# Ultrastructure and development of the body cavities in *Antedon bifida* (Pennant, 1777) (Comatulida, Crinoida)

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Hiermit erkläre ich, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe der Quelle gekennzeichnet.

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# i Abbreviations

| | |
|---|---|
| *A, B, C, D, E* | radii |
| *AB, BC, CD, DE, EA* | interradii |
| *a* | anterior |
| *abo* | aboral |
| *aboac* | aboral arm coelom |
| *abol* | aboral lacuna (***light coral***) |
| *abolsc* | oral somatocoel below oral lacunar meshwork (***black***) |
| *abons* | aboral nervous system (***citrus yellow***) |
| *abosec* | aboral section of the axial extra-coelomic space (***light coral***) |
| *abovm* | aboral vertical mesentery (***light coral***) |
| *ac* | axocoel (***yellow***) |
| *acd* | axocoelomic duct |
| *acf* | fused axocoel |
| *ahc* | axohydrocoel |
| *aj* | adherens junction |
| *an* | anus |
| *anc* | anal tube coelom |
| *ant* | anal tube |
| *ap* | adhesive pit |
| *arc* | arm coelom |
| *arch* | archenteron |
| *as* | axial sinus |
| *axc* | axial compartment |
| *axlsc* | axial extension of the oral somatocoel (***black***) |
| *axm* | axial mesentery |
| *axmus* | axial muscle (***crimson red***) |
| *bc* | main body cavity |
| *bl* | basal lamina |
| *bla* | blastocoel |
| *bp* | basal plate |
| *br1p* | first brachial plate |
| *bsi* | bullet shaped inclusion |
| *bw* | body wall (***grey***) |
| *bwb* | body wall bulges (***old rose***) |
| *bwp* | body wall protrusion (***old rose***) |
| *cf* | collagen fibrils |
| *chl* | centro-horizontal branch of the oral lacuna (***pale green***) |
| *cho* | (prospective) chambered organ (***red***) |
| *cir* | cirrus |
| *cp* | columnal plate |
| *csec* | central section of the axial extra-coelomic space |
| *d* | dorsal |
| *dmp* | distal mesenteric part of the axial extra-coelomic space |
| *dp* | deltoid (or oral) plate |
| *eahc* | enteroaxohydrocoel |
| *ecc* | cluster of large epithelial cells |
| *ecm* | extra-cellular matrix |
| *ect* | ectoderm |
| *end* | endoderm |
| *epi* | epidermis (***greyish***) |
| *epins* | epidermal nervous system (***warm yellow***) |
| *er* | endoplasmic reticulum |
| *es* | enteric sac (***green***) |
| *eso* | esophagus (***green***) |
| *fg* | food groove |
| *gao* | glandular axial organ |
| *gaol* | glandular axial organ lacuna |
| *gaos* | epithelial sheath of the glandular axial organ |
| *gaot* | glandular axial organ tubule (***turquoise***) |
| *hc* | hydrocoel (***blue***) |
| *hp* | hydropore (***yellow***) |
| *hs* | horizontal septum |
| *hw* | horizontal wall (***old rose***) |
| *hyns* | hyponeural nervous system (***greenish yellow***) |
| *int* | intestinal outgrowth/intestine |
| *l* | left |
| *lE* | left extension of the left/oral somatocoel (***black***) |

| | |
|---|---|
| *lL* | left lobe of the right/aboral somatocoel (***red***) |
| *lsc* | left/oral somatocoel (***black***) |
| *m* | mouth |
| *mes* | mesenchyme |
| *mesc* | mesenchyme cell |
| *mp* | madrepore (***yellow***) |
| *mt* | mitochondrium |
| *mus* | muscle |
| *nt* | neurite |
| *nu* | nucleus |
| *o* | oral |
| *oac* | oral arm coelom |
| *ol* | oral lacuna (***white***) |
| *olm* | oral lacunar meshwork (***old rose***) |
| *olsc* | oral somatocoel above oral lacunar meshwork (***dark grey***) |
| *osec* | oral section of axial extra-coelomic space (***pale grey***) |
| *ov* | oral valve |
| *ovm* | oral vertical “mesentery” |
| *p* | posterior |
| *pc* | peripheral compartment |
| *peril* | periesophageal lacuna (***pale yellow***) |
| *perimf* | periesophageal lacunar formation |
| *pmp* | proximal mesenteric part of the axial extra-coelomic space |
| *pod* | podium |
| *psc* | primary somatocoel |
| *r* | right |
| *rE* | right extension of the left/oral somatocoel (***black***) |
| *rL* | right lobe of the right/aboral somatocoel (***red***) |
| *rp* | radial plate |
| *rsc* | right/aboral somatocoel (***red***) |
| *sabovm* | secondary aboral vertical mesentery (***skin color***) |
| *sac* | sacculus |
| *snc* | sub-neural canal |
| *stc* | primary stone canal *and/or* secondary stone canals (***purple***) |
| *ste* | stereom |
| *sto* | stomach (***green***) |
| *svc* | small ventral coelom (***orange***) |
| *td* | *tegmenal duct* |
| *v* | ventral |
| *vb* | vestibulum (***white***) |
| *vl* | ventral lacuna (***pale blue***) |
| *vmf* | ventral mesenteric formation (***pale orange skin color***) |
| *ys* | yolk sphere |

# 1 Introduction

The postembryonic development of comatulid crinoids is characterized by a sequence of three different types of larval stages: (1) The bilaterally symmetric, lecitotrophic and swimming doliolaria stage. This attaches itself with its anterior pole and undergoes metamorphosis to (2) the stalked (sessile) non-feeding cystidean stage, inside of which both the intestinal tract as well as the first pentaradial-symmetric structures differentiate. As both the mouth and the anus of the larva become functional, the cystidean larva transforms into (3) the feeding pentacrinoid stage, which undergoes several morphological alterations, thereby consolidating the pentaradial symmetry; the pentacrinoid larva develops, amongst other structures, five arms, aboral cirri and an anal cone and finally detaches from the stalk. It thereby develops into the free-living juvenile stage, which is not considered a larva anymore as it has the lifestyle of an adult (Mladenov and Chia 1983, Lahaye and Jangoux 1987).

Previous comprehensive studies that describe crinoid larval coelomic development from dolioaria larva to juvenile stage have been carried out at the end of the nineteenth and in the early twentieth century using light microscopic data (Perrier 1886, Bury 1888, Seeliger 1893, Russo 1902, Mortensen 1920). These former authors gave highly valuable and detailed descriptions of their observations that were illustrated by detailed drawings of selected sections. Following these studies, four coelomic cavities, the axocoel, the hydrocoel, the left (=oral) and the right (=aboral) somatocoel are formed in the embryo. These coeloms are first arranged in a basically bilateral pattern around the enteric sac in the doliolaria. During the metamorphosis to the cystidean and the subsequent transformation to the pentacrinoid stage, the hydrocoel as well as the two somatocoelia undergo major displacing movements that lead to the pentaradial symmetry of the adult. The axocoel has been found to fuse with the somatocoelia, which, around the same time, merge as well. The illustrations of these former authors, however, were limited by the means of their time and for nowaday readers, it is difficult to understand the long, partly prosaic descriptive texts. Also, the use of terms varies between the old works and partially differs from those that are in use today, which complicates the reading of original literature enormously.

Several helpful and necessary attempts to review the older literature and to put them into a modern language have been carried out (Clark 1921, Dawydoff 1948, Hyman 1955, Nichols 1967, Holland 1991). However, unpreventably, detailed information – especially on coelomic cavity development - got simplified, lost or became ambiguous in this process. In addition, no modern illustrations based on original data have been created for these guides. Instead, as simplification was required, only the clearest and simplest figures were taken from the older literature, giving an idea about the location of the structure of interest, but leaving a lot of room for the reader's imagination concerning many details.

The most recent accounts on the embryonic development of *Oxycomanthus japonicus* (=formerly *Comanthus japonica*) using light microscopy, scanning electron microscopy and transmission electron microscopy are given by Holland (1976, 1978). Chia et al. (1986) describe the doliolaria of *Florometra serratissima*, with strong emphasis on ectodermal differentiations. The larval development has more recently been traced in several species: Holland and Kubota (1975) describe and illustrate the larval development of *O. japonicus* using SEM. Mladenov and Chia (1983) give a detailed overview on the full larval development of *F. serratissima* from zygote through sessile larval stages, comprising original light microscopic and scanning electron microscopic data on external morphology as well as larval behavior. Accordingly, the larval development of both *Antedon bifida* (Lahaye 1987; Lahaye and Jangoux 1985) and, most recently, *Antedon mediterranea* (Barbaglio et al. 2012) is described and illustrated using modern techniques. Kohtsuka and Nakano (2005) investigated the larval development of the colobometrid crinoid *Decametra tigrina* and Haig and Rouse (2008) that of *Aporometra wilsoni*.

Amongst these works, Barbaglio et al. (2012) represents the only careful analysis of crinoid development that also addresses the inner anatomy of the larva. These authors, however, do not trace the fate of the two somatocoelia individually, but merely indicate the position of the "two somatocoelomic canals" in general, thereby neglecting the fate of the axocoel. This approach shows the tremendous need to reinstate the results of the earlier works, in which the development of these coelomic cavities had already been revealed more extensively.

In addition to these works, several recent works focus on single aspects of crinoid larval development using electron microscopic methods: The ultrastructure of the doliolaria's epidermis (Chia et al. 1986) was investigated, as well as the larva's attachment process during metamorphosis (Lahaye and Jangoux 1991). Also, the development of the pentacrinoid larva's chambered organ in the stalk was described in ultrastructural detail (Grimmer et al. 1984), and scanning electron microscopic investigations on larval sclerite development (Lahaye and Jangoux 1987) and of the larval feeding-apparatus were carried out (Lahaye and Jangoux 1985a). However, none of all these recent works focuses on the development of the coelomic cavities in the calyx.

At the same time, in the seventies to nineties of the last century, the ultrastructure of the adult crinoid's *glandular axial organ* (Holland 1970, Balser and Ruppert 1993, Heinzeller and Welsch 1994; term used synonymous to "axial organ" (Bury 1888, Seeliger 1893) and "axial gland" (Hyman 1955, Holland 1970)) and the spongy body (Balser and Ruppert 1993, Heinzeller and Welsch 1994: spongy organ) came into focus.

These studies consistently found that the hemal spaces of both these hemal organs are confined by the basal lamina underlying the coelothelium that lines the axial sinus (=axial coelom; not to be confused with the "axial sinus" or "axial coelom" of Asteroids, Ophiuroids and Echinoids, which is formed by the axocoel). This sinus constitutes a (sub)cavity of the somatocoel located centrally within the intestinal coil. Both the stone canals and the madrepores (=tegmenal pores) that are both multiplied in crinoids open into this axial sinus. The rest of the somatocoelomic cavity that does not contribute to the axial sinus is secondarily subdivided by many septa which give the coelom the appearance to consist of a labyrinth of communicating clefts (Heinzeller and Welsch 1994).

The *glandular axial organ* consists of three main structural components; in its center locate (a) tubules of columnar epithelium, which are embedded in a (b) hemal space that is lined by the basal lamina of (c) the surrounding coelothel of the axial sinus. The cells of the tubule were found to presumably serve a glandular function.

The spongy body, however, is a "tangled mesh of blood vessels" underneath the tegmen, which is lined by the basal lamina of the axial sinus' epithelium. As the latter contains myocytes as well as podocytes, it presumably allows selective fluid transfer from the spongy body vessels into the axial sinus and may serve in excretion. In comasterid feather stars the spongy body consists of two continuous but distinct parts that reside in the axial sinus. The larger part of the spongy body locates lateral to the *glandular axial organ*, together with which it extends aborally from the circumesophageal hemal ring. The smaller part extends from multiple locations of the circumesophageal hemal ring (Balser and Ruppert 1993). This part thereby locates close to the multiple stone canals and madrepores.

These comprehensive studies aimed to form an ultrastructural basis for an inter-taxon comparison of both these organs with the components of the axial complex of other echinoderm groups. In this context, the question concerning the ontogenetic origin of the crinoid axial sinus was raised again. Based on the findings preserved in the old literature, the axial sinus was found to presumably be mainly derived from the somatocoel with a possible but unclear contribution of the axocoel to the axial sinus' oralmost region. The morphogenesis of the spongy body and axial sinus, and consequently of the *glandular axial organ*, was stated to be unknown (Balser and Ruppert 1993).

The present work is a new and comprehensive morphological analysis of *A. bifida* larval development that focuses on the development of the coeloms and their derived organs. Moreover, it aims to link the classical studies to recent research and to provide a uniform and overall picture of crinoid larval development, as well as a foundation for further comparative investigations.

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Since this work focuses on the contribution of the coelomic cavities to the organ systems found in adults, the youngest stage investigated in this work is a doliolaria larva, where all coelomic cavities are already formed. To understand the origin and nomenclature of these coeloms in the doliolaria larva, it is necessary to know their development during embryogenesis. Therefore, this development is described shortly in the following second part of the introduction.

### The development of the embryo until doliolaria stage

Since none of the more recent works focuses on or reinvestigated the coelomic development of crinoids, the first part of the following brief account on *Antedon* development is based on Hyman (1955: 75-76), who followed Seeliger's (1893) work on *A. mediterranea* (= *A. rosacea* = *A. adriatica*). The description is consistent with the results of the more recent works cited above. A very detailed summary of Seeliger (1893) is given in Clark (1921: 431ff.), who nearly translated Seeliger’s text into English. The part on doliolaria development of this summary, however, is directly based on Seeliger (1893).

Cleavage in *A. mediterranea* is undeterminate, holoblastic, nearly equal and radial. During gastrulation, cells of the blastula’s vegetative (=posterior) pole initially ingress into the blastocoel and, subsequently, are shifted epibolically inwards to form the archenteron (Figs. 1A-D). The epithelium at the archenteron’s animal pole becomes multilayered and mesenchyme cells delaminate into the blastocoel (Fig. 1C). The blastopore closes and the archenteron constricts completely, losing all contact to the ectoderm. While the mesenchyme cells continue to anteriorly delaminate from the archenteron, the latter divides into the posterior (primary) somatocoel and the anterior enteroaxohydrocoel (Fig. 1D). The (primary) somatocoel elongates laterally and becomes a dumbbell-like structure with expanded right and left ends (the prospective right and left somatocoel) and a constricted middle (Figs. 1E, 2A). The enteroaxohydrocoel, sending two horns posteriorly, bends anteriorly around this middle part in a plane at right angles to it (Fig. 2B). Anteriorly, it gives off the axohydrocoel, leaving the remains of the prospective enteric sac in a central position. Meanwhile, the delamination process of mesenchyme cells has decreased and mesenchyme cell formation proceeds mainly mitotically. The axohydrocoel subsequently differentiates into a ventro-anterior cavity, the hydrocoel, and a smaller cavity that lies dorsal to the hydrocoel, the axocoel. Both the right and the left somatocoel laterally flatten, increasing their size, and separate from each other (Fig. 2C). The enteric sac occupies the cleared space between the somatocoelia, fusing its two ventrally directed horns from anterior to posterior (Figs. 2B, 2C). At this point, the embryo has achieved an anterior-posteriorly elongated shape. The cluster of epithelially lined cavities achieves a posterior position in the embryo, while the anterior part of the primary body cavity is filled with mesenchyme.

Externally visible, the ciliated epidermis regionalizes and develops ciliary bands – four in *A. bifida* and five in *A. mediterranea*. Ventrally between ciliary band one and two (*A. bifida*) or two and three (*A. mediterranea*), the epidermis locally thickens forming a vestibulum (=stomodeum). Anteriorly, the apical organ forms, constituted by an apical tuft with underlying nerve plexus. Anteroventrally, an adhesive pit develops, residing anterior to the first ciliary band (*A. bifida*) or interrupting it (*A. mediterranea*). *A. mediterranea* doliolaria hatch from the egg membrane during the fifth day. Rarely, uniformly ciliated larvae have been seen to hatch after two days and develop further in the sea water (Barbaglio et al. 2012). Fully developed *A. bifida* doliolaria hatch 24 hours after fertilization, initially being rounded to slightly barrel-shaped but equipped with all ectodermal differentiations described above except for the adhesive pit,

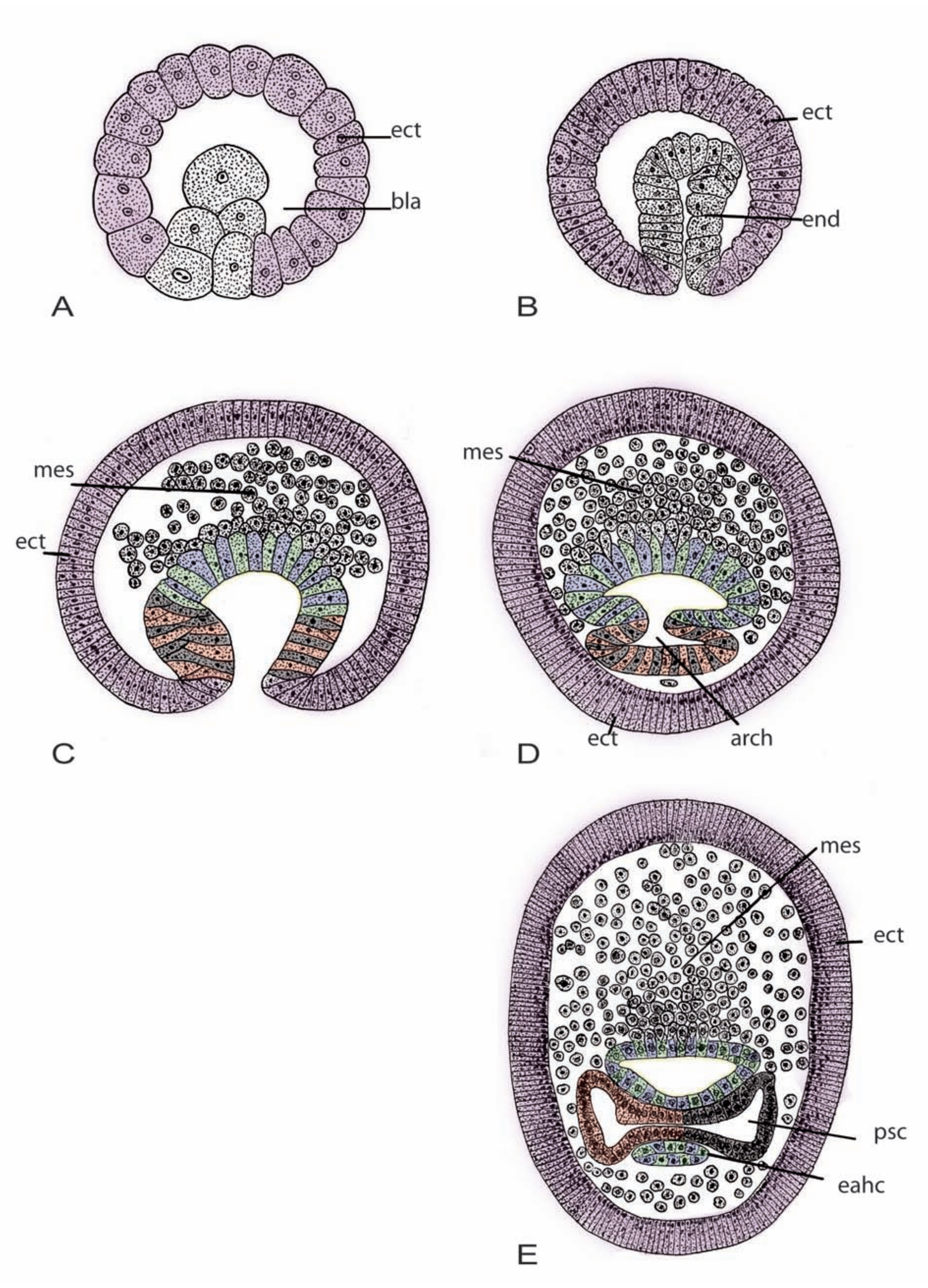


**Fig**. **1:** Embryogenesis I. Formation of the archenteron and its separation into primary somatocoel (*psc*) and enteroaxohydrocoel (*eahc*). Ventral view, anterior on the upper side. *arch* archenteron, *bla* blastocoel, *ect* ectoderm, *end* endoderm, *mes* mesoderm, *psc* primary somatocoel. Figures **A-E** after Seeliger, O. (1893): Studien zur Entwicklungsgeschichte der Crinoiden. Zoologische Jahrbücher Abteilung Anatomie und Ontogenie der Tiere. 6: Tafeln 13 (Fig. A =Fig. 25; Fig. B =Fig. 28), 14 (Fig. C = Fig. 40; Fig. D = Fig. 47) and 15 (Fig. E = Fig. 54). Figures have been taken from Hyman, L.H. (1955): The Invertebrates, Vol. 4: Echinodermata. MacGraw-Hill: New York, page 72 and have been colorized.

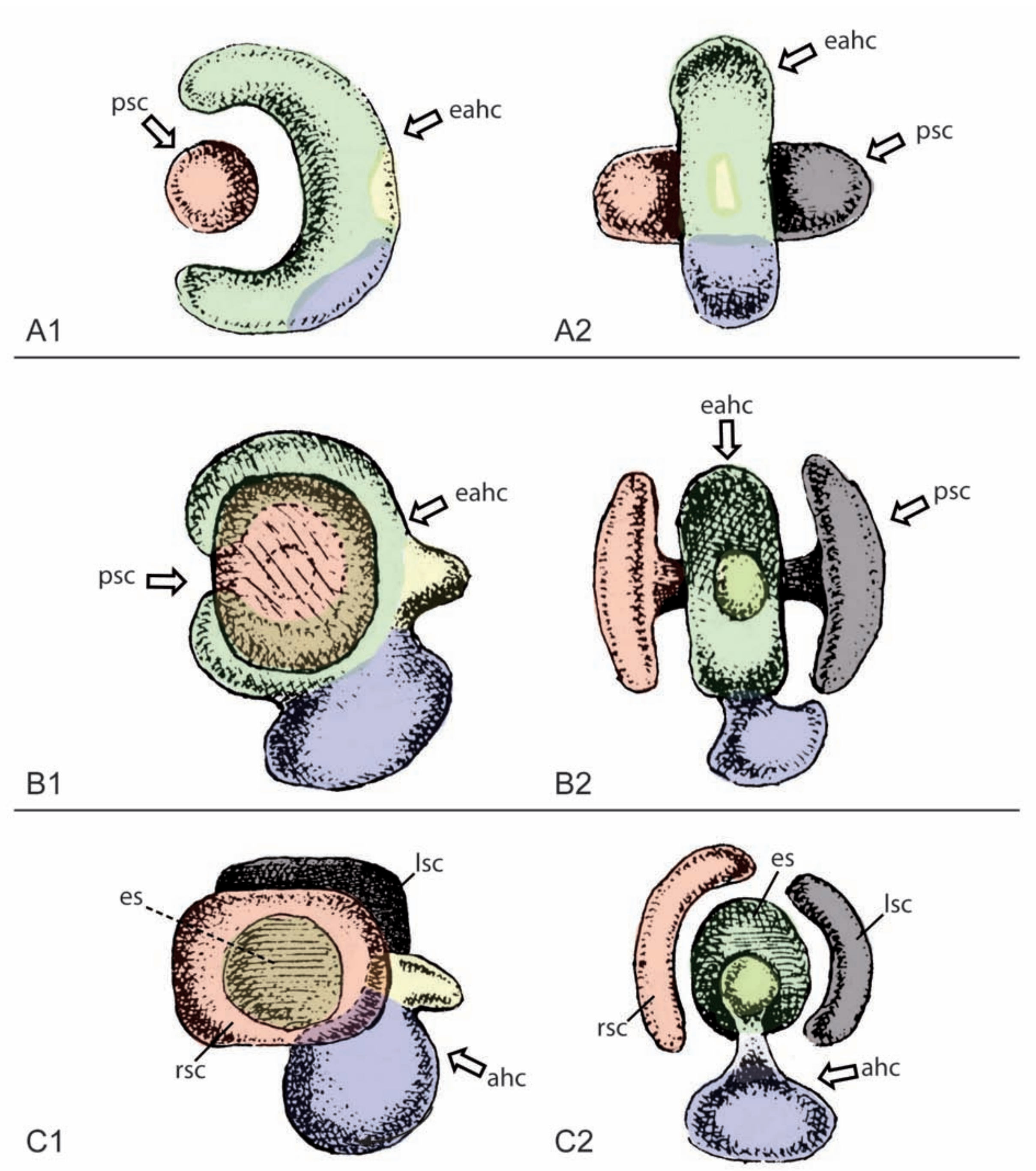


**Fig**. **2:** Embryogenesis II. Division of the primary somatocoel (*psc*) into left somatocoel (*lsc*) and right somatocoel (*rsc*) and division of the enteroaxohydrocoel (*eahc*) into enteric sac (*es*) and axohydrocoel (*ahc*). **A1**, **B1**, **C1** View onto the right side, anterior to the right, ventral on the lower side. **A2**, **B2 and C2** Same stage seen from anterior. All Figures are taken from Dawydoff, C. (1948): Grassé, P.-P. (ed.). Embryologie des Échinodermes. Traité de Zoologie, Anatomie, Systématique, Biologie, Tome XI. Masson, Paris, page 292. All Figures were colorized and **C1** was altered to match the description of Seeliger (1893).

which develops 48 hours after hatching (Lahaye and Jangoux 1985). The larvae swim freely for a few hours to a few days till they attach themselves anteriorly and start metamorphosis.

In the doliolaria, the epithelial cavities undergo further changes that, in short, lead to a state which matches the doliolaria described in the present thesis (Seeliger 1893: Fig. 67, see Fig. S67, appendix): The enteric sac flattens dorsoventrally. The left somatocoel achieves a more venteroposterior position around the enteric sac's posterior end, sending lateral processes anteriorly to each side. The right somatocoel moves into a rather dorsal position relative to the enteric sac; its posterior rim borders the anterodorsal rim of the left somatocoel. It forms two anteriorly directed lobes and sends out five anteriorly directed processes that extend between these lobes. The hydrocoel flattens dorsoventrally and achieves a position ventral to the enteric sac. It bends itself in a way that it becomes horizontally horseshoe-shaped. In freshly hatched larva, the right branch is somewhat larger than the left and the opening of the horseshoe resides postero-left. In the following, the "right branch develops rapidly at the expense of the left" (Clark 1921), meaning that the position of the horseshoe-opening successively relocates counterclockwise via the left side towards the anteroleft side of the larva. Therefore, that end of the hydrocoel-horseshoe which originally resided on the left side, is now on the right side and vice versa. In the older free swimming larvae, a dorsally directed process, the primary stone canal, develops from the (originally) left end of the hydrocoel horseshoe (Seeliger 1893: 254-256). Meanwhile, the main part of the axocoel, which is rather tubularly shaped, crosses the primary body cavity from right to left anteriorly to the hydrocoel. From here, the left end of the axocoel grows posteriorly to the left of, and parallel to, the border of hydrocoel and enteric sac. In *A. mediterranea*, it contacts the epidermis just before the fourth ciliated band (in *A. bifida* accordingly before the third), forming the hydropore. This happens at the end of, or shortly after, the embryonic stage (Seeliger 1893: 257). Also, a short hornlike process extends posteriorly on the other (right) side of the hydrocoel and enteric sac. This hornlike process exists only in quite young larvae and disappears in later stages. Third, an anterior process of the axocoel extends anteriorwards and slightly towards the left to before the second ciliary band underneath the apical pit. This process exists throughout the doliolaria stage and is described to be withdrawn "in older larvae"; it shortens and is probably incorporated into the main part of the axocoel. (Seeliger 1893: 258).

# 2 Material and Methods

## 2.1 Procurement and handling of larvae

An initial task of this work has been to establish a method suitable to get, handle and process the *Antedon bifida* larvae needed for this thesis. Therefore, three separate attempts were carried out in three different years at two different marine biological stations.

In early August 2005 and the middle of July 2006, adult *A. bifida* specimen and substratum (*Scrupocellaria sp*., algae and tunicates) taken from an *A. bifida* colony-area were kindly collected by Keith Hiscock of the Marine Biological Station (MBA) at Plymouth at Firestone Bay, Southeast coast of England, in ~20 m depth by means of SCUBA-diving. They were brought to the MBA and transferred into 2 – 3 liter tanks at constant cool temperature in a cool-storage room. The collected substratum was kept separately under the same conditions. At any time, filtered and previously well aerated sea water was used to keep adults, substratum and larva. All tanks were aerated except for Petri dishes. Free swimming doliolaria larvae were collected from water from the collection site. Most of the water was removed using a gravy pipette that was held into an open tube, to the bottom of which a finely woven mesh has been attached. The mesh prohibited suction of larvae. The remaining water was illuminated from the side and the few resulting swimming larvae were collected with a glass pipette and transferred into Petri dishes of 15 cm diameter. Adult animals and feeding larvae were fed daily with few drops of algae-solution. The water was changed twice a day with aerated filtered sea water using the gravy pipette-method in order to avoid loss of larvae. Stalked cystidean and pentacrinoid larvae were collected from substratum, mainly bryozoa of the taxon *Scrupocellaria sp*. (Figs. 3A-C), using a binocular, side-light, fine tweezers and a fine scissors. *Scrupocellaria sp*. turned out to be the best substratum for handling attached larvae. The larvae can be found on the substratum in high numbers and *Scrupocellaria sp*. can easily be cut into pieces around the larva’s attachment site so that damage of the larvae can be avoided. These small pieces of *Scrupocellaria sp*. were heavy enough to keep stalked cystidean and pentacrinoid larval stages at the bottom of the laboratory dishes during otherwise critical further treatments.

In 2006, two slightly different methods of artificial fertilization with naturally spawned eggs and dissected sperms were tested: Directly after collection, the sex of males and females with ripe (thickened) gonadal pinnules was identified by dissection of one gonadal pinnule. The sexes were separated, then washed with frequently changed filtered sea water and finally transferred into a third tank. The midday after catching, several females with ripe gonadal pinnu-

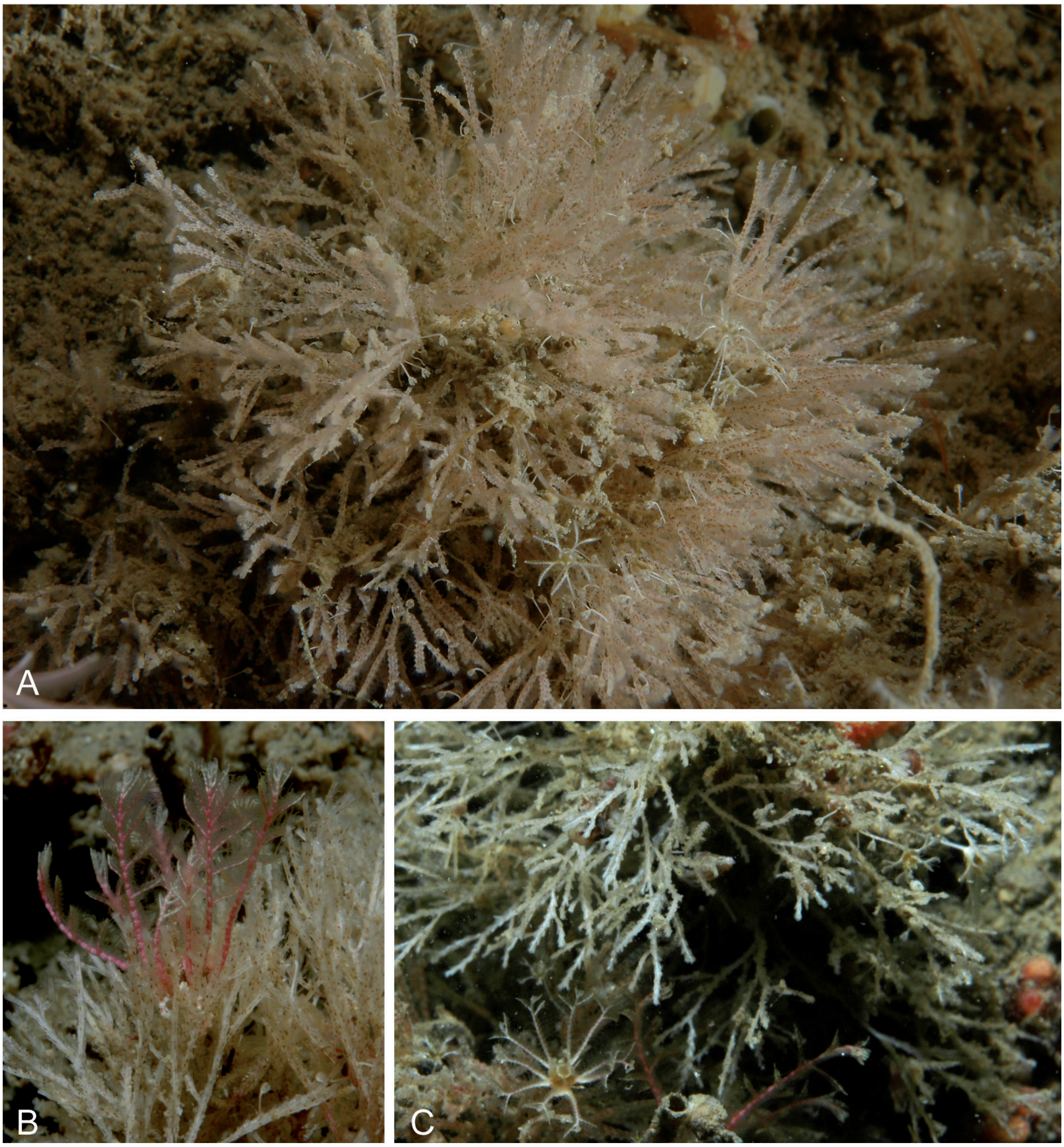


**Fig. 3: A – C**. Larvae and juveniles of *Antedon bifida* on *Scrupocellaria sp*. at Firestone Bay, Plymouth, in 20 m depth. The pictures were kindly taken by Keith Hiscock (MBA, Plymouth).

les showed spawned eggs attached to those pinnules. To get sperms, ripe gonadal pinnules of males were dissected in filtered sea water. A solution of agile dissected sperms was added to a) female animals with newly spawned eggs and b) whole cut off pinnules with newly spawned eggs attached. Approach a) was carried out in little tanks, approach b) in large Petri dishes. Both attempts were successful, leading to a small amount of larvae of defined age.

At the beginning of July 2008, *A. bifida* adults and substratum were collected by means of SCUBA-diving at Point d'Armorique (Ducs d'Albe) and transferred into open water circle tanks at the Station Biologique de Roscoff and substrate was examined for attached stages. These larvae were fixed in GPBS for SEM-studies.

**Table 1:** Summary of preparation procedures for individual larvae that are processed for sectioning in the present work. Numeration of the reference name relates to the red numbers given in Figure **3**, in which pictures of the specimens are given.

| Reference name | Short description | Date of catch at Firestone Bay | Fixative |
|---|---|---|---|
| **specimen 1** | doliolaria<br>(67h after fertilization) | middle of July 2006 | G-SCS |
| **specimen 2** | early cystidean<br>(130 h)<br>just settled | | G-SCS |
| **specimen 3** | early cystidean | | G-PBS |
| **specimen 4** | cystidean<br>intestinal tract winds<br>(167 h) | | G-PBS |
| **specimen 5** | cystidean<br>(vestibulum still closed) | begin of August 2005 | G-PBS |
| **specimen 6** | late cystidean<br>(vestibulum about to open) | | G-PBS |
| **specimen 7** | early pentacrinoid<br>(vestibulum just opened) | | G-PBS |
| **specimen 8** | pentacrinoid<br>(small arms, anus formed) | | G-PBS |
| **specimen 9** | pentacrinoid<br>anal tube forms<br>4 secondary stone canals form | | G-PBS |
| **specimen 10** | juvenile<br>stalkless, cirri,<br>5 pori (1 hydropore, 4 madrepori) | | G-PBS |

## 2.2 Fixation

Specimens (see Tab. 1, Fig. 5) were either fixed in 2.5% glutaraldehyde buffered in 0.05 M phosphate buffer with 0.3 M NaCl (G-PBS) OR in 2.5% glutaraldehyde buffered in 0.1 M sodium cacodylate with 0.2 M NaCl (G-SCS) at 4°C and pH 7.2 for 25-30 minutes (specimen 5 for 50 minutes). Ruthenium red was added to each fixative. After fixation, the specimens were rinsed in the same buffer used during fixation and stored therein until they were postfixed in 1% OsO4 buffered in either PBS or SCS, respectively. At that point of time, larval stages were documented using light microscopy (LM) (Fig. 4).

For transmission electron microscopy (TEM) and LM , larvae were dehydrated in an ethanol series and transferred to araldite via propylene oxide. In araldite, they were placed on microscope slides and imaged without cover glass for later reference (Fig. 5). Araldite-polimerization was started thereafter using BDMA (Benzyldimethylamin).

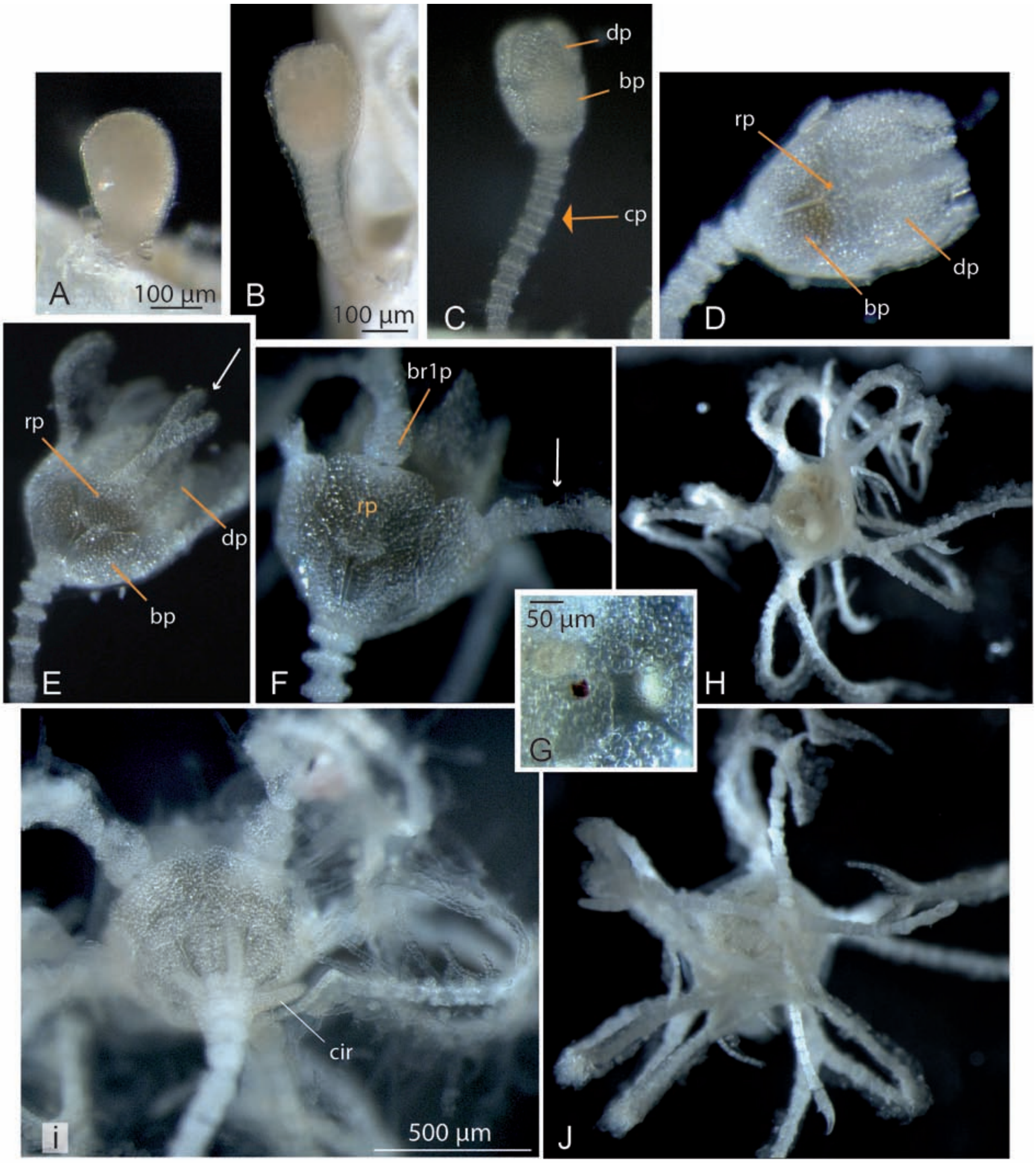


**Fig. 4:** Sequence of fixed *A. bifida* developmental stages in buffer, showing the larval skeleton. LM. **A** Metamorphosing larva after settlement with the anterior pole downwards. **B**, **C** Cystidean stages with closed vestibulum. **D – i** Pentacrinoid stages with **D** open vestibulum and **E**, **F**, **i**, outgrowing arms (*small arrow*). Note the outgrowing cirri (*cir*) in **i**. **G** Detail of the body wall with sclerites and granularly translucent mesenchymal cells between the sclerites. **H**, **J** Juveniles. Scale of **C** – **H**, **J** unknown, but can be inferred from Figures **5** and **6**. *bp* basal plate, *br1p* first brachial plate, *dp* deltoid (or oral) plate, *cp* columnal plate, *rp* radial plate.

Serial ultra-thin sections of ~70 nm thickness were made using diamond knives on a Leica Ultracut S ultramicrotome. They were collected on copper, formvar-covered single-slot grids, automatically stained with uranyl acetate and lead citrate with the Nanofilm TEM Stainer and examined either with a Philips BioTwin CM120 or with a Philips CM120 electron microscope.

Serial semi-thin sections of 500 nm were prepared using a diamond knife (Diatome Histo Jumbo) following the method described by Blumer et al. (2002). They were stained with toluidine

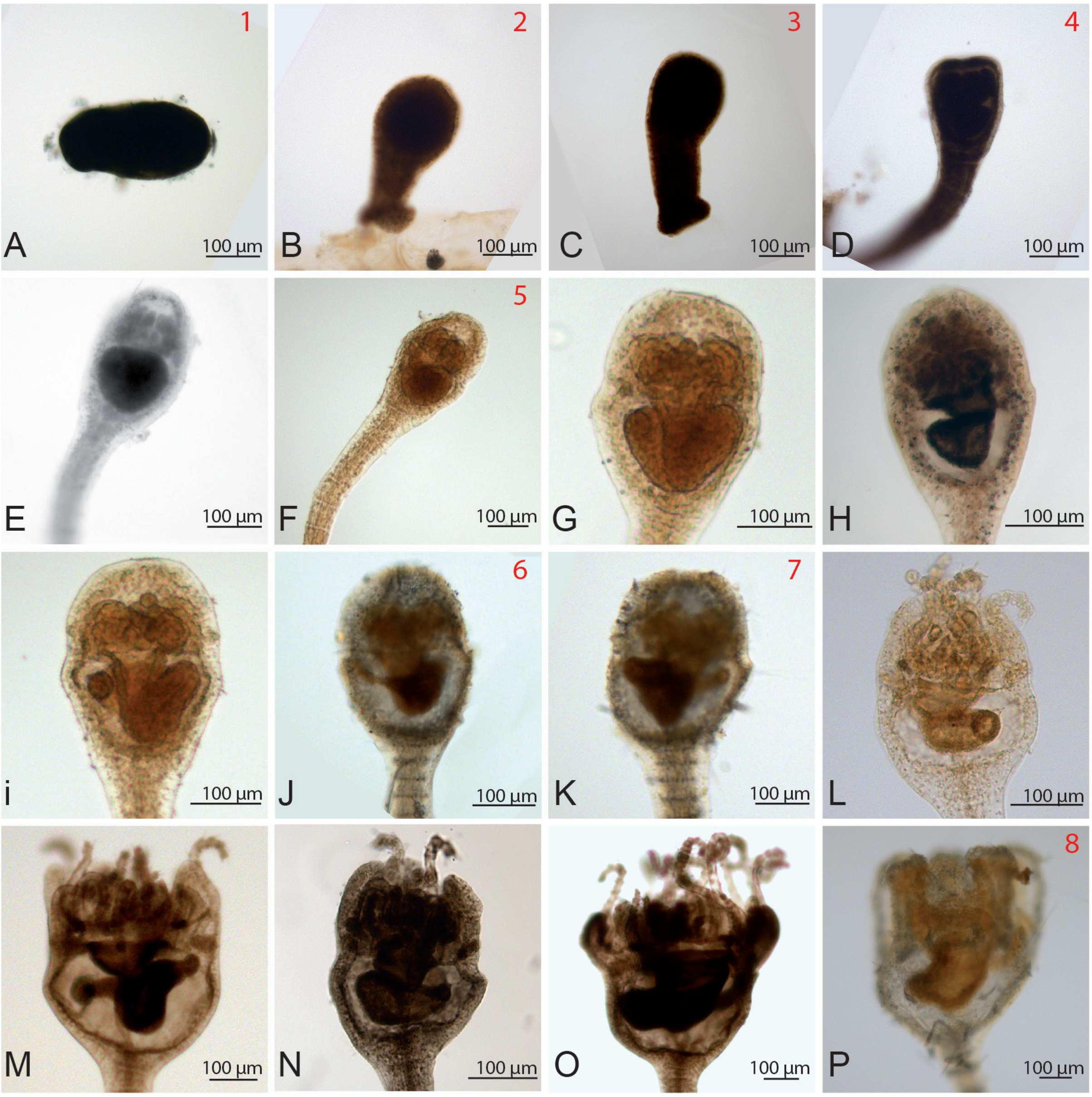


Fig. 5 part 1 of 2

**Fig. 5 (2 parts):** Sequence of fixed *A. bifida* developmental stages in fluid Araldit. LM. Red numbers indicate the specimen numbers of the larvae described in this thesis. **A** Doliolaria, anterior pole points to the left, ventral side downwards. **B** – **U** Stalked stages. **B** Larva after settlement with the anterior pole downwards. **C – J** Cystidean stages with closed vestibulum. Note the progressively diagonal bending of the intestinal tract (**G**, **H**) and the outgrowing intestine (**I** – **K**). **K** Vestibulum has just opened, as slits form between the oral plates. **K – U** Pentacrinoid stages with open vestibulum. **L** – **N** Larvae expose their primary podia into the water. **O** – **U** show successively outgrowing arms. **V** Juvenile, detached from the stalk.

blue (1% toluidine, 1% sodium-tetraborate and 20% saccharose) and mounted with Depex or Araldite. Light microscopy images of these sections were obtained with SIS color-View II camera on Olympus BX51 and BX 61 microscope in bright field mode.

For scanning electron microscopy (SEM), specimens were dehydrated in an alcohol series and critical-point dried in a Balzer CPD 030. They were covered with gold using a Balzer SCD 040 and examined with a FEI Quanta 200 SEM. Figure 6 shows SEM pictures of a developmental sequence of larval stages.

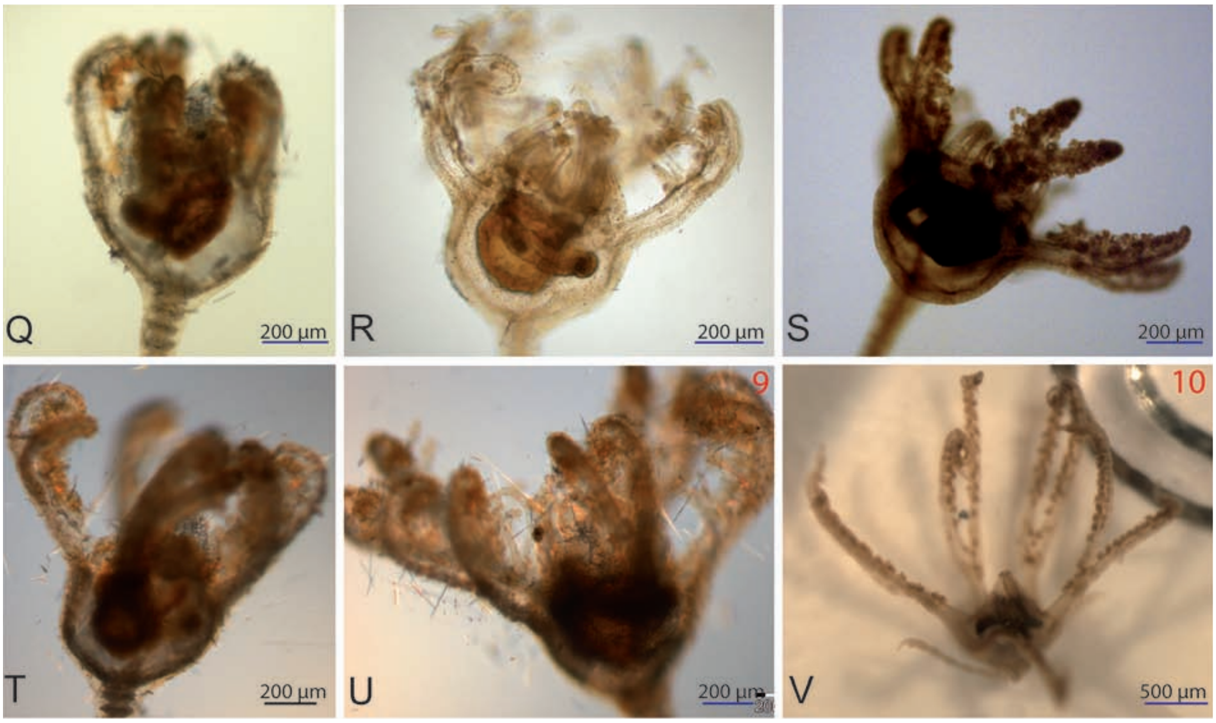


**Fig. 5 part 2 of 2**

## 3D-reconstruction

Pictures have been processed and enhanced using Photoshop CS2, 3, 4 and 5, respectively. The alignment of the serial section pictures has been done either by hand using Photoshop CS2, 3, 4 and 5, respectively (.tif-stacks), or with IMODalign.

For the reconstruction, picture stacks of down-sampled gray-scaled .tif-files have been loaded into Amira 5.2.2.. Structures were labeled using the Segmentation Editor and saved in Amira mesh file format (.am). Selected structure labels were computed using the Resample-tool, applying default settings. From these data, a surface was generated (Surface Gen-tool, unconstrained smoothing, default settings). Surfaces have been simplified once (Simplification Editor, default settings) and then computed using the Smooth Surface-tool (10 iterations). Surface View has been set up with optional “direct normals”.

Orthogonal views of aligned serial sections of specimen 5 have been generated with ImageJ using the orthogonal view tool and captured as screen-shots.

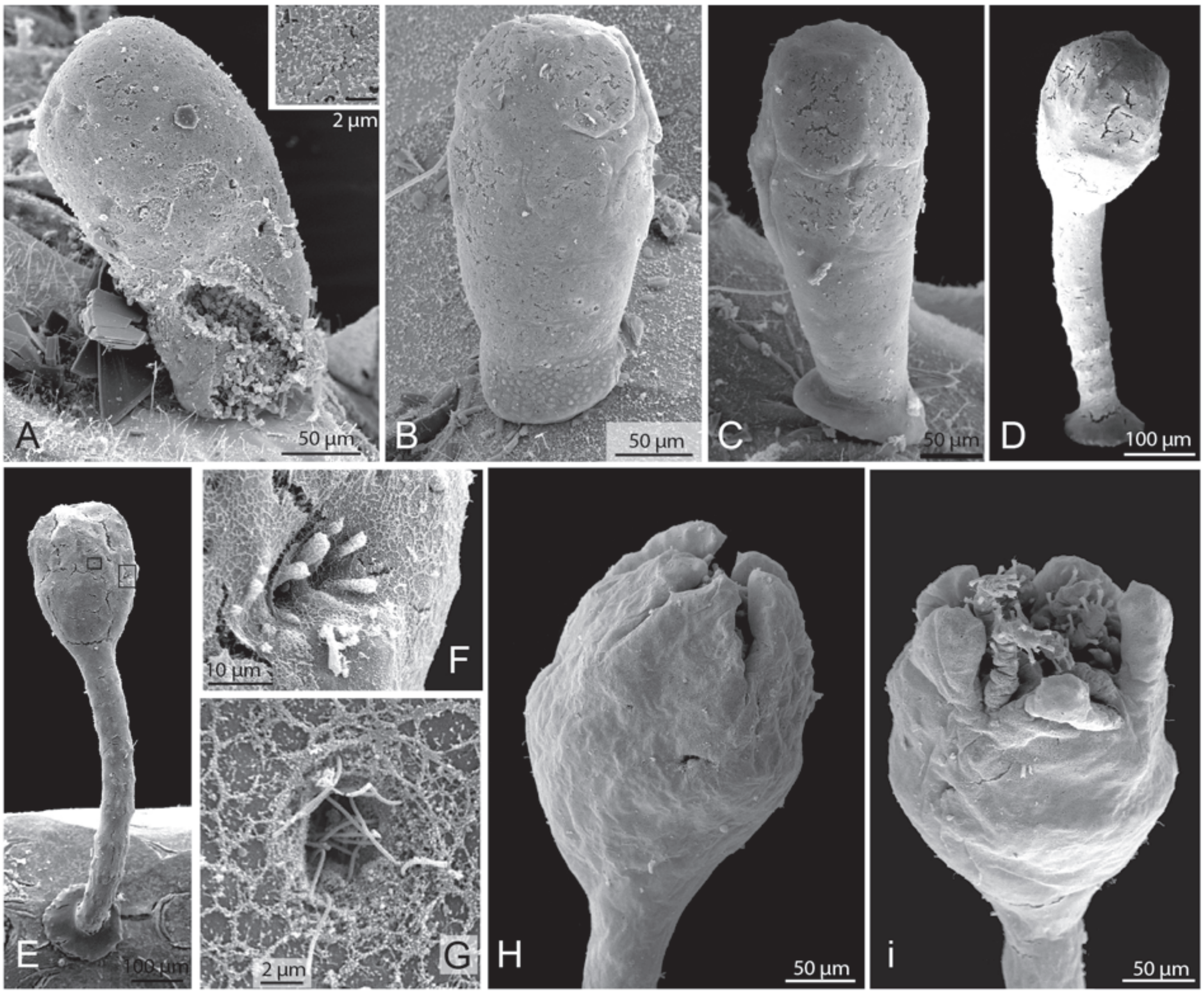


**Fig. 6 part 1 of 2**

**Fig**. **6** (**2 parts**)**:** Sequence of *A. bifida* developmental stages. SEM. **A**, **B** Recently settled metamorphosing larva, detail of epidermal surface in right upper corner. **C - E** Cystidean stages with closed vestibulum. **F** Developing anus, detail of **E** (large *square*). **G** Hydropore, detail of **E** (small *square*). **H – Q** Pentacrinoid stages with **H**, **i** open vestibulum and **J – N** and **P - Q** outgrowing arms (small *arrow*). Note the growing cirri (*cir*) in **Q**. **O** Detail of **N**, showing the developing anal tube, which is supportet by the anal plate. **R**, **S** Juveniles, same as in Figure **7**.

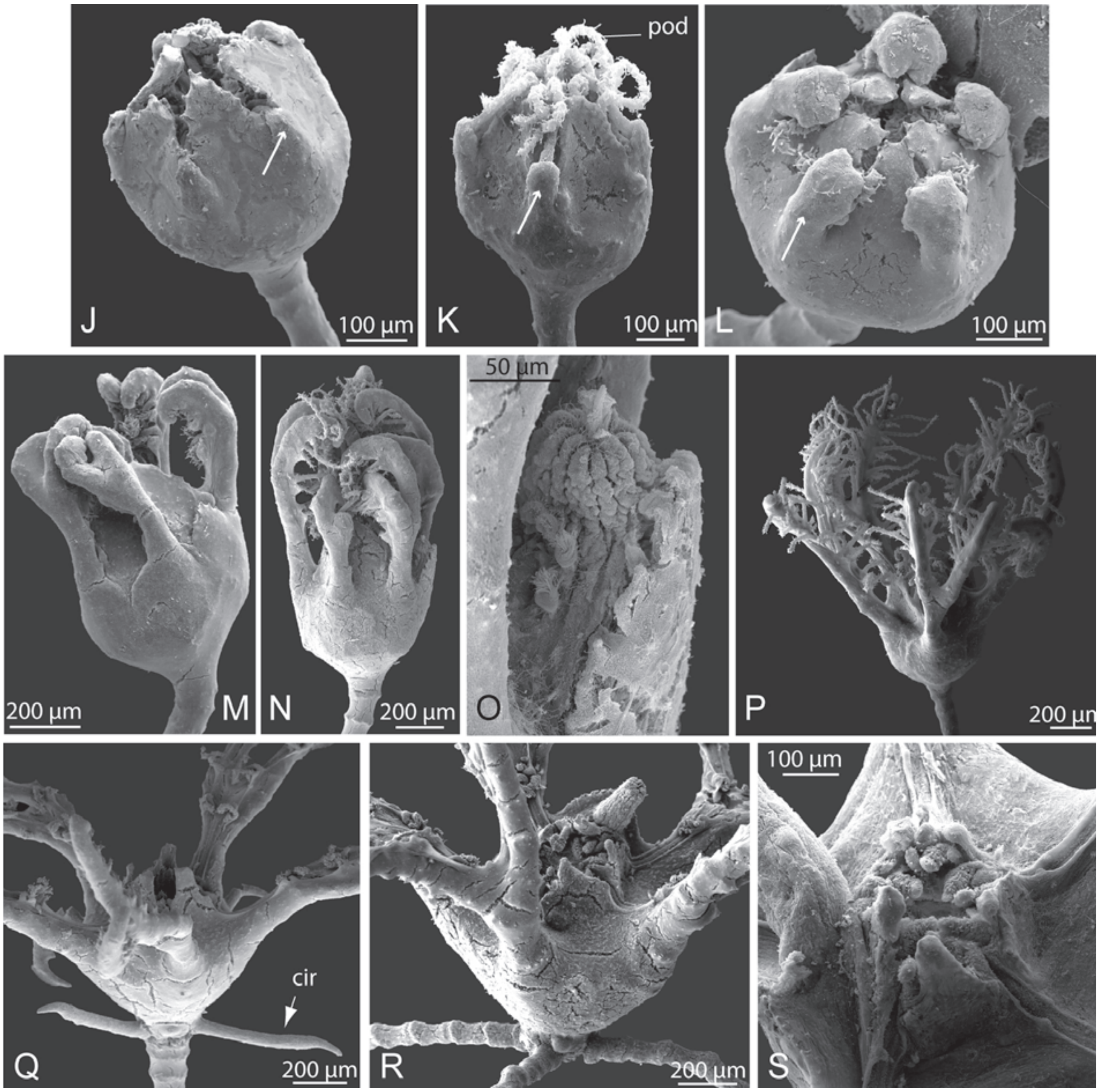


**Fig. 6 part 2 of 2**

# 3 Results

First, a detailed description of a 3D-reconstructed juvenile specimen of *A. bifida* will be given, focusing on the position of the *ecm* formed by the main body cavity. This will be followed by descriptions of the coelomic states of a sequence of selected larval stages, most of which have been 3D-reconstructed as well.

Thes results on the larval stages will be used to discuss the contribution of the axocoel and the two somatocoelia to the main body cavity and to the hemal organs in the juvenile.

## 3.1 Juvenile (Specimen 10)

The stalkless juvenile stage has been investigated using both SEM (Fig. 7) and a series of vertical semi-thin sections of one specimen (specimen 10) that has been reconstructed (Figs. 5V, 8).

The calyx of specimen 10 measures about 0.6 mm in diameter, and 0.5 mm in height (without cirri). The arms extend about 1.6 mm. The animal's major axis spans vertically between the oral and aboral side, respectively, that are defined by the position of the mouth opening, which is directed towards the water surface. This mouth is located at the calyx's oral center, being surrounded by five interradial triangular valves, each of them directing one tip towards a point above the mouth's center (Figs. 7A, 8B). Underneath the oral valves, twenty podia arise, bending into the mouth opening (Fig. 7B). The plane around the mouth opening is called the tegmen, the strongly convex aboral side of the calyx is named bowl (Fig. 7A). Towards the calyx' oral ends, five arms derive laterally from the calyx. They define the radii. Further distally, the arms divide horizontally into two branches (Figs. 7A, 5V). The calyx' areas between the arms are called interradii. In one interradius, a tube emerges from the oral body surface, bearing the juvenile's anus at its distal end (Fig. 7A). This anal tube is traditionally used to denominate the radial symmetric units in radial arms and interradial sections (e.g. Heinzeller and Welsch 1994): Looking at the oral side, the radii are clockwise assigned by the letters A to E, radius A by definition being positioned across the interradius CD, in which the anal tube arises (Fig. 7B). As the oral side derives from the larval posterior end, the (inter)radii AB to C form the right side of the animal and D to EA the left side. Along the oral side of each arm runs one strongly ciliated food groove, which continues on the tegmen, merging centrally into the opening of the mouth (Figs. 7B, 9). The food grooves are flanked by two orally directed lateral rows of developing podia, the crinoid tube feet, that stand in groups of three (triads). The first triads appear right before the splitting point of the arms. Towards the end of some arms, the first pin-

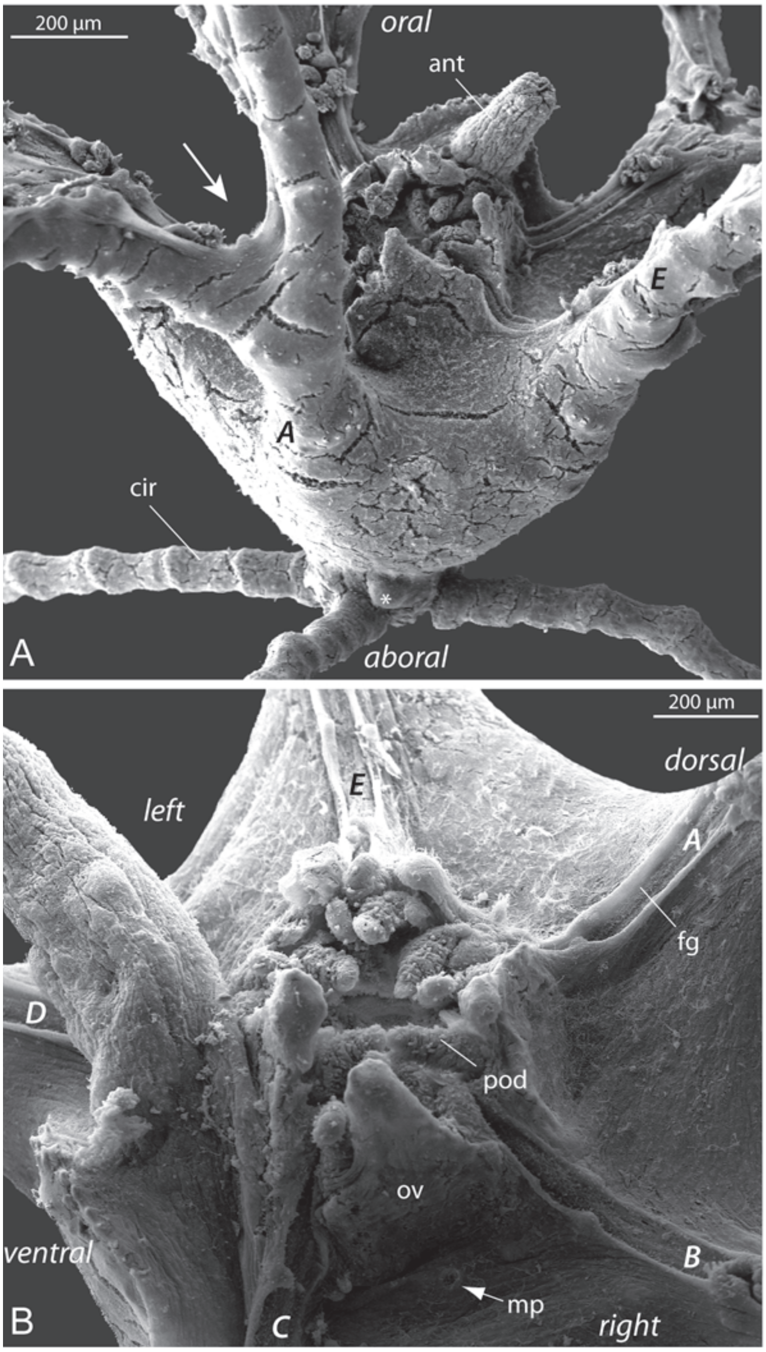


**Fig. 7:** SEM of two different juvenile stages. Radii marked with *capital italic letters*. **A** View from radius *A* (=dorsal) onto the anal tube (*ant*). Note the budding cirrus in interradius EA (*asterisk*), the podia (*pod*) around the mouth opening and the branching arms (*arrow*). **B** Oral view onto the tegmen. Note the madrepore (*mp*) in interradius *BC* and the food grooves (*fg*) that extend radially between the oral valves (*ov*).

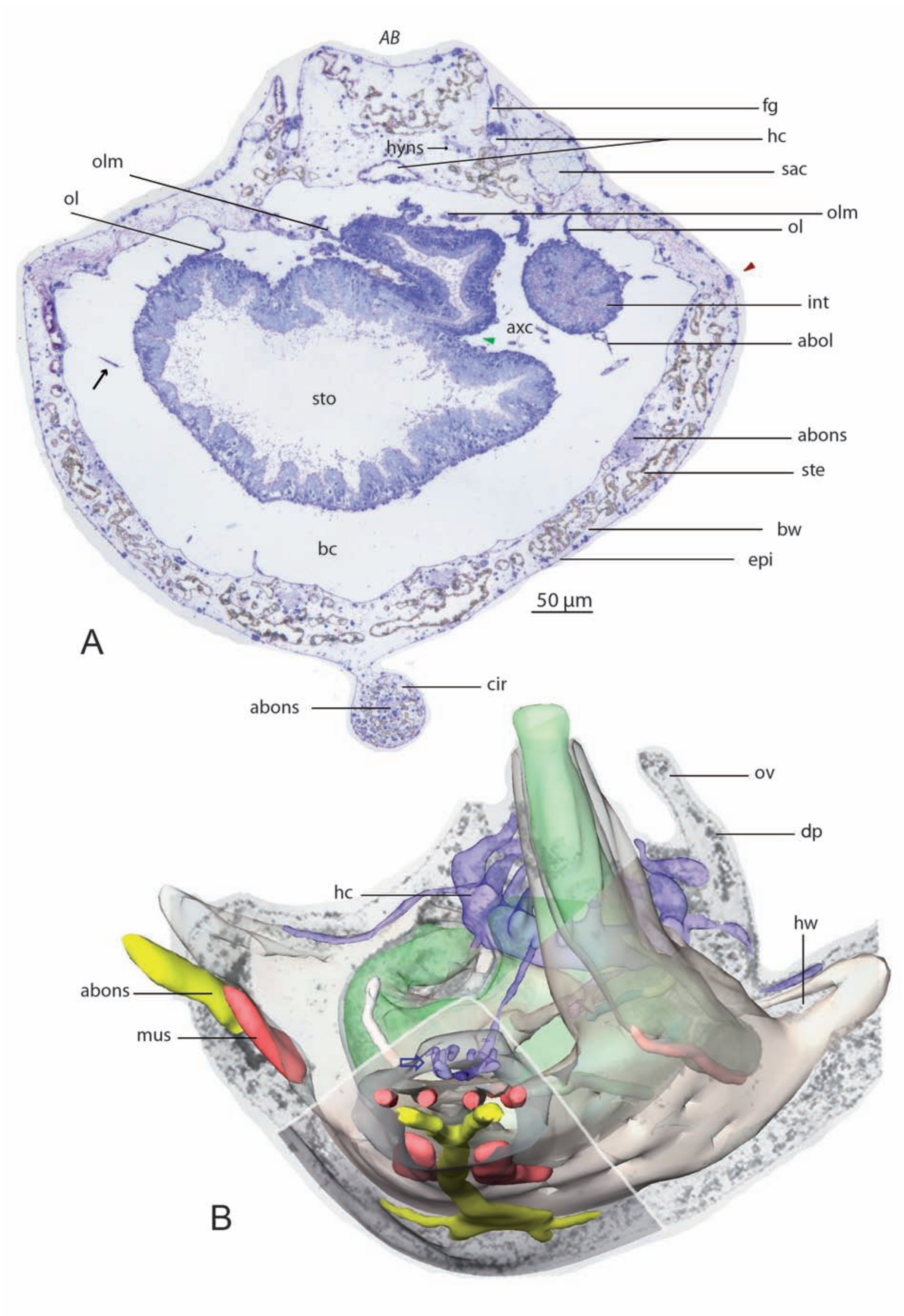
nulae develop (data not shown). Five pori reside on the tegmenal side; four madrepori are situated interradially at the bases of the triangular oral valves (Figs. 7B, 10E, 10F) that are located in those interradii without anal tube. The fifth, the hydropore, resides to the side of the base of the anal tube towards radius D. On the larva's aboral side, in the center of the calyx, long **cirri** and, possibly, one cirral bud reside radially (Fig. 7A). With these cirri, the crinoid attaches itself to the substrate.

### Epidermis

The epidermis is formed by ciliated cells that are shaped like a tack (Fig. 11); their distal parts are thinly flattened and the somatic part of the cell extends like the tack's pin into the body wall. Single somata are therefore usually distant from each other. Both the somata and the distal part are underlined by a continuous basal lamina. On the tegmen and on the oral side of the arms, bundles of several ciliated epidermal cells are abundant. They occur less frequent in the epidermis of the bowl.

### Intestinal tract

The intestinal tract can be subdivided into mouth, esophagus, stomach and intestine (Figs. 10A-E). The esophageal part of the intestinal tract descends from the large mouth opening aboralwards towards an enlarged compartment, the stomach, which fills up more than half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of the calyx's space. Seen from the oral side, the intestine (=intestinal outgrowth in larval stages without anus) emerges rather aborally from the stomach between radius A and interradius AB. From there, it takes half a clockwise and slightly orally directed turn around the stomach and lifts up in interradius CD into the anal tube, where the anus opens to the exterior at the tube‘s tip. The intestinal tract forms an orally directed pouch in radius E and a laterally directed one in radius D. As the stomach takes up the major part of the calyx and the intestine is of comparably smaller diameter, the space in the intestinal tract's bend resides on the larva's right side.

### Body wall and extracellular matrix

The extracellular matrix (*ecm*) forms a continuous framework between intestinal tract and epidermis, into which different body cavities and different cell types are embedded. The latter are primarily mesenchymal cells, sclerites, muscles, and nerve cells. The subepithelial basal laminae can be distinguished within the *ecm* by their stronger stainability. The juvenile's body wall consists of both the epidermis and a thick layer of *ecm*, into which the mentioned cell types are embedded (Figs. 8, 11). Basally, the body wall is limited by the basal lamina underlying the coelothel of the main body cavity (Fig. 11).

Since the *ecm* is continuous throughout the body, the body wall's loose matrix is also continuous with the intercoelomic matrix; it continues between hydrocoel and main body cavity, main body cavity and chambered organ, intestinal tract and hydrocoel, intestinal tract and main body cavity, and in the center of the chambers of the chambered organ. In the proximal part of the arms, a horizontal wall divides the arm coelom into an oral- and an aboral section. The wall consists of the aborally and orally located coelomic lining and the *ecm* in between, which again is continuous with the remaining *ecm* of the animal (Figs. 9A, 9B). The *oral lacunar meshwork* consists of interconnected strands of main body cavity coelothel enclosing some *ecm*. This *ecm*, again, is continuous with the periesophageal *ecm* and the loose matrix of the body wall (Fig. 18).

### Body cavities

Primary and secondary body cavities can be distinguished by their lining (Ax 1996). Secondary body cavities are lined by epithelia which here will be generalized as coelothelia. Primary body cavities are lined by matrix. Mesenteries represent potential structures for blood lacunae, since mesenteries consist of two coelothelial layers with *ecm* in between. Its *ecm* may be expanded to contain primary body cavities that allow fluid transport. Mesenteries are generally formed by

◂ **Fig. 8:** Juvenile *A. bifida*, specimen 10. **A** Vertical cross-section (similar to Fig. **16A**). LM. **B** Other section, interspersed with a partial 3D-reconstruction. Note the inset-section of radius D, in which the epidermal surface is reconstructed as well, illustrating the body wall (*bw*). *abol* aboral lacuna, *abons* aboral nervous system, *axc* axial compartment, *bc* main body cavity, *cir* cirrus, *dp* deltoid plate, *epi* epidermis, *fg* food groove, *hc* hydrocoel, *hw* horizontal wall in the arms, *hyns* hyponeural nervous system, *int* intestine, *mus* muscle, *ol* oral lacuna, *olm* oral lacunar meshwork, *ov* oral valve, *sac* sacculus, *ste* stereom, *sto* stomach, *arrow* trabeculum, *blue empty arrow* first podial triad, *green arrowhead* intestinal fold in which the centro-horizontal branch of the oral lacuna runs, *red arrowhead* bundle of ciliated cells, *AB* interradius AB.

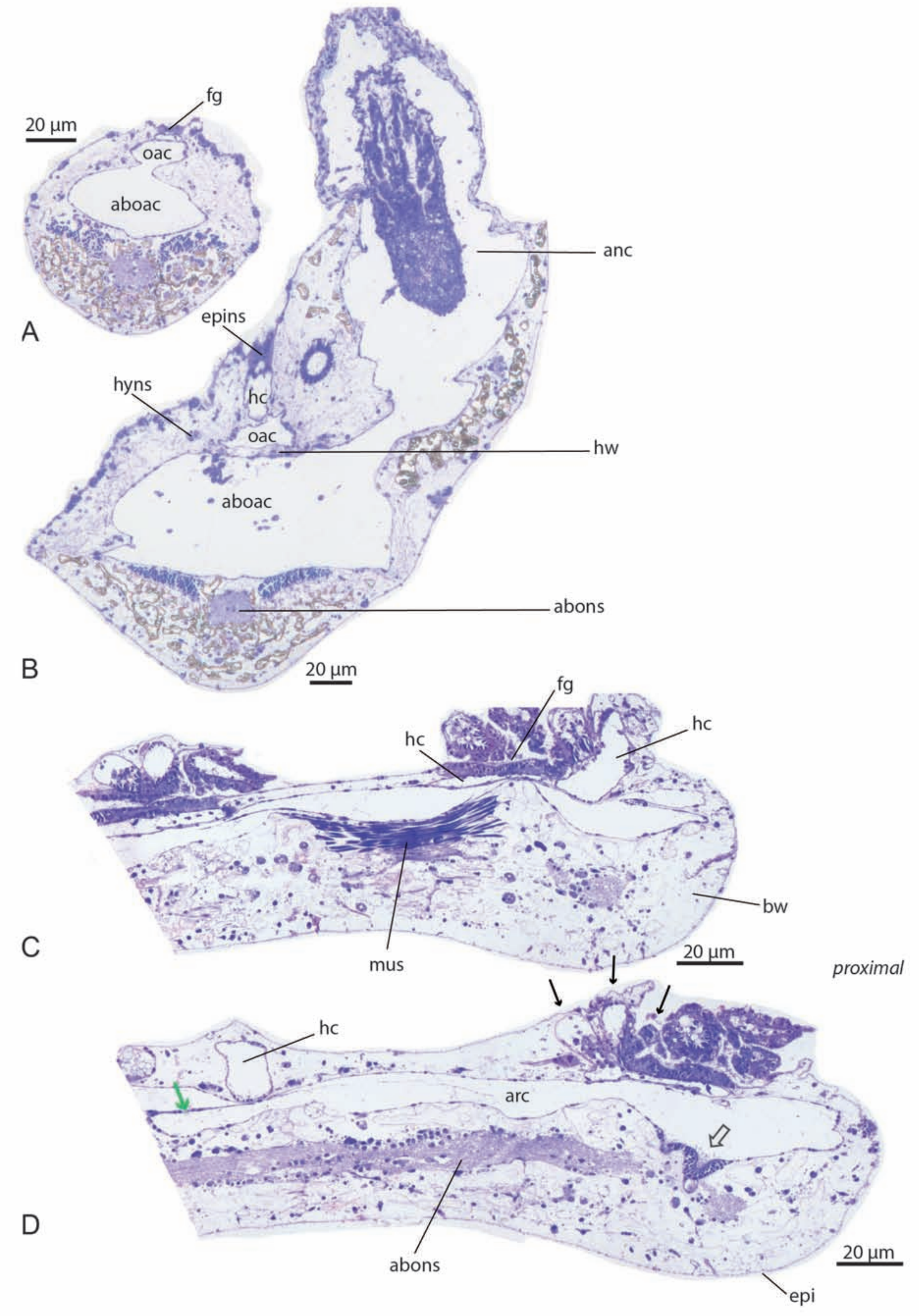


**Fig**. **9:** Juvenile *A. bifida*, specimen 10. **A**, **B** Cross-sections and **C**, **D** quasi-lateral sections of the arms. *aboac* aboral arm coelom, *abons* aboral nervous system, *anc* anal tube coelom, *arc* arm coelom, *epi* epidermis, *fg* food groove, *hc* hydrocoel (radial water canal), *hw* horizontal wall, *hyns* hyponeural nervous system, *mus* muscle, *oac* oral arm coelom, *black empty arrow* ciliated pit, *green arrow* horizontal septum, *small arrows* point to the podial triad that basally unite in an atrial enlargement.

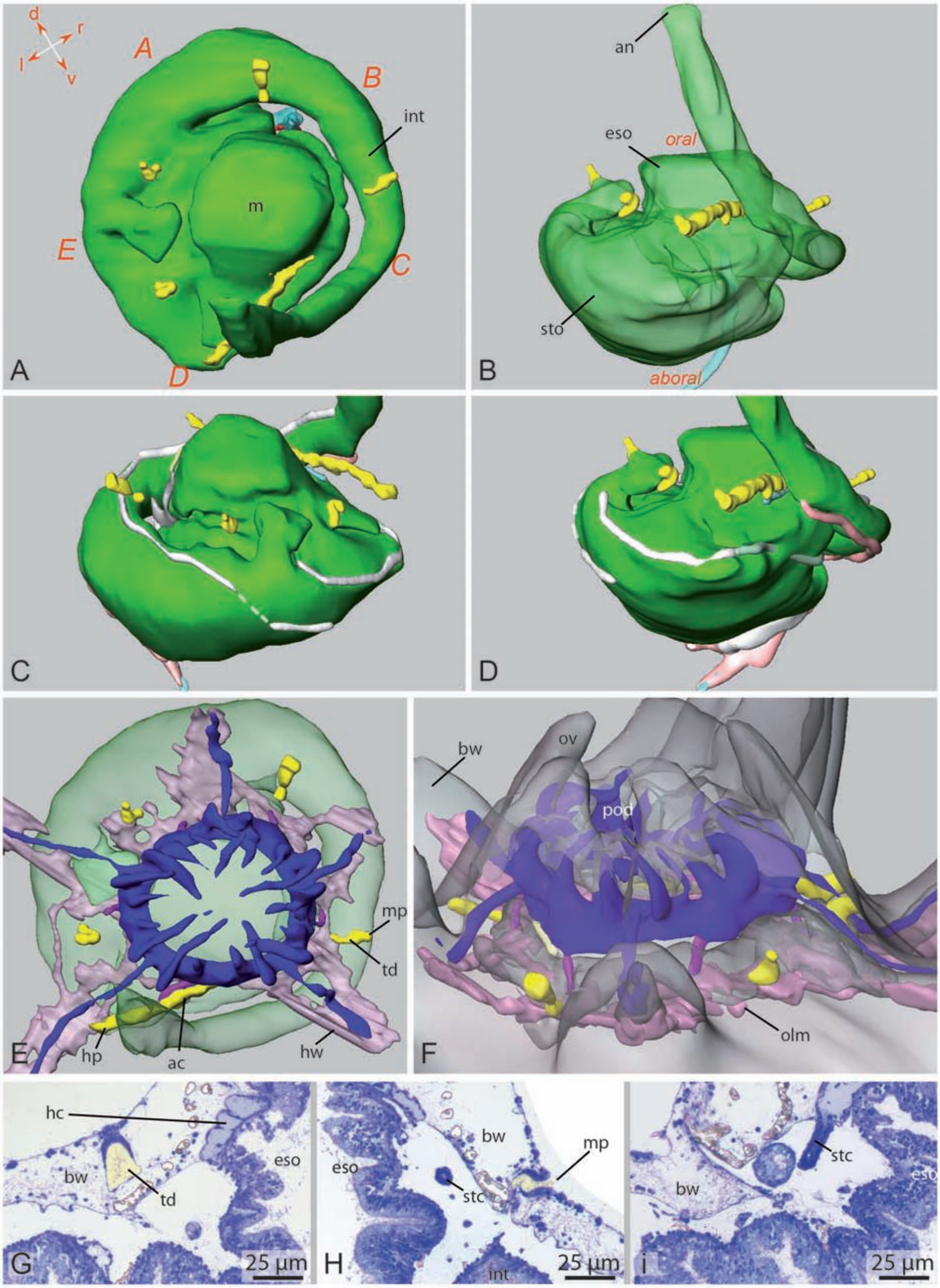


**Fig**. **10:** Juvenile *A. bifida*, specimen 10. **A – D** Intestinal tract (***green***) and **E - i** oral structures. **A** Oral view, position of axial organ tubule (***turquoise***) and axial muscle (***red***) within intestinal coil. **A**, **B** Symmetry nomenclature marked in *orange*. **B** Side view onto radius D. **C**, **D** position of lacunar system (compare Fig. **15**). **C** View onto interradius EA. **E - i** Position and structure of axocoel (*ac*, ***yellow***)(interradius CD) and tegmenal ducts (*td*, ***yellow***), *hc* hydrocoel (***blue***) and *stc* stone canals (***purple***) above the oral lacunar meshwork (*olm*, ***old rose***) and horizontal wall in arms (*hw*, ***old rose***). *an* anus, *bw* body wall (*gray*), *eso* esophagus, *int* intestine, *m* mouth, *mp* madrepore, *ov* oral valve, *pod* podium, *sto* stomach, *d* dorsal, *l* left, *r* right, *v* ventral.

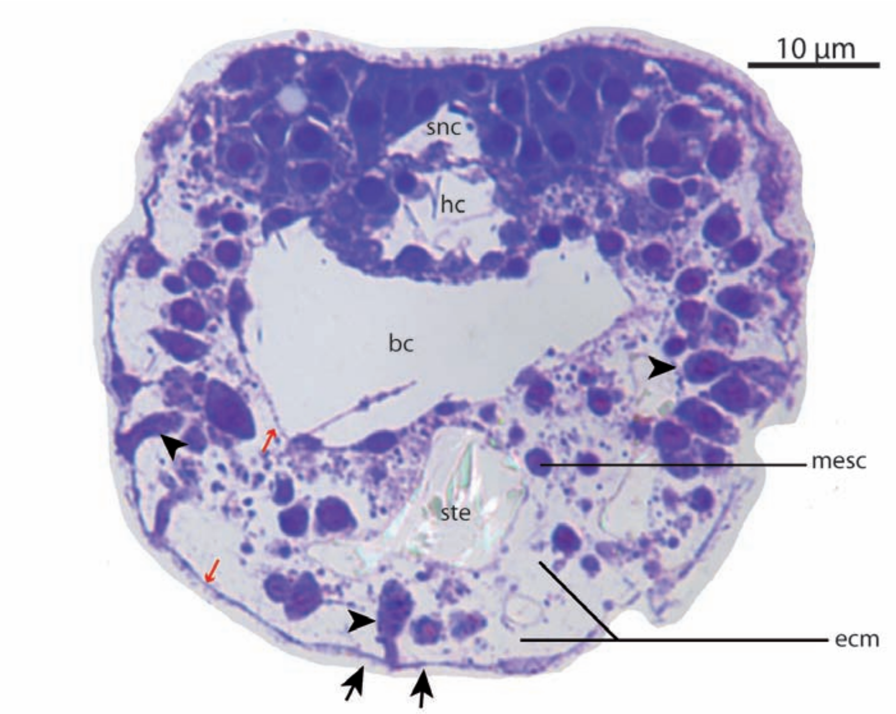


**Fig. 11:** Epidermis of juvenile *A. bifida,* specimen 10. Cross-section of the arm tip. *Arrows* mark the epidermis' thin flattened distal parts and *arrowheads* the epidermal somata. *Red arrows* point to the basal laminae of the main body cavity or that of the epidermis. *bc* main body cavity, *ecm* extracellular matrix, *hc* hydrocoel, *mesc* mesenchyme cell, *snc* sub-neural canal, *ste* stereom.

two juxtaposed somatocoelomic linings, while mesenteric formations are formed by any other combination of juxtaposed coelomic linings.

### Secondary body cavities

In *A. bifida*, axocoel, hydrocoel and two somatocoelia are traditionally said to be the only secondary body cavities. The main body cavity is a fusion product of the axocoel and the somatocoelia (Hyman 1955). However, an additional small coelomic cavity, the *small ventral coelom*, lies ventrally within the body wall protrusion.

### Hydrocoel

The hydrocoel forms a ring around the oralmost part of the esophagus from which it sends blindly ending extensions into the 20 podia that stand around the mouth (Figs. 7B, 8B, 10E, 10F). From the hydrocoel ring, five radial water canals extend underneath the basiepithelial nervous system, which underlies the food grooves, into the arms (Fig. 9). The radial water canals split together with the arms. Before the splitting point, the first podial triad appears (Figs. 5V, 8B, 7A). Underneath each triad, the radial water canal forms one atrial-like oral enlargement, from which the hydrocoel extends separately into each of the three podia (Fig. 9D). At the arm's distal ends, each radial water canal ends in the distalmost podium. Five interradial stone canals extend in aboral direction from the hydrocoel ring (Fig. 10F).

### Axocoel, stone canals, madrepori

The juvenile *A. bifida* specimen 10 has five pores, which traverse the tegmenal body wall and connect the main body cavity with the exterior (Fig. 10E). Four madrepori reside at the triangular valve‘s bases in interradii DE, EA, AB and BC (Fig. 7B). They are the openings of the tegmenal ducts that pass the body wall and open into the main body cavity above the *oral lacunar meshwork* (Figs. 10E-H). In proximity to the inner openings of these four tegmenal ducts, short tubes, the secondary stone canals, derive from the hydrocoel ring and open into the main body cavity above the *oral lacunar meshwork*, one in each interradius (Figs. 10E, 10F, 10i).

In interradius CD, both the pore and the primary stone canal differ from this pattern (Figs. 12, 13). The fifth pore, the hydropore, locates to the side of the base of the anal tube towards radius D (Fig. 12A). From here, the axocoelomic duct takes a comparatively long way through the body wall protrusion in interradius CD, following the latter’s orientation, and opens in interradius CD above the *oral lacunar meshwork* into a space that is semi-isolated from the main body cavity towards radius D by the body wall protrusion (Figs. 13A-C).

The fifth primary stone canal derives from the hydrocoel ring in interradius CD and passes the main body cavity above the *oral lacunar meshwork* (Figs. 12C, 12D, 13D-B). It enters the *body wall protrusion* in interradius CD, runs within it towards radius D and makes a distally directed turn to finally open into the main body cavity. It opens into the axocoel at the point where the axocoelomic duct opens into the main body cavity as well.

### Main body cavity

The large main body cavity is a coelomic cavity and resides in aboral position to the hydrocoel ring. It fills up the space between the body wall, the hydrocoel and the intestinal tract (Figs. 8, 12A). It is traversed by various trabecular and lacunar structures and is known to constitute a fusion product of the axocoel and the two somatocoelia of the larva (Hyman 1955, this thesis). In adults, the space within the intestinal coil is limited to a larger central coelomic space amidst a labyrinth of communicating somatocoelomic clefts formed by secondarily formed septa and trabeculae, which is called the axial sinus (Fig. 14, Heinzeller and Welsch 1994).

### Small ventral coelom

The *small ventral coelom* is a small epithelially lined cavity that resides within the body wall protrusion (Figs. 12, 13). It starts at the point where the primary stone canal and the axocoelomic duct merge with the main body cavity, and extends horizontally and parallel to the axocoel towards the intestinal tract. It is accompanied by the ventral lacuna on most of its way and concavely shapes around the latter, providing part of the lacuna’s lining. It ends near the proximal margin of the ventral lacuna, before the latter continues in the periesophageal lacuna.

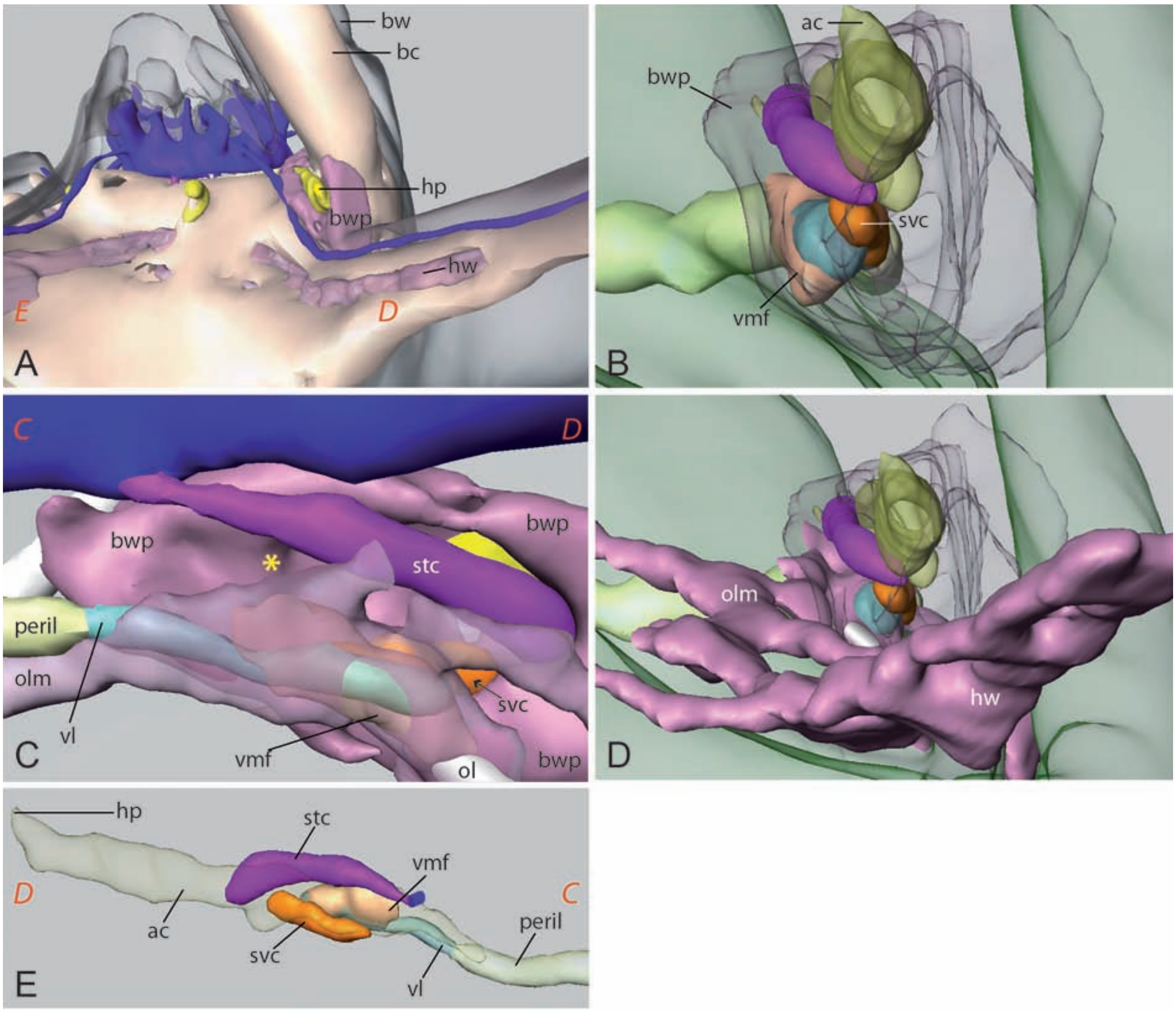


**Fig. 12:** Juvenile *A. bifida*, specimen 10. **A-E** Position of the axocoel (*ac*, ***yellow***), oral lacunar meshwork (*olm*, ***old rose***) and horizontal wall in arms (*hw*, ***old rose***), periesophageal lacuna (*peril*, ***pale yellow***), stone canal (*stc*, ***dark pink***), small ventral coelom (*svc*, ***orange***), ventral lacuna (*vl*, ***pale blue***), ventral mesenteric formation (*vmf*, ***light skin color***) relative to each other within the body wall protrusion (*bwp*) in interradius CD. **A**, **B**, **D** View onto interradius DE. Adjustment of **B** resembles that of the serial sections of Fig. **13**. **C** View from within the juvenile. Ventral mesenteric formation and ventral lacuna form part of the oral lacunar network, although they are distinct structures. *yellow asterisk* marks a semi-isolated space to the right of the axocoelomic duct. This space is continuous with the main body cavity aborally as well as to the right (radius C). To the left (radius D), it is mostly bordered by both the ventral mesenteric formation and the body wall protrusion, except for a small oral connection. **E** View onto selected structures from the outside onto interradius CD. *bc* main body cavity, *bw* body wall, *hp* hydropore, *ol* oral lacuna. Symmetry marked with *orange capital letters*.

## Primary body cavities and prominent matrix formations

Inside the matrix, presumably fluid filled interstices and lacunae represent the primary body cavities of the animal. These are three main intestinal lacunae and two lacunar side-branches, an axial lacuna, trabeculae, the *oral lacunar meshwork,* as well as the *ventral mesenteric formation* and the ventral lacuna. In addition, a prominent body wall protrusion in interradius CD and two conspicuous inwardly directed bulges of the body wall can be distinguished.

As the primary body cavities of echinoderms are, like in all coelomic organized animals, ontogenetically formed by the *ecm* between the coelomic cavities, they are all directly or indirectly interconnected – be it by mesenteries, lacunar structures or the smallest spaces between the

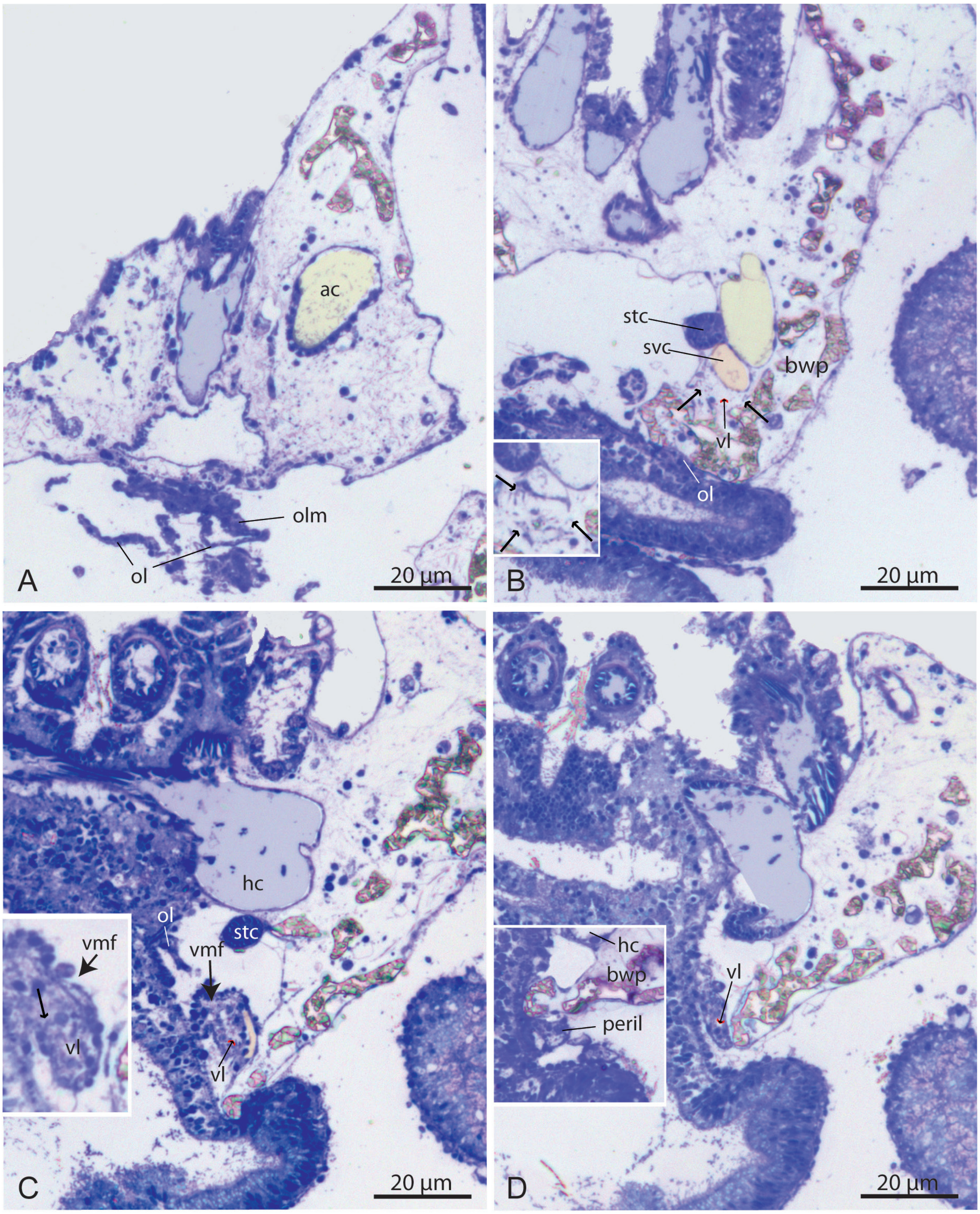


**Fig. 13 part 1 of 3**

**Fig. 13:** Juvenile *A. bifida*, specimen 10. **A-i** Sequence of serial sections, whose position in the animal is indicated by **J**. **A-D** Ventral structures in the body wall protrusion (*bwp*) in interradius CD and **E-F** the course of the periesophageal lacuna (*peril*). The *insets* in **B-D** show details on slightly later sections as the main pictures show. **B**, ***inset* B**, **inset C** Black arrows point to cellular protrusions sent out by cells of the small ventral coelom (*svc*) that surround the ventral lacuna (*vl*). **E-H** The periesophageal lacuna resides above the oral lacunar meshwork (*olm*), which, in this radius, is attached to the body wall above the oral arm coelom (*oac*). **i** The periesophageal lacuna merges with the oral end of the oral section of the axial mesentery (*osec*) and the oral lacunar meshwork. *ac* axocoel, *hc* hydrocoel, *hw* horizontal wall, *ol* oral lacuna, *stc* primary stone canal, *vmf* ventral mesenteric formation.

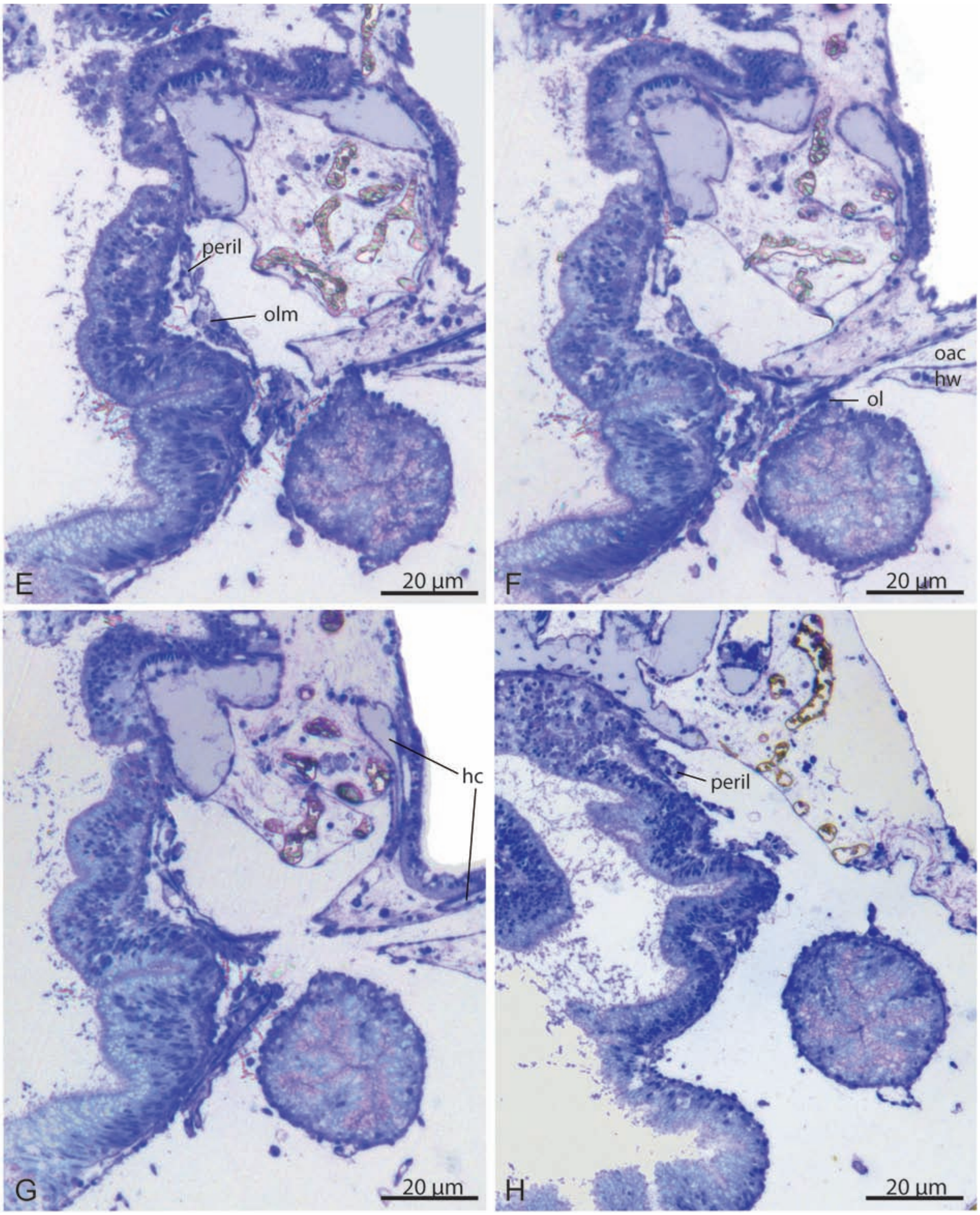


**Fig. 13 part 2 of 3**

basal laminae of adjacent coeloms. So they are interconnected primary body cavities or simply spaces inside the matrix.

The following description aims to describe all distinct structures one by one and to also name their observable direct (and important indirect) connections.

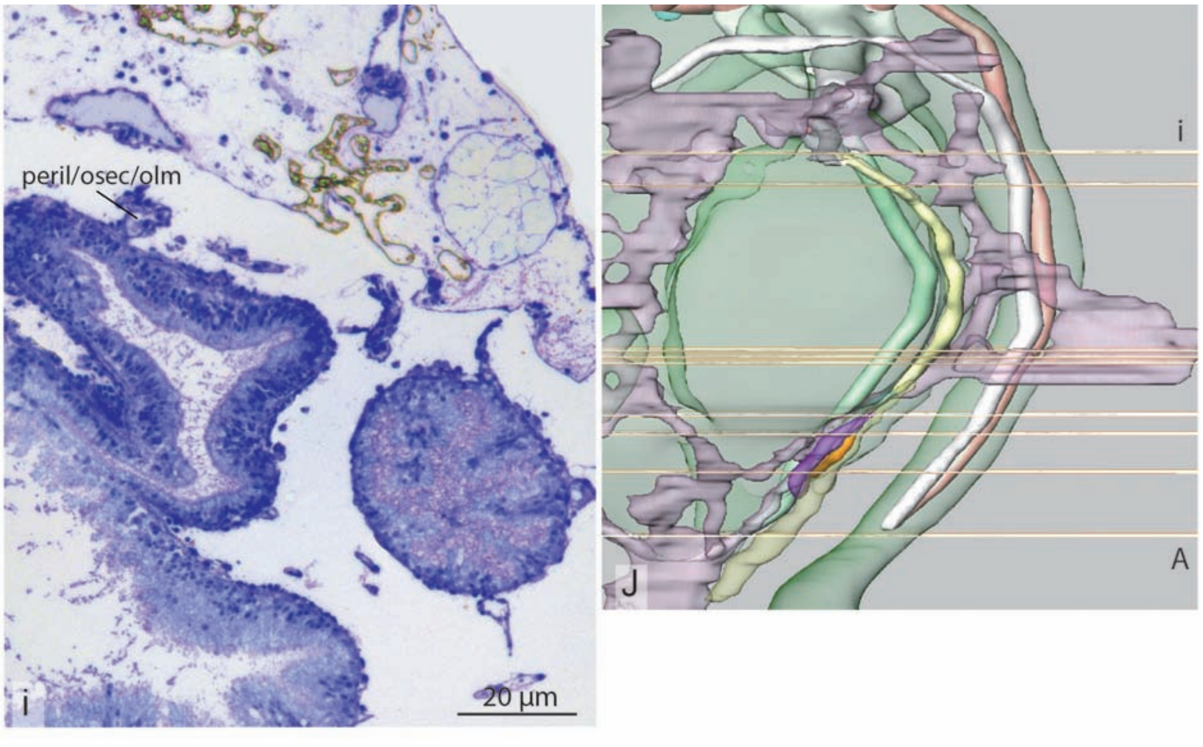


Fig. 13 part 3 of 3

## Intestinal lacunae

The intestinal tract is accompanied by three main lacunae: The oral lacuna, the aboral lacuna and the periesophageal lacuna (Figs. 15, 16). The former two can be traced running down the intestinal tract from the anus onwards at two opposite sites. They are continuous via a sequence of the proximal mesenteric part of the axial mesentery, the periesophageal lacuna, the ventral lacuna and the *ventral mesenteric formation*. The **oral lacuna** lies on the oral side of the intestinal tract and follows its course counterclockwise in a disto-oral position until radius D (Fig. 15). Its course is interrupted in Interradius DE, where the first part of the lacuna ends and a second part starts further aborally, winding slightly oralwards again (Figs. 15B, 15D). In radius D, the oral lacuna splits into an oral branch and a centro-horizontal branch that both continue counterclockwise around the stomach.

The centro-horizontal branch extends on the ventral side in an intestinal fold around the stomach and finally merges with the primary body cavity in the proximal mesenteric part of the *glandular axial organ* (Figs. 8A, 15F). As this branch is not visible in younger larval stages, it has to be considered as being secondarily formed during development.

The oral branch, however, continues until it becomes the *ventral mesenteric formation*, into which the ventral lacuna is embedded, that continues in the periesophageal lacuna (Figs. 12C, 13A-C).

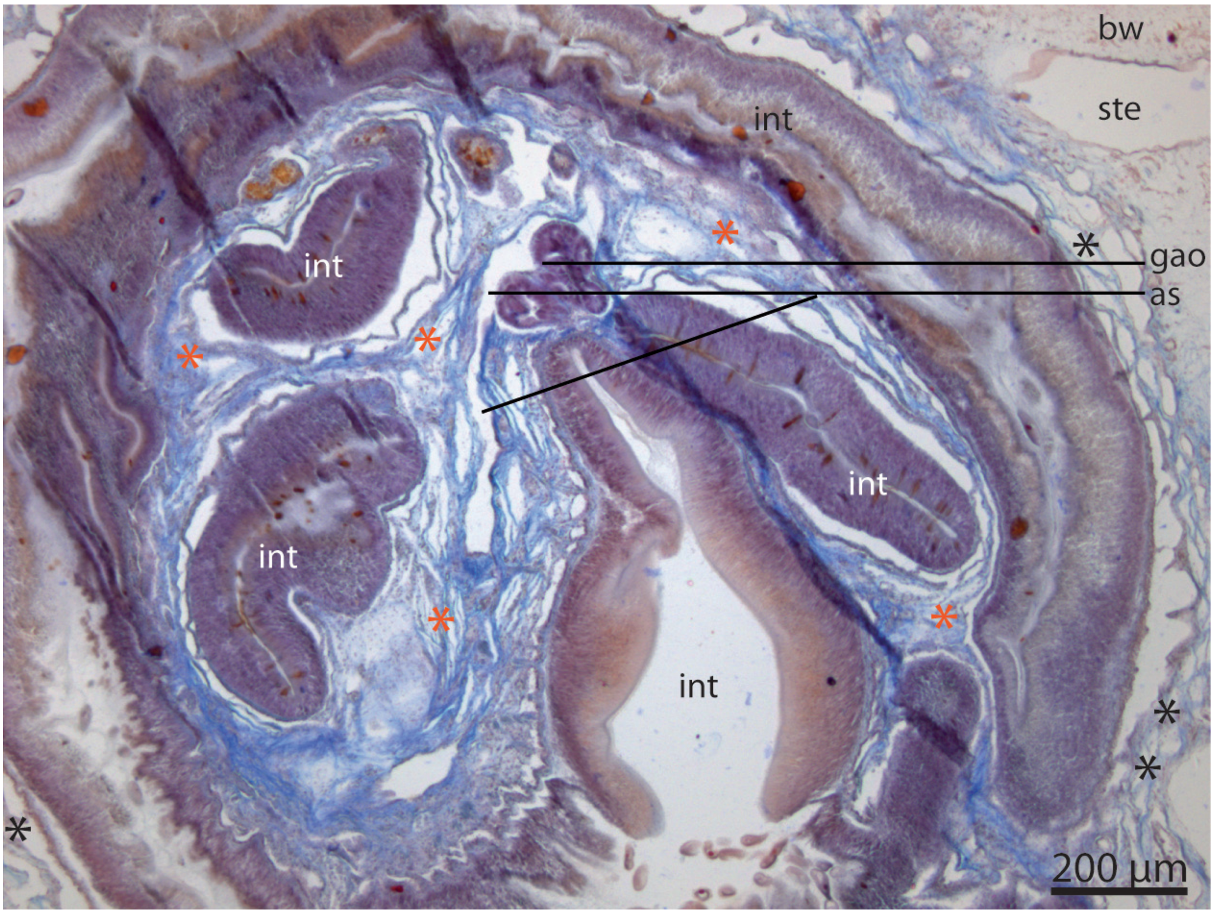


**Fig. 14:** Adult *Antedon petasus*. LM. Cross-section showing the position of the axial sinus (*as*) surrounding the glandular axial organ (*gao*) within the cleft main body cavity. *Orange asterisks* mark the clefts within the intestinal coil, *black asterisks* mark the clefts of the peripheral area of the main body cavity. *bw* body wall, *gao* glandular axial organ, *int* intestine, *ste* stereom.

The shape of the oral lacuna and its two branches varies. Where the intestine leaves the anal tube into the main body cavity, the covering epithelium of the oral lacuna is not just elevated, but in cross section sticks out like the loop of an omega. On the juvenile's right to dorsal side (radius C to E in counterclockwise direction), the oral lacuna is attached to the tegmenal body wall and possesses a mesenteric nature. On the left and ventral side and extending further around the stomach towards the bend of the intestine on the right, the visceral layer of the main body cavity is detached from the intestinal tract and forms the oral branch as well as the central-horizontal branch of the oral lacuna between the intestinal tract and the visceral layer of the main body cavity.

Starting at the anus, the **aboral lacuna** runs along the aboral side of the intestine until it reaches the intestinal tract‘s aboralmost location on the larva's right side (Fig. 15). Here, it makes a turn around the axis of the *glandular axial organ* and approaches the latter from the ventral side (interradius CD), uniting with the proximal mesenteric part of the *glandular axial organ ecm* as well. It performs this loop above the interradii AB and BC, going down in the former and rising up in the latter. Along the whole intestine, the aboral lacuna has the omega-shape described above (Fig. 16). Towards the proximal mesenteric part of the *glandular axial organ*, it widens and detaches from the intestinal tract (Fig. 16i).

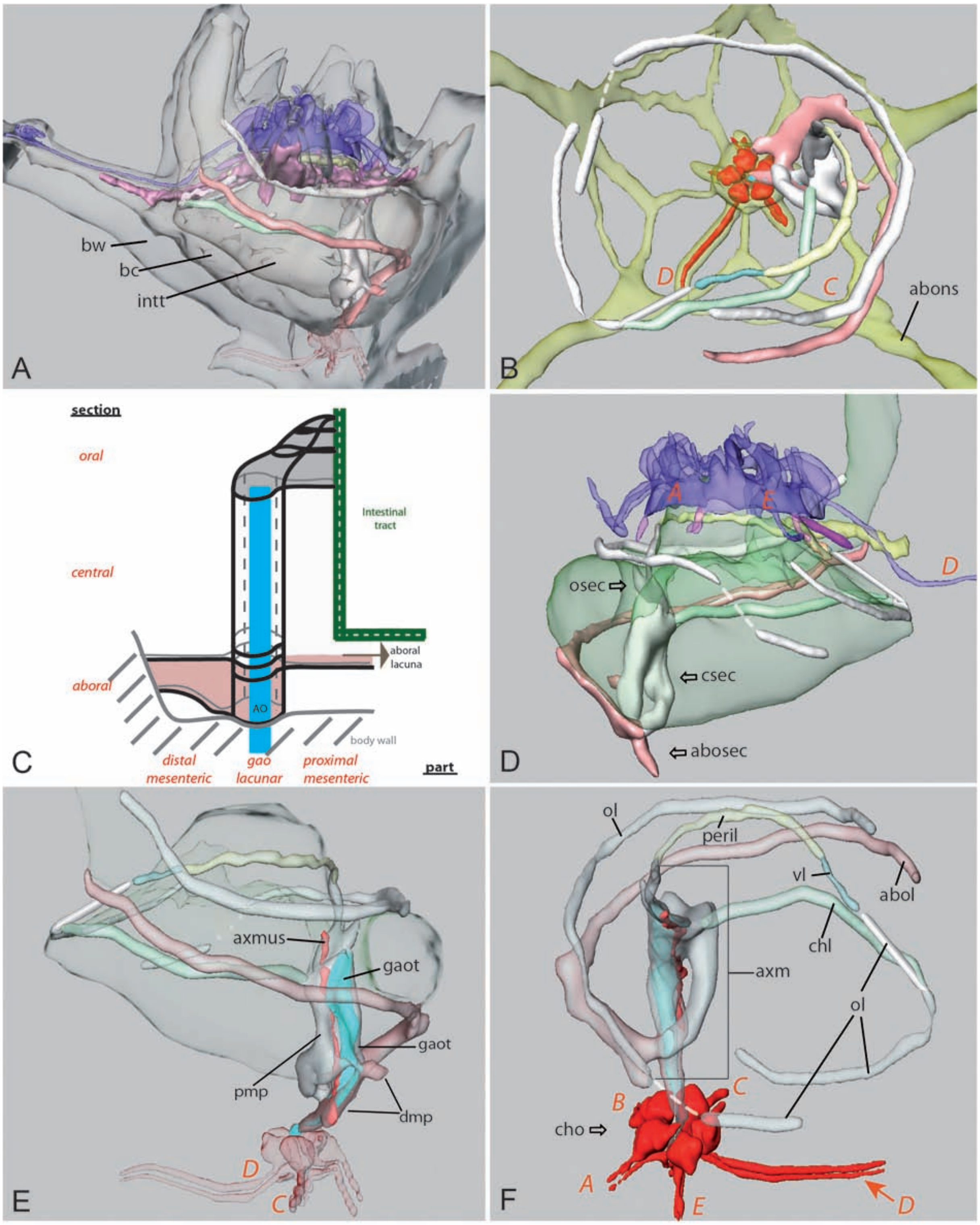


**Fig. 15:** Juvenile *A. bifida*, specimen 10. **A**, **B**, **D – F** Position of the extra-coelomic structures as explained in the text, view from different angles. Dotted white line indicates that the oral lacuna is continuous. **C** Schematic illustration showing the topology of the sections and parts of the axial mesentery (*axm*) between the body wall and the intestinal tract, ignoring the twist in the proximal mesenteric part of the central section of the axial mesentery (*csec*). View from *within* the intestinal tract's bent. Colors match those in the reconstruction. *abol* aboral lacuna (***light coral***), *abons* aboral nervous system, *abosec* aboral section of axial mesentery (***pale rose***), *axmus* axial muscle, *bc* main body cavity, *bw* body wall, *chl* centro-horizontal branch of the oral lacuna (***pale green***), *cho* chambered organ, *csec* central section of axial mesentery (***white***), *dmp* distal mesenteric part, *gaol* glandular axial organ lacuna, *gaot* glandular axial organ tubule (***turquoise***), *intt* intestinal tract (***green***), *ol* oral lacuna (***white***), *osec* oral section of axial mesentery (***pale gray***), *peril* periesophageal lacuna (***pale yellow***), *pmp* proximal mesenteric part, *vl* ventral lacuna (***pale blue***), ***blue*** hydrocoel, ***old rose*** both oral lacunar meshwork and horizontal wall in arms, ***purple*** stone canal. Symmetry marked with *orange capital letters*.

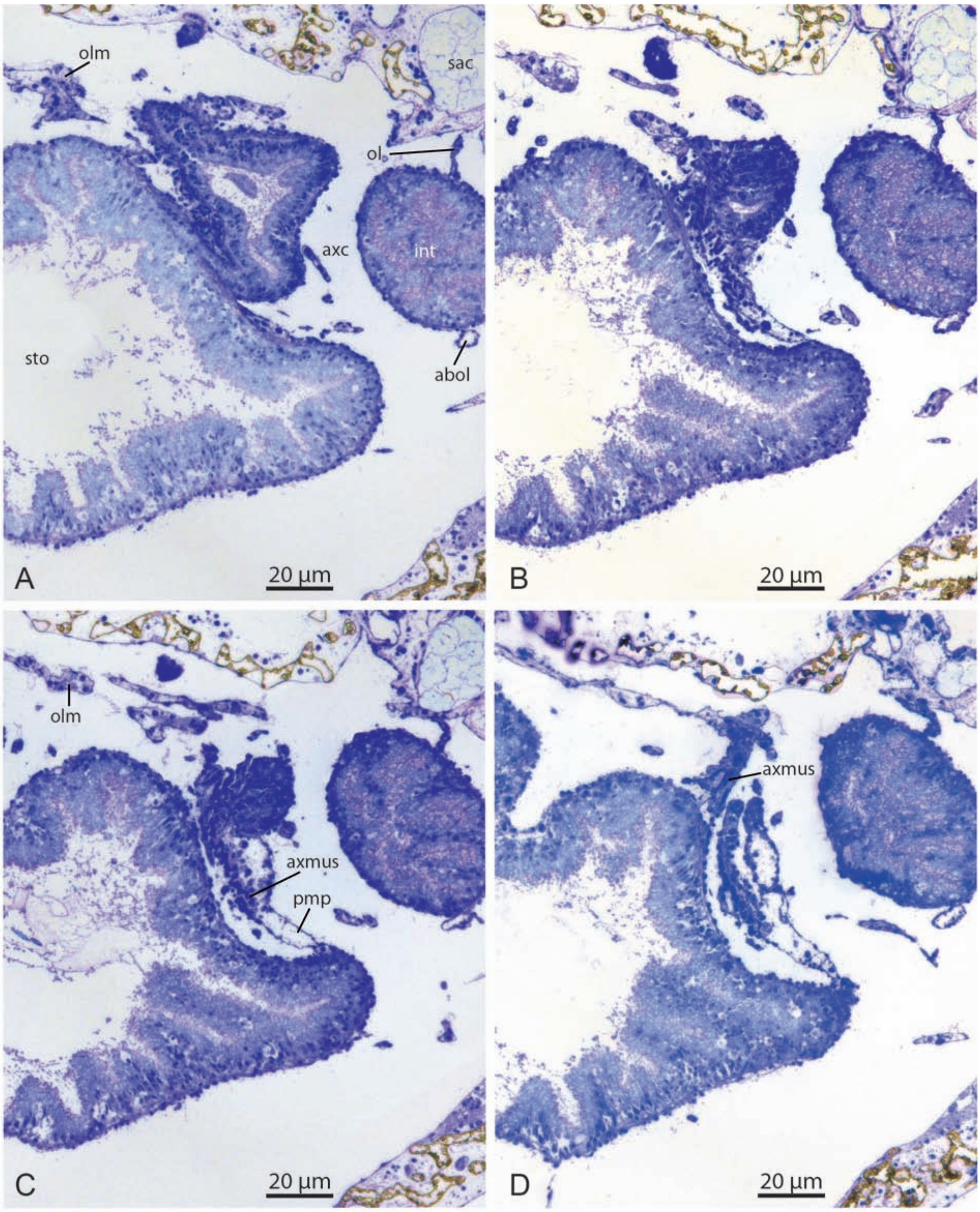


**Fig. 16 part 1 of 3**

**Fig**. **16 (3 parts):** Juvenile *A*. *bifida*, specimen 10. Glandular axial organ described as part of the axial mesentery (*axm*) within the axial compartment (*axc*). **A-i** Sequence of serial sections, whose position in the animal is indicated by **J**, **K**. *abol* aboral lacuna, *axmus* axial muscle, *dmp* distal mesenteric part of axial mesentery, *int* intestine, *gaot* glandular axial organ tubule, *ol* oral lacuna, *olm* oral lacunar meshwork, *pmp* proximal mesenteric part of axial mesentery, *sac* sacculus, *sto* stomach, *black-red arrow* further trend, *small black arrow* trabeculum, *small red arrow* mesenteric strand.

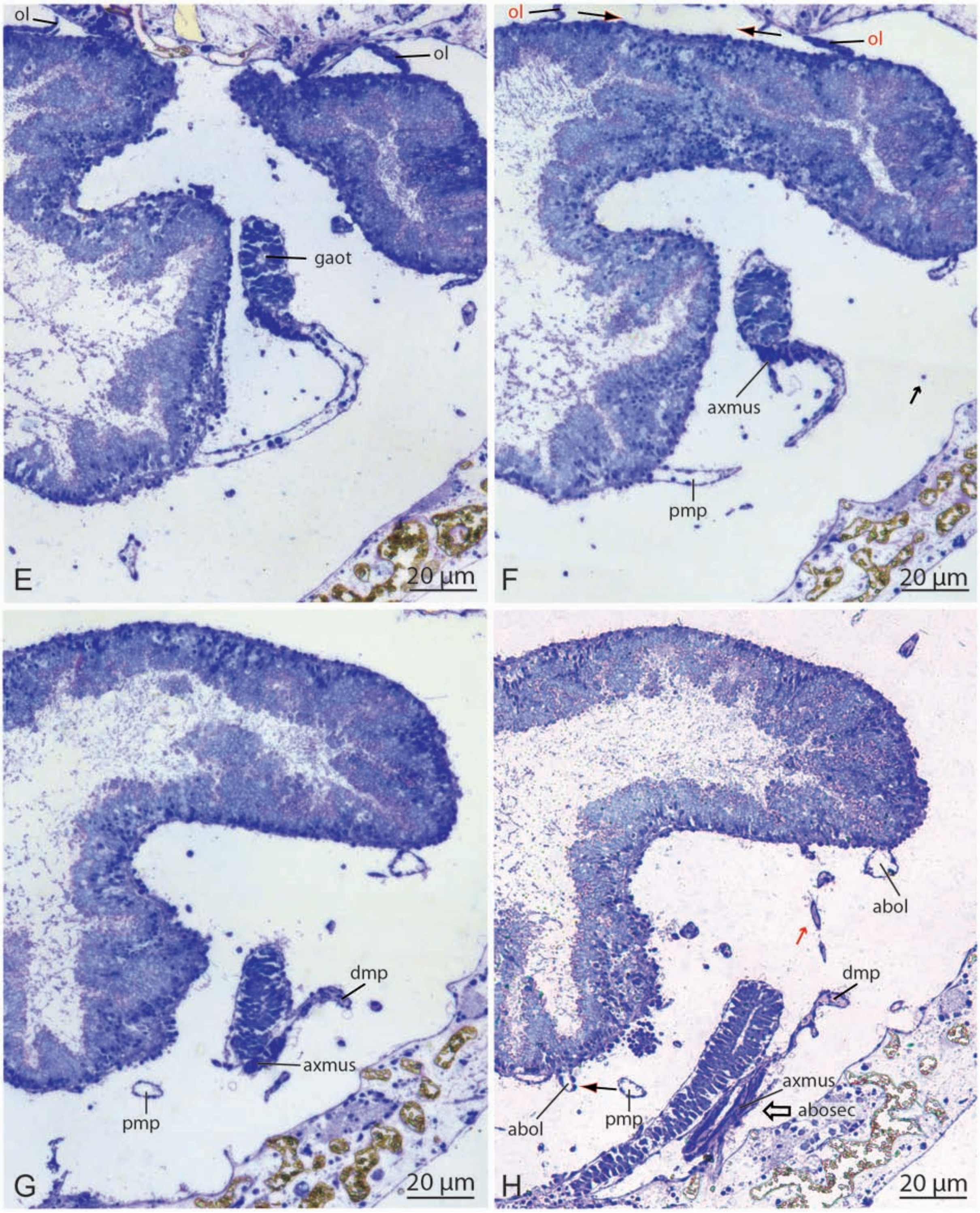


**Fig. 16 part 2 of 3**

The **periesophageal lacuna**, the covering epithelium of which sticks out like the loop of an omega as well (Figs. 13E-H), surrounds the esophagus on the ventroright side only (Figs. 15B, 15E, 15F, 18D). It continues from the right end of the ventral lacuna that is part of the *ventral mesenteric formation* and ends in connection to the oral part of the *glandular axial organ*'s lacunar space, which itself is associated with the *oral lacunar meshwork* (Fig. 18D).

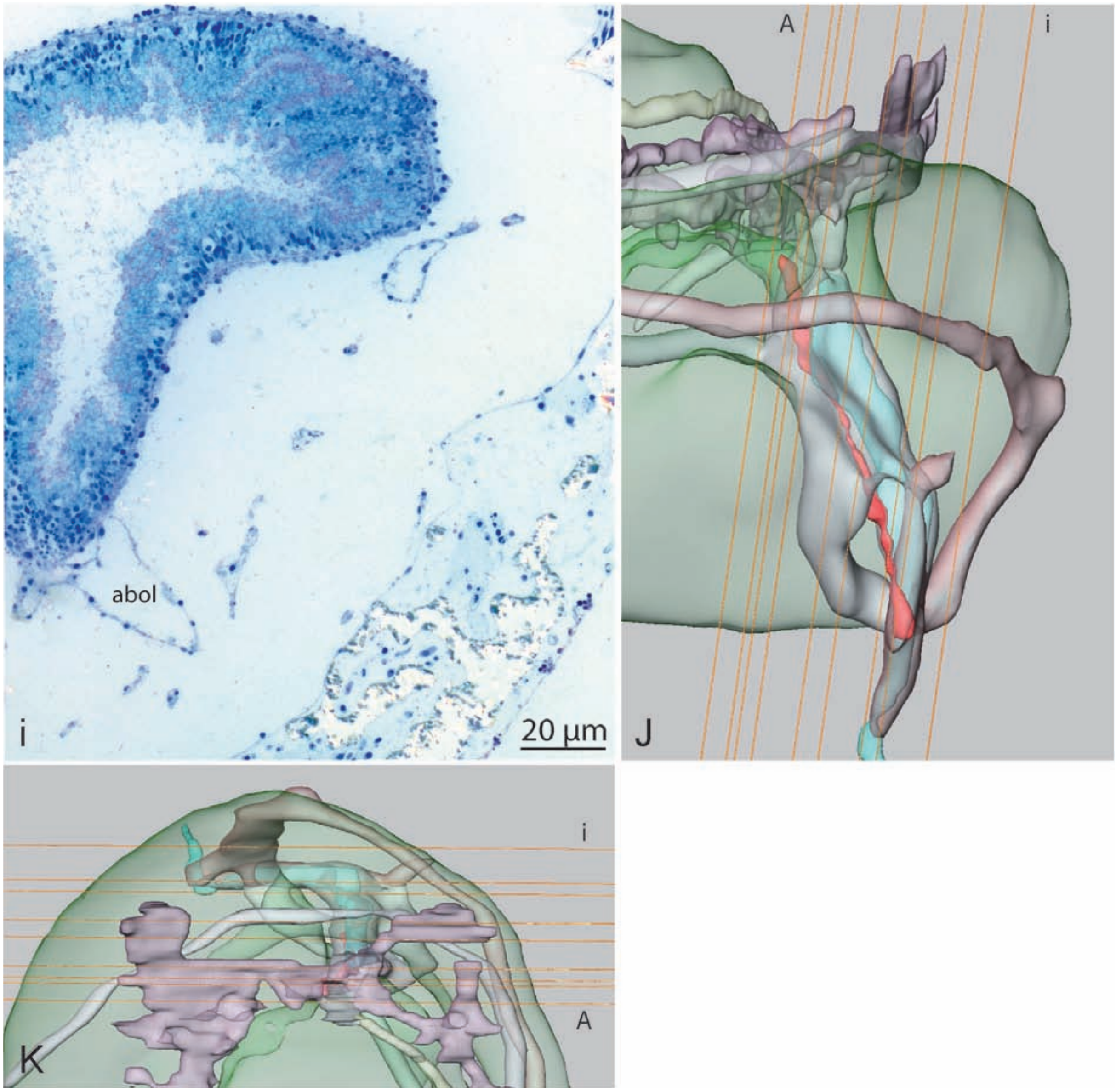


**Fig. 16 part 3 of 3**

### The axial mesentery

The main body cavity in the intestinal coil’s center is traversed by a large axially elongated mesentery, which spans between the aboralmost end of the body wall and the intestinal tract and is lined by the basal lamina of the coelomic lining of the main body cavity (Fig. 15). Part of it shares in the formation of the *glandular axial organ*. The axial mesentery can be axially subdivided into an **aboral section** underneath the intestinal tract, a **central section** and an **oral section** (Fig. 15C).

In the aboral section of the axial mesentery, the *glandular axial organ lacuna* emerges from the loose *ecm* of the body wall in interradius BC between the chambers of the chambered organ and rises oralwards, slightly shifting towards radius B on its oralmost way. The coelothelial lining of the axial mesentery splits into two mesenteric extensions that derive in radial direction and establish contact with the body wall in interradius BC. Therefore, the aboral section of the axial mesentery can be radially subdivided into a **(glandular axial organ) lacunar part** and a **distal mesenteric part** (Fig. 15C).

In the central section, the *glandular axial organ lacuna* is attached to the stomach via a slightly widened vertically elongated mesentery. Therefore, the cental section of the axial mesentery can be radially subdivided into a **proximal mesenteric part** and a **(glandular axial organ) lacunar part** (Fig. 15C). The mesenteric part is aborally in direct continuation with the aboral lacuna and orally with the centro-horizontal branch of the oral lacuna (colored in *pale green*).

Both the lacunar part of the aboral section and that of the central section house the tubules of the *glandular axial organ.* Tubules, surrounding hemal space and surrounding coelothelium of the axial mesentery, together form the *glandular axial organ* (Fig. 17B).

The oral section of the axial mesentery is a lacunar space in the periintestinal *ecm* of the esophagus (Figs. 15E, 17A) and represents the connection between the *glandular axial organ lacuna* and the periesophageal lacuna. It is also associated with the *oral lacunar meshwork* (Fig. 18D).

The hemal space of the *glandular axial organ lacuna* consists of a lightly woven fibrous matrix, into which single cells are locally embedded (Fig. 17). The mesenteric part is light-microscopically empty and only few cells reside within it. Those that do, reside in proximity near the basal lamina of the coelothelium. The matrix in the distal mesenteric part, however, resembles the texture of the body wall.

### Trabeculae

Scattered throughout the calyx, especially on the left to dorsal side between radii D to B, several trabeculae traverse the main body cavity (Figs. 8A, 16F). They are formed by squamous coelomic epithelium that surrounds a fragile strand of *ecm*, which extends between the body wall and the periintestinal matrix; no lacuna could be detected within the trabeculae. Most of these strands contain prominent central muscle fibers.

### The oral lacunar meshwork

The *"oral lacunar meshwork*" locates in the oralmost part of the main body cavity (Figs. 10E, 10F, 12, 13, 18), where it extends around the esophagus between the latter and the body wall. It consists of interconnected strands of main body cavity coelothel enclosing some *ecm*, which is continuous with both the periintestinal *ecm* and either with the loose *ecm* of the horizontal wall in the proximal part of the arms (radii D, E) *or* to the body wall above the oral arm coelom (radii A, B, C) (Figs. 18E, 18F).

On the esophagus' right side, the tissue strands insert right underneath the periesophageal lacuna (rarely above this lacuna) (Fig. 18F). They are also associated with the oral section of the axial mesentery. Further distally, the *oral lacunar meshwork* stretches *above* the intestinal tract's oral lacuna or attaches to the body wall *above* it (Figs. 13E-13H). On the esophagus' left side, it holds its position above the oral lacuna (Fig. 8A).

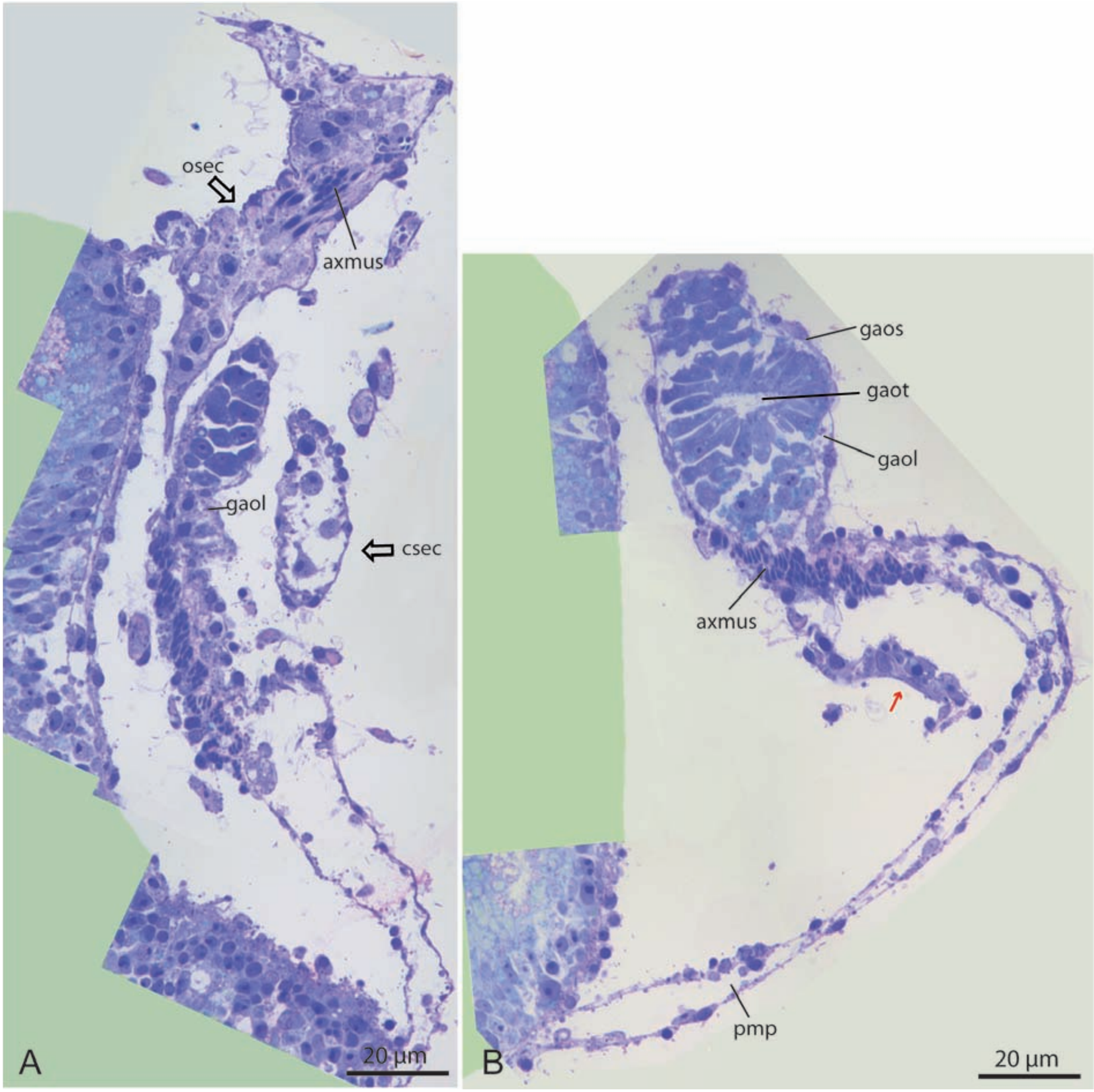


**Fig. 17:** Juvenile *A. bifida*, specimen 10. Glandular axial organ as part of the axial mesentery within the axial compartment. Details. **A** Section similar to Fig. **16D**. **B** Section between Figs. **16E** and **16F**. *axmus* axial muscle, *csec* central section of axial mesentery, *gaol* glandular axial organ lacunar part, *gaos* epithelial sheath of the glandular axial organ, *gaot* glandular axial organ tubule, *osec* oral section of the axial mesentery, *pmp* proximal mesenteric part of the axial mesentery, *small red arrow* mesenteric strand.

The radii B, C and D are traversed by two lateral *oral lacunar meshwork* tissue strands that are attached to the sides of the horizontal wall of each arm (Figs. 18C, 18D). Within the radii E and A, single broader strands extend to the horizontal wall in the respective arm. Several radial strands contain muscular strands and may be interconnected to each other in less regular ways via further tissue strands.

What is described as the *oral lacunar meshwork* is heterogeneously structured. Its matrix, however, is granularly filled, at least in most parts (Figs. 18E, 18F). Unlike the single trabeculae, the covering coelothelium consists of densely distributed somata and folds up locally. It contains single as well as locally coagglutinating multishaped or rounded cells and is pervaded by muscle strands that extend in various, but more or less horizontal directions. Obviously, the muscle

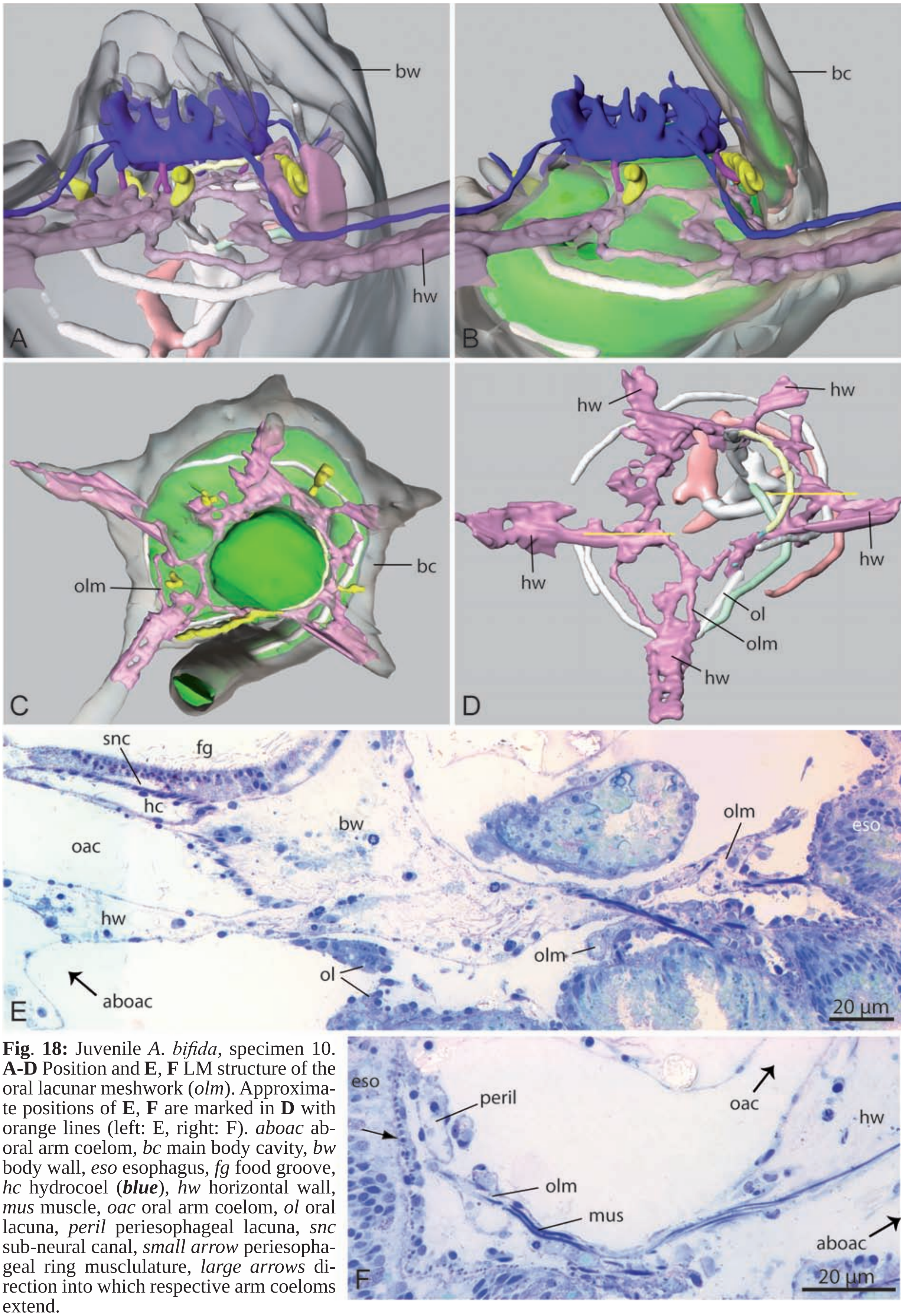


**Fig. 18:** Juvenile *A. bifida*, specimen 10. **A-D** Position and **E**, **F** LM structure of the oral lacunar meshwork (*olm*). Approximate positions of **E**, **F** are marked in **D** with orange lines (left: E, right: F). *aboac* aboral arm coelom, *bc* main body cavity, *bw* body wall, *eso* esophagus, *fg* food groove, *hc* hydrocoel (***blue***), *hw* horizontal wall, *mus* muscle, *oac* oral arm coelom, *ol* oral lacuna, *peril* periesophageal lacuna, *snc* sub-neural canal, *small arrow* periesophageal ring musclulature, *large arrows* direction into which respective arm coeloms extend.

strands are suitable to slightly alter the position of the juvenile‘s intestinal tract (and thereby agitate the body cavity’s coelomic fluid a bit).

### Inwardly directed bulges of the body wall

Starting at the aboral end of the *body wall protrusion* in interradius CD, the body wall forms a conspicuous bulge towards the intestinal tract (Fig. 19). The bulge can be traced running aboralwards in counterclockwise direction, opposing the aboral lacuna, towards the point, where the *glandular axial organ lacuna* emerges from the loose *ecm* of the body wall in interradius BC.

A similar local bulging of the body wall can be found further orally along the dorsal and left side of the larva, approximately opposing the non-attached part of the oral lacuna.

### Body wall protrusion in interradius CD.

In the calyx’ oral part in the section between radius D and interradius CD, the body wall forms an inwardly directed vertical protrusion that narrows towards the stomach and closely contacts the periintestinal *ecm* with its aboral tip (but does not unite with it) in interradius CD (Figs. 12, 13B-D, 19). It protrudes vertically into the space between the stomach and the intestine’s last section (that rises up into the anal cone) and separates the coelom of the anal cone from the main body cavity. This *body wall protrusion* also forms a kind of fundament that supports the anal cone’s proximal part.

The *body wall protrusion* is formed by lightly woven *ecm* whose texture correlates to that of the general body wall (Figs. 13B-D). It is connected with the periintestinal *ecm* via the ventral lacuna and the *ventral mesenteric formation* (Figs. 13D).

Within the *body wall protrusion*, the axocoelomic duct, the primary stone canal, the *small ventral coelom* and parts of the ventral lacuna can be found (Figs. 12, 13B).

### Ventral lacuna and ventral mesenteric formation

The *ventral mesenteric formation* connects the loose *ecm* of the *body wall protrusion* to the periintestinal *ecm* (Fig. 13C). It originates from the left end of the oral lacuna’s oral branch and extends, being a structurally different unit, from the point where the ventral lacuna leaves the *body wall protrusion* to where the ventral lacuna becomes the periesophageal lacuna (Figs. 12C, 13A-D).

The ventral lacuna starts within the *ecm* of the *body wall protrusion* (Fig. 13B, inset). At this left end, it stands in direct relation to the *small ventral coelom*, as it uses the basal side of the *small ventral coelom*’s epithelium as part of its outer limits. Within the ventral lacuna, the matrix is of a similar light optical quality as the matrix of the *body wall protrusion*. The other side of the *ventral lacuna* is formed by cell processes of the *small ventral coelom* that are sent out by distant areas of the latter and close around the ventral lacuna – a condition that, using a light-microscope, can only be noticed and understood knowing the species’ larval development. The ventral lacuna

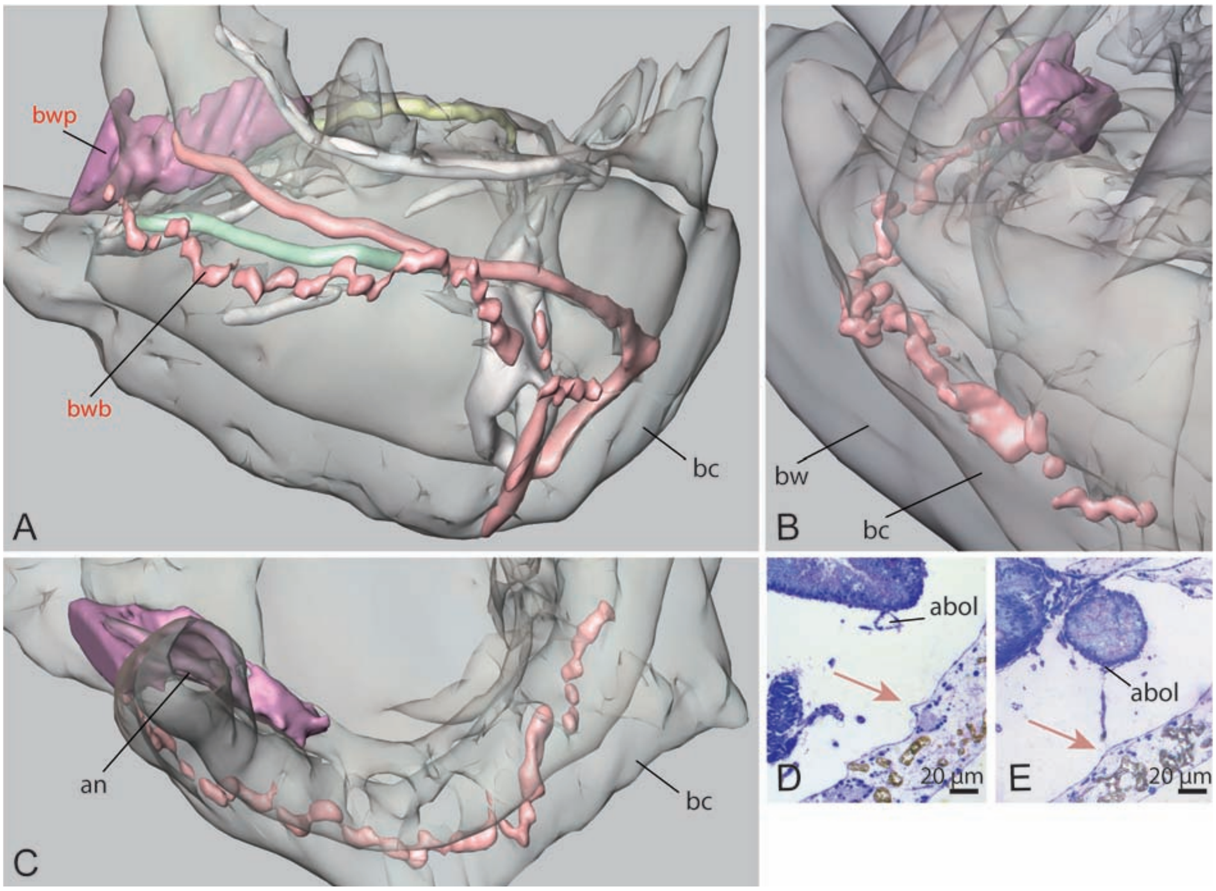


**Fig. 19:** Juvenile *A. bifida*, specimen 10. **A-C** Position of the inwardly directed body wall bulges (*bwb*) and the body wall protrusion (*bwp*). **D**, **E** LM. Examples for body wall bulges (*arrows*). *abol* aboral lacuna, *an* anus, *bc* main body cavity, *bw* body wall.

finally leaves the *body wall protrusion* at the latter's aboral tip, with the *small ventral coelom* as a separator between the two. The ventral lacuna then forms part of the *ventral mesenteric formation* (Figs. 13C, 13D), whose matrix has a similar light optical quality as the matrix of the *oral lacunar meshwork*. The ventral lacuna thereby remains subtly distinct from the *ecm* of the *ventral mesenteric formation* by means of elongated cell processes that, more or less prominently, cross the *ventral mesenteric formation* on its whole way. The *ventral mesenteric formation* shortens and as the *small ventral coelom* ends, the ventral lacuna loses contact to the *body wall protrusion* (Fig. 13C, inset). Finally, the ventral lacuna continues in the periesophageal lacuna (Figs. 13D, 13E).

## Structures in the ecm

### Aboral nervous system

The prominent aboral nervous system resides within the *ecm* of the body wall (Figs. 9, 20, 21). It encapsulates the chambered organ and sends interradial strands of nervous tissue, the interradial nerves, oralwards within the *ecm* of the body wall. After a short way, these strands bifurcate by 120 degree. The neighboring converging strands meet and thereby form radial nodes. These nodes are the origin of both the radial brachial nerves, which draw into the arms, and the ring

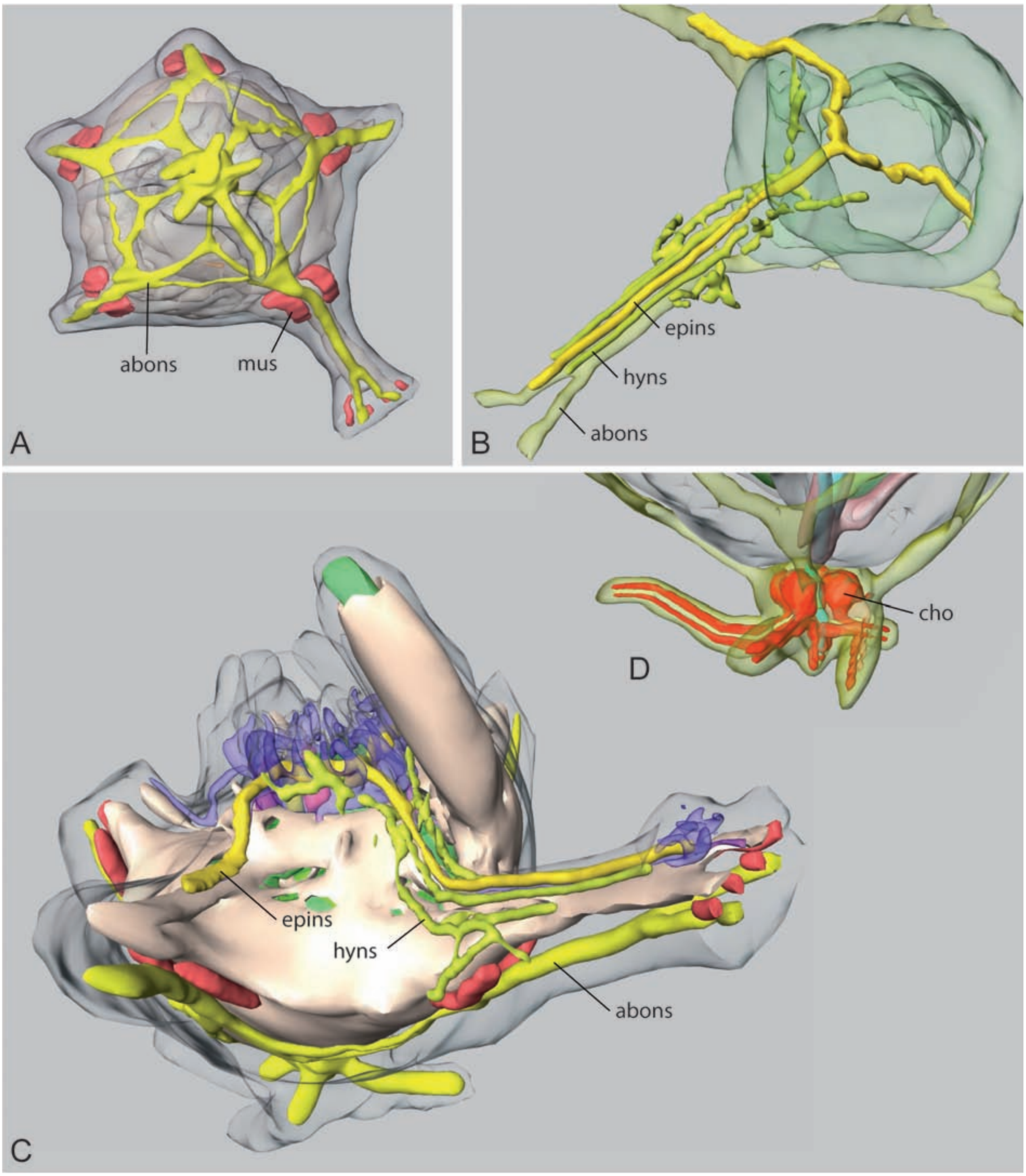


**Fig. 20:** Juvenile *A. bifida*, specimen 10. **A-D** Course of the centralized nervous systems and **A**, **C** position of the arm muscles (*mus*). **A** Aboral view onto the aboral nervous system (*abons*). **B** Oral view. **C** View onto interradius DE. **B**, **C** The epidermal nervous system (*epins*) forms a central nerve ring and radial nerves. The hyponeural nervous system (*hyns*) runs laterally within the mesoderm of the body wall and around the hydrocoel ring (***blue***). The lateral nerves form a commisure underneath the epidermal radial nerve. **D** The chambered organ (*cho*) is surrounded by the aboral nervous system.

nerve (=radial nerve commissure), which forms as the nodes of all neighboring radii are horizontally interconnected. Also, strands of the aboral nervous system extend from the central aboral capsule into the cirri, surrounding the cirral coeloms (Figs. 20D, 21B).

In the calyx' radii, the aboral nervous system is located between the skeletal elements and the peritoneal layer of the main body cavity (Fig. 8). Towards and in the arms, as well as in the

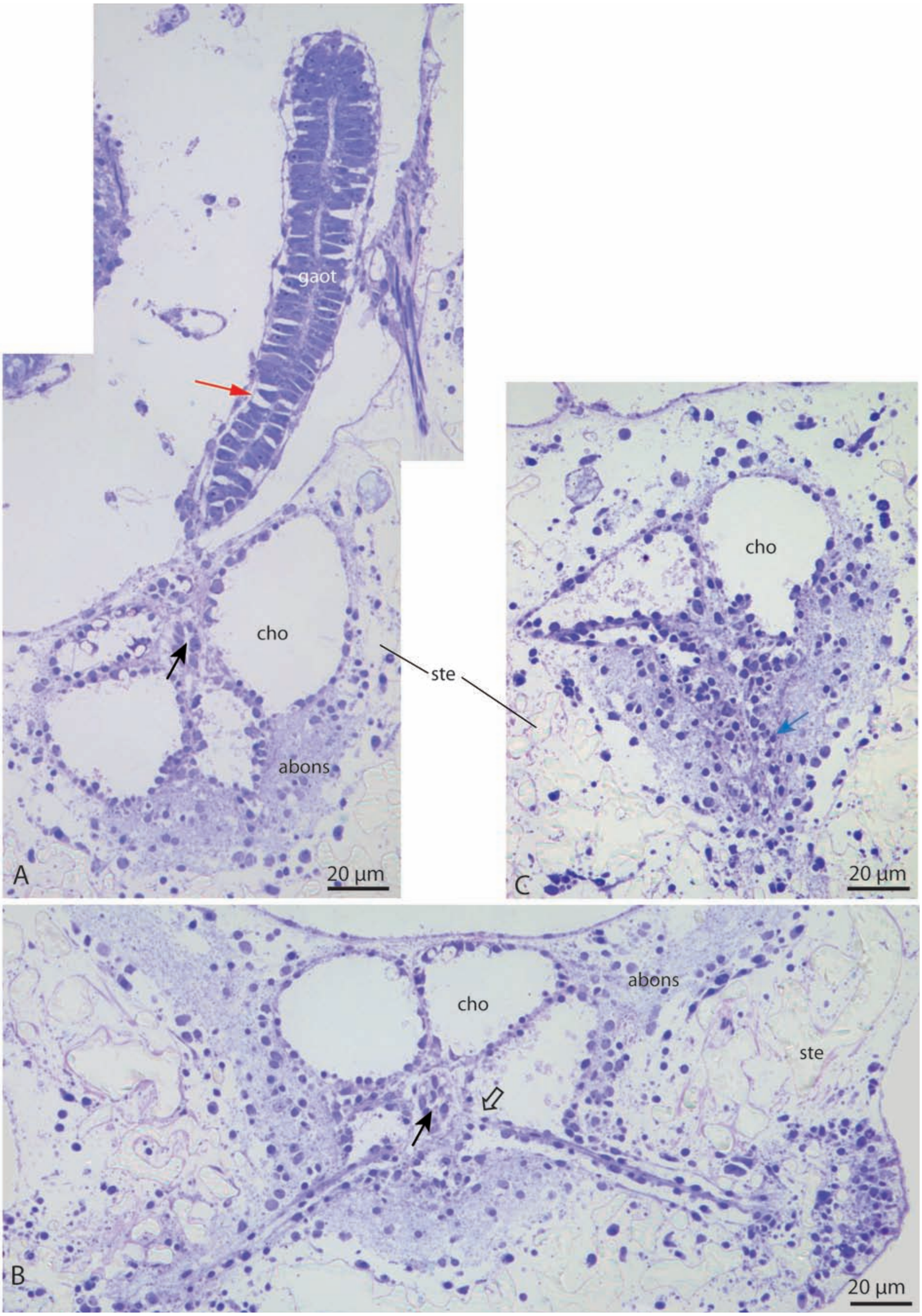


**Fig. 21:** Juvenile *A. bifida*, specimen 10. **A-C** LM structure of the glandular axial organ and the chambered organ (*cho*). *abons* aboral nervous system, *ste* stereom, *black arrows* tubule in the center of the chambered organ, *black empty arrow* foramen between the oral and aboral parts of the chamber, *blue arrow* aboral process, *red arrow* basal lamina of a glandular axial organ tubule (*gaot*).

cirri, it runs through a channel formed by the respective skeletal elements (cirrals and brachials) (Figs. 8, 9).

### Hyponeural nervous system

The hyponeural nervous system has not been completely reconstructed, but all prominent nervous structures indicating its arrangement around the mouth and all prominent structures of the arm in interradius D have been visualized (Fig. 20). It forms a simple network of thin subepithelial nervous tissue strands within the body wall's connective tissue, being mainly localized in the tegmen and in the oral half of the arms. It forms a ring distal to the hydrocoel ring from which two strands emerge on either side of the radial water canal into each arm. Another strand emanates orally into the oral valves, ending on half the latter's way. The strands in the arms subdivide again a few times further. The resulting radial strands have been observed to be locally interconnected. Local interconnections with both the basiepithelial and the aboral nervous system have been observed but have not been traced in detail.

### Basiepithelial nervous system

In larval stages (this thesis; Chia et al. 1986 for doliolaria) and adults (Heinzeller and Welsch 1994; Cobb 1987), all epithelia are underlain by a basiepithelial nerve plexus. The same has to be assumed for the present juvenile. As is the case in adults and as can be seen in the sections, the epidermal basiepithelial nervous system locally concentrates in the epidermis, forming the epidermal nervous system. The latter consists of the prominent ectoneural nerve ring around the mouth and of five ectoneural radial nerves, which derive from the nerve ring and run underneath the food grooves (Figs. 9, 20).

### Muscles

In the *ecm* of the body wall, prominent muscle bundles are located to the right and left side of each arm base (Figs. 20A, 20C). Further distally, after each arm‘s branching point, another pair of muscles can be found, on each of the two branches‘ right and left sides.

Muscle fibers in coelomic lining of the body cavity can be found in the *oral lacunar meshwork* and in the epithelial sheath of the *glandular axial organ*. In the *oral lacunar meshwork*, they extend horizontally, mainly within the radii, but strands that interconnect these radial ones can be observed as well (Figs. 18E, 18F.). In the epithelial sheath of the *glandular axial organ*, axially directed muscle fibers locate in that epithelial layer of the mesentery which is directed towards the intestinal tract's bend (Figs. 15E, 15F, 16J, 17B). This holds true for both the central and the aboral section. The muscle strand can be traced vertically along both mesenteric parts (aboral-distal and central-proximal), being anchored aborally in interradius BC between the related chambers. Orally, it extends within the oral section of the axial mesentery and connects to muscles of the *oral lacunar meshwork*.

Scattered muscle strands can be found within the *periintestinal ecm*. Around the esophagus, they are denser and form the periesophageal ring muscle (Fig. 18F).

### Coelomic spaces in arms

Orally in each arm, underneath the ciliated food groove, the comparatively small and narrow hydrocoelomic radial water canal is situated.

Underneath the hydrocoel resides a large coelomic cavity, which is continuous with the main body cavity in the calyx. Within the arm's most proximal segment, the cavity is divided by a horizontal wall (Figs. 9A, 9B, 18). The horizontal wall consists of loose *ecm*, which is laterally continuous with the arm's body wall and that is covered with coelomic epithelium. That way, an oral- and an aboral section of the arm coelom can be distinguished. The horizontal wall is pierced a few times by holes, so that the two coelomic sections are not fully separate from each other (Fig 12D). Further distally, the wall ends and the two sections unite to form one cavity until the distal ends of the arms (Figs. 9C, 9D). After some distance, the cavity divides together with the branching arms. Right before the division of the arms, the arm coelom starts to form local emarginations, the ciliated pits, on its aboral side (see Grimmer and Holland 1979 for ciliated pits of adult Crinoids). These are lined by cuboidal to columnar epithelial cells. The higher density of monociliated cells leads to a strong ciliation of this area. Distal to the division of the arms, the cavity is locally repeatedly divided into two areas by a thin horizontal septum, which is not in direct continuation with the horizontal wall just described. It spans the coelom in a more aboral position. Ciliated pits do not occur in this area.

The *ecm* and the covering epithelium of the horizontal wall in the arms are continuous with those of the *oral lacunar meshwork* in the calyx (Fig. 18).

### Chambered organ

Aborally underneath the main body cavity, five small radial coelomic chambers reside that are arranged like the chambers of an apple core: the mesenteries between each neighboring chambers are transversally stretched out, while the distal areas are rounded (Figs. 15F, 20D, 21). The chambers are separate from both each other and from the main body cavity. Each chamber is partially divided into an oral and a smaller aboral part by a horizontal septum; proximally, these parts are continuous via a foramen in the septum (as already described in detail by Grimmer et al. 1984). For technical reasons, this foramen has not been charted in the juvenile's 3D-reconstruction in order to illustrate the division of the coelomic tubes in the cirri. Figure 21B drafts a correction of this inaccuracy.

Each chamber's aboral part has a short, aborally directed process. Furthermore, both the oral and the aboral part of each chamber send out one coelomic process. These oral- and aboral coelomic tubes jointly pervade the related cirrus in the same radius as the chamber.

All chambers and cirral tubes described so far are fully surrounded by nervous tissue. The oral part of the chamber in radius D, however, sends out another single process, that is not surrounded by nervous tissue. Accordingly, the body wall is elevated towards the outside at this point, forming a cirral bud (compare to Fig. 7A).

### The tubules of the glandular axial organ

The juvenile’s *glandular axial organ* contains several central epithelially lined tubules that are vertically arranged in a row. One of the tubules originates aborally from the center of the chambered organ and extends straight orally through the aboral half of the *glandular axial organ* (Fig. 21). In the oral half of the *glandular axial organ*, there are further tubular entities that overlap each other with their ends (Fig. 17B). The tubules are formed by an epithelium of cuboidal to columnar monociliated cells, whose apical surfaces are directed towards the tubule‘s center and which are underlain by a continuous basal lamina on the tubules’ outside, towards the *glandular axial organ*’s hemal space.

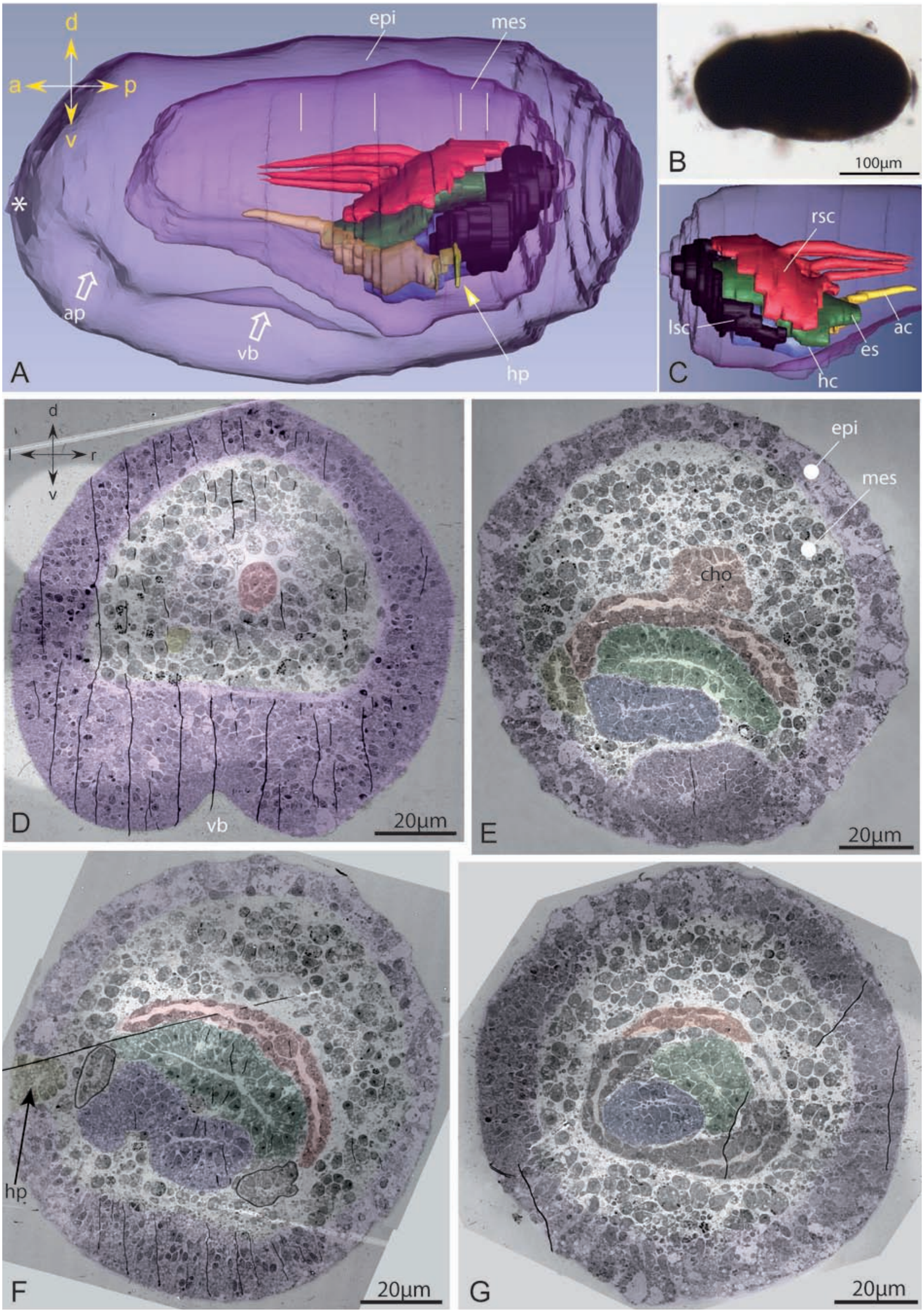
## 3.2 Doliolaria (Specimen 1)

### General morphology

The examined doliolaria larva has been cut from posterior to anterior. It is of 240 µm length and has a diameter of 100-150 µm. The anterior pole is marked by a densely ciliated region, the apical tuft, which points in the direction of movement (Fig. 22). The larva has four ciliary bands and two ciliated depressions of unequal size: the larger depression of the vestibulum, which resides ventrally in the anterior half of the larva and will be involved in forming the future mouth, as well as the smaller depression of the adhesive pit, which is situated between the vestibulum and the apical tuft (and will be involved in larval attachment).

Inside the posterior half of the larva resides a cluster of five epithelial cavities, comprising the four coelomic cavities, which are the axocoel, the hydrocoel and the two somatocoelia, as well as the enteric sac (the prospective intestinal tract). This cluster is surrounded by mesenchyme that consists of “free” cells, which are embedded in an extensive *ecm*. The mesenchyme is peripherally limited by the epidermal layer.

All cavities of the cluster reside collectively shifted by 30° clockwise to the right around the central axis of the larva when seen from posterior (Figs. 22F, 23C, 23i). However, to simplify matters, the following description idealizes the dimensional orientations of the cavities as ventral, dorsal or horizontal, respectively, though in truth they are shifted as described.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### Contents of coelothelic cells

With ongoing development, the cells of the different coelothelia vary gradually in their specific shape, but their ultrastructural composition remains similar to a large degree in all non-feeding stages. So, the coelothelia are not notably ultrastructurally specialized.

If not otherwise indicated, the following cell components can be found in all ultrastructurally investigated coelothelial cells: The cytoplasm is generally granular. The mainly euchromatic large nuclei are not restricted to a specific region of the cell and show one or two large nucleoli and a varying amount of central heterochromatic spots and irregularly thin heterochromatic ridges. Crescent shaped nuclei have occasionally been observed. All epithelial cells contain several to many yolk spheres of different size. They are usually dark-rimmed and contain differently textured electron-bright droplets of varying sizes. Rough Endoplasmic reticulum cisternae emerge from the perinuclear space and are usually mainly situated basally or laterally in the cell. In several cells, a Golgi apparatus could be detected apically; in some cases dictyosomes could be located between the nucleus and the root-structures of a cilium. Mitochondria are distributed throughout the cells but may locally occur in higher density as specifically stated. Optically empty vacuoles and vesicles, as well as vacuoles and vesicles that contain membranous structures (membrane-cored vesicles) or posses a granular, electron-optical dense core (dense-cored vesicles), can be found. Apically, the density of smaller empty vesicles is usually increased, many of them residing close to the apical cytoplasmic membrane.

### Hydrocoel

The ventralmost coelomic cavity, which can be shown to develop into the water-vascular system, is the hydrocoel (Figs. 22-24, 25D). It forms a slightly horizontally flattened horseshoe. The horseshoe-ends are overlapping on the left side (Figs. 23G, 23J). In a ventral view, the counter-clockwise directed end projects above/surmounts the clockwise end dorsally. Ventrally, slight evaginations butt against the proximal epithelial layer of the vestibulum, marking the prospective sites of the future primary podia.

The monolayered hydrocoelomic epithelium consists of cuboidal to columnar ciliated cells (Figs. 24, 25D). The ventral layer is thicker than the dorsal layer. The cells are apically connected by adherens junctions. Underneath the adherens junctions, some cells show further intercellular connections, whose type cannot be characterized ultrastructurally due to bad preservation. The epithelium is underlain by a basal lamina. Very few nerve cell processes reside in the basal interstitial spaces and between the basal lamina and the epithelial cells. In one case, a thin

◂ **Fig. 22:** Doliolaria of *A. bifida*, specimen 1. Orientation of the larva depicts a horizontal swimming position. **A**, **C** Reconstruction of the doliolaria in **B** based on ultra-thin cross-sections. **C** View onto the right side. **D-G** Selected colorized TEM pictures of cross-sections, the positions of which in the larva are indicated by vertical *white lines* in **A**. Colors match the related structures in the reconstruction. **D** Cross-section through ciliary band two and the vestibulum. *ac* axocoel (***yellow***), *ap* apical organ, *cho* prospective chambered organ (***red***), *epi* epidermis (***lilac-gray***), *es* enteric sac (***green***), *hc* hydrocoel (***blue***), *hp* hydropore (***yellow***), *lsc* left somatocoel (***black***), *mes* mesenchyme, *rsc* right somatocoel (***red***), *vb* vestibulum, *star* position of adhesive pit. *a* anterior, *d* dorsal, *l* left, *p* posterior, *r* right, *v* ventral. Orientation of **D** valid for **E-G** as well.

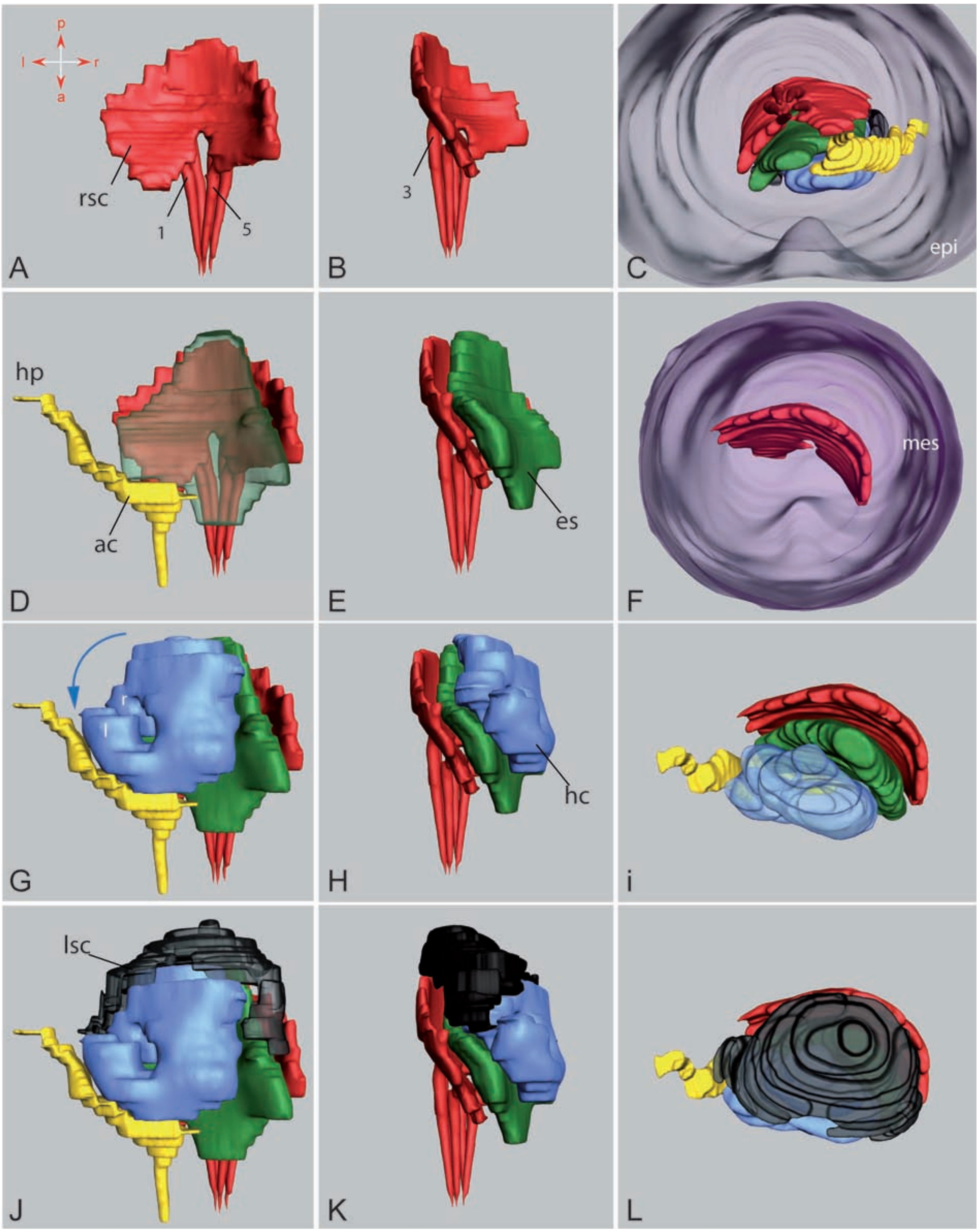


**Fig. 23:** Doliolaria of *A. bifida*, specimen 1. Orientation of the larva in the first two columns equates to the later vertical settlement position to facilitate a comparison with specimen 2. **A-L** Selected views onto varying compositions of the epithelial cavities. **A**, **D**, **G**, **J** ventral view. **B**, **E**, **H**, **K** view from left. **C** anterior view **F**, **i**, **L** posterior view. The opening of the hydrocoel horseshoe resides on the left, the direction of positional change as described by Seeliger (1893) is indicated with a *blue arrow*. Numbers in **A**, **B** give nomenclature as suggested by Seeliger (1893). *ac* axocoel (***yellow***), *ap* apical organ, prospective chambered organ (***red***), *epi* epidermis (***lilac-gray***), *es* enteric sac (***green***), *hc* hydrocoel (***blue***), *hp* hydropore (***yellow***), *lsc* left somatocoel (***black***), *mes* mesoderm (***lilac***), *rsc* right somatocoel (***red***).

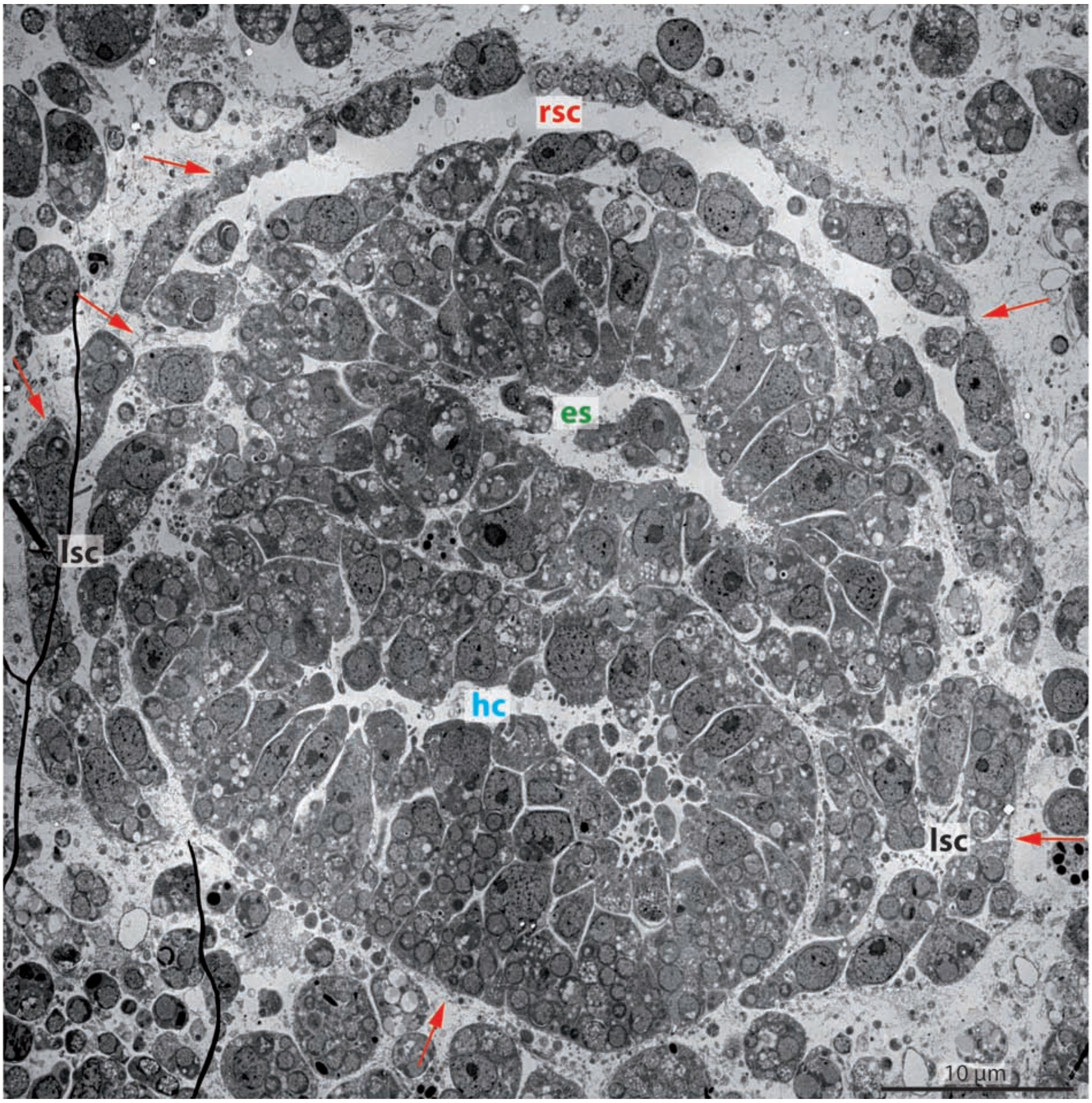


**Fig**. **24:** Doliolaria of *A. bifida*, specimen 1. TEM picture, showing a detailed overview of the epithelial cavities. Cross-section between Figs. **22F** and **22G**. *es* enteric sac, *hc* hydrocoel, *lsc* left somatocoel, *rsc* right somatocoel, *red arrows* point to the basal laminae, which delimit the mesenchyme basally. Note that the basal lamina of the right and left somatocoel is continuous (on the left).

process has been observed to be directly sent out by a hydrocoelomic cell. Underneath the basal lamina, mainly in a ventral position, cross-sections of cells of small diameter and of nerve fibers can be found in larger amounts. Cross-sections of cells are abundant in the hydrocoelomic cavity. It has not been determined whether they are cellular protrusions or free coelomocytes.

### Enteric sac

Dorsally of the hydrocoel lies a horizontally flattened epithelial cavity, which can be shown to develop into the intestine. This enteric sac is concavely bent around the hydrocoel (Figs. 22-24, 25B). Its right side overgrows the hydrocoel, whereas the left side does not cover the latter completely. The enteric sac has the same posterior extension as the hydrocoel, while

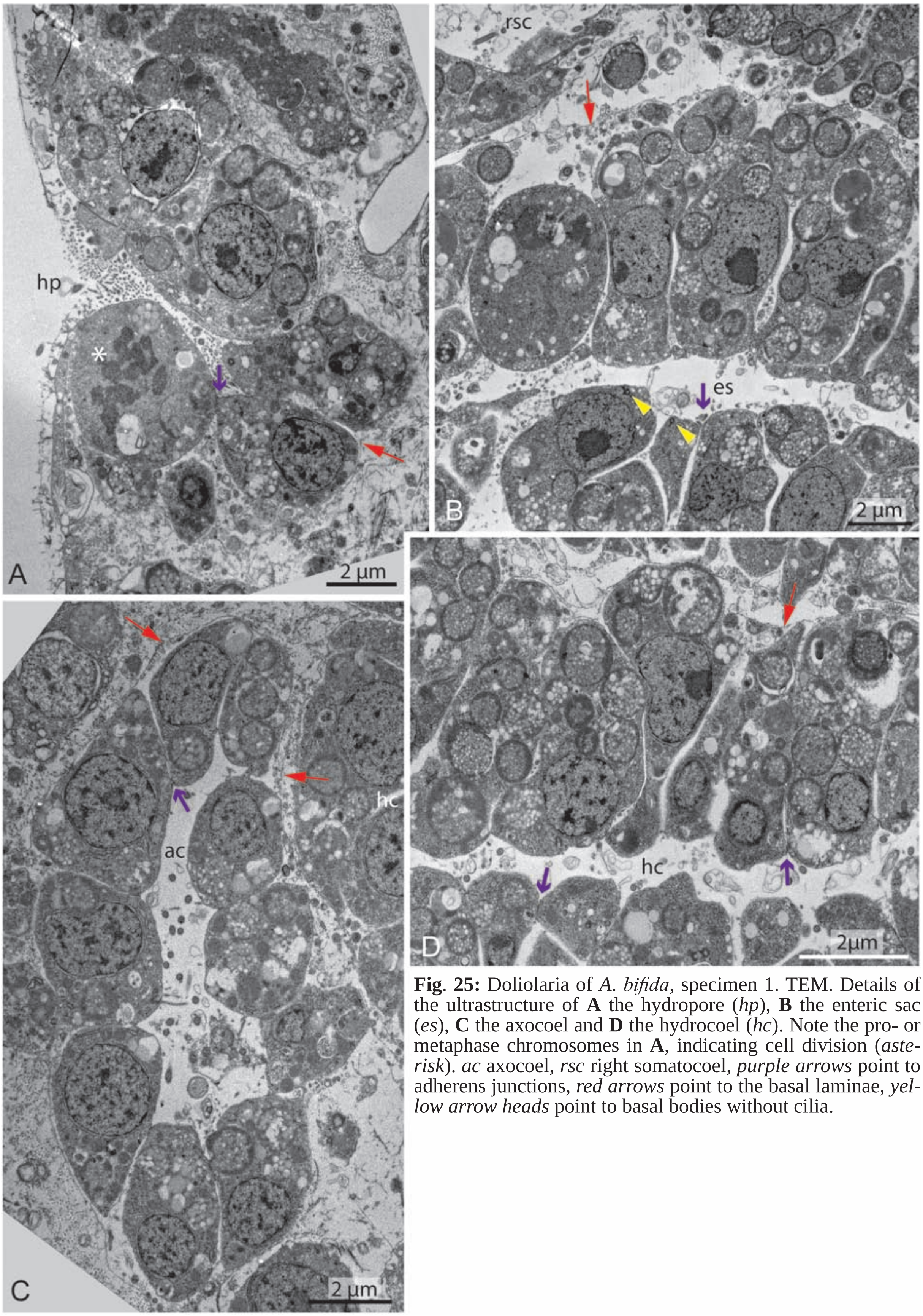


**Fig. 25:** Doliolaria of *A. bifida*, specimen 1. TEM. Details of the ultrastructure of **A** the hydropore (*hp*), **B** the enteric sac (*es*), **C** the axocoel and **D** the hydrocoel (*hc*). Note the pro- or metaphase chromosomes in **A**, indicating cell division (*asterisk*). *ac* axocoel, *rsc* right somatocoel, *purple arrows* point to adherens junctions, *red arrows* point to the basal laminae, *yellow arrow heads* point to basal bodies without cilia.

it extends further anterior than this precursor of the water-vascular system. While it is ventrally bordered by the hydrocoel, the enteric sac is dorsally covered by the right somatocoel and posteriorly capped by the left somatocoel.

The epithelium of the enteric sac consists of unciliated high cuboidal cells that are apically connected to each other by adherens junctions. Instead of full ciliary structures, basal bodies reside apically in the cells (Fig. 25B). At the basal part of the epithelial cells, neuronal processes are present that are further basally followed by a basal lamina. Within the cells, mitochondria exist in comparatively small amount. Some of the yolk spheres are noticeably bigger than in other epithelia.

### Axocoel and hydropore

The axocoel resides on the larva’s left side on one horizontal level with the contacting area of hydrocoel and enteric sac (Figs. 22, 23). It opens to the outside via the hydropore, which is situated on the larva’s left side posterior of the vestibulum and between ciliary bands two and three. Starting at this opening, the axocoel crosses the body wall, stretches anteriorly and bends around the sides of the hydrocoel. Where it reaches the same vertical axis as the tip of the right somatocoel’s left lobe, the axocoel first widens its lumen a bit and extends slightly towards the right, forming the right axocoelomic process. Centrally, the axocoel sends out a thin long anteriorly directed process that reaches about as far as the chambers of the chambered organ.

The axocoelomic epithelium consists of large simple squamous monociliated cells that are underlain by a delicate basal lamina and are apically connected to each other via adherens junctions (Fig. 25C). Nerve fibers are present between the basal lamina and the epithelial cells. The axocoelomic lumen contains some few free coelomocytes. Between the epithelial cells and their basal lamina, neurites are present. Podocytes have not been observed.

The hydropore‘s cuboidal epithelial cells are monociliated, connected to each other via adherens junctions and are underlain by a fragile basal lamina (Fig. 25A). They form the transition between the epidermis and the axocoelothel.

The hydropore cells that are closest to the larva’s epidermal surface are big and round. The apical surface of these big cells is beset with long microvilli, as microvillar cross-sections occur with high density in this region. The hydropore cells that proceed a bit deeper within the larva form a high cuboidal epithelium. Their nuclei reside clearly basally. The density of cilia increases slightly due to the denser packed cells. Many microvilli derive from these cells; however, the density of the microvillar cross-sections in the lumen is reduced compared to the distal section. The epidermal cuticle ends with the last flat epidermal cell at the point where the microvillar cross-sections start to be present.

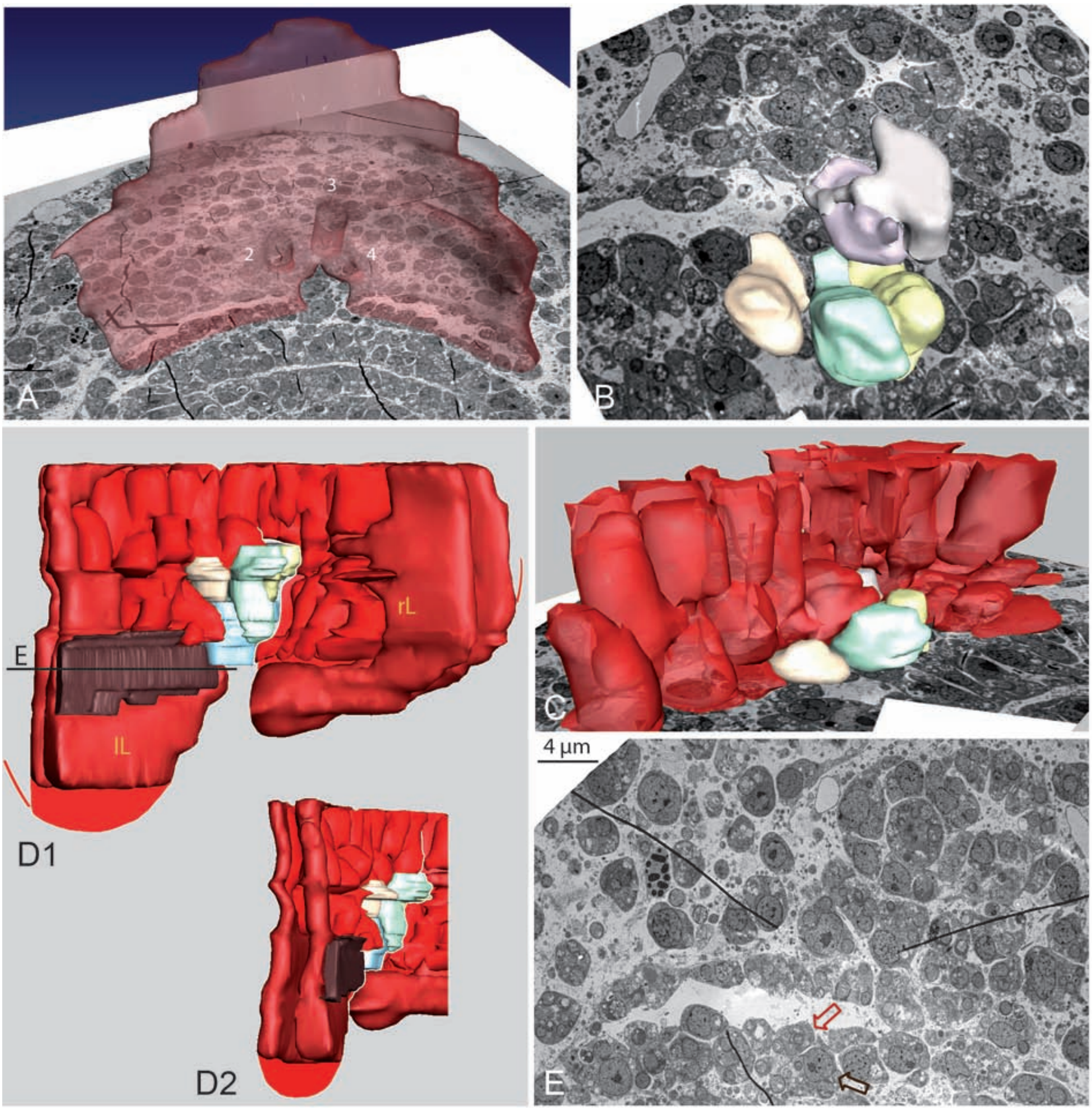


**Fig. 26:** Doliolaria of *A. bifida*, specimen 1. Delamination from the ventral layer of the right somatocoel's left lobe near the transitional zone to the prospective chambered organ. **A** Amira-screenshot of the 3d-reconstruction of the right somatocoel (***red***) interspersed with the related TEM picture of the transitional zone. Numbers give nomenclature as suggested by Seeliger (1893). **B** Similar section as in **A**, interspersed with individually reconstructed potentially delaminating cell candidates (***pale colors***). **C** Same arrangement as in **B**, adding the surrounding non-delaminating cells. **D1**, **D2** Left lobe (*lL*) and right lobe (*rL*) of the right somatocoel, reconstructed in semi-cellular resolution, showing the position of the delaminating cells shown in different angles. **E** Cross-section through the region indicated by the horizontal *black line* in **D1**. Note the second row of cells (*brown empty arrow*) ventral to the usual row of epithelially organized cells (*red empty arrow*).

## Right somatocoel and the prospective chambered organ

The dorsalmost coelomic space, which can be shown to develop into both the adult's aboral coelom and the chambered organ, is the right somatocoel. Its main part is a horizontally flattened cavity that covers the enteric sac dorsally (Figs. 22-24). In a dorsal view, the right somatocoel is heart shaped: posteriorly it is rounded and anteriorly it forms two lobes on each side, the left one being larger than the right one. Where the two anterior somatocoel-lobes meet, five tubes emerge: from the inner margins of each lobe (=transitional zone) two tubes project

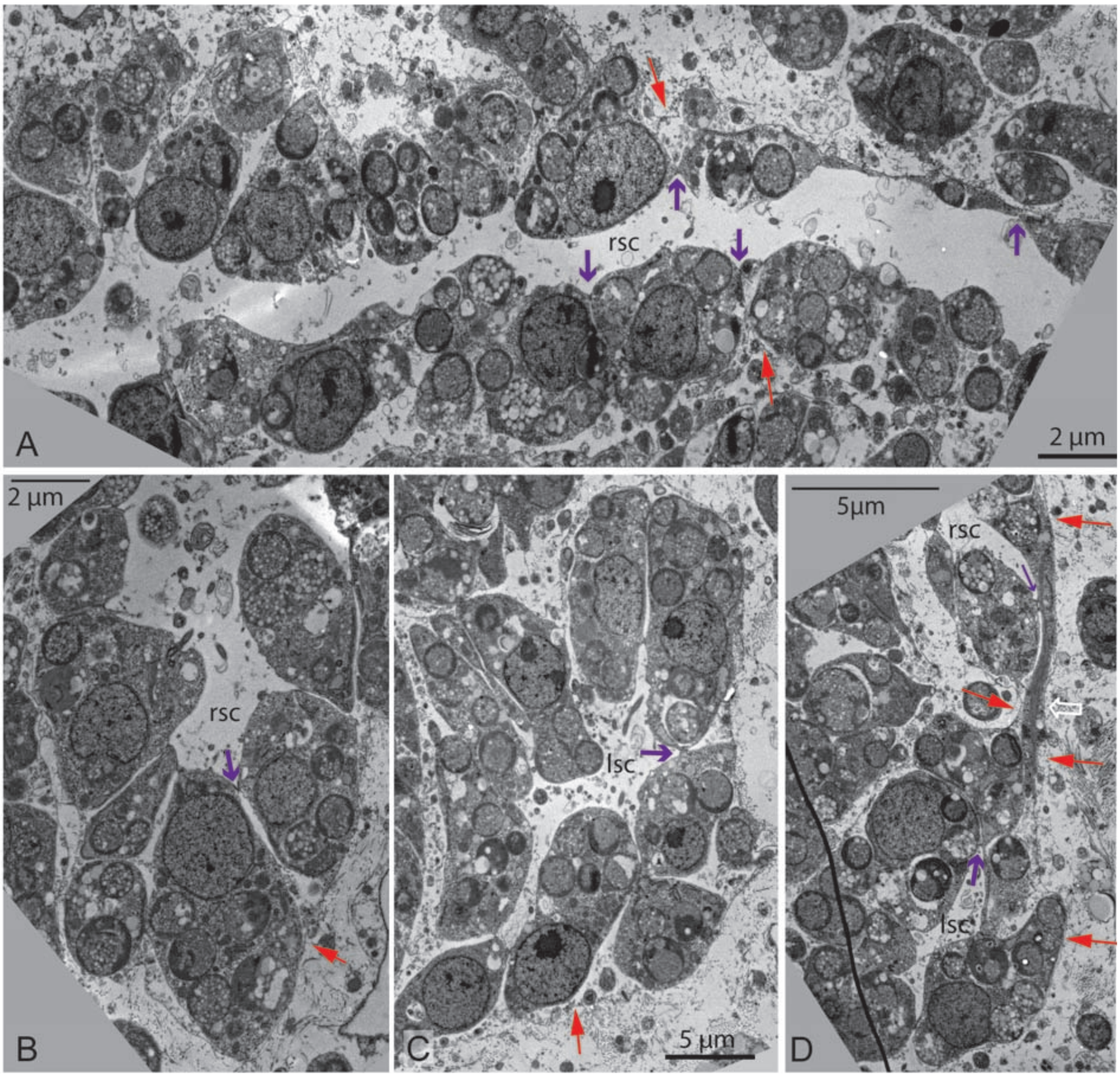


**Fig. 27:** Doliolaria of *A. bifida*, specimen 1. TEM. Details of **A** the right somatocoel (*rsc*), **B** the right somatocoel's right lobe's right margin and **C** the left somatocoel (*lsc*). **D** A cell of the right somatocoel locally contacts the left somatocoel (*white arrow*). *purple arrows* point to adherens junctions, *red arrows* point to the basal laminae.

slightly dorsally, while the fifth emerges dorsally between the lobes (Figs. 23, 26A). Assembling in a circle around a central longitudinal axis, the tubes project straight anteriorly. These tubes can be shown to develop into the chambers of the chambered organ. The tube that emerges most anteriorly from the left lobe will, as suggested by Seeliger (1893), be named with the number "1", the one emerging a bit more posteriorly with the number "2" and the one emerging between the lobes with "3". Accordingly, the tube emerging more posterior from the right lobe is number "4" and the more anterior one number "5" (Figs. 23A, 23B, 26A; see also Fig. 58).

The epithelium of the posterior part of the dorso-ventrally flattened right somatocoel consists of cuboidal to mainly densely distributed squamous monociliated cells. The cells are connected to each other via adherens junctions, and are underlain by a basal lamina. Nerve fibers and cell processes can be found in the basal interstitial spaces, between the epithelial cells and the basal lamina, and underneath the latter.

On the right somatocoel's right side, on one vertical level with the third tube's beginning, one of the right somatocoel's epithelial cells sends a lateral process towards the epithelium of the left somatocoel, and the basal laminae of both coelothelia merge locally (Fig. 27D). The process is longitudinally supported by actin filaments that are flanked by mitochondria. Close to the process' basal lamina, a dendrite resides and forms a synapse with the process across the basal lamina. This observation, however, shows a general disposition of the epithelia to interact and is of no further relevance for this thesis.

### a) Prospective chambered organ: Tubes and transitional zone

Within the transitional zone, each tube of the future chambered organ forms a ring of seven to eight monociliated cells in cross section (Fig. 28), which are interconnected by adherens junctions. Each tube's lumen is connected to the right somatocoel, since the cells in the transitional zone form adherens junctions with both the cells of the right somatocoel and those of the tubes. Hence, the tubes are clearly outgrowths of the right somatocoel. Some cells, which are part of the tube epithelium via cell contacts, form processes that extend into the tube coelom. Some free coelomocytes are present in the right somatocoel as well.

Posterior to the third tube of the future chambered organ, cells of the right somatocoel's distal epithelial layer contain actin filaments in their basal part, which run perpendicular to the larva's longitudinal axis. The actin filaments of neighboring cells are interconnected via cell contacts. As we will see, the tubes will separate from the right somatocoel. Therefore, the actin filaments are probably myofibers, indicating the development of myoepithelial cells that will assist in the constriction.

Towards their anterior ends, the tubes are formed by four to six smaller cells, which are apically connected to each other (Fig. 29). Ultrastructurally, these anterior cells of the tubes resemble the more posterior ones. The space in the center of the tubes decreases towards the tubes' tips. One tube is longer than all other tubes.

The entire group of tubes is surrounded by basal lamina; no such structure is present between individual tubes. This common basal lamina is continuous with that underlying the right somatocoel's dorsal coelothelial layer (Fig. 28). Nevertheless, fibrous structures reside in the *ecm* between the single tubes. These are probably neurites. The chambered organ's tip is surrounded by a basal lamina that is very fragile and hard to determine. So, a well defined basal lamina that is continuous with the right somatocoel's distal basal lamina surrounds all five tubes together as one cap.

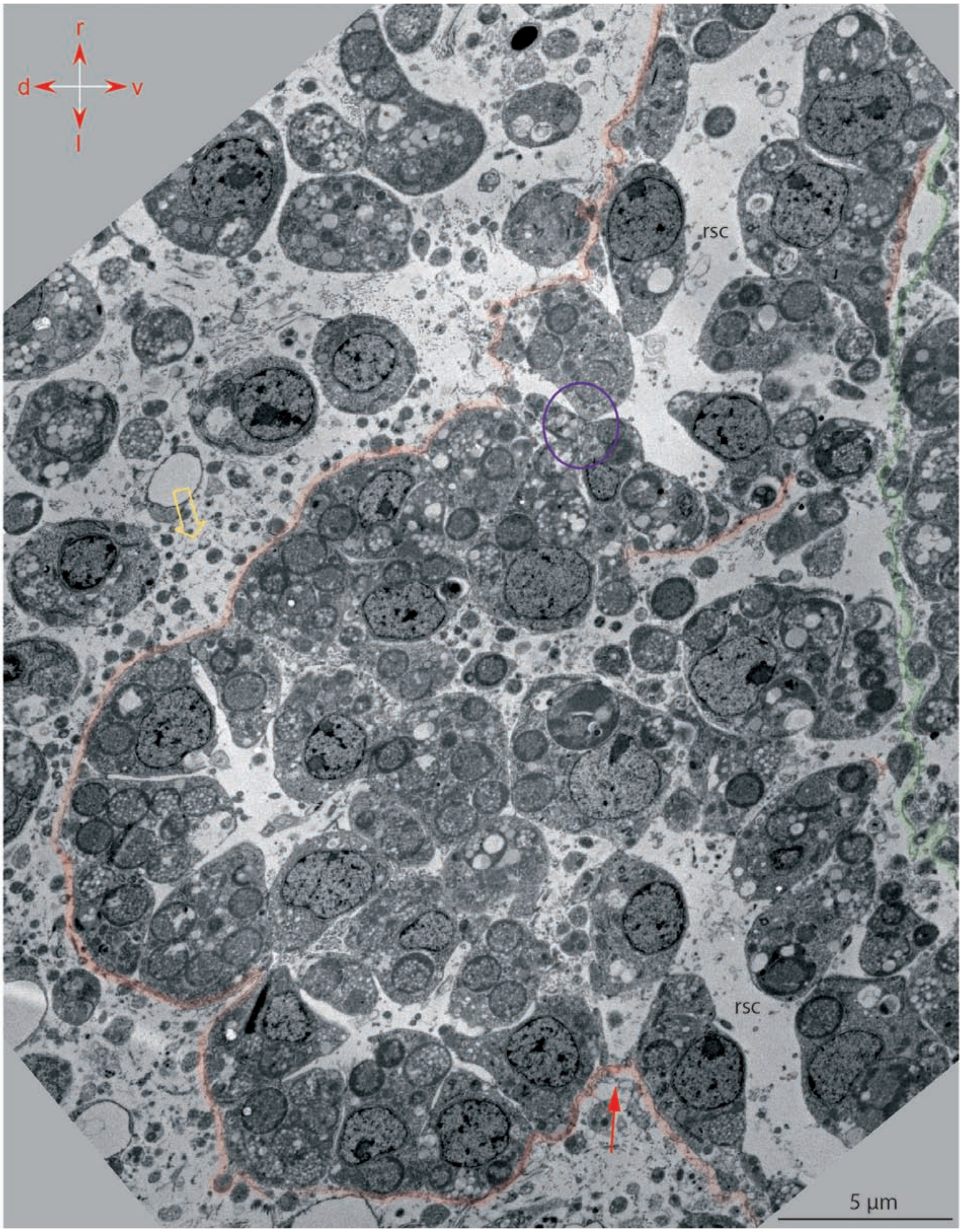


**Fig. 28:** Doliolaria of *A. bifida*, specimen 1. TEM. Right somatocoel's left lobe (lower half) in the transitional zone to the prospective chambered organ. The course of the basal lamina of the right somatocoel and the chambered organ tubes is marked in *orange*, that of the intestine in *green*. Note the absence of the basal lamina along the ventral and the inner margin of the right somatocoel's left lobe, while that of the right lobe is comparatively well defined. *red arrow* basal lamina of the right somatocoel and the tubes is continuous, *lilac circle* marks the apical junctions between cells of the right somatocoel and those of the fourth tube.

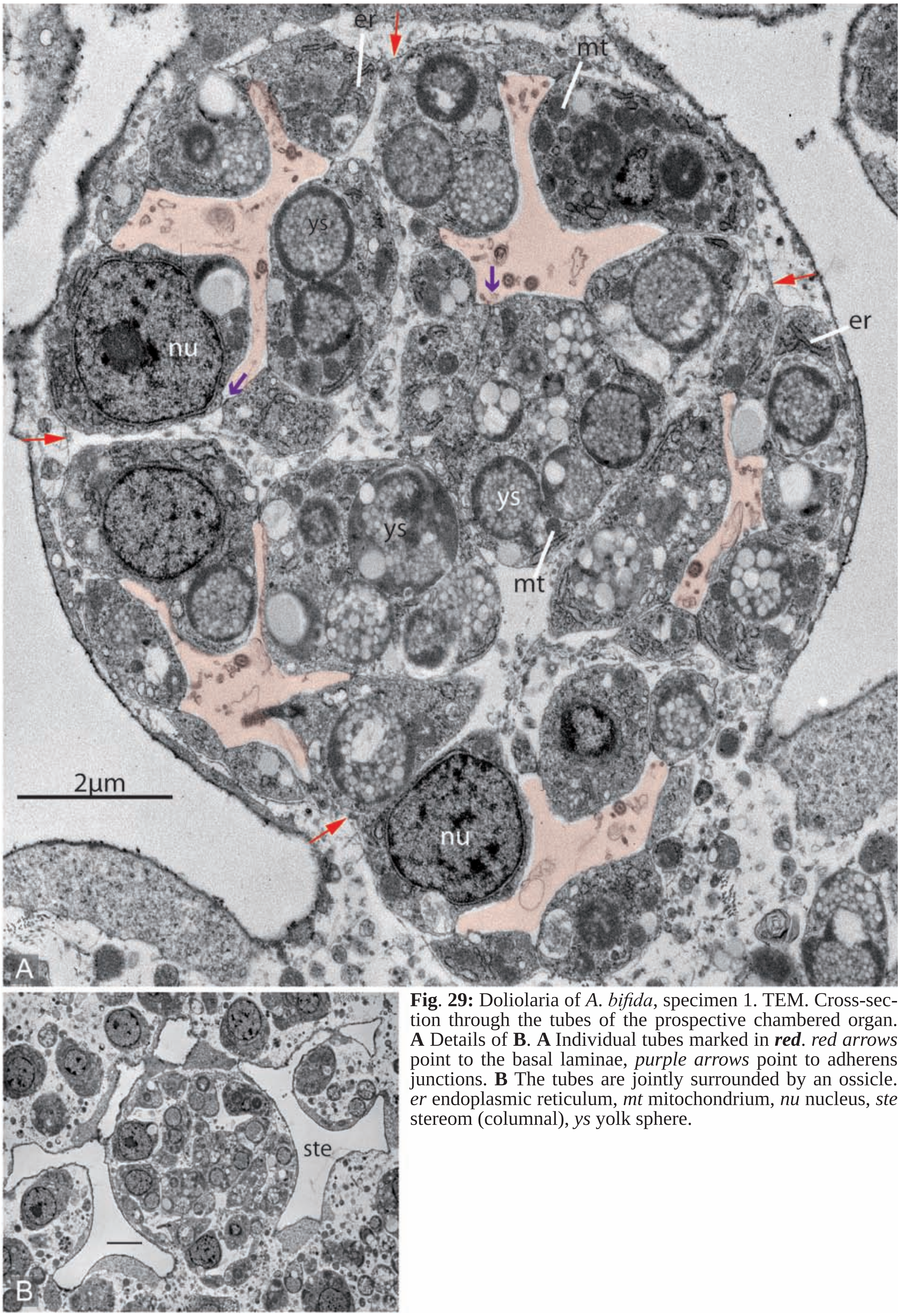


**Fig. 29:** Doliolaria of *A. bifida*, specimen 1. TEM. Cross-section through the tubes of the prospective chambered organ. **A** Details of **B**. **A** Individual tubes marked in ***red***. *red arrows* point to the basal laminae, *purple arrows* point to adherens junctions. **B** The tubes are jointly surrounded by an ossicle. *er* endoplasmic reticulum, *mt* mitochondrium, *nu* nucleus, *ste* stereom (columnal), *ys* yolk sphere.

In the center between the five tubes, at some locations, one or two cells reside, which are neither necessarily in contact with the tube's tissue nor with each other. They are probably free mesenchyme cells.

b) Right somatocoel: Right lobe

The right somatocoel's right lobe is formed by a monolayered epithelium that is underlain by a basal lamina. The ventral layer of the right lobe is thicker than the dorsal layer (Fig. 28). Single cells send processes into the right lobe's coelomic cavity or form basiepithelial lateral processes. Some free coelomocytes reside in the right lobe's cavity. Both layers of the right lobe are underlain by a continuous, well defined basal lamina.

c) Right somatocoel: Left lobe

The dorsal layer and most areas of the ventral layer of the right somatocoel's left lobe are formed by a monolayered epithelium that is underlain by a basal lamina. At its ventral inner margin, however, several cells (at least six) are partially freed from the epithelium (Figs. 26B-D). The basal parts of these cells are directed into the mesenchymal *ecm*. They remain part of the epithelium via slender apical cell parts that are attached to neighboring cells by means of adherens junctions. In this region, a continuous basal lamina cannot be traced though at few points a flimsy basal lamina might be present (Fig. 28).

Anterior of these delaminating cells, the proximal layer of the lobe is bilayered for a longitudinal distance of one or two cells. (Figs. 26D, 26E).

**Left somatocoel**

The left somatocoel, which can be shown to develop into the oral coelom, forms the posterior cap of the coelomic cluster (Figs. 22, 23). Dorsally, it is limited by the posterior margins of the right somatocoel. Ventrally, it merely covers the posteriormost part of the hydrocoel. It sends two lateral extensions anteriorly, which reach as far as the point where the third chamber of the future chambered organ arises from the right somatocoel. The extension on the right side reaches slightly further than that on the left side. So, the left somatocoel is limited by the right somatocoel at its dorsal margins and expands with two branches around the lateral sides of the enteric sac, thereby partly spreading between the hydrocoel and the right somatocoel, and covers the enteric sac posteriorly.

The left somatocoel consists of large simple squamous monociliated cells that are connected to each other by adherens junctions and are underlain by a basal lamina (Figs. 24, 27C, 27D). Some somatocoelomic cells send processes in the direction of the basal lamina. Some nerve cells and their fibers are present in the space between cell bases and their basal lamina. Very few free coelomocytes are distributed within the lumen of the left somatocoel.

### Epidermis and mesenchym

In the doliolaria larva the body wall consists of the epidermis, the underlying *ecm* with all embedded types of non-polarized cells, here simply called mesenchymal cells. Mesenchymal cells are all non-polarized cells within the *ecm* spanning between the subepidermal basal lamina and the basal laminae of epithelially lined cavities (coeloms) and the gut. The entire mass of mesenchymal cells plus *ecm* represents the mesenchyme

### Epidermis overview

The epidermis of the Doliolaria shows several functionally and morphologically distinguishable regions of different cell-types: The ciliary bands and the interciliary bands (in which the proper epidermis resides), as well as the vestibulum and two anterior epidermal organs, the sensory apical tuft and the adhesive pit (no data for these organs is shown in this thesis, see Chia et al. 1986).

### Epidermis proper

In the present larva, several but not all cells of the epidermis have been damaged during fixation. Nonetheless, the data show that the epidermis mainly consists of mushroom shaped monociliated cells (Figs. 30A, 30B, 31C). Microvilli protrude apically from the cells (Figs. 30E, 6A inset). A fibrous network of fine filamentous extra-cellular material, which is ambiguously called “cuticle” throughout literature (e.g. Heinzeller and Welsch 1994), interconnects the microvilli apically. That way, a space between the apical cell surface and this cuticle accrues. The umbrella of each mushroom shaped cell is formed by the apical part of an epidermal cell. This part is flatly stretched out and forms the larva‘s functional border to the exterior (Figs. 30B, 30E). It contains mitochondria, empty vacuoles and smaller vesicles. The umbrellas of the mushroom shaped cells are connected to each other via strong cell contacts. Rarely, electron-dark rivet-like structures exist that span between the umbrella‘s apical and basal side. The thick stems of the mushroom reach deeper into the *ecm* of the body wall. They represent the cell’s somata and contain the nuclei as well as the majority of the other organelles of the cells. Some of them form basal processes.

Since the cells’ umbrellas are limited in diameter and reside close together, comparatively few *ecm* resides between these stem-like cell somata. A joint basal lamina is stretched out under the basal ends of the mushroom-shaped cells and separates the epidermis from the mesenchyme (Fig. 30B). Between the mushroom-stems and above their basal lamina, neurites, free amoeboid cells and *fibrous elements* reside within the *ecm*. The neurites are mainly distributed close to the basal lamina as well as underneath the cell‘s umbrellas. They contain mitochondria, vesicles and dense-cored vesicles. The fibrous elements aggregate underneath the umbrella (Fig. 30E), but are present all over the epidermis‘ *ecm* (Fig. 30A). Often, these fibers run parallel to the umbrella.

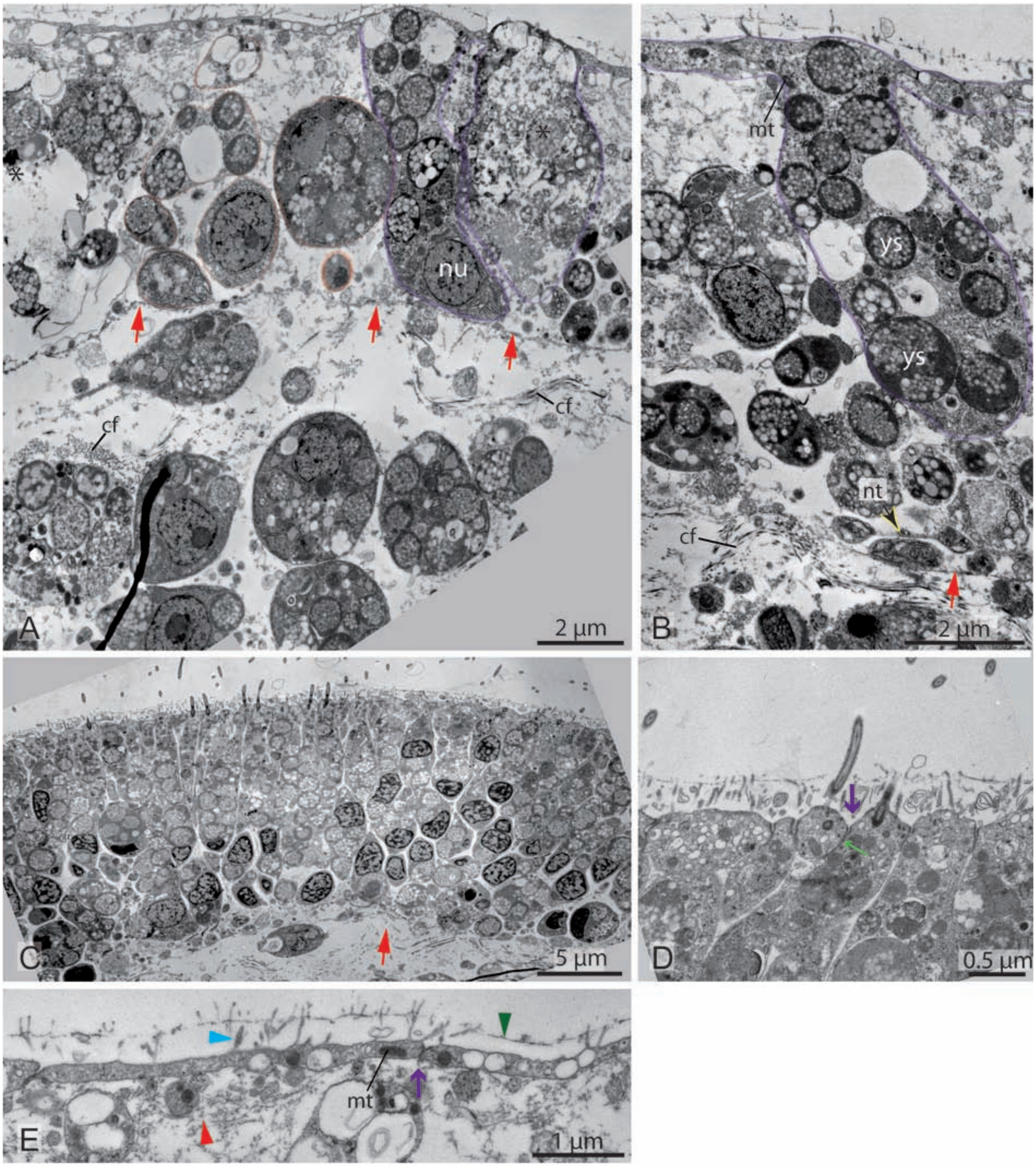


**Fig. 30:**. Doliolaria of *A. bifida*, specimen 1. TEM cross-sections. **A**, **B**, **E** Longitudinal sections through the epidermis proper and **C**, **D** a ciliary band. **D** detail of **C**. **E** detail of **A**. **A, B** Individual cells of unknown type circled individually. The basal lamina (*red arrows*) spans below the basal ends of the mushroom-shaped epidermal cells, whose somata keep distance to each other. **B** Tack-shaped cell and a basal neurite (*nt*). *cf* collagen fibrils, *er* endoplasmic reticulum, *mt* mitochondrium, *nu* nucleus, *ste* stereom (columnal), *ys* yolk sphere. *black asterisk* damaged cell, *blue arrow head* microvillus, *green arrow head* “cuticle”, *green arrow* septate junction, *purple arrows* point to adherens junctions, *red arrow head* subepidermal connective tissue fibrils.

## Ciliary bands

The ciliary bands consist of a densely packed palisade epithelium of monociliated cells (Figs. 30C, 30D). Microvilli protrude from the cells that are covered with a cuticle at their tip. The cells are apically connected to each other by broad adherens junctions and also septate junctions. All cells share a basal lamina that stretches straightly underneath their basal ends. Neu-

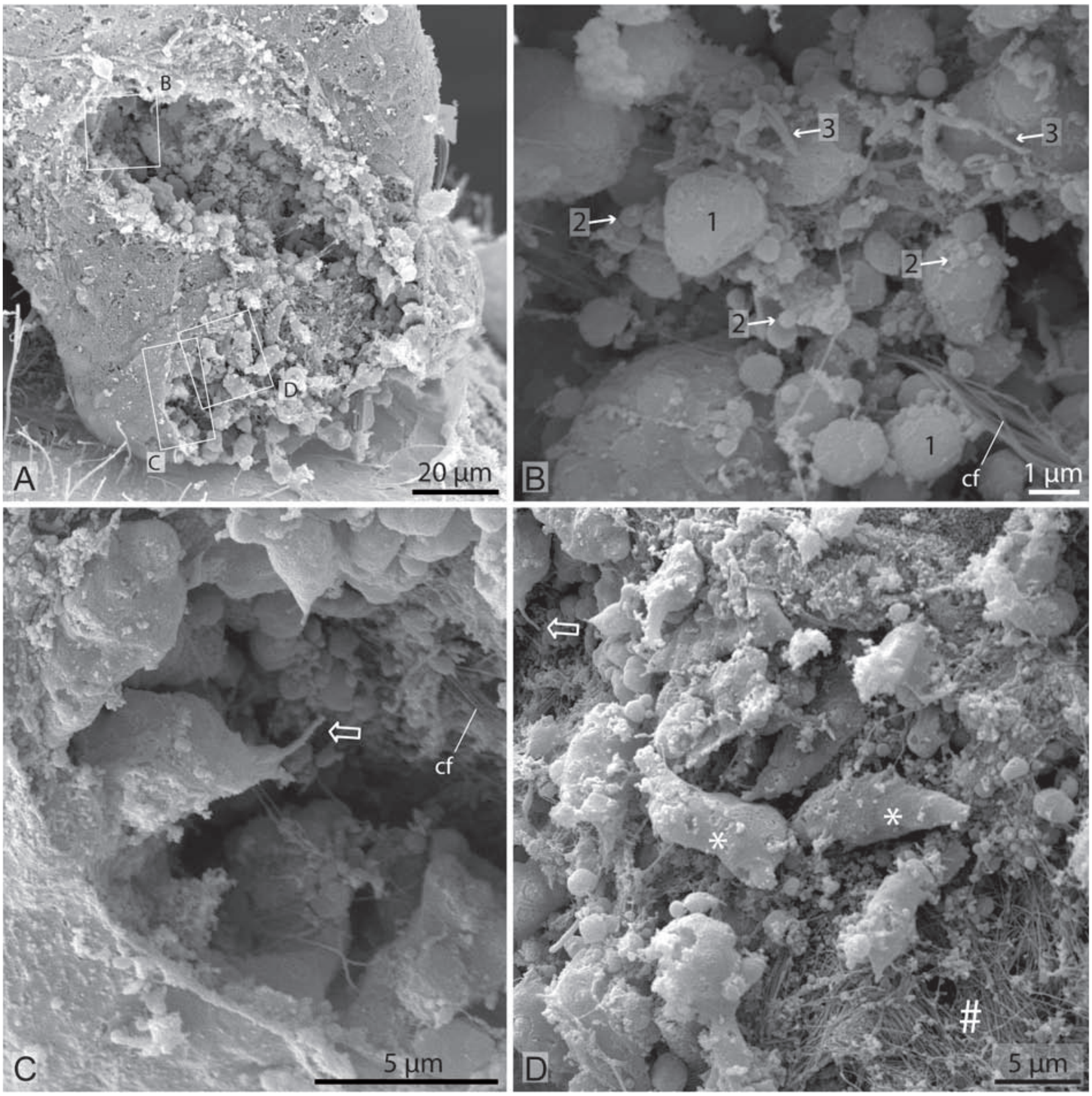


**Fig. 31: A** Slightly crushed recently attached larva of *A. bifida*. SEM. *Squares* indicate areas from which pictures of **B**, **C** and **D** have been taken. **B** Detail showing the three types of small mesenchymal cellular structures identified in this thesis. Classification is based on the relative size of the structure and does not reflect cell types. Assignment of numbers correlates with that of the structures distinguished in Fig. **33C**. The figure suggests that the structures are part of a network of neurites that are locally widenend by different types of organelles. **C** Epidermal cells. The *white empty arrow* points to an axon. **D** Large spherical cells (*asterisks*). *cf* collagen fibrils, # interwoven networks of neurites and collagen fibrils.

rites, dendrites and axons reside in the intercellular space between the basal lamina and the epidermal cells. Within the cells, yolk spheres are situated mainly in a basal position. Apically, a large amount of mitochondria, electron empty vesicles and small vacuoles, dense cored vesicles and membrane-filled vesicles are commonly found. Mitochondria also reside in other cell regions. The nuclei reside basally or centrally. They show many large heterochromatic electron-dark spots and their nucleoli are not discernible. Non-epithelially bound cells of the ciliary band epidermis that reside in a more basal position show a much electron-lighter nucleus.

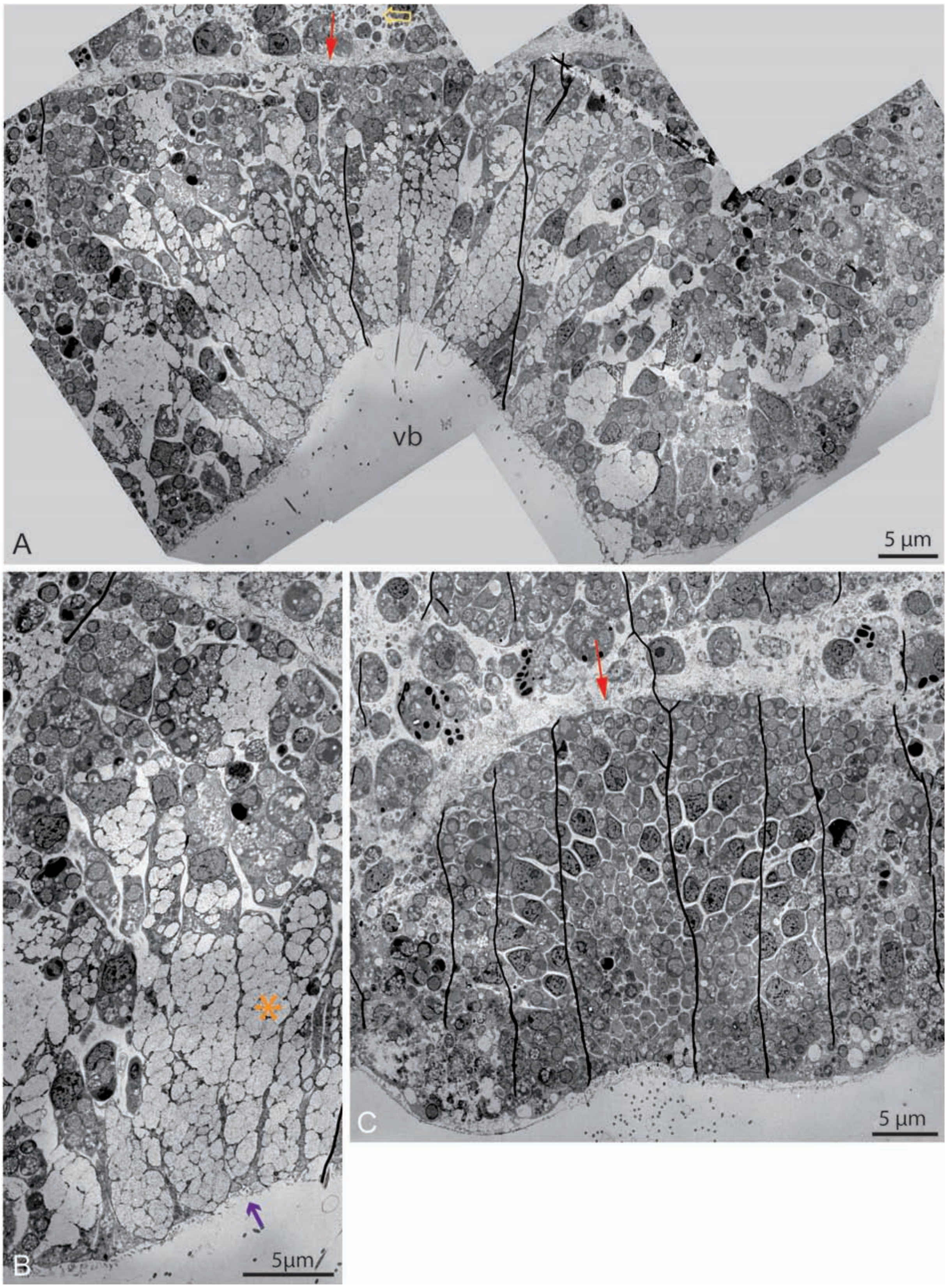


**Fig**. **32:** Doliolaria of *A. bifida*, specimen 1. TEM. Cross-sections through **A**, **B** the center and **C** through the posterior end of the vestibulum (*vb*). **B** detail of **A**. *purple arrow* points to adherens junctions, *red arrows* basal lamina, *yellow asterisk* membrane delimited agglomerates of floccular material, *yellow empty arrow* neurites.

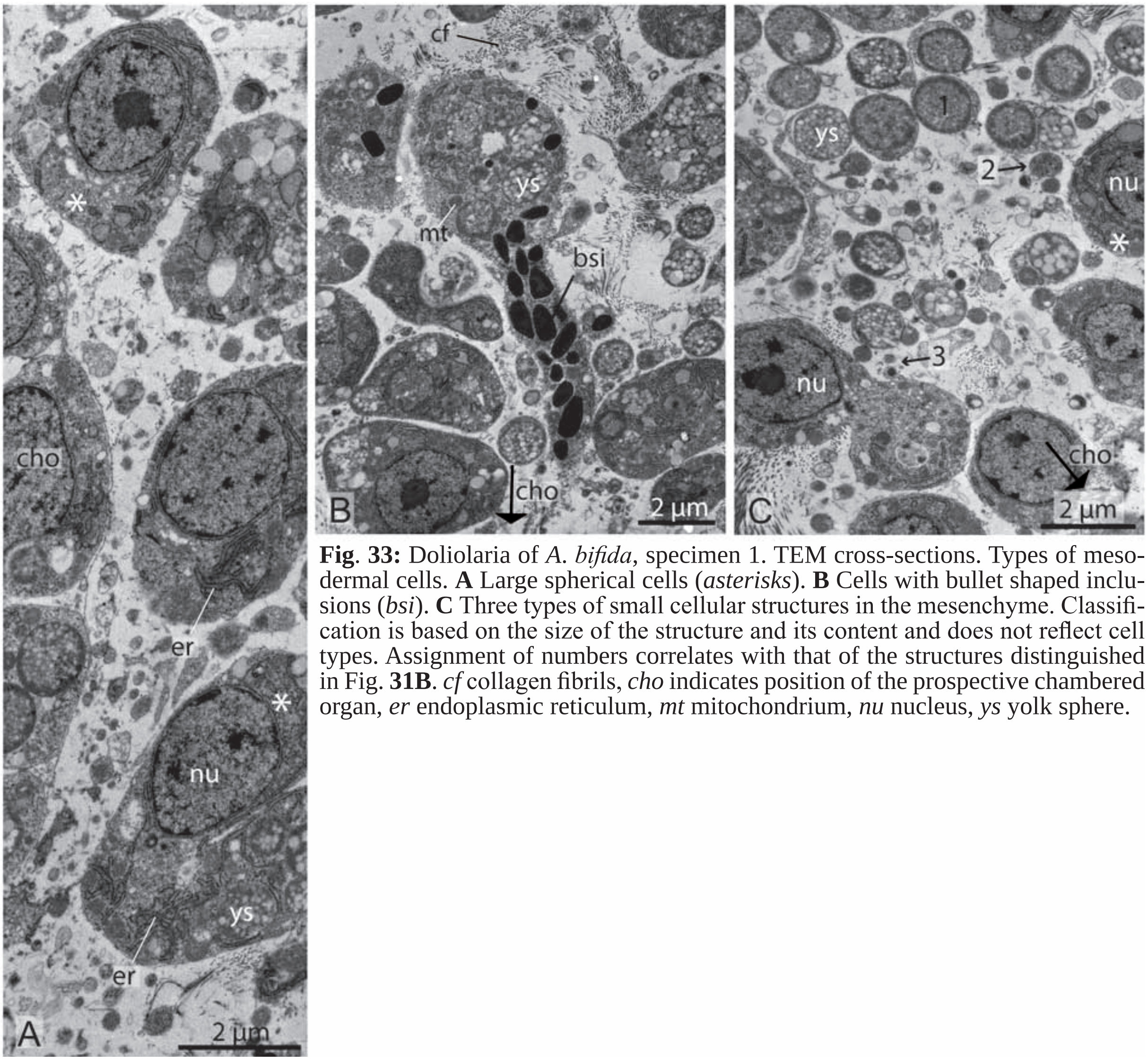


**Fig. 33:** Doliolaria of *A. bifida*, specimen 1. TEM cross-sections. Types of mesodermal cells. **A** Large spherical cells (*asterisks*). **B** Cells with bullet shaped inclusions (*bsi*). **C** Three types of small cellular structures in the mesenchyme. Classification is based on the size of the structure and its content and does not reflect cell types. Assignment of numbers correlates with that of the structures distinguished in Fig. **31B**. *cf* collagen fibrils, *cho* indicates position of the prospective chambered organ, *er* endoplasmic reticulum, *mt* mitochondrium, *nu* nucleus, *ys* yolk sphere.

**Vestibular epidermis**

The vestibular epidermis, which forms a large depression on the larval antero-ventral side, is mainly formed by 20-30 µm long, slender monociliated columnar flask shaped cells that concentrate in the center of the vestibulum (Fig. 32). They are apically connected by cell contacts and direct microvilli to the exterior, on which a so-called cuticle rests. The most prominent structures of these cells are membrane-delimited agglomerates of floccular material. Their nuclei are mainly electron-bright and have one or two nucleoli. Apart from these structures, they contain yolk spheres, as well as mainly apically positioned electron-light vesicles, membrane-filled vesicles and lateral rough Endoplasmic reticulum. Mitochondria are concentrated underneath the ciliary rootlets but also throughout the cell.

Towards the vestibulum's rim as well as at its anterior and posterior end, cuboidal to columnar cells that contain the usual arrangement of cell contents described for coelothelial cells and lack the floccular material filled organelles, are more common.

Neurites rest basiepithelially between the epithelial cells and their basal lamina. Underneath the latter, mainly longitudinally directed loosely bundled collagen fibers reside. To each side, the vestibular depression is flanked by lateral bulges that form as the larva's non vestibular epidermis is de-clined by up to 45° against the vestibular cells.

### Mesenchyme

Besides the *ecm* the mesenchyme contains different cells types. The most obvious cell type is represented by **large spherical cells** that are dendritic to spherical in shape and that are spread throughout the mesenchyme (Figs. 31D, 33A-C). Each cell possesses the composition of cell contents of coelothelic cells except for one detail; their most common characteristic is an abundant, prominent rough Endoplasmic reticulum, whose extent is larger than in any other cell type in the larva.

Some cells, which are often elongated or form a process, are **cells with bullet shaped inclusions** (Fig. 33B). These reside either in the whole cell or are, more commonly, mainly located in the process. The processes are frequently directed towards the epidermis, but may surpass the basal lamina rarely. Besides these inclusions, they contain the composition of cell contents of coelothelial cells described above.

Cross-sections of **smaller cellular spheres** are frequently present in the *ecm* (Fig. 33). They consist of yolk spheres that are surrounded by an additional (cellular) membrane. A SEM fracture preparation of a newly settled larva shows balls of comparative size that reside either singly or are interconnected by delicate fibers (Fig. 31B). The same SEM preparation also shows much smaller spherules, which can, in return, be assigned to TEM-structures of the same size that are mainly filled with cytoplasm as well as small amounts of rough Endoplasmic reticulum or few mitochondria. The third, next smaller type of cross-sectioned structures contains either cytoplasm or dense-cored vesicles. As SEM shows, these latter structures have a dendritic nature.

Distal to the cluster of epithelial cells reside anlagen of skeletal elements that are formed by **sclerocytes**. They are considerably smaller than the columnals formed in the larva's anterior part around the prospective chambered organ.

### Mesenchymal structures surrounding the chambered organ

Around the tubes of the chambered organ, the arrangement and abundance of mesenchymal elements differ significantly from that of the mesenchymal elements in other regions of the larva (Figs. 28, 29, compare to Fig. 24).

The chambered organ is surrounded by neurites that can be found in higher density in the *ecm*. The neurites are concentrated in the mesenchyme close to the chambered organ‘s basal lamina. They mainly run in the larva‘s main axis but are also interconnected with each other.

Among these neurites reside sclerocytes that are arranged around the tubes of the prospective chambered organ (Fig. 29) and form the columnals, the skeletal elements of the larva's stalk.

Bundles of collagen fibrils run axially alongside the chambered organ. Attachment of these bundles to sclerocytes can be observed. In addition to these longitudinal bundles, the *ecm* is abundantly traversed by collagen structures that extend in all directions. They locally occur in larger or smaller amounts forming loose bundles, but single fibrils may also cross the matrix individually. They can be frequently observed to be attached to the basal laminae of epithelia or mesenchyme cells.

## 3.3 Settled metamorphosing larva (Specimen 2)

The examined larva has just settled and is undergoing metamorphosis from the bilateral to the pentaradial stage. It is stoutly ninepin-shaped, with the smaller knob of the ninepin attached to the substratum and its larger part directed upright into the water (Fig. 5B). The long axis of the larva is about 300 µm long and the future calyx is up to 160 µm in diameter. The neck of the ninepin, which is developing into the stalk, is about 140 µm long and 60 µm in diameter. However, the prospective calyx and the developing stalk are not set apart, yet. The cluster of the epithelial cavities is situated in the larger part, the future calyx, of the larva (Fig. 34).

### Vestibular cavity

In this stage the vestibular invagination is detached from the surrounding epidermis and forms a closed and flattened vestibular cavity underneath the epidermis. It caps the cluster of epithelial cavities in a 45° angle to the horizontal plane and resides orally above the hydrocoel ring, its center still marking the ventral side (Figs. 35B, 36).

The distal and the proximal layer of the vestibular epithelium show regional differences: The vestibular cavity's distal layer lines the roof of the vestibular cavity underneath the epidermis. It is formed by mostly *un*microvillized and *un*ciliated squamous cells that are *not* covered by a cuticle and reside on a prominent fibrous basal lamina (Fig. 36A). The vestibular cavity's proximal layer forms an extension that reaches through the hydrocoel horseshoe's center towards the enteric sac (Figs. 34B, 36C). It is formed by unciliated flask shaped palisade cells that lack the doliolaria's floccular content (Fig. 36A). Their long axes are directed towards the ventral half of the flattened oral side of the enteric sac. Their apical microvilli are covered by a cuticle, hinting at the vestibular cavity's ectodermal origin. These apical cell structures also extend to the peripheral region of the vestibular cavity‘s distal layer (Fig. 36D). Basal bodies, remnants of the doliolaria's ciliary root structures, are frequent and prominent, but no cilium has been observed to extend into the vestibular cavity (Figs. 36D, 36E). The ultrastructural composition of organelles resembles that described for doliolaria coelothelia.

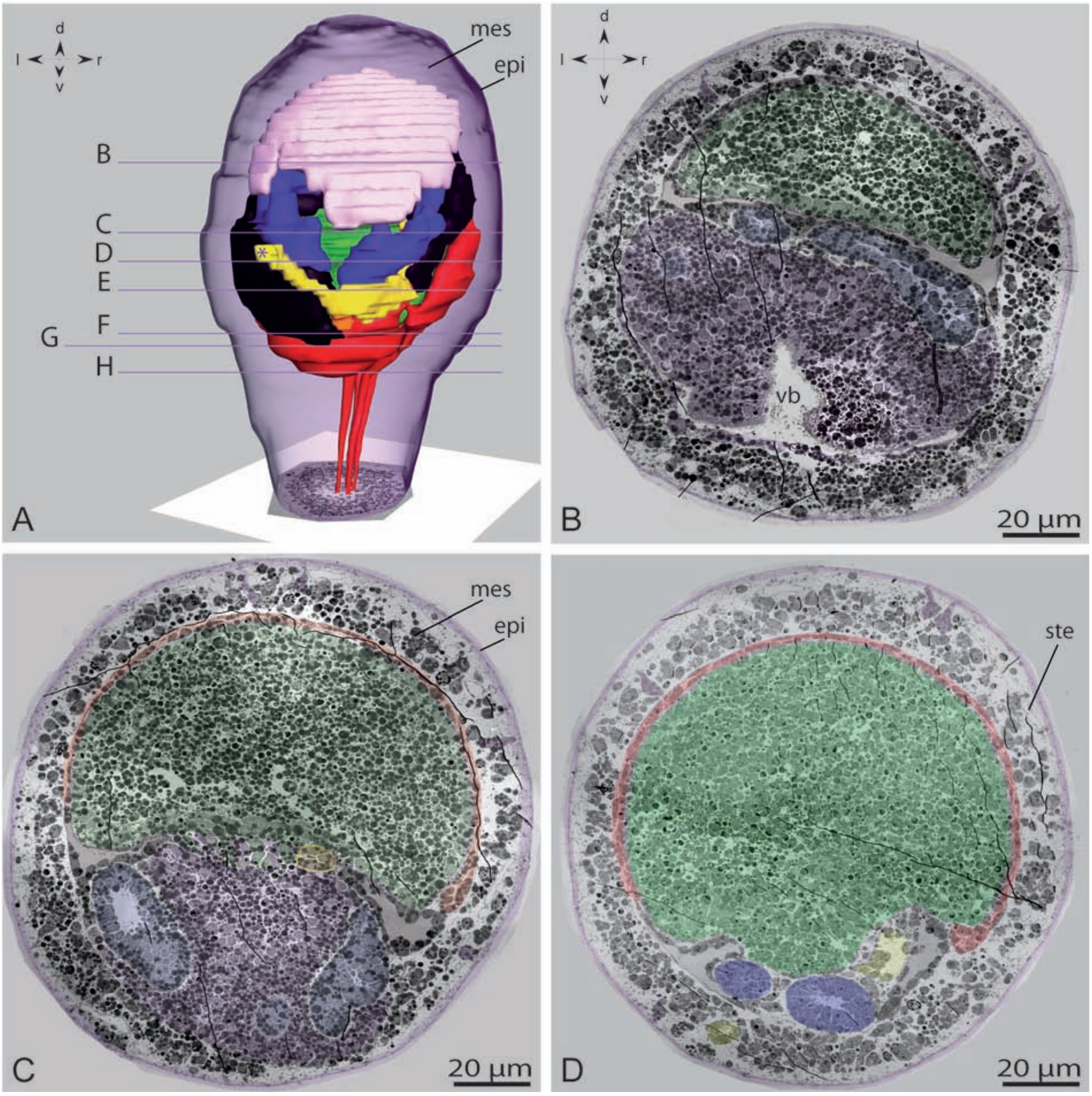


**Fig. 34 part 1 of 2**

**Fig**. **34 (2 parts):** Metamorphosing cystidean larva of *A. bifida*, specimen 2. **A** Reconstruction based on ultra-thin cross-sections. **B-H** Selected colorized TEM pictures of cross-sections, whose position in the larva is indicated by lines in **A**. Colors match the related structures in the reconstruction. *mes* mesenchyme, *ste* stereom, *epi* epidermis (***lilac-gray***), *gaot* tubule of the prospective glandular axial organ (***turquoise***), *vb* vestibular cavity (***white***), *cho* tubes of prospective chambered organ (***red***), ***black*** left somatocoel, ***blue*** hydrocoel, ***green*** enteric sac, ***orange*** small ventral coelom, ***red*** right somatocoel, ***yellow*** axocoel, *asterisk* (in **A**) hydropore, *pink arrow* proximal layer of the left somatocoel and distal layer of the right somatocoel consist of large cells (compare Fig. **40F**), *d* dorsal, *l* left, *r* right, *v* ventral. Orientation of **B** valid for **C-H** as well.

## Body wall: epidermis plus mesenchyme

The body wall of the larva’s future calyx is 10-20 µm thick. It consists of a thin monolayer of epidermal cells that is underlain by a thick *ecm* into which mesenchymal cells are embedded (Figs. 37A, 37E). Proximally, it is bordered by the basal laminae of the epithelial cluster‘s coelomic cavities.

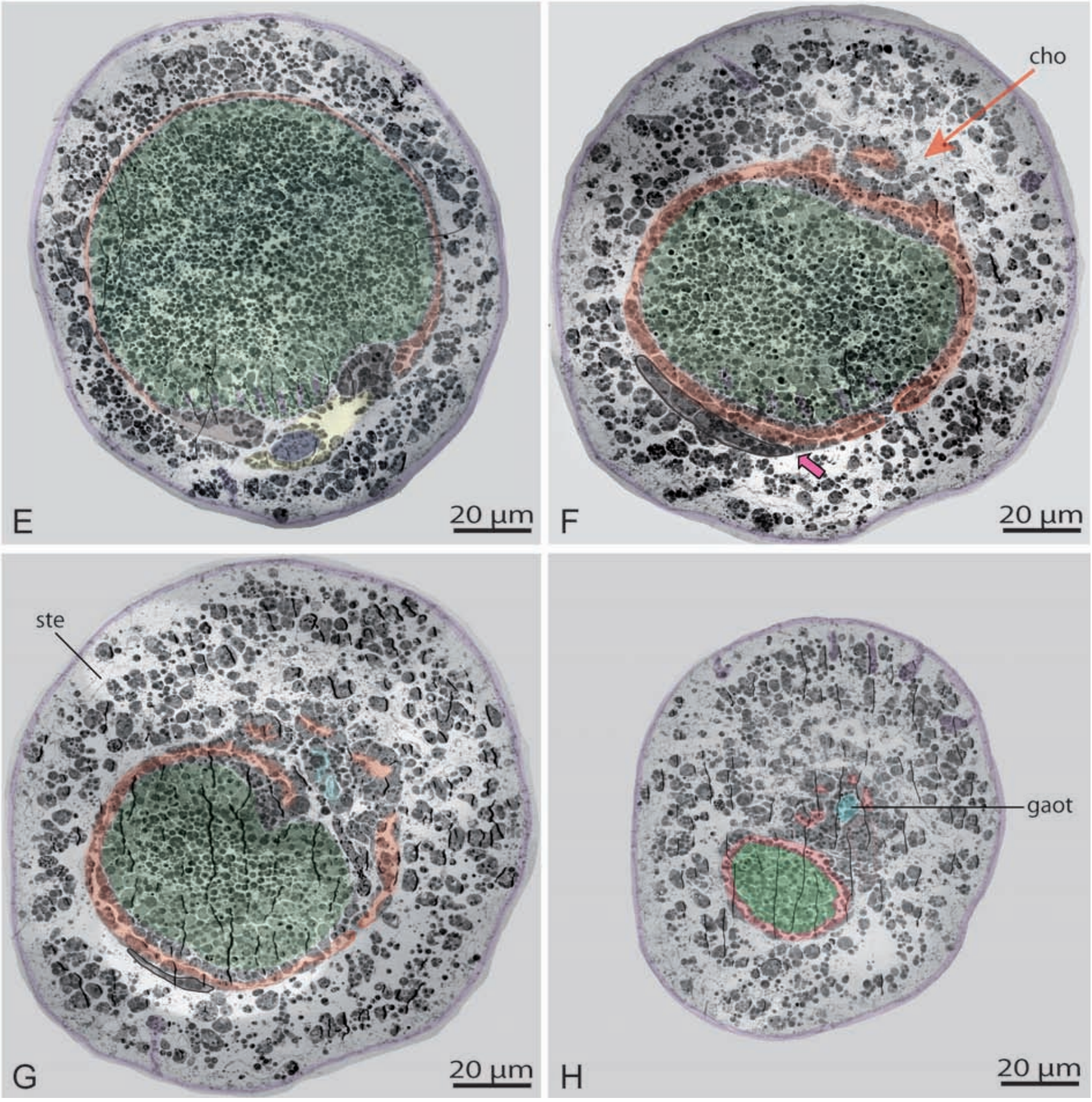
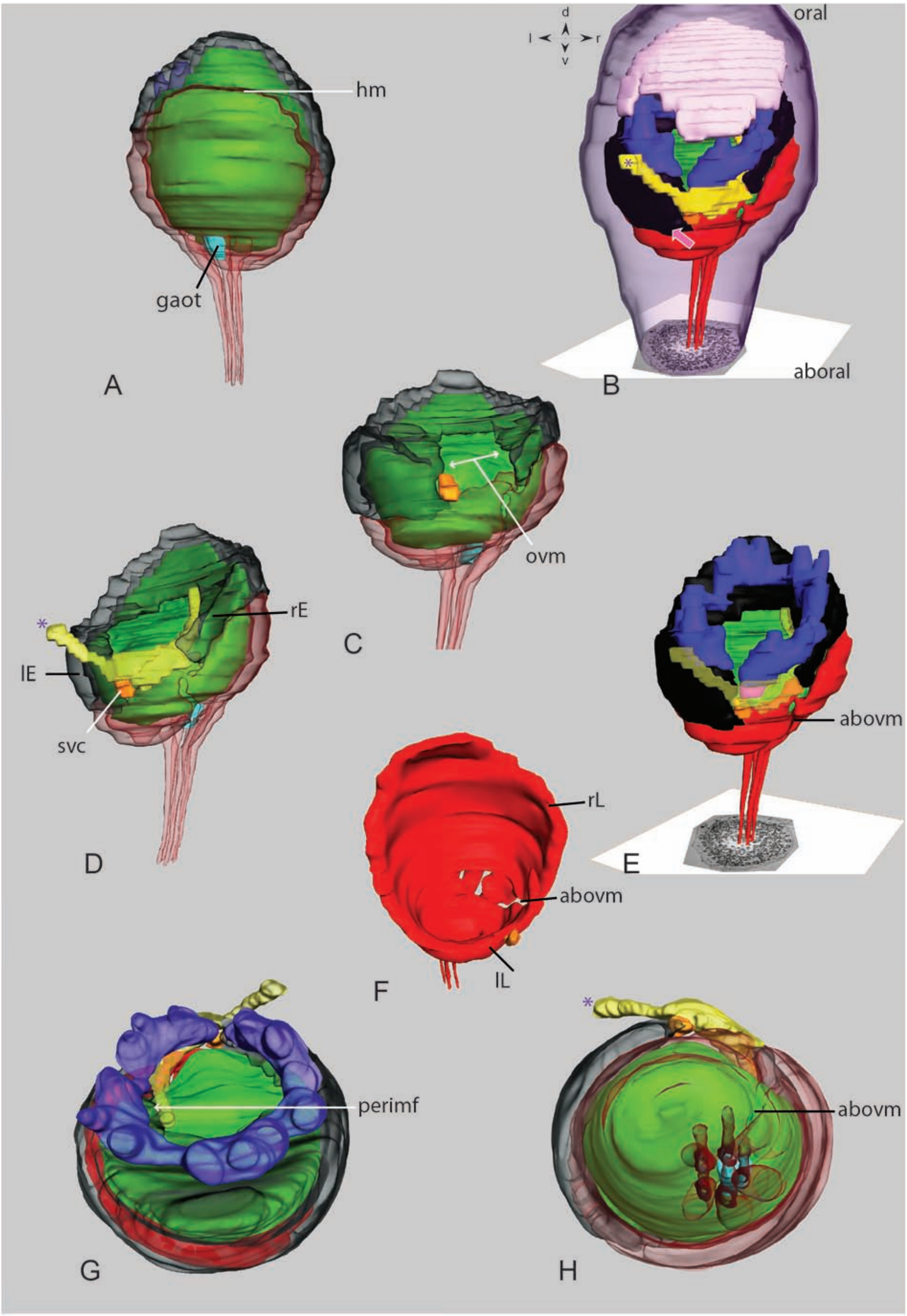


**Fig. 34 part 2 of 2**

The mushroom-shaped epidermal cells of the doliolaria are much thinner in the present larval stage so that their shape resembles much more that of a tack (Fig. 37E). The epidermis is made up of mostly *un*ciliated cells - only single cilia have been observed using SEM. The tacks' flat apical heads contain mainly cytoplasm, a few mitochondria and vacuoles. The latter may contain membranous or denser material. Frequently, the distal and the basal cell membrane of apical heads are tightly connected to each other by rivet-like structures. These are, however, more difficult to distinguish from adherens junctions than in adults (as described by Heinzeller and Welsch 1994). The single heads are laterally connected to each other by adherens junctions. The tack's pin, the cell soma, reaches vertically into the body wall's *ecm*. It contains the nucleus and most of the organelles. Microvilli emerge from the epidermis' apical surface. A delicate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so-called cuticle spans between these microvilli, leaving a space between the surface of the epidermal cells and the cuticle.

In the present larva, the epidermal somata are generally more distant from each other than in the doliolaria (Figs. 37A, 37E, 37F). The epidermis‘ basal lamina is fibrous and resides underneath the apical, surface-covering part of the epidermal cells. It turns inwards along the cell‘s soma for a short distance, but cannot be traced around the main part of the soma using TEM. Some of the epidermal cells possess basal processes, whose targets, however, have not been further investigated. The *ecm* consists of an electron-light matrix that is crossed by collagen fibers. A denser network of these fibers resides underneath and attaches to the epidermis. The network may not be distinguishable from the epidermis‘ basal lamina in some places.

The mesenchymal cells are distributed throughout the body wall‘s *ecm*. Their density varies. They are most prominently recognizable by a conspicuously abundant rough Endoplasmic reticulum. Nuclei with one or two nucleoli, yolk spheres, mitochondria and a Golgi complex can be recognized as well. Some of these cells also contain bullet shaped inclusions. The latter are more frequent in the mesenchyme of the stalk, but are present in the mesenchyme of the future calyx as well. The area directly underneath the epidermis, where the denser collagenous network resides, contains considerably less mesenchymal cells but mainly epidermal cell somata. Underneath this area, skeletal elements can be found all over the future calyx. Here, the epidermal cell somata are usually directed through the mesh-holes of the sclerites. The mesenchymal cells reside mainly proximal to the sclerites.

### Hydrocoel

The hydrocoel forms a nearly closed ring that runs underneath the vestibular cavity, positioned in the same 45° angle to the horizontal plane as the latter (Figs. 34, 35). Its opening, which has been on the right side in the doliolaria, is now situated ventrally at the “ring’s” most aboral point. The last section of the hydrocoel’s left end (on the larva’s right side, see Fig. 35E) is bent aborally and ends beneath the right end in direct vicinity of the axocoel. This left end, which represents the prospective primary stone canal, ends blindly (Fig. 40B). The hydrocoel horseshoe forms a bunch of orally directed bump-like outgrowths, the prospective podia (Figs. 35E, 35G). Literature states that temporarily fifteen podia develop, three in each radius (Hyman 1955). The number of developing bumps observed is surely close to fifteen. Several bumps noticeably form groups of three.

◂ **Fig. 35:** Metamorphosing cystidean larva of *A. bifida*, specimen 2. Reconstruction. **A-H** Selected views onto varying compositions of the epithelial cavities. **A** Dorsal view. Orientation for **C-F** given in **B**. Small ventral coelom (*svc*) marking the ventral side. **G** Oral view. **H** Aboral view. *abovm* aboral vertical mesentery, *gaot* tubule of the prospective glandular axial organ (***turquoise***), *hm* horizontal mesentery, *lE* left extension of the left somatocoel, *lL* left lobe of the right somatocoel, *ovm* "oral vertical mesentery", *perimf* periesophageal mesenteric formation, *rE* right extension of the left somatocoel, *rL* right lobe of the right somatocoel, ***black*** left somatocoel, ***blue*** hydrocoel, ***green*** enteric sac, ***lilac-gray*** epidermis, ***red*** both right somatocoel and tubes of prospective chambered organ, ***white*** vestibular cavity, ***yellow*** axocoel, *asterisk* hydropore, *pink arrow* proximal layer of the left somatocoel and distal layer of the right somatocoel consist of large cells (compare Fig. **40F**), *d* dorsal, *l* left, *r* right, *v* ventral.

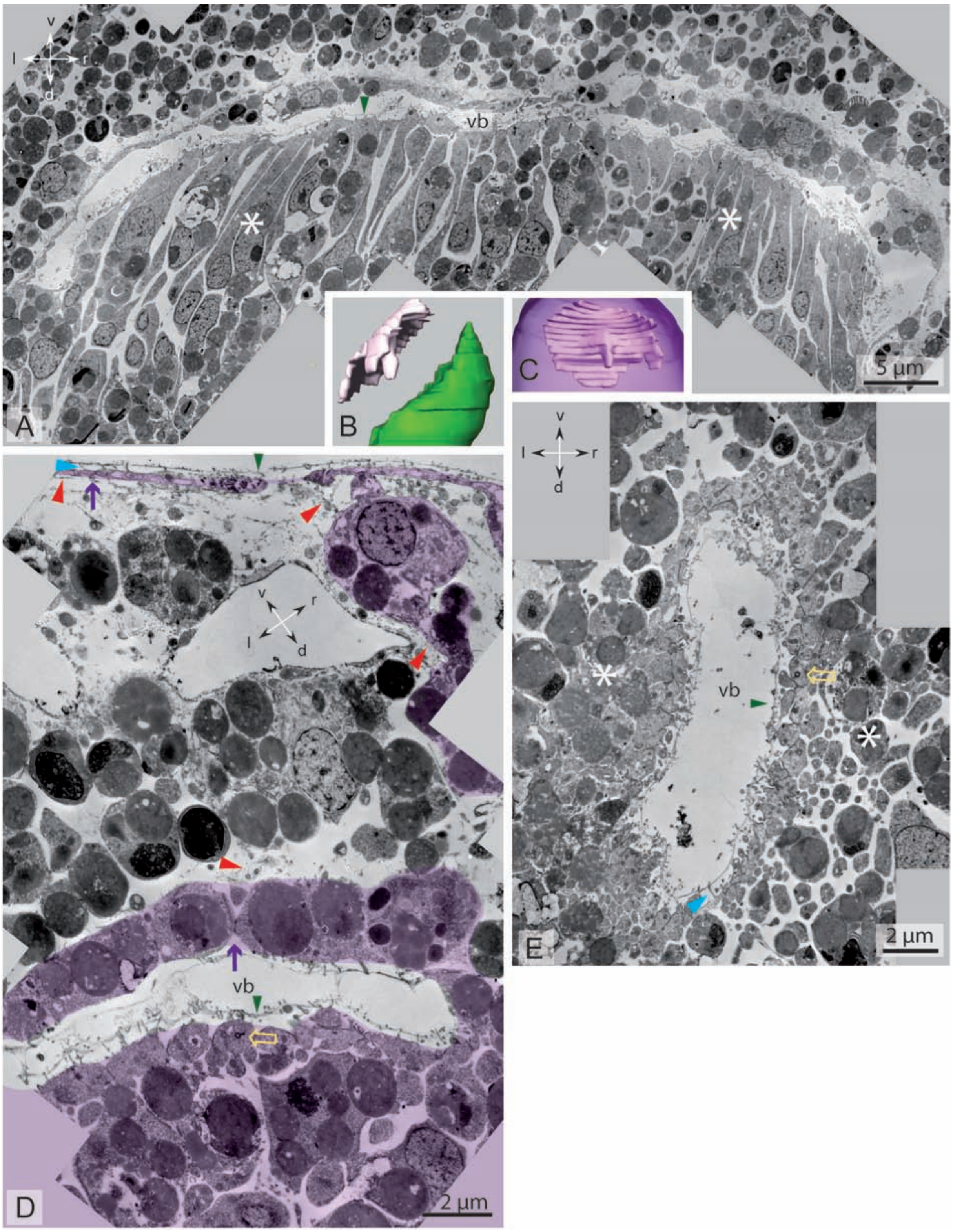


**Fig. 36:** Metamorphosing cystidean larva of *A. bifida*, specimen 2. **A**, **D**, **E** TEM cross-sections through the vestibular cavity (*vb*, ***white***). **A** Note the missing apical cell structures of the distal layer's cells. **B** Lateral and **C** dorsal view onto the vestibular cavity. **D** Vestibular cavity's peripheral region: The apical cell structures extend to the distal layer. Ectodermal cells marked in *lilac*, mesenchyme in between. **E** Cross-section through the extension of the vestibular cavity's proximal layer. *blue arrow heads* "cuticle", *green arrow heads* microvillus, *purple arrows* point to adherens junctions, *red arrow heads* fibrous basal lamina, *white asterisks* areas with flask shaped cells, *yellow empty arrows* basal body. *d* dorsal, *l* left, *r* right, *v* ventral.

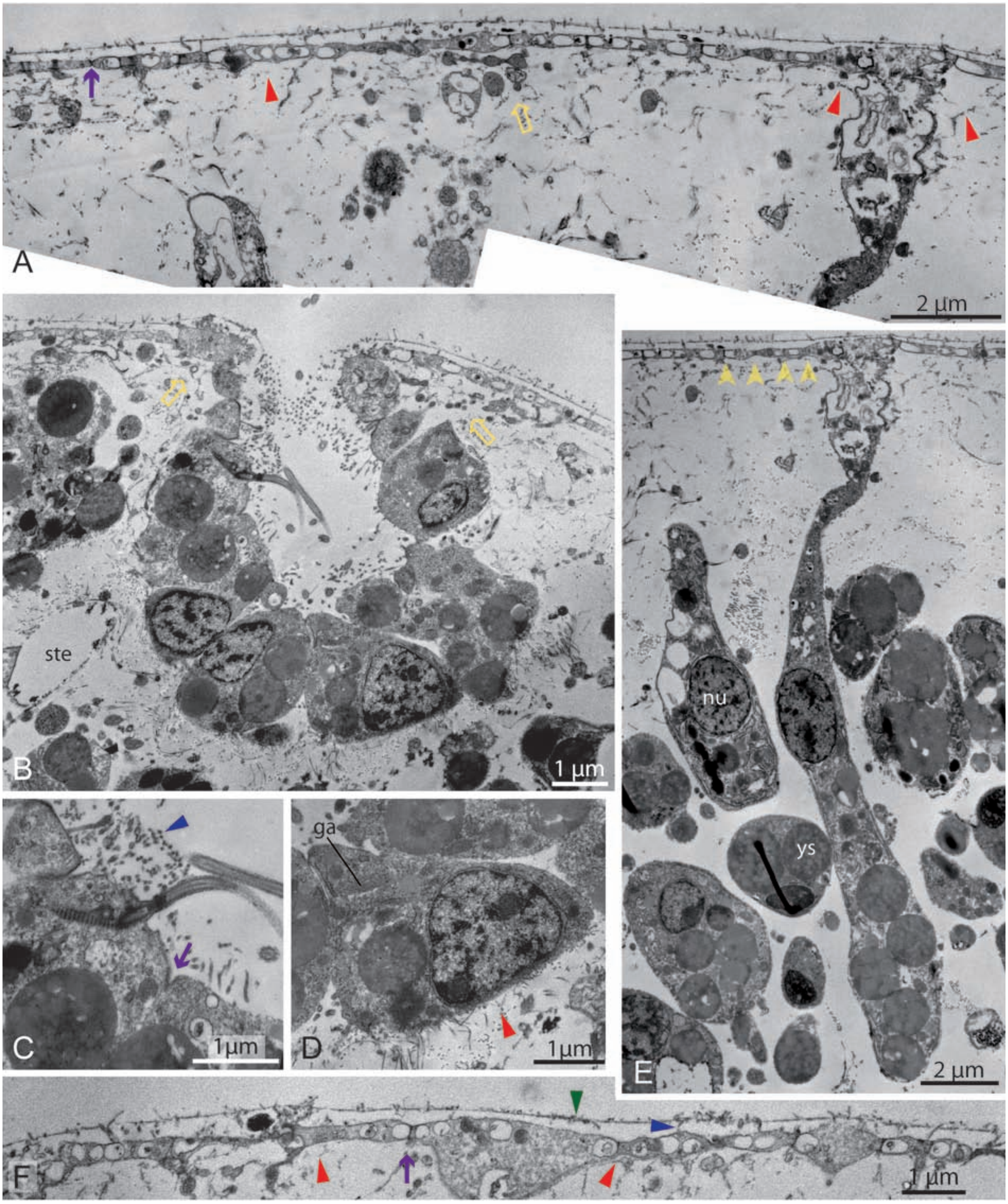
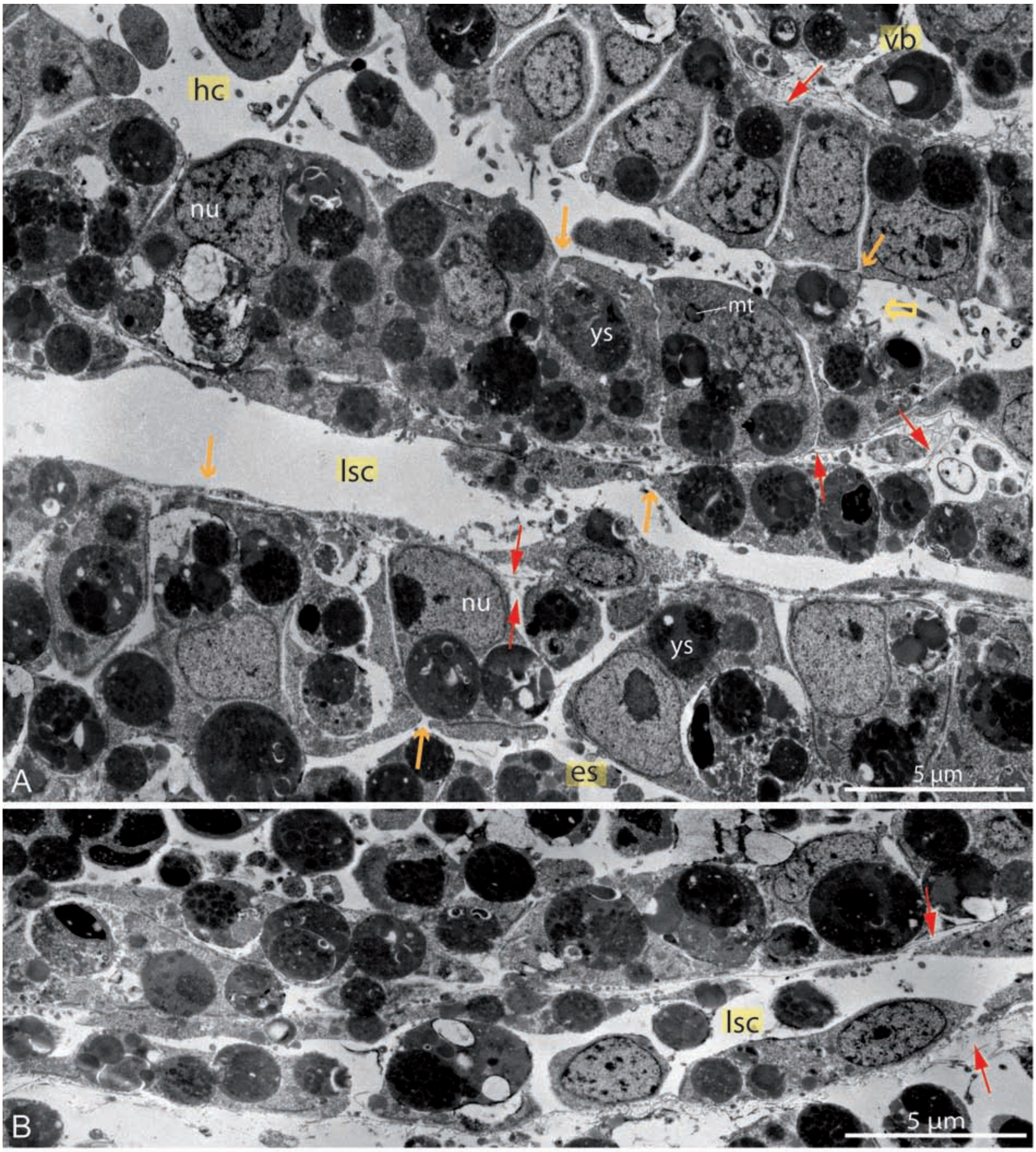


**Fig. 37:** Metamorphosing cystidean larva of *A. bifida*, specimen 2. TEM cross-sections. **A**, **E**, **F** Longitudinal section through the epidermis proper and **B-D** the hydropore. **D** detail of **B**. **E** Full extent of the cell soma of the tack-shaped cell on the right side of **A**. *ga* Golgi apparatus, *nu* nucleus, *ste* stereom, *ys* yolk sphere. *blue arrow heads* "cuticle", *green arrow head* microvillus, *purple arrows* adherens junctions, *red arrow heads* fibrous basal lamina, *yellow arrow heads* candidates for rivet-like structures, *yellow empty arrow* neurite.

The hydrocoelomic lining consists of cuboidal to columnar monociliated cells that are apically connected by adherens junctions and that are basally underlain by a basal lamina (Fig. 38A). The cell's ultrastructure did not change notably from that of the hydrocoel in the doliolaria. The epithelium of the prospective primary stone canal generally resembles that of the hydrocoel



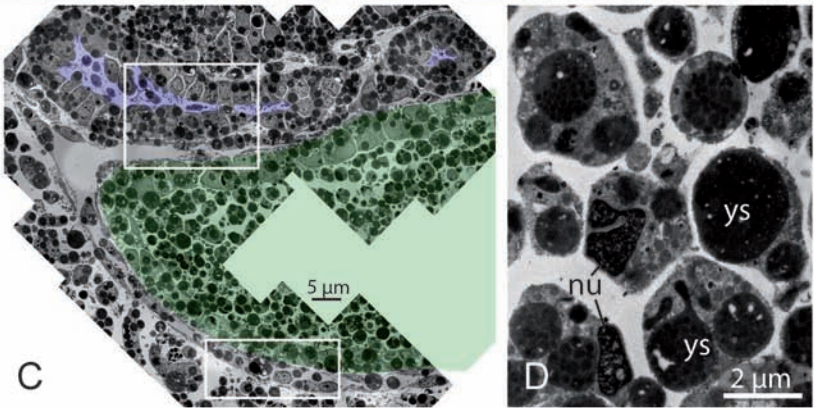


**Fig. 38:** Metamorphosing cystidean larva of *A. bifida*, specimen 2. TEM cross-sections cut orally of the horizontal mesentery (compare Fig. **35A**). **A**, **B** Detail of **C**. **A** Ultrastructure of the epithelia of the hydrocoel (*hc*), the left somatocoel (*lsc*) and the enteric sac (*es*). **B** Dorsal part of the left somatocoel. **D** Yolk cells. Note the highly heterochromatic nuclei (*nu*). *mt* mitochondrium, *vb* vestibular cells, *ys* yolk sphere, *orange arrows* adherens junctions, *red arrow heads* basal lamina, *yellow empty arrow* cell crossing the hydrocoel.

horseshoe (compare Figs. 38A and 40B). Many large cells reside in the hydrocoelomic lumen. Some of them form cell contacts that resemble adherens junctions with the epithelial cells (Fig. 38A).

### Enteric sac

The enteric sac is situated in the epithelial cluster’s center (Figs. 34, 35). Its form resembles a split pea, whose flat surface is directed anteriorly in a 45° angle to the horizontal plane. The enteric sac’s oral margin runs slightly lower but in parallel with the left somatocoel’s oral margin. Aborally, it is bordered by the right somatocoel. Compared with that of the doliolaria, the enteric sac is widened, especially in its anterior part.

The epithelium of the enteric sac consists of large cuboidal cells, whose ultrastructural composition largely resembles that of the doliolaria’s coelothelial cells (Fig. 38A). Neurites reside between the cells and the basal lamina.

The cavity of the intestinal sac is filled with loose comparatively small yolk cells (Fig. 38D). The cavity’s lumen is electron-lucent and free from *ecm* structures. The yolk cells contain small, highly heterochromatic nuclei as well as mitochondria and some rough Endoplasmic reticulum cisternae.

### Right (=aboral) somatocoel, future chambered organ

As in the doliolaria, five long tubes project aborally from the epithelial cluster (Figs. 34, 35). These precursors of the chambers of the chambered organ are directed vertically in the developing stalk, in the long-axis of the larva. They emerge from a flattened coelomic space, whose double-walls are forming a cap at the bottom of the cluster around the convex side of the enteric sac (Fig. 39). Due to the interrelation with these tubes, the coelomic cap can easily be recognized as the right somatocoel. Comparing the present larva’s right somatocoel with the heart-shaped one of the doliolaria, the former obviously achieved its cap-shape due to a strong enlargement of the right somatocoel’s *left* lobe, which grew around the enteric sac‘s aboral (formally anterior) end (Figs. 23A, 35F).

The coelothel of the right somatocoel consists of thin squamous monociliated cells that are apically attached to each other by adherens junctions and that are underlain by a basal lamina. At the oral margin of the left lobe’s distal layer, in vicinity to the *small ventral coelom* and across the left somatocoel’s left extension's proximal layer, the cells of the squamous epithelium are conspicuously larger than in other regions of this coelom (Figs. 34F, 40F).

As in the doliolaria, the five tubes are formed by monociliated epithelially arranged cells that are jointly encompassed by one basal lamina (Fig. 40A). Other than in the doliolaria, a fragile and not always continuous basal lamina is present in the extra-coelomic center between the tubes. Between the neighboring tubes, a basal lamina is locally present as well. The inner and the outer laminae are not contacting each other.

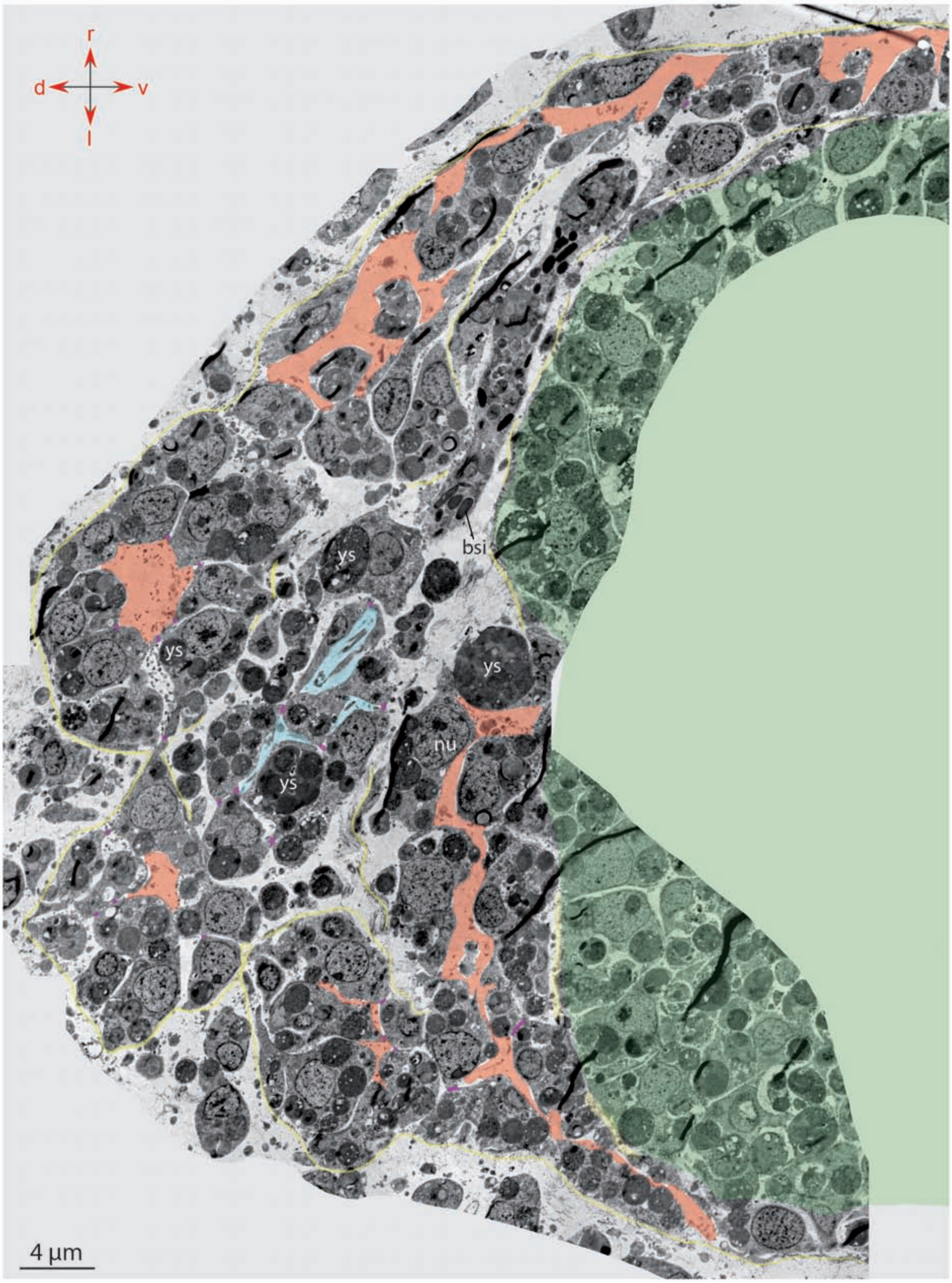


**Fig. 39:** Metamorphosing cystidean larva of *A. bifida*, specimen 2. TEM cross-section through the region, where the tubes of the prospective chambered organ emerge. *bsi* bullet shaped inclusions, *nu* nucleus, *ys* yolk spheres, adherens junctions (***lilac dots***), ***green*** enteric sac, ***red*** chambers of the chambered organ and right somatocoel, ***turquoise*** lumen of the prospective glandular axial organ tubule, ***yellow*** basal lamina.

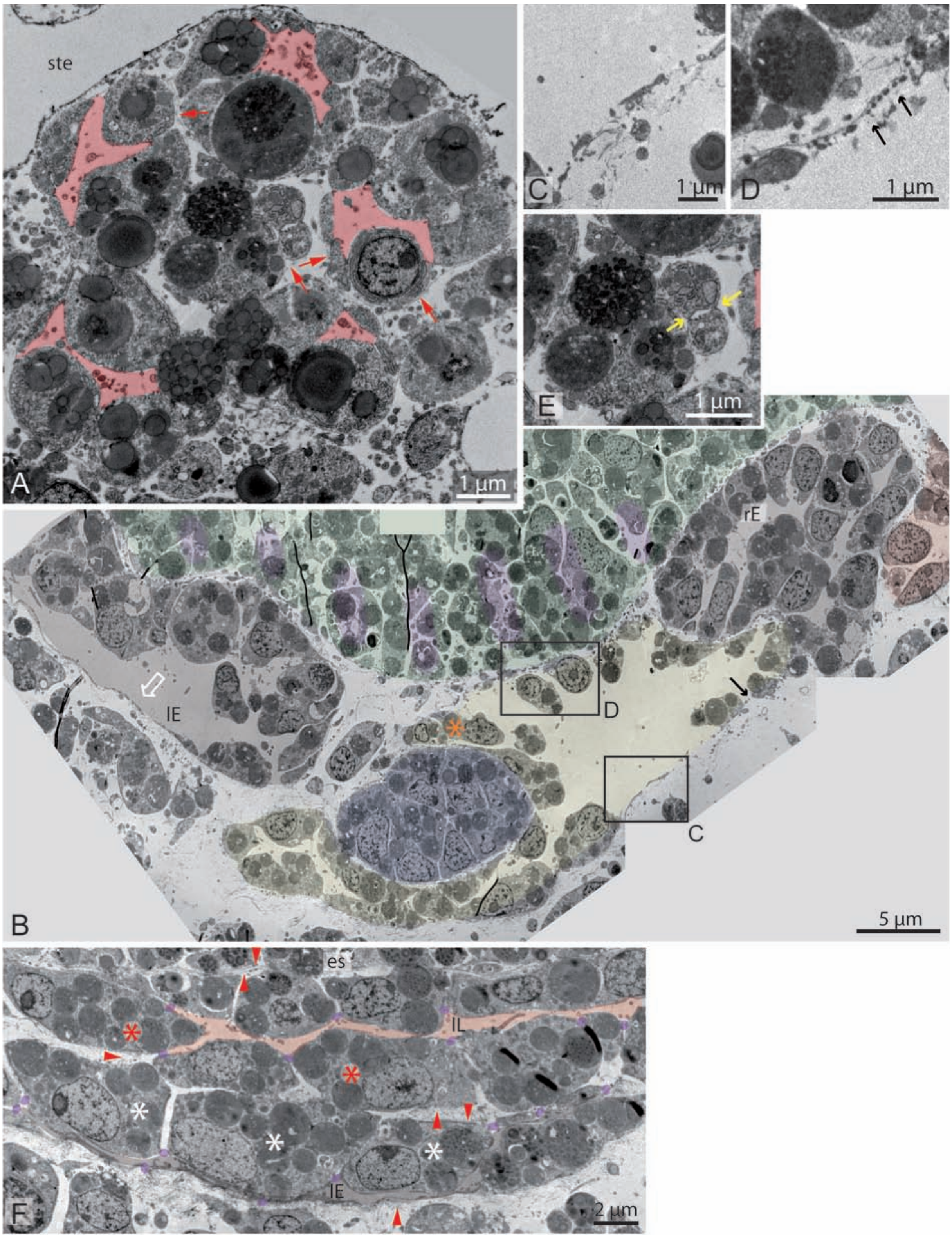


**Fig. 40:** Metamorphosing cystidean larva of *A. bifida*, specimen 2. TEM. **A** Cross-section through the tubes of the prospective chambered organ, cut in the middle of the stalk, **B** Cross-section right below Fig. **34E**. Below the *asterisk* starts the small ventral coelom. **C**, **D** Details of a nearby section of **B**, taken from a region similar to that marked with the *squares*. **C** Thin squamous epithelium. **D** Podocytes. **E** Detail of **A**, showing cell contacts (*yellow arrows*). Polarity of the cells additionally indicated by the basal lamina. Cilia have not been observed. **F** Detail of the area that is marked with a *pink arrow* in Figs. **34F** and **35B**. Large cells in the proximal layer of the left somatocoel (*white asterisks*) and in the distal layer of the right somatocoel (*red asterisks*). Note that the basal lamina is continuous around both somatocoelia on the left (horizontal *red arrow*). *es* enteric sac, *lE* left somatocoel left extension, *lL* right somatocoel left lobe, *rE* left somatocoel right extension, ***black*** left somatocoel, ***blue*** hydrocoelomic extension (prospective stone canal), ***green*** enteric sac, ***purple stripes*** cells of the vestibular cavity's epithelium, ***red*** right somatocoel, ***yellow*** axocoel, *purple markings* (in **F**) adherens junctions, *red arrows* and *red arrow heads* basal lamina, *white empty arrow* thin squamous epithelium.

### Tubule of the prospective glandular axial organ

At the oral end of the prospective chambered organ resides a small conical cavity in the center of the tubes, whose long axis is oriented in the larva's oral-aboral axis (Fig. 39). The cavity consists of cells that direct cilia into the cavity's lumen and that form adherens junctions. The cavity is not ensheathed by a basal lamina. The conical cavity's position is close to the respective area in the doliolaria, in which cells delaminated from the inner margin of the right somatocoel's left lobe (Fig. 26). As its position is homologous to the position of the tubule of the *glandular axial organ* in older stages, it is interpreted as being an initial stage of the latter.

Cross-sections of different distant levels of the larva reveal that one to a few cells reside in the center of the chambered organ. If more than one cell resides in the center, the cells form apical cell contacts that resemble adherens junctions (Figs. 40A, 40E).

### Left (=oral) somatocoel

The left somatocoel forms a crescent underneath the hydrocoel and is positioned in the same 45° angle to the horizontal plane as the latter (Fig. 35). The doliolaria's posterior cap now extends between the enteric sac and the hydrocoel and thereby stretches inwards over the dorso-oral side of the enteric sac (Figs. 38A, 38C). The doliolaria's left somatocoel's lateral extensions are lengthened around the outer oral margin of the enteric sac, forming the crescent mentioned above. The left extension broadens more in longitudinal direction than the right extension. The crescent's opening resides approximately on the ventral side.

That part of the left somatocoel which covers the epithelial sac dorsally is formed by monociliated cells that are connected to each other via adherens junctions and are underlain by a basal lamina (Fig. 38B). The basal lamina of the peritoneal layer is fibrous and that of the visceral layer delicate.

In different regions of the left somatocoel's lateral extensions, the cells are formed by different types of squamous cells: At the left extension's ventral end, the distal layer consists of extremely thin squamous cells and the proximal layer of very large squamous cells (Figs. 34E, 34F, 40B, 40F). The right extension's end, however, is fully formed by densely arranged large squamous cells. The cells of both extensions show the typical arrangement of organelles described for doliolaria coelothelial cells.

### Axocoel, hydropore, stone canal

The axocoel extends on the larva's ventroright side on one horizontal level with the primary stone canal. It is connected to the exterior via the hydropore, which resides on the ventroleft side a bit orally to the axocoelomic cavity (Figs. 34, 35). The hydropore is formed by large monociliated cuboidal cells that apically form long slender microvilli, which are not covered or connected by a cuticle (Figs. 37B-D). The epidermis‘ cuticle ends at the point where the hydroporus invaginates into the larval body. The epidermis' basal lamina follows the invagination, thereby achieving a more fibrous nature. Collagen fibers occur in higher density underneath the hydroporic invagination and attach to the basal lamina. In the bend between epidermis and hydroporic epithelium, a small plexus of several neurites is present.

Starting at the hydropore, a narrow axocoelomic duct, formed by a squamous to cuboidal epithelium passes the body wall diagonally in ventro-aboral direction (Figs. 35D, 40B). On one level with the prospective primary stone canal, the diameter of the axocoel increases and the epithelium flattens to a squamous epithelium. Some cells of the duct form basal dendritic extensions. Ventrally, a larger region of both the epithelium‘s distal and proximal layer is extremely flat. In the proximal layer, podocytes have been observed (Figs. 40D). The other epithelial areas are formed by a flat squamous epithelium. The cavity furthermore extends in counterclockwise direction distally around the primary stone canal’s anlage. It narrows again, passes towards the enteric sac and achieves a position between hydrocoel and left somatocoel. From here, it accompanies the course of the latter, narrows further, and ends as a slender process that extends around the location where the prospective esophagus will be formed, until it gets in proximity to the beginning of the left somatocoel’s cap (Figs. 34C-E, 35D). The epithelium that forms the slender process consists of squamous cells as well and is closely encompassed by the neighboring epithelia, the enteric sac’s epithelium being far more prominent and the oral somatocoel being more distinguished due to its larger cavity.

### Small ventral coelom

Underneath the primary stone canal, at the aboral end of the axocoel and to the right of the left somatocoel’s broad left extension, a small epithelially lined cavity can be found, which is named *small ventral coelom* in this thesis (Figs. 35, 41). Its monociliated cells are interconnected via adherens junctions so that the lumen of the cavity is independent from the neighboring coeloms. Some of the cells apically possess basal bodies instead of full ciliary root structures. A few of the cells possess microvilli and single epithelial cells cross the *small ventral coelom*’s lumen, remaining anchored within the epithelial tissue by adherens junctions (Fig. 41B).

Tracing the *small ventral coelom* from oral to aboral, single cells of the *small ventral coelom*'s oral end form apical cell contacts, which are adherens junctions, with the axocoelomic epithelium; these cells take part in the formation of both epithelia (Fig. 41B). Right below the axocoel, the *small ventral coelom* achieves its main characteristic: it is bluntly shaped towards the left somatocoel and tapers towards the latter’s far side (Figs. 41C, 41D). Some of its leftmost cells form cell processes that contact cell processes of the left somatocoel’s broad left extension (Fig. 41C). That way, a small basal lamina-lined *ecm* area, the ventral lacuna, is formed between the *small ventral coelom* and the left somatocoel’s broad left extension, which is locally continuous with the *ecm* between the *small ventral coelom* and the right somatocoel’s right lobe. The axocoel, the left somatocoel and the *small ventral coelom*, are jointly covered by a continuous basal lamina. Aboralmost, the *small ventral coelom* also gets in closer proximity to the epithelium of

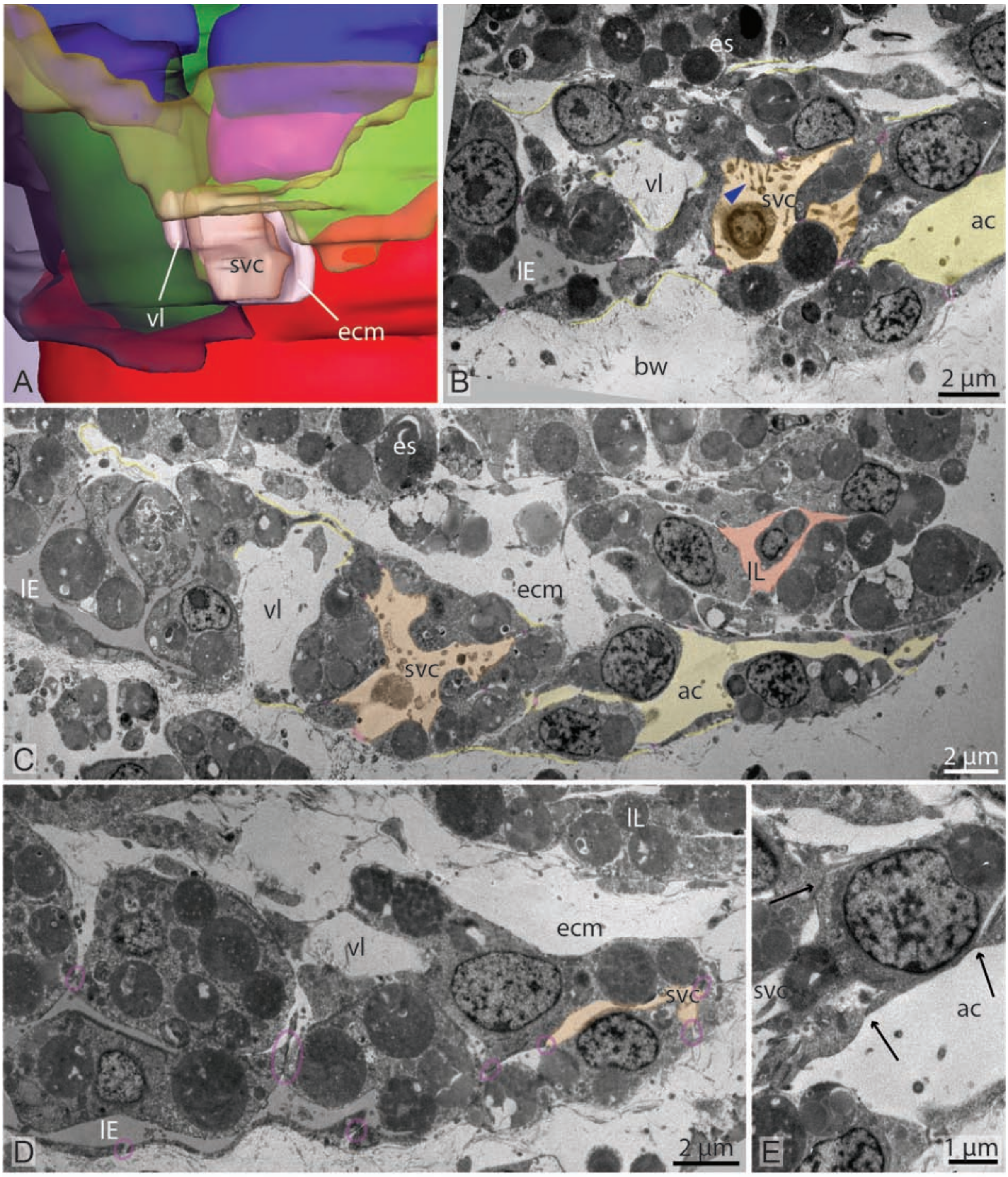


**Fig. 41:** Metamorphosing cystidean larva of *A. bifida*, specimen 2. **A** Detail of the reconstruction, ventral region. **B-D** Sequence of TEM cross-sections through the small ventral coelom (*svc*, ***orange***) from oral to aboral. **E** Detail of **B**. *ac* axocoel, *bw* body wall, *ecm* extracellular matrix, *es* enteric sac, *lE* left extension of the left somatocoel (***black***), *lL* left lobe of the right somatocoel (***red***), *vl* ventral lacuna. *blue arrow head* microvilli.

the right somatocoel's left lobe (Fig. 41D). However, processes following a specific pattern are not formed between these two cavities.

## Mesenteries

Where the two extensions of the left somatocoel are directed towards each other, they leave space for the axocoel and the primary stone canal. Traditionally, this region is referred to as the "*oral vertical mesentery*", although the epithelia do not effectively meet (Fig. 35C). A true mesentery, the *aboral vertical mesentery*, forms, where the right somatocoel's two lobes contact each other on the larva's right side (Figs. 35E, 35F, 35H). It is obliquely curved ventralwards when followed in oral direction. Where the left and the right somatocoel contact each other, the horizontal mesentery is formed (Fig. 35A). In the present larva, it is, of course, positioned in the same 45° angle to the horizontal plane as the hydrocoel.

These latter three mesenteries, true mesenteries or not, are complemented by another "untrue" locally confined mesentery, which forms where the epithelia of axocoel and left somatocoel's right extension get in closer contact. As it surrounds the prospective esophagus on the right side, I named it "the *periesophageal mesenteric formation*". Similarly, mesenteric formations exist ventrally between the axocoel and the left somatocoel's left extension and between the axocoel and the right somatocoel's left lobe.

### Nervous structures

Neurites are basi- and subepithelially associated with every epithelium of the metamorphosing cystidean larva. Neuronal structures can be found throughout the calyx's body wall. They are, however, much more abundant and organized in the developing stalk. Here, axially elongated cells and neurites accompany the tubes of the chambered organ. They are mainly located between the tubes and the sclerites, but can also be found surrounding the sclerites. Some neurites also reside in the center of the chambered organ.

## 3.4 Early cystidean larva (Specimen 3)

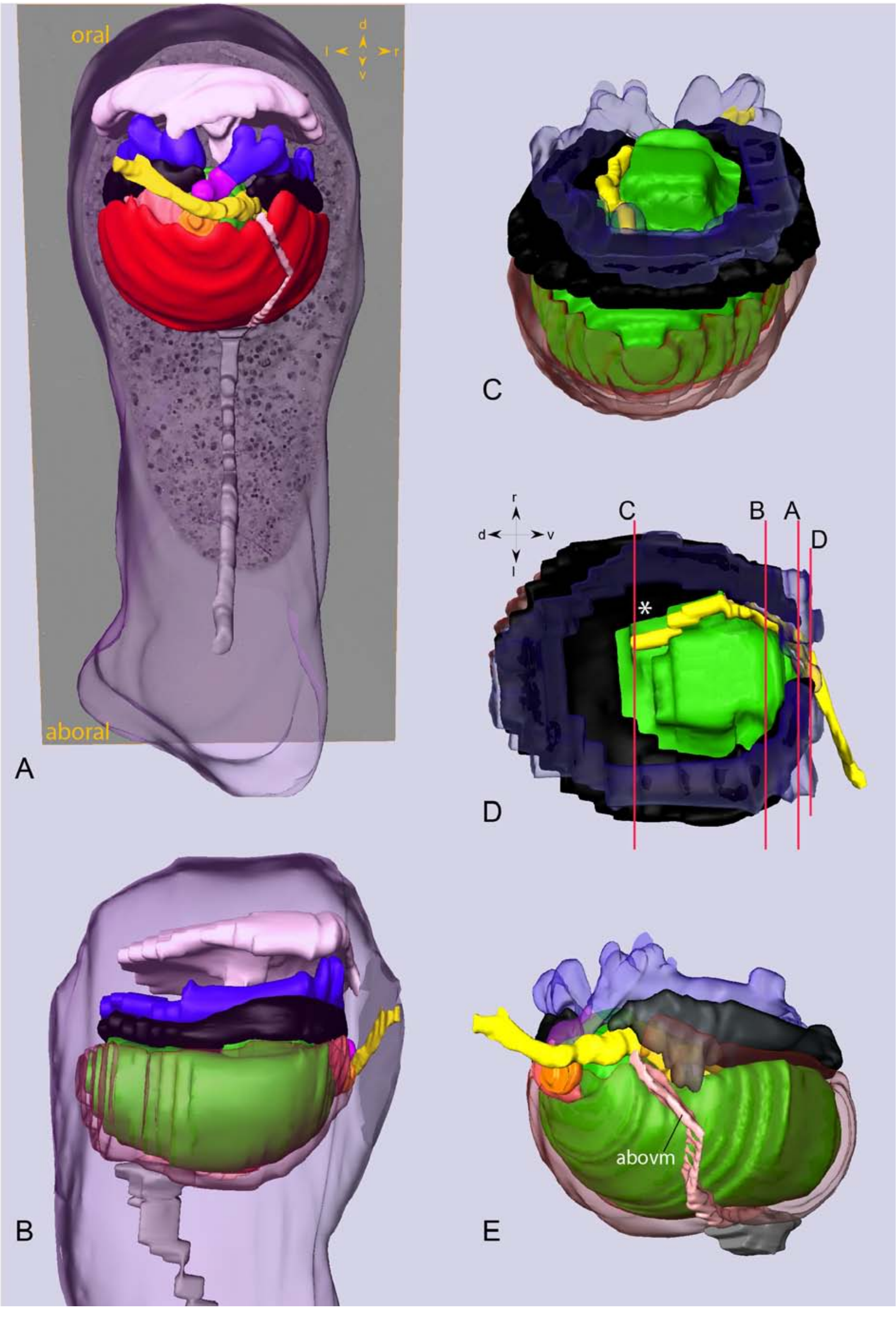
### General description

The next oldest larva has been cut in longitudinal semi-thin sections and investigated by means of light microscopy. It has a longer stalk than the larva previously described (Figs. 5C, 42).

The vestibular cavity, the hydrocoel and the oral (formerly left) somatocoel of the present larva take up fully horizontal positions (Fig. 42B). The axocoel, the hydrocoel and the somatocoelia are still separate from each other. The tubes of the chambered organ extend from the aboral somatocoel.

### Enteric sac

The hemispherically shaped enteric sac resides in the aboral half of the larva's calyx. The generally flattened oral side of the hemisphere extends horizontally and the aboral side is concavely bent. Although the border between the vestibular cavity and the enteric sac is difficult to determine, the enteric sac obviously forms an orally directed bump in direction to the aboral extension of the vestibular cavity (Figs. 42C, 42D). When seen from the oral side, the bump is formed in a ventro-left position of the enteric sac, where the latter is not covered by the oral so-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matocoel (Figs. 42C, 42D). To the bump's right side, the enteric sac's oral side is slightly emarginated. This emargination approximately starts anterior to the margin of the oral somatocoel's dorsoventral covering and ends in the area where the right extension of the oral somatocoel's crescent extends between the axocoel and the aboral (formerly right) somatocoel. The enteric sac is completely filled with non-epithelially organized yolk cells (Fig. 43).

### Vestibulum

The very flat vestibular cavity arches over the conglomerate of epithelial cavities, extending as far as the oral margin of the aboral somatocoel (Figs. 42A, 42B). Ventrocentrally, its proximal layer forms a thorn-like extension in the direction of the enteric sac's bump described above.

Between the enteric sac's bump and the vestibular cavity's aboral extension, a cell mass of mainly long, slender and axially directed cells reside (Fig. 43). They form the vestibulum's proximal layer. Lightmicroscopically, this epithelial part is difficult to distinguish from the enteric sac cells forming the bump, since dividing basal laminae cannot be identified.

### Oral somatocoel

In an oral view, the oral (former left) somatocoel forms a horizontal crescent (Fig. 42D). The gap between the two ends of the crescent, the so-called "*oral vertical mesentery"*, resides ventrally and is large enough to be passed by the primary stone canal (Fig. 42A). The right extension's end widens a bit in the aboral direction and, thereby, comes to lie between the aboral somatocoel and the axocoel (Fig. 42E). This latter formation is descriptively referred to as the *aboral outgrowth of the oral somatocoel's right extension* in this thesis. As in the previous larva, the oral somatocoel spans the dorsal half of the oral side on the enteric sac, but does not extend onto the dorsal side anymore (Figs. 42C, 42D).

### Aboral somatocoel

Similar to the larva previously described, the aboral (former right) somatocoel surrounds the enteric sac like a cup (Fig. 42). Together with the oral somatocoel, it forms the horizontal mesentery on one level with the oral end of the enteric sac.

The two lobes of the aboral somatocoel, the larger left lobe and the smaller right lobe, meet on the larva's right side to form the *aboral vertical mesentery* (Figs. 42A, 42E). The latter extends from the chambers of the chambered organ's oralmost end to the region where the axocoel meets the oral somatocoel. The *aboral vertical mesentery* bends ventrally on its oralmost side.

◂ **Fig. 42:** Early cystidean larva of *A. bifida*, specimen 3. Reconstruction based on semi-thin vertical sections. **A-E** Selected views onto varying compositions of the epithelial cavities. White lines in **D** indicate position of sections shown in Figs. **43A-D**. *abovm* aboral vertical mesentery, ***black*** left somatocoel, ***blue*** hydrocoel, ***dark pink*** primary stone canal, ***green*** enteric sac, ***orange*** small ventral coelom, ***pink*** cluster of large epithelial cells, ***red*** right somatocoel, ***white*** vestibular cavity, ***yellow*** axocoel, *asterisk* position of intestinal emargination, *d* dorsal, *l* left, *r* right, *v* ventral.

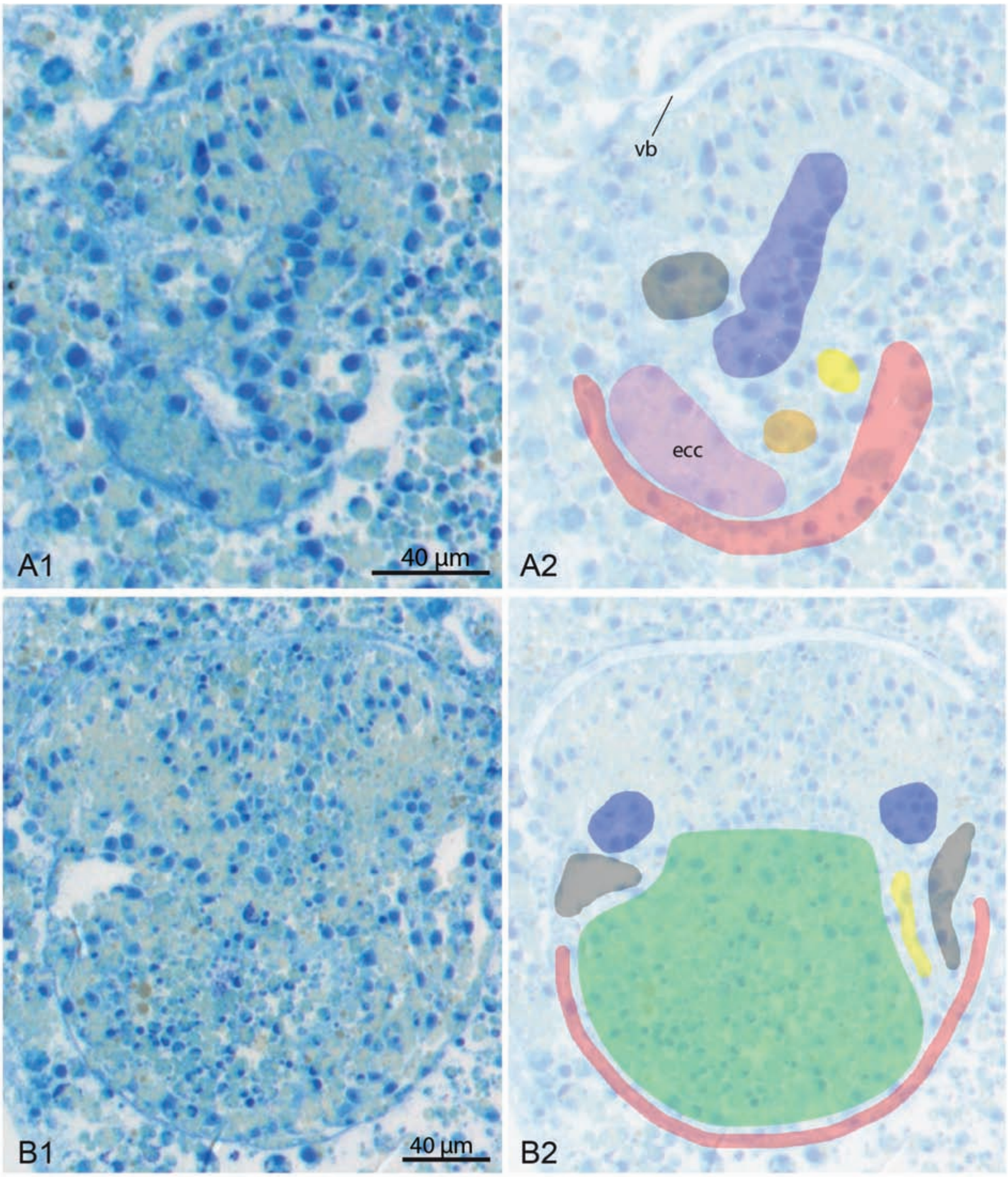


**Fig. 43 part 1 of 2**

**Fig**. **43 (2 parts):** Early cystidean larva of *A. bifida*, specimen 3. LM. **A1**, **B1**, **C1, D** Selected semi-thin vertical sections of the calyx whose position is marked in Fig. **42D**. **A2**, **B2**, **C2** Identical view of the related sections shown in reduced opacity. Different structures are individually labeled, showing their relative position and extent. **D** Detailed view of a section slightly ventral to **A**, showing a cluster of large epithelial cells (*ecc*, slightly colored in ***pink***) that forms the horizontal mesentery on the ventroleft side. Some of these cells are part of the left somatocoel (*black asterisks*) and others are, to a larger extent, part of the right somatocoel (*red asterisks*). *ac* axocoel (***yellow***), *hc* hydrocoel (***blue***), *lE* oral somatocoel's left extension, lL aboral somatocoel's left lobe, *rE* oral somatocoel's right extension, *stc* primary stone canal, *svc* small ventral coelom (***orange***), *vb* vestibular cavity (***white***), ***black*** left somatocoel, ***red*** right somatocoel.

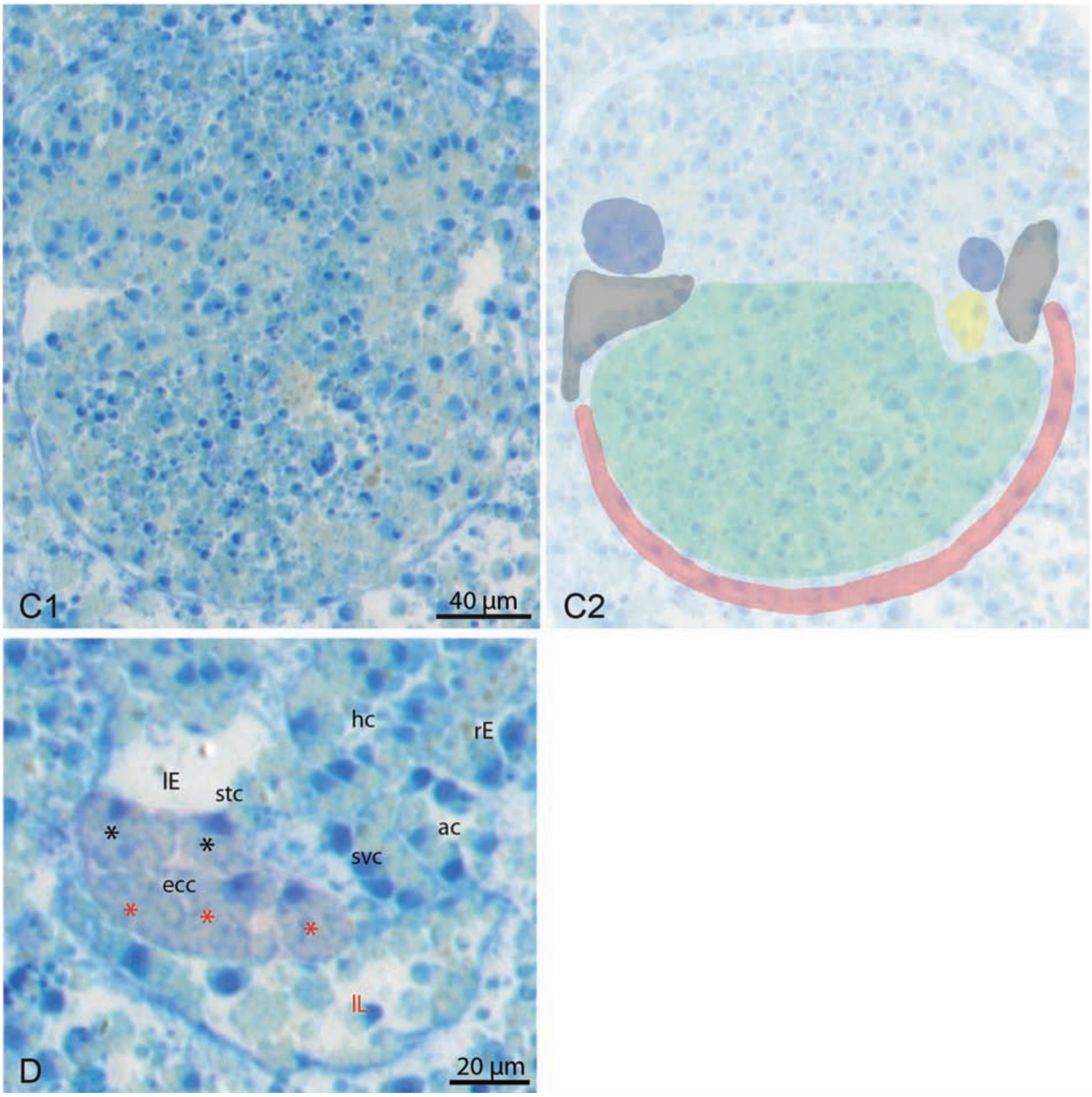


Fig. 43 part 2 of 2

## Prospective chambered organ, tubule of the glandular axial organ

The prospective chambered organ has not been investigated in detail in this larva. An actual connection between the delicate tubes of the prospective chambered organ and the aboral somatocoel could neither be confirmed nor denied with certainty by light microscopy. The position of the prospective chambered organ is therefore only grossly indicated in the reconstruction (Figs. 42A, 42B). A cylindrical, epithelially lined cavity that is formed by large cuboid cells resides between the chambers of the chambered organ and the enteric sac, its long axis being oriented from right to left. No continuous structure could be traced from it along the *aboral vertical mesentery*.

### Axocoel, hydropore

The hydropore is situated slightly to the left of the ventral side and slightly oral to the primary stone canal (Fig. 42). From there, the axocoel crosses the body wall slightly aborally in counterclockwise direction on the larva's ventral side and finds its way between the oral somatocoel's right extension and the enteric sac. It finally forms a slender process, which runs nearly half around the enteric sac's oral bump and ends with the beginning of the oral somatocoel's dorso-oral cap (Figs. 42C, 42D). Its epithelium thereby directly contacts the epithelia of both the oral somatocoel and the enteric sac (Fig. 43C2). The axocoel is separate from the oral somatocoel. It also is not connected to the hydrocoel, as the primary stone canal ends blindly.

### Hydrocoel

The hydrocoel resides on top of the outer margin of the oral somatocoel's crescent (Fig. 42). It is still horseshoe-shaped, the gap between the two ends lies ventrally. Its left end (on the right side) is aborally directed and passes through the "*oral vertical mesentery*". This end forms the primary stone canal, which ends blindly in intimate proximity to the axocoel. Also, about 15 orally directed outgrowths evaginate from the horseshoe, butting against the vestibulum. They will become the inner lining epithelium of the prospective podia.

### Small ventral coelom

An epithelially lined cavity locates ventrally, directly aboral of the location where the primary stone canal contacts (but does not merge with) the axocoel (Figs. 42A, 42E, 43A). This is the *small ventral coelom.*

### Large epithelial cells

The ventroleft end of the horizontal mesentery is formed by large cells that contain more yolk spheres than usual for cells of the coelothelia involved. Each of these cells also possesses a large nucleus with a prominent nucleolus. The cells are part of both the epithelium of the oral somatocoel's left extension's aboral layer and, to a considerably higher degree, the aboral somatocoel's left lobe's oral layer (Figs. 43A, 43D).

## 3.5 Cystidean stages with winding intestinal tract

A detailed comparison of wholemounts of all fixed cystidean larvae available gives the strong impression that the outgrowing of the enteric sac does not only involve a simple vertical carving of the latter, but that it also bends and tilts inwards around the central axis (Figs. 5G-I).

In all following larvae described, the enteric sac already differentiated the intestinal outgrowth on the right side. The larvae differ, however, in their developmental progress regarding the formation of mouth and anus, and regarding the advancement of coelomic fusion processes.

The following reconstruction (Fig. 44) is based on data of two cystidean larvae (A and B) that are described separately below. These two larvae share similarities regarding course and position of their intestinal tract, their overall coelomic arrangement and their *glandular axial organ*. The reconstruction's shape and its non-somatocoelomic arrangements are taken from the serial semi-thin sections of the older cystidean larva B. The horizontal mesentery of this larval stage, however, underwent a higher degree of histolysis, hampering a reliable interpretation of the stage's somatocoelomic state. These data have therefore been enriched with data on mesenteries confirmed by the serial ultra-thin sections of the younger cystidean larva A in order to identify the limits of the oral and aboral somatocoel. For both larvae, those aspects that contributed to the reconstruction will be emphasized in the following separate descriptions.

## 3.5.1 Cystidean larva A (Specimen 4)

### General morphology

Specimen 4 has been investigated ultrastructurally based on longitudinal sections cut from the ventroright to the dorsoleft side. Its oral end is flattened and the calyx is not fully distinct from the stalk (Fig. 5D). The stalk of the larva is about 500-600 µm long and less than 80 µm thick, while the head is about 150 µm in diameter. The larva has a closed vestibulum into which 10 podia reach; it possesses no anus. The five tubes of the chambered organ lost contact with the aboral somatocoel. The hydrocoel and the axocoel established contact via the primary stone canal.

### Intestinal tract

The larval intestinal tract can be subdivided into two regions, the prospective stomach, which locates at the former position of the enteric sac, and the intestinal outgrowth (Fig. 45B). The esophagus is not formed, yet, because the vestibular cavity and the intestinal tract have not merged. In this larva, the vestibular aboral extension and the successor of the oral bump above the stomach meet on one level with the horizontal mesentery (data not shown), implying that the prospective esophagus is formed by both ectoderm and endoderm. When looking from the oral side, the intestinal outgrowth extends from the stomach on the approximate dorso-right

▸ **Fig**. **44 (2 parts):** Cystidean stage. Reconstruction, based on semi-thin sections of specimen 5 (Fig. **48**) and details derived from specimen 4. **A-K** Selected views onto varying compositions of the epithelial cavities. **A**, **B**, **D**, **G**, **H**, **i** Ventral views slightly to the left. **G**, **i** Oral and aboral somatocoel and axocoel are depicted as part of the main body cavity (*bc*, ***grey***). **C**, **E**, **F** Oral views. **E** Note the fusion area (*acf*) of the left somatocoel's right extension and the axocoel. Pentaradial-symmetry marked with *orange letters* in **F**. **J**, **K** Details of the ventral area. The *white asterisk* indicates position of opening between primary stone canal and axocoel. *abovm* aboral vertical mesentery (***light coral***), *acd* axocoelomic duct (***yellow***), *axlsc* axial extension of the oral somatocoel, *axm* axial mesentery (***white***), *axmus* axial muscle, *cho* chambered organ (**red**), *ecc* position of cluster of large epithelial cells (***pink***), *epi* epidermis, *eso* esophagus (***green***), *gaot* glandular axial organ tubule (***turquoise***), *hc* hydrocoel (***blue***), *hm* horizontal mesentery (***white***), *int* intestinal outgrowth (***green***), *lL* aboral somatocoel left lobe, *lsc* oral somatocoel (***black***), *mes* mesenchyme, *ol* oral lacuna (***white***), *osec* oral section of the axial mesentery (***grey***), *pc* peripheral compartment, *peril* periesophageal lacuna (***pale yellow***), *rL* aboral somatocoel right lobe, *rsc* aboral somatocoel (***red***), *sabovm* secondary aboral vertical mesentery (***light coral***), *stc* primary stone canal (***purple***), *sto* stomach (***green***), *svc* small ventral coelom (***orange***), *vb* vestibulum, *vl* ventral lacuna (***pale blue***), *red cross* (=**white lines**) dissolved mesentery between left and right somatocoel.

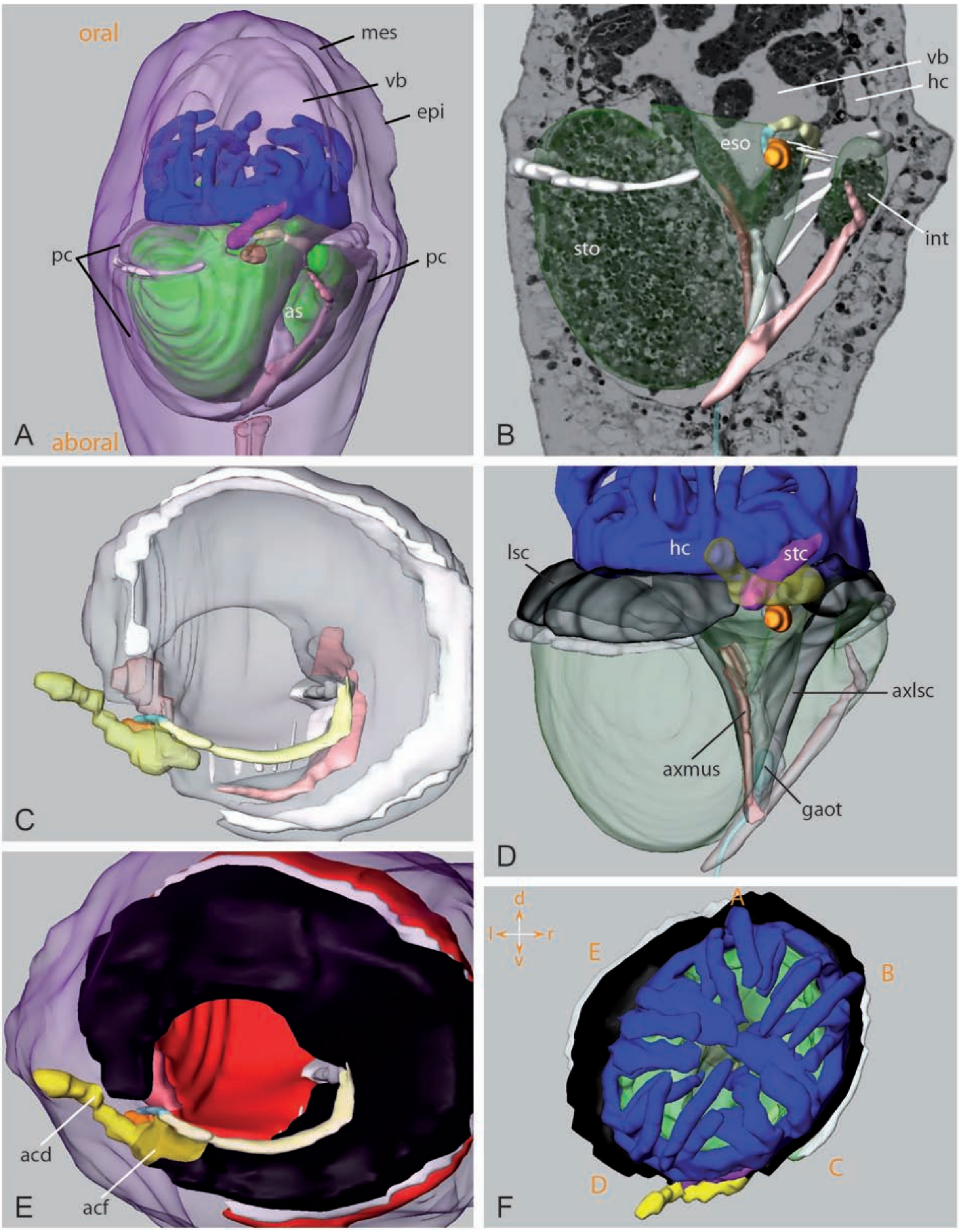


Fig. 44 part 1 of 2

side, being coiled in clockwise direction. The tip of the intestinal outgrowth contacts the body wall on the ventro-right side but an anus is not formed, yet (Fig. 45A). The intestinal epithelium consists of monociliated cuboidal and palisade cells that apically form several microvilli. The intestinal tract is empty to a large degree - only a few yolk cells can be observed in proximity to the epithelial cells.

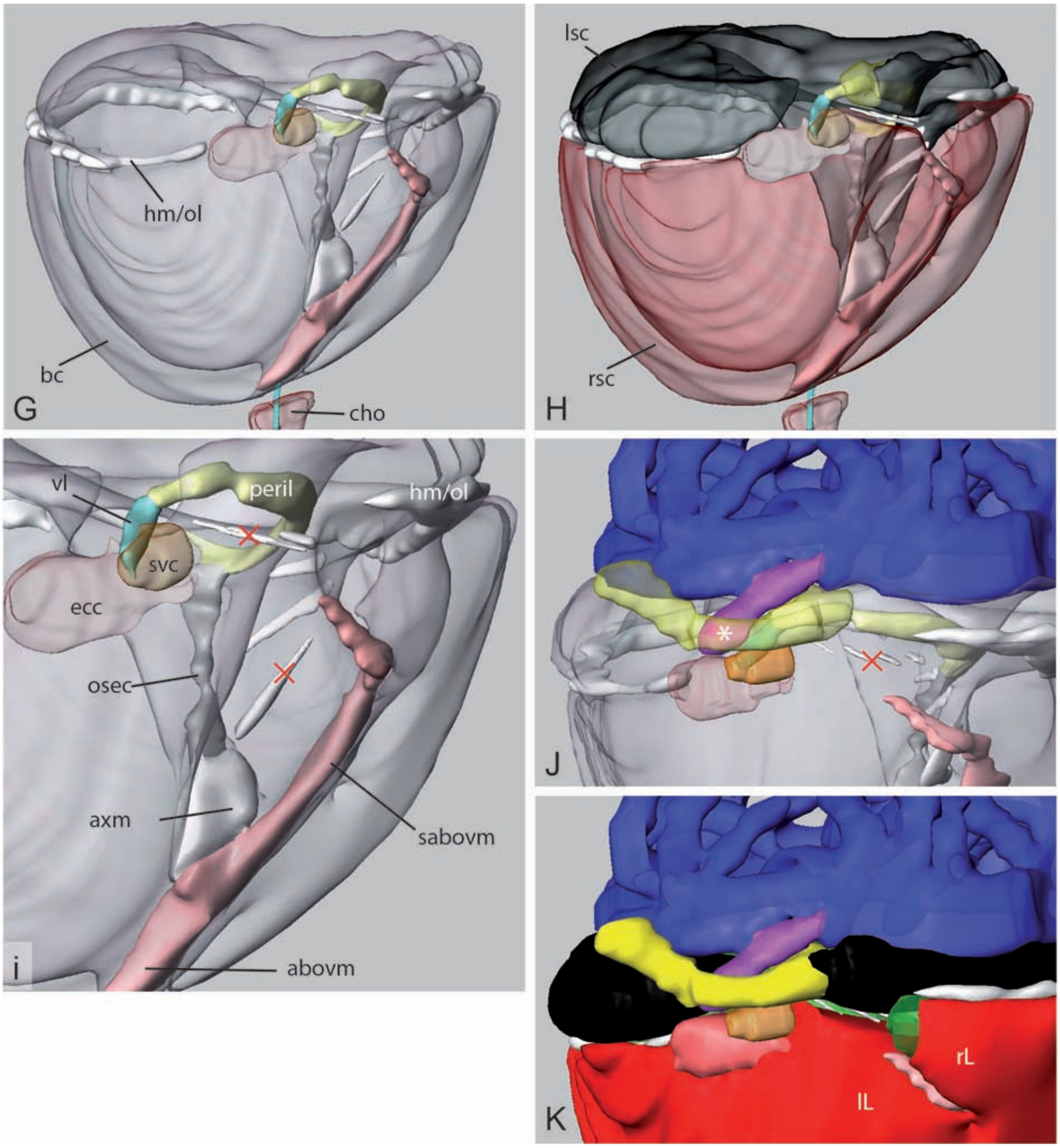


**Fig. 44 part 2 of 2**

As already stated, the vestibular cavity and the cavity of the intestinal tract are not connected. The cells of the vestibular cavity’s proximal layer in the region of the prospective esophagus are shorter than in the previous stage.

## Hydrocoel

Underneath the lateral margin of the vestibular cavity, the hydrocoel forms a horseshoe around the prospective esophagus (Fig. 45). The horseshoe’s ends are in close proximity, but not connected to each other. Its (originally) left end forms the primary stone canal, which opens into the axocoel. The hydrocoel sends oral extensions into all 10 podia of the larva. Between these, further evaginations butt against the proximal wall of the vestibulum. Due to the thick-

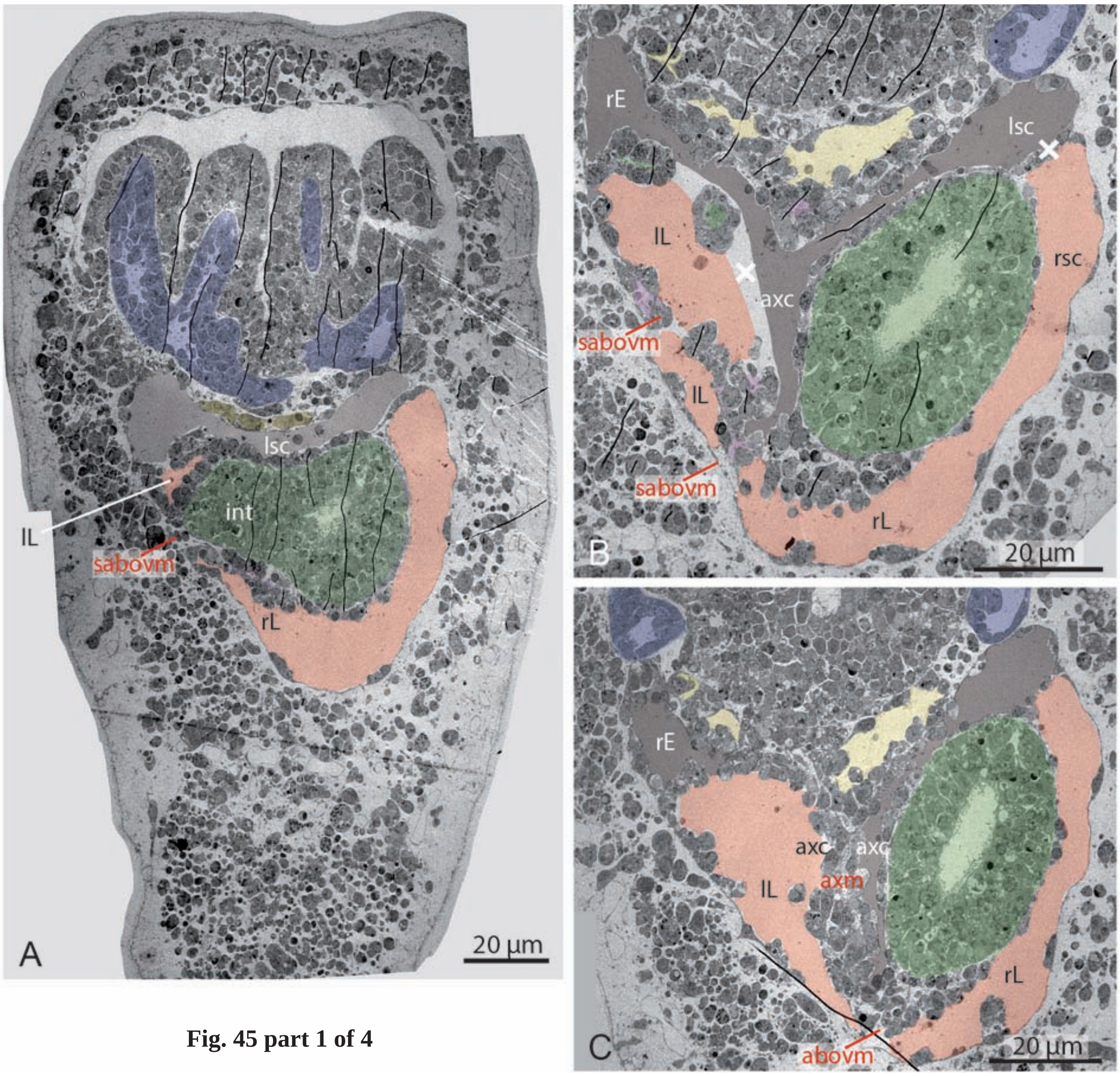


**Fig. 45 part 1 of 4**

**Fig. 45 (4 parts):** Cystidean larva A, specimen 4. TEM. **A-K** Sequence of serial vertical sections traced from right to left showing the axial aboral extension of the oral somatocoel in the axial compartment (*axc*) (**A-D**, **K**) and the ventral structures (**E-J**). Distances between sections vary. Mesenteries are mostly intact so that the coelomic spaces are distinguishable. Compare to Fig. **44**. Common structures are labeled only once and are thenceforth indicated by related color coding, unless specified otherwise. **A** The distalmost section of the intestinal outgrowth (*int*) is surrounded by the oral somatocoel (*lsc*) and both lobes of the aboral somatocoel (*rsc*). The left lobe (*lL*) and the right lobe (*rL*) of the aboral somatocoel are separated by the secondary aboral vertical mesentery (*sabovm*). **B** The horizontal mesentery (*white cross*) in the axial compartment is dissolved. The axocoel (*ac*) widens above the oral end of the glandular axial organ (*gao*). **C** The aboral vertical mesentery (*abovm*) resides aborally underneath the intestinal tract. The axocoel extends both ventrally and further dorsally. **D** To the left of the oral somatocoel's right extension (*rE*), the right end of the small ventral coelom (*svc*) resides distal to the axocoel. The axocoel enlarges close to the intestinal tract between the *rL* and the primary stone canal (*stc*). The *square* marks the ventral area, which is traced in **E-J**. **E** The axocoel extends underneath the primary stone canal and above the small ventral coelom. Note the contact between cuboidal cells of the aboral small ventral coelom-epithelium and those of the aboral somatocoel-epithelium. **F** The primary stone canal opens into the axocoel. The ventral lacuna (*vl*) opens up between small ventral coelom and aboral somatocoel, being limited by small ventral coelom’s cell processes. The left extension of the oral somatocoel (*lE*) extends between primary stone canal and axocoel. **G** The small ventral coelom protrusions continue. The small ventral coelom contacts the *lE* (*black asterisks*) and *rL* (*white asterisks*). **H** The small ventral coelom forms two lobes. The axocoel passes the body wall distally (axocoelomic duct). It probably also forms a proximal left extension around the esophagus. **i** The small ventral coelom still forms cell processes around the ventral lacuna. **J** The *rL*-epithelium locally consists of very large cells (*#*) that are contacted by cell processes, possibly neurites, sent out by the oral somatocoel (*red empty arrow*). **K** The axocoel opens to the outside via the hydropore (*hp*). Note the left and the dorsal extensions of the axocoel near the esophagus (marked in ***yellow***). **L** Detail of **J**. *axm* axial mesentery, *sac* sacculus, *sto* stomach, *black arrow* podocytes, *black empty arrow* processes sent out by cells of *svc*, *purple square* cell contacts, *red arrows* basal lamina, *red asterisks* region comparable to that which is marked with a red asterisk in Fig. **51** and **54**. *red question marks* indicate uncertainty, whether this part of the axocoelomic process extends from the left side of the axocoelomic cavity or constitutes the last section of that process, which is sent out towards the right side.

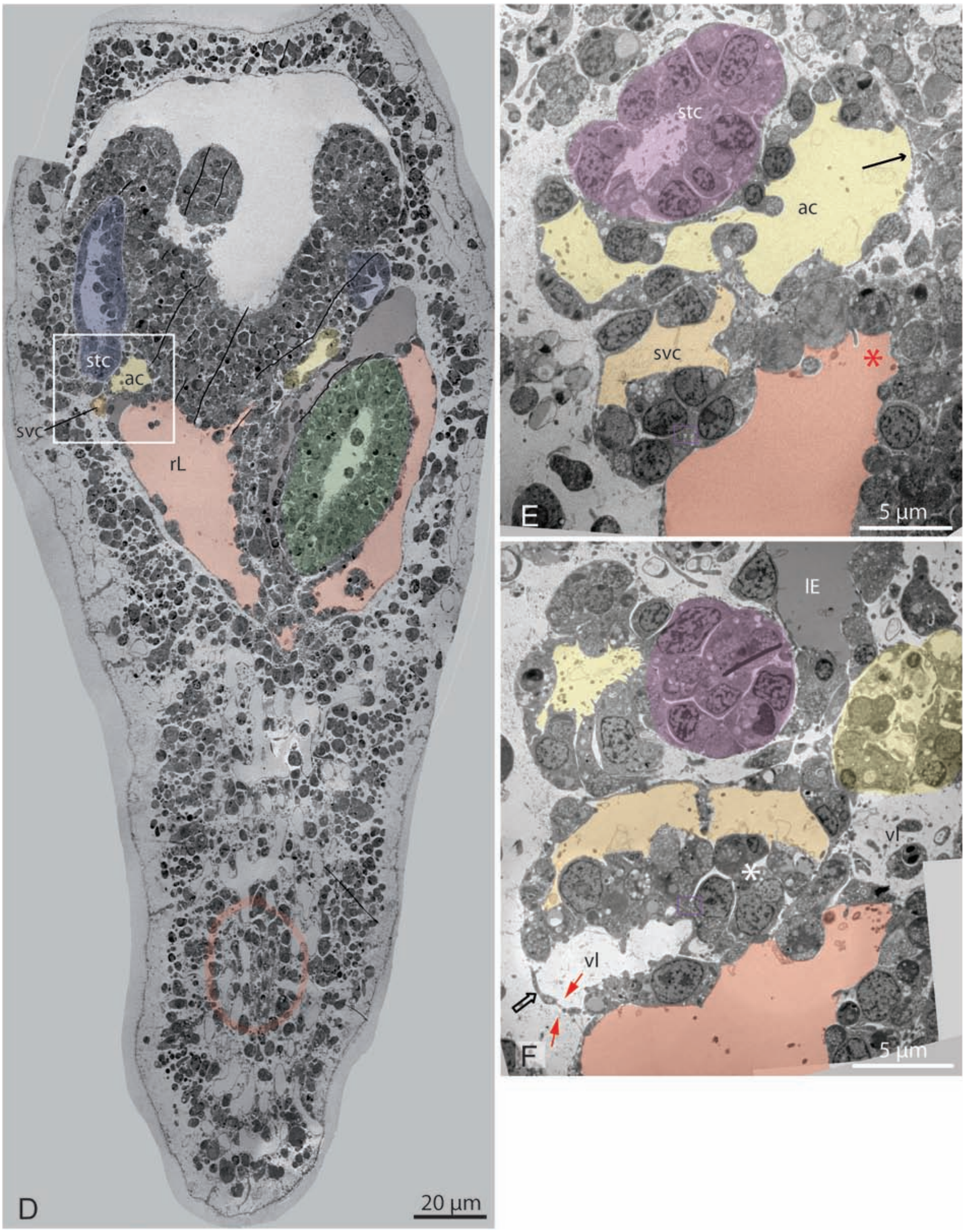


**Fig. 45 part 2 of 4**

ness of the latter's epithelium, they don't emerge into the cavity. The hydrocoelomic epithelium is formed by squamous to cuboidal monociliated cells. At few locations, these cells send out processes into the hydrocoelomic lumen. Some of these trabecular cell processes contain the nucleus.

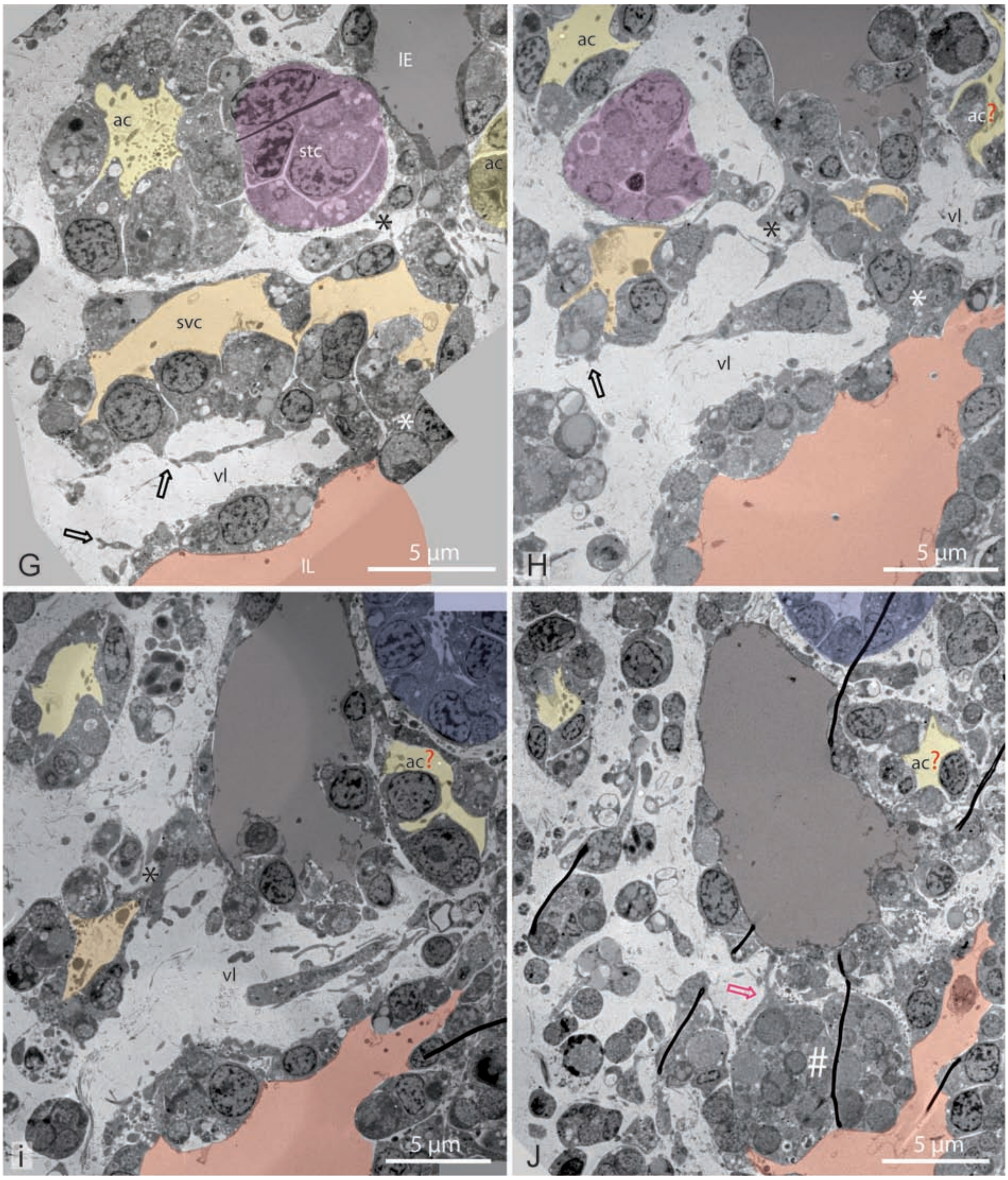


**Fig. 45 part 3 of 4**

## Axocoel

The results concerning this larva's axocoel have not (or at least not directly) been implemented in the reconstruction of Figure 44.

Starting at the hydropore, the axocoel passes the body wall counterclockwise ventrally and merges with the primary stone canal (Figs. 45K-F). From there it takes its way underneath the primary stone canal and above the *small ventral coelom* (i.e. also above the ventral lacuna)

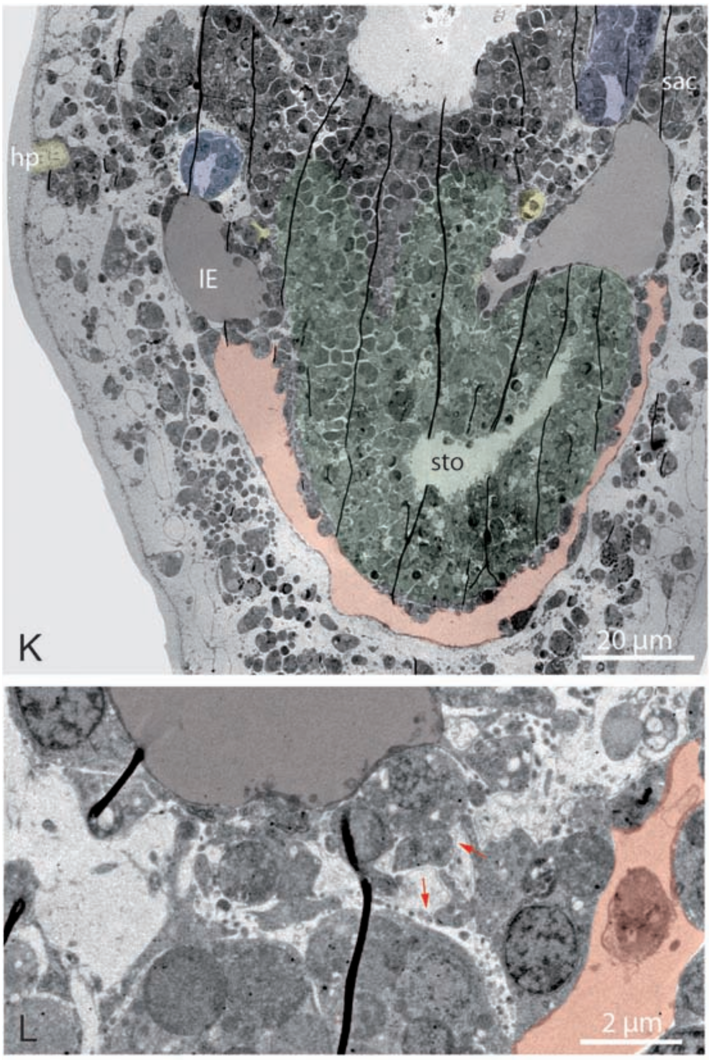


Fig. 45 part 4 of 4

and then further centralwards, where it enlarges (Figs. 45E, 45D). At the point where the axocoel passes the primary stone canal, podocytes are components of both the main axocoelomic cavity’s proximal epithelial layer (Fig. 45E) and of the axocoel’s distal layer as well, where their basal side lies against the basal side of the primary stone canal’s epithelium. The axocoel extends around the prospective esophagus towards the right side, whereby it comes to lie between the oral somatocoel and the intestinal tract (Figs. 45C-A), and achieves a considerable volume above the axial mesentery (Figs. 45D-A). The axocoel also extends a bit into the oral-most section of the axial mesentery (a bit further than shown in Fig. 45C; for axial mesentery see below). In this larva, the axocoel extends further around the esophagus than in the previous larva and becomes a slender process again on the larva’s dorsal side (Fig. 45K). Also, a second, presumably axocoelomic, process locates horizontally on the larva’s left side between the left extension of the oral somatocoel and the esophagus (Figs. 45K-G).

Both slender processes have not been traced to their end. Due to a lack of data, it could not be clarified whether the axocoel extends (1) from the hydropore once around the esophagus so that its end, coming from the left, meets the larger axocoelomic cavity underneath the primary stone canal, or whether this larger axocoelomic cavity (2) sends an (additional) outgrowth towards the left around the esophagus. Another interpretation would be that the left process is (3) independent from the axocoel, but this leaves the question for its origin unanswered. For positional reasons inferred from the serial sections, it was my strong impression that the second version has to be favored, the first version is possible and the third version speculative. In Figures 45H-J, the respective structure has therefore been colored yellow and marked with an additional question mark.

### Chambered organ

In the present larva, the tubes of the chambered organ lost contact to the aboral somatocoel so that, from now on, the organ will not be called "prospective" anymore. The tubes, which are formed by longitudinally elongated cells, are orally widened and pervade the stalk. They are surrounded by neurites (Figs. 46B, 46C).

### The axial compartment of the main body cavity forms during intestinal outgrowth

The outgrowth of the intestinal tract finds its way within the somatocoelomic mesenteries. Thereby, a new compartment is generated, which locates slightly to the right of the larva's center in the innermost bend of the winding intestinal tract between the stomach and the intestinal outgrowth. This axial area is called the "axial compartment" (Figs. 44B, 45B, 45C) in this thesis. The remaining peripheral area is referred to as the "peripheral compartment". Both areas are confluent.

As the axocoel and the hydrocoel can be identified as separate coelomic cavities, the remaining coelomic space of the main body cavity has to derive from the somatocoelomic cavities only.

In younger stages, the oral and aboral somatocoel surround the enteric sac only peripherally, forming three mesenteries, the "*oral vertical mesentery*", the horizontal mesentery and the *aboral vertical mesentery*. As we will see, a fourth mesenteric structure can be distinguished within the axial compartment from this larva (specimen 4) on, the axial mesentery, whose origin from the two somatocoelia will be shown in the following.

### Mesenteries defining the course of the oral and aboral somatocoel

The axial compartment is *orally* not limited by a hypointestinal horizontal mesenteric structure comparable to that which crosses the peripheral compartment. It is, however, pervaded by an axial mesentery that extends from the chambered organ towards the esophagus and is thereby attached to the stomach (Fig. 44B, 44G-i). In order to specify the origin of this axial mesentery and to determine the axial compartment's coelomic origin, a careful investigation of both the course and the identity of the somatocoelomic epithelial layers is initially required. Thereafter, the course of the mesenteries can be described.

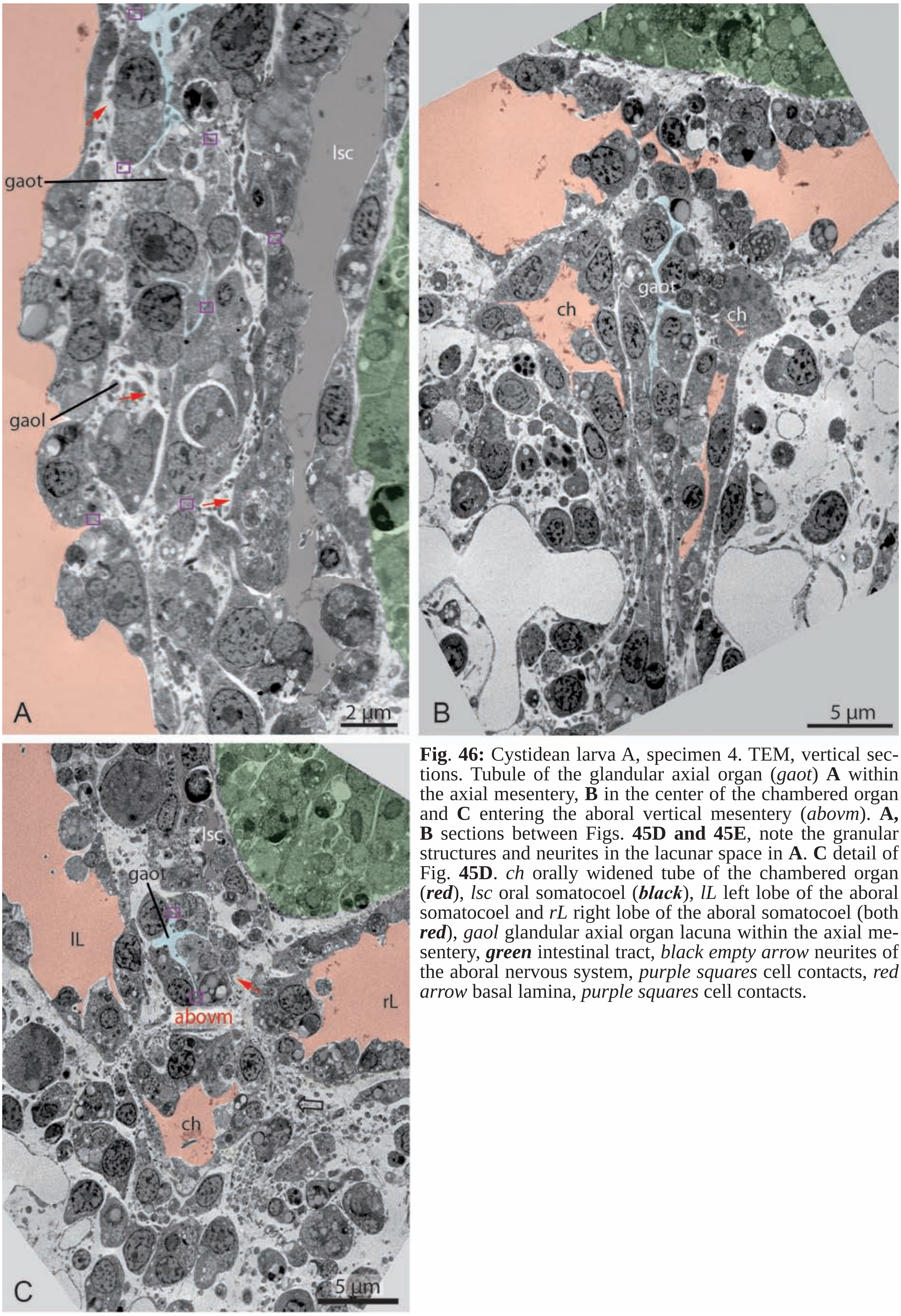


**Fig**. **46:** Cystidean larva A, specimen 4. TEM, vertical sections. Tubule of the glandular axial organ (*gaot*) **A** within the axial mesentery, **B** in the center of the chambered organ and **C** entering the aboral vertical mesentery (*abovm*). **A, B** sections between Figs. **45D and 45E**, note the granular structures and neurites in the lacunar space in **A**. **C** detail of Fig. **45D**. *ch* orally widened tube of the chambered organ (***red***), *lsc* oral somatocoel (***black***), *lL* left lobe of the aboral somatocoel and *rL* right lobe of the aboral somatocoel (both ***red***), *gaol* glandular axial organ lacuna within the axial mesentery, ***green*** intestinal tract, *black empty arrow* neurites of the aboral nervous system, *purple squares* cell contacts, *red arrow* basal lamina, *purple squares* cell contacts.

The axial compartment is lined by the following epithelial layers (as seen best in Fig. 45C): a) The oral epithelial layer of the oral somatocoel, which stretches underneath the hydrocoel, extends down into the axial compartment, forming the *central* visceral layer of the axial compartment along the esophagus (Fig. 45K) and around the *glandular axial organ*'s tubules (Fig. 45C). b) The oral somatocoelomic epithelium that forms the oral layer of the horizontal mesentery, extends centralwards around the intestinal outgrowth, forming the *distal* visceral layer of the axial compartment. The course of these two epithelial layers shows that the coelomic space of the axial compartment is continuous with the oral somatocoel. c) The aboral somatocoelomic epithelial layer that forms the aboral layer of the horizontal mesentery extends peripherally around the intestinal outgrowth, forming the visceral layer of the peripheral compartment. Aborally, it converges towards the aboralmost point of the calyx above the chambered organ. In other words, the aboral somatocoel resides aborally of the horizontal mesentery and is peripherally clasped around the winding intestinal tract, enclosing the axial compartment in its center. So, the visceral layer of the aboral somatocoel converges in the area underneath the intestinal tract, in which the tubule of the *glandular axial organ* arises, and forms the original remnant of the *aboral vertical mesentery.*

Tracing the aboral somatocoel's peritoneal layer from the larva's left side along the ventral side towards the right side shows that its left lobe extends between the stomach and the intestinal outgrowth towards the *glandular axial organ*'s tubules in the coil's center (Figs. 45A-D, 44H).

From these observations can be concluded that the intestinal outgrowth of the present larva is sandwiched by two epithelia of different coelomic origin: The oral somatocoel and the aboral somatocoel. The distalmost section of the intestinal outgrowth is surrounded by three different epithelia: (1) orally it is flanked by the oral somatocoel and (2) peripherally it is accompanied by the right lobe of the aboral somatocoel and (3) centrally by the aboral somatocoel's left lobe (Fig. 45A). Between the two lobes resides a mesentery that extends aboralwards from the intestinal outgrowth and is continuous with the *aboral vertical mesentery* underneath the intestinal tract. As it is formed by aboral mesenteric layers only but has its own developmental fate (as will be shown), it is named the *secondary aboral vertical mesentery* in this thesis, in order to distinguish it from the rather original part underneath the intestinal tract. The proximal section of the intestinal outgrowth is, contrary to the distal section, clasped between the axial extension of the oral somatocoel and the peripheral aboral somatocoel and resides in a structure topologically corresponding to the horizontal mesentery (Fig. 45C).

On the side towards the coil's center, the epithelial sheath of the *glandular axial organ* is formed by the oral somatocoel. There is, however, no fully intact continuous vertical mesenteric boundary between the left lobe of the aboral somatocoel and the axial compartment's oral somatocoelomic lining (white cross in Fig. 45B). As there is no fully intact vertical mesenteric boundary between the left lobe of the aboral somatocoel and the axial compartment's oral somatocoel, it is, strictly speaking, not unequivocally clear how far the former's lining epithelium within the axial compartment extended before the fusion occurred. However, at least part of the aboral somatocoel's left lobe's epithelium contributes to the *glandular axial organ*'s epithelial sheath (Fig. 45C). Therefore, the border of the aboral somatocoel within the axial compartment must have been located somewhere along the *glandular axial organ* or the *glandular axial organ* itself actually *represents* the remnant of this border.

Hence, the epithelial sheath of the *glandular axial organ* is hypothesized to be the axial mesentery, which is formed by the epithelial layers of the oral somatocoel (on the side towards the coil's center) and the aboral somatocoel (on the side towards the coil's opening) – a characteristic which it shares with the horizontal mesentery.

From this description finally follows that there are two mostly intact mesenteric structures that run along the intestinal outgrowth and extend in two opposite directions from it to the body wall (see also Fig. 44G): the horizontal mesentery and the *secondary aboral vertical mesentery*. The **horizontal mesentery** resides on one level with the oralmost border of the spiralized intestinal tract. Extending between the intestinal tract and the body wall, it spans the peripheral compartment. Starting at the tip of the intestinal outgrowth in counterclockwise direction, it runs along the whole larva's right, dorsal, and left side. It is slightly ruptured on the ventroright side, but its identity can never be doubted. The **secondary aboral vertical mesentery** extends somewhat vertically from the aboral side of the intestinal outgrowth towards the body wall. At the intestinal outgrowth's tip, it originates close to both the horizontal mesentery and the *"oral vertical mesentery"* and runs from the tip of the intestinal outgrowth in counterclockwise direction. It can be traced to the aboralmost point of the intestinal tract. Here, the *secondary aboral vertical mesentery* splits up into the **aboral vertical mesentery**, which extends towards the aboralmost end of the main body cavity, and the **axial mesentery**, which extends oralwards within the axial compartment and covers the tubules of the *glandular axial organ* that are embedded in the *glandular axial organ* lacuna. The *"oral vertical mesentery"* forms between the two ventral ends of the oral somatocoel (Fig. 44K).

### Glandular axial organ

The *ecm* within the axial mesentery is filled and crossed by granules and neurites, some of which have been observed to derive from the somatocoelothel (Fig. 46A). Within this *ecm*, an axially elongated tubular cavity is embedded, that consists of an epithelium of large squamous to cuboidal monociliated cells that direct their cilia into the tubule‘s center and are apically connected by adherens junctions, so that the cells' apical sides are directed inwards. The tubule's outside is covered by a basal lamina. The cells can frequently be observed being contacted by basal processes sent out by cells of the axial mesentery's epithelium, which probably represent synapses. In the axial mesentery's oral half, the lumen of the cavity is a bit wider than in the aboral half. The tubule can be traced aboralwards passing the *aboral vertical mesentery* underneath the intestinal tract and further down entering the space between the chambers of the chambered organ in the stalk (Fig. 46B). When passing the *aboral vertical mesentery*, the structure and position of the tubule equal that of the prospective tubule of the *glandular axial organ* observed in specimen 2. As already stated, it is my impression that the axocoel orally extends into the axial mesentery for some way.

### Small ventral coelom and prospective ventral lacuna

Ventrally, to the left of the oral somatocoel’s right extension and right above the aboral somatocoel’s right lobe resides a small horizontally flattened epithelially lined cavity (Figs. 44J, 44K, 45E-J). It locates underneath the merging area of the primary stone canal and the axocoel. The cells of the *small ventral coelom*’s aboral layer are cuboidal to squamous and densely packed; the cells of the oral layer are squamous. There are no podocytes in the epithelium of the *small ventral coelom*.

The cells on the right side of the *small ventral coelom* form cell contacts with the aboral somatocoel’s nearby cells (Figs. 45E, 45F). These cell contacts resemble those cell contacts that are identified as adherens junctions in other epithelia. In this area, both epithelia are jointly ensheathed by a continuous basal lamina. Towards the left side, the *small ventral coelom* detaches from the aboral somatocoel and a semi-isolated *ecm* area, the ventral lacuna, opens between these cavities (Figs. 45F-i). This cleft extends from distal to proximal when traced towards the left. Underneath the cavity, cells of the *small ventral coelom*’s aboral layer form basal processes that reach into this space. The processes either meet each other underneath the cavity, or extend farther and contact the aboral somatocoel (Fig. 45F). Here, a continuous basal lamina lines the ventral lacuna’s extra-cellular space, extending around the cell process as well. Further left, the *small ventral coelom* flattens horizontally and finally forks into two separate short lobes, a distal and a proximal one, as it is kidney dish- to banana-shaped (Fig. 45H). The more distally situated lobe ends underneath the merging area of the primary stone canal and the axocoel, from which it is clearly separated by their basal laminae. The proximal one spans towards the end of the oral somatocoel's left extension. Here, the epithelium of the *small ventral coelom* establishes cell contacts with both the oral somatocoel and with the aboral somatocoel. The latter cell contacts, again, resemble those that are identified as adherens junctions in other epithelia (Fig. 45i). Also, the basal lamina extends continuously around the respective cavities.

### Cluster of large epithelial cells

To the left of the *small ventral coelom*, at its oral margin, the epithelium of the aboral somatocoel’s left lobe consists of a few very large cells (Fig. 45J). These cells are associated with a comparatively well developed basiepithelial plexus, which again is interconnected with basiepithelial structures of the adjacent cells of the oral somatocoel’s left extension's end.

### Body wall

The body wall of the larva’s calyx consists of a thick *ecm* into which mesenchymal cells are embedded and an epidermal layer of tack-like cells, whose flattened apical parts direct micro-

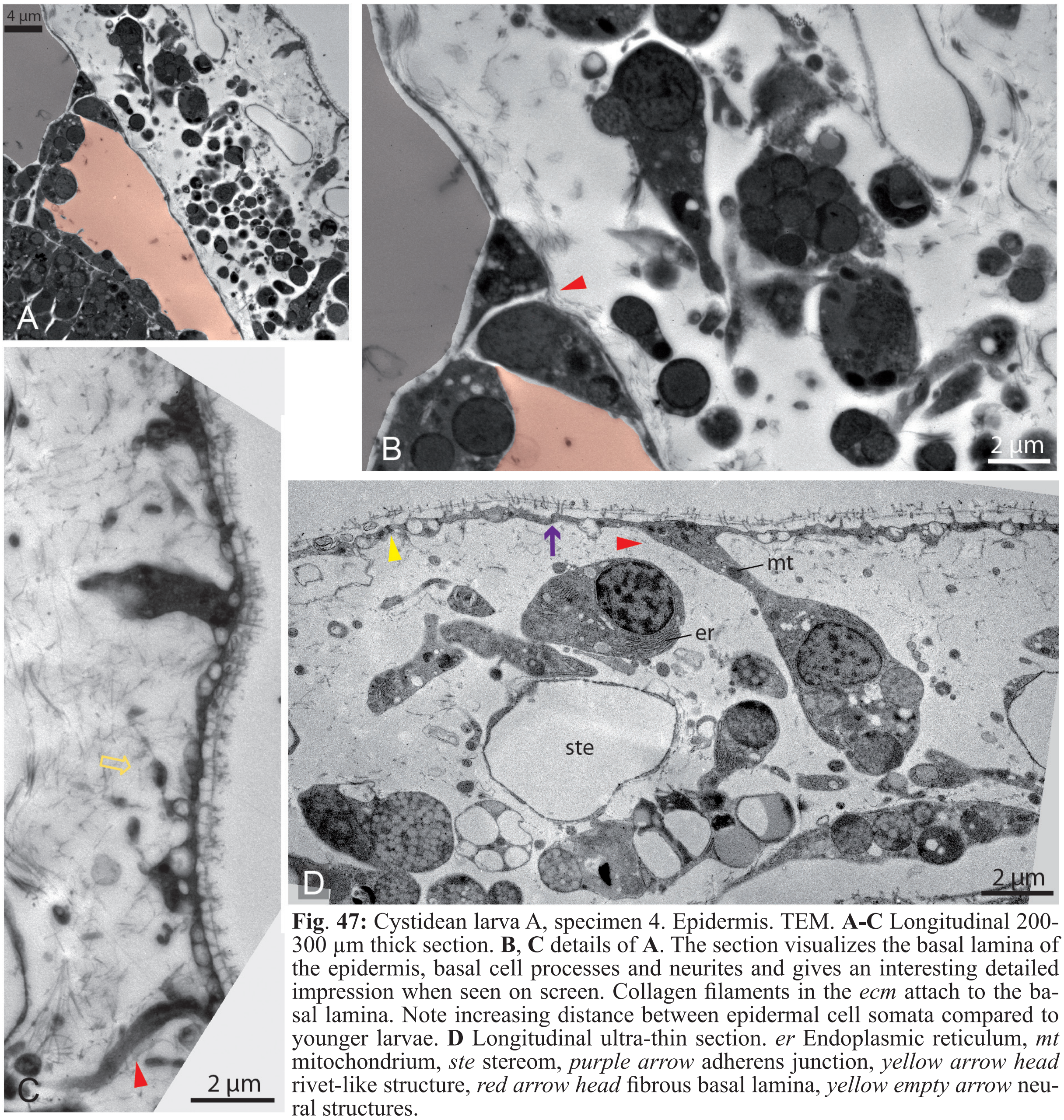


**Fig. 47:** Cystidean larva A, specimen 4. Epidermis. TEM. **A-C** Longitudinal 200-300 µm thick section. **B**, **C** details of **A**. The section visualizes the basal lamina of the epidermis, basal cell processes and neurites and gives an interesting detailed impression when seen on screen. Collagen filaments in the *ecm* attach to the basal lamina. Note increasing distance between epidermal cell somata compared to younger larvae. **D** Longitudinal ultra-thin section. *er* Endoplasmic reticulum, *mt* mitochondrium, *ste* stereom, *purple arrow* adherens junction, *yellow arrow head* rivet-like structure, *red arrow head* fibrous basal lamina, *yellow empty arrow* neural structures.

villi to the outside (Fig. 47). On the microvilli resides a delicate cuticle. The tack's somata are far distant from each other; their gaps are filled with *ecm*. The tack's heads are underlain with a varyingly prominent basal lamina while their pins, the cell somata, may be directly embedded in the body wall's *ecm*. TEM of a 200-300 µm thick section, however, revealed that single somata may possess a prominent basal lamina as well (Fig. 47C). Some of the cells possess basal processes, whose targets have not been investigated. The area between the somata, which comprises approximately the distal half of the body wall, is largely free from cell bodies. This holds true for both calyx and stalk. Skeletal elements can be found in the center of the body wall in the calyx and in the center of the stalk. In the calyx, the basal parts of the epidermal cell somata are frequently directed through the mesh-holes of the sclerites. The mesenchymal cells *sensu strictu* reside mainly underneath the sclerites (Fig. 47D). In the stalk, mesenchymal cells are located in the mesh holes of the columnals and are densely packed between the columnals and the epidermal cell somata (Fig. 45D). Apart from the usual composition of cell organelles described for doliolaria epithelial cells, the mesenchyme cells contain a prominent rough Endoplasmic reticulum. Some of these cells, which are distributed irregularly between the common mesenchymal cells, contain bullet shaped inclusions. On one level with the hydrocoel, sacculi are present in the body wall (Fig. 45K).

#### Vestibular cavity

The vestibular cavity is closed and has a prominent voluminous size (Figs. 45A, 45D). Its distal layer, bordering the oral body wall's basal side, consists of an unciliated, unmicrovillized squamous epithelium, while the proximal layer, forming the "outer" layer of the future podia and the linings of the stomodaeum, consists of a high columnar epithelium of monociliated cells, whose microvilli are covered by a cuticle.

### 3.5.2 Cystidean larva B (Specimen 5)

#### General morphology

The other (more advanced) larva that mainly contributed to reconstruction Figure 44, is slightly further developed and has been cut longitudinally into semi-thin sections, from the ventral to the dorsal side (Fig. 48). The larva's vestibular cavity is apically closed and 25 podia reach into it. The larva has one hydropore but neither an anus nor a mouth. The hydrocoel forms a ring. The axocoel, the oral somatocoel and the aboral somatocoel are largely fused, especially in the axial compartment and on the ventro-right side.

#### Vestibular cavity

The vestibular cavity extends aborally between the 25 podia in direction to the intestinal tract, forming the stomodaeum (Figs. 44A, 44B). The roof of the vestibular cavity is lined by a monociliated squamous epithelium, while podia and the stomodeal area are covered by monociliated cuboidal and palisade cells, respectively. The stomodeal cells and the proximal side of the podia form microvilli, on which a cuticle resides. The roof of the vestibular cavity and the distal side of the podia are not, or are significantly less, lined by these structures.

#### Intestinal tract

The intestinal tract is filled with yolk cells and takes a course similar to that of the cystidean larva A (Figs. 44A, 48): Starting with the stomodeum (the future esophagus) the intestinal tract first descends aborally, buckles and grows out on the right side coiling in clockwise direction. Seen from above, the intestinal outgrowth is a bit longer than in the previous larva. Intestinal tract and epidermis did not fuse to form the anus, yet.

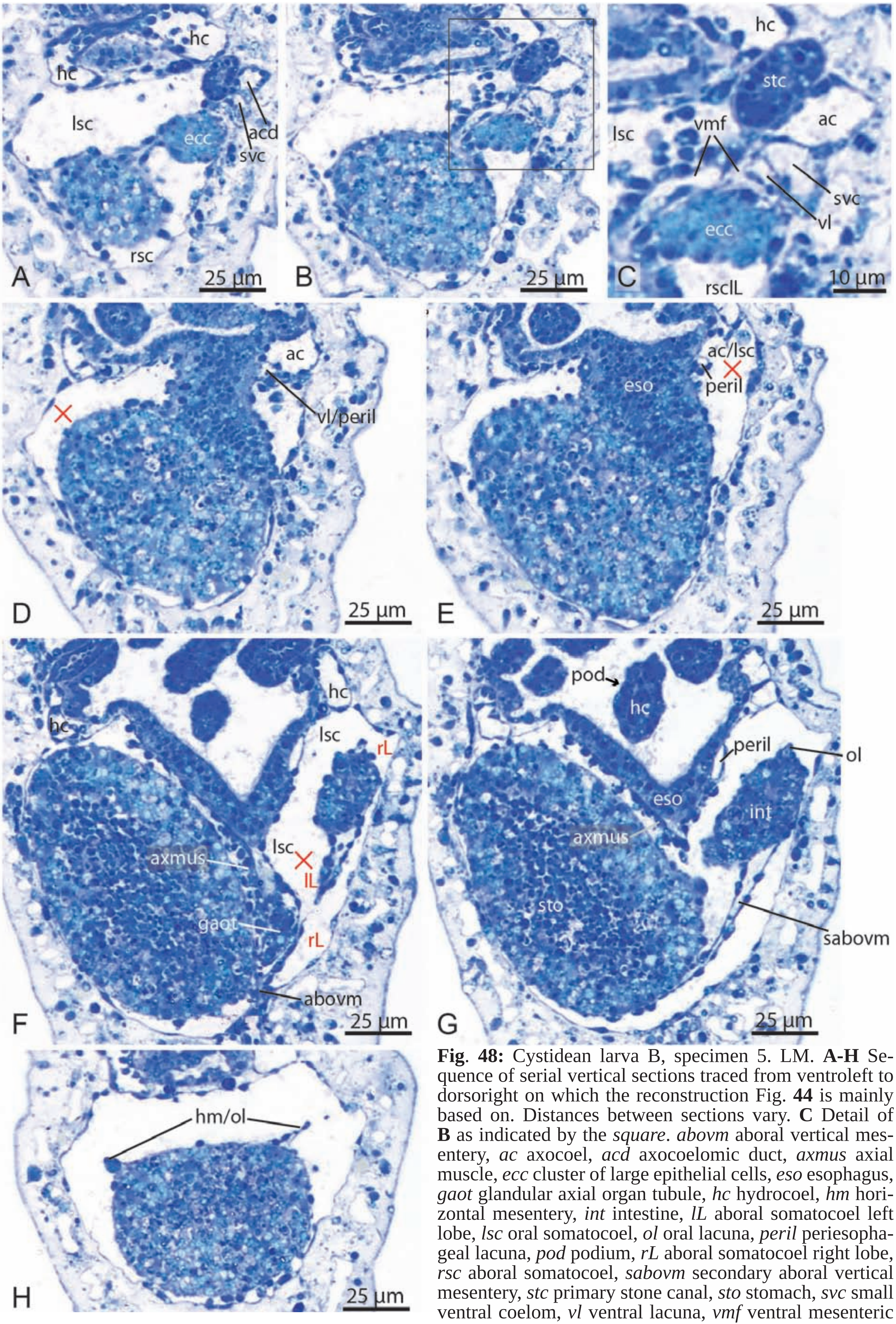


**Fig**. **48:** Cystidean larva B, specimen 5. LM. **A-H** Sequence of serial vertical sections traced from ventroleft to dorsoright on which the reconstruction Fig. **44** is mainly based on. Distances between sections vary. **C** Detail of **B** as indicated by the *square*. *abovm* aboral vertical mesentery, *ac* axocoel, *acd* axocoelomic duct, *axmus* axial muscle, *ecc* cluster of large epithelial cells, *eso* esophagus, *gaot* glandular axial organ tubule, *hc* hydrocoel, *hm* horizontal mesentery, *int* intestine, *lL* aboral somatocoel left lobe, *lsc* oral somatocoel, *ol* oral lacuna, *peril* periesophageal lacuna, *pod* podium, *rL* aboral somatocoel right lobe, *rsc* aboral somatocoel, *sabovm* secondary aboral vertical mesentery, *stc* primary stone canal, *sto* stomach, *svc* small ventral coelom, *vl* ventral lacuna, *vmf* ventral mesenteric formation, *red cross* position of dissolved mesentery between left and right somatocoel.

### Hydrocoel

Contrary to the previous larvae, the hydrocoel of the present larva forms a fully closed ring around the oral part of the stomodaeum (Figs. 44A, 44F). To the oral side, it extends into the 25 podia, forming their inner lining. Those (primary) podia that showed up first are the largest, they define the later position of the radii. Between each two neighboring primary podia, four smaller podia reside, so that five groups of five podia can be distinguished (Fig. 44F). The primary stone canal locates in interradius CD, emerging from the hydrocoel ring in aboral direction directly underneath the leftmost podium of the quintet in radius C. It passes the body wall and opens into the axocoel. The hydrocoel ring is horizontally traversed by trabeculae.

### Mesenteries, lacunae, trabeculae

As in the previous larva, there are two mesenteric structures that run along the intestinal outgrowth and extend in two opposite directions from the intestinal outgrowth to the body wall; the horizontal mesentery and the *secondary aboral vertical mesentery*. In addition, there are two axial components, the *aboral vertical mesentery* and the axial mesentery, in which the tubule of the *glandular axial organ* ascends (Figs. 44G, 44i, 48).

The horizontal mesentery resides on one level with the oralmost border of the spiralized intestinal tract and spans the peripheral compartment. It attaches the last segment of the intestinal outgrowth to the body wall on the ventroright side. Following the intestinal tract in counter-clockwise direction, the horizontal mesentery locally dissolves and a lacunar space can clearly be seen at the mesentery's original site alongside the outer margin of the intestinal outgrowth. The lacuna is formed between the intestinal epithelium and the visceral layer of the somatocoel(ia). On the larva's approximate dorsal to left side, the horizontal mesentery is nearly completely reduced and the related lacunar space is less prominent. On the larva's ventroleft side, the horizontal mesentery is, again, clearly preserved for a short distance on one level with the intestinal tract's oralmost end and finally becomes the *ventral mesenteric formation*. The horizontal mesentery does not extend further to the right into the axial compartment and it does not meet the axial mesentery.

Interpreting the lacunae as mesenteric remnants, the horizontal mesentery extends similarly as the one in the previous larva.

The *secondary aboral vertical mesentery* runs on the larva's right side from the tip of the intestinal outgrowth to the aboralmost point of the intestinal tract. It continues in the *aboral vertical mesentery*, which extends from the aboral side of the intestinal tract towards the body wall and forms the aboralmost epithelial sheath of the *glandular axial organ*'s tubule. The *aboral vertical mesentery* is clearly formed by the visceral aboral somatocoelomic epithelial layer only.

Based on the same observations a) - c) *et seq*. concerning the course and the identity of the somatocoelomic epithelial layers that were explained for the previous larva (p. 92), the coelomic space of the axial compartment is continuous with (and partially consists of) the oral so-

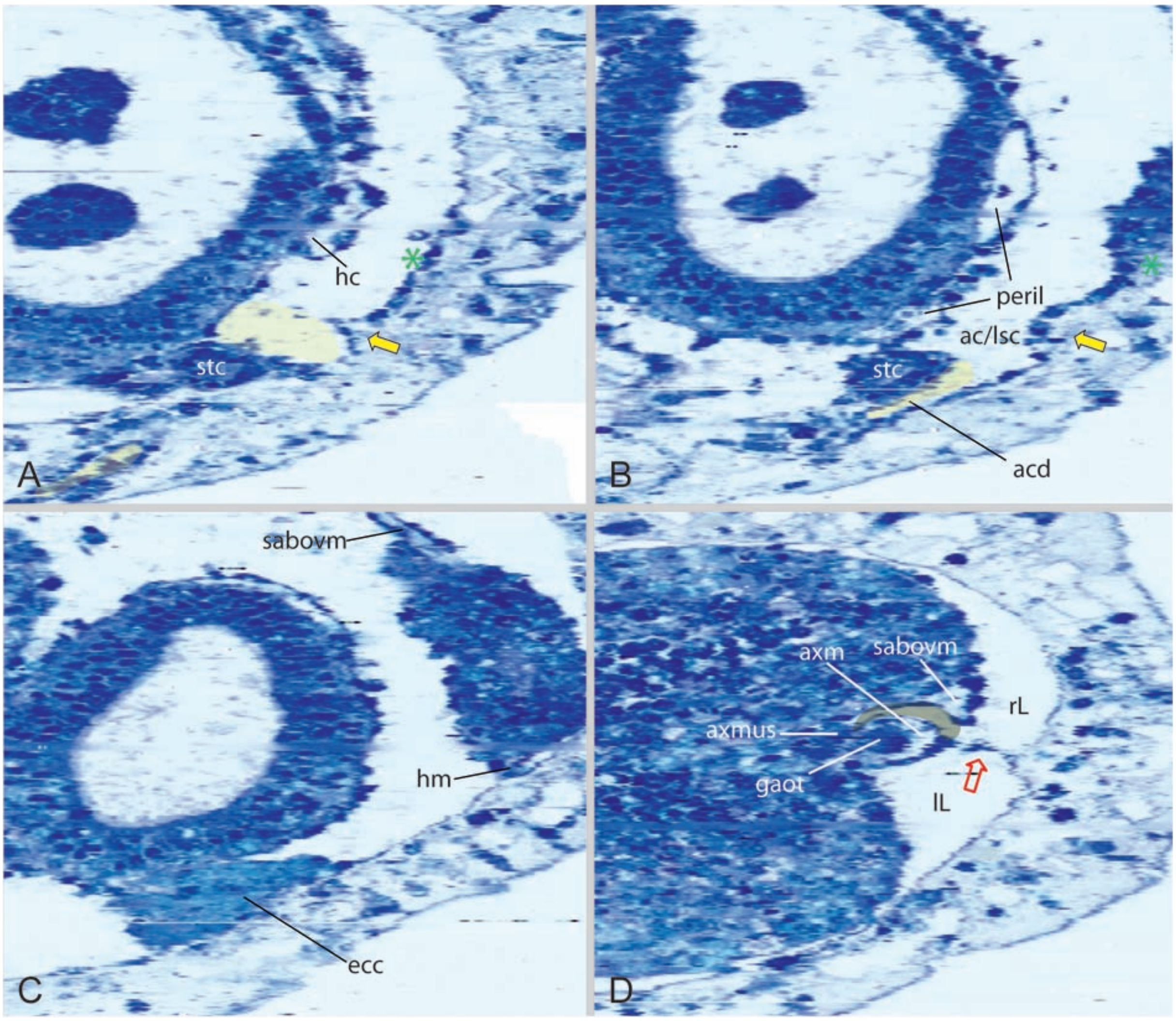


**Fig. 49:** Cystidean larva B, specimen 5. LM. **A-D** Horizontal views of the sequence of serial vertical sections presented in Fig. **48** (generated with ImageJ). **A**, **B** Note the body wall dent (*yellow arrow*) at the point, where the axocoelomic duct (*acd*) opens to the oral somatocoel (*lsc*) and note the mesenteric remnants that extend from it towards the esophagus. These structures presumably indicate the former border of the axocoel (*ac*) as marked in ***yellow***. **C** Impression of the horizontal extent of the cluster of large epithelial cells (*ecc*) along the oral margin of the aboral somatocoel's left lobe (compare Figs. **40F**, **44K**, **48C**, **51M**, **54J**). **D** View onto that horizontal level, on which the extent of the axial extension of the oral coelom (***green-black***) and the two lobes of the right somatocoel (*rL* right lobe, *lL* left lobe) can be depicted best. Below that level, the mesentery marked with the *red empty arrow* is in contact with both the axial mesentery (*axm*) and the secondary aboral vertical mesentery (*sabovm*) and continues to the aboralmost point of the main body cavity; above this level, it connects with and actually becomes the *sabovm* only. *axmus* axial muscle, *gaot* glandular axial organ tubule, *hc* hydrocoel, *hm* horizontal mesentery, *peril* periesophageal lacuna, *stc* primary stone canal, *green asterisk* location of the anus.

matocoel in the innermost intestinal coil. In the present larva, the oral somatocoelomic-lined part of the axial compartment does not meet the main body cavity's aboralmost end, but reaches down to the aboralmost point of the stomach, being axial compartment-outwards limited by the axial mesentery (Figs. 48F, 49D).

Thus, the bend-inwardly directed (inner) layer of the axial mesentery is of oral somatocoelomic origin and the bend-outwardly directed (outer) one is covered by the epithelial layer of the aboral somatocoel's left lobe.

The axial mesentery can be radially subdivided into a *glandular axial organ* part and a (very stout and comparatively wide) mesenteric part, which connects the former to the intestinal tract. The inner layer of the present larva’s mesenteric part contains an axial muscle strand that extends from the prospective esophagus to the body wall (Figs. 44D, 48F, 48G, 49D). The axial muscle strand is anchored in the body wall between the chambers 5 and 1 (corresponding to interradius BC).

In addition to all these mesenteries and lacunae, few trabeculae are formed randomly between the somatocoel’s visceral and peritoneal layer on several horizontal levels throughout the aboral somatocoel.

### Glandular axial organ

Close to the bend of the intestinal tract, the axial compartment is axially crossed by the *glandular axial organ*, which consists of one tubule that is embedded in the lacunar *ecm* and an axial mesenteric epithelial sheath, the axial mesentery, that surrounds the matrix together with the tubule. The tubule rises up in the calyx and ends halfway of the intestinal tract. Further orally, in its oral section, the axial lacunar space decreases in size, becomes closely attached to the esophagus, and is filled by *ecm* only (Figs. 44i, 48F). Down the stalk, the tubule extends into the central space of the chambered organ. Right underneath the chambered organ’s widened part, it transforms into an axial cord of fusiform axially elongated cells.

### Ventral mesenteric formation and the cluster of large cells

The structure of the horizontal mesentery’s ventroleft end differs from its other preserved areas. The two involved neighboring epithelial layers, that of the oral somatocoel’s left extension and that of the aboral somatocoel’s left lobe, do not lie against each other but are separated by a comparatively prominent *ecm* layer (Fig. 48C), which gives the oral lacuna a slightly vertical dimension.

As we will see, this vertical characteristic will be of major descriptive importance in older stages. As this structure constitutes no distinct mesentery but a part of the oral lacuna, I name this structure the *ventral mesenteric formation*.

In the present larva, the *ventral mesenteric formation* is associated with a prominent cluster of very large cells (Figs. 44G-K). According to light microscopic data, these cells are part of the oralmost lining of the aboral somatocoelomic epithelium (Figs. 48A-C, 49C). Being stained with Toluidin blue, their vast cytoplasm is stained turquoise. Their large nuclei contain one or two large nucleoli and reside in a more distal position within the cells of the cluster.

### Small ventral coelom

Right underneath the point where the primary stone canal opens into the axocoel, within the *”oral vertical mesentery”* and dextro-oral to the *ventral mesenteric formation* and the cluster of cells just described, a distinct epithelially lined cavity can be found that accompanies the axocoel for a short distance (Fig. 48A-C). This *small ventral coelom* is neither continuous with the axocoel nor with the oral somatocoel. The aboral lining of the cavity sinks into the cluster of large epithelially organized cells.

The *small ventral coelom* sends cell protrusions with which it forms a semi-isolated *ecm* area, the ventral lacuna, which resides within the *ecm* of the *ventral mesenteric formation* (Fig. 48C). This ventral lacuna continues in the periesophageal lacuna.

**Axocoel and periesophageal lacuna**

The hydropore resides a bit left to the ventral side and a bit oral to the oral somatocoel (Fig. 44K). From there, the axocoel passes the body wall towards the primary stone canal, which opens into the axocoel. To the left of the intestinal outgrowth's tip, the axocoel then enlarges and merges with the oral somatocoel's right extension (Figs. 44C, 44E, 49B). Since the horizontal mesentery is dissolved in this area as well, the aboral somatocoel cannot be distinguished from the oral somato-axocoelomic area (Fig. 48E).

A periesophageal lacuna originates from the ventral lacuna within the *ventral mesenteric formation's ecm* in counterclockwise direction. The lacuna extends around the stomodeum on the larva's right side and merges with the matrix space of the *glandular axial organ* on the dorsoright side.

## 3.6 Pentacrinoid stages

Light- and scanning electron microscopic photographies of stalked pentacrinoid stages show how the development of the radial symmetry, which externally starts to become visible with the opening of the vestibular cavity and under formation of five interradial oral valves, proceeds (Figs. 5L-U, 6H-Q). In its oral part, the larva develops five radial arms that bud off to the sides. The slits of the oral valves form on the radii, too. A radially directed food groove develops on the arm's oral side, originating from the mouth and passing through these slits (Fig. 6Q). Very early, the arms split off into two branches and, later, increase the length and the size of both the branches and the proximal unbranched segment. In all pentacrinoid larvae examined, twenty podia stand around the mouth, four between each food groove's origin (as in the juvenile Fig. 7B). The remaining five (radial primary) podia are integrated in the newly formed arms (Hyman 1955) - the hydrocoelomic extension within these podia is the fundament for the radial water canals within each arm.

Starting proximal to the arm‘s branching point, podia develop on the oral side of the arms on both sides along each food groove. Following the outgrowth of the arms, the radial cirri bud off right underneath the calyx on one level with and in the radius of the chambers of the chambered organ. As the arms stretch out to the side and the calyx‘ radius increases (while the oral valves‘ size remains about the same), the tegmen flattens and enlarges its diameter during pentacrinoid stage. In effect, the food grooves expand as well.

During pentacrinoid stage, the anus changes its position within the interradius CD from a marginal to the tegmenal side of the larva and an anal tube develops. The primary hydopore also relocates to the calyx‘ tegmenal side. Towards juvenile stage, four additional pores, the madrepores, develop in interradial positions on the tegmen.

Serial semi-thin sections of two pentacrinoid larvae (specimen 8 and 9) have been examined. In these, special attention has been given to both the ongoing fusion processes in interradius CD and to the structure of the axial mesentery, as well as to the development of the somatocoelomic cavities in the arms.

### 3.6.1 Pentacrinoid larva A (Specimen 8)

The pentacrinoid (Fig. 5P) was cut into serial semi-thin horizontal sections (Fig. 50). The hydrocoel of this larva is closed to a ring and its anus opens to the exterior. The horizontal mesentery is dissolved, so that the oral and aboral somatocoel fuse and (together with the axocoel) form the main body cavity; instead of the horizontal mesentery, an oral lacuna runs along the intestinal tract’s disto-oral margin. The *secondary aboral vertical mesentery* largely persists and extends vertically from the aboral side of the intestine to the body wall. As in the previous larvae, the *glandular axial organ* tubule ascends in the *aboral vertical mesentery* and further orally in the axial mesentery, in whose inwards directed layer the axial muscle is formed (Fig. 52).

#### Axocoel, ventral mesenteric formation and small ventral coelom

In the present larva, the anus locates to the side of the calyx in interradius CD on one level with the *small ventral coelom* (Fig. 50B). Proximal to the anus, the body wall is thicker than in the other radii and it extends somewhat raggedly towards the esophagus (*yellow arrow* in Fig. 50B). The right side of this thickened body wall area points towards the right in direction of the area between the esophagus and the last section of the intestine.

The hydropore, which connects the axocoel to the exterior, resides laterally on one level with the hydrocoel between interradius CD and radius D. From here, the axocoelomic duct runs counterclockwise and slightly aborally, merges with the primary stone canal (around Fig. 51G) and merges with the fused oral-aboral somatocoelomic region of the main body cavity above the point where the periesophageal lacuna becomes the ventral lacuna within the *ventral mesenteric formation* (Figs. 51C-i). The latter is a vertically elongated *ecm* area that extends between the body wall and the periintestinal *ecm* (Figs. 51H-L). It resides to the left of the merging area between the axocoelomic duct and the main body cavity. The ventral lacuna locates in the *ventral mesenteric formation*’s distal section (Figs. 51H-K). Above (and below) the *ventral mesenteric formation*, the main body cavity forms a continuous ring around the esophagus (above: Fig. 50A).

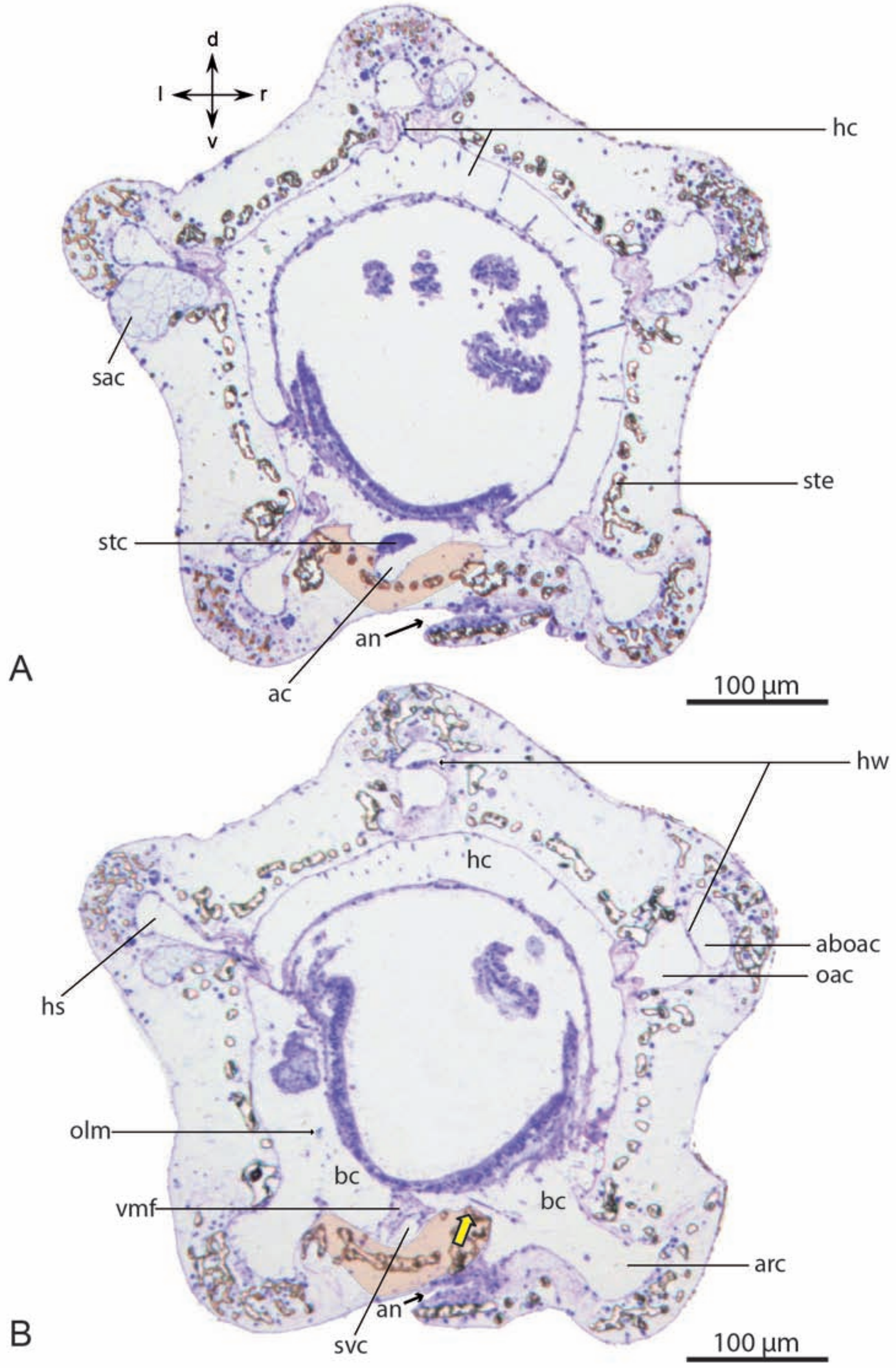


**Fig**. **50:** Pentacrinoid larva A, specimen 8. Overviews. LM. Cross-sections of the larva through **A** the hydrocoel (*hc*) and **B** the oralmost region of the main body cavity (*bc*). The area homologous to the body wall protrusion (*bwp*) of the juvenile is colorized in ***skin color***. *aboac* aboral arm coelom, *an* anus, *arc* arm coelom, *ac* axocoel, *hs* horizontal septum, *hw* horizontal wall, *oac* oral arm coelom, *olm* oral lacunar meshwork, *sac* sacculus, *stc* primary stone canal, *ste* stereom, *svc* small ventral coelom, *vmf* ventral mesenteric formation, *yellow arrow* dent in thickened body wall area marking the right end of the axocoelomic cavity.

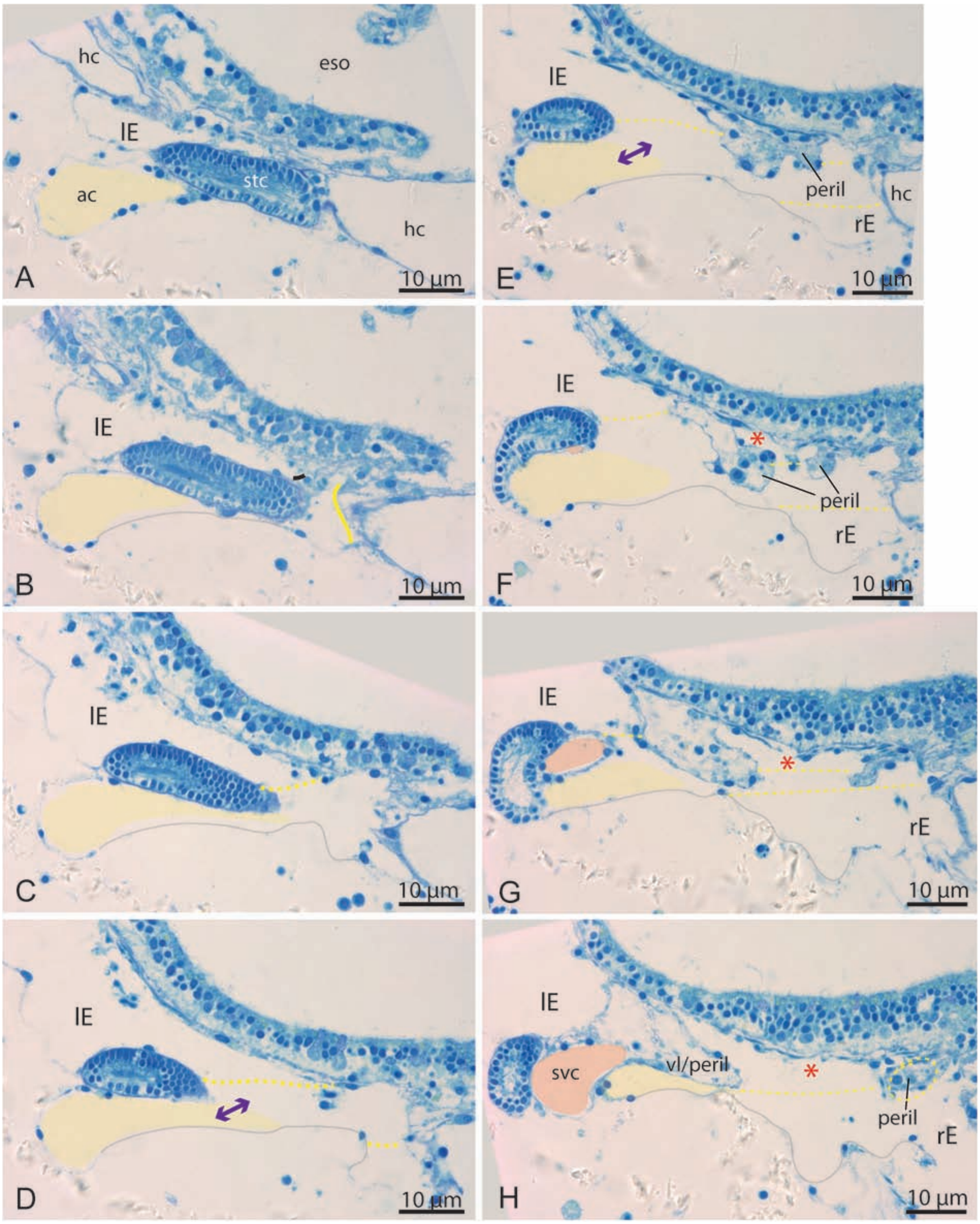


**Fig. 51 part 1 of 2**

The primary stone canal emerges from the closed hydrocoel ring in interradius CD, extends slightly aborally in clockwise direction, bends distally and gets in open contact to the axocoel (Figs. 51A-H). From within the primary stone canal's bend, a *small ventral coelom* extends aboralwards towards the end of the *ventral mesenteric formation* (Figs. 51F-K).

Tracing the ventral lacuna from aboral to oral (Fig. 51K-H), it extends from the *small ventral coelom*'s proximo-left side into the *ventral mesenteric formation*'s distal section. At the latter's

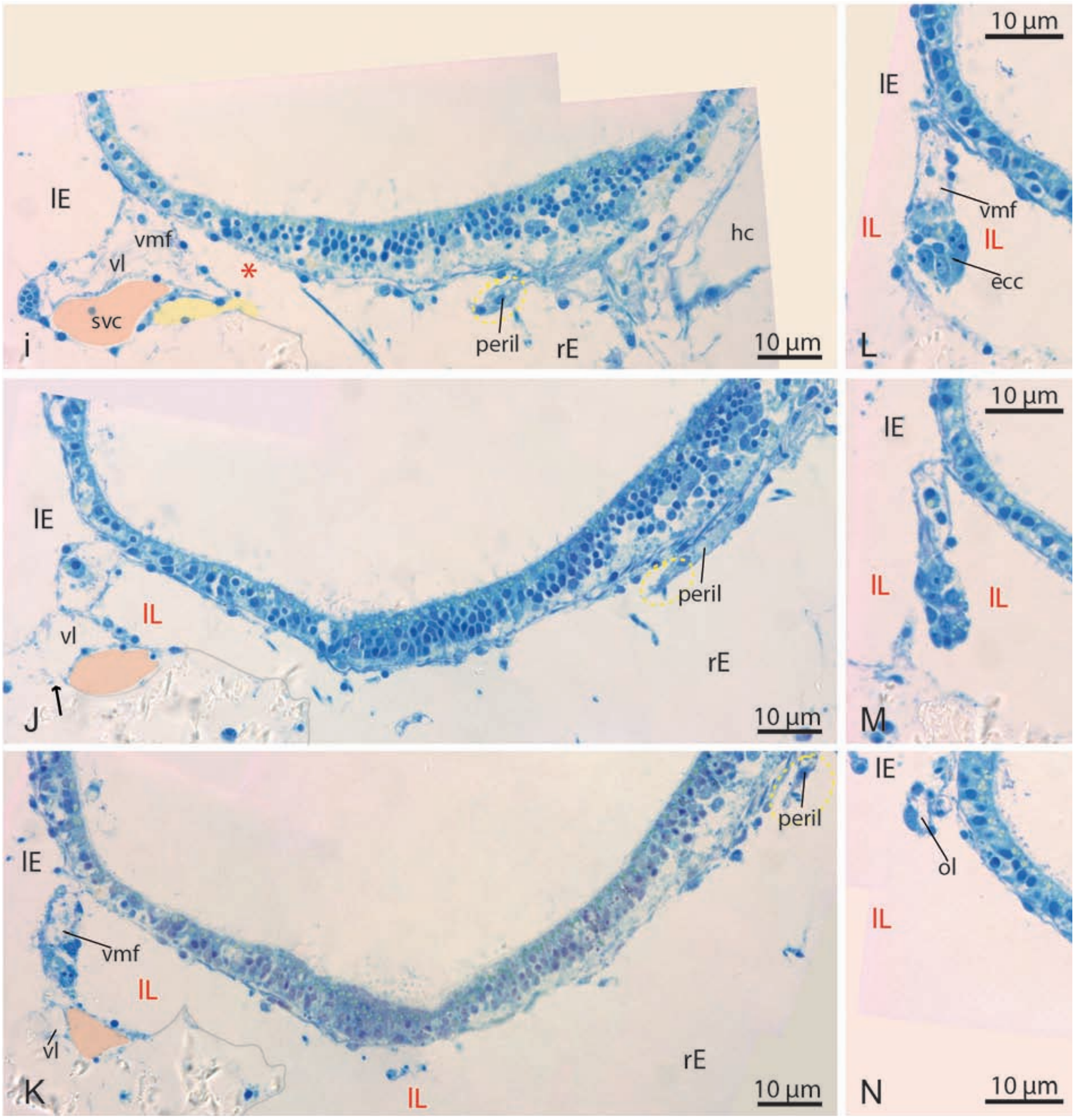


**Fig. 51 part 2 of 2**

**Fig**. **51 (2 parts):** Pentacrinoid larva A, specimen 8. LM. Series of semi-thin cross-sections through the ventral region in interradius CD, traced from oral to aboral. Distances between sections vary. **A-N** The somatocoelia and the axocoel are fused and form the main body cavity. Assignment of coelomic origins of its regions indicated is an approximation based on the markers given in Table 3 (p. 152). That part of the axocoel that crosses the body wall (=axocoelomic duct) is coloured in ***yellow***, the presumed former course of the axocoel is indicated with *dotted yellow lines*. *ac* axocoel, *ecc* cluster of large epithelial cells, *eso* esophagus, *hc* hydrocoel, *lE* left extension of the oral somatocoel, *lL* left lobe of the aboral somatocoel, *ol* oral lacuna, *peril* periesophageal lacuna, *rE* right extension of the oral somatocoel, *stc* stone canal, *svc* small ventral coelom (***orange***), *vl* ventral lacuna, *ol* oral lacuna, *vmf* ventral mesenteric formation. *black arrow* cellular protrusions sent out by cells of the small ventral coelom, *purple double arrow* area in which the axocoelomic duct fuses with the main body cavity, *red asterisk* ventrally open space underneath the periesophageal lacuna, which has to be interpreted as belonging to the left lobe of the aboral somatocoel, correlating to a region marked with a *red asterisk* in Fig. **45E**. The interpretive content of these figures is referred to in the discussion.

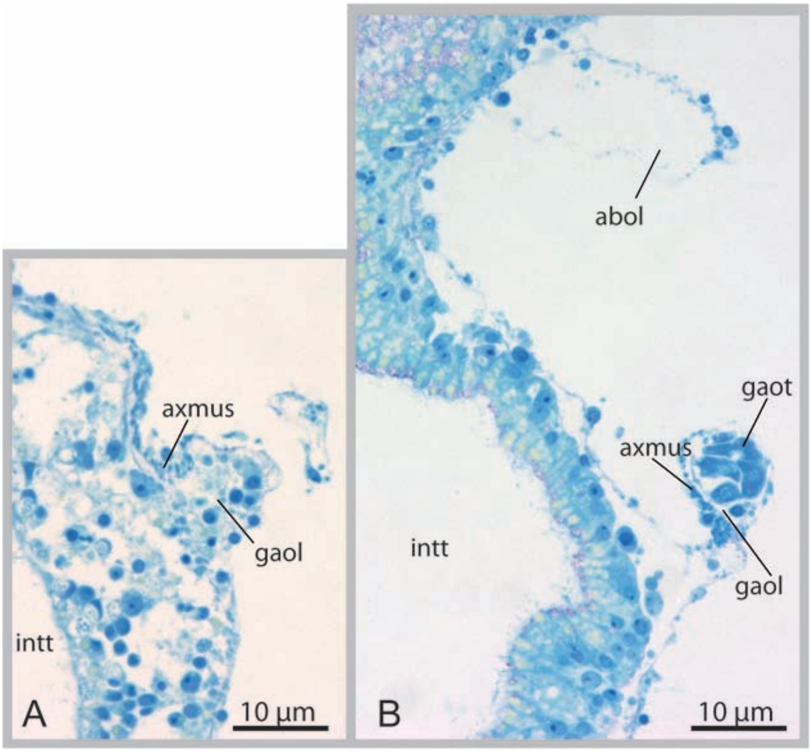


**Fig**. **52:** Pentacrinoid larva A, specimen 8. LM. Cross-sections through the glandular axial organ. **A** Oral section. **B** Central section close to the aboral end of the intestinal tract (*intt*). *abol* aboral lacuna, *axmus* axial muscle, *gaol* glandular axial organ lacuna, *gaot* glandular axial organ tubule.

oral end, the ventral lacuna is continuous with the periesophageal lacuna, which can be traced along the right side of the esophagus (Figs. 51F-K) until it merges with the *glandular axial organ* lacuna's oral end (Fig. 52A, merging not shown). So, on nearly its whole extent, the ventral lacuna is accompanied by the *small ventral coelom*. As in the previous stages, the latter is directly involved in ventral lacuna formation, as it sends cellular protrusions around it, forming a semi-isolated area within the *ecm* of the *ventral mesenteric formation* (Fig. 51J).

Underneath the ventral lacuna, *the vertical mesenteric formation* transforms into an epithelially organized mass of densely packed large cells that possess large nuclei (Figs. 51K-M). Based on light microscopic data, the vast majority of these cells seem to be epithelial in nature. Ultrastructural data, however, were necessary to verify this observation. Beneath the cell mass, the *ventral mesenteric formation* loses contact to the body wall and continues as a lacunar space which extends towards the left side (Fig. 51N) and represents the oral lacuna along the intestinal tract's left side.

### Arm coelom

The coelomic spaces in the arms of the present larva derive as one unit from the main body cavity (Fig. 50B):

As the axocoelomic part of the main body cavity has been ontogenetically shown to reside in interradius CD and proximally around the esophagus, the epithelium derived from the former axocoel does not share in the developing coelomic cavities of the arms.

The arm cavities derive directly underneath the hydrocoelomic space from the main body cavity. The oralmost part of the arm coelom locates on a level above the oral lacuna, indicating its left somatocoelomic origin. However, the aboralmost somatocoelomic area that is involved in arm coelom formation derives from an area underneath the oral lacuna and even below the *ventral mesenteric formation*.

In the proximal part of the arms in radii A, B and E, locally (at one spot each), the body wall grows inwards from the sides, forming a thin wall that divides the somatocoelomic arm cavity horizontally. Further distally within these arms, either close to the horizontal wall or considerably further distally, a fragile horizontal septum is formed that separates the aboral fourth to third of the somatocoelomic arm cavity from it's larger oral part. It resides on one level with the ingrowing wall, as if being in continuation with it. In the transitional zones of radii C and D, the arm's body wall is not grown inwards from the sides; however, the fragile horizontal septum is present.

### 3.6.2 Pentacrinoid larva B (Specimen 9)

The older pentacrinoid specimen 9 has been cut into serial, not exactly horizontal semi-thin sections (Fig. 53), the highest point residing in radius D. Its anus is lifted up onto an anal tube. The larva has one hydropore.

#### Axocoel, ventral mesenteric formation and small ventral coelom

The structures and their positions in interradius CD are, in principle, identical to those of the previous pentacrinoid larva (Fig. 54). The axocoel passes the body wall in counterclockwise direction starting with the hydropore. The *small ventral coelom* extends aborally, starting in the primary stone canal's bend (Figs. 54E-H). Horizontalizing the inclination of the cutting plane, the axocoelomic duct passes the body wall distally to the oral end of the *small ventral coelom*. After merging with the primary stone canal, the axocoel opens into the main body cavity above the *ventral mesenteric formation* (Figs. 54B-E). The ventral lacuna, which is formed by cell protrusions of the *small ventral coelom*, locates at the distal end within this *ventral mesenteric formation*. Both the ventral lacuna and the *small ventral coelom* extend vertically, their oral ends locating *below* the axo-somatocoelomic merging area of the main body cavity (Fig. 54G). The ventral lacuna continues towards the right in the periesophageal lacuna (Figs. 54D-G), the latter being continuous with the lacunar space of the *glandular axial organ*. Towards the aboral end of the *ventral mesenteric formation*, a compact cluster of epithelially organized large cells can be found (Figs. 54 i-J). As the aboral end of the *ventral mesenteric formation* loses contact with the body wall (Fig. 54J), it aborally continues as a lacunar lappet, which runs around the left side of the stomach and finally flattens on the intestinal tract. This lappet constitutes the oral lacuna.

The main difference between this and the previous pentacrinoid stage described is associated with the development of the anal cone and the differentiation of its anal cone coelom from the

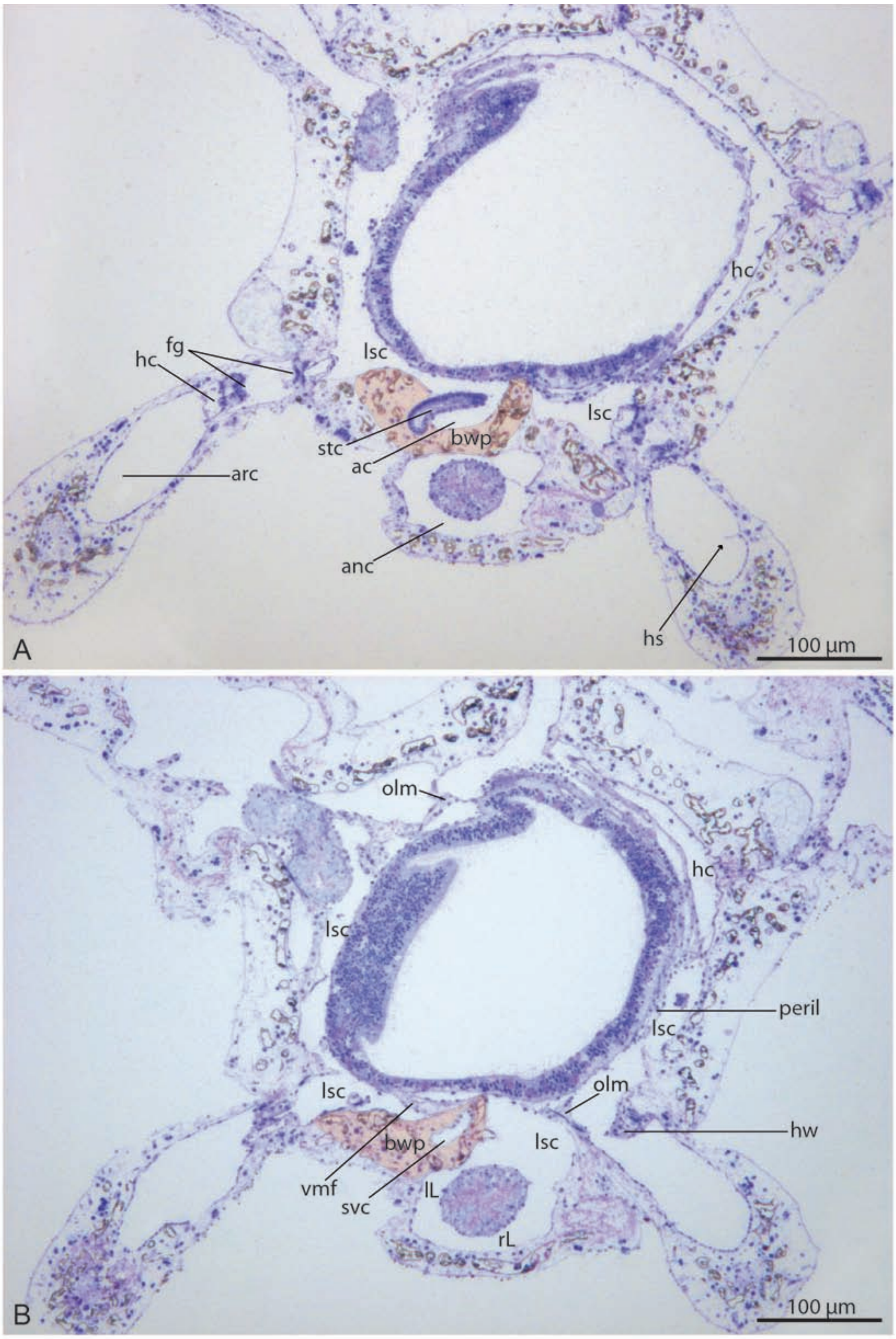


**Fig. 53 part 1 of 2**

**Fig**. **53 (2 parts):** Pentacrinoid larva B, specimen 9. Overviews, oral to aboral order. LM. Cross-sections of the larva through **A** the hydrocoel (*hc*) **B** the oralmost region of the main body cavity (*bc*) and **C** at the aboral end of the body wall protrusion (*bwp*). The area that will become the body wall protrusion of the juvenile is colorized in **skin color**. **C** Note the position of the secondary aboral vertical mesentery (*sabovm*) distal to the body wall protrusion. *abolsc* oral somatocoel below oral lacunar meshwork (***dark gray***), *ac* axocoel, *anc* anal tube coelom, *arc* arm coelom, *eso* esophagus, *fg* food groove, *hs* horizontal septum, *hw* horizontal wall, *intt* intestinal tract, *lL* right somatocoel left lobe (***red***), *lsc* oral somatocoel, *ol* oral lacuna, *olm* oral lacunar meshwork, *olsc* oral somatocoel above oral lacunar meshwork (***light gray***), *osec* oral section of axial mesentery, *peril* periesophageal lacuna, *rL* aboral somatocoel right lobe (***red***), *stc* primary stone canal, *svc* small ventral coelom, *vmf* ventral mesenteric formation.

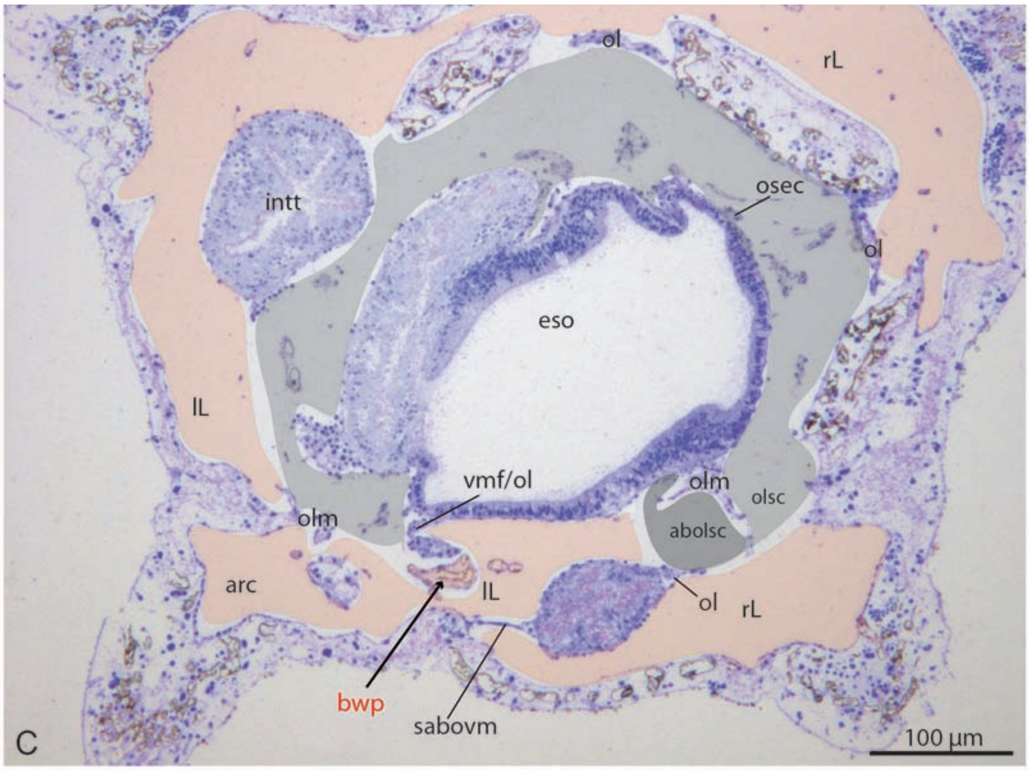


**Fig. 53 part 2 of 2**

main body cavity: Like a chimney that is attached to the outside wall of a house and opens above the roof, the anal cone forms *distally* to that body wall section in interradius CD, in which all these ventral structures are embedded. The main body cavity extends *underneath* all these structures into the anal cone, where it becomes the anal cone coelom. This leads to the fact that the ventral body wall section just specified becomes a vertical body wall protrusion that orally extends between the stomach and the intestine in interradius CD (Figs. 53A-C).

### Secondary stone canals

In addition to the primary stone canal, four further, secondarily formed stone canal anlagen, which consist of large cells similar to the primary stone canal, originate from the hydrocoel ring, one in each interradius, and open into the main body cavity (Fig. 55A). The secondary stone canal anlagen in interradii EA and AB are merely thickenings of the hydrocoel ring's aboral epithelium. The secondary stone canal anlagen in interradii DE and BC are comparatively far developed; they reach freely as an apically closed tube into the main body cavity somatocoel and do not open into it.

### Chambered organ and nearby structures

The chambered organ consists of five radially positioned tube-like coelomic cavities, which pervade the larva‘s stalk axially. The tube's oralmost ends are enlarged and form blindly ending chambers underneath the calyx's main body cavity. Towards their aboral end, chambers number 4 and 5 (in radii A and B) each form one distally directed process, which is locally laterally constricted. Two stacked slender processes extend from chamber number 3 (radius E).

The chambers are encapsulated by tissue of the aboral nervous system, which is surrounded by skeletal elements, respectively. Strands of nervous tissue derive interradially from this nervous tissue capsule. These strands take their way oralwards within the *ecm* of the body wall to a (radial) point above the chambers. The cirral processes are accompanied by nervous tissue as well.

Axially within the *ecm* in the center of the chambered organ's tubular part resides a cord of axially elongated fusiform cells. Where the chambers enlarge, this cord achieves a tubular nature before it raises further oralwards into the mesenteries of the calyx.

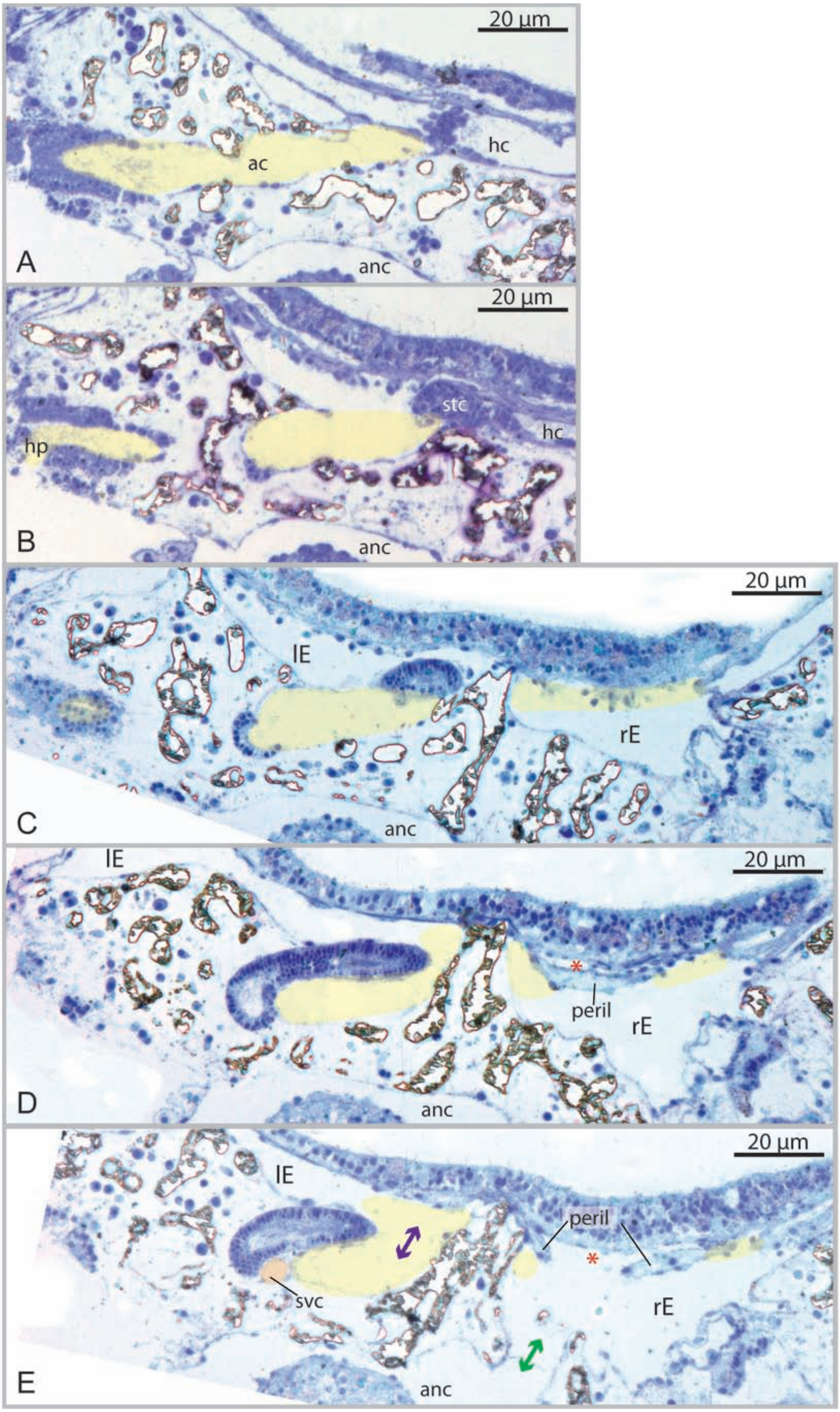
### Lacunar spaces and the tubules of the glandular axial organ

Close to the bend of the intestinal tract's coil, the axial compartment is longitudinally crossed by the *glandular axial organ*. It consists of a single epithelial tubule, an *ecm* in which the former is embedded (*glandular axial organ* lacuna), and a surrounding epithelial sheath. The tubule of the *glandular axial organ* originates axially from the center of the chambered organ and extends into the intestinal coil halfway of the intestinal tract. Oralmost, the axial mesenteric space is filled by *ecm* only and finally adapts to the esophagus.

Underneath the intestinal tract, the tubule first extends vertically within the ab*oral vertical mesentery* from the main body cavity's peritoneal layer to its visceral layer (Fig. 55). Aboralmost, this mesentery spans across both the interradii AB and BC (as indicated by the nervous tissue strands that originate between the related chambers of the chambered organ, Fig. 55C). As a consequence, the main body cavity aborally starts being fully divided into two distinct coelomic spaces. When contacting the intestinal tract's aboral end, the *aboral vertical mesentery* splits up into two structures; the epithelial sheath (=axial mesentery) of the *glandular axial organ* on the one hand and the aboral lacuna on the other hand (Fig. 55H). Both structures lose contact to the peritoneal lining of the body wall, the aboral lacuna first and the *glandular axial organ* further orally. The *glandular axial organ* remains attached to the visceral epithelium of the stomach and esophagus via a thin mesenteric structure.

In the *ecm* of the axial mesentery resides an axial muscle strand, which is anchored within the *ecm* in interradius BC aside of the chambered organ. From there it extends along the whole length of the axial mesentery up to the oral end of the *glandular axial organ* (as in Fig. 52). On

▸ **Fig. 54 (2 parts):** Pentacrinoid larva B, specimen 9. LM. Series of semi-thin cross-sections through the ventral region in interradius CD, traced from oral to aboral. Distances between sections vary. **A-J** The somatocoelia and the axocoel are fused and form the main body cavity. Assignment of coelomic origins of its regions indicated is an approximation based on the markers given in Table 3 (p. 152). Both the part of the axocoel that crosses the body wall (axocoelomic duct) as well as the presumed former course of the axocoel are coloured in ***yellow***. *ac* axocoel, *anc* anal coelom, *ecc* cluster of large epithelial cells, *hc* hydrocoel, *hp* hydropore, *lE* left extension of the oral somatocoel, *lL* left lobe of the aboral somatocoel, *olm* oral lacunar meshwork, *peril* periesophageal lacuna, *rE* right extension of the oral somatocoel, *stc* stone canal, *svc* small ventral coelom (***orange***), *vl* ventral lacuna, *ol* oral lacuna, *vmf* ventral mesenteric formation, *green double arrow* area in which the main body cavity communicates with the anal cone coelom, *purple double arrow* area in which the axocoelomic duct fuses with the main body cavity, *red asterisk* ventrally open space underneath the periesophageal lacuna, which has to be interpreted as belonging to the left lobe of the aboral somatocoel, correlating to a region marked with a red asterisk in Fig. **45E**.



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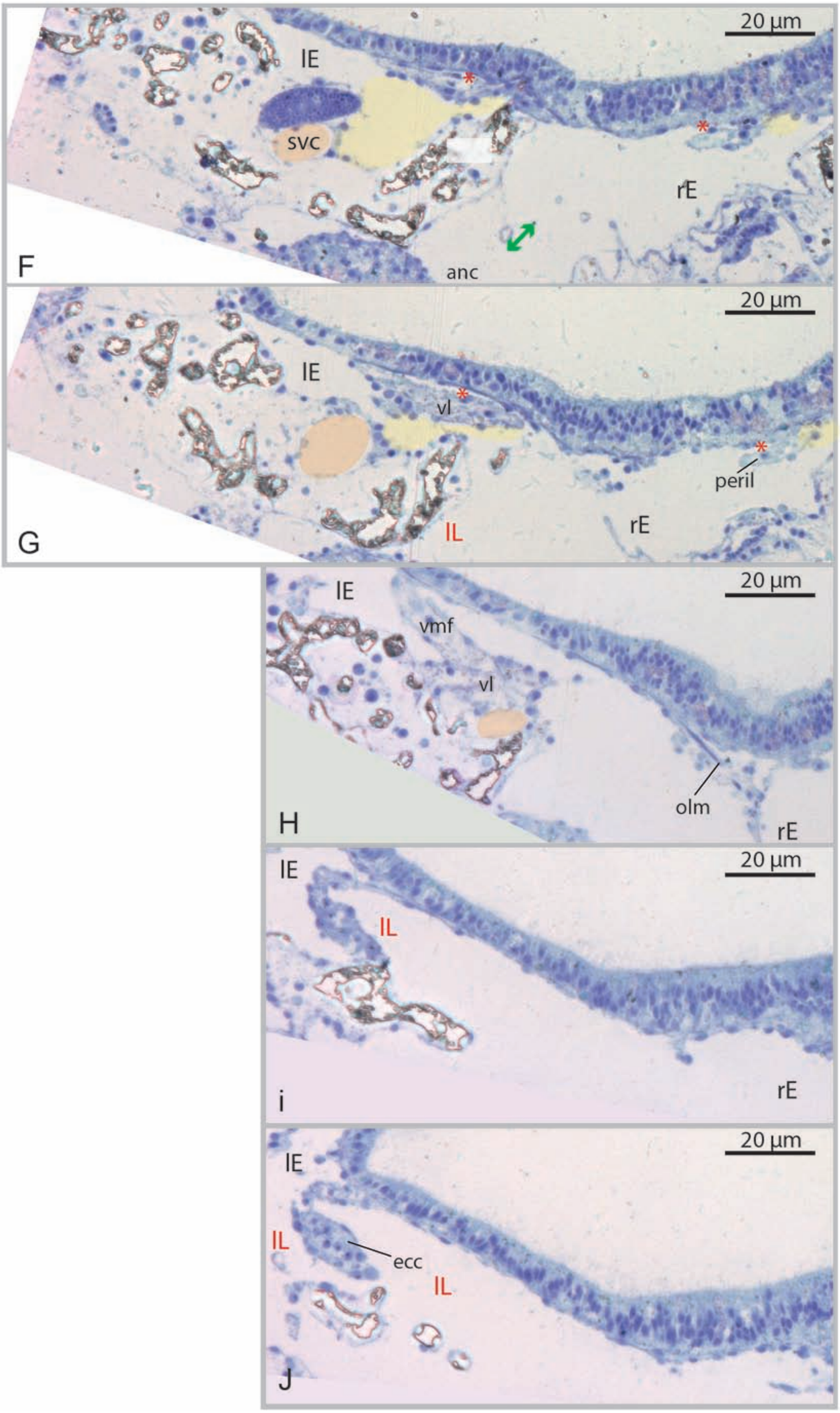

**Fig. 54 part 2 of 2**

most of its way, the muscle strand can be assigned to that epithelial layer of the axial mesentery that is directed towards the bend of the intestinal tract.

The aboral lacuna accompanies the intestine on its aboral side. The epithelium that covers the lacuna is detached from the intestinal tract and the resulting light-microscopically empty extra-cellular space is widened. It can be traced up to the anus. Locally, the aboral lacuna maintains a mesenteric character, i.e. the *secondary aboral vertical mesentery* is not fully resolved.

The intestinal tract is accompanied by a second lacuna, the oral lacuna, which runs along the intestinal tracts oral side. As previously mentioned, the oral lacuna ventrally continues in the *ventral mesenteric formation*. A peculiarity of the present larva is that dorsally, in (inter)radii E, EA, A, AB and B, the oral lacuna is associated to the tegmenal body wall, which projects aborally towards the intestinal tract (tegmenal projection, Fig. 53C). The epithelia of the tegmenal projection and the oral lacuna are continuous, implying that the horizontal mesentery of this specimen did not dissolve, yet, but just slightly widened its space.

On the larva's right side, both the oral lacuna and the aboral lacuna are clockwise continuously traceable along the intestinal tract until they enter the coelom of the anal cone. Within the anal cone, the lacunae (especially the aboral one) are less prominent.

When notionally extending these lacunae as mesenteries until the cone’s end, it becomes clear that the two lacuna running along the intestine in the anal cone, as expected, represent remnants of the former mesenteries; the oral lacuna derives from the horizontal mesentery between the oral and aboral somatocoel and the *secondary aboral vertical mesentery* derives from the two lobes of the aboral somatocoel. Therefore, the anal cone coelom's distal side unequivocally derives from the right lobe of the aboral somatocoel and the right extension of the oral somatocoel definitely forms *part of* the anal cone coelom's proximal side.

Even though there is no third lacuna along the proximal side of the intestinal outgrowth that would represent a former border between the right extension of the oral somatocoel and the left lobe of the aboral somatocoel, there are several hints that point to an involvement of the latter in the formation of the proximal side of the anal cone coelom. Firstly, the tip of the intestinal outgrowth of specimen 4 has been clearly shown to be ensheathed by this left lobe of the aboral somatocoel. Also, the position of the *ventral mesenteric formation*, which has been shown to mark the border between the oral somatocoel’s left extension and the aboral somatocoel’s left lobe, points towards the latter's involvement. Lastly, Figure 53C shows that aboral to the point where the intestine enters the anal cone, the locally preserved *secondary aboral vertical mesentery* (whose proximal side is by definition formed by the aboral somatocoel's left lobe) resides <u>distal</u> to the aboral end of the body wall protrusion, rendering it even more likely that the aboral somatocoel ascends in the anal cone coelom. So, the left part of the anal cone coelom’s proximal half is formed by the left lobe of the aboral somatocoel and the oral somatocoel extends merely along its proximal half"s right part.

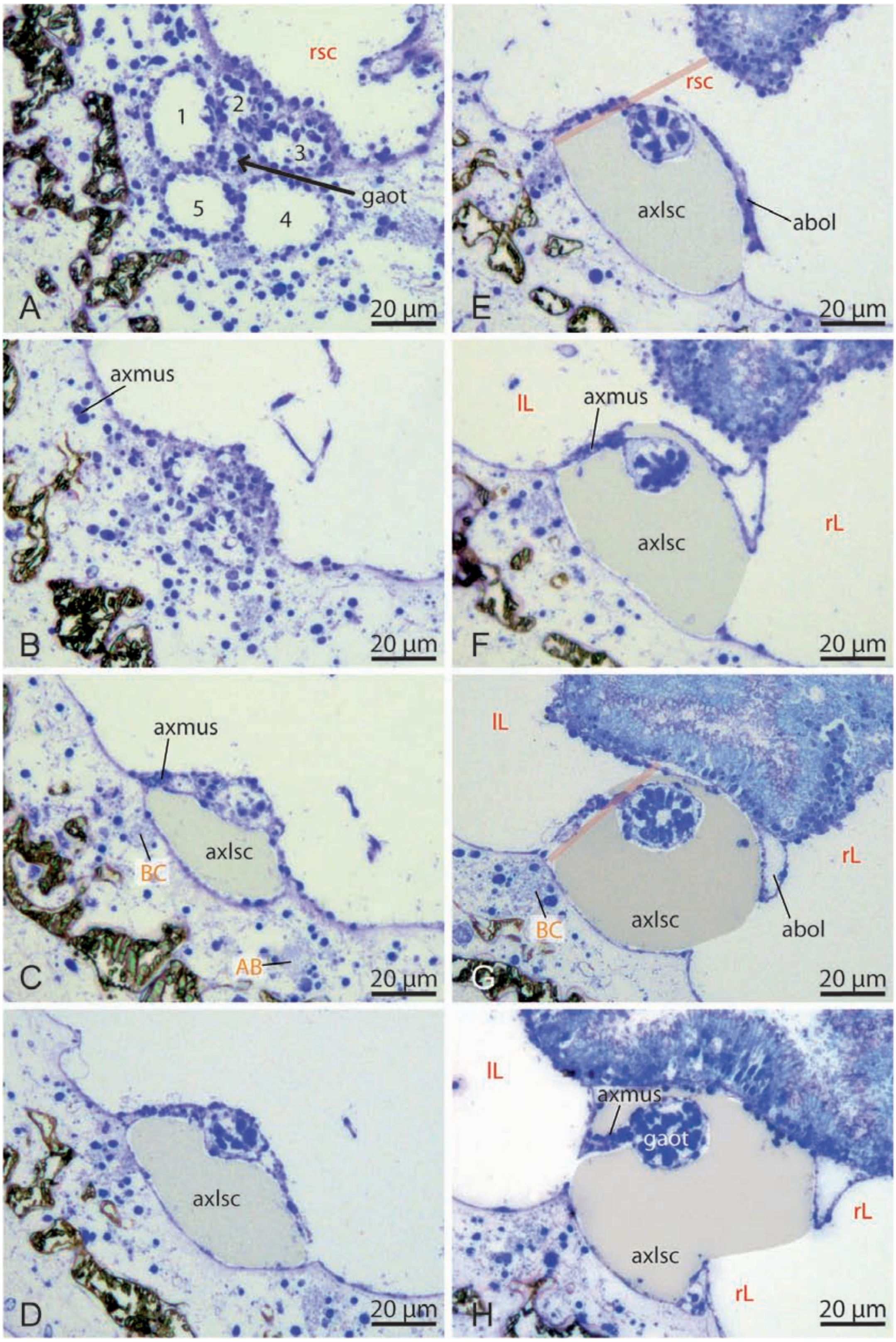

**Fig. 55:** Pentacrinoid larva B, specimen 9. LM. **A-H** Series of cross-sections (aboral to oral) through the aboral section of the aboral vertical mesentery (*abovm*), the axial extension of the oral somatocoel (*axlsc*) and the glandular axial organ tubule (*gaot*), respectively. Distances between sections vary. The aboral vertical mesentery forms between chambers 1 and 5 (*arabic numbers*) in interradius BC and extends between the body wall and the intestinal tract (position of *abovm* indicated by the *skin colored line*). In this larva, the epithelial layers of the aboral vertical mesentery are aborally split by the axial extension of the oral somatocoel. Note the course of the axial muscle (*axmus*) from its anchoring site in the body wall (**B**) through the aboral vertical mesentery (**C-H**) and in the prospective axial mesentery (**H**), respectively. *abol* aboral lacuna, *lL* left lobe aboral somatocoel, *rL* right lobe aboral somatocoel, *rsc* aboral somatocoel. *orange letters* interradii, indicated by the interradial strands of the aboral nervous system.

### Oral lacunar meshwork

A horizontal meshwork of lacunar tissue strands, the *oral lacunar meshwork*, extends around the esophagus from the periintestinal *ecm* to the calyx's body wall. It inserts above and to the side of the arm coelom's beginning (Fig. 53). Ventrally, it resides slightly above the *ventral mesenteric formation's* oral end. At the esophagus' right side, the tissue strands insert right underneath the periesophageal lacuna (rarely at or above this lacuna) till the latter reaches the *glandular axial organ*'s oral end. Further dorsally, the *oral lacunar meshwork* remains at the same level and stretches above the intestinal tract's oral lacuna without contacting it. On the left side, it keeps the same horizontal level as well. In the (inter)radii E, EA, A, AB and B, the *oral lacunar meshwork* inserts at the proximal side of the tegmenal projection, i. e. above the oral lacuna. The tissue strands are arranged in a way that two strands traverse each radius. They are attached to the sides of each armbase. They contain muscular strands and may be interconnected to each other in less regular ways via further tissue strands. The *oral lacunar meshwork* in radius D attaches to the body wall underneath the oral end of the arm's cavity. In all other radii, however, the *oral lacunar meshwork* attaches to the oral margin of the arm cavity's proximal end/beginning. The aboral part of the somatocoel is, in addition, traversed by scattered single threadlike trabeculae.

### Arm coelom

The arms' somatocoelomic cavity derives as one single unit from the main body cavity. Further distally, within the arms, the cavity becomes divided by a fragile horizontal septum (Fig. 53A).

Using the oral lacuna as a marker for the position of the former horizontal mesentery, the major part of the arm cavities resides on one level with that part of the main body cavity that can be assigned to the aboral somatocoel.

Proximally in each arm, a short horizontal wall separates an oral and an aboral section of the arm. This horizontal wall consists of loose *ecm*, which is laterally continuous with the arm's body wall. In radii E to C, the horizontal wall resides slightly above the oral lacuna. In radius D, it resides approximately on one level with the epithelially organized mass of densely packed large cells. The oral section of each arm cavity is continuous with the oralmost part of the main body cavity.

Considering the data on both pentacrinoid larvae described, the cavity of the present larva's arm has to be considered as potentially incorporating shares of both somatocoelomic domains of the main body cavity.

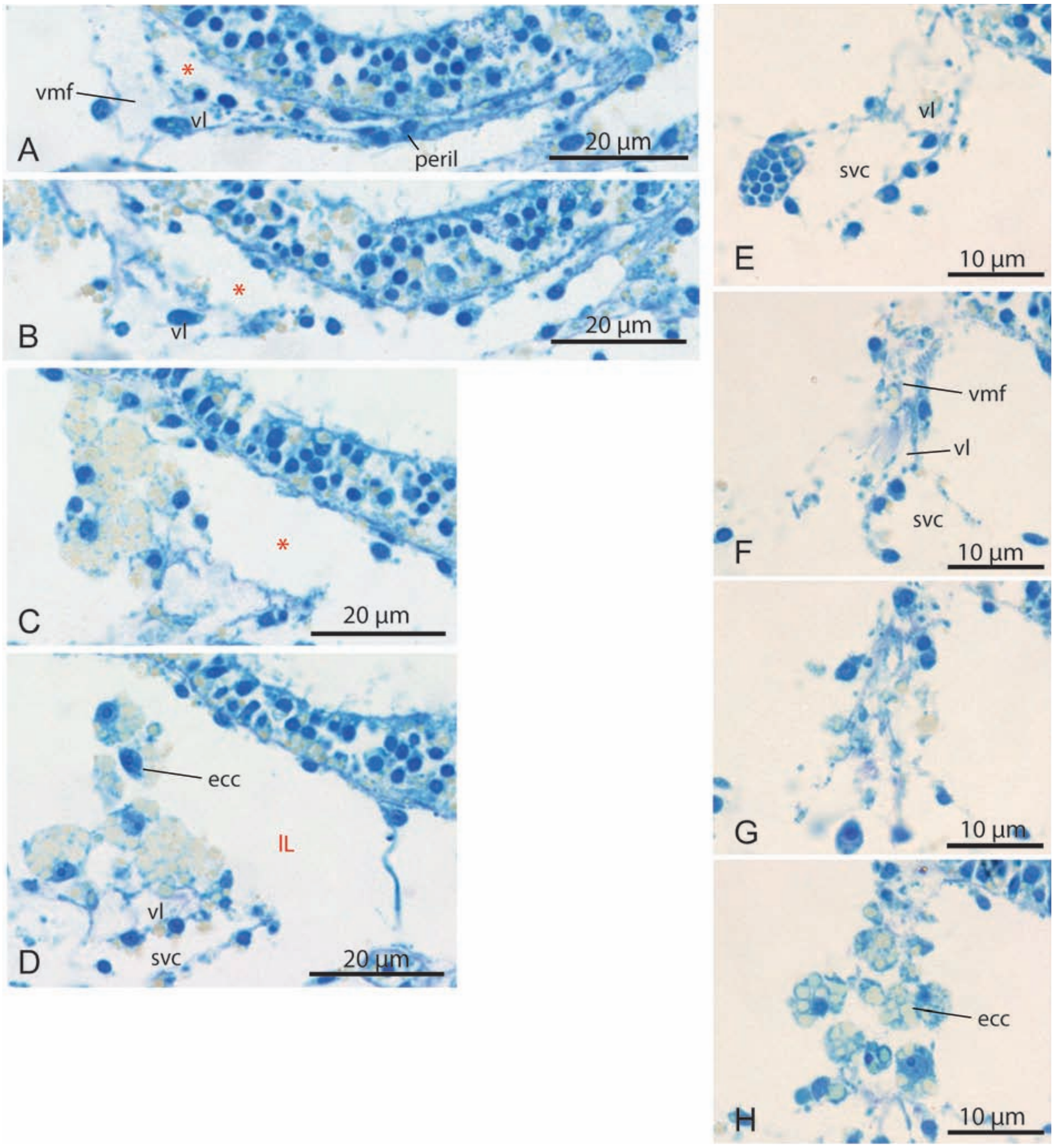

**Fig. 56: A-D** Late cystidean larva (specimen 6) and **E-H** early pentacrinoid larva (specimen 7). LM, cross sections through the ventral mesenteric formation (*vmf*) and the cluster of large epithelial cells (*ecc*). Note that the amount of large cells is reduced compared to the younger specimen 5 (Fig. **48C**), but the shape of the cluster is less compact than in the older specimens 8 and 9 (Figs. **51M**, **54J**). *lL* left lobe right somatocoel, *svc* small ventral coelom, *vl* ventral lacuna, *red asterisk* small ventrally open cavity underneath the periesophageal lacuna (*peril*), which has to be interpreted as belonging to the left lobe of the aboral somatocoel, correlating to a region marked with a *red asterisk* in Fig. **45E**.

## 3.7 Larval stages around the opening of the vestibular cavity (Specimen 6 and Specimen 7)

Two further larvae have been cut into serial horizontal semi-thin sections. The vestibulum of specimen 6 (Fig. 5J) is close to opening and that of specimen 7 (Fig. 5K) is already ruptured. The arrangement of the ventral structures in interradius CD equals that of the armed stages just described.

The main difference of these structures from those of the armed stages lies in the condition of the cluster of epithelial cells (Fig. 56). While the cluster reduced its size compared to that in the younger specimen 5, its cells are (still) loosely arranged compared to that in the older specimens 8 and 9. In both larvae (specimens 6 and 7), the cluster consists of very large yolk cells that have a large nucleus and a prominent nucleolus (Figs. 56D, 56H). Although “loosely” arranged due to their shape, the epithelial arrangement of these cells becomes obvious when “scrolling” through the serial sections; most of the cells are connected via cell contacts and basally lined by a basal lamina. In these larvae, the cell cluster forms the aboral end of the *ventral mesenteric formation*. The large cells are more numerous on the cluster's right side than on the left.

# 4 Discussion

The present work focuses on the coelomic development, so the epidermal regions have been examined for general epithelial characters only. However, comprehension of coelomic organ development improves with understanding the development of the *ecm* and the embedded mesenchyme. As the latter is delimited by the coelomic cavities and the epidermis, both epithelial borders need to be well defined.

## 4.1 Development of the epidermis

The epidermis of the doliolaria is regionalized into four ciliary bands, an anterior apical tuft, part of which is formed by the cells of the apical organ, an antero-ventral adhesive pit, a large ventral vestibular invagination, and the epidermis proper within the interband region (this thesis; Lahaye and Jangoux1985).

In the non-feeding doliolaria, the epidermis is the only functional organ of the larva and serves sensation and motility (Chia et al. 1986). It is underlain by a nerve plexus, which consists of both afferent and efferent components that are effective through sensory cells, secretory cells and cilia. The apical organ, which plays a major role in the selection of substratum, is an area at the anterior tip of the larva, in which all these components are concentrated (*A. mediterranea*: Barbaglio et al. 2012; *F. serratissima:* Mladenov and Chia 1983, Chia et al. 1986; *Oxycomanthus japonicus* and *Metacrinus rotundus*: Nakano et al. 2009). In addition, its nerve plexus is associated with a ganglion that stretches along the antero-dorsal side of the larva (Chia et al. 1986). During settlement, the larva attaches itself with the adhesive pit, which subsequently transforms into the attachment disc of the stalked larva. During metamorphosis, the ciliary bands and the apical organ disappear and the larva loses all cilia (Mladenov and Chia 1983, Barbaglio et al. 2012). The ganglion remains detectable in the cystidean larva but subsequently vanishes as well (Nakano et al. 2009).

As all these structures are transient, the focus in this work lies on the development of the proper epidermis of the doliolaria's interband region in the context of its contribution to the body wall.

In the doliolaria examined, the proper epidermal cells are monociliated, the stems of the mushroom-shaped cells stand close together, and their heads show rivet-like structures and apical microvilli that are covered by a so-called cuticle. The latter three structures can be observed in all later stages as well. As has been shown for doliolarias of all other crinoid species as well (Chia et al. 1986, Barbaglio et al. 2012), a basal lamina spans across the basal ends of the epidermal cells that basally limits the increasing intercellular spaces. A considerable amount of fibrous material resides basally in the *ecm* between the flat broadened heads of the epidermal cells and the basal lamina.

Comparing the epidermis of the doliolaria with that of the metamorphosing larva (specimen 2), the epidermal cells differentiate from the cloddy mushroom-shaped cells into the slender tack-shaped ones, whose pins are very distant from each other. With increasing distance between the single epidermal cell somata, the basal lamina is not directly attached to the epidermal cell's basal ends anymore and can also not be detected shaping around the epidermal cell's somata in the early metamorphosed larva. This prominent basal lamina obviously disappeared, so that the doliolaria's mesenchyme, which derived from the embryo's blastocoel, expands between the cell somata, in some sense uniting with the epidermis' intercellular space. Instead, fibrous extra-cellular structures reside underneath the flattened broad heads of the epidermal cells. They form part of a larger network of extra-cellular fibers that takes up the body wall's distalmost space, in which mesenchymal cells are rare. Neuritic structures cannot unequivocally be called basiepithelial anymore, as they mostly reside amongst the fibrous basal lamina and generally extend proximally into the body wall's *ecm*.

Throughout cystidean and pentacrinoid stage, the body wall's *ecm* between the somata of the epidermal cells increases. In adults, the basal lamina encompasses the whole epidermal cell (Heinzeller and Welsch 1994).

Based on the data available it therefore has to be concluded that the doliolaria's ectodermal basal lamina is reduced during development and is replaced by a newly formed basal lamina, which probably is formed by the epidermal cells. This process seems to start during early cystidean stage underneath the tack's heads from where the basal lamina expands around the somata thereafter. That way, the mesenchymal *ecm* unifies at least partly with the intercellular space that resided distal to the basal lamina between the epidermal cells in the doliolaria.

## 4.2 Development of the ecm

Topologically, the mesoderm comprises all cellular structures between the endoderm and the ectoderm. In crinoids (as in all other echinoderms), it derives from the endoderm and comprises the four coelomic cavities as well as the mesenchyme cells that delaminate from the anterior end of the archenteron into the blastocoels (Hyman 1955, Holland 1976). In the doliolaria, the coelomic cavities and the enteric sac form a cluster at the larva's posterior end, and the mesenchyme, comprising the mesenchymal cells and their surrounding *ecm*, fills the space between the basal laminae of the cluster of epithelial cells and the basal lamina of the epidermis.

The loose *ecm* consists of a matrix formed mainly by a network of collagen fibrils that are associated with glycosaminoglycans as well as glycoproteins; its most prominent structures are collagen fibrils that form the "mutable" collagen ligaments (Heinzeller and Welsch 1994). It extends into the spaces between the cavities formed by the meso- and the endoderm as well. In the doliolaria, many mesenchymal cells already differentiated from common mesenchymal cells into various cell types, e.g. sclerocytes, myocytes, nervous cells (of the hyponeural and aboral nervous system), but also free mesenchyme cells that are presumably undetermined and remain flexible in function. These latter cells may migrate throughout the body wall as well as within the interepithelial spaces.

The present data demonstrate clearly that, as the larva grows, the enteric sac and the coelomic cavities take up more and more space within the larva's calyx. The larval mesenchyme, including its sclerites, neuronal structures and further specialized cells, thereby becomes the main part of the body wall (or integument), which is limited to the outside by an extremely thin layer of epidermal cells that are anchored within the *ecm* with their somata.

So, the body wall is constituted by the epidermis and a subepidermal mesenchyme, whose *ecm* positionally directly derives from the embryonic blastocoel.

## 4.3 Development of the nervous system in the ecm

In the juvenile (as in adult), the aboral (=apical) nervous (sub-)system encapsulates the chambers of the chambered organ and forms prominent nerve strands, which form ganglia and a ring commissure in the pattern described above (p. 39ff.) and extend into the arms. The aboral nervous system forms within the *ecm*, probably deriving from mesodermal cells (Heinzeller and Welsch 1994, Cobb 1987).

The present data show that there are already nervous tissue structures around the prospective chambers of the chambered organ in the doliolaria, that have to be interpreted as precursor structures of the nervous tissue of the cystidean's stalk nerve.

This latter observation is interesting in the context of the study carried out by Nakano et al. (2009) on *Oxycomanthus japonicus*, which, using immunhistochemical methods showed that the aboral nervous system develops independently from the larval ectodermal nervous system. In *O. japonicus*, however, synaptotagmin-immunoreactivity of the stalk nerves, which belong to the aboral nervous system, was detectible only from a cystidean stage (1 day after settlement) on. The authors postulate a nerve cell center in the doliolaria that resides at the anterior border of both the hydrocoel and the right somatocoel (i.e. at the location, where the tubes of the prospective chambered organ derive), from which the adult aboral nervous system shall develop. The nerve cell center is postulated to be associated with the basal plate anlagen. The present results show, as postulated by these authors, that the stalk nerves of *A. bifida* are formed much earlier than in the cystidean stage, obviously in a doliolaria younger than the one investigated. The data do not show, however, whether these structures derive from a presumptive aboral nerve center as postulated or whether they form at the same time along the tubes of the chambered organ.

The hyponeural nervous (sub-)system has been found to exist in the juvenile, where it interacts with the aboral nervous system. It has, however, not been looked for in the pentacrinoid stages so that the present data are not suitable to shed light on its development.

## 4.4 Development of the vestibular cavity

The vestibular cavity is an epithelially lined cavity that exists only temporarily until the end of the cystidean stage. It provides a protective space, in which the 25 podia and the definitive mouth can develop.

It starts to form as an antero-ventral ectodermal depression (vestibulum or stomodeum) on the ventral side of the doliolaria. In the present study, the epidermis in the doliolaria's vestibular region is shown to mainly consist of long slender ciliated secretory cells, most of which contain vacuoles filled with floccular material. The vestibular cells form apical microvilli that are covered by a cuticle. Chia et al. (1986, *F. serratissima*) and Barbaglio et al. (2012, *A. mediterranea*) describe two types of cells for this region, secretory flask to spindle shaped cells and columnar cells. In *A. bifida*, the first type is abundant in the vestibulum's center and the second type mainly occurs towards the rim of the vestibular invagination.

The closure of the vestibulum has not been traced in this thesis. In specimen 2, the vestibular cavity is already closed but flattened and in specimen 3, the vestibular cavity achieved an oral position. In both larvae, its proximal layer forms an extension that reaches through the hydrocoel horseshoe's center and grows towards the orally directed extension of the enteric sac, marking the location of the future mouth. This proximal epithelial layer consists of long flask shaped cells that extend vertically, filling the space between the vestibular cavity and both the enteric sac and the hydrocoel. They possess apical microvilli that are covered by a cuticle, pointing to the vestibular cavity's ectodermal origin. It is not clear, however, why the distal layer that forms the vestibulum's roof and which is also ectodermal, lacks these structures.

The oral extensions of the hydrocoel horseshoe bump against the proximal vestibular epithelium and form the primary podia, which grow out into the vestibular cavity.

During transition from cystidean to pentacrinoid stage, the vestibular roof ruptures in all five radii, so that the triangular interradial valves are formed, and the vestibular cavity loses its identity. The twenty secondary podia will develop into podia around the mouth and the five primary podia will grow out and develop into the water canals of the arms. Due to this rupture, the proximal layer of the vestibular cavity becomes the larva's oral surface and the distal layer covers the inner side of the oral valves. The triangular valves are still visible around the mouth in the juvenile, in which they protect the stomodeum and the podia.

## 4.5 Transition from doliolaria to cystidean stage: change of body axes

In the bilateral doliolaria, the longitudinal axis takes up a horizontal position, with the tubes of the chambered organ being directed anteriorly, the body cavities at the posterior end and the vestibular invagination marking the ventral side. In late doliolaria to cystidean stage, the vestibulum closes from posterior to anterior and forms the vestibular cavity (Bury 1888: 273).

During settlement, the bilateral larva adjusts to the substratum with the anterior apical pit and lifts its posterior end 90° upright so that this main axis achieves a vertical position (Fig. 57). During the following metamorphosis, the larva reorganizes its body symmetry from bi-lateral to penta-radial, giving up its two direction-of-movement-related poles, anterior and posterior. As the mouth will later develop at the larval posterior end, being directed towards the free water column, the posterior side of the doliolaria is termed the oral side starting with cystidean stages. The former anterior end of the larva, which is now directed to the substratum, becomes the aboral side. The four lateral axes of the larva, dorsal, ventral, right and left, respectively, are given up in favor of five externally nearly identically arranged radii (Tab. 2).

The change of symmetry makes every description of metamorphosis hard to understand, since structures that are just developing into adult radial symmetry need to be described using bilateral symmetry markers up to a certain point, while bilateral-symmetry related structures still slightly change their place within the developing radial symmetry.

Just two remnants of bilateral larval structures remain as markers of the former bilateral symmetry in the calyx throughout cystidean and pentacrinoid larval development: the primary stone canal, which steadily marks the ventral side (later the interradius CD), but is only internally visible, and the hydropore, which changes its position slightly, but gives external orientation nonetheless.

The process, in which the coelomic cavities are rearranged during metamorphosis, can be subdivided into two different events: On the one hand, the hydrocoel and the two somatocoelia grow around the enteric sac to render radial symmetry possible. On the other hand, at the same time, all inner organs re-shift 90° from a vertical to a horizontal position as a kind of countermotion to settlement. It is helpful to see this movement being guided by the vestibular cavity, which moves along the postero-ventral side of the larva to its posteriormost (=oral) end. Since the vestibular cavity (generally speaking) keeps its position relative to the hydrocoel, the ring-forming left somatocoel, and to the right somatocoel, the latter three can be seen as shifting with it. However, the tubes of the prospective chambered organ retain their initial axial position in the larva, now reaching into the developing stalk. As a consequence, the right somatocoel takes up an aboral position and is therefore subsequently called aboral somatocoel. The left somatocoel comes to lie orally between the hydrocoel and the aboral somatocoel, and will be termed

**Tab. 2:** Directional terms before and after metamorphosis and their morphological markers. Identification of the body axes in the embryo is not trivial, as the assumption that the vestibulum resides ventrally is, in last consequence, arbitrary. An identification of both the left and right somatocoel can only be done by, first, tracing their developmental fates within the crinoid and, second, by a comparison of the latter's coelomic arrangement to that of other echinoderm groups (Peterson et al. 2000). This table summarizes the traditional interpretation of body axes which has been applied throughout the thesis.

| ***term*** | **marker** | | ***term*** | **marker** |
|---|---|---|---|---|
| | **embryo** | **doliolaria** | | **crinoid after metamorphosis** |
| *anterior* | animal pole,<br>location where the mesenchyme forms<br>later: axocoel | adhesive pit,<br>apical tuft,<br>chambered organ,<br>axocoel | *aboral* | chambered organ,<br>right (=aboral) coelom |
| *posterior* | vegetal pole<br>blastopore | left somatocoel | *oral* | mouth, (anus),<br>ambulacral fields,<br>hydrocoel,<br>which is followed by<br>left (=oral) somatocoel |
| *ventral* | convex side of the enteroaxohydrocoel;<br>orientation relative to the position of the left and right somatocoelia;<br>later: hydrocoel | vestibulum<br>hydrocoel | *ventral* | larva: stone canal, later anus<br>adult: interradius CD, in comatulids marked by the anal tube |
| *dorsal* | concave side of the enteroaxohydrocoel;<br>orientation relative to the position of the left and right somatocoelia | right somatocoel | *dorsal* | larva: proximally extending left somatocoel cap<br>adult: radius A, in comatulids opposite side of anal tube |
| *left* | identity traced backwards from position of the oral somatocoel in the adult as identified by a comparison with the left somatocoel in other echinoderm groups. | axocoelomic duct<br>hydropore | *left* | - |
| *right* | identity traced backwards from position of the aboral somatocoel in the adult as identified by a comparison with the right somatocoel in other echinoderm groups. | - | *right* | (*secondary*) *aboral vertical mesentery*,<br>*glandular axial organ* |
| *longitudinal* | a/p-axis<br>later: lsc/rsc- mesentery | mesentery between lobes of the right somatocoel, chambers of chambered organ | *vertical* | *aboral vertical mesentery*<br>*glandular axial organ*<br>until juvenile stage: chambered organ in stalk |
| *horizontal* | long-axis of the primary somatocoel (until it's division) | mesentery between left and right somatocoel | *horizontal* | horizontal mesentery |
| | | | *radial* | arms (radial water canals, arm nerves,...),<br>primary podia,<br>basalia,<br>primary cirry,<br>chambers of chambered organ |
| | | | *interradial* | interbasalia<br>aboral interradial nerves<br>water pores<br>stone canals |

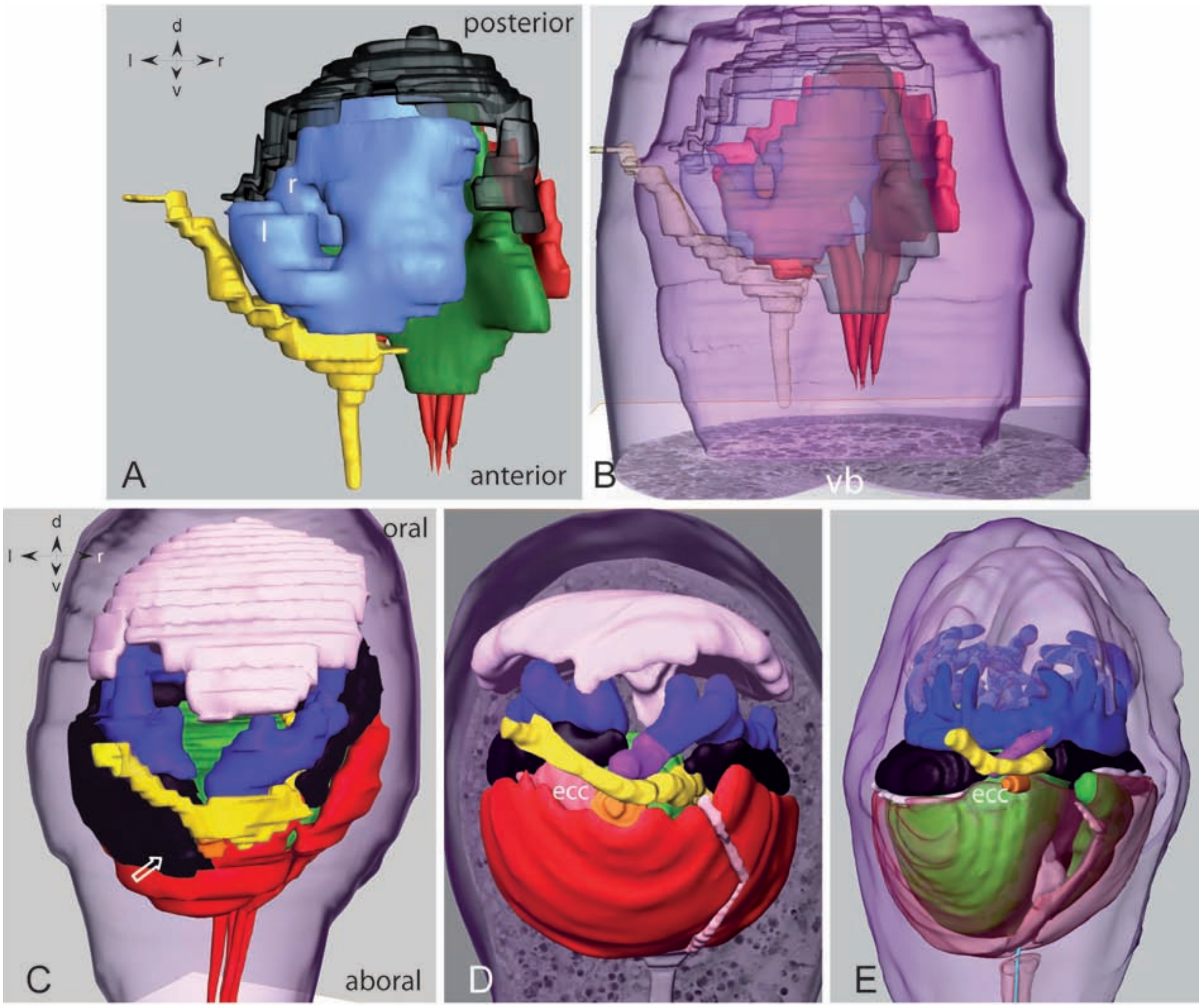

**Fig**. **57:** Ventral views of the reconstructions of **A**, **B** specimen 1, **C** specimen 2, **D** specimen 3 and **E** specimen 5 as a means to enable a direct comparison of the coeloms' position in the respective specimens. *ecc* cluster of large epithelial cells, *vb* vestibulum (**white**), ***blue*** hydrocoel, ***black*** left/oral somatocoel, ***red*** right/aboral somatocoel, ***yellow*** axocoel and hydropore, ***purple*** stone canal, ***orange*** small ventral coelom, *d* dorsal, *l* left, *r* right, *v* ventral, *white empty arrow* location, where the proximal layer of the left somatocoel and distal layer of the right somatocoel consist of large cells.

oral somatocoel. The axocoel joins the general movement and takes its place horizontally on one level with the oral somatocoel.

## 4.6 Transition from bilateral to pentaradial symmetry

The pentaradial symmetry nomenclature of adult crinoids can be derived from the bilateral symmetry of the swimming doliolaria larva as follows (Fig. 57): Based on the relative position of the right and left somatocoel during early embryo stage (Figs. 1, 2), the vestibulum marks the ventral side. In the doliolaria, the hydropore and the opening of the hydrocoel horseshoe are positioned on the left side. In the embryo and during early larval development, this opening changes its position by lengthening its right end, partly on the cost of the left end (Seeliger 1893). The present data support this observation.

Taking the vestibulum as the reference structure for the ventral side, the directional positions largely remain preserved in the metamorphosing larva, though the hydropore changes its position slightly ventrally. This movement could be explained with a 30 degree shift of the epithelial cluster around the longitudinal axis towards a horizontal orientation, but could also involve its further individual repositioning. So, the vestibular cavity, i.e. the ventral side, of the investigated metamorphosing larva can be localized in one radial plane with the primary stone canal and the *small ventral coelom* (Fig. 57C). Therefore, these latter structures constitute the new marker of the ventral side. In the early cystidean, whose epithelial cluster reached a horizontal position, the vestibular cavity already lost its marker function (Fig. 57D).

In the next oldest cystidean stage, which is characterized by a winding intestinal tract, the primary stone canal still remains between these two markers (Fig. 57E). As the five quintets of five podia around the mouth are formed in this stage, the relative position of both the primary stone canal and the hydropore to the podia can be described precisely: The primary stone canal emerges in aboral direction from the left end of the hydrocoel ring directly underneath the leftmost podium of the quintet to its right and merges with the axocoel underneath the rightmost podium of the quintet to its left. It therefore runs right between these two quintets (compare Figs. 44F, 58).

During pentacrinoid stage, the primary podia become the radial water canals in the arms, defining the pentaradial symmetry. As a consequence, the primary stone canal comes to lie in an interradial position. As the (dorsal) radius, which opposes the primary stone canal’s interradius, is conventionally called “A” and the radii are named in alphabetical order in clockwise direction, the primary stone canal comes to lie in the (ventral) interradius CD (Fig. 58). The primary stone canal and the hydropore will not be easily recognizable in the adult, as these structures get multiplied by further stone canals and tegmenal ducts during further development (Hyman 1955). As the anus comes to reside in interradius CD as well, the ventral side will, however, always remain easily recognizable in *Antedon* and other comatulid crinoids.

## 4.7 Outgrowing of the intestinal tract and formation of the mesenteries

### Enteric sac

The blastopore closes in early embryonic stage, leaving the archenteron cut off from the ectoderm (Seeliger 1893, Holland 1978). After all coelomic cavities are given off from the archenteron, the enteric sac still forms a flattened isolated cavity and the lecitothrophic doliolaria derives its energy from the yolk spheres’s constituents that can be found in all cells of the larva.

During metamorphosis, the larva’s enteric sac develops into a hemispherical cavity that is flattened towards the vestibular cavity. Its volume increases and it is filled with small round cells that possess highly heterochomatic and therefore inactive nuclei. These cells represent nutritional

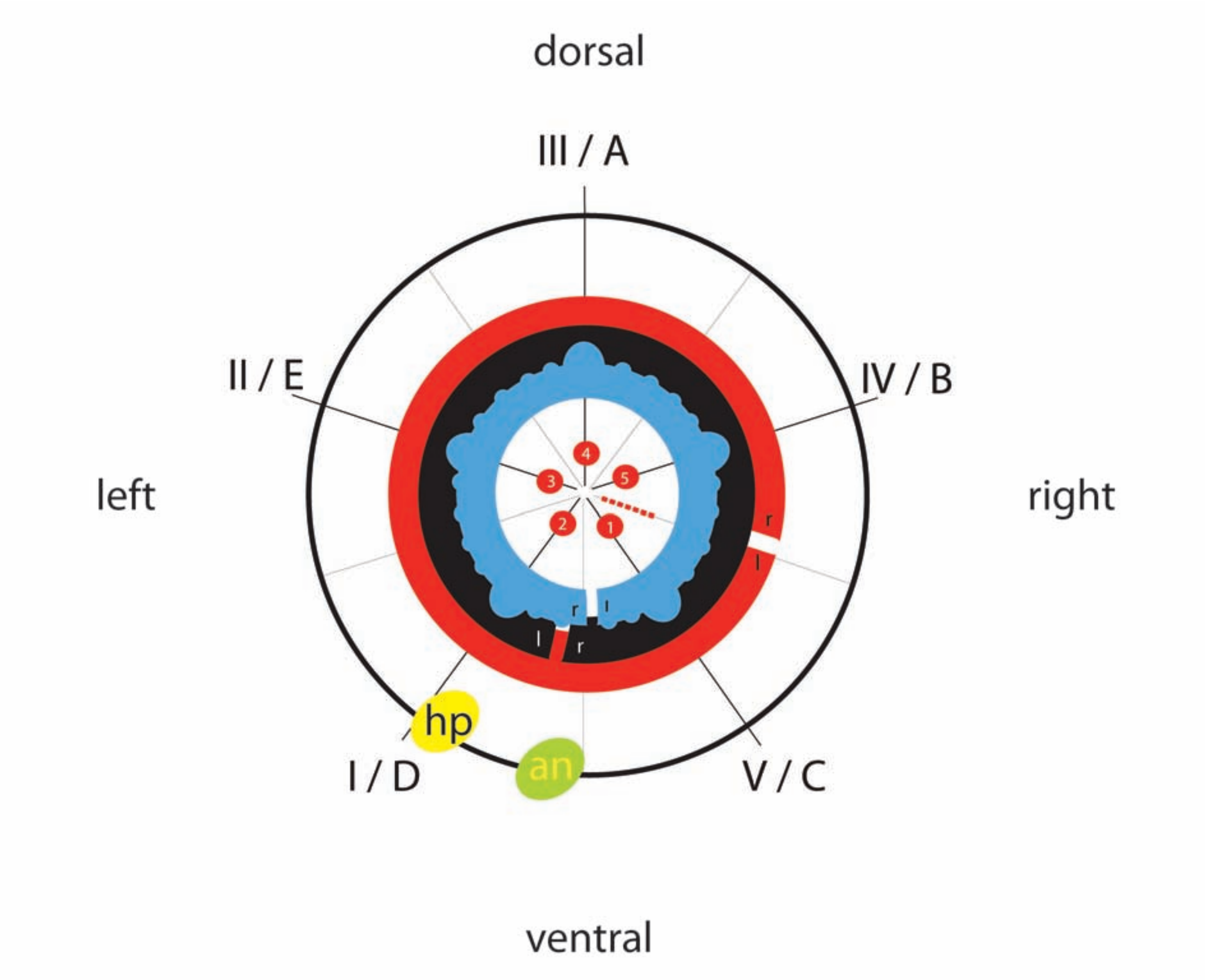

**Fig**. **58:** Schematic overview of the position of indicative structures in the pentaradial stalked larva in relation to the doliolaria's bilateral symmetry. Oral (=posterior) view. *Roman numbers* indicate the numeration of radii and *Arabic numbers* the numeration of the radially positioned chambers of the chambered organ (both as proposed by Seeliger 1893). *Roman letters* indicate the common nomenclature of radii for adult crinoids (Carpenter 1884), which equals the nomenclature of the radii in other echinoderms. Note: Since the originally right branch of the hydrocoel (***blue***) developed more intensely during development, its right end in the larva (which forms the primary stone canal) has been the left end of the hydrocoel in the embryo (Seeliger 1893). The five primary podia derive radially (*larger blue convexities*), and two sets of two further podia are formed to each of their sides (*smaller blue convexities*). Remark: Seeliger (1893) identified radius V or C, respectively, as the ventral side. *an* anus, *hp* hydroporus, ***black*** left/oral somatocoel, ***red*** right/aboral somatocoel, *red dotted line* aboral position of aboral vertical mesentery, *l* left, *r* right.

cells that are presumably going to be degraded (Hyman 1955). The present data clearly confirm that the left lobe of the aboral somatocoel grows around the antero-left side of the larva's enteric sac and meets the right lobe on the enteric sac's right side, forming the *aboral vertical mesentery*. The oral somatocoel caps the enteric sac dorso-orally, stretches on top of the dorsal half of the enteric sac, and sends two lateral extensions around the oral margin of the enteric sac that do not meet ventrally.

## Deformation of the enteric sac

Outgrowing of the intestinal tract starts soon after metamorphosis, probably in similarly far developed stages as cystidian stage specimen 3.

The detailed comparison of wholemounts of fixed cystidean larvae leaves the strong impression that the *enteric sac does not simply carve vertically but that it also bends and tilts inwards around the central axis* (Figs. 5G-i). This *in situ* observation provides the initial evidence for the hypothesis that the oral somatocoel, which stretches on top of the dorsal half of the enteric sac in specimen 3, reaches down into the axial compartment. In this context, the slight notch on the ventro-right side of specimen 3 may be interpreted as the location, where the center of this twist will be. The notch hypothetically carves down further, its ventral end tilting down, so that the enteric sac bends to a half-funnel, whose right section grows out further at its tip, forming the intestinal outgrowth.

The occurrences within the intestinal bend that are related to the initial curving have not been directly observed in the present thesis and are indirectly reconstructed from mesenteric and lacunar markers of older larvae. Any interpretation concerning the extent of both the oral and aboral somatocoel from specimen 4 on has therefore to be regarded as a hypothesis, while the mere positions of various *ecm*-cored structures that are formed by these somatocoelia and their further developmental fate represent observable and basically reproduceable facts. Regardless of the validity of the here presented hypothesis related to the formation of the *glandular axial organ*, the position of the horizontal mesentery, the aboral extension of the oral somatocoel and the origin of the periesophageal lacuna, these data can reliably be used as an orientation for further research.

Starting with specimen 4 of this thesis, the intestinal tract forms a horizontal halfspiral, which has two different parts, a voluminous part consisting of (both the ectodermal section of) the esophagus and the stomach as well as the intestinal outgrowth, which emerges from the stomach on the dorso-right side and grows out within the mesenteries. The outgrowth thereby extends within *ecm* located between the right extension of the oral somatocoel and the two lobes of the aboral somatocoel (Fig. 45A).

## 4.8 The fate of the somatocoelia: Formation of the axial mesentery and of the aboral lacuna

In the newly formed area within the center of the intestinal coil in specimen 4, which is called the axial compartment in this thesis, resides the *glandular axial organ*, which is formed by a tubular structure and its surrounding hemal space as well as the latter's covering coelothel, which constitutes the axial mesentery (Fig. 45C). The careful analysis of the epithelial layers (pp. 90-93) showed that the oral somatocoel is continuous with the innermost space formed between the *glandular axial organ* and the intestinal bend, and that the left lobe of the aboral somatocoel extends into the axial compartment. As there has been no horizontal mesentery in the axial compartment of specimen 4, the axial mesentery was initially assumed to represent a remnant of this horizontal mesentery.

The present data derived from specimens 4 and 5 clearly show that the *ventral mesenteric formation* and the oral lacuna, respectively, derive from the horizontal mesentery and that the aboral lacuna derives from the *secondary aboral vertical mesentery*. Furthermore, the oral lacuna is orally formed by the oral somatocoel and aborally by the aboral somatocoel, and it is also clear that the distal layer of the *secondary aboral vertical mesentery*, and hence that of the aboral lacuna, derives from aboral somatocoel as well.

Since the oral somatocoel extends varyingly far within the two cystidean specimens 4 and 5, there is, however, unclarity about both the origin of the proximal layer of the aboral lacuna and the origin of the axial mesentery. Depending on the contribution of the oral somatocoel to the main body cavity, these lacunae may derive from either oral or (the left lobe's) aboral somatocoelomic epithelium.

It has been the task of this thesis to find evidence for the one or other developmental origin for the aboral lacuna and the axial mesentery, i. e. for the course of the oral somatocoel in the juvenile. This evidence, however, lies subtly hidden in the course and position of the mesenteric remnants and can only be derived retrogressively from these latter, considering several lines of evidence.

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In the following, two hypotheses on the extent of the oral somatocoel are presented. The first hypothesis reflects the coelomic state of specimen 5, but does not explain certain details that can be observed in both specimen 9 and in the juvenile. These latter findings are applied in the second hypothesis, leading to an alternate interpretation of the juvenile's lacunar state than presented in the results of specimens 4 and 5 (Fig. 44).

### 4.8.1 Fate of the oral somatocoel – inferred from the cystidean stage

In specimen 5, the axial extension of the oral somatocoel does not reach to the aboralmost point of the calyx but extends only to the aboralmost end of the intestine (Fig. 48F). The *secondary aboral vertical mesentery* of this larva has therefore been interpreted as being formed by the aboral somatocoelothel only (Fig. 44H). The axial mesentery, hence, is formed by the lining of both the oral and the aboral somatocoel, representing a horizontal-mesentery-analogous structure. This arrangement allows the following conclusions on the oral somatocoel's axial extension:

(a) The intestine tilts together with the oral somatoel around the central axis, forming the axial compartment.

(b) The axial extension of the oral somatocoel extends within the axial compartment, between the axial mesentery and the intestinal bend.

(c) The tilt of the oral somatocoel thereby has the effect that the oral section (Figs. 15C, 44i) is formed by the oral somatocoel's clasping oral layer. This provides an explanation why the oral part of the axial mesentery does not have a mesenteric, but rather a lacunar character and extends vertically along the oral part of the esophagus, not taking part in the axial mesentery's twist.

(d) The axial muscle strand, which extends within the axial mesentery of all larvae older than specimen 4, is an indicator of the formerly orally situated layer of the oral somatocoelomic lining. In the aboral section, it continues within aboral somatocoel.

These conclusions form the basis for the reconstruction of the juvenile as presented in the results (Figs. 15-19), in which the coloration of the lacunae has been chosen to match that of specimen 5, allowing an easier initial comparison between the structures of interest and to initially facilitate nomenclature (Figs. 15 and 44G). In this reconstruction, mesenteries that might derive from the aboral somatocoel only have been colored *light coral* those that might form between both somatocoelia are colored white. This interpretation of the lacuna's mesenteric origin leads to an extent of the two somatocoelia as shown in the Figures 59 and 60, and to the lesser extent of the oral somatocoel depicted in Figure 61: the aboral somatocoel's left lobe extends far into the axial compartment and forms the outer layer of the axial mesentery while the oral somatocoel is restricted to the space behind the axial mesentery. It thereby has to be noted that the oral somatocoel is reconstructed to tip down to the left of the axial muscle. This course is the outcome of a combination of three different sources: First the initial assumption, that the axial mesentery represents the border between the oral and aboral somatocoel, second the fact that the axial mesentery in the juvenile, other than in specimen 5, is twisted in itself, and third the interpretation of the axial muscle strand as given in conclusion (d). Following this reconstruction, the axial mesentery topologically resembles the horizontal mesentery, as it is formed by layers of both somatocoelia.

### 4.8.2 Fate of the oral somatocoel – inferred from the late pentacrinoid stage

There is, however, evidence distributed throughout the other larvae investigated, that the mesenteric state of specimen 5 does not fully apply to the juvenile.

The first line of evidence is that the oral somatocoel in specimen 4 reaches down to the aboral end of the main body cavity. The same holds true for the late pentacrinoid, in which there are two distinct spaces at the bottom of the main body cavity (Fig. 55), which are, however, completely fused further orally towards the intestinal tract. Their fusion point marks the aboral end of both the axial mesentery and the aboral lacuna. These distinct spaces are separated by one continuous mesenteric structure that is attached to the body wall at *two* different sides. This highly organized aboral mesenteric structure has to derive from broken down tissue, since all trabeculae, which represent secondarily formed structures and locate in the aboral third of the calyx, extend rather unorganized and comparatively straight between different locations of the body wall. The occurrence of two different spaces that are separated as described can only be explained by the involvement of *two different* coelomic cavities. The first has to be the interradially clasped aboral somatocoel, and the second space has to be the oral somatocoel, which,

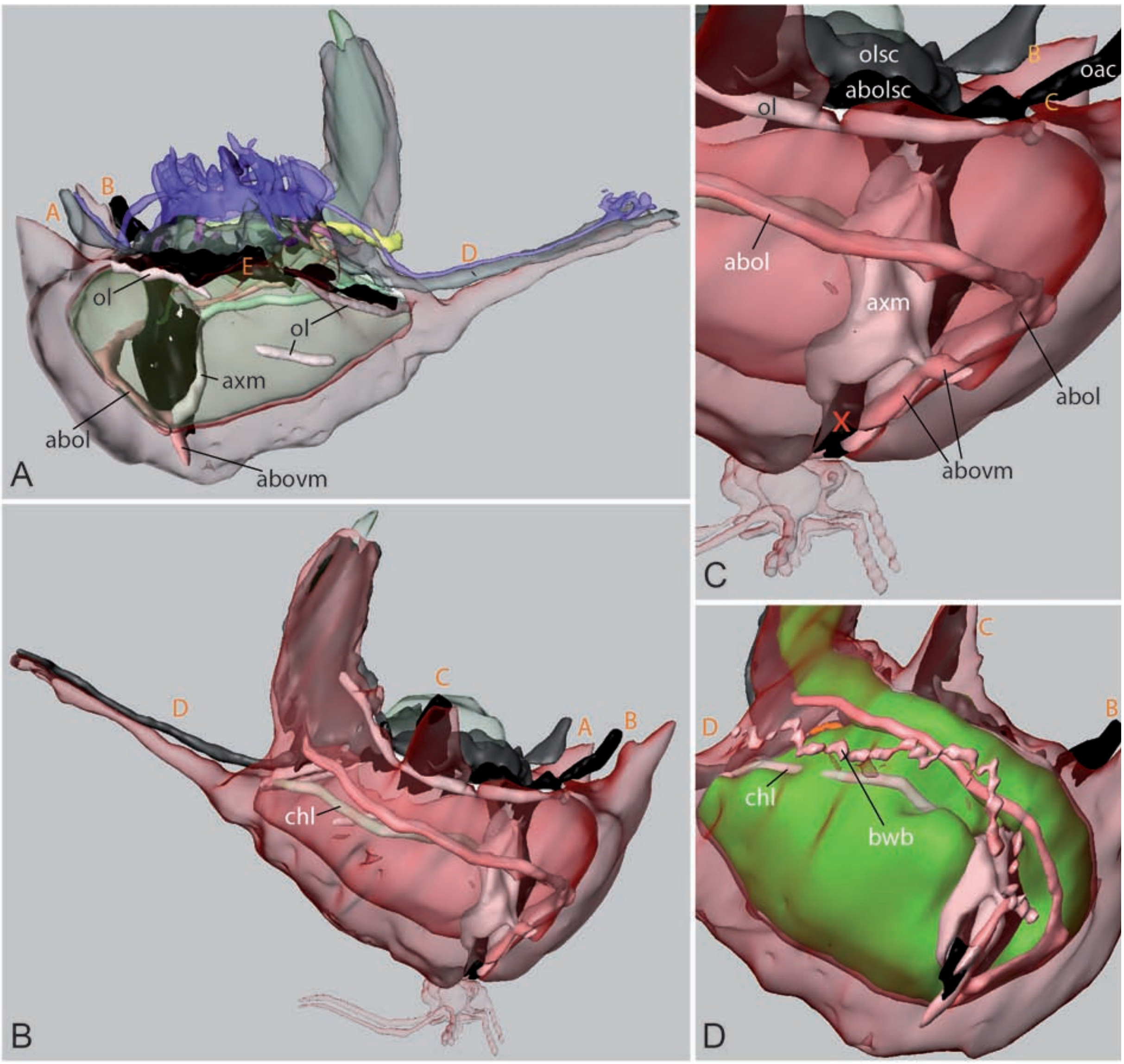

**Fig. 59:** Juvenile *A. bifida*, specimen 10. Course of the aboral somatocoel and oral somatocoel inferred from the cystidean specimen 5. *abol* aboral lacuna, *abolsc* oral somatocoel below oral lacunar meshwork (***black***), *abovm* aboral vertical mesentery, *axm* axial mesentery, *bwb* body wall bulb, *chl* centro-horizontal branch of the oral lacuna, *oac* oral section of the arm coelom, *ol* oral lacuna, *olsc* oral somatocoel above oral lacunar meshwork (***lighter gray***), aboral somatocoel (***red***), *red X* course of the oral somatocoel derived from the related hypothesis, *orange capital letters* radii.

hence has to be assumed to be inserted between the *aboral vertical mesentery*'s layers, i.e. spreading the latter.

A simple thought experiment, which starts with the mesenteric state of specimen 3, helps to understand this conclusion: The *aboral vertical mesentery* of this larva, being formed by the aboral somatocoel only, derives from the center of the chambered organ and extends from interradius BC oralwards along the enteric sac (Figs. 42E, 58). The exclusion of an involvement of a second cavity during intestinal tract formation led to only two potential aboral mesenteric patterns, which are both different from the pattern observed: Firstly, if the aboral somatocoel's left lobe merely extended towards the right lobe, repressing the latter, a (curved) mesentery would be formed, which still extended in distal direction from the center of the chambered organ. Even if, secondly, one of the two lobes tipped down behind an aborally unaltered section of the *ab-*

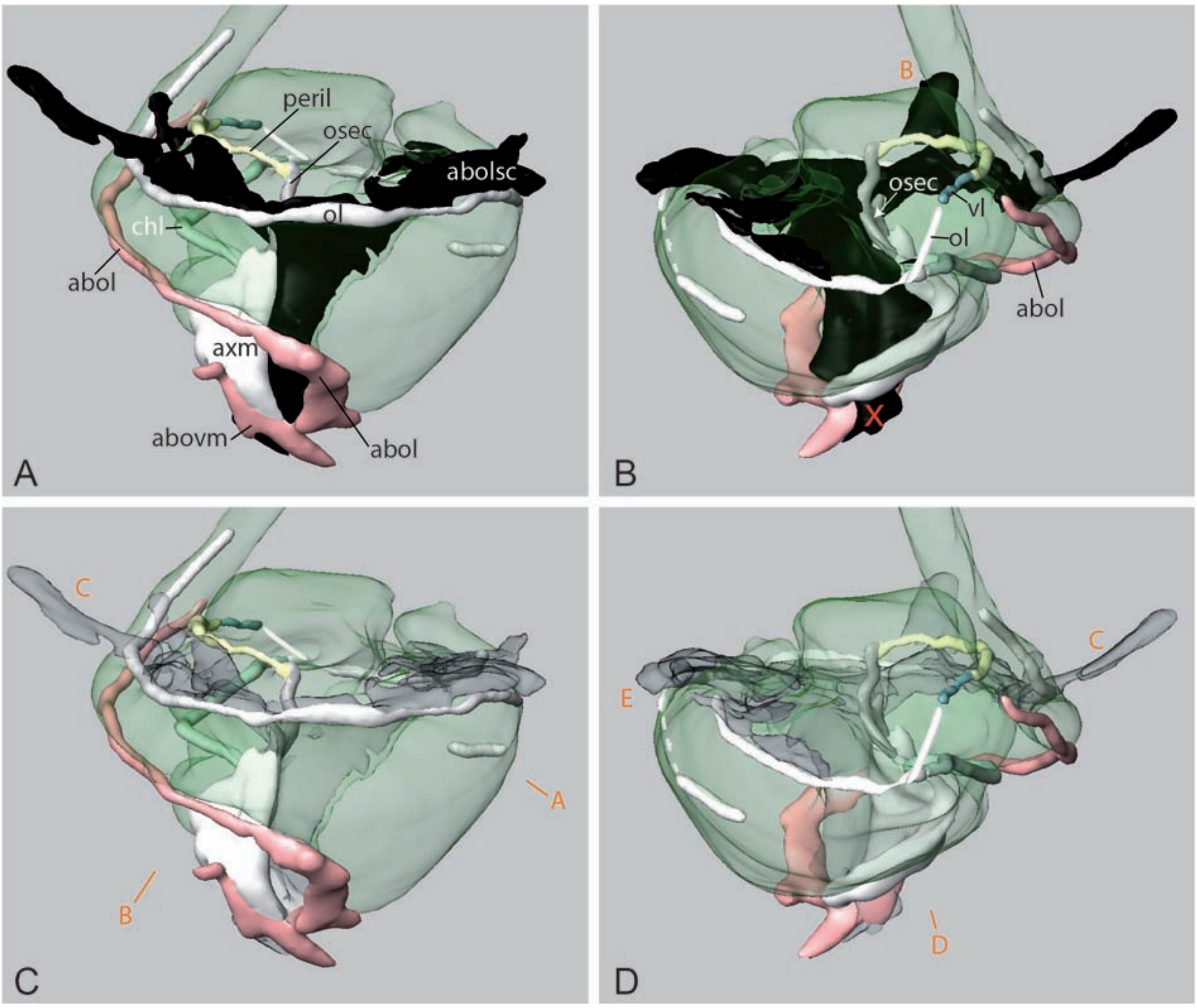

**Fig. 60:** Juvenile *A. bifida*, specimen 10. **A, C** Dorso-right view and **B, D** ventro-left view onto a reconstruction, in which the course of the aboral extension of the oral somatocoel is inferred from the cystidean specimen 5. The oral somatocoel above the oral lacunar meshwork is not shown. Note that the aboral lacuna is formed by aboral somatocoelothelium only along its whole way, therefore representing a remnant of the secondary aboral vertical mesentery. The central section of the axial mesentery, however, is formed by both somatocoelothelia and therefore represents a horizontal-mesentery-analogous structure. *abol* aboral lacuna, *abolsc* oral somatocoel below oral lacunar meshwork (***black***), *abovm* aboral vertical mesentery, *axm* axial mesentery, *chl* centro-horizontal branch of the oral lacuna, *ol* oral lacuna, *osec* oral section of the axial mesentery, *peril* periesophageal, lacuna, *vl* ventral lacuna, ***green*** intestinal tract, *red X* course of the oral somatocoel derived from the related hypothesis, *orange capital letters* radii.

*oral vertical mesentery*, one (curved) interradial line *and* a distinct ring (consisting of more than two coelothelial layers) had to be expected – and not a pattern as observed. These outcomes of the thought experiment, which are not supported by the present data, are depicted in the scheme provided in Figure 63, which is based on the developmental mode of the *glandular axial organ* that Seeliger (1893) suggested as will be explained below.

The second line of evidence for the alternative mesenteric state in the juvenile is provided by the axial muscle, which extends in the axial mesentery of all larvae older than specimen 4 and in the juvenile. It thereby seems to represent a landmark that allows identification of all mesenteric remnants, in which it occurs: In specimens 5, 9 and the juvenile (Figs. 15, 44D, 55), the muscle extends within that part of the mesentery, which stretches in interradius BC, i.e. in the original part of the *aboral vertical mesentery* or in the aboral section of the axial mesentery, respectively

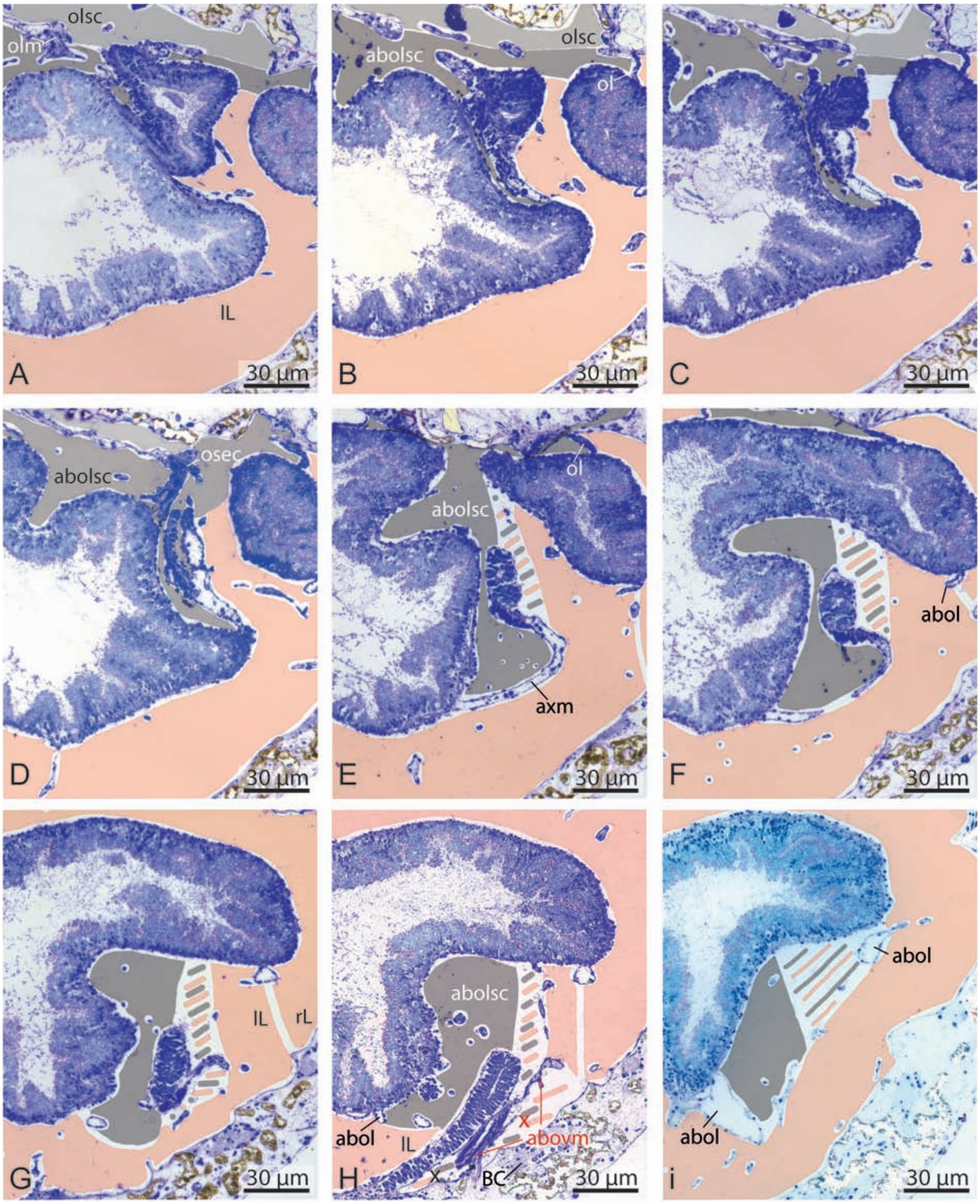

**Fig. 61:** Juvenile *A. bifida*, specimen 10. Interpretation of the former course of the fused coeloms of the main body cavity. **A-i** Sequence of serial sections, whose position in the animal is indicated by Figs. **16J, 16K**. Striped area indicates the optional assignment of the respective area depending on the hypothesized scenario. Lesser extent of the oral somatocoel thereby genererally represents the latter's area assumed for Figs. **59** and **60**, the larger extent represents its area assumed for Fig. **62**. **H** *red X* assignment of this area to the oral somatocoel represents the extent in Fig. **62**, *black X* assignment of this area to the oral somatocoel represents the extent in Figs. **59** and **60.** *abol* aboral lacuna, *abovm* aboral vertical mesentery, *axm* axial mesentery, *BC* interradius BC indicated by the nervous tissue strand, *abolsc* oral somatocoel below oral lacunar meshwork (***darker gray***), *lL* left lobe of the aboral somatocoel (***red***), *rL* right lobe of the aboral somatocoel (***red***), *ol* oral lacuna, *olm* oral lacunar meshwork, *olsc* oral somatocoel above oral lacunar meshwork (***lighter gray***).

(Fig. 15C). Further orally, it stretches within the proximal part of the axial mesentery's central section, in which it extends all the way up to the aboral end of the oral section.

Combining these two lines of evidence drawn from specimen 9 shows, that the end of the oral somatocoel extends around the axial mesentery and has to reside to the right of the axial mesentery – and not to its left, as initially concluded from specimen 5 (compare Figs. 55, 59C).

These observations have been incorporated in the reconstruction of the juvenile (as represented by the larger extent of the oral somatocoel depicted in Figure 61), leading to a mesenteric state, in which the axial mesentery is largely formed by the oral somatocoel (Fig. 62). Based on this reconstruction, the following alternative conclusions on the origin of the axial mesentery and the aboral lacuna can be drawn:

(a) The intestine tilts together with the covering oral somatocoel around the central axis, forming the axial compartment.

(b) The axial extension of the oral somatocoel extends within the axial compartment between the axial mesentery and the intestinal bend and may extent aborally *within* the *aboral vertical mesentery* as well.

(c) The oral section is *always* formed by oral somatocoel only.

(d) The axial muscle strand in fact is not necessarily an indicator of the presence of oral somatocoelothel. In the aboral section, it runs in a mesentery formed by either aboral and aboral somatocoelothel or oral and aboral somatocoelothel; in the central section it runs within either aboral and oral somatocoelothel or within oral somatocoelothel only.

(e) The aboral section of the axial mesentery is either identical to the *aboral vertical mesentery* (specimen 5), or develops from both somatocoelia, constituting a horizontal-mesentery-analogous structure (specimen 9, juvenile).

(f) The central section, however, is *never* formed by the (original) *aboral vertical mesentery*, i.e. by the aboral somatocoel only. Its distal part, in which the tubule of the *glandular axial organ* lies, may often be formed by the oral somatocoel only. Its proximal part, however, *may* partially constitute a horizontal-mesentery-analogous structure.

(g) The *secondary aboral vertical mesentery* and the aboral lacuna, respectively, do *not* necessarily derive from aboral somatocoelomic layers only. Instead, its aboralmost part may be formed by a horizontal-mesentery-analogous structure, whereas that part along the intestine that locates clockwise of interradius BC directly derives from the *aboral vertical mesentery*.

It is thereby intriguing, that the body wall bulges in the juvenile, which is interpreted to indicate the aboral end of the *secondary aboral vertical mesentery*, starts exactly where the border between these two parts is postulated – a fact that I discovered only *after* I reasoned the border from the statements (a) to (g). It may be also added that a subsequent horizontal view of the vertical sections of the juvenile (generated as projections similar to the pictures shown in

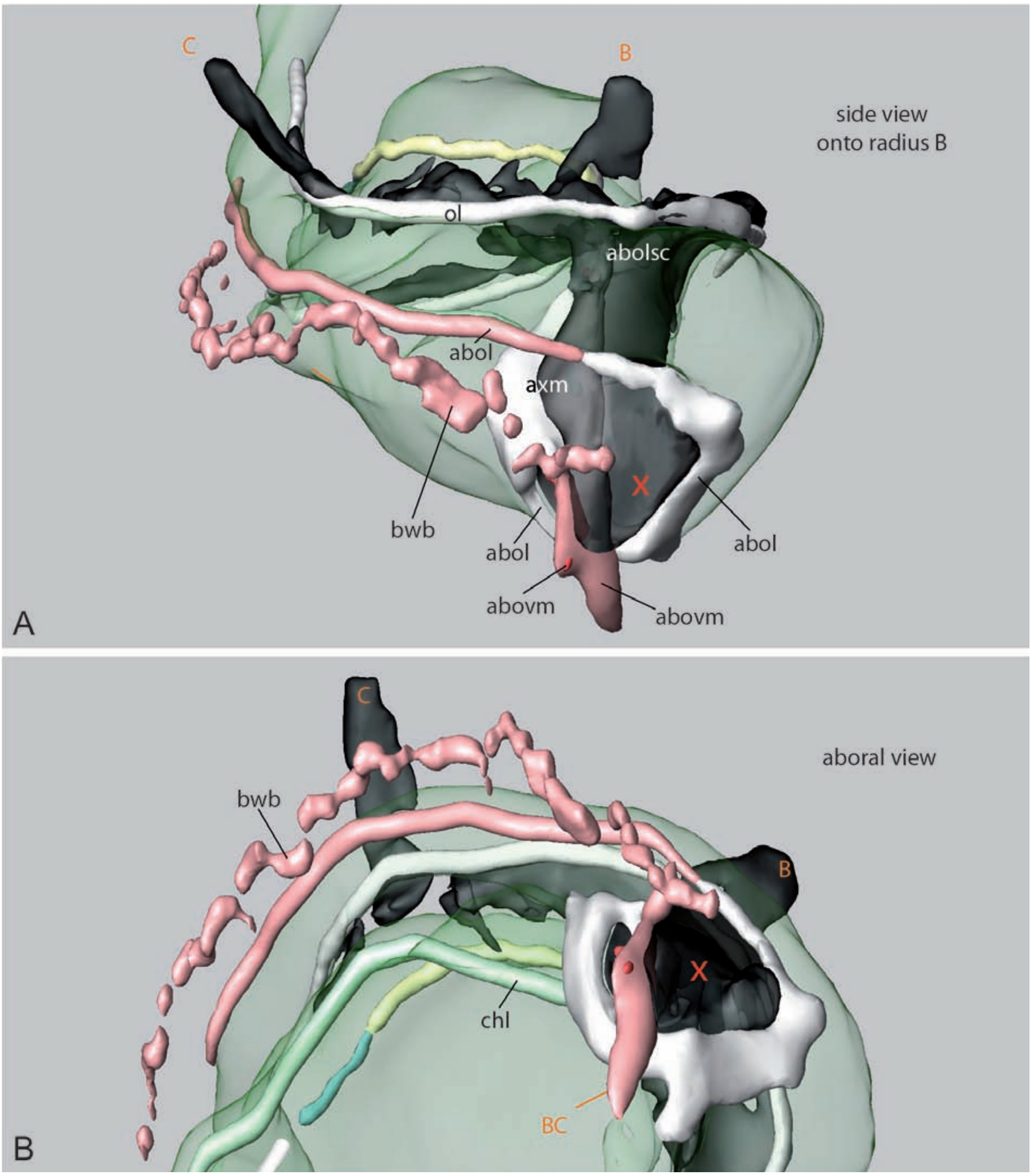

**Fig. 62:** Juvenile *A. bifida*, specimen 10. **A, B** Reconstruction based on the assumption that the aboral course of the oral somatocoel (***black***) equals that of specimen 9 (Fig. **55**). The axial muscle (***crimson red***) therefore is not a specific marker for the oral somatocoel'a oral layer. Note, that the extent of the oral somatocoel within the aboral vertical mesentery (*abovm*) has an impact on the aboral lacuna's (*abol*) coelomic origin, since it is formed by the oral somatocoel. The oral lacuna (*ol*) partially derives from a horizontal mesentery-anologous structure. *abolsc* oral somatocoel below oral lacunar meshwork, *axm* axial mesentery, *bwb* body wall bulb, *chl* centro-horizontal branch of the oral lacuna, ***green*** intestinal tract, *red X* course of the oral somatocoel derived from the related hypothesis, *orange capital letters* radii.

Fig. 49) showed aboral structures comparable to those in specimen 9, supporting this approach (data not shown).

The varying extent of the oral somatocoel found in cystidean stages of similar age (specimen 4 and 5) points to a general developmental plasticity concerning this feature. It may well be possible, that the formation and origin of the axial mesentery varies considerably amongst individual crinoids. This plasticity, however, supposedly does not interfere with the general finding that the oral somatocoel is involved in axial mesentery formation and hence, in the formation of the epithelial sheath of the *glandular axial organ*.

### Critical evaluation of Seeliger's (1893) description of the somatocoel

Either of the above hypotheses differs considerably from the observations that Seeliger (1893) described for *A. mediterranea*. Following him, the axial mesentery develops independently from the oral somatocoel, basically as a lacunar fold that develops *de novo* between the intestine and the visceral layer of the aboral somatocoel's left lobe. Not being able to verify or falsify his data *in toto* due to a lack of the stages necessary, it is nonetheless possible to evaluate most of his observations in the context of the data presented in this thesis.

Up to a larval stage similar to specimen 2, Seeliger's (1893) and the here presented data are congruent except for the presence of the axocoelomic process, which Seeliger (1893) did not recognize.

The first divergent data arise in stages similar to specimen 3. Following Seeliger (1893), the left lobe of the aboral somatocoel extends around the ventral side of the intestine, taking up the space between the latter and the aboral somatocoel's right lobe. Seeliger (1893) underlays this interpretation with a figure of a cross-section, derived in aboral position in the respective larvae (Fig. S120, appendix), and vertical sections of two different larvae of similar age (Figs. S129, S134, appendix). For none of these larvae does he explicitly state having seen the intestine having grown out. While his interpretation of the aboral somatocoel's left lobe in the cross section may be correct, he may have misinterpreted the left lobe's course further orally, as can be demonstrated on the vertical sections.

In these, Seeliger (1893: 294) claims having seen the aboral somatocoel altering considerably in its extent between individual larvae that have the same age. In the example related to his Figure 134 (Fig. S134, appendix) he states that he has seen the right lobe of the aboral somatocoel extending very far, namely as far as the primary stone canal. However, Seeliger (1893) locates this extension of the aboral somatocoel in a position, in which the present results locate the *small ventral coelom* – a cavity that he did not recognize in his work at all. The *small ventral coelom*, however, has been proven to exist in *A. mediterranea* by Russo (1902: *seno aborale*), who recognized it in cystidean stages that posses an open vestibulum (see below).

A similar contradiction between Seeliger's (1893: 295) observation and the results of this thesis opens up in the context presented in his Figure 129 (Fig. S129, appendix). Here, Seeliger (1893) describes that he has seen the oral somatocoel extending between the two lobes of the aboral somatocoel, i.e. into the *aboral vertical mesentery*, so that the aboral somatocoel's left lobe extends between the oral somatocoel and the stomach. In addition, he describes the *glandular axial organ* extending between the right extension of the oral somatocoel and the left lobe of the aboral somatocoel. This latter aspect related to the *glandular axial organ* will be discussed later.

However, Figure 42E of this thesis allows a different interpretation of his data, once again being based on a cavity, whose extent Seeliger (1893) recognized differently than the present data suggest, namely the axocoel. Following the here presented data, the axocoel extends around the esophagus' right side, between the latter and the aboral outgrowth of the oral somatocoel's right extension and may be of considerable size in the region of interest, as Figure 43B2 shows. This latter figure shows the optional interpretation of Seeliger's (1893) Figure 129, which opens up, if the presence of the axocoel in this area is assumed to exist in *A. mediterranea* as well. The sections of specimen 3 (Fig. 43) give a good impression on the real quality of the epithelia Seeliger (1893) must have been confronted with in the respective stages.

In addition to these stages, Seeliger (1893) describes a series of vertical sections (Figs. S124-S126, appendix), in which the oral somatocoel clearly resides in a position above the enteric sac similar to the one in specimen 3, stressing that the crescent of the oral somatocoel has a more disc-like appearance than in previous stages, since the oral somatocoelomic cover on top of the dorsal half of the enteric sac extends progressively towards the ventral side.

So, from stages of similar age, Seeliger (1893) got the impression that the oral somatocoel resides in oral position and that the aboral somatocoel extends along the enteric sac on the whole ventral side. If the overall picture, which Seeliger (1893) developed on the extent of the somatocoelia, and which builds the foundation of his description on the *glandular axial organ*, derived mainly from these data, he clearly might have misinterpreted the course of the aboral somatocoel in this young stage: All those data which Seeliger (1893) - based on his overall picture - presents as a developmental sequence in his work is represented by stages similar to specimen 3 in this thesis.

So, as has been just shown, Seeliger's (1893) potential misinterpretation leaves room for the here presented hypothesis that the oral somatocoel takes part in the formation of the axial compartment.

The next oldest stage that Seeliger (1893) investigated is 8 hours older than the previous ones. From this stage, Seeliger (1893) depicts a section of the middle intestinal region (Fig. S127, appendix), whose outline of the prospective *glandular axial organ* already resembles horizontal sections of specimen 5 in this region (as can be indirectly taken from Fig. 49D)

Seeliger (1893) comments this stage as being farther developed than the specimen depicted in his Fig. 120 (Fig. S120, appendix), not stating anything about the progress of its intestinal outgrowth, although a certain progress seems to have taken place in this stage. As there is no intermediate stage other than this depicted by Seeliger (1893), especially not one that has been cut vertically, it may, in fact, be *possible*, that *Seeliger's (1893) work contains a similar gap in data as this thesis does* – and that Seeliger (1893) closed this gap by presenting the data his way, applying the potentially wrong overall picture, which he derived form earlier stages.

The ages of the larvae observed by Seeliger (1893), however, show that both the formation of the postulated aboral extension of the oral somatocoel and the dissolution of the horizontal mesentery in the axial compartment must happen in less than 6 hours.

### 4.8.3 Corroboration of Seeliger's (1893) description of the initial formation of the glandular axial organ

The development of the *glandular axial organ* has last been described by Seeliger (1893), whose results were summarized in detail by Clark (1921) and later by Hyman (1955: 83), who summarized its development with one statement: "*The axial gland [=glandular axial organ]* is said to arise in the "*aboral vertical mesentery*", in which she did not mention his detailed remarks on the formation process.

The earliest stage in which Seeliger (1893) describes having seen the *glandular axial organ tubule* in *A. mediterranea* is a 12 hours old larva (age after hatching), obviously a recently settled cystidean (Fig. S117, appendix). Following him, the tubule of the *glandular axial organ* develops at the anteriormost end of the left lobe of the prospective *aboral vertical mesentery*, directly behind the area, in which the tubes of the prospective chambered organ arise. Initially, the cells shall be swollen, but he also observed initial stages of cell divisions, which lead him to think, that the region will soon become multilayered. The next stage Seeliger (1893) described is a dolilaria of 52h, which he found to be comparatively far developed regarding its *glandular axial organ*. In this stage, he found the left layer of the *aboral vertical mesentery* to be thickened and bilayered, and he saw a strand of cells that advanced towards the center of the chambered organ, namely between chambers 1 and 5 (Figs. 63A$_1$; S96B, appendix). He identified this cellular strand as the anterior (=aboral) end of the *glandular axial organ*, i.e. as its tubule, since it does not extend further between the tubes of the chambered organ.

Based on this description, the respective region in the *A. bifida* doliolaria has been investigated thoroughly. The data of specimen 1 show a bilayered area in the ventral layer of the right somatocoel's left lobe, at whose centralwards directed end delaminating cells reside (Fig. 26D). The locally dissolved basal lamina is an intriguing sign for a soon-to come detachment of the cells (Fig. 28). The similarity between these observations with those of Seeliger (1893) stress the view that the bilayered area and the delaminating cells together represent an initial stage of the cell strand. This is additionally supported by the results obtained in specimen 2 of this thesis, in which a small conical cavity can be found in the center of the chambered organ's tube's oral end. The related data show that very early, in late doliolaria to early cystidean stage, a cavity is formed, which kind of "rests" at the aboral end of the *aboral vertical mesentery*. The undifferentiated basal lamina of the metamorphosing larva, which will be seen around the tubule in later stages, points to its recent formation. So far, the here presented results on the doliolaria of *A. bifida* strongly stress the validity of Seeliger's (1893) observation.

The following findings, however, differ between his and the present study and lead to completely different conclusions.

### Critical evaluation of Seeliger's (1893) description of the further glandular axial organ formation

Seeliger (1893) documents his further results on the *glandular axial organ* development with several cross-sections of findings, which let him come to the conclusion, that the *glandular axial organ* develops by means of some kind of continuous delamination (or a constriction, since he uses the word "Abschnürung", which literally means constriction, but his following drawings give the impression that he must have somehow thought of delamination as well) from that mesenteric layer, which forms the left lobe of the aboral somatocoel. He describes this process as proceeding from aboral to oral, giving several examples on how the structures looked like at different places in the larva. Figure 63 provides a scheme that depicts the developmental mode Seeliger (1893) must have had in mind. Aborally, he describes a thickening of the mesenteric layer of the left lobe (Figs. $63A_2$; S120, appendix) and also a beaded rim in the center of the *aboral vertical mesentery* in another larva of similar age (Figs. $63A_2$, $63A_3$; S131, appendix), giving them the context of being successor-structures. This development leads to a stage, in which "the *glandular axial organ* occupies the entire extent of the ventral side of the inner wall of the aboral coelom. In the most anterior [=aboral] part, near the chambered organ, it lies as a solid cord of cells on the vertical mesentery [as in Fig. S131, appendix]. Further posteriorly it becomes more prominent, standing out as a pleatlike thickening lying on the intestinal tract [Figs. $63A_3$, $63A_4$; S127, S145, S146, appendix]. At the posterior [=oral] end is the youngest developmental stage" (Clark 1921, translating Seeliger 1893). Furthermore, Seeliger (1893) states that the whole structure is bent like a sausage.

Again, as already pointed out for the development of the somatocoelia, Seeliger (1893) thereby gives the impression that he *has* investigated larval stages of intermediate age *between* the here described specimen 3 and 4, and suggests that he has actually *seen* the aboral somatocoel being the only structure involved in axial organ formation. In order to investigate on which basis of data Seeliger (1893) founded his description, I decided to minimize confusion by arranging all available horizontal drawings in concert with their related original descriptive information, extracted from both the text and the explanation of the figures in a way that the relative age of the related larva (X) and the approximate horizontal level of the depicted structure within the larva (Y) are plotted against each other. Furthermore, I gathered all related information concerning their relative developmental progress to other larvae described. The resulting collages are attached in the appendix. This kind of presentation, again, shows clearly, that there is no proof that Seeliger (1893) *must* have seen larvae of intermediate age. All figures of young larvae have been drawn from sections of the aboralmost end and show structures that *could* be expected following the here presented oral-somatocoel-axial-extension-hypothesis at exactly this place as well. Since Seeliger (1893) tries to show a progression, he selects more oral sections from

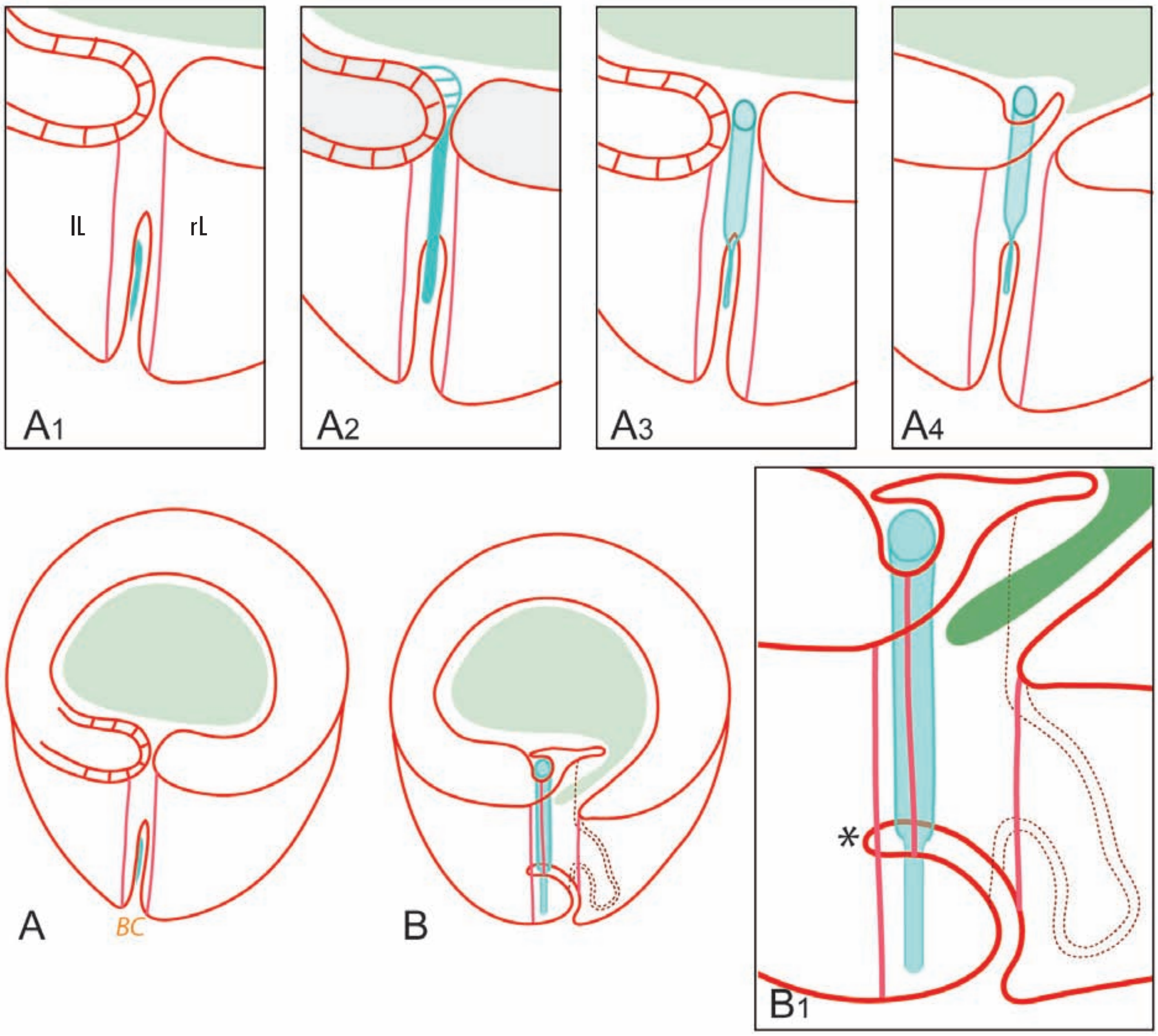

**Fig. 63:** Schematic illustration of the formation mode of the glandular axial organ as described by Seeliger (1893). Shown is the aboral part of the calyx below the oral somatocoel. Following Seeliger (1893), the glandular axial organ successively constricts in aboral to oral direction from the left lobe (*lL*) of the aboral somatocoel (***red***). The oral somatocoel does not extent into the axial compartment. **A**$_1$ (detail of **A**) The tubule (***turquoise***) is initially formed by delamination near the chambers of the chambered organ (chambers not shown; see also **26**, **S96B** (appendix)). **A**$_{2,}$ **A**$_3$ Delamination or constriction proceeds oralwards, the aboral part of the tubule is formed first (**S131**, **S120**, appendix). **A**$_4$ Later, the tubule has to be overgrown by the left lobe in order to achieve its observable position (**S127**, appendix). **B**$_1$ (detail of **B**) *black asterisk* When following Seeliger (1893), the growth of the oral somatocoel's left lobe towards the right side can only lead to a state, in which the aboral vertical mesentery extends in one direction from the center of the chambered organ, regardless of its exact shape. The resulting mesenteric pattern does not match the aboral pattern observed in **55**. *dotted line* thought-experiment, in which the left lobe grows over the aboralmost section of the aboral vertical mesentery and tips down behind the latter. This formation mode represents the only way, in which a second distinct space could form following the formation mode suggested by Seeliger (1893). It led, however, not to a mesentery consisting of two epithelial layers. *rL* right lobe of the aboral somatocoel, *BC* interradius, in which the aboral vertical mesentery occurs, ***green*** position and course of the intestinal tract.

older larvae. Again, the *glandular axial organ* in the latter stages looks very similar to the developmental state of the related regions found in the present thesis' older larval stages'.

The course of the *glandular axial organ* Seeliger (1893) depicts in his Figure 129 (Fig. S129, appendix), and which has already been mentioned above, is confusing. As already argued above, it can be doubted that the cavity, which resides between the *glandular axial organ* and the oral somatocoel in this figure, represents the aboral somatocoel, but rather the axocoelomic process. Regardless of the identity of this cavity, the course of the *glandular axial organ tubule* depicted by Seeliger (1893) simply doesn't make sense, not even in the context of the developmental mode Seeliger (1893) suggests for the *glandular axial organ* (Fig. 63). A scenario, in which the *glandular axial organ* forms within the *aboral vertical mesentery,* arises in a position between oral and aboral somatocoel as depicted, *and* finally comes to lie between the visceral layer of the aboral somatocoel and the intestine is highly unlikely.

What is fascinating is the discovery that Seeliger (1893) in fact did see the periesophageal lacuna, which he depicted in his Figures 145 and 146 (Figs. S145, S146, appendix) of a larva that seems to have about the same developmental progress as specimen 5, probably a bit less. In these specimens, however, he identified the periesophageal lacuna as the oralmost part of the *glandular axial organ* (the *"pleatlike thickening lying on the gut"* mentioned above refers to this section) and, very interesting, Seeliger (1893) had understood this part as deriving from the horizontal mesentery (Fig. S145, appendix), i.e. being partially formed by oral somatocoelomic tissue. So, contrary to the view presented in this thesis, Seeliger (1893) understands the periesophageal lacuna not only as *connected* to, but as *part of* the *glandular axial organ* (compare Fig. 44C).

Seeliger's (1893) findings related to his Figures 151, 152 and 153 (Figs. S151, S152, S153, appendix), however, prominently show the way he dealt with the available data, especially with his observations concerning the formation of the *glandular axial organ tubule*. He describes the younger developmental stages of the *glandular axial organ* as being located oralmost, and as represented by the pleatlike thickening cited above (the periesophageal lacuna). These stages shall only later be constricted from the thickening. In order to prove this, he depicts longitudinal cross-sections of the periesophageal lacuna which he describes in detail (Figs. S152, S153, appendix), and compares it to a cross-section, which he, to my opinion, seems to have taken from the oral section of the *glandular axial organ* of a much older stage (Fig. S151, appendix). The here presented data, however, show, that this developmental step from the periesophageal lacuna to the *glandular axial organ* never takes place that way within the older larvae.

I am therefore convinced that Seeliger (1893), although having correctly seen the delamination in the youngest larval stages, might have overinterpreted several of the structures observed, because he, probably not having seen intermediate stages, simply had no other explanation for their development. Being fully aware of the fact that my data possess as many gaps as Seeliger's (1893) and therefore certainly do not have the final say, I will, however rely on the advantage, which the recent methods provide, and therefore will not follow Seeliger's (1893) account and will present a new interpretation.

### 4.8.4 Development of the glandular axial organ within the axial mesentery

The data procured in this thesis suggest the following developmental mode for the tubule of the *glandular axial organ* after metamorphosis: If the oral-somatocoel-axial-extension-hypothesis were true, the only reasonable explanation for the rise of the tubule in the axial mesentery would require that the tubule remains in an aboral position until the axial extension is set up and that it extends into it (as well as into the stalk) thereafter. The observations that (1) the prospective tubule still resides in aboral position in specimen 3, not showing any sign of extending along the *aboral vertical mesentery*, and (2) that there is just one long continuous tubule within the axial mesentery in both specimen 4 and 5 as well as in the aboral part of the juvenile's *glandular axial organ*, stress this impression.

Beginning with specimen 4, the tubule also extends into the stalk, but it has not been investigated how far it reaches aborally. In specimen 2, halfway of the stalk, cells could be observed that were attached to each other by cell contacts and jointly surrounded by a basal lamina (Fig. 40A). These cells have not been there in the dolilaria. This formation, however, seemed to be independent from the prospective tubule at the chambered organ’s oral end. Based on the present data, it is, however, impossible to give any statement whether, and if so, to which degree, this structural formation contributes to the *glandular axial organ* of the detached juvenile. My impression, however, was, that the latter specimen’s tubule between the chambers consisted of only one piece, which was continuous with the tubule in the aboral half of the juvenile’s *glandular axial organ*.

## 4.9 Development of the axocoel and the axocoelomic periesophageal process.

In the doliolaria, the axocoel extends anteriorly from the hydropore along the larva’s ventro-left side, till it comes to lie in front of the cluster of epithelial cavities. In this anterior position, the axocoel is comparatively voluminous (Fig. 23). It sends a thin long process anteriorly, which runs more or less parallel to the chambers of the chambered organ. This thin anterior process is withdrawn in specimen 2 and its former position is probably represented by the axocoel’s aboralmost point to the right of the *small ventral coelom* (Fig. 35D). The right-most end of the voluminous part of the doliolaria's axocoel forms a short process, which has been depicted by Seeliger (1893) as well, without giving it further attention (Figs. 23; S68, appendix). (This right process seems to not be related to the short horn-like appendix, which is directed towards the right and posteriorly in very young doliolaria larvae of *A. mediterranea* (Seeliger 1893: 257), since the latter is reduced in older doliolaria larvae of this species.)

In the metamorphosing larva, a long slender periesophageal process appears around the enteric sac’s bump on the larva’s right side (Fig. 35). Based on its position, it must be interpreted as an extension of the short right process of the axocoel that has been seen in the doliolaria. This long slender periesophageal process has never been described before. In specimen 2 it is extremely thin and I would have missed it, if it hadn’t been as prominent in specimen 4. The course of the axocoel in specimen 4 let me search in specimen 3 and only as I clearly saw it continuously there, I was confident enough to search for it in specimen 2. The preservation of the latter specimen, however, is mediocre and the process’ course could not be traced in each and every section. Nonetheless, there were enough preserved localities along its way to state both its existence and approximate extent. There is no reason to believe that it is not continuous.

In specimen 3, the axocoel takes a nearly identical course as in specimen 2 and also forms the long slender periesophageal process. It may be important to note once again that it extends up to the enteric sac's oral somatocoelomic cover that resides dorsal to the bump. In specimen 4, in which the axial compartment is already formed and is passed by the prospective *glandular axial organ*, the process possesses a considerable size on the larva's right side. As I already pointed out in the related section of the results (pp. 88-90), there is still some uncertainty about its exact extent, as it extends on the larva's left side, too, and since it is not clear, whether there is a second clockwise directed process sent out from the axocoelomic process or not. But, one or other way, it seems to extend far around the esophagus and may even form a ring.

The prominence and extent of the axocoel in this larva has been a lucky incident (as it allowed me to discover the process in the younger larvae), but it also raises a lot of open questions which cannot be answered based on the data raised in this thesis.

It is, however, unclear how representative the observed axocoelomic state of specimen 4 for the development of all *A. bifida* larvae is. The comparatively empty intestinal tract, the unusual number of 10 tentacles as well as the flattened vestibular roof might direct towards an abnormally developed larva. On the other hand, the cells of the larva still contain yolk spheres, the larva possesses some additional orally directed hydrocoelomic extensions that initially bud against the vestibular cavity's proximal layer and, addressing the larva's shape, one also finds larvae of similar shape depicted by Perrier (1886) and Seeliger (1893), indicating that the "abnormality" might even be a stage-typical characteristic. Based on the data available it is, however, not possible to decide which developmental progress is finally vital or lethal. In any case, it has to be concluded, that the axocoel at least has the *potential* to extend like this in any larva.

Assuming that the extent of the axocoel in specimen 4 is basically normal for all *A. bifida* larvae, it is interesting to know its function or evolutionary reason. Of course, any ring-structure around the esophagus raises the attention towards the identification of the long-searched-for origin of gonadal structures. It would, however, be an incredible surprise if these were related to the axocoel. The wide extent of the axocoel therefore makes the most sense in the context of the multiple stone canals and madrepores that occur all around the esophagus. This issue will, however, be addressed again later in the discussions concerning the *oral lacunar meshwork* and the axial sinus development.

### Remark on a similarity with the Reichensperger's organ

Holland et al. (1991) describe the coexistence of two axial organs in the sea lily *Calamocrinus diomedae*; one is the *glandular axial organ* and the other the Reichensperger's organ, which has been found to exists in isocrinids as well (Reichensperger 1905, Heinzeller and Welsch 2009). The *glandular axial organ* of *C. diomedae* is described to have a similar extent and structure as the *glandular axial organ* in unstalked crinoids. In its oralmost part, the *glandular axial organ* is associated with the Reichensperger's organ in a way that both organs are lined up in one mesenteric structure, which is connected to the gut (Holland et al. 1991: Fig. 24); the Reichensperger's organ thereby comes to lie between the gut and the *glandular axial organ*. Between the two organs resides a “space”, which the authors interpret to possibly represent part of the axocoel. For this reason as well as for its histological arrangement, the authors suggest a homology to the axial organs of eleutherozoans.

In specimen 4 of this thesis, the axocoel has been found to slightly extend aboralwards within the oralmost part of the axial mesentery. This arrangement, in principle, represents a state similar to the arrangement in *C. diomedae*, since there is a coelomic space within the oralmost part of the same axial mesentery, in which the tubule of the *glandular axial organ* resides as well. This coelom thereby even locates between the tubule and the gut. The present findings therefore strongly support the interpretation of Holland et al. (1991) concerning the axocoelomic identity of the space between the two organs, since the coelom in the *A. bifida* larva clearly constitutes the axocoel. The fact that this arrangement of coelomic cavities is found in feather star larvae as well indicates that the Reichensperger's organ is reduced in feather stars.

## 4.10 The origin of the periesophageal lacuna

It has been clearly shown in this thesis that all pentacrinoid larvae older than specimen 4 as well as the juvenile possess a periesophageal lacuna, which extends along the right side of the esophagus. The periesophageal lacuna starts right underneath the point, where the axocoelomic duct fuses with the somatocoelia (and thereby contributes to the main body cavity), and extends until the oralmost end of the *glandular axial organ*. In specimen 5, the periesophageal lacuna locates on one level with the (prospective) oral lacuna that extends along the oral side of the intestinal outgrowth (Fig. 48).

As has already been discussed, the periesophageal lacuna has probably been seen, but not been recognized by Seeliger (1893). Russo (1902: 33-38) has been the first author, who put the periesophageal lacuna into the center of attention, and he already pointed out its connection to both the ventral lacuna and the *glandular axial organ*. He did, however, not recognize its developmental origin; in fact, he explicitly denied any developmental relationship between the two mesenteries (horizontal and aboral vertical) and the related intestinal lacunae in the pentacrinoid and stated that these lacunae form *de novo*.

As can be taken from the present data, there is a lot of evidence that all lacuna of *young* larval stages relate to former mesenteries, which themselves direct to the former extent of coelomic cavities. This initial formation mode can therefore also be assumed for the periesophageal lacuna.

There are two potential candidates that give rise to the periesophageal lacuna, either the horizontal mesentery or the *periesophageal mesenteric formation*. The former would, then, be assumed to extend between the later periesophageal lacuna and a position below the oral lacuna (position derived from Figs. 48F, 48G). The latter would probably form between the axocoelomic lining and the visceral layer of the oral somatocoelothel (position derived from Figs. 45B, 45C), but, due to potential developmental plasticity, the aboral somatocoelothel might also be involved in individual specimen.

In specimen 4, the axocoel is a fully intact coelomic cavity, while the horizontal mesentery is already largely dissolved in the axial compartment. In this specimen, there is no prominent structure, which can be interpreted as a potential prospective periesophageal lacuna. It can, however, not be excluded, that the positional information of the horizontal mesentery is maintained. Therefore, the reasoning for the one or other formation mode has been once again done retrogressively, considering the overall coelomic state of the juvenile and the various younger larvae.

Therefore, the – partially contradictory – arguments in favor of the one or other origin of the periesophageal lacuna will be listed, each argument followed by a brief discussion emphasizing the impacts of the respective line of arguments.

### 4.10.1 The horizontal mesentery as a potential candidate for the periesophageal lacuna

As there are no clearly traceable remnant structures of the horizontal mesentery in specimen 4, a developmental relatedness to the periesophageal lacuna cannot be discussed based on structural evidence. The two main arguments in favor of such an origin were, however, (1) the extent of the periseophageal lacuna, ending exactly at the *glandular axial organ* (since the axocoelomic process extends beyond), as well as (2) the periesophageal's position across the oral lacuna in specimen 5.

However, the assumption of a derivation of the periesophageal lacuna from the horizontal mesentery would basically exclude a derivation of the axial mesentery's oral section from mainly oral somatocoelothel, since the tilt were not likely to form anymore. Another counter-argument can be drawn from the juvenile, in which the periesophageal lacuna locates comparatively further oral to the oral lacuna than in specimen 5, indicating an asymmetric growth of different visceral coelothelial areas along the esophagus in respect to other areas of the animal. This successive oral dislocation can be traced in the subsequent larval stages (Figs. 13E-H, 48F, 51, 54). If the periesophageal lacuna derived from the horizontal mesentery, this asymmetric growth had an impact on the derivation of the *oral lacunar meshwork* (Fig. 64). On the larva's left side, the *oral lacunar meshwork* definitely extends within the former oral somatocoel, as the position of the oral lacuna (which locally still constitutes a mesentery) shows (Fig. 8A). On the right side, however, the *oral lacunar meshwork* had to then also extend through an area that had to

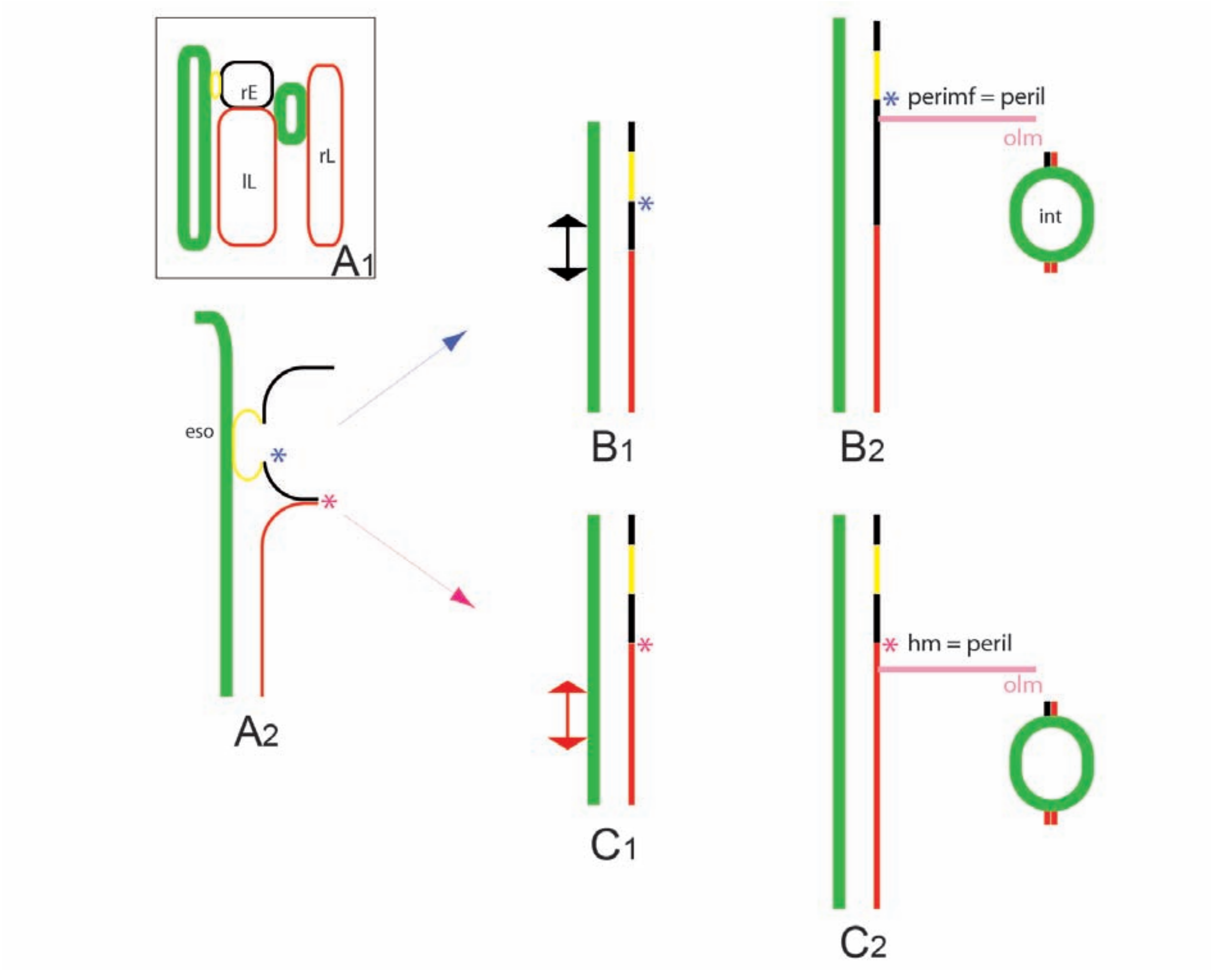

**Fig 64:** Schematic illustration of the two controversial origins of the perisophageal lacuna (*peril*) and their impact on the position of the oral lacunar meshwork (*olm*). **A**$_1$ Arrangement of the coelomic cavities on the larva's right side as derived from specimen 4 (Fig. **45C**), ventral view. **A**$_2$ Detail of **A**$_1$, showing both potential locations where the periesophageal lacuna could form from former mesenteric remnants. *blue asterisk* Location where the periesophageal lacuna derived from the periesophageal mesenteric formation's (*perimf*) aboral end, *pink asterisk* location where the periesophageal lacuna derived from the horizontal mesentery (*hm*). **B**$_1$ and **C**$_1$ Possible locations in which the asymmetric growth manifests depending on the origin (*double arrows*). An asymmetric growth as in **B**$_1$ leads to a more symmetric distribution of the oral somatocoel in the juvenile than that predicted in **C**$_1$. **B**$_2$ and **C**$_2$ The oral lacunar meshwork attaches underneath the perisophageal lacuna. Depending on the origin of the periesophageal lacuna, this leads to a different position and origin of the oral lacunar meshwork. Note the dislocation of the periesophageal lacuna relative to the oral lacuna's position compared to **A**$_1$. Position of the oral lacuna (black-red marking) and the aboral lacuna (red-red marking) are marked at the intestine (*int*). *eso* esophagus.

be attributed to the aboral somatocoel, since it attaches mainly below the periesophageal lacuna (Figs. 13G, 64C$_2$), i.e. to a region, which would then be derived from aboral somatocoelothel.

Since the findings on the aboral extension should not be ignored and as the consequential asymmetric extent of the *oral lacunar meshwork*, which is related to such an origin, had to be explained, there are substantial arguments against the horizontal mesentery being the origin of the periesophageal lacuna.

### 4.10.2 The periesophageal mesenteric formation as a potential candidate for the periesophageal lacuna

There is no trace left of the axocoelomic process in specimen 5. There are two different explanations on how the axocoelomic process of younger stages may vanish during development. One is, that it may be constricted from the larger axocoelomic cavity and be resorbed individually within the periintestinal *ecm*. The other is, that the mesenteric formation, which separates the axocoel from the somatocoel, may dissolve not only between the larger axocoelomic cavity and the somatocoel, but may also (continuously) dissolve between the axocoelomic process and the latter. As there is no evidence for the first explanation, the latter version will be followed in this discussion.

A mesenteric dissolution like that leads to two potential locations in which a lacuna may form; one resides at the formally oral border of the axocoelomic process and one at its aboral border (Fig. $64A_1$). The relative position and, especially, the lacuna's starting point underneath the axocoelomic cavity, match *exactly* the aboral border of both the axocoelomic cavity and the former axocoelomic process.

The main argument *against* a derivation of the periesophageal lacuna from the aboral limit of the *periesophageal mesenteric formation* would, most certainly, be the fact, that the former does not extend further than the *glandular axial organ*, although the axocoelomic process, at least in specimen 4, does.

However, assuming nonetheless that the periesophageal lacuna represents a remnant of the former axocoel (despite the differing extent of the axocoel in specimen 4 on the one hand and the periesophageal lacuna in older specimen on the other hand), the following conclusions can be drawn: In case the axocoelomic ring around the esophagus in specimen 4 constitutes a normal intermediate state, it would stand to reason that the periesophageal lacuna is *needed only* on the larva's right side, where it forms the shortest way between the ventral lacuna and the *glandular axial organ* in older stages. In case the axocoel is oversized in specimen 4 and usually does not form a ring around the esophagus, there is reason to postulate that its extent normally equals that of specimen 2 and 3. In both cases, there seemed to be a need for exactly *this* extent of the periesophageal lacuna, and no need for an extent around the whole esophagus, shedding light on the importance of this structure.

An indirect argument in favor of an origin from the *periesophageal mesenteric formation* is the missing lacunar imprint of the horizontal mesentery on the intestinal outgrowth in specimen 5. The absence of this structure shows that a complete dissolution of this structure along the intestinal tract in fact *is* possible.

Moreover, assuming the presently discussed origin, the oral somatocoel on the larva's right side would be free to expand symmetrically with that of the specimen's left side during later pentacrinoid towards juvenile stage, since only that part of the body cavity's visceral layer had

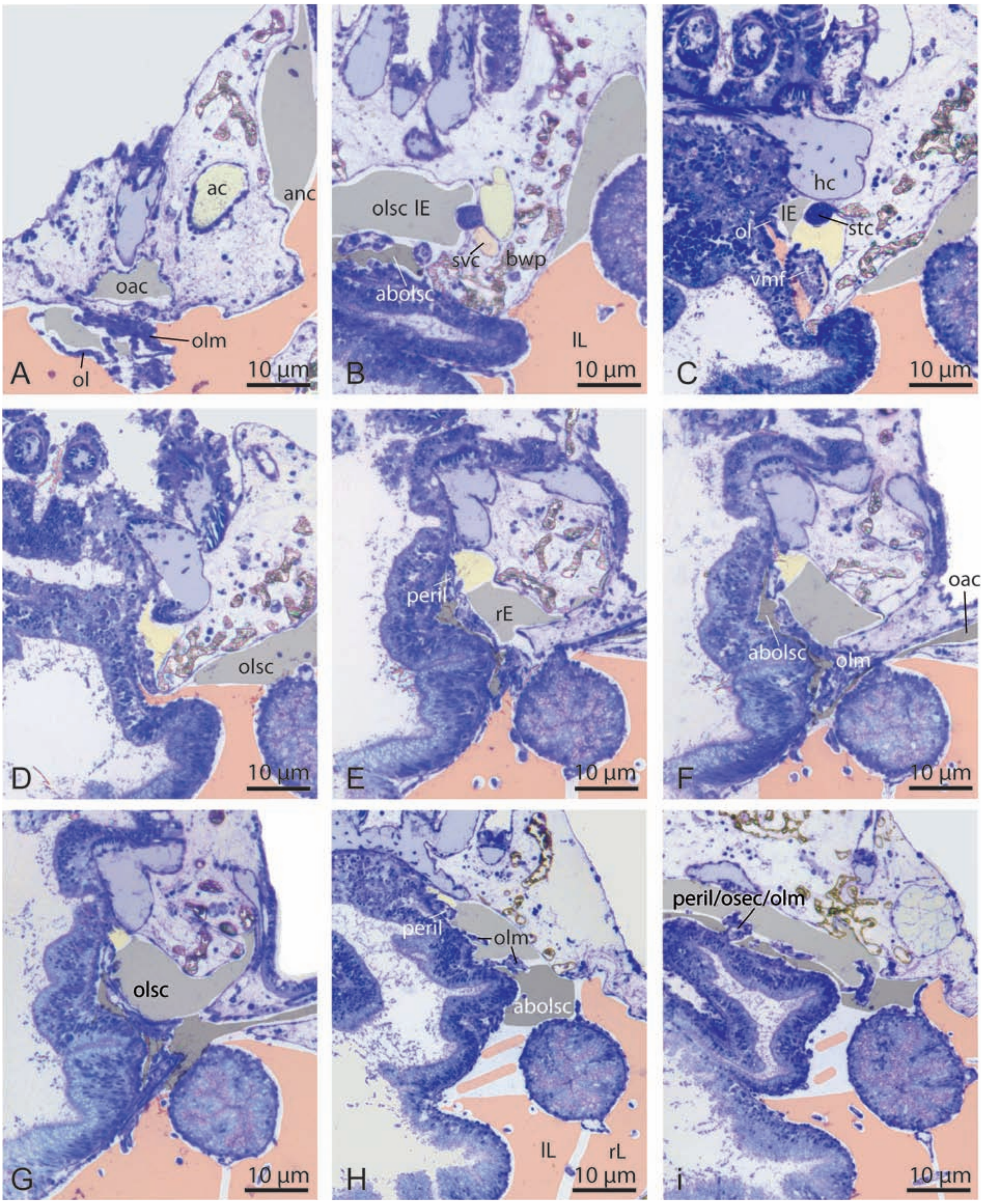

**Fig. 65:** Juvenile *A. bifida*, specimen 10. LM. Interpretation of the former course of the fused coeloms of the main body cavity on the larva's right side. **A-i** Sequence of serial sections, whose position in the animal is indicated by Fig. **13J**. Coloration indicates the hypothetical former extent of the axocoel (*ac*, ***yellow***), the oral somatocoel and the aboral somatocoel as indicated by the respective marker structures derived from the results of this thesis (Tab. 3). The periesophageal lacuna (*peril*) is thereby interpreted as a remnant of the periesophageal mesenteric formation's aboral end as shown in Figs. **64B$_1$**, **64B$_2$**. The oral lacunar meshwork (*olm*) is therefore formed by oral somatocoelothel and crosses the oral somatocoel, dividing the latter into two sections. **A-D** The primary stone canal (*stc*) opens into the axocoel. *abolsc* oral somatocoel below oral lacunar meshwork (***darker gray***), *anc* anal cone coelom, *hc* hydrocoel (***blue***), *lE* left extension of the oral somatocoel, *lL* left lobe of the aboral somatocoel (***red***), *oac* oral arm coelom, *ol* oral lacuna, *olsc* oral somatocoel above oral lacunar meshwork (***lighter gray***), *osec* oral section of the axial mesentery, *rE* right extension of the oral somatocoel, *rL* right lobe of the aboral somatocoel (***red***), *svc* small ventral coelom (***orange***), *vmf* ventral mesenteric formation.

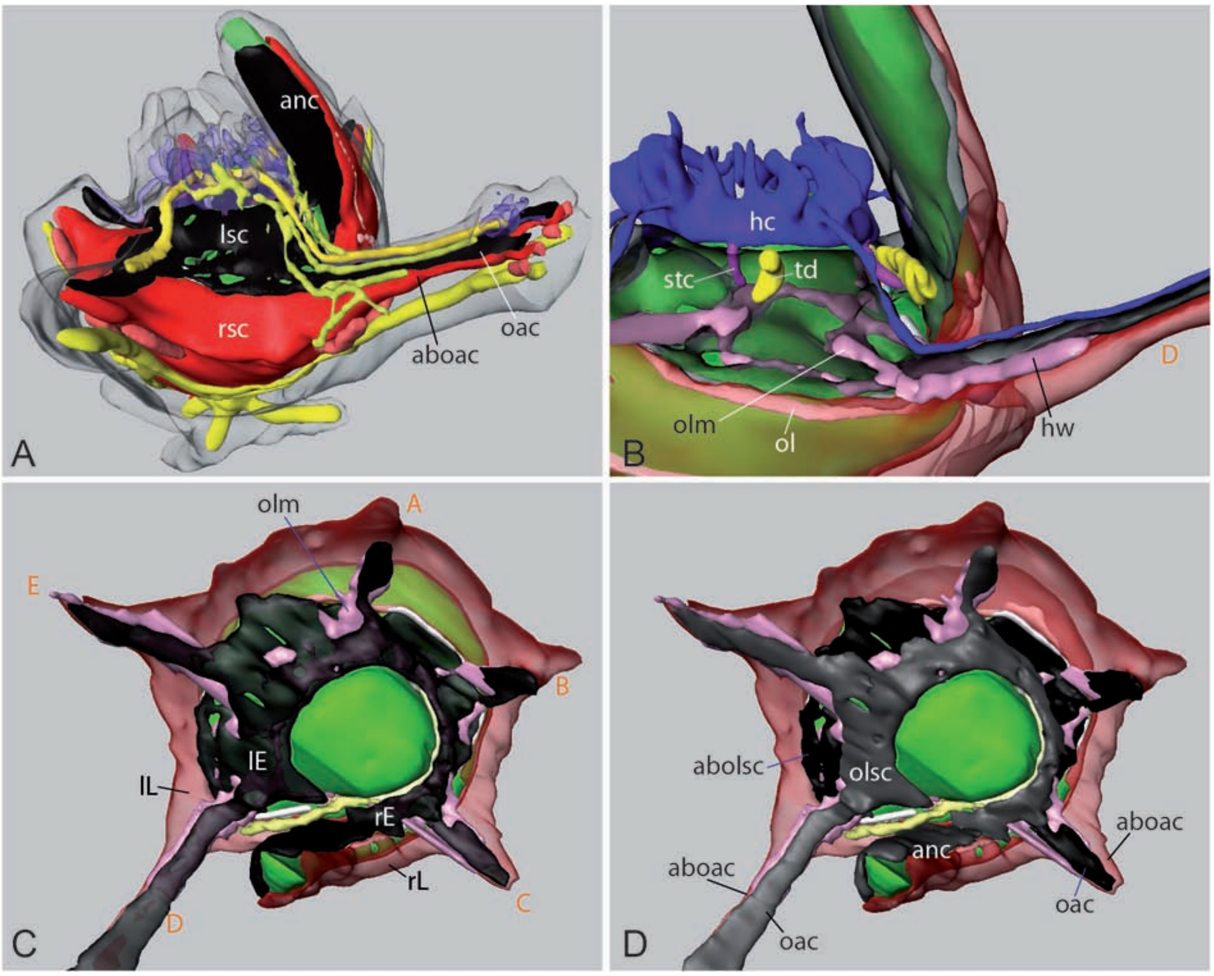

**Fig. 66:** Juvenile *A. bifida*, specimen 10. **A-D** Reconstruction of the somatocoelia based on the marker structures identified in this thesis (Tab. 3) assuming that the periesophageal lacuna marks the former aboral limit of the axocoelomic process. The oral lacunar meshwork (*olm*) is formed by oral somatocoelothel and crosses the oral somatocoel, dividing it into two sections. The *tegmenal ducts* (*td*) and all five stone canals (*stc*) open into the oralmost section. *aboac* aboral section of the arm coelom, *abolsc* oral somatocoel below oral lacunar meshwork (***darker gray***), *anc* anal cone coelom, *hc* hydrocoel, *hw* horizontal wall in the arms, *lE* left extension of the oral somatocoel, *lL* left lobe of the aboral somatocoel, *rE* right extension of the oral somatocoel, *rL* right lobe of the aboral somatocoel, *lsc* oral somatocoel, *oac* oral section of the arm coelom, *ol* oral lacuna, *olsc* oral somatocoel above oral lacunar meshwork (***lighter gray***), *rsc* aboral somatocoel.

to be comparatively uninvolved in growth, which then represented the axocoelothelial remnant (Figs. $64B_1$, $64C_1$)

Also, the oral somatocoel would be free to form the oral section of the axial mesentery, as indicated in the reconstruction of specimens 4 and 5 (Figs. 44E, 44H). The same holds true for the oral section of the juvenile, in which the required tilt of the oral somatocoel around the central axis *must* have taken place in order to explain the two distinct aboral spaces (Fig. 62).

If this assumption on the origin is true, the *oral lacunar meshwork* extends, in all radii, across an area, which can be attributed to the oral somatocoel throughout the animal, partitioning the oral somatocoel into one part above and one below it (Figs. 59, 61, $64B_2$, 65 and 66). Since the *oral lacunar meshwork* attaches right below the periesophageal lacuna (and on the larva's left side at about the same horizontal level), the meshwork's oral limit lies close to the aboral limit of the axocoelothelial lining around the esophagus. Since the madrepores and secondary stone canals open above the *oral lacunar meshwork*, these structures would open into a space, whose visceral lining derives from the axocoel.

This latter observation stresses the probability, that the axocoelomic ring in specimen 4 could represent a typical formation in all larvae of *A. bifida*, since the oralmost part of the main body cavity could then easily originate from axocoel all around the esophagus, irrespective of the presence and origin of the periesophageal lacuna.

Summing up, the overall impression obtained from several developmental stages leads one to conclude that it is the horizontal mesentery in the axial compartment that dissolves completely and that the periesophageal lacuna forms from the periintestinal mesenteric formation, rather than to assume that the hypothesis concerning the axial extent of the oral somatocoel is an illusion and that the juvenile grows asymmetrical**.**

The hypothetical former extent of the axocoel, which is derived from these arguments, has been already marked for the pentacrinoid stages in Figures 51 and 54, and is now marked for the juvenile in Figure 65 as well.

## 4.11 The oral lacuna and the “ventral structures” in interradius CD

As already pointed out, the oral lacuna has been shown to derive from that part of the horizontal mesentery, which extends between the intestine and the body wall. This latter part persists much longer in the larva than the mesentery’s central part, whose former position is, according to the present results, assumed to have been located somewhere (slightly) below the periesophageal lacuna. The horizontal mesentery or the oral lacuna, respectively, can be traced in all older specimens up to the juvenile stage from the tip of the intestine in counterclockwise direction along the intestinal tract, till it reaches the specimen’s ventro-left side. The ventro-left section of the horizontal mesentery dissolves latest during development and its ventralmost end persists at least until the juvenile stage observed, probably beyond that stage.

The present data clearly show that this last ventro-left end takes part in the formation of a cluster of large epithelial cells, which are formed by both the oral and the aboral somatocoelomic layer of the mesentery, but mostly by the latter one. This cluster, which has already been drawn by Seeliger (1893; Fig. S145, appendix) for *A. mediterranea*, has first been described in *A. mediterranea* by Russo (1902: 10-14), who homologized it with the gonad of holothuroids and the other echinoderm groups. Since its discovery, the cluster of epithelially arranged cells has been shown to exist at the same location in larvae of *Tropiometra carinata*, *Compsometra serrata*, *Isometra vivipara*, *Florometra serratissima* as well as in *A. bifida* (Mortensen 1920: 68).

This study shows the cluster’s existence in *A. bifida* from young cystidean (specimen 3) up to (at least) late pentacrinoid stage (specimen 9). It is, however, possible, that the larger cells in the ventral area of the presumptive horizontal mesentery in specimen 2 already point to the cluster’s formation. There is a clear sequence of developmental states which indicates that the cluster’s size increases towards late cystidean stage (specimen 4 represents an exception to this rule as there were only a few large cells present), reduces its size towards the opening of the vestibulum and becomes compact during pentacrinoid stage. In all stages, the large cells form part of the coelothelia. The cluster has not been distinctly identified in the juvenile. It can, however, not be excluded that individual remnant cells still inconspicuously reside within the coelothelium and cannot be identified due to the structural change of the *ventral mesenteric formation*. Since the cluster locates slightly to the left at the aboral end of the *ventral mesenteric formation* in younger larvae, these remaining cells had to reside at the latter's dextro-aboral end.

Russo (1902) showed the existence of the cluster only beginning with larvae of 5-6 days age, which already possess a long stalk and an open vestibulum, representing, hence, early pentacrinoid stages. Similar to the present results, he finds the cluster "to be in the process of reduction" with beginning arm growth and traces it up to those stages, in which the larvae have ”forked arms and a considerable length", pointing to a stage similar to specimen 9, in which, according to him, it shall be vanished. Mortensen's (1920) larvae are cystideans (*C. serrata*, *T. carinata*), young pentacrinoids (*F. serratissima*) and pentacrinoids with growing arms (*I. vivipara*) and he only remarked to have seen it for *A. bifida* as well. So, the present data generally confirm Russo's (1902) and Mortensen's (1920) findings.

The *ventral mesenteric formation* develops from the last (ventro-left) section of the larva's horizontal mesentery, being therefore formed by the oral somatocoel's left extension and the subjacent left lobe of the aboral somatocoel to the right of the epithelial cell cluster. In all specimens older than specimen 4, its dextro-oral limit locates exactly underneath the point, where the axocoelomic cavity opens into the main body cavity, so that its oral limit is formed by axocoelothel. During ongoing development, its initially loose *ecm* gets an increasingly dense fibrous to granular appearance.

Within the *ventral mesenteric formation* resides the ventral lacuna, which constitutes a semi-isolated area within the *ecm* of the *ventral mesenteric formation* that is formed by processes sent out by the *small ventral coelom*. The present data show that an initial developmental state of all these structures can already be found during metamorphosis, so that its formation might start around settlement. The ventral lacuna and the *small ventral coelom* can be traced up to juvenile stage.

Russo (1902, Figs. R8, R41, R42, appendix) describes a "*seno aborale*" whose position equals that of the *small ventral coelom* described in this thesis. In the metamorphosing larva (specimen 2), the *small ventral coelom* already locates right underneath the primary stone canal and to the left of the axocoel's aboralmost point. It forms cell contacts with the axocoel. Its shape and intimate connection with the oral somatocoel's left extension at its own aboral end, where it basically forms the latter's tip (Fig. 41D), however, point to a derivation from the oral somatocoel. The *ecm* that locates between the *small ventral coelom* and the oral somatocoel represents an initial stage of the ventral lacuna, which functions as a kind of wedge that opens up the space between its limiting cavities. The processes, which are formed by both the oral somatocoel and the *small ventral coelom*, thereby form the ventral lacuna's distal and proximal limits and connect the two cavities.

The ventral lacuna has been recognized by Russo (1902; Figs. R8, R41, R42, appendix) as well. In specimen 4, the ventral lacuna proceeds to extend (wedge-like) aboralwards and breaks through to the *ecm* underneath the *small ventral coelom* (Figs. 45H, 45i). The ventral lacuna is orally limited by both the primary stone canal and the *small ventral coelom*. The two connections between the oral somatocoel and the *small ventral coelom*, which is prominent in the younger specimen 2 (Fig. 41C), correspond to the connections that are established between the two horns of the *small ventral coelom* and the oral somatocoel in specimen 4 (Figs. 45H, 45i). In the latter, the connecting cells limit the ventral lacuna on the left together with the processes that extend between the oral somatocoel and the small number of large cells, which probably represent the cluster of epithelial cells (Figs. 45J, 45L). The cell contacts to the axocoel are retained as well (Fig. 45F). The *small ventral coelom* distally sends processes towards the aboral somatocoel's oral end (Fig. 45F) and establishes more intimate cell contacts with the aboral somatocoel on its right side (Figs. 45E, 45F). Therefore, the ventral lacuna is still limited distally and to the right. Aborally, the ventral lacuna is limited by the aboral somatocoel. Proximally, however, the ventral lacuna remains open towards the periesophageal *ecm*. Extending inwards, it passes between the axocoel and the aboral somatocoel.

During ongoing development, the ventral lacuna always remains delimited by processes that are (mainly) sent out by the *small ventral coelom*. The relative position of the ventral lacuna to all neighboring cavities is thereby maintained as described up to specimen 9, and in principle also until the juvenile stage. Since the size of the *ecm* around the ventral lacuna increases and the ventral lacuna is, however, partially reshaped, the respective contact areas of the ventral lacuna and the former coelomic areas become increasingly smaller and localize.

#### Summary of the position and origin of the identified marker structures

In the latter and in the previous sections of this thesis, the former course of both the horizontal mesentery and of the *aboral vertical mesentery*, as well as the fate of the axocoel have been traced. The related findings lead to the identification of several structures that can be used as marker structures to recognize the former coelomic borders in the juvenile, and possibly also in the adult. Table 3 gives a summary of these structures.

## 4.12 The oral lacunar meshwork – origin and further development

The results of the present thesis are not suitable to shed light on the *oral lacunar meshwork*'s initial formation; this meshwork does, however, certainly *not* directly develop from the horizontal mesentery, since the latter is basically reduced when the *oral lacunar meshwork* appears.

**Tab. 3:** Summary of the position and origin of the identified marker structures

| Structure | Position | origin/comments |
|---|---|---|
| ***abol***<br>**aboral lacuna** | runs along the aboral side of the intestinal tract in older developmental stages | remnant of the (*s*)*abovm* |
| **(*s*)*abovm***<br>**(secondary) aboral vertical mesentery** | runs along the aboral side of the intestinal tract in younger developmental stages | along the intestine, it derives from aboral somatocoelothel only;<br>underneath both the bend of the intestinal tract and the stomach, it may be formed by both aboral and oral somatocoelothel |
| ***ol***<br>**oral lacuna** | runs along the oral side of the intestinal tract in older developmental stages | remnant of the *hm* |
| ***hm***<br>**horizontal mesentery** | runs along the oral side of the intestinal tract in younger developmental stages | is formed by both aboral and oral somatocoelothel along its whole extent |
| ***vmf***<br>**ventral mesenteric formation** | locates to the left of interradius CD, where it extends slightly angularly (from right to left in oral-aboral direction); its oral end locates directly *underneath* the opening of the axocoelomic duct into the main body cavity | remnant of the *hm*, forms between the end of the oral somatocoel's left extension and the aboral somatocoel's left lobe, constituting the *hm*'s ventro-left end;<br>the oral end probably derives from axocoelothel |
| ***ecc***<br>**cluster of epithelial cells** | locates at the aboral end of the *vmf* between the end of the oral somatocoel's left extension and the aboral somatocoel's left lobe, to the left of the small ventral coelom | cells derive mainly from the epithelium of the aboral somatocoel but also from the oral somatocoel |
| ***vl***<br>**ventral lacuna** | semi-isolated area within the *ecm* of the *vmf* | is formed by protrusions sent out from cells of the small ventral coelom; continues in the *peril* |
| ***peril***<br>**periesophageal lacuna** | continues from the ventral lacuna's oral end towards the right around the esophagus; locates directly *underneath* the opening of the axocoelomic ducts into the main body cavity | this lacuna is hypothetised to form along the aboral end of the axocoelomic process by dissolution of the periesophageal mesenteric formation |

The *oral lacunar meshwork* extends from the visceral layer around the esophagus to the peritoneal layer of the body wall, inserting either to the sides of the horizontal walls in the arm bases, or at the horizontal walls itself. On the larva's right side, the *oral lacunar meshwork* generally extends from a location underneath the periesophageal lacuna, and stretches above the axial compartment and above the oral lacuna. On the left side, it keeps a similar level within the larva and the juvenile (below that of the periesophageal lacuna on the right side), spanning above the "stomach", whose oral side is covered by the visceral oral somatocoelothelium. Assuming that the periesophageal lacuna is a remnant of the aboral end of the *periesophageal mesenteric formation*, the *oral lacunar meshwork* crosses the oral somatocoel and, consequently, is covered by oral somatocoelothel (Fig. 64$B_2$).

The *oral lacunar meshwork* starts being formed during pentacrinoid stage beginning with the growth of the arms. Although it is described in all works in which older larval stages or juveniles were examined, it's prospective function, or that of individual parts of it, has not yet been sufficiently and systematically investigated (as can be seen in Perrier 1886, Russo 1902).

The granular nature of the *oral lacunar meshwork's* comparatively prominent *ecm* suggests a lacunar function. Amoebocytes and other wandering cells locate within the *ecm* and are potentially free to dislocate, e.g. from the lacunar space around the esophagus into the horizontal wall of the arms. The longitudinal muscular strands within the *oral lacunar meshwork* point to a certain contractability.

Constituting a presumably lacunar structure underneath the tegmen, the *oral lacunar meshwork* may, according to its position in the animal, develop into structures such as the periesophageal plexus (circumoral blood-vascular ring, circumesophageal hemal ring), the spongy body and the subtegmenal plexus (=annular lacuna of Chadwick 1907), which forms a ring outside the periphery of the periesophageal plexus, as well as the radial genital lacunae, which extend from the subtegmenal plexus (Chadwick 1907, Balser and Ruppert 1993, Heinzeller and Welsch 1994). Due to the interrelatedness and the basically horizontal arrangement of all these structures, it is highly likely that the *oral lacunar meshwork* is a precursor structure for *all* these lacunar blood vessels, which therefore have to differentiate subsequently from it during the later juvenile stage. In fact, the pattern of the *oral lacunar meshwork* in the here investigated juvenile already suggests a differentiation of two ring-structures: There is, on the one hand, a periesophageal ring-structure, into which the periesophageal lacuna is *not* integrated, which might develop into the periesophageal plexus. On the other hand, there is an anlage of an additional hemal ring in interradius DE, which might develop into the subtegmenal plexus (Fig. 18D).

Balser and Ruppert (1993) found "the epithelium overlying the spongy body vessels" to consist of a highly folded layer of monociliated podocytes and myocytes". The existence of myocytes thereby correlates with the occurrence of myocytes in the *oral lacunar meshwork* found in this thesis, stressing the developmental interrelatedness of the two structures.

In the present thesis, podocytes have been only found in the larval axocoelothel. However, understanding the spongy body as a derivative of the *oral lacunar meshwork* and assuming the latter's oral somatocoelomic origin, the spongy body has to, consequently, be assumed to be covered by oral somatocoelothel as well. So, the podocytes of the spongy body do not relate to those of the larva's axocoel, but are formed by somatocoelothel. The coelothelial covering of the gastric hemal tufts of asteroids, however, forms podocytes and derives from somatocoelothel as well (Beijnink and Voogt 1986). These hemal organs extend *between* the intestinal tract and the asteroid axial organ and are formed "by two mesenteries" (Beijnink and Voogt 1986), indicating, that they are probably not formed *de novo* as the *oral lacunar meshwork* and the spongy body, respectively, and that the gastric hemal tufts and the spongy body are therefore most likely not homologous. The presence of podocytes in the gastric hemal tufts, however, shows that the presence of podocytes in the spongy body does not hinder the latter's derivation from the oral somatocoel as hypothesized, since its derivation from axocoelothel is not mandatory.

The spongy body of adult crinoids consists of two continuous but distinct parts that extend into the *axial sinus*. The larger part of the spongy body locates lateral to the *glandular axial organ* and is continuous with both the *glandular axial organ* lacuna as well as with the periesophageal plexus. The smaller part generally derives at multiple places from all around the periesophageal plexus and locates beneath the oral body wall in proximity to the multiple stone canals (Chadwick 1907, Balser and Ruppert 1993, Heinzeller and Welsch 1994).

Comparing the topology of these two parts to the topology of the juvenile's lacunar system presented in this thesis, the data suggest that these two parts derive from two different locations.

Based on the present findings, the larger part of the adult spongy body probably originates from the oral section of the axial mesentery (or that area of the *oral lacunar meshwork* which is associated with this oral section) and the smaller part may originate from the *oral lacunar meshwork*'s periesophageal ring-structure.

## 4.13 Coelomic identity of the arm coeloms

The present data allow a reasonable suggestion on the developmental origin of the arm coelom. The horizontal mesentery largely dissolves before the arms are formed, as can be seen in Specimen 5. The main body cavity therefore extends into each arm as one piece. This, in principle, renders an assignment of the somatocoelia to the arm coelom difficult. However, the oral lacuna lies below the uppermost border of the united arm coelom, indicating that the oralmost area of the united arm coelom may derive from a region that is lined by oral somatocoelothel.

The body wall around the arm coelom grows subsequently in from the sides and forms the horizontal wall, which divides the arm coelom into an oral part and an aboral part. In the pentacrinoid specimens 8 and 9, this horizontal wall, where already formed, is contacted by the *oral lacunar meshwork's* threads laterally of the arm bases. Accordingly, in those arms, in which the horizontal wall is not yet formed, the threads of the *oral lacunar meshwork* contact the body wall at the sides of the proximal beginning of the arm coelom. In the juvenile, the threads contact the horizontal wall in each arm in a similar way, either directly or to its side, but on one level with it. Since the *oral lacunar meshwork* probably crosses and derives from the oral somatocoelothel, the horizontal wall, consequently, has to be assigned to the oral somatocoelomic lining as well. So, the oral part of the arm coelom can be assumed to mainly derive from the oral somatocoel, probably even *in toto*, while the aboral part mainly derives from aboral somatocoel, possibly also deriving in part from the oral somatocoel.

Since the latter possibility cannot be derived from any marker within the arms, the arm coelom of the juvenile has been reconstructed under the assumption that the oral somatocoel extends only into the oral part of the arms (Figs. 66D, 59B). From the same reconstruction can be argued, however, that in some arms, the oral part of the arm coelom derives clearly from an area above the *oral lacunar meshwork*, while in other arms its direct connection can be traced to an area below this meshwork (but still above the oral lacuna). This latter condition can, interestingly, be found in those radii, in which the axial compartment is formed and the course of the oral somatocoel may be influenced by the axial extension of the oral somatocoel. This is the same side on which the asymmetry concerning the periesophageal lacuna is detectable.

## 4.14 The origin and development of the axial sinus

In adults, the coelothelium of the axial sinus rests on the hemal space of both the *glandular axial organ*, along which it does not form podocytes, and of the spongy body, along which podocytes can be found. Moreover, the multiple stone canals and tegmenal ducts open into the axial sinus (Balser and Ruppert 1993, Heinzeller and Welsch 1994).

Balser and Ruppert (1993) raised the question of the origin of the axial sinus. They found two lines of evidence in the literature. The one is Carpenter (1878: 39), who found the axial sinus to represent “the limited space left by the coiling of the larval intestine around the stomach”, indicating that the axial sinus is a semi-isolated region of the perivisceral coelom. The other line of evidence is based on Bury (1888: 278) and Mortensen (1920: 66) and refers to Bury’s (1888) statement that "during the transition to the cystidean stage the walls of the parietal canal share in the general histolysis". This statement refers to the dissolution of the axocoelomic cavity only, which has been confirmed by Seeliger (1893), and does not refer to any axocoelomic process. Nonetheless, Balser and Ruppert (1993) concluded that the oral region of the axial coelom, in which the spongy body lies, could be interpreted to represent a derivative of the larval axocoel.

The present results are consistent with both lines of evidence and also Balser and Ruppert’s (1993) interpretations.

A comparison of the adult’s axial sinus (Fig. 14) with the findings on the mesenteric structures in the larval axial compartment clearly shows that the axial sinus around the *glandular axial organ* must form by a secondary ingrowth of coelothelial tissue, resulting in the formation of septa and trabeculae (Heinzeller and Welsch 1994). So, the axial sinus is formed by a reduction of the axial compartment. Considering both the presumed axial extent of the oral somatocoel in the different larval stages and in the juvenile and the positions, from which the ingrowth in the adult must have taken place, there is no doubt that a larger part of the axial sinus around the *glandular axial organ*, if not this whole part of the axial sinus, derives from oral somatocoelothel.

It has already been reasoned that the *oral lacunar meshwork* derives from oral somatocoel. The oral part of the axial sinus must therefore also derive mainly from oral somatocoelothel. Based on the observation that the axocoelomic process formed a ring around the esophagus of the early cystidean specimen 4 and as demanded by the assumed dissolution of the observed *periesophageal mesenteric formation* related to this process, the oralmost area of the axial sinus may in fact be formed by axocoelomic lining, at least along the visceral side of the main body cavity's peritoneum (Fig 65D-H). The secondary stone canals and the tegmenal ducts of the juvenile, however, do not open above the level of the periesophageal lacuna, but rather into an area, that has to be assigned to the oral somatocoel below the lacuna and above the *oral lacunar meshwork*. As they are not formed by the visceral lining of the main body cavity, they are presumably covered or lined by oral somatocoelothel.

So, the axial sinus is mainly formed in areas, in which the coelothel covering the respective structural elements originates from the oral somatocoel. This holds true for both the *glandular axial organ*, which is partially formed by the oral somatocoel of the axial compartment (Fig. 62) and for the spongy body, represented by parts of the *oral lacunar meshwork* (Fig. 66), as well as for the multiple stone canals. It can, of course, not be excluded that septa, which form from the coelothelial lining of the main body cavity and reduce the axial compartment as well as the oral somatocoel above the intestinal tract, also extend from areas that can be assigned to aboral somatocoel.

### Formation of the tegmenal ducts and secondary stone canals

It can be clearly stated from the present data on the late pentacrinoid that the secondary stone canals develop as interradial protrusions of the aboral hydrocoelomic lining and therefore develop independently from the primary stone canal. It furthermore seems to be highly unlikely, that the tegmenal ducts shall form by a division of the axocoelomic duct as suggested by Balser and Ruppert (1993). In the late pentacrinoid, no tegmenal duct is formed and in the juvenile, the ducts already reside interradially at the basis of the oral valves. The huge distance between the axocoelomic duct and the prospective locations of the tegmenal ducts in the late pentacrinoid, especially the prospective locations in the interradii EA and AB, rather suggest their *de novo* formation.

### The term glandular axial organ and the organ's homology to the axial organ of asteroids, ophiuroids and echinoids

An organ is regarded as a collection of tissues joined in structural unit to serve a common function. For the *glandular axial organ*, this definition is satisfied only for a complex consisting of three structural units, the tubules, the lacunar space and the somatocoelothelial sheath. There are, however, two strands of evidence that are reflected throughout literature: its function and its evolutionary origin. On the one hand, the *glandular axial organ* shares components with the axial organ of Asteroids, Ophiuroids and Echinoids (AOE), namely the somatocoelothelial cover and the hemal vessel (Balser and Ruppert 1993, Fedotov 1930, Erber 1983). On the other hand, however, the *glandular axial organ tubule* is unique to crinoids that possess either a stalk or a centrodorsale (Heinzeller and Welsch 2009) and can therefore not be homologized to the

AOE axial organ (Balser and Ruppert 1993). These two methods of approach are reflected by the two differing main names used for the *glandular axial organ*, namely “axial gland” (Holland 1970, Hyman 1955) and “axial organ” (Bury 1888, Seeliger 1893, Balser and Ruppert 1993, Heinzeller and Welsch 1994, 2009), whereby the most recent articles that use the term axial organ additionally emphasize its glandular function (Heinzeller and Welsch 1994, 2009). The term “axial organ”, however, leads to an overlap with the term used for the AOE echinoderms, providing a major source of confusion. Therefore, the compound name *glandular axial organ*, which was introduced by Holland et al. 1991, has been chosen in this thesis again in order to reflect both lines of evidence at once. When used consistently as *one term*, this term allows a sufficiently clear distinction between the “axial organ” (of AOE echinoderms) and the “*glandular axial organ*” (of crinoids) and provides a link between the two organs at the same time.

### The crinoid axial complex inferred from the present data

The axial complex of AOE echinoderms and that of holothuroids consists of (1) a protocoel ampulla and the axial sinus (left protocoel = left axocoel), (2) the dorsal sac (right protocoel, reduced in crinoids), (3) the stone canal (extending from the left hydrocoel ring), (4) the axial gland, constituting of a hemal system that also extends into the genital sinus, which itself is a derivative of the left metacoel = left somatocoel and of (5) an oral metacoel ring (hyponeural canal, derivative of the left somatocoel) (Fedotov 1930 refined by Erber 1983).

As proposed by Fedotov (1930) and refined by Erber (1983), all individual components of the AOE axial complex are present in crinoids as well (unless reduced) and can be individually homologized with the respective structures. The components are, however, disaggregated. Fedotov (1930) homologizes the AOE ampulla with the crinoid larva’s axocoelomic cavity and notes correctly, that its lining is dissolved and an axial sinus not present, resulting in an opening of the multiple stone canals into the main body cavity (or axial sinus of adult crinoids), which therefore serves as the ampulla. Furthermore, he homologized the *glandular axial organ* lacuna with the AOE hemal sinus of the axial gland. The present data strengthen this view: The closeness of the axocoelomic process to the oral end of the *glandular axial organ* lacuna (specimen 4), into which it slightly extends, reminding of the Reichensperger’s organ (see pages 141f.), point to this lost relationship. At the same time, the proposed homology of the Reichensperger’s organ to the AOE axial gland (Holland et al. 1991, Balser and Ruppert 1993) is supported as well, suggesting the Reichensperger’s organ to represent a plesiomorphic character in isocrinids and *C. diomedae*.

Fedotov (1930) distinguished two parts of the *glandular axial organ*; an oral and an aboral part, which he homologized to the oral and aboral part of the axial organ that he found in other echinoderm taxa. In his text, Fedotov (1930) defines the oral part to be represented by the bundle of blood vessels, which extends from the spongy body (‘labiales Blutgefäßnetz’) to the *glandular axial organ*. His figures suggest, that he, in the context of Seeliger’s (1893) description, interpreted the *ventral mesenteric formation* (including the ventral lacuna) as well as the periesophageal lacuna as the oral part of the axial complex. Following him, the *glandular axial organ* lacuna represents the aboral part of the crinoid axial complex, this view being stressed by the continuity of the *glandular axial organ* lacuna with the genital lacuna that is found in the AOE axial complex as well.

The here presented view that the periesophageal lacuna represents a remnant of the *periesophageal mesenteric formation* stresses the interpretation concerning the oral part and refines it at the same time, since the coelomic cover of Fedotov's (1930) 'oral part' of the axial complex represents a fusion product between the axocoel and the main body cavity and is therefore interpreted as being part of the ampulla. The hemal core of the periesophageal lacuna therefore may in fact represent the corresponding structure to the hemal sinus of the AOE axial complex' oral part.

In holothuroids, the axial complex as well as the primordial germ cells (PGCs) and lie in the dorsal mesentery in interradius CD, which is formed between the left and the right somatocoel (Russo 1902, Hyman 1955, Erber 1983, Smiley et al. 1991), representing a structure corresponding to the horizontal mesentery. The vertical mesentery of asteroids, ophiuroids and echinoids lies in the interradius CD. In it, both the axial complex and the PGCs are formed. The AOE vertical mesentery is, however, described as being formed by two vertically stacked mesenteries, one that is formed by the two ventral ends of the left somatocoel and one that is formed by the two ends of the right somatocoel (Hyman 1955, Dawydoff 1948, Giese et al. 1991). This arrangement corresponds to the *aboral vertical mesentery* and the "oral vertical mesentery" in crinoids, the latter of which encloses the primary stone canal and the axocoel. In these echinoderm taxa, the left somatocoel constitutes the larger body cavity, and the contribution of the right somatocoel is rather minor (Hyman 1955). Literature does not provide a uniform picture on the contribution of the two somatocoelia to the peritoneal covering of the axial complex, also not within the main echinoderm taxa, but due to the left somatocoel's larger extent, the AOE axial complex seems to be mainly covered by left somatocoelothel in all AOE echinoderms (Dawydoff 1948, Hyman 1955, Goldschmidt 2007). It is noteworthy, that the *glandular axial organ*, as suggested in this thesis, is also formed by left somatocoel along its whole way, although the left somatocoel, unique to crinoids within echinoderms, has a comparatively small extent. Were one to follow Seeliger's (1893) point of view on the formation of the *glandular axial organ*, the latter's tubules would be surrounded by the aboral somatocoel and its hemal sinus less likely homologous to the hemal sinus in the AOE axial complex. The aboral extension of the oral somatocoel may thereby be interpreted as a way to increase or maintain the volume of the hemal lacuna while reducing the extent of the left somatocoel. At the same time, it allows to "pick up" the *glandular axial organ tubule* – a structure unique to crinoids and closely related to the stalk. Summing up, according to the present hypothesis, all components of the crinoid axial complex are covered by the oral somatocoelothel: The primary stone canal and the axocoelomic cavity, as well as the hemal sinus (represented by the *glandular axial organ* lacuna), and also the later formed secondary stone canals and tegmenal ducts. The axial and the ventral location of the axial complex are separated or connected, respectively, by a semi-circularly stretched remnant of the ampulla, the periesophageal lacuna, whose coelothelial linings are fused with the main body cavity. In some sense, the axial extension can be regarded as constituting a dislocated prolongation of the “oral vertical mesentery”, in which the axial complex is continued.

## 4.15 Assumptions on the origin of the crinoid gonads

PGCs of echinoderm larvae are “large cells with specific cytoplasmic inclusions and one conspicuous nucleolus in the prominent nucleus” (Gosselin 2003, see also Giese et al. 1991). As already stated, they develop in the mesentery of interradius CD in all non-crinoid echinoderm groups. In holothuroids, this mesentery clearly constitutes a horizontal mesentery-corresponding structure (Russo 1902, Hyman 1955), whereas in AOE echinoderms, the mesentery of the interradius CD is usually referred to as consisting of the two vertically stacked vertical mesenteries formed between the ends of the left and the right somatocoel, respectively (Hyman 1955, Dawydoff 1948, Giese et al. 1991).

The cluster of large epithelial cells described in this thesis consists of cells that satisfy the basic requirements of PGCs, as already pointed out by Russo (1902). Like in holothuroids, they are formed in the horizontal mesentery (Russo 1902). They are, however, also formed at the right end of the aboral side of the left somatocoel’s left extension, i.e. in ultimate proximity to the mesentery mainly referred to as the vertical mesentery in AOE echinoderms. Since the right and left somatocoel in AOE echinoderms also form a horizontal mesentery and since this must also meet the vertical mesenteries in interradius CD, the question is raised, whether the PGCs in AOE echinoderms are also formed in the horizontal mesentery very close to the vertical mesenteries and not within the vertical mesentery themselves. However, recent literature (e.g. Giese et al. 1991) does not always allow differentiation between these two possible locations, leading to certain confusion. A modern reinvestigation of this issue seems to be necessary.

The fate of the PGCs in crinoids is still unanswered. Russo (1902) suggested that the primary gonad he observed atrophies and a second gonadal structure is formed. According to Fedotov (1930), the cells of the epithelial cluster merely relocate and form the beginning of the genital strands in the arms. The present data suggest that if Fedotov (1930) is right, the PGCs of the cell cluster were free to move via the ventral lacuna, the periesophageal lacuna and the *oral lacunar meshwork* into the arms. This way could be taken individually or jointly by the cells. The time frame in which this relocation would happen cannot be stated yet; it must, however take place in very late pentacrinoids or juveniles. The next question not yet answered is the formation of the gonocoel in crinoids and how the genital cord of the adult finds its way into it. Since the gonocoel is frequently interconnected with both the two oral parts and the aboral part of the arm coelom (Grimmer and Holland 1979), it might form as an infolding of the horizontal wall.

The function of the *small ventral coelom*, whose origin from left somatocoel suggested by Russo (1902) has been confirmed in this thesis, remains unclear. It is, however, surely involved in the formation of the ventral lacuna and thereby, possibly, in the formation of a channel for the PGCs. In the echinoid *Paracentrotus lividus*, the gonad is formed by an overgrowth of the juvenile's germinal rachis (that originates 'in interradius CD') by the aboral ring, which constitutes the echinoid gonocoel (Gosselin et al. 2003). The aboral ring is a derivative of the left somatocoel (Goldschmidt 2007). In asteroids, "the primordial germ cells are enclosed as a genital rachis within a vesicle that pinches off from the left somatocoel" (Pearse and Cameron 1991, citing Delage and Hérouard 1903). It is therefore well possible that the *small ventral coelom* constitutes a remnant of the echinoderm gonocoel that may have lost its function in the adult (as already suggested by Russo 1902), whether it persists or not.

# 5 Summary

The study presented here shows the development of *Antedon bifida* starting with the doliolaria larva until juvenile stage based on ultrastructural and lightmicroscopical data. 3d-reconstructions of a doliolaria and of three young cystidean stages as well as a juvenile have been done. The related data have been complemented by serial sections of older cystidean and pentacrinoid stages. Special attention has been given to the development of the "ventral structures" in interradius CD, to the development of the axocoel and the fate of both the left and the right somatocoel. The fusion processes of these three coeloms as well as their potential contribution to the formation of the axial sinus (=axial coelom) and its associated hemal organs of the adult are investigated.

Part of the "ventral structures" in interradius CD are the primary stone canal, which is sent out by the hydrocoel, as well as the axocoel and the small ventral coelom, whose formation is traced in this thesis. Furthermore, there is a *ventral mesenteric formation*, which derives from the horizontal mesentery and from whose coelothelia the potential primary gonadal cells (PGCs) are formed. Enclosed in the *ventral mesenteric formation* resides the ventral lacuna, which is partially formed by cell protrusions sent out by the small ventral coelom's coelothel and also bordered by the nearby coeloms. Structural and positional characteristics of the PGCs, the small ventral coelom and the ventral lacuna support the assumption that these are parts of a primary gonad anlage, whose fate in the adult is, however, unclear.

In contrast to former findings in *Antedon mediterranea*, the axocoel in the cystidean larva of *A. bifida* forms a process around the esophagus that may even become annular. The possibility is discussed in this thesis that this axocoelomic process takes part in fusion processes and that it is involved in formation of the periesophageal lacuna that extends on the right side of the esophagus from the ventral lacuna to the oral end of the *glandular axial organ*. The intimate proximity of the axocoelomic periesophageal process to the oral end of the *glandular axial organ*'s hemal sinus corroborates the widely accepted assumption that the latter is homologous to the axial organ of asterioids, ophiuroids and echinoids (AOE) and also supports an homology of the AOE axial organ to the Reichensperger's organ of certain sea lily species (Isocrinida, *Calamocrinus diomedae*).

The primarily formed larval lacunae can be traced back to the mesenteries dissolved during the fusion processes. In this thesis, marker structures are defined, that allow tracing the development and the fate of the coeloms up to juvenile stage and may possibly also give an orientation in the adult. The detailed investigation of mesenteric remnants in older larval stages and in

the juvenile furthermore shows that the left somatocoel extends aborally within the innermost intestinal coil (axial compartment). This is probably enabled by an inward directed tilting of the gut during intestinal outgrowth. Where the left and the right somatocoel come to lie against each other, a new mesentery forms, from whose extracellular matrix the hemal sinus originates. The present data support the heretofore proposed initial formation of the epithelial tubule of the *glandular axial organ* as a delamination of a cell strand from the coelothelium of the right somatocoel in intimate proximity to the proximal end of the prospective chambered organ in the doliolaria. However, a continued delamination from the right somatocoelothel, as assumed until now, is highly unlikely. The present data suggest that the tubule achieves an epithelial organization in the oralmost part of the prospective chambered organ and grows successively orally into the axial mesentery as well as aborally into the stalk.

Based on the origin discussed for both the periesophageal lacuna and the *glandular axial organ* it can be shown, that the axial sinus is largely formed by the left somatocoel. Based on the present results, the coelothelium that rests on the hemal space of the spongy body, also derives from the left somatocoel.

# 6 Zusammenfassung

Die vorliegende Arbeit untersucht die Entwicklung von *Antedon bifida* von der Doliolarialarve bis zum Juvenilstadium basierend auf ultrastrukturellen und lichtmikroskopischen Daten. 3D-Rekonstruktionen einer Doliolaria und dreier junger Cystidienstadien sowie eines Juvenilstadiums wurden angefertigt und die zugehörigen Daten durch serielle Schnittserien von älteren Cystidien- und Pentacrinoidenstadien ergänzt. Besondere Beachtung finden die Entwicklung der „ventralen Strukturen“ im Interradius CD, die Entwicklung des Axocoels sowie das Schicksal des larvalen linken und rechten Somatocoels. Die Fusionsprozesse dieser drei Coelome sowie ihr möglicher Beitrag zur Bildung des Axialsinus (=Axialcoelom) und seiner assoziierten Hämalorgane im Adulttier werden untersucht.

Zu den „ventralen Strukturen“ im Interradius CD gehören der vom Hydrocoel gebildete Steinkanal und das Axocoel, sowie ein kleines ventrales Coelom, dessen Bildung in dieser Arbeit nachvollzogen wird. Weiterhin findet sich hier eine ventrale mesenteriale Bildung, die sich vom horizontalen Mesenterium ableitet und aus deren Coelothelien die potentiellen primären Geschlechtszellen (PGCs) gebildet werden. In die ventrale mesenteriale Bildung eingebettet befindet sich die ventrale Lakune, die teilweise von Zellausläufern des Coelothels des kleinen ventralen Coeloms und zudem von den benachbarten Coelomen begrenzt wird. Strukturelle und räumliche Merkmale der PGCs, des kleinen ventralen Coeloms und der ventralen Lakune unterstützen die These, dass diese Teile einer primären Gonadenanlage sind, deren Verbleib im Adultus jedoch ungeklärt ist.

Anders als bisher für *Antedon mediterranea* beschrieben, bildet das Axocoel in der Cystidienlarve von *A. bifida* einen Fortsatz um den Ösophagus, der vermutlich ringförmig auswächst. In der vorliegenden Arbeit wird die Möglichkeit diskutiert, dass dieser Axocoelfortsatz in die Verschmelzungsprozesse der Coelome der Larve mit einbezogen wird und an der Bildung der Perioesophageallakune beteiligt ist, welche auf der rechten Seite des Oesophagus von der Ventrallakune bis zum oralen Ende des glandulären Axialorganes verläuft. Die unmittelbare Nähe dieses Fortsatzes zum oberen Ende des Hämalsinus des glandulären Axialorganes untermauert die bestehende Vermutung, dass letzterer dem Hämalsinus des Axialorganes in Asteriden, Ophiuriden und Echiniden (AOE) homolog ist und unterstützt zudem die These einer Homologie des AOE-Axialorganes zum Reichensperger´schen Organ einiger Seelilienarten (Isocrinida, *Calamomocrinus diomedae*).

Die zuerst auftretenden larvalen Lakunen können auf die während des Fusionsprozesses aufgelöste Mesenterien zurückgeführt werden. In der Arbeit werden Markerstrukturen definiert, anhand derer sich die Entwicklung und das Schicksal der Coelomräume bis zum Juvenilstadium verfolgen lassen und welche möglicherweise auch eine Orientierung im Adulttier geben können. Die eingehende Untersuchung der mesenterialen Überreste in älteren Larvenstadien und im Juvenilstadium zeigt zudem, dass das linke Somatocoel im Inneren der ersten intestinalen Windung (axiales Kompartiment) nach aboral, in Richtung gekammertes Organ reicht. Dies wird vermutlich durch eine in Totalpräparaten beobachtbare, nach innen gerichtete Kippung des Darmes während des Auswachsens ermöglicht. Dort wo das linke an das rechte Somatocoel angrenzt, bildet sich ein neues axiales Mesenterium, in dessen extracellulärer Matrix der Hämalsinus des Axialorganes geformt wird. Die vorliegenden Daten stützen die bereits beschriebene Bildung des epithelialen Schlauches des glandulären Axialorgans durch Delamination eines Zellstranges aus dem Coelothel des rechten Somatocoels am proximalen Ende des entstehenden gekammerten Organes in der Doliolaria. Allerdings ist eine fortschreitende Bildung aus der Wand des rechten Somatocoels, wie bisher angenommen, höchst unwahrscheinlich. Die vorliegenden Daten legen hingegen nahe, dass sich die Schlauchanlage zunächst im obersten Abschnitt des gekammerten Organes epithelial organisiert und dann sukzessive nach oral in das axiale Mesenterium und nach aboral in den Stiel auswächst.

Aufgrund der diskutierten Herkunft der Perioesophageallakune und des glandulären Axialorganes lässt sich zeigen, dass der Axialsinus weitgehend vom Coelothel des linken Somatocoels gebildet wird. Das Coelothel, das dem den Axialsinus durchziehenden spongy body aufliegt, ist nach den vorliegenden Ergebnissen ebenfalls linken somatocoelen Ursprungs.

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Seeliger, O. (1893): Studien zur Entwicklungsgeschichte der Crinoiden. Zoologische Jahrbücher Abteilung Anatomie und Ontogenie der Tiere. **6**: 161-444.

Smiley, S., McEuen, F.S., Chaffe, C. and Krishnan, S. (1991): Echinodermata: Holothuroidea. In: Giese, C., Pearse, J.S., Pearse, V.B. (eds.), Reproduction of marine invertebrates, Volume VI: Echinoderms and Lophophorates. pp. 663-750. Boxwood Press, Pacific Grove, California.

# 8 Appendix

## 8.1 Annotated figures from Seeliger (1893)

The following index and figures (pages 169-172) are taken from Seeliger, O. (1893): Studien zur Entwicklungsgeschichte der Crinoiden. Zoologische Jahrbücher Abteilung Anatomie und Ontogenie der Tiere. 6: 161-444 and have been colorized.

The “Tafelerklaerung” can be found on page 427 in Seeliger (1993). The respective expressions have been complemented by the related abbreviations used in this thesis.

The figures in the appendix were named retaining Seeliger’s (1993) original numeration preceding an “S”:

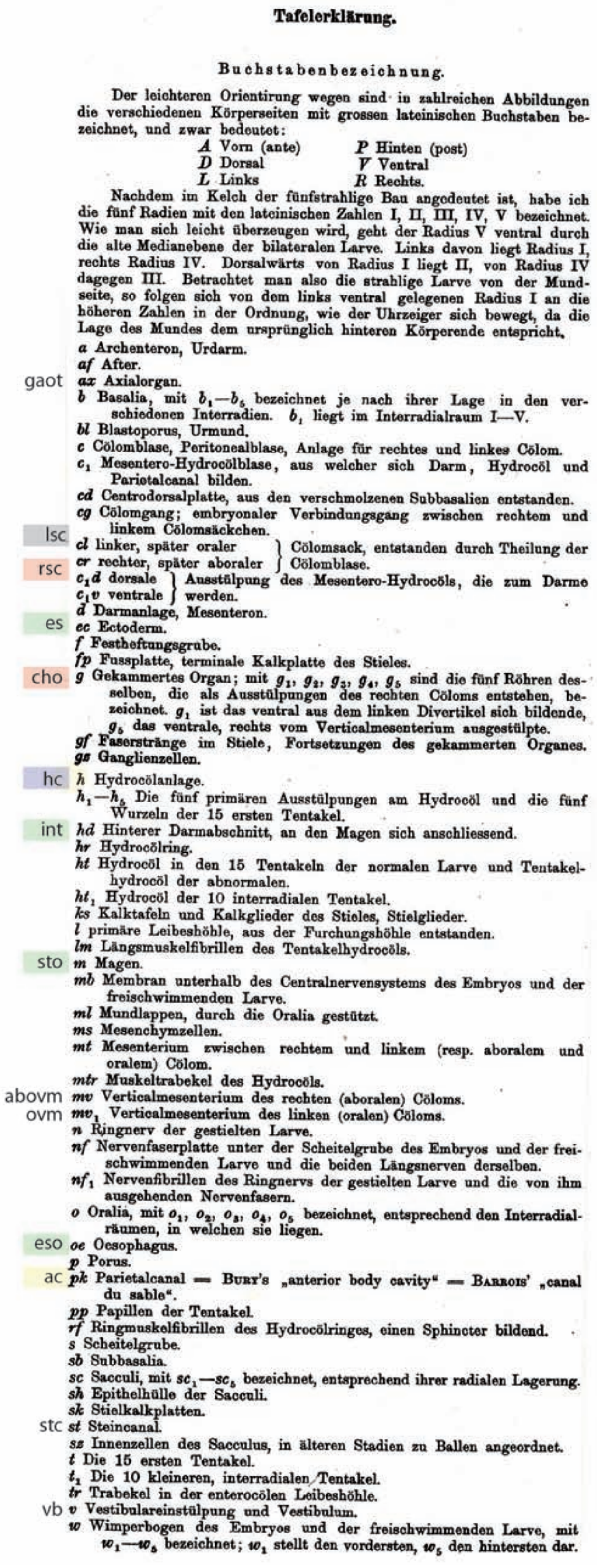
Figs. S67, S68 (=Fig. 67, 68) can be found on Seeliger’s (1893) Taf. 16;

Fig. S96B (=Fig. 96B) is taken from his Taf. 17;

Figs. S102, S110 (=Figs. 102, 110) are taken from his Taf. 18;

Figs. S114, S119, S124, S125, S126, S129, S134 (=Figs. 114, 119, 124, 125, 126, 129, 134) are taken from his Taf.19;

Figs. S117, S120, S127, S131, S138, S141, S142, S144, S145, S146, S151, S152, S153 (=Figs. 117, 120, 127, 131, 138, 141, 142, 144, 145, 146, 151, 152, 153) are taken from Seeliger’s (1893) Taf. 20.



**Tafelerklärung.**

**Buchstabenbezeichnung.**

Der leichteren Orientirung wegen sind in zahlreichen Abbildungen die verschiedenen Körperseiten mit grossen lateinischen Buchstaben bezeichnet, und zwar bedeutet:

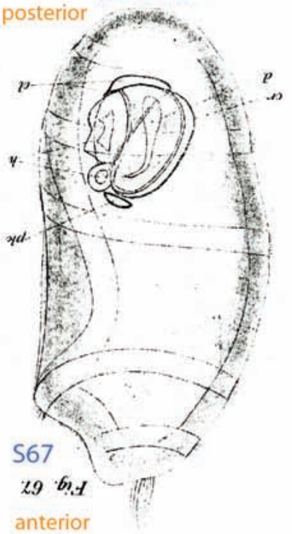
*A* Vorn (ante) *P* Hinten (post)
*D* Dorsal *V* Ventral
*L* Links *R* Rechts.

Nachdem im Kelch der fünfstrahlige Bau angedeutet ist, habe ich die fünf Radien mit den lateinischen Zahlen I, II, III, IV, V bezeichnet. Wie man sich leicht überzeugen wird, geht der Radius V ventral durch die alte Medianebene der bilateralen Larve. Links davon liegt Radius I, rechts Radius IV. Dorsalwärts von Radius I liegt II, von Radius IV dagegen III. Betrachtet man also die strahlige Larve von der Mundseite, so folgen sich von dem links ventral gelegenen Radius I an die höheren Zahlen in der Ordnung, wie der Uhrzeiger sich bewegt, da die Lage des Mundes dem ursprünglich hinteren Körperende entspricht.

*a* Archenteron, Urdarm.
*af* After.
gaot *ax* Axialorgan.
*b* Basalia, mit $b_1$—$b_5$ bezeichnet je nach ihrer Lage in den verschiedenen Interradien. $b_1$ liegt im Interradialraum I—V.
*bl* Blastoporus, Urmund.
*c* Cölomblase, Peritonealblase, Anlage für rechtes und linkes Cölom.
$c_1$ Mesentero-Hydrocölblase, aus welcher sich Darm, Hydrocöl und Parietalcanal bilden.
*cd* Centrodorsalplatte, aus den verschmolzenen Subbasalien entstanden.
*cg* Cölomgang; embryonaler Verbindungsgang zwischen rechtem und linkem Cölomsäckchen.
lsc *cl* linker, später oraler } Cölomsack, entstanden durch Theilung der
rsc *cr* rechter, später aboraler } Cölomblase.
$c_1d$ dorsale } Ausstülpung des Mesentero-Hydrocöls, die zum Darme
$c_1v$ ventrale } werden.
*d* Darmanlage, Mesenteron.
es *ec* Ectoderm.
*f* Festheftungsgrube.
*fp* Fussplatte, terminale Kalkplatte des Stieles.
cho *g* Gekammertes Organ; mit $g_1$, $g_2$, $g_3$, $g_4$, $g_5$ sind die fünf Röhren desselben, die als Ausstülpungen des rechten Cöloms entstehen, bezeichnet. $g_1$ ist das ventral aus dem linken Divertikel sich bildende, $g_5$ das ventrale, rechts vom Verticalmesenterium ausgestülpte.
*gf* Faserstränge im Stiele, Fortsetzungen des gekammerten Organes.
*gz* Ganglienzellen.
hc *h* Hydrocölanlage.
$h_1$—$h_5$ Die fünf primären Ausstülpungen am Hydrocöl und die fünf Wurzeln der 15 ersten Tentakel.
int *hd* Hinterer Darmabschnitt, an den Magen sich anschliessend.
*hr* Hydrocölring.
*ht* Hydrocöl in den 15 Tentakeln der normalen Larve und Tentakelhydrocöl der abnormalen.
$ht_1$ Hydrocöl der 10 interradialen Tentakel.
*ks* Kalktafeln und Kalkglieder des Stieles, Stielglieder.
*l* primäre Leibeshöhle, aus der Furchungshöhle entstanden.
*lm* Längsmuskelfibrillen des Tentakelhydrocöls.
sto *m* Magen.
*mb* Membran unterhalb des Centralnervensystems des Embryos und der freischwimmenden Larve.
*ml* Mundlappen, durch die Oralia gestützt.
*ms* Mesenchymzellen.
*mt* Mesenterium zwischen rechtem und linkem (resp. aboralem und oralem) Cölom.
*mtr* Muskeltrabekel des Hydrocöls.
abovm *mv* Verticalmesenterium des rechten (aboralen) Cöloms.
ovm $mv_1$ Verticalmesenterium des linken (oralen) Cöloms.
*n* Ringnerv der gestielten Larve.
*nf* Nervenfaserplatte unter der Scheitelgrube des Embryos und der freischwimmenden Larve und die beiden Längsnerven derselben.
$nf_1$ Nervenfibrillen des Ringnervs der gestielten Larve und die von ihm ausgehenden Nervenfasern.
*o* Oralia, mit $o_1$, $o_2$, $o_3$, $o_4$, $o_5$ bezeichnet, entsprechend den Interradialräumen, in welchen sie liegen.
eso *oe* Oesophagus.
*p* Porus.
ac *pk* Parietalcanal = BURY's „anterior body cavity“ = BARROIS' „canal du sable“.
*pp* Papillen der Tentakel.
*rf* Ringmuskelfibrillen des Hydrocölringes, einen Sphincter bildend.
*s* Scheitelgrube.
*sb* Subbasalia.
*sc* Sacculi, mit $sc_1$—$sc_5$ bezeichnet, entsprechend ihrer radialen Lagerung.
*sh* Epithelhülle der Sacculi.
*sk* Stielkalkplatten.
stc *st* Steincanal.
*sz* Innenzellen des Sacculus, in älteren Stadien zu Ballen angeordnet.
*t* Die 15 ersten Tentakel.
$t_1$ Die 10 kleineren, interradialen Tentakel.
*tr* Trabekel in der enterocölen Leibeshöhle.
vb *v* Vestibulareinstülpung und Vestibulum.
*w* Wimperbogen des Embryos und der freischwimmenden Larve, mit $w_1$—$w_5$ bezeichnet; $w_1$ stellt den vordersten, $w_5$ den hintersten dar.

doliolaria

posterior

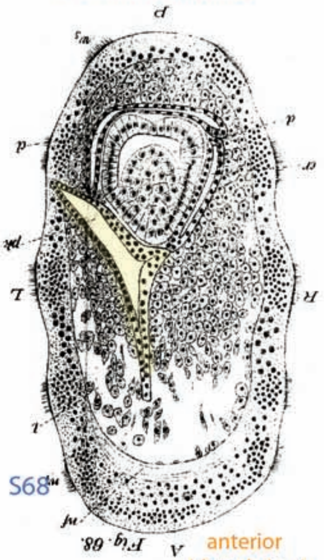


S67

Fig. 67.

anterior
(right view)

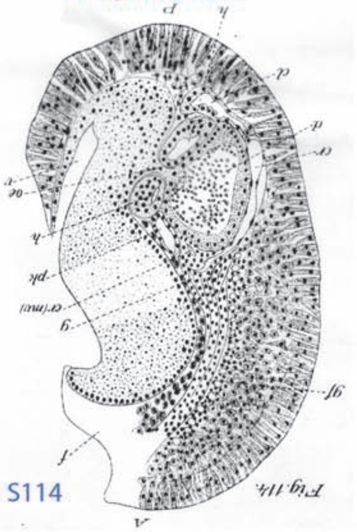
recently hatched



S68

anterior
(dorsal view)

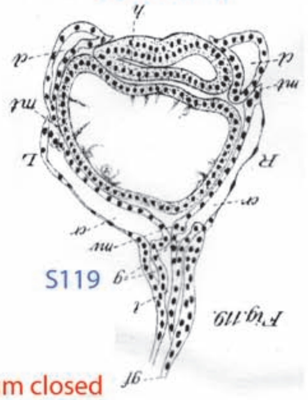
28h, just settled



S114

anterior
(right view)

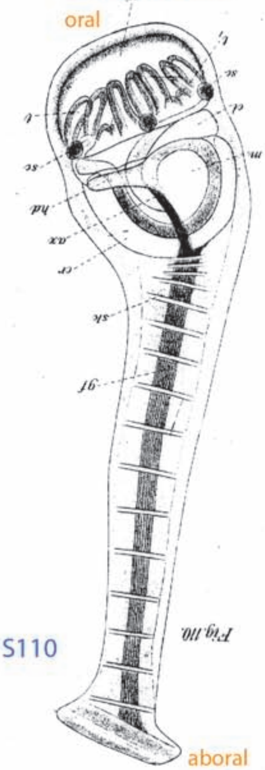
28h, settled



S119

84h, 25 podia

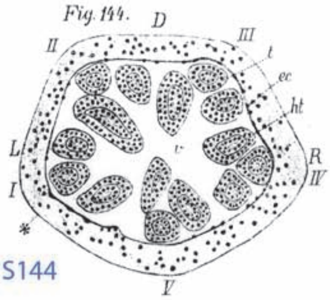
oral



S110

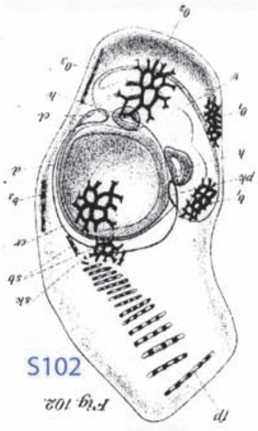
aboral
(right view)

108h, vestibular cavity opens soon



S144

48h, vestibulum closed



S102

aboral
(left view)

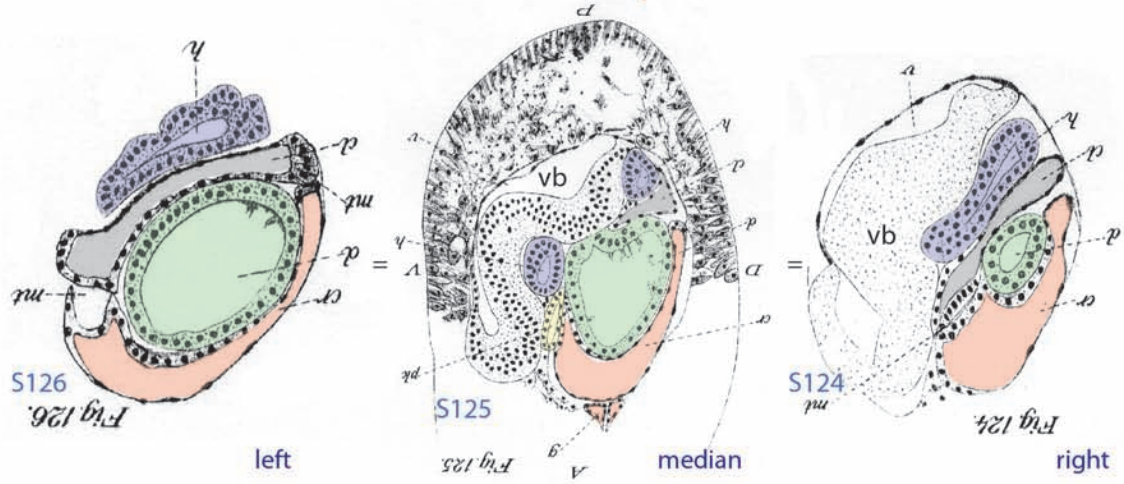
15. sfp (21. sfp shows hp)

At this stage the axial organ occupies the entire extent of the ventral side of the inner wall of the aboral coelome. In the most anterior [=aboral] part, near the chambered organ, it lies as a solid chord of cells on the vertical mesentery (as in Fig. S131). Further posteriorly it becomes more prominet, standing out as a pleat like thickening lying on the gut (as in Figs. S127, S145, S146). At the posterior [=oral] end is the youngest develompental stage (Clark 1921 closely following Seeliger 1892).

sfp = section from posterior

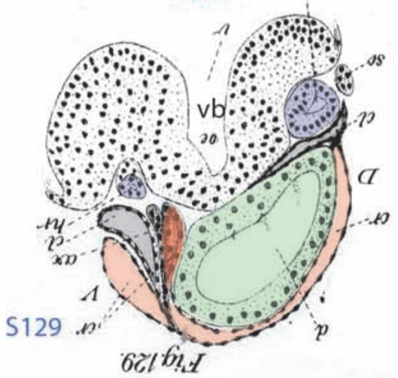
orientation of the figures matches the orientation chosen in this thesis

52h, a bit further developed than S102

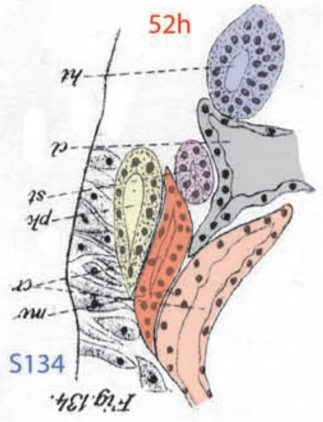


Seeliger does not state an intestinal outgrowth in this specimen.
The lateral sections are cut through the length of the former extensions of the left lobe and show, that they reside between enteric sac and hydrocoel. Hence, the crescent got a more disc-like appearence. At the location, where the extensions once pointed to each other, an aperture/neckline [=Ausschnitt] can now be found, in which the axocoel, primary stone canal and the esophagus reside.

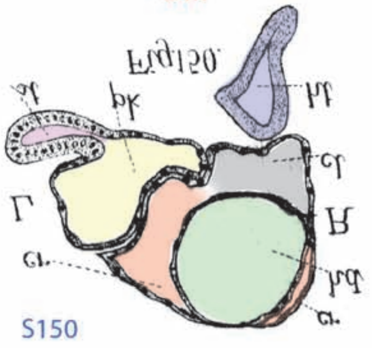
52h



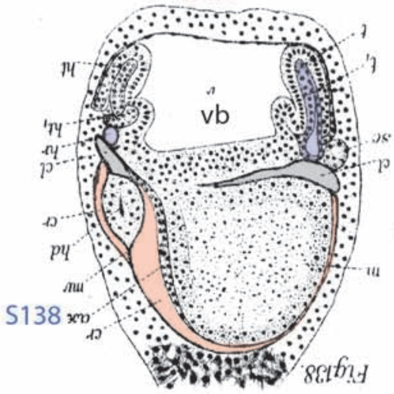
52h



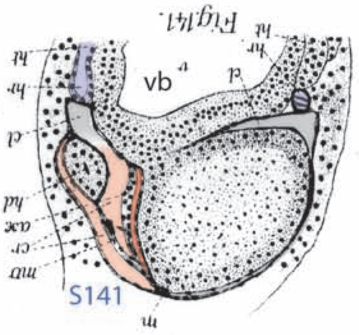
84h



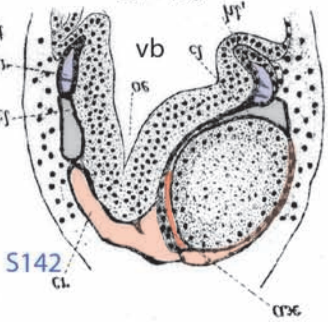
84h



84h



84h



orientation of the figures matches the orientation chosen in this thesis posterior / oral

84h, similar to S110

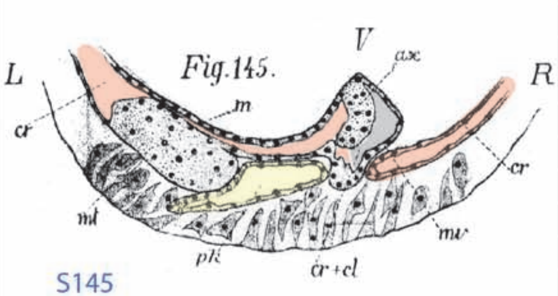


Fig. 145.

S145

84h, a bit further developed than S145

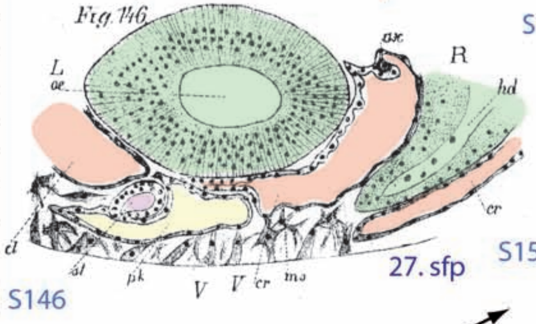


Fig. 146

S146

27. sfp

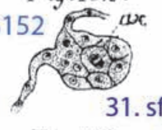


Fig. 152.

S152

31. sfp

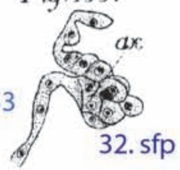


Fig. 153.

S153

32. sfp

108h, (same larva as S144)

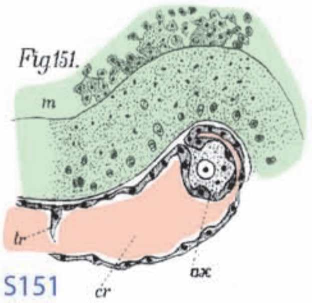


Fig 151.

S151

The bead of cells differentiates into a peripheral layer, representing the coelomic tissue and a central strand. These, however, separate only later.

34. sfp (21. sfp shows porus)

#

60h, further developed than S124-S126

Further posteriorly it (=the *gao*) becomes more prominet, standing out as a pleat like thickening lying on the gut (as in Figs. S127, S145, S146) (Clark 1921 closely following Seeliger 1892).

Under high magnification (Figs. S152, S153) it can be easily seen how the flat coelomic epithelium juts out into the body cavity and the cells involved become richer in plasma and divide so that a several layered swelling results among the cells of which there very soon appears a differentiation of the nuclei, some rapidly increasing in size and becoming vesicular. In individual larvae soon after the first formatiion of the axial organ strikingly large vesicular nuclei appear which have quite the aspect of germ vesicles. They surround themselves with an especially thick plasma zone and their bulk appears so considerable in contrast to that of neighboring cells that they give the impression of young egg cells (Clark 1921 closely following Seeliger 1892).

The axial organ does not run in an absolutely straight line; clinging to the curve of the stomach and esophagus, it is more or less sausage-shaped. The posterior end lies on the border of the oral and aboral coelome, while the anterior proceeds into the column where it becomes extremely difficult to follow (Clark 1921 closely following Seeliger 1892).

52h, a bit further developed than S102

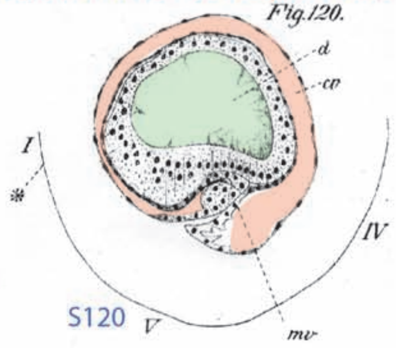


Fig. 120.

S120

- anterior region of the intestine
- *gao* (=*ax*) lies in the aboral somatocoel's left lobe
- forms as thickening of the latter's mesenteric layer

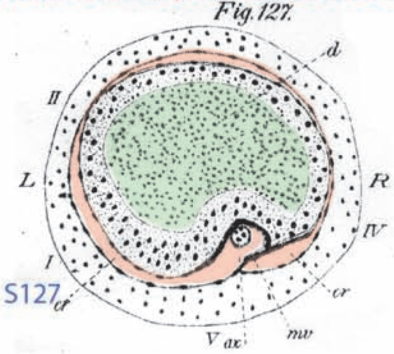


Fig. 127.

S127

- middle region of the intestine
- vertical mesentery develops, as the right lobe lies itself over the left lobe

12h

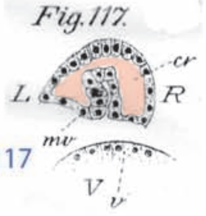


Fig. 117.

S117

52h

Fig. 96.

S96B

52h

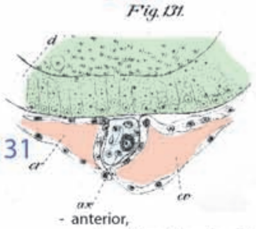


Fig. 131.

S131

- anterior,
- *gaot* conjoined locally with mesenteric layer at its posterior end

time (age after hatching)

sfp = section from posterior

anterior / aboral

#

## 8.2 Selected figures from Russo (1902)

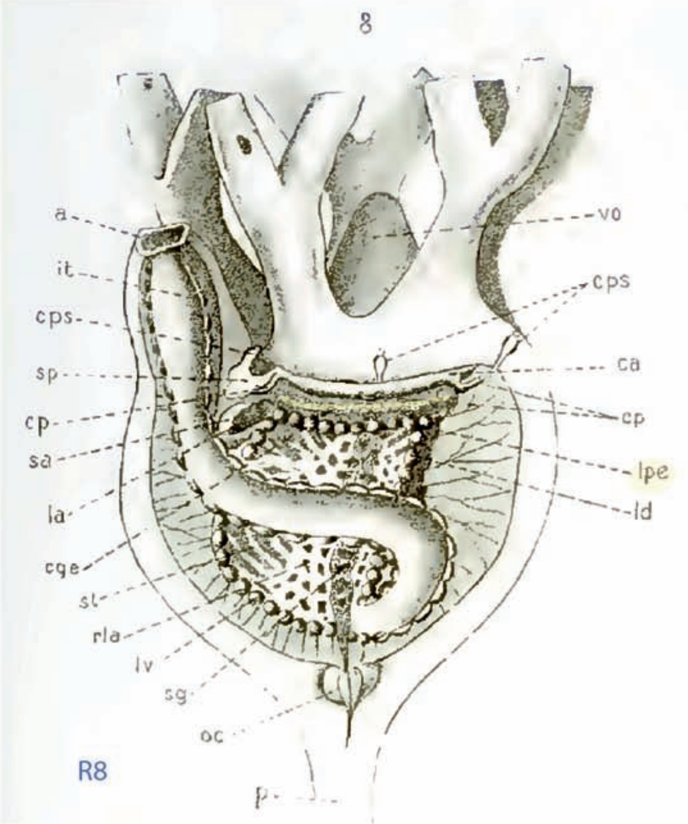
The following index and figures (pages 173-174) are taken from Russo, A. (1902): Studii su gli Echinodermi. Atti dell' Accademia Gioenia di Scienze Naturali in Catania, Ser. 4, 15(7): 1-93, plates I-III.

The “lettere communi a tutte le figure” can be found on page 89. The respective expressions have been complemented by the related abbreviations used in this thesis.

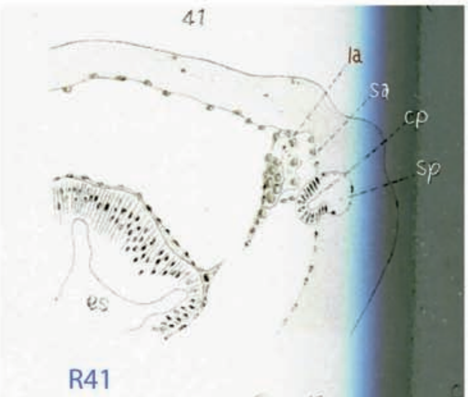
Figures were named retaining Russo’s (1902) original numeration preceding an “R”:
Fig. R8 (=Fig. 8) can be found on Russo’s (1902) Tav. I;
Figs. R23, R24, R41, R42 (=Figs. 23, 24, 41, 42) are taken from his Tav. II.

**Lettere comuni a tutte le figure**

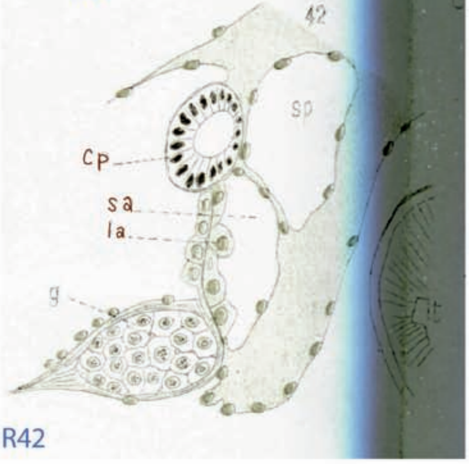
*a* — apertura anale
*ai* — assorbente intestinale
*an* — anello nervoso peribboccale
*b* — apertura boccale
*br* — braccia
*ca* — cerchio acquifero
*cd* — vescicola enterocelica destra
olm *cge* — cordone genitale periesofageo
*ci* — cirri
olm *cg* — cordoni genitali
*cl* — cloaca
stc *cp* — canale petroso primario (prim. + sec.)
*cpa* — canale petroso atrofico
acd, mp *cps* — canale petroso secondario
*cs* — vescicola enterocelica sinistra
*es* — esofago
*fmi* -- sviluppo della madreporite interna
*fsa* — sviluppo del seno aborale
ecc *g* — gonade
*gi* — gemma lacunare intestinale
*gd* — gonodutto
*gla* — gemma proveniente dalla lacuna aborale
*gpc* — origine gonade periesofagea
*i* — idroporo
*idr* — idrocele
*ig* — inizio della gonade
*int* — tubo intestinale
*ip* — idroporo primario
*it* — intestino terminale
*ioc* — sviluppo dell' organo camerato
*ir* — invaginazione vestibolare
*l* — lacuna
vl *la* -- lacuna aborale
ol *ld* — lacuna intestinale dorsale
*li* — lacune intestinali
*lmd* — lamina mesenterica dorsale
*lma* — lamina mesenterica accessoria
peril *lpc* — lacune periesofagee
abol *lv* — lacuna intestinale ventrale
*ma* — madreporite interna
*mr* — muscoli radiali
*nr* — nervo radiale
cho *oc* — organo camerato
*org* — origine della gonade
*og* — organo genitale
*p* — peduncolo
*pc* — pedicelli ambulacrali
*pi* -- papilla dello sbocco del canale petroso atrofico
*ri* — resto dell' idroporo
*rla* — rete lacunare assile
*rli* — rete lacunare intestinale
svc *sa* — seno aborale
*sg* — stolone genitale
*sgd* — sviluppo del gonodutto
ac *sp* — seno parietale (cavity)
*ss* — spazii schizocelici
*st* — stomaco
*svd* — sviluppo del gonodutto
*tc* — tentacoli
*tp* — tentacoli primarii
*ts* — tentacoli secondarii
*vc* — vestibolo
*vea* — vescicola enterocelica anteriore
*vo* — valve orali
*vP* — vescicola di Poli



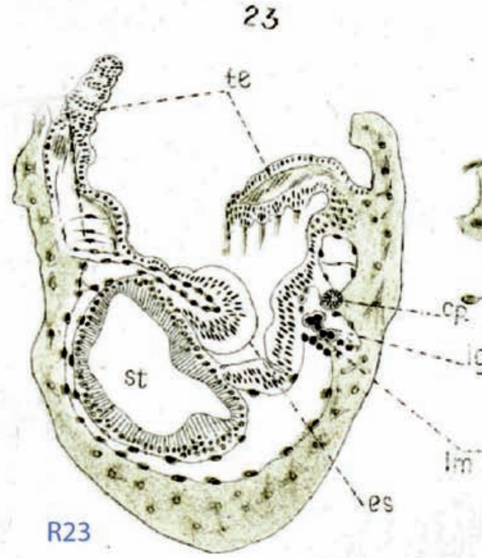
R8



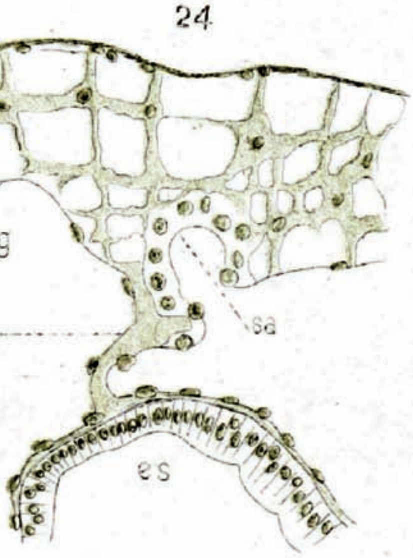
R41



R42



R23



R24