

Chapter 3

Ultrastructure of the body cavities in phylactolaemata Bryozoa

Abstract - Only species belonging to the bryozoan subtaxon Phylactolaemata possess an epistome. In order to test whether there is a specific coelomic cavity inside the epistome *Fredericella sultana*, *Plumatella emarginata* and *Lophopus crystallinus* were studied on the ultrastructural level. In *Fredericella sultana* and *Plumatella emarginata* the epistome contains a coelomic cavity. The cavity is confluent with the trunk coelom and lined by peritoneal and myoepithelial cells. The lophophore coelom extends into the tentacles and is connected to the trunk coelom by two weakly ciliated coelomic ducts on either side of the rectum. The lophophore coelom passes the epistome coelom on its anterior side. This region has traditionally been called forked canal and hypothesized to represent the site of excretion. *Lophopus crystallinus* lacks an epistome. There is a simple ciliated field where an epistome is situated in the other species. Underneath this field the forked canal is situated. Compared to the other species it is pronounced and exhibits a dense ciliation. Despite the occurrence of podocytes which are prerequisites for a selected fluid transfer, there is no indication for an excretory function of the forked canal, especially as no excretory porus was found.

Introduction

The phylogenetic position of the three lophophorate taxa is still uncertain. Recent phylogenetic analyses have rendered the long-held view of a monophyly of these taxa, as Lophophorata or Tentaculata as unlikely (Passamaneck and Halanych 2004, 2006, Halanych 2004, Dunn et al. 2008). However, the striking similarity of the tentacular apparatus in Bryozoa, Brachiopoda, and Phoronida with its ciliation and support by a secondary body-cavity has attracted the attention of comparative morphologists for a long time. The current phylogenetic hypotheses clearly show no evidence for the lophophore being a synapomorphic feature of the lophophorate taxa. However, the possibility still exists, that it might be a homologous, plesiomorphic feature. A second structure that is likely to be functionally coupled to the lophophore is the epistome. The epistome is a flap-like upper-lip organ that is found on the anal side of the mouth opening in the bryozoan subtaxon Phylactolaemata as well as in phoronids and brachiopods. It is likely to be involved in feeding, but the exact

function is unclear. Perhaps even more than the lophophore, the epistome had been assigned a disproportionately high phylogenetic significance by some earlier investigators. For example, proponents of the archicoelomate conception have regarded the epistome to represent the anterior coelomic body compartment of a hypothetical tripartite bilaterian ancestor (e.g., Remane 1950, Siewing 1980). In contrast to such far-reaching hypotheses, the organ itself remained relatively poorly studied. In Phoronida as well as Brachiopoda ultrastructural studies have found no support for a separate coelomic cavity in the epistome in representatives of these taxa (Lüter 1996, Bartolomaeus 2001, Gruhl et al. 2005), although this had been suggested by earlier investigations (e.g., Siewing 1973, Emig and Siewing 1975, Herrmann 1980). In phylactolaemate bryozoans there is clearly no evidence for a tripartition of the coelom at any time during embryonic development (Mukai 1982, Nielsen 2001). However, the exact structure and function of the epistome is unclear, as especially ultrastructural studies of this body region are lacking. Many early workers have found a cavity inside the epistome, but it remained ambiguous whether this represents a primary or a secondary body cavity and, in the latter case, whether it is separate or connected to the remaining body cavity (Zimmer 1973, Brusca and Brusca 2003, Ruppert et al. 2004). Some authors regard the epistome coelom to be confluent with the lophophoral coelom (Ryland 1970, Mukai et al. 1997)

Another unsolved issue is the mode of excretion in Bryozoa. Metanephridial organs drain primary urine out of the trunk coelom in Phoronida (Bartolomaeus 1989), or discharge coelomocytes in Brachiopoda (Lüter 1995, James 1997). No comparable excretory organs have been found in Bryozoa so far. Additionally, metanephridial systems usually require a second compartment, typically a primary body cavity, such as a blood vessel, that is separated from the coelom by an epithelial lining containing podocytes (Ruppert and Smith 1988). Blood vessels are lacking in Bryozoa and nutrient transport is likely to be accomplished by the coelom itself, but the funiculus has been considered to be a blood vessel homologue (Carle and Ruppert 1983). Some authors have suggested the epistome and/or the forked canal, which is the part of the inner arc of the lophophoral coelom that is situated between mouth and anus, to serve excretory functions (Verworn 1887, Cori 1893, Rogick 1937). However, these considerations remained rather speculative as no convincing mechanism could be proposed. Openings of the forked canal to the exterior have been described at various locations (Braem 1890, Cori 1893), but none of these have been proved by more detailed analyses.

Apart from the intent to deliver further data on the fine-structural organization of Phylactolaemata, the present paper has two central aims: Studying three species of the Phylactolaemata, *Fredericella sultana* (Blumenbach, 1779), *Plumatella emarginata* Allman, 1844 and *Lophophus crystallinus* (Pallas, 1768), we firstly try to ascertain whether the cavity inside the epistome is actually a coelom. As coelomic cavities are lined by an epithelium with polarized cells, electron microscopy is used to identify reliably the epithelial character of the lining cells of the cavity inside the epistome. The second aim is to find ultrastructural

evidence for possible excretory processes in the epistome and the forked canal. Although ring canal and forked canal are part of the lophophore coelom and the latter is confluent with the trunk coelom, these terms will be kept for comparative purposes.

Material and methods

Colonies of *Fredericella sultana* (Blumenbach, 1779) and *Lophopus crystallinus* (Pallas, 1768) were collected from the underside of stones in the Salzgitter Canal near Braunschweig, Germany in October 2000. Additional specimens of *F. sultana* were collected in the Teltow canal, Berlin, Germany in summer 2004 and 2005. *Plumatella emarginata* Allman, 1844 was collected in the lake Lehnitzsee, near Potsdam, Germany during October 2004. Colonies were fixed with 1-2.5% glutaraldehyde in distilled water or buffered in 0.01 M sodium cacodylate for 1 h at 4°C and rinsed several times in the same buffer. During fixation ruthenium red was added to the primary fixative. The postfixation was carried out with 1% OsO₄ for 1 h at 4°C. The specimens used for transmission electron microscopy (TEM) were dehydrated in an acetone or ethanol series, embedded into Araldite and sectioned with diamond knives on a Leica Ultracut microtome. Series of silver interference colored sections were placed on formvar-covered single-slot copper grids and automatically stained with uranyl acetate and lead citrate (Nanofilm TEM Stainer). The objects were examined in a Phillips CM 120 transmission electron microscope. Colonies of *Fredericella sultana* were removed from their chitinous tubes within the Araldite. For scanning electron microscopy (SEM), zooids were relaxed with 7% MgCl₂, fixed with Bouin standard solution in an evaginated condition, dehydrated in an alcohol series, automatically dried according to critical-point method (BAL-TEC CPD 030), and sputtered with gold (BAL-TEC CSD 005). A Hitachi S 450 scanning electron microscope was used for examination.

Results

The general organization in the three species examined here is comparable in most respects. Thus a detailed description is given chiefly for *Plumatella emarginata*. In the remaining species mainly the differences are pointed out.

Plumatella emarginata

Plumatella emarginata forms branching colonies of various appearances. The tube-like zooids are up to 300 µm in diameter and cystides are covered by a thin cuticle. The cuticle

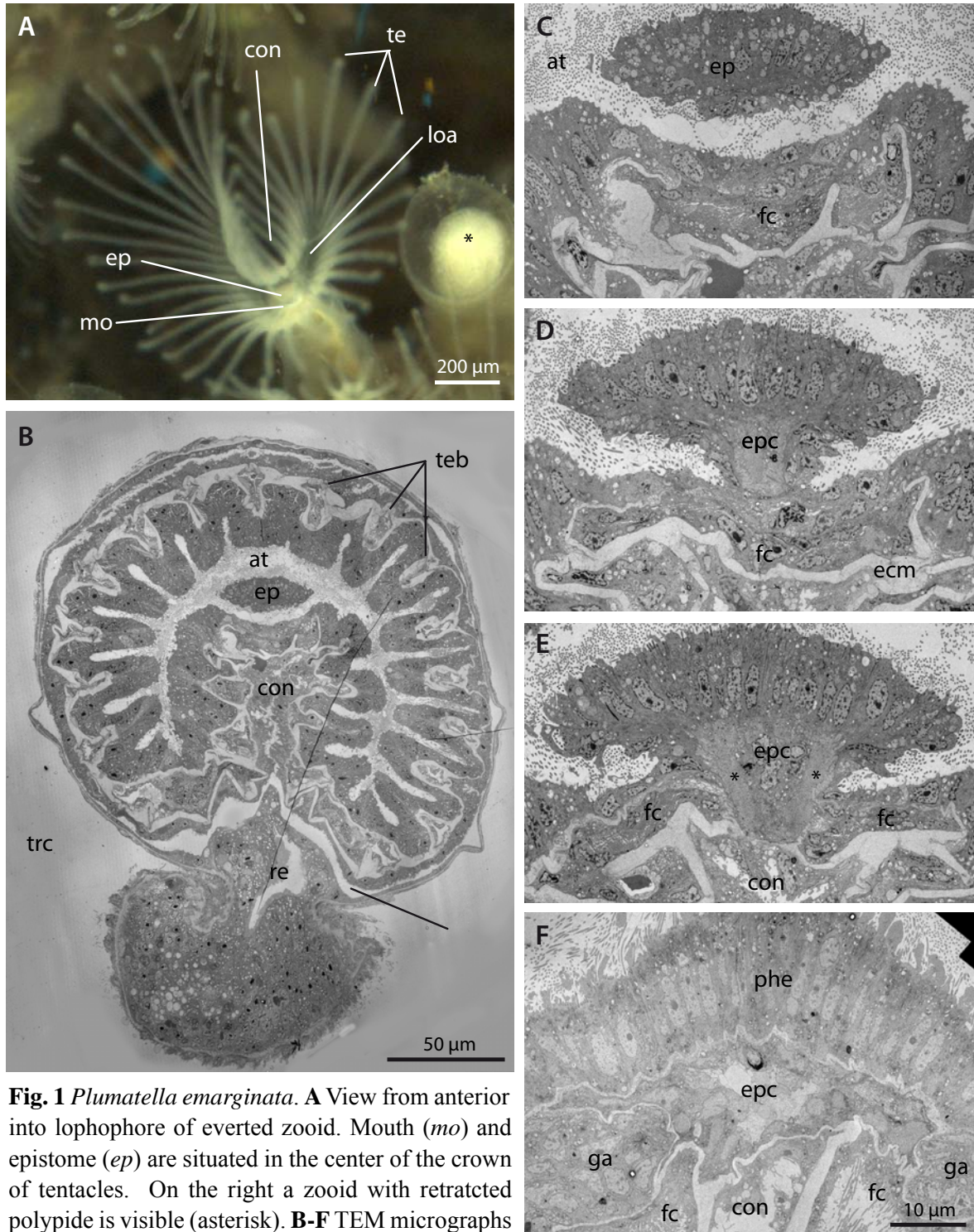


Fig. 1 *Plumatella emarginata*. **A** View from anterior into lophophore of everted zooid. Mouth (*mo*) and epistome (*ep*) are situated in the center of the crown of tentacles. On the right a zooid with retracted polypide is visible (asterisk). **B-F** TEM micrographs of cross sections. **B** Overview, cross section in height of epistome tip. The zooid is in slightly retracted state, thus a layer of tentacle sheath integument surrounds it. **C-F** Series of representative cross-sections of same zooid. **C** Enlargement of **B**. The epistome is sectioned at its tip. The forked canal (*fc*) is situated on the anal side of the epistome. It is ciliated and connects, running transversally the bases of the tentacles situated laterally behind the epistome. **D** A few (?) μm deeper a central compartment of the epistome is visible. **E** The epistome coelom runs beneath the forked canal in anal direction. It bears two large lateral muscle blocks (asterisks) **F** Basally, the epistome compartment bears a cavity. The epistome coelom is adjacent to the ganglion (*ga*). It extends in anal direction, where it merges with the trunk coelom. *at* atrium, *con* lophophoral concavity, *loa* lophophore arm, *te* tentacle, *teb* tentacle base, *trc* trunk coelom

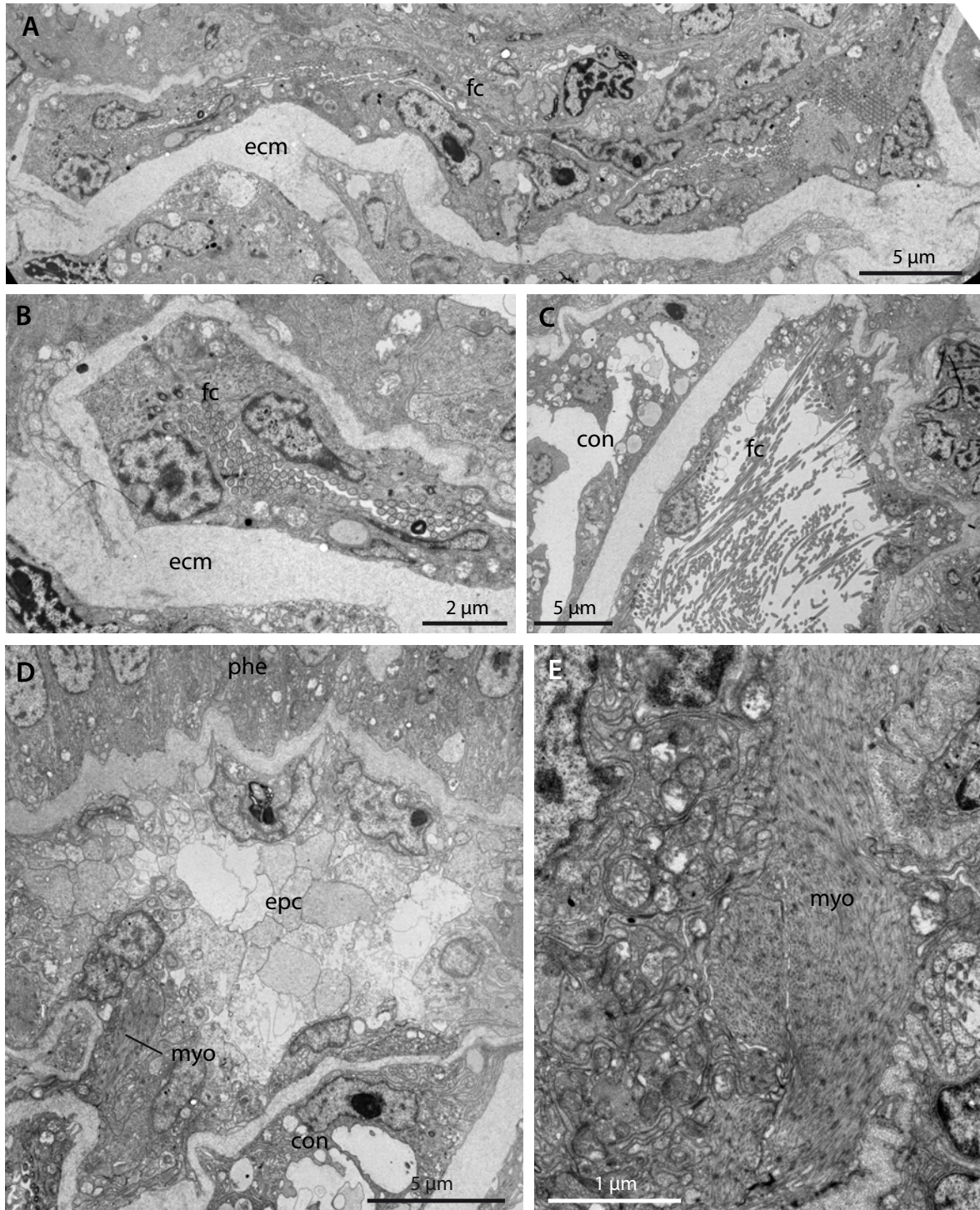


Fig. 2 *Plumatella emarginata*, details of forked canal and epistome, TEM micrographs. **A** Enlargement of forked canal as seen in Fig. 1D. The canal has a narrow lumen lined by an epithelium consisting of ciliated cells. Numbers of cilia per cell vary between 5 and 20. The ECM underneath the epithelium is much thicker on the side facing the exterior than on the side facing the epistome. **B** Detail of ciliated cells in forked canal **C** Ciliary field in the part of the forked canal that connects to the trunk coelom. **D** Lumen of the epistome coelom, enlargement of Fig. 1F **E** Muscle cell laterally in the epistome coelom, enlargement of Fig. 1E. *con* lophophoral concavity, *ecm* extracellular matrix, *fc* forked canal, *myo* myofilaments, *phe* pharynx epithelium

is pigmented except in the anteriormost region. *P. emarginata* has a prominent horseshoe-shaped lophophore with 40-50 tentacles.

The epistome is situated within the ring of tentacles, at the bottom of the lophophore (Figs. 1A, 7). It resembles a semicircular lid inserting between the mouth opening and the inner arc of the lophophore. Lateral as well as longitudinal dimensions are about 50 μm . The tip of the epistome is biconvex in cross-section comprising only multiciliated epidermal cells (Fig. 1B, C). At the oral side, which faces the mouth opening, the epidermis of the epistome is columnar, with the cells up to 12 μm in height. On the anal side of the epistome, the epidermal cells are more cuboidal, about 4 μm high and slightly irregular in shape. Following a series of cross-sections of the epistome in posterior direction, a central subepidermal compartment becomes visible at the anal side, where the epistome is attached to the lophophore base (Fig. 1D). The internal compartment is lined by an ECM which is rather thin in comparison to the ECM that lines the tentacle bases. In these sections, the compartment appears lumen-less being completely filled with cells. Laterally, thick bundles of myofilaments are situated. These are oriented in anal-oral direction, slightly diverging towards the oral side. The myofilaments show an irregular striation pattern (Fig. 2E). More posteriorly, the epistome is attached to the lophophore base to a greater extent. The central compartment is wider and extends in anal direction (Fig. 1E). It proceeds beneath the transverse, unpaired section of the forked canal and neighbors the aboral epidermis facing the lophophoral concavity. Further in posterior direction the internal compartment becomes wider and a central lumen appears between the cells (Fig. 1F, 2D). There are still muscle strands situated peripherally, but also non-muscular peritoneal cells are found. The lining cells are interconnected by adherens junctions, thus form an epithelium. The lumen contains coelomocytes. The epistomal cavity is directly adjacent to the ganglion, between the lateral ganglion horns and anterior to the central mass of the ganglion. The cavity encompasses the ganglion centrally in anal direction and fuses with the trunk coelom (Figs. 2D, 7).

The forked canal (Fig. 1C, D, 2A) comprises the portion of the inner arc of the lophophore coelom that is situated between epistome and anus. It is formed by a merger of the bases of those tentacles that are situated most closely to the epistome. It is a heavily ciliated canal on the side of lophophore which faces the epistome, passing above the epistome's inner compartment. Underneath those tentacles that are situated right and left of the attachment point of the epistome, a short, also heavily ciliated branch of the canal projects in anal/posterior direction, connecting the lophophore cavity to the large body coelom. The lining epithelium of the forked canal is thicker than that in the remaining parts of the body cavity. It is heavily ciliated, especially in the transverse part directly behind the epistome (Fig. 2A, B), and in dense fields at the connection to the trunk coelom (Fig. 2C).

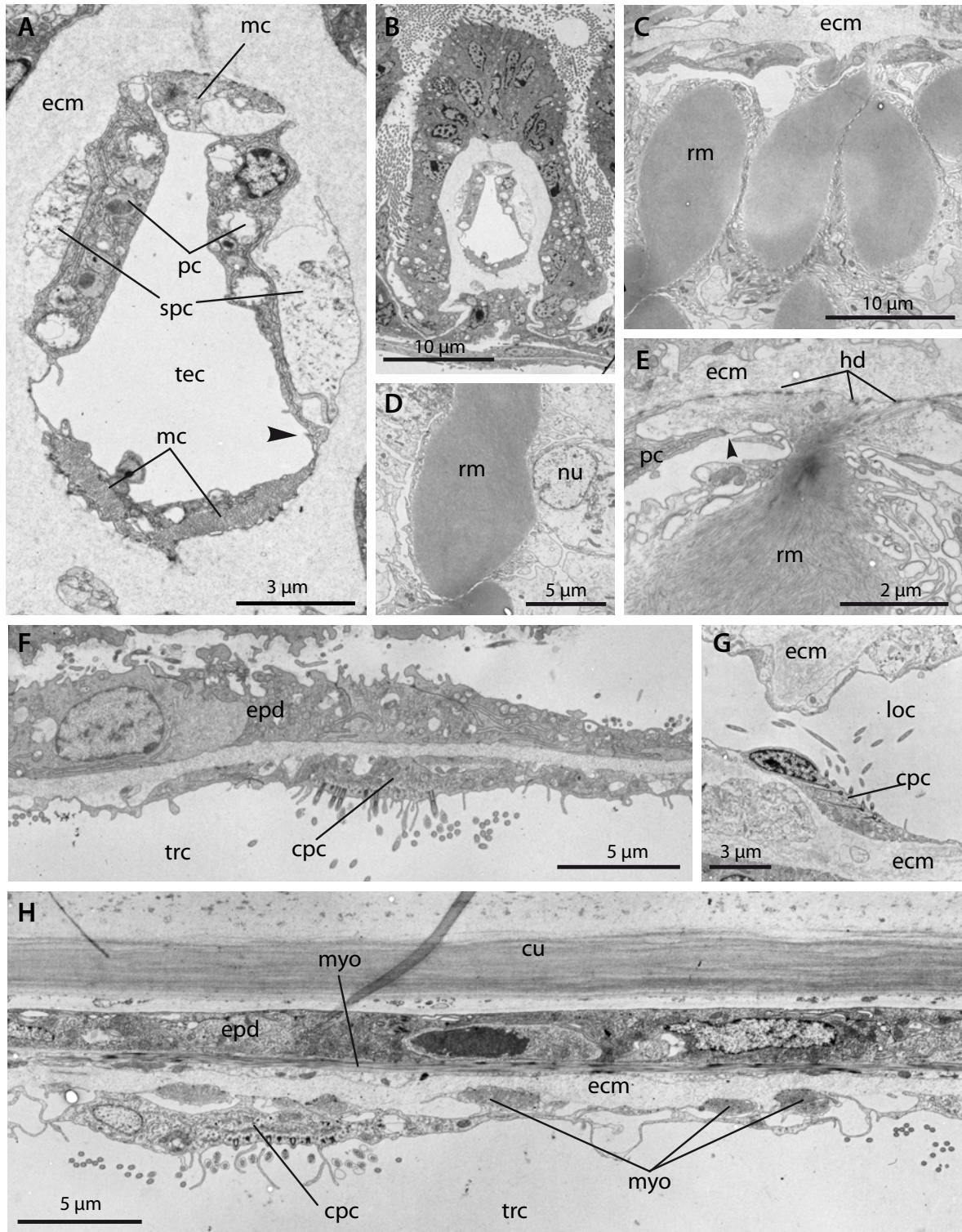


Fig. 3 *Plumatella emarginata*, details of tentacle and trunk coelom, TEM micrographs. **A** Tentacle coelom (*tec*), lined by myoepithelial cells (*mc*), peritoneal cells (*pc*) and subperitoneal cells (*spc*) **B** Tentacle base **C** Several retractor muscles (*rm*) traverse the trunk coelom. **D** Retractor muscle cell with nucleus (*nu*) **E** The retractor muscles insert directly on the ecm and are connecte to surrounding peritoneal cells by adherens junctions (*arrowhead*) **F** Integument of polypide (lophophore base). The epidermis (*epd*) lacks cuticle and glycocalyx. The lining of the trunk coelom (*trc*) is formed by

The tentacles, triangular to trapezoid in cross-section, contain a coelomic cavity (Fig. 1B, 3A, B). The lining cells, usually 6-7 in one cross-section, are unciliated and of three types. On the frontal and abfrontal side myoepithelial cells are present. Their bundles of myofilaments are oriented longitudinally. The cells are anchored to the prominent, up to 2 μm thick ECM via hemidesmosomes. On each lateral side one peritoneal cell is located. These do not reside entirely on the ECM, but cover each one subperitoneal cell, which differs from the former in its cytoplasmic composition. The cytoplasm is electron-lucent, without conspicuous amounts of ribosomes or other organelles. Nuclei of the subperitoneal cells have been found only near the base of the tentacle. Because not the entire tentacle was serial-sectioned, it is not clear whether one cell spans the entire length of tentacle. The lateral cells as well as the frontal and abfrontal cells are thickest in their central region. They meet with very delicate processes. However, adherens junctions are present at the ends of these processes. At the base of the tentacles where the blindly ending tentacle coeloms merge with the ring canal or the forked canal, ciliated cells occur (Fig. 3G). Similar cells are found widely distributed in the linings of the lophophore coelom and trunk coelom.

The lining of the trunk coelom is mostly very delicate (Fig. 3F,H), but forms a continuous epithelial cell layer. The membranes of the lining cells are extensively folded and bear plenty of fine protrusions. Most cells are ciliated cells, bearing 10-20 cilia that have inconspicuous rootlets and are arranged in a row- or fan-like fashion. Other cells bear long myofilamentous processes that are oriented longitudinally and protrude beneath the other lining cells. The processes proceed on the surface of the ECM and do not penetrate it. Embedded ring muscle cells are only found in the ECM around the intestinal tract and not in the outer body wall (not shown here, see Gruhl and Bartolomaeus 2008). However, the ring musculature of the cystide body wall is formed by epidermal epitheliomuscular cells that have long basiepithelial muscle processes. Very conspicuous muscles are the large retractors (Fig. 3C-E) that pass through the entire body cavity longitudinally. In the polypide they insert mostly near the ganglion. The terminal parts of the cells are integrated into the coelothelial lining, as they are anchored directly to the ECM and connected to the neighboring peritoneal cells via adherens junctions (Fig. 3E). Each muscle cell forms one strand with a large central bundle of myofilaments. Myofilament-free cytoplasm and nucleus are situated peripherally (Fig. 3D).



peritoneal cells. Some of these bear rows of cilia. **G** Ciliated peritoneal cell (*cpc*) at tentacle base. **H** Integument of cystid with ectocyst/cuticula (*cu*). Epidermal cells of the cystide are myoepithelial cells that form ring musculature. Longitudinal musculature is formed by myoepithelial cells of coelomic lining. *cpc* ciliated peritoneal cell, *ecm* extracellular matrix, *epd* epidermis, *hd* hemidesmosome, *myo* myofilaments

Lophopus crystallinus

Lophopus crystallinus (Fig. 4A) forms gelatinous and transparent fan-shaped colonies consisting of up to about 30 zooids. Colonies that grow larger, undergo fission. In this species 3 to 7 zooids house a single lobus which has the shape of a glove with a central coelomic cavity. All individuals of this lobus can be withdrawn into this cavity. Each zooid has a large and prominent horseshoe-shaped lophophore with up to 70 tentacles.

Zooids of *L. crystallinus* do not possess an epistome. Neither on the light microscopical level nor with electron microscopical techniques could such structure be recognized. In the region where the epistome is situated in *P. emarginata*, a densely ciliated field is found in *L. crystallinus* (Fig. 4B). This region, however, is morphologically not clearly distinct from the remaining epidermis of the lophophore base.

Beneath the ciliated epidermis (in the epistomal region) the forked canal is found. Similar as in *P. emarginata*, it is formed by the fusion of the tentacle bases of the inner lophophore arc. In contrast to the former species the canal is much wider and bears a much denser ciliation. A dense ciliary flame (Fig 4C) is situated centrally in the forked canal underneath epidermal ciliated field. It is formed by several peritoneal cells each bearing up to 50 cilia. The flame bifurcates and extends in both the right and left branch of the forked canal. In the anterior part of the forked canal the cilia are surrounded by some electron denser material (Fig. 4D). The coelomic lining is formed by ciliated and unciliated peritoneal cells and myoepithelial cells. Some peritoneal cells near the ciliary flame exhibit large homogeneously electron dense vacuoles. Other cells have very thin protrusions and thus appear podocyte-like (Fig. 4E). As in *P. emarginata*, the forked canal opens near the ganglion in posterior direction into the trunk coelom (Fig. 4F). These parts of the forked canal are only sparsely ciliated.

Fredericella sultana

Fredericella sultana colonies are antler-shaped, with the chitinous tubes usually erect from the surface of the substrate. The zooids measure about 200 µm in diameter. The cuticle of the cystid is dark brown and often small sand grains are found cemented to it. In contrast to *P. emarginata* and *L. crystallinus*, the lophophore of *F. sultana* zooids is nearly circular, exhibiting only a slight concavity between mouth and anus. About 20 tentacles are present.

The epistome of *Fredericella sultana* is clearly visible at the lophophore base, between mouth and anus (Fig. 5A, B). Its epidermis is ciliated. As in *P. emarginata*, the epistome has a central cavity lined by an epithelium (Fig. 5D). The lining consists of myoepithelial cells and peritoneal cells. All lining cells are connected by apical adherens junctions and rest on a subepidermal extracellular matrix (Fig. 5C). The myoepithelial cells form large longitudinal muscle tracts that insert chiefly in the lateral regions of the cavity. Several peritoneal cells exhibit a podocyte-like appearance with pedicels connected by diaphragmata consisting of

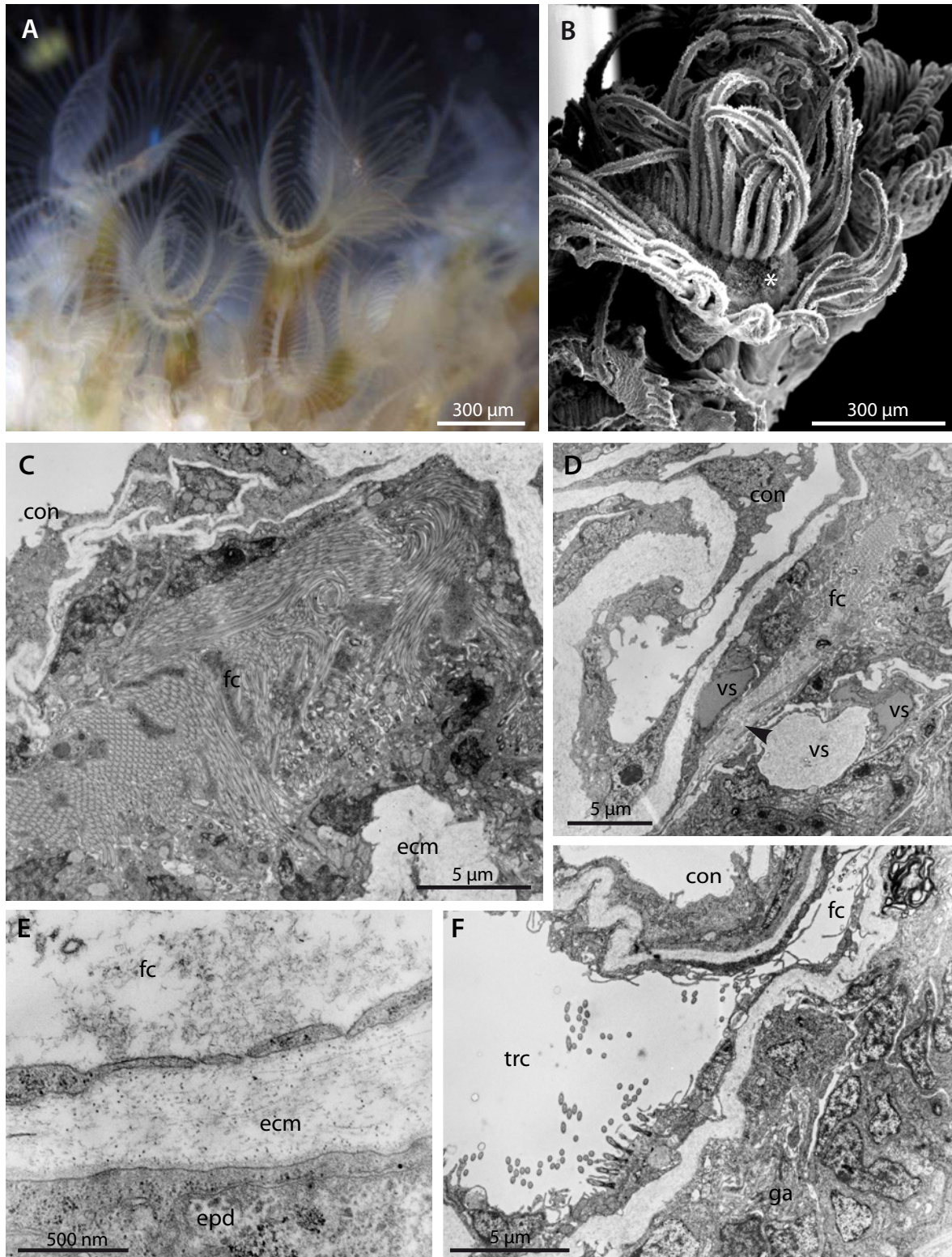


Fig. 4 *Lophopus crystallinus*. **A** Part of colony with everted polypides. **B** SEM micrograph of lophophore. At the side where the epistome is situated in other phylactolaemate species (*asterisk*) a ciliated epidermal field is found. **C-F** TEM micrographs **C** Central region of the forked canal (*fc*), situated subepidermally below the ciliated region marked in **B**. A dense ciliary flame originates there and extends into both legs of the forked canal. **D** Near the ciliary flame the coelomic fluid in the forked canal is electron dense (*arrowhead*) and some coelothelial cells exhibit vacuoles/vesicles (*vs*) **E** Some peritoneal cells (*pc*) of the coelomic lining of the forked canal have a podocyte-like appearance. **F** Below the tentacles near the peak of the inner arc of the lophophore, the forked canal branches in posterior direction opening into the trunk coelom (*trc*). *con* lophophoral concavity, *ecm* extracellular matrix, *epd* epidermis, *ga* ganglion.

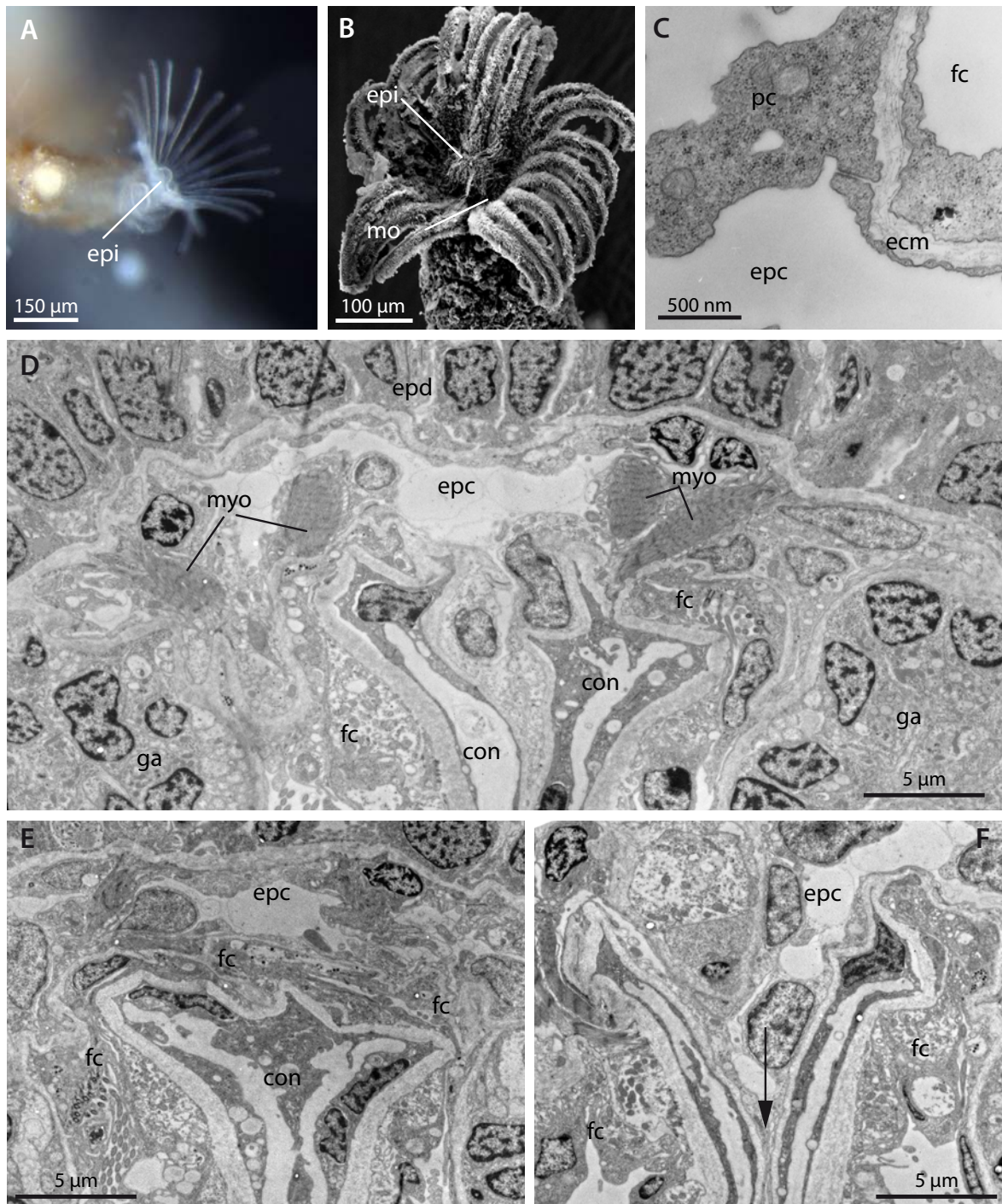


Fig. 5 *Fredericella sultana*. **A** Zooid with everted lophophore. **B** SEM micrograph, view into lophophore. The epistome (*epi*) is a ciliated flap on the anal side of the mouth opening (*mo*). **C-F** TEM micrographs. **C** Cells of the lining of the epistome coelom (*epc*) are connect via adherens junctions (arrowhead). **D** Cross section of the base of the epistome. In the lateral parts of the epistome coelom myoepithelial cells are part of the coelomic lining. **E** Cross section of the epistome xxμm anterior to the plane of section of **D**. The forked canal traverses behind the epistome. **F** Cross section of the epistome xxμm posterior to the plane of section of **D**. The epistome coelom proceeds in anal direction opening into the trunk coelom (arrow). *con* lophophoral concavity, *ecm* extracellular matrix, *fc* forked canal, *ga* ganglion, *myo* myofilaments, *pc* peritoneal cell.

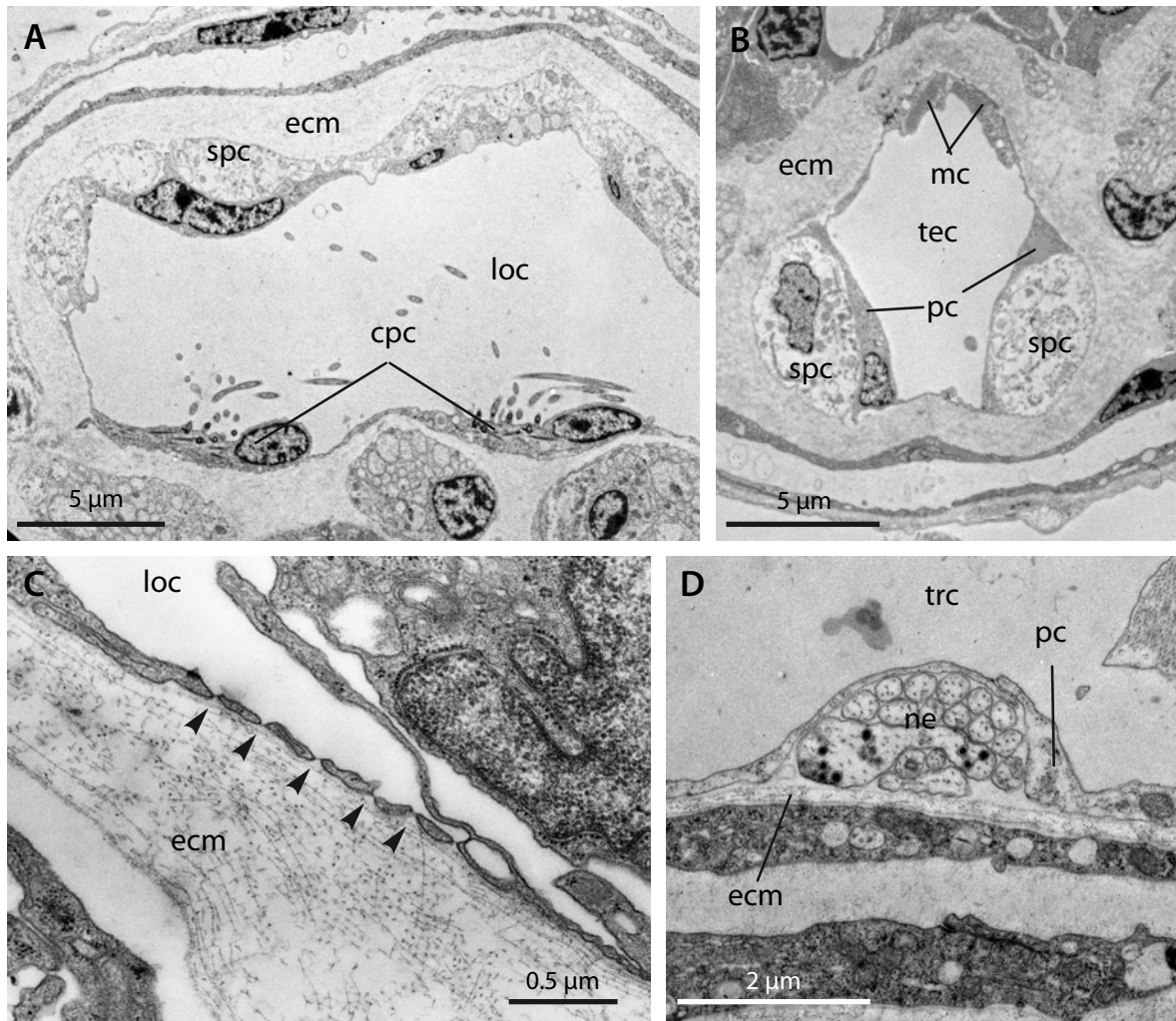


Fig. 6 *Fredericella sultana*. TEM micrographs. **A** At the base of the tentacles, where the tentacle coeloms fuse into the lophophoral coelom (ring canal or forked canal) (*loc*), ciliated peritoneal cells (*cpc*) are situated. **B** Cross section through tentacle base. The tentacle coelom (*tec*) is lined by myoepithelial cells (*mc*), peritoneal cells (*pc*) and subperitoneal cells (*spc*). **C** Some peritoneal cells in the lophophore coelom appear podocyte-like. They bear long protrusions that are interconnected by electron-dense diaphragmata (arrowheads). **D** Nerve fibers (*ne*) run beneath the lining of the trunk coelom (*trc*). *ecm* extracellular matrix, *ga* ganglion *hd* hemidesmosome.

electron dense material (Fig. 6C). The epistomal cavity encompasses the ganglion centrally running in anal direction and opens into the trunk coelom.

The general organization of the forked canal is similar as in the two other species. However, the large ciliary flame that was found *Lophopus crystallinus* is absent here. The lumen of the canal is narrower and less ciliated than in *P. emarginata*. Especially the part traversing behind the epistome is relatively narrow.

The tentacle coelom (Fig. 6B) resembles that in *P. emarginata*, but in contrast, longitudinal muscles are only present at the frontal side of the tentacles. The coelomic epithelia are essentially similar, consisting of ciliated and unciliated peritoneal as well as myoepithelial

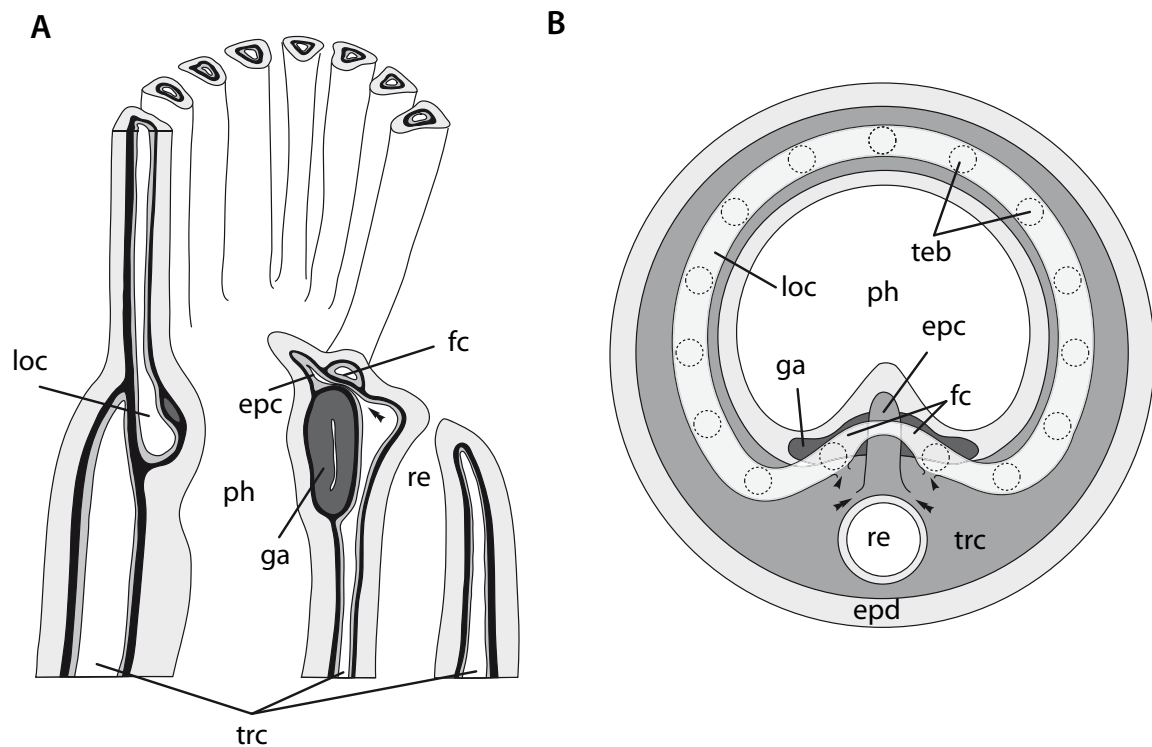


Fig. 7 Schematic representations illustrating coelomic organisation as in *Fredericella sultana* and *Plumatella emarginata*. **A** Sagittal section **B** View from anterior. *arrowheads* opening of the forked canal into the trunk coelom, *double arrowheads* opening of the epistome coelom into the trunk coelom, *epc* epistome coelom, *epd* epidermis, *fc* forked canal, *ga* ganglion, *loc* lophophore coelom, *ph* pharynx *re* rectum, *teb* tentacle basis, *trc* trunk coelom

cells. More often than in the former two species, nerves were found underneath the trunk epithelium (Fig. 6D).

Discussion

All coelomic spaces in the investigated species are confluent and lined by a continuous epithelium, which consists, to various percentages, of ciliated and unciliated peritoneal cells and myoepithelial cells. Ciliation is concentrated in certain areas, but lacking in the tentacle coeloms. In the trunk coelom conspicuous retractor muscles occur.

Epistome cavity

In contrast to *Fredericella sultana* and *Plumatella emarginata*, *Lophopus crystallinus* lacks an epistome. In his detailed account on *L. crystallinus*, Marcus (1934) described presence of an epistome, but recognized a considerable difference to that previously described in *C. mucedo*. As he found the epistome in *L. crystallinus* to resemble a bulge rather than a flap, he stated *L. crystallinus* to be “nearly gymnolaemate” in that respect. The present study suggests

that there is actually no epistome, and that the bulge Marcus (1934) recognized, might result from the pronounced central region of the forked canal. In most general accounts on phylactolaemate morphology the epistome is stated to be commonly present (Mukai et al. 1997), however, Reed (1991) reported absence of an epistome in *Fredericella*. In *F. sultana* and *P. emarginata*, the epistome contains a coelomic cavity. The cavity is lined by unciliated peritoneal cells and myoepithelial cells. On the anal side the cavity is confluent with the trunk coelom. There is no direct connection of the epistome coelom to the lophophore coelom. The latter, however, has a connection to the trunk coelom, but this is separate from the connection of the epistome. Instead, the forked canal passes the epistome coelom anteriorly as it traverses from one side to the other behind the epistome. These results substantiate most earlier descriptions, e.g. by (Braem 1890).

The functional significance of the epistome is not completely understood. Based on the model of feeding suggested by Bullivant (1968), Gilmour (1978), proposed the epistome to serve for segregating and rejecting inedible particles. Incoming particles that impinge on the upper (aboral) side of the epistome are propelled away from the mouth opening by cilia beating toward the tentacle bases. Cilia located on the oral side are described to beat in direction toward the mouth opening and thus transport potentially edible particles into the intestinal tract. Gilmour (1978) found evidence for this mechanism in phoronids and brachiopods as well, leading him to regard the epistomes in these groups as homologous. However, the precise feeding mechanisms in phylactolaemates as well as in the remaining lophophorate groups is still under discussion and might also be very different (see, for example, Strathmann 1973, 1982, Riisgard et al. 2004). From the data presented here, it can only be concluded, that hydrostatic pressure of the trunk coelom can be directed into the epistome cavity. Thus the latter forms a hydroskeleton, which might function as an antagonist to the muscle strands inside the epistome. As it is also ciliated, it might contribute to the transport of particles towards the mouth opening or away from it. The arrangement of the musculature the epistome in form of two lateral bundles suggests that it can accomplish vertical as well lateral movements. The latter case would require that the muscles of each side can contract independently from those on the other side. It is not fully clear to what extent the epistome can lower down, e.g. if it could cover the mouth opening completely.

In Brachiopoda an epistome occurs chiefly in the Lingulida and Discinida. In earlier developmental stages it superficially resembles the organs in Phoronida and Phylactolaemata, but during further development it grows larger to form the brachial fold (Williams et al. 1997). However, no coelom but only ECM and isolated muscle cells are found in these structures (Lüter 1996, 2000). In *P. mülleri* as well as in *P. ovalis* the epistome does not contain a coelomic cavity, but only extracellular matrix and muscles processes (Bartolomaeus 2001, Gruhl et al. 2005). Thus concerning the coelomic organization, no further argument can be given for primary homology of the epistome in phylactolaemate Bryozoa and Phoronida

(Brachiopoda). This renders an independent origin of the epistome structure in these groups possible. Thus, though Phylactolaemata are probably the sistergroup to the remaining Bryozoa, it can not be decided whether the epistome is an ancestral, plesiomorphic feature that has been lost in the Gymnolaemata or an apomorphy of Phylactolaemata that has arisen because of selection pressures similar to those that might have driven evolution of this character in Phoronida or Brachiopoda.

Forked canal and excretory function

Although the term “forked canal” was introduced by Braem (1890), Verworn (1887) was the first one to recognize this structure. Verworn investigated *Cristatella mucedo* and assumed an excretory function. He correlated the organ to nephridia in Kamptozoa and Annelida, because he found a small pore through which the channel opens to the exterior. This pore is described as located at the basis of those tentacles exactly opposite to the epistome. In a study by Cori (1893), an excretory function was assumed, but found the pore located on the opposite site in *Cristatella*. When Braem (1890) studied species of *Fredericella*, *Plumatella* and *Cristatella*, he could not confirm an opening of the forked canal, thus he concluded that Phylactolaemata lack excretory organs.

Later, Schulz (1901) agreed only in part to this statement. He denied the excretory function of the forked canal for *Plumatella* species, but he stated that the great extension of the unpaired part of the forked canal of *Cristatella* species may have an excretory function. Because this structure lacks a pore, he believed that the excretates accumulate within the lumen and are deposited there until zooid senescence. Kraepelin (1887), Gerwerzhagen (1913), and Marcus (1926) believed that the coelomic ciliation might be involved into transport of substances, but that excretion takes place via the tentacle ectoderm cells. None of the authors found evidence for an opening of the forked canal, but Marcus described a gap in the basement membrane of the anal wall of the forked canal. Rogick (1937) found no confirmation for a gap or an opening. However, she stated the excretory parts of the forked canal to reside within the epistome.

The present study shows that the forked canal neither communicates directly to the epistome coelom nor opens to the exterior. Some evidence has been presented here that the coelomic lining in *L. crystallinus* and *F. sultana* contains podocytes. These are connected by diaphragmata which form an unspecific molecular sieve possibly functioning as an ultrafiltration unit. The cilia of the forked canal and especially the strong ciliary flame of *L. crystallinus* could cause the necessary negative pressure for an unspecific filtration. However, as these cells generally rest on the subepidermal matrix, merely fluid inside the matrix could be ultrafiltrated when passing the diaphragmata of the podocytes. As no larger fluid filled spaces are found in this matrix, this seems unlikely. The actual function of the podocytes remains

unclear, but their involvement into an excretory process, where they act as ultrafilters, seems unlikely in the framework of the general organization of the Bryozoa. Even if the funiculus should be a blood vessel homologue (Carle and Ruppert 1983), an excretory function of the forked canal seems highly unlikely.

Acknowledgements

We would like to thank Dr. Carsten Lüter (Berlin) for valuable comments and helpful advice on the manuscript. Thanks also to Renate Feist (Bielefeld) for technical support. Financial support by the Deutsche Forschungsgemeinschaft (Ba 1520/1-3) is gratefully acknowledged.

References

- Bartolomaeus T (1989) Ultrastructure and relationship between protonephridia and metanephridia in *Phoronis muelleri* (Phoronida). *Zoomorphology* 109:113-122
- Bartolomaeus T (2001) Ultrastructure and formation of the body cavity lining in *Phoronis muelleri* (Phoronida, Lophophorata). *Zoomorphology* 120:135-148
- Braem F (1890) Untersuchungen über die Bryozoen des süßen Wassers. *Zoologica* 2:1-134
- Brusca RC, Brusca GJ (2003) Invertebrates. Sinauer Associates, Sunderland, Mass
- Bullivant JS (1968) The method of feeding of lophophorates (Bryozoa, Phoronida, Brachiopoda). *New Zeal J Mar Fresh* 2:135-146
- Carle KJ, Ruppert EE (1983) Comparative ultrastructure of the bryozoan funiculus: A blood vessel homologue. *Z zool Syst Evol -forsch* 21:181-193
- Cori CI (1893) Die Nephridien der *Cristatella*. *Z wiss Zool* 55:626-644
- Dunn CW, Hejnal A, Matus DQ, Pang K, Browne WE, Smith SA, Seaver E, Rouse GW, Obst M, Edgecombe GD, Sorensen MV, Haddock SHD, Schmidt-Rhaesa A, Okusu A, Kristensen RM, Wheeler WC, Martindale MQ, Giribet G (2008) Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature* 452:745-750
- Emig CC, Siewing R (1975) The epistome of *Phoronis psammophila* (Phoronida). *Zool Anz* 194:47-54
- Gerwerzhagen A (1913) Untersuchungen an Bryozoen. *Sitzungs Heidelb Adad Wiss Math Nat Kl Abt B* 9:1-16
- Gilmour THJ (1978) Ciliation and function of the food-collecting and waste-rejecting organs of lophophorates. *Can J Zool* 56:2142-2155
- Gruhl A, Bartolomaeus T (2008) Ganglion ultrastructure in phylactolaemate Bryozoa: Evidence for a neuroepithelium. *J Morphol* 269:594-603
- Gruhl A, Grobe P, Bartolomaeus T (2005) Fine structure of the epistome in *Phoronis ovalis*: significance for the coelomic organization in Phoronida. *Inv Biol* 124:332-343
- Halanych KM (2004) The new view of animal phylogeny. *Ann Rev Ecol Syst* 35:229-256

- Herrmann K (1980) Die archimere Gliederung bei *Phoronis muelleri* (Tentaculata). Zool Jb Anat Ontog Tiere 103:234-249
- James MA (1997) Brachiopoda: Internal Anatomy, Embryology, and Development. In: Harrison FW, Woollacott RM (eds) Microscopic Anatomy of Invertebrates Vol 13: Lophophorates, Entoprocta and Cycliophora. Wiley-Liss, New York, pp 298-407
- Kraepelin K (1887) Die Deutschen Süßwasser-Bryozoen. Eine Monographie. I. Anatomisch-systematischer Teil. Abhandlungen des naturwissenschaftlichen Vereins Hamburg 10:1-168
- Lüter C (1995) Ultrastructure of the metanephridia of *Terebratulina retusa* and *Crania anomala* (Brachiopoda). Zoomorphology 115:99-107
- Lüter C (1996) The median tentacle of the larva of *Lingula anatina* (Brachiopoda) from Queensland, Australia. Aust J Zool 44:355-366
- Lüter C (2000) The origin of the coelom in Brachiopoda and its phylogenetic significance. Zoomorphology 120:15-28
- Marcus E (1934) Über *Lophopus cristallinus* (Pall.). Zool Jb Anat Ontog Tiere 58:501-606
- Marcus E (1926) Beobachtungen und Versuche an lebenden Süßwasserbryozoen. Zool Jb (Abt Syst Ökol Geogr Tiere) 52:279-350
- Mukai H (1982) Development of freshwater bryozoans (Phylactolaemata). In: Harrison FW, Cowden RR (eds) Developmental Biology of Freshwater Invertebrates. Liss, New York, pp 535-576
- Mukai H, Terakado K, Reed CG (1997) Bryozoa. In: Harrison FW, Woollacott RM (eds) Microscopic Anatomy of Invertebrates Vol. 13: Lophophorates, Entoprocta and Cycliophora. Wiley-Liss, New York, pp 45-206
- Nielsen C (2001) Animal evolution. Interrelationships of the living phyla. Oxford University Press,
- Passamaneck YJ, Halanych KM (2004) Evidence from Hox genes that bryozoans are lophotrochozoans. Evol Dev 6:275-281
- Passamaneck YJ, Halanych KM (2006) Lophotrochozoan phylogeny assessed with LSU and SSU data: Evidence of lophophorate polyphyly. Mol Phylogenet Evol 40:20-28
- Reed CG (1991) Bryozoa. In: Giese AC, Pearse JS, Pearse VB (eds) Reproduction of Marine Invertebrates VI Echinoderms and Lophophorates. Boxwood Press, Pacific Grove, California, pp 85-245
- Remane A (1950) Die Entstehung der Metamerie der Wirbellosen. Zool Anz Suppl 42:16-23
- Riisgard HU, Nielsen KK, Fuchs J, Rasmussen BF, Obst M, Funch P (2004) Ciliary feeding structures and particle capture mechanism in the freshwater bryozoan *Plumatella repens* (Phylactolaemata). Inv Biol 123:156-167
- Rogick M (1937) Studies on fresh-water Bryozoa VI. Finer anatomy of *Lophopodella cart-eri*. Trans Am Microsc Soc 56:
- Ruppert EE, Fox RS, Barnes RD (2004) Invertebrate Zoology. A Functional Evolutionary Approach. Thomson Brooks/Cole,
- Ruppert EE, Smith PR (1988) The functional organization of filtration nephridia. Biological Reviews of the Cambridge Philosophical Society 63:231-258

- Ryland JS (1970) Bryozoans. Hutchinson University Library, London
- Schulz K (1901) Untersuchungen über den Bau der Bryozoen mit besonderer Berücksichtigung der Exkretionsorgane. Nicolaische Verlags-Buchhandlung, Berlin
- Siewing R (1980) Das Archicoelomatenkonzept. Zool Jb Anat Ontog Tiere 103:439-482
- Siewing R (1973) Morphologische Untersuchungen zum Archicoelomatenproblem 1: Die Körpergliederung bei *Phoronis ijimai* Oka (Phoronidea). Z f Morphol u Ökol d Tiere 74:17-36
- Strathmann RR (1973) Function of lateral cilia in suspension feeding lophophorates (Brachiopoda, Phoronida, Ectoprocta). Mar Biol 23:129-136
- Strathmann RR (1982) Comment on Dr. Gilmours views on feeding by hemichordates and lophophorates. Can J Zool 60:3466-3468
- Verworn M (1887) Beiträge zur Kenntnis der Süßwasserbryozoen. Z wiss Zool 46:7-128
- Waeschenbach A, Telford MJ, Porter JS, Littlewood DTJ (2006) The complete mitochondrial genome of *Flustrellidra hispida* and the phylogenetic position of Bryozoa among the Metazoa. Mol Phylogenet Evol 40:195-207
- Williams A, Kaesler RL, Moore RC (1997) Treatise on invertebrate paleontology Part H, Brachiopoda, Volume 1. Geological Society of America, Boulder, Colorado