

The non-brain anterior nerve center and tentacle crown structure of *Owenia borealis* (Annelida, Oweniidae): the evolution of the nervous system and tentacles in Bilateria

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1 **The non-brain anterior nerve center and tentacle crown structure of *Owenia borealis***
2 **(Annelida, Oweniidae): the evolution of the nervous system and tentacles in Bilateria**

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21 **Abstract**

22 The Oweniidae are marine annelids with many unusual features of organ system,
23 development, morphology, and ultrastructure. Together with magelionds, oweniids have been
24 placed within the Palaeoannelida, a sister group to all remaining annelids. The study of this
25 group may increase our understanding of the early evolution of annelids (including their
26 radiation and diversification) and of the morphology of the last common bilaterian ancestor.
27 In the current research, scanning electron microscopy revealed that the tentacle apparatus
28 consists of 10 branched arms. The tentacles are covered by monociliary cells that form a
29 ciliar groove that extends along the oral side of the arm base. Light, confocal, and
30 transmission electron microscopy revealed that head region contains two circular
31 intraepidermal nerves (outer and inner) that give rise to the neurites of each tentacle, i.e.,
32 intertentacular neurites are absent. Each tentacle contains a coelomic cavity with a network of
33 blood capillaries. Monociliar myoepithelial cells of the tentacle coelomic cavity form both
34 the longitudinal and the circular muscles. The structure of this myoepithelium is intermediate
35 between simple and pseudo-stratified myepithelium. Overall, tentacles lack prominent
36 zonation, i.e., co-localization of ciliary zones, neurite bundles, and muscles. This
37 organization, which indicates a non-specialized tentacle crown in *O. borealis* and other
38 oweniids with tentacles, is probably ancestral for annelids and for all Bilateria. The outer
39 circular nerve of *O. borealis* is a dorsal medullary commissure that apparently functions as an
40 anterior nerve center and is organized at the ultrastructural level as a stratified
41 neuroepithelium. Given the hypothesis that the anterior nerve center of the last bilateral
42 ancestor might be a diffuse neural plexus network, these results suggest that the ultra
43 anatomy of that plexus brain might be a stratified neuroepithelium. Alternatively, the results
44 could reflect the simplification of structure of the anterior nerve center in some bilaterian
45 lineages.

46 **Keywords:** medullary dorsal commissure, stratified neuroepithelium,

48 **Background**

49 The Annelida is a phylum of bilaterian animals and is the central clade of the
50 Lophotrochozoa superphylum. Annelids exhibit extremely wide patterns of organ system
51 anatomy and ultrastructure (1). According to recent data, the Annelida can be divided into
52 two large clades, Errantia and Sedentaria, and also includes several sister groups, so-called
53 basal branching lineages, including oweniids, chaetopterids, amphinomids, sipunculids, etc.
54 (2–7). Members of the family Oweniidae have many unusual morphological, ultrastructural,
55 and developmental characteristics (8–16). Oweniids together with magelonids have been
56 recently placed among the Palaeoannelida, a sister group to all remaining annelids (5,7). The
57 study of oweniids may increase our understanding of the evolution of annelids, including
58 their radiation and diversification. An improved understanding of annelid evolution should
59 increase our understanding of the morphology of the last common bilaterian ancestor
60 (LCBA).

61 At present, there are two main hypotheses regarding the structure of the anterior nerve center
62 of the LCBA: it consisted of either a ganglionic accumulation of neurons or a diffuse nerve
63 plexus (17,18). The first hypothesis suggests that the LCBA could be simple ganglia, or even
64 an elaborated brain, defined as a central collection of neuronal centers with distributed and
65 hierarchical functions (19,20). The organizations of the ganglia and brains have been well
66 studied (21–23). The second hypothesis suggests that the anterior nerve center is organized as
67 a nerve plexus, or a non-ganglionic intraepidermal anterior nerve center (7,15,23–30).

68 Unfortunately, the detailed structure of the non-ganglionic intraepidermal anterior nerve
69 center has not been described for bilaterians, especially for annelids, which form the central
70 clade of the Lophotrochozoa.

71 One interesting question concerning the LCBA is whether it had tentacle-like appendages. In
72 recent metazoans, tentacles are used for food collection by cnidarians and ctenophores, as
73 well as by many bilaterian groups including phoronids, brachiopods, bryozoans, entoprocts,

74 annelids, mollusks, hemichordates, echinoderms, and chordates (1,31). The presence of
75 tentacles in many groups suggests that the LCBA may have also had tentacles. If tentacles are
76 inherited from the LCBA, they must have evolved in different directions among bilaterians.
77 Although the directions of tentacle evolution remain uncertain, we know that some organisms
78 have specialized tentacles (32–38). This specialization is expressed in the zonation and co-
79 localization of several organ systems: ciliary bands, nerve cords, and muscles (39–47). Such
80 specialized tentacles are present in the lophophorates (48–51). To increase our understanding
81 of how tentacles have evolved among the Bilateria, we require detailed data on the
82 organization and development of tentacles from different groups of recent bilaterians.

83 All oweniids have an intraepidermal non-ganglionic nerve center (11–13,15,26), but its
84 ultrastructural organization remains unclear. The family Oweniidae includes genera that have
85 tentacles (*Owenia* and *Myriowenia*) and those that lack tentacles (*Galathowenia* and
86 *Myriochele*) (52). Tentacles of *Owenia fusiformis* were briefly studied in the past, i.e., the
87 cells on the oral side of the tentacles have been described (53,54). Considering that the
88 morphology of oweniids is highly relevant to discussions of the structure of the last common
89 ancestor of the Annelida, in the current report we provide a detailed description of the
90 anatomy and ultra-anatomy of the head and tentacle apparatus of *Owenia borealis*. We also
91 consider the relevance of the data to the evolution of the structure of the nerve center and
92 tentacles in Bilateria.

93 **Materials and Methods**

94 About 20 adults of *Owenia borealis* Koh, Bhaud & Jirkov, 2003 (55) were collected in
95 September 2018 near the Espeyrend Marine Biological Station, University of Bergen,
96 Norway. Live adults were extracted from their tubes and were used for the research.

97 ***Scanning electron microscopy (SEM)***

98 The structure of the head was studied by scanning electron microscopy (SEM). The head
99 fragments were postfixed in 1% OsO₄ and dehydrated in an ascending ethanol and acetone
100 series, critical point dried, and then sputter coated with platinum-palladium. Specimens were
101 examined with a JEOL JSM-6380LA (JEOL Ltd., Tokyo, Japan) microscope at operating
102 voltages of 15–20 kV at Lomonosov Moscow State University.

103 ***Transmission electron microscopy (TEM)***

104 The head regions with tentacles were fixed overnight at 4°C in a 2.5% solution of
105 glutaraldehyde in 0.2 M phosphate buffer (PBS). The heads were then washed in 0.2 M PBS
106 for 4 h with three changes and postfixed in 1% OsO₄ in 0.2 M PBS for 3 h at room
107 temperature (RT) with gentle rotation. The specimens were then dehydrated in an increasing
108 series of ethanol concentrations (from 15 to 96%) and isopropanol. They were subsequently
109 infiltrated in a mixture of isopropanol and Spurr resin for 3 days and then embedded in pure
110 Spurr resin at 60°C for 24 h.

111 The anterior part of the body of two adults embedded in resin were used to prepare a
112 complete series of 1- μ m (semi-thin) and 70-nm (thin) resin sections with a Leica UC 7
113 ultramicrotome (Leica Microsystems, Wetzlar, Germany). The semi-thin sections were
114 stained with methylene blue and examined with a Zeiss Axioplan2 light microscope equipped
115 with an AxioCam HRm camera (Carl Zeiss Microscopy, LLC, USA). Semi-thin sections
116 were used for description of gross anatomy and for 3D reconstructions. The thin sections
117 were stained with uranyl acetate and lead citrate and were examined with a JEM-1011 JEOL
118 or a JEM-100 B-1 JEOL transmission electron microscope (JEOL, Akishima, Japan).

119 ***Whole-mount immunostaining and confocal laser scanning microscopy (CLSM)***

120 Adults were fixed in a 4% paraformaldehyde solution in PBS (pH 7.4) (ThermoFisher
121 Scientific, Pittsburgh, PA, USA) for 8 h at 4°C and then were washed three times (30 min
122 each time) in PBS with 1% Triton X-100 (PBT) (ThermoFisher Scientific). The specimens

123 were then placed in a mixture of normal goat serum and PBT (NGS 15%) for 2 h to block the
124 sites of unspecific staining. For immunostaining, solution of primary antibodies (Abs) anti-
125 rabbit 5-HT (Immunostar, 20080, 1:1000) alone or in combination with anti- α -acetylated
126 mouse tubulin (Santa Cruz, sc-23950, 1:1000) in PBT were used. The animals were incubated
127 in primary Abs for 24 h at 4°C with rotation, followed by triple rinses with PBT. The
128 secondary antibody mixtures consisted of donkey anti-rabbit (DAR) 488 (Life Technologies,
129 A21206, 1:1000) with donkey anti-mouse (DAM) 555 (Life Technologies, A31572, 1:1000)
130 in PBT, for 24 h at 4°C. After antibodies labeling, specimens were placed in a 1:30 dilution
131 of AlexaFluor 488 phalloidin for detection F-actin together with 1:100 dilution of 4', 6-
132 diamidino-2-phenylindole (DAPI, Molecular Probes, USA,) in PBT for 4 h at RT. As a
133 control for non-specific immunorecognition, we performed immunohistochemical staining
134 without the primary antibodies, adding only the secondary antibodies or normal (non-
135 immunized) immunoglobulin G (1:500-1:1000; Sigma-Aldrich; I5006, I5381). The
136 specimens were then washed three times in PBS, washed for several minutes in increasing
137 concentrations of isopropanol, and embedded in Murray Clear (a 50/50 mixture of benzyl
138 benzoate and benzyl alcohol) at RT. Specimens were observed with a Zeiss LSM 780
139 confocal microscope (Far Eastern Center of Electron Microscopy, A.V. Zhirmunsky National
140 Scientific Center of Marine Biology, Far Eastern Branch of the Russian Academy of
141 Sciences, Vladivostok, Russia) and with with a Nikon Eclipse Ti confocal microscope (Nikon
142 Corporation, Tokyo, Japan) at Lomonosov Moscow State University, Moscow, Russia.

143 ***Image processing***

144 Z-projections were prepared using ImageJ software (56). Volume renderings were prepared
145 with Amira version 5.2.2 software (ThermoFisher Scientific, MA, USA). Images were
146 processed in Adobe Photoshop CS3 (Adobe Systems, San Jose, CA, USA). Three-
147 dimensional reconstructions were prepared with Imaris 7.2.1 software (ThermoFisher
148 Scientific, MA, USA).

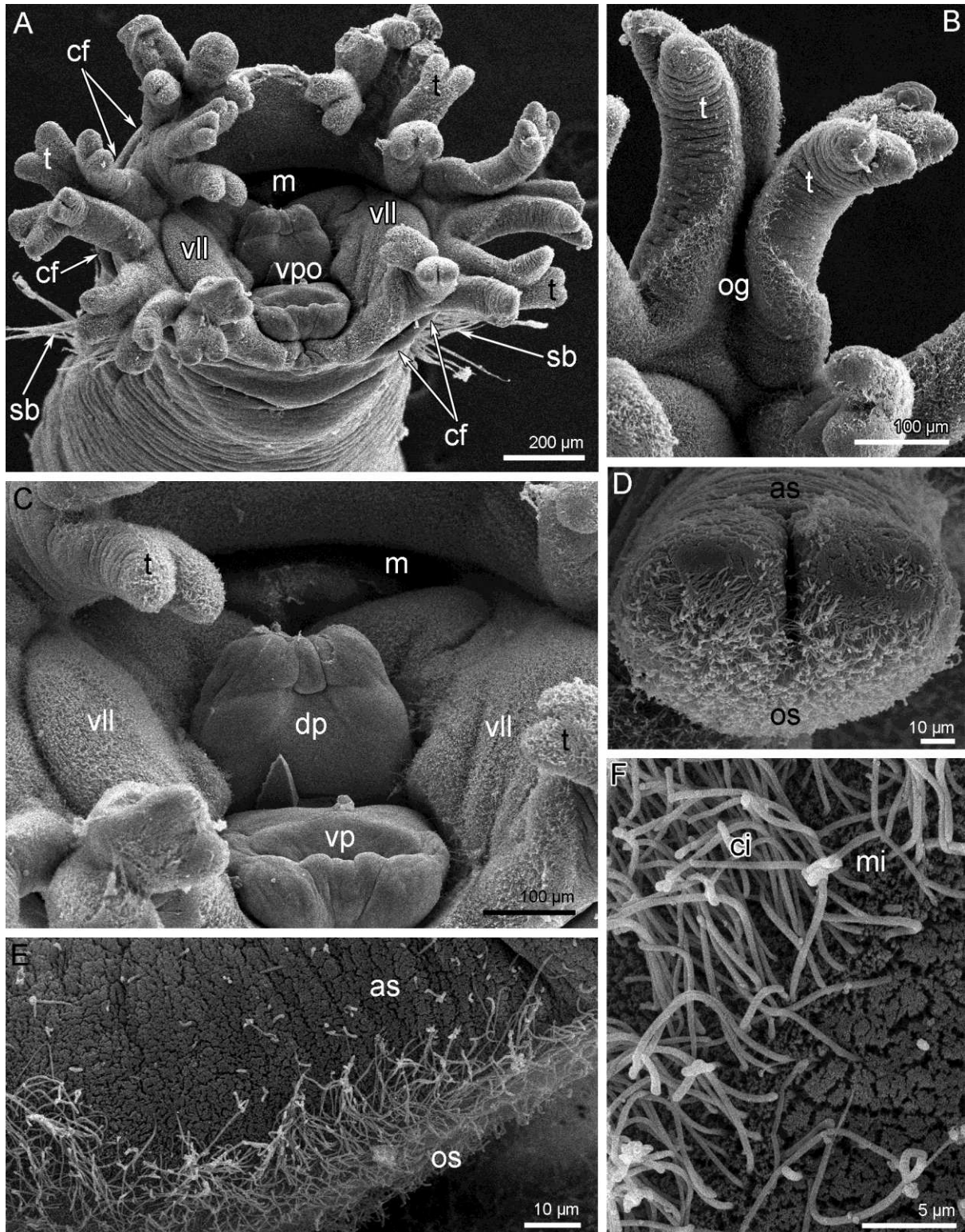
149 **Results**

150 ***Morphology of the head and tentacles***

151 Our observations indicated that the tentacle crown of *O. borealis* is not symmetrical and is
152 formed by two lateral groups, which are separated on the dorsal and ventral sides. Each
153 lateral group is represented by five short tentacles, which included (from the ventral to the
154 dorsal side) 2 double tentacles, 1 quadruple tentacle, and 1 double tentacle on the right side or
155 1 triple tentacle and 1 quadruple tentacle on the left side (Figure 1A). There are two levels of
156 tentacle ramification: for the first level, each tentacle arm splits into two or four branches; for
157 the second level, each tentacle is split into bifid tips. Tentacles and their arms are covered by
158 cilia, which are abundant on the oral side and are almost absent on the aboral side (Fig. 1D-F,
159 2A). The base of the tentacles has a deep groove extending along the oral side (Fig. 2B, C).
160 This groove is prominent at the base of the tentacle arm (Fig. 2C). The base of the tentacle
161 crown forms a collar that extends along external side of the head (Fig. 1C). The ventral
162 pharyngeal organ, consisting of the dorsal and ventral lips, is very large and is located at the
163 ventral side of the tentacle crown. Two ventrolateral lips are adjacent to the ventral
164 pharyngeal organ and are covered by cilia (Fig. 1C). The mouth resembles a crescent slit
165 (Fig. 1A). The base of the tentacle apparatus is surrounded by a thin collar fold from the
166 outside of the tentacle crown (Fig. 1A).

167 ***Histology and ultrastructure of the tentacles and head***

168 Epithelium. Each tentacle is covered by ciliated epithelial cells and contains a coelomic
169 cavity that contains muscles and blood vessels (Fig. 3A, B). The aboral epithelium is formed
170 by large monociliated cells, which are filled with many vesicles of different diameter (Fig.
171 4A). These cells form the basal thin projections that contain electron-dense filaments,
172 surround the neurite bundles, and attach to the basal lamina (Fig. 4A). Large gland cells of
173 different types are scattered in the epithelium of the aboral side (Fig. 2A, C). Some of these
174 cells have large vacuoles and electron-lucent content (Fig. 2A, C), and others have many



175
 176 **Figure 1.** Morphology of the head and tentacles of *Owenia borealis* (SEM). (A) The head viewed from
 177 the top. (B) Tentacle. (C) Ventral pharyngeal organ. (D) Forked tip of tentacle. (E) Ciliated oral and
 178 non-ciliated aboral sides of tentacle. (F) A portion of the oral side of tentacle. Abbreviations: as –
 179 aboral side; cf – collar fold; ci – cilia; dp – dorsal part of pharyngeal organ; m – mouth; mi –
 180 microvilli; og – oral groove; os – oral side; sb – setae bundle; t – tentacle; vll – ventrolateral lip; vp –
 181 ventral part of pharyngeal organ.
 182

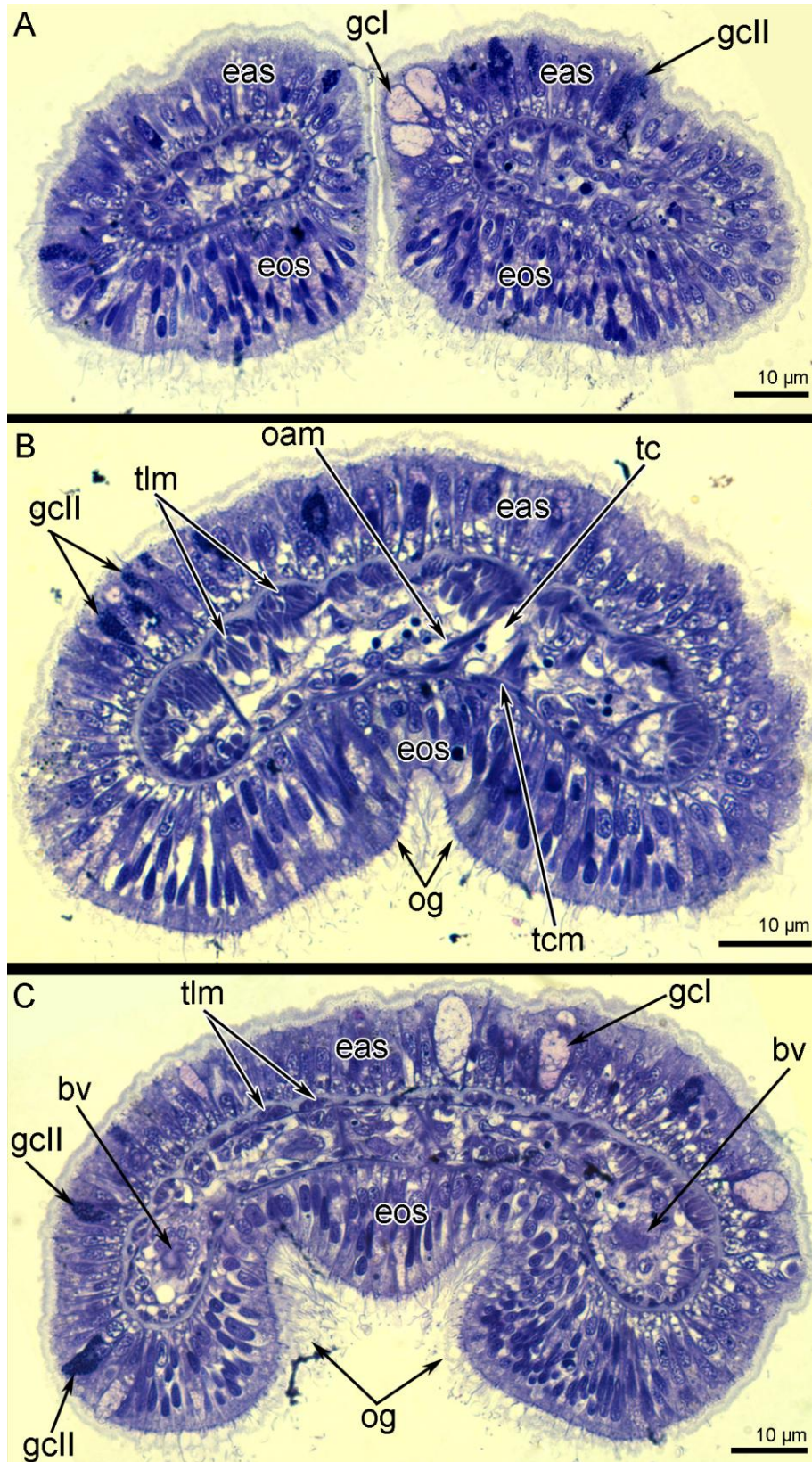
183 small and dense granules in the cytoplasm (Fig. 2B, C). Longitudinal neurite bundles extend
 184 along the base of the aboral epithelium, which also contains cells with different organization

185 (Fig. 4A, B). Some of these basal cells are small roundish perikarya, which cytoplasm
186 contains synaptic vesicles. Other basal cells contain ovoid electron-dense granules, therefore
187 their projections with the same granules can be easily recognized between neurite bundles
188 (Fig. 4B). Other cells form large thin and thick projections, which contain electron-dense
189 intermediate filaments and synaptic vesicles (Fig. 4B).

190 The epithelium of the oral side consists of slender high cells, which bear cilium and do not
191 form prominent basal projections (Fig. 4C). The cytoplasm of these cells contains prominent,
192 apical transverse and longitudinal electron-dense fibers. The epithelium of the oral side
193 contains many glandular cells, whose cytoplasm is filled with roundish vesicles with
194 flocculent content (Fig. 3A). The secretory apparatus and nucleus are located in the basal part
195 of the glandular cells. Neurite bundles, which extend between the basal parts of the epithelial
196 cells, are less numerous than in aboral epithelium (Fig. 4C). Perikarya and cells with ovoid
197 electron-dense granules are scattered in the basal portion of the epithelium of the oral side
198 (Fig. 4C).

199 The epithelium lies on the extracellular matrix layer (ECM) (Fig. 3A, B). On the aboral side,
200 the ECM has waves and forms invaginations that contain the bundles of muscles. The aboral
201 ECM is up to 2 μm thick (Fig. 3B). The ECM is 2-3 times thinner on the oral side than on the
202 aboral side of the tentacle (Fig. 3A).

203 Coelomic cavity and musculature. The coelomic cavity of the tentacles is connected to the
204 voluminous cavity of first body segment (i.e., the head cavity), which is formed by the
205 prostomium and peristomium (Figs. 5A, 6A). The lower border of the head cavity forms
206 ventral and dorsal projections (Fig. 5A). On the ventral side, the head cavity is occupied by a
207 large ventral pharyngeal organ that extends to the lower border of the head cavity (Fig. 5B).
208 On the dorsal side, the head cavity is occupied by voluminous folds of the pharynx (Fig. 5C).
209 Each tentacle contains a coelomic cavity lined with a coelomic epithelium. The epithelial
210 cells form outgrowths that extend into the cavity and that connect the lining of the coelom of

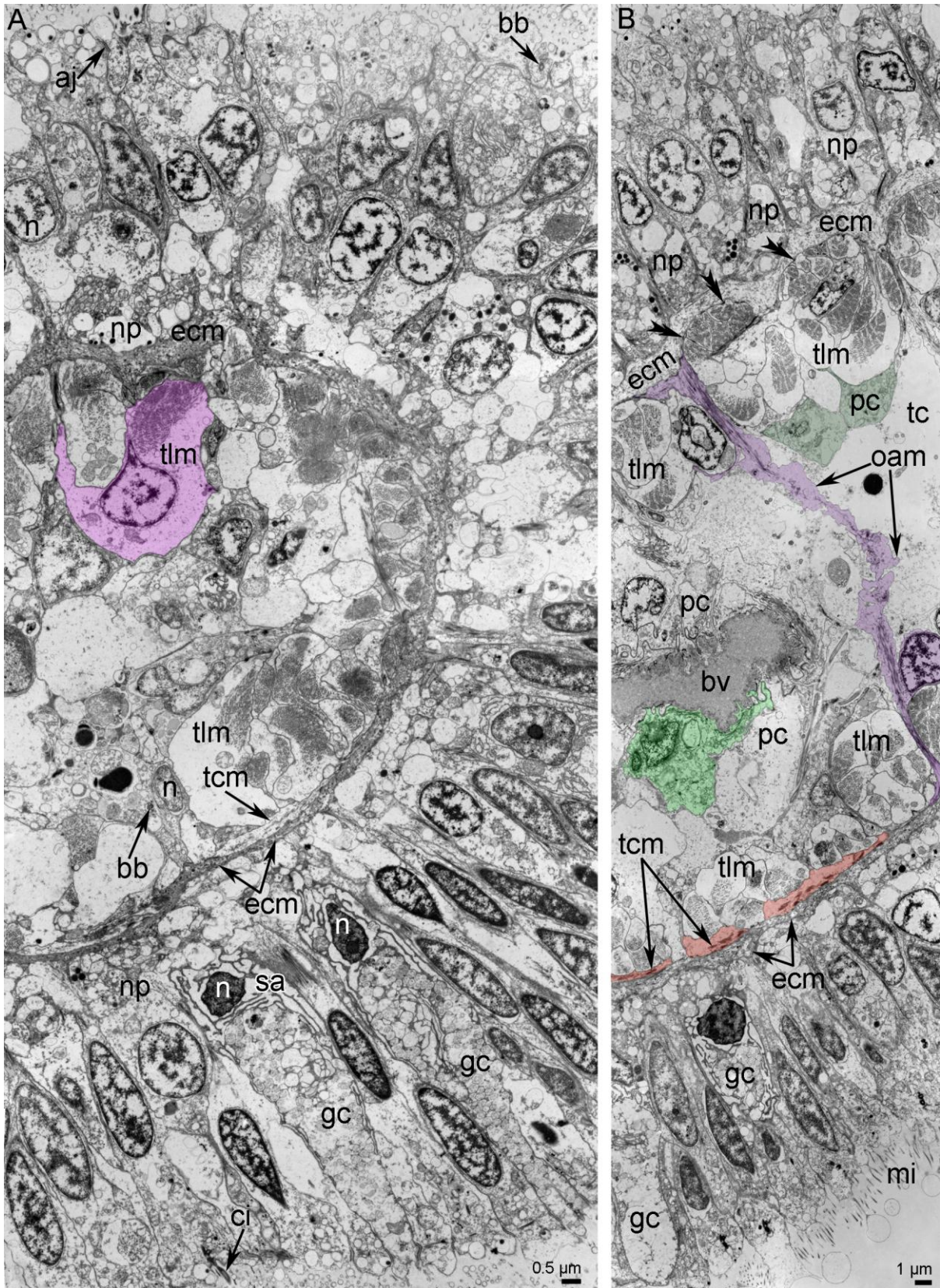


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Figure 2. Organization of tentacles of *Owenia borealis*. Transverse semi-thin sections at different levels of tentacles. (A) Forked tip of tentacle. (B) Middle portion of tentacle. (C) Base of tentacle. Abbreviations: as – aboral side; bv – blood vessel; eas – epithelium of aboral side; eos – epithelium of oral side; gcl – gland cell of first type; gcll – gland cell of second type; oam – oral-aboral muscles; og – oral groove; tc – tentacle coelom; tlm – tentacle longitudinal muscles.

218 the aboral and oral sides of the tentacles (Figs. 2, 3B). The coelomic lining is composed of
219 myoepithelial cells that form the musculature of the tentacles and the wall of the blood vessel
220 (Figs. 3A, B; 4D). The musculature of each tentacle includes longitudinal, circular, and oral-
221 aboral muscles (Figs. 2, 3). Circular muscles form a thin layer that is only present on the oral
222 side of the tentacles (Figs. 2B, 3B). The strands of the longitudinal muscles are much thicker
223 (due to an increased number of cells) on the aboral than on the oral side of the tentacle (Fig.
224 2B; 3B). Cells of all types of muscles are attached to the extracellular matrix via
225 hemidesmosomes (Fig. 3B). The head cavity is lined by coelomic epithelium, which is
226 formed by different types of cells (Fig. 6B, C). Most of these cells are the myoepithelial
227 monociliated cells that form the longitudinal musculature of the head (Fig. 6A, C). Each
228 myoepithelial monociliated cell bears one long cilium, at the base of which the basal body
229 and accessory centriole are located (Fig. 6C). These cells contact each other via apical
230 adhering junctions, which are usually located on the thin apical projections (Fig. 6C). A large
231 nucleus occupies the apical portion of the cell, whereas myofilaments extend into the basal
232 portion of the cell. Myofilaments are organized as in cross-striated muscles: in cross section,
233 there are light and dense areas that correspond to aggregations of actin and myosin filaments
234 (Fig. 6C). The cells are anchored to the basal lamina by hemidesmosomes (Fig. 6C). The
235 myoepithelial monociliated cells of the coelomic lining form the walls of blood vessels of the
236 head (Fig. 6D). These cells contain a few basal myofilaments that extend longitudinally (Fig.
237 6D). Portions of the coelomic lining are formed by a typical peritoneal coelothelium that
238 covers the longitudinal and circular musculature (Fig. 6B). The peritoneal cells lack
239 myofilaments; they are attached to the basal lamina between muscle cells. Peritoneal cells are
240 connected to each other and to the muscle cells by adhering junctions (Fig. 6B).

241 Blood vessels. Three-dimensional modelling revealed that the ventral and dorsal blood
242 vessels give rise to numerous blood vessels in the head and tentacles (Fig. 5D). At the border
243 between the first trunk segment and the head, the ventral blood vessel splits into two lateral
244 efferent branches, which give rise to two prominent lateral blood plexuses (Fig. 5D). The



245
 246 **Figure 3.** Details of tentacle ultrastructure of *Owenia borealis* (TEM). (A) General view of epithelia
 247 and coelomic cavity of tentacle. Cell of longitudinal muscle is shown in pink. (B) Different muscles of
 248 tentacle: oral-aboral muscle is shown in violet, tentacle cross muscles are shown in orange.
 249 Peritoneal cells, which cover longitudinal muscles, are shown in dark green. Cell, which includes into
 250 wall of blood vessel, is shown in light green. Hemidesmosomes are indicated by double arrowheads.
 251 Abbreviations: aj – adherence junction; bb – basal body; bv – blood vessel; ci – cilium; ecm –
 252 extracellular matrix; gc – gland cell; n – nucleus; np – neuropil; oam – oral-aboral muscle; pc –
 253 peritoneal cells; sa – secretory area of the gland cell; tcm – tentacle circular muscles; tlm – tentacle
 254 longitudinal muscles.

255 dorsal blood vessel splits into two lateral afferent vessels at the middle of the head (Fig. 5D).
256 In each tentacle, there are three longitudinal blood vessels that are connected to each other
257 (Fig. 5E). Together, they form the tentacular blood plexus.

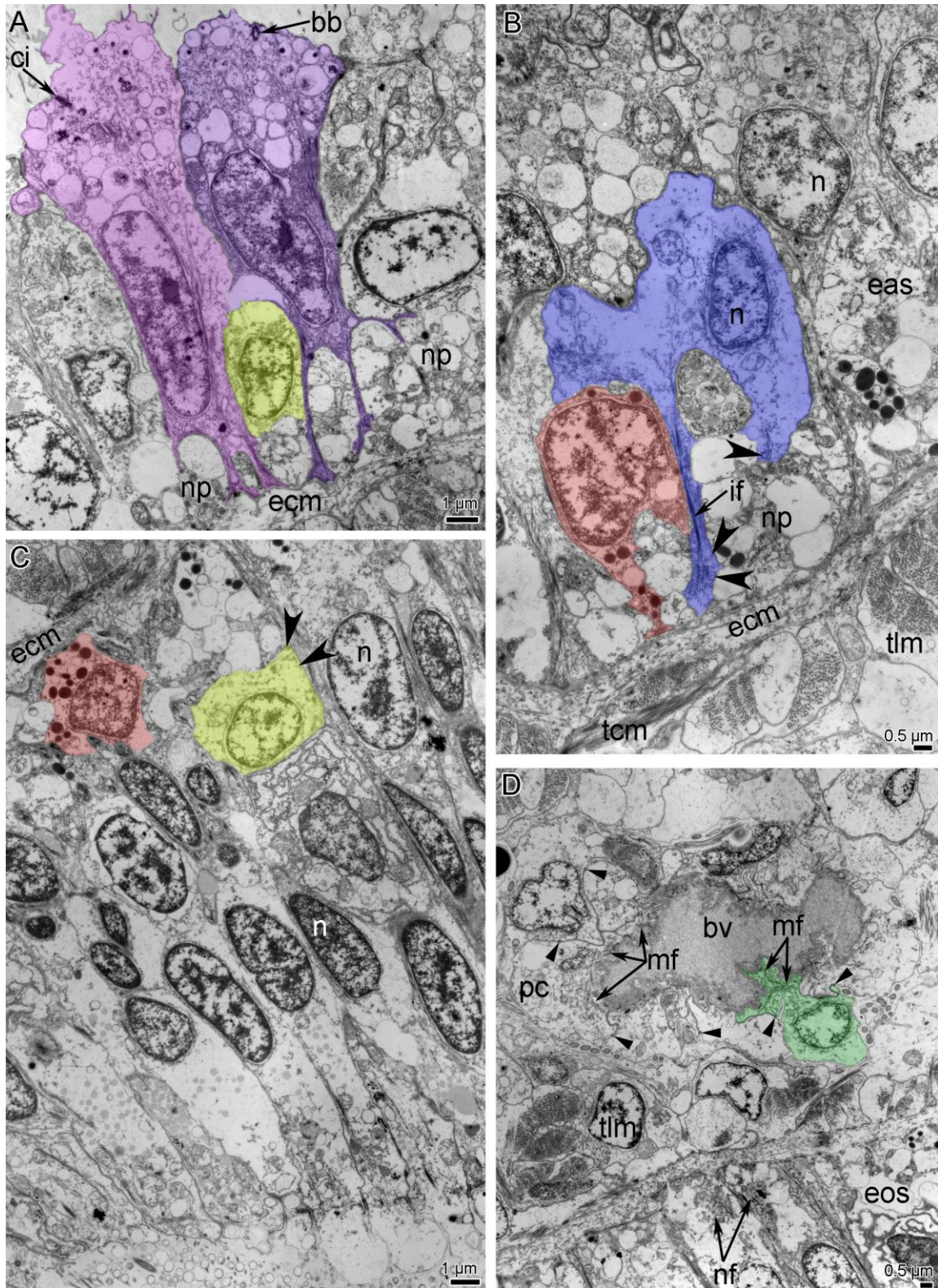
258 Myoepithelial cells form the wall of blood vessels (Figs. 3B; 4D). The basal parts of these
259 cells bear myofilaments and form numerous plasmatic projections, which extend into the
260 lumen of the vessel (Fig. 4D).

261 *Neural elements of the head*

262 Anatomy. The head contains the main elements of the nervous system: the nerve center as a
263 medullary commissure, two circumesophageal connectives with a pair of lateral medullary
264 cords with a single commissure in between, the circumoral nerve ring, two ventrolateral roots
265 of the circumoral nerve ring, and dorsal circular neurites that form a nerve net of the collar
266 fold (Figs. 7A, B; 8A, B). All other nerve elements, such as the ventral medular nerve cord
267 and the dorsal and lateral neurite bundles, are located in the first and other chaetigers (Fig.
268 8A, B).

269 Immunocytochemistry. Many of the neurite bundles exhibit acetylated alpha-tubulin-like
270 immunoreactivity (-lir) (Fig. 9A, C, F) and serotonin-lir (Figs. 7B, 9B, D, E). Interestingly,
271 labelling with both serotonin and acetylated alpha-tubulin antibodies revealed that the
272 anterior nerve center has two parts: an anterior and posterior part (Fig. 9E). Anti-acetylated
273 alpha-tubulin antibody staining was less intense in the anterior part than in the posterior part
274 of the nerve center (Figs. 9E). The intensity of anti-serotonin antibody staining, in contrast,
275 was similar in the anterior and posterior parts of the nerve center, but an area between the
276 anterior and posterior parts is formed by neurites that do not exhibit serotonin-lir (Fig. 9E).

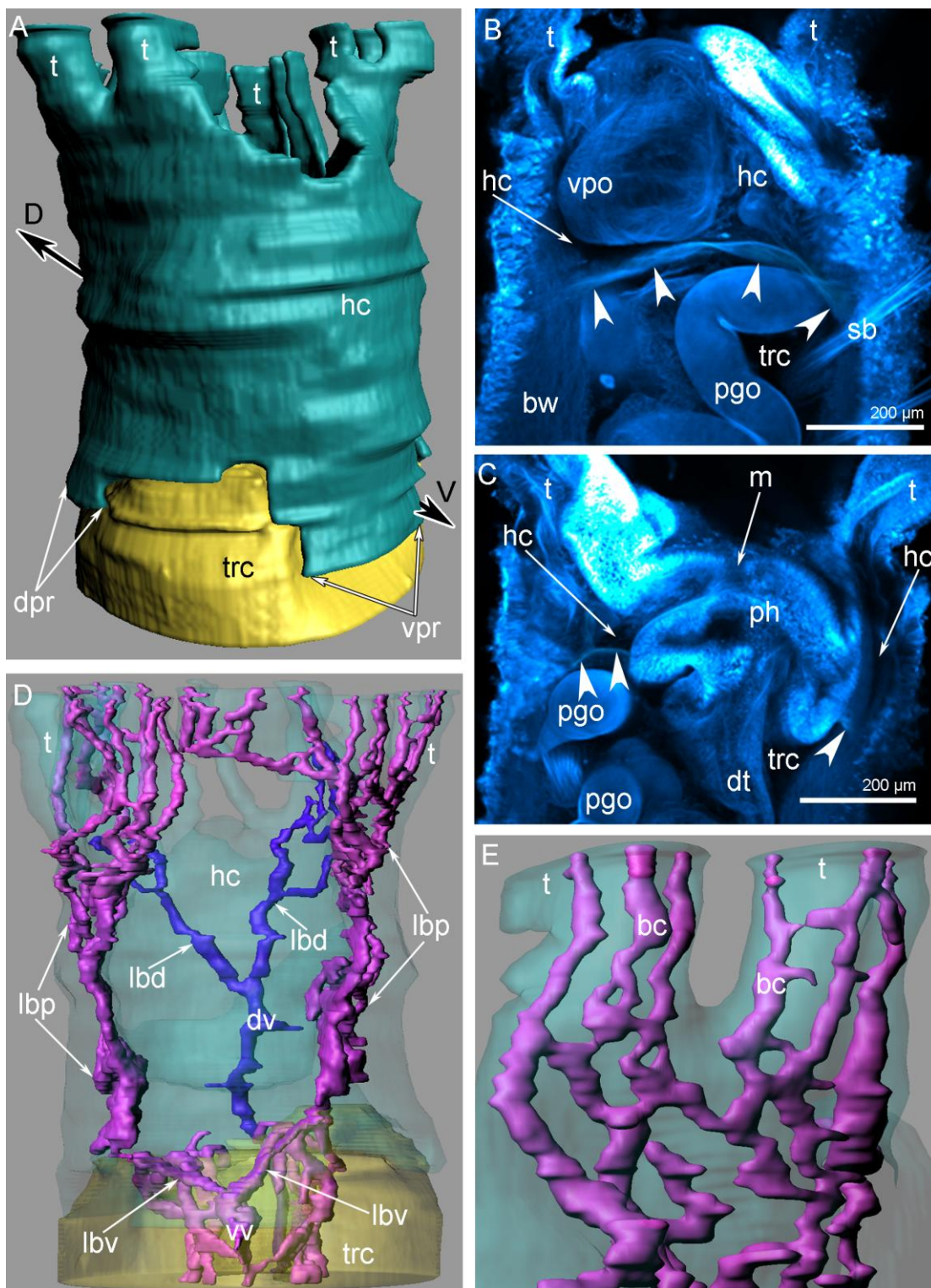
277 As a medullary dorsal commissure, the nerve center gives rise to the aboral tentacular neurite
278 bundles (Fig. 9A). Four or five thick neurite bundles extend into each tentacle, where they
279 spilt into many thin neurites that run along the aboral side of the tentacles. Some of these



280
 281 **Figure 4.** Ultrastructure of tentacle nerves and blood vessel of *Owenia borealis* (TEM). (A) Epithelium
 282 of aboral side: different types of cells. Two supportive cells are shown by different colors; perikaryon
 283 is shown in yellow. (B) The base of epithelium of aboral side: two types of glial cells are shown by red
 284 and blue. (C) Epithelium of oral side with perikaryon (yellow) and glial cell (red). Synaptic vesicles are
 285 indicated by concaved arrowheads. (D) Myoepithelial cells (green) of coelomic lining, which form the
 286 wall of blood vessel. Adherence junctions between cells are indicated by straight arrowheads.
 287 Abbreviations: bb – basal body; bv – blood vessel; ci – cilium; ecm – extracellular matrix; eas –
 288 epithelium of aboral side; eos – epithelium of oral side; if – intermediate filaments; mf –
 289 myofilaments; n – nucleus; np – neuropil; pc – peritoneal cells; tcm – tentacle circular muscles; tlm –
 290 tentacle longitudinal muscles.

292 neurites exhibit serotonin-lir and are associated with epidermal sensory cells (Figs. 7A, B;
 293 9B).

294 On the ventro-lateral sides of the head, the circumesophageal connectives that connect the
 295 medullary dorsal commissure with the ventral nerve cord are simple, i.e., they do not divide
 296 into dorsal and ventral roots before reaching the brain. Because the transition from the



298 **Figure 5.** Organization of the coelom of the head of *Owenia borealis*. (A) Three-dimensional
299 reconstruction of head (green) and trunk (yellow) coeloms. (B) Z-projection of head after staining
300 with DAPI: dissepiment between head and trunk coeloms is indicated by arrowheads. (C) Z-
301 projection of head and trunk cavity at sagittal optical section after staining with DAPI: dissepiment
302 between head and trunk coeloms is indicated by arrowheads. (D) Three-dimensional reconstruction
303 of blood vessels in head and part of trunk. Trunk and head coeloms are partly transparent. (E) Three-
304 dimensional reconstruction of blood capillaries in tentacles. Abbreviations: bc – blood capillary; bw –
305 body wall; dpr – dorsal protrusion; dt – digestive tube; dv – dorsal blood vessel; hc – head coelom;
306 lbd – lateral branch of dorsal blood vessel; lbv – lateral branch of ventral blood vessel; lbp – lateral
307 blood plexus; m – mouth; pgo – parapodial glandular organ; ph – parynx; t – tentacle; sb – setae
308 bundle; trc – trunk coelom; vpo – ventral pharyngeal organ; vpr – ventral protrusion; vv – ventral
309 blood vessel.

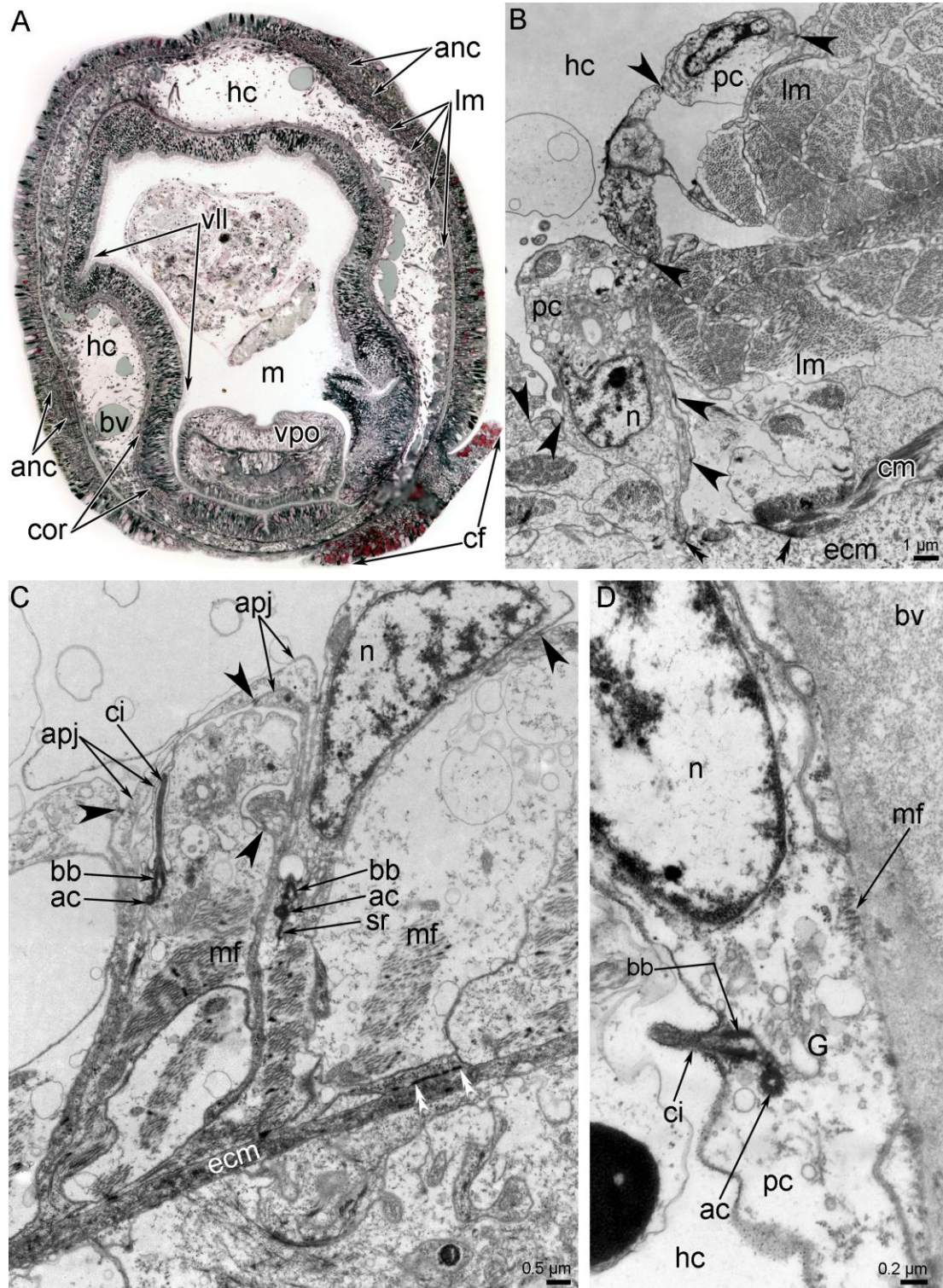
310
311 medullary dorsal commissure to the circumesophageal connectives is diffuse (Fig. 10A), it is
312 impossible to say where one ends and the other begins.

313 The circumesophageal connectives give rise to two ventro-lateral medullar nerve cords that
314 fuse together at the border between the head and the first chaetiger (Figs. 8A, 10A). Each
315 ventro-lateral medullar cord gives rise to a root that skirts a tentacle on the oral side and
316 connect the outer and inner nerve rings (Fig. 9C-F). The inner nerve ring gives rise to a few
317 thin neurites that extend along the oral side of the tentacles (Fig. 9A).

318 On the ventral side of the body, two ventro-lateral medular cords are connected via a single,
319 thin commissure (Figs. 7A,B; 9E, F; 10C). Each ventro-lateral medular cord forms several
320 dorsal neurite bundles that fuse together on the dorsal side to form a nerve plexus (Fig. 10B,
321 C). Several thin neurites extend along the dorsal side of the body (Fig. 10C). Thick lateral
322 nerve tracts extend from the head region along both lateral sides of the body (Fig. 10D).
323 Above the neuropodium of each segment, these nerve tracts form prominent varicoses (Fig.
324 10C). The medular ventral nerve cord consists of two pairs of nerve tracts: left and right (Fig.
325 10B). In each pair, prominent thick central and thin peripheral zones can be distinguished
326 (Fig. 10C).

327 Histology and ultrastructure. In the head, all nerve elements are located basiepidermally:
328 perikarya and neurite bundles are located between the somata of the epidermal cells and the
329 layer of the extracellular matrix (Figs. 7C, D; 11A, B). As a consequence, each nerve element
330 has a stratified structure, in which the cellular components form three layers, i.e., the upper,

331 middle, and lower layers (Figs. 11A, B). The upper layer is formed by the somata of the
 332 epithelial cells. The middle layer is formed by the perikarya of the neurons. The lower layer
 333 is formed by nerve projections and projections of epithelial cells (Figs. 7C, D; 11A, B).



334 **Figure 6.** Histology and ultrastructure of the coelom of the head of *Owenia borealis*. (A) Semi-thin
 335 transverse section of the head: the spacious head coelom is visible. (B) Ultrastructure of a part of
 336 coelomic lining, which is formed by alternating peritoneal and myoepithelial cells. Adherence
 337 junctions between cells are indicated by arrowheads. Hemidesmosomes are indicated by double
 338 arrowheads. (C) Coelomic lining, which is formed by monociliated myoepithelial cells. Adherence
 339 junctions between cells are indicated by arrowheads. Hemidesmosomes are indicated by double
 340

341 arrowheads. (D) A part of wall of blood vessel, which consists of monociliated myoepithelial cells.
342 Abbreviations: ac – accessory centriole; anc – anterior nerve center; apj – adherence junction; bb –
343 basal body; bv – blood vessel; ci – cilium; cf – collar fold; cm – circular muscles; cor – circumoral
344 nerve ring; ecm – extracellular matrix; G – golgi apparatus; hc – head coelom; lm – longitudinal
345 muscle; m – moth; mf – myofilaments; n – nucleus; pc – peritoneal cell; sr – striated rootlet; vll –
346 ventrolateral lip; vpo – ventral pharyngeal organ.

347

348 As a medullary commissure, the nerve center lies at the tentacle base, in the outer epidermis
349 of the head (Figs. 7C, 11A, B). The epithelium, which includes the nerve center, is up to
350 45µm in height (Figs. 7C, 11A). The neurite bundles, which make up the largest portion of
351 the nerve center, form a layer that is up to 30 µm thick (Fig. 7C). According to TEM, the
352 epithelium, which contains the nerve center, is formed by monociliated cells and has a wide
353 apical part and a narrow basal part that is transformed into a long thin process. The apical
354 surface of the monociliated cells bears thin long microvilli, whose tips are electron dense
355 (Fig. 11A). A thick layer of cuticle is located between the microvilli and cilia. The basal body
356 and accessory centriole are located at the base of the cilium. The cytoplasm of the
357 monociliated cells has many large vesicles and an electron-lucent content (Fig. 11A).
358 Electron-dense bundles of intermediate filaments extend into the apical cytoplasm, where
359 they fuse with each other and occupy the long basal projection; the latter projection extends
360 between the neurite bundles of the nerve center and adheres to the basal lamina via
361 hemidesmosomes (Fig. 11A). The epithelium of the nerve center contains many gland cells of
362 two types, which are similar to those of gland cells in the tentacle epithelium: cells of first
363 type contain large vesicles with mucous content and cells of second type are filled with small
364 dense granules (Fig. 7C). In the nerve center, perikarya are scattered between the somata of
365 the epithelial cells, above the neuropil (Fig. 11A). These perikarya are small (diameter ~ 5
366 µm) (Fig. 12A). The nucleus in these cells has an irregular shape, lacks a nucleolus, has
367 electron-lucent karyoplasm, and contains large aggregations of heterochromatin in the centre
368 (Fig. 12A). The cytoplasm of the perikarya contains many synaptic vesicles that differ in
369 diameter and content. The cytoplasm also contains mitochondria that are small, not abundant,
370 and have an electron-dense matrix (Fig. 12A). The neuropil, which is formed by numerous
371 neurites, is located between the perikarya and the extracellular matrix. In the nerve center,

372 two components of the neuropil can be distinguished at the ultrastructural level. The first
373 component is the upper portion of the neuropil, which is mostly formed by circular neurites
374 that are cut longitudinally in transverse sections of the nerve center (Fig. 11A). The second
375 component is the lower layer, which is mostly formed by longitudinal neurites that are cut
376 transversally in transverse sections of the nerve center (Fig. 11A). In the nerve center, the
377 neuropil consists of neurites that differ in structure. Some of these neurites have small
378 diameters and electron-dense cytoplasm, which regularly form wide varicoses with electron-
379 lucent cytoplasm. The cytoplasm of these small neurites contains synaptic vesicles with
380 electron-dense content and with content of intermediate electron density (Fig. 12A, B). Other
381 neurites of the neuropil usually have large diameters and electron-lucent cytoplasm, and
382 contain dense-core synaptic vesicles and vesicles with electron-lucent content (Fig. 12B). In
383 addition to these two types of neurites, the neuropil contains projections of cells that contain
384 ovoid electron-dense granules (Fig. 12A, B).

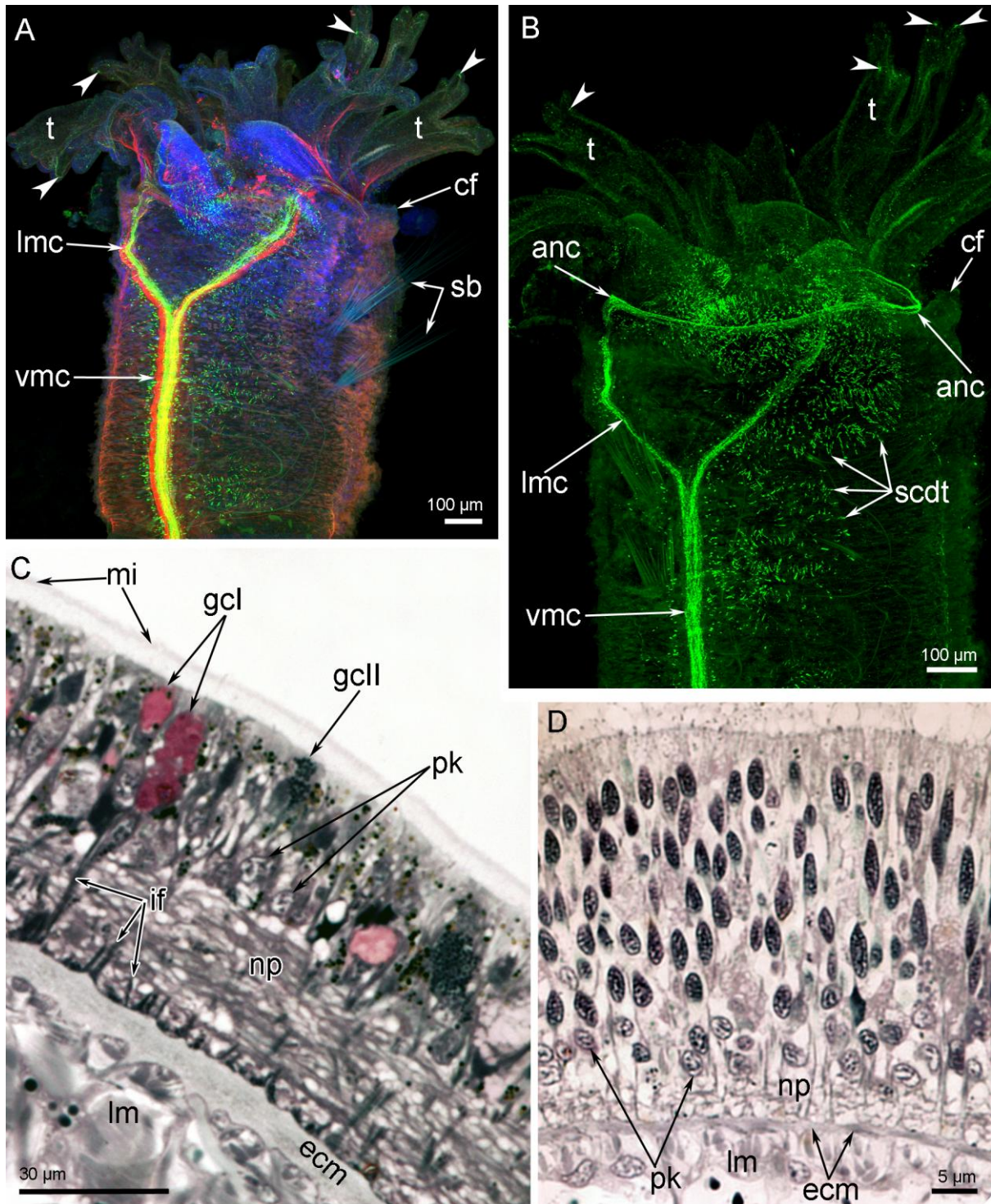
385 **Discussion**

386 In the current research, we used TEM and immunocytochemistry coupled with CLSM to
387 study the anatomy and ultra-anatomy of the anterior nerve center. We report the absence of a
388 brain-like structure in *O. borealis*. We also used histology, TEM, SEM, and 3D modelling to
389 examine the organization of the organ system of the tentacle crown of *O. borealis* with the
390 goal of gaining insight into the evolution of Bilateria nervous system and feeding
391 apparatuses.

392 **The stratified neuroepithelium of *O. borealis* as a trait of the bilaterian anterior nerve** 393 **center**

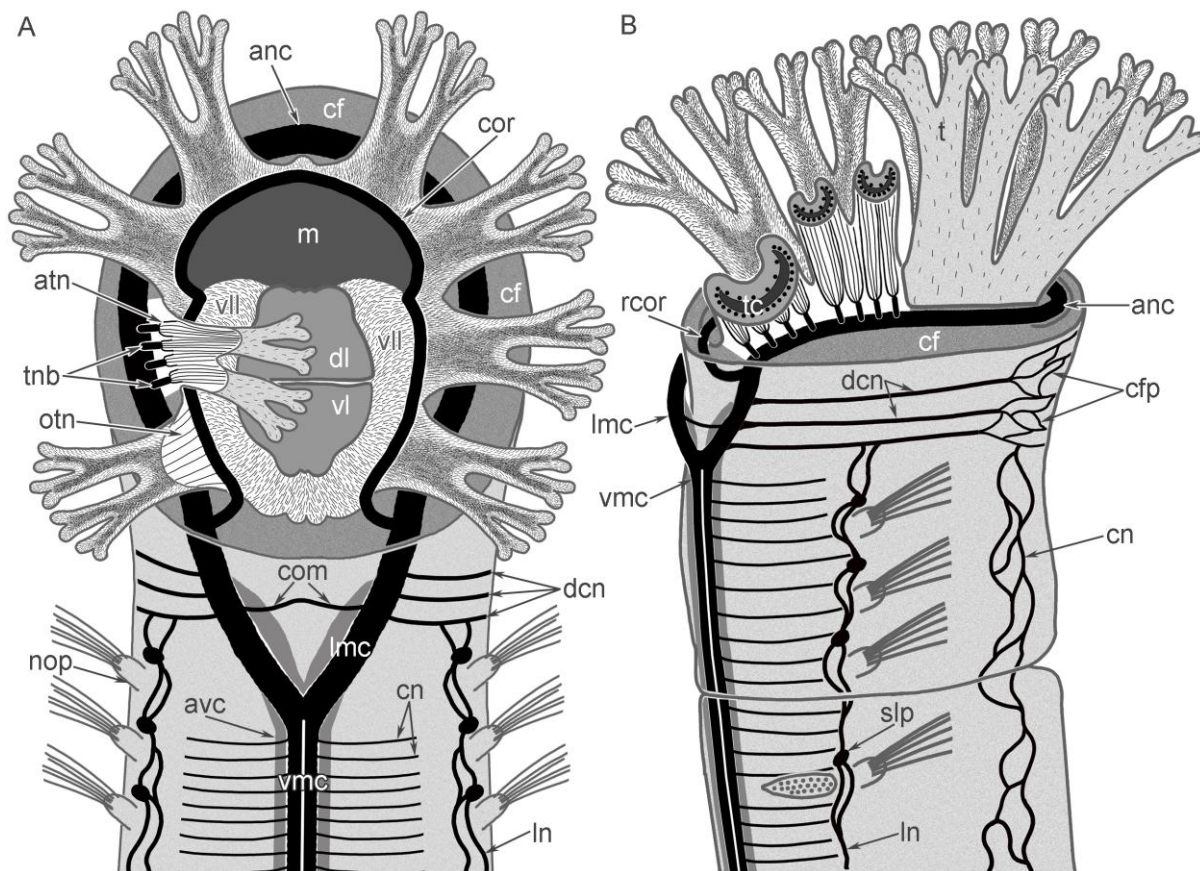
394 The supraesophageal ganglion, or brain, is considered to comprise a compact central mass of
395 neuropil surrounded by a cell cortex (22). Our study revealed the absence of a ganglionic
396 organization of the anterior nerve center in *O. borealis*. In *O. borealis*, serotonin-lir somata
397 do not form a compact cell cortex, and tubulin-lir neurite bundles do not form a swelling

398 within the dorsal commissure. According to Richter et al. (22), nerve cords or neurite bundles
 399 that are surrounded by a cellular cortex are commonly termed medullary nerve cords or



400
 401 **Figure 7.** Organization of the nervous system of the head of *Owenia borealis*. (A) General anatomy of
 402 the nervous system viewed from the ventral side: Z-projection after double immunostaining against
 403 acetylated alpha-tubulin (red) and serotonin (green) and staining with DAPI (blue). (B) Serotonin-lir
 404 nerve elements of the head viewed from the ventral side; Z-projection after immunostaining against
 405 serotonin (green). Some serotonin-lir cells in the epithelium of tentacles are indicated by
 406 arrowheads. (C) Semi-thin transverse section of the anterior nerve center. (D) Semi-thin transverse
 407 section of the circumoral nerve ring. Abbreviations: anc – anterior nerve center; cf – collar fold; ecm –
 408 extracellular matrix; gcl – gland cell of first type; gcll – gland cell of first type; if – intermeditated
 409 filaments; lm – longitudinal muscles; lmc – lateral medullary cord; mi – microvilli; np – neuropil; pk –

410 perikarya; sb – setae bundle; scdt – serotonin-lir cells of the digestive tract; t – tentacle; vmc –
 411 ventral medullary cord.
 412



413 **Figure 8.** Schemes of the nervous system organization of the anterior body part of *Owenia borealis*.
 414 All nerve elements are shown in black. (A) Ventral view. (B) Lateral view. Abbreviations: anc –
 415 anterior nerve center; atn – aboral tentacle nerves; avc – additional ventral cord; cf – collar fold; cfp
 416 – collar fold dorsal nerve plexus; cn – cross nerve; com – ventral commissure; cor – circumoral nerve
 417 ring; dcn – dorsal cross nerve; dl – dorsal lip of the ventral pharyngeal organ; dn – dorsal neurites;
 418 ldn – laterodorsal nerve; lmc – lateral medullary cord; m – mouth; nep – neuropodia; nop –
 419 notopodia; otn – oral tentacle nerves; pbr – posterior portion of the brain; ph – pharynx; rcor – root
 420 of circumoral nerve ring; slp – serotonin-lir perikarya; stn – serotonin-lir neurites of tentacles; t –
 421 tentacle; tbn – tentacle neurite bundle; vl – ventral lip of the ventral pharyngeal organ; vmc – ventral
 422 medullary cord.
 423

424
 425 bundles. It follows that the anterior nerve center of *O. borealis* could be termed a “medullary
 426 dorsal commissure”. The medullary dorsal commissure has also been documented in adults of
 427 three other oweniid species, i.e., *Owenia fusiformis*, *Galatowenia oculata*, and *Myriowenia*
 428 sp. (12,13,15,26). We therefore suggest that the anterior nerve center in the entire Oweniidae
 429 clade can be termed the “medullary dorsal commissure”.

430 In a previous study, electron microscopy revealed that *O. borealis* has a stratified
 431 neuroepithelium, similar to that in the brachiopod *Coptothyris grayi* (57). A stratified

432 neuroepithelium has also been described based on TEM in *O. fusiformis* and *G. oculata* (see
433 Fig 8A in (15) and Fig 3D in (13)). We therefore suggest that all oweniids probably have the
434 dorsal medullary commissure, which is organized as a stratified neuroepithelium.

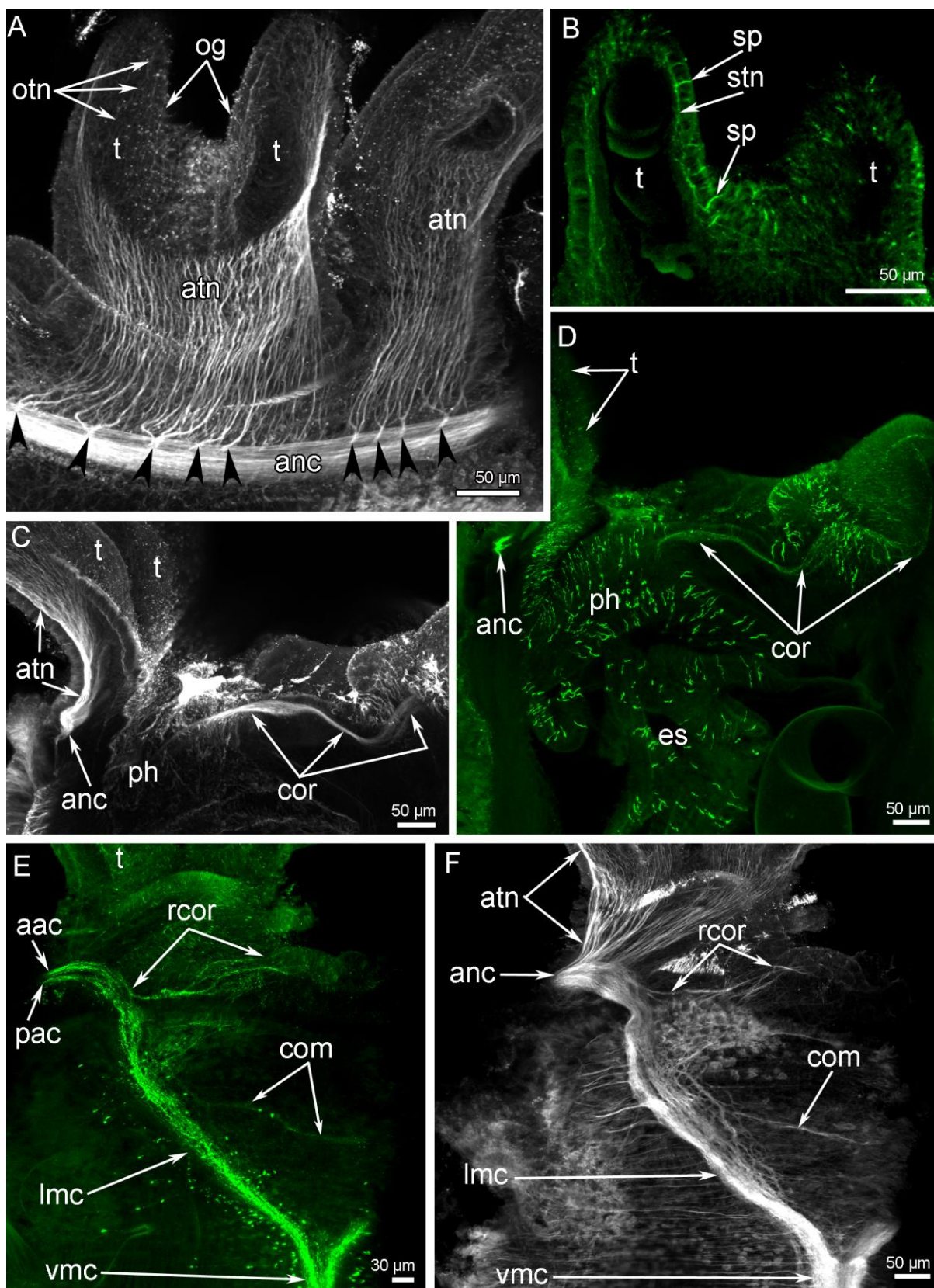
435 The stratified neuroepithelium in *O. borealis* consists of three layers: somata of glial cells,
436 perykaria of neurons, and the neuropil. A similar organization can be found in in protostomes
437 such as brachiopods (57), phoronids (38,58), oweniid annelids (this study; (15)), and echiurid
438 annelids (Temereva, Kuznetsov, personal observation), and in priapulids (29). The
439 organization can also be found in deuterostomes such as enteropneust hemichordates (29) and
440 echinoderms (59), as well as in the sister group to all remaining bilateria, the nemetodermatid
441 acoelomorph (see Fig. 5F' in (60)). Thus, a stratified neuroepithelium may represent the
442 ancestral trait of the anterior nerve center in all bilaterians. That possibility is consistent with
443 earlier suggestions regarding the ancestral state of the intraepidermal and non-ganglionic
444 anterior nerve center of bilaterians (7,15,23–29).

445

446 **The non-specialized tentacle crown in oweniids**

447 Oweniids primarily feed on the surfaces of substrates, and those species with a
448 tentacle crown also use it in suspension feeding (61). The degree of specialization in feeding
449 probably determines the architecture of the tentacle crown, which may differ in number of
450 tentacles, crown length, ramification from the base, rate of branching, and the shape of
451 grooves that conduct particles from the tentacle tips to the mouth (62–66). The tentacle crown
452 of *O. borealis* branches from its base and is formed by five tentacles on the left and right
453 sides of the head. All tentacles consist of four branches, except the most ventral pair, which
454 consist of two branches (Fig. 1A). Oral tentacle grooves in *O. borealis* are 50-60 um in
455 diameter and are formed by fused tentacle branches. The tentacles are covered by a
456 monociliated epithelium on both aboral and oral sides. Ciliation is denser on the oral side due
457 to the columnar epidermal cells on the oral side. Taking into account all these traits, we

458 consider that *O. borealis* is not specialized in its mode of feeding, but instead uses its tentacle
 459 crown for feeding on suspended particles and on particles on the surface of the substrate.



460
 461 **Figure 9.** Details of innervation of tentacle crown and oral area in *Owenia borealis*. CLSM data: Z-
 462 projections after immunostaining against acetylated alpha-tubulin (grey) and serotonin (green). (A)
 463 The aboral side of tentacle base: several short thick nerves (arrowheads) extend from the anterior
 464 nerve center and give rise to the oral neurite bundles of tentacles. (B) Serotonin-lir perikarya and

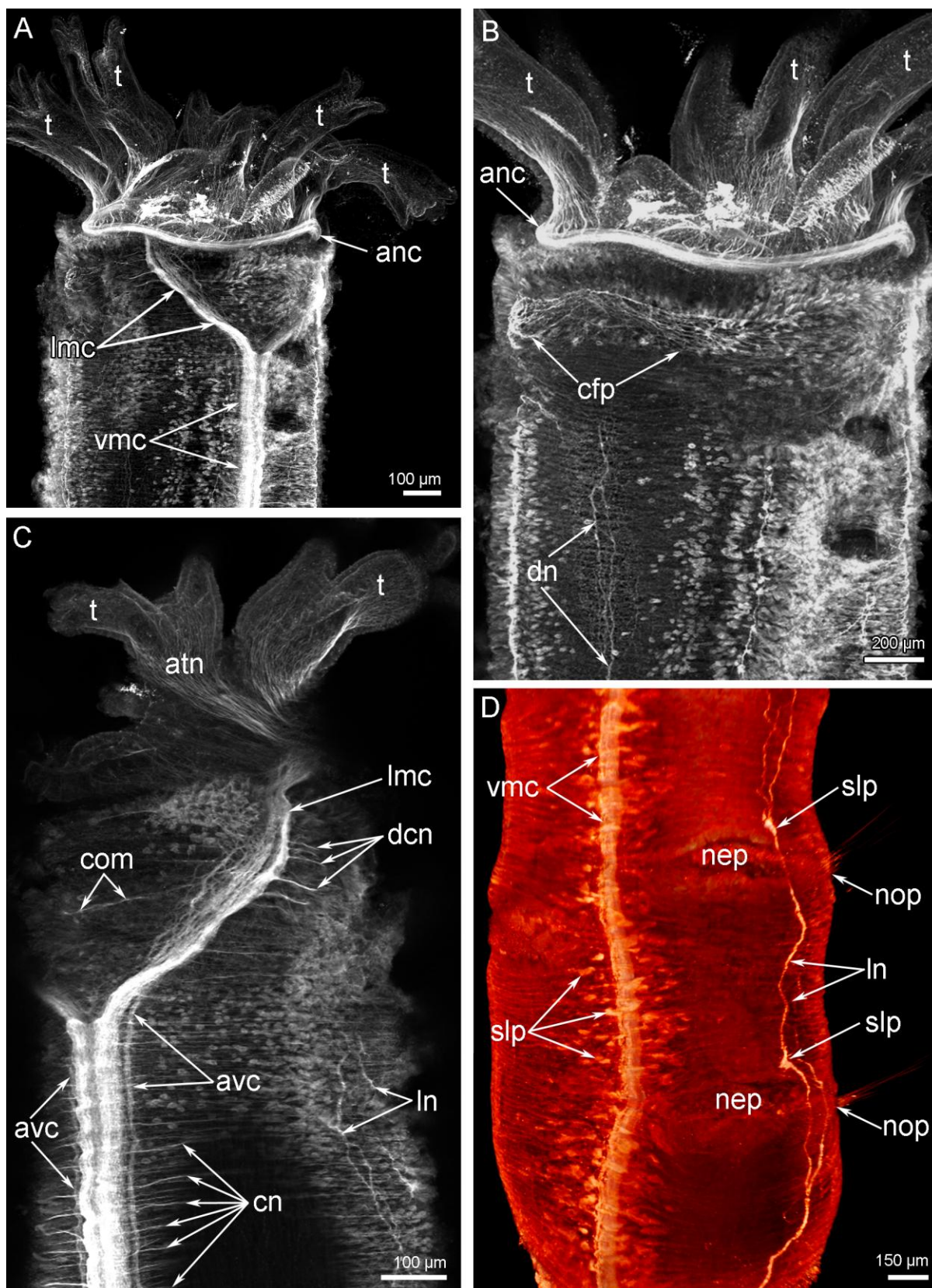
465 neurites in the epithelium of tentacle. (C) Central portion of the head: circumoral nerve ring is
466 visible. (D) Central portion the head: serotonin-lir neurites are visible in the circumoral nerve ring.
467 The epithelium of pharynx contains numerous serotonin-lir cells. (E) A portion of the head viewed
468 from the ventral side. There are anterior and posterior portions of the anterior nerve center. Right
469 ventrolateral root of the circumoral nerve ring and ventral commissure are visible. (F) Right portion
470 of the head viewed from the ventral side. Abbreviations: aac – anterior portion of the anterior nerve
471 center; anc – anterior nerve center; atn – aboral tentacle nerves; com – ventral commissure; cor –
472 circumoral nerve ring; es – esophagus; lmc – lateral medullary cord; og – oral groove; otn – oral
473 tentacle nerves; pac – posterior portion of the the anterior nerve center; ph – pharynx; rcor – root of
474 circumoral nerve ring; sp – serotonin-lir perikarya; stn – serotonin-lir neurites of tentacles; t –
475 tentacle; vmc – ventral medullary cord.

476
477 Innervation of the tentacle crown. Recent studies have shown that the nervous system

478 anatomy of the tentacle crown of the lophophorates, which are highly specialized filter
479 feeders, has a characteristic organization. The tentacle crown of the lophophorates contains
480 two nerve centers and two circular nerves that give rise to the intertentacular nerves that
481 innervate two adjacent tentacles (42,58). Together, all of these neural structures may
482 represent a ground pattern of the tentacle crown innervation of the phoronid-like ancestor of
483 lophophorates (51). Interestingly, at the base of the tentacle crown of the annelid *O. borealis*,
484 we documented both the external circular nerve (which is known in other oweniids
485 (12,13,15), and the complete internal circular nerve (which is only partly represented in
486 *Galathowenia oculata* (13). Other features of the tentacle crown innervation pattern that are
487 present in the highly specialized filter feeders are absent in *O. borealis*. For example, *O.*
488 *borealis* lacks a ganglionic nerve center (see the Discussion above about the stratified
489 neuroepithelium) and lacks intertentacular nerves, i.e., each tentacle is independently
490 innervated. The presence of intertentacular nerves, which innervate adjacent tentacles, is an
491 important character filter-feeders such as lophophorates (46). Among annelids, the highly
492 specialized filter feeders, the sabellids, have these nerves (61).

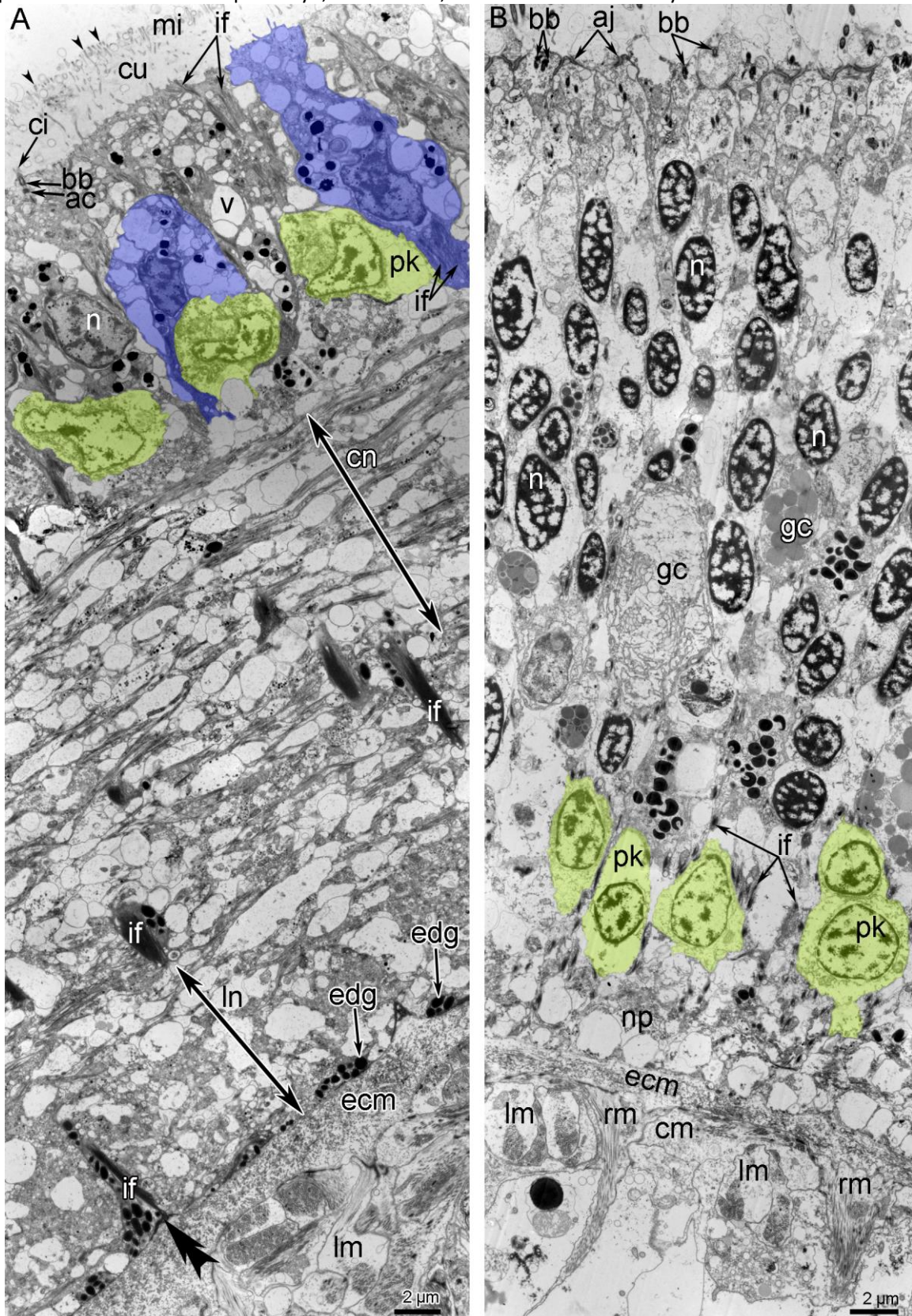
493 Musculature of the tentacle crown. The muscles of the tentacle crown in *O. borealis*
494 are mainly represented by longitudinal bundles, most of which are located on the crown's
495 aboral side (Figs. 2B, C, 3B). Contraction of the longitudinal muscles pulls the tentacles
496 outward and opens the tentacle crown. The longitudinal musculature in *O. borealis* thereby
497 helps the tentacle crown to collect particles that are suspended in the water column or that

498 have settled on the substrate (61,63,67–69). The reverse folding of the tentacles likely occurs
 499 due to the deformed layer of the ECM, which is much thicker on the aboral side than on the



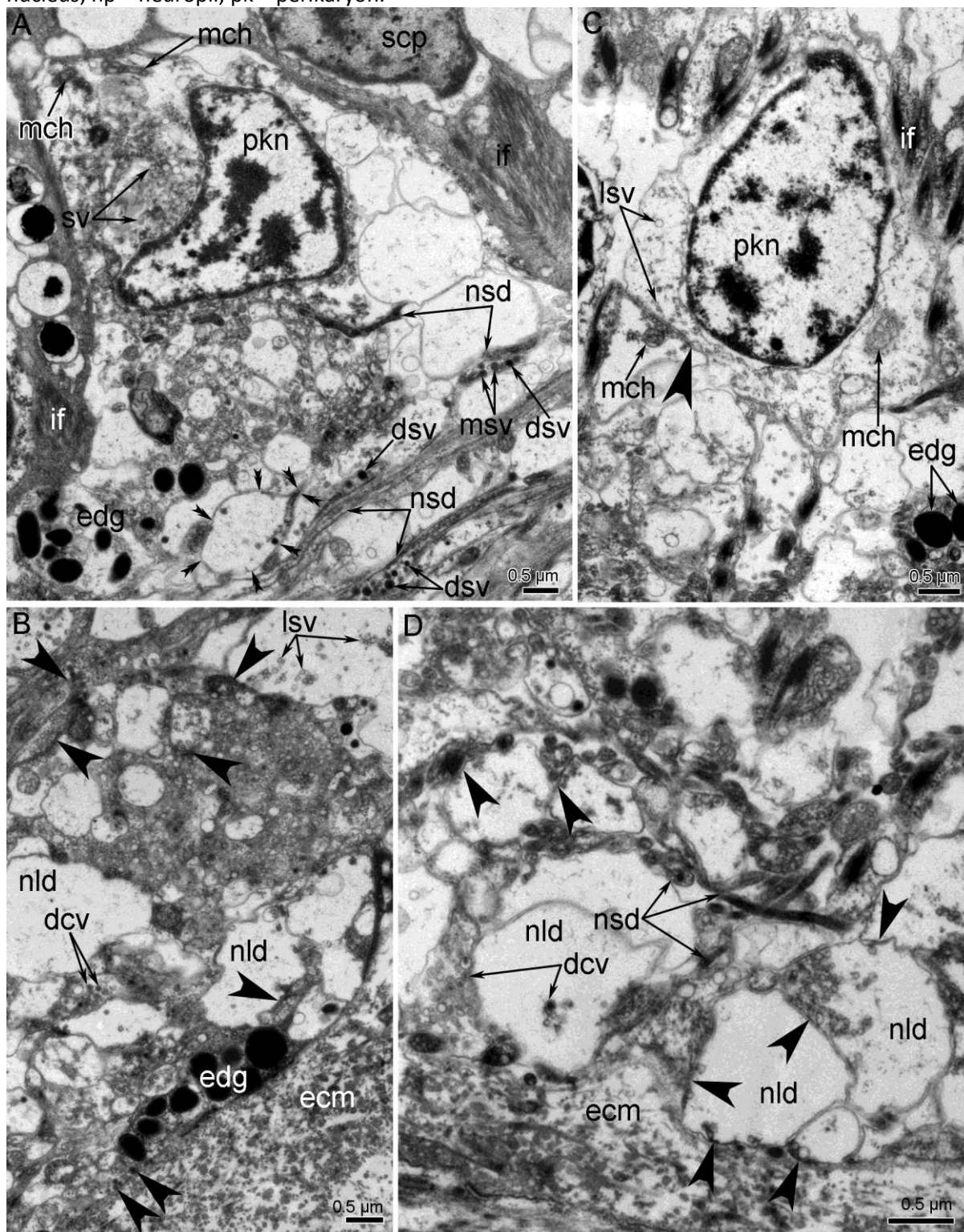
500
 501 **Figure 10.** Details of the nerve element location in the head and adjacent segments of *Owenia*
 502 *borealis*. CLSM data: Z-projections (A-C) and volume rendering (D) after immunostaining against
 503 acetylated alpha-tubulin (grey and orange). (A) A head with tentacle crown viewed from the ventral
 504 side. (B) A head with some tentacles viewed from the dorsal side. (C) Left portion of the head viewed

505 from the ventral side. (D) A portion of the body near the head viewed from the left. Abbreviations:
 506 anc – anterior nerve center; atn – aboral tentacle nerves; avc – additional ventral cord; com – ventral
 507 commissure; cfp – collar fold dorsal nerve plexus; cn – cross nerve; dcn – dorsal cross nerve; dn –
 508 dorsal neurites; lmc – lateral medullary cord; ln – lateral nerve; nep – neuropodia; nop – notopodia;
 509 slp – serotonin-like lateral perikarya; t – tentacle; vmc – ventral medullary cord.



510
 511 **Figure 11.** Ultrastructure of head nerve elements in *Owenia borealis*. Ultra-thin transverse sections.
 512 (A) A portion of the ring-shaped anterior nerve center. (B) A portion of the circumoral nerve ring.
 513 Supportive cells (= radial glial cells) are shown in blue; perikarya are shown in yellow.
 514 Hemidesmosome is indicated by double arrowhead. Dense tips of microvilli are indicated by

515 arrowheads. Abbreviations: ac – accessory centriole; aj – adherence junction; bb – basal body; cn –
 516 cross extended neurites; cu – cuticle; ecm – extracellular matrix; edg – electron dense granules; gc –
 517 gland cell; if – intermediate filaments; ln – longitudinally extended neurites; mi – microvilli; n –
 518 nucleus; np – neuropil; pk – perikaryon.



519 **Figure 12.** Ultrastructural details of the anterior nerve center (A, B) and circumoral nerve ring (C, D)
 520 of *Owenia borealis*. (A, C) Perikarya, which are surrounded by supportive cells and their projections.
 521 (D, E) The basal portions of neuropil. Synaptic-like structures, which are characterized by cell
 522 membrane density and by synaptic vesicle concentration, are indicated by arrowheads.
 523 Abbreviations: dcv – dense-core synaptic vesicle; dsv – dense synaptic vesicles; ecm – extracellular
 524 matrix; edg – electron dense granules; if – intermediate filaments; lsv – light synaptic vesicle; msv –
 525 synaptic vesicle with content of middle electron density; nld – neurite of large diameter; nsd –
 526 neurite of small diameter; pkn – nucleus of perikaryon; scp – supportive cell; sv – synaptic vesicle.
 527
 528

529 oral side of the tentacles (Fig. 3A). In those annelids that are specialized filter feeders, the
530 outward expansion of the fan of tentacles also occurs due to the contraction of the aboral
531 longitudinal muscles. Those filter feeders, however, also have a cartilaginous skeleton as well
532 as muscles at the base of the tentacular crown that serve as antagonists of the aboral
533 longitudinal muscles, i.e., that enable the organism to withdraw the tentacles and move the
534 captured particles to the mouth (70).

535 In the tentacles of *O. borealis*, the circular muscle layer is very thin. Although this muscle
536 layer may represent only short fragments of individual muscle filaments, we suspect that it
537 represents a complete muscle ring (Fig. 2B, 3A). In the tentacles of the specialized filter
538 feeders such as the lophophorates and annelids Sabellidae and Serpulidae, a complete
539 reduction of the circular muscles occurs (70–73). That *O. borealis* apparently retains the
540 circular muscles in the tentacles is consistent with the inference of a non-specialized mode of
541 feeding.

542 Coelomic lining of the tentacle crown. Four types of coelomic myoepithelium have
543 been described in echinoderms, various annelids, and lophophorates: simple, pseudostratified,
544 bipartite pseudostratified, and stratified (74–77). The coelomic epithelium in *O. borealis* is
545 intermediate between the simple and the pseudostratified myoepithelium. The
546 pseudostratified myoepithelium is known for echinoderms (74,78), brachiopods (77,79),
547 phoronids (58,72), and sedenterian and errantian annelids (75,76). The cells of a
548 pseudostratified myoepithelium are arranged in two rows: internal myoepithelial cells and
549 external non-muscular epithelial cells (i.e., cells without myofilaments). In *O. borealis*, both
550 rows of cells have myofilaments. The internal myoepithelial cells are mainly used for
551 contraction, and their wide basal parts contain longitudinal or rarely circular myofilaments
552 (Figs. 3, 6C). Because both types of cells in *O. borealis* are myoepithelial, the
553 pseudostratified myoepithelium of *O. borealis* differs from the pseudostratified
554 myoepithelium described by Rieger and Lombardi (74). We propose that the coelomic lining

555 of the head and tentacles of *O. borealis* is intermediate between the simple and
556 pseudostratified evolutionary stages of the coelomic epithelium of Bilateria.

557 The pseudostratified myoepithelium is considered to be associated with the basiepidermal
558 nervous system: each myoepithelial cell receives a signal from neurotransmitters in the
559 immediate vicinity of the neurons (75). Together, the pseudostratified myoepithelium and the
560 basiepidermal nervous system in *O. borealis* have been recognized as plesiomorphic traits of
561 the epithelia of the body wall in annelids (15,26,27) and possibly in Spiralia (7). It follows
562 that, on the one hand, the myoepithelium of the coelomic lining and the basi-epidermal
563 nervous system co-evolved in *Owenia*. On the other hand, the myoepithelial cells of the
564 coelom do not specialize in performing various functions, all cells carry myofilaments, and
565 there is no typical pseudo-stratified myoepithelium.

566 To summarize this part of the Discussion, we observed the presence of a non-specialized
567 tentacle crown in *O. borealis* and note that such crowns have also been observed in other
568 oweniids with tentacles. The lack of a specialized tentacle crown corresponds with the
569 structural elements of the nervous, muscular, coelomic, and circulatory systems. *O. borealis*
570 lacks a dorsal brain but instead has an anterior nerve center in the form of the dorsal
571 medullary commissure. There are no intertentacular nerves. Myoepithelial cells of the
572 coelomic cavity are not specialized and represent an intermediate stage between simple and
573 pseudostratified myoepithelium. The blood vessels form a complex network of capillaries in
574 which the afferent and efferent vessels cannot be traced. The circular muscles remain, and
575 there are no muscles that are antagonistic to the longitudinal muscles that open the tentacle
576 crown.

577 *Evolution of the tentacle apparatuses*

578 It is assumed that various bilaterians, including annelids, cephalopods, onychophorans,
579 echinoderms, and ascidians, have the same genetic program defining the coordinate grid of
580 the various appendages or outgrowths of the body (80–82). At the same time, these

581 appendages of the body are not homologous to each other, have different morphologies and
582 perform completely different functions, for example, sensitive perception, nutrition,
583 movement, etc. Interestingly, in different groups of the bilaterians, including annelids, the
584 anterior outgrowths of the body specialized in parallel in capture of the food particles, the so-
585 called tentacular apparatuses. Here, we consider the evolutionary trends of the organization of
586 tentacular apparatus that are used for suspension and filter feeding.

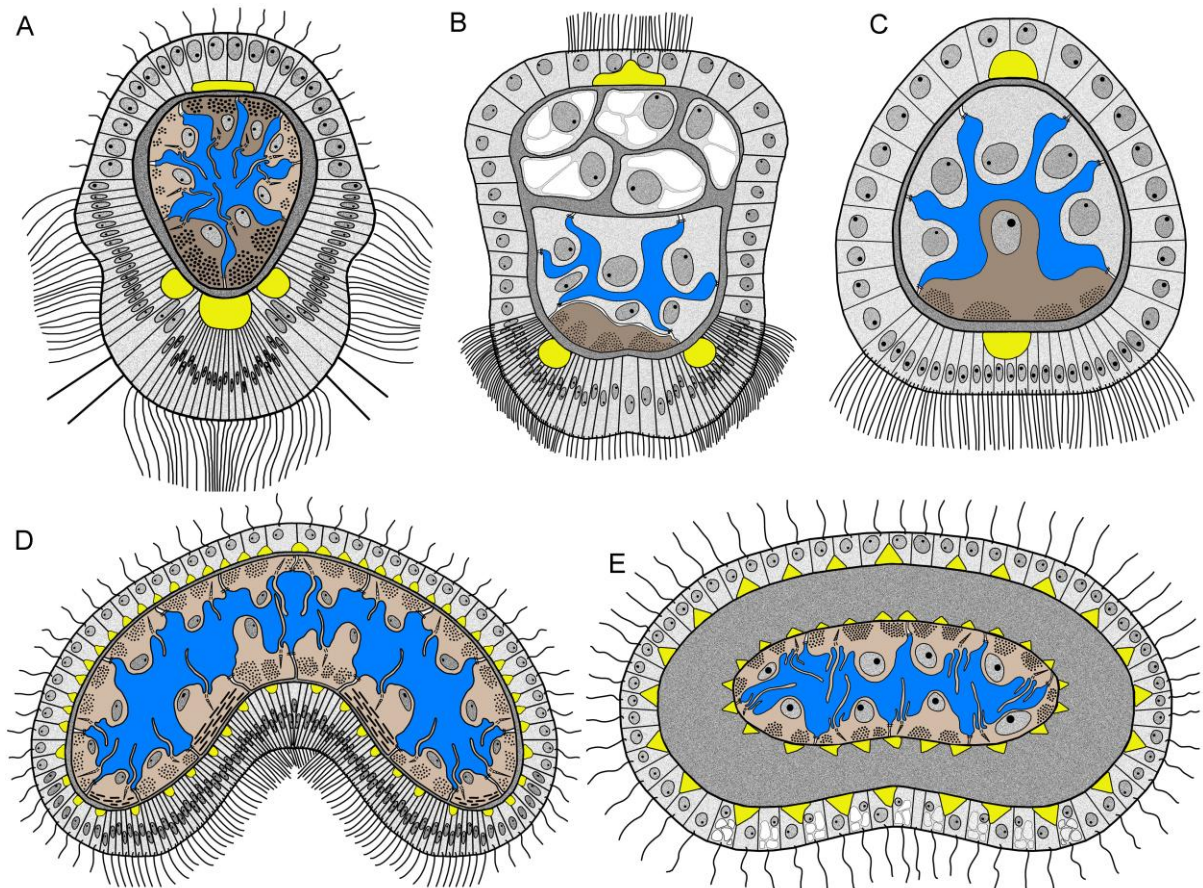
587 *O. borealis* is one of the various annelids that has a tentacle apparatus or anterior appendages.
588 A comparative analysis of the organization of tentacles in different groups of Bilateria reveals
589 three main patterns of the tentacle specialization (Fig. 13). The first pattern is represented by
590 highly specialized tentacles with a zonality of the epithelium that is co-localized with nerve
591 tracts and muscle bundles. Specialized tentacles are found in some filter feeders including
592 annelids in the families Serpullidae and Sabellidae (83), all lophophorates (Phoronida,
593 Brachiopoda, and Bryozoa) (32,48,51), and Kamptozoans (=Entoprocta) (34,84). Specialized
594 tentacles always have at least four zones: one oral, one aboral, and two lateral (Fig. 13A, B).
595 Oral and lateral zones are heavily ciliated, whereas cilia are rare or even absent in the aboral
596 zone. The aboral zone can undergo specialization involving the presence of additional
597 skeletal structures and gland cells. These four zones are innervated by different nerve tracts.
598 The epidermal zones and nerve tracts are co-localized. The muscle bundles are also co-
599 localized with certain zones of tentacle. There are usually two muscular bundles, oral and
600 aboral, which allow each tentacle to bend in two directions. Among all filter feeders,
601 lophophorates have the most specialized tentacles, each of which bears eight zones: one
602 frontal (oral), one abfrontal (aboral), two lateral, two laterofrontal, and two lateroabfrontal.
603 Each of these zones is innervated by a specific nerve tract and has a specific function (51).
604 The second pattern of tentacle organization is less specialized tentacles, which have at least
605 two zones: heavy ciliated oral and less ciliated aboral. In the second pattern, the innervation
606 of each zone is provided by a specific nerve tract. Less specialized tentacles occur in some

607 annelids that are not specialized filter-feeders, i.e., the Fabriciidae and some Serpulidae
608 (71,83) (Fig. 13C). Although the information is scarce, fabriciids could be deposit and/ or
609 suspension feeders (61,83,85).

610 The third pattern of the tentacle organization is the non-specialized tentacles. Such tentacles
611 lack zonality of the epidermis and co-localization of ciliary cells, nerve tracts, and muscular
612 tracts (Fig. 13D, E). In these non-specialized tentacles, all sides are evenly ciliated. If present,
613 ciliary zones are not co-localized with the nerve tracts or neurite bundles, which are evenly
614 scattered in the tentacle. In the tentacles of the third type, muscle cells do not form bundles,
615 and they are evenly distributed in the tentacle. The non-specialized tentacles can be found in
616 oweniids (this study) and in some holothurians (86). Holothurians are deposit or suspension
617 feeders and are able to attach deposited particles to tentacles due to the secretion of a glue by
618 gland cells (87–89). The presence of many gland cells can be regarded as a kind of
619 specialization but cannot be compared with the zonality of the highly specialized tentacles for
620 filter-feeding.

621 If we assume that tentacles have been inherited from the LCBA, we can suggest the evolution
622 of tentacle apparatuses from the non-specialized tentacles of deposit/suspension feeders to the
623 highly specialized tentacles of the filter-feeders. This idea may be supported by data on
624 morphology and diet of the Sabellidae, whose tentacles evolved from less specialized in the
625 Fabriciidae to highly specialized in the Sabellidae (83). In the hypothetical order of tentacle
626 evolution, *O. borealis* exhibits the least specialized tentacle apparatus, which can be regarded
627 as ancestral for all bilaterians. On the other hand, we must keep in mind that tentacles may
628 have appeared independently in different groups and evolved according to the mode of
629 feeding.

630



631
 632 **Figure 13.** Schemes of transverse section of tentacles of different types. A-B: highly specialized
 633 tentacles; C – less specialized tentacle; D-E – non-specialized tentacles. (A) Generalized tentacle of
 634 lophophorates (based on 32,48,51). (B) Generalized tentacle of Serpullidae and Sabellidae (based on
 635 83). (C) Generalized tentacle of Fabriciidae (based on 83) and serpulid *Pomatoceros triqueter* (based
 636 on 71). (D) Tentacle of *Owenia borealis* (this study). (E) Tentacle of holothuria *Holothuria forskali*
 637 (based on 86). Color code: yellow – nerve elements; blue – coelomic cavity; light brown –
 638 myoepithelial cells; dark brown – muscle cells and myoepithelial cells with numerous myofilaments;
 639 dark grey – extracellular matrix; light grey – epithelial cells.

640
 641 **Conclusions**

642 In this report, we described the anatomy and ultra-anatomy of the tentacular crown of *Owenia*
 643 *borealis*. Because they belong to the clade of palaeoannelids (3,5), the Oweniidae are
 644 important for studies of the morphological traits of annelid ancestors or even of bilaterian
 645 metazoan ancestors. In *O. borealis*, the anterior nerve center is represented by stratified
 646 neuroepithelium and consists of three layers: apical somata of the glial cells, perikarya of
 647 neurons, and the basal neuropil between the thin projections of the glial cells. Based on the
 648 available data on the structure of the various bilaterian lineages (15,29,38,57–60), we suggest
 649 that the anterior nerve center of the last common ancestor of annelids and possibly of all
 650 bilaterian metazoans was a basiepidermal stratified neuroepithelium. After describing the

651 architecture of the tentacle crown of *O. borealis*, and its innervation, musculature, blood
652 system, and coelomic myoepithelial lining, we compared the tentacle crown of *O. borealis*
653 with the tentacular apparatuses of the other bilaterian metazoans. These groups have three
654 patterns of tentacle organization: highly specialized tentacles, less specialized tentacles, and
655 non-specialized tentacles. Our anatomical and ultra-anatomical data suggest that *O. borealis*
656 has the least specialized tentacle apparatus, which can be regarded as an ancestral trait. We
657 propose that the tentacle apparatuses in the Bilateria evolved from the non-specialized
658 tentacles of deposit/suspension feeders to the highly specialized tentacles of filter-feeders.

659

660 **Data availability statement**

661 The data sets analyzed during this study are available from ET upon request.

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877

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893 **Author information**

894 *Contributions*

895 NR collected and fixed the animals and wrote the Discussion. VD performed the confocal
896 laser scanning microscopical studies. ET designed the study, wrote the manuscript, and
897 prepared all figures.

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909 **Ethics declarations**

910 *Ethics approval and consent to participate*

911 Not applicable. The field sampling did not involve endangered or protected species. The use
912 of annelids in the laboratory does not raise any ethical issues.

913 *Consent for publication*

914 All authors read and approved the manuscript.

915 *Competing interests*

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918 **Additional information**

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