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Observations on the histology and histochemistry of *Penetrocephalus* plerocercoid (Pseudophyllidea: Cestoda)

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Abstract. The histology of plerocercoid of *Penetrocephalus* sp. reveal that the body surface consists of tegument, basement membrane, epidermal longitudinal musculature, parenchymatic longitudinal musculature, transverse and dorsoventral muscle fibres. Three types of glands could be recognized from the scolex of the plerocercoid. The musculature of the plerocercoid consists of glycogen, acid, sulfated, neutral and carboxylated mucopolysaccharides: basic proteins containing tyrosine, S-H and S-S groups, protein bound amino groups, sulfhydril groups, glycoprotein and lipid.

The frontal glands contain carbohydrates (1, 2 glycols, both acid and neutral mucopolysaccharides), basic protein (tyrosine, S-H group, protein bound amino group) and phospholipids. The structure, organization and histochemistry of the plerocercoid is discussed.

Keywords. Histology; histochemistry; *Penetrocephalus*; plerocercoid; cestoda.

1. Introduction

Hanumantha Rao (1960a) erected the genus *Penetrocephalus* for the form described by him earlier as *Bothriocephalus ganapatii* (Hanumantha Rao 1954), recovered from a teleost fish *Saurida tumbil* (Bloch). In his later paper (Hanumantha Rao 1960b) observations on histochemistry and egg formation were furnished. Rama Devi (1970) studied the histology and some aspects of histochemistry. But histochemical work on the plerocercoid of *Penetrocephalus* has not been carried out so far.

Histochemical investigation on pseudophyllidean cestodes probably started with the work of Takahashi (1959) on *Diphyllobothrium* (*Spirometra*) *mansoni* and then Arme (1966) on *Ligula intestinalis*. But little attention has been paid to larval stages. Ohman (1968) was the first to investigate the histochemistry of the larva of *D. detriticum* and concluded that one cannot rely solely on morphological descriptions to solve the taxonomic problems in *Diphyllobothrium*.

The present work deals with the histology and histochemistry of plerocercoid of *Penetrocephalus* collected from a number of teleost fishes.

2. Material and methods

Plerocercoids of *Penetrocephalus* were recovered from various locations in the body cavity of *Saurida tumbil* and 13 other species of teleosts, collected from the off-shore

fishing station, Visakhapatnam and from local fish markets. The larvae were fixed in alcoholic Bouin's, Susa, Carnoy and formal calcium, passed through grades of alcohol, cleared, embedded in paraffin wax (m.p. 58°C) and sections cut at 10–12 μ thickness.

Heidenhain's azan, and Mallory's triple stains were used for histological studies. The histochemical tests employed were periodic acid schiff (PAS) technique for carbohydrate containing groups, PAS saliva for glycogen, PAS after acetylation and deacetylation for 1 : 2 glycol groups. Alcian blue (AB) 1 pH and AB 2·5 pH for acid mucins, AB (1 pH)/PAS to detect sulphate free sialic acid containing mucins, AB (2·5 pH)/PAS to distinguish neutral from acid mucopolysaccharide and toluidine blue for the demonstration of acid mucopolysaccharides. Aldehyde fuchsin (AF)/AB was employed to distinguish between sulphated and non-sulphated mucosubstances and, to confirm the presence of sulphated mucins, AB/safranin was performed. For basic proteins, mercury bromophenol blue was used and, potassium permanganate/alcian blue ($KMnO_4$/AB) for disulphide, ferric ferricyanide for sulfhydril group and Congo red for glycoprotein. To demonstrate lipids-Sudan black B and for phospholipids copper phthalocyanin techniques were employed. Most of the procedures for histochemical tests were adopted from Pearse (1968).

3. Observations

Live specimens were obtained from the stomach wall, liver, muscles and other regions of the alimentary canal of the hosts. The worms appeared flat, elongated and milky-white in colour. The scolex was invaginated.

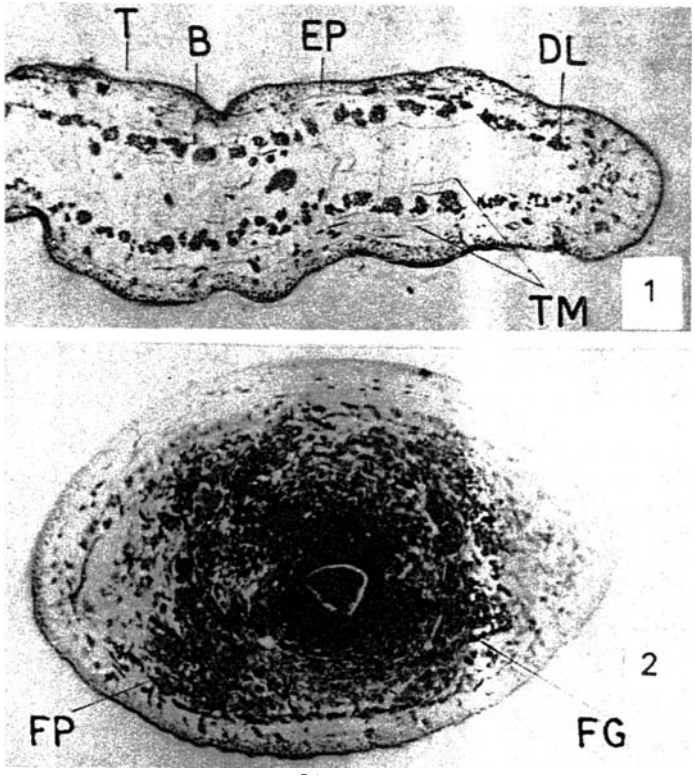
3.1 *Histology*

The outer layer of the larval worms of *Penetrocephalus* sp. is a thick tegument. It is smooth, but not uniform throughout the body. Beneath the tegument is the basement membrane. This layer separates the inner muscular layer. It is single layered with no vacuoles, reticulations etc. Below the basement membrane, lies the epidermal longitudinal musculature. The cells of this region are not widely spaced nor densely packed. A deeper parenchymal longitudinal musculature is also present. Transverse muscles are situated between two rows of longitudinal muscles (figure 1). They are also found in the central region running towards periphery, but are more distinct in the scolex.

In the invaginated anterior region of the larva, numerous frontal glands occur in the medular parenchyma. They are irregular and of various shapes. Three types of glands could be distinguished, though one type occupies the major part of the scolex. In the central region there is a frontal pit filled with secretions. The entire part is covered with thin fibrous parenchymal musculature (figure 2).

3.2 *Histochemistry*

The staining and histochemical reactions of the various anatomical regions especially the tegument and the frontal glands are summarized in table 1 and the reactions on the various regions are shown in figures 3–8.



Figures 1–2. 1. Cross-section of the body showing general musculature (Azan). 2. Cross-section through the scolex showing frontal glands (Azan).

From the ensemble of histochemical tests it could be stated that the tegument is charged with acid, sulfated, carboxylated mucopolysaccharides with hyaluronic acid, basic proteins containing tyrosine, S–S group, S–H group and lipids especially phospholipids.

The muscles display carbohydrate containing glycogen, proteins containing tyrosine, protein bound S–H and NH_2 groups and lipids.

The frontal glands contain carbohydrates (1, 2 glycol groups and both acid and neutral mucopolysaccharides), proteins (S–H, NH_2 groups and tyrosine) and phospholipids.

4. Discussion

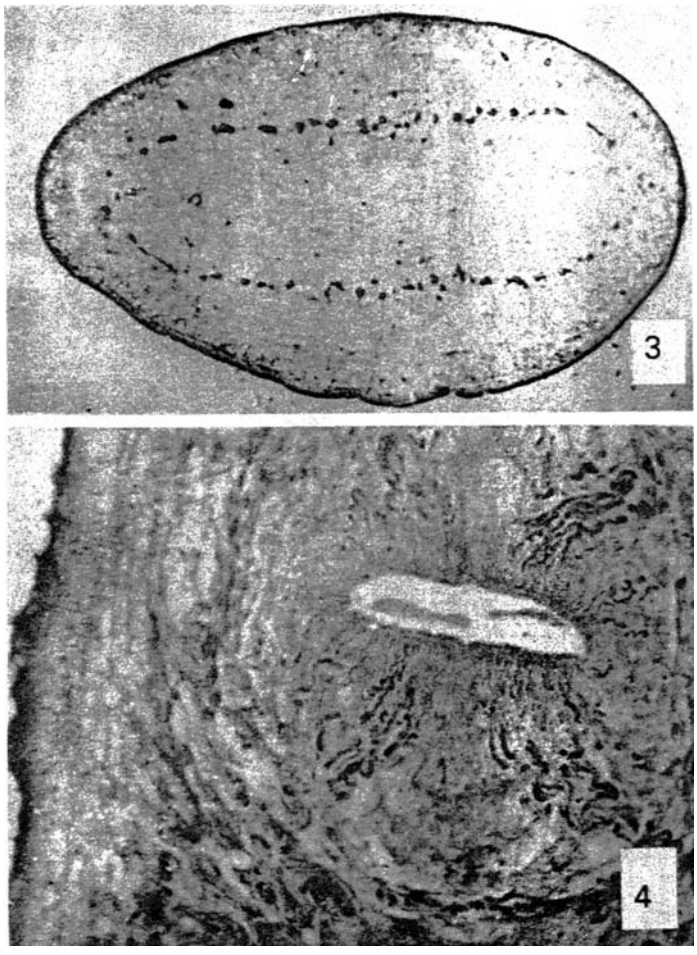
The results of the study on the histology and histochemistry of the plerocercoid of *Penetrocephalus* sp. agree to some extent with those obtained for the adult of *Penetrocephalus ganapatii*.

The histochemical composition of the tegument has been studied in detail, more in cyclophyllidean cestodes than in pseudophyllideans. Bogitsh (1963) found PAS positive material in the tegument of *Hymenolepis microstoma* and he stated that it could probably be a mucoprotein. Lumsden (1975) stated that the external limiting

Table 1. Results of histochemical reactions of *Penetrocephalus* plerocercoid.

Histochemical tests applied	Tegument	Basement membrane	Epidermal longitudinal muscle	Parenchymal longitudinal muscle	Frontal glands
Periodic acid/Schiff (PAS)	+ +	+	±	+ +	+ +
PAS/saliva	–	–	–	–	+ +
Acetylation	+	–	–	–	–
Deacetylation	+ +	–	–	–	+ +
Alcian blue (AB) 1 pH	+ +	–	–	–	+
AB 2·5 pH	+ + +	–	–	–	+
AB 1 pH/PAS	Purple	–	–	–	Red
AB 2·5 pH/PAS	+ + + Blue	–	–	–	Blue purple
Toluidine blue	γ-metachromasia	–	–	–	γ-metachromasia
Aldehyde fuchsin (AF)/AB	+ Blue	–	–	–	Blue purple
AB/Safranin	Red	–	–	–	Red
Bromophenol blue	+	+ +	+	+ +	+ +
$KMnO_4$/AB	+ +	–	–	–	–
Ferric ferricyanide	+ +	+ +	±	+	+
Congo red	+ + +	+ +	+	+ + +	+ + +
Sudan black B	+ +	+ + +	+ +	+ + +	+ + +
Copper phthalocyanin	+ +	+ +	+	+ + +	+ +

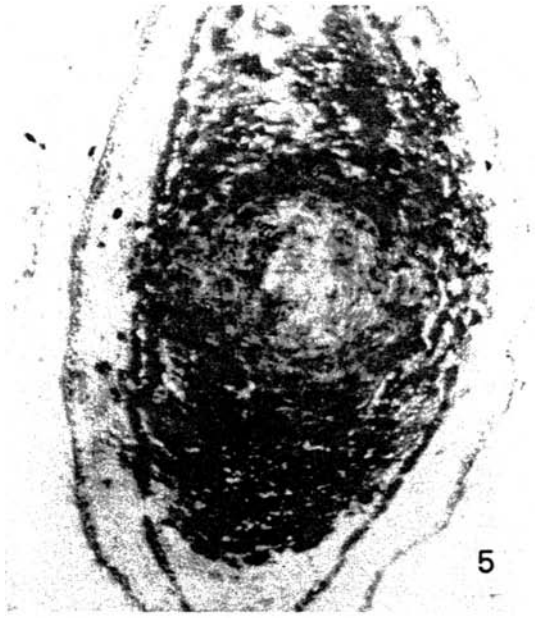
+ + + intensely positive; + + strongly positive; + moderately positive; ± faintly positive; – negative.

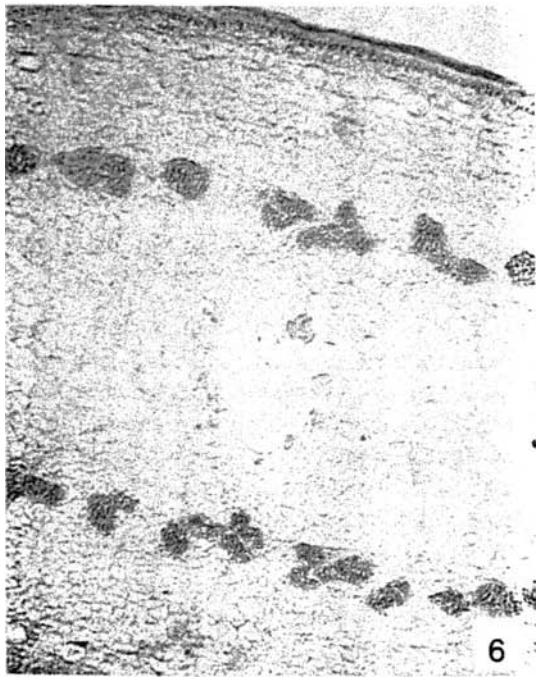


Figures 3–4. **3.** Tegument and the deeper longitudinal musculature showing positivity towards PAS. **4.** Frontal glands showing positivity towards PAS.

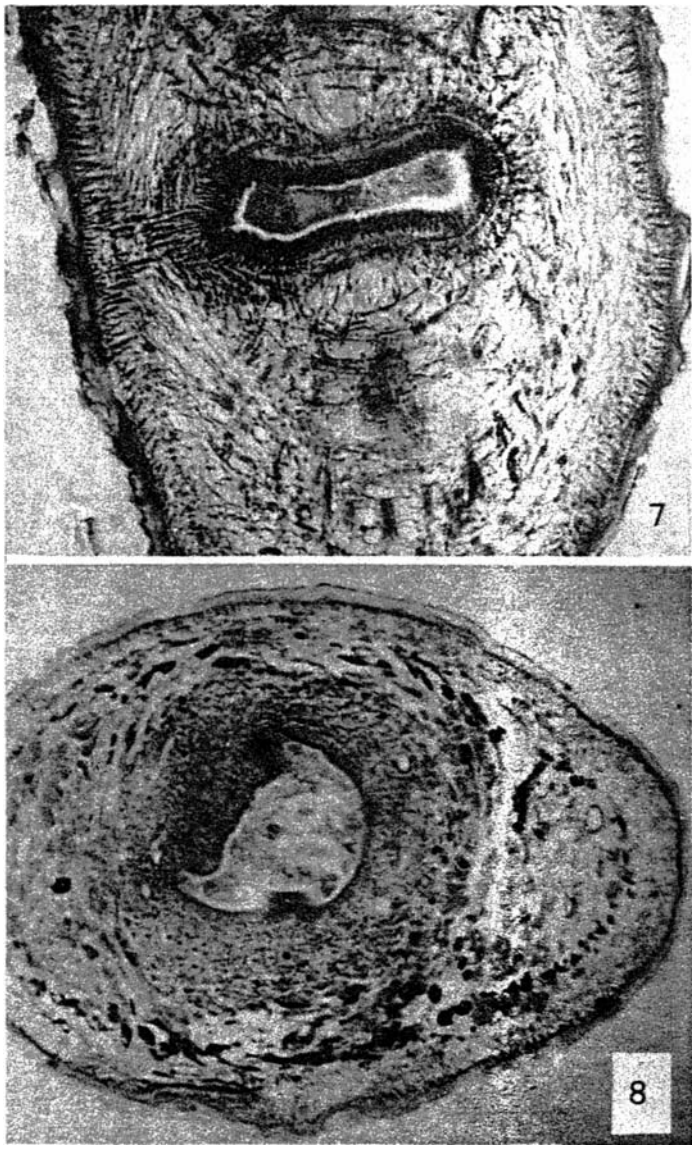
membrane is coated with a layer of carbohydrate-rich polyelectrolyte, namely, a glycocalyx which serves as a binding surface for inorganic ions and higher molecular weight organic compounds including host enzymes. Hanumantha Rao (1960a) also mentioned that the tegument of *P. ganapatii* is PAS positive and fast to saline. Trimble and Lumsden (1975) found the tegument of larval *Taenia crassiceps* to possess a surface coat rich in both neutral and acidic carbohydrates. Rama Devi (1970) worked on six species of pseudophyllidean tapeworms and reported similar findings. She also detected the presence of lipids. In *Ptychobothrium cypseluri*, *Bothriocephalus manubriformis*, *Oncodiscus fimbriatus* and *Bothriocephalus indicus* the tegument was found to possess acid mucopolysaccharides and glycogen (Rama Devi 1970). The present observations reveal the tegument to consist of basic proteins containing tyrosine, glycoprotein, S–H, S–S etc., in addition to mucopolysaccharides.

The basement membrane is composed of basic proteins containing tyrosine, S–H groups, NH_2 groups, protein bound amino acid groups and glycoproteins and lipids bearing phospholipids. The epidermal and the parenchymal musculature contain basic





Figures 5–6. **5.** Frontal glands showing metachromasia with toluidine blue. **6.** Basement membrane and the deeper longitudinal muscles showing the presence of basic proteins (bromophenol blue).



Figures 7–8. 7. Frontal glands indicating the presence of basic proteins (bromophenol blue). 8. Frontal glands showing glycoproteins (Congo red).

Abbreviations. B – Basement membrane; Dl – Deeper longitudinal musculature; EP – Epidermal longitudinal musculature; FG – Frontal glands; FP – Frontal pit; T – Tegument; TM – Transverse muscle.

proteins with tyrosine, S–H groups, very little quantities of protein bound amino groups and lipids.

The presence of glycogen in the parenchyma in various pseudophyllids was demonstrated by many workers (Smyth 1946, 1947; Hanumantha Rao 1960a; Rama

Devi 1970). In the present study also glycogen was detected in the parenchyma and muscles.

In addition to glycogen, both epidermal and parenchymal musculature of the plerocercoid contain lipids especially phospholipids. Smyth (1946) reported the presence of lipid droplets of various sizes scattered throughout the parenchyma of *Ligula intestinalis*. Rama Devi (1970) also reported the presence of lipid droplets in the parenchyma of pseudophyllidean cestodes. Lipids may also be considered as break down products of metabolism (Rama Devi 1970).

Baer (1956) reported that the scolex of *Monorygma perfectum* displays a 'deep staining granular mass' which was considered to evoke a pronounced host tissue reaction. In Lecanicephalidae, a similar 'glandular complex' was described in *Polypocephalus rhinobatides* and in *P. radiatus* by Subhapradha (1951). Smyth (1964) reported the occurrence of glands in the restollum of *Echinococcus granulosus*. He stated that the secretion of the gland is PAS negative and concluded that it is probably an extremely labile lipoprotein or lipid-protein coacervate. In the present investigation on the plerocercoid of *Penetrocephalus* sp. three types of gland cells could be recognized. The glands are PAS positive. Rama Devi (1970) also recognized three types of glands in the scolex of adult *Penetrocephalus ganapatii*. Wolffhugel (1938) reported the occurrence of three types of gland cells in the scolex of *Nematoparataenia southwelli*.

Many speculations have been made as to the function of these glands. However, Smyth (1964) mentioned that the glands cause contraction of the villi which can assist the orientation of the scolex. Wolffhugel (1938) suggested that the enzymic secretion of the gland assists the cellular digestion. He also assumed that the secretion was hormonal in nature, related to the regulation of growth and maturation of the strobila. According to Rama Devi (1970) the glands of *P. ganapatii* assist in penetration activities.

Rawson and Rigby (1960) observed that in the cysticercoid of *Choanotaenia crassiscolex* these glands secrete a lubricant into the rostellar sac to help movement of the rostellum. Therefore, the gland cells in different cestodes may vary in structure, anatomy, chemical nature and function.

It is believed that in *Penetrocephalus* also the scolex glands aid in the penetration activities.

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