CHAPTER-9

Staining & Mounting of Any Biota from Gut of Cockroach (Protozoa / Helminth)

Aim: Staining and mounting of any biota (Protozoa/Helminth) from gut of cockroach.

Materials Required:

a) Sample: Adult cockroach (*Periplaneta americana*)

b) Reagents: i. 0.5% NaCl solution , ii. Schaudinn's fixative, iii. Iodinated alcohol, iv. Distilled water, v. Heidenhain's haematoxylin, vi. 3% iron alum, vii. 1% iron alum, viii. Ascending and descending grades of ethanol, ix. Xylene, x. DPX.

c) Glasswares: i. Glass dropper/ Pasteur pipette, ii. 100 ml measuring cylinder, iii. 50 ml and 100 ml beaker, iv. Petridish with cover, v. Glass rod, vi. Dropping bottle, vii. Coupling jar, viii. Grease free glass slides, ix. Cover slips.

Preparation of Reagents:

Reagents	Components	Method of preparation
i. 0.5% NaCl solution	• NaCl – 0.5g,. • Distilled water – 100ml.	0.5g of NaCl wax dissolved in 100ml of distilled water and mixed thoroughly.
ii. Schaudinn's fixative	• Saturated aqueous mercuric chloride solution (2 parts), • Absolute ethanol (1 part). • Glacial acetic acid	The saturated aq. Solution of mercuric chloride was mixed with ethanol in the ratio 2:1. It was mixed thoroughly and kept in a glass container covered.
iii. Iodinated alcohol	• A few crystals of iodine, • 70% ethanol – 50ml.	The two reagents were mixed and kept in a coupling jar.
iv. Heidenhain's haematoxylin (mordant free)	• Haematoxylin powder – 0.5g, • Distilled water – 100ml, • 95% ethanol – 10ml.	The haematoxylin powder was dissolved in 10 ml 95% ethanol and then distilled water was added. The stain was then ready for use.
v. 3% iron alum (mordant)	• Ferric ammonium sulphate – 3gm, • Distilled water – 100ml.	3gm of Ferric ammonium sulphate was dissolved in 100ml distilled water and stirred and kept as a coupling jar
vi. 1% iron alum (differentiating reagent)	• Ferric ammonium sulphate – 3gm, • Distilled water – 100ml.	1gm of Ferric ammonium sulphate was dissolved in 100ml distilled water and stirred and kept as a coupling jar.

Procedure:

- Specimen is anesthetized.
- Dead specimens are avoided to avoid any damage to the parasites of our interest.
- Another method is by submerging the specimen for a minute in a more or less concentrated solution of domestic detergent (dishwasher). The detergent is immediately absorbed by the tracheal system and it kills quickly without affecting the hosts of the digestive tract.
- Head is first severed out, and next are the legs, with the help of fine pointed forceps and scissors. Next the body must be pinned to a small dissection tray, ventral side facing upwards with thin but rigid pins (the kind used by entomologists).
- With the scissors cut the ligaments on the right hand side of the abdominal sternites, beginning at the rear end, and the ventral plate so released is hinged towards the left side, clearing its adhesions to the internal organs with sharp needles or with a microscalpel, and is discarded or pinned down.

Fig. 9.1 Cockroach (Ventral plate)

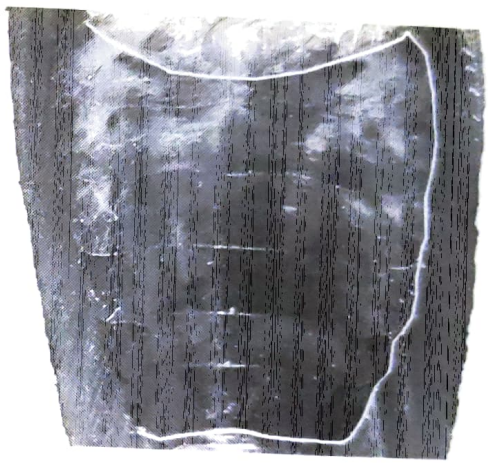

Fig. 9.1 Cut along the white line

- All this can be done in the air, but it give better results if they are made in a physiological saline solution (0.7% NaCl) to avoid damages to tissues by osmotic pressure differentials.
- With thin and sharp teasing needles and very fine pointed forceps, the fat that surrounds the abdominal organs is removed. The alimentary canal is easily isolated and it can be set free from its ties. The gizzard is moved with the tweezers until the esophagus is seen and then it must be cut distally, the rest of the digestive tract can now be liberated with the needles, and cut at the other end, at the level of the anus (or cloaca).
- The gizzard and crop normally do not have parasites of interest, thus there is need to separate only the intestine and the rectum.
- Identify the intestinal cecae. With the help of the forceps and the scissors, make two cuts in the intestine: one below the cecae, and another one at the level of the cloaca. The separated intestine is transferred to a petridish with clean physiological solution. With the two rigid teasing needles, the intestine must be open alongside, releasing its content.

Micro-scalpel should not be used as there is a risk of cutting in pieces the parasites. With due care remove the intestinal tissues.

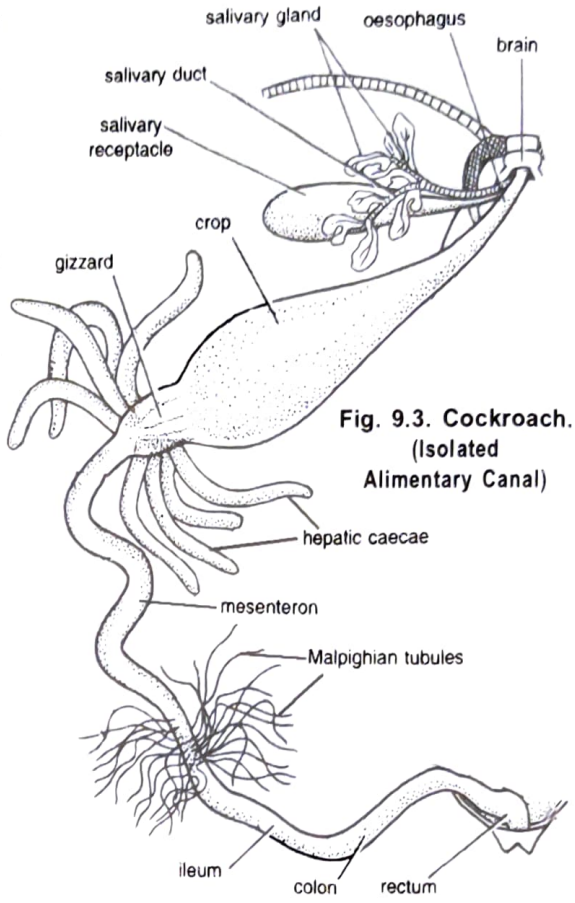

Fig. 9.3. Cockroach. (Isolated Alimentary Canal)

COLLECTING THE PARASITES

- The gut is preferably examined over a matt dark background, this will make it easy to see the nematodes which are wriggling most of the time, or the ciliates swimming in the bottom of the petridish.
- Separate the parasites, with a fine pointed pipette or a fine pointed brush, with the help of a magnifying glass of 6 to 10 powers (or under a dissecting microscope).
- Collect the nematodes in a watch glass with a few of millilitres of physiological solution.
- An useful method to fix nematodes is to warm water or alcohol 50%, in a small test tube over a low flame.
- Collect the nematodes with a fine brush and submerge them quickly in the fixative. The organisms are thus fixed.
- Now pour off most of the liquid without losing nematodes and replace it with AGA (alcohol 70% with 10% of glycerin and 1% of acetic acid). Set the specimens in a well stopped vial for examination.
- The sediment in the watch glass is sampled to investigate the parasitic protozoa.
- Transfer the macroscopic objects from the watch glass to a glass slide containing a drop of normal saline, with the help of a brush or a pair of fine forceps. Spread those on the slide with needles and examine under a powerful dissecting binocular or low power (under 40x and even 100x objectives) of a microscope. Fix the objects with Schaudinn's fluid for 10-15 minutes and make stained, temporary or permanent preparation.
- Make your drawings, and notes.
- To examine in detail the nematodes, you can pass after an hour the fixed material to glycerine 20%, and after another hour to 30% glycerin.

RESULT/OBSERVATION

At least 30 species of protozoans (genera: *Balantidium, Endamoeba, Endolimax, Entamoeba, Iodamoeba, Lophomonas, Nyctoterus, Isotricha, Polymastix, Gregarina* and others) and more than 10 species of helminths (genera: *Binema, Gordius, Neoplectana,*

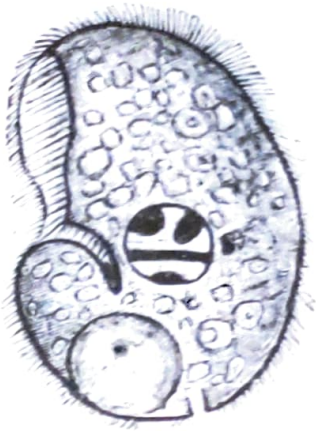

Fig. 9.4 *Nyctotherus* Sp.

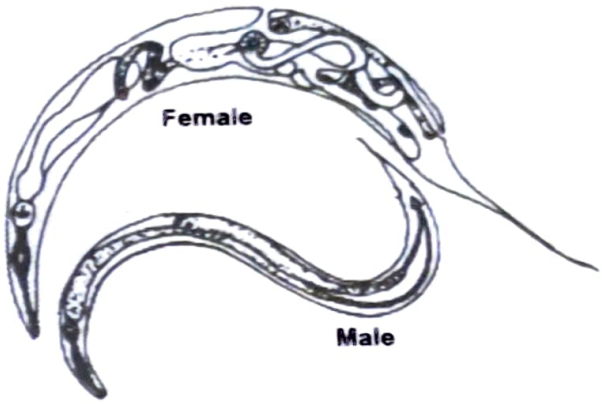

Fig. 9.5 *Leidynema* Sp.

Thelastoma, Hammerschmidtiella, Leidynema, Prostrellus) have been isolated from field collected American cockroaches as a primary host.

The most common parasites are *Nyctotherus* sp., *Balantidium* sp. (Ciliaphora); *Gregarina* sp. (Apicomplexa); *Entamoeba* sp. (Sarcomastigophora); *Leidynema* sp. (Ascaridida), etc.

Smear Preparation: The smear of the gut content was made on a very thin, grease free slide with the help of 0.5% NaCl solution.

Fixation: The smear was then fixed in Schaudinn's fixative for 10-15 minutes.

Staining:

i. The slide was dipped in iodinated alcohol for 1 minute.
ii. The slide was passed through descending grades of ethanol 70%, 50%, 30% each for 1 minute.
iii. Next, it was dipped in distilled water.
iv. The smear was then stained in Heidenhain's haematoxylin followed by a dip in distilled water. It was then observed under a microscope.
v. If proper staining has taken place, the slide was dipped in 1% iron alum for differentiation.
vi. After proper differentiation the slide was rinsed in distilled water and then dehydrated by passing through ascending grades of alcohol for 2-3 min each.
vii. It was then quickly passed through xylene and thereafter the slide was mounted in DPX and observed under microscope.

NYCTOTHERUS SP.

Systematic Position:

Subkingdom – Protozoa
Phylum – Ciliophora

Class – Polymenophora
Subclass – Spirotrichia
Order – Heterotrichida
Genus – *Nyctotherus*

Comments :

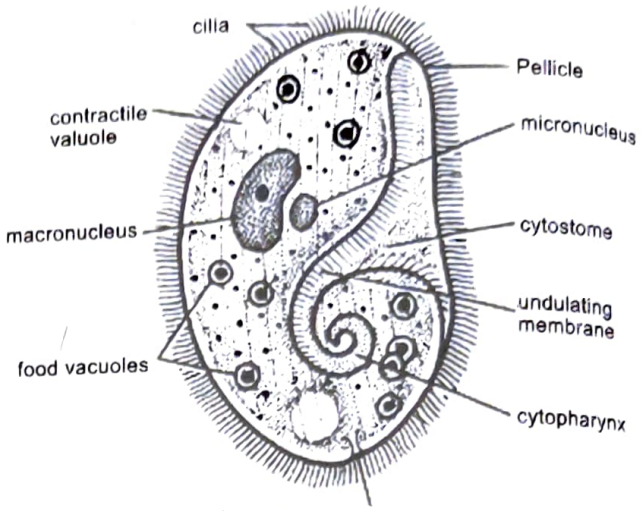

Fig. 9.6 ***Nyctotherus***

1. Body somewhat bean-shaped and bears uniform ciliation all over.
2. Cytoplasm well differentiated into ectoplasm and granular endoplasm.
3. The ectoplasm is having oblique rows of myonemes.
4. Endoplasm is having a large and somewhat kidney-shaped macronucleus, a small micronucleus attached to it, a large eccentric contractile vacuole and numerous small food vacuoles.
5. On one side it bears a deep oral groove which, near the middle of the animal, communicates through a wide cytostome continuing into a deep and coiled tube-the cytopharynx.
6. Near the posterior end is present a small cytopyge or the anus.
7. The oral groove bears rows of aboral cilia and cytopharynx bears undulating membrane.
8. Nutrition is generally saprozoic.
9. It reproduces through multiple fission, encystment and conjugation.

ENDAMOEBA SP.

1. *Endamoeba,* protozoan genus of the rhizopodan order Amoebida that inhabits the intestines of invertebrates.
2. *Endamoeba blattae,* a representative species, lives in the cockroach intestine.
3. In *Endamoeba* the nucleus, has a thick membrane and is divided into a granular outer ring and a coarsely netted centre.
4. The outer ring and the centre are separated by a number of nuclear bodies called endosomes.
5. *Endamoeba blattae* is a big species, easy to observe, with a clear and homogenous cytoplasm and a globular nucleus with a wide peripheral clear ring and a darker center.

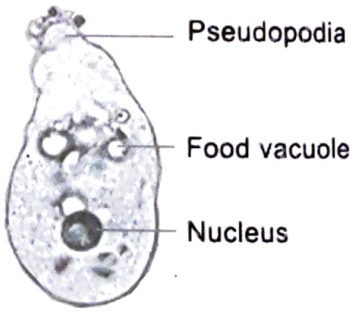

Fig. 9.7 ***Endamoeba***

BALLANTIDIUM SP.

Systematic Position:

Subkingdom – Protozoa
Phylum – Ciliophora
Class – Litostomatea
Order – Vestibuliferida
Genus – Ballantidium

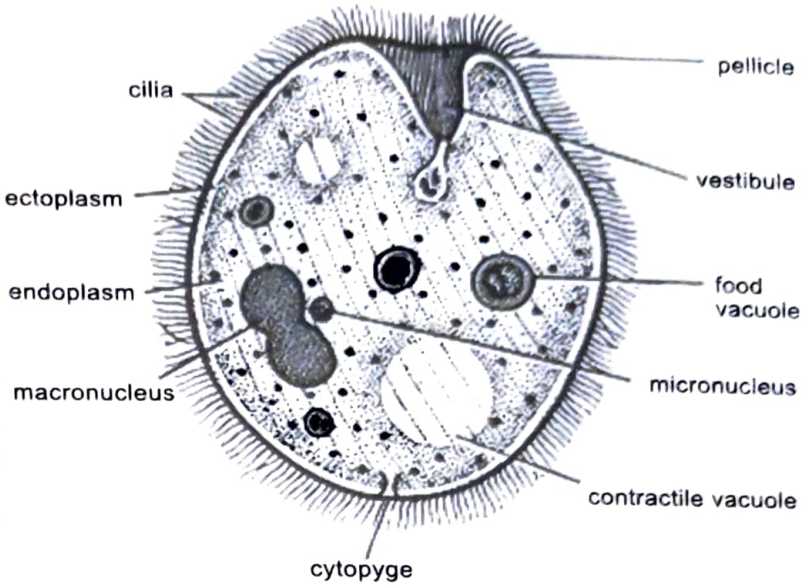

Fig. 9.8 *Ballantidium*

1. *Balantidium* is a ciliate parasitic in the large intestine of pigs, monkeys, and man, some species are parasitic in frog, fish, cockroach and horse.
2. It is an egg- shaped animal pointed at the anterior end and rounded posteriorly.
3. Outer lining covered by longitudinal row of small cilia.
4. Vestibule at the anterior end that leads to cytosome.
5. There is a large sausage-shaped macronucleus obliquely in the middle of the body, and in its concavity near it is a small micronucleus.
6. Unlike most parasitic Protozoa there are two contractile vestibule vacuoles, one near the middle and a larger one at the posterior end.
7. Presence of food vacuole containing cell fragments, starch and yeast from the colon of the host.
8. Presence of cytopyge at the posterior end.
9. Reproduction is by transverse binary fission.

GREGARINA SP.

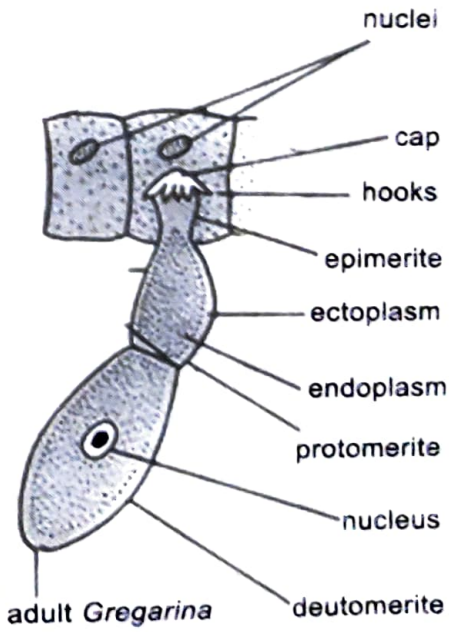

Fig. 9.9 *Gregarina*

1. Gregarina is a sporozoan parasite into the intestine or body cavity of insects or Annelida.
2. The adult or trophozoite is extracellular, it has a thick cuticle.
3. The ectoplasm has myonemes which grow in and divide the body into two parts, an anterior protomerite and a posterior deutomerite which contains a nucleus.
4. When the trophozoite is attached to the gut it acquires an anterior epimerite having radiating spines, it gets attached by the epimerite, but the epimerite is lost when the trophozoite comes to the lumen of the gut.

LEIDYNEMA SP.

Diagnostic Characters of the Genus *Leidynema*

1. Mouth surrounded by very large sub median labiopapillae, amphids or lateral organs appearing externally as small circular openings.
2. Esophagus of female consisting of an anterior part which is subdivided into cylindrical parts of which the posterior is somewhat larger in diameter than the anterior, a distinct isthmus, and a valvular bulb.
3. Excretory pore situated posterior to base of esophagus. Intestine may or may not be enlarged anteriorly to form a cardia; a posteriorly directed caecum may or may not be present; the intestine may have a loop in the posterior part of the body.

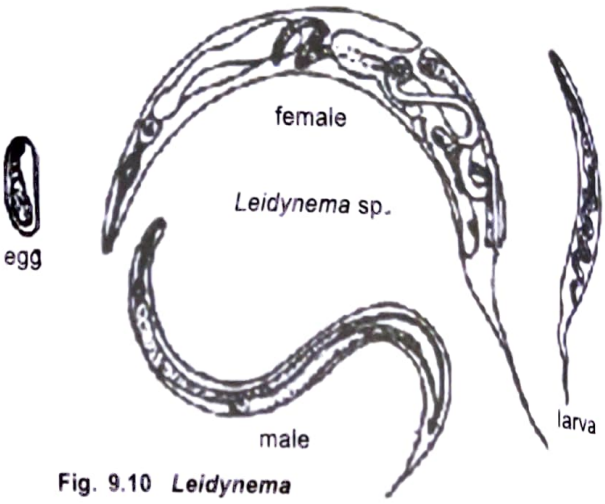

Fig. 9.10 *Leidynema*

4. Vulva near middle of body; amphidelphic.
5. Eggs oval, elongate and flattened slightly on one side.
6. Esophagus of male without distinct posterior swelling.
7. Tail of female attenuated or filiform.
8. Tail of male short, rounded, filiform.
9. One pair of large preanal caudal papillae, two pairs of postanal papillae.
10. One spicule.

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