Mesodermal pattern and pattern repeats in the starfish bipinnaria larva, and related patterns in other deuterostome larvae and chordates

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SUMMARY

The arrangement of the body-wall musculature and supporting mesenchyme of the bipinnaria larva of *Pisaster ochraceus* is described, based on an EM analysis of *Pisaster ochraceus* larvae. There are four main cell types: (i) a generalized mesenchyme that links the ectoderm with internal structures, e.g. coelom and gut; (ii) dorsal longitudinal muscles, which flex the body; (iii) oral-field muscles, which flex the larval lobes individually; and (iv) cords of subtrochal cells, a distinctive type of mesenchyme located beneath the ciliary band. Processes from the subtrochal cells insert into the band and associate closely with the ciliary nerve. The function the subtrochal cells perform is not obvious, but a role in body support is proposed, with the cells acting as tensile elements to increase the structural stability of the band. The insertions formed by the subtrochal cells could be attachment structures or, assuming the cells are contractile, they could be innervation sites.

The larval lobes in the bipinnaria are arranged in series along the body, and the muscles that supply them are also arranged in series. Similar patterns of repeating elements occur in other echinoderm larvae, in the hemichordate tornaria larva, and also in chordates. In particular, there are marked similarities between (i) the arrangement of the oral-field muscles in the bipinnaria and the muscle bands of pelagic tunicates, and (ii) the subtrochal cells in the bipinnaria and the myotomes in *Amphioxus*. These similarities are suggestive; whether they are due to homology is an open question. On the assumption that they may, a hypothesis is proposed to explain the differences between anterior and posterior mesodermal repeats in vertebrates (somitomeres and somites) based on differences between anterior and posterior lobes in the bipinnaria.

1. INTRODUCTION

The starfish bipinnaria larva, together with the pluteus, auricularia and tornaria, comprise a set of related planktonic larval forms known collectively as dipleurula larvae (reviewed by Nielsen 1995). These larvae are interesting organisms in their own right, for their diverse adaptations to planktonic life, but they are of special interest from an evolutionary standpoint as possible antecedents of chordates. This is because one of the few plausible explanations we have for chordate origins, Garstang's auricularia hypothesis (Garstang 1894), takes the dipleurula larva as a starting point. The auricularia hypothesis derives the chordate nerve cord by convergence from the larval ciliary bands, but it is otherwise incomplete; it fails to account for a number of other key chordate features, including mesodermal structures like the somites and notochord.

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These structures have no obvious counterparts in the dipleurula except, perhaps, in the larval coeloms. Dipleurula larvae typically have three pairs of coeloms, so if coeloms are antecedents of somites, evolution has simply extended the series, from three pairs in the dipleurula, to many, in chordates. This does not account for the much greater complexity of the somites, however, which combine muscle, support structures and coelomic elements in a characteristic fashion that, again, has no obvious counterpart in the dipleurula.

For any meaningful comparisons with chordates, other components of the larval mesoderm need to be considered, not just the coeloms. This paper examines mesoderm organization in the starfish bipinnaria, chosen because it is among the largest and most muscular of extant dipleurula larvae. This means the mesoderm is especially well developed, performs more functions, and may therefore be more revealing than in other dipleurula types. Special attention is paid to structures that occur in series. The results, described in §3, provide a point of departure for a discussion of some broader issues. The occurrence of similar patterns of serial repeats in related larvae, suggesting a common underlying plan, is discussed in §4; similarities in the way that the mesoderm is organized and deployed in the bipinnaria in comparison with various chordates, and the homologies these similarities suggest, are discussed in §5.

2. METHODS

The results reported are on *Pisaster ochraceus* larvae from laboratory cultures maintained by T. H. J. Gilmour for studies of feeding behaviour and metamorphosis. Methods for culture, EM fixation and behavioural observations are as previously described (Gilmour 1988; Lacalli *et al.* 1990). The current study examines larvae between 20 and 30 days old, the youngest being roughly equivalent to that shown in figure 1*b*, the oldest to that shown in figure 2. Specimens were examined whole by light microscopy, and by scanning EM (SEM), which was used also to survey the inside surface of the body in fractured specimens. A gel-like matrix fills the blastocoel, however, and this is difficult to remove, which makes the fractured specimens less informative than they would otherwise be.

Transmission EM (TEM) section series were prepared through selected regions of the body. Because the larvae are too large for full serial reconstruction, sections were routinely collected at quarter- or half-micrometre intervals. Three interval series of this type were examined: (i) a 350 μm transverse series through a 20 d larva extending from its apex through the anterodorsal ridge; (ii) a 50 μm transverse series through the anterodorsal ridge zone in a 24 d larva, with serial sections through the ridge itself; and (iii) a 300 μm series cut transversely through the posterolateral lobe of a 24 d larva, from tip to base, with serial sections at the tip. Three-dimensional reconstructions were prepared from selected parts of the series using Skandha, a reconstruction software package, as described by Lacalli *et al.* (1994).

3. BIPINNARIA MUSCULATURE AND MESENCHYME

Figures 1 and 2 show a selection of *Pisaster* bipinnaria and brachiolaria larvae illustrating their complex morphology. Since the larvae collapse somewhat in SEM preparations, the stereo image (figure 2) better approximates the real shape of the living larvae. The body surface is divided into three domains by the ciliary band. These are: (i) the oral field, which surrounds the mouth; (ii) the aboral epithelium, which wraps around the posterior end of the larva so as to cover both the dorsal surface and the postoral part of the ventral surface; and (iii) the shield-like preoral field, which is enclosed by an extension of the dorsal ciliary band that separates from the latter secondarily to form an independent domain. The ciliary band consists of a close-packed array of columnar cells, and

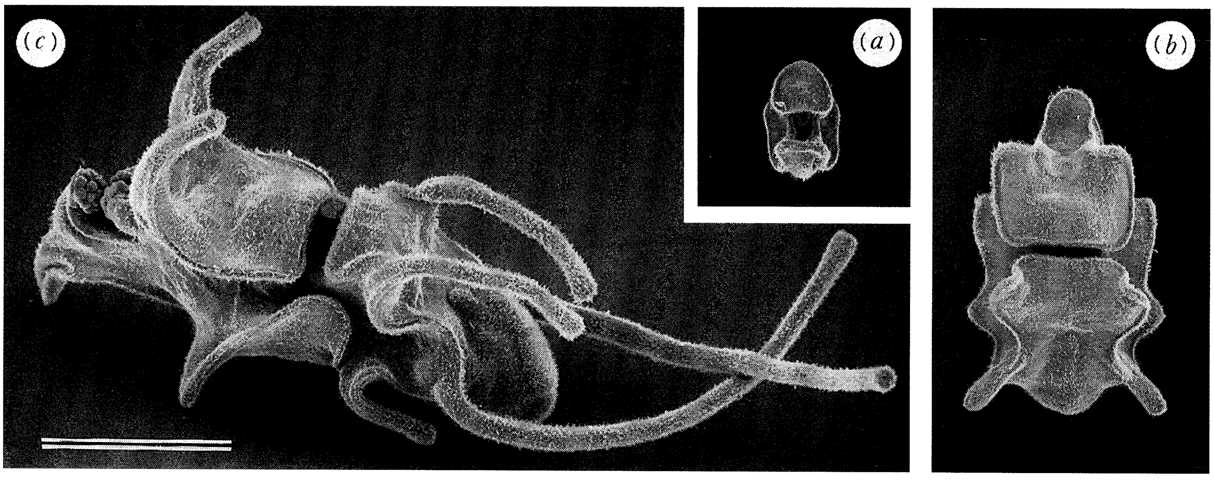


Figure 1. *P. ochcraceus* larvae, a range of stages all to the same scale. (*a*) A young larva at 8 d. (*b*) A 20 d bipinnaria at the beginning of the period of lobe growth. (*c*) A 40 d brachiolaria from a culture in which rudiment development was delayed; the lobes have nevertheless continued to grow. Specimens prepared by T. H. J. Gilmour, see Gilmour (1988). Scale bar 500 μm.

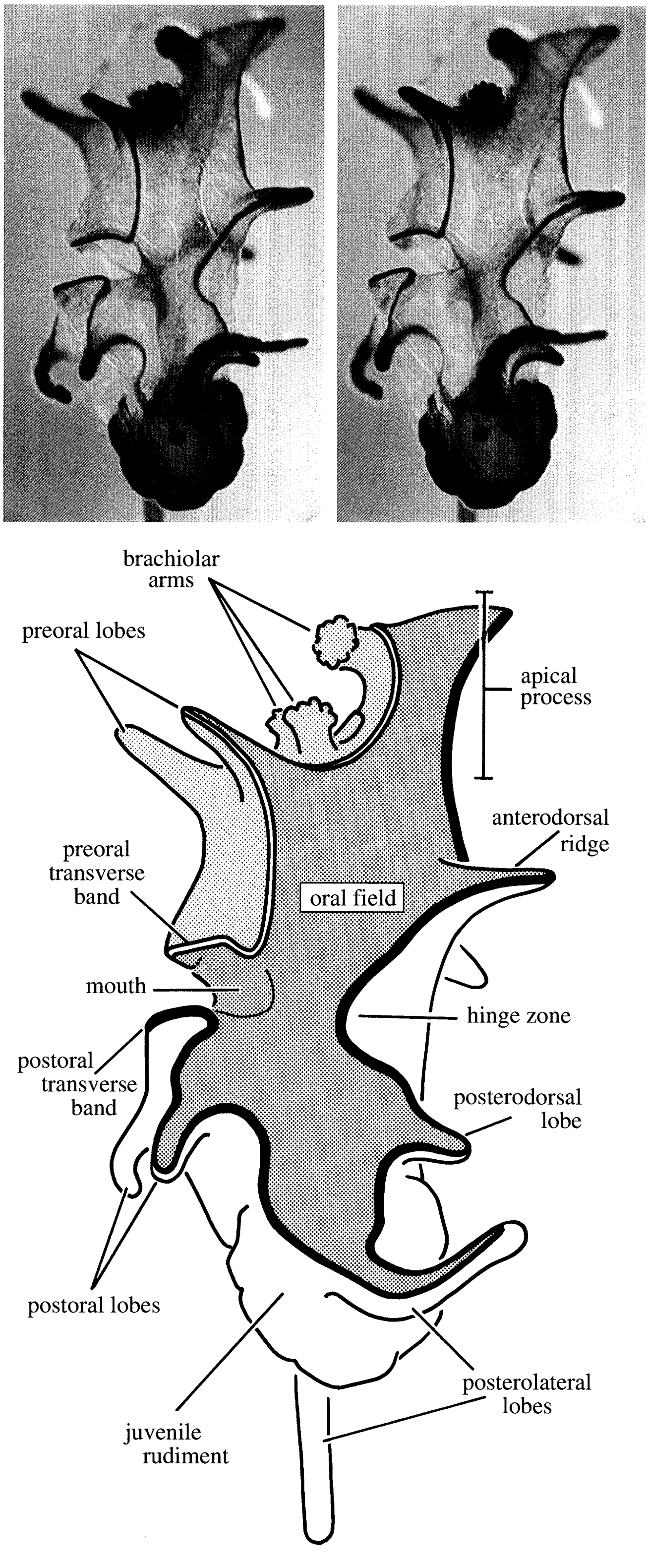
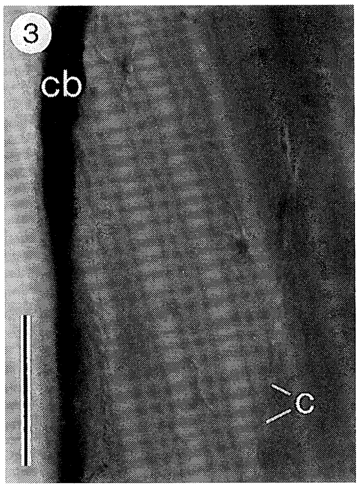
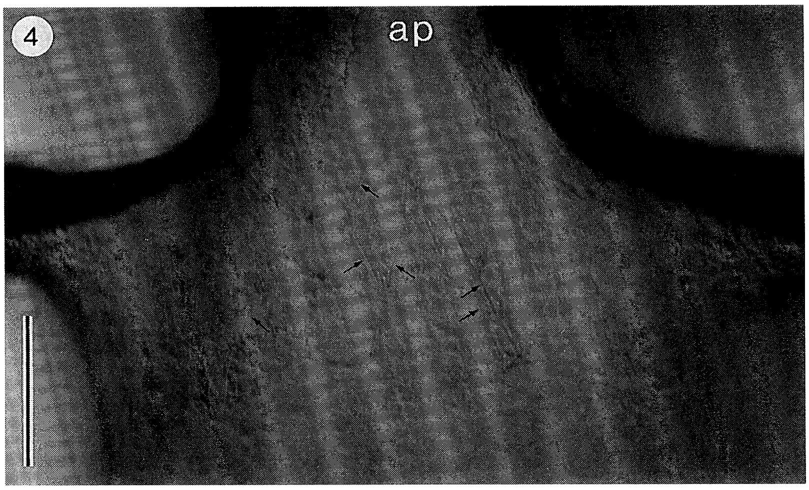


Figure 2. A 30 d brachiolaria. The top panel shows an osmium-stained whole mount in stereo; the surfaces of the gut and coelom can be seen inside, and the juvenile rudiment is visible near the posterior end. The main structures and subdivisions of the body surface are indicated in the drawing. The ectoderm is divided into three domains: the aboral epithelium (unshaded) is separated from the oral field (dark shading) by the main loop of the ciliary band (solid line), which runs dorsolaterally along both sides of the body, from the apex to the posterior end, then forward ventrally to the postoral region, where the two sides are joined by the postoral transverse band. A separate preoral loop of ciliary band (doubled line) encloses the ventral preoral field (light shading) and separates it from the oral field.

has a characteristic wedge-shaped profile in section (figure 12). The epithelium between the bands is thin and very delicate. In older larvae, much of the aboral epithelium bulges slightly outward, and so is flat or slightly convex where it meets the band. The oral field epithelium, in contrast, is often inwardly curved





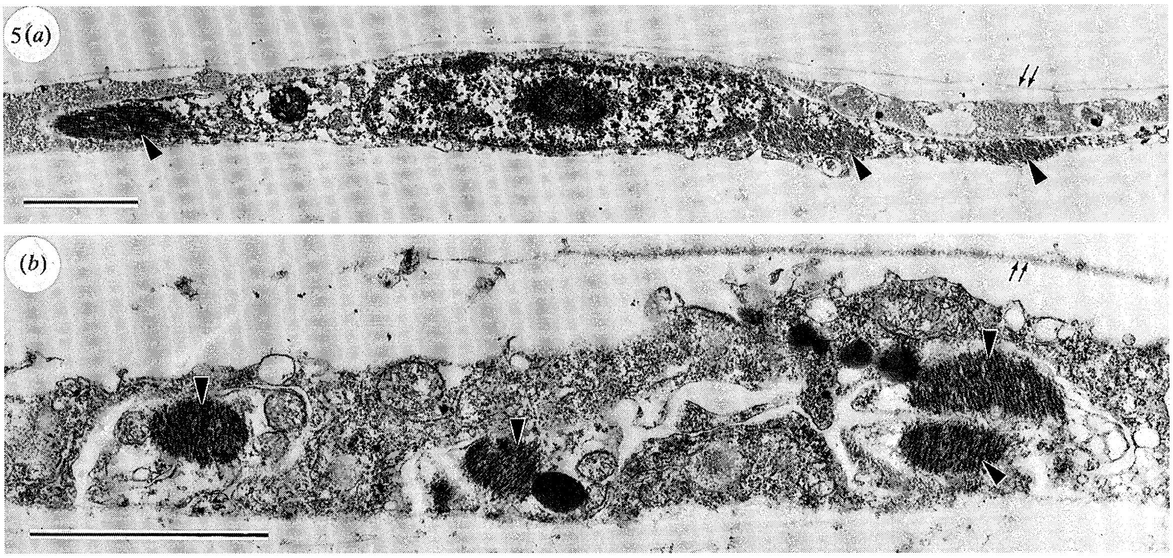


Figure 3. Typical stellate mesenchyme cells in a whole mount, spanning the space between the ectoderm and ciliary band (cb) and the coelomic epithelium (c). Scale bar 100 µm.

Figure 4. Fibres (arrow) belonging to the dorsal muscle cells. This is a dorsal view of a 30 d larva just below the apical process (ap) at the level of the anterodorsal ridges, which are visible on each side. Scale bar 100 µm.

Figure 5. Sections of the dorsal epithelium showing dorsal muscle cells; they are oriented with the external, cuticular surface (small double arrows) facing up. (*a*) The cell body and nucleus of one such cell, which contains myofibril bundles (arrowheads). (*b*) Individual muscle fibres (arrowheads) enclosed within the epithelium. The basal lamina is very delicate; only scattered bits of fibrous material are visible in this section. Scale bars 2 µm.

(concave) where it meets the band. The possible significance of this arrangement is discussed in §3*c*, and relates to the role the band may play as a structural support element and anchoring point for muscles. The internal organs of the body will not be dealt with in detail. They comprise a tripartite gut, with an oesophagus, stomach and intestine, and coelomic compartments, the largest being the fused left and right anterior coeloms (visible in figure 2 through the epithelium), which will be referred to simply as 'the coelom'.

(*a*) *Cell types, organization and innervation*

Four types of contractile and support cells associate with the body wall. There are two types of muscle, and two cell types that lack obvious myofilament bundles and are therefore assumed to be mesenchyme. Mesenchymal cells provide much of the contractile driving force for morphogenesis in echinoderms, and the absence of obvious myofibrils in a given cell type does not preclude its having a significant force-generating function. The main characteristics of the four cell types are summarized below, and in diagrammatic form in figure 16.

(i) *Stellate mesenchyme*

These are multipolar or bipolar cells that span the blastocoel between the epithelium and internal structures at various points (figure 3). In older larvae, they are scattered more-or-less evenly through the blastocoel. Attachments show close apposition to the basal lamina and specialized point contacts similar to those formed by the other mesodermal cell types, as described below.

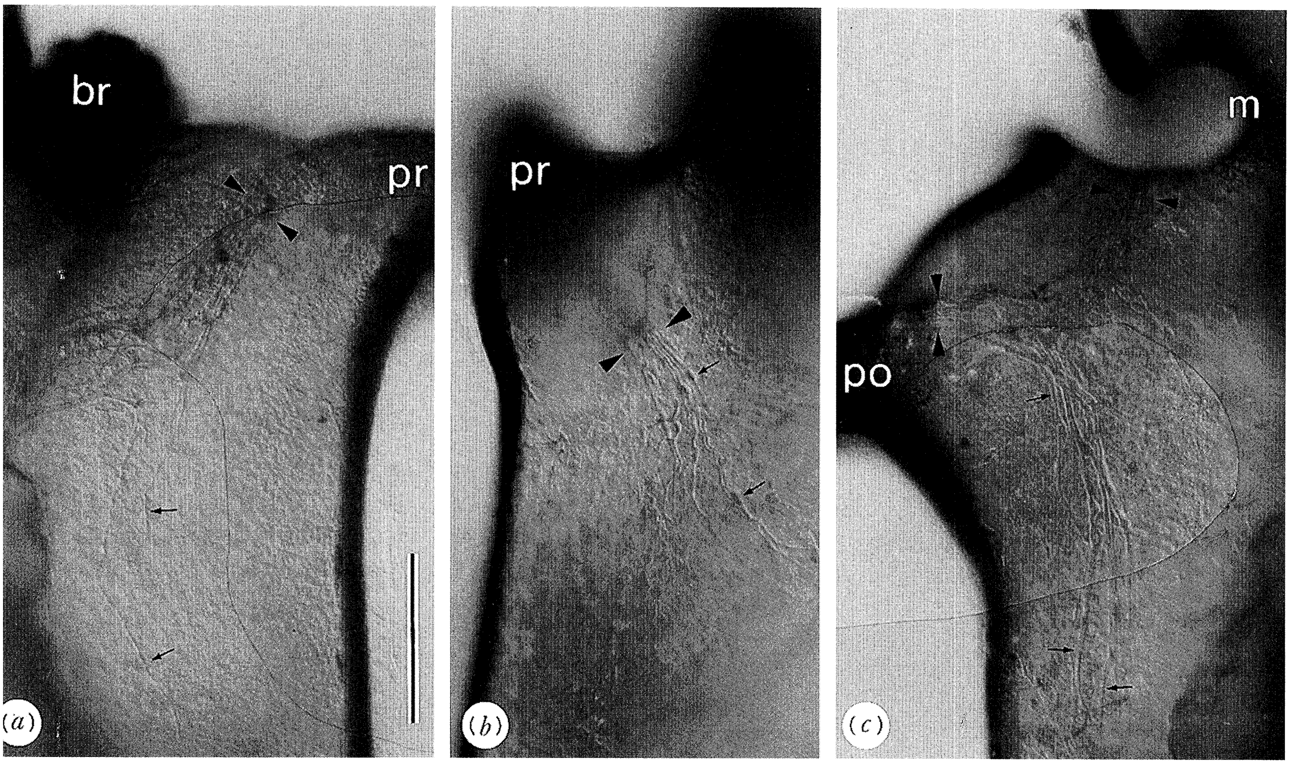


Figure 6. Muscle bands supplying the lobes in 25 to 30 d larvae. The bands are typically narrowest at the bases of the lobes (between arrowheads) and then splay out across the oral field (small arrows mark individual fibres). (*a*) A side view of the oral field at the base of a preoral (pr) lobe; one of the lower brachiolar arms (br) is visible. (*b*) Ventral view of the right side of the preoral field showing fibres associated with the right preoral lobe, out of focus in this view. (*c*) Side view of the oral field below the mouth (m) showing fibres associated with the postoral (po) lobe. Scale bar 100 μm.

(ii) *Dorsal muscles*

A pattern of faint lines is visible on the dorsal surface of the larvae at about the level of the anterodorsal ridges (figure 4). These are best defined near midline, becoming fainter and disappearing more laterally. Sections (figure 5) show the lines to be small muscle fibres buried in the epithelium; some are basal, others are enclosed entirely by epithelial cells. They radiate from cell bodies located near the dorsal midline, several fibres from each cell. The cell bodies are flattened and are not fully enclosed by the basal lamina, which is, in any case, delicate and easily disrupted in their vicinity.

The dorsal muscles are very likely the cells responsible for dorsal flexure, a response that develops long before the lobes develop (§3*b*). This may account for their more intimate association with the epithelium in comparison with the muscle bands, which develop much later, and are always separated from the epithelium by the basal lamina. Various small nerves pass irregularly through the dorsal epithelium, but no specific contacts were found between these and nearby muscles. The nerves typically each consist of 3–4 fibres, some containing scattered dense-core vesicles. Some of the fibres are evidently serotonergic: the presence of a loose aboral network of serotonergic cells has been demonstrated by Nakajima (1988), and the flexure response, which is cholinergic and nicotinic, is blocked by serotonin (Lacalli *et al.* 1990).

(iii) *Muscle bands*

The muscle bands are well developed in late-stage larvae. They can be easily traced in whole mounts across the oral field and preoral shield into the lobes (figures 6 and 7). They are absent from the dorsal epithelium except, in the brachiolaria stage, for a band that develops near the tip of the apical process (figure 7*b*), and a small cord of cells that develops on the aboral surface near the base of the posterolateral lobes. In young larvae, prior to lobe formation, few definite fibres are visible in whole mounts. Sections of 20 d larvae show, however, that a population of oral-field muscle cells has developed by this stage. The cells are elongated and rather irregular in shape. They typically run perpendicular to the band across the oral field and terminate on the oral side of the band, without actually reaching it. Such cells were most commonly encountered in areas that later form lobes, e.g. along the anterodorsal ridge. They may, in fact, be pioneer fibres for the muscle bands.

When fully formed, the muscle bands in the lobes have a characteristic proximodistal organization. Only a few fibres extend close to the tips of the lobes (figure 9*a* and *b*), and even these stop short of the tip. More proximally, the band enlarges as fibres from more cells are added to it (figure 9*c*), it narrows at the base of the lobe (figure 9*d*), and then splays out irregularly across the surface of the oral field (figures 6 and 7).

The cells of the muscle bands are attached to the basal lamina along most of their length via zones of contact with epithelial cells and point contacts with

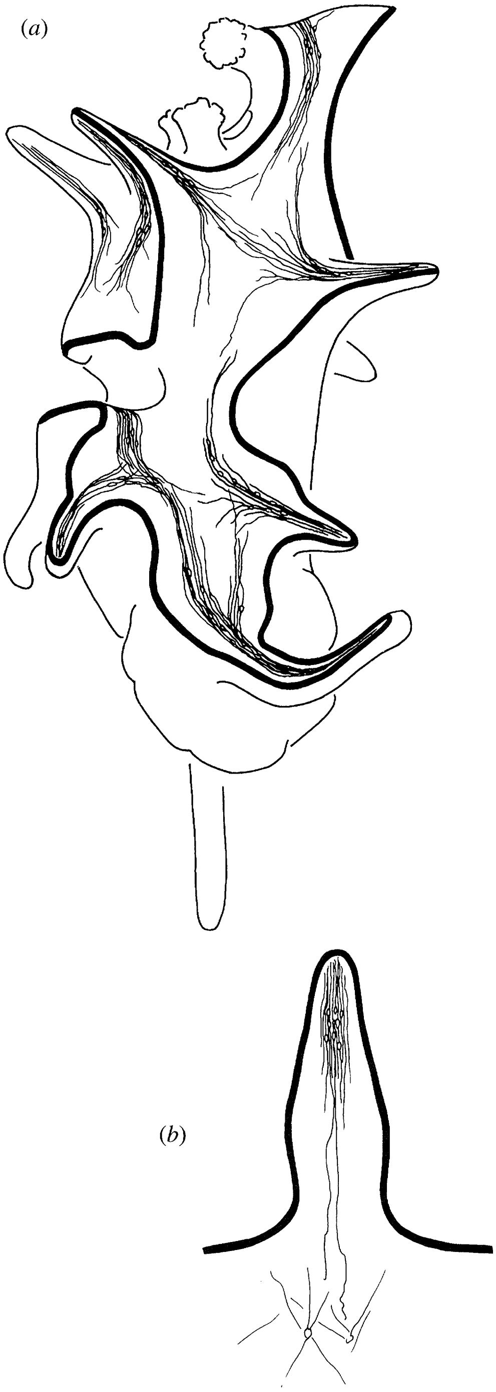


Figure 7. Summary diagram to show (*a*) the arrangement of muscle bands of a 30 d larva in side view and (*b*) the muscle bands in the apical process, seen in dorsal view.

small basal epithelial cell processes (figures 8 and 9*b*). Bundles of such processes run beneath the epithelium on both sides of the ciliary band; from sections of the 20 d larva, they are about twice as numerous on the oral side of the band. In no instance, either in lobes or between them, were muscles seen to extend beneath the band itself. Instead, they attach near it along its oral margin.

It is not clear how the muscle bands are innervated. Bundles of nerve fibres were seen to leave the band on the oral side at various points, near the tips of the posterolateral lobe for example, and along the anterodorsal ridges. These nerves are typically larger than the aboral nerves and contain dense-staining fibres not found in the latter. One especially large nerve leaves the band near the base of the apical process, as shown in figure 11. No specific contacts or junctions were found between the nerves and adjacent oral field muscles, however, despite repeated searching.

(iv) *Subtrochal cells*

Subtrochal cells are a distinct subclass of the mesenchyme that associates in a characteristic way with the ciliary bands. The cells are multipolar or bipolar, often with very long processes; they form closely linked cords that run along the underside of the ciliary bands (figures 10–15). Judging from the fractured SEM specimens examined, subtrochal cells seem to occur along most of the length of the band. Exceptions include the posterolateral lobes where, from TEM data, they occur only near the lobe base, and the preoral and postoral transverse bands, where they are rather sparse. From the data currently available, the subtrochal system would seem to be best developed in larvae with well-developed lobes, and in regions where muscle attachments are numerous. The system appeared to be especially well developed along the anterodorsal ridges, and this region was therefore examined in some detail.

Unlike the muscle cells, subtrochal cells are attached at discrete points to the basal lamina (figures 11 and 12) rather than being closely apposed to it. Points of contact were less numerous in the youngest larva examined (e.g. figure 11) compared with older ones. Adjacent subtrochal cells form extensive zones of contact with one another, however (figure 13*c* and *d*), which means that only comparatively few contacts with the overlying epithelium would be needed to anchor the bundle as a whole quite firmly.

The most unusual feature of the subtrochal system are the cells' insertions, which penetrate into the ciliary band through the basal lamina and, in all cases examined, terminate adjacent to the ciliary nerve or branches from it. Examples are shown in figure 13 and in reconstruction (figures 14 and 15); they range from individual small ones (figure 13*a*), to clusters or rows (figure 13*c* and *d*), to large irregular ones (figure 13*e*). Their associations with the nerves are not particularly revealing: some insertions terminated near vesicle-filled fibres (figure 13*a*), but specialized contacts were not found. It general, the large insertions were associated with small nerves (e.g. figure 13*e*), and small insertions with the large nerve tracts.

Only limited data on the distribution of insertions were obtained, because they are difficult to find in long section series. They were, in general, sparse in the youngest specimen (figure 11), but more numerous in the older specimens, where they were found in the apical process, posterolateral lobes and anterodorsal ridge. Along this last structure, which was examined quite thoroughly, every subtrochal cell had at least one insertion; most had several.

(v) *Summary*

Figure 16 provides a summary diagram of the four muscle and support systems described above. Of these,

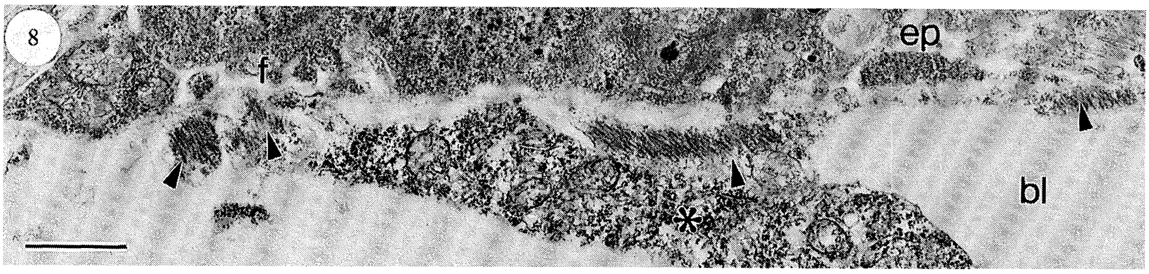


Figure 8. Typical zone of muscle attachment to the epithelium (ep) of the oral field near the ciliary band. Facing the blastocoel (bl) are parts of one muscle cell (*) and four individual fibres (arrows). The muscle cells attach to the basal lamina along the underside of the epithelial cells and to their basal processes; examples of the latter are marked (f). Scale bar 1 μm.

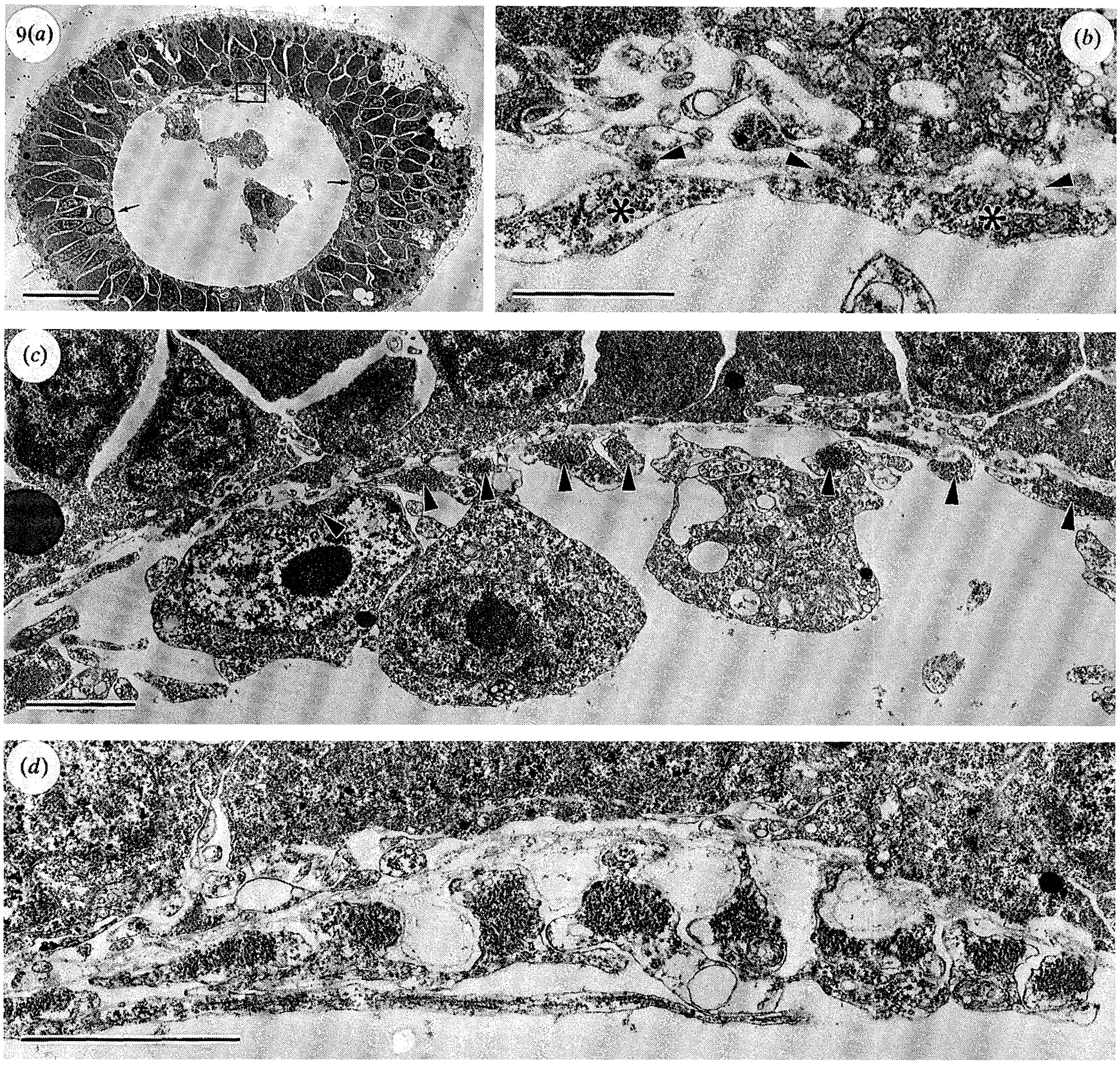


Figure 9. Transverse sections through the posterolateral lobe of a 25 d larva to show the muscle band; all are oriented with the oral field up. (*a*) A section near the lobe tip; scattered mesenchyme cells are visible in the blastocoel with, at the top, flattened terminal processes belonging to several oral field muscle cells. The ciliary nerves lie at either side (circled, small arrows). Scale bar 10 μm. (*b*) A detail showing the boxed region in (*a*), where the terminal portions of an oral field muscle cell (*) attaches to the epithelium. Contacts are formed with individual basal processes (left arrow) and along the undersides of the epithelial cells (between the two arrows on the right). Scale bar 1 μm. (*c*) A section of the muscle band about midway down the lobe. There are eight muscle fibres (arrows) at this point, and the cell bodies also belong to muscle cells. Scale bar 2 μm. (*d*) Muscle band at the base of the lobe, which is its narrowest point. Scale bar 2 μm.

the dorsal muscles are distinctive for being incorporated into the epithelium. This may simply be a matter of when they develop; they function very early and probably differentiate before the basal lamina is well established. The later-developing muscles all lie beneath the latter and attach to it in a conventional fashion. This includes the individual cells of the early oral field muscles, and the fully developed bands that

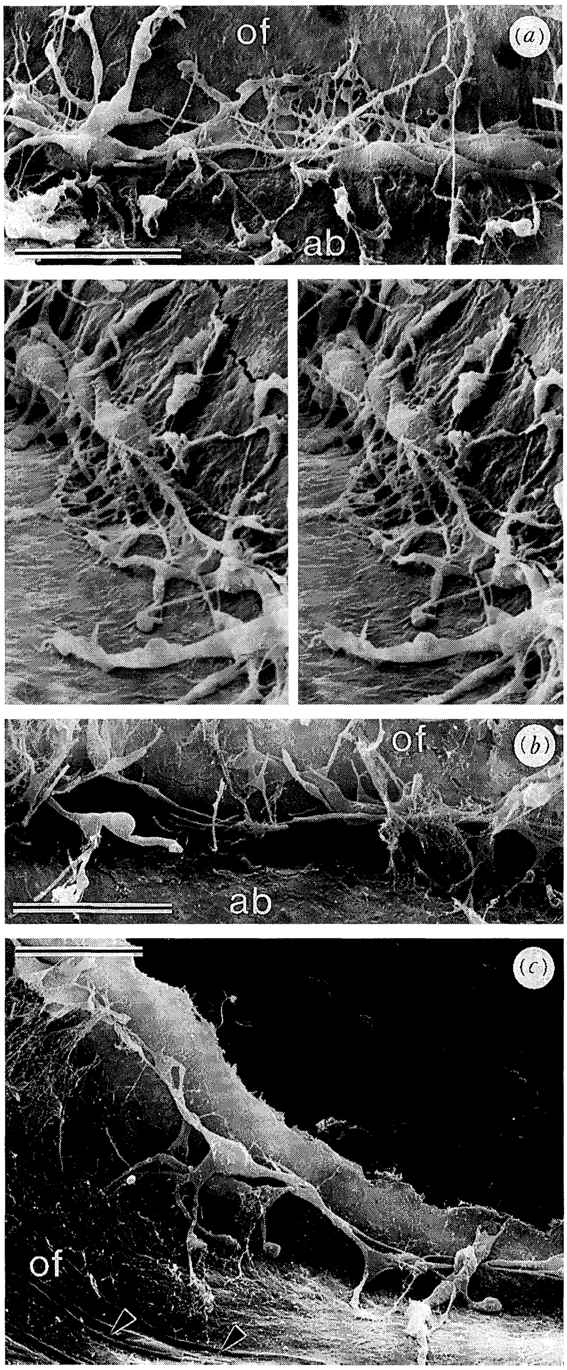


Figure 10. Subtrochal cells in SEM images of the inside surface of fractured 25–30 d larvae. The cells run along the underside of the band, which separates the oral field (of) from the aboral surface (ab). (*a*) The top panel shows a face-on view of the inside surface of the anterodorsal ridge, approximately the same region shown in figure 13*b–d* and the three-dimensional reconstructions. The lower panels show the same cells in stereo, as seen from the left of the top panel; the aboral surface is inclined upward on the right in the stereo view. (*b*) Subtrochal cells along the main band near the base of the apical process. (*c*) Similar cells along the main band in the hinge zone; the aboral epithelium has torn away along the curved margin where it meets the band. Note the parallel fibres of the oral field muscles (arrows). Scale bars 20 μm.

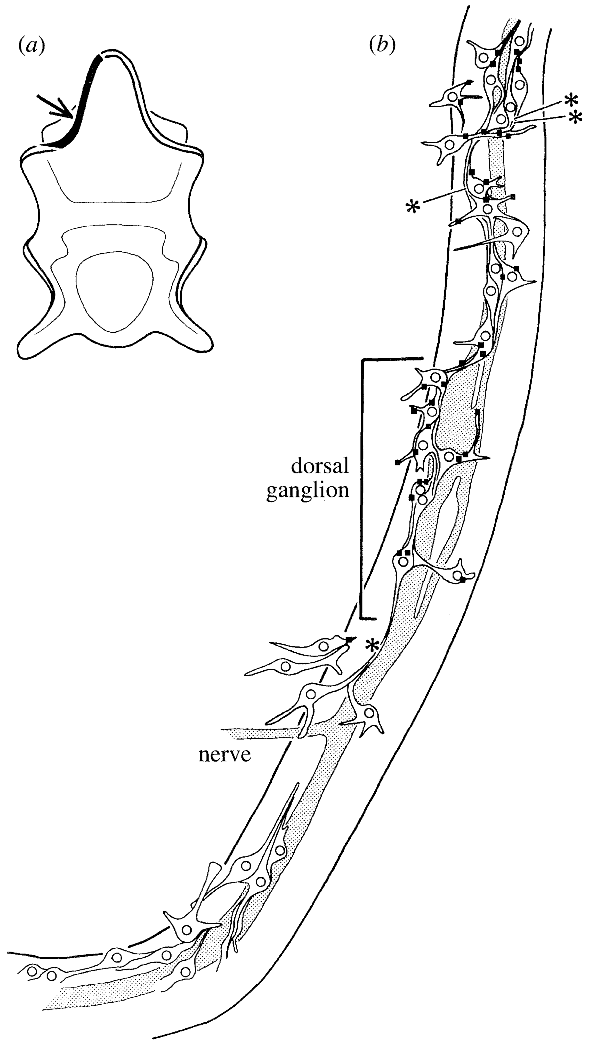
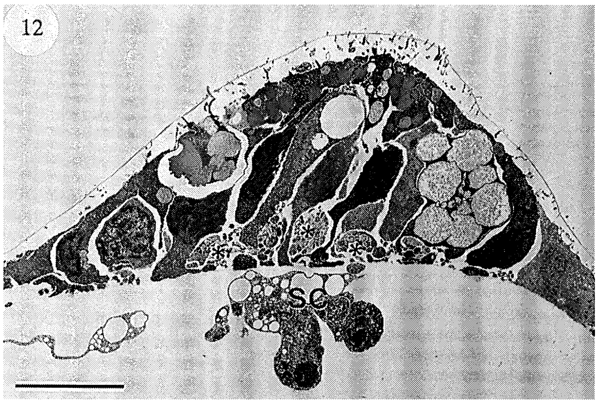
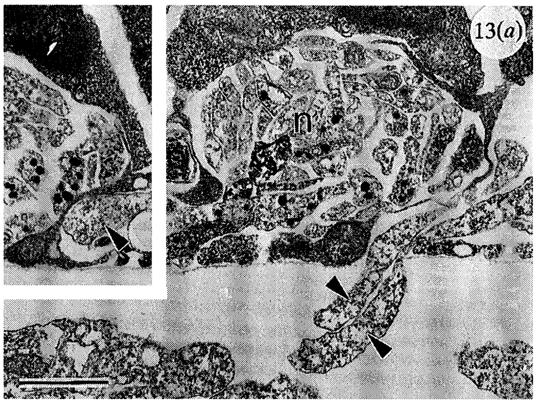


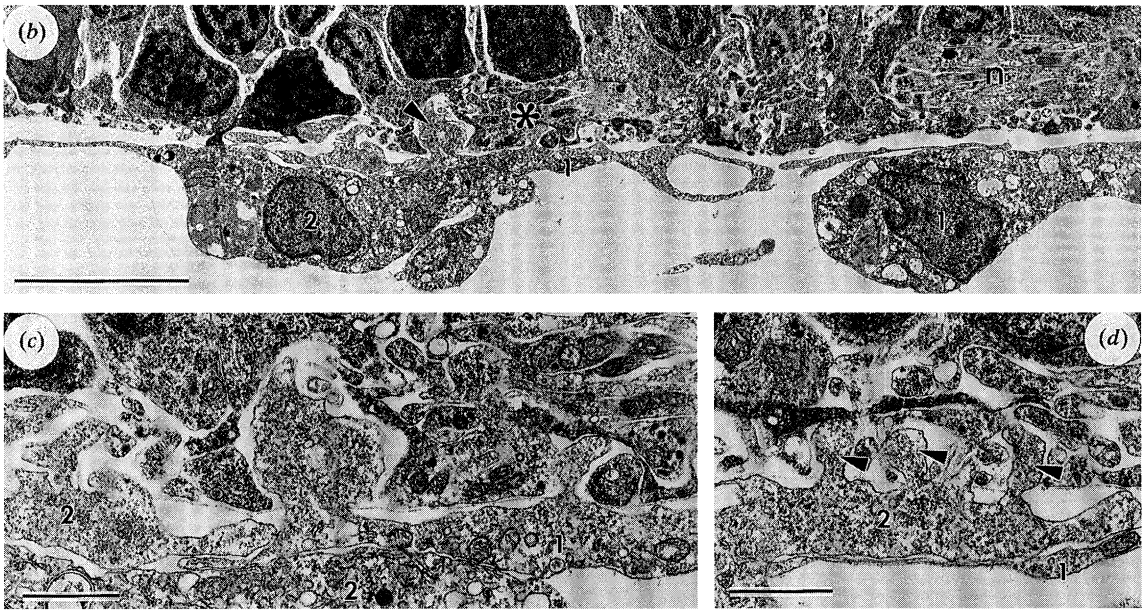
Figure 11. The anterior portion of the band system in a 20 d larva, showing the subtrochal cells, reconstructed from a section series examined at 0.5 μm intervals. (*a*) A dorsal view of the larva indicating (arrow) the portion of band shown in (*b*). (*b*) The ciliary band, subtrochal cells and the ciliary nerve (shaded). Solid squares show the points of attachment to the basal lamina; insertions are indicated by *. Only the region anterior to the lateral nerve was examined in detail for contacts and insertions. The figure probably underestimates the former by 40–50 %, since point contacts at the tips of cell processes were often missed. The ciliary nerve enlarges in the zone occupied by the dorsal ganglion (see Moss *et al.* 1994), and here the subtrochal cells are displaced to the oral side of the band.

supply the lobes. Organized muscle bands occur in the aboral epithelium at a few specific locations, but generally the main muscle fibres and bands in late-stage larvae are associated either with the oral field or the preoral shield. This suggests the main epithelial domains into which the body is divided differ with respect to their roles in locomotion and body support. In particular, the dorsal epithelium, which is the most delicate of the three, is also least preferred as a site for muscle attachment.

Among the non-muscle mesenchyme or support cells, the subtrochal cells form a distinctive sub-category. Because they run along the band, while the oral field muscles run predominantly perpendicular to







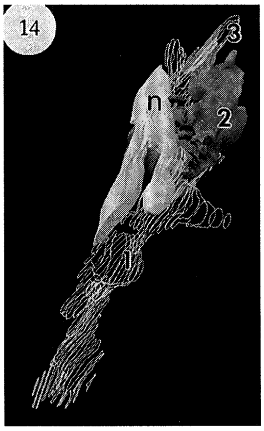


Figure 12. Transverse section through the ciliary band showing the subtrochal cells (sc) associated with it, and the ciliary nerve, which is broken into several subsidiary tracts (*) at this point. The oral field is to the left; the aboral surface is to the right. Scale bar 5 μm.

Figure 13. Subtrochal cell attachments and insertions. Scale bars 1 μm except in (*b*). (*a*) Transverse views of two subtrochal cell processes (arrows) crossing the basal lamina near the ciliary nerve (n). One of these terminates adjacent to the nerve (arrow in inset) in close proximity to a vesicle-filled terminal. (*b*) A section along the band in the anterodorsal ridge area, and two of the subtrochal cells from the reconstruction, indicated by number. Cell 1 forms an insertion (arrow) that penetrates adjacent to a branch (*) of the ciliary nerve (n). Scale bar 5 μm. (*c*) The insertion from (*b*). (*d*) A nearby section with multiple small insertions (arrows); note adjacent neurites and the closely apposed processes of cells 1 and 2. (*e*) A transverse section through the band where the nerve (n) has three subsidiary branches (*). A large insertion (arrow) surrounds the smallest of these, on the oral side of the ciliary band.

Figure 14. Reconstruction of the two subtrochal cells shown in figure 13*b* (cells 1 and 2), with part of a third cell; a key for interpreting figure 15. The position of the ciliary nerve (n) is shown, including one branch; smaller branches also occur but are not shown.

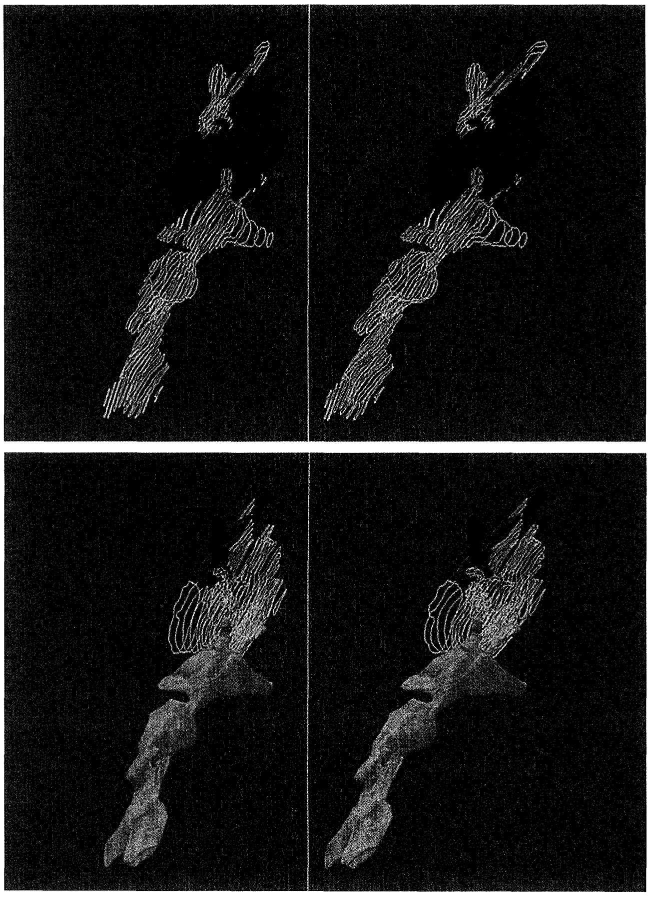


Figure 15. Stereo views of the cells in figure 14. Cells 1 and 3 are shown in outline in the top panel, and cell 2 is solid; this is reversed in the bottom panel, to show how the flattened processes from adjacent cells interleave. The insertions project upward in this view.

it, the two systems are necessarily orthogonal. The subtrochal cells are unusual in forming insertions into the ciliary band. If these cells are, in fact, contractile, e.g. if they act in effect as low-grade muscles, they might be expected to be subject to neural control. It is therefore tempting to suggest that the insertions may provide sites for innervation, but the morphological evidence is so far only circumstantial. In fact, there is even less evidence to indicate how the major muscles and muscle bands are controlled, since no morphologically identifiable neuromuscular contacts were found. Synapses and other specialized junctions are rare in echinoderm nervous tissue in any case, however (Cobb 1987), and are essentially unknown in larvae.

(*b*) *Behaviour*

Contractile mesenchyme cells are present from gastrulation onward in echinoderm larvae, and provide at least some of the driving force behind morphogenesis during the embryonic period. The first obvious response in starfish larvae attributable to differentiated muscle is the dorsal flexure. This acts to raise the hood-like preoral region and increase the distance between the preoral and postoral transverse bands (figure 17*a*). It is generally considered to be a method for clearing debris from the mouth (Strathmann 1971) and is observed in even very young larvae (Lacalli *et al.* 1990). The dorsal muscles are the only muscles suitably positioned to generate a flexure of this type. They also illustrate the way bipinnaria muscles are designed to function. Since they are embedded in the epithelium along their whole length, the epithelium must shorten in the same direction when the muscles contract. Body volume remains constant, so the surface to which the muscles are attached becomes increasingly concave, while the opposite side of the body is stretched. The opposite result would occur if the muscles spanned the blastocoel and attached only at each end, but this does not occur. Instead, all the muscles attached at the body surface appear to act in essentially the same way; they flex structures by shortening the epithelial surface to which they are attached.

The other feature relevant to the flexure response is the need for a weak point to act as a hinge. This is evidently provided by the deeply curved portion of the dorsal ciliary band lateral to the mouth (the 'hinge zone', figure 2), which is located precisely at the point of maximum flexure. A similar hinge zone is present also in the auricularia larva.

When the bipinnaria lobes flex, they bend forward towards the oral field (figure 17*b*), again, because the muscles are shortening the surface (the oral field) to which they are attached. The long posterolateral lobes are recruited first in this type of response, so that both lobes twitch forward together. A stronger stimulus evokes a response from other lobes, first the nearby postoral or posterodorsal lobes, then more distant

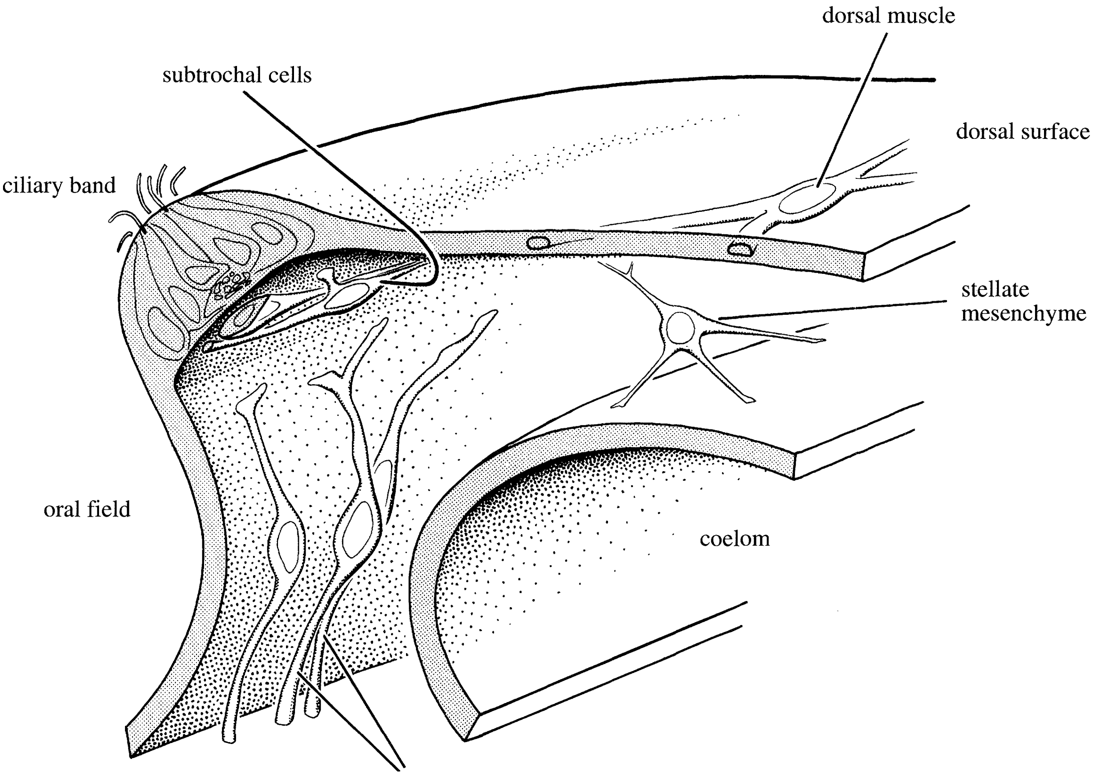


Figure 16. Summary diagram showing the characteristic arrangement of the four types of contractile and support cells described in this account.

anterior ones. With repeated stimuli, all of the lobes may finally curl forward, as in figure 17*b*.

The posterolateral lobes can also wave rhythmically back-and-forth for extended periods of time (figure 17*c* and *d*). When the larvae are suspended vertically in the feeding posture, this action causes them to rotate on axis. This may be useful in feeding as a means of increasing the volume of surrounding fluid that can be sampled by the ciliary currents generated by the band. During both the forward and recovery strokes, the greatest change in lobe curvature occurs near the base of the lobes. The lobe tips trail at first, and change curvature only near the end of each sweep. Muscle contraction is thus initiated and is strongest at the base of the lobes, where the muscle bands are best developed; more distal parts of the lobe contract later and more weakly.

Besides the posterolateral lobes, there are only two other places where the muscles attached to the oral field are specifically antagonized by muscles on an opposing surface. (i) Muscles on the dorsal surface of the apical process are involved in drawing back the apical tip of the larva. This is not a very pronounced behaviour in *Pisaster*. It is much more so in large *Luidia* larvae. These have a much enlarged apical process that is very muscular and active, due to massively developed muscles along the dorsal surface of the apical process (Tattersall & Sheppard 1934) that occupy much the same position as those shown in figure 7*b*. (ii) There are muscles on both surfaces of the preoral lobes, which may relate to the fact that the preoral shield, in section, is thicker than the dorsal epithelium, which may make it a better attachment site. The lobes themselves nevertheless behave much like the other small lobes; they curl forward over the oral field, and lack an obvious recovery phase.

(*c*) *Body support*

Larval musculature and behaviour in *Pisaster* correlate comparatively well, that is, most behaviours are attributable to specific, identifiable muscles. Explaining how the body as a whole is supported is more difficult. The epithelium is very delicate, which raises the question of how it can possibly be stiff enough to serve as a stable attachment point for muscles, and how it resists deformation and collapse. Several factors are evidently involved. First, the body clearly has a positive internal pressure with respect to the outside, because the lobes bend when muscles contract rather than collapsing. The pressure differential between the blastocoel and the outside is probably not very great, however. Bipinnaria larvae are not particularly inflated in appearance, as would be expected if the pressure differential were large, and have regions of both positive and negative surface curvature. Other dipleurula-type larvae (e.g. the tornaria) are more uniformly convex in outline, which may be an indication of higher internal pressure. The blastocoel supports the epithelium in a second way, by virtue of the semi-solid gel-like matrix substance it contains (Strathmann 1989). A supporting gel could compensate for the low blastocoelic pressure if indeed this is a problem for the larva. The gel is either not completely rigid, or fails to penetrate fully throughout the larva, however, as the lobes would otherwise not

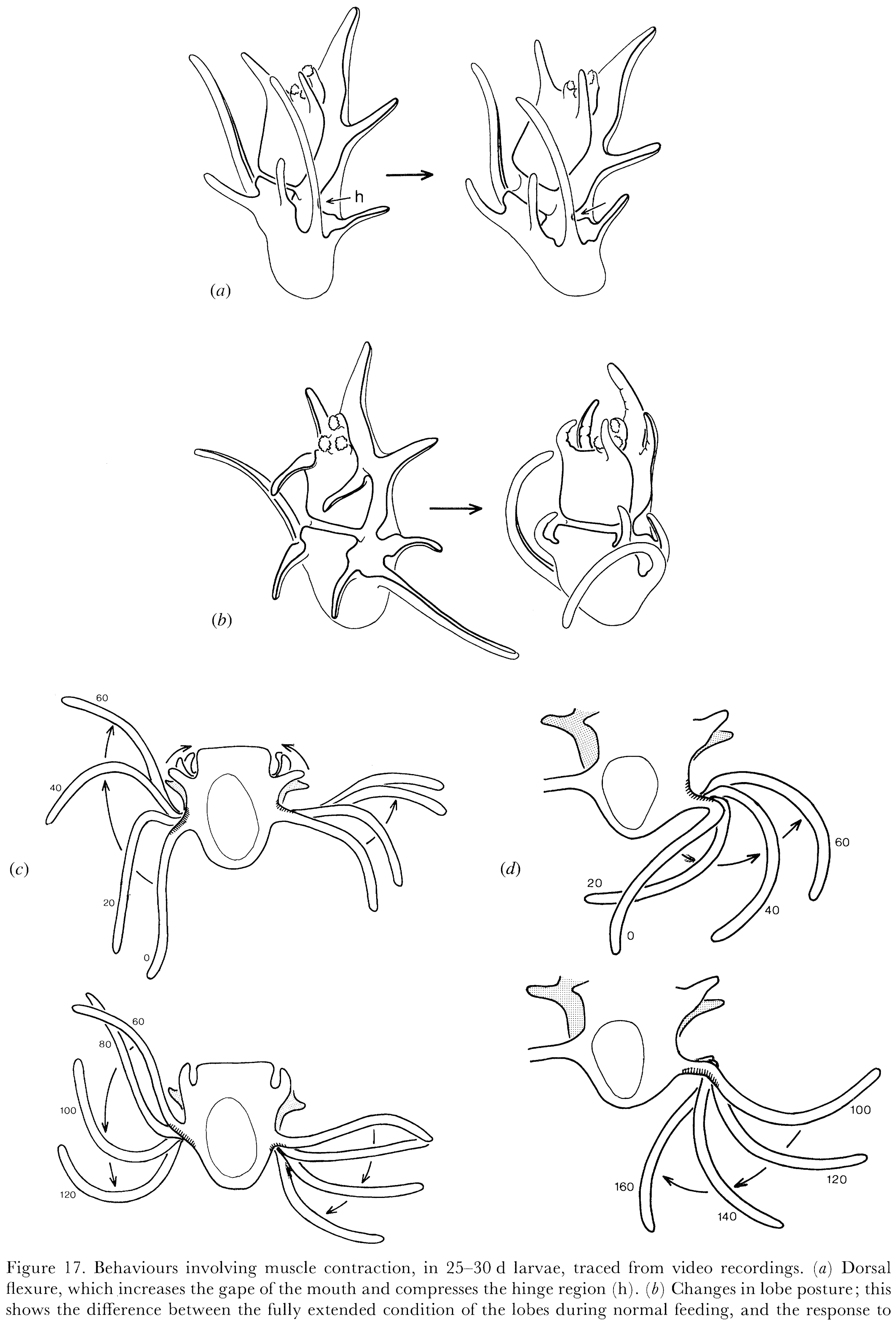


Figure 17. Behaviours involving muscle contraction, in 25–30 d larvae, traced from video recordings. (*a*) Dorsal flexure, which increases the gape of the mouth and compresses the hinge region (h). (*b*) Changes in lobe posture; this shows the difference between the fully extended condition of the lobes during normal feeding, and the response to repeated disturbances, which causes the lobes to curl inwards. (*c*) Coordinated contraction of the posterolateral lobes from a motionless starting point. Profiles are numbered by video frame, at 30 frames per second, from the initial contraction, at 0. Note that both of the posterolateral lobes and the pair of small postoral lobes initially contract together, but only the former have a reverse stroke. Hatching marks the zone of maximal flexure. (*d*) Part of a prolonged cycle of rhythmic back-and-forth waving of the posterolateral lobes due to alternating contraction of

flex so easily. The coelom is the only structure in the bipinnaria that seems to be maintained in a fully turgid state by hydrostatic pressure, which may explain why it is the main attachment site for the mesenchyme cells that link the body surface with internal structures.

The other structural feature relevant to the issue of body support is the ciliary band. The bands are substantial pieces of tissue, in terms of cell number and volume, compared with the rest of the epithelium, and they could, therefore, provide a supporting framework for more delicate tissues. The shape of the larva is essentially defined by the shape of the band; changes in surface contour (e.g. from convex to concave) occur only at bands, and projecting structures, e.g. lobes and ridges, always incorporate a segment of the band. The brachiolar arms are exceptions, but they are directly supported by coelomic diverticula, and so are under independent hydrostatic control. If the bands are functional support elements, it is logical to suppose they would be the preferred sites for muscle attachment, as indeed they are in the bipinnaria. The muscles attach along the margin of the band, however, not directly to it, and only on the oral side. This may explain why there are more basal-cell processes on the oral side of the band than the aboral one. By forming a meshwork linking adjacent cells, such processes could conceivably provide a degree of rigidity in this region that would help maintain the curvature of the epithelium, as well as stabilizing muscle attachments.

There is then the question of what function the subtrochal cells perform, if any. One possibility is that they are required to stabilize the band late in development, when the larval muscles are themselves better developed and contract with greater force. This is supported by the observation that increasing body size and musculature correlates generally with an increase in the number of subtrochal cells, and increasing numbers of attachments and insertions. The observations reported here are from one species only, but tracts of mesenchymal cells have been reported beneath the ciliary band in *Luidia* larvae (Tattersall & Sheppard 1934), which are very muscular, and essentially move by flexing the whole anterior part of the body. Subtrochal cells also occur in auricularia larvae (T. C. L., unpublished results), where they have so far been found beneath the ridge elements and along the underside of the anterior part of the dorsal bands. These areas are especially resistant to deformation and collapse in SEM preparations, which indicates their structural strength in fixed specimens at least, if not in live larvae. The auricularia cells also form insertions, which indicates that this curious feature is not restricted to bipinnaria larvae.

The above provides at least circumstantial evidence that subtrochal cells may be involved in improving the load-bearing characteristics of the band. Figure 18 shows, from engineering considerations (Gordon 1978), how this could, in principle, be achieved. As shown in figure 18*a* and *b*, the angle between the band and the adjacent epithelium is variable, but is typically quite small on the oral side owing to the concavity of the oral field. Various forces act on the band; those directed inward could potentially buckle it. Consider, for example, a segment of band subject to an external load, and seen in longitudinal section, as in figure 18*c*. The situation is analogous to a horizontal beam supported at each end and loaded in the centre: the load acts to compress the top surface of the beam, while the opposite (bottom) surface is stretched. The beam will sag, and may eventually give way. Opposing the force of the load is the beam's intrinsic resistance to compression, along its top surface, and its tensile strength, i.e. resistance to stretch, along the bottom. In the case of the ciliary band, the load may be applied from the outside, or it could arise internally from the force of the muscles pulling on it. Regardless, the direction of the force will be inwards, so the effect is the same. If the cells in the band are turgid and adhere more-or-less firmly to one another, the band could be expected to form a reasonably stable, incompressible unit, and so provide some intrinsic resistance to compressive forces. Strong adhesion between cells and a sturdy basal lamina undoubtedly contribute to the tensile strength of its inner surface, but an obvious way to augment this would be to deploy a series of contractile cells along the underside of the band that would hold it under tension. Assuming they are capable of exerting a contractile force, the subtrochal cells are ideally positioned to accomplish this task. In a mechanical structure, this would be equivalent to adding guy wires or springs to stiffen a light framework, and so increase the tensile strength of the framework as a whole.

Because they are positioned directly beneath the band, the subtrochal cells would be effective primarily against forces directed normal to it. This is, in fact, precisely what is required to resist the deforming effects of muscle contraction. This is because the oral field, to which the muscles are attached, typically meets the band at an angle close to 90°, so most of the force of contraction will be directed normal to the band (F_n in figure 18*d*). The intervening tissue must also be sufficiently stiff to transfer the force from the point of attachment to the band. Why so few muscles attach to the aboral epithelium is not clear. It may be intrinsically less suitable for structural reasons, or there may be other limitations, e.g. in the nature of the available innervation.

In summary, this section argues that the ciliary band acts as a semi-rigid framework that supports the epithelium and provides sites for muscle attachment, and that the subtrochal cells contribute an added degree of stability to that framework. Other means of stabilization are possible, for example, blastocoelic pressure could be increased, but this could have other disadvantages. It could constrain body shape in an

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muscles on the oral and aboral surface. In this instance an atypical but perisitent kink has developed on the aboral side distal to the lobe base, and this shows that local contraction, which is maximal at the kink, can effectively move the lobe as a whole.

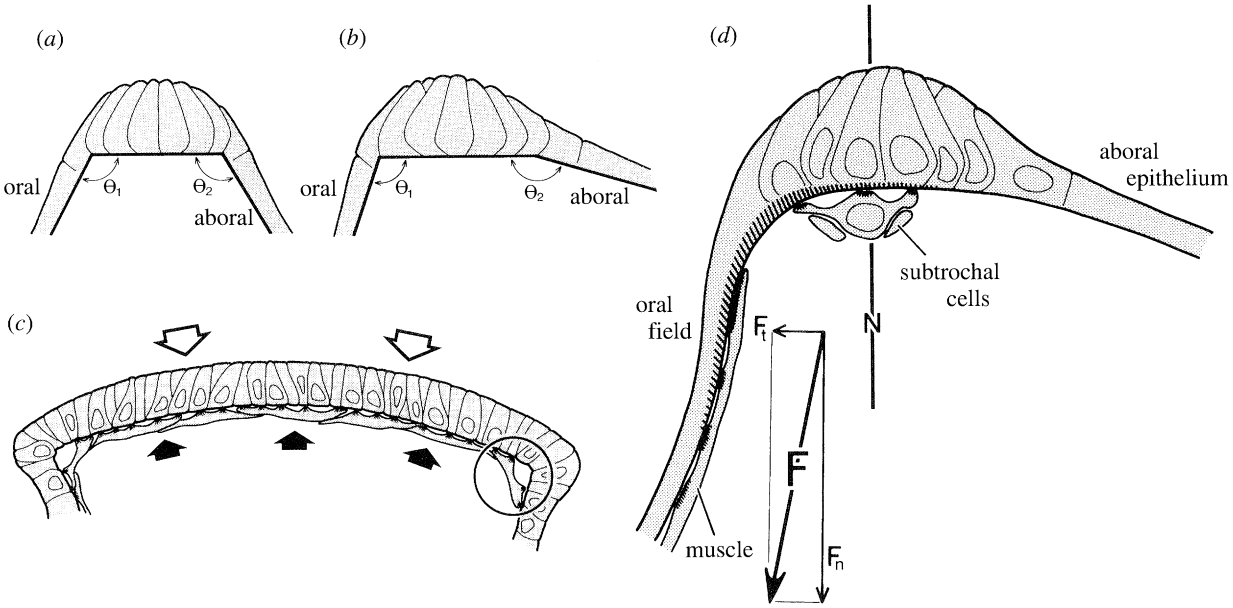

Figure 18. Ciliary bands as body-support elements, a proposal for how the subtrochal cells may act to increase the resistance of the ciliary band to local deformation. (*a*) and (*b*) show bands in transverse section; the angle formed between the band and the adjacent epithelium on the oral (Θ_1) and aboral (Θ_2) sides varies depending on the stage and location. The situation shown in (*a*), where the angles are similar and close to 90° is typical of young larvae (cf. figure 1 (*a*) and some regions of older ones, e.g. the hinge zone. The situation along most of the band in older larvae is closer to that shown in (*b*), i.e. Θ_2 is larger than Θ_1 with the former close to 180°. (*c*) A schematic side view of a segment of band, cut lengthwise, to show a file of trochal cells, with subtrochal cells beneath. Various forces act on the band. Whether these originate outside the larva or on the inside, from the action of muscles, the force is directed inward (upper arrows). It is opposed by the gel-like contents and internal pressure of the blastocoel (solid arrows), and the structural strength of the band itself. The latter stems in part from the band's incompressibility, i.e. it resists compression. The position of the subtrochal cells is such that they could, in principle, provide an added degree of tensile strength to the structure as a whole, stabilizing the band by holding its inner surface under tension. This reduces the tendency of any structure to stretch under an external load. The subtrochal cells could potentially also be involved in stabilizing points of high curvature (e.g. the circled region), and thus have an active role in shaping the band, but there is currently no specific evidence to support this idea. (*d*) A transverse section of the band showing how the contraction of the oral field muscles would be expected to act on it. The subtrochal cells are positioned to provide resistance to any inward force directed normal to the band, i.e. along line N. The angle the oral surface forms with the band ensures that when the oral field muscles contract, a major component of the force (F) they exert is also directed normal to the band (F_n), whereas the tangential component (F_t) is much smaller. This should maximize the band's resistance to being locally deformed by the contraction of nearby muscles, but requires that the intervening tissue be sufficiently rigid to carry the force without being stretched or deformed. The expectation is that tissue on the oral side of the band (hatched zone) should be reinforced in some way, which may explain the network of basal fibres found in this area.

unacceptable way, for example, or interfere with other functions, e.g. swallowing and food passage through the gut. Strengthening the external framework seems, instead, to be the preferred solution.

4. PATTERN REPEATS IN RELATED DEUTEROSTOME LARVAE

Echinoderm larvae and the tornaria larva of enteropneust hemichordates share a common topological plan, in the way the ciliary bands are arranged with respect to the mouth, for example, and other features of ciliary band structure and innervation (Nielsen 1987, 1995; Lacalli 1993). In late-stage larvae, the ciliary band typically undulates over the surface of the body and is drawn out, at various points, to form projecting lobes, ridges or tentacle-like structures. These occur at a roughly regular intervals along the band, and similarities in position among the different larval types, at least among echinoderms, suggest there is an underlying common plan. The most consistent feature, in the echinoderms, is the similar arrangement of the three posterior-most elements; these are postoral, posterodorsal, and posterolateral in position (po, pd and pl, respectively, in figure 19). They are developed as lobes in starfish larvae (figure 19*a* and *b*), as projecting ridges in holothurian auricularia larvae (figures 19*c* and 20), and as epaulettes or vibratile lobes in pluteus larvae (not shown, see Lacalli 1993 for examples). The pluteus is better known for its larval arms, which are supported by skeletal rods, but these form a second series out-of-phase with (between) the epaulettes.

The anterior, preoral part of the larval body is more variable. Anterior to the hinge zone, auricularia larvae have two dorsal ridges and two ventral ones. *Pisaster* larvae, in common with most starfish larvae (e.g. see Chia *et al.* 1993), have a dorsal ridge that produces a single lobe. This is matched on the ventral surface by

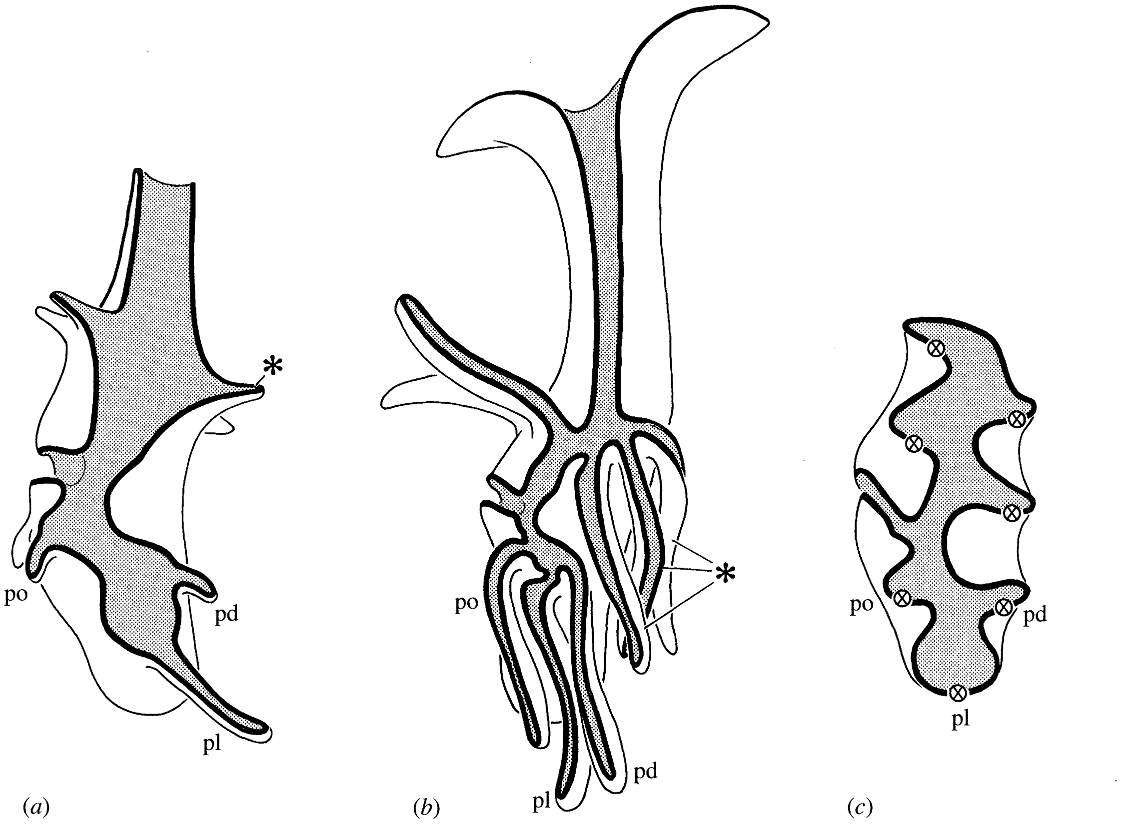

Figure 19. A selection of echinoderm larvae showing the arrangement of projecting lobes and ridges; the oral field is shaded. Bipinnaria larvae of (*a*) *Pisaster*, (*b*) *Luidia* (adapted from Nielsen 1995), and (*c*) a holothurian auricularia larva, with the position of each of the projecting ridges marked by a circled X. The three-dimensional shape of the auricularia is better appreciated in SEM images (e.g. Lacalli 1993). Note that all three larvae have the same characteristic triad of posterior elements: postoral (po), posterolateral (pl), and posterodorsal (pd). The anterodorsal ridge in the two bipinnaria larvae is marked by *; it produces a single lobe in *Pisaster*, but multiple lobes in large *Luidia* larvae (the example shown is drawn about half-size in relation to the other two larvae).

the preoral lobe. The largest known *Luidia* larvae generate multiple lobes from the anterodorsal ridge (figure 19*b*), which shows that at least some bipinnaria larvae can replicate the lobes in series.

While the actual outgrowth in the above examples is ectodermal, mesodermal components of various types must necessarily be involved. For structures that move, muscles are required, as in the case of the bipinnaria lobes. Ridge elements in the auricularia have a complement of mesenchyme, which includes subtrochal cells (T. C. L., unpublished results) and, in late-stage larvae, hyaline spheres. The latter consist of scattered mesenchyme embedded in a spherical ball of extracellular secretion (figure 20*b*); their function is unknown. The periodic repeat of structures observed at the gross morphological level thus implies a parallel repeating pattern in the arrangement of the mesoderm. Whether this is a causative agent, i.e. whether mesodermal periodicity is imposed on the ectoderm or vice versa, is not clear.

Figure 21 shows a tornaria larva, with the band developed into a series of tentacle-like projections. These are characteristic of large, late-stage larvae, and may be blunt, or broad and flat, depending on species. Various examples are illustrated by Damas & Stiasny (1961). The Bahamian tornaria of Morgan (1894) is particularly impressive, but the *Ptychodera* larvae described by Strathmann & Bonar (1976) are essentially similar. The tentacles tend to lie flat over the oral field, but can be elevated (figure 21*b*), so they clearly contain muscles and other support elements; SEM images (e.g. figure 32 in Nielsen 1987) show the three-dimensional arrangement more clearly. The important point is that the anterior part of the ciliary band system, which generates only a few repeats in echinoderm larvae, is further elaborated in the tornaria to form an extended series of structures. Posteriorly, there is a small blind pocket, the lateral bay, that continues the process in a more modest fashion. Initially it has a single pair of lobes, which becomes a series in late stages in some species (figure 21*c*). Whether this region is related to the posterior part of the echinoderm system, with its stereotyped triad of structures, is not clear, but it is possible. Comparing ciliary band patterns in the two phyla, the posterior part of the body does appear generally to be less modified than the anterior part.

In summary, examples of serially repeating structures are known from all the basic dipleurula types. In the present context, the differences between the bipinnaria and other dipleurula types are less significant than the fact that they appear to share an intrinsic capacity to replicate contractile and mesodermal support elements in series.

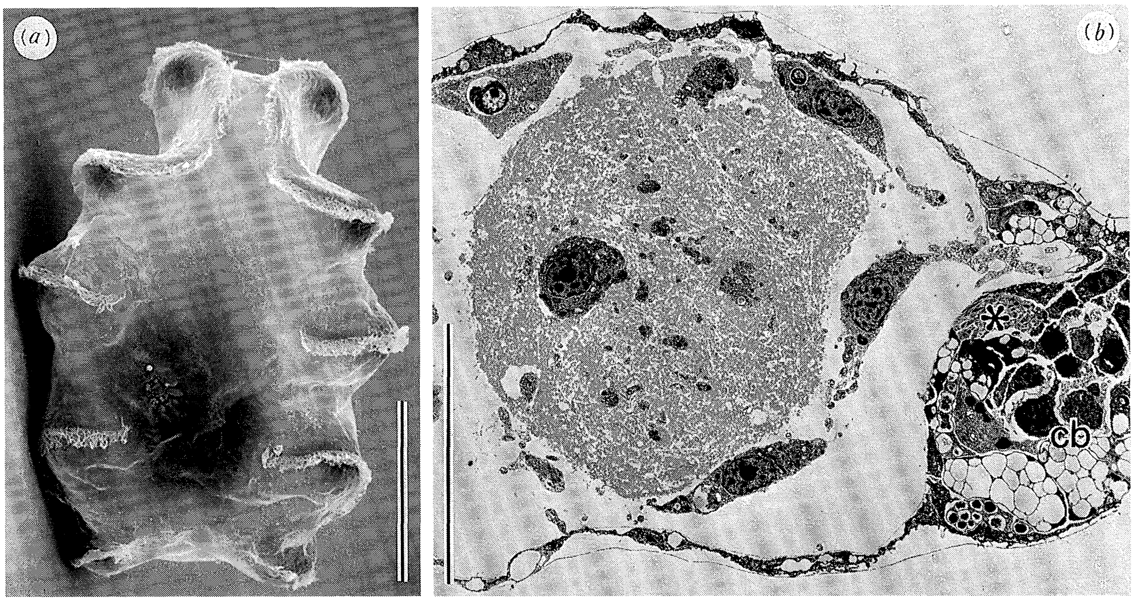

Figure 20. Ridge elements in the auricularia larva of the holothurian *Stichopus californicus* (for methods, see Lacalli 1993). (*a*) Dorsal view of a larva beginning metamorphosis. The band fragments during this process, but the ridges persist and form a regular dorsolateral series. The hyaline spheres are visible as dark spots under each ridge. Scale bar 200 μm. (*b*) A hyaline sphere in section; showing the mesenchyme cells that surround it (others are embedded inside), the adjacent ciliary band (cb), and the ciliary nerve (*). Scale bar 20 μm.

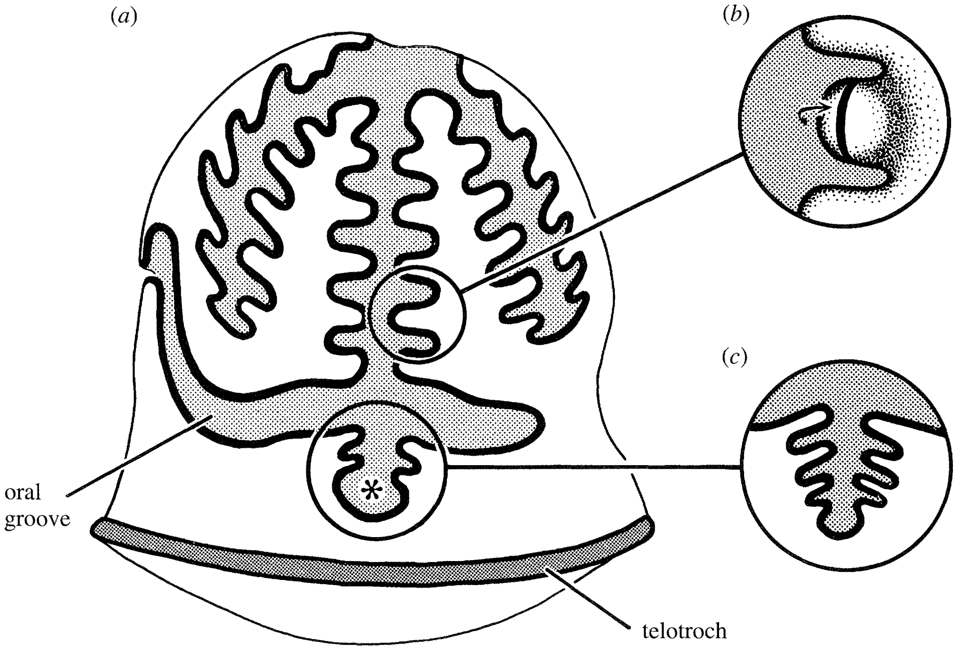

Figure 21. A tentaculate tornaria larva, showing the arrangement of tentacles along the ciliary band. (*a*) A side view with the mouth on the left; the oral field is shaded. The tentacles are arranged in a regular series, indicated by the undulating heavy line. Each excursion into the oral field is intended to represent a free-standing tentacle that can be lifted away from the oral field, as in (*b*). A blind posterior extension of the oral field, the lateral bay (*), is also characteristic, and is variously developed in different species. In some, in advanced stages, a series of small tentacles develop along both sides, as in (*c*).

5. COMPARISONS WITH CHORDATES

Echinoderms are related to chordates, but the gulf separating the two phyla is substantial and poorly understood. The embryonic and larval stages show the relationship most clearly, which led Garstang to propose a means of deriving chordates by modification of an ancestral larva resembling the dipleurula (Garstang 1894). Garstang was chiefly concerned with the origin of the chordate nerve cord, which he derived from the dipleurula ciliary bands. The arrangement of nerve cells within the bipinnaria ciliary band (see figure 23) provides some circumstantial support for this idea. Accounting for the characteristic features of chordate mesoderm (e.g. notochord and somites) is more difficult. The mesoderm in dipleurula larvae typically forms scattered mesenchyme, from which the larval muscles develop, and three pairs of coelomic compartments. The stomochord in enteropneusts is considered a possible notochord homologue (reviewed by Balser & Ruppert 1990), but it is not present in the larva, and there are no other obvious notochord-like structures in other dipleurula types. Nor are there obvious counterparts of somites, unless the coeloms represent a rudimentary version of the somite series, but that would still leave many features of somite structure and organization unexplained. The origin of the somites and, hence, of the 'segmental' nature of the body in advanced chordates is thus still largely a mystery.

The sections below highlight features that the mesoderm in the bipinnaria share with that of chordates. The emphasis is on structures arranged in series, and how these might relate to serially repeated structures in chordates.

(*a*) *Muscle bands in pelagic tunicates*

Assuming the main muscle bands of a primitive dipleurula were organized perpendicular to the ciliary band, as in the bipinnaria, internalizing the ciliary band and aboral ectoderm as suggested by Garstang (figure 22*a*) would generate a barrel-shaped organism with circumferential muscles arranged in series (figure 22*b*). The muscles in tunicates that meet at the dorsal midline between the siphons have a somewhat similar arrangement. This is most clearly seen in two pelagic groups, the doliolids and salps, where these muscles form a regular series of distinct bands around the cylindrical body. The muscle bands are not generally considered to be related to somites which, if present in tunicates at all, occur only as vestiges in the larval tail (Crowther & Whittaker 1994). Tunicates depend for body support on a semi-rigid external envelope, a tunic or test, to which muscles attach and against which they

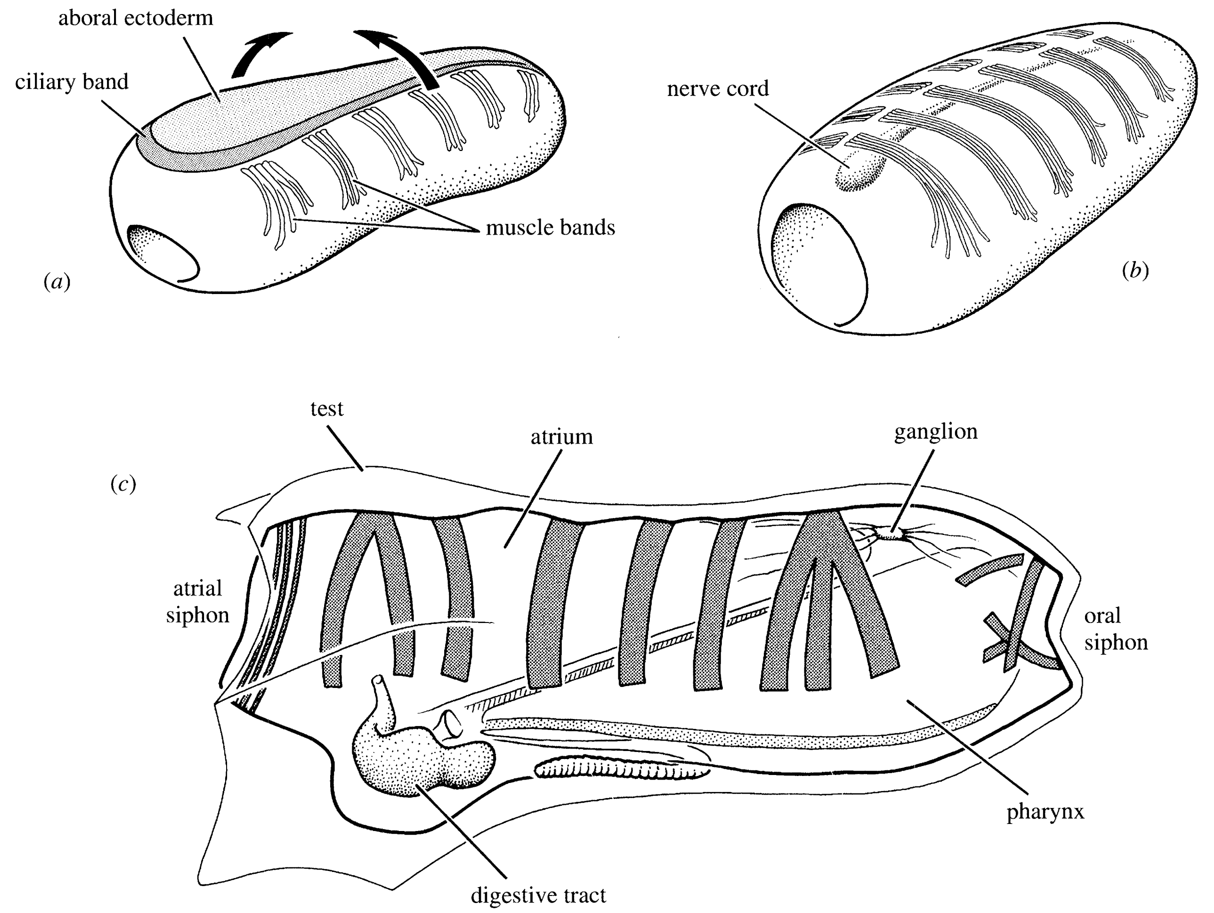

Figure 22. Following Garstang's scheme, (*a*) and (*b*) show how the dorsal ciliary bands and aboral ectoderm of a dipleurula larva, in (*a*), could be transformed into the nerve cord of a barrel-shaped, filter-feeding chordate (*b*). Note that the dipleurula oral field becomes the definitive body surface in the chordate, and that muscle bands oriented perpendicular to the band, like those described here for the bipinnaria, become circumferential, and terminate at or near the dorsal midline. For comparison, (*c*) shows the solitary stage of a typical salp, *Salpa fusiformis*, adapted from Berrill (1950*a*, *b*). Salps have a regular series of muscle bands (shaded) that meet at the dorsal midline and are innervated by nerves from the dorsal ganglion. There is no nerve cord except in the stolon, which projects forward along the ventral surface just in front of the digestive organs in this species, and then loops back on itself. The specimen shown would be about 6–7 cm long. There are both larger and smaller species; the smallest fall roughly in the same size range as large dipleurula larvae.

act. Among the pelagic forms, doliolids typically have eight or nine muscle bands that form complete rings around the body; salps (figure 22*c*) are more variable, both in the number of bands (up to 20) and how complete they are. Internal support structures like those found in *Amphioxus* and vertebrates are absent; nor are there large enclosed hydrostatic chambers like the coelom and blastocoel, gel-filled or otherwise, on which dipleurula larvae depend. In consequence, the internal space can be devoted instead to housing the spacious pharynx and atrium. In salps, when the muscle bands contract, a jet of water is forced out of one or other of the siphons (Bone & Ryan 1973), which provides a much more effective means of rapid locomotion than that employed by dipleurula larvae. The way the contractile elements are arranged is nevertheless comparable to that in the bipinnaria. In both, the muscles attach along the inner surface of an external epithelium, and locomotion depends on controlled deformation of the body surface.

If, among the pelagic tunicates, there were undoubted primitive forms closely related to echinoderms, it might be possible to make a reasonably convincing case for homology between the muscle bands in the two groups. Interrelationships within the tunicates are uncertain, however. The traditional view is that the pelagic forms evolved from sedentary ancestors resembling ascidians (Garstang 1928; Berrill 1950*a*), which would make the pelagic habit derived rather than primitive. More recent work supports the idea that appendicularians are probably the earliest offshoot from the tunicate lineage (Holland *et al.* 1988; Crowther & Whittaker 1992; Wada & Satoh 1994), and appendicularians are entirely pelagic. However, they also differ substantially from other tunicates both in body form (they are seen as being derived by neoteny from the tadpole larva) and feeding mechanism (they employ a disposable mucus 'house' as a filter). Therefore, despite their early divergence, modern appendicularians are probably much modified from the ancestral form and poor models for what that ancestor might have been like. Of the remaining groups, salps and pyrosomes (i.e. *Pyrosoma*) are better candidates. Their simple gill arrangement and the structure of the stolon are considered to be primitive, compared with the condition seen in ascidians (Godeaux 1957, 1989), and the stolon structure, in particular, separates them quite clearly from ascidians and doliolids (Berrill 1950*b*). Both salps and pyrosomes are pelagic, so if either group is close to the ancestral form, a holopelagic ancestor becomes much more likely. Even if they are not, it is still possible that forms like salps and doliolids adapted secondarily to pelagic life by elaborating structures derived from more distant ancestral forms that were, themselves, pelagic. This is not a particularly strong argument, but it is nevertheless possible that the similarities between pelagic tunicates and the bipinnaria in the arrangement of the muscles bands, in series, and in the way the muscles operate, are due to more than just convergence.

(*b*) *Muscle attachment and innervation in Amphioxus*

Turning the dipleurula ciliary band into a nerve cord, as suggested by Garstang, changes relative cell position in a predictable way, as shown in figure 23*a* and *b*. In particular, if the subtrochal cells were to maintain their close association with the ciliary band through this transformation, their final position would be roughly the same as that occupied by the myotome in *Amphioxus* and vertebrates. Subtrochal cells in the bipinnaria attach to the band, and also form insertions that penetrate to the ciliary nerve. In *Amphioxus*, the myotomal cells attach directly to the nerve cord via specialized processes (Flood 1966), and they are innervated at the point of attachment, as shown in figure 23*c*. As the figure also shows, upward growth of the ventral part of the myocoel effectively separates the myotome and notochord over most of the length of each somite (e.g. see Holland & Holland 1990). Having the myotome attached more firmly to the nerve cord than the notochord may, at first, seem surprising. The sheet of basement membrane that joins the front and back surfaces of adjacent myotomes to each other is, however, firmly fixed to the notochordal sheath, and this evidently acts as the principal load-bearing connection between the muscles and notochord.

The attachment of subtrochal cells to the ciliary band differs in detail from the arrangement in *Amphioxus*. Subtrochal insertions penetrate the basement membrane, for example, whereas myotomal processes do not. The association with the adjacent epithelium is, however, specific and very close in both cases, which is suggestive. Whether the two situations are sufficiently similar to indicate homology is an open question, but it would certainly help to account for the *Amphioxus* condition, which is otherwise difficult to explain. The subtrochal cells would be more convincing as somite homologues if they formed clusters arranged in series. The larval lobes and muscle bands do occur in series, but subtrochal cells in the bipinnaria appear to be uniformly distributed along the band, at least until the lobes form. If the subtrochal cells then associated in a specific way with each lobe, they would form a repeating series as well, either in-phase or out-of-phase with the muscle bands supplying each lobe. Preliminary evidence suggests this may occur in the auricularia. At metamorphosis, the auricularia band fragments, and only the ridge elements remain (Lacalli 1993). For a brief period, before these reorganize, the ridges form a regularly spaced, paired series on the dorsal surface (figure 20*a*), and each ridge has its own hyaline sphere and complement of mesenchyme. In the bipinnaria, the evidence so far indicates that the subtrochal cells cluster at the base of the posterolateral lobes where the ciliary band enters and leaves. If this was the basic pattern, then subtrochal cell clusters would be expected to occur in pairs, two for each lobe. There are, in short, various possibilities that need further investigation.

The close association between the ciliary band and subtrochal cells is suggested above (§3*c*) to be a way of

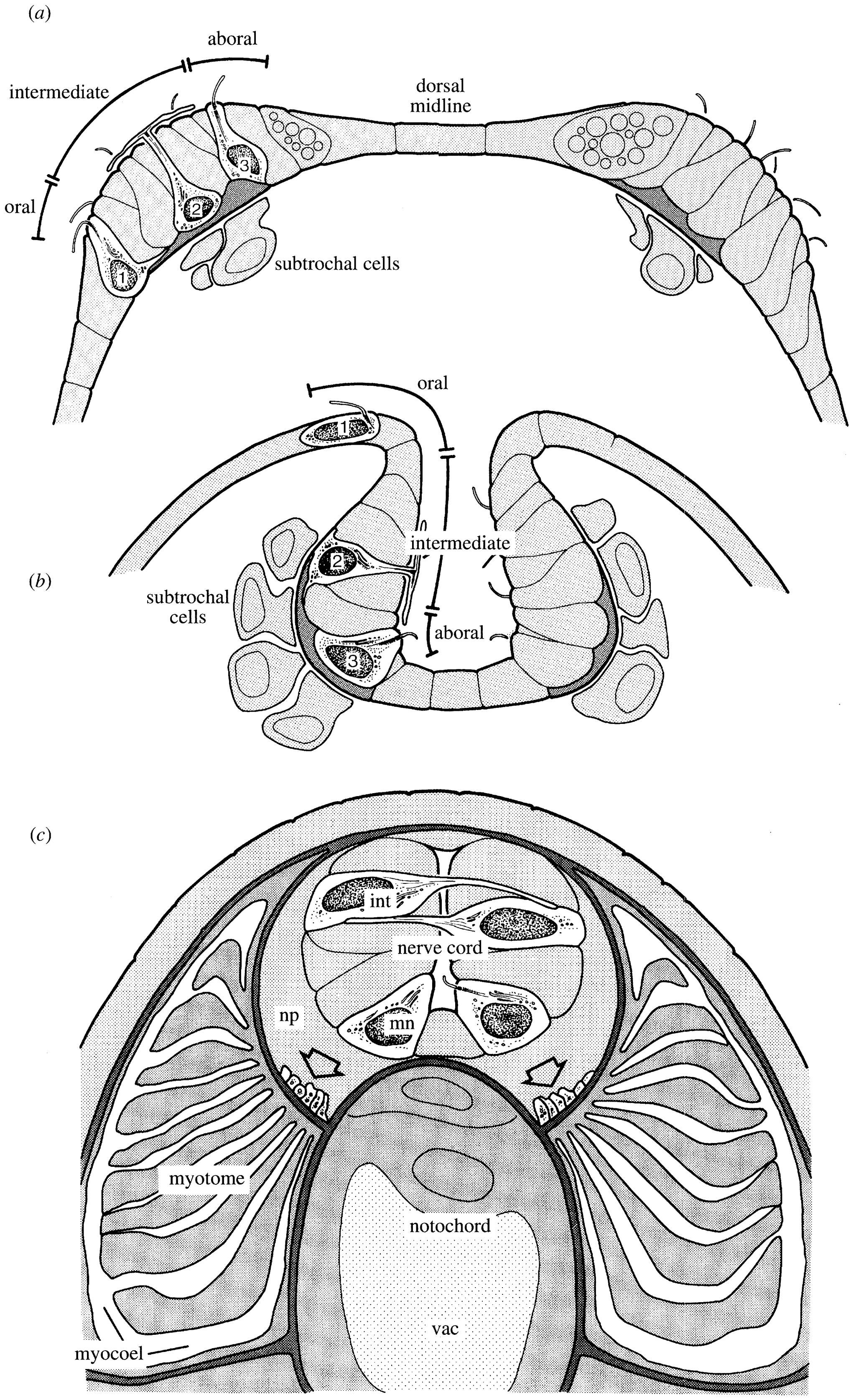

Figure 23. Further consequences of Garstang's hypothesis: (*a*) and (*b*) show, in transverse section, how the relative positions of particular cell types change as the ciliary bands are converted into a neural tube. Lacalli *et al.* (1990) found three different nerve–cell types in the bipinnaria band (numbered in sequence), and these occupy characteristic locations. Sensory cells (#1) lie along the oral margin and in the adjacent oral field; the main tract of the ciliary nerve (darker shading) and cells that contribute to it (#3) lie near the aboral margin. Between these is an intermediate zone containing multipolar cells (#2) with extensive apical surface processes. Such cells occur widely in dipleurula larvae

[*legend continues on p. 1756*

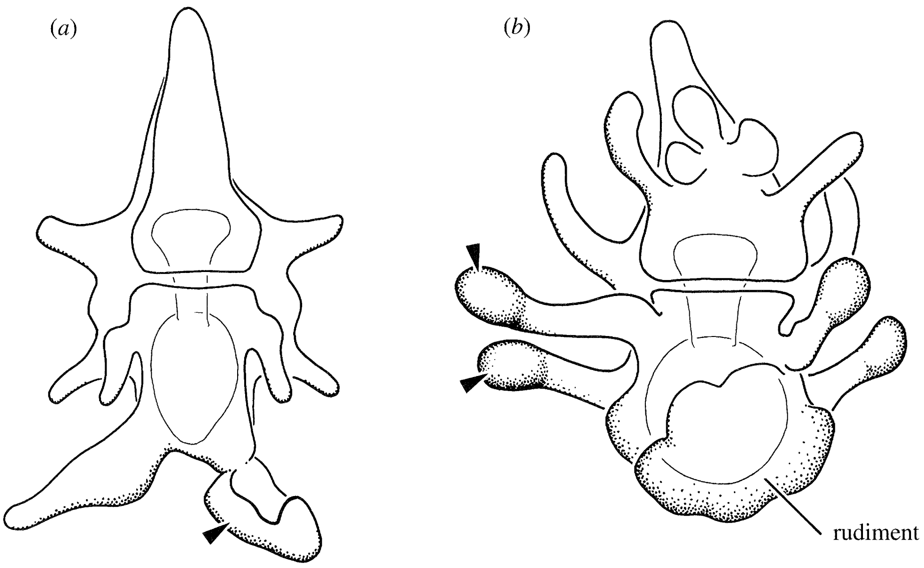

Figure 24. Asexual budding in oceanic bipinnaria larvae, from Jaeckle (1994). (*a*) A *Luidia* larva, with a secondary larva (arrow) forming from one of the posterolateral lobes. (*b*) An unidentified species, with rudimentary embryos forming at the swollen tips of the postoral and posterodorsal lobes (arrows). These detach and develop independently in the plankton.

strengthening the band to improve its load-bearing capabilities. If this association remained intact while the ciliary band was transformed into a nerve cord, the cord would be expected also to be capable of providing a degree of structural support. For example, the incipient nerve cord shown in figure 23*b* is supplied with a complement of subtrochal cells on each side. This would allow it, in principle, to resist side-to-side bending, even in the absence of a notochord. The notochord could, in fact, have evolved quite late as a means of providing greater support, with the evolution of a more powerful musculature than was possible using the nerve cord alone. Very little is known about how the notochord actually originated, but in *Amphioxus* the notochordal cells are modified muscle and receive their own innervation (Flood *et al.* 1969). In vertebrates the default state for notochord tissue, in the absence of key genes, is also muscle (Talbot *et al.* 1995). In sea urchin embryos, the homologue of the gene most characteristic of vertebrate notochord, *Brachyury*, is expressed in secondary mesenchyme (Harada *et al.* 1995). Secondary mesenchyme derivatives in the bipinnaria include medial structures such as the dorsal muscles, the coelom and mesenchyme cells. Any or all of these could be notochord homologues.

(*c*) ***Vertebrate somites and somitomeres***

The mesodermal repeats (somites) in *Amphioxus* are all similar enough that they are generally considered as a single series of essentially equivalent elements. This is clearly not true of the repeating elements in vertebrates, which are of two different types, cranial somitomeres (head cavities in some lower vertebrates) and postcranial somites. The former are transient metameres formed by paraxial mesoderm in the head. A similar but not strictly equivalent metameric pattern is observed in the adjacent nerve cord (rhombomeres), in the pattern of outgrowth of cranial nerves, and in the neural crest and epidermal placodes (Jacobson 1988; Hunt *et al.* 1991; Gilland & Baker 1993). The postcranial mesoderm, in contrast, forms somites. Somites are persistent structures, and each gives rise to a characteristic array of muscle elements, dermis, skeleton, coelom, excretory tubules and, in some cases,

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(Lacalli & West 1993), but their function is unknown. The subtrochal cells lie beneath the band. After neurulation, the ciliary band cells and subtrochal mesenchyme would be positioned as shown in (*b*). Since sensory cells occur both in the band and nearby in the oral field, it is not obvious whether they should be located inside the neural tube or outside it, as shown. For comparison, (*c*) shows how the nerve cord, notochord and myotomes are arranged in an *Amphioxus* larva, traced from 8–14 d larvae (see Lacalli *et al.* 1994). The basement membrane is indicated by dark shading, and mesodermal and ectodermal derivatives by progressively lighter shading. The main motor neurons (mn) at this stage lie in the ventral part of the cord, adjacent to the floorplate; their fibres enter the neuropile (np), and form axial nerve tracts that synapse with adjacent muscle cell processes (arrows) attached to the surface of the cord. The association between the myotomes and nerve cord in *Amphioxus* thus resembles, at least superficially, the subtrochal cell/ciliary band association in (*b*). Interneurons (int) in *Amphioxus* larvae are generally located in the dorsal and dorsolateral parts of the cord; many have long transluminal processes (Lacalli & West 1993), so they resemble the multipolar cells in (*b*) in both morphology and relative position. Sensory cells are not shown in (*c*); columns of putative sensory cells occur both within the cord (dorsal bipolar cells, Bone 1959) and in the adjacent epithelium (T.C.L., unpublished results), but the early development and basic features of the sensory system are poorly understood.

gonadal tissue. The adjacent nerve cord is evidently not intrinsically metameric, but becomes so under the influence of the somites (Stern *et al.* 1991; Wilkinson 1993). Comparative anatomists have made various attempts to devise a single basic segmental plan that would combine the somitomeres, rhombomeres and somites in a single series with all elements in register. Variation in the numbers of elements and overlaps in the innervation patterns, mainly in the cranial region, and various positional shifts, make this difficult. Nor is it easy to derive either one of the series in simple fashion by extension of the other (Northcutt 1993).

The two important features of vertebrate segmentation in the present context are that (i) the anterior and posterior series (somitomeres and somites) are qualitatively different, and (ii) the posterior series forms extra structures not represented in the head. In the bipinnaria, the principal repeating structures are the larval lobes and their supporting mesoderm, and here also there is evidence for a qualitative difference between the anterior and posterior members of the series. This comes from recent observations on asexual reproduction by budding in oceanic bipinnaria and brachiolaria larvae, some of unknown species (Bosch *et al.* 1989; Jaeckle 1994). Figure 24 illustrates two examples: buds are formed either on the posterolateral lobes (figure 24*a*) or on the postoral and posterodorsal lobes (figure 24*b*). In the former case, the lobes expand and form secondary larvae that develop to an early bipinnaria stage before separating from the parent larva. In the latter, the buds separate very early from the tips of lobes and develop as free-swimming blastulae that develop further in normal fashion. Thus, on current evidence, posterior lobes bud, whereas anterior ones do not.

Assuming that the buds grow normally to settlement and adulthood, each must eventually generate a complete set of adult structures, including excretory organs, gonads and a nervous system. If, at some point in evolution, a species that propagated in this fashion was to suppress the asexual phase, lobes previously involved in the propagative process might still retain the capacity to differentiate structures derived from each propagule. Some of these might then become incorporated into the body of the parent larva, as part of whatever vestige remained of each lobe. They would form a series if, in addition, the lobes were originally generated in series, for example, by a posterior growth zone of some type. This would give an extended posterior series of structures, each of which, like a somite, would produce a characteristic assemblage of subsidiary structures. Complex life histories and propagative stolons of various types occur widely in tunicates, which suggests a period of active experimentation with different modes of asexual propagation occurred early in chordate evolution. If so, it is possible that the vertebrate trunk, with its extended series of repeating elements, arose as a by-product of this experimentation, which would make it, in effect, a modified reproductive stolon.

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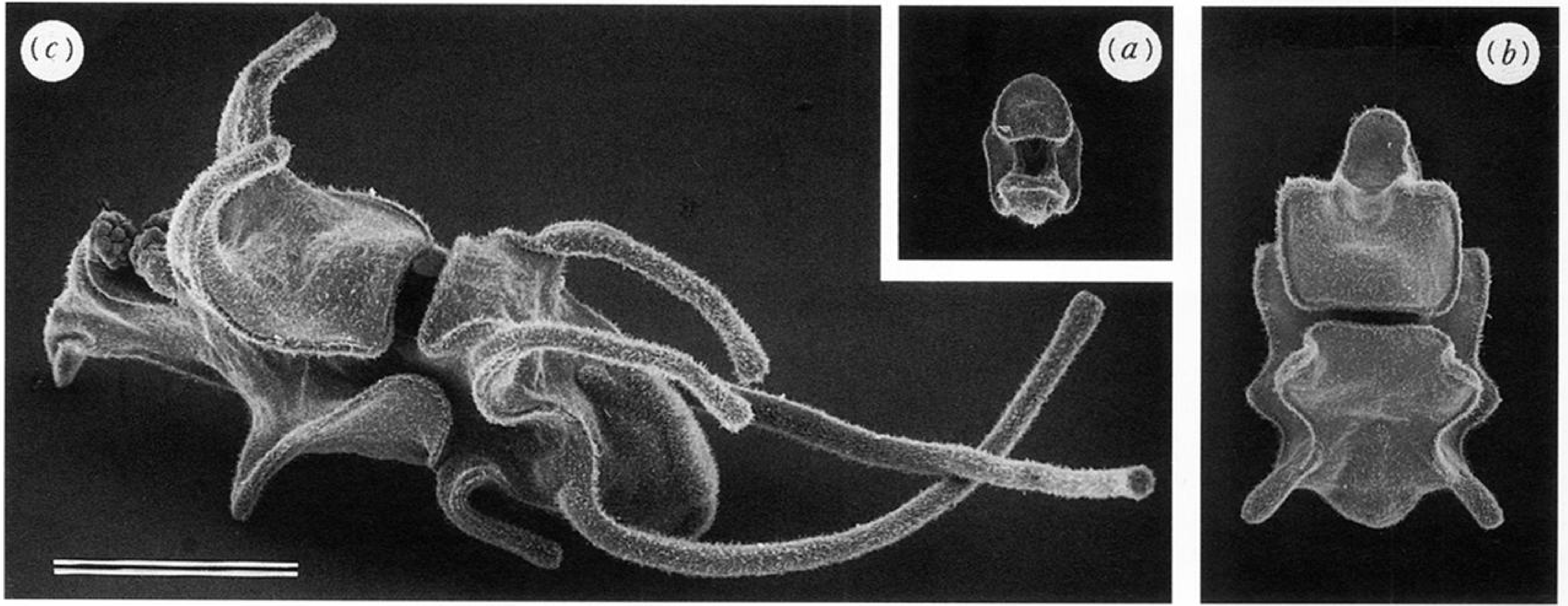


Figure 1. *P. ochcraceus* larvae, a range of stages all to the same scale. (*a*) A young larva at 8 d. (*b*) A 20 d bipinnaria at the beginning of the period of lobe growth. (*c*) A 40 d brachiolaria from a culture in which rudiment development was delayed; the lobes have nevertheless continued to grow. Specimens prepared by T. H. J. Gilmour, see Gilmour (1988). Scale bar 500 µm.

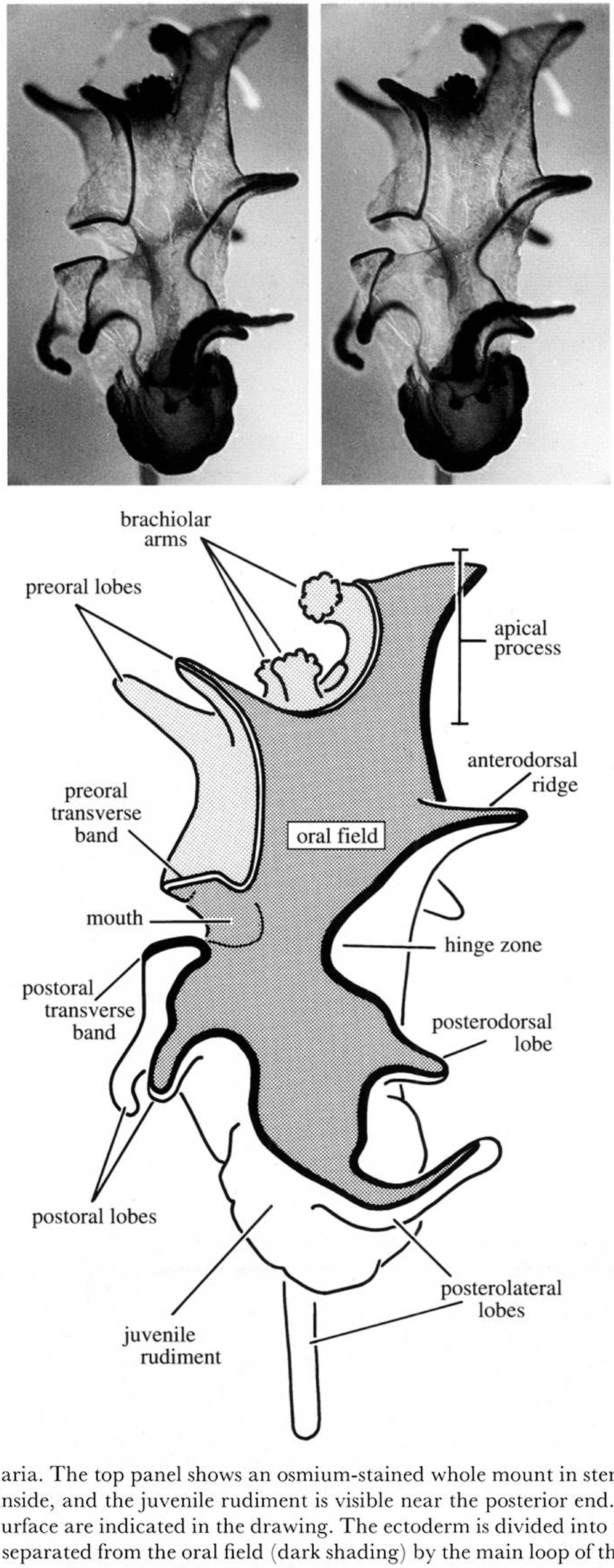
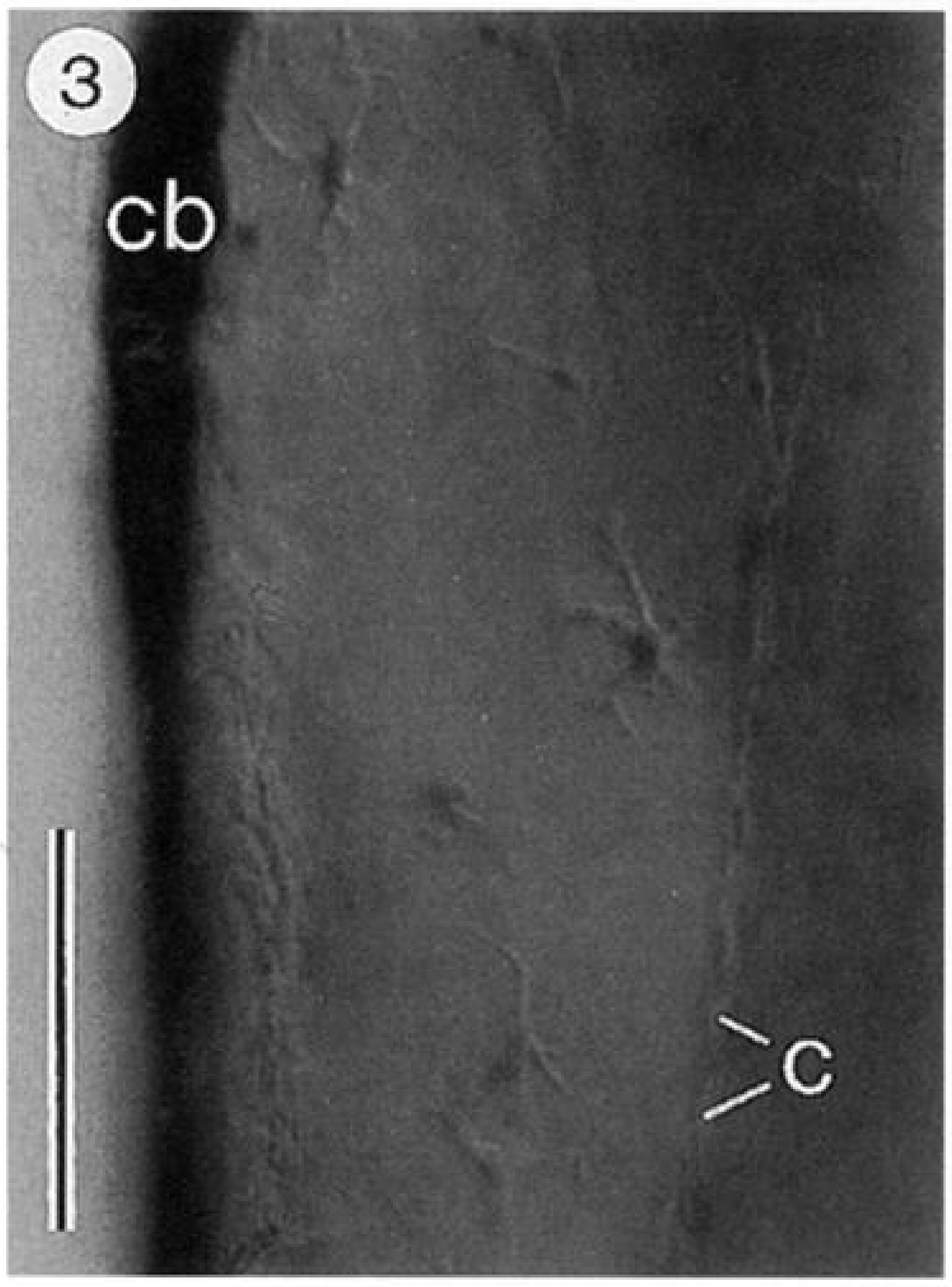
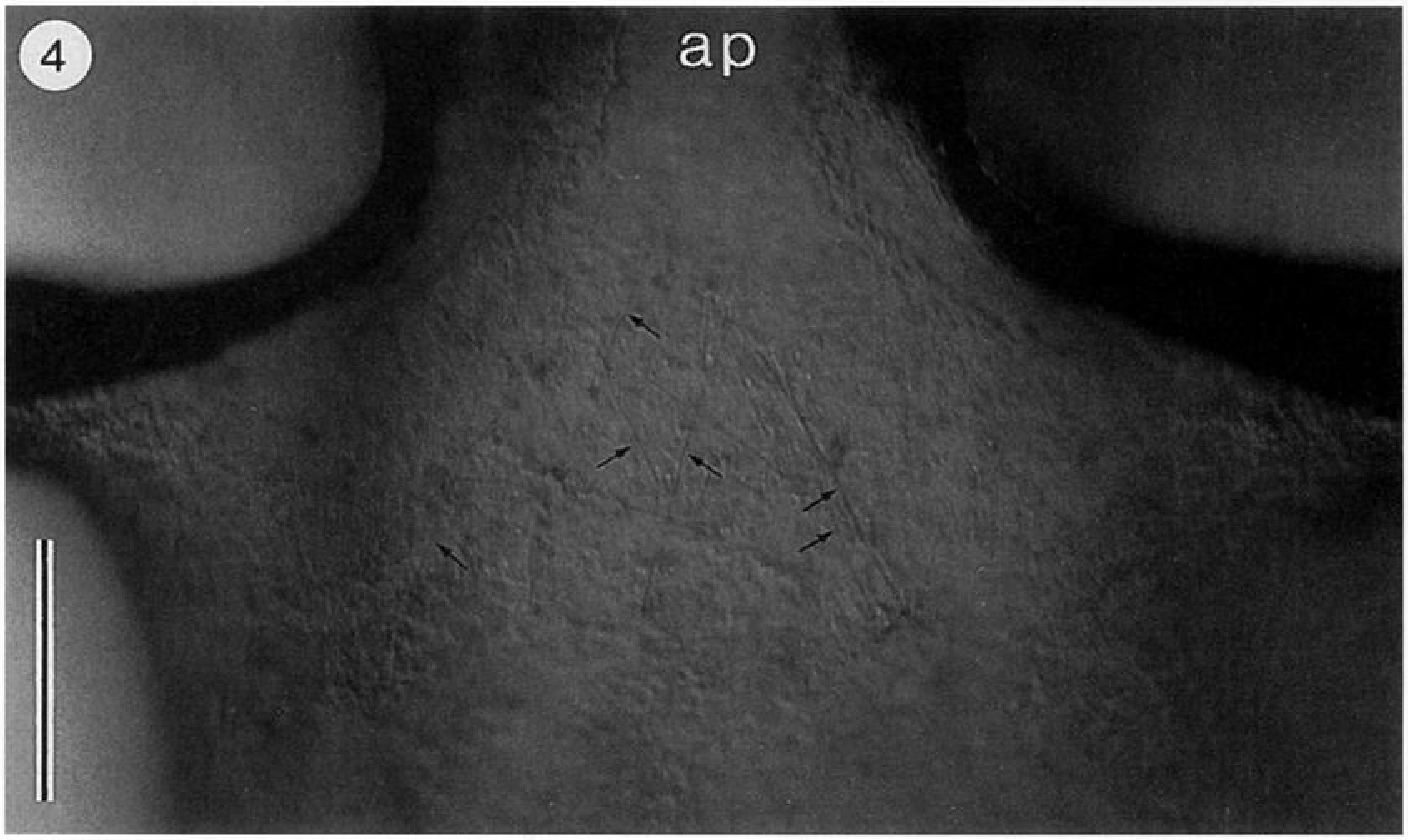


Figure 2. A 30 d brachiolaria. The top panel shows an osmium-stained whole mount in stereo; the surfaces of the gut and coelom can be seen inside, and the juvenile rudiment is visible near the posterior end. The main structures and subdivisions of the body surface are indicated in the drawing. The ectoderm is divided into three domains: the aboral epithelium (unshaded) is separated from the oral field (dark shading) by the main loop of the ciliary band (solid line), which runs dorsolaterally along both sides of the body, from the apex to the posterior end, then forward ventrally to the postoral region, where the two sides are joined by the postoral transverse band. A separate preoral loop of ciliary band (doubled line) encloses the ventral preoral field (light shading) and separates it from the oral field.





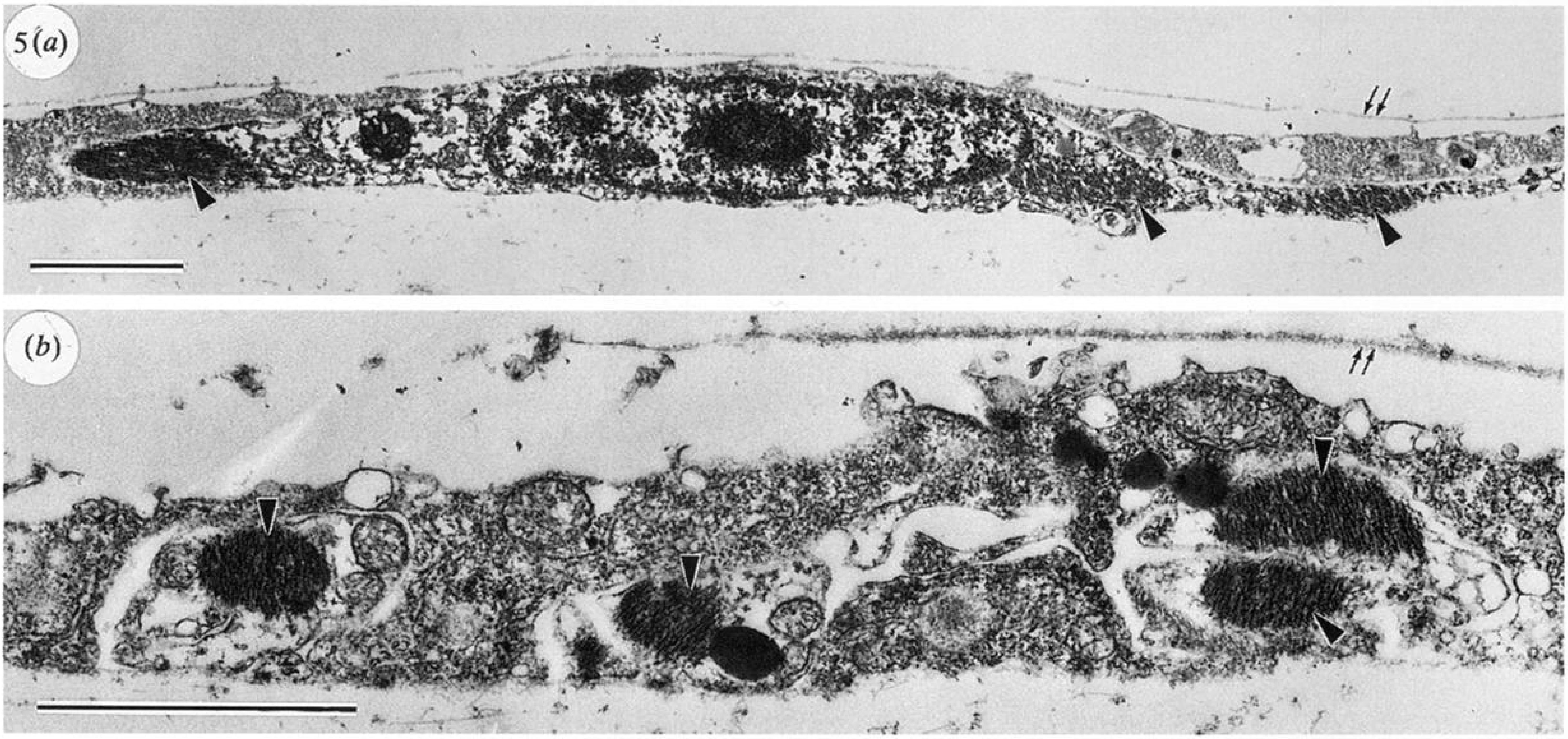


Figure 3. Typical stellate mesenchyme cells in a whole mount, spanning the space between the ectoderm and ciliary band (cb) and the coelomic epithelium (c). Scale bar 100 μm.

Figure 4. Fibres (arrow) belonging to the dorsal muscle cells. This is a dorsal view of a 30 d larva just below the apical process (ap) at the level of the anterodorsal ridges, which are visible on each side. Scale bar 100 μm.

Figure 5. Sections of the dorsal epithelium showing dorsal muscle cells; they are oriented with the external, cuticular surface (small double arrows) facing up. (*a*) The cell body and nucleus of one such cell, which contains myofibril bundles (arrowheads). (*b*) Individual muscle fibres (arrowheads) enclosed within the epithelium. The basal lamina is very delicate; only scattered bits of fibrous material are visible in this section. Scale bars 2 μm.

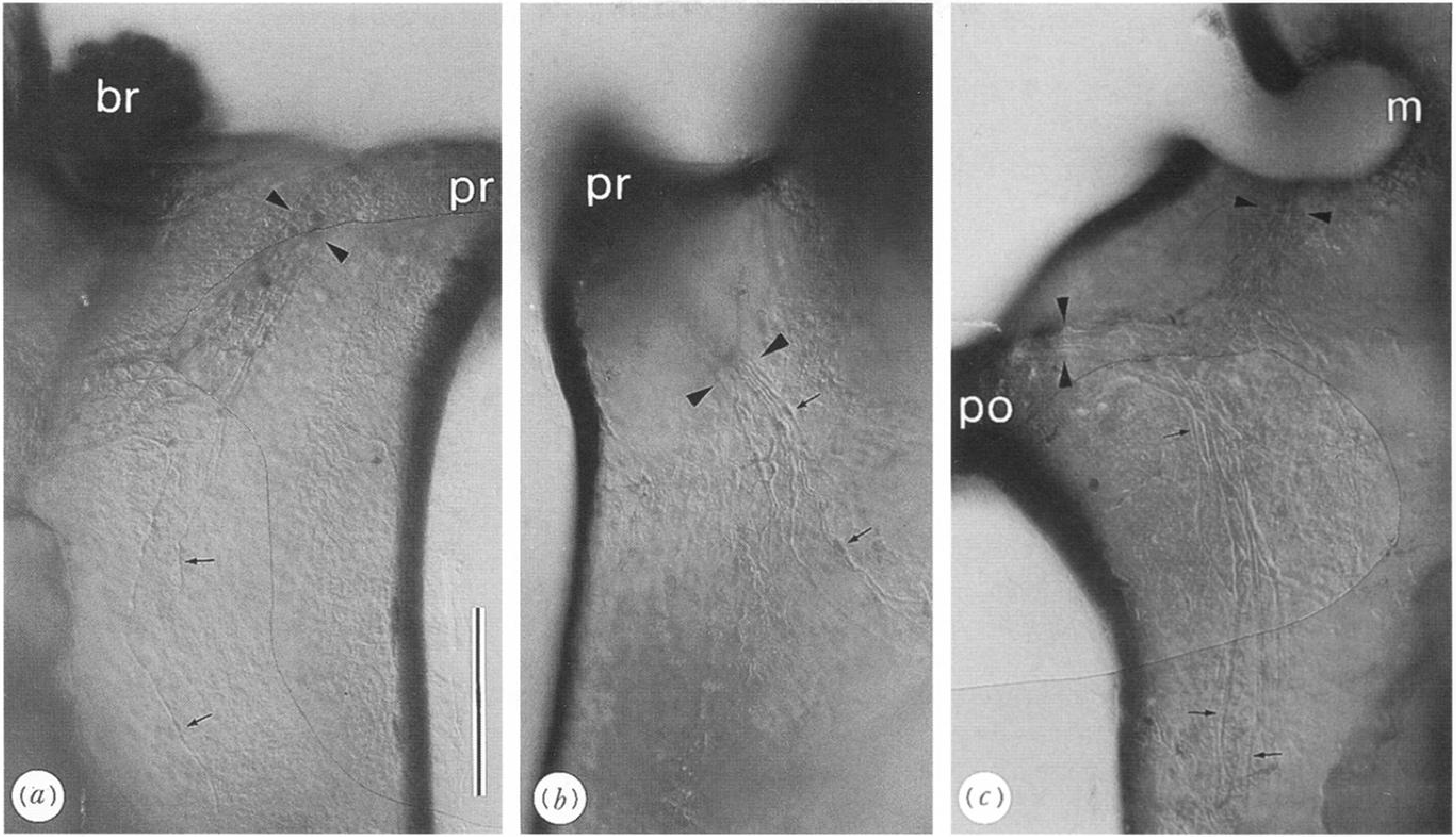
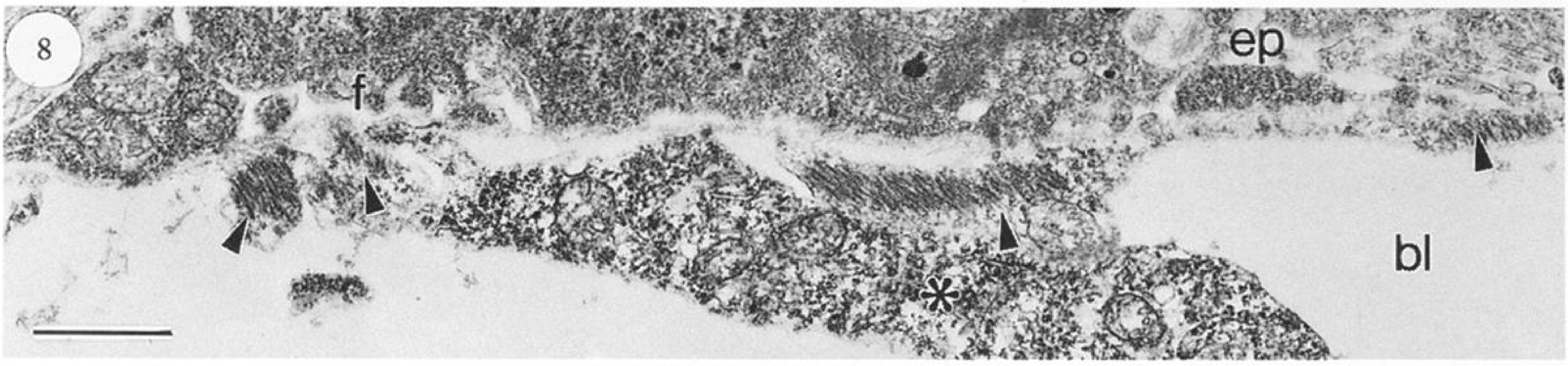


Figure 6. Muscle bands supplying the lobes in 25 to 30 d larvae. The bands are typically narrowest at the bases of the lobes (between arrowheads) and then splay out across the oral field (small arrows mark individual fibres). (*a*) A side view of the oral field at the base of a preoral (pr) lobe; one of the lower brachiolar arms (br) is visible. (*b*) Ventral view of the right side of the preoral field showing fibres associated with the right preoral lobe, out of focus in this view. (*c*) Side view of the oral field below the mouth (m) showing fibres associated with the postoral (po) lobe. Scale bar 100 μm.



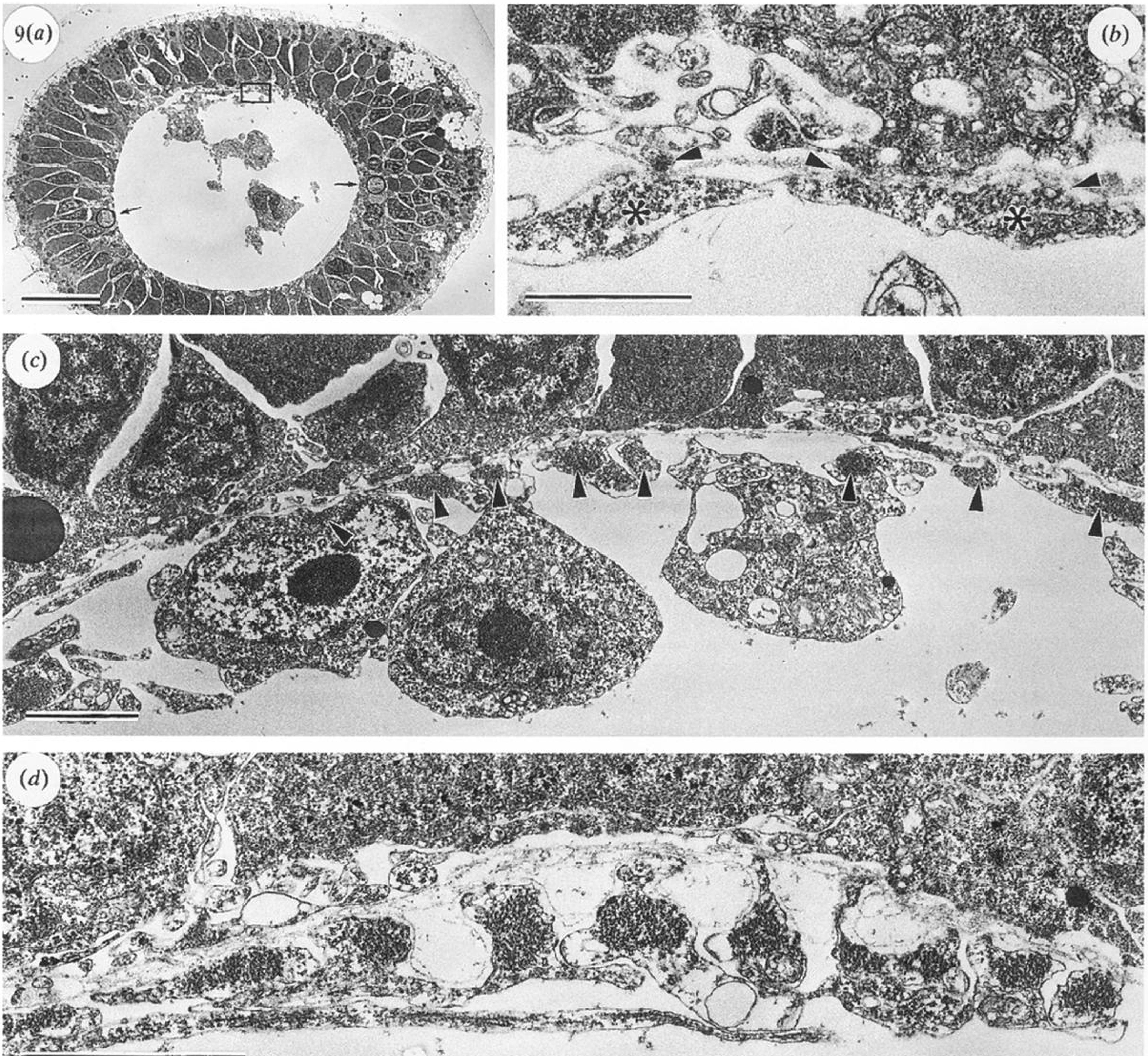


Figure 8. Typical zone of muscle attachment to the epithelium (ep) of the oral field near the ciliary band. Facing the blastocoel (bl) are parts of one muscle cell (*) and four individual fibres (arrows). The muscle cells attach to the basal lamina along the underside of the epithelial cells and to their basal processes; examples of the latter are marked (f). Scale bar 1 μm.

Figure 9. Transverse sections through the posterolateral lobe of a 25 d larva to show the muscle band; all are oriented with the oral field up. (*a*) A section near the lobe tip; scattered mesenchyme cells are visible in the blastocoel with, at the top, flattened terminal processes belonging to several oral field muscle cells. The ciliary nerves lie at either side (circled, small arrows). Scale bar 10 μm. (*b*) A detail showing the boxed region in (*a*), where the terminal portions of an oral field muscle cell (*) attaches to the epithelium. Contacts are formed with individual basal processes (left arrow) and along the undersides of the epithelial cells (between the two arrows on the right). Scale bar 1 μm. (*c*) A section of the muscle band about midway down the lobe. There are eight muscle fibres (arrows) at this point, and the cell bodies also belong to muscle cells. Scale bar 2 μm. (*d*) Muscle band at the base of the lobe, which is its narrowest point. Scale bar 2 μm.

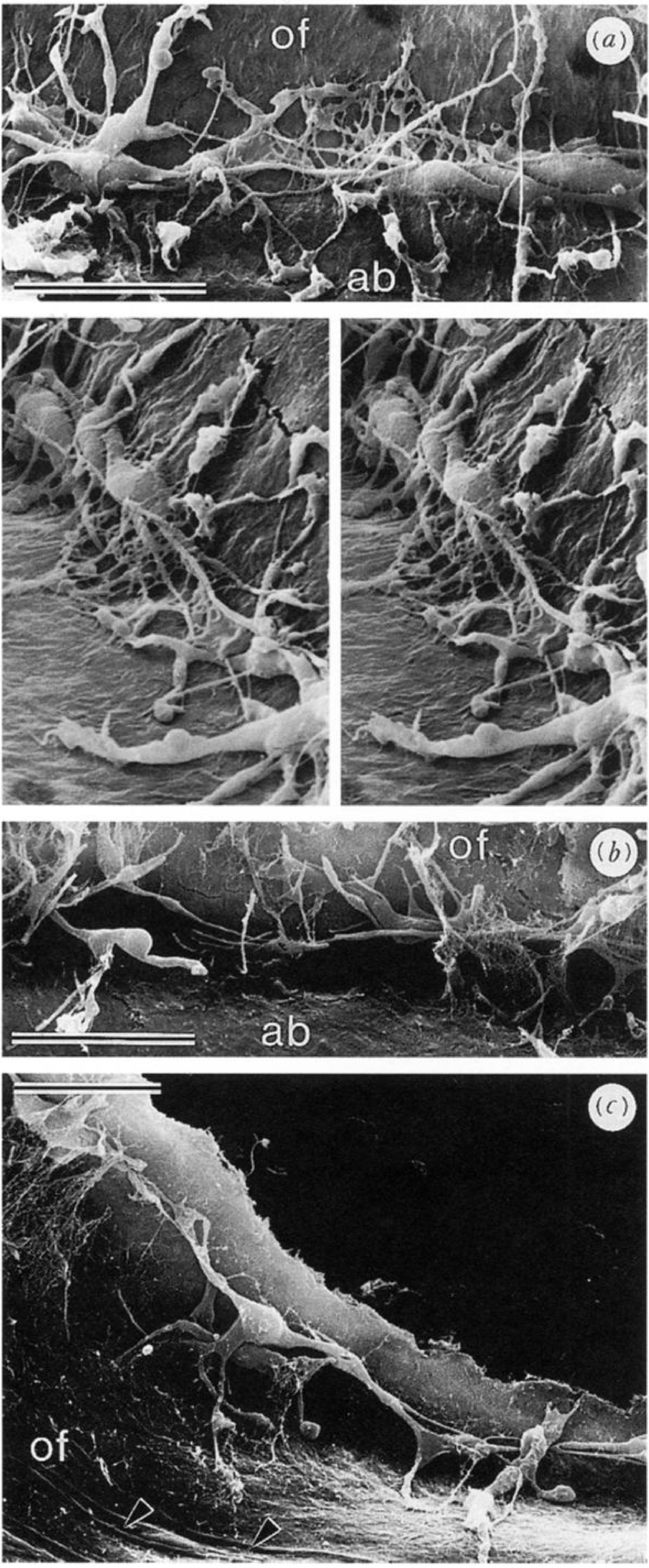
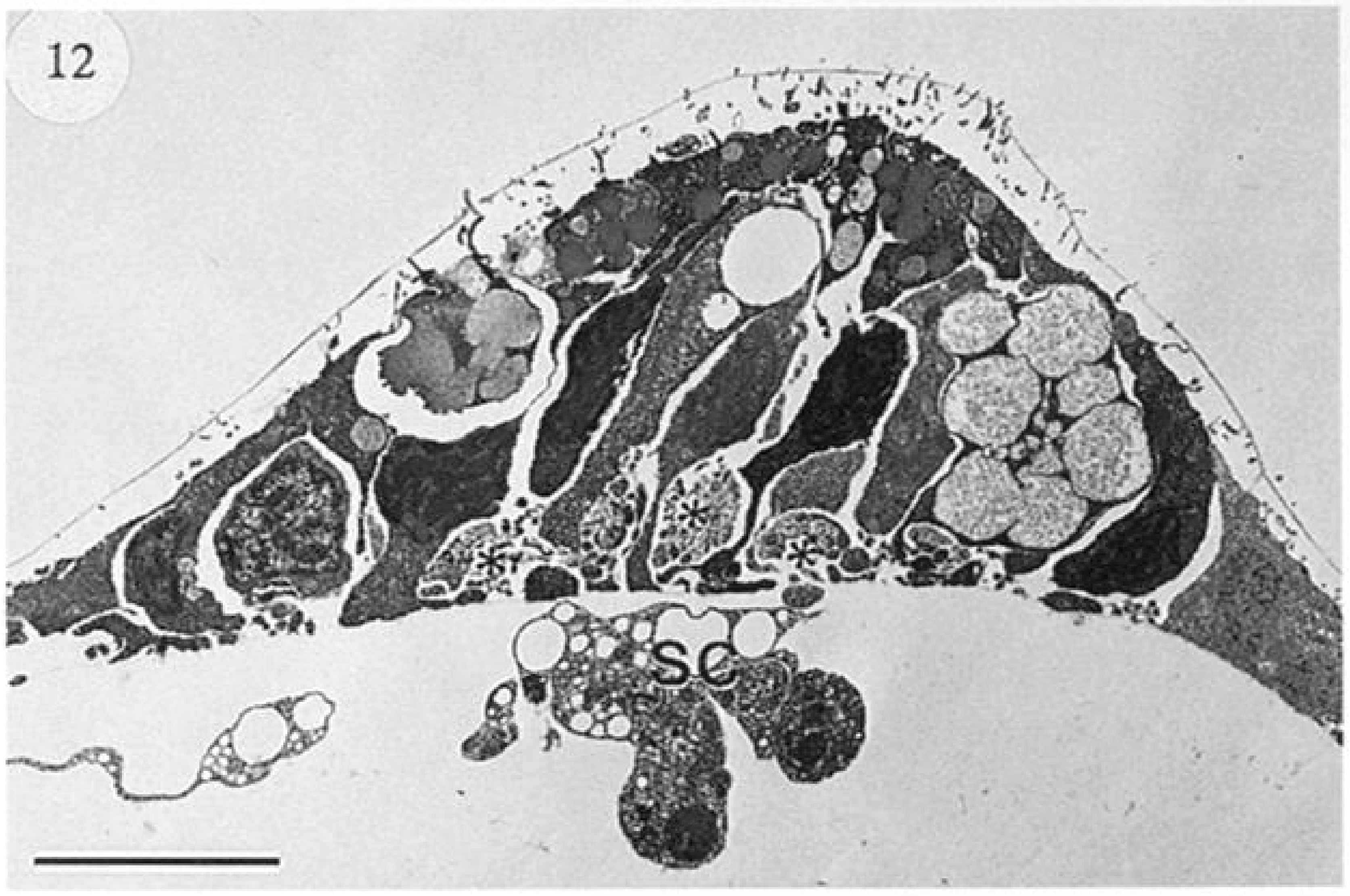
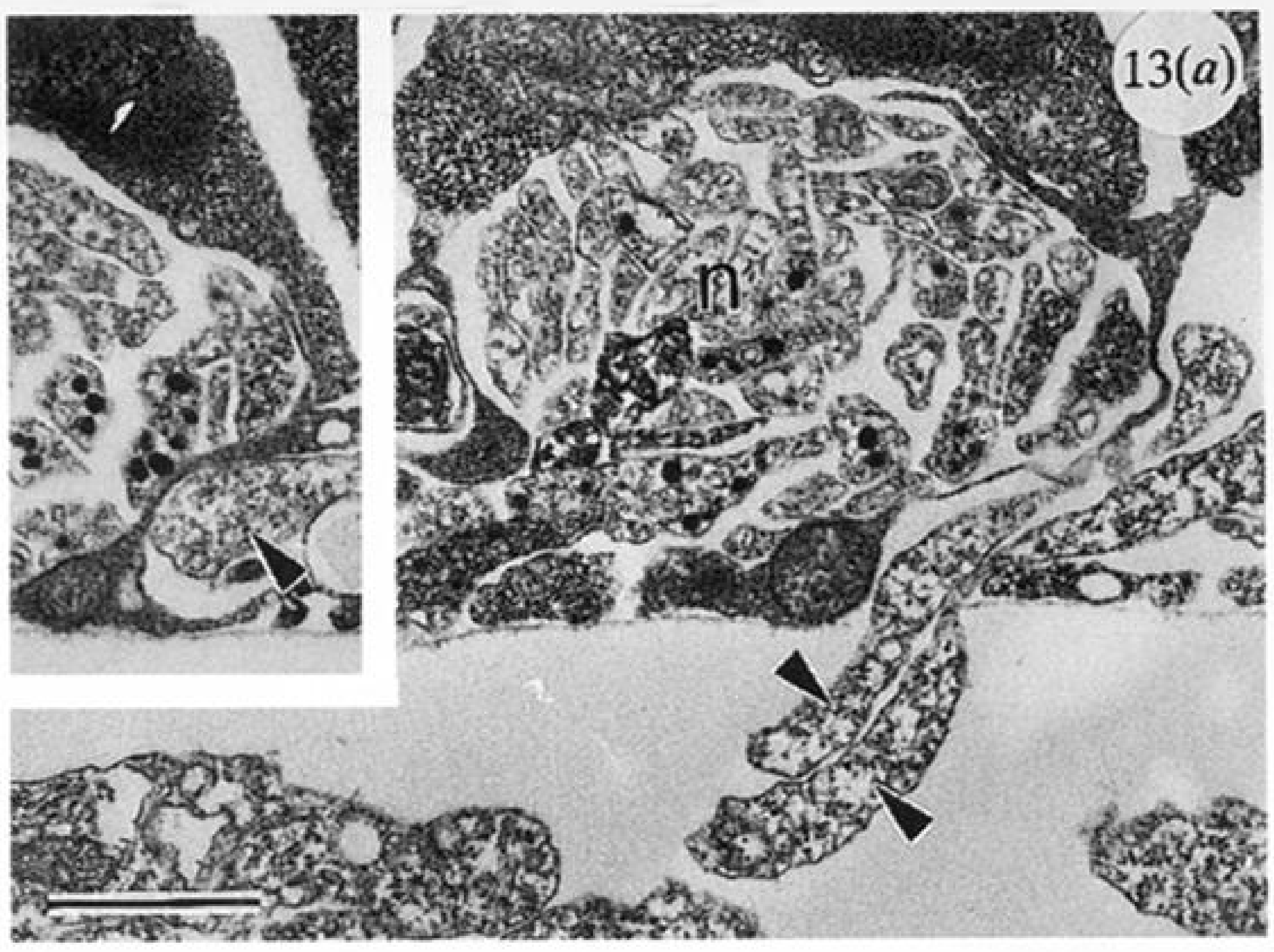
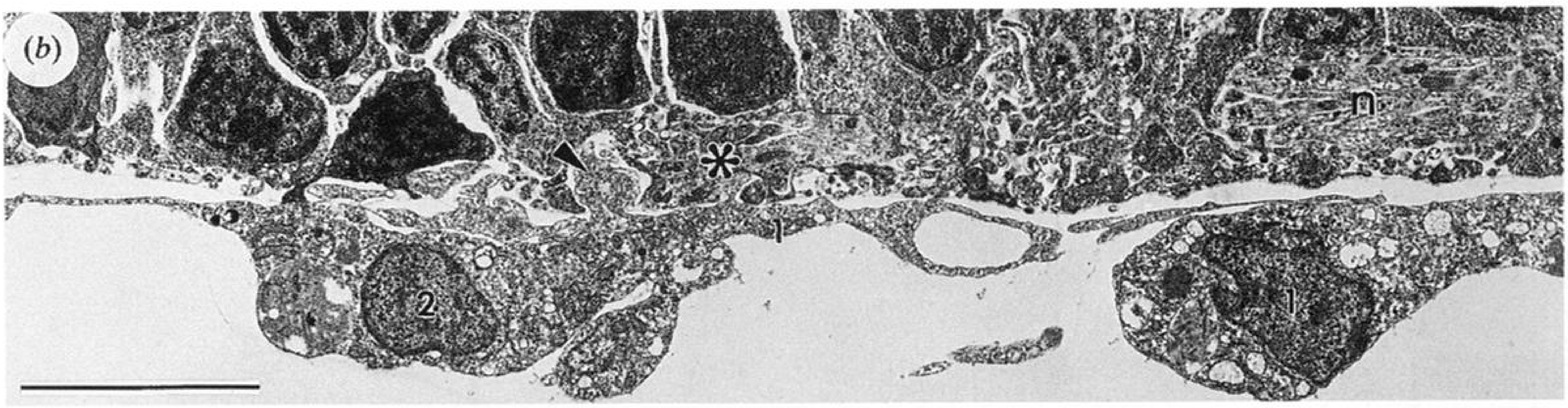
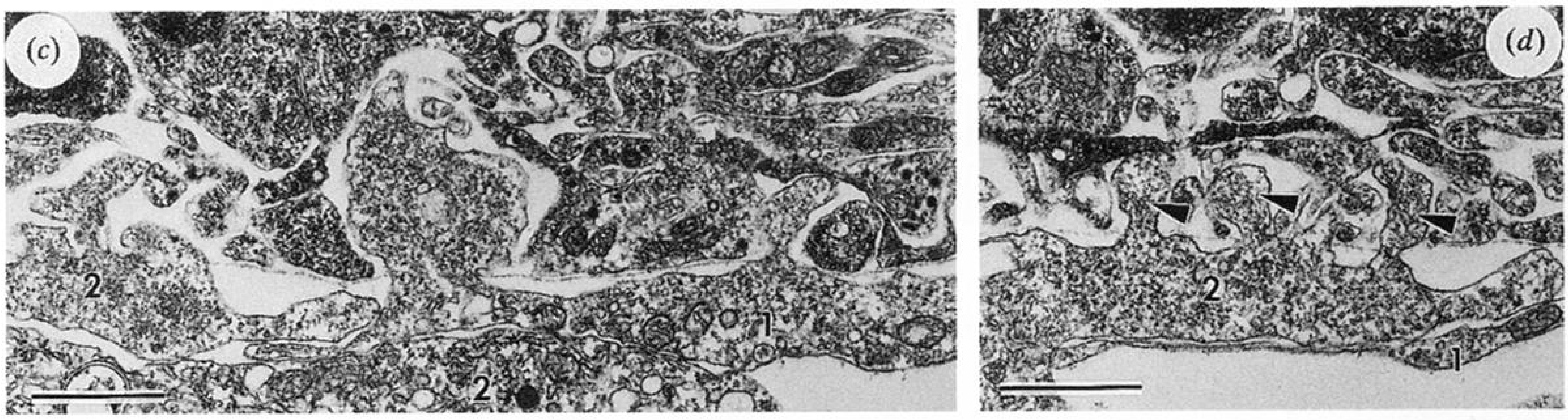


Figure 10. Subtrochal cells in SEM images of the inside surface of fractured 25–30 d larvae. The cells run along the underside of the band, which separates the oral field (of) from the aboral surface (ab). (*a*) The top panel shows a face–on view of the inside surface of the anterodorsal ridge, approximately the same region shown in figure 13*b*–*d* and the three-dimensional reconstructions. The lower panels show the same cells in stereo, as seen from the left of the top panel; the aboral surface is inclined upward on the right in the stereo view. (*b*) Subtrochal cells along the main band near the base of the apical process. (*c*) Similar cells along the main band in the hinge zone; the aboral epithelium has torn away along the curved margin where it meets the band. Note the parallel fibres of the oral field muscles (arrows). Scale bars 20 μm.









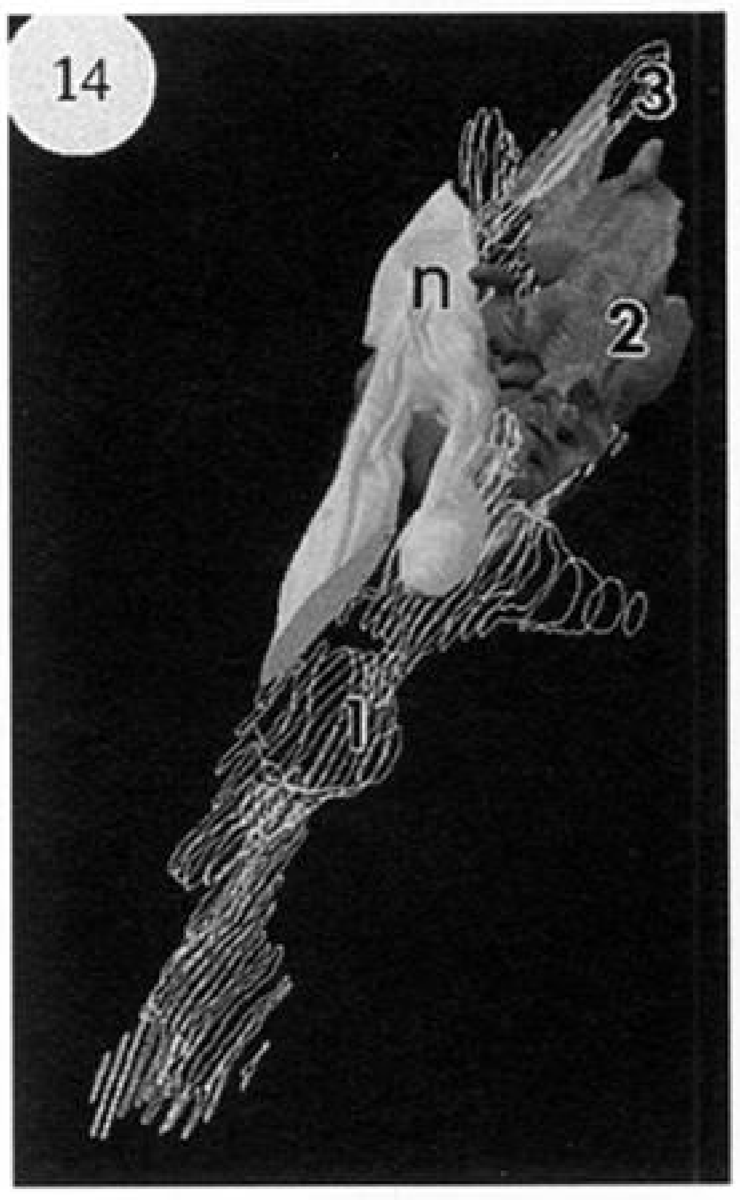


Figure 12. Transverse section through the ciliary band showing the subtrochal cells (sc) associated with it, and the ciliary nerve, which is broken into several subsidiary tracts (*) at this point. The oral field is to the left; the aboral surface is to the right. Scale bar 5 μm.

Figure 13. Subtrochal cell attachments and insertions. Scale bars 1 μm except in (*b*). (*a*) Transverse views of two subtrochal cell processes (arrows) crossing the basal lamina near the ciliary nerve (n). One of these terminates adjacent to the nerve (arrow in inset) in close proximity to a vesicle-filled terminal. (*b*) A section along the band in the anterodorsal ridge area, and two of the subtrochal cells from the reconstruction, indicated by number. Cell 1 forms an insertion (arrow) that penetrates adjacent to a branch (*) of the ciliary nerve (n). Scale bar 5 μm. (*c*) The insertion from (*b*). (*d*) A nearby section with multiple small insertions (arrows); note adjacent neurites and the closely apposed processes of cells 1 and 2. (*e*) A transverse section through the band where the nerve (n) has three subsidiary branches (*). A large insertion (arrow) surrounds the smallest of these, on the oral side of the ciliary band.

Figure 14. Reconstruction of the two subtrochal cells shown in figure 13*b* (cells 1 and 2), with part of a third cell; a key for interpreting figure 15. The position of the ciliary nerve (n) is shown, including one branch; smaller branches also occur but are not shown.

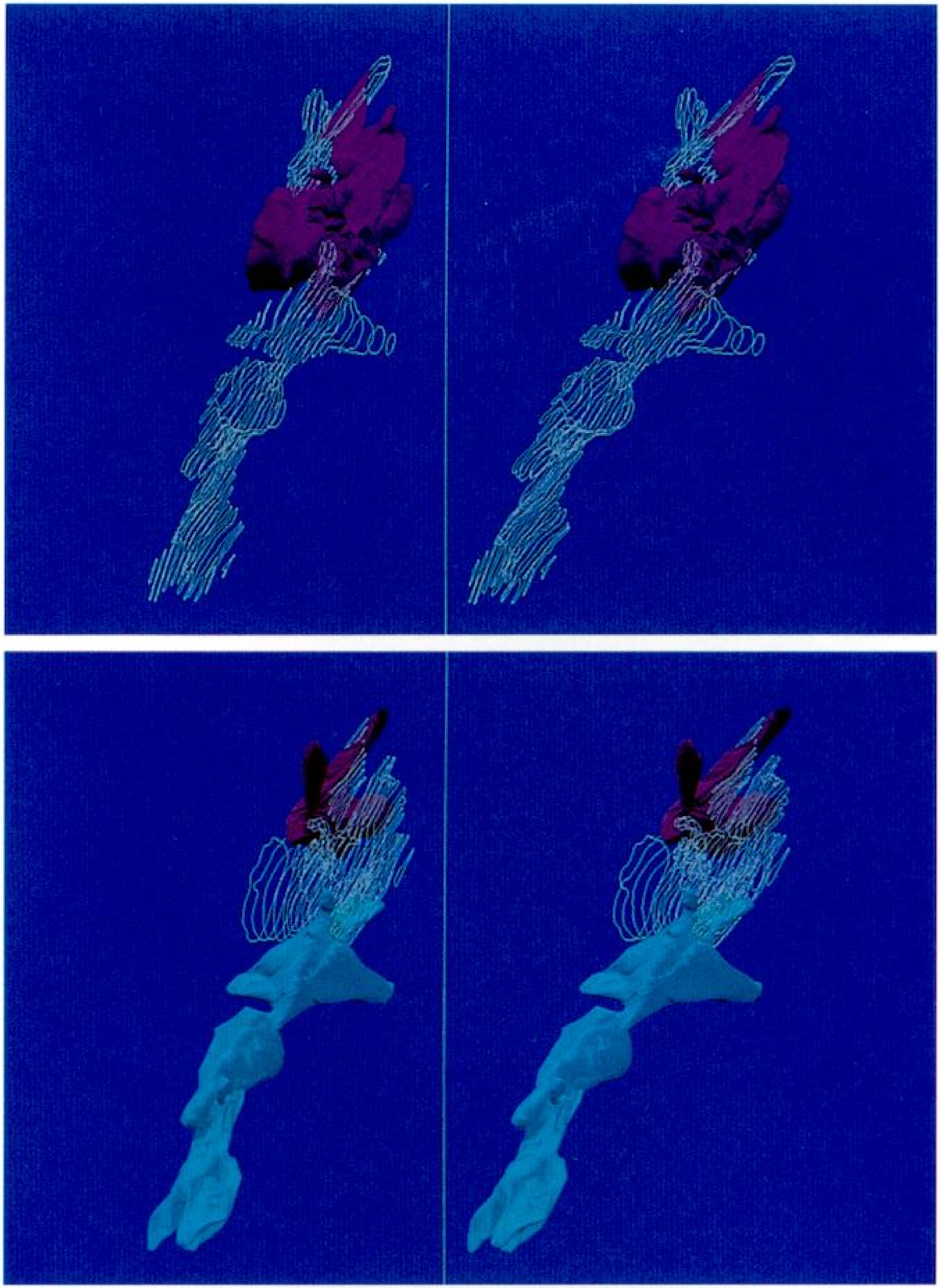


Figure 15. Stereo views of the cells in figure 14. Cells 1 and 3 are shown in outline in the top panel, and cell 2 is solid; this is reversed in the bottom panel, to show how the flattened processes from adjacent cells interleave. The insertions project upward in this view.

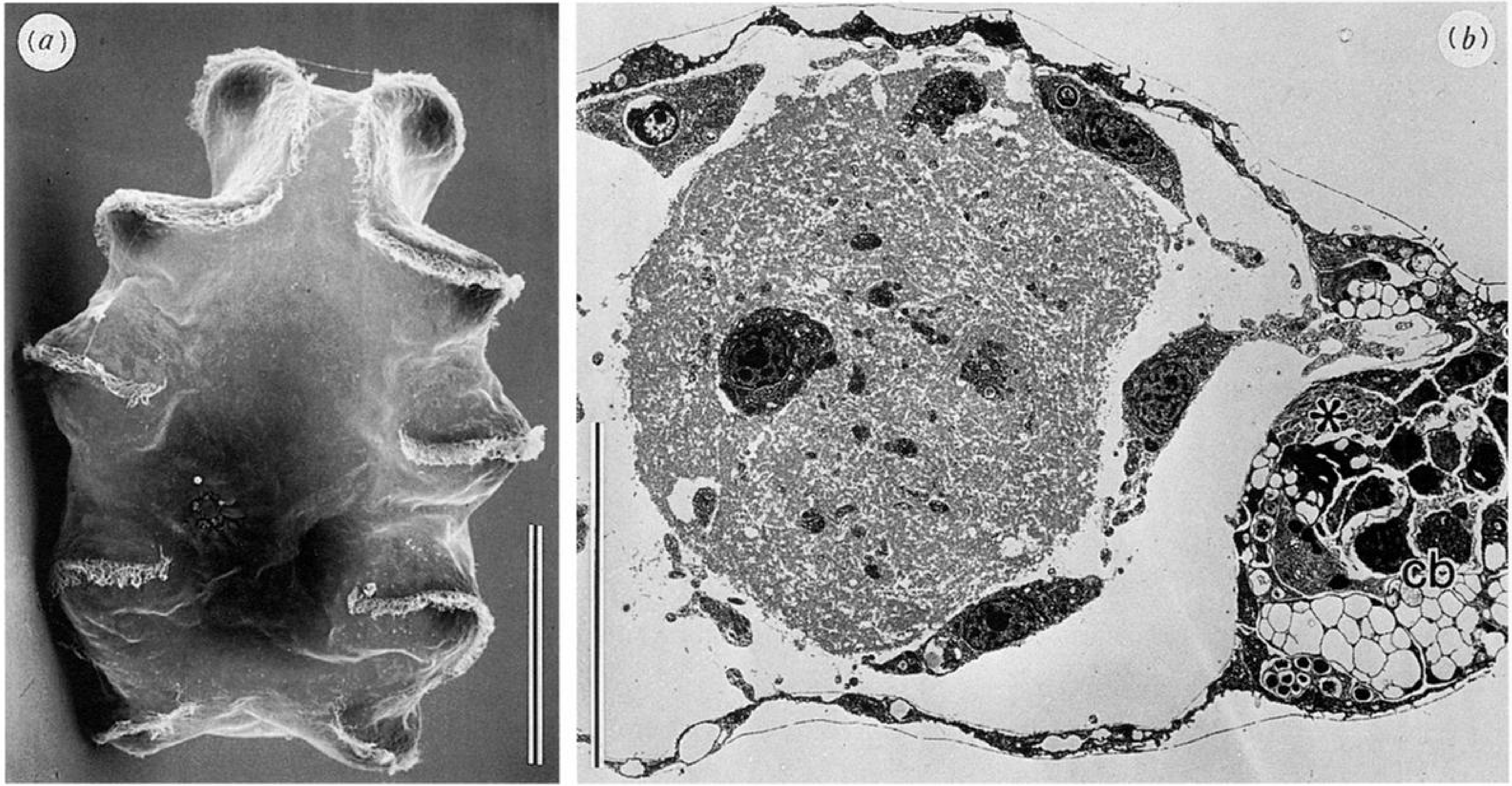


Figure 20. Ridge elements in the auricularia larva of the holothurian *Stichopus californicus* (for methods, see Lacalli 1993). (*a*) Dorsal view of a larva beginning metamorphosis. The band fragments during this process, but the ridges persist and form a regular dorsolateral series. The hyaline spheres are visible as dark spots under each ridge. Scale bar 200 µm. (*b*) A hyaline sphere in section; showing the mesenchyme cells that surround it (others are embedded inside), the adjacent ciliary band (cb), and the ciliary nerve (*). Scale bar 20 µm.