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Metamorphosis of the Marine Bryozoan *Membranipora membranacea*: An Ultrastructural Study of Rapid Morphogenetic Movements

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ABSTRACT The colonial marine bryozoan *Membranipora membranacea* produces a planktotrophic cyphonautes larva that is encased by a triangular bivalved shell. Following a relatively long free-swimming phase, the cyphonautes larva settles and undergoes a dramatic metamorphosis to become the sessile progenitor of the colony, referred to as the ancestrula. This paper examines the initial morphogenetic movements of metamorphosis that transform the cyphonautes larva into an incipient ancestrula. At the onset of metamorphosis, contractions of the striated median muscles situated along the anterior and posterior margins of the larva cause a retraction of the larval apical organ and a centripetal movement of the anterior and posterior ends of the larva. Concurrently, the ciliated corona at the base of the larva is pulled within the shell by contractions of the striated lateral muscles. As the larva assumes a more spherical shape, the posterior margins of the shell are spread apart, and the internal sac is everted. Eversion of the sac is apparently achieved by contractions of the lateral muscles that cause a buckling of the shell in the apical-basal direction. The neck region of the everted sac secretes adhesive granules that attach the larva to the substratum. Subsequently, contractions of the nonstriated sac muscles fold the valves of the shell over each other and draw the larva toward the substratum. The initial events of metamorphosis that culminate in the attachment and flattening of the larva are completed in 10–15 seconds. In the subsequent few minutes, the lateral edges of the everted sac fuse with the neighboring margins of the aboral epithelium underlying the shell and thus form the fully sealed body wall of the incipient ancestrula.

The phylum Bryozoa is composed of sessile colonial animals that characteristically produce short-lived lecithotrophic larvae (Ryland, '70, '74). In contrast to such nonfeeding larvae, a long-lived planktotrophic form, called the cyphonautes larva, develops in a few marine species belonging to the class Gymnolaemata (Zimmer and Woollacott, '77a; Stricker et al., '88a,b). The lecithotrophic larvae of bryozoans tend to be ovoid and covered by a densely ciliated epidermis, whereas the cyphonautes larva is laterally compressed and encased by a chitinous shell. At the conclusion of the free-swimming phase, both types of larvae settle and undergo a dramatic metamorphosis that is characterized by an initial rapid change in larval morphology followed by a more protracted period of histolysis and histogenesis (Zimmer and Woollacott, '77b).

The first stage of metamorphosis in bryozoans usually lasts only a few minutes and consists of rapid morphogenetic movements that transform the larva into a sessile "preancestrula" (Zimmer and Woollacott, '77b). The second stage of metamorphosis occurs over a period of several days and involves the histolysis of ephemeral larval tissues as well as the differentiation of presumptive adult structures. Such processes result in the gradual transformation of the preancestrula into the initial zooid of the colony, referred to as the "ancestrula" (Zimmer and Woollacott, '77b).

The rapid morphogenetic movements involved in the transformation of the larva into a preancestrula have been extensively studied in several species of bryozoans that produce short-lived lecithotrophic larvae (e.g., d'Hondt, '77; Woollacott and Zimmer, '78; Reed, '85). By contrast, little is known about the early events of metamorphosis in gymnolaemates that form cyphonautes larvae. Kupelwieser ('05) presents an elegant histo-

logical account of the free-swimming cyphonautes larva of *Electra pilosa* and describes aspects of settlement and metamorphosis. Subsequent investigations related to the metamorphosis of cyphonautes larvae, however, deal mainly with the gross anatomy of the preancestrula and the patterns of ancestrular budding (e.g., O'Donoghue, '27; Atkins, '55: Mawatari, '73; Mawatari and Mawatari, '74). The cytological changes that occur during the transformation of the cyphonautes larva into a preancestrula remain poorly understood.

The present paper describes the early events in the metamorphosis of the cyphonautes larva produced by *Membranipora membranacea* (Class Gymnolaemata, Order Cheilostomata). The mechanisms by which the larva attaches to the substratum and rapidly changes its morphology during the initial stage of metamorphosis are investigated by light and electron microscopy. In addition, the transformation of the free-swimming cyphonautes larva into a preancestrula is compared to the patterns previously described for other bryozoans, which produce lecithotrophic larvae.

MATERIALS AND METHODS

Larvae were collected from the plankton in the vicinity of the Friday Harbor Laboratories, San Juan Island, WA during the summers of 1985 and 1986. Specimens were kept in glass culture dishes containing unfiltered seawater for up to 1 week before being offered suitable substrata for metamorphosis.

As discussed by Stricker ('87) and Stricker et al. ('88a), the larvae were identified as belonging to the genus *Membranipora* based on the formation of a twin ancestrula stage after metamorphosis. The common membraniporid in the vicinity of Friday Harbor Laboratories is believed to be *M. membranacea* (Kozloff, '83; Strathmann and McEdward, '86), but the systematics of this genus is currently in a state of flux (Yoskioka, '82; Harvell, '84). Thus, the larvae examined in this study are referred to *sensu latu* as *M. membranacea.*

To induce settlement and metamorphosis, small discs (~1 cm diameter) were cut from the proximal ends of the brown algae *Laminaria groenlandica* and *L. saccharina*, which serve as preferred sites for metamorphosis of cyphonautes larvae in the field. The algal discs were washed in running seawater overnight to remove mucilaginous secretions emanating from the cut edges. Subsequently, the pieces of algae were placed at the bottom of depression wells in a plastic culture dish (Falcon No. 3047). A small volume of seawater was pipetted over the algal discs, and five to ten larvae were introduced into each well.

As soon as one of the larvae began to metamorphose, an aliquot of primary fixative was squirted directly over the metamorphosing specimen. The primary fixative consisted of either a cacodylate-buffered solution of glutaraldehyde containing trace amounts of ruthenium red (Cavey and Cloney, '72) or a bicarbonate-buffered mixture of osmium tetroxide (Stricker and Reed, '81). Specimens that remained firmly affixed to the alga were cut out with a razor blade and reimmersed in fresh fixative. All glutaraldehyde-fixed larvae were postfixed in bicarbonate-buffered osmium tetroxide, embedded in LX-112 plastic resin (Ladd Research, Inc.), and processed for light and electron microscopy according to the methods of Stricker and Reed ('85). Stages in larval metamorphosis on algal substrata were also recorded on Tri-X film using a Wild Photomacroscope equipped with a strobe light flash unit. Unless stated otherwise, all micrographs are of material initially fixed in the ruthenium red/ sodium cacodylate solution of glutaraldehyde.

RESULTS

Morphology of the free-swimming larva

The free-swimming cyphonautes larva of *Membranipora membranacea* is covered by a triangular bivalved shell and typically measures 400–550 μm in length (Figs. 1, 2, 11). The following descriptions pertain to organs that are involved in settlement and metamorphosis. Further details concerning larval anatomy are contained in Stricker et al. ('88a,b).

The outer layer of the body wall underlying the shell is referred to as the aboral epithelium. At the apex of the larva, the aboral epithelium forms a knob-shaped sensory structure, or "apical organ" (Stricker, '87). The apical organ protrudes through an ovoid opening between the two valves of the shell

Fig. 1. Photomicrograph of a right lateral view of a living cyphonautes larva of *Membranipora membranacea*. Differential interference contrast optics. Bar = 100 μm.

Fig. 2. Photomicrograph of a 1-μm sagittal section of a cyphonautes larva. ao, apical organ; co, corona, cr, ciliary ridge in vestibule; g, gut; is, internal sac; po, pyriform organ; sh, shell. Bar = 100 μm.

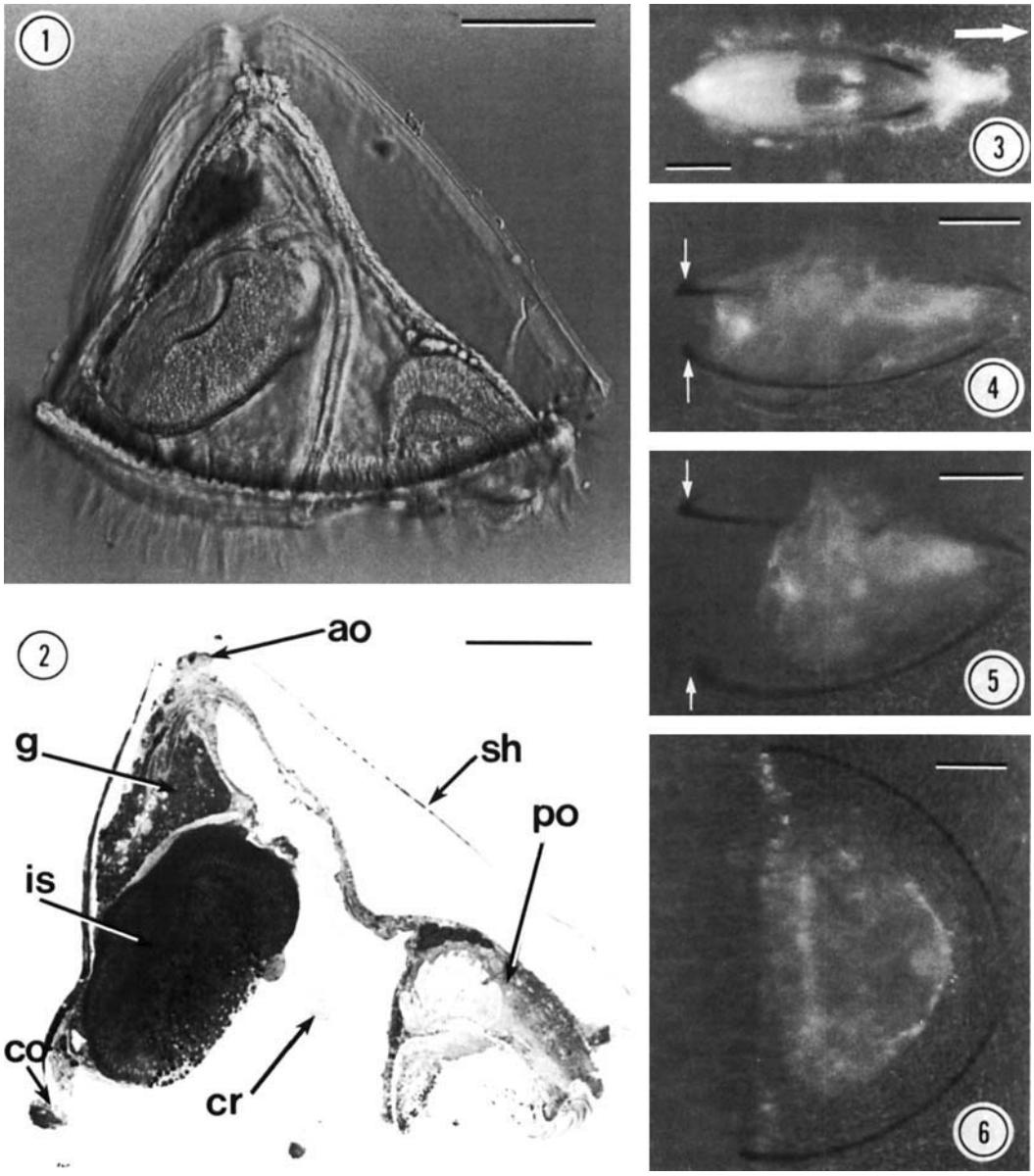


Fig. 3. Photomacrograph of an apical view of a living larva in an upright position over the algal substratum during the so-called exploratory phase of settlement prior to metamorphosis. The arrow marks the direction of locomotion. Note the protruded pyriform organ at the anterior end of the larva. Bar = 100 μm.

Fig. 4. Photomacrograph of an apical view of a living larva that had just begun to metamorphose. The posterior margins of the shell are beginning to spread apart (arrows). Bar = 100 μm.

Fig. 5. Photomacrograph of a slightly more advanced stage of metamorphosis (approximately 2 seconds after the stage depicted in Fig. 4). The posterior end of the shell is spread further apart (arrows), and the larva has assumed a more spherical shape. Bar = 100 μm.

Fig. 6. Photomacrograph of an apical view of a living larva immediately after it had flattened itself against the algal substratum. The former posterior end of the free-swimming larva occurs toward the left side of the figure. The dark, curved edges of the overlapped valves that cover the flattened larva correspond to the basal margins of the larval shell. Bar = 100 μm.

and differs from the neighboring regions of the aboral epithelium in possessing a core of neurons and an overlying cuticle. Toward the basal half of each valve, the aboral epithelium is filled with granule-containing "polster cells" that are tightly associated with the shell (Fig. 8). The mass of polster cells is in turn connected to a densely ciliated "corona" that protrudes beyond the basal margin of the shell and serves as the larval locomotory organ. Anteriorly, the larva contains a neuroglandular complex, termed the pyriform organ. Posterior to the pyriform organ is a large mantle cavity, or vestibule, that contains a pair of ciliated ridges.

An invaginated ovoid organ, called the internal sac, occurs anterior to the gut in the posterior half of the larva. The long axis of the sac is oriented at an oblique angle relative to the base of the larva. When fully developed, the sac consists of a posterior roof region, an anterior neck region, and an intermediate wall region (Fig. 11). The roof and wall regions are composed of essentially nonglandular cells, whereas the neck region comprises granule-containing cells that are folded posteriorly into the cavity surrounded by the roof and wall regions. A small anterior pore occurs near the midfrontal plane of the sac and leads into the lumen enveloped by the infolded neck cells. The anterolateral edges of the sac connect with the epithelium that lines the vestibule (for topographic relationships, see Stricker et al., '88b).

Four major sets of muscles occur within the larva: i) a nonstriated adductor muscle, ii) a pair of nonstriated sac muscles, iii) two striated median muscles, and iv) two groups of striated lateral muscles (Fig. 11). The adductor muscle lies directly anterior to the internal sac near the midfrontal plane of the larva and extends across the larva to attach to the two valves of the shell. A sac muscle originates in the mesodermal compartment of the body wall underlying the central region of each valve of the larval shell. The muscles drape over the internal sac and insert in the roof region of the sac. The posterior and anterior margins of the aboral epithelium are underlain by a median band of muscles. The posterior median muscle attaches to the basal lamina underlying the apical organ and extends to the posterior edge of the corona. The anterior median muscle runs between the apical organ and the posterior end of the pyriform organ. Each group of lateral muscles originates in the aboral epithelium directly anterior to the origin of the neighboring sac muscle. The myofibers of the lateral muscle complexes ramify along both sides of the larva and insert along the margins of the corona.

Overview of the early events of metamorphosis

In accordance with the terminology proposed by Chia ('78), settlement is regarded as the behavioral change wherein the free-swimming cyphonautes larva adopts a benthic existence. Metamorphosis, on the other hand, is viewed as the actual morphological and physiological alterations that transform the benthic larva into a sessile ancestrula.

Cyphonautes larvae maintained in glass containers for a week seldom metamorphose, but metamorphosis occurs fairly frequently when the larvae are kept in plastic dishes. Larvae usually metamorphose along the sides or on the bottoms of these dishes, but a few specimens attempt to metamorphose on the undersurface of the air-water interface. When the appropriate algal substratum is added to a culture dish, a marked increase occurs in the number of competent larvae that settle and metamorphose. Metamorphosis usually begins a few minutes to several hours after the larva settles on the alga.

Before metamorphosis commences, the larva characteristically spends a variable period moving directly over the algal substratum during the so-called exploratory phase of settlement (Fig. 3). As the larva glides in an upright position over the alga, the pyriform organ extends beyond the anterior edge of the shell and is thought to test the suitability of the substratum for metamorphosis (Kupelwieser, '05; Stricker et al., '87b). The pyriform organ also secretes a thin mucous sheet that aids locomotion and helps to fasten the larva temporarily to the substratum (Stricker et al., '87b).

At the onset of metamorphosis, the laterally compressed larva assumes a more spherical shape. The rounding up of the larva involves a retraction of the apical organ and corona, as well as a centripetal movement of the anterior and posterior ends of the body. Concurrently, the apices of the two valves are drawn together, and the posterior margins of the shell are spread apart (Figs. 4, 5). As these events occur, the internal sac everts and attaches the larva to the substratum. The sac is fully everted by 5–10 seconds following the onset of metamorphosis.

Once the internal sac is everted, there is often a pause lasting up to a few seconds. The larva then rapidly flattens itself against

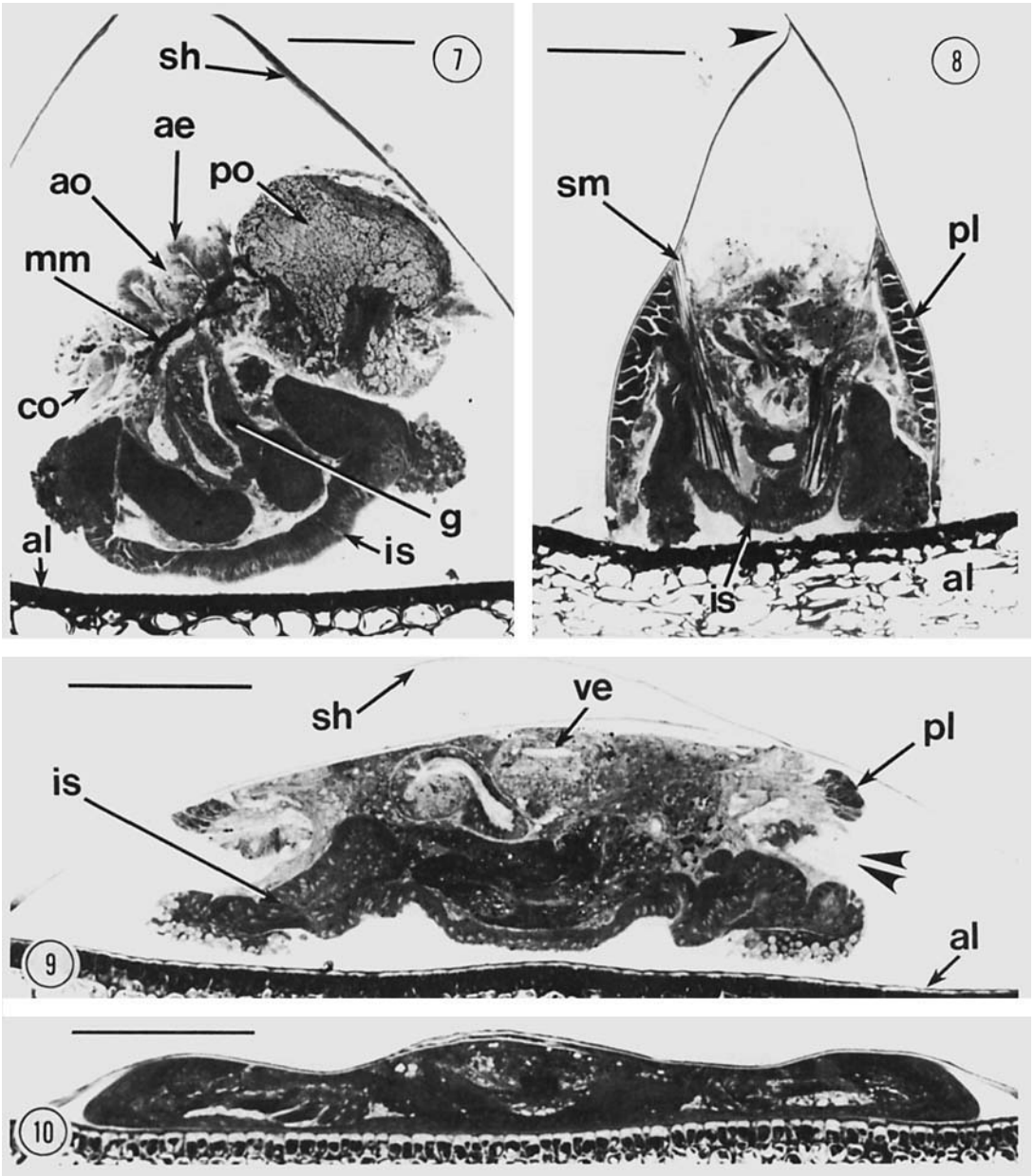


Fig. 7. Photomicrograph of a 1-μm sagittal section through a larva at the beginning of metamorphosis. ae, aboral epithelium; al, alga; ao, apical organ; co, corona; g, gut; is, internal sac; mm, median muscles; po, pyriform organ; sh, shell. Osmium tetroxide fixation. Bar = 100 μm.

Fig. 8. Photomicrograph of a 1-μm transverse section through a larva at the beginning of metamorphosis. al, alga; is, internal sac; pl, polster cells; sm, sac muscle. Note that the opening at the apex of the shell is no longer present (arrowhead). Osmium tetroxide fixation. Bar = 100 μm.

Fig. 9. Photomicrograph of a 1-μm transverse section of a larva approximately midway through the initial phase of metamorphosis. al, alga; is; internal sac; pl, polster cells: sh, shell; ve, vesicle above neural core of apical organ. The double arrowheads mark the gap between the aboral epithelium and everted internal sac. Bar = 100 μm.

Fig. 10. Photomicrograph of a 1-μm transverse section through a fully flattened larva about 1 min after the onset of metamorphosis. Note that the lateral margins of the body wall have fused. Bar = 100 μm.

the substratum (Figs. 6–14). Concomitantly, the two valves of the shell are folded over each other along their anterior margins and drawn toward the flattened larva (Figs. 9, 10, 13, 14).

Immediately after the larva is covered by the overlapped valves, it shifts laterally to each side. Such movements spread the larva maximally over the substratum and mark the completion of the initial rapid morphogenetic movements of metamorphosis. Most larvae lie completely flat against the substratum by 10–15 seconds after the onset of metamorphosis. In the subsequent few minutes, the flattened larva undergoes a subtle morphological change that involves the fusion of oral and aboral epithelia to form a completely sealed body wall.

Specimens fixed with glutaraldehyde directly before the onset of metamorphosis proceed to round up. The larvae also spread apart the posterior margins of the their shell, but they do not usually fasten themselves to the substratum. If a specimen is allowed to begin metamorphosis before the glutaraldehyde fixative is introduced, the larva tends to attach itself to the substratum and continue to metamorphose to a flattened state. Thus, glutaraldehyde-fixed specimens are typically attained either before attachment to the substratum is achieved, or after flattening is completed. Only larvae fixed with the osmium tetroxide solution can be routinely preserved in an attached, yet nonflattened, state.

Morphogenetic movements at the onset of metamorphosis

Metamorphosis is initiated by a synchronous contraction of the striated muscles within the larva (i.e., the median and lateral muscles). When fully contracted, each myofiber of the median muscles contains a compact mass of myofilaments flanked by a vacuolated sarcoplasm (Figs. 15, 16). As the median muscles contract, the apical organ is retracted away from the ovoid opening at the apex of the shell. Contractions of the median muscles and the centrally located myoepithelial cell in the apical organ (Stricker, '87) draw the organ toward the center of the larval body and cause the aboral epithelium to fold over the retracted apical organ (Fig. 17). As these events occur, the median muscles also pull the pyriform organ toward the apical organ and tilt the posterior end of the larva apically such that the long axis of the internal sac becomes oriented parallel to the substratum (Figs. 7, 12). The mass of polster cells under each valve remains in the same position relative to the overlying shell.

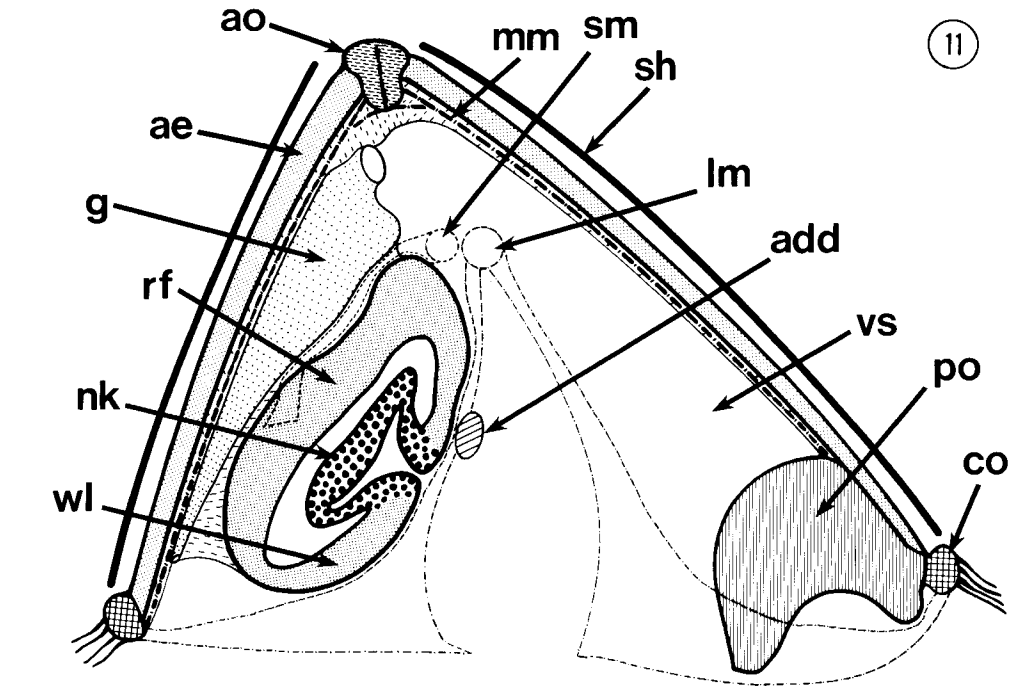
As the median muscles draw the anterior and posterior ends of the larva toward the retracted apical organ, the lateral muscles contract and pull the corona within the shell (Fig. 12). In addition, contractions of the lateral muscles seem to cause the shell to buckle along the apical-basal axis of the larva. As the shell buckles, the apices of the two valves of the shell are drawn together, and the posterior margins of the shell are spread apart (Figs. 18, 19). The buckling of the shell apparently occurs because the lateral muscles pull on the inner surface of each valve between the apical origins of the muscles and the basal edge of the shell. The forces generated by the contracting lateral muscles during retraction of the corona are presumably transmitted to the basal margin of the shell by the polster cells that lie between the shell and the corona.

Sections of free-swimming larvae and specimens undergoing the first phase of metamorphosis indicate that the apical opening between the two valves measures up to 60 μm in diameter prior to metamorphosis but is completely obliterated after the valves are spread apart (Figs. 18, 19). Accordingly, the distance between the center of the two valves at the midtransverse plane is typically 80–120 μm in free-swimming specimens, but the space between the valves increases by as much as 130 μm during the first phase of metamorphosis (Figs. 18, 19).

Eversion of the internal sac and attachment to the substratum

As the lateral muscles contract and the valves of the shell are spread apart, the neck region of the internal sac everts (Figs. 19, 20). Eversion apparently occurs because the lateral margins of the wall region in the sac are pulled outward by the movements of the shell valves. This in turn causes the anterior pore of the sac to widen and forces the infolded neck region to evert toward the substratum.

Immediately after eversion, the sac is a bilayered discoid structure (Fig. 20). The neck region is oriented toward the substratum, and the wall and roof regions are situated lateral to, and above, the neck, respectively. The invaginated portion of the neck measures about 150 μm long in free-swimming larvae. Thus, the 130-μm increase observed



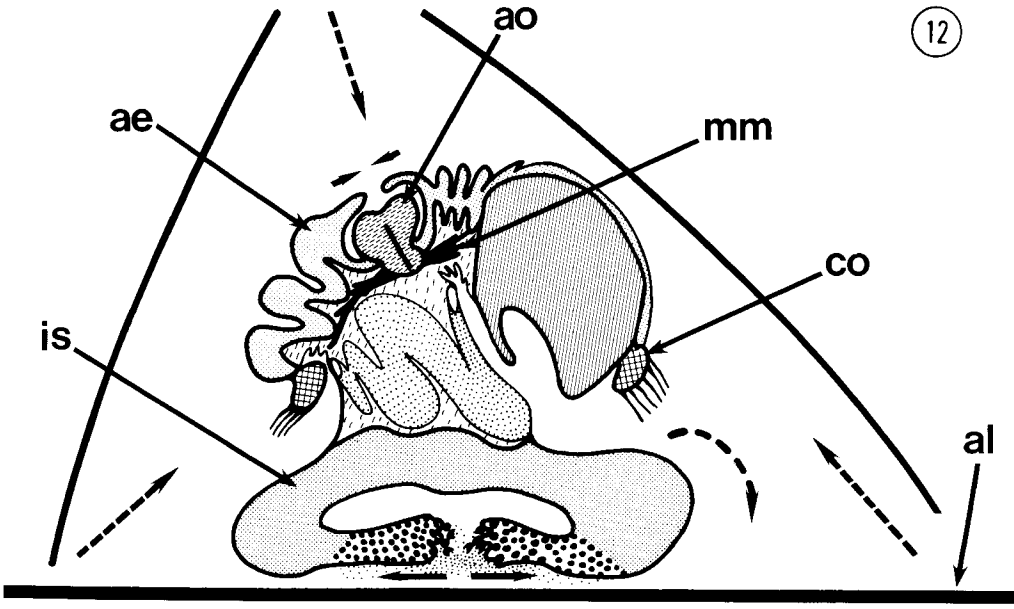
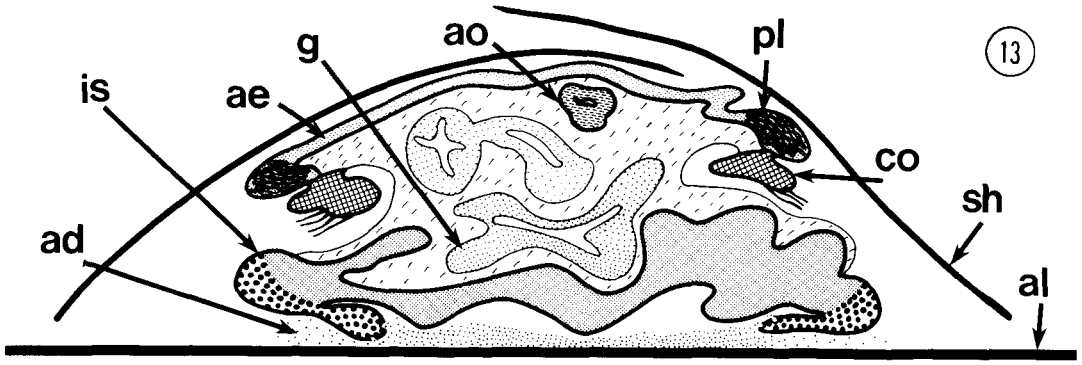


Fig. 11. Diagram of a midsagittal section through a free-swimming cyphonautes larva. The positions of a lateral set of muscles (lm) and a sac muscle (sm) are indicated by the dashed lines. add, adductor muscle; ae, aboral epithelium; ao, apical organ; co, corona; g, gut; mm, median muscle; nk, neck region of internal sac; po, pyriform organ; rf, roof region of internal sac; sh, shell; vs. vestibule; wl, wall region of internal sac.

Fig. 12. Diagram of a sagittal section through a larva at the beginning of metamorphosis. The arrows mark the movements of larval tissues during the first few seconds of metamorphosis. ae, aboral epithelium; al, alga; ao, apical organ; co, corona; is, internal sac; mm, median muscle.



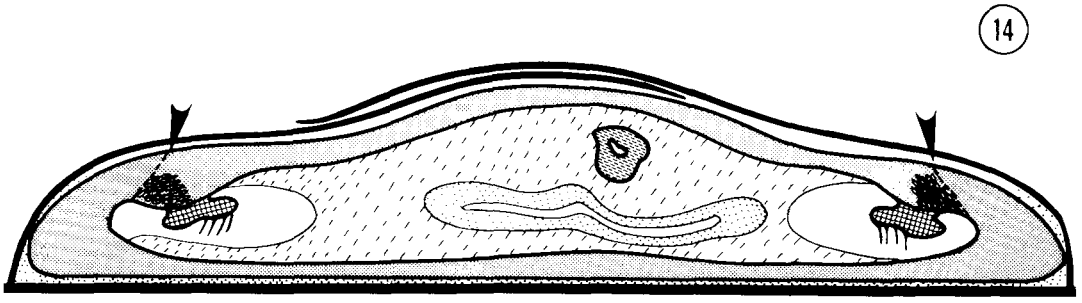


Fig. 13. Diagram of a transverse section of a larva at about 10 seconds after the onset of metamorphosis. ad, adhesive material secreted by neck cells of internal sac; ae, aboral epithelium; al, alga; ao, apical organ; co, corona; g, gut; is, internal sac; pl, polster cells; sh, shell.

Fig. 14. Diagram of a transverse section of a larva about 1 minute after the onset of metamorphosis. The lateral margins of the everted internal sac have fused to the aboral epithelium (arrowheads).

in the distance separating the two valves following contraction of the lateral muscles coincides fairly well with the length of the infolded neck region that is everted during metamorphosis.

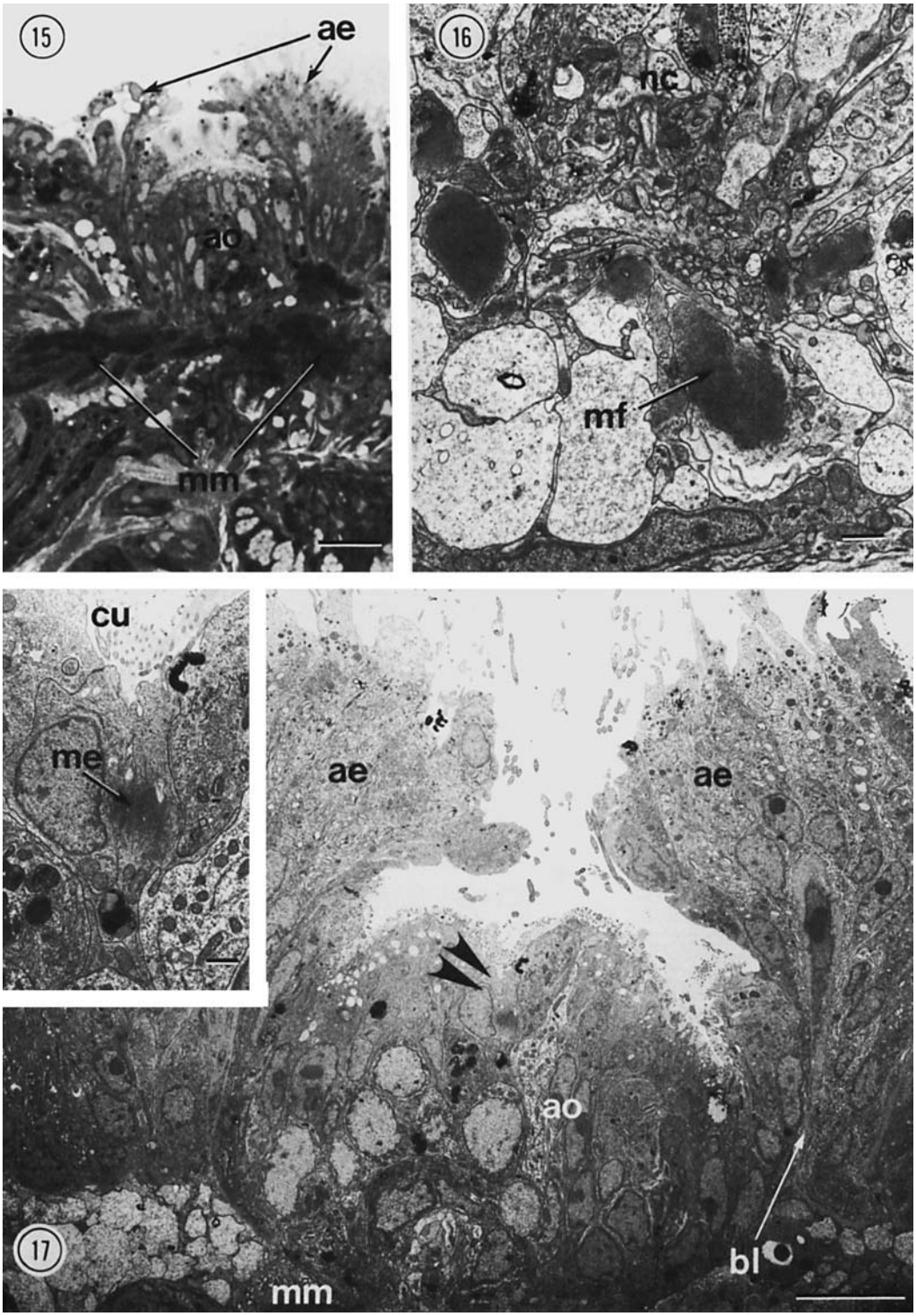
As the internal sac everts, the neck cells undergo a rapid holocrine secretion (Figs. 21, 24, 25). The exocytosed granules fuse together to form a sheet of flocculent material. After the neck granules are released, a few apically situated vesicles are secreted in a merocrine fashion by some of the cells in the roof and wall regions of the internal sac.

Exocytosis of the granules by the cells of the neck region completely obliterates each secretory cell and leaves small bits of cellular debris scattered throughout the adhesive sheet (Fig. 24). As more cells secrete their granules, a large hole is generated in the central region of the everted neck (Figs. 22, 23). Concurrently, the nonstriated sac muscles begin to contract and thus pull the intact parts of the neck toward the periphery of the sac. Following these events, the roof and wall regions of the sac become cemented to the substratum by the sticky sheet emanating from the neck cells. The roof cells lie within a few micrometers of the alga and possess apical hemidesmosomes that are attached to bundles of tonofilaments (Figs. 23, 26).

Fig. 15. Photomicrograph of a 1-μm sagittal section through the apical end of a larva that had just begun to metamorphose. The median muscles (mm) have contracted to fold the aboral epithelium (ae) over the apical organ (ao). Bar = 10 μm.

Fig. 16. Transmission electron micrograph (TEM) of a transverse section through the contracted median muscles. mf, myofilaments; nc, neural core of apical organ. Bar = 1 μm.

Fig. 17. TEM of a sagittal section through the retracted apical organ (ao) and overlying folds of the aboral epithelium (ae) at the onset of metamorphosis. bl, basal lamina; mm, median muscles. The double arrowheads mark the region shown at higher magnification in the inset. Bar = 10 μm. **Inset**: Contracted myofibril in the centrally located myoepithelial cell (me) of the apical organ. cu, cuticle. Bar = 1 μm.



Figures 15–17

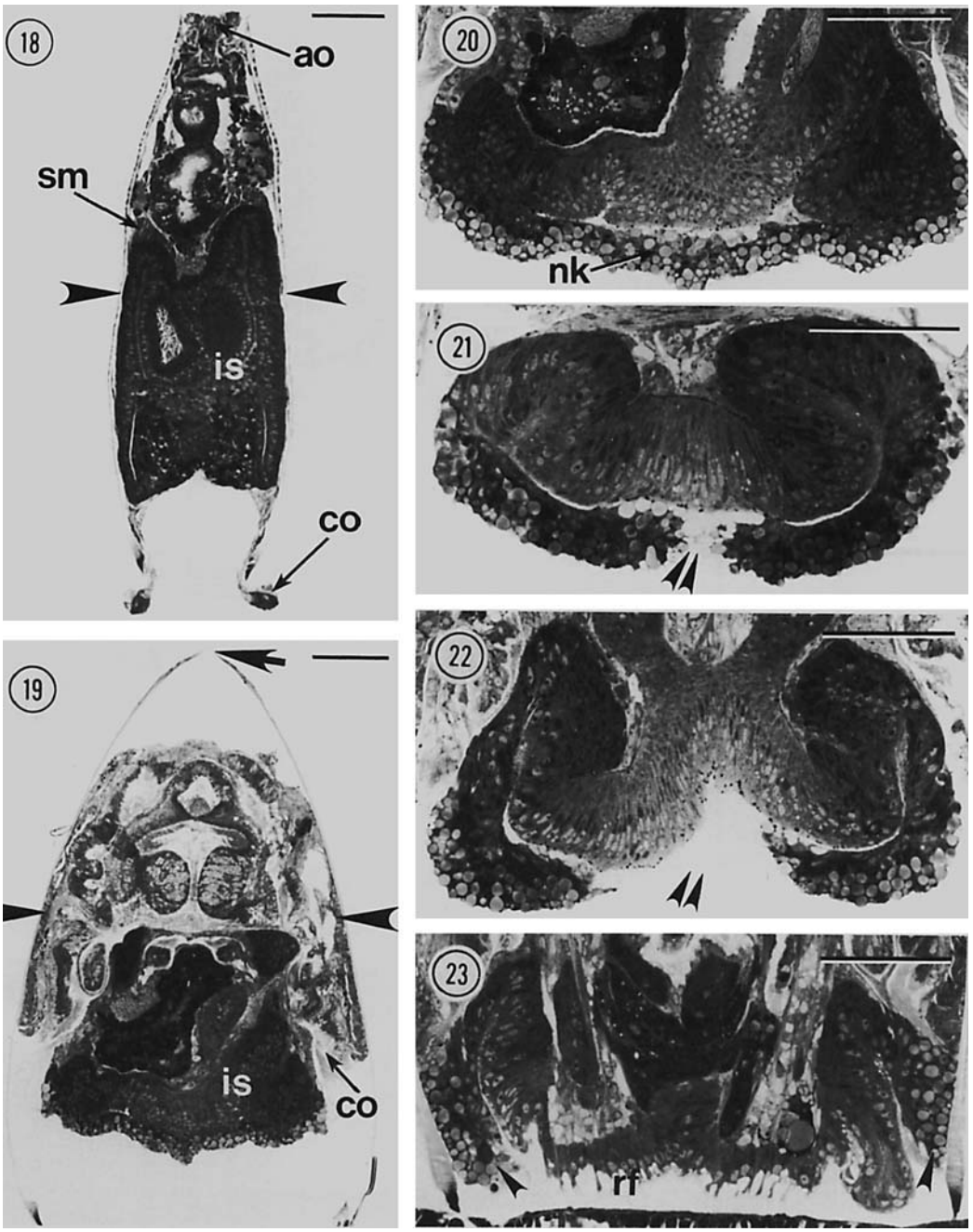


Fig. 18. Photomicrograph of a transverse section through a free-swimming larva. ao, apical organ; co, corona; is, internal sac; sm, sac muscle. The arrowheads mark the distance across the larva (cf. Figure 19). Osmium tetroxide fixation. Bar = 50 μm.

Flattening of the larva

After the globose larva has attached its everted internal sac to the substratum, the sac muscles undergo further contractions. Each muscle originates beneath a shell valve and inserts in the roof region of the internal sac. Since the roof region of the fully everted sac is cemented to the substratum by the adhesive sheet produced by the neck region, continued contractions of the sac muscles tend to draw the larva and its overlying shell toward the substratum.

As the sac muscles contract, the attachments of the adductor muscle to the shell valves are broken. Subsequently, the valves are folded over each other and rapidly pulled toward the substratum. As the shell is drawn over the larva, the corona becomes positioned above the lateral margins of the everted internal sac. The flattened larva then shifts laterally and spreads itself over the substratum. The exact mechanism by which these lateral movements occur remains obscure, but it apparently involves a temporary relaxation of the sac muscle on each side of the larva.

Morphology of the preancestrula

The flattened larva generated by the initial morphogenetic movements of metamorphosis comprises a thin disc that measures 450–600 μm in diameter and about 60 μm high. The basal surface of the larva is attached to the substratum and consists of a large central area corresponding to the former roof region of the internal sac. The roof is in turn surrounded by a rim of cells originally located in the wall region of the sac. The former wall cells also extend along the sides of the larva to form the lateral body wall. A compacted layer of adhesive material arising from the exocytosed neck granules lies between the basal surface of the larva and the substratum (Fig. 27). In addition, a sheet of similar appearing material extends over the lateral body wall (Fig. 30).

The frontal surface of the discoid larva occurs opposite to the substratum and is covered by the overlapped valves of the shell. The broadened, straight end of the overlapped valves corresponds to the former posterior margins of the larval shell, whereas the curved edges of the flattened shell that possess brownish tubercles represent the former basal margins of the shell in free-swimming specimens (Fig. 6).

The epidermis of the frontal body wall is derived from the aboral epithelium of the free-swimming larva. After the larva flattens itself against the substratum, the folded aboral epithelium at the former apex of the larva fuses together over the retracted apical organ. Thus, the organ comes to lie beneath the frontal body wall in flattened specimens (Fig. 28). The retraction of the apical organ and the subsequent overlayering of the organ by the aboral epithelium create a vesicle with a narrow lumen in the region directly above the neural core of the apical organ (Fig. 9). Concurrently, the dense clusters of undifferentiated mesodermal cells that lie beneath the apical organ in free-swimming larvae are drawn toward the frontal body wall to form a large aggregate of cells on either side of the retracted apical organ (Fig. 28).

Immediately after the larva flattens itself against the substratum, a narrow gap separates the frontal body wall from the lateral margin of the internal sac (Figs. 13, 29). The retracted corona lies within this space at the edge of the incipient preancestrula. The peripheral gap is subsequently obiterated as the lateral edge of the internal sac fuses with the neighboring margins of the aboral epithelium (Figs. 14, 30). After fusion occurs, no neck region is detectable between the aboral

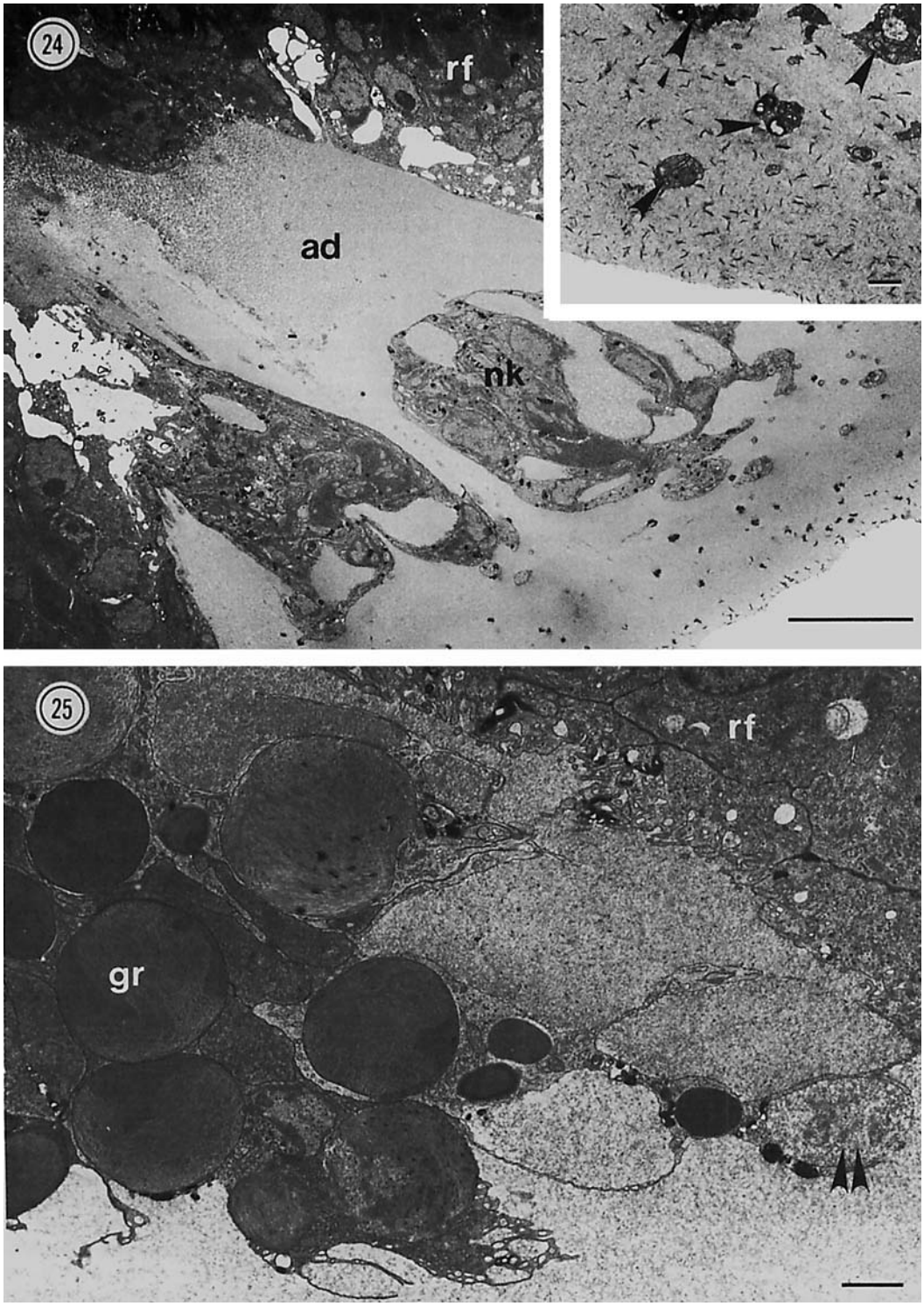
Fig. 19. Photomicrograph of a transverse section through a larva that had just begun to metamorphose. Note the increase in width (between arrowheads) owing to a buckling of the shell along the apical-basal axis (cf. Fig. 18). The apical end of the larva has been retracted from its former position (arrow), and the corona (co) lies above the everted internal sac (is). Bar = 50 μm.

Fig. 20. Photomicrograph of a transverse section through the internal sac directly after eversion of the neck region (nk). Bar = 50 μm.

Fig. 21. Photomicrograph of a transverse section through exocytosing neck cells. Secretion of the neck granules gives rise to a layer of adhesive materal that cements the larva to the substratum. As the neck cells undergo exocytosis, a hole forms in the center of the everted neck region (double arrowheads). Bar = 50 μm.

Fig. 22. Photomicrograph of a transverse section through the enlarged space in the center of the everted neck region (double arrowheads). Bar = 50 μm.

Fig. 23. Photomicrograph of a transverse section of an attached larva prior to flattening. The roof region (rf) of the everted internal sac lies next to the subtratum. Note the position of the neck cells at the periphery of the sac (arrowheads). Bar = 50 μm.



Figures 24–25

epithelium and former wall region. In most cases, fusion is completed with a minute after the larva flattens itself against the substratum, but a few specimens still possess unfused epithelia by 5 minutes postflattening.

Fusion of the two epithelia forces the granule-containing cytoplasm of the polster cells away from the shell and further internalizes the corona. When fully joined, the border between the aboral epithelium and internal sac is difficult to discern by light microscopy. The exact site of the fusion between the internal sac and aboral epithelium varies along the anteroposterior axis of the flattened larva, but it typically lies 30 μm from the lateral margin of the flattened larva. In some regions, the internal sac is situated above the aboral epithelium at the site of fusion, whereas in others the aboral epithelium overlies the internal sac. After the fusion is completed, the flattened larva corresponds to a preancestrula.

DISCUSSION

General patterns of settlement and metamorphosis in gymnolaemates

As opposed to the long-lived planktotrophic larva of *Membranipora membranacea* that apparently remains free-swimming for a month or more (Stricker et al., '88a), the lecithotrophic larvae produced by the vast majority of gymnolaemate bryozoans are usually planktonic for only 15 minutes to 8 hours (Woollacott and Zimmer, '78). Following adoption of the demersal habit, both types of larvae initiate an active exploratory phase. During this period, the pyriform organ remains in intimate contact with the substratum and apparently serves as a sensory organ that helps select a suitable site for metamorphosis (Stricker et al., '88a). In addition, the pyriform organ forms temporary attachments to the substratum and secretes a mucous sheet the aids ciliary gliding (Reed and Woollacott, '82; Stricker et al., '88b).

In *M. membranacea* and other bryozoans examined, permanent attachment to the substratum during metamorphosis involves a rapid eversion of the internal sac (Zimmer and Woollacott, '77b; Reed and Cloney, '82b; Reed, '84). The internal sac of gymnolaemates varies from a simple nonregionated structure such as found in vesicularioid ctenostomes (Reed and Cloney, '82a) to a massive tripartite organ such as occurs in the cyphonautes larva and in the lecithotrophic larvae of cellularioid cheilostomes (Woollacott and Zimmer, '78). In the vesicularioid *Bowerbankia gracilis* and the cellularioid *Bugula neritina*, eversion of the sac takes about 1 minute (Reed and Cloney, '82b; Reed and Woollacott, '82), whereas the cyphonautes larva everts its internal sac in only 5–10 seconds.

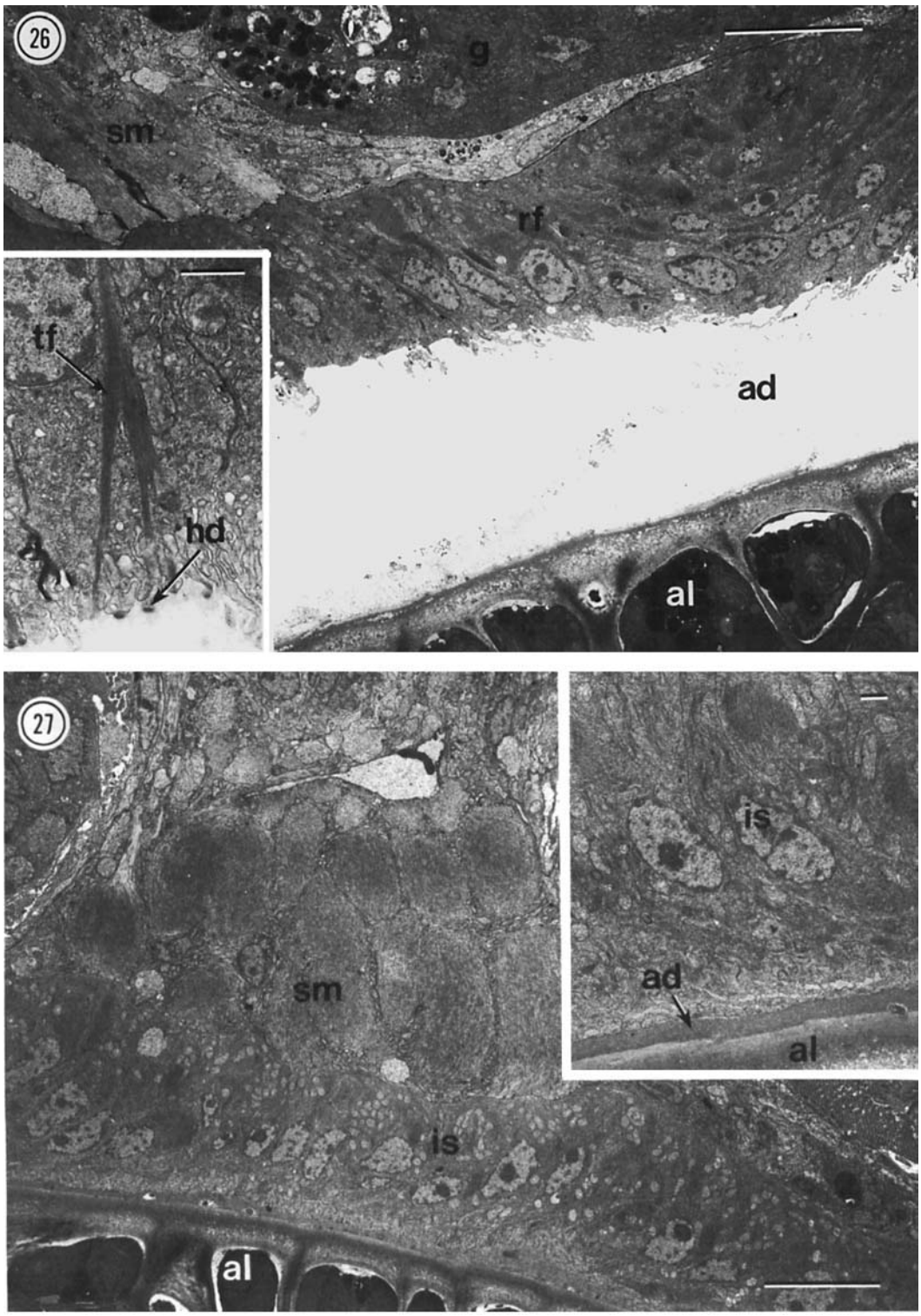
Two major sets of morphogenetic movements accompany the eversion of the internal sac during the initial phase of metamorphosis in gymnolaemates. These movements result in i) the retraction and subsequent internalization of the apical disc region, and ii) the involution of the corona into the interior of the metamorphosing larva (Zimmer and Woollacott, '77b). Retraction of the apical disc and involution of the corona take only a few seconds in *M. membranacea*, whereas 1.5–4.5 minutes are required to complete these processes in other gymnolaemates (Reed and Cloney, '82b; Reed and Woollacott, '83). Thus, the eversion of the internal sac and the accompanying morphogenetic movements that occur at the onset of metamorphosis are completed at least ten times faster in *M. membranacea* than in other bryozoans. The larvae of *Bowerbankia gracilis* and *Bugula neritina* retract their apical discs and involute their coronas after everting their internal sacs (Reed and Cloney, '82b; Reed and Woollacott, '83), but the extreme rapidity of metamorphosis in *M. membranacea* precludes any precise staging of these three events.

As the cyphonautes larva retracts its apical organ, the anterior and posterior ends of the body move centripetally. The distances moved by the apical organ and the anterior and posterior ends of the larva roughly correspond to the "polster-free zones" in the aboral epithelium underlying the shell in free-swimming specimens (Stricker et al., '88a).

In most gymnolaemates, the initial rapid morphogenetic movements described above are followed by the fusion of the lateral edges of the internal sac to the nearby margins of

Fig. 24. TEM of a sagittal section through the everted internal sac, showing the adhesive sheet (ad) secreted by the neck region (nk). rf, roof region of internal sac. Bar = 10 μm. **Inset**: Higher-magnification view of the adhesive sheet secreted by the neck cells. Note the bits of cellular debris (arrowheads) embedded in the sheet. Bar = 1 μm.

Fig. 25. TEM of the adhesive granules (gr) of the neck region in the process of being exocytosed (double arrowheads). rf, roof. Bar = 1 μm.



Figures 26–27

the former aboral epithelium of the larva. The cyphonautes larva of *M. membranacea* undergoes such a fusion within a few minutes after flattening against the substratum is completed and thus attains the preancestrula stage. The larva of *Bowerbankia gracilis* is unusual in that the internal sac retracts away from the substratum after having formed an initial attachment and does not subsequently fuse to the aboral epithelium (Reed and Cloney, '82b).

The preancestrula that is generated by the initial morphogenetic movements of metamorphosis in gymnolaemates generally differentiates into a functional, feeding ancestrula within 1–6 days (Hyman, '59). In *M. membranacea*, a twin ancestrula develops from each preancestrula (O'Donoghue, '27). The two zooids of the twin ancestrula extend their lophophores and begin to feed by 3–4 days after the onset of metamorphosis (Stricker, unpubl. observ.).

Comparative mechanisms of the morphogenetic movements of metamorphosis

Eversion of the internal sac

In *Bowerbankia gracilis*, the relatively small, monopartite internal sac of the larva is everted by the contraction of an overlying sheath of smooth muscle fibers called the rete muscularis (Reed, '84). Eversion of the large, tripartite sac in the larva of *Bugula neritina* involves two sets of muscles (Reed and Woollacott, '82). At the onset of eversion, a ring of smooth muscles situated in an equatorial position within the larva contracts to evert the neck and wall regions of the sac. Concurrently, a large axial bundle of smooth myofibers that runs between the apical disc and the roof region contracts to pull the roof toward the apical disc. Following relaxation of the axial muscle, the roof region is drawn toward the substratum by the continued contraction of the equatorial muscles.

Fig. 26. TEM of a sagittal section through the everted sac in a larva that had attached to the alga (al) but had not yet fully flattened itself against the substratum. ad, adhesive; g, gut; rf, roof region of internal sac; sm, sac muscle. Bar = 10 μm. **Inset:** TEM of the tonofilaments (tf) and hemidesmosomes (hd) in the roof cells. Bar = 1 μm.

Fig. 27. TEM of a transverse section through a larva that flattened itself against the algal substratum (al). Note the highly contracted sac muscle (sm). is, internal sac. Bar = 10 μm. **Inset:** Higher-magnification view of the basal surface of a flattened larva, showing the compacted adhesive layer (ad) situated between the internal sac (is) and the algal substratum (al). Bar = 1 μm.

Eversion of the internal sac in the cyphonautes larva of *M. membranacea* is also mediated by muscular contractions, but the muscles implicated in this process differ somewhat from those described for lecithotrophic larvae. Tests conducted on paper models of the cyphonautes shell equipped with elastic bands at sites where the lateral muscles are located support the notion that the contractions of the lateral muscles cause a buckling of the shell, which in turn leads to the eversion of the internal sac. The cross-striated nature of the lateral muscle myofibrils and the fact that these muscles are attached to an external shell probably account for the extremely rapid eversion that is observed in this species. The internal sac of the free-swimming cyphonautes larva is not attached to muscles comparable to the rete muscularis of *Bowerbankia gracilis* or the axial muscle of *Bugula neritina*. Only the sac muscles of the cyphonautes larva actually insert in the internal sac, but these muscles do not contract significantly until after the neck region of the sac is everted.

In other bryozoans, the pore of the internal sac occurs near the oral end of the larva and is directed toward the substratum during eversion of the sac (Zimmer and Woollacott, '77a,b). The internal sac of the cyphonautes larva is oriented away from the oral end of the larva in free-swimming specimens and does not face the underlying substratum during the exploratory phase of settlement. Contractions of the median muscles at the onset of metamorphosis, however, tilt the internal sac so that the pore is oriented toward the substratum.

In the free-swimming cyphonautes larva, the neck region of the internal sac is folded into the lumen surrounded by the roof and wall regions. Thus, the fully everted sac initially comprises a bilayered structure with the everted neck region occurring between the roof and the substratum. In other gymnolaemates that have been examined, the neck region constitutes either the entire sac (Reed, '78; Reed and Cloney, '82a) or a small region directly surrounding the anterior pore of the sac. Accordingly, the fully everted sac in these species constitutes a monolayered disc.

After the sac everts to form a bilayered organ in *M. membranacea*, exocytosis of the neck granules completely obliterates each secretory cell. This process in turn causes the central part of the neck to disappear. Concur-

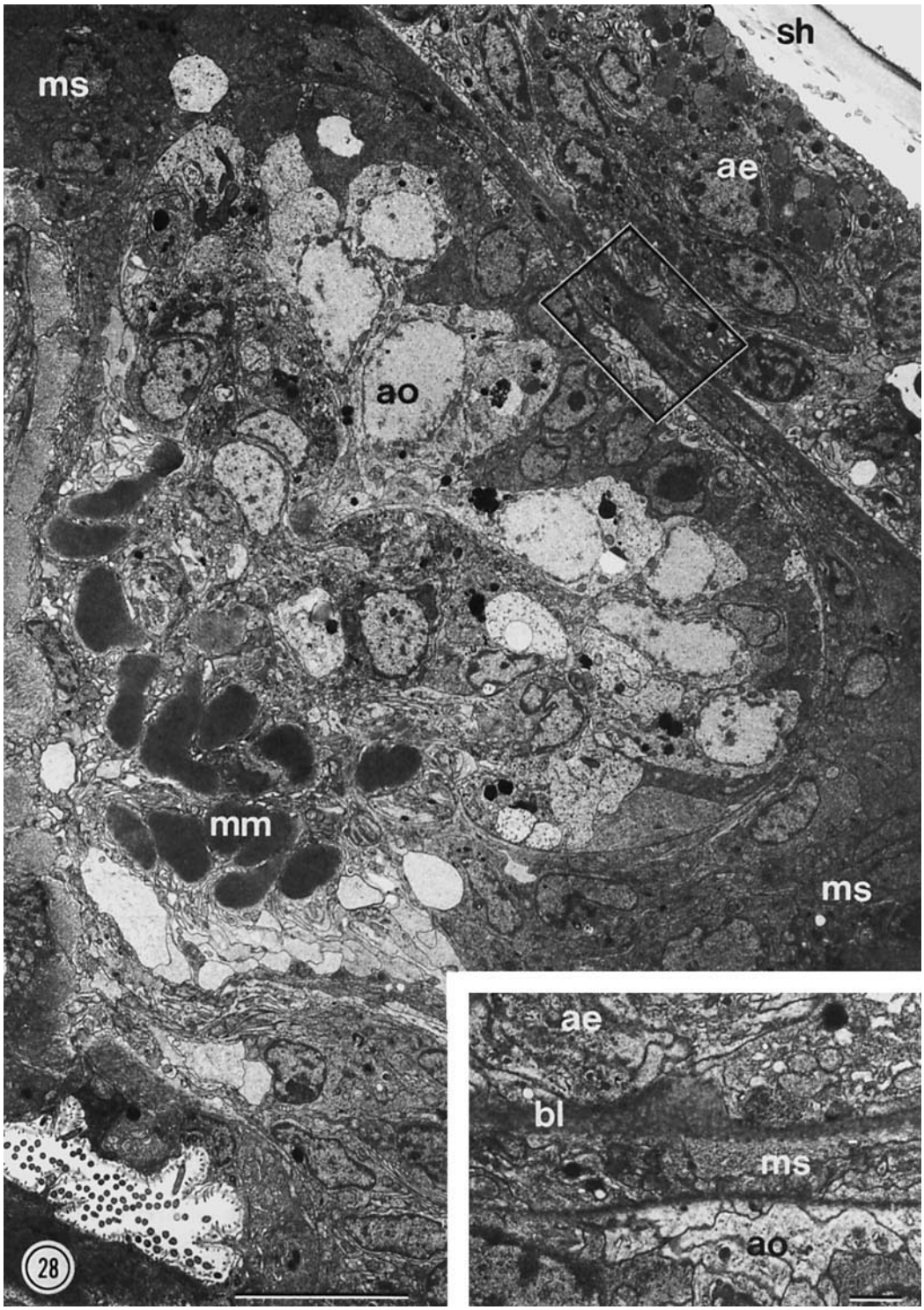


Figure 28

rently, the sac muscles pull the intact parts of the neck peripherally. Such movements result in the formation of a monolayered sac with its former wall and roof lying next to the substratum.

Attachment to the substratum

In gymnolaemate bryozoans, attachment to the substratum involves the secretion of adhesive material by cells in the neck region of the internal sac (Zimmer and Woollacott, '77b; Reed, '84). Each fully formed granule in the neck region of gymnolaemate larvae usually has a bipartite structure or patchy appearance when viewed by TEM (Stricker et al., '88b). In *M. membranacea*, the exocytosed granules rapidly form a sticky sheet. Apical vesicles are also secreted by the cells of the roof and wall regions of the sac during attachment to the substratum. Whether or not these vesicles contribute to the adhesive properties of the secreted sheet in a manner similar to the "duo-gland" products of various marine invertebrates (Hermans, '83) remains to be determined.

In other gymnolaemates, the material secreted by the neck region not only attaches the basal surface of the larva to the substratum, but it also forms a tentlike pellicle over the metamorphosing larva (Reed and Cloney, '82b; Reed and Woollacott, '82). In *M. serrilamella*, a hyaline sheet that may represent a rudimentary pellicle directly surrounds the metamorphosing larva (Mawatari and Mawatari, '74). This refractile covering probably corresponds to the layer of secretory material that extends over the lateral body wall of the incipient preancestrula in *M. membranacea*.

Retraction of the apical disc and involution of the corona

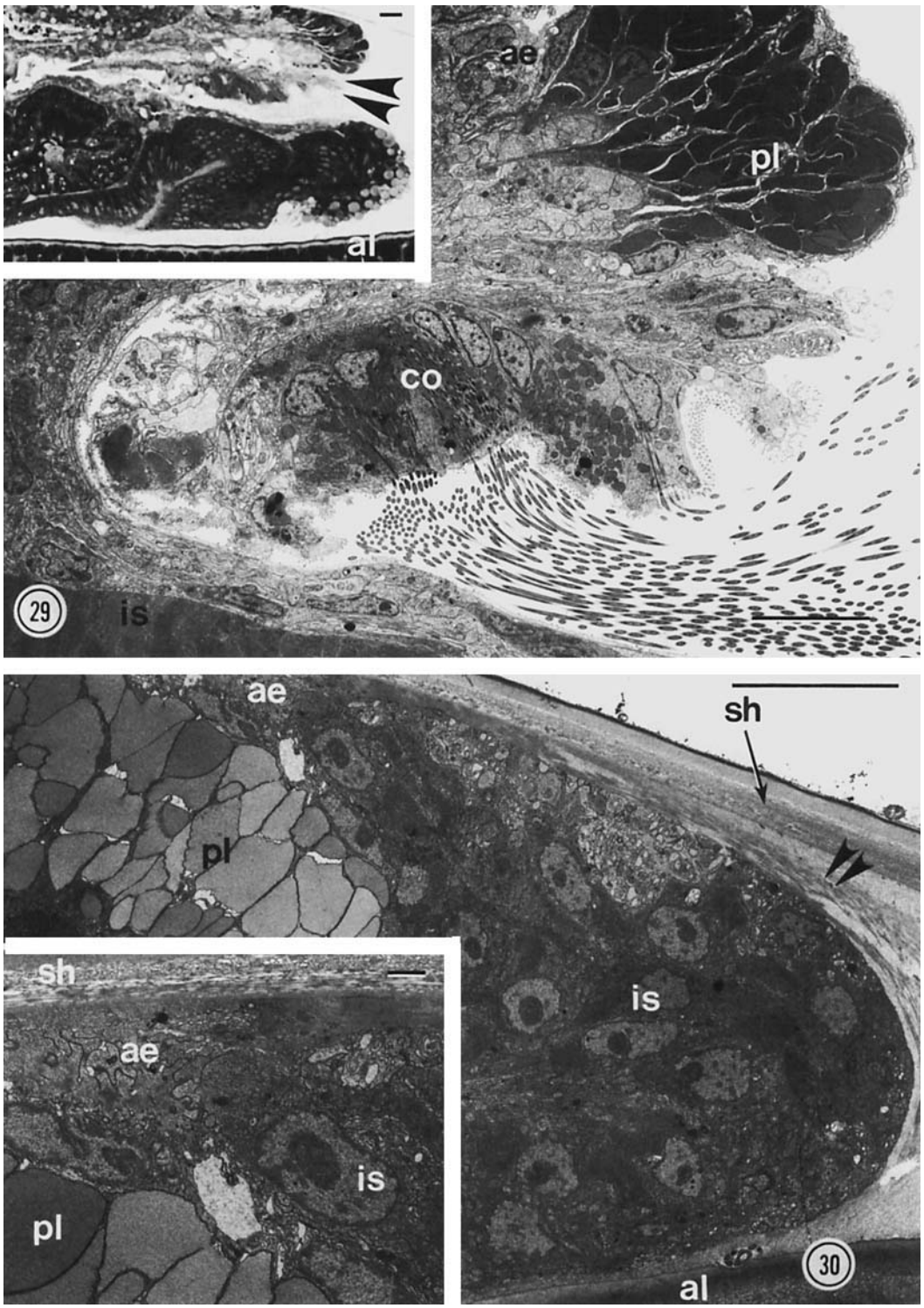
Retraction of the apical disc in *Bowerbankia gracilis* and *Bugula neritina* involves the contraction of nonstriated muscles that are attached to the basal surface of the disc (Reed and Cloney, '82b; Reed and Woollacott, '83). Retraction of the disc occurs directly after the sac everts, and in the case of *B. neritina*, a second retraction takes place approximately 1 minute after the initial, temporary withdrawal of the apical disc occurs (Reed and Woollacott, '83). Similarly, retraction of apical organ in *M. membranacea* is achieved around the same time that the internal sac everts. Retraction of the apical organ in *Membranipora* involves the contractions of the striated median muscles that are attached to the basal surface of the organ, as well as the contraction of a myoepithelial cell in the central region of the organ (Stricker, '87).

Following retraction of the apical organ, the aboral epithelium fuses over the organ, and a small vesicle is created above the neural core region. Fusion of aboral epithelia and formation of an apical vesicle have also been reported for other gymnolaemates (Zimmer and Woollacott, '77b). The apical vesicle that develops in the preancestrulae of other gymnolaemates consists of cells derived from an epidermal and a mesodermal blastema located at the aboral end of the larva (Zimmer and Woollacott, '77b). Subsequently, this vesicle differentiates into the polypide of the ancestrula (Zimmer and Woollacott, '77b). Whether or not the vesicle in *M. membranacea* comprises similar blastemal cells and gives rise to the two polypides of the twin ancestrula remain to be determined.

Involution of the corona in *Bowerbankia gracilis* occurs by a complex mechanism (Reed, '85). At the onset of metamorphosis, the coronal cilia initially reverse the direction of their beat and thus cause some of the fluid secretions of the neck cells to be wafted over the surface of the larva. After this material has hardened to form the tentlike pellicle, the continued beating of the coronal cilia against the inner surface of the pellicle propels the corona in the direction opposite to that of the effective strokes and thus involutes the corona into the interior of the larvae. The mechanism by which the corona is involved in *Bugula neritina* is not completely understood, but the process may also involve an interaction between the coronal cilia and the overlying pellicle (Reed and Woollacott, '83).

In *M. membranacea*, coronal involution appears to be achieved by the contraction of the lateral muscle complex on each side of the larva. According to Kuplewieser ('05), the involution of the corona of the cyphonautes

Fig. 28. TEM of a transverse section through the apical organ (ao) at about 10 seconds after the onset of metamorphosis. The organ lies completely beneath the frontal body wall. No conspicuous vesicle is visible in this section. The box outlines the region shown at higher magnification in the inset. ae, aboral epithelium; mm, median muscles; ms, mesodermal cells; sh, shell. Bar = 10 μm. **Inset:** TEM of the tissue layers covering the retracted apical organ (ao). ae, aboral epithelium; bl, basal lamina, ms, mesodermal cells. Bar = 1 μm.



Figures 29–30

larva produced by *Electra pilosa* may also be assisted by a vacuum that develops when the larva flattens itself against the substratum.

Fusion of epithelia in the body wall of the incipient preancestrula

As in many gymnolaemates, the lateral edges of the aboral epithelium in the incipient preancestrula of *M. membranacea* fuse to the neighboring margins of the everted internal sac after the initial rapid morphogenetic movements of metamorphosis are completed. The fusion probably involves secretion of the remaining neck granules occurring at the periphery of the everted sac, judging from the absence of a discrete neck region in the preancestrula. Once fusion occurs, the transient tissues of the larva and the differentiating rudiments of adult internal organs (="polypide") become enclosed in a fully sealed body wall, called the cystid (Reed, '87).

As in *M. membranacea*, the body wall of the ancestrula of *Watersipora cucullata* consists of the former internal sac fused to the aboral epithelium (referred to as the "pallial epithelium") (Lyke et al., '83). In *Bowerbankia gracilis*, however, the ancestrular body wall is derived solely from the pallial epithelium (Reed and Cloney, '82b). Fusion of the internal sac to the pallial epithelium occurs during the early phase of metamorphosis in *Bugula neritina*. Subsequently, however, microfilaments in the pallial epithelium contract and thereby cover the preancestrula with the roof and wall region of the internal sac (Reed and Woollacott, '83). Preliminary studies suggest that a complementary origin of the preancestrular cystid from both the internal sac and aboral epithelium such as occurs in *Membranipora* and *Watersipora* represents the primitive mode of body wall formation within the phylum Bryozoa (Reed, '87).

Fig. 29. TEM of a transverse section through the lateral edge of a metamorphosing larva. The larva had attached to the substratum but had not yet flattened itself completely. Note the space between the aboral epithelium (ae) and internal sac (is). co, corona; pl, polster cells. Bar = 10 μm. **Inset:** Photomicrograph of the tissues surrounding the peripheral gap (double arrowheads) depicted in the TEM. al, alga. Bar = 10 μm.

Fig. 30. TEM of a transverse section through a newly formed preancestrula approximately 1 minute after the onset of metamorphosis. The internal sac (is) has fused to the aboral epithelium (ae). The double arrowheads mark adhesive material covering the lateral body wall. al, algal; pl, polster cells; sh, shell. Bar = 10 μm. **Inset:** Higher-magnification view of the site of fusion between the internal sac (is) and aboral epithelium (ae). pl, polster cells; sh, shell. Bar = 1 μm.

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