

The fate of the larval epidermis in the Desor-larva of *Lineus viridis* (Pilidiophora, Nemertea) displays a historically constrained functional shift from planktotrophy to lecithotrophy

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Abstract Pilidiophora constitutes a clade of nemerteans characterized by a peculiar larval type, the pilidium. A characteristic of this larva is the transitory epidermis in which the juvenile develops from imaginal discs. The primary function of this larval envelope is assumed to be feeding and dispersal. When juvenile development is complete, the larval epidermis is ruptured and swallowed by the juvenile. According to recent cladistic and molecular analyses of the Nemertea, the intracapsular Desor-larva of the sibling species *Lineus viridis* and *L. ruber* is thought to have evolved from a pelagic pilidium. The general course of development has been demonstrated to be similar to that of the pilidium, in which the juvenile forms from imaginal discs under the larval epidermis. The two *Lineus* species, however, differ in their mode of larval feeding: *L. ruber* being ootrophic and *L. viridis* being lecithotrophic. In order to elucidate the transition from the planktotrophic pilidium to lecithotrophic development, I studied the early cleavage and metamorphosis from intracapsular Desor-larva to juvenile stages in *L. viridis* from the island of Sylt, using light microscopical, electron microscopical, and fluorescent staining methods. Due to the specific cleavage pattern with equally sized 1st quartet animal blastomeres and vegetal blastomeres in *L. viridis*, the larval epidermis later contains a considerable amount of the yolk reserve. During metamorphosis, the larval epidermis is ingested by the juvenile thus displaying behavior similar to that of the pilidium

larva. In contrast to the pilidium, the function of the larval epidermis of the Desor-larva has shifted from feeding and dispersal to direct food supply. Thus, the development of *L. viridis* is a perfect example for strong historical constraints that prevent ancestral larval structures from being lost.

Keywords Metamorphosis · Larval ecology · Lophotrochozoa · Ultrastructure · Spiralia

Introduction

Nemertea is a spiralian taxon that comprises approximately 1,275 species of unsegmented, predominantly marine worms (Gibson 1995; Kajihara et al. 2008). Most members of this taxon develop via a pelagic, planktotrophic larval stage (Gibson 1972; Friedrich 1979; Maslakova et al. 2004; Maslakova 2010a, b). The nemertean subtaxon Pilidiophora, however, forms a group that displays remarkable differences in its mode of development with regard to the metamorphosis of larva to juvenile worm (Tholleson and Norenburg 2003). In the larval stage, known as the pilidium, the juvenile worm is formed inside a larval envelope from so-called imaginal discs. The pilidium is characterized by a dome-shaped body, which is equipped with four perioral lobes that are adorned with a band of long cilia (Lacalli 2005). The pilidial envelope thus constitutes a structure that primarily enables feeding and dispersal of the developing juvenile inside. At the end of development, the juvenile performs a catastrophic metamorphosis by escaping from the pilidial envelope and eating it (Cantell 1966; Maslakova 2010b).

Within the heteronemertean pilidiophorans, there are a few derived modifications to this larval type (Schwartz 2009). Pelagic but lecithotrophic larvae that lack feeding

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structures are reported from *Micrura* species, namely *Micrura akkeshiensis* Yamaoka, 1940, *Micrura rubramaculosa* Schwartz and Norenburg, 2005, and *Micrura verrilli* Coe, 1901 (Iwata 1958; Schwartz and Norenburg 2005; Schwartz 2009). In two closely related species, *Lineus viridis* (Müller, 1774) and *Lineus ruber* (Müller, 1774), larval development diverges from the typical pilidium development to the extent that the larval stage is not pelagic but intracapsular (Bürger 1895; Nusbaum and Oxner 1913; Schmidt 1964). Additionally, these two species are characterized by pseudocopulation, internal fertilization, and a modified sperm type (Gontcharoff 1951; Bartolomaeus 1984; Franzén 1983; Döhren and Bartolomaeus 2006).

In animals that develop via a planktotrophic larval stage, the shift to intracapsular development is typically faced with the problem of supplying food for larvae within the confined space of the clutch. This is usually achieved by provisioning the eggs with a yolk reserve sufficient to ensure proper development. The yolk supply not only alters the size of the egg but may also alter the developmental mode even to the extent that the larval stage is completely absent (e.g., Cephalopoda, Clitellata) (see Fioroni 1978; Dohle 1999; Bergter et al. 2004). Most species, however, show a recapitulation of larval structures during development within the egg (e.g., Moran 1999; Pernet 2003). The latter statement also holds true for pilidiophorans with intracapsular development such as *L. viridis* and *L. ruber*: After total cleavage of the spiral type, a larva develops that, apart from the formation of the juvenile from imaginal discs inside a larval envelope, lacks most features of a pilidium larva, such as perioral lobes with long cilia and an apical tuft (Nusbaum and Oxner 1913; Schmidt 1964). This larval type has been termed Desor-larva. However, the embryogenesis of these two species shows remarkable differences with regard to the provisioning of the larvae. While the Desor-larva of *L. ruber* has been shown to feed on unfertilized eggs within the same clutch, it has been stated that *L. viridis* does not show this behavior (Nusbaum and Oxner 1913; Gontcharoff 1951, 1960; Schmidt 1964). Although apparently not able to take up food, Nusbaum and Oxner (1913) reported that the larva in *L. viridis* utilizes the yolk situated inside the larval epidermis. The larger yolk reserves found in *L. viridis* compared with those in *L. ruber* result in higher numbers of fertilized eggs and thus higher numbers of offspring per female in *L. viridis* (Gontcharoff 1960). A detailed description of utilization of yolk in *L. viridis*, however, is not given. Provided that the Desor-larva of *L. viridis* is derived from a free swimming pilidium, as has been stated by Tholleson and Norenburg (2003), it is expected that the pattern of larval envelope ingestion by the juvenile at the end of metamorphosis is retained. This would comply with observations in other animals that, due to historical constraints, exhibit structures from pelagic

larval stages that are retained in intracapsular development. In this respect, the function of the larval epidermis has changed from a feeding and dispersal structure to an additional yolk supply that is necessary to complete the development of the juvenile in *L. viridis*. To test this hypothesis, the development of *L. viridis* was examined with respect to the structure and fate of the larval epidermis before and after metamorphosis.

Materials and methods

Animals and collecting sites

Sexually mature adult males and females of *Lineus viridis* (Müller, 1774) (Heteronemertea, Pilidiophora) were collected in March of 2006 and February of 2008 during nocturnal low tide from sand flats near the Wadden Sea Station in List, Germany, on the island of Sylt. Details on culturing and reproduction of *L. viridis* can be taken from Döhren et al. (2011). For development, the deposited eggstrings were kept at 17°C in petri-dishes filled with artificial sea water that was changed every fourth day.

Fluorescent staining and confocal laser scanning microscopy

Parts of the clutch comprising approximately 20 egg capsules were fixed in 4% Paraformaldehyde buffered in 0.05 M PBS with 0.3 M NaCl. After fixation for 20 h at 4°C, the samples were washed in three changes of PBT/BSA (1% Triton X-100 (Sigma) and 0.05% BSA (Serva) in 0.05 M PBS with 0.3 M NaCl) for 15 min. Subsequently, the samples were incubated in Phalloidin Alexa Fluor[®] 568 (Molecular Probes, Cat. No. A 12380) at a dilution of 1:200 in PBT for 4 h at room temperature optionally followed by addition of Sytox Green (Molecular Probes, Cat. No. S 7020) at a dilution of 1:1000 and incubation for 1 h at room temperature. The subsequent washing procedure was carried out over 3 h with 3 changes of PBS. The samples were then quickly dehydrated in a isopropanol series (40%, 70%, 85%, 95%, two times 100%, 1 min each) and cleared in Murray-Clear (1 part benzyl-alcohol: 2 parts benzyl-benzoate), mounted on hollow ground microscope slides and examined in a Leica Confocal Laser Scanning Microscope (TCS SPE) using the 488- and 532-nm excitation laser lines. Resulting stacks were projected to maximum z-projections with ImageJ (ImageJ 1.38w).

Electron microscopy

Larvae and juveniles of according developmental stages as for immunohistochemistry were fixed as a whole in 1.25%

glutaraldehyde buffered in 0.05 M phosphate with 0.3 M sodium-chloride buffer (pH 7.2, 4°C) with traces of Ruthenium red for 60 min. Afterward, they were washed in the same buffer and postfixed in 1% OsO₄ buffered in 0.05 M phosphate with 0.3 M sodium-chloride (4°C) for 60 min. Subsequently, they were dehydrated in an acetone series, embedded in araldite, and sectioned into silver interference-colored sections (60 nm) with a diamond knife (Diatome) on a REICHERT ULTRACUT S microtome. The sections were mounted on formvar covered single slot copper grids, automatically stained with uranyl acetate and lead citrate in a TEM-Stainer (Nanofilm) and examined in a Philips CM120 BioTWIN electron microscope (TEM). Pictures were digitally recorded on Ditabis Image Plates.

For Scanning Electron Microscopy, the samples were fixed, washed, and dehydrated as for transmission electron microscopy. Subsequently, they were dried with the critical point drying method (CPD 030; Bal-Tec), mounted on stubs, and sputtered with gold (SCD 040, Balzers Union). The samples were then examined in a Quanta 200 Scanning Electron Microscope (Fei).

Light microscopy

For histological series, 500 nm semi-thin sections of the developmental stages were made with a diamond knife (Diatome). The sections were mounted on glass slides and stained with 1% aqueous Toluidine-blue (1% Toluidine-blue, 1% Sodium tetraborate, 20% Sucrose) for 2 min at 60°C and rinsed with distilled water. Whole mount preparations were made from samples fixed in 4% PFA (s.a.) that were washed in three changes of PBS (0.05 M PBS with 0.3 M NaCl) and subsequently stained with 1% OsO₄ buffered in 0.05 M phosphate with 0.3 M sodium-chloride (4°C) for 30 min. After dehydration in an acetone series, the samples were transferred to pure ethanol and then to Murray Clear (s.a.) for clearing and mounted on hollow ground microscope slides. Both histological sections and whole mount preparations were examined with an Olympus BX 51 Light Microscope with a mounted digital camera (Colorview Soft Imaging System Camera). Pictures were digitally processed with AnalySIS (Soft Imaging Systems).

Results

Development from cleavage to premetamorphic Desor-larva

Development in *L. viridis* is highly dependent on temperature. It takes 26 days at 17°C from the laying of the eggstrings by the female to the first juveniles leaving the clutch. At 4°C, development is almost completely

suspended. Development is not completely synchronous within a single eggstring; the given duration represents an average at 17°C, but some individuals diverge by up to 2 days. The cleavage that starts soon after deposition of the eggstrings is equal. In the 8-cell stage, the blastomeres are roughly of the same size, with the animal blastomeres being slightly larger (vegetal blastomeres about 90% the size of animal blastomeres, $n = 6$) (Fig. 1a). Gastrulation starts on the 3rd day after fertilization and is completed on the 5th day. The larva has then attained a discoid, dorso-ventrally flattened shape. On the 7th day, the larval body begins to elongate, with the caudal region becoming more elongated than the frontal region so that the larva is rhomboid in shape by the end of the 12th day of development. It has an overall body length of $500 \pm 45 \mu\text{m}$ ($n = 8$) and is $300 \pm 25 \mu\text{m}$ ($n = 8$) wide in the widest part of the body, the mouth area. The mouth is crescent shaped with the convex curve facing frontally (Fig. 1b).

Structure of the tissues of the premetamorphic Desor-larva

In the premetamorphic Desor-larva, the epidermis is evenly ciliated with neither perioral lobes nor tufts or bands of long cilia (Fig. 1b). The larva possesses a sac-like gut that consists of a monolayered epithelium comprising large yolk-rich cells (Figs. 1d, 2a). An anus is missing (Fig. 1d, e). By this time, the definitive epidermis has been formed beneath the larval epidermis (Figs. 1c–e, 2a, c, e). At the end of the 13th day, the larval epidermis is completely detached from the definitive epidermis of the juvenile, forming an envelope that is only attached to the juvenile's buccal epithelium (Fig. 1e). The body wall musculature of the juvenile inside the larval envelope consists of an outer ring and an inner longitudinal muscle layer that is especially pronounced caudally of the mouth opening (Fig. 1c, e).

The larval epidermis in *L. viridis* developmental stages varies between 10 and 20 μm in width and consists of two cell types (Fig. 2a–c). The first type that constitutes most of the tissue is a monolayered, epithelial cell type with a distinct polarity (Fig. 2b, e). It is comparably large and flat and has a single nucleus with only little heterochromatin (Fig. 2e). The cell type possesses apical microvilli and cilia that have a 9 + 2 pattern axoneme (Fig. 2b). There is no ciliogenesis detectable in this cell type. The basal body of the cilia shows a conspicuous lateral anchoring device and two striated ciliary rootlets of different size. The larger rootlet parallels the apical cellular membrane, while the smaller rootlet is oriented perpendicularly toward the inside of the cell. Adhaerens junctions are present both apically and basally between the cells (Fig. 2c, d). The electron-lucent staining cytoplasm contains numerous large electron

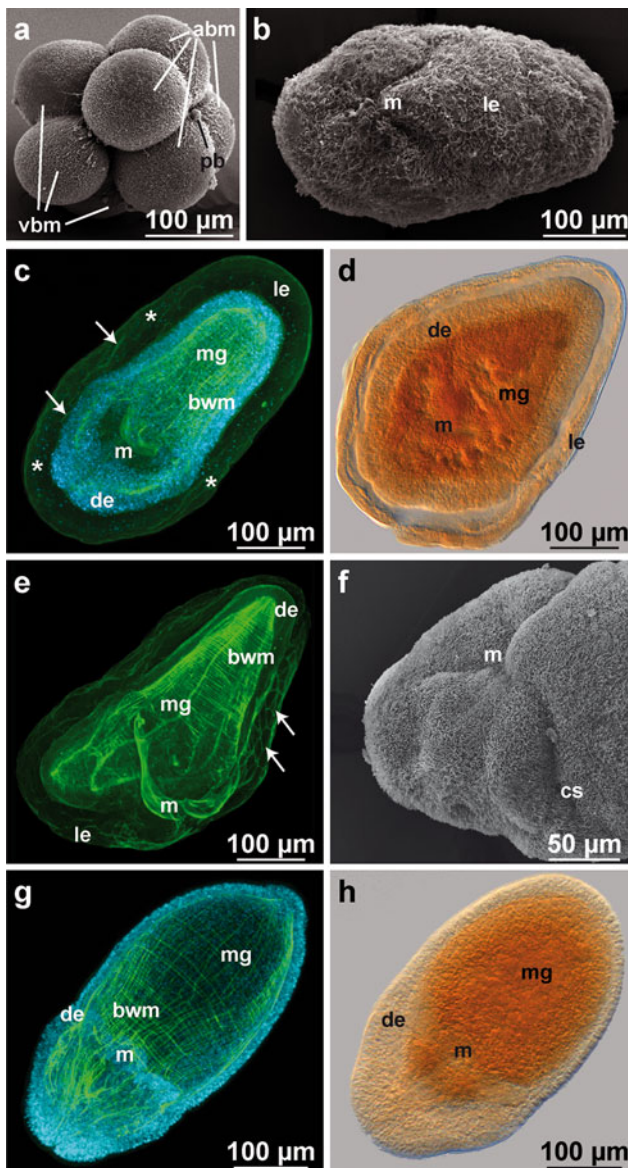


Fig. 1 Whole mount preparations of *L. viridis* developmental stages. **a** 8-cell stage, note that all blastomeres are of equal size; **b** advanced Desor-larva (12 days old), the crescent-shaped mouth is visible on the ventral side of the body which is characteristically rhomboid in shape; **c** advanced Desor-larva (similar stage as in **c**), note the densely spaced nuclei of definitive epidermis in contrast to widely spaced nuclei of the larval epidermis, nuclei of larval epidermal cells marked by *asterisks*; **d** advanced Desor-larva (similar stage as in **b**), note that the larval epidermis is separated from the juvenile developing inside; **e** Desor-larva (14th–15th day) at the onset of metamorphosis, note the larval epidermis being continuous with the foregut epithelium; **f** rostral part of postmetamorphic juvenile (15 days old), note the mouth opening being closed. **g** postmetamorphic juvenile worm (same stage as in **f**), note the bulging midgut caused by the swallowed larval epidermis; **h** juvenile worm (similar stage as in **f**), note the midgut being packed with yolk material. **a**, **b**, **f** SEM pictures; **c**, **e**, **g** CLSM projections, nuclei: cyan, f-actin: green,—*arrows* indicate adherens junctions of larval epidermal cells; **d**, **h** DIC pictures stained with OsO_4 , specimens slightly compressed. *abm* 1st quartet animal blastomeres, *bwm* body wall musculature, *cs* cephalic slit, *de* definitive epidermis, *le* larval epidermis, *m* mouth, *mg* midgut, *vbm* vegetal blastomeres

densely staining vesicles that contain the yolk reserve of the larval epidermis (Fig. 2b, e). Besides those, only few other types of vesicles and organelles are found. Mitochondria are located in the apical part of the cell among the ciliary rootlets along with several small, differently staining vesicles of unclear function (Fig. 2b). The second type of cells in the larval epidermis is an apolar flat cell with numerous microtubules inside that is sitting basally between the ciliated epidermal cells (Fig. 2e, f). These cells are identified as neurons. No other types of cells, e.g., muscular or gland cells are observable in this layer. The larval epidermis is not underlain by an extracellular matrix (*ecm*), instead an open space separates the larval from the definitive epidermis (Fig. 2a, c, e).

The definitive juvenile epidermis has a width of 30 μm and consists of slender, ciliated epithelial cells that are of a mushroom or flask-like shape (Fig. 2e). Apically, the surface of this epithelium seems to fold in from the main apical outline, increasing the circumference of the cell layer (Fig. 2e). Among the ciliated cells, there are many cells that show a peculiarly lobed surface as well as a densely staining cyto- and karyoplasm (Fig. 2e). The ciliated cells are more slender than those of the larval epidermis and their cilia are more densely set (Fig. 2c, e). The two ciliary rootlets of the cilia seem to be inversed compared with the cilia of the larval epidermis as the perpendicular ciliary rootlet seems to be the larger one. The cells possess apical microvilli and adherens junctions that are not different from the respective structures of the larval cells (Fig. 2b, c). Basally, all cells adhere to the underlying basal *ecm* (Fig. 2e). Inside, the definitive epidermis cells show some remarkable differences from the larval epidermal cells. Their cytoplasm is of a coarse, electron densely staining texture containing a wide variety of inclusions such as centrioles in large numbers (Fig. 2c) hinting at ciliogenesis and numerous differentially staining vesicles of various sizes (Fig. 2c, e). The number of large lipid vesicles, however, is notably smaller (Fig. 2e) than that observed in the larval epidermis. Mitochondria are found near the apical cell pole among the ciliary rootlets, but in larger numbers than in the larval epidermal cells (Fig. 2c). There is no hint at neuronal cell processes inside this epithelium, but there are gland cells with different content interspersed among the ciliated cells.

Underneath the epidermal basal *ecm* a layer of mesodermal tissues of less than 10 μm width is present. It consists mainly of muscle cells that constitute the body wall musculature of the juvenile (Fig. 2e). The mesodermal tissue layer is delimited by a basal *ecm* that underlies the intestinal epithelium. This epithelium consists of large cells that contain an electron-lucent cytoplasm as well as several yolk vesicles per cell. Compared with the larval epidermis, the number of yolk vesicles is considerably smaller (Fig. 2e).

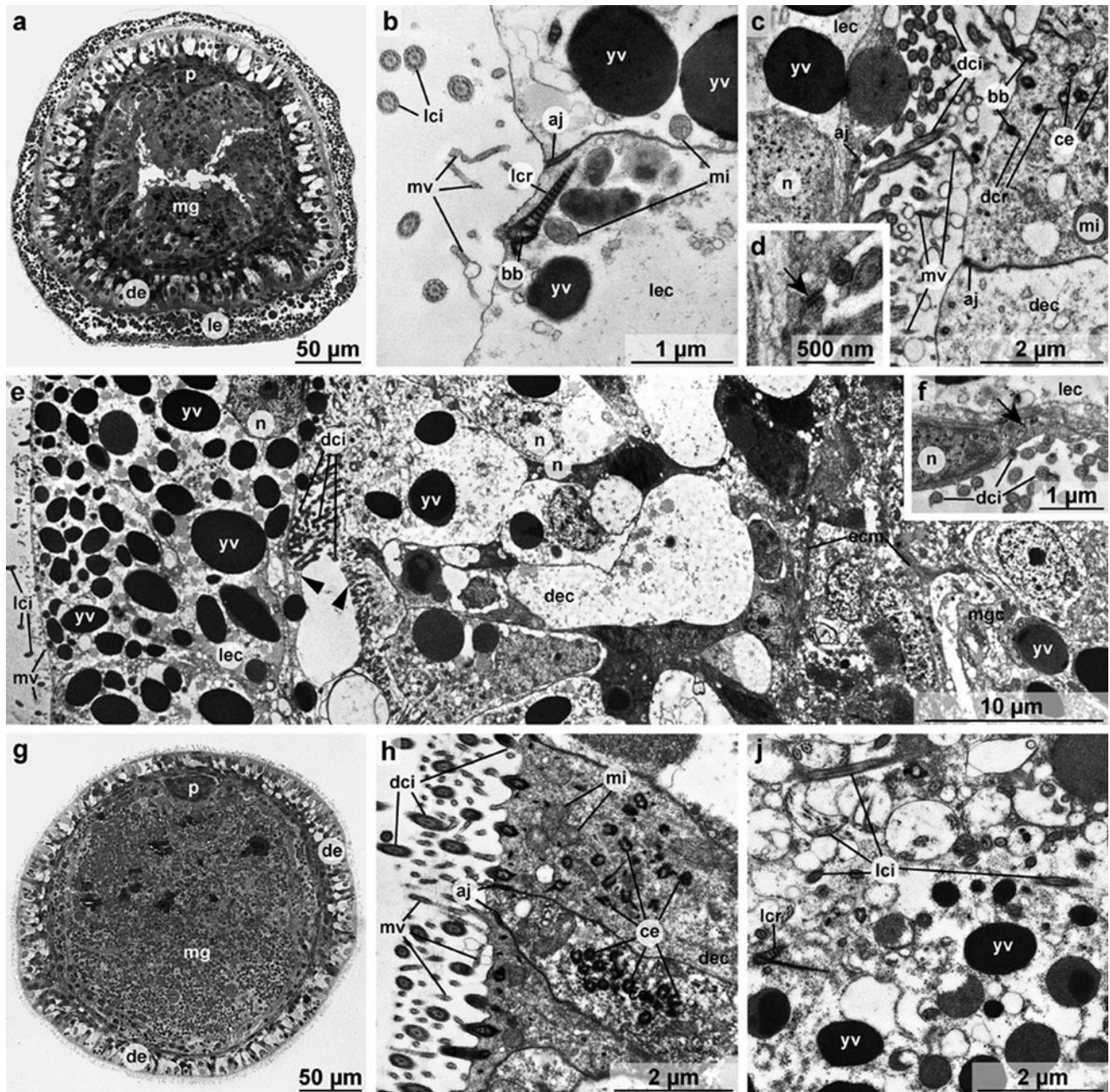


Fig. 2 Sections of *L. viridis* developmental stages. **a** Cross-section through a premetamorphic Desor-larva caudal of mouth region, the larval epidermis (*le*) is visible distally of the definitive epidermis (*de*), gut (*mg*) with clearly visible lumen; **b** apical part of the larval epidermis—cells possess cilia (*lci*) and microvilli (*mv*) as well as apical adherens junctions (*aj*), note large electron densely staining yolk vesicles (*yv*); **c** larval and definitive epidermis in a premetamorphic larva—note coarsely staining cytoplasm and lack of large darkly staining yolk vesicles in the definitive epidermis, arrows mark neuronal cell processes inside the larval epidermis; **d** inset of (**c**) showing basal adherens junction (arrow) of the larval epidermis; **e** section through the premetamorphic Desor-larva—the distal larval epidermis is underlain by the definitive epidermis (arrowhead marks a fold of the definitive epidermis), note size and shape of different epidermal cells as well as differing distribution of large electron densely staining yolk vesicles (*yv*); **f** neuronal cell (arrow) basal to larval epidermal cell

(*lec*); **g** cross-section of a postmetamorphic juvenile caudal of the mouth region—distally, the definitive epidermis (*de*) is visible, the gut (*mg*) is packed with the swallowed larval epidermis; **h** apical part of the definitive epidermis in the postmetamorphic juvenile—the cells show dense ciliation, microvilli and apical adherens junctions (*aj*), inside, numerous centrioles (*ce*) hint at ongoing ciliogenesis; **j** section through the midgut region of a postmetamorphic juvenile—some of the structures such as cilia (*lci*) and electron densely staining yolk vesicles (*yv*) from the larval epidermis are still discernible. **a**, **g** semi-thin sections stained with 1% Toluidine-blue; **b–f**, **h**, **j** ultra-thin sections, TEM. *bb* basal body, *dci* cilia of definitive epidermis, *dcr* ciliary rootlet of definitive epidermis cilium, *dec* definitive epidermis cell, *ecm* basal extracellular matrix *lci* cilia of larval epidermis, *lcr* ciliary rootlet of larval epidermis cilium, *lec* larval epidermis cell, *mg* midgut, *mge* cells of midgut epithelium, *mi* mitochondrium, *mv* microvilli, *n* nucleus, *p* anlage of proociscis apparatus

Metamorphosis and postmetamorphic juvenile

On the 14th day, after fertilization, the developmental stages of *L. viridis* undergo metamorphosis. Upon doing so, their appearance changes drastically. The outer shape transforms from rhomboid to oval with more or less equal width throughout the length of the animal. On the lateral sides of the head, the cephalic slits are visible while ventrally the mouth opening is sealed only leaving a shallow dimple (Fig. 1f).

In postmetamorphic juveniles, the gut is densely packed with yolk-rich, cellular material (Figs. 1h, 2g) so that it bulges, giving the animal its more or less round cross-section (Figs. 1g, h, 2g). The body wall musculature of the juvenile is now stretching over the entire length of the animal (Fig. 1g).

The epidermis consists of an evenly ciliated monolayered epithelium. The cells are slender with densely set cilia and microvilli projecting from the epidermal surface. Inside, the coarse electron dense cytoplasm and numerous centrioles hint at high biochemical activity and ongoing ciliogenesis (Fig. 2h). All cells rest on an underlying basal *ecm* (not shown). There is no gap between the basal *ecm* of this epithelium and the underlying tissue. This epithelium exhibits the characteristics of the inner (definitive) epidermis described for the premetamorphic Desor-larva, i.e., that they are identical. The contents of the gut consist of densely packed cellular debris that contains numerous large yolk vesicles similar to those found in the larval epidermis of the premetamorphic Desor-larva. Among the cellular debris, there are unordered, detached cilia presumably identical with those of the larval epidermis (Fig. 2j). The cellular material inside the juvenile gut is the detached larval epidermis that has been ingested by the juvenile during metamorphosis.

On the 19th day of development, the juveniles increase in body length and begin to thin. They leave their pear-shaped egg capsules and begin to crawl around in the gelatinous layer of the eggstring. This continues until day 26, when the juveniles finally leave the eggstring completely, their guts still containing small rests of the yolk from the larval epidermis cells. They have now reached a body length of about 1,000 μm .

Discussion

Due to the phylogenetic position of *L. viridis* being nested within Pilidiophora (Tholleson and Norenburg 2003; Andrade et al. 2011), its intracapsular Desor-larva is presently thought to be derived. The ancestral larval type of this taxon is the pilidium, a pelagic, planktotrophic larva (Tholleson and Norenburg 2003; Maslakova and Norenburg

2001; Maslakova 2010a, b). The mode of development in the intracapsular Desor-larva has been demonstrated to be very similar to the development of the pilidium (Nusbaum and Oxner 1913; Schmidt 1964; Maslakova 2010a, b). The juvenile worm develops from several imaginal discs inside the larval epidermis. During the development of the juvenile inside the pilidial epidermis, the larva is planktotrophic (Maslakova 2010b). When morphogenesis of the juvenile is completed, the fully developed juvenile hatches from its envelope by means of a catastrophic metamorphosis during which the larval envelope is ruptured and subsequently eaten by the juvenile (Cantell 1966; Maslakova 2010b). The pilidial envelope thus primarily constitutes a device for food capture and dispersal and represents the first food of the juvenile worm.

While the pilidium is dependent on external food sources during its development (e.g., Maslakova 2010b), the Desor-larva of *L. ruber* has been reported to be non-feeding (Nusbaum and Oxner 1913). The yolk supply that is situated in the larval epidermis is claimed to pass through the mesodermal tissues of the developing juvenile to be resorbed in the gut. This is contradicted by Schmidt (1964), who points out that *L. ruber* larvae are adelphophagic, feeding on abortive embryos within their egg string, whereas *L. viridis* (as *L. desori*) is lecithotrophic, relying entirely on the internal yolk supply of the egg. The exact process of the uptake of the yolk reserve, however, is not described. Moreover, neither of the cited authors has described metamorphosis and the fate of the larval epidermis. The present study demonstrates the actual process of metamorphosis and shows that it is similar to that found in the pilidium larva. The larval epidermis, which, due to the mode of cleavage, contains the majority of the yolk supply of the zygote, is ruptured and swallowed by the juvenile. Furthermore, in reviewing the data on the nourishment of *L. ruber*, it is very likely that this kind of metamorphosis is also present in this species. Although Schmidt (1964) states that *L. ruber* already feeds on abortive embryos within the same egg capsule during its larval stage, his published images (Schmidt 1964: Figs. 3, 14) show entire ingested sibling embryos inside a postmetamorphic juvenile (the larval epidermis is obviously absent). The latter interpretation is supported by the account of Gontcharoff (1960), in which she states that *L. ruber* does not feed before having undergone metamorphosis. Moreover, in contrast to *L. viridis*, in which the mouth opening is sealed after metamorphosis, the mouth opening and digestive tract of *L. ruber* become functional when the animal is still within the egg capsule (Schmidt 1964). Thus, the main difference between the two species is the feeding behavior of the postmetamorphic juveniles (reviewed by Friedrich 1979).

The differing modes of feeding during development in *L. viridis* and *L. ruber* are due to the different yolk content of the eggs (Schmidt 1964). While the yolk content of the

egg and consequently of the larval epidermis in *L. viridis* enables complete development until hatching (lecitotrophy), the juveniles of *L. ruber* have to take up additional food until they are able to hatch (adelphophagic ootrophy). The interpretation by Schmidt (1964) that *L. ruber* is more derived than *L. viridis* has to be doubted. The ancestral developmental mode of Pilidiophora is by a larval stage in which the yolk supply is so little that the larva has to feed (planktotrophic pilidium). Compared with the ancestral state of yolk content in the pilidium, lecithotrophy in *L. viridis* has to be regarded as more derived than ootrophy in *L. ruber*. To supply the larger yolk reserve, oogenesis lasts much longer in females of *L. viridis* (9–10 months), in contrast to *L. ruber*, in which oogenesis only takes 5–6 months (Bierne 1983 and references therein; Döhren et al. 2011). The adaptive advantage for *L. viridis* is to have more offspring per clutch compared with *L. ruber* (Gontcharoff 1960).

Conclusion

Development in *L. viridis* is another example of larval trait retention within a modified type of development. It has been demonstrated that certain gastropods or polychaetes that undergo intracapsular development pass through the developmental stages that exhibit larval structures, e.g., trochophore stages (see Pechenik 1999, 270, for references). In *L. viridis*, the formation of a larval epidermis is obligatory for further development although it is not used as a device for food capture and dispersal. Instead, its main function has shifted to a food supply for the postmetamorphic juvenile within the confined space of the egg capsule. There has been an observable adaptation to distribute a larger portion of the yolk supply to the prospective intestinal cells by a relative enlargement of the 1st quartet vegetal blastomeres IA–D in *L. viridis* compared with the vegetal blastomeres of the same cleavage stage in *L. ruber* (Schmidt 1964) to make more yolk useable for premetamorphic larval nourishment. Nevertheless, the historical constraint of pilidiophoran development (as outlined most recently by Maslakova 2010b) prevents the total reduction in the larval ectoderm in *L. viridis*. Retaining specific larval traits when the yolk supply is increased to the extent described herein is somewhat unusual in Spiralia. In clitellates and cephalopods, the cleavage pattern is highly modified and the larva is generally reduced when yolk supply exceeds a certain level (Fioroni 1978; Dohle 1999; Bergter et al. 2004).

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