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ULTRASTRUCTURE OF THE METACESTODE *APLOPARAKSIS SHIGINI* BONDARENKO ET KONTRIMAVICHUS, 2006 (CESTODA: APLOPARAKSIDAE)

© 2021 N. A. Pospekhova[a,*], K. V. Regel[a]

[a]Institute of Biological Problems of the North,
Far Eastern Branch of the Russian Academy of Sciences,
Magadan, 685000 Russia
**e-mail*: *posna@ibpn.ru*

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The fine structure of the metacestode *Aploparaksis shigini* Bondarenko et Kontrimavichus, 2006 from the predatory leeches *Erpobdella octoculata* L. from the lakes of the Upper Kolyma River basin was studied for the first time. The cysticercoid is similar to the floricercus, i.e. has an open cellular exocyst and many processes at its base, however, it is distinguished by a long tail. The exocyst and all its outgrowths (including the caudal process) are covered with long and thick microvilli. Excretory canals of different diameter are noted in the exocyst, near the base of the caudal process. The structure of the endocyst is typical of hymenolepidid metacestodes; the glycocalyx has a characteristic reticular structure.

Keywords: metacestode, ultrastructure, floricercus, exocyst, excretory canals

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Cysticercoids *Aploparaksis shigini* represent one of three species of metacestodes found in the predatory pharyngeal leeches *Erpobdella octoculata* L. from the lakes of the Upper Kolyma basin (Regel, 2016). Small aploparaksid metacestodes were freely located in the fluid of lateral lacunae – the rudiments of the coelom, while the larger hymenolepidids from the genus *Kowalewskius* Yamaguti, 1959 were found in the thickness of the botryoid tissue of leeches (Regel, 2010). Following morphological features of *K. formosus* cysticercoids were noted: closeness to the modification of cyclocercus and the presence of long, thick microvilli on the tail appendage twining the cyst around (Regel, Pospekhova, 2019).

The structural features of the *A. shigini* metacestodes do not allow attributing them to any of the known modifications. The presence of a bowl-shaped outgrowth of the tail appendage, together with numerous lobes at the base of the endocyst, makes it similar to the floricercus (Bondarenko, Krasnoshchekov, 1978; Bondarenko, Kontrimavichus, 2006). However, the floricercus has a relatively short tail, whereas metacestodes from leech has a long tail, forming a single conglomerate with the caudal processes of other metacestodes (Regel, 2016). Similar structure of the caudal process was noted in metacestodes of the

"marine" species of aploparaksids, *Wardium cirrosa* (Krabbe, 1869) (Greben et al., 2019). The authors could not attribute that metacestode to any of the known modifications as well.

The aim of our research is to identify the characteristic features of the fine morphology of the cysticercoid *A. shigini*, which could be useful for specifying of the classification system of metacestodes, as well as for establishing the generic relations with other representatives of the family.

MATERIAL AND METHODS

The original material was obtained by dissection of leeches *Erpobdella octoculata* L. from the lakes of the Upper Kolyma basin. Metacestodes were fixed in a 2% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.2) at a temperature of about 4 °C. After fixation the material was postfixed in a 2 % OsO4 solution in a 0.2 M phosphate buffer (pH 7.2) for 12 hours, dehydrated, and embedded in an EPON-araldite mixture. During dehydration, the specimens were stained with a saturated uranyl acetate solution in 70 % ethanol for a night. Ultra-thin sections (90 nm), obtained on an LKB ultratome (Sweden), were viewed in JEM-1011 (JEOL, Japan) operating at 80 kV and JEM-1400Plus (JEOL, Japan) operating at 120 kV transmission electron microscopes.

RESULTS

The location of the exocyst and endocyst on the sections is close to that observed in living metacestodes, although preparation for electron microscopic examination leads to a change of the envelopes' form (fig. 1*A,* 1*B*). On the sections, the bowl-shaped profile of the exocyst is located around the endocyst, and the long and dense microvilli of the exocyst form a continuous zone around it (fig. 1*B,* 1*C*). The width of this zone sometimes exceeds 10 μm. At a distance of up to 2 μm from the tegument surface, the microvilli retain linear outlines (fig. 1*C*), while the ends of the microvilli bend, forming numerous profiles on the sections along the border periphery, which makes it difficult to measure the length of separate microvilli. The transverse sections of the microvilli allow watching the fine fibers that bind them into a single border.

Long (up to 100 μm) radial rays, discernible at the surface of the exocyst of some living metacestodes, can be microvilli that shrivel up when processing the material and turn into this border.

The exocyst tegument, containing microvilli, has a thin distal cytoplasm connected with the underlying cytons. They are distinguished by dense cytoplasm and looser karyoplasm with the large amount of heterochromatin (fig. 1*C*). The tegument of the inner surface of the exocyst, at the base of the endocyst, secretes dense material. The latter is accumulated in the surface cytoplasm, and rounded or oblong bodies are separated from it (fig. 1*D*, 1*E*).

Muscle cells with expanded channels of the granular endoplasmic reticulum were not found, although muscle fibers of a small section were noted in the subtegument.

Excretory canals of different diameters are located in the area adjacent to the base of the tail. It was not possible to trace the exact topography of the canals, however, larger canals are located at the base of the bowl-shaped outgrowth of the exocyst; small canals are located outside of large ones. The canal walls are formed by thin syncytium lined with typical round microvilli. In the lumen of the canals, dense bodies and myelin figures are occasionally observed (fig. 1*E*).

In the contact places of exocyst microvilli and the endocyst glycocalyx, the latter adheres to the microvilli and separates from the endocyst, leaving only an inner homogeneous glycocalyx layer on its surface (fig. 1*F*).

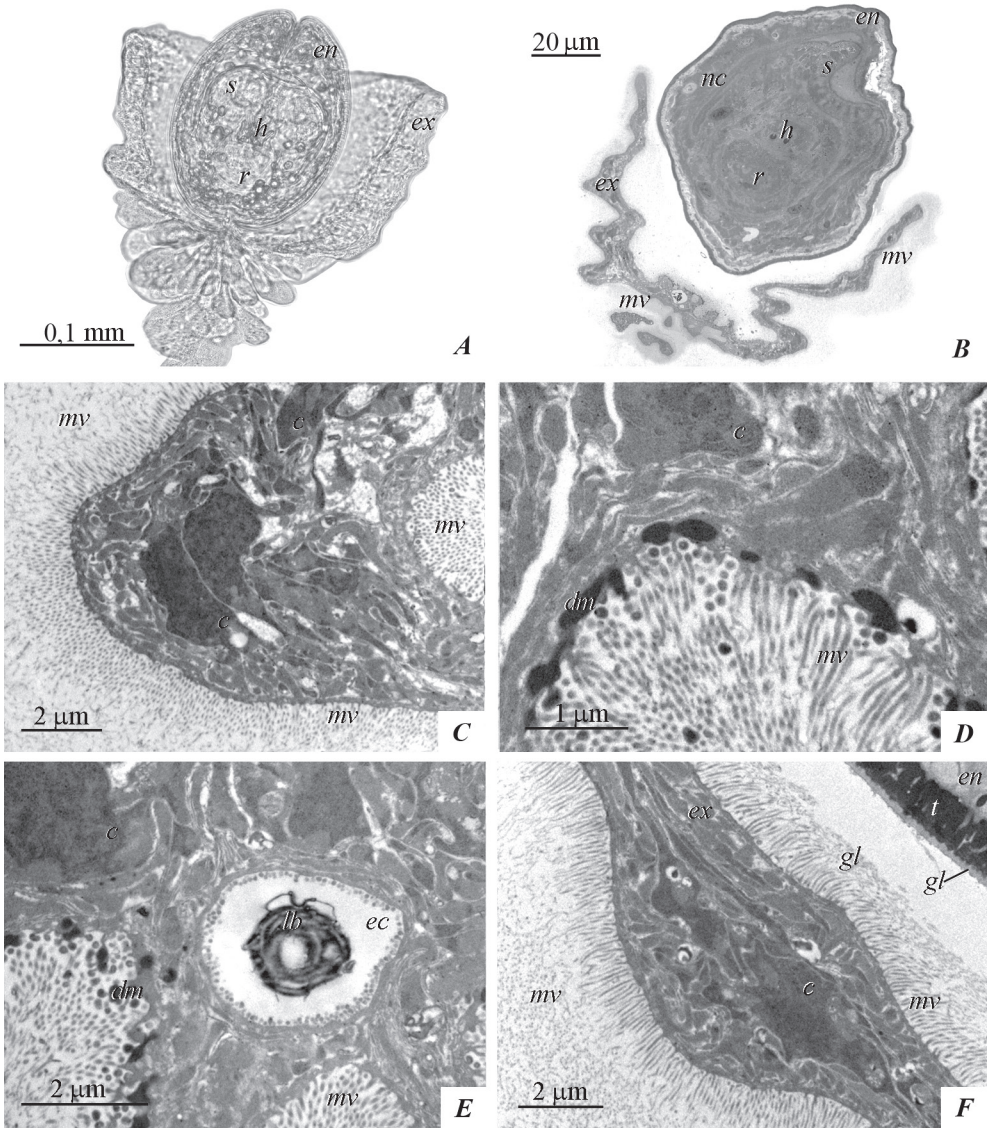

Figure 1. Metacestode of *Aploparaksis shigini*: exocyst.
A – living metacestode,
B – a thin section of the metacestode (tail process is outside the field of view),
C – microvilli of the exocyst tegument,
D – secretion of dense material from the inner surface of the exocyst,
E – excretory canal with a lamellar body,
F – adhesion of endocyst glycocalyx and exocyst microvilli. Abbreviations: *c* – cyton, *dm* – dense material, *ec* – excretory canal, *en* – endocyst, *ex* – exocyst, *gl* – glycocalyx, *h* – rostellar hook, *lb* – lamellar body, *mv* – microvilli, *nc* – neck, *r* – rostellum, *s* – sucker, *t* – distal cytoplasm of tegument.

The endocyst wall structure of *A. shigini* is shown in figs. 2*A*–2*C*. The endocyst tegument is covered with a tubular-fibrous glycocalyx up to 6-7 μm thick; its inner layer consists of a homogeneous material of about 200 nm thick (fig. 2*B*). The tubules form a reticular structure, interspersed with fine fibers and vesicles; there is usually a narrow light zone in the area of attachment of the outer part of the glycocalyx to the inner homogeneous layer (fig. 2*A*, 2*B*). The distal cytoplasm of the endocyst, about 1.5 μm thick, is filled with dense material with small cavities (fig. 2*A*–2*C*). Fibrous-muscular layers are located under the basal plate: external circular one (with the inclusion of muscle fibers of the same orientation) and internal longitudinal one with longitudinal muscle fibers. The deeper layer of cellular elements (muscle cells, excretory canals and cells whose processes form the pseudomyelin layer at the endocyst border) usually constitutes about ¼ of the endocyst wall, in rare cases – up to half of it (fig. 2*C*).

Large oval calcareous bodies, up to 8-9 μm in diameter, lie in the parietal part of the neck and at the base of the scolex, under the suckers and rostellar sac (figs1*B*, 2*F*). The neck and scolex are covered with microtriches, which are longer in the area of the suckers. In the tegument of the suckers, non-cilia sensory endings can be distinguished; among the muscles of the suckers, there are cells containing numerous light vesicles, about 100 nm in diameter. The endocyst cavity is filled with flaky material and vesicles. The retracted pyriform rostellum with hooks is surrounded behind by the rostellar sac with cords of cells without visible synthetic activity.

DISCUSSION

For the first time, conglomerates of metacestodes with intertwining caudal processes ("larvophores") were noted in the caudate diplocysts *Wardium fryei* Mayhew, 1925; the author suggested the possibility of asexual proliferation of metacestodes from bud-like outgrowths on the tail appendage (Bondarenko, 1997). The presence of long intertwined caudal processes with rounded thickenings was noted in *A. shigini* by Regel (2016), and subsequently in *W. cirrosa* by Greben et al. (2019), and in the latter case, the authors also suggested the possibility of the development of several cysticeroids from one oncosphere.

No evidence of asexual reproduction of *A. shigini* by budding was found in our material, although we do not exclude this possibility. The cellular exocyst of *A. shigini*, together with all its processes, is a modified tail appendage (cercomer according to Freeman, 1973; Gulyaev, 1989; Chervy, 2002; Greben et al., 2019 and other authors). In some cyclophyllids, it is the homologues of the tail appendage that contain poorly differentiated cells capable of forming new individuals, as noted, for example, in the multicercus *Mircia shigini* (Gulyaev et Konyaev, 2006) (Gulyaev, 1989; Regel, Pospekhova, 2012; Pospekhova, Regel, 2015).

The presence of a thick border of long microvilli is characteristic of the tail appendage and its homoloque in two examined (by electron microscopy) metacestodes from leeches *E. octoculata* (*K. formosus* and *A. shigini*), although they have different localization (tissue and cavity), belong to different families (Hymenolepididae and Aploparaksidae), are close to different morphological modifications (cyclocercus and floricercus) and parasitize in different definitive hosts (Laridae and Anatidae) (Regel, 2016). The only common feature of these metacestodes is the same intermediate host - the pharyngeal leech *E. octoculata*. Supposedly, long dense microvilli, which are a kind of frame for the glycocalyx of the tail appendage tegument, is a general adaptation of various modifications of cyclophyllids metacestodes in the body of pharyngeal leeches.

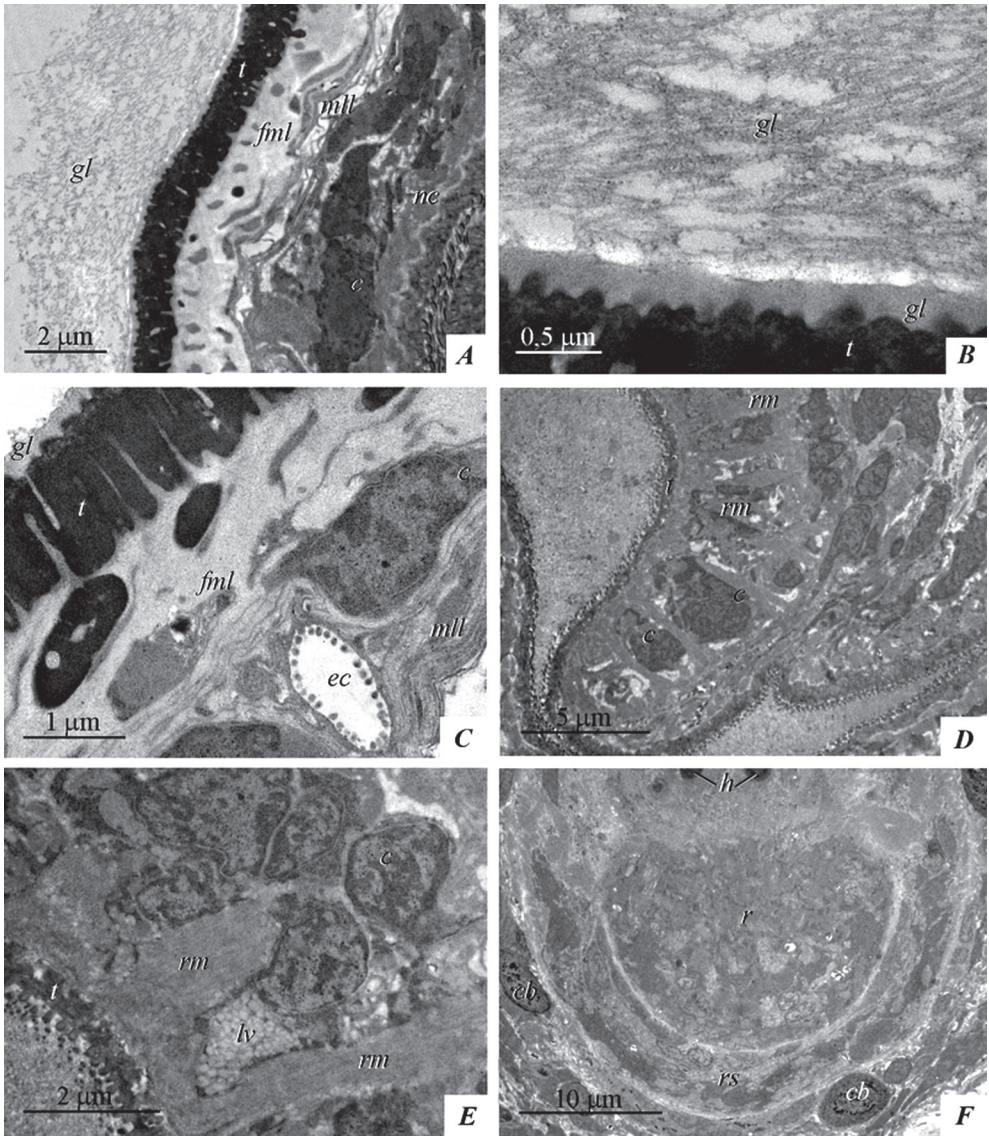

Figure 2. Metacestode of *Aploparaksis shigini*: endocyst and definitive part.
A – endocyst and parietal part of the neck,
B – endocyst glycocalyx,
C – endocyst wall,
D – sucker,
E – a cell with light vesicles in a sucker,
F – rostellum and rostellar sac.
Abbreviations: *c* – cyton, *cb* – calcereous body, *ec* – excretory canal, *en* – endocyst, *fml* – fibrous–muscular layer, *gl* – glycocalyx, *h* – rostellar hook, *lb* – lamellar body, *lv* – light vesicles, *mll* – myelin-like layer, *nc* – neck, *r* – rostellum, *rm* – radial muscles, *rs* – rostellar sac, *s* – sucker, *t* – distal cytoplasm of tegument.

A possible protective role of exocyst long microvilli has been noted for a typical diplocyst *Aploparaksis bulbocirrus* Deblock et Rausch, 1968: in the area of the exocyst outlet, the microvilli are denser and longer, comparing to those, on the lateral surface. They intertwine and form a "plug" (Nikishin, 2009). It is possible that this "plug" prevents the penetration of host immune cells into the exocyst cavity.

Studying the postembryonic development of aploparaksids at the light-microscopic level, the presence of excretory canals and cyrtocytes in the cellular exocyst of *A. birulai* floricercus, typical diplocysts *A. scolopacis*, and caudate diplocysts *W. friey* was noted (Bondarenko, Krasnoshchekov, 1978; Bondarenko, 1993, 1997). Our finding of excretory canals in the exocyst of *A. shigini* and the absence of similar data in ultrastructural studies of other cysticercoids of the Aploparaksidae family (Nikishin, Krasnoshchekov, 1979; Nikishin, 2009) may indicate only local functioning period the of the exocyst excretory system in postembryonic development, or be associated with specific structural features of a particular type of cestodes in the intermediate host.

The endocyst walls of the studied species have the same morphological features that are noted for the endocysts of hymenolepidids: the distal cytoplasm of the tegument is filled with dense material, the muscle fibers of the subtegumental muscles are embedded into fibrous layers of the same orientation, the wall of the endocyst is separated from the parietal part of the neck by the so-called pseudomyelin (myelin-like) layer (Caley, 1974). However, the endocyst glycocalyx of *A. shigini* is quite different from those previously described (see review by Nikishin, 2017). It also differs from the glycocalyx of another species of metacestodes from leeches, *K. formosus* (Regel, Pospekhova, 2019). In the latter case, the endocyst glycocalyx has a fibrous structure typical of hymenolepidids, which is denser near the tegument surface.

Analysis of the available literature and our observations show that the main role in the formation of metacestodes morphological polymorphism belongs to temporary larval structures, which undergo significant changes during the postembryonic development. This is especially evident in metacestodes of the Aploparaksidae family. Such features, as the degree of immersion of the endocyst into the exocyst, the shape of the exocyst, and the length of the tail process can vary depending on the stage of development, the intensity of invasion, and many other factors, which are still unknown.

An even more significant (in our opinion) difference in the postembryonic development of some aploparaksids is the presence of two invaginations (most cyclophyllids have only one), as well as the displacement of the primary lacuna into the tail appendage primordium (Gulyaev, 1977; Bondarenko, 1978; Bondarenko, Kontrimavichus, 2006; Nikishin, 2009; Pospekhova, 2017).

Considering these circumstances, as well as a significant prevalence of molecular genetic research methods in modern helminthology, the study of life cycles (Blasco-Costa, Poulin, 2017) and the morphological characteristics of various modifications of metacestodes is of particular importance, since it is obvious that to obtain a complete picture "… it is necessary to study all existing modifications of cestodes' larvae" (Mrazek, 1927).

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УЛЬТРАСТРУКТУРА МЕТАЦЕСТОДЫ *APLOPARAKSIS SHIGINI* BONDARENKO ET KONTRIMAVICHUS, 2006 (CESTODA: APLOPARAKSIDAE)

Н. А. Поспехова, К. В. Регель

Ключевые слова: метацестода, ультраструктура, экзоциста, флорицерк

РЕЗЮМЕ

Впервые изучено тонкое строение метацестоды *Aploparaksis shigini* Bondarenko et Kontrimavichus, 2006 от глоточных пиявок *Erpobdella octoculata* L. из озёр бассейна Верхней Колымы. Цистицеркоид схож с флорицерком, т.е. имеет незамкнутую клеточную экзоцисту и множество отростков у её основания, однако отличается длинным хвостом. Экзоциста и все её выросты (включая хвостовой отросток) покрыты длинными, густыми микроворсинками. Экскреторные каналы разного диаметра отмечены в экзоцисте, вблизи основания хвостового отростка. Структура эндоцисты типична для гименолепидидных метацестод, гликокаликс имеет характерное сетчатое строение.