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MORPHOLOGY AND ULTRASTRUCTURE OF TWO SCHISTOTAENIID CYSTICERCOIDS (CESTODA: CYCLOPHYLLIDEA) FROM THE HAEMOCOELE OF THE DRAGONFLY LARVAE[1]

© N. A. Pospekhova,* K. V. Regel

Institute of Biological Problems of the North FEB RAS
Magadan, 685000
* E-mail: posna@ibpn.ru
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Two cysticercoids, belonging to ascocercus type, namely euascocercus and multicercus, were found in haemocoele of dragonfly larvae of the genus *Aeshna* from the lakes of the Magadan Province. The cysticercoid of *Schistotaenia srivastavai* Raush, 1970 (euascocercus) is formed of the outer (exocyst) and inner (endocyst) envelopes, containing the scolex and larval strobila. The outer and inner surfaces of the exocyst are represented by the tegument covered with microvilli. The microvilli of the outer tegument are restricted by the surface layer, consisting of granular and fibrillar material, and possess different structures at different stages of post-embryonic development. The multicercus of *Mircia shigini* (Konyaev et Gulyaev, 2006) is able to multiply asexually by the endogenous budding. The daughters' individuals are formed in the envelope of the multicercus that represents the tegument bearing microvilli. These microvilli are also restricted by the surface layer. The morphology and development of each individual cysticercoid of the multicercus is similar to those of euascocercus. The production of a great amount of cysticercoids, and the presence of the surface layer resembling the laminated layer of *Echinococcus*, relates multicercus to hydatid cysts.

Key words: Cyclophyllidea, Schistotaeniidae, metacestode, ascocercus, euascocercus, multicercus, exocyst, endocyst, laminated layer, surface layer, ultrastructure.

[1] Dedicated to the memory of Florence Gwendolen Rees, President of the British Society for Parasitology (1972—1974).

МОРФОЛОГИЯ И УЛЬТРАСТРУКТУРА ДВУХ ШИСТОТЕНИИДНЫХ ЦИСТИЦЕРКОИДОВ (CESTODA: CYCLOPHYLLIDEA) ИЗ ГЕМОЦЕЛИ ЛИЧИНОК СТРЕКОЗ

© Н. А. Поспехова,* К. В. Регель

Институт биологических проблем Севера
Магадан, 685000
* E-mail: posna@ibpn.ru
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В гемоцели личинок стрекоз рода *Aeshna* из озер Магаданской обл. были обнаружены два цистицеркоида, относящиеся к типу аскоцерк — эуаскоцерк и мультицерк. Цистицеркоид *Schistotaenia srivastavai* Raush, 1970 (эуаскоцерк) состоит из внешней (экзоциста) и внутренней (эндоциста) оболочек, содержащих сколекс и личиночную стробилу. Внешняя и внутренняя поверхности экзоцисты представляют собой тегумент, покрытый микроворсинками. Микроворсинки наружного тегумента ограничены поверхностным слоем, состоящим из гранулярного и фибриллярного материала, и имеющим разную структуру на разных этапах постэмбрионального развития. Мультицерк *Mircia shigini* (Konyaev et Gulyaev, 2006) обладает способностью к бесполому размножению путём эндогенного почкования. Источником дочерних особей является оболочка мультицерка, которая представляет собой тегумент, покрытый микроворсинками. Они также ограничены поверхностным слоем. Строение и развитие каждого отдельного цистицеркоида мультицерка сходно с таковыми эуаскоцерка. Продукция большого количества цистицеркоидов и наличие поверхностного слоя, напоминающего laminated layer эхинококков, сближает мультицерк с гидатидной цистой.

Ключевые слова: Cyclophyllidea, Schistotaeniidae, метацестода, аскоцерк, эуаскоцерк, мультицерк, экзоциста, эндоциста, laminated layer, поверхностный слой, ультраструктура.

Dragonfly larvae (Odonata) serve as intermediate hosts for the majority of cestodes of the family Schistotaeniidae Johri, 1959, specific parasites of the grebes (Podicipediformes) (Linstow, 1892; Lühe, 1910; Mrázek, 1927; Yamaguty, 1942; Golikova, 1960; Tkachev, 1969; Rees, 1973a; Boertje, 1975; Kukashev, 1985, 1989; Gulyaev, 1989; Gulyaev et al., 2010; Regel, Pospekhova, 2012). All of the above mentioned authors noted the singularity of metacestodes from the dragonfly larvae, and only reviews referred them to the known types of cysticercoids (Ryzhikov, Tolkatcheva, 1981; Storer, 2000; Chervy, 2002).

Gulyaev (1989) was the first to suggest the term 'ascocercus' to note the independent morphological type of cysticercoids from the subfamily Schistotaeniinae Johri, 1959. According to this author, the main features of ascocercus are the following: early separation of the cystoscolex primordium (1), lacking the primary lacuna (2), and its development in the closed cavity of the exocyst (3), which possesses the developed musculature (4). Besides, the anterior closing valve of the endocyst is opened, and scolex freely protrudes into the exocyst cavity (5) (Gulyaev, 1989).

Another morphological type of cysticercoids from dragonfly larvae, the multicercus, was described for polycephalic schistotaeniid metacestodes *Joyeuxilepis uralensis* Gulyaev, 1989 (Gulyaev, 1989). Later *J. uralensis* was included

into the genus *Mircia* Konyaev et Gulyaev, 2006 with the type species *M. shigini* (Konyaev, Gulyaev, 2006). In the Magadan Province of Russia, multicerci of *M. shigini* are mass parasites of dragonfly larvae, and the development of filial individuals of the multicercus is virtually identical to that of the ascocercus (Gulyaev et al., 2010; Regel, Pospekhova, 2012, 2014).

The third type of cysticercoids from the dragonfly larvae was named the megalocercus (Regel et al., 2013). The latter was described for the unusually large metacestode *Dioecocestus asper* (Mehlis, 1831) (Dioecocestidae), which develops according to the ascocercus type. Cysticercoids from dragonfly larvae of the similar size and morphology were described previously as strobilocercoids (Boertje, 1975).

Considering the similarity of postembryonic development and species biology, different modifications of metacestodes of Schistotaeniidae and Dioecocestidae were assumed to be integrated into a single morpho-ecological group of ascocerci (Regel et al., 2013). The use of the term 'ascocercus' as the nominal name for the group of larvocysts determined the change of the name of the ascocercus of Schistotaeniidae into the 'euascocercus' (Regel et al., 2013).

In the present work we present the data on the morphology and ultrastructure of two species of ascocerci — the euascocercus, with the example of *Schistotaenia srivastavai*, and the multicercus (the genus *Mircia*). The biology and ultrastructure of the megalocercus was described in our previous works (Regel et al., 2013; Pospekhova et al., 2014).

The study of the postembryonic development and fine structure of euascocercus of *S. srivastavai* (Regel, Pospekhova, 2012, 2014) had demonstrated that they are rather similar to those described for cysticercoids *Tatria* s.l. (Rees, 1973a, b). Therefore, we give only the description of the metamere stage and completely developed euascocercus, focusing on the structure of polycephalic metacestodes (multicerci) from the genus *Mircia*, which have not been studied yet.

MATERIALS AND METHODS

The original material was obtained by dissection of dragonfly larvae (Anizoptera) of the genus *Aeshna* from lakes of the Upper Kolyma basin. Metacestodes were fixed in a 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 % OsO_4 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 Libra-120 (Carl Zeiss, Germany) operating at 120 kV transmission electron microscopes. Semi-thin sections (1—2 μm) were stained with methylene blue using the method of Morgenstern (1969), and analysed and photographed using an Olympus CX41 (Olympus Corporation, Japan) optical microscope with an Olympus E-420 digital camera.

RESULTS

Schistotaenia srivastavai Rausch, 1970. At the metamere stage, the euascocercus of *S. srivastavai* consists of the exocyst, in which the elongated primordium of the cystoscolex, still attached to the exocyst, is located (fig. 1, *a*, see inset). The latter is oval, and it is attached to the intestine of the dragonfly larvae by a short pedicle.

The exocyst of the euascocercus posesses the cellular structure (fig. 1, *b*). Its outer and inner surfaces are formed of the tegument, covered with microvilli (fig. 1, *c*). Microvilli of the outer tegument are limited by the surface layer, and their apical ends are immersed in the latter (fig. 1, *d*). The surface layer, 100—800 nm thick, consists of granular material, vesicles and fibrils. The densest granular material is found on the surface of the layer, vesicles are scattered throughout the thickness, and free fibrils are arranged chaotically over the thickness and between microvilli. Bundles of fibrils lay in the thickness of the layer at different angles; near the outer surface they are oriented longitudinally.

Elements of the host respiratory system can be observed on the outer surface of the exocyst. Branches of visceral tracheae supplying the intestine run as far as the surface of the ascocercus and ramify, forming large number of small tracheoles tightly adjusting the surface layer (fig. 1, *e*). The maximum density of fibril bundles is observed in the surface layer of the exocyst (up to formation of a solid layer) near the tracheole area. The outer surface of tracheal cells is covered with a thin layer of granular material similar to that located in the outer side of the surface layer.

The dense distal cytoplasm of the outer tegument, 3—5 μm thick, is rich in vesicles and large vacuoles, containing vesicles and fibrils (fig. 1, *f*). It is underlied by the basal matrix, 200—300 nm, and circular and longitudinal muscle fibres. Subtegumental cells (cytons) also possess dense cytoplasm, nuclei with large nucleoli, and long processes. Cytons contain multiple twisted channels of the rough endoplasmic reticulum (RER). Muscle cells with extended RER cisternae connected with subtegumental musculature by processes are situated between the cytons (fig. 1, *g*). The distal cytoplasm and cytoplasm of cytons of inner tegument possess lower density in comparison with the tegument (fig. 1, *h*). The basal matrix, as thick as 500 nm, is located close to the circular subtegumental musculature. Longitudinal muscle fibres are situated at a distance of about 10 μm from each other. Muscle cells and small dense cells with a rounded outline, which contain isolated mitochondria, are situated among the cytons (fig. 1, *c*). Microvilli of the inner tegument are arranged sparsely, frequently branching in their basal part, and are of different lengths (fig. 1, *c*, *h*).

At the stage of the metamere, the primordium of the cystoscolex represents a dense elongated mass of poorly differentiated cells (fig. 1, a, b). The surface of the primordium is covered with the tegument with sparse short microvilli (fig. 1, *i*). Its distal cytoplasm, 200—600 nm thick, is separated from the subtegumental musculature and subadjacent cytons of the tegument with a fine basal matrix.

Completely developed euascocercus of *S. srivastavai* consists of the exocyst and endocyst with the enclosed definitive part (fig. 2, *a*, see inset). The exocyst is attached to the wall of the dragonfly larva midgut by a fine pedicle.

After fixation, the metacestode becomes compact, envelopes become folded, the scolex is retracted into the endocyst, the anterior part of the rostellum turns

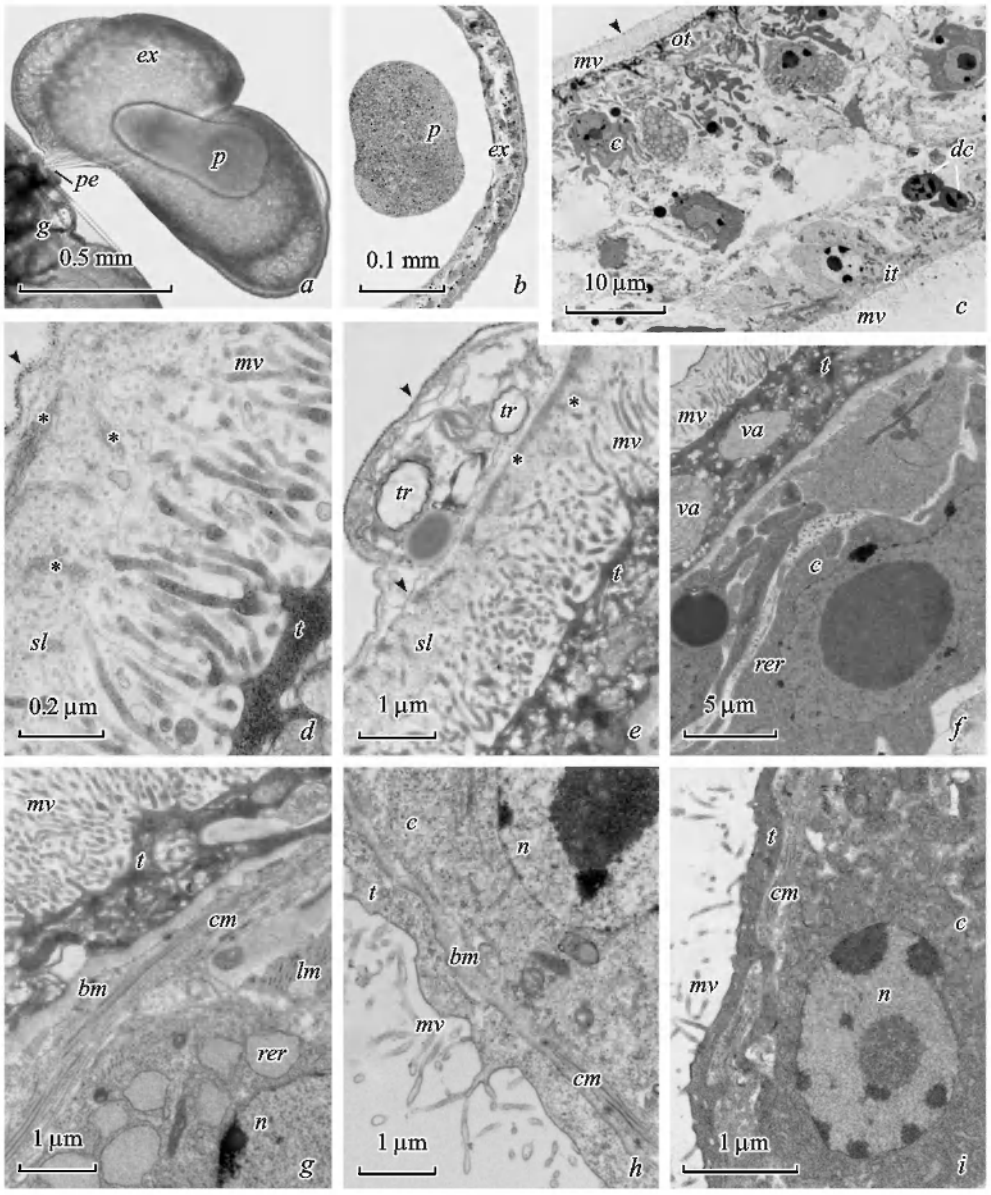

Fig. 1. Euascocercus of *Schistotaenia srivastavai*, metamere stage.

a — living metacestode, *b* — semi-thin transverse section, *c* — electron micrograph of exocyst with surface layer (arrowhead), *d* — surface layer of exocyst with dense granular material (arrowhead), *e* — tracheal cell on the surface layer, *f* — outer tegument of exocyst, *g* — muscle cell, *h* — inner tegument of exocyst, *i* — detail of cystoscolex' primordium. *bm* — basal matrix, *c* — cyton, *cm* — circular muscle, *dc* — dark cell, *ex* — exocyst, *g* — host gut, *it* — inner tegument, *lm* — longitudinal muscle, *mv* — microvilli, *n* — nucleus, *ot* — outer tegument, *p* — primordium of cystoscolex, *pe* — pedicle, *rer* — rough endoplasmic reticulum, *sl* — surface layer, *t* — distal cytoplasm of tegument, *tr* — trachea and tracheole, *va* — vacuole, asterisks — fibrillar bundles.

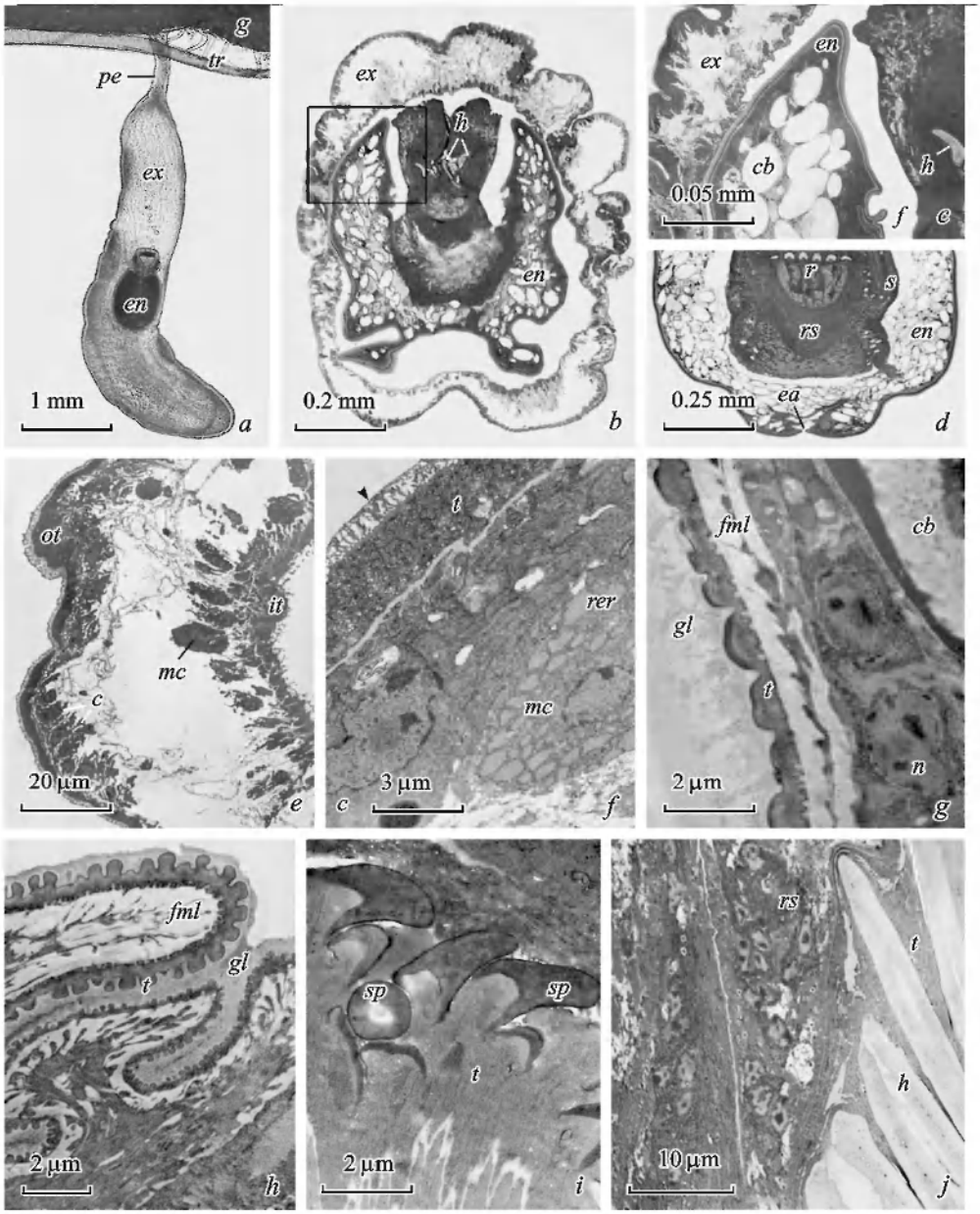

Fig. 2. Fully developed euascocercus of *Schistotaenia srivastavai* (*b*, *c*, *d* — semi-thin sections; *e*—*j* — electron micrographs).

a — living cysticercoid, *b* — longitudinal section, *c* — enlargement of *b*, *d* — endocyst with excretory atrium, *e* — exocyst, *f* — outer tegument of exocyst, *g* — endocyst wall, *h* — change of endocyst tegument in a fold area, *i* — tegument of rostellum, *j* — detail of retracted rostellum in rostellar sac. *bm* — basal matrix, *c* — cyton, *cb* — calcereous body, *cm* — circular muscle, *ea* — excretory atrium, *en* — endocyst, *ex* — exocyst, *f* — fold, *fml* — fibrous-muscle layer, *g* — host gut, *gl* — glycocalyx, *h* — rostellar hook, *it* — inner tegument, *mc* — muscle cell, *n* — nucleus, *ot* — outer tegument, *pe* — pedicle, *r* — rostellum, *rer* — rough endoplasmic tericulum, *rs* — rostellar sac, *s* — sucker, *sp* — spinule, *t* — distal cytoplasm of tegument, *tr* — trachea and tracheole, arrowhead — surface layer.

inside, and the blades of the hooks point forward (fig. 2, *b*, *c*). In semi-thin sections, the most evident details of cysticercoids are represented by the thick folded exocyst and multiple calcareous corpuscles of endocyst walls (fig. 2, *b*, *c*, *d*). Microvilli of the outer exocyst's tegument are limited to a thin 100 nm thick layer of granular material (fig. 2, *e*, *f*). Distal cytoplasm, 0.5—3 μm thick, contains multiple mitochondria and pale vesicles formed of cytons of the tegument. Under the basal matrix (200 μm thick), sparse muscle fibrils, 0.6—0.8 μm in diameter, are located. The microvillar distal cytoplasm of the inner tegument is about 1 μm thick and is similar in its width to the subadjacent basal matrix. Circular and longitudinal muscle fibres, up to 2 μm in diameter, are located deeper.

Cytons of the outer and inner tegument form parietal layers, whereas separate cells with widened RER cisternae and multiple appendages with no visible content are located in the medullar zone (fig. 2, *e*).

The endocyst of *S. srivastavai* is covered with a 4 μm thick glycocalyx (fig. 2, *g*). The thick of the distal cytoplasm of the tegument never exceeds 1.5 μm. The basal matrix is indistinct and fibrous layers, including circular and longitudinal muscles, are about 3 μm thick. Immediately under these structures, cytons of the tegument of the endocyst, muscle cells with widened cisternae of RER, and excretory channels and flame cells are located. This cellular layer is compressed by multiple calcareous corpuscles at different stages of formation. In sections running through the posterior pole of the endocyst, an excretory atrium with excretory pore is visible (fig. 2, *d*).

The anterior half of the inner surface of the endocyst is lined with the same tegument on the outside. Then, a folded area where the distal cytoplasm of the tegument gets thinner is situated (fig. 2, *c*, *h*). Posteriorly to foldings, the distal cytoplasm of the tegument possesses microvilli. More deeply, microtriches appear between the microvilli that become bigger. A short strobila protrudes from the bottom part of the endocyst covered with the tegument with microtrichiae, typical of the anterior part. The longest microtriches with the apical ends pointed backwards are located on the sucker surface. The tegument of the retracted rostellum under rostellar hooks is covered with small spinules, up to 5 μm long (fig. 2, *i*). In cross sections, rostellar hooks demonstrate a denser outer layer and less dense core (fig. 2, *j*).

The muscle walls of the rostellum and rostellar sac consist of two layers: the inner circular and outer longitudinal ones. The front part of the rostellum is crossed by retractor muscles of rostellar hooks running longitudinally from the root of the hooks to rostellum walls. Tegumental cytons of the rostellum and separate neurons are located between muscles. The rostellar sac also contains tegumental cytons that produce rounded granules coming to the distal cytoplasm of the tegument. Nerve cells, surrounding islets of the neuropile, were found at the base of the rostellar sac and the scolex under the suckers.

Mircia shigini Konyaev et Gulyaev, 2006. The multicercus is represented by accumulation of separate cysticercoids under the common envelope, the source of their formation (fig. 3, *a*, see inset). The multicercus can change its shape and size depending on the developmental stage: young multicerci are rounded and small in diameter, while mature multicerci are significantly enbranched, reaching 5 sm in length. Round buds that give separate cysticercoids are localized in the envelope of young multicerci (fig. 3, *b*). With the development of the multicercus, cystoscolex' primordia separate inside and fill the cavity of multicercus.

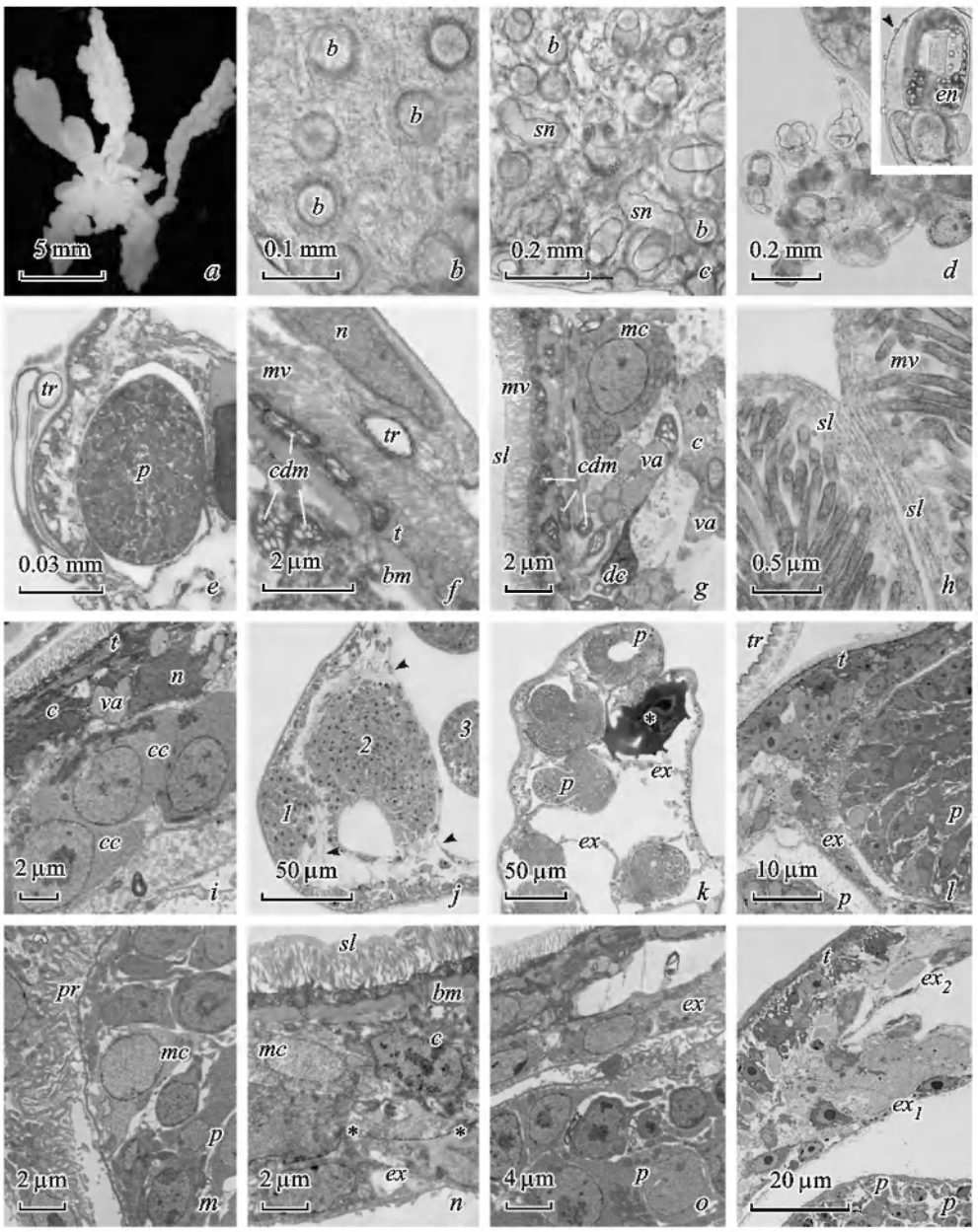

Fig. 3. Multicercus of *Mircia shigini*.

a — living developed multicercus, *b*, *c* — different developing stages of multicercus, *d* — releasing of developed individuals (insert — exocyst's cell bodies, arrowhead), *e* — tracheole on the multicercus surface, *f* — tracheal cell on the tegument, *g* — muscle cell of outer tegument, *h* — surface layer of the developed multicercus, *i* — clear cells near the tegument cytons, *j* — formation stages of cystoscolex primordium, separated by exocyst (arrowheads), *k* — multicercus with one developed cysticercoid, surrounded by dense fluid (asterisk), *l* — separation of primordium and exocyst, *m* — enlargement of *l*, *n* — exocyst before separation (asterisks — condensed cellular membranes), *o* — separation of primordium, exocyst and tegument, *p* — formation of the first (ex_1) and second (ex_2) exocysts of developing primordia. *b* — bud, *bm* — basal matrux, *c* — cyton, *cc* — clear cell, *cdm* — condensed mitochondria, *dc* — dark cell, *en* — endocyst, *ex* — exocyst, *mc* — muscle cell, *mv* — microvilli, *n* — nucleus, *p* — primordium of cystoscolex, *sl* — surface layer, *sn* — scolexogenesis stage, *t* — distal cytoplasm of tegument, *tr* — trachea and tracheole, *va* — vacuole.

The characteristics of cysticercoid stages are traced in the development of each daughter individual: from early (including metameres), to the stage of scolexogenesis (fig. 3, *c*), and later to completely developed cysticercoids. If the common envelope of the multicercus is damaged, separate cysticercoids are released to the external environment, each in their own one-layer envelope making them look an oval formation (fig. 3, *d*).

One of the more prominent and frequent peculiarities of multicerci is their intimate contact with the tracheal system of the host — dragonfly larva. During dissection of the abdominal region of the naiad, many multicerci appear to be attached to visceral trachea with one branch connected to the surface of the multicercus. The dense contact of tracheal cells with the multicercus is not lost during fixation and embedding (fig. 3, *e*). An ultrastructural study of the contact zone showed the location of the tracheole lumen to be situated maximally close to the multicercus surface, driving back the nuclei of the tracheal cells to the periphery (fig. 3, *f*). In the cytoplasm of the multicercus envelope, close to the adjacent tracheoles, a great number of large (up to 2 μm length) mitochondria with the dense matrix and expanded cristae (condensed mitochondria) are observed (fig. 3, *f*, *g*); on the sites distant from the contact zone, mitochondria possess their usual structure.

The multicercus envelope represents the tegument, and consists of the dense distal cytoplasm, covered with microvilli, subadjacent basal matrix and submerged cytons, connected to the distal cytoplasm by short processes. The distal cytoplasm of the tegument, 0.5—0.8 μm thick, contains large number of vesicles coming from tegumental cytons. Vesicles are produced in the Golgi apparatus and can merge, forming vacuoles (fig. 3, *g*). Muscle cells, connected with the subtegumental musculature, and small dark rounded cells with one to two processes are located among the cytons (fig. 3, *g*).

Microvilli, 1—2 μm long, cover the envelope. They are restricted by the surface layer of granular material, where apical ends of microvilli are submerged (fig. 3, *g*). Vesicles and fibrils are regularly noted in the thickness of the layer. The surface layer of developed multicerci, 200—400 nm thick, mainly consists of fibrils arranged in four to five plates (fig. 3, *h*).

Tegumental cytons of multicerci and muscle cells, integrated among them, are usually located in a line. However, this unicellular layer possesses multiple thickenings. The smallest thickenings are represented by groups from 2—3 light cells, situated deeper than the tegumental cytons of the multicercus (fig. 3, *i*). These cells contain a large rounded nucleus with traces of heterochromatin and few mitochondria. Cytons, lying close to light cells, have large angular nuclei with a low content of heterochromatin, surrounded by the narrow band of dense cytoplasm. Their processes contain oval vacuoles filled with fibrillar material (fig. 3, *g*, *i*).

Larger multicellular thickenings of multicercus tegument (buds) are rounded. The next stage of the development of separate cysticercoids is submerging of the cellular mass of the bud into the multicercus cavity with the simultaneous initiation of differentiation of the cystoscolex primordium (fig. 3, *j*, *k*). At this stage, the cellular mass is already separated from the neighbouring cellular masses by a thin layer — the forming exocyst. At the stage of early scolexogenesis, the cystoscolex primordium separates from the exocyst. One gets an impression that multiple thin processes tear off, and after separation of the exocyst they

look like short microvilli on the inner surface of the exocyst and the outer surface of the primordium (fig. 3, *l*, *m*). At this stage the exocyst is an integral part of the multicercus tegument and lateral surfaces of the neighbouring cysticercoids' exocysts. Condensation of membranes in the cellular layer of the tegument shows the place of future separation of the exocyst (fig. 3, *n*). After separation of the exocyst the cellular layer of the multicercus tegument returns to its initial thickness (fig. 3, *o*). However, most frequently separation of a single exocyst from the multicercus tegument is accompanied by formation of the exocyst of the next-developing cysticercoids (fig. 3, *o*, *p*).

Separated exocysts represent the tegument with an underdeveloped distal cytoplasm, covered with sparse, short microvilli. They are pointed inwards, to the surface of the cystoscolex primordium. Under the distal cytoplasm of the exocyst, which is 100—300 nm thick, the thinner basal matrix, loose subtegumental musculature (0.5—1 μm in diameter), cytons and muscle cells with widened RER cisternae, are located (fig. 4, *a*, *b*, *c*, see inset). Exocysts of cysticercoids, submerged into the cavity of the multicercus, are in contact with each other, and the same cell (cyton or muscle cell) can be connected to both distal cytoplasms (fig. 4, *a*, *b*).

The primordium of the cystoscolex during separation from the exocyst forms a tegument with a fine distal cytoplasm (fig. 3, *m*, *o*, *p*). At this period, separate muscle cells (fig. 3, *m*) and flame cells (fig. 4, *d*) are formed in the cellular mass, constituting the primordium. Subsequent stages of cysticercoids' development follow the general plan, including differentiation of the anterior end into the scolex and larval strobila, and the posterior end, into the endocyst. As the cysticercoids develop, the exocyst cavity is filled with liquid. In the semi-thin sections of young cysticercoids stained with methilene blue, this liquid looks pale blue; developed cysticercoids are surrounded by liquid of a dark blue color (fig. 3, *k*). In electron micrographs the liquid in the exocyst cavity looks like more or less dense accumulation of granular material and vesicles (fig. 4, *b*).

Developed cysticercoids move freely into the exocyst cavity that, however, can be deformed significantly by neighbouring cysticercoids. Oval outlines of the exocyst can be seen only under a relatively free distribution of cysticercoids in the cavity of multicercus (fig. 3, *c*), or during the release of cysticercoids into the external environment (fig. 3, *d*). In the latter case, cellular bodies of the exocyst tegument are directed outwards and are clearly defined on the surface as rounded projections (fig. 3, *d*).

The endocyst of the cysticercoid possesses the unlocked anterior valve, allowing scolex to be retracted or protruded into the exocyst cavity (fig. 4, *e*). The endocyst wall consists of the tegument, covered with double-layered reticular glycocalyx up to 3.5 μm thick (fig. 4, *e*). The dense distal cytoplasm of the tegument is 0.2—1.0 μm thick. A thin basal matrix and fibro-muscular layer, consisting of circular and longitudinal fibres, enclosing circular and longitudinal subtegumental musculature, underlies this cytoplasm. Tegumental cytons, muscle cells, and flame cells are situated deeper (fig. 4, *f*). The zone of gradual change of the tegument of the outer surface into the tegument with microvilli is located in the area of folds, which protrude into the endocyst cavity (fig. 4, *e*, *g*).

Larval strobila, going from the base of the endocyst, has no distinct segments. When the strobila is retracted into the endocyst its surface is covered

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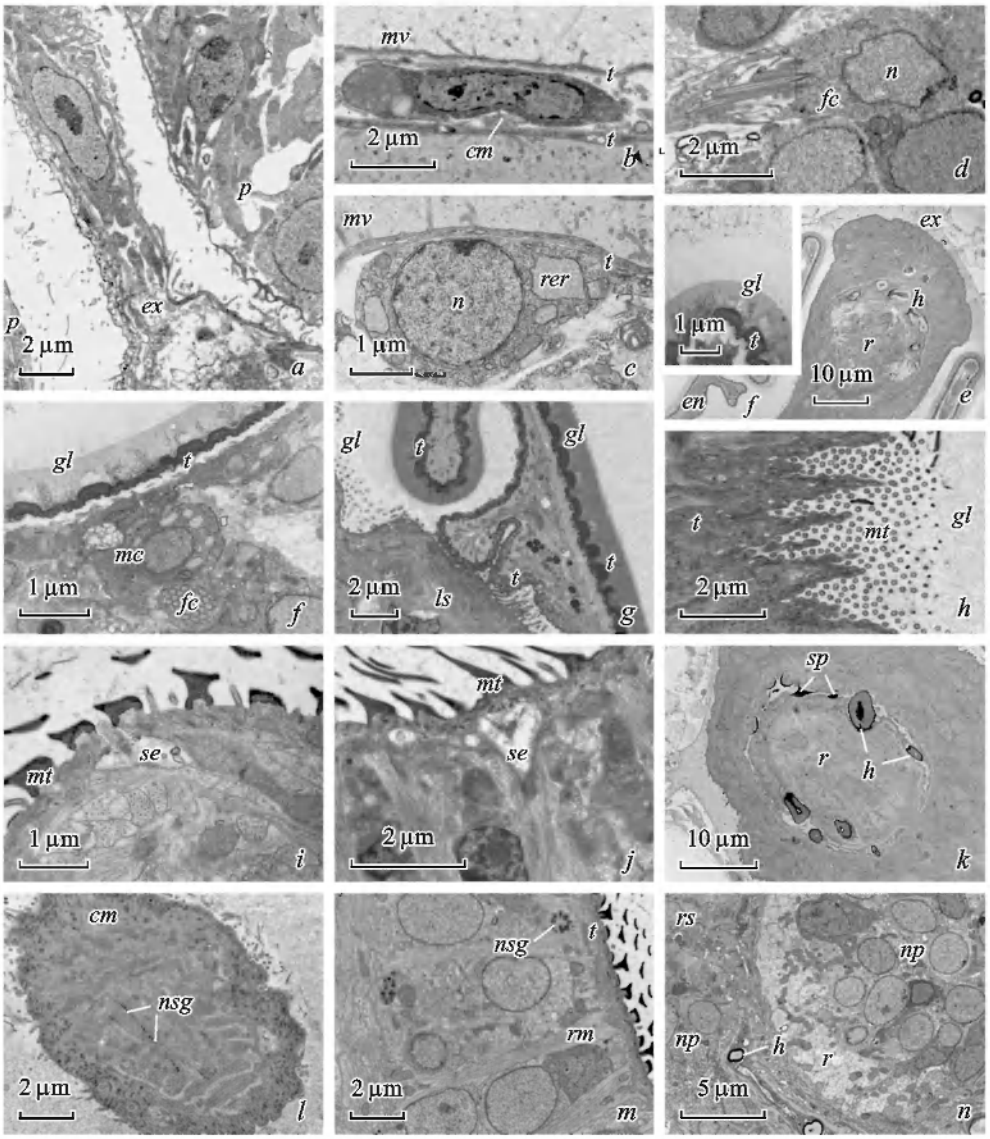

Fig. 4. Details of multicercus of *Mircia shigini*, electron micrographs.

a, *b* — common cytons of two different exocysts of individual cysticercoid, *c* — muscle cell belonging to exocyst, *d* — detail of primordium with flame cell, *e* — developed cysticercoid with retracted scolex (insert — double-layered glycocalyx of endocyst), *f* — endocyst wall, *g* — fold area of endocyst, *h* — part of retracted larval strobila, *i* — sucker's tegument: ciliated sensory ending, *j* — non-ciliated sensory ending, *k* — retracted rostellum, *l* — transverse section of rostellum, *m* — neurosecretory granules in sucker, *n* — nerve ganglia in rostellum and rostellar sac. *cm* — circular muscle, *en* — endocyst, *ex* — exocyst, *f* — fold, *fc* — flame cell, *gl* — glycocalyx, *h* — rostellar hook, *ls* — larval strobila, *mc* — muscle cell, *mt* — microtrix, *mv* — microvilli, *n* — nucleus, *nsg* — neuro-secretory granules, *np* — neuropile, *p* — primordium of cystoscolex, *r* — rostellum, *rer* — rough endoplasmic reticulum, *rm* — radial muscle, *rs* — rostellar sac, *se* — sensory ending, *sp* — spinule, *t* — distal cytoplasm of tegument.

with folds (fig. 4, *h*). The tegument of the strobila is covered with microtrichiae bounded to each other by a fibrous glycocalyx, forming a reticular structure (fig. 4, *g*, *h*). The largest microtrichiae are located in the tegument of the suckers, where ciliate and nonciliate sensory endings are found (fig. 4, *i*, *j*).

The apical part of the rostellum has no microtrichiae. Spinules representing large modified microtriches are visible below rostellar hooks. When the rostellum is retracted, they are located on the inner surface of the retraction channel (fig. 4, *k*). The musculature of the rostellum and rostellar sac is well-developed, consisting of two (circular and longitudinal) muscle layers and separated from the adjacent tissues by the thin basal matrix. Nerve processes with granules are frequently found among muscle fibres of suckers and the rostellum (fig. 4, *l*, *m*). Nerve ganglia in the shape of two lobes are located at the base of the scolex (cerebral ganglion) and in the rostellar sac (ganglion of rostellar sac) (fig. 4, *n*). The rounded rostellar ganglion lies at the base of the widened part of the rostellum.

DISCUSSION

According to previous studies (Mrazek, 1927; Rees, 1973a, b; Boertje, 1975; Gulyaev, 1989; Regel, Pospekhova, 2012, 2014), postembryonic development of ascocerci includes two invaginations. These two invaginations result in the formation of two separate envelopes, although Rees underlines that all the surfaces were originally continuous (Rees, 1973b). The scheme of the development of cysticercoids of *Tatria* s.l. restored by Rees (1973a, b), is still the most comprehensive study of schistotaeniidae and, undoubtedly, corresponds with our conceptions of development of 'euascocysticercoids' (Regel, Pospekhova, 2014).

Ultrastructural investigations of another metacestode species from the dragonfly larvae revealed some differences in the structure of the ascocercus compared with the data of Rees (1973b). The first difference concerns the structure of the subtegumental muscles of the exocyst. Rees (1973b) noted that subtegumental muscles of the outer surface of the *Tatria* s.l. exocyst consist only of circular fibres, while muscles of the inner surface consist of longitudinal ones. Euascocercus *S. srivastavai*, multicercus *M. shigini* (present paper) and megalocercus *D. asper* (Pospekhova et al., 2014) possess subtegumental muscles of both surfaces of the exocyst consisting of circular and longitudinal muscles. However, longitudinal muscle fibres are located at significant distances from each other, especially under stretching of the exocyst wall.

One more difference was found in the structure of the surface layer (the capsule, according to Rees, 1973b), where apical ends of microvilli of the outer surface of the exocyst are immersed. Completely developed cysticercoids of *S. srivastavai* has a surface layer practically identical to that described by Rees (1973b), which is called a serous capsule. At the electron-microscopic level it is represented by a thin layer of granular material, lying at ends of microvilli. However, the surface layer of the developing cysticercoid of *S. srivastavai* is of considerable thickness, and, besides, possesses more complicated structure. The presence of multiple vesicles and vacuoles with fibrous material in the distal cytoplasm of the exocyst, and the developed synthetic apparatus of cytons, allows assuming that the formation of the surface layer is the function of the outer tegument of the exocyst.

The surface layer of ascocerci is probably homologous to glycocalyx, as well as the laminated layer of *Echinococcus* metacestodes (Richards et al., 1983). We do not exclude that the dense granular material, restricting the surface layer is of the host origin, and thus represents the capsule, as was supposed by Rees (1973b). It is quite possible that only this component of the surface layer remains in the developed euascocercus; however, the earlier developmental stages need more profound protection. At the stage of the metamere, fibrous bundles in the surface layer of *S. srivastavai* line up in places where the cytoplasm of tracheal cells is adjacent to the exocyst surface. As for the surface layer of the *M. shigini* multicercus, developed specimens have the fibrous material of the layer organized in four to five plates, which resembles, to some extent, the structure of the laminated layer of hydatid cysts (Morseth, 1967; Richards et al., 1983; Sakamoto, Sugimura, 1970; Ingold et al., 2000). According to the review of Diaz with co-authors (2011), the laminated layer is considered to be a derivative of the germinal layer and one of the main protective structures of larval *Echinococcus*. Despite considerable differences, both laminated and surface layers separate the parasite from the organism of the intermediate host and probably possess similar origin and function.

The host origin of the outer granular part of the ascocerci' surface layer is supported by enbranched host tracheoles on its surface that are especially numerous in multicerci (Pospekhova, Regel, 2010). This phenomenon can be explained in the case where tracheal cells perceive the multicercus surface as that of the host gut. This is possible if: 1) the outer part of the surface layer is the capsule (derivative of the host tissues); 2) it is the derivative of the parasite, but it has the same antigenic determinants as the dragonfly gut. Evidently, the question about the parasite or host belonging of the surface layer (or its belonging to both of them) needs special investigation.

The presence of condensed mitochondria in the tegument of multicerci near the host tracheoles, probably, reflects the specificity of respiration and energy metabolism in the given area of the multicercus surface.

The obtained data on the ultrastructure of the ascocercus confirm morphological similarity of exocysts of ascocerci and typical diplocysts, as noted earlier (Nikishin, 2009). Both envelopes consist of outer and inner tegument bearing microvilli. Both envelopes possess muscle cells and subtegumental muscle fibres, and also dark cells presumably undifferentiated. The ascocercus exocyst, however, separates from cystoscolex primordium at the stage of early scolexogenesis (Rees, 1973a; Regel, Pospekhova, 2014), whereas the exocyst of the diplocyst is connected to the endocyst up to getting into the organism of the definitive host. Besides, the exocyst of the diplocysts is not closed envelope, by contrast to the exocyst of ascocerci, where the content of the endocyst excretory vessels is released into the closed cavity of the exocyst. This should considerably diminish the number of antigens getting into the host organism, which is of special importance for the parasite that has a long period of development in the intermediate host (Regel, Pospekhova, 2012, 2014).

Exocysts of different ascocerci (euascocerci, multicerci and megalocerci) also possess morphological differences despite the basic structural similarity. Thus, the exocyst of the megalocercus of *Dioecocestus asper* possesses the well-developed protonephridial system, consisting of flame cells, collecting ducts and large vessels, which is an unique character, not found at other meta-

cestodes (Regel, Pospekhova, 2012; Pospekhova et al., 2014). At the same time, the exocyst of the megalocercus lacks the surface layer, which is the characteristic feature of the ascocerci of Schistotaeniidae (Pospekhova et al., 2014; present work).

The outer envelope of the multicercus provides the process of asexual multiplication. Comparison of the ultrastructure of the exocyst of the young euascocercus and envelope of multicercus at the stage of bud formation shows their obvious significant similarity. In both cases we can see the outer and inner teguments, which are in contact with their cellular layers, having dark small cells, which are apparently poorly differentiated. The outer tegument looks more developed in both cases. It has thicker distal cytoplasm, and larger and more numerous cells. Its distal cytoplasm has a dense layer of microvilli, which are restricted by the surface layer.

We assume that the envelope (the tegument) of multicercus is analogous to the outer tegument of the euascocercus exocyst, while the inner tegument of the euascocercus exocyst is analogous to the one-layered exocyst of separate cysticercoids of multicercus. For convenience we have denoted the one-layered envelope of separate cysticercoids as the exocyst, although, if taking the abovementioned reasons into account, one should consider multicercus tegument together with a one-layered envelope of separate cysticercoids to be an exocyst. In this case, a united exocyst of the multicercus exists only for a short time, from the beginning of bud formation till the separation of the cysticercoid from the outer tegument.

Sparse microvilli of the inner tegument of the ascocerci' exocysts are pointed inside. Such orientation of the tegument is characteristic of the inner surface of the exocyst of diplocysts (Nikishin, 2009) and for brood capsules of *Echinococcus* (Sakamoto, Sugimura, 1970; Thompson, 1976; Richards et al., 1983). Growth of the surface of the brood capsule in *Echinococcus* occurs as a result of the integration of poorly differentiated cells into the cellular layer, which later forms connections with the distal cytoplasm of the tegument (Mehlhorn et al., 1983). The important role of tegumental cytons in metacestode proliferation was noted by Bilqees (1970). Whitfield and Evans (1983) resume in their review, '...the 'germinal (or germinative) membrane' of the light microscopists is, in all essential respects, a normal tegumentary body wall'. We suppose that the tegument (namely — the exocyst' tegument of ascocerci) is a multifunctional formation that can combine functions of nutrition, protection, excretion and asexual multiplication at different ascocerci and the last function is carried out, probably, with the participation of poorly differentiated cells of exocyst' tegument.

Asexual multiplication at the larval stage among Cyclophyllidea that is mainly characteristic of the family Taeniidae, was found almost exclusively in terrestrial intermediate hosts and supposedly has arisen independently in the Mesocestoididae, Dilepidae, Hymenolepidae and Taeniidae in response to shared selection pressure associated with transmission under terrestrial conditions (Whitfield, Evans, 1983). The intermediate host of multicercus is dragonfly larvae, an aquatic dweller. This circumstance violates the tendency noted by Whitfield, Evans (1983) but agrees with their observations about parasitological under-recording of marine (and probably freshwater) environment.

One of the best known examples of terrestrial polycephalic non-taeniid metacestodes is the polycercus *Paricterotaenia paradoxa* (Rudolphi, 1802), which

was firstly found by Metchnikov (1868) in the earthworm. The first detailed morphological study of this metacestode, named *Polycercus lumbrici* Villot, 1883, was performed at the light-microscopic level (Scott, 1965). Electron-microscopic studies showed that the polycercus is a polycephalic form of the monocercus (MacKinnon, Burt, 1984), while the study of the budding processes of the polycercus allowed identifying its ability for virtually unrestricted production of new individuals (Gulyaev, 2000). The multicercus represents a polycephalic form of the ascocercus, and the amount of cysticercoids in multicercus is, evidently, limited only by the lifespan of the intermediate host. This ability, together with the presence of the surface layer, brings together multicercus and hydatid cysts, making it possible to consider multicercus as 'dragonfly' *Echinococcus*. The structure of the multicercus is simpler; thus, it does not produce brood capsules, but immediately produces new individuals. Taking into account the evolutionary antiquity of its intermediate hosts (Odonata), we do not exclude that the multicercus appeared much earlier than hydatid cysts, and their resemblance is the manifestation of convergence.

There is a great deal of papers devoted to morphology (as well as fine morphology) of metacestodes; however, new unexplored peculiarities of their construction are still being revealed. This is why the nearly century-old remark '...jest jisto, že ku konečnému obrazu bude jeste treba zevrubne vyšetření všech vůbec mošných, to jest skutečne existujicich modifikaci larvy Cestodů ('...it is obvious that in order to achieve a complete picture it is necessary to study all existing modifications of cestode larvae') remains relevant today (Mrazek, 1927).

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