Journal of Morphology

WILEY

JOURNAL OF morphology

RESEARCH ARTICLE

Complex Brain Morphology Discovered in the Shark Parasite *Nybelinia surmenicola* (Cestoda: Trypanorhyncha)

Natalia M. Biserova | Anna A. Margarit

Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia

Correspondence: Natalia M. Biserova (nbiserova@yandex.ru)

Received: 2 August 2024 | **Revised:** 30 September 2024 | **Accepted:** 4 October 2024

Funding: This study was supported by the RSF grant no. 23-24-00118 and project no. 121032300121-0.

Keywords: frontal lobes | giant axons | glia | neural lamella | neuron | ultrastructure

ABSTRACT

The ultrastructure of the nervous system has been studied in sexually mature *Nybelinia surmenicola* (Cestoda: Trypanorhyncha) from the intestine of a shark *Lamna ditropis*. The central nervous system (CNS) reveals a complex organization within cestodes and corresponds to the trypanorhynch pattern of brain architecture. The brain of *N. surmenicola* is differentiated into nine clearly defined lobes and semicircular, median, and X-shaped cruciate commissures. A specific feature is the presence of a powerful extracellular capsule that surrounds the brain lobes with the cortical glial cells. Moreover, the architecture of the anterior lobes clearly distinguishes the species of Tentacularioidea. The neurons of the anterior lobes form compact groups looking like frontal horns. There are approximately 120 neurons in the anterior lobes and a preliminary estimate of more than 300 perikarya in the brain. Several ultrastructural types of neurons have been identified, differing in the size and shape of the soma, the density of the cytoplasm, and the ultrastructure of synaptic vesicles. Numerous synapses involving clear and electron-dense vesicles have been observed in neuropils. Two types of glial cells have been found in the brain that participate in neuronal metabolism and wrap around the giant axons, brain lobes, neuropil compartments, and the main nerve cords. Such a powerful extracellular fibrillar brain capsule has not been observed in the brain of other studied cestodes and has been demonstrated in this study for the first time. The differentiation of the brain lobes reveals the important role of the rhyncheal system in the evolution of cestodes and correlates with their behavior. The anterior nerves arising from the anterior lobes innervate the radial muscles stabilizing the position of the tentacle sheaths and movements of the attachment organs. The nervous system anatomy and the brain architecture may reflect the morphofunctional aspects of the tapeworm evolution.

1 | Introduction

Research of the nervous system of cestodes is a trend in modern cell biology due to novel insight of the complex organization of the brain and the structural diversity of neurons in tapeworms (Barčák et al. 2023; Biserova, Mustafina, and Raikova 2022, Biserova et al. 2023; Koziol, Krohne, and Brehm 2013; Liu et al. 1996; Montagne, Preza, and Koziol 2023; Terenina et al. 2009).

For a long time, the nervous system of cestodes was considered secondarily simplified due to the intransition to a completely parasitic lifestyle (Beklemishev 1964; Kuperman 1988) and was described as a simply arranged orthogon (Kotikova 1976). However, detailed studies of the nervous system of representatives of the Trypanorhyncha have radically changed our understanding of the cestode nervous system (Biserova 2016; Biserova and Korneva 2012; Biserova, Korneva, and Polyakova 2020; Crangle et al. 1995; Halton et al. 1994;

Rees 1941b, 1950, 1988). The presence of a complexly organized accumulation of neurons and neuropils on the trypanorhynch scolex is consistent with the definition of a brain in bilaterian. The term "brain" is used as taxon-independent and free of homology assumptions, precise and consistent terminology (Richter et al. 2010). Concerning cestodes, a brain is a cluster of neurons, which is the most prominent anterior condensation of neurons and glial cells in the scolex. In other parts of cestode body, there are ganglia; for example, bulbar ganglia (in Trypanorhyncha) or caudal ganglia (in Amphilinida).

Interest in the morphology of the nervous system of trypanorhynch cestodes is due to the unique morphology of the scolex, their position in the phylogenetic tree and their connection (parasitizing) with ancient representatives of the ichthyofauna, sharks and rays, as definitive hosts (Beveridge et al. 2017; Caira and Jensen 2014; Campbell and Beveridge 1994; Palm et al. 2009; Waeschenbach and Littlewood 2017). The trypanorhynch scolex is divided into four regions (Figure 1): *pars bothrialis*—the region of bothria, *pars vaginalis*—the region of the proboscis sheaths, *pars bulbosa*—the region of the muscular proboscis bulbs, *pars postbulbosa*—the region of the scolex behind the bulbs. The position of the Trypanorhyncha in the phylogenetic tree of cestodes is in discussion. Morphological evidence strongly supports the monophyly of the Trypanorhyncha (Campbell and Beveridge 1994; Palm 2004). Some molecular data provide evidence for the monophyly of the Trypanorhyncha (Waeschenbach et al. 2007), in contrast to previous molecular studies (Olson and Caira 1999). However, recent molecular data have supported the existence of two independent trypanorhynch lineages, Trypanobatoida and Trypanoselachoida, that is, paraphyly (Olson et al. 2010). Studies of the nervous system and the brain architecture in different taxa may reveal morphofunctional aspects of the trypanorhynch evolution.

The microscopic anatomy of the trypanorhynch nervous system was described with varying degrees of details in *Aporhynchus norvegicus* (Rees 1941a), *Dibothriorhynchus grossum* (Rees 1941b), *Grillotia heptanchi* (Rees 1950), *G. erinaceus* (Johnstone 1911; Biserova 1991, Biserova, Korneva, and Polyakova 2020), *Callitetrarhynchus gracilis* (Rees 1988), *Parachristianella sp*. (Biserova and Korneva 2012), *Dollfusiella aculeata* (Biserova, Korneva, and Polyakova 2020), and in the plerocercoid stage in *N. surmenicola* (Gordeev 2016). The nervous system of *G. erinaceus* belonging to the Lacistorhynchoidea has been studied both by immunocytochemical (Crangle et al. 1995; Halton et al. 1994) and ultrastructural methods; several types of neurons, sensory organs, giant axons and several types of glial cells have been discovered (Biserova 2008a, 2008b).

Studies on the ultrastructural features of the Trypanobatoidea are few and are largely restricted to investigations of the rhyncheal system (Beveridge and Smith 1988), musculature, tegument, and scolex glands (Jones and Beveridge 1998). Representatives of the Tentacularioidea (Trypanorhyncha: Trypanobatoidea) differ from other trypanorhynch cestodes in many respects in scolex and strobilar morphology (Beveridge et al. 2017). They are characterized by a compact craspedote

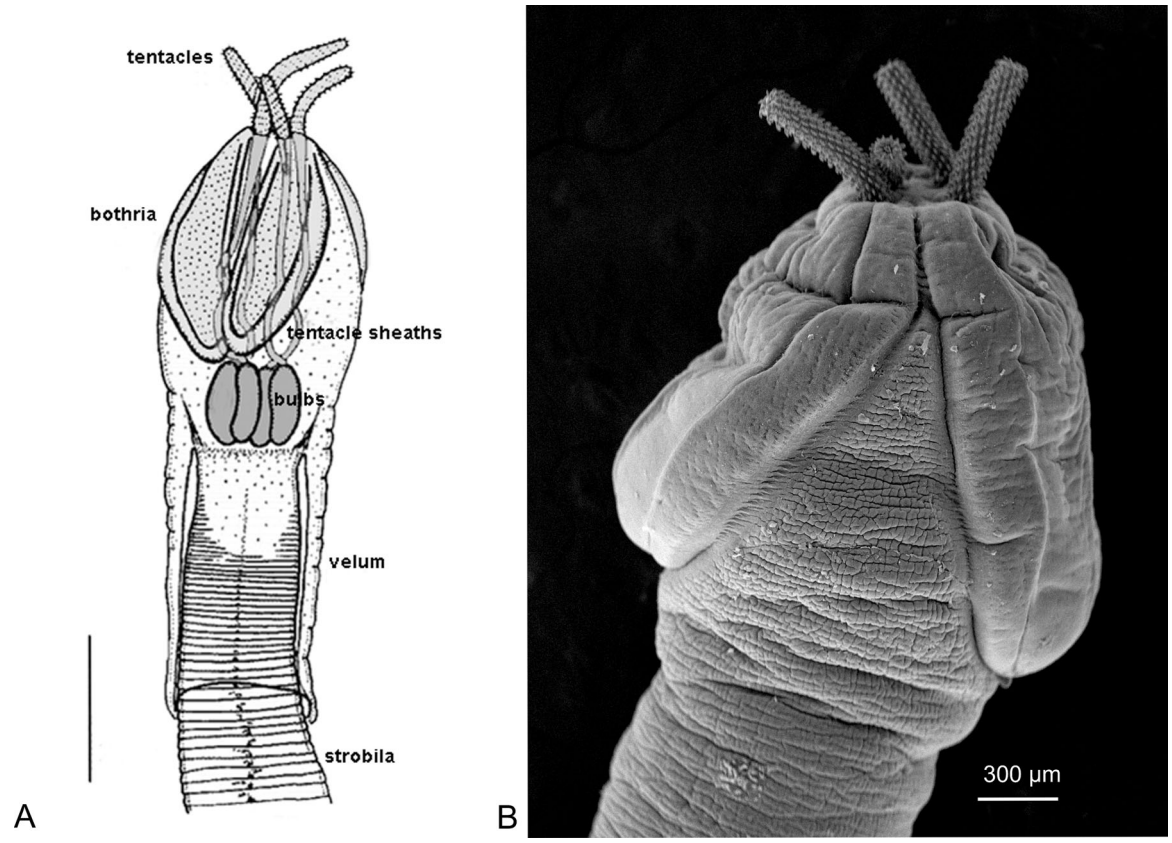

FIGURE 1 | *Nybelinia surmenicola* scolex. (A) Schematic drawing of the scolex, dorsal view with tentacles, bothria, tentacle sheaths, muscle bulbs, velum and the anterior part of the strobila; scale: 1000 µm. (B) Scanning electron microscopy of the scolex lateral view.

scolex, the *pars bothrialis* is close to the *pars bulbosa* (Figure 1A,B), because the *pars vaginalis* is short (Palm 2004). The musculature of the scolex and the rhyncheal system has been studied in two species of *Nybelinia*; and many specific features of muscle ultrastructure have been found (Jones and Beveridge 1998; Gordeev 2016). The tegument of *Nybelinia* species has several ultrastructural features which make it considerably different from the Eutetrarhynchoidea and Lacistorhynchoidea. Species of the genus *Nybelinia* have no palmate microtriches (Biserova, Gordeev, and Korneva 2016; Palm 2004). It has been hypothesized that the tegument of tentaculariids lacks ciliary sensory structures, since they were not found in the scolex of three species: the plerocercoid of *Tentacularia coryphaenae* (Palm, Mundt, and Overstreet 2000), the plerocercoid of *N. surmenicola* (Biserova, Gordeev, and Korneva 2016) and adult *N. queenslandensis* (Jones and Beveridge 1998). At the same time, in the plerocercoids of *N. surmenicola*, the presence of complex motor activity has been established, including the active tentacle ejection on the first contact with the substrate, a rapid forward movement of the "swimming" type, or sliding along the substrate with the participation of bothria, turns to the right and to the left, and other patterns (Gordeev and Biserova 2016). Such motor patterns should correlate with a complex organization of the nervous system, which is involved in processing incoming information and regulating the coordinated muscle contraction of the scolex and tentacles in the process of attachment to the host tissues. The anatomy of muscular, excretory, and nervous systems has previously been investigated in *N. surmenicola* plerocercoids from bony fishes (Gordeev 2016). At the anatomical level, it has been found that the plerocercoid nervous system has a median commissure, anterior commissures and lobes, central lobe and central nerve, and main lateral nerve cords. At the ultrastructural level, thin neurites in the subtegument, giant axons, and the envelopes of the central nerve have been shown (Gordeev 2016). The nervous system of sexually mature *N. surmenicola* from sharks as well as in other Tentacularioidea has never ever been studied.

Our research addressed the ultrastructure of the adult brain of sexually mature *N. surmenicola* for the purpose of a detailed description of the structure and morphofunctional features of the nervous system organization in Tentacularioidea.

2 | Material and Methods

Nybelinia surmenicola Okada, 1929 (Cestoda: Trypanorhyncha Diesing, 1863; Tentacullariidae Poche, 1926) were collected from the stomach and spiral valve of a shark, *Lamna ditropis*, caught in the open northwestern waters of the Pacific Ocean (42°35′ N 148°50′ v. d.). The material was collected in June 2018 on board the research vessel "Professor Kaganovsky." More than 20 specimens of worms were extracted from one shark for research.

For transmission electron microscopy (TEM) cestodes were fixed with 2.5% glutaraldehyde (SERVA, Germany) in 0.1 mol L^{-1} PBS, pH 7.4, washed with the same buffer for 10 min, and then postfixed with a 1% OsO_4 (Moscow Chemical Factory) in the same buffer for 1 h, dehydrated in gradient series ethanol and acetone, impregnated for 3 days in epoxy resin Embed 812 (SIGMA) and polymerized at 37°C (24 h) and 60°C (48 h).

The microscopic anatomy of the brain was studied in a series of transverse semi-thin (1–2 μm) sections of the adult scolex made on a Leica UC7 ultracut, stained with 1% methylene blue and photographed using a Leica DM5000 light microscope.

The ultrastructure of the brain was studied on serial sections prepared on a Leica UC7 ultracut. 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 tissues was studied on a JEM-1011 and JEM-1400 (JEOL) microscope at an accelerating voltage of 80 kV at the Center for Collective Use of Electron Microscopy, Faculty of Biology, Lomonosov Moscow State University and at the Center for Collective Use of Electron Microscopy at the Papanin Institute for Biology of Inland Waters, the Russian Academy of Sciences.

3 | Results

3.1 | Microscopic Anatomy

The brain is located medially in *pars bothrialis* and *pars vaginalis*, under the cruciate muscle bundles that stabilize the position of the sheaths in the upper part of the scolex and is surrounded by four tentacle sheaths (Figures 1B and 2). The brain of adult *N. surmenicola* has a complex architecture, includes nine lobes, and consists of two pairs of anterior, one unpaired central, and two pairs of lateral lobes. The pair of lateral nerve cords and a central nerve innervating the bulbar ganglia run from the brain caudally. The anterior lobes are united by the dorsal and ventral semicircular commissures; the lateral lobes are connected by the X-shaped cruciate and median commissures. Dorsal, ventral, and lateral paired roots emerge from each pair of brain lobes. The excretory system canals pass through the brain: paired dorsal and paired ventral vessels; in the center of the brain, there is a median anastomosis connecting the ventral excretory vessels. The central zone of the brain is located proximal to the paired excretory vessels.

3.1.1 | The Anterior Lobes of the Brain

The four anterior (or frontal) lobes of *N. surmenicola* lie under the cruciate muscles controlling the dorsal, ventral, and lateral right and left sides of the scolex apical part (Figure 3A). The anterior lobes have a complex organization including considerable neurites compartments and neuron perikarya compartments located separately (Figure 3B). Anterior lobes are organized as dorsal and ventral pairs; two muscle bundles extend in the lateral direction between the lobe pairs. Neuropils and semicircular commissures make up the main volume of the anterior lobes; the neuron perikarya compartments are located frontally in each lobe. They form *frontal clusters/horns* above the semicircular commissures, which unite the right and left lobes (Figure 3B–D). Thus, the *frontal neurons* do not form a cortical layer around the neuropil; the anterior lobe neuropil

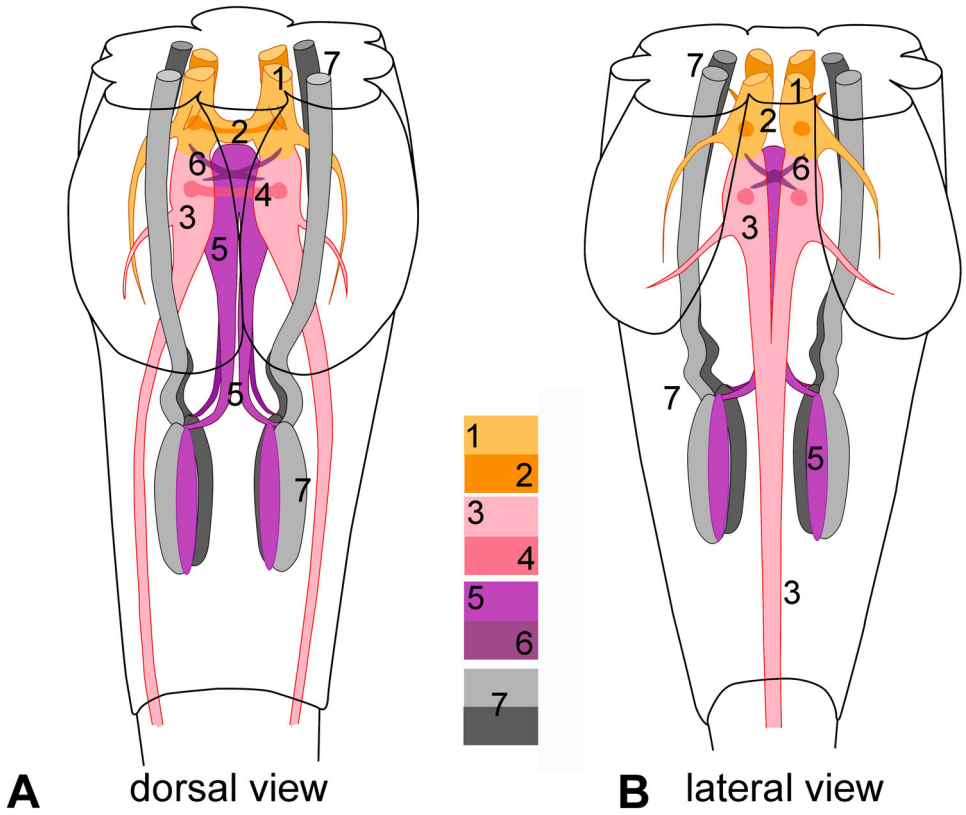

FIGURE 2 | *Nybelinia surmenicola* brain architecture. (A) Dorsal view. (B) Lateral view. 1, anterior lobes; 2, semicircular commissures and neuropils; 3, lateral lobes and main lateral cords; 4, median commissure and neuropils of the lateral lobes; 5, central lobe, central nerve, bulbar ganglia; 6, X-shaped commissure; 7, tentacle sheaths and muscular bulbs.

occupies a lateral position and is separated from the perikarya. Each frontal neuron cluster includes up to 30 perikarya and totally more than 120 neurons located in the frontal horns of the anterior lobes. In addition, individual neurons were found among the nerve processes and in the neuropils; probably they are interneurons.

Four neuropils of the anterior lobes are located at the corners of an elongated quadrangle formed by powerful dorsal and ventral semicircular commissures (Figure 3D,E). The anterior neuropils give rise to paired ventral, dorsal and lateral roots; the lateral roots go laterally around the tentacle sheath. In addition, two pairs of anterior nerves extend forward to the apical part of the scolex. The anterior nerves pass along the tentacle sheaths' inner side (Figure 2).

3.1.2 | The Central Lobe

The central unpaired lobe begins under the semicircular commissures of the anterior lobes and extends caudally beyond the lateral lobes. It includes large neurons with clearly visible nuclei and many nerve fibers. The perikarya of the neurons lie in the middle of the central lobe (Figure 4). Two symmetrical groups of giant neurons (three cell bodies in each) lie in the center, between the dorsal and the ventral pairs of the anterior lobes (Figure 4B). In the area of the cruciate muscle intersection, we find motor neuropils on the surface of the cruciate muscles. Here, is a group of giant unipolar neurons, their neurites form the X-shaped cruciate commissure, connecting the neuropils diagonally. The X-shaped commissure contains giant axons reaching 10–15 μm in diameter. Their perikarya have rounded nuclei and small nucleoli. The X-shaped commissure connects the four neuropils of the lateral lobes (Figure 4A,B).

Deeper to the X-shaped commissure, there is the median commissure, connecting the right and the left pairs of neuropils of the lateral lobes (Figure 4C,D). The median commissure consists of axons of different diameters, and it is surrounded by large neurons. There are giant bipolar and giant tripolar neurons reaching 40×20 μm in soma size with oval nuclei of 13×6 μm in diameter (Figure 4E).

In addition to the neurons, the central lobe includes four compact groups of giant axons, strictly oriented longitudinally, which extend to the posterior end of the lobe. Clusters of giant axons are located outside the cell bodies of the X-shaped commissure, forming the dorsal and the ventral pairs. At the exit from the central lobe, they give rise to the central nerve (Figure 4C,D).

3.1.3 | The Central Nerve

The central nerve consists of four bundles of nerve processes extending back from the central lobe to the muscular bulbs of the tentacles (Figure 4E–G). Four compact neurite bundles

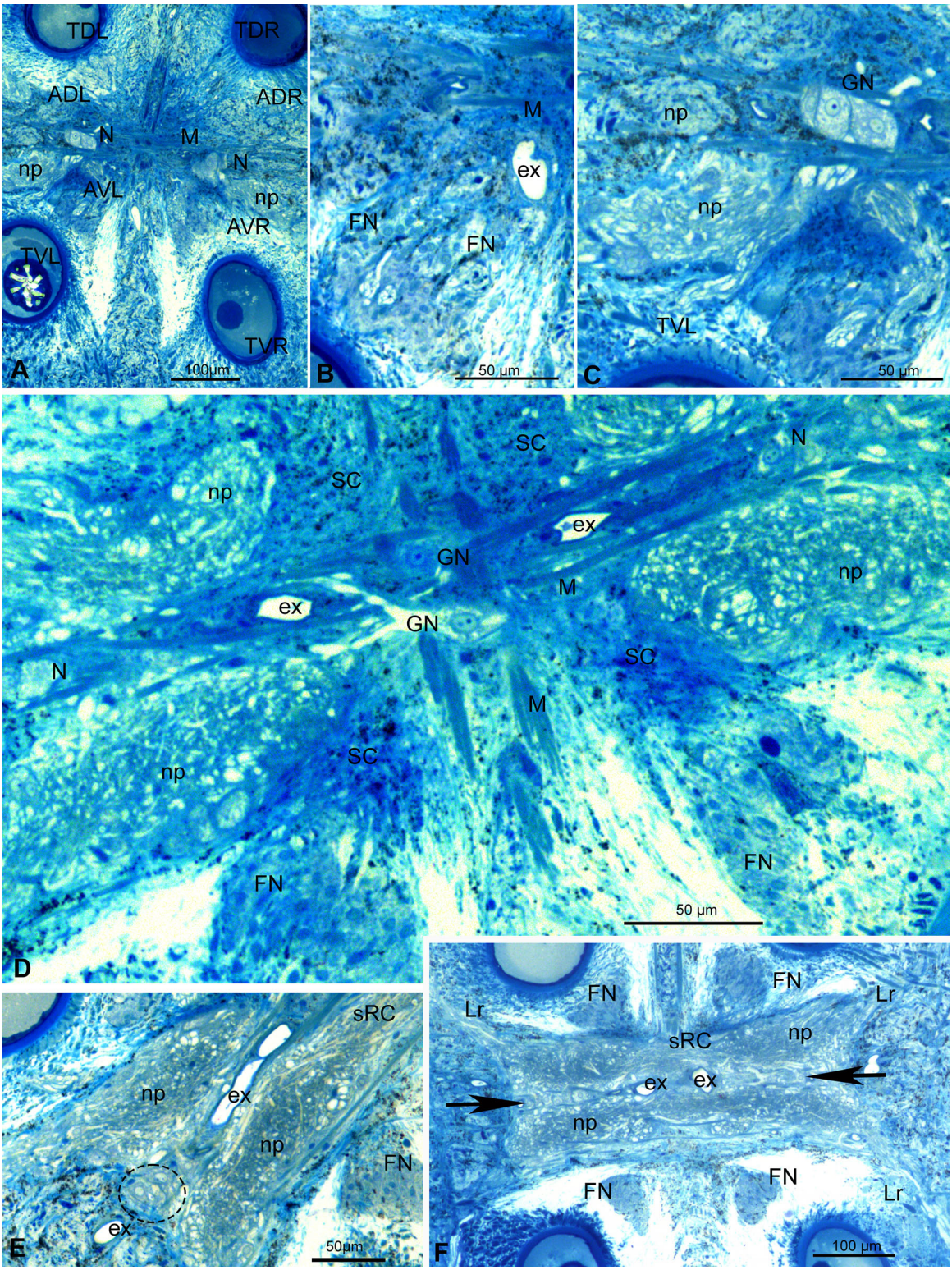

FIGURE 3 | Anterior lobes microscopic anatomy; cross sections; methylene blue; Leica DM5000. (A) Crisscross muscles and anterior lobes in the frontal part of the scolex; (B) frontal neurons of the anterior ventral lobe; (C) the giant neurons in the middle part of the anterior lobes (the left pair); (D) common view of the anterior brain region; the unipolar neuron with the giant axon and large neurons are situated in the crisscross muscle area; the frontal neuron perikarya and secretory cells are shown; (E) the left pair of the anterior lobes with semiring commissures and cluster of neurons (the dotted circle) located in the median line; (F) the dorsal and ventral pairs of the anterior lobes; two semiring commissures and four neuropils of the anterior brain region; the frontal horns of neuron pericarya are shown; arrows point to midline neurons. ADL, ADR, AVL, AVR, anterior dorsal and ventral left and right lobes; ex, excretory vessel; FN, frontal neuron perikarya; GN, giant neuron; Lr, lateral brain rootlet; M, muscle; N, neuron perikaryon; np - neuropil; TDL, TDR, TVL, TVR, dorsal and ventral left and right tentacle sheath; SC, secretory cells; sRC, semiring commissure.

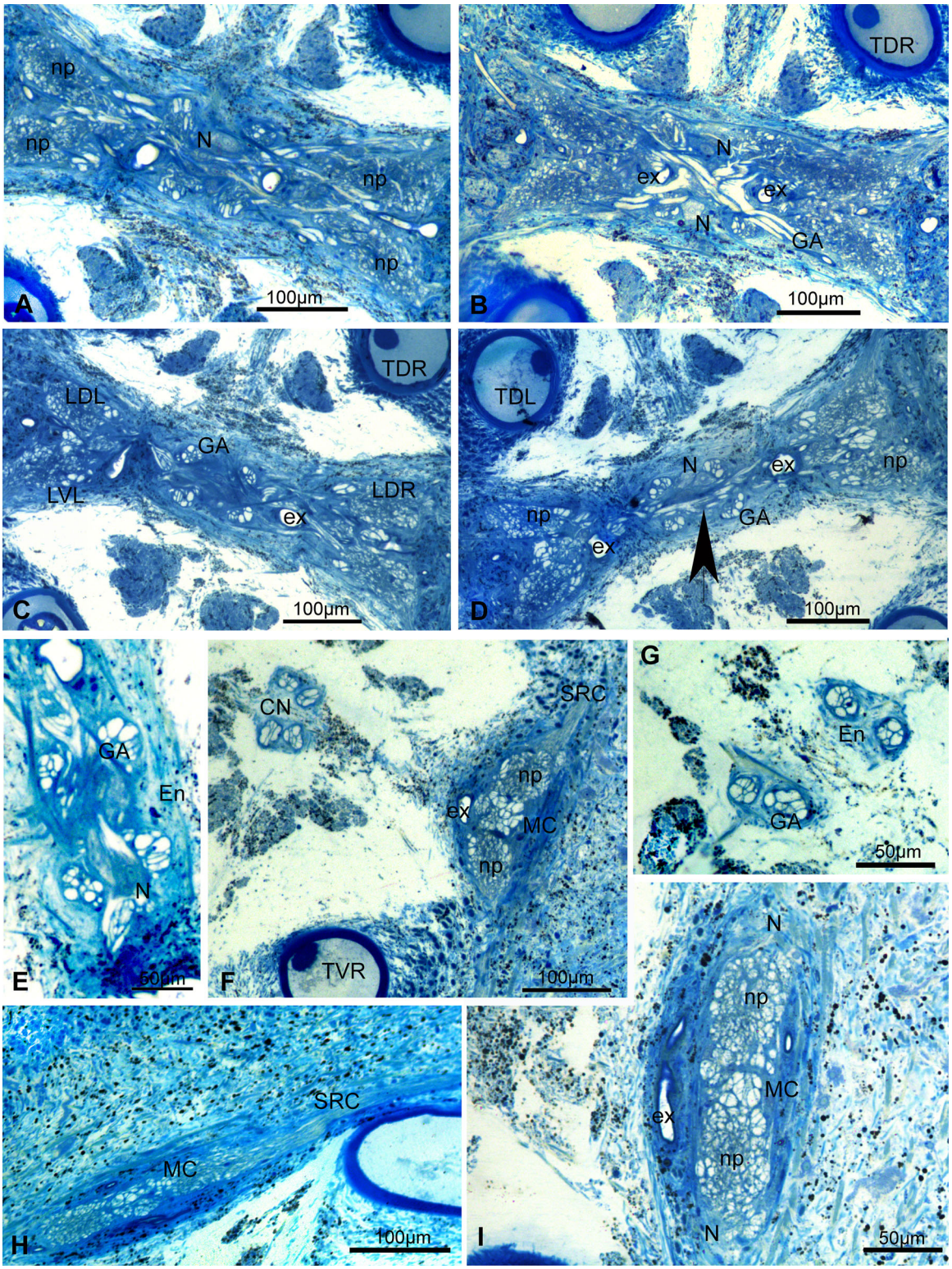

FIGURE 4 | Microscopic anatomy of the brain central and lateral lobes and the main nerve cords; cross sections; methylene blue; Leica DM5000. (A, B) The X-shaped commissure with neuron nuclei in the central lobe; four neuropils of the lateral lobes are connected by the X-shaped commissural giant axons; part of giant axons are arranged in compact clusters which will develop in the central nerve; (C) the four lateral lobes are located under the X-shaped commissure; giant axons organize in four clusters surrounded by glia-like envelope cells; (D) the median commissure with neurons of the central lobe (arrow); (E) posterior part of the central lobe with four clusters of giant axons; the neurons and glia-like envelope cells are also shown on the figure; (F) four clusters of giant axons join in dorsal and ventral pairs; the caudal part of the right lateral lobe continues in the main lateral cord; (G) the central nerve in the *pars vaginalis* area surrounded by the glia-like envelope; (H, I) the main cord with axons, neurons and envelope cells. CN, central nerve; GA, giant axon; En, glia-like envelope cells; ex, excretory vessel; LDL, LDR, LVL, LVR, lateral dorsal and ventral left and right lobes of the brain; MC, main lateral nerve cord; N, neuron perikaryon; np, neuropil; SRC, semiring commissure; TDL, TDR, TVR, dorsal and ventral left and right tentacle sheath.

(25–30 μm each) include 2–3 giant axons 10–12 μm in diameter. In the central lobe each cluster of giant axons is surrounded by its own sheath; deeper (caudally) they are united by a common sheath into the central nerve, extending back to the bulbar ganglia. On the dorsal and ventral sides, the neurites are brought together in pairs and combined into dorsal and ventral pairs. They diverge closer to the bulbs and form 4 bulbar nerves, one nerve to each bulb. It is important to note that the central nerve lacks neuronal perikarya.

Close to each muscular bulb, powerful bulbar ganglia are located, consisting of large neurons. These ganglia continue along the muscular wall to the posterior end of the bulbs and innervate the proboscis apparatus.

3.1.4 | The Lateral Lobes

The four lateral lobes are formed from the marginal parts of the anterior lobes below the semicircular commissures. They represent four dense clusters of neurites and cortical neurons. The lateral lobes include the axons of the X-shaped and median commissures (Figure 4D,F,I). The neurons of the lateral lobes lie outside the neuropils and form symmetrical compact groups: the paired lateral ones (four groups) and the two groups of neurons located medially. The paired neuropils of the lateral lobes are designated as the right and left dorsal, and the right and left ventral neuropils (Figure 4D,F,I). Neuropils have a diameter of about 25 μm and each contains three giant axons. The sizes of neurons in the lateral lobes are smaller than those in the central lobe. Below the median commissure, the four lateral neuropils merge in two: the right and the left lateral neuropils. Together with the cortical neurons, they form the double right and left lateral lobes, extending caudal to the *pars bothrialis*. Gradually, the lobes decrease in diameter and extend in the dorsoventral direction, maintaining the double structure of the neuropils. At the middle level of the bothrium, dorsal and ventral roots depart from the lateral lobes, encircling the proboscis sheaths in the form of semicircular nerves (the first semicircular nerve). These nerves innervate the scolex muscle (Figure 4F). The second semicircular nerve emerges from the main cords in front of the muscular bulbs; it is also formed by the ventral and dorsal nerve roots (Figure 4H).

The lateral lobes smoothly transform into the main lateral cords, lose their clearly dual structure, and have a rounded neuropil in cross section, which is surrounded at the periphery by small neurons.

3.1.5 | The Main Lateral Cords

The main lateral cords are a direct continuation of the neuropils of the lateral lobes of the brain (Figure 4F,H,I). The main cords are located on the median line, distal to the main longitudinal excretory vessels.

Each cord consists of dense clusters of neural processes measuring approximately 50 × 25 μm. Throughout the scolex, the main cords retain paired neuropils. The neurons are located both among the neurites and in the cortical layer around the conducting central neurites. Outside, the lateral cords are surrounded by electrondense cells with elongated large nuclei. Anterior to the muscular bulbs, the second semicircular nerve emerges from the main cords; it, like the first, is formed by the ventral and dorsal roots extending from the neuropils of the main cords (Figure 4H).

The peripheral nervous system is represented by numerous nerves in the cortical parenchyma. Posterior to the *pars bothrialis*, many small peripheral nerves innervate the subtegumental ring and longitudinal muscles of the scolex and body. Sensory neurons have been found in the subtegument, with free dendrites terminating in the bothrial tegument. In the posterior fold behind the scolex, thin longitudinal nerves pass along the longitudinal excretory canals. The peripheral nerves of the velum are surrounded by elongated cells with long thin processes that wrap around the nerve; they contain large mitochondria (1.5–2 μm).

3.2 | Ultrastructural Organization of the Brain

A feature of the ultrastructure of adult *N. surmenicola* is a clear compartmentalization of the brain, where each part is surrounded by a fibrillar membrane. A synaptic apparatus is well developed. There are numerous synapses in lobe' neuropils. The central lobe has several neuropil clusters. In different parts of the central lobe there are compact clusters of thin neurites and large axons that form synaptic glomeruli with asymmetrical synapses with different types of vesicles. Also, there are motor neuropils with numerous neuromuscular junctions on the cruciate scolex muscles and on the radial muscles stabilizing tentacle sheath.

The ultrastructure of the nervous system of *N. surmenicola* shows a wide variety of ultrastructural types of neurons, which differ in the shape and size of the soma, nucleus, and the cytoplasm, as well as in vesicle size and structure (Figures 5 and 6). *Small, large*, and *giant neurons* are distinguished by size. Giant neurons reach 20–30 μm in soma diameter and are located in the central lobe, median and cruciate commissures, and in the bulbar ganglia. Large (10–15 μm) and small (from 3–10 μm) neurons are found in each lobe of the brain. Small neurons are located mostly in the main lateral cords in the areas of the *pars bulbosa* and *pars postbulbosa*, and also occur in the peripheral subtegumental plexus.

Based on the structure of the cytoplasm, neurons are divided into light and dark. The cytoplasm of *light neurons* is electron-translucent and does not contain free ribosomes and β-glycogen; granular endoplasmic reticulum is poorly developed, located mainly around the nucleus where small oval mitochondria are found; neurites contain microtubules and rare cisternae of smooth ER. Few vesicles are found in the area of the Golgi complex and near synaptic contacts. The cytoplasm of *dark neurons* is dense, filled with clusters of ribosomes, rough ER, and β-glycogen granules. At the periphery of the perikaryon, there are small round mitochondria (0.3–0.4 μm) and microtubules. The neurites possess numerous synaptic contacts, mainly collected in the neuropils of the lobes, as well as in the conducting zone of the main cords and exiting roots of the brain (Figure 7).

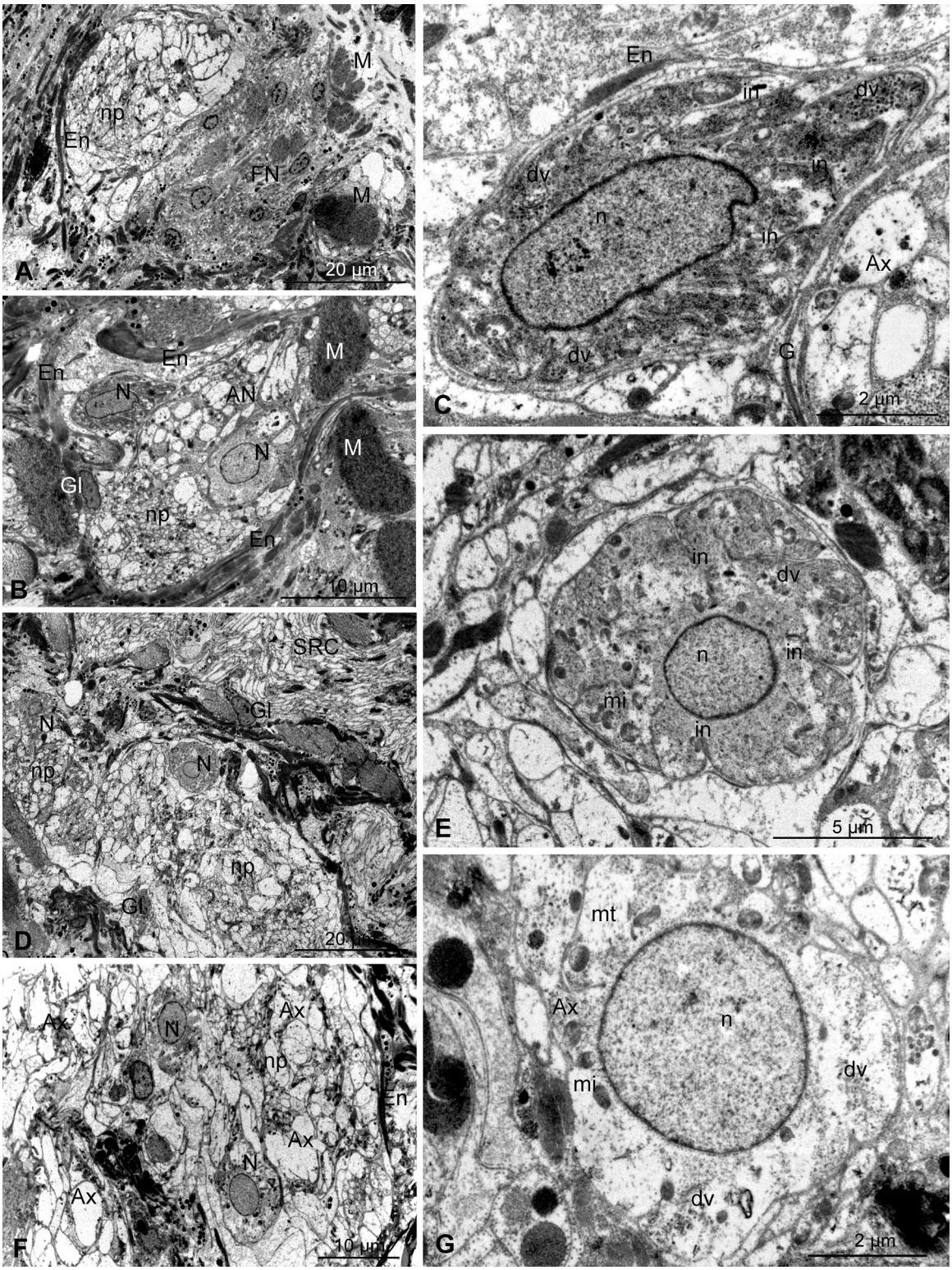

FIGURE 5 | Ultrastructural organization of the anterior lobes; TEM. (A) The frontal neurons of the anterior lobe (in level of Figure 3B,C,E); neuropil and perikarya of the frontal horn; (B) an apical part of the anterior lobe with two neurons, a glia-like cell, an extracellular envelope and the compartment of neurites giving rise to the anterior nerve; (C) the dark neuron (second type) with dense vesicles and deep invaginations of the neurilemma; (D) part of the semicircular commissure (SRC) and neuropil compartments in the anterior lobe; (E) a large neuron, ultrastructure from Figure 5D; (F) pyriform neurons (type 1) in the anterior lobes; (G) the ultrastructure of the light unipolar neuron (type1) with the main axon. AN, anterior neurites; Ax, axon; dv, dense vesicles; En, extracellular envelope; FN, frontal neurons perikarya; G, glial processes; Gl, glia-like cell; in, neurilemma invagination; M, muscle; mi, mitochondria; mt, microtubule; N, neuron; n, nucleus; np, neuropil.

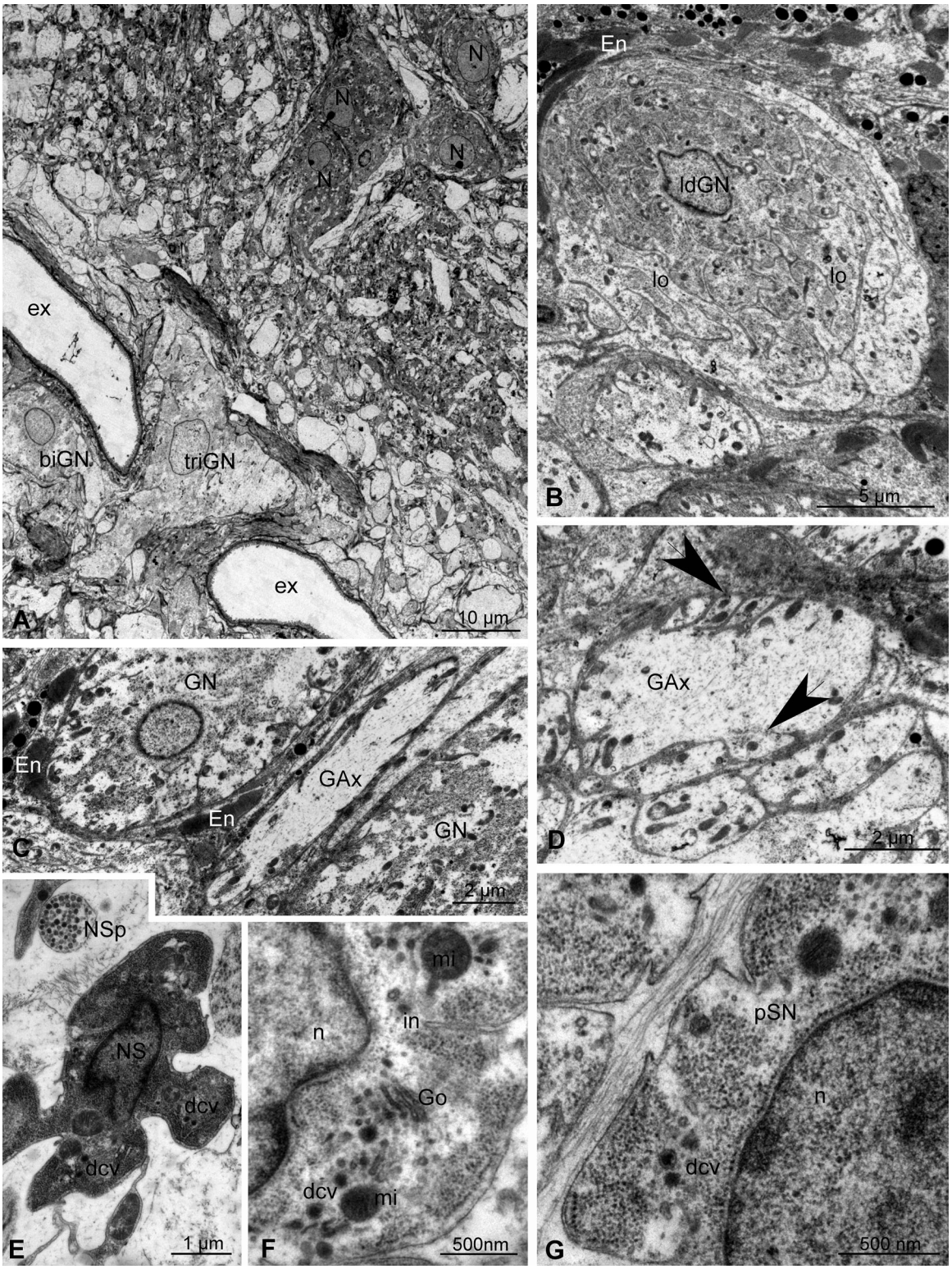

FIGURE 6 | Ultrastructural organization of the neurons; TEM. (A) The central lobe neurons with the tripolar giant neuron belonging to the X-shaped commissure; (B) a giant lobate dark neuron; (C) a giant light neuron and giant axons; (D) the median commissure axons with several lateral outgrowths (arrowheads); (E, F) peripheral neurosecretory neurons with dense-cored vesicles; (G) a peripheral sensory neuron. dcv, dense-cored vesicle; ex, excretory vessel; En, extracellular envelope; GAx, giant axon; GN, giant neuron; ldGN, lobate dark giant neuron; triGN, tripolar GN; biGN, bipolar GN; Go, Golgi complex; in, invagination of neurilemma; mi, mitochondria; lo, neuron lobes; N, neuron; n, nucleus; NS, neurosecretory neuron; NSp, neurosecretory process with electrondense vesicles; pSN, peripheral sensory neuron.

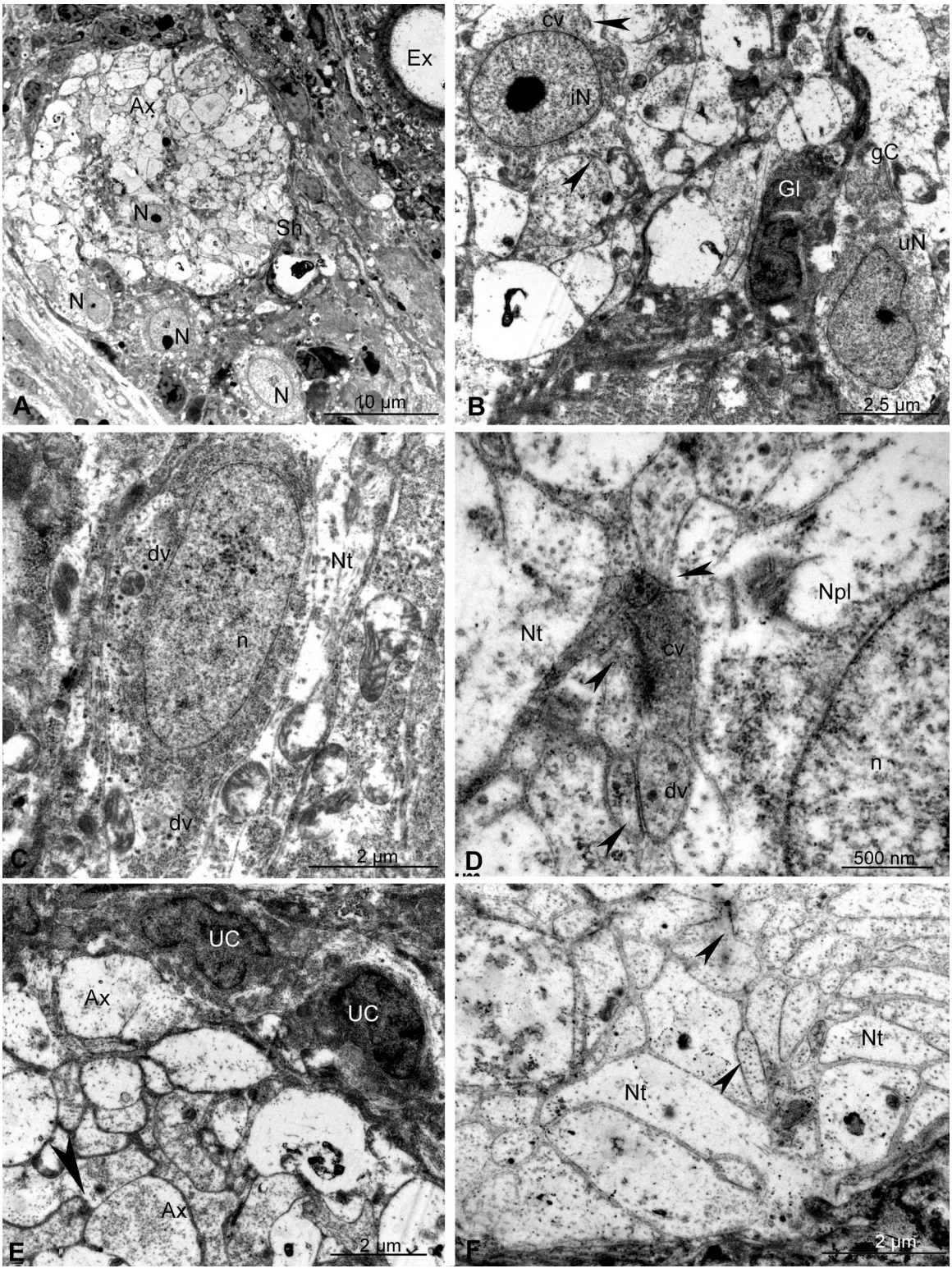

FIGURE 7 | Longitudinal nerve cords in the scolex posterior part; TEM. (A) Cross section of the main lateral nerve cord; neuron somas, compact neuropil, outer sheath, and excretory vessels are shown; (B) a light interneuron with synaptic contacts on the perikaryon, and a poorly differentiated neuron with a growth axon cone; (C) a differentiated bipolar neuron with dense vesicles located on the periphery of the conducting part of the main cord; (D) the ultrastructure of synaptic contacts located in the interneuron zone in the main cord; (E) a marginal area of the neuropil of the main cord with electron-dense poorly differentiated cells; (F) a bundle of neurites in the zone of the velum fold. Ax, axon; cv, clear vesicle; dv, dense vesicle; gC, growth cone; Gl, glia-like cell; iN, interneuron; N, neuron; n, nucleus; Npl, neuroplasm; Nt, neurite; Ex, main lateral excretory vessel; uN, undifferentiated neuron; UC, undifferentiated cell; arrowheads note synaptic junctions.

Type 1. Light unipolar neurons of the anterior lobes. Pear-shaped neurons form large clusters in the anterior lobes (Figure 5A,B). The perikaryon diameter is 5–9 μm, with large round nuclei filled with euchromatin and heterochromatin at the nuclear membrane and 2–3 nucleoli. The outer plasma membrane invaginates deeply to the cytoplasm, almost reaching the nucleus and dividing the perikaryon into lobes. The lobes are pressed tightly against each other and diverge only at the elongated end of the neuron (Figure 5F,G). The light cytoplasm contains free ribosomes and β-glycogen granules, microtubules, small oval mitochondria, and small electron-dense vesicles (dv) with a diameter of 70–80 nm. Perikarya are located on the periphery of the conducting part of the anterior lobes, forming cluster of *frontal* "horns" (Figure 3). On the outside, neurons are surrounded by a fibrillar matrix; inside, they are adjacent to a cluster of neurites with electron-dense granules of irregular shape and different sizes from 0.15 to 0.3 μm.

Type 2. Dark lobed neurons. Dark neurons have electron-dense cytoplasm; they are about 6–10 μm in size. The outer plasma membrane forms deep invaginations, almost reaching the nucleus, and forms wide lobes (Figure 5B–E). The nuclei are oval, filled with homogeneous euchromatin. The dark cytoplasm is filled with free ribosomes and β-glycogen granules and numerous mitochondria of different shapes and sizes. Electron-dense round or oval vesicles with a diameter of 70 nm (dv) are confined to the periphery of the lobes. Neurons occupy a predominantly cortical position and are surrounded externally by a densely packed fibrillar matrix.

Type 3. Giant neurons. They are found in the X-shaped cruciate commissure and bulbar ganglia. Sizes of perikarya vary from 20 to 30 μm (Figure 6A–C). Bi- and tripolar giant neurons were found in the cruciate commissure. Giant neurons are distinguished by the light cytoplasm of the perikaryon, round nuclei, and regular, short, radial invaginations of the outer membrane. Membrane invaginations do not reach the nucleus and do not form regular lobes. Oval mitochondria (0.3 μm) are numerous in the perikaryon zone; in neurites, mitochondria are confined to the axolemma. The neurites of giant neurons contain numerous microtubules oriented longitudinally to axon axis; in cross section, they have regular shallow invaginations of the membrane, to which mitochondria and vesicles are confined. Giant neurons produce round light vesicles with a diameter of 60–70 nm and more numerous electron-dense round vesicles with a diameter of 30–40 nm.

Type 4. Neurosecretory neurons. Neurosecretory neurons up to 4–5 μm in size were found at the level of the anterior lobes of the brain close to the tentacle sheaths. They have an active nucleus with a prominent nucleolus (Figure 6E,F). The perikaryon is irregular in shape and forms wide lobes. The cytoplasm is heterogeneous, containing ribosomes, round mitochondria, and Golgi complex producing dense-core vesicles (dcv) with a diameter of 100 nm.

Type 5. Peripheral sensory neurons. In the apical part of the scolex, among subtegumental cells, sensory neurons have been found that send neurites to the tegument. These are small cells with a diameter of about 3–5 μm with large round nucleus occupying more than 2/3 of the perikaryon volume (Figure 6G). The outer membrane forms shallow invaginations. The cytoplasm contains many ribosomes and rough ER. Neurons produce round dense-cored vesicles with a diameter of 80–100 nm and small clear vesicles (cv) 50–70 nm in diameter.

Ultrastructure of longitudinal nerve cords. In the scolex, in the *pars vaginalis* and *pars bulbosa* zones, the longitudinal nerve cords are represented by a pair of main lateral cords running along the main lateral excretory vessels and minor longitudinal nerves in the subtegumental area of the velum fold (Figure 7). The main cords include a compact bundle of neurites with bipolar neurons located on the surface, and individual interneurons within the neuropil. The cord is surrounded by an electron-dense sheath of small electron-dense cortical cells. Dark and light bipolars have been found, with a soma diameter of about 5 μm. Dense vesicles (dv) with diameter of 50 nm have been found in the perikaryon. In addition to mature neurons, poorly differentiated neurons with granular cytoplasm and undeveloped neurite have been found on the surface of the cord. Figure 7B shows the perikaryon of a poorly differentiated neuron with a growing axon cone; synaptic vesicles are absent. There are numerous synapses in the central part of the main cord, including those at the surface of the interneuronal soma (Figure 7B,D,E). Synapses contain single electron-dense vesicles and microtubules. The postsynaptic membrane carries an accumulation of dense fibrillar material. In addition to the main longitudinal cords, there are compact clusters of neurites and thin longitudinal nerves (Figure 7F). Thin nerves lack neurons; the neurites contain numerous microtubules and form numerous chemical and electrical synapses within the bundle.

3.3 | Glial Cells and an Extracellular Envelope of the Brain

In light microscopic semithin sections, the brain, central nerve, and main nerve cords are clearly delimited from the surrounding tissues by a dark, dense thin membrane (Figures 4 and 8). At the ultrastructural level, many dark cortical cells were found in the brain and main cords, that form a thin sheath around neurons, neuropils, and giant axons. In different parts of the nervous system, the envelope may have a different structure. Around the brain lobes and commissures, the envelope is represented by a thick layer of regular extracellular fibrils, forming the outer border of the brain. Within the brain lobes and neuropils, compartmentalization is carried out by thin processes that fit tightly with each other and surround the neuropil compartment or group of neurons. These envelop structures are formed by different types of glia-like cells.

Small dark cells up to 1 μm in diameter were found at the periphery of the brain lobes and in the lateral cords; they have small, elongated nuclei and dense cytoplasm (Figure 8). In the brain lobes, we found small electron-dense cells with strongly ramified cytoplasm. They differ from neurons by having small nuclei and do not have axons. They do not produce synaptic vesicles and do not have synapses. The cytoplasm of these cells contains numerous mitochondria around the nucleus. These cells develop long, thin

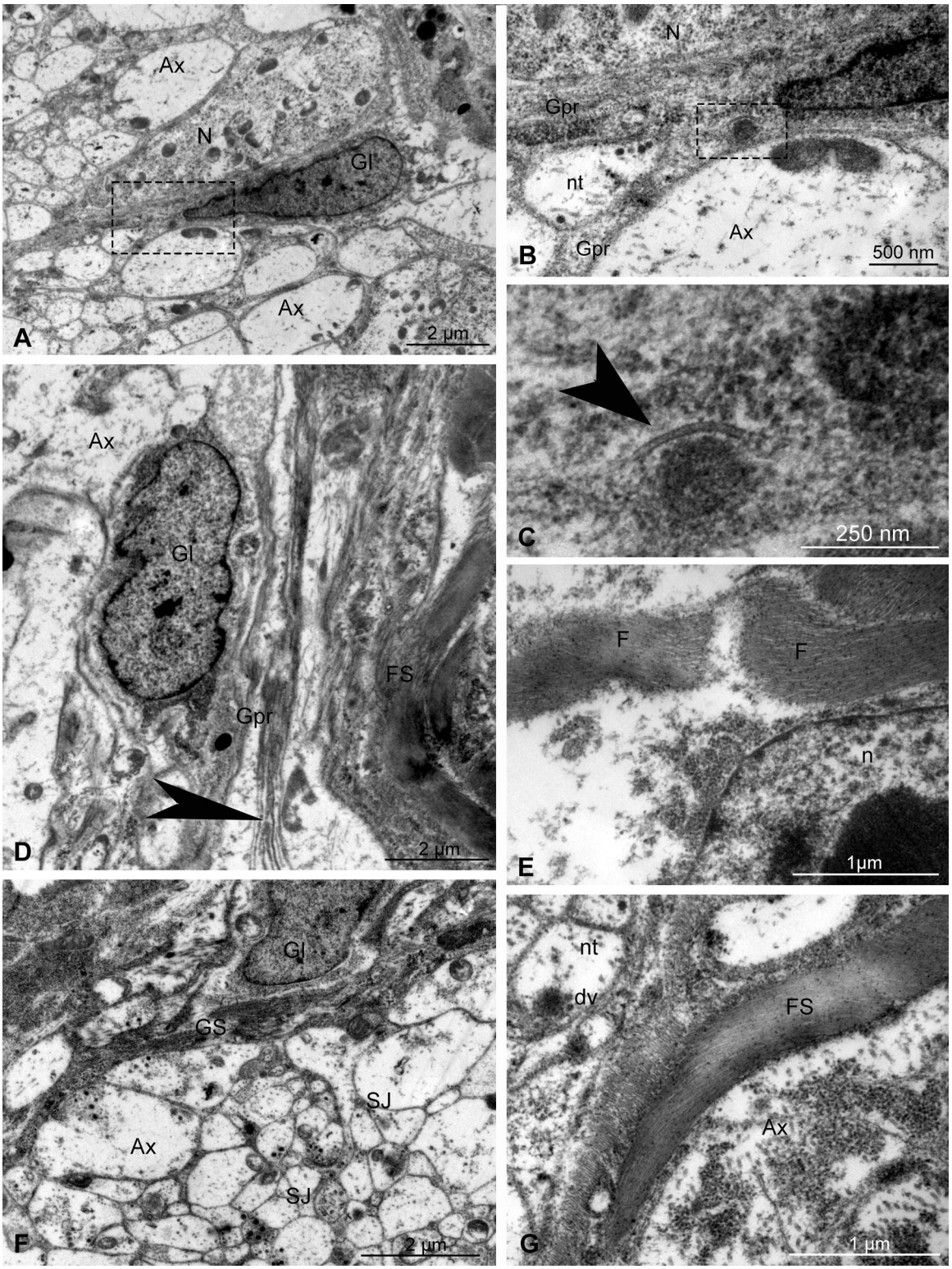

FIGURE 8 | Glial cells and an extracellular envelope of the brain. (A–C) Glial cell (type 1) as element of the neuropil of the anterior lobe; (B) at higher magnification, processes with dense cytoplasm and (C) the intercellular junction are shown by arrowhead; (D) the glial cell forms thin projections and lamellae (arrowhead) extending into a neuropil; a strong fibrillar sheath is shown outside the lobe; (E) the cell with fibrillar material in the cytoplasm; (F) an outer fibrillar sheath of the neuropil; (G) an extracellular layer of fibrils separates two zones of the neuropil. Ax, axon; dv, dense vesicles; F, fibrils; FS, fibrillar sheath; Gl, glia-like cell; Gpr, processes of the glial cell; GS, glial sheath; N, neuron; n, nucleus; nt, neurit; SJ, synaptic junction.

projections and lamellae around individual neurons and clusters of neurites, isolating individual compartments of nervous tissue from each other. We designate them as glial cells type 1—multilamellar cells.

The outer fibrillar layer of the brain has the form of a thick wall that borders the anterior parts of the brain or neuropil. The envelope around the anterior lobes can consist of several layers of strong oriented fibrils and reach 1 µm in thickness.

Extracellular fibrils of the brain envelope are synthesized by specialized cells located on the periphery of the brain and nerves.

The cells that form the fibrillar wall of the brain probably come from different sources. First of all, there are myocytes that secrete the fibrils of the extracellular matrix onto the membrane surface (Figure 5B). According to our data, the outermost layer of the fibrillar membrane of the brain is formed from these cells. However, directly adjacent to the neurons are the cells that do not have myofibrils. The cells' cytoplasm is filled with thin fibers, some of the fibers are located extracellularly (Figure 8E). These cells are more like fibroblasts. We designate them as glial cells type 2—fibroblast-like cells.

4 | Discussion

The anatomy of the trypanrhynch nervous system has been studied since the mid-20th century at the histological (Rees 1941a, 1941b, 1950, 1988) and ultrastructural level (Biserova 1991, 2008a, 2008b, 2016; Biserova et al. 2010, 2016; Biserova and Korneva 2012). A comparative analysis showed that the architecture of the trypanorhynch brain is quite uniform: the species studied had paired anterior and paired lateral (or posterior) lobes connected by the anterior semicircular and median commissure. The anterior nerves, lateral, dorsal, and ventral paired brain roots emerge from the brain, innervating the scolex muscles; bulbar nerves with giant axons connected with the ganglia of the muscular bulbs and the main nerve cords exit posterior from the brain (Biserova, Korneva, and Polyakova 2020). Approximately 200 neurons have been found in the brain of *Parachristianella* sp. (Biserova and Korneva 2012).

Some information about microscopic anatomy and tegument ultrastructure is known for 4 species of the family Tentaculariidae: *N. surmenicola* (Palm 2004; Hyeon-Cheol Kim et al. 2015; Gordeev 2016; Biserova, Gordeev, and Korneva 2016), *N. queenslandensis* (Jones and Beveridge 1998), *Heteronybelinia alloiotica* (Palm, Mundt, and Overstreet 2000), and *Tentacularia coryphaenae* (*bicolor*) (Al-Bassel 2004). Microscopic anatomy of the nervous system has been partly studied in the plerocercoid *N. surmenicola* from the stomach of a greenling *Pleurogrammus azonus* (Gordeev 2016) The plerocercoid brain has been shown to have well-developed anterior lobes and a central azygos lobe. The main nerve cords develop from the lateral lobes, the central nerve—from the central lobe (Figure 9). The central nerve ultrastructure in the plerocercoid closely corresponds to the structure of the central nerve in the adult cestode. The plerocercoid, like the adult, has semicircular dorsal and ventral commissures of the anterior lobes of the brain. The X-shaped cruciate and median commissures pass into the central lobe of the plerocercoid brain (Figure 9B). In the X-shaped cruciate commissure, it has a giant four-polar neuron (soma size 21 μm, nucleus—6 μm).

Also, it has been noted at anatomical level, that the brain of *N. surmenicola* plerocercoids is surrounded by a muscular corset and a liquid intercellular medium containing elements of a

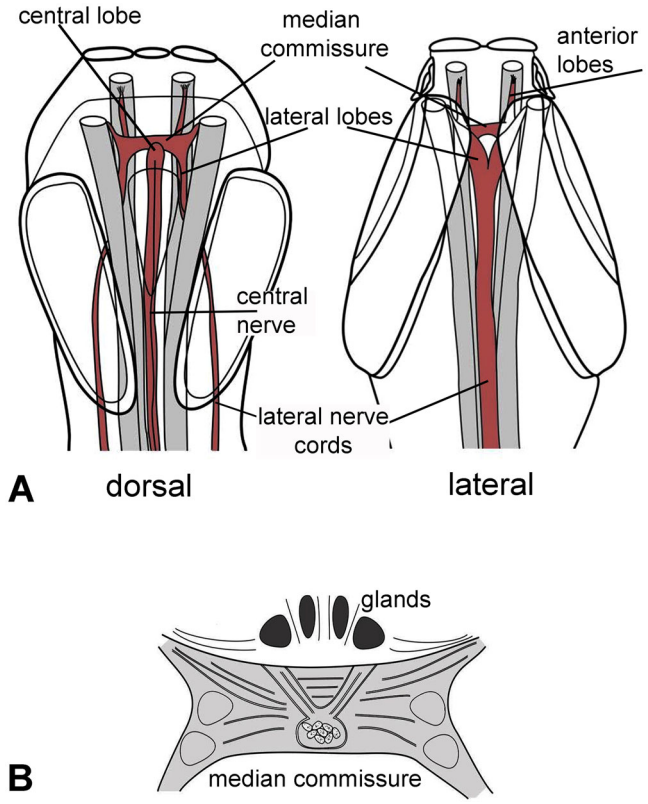

FIGURE 9 | Brain anatomy in *Nybelinia surmenicola* plerocercoid (modified after Gordeev 2016; with permission). (A) Schematic drawing of scolex nervous system (red color); dorsal and lateral view; (B) schematic drawing of the median commissure cross section.

fibrillar matrix that protect the brain during sudden muscle contractions and movements of the scolex (Gordeev 2016). Such structures were not found in species of Lacistorhynchoidea and Eutetrarhynchoidea (Beveridge and Smith 1988; Mutti and Ivanov 2020).

The architecture of the plerocercoid brain (Figure 9) in terms of a number of lobes and commissures corresponds to the adult stage, but the lobes of the brain are poorly differentiated. The adult brain (Figures 2 and 10) has complexly differentiated lobes with distinct neuropile zones and neuron perikarya zones. Number, volume, and size of neurons and glial cells in each of the nine lobes is significantly greater in adult worms. The adult brain has a developed extracellular capsule surrounding the lobes and individual neuropile zones in the form of a *neural lamella*.

The Anterior/frontal Lobes. The peculiar architecture of the anterior lobes in species studied discussed in more detail. The anterior/frontal lobes of *Grillotia erinaceus* and *Parachristianella* sp. have a relatively loose structure, the neurons locate in small groups of 3–5 perikarya, which surround the centrally located neuropil. The neurites form the anterior nerves, which in *G. erinaceus* also include the neurons perikarya extending along the nerves and directed toward the apical zone of the scolex. The anterior lobes of the brain of *N. surmenicola* (Figure 10) are organized differently from *G. erinaceus*. The neurons are located in compact groups and include up to 30 perikarya each. They are situated above the semicircular

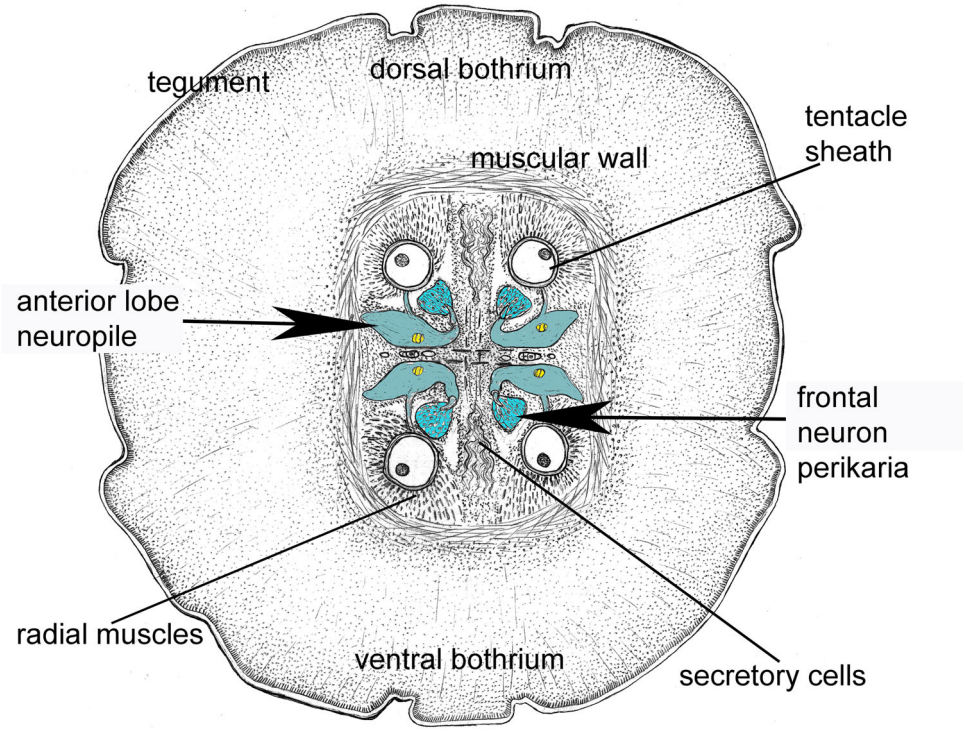

FIGURE 10 | A schematic drawing of the anterior brain lobe (blue color) in the scolex; cross-section view; the brain lobes lie between the four tentacle sheaths, that are surrounded by the radial and semiring musculature stabilizing the proboscis apparatus.

commissures like frontal horns. As in the plerocercoid, the anterior nerves arising from the anterior lobes innervate the radial muscles stabilizing the position of the tentacle sheaths (Figure 10). A similar configuration of anterior lobes has not been noted in Lacistirhynchoidea or Eutetrarhynchoidea and, perhaps, characterizes representatives of the Tentacleriidae only.

The X-shaped cruciate commissure within the trypanorhynch was discovered after ultrastructural reconstruction in *Parachristianella* sp. (Biserova and Korneva 2012), and then was identified in *Dollfusiella aculeata* with ultrastructural and immunocytochemical methods (Biserova, Korneva, and Polyakova 2020). Our recent study has shown that in *N. surmenicola*, the X-shaped cruciate commissure has a complex organization. The X-shaped commissure includes axons of neurons localized in different parts of the brain in the adult cestode. These are mainly neurons of the central unpaired lobe, which give rise to neurites in the central nerve and bulbar ganglia, and also to neurites running to the lateral lobes. Functionally, this means coordination of the movements of bothria and proboscis, which are involved in the forward movement of plerocercoids (Gordeev and Biserova 2016), as well as in the process of attachment of a mature cestode in the shark intestine.

The central lobe of the trypanorhynch brain acts as the integrative functioning center of the proboscis apparatus. This shows the important role of the attachment apparatus in the evolution of the brain and nervous system of cestodes. The presence of the X-shaped commissure suggests that neurons in the cruciate commissure take part in the coordinated control of the scolex muscles. In the central lobe of *N. surmenicola*, there is no distinct single neuropil as is characteristic in *Parachristianella* sp. (Biserova and Korneva 2012) and *D. aculeata* (Biserova, Korneva, and Polyakova 2020). Instead, in different parts of the central lobe there are compact clusters of thin neurites and large axons that form synaptic glomeruli with different types of synapses. In addition, there are dedicated motor neuropils on the surface of the scolex cruciate muscles and on the radial tentacle sheath muscles. These features are associated with the scolex musculature and proboscis apparatus of tentacleriids, in contrast to the previously studied lacistirhynchoidean or eutetrarhynchoidea species. The muscular corset and a liquid intercellular medium surrounding the brain in the plerocercoid (Gordeev 2016) can also support the complex brain of an adult cestode.

The median commissure is characteristic of the brain of all cestodes and it has been noted in all trypanorhynch species studied. It communicates between the right and left sides of the body and is the earliest evolutionary acquisition. Thus, in the ontogenesis of *Triaenophorus nodulosus* (Bothriocephallidea) at the early procercoid stage, the formation of a median commissure from the contralateral neurites of two pioneer neurons has been shown (Biserova and Korneva 2006).

Like in all cestodes, the lateral lobes of *N. surmenicola*, merging into the dorsal and the ventral neuropils, form two powerful main lateral cords, which continue from the scolex to the strobila. The main lateral cords in flatworms belong to the central nervous system and include numerous neuronal perikarya. The population of neurons in the main cords of *N. surmenicola* is replenished from undifferentiated cells in the proliferation zone in the posterior part of the scolex and neck. Poorly differentiated neurons occupy a marginal position in the lateral cords. Mature neurons are represented mainly by bipolar forms in the main cords. Similar processes of the main nerve

cords growth have been described in *T. nodulosus*: poorly differentiated neurons without axons are located on the surface of the neurite bundle and with growth of the strobila are gradually integrated, turning into bipolar neurons (Biserova and Salnikova 2002). In the zone of the cortical parenchyma of the *pars postbulbosa*, there are peripheral longitudinal nerves, including numerous neurites and axons. There are no neuronal perikarya in the small longitudinal nerves. It should be noted that the neurites have a compact arrangement, in cross section, in the form of a regular circle, bounded by an electron-dense envelope of unknown cell processes.

The central nerve of *N. surmenicola* is a homolog of the bulbar nerves of other Trypanorhyncha studied, emerging from the brain towards the posterior end of the scolex and innervating the muscular bulbs. The central nerve includes four groups of wrapped giant axons, the same as four bulbar nerves in *Grillotia erinaceus* (Biserova 2008b; Biserova, Korneva, and Polyakova 2020) and *Parachristianella* sp. (Biserova and Korneva 2012). The central nerve does not contain neuron perikarya in all species studied. It is important to note that the brain of *Aporhynchus norvegicus* (Trypanorhyncha) is devoid of the cruciate commissure, central lobe, bulbar, and proboscis nerves because the scolex lacks the proboscis apparatus (Rees 1941a).

Glia. In the brain of *N. surmenicola*, we found two types of cells involved in the formation of the protective sheath and isolating neuropil compartments, groups of neurons, the central nerve, as well as the main nerve cords. The first type is multilamellar glial cells; they give off numerous processes and form layered structures surrounding neuropils and neurons. Such cells have been previously noted in the brains of free-living flatworms, trematodes, and cestodes (Sukhdeo and Sukhdeo 1990; Biserova 2000, 2008a, 2008b; Wang et al. 2016). The second type is fibroblast-like glial cells, secreting extracellular fibrils and forming an outer fibrillar layer as a protective sheath. This type of glia has been described in many invertebrates (Quiroga et al. 2015; Verkhratsky et al. 2022).

In cestodes, multilamellar glial cells have been described in the main nerve cords of *Amphilina foliacea* (Amphilinida; Biserova 2000) and *G. erinaceus* (Biserova 2008b; Biserova et al. 2010). In the nervous system of *G. erinaceus*, glial cells have different structures, confined to different parts of the nervous system: the brain, nerves and main cords. Thus, fibroblast-like cells are associated with the brain of *G. erinaceus*. In *N. surmenicola*, fibroblast-like cells are also associated with the brain, but unlike *G. erinaceus*, they form a thick wall of ordered fibrils more than 1 μm thick. Such a membrane can be attributed to the brain capsule, characteristical for the brain of annelids and nemerteans (Baskin 1971a, 1971b; Riehl and Schlue 1998).

It is known that the formation of a protective capsule is one of the stages in brain evolution. In annelids, higher invertebrates, and vertebrates, the brain is characterized by the presence of a connective tissue membrane; in the simplest case, it is the neural lamella, or fibrillar plate, separating the brain from the surrounding tissues. Thin fibrillar plate/neural lamella was revealed in the CNS of some cestode species (Biserova et al. 2010). Most often the basal matrix, which limits cortical surface of brain or main cords, is quite loose and is not expressed as an independent layer. A clearly expressed fibrillar layer, which limits both the brain and main nerve cords, is present in CNS of plerocercoid and adult *T. nodulosus* (Biserova and Salnikova 2002), the cortical envelopes of which are formed by outgrowths of epithelial cells of excretory vessels. Analysis of the ultrastructure of the nervous system of other parasitic and free-living flatworms indicates the presence of a well-developed connective tissue membrane, or capsule, which surrounds the brain, in representatives of acantacephallides and the nemerteans. In Nemertea, brain and medullary cords are ensheathed by a dense layer of extracellular matrix (*ecm*) traditionally termed outer neurilemma (brain and lateral nerve cord capsule). Neuronal somata and the neuropil may be separated from each other by a similar layer of *ecm*, which has been termed inner neurilemma (Beckers and Döhren 2016). The glial cells of the cortex are located under the fibrillar plate. In *N. surmenicola*, the brain extracellular capsule is many times thicker than the fibrillar matrix surrounding neurons in *G. erinaceus* and *Parachristianella* sp., as well as in all known descriptions of free-living and parasitic flatworms. Fibroblast-like cells can be classified as cortical glia, because they are always located on the outer border of the brain to protect and separate the lobes. An indirect argument in favor of the protective function of the powerful extracellular capsule around the brain of *N. surmenicola* can be the discovery of a similar structure around the brain of *Branchinotogluma japonica* (Polynoidae), a deep-sea polychaeta, endemic to hydrothermal vents (Shigeno et al. 2015).

Multilamellar glial cells of *N. surmenicola* are always located under the fibrillar sheath. They give off processes inside the lobes and neuropils and form multilayer wraps around giant axons. This type probably represents a stage of medullary glia differentiation, although we have not detected these cells in the deep regions of the brain. In the nervous system of *G. erinaceus*, they form myelin-like wraps around giant axons and are characterized by long membranous structures in the cytoplasm; in the bulbar nerves, the number of glial cells exceeds the number of neurons (Biserova 2008a, 2008b). The central nerve of *N. surmenicola* also includes giant axons with wraps formed by multilamellar glial cells.

Glial cells of a different structure have been found in the brain in the trypanorhynch cestodes *Dolfusiella aculeata* and *Parachristianella* sp. (Biserova, Korneva, and Polyakova 2020). These cells wrap the giant axons in the central lobe and in the X-shaped commissure. Glial processes contact the axons near the perikaryon or axon hill. The processes contain electron-dense agglomerates of irregular shape and mitochondria. Tiny glia-like cell processes with irregular electron-dense material possess intimate contacts with neurites of the X-shaped criss-cross commissure.

Multilamellar glial cells of *N. surmenicola* develop gap junctions between their processes, which apparently contributes to the creation of the internal environment of the brain. Gap junctions between processes are characteristic for glia in higher animals; their presence indicates participation in neuronal trophism.

Two basic mechanisms for increasing the conduction speed of the electrical impulse are known: (1) axon gigantism using axons

several times larger in diameter than the norm for other large axons and (2) encasing axons in helical or concentrically wrapped multilamellar sheets of insulating plasma membrane—the myelin sheath (Hartline and Colman 2007). Speed of nerve impulse conduction is greatly increased by myelin, a multi-layered membranous sheath surrounding axons. Myelinated axons are ubiquitous among the vertebrates, but relatively rare among the invertebrates. The highly organized myelin is characterized by the complete exclusion of cytoplasm from the intracellular spaces of the cell generating it. Myelin-like wrapping of nerve fibers and the presence of nodes have been described at the electron microscope level in annelids (Hartline and Colman 2007). Both mechanisms have been found in *N. surmenicola*. Thus, the brain envelopes and giant axons probably provide rapid signal transmission from the scolex apical zone to the muscular bulbs, which reflects in behavioral reactions; when the apical zone of the scolex contacts the surface, the tentacles are instantly ejected.

5 | Conclusion

The structure of the nervous system of *N. surmenicola* demonstrates a complex morphology within the class of cestodes and corresponds to the known trypanorhynch brain architecture. The brain of *N. surmenicola* is differentiated into nine clearly defined lobes and semicircular, median, and X-shaped cruciate commissures. There are approximately 120 neurons in the anterior lobes alone. The exact number of neurons in the brain has not yet been established, but according to our estimates there are more than 300 perikarya. Several ultrastructural types of neurons have been identified in the brain, differing in the size and shape of the soma, the density of the cytoplasm, and the ultrastructure of synaptic vesicles. Numerous synaptic contacts involving clear and electron-dense vesicles have been found in neuropils. An important feature of the ultrastructural organization of the adult *N. surmenicola* brain is the presence of an extracellular fibrillar capsule surrounding neurons, neuropils, and brain roots. Two types of glial cells have been found in the brain that participate in neuronal metabolism and developing of multilayered sheaths of the giant axons, brain lobes, neuropil compartments, and the main cords.

The complex brain reflects the important role of the rhyncheal system in the evolution of Trypanorhyncha and correlates with cestode behavior. Movements of the attachment organs are coordinated by the nervous system and a complex repertoire of motor activity must correlate with the brain architecture. Correlation with brain lobes and other aspects have been reviewed in greater detail in diphyllobothriidean and trypanorhynchs tapeworms (Biserova, Korneva, and Polyakova 2020). We believe that our new data about size and architecture of the cestodes brain as well as the ultrastructural organization of giant axons and neurons represents an advance tapeworm neuromorphology. Also, the phylogeny of the Trypanorhyncha is still under discussion. The main challenge that remains is the resolution of the polytomies amongst and within the major lineages of the Eutetrarhynchoidea, Tentacularioidea, Gymnorhynchoidea, and Lacistorhynchoidea (Beveridge et al. 2017; Waeschenbach and Littlewood 2017). Comparing the brain of *G. erinaceus* (Lacistorhynchoidea) and *N. surmenicola* (Tentacularioidea), one notices the powerful development of the anterior lobes in *N. surmenicola* and the participation of the anterior nerves in the innervation of the radial muscles stabilizing the position of the tentacle sheaths. The anterior lobes of *G. erinaceus* are poorly developed compared to *N. surmenicola*. Moreover, a specific feature of *N. surmenicola* brain is the presence of a powerful extracellular capsule that ties the brain lobes together with the cortical glial cells.

The complex morphology of the trypanorhynch brain has some similarities with representatives of free-living worms, such as nemerteans and annelids, both in the presence of an extracellular capsule and in the distinct differentiation of lobes. The Trypanorhyncha brain is specialized on the innervation of the muscular apparatus of the mobile tentacles. At the same time, the Trypanorhyncha brain differs significantly from the brain of Diphyllobothriidea, which has weakly differentiated two or three lobes and a median commissure (Gustafsson 1990; Barčák et al. 2023; Biserova, Korneva, and Polyakova 2020). The brain of diphyllobothriids, specialized on the innervation of the glandular apparatus, the neuro-glandular morpho-functional brain complex, has been suggested to be a model for Diphyllobothriidae family (Biserova, Mustafina, and Raikova 2022). Nervous system anatomy and brain architecture may reflect the morphofunctional aspects of the tapeworm evolution.

Author Contributions

Natalia M. Biserova: conceptualization; investigation; funding acquisition; writing–original draft; supervision; resources; methodology; validation; writing–review and editing. **Anna A. Margarit:** visualization; investigation; writing–original draft; methodology.

Acknowledgments

The authors are grateful to our colleague Ilia I. Gordeev for collecting material. The authors cordially thank the staff of the Laboratory of Electron Microscopy of the Faculty of Biology, Moscow State University, and the staff of the Electron Microscopy Center for Collective Use, IBIW RAS (Borok).

This study was supported by the RSF grant no. 23-24-00118 and project no. 121032300121-0.

Ethics Statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. In accordance with Directive 2010/63/EU of September 22, 2010 on the protection of animals used for scientific purposes, Chapter 1, Paragraph 3. The requirements of bioethics do not apply to cestodes, the object of this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/jmor.70002.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. All photographs obtained by the authors as a result of the study (LM, TEM, SEM,) are stored in the personal database of Natalia M. Biserova and are available upon personal request.

References

Al-Bassel, D. A. 2004. "Surface Ultrastructure of the Scolex of the Postlarva of *Tentacularia bicolor* (Cestoda: Trypanorhyncha)." *Journal of the Egyptian-German Society of Zoology* 44D: 53–67.

Barčák, D., A. Alexovič Matiašová, E. Čisovská Bazsalovicsová, M. Soldánová, M. Oros, and I. Králová-Hromadová. 2023. "An Examination of Nervous System Revealed Unexpected Immunoreactivity of Both Secretory Apparatus and Excretory Canals in Plerocercoids of Two Broad Tapeworms (Cestoda: Diphyllobothriidea)." *Parasitology* 150, no. 7: 612–622. https://doi.org/10.1017/S0031182023000306.

Baskin, D. G. 1971a. "Fine Structure, Functional Organization and Supportive Role of Neuroglia in *Nereis*." *Tissue and Cell* 3, no. 4: 579–587.

Baskin, D. G. 1971b. "The Fine Structure of Neuroglia in the Central Nervous System of Nereid Polychaetes." *Cell Tissue Research* 119, no. 3: 295–308.

Beckers, P., and J. Döhren. 2016. "Nemertea (Nemertini)." In *Structure and Evolution of Invertebrate Nervous Systems*, edited by A. Schmidt-Rhaesa, S. Harzsch, and G. Purschke, 148–165. Oxford, UK: Oxford University Press.

Beklemishev, V. N. 1964. *Bases of Comparative Anatomy of Invertebrates. V. 2. Organology*, 3rd ed., 1–448. Moscow: Nauka. http://booksshare.net/index.php?id1=4&category=biol&author=beklemishev-vn&book=19642.

Beveridge, I., M. Haseli, V. A. Ivanov, A. Menoret, and B. C. Schaeffner. 2017. "Trypanorhyncha Diesing, 1863." In *Planetary Biodiversity Inventory (2008–2017): Tapeworms From Vertebrate Bowels of the Earth*, edited by J. N. Caira and K. Jensen, 401–429. Lawrence: The University of Kansas, Natural History Museum.

Beveridge, I., and K. Smith. 1988. "Ultrastructure of the Rhyncheal System of *Trimacracanthus aetobatidis* (Cestoda: Trypanorhyncha)." *International Journal for Parasitology* 18, no. 5: 623–632.

Biserova, N. M. 1991. "Distribution of Receptors and Peculiarities of Ultrathin Structure of Nervous System in Representatives of Three Orders of Lower Cestoda." *Journal of General Biology* 52: 551–563.

Biserova, N. M. 2000. "The Ultrastructure of Glia-Like Cells in Lateral Nerve Cords of Adult *Amphilina foliacea* (Amphilinida)." *Acta Biologica Hungarica* 51: 439–442.

Biserova, N. M. 2008a. "Do Glial Cells Exist in the Nervous System of Parasitic and Free-Living Flatworms? An Ultrastructural and Immunocytochemical Investigation." Supplement, *Acta Biologica Hungarica* 60, no. S30: 208–219.

Biserova, N. M. 2008b. "Ultrastructure of Glial Cells in the Nervous System of *Grillotia erinaceus*." *Cell & Tissue Biology* 2, no. 3: 253–264.

Biserova, N. M. 2016. "Platyhelminthes: Neodermata." In *Structure and Evolution of Invertebrate Nervous Systems*, edited by A. Schmidt-Rhaesa, S. Harzsch, and G. Purschke, 93–117. Oxford, UK: Oxford University Press.

Biserova, N. M., I. I. Gordeev, and J. V. Korneva. 2016. "Where Are the Sensory Organs of *Nybelinia surmenicola* (Trypanorhyncha)? A Comparative Analysis With *Parachristianella* sp. and Other Trypanorhynchean Cestodes." *Parasitology Research* 115, no. 1: 131–141.

Biserova, N. M., I. I. Gordeev, J. V. Korneva, and M. M. Salnikova. 2010. "Structure of the Glial Cells in the Nervous System of Parasitic and Free-Living Flatworms." *Biology Bulletin* 37, no. 3: 277–287.

Biserova, N. M., and J. B. Korneva. 2006. "The Nervous System Ontogeny in Cestodes and Amphilinids." *Invertebrate. Zoology* 3: 157–184. [In Russian].

Biserova, N. M., and J. V. Korneva. 2012. "Reconstruction of the Cerebral Ganglion Fine Structure in *Parachristianella* sp. (Cestoda, Trypanorhyncha)." *Zoologicheskii Zhurnal* 91, no. 3: 259–272. [In Russian].

Biserova, N. M., J. V. Korneva, and T. A. Polyakova. 2020. "The Brain Structure of Selected Trypanorhynch Tapeworms." *Journal of Morphology* 281: 893–913. https://doi.org/10.1002/jmor.21145.

Biserova, N. M., I. A. Kutyrev, V. R. Saitov, and I. A. Kolesnikov. 2023. "The Neuro-Exocrine Secretion: A New Type of Gland in Tapeworms?" *Zoology* 160: 126119. https://doi.org/10.1016/j.zool.2023.126119.

Biserova, N. M., A. R. Mustafina, and O. I. Raikova. 2022. "The Neuro-Glandular Brain of the *Pyramicocephalus phocarum* Plerocercoid (Cestoda, Diphyllobothriidea): Immunocytochemical and Ultrastructural Study." *Zoology* 152: 126012. https://doi.org/10.1016/j.zool.2022.126012.

Biserova, N. M., and M. M. Salnikova. 2002. "The Ultrastructure of Main Lateral Nerves Cords and Associated Cell Elements in *Triaenophorus Nodulosus* (Cestoda: Pseudophyllidea)." *Tsitologiya* 44: 611–622. [In Russian].

Caira, J. N., and K. Jensen. 2014. "A Digest of Elasmobranch Tapeworms." *Journal of Parasitology* 100, no. 4: 373–391.

Campbell, R. A., and I. Beveridge (1994). "Order Trypanorhyncha Diesing, 1863." In *Keys to the Cestode Parasites of Vertebrates*, edited by L. F. Khalil, L. A. Joes, and R. A. Bray, 51–148. Wallingford: CAB International.

Crangle, K. D., G. McKerr, J. M. Allen, C. V. Howard, and O. Johansson. 1995. "The Central Nervous System of *Grillotia erinaceus* (Cestoda: Trypanorhyncha) as Revealed by Immunocytochemistry and Neural Tracing." *Parasitology Research* 81, no. 2: 152–157.

Gordeev, I. I. 2016. "Microscopic Anatomy of the Plerocercoids of the Cestodes in the Orders Trypanorhyncha and Diphyllobothriidea." PhD diss., Moscow State University. [In Russian].

Gordeev, I. I., and N. M. Biserova. 2016. "Morpho-Functional Analysis of *Nybelinia surmenicola* (Trypanorhyncha: Tentacularioidea) Forward Movement." In *Modern Problems of Theoretical and Marine Parasitology*, edited by K. V. Galaktionov and A. V. Gaevskaya, 168–169. Sevastopol: Publishing House Bondarenko N. Yu. [In Russian].

Gustafsson, M. K. S. 1990. "The Cells of a Cestode. *Diphyllobothrium dendriticum* as a Model in Cell Biology." In *The Early Brain*, vol. 50, no. 4, Acta Academiae Aboensis, Åbo, edited by M. K. S. Gustafsson and M. Reuter, 13–44. Academy Press.

Halton, D. W., A. G. Maule, G. P. Brennan, C. Shaw, S. R. Stoitsova, and C. F. Johnston. 1994. "*Grillotia erinaceus* (Cestoda, Trypanorhyncha): Localization of Neuroactive Substances in the Plerocercoid, Using Confocal and Electron-Microscopic Immunocytochemistry." *Experimental Parasitology* 79: 410–423.

Hartline, D. K., and D. R. Colman. 2007. "Rapid Conduction and the Evolution of Giant Axons and Myelinated Fibers." *Current Biology* 17: R29–R35.

Johnstone, J. 1911. "Tetrarhynchus Erinaceusvan Beneden. I. Structure of Larva and Adult Worm." *Parasitology* 4: 364–415.

Jones, M. K., and I. Beveridge. 1998. "*Nybelinia queenslandensis* sp. n. (Cestoda: Trypanorhyncha) Parasitic in *Carcharhinus melanopterus*, From Australia, With Observations on the Fine Structure of the Scolex Including the Rhyncheal System." *Folia Parasitologica* 45: 295–311.

Kim, H. C., J. Park, D. H. Yu, et al. 2015. "A Survey and Morphological Study of Plerocercoidal *Nybelinia surmenicola* (Cestoda: Tentacularriidae) in the Common Squid From the Korean Coastal Waters." *Journal of Veterinary Clinics* 32, no. 3: 239–242.

Kotikova, E. A. 1976. "Patterns of evolution of the nervous system in cestodes." In *Evolutionary Morphology of Invertebrate Animals*, edited by Y. V. Mamkaev, 33–34.Leningrad: Nauka. [In Russian].

Koziol, U., G. Krohne, and K. Brehm. 2013. "Anatomy and Development of the Larval Nervous System in *Echinococcus multilocularis*." *Frontiers in zoology* 10, no. 24: 24. https://doi.org/10.1186/1742-9994-10-24.

Kuperman, B. I. 1988. *Functional Morphology of Lower Cestodes: Ontogenetic and Evolutionary Aspects*. Leningrad: Nauka [In Russian].

Liu, B., H. Wakuri, K. Mutoh, and K. Taniguchi. 1996. "Ultrastructural Study of Neurosecretory Cells in the Nervous System in the Cestode (*Taenia hydatigena*)." *Okajimas Folia Anatomica Japonica* 73: 195–203. https://doi.org/10.2535/ofaj1936.73.4_195.

Montagne, J., M. Preza, and U. Koziol. 2023. "Stem Cell Proliferation and Differentiation during Larval Metamorphosis of the Model Tapeworm *Hymenolepis microstoma*." *Frontiers in Cellular and Infection Microbiology* 13: 1286190. https://doi.org/10.3389/fcimb.2023.1286190.

Mutti, L. D., and V. A. Ivanov. 2020. "Ultrastructure of the Rhyncheal Apparatus and Other Structures of the Scolex of *Grillotia (Christianella) carvajalregorum* (Cestoda: Trypanorhyncha)." *Journal of Morphology* 282, no. 2: 309–324. https://doi.org/10.1002/jmor.21305.

Olson, P. D., and J. N. Caira. 1999. "Evolution of the Major Lineages of Tapeworms (Platyhelminthes: Cestoidea) Inferred From 18S Ribosomal DNA and Elongation Factor-1a." *Journal of Parasitology* 85: 1134–1159.

Olson, P. D., J. N. Caira, K. Jensen, R. M. Overstreet, H. W. Palm, and I. Beveridge. 2010. "Evolution of the Trypanorhynch Tapeworms: Parasite Phylogeny Supports Independent Lineages of Sharks and Rays." *International Journal for Parasitology* 40, no. 2: 223–242.

Palm, H. W. 2004. *The Trypanorhyncha Diesing, 1863*. Bogor: PKSPL-IPB Press.

Palm, H. W., U. Mundt, and R. Overstreet. 2000. "Sensory Receptors and Surface Ultrastructure of Trypanorhynch Cestodes." *Parasitology Research* 86, no. 10: 821–833.

Palm, H. W., A. Waeschenbach, P. D. Olson, and D. T. J. Littlewood. 2009. "Molecular Phylogeny and Evolution of the Trypanorhyncha Diesing, 1863 (Platyhelminthes: Cestoda)." *Molecular Phylogenetics and Evolution* 52: 351–367.

Quiroga, S. Y., E. Carolina Bonilla, D. Marcela Bolaños, F. Carbayo, M. K. Litvaitis, and F. D. Brown. 2015. "Evolution of Flatworm Central Nervous Systems: Insights From Polyclads." *Genetics and Molecular Biology* 38, no. 3: 233–248.

Rees, F. G. 1941a. "The Scolex of *Aporhynchus norvegicus* (Olss.)." *Parasitology* 33, no. 4: 433–438.

Rees, F. G. 1941b. "The Musculature and Nervous System of the Plerocercoid Larva of *Dibothriorhynchus grossum* (Rud.)." *Parasitology* 33, no. 4: 373.

Rees, F. G. 1988. "The Muscle, Nervous and Excretory Systems of the Plerocercoid of *Callitetrarhynchus gracilis* (Rud., 1819) (Pintner 1931) (Cestoda: Trypanorhyncha) From Bermuda Fishes." *Parasitology* 96: 337–351.

Rees, G. 1950. "The Plerocercoid Larva of *Grillotia heptanchi* (Vaullegeard)." *Parasitology* 40, no. 34: 265–272.

Richter, S., R. Loesel, G. Purschke, et al. 2010. "Invertebrate Neurophylogeny: Suggested Terms and Definitions for a Neuroanatomical Glossary." *Frontiers in Zoology* 7, no. 29: 29. http://www.frontiersinzoology.com/content/7/1/29.

Riehl, B., and W. R. Schlue. 1998. "Morphological Organization of Neuropile Glial Cells in the Central Nervous System of the Medicinal Leech (*Hirudo medicinalis*)." *Tissue and Cell* 30, no. 2: 177–186.

Shigeno, S., A. Tame, K. Uematsu, T. Miura, S. Tsuchida, and K. Fujikura. 2015. "Dual Cellular Supporters: Multi-Layer Glial Wrapping and the Penetrative Matrix Specialized in Deep-Sea Hydrothermal Vent Endemic Scale-Worms." *Biological Bulletin* 228: 217–226. https://doi.org/10.1086/Bblv228n3p217.

Sukhdeo, S. C., and M. V. K. Sukhdeo. 1990. "Ontogenetic Development of the Brain of the Platyhelminth *Fasciola hepatica*." *Tissue and Cell* 22, no. 1: 39–50.

Terenina, N. B., L. G. Poddubnaya, O. O. Tolstenkov, and M. K. S. Gustafsson. 2009. "An Immunocytochemical, Histochemical and Ultrastructural Study of the Nervous System of the Tapeworm *Cyathocephalus truncatus* (Cestoda, Spathebothriidea)." *Parasitology Research* 104: 267–275. https://doi.org/10.1007/s00436-008-1187-x.

Verkhratsky, A., A. M. Arranz, K. Ciuba, and A. Pękowska. 2022. "Evolution of Neuroglia." *Annals of the New York Academy of Sciences* 1518, no. 1: 120–130. https://doi.org/10.1111/nyas.14917.

Waeschenbach, A., and D. T. J. Littlewood. 2017. "A Molecular Framework for the Cestoda." In *Planetary Biodiversity Inventory (2008–2017): Tapeworms From Vertebrate Bowels of the Earth*, edited by J. N. Caira, and K. Jensen, 431–451. Lawrence: The University of Kansas, Natural History Museum.

Waeschenbach, A., B. L. Webster, R. A. Bray, and D. T. J. Littlewood. 2007. "Added Resolution Among Ordinal Level Relationships of Tapeworms (Platyhelminthes: Cestoda) With Complete Small and Large Subunit Nuclear Ribosomal RNA Genes." *Molecular Phylogenetics and Evolution* 45: 311–325.

Wang, I. E., S. W. Lapan, M. L. Scimone, T. R. Clandinin, and P. W. Reddien. 2016. "Hedgehog Signaling Regulates Gene Expression in Planarian Glia." *eLife* 5: e16996.