
POSBMAR19	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Adult***female	*R. prolixus* haplotype 1
POSBMAR2	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1
POSBMAR4	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1
POSBMAR5	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1
POSBMAR6	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	*R. prolixus* haplotype 1
POSBMAR9	Portuguesa	San Bartolo	Domestic	House 2	N09°27.218' W069°32.745'	03/07/2002	Nymph***	-
POSL89A	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Nymph***	*R. prolixus* haplotype 9
POSL89B	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Nymph***	*R. prolixus* haplotype 9
POSL89C	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Adult***	*R. prolixus* haplotype 9
POSL89D	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Adult***female	*R. prolixus* haplotype 9
POSL89E	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Adult***female	*R. prolixus* haplotype 9
POSL89F	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Nymph***	*R. prolixus* haplotype 9
POSL89G	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Nymph***	*R. prolixus* haplotype 9
POSL89H	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Nymph***	*R. prolixus* haplotype 9
POSL89J	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Nymph***	*R. prolixus* haplotype 9
POSL89K	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Adult***female	*R. prolixus* haplotype 9
POSL89L	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Adult***female	*R. prolixus* haplotype 9
POSL89M	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Nymph***	*R. prolixus* haplotype 1
POSL89N	Portuguesa	Santa Lucia	Domestic	House 89	N 9.4102 W -69.575	01/07/2002	Adult***female	*R. prolixus* haplotype 1
CP7	Portuguesa	Casa Rena	Domestic	M. Barreto	N9.6032 W-69.36	Dec-03	Nymph***	*R. robustus* haplotype 3
CP8	Portuguesa	Casa Rena	Domestic	M. Barreto	N9.6032 W-69.36	Dec-03	Nymph***	*R. robustus* haplotype 3
CP9	Portuguesa	Casa Rena	Domestic	M. Barreto	N9.6032 W-69.36	Dec-03	Nymph***	*R. robustus* haplotype 3
CP10	Portuguesa	Casa Rena	Domestic	M. Barreto	N9.6032 W-69.36	Dec-03	Adult***female	*R. robustus* haplotype 3

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage	Cytb haplotype
CP11	Portuguesa	Casa Rena	Domestic	M. Barreto	N9.6032 W-69.36	Dec-03	Adult***female	*R. robustus* haplotype 3
CP12	Portuguesa	Casa Rena	Domestic	M. Barreto	N9.6032 W-69.36	Dec-03	Adult***	-
CP13	Portuguesa	Casa Rena	Domestic	M. Barreto	N9.6032 W-69.36	Dec-03	Adult***	*R. robustus* haplotype 3
CP14	Portuguesa	Casa Rena	Domestic	M. Barreto	N9.6032 W-69.36	Dec-03	Adult***female	*R. robustus* haplotype 3
CP15	Portuguesa	Casa Rena	Domestic	M. Barreto	N9.6032 W-69.36	Dec-03	Adult***female	*R. robustus* haplotype 3
CP16	Portuguesa	Casa Rena	Domestic	M. Barreto	N9.6032 W-69.36	Dec-03	Adult***female	*R. robustus* haplotype 3
CP17	Portuguesa	Casa Rena	Domestic	M. Barreto	N9.6032 W-69.36	Dec-03	Adult***female	*R. robustus* haplotype 3
L2P1	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-
L2P10	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-
L2P11	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-
L2P12	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-
L2P13	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	nymph***	-
L2P14	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-
L2P15	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-
L2P16	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-
L2P17	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	nymph***	-
L2P18	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-
L2P19	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-
L2P20	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-
L2P21	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	nymph***	-
L2P22	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-
L2P24	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-
L2P25	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-
L2P26	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	nymph***	-
L2P4	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-
L2P7	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-
L2P8	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-
L2P2	Portuguesa	Laurianito	Peridomestic	House 1	MC*	Dec-03	Adult***	-

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage	Cytb haplotype
109 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph*	-
110 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph* female	*R. prolixus* haplotype 1
117 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph*	-
121 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph* female	*R. robustus* haplotype 3
138 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph*	-
147 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph* female	*R. robustus* haplotype 3
149 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph* male	*R. prolixus* haplotype 1
15 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph* female	*R. prolixus* haplotype 1
153 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph* female	*R. prolixus* haplotype 1
167 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph*	-
169 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph*	-
178 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph*	-
185 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph* female	*R. robustus* haplotype 3
189 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph*	-
190 PALO	Portuguesa	Palo Gacho	Silvatic Palm	Not near house	N09°35.325' W069°20.737'	17/07/2001	Nymph*	-
RASH1	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Adult male	*R. prolixus* haplotype 1
RASH2	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH3	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH4	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH5	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH6	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH7	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 2
RASH8	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH9	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH10	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH11	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH12	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage	Cytb haplotype
RASH13	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	-
RASH14	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH15	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	-
RASH16	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH17	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH18	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH19	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH20	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 2
RASH21	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH22	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH23	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASH24	Portuguesa	Las Rastrojas	Domestic	J. P. Delgado	N0917.636 W06958.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASP1	Portuguesa	Las Rastrojas	Silvatic Palm	J. P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASP2	Portuguesa	Las Rastrojas	Silvatic Palm	J. P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASP3	Portuguesa	Las Rastrojas	Silvatic Palm	J. P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASP4	Portuguesa	Las Rastrojas	Silvatic Palm	J. P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASP5	Portuguesa	Las Rastrojas	Silvatic Palm	J. P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASP6	Portuguesa	Las Rastrojas	Silvatic Palm	J. P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASP7	Portuguesa	Las Rastrojas	Silvatic Palm	J. P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASP8	Portuguesa	Las Rastrojas	Silvatic Palm	J. P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASP9	Portuguesa	Las Rastrojas	Silvatic Palm	J. P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	-
RASP10	Portuguesa	Las Rastrojas	Silvatic Palm	J. P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASP11	Portuguesa	Las Rastrojas	Silvatic Palm	J. P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	*R. prolixus* haplotype 1
RASP12	Portuguesa	Las Rastrojas	Silvatic Palm	J. P. Delgado	N09 17.627 W069 58.615	06/10/2004	Nymph***	-
Lara								
102 Lara	Lara State	Salvador	Domestic	house 2	N09°45.858' W069°20.757'	02/08/2001	Adult***male	*R. prolixus* haplotype 1
103 Lara	Lara State	Salvador	Domestic	house 2	N09°45.858' W069°20.757'	02/08/2002	Adult***male	*R. prolixus* haplotype 1
122 Lara	Lara State	Guamito	Domestic	house 1	N09°45.858' W069°20.757'	01/08/2001	Nymph* female	*R. prolixus* haplotype 1
123 Lara	Lara State	Guamito	Domestic	house 1	N09°45.858' W069°20.757'	01/08/2001	Nymph* female	*R. prolixus* haplotype 1

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage	Cytb haplotype
125 Lara	Lara State	Guamito	Domestic	house 1	N09°45.858' W069°20.757'	01/08/2001	Nymph* male	*R. prolixus* haplotype 1
126 Lara	Lara State	Guamito	Domestic	house 1	N09°45.858' W069°20.757'	01/08/2001	Nymph* male	*R. prolixus* haplotype 1
129 Lara	Lara State	Guamito	Domestic	house 1	N09°45.858' W069°20.757'	01/08/2001	Nymph* male	*R. prolixus* haplotype 1
130 Lara	Lara State	Guamito	Domestic	house 1	N09°45.858' W069°20.757'	01/08/2001	Nymph* female	*R. prolixus* haplotype 1
131 Lara	Lara State	Guamito	Domestic	house 1	N09°45.858' W069°20.757'	01/08/2001	Nymph* female	*R. prolixus* haplotype 1
79 Lara	Lara State	Guamito	Domestic	house 1	N09°45.858' W069°20.757'	01/08/2001	Adult***male	*R. prolixus* haplotype 1
81 Lara	Lara State	Guamito	Domestic	house 1	N09°45.858' W069°20.757'	01/08/2001	Adult***female	*R. prolixus* haplotype 1
84 Lara	Lara State	Guamito	Domestic	house 1	N09°45.858' W069°20.757'	01/08/2001	Adult***female	*R. prolixus* haplotype 1
90 Lara	Lara State	Guamito	Domestic	house 1	N09°45.858' W069°20.757'	01/08/2001	Adult***female	*R. prolixus* haplotype 1
94 Lara	Lara State	Guamito	Domestic	house 1	N09°45.858' W069°20.757'	01/08/2001	Adult***female	*R. prolixus* haplotype 1
95 Lara	Lara State	Guamito	Domestic	house 1	N09°45.858' W069°20.757'	01/08/2001	Adult***male	*R. prolixus* haplotype 1
96 Lara	Lara State	Guamito	Domestic	house 1	N09°45.858' W069°20.757'	01/08/2001	Adult***male	*R. prolixus* haplotype 1
98 Lara	Lara State	Guamito	Domestic	house 1	N09°45.858' W069°20.757'	01/08/2001	Adult***male	*R. prolixus* haplotype 1
Cojedes								
COJ1P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Adult***female	*R. prolixus* haplotype 1
COJ2P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ3P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Adult***male	*R. prolixus* haplotype 1
COJ4P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Adult***male	*R. prolixus* haplotype 1
COJ5P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Adult***female	*R. prolixus* haplotype 1
COJ6P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Adult***male	*R. prolixus* haplotype 1
C0J7P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Adult***male	*R. prolixus* haplotype 1
C0J8P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Adult***female	*R. robustus* haplotype 3
COJ9P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Adult***female	*R. prolixus* haplotype 1
COJ10P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Adult***male	*R. prolixus* haplotype 1
COJ11P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Adult***male	*R. prolixus* haplotype 1
COJ12P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ13P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ14P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ15P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Adult***female	*R. prolixus* haplotype 1

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage	Cytb haplotype
COJ16P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Adult***male	*R. prolixus* haplotype 1
COJ17P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Adult***male	*R. prolixus* haplotype 1
COJ18P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1
C0J19P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Adult***male	*R. prolixus* haplotype 1
C0J20P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Adult***male	*R. robustus* haplotype 3
COJ21P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ22P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ23P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ24P	Cojedes	Las Queseras	Silvatic Palm	J. P Arraez	N09°47.304 W068°19.916	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ1H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Adult***male	*R. prolixus* haplotype 1
COJ2H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	-
COJ3H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Adult***male	*R. prolixus* haplotype 1
COJ4H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ5H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph** female	*R. prolixus* haplotype 1
COJ6H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph**male	*R. prolixus* haplotype 1
C0J7H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph**male	*R. prolixus* haplotype 1
C0J8H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ9H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ10H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ11H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Adult***male	*R. prolixus* haplotype 1
COJ12H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Adult***female	*R. prolixus* haplotype 1
COJ13H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ14H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ15H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ16H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	-
COJ17H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ18H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1
C0J19H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1
C0J20H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage	Cytb haplotype
COJ21H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ22H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ23H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1
COJ24H	Cojedes	Las Queseras	Domestic	J. P Arraez	N09°47.301 W068°19.892	16/09/2004	Nymph***	*R. prolixus* haplotype 1
Trujillo								
LDAT1	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult female	*R. robustus* haplotype 16
LDAT10	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***male	*R. prolixus* haplotype 5
LDAT11	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	-
LDAT12	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***male	*R. prolixus* haplotype 5
LDAT13	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***female	*R. prolixus* haplotype 5
LDAT14	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***female	*R. prolixus* haplotype 5
LDAT15	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	-
LDAT16	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***	-
LDAT17	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	*R. prolixus* haplotype 5
LDAT18	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	*R. prolixus* haplotype 5
LDAT19	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***female	*R. prolixus* haplotype 5
LDAT2	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	*R. prolixus* haplotype 5
LDAT20	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***male	*R. prolixus* haplotype 5
LDAT21	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***male	*R. prolixus* haplotype 5
LDAT22	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***male	*R. prolixus* haplotype 5
LDAT23	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***male	*R. prolixus* haplotype 5
LDAT24	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***female	*R. prolixus* haplotype 5
LDAT25	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***male	*R. prolixus* haplotype 5
LDAT26	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***male	*R. prolixus* haplotype 5
LDAT3	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	*R. prolixus* haplotype 5
LDAT4	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	*R. prolixus* haplotype 5
LDAT5	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	-
LDAT6	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	*R. prolixus* haplotype 5
LDAT7	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	*R. prolixus* haplotype 5

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage	Cytb haplotype
LDAT8	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Adult***	-
LDAT9	Trujillo	Loma de Amarillo	Domestic	House 1	N 09.5 W -70.417	10/11/2003	Nymph***	*R. prolixus* haplotype 5
Barinas								
01071fpd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Adult Female	*R. prolixus* haplotype 1
01072fpd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Adult female	*R. prolixus* haplotype 1
01073fpd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Adult female	*R. prolixus* haplotype 1
01075fpd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Adult female	*R. prolixus* haplotype 1
01076fpd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Adult female	*R. prolixus* haplotype 2
01077fpd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Adult female	*R. prolixus* haplotype 1
010710mpd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Adult male	-
01071npd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Nymph***	-
01072npd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Nymph***	*R. prolixus* haplotype 2
01074npd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Nymph***	*R. prolixus* haplotype 4
01075npd	Barinas	Cascabel	Peridomestic	House 7	N 8.174181 W -69.74433	28-4-03	Nymph***	*R. prolixus* haplotype 2
01231fh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult female	*R. prolixus* haplotype 1
01232fh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult female	*R. prolixus* haplotype 1
01233fh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult female	*R. prolixus* haplotype 1
01234fh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult female	*R. prolixus* haplotype 5
01235fh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult female	-
01236mh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult male	*R. prolixus* haplotype 1
01237mh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult male	*R. prolixus* haplotype 1
01238mh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult male	*R. prolixus* haplotype 2
01239mh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Adult male	*R. prolixus* haplotype 1
01231nh	Barinas	Cascabel	Domestic	House 23	N 8.174181 W -69.74433	29-4-03	Nymph***	-
01p1f2	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Adult female	*R. prolixus* haplotype 1
01p1f4	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Adult female	*R. prolixus* haplotype 2
01p1m5	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Adult male	*R. prolixus* haplotype 1
01p1m6	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Adult male	*R. prolixus* haplotype 2
01p1m7	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Adult male	*R. prolixus* haplotype 1

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage	Cytb haplotype
01p1tm1	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Adult male	*R. prolixus* haplotype 1
01p1n1	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	-
01p1n2	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	-
01p1n3	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 1
01p1n4	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 12
01p1n5	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 1
01p1n6	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 2
01p1n7	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 5
01p1n25	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 2
01p1n8	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 1
01p1n9	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 2
01p1n10	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	-
01p1n11	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	*R. prolixus* haplotype 2
01p1n12	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	-
01p1n13	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	-
01p1n14	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	-
01p1n15	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	-
01p1n16	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	-
01p1n17	Barinas	Cascabel	Silvatic Palm	House 2 Palm 1	N 8.174181 W -69.74433	8-10-03	Nymph***	-
06091fh	Barinas	Guaranda	Domestic	House 9	N8.084781 W-69.7035	-	Adult female	-
06092fh	Barinas	Guaranda	Domestic	House 9	N8.084781 W-69.7035	-	Adult female	-
06093fh	Barinas	Guaranda	Domestic	House 9	N8.084781 W-69.7035	-	Adult female	-
06094fh	Barinas	Guaranda	Domestic	House 9	N8.084781 W-69.7035	-	Adult female	-
06095fh	Barinas	Guaranda	Domestic	House 9	N8.084781 W-69.7035	-	Adult female	*R. prolixus* haplotype 1
06096fh	Barinas	Guaranda	Domestic	House 9	N8.084781 W-69.7035	-	Adult female	-
06097fh	Barinas	Guaranda	Domestic	House 9	N8.084781 W-69.7035	-	Adult female	*R. prolixus* haplotype 1
06098fh	Barinas	Guaranda	Domestic	House 9	N8.084781 W-69.7035	-	Adult female	*R. prolixus* haplotype 1
06091nh	Barinas	Guaranda	Domestic	House 9	N8.084781 W-69.7035	-	Nymph***	*R. prolixus* haplotype 1

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06092nh	Barinas	Guaranda	Domestic	House 9	N8.084781 W-69.7035	-	Nymph***	*R. prolixus* haplotype 1
06093nh	Barinas	Guaranda	Domestic	House 9	N8.084781 W-69.7035	-	Nymph***	-
06p2f1	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Adult female	-
06p2m2	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Adult male	*R. prolixus* haplotype 1
06p2m3	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Adult male	*R. prolixus* haplotype 1
06p2m4	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Adult male	*R. prolixus* haplotype 1
06p2m5	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Adult male	*R. prolixus* haplotype 10
06p2n1	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	*R. prolixus* haplotype 1
06p2n3	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	*R. prolixus* haplotype 2
06p2n4	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	*R. prolixus* haplotype 1
06p2n5	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	*R. prolixus* haplotype 1
06p2n7	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	*R. prolixus* haplotype 1
06p2n8	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	*R. prolixus* haplotype 1
06p2n9	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	*R. prolixus* haplotype 1
06p2n10	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	-
06p2n11	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	-
06p2n12	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	-
06p2n14	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	-
06p2n15	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	-
06p2n16	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	-
06p2n17	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	-
06p2n18	Barinas	Guaranda	Silvatic Palm	House Quintero	N8.084781 W-69.7035	24-10-03	Nymph***	-
07031MH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult male	-
07032MH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult male	*R. prolixus* haplotype 1
07033MH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult male	*R. prolixus* haplotype 1
07034MH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult male	*R. prolixus* haplotype 5
07035MH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult male	-
07036MH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult male	*R. prolixus* haplotype 1

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07038FH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult female	*R. prolixus* haplotype 1
07039FH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult female	-
070310FH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult female	-
070311FH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult female	*R. prolixus* haplotype 1
070312FH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult female	*R. prolixus* haplotype 1
070313MH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult male	*R. prolixus* haplotype 1
070314MH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult male	*R. prolixus* haplotype 1
070315FH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult female	*R. prolixus* haplotype 1
070317FH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult female	-
070318FH	Barinas	Laguna Hermosa	Domestic	House 3	N 8.089 W-69.8314	29-1-03	Adult female	-
07P2F1	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Adult female	*R. prolixus* haplotype 1
07P2F2	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Adult female	*R. prolixus* haplotype 5
07P2F3	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Adult female	*R. prolixus* haplotype 1
07P2F4	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Adult female	-
07P2N1	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	-
07P2N2	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	*R. prolixus* haplotype 2
07P2N3	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	*R. prolixus* haplotype 1
07P2N4	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	*R. prolixus* haplotype 1
07P2N5	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	*R. prolixus* haplotype 1
07P2N6	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	*R. prolixus* haplotype 11
07P2N7	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	-
07P2N8	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	*R. prolixus* haplotype 1
07P2N9	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	-
07P2N10	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	-
07P2N11	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	-
07P2N12	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	-
07P2N13	Barinas	Laguna Hermosa	Silvatic palm	House Monagas	N 8.089 W-69.8314	29-10-03	Nymph***	-
07292FPD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Adult female	-
07293FPD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Adult female	*R. prolixus* haplotype 1

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07294FPD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Adult female	-
07295MPD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Adult male	*R. prolixus* haplotype 1
07296MPD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Adult male	*R. prolixus* haplotype 12
07297MPD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Adult male	*R. prolixus* haplotype 5
0729N1PD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Nymph***	*R. prolixus* haplotype 1
0729N2PD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Nymph***	*R. prolixus* haplotype 1
0729N3PD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Nymph***	*R. prolixus* haplotype 1
0729N4PD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Nymph***	-
0729N5PD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Nymph***	-
0729N6PD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Nymph***	-
0729N7PD	Barinas	Laguna Hermosa	Peridomestic	House 29	N 8.089 W-69.8314	-	Nymph***	-
12111FH	Barinas	G. Paraguey	Domestic	House 1	N8.1531 W-69.919	-	Adult female	-
12112FH	Barinas	G. Paraguey	Domestic	House 1	N8.1531 W-69.919	-	Adult female	*R. prolixus* haplotype 1
12113FH	Barinas	G. Paraguey	Domestic	House 1	N8.1531 W-69.919	-	Adult female	-
12114FH	Barinas	G. Paraguey	Domestic	House 1	N8.1531 W-69.919	-	Adult female	*R. prolixus* haplotype 1
12116FH	Barinas	G. Paraguey	Domestic	House 1	N8.1531 W-69.919	-	Adult female	*R. prolixus* haplotype 1
12117FH	Barinas	G. Paraguey	Domestic	House 1	N8.1531 W-69.919	-	Adult female	-
12118FH	Barinas	G. Paraguey	Domestic	House 1	N8.1531 W-69.919	-	Adult female	-
12119FH	Barinas	G. Paraguey	Domestic	House 1	N8.1531 W-69.919	-	Adult female	-
121110FH	Barinas	G. Paraguey	Domestic	House 1	N8.1531 W-69.919	-	Adult female	-
121111FH	Barinas	G. Paraguey	Domestic	House 1	N8.1531 W-69.919	-	Adult female	-
121113FH	Barinas	G. Paraguey	Domestic	House 1	N8.1531 W-69.919	-	Adult female	-
12131FH	Barinas	G. Paraguey	Domestic	House 2	N8.1531 W-69.919	-	Adult female	*R. prolixus* haplotype 2
12132FH	Barinas	G. Paraguey	Domestic	House 2	N8.1531 W-69.919	-	Adult female	*R. prolixus* haplotype 1
12133FH	Barinas	G. Paraguey	Domestic	House 2	N8.1531 W-69.919	-	Adult female	-
12134FH	Barinas	G. Paraguey	Domestic	House 2	N8.1531 W-69.919	-	Adult female	*R. prolixus* haplotype 1
12135FH	Barinas	G. Paraguey	Domestic	House 2	N8.1531 W-69.919	-	Adult female	*R. prolixus* haplotype 5
12136FH	Barinas	G. Paraguey	Domestic	House 2	N8.1531 W-69.919	-	Adult female	*R. prolixus* haplotype 1

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage	Cytb haplotype
12137FH	Barinas	G. Paraguey	Domestic	House 2	N8.1531 W-69.919	-	Adult female	-
12138FH	Barinas	G. Paraguey	Domestic	House 2	N8.1531 W-69.919	-	Adult female	-
12139FH	Barinas	G. Paraguey	Domestic	House 2	N8.1531 W-69.919	-	Adult female	-
121310MH	Barinas	G. Paraguey	Domestic	House 2	N8.1531 W-69.919	-	Adult male	-
121311MH	Barinas	G. Paraguey	Domestic	House 2	N8.1531 W-69.919	-	Adult male	-
121312MH	Barinas	G. Paraguey	Domestic	House 2	N8.1531 W-69.919	-	Adult male	-
12P1TN1	Barinas	G. Paraguey	Silvatic palm	Palm 1	N8.1531 W-69.919	-	Nymph***	-
12P1TN2	Barinas	G. Paraguey	Silvatic palm	Palm 1	N8.1531 W-69.919	-	Nymph***	-
12P1TN3	Barinas	G. Paraguey	Silvatic palm	Palm 1	N8.1531 W-69.919	-	Nymph***	-
12P1TN4	Barinas	G. Paraguey	Silvatic palm	Palm 1	N8.1531 W-69.919	-	Nymph***	-
12P1TN5	Barinas	G. Paraguey	Silvatic palm	Palm 1	N8.1531 W-69.919	-	Nymph***	-
12P1N6	Barinas	G. Paraguey	Silvatic palm	Palm 1	N8.1531 W-69.919	-	Nymph***	-
12P1N7	Barinas	G. Paraguey	Silvatic palm	Palm 1	N8.1531 W-69.919	-	Nymph***	-
12P1N8	Barinas	G. Paraguey	Silvatic palm	Palm 1	N8.1531 W-69.919	-	Nymph***	-
12P1N9	Barinas	G. Paraguey	Silvatic palm	Palm 1	N8.1531 W-69.919	-	Nymph***	-
12P1N10	Barinas	G. Paraguey	Silvatic palm	Palm 1	N8.1531 W-69.919	-	Nymph***	-
12P1N11	Barinas	G. Paraguey	Silvatic palm	Palm 1	N8.1531 W-69.919	-	Nymph***	-
12P1N12	Barinas	G. Paraguey	Silvatic palm	Palm 1	N8.1531 W-69.919	-	Nymph***	-
12P2N1	Barinas	G. Paraguey	Silvatic palm	Palm 2	N8.1531 W-69.919	-	Nymph***	-
12P2N2	Barinas	G. Paraguey	Silvatic palm	Palm 2	N8.1531 W-69.919	-	Nymph***	-
12P2N3	Barinas	G. Paraguey	Silvatic palm	Palm 2	N8.1531 W-69.919	-	Nymph***	-
12P2N4	Barinas	G. Paraguey	Silvatic palm	Palm 2	N8.1531 W-69.919	-	Nymph***	-
12P2N5	Barinas	G. Paraguey	Silvatic palm	Palm 2	N8.1531 W-69.919	-	Nymph***	-
12P2N6	Barinas	G. Paraguey	Silvatic palm	Palm 2	N8.1531 W-69.919	-	Nymph***	-
12P2N7	Barinas	G. Paraguey	Silvatic palm	Palm 2	N8.1531 W-69.919	-	Nymph***	-
12P2N8	Barinas	G. Paraguey	Silvatic palm	Palm 2	N8.1531 W-69.919	-	Nymph***	-
12P2N9	Barinas	G. Paraguey	Silvatic palm	Palm 2	N8.1531 W-69.919	-	Nymph***	-
12P2N11	Barinas	G. Paraguey	Silvatic palm	Palm 2	N8.1531 W-69.919	-	Nymph***	-
12P2F1	Barinas	G. Paraguey	Silvatic palm	Palm 2	N8.1531 W-69.919	-	Nymph***	-

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage	Cytb haplotype
13121fpd	Barinas	19 Abril	Domestic	House 12	N8.09622 W-70.3829	-	Adult female	*R. prolixus* haplotype 14
13122fpd	Barinas	19 Abril	Domestic	House 12	N8.09622 W-70.3829	-	Adult female	*R. prolixus* haplotype 14
13123fpd	Barinas	19 Abril	Domestic	House 12	N8.09622 W-70.3829	-	Adult female	*R. prolixus* haplotype 14
13124fpd	Barinas	19 Abril	Domestic	House 12	N8.09622 W-70.3829	-	Adult female	*R. prolixus* haplotype 14
13125fpd	Barinas	19 Abril	Domestic	House 12	N8.09622 W-70.3829	-	Adult female	*R. prolixus* haplotype 14
13126mpd	Barinas	19 Abril	Domestic	House 12	N8.09622 W-70.3829	-	Adult male	*R. prolixus* haplotype 14
1312N1PD	Barinas	19 Abril	Domestic	House 12	N8.09622 W-70.3829	-	Nymph***	*R. prolixus* haplotype 14
1312N2PD	Barinas	19 Abril	Domestic	House 12	N8.09622 W-70.3829	-	Nymph***	*R. prolixus* haplotype 14
1312N3PD	Barinas	19 Abril	Domestic	House 12	N8.09622 W-70.3829	-	Nymph***	-
1312N4PD	Barinas	19 Abril	Domestic	House 12	N8.09622 W-70.3829	-	Nymph***	-
1312N6PD	Barinas	19 Abril	Domestic	House 12	N8.09622 W-70.3829	-	Nymph***	-
1312N7PD	Barinas	19 Abril	Domestic	House 12	N8.09622 W-70.3829	-	Nymph***	*R. prolixus* haplotype 14
1312N8PD	Barinas	19 Abril	Domestic	House 12	N8.09622 W-70.3829	-	Nymph***	*R. prolixus* haplotype 14
13P2F1	Barinas	Parcelamiento	Silvatic palm	House Peralta	N8.09622 W-70.3829	20-1-03	Adult female	*R. prolixus* haplotype 14
13P2F2	Barinas	Parcelamiento	Silvatic palm	House Peralta	N8.09622 W-70.3829	20-1-03	Adult female	*R. prolixus* haplotype 1
13P2N1	Barinas	Parcelamiento	Silvatic palm	House Peralta	N8.09622 W-70.3829	20-1-03	Nymph***	*R. prolixus* haplotype 1
13P2N2	Barinas	Parcelamiento	Silvatic palm	House Peralta	N8.09622 W-70.3829	20-1-03	Nymph***	*R. prolixus* haplotype 1
13P2N3	Barinas	Parcelamiento	Silvatic palm	House Peralta	N8.09622 W-70.3829	20-1-03	Nymph***	*R. prolixus* haplotype 14
13P2N4	Barinas	Parcelamiento	Silvatic palm	House Peralta	N8.09622 W-70.3829	20-1-03	Nymph***	*R. prolixus* haplotype 1
13P2N5	Barinas	Parcelamiento	Silvatic palm	House Peralta	N8.09622 W-70.3829	20-1-03	Nymph***	-
13P2N6	Barinas	Parcelamiento	Silvatic palm	House Peralta	N8.09622 W-70.3829	20-1-03	Nymph***	*R. prolixus* haplotype 1
13P2N8	Barinas	Parcelamiento	Silvatic palm	House Peralta	N8.09622 W-70.3829	20-1-03	Nymph***	-
13P2N9	Barinas	Parcelamiento	Silvatic palm	House Peralta	N8.09622 W-70.3829	20-1-03	Nymph***	-
13P2N10	Barinas	Parcelamiento	Silvatic palm	House Peralta	N8.09622 W-70.3829	20-1-03	Nymph***	-
13P2N11	Barinas	Parcelamiento	Silvatic palm	House Peralta	N8.09622 W-70.3829	20-1-03	Nymph***	-
13P2N12	Barinas	Parcelamiento	Silvatic palm	House Peralta	N8.09622 W-70.3829	20-1-03	Nymph***	-

Label	State	Locality	Ecotope	Location	GIS coordinates ^*	Collection date	Life Stage	Cytb haplotype
18071FPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Adult female	*R. prolixus* haplotype 1
18072FPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Adult female	-
18074FPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Adult female	-
18078FPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Adult female	*R. prolixus* haplotype 1
18079FPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Adult female	-
180711FPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Adult female	-
180713FPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Adult female	-
180714FPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Adult female	-
180717MPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Adult male	-
180719MPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Adult male	-
180720MPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Adult male	-
180722MPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Adult male	-
180724MPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Adult male	-
18071NPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Nymph***	*R. prolixus* haplotype 2
18072NPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Nymph***	*R. prolixus* haplotype 1
18073NPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Nymph***	*R. prolixus* haplotype 1
18074nPD	Barinas	Rio Bravo II	Peridomestic	House 7	N8.08521 W-69.7339	2002	Nymph***	*R. prolixus* haplotype 1
18P1F1	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	2002	Adult female	-
18P1F2	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	2002	Adult female	*R. prolixus* haplotype 2
18P1F3	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	2002	Adult female	*R. prolixus* haplotype 1
18P1F4	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	2002	Adult female	*R. prolixus* haplotype 1
18P1M6	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	2002	Adult male	*R. prolixus* haplotype 1
18P1M7	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	2002	Adult male	*R. prolixus* haplotype 2
18P1M8	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	2002	Adult male	*R. prolixus* haplotype 1
18P1M1	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	2002	Adult male	-
18P2N1	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	2002	Nymph***	*R. prolixus* haplotype 1
18P2N2	Barinas	Rio Bravo II	Silvatic palm	House 7	N8.08521 W-69.7339	2002	Nymph***	*R. prolixus* haplotype 1

^ Coordinates not always exact point of collection but within locality, some coordinates in decimal degrees. MC =no coordinate data available, ++ life stage at point of analysis not always known some adults may have been collected as nymphs, some adults sex not known. Nymph*= matured in lab to Adult prior to analysis, Nymph**= mature Nymphs on collection emerged as adults shortly after collection, Nymph***=analysed as Nymphs, Adult*** adults may have emerged from late stage nymphs, as adults were not kept separate from nymphs.

Table 65. All 52 primer pairs designed, ordered (MWG Biotech) and tested by PCR

Primer sequence	Sequence (5'-3')	Cloned allele size (bp)	Microsatellite Library	Repeat motif	Annealing temp (C°)
List14-001F *	CCC AAT ACA ACA CCC AAT ACC	-	Library 2 plate 1	GT	52
List14-001R *	GTG ACG GTG CCA TGT TAG G				
List14-002F	GCT GAA AAT GAG CAA AAA CGG	211	Library 3 plate 1	[CTT] N16 [GTT]6	53
List14-002R	ATC GAG ACC CCC AAA AGG C				
List14-003F	ACT TCT CTT GCC TTT GAC TCC	189	Library 3 plate 1	[CTT]7[TCT]18	54
List14-003R	CGG AAG GAA GTC TAA AAC TCG				
List14-004F	ATA CCT ACC CAG TAA AAA GC	211	Library 3 plate 1	[GT]18	52
List14-004R	GAG AAG TTG AAA AGT TGA CC				
List14-005F	TCG GTT CAC GCG TTT AGG CG	233	Library 3 plate 1	[CAA]5[GAA]12	Failed at 50 52 53 55
List14-005R	GAT CTC AGG AGG TAG TTC AGG				
List14-007F	CTTTTCCGTCTTGCAGGAAGG	185	Library 1 plate 1	[CT]16 [CA]14	55
List14-007R	CCGGTTGACCCGTTAGTTGG				
List14-009F	ATGGTAAAAGTCGCAAAGCCGG	213	Library 1plate 3	[GT]12[AT]2	55
List14-009R	TTTCTGCTAAAGCTGTGCCCGG				
List14-010F	AATGATGACTGTATTGATGGGC	322	Library1 plate 3	[CA]9	55
List14-010R	TTCGACCAACAACAACTTCCC				
List14-013F	CATACTACACGCACACAAGACC	341	Library1 plate 1	[AC]10	55
List14-013R	ATACTCGCATCAAGCCATTTGG				
List14-014F	TTCTGTTTCTCTGATTCCAGG	301	Library1 plate 1	[CA]18	55
List14-014R	ACGTGTTGTGGTCTCTCG				
List14-016F	ATAATACTAAAGGTGCCGATGG	206	Library1 plate 3	[AC]24	Failed at 50 52 53 55
List14-016R	TGTATTGTCTCAGTTGAACACC				
List14-017F	ATTGAAGGTTACTACTTGCTGC	161	Library1 plate 1	[TG]12	55
List14-017R	ACGCTGCTTCATTTTTTAGTGG				

Primer sequence	Sequence (5'-3')	Cloned allele size (bp)	Microsatellite Library	Repeat motif	Annealing temp (C°)
List14-019F	CTCTGTTAGTAGATTGTGGAGG	149	Library 1plate 3	[GT]11	55
List14-019R	CGCAACTGCTTTGGGTTTAGC				
List14-021F	AACCTCTGAACACATCAAATGG	297	Library 1 plate 1	[TG]10	55
List14-021R	AGCTACCTCTTGCCTCTACG				
List14-025F	CCGCTCTATCAACTACTCC	180	library 1 plate 3	$[TC]^{9}$[AC]7N8[AC]7	50
List14-025R	GATCCCTTATGTTTCTCAGC				
List14-028F	AAATAGAGCAGCGTTGGACG	293	Library 1 plate 1	[TG]19	55
List14-028R	CTTGCAGACAGGGAATCACC				
List14-029F	ATCAAGCTGAACGCCTTAGG	300	Library 1 plate 1	[TG]25	55
List14-029R	TCAGCATAGTTAGGATGGAACC				
List14-031F	AGAGAGCGTAGAAGTGGC	269	Library 1 plate 1	[AC]17N2[AC]8 N2 [AC]6	50
List14-031R	TTCGGGTCCGTAGTTTGG				
List14-032F	GTTGTCCAGCACTTTGTTGG	248	library 1 plate 3	[GT]22	50
List14-032R	TTTTTAGTAGGCTTGTAGGC				
List14-035F	TTACAGATAAAACAGTAGCCGC	218	Library 1 plate 1	[TG]12	55
List14-035R	GGTGTCCCATCCTAACATCG				
List14-037F	GGCGACACCCCATAGAAACC	239	Library 1 plate 1	[GT]8	55
List14-037R	ATTAAAGAACGGAAACCCCACC				
List14-039F	ATTGAAGGTTACTACTTGCTGC	161	Library 1 plate 3	[TG]12	50
List14-039R	ACGCTGCTTCATTTTTTAGTGG				
List14-041F	CCAATACAACACATACACTCG	160	Library 1 plate 1	[CA]17	50
List14-041R	ATCTGACACGACGTGATTCC				
List14-042F	TACTTCCGACTGACAACCG	170	Library 1 plate 1	[GT]9	50
List14-042R	GGTTTTAGTTCACCAATAGC				
List14-044F	CTTATGTTTGCTCAGAGGC	242	Library 1 plate 1	[TG]15 N2 [TTG]8	Failed at 50 52 53 55 c
List14-044R	AGAAGGCCAGCCATTTCC				
List14-047F	CACTCGTATCCGAATATAGC	225	Library 1 plate 3	[CA]19	55
List14-047R	GGATGAAGAATGTTGCGGTGG				

Primer sequence	Sequence (5'-3')	Cloned allele size (bp)	Microsatellite Library	Repeat motif	Annealing temp (C°)
List14-050F	CGGACACATTTTCGGTTGG	201	Library 1 plate 1	[CA]8	50
List14-050R	ATACAAGTTTTGAAGCCACC				
List14-052F	GAAAACTGAAGATGAAGAAGGC	288	Library 1 plate 1	[AC]10	Failed at 50 52 53 55 c
List14-052R	GAGGACAATAGGCCTGTGTGG				
List14-053F	GACCAAGCAGATAGATAGC	127	Library 1 plate 1	[GT]15	Failed at 50 52 53 55 c
List14-053R	AGAAGGCCAGCCATTTCC				
List14-055F	CTTATAGAATGGAGACGTCC	157	Library 1 plate 2	[GT]23	50
List14-055R	CAGAGGTAGTTGATGTGTGC				
List14-056F	TTTCCATTTGGCTCGTTTTGC	167	Library 1 plate 2	[CA]16 N14 [CT]6	50
List14-056R	GATAGTGCGATACATTTTGC				
List14-061F	ATTTAGTGGACCAACCTCTAGC	91	Library 1 plate 2	[TG]13	55
List14-061R	CTCCTACAACCATTCCGCCC				
List14-064F	AGAAAATGAGCAAAACGGCC	242	Library 1 plate 2	[GT]10	50
List14-064R	ACAGGCAAACAACTATGACG				
List14-067F	CTGTGGAAAGCGACTCCCTGG	198	Library 1 plate 4	[AC]12	55
List14-067R	GCTTTGGGCACCTGGCAGATGG				
List14-069F	TCGCTTTATTGTTAGGTAGGGG	342	Library 3 plate 1 wi	[GAA]9	55
List14-069R	TGCCGAAAATGAGCAAAAACGG				
List14-072F	ACATAAAGGGGGCTAACTCC	258	Library 3 plate 1 wi	[GAA]16	multiple bands 50-55
List14-072R	CGAAAATGAGCAAAAACGGC				
List14-073F	ACCAGCGTCCTTTTAAATGACG	186	Library 1 plate 4	[GT]13	failed at 53
List14-073R	TGCAGAATCCTCACACAATACC				
List14-074F	TTCTCATTGGGCAAAATACC	299	Library 1 plate 4	[CT]6 n22 [TG]14	53
List14-074R	GCAAAACATTCCTGATAACC				
List14-075F	GCCCCTAAAAAATCTTGAATGC	131	Library 1 plate 4	[TG]16	53
List14-075R	AAATAACCGCTCGGCTACCG				
List14-076F	AGATAGTGCGATACATTTTGCG	218	Library 1 plate 4	[TG]17	55
List14-076R	GTTAGAGTTGTCCTCAAGAAGC				

Primer sequence	Sequence (5'-3')	Cloned allele size (bp)	Microsatellite Library	Repeat motif	Annealing temp (C°)
List14-077F	GCACACTAAATACCACTGAGG	284	Library 1 plate 4	[GT]9	55
List14-077R	AACCCTGTCACCACTACACACG				
List14-078F	GCAGTTCTATGGAATCTCC	292	Library 1 plate 4	[GT]18	53
List14-078R	TAAGGCGTGACATTAGTGC				
List14-079F	TAGAGTTTTTGCTCCTGTTAGC	314	Library 1 plate 4	[CA]7 N2 [CA]10	53
List14-079R	TCCTATCTTTCGGTAAGTCCG				
List14-080F	CGCTTTAATGTAACGTAGGGG	366	Library 3 plate 1	[GAA]15	multiple bands 50-60
List14-080R	CCGAAAATGAGCAAAAACGGC				
List14-081F	GCAAAACAGCAACAAACACACC	95	Library 1 plate 4	[CA]12	failed at 53
List14-081R	ATACAAAACACGGGTTATCTCG				
List14-082F	CGTCACCCATACTTCAGAGG	242	Library 1 plate 4	[AC]10	failed at 55, 57
List14-082R	ATTGGCCAGAGACCCCAAGC				
List14-083F	ATAATACTAAAGGTGCCGATGG	269	Library 1 plate 3	[CA]17	55
List14-083R	GATCTGACACGACGTGATTCC				
List14-084F	AGTTTTGAAGCCACCTGTGTGC	305	Library 1 plate 3	[GT]11	failed at 50-60
List14-084R	ATTGGTAGTTGGACGATAAGCC				
List14-085F	AAAGTCTGACACTACCGCAGG	165	Library 1 plate 3	[AC]31	52
List14-085R	CCTGATTGATACATACAGCACC				
List14-086F	TACAGGAGGCGCAGTTAGTGG	320	Library 1 plate 3	[CA]17	57
List14-086R	ATGCACCGCTTCTATTCAACCG				
List14-087F	CTTGTTCCTATTGCTAAACTGG	254	Library 1 plate 3	[CA]17	55
List14-087R	CTCTGCCAATCAAATTCTTAGC				
List14-088F	GCAGTTCTATGGAATCTCC	145	Library 1 plate 3	[GT]18	failed at 50-60
List14-088R	GAGTCCCAGTTATTTACAGC				

* File misplace exact number repeats unknown, F=forward, R=reverse

Table 66. Extra primers produced but not tested by PCR.

Extra primers	Primer sequence	Extra primers	Primer sequence
List14-002F	TCTTACTCCAAACCCTCC	List14-044R	AGAGAAGGCCAGCCATTTCC
List14-002F	AAATCTTACTCCAAACCCTCC	List14-044R	CGTATGGCCAAAGAGAAGGCC
List14-002R	ACATAACATTCCGACTCG	List14-047F	CGGATCTCTGTTAATTTACG
List14-002R	ATCGAGACCCCCAAAAGG	List14-047F	TTACGATCTCCACTCGTATCCG
List14-003F	CTTCTCTTGCCTTTGACTCCC	List14-047F	TCCACTCGTATCCGAATATAGC
List14-003F	TTCTCTTGCCTTTGACTCCC	List14-047R	CAGGATGAAGAATGTTGCG
List14-003F	CTCTTGCCTTTGACTCCC	List14-047R	AACTCGGCACTTGGAAGG
List14-003R	GGAAGGAAGTCTAAAACTCGC	List14-047R	ACAGGATGAAGAATGTTGCGG
List14-003R	AAAGAAGAGAAGTAGTGGAGG	List14-047R	CACTCTCTCCTACCGTAAAGG
List14-003R	GTAAGAAGGAAGTAGTTTGG	List14-050F	TTCGGACACATTTTCGGTTGG
List14-003R	GTAAAGAAGAGAAGTAGTGG	List14-050F	TCAGATGTTGACTTTCCGG
List14-004F	TACCTACCCAGTAAAAAGC	List14-050F	AGTTAAGCTGACCTTATGTGG
List14-004F	TACAACACCCAATACCTACCC	List14-050F	TTCGGACACATTTTCGGTTGGC
List14-004R	ATGTTCTAGTGACGGTGCC	List14-050F	GGACACATTTTCGGTTGGCC
list14-004R	GGAGAAGTTGAAAAGTTGACC	List14-050R	TTTTGAAGCCACCTGTGTTCG
List14-005F	AGGGGGCCTTACATAAAGGG	List14-050R	TTTGAAGCCACCTGTGTTCG
List14-005F	TACATAAAGGGGGCTAACTCC	List14-050R	GTTTTGAAGCCACCTGTGTTCG
List14-005F	TAACTCCTCTCCCATTCG	List14-052F	ACTGTCAGACAAGTTAAACG
List14-005R	CCCACGACTGAGCAAAAACG	List14-052R	GAAAGTTTGGCCCATTTTCC
List14-005R	CCATTTACCATCACTTTGG	List14-053F	GAATCTTATGTTTGCTCAGAGG
List14-007F	GGAAGTATAGTAATCGAGC	List14-053F	ACAGACCAAGCAGATAGATAGC
List14-007F	AGTAATCGAGCAGTTTTTCG	List14-053R	TTATCAAAATCCGTATGGCC
List14-007F	CGGTTATACTTTTCCGTCTTGC	List14-055F	CGTCCGTAAAGTGTACAACG
List14-007R	GGTTGACCCGTTAGTTGGG	List14-055R	TATTTGGGCTTAGCGAACC
List14-007R	GAATGTAGAAGTCGTTTTCG	List14-055R	CCACAGGCAAACTTGAGC
List14-007R	TCCGGTTGACCCGTTAGTTGG	List14-055R	TGCAACTATTTGGGCTTAGC
List14-009F	GAATGGTAAAAGTCGCAAAGC	List14-056F	CTATTGAAGGATGTTAGAGCC

Extra primers	Primer sequence	Extra primers	Primer sequence
List14-009F	GAATGGTAAAAGTCGCAAAGCC	List14-056F	CTATTGAAGGATGTTAGAGCCG
List14-009F	TGGTAAAAGTCGCAAAGCC	List14-056F	CATTTGGCTCGTTTTGCCC
List14-009R	CTCTGTATGTGTTAGAAACC	List14-056F	AACTCTGCATTTTTCGTCAGC
List14-009R	TCTGCTAAAGCTGTGCCCGG	List14-056R	AGTGATTGGCCAGAGACCC
List14-010F	ATGATGACTGTATTGATGGG	List14-056R	CTCCCATATGGTCGACCTGC
List14-010F	TGTGATGGTCAGTTAGAAGC	List14-056R	ATATGGTCGACCTGCAGGC
List14-010F	GAAACAGGACTTTTACCTTCC	List14-056R	ATAGTGCGATACATTTTGCG
List14-010R	TCGACCAACAACAACTTCC	List14-061F	CTGGAAATACAAGAGTTCAGC
List14-010R	CCAAGCAGGAAAAATACTCG	List14-061F	TGGAAATACAAGAGTTCAGC
List14-010R	CAACAACTTCCCAAGCAGG	List14-061F	AAGAGTATTTAGTGGACCAACC
List14-013F	TAAGGGGCAGCAGCACTTCC	List14-061F	AGAGTATTTAGTGGACCAACC
List14-013F	TAATCATAAGGGGCAGCAGC	List14-061R	TAGCCACCTCCTACAACC
List14-013F	CCATTGGATACACGACCTCC	List14-061R	CTTGCTTTTACCGCCTCC
List14-013R	ACTCGCATCAAGCCATTTGG	List14-061R	CACCTCCTACAACCATTCC
List14-013R	ACTGGCAGAGTTTAAATACTCG	List14-061R	ACCTCCTACAACCATTCCGC
List14-014F	ACTTCTGTTTCTCTGATTCC	List14-061R	GTCACGCTTCTTGCTTTTACC
List14-016F	ACCAATACAACACATACACTCG	List14-061R	CTTCTTGCTTTTACCGCCTCC
List14-016R	GATCTGACACGACGTGATTCC	List14-064F	ATCGCTACAAGAAAATGAGC
List14-017F	TGAAGGTTACTACTTGCTGC	List14-064F	CAAGAAAATGAGCAAAACGGCC
List14-017R	CGCTGCTTCATTTTTTAGTGG	List14-064R	ACTGTCTGGTTGGCACACC
List14-019F	ACTCTGTTAGTAGATTGTGG	List14-064R	TCACAGGCAAACAACTATGACG
List14-019F	TCTGTTAGTAGATTGTGGAGGG	List14-067F	TGGAAAGCGACTCCCTGGG
List14-019R	AACTGCTTTGGGTTTAGCG	List14-067F	CTGTGGAAAGCGACTCCC
List14-019R	CCATATGGTCGACTTGCAGGC	List14-067F	TGTGGAAAGCGACTCCCTGG
List14-019R	AGACCCCAAGCTTCGTATCCC	List14-067R	TTGGGCACCTGGCAGATGG
List14-021R	GAGTTATAAATACGAACTGGGG	List14-067R	TGTAGGTCTGTTAACTGAGGCC
List14-021R	GCCTCTACGTGCAGTTATTGCC	List14-067R	GGCACCTGGCAGATGGATAGC
List14-025F	AGGTTATTGATTGTGGCAGG	List14-067R	CCTGGCAGATGGATAGCC
List14-025F	CAGTATTGACTATCTGGCC	List14-067R	TGCAGGAAGAGAAGGAAACG

Extra primers	Primer sequence	Extra primers	Primer sequence
List14-025F	ATCCGCTCTATCAACTACTCC	List14-069F	CCTAACCCTATCATCCTACC
List14-025F	CGGGTAAGGTTATTGATTGTGG	List14-069F	TCGCTTTATTGTTAGGTAGGG
List14-025R	TCAGCTTGACTCTATTAAGTGC	List14-069R	TTCGAGAAAAAAAAGTTGCCG
List14-028F	ACAAAATAGAGCAGCGTTGG	List14-069R	CCGAAAATGAGCAAAAACGGC
List14-028R	CTAACTTCTTGCAGACAGGG	List14-072R	GAGCCCTAACCCTATCATCC
List14-029F	TCAAGCTGAACGCCTTAGG	List14-073F	CCAGCGTCCTTTTAAATGACG
List14-029R	GCATAGTTAGGATGGAACCTGG	List14-073R	TAACAAGTCACTACTACCCCC
List14-029R	TCAGCATAGTTAGGATGGAACC	List14-074F	CTCTCTCTCTCTTCACCC
List14-031F	GAGAGAGAGCGTAGAAGTGGC	List14-074F	GTTCAAAGTCCCCACCCTCC
List14-031F	GGTGAAAGATGAGAGAGAGCG	List14-074R	GCAAAACATTCCTGATAACC
List14-031R	TGTGCTTATTTGGTGAGC	List14-075F	GCTGACTGTTTGGTGTGG
List14-031R	CTTGTGCTTATTTGGTGAGC	List14-075F	TTCCTCAAAAATGTTCTCCTGC
List14-031R	TGCTTGTGCTTATTTGGTGAGC	List14-075R	AATAACCGCTCGGCTACC
List14-031R	GGGTGTATTGAATGTCTGTTGG	List14-075R	TTAAATAACCGCTCGGCTACC
List14-031R	GCTTCGGGTCCGTAGTTTGG	List14-076R	TTCCATTTGGCTCGTTTTGCC
List14-032F	GAGTGATTAAGGTGATGTGG	List14-076R	TGAAGGATGTTAGAGCCGCC
List14-032R	ATCGAAGGCGAGTGAACC	List14-077F	CACTACAATGTGGTCGTTGG
List14-032R	AGGCTTGTAGGCATCGAAGG	List14-077F	AGTGAGCCAAAGCGATAAGG
List14-032R	GCTTGTAGGCATCGAAGGCG	List14-077R	ACTAGAGGTAAGACGGGGG
List14-035F	AGCTAGCATGAAAGCACAGTGG	List14-077R	GACGGGGGACTTTTTTGTTACC
List14-035F	AGTTTAGCTAGCATGAAAGC	List14-078F	AGTTCTATGGAATCTCCACG
List14-035F	GCATGAAAGCACAGTGGTTTCC	List14-078R	CCTCTCTCATCATTAGTGG
List14-035R	CGGGTGTCCCATCCTAACATCG	List14-078R	CACAGTTGGCATTAAACACC
List14-035R	GGTTTATTGAAGCCACACG	List14-078R	GAGTCCCAGTTATTTACAGC
List14-035R	CTTGGTTTATTGAAGCCACACG	List14-079F	TTCTGCTCTTTGGCTGTTATCC
List14-037F	ATTTATTAGGGCGACACCC	List14-079F	CGCCCTTGAATAAACGGG
List14-037F	ATTTATTAGGGCGACACCCC	List14-079F	CGCCCTTGAATAAACGGGG
List14-037F	AGGGCGACACCCCATAGAAACC	List14-079F	TTGAATAAACGGGGCTGTATGG
List14-037F	CGACACCCCATAGAAACC	List14-079F	TCCTATCTTTCGGTAAGTCCGC

Extra primers	Primer sequence	Extra primers	Primer sequence
List14-037R	TTAAAGAACGGAAACCCCACC	List14-079R	CCTATCTTTCGGTAAGTCC
List14-037R	AAGAACGGAAACCCCACC	List14-079R	CCTATCTTTCGGTAAGTCCG
List14-037R	CCTTAGAAAAACAAAAGACGGG	List14-079R	CTCCTATCTTTCGGTAAGTCC
List14-037R	TCCTTTTGATGAAGAAGTGG	List14-080F	GGAGCCCTAACCCTATCATCC
List14-039F	TCCCTTAAACTTCGAGTTCC	List14-081F	CTACTGCCAAAGACAGACAAGC
List14-039F	GAAGGTTACTACTTGCTGC	List14-081F	CAAAACAGCAACAAACACAC
List14-039R	CGCTGCTTCATTTTTTAGTGG	List14-081R	TACAAAACACGGGTTATCTCG
List14-039R	GCTGCTTCATTTTTTAGTGGC	List14-082 R	ATATGTTCGACCTGCAGGCG
List14-039R	GCTGCTTCATTTTTTAGTGG	List14-083	TCAGATATGTACGCAGGCGC
List14-041F	GCGTCTGAAGAGTAAATCTAGC	List14-083	GTATTTGTCTCAGTTGAACACC
List14-041F	GTCTGAAGAGTAAATCTAGC	List14-084	GTTTTGAAGCCACCTGTGTGC
List14-041F	ACCAATACAACACATACACTCG	List14-084	GGCCCATCTTGACCAATAGC
List14-041R	GTATTTGTCTCAGTTGAACACC	List14-085F	GCAGGTCGTAGTGAATGC
List14-041R	TTCGGCTCATGTATTGGC	List14-085R	ACCTGATTGATACATACAGC
List14-041R	CTGACACGACGTGATTCC	List14-086F	CACGTGTATGTACAGGTACAGG
List14-041R	TGTATTGGCACACTCGTGACG	List14-086F	ATGGGGATTCATGAACTCTCC
List14-042F	ACTTCCGACTGACAACCG	List14-086R	TGGCCCACAGGATTATCTCC
List14-042R	TTCACCAATAGCTTCTATGG	List14-087F	TCCATATTATGTTTTCCGCG
List14-042R	TTGATGTGTAGCTTAAGCG	List14-087R	CTGCCAATCAAATTCTTAGC
List14-042R	TTGATGTGTAGCTTAAGCGC	List14-088F	AGTGCAGTTCTATGGAATCTCC
List14-044F	GAATCTTATGTTTGCTCAGAGG	List14-088R	TGGAGTCCCAGTTATTTACAGC
List14-044F	ATCTTATGTTTGCTCAGAGGCC	List14-088R	TAAGGCGTGACATTAGTGC
List14-044F	ACAGACCAAGCAGATAGATAGC		

F=forward, R=reverse.

Table 67. Seven fluorescent primer pairs were subsequently dropped due to PCR problems.

Primer	Sequence 5'-6'	Fluorescent Dye	Problem
LIST14-39F	ATTGAAGGTTACTACTTGCTGC	NED	Multiple banding in PCR
LIST14-39R	ACGCTGCTTCATTTTTTAGTGG		
List14-061F	ATTTAGTGGACCAACCTCTAGC	NED	Multiple banding in PCR
List14-061R	CTCCTACAACCATTCCGCCC		
LIST14-55F	CTTATAGAATGGAGACGTCC	VIC	Multiple banding in PCR
LIST14-55R	CAGAGGTAGTTGATGTGTGC		
LIST14-19F	CTCTGTTAGTAGATTGTGGAGG	PET	Multiple banding in PCR
LIST14-19R	CGCAACTGCTTTGGGTTTAGC		
List14-067F	CTGTGGAAAGCGACTCCCTGG	PET	Multiple banding in PCR
List14-067R	GCTTTGGGCACCTGGCAGATGG		
List14-050F	CGGACACATTTTCGGTTGG	VIC	Multiple banding in PCR
List14-050R	ATACAAGTTTTGAAGCCACC		
LIST14-007F	CTTTTCCGTCTTGCAGGAAGG	NED	Inconsistent amplification
LIST14-007R	CCGGTTGACCCGTTAGTTGG		

F=forward, R=reverse

List of populations for Appendix Table

Pop 1 Terronal H1 01
Pop 2 Terronal H2 01
Pop 3 Terronal H2 p01
Pop 4 Terronal H1 03
Pop 5 Terronal H2 p03
Pop 6 San Bartolo h
Pop 7 San Bartolo h
Pop 8 Santa Lucia h
Pop 9 Casa Rena h
Pop 10Laurianito pd
Pop 11Palo Gacho p
Pop 12Los Rastrojos P
Pop 13Los Rastrojos H
Pop 14Lara
Pop 15Cojedes p
Pop 16Cojedes h
Pop 17Trujillo h
Pop 18Cascabel pd
Pop 19Cascabel h
Pop 20Cascabel p
Pop 21Guaranda h
Pop 22Guaranda p
Pop 23Laguna Hermosa h
Pop 24Laguna Hermosa p
Pop 25Laguna Hermosa pd
Pop 26G. Paraguey h1
Pop 27G. Paraguey h2
Pop 28G. Paraguey p1
Pop 29G. Paraguey p2
Pop 3019 Abril pd
Pop 31Parcelamiento p
Pop 32Rio Bravo II pd
Pop 33Rio Bravo II p

Table 68. Pairwise R_{ST} indices (below diagonal) for pairwise comparisons of specimens grouped by State divided by ecotope (p-values above) (microsatellite set 1)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Pop 1		0.26	0.01	0.24	0.03	0.01	0.00	0.01	0.07	0.54	0.66	0.00	0.02	0.00	0.00	0.01	0.00	0.28	0.03	0.01	0.04	0.02	0.00	0.27	0.85	0.15	0.39	0.11	0.02	0.00	0.22	0.06	0.01
Pop 2	0.02		0.00	0.57	0.31	0.00	0.00	0.00	0.11	0.24	0.29	0.01	0.07	0.00	0.01	0.01	0.00	0.14	0.02	0.01	0.01	0.00	0.00	0.16	0.62	0.02	0.37	0.12	0.02	0.00	0.02	0.00	0.02
Pop 3	0.06	**0.12**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.02	0.00	0.00	0.00	0.00	0.01	0.00	0.00
Pop 4	0.02	-0.01	0.12		0.10	0.01	0.00	0.00	0.00	0.25	0.20	0.04	0.15	0.00	0.02	0.01	0.00	0.11	0.05	0.08	0.03	0.02	0.00	0.50	0.78	0.04	0.73	0.23	0.10	0.00	0.06	0.01	0.09
Pop 5	0.04	0.01	**0.10**	0.03		0.00	0.00	0.00	0.09	0.00	0.08	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.03	0.21	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Pop 6	0.08	**0.12**	**0.15**	0.10	**0.11**		0.96	0.75	0.01	0.00	0.03	0.00	0.00	0.00	0.00	0.01	0.00	0.20	0.01	0.40	0.12	0.00	0.00	0.24	0.08	0.81	0.12	0.07	0.00	0.00	0.28	0.07	0.00
Pop 7	0.12	**0.18**	**0.25**	0.16	**0.19**	-0.02		0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.01	0.15	0.11	0.01	0.01	0.07	0.01	0.25	0.05	0.03	0.00	0.00	0.14	0.07	0.00
Pop 8	0.11	**0.16**	**0.24**	**0.14**	**0.17**	-0.01	0.00		0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.03	0.01	0.00	0.00	0.01	0.00	0.11	0.01	0.00	0.00	0.00	0.02	0.01	0.00
Pop 9	0.06	0.05	**0.13**	**0.12**	0.03	0.12	**0.24**	**0.23**		0.03	0.41	0.00	0.00	0.00	0.00	0.06	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.24	0.02	0.02	0.00	0.00	0.00	0.00	0.01	0.00
Pop 10	0.00	0.01	**0.07**	0.01	0.06	**0.09**	**0.12**	**0.11**	0.05		0.71	0.00	0.02	0.00	0.00	0.00	0.00	0.09	0.10	0.01	0.02	0.07	0.00	0.11	0.92	0.02	0.52	0.18	0.10	0.00	0.17	0.02	0.05
Pop 11	-0.01	0.01	0.02	0.03	0.03	0.09	**0.13**	0.11	0.00	-0.01		0.00	0.01	0.04	0.00	0.02	0.03	0.09	0.11	0.01	0.10	0.08	0.00	0.09	0.96	0.13	0.26	0.07	0.07	0.00	0.28	0.07	0.06
Pop 12	**0.19**	0.10	**0.32**	0.08	**0.21**	**0.26**	**0.29**	**0.29**	**0.27**	**0.15**	**0.17**		0.17	0.00	0.08	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.01	0.00	0.00	0.00	0.02
Pop 13	0.10	0.06	**0.27**	0.03	**0.15**	0.12	**0.15**	**0.16**	**0.19**	0.07	0.11	0.02		0.00	0.10	0.00	0.00	0.14	0.08	0.06	0.02	0.01	0.00	0.06	0.03	0.01	0.24	0.46	0.11	0.00	0.01	0.01	0.19
Pop 14	**0.15**	**0.17**	**0.20**	**0.15**	**0.25**	**0.21**	**0.21**	**0.22**	**0.21**	0.09	0.08	**0.22**	**0.17**		0.00	0.00	0.00	0.00	0.41	0.00	0.07	0.07	0.02	0.00	0.04	0.00	0.01	0.00	0.32	0.03	0.00	0.04	0.12
Pop 15	**0.15**	0.09	**0.26**	0.09	**0.17**	**0.21**	**0.24**	**0.24**	**0.19**	**0.13**	0.14	0.03	0.03	**0.20**		0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.02	0.03	0.00	0.00	0.00	0.00	0.04
Pop 16	0.07	0.08	**0.12**	0.11	0.05	0.09	**0.17**	**0.19**	0.06	**0.10**	0.06	**0.24**	**0.18**	**0.25**	**0.16**		0.02	0.03	0.00	0.00	0.00	0.00	0.00	0.02	0.07	0.15	0.01	0.00	0.00	0 00	0.03	0.00	0.00
Pop 17	**0.08**	**0.12**	**0.17**	**0.19**	**0.09**	**0.14**	**0.21**	**0.27**	0.07	**0.09**	0.05	**0.32**	**0.24**	**0.24**	**0.23**	0.05		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.01	0.00	0.00
Pop 18	0.02	0.04	**0.19**	0.04	0.08	0.03	0.07	0.11	**0.16**	0.03	0.05	0.16	0.03	**0.18**	0.09	0.07	0.10		0.07	0.64	0.07	0.09	0.01	0.78	0.05	0.19	0.62	0.69	0.06	0.00	0.08	0.28	0.07
Pop 19	0.08	0.10	**0.20**	0.07	**0.19**	0.12	0.11	**0.15**	**0.17**	0.04	0.05	0.13	0.05	0.00	0.10	**0.18**	**0.17**	0.06		0.04	0.62	0.99	0.36	0.04	0.12	0.01	0.39	0.41	0.91	0.09	0.08	0.58	0.84
Pop 20	0.05	0.06	**0.13**	0.03	**0.08**	0.01	0.02	0.05	**0.09**	0.05	0.07	**0.15**	0.04	**0.16**	**0.12**	**0.08**	**0.09**	-0.01	0.06		0.09	0.02	0.00	0.70	0.05	0.17	0.57	0.54	0.02	0.00	0.13	0.03	0.03
Pop 21	0.06	0.10	**0.16**	0.07	**0.15**	0.05	0.05	0.10	**0.13**	0.06	0.05	**0.17**	0.09	0.05	0.13	**0.10**	**0.11**	0.05	-0.01	0.03		0.82	0.14	0.12	0.14	0.09	0.38	0.25	0.51	0.02	0 60	0.69	0.28
Pop 22	0.05	0.08	**0.13**	0.06	**0.13**	0.08	0.07	**0.10**	**0.10**	0.02	0.03	**0.15**	0.07	0.03	**0.13**	**0.12**	**0.09**	0.03	-0.04	0.04	-0.02		0.10	0.06	0.13	0.01	0.40	0.27	0.39	0.01	0.16	0.23	0.40
Pop 23	**0.14**	**0.19**	**0.19**	**0.16**	**0.24**	0.12	0.09	**0.15**	**0.17**	**0.11**	0.11	**0.28**	**0.17**	0.06	**0.21**	**0.19**	**0.16**	0.09	0.00	0.10	0.03	0.02		0.00	0.01	0.01	0.01	0.01	0.12	0.20	0.05	0.23	0.19
Pop 24	0.01	0.02	**0.11**	0.00	0.04	0.02	0.05	0.08	0.08	0.02	0.04	**0.14**	0.04	**0.16**	**0.11**	0.05	**0.07**	-0.02	0.06	-0.01	0.03	0.03	**0.10**		0.30	0.36	0.94	0.66	0.04	0.00	0.29	0.09	0.03
Pop 25	-0.02	-0.01	0.02	-0.02	0.02	0.07	0.12	**0.13**	0.03	-0.02	-0.03	0.14	0.08	0.08	0.10	0.04	0.08	0.06	0.05	0.05	0.04	0.03	0.10	0.02		0.28	0.63	0.17	0.15	0.00	0.35	0.17	0.10
Pop 26	0.04	0.09	0.07	0.07	0.07	-0.01	0.04	0.06	0.09	0.06	0.05	**0.25**	0.14	**0.20**	**0.18**	0.04	0.09	0.03	0.12	0.02	0.05	0.08	0.12	0.01	0.03		0.15	0.04	0.00	0.00	0.58	0.07	0.01
Pop 27	0.01	0.01	0.13	-0.02	0.05	0.05	0.06	0.09	0.09	0.00	0.02	0.08	0.01	0.10	0.08	0.08	**0.10**	-0.01	0.01	-0.01	0.01	0.00	0.09	-0.02	-0.01	0.04		0.96	0.27	0.00	0.44	0.12	0.28
Pop 28	0.03	0.03	**0.18**	0.01	**0.10**	0.05	0.06	**0.10**	**0.13**	0.01	0.05	0.08	-0.01	0.11	0.06	**0.10**	**0.13**	-0.02	0.00	-0.01	0.01	0.00	0.08	-0.01	0.03	0.06	-0.03		0.28	0.00	0.18	0.08	0.45
Pop 29	0.10	0.11	**0.18**	0.06	**0.20**	**0.14**	**0.13**	**0.15**	**0.17**	0.04	0.07	0.13	0.05	0.01	0.12	**0.20**	**0.20**	0.07	-0.04	0.07	0.00	0.00	0.04	0.07	0.05	**0.13**	0.02	0.01		0.08	0.21	0.16	0.74
Pop 30	**0.29**	**0.32**	**0.34**	**0.28**	**0.39**	**0.24**	**0.22**	**0.27**	**0.34**	**0.22**	**0.21**	**0.36**	**0.24**	0.10	**0.29**	**0.35**	**0.34**	**0.23**	0.07	**0.20**	0.11	0.10	0.02	**0.23**	0.23	**0.26**	**0.20**	**0.17**	0.06		0.00	0.03	0.06
Pop 31	0.02	0.08	0.07	0.05	**0.10**	0.03	0.04	0.09	**0.10**	0.02	0.02	**0.20**	0.10	0.11	**0.14**	0.06	0.07	0.04	0.05	0.02	-0.01	0.02	0.05	0.01	0.01	0.00	0.01	0.02	0.04	0.17		0.66	0.07
Pop 32	0.03	**0.09**	**0.08**	0.07	**0.12**	0.04	0.03	0.05	0.08	0.04	0.03	**0.20**	0.09	0.04	**0.15**	**0.10**	**0.08**	0.00	-0.02	0.04	-0.02	0.00	0.00	0.02	0.02	0.03	0.02	0.03	0.02	0.07	-0.02		0.24
Pop 33	0.12	0 11	**0.23**	0 07	**0.22**	0.16	**0.16**	**0.20**	**0.20**	0.06	0.08	0.10	0.03	0.05	0.06	**0.19**	**0.21**	0.07	-0.03	0.07	0.02	0.00	0.03	0.07	0.07	0.15	0.02	0.00	-0.02	0 08	0 07	0.01	

Values in Bold significant after Bonferroni correction (p-value <0.05).

Table 69. Genetic distances between populations **DwS** (below diagonal) and **Dmu** (above diagonal).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1 Terronal h1 01		0.04	0.06	0.04	0.07	0.14	0.18	0.15	0.07	0.00	-0.02	0.58	0.29	0.33	0.60	0.13	0.09	0.03	0.16	0.11	0.14	0.11	0.42	0.02	-0.02	0.05	0.00	0.07	0.24	0.81	0.04	0.06	0.25
2 Terronal h2 01	0.02		0.19	-0.03	0.01	0.31	0.41	0.34	0.10	0 03	0.03	0.32	0.17	0.54	0.37	0.17	0.19	0.14	0.33	0.18	0.35	0.25	0.79	0.08	0.03	0.19	0.01	0.09	0.36	1.26	0.22	0.33	0.35
3 Terronal h2 p01	0.06	0.10		0.15	0.14	0.24	0.31	0.26	0.10	0.11	0.04	0.91	0.62	0.41	0.94	0.19	0.16	0.19	0.37	0.32	0.28	0.33	0.53	0.18	0.03	0.07	0 21	0.32	0.39	0.87	0.07	0.15	0.49
4 Terronal h1 03	0.02	0.03	0.04		0.05	0.25	0.34	0.27	0.20	0.02	0.07	0.27	0.13	0.51	0.45	0.26	0.29	0.13	0.31	0.13	0.33	0.25	0.76	0.03	0.01	0.15	-0 05	0.05	0.27	1.20	0.18	0.30	0.32
5 Terronal h2 p03	0.03	0.00	0.09	0.04		0.23	0.37	0.31	0.05	0.11	0.07	0.64	0.40	0.71	0.63	0.09	0.13	0.14	0.50	0.20	0 39	0.38	0.83	0.07	0.06	0.11	0.10	0.22	0.57	1.38	0.20	0.34	0.59
6 San Bartolo h1	0.06	0.11	0.17	0.09	0.11		-0.04	-0.01	0.21	0.22	0.23	1.03	0.45	0.67	1.05	0.22	0.22	0.05	0.36	0.02	0 16	0.29	0.49	0.04	0.21	-0.01	0.14	0.18	0.49	0.88	0.05	0.11	0.54
7 San Bartolo h2	0.05	0.12	0.16	0.10	0.13	0.00		0.00	0.31	0.24	0.28	1.09	0.47	0.55	1.15	0.37	0.29	0.08	0.25	0.06	0.13	0.22	0.35	0.10	0.26	0.06	0.17	0.17	0.38	0.64	0.07	0.05	0.44
8 Santa Lucia h1	0.08	0.12	0.14	0.11	0.14	0.08	0.07		0.25	0.21	0.23	1.03	0.47	0.56	1.15	0.39	0.34	0.12	0.35	0.13	0 23	0.31	0.54	0.16	0.22	0.08	0.20	0.24	0.45	0.81	0.13	0.13	0.57
9 Casa Rena h	0.03	0.02	0.10	0.08	0.02	0.11	0.12	0.12		0.10	0.02	0.92	0.59	0.51	0.88	0.11	0.06	0.17	0.40	0.29	0.30	0.31	0.63	0.15	0 04	0.10	0.19	0.31	0.51	1.07	0.14	0.22	0.57
10 Laurianito pd	0 01	0.01	0.06	0.02	0.03	0 08	0.08	0 12	0.02		-0.03	0.46	0.21	0.24	0 53	0.23	0.14	0.08	0.09	0.15	0.16	0.07	0.40	0.06	-0.02	0.13	-0.02	0.03	0.09	0.70	0.07	0.09	0.13
11 Palo Gacho p	0.02	0.00	0.07	0.04	0.02	0.10	0.12	0.09	0.01	0.01		0.61	0.39	0.21	0.64	0.14	0.07	0.12	0.14	0.23	0.14	0 11	0.42	0.10	-0.06	0.10	0.05	0.14	0.21	0.77	0.05	0.08	0.24
12 Los Rastrojos h	0.12	0.07	0.24	0.11	0.14	0.22	0.23	0.18	0.15	0.11	0.11		0.07	0.95	0.10	0.83	0.93	0.65	0.63	0.57	0.83	0.62	1.43	0.57	0.58	0.90	0.28	0.27	0.54	1.81	0.84	0.96	0.41
13 Los Rastrojos p	0.08	0.05	0.19	0.10	0.09	0.13	0.12	0.09	0.10	0.07	0.09	0.04		0.77	0.14	0.55	0.57	0.20	0.34	0.12	0.50	0.31	0.90	0.21	0 40	0.46	0.05	-0.05	0.27	1.20	0 45	0.50	0.19
14 Lara h	0.09	0.10	0.14	0.08	0.14	0.12	0.14	0.13	0.13	0.08	0.05	0.17	0.18		1.17	0.76	0.49	0.52	-0.02	0.66	0.15	0.10	0.25	0.52	0.23	0.57	0.34	0.40	0.06	0.30	0.28	0.15	0.14
15 Cojedes p	0 14	0.10	0.26	0.16	0.14	0 23	0.25	0.29	0.16	0.13	0.15	0.09	0.09	0.25		0 62	0.79	0.58	0.73	0.57	0.89	0.68	1.34	0.60	0.69	0.87	0.38	0.31	0.74	1.84	0.85	0.95	0.42
16 Cojedes h	0 09	0.09	0.16	0.15	0.07	0.15	0.16	0.22	0.06	0.10	0.11	0.22	0.13	0.24	0.13		0.07	0.14	0.50	0.21	0.27	0.40	0.71	0.12	0.15	0 07	0.19	0.28	0.68	1.27	0.14	0.28	0.57
17 Trujillo h	0.09	0.08	0.19	0.17	0.08	0.15	0.16	0.23	0.06	0.10	0.09	0.20	0.13	0.20	0.18	0.08		0.09	0.27	0.22	0.16	0.21	0.41	0.09	0.10	0.10	0.16	0.22	0.42	0 85	0.09	0.13	0.40
18 Cascabel pd	0.02	0.07	0.10	0.07	0.08	0.06	0.04	0.12	0.08	0.05	0.08	0.16	0.08	0.16	0.16	0.11	0.08		0.16	-0.03	0.12	0.11	0 36	-0 04	0 12	0.03	-0 01	-0.02	0.27	0.76	0.06	0.04	0.24
19 Cascabel h	0.05	0.05	0.12	0.06	0.06	0.08	0.08	0.11	0.07	0.02	0.02	0.12	0.07	0.03	0.15	0.12	0.11	0.05		0.24	-0.05	-0.13	0.03	0.18	0 14	0.34	0.04	0.02	-0.10	0.21	0.11	-0.04	-0.14
20 Cascabel p	0.07	0.08	0.14	0.08	0.07	0 08	0.07	0.12	0.10	0.06	0.08	0.17	0.06	0.16	0.17	0.11	0.13	0.03	0.01		0.15	0.17	0.50	-0 04	0.20	0.06	-0.02	-0.04	0.32	0.92	0.10	0.14	0.28
21 Guaranda h	0.05	0.06	0.12	0.09	0.06	0 07	0.07	0.12	0.06	0.05	0.04	0.16	0.08	0.08	0.15	0.08	0.06	0.04	0.00	0.03		-0.02	0.14	0.10	0.12	0.13	0.07	0.11	0.15	0.43	-0.01	-0.05	0.11
22 Guaranda p	0.05	0.05	0.13	0.06	0.05	0.10	0.10	0.14	0.07	0.04	0.04	0.15	0.08	0.08	0.14	0 10	0.10	0.05	0.00	0.02	0.00		0.10	0.11	0.11	0.27	0.00	0 01	0.00	0.39	0.10	-0.02	-0.06
23 L. Hermosa h	0.11	0.15	0.18	0.16	0.14	0.14	0.13	0.20	0.14	0.10	0.12	0.28	0.16	0.16	0.24	0.16	0.16	0.08	0.01	0.05	0.05	0.03		0.43	0.44	0.47	0.40	0.38	0.20	0.07	0.22	-0.01	0.10
24 L. Hermosa p	0.04	0.05	0.11	0.05	0.04	0 07	0.07	0.13	0.06	0.04	0.06	0.15	0.07	0.15	0.15	0.07	0.09	0.01	0.01	0.00	0 02	0.01	0.05		0.05	0.02	-0.07	-0.02	0.27	0.91	0.04	0.09	0.25
25 L. Hermosa pd	0.03	0 03	0.07	0.03	0.02	0 10	0.11	0.09	0.04	0 04	0.01	0.12	0.11	0.05	0.18	0.09	0.12	0.09	0.02	0.06	0.04	0.02	0.10	0.03		0.08	0.00	0.13	0.21	0.84	0.03	0.09	0.26
26 G. Paraguey h1	0.01	0.06	0.05	0.02	0.04	0 03	0.03	0.05	0.05	0.04	0.04	0.16	0.09	0.10	0.17	0.08	0.11	0.02	0.04	0.03	0 03	0.05	0.09	0.02	0.02		0.10	0.17	0.44	0.88	-0.01	0.08	0.47
27 G. Paraguey h2	0.04	0.04	0.11	0.04	0.05	0.08	0.08	0.10	0.06	0.04	0.05	0.09	0.04	0.11	0.13	0.07	0.11	0.03	0.00	0.00	0.03	0.01	0.05	0.00	0.01	0.02		-0.11	0.10	0.83	0.05	0.09	0.05
28 G. Paraguey p1	0.03	0.03	0.12	0.04	0.05	0 05	0.05	0.11	0.06	0.02	0.05	0.09	0.03	0.12	0.11	0.09	0.08	0.00	0.00	0.00	0 01	0.00	0.05	0.00	0.04	0.03	0.00		0.05	0.71	0.11	0.11	-0.01
29 G. Paraguey p2	0 08	0.07	0 14	0.07	0 09	0 11	0 11	0.14	0.10	0 04	0 05	0.13	0.07	0 04	0.18	0 17	0 14	0 08	0 00	0 04	0 02	0 01	0 05	0 04	0 04	0.07	0 02	0 01		0 22	0.19	0.11	-0.12

30 19 Abril pd	0.19	0.24	0.20	0.24	0.24	0.21	0.19	0.26	0.22	0.15	0.18	0.40	0.24	0.19	0.38	0.27	0.25	0.16	0.07	0.13	0.11	0.12	0.05	0.16	0.20	0.17	0.16	0.12	0.07		0.52	0.26	0.30
31 Parcelamiento p	0.03	0.05	0.05	0.04	0.05	0.06	0.06	0.09	0.04	0.02	0.03	0.16	0.09	0.08	0.17	0.09	0 07	0.04	0.01	0.03	0 00	0.02	0.06	0.02	0.03	0.00	0.02	0.02	0.03	0.11		-0.04	0.20
32 Rio Bravo II pd	0.09	0.12	0.17	0.12	0.12	0.14	0.13	0.17	0.16	0.09	0.10	0.19	0.11	0.16	0.22	0.17	0.17	0.07	0.04	0.05	0 07	0.06	0.03	0.04	0.09	0.08	0.05	0.04	0.06	0.12	0.09		0.10
33 Rio Bravo II p	0 09	0.09	0.18	0.09	0 10	0.12	0.13	0.20	0 13	0.06	0 08	0.16	0 10	0 10	0 14	0.15	0.17	0.07	0.00	0.02	0 04	0 00	0 00	0.02	0.06	0 08	0.00	0 00	0 00	0.09	0 06	0 03	

p-palm, pd-peridomestic, h=house, 01-2001, 03=2003

Table 70. Specimens used in morphometric analysis by Terronal, Portuguesa and State, also for global ecotope analysis minus peridomestic specimens (all Adults).

Label	State	Locality	Location	Ecotope
Portuguesa				
14 TERR	Portuguesa	Terronal	House 2 01	Domestic
15 TERR	Portuguesa	Terronal	House 2 01	Domestic
16 TERR	Portuguesa	Terronal	House 2 01	Domestic
1 TERR	Portuguesa	Terronal	House 2 01	Domestic
2 TERR	Portuguesa	Terronal	House 2 01	Domestic
53 TERR	Portuguesa	Terronal	House 2 01	Domestic
9 TERR	Portuguesa	Terronal	House 2 01	Domestic
10 TERR	Portuguesa	Terronal	House 2 01	Domestic
11 TERR	Portuguesa	Terronal	House 2 01	Domestic
17 TERR	Portuguesa	Terronal	House 2 01	Domestic
54 TERR	Portuguesa	Terronal	House 2 01	Domestic
5 TERR	Portuguesa	Terronal	House 2 01	Domestic
6 TERR	Portuguesa	Terronal	House 2 01	Domestic
7 TERR	Portuguesa	Terronal	House 2 01	Domestic
8 TERR	Portuguesa	Terronal	House 2 01	Domestic
18 TERR	Portuguesa	Terronal	House 1 01	Domestic
19 TERR	Portuguesa	Terronal	House 1 01	Domestic
21 TERR	Portuguesa	Terronal	House 1 01	Domestic
22 TERR	Portuguesa	Terronal	House 1 01	Domestic

Label	State	Locality	Location	Ecotope
23 TERR	Portuguesa	Terronal	House 1 01	Domestic
25 TERR	Portuguesa	Terronal	House 1 01	Domestic
26 TERR	Portuguesa	Terronal	House 1 01	Domestic
27 TERR	Portuguesa	Terronal	House 1 01	Domestic
33 TERR	Portuguesa	Terronal	House 1 01	Domestic
35 TERR	Portuguesa	Terronal	House 1 01	Domestic
36 TERR	Portuguesa	Terronal	House 1 01	Domestic
55 TERR	Portuguesa	Terronal	House 1 01	Domestic
57 TERR	Portuguesa	Terronal	House 1 01	Domestic
58 TERR	Portuguesa	Terronal	House 1 01	Domestic
20 TERR	Portuguesa	Terronal	House 1 01	Domestic
23 TERR	Portuguesa	Terronal	House 1 01	Domestic
24 TERR	Portuguesa	Terronal	House 1 01	Domestic
28 TERR	Portuguesa	Terronal	House 1 01	Domestic
30 TERR	Portuguesa	Terronal	House 1 01	Domestic
31 TERR	Portuguesa	Terronal	House 1 01	Domestic
32 TERR	Portuguesa	Terronal	House 1 01	Domestic
34 TERR	Portuguesa	Terronal	House 1 01	Domestic
48 TERR	Portuguesa	Terronal	House 1 01	Domestic
49 TERR	Portuguesa	Terronal	House 1 01	Domestic
59 TERR	Portuguesa	Terronal	House 1 01	Domestic
133 TERR	Portuguesa	Terronal	House 1 01	Domestic
43 terfp	Portuguesa	Terronal	House 2 01	Silvatic palm
44 terfp	Portuguesa	Terronal	House 2 01	Silvatic palm
45 terfp	Portuguesa	Terronal	House 2 01	Silvatic palm
108 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
114 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
116 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm

Label	State	Locality	Location	Ecotope
132 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
134 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
135 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
136 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
140 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
141 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
143 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
144 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
145 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
146 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
148 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
150 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
151TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
152 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
154 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
155 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
156 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
158 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
162 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
163 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
164 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
165 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
168 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
170 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
173 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
174 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
176 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
177 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
179 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm

Label	State	Locality	Location	Ecotope
183 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
186 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
187 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
188 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm
38 terfp	Portuguesa	Terronal	House 2 01	Silvatic palm
T1P12	Portuguesa	Terronal	House 1 03	Domestic
T1P13	Portuguesa	Terronal	House 1 03	Domestic
T1P15	Portuguesa	Terronal	House 1 03	Domestic
T1P16	Portuguesa	Terronal	House 1 03	Domestic
T1P17	Portuguesa	Terronal	House 1 03	Domestic
T1P5	Portuguesa	Terronal	House 1 03	Domestic
T1P6	Portuguesa	Terronal	House 1 03	Domestic
T1P7	Portuguesa	Terronal	House 1 03	Domestic
T2P15	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P18	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P1	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P20	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P21	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P23	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P25	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P26	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P27	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P29	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P2	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P30	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P31	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P32	Portuguesa	Terronal	House 2 03	Silvatic palm

Label	State	Locality	Location	Ecotope
T2P35	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P36	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P38	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P37	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P38	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P40	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P41	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P39	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P17	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P19	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P42	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P22	Portuguesa	Terronal	House 2 03	Silvatic palm
T2P24	Portuguesa	Terronal	House 2 03	Silvatic palm
POSB19a	Portuguesa	San Bartolo	House 1	Domestic
POSB19b	Portuguesa	San Bartolo	House 1	Domestic
POSB19c	Portuguesa	San Bartolo	House 1	Domestic
POSB19e	Portuguesa	San Bartolo	House 1	Domestic
POSB19f	Portuguesa	San Bartolo	House 1	Domestic
POSB19g	Portuguesa	San Bartolo	House 1	Domestic
POSB19k	Portuguesa	San Bartolo	House 1	Domestic
POSB20a	Portuguesa	San Bartolo	House 20	Domestic
POSB25a	Portuguesa	San Bartolo	House 25	Domestic
POSBMAR18	Portuguesa	San Bartolo	House 1	Domestic
POSBMAR19	Portuguesa	San Bartolo	House 1	Domestic
POSBMAR14	Portuguesa	San Bartolo	House 1	Domestic
POSBMAR16	Portuguesa	San Bartolo	House 1	Domestic
POSBMAR15	Portuguesa	San Bartolo	House 1	Domestic
POSBMAR17	Portuguesa	San Bartolo	House 1	Domestic

Label	State	Locality	Location	Ecotope
CP10	Portuguesa	Casa Rena	House 1	Domestic
CP11	Portuguesa	Casa Rena	House 1	Domestic
CP12	Portuguesa	Casa Rena	House 1	Domestic
CP13	Portuguesa	Casa Rena	House 1	Domestic
CP14	Portuguesa	Casa Rena	House 1	Domestic
CP15	Portuguesa	Casa Rena	House 1	Domestic
CP16	Portuguesa	Casa Rena	House 1	Domestic
CP17	Portuguesa	Casa Rena	House 1	Domestic
POMO10.1a	Portuguesa	Morichal	House 10.1	Domestic
POMO10.1b	Portuguesa	Morichal	House 10.1	Domestic
POMO10.1c	Portuguesa	Morichal	House 10.1	Domestic
POMO10.1d	Portuguesa	Morichal	House 10.1	Domestic
POMO10.1e	Portuguesa	Morichal	House 10.1	Domestic
POMO10.1f	Portuguesa	Morichal	House 10.1	Domestic
POMO10.1g	Portuguesa	Morichal	House 10.1	Domestic
POMO10a	Portuguesa	Morichal	House 10	Domestic
POMO9	Portuguesa	Morichal	House 9	Domestic
POMO21a	Portuguesa	El Mosquito	House 21	Domestic
POMO21b	Portuguesa	El Mosquito	House 22	Domestic
POMO21c	Portuguesa	El Mosquito	House 23	Domestic
POMO40a	Portuguesa	El Mosquito	House 40	Domestic
POMO48a	Portuguesa	El Mosquito	House 48	Domestic
POMO59a	Portuguesa	El Mosquito	House 59	Domestic
POMO59b	Portuguesa	El Mosquito	House 59	Domestic
POMO8a	Portuguesa	El Mosquito	House 8	Domestic
POMO8b	Portuguesa	El Mosquito	House 8	Domestic

Label	State	Locality	Location	Ecotope
POMO8c	Portuguesa	El Mosquito	House 8	Domestic
POQN20a	Portuguesa	Qdra Negra	House 20	Domestic
POQN20b	Portuguesa	Qdra Negra	House 20	Domestic
POQN20c	Portuguesa	Qdra Negra	House 20	Domestic
POQN20d	Portuguesa	Qdra Negra	House 20	Domestic
POQN20e	Portuguesa	Qdra Negra	House 20	Domestic
POQN20f	Portuguesa	Qdra Negra	House 20	Domestic
POQN22.1a	Portuguesa	Qdra Negra	House 22.1	Domestic
POQN114a	Portuguesa	Qdra Negra	House 114	Domestic
POQN26a	Portuguesa	Qdra Negra	House 26	Domestic
POQN27a	Portuguesa	Qdra Negra	House 27	Domestic
POQN27b	Portuguesa	Qdra Negra	House 27	Domestic
POQN22.1b	Portuguesa	Qdra Negra	House 22.1	Domestic
POQN30.1a	Portuguesa	Qdra Negra	House 30.1	Domestic
POQN30.1b	Portuguesa	Qdra Negra	House 30.1	Domestic
104 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
110 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
117 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
121 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
115 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
117 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
121 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
137 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
138 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
139 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
147 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
149 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm

Label	State	Locality	Location	Ecotope
153 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
161 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
167 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
169 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
178 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
185 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
189 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
190 PALO	Portuguesa	Palo Gacho	Not near House	Silvatic palm
Merida				
merpa	Merida	-	Not near House	Silvatic palm
merpa2	Merida	-	Not near House	Silvatic palm
merpb11	Merida	-	Not near House	Silvatic palm
merpb12	Merida	-	Not near House	Silvatic palm
merpb1	Merida	-	Not near House	Silvatic palm
Lara				
102 lara	Lara	Salvador	House 2	Domestic
103 lara	Lara	Salvador	House 2	Domestic
125 lara	Lara	Guamarito	House 1	Domestic
122 lara	Lara	Guamarito	House 1	Domestic
126 lara	Lara	Guamarito	House 1	Domestic
129 lara	Lara	Guamarito	House 1	Domestic
130 lara	Lara	Guamarito	House 1	Domestic
131 lara	Lara	Guamarito	House 1	Domestic
69 lara	Lara	Guamarito	House 1	Domestic
94 lara	Lara	Guamarito	House 1	Domestic
95 lara	Lara	Guamarito	House 1	Domestic
96 lara	Lara	Guamarito	House 1	Domestic
84 lara	Lara	Guamarito	House 1	Domestic
107 lara	Lara	Guamarito	House 1	Domestic

Label	State	Locality	Location	Ecotope
90 lara	Lara	Guamarito	House 1	Domestic
74 lara	Lara	Guamarito	House 1	Domestic
75 lara	Lara	Guamarito	House 1	Domestic
76 lara	Lara	Guamarito	House 1	Domestic
79 lara	Lara	Guamarito	House 1	Domestic
Guarico				
66 gua	Guarico	El Sombero	Not near House	Silvatic palm
105 gua	Guarico		Not near House	Silvatic palm
106 gua	Guarico	El Sombero	Not near House	Silvatic palm
111 gua	Guarico	El Sombero	Not near House	Silvatic palm
112 gua	Guarico	El Manguito	Not near House	Silvatic palm
113 gua	Guarico	El Manguito	Not near House	Silvatic palm
118 gua	Guarico	El Manguito	Not near House	Silvatic palm
119 gua	Guarico	El Manguito	Not near House	Silvatic palm
120 gua	Guarico	El Manguito	Not near House	Silvatic palm
142 gua	Guarico		Not near House	Silvatic palm
159 gua	Guarico	Bravero	Not near House	Silvatic palm
160 gua	Guarico		Not near House	Silvatic palm
166 gua	Guarico		Not near House	Silvatic palm
171 gua	Guarico	Bravero	Not near House	Silvatic palm
172 gua	Guarico		Not near House	Silvatic palm
175 gua	Guarico		Not near House	Silvatic palm
182 gua	Guarico	El Manguito	Not near House	Silvatic palm
184 gua	Guarico		Not near House	Silvatic palm
62 ortiz	Guarico	Bravero	Not near House	Silvatic palm
63 ortiz	Guarico	Bravero	Not near House	Silvatic palm
Trujillo				
LDAT 10	Trujillo	Loma de Amarillo	House 1	Domestic
LDAT 11	Trujillo	Loma de Amarillo	House 1	Domestic

Label	State	Locality	Location	Ecotope
LDAT 12	Trujillo	Loma de Amarillo	House 1	Domestic
LDAT 13	Trujillo	Loma de Amarillo	House 1	Domestic
LDAT 14	Trujillo	Loma de Amarillo	House 1	Domestic
LDAT 16	Trujillo	Loma de Amarillo	House 1	Domestic
LDAT 19	Trujillo	Loma de Amarillo	House 1	Domestic
LDAT 1	Trujillo	Loma de Amarillo	House 1	Domestic
LDAT 20	Trujillo	Loma de Amarillo	House 1	Domestic
LDAT 21	Trujillo	Loma de Amarillo	House 1	Domestic
LDAT 22	Trujillo	Loma de Amarillo	House 1	Domestic
LDAT 23	Trujillo	Loma de Amarillo	House 1	Domestic
LDAT 24	Trujillo	Loma de Amarillo	House 1	Domestic
LDAT 25	Trujillo	Loma de Amarillo	House 1	Domestic
LDAT 26	Trujillo	Loma de Amarillo	House 1	Domestic
LDAT 8	Trujillo	Loma de Amarillo	House 1	Domestic
PRT1	Trujillo	Palma real	House	Domestic
INS1	Trujillo	Insectary	Insectary	Insectary
INS2	Trujillo	Insectary	Insectary	Insectary
INS3	Trujillo	Insectary	Insectary	Insectary
INS10	Trujillo	Insectary	Insectary	Insectary
INS11	Trujillo	Insectary	Insectary	Insectary
INS12	Trujillo	Insectary	Insectary	Insectary
INS13	Trujillo	Insectary	Insectary	Insectary
INS12	Trujillo	Insectary	Insectary	Insectary
INS15	Trujillo	Insectary	Insectary	Insectary
INS16	Trujillo	Insectary	Insectary	Insectary
INS17	Trujillo	Insectary	Insectary	Insectary
LJT2	Trujillo	La Juventud	Palm 1	Silvatic palm
LJT3	Trujillo	La Juventud	Palm 1	Silvatic palm
LJT4	Trujillo	La Juventud	Palm 1	Silvatic palm

Label	State	Locality	Location	Ecotope
Barinas				
CAR2b1	Barinas	Carreteron	House 2	Domestic
CAR2b2	Barinas	Carreteron	House 2	Domestic
CAR2b3	Barinas	Carreteron	House 2	Domestic
CAR2b4	Barinas	Carreteron	House 2	Domestic
CAR2b5	Barinas	Carreteron	House 2	Domestic
CAR2b6	Barinas	Carreteron	House 2	Domestic
CAR2b7	Barinas	Carreteron	House 2	Domestic
CAR2b8	Barinas	Carreteron	House 2	Domestic
CAR3b2	Barinas	Carreteron	House 3	Domestic
CAR3b1	Barinas	Carreteron	House 3	Domestic
CAR4b1	Barinas	Carreteron	House 4	Domestic
CAR4b2	Barinas	Carreteron	House 4	Domestic
CAR4b3	Barinas	Carreteron	House 4	Domestic
CAR4b5	Barinas	Carreteron	House 4	Domestic
CAR5b1	Barinas	Carreteron	House 5	Domestic
CAR6b1	Barinas	Carreteron	House 6	Domestic
CAR1b10	Barinas	Carreteron	House 1	Domestic
CAR1b11	Barinas	Carreteron	House 1	Domestic
Cojedes				
COJ12h	Cojedes	Las Queseras	J. P. Arraez	Domestic
COJ2h	Cojedes	Las Queseras	J. P. Arraez	Domestic
COJ3h	Cojedes	Las Queseras	J. P. Arraez	Domestic
COJ4p	Cojedes	Las Queseras	J. P. Arraez	Domestic
COJ5h	Cojedes	Las Queseras	J. P. Arraez	Domestic
COJ6h	Cojedes	Las Queseras	J. P. Arraez	Domestic
COJ7h	Cojedes	Las Queseras	J. P. Arraez	Domestic
COJ10p	Cojedes	Las Queseras	J. P. Arraez	Silvatic palm
COJ11p	Cojedes	Las Queseras	J. P. Arraez	Silvatic palm

Label	State	Locality	Location	Ecotope
COJ15p	Cojedes	Las Queseras	J. P. Arraez	Silvatic palm
COJ16p	Cojedes	Las Queseras	J. P. Arraez	Silvatic palm
COJ17p	Cojedes	Las Queseras	J. P. Arraez	Silvatic palm
COJ19p	Cojedes	Las Queseras	J. P. Arraez	Silvatic palm
COJ1p	Cojedes	Las Queseras	J. P. Arraez	Silvatic palm
COJ20p	Cojedes	Las Queseras	J. P. Arraez	Silvatic palm
COJ3p	Cojedes	Las Queseras	J. P. Arraez	Silvatic palm
COJ5p	Cojedes	Las Queseras	J. P. Arraez	Silvatic palm
COJ6p	Cojedes	Las Queseras	J. P. Arraez	Silvatic palm
COJ7p	Cojedes	Las Queseras	J. P. Arraez	Silvatic palm
COJ8p	Cojedes	Las Queseras	J. P. Arraez	Silvatic palm
COJ9p	Cojedes	Las Queseras	J. P. Arraez	Silvatic palm

Table 71. Specimens used in haplotype group analysis.

Label	State	Locality	Location	Ecotope	Cytb
CAR2b3	Barinas	Carreteron	House 2	Domestic	*R. prolixus* haplotype 1
CAR2b4	Barinas	Carreteron	House 2	Domestic	*R. prolixus* haplotype 1
CAR2b7	Barinas	Carreteron	House 2	Domestic	*R. prolixus* haplotype 1
CAR3b2	Barinas	Carreteron	House 3	Domestic	*R. prolixus* haplotype 1
CAR4b3	Barinas	Carreteron	House 4	Domestic	*R. prolixus* haplotype 1
CAR4b4	Barinas	Carreteron	House 4	Domestic	*R. prolixus* haplotype 1
CAR6b1	Barinas	Carreteron	House 6	Domestic	*R. prolixus* haplotype 1
CAR1b10	Barinas	Carreteron	House 1	Domestic	*R. prolixus* haplotype 1
CAR1b11	Barinas	Carreteron	House 1	Domestic	*R. prolixus* haplotype 1
COJ12h	Cojedes	Las Queseras	J. P. Arraez	Domestic	*R. prolixus* haplotype 1
COJ3h	Cojedes	Las Queseras	J. P. Arraez	Domestic	*R. prolixus* haplotype 1
COJ4p	Cojedes	Las Queseras	J. P. Arraez	Domestic	*R. prolixus* haplotype 1
COJ5h	Cojedes	Las Queseras	J. P. Arraez	Domestic	*R. prolixus* haplotype 1

Label	State	Locality	Location	Ecotope	Cytb
COJ6h	Cojedes	Las Queseras	J. P. Arraez	Domestic	*R. prolixus* haplotype 1
COJ7h	Cojedes	Las Queseras	J. P. Arraez	Domestic	*R. prolixus* haplotype 1
102 lara	lara	Salvador	House 2	Domestic	*R. prolixus* haplotype 1
103 lara	lara	Salvador	House 2	Domestic	*R. prolixus* haplotype 1
125 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
122 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
126 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
129 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
130 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
131 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
69 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
94 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
95 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
96 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
84 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
107 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
90 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
74 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
75 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
76 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
79 lara	lara	Guamarito	House 1	Domestic	*R. prolixus* haplotype 1
16 TERR	Portuguesa	Terronal	House 2 01	Domestic	*R. prolixus* haplotype 1
1 Terr	Portuguesa	Terronal	House 2 01	Domestic	*R. prolixus* haplotype 1
2 TERR	Portuguesa	Terronal	House 2 01	Domestic	*R. prolixus* haplotype 1
53 TERR	Portuguesa	Terronal	House 2 01	Domestic	*R. prolixus* haplotype 1
9 TERR	Portuguesa	Terronal	House 2 01	Domestic	*R. prolixus* haplotype 1
10 TERR	Portuguesa	Terronal	House 2 01	Domestic	*R. prolixus* haplotype 1
5 TERR	Portuguesa	Terronal	House 2 01	Domestic	*R. prolixus* haplotype 1
7 TERR	Portuguesa	Terronal	House 2 01	Domestic	*R. prolixus* haplotype 1
21 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. prolixus* haplotype 1

Label	State	Locality	Location	Ecotope	Cytb
23 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. prolixus* haplotype 1
25 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. prolixus* haplotype 1
33 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. prolixus* haplotype 1
57 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. prolixus* haplotype 1
23 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. prolixus* haplotype 1
48 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. prolixus* haplotype 1
39 pen	Portuguesa	Peña Negra	House 1	Domestic	*R. prolixus* haplotype 1
40 pen	Portuguesa	Peña Negra	House 1	Domestic	*R. prolixus* haplotype 1
41pen	Portuguesa	Peña Negra	House 1	Domestic	*R. prolixus* haplotype 1
42 pen	Portuguesa	Peña Negra	House 1	Domestic	*R. prolixus* haplotype 1
T2P33	Portuguesa	Terronal	House 2 03	Domestic	*R. prolixus* haplotype 1
T2P34	Portuguesa	Terronal	House 2 03	Domestic	*R. prolixus* haplotype 1
POMO10.1b	Portuguesa	Morichal	House 10.1	Domestic	*R. prolixus* haplotype 1
POMO10.1c	Portuguesa	Morichal	House 10.1	Domestic	*R. prolixus* haplotype 1
POMO10.1f	Portuguesa	Morichal	House 10.1	Domestic	*R. prolixus* haplotype 1
POMO10.1g	Portuguesa	Morichal	House 10.1	Domestic	*R. prolixus* haplotype 1
POMO21a	Portuguesa	El Mosquito	House 21	Domestic	*R. prolixus* haplotype 1
POMO21c	Portuguesa	El Mosquito	House 23	Domestic	*R. prolixus* haplotype 1
POMO40a	Portuguesa	El Mosquito	House 40	Domestic	*R. prolixus* haplotype 1
POMO48a	Portuguesa	El Mosquito	House 48	Domestic	*R. prolixus* haplotype 1
POMO8a	Portuguesa	El Mosquito	House 8	Domestic	*R. prolixus* haplotype 1
POQN20b	Portuguesa	Qdra Negra	House 20	Domestic	*R. prolixus* haplotype 1
POQN20c	Portuguesa	Qdra Negra	House 20	Domestic	*R. prolixus* haplotype 1
POQN114a	Portuguesa	Qdra Negra	House 114	Domestic	*R. prolixus* haplotype 1
POQN26a	Portuguesa	Qdra Negra	House 26	Domestic	*R. prolixus* haplotype 1
POQN27a	Portuguesa	Qdra Negra	House 27	Domestic	*R. prolixus* haplotype 1
POQN27b	Portuguesa	Qdra Negra	House 27	Domestic	*R. prolixus* haplotype 1
POQN22.1b	Portuguesa	Qdra Negra	House 22.1	Domestic	*R. prolixus* haplotype 1
POQN30.1a	Portuguesa	Qdra Negra	House 30.1	Domestic	*R. prolixus* haplotype 1
T1P15	Portuguesa	Terronal	House 1 03	Domestic	*R. prolixus* haplotype 1

Label	State	Locality	Location	Ecotope	Cytb
POSB19a	Portuguesa	San Bartolo	House 19	Domestic	*R. prolixus* haplotype 1
POSB19b	Portuguesa	San Bartolo	House 19	Domestic	*R. prolixus* haplotype 1
POSB19c	Portuguesa	San Bartolo	House 19	Domestic	*R. prolixus* haplotype 1
POSB19e	Portuguesa	San Bartolo	House 19	Domestic	*R. prolixus* haplotype 1
POSB19f	Portuguesa	San Bartolo	House 19	Domestic	*R. prolixus* haplotype 1
POSB19g	Portuguesa	San Bartolo	House 19	Domestic	*R. prolixus* haplotype 1
POSB19k	Portuguesa	San Bartolo	House 19	Domestic	*R. prolixus* haplotype 1
POSB20a	Portuguesa	San Bartolo	House 20	Domestic	*R. prolixus* haplotype 1
POSB25a	Portuguesa	San Bartolo	House 25	Domestic	*R. prolixus* haplotype 1
POSBMAR19	Portuguesa	San Bartolo	House 2	Domestic	*R. prolixus* haplotype 1
POSBMAR15	Portuguesa	San Bartolo	House 2	Domestic	*R. prolixus* haplotype 1
POSBMAR17	Portuguesa	San Bartolo	House 2	Domestic	*R. prolixus* haplotype 1
POSL89n	Portuguesa	Santa Lucia	House	Domestic	*R. prolixus* haplotype 1
POSL89l	Portuguesa	Santa Lucia	House	Domestic	*R. prolixus* haplotype 1
43TERRfp	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
45terfp	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
108 TERRp	Portuguesa	Terronal	House 2 0102	Silvatic palm	*R. prolixus* haplotype 1
116 TERRp	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
140 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
143 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
144 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
146 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
151 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
152 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
154 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
155 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
156 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
158 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
162 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
163 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1

Label	State	Locality	Location	Ecotope	Cytb
164 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
168 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
170 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
174 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
176 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
179 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
183 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
186 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
188 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. prolixus* haplotype 1
159 gua	Guarico	Bravero	Not near house	Silvatic palm	*R. prolixus* haplotype 1
171 gua	Guarico	Bravero	Not near house	Silvatic palm	*R. prolixus* haplotype 1
172 gua	Guarico	Bravero	Not near house	Silvatic palm	*R. prolixus* haplotype 1
62 ortiz	Guarico	Bravero	Not near house	Silvatic palm	*R. prolixus* haplotype 1
63 ortiz	Guarico	Bravero	Not near house	Silvatic palm	*R. prolixus* haplotype 1
CP2	Portuguesa	Casa Rena	Palm 1	Silvatic palm	*R. prolixus* haplotype 1
CP6	Portuguesa	Casa Rena	Palm 1	Silvatic palm	*R. prolixus* haplotype 1
T2P3	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P15	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P18	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P1	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P20	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P21	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P23	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P25	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P26	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P27	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P29	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P2	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P30	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P31	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1

Label	State	Locality	Location	Ecotope	Cytb
T2P32	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P35	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P36	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P38	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P37	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P38	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P40	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P41	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
T2P39	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 1
CAR1b1	Barinas	Carreteron	House 1	Silvatic palm	*R. prolixus* haplotype 1
110 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	*R. prolixus* haplotype 1
115 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	*R. prolixus* haplotype 1
139 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	*R. prolixus* haplotype 1
149 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	*R. prolixus* haplotype 1
153 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	*R. prolixus* haplotype 1
161 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	*R. prolixus* haplotype 1
COJ10P	Cojedes	Las Queseras	J.P. Arraez	Silvatic palm	*R. prolixus* haplotype 1
COJ11P	Cojedes	Las Queseras	J.P. Arraez	Silvatic palm	*R. prolixus* haplotype 1
COJ15P	Cojedes	Las Queseras	J.P. Arraez	Silvatic palm	*R. prolixus* haplotype 1
COJ16P	Cojedes	Las Queseras	J.P. Arraez	Silvatic palm	*R. prolixus* haplotype 1
COJ17P	Cojedes	Las Queseras	J.P. Arraez	Silvatic palm	*R. prolixus* haplotype 1
COJ19P	Cojedes	Las Queseras	J.P. Arraez	Silvatic palm	*R. prolixus* haplotype 1
COJ1P	Cojedes	Las Queseras	J.P. Arraez	Silvatic palm	*R. prolixus* haplotype 1
COJ3P	Cojedes	Las Queseras	J.P. Arraez	Silvatic palm	*R. prolixus* haplotype 1
COJ5P	Cojedes	Las Queseras	J.P. Arraez	Silvatic palm	*R. prolixus* haplotype 1
COJ6P	Cojedes	Las Queseras	J.P. Arraez	Silvatic palm	*R. prolixus* haplotype 1
COJ7P	Cojedes	Las Queseras	J.P. Arraez	Silvatic palm	*R. prolixus* haplotype 1
COJ9P	Cojedes	Las Queseras	J.P. Arraez	Silvatic palm	*R. prolixus* haplotype 1
T1P1	Portuguesa	Terronal	House 1 03	Silvatic palm	*R. prolixus* haplotype 1
44 TERFP	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. prolixus* haplotype 2

Label	State	Locality	Location	Ecotope	Cytb
66 gua	Guarico	El Sombero	Not near house	Silvatic palm	*R. prolixus* haplotype 2
106 gua	Guarico	El Sombero	Not near house	Silvatic palm	*R. prolixus* haplotype 2
111 gua	Guarico	El Sombero	Not near house	Silvatic palm	*R. prolixus* haplotype 2
112 gua	Guarico	El Manguito	Not near house	Silvatic palm	*R. prolixus* haplotype 2
113 gua	Guarico	El Manguito	Not near house	Silvatic palm	*R. prolixus* haplotype 2
118 gua	Guarico	El Manguito	Not near house	Silvatic palm	*R. prolixus* haplotype 2
119 gua	Guarico	El Manguito	Not near house	Silvatic palm	*R. prolixus* haplotype 2
120 gua	Guarico	El Manguito	Not near house	Silvatic palm	*R. prolixus* haplotype 2
160 gua	Guarico		Not near house	Silvatic palm	*R. prolixus* haplotype 2
182 gua	Guarico	El Manguito	Not near house	Silvatic palm	*R. prolixus* haplotype 2
184 gua	Guarico		Not near house	Silvatic palm	*R. prolixus* haplotype 2
15 TERR	Portuguesa	Terronal	House 2 01	Domestic	*R. robustus* haplotype 3
11 TERR	Portuguesa	Terronal	House 2 01	Domestic	*R. robustus* haplotype 3
17 TERR	Portuguesa	Terronal	House 2 01	Domestic	*R. robustus* haplotype 3
54 TERR	Portuguesa	Terronal	House 2 01	Domestic	*R. robustus* haplotype 3
6 TERR	Portuguesa	Terronal	House 2 01	Domestic	*R. robustus* haplotype 3
8 TERR	Portuguesa	Terronal	House 2 01	Domestic	*R. robustus* haplotype 3
18 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. robustus* haplotype 3
19 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. robustus* haplotype 3
22 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. robustus* haplotype 3
27 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. robustus* haplotype 3
55 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. robustus* haplotype 3
20 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. robustus* haplotype 3
24 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. robustus* haplotype 3
28 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. robustus* haplotype 3
30 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. robustus* haplotype 3
31 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. robustus* haplotype 3
49 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. robustus* haplotype 3
133 TERR	Portuguesa	Terronal	House 1 01	Domestic	*R. robustus* haplotype 3
CP101	Portuguesa	Casa rena	House	Domestic	*R. robustus* haplotype 3

Label	State	Locality	Location	Ecotope	Cytb
CP11	Portuguesa	Casa rena	House	Domestic	*R. robustus* haplotype 3
CP13	Portuguesa	Casa rena	House	Domestic	*R. robustus* haplotype 3
CP14	Portuguesa	Casa rena	House	Domestic	*R. robustus* haplotype 3
CP15	Portuguesa	Casa rena	House	Domestic	*R. robustus* haplotype 3
CP16	Portuguesa	Casa rena	House	Domestic	*R. robustus* haplotype 3
CP17	Portuguesa	Casa rena	House	Domestic	*R. robustus* haplotype 3
T2P16	Portuguesa	Terronal	House 2 03	Domestic	*R. robustus* haplotype 3
CAR2b8	Barinas	Carreteron	House 2	Domestic	*R. robustus* haplotype 3
POMO10A	Portuguesa	Morichal	House 10	Domestic	*R. robustus* haplotype 3
T1P12	Portuguesa	Terronal	House 1 03	Domestic	*R. robustus* haplotype 3
T1P13	Portuguesa	Terronal	House 1 03	Domestic	*R. robustus* haplotype 3
T1P16	Portuguesa	Terronal	House 1 03	Domestic	*R. robustus* haplotype 3
T1P5	Portuguesa	Terronal	House 1 03	Domestic	*R. robustus* haplotype 3
T1P6	Portuguesa	Terronal	House 1 03	Domestic	*R. robustus* haplotype 3
T1P7	Portuguesa	Terronal	House 1 03	Domestic	*R. robustus* haplotype 3
134 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. robustus* haplotype 3
136 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. robustus* haplotype 3
145 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. robustus* haplotype 3
173 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. robustus* haplotype 3
38 TERFP	Portuguesa	Terronal	House 2 01	Silvatic palm	*R. robustus* haplotype 3
CP11	Portuguesa	Casa Rena	Palm	Silvatic palm	*R. robustus* haplotype 3
T2P17	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. robustus* haplotype 3
T2P19	Portuguesa	Terronal	House 2 03	Silvatic palm	*R. robustus* haplotype 3
121 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	*R. robustus* haplotype 3
137 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	*R. robustus* haplotype 3
147 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	*R. robustus* haplotype 3
185 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	*R. robustus* haplotype 3
COJ20P	Cojedes	Las Queseras	J.P. Arraez	Silvatic palm	*R. robustus* haplotype 3
COJ8P	Cojedes	Las Queseras	J.P. Arraez	Silvatic palm	*R. robustus* haplotype 3
LDAT10	Trujillo	Loma de Amarillo	House 1	Domestic	*R. prolixus* haplotype 5

Label	State	Locality	Location	Ecotope	Cytb
LDAT12	Trujillo	Loma de Amarillo	House 1	Domestic	*R. prolixus* haplotype 5
LDAT13	Trujillo	Loma de Amarillo	House 1	Domestic	*R. prolixus* haplotype 5
LDAT14	Trujillo	Loma de Amarillo	House 1	Domestic	*R. prolixus* haplotype 5
LDAT19	Trujillo	Loma de Amarillo	House 1	Domestic	*R. prolixus* haplotype 5
LDAT20	Trujillo	Loma de Amarillo	House 1	Domestic	*R. prolixus* haplotype 5
LDAT21	Trujillo	Loma de Amarillo	House 1	Domestic	*R. prolixus* haplotype 5
LDAT22	Trujillo	Loma de Amarillo	House 1	Domestic	*R. prolixus* haplotype 5
LDAT23	Trujillo	Loma de Amarillo	House 1	Domestic	*R. prolixus* haplotype 5
LDAT24	Trujillo	Loma de Amarillo	House 1	Domestic	*R. prolixus* haplotype 5
LDAT25	Trujillo	Loma de Amarillo	House 1	Domestic	*R. prolixus* haplotype 5
LDAT26	Trujillo	Loma de Amarillo	House 1	Domestic	*R. prolixus* haplotype 5
CAR2b5	Barinas	Carreteron	House 2	Domestic	*R. prolixus* haplotype 5
POQN20d	Portuguesa	Qdra Negra	House 20	Domestic	*R. prolixus* haplotype 5
POQN 20e	Portuguesa	Qdra Negra	House 20	Domestic	*R. prolixus* haplotype 5

Table 72. Population subgroups analysed by both morphometrics and *cytb*.

Label	State	Locality	Location	Ecotope	Haplotype
16 TERR	Portuguesa	Terronal	House 2 01	Domestic	Haplotype 1 *R. prolixus*
1 TERR	Portuguesa	Terronal	House 2 01	Domestic	Haplotype 1 *R. prolixus*
2 TERR	Portuguesa	Terronal	House 2 01	Domestic	Haplotype 1 *R. prolixus*
53 TERR	Portuguesa	Terronal	House 2 01	Domestic	Haplotype 1 *R. prolixus*
9 TERR	Portuguesa	Terronal	House 2 01	Domestic	Haplotype 1 *R. prolixus*
10 TERR	Portuguesa	Terronal	House 2 01	Domestic	Haplotype 1 *R. prolixus*
5 TERR	Portuguesa	Terronal	House 2 01	Domestic	Haplotype 1 *R. prolixus*
7 TERR	Portuguesa	Terronal	House 2 01	Domestic	Haplotype 1 *R. prolixus*
15 TERR	Portuguesa	Terronal	House 2 01	Domestic	Haplotype 3 *R. robustus*
11 TERR	Portuguesa	Terronal	House 2 01	Domestic	Haplotype 3 *R. robustus*
17 TERR	Portuguesa	Terronal	House 2 01	Domestic	Haplotype 3 *R. robustus*

Label	State	Locality	Location	Ecotope	Haplotype
54 TERR	Portuguesa	Terronal	House 2 01	Domestic	Haplotype 3 *R. robustus*
6 TERR	Portuguesa	Terronal	House 2 01	Domestic	Haplotype 3 *R. robustus*
8 TERR	Portuguesa	Terronal	House 2 01	Domestic	Haplotype 3 *R. robustus*
21 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 1 *R. prolixus*
23 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 1 *R. prolixus*
25 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 1 *R. prolixus*
33 TERR1	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 1 *R. prolixus*
57 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 1 *R. prolixus*
23 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 1 *R. prolixus*
48 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 1 *R. prolixus*
18 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 3 *R. robustus*
19 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 3 *R. robustus*
22 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 3 *R. robustus*
27 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 3 *R. robustus*
55 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 3 *R. robustus*
20 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 3 *R. robustus*
24 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 3 *R. robustus*
28 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 3 *R. robustus*
31 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 3 *R. robustus*
49 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 3 *R. robustus*
133 TERR	Portuguesa	Terronal	House 1 01	Domestic	Haplotype 3 *R. robustus*
43 TERRfp	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
45 terfp	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
108 TERRp	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
116 TERRp	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
140 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
143 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
144 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
146 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*

Label	State	Locality	Location	Ecotope	Haplotype
151 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
152 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
154 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
155 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
156 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
158 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
162 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
163 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
164 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
168 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
170 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
174 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
176 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
183 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
186 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
188 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 1 *R. prolixus*
44terfp	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 3 *R. prolixus*
134 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 3 *R. robustus*
136 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 3 *R. robustus*
145 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 3 *R. robustus*
173 TERR	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 3 *R. robustus*
38TERRfp	Portuguesa	Terronal	House 2 01	Silvatic palm	Haplotype 3 *R. robustus*
102 lara	lara	Salvador	House 2	Domestic	Haplotype 1 *R. prolixus*
103 lara	lara	Salvador	House 2	Domestic	Haplotype 1 *R. prolixus*
125 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*
122 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*
126 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*
129 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*
130 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*

Label	State	Locality	Location	Ecotope	Haplotype
131 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*
69 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*
94 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*
95 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*
96 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*
84 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*
107 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*
90 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*
74 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*
75 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*
76 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*
79 lara	lara	Guamarito	House 1	Domestic	Haplotype 1 *R. prolixus*
159 gua	Guarico	Bravero	Not near house	Silvatic palm	Haplotype 1 *R. prolixus*
171 gua	Guarico	Bravero	Not near house	Silvatic palm	Haplotype 1 *R. prolixus*
172 gua	Guarico	Bravero	Not near house	Silvatic palm	Haplotype 1 *R. prolixus*
62ortiz	Guarico	Bravero	Not near house	Silvatic palm	Haplotype 1 *R. prolixus*
63ortiz	Guarico	Bravero	Not near house	Silvatic palm	Haplotype 1 *R. prolixus*
66 gua	Guarico	El Sombero	Not near house	Silvatic palm	Haplotype 2 *R. prolixus*
106 gua	Guarico	El Sombero	Not near house	Silvatic palm	Haplotype 2 *R. prolixus*
111 gua	Guarico	El Sombero	Not near house	Silvatic palm	Haplotype 2 *R. prolixus*
112 gua	Guarico	El Manguito	Not near house	Silvatic palm	Haplotype 2 *R. prolixus*
113 gua	Guarico	El Manguito	Not near house	Silvatic palm	Haplotype 2 *R. prolixus*
118 gua	Guarico	El Manguito	Not near house	Silvatic palm	Haplotype 2 *R. prolixus*
119 gua	Guarico	El Manguito	Not near house	Silvatic palm	Haplotype 2 *R. prolixus*
120 gua	Guarico	El Manguito	Not near house	Silvatic palm	Haplotype 2 *R. prolixus*
160 gua	Guarico		Not near house	Silvatic palm	Haplotype 2 *R. prolixus*
182 gua	Guarico	El Manguito	Not near house	Silvatic palm	Haplotype 2 *R. prolixus*
184 gua	Guarico		Not near house	Silvatic palm	Haplotype 2 *R. prolixus*
LDAT10	Trujillo	Loma de Amarillo	House 1	Domestic	Haplotype 5 *R. prolixus*

Label	State	Locality	Location	Ecotope	Haplotype
LDAT12	Trujillo	Loma de Amarillo	House 1	Domestic	Haplotype 5 *R. prolixus*
LDAT13	Trujillo	Loma de Amarillo	House 1	Domestic	Haplotype 5 *R. prolixus*
LDAT14	Trujillo	Loma de Amarillo	House 1	Domestic	Haplotype 5 *R. prolixus*
LDAT19	Trujillo	Loma de Amarillo	House 1	Domestic	Haplotype 5 *R. prolixus*
LDAT1	Trujillo	Loma de Amarillo	House 1	Domestic	Haplotype 16 *R. robustus*
LDAT20	Trujillo	Loma de Amarillo	House 1	Domestic	Haplotype 5 *R. prolixus*
LDAT21	Trujillo	Loma de Amarillo	House 1	Domestic	Haplotype 5 *R. prolixus*
LDAT22	Trujillo	Loma de Amarillo	House 1	Domestic	Haplotype 5 *R. prolixus*
LDAT23	Trujillo	Loma de Amarillo	House 1	Domestic	Haplotype 5 *R. prolixus*
LDAT24	Trujillo	Loma de Amarillo	House 1	Domestic	Haplotype 5 *R. prolixus*
LDAT25	Trujillo	Loma de Amarillo	House 1	Domestic	Haplotype 5 *R. prolixus*
LDAT26	Trujillo	Loma de Amarillo	House 1	Domestic	Haplotype 5 *R. prolixus*
INS1	Trujillo	Insectary	Insectary	Insectary	Haplotype 16 *R. robustus*
INS2	Trujillo	Insectary	Insectary	Insectary	Haplotype 16 *R. robustus*
INS3	Trujillo	Insectary	Insectary	Insectary	Haplotype 18 *R. robustus*
LJT2	Trujillo	La Juventud	Palm 1	Silvatic palm	Haplotype 17 *R. robustus*
LJT3	Trujillo	La Juventud	Palm 1	Silvatic palm	Haplotype 16 *R. robustus*
LJT4	Trujillo	La Juventud	Palm 1	Silvatic palm	Haplotype 17 *R. robustus*
CP10	Portuguesa	Casa Rena	House 1	Domestic	Haplotype 3 *R. robustus*
CP11	Portuguesa	Casa Rena	House 1	Domestic	Haplotype 3 *R. robustus*
CP13	Portuguesa	Casa Rena	House 1	Domestic	Haplotype 3 *R. robustus*
CP14	Portuguesa	Casa Rena	House 1	Domestic	Haplotype 3 *R. robustus*
CP15	Portuguesa	Casa Rena	House 1	Domestic	Haplotype 3 *R. robustus*
CP16	Portuguesa	Casa Rena	House 1	Domestic	Haplotype 3 *R. robustus*
CP17	Portuguesa	Casa Rena	House 1	Domestic	Haplotype 3 *R. robustus*
T2P15	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P18	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P1	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P20	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*

Label	State	Locality	Location	Ecotope	Haplotype
T2P21	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P23	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P25	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P26	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P27	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P29	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P2	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P30	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P31	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P32	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P35	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P36	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P38	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P37	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P38	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P40	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P41	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P39	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 1 *R. prolixus*
T2P17	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 3 *R. robustus*
T2P19	Portuguesa	Terronal	House 2 03	Silvatic palm	Haplotype 3 *R. robustus*
CAR2b3	Barinas	Carreteron	House 2	Domestic	Haplotype 1 *R. prolixus*
CAR2b4	Barinas	Carreteron	House 2	Domestic	Haplotype 1 *R. prolixus*
CAR2b7	Barinas	Carreteron	House 2	Domestic	Haplotype 1 *R. prolixus*
CAR3b2	Barinas	Carreteron	House 3	Domestic	Haplotype 1 *R. prolixus*
CAR4b3	Barinas	Carreteron	House 4	Domestic	Haplotype 1 *R. prolixus*
CAR4b5	Barinas	Carreteron	House 4	Domestic	Haplotype 1 *R. prolixus*
CAR6b1	Barinas	Carreteron	House 6	Domestic	Haplotype 1 *R. prolixus*
CAR1b10	Barinas	Carreteron	House 1	Domestic	Haplotype 1 *R. prolixus*
CAR1b11	Barinas	Carreteron	House 1	Domestic	Haplotype 1 *R. prolixus*

Label	State	Locality	Location	Ecotope	Haplotype
CAR2b8	Barinas	Carreteron	House 2	Domestic	Haplotype 3 *R. robustus*
CAR2b2	Barinas	Carreteron	House 2	Domestic	Haplotype 4 *R. prolixus*
CAR2b6	Barinas	Carreteron	House 2	Domestic	Haplotype 4 *R. prolixus*
CAR2b5	Barinas	Carreteron	House 2	Domestic	Haplotype 5 *R. prolixus*
CAR5b1	Barinas	Carreteron	House 5	Domestic	Haplotype 7 *R. prolixus*
110 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	Haplotype 1 *R. prolixus*
115 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	Haplotype 1 *R. prolixus*
139 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	Haplotype 1 *R. prolixus*
149 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	Haplotype 1 *R. prolixus*
153 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	Haplotype 1 *R. prolixus*
161 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	Haplotype 1 *R. prolixus*
121 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	Haplotype 3 *R. robustus*
137 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	Haplotype 3 *R. robustus*
147 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	Haplotype 3 *R. robustus*
185 PALO	Portuguesa	Palo Gacho	Not near house	Silvatic palm	Haplotype 3 *R. robustus*
COJ12h	Cojedes	Las Queseras	J.P. Arreaz	Domestic	Haplotype 1 *R. prolixus*
COJ3h	Cojedes	Las Queseras	J.P. Arreaz	Domestic	Haplotype 1 *R. prolixus*
COJ4p	Cojedes	Las Queseras	J.P. Arreaz	Domestic	Haplotype 1 *R. prolixus*
COJ5h	Cojedes	Las Queseras	J.P. Arreaz	Domestic	Haplotype 1 *R. prolixus*
COJ6h	Cojedes	Las Queseras	J.P. Arreaz	Domestic	Haplotype 1 *R. prolixus*
COJ7h	Cojedes	Las Queseras	J.P. Arreaz	Domestic	Haplotype 1 *R. prolixus*
COJ10p	Cojedes	Las Queseras	J.P. Arreaz	Silvatic palm	Haplotype 1 *R. prolixus*
COJ11p	Cojedes	Las Queseras	J.P. Arreaz	Silvatic palm	Haplotype 1 *R. prolixus*
COJ15p	Cojedes	Las Queseras	J.P. Arreaz	Silvatic palm	Haplotype 1 *R. prolixus*
COJ16p	Cojedes	Las Queseras	J.P. Arreaz	Silvatic palm	Haplotype 1 *R. prolixus*
COJ17p	Cojedes	Las Queseras	J.P. Arreaz	Silvatic palm	Haplotype 1 *R. prolixus*
COJ19p	Cojedes	Las Queseras	J.P. Arreaz	Silvatic palm	Haplotype 1 *R. prolixus*
COJ1p	Cojedes	Las Queseras	J.P. Arreaz	Silvatic palm	Haplotype 1 *R. prolixus*
COJ20p	Cojedes	Las Queseras	J.P. Arreaz	Silvatic palm	Haplotype 3 *R. robustus*

Label	State	Locality	Location	Ecotope	Haplotype
COJ3p	Cojedes	Las Queseras	J.P. Arreaz	Silvatic palm	Haplotype 1 *R. prolixus*
COJ5p	Cojedes	Las Queseras	J.P. Arreaz	Silvatic palm	Haplotype 1 *R. prolixus*
COJ6p	Cojedes	Las Queseras	J.P. Arreaz	Silvatic palm	Haplotype 1 *R. prolixus*
COJ7p	Cojedes	Las Queseras	J.P. Arreaz	Silvatic palm	Haplotype 1 *R. prolixus*
COJ8p	Cojedes	Las Queseras	J.P. Arreaz	Silvatic palm	Haplotype 3 *R. robustus*
COJ9p	Cojedes	Las Queseras	J.P. Arreaz	Silvatic palm	Haplotype 1 *R. prolixus*
POMO10.1b	Portuguesa	Morichal	House 10.1	Domestic	Haplotype 1 *R. prolixus*
POMO10.1cl	Portuguesa	Morichal	House 10.1	Domestic	Haplotype 1 *R. prolixus*
POMO10.1f	Portuguesa	Morichal	House 10.1	Domestic	Haplotype 1 *R. prolixus*
POMO10.1g	Portuguesa	Morichal	House 10.1	Domestic	Haplotype 1 *R. prolixus*
POMO10a	Portuguesa	Morichal	House 10	Domestic	Haplotype 3 *R. robustus*
POMO21a	Portuguesa	El Mosquito	House 21	Domestic	Haplotype 1 *R. prolixus*
POMO21bl	Portuguesa	El Mosquito	House 22	Domestic	Haplotype 2 *R. prolixus*
POMO21cl	Portuguesa	El Mosquito	House 23	Domestic	Haplotype 1 *R. prolixus*
POMO40a	Portuguesa	El Mosquito	House 40	Domestic	Haplotype 1 *R. prolixus*
POMO48a	Portuguesa	El Mosquito	House 48	Domestic	Haplotype 1 *R. prolixus*
POMO8a	Portuguesa	El Mosquito	House 8	Domestic	Haplotype 1 *R. prolixus*
POQN20b	Portuguesa	Qdra Negra	House 20	Domestic	Haplotype 1 *R. prolixus*
POQN20cl	Portuguesa	Qdra Negra	House 20	Domestic	Haplotype 1 *R. prolixus*
POQN114a	Portuguesa	Qdra Negra	House 114	Domestic	Haplotype 1 *R. prolixus*
POQN26al	Portuguesa	Qdra Negra	House 26	Domestic	Haplotype 1 *R. prolixus*
POQN27a	Portuguesa	Qdra Negra	House 27	Domestic	Haplotype 1 *R. prolixus*
POQN27b	Portuguesa	Qdra Negra	House 27	Domestic	Haplotype 1 *R. prolixus*
POQN22.1b	Portuguesa	Qdra Negra	House 22.1	Domestic	Haplotype 1 *R. prolixus*
POQN30.1a	Portuguesa	Qdra Negra	House 30.1	Domestic	Haplotype 1 *R. prolixus*
POQN20f	Portuguesa	Qdra Negra	House 20	Domestic	Haplotype 2 *R. prolixus*
POQN20d	Portuguesa	Qdra Negra	House 20	Domestic	Haplotype 5 *R. prolixus*
POQN20e	Portuguesa	Qdra Negra	House 20	Domestic	Haplotype 5 *R. prolixus*
POQN22.1a	Portuguesa	Qdra Negra	House 22.1	Domestic	Haplotype 8 *R. prolixus*

Label	State	Locality	Location	Ecotope	Haplotype
T1P15	Portuguesa	Terronal	House 1 03	Domestic	Haplotype 1 *R. prolixus*
T1P12	Portuguesa	Terronal	House 1 03	Domestic	Haplotype 3 *R. robustus*
T1P13	Portuguesa	Terronal	House 1 03	Domestic	Haplotype 3 *R. robustus*
T1P16	Portuguesa	Terronal	House 1 03	Domestic	Haplotype 3 *R. robustus*
T1P5	Portuguesa	Terronal	House 1 03	Domestic	Haplotype 3 *R. robustus*
T1P6	Portuguesa	Terronal	House 1 03	Domestic	Haplotype 3 *R. robustus*
T1P7	Portuguesa	Terronal	House 1 03	Domestic	Haplotype 3 *R. robustus*
POSB19a	Portuguesa	San Bartolo	House 1	Domestic	Haplotype 1 *R. prolixus*
POSB19b	Portuguesa	San Bartolo	House 1	Domestic	Haplotype 1 *R. prolixus*
POSB19cl	Portuguesa	San Bartolo	House 1	Domestic	Haplotype 1 *R. prolixus*
POSB19e	Portuguesa	San Bartolo	House 1	Domestic	Haplotype 1 *R. prolixus*
POSB19f	Portuguesa	San Bartolo	House 1	Domestic	Haplotype 1 *R. prolixus*
POSB19g	Portuguesa	San Bartolo	House 1	Domestic	Haplotype 1 *R. prolixus*
POSB19k	Portuguesa	San Bartolo	House 1	Domestic	Haplotype 1 *R. prolixus*
POSB20ar	Portuguesa	San Bartolo	House 20	Domestic	Haplotype 1 *R. prolixus*
POSB25a	Portuguesa	San Bartolo	House 25	Domestic	Haplotype 1 *R. prolixus*
POSBMAR19	Portuguesa	San Bartolo	House 2	Domestic	Haplotype 1 *R. prolixus*
POSBMAR15	Portuguesa	San Bartolo	House 2	Domestic	Haplotype 1 *R. prolixus*
POSBMAR17	Portuguesa	San Bartolo	House 2	Domestic	Haplotype 1 *R. prolixus*

Table 73. Population subgroups analysed by both morphometrics and microsatellites.

Label	State	Locality	Ecotope	Location
Lara				
122 lara	Lara	Guamarito	Domestic	House 1
126 lara	Lara	Guamarito	Domestic	House 1
129 lara	Lara	Guamarito	Domestic	House 1
130 lara	Lara	Guamarito	Domestic	House 1
131 lara	Lara	Guamarito	Domestic	House 1

Label	State	Locality	Ecotope	Location
94 lara	Lara	Guamarito	Domestic	House 1
95 lara	Lara	Guamarito	Domestic	House 1
96 lara	Lara	Guamarito	Domestic	House 1
84 lara	Lara	Guamarito	Domestic	House 1
90 lara	Lara	Guamarito	Domestic	House 1
79 lara	Lara	Guamarito	Domestic	House 1
102 lara	Lara	Salvador	Domestic	House 2
103 lara	Lara	Salvador	Domestic	House 2
Cojedes				
COJ12h	Cojedes	Las Queseras	Domestic	J. P. Arraez
COJ2h	Cojedes	Las Queseras	Domestic	J. P. Arraez
COJ3h	Cojedes	Las Queseras	Domestic	J. P. Arraez
COJ4p	Cojedes	Las Queseras	Domestic	J. P. Arraez
COJ5h	Cojedes	Las Queseras	Domestic	J. P. Arraez
COJ6h	Cojedes	Las Queseras	Domestic	J. P. Arraez
COJ7h	Cojedes	Las Queseras	Domestic	J. P. Arraez
COJ10p	Cojedes	Las Queseras	Silvatic palm	J. P. Arraez
COJ11p	Cojedes	Las Queseras	Silvatic palm	J. P. Arraez
COJ15p	Cojedes	Las Queseras	Silvatic palm	J. P. Arraez
COJ16p	Cojedes	Las Queseras	Silvatic palm	J. P. Arraez
COJ17p	Cojedes	Las Queseras	Silvatic palm	J. P. Arraez
COJ19p	Cojedes	Las Queseras	Silvatic palm	J. P. Arraez
COJ1p	Cojedes	Las Queseras	Silvatic palm	J. P. Arraez
COJ20p	Cojedes	Las Queseras	Silvatic palm	J. P. Arraez
COJ3p	Cojedes	Las Queseras	Silvatic palm	J. P. Arraez
COJ5p	Cojedes	Las Queseras	Silvatic palm	J. P. Arraez
COJ6p	Cojedes	Las Queseras	Silvatic palm	J. P. Arraez

Label	State	Locality	Ecotope	Location
COJ7p	Cojedes	Las Queseras	Silvatic palm	J. P. Arraez
COJ8p	Cojedes	Las Queseras	Silvatic palm	J. P. Arraez
COJ9p	Cojedes	Las Queseras	Silvatic palm	J. P. Arraez
Trujillo				
LDAT10	Trujillo	Loma de Amarillo	Domestic	House 1
LDAT12	Trujillo	Loma de Amarillo	Domestic	House 1
LDAT13	Trujillo	Loma de Amarillo	Domestic	House 1
LDAT14	Trujillo	Loma de Amarillo	Domestic	House 1
LDAT16	Trujillo	Loma de Amarillo	Domestic	House 1
LDAT19	Trujillo	Loma de Amarillo	Domestic	House 1
LDAT1	Trujillo	Loma de Amarillo	Domestic	House 1
LDAT20	Trujillo	Loma de Amarillo	Domestic	House 1
LDAT21	Trujillo	Loma de Amarillo	Domestic	House 1
LDAT22	Trujillo	Loma de Amarillo	Domestic	House 1
LDAT23	Trujillo	Loma de Amarillo	Domestic	House 1
LDAT24	Trujillo	Loma de Amarillo	Domestic	House 1
LDAT25	Trujillo	Loma de Amarillo	Domestic	House 1
LDAT26	Trujillo	Loma de Amarillo	Domestic	House 1
LDAT8	Trujillo	Loma de Amarillo	Domestic	House 1
LDAT11	Trujillo	Loma de Amarillo	Domestic	House 1
Portuguesa				
L2P11	Portuguesa	Laurianito pd	Peridomestic	House 1
L2P12	Portuguesa	Laurianito pd	Peridomestic	House 1
L2P14	Portuguesa	Laurianito pd	Peridomestic	House 1
L2P15	Portuguesa	Laurianito pd	Peridomestic	House 1
L2P16	Portuguesa	Laurianito pd	Peridomestic	House 1
L2P18	Portuguesa	Laurianito pd	Peridomestic	House 1
L2P19	Portuguesa	Laurianito pd	Peridomestic	House 1
L2P1	Portuguesa	Laurianito pd	Peridomestic	House 1

Label	State	Locality	Ecotope	Location
L2P20	Portuguesa	Laurianito pd	Peridomestic	House 1
L2P22	Portuguesa	Laurianito pd	Peridomestic	House 1
L2P24	Portuguesa	Laurianito pd	Peridomestic	House 1
L2P25	Portuguesa	Laurianito pd	Peridomestic	House 1
L2P4	Portuguesa	Laurianito pd	Peridomestic	House 1
L2P7	Portuguesa	Laurianito pd	Peridomestic	House 1
L2P8	Portuguesa	Laurianito pd	Peridomestic	House 1
L2P10	Portuguesa	Laurianito pd	Peridomestic	House 1
110 PALO	Portuguesa	Palo Gacho	Silvatic palm	Not near House
117 PALO	Portuguesa	Palo Gacho	Silvatic palm	Not near House
121 PALO	Portuguesa	Palo Gacho	Silvatic palm	Not near House
115 PALO	Portuguesa	Palo Gacho	Silvatic palm	Not near House
147 PALO	Portuguesa	Palo Gacho	Silvatic palm	Not near House
149 PALO	Portuguesa	Palo Gacho	Silvatic palm	Not near House
153 PALO	Portuguesa	Palo Gacho	Silvatic palm	Not near House
167 PALO	Portuguesa	Palo Gacho	Silvatic palm	Not near House
169 PALO	Portuguesa	Palo Gacho	Silvatic palm	Not near House
178 PALO	Portuguesa	Palo Gacho	Silvatic palm	Not near House
185 PALO	Portuguesa	Palo Gacho	Silvatic palm	Not near House
189 PALO	Portuguesa	Palo Gacho	Silvatic palm	Not near House
190 PALO	Portuguesa	Palo Gacho	Silvatic palm	Not near House
POSB19a	Portuguesa	San Bartolo	Domestic	House 1
POSB19b	Portuguesa	San Bartolo	Domestic	House 1
POSB19c	Portuguesa	San Bartolo	Domestic	House 1
POSB19e	Portuguesa	San Bartolo	Domestic	House 1
POSB19f	Portuguesa	San Bartolo	Domestic	House 1
POSB19g	Portuguesa	San Bartolo	Domestic	House 1
POSB19k	Portuguesa	San Bartolo	Domestic	House 1

Label	State	Locality	Ecotope	Location
POSBMAR18	Portuguesa	San Bartolo	Domestic	House 2
POSBMAR14	Portuguesa	San Bartolo	Domestic	House 2
POSBMAR16	Portuguesa	San Bartolo	Domestic	House 2
POSBMAR15	Portuguesa	San Bartolo	Domestic	House 2
POSBMAR17	Portuguesa	San Bartolo	Domestic	House 2
18 TERR	Portuguesa	Terronal	Domestic	House 1 01
19 TERR	Portuguesa	Terronal	Domestic	House 1 01
21 TERR	Portuguesa	Terronal	Domestic	House 1 01
22 TERR	Portuguesa	Terronal	Domestic	House 1 01
23 TERR	Portuguesa	Terronal	Domestic	House 1 01
25 TERR	Portuguesa	Terronal	Domestic	House 1 01
26 TERR	Portuguesa	Terronal	Domestic	House 1 01
27 TERR	Portuguesa	Terronal	Domestic	House 1 01
33 TERR	Portuguesa	Terronal	Domestic	House 1 01
35 TERR	Portuguesa	Terronal	Domestic	House 1 01
36 TERR	Portuguesa	Terronal	Domestic	House 1 01
55 TERR	Portuguesa	Terronal	Domestic	House 1 01
57 TERR	Portuguesa	Terronal	Domestic	House 1 01
58 TERR	Portuguesa	Terronal	Domestic	House 1 01
20 TERR	Portuguesa	Terronal	Domestic	House 1 01
24 TERRr	Portuguesa	Terronal	Domestic	House 1 01
28 TERR	Portuguesa	Terronal	Domestic	House 1 01
30 TERR	Portuguesa	Terronal	Domestic	House 1 01
31 TERR	Portuguesa	Terronal	Domestic	House 1 01
32 TERR	Portuguesa	Terronal	Domestic	House 1 01
34 TERR	Portuguesa	Terronal	Domestic	House 1 01
48 TERR	Portuguesa	Terronal	Domestic	House 1 01
49 TERR	Portuguesa	Terronal	Domestic	House 1 01

Label	State	Locality	Ecotope	Location
T1P13	Portuguesa	Terronal	Domestic	House 1 03
T1P15	Portuguesa	Terronal	Domestic	House 1 03
T1P16	Portuguesa	Terronal	Domestic	House 1 03
T1P5	Portuguesa	Terronal	Domestic	House 1 03
T1P6	Portuguesa	Terronal	Domestic	House 1 03
T1P7	Portuguesa	Terronal	Domestic	House 1 03
T1P12	Portuguesa	Terronal	Domestic	House 1 03
14 TERR	Portuguesa	Terronal	Domestic	House 2 01
15 TERR	Portuguesa	Terronal	Domestic	House 2 01
2 TERR	Portuguesa	Terronal	Domestic	House 2 01
53 TERR	Portuguesa	Terronal	Domestic	House 2 01
9 TERR	Portuguesa	Terronal	Domestic	House 2 01
10 TERR	Portuguesa	Terronal	Domestic	House 2 01
11 TERR	Portuguesa	Terronal	Domestic	House 2 01
17 TERRr	Portuguesa	Terronal	Domestic	House 2 01
5 TERR	Portuguesa	Terronal	Domestic	House 2 01
6 TERR	Portuguesa	Terronal	Domestic	House 2 01
7 TERR	Portuguesa	Terronal	Domestic	House 2 01
8 TERR	Portuguesa	Terronal	Domestic	House 2 01
134 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
136 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
141 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
144 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
145 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
148 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
150 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
152 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
154 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01

Label	State	Locality	Ecotope	Location
155 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
156 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
158 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
162 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
163 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
164 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
168 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
170 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
173 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
174 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
176 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
179 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
183 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
187 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
188 TERR	Portuguesa	Terronal	Silvatic palm	House 2 01
T2P15	Portuguesa	Terronal	Silvatic palm	House 2 03
T2P18	Portuguesa	Terronal	Silvatic palm	House 2 03
T2P1	Portuguesa	Terronal	Silvatic palm	House 2 03
T2P20	Portuguesa	Terronal	Silvatic palm	House 2 03
T2P21	Portuguesa	Terronal	Silvatic palm	House 2 03
T2P23	Portuguesa	Terronal	Silvatic palm	House 2 03
T2P25	Portuguesa	Terronal	Silvatic palm	House 2 03
T2P26	Portuguesa	Terronal	Silvatic palm	House 2 03
T2P27	Portuguesa	Terronal	Silvatic palm	House 2 03
T2P29	Portuguesa	Terronal	Silvatic palm	House 2 03
T2P2	Portuguesa	Terronal	Silvatic palm	House 2 03
T2P30	Portuguesa	Terronal	Silvatic palm	House 2 03
T2P31	Portuguesa	Terronal	Silvatic palm	House 2 03
T2P32	Portuguesa	Terronal	Silvatic palm	House 2 03