Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

How to build the best house: habitat formation by the boring arctic phoronid Phoronis ovalis

Elena N. Temereva

temereva@mail.ru

Lomonosov Moscow State University

Research Article

Keywords: tube, inner organic cylinder, soft sediment, hard substratum, chitin, annelids, bacteria

Posted Date: November 17th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11737560/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

How to build the best house: habitat formation by the boring arctic phoronid *Phoronis ovalis*

Elena Temereva[1,2]

[1]119991, Russia, Moscow, Lomonosov Moscow State University, Biological Faculty, Dept. Evolutionary Biology, temereva@mail.ru

[2]101000, Russia, Moscow, Faculty of Biology and Biotechnology, National Research University "Higher School of Economics", Myasnitskaya Street, 20

For correspondence:

Elena N. Temereva

119991, Russia, Moscow, Leninskie Gory, 1-12, Moscow State University, Biological Faculty

Tel.: +7(495)939-44-95

Fax.: +7(495)939-56-95

Abstract

Boring marine invertebrates are key ecosystem engineers: they modify hard substrates, create microhabitats, drive nutrient cycling, and can harm aquaculture by damaging commercial molluscs. Phoronids are ecologically important, but their biology is poorly known. This study examines *Phoronis ovalis*, a boring phoronid inhabiting empty bivalve shells in the White Sea. Using electron microscopy, confocal laser scanning microscopy with Calcofluor White staining (to detect chitin), and micro‑CT with 3D reconstruction, we characterized the animal's morphology, burrows, and tube. In the valve of scallop shell *Chlamys islandica*, burrows of different *P. ovalis* individuals form an interconnected network with multiple openings. This likely reflects asexual reproduction and supports ecological flexibility, including efficient lophophore exposure and potential coordination of gamete release. The calcis material of the valve is structurally modified through 45-47 μm in thickness from the burrow lumen: it is represented by numerous small spines. This layer undergoes the chemical influence of the secretion of epithelial glands, which probably release chelating agent. The burrows are lined by organic material, which forms the tube, which secretion is supplied by body wall epithelium. Experiments with Calcofluor White revealed for the first time the presence of chitinous material in epithelium of body wall and in the tube. The tube is multilayered; its structure differs from that of other phoronids in thickness of the entire tube and separated fibers and in organization of each layer, which forms by tightly compressed fibers. A diverse bacterial community (multiple size groups, Gram negative) is consistently associated with *P. ovalis*, abundant in the cuticle, between tube layers, and on the tube surface. Our findings highlight *P. ovalis*' adaptations to a boring lifestyle and underscore the need for further research into substrate erosion, tube remodelling, and host–microbiome interactions in phoronids.

Key words: tube, inner organic cylinder, soft sediment, hard substratum, chitin, annelids, bacteria

Introduction

Adult phoronids are marine benthic animals, which have worldwide distribution and are able to live in the mouth of large rivers (Emig, 1982). In some areas, phoronid populations may reach up to 150.000 ind/m^2 or even higher (Vyshkvartzev et al., 1990; Emig, 1982; Neklyudov et al., 2026; Turanov, 2026) and play an important role in benthic communities as ecosystem engineers (Larson, Stachowicz, 2009).

Adult phoronids live in soft sediments or in hard substrata (Emig, 1982). Due to the substratum characteristics all phoronids can be divided into two groups: digging and boring. Specific type of substratum is known for *Phoronis svetlanae* (Temereva, Malakhov, 1999), which lives on the surface of the hard substratum, and in *Phoronis australis*, which lives in tube of ceriantharia. The type of substratum has been traditionally regarded as an ecological characteristic; however the cladistic analysis revealed the taxonomical value of this criterion (Temereva, Neklyudov, 2018). The most abundance phoronids reach up in soft sediments: silt, sand, and combination of silt and sand. For example, abundant populations of digging phoronid are known in Bodega harbor (USA) and in Vostok bay (Russia) (Larson, Stachowicz, 2009; Turanov, 2026; Neklyudov, Temereva, 2027). However, boring phoronids are also abundant in some areas: accordingly to data base iNaturalis exactly boring phoronid species are the most observed species the last month this year, 2026 (https://www.inaturalist.org/taxa/246126-Phoronidae#highlights-tab). Phoronids can be recognized because of presence of specific tentacle crown, the lophophore, which gives rise to the mane of this phylum “horseshoe worms”. Whole worm-like phoronid body is submerged into the substratum and the only anterior part of the body with the lophophore is exposed into the water. The lophophore has specific morphology, which in some cases can be assigned to certain phoronid species (as helicoidal lophophore of *Phoronopsis californica*). In many other cases, the phoronid species cannot be established by the lophophore morphology (Temereva et al., 2016).

Among all other invertebrates, phoronida is a unique phylum, all species of which live in the tubes formed by secretion of epidermal cells. The organization of tubes formed by different phoronid species has been described at morphological level (Temereva et al., 2020). The tubes formed by phoronids have common structure, which is not species specific, but depends on kind of substratum. Thus, specimens, which belong to one species and live in silt and in sand, have different structure of the tube (Neklyudov, Temereva, 2027). The difference concerns the thickness of organic cylinder, thickness of a single fiber produced by epidermal cell, character of the outer layer formed by particles of substratum. Accordingly to fine morphology, phoronid

tubes differ from that of annelids by several criteria that can be used to distinguish phoronid and annelid tubes in hydrobiological samples and in paleontological material (Temereva et al., 2020). The secretion of tube material supplies by glandular cells, which are represented by several kinds and are abundant in epidermis of the body wall (Pourreau, 1979; Aguirre et al., 1993; Fernández et al., 1996; Temereva, 2024). The chemical composition of phoronid tube is still not strictly established. Without any experimental data phoronid tubes are alleged to be formed by chitinous material (Hyman, 1958). Accordingly to histology, the tube of *Phoronis hippocrtpia*, which lives in soft sediment, includes inner basophilic and outer acidophilic parts (Pourreau, 1979). This fact supports the mixed nature of tube in at least some phoronid species.

Another one question concerning phoronid tubes is the growth of the tube. In all phoronids, tubes are slightly swollen in that part, which is submerged into the substrate; whereas the upper portion of tube is narrow (Temereva et al., 2020). Such shape probably means that the tube growths at the posterior part of the body. At the same time, in some phoronids, the tube is closed in the posterior end. Thus, animal should firstly dissolve the old tube and then produce new portion of the tube.

The phenomenon of drilling of hard calcic substratum is known for many invertebrates: sponges, molluscs, bryozoans, annelids, ect. In these different groups, the ability to drill the calcic substratum appeared independently and several times in certain group (Radashevsky, 2012, 2022). Little is known about details of this process: some data are obtained for annelids (Zottoli, Carriker, 1974a, 1974b; Sato-Okoshi, Okoshi, 2000; Ehrlich et al., 2009). Chemical and mechanical ruts of drilling mechanism are unknown for boring phoronids, which are able to live in the shell of scallops, limpets, oysters, mussels and other economically important mollusks. *Phoronis ovalis* is one of boring phoronids, which the only phoronid species that is explored the arctic seas. Many details of biology of this species are still vague and therefore cannot be incorporate into comparative analysis of phoronid taxonomy, evolution, and embryology.

In this study, the detailed description of tubes and burrows, which a formed by *P. ovalis*, are given by data on electron microscopy, cytochemistry with confocal laser scanning microscopy, and computer micro-tomography.

Materials and Methods

Habitat and sampling. Adult phoronids were collected in August 2024 in Onega Bay of the White Sea (64º51′21.9″ N 35º19′50″ E) with the trawl from depth 34.8 m (Fig. 1a, b). *Phoronis ovalis* lives in dead shells of scallop *Chlamys islandica* and mussels *Mytilus edulus*

(Bivalvia). Numerous valves were kept in large basins with sea water together with other animals from the sample: *Hyas* sp. (Decapoda), *Crossaster* sp. (Asteroidea), *Mitylus edulus* (Bivalvia), *Strongylocentrotus drobachiensis* (Echinoidea), and many others (Fig. 1c, d). These basins were transported to the White Sea Biological station (66° 34′ N, 33° 08′ E), in Kandalksha Bay. Here, mollusk shells were observed and live phoronids were photographed with a digital camera mounted on a Leica MZ12.5 stereomicroscope (Fig. 2a). About 90 live specimens of *P. ovalis* were taken out the shells due to crash of the valves (Fig. 2b, c) and then fixed for the study by different methods.

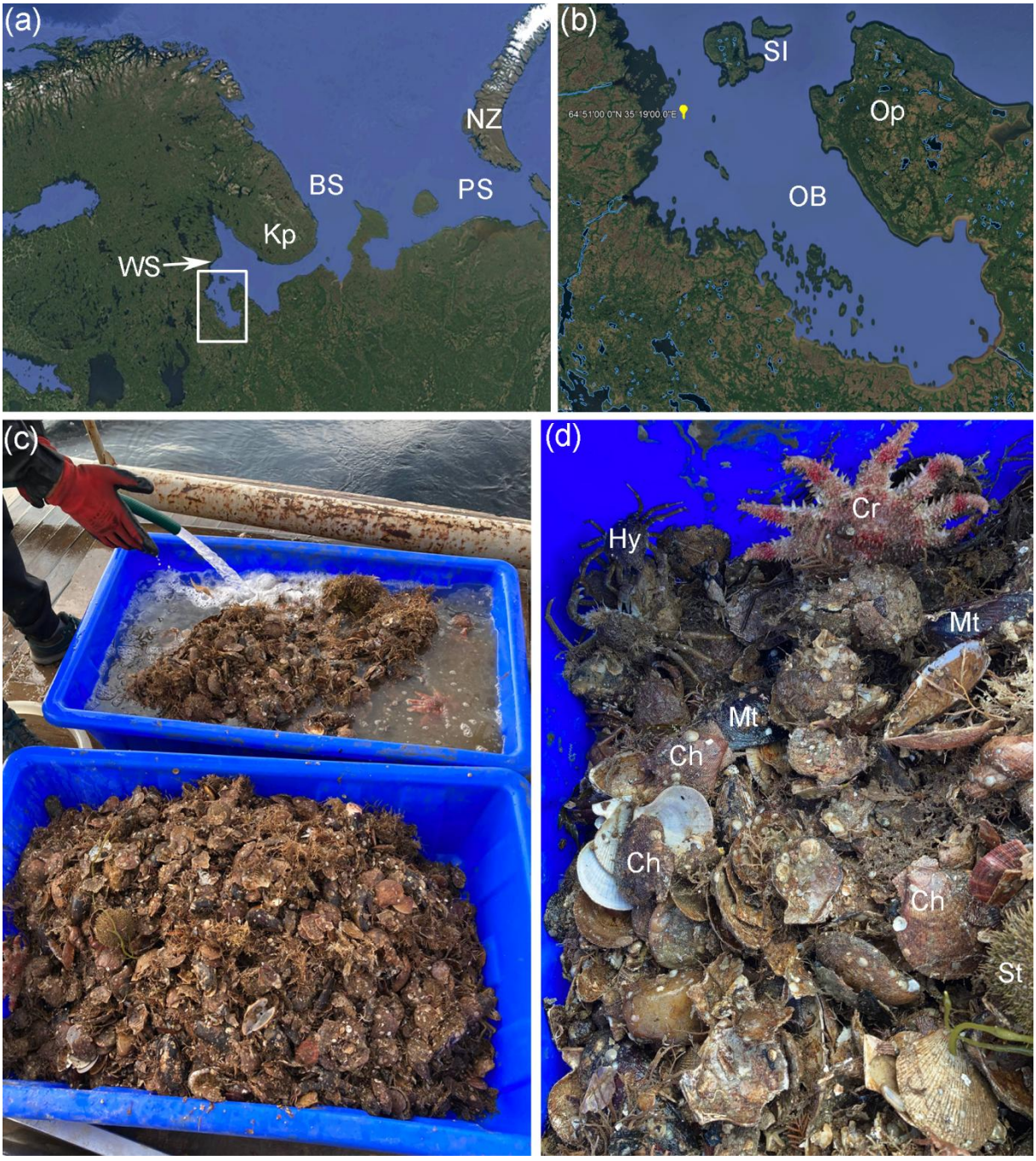


Fig. 1. The area and collection of the material: bivalve shells with *Phoronis ovalis*. (a) General map: the rectangle indicates the area of material collection. (b) The Onega Bay: yellow pin indicates the certain point of the material collection. (c) Trawl collection in two large tanks; sea water is added into the tanks. Courtesy by A. Kokorin. (d) Certain content of the trawl collection. Courtesy by A. Kokorin. Abbreviations: Bs, the Barents Sea; Ch, valve of *Chlamys islandica*; Cr, *Crossaster* sp.; Hy, *Hyas* sp.; KP, Kola peninsula; Mt, valve of *Mytilus edulus*; NZ, Novaya Zemlya; OB, Onega Bay; Op, Onega peninsula; PS, the Pechora Sea; SI, Solovetsky Islands; St, *Strongylocentrotus drobachiensis*; WS, the White Sea.

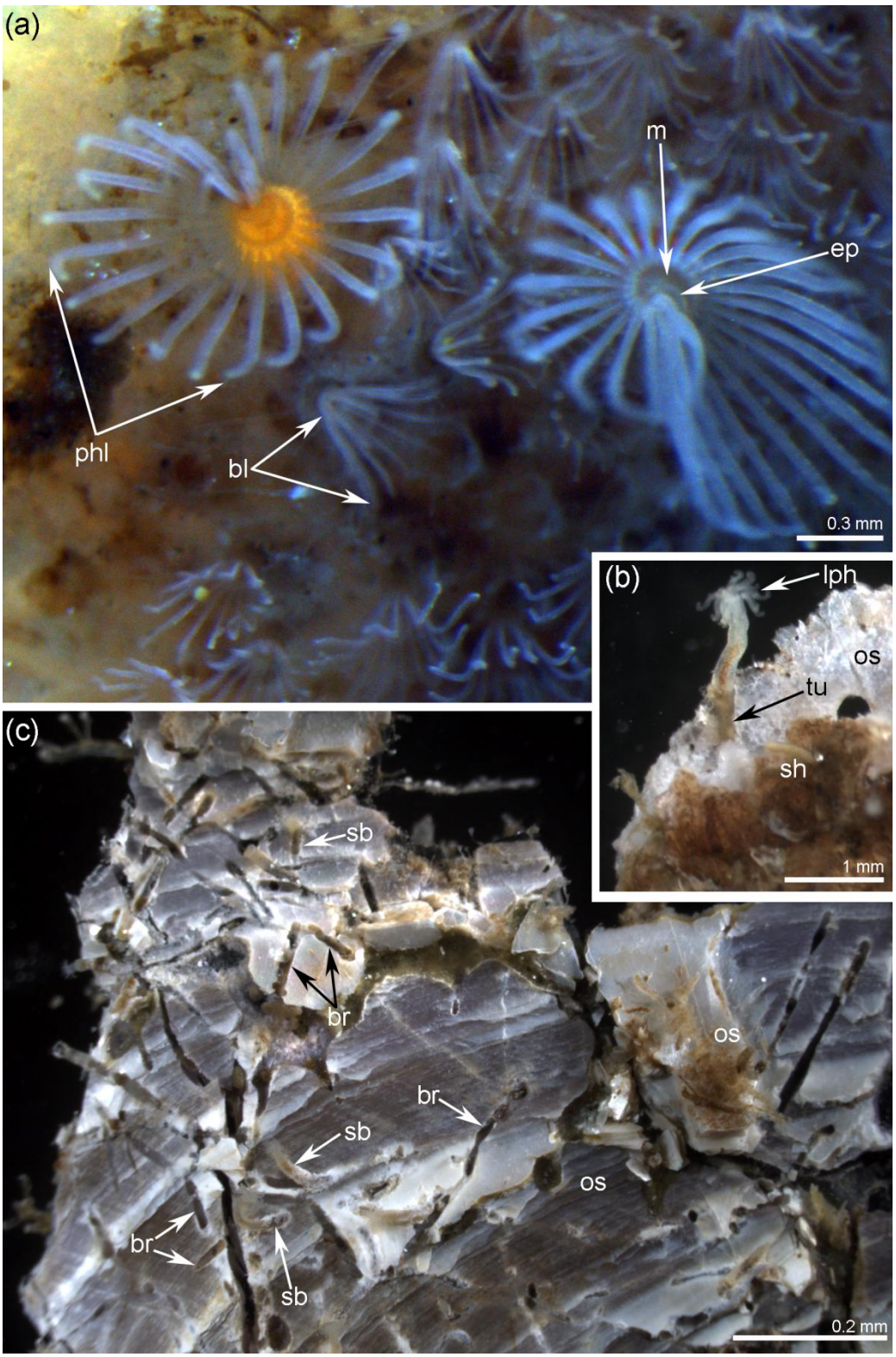


Fig. 2. *Phoronis ovalis* in the mollusks shell; live animals. (a) Two lophophores of *P.ovalis* and many small lophophores of a cheilostome bryozoa. (b) Portion of the soft body extruded from the shell. (c) Crashed valve of mollusk with numerous phoronid burrows inside. Abbreviations: bl, bryozoa lophophore; br, phoronid burrow; ep, epistome; lph, lophophore; m, mouth; phl, phoronid lophophore; os, shell ostracum; sb, soft body; sh, shell; tu, tube.

Histology and electron microscopy. For histology and electron microscopy, adult animals and part of shells with phoronids inside were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer for 8 hours at 4˚C. Animals were then washed several times in 0.1 M cacodylate buffer and postfixed in 1% osmium tetroxide in the same buffer.

Then, for histology and transmission electron microscopy (TEM), the specimens were dehydrated in ethanol followed by an isopropanol series and were then embedded in EMBed-812. Semithin (500 nm) and ultrathin (30 nm) sections were prepared with a Leica UC7 ultramicrotome (Leica Microsystems GmbH, Wetzlar, Germany). Semithin sections of the animals in tubes were stained with methylene blue and acid fuchsine, observed with Olympus BX51 microscope (Olympus), and photographed using a Toupcam camera (ToupTek Photonics Co. Ltd.). Ultrathin sections were prepared and placed on grids. These sections were then stained with 1% uranyl acetate and 2% lead citrate, and examined using a JEM-1011 transmission electron microscope (JEOL, Akishima, Japan). For study of ultrastructure, the upper parts of the bodies were used, because the epithelium and the tube in this part are usually intact (undamaged).

For scanning electron microscopy (SEM), adult animals and parts of shells were dehydrated in ethanol and an acetone series, critical point dried, mount of the stubs, plated with platinum-palladium. Parts of shells were crashed before the mount on stubs. The specimens were examined on a JEOL JSM-6380L scanning electron microscope (JEOL Ltd., Tokyo, Japan).

Computer microtomography (µ-CT) and 3D reconstruction. After postfixation in 1% osmium tetroxide, **t**hree parts of mollusk shells with phoronid inside were rinsed in distilled water, dehydrated in a series of ethanol followed by acetone, and then critical point dried. µ-CT was performed using an X-ray tomograph SkyScan 1272 (Bruker Corporation). The source current was 145 µA, the source voltage was 39 kV, and the isotropic voxel size was 0.5 µm. The 360° rotation and frame averaging modes were on. The image stacks were created using the NRecon software (Bruker Corporation). Z-projections were done using CTVox micro-CT Volume Rendering Software (Bruker, Billerica, USA); 3D reconstructions were prepared in Amira ver.5.2.2.

Cytochemistry and confocal laser scanning microscopy (CLSM). For examination of chemical composition of the tube, five specimens in tubes and three empty tubes were used. Live animals were relaxed in the isotonic MgCl2 solution for 10 minutes at room temperature, fixed in 4 % paraformaldehyde diluted in 0.1 M PBS (phosphate saline buffer, pH 7.2–7.4) for 8h at a temperature of 4–5C, washed three times for 10 min in the same buffer. Then the specimens were exposed in PBT (0.1M

PBS containing 1% Triton X-100 [Sigma–Aldrich, St. Louis, USA]) and stained for 2h at 4–5C with phalloidin (AlexaFluor 488 phalloidin – Molecular Probes, Eugene, OR) (diluted 1:50 in PBT) for detecting actin filaments in muscles and Calcofluor White (Fluorescent Brightener 28, F3543, Sigma–Aldrich, St. Louis, USA) (diluted 1:1000 in PBT) to visualize chitin. The use of Calcofluor White for visualization of chitinous structures in invertebrates is done in many studies of molluscs radula and jaws in adults and larvae (Vortsepneva, 2020; Vortsepneva et al., 2023; Mikhlina et al., 2024), as well as in some phoronids (Temereva, Sokolova, 2025). After staining, the animals were rinsed in PBS for 3 × 5 min and mounted in glycerol. The samples were analyzed using Nikon Eclipse Ti confocal microscope (Nikon, ThermoFisher Scientific, Waltham, MA). Z-projections were prepared in ImageJ version 1.43.

Results

Morphology of the soft body

The body is subdivided into the trunk and the lophophore, specific organ, which bears 24-26 ciliated tentacles (Fig. 2a, b). The lophophore is exposed into the water and collects food particles. The trunk is submerged in to the thickness of mollusk ostracum, which is penetrated by numerous phoronid burrows (Fig. 2c). These burrows are especially numerous along the edge of the shells, where the ostracum is thinner than in the other parts. The phoronid trunk is subdivided into several parts, which differ from each other in density of the integument and coloration. There are anterior trunk region, which has relatively dense opaque integument that bears many large white spots (Fig. 3a, b). The posterior trunk region has thin transparent integument, which about lacks the white spots. The terminal part of the body is usually swollen and forms the ampulla; it is a bulb, which is able to change in diameter (Fig. 3a, d). In many individuals, the ampulla is not well pronounced.

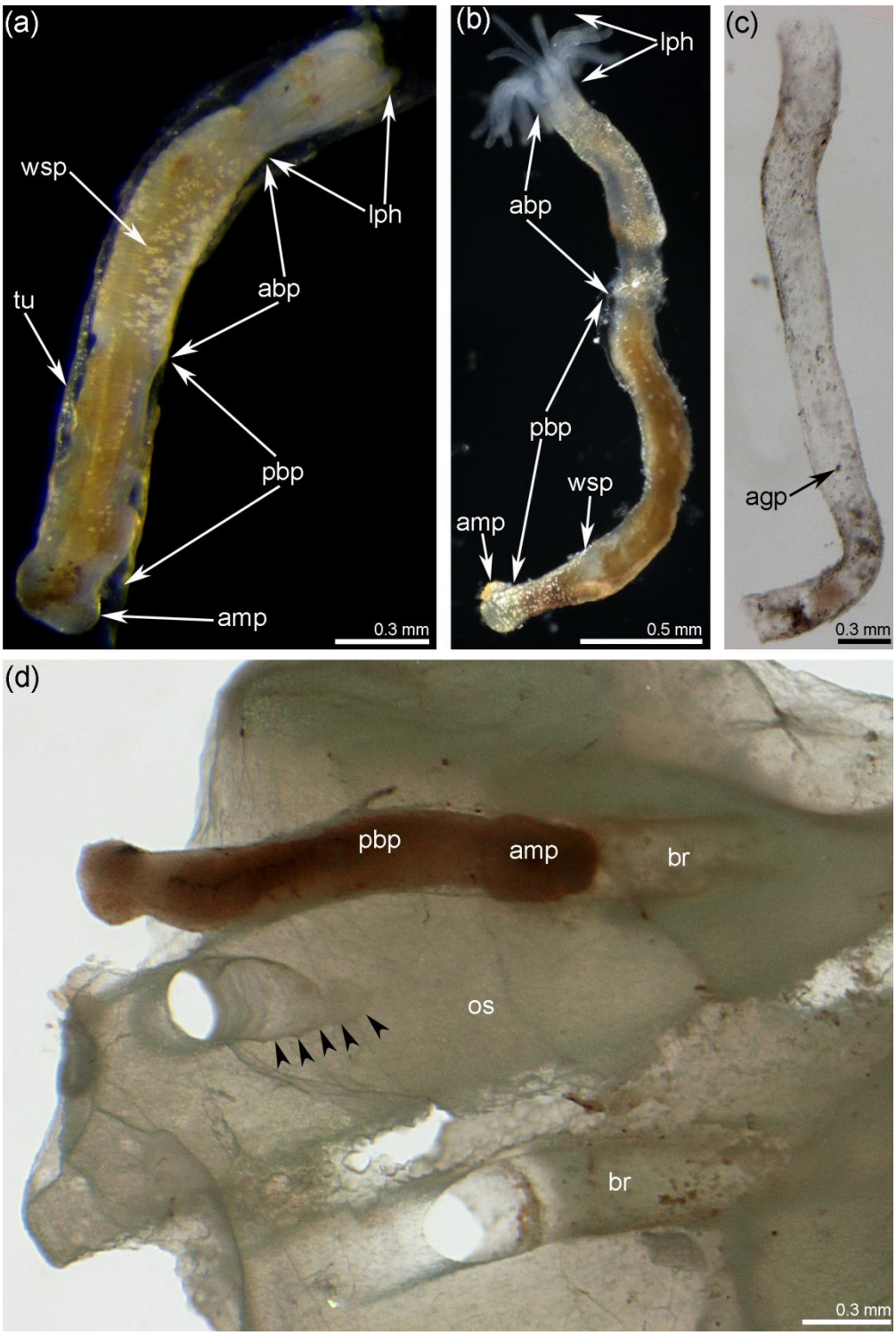


Fig. 3. *Phoronis ovalis*: soft body and tube; light microscopy. (a) Live specimen in the tube. (b) Live specimen without tube. (c) Empty tube. (d) Several phoronid burrows (some with the phoronid soft body) in shell of *Chlamys islandica*: arrowheads point the distal portion of the burrow, which terminates in the ostracum. Abbreviations: agp, agglutinated particle; amp, ampulla; abp, anterior body part; br, burrow; lph, lophophore; os, shell ostracum; pbp, posterior body part; wsp, white spots in the epithelium.

Structure of the burrows and tubes

The burrows, which *P. ovalis* drills in the shell, extend in the ostracum and have fanciful shape (Figs 4a, B; 5a, b). Each burrow forms several branches, which extend in different directions (Figs 4a; 5b). Some burrows have complicated architecture and include several parts, which extend parallel to each other (Fig. 4a). Most burrows connect each other and together form complex net inside the shell. However, some burrows do not communicate with the others. Each burrow has several openings, which are located on both outer (Fig. 4b) and inner (Fig. 4c) sides of the shell. The inner side of the shell is usually incrusted by different sedentary animals such as bryozoan colonies, sponges, and others. The openings of *P. ovalis* burrows are evidently located exactly inside the bryozoan colony (Fig. 4c). The length of burrows terminates by the shell diameter (about 6 cm); the burrow diameter varies in its different parts, but never exceeds 250 nm.

Accordingly to SEM data, the terminal end of burrow is similar to the ampulla of the soft body in shape and size (Fig. 5a). The terminal end of the burrow exhibits annulate microstricture (Fig. 5c). The edges of the burrows look the darkest in comparison with the lumen in tomographic photographs (Fig. 4d, e). The calcis material of the shell is structurally modified through 45-47 μm in thickness from the burrow lumen (Fig. 4d). Moreover, the thin dark line underlines the lumen of the burrows (Fig. 4d). This line is probably corresponds to the chitinous tube (see below). In SEM photographs, the edges of the burrows look lighter in comparison with the intact shell structures (Fig. 5d). The edges of the burrows consist of many tiny spines, whereas intact shell is formed by laminae (Fig. 5e).

The burrows are lined by the tube, which is transparent and agglutinated by some mineral particles (Fig. 3c). The tube has outer and inner surfaces: the outer contact the burrow wall, whereas the inner is faced to the soft body (Fig. 6a). The outer surface of the tube is usually covered by numerous spines, which are incorporated into the tube from the edge of the burrow (Fig. 6b). The outer surface of the tube is also covered by numerous bacteria: these are rod-like cells, which are about 3μ in length and 340 nm in diameter (Fig. 5f). Some rod-like bacteria are slightly curved. Tube is multilayered and, accordingly to SEM, bacteria are also present between layers and in the innermost layer, which is faced to the soft body (Fig. 6c). On the surface of the innermost layer roundish bodies with diameter about 0.6μ are located. The similar roundish bodies can be observed on the surface of the body integument, which is composed of nonciliated cells that bear numerous short microvilli (Fig. 6d). The innermost layer consists of thick net of fiber, which diameter varies from 60 to 70 nm (Fig. 6e). In other layers, such net cannot be

observed: they have a solid structure with permanent hollows of different shape and diameter (Fig. 6f).

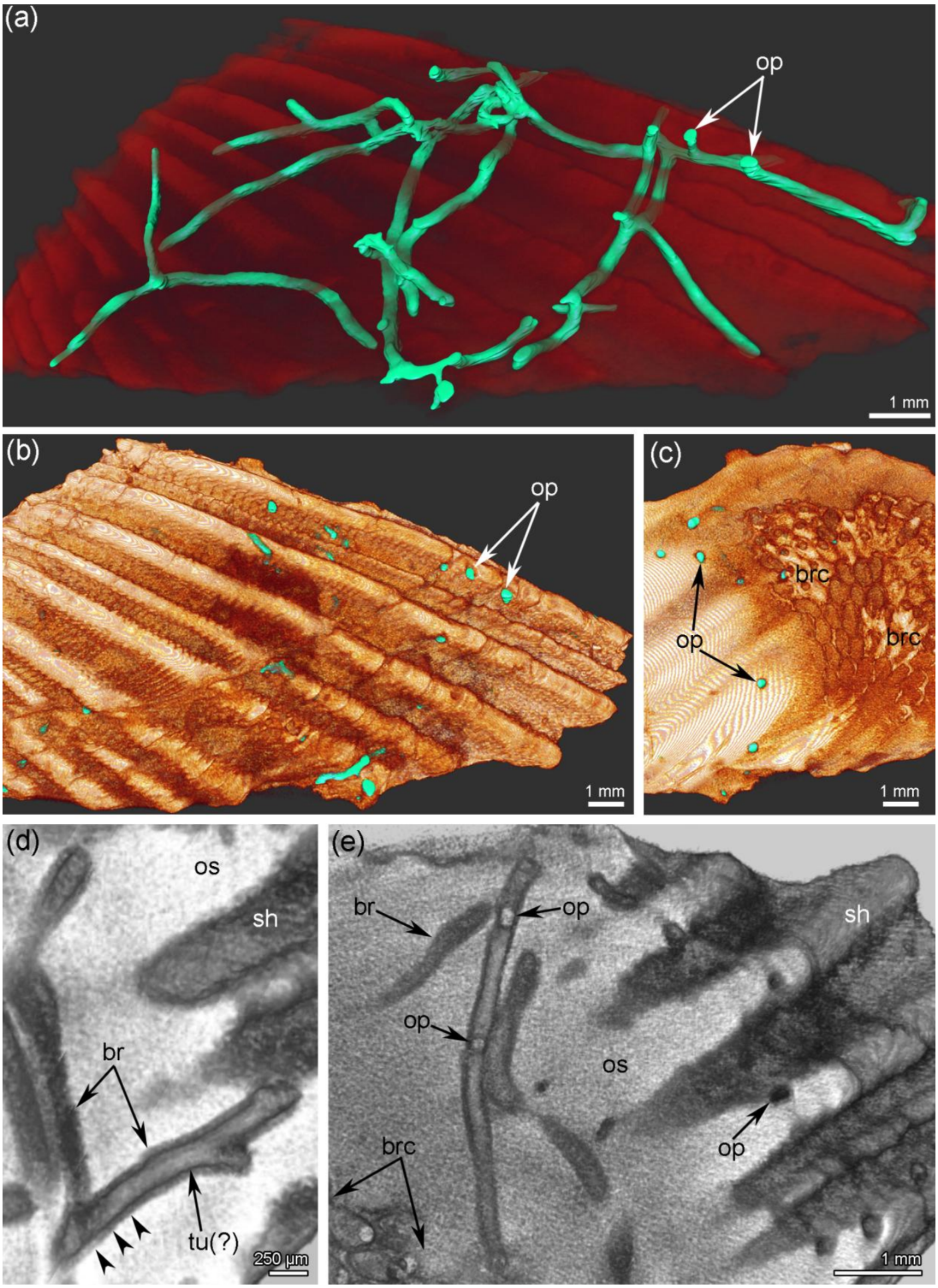


Fig. 4. Organization of *Phoronis ovalis* burrows; µ-CT. (a) 3D reconstruction of burrows (green) in the shell (red). (b) Volume rendering of outer surface of the shell with the openings (green) of phoronid burrows. (c) Volume rendering of inner surface of the shell with the openings (green) of phoronid burrows. (d) Optical section of the shell with burrows inside: heterogeneous structure of the burrow is evident. Arrowheads point the electron-dense edges of the burrow. (e) Optical longitudinal section of the burrows: several openings in one burrow are evident.

Abbreviations: br, burrow; brc, bryozoan colony; op, opening; os, shell ostracum of shell; sh, shell; tu(?), electon-dense layer of the burrow that probably corresponds to the tube.

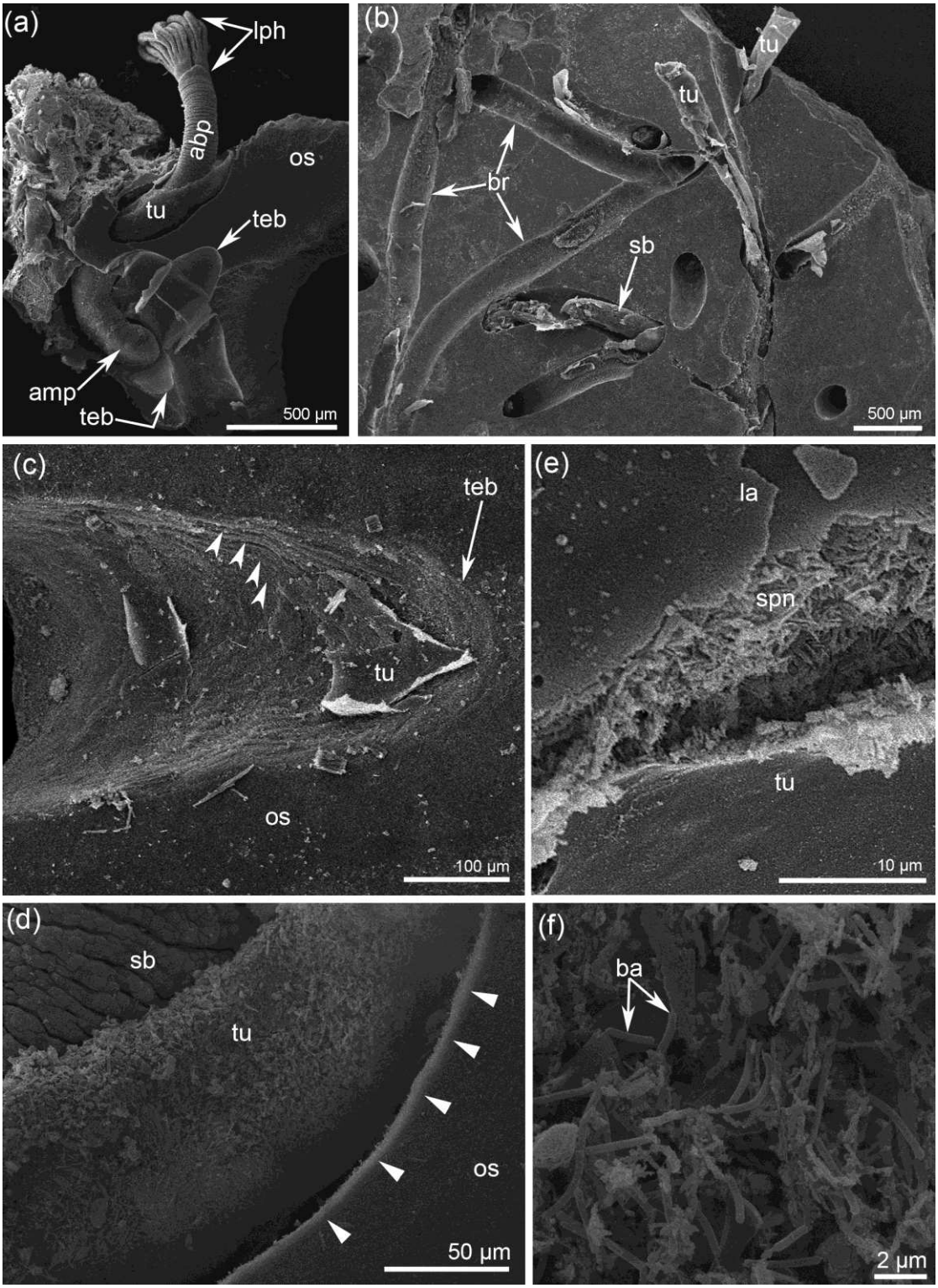


Fig. 5. Fine morphology of *Phoronis ovalis* burrows; SEM. (a) Whole animal inside the piece of the mollusk shell. (b) Piece of mollusk shell with numerous branched phoronid burrows. (c) The terminal end of the phoronid burrow: annulated structure is visible. Annuli are pointed by concaved arrowheads. (d) A portion of the burrow with tube and animal inside: light edge of the burrow is pointed by straight arrowheads. (e) The edge of the burrow and tube. (f) Numerous

bacteria between tube and burrow wall. Abbreviations: abp, anterior body part; ba, bacteria; br, burrow; la, lamella of shell ostracum; lph, lophophore; os, shell ostracum; sb, soft body; spn, spines of calcic material; teb, terminal end of tube; tu, tube.

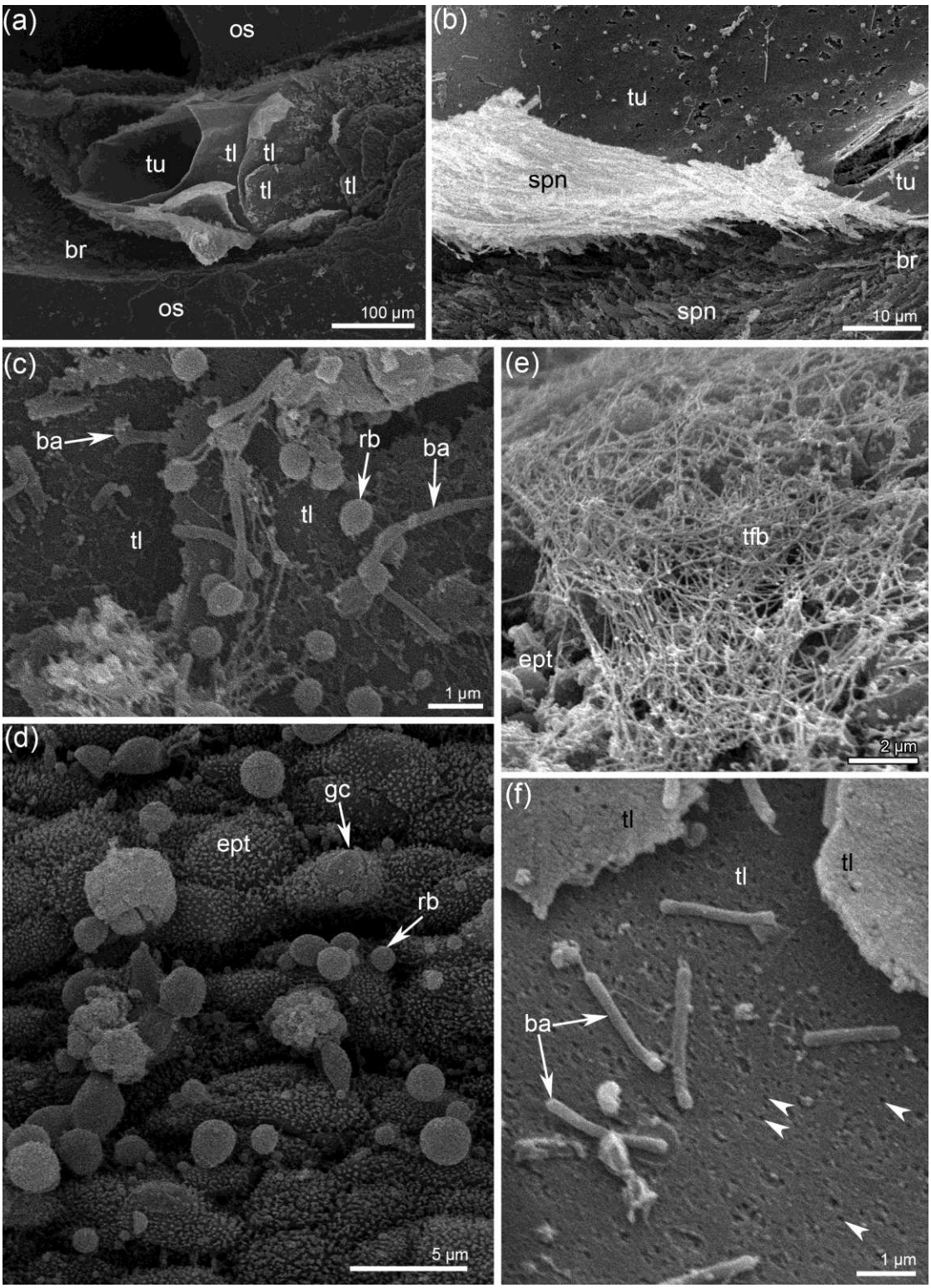


Fig. 6. Fine structure of the tube of *Phoronis ovalis;* SEM. (a) Empty tube inside the burrow. (b) The outermost layer of the tube, which faces to the burrow wall. (c) Two middle layers of the tube. (d) The surface of the body epithelium. (e) The innermost layer of the tube: numerous fibers are visible. (f) One of the tube layer (film) with the permanent hollows of different shape

and diameter (arrowheads). Abbreviations: ba, bacteria; br, burrow; ept, body wall epithelium; gc, glandular cell; os, shell ostracum; rb, roundish body; spn, spines of calcic material; tfb, tube fibers; tl, tube layer (film); tu, tube.

Accordingly to histology and TEM, the tube consists of many layers, which differ from each other in thickness, density, and fine structure (Fig. 7a-c). Layers can be separated from each other that influences on the thickness of the tube. In places, where all layers tightly connect each other, the entire thickness of the tube is about 6.5 μ (Fig. 7a). However, in most cases, the thickness of the tube is bigger and can reach up 14-15μ (Fig. 7a, b) and 19μ (Fig. 7c). Double staining with methylene blue and acid fuchsine revealed that the tube includes both basophilic and acidophilic components (Fig. 7a). The outermost layers exhibit much acidophilic reaction in comparison with mostly basophilic innermost layers. The epithelium of the upper part of the body is glandular and contains many intraepidermal glands (Fig. 7a, b). The epithelium consists of columnar cells with height about 20-24 μ. The most abundant glandular cells are spindle-like cells with narrow basal and apical parts and expanded middle part, which contains granules of secretion. The apical parts are slightly extruded above the epithelium surface (Fig. 7a). The cytoplasm of glandular cells is filled with numerous granules of different diameters (2.2–2.7 μ), which has heterogeneous content: in electron dense material several roundish inclusions are incorporated (Fig. 7b). There are electron light border between electron dense content and inclusions.

The analysis of TEM data revealed that the fine organization of the tube lacks certain structure along its entire length. The common characteristic of all parts of the tube is the presence of thin layers of high electron density and thick layers, which are formed by amorphous material of middle electron density (Fig. 7b, c). The innermost layer is always formed by flocculent and fibrillar material. Bacteria of different diameter are located between the tube and the body, between the tube layers, and along the outer surface of the outermost layer. Bacteria of three size-groups are found in all studied animals. Bacteria of large diameter size (800–900 nm) are located in the innermost and outermost layers (Fig. 7b-d). Bacteria of middle diameter size (340–400 nm) are the most numerous and usually distributed between layers (Fig. 7c, e), but are also abundant on the outermost surface of the tube (Fig. 6f). Bacteria of small diameter size (200–230 nm) are observed in the innermost layer and between layers (Fig. 7c, f). All bacteria have two cell walls and therefore they can be regarded as gram-negative. Together with bacteria, the membranous vesicles of different diameter and remnants of cilia are located between tube layers (Fig. 7c).

293 The results of exposing animals and tubes to Calcofluor White revealed the presence of chitin in
294 the tube and in the epithelial cells (Fig. 8). The epithelium of the anterior trunk region is
295 relatively thicker and is dyed by Calcofluor White more intensively in comparison with the
296 thinner epithelium of the posterior trunk region (Fig. 8a). The innermost layer of the tube, which
297 contacts the body epithelium, exhibits relatively strong coloration with Calcofluor White (Fig.
298 8b, c). Here, the chitinous material appears granular (Fig. 8b). Not all tube layers contain
299 Calcofluor White: the outermost and some middle layers seem to lack coloration (Fig. 8d).
300 However, conglomerates of amorphous dyed material are associated with these layers as well
301 (Fig. 8d). Numerous dense inclusions are visible between layers. High resolution allowed us to
302 visualize chitinous granules in the apical cytoplasm of epidermal cells (Fig. 8e). In some parts, the
303 tube looks like entire solid layer (Fig. 8e), whereas some other parts are evidently laminated and the
304 outermost layer forms some folds (Fig. 8f).

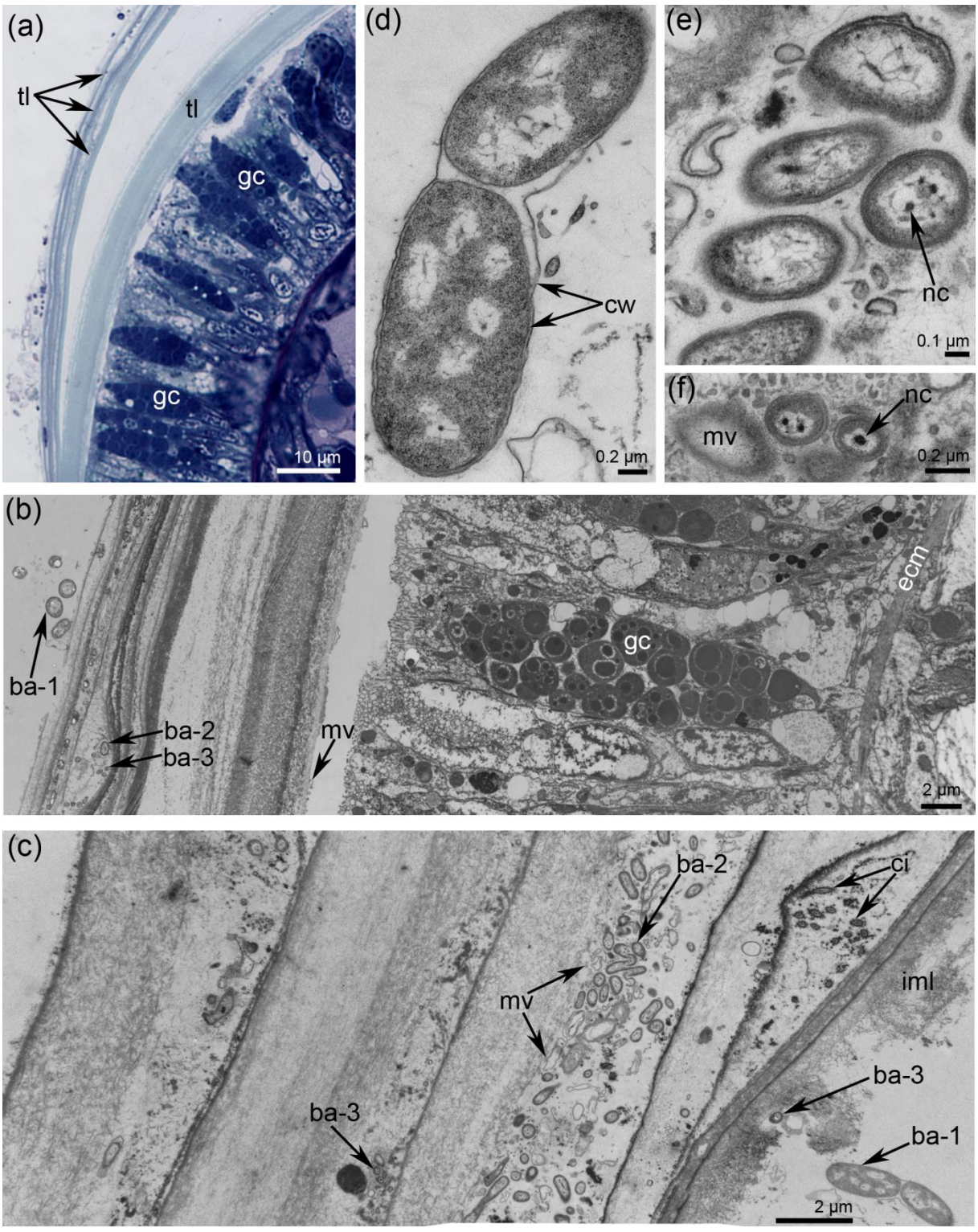


Fig. 7. Ultrastructure of *Phoronis ovalis* tubes. (a) A portion of the body and tube; semithin section. (b) A portion of the body and tube; ultrathin section. (c) A portion of the tube; outermost layer on the left. (d) Bacteria of large diameter (ba-1); ultrathin section. (e) Bacteria of middle size (ba-2); ultrathin section. (f) Bacteria of small size (ba-3); ultrathin section. Abbreviations: ci, cilia; cw, cell wall; ecm, extracellular matrix; iml, innermost layer; gc, glandular cell; mv, membranous vesicle; nc, nucleoid; tl, tube layer

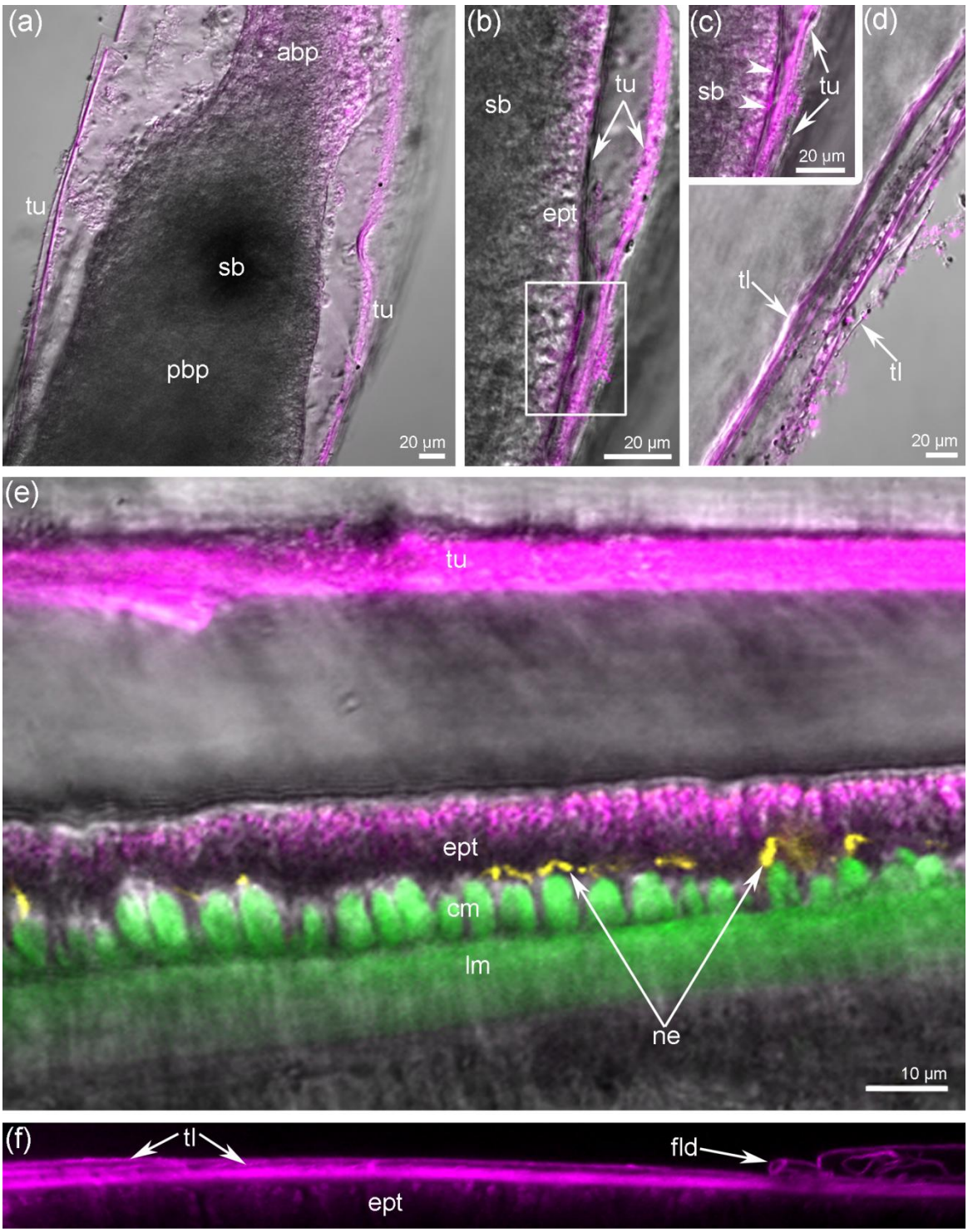


Fig. 8. Organization of *Phoronis ovalis* tubes accordingly to CLSM data with Calcofluor White (magenta) staining; combination of Z-projection and optical section of soft body. (a) A portion of the animal soft body with tube. (b) Magnified portion of the animal soft body with tube. The rectangle indicates figure 8c. (c) Magnified portion of the body wall epithelium and tube. Arrowheads point the secretion of the tube innermost layer. (d) A portion of the tube: separated layers are evident. (e) A portion of the body wall and tube. (f) A portion of the tube: thin layers and folds of outermost layer are evident. Abbreviations: abp, anterior body part; cm, transverse musculature; ept, epithelium; fld, folds of outermost tube layer; lm, longitudinal musculature; ne, neurites; pbp, posterior body part; sb, soft body; tl, tube layer; tu, tube.

Discussion

Soft body and burrow structure

Phoronis ovalis is one of the smallest phoronid species: accordingly to some data the body length is not more than 15 mm (Emig, 1979). However, our results evidence that mature adults of this species from the White Sea are mostly about 3-5 mm in length (Temereva, 2017, 2019; Alexandrova, Temereva, 2026; Temereva, Yurchenko, 2026). The body division of *P. ovalis* is typical for other phoronids (for summary see Temereva, 2024); however, the ampulla is not well pronounced and in some *P. ovalis* specimens cannot be certainly distinguished (Temereva, 2017, 2019). On the other hand, the terminal part of the body (the ampulla) possesses the thick epithelium, which contains acidophilic and basophilic glandular cells (Marcus, 1949), and typical for other phoronids inversion of the muscular layers: the circular muscles extend above the longitudinal muscles (Temereva, 2019). Thus, although the ampulla of *P. ovalis* is not expressed morphologically, the terminal part of the trunk exhibits anatomical features, which are typical for ampulla of other phoronids. Moreover, exactly the ampulla, apparently, supplies the formation of the burrow. This suggestion can be supported by specific micro sculpture of the terminal end of the *P. ovalis* burrow: it is annulated and the annuli may be formed due to gradual dissolving of the calcic material of the shell. Similar concentric lines are typical in the inner surface of burrows in different species of boring annelids, e.g. *Polydora* (Sato-Okoshi, Okoshi, 1996, 2000). The dissolving of the calcitic material leads to the changes of structure of the host shell: the lamellar micro structure turns into spines. Such transformation of the shell microstructure has been established for several annelid species of *Polydora* and *Dipolydora*, which burrow different mollusc shells consisting of different types of microstructure (foliated, nacreous, prismatic, and crossed lamellar) (Zottoli, Carriker, 1974a, 1974b; Sato-Okoshi, Okoshi, 2000).

The exact mechanism of boring has not been fully elucidated. Many invertebrates from different phyla are able to make burrows in calcic substrates: stones, mollusk shells, crustacean carapaces, etc. The ability to burrow into calcic substrates apparently arose independently in different groups and, moreover, in different species within a single large clade (Radashevsky, 2012, 2022). Boring organisms are known among Porifera, Crustacea, Annelida (including Sipunculida and Echiurida), Gastropoda, Bivalvia, Bryozoa, and Phoronida. In general, the burrowing mechanism combines several actions: (1) mechanical impact, (2) production of inorganic acids, and (3) production of chelating agents (Carriker, Williams, 1978). Some animals use all of these processes for boring, whereas others use a combination of only one or two. Thus, the most studied invasive annelid, *Polydora*, is traditionally regarded as using both mechanical and

chemical actions; however, experiments involving the removal of chaetae revealed the prevalence of predominantly chemical erosion of the host shell, in which the large specialized chaetae are not involved (Haigler, 1969). Phoronids do not have any hard structures that would be able to destroy a calcic substratum mechanically. Therefore, phoronids are able to use only the chemical mechanism of substratum boring, in combination with active movements of the ampulla.

Among phoronids, several boring species are known to inhabit gastropod and bivalve shells, crustacean shelters, and rocky substrates; these include *P. ovalis*, *P. ijimai*, and *P. hippocrepia* (Emig, 1982). The precise mechanisms underlying their boring activity remain to be elucidated. Notably, *P. hippocrepia* is capable of inhabiting soft sediments (sand and sand-silt mixtures) and of burrowing into calcified substrata (Emig, 1982). This dual capacity suggests that the physiological and morphological mechanisms involved in boring and digging may be functionally similar in phoronids. However, this hypothesis requires empirical validation; in particular, a comparative histological analysis of the body epithelium should be conducted in both boring and digging specimens of *P. hippocrepia*. Alternatively, the boring and digging morphotypes of *P. hippocrepia* might represent distinct species exhibiting differential capacities for substratum exploration.

The morphology of burrows constructed by phoronids in hard substrata has not yet been systematically investigated. According to our new data, most burrows are interconnected and feature multiple openings on opposite sides of the shell. This architectural pattern may reflect the influence of asexual reproduction, which is well documented in *P. ovalis* (Marcus, 1949), wherein newly formed individuals excavate their own burrows. Moreover, the network of interconnected burrows may facilitate communication among individuals: within this complex burrow system, gametes (oocytes and sperm) are probably released (Temereva and Yurchenko, 2026). The presence of several openings located on different sides of the shell enables the lophophore to be extended into the water column irrespective of the shell's orientation. This feature is particularly significant given that *P. ovalis* predominantly colonizes empty bivalve shells.

The ability to burrow the calcitic substratum makes the boring organisms very important components of marine ecosystems, because they create the specific environment for many other organisms, promote the recycling of nutrients, and form the sediment deposit (Mokievsky, 2021; Turicchia et al., 2022). Moreover, some boring organisms (sponges, annelids, phoronids), which prefer to live in shells of marketable mollusks, spoil the appearance of these mollusks and even leads to their death (Podolskaya et al., 2025). Thus, boring organisms, in general, and boring

phoroniods, in particular, play great fundamental and practical role in ecosystems and in human economical activity.

Structure of the tubes

Regardless the kind of substratum all phoronids produce the tube (Temereva et al., 2020). The structure of the tube – size, shape, and thickness – depends on the soft body characteristics and on type of substratum where phoronids live. In contrast with annelids, the structure of phoronid tubes does not have species-specific impact: specimens of one species, which live in different substrata, have different structure of the tube. For example, in Vostok Bay, the Sea of Japan, *Phoronopsis harmeri* form two populations: specimens from the silt have relatively thin and flexible tubes, whereas specimens from the sand have straight and rigid tubes (Neklyudov, Temereva, 2027).

Usually, phoronids possess tubes that are wider at the distal end compared to the proximal end (Temereva et al., 2020). Furthermore, in some species, the distal end of the tube is closed (Temereva et al., 2020; Temereva, 2025). This morphological arrangement raises the question of how the tubes accommodate the growth of the soft body. It has been suggested that phoronids are able to dissolve the posterior portion of the tube, allowing the soft body to extend deeper into the substratum (Temereva et al., 2020). Notably, the most distal region of the body – the ampulla – bears specific intraepidermal glands that are absent in other body regions and likely play a crucial role in the dissolution of the tube (Temereva et al., 2001; Temereva, 2024). In *P. ovalis* and probably in other boring phoronids, the tube remains open at the distal end, with the tube space being limited only by the burrow. I cautiously propose that similar (or possibly identical) intraepidermal glands are involved in dissolving the tube at its distal end in digging phoronids and in degrading the organic material of the mollusc shell in boring phoronids. The degradation of the organic matrix represents a key initial step in the boring process (Ehrlich et al., 2009). Overall, the capacity of phoronids to manipulate both the organic matrix and calcified material likely reflects a common trait among lophophorates, most of which are capable of biomineralisation (Yang et al., 2026).

The chemical structure of phoronid tubes has never been scientifically investigated: in some studies the presence of chitin in the phoronid tubes has been declared (Hyman, 1958; Santagata, 2002). Accordingly to our experiment with the Calcofluor White, chitin presents in epidermal cells and in layers of the tube *P. ovalis*. The presence of chitin can explain the acidophilic reaction of the tube in histological sections. Structurally the tube consists of numerous fibers, which are produced by epidermal glands. The diameter of fibers probably depends on diameter

of glandular cell opening and varies in wide diapason in phoronids: from 220 nm to 40 nm (Temereva et al., 2020). Interesting, in most phoronids, the diameter of chitinous fibers is in twice smaller than that in most annelids. Accordingly to literature data, so called non-acidic mucus cells (NAM-I) play the main role in the tube secretion (Fernández et al., 1991; Aguirre et al., 1993; Temereva, 2024). These cells contain large granules with heterogeneous structure of the secret: they contain several electron-dense cores (Fernández et al., 1991; Aguirre et al., 1993). Cells of similar ultrastructure are described in *P. ovalis* and are probably involved into the tube secretion.

Bacteria

The presence of bacteria associated with phoronids is mentioned in many papers: bacteria were found in phoronid cuticule (Temereva et al., 2001; Temereva, 2024), in the epithelium of lophophoral organs (Temereva, Malakhov, 2006), in phoronid larvae (Temereva, 2017b), and in the phoronid tubes (Temereva et al., 2020). Usually, bacteria are numerous in the cuticule and between layers of the tubes, formed by phoronids (Temereva et al., 2020) in contrast with annelids, which usually do not contain bacteria inside the tube: for some of annelids, the presence of antibacterial peptides in the secretion of epidermal glands has been documented (Panteleev et al., 2018).

The microbiomes of *Phoronis vancouverensis* and *Phoronopsis harmeri* has been studied by molecular methods and revealed the presence of bacteria from many different groups (Holt et al., 2023; Ettinger, Eisen, 2024). Structure of microbiome of *P. vancouverensis* differs from that of substratum and surrounding water; in additional, the microbiome changes during several months (Holt et al., 2023). The difference in microbiome of the tubes and sediment has also been described in annelid *Terebellides stroemii* and probably is typical for animals, which live in soft sediments (Tzetlin at el., 2023). The function of phoronid microbiome is not evident. Accordingly to some data, phoronids are able defense against local epibenthic predators (Larson, Stachowicz, 2009): probably this defense is supplied by bacterial activity. Many of bacteria from phoronid microbiomes are involved into sulfur cycle: the microbiome of *P. harmeri* is dominated by bacteria from the orders Campylobacterales, Desulfobulbales, and Desulfobacterales (Ettinger, Eisen, 2024). Because most of sulfate‑reducing bacteria are Gramm-negative, I can suppose that in P. ovalis tube exactly such kind of bacteria are present. Metagenomic analysis has revealed that the community contains genes necessary for various stages of the sulfur cycle: some strains are capable of sulfur oxidation; others can oxidize sulfite and reduce sulfate and sulfite; yet others can reduce sulfite. For the phoronid, this may be beneficial: such reactions help

process organic debris in or around the tube, making certain substances more accessible to the host (e.g., in a form suitable for uptake). In marine ecosystems, the sulfur cycle is critically important, and the microbiome acts as an additional metabolic "workshop" for the host. Thus, the absorption of nutrients has been suggested for *Phoronis embryolabi* epithelium in zone, where the Gramm-negative bacteria of large diameter have been observed (Temereva, 2024). For instance, the degree of integration between the host's and the microbial metabolic pathways remains incompletely elucidated, including whether direct metabolite exchange occurs. Furthermore, the regulatory mechanisms governing community composition and the specific molecular mediators of protective functions are not yet fully defined.

Conclusion

This study advances the understanding of key biological traits in *Phoronis ovalis*, focusing on body morphology, burrowing behaviour, tube organization, and host–microbiome interactions. Morphologically, the terminal body region of *P. ovalis* retains anatomical features typical of the ampulla in other phoronids despite its reduced prominence, indicating a conserved functional role of the ampulla across the group. The observed network of interconnected burrows with multiple openings reflects links between reproductive strategies (including asexual reproduction) and ecological adaptation, enabling flexible lophophore exposure and potentially supporting communication and gamete release. *P. ovalis* in particular and all phoronids in general appears to rely primarily on chemical erosion rather than mechanical action for boring into calcic substrates, likely mediated by glandular secretions. This mechanism may share a common physiological basis with the process of tube remodeling, representing a broader adaptation among lophophorates. Structure of tubes, formed by phoronids of different ecological groups, shows high environmental plasticity: it varies with the substratum rather than being strictly species-specific, highlighting the adaptive value of this trait. A stable association between phoronids and their microbiome is evident, with microbial communities enriched in taxa involved in the sulfur cycle. These microbes likely benefit the host by processing organic matter and may contribute to predator defense, though the extent of metabolic integration between host and microbiome remains unclear. Boring phoronids significantly influence marine ecosystems by modifying habitats, promoting nutrient cycling, and affecting sediment dynamics; some species also have economic relevance due to their impact on commercial molluscs. Future research should target the biochemical basis of boring and tube remodelling, comparative histology across

ecological morphotypes, and the molecular underpinnings of phoronid–microbiome integration to further elucidate the ecological role of these organisms.

Acknowledgements

The author expresses her gratitude to all the staff of the White Sea Biological Station of Moscow State University for collecting animals and the opportunity to work with them in the laboratory. My special thanks are devoted to the crew of ship “Akademik Zenkevitch”, as well as to Alexander Kokorin and Polina Belova. The study was conducted under the state assignment of Lomonosov Moscow State University.

Fubding

The study was done with support from the Russian Science Foundation (#23-14-00020-P).

Author Contributions Statement

N/A

Competing interests

The author declares that she has no competing interests.

Data availability statement

The data sets analyzed during this study are available from the author upon request.

Ethics statement

The field sampling did not involve endangered or protected species. The use of phoronids in the laboratory does not raise any ethical issues.

References:

Aguirre, A., Fernandez, F. P., Roldan, C., & Benito, J. (1993). Further ultrastructural observation on the epidermis of phoronids: *Phoronis australis* and *Phoronis hippocrepia*. Transactions of the American Microscopical Society, 112(3), 280–291.

Alexandrova, Y., & Temereva, E. (2026). Oogenesis in *Phoronis ovalis*: First description of Balbiani bodies, non-follicular oogenesis, and yolk heterosynthesis in phoronids. Tissue and Cell, 104(2), 103860.

Carriker, M. R., & Williams, L. G. (1978). The chemical mechanism of shell dissolution by predatory boring gastropods: A review and an hypothesis. Malacologia, 17(1), 143–156.

519 Ehrlich, H., Koutsoukos, P. G., Demadis, K. D., & Pokrovsky, O. S. (2009). Principles of
520 demineralization: Modern strategies for the isolation of organic frameworks. Part II.
521 Decalcification. Micron, 40(2), 169–193.

522 Emig, C. C. (1979). British and Other Phoronides (Vol. 13, Synopsis of the British Fauna).
523 Linnean Society of London.

524 Emig, C. C. (1982). The biology of Phoronida. Advances in Marine Biology, 19, 2–90.

525 Ettinger, C. L., & Eisen, J. A. (2024). Phoronids and their tubes harbor distinct microbiomes
526 compared to surrounding sediment. bioRxiv.

527 Fernández, I., Aguirre, A., Pardos, F., Roldan, C., Benito, J., & Emig, C. (1991). The epidermis
528 of *Phoronis psammophila* Cori (Phoronida, Lophophorata): An ultrastructural and histochemical
529 study. Canadian Journal of Zoology, 69(9), 2414–2422.

530 Fernández, I., Pardos, F., Benito, J., & Roldan, C. (1996). Ultrastructural observation on the
531 phoronid nervous system. Journal of Morphology, 230(3), 265–281.

532 Haigler, S. A. (1969). Boring mechanism of *Polydora websteri* inhabiting *Crassostrea virginica*.
533 American Zoologist, 9(3), 821–828.

534 Holt, C. C., Dhaliwal, S., Na, I., Mtawali, M., Boscaro, V., & Keeling, P. (2023). Spatial
535 compartmentalisation of bacteria in phoronid microbiomes. Scientific Reports, 13, 18612.

536 Hyman, L. H. (1958). The occurrence of chitin in the lophophorate phyla. Biological Bulletin,
537 114(1), 106–112.

538 iNaturalist. (n.d.). Phoronidae. Retrieved from https://www.inaturalist.org/taxa/246126-
539 Phoronidae#highlights-tab

540 Larson, A. A., & Stachowicz, J. J. (2009). Chemical defense of a soft sediment dwelling
541 phoronid against local epibenthic predators. Marine Ecology Progress Series, 374, 101–111.

542 Marcus, E. (1949). *Phoronis ovalis* from Brazil. Zoologia (São Paulo), 14, 157–170.

Mikhlina, A., Khramova, Y., Nikitenko, E., & Vortsepneva, E. (2024). Radula formation and development in *Limapontia senestra* (Quatrefages, 1844) (Heterobranchia, Gastropoda) from the White Sea. Zoologischer Anzeiger, 311, 110–122.

Mokievsky, V. O. (2021). Marine biogeomorphology: Biotic transformation of marine bottom landforms. Geomorfologiya, 52(3), 3–23. (In Russian)

Neklyudov, A. V., & Temereva, E. N. (2027). The same, but different: Two populations of phoronid *Phoronopsis harmeri* from the Vostok Bay, Sea of Japan as an example of phoronidization of the ocean communities. Marine and Freshwater Research. In press.

Neklyudov, B., Osipova, Z., Galkin, S., Belan, T., & Temereva, E. (2026). First record of deep-sea phoronids from the Sea of Okhotsk with description of *Phoronopsis deprofundis* sp. nov. and phoronidization of the ocean communities. Progress in Oceanography, 240, 103592.

Panteleev, P. V., Tsarev, A. V., Bolosov, I. A., Paramonov, A. S., Marggraf, M. B., Sychev, S. V., Shenkarev, Z. O., & Ovchinnikova, T. V. (2018). Novel antimicrobial peptides from the Arctic polychaete *Nicomache minor* provide new molecular insight into biological role of the BRICHOS domain. Marine Drugs, 16(11), 401.

Podolskaya, M. S., Lavrichenko, D. S., Chelebieva, E. S., & Kladchenko, E. S. (2025). Infestation of oyster shells by boring sponges of the family Clionaidae: A threat to aquaculture. Biodiversity and Sustainable Development, 10(2), 61–84.

Pourreau, C. (1979). Morphology, distribution and role of the epidermal gland cells in *Phoronis psammophila* Cori. Téthys, 9, 133–136.

Radashevsky, V. I. (2012). Spionidae (Annelida) from shallow waters around the British Islands: An identification guide for the NMBAQC Scheme with an overview of spionid morphology and biology. Zootaxa, 3152, 1–35.

Radashevsky, V. I. (2022). Three new species of shell-boring *Dipolydora* (Annelida: Spionidae: Polydorini) from the South China Sea and the Gulf of Thailand, Vietnam, with comments on the modified spines in posterior notopodia and sperm morphology in polydorins. Zootaxa, 5162(2), 101–119.

570 Santagata, S. (2002). Structure and metamorphic remodeling of the larval nervous system and
571 musculature of *Phoronis pallida* (Phoronida). Evolution & Development, 4(1), 28–40.

572 Sato-Okoshi, W., & Okoshi, K. (1996). Microstructure of scallop shells infested with young
573 boring polychaete, *Polydora variegata*. Journal of the Marine Biological Association of the
574 United Kingdom, 76(3), 627–640.

575 Sato-Okoshi, W., & Okoshi, K. (2000). Structural characteristics of self-excavated burrows by
576 boring polydorid species (Polychaeta, Spionidae). Bulletin of Marine Science, 67(1), 235–248.

577 Temereva, E. N. (1999). A new rock-dwelling phoronid species, *Phoronis svetlanae*
578 (Lophophorata, Phoronida) from the Sea of Japan. Zoologichesky Zhurnal, 78(5), 626–630.

579 Temereva, E. N. (2001). Histology and ultrastructure of the body wall in the phoronid
580 *Phoronopsis harmeri*. Russian Journal of Marine Biology, 27(3), 156–167.

581 Temereva, E. N. (2006). Microscopical anatomy and ultrastructure of the lophophoral organs and
582 adjacent epitheliums of the lophophoral concavity and anal papilla of *Phoronopsis harmeri*
583 Pixell, 1912 (Lophophorata, Phoronida). Russian Journal of Marine Biology, 32(6), 134–141.

584 Temereva, E. N. (2017a). Innervation of the lophophore suggests that the phoronid *Phoronis*
585 *ovalis* is a link between phoronids and bryozoans. Scientific Reports, 7, 14440.

586 Temereva, E. N. (2017b). Ground plan of the larval nervous system in phoronids: Evidence from
587 larvae of viviparous phoronid. Evolution & Development, 19(4–5), 171–189.

588 Temereva, E. N. (2019). Myoanatomy of the phoronid *Phoronis ovalis*: Functional and
589 phylogenetic implications. Zoology, 133, 27–39.

590 Temereva, E. N. (2024). Unusual body division and epithelium structure in unusual phoronid
591 *Phoronis embryolabi*. Zoology, 167, 126221.

592 Temereva, E. N. (2025). New data on the musculature organization of the atypical phoronid
593 *Phoronis embryolabi* allow reconstruction of its distinctive lifestyle characteristics. Journal of
594 Morphology, 288(3), 512–528.

595 Temereva, E., & Neklyudov, B. (2018). A new phoronid species, *Phoronis savinkini* sp. n., from
596 the South China Sea and an analysis of the taxonomic diversity of Phoronida. Biology Bulletin,
597 45(7), 1–23.

598 Temereva, E., & Sokolova, Y. (2025). Host–parasite interplay within a phoronid–microsporidia
599 system: Anti-parasitic defense in Lophophorata. Journal of Invertebrate Pathology, 128, 108123.

600 Temereva, E., & Yurchenko, O. (2026). Spermatogenesis and spermatozoon structure shed light
601 on the reproductive biology of *Phoronis ovalis*, phoronid species with lecitotrophic larva. Tissue
602 and Cell, 104(3), 104054.

603 Turanov, S. V. (2026). Environmental DNA metabarcoding of a seagrass meadow in Vostok Bay
604 (Peter the Great Bay, Sea of Japan): A COI snapshot of coastal biodiversity and its limitations.
605 Diversity, 18(5), 120.

606 Turicchia, E., Abbiati, M., Bettuzzi, M., Calcinai, B., Morigi, M. P., Summers, A. P., & Ponti,
607 M. (2022). Bioconstruction and bioerosion in the northern Adriatic coralligenous reefs quantified
608 by X-ray computed tomography. Frontiers in Marine Science, 8, 790869.

609 Tzetlin, A. B., Klyukina, A. A., Elcheninov, A. G., Shcherbakova, P. A., Gavirova, L. A.,
610 Shestakov, A. I., Vortsepneva, E. V., Zhadan, A. E., & Kublanov, I. V. (2023). The study of
611 microbial associations helps us understand the lifestyle of *Terebellides* cf. *stroemii* (Annelida,
612 Terebelliformia, Trichobranchidae) in the White Sea. Zoologicheskii Zhurnal, 102(12). (In
613 Russian)

614 Vortsepneva, E. V. (2020). Radula morphology of *Clione limacina* (Phipps, 1774) (Gastropoda:
615 Heterobranchia: Gymnosomata). Invertebrate Zoology, 17(3), 291–309.

616 Vortsepneva, E., Herbert, D. G., & Kantor, Y. (2023). Development of *Lacuna pallidula* (da
617 Costa, 1778) from the White Sea (Caenogastropoda: Littorinimorpha) with emphasis on radula
618 formation. Journal of Molluscan Studies, 89(1), eyad004.

619 Yang, M., Butler, A., López Carranza, N., Vinther, J., Whittle, R., Henkel, D., Jurikova, H.,
620 Wörheide, G., Giacomelli, M., Donoghue, P. C. J., Pisani, D., Carlson, S. J., & Sperling, E. A.
621 (2026). A comprehensive phylogenomic framework for brachiopod evolution among
622 lophotrochozoans. Science Advances, 12, eaeh5363.

623 Zottoli, R. A., & Carriker, M. R. (1974a). Burrow morphology, tube formation, and
624 microarchitecture of shell dissolution by the spionid polychaete *Polydora websteri*. Marine
625 Biology (Berlin), 27(4), 307–316.

626 Zottoli, R. A., & Carriker, M. R. (1974b). External release of protease by stationary burrow-
627 dwelling polychaetes. Journal of Marine Research, 32(3), 331–342.

628

629