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Excretory system ultrastructure of diphyllobothriid tapeworm *Pyramicocephalus phocarum* (Cestoda) with cytochemical and functional implication

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ABSTRACT: The article describes the architecture and ultrastructure of the excretory system (protonephridial system) of the plerocercoid *Pyramicocephalus phocarum* (Fabricius, 1780), a parasite of the liver of the cod *Gadus morhua* Linneaus, 1758. The excretory system consists of cyrtocytes and a syncytial excretory epithelium, which forms the protonephridial funnels, the canal system, and the excretory bladder. Our immunocytochemical studies have shown that cyrtocytes are immunoreactive to α-tubulin, the protonephridial complexes are reactive to fibrillar actin. The excretory epithelium consists of differentiated apical cytoplasm specializing at secretion and reabsorption, and submerged cytons/perikarya. Architecture of the excretory system includes syncytial canals of various diameters and positions within the body. The 1st and the 2nd order canals form a complex three-dimensional peripheral network. The longitudinal central (main) excretory canals branch dichotomously at the posterior end of the body and flow into the excretory bladder. The main excretory canals possess a thick muscular wall innervated by the central nervous system. In addition, the ultrastructure of the excretory bladder, nephropore, terminal excretory pore are described. A close connection between the excretory and nervous systems occurs in the plerocercoid body. In the scolex, the main excretory canals and transverse excretory anastomoses pierce the brain, and underlie the transverse cerebral commissure. Neurites of the main nerve cords innervate the main excretory channels. Apart from this, there are unciliated sensory organs in the wall of the terminal excretory pore. The functional significance of the described structures is discussed. Based on the obtained ultrastructural and immunocytochemical data, we support the hypothesis of primary ultrafiltration through a molecular sieve of the glycocalyx, covering the weir ribs of protonephridia.

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KEY WORDS: Cestoda, cyrtocyte, excretory bladder, flame cell, filtration, junctions, immunocytochemistry, nephropore, protonephridia, syncytium, ultrastructure.

Ультраструктура выделительной системы ленточного червя *Pyramicocephalus phocarum* (Cestoda) с обсуждением цитохимического и функционального значения

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РЕЗЮМЕ: Изучено строение и ультраструктура выделительной системы (протонефридиальной системы) плероцеркоида *Pyramicocephalus phocarum* (Fabricius, 1780), паразита печени трески *Gadus morhua* Linneaus, 1758. Выделительная система состоит из циртоцитов и синцитиального экскреторного эпителия, образующего протонефридиальные воронки, систему каналов и экскреторный пузырь. Иммуноцитохимические исследования показали, что циртоциты обладают иммунореактивностью к α-тубулину, протонефридиальный комплекс реактивен к фибриллярному актину. Экскреторный эпителий состоит из дифференцированной апикальной цитоплазмы, специализированной для секреции и реабсорбции, и погруженных цитонов/перикарионов. Архитектура выделительной системы включает синцитиальные каналы различного диаметра и положения в организме. Каналы 1-го и 2-го порядка образуют сложную трехмерную периферическую сеть. Продольные центральные (главные) экскреторные каналы дихотомически разветвляются на заднем конце тела и впадают в мочевой пузырь. Главные экскреторные каналы имеют толстую мышечную стенку, иннервируемую центральной нервной системой. Описана ультраструктура мочевого пузыря, нефропора, терминальной экскреторной поры. Тесная связь между выделительной и нервной системами отмечена в теле плероцеркоида. В сколексе, через мозг проходят главные экскреторные каналы и поперечные экскреторные анастомозы, которые подстилают медианную мозговую комиссуру. Нейриты главных нервных стволов иннервируют мускулатуру главных выделительных каналов; в стенке терминальной поры имеются безресничные сенсорные органы. Обсуждается функциональное значение описанных структур. На основании полученных ультраструктурных и иммуноцитохимических данных, мы поддерживаем гипотезу первичной ультрафильтрации через молекулярное сито гликокаликса, покрывающего микроворсинки верши протонефридия.
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КЛЮЧЕВЫЕ СЛОВА: Цестоды, циртоцит, экскреторный пузырь, пламенная клетка, фильтрация, контакты, иммуноцитохимия, нефропор, протонефридий, синцитий, ультраструктура.

Introduction

Neodermatan flatworms Cestoda lack a coelom, digestive, blood-vascular, and respiratory systems. The neodermis (or tegument), muscular and excretory systems have a syncytial organization (Kuperman, 1988; Korneva, 2013). The excretory system (protonephridial system) has an epithelial lining and forms a closed vascular system. The excretory system (protonephridial system) of cestodes is important for the comparative morphology of tapeworm taxa, as well as for the system functioning in parasitic lifestyle (Malmberg, 1971; Lindroos, Gardberg, 1982; Kuperman, 1988; Korneva *et al*., 1998; Poddubnaya *et al*., 2020).

Cestoda protonephridial filtration apparatus consist of two cells, the flame cell and the first canal cell (Wilson, Webster, 1974; Xylander 1987, 1992; McCullough, Faiweather, 1991; Biserova *et al*., 2021). At the same time, there is an opposite hypothesis, which rejects the bicellularity of the filtration apparatus in some species of tapeworms. According to data of B.I. Kuperman, in lower cestodes the flame cell and canal epithelium forms a united syncytium of the protonephridia (Kuperman, 1988).

During ontogeny of cestodes, the excretory system is formed several times. Oncospheres of cyclophyllid cestodes lack cyrtocytes (Coil, 1991; Swiderski, Tkach, 2002), whereas there are 2 cyrtocytes in free-living licophores of amphilinids or gyrocotylids, and coracidia of Pseudophyllidea *sensu lato*. Each of the cyrtocytes contacts the excretory canal, which in turn contacts the cell that forms the excretory pore of the larva (Korneva, 1994, 2001, 2004; Swiderski, Mackievicz, 2004). Thus, three cells from each side build the primary excretory system of coracidia. The primary excretory system is destroyed in procercoids in the first intermediate host, and then the secondary excretory system is formed. Thus, the secondary excretory system develops *de novo* from undifferentiated cells and functions independently of the primary excretory system (Malmberg, 1971, 1972, 1974; Korneva, 2004). The secondary excretory system is inherited by plerocercoids, the next stage developing in a second intermediate host, and is passed on to adult cestodes. The excretory system of plerocercoids and adult cestodes is highly complicated due to a large size of the body. It is represented by the extended canals of various architecture in different taxa (Dubinina, 1982; Rohde, Watson, 1991; Rohde *et al*., 1992; Xylander, 1992; Korneva *et al*., 1998; Poddubnaya *et al*., 2020). A significant part of excretory system has syncytial organization (Kuperman, 1988; Pospekhova *et al*., 1993; Kabbany, 2009; Korneva, 2013).

The canal part of the excretory system consists of longitudinal main canals and a peripheral net of smaller canals in different representatives of lower cestodes (Malmberg, 1974; Lindroos, Gardberg, 1982; Lindroos, 1983; Kuperman, 1988). In the plerocercoids of *Diphyllobothrium dendriticum** (Nitzsch, 1824) (Diphyllobothriidea) the excretory system consists of a peripheral net of canals, a central system consisting of two main longitudinal canals which communicate with each other via transverse canals, as well as a dorsal and ventral capillary plexus in the scolex and a 4-lobated bladder with an excretory pore at the posterior end of the plerocercoid body. Cyclophyllidea possess four main longitudinal canals (two dorsal and two ventral); the ventral pair is connected by regular transverse anastomoses (Coil, 1991; Rozario, Newmark 2015). Though currently there is a lot of contradictory data and conflict terms concerning the structure, position, and interconnection of the caudal parts of the cestode excretory system, there are several questions to be studied thoroughly. The issue to be primarily considered is what the bladder in cestodes actually is and whether or not the cestode bladder can be regarded as a structure with a tegumental lining (Malmberg, 1972) or as a reservoir with excretory epithelium lining (Korneva *et al*., 1998). Another issue to consider is that the connection

* For citation, we use the species name as noted in the references; for example, *Diphyllobothrium dendriticum* and *D. latum* instead of current suggested name *Dibothriocephalus dendriticus* and *D. latus,* although genus *Diphyllobothrium* was divided into two genera: *Dibothriocephalus* and *Diphyllobothrium* (Waeschenbach *et al*., 2017).

between the main longitudinal canals and the excretory bladder is not reliably shown in diphyllobothriidean tapeworms (Lindroos, Gardberg, 1982). Furthermore, there is no clear definition of such structures as nephropores (or nephridiopores), and excretory pores (Xylander, 1992; Korneva *et al*., 1998; Lindroos, Gardberg, 1982). Functional significance of the above mentioned structures also seems to be a controversial issue (Wilson, Webster, 1974; Lindroos, Gardberg, 1982; Lindroos, 1983; Pospekhova *et al*., 1993; Korneva *et al*., 1998).

Pyramicocephalus phocarum (Fabricius, 1780) studied in this work belongs to the order Diphyllobothriidea and is the only representative of the genus. Diphyllobothriids are widespread in natural habitats parasitizing at their adult stage on warm-blooded animals and humans. The plerocercoids of *P. phocarum* infect the liver of the White Sea cod *Gadus morhua* (Linnaeus, 1758), a valuable human food product. The *P. phocarum* larvae enter along with the food the digestive tract of their definitive hosts — seals (Delamure, 1961; Rausch *et al*., 2010). In addition, the plerocercoids themselves significantly harm the organism of the intermediate host, i.e. commercial fish. Importantly, there is very little information on the morphology and ultrastructural organization of a tegument, glandular and nervous system of the *P. phocarum* plerocercoid (Mustafina, Biserova, 2017; Mustafina, 2017, 2021; Biserova *et al*., 2021, 2022). Thus, the aim of this research is to study the architecture and fine structure of the excretory system of the *P. phocarum* plerocercoid in order to resolve the contradictions in the literature on the topic and critically review the data in terms of possible functional significance.

Material and methods

Plerocercoids of *P. phocarum* were obtained from the body cavity of *Gadus morhua* (White Sea, the WSBS MSU, Russia), fixed in 2.5% glutaraldehyde and post-fixed in 1% OsO_4 in PBS, dehydrated and embedded in Araldite resin at 60 °C (Biserova, 2013; Biserova *et al*., 2022). Semithin sections were stained with methylene blue; and ultrathin sections were stained with 4% uranyl acetate and 0.4% lead citrate and examined under a Jeol JEM-1011 (TEM). For scanning electron microscopy (SEM), specimens were fixed and dehydrated as noted above and then, undergone critical-point drying, coated with gold, and examined under a scanning electron microscope JSM35S JEOL. To undergo confocal microscopy, the specimens were fixed in 4% paraformaldehyde (PF) in 0.1 M phosphate buffer (PBS), washed in 0.1 M PBS + 0.03% sodium azide (NaN_3). Subsequently, the specimens were embedded in Tissue-Tek (Sakura Finetek, Torrance, California) and cut with a cryotome (Leica CM1850UV, Leica Microsystems GmbH, Wetzlar, Germany). Sections at a thickness of 8–10 μm were placed on poly-L-lysine slides, treated with 1% Triton X100 in 0.01M PBS, for 1–2 hr and pre-incubated in a mixture of 1% Triton X100 + 1% bovine serum albumin (BSA) in 0.01 M PBS for 3 hr. The prepared tissue was incubated in a solution of monoclonal antibodies against acetylated tubulin (α-Tub, product number: T6793). Part of the material was additionally stained with Phalloidin TRITC (Sigma P1951).

The following protocols (Biserova, 2013) were used for incubation in primary antibodies at 4°C on a shaker: anti-tubulin mouse 1:1000 in 0.01 M PBS + 1% TritonX100 + 1% NGS + 0.003% NaN3. The incubation time in primary antibodies varied from 10 to 25 h. Washing after incubation with primary antibodies: 0.1 M PBS + 1% TritonX100 + 1% NGS, 6 times for 15 min on a shaker at 4°C. As secondary antibodies, we used Alexa 488, 532, 635 against mouse in various combinations at a 1:800 dilution in 0.01 M PBS + 1% Triton for 2 h at 4°C; followed by staining with Phalloidin-TRITC. Phalloidin TRITC 1: 1000 in 0.1 M PBS + 1% Triton X100, 1h. Sections were washed in 0.1 M PBS, 6 times for 15 minutes. The obtained preparations were embedded in glycerol adding 0.1 M PBS (1: 1), edged and stored at –20°C. The samples were examined under MSU Nikon confocal laser system (Japan).

The terminology of the cestode excretory system used in our article is presented in the Table 1.

Table 1. List of terms of the excretory system elements using for *Pyramicocephalus phocarum* plerocercoid.
Таблица 1. Список терминов, используемых для описания выделительной системы плероцеркоида *Pyramicocephalus phocarum*.

Terms	Definition
Cyrtocyte	Flame cell is characterized by multiple cilia forming ciliary tuft
Leptotriches	Long thin cytoplasmic outgrowths of the cyrtocyte membrane
Protonephridial funnel	The initial part of an excretory funnel-shaped canal. The extended part of a funnel has long finger-shaped outgrowths (ribs, see below) forming an ultrafiltration complex
Ribs (cyrtocyte ribs, funnel ribs)	Long finger-shaped outgrowths of cyrtocyte cytoplasm and a funnel cytoplasmic membrane. Ribs form an ordered ultrafiltration structure
Weir	Ordered ultrafiltration structure formed by cyrtocyte ribs and funnel ribs
Zip-contact	Connection of superficial extracellular structures of ribs in a form of a closed 'zipper', which connects of the opposite ribs membranes
Excretory epithelium	Syncytial epithelium with apical surface possesses microvilli. Basal membrane of epithelium has a varying degree submerged cytons
Excretory canal system	Canal part of the excretory system, including the protonephridial funnels, canals of the 1st and 2nd orders, central canals, anastomoses, and the excretory bladder (see below). Excretory canal system is formed by excretory syncytial epithelium
Excretory bladder	Lobular reservoir in the caudal body part associated with the main canals and formed by the excretory epithelium. The excretory bladder connects with the reservoir of the terminal excretory pore through the nephropore (see below)
Nephropore	Contact of the excretory epithelium with the tegument, supported by the annular septate junction
Terminal excretory pore	An opening at the posterior end of the body, formed by a fold of the tegument and connected to excretory canal system through nephropores
Excretory pore reservoir	Tegumental canal / invagination, into which the nephropores of the bladder and peripheral canals open

Results

Series of semi-thin and ultrathin sections show that the excretory system of the *P. phocarum* plerocercoid consists of two independent structural elements: cyrtocytes, or flame cells, and an excretory epithelium. The excretory epithelium, in turn, forms the protonephridial funnels, system of canals of various orders and the excretory bladder (Fig. 1A–E). The weir consists of the cytoplasmic ribs of cyrtocytes and the cytoplasmic ribs of the protonephridial funnel (Fig. 2A). The terminal pore is the final part of the excretory system. It is an opening at the posterior end of the body formed by a deep fold of the tegument (Fig. 1D, E).

Cyrtocytes and protonephridial complex. Cyrtocytes, or flame cells, are found along the entire length of the *P. phocarum* body. Confocal microscopy and staining with phalloidin and α-tubulin, reveal numerous cyrtocytes located

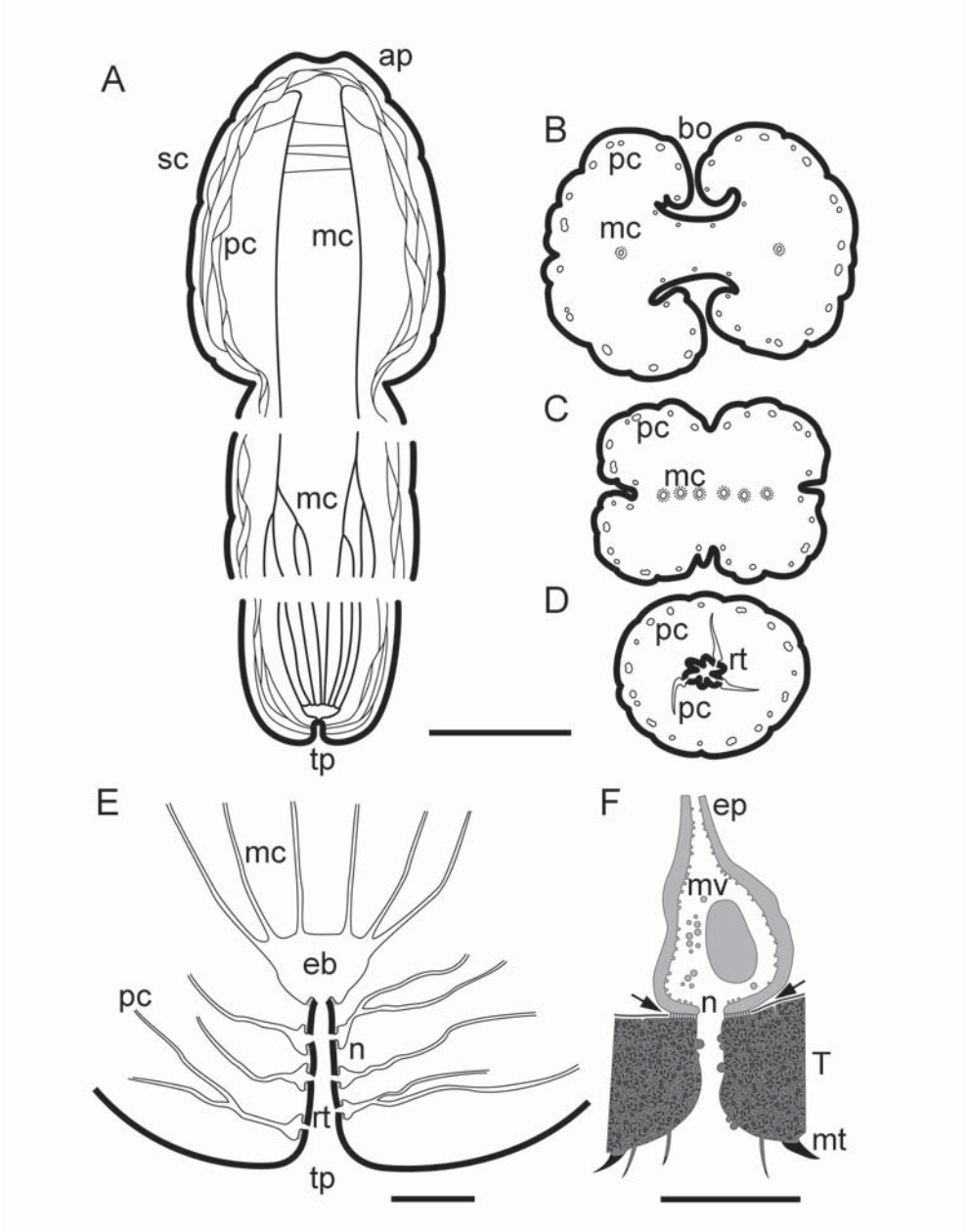

Fig. 1. The excretory system architecture of *Pyramicocephalus phocarum* plerocercoid. A — frontal view of the excretory system; B–D — cross section at the level of scolex (B), middle body (C) and posterior end (D); E — the excretory bladder and terminal excretory pore with numerous nephropores; F —ultrastructural organization of the nephropore; arrows mark the septate junction between excretory epithelium (ep) and tegument (T).

Abbreviations*:* ap — apical part of the scolex; bo — bothria; ep — excretory epithelium; mc — main canal; mv — microvilli; mt — microtriches; n — nephropore; pc — peripheral net of the 2nd order canals; rt — reservoir of the terminal pore; sc — scolex; T — tegument; tp — terminal excretory pore; eb — excretory bladder. Scale bars: A–D — 1 mm, E — 20 μm, F — 5 μm.

mainly in the subtegument and cortical parenchyma of the plerocercoid. The nucleus is located at one pole of the cyrtocyte; at the other pole there is the bundle of cilia (Figs 2A; 3A; 4A). Generally, flame cells lie with their nucleus oriented towards the tegument. Their ciliary flame, intensely stained with α-tubulin, is directed into the protonephridial funnel formed by the excretory epithelium. An intense f-actin reactivity of flame cells is exhibited (Fig. 3G). The soma, ciliary rootlets of the flame cell and weir ribs are brightly colored with Phalloidin-TRITC. The protonephridial funnel was less intensively stained with Phalloidin-TRITC.

Cyrtocytes are scattered in loose intercellular matrix of the parenchyma. The diameter of the cyrtocyte soma is noticeably smaller than the diameter of the surrounding cells. The cyrtocyte is cylinder-shaped (diameter about 3 μm) with a conical bundle of cilia inside, which gradually narrows towards the protonephridial funnel. The cyrtocyte nucleus is surrounded by a small layer of cytoplasm that contains mitochondria, vesicles, the Golgi apparatus, and a set of basal bodies (Fig. 3A, B). The outer cytoplasmic membrane of the perikarya forms irregular outgrowths protruding into the surrounding parenchyma. Among other elements are free ribosomes, vacuoles with light heterogeneous content and those with dark homogeneous content sized (together with the outgrowths) up to 120 nm. These processes sometimes form short gap junctions with the cells of the surrounding parenchyma; in particular, a certain connection between cyrtocytes and the muscle cells accumulating glycogen was revealed. The membrane of cyrtocytes forms a regular row of long finger-shaped ribs and thin internal leptotrichia surrounding cilia bundle at the ciliary pole (Fig. 3A–E). The cilia have a normal axoneme with 9 + 2 microtubule arrangement about 9 microns long and 270 nm in diameter, covered with an outer cytoplasmic membrane (Fig. 3A). Internal leptotrichia, thin (70 nm) cytoplasmic outgrowths of the outer membrane of the cyrtocyte, form two rows (Fig. 3D). Leptotrichia are surrounded by a row of long finger-shaped ribs of regular cylindrical shape, 140–150 nm in diameter. The ribs contain thin filaments or fibers in cytoplasm and probably include one central microtubule, which, though, can be seen in large magnification solely (Fig. 3D). The superficial ribs membrane bears a pronounced layer of fine extracellular filaments lying 22 nm deep (Fig. 3F).

The protonephridial funnel is the second structural unit of the protonephridium. The protonephridial funnel has a widened end with a row of long finger-like ribs 180–190 nm in diameter, and a narrowed thin-tubule part extending into the canal system (Figs 3H, I, 4A). The ciliary flame of the cyrtocyte is located inside the funnel. The walls of the protonephridial funnel continue into the walls of the canals and are formed by the excretory epithelium. The outer row of the protonephridial funnel ribs adjoins the inner row of cyrtocyte ribs, so they both form the weir, or filtration apparatus of the protonephridium. An electron-dense material is located in the ribs cytoplasm at the contact points of opposite ribs (Fig. 3D, E). A 22 nm thick layer of extracellular filaments covers the opposing membranes. Filaments are located in a crisscross pattern along the zipper-like contact with the opposing membrane (Fig. 3F). As Phalloidin-TRITC staining shows, fibrillar actin collar is present in this area of the protonephridium (Fig. 3G).

Рис. 1. Архитектура выделительной системы плероцеркоида *Pyramicocephalus phocarum*. A — фронтальный вид выделительной системы; B–D — поперечный срез на уровне сколекса (B), середины телы (C) и заднего конца тела (D); E — мочевой пузырь и терминальная экскреторная пора с многочисленными нефропорами; F — ультраструктурная организация нефропора; стрелки указывают на септированные контакты между экскреторным эпителием (ep) и тегументом (T).

Обозначения: ap — апикальная часть сколекса; bo — ботрия; ep — экскреторный эпителий; mc — главный канал; mv — микроворсинки; mt — микротрихии; n — нефропор; pc — периферическая сеть каналов 2-го порядка; rt — резервуар терминальной поры; sc — сколекс; T — тегумент; tp — терминальная экскреторная пора; eb — мочевой пузырь. Масштаб: A–D — 1 mm, E — 20 μm, F — 5 μm.

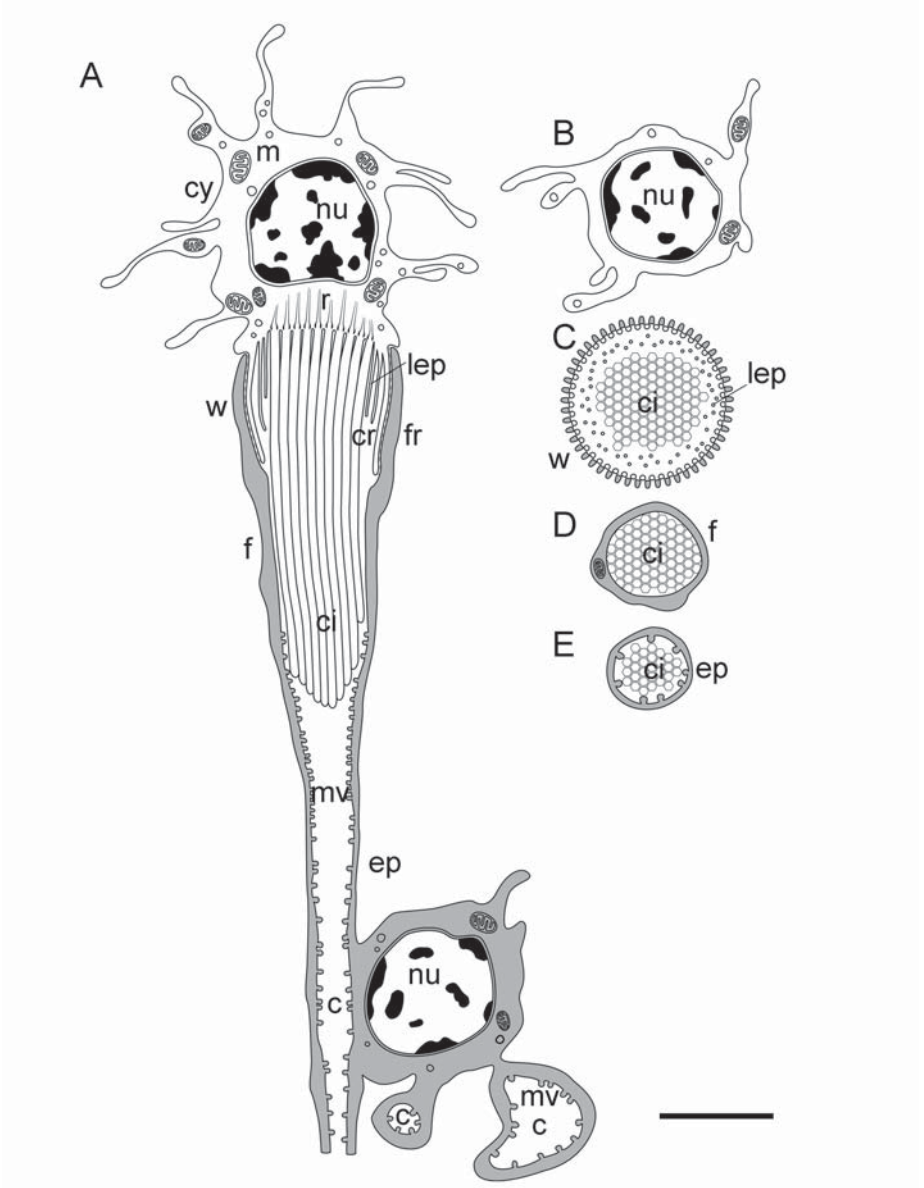

Fig. 2. Illustration of cyrtocyte and protonephridial funnel of *Pyramicocephalus phocarum* plerocercoid. A — protonephridium longitudinal section; B–E — cross sections; B — perikarya; C —weir; D —protonephridial funnel; E — the 1st order canal.

Abbreviations: c — the 1st order canal; cy — cytrocyte; ci — cilia; cr — cyrtocyte ribs; ep — epithelium of the excretory canal; f — protonephridial funnel; fr — funnel ribs; lep — leptotriches; m — mitochondria; mv — round microvilli in the excretory canal; nu — nucleus; r — rootlets; w — weir. Scale bar 2 μm.

Рис. 2. Иллюстрация циртоцита и протонефридиальной воронки плероцеркоида *Pyramicocephalus phocarum*. A — продольный срез протонефридия; B–E — поперечные срезы; B — перикарион; C — верша; D — протонефридиальная воронка; E — канал первого порядка.

Обозначения: c — канал первого порядка; cy — циртоцит; ci — реснички; cr — микроворсинки циртоцита; ep — экскреторный эпителий; f — протонефридиальная воронка; fr — микроворсинки воронки; lep — лептотрихии; m — митохондрия; mv — микроворсинки в экскреторном канале; nu — ядро; r — корешки ресничек; w — верша. Масштаб 2 μm.

The electron-dense cytoplasm of the protonephridial funnel contains a lot of fibrils located concentrically (Fig. 3H); sometimes single small mitochondria may occur in the basal layer of the funnel epithelium. The surface membrane of the funnel is smooth in the broad part. Deeper in the canal, the membrane bears round microvilli up to 100 nm in diameter with electron-dense rounded particles (Fig. 3I). The protonephridial funnel continues into the canal of the 1st order.

The architecture of the canals of the excretory system. We examined a series of histological frozen sections with phalloidin histochemical and immunocytochemical staining for CSLM for reconstruction of the plerocercoid canal system; we also examined series of semi-thin cross-sections and a series of fractures for scanning electron microscopy in the longitudinal and cross direction.

We were found that the excretory system includes the canals of various diameters and positions in the body of the plerocercoid (Figs 1A–E; 4; 5; 6; 7; Tab. 1). In the present study we distinguish between the canals of the 1st order and those of the 2nd order, which form a complex three-dimensional peripheral network in the subtegument and cortical parenchyma, on the one hand, and longitudinal central (main) excretory canals connected by the transverse anastomoses, on the other hand. In the scolex, the main canals pass through the lateral lobes of the brain, proximal to the neuropils. The transverse anastomosis of the excretory system underlies the nerve fibers of the median brain commissure (Fig. 6D). The longitudinal main excretory canals branch dichotomously at the posterior end of the body and flow into the excretory bladder (Figs 1C, E; 7A). The excretory bladder is connected by a nephropore with a long, convoluted tegumental reservoir of the terminal excretory pore. The excretory pore opens at the posterior end of the plerocercoid (Figs 1E; 4G). In addition, the canals of the 2nd order in the posterior end of the body open into the reservoir of the terminal pore by multiple nephropores (Fig. 1F).

A pair of main canals is located at the border of the muscle layers of the medullary and cortical parenchyma (Fig. 6A). The main canals are closely associated with the main nerve cords, which run distally, closer to the tegument (Fig. 6A). The diameter of the lumen of the main canal reaches up to 22 μm in the body of the plerocercoid. Several layers of muscle fibers were revealed in the wall of the main canals using histochemical Phalloidin TRITC staining. Muscles are oriented mainly longitudinally and cover the canal in a spiral (Fig. 6A). Rounded perikariya of excretory epithelium of the main canals are located under the layers of myofibrils (Fig. 6B). In addition to fibrillar actin reactivity, we found α-tubulin reactivity in the muscle layers of the wall of the main excretory canal. The intense immunoreaction to α-tubulin is found in the nerve processes closely associated with Phalloidin TRITC stained muscle fibers around the main canals wall (Fig. 6A). On ultrastructural level, the processes of neurons are found in the vicinity of the main excretory canal and transverse anastomosis (Fig. 6D); their neurites terminate in the canal wall. Innervation of the canal wall muscles suggests the possibility of coordinated contraction of the lumen/diameter of the excretory canals under the control of the nervous system. No phalloidin staining label was found in the peripheral canals of the 1st and 2nd orders. Thus, the only main canals are capable of peristaltic contractions due to the musculature of the wall and innervation from the main nerve cords, while the peripheral canals change their diameter only passively, ensured by contractions of the subtegumental musculature.

Ultrastructure of the excretory epithelium. All canals of the excretory system including protonephridial funnel, canals of various orders, and the excretory bladder are lined with syncytial excretory epithelium. The 1st-order thin canals are located in different areas of the body and can be of irregular shape, size, and direction (Fig. 5A). They are formed in epithelial cells expanded processes as small intracellular vacuoles with round microvilli inside. The wall thickness does not exceed 0.5 μm. The basal surface of the 1st-order canal wall is the

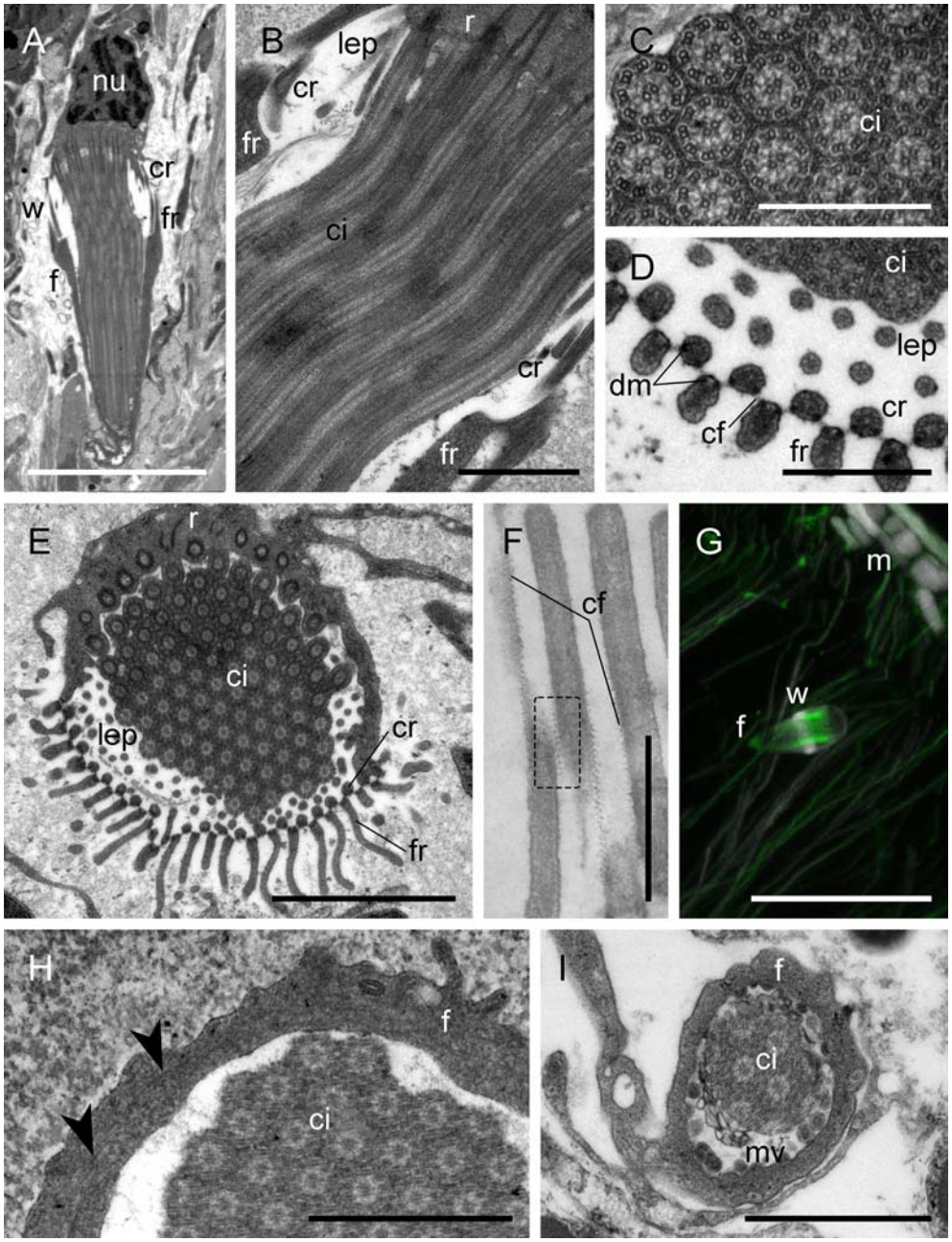

Fig. 3. Ultrastructure of the cyrtocyte and protonephridial funnel of *Pyramicocephalus phocarum* (TEM). A — longitudinal section through protonephridial filtration complex; B — longitudinal section of cilia with rootlets; C — cross section through cilia; D — cross section of the weir; E — cross-oblique section of protonephridial filtration complex at the level of weir; F — longitudinal section of the weir ribs, frame shows the spot where weir ribs are interconnected by zip-contact; G — immunocytochemistry of the protonephridium in subtegument (tubulin — green, phalloidin — gray; confocal microscopy; full colour version see online); H — cross section of the protonephridial funnel with cilia tuft, arrowheads note actin filaments in the cytoplasm; I — cross section of the canal part of the funnel.

outer basal plasma membrane of the excretory syncytial epithelium. The basal plasma membrane forms folds and contacts with other cells and lack morphological distinct electron-dense layer of *lamina densa* (Figs 3I; 5A). Filamentous extracellular matrix (ECM) adheres to the basal plasma membrane of the canal wall. Round microvilli are located on the surface of the membrane facing the lumen of the 1st-order canal (the apical membrane) (Fig. 4B). Round microvilli (up to 100 nm in diameter) contain electron-dense rounded particles up to 60–70 nm in size. The nuclei of epithelial cells are rich in the euchromatin that are surrounded by dense cytoplasm, including mitochondria and glycogen granules; closer to the apical membrane, rare rod-shaped dense bodies may sometimes be found. As the diameter expands, the 1st-order canal cytoplasm is divided into 2 layers: the apical layer of the cytoplasm is homogeneous, electron-dense, without organelles; the basal layer of the cytoplasm is thin, light and contains a large number of organelles (mitochondria, rare rod-shaped dense bodies, various size vesicles, ribosomes).

The 2nd order canals continue the 1st order canals. They are located predominantly along the body. The 2nd order canals have frequent anastomoses among themselves (Fig. 1A). In bothria, the canals of the 2nd order form an ordered network: there are strictly longitudinal and strictly transverse anastomosing ducts (Fig. 4E). In the scolex, the 2nd order canals occasionally connect to the main protonephridial canals (Figs 1A; 6D). At the posterior end of the body, the 2nd order canals are most numerous: they are secularly widened and make contacts with the tegument of the terminal pore (Figs 1F; 5D, E).

The nuclei of the excretory epithelium of the 2nd order canals are slightly immersed in parenchyma and connected to the canal wall by means of short cytoplasmic processes (Fig. 5B). The ultrastructure of the perikarya is similar to the structure of the 1st order canals perikarya. Differences in ultrastructure between the apical cytoplasm in the 1st and the 2nd order canals wall were revealed. The wall of the 2nd order canals is differentiated into 3 layers with different contents: basal, medial, and apical layer (Fig. 5C). The basal layer (about 0.8 μm thick) is light, rich in organelles, including mitochondria, ribosomes, glycogen granules, and small light vesicles up to 50 nm in diameter. The medial layer (0.3 μm thick) contains numerous rod-shaped dense bodies oriented along the apical surface of the canal. The apical layer of the cytoplasm (about 0.3 μm thick) is homogeneous, electron-dense, and does not contain organelles; the apical membrane forms round microvilli like those described above. Occasionally, some areas of the smooth-surfaced membrane epithelium without microvilli can be detected. In the lumen of the canal there are rare protrusions of the apical cytoplasm layer with-

Abbreviations: ci — cilia; cf — surface membrane filaments; cr — cyrtocyte ribs; dm — dense material in ribs cytoplasm; f — protonephridial funnel; fr — funnel ribs; lep — leptotriches; m — subtegumental muscles; mv — round microvilli; nu — nucleus; r — ciliary rootlets; w — weir. Scale bars: A — 5 μm, B, H, I — 1 μm, C, D — 0.5 μm, E — 2 μm, F — 500 nm, G — 10 μm.

Рис. 3. Ультраструктура циртоцита и протонефридиальной воронки плероцеркоида *Pyramicocephalus phocarum* (ТЭМ). A — продольный срез через протонефридиальный фильтрационный комплекс; B — продольный срез ресничек с корешками; C — поперечный срез ресничек; D — поперечный срез верши; E — поперечно-косой срез фильтрационного комплекса на уровне верши; F — продольный срез верши, рамкой отмечено место, где микроворсинки верши сомкнуты с помощью zip-контакта; G — иммуногистохимия протонефридия в субтегументе (тубулин — зеленый, фаллоидин — серый; конфокальная микроскопия; цветную версию см. онлайн); H — поперечный срез протонефридиальной воронки с пучком ресничек, стрелки указывают на актиновые филаменты в цитоплазме; I — поперечный срез канальной части воронки.

Обозначения: ci — реснички; cf — филаменты на поверхности мембраны; cr — микроворсинки циртоцита; dm — плотный материал в микроворсинках верши; f — protonephridial funnel; fr — микроворсинки воронки; lep — лептотрихии; m — субтегументальная мускулатура; mv — округлые микроворсинки; nu — ядро; r — корешки ресничек; w — верша. Масштаб: A — 5 μm, B, H, I — 1 μm, C, D — 0.5 μm, E — 2 μm, F — 500 nm, G — 10 μm.

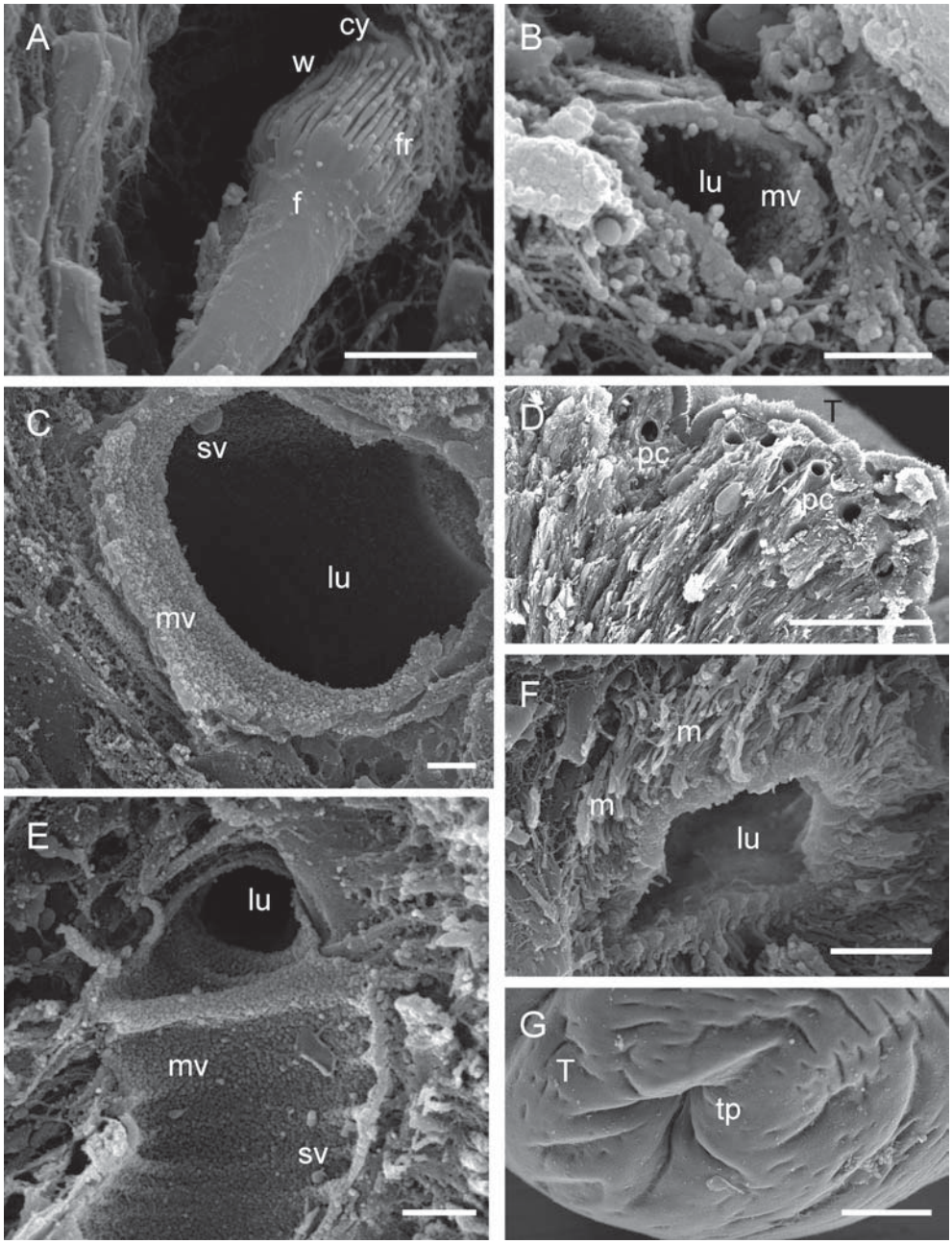

Fig. 4. Elements of excretory system of *Pyramicocephalus phocarum* (SEM). A — the protonephridium located in the parenchyma; B — the 1st order canal; C — the peripheral 2nd order canal with microvilli (mv) and secretory vacuoles (sv); D — peripheral 2nd order canals in subtegument; E — mutually perpendicular 2nd order canals in the scolex; F — the main excretory canal with muscular sheath; G — terminal pore.
Abbreviations: pc — 2nd order canals; cy — cyrtocyte; f — protonephridial funnel; fr — funnel ribs; lu — lumen; m — muscles of the main excretory canal; mv — microvilli; sv — secretory vacuole; T — tegument; tp — terminal pore; w — weir. Scale bars: A–C, E — 2 μm, D — 50 μm, F — 5 μm, G — 1 mm.

out organelles and numerous detached vacuoles (which sometimes may be very large, up to 4 μm in diameter). The basal plasma membrane of the epithelium of the 2nd order canals is folded. ECM adheres plasma membrane; it consists of thin fibers and forms a thick layer (0.8–2 μm) which may be noted as *lamina reticularis.* The *lamina densa* are not observed. Thin irregular muscle bundles are found under the basal plate.

At the posterior section of the body the 2nd order canals open into the reservoir of the terminal pore. At this point, the membranes of the excretory epithelium and the tegument of the terminal pore form an annular septate junction surrounding each excretory pore (nephropore) (Fig. 5D, E). Each canal permeates the tegument, from which it is separated by an annular septate junction contact. The 2nd order canals pour their contents through nephropores into the reservoir of the terminal pore. From this pore the liquid is excreted into the host.

Two main excretory canals occupy a lateral position at the border of the central parenchyma and extend along the entire body of the plerocercoid (Fig. 6A, B). In the scolex, the central canals are interconnected by rare transverse anastomoses (Figs 1A; 6D). The central canals are closely associated with the nerve cords and the brain (Fig. 6A, D): they are always located medial to the nerve cords. The neurons of the brain lobes may adjoin the canal wall in the most anterior part of the scolex. The lumen of the central canals varies from 4 to 22 μm in diameter, has an oval shape and thickened walls. The apical surface of the canal is folded.

The nuclei of the excretory epithelium of the central canals are immersed in the parenchyma up to 5 μm in depth, and have long cytoplasmic processes connecting them with the apical cytoplasm (Fig. 6E, F). The cytoplasm of cytones contains rod-shaped electron-dense bodies, mitochondria, glycogen granules, ribosomes, and vesicles. Cytoplasmic processes are filled with rod-shaped electron-dense bodies. Perikaryon cytoplasm give rise numerous basal processes of different diameters, which form the extended gap junctions with parenchymal cells.

The anuclear cytoplasm of the canal wall is differentiated into two distinct layers, apical and basal (Fig. 6F). The apical cytoplasmic layer is electron-dense, homogeneous, 0.16–1.1 μm thick. The basal layer contains numerous rod-shaped electron-dense bodies oriented parallel to the basal membrane. The thickness of basal layer is about 0.5 μm. The apical membrane forms round microvilli; at the same time, in many areas of the apical membrane round microvilli are absent.

The basal membrane of the epithelium is slightly folded. ECM is represented by loose fibers and filaments and forms the 0.3 μm thick layer of lamina fibroreticularis. The lamina densa is not observed. Several layers of myofibrils are located under the basal lamina; the myofibrils diameter varies from 1 to 7 μm. Muscle fibres contain thick myosin fibrils and thin actin filaments. The actin myosin ratio is not regular; moreover, there are only actin filaments in some myocytoplasm areas.

At the posterior end of the body, the main excretory canals lose part of the myofibrils, the epithelium of the canal wall is surrounded by irregular and loose muscle bundles (Fig. 7A, B). The cytones of the excretory epithelium in the caudal zone are submerged to a depth of 2–3 μm from the canal wall. The posterior canal cytoplasm is also differentiated into 2 layers as in the anterior or central body parts (Fig. 6A).

Рис. 4. Элементы экскреторной системы плероцеркоида *Pyramicocephalus phocarum* (СЭМ). A — протонефридий в паренхиме; B — канал 1-го порядка; C — периферический канал 2-го порядка с округлыми микроворсинками (mv) и секреторными вакуолями (sv); D — периферические каналы 2-го порядка в субтегументе; E — взаимоперпендикулярные каналы 2-го порядка в сколексе; F — главный экскреторный канал с мышечной обкладкой; G — терминальная пора.

Обозначения: pc — периферический канал 2-го порядка; cy — циртоцит; f — протонефридиальная воронка; fr — микроворсинки воронки; lu — просвет канала; m — мышцы главного экскреторного канала; mv — микроворсинки; sv — секреторная вакуоль; T — тегумент; tp — терминальная пора; w — верша. Масштаб: A–C, E — 2 μm, D — 50 μm, F — 5 μm, G — 1 mm.

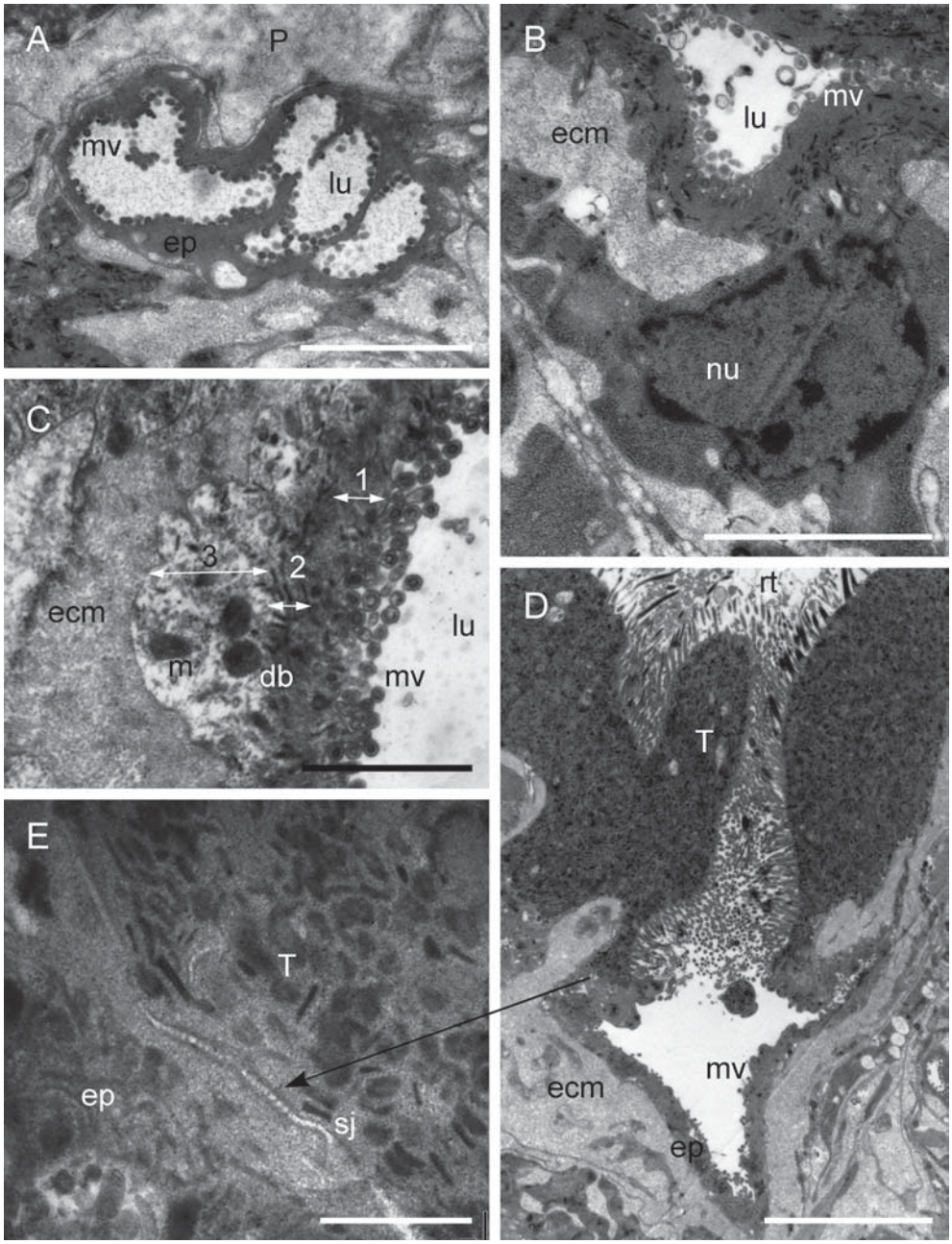

Fig. 5. Ultrastructure of the peripheral canal net in the middle and caudal body parts of *Pyramicocephalus phocarum* (TEM). A — the narrow 1st order canal with thin epithelial wall and numerous microvilli; B — the perikarya of the 2nd order canal; C — the stratification of the wall cytoplasm into the apical (1), medial (2) and basal (3) layers in the 2nd order canal; D — the nephropore ultrastructure: the connection between the peripheral canal and terminal pore reservoir tegument; E — the septate junction of the nephropore. Abbreviations: db — rod-shaped dense bodies; ecm — basal plate; ep — excretory epithelium; lu — lumen; m — mitochondria; mv — round microvilli; nu — nucleus; P — cells in parenchyma; rt — terminal pore reservoir; sj — septate junction; T — terminal pore tegument. Scale bars: A–B — 2 μm, C — 1 μm, D — 5 μm, E — 50 nm.

Excretory bladder. The main excretory canals are connected to the excretory bladder in the posterior part of the body (Fig. 1E). Syncytial epithelium lines the wall of the excretory bladder (Fig. 7C). The cytoplasm of the excretory bladder is differentiated into 2 layers: the basal layer contains numerous rod-shaped electron-dense bodies; the apical layer is homogeneous, electron-dense, lacking organelles and occasionally containing round microvilli. There are extended areas without microvilli, with a smooth apical membrane. Thin layer of ECM occurs beneath a basal plasma membrane; lamina densa is not observed. The nuclei of the excretory epithelium lie at a depth of 1–2 μm; they are less deeply immersed in the parenchyma than the nuclei of the main canals. There is no regular muscular sheath.

The excretory bladder opens with a nephropore into a folded reservoir of the terminal pore (Figs 1E; 7D, E). The excretory epithelium of the bladder contacts with the tegument of the terminal pore through the annular septate junction surrounding the nephropore.

Terminal excretory pore. There is one terminal pore (Fig. 4G) at the posterior end of the *P. phocarum* plerocercoid. The terminal pore leads into a dilated terminal reservoir lined with tegument (Fig. 7D). The observation of live plerocercoids revealed that the excretory bladder and the terminal reservoir can expand significantly, once getting filled with liquid, and then shrink tightly freeing itself from it. The terminal pore can tightly close and separate the reservoir from the external environment (Fig. 4G). The inner surface of the reservoir is folded. The tegument has numerous cylindrical microtriches (filitriches) and rare conical hook-shaped ones (spinithrix microtriches). The distal cytoplasm of the tegument has a typical structure, including rod-shaped and disc-shaped electron-dense bodies. However, the reservoir tegument lacks mitochondria and vacuoles. The tegument of the terminal pore often forms cytoplasmic protrusions into the reservoir lumen, about 0.3–0.6 μm in size, devoid of any organoids.

A sensory organ was found in the tegument of the terminal pore (Fig. 7F). The unciliated receptor has an expanded bulb, a kinetosome, a wide complex root, and mitochondria. The membrane of the receptor bulb forms a septate junction with the basal membrane of the tegument. There is a thick supporting ring under the septate junction.

Discussion

Cyrtocytes. Cyrtocyte, or a flame cell, is the initial segment of the protonephridial excretory system in the cestodes. Cyrtocytes have a common structure within different groups of neodermata (Wilson, Webster, 1974; Rohde, 1990; Xylander, 2001; Swiderski *et al*., 2007; Poddubnaya *et al.,* 2020; Biserova *et al*., 2021); however, at the ultrastructural level the degree of plasticity is high. Previous investigations (Biserova *et al*., 2021) showed that the cyrtocytes of the *P. phocarum* plerocercoid have one bundle of cilia, as in other diphyllobothriids, for instance *D. dendriticum* (Lindroos, 1983; Kutyrev *et al*., 2017), *D. latus* (Barcak *et al*., 2019). Single ciliary complexes are described in cestodes of Pseudophyllidea *sensu lata* (Kuperman, 1988), Bothriocephalidea (Korneva, 1994), Tetraphyllidea (McCullough, Faiweather, 1991),

Рис. 5. Ультраструктура периферической сети каналов в средней и задней частях тела *Pyramicocephalus phocarum* (ТЭМ). A — мелкие каналы 1-го порядка с эпителиальной стенкой и многочисленными микроворсинками; B — перикарион канала 2-го порядка; C — стратификация цитоплазмы стенки канала 2-го порядка на апикальный (1), медиальный (2) и базальный (3) слои; D — строение нефропора: связь между периферическим каналом и тегументом резервуара терминальной поры; E — септированный контакт нефропора.

Обозначения: db — палочковидные плотные тела; ecm — базальная пластинка; ep — экскреторный эпителий; lu — просвет канала; m — митохондрия; mv — округлые микроворсинки; nu — ядро; P — клетки паренхимы; rt — резервуар терминальной поры; sj — септированный контакт; T — тегумент терминальной поры. Масштаб: A–B — 2 μm, C — 1 μm, D — 5 μm, E — 50 nm.

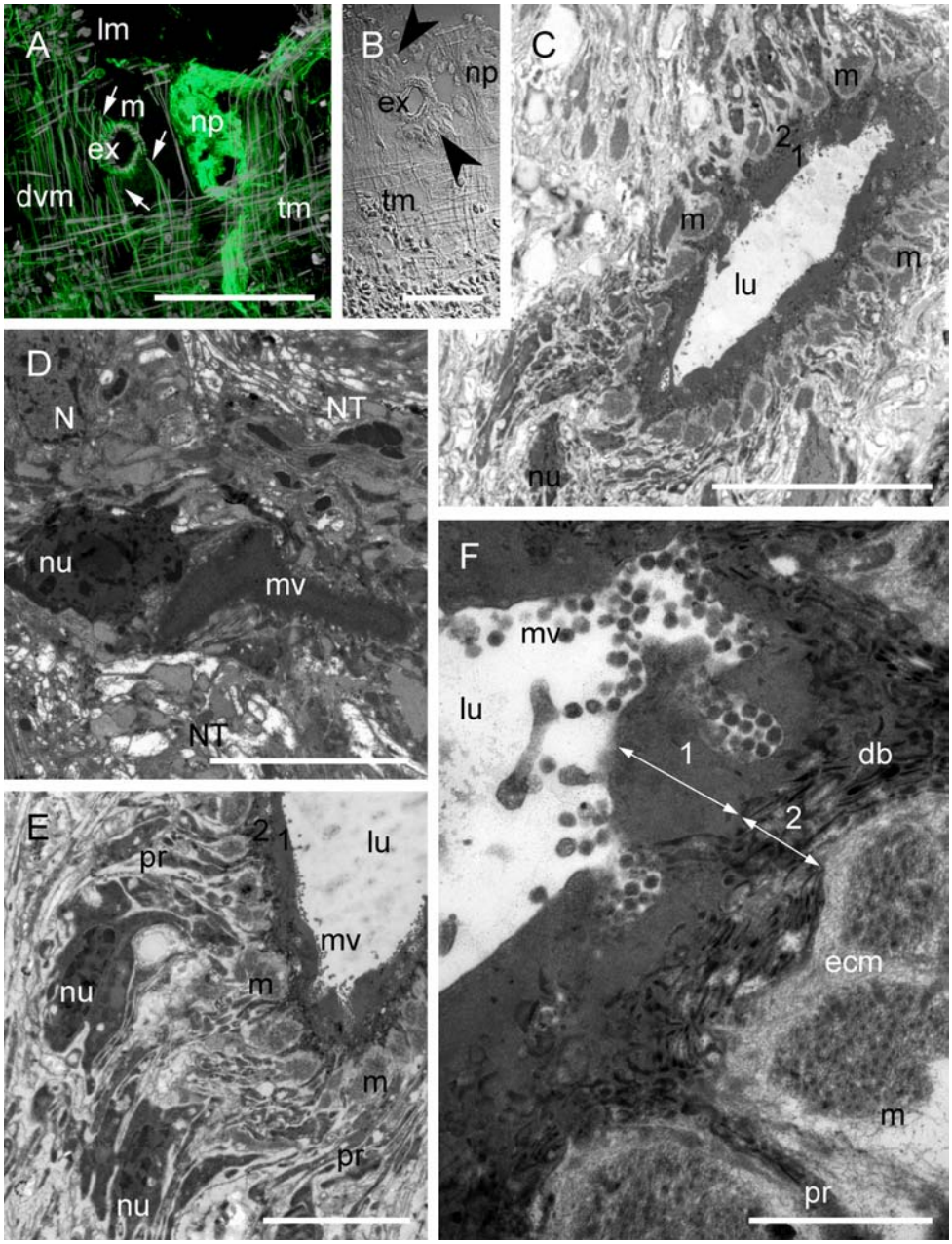

Fig. 6. Structure of the main excretory canals of *Pyramicocephalus phocarum* plerocercoid. A — muscular (grey) and nervous (green) fibres in the wall of the main canal in the scolex (phalloidin — grey, tubulin — green, confocal microscopy, maximum projection; full-colour version see online); arrows mark neurites innervating main canal muscles; B — the wall structure; note muscle fibres and nuclei of epithelial syncytium (arrowheads) of the main canal (DIC light microscopy); C — cross section of the main excretory canal with thick muscular fibres in the wall (TEM); D — the transverse anastomosis underlying the median brain commissure in the scolex with numerous neurites in the wall (TEM); E–F — ultrastructure of the excretory epithelium with bilayered apical cytoplasm (1,2), submerged cytones with long processes, the basal plate and muscular fibres (TEM).

Litobothriidea (Gallagher *et al*., 2017), and Cyclophyllidea (Howells, 1969; Coil, 1991; Swiderski *et al*., 2007; Valverde-Islas *et al*., 2011; Rozario, Newmark, 2015). Cyrtocytes with several ciliary complexes are found in the basal groups of cestodes. For example, *Khawia armeniaca* (Caryophyllidea) has cyrtocytes with 4 ciliary complexes (Poddubnaya, 2003). In adult *Amphilina foliacea* regular cyrtocytes with one ciliary tuft can be found (Xylander, 1992) and those with star-like cyrtocytes, which have up to 6 ciliary complexes per cell (Poddubnaya *et al*., 2020). Significant differences in the structure of cyrtocytes of different cestode taxa mainly concern the set of organelles in the soma, the structure of the rootlet system, the number and length of cilia, the presence or absence of external and internal leptotriches (Xylander, 1992; McCullough, Faiweather, 1991; Pospekhova *et al*., 1993; Poddubnaya, 2003; Poddubnaya *et al*., 2020). Cyrtocytes of some cyclophyllids are located in groups (Pospekhova *et al*., 1993). Cyrtocytes of *P. phocarum* plerocercoid are located separately; each cell forms one weir with one ciliary tuft. No differences in the ultrastructure of cyrtocytes were found in anterior and posterior body parts of *P. phocarum*. The presence of cyrtocytes with several ciliary complexes was interpreted some authors as a tendency towards organ polymerization (Poddubnaya, 2003). Given the ability of tapeworms to constantly grow and increase the linear dimensions of both larvae and adult worms, the mechanism of doubling the basal bodies and axonemes of ciliary cells should be kept in mind.

Currently, the main subject for scientific debate is the way the cyrtocyte is connected to the protonephridial canal. For the *Moniezia expansa* (Cyclophyllidea), Howells (1969) have showed, that the weir ribs are interconnected by desmosomes, and in the same time, between the duct ribs and the surface of the cyrtocyte velum there is a triangular pore, or nephrostome, which extends from the intercellular space of the parenchyma to the lower limit of the velum, and hence into the terminal chamber. These pores may be considered nephrostomes and the system therefore is not protonephridial as defined before (Howells, 1969). In more recent studies of the protonephridial complex ultrastructure in other cestodes do not have data on nephrosomes. Septate junctions have been described in Gyrocotylidea (Poddubnaya *et al*., 2020) and Cyclophyllidea (Pospekhova *et al*., 1993). In contrast, in the weir of the *P. phocarum* plerocercoid lack the septate junctions and desmosomes. The intercellular connection of the weir ribs is ensured by zip-like connected fibrillar components on

Abbreviations: 1 — apical layer of cytoplasm; 2 — basal layer of cytoplasm; db — rod-shaped dense bodies in excretory epithelium; dvm — dorso-ventral muscles of the scolex; ecm — basal plate; lu — lumen; lm — longitudinal muscles of the scolex; mv — rounded microvilli; m — canal muscles; N — neuron; nu — nucleus of epithelial cyton; np — neuropile of the nerve cord; NT — neurites of the brain commissure; pr — epithelium cyton processes; tm — transverse muscles of the scolex. Scale bars: A, C — 10 μm, B, D, E — 5 μm, F — 1 μm.

Рис. 6. Строение главных экскреторных каналов плероцеркоида *Pyramicocephalus phocarum*. A — мышечные (серые) и нервные (зеленые) волокна в стенке главных каналов в сколексе (фаллоидин — серый, тубулин — зеленый, конфокальная микроскопия, максимальная проекция; см. цветную версию онлайн); стрелки указывают на нейриты, иннервирующие мышцы главных каналов; B — строение стенки; изображены мышечные волокна и ядра эпителиального синцития (стрелки) главных каналов (DIC световая микроскопия); C — поперечный срез главных экскреторных каналов с толстыми мышечными волокнами в стенке (ТЭМ); D — поперечный анастомоз, подстилающий медианную мозговую комиссуру в сколексе с многочисленными нейритами в стенке (ТЭМ); E–F — ультраструктура экскреторного эпителия с двуслойной апикальной цитолазмой (1, 2), погруженными цитонами с длинными отростками, базальной пластинкой и мышечными волокнами (ТЭМ).

Обозначения: 1 — апикальный слой цитоплазмы; 2 — базальный слой цитоплазмы; db — палочковидные плотные тела в экскреторном эпителии; dvm — дорсо-вентральные мышцы в сколексе; ecm — базальная пластинка; lu — просвет канала; lm — продольные мышцы в сколексе; mv — округлые микроворсинки; m — мышцы канала; N — нейрон; nu — ядро цитона экскреторного эпителия; np — нейропиль нервного ствола; NT — нейриты мозговой комиссуры; pr — отросток цитона экскреторного эпителия; tm — поперечные мышцы сколекса. Масштаб: A, C — 10 μm, B, D, E — 5 μm, F — 1 μm.

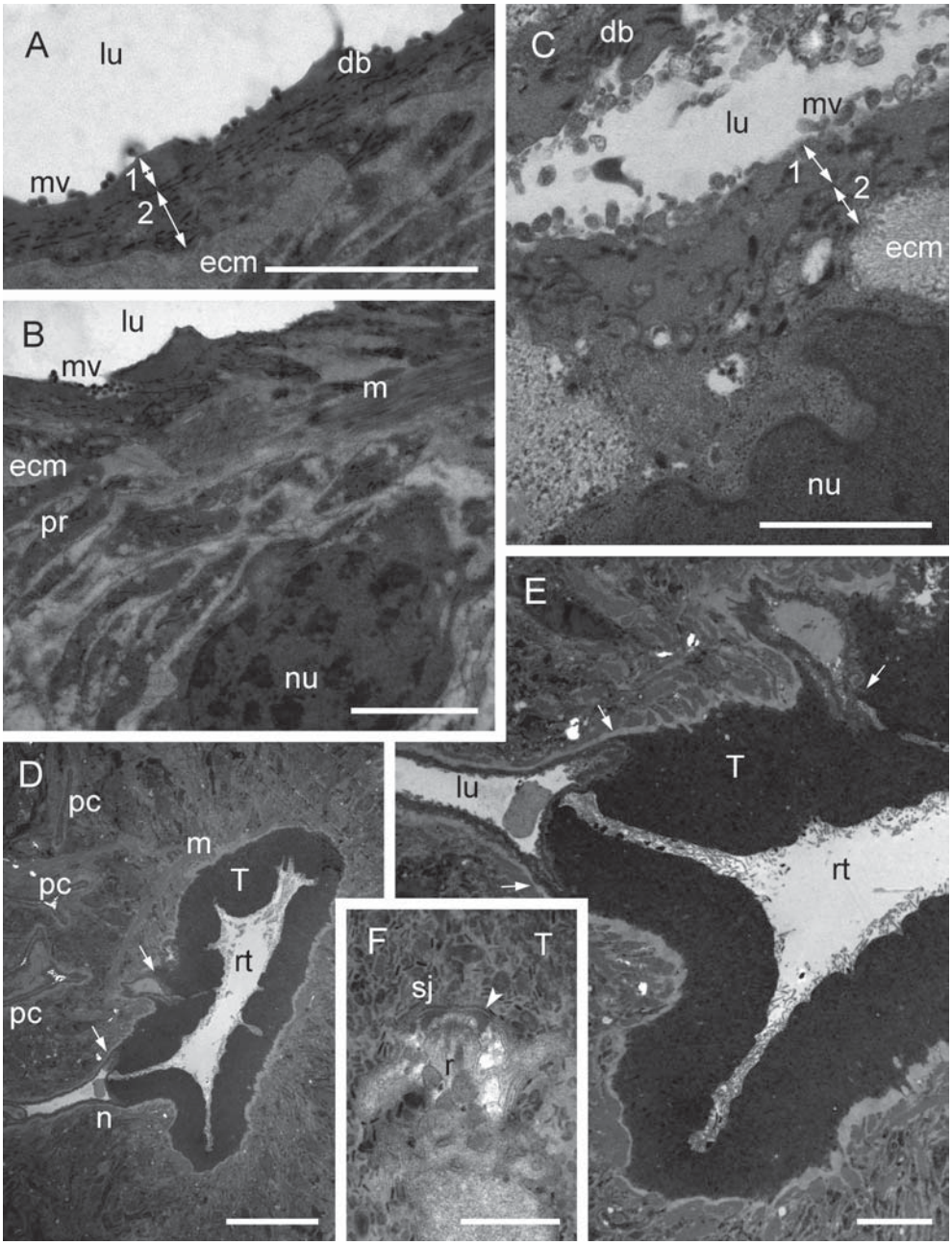

Fig. 7. Caudal (terminal) part of the excretory system of *Pyramicocephalus phocarum* plerocercoid (TEM). A–B — main excretory canal above fusion with excretory bladder; cytoplasm has two layers with rare microvilli and poorly developed muscles in the canal wall; C — epithelium of the excretory bladder, differentiated into 2 layers; D — reservoir of the terminal pore on cross section; E — magnified part of figure 7D, two nephropores (arrows) in the tegument; F — unciliated receptor with electron dense supporting ring (arrowhead) and rootlet in the tegument of the excretory pore;

Abbreviations: 1 — apical layer of cytoplasm; 2 — basal layer of cytoplasm; db — rod-shaped dense bodies in excretory epithelium; ecm — basal plate; lu — lumen; m — muscles; mv — rounded microvilli; nu — nucleus; pc — the 2nd order

the membrane surface (Biserova *et al*., 2021). This fibrillary layer (22 nm) forms the so-called *zonula adherens* between the ribs membranes and correspond an actin ring of the protonephridium (Fig. 3G). Actin ring in protonephridium was found also in *D. dendriticum* (Wahlberg, 1998). *Zonula adherens* was found between the flame cell and adjoining duct in *Trilocularia acanthiaevulgaris* (McCullough, Fairweather, 1991) and *Gyrocotyle urna* (Poddubnaya *et al*., 2020). The abovementioned data confirm that cyrtocytes are separate cells and that the protonephridial complex is bi-cellular, which contradicts the syncytial hypothesis by Kuperman (1988).

Cyrtocyte function. The mechanism of cestode cyrtocyte functioning has not yet received all the attention it deserves. According to Valverde-Islas and co-authors (Valverde-Islas *et al*., 2011), who studied *Taenia solium* larvae, fluid gets transported from the parenchyma to the excretory canal directly through the cytoplasm of cyrtocytes. The authors suggested that the excretory vesicles are released into the space between the basal bodies of the cilia. In *P. phocarum* cyrtocytes, vesicle exocytosis into the weir cavity has been observed neither from the surface of leptotrichia nor from the surface of the ribs. Since there is no free membrane space between cilia, and the bundle of axonemes has a very dense arrangement, the existence of exocytosis at the base of the cilia is physically unlikely. Therefore, the mechanism of cyrtocyte functioning described by Valverde-Islas *et al*. (2011) does not seem viable. Another point to be considered is the active beating of the cilia located inside the weir. At the same time, serotonin contributing to the functioning of cyrtocytes (Biserova *et al*., 2021) and the actin collar surrounding the weir allows us to suggest a different mechanism. According to our hypothesis, the cyrtocytes of *P. phocarum* are located freely in the intercellular space of parenchyma; intercellular fluid filters through a 22 nm thick molecular sieve of the glycocalyx, covering the ribs of the weir (Biserova *et al*., 2021). That mechanism is quite consistent with the previous hypothesis concerning ultrafiltration of the intercellular fluid through the weir presupposing the separation of water and low-molecular-weight compounds from macronutrients (Wilson, Webster, 1974; Ruppert, Smith, 1988; Swiderski *et al*., 2007). Such an ultrafiltration mechanism presupposes that the weir cavity should be completely isolated from the extracellular space, which is achieved by closing the opposed membranes of the ribs on the principle of zip-connection. The collar of polymerized fibrillar actin appears to maintain the diameter of the protonephridial funnel unchanged independently from the active ciliary flame beating. Obviously, the main function of the constant volume of the weir and protonephridial funnel preserves the negative fluid pressure, which is created by the cilia's beating. The immunoreactivity of cyrtocytes to serotonin is probably associated with the role of serotonin in ensuring the movement of the cilia, which is typical mainly of invertebrates. Apart from that, we

canal; pr — epithelium cyton processes; r — rootlet; rt — terminal pore reservoir; sj — septate junction; t — terminal pore tegument. Scale bars: A, B — 2 μm, C, F — 1 μm, D — 20 μm, E — 5 μm.

Рис. 7. Каудальная (терминальная) часть экскреторной системы плероцеркоида *Pyramicocephalus phocarum* (ТЭМ). A–B — главный экскреторный канал над местом слияния с мочевым пузырем; цитоплазма двуслойная с редкими микроворсинками и слабо развитой мускулатурой стенки канала; C — эпителий мочевого пузыря с цитоплазмой, дифференцированной на 2 слоя; D — резервуар терминальной поры на поперечном срезе; E — увеличенный фрагмент изображения 7D, два нефропора (стрелки) в тегументе; F — безресничный рецептор с электронноплотным опорным кольцом (стрелка) и корешком в тегументе экскреторной поры.

Обозначения: 1 — апикальный слой цитоплазмы; 2 — базальный слой цитоплазмы; ecm — базальная пластинка; db — палочковидный плотные тела в экскреторном эпителии; lu — просвет канала; m — мышцы; mv — округлые мироворсинки; nu — ядро; pc — каналы 2-го порядка; pr — отростки цитонов; r — корешок; rt — резервуар терминальной поры; sj — септированный контакт; t — тегумент терминальной поры. Масштаб: A, B — 2 μm, C, F — 1 μm, D — 20 μm, E — 5 μm.

hypothesize that prostaglandins found in *D. dendriticum* cyrtocytes (Kutyrev *et al*., 2017) can act as a vasodilator and contribute to an increase in the lumen of the small 1st order canals. The actin filament collar in the protonephridium has also been described in *D. dendriticum* (Wahlberg, 1998; Kutyrev *et al*., 2017) and *T. solium* (Valverde-Islas *et al*., 2011).

Canal architecture. In the basal groups of cestodes, there is no common architecture of large canals. In amphilinids, a very complex net of anastomosing canals is observed throughout the body. Though the main longitudinal canals are not distinguished, in different genera there is both a network of canals (*A. foliacea*) and a set of longitudinally oriented canals of excretory system (*Schizochoerus liguloideus*); at the same time, in the *Gephyrolina paragonophora* two lateral canals are connected by transverse canals (Dubinina, 1982; Xylander, 1992). *G. urna* also lacks main canals: its canal system consists of two types of position and ultrastructure. The superficial (peripheral) network of canals is characterized by the presence of lateral ciliary tufts in the epithelial wall (Xylander, 1992; Poddubnaya *et al*., 2020). The deeper (proximal) network of canals has terminal flame cells, and there are lamellae on the inner surface of the canal epithelium. Thus, the canal system of gyrocotylids differs significantly from amphilinids and eucestodes, which lack the ciliated epithelium at every stage of development. The structure of the excretory system was distinguished by some authors as one of the cestodes autapomorphies (Xylander, 1992, 2001).

According to Coil (1991), the simplest type of the excretory canal architecture among eucestodes was found in cyclophyllids. In the adult *M. expansa*, longitudinal, transverse, and primary collecting canals, as well as thin terminal tubules were described (Howells, 1969). In *Cysticercus taeniae-taeniaeformis* (larvae of *Taenia taeniaeformis*), dorsal and ventral excretory canals and transverse canals in the body were outlined (Rees, 1951). In the scolex, the longitudinal canals are interconnected and form a complex network. In cysticercoid bladder the ventral and dorsal canals branch also to form a network. In *Hymenolepis diminuta* transverse anastomoses were found to connect only the ventral longitudinal canals. The ventral canals connect with a network of thin terminal tubules, each terminal tubule ends with a flame cell (Rozario, Newmark, 2015). Paired ventral and dorsal canals were found in representatives of different orders of eucestodes, such as Trypanorhyncha (Rees, 1988), Tetraphyllidea (Rees, 1953; McCullough, Fairweather, 1991) and Litobothriidea (Gallagher *et al*., 2017). They branch in the scolex into a network of smaller canals. The degree of development of small canals network is associated with the development of attachment organs in a particular group of cestodes. The connection of the ventral and dorsal canals in the scolex can be carried out by both transverse and dorsoventral anastomoses. *D. dendriticum* plerocercoid includes three compartments of the canal system at the macrolevel: the peripheral network of canals, the capillary network in the scolex, and the main excretory canals connected to the excretory bladder (Lindroos, Gardberg, 1982). The canal system of the *P. phocarum* plerocercoid is also represented by the capillary network of the 1st order canals, a peripheral network of the 2nd order canals, and longitudinal main canals, and, in general, exhibits an architecture typical of diphyllobothriids. Thus, diphyllobothriidean tapeworms have a more differentiated excretory system as compared to the basal groups of cestodes (Amphilinidea and Gyrocotilidea); moreover, this group has both a peripheral network and longitudinal main excretory canals with a muscular support.

Caudal elements of the excretory system. Caudal parts of the excretory system include the excretory bladder, nephropores, and terminal excretory pore. Various terms are used in the literature to refer to the caudal parts of the excretory system: *nephropores* (Biserova *et al*., 2021) or *nephridiopores* (Xylander, 1992; Korneva *et al*., 1998), as well as *terminal excretory pore* and *bladder* (Lindroos, Gardberg, 1982, 1983; Biserova *et al*., 2021). Moreover, these terms are defined differently in different arti-

cles. In cestodes, for instance, different structures are called the *excretory bladder*. Thus, the excretory bladder of the procercoids of *D. latum**, *D. dendriticum, D. osmeri* (Malmberg, 1972) indicates the fold/invagination of the tegument at the posterior end of the body. At the same time, however, in the procercoid of *Triaenophorus nodulosus,* the excretory bladder is a saccular dilatation of the excretory system canals lined with excretory epithelium (Korneva *et al*., 1998). The term *nephropore* or *nephridiopore* is also ambiguous. For example, Xylander (1992) describes nephropores as openings on the surface of the body and considers them synonymous with excretory pores. Lindroos and Gardberg (1982) in their descriptions confine themself by using the term *excretory pore*, which is understood as an opening on the surface of the body. In early larvae (coracidium) *nephropore* is a canal formed by a special cell, in procercoid it is an opening on the body surface (Korneva *et al*., 1998). Several representatives of diphyllobothriids (*D. latum**, *D. osmeri, D. dendriticum*) studied by light microscopy have several nephropores described (Malmberg, 1971).

The present study shows that the caudal parts of the excretory system of the plerocercoid of *P. phocarum* are more complex than it was previously thought. The research proves that the excretory bladder is a saccular expansion formed by the excretory epithelium at the posterior end of the plerocercoid body. The nephropore, or nephridiopore, is the point where the excretory epithelium and the tegument contact with each other. The terminal excretory pore is a terminal opening at the posterior end of the body, formed by deep invagination of the tegument. The excretory bladder and nephropores open into the reservoir of the terminal pore.

The number and position of terminal excretory pores vary in different groups of cestodes. For example, gyrocotylids have a pair of excretory pores protruded onto the surface of the tegument near the anterior end of the body (Malmberg, 1974; Xylander, 1992). Amphilinids (*A. foliacea*) have only one excretory pore at the posterior end of the body. The plerocercoid of *D. dendriticum* has the excretory pore at the posterior end of the body (Lindroos, Gardberg, 1982). According to G. Rees, *Cysticercus taeniae-taeniaeformis* lacks the terminal excretory pore; instead, the dorsal and ventral canal networks communicate with the exterior by a few small irregularly placed excretory pores (Rees, 1951). The procercoid of *T. nodulosus*, which has its secondary excretory system formed, has only one single nephropore near the cercomer (Korneva *et al*., 1998). Malmberg (1971) has shown that procercoids of diphyllobothriids (*D. dendriticum, D. latum**, *D. osmeri*), once getting their secondary excretory system completed, acquire several canals that communicate with the lateral and medial surfaces of the body. The excretory bladder is not yet formed at the procercoid stage; it forms at the plerocercoid stage (Lindroos, Gardberg, 1982). If the same mechanism of the formation of the secondary excretory system takes place in the procercoid of *P. phocarum*, it could be assumed that after the cercomer is separated during the plerocercoid development, the nephropores that were initially located on the caudal surface may become invaginated inward together with the tegument. As a result, a reservoir of terminal excretory pore with several nephropores on the inner surface could be formed. The invagination of the caudal tegument with the formation of a reservoir is also found in other cestodes (Malmberg, 1972).

The excretory epithelium. The epithelium of the protonephridial funnel, peripheral and central excretory canals of the *P. phocarum* plerocercoid is a common syncytium. We have not found any evidences of intercellular contacts within the canal system of *P. phocarum* plerocercoid as it was shown in *D. denriticum* (Lindroos, 1983). It is known that the canal epithelium of *T. nodulosus* is formed in a procercoid from separate undifferentiated cells with cell contacts. These cells subsequently fuse with each other and form the syncytial layer of the canal epithelium (Korneva *et al*., 1998). The question whether cell contacts in the excretory epithelium in plerocercoids and adult cestodes do take place still remains controversial. Amphilinids (Xylander, 1992), tetraphyllides (Mc-

Cullough, Fairweather, 1991), and bothriocephalids (Kuperman, 1988; Korneva, 2007, 2013) have a syncytial lining of canals with nuclei submerged under the basement membrane. Some adult cyclophyllidean cestodes have intercellular contacts between different canals (Pospekhova *et al*., 1993), while other authors, on the contrary, state the absence of such contacts (Kabbany, 2009). The canal epithelium of plerocercoid *D. dendriticum* is syncytial, although some authors note that desmosomes are located at the fusion of small canals with larger ones; this fact can be explained by larval growth (Lindroos, 1983). It is to be suggested that such differences in structure of the excretory epithelium at different stages of development may indicate a high level of tissue plasticity of cestodes (Korneva, 2007). On the other hand, during larval growth enlargement of the protonephridial duct can either happen by extending a syncytium or by integration differentiating stem cells into the syncytium. The latter would require cell junctions, at least for a short while, before cell membranes initiate integrating stem cells, that differentiated into protonephridial canal cells, into the syncytium. We have never seen undifferentiated cells in the wall of the 2nd order or main excretory canals of *P. phocarum* plerocercoid. Cell junction, thus, may therefore be only temporary structures that are related to growth in early plerocercoid development.

The cytoplasm of the excretory epithelium of the *P. phocarum* plerocercoid is vertically stratified. Moreover, the nature of the vertical stratification of the cytoplasm in the plerocercoid *P. phocarum* changes from the protonephridial funnel to the nephropore. In addition, along with the vertical stratification of the cytoplasm, the contents of the canal lumen and the density of the round microvilli on the canal surface are gradually changing. Microvilli may take place in all parts of the excretory epithelium yet largely concentrate in the 2nd order canals in the rostral part of the body. Our study reveals a high secretory activity of the epithelium; in the caudal segment of the canal system, there are detachable vacuoles that are located freely in the lumen of the canal. The immersion depth of perikarya in the parenchyma also changes; the most distant cytons are located in the central longitudinal canals; such a location depends on the development of the multilayer muscular sheath of the canals.

Excretory epithelium function. It has been shown that the contents of the canals of *H. diminuta* have a high concentration of lactic acid, which proves the excretory function of the canal epithelium (Webster, Wilson, 1970). According to the abovementioned authors, the excretory system has lost its ability to osmoregulation because no evidence of hypoosmotic fluid was found in the system. It might correlate with the osmotically constant environment of the host, which a plerocercoid inhabits. The role of osmoregulation is provided by the tegument (Vinogradov *et al*., 1982). The way distribution (Lindroos, Gardberg, 1982; Kuperman, 1988) or the drainage function of the epithelium (Pospekhova *et al*., 1993) are performed has also been put forward. The analysis of the ultrastructure of the canals suggests that the excretory epithelium may combine several different functions. They could replace each other as the fluid moves from protonephridium to the excretory bladder. This assumption is supported by the histochemical studies of various parts of the excretory system. Thus, the collecting tubules of *Raillietina cesticillus* show an intense reaction to alkaline phosphatase and ATPase (Porshad, Guraya, 1977). In addition, the absorption of glucose and the transport of lactate and uric acid in various parts of the excretory system has been described by Webster (1972). Change of function during fluid moves through the canals, associated with the structure of the walls of these channels, has also been shown for other types of animals — primarily for vertebrates (Gambarian, 1985; Ojeda *et al*., 2006).

In addition, the importance of circulatory function of the excretory system should be highlighted (Kuperman, 1988). The pair of main longitudinal canals of the plerocercoid *P. phocarum* has a well-developed muscular wall. The main canals are suggested to play the

role of the main distribution pathways creating a directed flow to the excretory bladder. The muscular sheath formed by several layers of subtegumental muscles is present close to nephropores, as well as under the tegument of the terminal pore. The lumen of the terminal excretory pore gets compressed and then released from the contents owing to the contraction of subtegumental muscles. Plerocercoid of *D. dendriticum* is also characterized by powerful musculature of the central longitudinal canals, and poorly developed musculature in the wall of the peripheral excretory canals (Wahlberg, 1998; Biserova *et al*., 2014).

Innervation of the excretory system. In the course of our study free nerve endings in the form of unciliated receptors were found in the tegument of the *P. phocarum* terminal pore. Similar structures were detected in the scolex tegument (Mustafina, Biserova, 2017; Biserova *et al*., 2022) which may act as mechano-tactile sensory organs sensitive to tegument stretching. The musculature of the central canals apparently may be immediately regulated by the central nervous system. For example, a GABA-like IR was found in the wall of the main canals of *D. dendriticum* (Biserova *et al*., 2014; Biserova, Kutyrev, 2014). In *P. phocarum* plerocercoid the musculature of the main canals gets innervated by the neurites of the main nerve cords (Fig. 6D). A close relation of the main canals with the nervous system and brain architecture was found in many cestodes (Rees, Williams, 1965; Rees, 1966, 1988; Biserova, 1997; Biserova, Salnikova, 2002; Biserova, Gordeev, 2010; Biserova, Korneva, 2012). The basal processes of excretory epithelium pericarya often surround nerve cells, participate in brain metabolism and serve as glia-like structures in the nervous system of some cestodes (Biserova *et al*., 2010).

Thus, the excretory system of the *P. phocarum* plerocercoid contains two types of structures at the cytological level. It includes cyrtocytes (flame cells) and the syncytial excretory epithelium. The excretory system is isolated from other cells by a layer of loose extracellular matrix. Filtration begins in the protonephridial two-cell complex. The flame cell and the protonephridial funnel are separated by intercellular filaments forming the zonula adherens. The filtration fluid moves through the canals of different diameters and orders into the excretory bladder, which opens with a nephropore into the terminal excretory pore at the posterior end of the body and is discharged outside, in the host. The characteristic feature of the excretory system of *P. phocarum* plerocercoid is the presence of independent nephropores of the 2nd order peripheral canals. All detected nephropores are separated by annular septate junctions from the tegument. The close connection between the excretory system and the central nervous system is observed throughout the body. Transverse anastomoses and the main excretory canals pass through the lateral brain lobes and underlie the median brain commissure in the scolex. The main nerve cords are co-localized with the main canals in the body. The processes of neurons innervate the muscular wall of the main canals. There are sensory organs in the form of unciliated receptors in the wall of the terminal pore. Thus, several functions, such as reabsorption, circulation, secretion, and excretion, might be presented in the protonephridial system of *P. phocarum*.

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