CHAPTER 5

SCANNING ELECTRON MICROSCOPY OF SOME PLATYHELMINTH PARASITES OF AMPHIBIA

INTRODUCTION

Platyhelminths exhibit a complex and highly diverse micromorphological features of the tegumental surface which provide valuable taxonomic information of high systematic significance (Bakke, 1976 a & b and Otubanjo, 1985). Some previous workers (Fischoeder, 1903; Stiles & Goldberger, 1910; Leiper, 1910; Dollfus, 1950; Tandon, 1955; Dinnik, 1961, 1962 and 1964; Gretillat, 1964; Velazques-Maldonado, 1976 and Sey, 1983) have described tegumental papillae with light microscopy. However, due to the limitations of light optics, the exact nature and pattern of deposition of tegumental papillae is not elucidated with certainty. Recently, scanning electron microscopy (SEM) has been successfully
SEM micrographs (1-4) of *Ganeo tigrinus* Mehra & Negi, 1928.

**EM.1.** Entire view seen from the ventral side.

**EM.2.** Pre-acetabular tegument showing pitted appearance.

**EM.3.** Same as above but at higher magnification showing details of papillae with bulb-like branches.

**EM.4.** Oral sucker showing dome-shaped aciliated papillae.
SEM micrographs (5-7) of *Ganeo tigrinus* Mehra & Negi, 1928.

EM.5. Genital pore with protruding cirrus.

EM.6. Cirrus at higher magnification.

EM.7. Ventral sucker.
SEM micrographs (8-10) of *Prosotocus himalayai* Pande, 1938.

**Em. 8.** Entire view seen from the ventral side.

**Em. 9.** Oral sucker and pre-acetabular tegument showing rhomboidal depression.

**Em. 10.** Pre-acetabular tegument showing large dome-shaped papillae.
SEM micrographs (11-13) of *Plagiorchis himalavii* (Jordan, 1930).

**Em. 11.** Entire view seen from ventrolateral side.

**Em. 12 & 13.** Post-acetabular tegument showing crater-like papillae.

**Phms. 112 & 113.** Morphology of *P. himalavii* as seen in light microscope. Semichon's carmine stained specimens.
SEM micrographs (14-18) of *Plagiorchis himalavii* (Jordan, 1930).

**EM. 14.** Oral sucker and pre-acetabular tegument. Note a characteristic slit-like depression anterior to oral sucker.

**EM. 15.** Ventral sucker showing dome-shaped aciliated papillae.

**EMs. 16 & 17.** Genital pore in relationship to ventral sucker.

**EM. 18.** Posterior end showing excretory pore.
SEM micrograph of *Naematotaenia kashmiriensis* Fotedar, 1966.

Em. 19. Scolex with well developed suckers.

Phm. 114. Scolex of *N. kashmiriensis* as seen in light microscope. Semichon's carmine stained specimen.
used to understand the exact nature of these tegumental papillae at ultra structural level (Berger & Mettrick, 1971; Race et al., 1971; Miller et al., 1972; Harris et al., 1974; Nollen & Nadakavukaren, 1974; Bennett, 1975; Lumsden, 1975; Nadakavukaren & Nollen, 1975; Bakke, 1977 and 1978; Edwards et al., 1977; Øie, 1977; Bakke & Lien, 1978; Kechemir, 1978; Fujino et al., 1979; Eduardo, 1980 and 1982; Font & Wittrock, 1980; Thulin, 1980; Tandon & Maitra, 1981, 1982 and 1983; Oliver et al., 1984 and Sey, 1984). Some authors (e.g., Tandon & Maitra, 1981) have stressed the physiological value of tegumental papillae, whereas, numerous other authors (e.g., Eduardo, 1980 & 1982 and Sey, 1984) have emphasized their use at various taxonomical levels. The present investigation was undertaken in order to elucidate and compare the ultrastructural features of the tegument of Ganeo tigrinus Mehra & Negi, 1928; Prosotocus himalayai Pande, 1937; Plagiorchis himalayii (Jordan, 1930) and Naematotaenia kashmirensis Fotedar, 1966.

MATERIALS AND METHODS

Sexually mature trematodes, viz., Ganeo tigrinus Mehra & Negi, 1928 and Prosotocus himalayai Pande, 1937 were recovered from the gut of naturally infected frog, Rana (Dicroglossus) cyanophlyctis Schneider, 1799 collected from Dal Lake in the vicinity of Telbal nallah. The trematode, Plagiorchis himalayii (Jordon, 1930) and the cestode, Naematotaenia kashmirensis Fotedar, 1966 were obtained from the intestines of the toad, Bufo viridis.
Laurenti, 1768 which were collected from the grassfields in the University Campus, Nazratbal.

Prior to processing the helminths, namely, Ganeo tigrinus and Prosotocus himalayai for SEM studies different morphological types of both the taxa were selected by observing living worms under light optics with the aid of phase-contrast microscope.

All the helminths were thoroughly rinsed in 0.65% NaCl solution, killed and fixed in hot (approx. 70°C) 10% formalin and were dehydrated through ascending series of ethanol. The worms were placed for 5 minutes each in two changes of acetone and were later critical point dried using carbon dioxide (CO₂) and acetone system. The worms were then mounted on the aluminium stubs with the aid of conductive silver paint and were coated with gold using high vacuum evaporator coating apparatus (Model Hitachi, HUS-5 GB) at a negative pressure of 10⁻⁴ to 10⁻⁵ torr. In order to achieve uniform coating, the specimens were rotated rapidly during coating. Finally, the specimens were examined with Hitachi Scanning Electron Microscope Model S-510, at an accelerating voltage of 15-25 kV and a filament current of 90 amperes.

RESULTS

Ganeo tigrinus Mehra & Negi, 1928 (EM 1-7).

The body of Ganeo tigrinus is linguiform with a distinct round anterior end (EM 1). The oral sucker is
subterminal, round with a conspicuous mouth opening. The pre-equatorial acetabulum is almost equal in size to the oral sucker. At low magnification the surface of this lecithodendrid appears to be more or less smooth. However, in the post-acetabular region the uterine slings are clearly visible especially towards the hinder portion of the worm (EM 1). At higher magnifications the tegumental surface of the worm is seen to be composed of depressions and ridges giving the tegument a pitted appearance (EM 2). The ridges are composed of numerous long papillae with bulb-like branches (EM 3). However, no spines are observed on either the ventral or dorsal tegumentary surface of the fluke. The oral sucker which surrounds the mouth has the same tegumental ultrastructure as rest of the body. However, few dome-shaped non-ciliated papillae (about 7.29 μm average basal diameter) are found along the inner margin of the oral sucker (EM 4).

The genital pore is situated on the left margin of the body in the pre-equatorial region (Fig. 5). The genital atrium appears as a transverse slit-like aperture surrounded by thick lip-like rim. Numerous, non-branching long cone-shaped, non-ciliated sensory papillae are seen in the vicinity of the genital aperture (EM 5). A distinct dome-shaped eversible cirrus is seen at the corner of the genital atrium (EM 5). At a high magnification distinct non-ciliated dome-shaped papillae are seen regularly arranged on the surface of the cirrus. The distal tip of the cirrus bears characteristic small dome-shaped
protuberance at the centre which seems to emerge from a depression. Similar 4 - 6 dome-shaped papillae are found around the periphery of the cirrus at regular intervals. These smaller domes in turn also bear small sensory papillae. The cirral papillae are restricted to the anterior tegument of the everted cirrus only and measure upto 0.75 \( \mu m \) in average diameter and are not found on the other parts of the body surface.

**Prosotocus himalayai** Pande, 1937 (EM 8 - 10)

The body of *P. himalayai* is elliptical to obovate. The oral sucker is subterminal round with conspicuous mouth opening which is more or less oval. At low magnification the body surface appears to be smooth. However, at higher magnification the body surface is composed of rhomboidal depression and ridges giving the body a pitted appearance (Fig. 9). No spines are seen on the tegumental surface. However, the ridges of the tegument bear non-ciliated dome-shaped papillae which do not exceed 2.6 \( \mu m \) in diameter. A few dome-shaped but larger (5 - 6 \( \mu m \) in average diameter) non ciliated papillae are found associated with the inner margin of the oral sucker.

**Plagiorchis himalayii** (Jordan, 1930) (EM 11 - 18).

The body of *P. himalayii* is typically cylindrical and elongated in shape (Fig. 11). The posterior end of the body is blunt and is distinctly narrower as compared
to the rest of the body. The oral sucker is subterminal surrounding a more or less longitudinally deposited oval aperture. The acetabulum is preequatorial in position and is distinctly smaller in size as compared to the oral sucker. At low magnification a distinct longitudinal groove is seen midventrally in pre and post acetabular regions (EM). The tegument also appears to be more or less smooth except that a few wrinkles are seen on the tegumental surface. At higher magnification the tegument is seen to carry numerous closely packed and regularly arranged columnar papillae with crater-like depressions in the centre. These papillae have an average diameter of 2.14 μm. The oral sucker has a characteristic topography (EM 14) and is provided with numerous dome-shaped non ciliated papillae which are only slightly elevated from the tegumental surface. These papillae measure 3.8 μm in average diameter. A characteristic slit-like depression is present at the anterior end of the oral sucker (EM 14). The acetabulum or ventral sucker is also provided with numerous dome-shaped non ciliated papillae which are markedly elevated from the tegumental surface (in contrast to those associated with the oral sucker) and measure 4.2 μm in average diameter.

The genital pore is situated in front of the acetabulum slightly to the left. Five to six elongated cone-shaped papillae are found in the inner margin of the genital pore and measure about 19 μm in average diameter.
SEM micrographs (20-21) of *Naematotaenia kashmirensis* Fotedar, 1966.

Ems. 20 & 21. Tegument of the neck region.

At the posterior end of the worm a circular excretory pore is also visible (EM).

*Naematotaenia kashmirensis* Fotedar, 1966 (EM 19 - 20)

The anterior region of this cestode has a characteristic scolex provided with four well developed suckers. In contrast to light microscopic observations (Phm.115) the anterior portion of this cestode is found to be segmented although the segmentation is incomplete (EM 20). The tegument is perforated at numerous places and carries numerous long filamentous structures or microvilli (EM 21).

**DISCUSSION**

The results of the present investigation show that the tegumental papillae are characteristics of digenean trematodes. Sey (1984) has described 8 types of papillae. These are:

1. Dome to conical non-ciliated papillae,
   a) papillae hardly elevated from tegumental surface,
   b) papillae elevated regularly,
2. Dome to conical ciliated papillae,
3. Short and stumpy papillae covered with hair-like processes,
4. Short and stumpy papillae resting on a tegumental elevation,
In the present investigation only four types of papillae were observed. These are:

i) dome to conical non-ciliated papillae (type 1 a & b),

ii) long non-branching and non-ciliate papillae (type 5).

iii) long papillae with bulb like branches (type 6),

and

iv) crateriform papillae (type 7).

The dome to conical non-ciliated papillae are considered to be sensory receptors having tactile and rheoreceptor functions (Bennett, 1975). This can also explain the abundance of these papillae around the oral sucker and genital region (Otubanjo, 1985). As in case of Ganeo tigrinus studied in the present investigation Bakke (1976 b) has also observed aggregation of sensory papillae associated with the everted cirrus surface of the trematode Leucochloridium species. According to Otubanjo (1985), "cirral papillae may play a significant role in the location of genital atrium and in copulation".
Numerous workers (Yamaguti, 1971 and Prudhoe & Bray, 1982) have described the body surface of lecithodendriids as spined. However, the present investigation indicates that no such spines exist. This discrepancy may be due to the limitations of light microscopic resolution. Tandon & Maitra (1981) have also reported the absence of spines in adult digeneans viz., Gastrothylax cruminifer and Parempistomum epiclitum. According to Otubanjo (1985), "the absence of spines on the body surface probably allows for smooth movement and less physical damage and abrasion of the host tissue".

The scanning electron micrographs of the various helminths studied in the present investigation reveal that no two species have same micromorphological pattern. Therefore, the tegumental features have strong taxonomic value. This is also evident from the fact that the numerous specimens examined in the present study had constant nature of tegumental topography especially the number and pattern of papillary distribution. Eduardo (1982 a) has also reported the consistency and uniformity of papillae in mammalian amphistome species. Besides, the shape and topography of the genital opening and especially that of the cirrus can also be used for making phylogenetic inferences because of their complex structure. Complex structures especially those which have low phenotypic variability are given more taxonomic weight than simple structures which are more variable (Mayr, 1969). According to Mayr (1969) the complex structures are more useful from the taxonomic point of view because "the probability that
species which are only distally related would become similar in such (complex) structure by convergence is extremely low". Otubanjo (1985) has also noted the complex nature of cirrus in case of dicrocoelid trematode, Concinnum epomopis Sandground, 1973. According to him the everted cirrus is cylindrical and is covered with anastomosing tegumentary lamellae forming a reticulate network. He also described the presence of closely packed protuberances interspersed between the lamellae.

The tegument of the cestode, Naematotaenia kashmirensis seems to consist of numerous microvilli in contrast to the tegumental pattern of the trematodes. This may be an adaptation to increase the absorptive surface area.

In the end, it may be concluded that: (1) SEM can be successfully used as corroborative data to supplement the light microscopical observations and also to elucidate numerous new character-states which are not resolved by light microscopy and (2) Since ultrastructural features of the tegument in genetically close species show close resemblance (e.g., Ganeo tigrinus shows more close resemblance with Prosotocus himalavai than with Plagiorchis himalavii which belongs to different family), therefore, these features can be used for estimating phylogenetic relationship.