

1.8 Protozoan parasites of the intestinal tract of the cockroach, *Periplaneta americana*

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Aims and objectives

This exercise is designed to:

1. Examine the protozoan parasites found in the various regions of the cockroach intestine and to draw and attempt to identify those found.
2. Make detailed drawings of the internal and external arrangement of each cell type.
3. Identify the organisms.

Introduction

A large number of Protozoa are found in the intestinal tract of almost all species of animals; most of those present in the cockroach intestine are flagellates but amoebae and ciliates also occur.

Laboratory equipment and consumables

(per student or group)

Equipment

Compound microscope	Sharp-pointed scissors
×10, ×40, ×100 objectives	Fine-point forceps
Wax-filled dissection dish	

Consumables

Pasteur pipettes and bulbs	22 × 22 mm coverslips
Glass microscope slides	Physiological saline (0.9% NaCl)
Lugol's iodine (or other bacteriological iodine solution)*	

*Dissolve 2 g potassium iodide in approximately 30 ml distilled water. Add 1 g iodine crystals, dissolve and make up to 100 ml.

Sources of parasite material

Freshly killed laboratory-reared cockroaches, *Periplaneta americana* (the bigger the better!) are the usual source, but other species, e.g. *P. blatta*, may be used.

Safety

Other than normal laboratory safety procedures, no special precautions are required. The only aspect worth pointing out is that some students may be allergic to insects and appropriate precautions should be taken for such persons.

Instructions for staff

Cockroaches should be killed in a jar containing ethyl acetate or chloroform and fixed ventral side up in a small dish of wax. This is best done for the students as there is a potential for numerous cockroach 'escapees' in the class.

Instructions for students

1. Before beginning to examine the cockroach intestine, ensure that the microscopes are set up in bright-field illumination and that the condenser iris is closed down to a very small aperture. Doing this will increase the contrast of the image obtained and allow more of the structures of the various parasites to be observed.
2. Open up the abdomen using fine scissors and forceps and squeeze a drop of rectal contents onto a slide; mix with physiological saline. Make sure that the gut contents do not dry during preparation of the smears, as the organisms are very susceptible to desiccation. Examine using wet film technique.
3. Pull out the intestine and Malpighian tubules and examine the gut contents at various points along the length, making wet preparations with saline to help visualise the parasites. Examine for amoebae, flagellates and ciliates. Examples of some of the protozoans you may encounter are illustrated in Fig. 1.8.1.
4. Make a note of which part of the gut the different organisms are found. Using the diagrams, try to identify what you find.
5. Large numbers of motile bacteria will be found in the preparations and these should not be confused with protozoan flag-

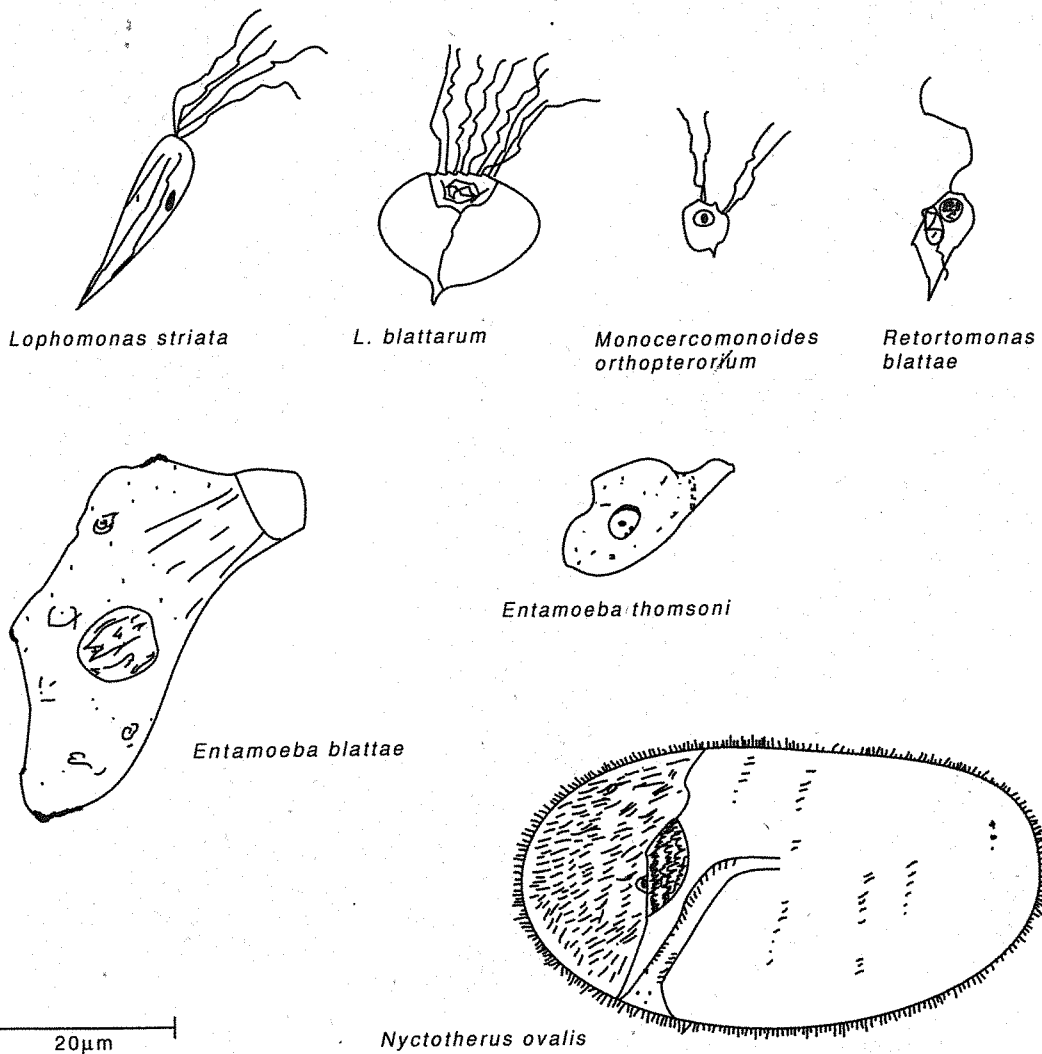


Fig. 1.8.1 Examples of protozoans likely to be found in the cockroach intestine.

ellates. Some of the bacteria are very large, rod-shaped with fibrils or flagella at both ends of the body and these can be differentiated from smaller protozoan flagellates by addition of Lugol's iodine to the preparation, which will kill any of the organisms present and stain their internal and external structures.

Ideas for further exploration

- This practical can be expanded to include permanently stained preparations of the various organisms found in the intestine.

- Gut contents can be placed in sodium acetate–acetic acid–formalin (SAF) fixative and smears made after fixation, which can then be stained using an iron haematoxylin stain to show morphology.
- Alternatively, thin smears can be made direct from the gut contents and fixed with methanol and stained using Giemsa; this is not so good for amoebae but does work reasonably well with flagellates.

Additional information

In the USA, live cockroaches (*Periplaneta* and *Blatta*) are supplied commercially by Carolina, Wards and other commercial suppliers. Woodroaches and termites (from Carolina) are also a good source of *Trichonympha* and related hypertrichs. You should request a catalogue from Carolina, Wards or your local supplier for recent information on availability before ordering.

REFERENCES

Mackinnon, D. L. & Hawes, R. S. J. (1961). *An Introduction to the Study of Protozoa*. Oxford, Clarendon Press.