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ULTRASTRUCTURE MORPHOLOGY OF THE PSEUDOPHYLLIDEAN CESTODE *BOTHRIOCEPHALUS ACHILOGNATHI*, FROM *SCHIZOTHORAX* SPECIES OF KASHMIR

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ABSTRACT
The ulrastructural morphology of the cestode *Bothriocephalus acheilognathi* (Yamaguti, 1934) has been studied by SEM (scanning electron microscopy) and TEM (transmission electron microscopy). This parasite was isolated from *the Schizothorax* species from fresh water bodies of Kashmir. Adult *Bothriocephalus acheilognathi* ranged from 3.5 to 13.5 cm long and 0.36 to 1.44 mm wide. This cestode presents three types of microtriches., the first type corresponds to a large and thin structure, the second type is short and wide, both types are found on the scolex and the strobila. The third type is observed exclusively at the bottom of the bothria and is larger and thinner than the first type. Sensilla are located at the tegument surface. These sensitive organs are distributed on the scolex and stroblia., their cilia never protrude higher than the surrounding microtriches. The bulb of the sensilla is connected to the dystal cytoplasm by septate desmosomes and a single electodense collar. Other structures of the tegument are the dome shaped tumuli, present exclusively on the scolex and containing dense staining inclusions. They are discharging structures originated at the unicellular glands of the perinuclear cytoplasm. Muscle bundles are present in the perinuclear region below the basal membrane. These smooth muscle fibers present two orientations. Between the muscular zone and the perinuclear cytoplasm there is an intermediate zone clearly observable at the scolex and less defined but present at the proglottids. This zone is packed with cytoplasmic projections, which contain rabdiform bodies, mitochondria and abundant glycogen. The Golgi bodies indicate a cell actively secreting proteins. In the region of the perinuclear cytoplasm, in addition to the unicellular glands and the mucous secretory cells, the main organs are vitellogenic glands, protonephridial system, gonads and accessory structures. Storage structures rich in reserve material are also observed.

Key Words: cestode ultrastructure, *Bothriocephalus*, freshwater fish, *Schizothorax*

INTRODUCTION

The present work is part of a general study on the morphology of the cestode *Bothriocephalus acheilognathi.* Some previous ultrastructural studies (Granath *et al*., 1983., Pool, 1984) have been made for the genus *Bothriocephalus.* We considered important study of this endoparasite, as in Kashmir it infects several species of *Schizothorax* species. The first description of *Bothriocephalus acheilognathi* isolated from fish of the Cyprinidae family was made by Yamaguti, quoted by Korting (1975) who also made a list of synonyms. A study of the intermediate host for this parasite was made Hoffman (1976), Bauer *et al*., (1969, 1987) and Musselius (1962). A definitive host can only become infected by ingesting cyclopoid copepods (Chubb, 1981), usually from the genera *Acantocyclops, Macrocyclops, Mesocyclops, Tropocyclops* and *Diacyclops.* The parasitosis induced by *Bothriocephalus acheilognathi* is exclusively limited to freshwater fish, especially those localized in the ponds and water locks in which they are grown as an ecological control for water weeds, and to provide a source of fish proteins for human consumption. The world distribution of the parasite includes developed and underdeveloped countries (Korting, 1984; Sopinska, 1985; Leong, 1986; Kritscher, 1986; Riggs and Esch, 1987; Alarcon – Gonzalez, 1988).

Although a species of parasite may infect many species of hosts in an ecosystem, the maintenance of a local suprapopulation is usually dependent on a few species of ‘’required hosts’’ (Holmes and Price, 1986). *Bothriocephalus acheilognathi* is notable among fish cestodes for its relative lack of specificity for the definitive host. It has been recovered from the intestine of more than 40 species of freshwater fish, most of which are cyprinids (Riggs and Esch, 1987). The degree of infection determines the growth rate of fish. Mortality depends to a high degree on the amount of parasites that develop in the infected fish. It can also be due to mechanical intestinal obstruction, competition by essential nutrients or the degree and type of lesions developed at the attachment site of the bothria to the intestinal mucosa. Another consequence of parasitosis is the disease of hemoglobin content in the fish blood; this diminution can be of more of 28%, a situation that forces the fish to swim at the water surface where the oxygen concentration is higher. The most obvious economic damage is the lowering of muscle protein content, which in turn will give a poor yield in fish production (Alarcon – Gonzalez, 1988).

MATERIALS AND METHODS

Adult forms of the parasite were obtained from the final host of the *Schizothorax* species grown in the water bodies of the Kashmir. The location of worms within the gut is in most cases 3 to 5 mm from the intestinal opening of the bile duct., however, in massive infections it was possible to localize parasites in the lower part of the intestine. The bothria act as elongated pincers on secondary folds of intestinal mucosa. Living *Schizothorax* species were transported from the water bodies to the laboratory in the same water medium. After a humanitarian sacrifice, adult worms were collected by

dissection of the fish intestine. A total of 250 fish were dissected. Immediately after isolation, specimens were fixed for two hours in a 3% glutaraldehyde solution in 0.1M phosphate buffer, pH 7.4 at 4^0C and rinsed overnight in a 0.25M sucrose solution in the same buffer at 4°C. Post fixation was made in 2% osmium tetra oxide in 0.1M phosphate buffer pH 7.4 at 4^0C for two hours., after post fixation, samples were serially dehydrated in acetone at room temperature. Infiltration was made in a 1 : 1 acetone – Epon mixture for 24 hours. Samples were embedded in an undiluted Epon mixture as described by Luft (1961).

Ultrathin sections were obtained by an ultra-microtome with a diamond knife, stained with uranyl acetate (Hayat, 1970) and lead citrate (Reynolds, 1963) and observed in an electron microscope operated at 60 kv. Thin sections (μm) were obtained and stained with 2% toluidine bleu-borate buffer for light microscopy. For the scanning electron microscopy, after dehydration cestodes were dried to the critical point. The critical point was maintained 5 minutes at 31°C. The cestode was fixed to the cylinder that holds the sample with the silver paint and covered with 30nm uniform film of carbon and gold. Samples were observed with scanning electron microscope.

RESULTS

From the 250 dissected fish, 78 (31.2%) were infected. Most tapeworms (91%) were recovered from the first half of the intestine. We acknowledge the preference of young worms for the anterior gut region, whereas larger worms were located further back. The size of *Bothriocephalus acheilognathi* ranged from 3.5 to 13.5 cm, the size of the heart – shaped scolex was 0.8 to 1.61 mm with two lateral, deep bothria as described for other species (Lopez – Jimenez, 1980; *Granata et al*., 1983). The bottom of the bothria showed a special arrangement of microtriches and the neck was absent. Dome shaped tumuli (viewed with SEM) are numerous and more or less uniformly spaced on the scolex. No differences were observed in microthrix or tumulus density among the scolices of gravid, segmented or unsegmented worms, however within the bothria of all those developmental stages, microtriches are more slender in appearance. Tumuli of the scolex contain dense – staining inclusions.

Attachment of the parasite to the host intestine is insured through the bothria. Each one encircling one or two intestinal folds, and as described for other species, with strobila wedged and coiled against the intestinal wall (Scott and Grizzle, 1977). Small specimens were less intimately associated with the intestinal mucosa than were larger specimens, indicating their greater reliance on the bothria for attachment. The scolex penetrated into the host gut wall as for as the muscle underlying the sub mucosa, the host reacted by depositing connective tissue around the scolex, forming a swelling in the gut wall. Villi from this area disappeared and there was marked fibrosis. Higher magnification of the host – parasite interaction area at the tip of the scolex showed a layer of vesicles within the parenchyma, suggesting an area of secretory activity. The intestinal mucosa engulfed by the bothria can be damaged, resulting in desquamation, hemorrhagic enteritis or necrosis. The host response consisted also in numerous macrophages bound to the scolex of the parasite (seen by SEM).

TEGUMENT ULTRASTRUCTURE

The strobila and the scolex of this parasite are covered by a syncityal tegument as described for other members of the class Cestoidea. Special features displayed by this plasma membrane are the digitiform projections or ''microtriches'' (Rothman, 1959). Three types of microtriches (Fig. 1) were found in *Bothriocephalus acheilognathi.,* the first type corresponds to a large and thin structure, the second type is short and wide. Both types are found on the strobila as well as the scolex, without preferential localization or distribution. The third type corresponds to those observed exclusively at the bottom of the bothria, as revealed by scanning electron microscopy (not shown here). this microtriches is larger and thinner than the first type mentioned and occur in bundles. All types of microtriches show an electron dense ''spine'', curved to the posterior end of the parasitic body. At the interior of the microthrix a number of longitudinally arranged microfilaments are observed. The absorptive zone of the microthrix is limited by a continuous plasma membrane, and is covered by glycocalyx (inset Fig. 1). The distal cytoplasm is separated from the perinuclear (perikarya) cytoplasm by a proximal plasma membrane which in turn, is the limit of the basal membrane. This basal lamina is constituted by a dense sheet of about 0.28 μm in thickness, which is either in contact, or is the attachment site of numerous fibers.

The distal cytoplasm shows numerous mitochondria and rabdiform bodies. Also different types of pore- like structures open to the exterior surface (Fig. 2). As shown in Fig. 2A, the dense sheet of the basal lamina is interrupted, generating a pore like structure with clear connection between the interstitial space and the external surface of the parasite. An increased amount of plastic fibers attached to the basal lamina of the pore – like structures (PLS) can also be observed, the PLS itself is clearly separated from the distal cytoplasm by a low electron dense thick wall and a membrane that limits the lumen of the conduct. In figure 2 the vesicles at the terminal end of the conduct may be observed. A possible function of the elastic fibers observed in figure 2A is also presented.

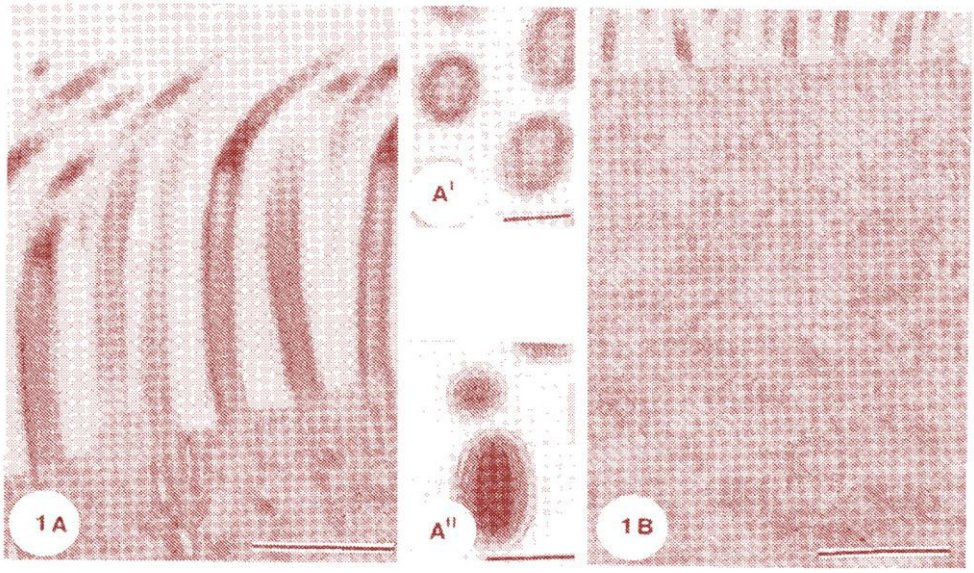

Figure 1. A) Micrograph of microtriches of the tegument of *Bothriocephalus acheilognathi*. Two structural components are observed: the so called ''absorptive zone'' (a transverse section is shown in the lower inset) covered by glycocalyx, and in the upper inset the electron dense ''spine'', curved towards the end of the parasite body is shown, 25000 X (Barr = 1.0 µm).

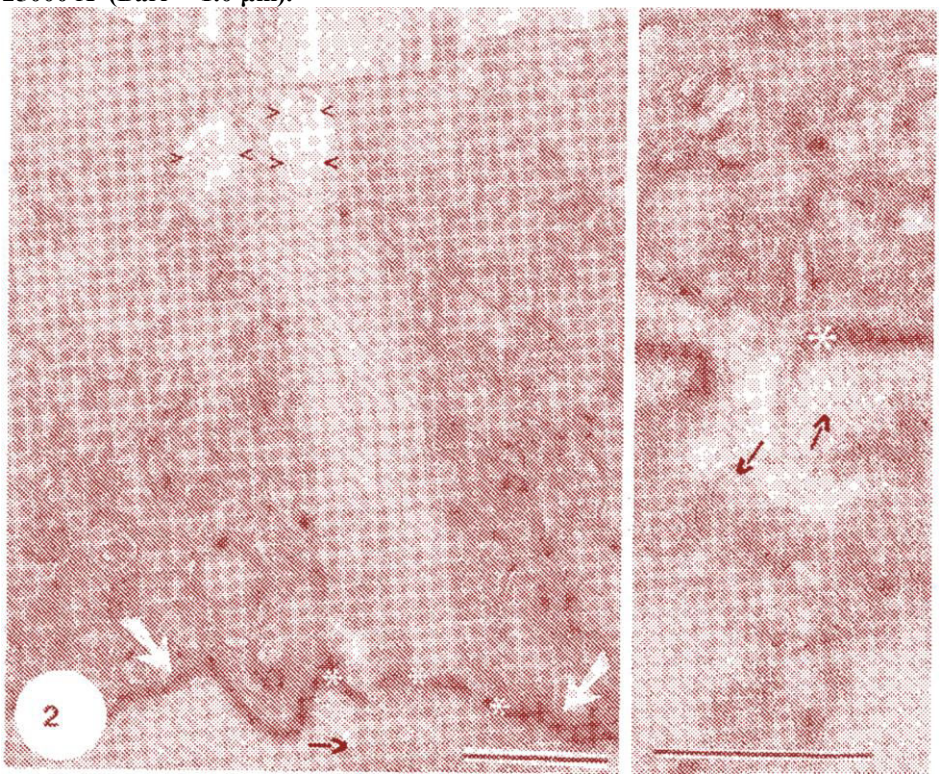

Figure 2. A) Morphology of the distal cytoplasm of *Bothriocephalus acheilognathi*. The ultrastructure of the dense limiting sheet of the basal lamina (arrows), localized between the perinuclear cytoplasm and the tegument is shown. The distal cytoplasm is interrupted (*) at several places with membrane limited conducts, inside them it is possible to observe empty vesicles (arrow heads). Numerous elastic fibres at the basement membrane (heavy arrow), resembles tendinous tissue for muscle attachment. Inset shows a magnification of an aperture of the dense lamina (cytoplasmic bridge). 20000 X (Barr = 1.04 µm).

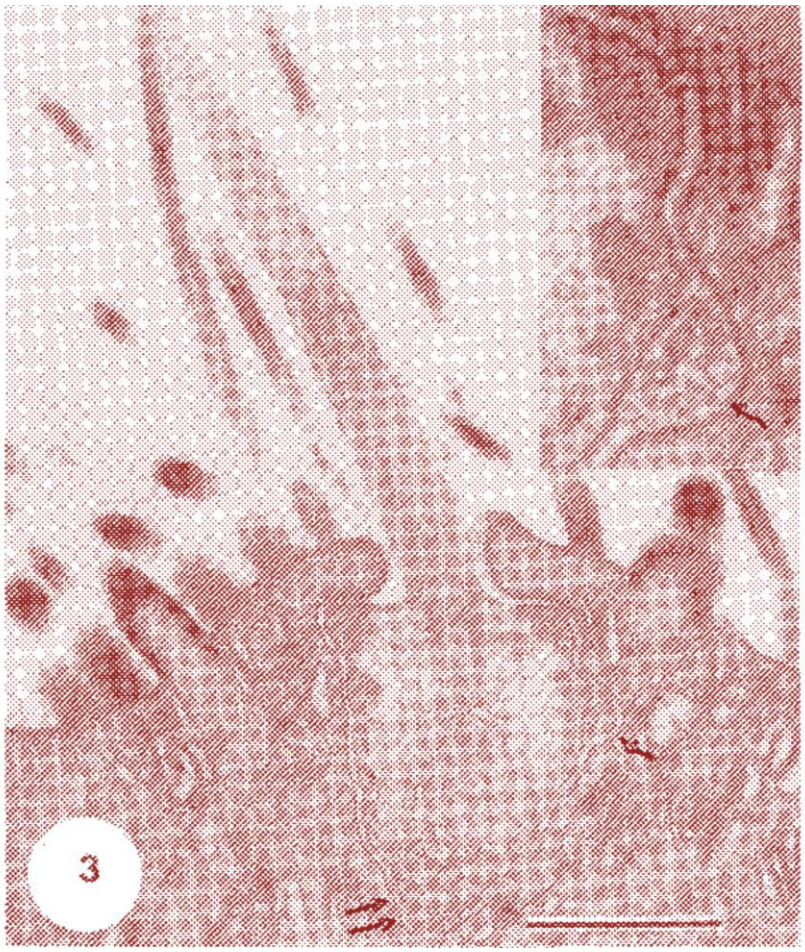

Figure 3. Morphology of the ciliated sense organ (sensilla). The elements of this organ are similar to the cellular microvillus of a microthrix. The absence of the 'spine' is apparent. The basal pieces of the sensilla bound to the distal cytoplasm, shows similar ultrastructure to the synaptic junction of mammals (I.e. presence of micro vesicles (arrows). The sensilla is isolated from the exterior by means of a circular septate desmosomes, 42000 X (Barr = 0.5 µm).

Another structure observed at the tegumental surface are the sensory cilia or sensilla (Fig. 3). These sensitive organs are distributed along the parasite body, scolex and strobila. The distribution disagrees with that reported by other authors (Morseth, 1967., Jones, 1975., Granath *et al*., 1983) for this species and other related species. The cilium emerges through a dendritic bulb and protrudes through the tegument. The cilia of these structures never protrude higher than the surrounding microtriches. The spine is absent and the tubule content is better defined that in the microthrix. This organ ends in the proximal bud that contains numerous electrons – luscent vesicles which provide connection with a nerve ending that also contains oval membrane – bound vesicles. The bulb of the sensilla is connected to the dystal cytoplasm by separate desmosomes; under the desmosomes is a single electrodense collar. The cilium extends 1.0 to 1.3 µm from the basal plate.

Other structures of the tegument are numerous projecting buds or tumuli (Fig. 4) localized mainly at the scolex, but also on the proglottids (few). These tumuli are discharging structures originated at the unicellular glands of the perinuclear cytoplasm with cytoplasmic projections towards the surface of the parasite. The basal lamina, together with the cytoplasmic projected out of the cell and penetrates the tegument. Besides, some muscle fibers are located between the conduct and the basal lamina. The limiting membrane of the basal lamina apparently continuous with the channel membranes, during the evagination of the cytoplasmic projection through the distal cytoplasm. The rabdiform bodies are mixed with the dense secretion granules.

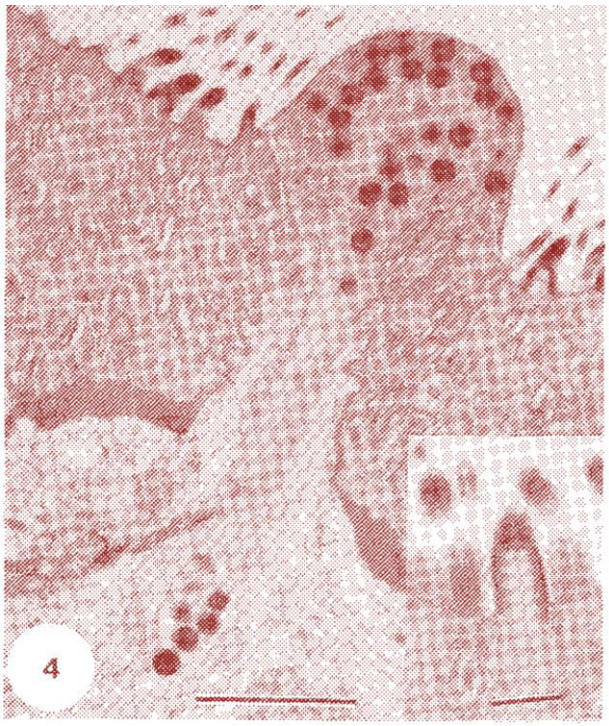

Figure 4. Secretory receptor (tumulus). This figure shows the dense granules of the secretory process, synthesized in the ''unicellular endocrine glands'' localized deeply at the parenchyma. These glands are profusely ramified towards the parasite tegument. We can observe short and wide microtriches around the tumulus. 22500 X (Barr = 0.5 µm).

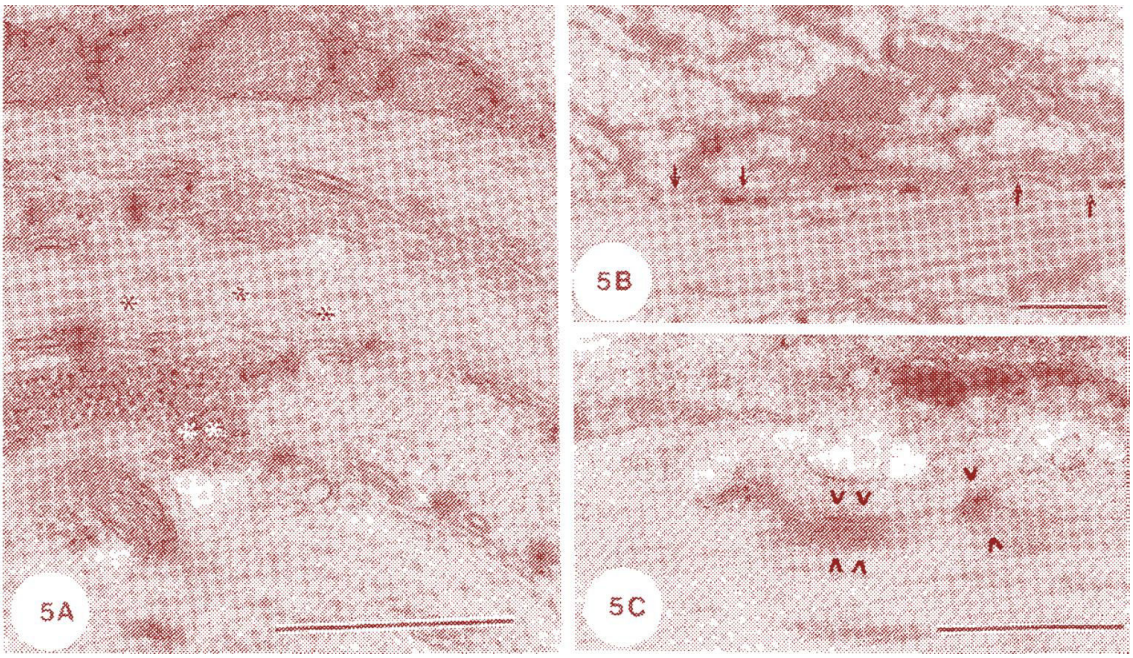

Figure 5. Muscle tissue. In A can be seen the morphology of the muscle bundles with some common characteristics like mitochondria and sarcoplasmic reticulum. We can observe the membrane spealizations (marked * in the figure)., and abundant glycogen (**) (38500 X, Barr = 1.0 µm). The nerve perikaryon (arrows), contains many dense core granules and some vesicles with variable density, some of them are empty (15000 X, Barr = 1.0 µm). Also in this figure (C), another type of electric connection seems to be present. This structure apparently is another type of innervations, and resembles the striated muscle neuromuscular plate (arrow heads), 30000 X, Barr =1.0 µm).

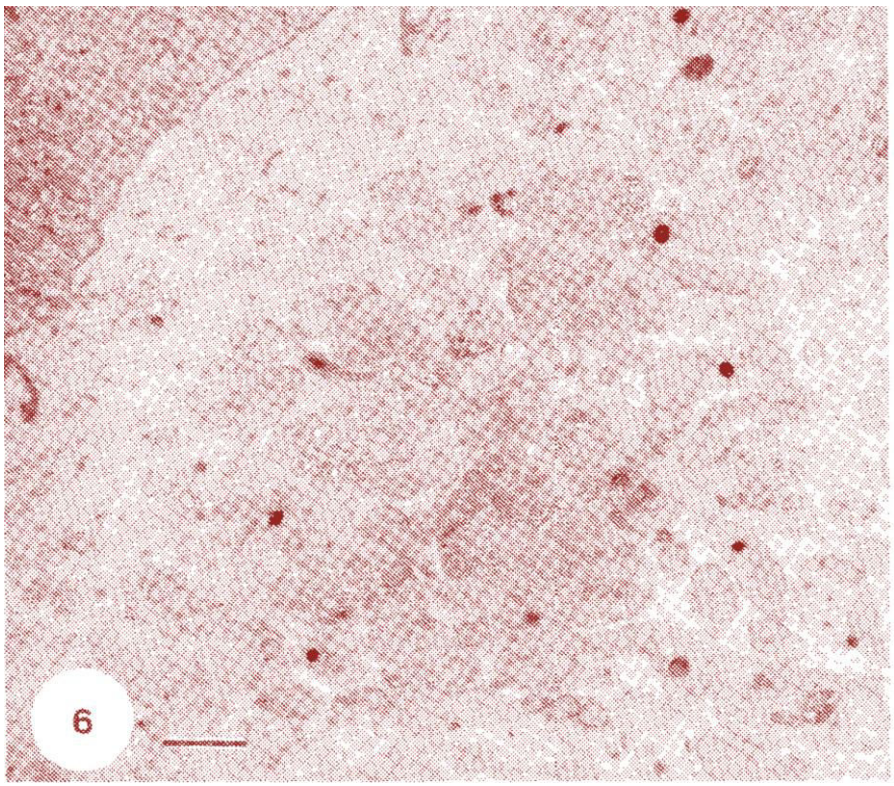

Figure 6. Ultrastructure of the intermediate zone. This tissue is localized between distal and peripheral muscle bundles. In this zone, cytoplasmic projections of the rabdiform bodies of the synthesizing cell and perinuclear or proximal cytoplasm are numerous. In this projections glycogen and mitochondria are abundant., also cytoplasmic projections of mucous secretory cells and those originated from the unicellular endocrine glands are important components of this area. This zone is better defined at the scolex than at the proglottids, but it is not absent at any part of the parasite body. 5000 X (Barr = 0.2 µm)

THE SPACE BETWEEN THE BASAL LAMINA AND THE PERINUCLEAR CYTOPLASM

Smooth muscle fibers arranged in layers (bundles) show two orientations (Fig. 5A) , longitudinal cones directly in contact with the basal lamina and transversal ones in connection with the perinuclear cytoplasm. These muscles are composed of micro fibrils, with thick (myosin) and thin filaments (actins). The muscular innervations (Fig. 5B) cannot be described in the classical terms of a neuromuscular plate or electrical synapses. Five cytoplasmic projections from the unicellular glands that run parallel and in close contact with the muscular fibers are also observed (Fig. 5B). these cellular projections contain vesicles with different electron densities (or affinity to the staining chemicals). Another type of innervations observed consisted in a dense area between the muscular fibers and a cytoplasmic cell projection, this structure resembles more the structure of a vertebrate neuromuscular plate (Fig 5C).

Between the muscular zone and the perinuclear cytoplasm there is an intermediate zone (fig. 6), clearly observable at the scolex and less well defined, but present at the proglottids. This zone is packed with cytoplasm projections or internuncial processes connecting distal and perinuclear cytoplasm. These projections contain in addition to the rabdiform bodies, mitochondria (0.97 – 1.22 µm) and abundant glycogen granules. Other type of cytoplasmic projections located at this level are those originated in the unicellular glands and in the mucous secretory cells (Fig. 7). The type of cytoplasmic projections are differentiated by the type and morphological characteristics of the granules that contain (compare Figs. 4 and 5 with fig. 7). The type of granules in the cytoplasmic projections of the mucous secretory cells are larger and show lower electron density and a well defined limiting membrane. The relationship of the limiting basal membrane and the cytoplasmic projection of the mucous secretory cells show a different pattern., in this case the basal lamina is projected towards the perinuclear cytoplasm (Fig. 7) forming a neck type structure that probably, functioning like a sphincter, regulates the number of granules that can migrate to the tumuli at the surface of the parasite. Other characteristic elements in this cytoplasmic projection are the microtubules, whose function is probably to orientate the direction of granule migration towards the parasite surface. As described above for the sensilla, this projection is also isolated from the exterior by means of clearly defined desmosomes.

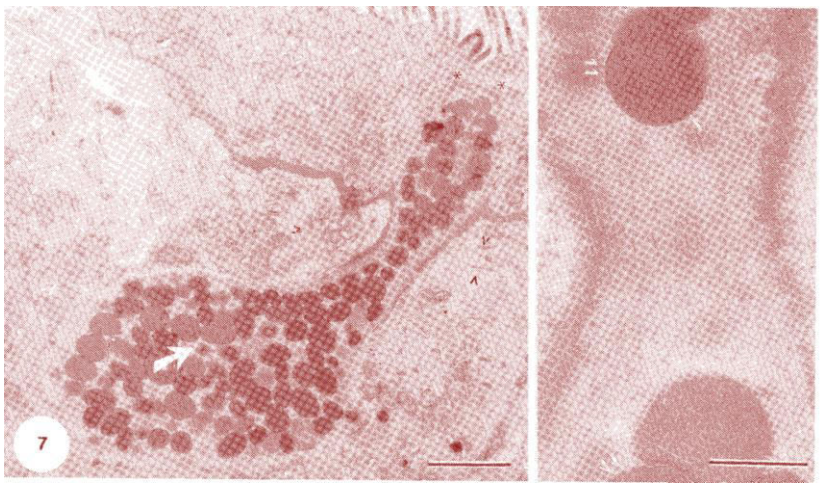

Figure 7. Mucous like secretory cells. The ''head'' of the projection is limited by a desmosomes (*), secretory granules are defined by a clear unit membrane (arrow). The dense basal lamina adjacent to the distal cytoplasm is projected towards the distal cytoplasm, forming a ''neck'' which might regulate the number of granules passing through, and perhaps functioning like a sphincter together with the muscle fibers (arrow heads) that surround the structure. The inset (23000 X, Barr = 1.0 µm) shows a high magnification of the ''neck''. The presence of microfilaments whose function could be the regulation of the sense of migration of the granules in the direction of the parasite tegument is evident, 18000 X (Barr = 1.0 µm).

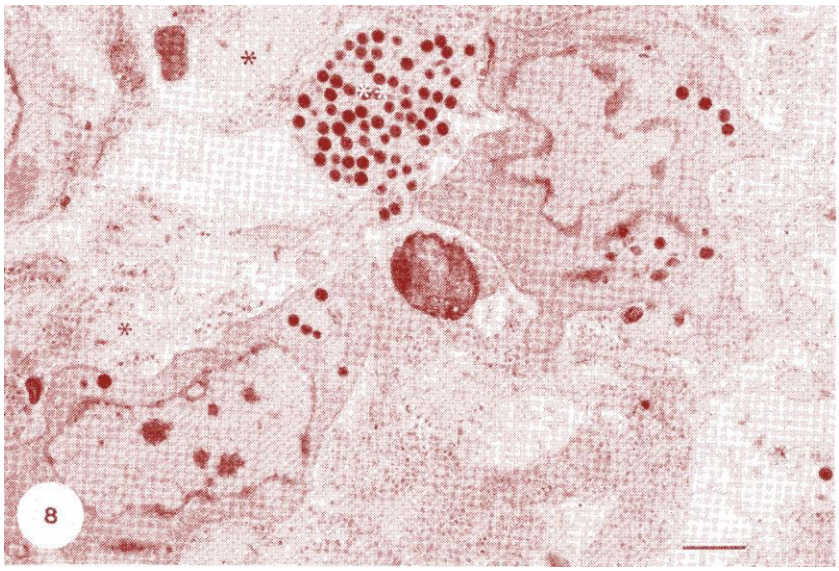

Figure 8. Ultrastructure of the perinuclear cytoplasm. This zone of the parasite body presents a high cellular density, muscular fibers (*) and cytoplasmic projections (**) of cells localized at the center of the cestode body (I.e. unicellular endocrine gland). 12000 X (Barr = 1.0 µm).

THE REGION OF THE PERINUCLEAR CYTOPLASM

In the region of the perinuclear cytoplasm (Fig. 8), in addition to the unicellular glands and the mucous secreting cells, the main organ of the parasite are : vitellogenic glands, protonephridial system, gonads and their accessory structures which are not described here. The unicellular glands (Fig. 8) are identified by the type of granules containing in the cytoplasm. Some cells show a dense cytoplasm with several actively secreting Golgi bodies., this is in agreement with the secretory activity of this type of cells which is also supported by a high ribosome content . the cytoplasmic projections of the cell are packed with characteristic granules and a number of empty vesicles. These cellular projections showed two types of orientations, one runs along the muscular fibers, and the other towards the distal cytoplasm as described above (Fig. 4 and 5).

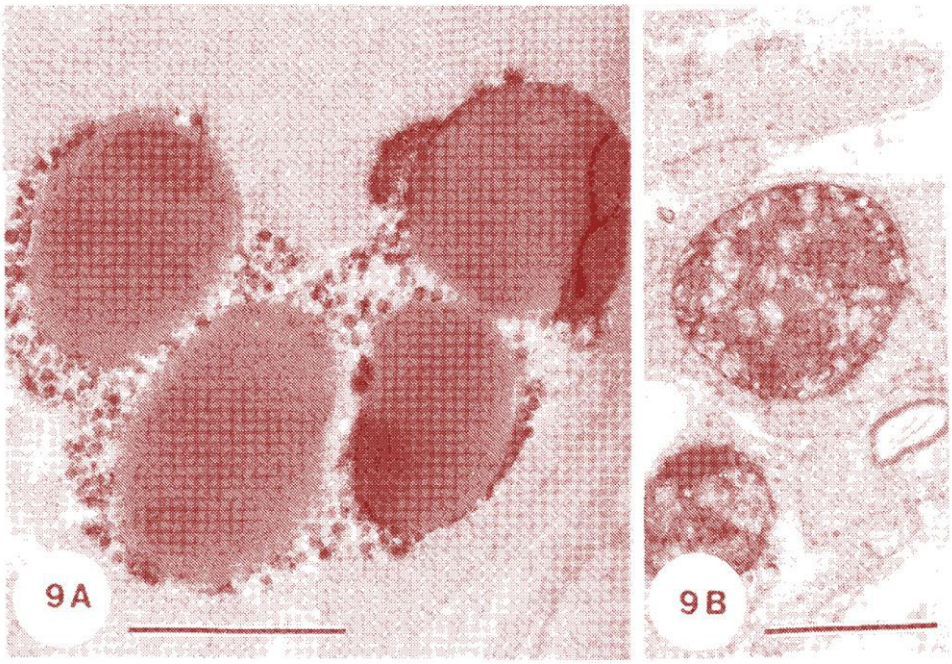

Figure 9. Lipid storage bodies are shown at the cytoplasm of a cell with high content of free ribosome's and also a high content of granular endoplasmic reticulum. A : 23000 X (Barr = 1.0 µm). In B the residual bodies are shown. B : 22000 X (Barr = 1.0 µm).

The perinuclear cytoplasm is also profusely ramified. Ramifications connect these cells with different types of organs, glands and other cells found in the proglottids. Storage structures rich in reserve materials, like glycogen and lipid droplets are also observed, (Fig. 9). Materials generated by the active metabolism of the reserve materials are present, especially those derived from lipids (myelin figures and residual bodies).

DISCUSSION

Bothriocephalus acheilognathi has been described as a parasite of several families of fish : Siluriform, Poeciliidae, Cyprinidae, Acipenseridae and Atherinidae. In Mexico, it has been found mainly in the grass carp (*Ctenopharyngodon idellus*) (Lopez – Jimenez, 1978, 1980), in *Carassius auratus* (Alarcon – Ganzalez, 1988) and the white fish (*Chirostoma estor*), (Osorio – Sarabia *et al*., 1986). The fine structure of *Bothriocephalus acheilognathi* is similar in many aspects to that of other Pseudophyllidean tapeworms. Among the species of *Bothriocephalus* found in North America only *Bothriocephalus acheilognathi* has a heart shaped scolex, the other species present elongated scolices. So as mentioned by Hoffman (1980), this characteristic is a diagnostic feature for *Bothriocephalus acheilognathi* in North America. Tumuli are more or less uniformly distributed on the surface of the scolex of *Bothriocephalus acheilognathi.* These structures were first described by Boyce (1976) on adult *Eubothrium salvelini*, *Clestobothrium crassiceps* and *Bothriocephalus scorpii*. Later, Tedesco and Coggins (1980) made a TEM study of the tegument of adult *Eubothrium salvelini* which revealed the presence of electron – dense inclusions within the tumuli. Their work showed that the inclusions were manufactured by the endoplasmic reticulum, packaged by the Golgi bodies within the perinuclear cytoplasm and transported via ducts to the parasite surface. Although this study did not follow the inclusions from synthesis through deposition in the tumuli, the observations made are consistent with those of Tedesco and Coggins (1980).

The plerocercoid of *Eubothrium salvelini* do not posses tumuli, even though they are present on the tegument of adult of this species (Boyce, 1976)., contrary, recently recruited immature *Bothriocephalus acheilognathi* as well as mature specimens, possess tumuli. Tedesco and Coggins (1980) mention that tumuli may serve in eccrine secretion, however the functional significance has not been elucidated. Granath *et al*., (1983) acknowledge also the presence and distribution of tumuli in the scolex and strobila of *Bothriocephalus acheilognathi* but they do not mention their possible function. Since *Bothriocephalus acheilognathi* does not have a plerocercoid stage any comparison are difficult. The tegument of *Bothriocephalus acheilognathi* was composed of a dense layer of microtriches, their morphology varies between the scolex and the proglottids. Generally, scolex microtriches are larger and slender than those on the strobila, nevertheless the thinnest microtriches are found within the bothria. These observations are consistent with those made by Granath *et al.,* (1983). Andersen (1975) also noted slender microtriches within the bothria of three species of *Diphyllobothrium*. Gland cells in the bothria of Pseudophyllidean cestodes have been through to produce secretions for

adhesion rather than penetration. However, the granular nature of the scolex tissue and the vesicles in the distal parenchyma of some species of *Bothriocephalus* suggests some kind of secretory activity which may help the penetration of the gut wall of the host. Sensilla are distributed between the microtriches of the scolex and strobila. This observation disagrees with that reported by Granath *et al.,* (1983) for *Bothriocephalus acheilognathi,* who found them only in the tegument of proglottids, and is also different from those described by Morseth (1967) for *Eichinococcus granulosus* and Jones (1975) for *Bothriocephalus scorpii* in which an exclusive localization of these sensory cilia at the scolex was reported. Further observations in this species and other species of this genus are needed.

The sensory cilia found in *Bothriocephalus acheilognathi* most closely resemble the sensilla described from the scolex of *Hymenolepsis microstoma* (Webb and Davey, 1974), both are characterized by a rather long cilium and the absence of ciliary rootlets. Two elecrro-dense collar can be seen within the dendritic bulb of the sensilla of *Hymenolepsis microstoma*, but only one is present in the dendritic bulb of *Bothriocephalus acheilognathi.* Sensilla of *Bothriocephalus acheilognathi* possess microtubules. Granath *et al* (1983) mention that electron-dense collars are a common feature of the sensilla of parasitic flatworms and, that the number present is related to the size of the cilium. in fact, such collars serve to support the cilium and septate desmosomes serve to attach the bulb to the tegument. The tegument of *Bothriocephalus acheilognathi* presents an arrangement typical of all cestode species studied to date., it is composed of an external syncytial layer with underlying perikarya. Within the syncytium we observed mitochondria and membrane bound vesicles. These vesicles are probably pinosomes, since it has already being demonstrated in *Schistocephalus solidus* and *Ligula intestinalis* (Threadgold and Hopkins, 1981) that the cestode tegument can take up macromolecules by pinocytosis. The vesicles described by these authors are similar (size and Shape) to the ones we observed in *Bothriocephalus acheilognathi,* so we assume they are involved in pinocytosis. Muscle bundles are present in the perinuclear region, below the basal membrane. They are comparable to those described from other cestode species (Hess, 1980). The cells of *Bothriocephalus acheilognathi* have a prominent nucleus, a high ribosome content, activity secreting Golgi bodies, mitochondria and glycogen. The state of the Golgi bodies and endoplasmic reticulum indicates a cell actively synthesizing proteins. The fate of the proteins is not known, although some authors thick that the inclusions found within the tumuli are synthesized in these cells (Tedesco and coggins, 1980). Other hypothesis is that some of these vesicles contain enzymes, or contribute to the formation of the glycocalyx (Smith, 1969).

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