Chapter 4

Muscular systems in gymnolaemate bryozoan larvae

Abstract - Gymnolaemate bryozoan species exhibit larval forms that can either be planktotrophic cyphonautes or one of the several types of lecithotrophic larvae. Due to the lack of both a phylogenetic system of the Bryozoa and detailed information about larval morphology for many species, it is difficult to elaborate hypotheses about the evolution of larval forms in this group at the moment. In this study, phalloidin staining and confocal laser scanning microscopy are used to provide detailed morphological descriptions of the three-dimensional structure of muscular systems in the larvae of *Flustrellidra hispida*, *Alcyonidium gelatinosum*, *Membranipora membranacea*, *Bugula fulva* and *Bowerbankia gracilis*. A comparison to the available data from the literature is added. The results show muscular systems to be much more complex than previously recognized. In all but one species, an anterior median muscle inserts with one end at the basal side of the apical organ and with its other end at the papilla of the pyriform organ. This muscle can be paired or unpaired and was referred to as "neuromuscular strand" in earlier descriptions. All species except *Bugula fulva* exhibit a coronal ring muscle running concentric to the larva's main locomotory ciliary band. Lateral muscle strands that connect the pallial epithelium to either the corona or oral epithelium are found mainly in the cyphonautes and pseudocyphonautes larvae and, in modified shape though, also in some coronate larvae. Transversal or shell adductor muscles can be shown to co-occur with lateral muscles and possibly originate from the same source. The findings are in accordance with the idea of a multiple origin of lecithotrophic forms within gymnolaemate Bryozoa.

Introduction

Bryozoa or Ectoprocta are colonial, sessile suspension-feeders occurring in both marine and limnetic habitats. Commonly three major subtaxa are recognized: Phylactolaemata, Stenolaemata, and Gymnolaemata. The Phylactolaemata exclusively inhabit freshwater with about 60 species worldwide. Stenolaemata with its only recent subtaxon Cyclostomata are calified and occur in the oceans. Gymnolaemata consist of Ctenostomata, which are uncalcified and exhibit marine and a few brackish-water and freshwater species, and the calcified

Cheilostomata representing the dominating group of bryozoans in the oceans. Recent data argue for Ctenostomata to be a paraphyletic assemblage including possible sister groups of both Cheilostomata and Cyclostomata (2000, Ernst and Schäfer 2006).

Whereas bryozoan colonies grow through the asexual process of budding, new colonies (genets) arise sexually in most cases. Most bryozoans are colonial hermaphrodites with their reproducing zooids being either gonochorists or proterandrous hermaphrodites. Fertilization seems to be generally internal, as shown at least for gymnolaemates (Temkin 1994, 1996). Zygotes are shed through the supraneural canal into the water in species with planktotrophic larvae. In species with lecithotrophic larvae, eggs are either placed into external brood chambers or retained in the body cavity, in such cases where extraembryonic nutrition occurs (Ström 1977).

Gymnolaemate larvae are diverse in many aspects but share a general body organization, consisting of a defined set of organs and tissues: The most prominent structures are the corona, which is the locomotory ciliary band, and a ciliated apical sense organ. The pyriform organ consists of glands and sensory cells and is involved in settlement. The internal sac is an invaginated epithelium that is expelled at the onset of metamorphosis (see Fig. 1 for a brief overview; see Nielsen 1971, Zimmer and Woollacott 1977, Reed 1991 for comprehensive reviews). There have been several attempts to classify the different larval forms, the most well known of which might be from Zimmer and Woollacott (1977). These authors differentiate between seven types of gymnolaemate larvae including the well known cyphonautes, shelled lecithotrophic (pseudocyphonautes) larvae, as well as five types of so-called coronate larvae (Type O, E, AE, AEO/ps and AEO/PS). The latter types resemble each other in being lecithotrophic and unshelled. They are distinguished mainly by position and extension of the corona (although the types share more characters): Type O and E have rather narrow coronae that are located either orally (O) or equatorially (E). In type AE the corona is expanded and covers the equatorial and aboral part of the larva. The aboral epidermis is invaginated and forms a so-called pallial sinus. AEO/ps larvae have expanded coronae that form most of the larval surface and a small pallial sinus, whereas that of the oral-aborally elongated AEO/PS larvae is large.

When trying to correlate larval types with higher level taxa in traditional bryozoan classification (Boardman et al. 1983, Hayward 1985, McKinney and Jackson 1989, Hayward and Ryland 1998), it turns out that many types seem to be "randomly" distributed, suggesting that they have arisen independently multiple times within Bryozoa. However, assumptions like these can only be drawn reliably when a sufficient amount of background knowledge is available. This would include a reliable phylogenetic system, which is unfortunately not available at present. Furthermore, sufficiently detailed morphological data are required for as many taxa as possible in order to make confident decisions about primary homology of
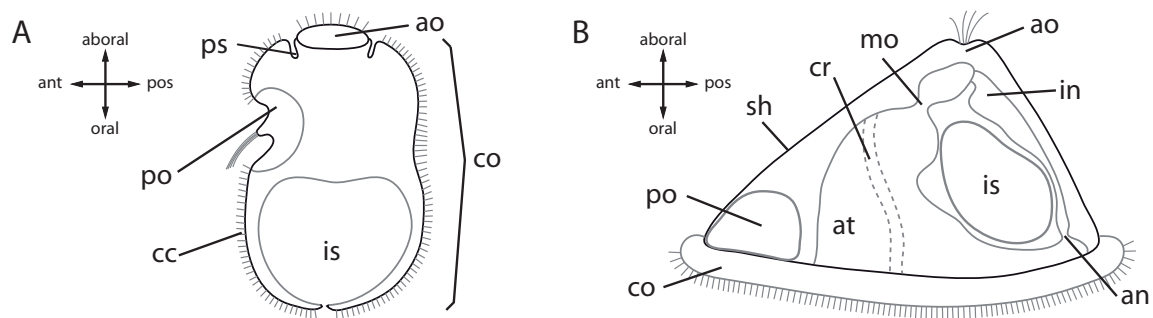
Fig. 1 General morphology of bryozoan larvae, represented here by two types. Because the terms dorsal and ventral can be misleading in bryozoan larvae, body axes are termed by convention (Zimmer and Woollacott 1977, Reed 1991) aboral or apical for the former animal region of the embryo bearing the apical organ and oral for the opposite side, that is derived from the vegetal region. The anterior (*ant*) region is defined by the presence of the pyriform organ, the opposite region is posterior (*pos*). A ciliary band, the corona (*co*), consisting of multiciliated cells is the main organ for swimming. All larvae bear an apical organ (*ao*), that can be either simple, that is, consisting of a small number of cells, or complex. In the latter case it is usually referred to as apical disc. The pyriform organ (*po*), a complex of ciliated and glandular cells is situated anteriorly. At the posterior side, the internal sac (*is*), an epithelial invagination, functioning as adhesive organ during setttlement, can be found. **A** Coronate larva are lecithotrophic. The corona forms the largest part of the larval surface. The gut is either absent or vestigial. The aboral epithelium is invaginated in some cases, forming the so-called pallial sinus (*ps*). Between pyriform organ and internal sac a small ciliated cleft (*cc*) is located. **B** Cyphonautes larvae are planktotrophic and have a functional digestive tract (*in*). The aboral epithelium secretes the lateral shells (*sh*). The oral surface is densely ciliated and invaginated forming the atrium (*at*) with its ciliated ridges (*cr*), where plankton particles are captured. *an* anus, *mo* mouth.

structures. The currently available descriptions of gymnolaemate larvae differ tremendously in their level of detail. Therefore a comparative approach on certain larval characters might have the potential to bring new light into an evolutionary discussion. A useful character should be easily accessible, be complex, and show a certain amount of variation in order to be informative for phylogenetic analyses (Jenner and Schram 1999). Musculature has been widely ignored in most studies on larval morphology so far, mainly because of methodic difficulties. However since methods like fluorescence staining and confocal laser scanning microscopy (CLSM) are available, musculature meets these requirements, as already shown in many other metazoan groups (e.g., Wanninger 2004, Müller and Worsaae 2005, Wanninger et al. 2005, Fuchs et al. 2006, Leasi et al. 2006, Schmidt-Rhaesa and Kulessa 2007). Three-dimensional analyses of muscular anatomy have already contributed to solve evolutionary or phylogenetic problems (Haszprunar and Wanninger 2000, Purschke and Müller 2006). Morphology of the larval musculature in Bryozoa is closely linked to larval biology and metamorphosis (Reed 1985) so that many differences between the larvae should be reflected in their musculature.

The aim of this study is to compare muscular systems of gymnolaemate larvae. Musculature of five gymnolaemate species is examined by means of phalloidin staining and CLSM.

Species were chosen, for which detailed ultrastructural information is already available from the literature, if not for the same species, at least for congenerics. This is done to validate comparisons between data obtained by CLSM and by transmission electron microscopy (TEM). The obtained results are then extended by results from the literature and discussed in a phylogenetic context. While finishing this manuscript, a partially overlapping study was published by Santagata (2008).

Materials and methods

Collection of larvae

Reproducing colonies of *Flustrellidra hispida* (Fabricius, 1780) and *Alcyonidium gelatinosum* (Linnaeus, 1761) were collected in the rocky intertidal of Concarneau (Bretagne/France) between 2005 and 2006. Both species prefer thalli of *Fucus serratus*, whereas *F. hispida* occurs on the lower parts of the phylloids, and *A. gelatinosum* inhabits the tips. *Bugula fulva* Ryland, 1960 was collected in the harbour of Brest (Bretagne, France) on the undersides of floating docks in May 2007. *Bowerbankia gracilis* Leidy, 1855 was collected in the harbour of Helgoland (Germany) in June 2006. Cyphonautes larvae of *Membranipora membranacea* (Linnaeus, 1767) were collected in *Laminaria* forests in Helgoland (Germany) in June 2006 using a 70-μm plankton net. As it is not possible to determine the cyphonautes larvae safely, about half of the collected larvae were allowed to settle in glass dishes and observed over a period of 3 days. All formed twin-ancestrulae, which are unique for *M. membranacea.* Colonies of *F. hispida* and *A. gelatinosum* were kept in vessels with a constant current of fresh seawater entering the vessel at its base. The overflow at the top of the vessel was directed into a chamber with a 100-μm plankton net. Shed larvae were obtained from the plankton net twice daily whereas larval release was highest during the morning hours. Colonies of *B. fulva* and *B. gracilis* were kept in 300-ml glass dishes. Emerging larvae are strongly phototactic and were attracted to the vessel wall by fiber-optics illumination. Clouds of larvae were then transferred into smaller dishes for fixation using a glass pipette. Live observations of larvae were accomplished with a Leica MZ 16A dissecting microscope and an Olympus BZ51 compound light microscope.

Phalloidin staining

Specimens were fixed in 4% paraformaldehyde in 0.05 M PBS containing 0.33 M NaCl. Duration of fixation was from 30 min to 6 h at room temperature. Some larvae were relaxed using a 7% $MgCl_2$ solution prior to fixation. Phalloidin staining was accomplished by incubating permeabilized (0.1 % Triton-X 100 in PBS, 2 hours) specimens with Alexa-Fluor-568

labeled phalloidin at a final dilution of 1 U/500 µl for 6-12 h. Specimens were rinsed in PBS and mounted on slides using Citiflour Glycerol solution. Confocal image stacks were taken on a Zeiss LSM 410 and a Leica TCS SPE. Altogether 24 specimens of *F.hispida*, 11 of *A. gelatinosum*, 10 of *M. membranacea,* 14 of *B. fulva*, and 9 of *B. gracilis* were examined. ImageJ with WCIF plugin bundle was used to process digital image stacks and to generate projection views. Three-dimensional inspection was done by volume rendering using Voxx2.

Scanning Electron Microscopy

Specimens were fixed either with 2.5% Glutaraldehyde in 0.05 M PBS containing 0.33 M NaCl (1h, 4°C) or with 1% OsO_4 in seawater. Fixative was removed by washing several times with PBS. Specimens were dehydrated in a graded ethanol series, critical point dried, mounted, and subsequently sputter-coated with gold. Specimens were examined using a FEI Quanta 200 SEM at 15 kV.

Results

Flustrellidra hispida (Ctenostomata, Flustrellidridae)

The larva of *F. hispida* is of the shelled lecithotrophic type, sometimes also referred to as pseudocyphonautes, indicating its morphological resemblance to the planktotrophic cyphonautes. Descriptions on its morphology are found in Barrois (1877), Prouho (1890), and Pace (1906). Ultrastructural details have been added by d'Hondt (1977a).

The larva (Fig. 2A) is of elongate shape and nearly triangular in cross-section. It bears two chitinous shells that completely cover the lateral sides of the body. The oral surface is heavily ciliated and a pronounced ciliary band, the corona, runs at the rim of the oral surface. Anteriorly the pyriform organ is located, visible from exterior by the occurrence of several tufts of cilia (collectively referred to as vibratile plume). The larval mouth is situated directly behind the pyriform organ, leading into a lumen-less gut. The internal sac appears as a deep invagination posterior to the larval mouth. It is made up of a thickened glandular epithelium that shows clear regionalization. At the posterior end of the larva, a field of elongate cilia is present. At the aboral side, where the shells converge, a narrow longitudinal groove is left uncovered (Fig. 2A). The epidermis in this groove is not ciliated. In the middle of the groove, the ciliated apical organ is situated. It consists of several types of ciliated cells and can be retracted by muscular contraction.

Concentric to the corona runs one *ring muscle* (ri, Fig. 2C,E). It is approximately 5µm in diameter and composed of about five smooth bands. The muscle is situated beneath the corona
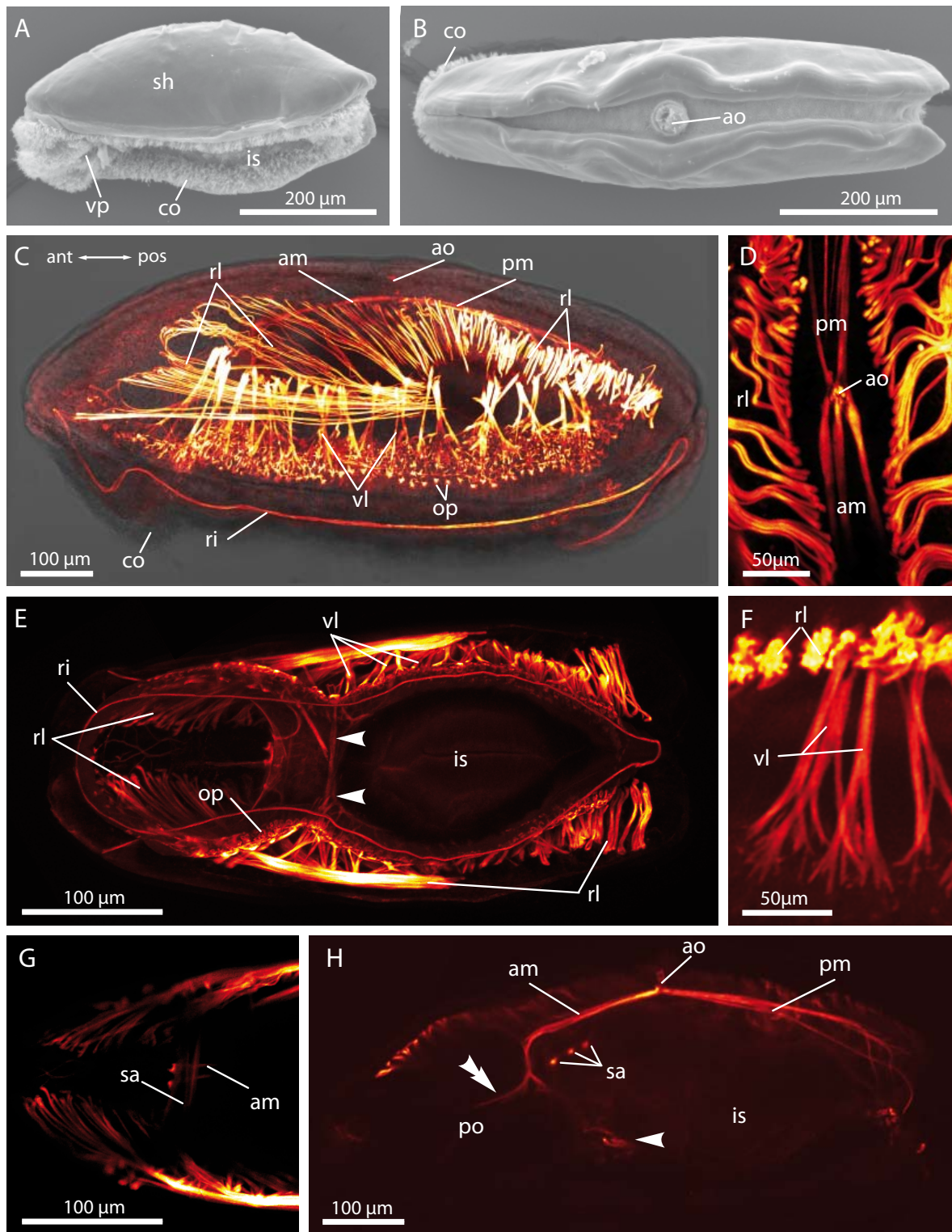
Fig. 2 *Flustrellidra hispida.* Except in **D,** anterior end is always oriented to the left as indicated in **C**. **A, B** SEM micrographs of larva. **A** View from lateral/oral. **B** View from aboral, the apical organ is situated between the two shells (*sh*). **C-H** Phalloidin-stained larval muscular system, projections of confocal image stacks. **c** View from lateral, merged with light micrograph. For better visibility, only muscles of right half of the larva are shown. The main muscle groups are visible. **D** View from aboral (like in b). Anterior median muscle (*am*) and posterior median muscles (*pm*) insert at the base of the apical organ (*ao*). Radial lateral muscles (*rl*) are connected to the shells' margins. **E** View from oral. The oral side of the larva is surrounded by the corona, represented through its ring musle (*ri*)

➤

cells. It obviously works in contracting the corona, a behavior that can often be observed in swimming larvae, when these experience mechanical stimuli.

About 15-20 pairs of *vertical lateral muscles* (vl, Fig. 2C) underlie the shells. They are serially arranged and each muscle inserts aborally at the epithelium underlying the inner surface of the shell. The muscles run in oral direction, branching into single fibers before reaching the oral epithelium (Fig. 2C, F). Here they are attached to papillae made up of several ciliated cells. The papillae themselves measure about 10 μm in diameter and contain rings of myofilaments. The most anterior pair of vertical lateral muscles is arranged more obliquely with some branches embracing the pyriform organ on the anterior side and running parallel to the corona ring muscle there (Fig. 2E). The pairs situated between the internal sac and the pyriform organ do not run directly to the oral papillae but send their branches nearly horizontal underneath the oral epithelium towards the opposite side (Fig. 2E). The ends of both the right and left muscles interdigitate.

Shell adductor muscles (sa, Fig. 2G, H) traverse the larva horizontally, connecting the left to the right shell. Usually 3-5 strands are situated between pyriform organ and internal sac. They have their origins at the underlying epithelium of the shells in close proximity to the aboral points of insertion of the vertical lateral muscles. They resemble the vertical lateral muscles in their overall appearance, with the difference of being arranged horizontally rather than vertically. Contraction of theses muscles leads to lateral compression of the larva.

Radial lateral muscles (rl, Fig. 2C, D) also insert at the inner surface of the shells. On each side numerous thin bands, 1-3μm in diameter can be found. They run in a radiating fashion anteriorly and aborally and end close to the aboral margins of the shells (Fig. 2D). The anteriorly directed strands attach mainly at one point in the middle of the shell. The aborally and posteriorly directed strands attach each along a line towards the posterior end of the shell near the aboral points of insertion of the vertical lateral muscles (Fig. 2F). The bands are usually unbranched. In some areas they appear as loosely associated bundles, each consisting of 3-10 bands. The radial lateral muscles cannot be brought into connection to any movements of the larva while either swimming or creeping. They seem to be involved into morphogenetic movements during metamorphosis.

← ───

here. Vertical lateral muscles (*vl*) form horizontal processes (*arrowheads*) on the oral side, between pyriform organ and internal sac. **F** View from lateral, detail of vertical lateral muscles (*vl*) and insertion of radial lateral muscles (*rl*). **G** View from oral. Detail of shell adductor muscle (*sa*) running transversely on the oral side of the anterior median muscle (*am*). **H** Lateral view. Projection of central sagittal optical sections showing anterior and posterior median muscle as well as, in cross section, shell adductor muscle (*sa*) and horizontal projections of vertical lateral muscles (*arrowhead*). Some fibers of the anterior median muscle extend into the pyriform organ (*double arrowhead*). *ant* anterior, *co* corona, *is* internal sac, *op* oral papillae, *po* pyriform organ, *pos* posterior, *vp* vibratile plume

An *anterior median muscle* (am, Fig. 2C, D, H) connects the apical organ to the pyriform organ. Three compact bundles insert at the base of the apical organ. They run parallelly in anterior direction towards the pyriform organ where they branch into numerous fibers. Some of the fibers attach to the posterior side of the pyriform organ or surround it as delicate fibers. Others proceed further into the pyriform organ and run between the superior and inferior glandular field towards the papilla.

A *posterior median muscle* (pm, Fig. 2C, D, H), consisting of several strands, also originates at the basal side of the apical organ. It runs in posterior direction, branching into several fibers. These attach at the posterior end of the larva near the corona.

The *apical organ* (ao, Fig. 2D, H) bears a myoepithelial cell, whose myofilaments are arranged in aboral-oral direction. When it contracts, the central cells of the organ become flattened.

Alcyonidium gelatinosum (Ctenostomata, Alcyonidiidae)

The larva of *A. gelatinosum* belongs to type E according to Zimmer and Woollacott (1977). Descriptions of its morphology are found in Calvet (1900), Seeliger (1906), and d'Hondt (1973a, 1975).

Its narrow corona encircles the roundish larva (Fig. 3A) equatorially. The aboral epithelium is unshelled. A small part of the aboral epithelium is invaginated and forms, concentric to the corona, the circular pallial sinus. The apical organ is situated in the anterior part of the aboral hemisphere. Like *F. hispida* it has a rudimentary gut beginning with a mouth at the oral surface between pyriform organ and internal sac. The latter comprises a deep invagination of thickened oral epithelium.

There is a *coronal ring muscle* (ri, Fig. 3B) encircling the larva underneath the coronal ciliary band. It is not one continuous strand, but consists of several shorter sequentially arranged muscle bands, that measure about 20µm in length and 3µm in thickness. During fixation and sample preparation the ring muscle often gets disrupted at the junctions between these bands.

Several bundles of *lateral muscles* (la, Fig. 3B) insert at the aboral epithelium. They protrude orally towards the corona and the oral epithelium. A few delicate fibers also cross the larval body transversally.

Paired *anterior median muscles* (am, Fig. 3B) connect the apical organ and the pyriform organ. Each strand consists of several muscle fibers, inserting at the basal side of the apical organ and terminating laterally at the pyriform organ. They resemble very much the lateral muscles in their overall appearance, but can be to distinguished from the latter by the different points of insertion and their higher length.
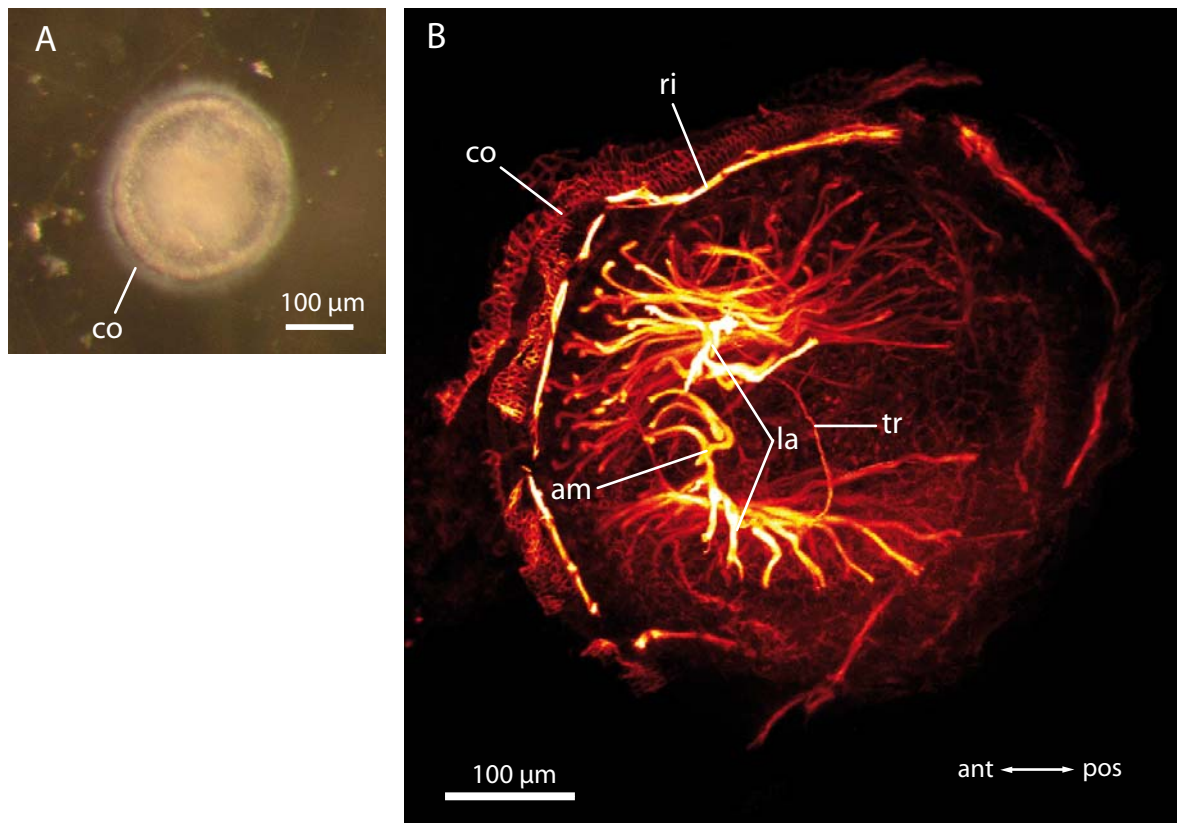
Fig. 3 *Alcyonidium gelatinosum.* **A** Light micrograph of larva. **B** Phalloidin staining of musculature, projection of confocal image stack. View from aboral. Underneath the corona (*co*) runs a ring muscle (*ri*) that is composed of several elements. Lateral muscles (*la*) insert at the pallial epithelium in the aboral hemisphere and project anteriorly towards the oral epithelium. An anterior median muscle (*am*) connects apical organ and pyriform organ. A delicate transverse muscle (*tr*) connects right and left pallial epithelium. *ant* anterior, *pos* posterior

Membranipora membranacea (**Cheilostomata, Membraniporidae**)

Cyphonautes larvae have been described by Prouho (1892), Kupelwieser (1905), and Atkins (1955). An ultrastructural study of the larva of *M. membranipora* has been conducted by Stricker et al. (1988a, 1988b).

The cyphonautes larva of *M. membranacea* (Fig. 4A) is laterally compressed and triangular-shaped in side-view. The shells completely cover the lateral surfaces. The apical organ is located at the tip of the larva. The oral epidermis is deeply invaginated into the larva, forming the so-called atrium. Ciliary ridges inside the atrium are responsible for particle capturing. The mouth opening is situated at the roof of the atrium. The digestive tract encompasses the internal sac and terminates with an anus in the atrium's posterior angle. Anteriorly on the oral side the pyriform organ is situated.

A *ring muscle* (ri, Fig. 4B, D) encircles the atrial opening underneath the coronal cells. It is about 5µm thick. When looking at it in closer detail, it appears that the muscle consists of a row of discrete packages, each about 15µm in length.
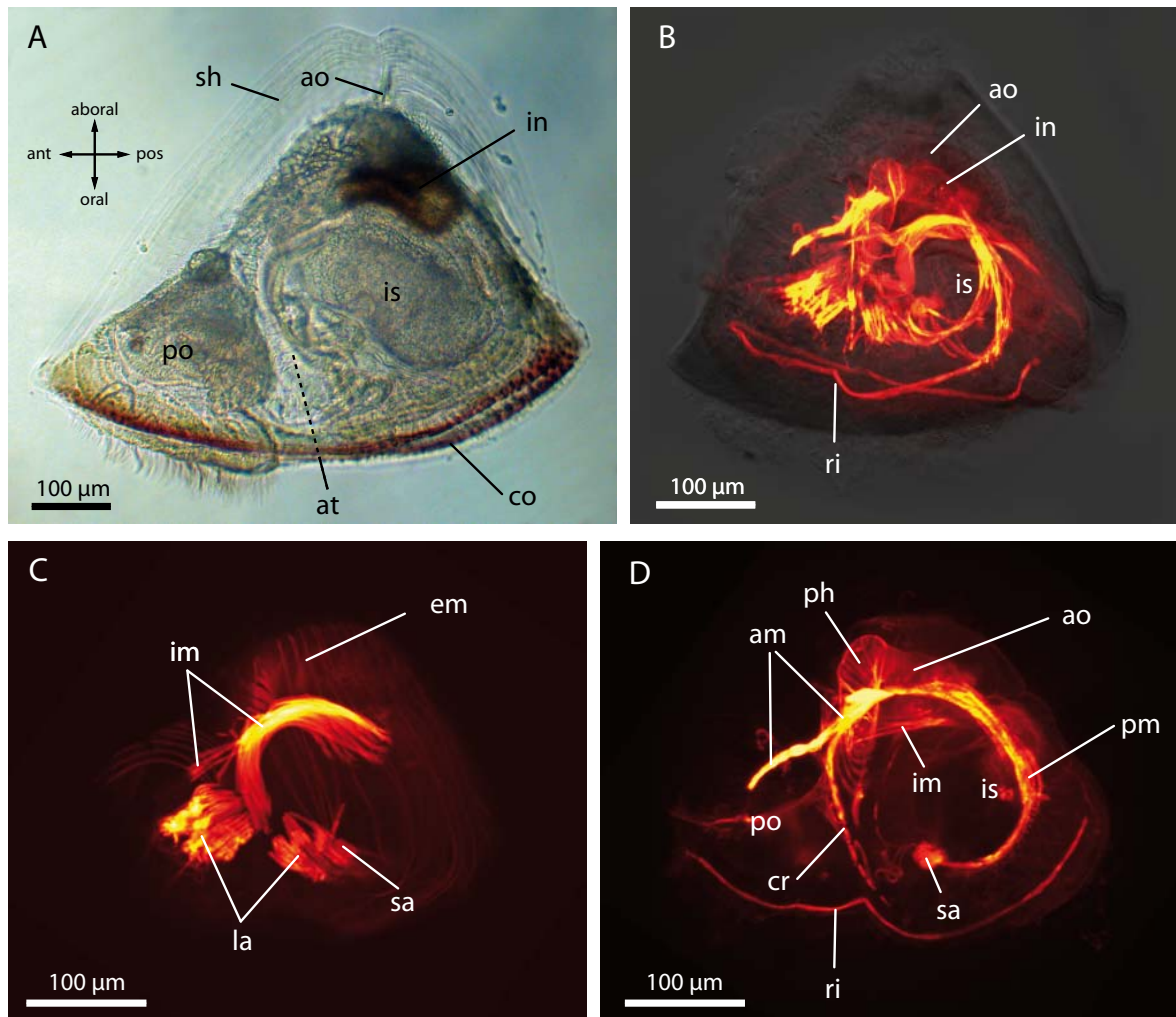
Fig. 4 *Membranipora membranacea.* **A** Lightmicrograph of cyphonautes larva, lateral view. **B-D** Phalloidin staining of larval musculature, projections of confocal image stacks. Lateral view, orientation like in **A. B** Whole stack, merged with transmission image. **C** Projection of peripheral optical sections, showing muscles that insert directly underneath the shell. Outermost are delicate epidermal muscle fibers (*em*). Five muscle insert at the center of the shell: anterior and posterior internal sac muscle (*im*), anterior and posterior lateral muscle (*la*), shell adductor muscle (*sa*). **D** Projection of central optical sections. Anterior (*am*) and posterior (*pm*) median muscles insert at the apical organ (*ao,* slightly retracted here). Ciliated ridge muscle (*cr*) branches from anterior median muscle, latter proceeds towards papilla of pyriform organ. Shell adductor muscle inserts at the inside of the shell in the height of the opening of the internal sac. *ant* anterior, *at* atrium, *co* corona, *in* intestine, *ph* pharynx, *pos* posterior, *ri* corona ring muscle, *sh* shell.

An *anterior median muscle* (am, Fig. 4D) connects the pyriform organ and the apical organ. The muscle is about 12μm in diameter and composed of numerous muscle bands. While proceeding in oral direction it branches off two smaller strands that run towards the ciliated ridges on both sides of the atrium. Inside the ridges is a muscle that is composed of discrete packages, each 3μm thick and 15μm long. The central part of the anterior median muscle proceeds towards the pyriform organ. There it continues as a conspicuous central muscle that

runs towards the vibratile plume of the pyriform organ. Numerous fine fibers are found in the pyriform organ. These are also likely to originate from the anterior median strand.

The *posterior median muscle* (pm, Fig. 4D) runs, in opposite direction to the anterior strand, underneath the pallial epithelium between the two shells. Like the anterior median muscle it is conspicuous and composed of several muscle bands. It branches shortly after the apical organ, giving rise to two strands that encompass the internal sac towards its opening inside the atrium.

Underneath the shells there are delicate *epidermal muscles* (em, Fig. 4C) composed of single fibers that form a striped pattern. They are pronounced at the pyriform organ and at the aboral posterior side of the shell.

At the inside of the center of the shells there insert five muscles on each side: One anterior and one posterior bundle of *lateral muscles* (la, Fig. 4C) run in oral direction towards the corona and the oral epithelium, where they insert after branching widely. Each is composed of a large number (> 20) muscle bands.

Additionally two strands protrude backwards to the roof of the internal sac. These are termed *internal sac muscles* (im, Fig. 4C, D). The bigger one inserts between the lateral muscles and runs in a curved way. It is very elaborate, about 25 µm in diameter and consists of about 20 muscle fibers. The smaller one consists of only about five muscle bands that insert more anteriorly, above the anterior lateral muscle and runs straight to the roof of internal sac.

On the inside of the shell there also inserts the *shell adductor muscle* (sa, Fig. 4D) connecting both the shells by running transversely through the larva in the area of the internal sac. It is a compact muscle around 15µm in diameter and composed of about ten muscle bands.

Circular and helicoidal muscle fibers surround the pharynx, the first section of the digestive tract (Fig. 4C, D). They form a basket-like structure when viewed from lateral.

Bugula fulva (Cheilostomata, Bugulidae)

This species has a barrel-shaped coronate larva of type AEO/ps (Zimmer and Woollacott 1977). Larval anatomy of the members of the bryozoan superfamily Celllarioidea has been described by Barrois (1877) and Calvet (1900). Reed and Woollacott (1982) investigated *B. neritina* in more detail.

The majority of the larval surface in *B.fulva* is formed by the corona (Fig. 5A). Only the apical organ and the small oral surface including the anterior pyriform organ and the posterior invagination site of the internal sac are distinguishable when viewed from exterior. On the anterior surface, between pyriform organ and internal sac, there is a small ciliated area, the ciliated cleft. The extensive apical disc is densely ciliated. Around the apical disc, the pallial
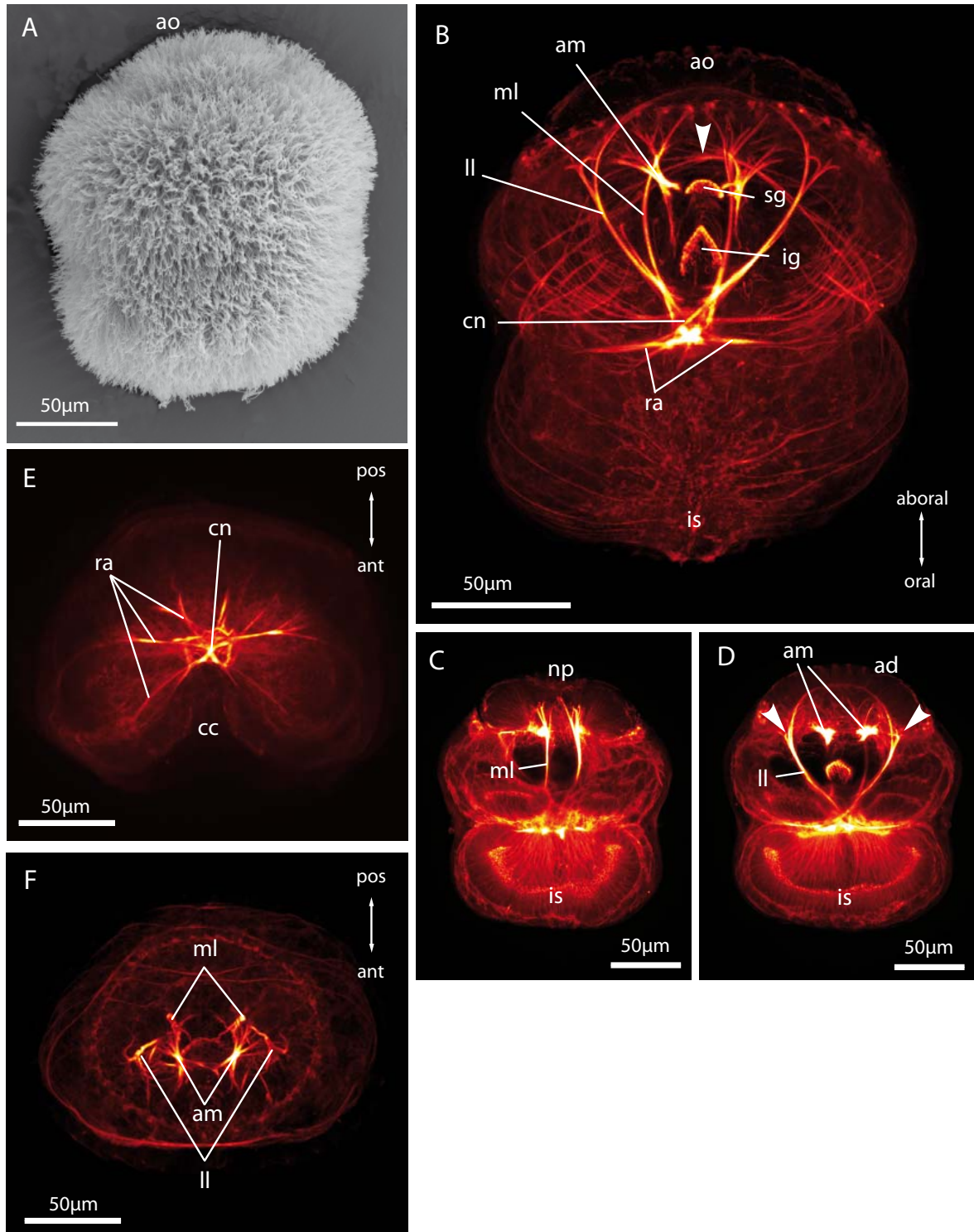
Fig. 5 *Bugula fulva*, larva. **A** SEM, View from posterior. **B-F** Phalloidin staining of larval musculature, projections of confocal image stacks. **B** View from posterior. A central muscular node (*cn*) gives rise to radial (*ra*) as well as to two pairs of longitudinal muscles (*ll, ml*). Paired anterior median muscles (*am*) connect apical organ (*ao*) and pyriform organ. Several sets of delicate circular fibers are found, especially in the aboral hemisphere, where they are arranged orthogonally in some areas. A concentration of circular fibers (*arrowhead*) is found anteriorly, between apical disc and pyriform organ. **c** Projection of horizontal optical sections in posterior part of larva, orientation of larva as in **b**. Median longitudinal muscles (*ml*) project deep into the apical disc (*ad*) towards the neural plate (*np*),

➤

epithelium is invaginated, forming a pallial sinus. The larva usually appears with the corona slightly constricted to a "waistline", dividing the body into an aboral and oral hemisphere.

A conspicuous subepidermal coronal *ring muscle* could not be observed, however several sets of delicate fibers encircle the larva (Fig. 5B). Some of them were given rise from three pairs of *radial muscles* (ra, Fig. 5B, E). The radial muscles originate at a central muscular node from where they at first protrude laterally towards the surface (Fig 5E), before diverging into several fine fibers. The anterior pair branches into fibers that encircle the aboral half of the larva. The middle pair gives branches to the anterior as well as to the posterior side. The anterior branches also encircle the larva's aboral hemisphere, running perpendicular to the first set of circular fibers. The fibers concentrate to a conspicuous strand between pyriform organ and apical organ (Fig. 5B). The posterior branches embrace the internal sac in the oral hemisphere. The anterior pair of radial muscles curve around the internal sac on its posterior side, terminating at its opening.

The paired *anterior median muscles* (am, Fig. 5B, D, F) are comparatively short. They insert right and left at the anterior side of the basis of the apical organ, from where they protrude towards the pyriform organ. Here they are attached laterally between superior and inferior glandular field. They are composed of several muscle bands that diverge considerably near the points of insertion.

The *median longitudinal muscles* (ml, Fig. 5B, C) originate at the central node and run towards the apical organ encompassing the pyriform organ on its posterior side. When reaching the center of the apical organ they branch into 4-6 fibers that attach to the basis of the neural plate (Fig. 5C, F).

The *lateral longitudinal muscles* (ll, Fig. 5B, D) also run from the central node towards the apical organ, but pass the pyriform organ laterally. Each muscle is composed of around five fibers. When they reach the lateral basis of the apical disc, some of the fibers insert there (Fig. 5F). The other fibers form an arc-like structure inside the apical disc, just anterior to the neural plate (Fig. 5D).

---

the central part of the apical disc. **D** Projection of median horizontal optical sections. Lateral longitudinal muscles (*ll*) branch at the base of the apical disc: Some fibers project in arc-like fashion into the apical disc, whereas some insert laterally at the base of the apical disc (*arrowheads*). **E** Projection of central optical cross-sections. Radial muscles (*ra*) originating at the central node (*cn*), above the ciliated cleft (*cc*), branch into more delicate fibers. Those coming from the anterior and median pairs encirle the larva's aboral hemisphere, those from the posterior pair encompass the internal sac on its posterior side towards its opening. **f** Projection of aboral optical cross-sections, showing the points of insertion of anterior median (*am*), lateral (*ll*) and median (*ml*) longitudinal muscles at the apical disc. *ant* anterior, *ig* inferior glandular field of pyriform organ, *is* internal sac, *pos* posterior, *sg* superior glandular field of pyriform organ
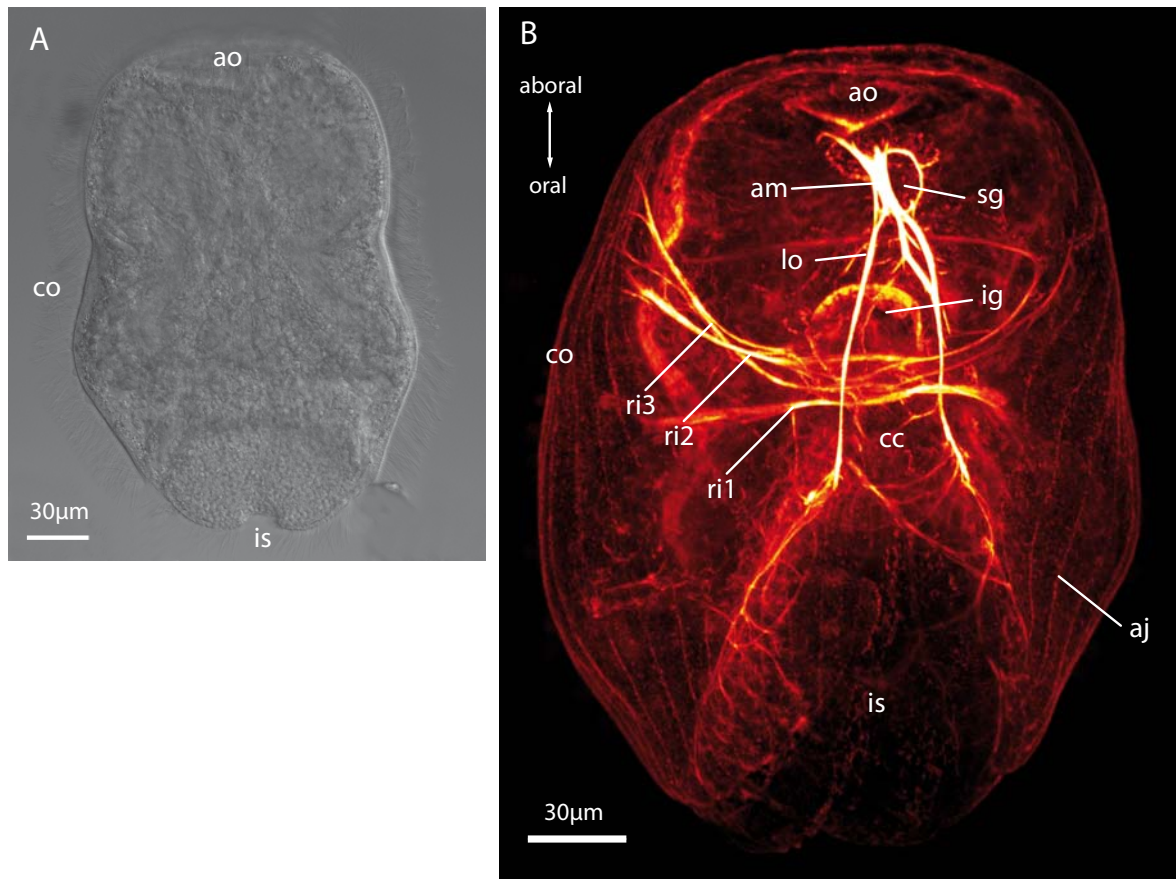
Fig. 6 *Bowerbankia gracilis* larva. **A** LM, view from posterior. **B** Phalloidin staining of larval muscu-
lature, projection of confocal image stack. View from anterior. Three ring muscles encircle the larva,
running parallelly on the anterior side. *ri1* parallels the corona, *ri2* runs more aborally on the poste-
rior side. *ri3* runs toward the apical organ, but nearly fades on the posterior side. An unpaired anterior
median strand (*am*) connects apical organ (*ao*) and pyriform organ. Paired longitudinal muscles (*lo*)
run from pyriform organ on both sides of the ciliated cleft (*cc*) towarts the internal sac. Apical adher-
ens junctions (*aj*) between corona (*co*) cells are visible. *ig* inferior glandular field of pyriform organ,
is internal sac, *sg* superior glandular field of pyriform organ

Bowerbankia gracilis (Ctenostomata, Vesiculariidae)

Morphology of the AEO/PS coronate larvae of the vesiculariid ctenostome *B. gracilis* (Fig.
6A) has been initially described by Repiachoff (1878) and investigated in greater detail es-
pecially by Reed (1980) and Reed and Cloney (1982a).

Though resembling the AEO/ps larvae in overall appearance, larvae of *B. gracilis* are char-
acterized through their smaller apical discs. The internal sac is also small and simple. It is
only slightly invaginated, consisting of thick columnar cells. In contrast, the pallial sinus is
much more pronounced. The larvae usually appeared a little elongate and often constricted
equatorially.

Three *ring muscles* (ri1-3, Fig. 6B) can be found. One circular muscle encompasses the
larva equatorially beyond the corona. A second ring muscle runs parallel to the coronal ring
muscle at the anterior side but more aborally on the posterior side. An additional incomplete

ring protrudes towards the apical organ, but fades on the posterior side. A small ring muscle encompasses the superior glandular field of the pyriform organ. Each ring muscle consists of a few muscle bands, that are usually more condensed on the anterior side of the larva.

A short unpaired *anterior median muscle* (am, Fig. 6B) runs from the center of the basis of the apical organ to the pyriform organ, where it inserts centrally between superior and inferior glandular field. This muscle has the largest diameter (4μm) of all the muscles found in the larva. It branches into single fibers at the points of insertion.

At the same point of the pyriform organ there insert two strands of *longitudinal muscles* (lo, Fig. 6B) that run on the anterior side of the larva, passing the ciliated cleft laterally from the pyriform organ towards the anterior side of the internal sac. They terminate at the basal lamina of the internal sac epithelium. The latter is underlain by a fine network of muscular fibers that concentrates towards the point of insertion of the longitudinal muscle.

Discussion

There appear certain discrepancies between my results on *Flustrellidra hispida* and a histological study conducted by Prouho (1890): Prouho observed a longitudinal muscle originating at the anterior aboral rims of the shells and terminating posteriorly at the corona. This could be a misinterpretation of the lateral radial muscles, which in fact terminate at the inner side of the shell. He found lateral vertical muscles only in the anterior part of the larva. Based on ultrastructural investigations, d'Hondt (1977a) described both the "axial muscle" (corresponding to the anterior median muscle in the present study) and the coronal ring muscle as striated. Furthermore the author found the axial muscle to consist of four strands each of which is formed by three to six muscle fibers. They form a sheath around the paired anterior nerve.

In the larva of *Alcyonidium polyoum* (Hassall, 1841), d'Hondt (1973a, 1973b) identified a striated anterior median muscle as well as a coronal ring muscle. Although I could not differentiate striated and smooth muscles, this resembles my results. *A. gelatinosum* and *A. polyoum* are very similar on morphological aspects and are regarded as closely related, although there has been considerable confusion in the taxonomy of the genus *Alcyonidium* in the past (see Ryland and Porter 2003, 2006).

Detailed ultrastructural information about the musculature in the cyphonautes of *Membranipora membranacea* is given in Stricker et al. (1988a, 1988b). The authors differentiate between four muscle groups. In addition to these groups, my data show delicate epidermal fibers and a second internal sac retractor. The posterior median muscle was reported to terminate at the larva's posterior end, but was observed to encompass the internal

sac on the oral side in my study. These additional data are only partly confirmed by Santagata (2008), who also described a second sac muscle, but located on the oral side of the gut.

Larva and metamorphosis in *Bugula neritina* (Linnaeus, 1758) have been described ultra-structurally by Reed and Woollacott (1982, 1983). The authors found a pair of muscles running from the apical disc to the papilla. On the aboral side these are connected to radial muscles extending into the apical organ. According to my results it seems more likely that the muscles are heavily branching when reaching the apical disc. Furthermore paired axial muscles are present that run from the apical disc towards the central nerve plexus. These seem to represent my lateral longitudinal muscles. The median longitudinal muscles were not found. The incongruences can be explained by different resolutions of the applied methods, but as the results are from different but closely related species, variation on the genus level can not be excluded. This is also the case for the data on *B. stolonifera* Ryland, 1960 and *B. neritina* obtained by Santagata (2008): Apart from an epidermal meshwork only one pair of muscles between apical disc and internal sac has been found in these two species.

Larval morphology of *Bowerbankia gracilis* has been described by Reed (1980) and Reed and Cloney (1982a), *Bowerbankia imbricata* (Adams, 1798) by d'Hondt (1977c). The musculature as reconstructed by Reed closely resembles the situation found here. The only difference is in the exact position of the aboral muscle ring, which is located more aborally in Reeds results. Interestingly he finds the anterior median muscle and the axial muscle each consisting of one large muscle cell.

Additional data from literature

Zimmer and Reed (1994) investigated *Tanganella muelleri* Kraepelin, 1887 (Cheilostomata, Victorellidae) (Fig. 8A). Interestingly, no axial muscular elements were found. The musculature mainly comprises two annular muscles, which are formed by supra- and infrapallial cells that contain myofilaments in their apical region. Furthermore, there is an equatorial muscle ring and a plexus of smooth fibers situated underneath the undifferentiated oral epidermis, anterior to the rudimentary gut. Results by Santagata (2008) on the victorellid *Nollela stipata* reveal a delicate apical disc retractor muscle and a further longitudinal muscle, suggesting considerable variation within this family.

In a study on the larva of the vesiculariid *Amathia vidovici* Heller, 1867 (Ctenostomata, Vesiculariidae) (Fig. 8B), Zimmer and Woollacott (1993) found an equatorial (coronal) as well as an aboral ring muscle. A massive apical retractor muscle connects the apical organ with the ciliated plaque of the pyriform organ. Furthermore there is an anterior constrictor muscle that runs backward from the epidermis on either side of the ciliated cleft. The paired strands converge towards the larval mid-line at the level of the ciliated plaque. Here they are

coupled to circumferential muscles enmeshing the cupiform layer and the internal sac. These results have also been confirmed by means of fluorescence staining by Santagata (2008).

In *Watersipora arcuata* Banta, 1969 (Cheilostomata, Watersiporidae) (Fig. 8C) Zimmer and Woollacott (1989) found this situation: A supracoronal ring muscle consisting of two to three annuli as well as a coronal ring muscle consisting of several annuli is present. Small fibers extend radially inwards from the coronal ring muscle. Paired paraxial muscles originate at the base of the apical organ where they are formed by converging fibers emanating from the apical disc. They connect to the epithelium of the ciliated groove of the pyriform complex. There occur further axial muscles: a pair of parietal muscles jointed to the ciliated ray cells of the apical complex extends towards the ciliated plaque epithelium. One or two pairs of oral aboral fibers occur near the anterior side. Additionally there are delicate parietal fibers running from the apical organ to the corona and a number of small fibers from the internal sac to the apical organ. From the paired axial muscle cords, anteroposterior connectives pass above the internal sac. A transverse muscle inserts beneath coronal cell four and five on each side. The authors also describe a "dendromuscular sheath", a meshwork of fibers giving rise to a body wall musculature.

Comparison of muscle groups

The use of confocal microscopy and phalloidin staining reveals that musculature is surprisingly complex in gymnolaemate larvae. Furthermore it shows tremendous differences among the five species studied here. Taking into account that this is only a small choice of representatives, and that larvae have been described in detail only for a weak percentage of known bryozoan species so far, it is suggested that much more variety still waits to be discovered. Despite these differences, it is possible to identify muscles that occur, sometimes in modified shape, in different larval types and/or bryozoan subtaxa.

Below, I define muscle groups that are likely to represent identical structures in gymnolaemate larvae (Figs. 7, 8). These definitions should be understood as representing primary homology hypotheses (de Pinna 1991), that are open to congruence tests, as soon as a phylogenetic tree is available. The muscle groups are primarily defined according to their position within the larva. I do not attach much weight on muscle striation patterns here, as it is not clear whether these are phylogenetically informative (see discussion in Schmidt-Rhaesa 2007 pp 80-84).

Anterior median muscle (AMM): paired or unpaired muscle connecting the apical organ and the pyriform organ.

This muscle is found in all species examined in this study as well as in the earlier ultrastructural studies discussed above except for *Tanganella muelleri*. It is also described in
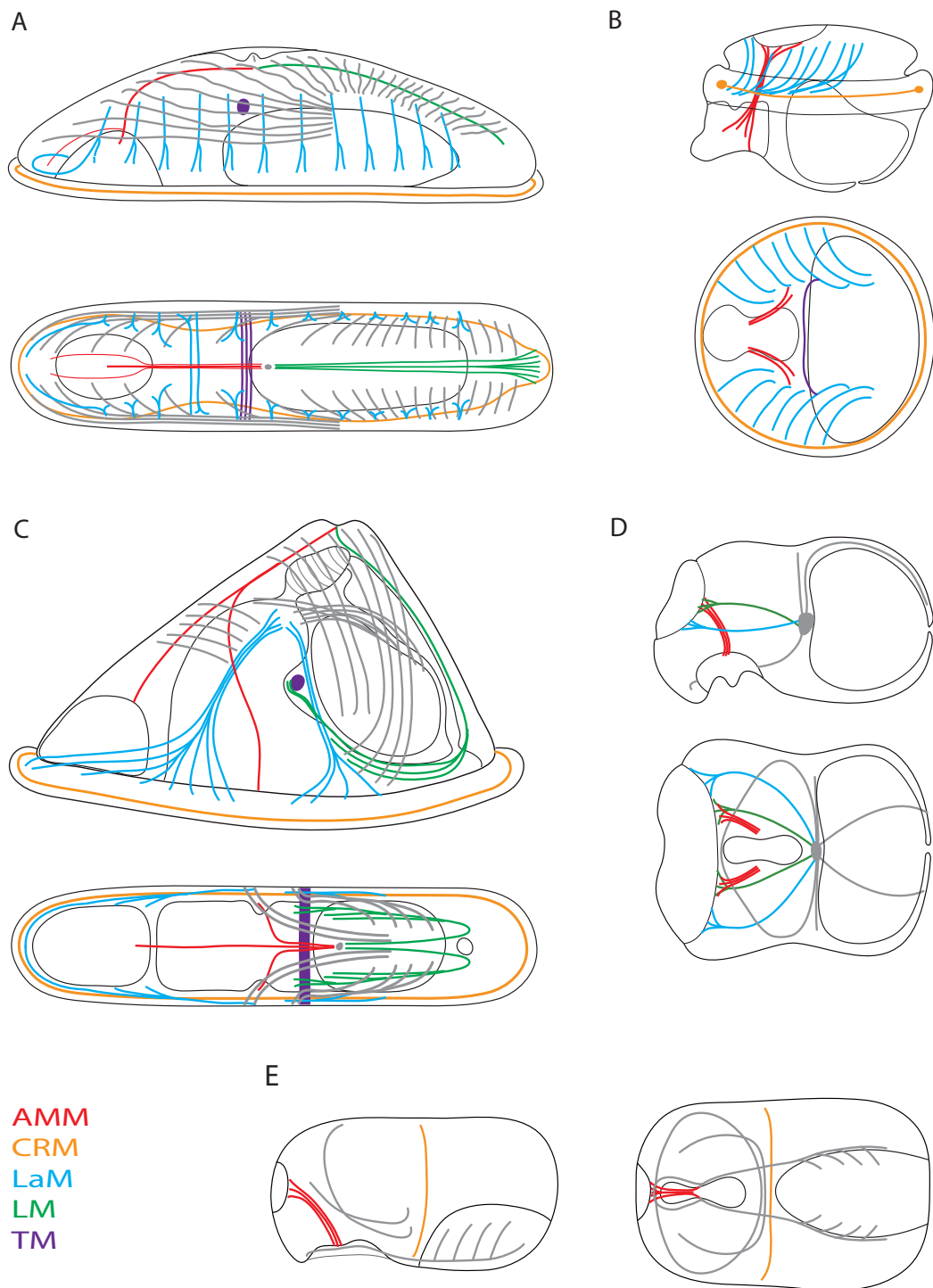
Fig. 7 Schematic representations summarizing results from this study. Color codes illustrate possible homology relationships between muscles of different larvae (see text for explanation and also Fig. 8). Gray lines indicate muscles for which homology is uncertain. **A** *Flustrellidra hispida,* top: view from lateral, bottom: view from aboral **B** *Alcyonidium gelatinosum,* top: view from lateral, bottom: view from aboral **C** *Membranipora membranacea,* top: view from lateral, bottom: view from aboral **D** *Bugula fulva,* top: view from lateral, bottom: view from posterior **E** *Bowerbankia gracilis* left: view from lateral, right: view from posterior. *AMM* anterior median muscle, *CRM* coronal ring muscle, *LaM* lateral muscles, *LM* longitudinal muscles, *TM* transversal muscle.
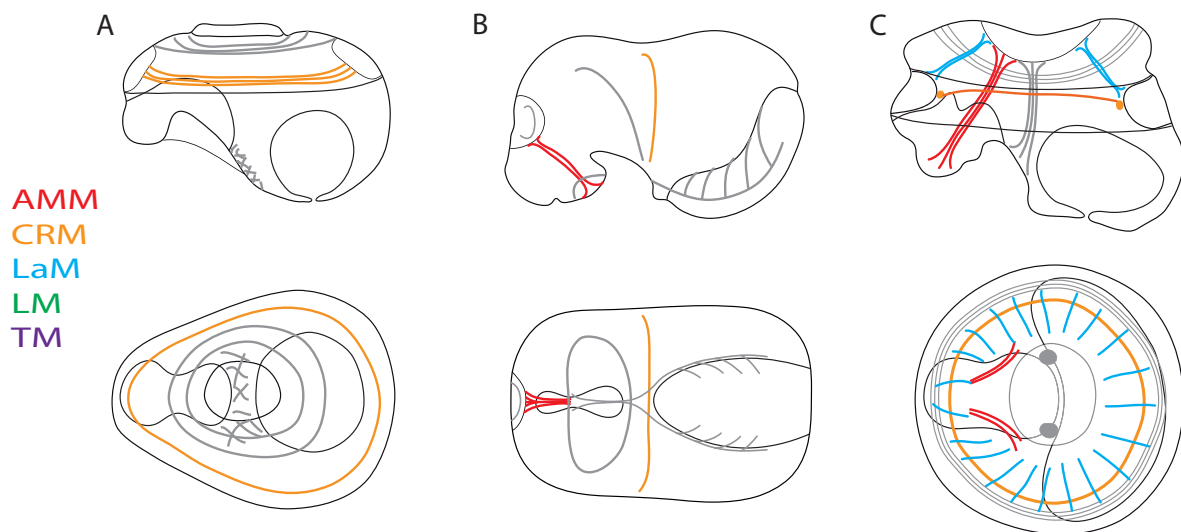
Fig. 8 Schematic representations illustrating data from descriptive literature. Color codes illustrate possible homology relationships between muscles of different larvae (see text for explanation and also Fig. 7). Gray lines indicate muscles for which homology is uncertain. **A** *Tanganella muelleri,* modified after Zimmer and Reed (1994), top: view from lateral, bottom: view from aboral. **B** *Amathia vidovic*i, modified after Zimmer and Woollacott (1993), top: view from lateral, bottom: view from posterior. **C** *Watersipora arcuata*, modified after Zimmer and Woollacott (1989), top: view from lateral, bottom: view from aboral. *AMM* anterior median muscle, *CRM* coronal ring muscle, *LaM* lateral muscles, *LM* longitudinal muscles, *TM* transversal muscle.

earlier histological descriptions of gymnolaemate larvae (Prouho 1890, 1892, Calvet 1900, Kupelwieser 1905). The AMM is mostly referred to as "neuromuscular strand", as it is often closely arranged with a nerve coming from the apical organ. However, this term implies that both elements, nerves and muscles, are intrinsically tied to each other, which is not the case for some larvae. This is the reason why I suggest using the neutral term AMM here. The AMM always originates at the base of the apical organ. Of course apical organs show considerable differences between the species, therefore it could help to elucidate in more detail where the muscles are attached exactly. On the oral side of the larva the AMM is always connected to the pyriform organ. It inserts either centrally at the papilla (the part of the pyriform organ that gives rise to the vibratile plume), or laterally in case it is a paired muscle. Slight differences in the points of insertion can be explained by variances in the structure of the pyriform organ in the different larvae. In some cases additional muscles insert at the pyriform organ, that concert with the AMM to mediate movements of the pyriform organ. The AMM can be either paired or unpaired, but in both cases it consist of several muscle fibers. Only Reed (1980) describes the AMM in *Bowerbankia gracilis* to consist of one single muscle cell. Since pyriform and apical organs are present and certainly homologous in all gymnolaemate larvae, homology of the anterior median muscle is very likely. Santagata (2008) in his study defines an apical disc retractor, based on the presumed function of this muscle during metamorphosis. However the retraction of the apical disc is only in some

species accomplished by the muscle between apical organ and pyriform organ, for example in *Membranipora membranacea,* (Stricker 1988) and in *Bowerbankia gracilis* (Reed and Cloney 1982b). In others, for example *Bugula neritina* (Reed and Woollacott 1982) longitudinal muscles between apical organ and internal sac perform this movement.

Coronal ring muscle CRM: circular muscle concentric to the corona or in case of expanded coronal cells, the ring of nuclear regions of the coronal cells.

The CRM is present in all the species treated here, except *Bugula fulva* where only delicate circular fibers exist basiepidermally. However, these could correspond to the CRM in other species. They could be reduced in this species, because here radial muscles occur that can serve a similar function: an equatorial constriction of the larva. Where the corona is expanded like in the *Bowerbankia gracilis* the CRM runs concentric to the nuclear regions of the coronal cells. It also runs parallel to a ring nerve beyond the corona. There are obvious structural differences among some of the mentioned species. These regard the nature and origin of the cells that make up the muscle. In most cases the coronal ring muscle consists of continuous bands, indicating its origin from mesodermal, unpolarized muscle cells. In *Membranipora membranacea* and *Alcyonidium gelatinosum,* the ring muscle seems to consist of concatenated muscle blocks, indicating its origin from myoepithelial cells. Stricker et al. (1988a) in their ultrastructural study on *M. membranacea* came to similar results and furthermore identified supracoronal cells to form the muscle. This is hard to interpret in a phylogenetic way. It is not clear whether these differences argue for a convergent origin in the mentioned species. As long as there is no further information, I hypothesize the CRM as homologous.

Lateral muscles LaM: muscle strands inserting at the pallial epithelium (aboral epidermis) on one side and at the corona or at the oral epithelium on the other side.

Comparable LaM occur in *M. membranacea*, *F. hispida*, and *A. gelatinosum*. The muscles insert at the aboral epithelium (which itself is attached to the shells in *F. hispida* and *M. membranacea*). They head towards the oral side where they insert at the corona or in close proximity, at the oral epithelium. In *Watersipora arcuata* alike muscles occur, that run peripherally from the apical disc to the corona (Zimmer and Woollacott 1989). The lateral longitudinal muscles in *B. fulva* also originate laterally from the apical disc and project towards the central node. The apical discs are complex in both the latter species so the aboral insertion points could have shifted from pallial epithelium to apical disc. In the larvae of the ctenostomes *Bowerbankia gracilis* (this study) and *Tanganella muelleri* (Zimmer and Reed 1994) no resembling muscles can are found. In the three first mentioned species, that exhibit lateral muscles, there occur also transversal muscles, either in the form of shell adductors like in *M. membranacea* and *F. hispida* or as muscle connecting right and left side of the

pallial epithelium like in *A. gelatinosum*. These muscles always originate near the points of insertion of the LM, suggesting that they have the same muscular precursors.

Longitudinal muscles LM: Muscles that run from the apical organ towards the internal sac.

Muscles that parallel the larva's sagittal plane are present in all larvae, but occur in numerous shapes. In *F. hispida* and *M. membranacea*, posterior median muscles can be found. Obviously identical here, it is difficult to find resembling structures in the other larvae. Only in *B. fulva*, the median longitudinal muscle could represent a homologue: It runs nearly posteriorly from the apical organ towards the internal sac. In *Watersipora arcuata*, a pair of muscles runs from the apical organ towards the oral epithelium between pyriform organ and internal sac (Zimmer and Woollacott 1989). In larvae of both the vesiculariid species, *B. gracilis* (this study) and *A. vidovici* (Zimmer and Woollacott 1993), a longitudinal strand connects pyriform organ and internal sac on the anterior side. Although obviously of homologous shape in these two closely related species, it can hardly be brought into resemblance to the LM as mentioned above. In *Alcyonidium gelatinosum* (this study) and *Tanganella muelleri* (Zimmer and Reed 1994) no equivalent longitudinal muscles are present.

Musculature and evolution of bryozoan larval types

As outlined above, there are presumably phylogenetically significant similarities in the muscular arrangement in different bryozoan larvae. Despite a still insufficient taxon sampling, some conclusions can be drawn: The cyphonautes larva shows the greatest diversity of muscles, obviously due to its complex morphology with feeding structures and functional digestive tract. Muscular systems in coronate forms are, though more complex than expected, still much simpler. In many cases, potential homologues of the muscle groups of cyphonautes larvae can be identified in coronate larvae. Most of these muscles are involved in morphogenetic movements during metamorphosis (Reed 1991). Furthermore, superficially similar larvae like e.g. buguliform (Fig. 7D) and vesiculariid (Figs. 7E, 8D) larvae show strong differences in their muscular arrangement. Musculature of shelled lecithotrophic larvae of *Flustrellidra hispida* and type E coronate larvae of *Alcyonidium gelatinosum* shows much more correspondence to cyphonautes larvae than to all other coronate forms.

Evolution of gymnolaemate larvae is still controversially discussed. Most authors regard the cyphonautes as the ancestral larval form for Gymnolaemata, especially because of its occurence in both Ctenostomata and Cheilostomata (e.g., Nielsen 1971, Strathmann 1978). Lecithotrophic forms therefore must have originated at least twice within Gymnolaemata. This view is strongly supported by the present study since especially ctenostome and cheilostome coronate larvae show considerable differences in their musculature. For cheilostome bryozoans. Taylor (1988) postulated, based on fossils, a singular origin of lecithotrophy. Ctenostomes unfortunately lack an extensive fossil record. However, the differences found

in larval musculature of coronate forms within both Cheilostomata and Ctenostomata could as well argue for a multiple origin of this larval type. While, for the above-mentioned reasons, it is parsimonous to assume a cyphonautes type larva as ancestral for Gymnolaemata, it is hard to find similarities to the larval musculature in Cyclostomata and Phylactolaemata:

Cyclostomes have gutless spherical larvae lacking both the apical and the pyriform organ. One end bears an epidermal invagination possibly identical to the pallial sinus in gymnolaemates. On the opposite side an internal sac is present. The remaining epidermis is uniformly ciliated (Nielsen 1970). Simplicity of cyclostome larvae is usually interpreted in correlation to the highly derived type of reproduction and embryogenesis in this group (Reed 1991). In *Crisia denticulata* (Lamarck, 1816) d'Hondt (1977b) described two lateral smooth strands connecting the periphery of the pallial sinus to the oral side of the internal sac. Santagata (2008) found the musculature in the larva of *Crisia elongata* Milne Edwards, 1838 represented by about 20 thin fibers that insert at the center of the invaginated pallial sinus and embrace the internal sac. These muscles could therefore either correspond to lateral muscles (LaM) or longitudinal muscles (LM) in gymnolaemate larvae.

Phylactolaemates have completely different larval stages that can hardly be compared to stenolaemate or gymnolaemate larvae. These resemble more or less elongated ovoids that already bear one or several invaginated polypid anlagen. Their epidermis is completely ciliated, but a distinct corona is lacking (Braem 1897, 1908, Rogick 1939). Homologues of the organs characteristic for gymnolaemate larvae cannot be found. On the opposite side of the polypid bud a concentration of nerve cells is found (Marcus 1926, Franzén and Sensenbaugh 1983), but homology to the apical organ in gymnolaemate larva remains doubtful. Several muscles are found within the larva (Rogick 1939), but these are either precursors of the adult muscles, like polypid retractors or incomparable to gymnolaemate larval muscles because of the lack of positional markers as described above. However, a detailed analysis of phylactolaemate larval musculature is still missing.

There is now condensing evidence that Bryozoa group somewhere in the Lophotrochozoa (Passamaneck and Halanych 2004, Philippe et al. 2005,Passamaneck and Halanych 2006, Waeschenbach et al. 2006). However, it is quite difficult to try to homologize the elements of the bryozoan muscular system to that of other lophotrochozoan larvae: Phoronid actinotrocha larvae show a completely different musculature (Santagata and Zimmer 2002). Prototroch ring muscles are present in trochophore larvae of capitellid (Hill and Boyer 2001) and serpulid polychaetes (McDougall et al. 2006) as well as in aplacophoran (Nielsen et al. 2007), gastropod (Wanninger et al. 1999), and polyplacophoran (Wanninger and Haszprunar 2002) mollusc larvae. (Haszprunar and Wanninger in press) found a row of myoepithelial cells in the prototroch of the larva of the entoproct *Loxosomella murmanica* (Nilus, 1909).

Nemertean pilidium larvae seem to have retractor muscles associated with their apical organs (Lacalli and West 1985).

The present study shows a complex musculature in all types of gymnolaemate larvae. Considerable differences especially between the various coronate forms argue for their convergent origin. Many functional aspects of larval muscles, for example their function during metamorphosis need be elucidated in more detail.

Acknowledgments

References

Atkins D (1955) The cyphonautes larvae of the Plymouth area and the metamorphosis of *Membranipora membranacea*. J Marine Biol Assoc UK 34:441-449

Barrois J (1877) Recherches sur l' embryologie des Bryozoaires. Travaux de l'institut zoologique de Lille Fasc. 1:1-307 + 16 pls

Boardman RS, Cheetham AH, Blake DB, Utgaard J, Karklins OL, Cook PL, Sandberg PA, Lutaud G, Wood TS (1983) Bryozoa. Treatise on invertebrate palaeontology, part G. Geological Society of America and University of Kansas Press, Kansas

Braem F (1897) Die geschlechtliche Entwicklung von *Plumatella fungosa*. Zoologica 10:1-96

Braem F (1908) Die geschlechtliche Entwicklung von *Fredericella sultana*. Zoologica 52:1-37

Calvet L (1900) Contribution à l'histoire naturelle des bryozoaires ectoproctes marins. Trav Inst Zool Univ Montpellier 8:1-488

de Pinna MCC (1991) Concepts and tests of homology in the cladistic paradigm. Cladistics 7:367-394

d`Hondt JL (1973a) Etude anatomique, histologique, et cytologique de la larve d'*Alcyonidium polyoum* (Hassal, 1841). Arch zool exp gén 114:537-602

d`Hondt JL (1973b) La musculature de la larve d'*Alcyonidium polyoum* (Hassall), Bryozoaire Cténostome. Comparaison avec la musculature larvaire d'autres Bryozoaires Eurystomes. C R Acad Sci (Paris) Ser D 276:1449-1452

d`Hondt JL (1975) La metamorphose larvaire et la realisation du "cystide" chez *Alcyonidium polyoum* (Hassal 1841). Arch zool exp gén 115:577-605

d`Hondt JL (1977a) Larval structure and post-larval organogenesis of *Flustrellidra hispida* (Fabricius, 1780), Bryozoa, Ctenostomata. Zoomorphologie 87:165-189

d`Hondt JL (1977b) Structure larvaire et histogenèse post-larvaire chez *Crisia denticulata* (Lamarck) (Bryozoa, Cyclostomata, Articulata). Zool Scr 6:55-60

d`Hondt JL (1977c) Structure larvaire et histogenese postlarvaire chez *Bowerbankia imbricata* (Adams 1798). Arch zool exp gén 118:211-243

Ernst A, Schäfer P (2006) Palaeozoic vs. post-Palaezoic Stenolaemata: phylogenetic relationship or morphological convergence? Courier Forsch -Inst Senckenberg 257:49-63

Franzén Å, Sensenbaugh T (1983) Fine structure of the apical plate in the larva of the freshwater Bryozoan *Plumatella fungosa* (Pallas) (Bryozoa: Phylactolaemata). Zoomorphology 102:87-98

Fuchs J, Bright M, Funch P, Wanninger A (2006) Immunocytochemistry of the neuromuscular systems of *Loxosomella vivipara* and *L. parguerensis* (Entoprocta: Loxosomatidae). J Morphol 267:866-883

Haszprunar G, Wanninger A (2000) Molluscan muscle systems in development and evolution. J Zool Syst Evol Res 38:157-163

Haszprunar G, Wanninger A (2008) On the fine structure of the creeping larva of *Loxosomella murmanica*: additional evidence for a clade of Kamptozoa (Entoprocta) and Mollusca. Acta Zool in press:

Hayward PJ (1985) Ctenostome Bryozoans. E.J. Brill/Dr.W. Backhuys, London

Hayward PJ, Ryland JS (1998) Cheilostomatous Bryozoa. Field Studies Council, Shrewsbury

Hill SD, Boyer BC (2001) Phalloidin labeling of developing muscle in embryos of the polychaete Capitella sp I. Biol Bull 201:257-258

Jenner RA, Schram FR (1999) The grand game of metazoan phylogeny: rules and strategies. Biol Rev 74:121-142

Kupelwieser H (1905) Untersuchungen über den feineren Bau und die Metamorphose des Cyphonautes. Zoologica 47:1-50

Lacalli TC, West JE (1985) The Nervous-System of A Pilidium Larva - Evidence from Electron-Microscope Reconstructions. Can J Zool 63:1909-1916

Leasi F, Rothe BH, Schmidt-Rhaesa A, Todaro MA (2006) The musculature of three species of gastrotrichs surveyed with confocal laser scanning microscopy (CLSM). Acta Zool 87:171-180

Marcus E (1926) Sinnesphysiologie und Nervensystem der Larve von *Plumatella fungosa* (Pall.). Verh Dtsch Zool Ges 31:86-90

McDougall C, Chen WC, Shimeld SM, Ferrier DE (2006) The development of the larval nervous system, musculature and ciliary bands of *Pomatoceros lamarckii* (Annelida): heterochrony in polychaetes. Front Zool 3:16-

McKinney FK, Jackson JBC (1989) Bryozoan life histories. In: Bryozoan evolution. Unwin Hyman, Boston, pp 97-118

Müller MCM, Worsaae K (2005) CLSM Analysis of the phalloidin-stained muscle system in *Nerilla antennata, Nerillidium* sp. and *Trochonerilla mobilis* (Polychaeta; Nerillidae). J Morphol 267:885-896

Nielsen C (1970) On metamorphosis and ancestrula formation in cylostomatous bryzoans. Ophelia 7:217-256

Nielsen C (1971) Entoproct life-cycles and the entoproct/ectoproct relationship. Ophelia 9:209-341

Nielsen C, Haszprunar G, Ruthensteiner B, Wanninger A (2007) Early development of the aplacophoran mollusc *Chaetoderma*. Acta Zool 88:231-247

Pace RM (1906) On the early stages in the development of Flustrella hispida (Fabricius), and on the existence of a -yolk nucleus- in the egg of this form. Q J Microsc Sci 50:435-478 + 4 pls.

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

Philippe H, Lartillot N, Brinkmann H (2005) Multigene analyses of bilaterian animals corroborate the monophyly of Ecdysozoa, Lophotrochozoa and Protostomia. Mol Biol Evol 22:1246-1253

Prouho H (1890) Recherches sur la larve de *Flustrellidra hispida*: Structure et metamorphose. Arch Zool Exp Gén 8 (2. Ser.):409-459

Prouho H (1892) Conribution a l'histoire des bryozoaires. Arch zool exp gén 10 (2.Ser.):557-656

Purschke G, Müller MCM (2006) Evolution of body wall musculature. Integ Comp Biol 46:497-507

Reed,CG (1980) The reproductive biology, larval morphology, and metamorphosis of the marine bryozoan, *Bowerbankia gracilis* (Vesicularioidea, Ctenostomata). PhD Thesis, University of Washington, Seattle, WA, USA

Reed CG (1985) The many motors of morphogenesis. The roles of muscles, cilia, and microfilaments in the metamorphosis of marine bryozoans. In: Sawyer RH, Showman RM (eds) The Cellular and Molecular Biology of Invertebrate Development. University of South Carolina Press, Columbia, pp 197-219

Reed CG (1991) Bryozoa. In: Giese AC, Pearse JS, Pearse VB (eds) Reproduction of Marine Invertebrates VI Echinoderms and Lophophorates. Boxwood Press, Pacific Groove, California, pp 85-245

Reed CG, Cloney RA (1982a) The larval morphology of the marine bryozoan *Bowerbankia gracilis* (Ctenostomata: Vesicularioidea). Zoomorphology 100:23-54

Reed CG, Cloney RA (1982b) The settlement and metamorphosis of the marine bryozoan *Bowerbankia gracilis* (Ctenostomata: Vesicularioidea). Zoomorphology 101:103-132

Reed CG, Woollacott RM (1982) Mechanisms of rapid morphogenetic movements in the metamorphosis of the bryozoan *Bugula neritina* (Cheilostomata, Cellularioidea) I. Attachment to the substratum. J Morphol 172:335-348

Reed CG, Woollacott RM (1983) Mechanisms of rapid morphogenetic movements in the metamorphosis of the bryozoan *Bugula neritina* (Cheilostomata, Cellularioidea) II. The role of dynamic assembages of microfilaments in the pallial epithelium. J Morphol 177:127-143

Repiachoff W (1878) Zur Embryologie der *Bowerbankia*. Zool Anz 1-4

Rogick M (1939) Studies on fresh-water Bryozoa VIII. Larvae of *Hyalinella punctata*. Trans Am Microsc Soc 58:199-209

Ryland JS, Porter JS (2003) The identity of Alcyonidium gelatinosum (Linnaeus, 1761) (Bryozoa : Ctenostomatida). J Nat Hist 37:2179-2189

Ryland JS, Porter JS (2006) The identification, distribution and biology of encrusting species of *Alcyonidium* (Bryozoa, Ctenostomatida) around the coast of Ireland. Biol Environ 106B:19-33

Santagata S (2008) The morphology and evolutionary significance of the ciliary fields and musculature among marine bryozoan larvae. J Morphol in press:

Santagata S, Zimmer RL (2002) Comparison of the neuromuscular systems among actinotroch larvae: systematic and evolutionary implications. Evol Dev 4:43-55

Schmidt-Rhaesa A (2007) The evolution of organ systems. Oxford University Press, New York

Schmidt-Rhaesa A, Kulessa J (2007) Muscular architecture of Milnesium tardigradum and Hypsibius sp. (Eutardigrada, Tardigrada) with some data on Ramazottius oberhaeuseri. Zoomorphology 126:265-281

Seeliger O (1906) Über die Larven und Verwandschaftsbeziehungen der Bryozoen. Z wiss Zool 84:1-78

Strathmann RR (1978) The evolution and loss of feeding larval stages of marine invertebrates. Evolution 32:894-906

Stricker SA (1988) Metamorphosis of the-marine bryozoan *Membranipora membranacea*: an ultrastructural study of rapid morphogenetic movements. J Morphol 196:53-72

Stricker SA, Reed CG, Zimmer RL (1988a) The cyphonautes larva of the marine bryozoan *Membranipora membranacea*. I. General morphology, body wall, and gut. Can J Zool 66:368-383

Stricker SA, Reed CG, Zimmer RL (1988b) The cyphonautes larva of the marine bryozoan *Membranipora membranacea*. II. Internal sac, musculature, and pyriform organ. Can J Zool 66:384-398

Ström R (1977) Brooding patterns of bryozoans. In: Woollacott RM, Zimmer RL (eds) Biology of bryozoans. Academic Press, New York, pp 23-55

Taylor PD (1988) Major radiations of Cheilostome Bryozoans: triggered by a new larval type? Hist Biol 1:45-64

Temkin MH (1994) Gamete spawning and fertilization in the gymnolaemate bryozoan *Membranipora membranacea*. Biol Bull Woods Hole 187:143-155

Temkin MH (1996) Comparative fertilization biology of gymnolaemate bryozoans. Mar Biol 127:329-339

Todd JA (2000) The central role of ctenostomes in bryozoan phylogeny. In: Herrera-Cubilla A, Jackson JBC (eds) Proceedings of the 11th International Bryozoology Association Conference. Smithsonian Tropical Research Institute, Balboa, R.P., pp 105-135

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

Wanninger A (2004) Myo-anatomy of juvenile and adult loxosomatid entoprocta and the use of muscular body plans for phylogenetic inferences. J Morphol 261:249-257

Wanninger A, Haszprunar G (2002) Chiton myogenesis: Perspectives for the development and evolution of larval and adult muscle systems in molluscs. J Morphol 251:103-113

Wanninger A, Koop D, Bromham LD, Noonan E, Degnan BM (2005) Nervous and muscle system development in *Phascolion strombus* (Sipuncula). Dev Genes Evol 215:509-518

Wanninger A, Ruthensteiner B, Lobenwein S, Salvenmoser W, Dictus WJAG, Haszprunar G (1999) Development of the musculature in the limpet *Patella* (Mollusca, Patellogastropoda). Dev Genes Evol 209:226-238

Zimmer RL, Reed CG (1994) Morphology and ultrastructure of the larva of the bryozoan *Tanganella muelleri* (Ctenostomata: Victorellidae). In: Wilson WH (eds) Reproduction and development of marine invertebrates : Papers from a symposium held at Friday Harbor Laboratories of the University of Washington, June 9 - 11. Johns Hopkins Univ. Press, Baltimore, Maryland, pp 224-245

Zimmer RL, Woollacott RM (1977) Structure and classification of gymnolaemate larvae. In: Woollacott RM, Zimmer RL (eds) Biology of bryozoans. Academic Press, New York, pp 57-89

Zimmer RL, Woollacott RM (1989) Larval morphology of the bryozoan *Watersipora arcuata* (Cheilostomata: Ascophora). J Morphol 199:125-150

Zimmer RL, Woollacott RM (1993) Anatomy of the larva of *Amathia vidovici* (Bryozoa: Ctenostomata) and phylogenetic significance of vesiculariform larva. J Morphol 215:1-29