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The neuro-glandular brain of the *Pyramicocephalus phocarum* plerocercoid (Cestoda, Diphyllobothriidea): Immunocytochemical and ultrastructural study

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ABSTRACT

A novel type of a complex neuro-glandular brain structure including both nervous and glandular elements and associated with sensory ones is detected in *Pyramicocephalus phocarum* plerocercoid (Cestoda: Diphyllobothriidea), parasite of *Gadus morua* from the White Sea. The brain has two lateral lobes connected by a long cellular median commissure. The brain is tightly surrounded by glandular cells, which receive numerous synapses from the brain neurons. A complex of sensory organs associated with ducts and terminal pores of the frontal glands lies in the scolex tegument. Serotonin, FMRFamide- and GABA-like immunoreactive (IR) neurons are found in the brain, the main nerve cords, and the plexus of the plerocercoid. The innervation of the frontal gland ducts by FMRFamide-IR neurites is detected for the first time proving that they function under control of the nervous system and thus evidencing the eccrine nature of the secretion mechanism. Ultrastructural data show that light, dark and neurosecretory neurons are present in the brain lobes. The median commissure consists of loosely arranged thin parallel axons and several giant and small neurons. The commissure is stratified and penetrated by frontal glandular cells and their processes. Such neuro-glandular morpho-functional brain complex is suggested as a model for Diphyllobothriidae family. Five structural types of sensory organs are described in the scolex of *P. phocarum*; their colocalization with eccrine gland terminals is supposedly specific for Diphyllobothriidae family. Within the order Diphyllobothriidea, there are significant differences in the architecture of the plerocercoid brain at the family level. We suppose homology of giant commissural neurons among Diphyllobothriidea. Differences between diphyllobothriidean nervous system and that of other cestodes are discussed.

1. Introduction

Tapeworms from the order Diphyllobothriidea are widespread in natural habitats parasitizing at their adult stage on warm-blooded animals and humans. The order includes several families and a significant number of cestode species infecting a wide range of host species during their life cycle. Diphyllobothriidean larvae often inhabit muscles and caviar of commercial teleost fish. These tapeworms are the causative agent of diphyllobothriasis with an estimated 20 million cases worldwide (Chai et al., 2005; Dupouy-Camet and Year, (2009); Kutyrev et al., 2017; Scholz et al., 2019). The plerocercoids of *Pyramicocephalus phocarum* (Fabricius, 1780) 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, but cases of human infection have also been reported (Delamure, 1961; Rausch et al., 2010). In addition, the plerocercoids themselves cause significant harm to the organism of the intermediate host - commercial fish.

Tapeworms belong to the Neodermata group; they lack a coelom, as well as digestive, circulatory and respiratory systems. Neodermis (or tegument), muscular and excretory systems have a syncytial organization (Kuperman, 1988; Korneva, 2013). In tapeworm parenchyma, cells of different functional modalities contact each other without forming mono-structural layers. When describing cestode fine morphology, authors usually distinguish the distal cytoplasm of the tegument, the subtegument with submerged cytons, the layers of the integumentary muscles, and the longitudinal and transverse muscles of the body (Coil, 1991).

Fine morphology and architecture of the brain is an important morphological criterion for the evolution of Cestoda and Neodermata in

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general. The absence of clear boundaries between different tissues significantly complicates the study of the architecture and cellular composition of the cestode nervous system. Early studies detected a weakly concentrated orthogon and subtegumental and submuscular plexuses in the tapeworm nervous system (Kotikova, 1976; Kotikova and Kuperman, 1978). Later, detailed studies of cestode histology, ultrastructure and immunocytochemistry (Rees, 1966; Webb and Davey, 1975; Gustafsson and Wikgren,1981; Fairweather and Threadgold, 1983; Gustafsson, 1984; 1990; Biserova et al., 1996; 2000; Halton and Gustafsson, 1996; Halton and Maule, 2004) revealed a complex accumulation of neurons and neuropils which correspond to the brain of Bilateria according to the neuroanatomical glossary (Richter et al., 2010). Data analysis based on 56 species (Biserova, 2016), singled out more prominent concentrations of neurons located in the scolex around the median commissure connecting left and right lateral lobes (*ganglia*). The lateral lobes are the largest lobes in the brain of all cestode species studied. Somas are located on the surface or at some depth in the neuropil. They send their neurites to the median commissure, to the anterior and posterior nerves or *ganglia*, and also to dorsal, ventral and lateral rootlets of the brain. Scoleces with 4 sets of attachment organs (e.g. Trypanorhyncha, Tetraphyllidea, Cyclophyllidea) have distinct double lateral lobes, i.e. four, with two pairs of main neuropils (4). The most complicated brain consisting of 9 lobes occurs in Trypanorhyncha (Rees, 1950; 1988; Biserova and Korneva, 2012; Biserova et al., 2020).

The peripheral nervous system (PNS) is well developed in cestodes (Kotikova, 1976; Kotikova and Kuperman, 1978; Kuperman, 1988). The PNS of eucestodes comprises at least 4 pairs of minor cords (2 dorsal, 2 ventral and 4 sublateral) and nerve plexuses. Well-developed nerve nets exist on the surface of the subtegumental muscles and the longitudinal body musculature, as well as on the dorsoventral and transverse muscles of the central parenchyma in all species studied (Halton and Gustafsson, 1996; Halton and Maule, 2004; Biserova, 2016). These plexuses include both the projections of neurons from the CNS (MCs) and from peripheral neurons situated on the muscle layers and organs. Peripheral neuropils associated with the minor longitudinal cords have been found on the muscular layers of *D. dendriticum* (Gustafsson et al., 1994; Biserova et al., 2014). Muscle innervation is achieved both by peripheral neurons forming a plexus and also by central neurons located in the brain and the MCs. Many peripheral neurons form a rich plexus on the surface of the reproductive system muscles (Gustafsson, 1990; Gustafsson. et al., 1985; 1994; Halton and Gustafsson, 1996). Nerve plexuses of attachment organs of the scoleces originate from the brain and the MCs nerve projections (Fairweather et al., 1990; Halton and Gustafsson, 1996; Halton and Maule, 2004).

Modern studies of diphyllobothriidean tapeworms are mostly dedicated to the study of their morphology (Braten, 1968; Malmberg, 1971; Andersen, 1975; 1977; Lindroos and Gardberg, 1982; Kuperman, 1988; Biserova and Kemaeva, 2012; Mustafina and Biserova, 2017; Yoneva et al., 2018; Barčák et al., 2019; Biserova et al., 2021), the peculiarities of the cytochemical organization of the nervous system (Ohman-James, 1973; Gustafsson, 1990; Biserova et al., 2014), and the molecular aspects of the interaction of the parasite with the host (Biserova et al., 2011; Scharsack et al., 2013; Biserova and Kutyrev, 2014; Kutyrev et al., 2014; 2017; 2019; 2021). Unfortunately, most of the morpho-functional studies were carried out on a very limited range of species, making them unsuitable for a comparative analysis of the nervous system of Diphyllobothriidea within Eucestoda.

The functional aspect of the cestode nervous system remains the least studied (Fairweather and Halton, 1991; Brennan et al., 1993; Halton and Gustafsson, 1996; Halton and Maule, 2004). However, an understanding of the peculiarities of the functioning of the cestode nervous system is necessary to clarify the tapeworm's interaction with the host at the different stages of development. For diphyllobothriideans, dangerous parasites of humans and animals, it is especially important to understand the role of the nervous system in manipulating the host and parasite behavior (Biserova et al., 2011). The tapeworms can influence the host by different means, most noticeably by active molecules secreted by the plerocercoid into the host tissues, first of all secretions of the eccrine glands, and secretory products of the tegument (Kutyrev et al., 2021). Also, there is information about the release of active molecules from the surface of ciliary and non-ciliary free nerve terminals (Biserova and Korneva, 1999; Kutyrev et al., 2017).

The mysterious genus *Pyramicocephalus* Monticelli 1890 of the family Diphyllobothriidae Lühe, 1910 represented by a single species *P. phocarum* is the least studied. There is very little information about the biology and morphology of this species (Rausch and Adams, 2000; Mustafina, 2017a, 2017b). In the scolex of the plerocercoid of *P. phocarum* frontal glands are detected (Mustafina, 2017b); these glands are common for all the species of Diphyllobothriidae (Andersen,1975; Kuperman, 1988; Biserova and Kemaeva, 2012; Kutyrev et al., 2017; Barčák et al., 2019). The frontal glands of *P. focarum* occupy most of the volume of the scolex; their perikarya and reservoirs are packed with secretory granules. The long secretory ducts of the frontal glands are oriented towards the bothrial folds and open through specialized pores (Mustafina and Biserova, 2017). The frontal gland ducts in different cestode species are lined by microtubules and thus show anti-tubulin IR (Davydov and Biserova, 1985; Moreno et al., 2001; Barčák et al., 2019).

In invertebrates, glands are defined as "eccrine" if they are innervated by nerve terminals and release secretions under direct control of the nervous system. Many groups of flatworms have an assembly of glands opening at the frontal end of the body - frontal glands. Tapeworms possess frontal glands in the scolex during their life cycle (Gustafsson and Vaihela, 1981; Kuperman and Davydov, 1982a; b; Davydov and Biserova, 1985; Davydov and Poddubnaya, 1988; Žd'árská and Nebesárová, 1997; Žd'árská et al., 2004; Brunanská, et al., 2000). However, the eccrine nature of these glands is not yet fully proven. Sensory organs co-localized with terminal pores of the frontal glands were found in the tegument of *P. phocarum* (Mustafina and Biserova, 2017) and in other tapeworms (Davydov and Biserova, 1985; Kutyrev et al., 2017; Barčák et al., 2019). But it still remains unknown which neurons control the synthesis of secretory products of the frontal glands and their excretion through the tegument.

The presence of pores and ordered peripheral microtubules in the secretory ducts distinguish the frontal glands of the eccrine type from tegumental secretion. It is assumed that the frontal glands of Neodermata, in particular Monogenea and Cestoda, emit secretions under the control of the nervous system during attachment to the host (Lyons, 1972; El-Naggar and Kearn, 1983; Biserova and Kemaeva, 2012). Considering the role of the frontal glands in the penetration of helminths into the host, this issue is of fundamental importance for the development of new drugs against cestodosis.

The aim of this work is to study the ultrastructural and cytochemical organization of the nervous system of the *P. phocarum* scolex. Such study is necessary for clarifying the morphological characteristics of the genus, represented by a single species, as well as for understanding the evolution of the nervous system of Diphyllobothriidea within Cestoda. In addition, it is important to prove the existence of an innervation of the frontal glands (with presumable eccrine mechanism of secretion), to determine the neurotransmitters specific for these neurons, and to investigate the ultrastructure of synaptic contacts of the neurons with effectors.

2. Materials and methods

The plerocercoids of *Pyramicocephalus phocarum* were extracted from the liver and abdominal cavity of the White Sea cod *Gadus morhua*, caught at the N.A. Pertsov White Sea Biological Station of the Moscow State University and fixed in accordance with the previously developed protocol (Biserova, 2013).

2.1. Immunocytochemistry

Live worms (11 plerocercoids) were fixed in 4% paraformaldehyde (PF) in 0.1 M phosphate buffer (PBS), pH = 7.3 for 10 h at 4 °C; the fixed worms were cut into pieces with a volume of 1–2 mm^3 oriented transversely or longitudinally. The pieces were washed in 0.1 M PBS + 0.03% sodium azide (NaN_3) at 4 °C on a shaker twice for 1 h; then in 0.01 M PBS + 3% Triton x100 + 0.003% NaN_3 at 4 °C on a shaker for 6 h. Pre-incubation was carried out in 5% normal goat serum (NGS) in 0.01 M PBS + 3% Triton x100 + 0.003% NaN_3, 15 h, 4 °C. The prepared tissue was incubated in a solution of antibodies against serotonin (5-HT), FMRF-amide, gamma-aminobutyric acid (GABA), acetylated α-tubulin (αTub) to identify neuroactive molecules and visualize the nervous system.

The following primary antibodies (from SIGMA, USA) were used:

1. Anti-serotonin developed in rabbit (whole antiserum), product number: S5545;
2. Anti-FMRFamide developed in rabbit polyclonal, product number: AB15348;
3. Monoclonal anti-GABA, clone GB-69 mouse ascites fluid, product number: A0310;
4. Monoclonal anti-acetylated-tubulin, clone 6–11B-1, product number: T6793;

Part of the material was additionally stained with Phalloidin TRITC (Sigma P1951) to detect fibrillar actin and visualize actin myofibrils. Part of the fixed material (4 plerocercoids) was cut with a cryostat (Zeiss). Serial frozen 7 μm thick sections were laid out on glasses, dried (1 day), then stained with antibodies against serotonin (5-HT), RF neuropeptides (FMRFamide), acetylated α-tubulin (αTub), and with phalloidin-TRITC to identify specific compartments of the nervous system and areas of innervation of muscle fibers.

The following protocols (Biserova, 2013) were used for incubation in a cocktail of primary antibodies at 4 °C on a shaker:

a) anti 5-HT (serotonin) rabbit 1: 7000, + anti-tubulin mouse 1: 1000 in 0.01 M PBS + 1% TritonX100 + 1% NGS + 0.003% NaN_3
b) anti FMRFamide rabbit 1: 5000, + anti-tubulin mouse 1: 1000 in 0.01 M PBS + 1% TritonX100 + 1% NGS + 0.003% NaN_3
c) anti GABA mouse 1: 200 + anti FMRFamide rabbit 1: 5000 in 0.01 M PBS + 1% TritonX100 + 1% NGS + 0.003% NaN_3

The incubation time in primary antibodies varied from 1 to 5 days.

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 405, 488, 532, 633, 635 against a rabbit or a mouse in various combinations at a 1: 800 dilution in 0.01 M PBS + 1% Triton for 7–12 h at 4 °C; followed by staining with Phalloidin-TRITC.

Phalloidin TRITC 1: 1000 in 0.1 M PBS + 1% Triton X100, 1 h. Pieces of tissue were washed in 0.1 M PBS, 6 times for 15 min. The preparations obtained were embedded in glycerol with the addition of 0.1 M PBS (1: 1), edged and stored at – 20 °C. Later, the samples were examined under MSU Nikon confocal laser system (Japan).

2.2. Ultrastructure

Seven plerocercoids were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 h and then post-fixed in 1% OsO_4 in the same buffer both for scanning (SEM) and transmission (TEM) electron microscopy. For TEM, the fixed samples were dehydrated in a series of solutions with increasing ethanol concentration, then the alcohol was replaced with pure acetone, gradually impregnated with Epon or Srurr epoxy resin, and polymerized at 37 °C and 60 °C. The resulting blocks were oriented and cut into semi-thin series; the desired areas of the body and scolex were used for ultra-fine cutting. Sections were made on an LKB 6 microtome and a Leica UC7 ultramicrotome. Semi-thin Sections (1–2 μm) were stained with methylene blue (Biserova, 2013); ultrathin sections with a thickness of 70 nm were mounted on formvar coated slots and contrasted with 4% aqueous uranyl acetate and 0.4% lead citrate. The ultrastructure of plerocercoid tissues was studied under a transmission electron microscope (JEM-1011 and JEM-1400) at the LEM Faculty of Biology, Lomonosov Moscow State University and the Center for Collective Use of the IBIW RAS. For SEM, two specimens were dehydrated in a graded ethanol series with a final change in absolute ethanol, and then, at the critical point, they were dried using liquid CO_2. The specimens were mounted on stubs, sputter-coated with gold–palladium and examined using a JSM 6380LA JEOL scanning electron microscope, MSU.

3. Results

3.1. External morphology and anatomy of the nervous system (Figs. 1–2)

The size of the plerocercoids of *P. phocarum* varies from 10 to 40 mm. In physiological saline with osmolarity equal to that of the cod blood the worms show low mobility. The animal is characterized by regular peristaltic contractions of the scolex, body and tail section; extension and contraction of the scolex significantly changes the external morphology of bothria and apex (Fig. 1 A, B). Scolex bears one dorsal and one ventral bothrium. Bothria have the form of folded scallops with deep midline groove and the lateral scolex surface also has many folds (Fig. 1 C, D). The pyramidal shape of the scolex is clearly visible in an elongated state, with the apex extending a significant distance forward.

The study of the anatomy of the nervous system of the scolex was carried out on a series of semi-thin cross-sections stained with methylene blue (Fig. 2). Distinct neuropils were detected at a distance of one third from the apex of the scolex. They are located on the border of the medullary and cortical parenchyma, distally from the protonephridial canals. The neuropils are very dense and surrounded by faintly stained cells. Dorsally and ventrally, neuropils send nerves into the bothrial folds (dorso-ventral roots) (Fig. 2, big arrows); several thin median commissural nerves extend into the medullary zone (Fig. 2, arrowheads), forming a loose median commissure. At the bottom of the bothria, thin nerves are found (Fig. 2, double thin arrows) forming the subtegumental plexus.

3.2. Immunocytochemistry (Figs. 3–8)

3.2.1. Acetylated tubulin immunoreactivity (acTub-IR)

Anti acetylated tubulin antibody is a marker of various microtubules that are always composed of alpha and beta tubulin dimers. In the present study the anti-tubulin antibody marks microtubules lining the gland ducts and neurotubules in the nerve cells. Since the zones of the perikarya contain fewer neurotubules as compared to the nerve processes, they are weakly stained and are visible only at high magnification on separate optical sections. The nerve processes containing numerous neurotubules are usually strongly stained. In the present work αTub-IR was used for visualization of the central and peripheral nervous system and eccrine glands (Fig. 3). αTub-IR was detected in the neurons, nerves, nerve endings in the tegument, in the secretory terminals of the eccrine glands and in the flame cells. The architecture of the main and minor longitudinal nerve cords was traced as well as commissures and peripheral plexus. Also the location of the ducts of the eccrine glands was visualized in the scolex (Fig. 3).

The lateral lobes of the brain are located in the middle part of the scolex and they gradually continue into the main lateral nerve cords. The lateral lobes are widely spaced, oval to elongated, with the densely

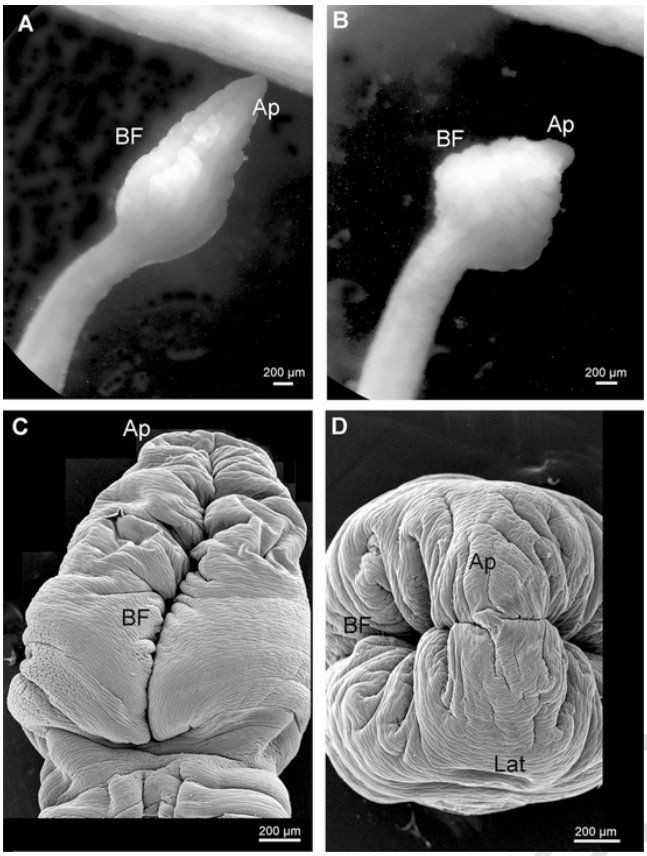

Fig. 1. Scolex of the *P. phocarum* plerocercoid. A, B – pictures of live animals with extended (A) and contracted (B) scolex. C – dorsal view of the scolex with bothrial folds; scanning electron microscopy (SEM); D – frontal view of the scolex; an apex is retracted. Note scolex lateral surface and dorso-ventral bothrial folds; SEM. *Abbreviations:* Ap, apex; BF, bothrial folds; Lat, lateral surface.

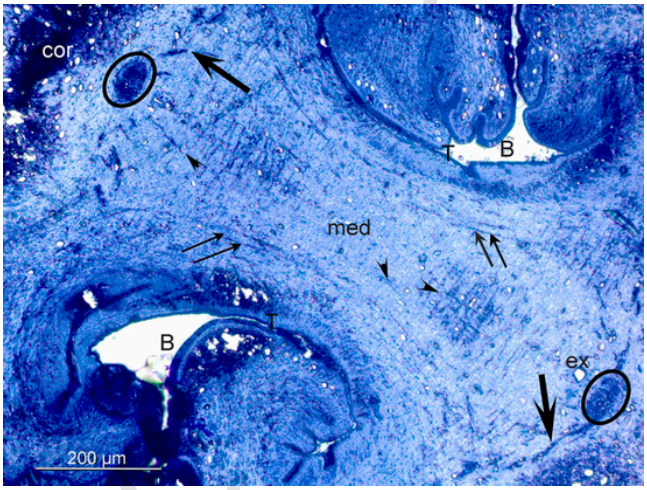

Fig. 2. Scolex of *P. phocarum* plerocercoid in cross section; semi-thin Section (2 μm) stained by methylene blue; LM. Note two lateral lobes (neuropils) marked by black rings, proximal position of the protonephridial canal (ex); extended dorsal and ventral rootlets (big arrows), median nerve bundle (arrowhead); subtegumental nerve plexus (double thin arrows). *Abbreviations:* B, bothrial folds; cor, cortical parenchyma; ex, protonephridial canal; med, medullar part; T, tegument.

packed αTub-IR central zone (Fig. 4). Numerous neurites extend into the subtegument zone from each lobe (distal surface) (Fig. 4 A-C, K-N); the proximal surface of each lobe faces the main (lateral) protonephridial canal. Numerous median commissural nerves emerge from the neuropils, connecting both the right and left lobes (Fig. 4 O-R). The central region of each lobe is represented by a massive neuropil extended in the dorso-ventral direction; the size of each neuropil is about 58 × 18 μm (Fig. 4 A, E-G). The brain roots are detected in each lobe; they are directed dorsally and ventrally, following the course of the dorso-ventral muscle bundles (Figs. 2, 4A, E-G). In the brain roots, the neuron perikarya are occasionally found at some distance from the lobe. Additionally, the rich innervation of the muscle wall of the main protonephridial canals should be noted (Fig. 3 C); it is carried out directly by the neurites of the main nerve cords.

The peripheral nervous system (PNS) is represented by minor longitudinal nerve cords, annular and radial fibers of the rough plexus, and thin fibers of the subtegumental plexus. PNS usually locates in the cortical parenchyma of the body, outside of the main nerve cords. On a cross section of the body, we find the peripheral neuropils with a distinct αTub-IR zone occurring at regular intervals. The peripheral neuropils are located at the cross points of the minor cords and annular and radial fibers of the plexus (Fig. 3 D; 5 E). A thin αTub-IR nerve bundle leaves each peripheral neuropil towards the tegument; together they form the subtegumental nerve plexus. The plexus contains both serotoninergic and peptidergic fibers; bodies of FMRFamide- and 5-HT-IR neurons lie outside the neuropils (Fig. 3 E; 5 E, G).

The frontal (eccrine) glands, which have ducts reinforced by microtubules, were also visualized using αTub-IR. In the center of the scolex and bothria, numerous αTub-IR terminals were found, reaching the bothria tegument (Fig. 3). More often, the glandular terminals open on the inner surface of the bothrial groove.

3.2.2. *FMRFamide-like immunoreactivity (FMRFamide-IR)*

FMRFamide-IR was detected in the lateral lobes of the brain, median commissural nerves, main nerve cords, and peripheral plexuses.

Lateral lobes and the main cords. In the neuropils of the lateral lobes and the MCs, an intense immunoreaction towards FMRFamide was found both in the processes and in the bodies of neurons (Figs. 4, 5). The somas of FMRFamide-IR neurons occupy a cortical position in the neuropil, located on its surface. Neurons can be directly adjacent to the neuropil, or lie at a short distance, sending processes into the neuropil. Neurons have an oval shape; reach 10–12 μm in length and 6.5 μm in width; nuclei are large, 6 × 3 μm; neurites (2–4) are extended in dorsal and ventral direction and run into the roots. In the plane of one transverse section with a thickness of 5–7 μm, usually 3–4 neurons are revealed around neuropil. Thus, Fig. 4E, F, G shows four FMRFamide neurons in the MC2 right neuropil, and one more neuron located between them has only αTub-IR. In the neuropil MC1 left (Fig. 4 H, I, J) on the distal side lies one FMRFamide-IR neuron with 4 processes, size 11 × 5 μm, nucleus 5 × 3 μm; and one distinct bipolar neuron on the proximal side. Most of the FMRFamide neurons are located on the surface of the lobes and main cords. The cortical position is clearly visible in the longitudinal section (Fig. 4 K-N; 5D). Neurites of FMRFamide neurons look like a string of beads (Fig. 5 D, E). The FMRFamide-IR neurites innervate the musculature of bothria, the longitudinal muscles in the scolex and the body; FMRFamide terminals are numerous in the subtegument (Fig. 5 H).

The dorsoventral roots extending from the lateral brain lobes (Fig. 4 A-D) are very powerful. Each one consists of 4 or more neurite bundles, which further branch into thinner nerves in the area of the cortical parenchyma and subtegument. The first pair of nerves (1 on the scheme Fig. 8) that leaves the neuropil proximally, closer to the protonephridial canal, innervates the bottom of the bothria (dorsal and ventral regions of the scolex); the remaining three pairs of FMRFamide-IR nerves branch in the distal zone, innervating the inner (2) and outer (3) sur-

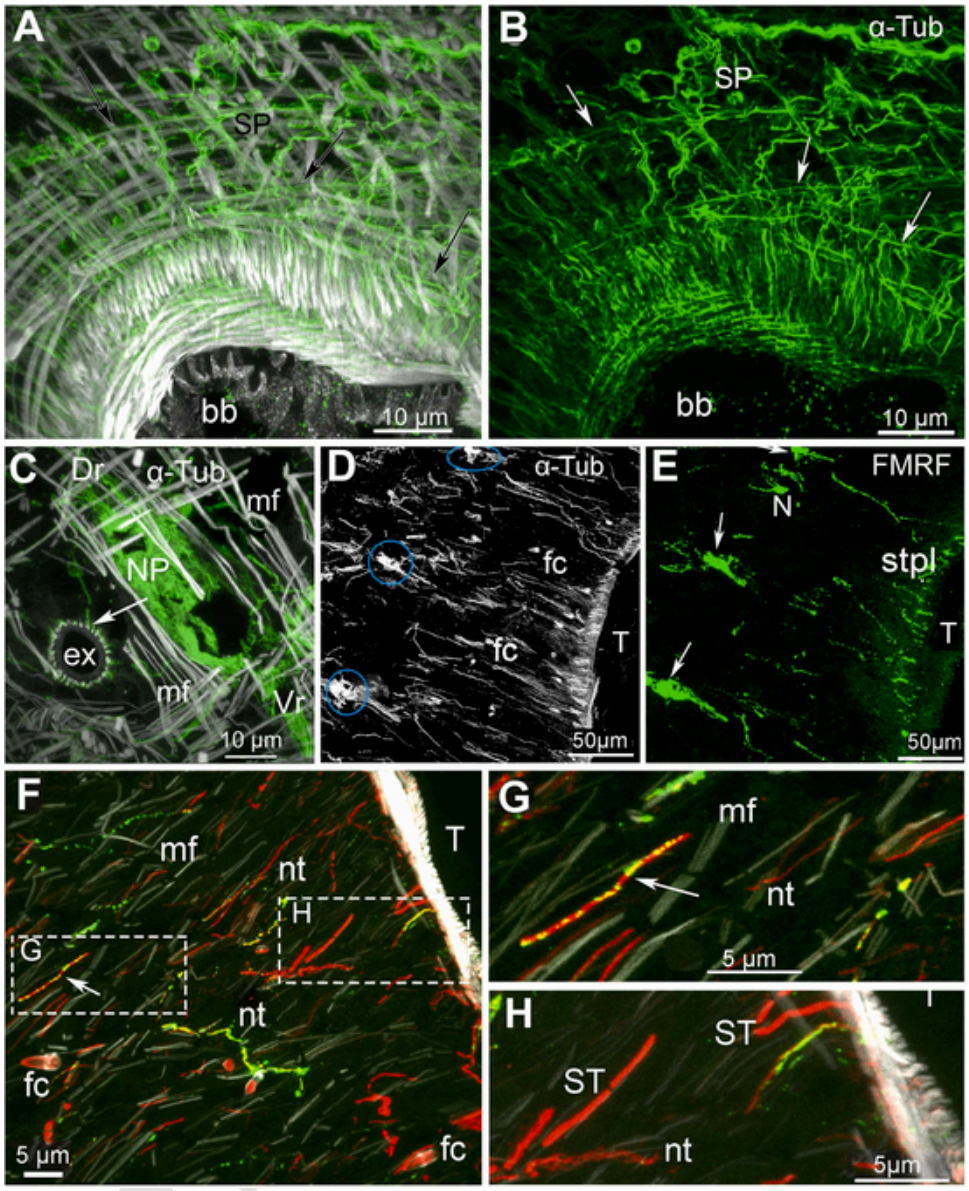

Fig. 3. Visualization of the nervous system and eccrine glands in the scolex and plerocercoid body by acTub-IR, FMRFamide-IR and phalloidin TRITC staining; cross sections; confocal laser scanning microscopy (CLSM). A, B – bottom zone of the bothrial fold; αTub-IR shown in green, F-actin shown in gray; multilayered musculature is richly innervated by nerve terminals; numerous terminals of the eccrine glands reach the tegument; the nerve terminals look like separate thin fibers (arrows), while the secretory ducts look like highly convoluted, thicker fibers; confocal pair, CLSM. C – cross section of the lateral lobe neuropil in its posterior part with emerging dorsal and ventral roots. αTub-IR shown in green, arrow points to αTub-IR in the wall of the main protonephridial canal. D, E – αTub- and FMRFamide-IR in lateral zone of plerocercoid; confocal pair; cross section of the lateral scolex zone. αTub-IR regular peripheral neuropils in the subtegument are circled (D), FMRFamide-IR plexus in subtegument (E); somas of neurons (N) and peripheral neuropils (arrows) do not exactly match in the cortical layer of the parenchyma. F-H – Extended terminals of the secretory ducts in the subtegument have numerous FMRFamide-IR nerve terminals on their surface; intense αTub-IR (red) in the secretory terminals is combined with FMRFamide-IR (green); muscle fibrillar actin (Phalloidin TRITC, gray); a cross section; G – high magnification of a frame in Fig. 3F; the arrow points to the secretory duct (red), which is innervated by FMRFamide-IR neurite (green); H – high magnification of another frame in Fig. 3F, arrow; secretory terminals have a high intensity of αTub-IR (red) and a characteristic rod-shaped form. *Abbreviations:* bb, bothrial bottom; Dr, dorsal root; ex, protonephridial canal; fc, flame cell; mf, myofibril; N, neuron; NP, neuropil; nt, nerve terminals; SP, secretory processes; ST, secretory terminals; stpl, subtegumental plexus; T, tegument; Vr, ventral root.

faces of bothria and the lateral (4) zone of the scolex (Fig. 8). Peripheral FMRFamide-IR apparently sensory neurons associated with the roots are numerous, 5–8 in the field of view, bipolar somas and large varicose neurites lie in the subtegument (Fig. 4 A-D, arrows).

The median commissural nerves connecting the lateral lobes of the brain are represented by individual FMRFamide-IR neurites passing through the central zone of the scolex. They are numerous, loosely located and do not form a compact bundle (Fig. 4 O, P, R).

Peripheral nervous system is represented by minor longitudinal nerve cords, annular and radial fibers of the plexus, and peripheral neuropils. FMRFamide-IR part of the PNS is richly represented in the subtegumental plexus in the form of annular and radial nerves (Fig. 3; 5 E-H). Peripheral neurons are numerous and they have the same size (10 μm). The perikarya have rich intensive FMRFamide-IR. Fig. 5 E shows a peripheral neuron with an oval soma and round nucleus; two branching dendrites are directed into the subtegument; the axon is directed towards the main neuropil (MC1). The neuron lies at the

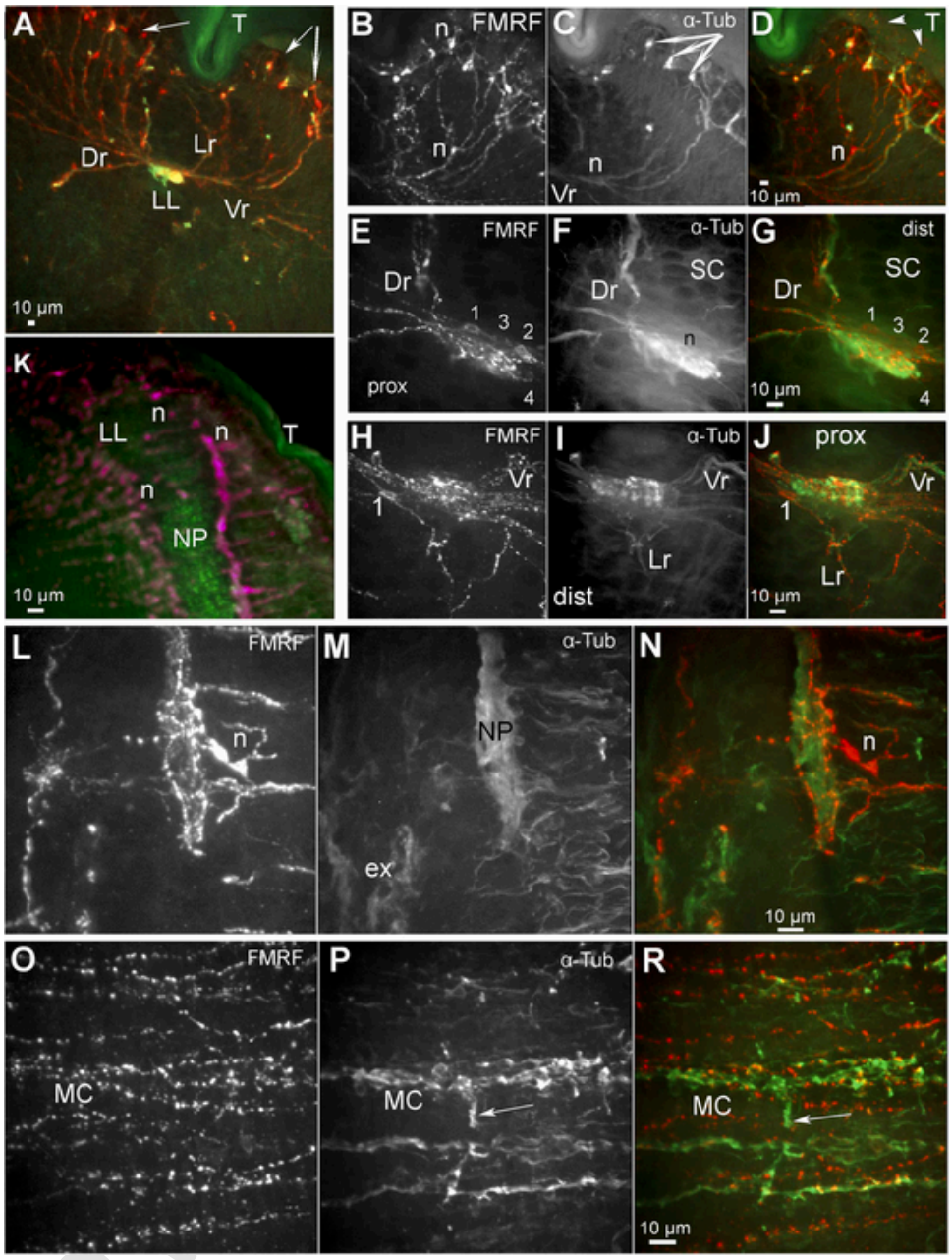

Fig. 4. The peptidergic (FMRFamide-IR) compartment in the lateral brain lobes and median commissure in the scolex of *P. phocarum* plerocercoid. A-D - transversal section of the lateral lobe (LL) with neuropil and outgoing dorso-ventral (Dr, Vr) and lateral (Lr) roots; arrows point to presumable sensory neurons of the lateral subtegumental zone of the scolex. Superposition of FMRFamide-IR (red) and αTub-IR (green). A – overview of the cross section in the middle of bothria; arrows point to subtegumental neurons; B– FMRFamide-IR in nerve terminals and neurons of the subtegument; C – acTub-IR in the same area: a large neuron being part of the ventral root (n); arrows point to subtegumental neurons; D – superposition of FMRFamide-IR (red) and αTub-IR (green), note apparently sensory terminals in the tegument (arrowheads); E-J – superposition of FMRFamide-IR (red) and αTub-IR (green), transverse section; E, F, G –neuropil of the right lateral lobe (MC2); H, I, J – neuropil of the left lateral lobe (MC1); E,H - FMRFamide-IR; F,I – αTub-IR; FMRFamide-IR neurons in the neuropil are indicated by numbers 1–4; K – overview of the lateral brain lobe in longitudinal direction; FMRF-IR (magenta) and αTub-IR (green); neurons surround a huge neuropil; L-N – longitudinal section of the lateral brain lobe with FMRFamide-IR neurons (n); confocal pair, superposition of FMRFamide-IR (red) and αTub-IR (green); O-R – median commissure zone in the center of the scolex; arrow points to the dorso-ventral nerve; scolex transverse section, confocal pair, superposition of FMRFamide-IR (red) and αTub-IR (green); *Abbreviations:* dist – distal zone; Dr, dorsal brain root; ex, protonephridial canal; LL, lateral lobe; MC, median commissure; n, neuron; NP, neuropil; prox, proximal zone near the protonephridial canal; R, rootlets; SC, secretory glandular cells; T, tegument; Vr, ventral brain root.

intersection of the annular and radial nerves of the peripheral plexus. It is important to note that the αTub-IR plexus bundles do not always co-localize with the FMRFamide-IR: some of the nerves show only αTub-IR. In this case, annular and radial nerves have predominantly αTub-IR (Fig. 3 D; 5). The annular nerve runs into the cortical parenchyma; at the intersection with the radial bundle peripheral neuropils with pronounced αTub-IR are regularly observed. Here, FMRFamide-IR neurons can also be found sending processes along the annular nerve. The most intense development of the FMRFamide-ergic plexus was observed in the zone of the bothrial bottom (Fig. 5F) and also in the subtegument of the scolex lateral zone (Fig. 5 G, H).

In bothrium groove, the secretory ducts with intense αTub-IR are often accompanied by the FMRFamide-IR nerve terminals which form numerous varicosities on the surface of the secretory ducts. (Fig. 3 F, G,

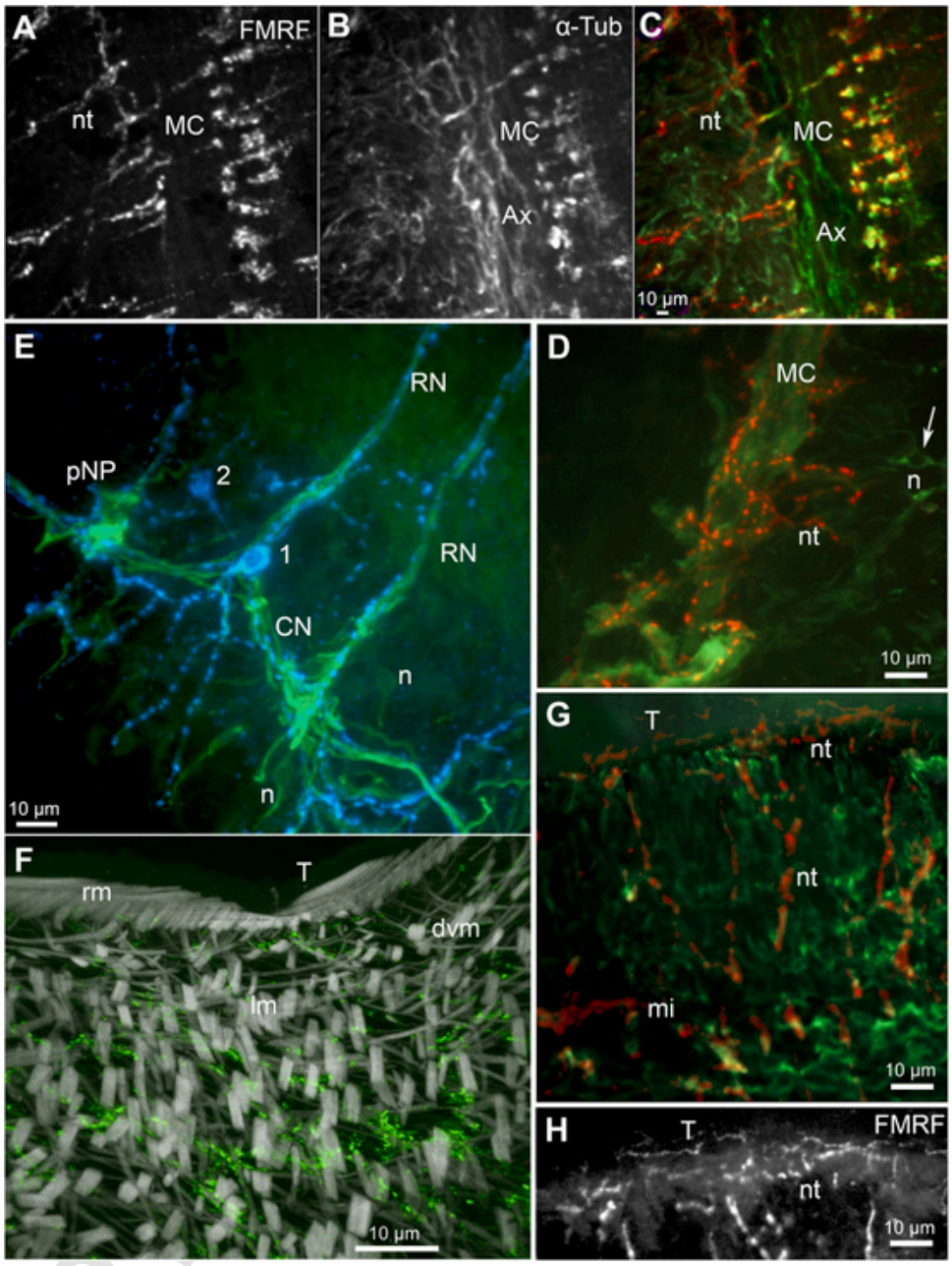

Fig. 5. The FMRFamide-IR compartment in the main nerve cords and the peripheral NS A-C – longitudinal section of the main nerve cord in the posterior part of scolex; FMRFamide -IR neurites (nt, red) run in distal and proximal directions; axons (Ax, green) run along the center of the MC; confocal pair, superposition of FMRFamide-IR (red) and αTub-IR (green); D - main nerve cord in plerocercoid body, longitudinal section. FMRFamide -IR neurites (nt, red) are directed distally; arrow points to tripolar αTub-IR neuron closely associated with the MC. E – part of peripheral plexus in cortical parenchyma, cros ssection. Note the radial nerves (RN), the circular nerve (CN), peripheral neuropils (pNP), FMRFamide-IR neurons (1,2, cyan) and αTub-IR bipolar neurons (n, green). F – FMRFamide innervation of the musculature at the bottom of the bothria, middle scolex cross section; F-actin, gray; FMRFamide-IR, green. G –innervation of the subtegument and cortical parenchyma; longitudinal minor cord (mi) and radial nerves with FMRFamide -IR neurites (nt, red) and αTub-IR (green). H - innervation of the tegument; part of Fig. 4G, one channel *Abbreviations:* Ax, axons; CN, circular nerve; dvm, dorso-ventral muscle; lm, longitudinal muscle; MC, main nerve cord; mi, minor cord; n, neuron; nt, neurites; pNP, peripheral neuropils; rm, ring muscle; RN, radial nerves; T, tegument.

H). These FMRFamide-IR nerve terminals found in close association and on the surface of the secretory gland ducts serve as evidence that the frontal glands function under the direct control of the nervous system.

3.2.3. Serotonin immunoreactivity (5HT-IR)

5HT-IR is detected in the lateral brain lobes, in the median commissure, in the main cords, and in the peripheral nerve plexus (Fig. 6).

In the anterior part of the scolex, serotonergic neurons are located around the lateral lobes, and their somas lie at some distance from the compact part of the neuropil. 5-HT-IR neurons with presumably sensory function are found in this area (Fig. 6 A, C). They have large oval somas (15 μm); the main axon is directed towards the neuropil of the lateral lobe, and the branching dendrite is at the opposite pole of the soma. Dendrite branches are directed towards the subtegument and tegument. Other neurons are usually bipolar, their somas (15 μm) are located near the neuropil of the main cords, to which one of the neurites is connected, and the second one goes to the annular nerve or to the radial nerve of the peripheral plexus (Fig. 6 A, B). Many bipolar neurons are located in the main nerve cord. A tripolar neuron with a triangular

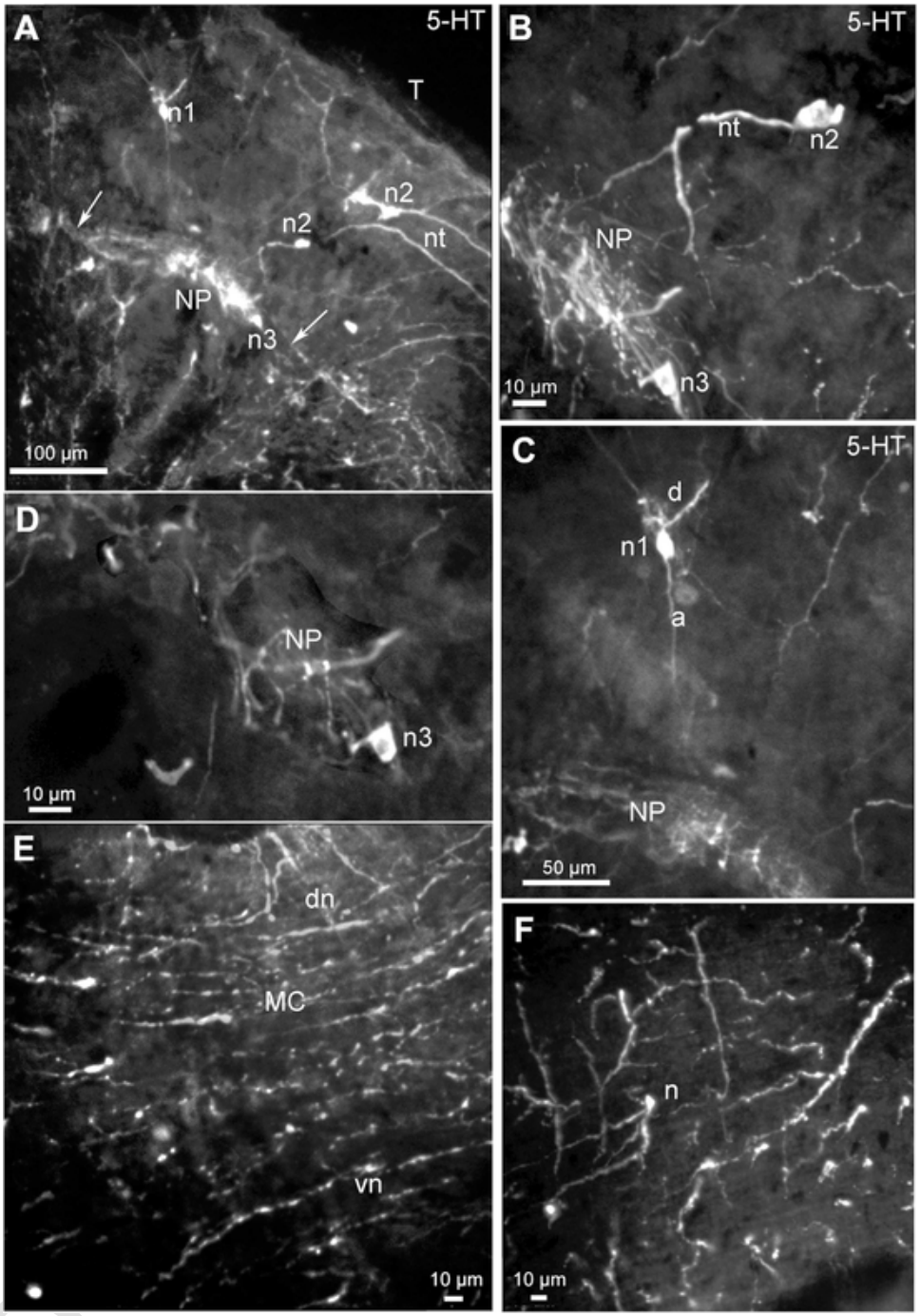

Fig. 6. The serotonergic (5-HT-IR) compartment of the nervous system in the scolex of *P. phocarum*. A-D –neuropil and neurons in the lateral lobe; cross section of the scolex anterior third; A – overview; arrows point to the dorsal and ventral nerve roots; B – bipolar neuron (n2) with proximal bifurcated neurite and tripolar neuron (n3) in the neuropil; C –unipolar neuron(n1) associated with the lateral neuropil lobe; dendrite tree located in the subtegument; D - tripolar serotonergic neuron (n3) with round nucleus in the lateral lobe of the neuropil; E - serotonergic nerves in the middle part of scolex; the median commissure (MC) and additional subtegumental dorsal (dn) and ventral (vn) nerve bundles; scolex cross section; F –5-HT nerves in the bothrial folds, scolex cross section. *Abbreviations*: dn, dorsal nerve; MC, median commissure; n, neuron; nt, neurite; NP, lateral lobe neuropil; T – tegument; vn, ventral nerve.

soma 8 × 10 µm in size and a wide main neurite in the dorsoventral root is identified directly within the neuropil (Fig. 6 D). The neuropils of the lateral lobes have a high intensity of 5-HT-IR and are represented by numerous neurites that form a dense network of intertwined processes.

In the medullar (central) zone of the scolex, 5-HT-IR neurons lie diffusely, numerous neurites run medianly from one lobe to another, and branch out to fine-fibrous plexus in the bothria subtegument (Fig. 6 E). Frequently, there are individual nerve fibers passing laterally and connecting the branching zones of the roots and peripheral neuropils. Powerful 5-HT-IR nerve terminals are found in the bothrial bottom zone. All serotonergic neurons are characterized by an oval soma with well-defined border and smooth neurites, often coinciding with αTub-IR. In the main cords, 5-HT-IR neurons are located in the cortical layer, mainly distal to the neuropil and protonephridial canal, innervating the lateral zone of the scolex and the body.

3.2.4. Gamma-aminobutyric acid-like immunoreactivity (GABA-like IR)

GABA-like IR elements (Fig. 7) are detected mainly in the peripheral nervous system: in neurons associated with the minor cords (Fig. 7 A) in the peripheral plexus, as well as in numerous cells on the surface of muscle layers (Fig. 7 D), both in the medullar and cortical regions and in the subtegument. On the surface of the longitudinal muscles numerous small neurons with intense GABA-like IR are identified (Fig. 7 D). GABA-like IR perikarya have a weakly expressed immunoreaction, while the processes of these neurons are intensely stained. Fig. 7A illustrates an intimate relation of the muscle fibers with GABA-like and FMRFamide-IR neurites. No co-localization of two neuroactive substances in one neuron has been observed. GABA-like IR neurons in the cords do not show any immunoreaction to FMRFamide (Fig. 7).

3.3. Ultrastructure

3.3.1. Brain architecture

Examination of serial semi-thin and ultrathin sections of *P. phocarum* has revealed an accumulation of neurons surrounded by the frontal glandular cells in the anterior third of the plerocercoid scolex (Figs. 8, 9). The neurons are located in *paired lateral lobes* connected by a long slim median commissure composed of both very large (giant) and small neurons. Each lateral lobe contains neuron somas and a compact neuropil. In the neuropil, many nerve processes contact each other and form synapses. Both left and right neuropils are elongated in the dorsal and ventral direction. The neurons are arranged in groups and located outside the neuropil. In each lobe 11–18 neurons are observed in one section. Large glandular cells and their reservoirs with secretory granules surround the lateral lobes and median commissure (Figs. 9, 10). Powerful paired *dorsal and ventral roots* start from the lobes and form the dorsal and ventral bothria nerves. The root nerves (1–4 on Fig. 8) innervate the bothrial musculature, processes of the glandular cells with terminals in the tegument, and include the processes of apparently sensory neurons located in the subtegument of the bothrial folds (Fig. 8).

The median commissure is long and consists of many light thin parallel axons and several neurons (Fig. 9). Large neurons are found in the commissure central zone and small ones are located in lateral positions close to the neuropils. In the central zone, the median commissure is stratified being penetrated by numerous frontal gland ducts (Figs. 9, 10 A). Perikarya of glandular cells and large reservoirs with secretory granules occupy most of the parenchyma around the median commissure. Between the glandular cells, there are many individual thin neurites running parallel to the commissure bundles and thin submedian nerves (black arrows on Fig. 9B) are connecting the branching zones of the dorsal and ventral roots. In addition, the dorsoventral muscle fibers cross the median commissure.

The lateral lobes of the brain smoothly continue into the *main nerve cords* (MCs). It should be noted that all neurites composing the neuropils, the median commissure and the MCs of *P. phocarum* plerocercoid have a small diameter (Figs. 9, 10). The MCs include cortical neurons and the neurite bundle in the central conducting part. Sometimes bipolar and undifferentiated cells are found in the center of MCs. Bipolar neurons have two main neurites extending along the cord in the rostral and caudal directions. Also, nerve processes are often found to extend in perpendicular plane to the main cord and run towards the subtegument zone. These numerous thin neurites form nerves running along the muscle layers of the scolex posterior part. In addition, numerous neurites extend into the medullar parenchyma.

3.3.2. Neurons

The brain neurons have rounded nuclei containing eu- and heterochromatin. Perikarya (3–4.5 μm in maximum diameter) form thin processes - neurites. According to the structure of the perikaryon cyto-

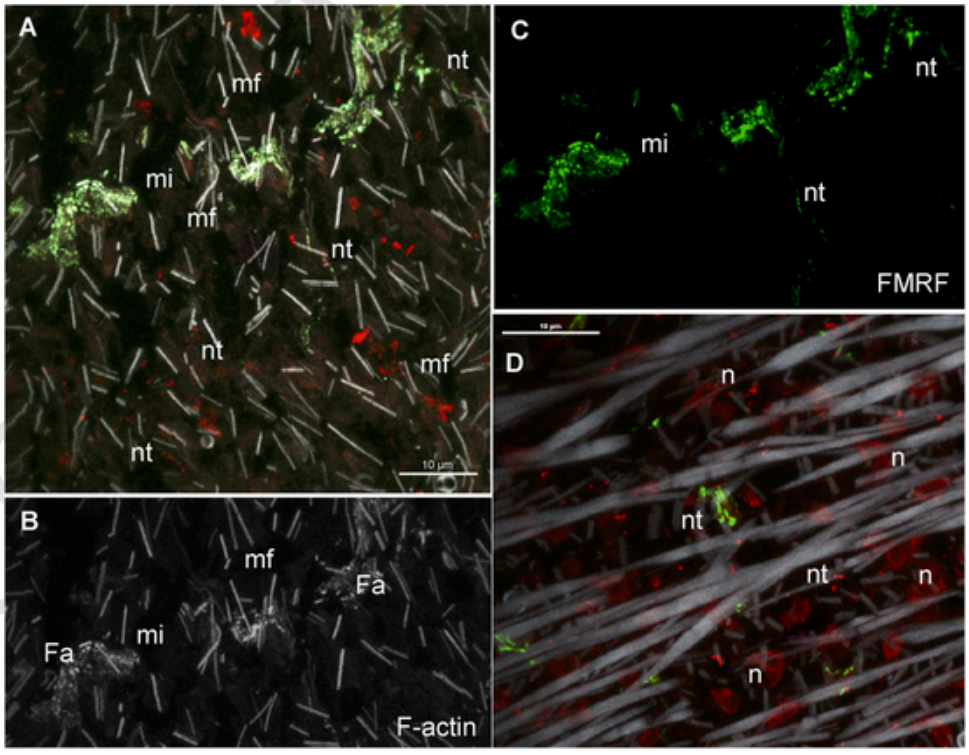

Fig. 7. GABA-like IR in a minor nerve cord and muscles innervation. A-C, scolex oblique sections; confocal pairs, three channels; maximum projection of 32 optical sections A–minor nerve cord and surroundings tissues; visualization of the GABA-like IR (red), FMRFamide (green) and F-actin in the muscles (gray); B,C, confocal pairs; B – F-actin in the muscle cells; C – FMRFamide-IR in the minor cord and neurites; D – GABA-like IR (red) and FMRFamide-IR (green) on the surface of the longitudinal body musculature (gray); maximum projection of 44 optical sections. *Abbreviations*: Fa, F-actin; mi, minor nerve cord; mf, myofibrils; n, neuron; nt, neurite;.

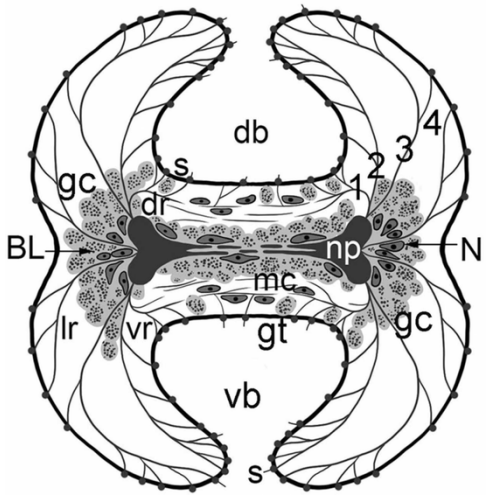

Fig. 8. Schematic illustration of the neuro-glandular brain of *P. phocarum* pelagocercoid. *Abbreviations*: BL, brain lobe; db, dorsal bothrium; dr, dorsal brain root; gc, frontal gland cells; gt, glandular terminals; mc, median commissure; lr, lateral brain root; N, neurons; s, sensory organs; vb, ventral bothrium; vr, ventral brain root; 1–4, branches of brain root.

plasm, neurons can be subdivided into "light" and "dark" ones (Fig. 10 B; 11).

Light neurons are characterized by a small number of free ribosomes and poor development of granular endoplasmic reticulum (GER). They contain mostly light round vesicles in the perikaryon; occasionally round electron-dense vesicles are found (Fig. 10 B; 11B). The lucent cytoplasm contains neurotubules in the perikaryon and in the neurites; the soma membrane shows numerous incoming synapses formed by thin neurites; often an outgoing synapse with a muscle fiber can be seen.

Dark neurons are distinguished by a dense cytoplasm containing ribosomes, β-glycogen granules, mitochondria, and electron-dense vesicles of various diameters in the Golgi complex zone. The Fig. 11 D shows a unipolar dark neuron with an oval nucleus and a soma; there are electron-dense round vesicles measuring 100 nm in the perikaryon; numerous microtubules are visible in the axon. In addition, some dark neurons with small electron-dense vesicles (10–20 nm) lying by the Golgi complex occur. Their cytoplasm contains small mitochondria with a dense matrix, neurotubules and free ribosomes.

Neurosecretory cells constitute a separate cluster of neurons in the scolex of *P. phocarum*. Their cytoplasm is filled with numerous oval electron-dense vesicles 150–175 × 90 nm in size. Secretory vesicles fill the cytoplasm around the nucleus and in the outgoing processes. These neurons lie at a short distance from the neuropils of the lateral lobes and close to the muscle cells (Fig. 11C).

The giant median commissure neurons are characterized by larger sizes as compared to the neurons of the lateral lobes (Figs. 9 A; 11A). Their somas are rounded, reaching 12 μm, with a round nucleus (diameter 5.8–6.5 μm) and a large round nucleolus, 2.5 μm in diameter. The cytoplasm of the perikaryon has numerous invaginations, contains light and dark areas, which gives it a "foamy" appearance; the cytoplasm contains numerous electron-dense vesicles 80 nm in diameter and microtubules (Fig. 11 A).

3.3.3. Synapses

Numerous synaptic junctions are detected in the neuropils of the lateral lobes (Fig. 12). Asymmetric chemical synapses are formed by thin neurites containing both clear (cv) and electron-dense (dv) round vesicles in the presynaptic zone. In addition, there are presynaptic terminals with only clear round vesicles (Fig. 12 B), probably containing acetylcholine. Shared synapses are common in the brain neuropil; in this case, the presynaptic terminal makes contact with membranes of two postsynaptic neurites. Numerous thin neurites containing electron-dense vesicles (70–80 nm) form junctions with the dorsoventral muscle

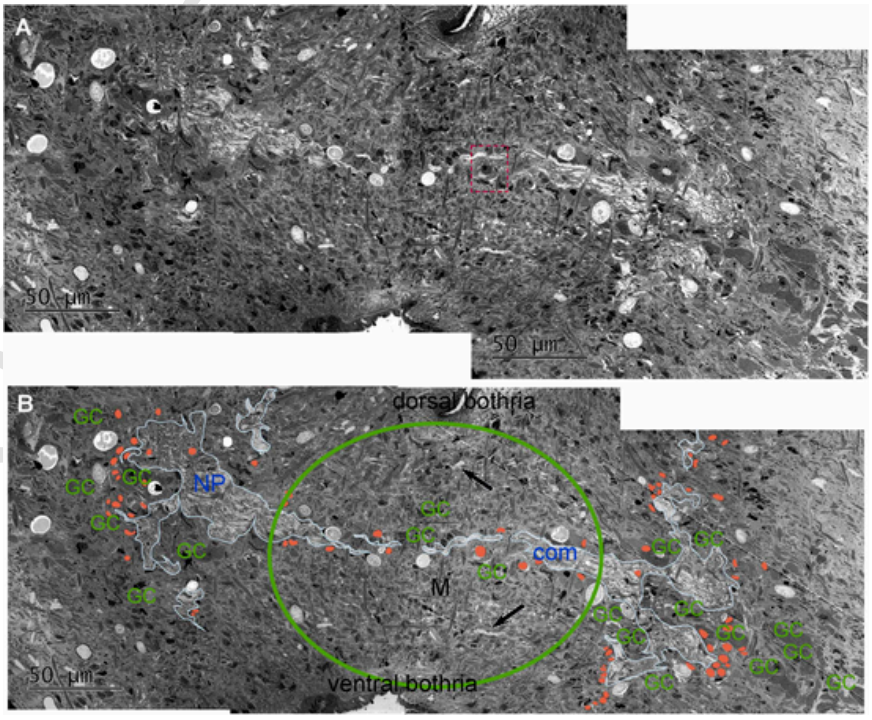

Fig. 9. Overview of the brain at the level of the median commissure. Scolex cross section. TEM. A – original TEM photograph; the red frame marks the giant neuron, the same as in Fig. 11A; B – the same photograph with labels; neurons are marked in red; light blue line surrounds axon bundles, neuropils, commissure and roots; green circle marks a central part with most concentrated frontal glandular cells partly shown in the Fig. 10A; black arrows point to submedian thin nerves. *Abbreviation:* com, median commissure axons; GC, glandular cells and reservoirs with secretory granules; M, musculature; NP, neuropil.

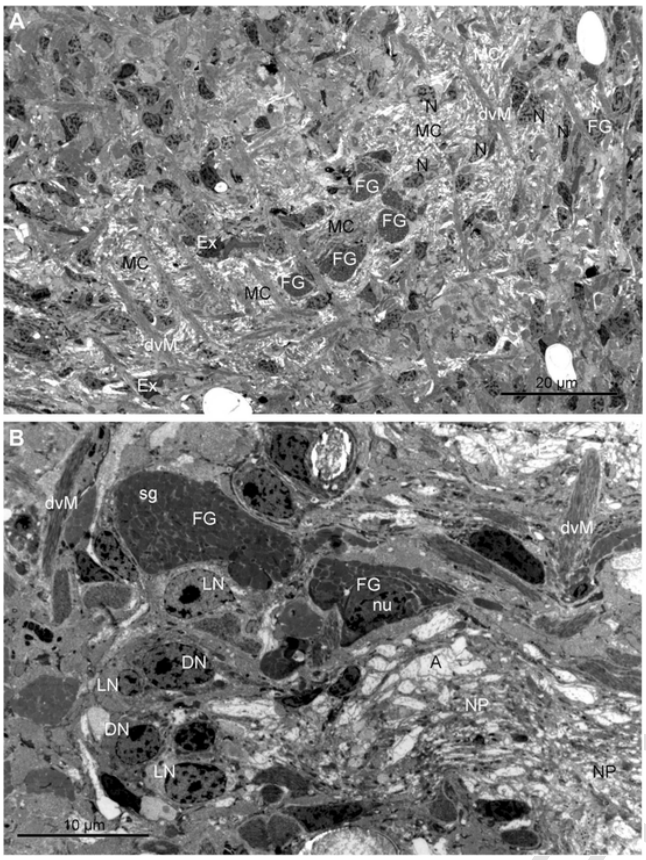

Fig. 10. Ultrastructure of the neuro-glandular complex of the median commissure and lateral lobe of the brain A – Central part of the median commissure; transversal section; thin neurites of the median commissure contact with frontal glandular cells and dorsoventral muscles; B – cluster of "light" and "dark" neurons, frontal gland cells and a neuropil in the lateral lobe of the brain; *Abbreviations*: A, axons; DN, dark neurons; dvM, dorsoventral muscle; Ex, protonephridial canal; FG, frontal gland cells; LN, light neuron; MC, median commissure neurites; N, neurons; NP, neuropil; nu, nucleus; sg, secretory granules in the reservoir of the glandular cell.

fibres (Fig. 10 A; 12 B, C). This musculature is responsible for the bothrial attachment: when the dorsoventral muscles of the scolex contract, the bothrial grooves deepen; when the muscles relax, the grooves flatten. Thus, the neurites of the median commissure forming neuromuscular synapses on the membranes of the dorsoventral muscles coordinate the movements of the scolex.

3.3.4. Neuro-glandular and sensory structural complex

Secretory cells of frontal glands are located around the brain lobes and the median commissure, distal to the neuropils of the lateral lobes, giving them a horseshoe shape (Fig. 8). Small light neurons are found adjacent to the glandular cells and their processes with secretory granules (Figs. 9–12). Numerous neuro-glandular synapses occur in the brain. We find the junction of the axon with clear vesicles with the gland cell's membrane (Fig. 12 C, cv), and also neighboring nerve processes with electron-dense small round vesicles (Fig. 12 C, dv) that may have modulatory function toward neuro-glandular synapse. These facts serve as evidence of stimulation of the glandular cells by the brain neurons. Thus, functioning of the frontal gland cells might be supported by two different neuroactive substances.

The membrane of the secretory ducts is reinforced with microtubules that help secretory granules move to the tegument surface. In subtegument, the secretory ducts make contact with muscle processes and neuromuscular junctions are present on these muscles. In the tegument, secretory terminals have pores surrounded by annular septate junction and one supported by an electron-dense ring.

Numerous ciliated and unciliated *sensory organs* were revealed in the bothria tegument in close connection with the frontal gland terminals and pores (Fig. 13).

Type 1. Ciliated receptor with a long cilium, well developed kinetosome and a short rootlet. The base of the cilium is tightly surrounded by a ring of the adjacent tegument. There is an annular septate junction in the form of a wide ring including up to 10 septae. Inside the sensory bulb there are two powerful supporting rings, small mitochondria, small light vesicles (cv, 30–40 nm) and microtubules. Microtubules continue into a thin convoluted dendrite (Fig. 13A).

Type 2. Ciliated receptor with a short cilium (0.36 ×0.19 μm) located at the level of the base of the tegument. The cilium lacks kinetosome and rootlet. There is the annular septate junction and one supporting ring. The cytoplasm contains numerous microtubules and irregular vesicles. Thin capillary connects the sensory ending with host environment (Fig. 13 B).

Type 3. An unciliated nerve ending is located in the basal layer of the distal cytoplasm of the tegument. It does not connect with the tegument surface and host environment. The sensory ending has an inverted cone shape 1–1.2 μm in diameter. It has a wide septate junction on the apical surface with two supporting rings, which unite into a single complex in the basal region. The kinetosome is absent. The wide "umbrella-shaped" striated root is represented by a central bundle of thin filaments with transverse striation, and peripheral bundles in the form of diverging spokes, united by annular bridges. Around the root, elongated mitochondria and microtubules are located; the same mitochondria fill a thin dendrite. The bulb has an asymmetric slim process directed along the basal tegumental membrane. Type 3 sensory organs are located near the secretory ducts of the frontal glands together with other sensory endings (Fig. 13 C, D).

Type 4. An unciliated free ending of the dendrite; kinetosome and rootlet are absent. There is an annular septate junction and one supporting ring. The cylindrical process has 0.72 μm in diameter and lies in the middle layer of the tegument. The ending is connected to the tegument surface by a thin capillary. The free ending is filled with transparent oval vesicles 40–50 nm in diameter. The vesicles are secreted outward into the capillary through the center of the apical surface. Such endings are regularly found together with the secretory ducts of the frontal glands and the 3rd type receptors (Fig. 13 C, D).

Additionally, another type of thin terminals (type 5) connected to the surface by a thin capillary is detected in the bothrium tegument. The free terminals are surrounded by an annular septate junction underlain by a weakly developed electron-dense ring. The diameter of such terminals is very small and does not exceed 0.43–0.58 μm, which is significantly smaller in comparison with typical sensory terminals. They are located in the middle of the distal cytoplasm and are often found in groups of 3–4 terminals. The terminal usually contains scattered microtubules and in some cases vesicles. There is often a small cavity above the apical surface (Fig. 13 D, E). Cells forming these free terminals were not identified.

4. Discussion

4.1. The neuro-glandular brain

The microscopic anatomy of the nervous system of diphyllobothriidean cestodes was studied mostly in plerocercoid larvae, especially by immunocytochemical visualization of the nervous system of the *Diphyllobothrium dendriticum* plerocercoid (Gustafsson and Wikgren, 1981; Wikgren, 1986; Gustafsson, 1990; Gustafsson et al., 1993, 1994; Gustafsson and Eriksson, 1991, 1992). Additional ultrastructural data provided descriptions of individual neurons and synapses (Gustafsson, 1984). Despite a large number of publications, the architecture of the

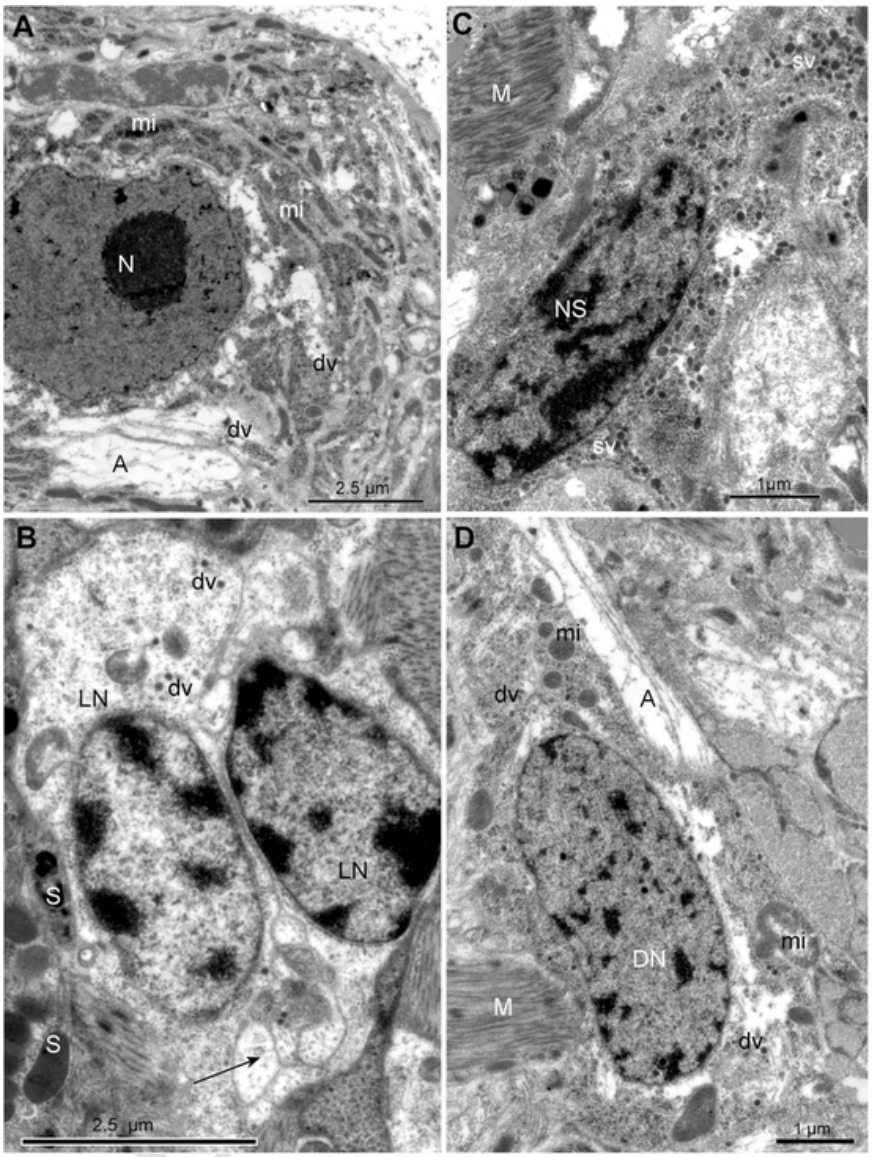

Fig. 11. Brain neurons ultrastructure. A – part of the giant median commissure neuron with "foamy" cytoplasm, numerous invaginations of the membrane, mitochondria and electron-dense vesicles; B – two "light" neurons with lucent cytoplasm and small electron-dense vesicles closely adjacent to the secretory cells; arrow points to synaptic glomerula with synapses; C – secretory neuron containing numerous electron-dense secretory vesicles 150–175 x. 90 nm in size; D – "dark" unipolar neuron with electron-dense perikaryon, ribosomes and small vesicles in cytoplasm; axon contains neurotubules. *Abbreviations*: A, axon; dv, dense vesicle; DN, dark neuron; FG, frontal gland processes; M, muscle cell; mi, mitochondria; LN, light neuron; N, nucleolus; NS, neuro-secretory neuron; S, secretory granules of the eccrine glands; sv, secretory vesicle.

brain of *D. dendriticum* remains unclear and can hardly be useful for comparative morphological analysis within Eucestoda. Significant differences in the architecture of the brain among representatives of different families of diphyllobothriidean cestodes are evidenced by studies of the fine morphology of the brain in *Ligula intestinalis* (Biserova and Gordeev, 2010) and the broad tapeworm (Barčák et al., 2019). In the plerocercoid of *L. intestinalis* (family Ligulidae), three lobes in the brain (two lateral and one unpaired central) were found, as well as dorsoventral asymmetry in the arrangement of neurons (Biserova and Gordeev, 2010).

The most noticeable accumulations of nerve elements at the anterior end of *P. phocarum* have the appearance of two large lateral neuropils with associated neurons interconnected by numerous neurite bundles (Figs. 2, 8). The median neurite bundles meet at a considerable distance towards the posterior end of the lateral lobes and form the median brain commissure. The dorsal and ventral roots emerging from the neuropils innervate the folds and the bottom of the bothria, forming a submuscular plexus, which also extends over a considerable distance in the caudal direction. The neuropils of the lateral lobes give rise to the main lateral cords, while the emerging roots continue into paired sublateral, dorsal and ventral minor cords in the body of the plerocercoid. Neurons with supposedly sensory function are located in the peripheral part of the nervous system, in the deep subtegument layer. We consider a neuron to be sensory when its soma lies in the subtegument zone, several thin processes are directed towards the tegument, and one main process goes to the neuropil. Thus, a distinct polarity of such neurons and their position provide evidence of their function.

We identified both similarities and differences in morphology of the nervous system in the Diphyllobothriidae family. The similarity of the brain between *Pyramicocephalus* and *Diphyllobothrium* consists in the same number of lobes (two), in their elongated shape, as well as in their symmetrical arrangement in the scolex (Gustafsson and Wikgren, 1981; Wikgren, 1986; Gustafsson, 1990; Biserova and Kutyrev, 2014; Biserova and Kemaeva, 2012; Barčák et al., 2019).

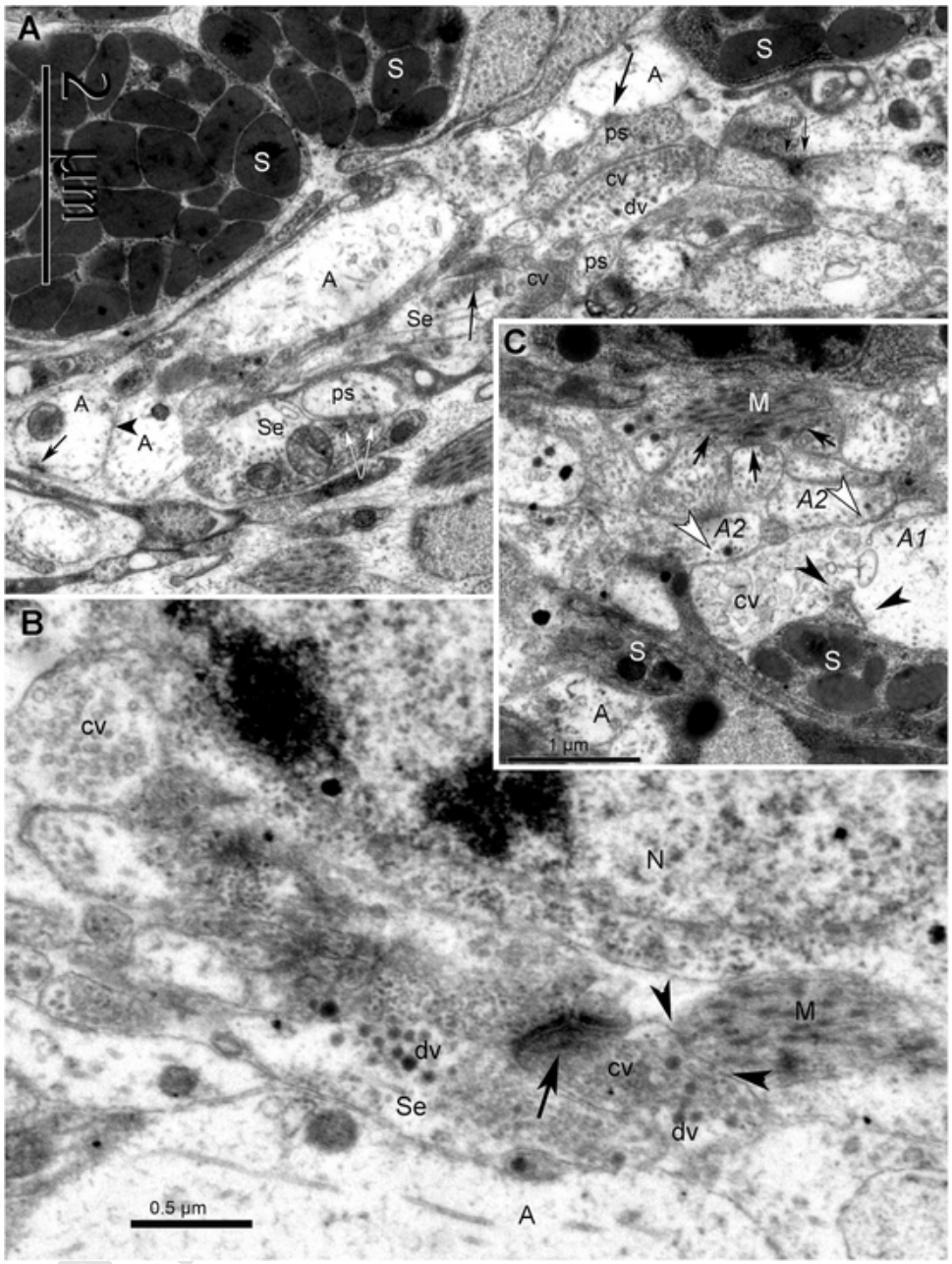

Fig. 12. Ultrastructure of the synaptic junctions in the brain. A – a part of the neuropile with axons (A) and synaptic endings (Se), postsynaptic membrane structures (ps), clear and dense synaptic vesicles; black and white arrows mark presynaptic membrane density; arrowhead points to axo-axonal junction; S, secretory granules of the eccrine glands. B – synapses located on the neuron soma; synaptic ending with two types of site release; arrow marks the pre-synapse with membrane density in active zone; arrowheads point to neuromuscular junctions; C – neuromuscular (short arrows) and neuro-glandular (arrowheads) junctions in the median commissure of the brain; *Abbreviations*: A, axon; *A1,A2*, axon terminals; cv, clear vesicles; dv, dense-core vesicles; M, muscle; N, nucleus; ps, postsynaptic membrane structures; Se, synaptic ending; S, secretory granules of the eccrine glands.

The main peculiarity of the *Pyramicocephalus* brain architecture is the loose arrangement of the median commissure neurites. The structure and position of the cerebral commissure in the scolex of cestodes is one of the important morphological criteria for brain concentration in flatworms. The median (= non-circular) cerebral commissure connecting the right and left lobes of the brain is present in all cestodes and other groups of Neodermata studied in this respect (Biserova, 2016). Among diphyllobothriideans, the presence of a compact median commissure was noted for *D. dendriticum* (Gustafsson and Wikgren, 1981; Wikgren, 1986; Gustafsson, 1990; Biserova and Kutyrev, 2014; Biserova et al., 2020), *D. ditremum* (Biserova and Kemaeva, 2012), *Ligula intestinalis* (Biserova and Gordeev, 2010). In *Dibothriocephalus latus,* two commissures were noted connecting the right and left lobes (Barčák et al., 2019). However, the absence of photographs of cross sections of the *D. latus* scolex does not allow us to understand their location in the brain. It remains unclear whether these commissures have a median or distal (semicircular) position.

As we show by ICC and ultrastructural methods, the loose arrangement of the median commissure neurites in the *P. phocarum* brain is due to the fact that in the central zone it splits into numerous neurites that surround the frontal glands. It is known that frontal glands are present in the scolex of all studied diphyllobothriidean cestodes (Braten, 1968; Andersen, 1975; 1977; Gustafsson and Vaihela,1981; Kuperman and Davydov, 1982a, b; Kuperman, 1988; Biserova and Kemaeva, 2012). In the *P. phocarum* plerocercoid, the frontal glands are very strongly developed (Mustafina, 2017b; Mustafina and Biserova, 2017), their

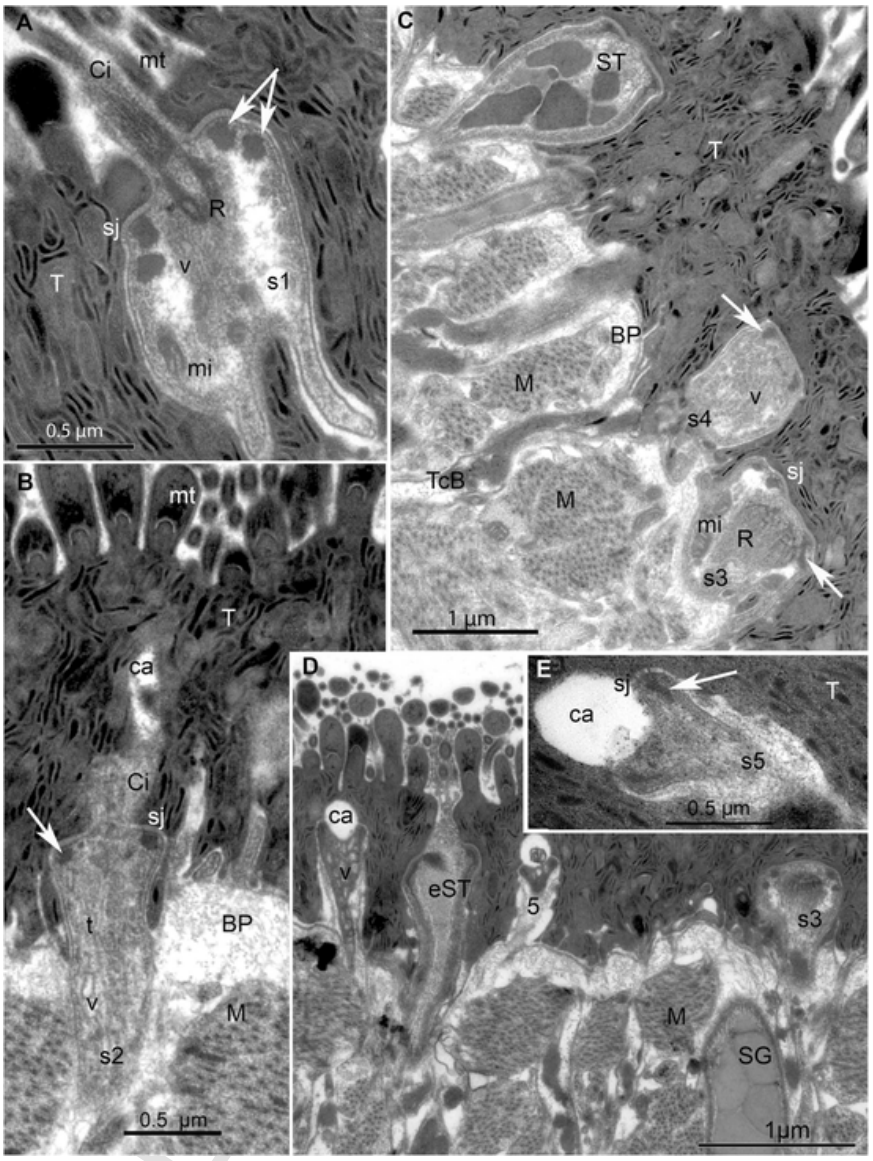

Fig. 13. Sensory-glandular complex in the tegument of the *P. phocarum*. A –ciliated sensory ending of the 1st type with a cilium and two supporting rings (white arrows); B – ciliated sensory ending of the 2nd type with a cilium and one supporting ring lying deep within the tegument; C – part of the tegument with 3rd and 4th type of unciliated nerve endings and the secretory terminal of the eccrine gland; D –part of the tegument with 3rd and 4th type of unciliated nerve endings and the secretory terminal of the eccrine gland; E – unciliated sensory terminal of the 5th type in the tegument; *Abbreviations*: BP, basal plate; ca, cavity and capillary; Ci, cilia; eST, empty terminal of the eccrine glands; M, muscle; mi, mitochondria; mt, microtriches; R, ciliary rootlet; sj, septate junction; SG, secretory granules in the eccrine terminal; ST, secretory terminal of the eccrine glands; T, tegument; t, tubules; v, vesicles; white arrows indicate supporting rings.

perikarya and ducts occupy a large part of the scolex, both in the median region and in the subtegument.

Numerous assumptions have been made regarding the functioning and role of the frontal eccrine glands, none of which have been tested and proven. In this study, we present ultrastructural and immunocytochemical evidence of the connection between the nervous and glandular systems in cestodes. In the brain of *P. phocarum,* the lateral lobes and neurites of the median commissure are densely surrounded by glandular cells. This morphological feature determines the transiting of nerve terminals between the secretory ducts and the cell bodies of the frontal glands, which acquire additional zones of synaptic contacts. Thus, stimulation of the eccrine glands of the plerocercoid of *P. phocarum* is carried out directly by the neurons of the lateral lobes and the median commissure. Membranes of glandular cells receive synaptic contacts directly from neurons in the brain containing clear vesicles in the presynapse. On the periphery, the secretory ducts with intense αTub-IR are often accompanied by FMRFamide-IR nerve terminals. The FMRFamidergic axons form beaded fibers along glandular ducts where the boutons or varicosities terminate on the surface of the secretory duct. Earlier, neuro-glandular synaptic contacts were described in the brain of *D. ditremum* plerocercoid (Biserova and Kemaeva, 2012) and *D. dendriticum* (Kutyrev et al., 2017). The brain of the plerocercoids of diphyllobothriideans *P. phocarum, D. ditremum, D. dendriticum* consists of a complex of neurons and glandular cells that closely interact via synaptic contacts. We introduce the term "neuro-glandular brain" for such type of structure common for diphyllobothriideans.

4.2. Cytochemical and cytomorphological organization of the nervous system

In diphyllobothriidean cestodes, the cytochemical and cytomorphological organization of the central nervous system is very similar. In three species of *Diphyllobothrium*: *D. dendriticum*, *D. latus*, *D. ditremum* (Ohman-James, 1973; Gustafsson and Wikgren, 1981; Gustafsson and

Eriksson, 1991; Gustafsson et al., 1993; Biserova and Kutyrev, 2014; Biserova et al., 2014; Barčák et al., 2019) and in *P. phocarum* described in this work, serotonergic neurons are larger cells with a regular oval soma shape. The number of serotonergic neurons detected in the brain and the main cords is less than peptidergic ones. In the brain lobes and MCs of *P. phocarum*, serotonergic neurons are located in the cortical layer, on the distal surface in the lateral zone of the body. In general, this coincides with the position of 5-HTergic neurons in the MCs of the plerocercoid of *D. dendriticum*. In *P. phocarum* 5-HTergic neurons lie at some distance from the neuropil (Fig. 6), while in *D. dendriticum* 5-HTergic neurons lie directly on the surface of the neuropil (Biserova and Kutyrev, 2014). In the scolex of *P. phocarum* we found peripheral 5-HT-IR neurons located at a considerable distance from the lateral lobes, but they are connected with the neuropil by the main neurite (axon). Thin processes of the dendritic tree of such a neuron were traced to the bothria subtegument. We hypothesize the sensory function of these serotonergic neurons. Peripheral 5-HT-IR neurons were also noted in the bothria of *D. latus* (Barčák et al., 2019).

FMRFamide-IR neurons of *P. phocarum* have a common morphological feature: their somas are often irregular in shape, with 3–4 neurites, without the main axon, and the processes look like a string of beads. The cytomorphology of FMRFamide-IR neurons in P. *phocarum* and *D. dendriticum* is similar. Differences are found in the arrangement of neurons more confined to the proximal and medullar areas of the body of *D. dendriticum* (Gustafsson and Wikgren, 1981; Gustafsson et al., 1985; Wikgren, 1986; Biserova et al., 2014). In contrast, in *P. phocarum*, FMRFamide-IR neurons and processes are richly represented in the cortical parenchyma and subtegument. At the same time, FMRFamide-IR neurons with a main axon directed to the radial nerve associated with the main cord are identified in the peripheral plexus of *P. phocarum*. The dendritic tree is developed on the opposite pole of these neurons. Probably FMRFamide-IR neurons of this type have sensory function associated with the subtegumental muscles.

Both 5-HT-IR and FMRFamide-IR neurons have been found in the median commissure midline in *D. dendriticum* plerocercoid (Gustafsson et al., 1985; Biserova et al., 2014; Biserova and Kutyrev, 2014). In *P. phocarum*, neither serotonin nor FMRFamide IR neurites form a single central bundle of the cerebral commissure. The immunoreactive neurons are located loosely, closer to the lateral neuropils.

In addition to the above, GABA-like IR neurons have been identified in the central and peripheral nervous system of *P. phocarum*. The presence of GABA-like IR in tapeworms has long been questioned, but finally it has been identified in the nervous system of *Moniezia expansa* (Eriksson et al., 1995), *Caryophyllaeus laticeps* and *D. dendriticum* (Biserova et al., 1998; 2014), *Ligula intestinalis* and *Triaenophorus nodulosus* (Biserova et al., 2007). In *Moniezia expansa*, GABA-like-IR is present in nerve nets closely associated with the body wall musculature and in the longitudinal nerve cords. In *D. dendriticum*, GABA-like IR neurons are usually unipolar and have more intensive IR in the neurite than in the perikaryon. In *C. laticeps,* two types of GABA--like IR neurons occur: small neurons of the CNS (MCs), and large unipolar neurons of the PNS associated with the longitudinal muscles. The location of GABA-like IR neurites is associated with the longitudinal, transverse and dorsoventral muscles layers, including intensely stained peripheral GABA-like IR neurites in the subtegumental zone (Biserova et al., 2014). The distribution pattern of GABA-like IR in the nervous system of the plerocercoid of *P. phocarum* is similar to that of the plerocercoid of *D. dendriticum*. GABA-like IR is detected in both the central and peripheral NS of the plerocercoids. At the periphery, all muscle layers have accompanying GABA-like IR neurons. It is important to note that as in the plerocercoid of *D. dendriticum* the muscle fibers of the longitudinal muscles of *P. phocarum* are jointly innervated by GABA-like and FMRFamide-IR neurites (Fig. 7). Thus, these neuroactive substances could have opposite functions in cestodes. In addition, GABA-like IR substance may act as an independent transmitter in the PNS, where it may be involved in the function of the subtegumental muscles as well as the dorsoventral and transverse musculature.

Opposite to immunocytochemical data, some molecular studies indicate that tapeworms have lost the genes that are specifically required in other animals for synaptic signaling using the classical neurotransmitters dopamine, tyramine, octopamine, histamine and gamma-aminobutyric acid (Preza et al., 2018). Authors hypothesize that these signaling pathways are either absent in these parasites, or that they require completely different molecular components in comparison with other animals. We believe that tapeworms may use not classic, but some other machinery for synaptic signaling. Moreover, parasites acquire from their hosts many chemical substances, including neuroactive molecules, which may be used in their metabolism. In *Moniezia expansa* the presence of GABA was verified using high-pressure liquid chromatography coupled with fluorescence detection. The concentration of GABA (mean ± S.D.) in *M. expansa* anterior region was 124·8 ± 15·3 picomole/mg wet weight (Eriksson et al., 1995). During immunocytochemical experiments, specific antibodies (monoclonal anti-GABA, clone GB-69 mouse ascites fluid, product number: A0310; Sigma-Aldrich Logistik GmbH) reacted in a similar way in different cestode species (*T. nodulosus; C. laticeps; L. intestinalis; D. dendriticum; P. focarum*), labeling small, predominantly bipolar cells more numerous on the periphery than in the CNS. Therefore, the question of the presence and origin of GABA in cestodes remains open. The features of the neurochemical organization and functioning of the brain in tapeworms require further studies.

Peripheral nervous system. The ordered αTub-IR plexus with regular annular and radial nerves distinguishes the peripheral NS of *P. phocarum* from other diphyllobothriidean cestodes studied. Regular peripheral neuropils were found in the area of the subtegument and cortical parenchyma of *P. phocarum*. In Cestoda, in addition to the brain, neuropils as cluster of neurites with synapses but without neuronal somata (according to the definition by Richter et al., 2010) occur in the main cords (CNS), minor cords and at cross-points of minor cords and ring commissures or radial/lateral nerves (Gustafsson, 1984; Gustafsson et al., 1994; Biserova et al., 2014, and review Biserova, 2016). Somas are usually absent in peripheral neuropils, but αTub-IR neurites form compact areas, regularly distributed in the cortical body layer (Biserova et al., 2014). Ultrastructure of such clusters is described in diphyllobothriidean *Ligula intestinalis* plerocercoid (Biserova and Gordeev, 2010). The development of a regular ordered plexus is most likely associated with the development of thick layers of longitudinal muscles of the body, muscles of the body wall, as well as the powerful dorso-ventral and transverse muscles of the *P. phocarum* scolex.

Three types of muscle innervation have been found in cestodes: a) by peripheral neurons (Jones, 1989; Gustafsson, 1990; Biserova and Salnikova, 2002; Biserova et al., 2000), b) by brain neurons and anterior nerves emerging from the brain and commissures (Webb and Davey, 1975; Fairweather and Threadgold, 1983; Fairweather et al., 1988; 1990; Biserova and Gordeev, 2010), and c) by sarco-neural contacts in the main nerve cords (Biserova et al., 1996; 2000; Biserova, 1997; Biserova and Salnikova, 2002; Hartenstein, 2016). In trypanorhynchs, the muscle bulbs of the tentacles are innervated by bulbar nerves extending from the central lobe of the brain and by giant axons (Rees, 1950; 1988; (Halton, 1994)Halton et al., 1994; Crangle, et al., 1995; Biserova et al., 2020; Biserova, 2021).

In the scolex of *P. phocarum* plerocercoid, the musculature of the botria is innervated by the nerves of the cerebral roots, while the dorsoventral muscles receive contacts directly from the axons of the median commissure belonging to the CNS. In the lateral zone of the scolex and in the body of *P. phocarum*, the muscle layers are accompanied by both neurites of the main nerve cords and the submuscular plexus. The ultrastructure of the neuromuscular contacts is yet insufficiently studied. Neuromuscular contacts with small electron-dense vesicles in the presy-

napse have been described in *Echinoccocus granulosus*(Morseth, 1967) (), *T. nodulosus* (Biserova and Korneva, 2006), *L. intestinalis* (Biserova and Gordeev, 2010) and their ultrastructure is similar to the neuromuscular junctions observed in *P. focarum* median commissure. Paracrine contacts on myocytes with the participation of large vesicles have been described in *Hymenolepis nana, D. dendriticum*, and *T. nodulosus* (Fairweather and Threadgold, 1983; Gustafsson, 1990; Biserova et al., 1996; Biserova, 1997). The ultrastructure of synapses in the brain neuropils of *P. phocarum* corresponds to that in the plerocercoids of *D. dendriticum* (Gustafsson, 1984) and *T. nodulosus* (Biserova and Korneva, 2006).

Intense 5-HT-, FMRFamide-, and αTub-IR at the bottom of the *P. phocarum* bothria is caused by rich innervation of muscles, glandular ducts, and the presence of numerous apparently sensory neurons. The dense plexus of the bothrium bottom shows some semblance to additional dorsal and ventral commissures. A similar pattern at the bottom of bothria was revealed in *D. dendriticum* in relation to cholinesterase activity (Kotikova and Kuperman, 1978); however, the 5-HT or FMRFamide immunoreaction was not expressed (Biserova and Kutyrev, 2014). Both species are characterized by intense αTub-IR in the central part of the scolex and in bothria, associated with the presence of frontal glands. Intense αTub-IR in the secretory terminals of *P. phocarum* is combined with FMRFamide-IR (Fig. 3). It is demonstrated for the first time that the extended terminals of the secretory ducts have numerous FMRFamide-IR nerve terminals on their surface, which confirms that they function under control of the nervous system.

4.3. Nervous system ultrastructure

The nervous system has been investigated by ultrastructural methods in several species of Diphyllobothriidea, i.e. *D. dendriticum, D. ditremum, D. latus, Spirometra mansoni, L. intestinalis.* The ultrastructure of *P. phocarum* plerocercoid neurons demonstrates a high similarity with that of *D. dendriticum* (Gustafsson and Wikgren, 1981; Wikgren, 1986; Kutyrev et al., 2017), as well as a certain similarity with the neurons of tapeworms from the orders Bothriocephalidea, Trypanorhyncha, and Cyclophyllidea studied in this respect (Webb and Davey, 1975; Fairweather and Threadgold, 1983; Biserova et al., 1996; Biserova, 1997; Biserova et al., 2020). As in most species, the dark, light and neurosecretory neurons are identified in the brain and main cords. The median commissure of *P. phocarum* contains large commissural neurons, which are similar in their ultrastructural characteristics to the giant commissural neurons of *Ligula intestinalis* (Biserova and Gordeev, 2010) and *D. ditremum* (Biserova and Kemaeva, 2012). Commissural neurons are distinguished by very large nuclei (more than 6 μm in all three species), deep invaginations of the outer membrane, foamy cytoplasm and small electron-dense vesicles. This type of neurons has not been found in the median commissure of *Triaenophorus nodulosus* (Bothriocephalidea) (Biserova et al., 1996) or in Trypanorhyncha (Biserova et al., 2020). The finding of this type of neurons in *P. phocarum*, in addition to those previously described in the other two species of diphyllibothriidean plerocercoids, makes it possible to assume homology of giant commissural neurons among Diphyllobothriidea.

The nervous system of the *P. phocarum* plerocercoid is characterized by a loose arrangement of neurons and small diameter of neurites both in the neuropils and in the main cords and peripheral nerves. We did not observe a clear bordering of the brain lobes from the parenchyma, or specialized envelopes around the neuropils. The processes of the excretory epithelium cells penetrate between neurons, but do not form specialized envelopes, as it was found in the brain lobes and main cords of *Triaenophorus nodulosus* (Bothriocephalidea) (Biserova et al., 1996). In this species, the epithelium cells of the protonephridial canal can assume glial function and form unique envelopes of the cerebral ganglion and MC's (Biserova and Salnikova, 2002). In *T. nodulosus*, isolation of the central nervous system compartments starts from the early stages of cestode ontogeny. In the procercoid stage the envelopes are not completely formed yet; in the plerocercoid stage, however, the specialized envelope is completely evolved; it is formed by excretory epithelium processes and by intracellular fibrillar matrix (Biserova and Korneva, 2006). In *L. intestinalis* plerocercoid (Diphyllobothriidea) the glia-like cells have been found in the main cords, as confirmed by immunocytochemical evidence (Biserova, 2008). The envelopes separating neurons from the parenchyma are well developed in trypanorhynchean tapeworms, in which several types of glial cells are found in the brain, nerves and MCs (Biserova, 2008) Biserova et al., 2020). Also, cortical and medullar glia are composed of several types of glial cells in the nervous system of Neodermata (Biserova et al., 2010; Biserova, 2016). Contrary to the above, the glia-like cells are not yet found in the nervous system of *P. phocarum* plerocercoid.

To summarise the above, the ultrastructure of the neurons of diphyllobothriideans has a high degree of similarity. Giant neurons of the median commissure are probably homologous within the order Diphyllobothriidea.

4.4. Sensory organs ultrastructure

Sensory organs have been studied by TEM in several species of diphyllobothriidean tapeworms. Sensory papillae were described in the tegument of an adult *Spirometra mansoni* (Okino and Hatsushika, 1994). Two types of nonciliated sensory receptors were observed. The dome-like sensory receptor contained two electron-dense collars and four rootlets surrounded by numerous thin filaments. The type II receptor was found arranged in groups and the apical end was exposed to the external environment. This simple, club-like sensory receptor contained electron-lucent vesicles and microtubules (Okino and Hatsushika, 1994). Also, three types of sensory organs were found in the tegument of *L. intestinalis* plerocercoid: one type of a ciliated receptor and two types of unciliated ones (Biserova and Gordeev, 2010). Previously, six types of sensory organs have been found in *P. phocarum* plerocercoid (Mustafina and Biserova, 2017). In the present study, the unciliated sensory organ of the 3rd type presumably has a mechano-tactile function and corresponds to a mechano-tactile free nerve ending type 6 in the early publication of Mustafina and Biserova (2017) where the receptor was illustrated in detail. The ciliated sensory organ type 2 with short cilia in *P. phocarum* has the same structure with the type 4 receptor in *D. dendriticum* (Kutyrev et al., 2017), type 1 receptor in *L. intestinalis* (Biserova and Gordeev, 2010), type 1 receptor in plerocercoid of *T. nodulosus* (Biserova and Korneva, 1999), and one type of ciliated receptor in an adult *Hymenolepis nana* (Fairweather and Threadgold, 1983). These sensory organs can be characterized by short cilia, one supporting ring under the septate junction and clear vesicles in the bulb; a kinetosome is present; the roots are mostly reduced. Presumably, this type of nerve endings belongs to chemoreceptors (Vinnikov, 1982).

Ciliary endings with a long cilium, two supporting rings, a kinetosome and a rootlet (type 1 in *P. phocarum*) are found in all cestodes studied in this respect (Webb and Davey, 1974). However, it is impossible to talk about the uniformity of the structure and function of these receptors; in each species, there are some differences in the structure of the vesicles, the presence of mitochondria, the degree of root development, the shape of the cilium tip and etc. Fairweather and Threadgold (1983) described in adult *Hymenolepis nana* sensory organs with the same ultrastructure as we find in *P. phocarum* plerocercoid (free endings, type 5). Also, similar free endings were found in the tegument of other cestodes, but sources of these free ending were not found yet.

Specific for diphyllobothriidean sensory organs distribution on the scolex is the colocalization with eccrine glands terminals in the tegument. This colocalization was found in *P. phocarum, D. dendriticum, D. latus, D. ditremum* (Mustafina, 2017b; Mustafina and Biserova, 2017; Kutyrev et al., 2017; Barčák et al., 2019; Biserova and Kemaeva, 2012). Such complexes may be similar in function to the cephalic sensillae of

monogeneans, regulating the secretions release at the frontal end of the body (Lyons, 1972; El-Naggar, Kearn, 1983). Frontal gland apparatus is highly developed in Diphyllobothriidea. The discharge of secretory products into host environment is regulated by the eccrine mechanism and correlates with sensory input and immunomodulatory answer of the plerocercoid (Kutyrev et al., 2021).

Thus, all studied Diphyllobothriidae are characterized by the formation of a structural complex of sensory organs associated with ducts and terminal pores of the frontal glands in the scolex tegument. Such complexes may be similar in function to the cephalic sensillae of monogeneans, regulating the eccrine secretions release at the frontal end of the body.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

All photographs obtained by the authors as a result of the study (LM, TEM, SEM, CLSM) are stored in the personal database of N.M. Biserova and are available upon personal request.

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Compliance with ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

Andersen, K.I., 1975. Ultrastructural studies on *Diphyllobothrium ditremum* and *Diphyllobothrium dendriticum* (Cestoda, Pseudophyllidea). Parasitol. Res. 46, 253–264.

Andersen, K.I., 1977. A marine *Diphyllobothrium* plerocercoid (Cestoda) from blue whiting *Micromestius poutasson*. Parasitol. Res. 52, 289–296.

Barčák, D., Yoneva, A., Sehadová, H., Oros, M., Gustinelli, A., Kuchta, R., 2019. Complex insight on microanatomy of larval "human broad tapeworm" *Dibothriocephalus latus* (Cestoda: Diphyllobothriidea). Parasites Vectors 12, 408.

Biserova, N.M., 1997. Structure of a scolex nervous system of the *Triaenophorus nodulosus* (Cestoda: Pseudophyllidea). Parazitologiya 31, 249–259.

Biserova, M., N., 2008. Ultrastructure of glial cells in the nervous system of *Grillotia erinaceus*. Cell and Tissue Biology 2 (3), 253–264. https://doi.org/10.1134/S1990519X08030061.

Biserova, N.M., 2013. Methods of Visualization for Biological Ultrastructures. Manual for Electron and Confocal Microscopy. A Practical Guideline for Biologists. Publishing House KMK, Moscow.

Biserova, N.M., 2016. Platyhelminthes: Neodermata. In: Schmidt-Rhaesa, A., Harzsch, S., Purschke, G. (Eds.), Structure and Evolution of Invertebrate Nervous Systems. Oxford University Press, Oxford, pp. 93–117.

Biserova, N.M., 2021. Tapeworm *Grillotia erinaceus* (van Beneden, 1858) (Cestoda: trypanorhyncha) ultrastructural organization of the surface tegument and rhyncheal system: skeletal system. Microscopic Anatomy of the Animals. Wiley. https://doi.org/10.1002/9781118158036.maa20180116.

Biserova, N.M., Dudicheva, V.A., Terenina, N.B., Reuter, M., Halton, D.W., Maule, A.G., Johnston, R., Gustafsson, M.K.S., 2000. The nervous system of *Amphilina foliacea* (Platyhelminthes, Amphilinidea). An immunocytochemical, ultrastructural and spectrofluoromentrical study. Parasitology 121, 441–453.

Biserova, N.M., Gordeev, I.I., 2010. Fine structure of the nervous system in *Ligula intestinalis* plerocercoid (Cestoda: Diphyllobothriidea). Invertebr. Zool 7, 133–154.

Biserova, N.M., Kemaeva, A.A., 2012. The innervations of the frontal glands in the scolex of the plerocercoid *Diphyllobothrium ditremum* (Cestoda: Diphyllobothriidea). In: Galkin, A.K., Dubinina, E.V., Poddubnaya, L.G. (Eds.), The Problems of Cestodology IV. Nauka, St.-Petersburg, pp. 13–33.

Biserova, N.M., Korneva, Zh.V., 1999. Sensory apparatus and particularities of the nervous system formation in the ontogenesis of *Triaenophorus nodulosus* (Cestoda). Parazitologiya 33, 39–48.

Biserova, N.M., Korneva, J.V., 2006. The nervous system ontogeny in cestodes and amphilinids. Invertebr. Zool 3, 157–184.

Biserova, N.M., Kutyrev, I.A., 2014. Localization of prostaglandin E2, γ-aminobutyric acid, and other potential immunomodulators in the plerocercoid *Diphyllobothrium dendriticum* (Cestoda). Biol. Bull. 41, 242–250.

Biserova, N.M., Salnikova, M.M., 2002. The ultrastructure of main lateral nerves cords and associated cell elements in *Triaenophorus nodulosus* (Cestoda: Pseudophyllidea). Tsitologiya 44, 611–622.

Biserova, N.M., Gustafsson, M.K.S., Reuter, M., Terenina, N.B., 1996. The nervous system of the pike-tapeworm *Triaenophorus nodulosus* (Cestoda: Pseudophyllidea) - ultrastructure and immunocitochemical mapping aminergic and peptidergic elements. Invertebr. Biol. 115, 273–285.

Biserova, N.M., Korneva, J.V., Polyakova, T.A., 2020. The brain structure of selected trypanorhynch tapeworms. J. Morphol 281, 1–21.

Biserova, N.M., Kutyrev, I.A., Malakhov, V.V., 2011. Tapeworm *Diphyllobothrium dendriticum* (Cestoda) produces prostaglandin E_2 – regulator of host immunity. Dokl. Biol. Sci. 441, 367–369.

Biserova, N.M., Kutyrev, I.A., Jensen, K., 2014. GABA in the nervous system of the cestodes *Diphyllobothrium dendriticum* (Diphyllobothriidea) and *Caryophyllaeus laticeps* (Caryophyllidea), with comparative analysis of muscle innervation. J. Parasitol 100, 411–421.

Biserova, N.M., Mustafina, A.R., Malakhov, V.V., 2021. Structure of the excretory system of the plerocercoid *Pyramicocephalus phocarum* (Cestoda: Diphyllobothriidea): proof for the existence of independent terminal cells. Dokl. Biol. Sci. 496, 17–20.

Biserova, N.M., Reuter, M., Gustafsson, M.K.S., 1998. GABA-and 5-HT-immunoreactivity in nervous system of *Caryophyllaeus laticeps* (Cestoda: Caryophyllaeidea). Wiad. Parazytol. 44, 429.

Biserova, N.M., Salnikova, M.M., Gordeev, I.I., 2007. Immunocytochemical identification and localization of GABA-like compartment of the nervous system in fish helminthes. Proc. Kazan Univ. Nat. Sci. 149, 93–98.

Braten, T., 1968. The fine structure of the tegument of *Diphyllobothrium latum*. A comparison of the plerocercoid and adult stages. Parasitol. Res. 30, 104–112.

Brennan, G.P., Halton, D.W., Maule, A.G., Shaw, C., 1993. Electron immunogold labeling of regulatory peptide immunoreactivity in the nervous system of *Moniezia expansa* (Cestoda: Cyclophyllidea). Parasitol. Res. 79, 409–415.

Brunanská, M., Fagerholm, H.P., Gustafsson, M.K.S., 2000. Ultrastructure studies of *Proteocephalus longicollis* (Cestoda, Proteocephalidea): transmission electron microscopy of scolex glands. Parasit. Res. 86, 717–723.

Chai, J.Y., Murrell, K.D., Lymbery, A.J., 2005. Fish-borne parasitic zoonoses: status and issues. Int. J. Parasitol 35, 1233–1254.

Coil, W., 1991. Platyhelminthes: Cestoidea. In: Harrisson, F.W., Bogitsh, B.J. (Eds.)Microscopic Anatomy of Invertebrates, Vol 3. Wiley-Liss, Inc, pp. 211–283 ISBN 0471568430, 9780471568438.

Crangle, K.D., McKerr, G., Allen, J.M., Howard, C.V., Johansson, O., 1995. The central nervous system of *Grillotia erinaceus* (Cestoda: Trypanorhyncha) as revealed by immunocytochemistry and neural tracing. Parasitol. Res. 81, 152–157.

Davydov, V.G., Biserova, N.M., 1985. The morphology of two types of frontal glands of *Grillotia erinaceus* (Cestoda: Trypanorhyncha). Parazitologiya 1, 32–37.

Davydov, V.G., Poddubnaya, L.G., 1988. Functional morphology of frontal and uterine glands in cestodes of the order Caryophyllidea. Parazitologiya 22, 449–457.

Delamure, S.L., 1961. Ecological and geographical survey of helmintofauna of the northern fur seal. Helmintol 3, 73–79.

Dupouy-Camet, J., Yera, H., 2009. *Diphyllobothrium*. In: Don, L. (Ed.), Molecular Detection of Foodborne Pathogens. Taylor and Francis CRC Press, NewYork, pp. 781–788.

El-Naggar, M.M., Kearn, G.C., 1983. Glands associated with the anterior adhesive areas and body margins in the skin-parasitic monogenean *Entobdella soleae*. Int. J. Parasitol 13 (1), 67–81.

Eriksson, K.S., Maule, A.G., Halton, D.W., Panula, P., Shaw, C., 1995. GABA in the nervous system of parasitic flatworms. Parasitology 110, 339–346.

Fairweather, I., Halton, D.W., 1991. Neuropeptides in platyhelminths. Parasitology 102, 77–92.

Fairweather, I., Threadgold, L.T., 1983. *Hymenolepis nana*: the fine structure of the adult nervous system. Parasitology 86, 89–103.

Fairweather, I., Macartney, G.A., Jonston, C.F., Halton, D.W., Buchanan, K.D., 1988. Immunocytochemical demonstration of 5-hydroxytryptamide (serotonin) and vertebrate neuropeptides in the nervous system of excysted cysticercoid larvae of the rat tapeworm, *Hymenolepis diminuta* (Cestoda, Cyclophillidea). Parasitol. Res. 74, 371–379.

Fairweather, I., Mahendrasingam, S., Johnston, C.F., Halton, D.W., McCullough, J.S., Shaw, C., 1990. An ontogenetic study of the cholinergic and serotoninergic nervous systems in *Trilocularia acanthiaevulgaris* (Cestoda, Tetraphyllidea). Parasitol. Res. 76, 487–496.

Gustafsson, M.K.S., 1984. Synapses in *Diphyllobothrium dendriticum* (Cestoda). An electron microscopical study. Ann. Zool. Fenn. 21, 167–175.

Gustafsson, M.K.S., 1990. The cells of a cestode. *Diphyllobothrium dendriticum* as a model in cell biology. In: Gustafsson, M.K.S., Reuter, M. (Eds.), The Early Brain. Acta Academiae Aboensis, Åbo, pp. 13–44.
Gustafsson, M.K.S., Eriksson, K., 1991. Localization and identification of catecholamines in the nervous system of *Diphyllobothrium dendriticum* (Cestoda). Parasitol. Res. 77, 498–502.
Gustafsson, M.K.S., Eriksson, K., 1992. Never ending growth and a growth factor. I. Immunocytochemical evidence for the presence of basic fibroblast growth factor in a tapeworm. Growth Factors 7, 327–334.
Gustafsson, M.K.S., Wikgren, M.C., 1981. Peptidergic and aminergic neurons in adult *Diphyllobothrium dendriticum* Nitzsch, 1824 (Cestoda, Pseudophyllidea). Z. Parasitenk 64, 121–134.
Gustafsson, M.K.S., Halton, D.W., Maule, A.G., Reuter, M., Shaw, C., 1994. The gull-tapeworm, *Diphyllobothrium dendriticum* and neuropeptide F: an immunocytochemical study. Parasitology 109, 599–609.
Gustafsson, M.K.S., Nassel, D., Kuusisto, A., 1993. Immunocytochemical evidence for the presence of P-like peptide in *Diphyllobothrium dendriticum*. Parasitology 106, 83–89.
Gustafsson, M.K.S., Vaihela, B., 1981. Two types of frontal glands in *Diphyllobothrium dendriticum* (Cestoda, Pseudophyllidea) and their fate during the maturation of the worm. Z. Parasitenk 66, 145–154.
Gustafsson, M.K.S., Wikgren, M.C., Karhi, T.J., Schot, L.P.C., 1985. Immunocytochemical demonstration of neuropeptides and serotonin in the tapeworm *Diphyllobothrium dendriticum*. Cell Tissue Res. 240, 255–260.
Halton, W, D, Maule, A.G., Brennan, G.P., Shaw C., Stoitsova, S.R., & Jonston, C. F., 1994. *Grillotia erinaceus* (Cestoda, Trypanorhyncha): Localisation of neuroactive substances in the plerocercoid, using confocal and electron microscopic immunocytochemistry. Experimental Parasitology 79, 410–423.
Halton, D.W., Gustafsson, M.K.S., 1996. Functional morphology of the platyhelminth nervous system. Parasitology 113, 47–72.
Halton, D.W., Maule, A.G., 2004. Flatworm nerve-muscle: structural and functional analysis. Can. J. Zool. 82 (2), 316–333.
Hartenstein, V., 2016. The nervous system of Platyhelminthes. In: Schmidt-Rhaesa, A., Harzsch, S., Purschke, G. (Eds.), Structure and Evolution of Invertebrate Nervous Systems. Oxford University Press, Oxford, pp. 74–92.
Korneva, Zh.V., 2013. Characterization of cestoda tissue organization. Biol. Bull. 40, 146–157.
Kotikova, E.A., 1976. Patterns of evolution of the nervous system in cestodes. In: Mamkaev, Yu.V. (Ed.), Evolutionary Morphology of Invertebrate Animals. Nauka, Leningrad, pp. 33–34.
Kotikova, E.A., Kuperman, B.I., 1978. Anatomy of the nervous apparatus of cestodes in family *Amphicotylidae* and *Diphyllobothriidae* (Pseudophyllidea). Parazitologiya 12, 210–217.
Kuperman, B.I., 1988. Functional Morphology of Lower Cestodes: Ontogenetic and Evolutionary Aspects. Nauka, Leningrad.
Kuperman, B.I., Davydov, V.G., 1982a. The fine structure of glands in oncospheres, procercoids and plerocercoids of Pseudophyllidea. Int. J. Parasitol 12, 135–144.
Kuperman, B.I., Davydov, V.G., 1982b. The fine structure of frontal glands in adult Cestodes. Int. J. Parasitol. 12, 285–293.
Kutyrev, I.A., Franke, F., Büscher, J., Kurtz, J., Scharsack, J.P., 2014. *In vitro* effects of prostaglandin E_2 on leucocytes from sticklebacks (*Gasterosteus aculeatus*) infected and not infected with the cestode *Schistocephalus solidus*. Fish Shellfish Immun 41, 473–481.
Kutyrev, I.A., Biserova, N.M., Olennikov, D.N., Korneva, J.V., Mazur, O.E., 2017. Prostaglandins E2 and D2 – regulators of host immunity in model parasite *Diphyllobothrium dendriticum*: immunocytochemical and biochemical study. Mol. Biochem. Parasitol. 212, 33–45.
Kutyrev, I.A., Franke, F., Kurtz, J., Scharsack, J.P., 2019. In vitro effects of the neuroactive substances serotonin and γ-aminobutyric acid on leucocytes from sticklebacks (*Gasterosteus aculeatus*). Fish Shellfish Immun 87, 286–296.
Kutyrev, I.A., Biserova, N.M., Mazur, O.E., Dugarov Zh, N., 2021. Experimental study of ultrastructural mechanisms and kinetics of tegumental secretion in cestodes parasiting fish (Cestoda: Diphyllobothriidea). J. Fish Dis. 44, 1237–1254. https://doi.org/10.1111/jfd.13386.
Lyons, K.M., 1972. Sense organs of monogeneans. In: Canning, E., Wright, C. (Eds.), Behavioural aspects of parasite transmission. Zool. J. Linn. Soc. 51, 181–199.
Lindroos, P., Gardberg, T., 1982. The excretory system of *Diphyllobothrium dendriticum* (Nitzsch 1824) plerocercoids as revealed by an injection technique. Parasitol. Res. 67, 289–297.
Malmberg, G., 1971. On the procercoid protonephridial systems of the *Diphyllobothrium* species (Cestoda, Pseudophyllidea) and cercomer theory. Zool. Scr. 1, 43–56.
Moreno, M.J., Casado, N., Urrea-París, M.A., Rodriguez-Caabeiro, F., 2001. Evidence of tubulin in the scolex gland ducts of *Gymnorhynchus gigas* plerocercoid (Cestoda: Trypanorhyncha). Folia Parasit 48, 163–164.
Morseth, J, D, 1967. Observations on the fine structure of the nervous of *Echinococcus granulosus*. J. Parasitol 53, 492–500.
Mustafina, A., 2017a. The ultrastructure of the excretory system of *Pyramicocephalus phocarum* (Cestoda: Diphyllobothriidea). 4th Int. Congress Invertebr. Morphol. 187–188.
Mustafina, A., 2017b. The ultrastructure of the tegument, glands and sensory organs of *Pyramicocephalus phocarum* (Cestoda: Diphyllobothriidea). 4th Int. Congress Invertebrate Morphol. 188.
Mustafina, A.R., Biserova, N.M., 2017. *Pyramicocephalus phocarum* (Cestoda: Diphyllobothriidea): the ultrastructure of the tegument, glands, and sensory organs. Invertebr. Zool. 14, 154–161.
Ohman-James, C., 1973. Cytology and cytochemistry of the scolex gland cells in *Diphyllobothrium ditremum* (Creplin, 1825). Z. Parasitenk 42 (1), 77–86.
Okino, T., Hatsushika, R., 1994. Ultrastructure studies on the papillae and the nonciliated sensory receptors of adult *Spirometra erinacei* (Cestoda, Pseudophyllidea). Parasitol. Res. 80, 454–458.
Preza, M., Montagne, J., Costábile, A., Iriarte, A., Castillo, E., Koziol, U., 2018. Analysis of classical neurotransmitter markers in tapeworms: Evidence for extensive loss of neurotransmitter pathways. Int. J. Parasitol 48, 979–992. https://doi.org/10.1016/j.ijpara.2018.06.004.
Rausch, R.L., Adams, A.M., 2000. Natural transfer of helminths of marine origin to freshwater fishes, with observations on the development of *Diphyllobothrium alascense*. J. Parasitol. 86, 319–327.
Rausch, R.L., Adams, A.M., Margolis, L., 2010. Identity of *Diphyllobothrium* spp. (Cestoda: Diphyllobothriidae) from sea lions and people along the Pacific coast of South America. J. Parasitol 96, 359–365.
Rees, G., 1950. The plerocercoid larva of *Grillotia heptanchi* (Vaullegeard). Parasitology 40, 265–272.
Rees, G., 1966. Nerve cells in *Acanthobothrium coronatum* (Rud.) (Cestoda:Tetraphyllidea). Parasitology 56, 45–54.
Rees, F.G., 1988. The muscle, nervous and excretory systems of the plerocercoid of *Callitetrarhynchus gracilis* (Rud 1819) (Pinter 1931) (Cestoda: Trypanorhyncha) from Bermuda fishes. Parasitology 96, 337–351.
Richter, S., Loesel, R., Purschke, G., Schmidt-Rhaesa, A., Scholtz, G., Stach, T., Vogt, L., Wanninger, A., Brenneis, G., Döring, C., Faller, S., Fritsch, M., Grobe, P., Heuer, C.M., Kaul, S., Møller, O.S., Müller, C.H.G., Rieger, V., Rothe, B.H., Stegner, M.E.J., Harzsch, S., 2010. Invertebrate neurophylogeny: suggested terms and definitions for a neuroanatomical glossary. Front. Zool. 7, 29. ⟨http://www.frontiersinzoology.com/content/7/1/29⟩.
Scharsack, J.P., Gossens, A., Franke, F., Kurtz, J., 2013. Excretory products of the cestode, *Schistocephalus solidus*, modulate in vitro responses of leukocytes from its specific host, the three-spined stickleback (*Gasterosteus aculeatus*). Fish Shellfish Immunol. 35, 1779–1787.
Scholz, T., Kuchta, R., Brabeca, J., 2019. Broad tapeworms (Diphyllobothriidae), parasites of wildlife and humans: recent progress and future challenges. Int. J. Parasitol. Parasites Wildlife. 9, 359–369.
Vinnikov, Ya.A., 1982. Evolution of receptor cells: cytological. Membranous and Molecular Levels (Translated from Russian by Nicholas Bobrov, with a Foreword by Steven Price). Springer-Verlag, New York.
Webb, R.A., Davey, K.G., 1974. Ciliated sensory receptors of the unactivated metacestode of *Hymenolepis microstoma*. Tissue Cell 6, 587–598.
Webb, R.A., Davey, K.G., 1975. The gross anatomy and histology of the nervous system of the metacestode of *Hymenolepis microstoma*. Can. J. Zool 53, 661–677.
Wikgren, M.C., 1986. The nervous system of early larval stages of the cestode *Diphyllobothrium dendriticum*. Acta Zool 67, 155–163.
Yoneva, A., Scholz, T., Kuchta, R., 2018. Comparative morphology of surface ultrastructure of diphyllobothriidean tapeworms (Cestoda: Diphyllobothriidea). Invertebr. Biol. 137, 38–48.
Žďárská, Z., Nebesárová, J., 1997. Ultrastructure of three types of scolex gland cells in adult *Bothriocephalus claviceps* (Cestoda: Pseudophyllidea). Folia Parasitol. 44, 139–146.
Žďárská, Z., Scholz, T., Nebesárová, J., 2004. Ultrastructure of the apical glandular region of the scolex of *Proteocephalus torulosus* (Cestoda: Proteocephalidae). Folia Parasitol. 51, 333–338.