

## ULTRASTRUCTURE OF THE PSEUDOPHYLLIDEAN CESTODE *BOTHRIOCEPHALUS ACHEILOGNATHI*, PARASITE OF FRESHWATER FISH OF COMMERCIAL IMPORTANCE

V. DÍAZ-CASTAÑEDA\*  
A. CARABEZ-TREJO\*\*  
R. LAMOTHE-ARGUMEDO\*\*\*

### RESUMEN

La ultraestructura del céstodo *Bothriocephalus acheilognathi* (Yamaguti, 1934) ha sido estudiada por microscopía electrónica de barrido (SEM) y principalmente por microscopía electrónica de transmisión (TEM). Este parásito se aisló de carpas herbívoras (*Ctenopharyngodon idellus*) provenientes de estanques y presas de dos estados de México (Quéretaro e Hidalgo).

Los adultos de *B. acheilognathi* medían entre 3.5 y 13.5 cm de largo y 0.36 a 1.44 mm de ancho. Este céstodo presenta tres tipos de microtricos. El primero corresponde a estructuras largas y delgadas; el segundo es corto y ancho, y el tercero se observa exclusivamente al fondo de los botrios y es más largo y delgado que el primer tipo.

En la superficie del tegumento se encuentran los cilios sensoriales. Estos órganos están distribuidos en el escólex y estróbilo del céstodo. Los cilios sensoriales nunca sobresalen por arriba de los microtricos que los rodean. El bulbo está conectado al citoplasma distal por medio de desmosomas septados y un solo collar electrodenso. Otras estructuras del tegumento son los túmulos en forma de domo, localizados exclusivamente en el escólex y conteniendo inclusiones teñidas densamente. Son estructuras de descarga que se originan en las glándulas unicelulares del citoplasma perinuclear.

En la región perinuclear abajo de la membrana basal, existen fibras musculares lisas que presentan dos orientaciones. Entre la zona muscular y el citoplasma perinuclear hay una zona intermedia claramente observable en el escólex y menos definida pero presente en los proglótidos. Esta zona presenta

\* Centro de Investigación Científica y Estudios Superiores de Ensenada, B.C. Apartado postal 2732, 22800 Ensenada, B. C. México.

\*\* Facultad de Medicina, UNAM, Departamento de Histología, Apartado postal 70-138, 04510 México, D.F.

\*\*\* Instituto de Biología, UNAM, Laboratorio de Helminología, Apartado postal 70-153, 04510 México, D.F.

proyecciones citoplásmicas, que contienen cuerpos rabdiformes mitocondrias y abundantes gránulos de glicógeno. El estado de los aparatos de Golgi indica células sintetizando proteínas activamente.

En la región del citoplasma perinuclear, además de las glándulas unicelulares y las células secretoras de mucus, los órganos principales son: glándulas vitelógenas, sistema protonefridial, gónadas y sus estructuras accesorias. También se observan formas de almacenamiento ricas en materiales de reserva.

Palabras clave: Ultraestructura céstodo, *Bothriocephalus*, peces dulceacuícolas, ciprínidos.

#### ABSTRACT

The ultrastructural morphology of the cestode *Bothriocephalus acheilognathi* (Yamaguti, 1934) has been studied by SEM (scanning electron microscopy) and principally by TEM (transmission electron microscopy). This parasite was isolated from the grass carp *Ctenopharyngodon idellus* from fresh water ponds and water locks in two states of Mexico (Querétaro and Hidalgo).

Adult *B. 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 strobila; their cilia never protrude higher than the surrounding microtriches. The bulb of the sensilla is connected to the distal cytoplasm by septate desmosomes and a single electrodense 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 their accessory structures. Storage structures rich in reserve materials are also observed.

Key words: Cestode ultrastructure, *Bothriocephalus*, freshwater fish, cyprinids.

## INTRODUCTION

The present work is part of a general study on the life cycle and 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 the study of this endoparasite as in Mexico it infects several species of cyprinids and *Chirostoma estor* (white fish), an endemic species from Patzcuaro lake (Michoacan).

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 by 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*, *Macrocyclus*, *Mesocyclops*, *Tropocyclops* and *Diacyclops*. The parasitosis induced by *B. acheilognathi* is exclusively limited to freshwater fish, specially 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 & Esch, 1987; Alarcón-González, 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 & Price, 1986).

*B. acheilognathi* is notable among fish cestodes for its relative lack of specificity for the definitive host. It has been recovered from the intestines of more than 40 species of freshwater fish, most of which are cyprinids (Riggs & Esch, 1987).

This pseudophyllidean tapeworm passes from a free phase called *coracidium* to the infective form: the proceroid, which inhabits the intermediary host, usually a copepod or a cladoceran where they feed on hemolymph; and finally an adult phase in the intestine of some freshwater fish. They do not have the plerocercoid stage. Fish get infected when they feed in zooplankton organisms containing fully developed proceroids. In Mexico the intermediary host is usually a copepod of the genus *Mesocyclops*. It can not be transmitted to humans but it affects acuaculture farms, so there is an important economic impact.

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 decrease 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 (Alarcón-González, 1988).

## MATERIALS AND METHODS

Adult forms of the parasite were obtained from the final host, the grass carp *Ctenopharyngodon idellus*, originally imported from China and grown in the water ponds of the Centro Acuícola de Tezontepec, Hidalgo.

The location of worms within the guts 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 grass carps were transported from the water ponds 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 0.1 M phosphate buffer, pH 7.4 at 4° C and rinsed overnight in a 0.25 M sucrose solution in the same buffer at 4° C. Post-fixation was made in 2% osmium tetroxide in 0.1 M phosphate buffer pH 7.4 at 4° C 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 in a Reichert UmO3 ultramicrotome with a diamond knife (E.I. Dupont de Nemours), stained with uranyl acetate (Hayat, 1970) and lead citrate (Reynolds, 1963), and observed in a JEOL 100 B electron microscope operated at 60 kV. Thin sections ( $\mu\text{m}$ ) were obtained and stained with 2% toluidine blue-borate buffer for light microscopy.

For the scanning electron microscopy, after dehydration cestodes were dried to the critical point with carbon dioxide in a dryer Technics CPA-11. The critical point was maintained 5 minutes at 31°C and 1070 p.s.i. The cestode was fixed to the cylinder that holds the sample with a silver paint and covered with a 30 nm uniform film of carbon and gold with the JEOL model JFC-1100. Samples were observed with an scanning electron microscope JEOL JSM-35.

## 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 adult *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 (López-Jiménez, 1980; Granath *et al.*, 1983). The bottom of the bothria showed a special arrangement of microtriches and the neck was absent.

Dome shaped tumuli (viewed with the 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 these 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 & 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 far as the muscle underlying the submucosa, 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, haemorrhagic 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 syncytial 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 *B. 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 microtrich 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 parasite 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 sheat of about 0.28  $\mu\text{m}$  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 sheat of the basal lamina is interrupted, generating a

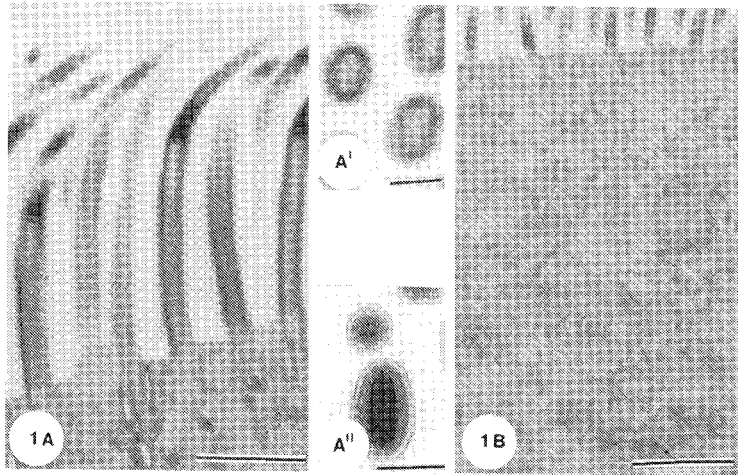


Fig. 1. A) Micrograph of microtriches of the tegument of *B. acheilognathi*. Two structural components are observed: the so called "absorptive zone" (a transverse section is shown in the lower inset) covered by glycocalix, and in the upper inset the electron dense "spine", curved towards the end of the parasite body is shown, 25 000X (Barr = 1.0  $\mu$ m).

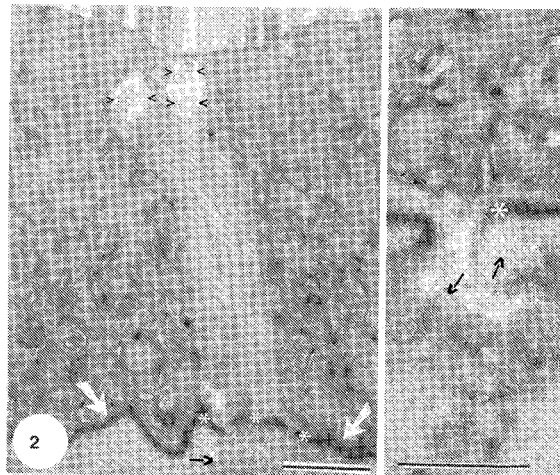


Fig. 2. Morphology of the distal cytoplasm of *B. acheilognathi*. The ultrastructure of the dense limiting sheat of the basal lamina (arrows), localized between the perinuclear cytoplasm and the tegument is shown. The distal cytoplasm is interrupted (\*) at several places with membrane limited conducts, inside them it is possible to observe empty vesicles (arrow heads). Numerous elastic fibers at the basement membrane (heavy arrows), resembles tendinous tissue for muscle attachment. Inset shows a magnification of an aperture of the dense lamina (cytoplasmic bridge). 20 000X (Barr = 1.0  $\mu$ m).

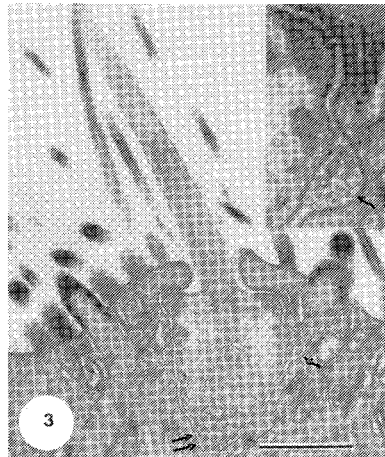


Fig. 3. Morphology of the ciliated sense organ (sensilla). The elements of this organ are similar to the cellular microvillus of a microtrich. The absence of the "spine" is apparent. The basal piece of the sensilla bound to the distal cytoplasm, shows similar ultrastructure to the synaptic junction of mammals (i.e. presence of microvesicles (arrows), see inset, 98 000X). The sensilla is isolated from the exterior by means of a circular septated desmosome. 42 000X (Barr = 0.5  $\mu$ m).

pore-like structure with clear connections between the interstitial space and the external surface of the parasite. An increased amount of elastic 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.

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 protrudes higher than the surrounding microtriches. The spine is absent and the tubule content is better defined than in the microtrich. This organ ends in a proximal bud, that contains numerous electron-lucent vesicles which provide connection with a nerve ending that also contains oval membrane-bound vesicles. The bulb of the sensilla is connected to the distal cytoplasm by septate desmosomes; under the desmosomes is a single electron-dense collar. The cilia extend 1.0 to 1.3  $\mu$ m 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 tu-



Fig. 4. Secretory receptor (tumulus). This figure shows the dense granules of the secretory process, synthesized in the "unicellular endocrine gland" localized deeply at the parenchima. These glands are profusely ramified towards the parasite tegument. We can observe short and wide microtriches around the tumulus. In the inset (42 000X, Barr = 0.25  $\mu$ m) a closer view of one of those microtriches is shown. 22 500X (Barr = 0.5  $\mu$ m).

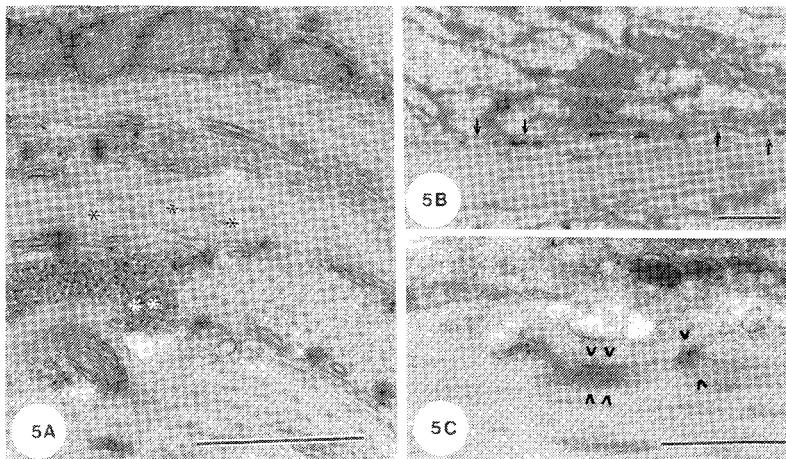


Fig. 5. Muscle tissue. In A can be seen the morphology of the muscle bundles with some common characteristics like mitochondria and sarcoplasmic reticulum. We can observe the membrane specialization (marked \* in the figure); and abundant glycogen (\*\*) (38 500X, Barr = 1.0  $\mu$ m). The nerve perikaryon (arrows), contains many dense core granules and some vesicles with variable density, some of them are empty (15 000X, Barr = 1.0  $\mu$ m). Also in this figure (C), another type of electric connection seems to be present. This structure apparently is another type of innervation, and resembles the striated muscle neuromuscular plate (arrow heads), 30 000X, Barr = 1.0  $\mu$ m.



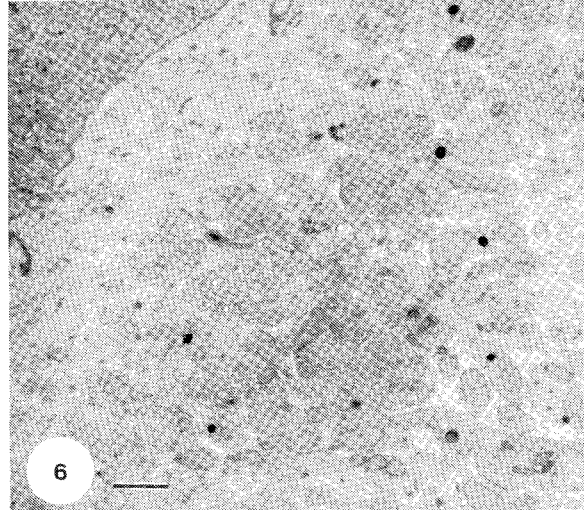


Fig. 6. Ultrastructure of the intermediate zone. This tissue is localized between distal and peripheral muscle bundles. In this zone, cytoplasmic projections of the rabdiform bodies of the synthesizing cell and perinuclear or proximal cytoplasm are numerous. In this projections glycogen and mitochondria 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. 5 000X (Barr = 0.2  $\mu$ m).

muli 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 cytoplasm is 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 membrane of the process, which may indicate fusion of both membranes during the evagination of the cytoplasmic projection through the distal cytoplasm; the rabdiform bodies are mixed with the dense secretion granules.

#### **The space between the basal lamina and the perinuclear cytoplasm**

Smooth muscle fibers arranged in layers (bundles) show two orientations (Fig. 5A), longitudinal ones directly in contact with the basal lamina and transversal ones in connection with the perinuclear cytoplasm. These muscles are composed of miofibrils, with thick (myosin) and thin filaments (actin).

The muscular innervation (Fig. 5B) cannot be described in the classical terms of a neuromuscular plate or electrical synapsis. Fine cytoplasmic projections from the unicellular glands that run parallel and in close contact with the muscular fi-

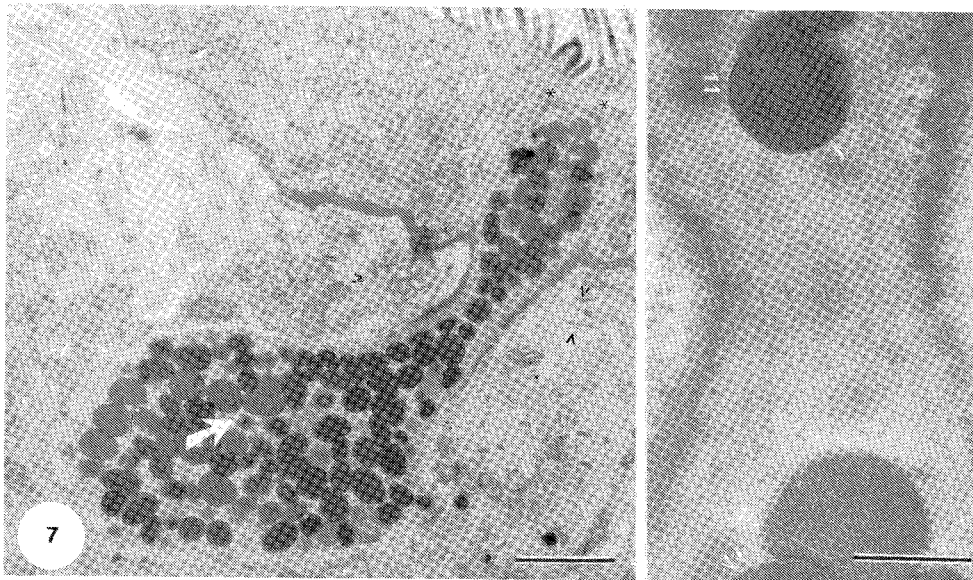


Fig. 7. Mucous like secretory cells. The "head" of the projection is limited by a desmosome (\*), secretory granules are defined by a clear unit membrane (arrow). The dense basal lamina adjacent to the distal cytoplasm is projected towards the distal cytoplasm, forming a "neck" which might regulate the number of granules passing through, and perhaps functioning like a sphincter together with the muscle fibers (arrow heads) that surround the structure. The inset (23 000X, Barr = 1.0  $\mu$ m) 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. 18 000X (Barr = 1.0  $\mu$ m).

bers are also observed (Fig. 5B). These cellular projections contain vesicles with different electron densities (or affinity to the staining chemicals). Another type of innervation observed consisted in a dense area between the muscular fiber 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 cytoplasmic projections or internuncial processes connecting distal and perinuclear cytoplasm. These projections contain in addition to the rabdiform bodies, mitochondria (0.97-1.22 $\mu$ m) 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 mem-

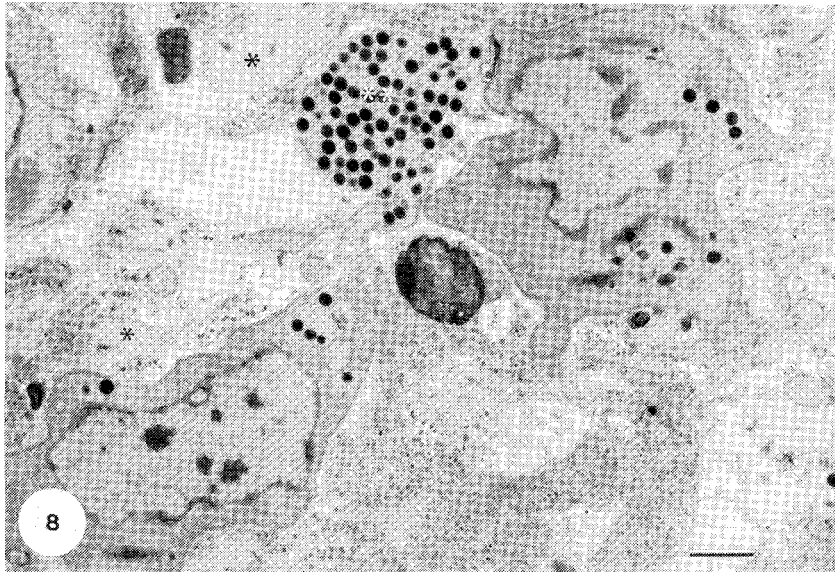


Fig. 8. Ultrastructure of the perinuclear cytoplasm. This zone of the parasite body presents a high cellular density, muscular fibers (\*) and cytoplasmic projections (\*\*) of cells localized at the center of the cestode body (i.e. unicellular endocrine glands). 12 000X (Barr = 1.0  $\mu$ m).

brane. 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.

### The region of the perinuclear cytoplasm

In the region of the perinuclear cytoplasm (Fig. 8), in addition to the unicellular glands and the mucous secretory cells, the main organs 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 contained 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

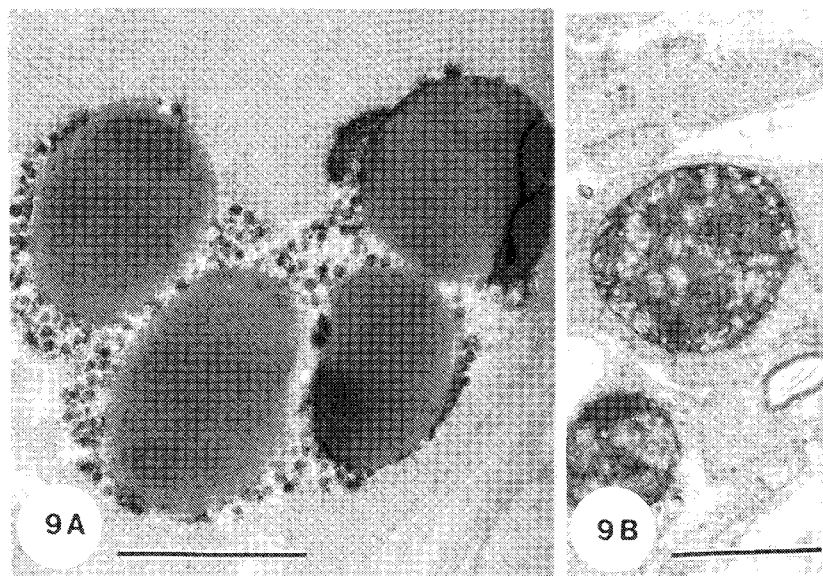


Fig: 9. Lipid storage bodies are shown at the cytoplasm of a cell with high content of free ribosomes and also a high content of granular endoplasmic reticulum. A: 23 000X (Barr = 1.0  $\mu$ m). In B the residual bodies are shown. B: 22000X (Barr = 1.0  $\mu$ m).

packed with characteristic granules and a number of empty vesicles. These cellular projections showed two types of orientation, one runs along the muscular fibers, and the other towards the distal cytoplasm as described above (Fig. 4 and 5).

The perinuclear cytoplasm is also profusely ramified. Ramifications connect these cells with different types of organs, glands and other cells found in the proglottid. 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: Siluridae, Poeciliidae, Cyprinidae, Acipenseridae and Atherinidae. In Mexico, it has been found mainly in the grass carp (*Ctenopharyngodon idellus*) (López-Jiménez, 1978, 1980), in *Carassius auratus* (Alarcón-González, 1988) and the white fish (*Chirostoma estor*), (Osorio-Sarabia *et al.*, 1986).

The fine structure of *B. acheilognathi* is similar in many aspects to that of other pseudophyllidean tapeworms. Among the species of *Bothriocephalus* found in

North America only *B. 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 *B. acheilognathi* in North America.

Tumuli are more or less uniformly distributed on the surface of the scolex of *B. acheilognathi*. These structures were first described by Boyce (1976) on adult *Eubothrium salvelini*, *Clestopothrium crassiceps* and *Bothriocephalus scorpii*. Later, Tedesco & Coggins (1980) made a TEM study of the tegument of adult *E. 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 & Coggins (1980).

The plerocercoids of *E. salvelini* do not possess tumuli, even though they are present on the tegument of adults of this species (Boyce, 1976); contrary, recently recruited immature *B. acheilognathi* as well as mature specimens, possess tumuli. Tedesco & 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 *B. acheilognathi* but they do not mention their possible function. Since *B. acheilognathi* does not have a plerocercoid stage any comparisons are difficult.

The tegument of *B. acheilognathi* was composed of a dense layer of microtriches, their morphology varies between the scolex and the proglottids. Generally, scolex microtriches are longer 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 thought to produce secretions for adhesion rather than for penetration. However, the granular nature of the scolex tissue and the vesicles in the distal parenchyma of some species of *Bothriocephalus* suggest 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 *B. acheilognathi*, who found them only in the tegument of proglottids, and is also different from those described by Morseth (1967) for *E. granulosus* and Jones (1975) for *B. 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 *B. acheilognathi* most closely resemble the sensilla described from the scolex of *Hymenolepis microstoma* (Webb & Davey, 1974); both are characterized by a rather long cilium and the absence of ciliary rootlets. Two electron-dense collars can be seen within the dendritic bulb of the sensilla of *H. micros-*

*toma*, but only one is present in the dendritic bulb of *B. acheilognathi*. Sensilla of *B. 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 *B. acheilognathi* present 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 been demonstrated in *Schistocephalus solidus* and *Ligula intestinalis* (Threadgold & 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 *B. acheilognathi*, so we assume they are involved in pinocytosis.

Muscles 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 *B. acheilognathi* have a prominent nucleus, a high ribosome content, actively secreting Golgi bodies mitochondria and glycogen. The state of the Golgi bodies and endoplasmic reticulum indicates a cell actively synthesizing proteins. The fate of these proteins is not known, although some authors think that the inclusions found within the tumuli are synthesized in these cells (Tedesco & Coggins, 1980). Other hypothesis is that some of these vesicles contain enzymes, or contribute to the formation of the glycocalyx (Smith, 1969).

#### ACKNOWLEDGEMENTS

We are indebted to QFB. Francisca Sandoval and to Dr. Silvia Antuna for expert technical assistance. This work was partially supported by a grant from CONACYT (PCNB-209) to A. Cárabez-Trejo.

#### LITERATURE CITED

- ALARCÓN GONZÁLEZ, C. 1988. Diagnosis of a helminthiasis in *Carassius carassius*, in a fish culture center at San Jose Atlangatepec, Tlaxcala, México. *Rev. Latinoam. Microbiol.* 30 (3): 297-298.
- AMIN, M.O. 1978. Intestinal helminths of some Nile fishes taken near Cairo, Egypt. *J. Parasitology* 64 (1): 93-101.
- ANDERSEN, K. 1975. Comparison of surface topography of three species of *Diphyllbothrium* (Cestoda: Pseudophyllidea) by scanning electron microscopy. *Int. J. Parasitol.* 5:293-300.
- BAUER, O.N. V.A. MUSSELIUS & V.A. STRELKOV, 1969. *In: Diseases of pond fish*. Israel Program for Scientific Translations, Jerusalem. pp. 115-122.
- BAUER, O.N. MUSSELIUS & J. STRELKOV, 1987. Parasites and diseases of fish in cages. *Adv. Fish. Sci.* 5-6: 121-125.

- BOYCE, N. 1976. A new organ in cestode surface ultrastructure. *Can. J. Zool.* 54:610-613.
- CHUBB, J.C. 1981. The chinese tapeworm *Bothriocephalus acheilognathi* Yamaguti, 1934 (synonym *B. gowkongensis* Yeh, 1955) in Britain. *Proceed. 2nd British Freshwater Fish Conference*. pp:40-51.
- GRANATH, W.O. JR., J.C. LEWIS & G.W. ESCH 1983. An ultrastructural examination of the scolex and tegument of *Bothriocephalus acheilognathi* (Cestoda: Pseudophyllidea). *Trans. Am. Microsc. Soc.* 102: 240-250.
- HAYAT, M.A. 1970. *Principles and techniques of electron microscopy. Biological Applications*. Vol. 1. Van Nostrand Reinhold Co., New York and Toronto. 274 p.
- HESS, E. 1980. Ultrastructural study of *Mesocestoides corti* Hoespli, 1925: tegument and parenchyma, *Z. Parasitenkd.* 61: 135-159.
- HOFFMAN, G.L. 1976. The Asian tapeworm, *Bothriocephalus gowkongensis* in the United States and research needs in fish parasitology. In: *Proc. Fish Farming Conference*.
- HOFFMAN, G.L. 1980. Asian tapeworm, *Bothriocephalus acheilognathi* in North America. *Fish Umwelt* 8: 69-75.
- HOLMES J. & P.W. PRICE. 1986. Communities of parasites. In: D. J. Anderson & Kikkawa (eds.) *Community ecology*. Blackwell Scientific Publishers, Oxford, pp. 187-213.
- HSIANG-HUA, L. & S. LEU-CHANG. 1956. Contributions to the biology and control of *Bothriocephalus gowkongensis* Yeh, a tapeworm parasitic in the young grass carp (*Ctenopharyngodon idellus* C. and V). *Acta Zool. Sin.*: 182-195.
- JONES, A. 1975. The morphology of *Bothriocephalus scorpii* (Muller) (Pseudophyllides, Bothriocephalidae) from littoral fish in Britain. *J. Helminth.* 49 (4): 251-261.
- KORTING, W. 1975. Larval development of *Bothriocephalus* sp. (Cestoda: Pseudophyllidea) from carp (*Cyprinus carpio* L.) in Germany. *J. Fish. Biol.* 7:727-733.
- KORTING, W. 1984. The economic importance of helminths parasitic in fresh water fish. *Fourth European Multicolloquium of Parasitology. Turkey. Abstracts*, p.251.
- KRITSCHER, E. 1986. Die fische des Neusiedlersees und ihre parasiten VI. Cestoidea. *Ann. Naturhist. Mus. Wien*, 90:183-192.
- LEONG T.S. 1986. Seasonal occurrence of metazoan parasites of *Puntius binotatus* in an irrigation canal, Pulau Pinang, Malaysia. *J. Fish Biol.* 28: 9-16.
- LÓPEZ-JIMÉNEZ, S. 1978. *Presencia en México de Bothriocephalus (Clestobothrium) acheilognathi en peces introducidos del lejano Oriente*. Depto. de Pesca, México.
- LÓPEZ-JIMÉNEZ, S. 1980. Céstodos de Peces I. *Bothriocephalus acheilognathi* (Cestoda: Bothriocephalidae). *Anales Inst. Biol. Univ. Nac. Autón. de México. Ser. Zool.* 51: 69-84.
- LUFT, J. 1961. Improvement in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* 9: 409-411.
- MARGOLIS, L., G. ESCH, J.C. HOLMES, A.M. KURIS & G. SCHAD. 1982. The use of ecological terms in parasitology. *J. Parasitol.* 68: 131-133.
- MOLNAR, K. 1977. On the synonymy of *Bothriocephalus acheilognathi* Yamaguti (1934). *Parasit. Hung.* 10:61-62.
- MORSETH, D.J. 1967. Observations on the fine structure of the nervous system of *Echinococcus granulosus*. *J. Parasitol.* 53 (3): 492-500.
- MUSSELIUS, V.A. 1962. Trad: The bothriocephalosis of carp fingerlings and its control. 11:78-88 (traducido del ruso).
- OSORIO-SARABIA, D., G. PONCE DE LEÓN & G. SALGADO MALDONADO. 1986. Helminths of fishes of Lago de Pátzcuaro, Michoacán. *Anales Inst. Biol. Univ. Nac. Autón. México. Ser. Zool.* 57(1):61-92.

- POOL, D. 1984. A scanning electron microscope study of the life cycle of *Bothriocephalus acheilognathi* Yamaguti (1934). *J. Fish Biol.* 25: 361-364.
- REYNOLDS, E.S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell. Biol.* 17: 208-212.
- RIGGS, M. & G. ESCH. 1987. The suprapopulation dynamics of *Bothriocephalus acheilognathi* in North Carolina reservoir: abundance, dispersion and prevalence. *J. Parasitol.*, 73(5): 877-892.
- RIGGS, M., A.D. LEMLY & G. ESCH. 1987. The growth biomass and fecundity of *Bothriocephalus acheilognathi* in a North Carolina cooling reservoir. *J. Parasitol.*, 73(5): 893-900.
- SCOTT, A.L. & J.M. GRIZZLE. 1977. Pathology of cyprinid Fish caused by *Bothriocephalus gowkongensis* (Cestoda: Pseudophyllidae). *Journal of Fish Disease* 2(1): 65-73.
- SMYTH, J.D. 1969. *The Physiology of Cestodes*. WH. Freeman and Co. San Francisco, USA. 279 p.
- SOPINSKA, A. 1985. Effects of physiological factors, stress and disease on hematologic parameters of carp, with a particular reference to the leukocyte patterns. 3. Changes in blood accompanying branchinecrosis and bothriocephalosis. *Acta Ichtyol. Pisc.* 15 (2): 141-170.
- TEDESCO, J. & J.R. COGGINS. 1980. Electron microscopy of the tumulus and origin of associated structures within the tegument of *Eubothrium salvelini* Schrank, 1790 (Cestoda: Pseudophyllidea). *Int. J. Parasitol.* 10:275-280.
- THREADGOLD, L & C.A. HOPKINS. 1981. *Schistocephalus solidus* and *Ligula intestinalis*: pinocytosis by the tegument. *Exp. Parasitol* 51: 444-456.
- WEBB, R. & K.G. DAVERY, 1974. Ciliated sensory receptors of the unactivated metacestodes of *Hymenolepis microstoma*. *Tissue Cell* 6: 587-598.