Ultrastructure Study Of The Scolex And Tegument Of *Bothriocephalus Acheilognathi* Yamaguti, 1934 (Cestoda: Pseudophyllidea) From *Schizothorax* Species Of Kashmir

Tanveer A. Sofi* And Fayaz Ahmad

P.G. Department of Zoology, University of Kashmir, Srinagar – 190006
* stanveer96@gmail.com

Abstract: Immature, mature, and gravid specimens of *Bothriocephalus acheilognathi* from *Schizothorax* spps. were studied by scanning (SEM) and transmission electron microscopy (TEM). SEM of the heart-shaped scolex revealed long, deep, pear-shaped bothria. A bilobed apical disc was present, although it was not prominent. The scolex tegument possessed microtriches which were morphologically distinct from those of the strobila. Microtriches had a more slender appearance within the bothria than on the surrounding tegument. SEM also revealed the presence of tumuli which were numerous and uniformly spaced on the scolex, but became less abundant posteriorly along the strobila. TEM revealed that tumuli contained dense-staining inclusions. Sensory cilia extended through the tegument of mature and gravid proglottids. The distal cytoplasmic layer was connected to the perikarya by cytoplasmic bridges. Muscle bundles were observed in longitudinal and cross-sections within the perinuclear region. Various organelles, including ribosomes, endoplasmic reticulum, and Golgi bodies, were present and within the cytons.
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Key words: *Bothriocephalus acheilognathi, Schizothorax*, SEM, TEM, Scolex, Tegument, Bothria.

INTRODUCTION

Ultrastructural aspects of cestodes have received the attention of many investigators during the last two decades. Many of these studies have focused on cestodes of medical or veterinary importance (e.g., Jha & Smyth, 1971; Mehlhorn *et al*., 1981; Rothman, 1963; Specian & Lumsden, 1980; Thompson *et al*., 1979), although other cestodes have not been ignored entirely (e.g., Hayunga & Mackiewicz, 1975; McVicar, 1972; Tedesco & Coggins, 1980). Generally, these studies have concentrated on a few specific microanatomical features of the parasite in question (e.g., Berger & Mettrick, 1971; Coggins, 1980; Lumsden & Byam, 1967; Morseth, 1967; Specian *et al*., 1979); few exhaustive studies have been undertaken.

The present study was part of an investigation of the biology of *Bothriocephalus acheilognathi* Yamaguti, 1934, a parasite of *Schizothorax* spps. in a Kashmir region. *B. acheilognathi* was introduced into the United States via grass carp in the early 1970's and since has become well established in the mid-south and southeastern states (Hoffman, 1980). Previous light microscopic studies have described the general morphological features of *B. acheilognathi* (Molnar & Murai, 1973; Yamaguti, 1934; Yeh, 1955), but the ultrastructure of this species has not been described. In the present study, both scanning electron microscopy (SEM) and trans-mission electron microscopy (TEM) were used to study the scolex and tegument of immature, mature, and gravid forms of *B. acheilognathi*. Results are compared with similar observations made on other cestode species.

MATERIALS AND METHODS

Bothriocephalus acheilognathi (at least 10 specimens each of non-segmented, segmented, and gravid stages) were recovered from *Schizothorax* spps. and rinsed in a 0.65% saline solution, fixed for 1, 2, or 12 h in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.1), transferred to a buffer rinse, and dehydrated in an ascending series of ethanol solutions. Specimens were critical-point dried, mounted on aluminum stubs with double-stick cellophane tape, sputter-coated with gold-palladium, and viewed with a Philips SEM 501. Worms for TEM were fixed as above prior to secondary fixation in 1% osmium tetroxide and dehydration in an ascending series of ethanol solutions. The specimens were embedded in epoxy resin, sectioned with a Sorvall MT- 2B ultramicrotome, mounted, double-stained with uranyl acetate and lead citrate, and viewed with a Philips TEM 400.

RESULTS

Scanning Electron Microscopy

The anterior portion of *Bothriocephalus acheilognathi*, when viewed in the SEM, is composed of a heart-shaped scolex with two long, deep, pear-shaped bothria (Fig. 1). A bilobed apical disc also is present, although it is not a prominent feature (Fig. 2). The outer portion of the scolex tegument is uniformly and densely composed of a single morphologically distinct type of microthrix. Dome-shaped tumuli, approximately 1.35 µm wide, also are numerous and

uniformly spaced at a distance of 2.4-4.5 µm on the scolex (Fig. 3). No obvious differences were observed in microthrix or tumulus density among the scolices of gravid, segmented, or non-segmented worms; however, within the bothria on the scolices of all of these developmental stages, mi-crotriches are more slender in appearance. Moreover, tumuli (0.6-0.7 µm) are less wide than on the outer surface of the scolex (Fig. 4). No other surface structures were observed in association with the tumuli.

Posteriorly, tumuli are less numerous (5.2-13.2 µm apart) on immature seg-ments (Fig. 5) and are entirely absent from mature and gravid proglottids. Sensory structures were not discernible on the surface of these proglottids using SEM, but they were evident in sections examined by TEM.

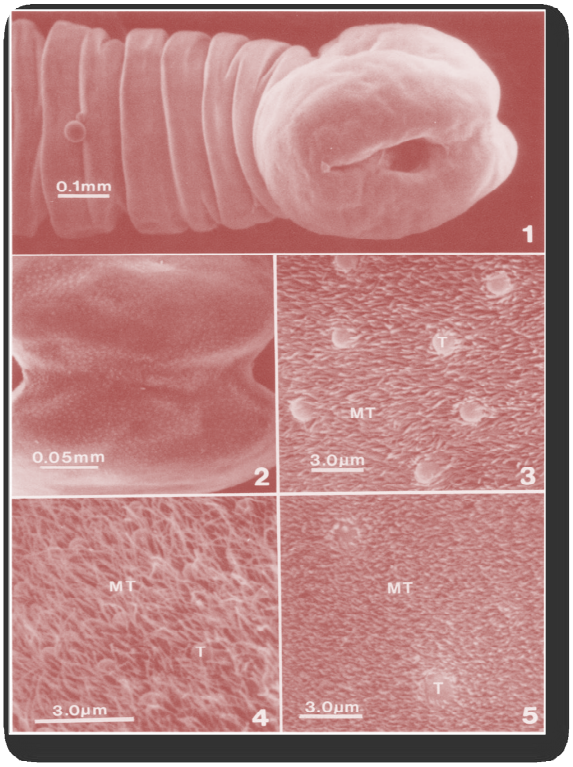
Transmission Electron Microscopy

Tumuli of the scolex contain dense-staining inclusions (Fig. 6). The basal lamina is evaginated and appears to form a duct through the tumulus. This duct also contains dense-staining inclusions. Scolex microtriches are rather long (0.8-1.1, µm) and thin (87-113 nm) (Fig. 6). A single electron-dense base plate is evident near the center of these microtriches; the shaft is long and spine-like in nature.

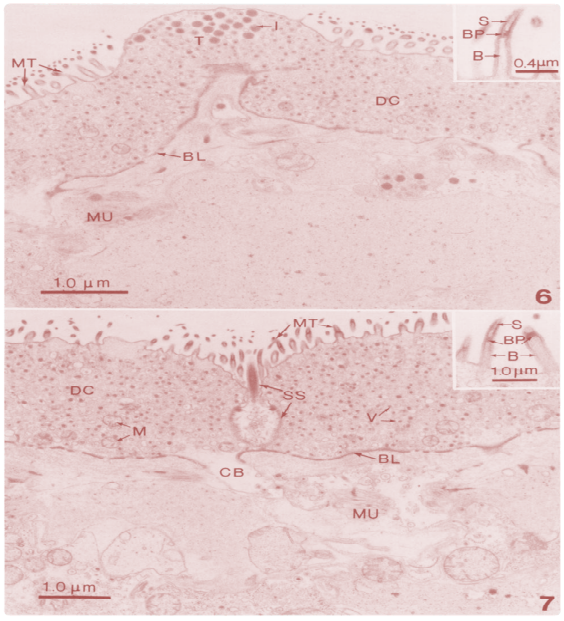
The distal cytoplasmic layer is located between the surface plasma membrane and the basement membrane and is rich in membrane-bound vesicles (Figs. 6, 7). Small mitochondria (0.24-0.38 µm) are numerous in the proximal one-third of the syncytial layer and cytoplasmic bridges connect this layer to the perikarya (Fig. 7).

Microtriches on mature and gravid segments measured 0.12-0.16, µm wide by 0.5-0.8 µm long. They possess a clearly distinguishable base and shaft, and a prominent base plate (Fig. 7). The plate is located near the center of the microthrix and is comprised of a single, straight, electron-dense layer in contact with the plasma membrane.

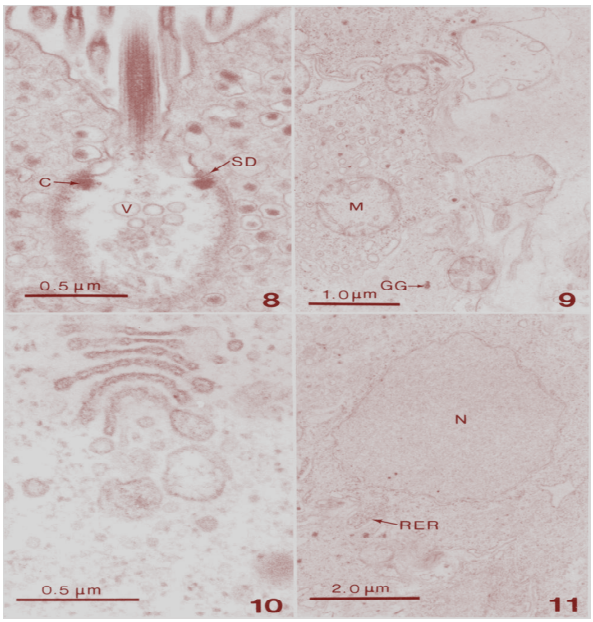
Sensory cilia were observed along the tegumental surface of the strobila (Figs. 7, 8). The cilium emerges through a dendritic bulb and protrudes through the tegument. The cilia of these structures never protrudes higher than the surrounding microtriches, which probably accounts for their not being observed by SEM. The bulb of the sensilla is connected to the distal cytoplasm by septate desmosomes. Directly below the desmosomes is a single electron-dense collar. Most of the bulb is filled with oval, membrane-bound, electron-lucent vesicles. The cilia extend 1.1-1.2 µm from the basal plate; a rootlet system was not observed (Fig. 8).



FIGS. 1-5. *Bothriocephalus acheilognathi*; SEM. **Fig. 1**. Scolex showing pear-shaped bothrium. **Fig. 2**. Bilobed apical disc on the scolex. **Fig. 3**. Scolex tegument showing microtriches (MT) and tumuli (T). **Fig. 4**. Tegument within a bothrium showing slender microtriches (MT) and small tumuli (T). **Fig. 5**. Immature proglottid showing microtriches (MT) and tumuli (T).



FIGS. 6, 7. *Bothriocephalus acheilognathi*; TEM. **Fig. 6.** Scolex tegument showing a tumulus (T), microtriches (MT), distal cytoplasm (DC), dense-staining inclusions (I), basal lamina (BL), and muscles (MU). Inset shows detail of a scolex microthrix including the shaft (S), base (B), and base plate (BP). **Fig. 7**. Tegument of a mature segment showing a sensory structure (SS), microtriches (MT), distal cytoplasm (DC), vesicles (V), mitochondria (M), basal lamina (BL), cytoplasmic bridge (CB), and muscle (MU). Inset shows detail of a strobilar microthrix including the shaft (S), base (B), and base plate (BP).



FIGS. 8-11. *Bothriocephalus acheilognathi*; TEM. **Fig. 8.** Sensilla within the tegument of a mature segment showing a single electron-dense collar (C), septate desmosomes (SD), and vesicles (V) within the dendritic bulb. **Fig. 9**. Perinuclear cytoplasm showing large mitochondria (M) and glycogen granules (GG). **Fig. 10**. Golgi body within the perinuclear cytoplasm. **Fig. 11**. A large nucleus (N) and rough endoplasmic reticulum (RER) within the perinuclear cytoplasm.

Muscle bundles were observed within the perinuclear region of the cestode (Figs. 6, 7). These muscles are composed of myofibrils, with the thick filaments (myosin) measuring 20.1-25.3 nm in diameter and the thin filaments (actin) measuring 4.4-4.6 nm in diameter. The cytons contain large mitochondria (0.98-1.21, µm), glycogen granules (Fig. 9), actively secreting Golgi bodies (Fig. 10), rough endoplasmic reticulum, and large nuclei (Fig. 11).

DISCUSSION

The fine structure of *Bothriocephalus acheilognathi* is similar in many respects to that of other pseudophyllidean tapeworms. The heart-shaped scolex, without a prominent apical disc, characterizes several species, including *Eubothrium parvum* (see Andersen, 1979), *Diphyllobothrium dentriticum*, *D. latum, D. ditremum* (see Andersen, 1975), and *Bothriocephalus scorpii* (see Jones, 1975). However, among the species of *Bothriocephalus* found in the Kashmir region, only *B. acheilognathi* has a heart-shaped scolex; the other six species have elongated scolices. As pointed out by Hoffman (1976, 1980), this characteristic is a diagnostic feature for *B. acheilognathi* in the Kashmir region.

Dome-shaped tumuli are uniformly spaced on the surface of the scolex of *B. acheilognathi*. Tumuli were first described by Boyce (1976) who observed them on adult *Eubothrium salvelini*, *Clestobothrium crassiceps*, and *Both-riocephalus scorpii,* all pseudophyllidean tapeworms. Arme & Threadgold (1976) noted the presence of tumulus-like structures on *Eubothrium crassum*, also a pseudophyllidean of fish. Subsequently, Tedesco & Coggins (1980) conducted a TEM study of the tegument of adult *E. salvelini* which revealed the presence of electron-dense inclusions within the tumuli. Their work indicated that the inclusions were manufactured by the endoplasmic reticulum, packaged by the Golgi apparatus within the perinuclear cytoplasm, and transported via ducts to the surface. Although the present study did not follow the inclusions from synthesis through deposition in the tumuli, the observations that were made are consistent with those of Tedesco & Coggins (1980). Thus, the Golgi apparatus was active within the perinuclear cytoplasm, and the inclusions, apparently packaged by these organelles, were of similar size to the structure observed within the tumuli of *E. salvelini*. Other morphological features of tumuli on *B. acheilognathi* were consistent with the previous descriptions of tumuli on other species.

Tumuli have been described previously only from adult pseudophyllideans recovered from fish hosts (Boyce, 1976; Tedesco & Coggins, 1980). The plerocercoids of *E. salvelini* and *Diphyllobothrium* sp. do not possess tumuli, even though they are present on the tegument of adults of these species (Boyce, 1976). By contrast, newly recruited immature *B. acheilognathi*, as well as mature and gravid specimens, possess tumuli. Previous studies did not examine immature worms within the definitive host, and since *B. acheilognathi* does not have a plerocercoid stage (Freeman, 1973), any comparisons are difficult. However, tumuli must develop soon after the parasite reaches the definitive host since these structures appear on very young individuals of (four days old) *B. acheilognathi*.

The distribution of tumuli varies greatly among scolex, immature, mature, and gravid segments of *B. acheilognathi,* the structures being most dense on the scolex, fewer in number on immature segments, and completely absent from mature and gravid proglottids. Boyce (1976) noted a similar pattern in the distribution of tumuli on the cestodes he studied, but Tedesco & Coggins (1980) made no mention of their distribution on *E. salvelini*. Tedesco & Coggins (1980) speculated that tumuli may serve in eccrine secretion. However, the functional significance of tumuli has not been elucidated, so it is difficult to explain the distribution of these structures along the strobila.

The outer portion of the tegument of *B. acheilognathi* was composed of a dense layer of microtriches, and while their morphology is somewhat

different between the scolex and the proglottids, each area possesses microtriches of a single form. Scolex microtriches are longer and thinner than those on the strobila. This apparent dimorphism, however, is not consistent for all cestode species. For example, Thompson *et al*. (1980) observed morphologic and size differences among microtriches on the strobila and scolex, as well as differences within the same region of *Proteocephalus tidswelli*. Several investigators have reported on the distribution of two types of microtriches found on *Mesocestoides corti* (Hess, 1981; Hess & Guggenheim, 1977; Voge *et al*., 1979). Both filamentous and blade-like microtriches occur behind the suckers; only filamentous forms are found at the posterior end of the strobila. Berger & Mettrick (1971) reported that *Hymenolepis diminuta* has two morphologically distinct types of microtriches on the mature and gravid segments. Similar patterns of microthrix distribution occur on *H. nana* and *H. microstoma*, although the direction in which these structures bend varies among the three species.

Microtriches within the bothria of *B. acheilognathi* are somewhat more slender in appearance than microtriches on the adjacent outer tegument. Andersen (1975) also noted a similar appearance of the microtriches within the bothria of *Diphyllobothrium dendriticum, D. ditremum,* and *D. latum*, but the functional significance of these slender structures is not known. Sensory cilia (sensilla) are spaced between microtriches of the strobila. These structures were observed only in the tegument of proglottids and were not seen within the scolex tegument. This observation is in contrast to that of Jones (1975), who found sensory cilia in the tegument of the scolex of *Bothriocephalus scorpii* but not on the strobila.

Webb & Davey (1974) conducted a thorough ultrastructural study of sensory receptors in a cestode species. These investigators described two different types of sensory structures from *Hymenolepis microstoma*. The sensory cilia found in the tegument of the strobila of *B. acheilognathi* most closely resemble the sensilla described from the scolex of *H. microstoma*. Both are char-acterized by a rather long cilium and the absence of ciliary rootlets. There are two electron-dense collars within the dendritic bulb of the sensilla of *H. microstoma*, but only one such collar is present in the bulb of *B. acheilognathi*. Moreover, sensilla of *B. acheilognathi* possess microtubules and a few electron-lucent vesicles. Sensory bulbs of *H. microstoma* are packed with such vesicles; microtubules were not discernible. The dendritic bulb of *Raillietina cesticillus* possesses both electron-lucent vesicles and microtubules (Blitz & Smyth, 1973).

Electron-dense collars appear to be a common feature of the sensilla of parasitic flatworms and, apparently, the number present is related to the size of the cilium (Lyons, 1969). If the cilium is very long, many collars will be present and the rootlet system will be well-developed. No doubt, such collars serve to support the cilium. Septate desmosomes are evident in the sensilla of the cestodes described earlier, as well as in *B. acheilognathi*, and serve to attach the bulb to the tegument.

The tegument of *B. acheilognathi* is composed of an outer syncytial layer with underlaying perikarya; such an arrangement is typical of all cestode species examined to date. Small mitochondria are abundant in the lower one-half of the syncytial layer, whereas large mitochondria are prominent in the cytons. The functional significance of this size difference is not known. Also prominent within the syncytium are membrane-bound vesicles which probably are pinocytotic (pinosomes). Although it has been long speculated that the cestode tegument could take up macromolecules by pinocytosis, such a phenomenon has been demonstrated only recently. Hopkins *et al.* (1978) showed that pinocytosis occurs in the tegument of the plerocercoid of *Schistocephalus solidus* and Threadgold & Hopkins (1981) reported the same phenomenon in the tegument of adult *S. solidus* and *Ligula intestinalis*. The pinosomes described by these investigators are remarkably similar in size and shape to the vesicles seen within the syncytium of *B. acheilognathi*; it seems reasonable to assume that these vesicles are involved in the same process.

Muscle bundles occur within the perinuclear region and just below the basal membrane in *B. acheilognathi*. These muscles are comparable in morphology to those described from other cestode species (Hess, 1980; Lumsden & Byam, 1967). The cytons of *B. acheilognathi* contain a prominent nucleus, actively secreting Golgi bodies, ribosome-rich endoplasmic reticulum vesicles, glycogen granules, and large mitochondria. The condition of the Golgi apparatus and endoplasmic reticulum indicates a cell that is synthesizing protein actively. The fate of this protein, however, is not known. Tedesco & Coggins (1980) indicated that the inclusions found within tumuli are synthesized within the cytons. Another possibility is that some of these vesicles may contain enzymes, or may contribute to the glycocalyx which covers the external plasma membrane (Smyth, 1969).

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