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Ultrastructure of the osphradium of the Tertiary relict snail, *Campanile symbolicum* Iredale (Mollusca, Streptoneura)

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SUMMARY

The osphradium of *Campanile symbolicum* Iredale, 1917 is a gill-like, bipectinate sense organ, which is located at the left side of the mantle roof. The mass of the deeply clefted sensory epithelium of the leaflets is built up by sensory cells, which are provided with deeply invaginated aberrant cilia and large cytosomes containing pigment formations. In addition, many free nerve processes are present, bearing a single or few sensory cilia with accessory centrioles. Polyciliary cells are interspersed. A cell type with net-like or concentrically arranged smooth endoplasmic reticulum is commonly found near the central axis of the osphradium. The central zone of each leaflet includes nervous tissue and a complicated muscular grid, with pore cells and fibroblasts also present.

Based on the fine-structural data the functional and ecological significance of the osphradium of *Campanile symbolicum* is discussed. The combination of herbivory and a lamellar osphradium is rare among the Gastropoda, suggesting that the osphradium of *Campanile* might also be involved in reproductive biology.

Many fine-structural features of the osphradium of *Campanile symbolicum* are unique among the gastropods and reflect the phylogenetic isolation of this relict snail. The net-like cell type, however, is probably homologous with the so-called Si4 cell in the remaining caenogastropods, for which a largely different osphradial fine-structure is diagnostic. The affinities of *Campanile symbolicum* are probably closer to the Caenogastropoda than to the Allogastropoda and Euthyneura. With present knowledge it might be best classified near the base or even as the first clade within the Caenogastropoda.

1. INTRODUCTION

The large (up to 25 cm), cerithioid-like streptoneuran gastropod *Campanile symbolicum* Iredale, 1917 is regarded as a 'living fossil', being the only extant member of its family (Campanilidae Douville 1904), which was a species-rich group in the Tethys Sea (Houbrick 1981, 1984). The species is a subtidal, shallow water dweller and is quite common in Western Australia.

The anatomy of *Campanile symbolicum* was briefly studied by Quoy & Gaimard (1834), in more detail by Bouvier (1887) and most recently described in detail by Houbrick (1981). The last author noted the differences between *Campanile symbolicum* and other cerithioids and classified the species in its own family, which was originally erected for the fossils.

Because of certain similarities in the pallial roof, genital system, 'opisthobranch-like' (Houbrick 1981) spawn, and of the osphradium, I have proposed a closer relationship between *Campanile* and the Architectonicoidea, the latter being regarded as connecting links between the streptoneuran and the euthyneuran level of gastropod organization (Robertson 1974, 1985; Haszprunar 1985*b–e*, 1988*a*,*b*; Salvini-Plawen & Haszprunar 1987; Ponder & Warén 1988). This view was in particular supported by the assumed presence of campanilid chalazae, egg-connecting strings which are otherwise characteristic of Architectonicoidea, Pyramidelloidea and primitive Euthyneura.

The fine-structural studies by Healy (1986) on eu- and paraspermatozoa of *Campanile symbolicum* revealed a new data-field to be considered. Whereas the paraspermatozoa are of the type occurring in the Architaenioglossa and Cerithioidea, the structure of the euspermatozoa is highly unusual and again reflects an isolated position of *Campanile*. Healy (1986) concluded 'that the Campanilidae probably diverged at an early stage from the primitive cerithiacean stock'. The same conclusion was reached by Ponder & Warén (1988), who doubted the proposed transition from a campanilid-like ancestor to the early heterobranchs and classified *Campanile* within the Cerithioidea, whereas Houbrick (1988) stated in his cladistic analysis of the Cerithioidea: 'The Campanilidae . . . may also be considered as an outgroup'. Indeed, the assumed synapomorphies between Campanilidae and the remaining Cerithioidea may be due to convergence or are but symplesiomorphies (see Discussion).

Recently Houbrick (1989) has added several new and interesting data on *Campanile symbolicum*, in particular concerning the alimentary and excretory

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system. He emphasized that the campanilid chalazae connect egg-capsules with 1–3 eggs, thus differing from the allogastropod and euthyneuran chalazae, which always interconnect single eggs. Thus, the formerly assumed affinity of *Campanile* with the allogastropod–euthyneuran level of organization (Haszprunar 1985*e*, 1988*a*,*b*; Salvini-Plawen & Haszprunar 1987) is weakened. On the other hand, Houbrick (1989) also concludes that 'the Campanilidae should no longer be considered as cerithioidean gastropods'.

As outlined in detail by Haszprunar (1985*a*,*b*, 1986) the fine-structure of the Caenogastropoda (including the Cerithioidea) osphradium is diagnostic: Largely unaffected by different habitat (marine, freshwater, terrestrial) and habit (herbivorous, carnivorous, filter-feeders, parasites) the osphradial epithelium of Caenogastropoda is characterized by the presence of three specialized cell-types (so-called Si1, Si2, and Si4 cells) in constant mutual position. Lamellar osphradia of cerithioideans are further characterized in that the Si1–Si2 zone is restricted to the main axis of the osphradium, whereas this zone extends towards the osphradial leaflets in the remaining caenogastropods with lamellar osphradium. In contrast, the Architectonicoidea have a different osphradial type resembling in certain characters that of the Euthyneura (Haszprunar 1985b, 1986). Therefore the problem of the systematic position of *Campanile symbolicum* focuses towards the question: Which osphradial type is present, the caenogastropod one, the cerithioid one, the architectonicoid one, or a new one?

2. MATERIAL AND METHODS

Total osphradia of *Campanile symbolicum* were sent in the primary fixative (3% glutaraldehyde in 0.1 M cacodylate buffer at pH = 7.3, 10% sucrose added for osmolarity) from Australia to Innsbruck (see Acknowledgements).

Small pieces with several leaflets were postfixed in 1% buffered osmium tetroxid for two hours. After dehydration in a methanol series and propylenoxide as an intermedium, the pieces were embedded in Durcupan. Semithin sections (0.5 μm) were made with glass knives and stained with methylene blue (Richardson *et al.* 1960). Ultrathin sectioning (60–80 nm) was done with diamond knives, and standard staining was with uranyl acetate and lead citrate. The ultrasections were studied in a ZEISS EM 902.

For scanning electron microscopy, osphradial pieces were fixed as described above, dehydrated in a methanol series and critically point dried with carbondioxide as intermedium. After sputtering the objects were studied in a ZEISS DSM 950.

In addition, larger pieces were dehydrated in an ethanol series and embedded in GMA (= glycolmethacrylate = 2,hydroxyethylmethacrylate) after Bennett *et al.* (1976; see also Gerrits 1984). Longitudinal and transversal sections (3 μm thick) were cut with a REICHERT Autocut to study the structure of whole leaflets. Staining was by various methods (Cason 1950; Richardson *et al.* 1960; Movat 1961; Reempts & Borgen 1975; Gerrits 1984).

3. RESULTS

(*a*) *General remarks*

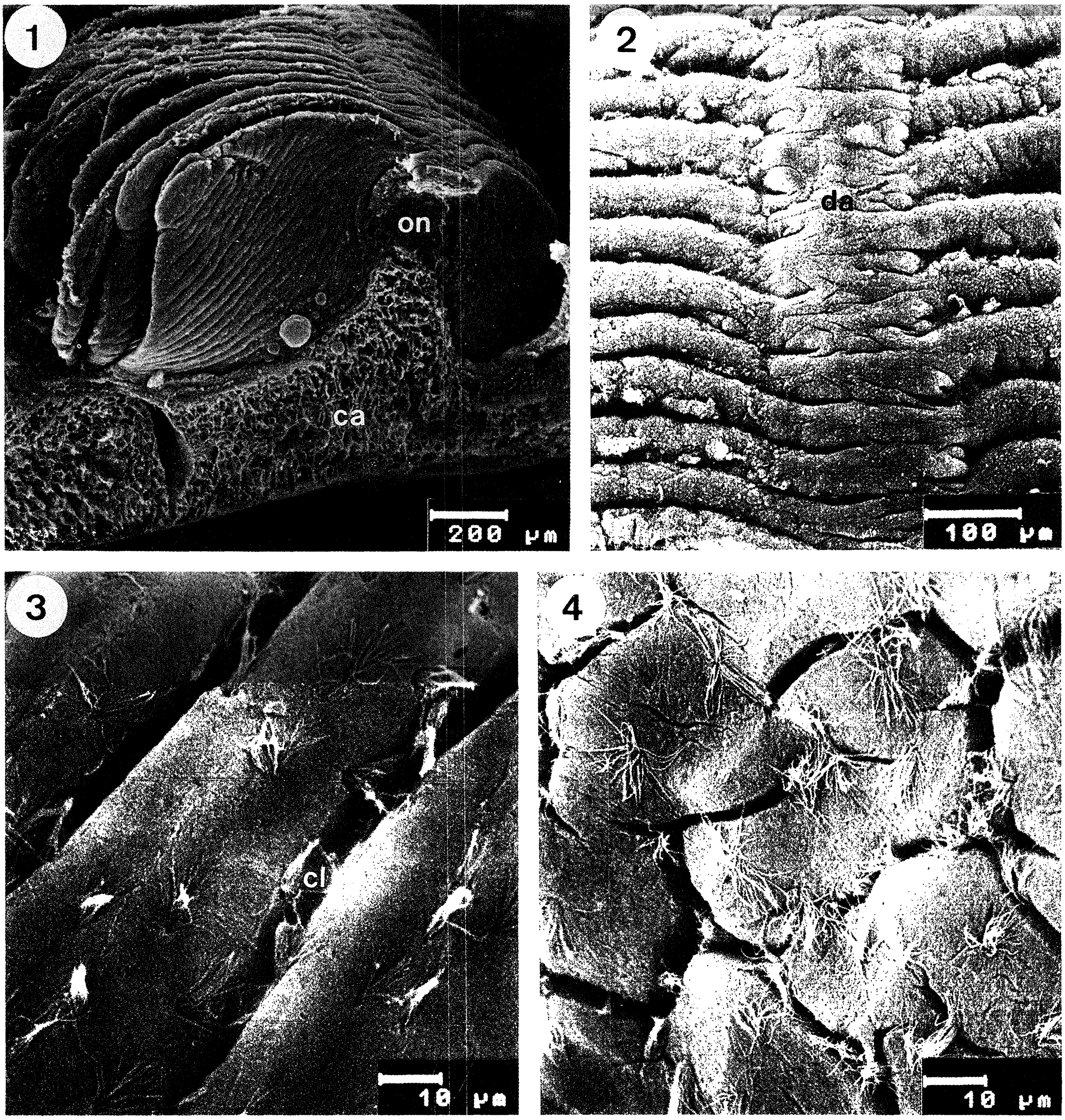
As in my foregoing papers on osphradial fine-structure (Haszprunar 1985*a*,*b*, 1986, 1987*a*,*b*), the osphradium is orientated so that the distal–proximal sides with respect to the mantle roof are called 'dorsal–ventral', the leaflets are 'ctenidial' or 'actenidial' with respect to the central axis (figure 1). This will facilitate comparison with other osphradia described. The correct position and orientation of the osphradium of *Campanile* is described below.

(*b*) *Position, orientation and external morphology*

As described by Bouvier (1887) and in more detail by Houbrick (1981) the osphradium of *Campanile symbolicum* is situated at the left side of the anterior mantle roof adjacent to the ctenidium. Functionally the osphradium lies near the entrance of the water current. The osphradium itself is a bipectinate structure of about 20 mm length in adult animals, the leaflets are alternately arranged (figures 1 and 2). Houbrick's (1981) figures show uniform leaflets, however, the 'actenidial' leaflets are only half as large as the 'ctenidial' ones (figure 1). In addition, there are many more osphradial leaflets (up to 200 on each side) than figured by Houbrick (1981). The thickness of the leaflets varies between 40 to 60 μm. Scanned pieces show that the epithelium of each 'ctenidial' osphradial leaflet is regularly clefted in an oblique orientation (figures 1 and 3), the distance between such clefts ranges between 20 to 30 μm, whereas the 'actenidial' leaflets show irregularly orientated clefts (figures 1 and 4). In both cases the whole area of each leaflet is characterized by regularly distributed ciliary tufts (figures 3 and 4). Otherwise there is no zonation visible from external view and I could not detect any ciliary band along the main axis. The edges of the leaflets are different in showing a thin ciliary band and an extended microvillous border.

(*c*) *The central axis*

The central axis of the osphradium is quite broad at its base and decreases continuously in diameter towards its distal portion. Basally the axis is formed by a cartilage (figure 1), the osphradial nerve (diameter about 150 μm) is situated distally. The latter is composed mainly of neural fibres. Nuclei of neural or glial cells are rarely found within the neuropile and are concentrated laterally at the periphery of the nerve. The osphradial nerve sends thick branches into each leaflet (figure 7). Below each emergence point a specific layer of neural cells is present and the covering epithelium is innervated by many tiny fibres. As a whole the appearance of the osphradial nerve closely resembles that of *Conus flavidus* (see Alexander 1973). Distally a thick bundle of longitudinal muscle fibres covers the osphradial nerve.



Figures 1–4. Scanning electron microscope (SEM) photographs of the osphradium.

Figure 1. Transverse section through the main osphradial axis (posterior view) showing the different sorts of osphradial leaflets and the underlying cartilage (ca). The central osphradial nerve (on) mainly contains a neuropile, the 'ctenidial' osphradial leaflets (to the left) are larger and show parallel alignment of clefts, whereas the 'actenidial' leaflets (to the right) show a reticulate pattern of clefts.

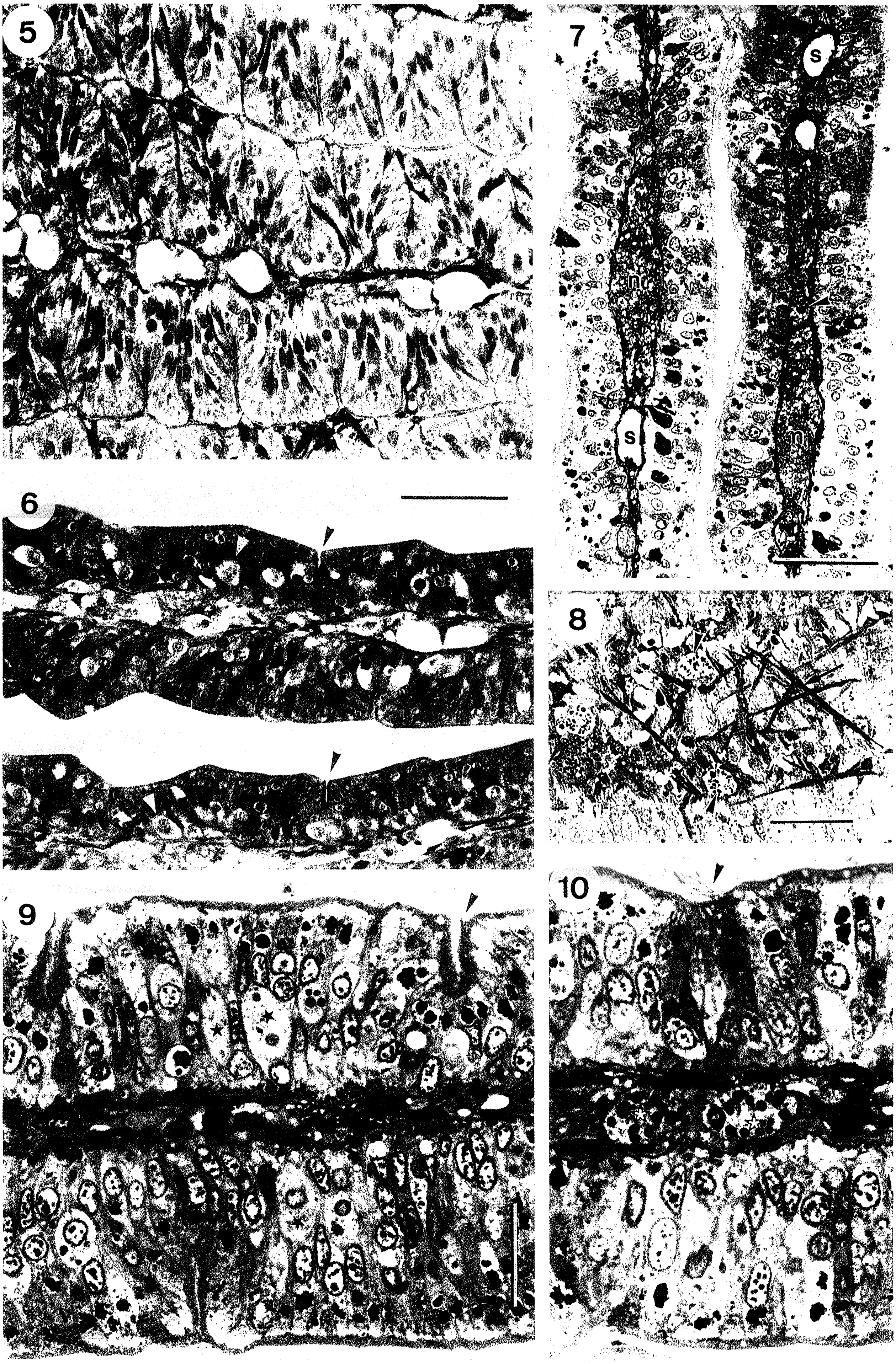
Figure 2. View of the distal main axis (da) of the osphradium showing the alternately arranged leaflets.

Figure 3. Detail of a 'ctenidial' leaflet showing parallel alignment of epithelial clefts (cl) and many ciliary tufts.

Figure 4. Detail of an 'actenidial' leaflet showing a reticulum of epithelial clefts and many ciliary tufts.

(*d*) *Histology of osphradial leaflets*

Although the osphradial leaflets of each side differ in their lengths, their histology is largely constant throughout. In general the composition of the epithelia of each leaflet changes gradually from dorsal to ventral and from central to lateral. However, this concerns only the distribution and density of cell types. The epithelium is up to 40 µm high but considerably less (15–20 µm) in the deep clefts (figures 5 and 9). It is mainly built up by pigmented cells, and ciliary cells (figure 10) with nerve processes interspersed. Basally, a weakly-stained cell type (Si4 cell, see figure 9 and below) occurs with increasing abundance towards the ventral area (figure 6). Mucous cells are generally lacking. The basement membrane



Figures 5–10. For description see opposite.

of the epithelium of the leaflets is well developed and folded (figure 10).

The central zone of each leaflet is composed of a highly complicated muscular grid, which is elaborated immediately below the basement membrane (figure 8). The respective muscle fibres run from the leaflet into the connective tissue of the main axis, where they show again a complicated pattern. Many pore cells are found interspersed in the central zone (figures 7, 10, 11, 22). Numerous nerve bundles, which are encircled by a thin basement membrane, run in the central zone of each leaflet (figure 7) and penetrate the basement membrane of the leaflet's epithelium.

(*e*) *Ultrastructure of osphradial cell-types* (figure 11)

Pigmented cells are by far the most common cell type of the osphradial epithelium and the mass of the tissue is built up from them. The cells reach from the basement membrane up to the surface of the epithelium and have a diameter of about 3–5 μm. Basally they are continued by axons, which run through the basement membrane and contact nervous processes in the central zone. The oval to elongate nuclei (about 9 μm × 4 μm) are variably situated. The cytoplasm shows a tubular endoplasmic reticulum which forms a labyrinth throughout the cell. One or two dictyosomes (Golgi) are regularly present and are always situated distally of the nucleus. A large cytosome is positioned in the distal area of each cell. It has a distinct membrane and includes several large pigment granules. Some cytosomes contain a more continuous, yet heterogenous mass (figure 14). The cytoplasm of the pigmented cells contains many multivesicular bodies (diameter 0.5 μm), most of which are also situated distally. These spherical bodies include several small vesicles (figure 16) which are also found free in the cytoplasm. Each pigmented cell bears a high (up to 3 μm) and dense microvillous border, which is covered by a weak but distinct glycocalyx layer. Occasionally single bacteria are found within the microvillous border (figure 12). In cells, which are situated near the main axis of the osphradium, the microvilli partly fuse and form larger entities, but their tips remain distinct. In addition, each pigmented cell bears one to three cilia, which are situated in a deep cleft of the cell's surface. These 'hidden' cilia are extremely short in lacking a distinct ciliary axis (figures 12 and 13). They do not reach the surface of the epithelium. Often a second centriole is found which is always orientated in an angle of 90 degrees to the primary centriole. Sometimes additional centrioles are found far below of the cell's surface in the cytoplasm. The cilium has a very weak, straight rootlet only (figure 13).

Supporting cells are found rarely interspersed between the pigmented cells. These are more slender (diameter 1–2 μm), lack pigment bodies and are characterized by a quite elongate nucleus (about 10 μm × 1.5 μm). Multivesicular bodies are also present, but cilia are lacking.

Ciliary cells are found dispersed throughout the osphradial epithelium. The oval nucleus of the ciliated cells is basally situated. The cytoplasm is slightly more electron-dark than that of neighbouring pigmented cells. Cytosomes do not occur, and multivesicular bodies are rarely present. Numerous mitochondria are found within the cell and are concentrated distally in the region of the ciliary roots (figure 17). Each cell bears up to 50 cilia of the usual 9 + 2 pattern. These cilia are arranged in parallel, at some height of the ciliary shaft an electron-dense mass is found associated with the ciliary axis in various orientations (figure 18). The cilia have a single basal body, with one root orientated straight downwards, and a second slightly oblique (figure 17). Synaptic contacts could not be detected.

Cells with net-like cytoplasm are found predominantly at the base of the epithelium near the main axis of the osphradium (figure 19). They contact the basement membrane, but most of them do not appear to reach the surface of the epithelium. However, they do reach the surface in the region of epithelial clefts. Their round nuclei (diameter 6–7 μm) are situated

Figures 5–10. Semithin sections of the osphradium.

Figure 5. Transverse section of a 'ctenidial' osphradial leaflet near the distal end showing a regular series of narrow epithelial clefts. 3 μm thick section, staining after Cason (1950). Scale as in figure 6.

Figure 6. Transverse section of the same leaflet as in figure 5 near the basal end showing a darker and lower epithelium, fewer epithelial clefts (black arrowheads) and more round and bright Si4 cells (white arrowheads). 3 μm thick section, staining after Cason (1950). Scale bar 50 μm.

Figure 7. Longitudinal section of two osphradial leaflets. Nerves (n), blood sinuses (s) and a pore cells (arrowhead) are clearly visible in the central zone, and the epithelium consists mainly of sensory cells (with pigmented cytosomes). 0.5 μm thick section, methylene-blue staining. Scale bar 50 μm.

Figure 8. Oblique section through the central zone of an osphradial leaflet showing the complex arrangement of muscle fibres (arrow) and several pore cells (arrowheads). 0.5 μm thick section, methylene-blue staining. Scale bar 50 μm.

Figure 9. Transverse section through the central area of an osphradial leaflet showing few and broad epithelial clefts (arrow), sensory cells with their pigment bodies and a Si4 cell with net-like plasma (asterisc). 0.5 μm section, methylene-blue staining. Scale bar 20 μm.

Figure 10. Transverse section through an osphradial leaflet showing a epithelial ciliary cell (arrowhead) and two pore cells (asterisks) with dark granules in the central zone. 0.5 μm thick section, methylene-blue staining. Scale bar as in figure 9.

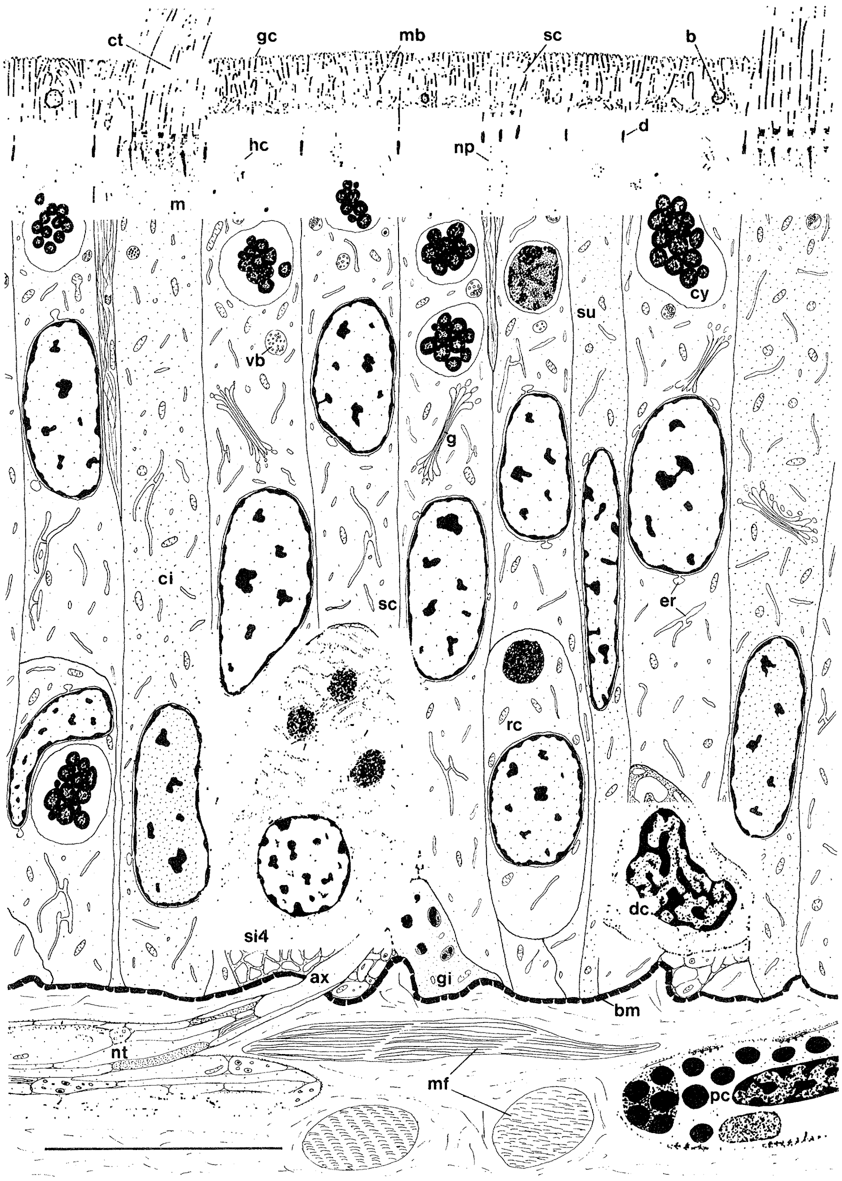


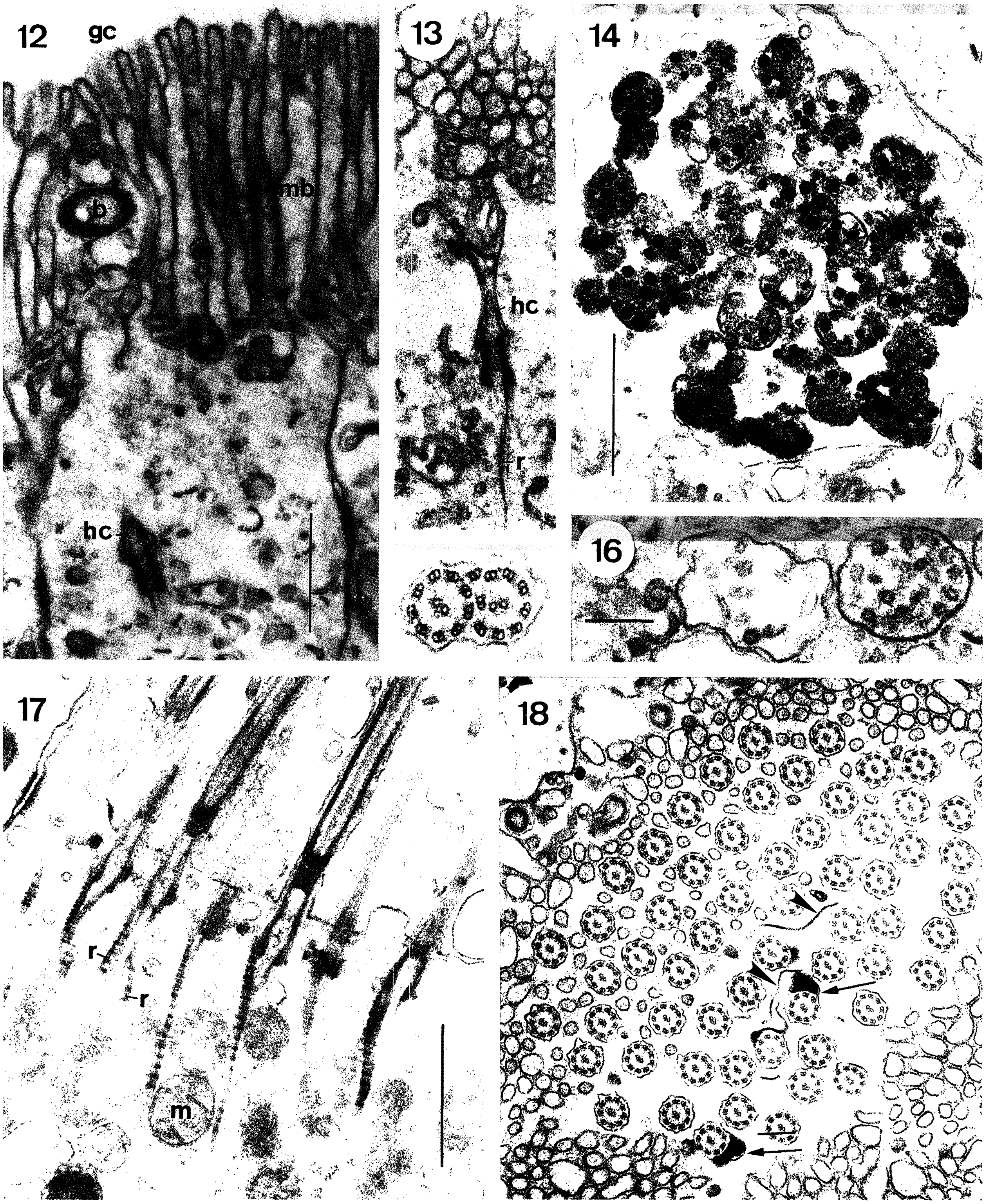
Figure 11. Semischematic drawing of the osphradial epithelium and certain elements of the central zone. Abbreviations: ax, axoneme of sensory cell; b, bacterium in microvillous border; bm, basement membrane; ci, ciliary cell; cy, cytosome with electron-dense granules; ct, ciliary tuft; d, belt desmosome (proximally followed by septate junction); dc, 'dark' (degenerating?) cell; er, tubular endoplasmic reticulum; g, Golgi apparatus; gc, glycocalix; gi, elements of the gliointerstitial system; hc, 'hidden' cilium of sensory cells; mb, microvillous border; mf, muscle fibres; np, neural process; nt, nervous tissue; pc, pore cell with large cytosomes, 'pores', and surrounding sheath of collagen matrix; rc, replacing cell; sc, sensory cilium of neural process; se, sensory cell; Si4, 'Sinneszelle Typ 4' (after Welsch & Storch 1969) with net-like cytoplasm; su, supporting cell; vb, multivesicular body. Scale bar 10 μm.

more or less in the centre of the cells. The smooth endoplasmatic reticulum has a net-like appearance, but there is uninterrupted transition between the typical 'net'-cell to those with an endoplasmic reticulum forming concentric aggregates (figure 19). Mitochondria are rarely found. Electron-dense cytosomes are also present and are concentrated distally, these cytosomes are much smaller and much more compact than those of the pigmented cells. Multivesicular bodies sometimes occur.

'Dark' cells are found interspersed in the epithelium of the leaflets. They do not contact the surface of the epithelium. Their nuclei show all degrees between clearly visible heterochromatin and amorphous chromatin, and the nucleic membrane is often irregularly widened. In general, the cytoplasm is electron dark and shows many 'empty vesicles', which is in fact widened endoplasmic reticulum. In contrast, the mitochondria appear not to be degenerated and show normally formed cristae (figure 21).

Spherical cells with several rather small pigment granules are found rarely at the base of the osphradial epithelium (figure 11: rc).

Nerve processes are situated between the pigmented cells and are found in great numbers particularly in the clefts of the epithelium. The slender (diameter 2–3 μm) processes are characterized by numerous neurotubules in their cytoplasm, and centrioles may occur in all positions. Distally they bear one or two cilia which resemble those of the pigmented cells in



Figures 12–18. Transmission electron microscopy (TEM) photographs of details of the osphradial epithelium.

Figure 12. Longitudinal sections of the apical area of a sensory cell with a hidden cilium (hc), microvillous border (mb) with glycocalix (gc) and bacterium (b). Scale bar 1 μm.

Figure 13. Oblique section of the apical area of a sensory cell. The hidden cilium (hc) has a single, very weak rootlet (r) and lacks a ciliary shaft . Scale bar as in figure 12.

Figure 14. Cytosome of a sensory cell showing the pigment granules. Scale bar 1 μm.

Figure 15 (not labelled). Example of an aberrant (pathogenic?) ciliary aggregate of two regular cilia. Scale bar as in figure 16.

Figure 16. Two multivesicular bodies of a sensory cell. Scale bar 0.2 μm.

Figure 17. Longitudinal section of a ciliary cell showing two roots (r) and mitochondria (m). Scale bar 1 μm.

Figure 18. Transverse section of a ciliary tuft showing ciliary shafts arranged in parallel, the direction of main beat is to 'two o'clock'. At a particular height electron dense bulges (arrows) and 'wings' (arrowheads) occur. Microvilli covered with glycocalyx are interspersed basally. Scale bar as in figure 17.

having a very weak rootlet system and often a second centromere. Fixation artifacts of the ciliary membrane of these cilia are often found, resulting in ornamented membrane patterns (figure 20). The cilia are situated within the microvillous border and have a normal 9+2 microtubule pattern. Very rarely double-cilia are found (figure 15). Occasionally, a perikaryon with an oval nucleus, which has very regularly distributed heterochromatin, was found within the epithelium.

Cells which reach the surface of the epithelium, i.e. pigment cells, ciliated cells, supporting cells, nerve processes and certain net-like cells, are distally connected by typical belt-desmosomes, which are continued downwards by septate junctions. In general, lateral intercellular clefts are extremely narrow, gap junctions are found between all cell types (except of belt desmosomes, junctions and epithelial intercellular spaces are omitted in figure 11).

At the base of the epithelium elements of the glio-interstitial system are regularly found. These elements are characterized by quite large electron-dense vesicles (figure 11).

The basement membrane (terminology after Pedersen (1991)) is very prominent. Below the cell membranes of the epithelial cells a thick (about 0.1 μm) electron dense matrix is present. This matrix is regularly interrupted resulting in an image of a broken line (figures 22 and 25). The basement membrane is penetrated by the axons of the pigmented cells as well as by neurons running to the surface of the epithelium.

Pore cells are found in great numbers in the central zone of each leaflet as well as in the connective tissue below the osphradial nerve (figures 7 and 10). These large, round (diameter up to 30 μm) cells are characterized by the sieve structures of their surface. The 'pores' are in fact slit-like openings of the cell membrane with a meandering pattern (figure 23). They are found all around the cell. Below the slits so-called subsurface cisternae are formed. Small vesicles contact these cisternal cavities (figure 24). Each cell is surrounded by a weak but distinct and complex basement membrane and is embedded in a fibrillar extracellular matrix (figure 23). Pore cells do not have contact with any other cell in the central zone. The pore cells have round or oval nuclei (diameter 3–4 μm), a well-developed tubulous endoplasmic reticulum and a golgi apparatus. Large pigment granules of irregular form occur in the cytoplasm, making this cell type clearly visible in light microscope sections (figures 7, 10, 22).

Smooth muscle cells are present in great numbers in the central zone of each leaflet (figures 11 and 25). Like those in the dorsal muscle fibers of the central axis they are not in contact with each other, but are connected via hemidesmosomes with the surrounding matrix.

This matrix contains many collagen fibres (figure 25), which are produced by fibroblasts, the latter are found sparsely distributed in the central zone of the leaflets.

Granular cells occur sparsely in the central zone. They are characterized by a very elongated nucleus, which is peripherically positioned, and a mass of electron-dense granules (diameter about 1 μm). Occasionally deposits of glycogen are found in these cells.

Nervous processes occur in great numbers in the central zone. The main nerve is surrounded by a weak but distinct basement membrane. The axons include all kinds of typical neuronal vesicles such as neurotubules, dense vescicles or dense core vesicles. Rarely glial cells are found, which send fine processes between the nerve fibres and surround them. The glial cells have elongated nuclei and their cytoplasm typically contains small glycogen rosettes (figure 26).

4. DISCUSSION

(*a*) *Histology of the osphradium*

The assumption that the pigmented cells are sensory is based on several facts. Firstly, the pigmented cells are equipped with axons which contact the nervous tissue of the central zone. Secondly, the occurrence of

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Figures 19–21. TEM photographs of details of the osphradial epithelium.

Figure 19. Si4 cell with net-like plasma and cytosomes. Scale bar 5 μm.

Figure 20. Apical area of a sensory process showing artificially extended ciliary membrane and many neurotubules in the cytoplasm. Scale bar 1 μm.

Figure 21. 'Dark cell' showing an electron dense cytoplasm with many vacuoles (extended nucleus membrane and endoplasmic reticulum). Scale bar 1 μm.

Figure 22. Pore cell (pc) with large pigment granules (asterisks) and numerous slits (i.e. 'pores', cf. figure 23) (arrowheads) of the central zone below of the basement membrane (bm). Scale bar 1 μm.

Figure 23. Nearly tangential section of the slit zone of a pore cell showing meander-like slits (arrowheads) and covering fibres of connective tissue (asterisks). Scale bar 1 μm.

Figure 24. Longitudinal section of the slit zone of a pore cell showing the pore-like slits (arrowheads) and the associated cisternae (points). Scale bar 1 μm.

Figure 25. Basement membrane (bm) with underlying connective tissue. The arrowheads mark a longitudinal section of a collagen fibre showing clear periodicity. A muscle fibre (m) is associated. Scale bar 0.5 μm.

Figure 26. Glial cell (g) with elongated nucleus and glycogen aggregates in the main osphradial nerve enveloping nervous fibres (n) with its cytoplasmatic processes (asterisks). Scale bar 1 μm.

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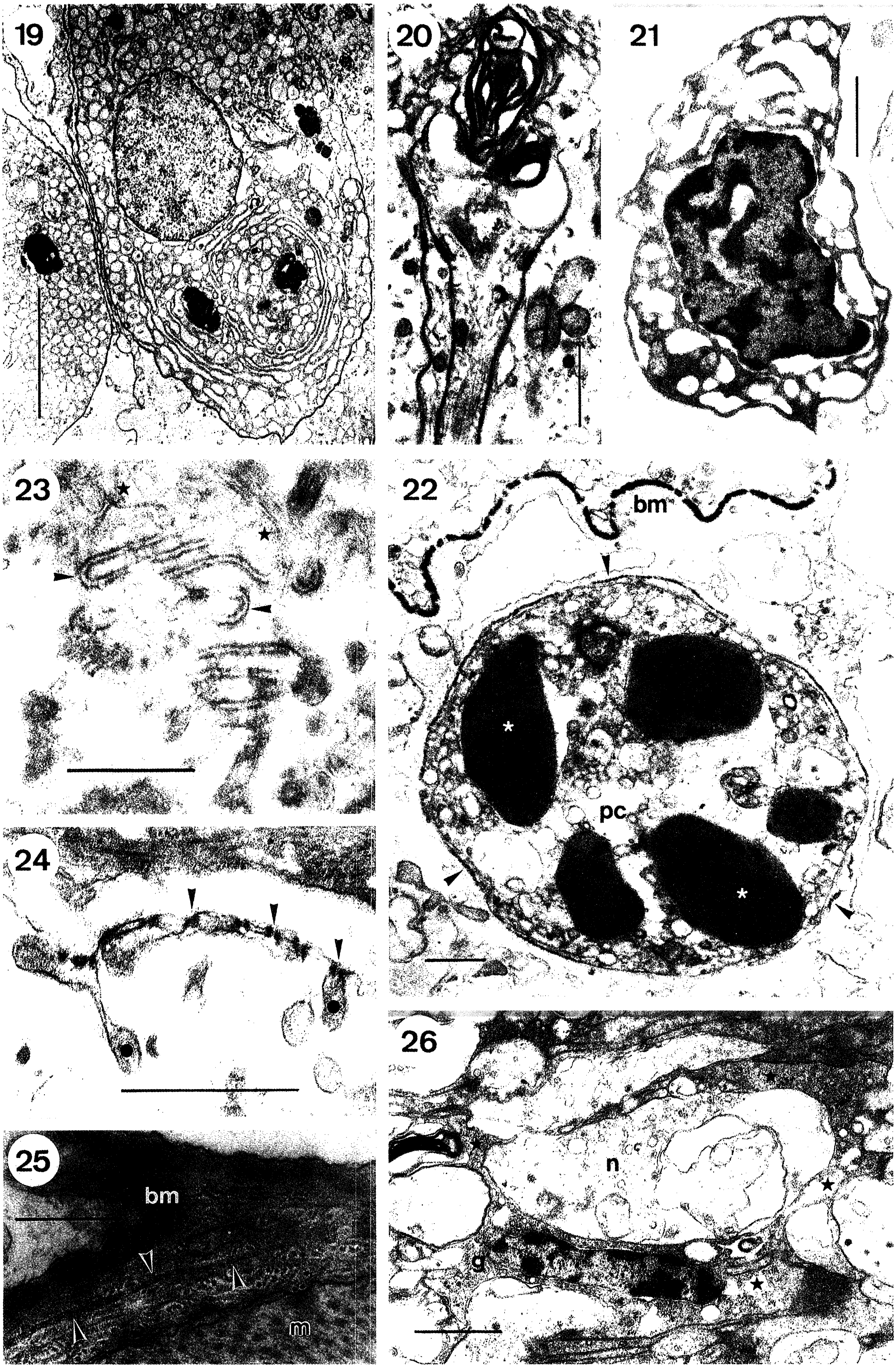

Figures 19–26. For description see opposite

largely modified and deeply sunken cilia supports this hypothesis. Finally, the presence of many multivesicular bodies calls for a function in neurosecretion or exo- or endocrinology.

Pigment bodies are regularly seen in the sensory cells of molluscan osphradia (e.g. Welsch & Storch 1969; Benjamin & Peat 1971; Crisp 1973; Alexander & Weldon 1975; Newell & Brown 1977; Garton *et al.* 1984; Haszprunar 1985*a*,*b*, 1987*a*,*b*, unpublished results; Theler *et al.* 1986) and are also often present in sensory cells of other molluscan chemoreceptive organs (e.g. Emery & Audesirk 1978, Totàro *et al.* 1984). This suggests a possible metabolic function of such pigments in chemoreceptive cells (Totàro *et al.* 1984). On the other hand pigments do not occur in the ciliated nerve endings indicating that the metabolism of this cell type is quite different from that of the pigmented cells. Such a difference might also suggest that the mode of reception of both sensory cell types differs considerably.

The typical structure of the cilia in the ciliary cells, and the mass of distal mitochondria, suggests that they are motile, while their sparse distribution suggests a role in cleaning of the epithelium rather than in water movement (see also below).

The bright cell with its net-like smooth endoplasmatic reticulum in *Campanile symbolicum* closely resembles the so-called 'Si4 cell' ('Sinneszelle Typ 4') described from caenogastropod osphradia (Welsch & Storch 1969; Crisp 1973; Newell & Brown 1977; Haszprunar 1985*a*). According to Welsch & Storch (1969) the cytostomes include lipids. Based on the similarity with a mechanoreceptive cell type (Merkel's cell) in vertebrates, Welsch & Storch (1969) considered a mechanoreceptive role. However, these similarities appear superficial (smooth endoplasmic reticulum versus cell membranes), and up to now the distinct function of this cell type remains obscure.

The condensed nuclei and cytoplasm with enlarged endoplasmic reticulum of the dark cells seem to indicate a degenerating epithelial cell type, but the non-degenerated mitochondria argue against such an interpretation. In contrast, the basal cells with their small pigment granules and a high nucleus–cytoplasm ratio might represent developing sensory cells. This interpretation is supported by the presence of cells which are somewhat intermediate in morphology between basal cells and pigmented cells, hence this type would reflect a constant turn-over of cells in the osphradial epithelium.

The complexity of the muscle system in the leaflets and the dorsally situated thick longitudinal muscle in the central axis suggests an important role this system. Because of the dense arrangement of the osphradial leaflets and their deeply ridged epithelium, the sparsely distributed ciliary tufts appear too weak for a significant contribution to water exchange. Contraction of the osphradium as a whole or movement of osphradial leaflets may be important for rapid exchange of the surrounding water body. Indeed, published scanning electron microscopy (SEM) photos of the osphradium of the muricid *Thais haemastoma* (Garton *et al.* 1984) suggest motility of osphradial leaflets. However, observation of living animal is necessary to confirm this view.

The presence of many pore cells in the central zone of the osphradial leaflets as well as in the underlying connective tissue calls for attention. It cannot be unequivocally decided, whether the vesicles, which contact the subsurface cisternae, represent exo- or endocytosis. Judging from the presence of a surrounding basement membrane and from the example of the similarly structured podocytes (Boer & Sminia 1976), the latter version appears more probable. As reviewed by Simkiss & Mason (1983) and Mason *et al.* (1984), pore cells are thought to act in haemocyanin synthesis, in phagocytosis, or in recycling of respiratory pigments. All these assumed functions do not make much sense in a sensory organ, however. On the other hand, pore cells occur in the connective tissue or blood sinuses of all parts of the body in *Littorina* (Martoja *et al.* 1980; Mason *et al.* 1984; Brough & White 1990), thus they might necessarily be present in association with connective tissue or blood sinuses.

The lack of large numbers of nerve cells or of an osphradial ganglion and the formation of an osphradial plexus is in accordance with conditions described for neogastropods such as *Conus* (Alexander 1973). However, the physiological significance of such features is still unknown.

(*b*) *Functional significance*

Whereas the bipectinate morphology of the osphradium can be easily understood to increase the sensory surface, the difference in size and morphology between ctenidial and actenidial leaflets is difficult to interpret. Bipectinate osphradia of *Nassarius reticulatus* or *Polinices tumidus* also show such asymmetry, whereas the great majority of species have symmetrical osphradia (e.g. Spengel 1881; Bernard 1890; Welsch & Storch 1969; Crisp 1973; Maeda 1983, 1986, 1988, 1990; Garton *et al.* 1984; Kamardin 1984; Haszprunar 1985*a*; Taylor & Miller 1989). Unfortunately, the fine-structure does not help as it is the same in symmetric and asymmetry osphradia.

According to the structural details of the osphradial epithelial cells there is little doubt that the osphradium of *Campanile symbolicum* is essentially a sensory organ, as is usual among streptoneuran gastropods. Structural data suggest a chemoreceptive function, because it is very unlikely that the deeply sunken sensory cilia of the pigmented cells act in mechanoreception. Also the cilia of the nerve endings, which are situated within the microvillous border, probably are chemoreceptors. In addition, typical mechanoreceptors such as collar receptors (see Haszprunar (1985*f*) for comparative discussion among molluscs) have not been found.

(*c*) *Ecological significance*

For the osphradia of streptoneuran gastropods which have been studied comparatively (cf. Bernard 1890; Yonge 1947; Fretter & Graham 1962; Maeda 1983, 1986, 1988, 1990; Haszprunar 1985*a*,*b*; Taylor

& Miller 1989), there is good correlation in most species investigated between the mode of nourishment and the external feature of the respective osphradium. Whereas most herbivorous and detritivorous species show a simple, ridge-like osphradium, carnivorous species have a highly elaborated osphradium resembling a small, bipectinate gill. Filter feeders (e.g. *Crepidula*) and commensals (e.g. *Capulus hungaricus*) may range between these extremes. Cypraeidae with a big tripectinate osphradium are omnivorous browsers which feed on algae as well as on sponges or bryozoans. *Campanile symbolicum* combines herbivory (Houbrick 1981, 1984, 1989) with a big, lamellate osphradium. Naturally the question arises, why herbivorous gastropods need such an elaborate sensory structure.

Explanation of a particular biological structure needs two aspects: function and history. Concerning function one should consider that the alimentary system of *Campanile symbolicum* is highly aberrant in that it exhibits many unique features (Houbrick 1981, 1989), which might reflect a highly specialized herbivory. However, stomach contents (pieces of *Posidonia*, fragment of various algae; see Houbrick 1989) suggest a broad spectrum of food. Concerning history, one might assume a secondary herbivory from a carnivorous ancestor. However, there are no living close relatives of *Campanile*, and the feeding biology of the fossil congeners cannot be known. The conclusion is that at present the role of the osphradium in the feeding biology and the reasons for the aberrant features of the alimentary tract of *Campanile* remain obscure.

I have emphasized a possible role of the osphradium in reproductive biology of molluscs to coordinate spawning or to find a sexual partner (Haszprunar 1987*a*). The reproductive biology of *Campanile symbolicum* is poorly understood (Houbrick 1981). A trail following mechanism to find a partner, which has been described for many gastropods (see Wells & Buckley 1972; Croll 1983; Sterling & Hamilton 1986) cannot be excluded. However, noting the different chemoreceptive cells in the osphradial epithelium, in particular the pigmented cells and the nerve endings, a multiple function of the osphradium appears likely. Possibly the osphradium of *Campanile symbolicum* acts in feeding and sexual biology. Detailed physiological studies are needed to clear up the matter.

(*d*) ***Evolutionary significance***

In the former papers on gastropod osphradia (Haszprunar 1985*a*, 1986) I concluded that the lamellar type of osphradium has been convergently evolved several times among the Streptoneura. This view was based mainly on the different arrangement of ciliary bands (the so-called lateral zones) in the osphradial leaflets of the various caenogastropod groups. Meanwhile Maeda (1988, 1990) has shown by light-microscope sectioning a great variability in the osphradial leaflets among the Naticidae. Furthermore Taylor & Miller (1989) used SEM-technique to show that the leaflet types of higher Neotaenioglossa (in particular members of the superfamilies Cypraeoidea, Calyptraeoidea and Naticoidea) are more similar to those of stenoglossans (Neogastropoda) than previously assumed. Assumption of a closer relationship of the mentioned neotaenioglossan superfamilies and the Stenoglossa is supported by sperm-structure (Taylor & Morris 1988; Healy 1988; Haszprunar 1988*a*,*b*), therefore a common origin of this lamellar type might be accepted.

However, the quite different conditions among certain Cerithioidea with a semi-lamellar osphradium (only the central zone is lamellate, e.g. *Cerithium*, *Rhinoclavis*; cf. Bernard 1890, Taylor & Miller 1989) and the occurrence of both types, the simple ridge-like (e.g. *Aporrhais*) and the lamellar type (e.g. *Terebella*) within the Stromboidea suggest parallel evolution of the lamellar type in these groups. Such independent evolution of the lamellar type is also very likely within the Architectonicoidea and of *Campanile*, both groups showing very peculiar osphradial types.

(*e*) ***Systematic significance***

It has been shown that occurrence of osphradial cell types reflect phylogenetic relationships rather than ecological or functional correlations among the streptoneuran Gastropoda (Haszprunar 1985*a*, 1986). Bearing in mind the isolated position of *Campanile* with respect to anatomy and sperm structure, osphradial fine structure may help to clarify the systematic position of the species.

There are no special similarities between the osphradia of *Campanile* and those of the Architaenioglossa (cf. Haszprunar 1985*a*; Kamardin 1986).

The osphradium of *Campanile* shares some characters with the osphradia of the Allogastropoda and primitive Opisthobranchia. These mainly concern the external shape and the presence of an uniformly structured and clefted osphradial epithelium (Edlinger 1980; Haszprunar 1985*b*). However, the structure of the mathildid osphradium, which is also bipectinate (Haszprunar 1985*e*), is unknown, and the osphradial fine structure of Architectonicidae, Pyramidellidae and primitive Opisthobranchia (cf. Haszprunar 1985*b*) differs considerably from the campanilid type.

Most bipectinate osphradia of caenogastropods show a ciliated band on their lamellae (Crisp 1973; Maeda 1983, 1986, 1988, 1990; Haszprunar 1985*a*; Taylor & Miller 1989). This is in clear contrast to the conditions found in *Campanile*, where only ciliary tufts are present on the osphradial lamellae. The latter condition is present in certain Cerithioidea (*Rhinoclavis* and *Cerithium*), where the ciliary band is restricted to the main axis of the osphradium and is not extended on to the osphradial lamellae (Taylor & Miller 1989). However, no ciliary bands are present along the central axis of the *Campanile* osphradium.

The presence of three special osphradial cell types in constant mutual position is regarded as a diagnostic autapomorphy for the Caenogastropoda (Haszprunar 1985*a*, 1986, 1988*b*). Two cell types (called Si1 and Si2 cells after Welsch & Storch (1969)) are provided with specialized 'columnar' microvilli with short stalks. Few cells in the osphradial epithelium of *Campanile symbolicum* show extended microvilli. However, (i) the tips of the microvilli are regularly formed, (ii) there are no stalks, and (iii) these cells are never ciliated. These differences suggest that a homology of these cell types between *Campanile* and the Caenogastropoda cannot be established.

Net-like cells, i.e. cells with concentrically arranged endoplasmatic reticulum are present in the osphradial epithelium of *Campanile*. These cells closely resemble the Si4 cells at the central zone of caenogastropod osphradia (Welsch & Storch 1969, Crisp 1973, Haszprunar 1985*a*, 1986). Also the presence of pigment granules and the contact with the epithelial surface via thin processes is shared in both cases. Therefore a homology between the cells of *Campanile* and those of the Caenogastropoda can be reasonably assumed. In contrast, the 'Si4 cell' described by Edlinger (1980) in the osphradia and Hancock's organ of the bullomorph opisthobranch *Haminea navicula*, differs considerably from the true Si4 cell in having a rough endoplasmatic reticulum, which suggests a entirely different function.

The remaining cell types of the osphradial leaflets of *Campanile* differ fundamentally from those of the caenogastropod osphradial central zones as well as from osphradia of allogastropods or euthyneurans.

In summary, the osphradial ultrastructure of *Campanile symbolicum* indeed provides some evidence that assists in establishing the relationships of this enigmatic species. The many peculiarities of the osphradial epithelium strengthen the isolated position of this species. The assumed similarities (Haszprunar 1988*a*,*b*) with the osphradia of the Architectonicidae (respectively Allogastropoda) are shown to be superficial. Osphradial similarities exist between *Campanile* and the Caenogastropoda in particular based on the commmon presence of Si4 cells, yet there are fundamental differences between the osphradia of both groups.

Accordingly, the proposed affinities of *Campanile* with the allogastropod–euthyneuran line (Haszprunar 1985*e*, 1988*a*,*b*; Salvini-Plawen & Haszprunar 1987) can no longer be supported. Based on sperm structure (Healy 1986) there are affinities with the architaenioglossate groups and with the Cerithioidea, the latter being regarded as the earliest offshoot of the Caenogastropoda (Haszprunar 1988*b*, Ponder & Warén 1988). At the present stage of knowledge the Tertiary relict snail *Campanile symbolicum* Iredale is best placed in its own clade near the base of, or even within, the Caenogastropoda.

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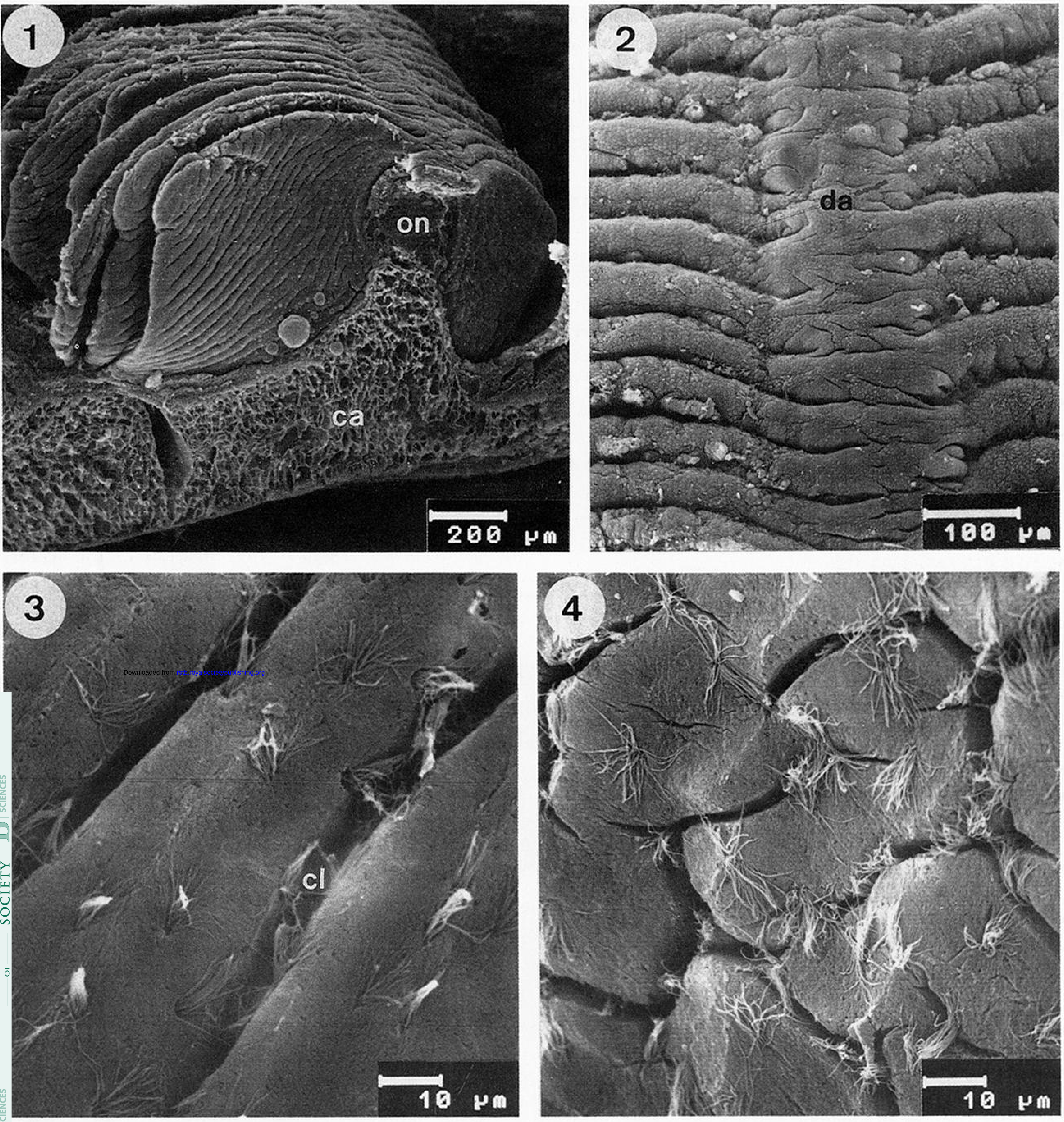


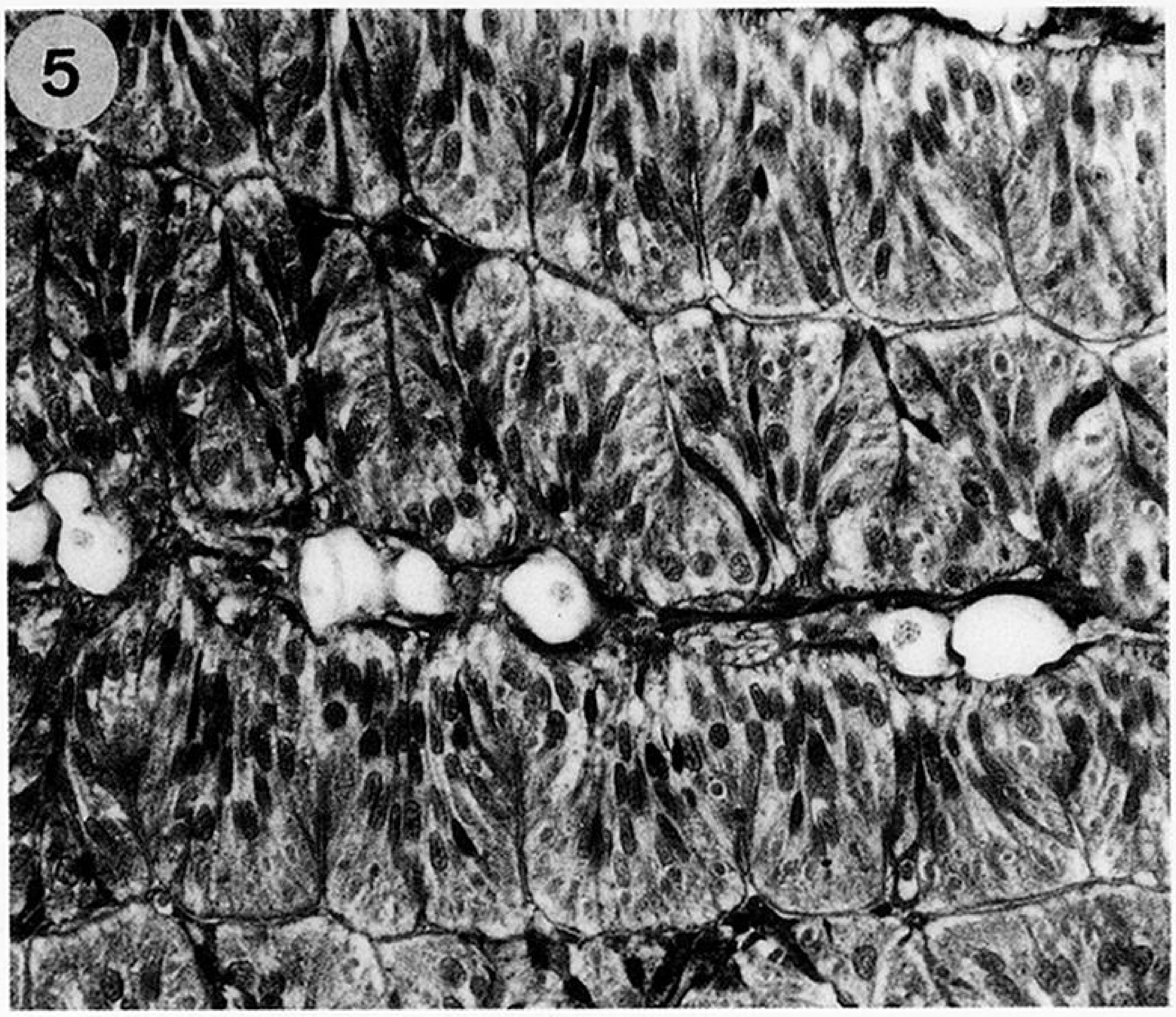
Figures 1–4. Scanning electron microscope (SEM) photographs of the osphradium.

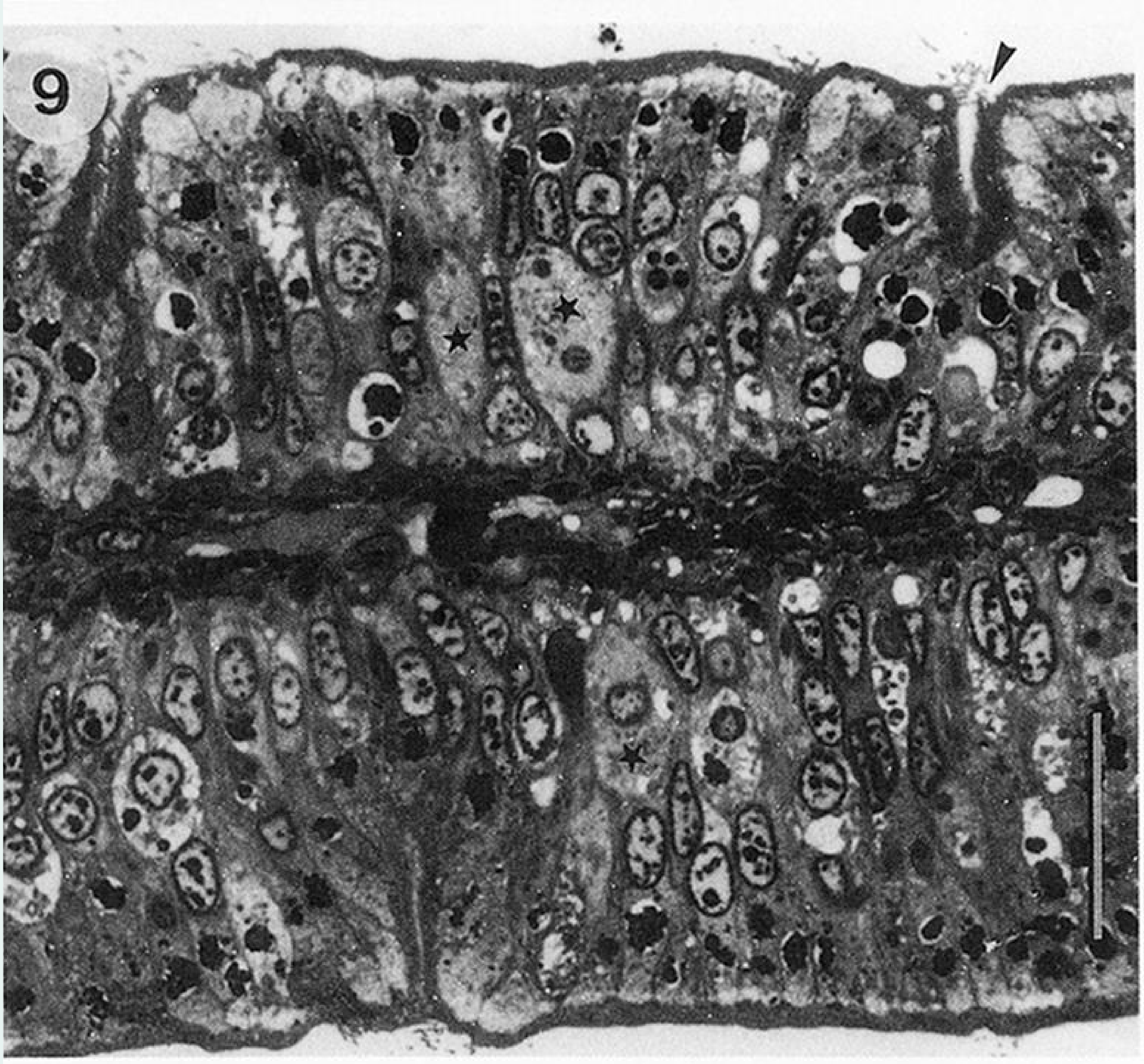
Figure 1. Transverse section through the main osphradial axis (posterior view) showing the different sorts of osphradial leaflets and the underlying cartilage (ca). The central osphradial nerve (on) mainly contains a neuropile, the 'ctenidial' osphradial leaflets (to the left) are larger and show parallel alignment of clefts, whereas the 'actenidial' leaflets (to the right) show a reticulate pattern of clefts.

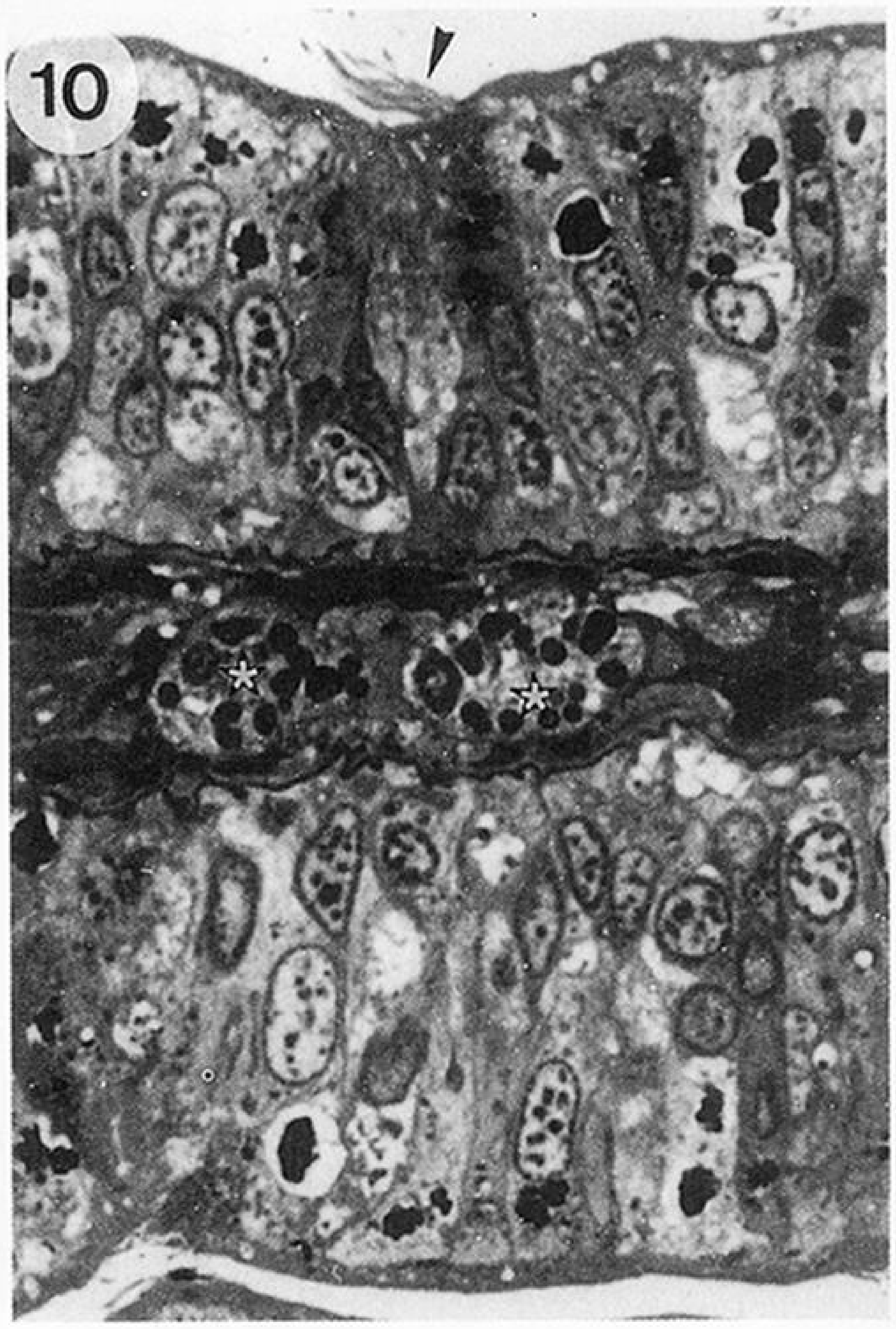
Figure 2. View of the distal main axis (da) of the osphradium showing the alternately arranged leaflets.

Figure 3. Detail of a 'ctenidial' leaflet showing parallel alignment of epithelial clefts (cl) and many ciliary tufts.

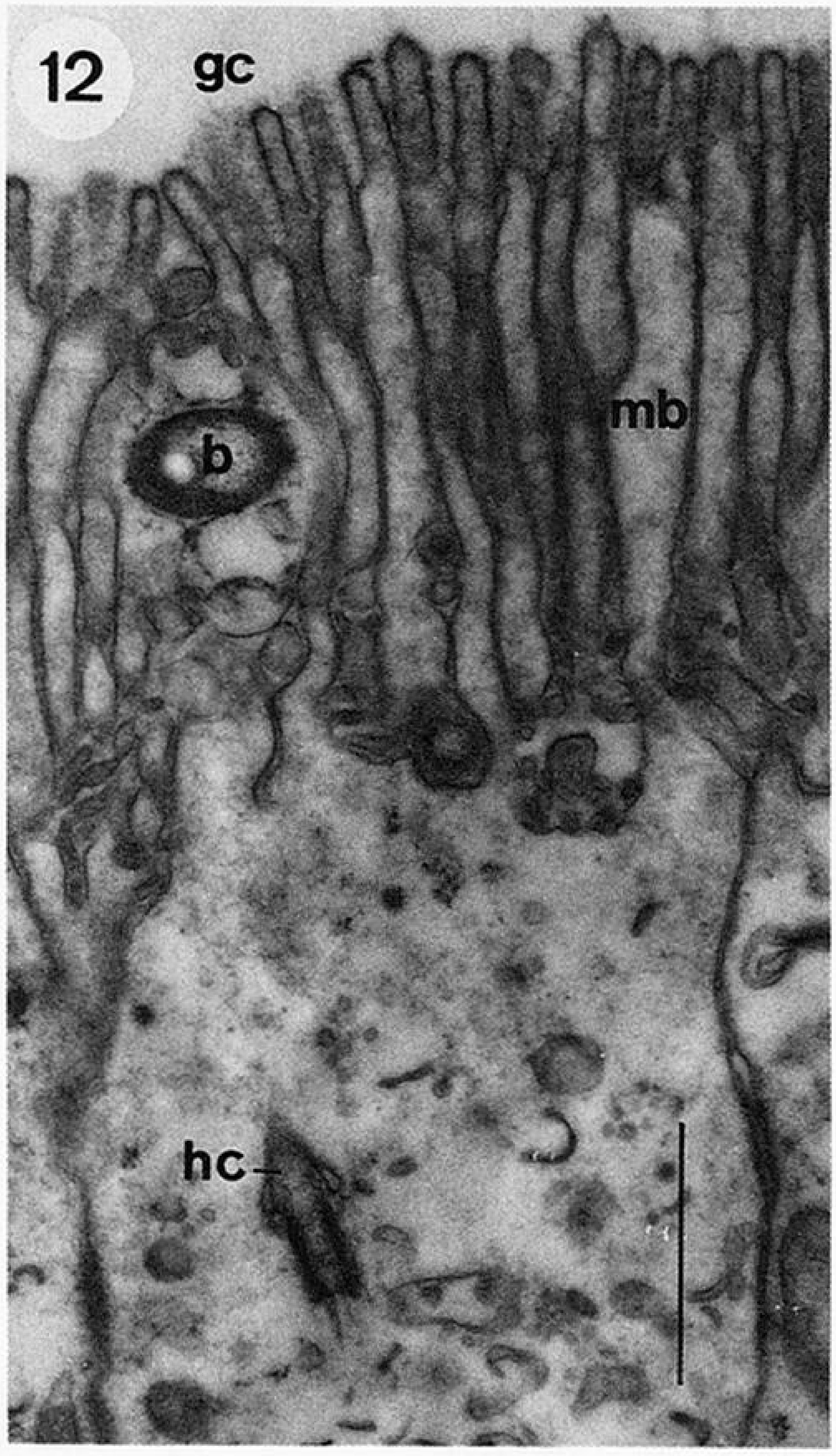
Figure 4. Detail of an 'actenidial' leaflet showing a reticulum of epithelial clefts and many ciliary tufts.

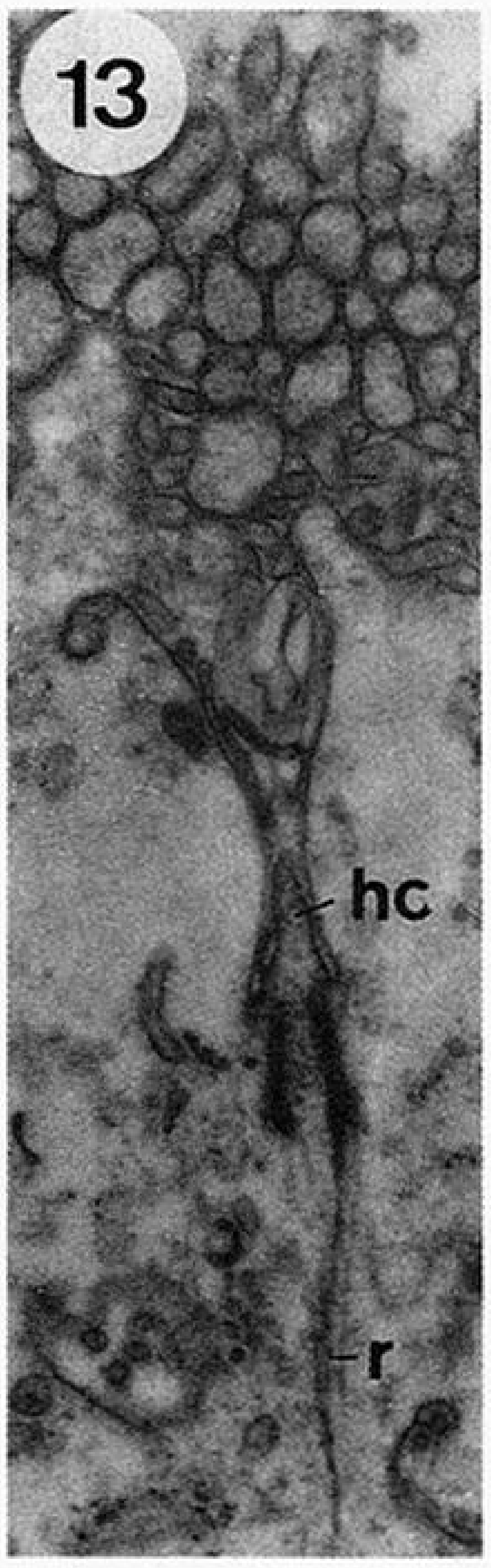


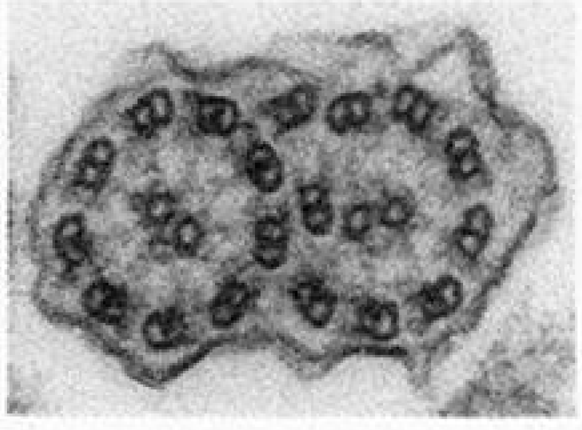


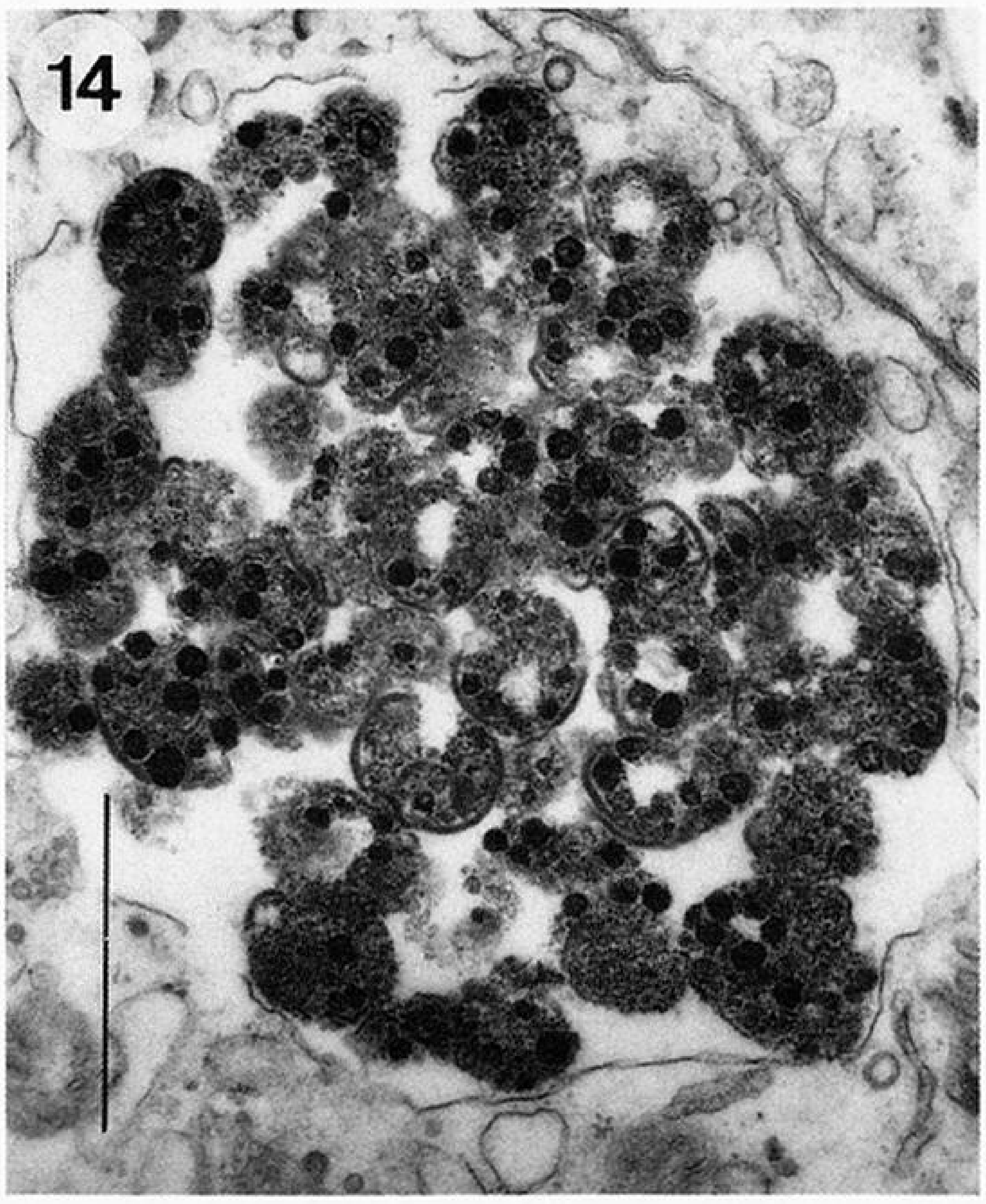


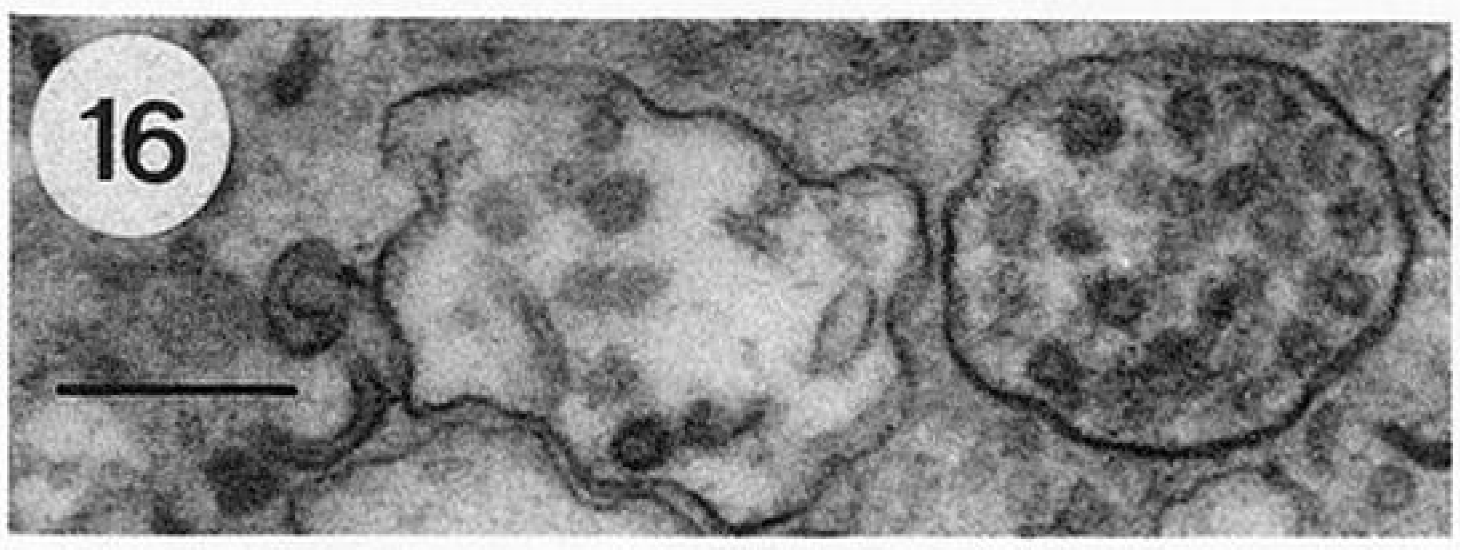
Figures 5–10. For description see opposite.

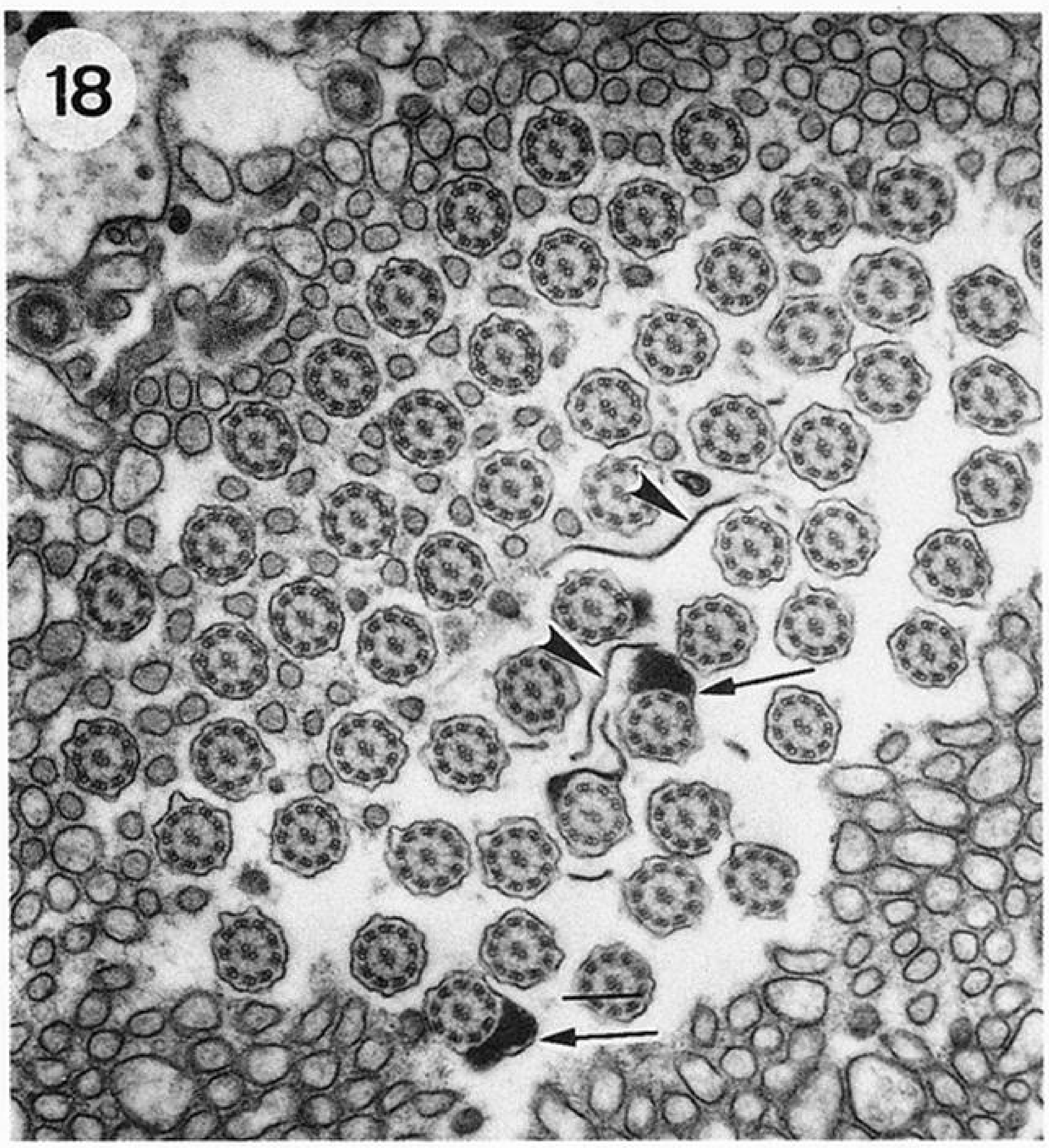












Figures 12–18. Transmission electron microscopy (TEM) photographs of details of the osphradial epithelium.

Figure 12. Longitudinal sections of the apical area of a sensory cell with a hidden cilium (hc), microvillous border (mb) with glycocalix (gc) and bacterium (b). Scale bar 1 μm.

Figure 13. Oblique section of the apical area of a sensory cell. The hidden cilium (hc) has a single, very weak rootlet (r) and lacks a ciliary shaft . Scale bar as in figure 12.

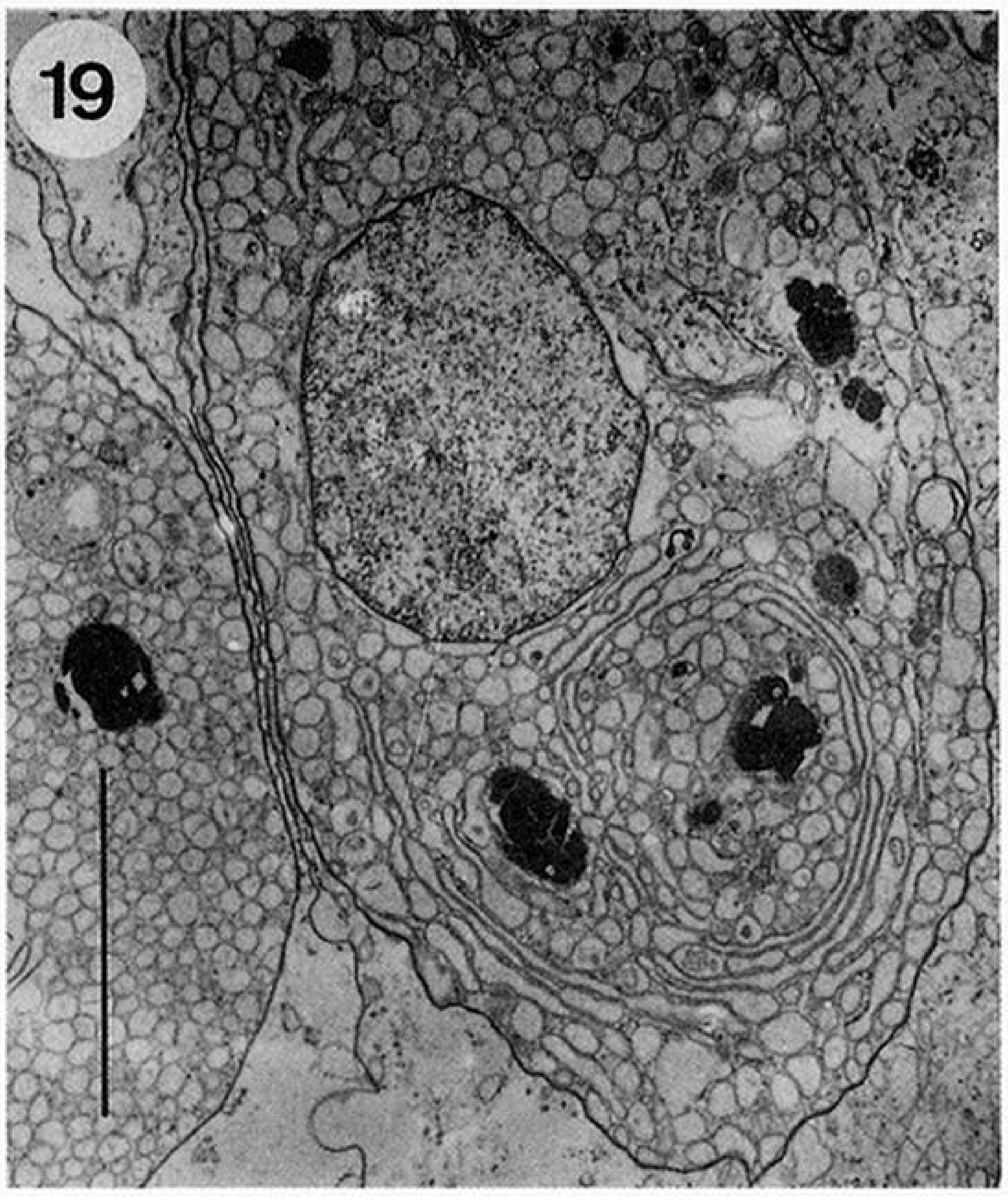
Figure 14. Cytosome of a sensory cell showing the pigment granules. Scale bar 1 μm.

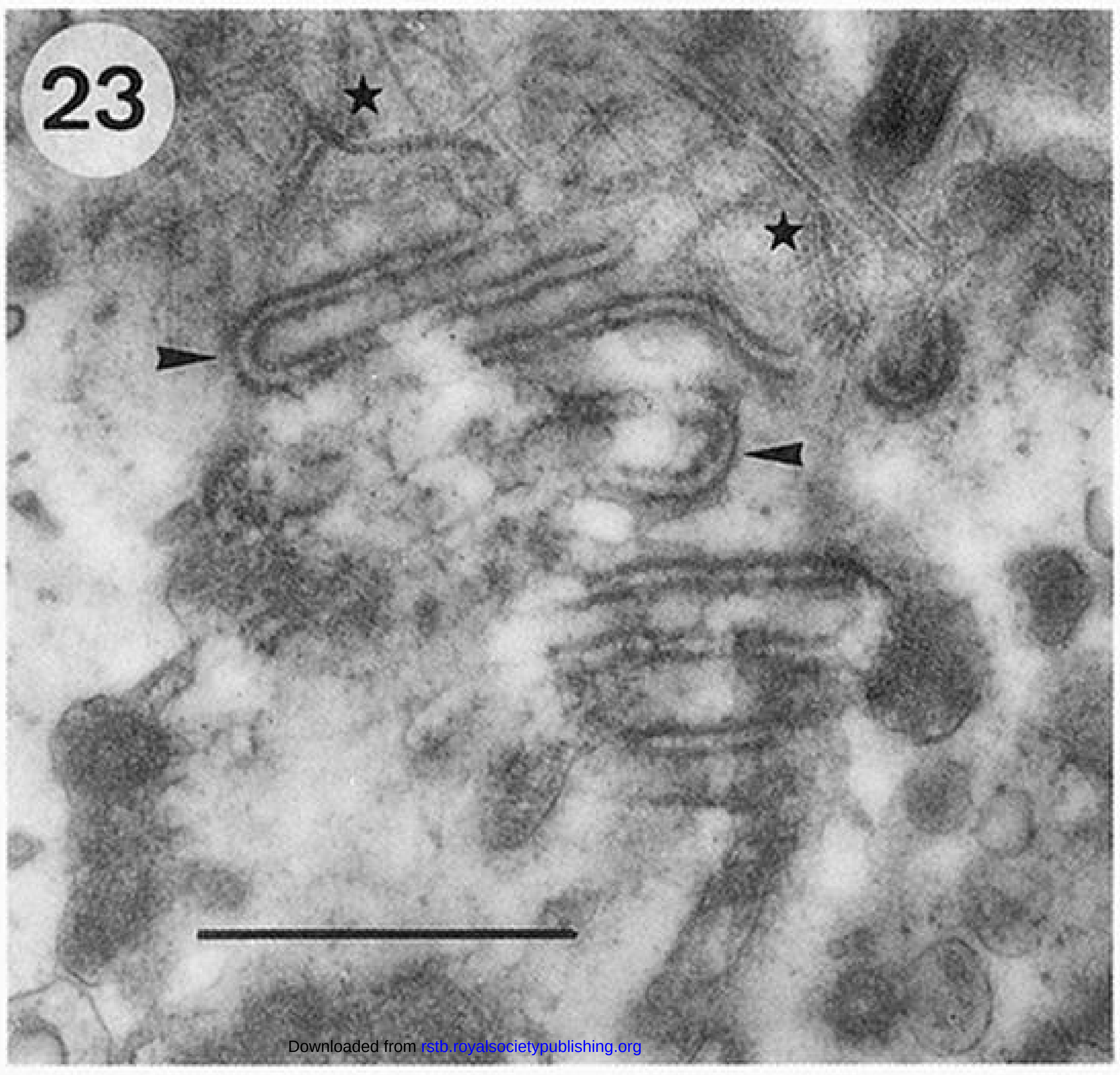
Figure 15 (not labelled). Example of an aberrant (pathogenic?) ciliary aggregate of two regular cilia. Scale bar as in figure 16.

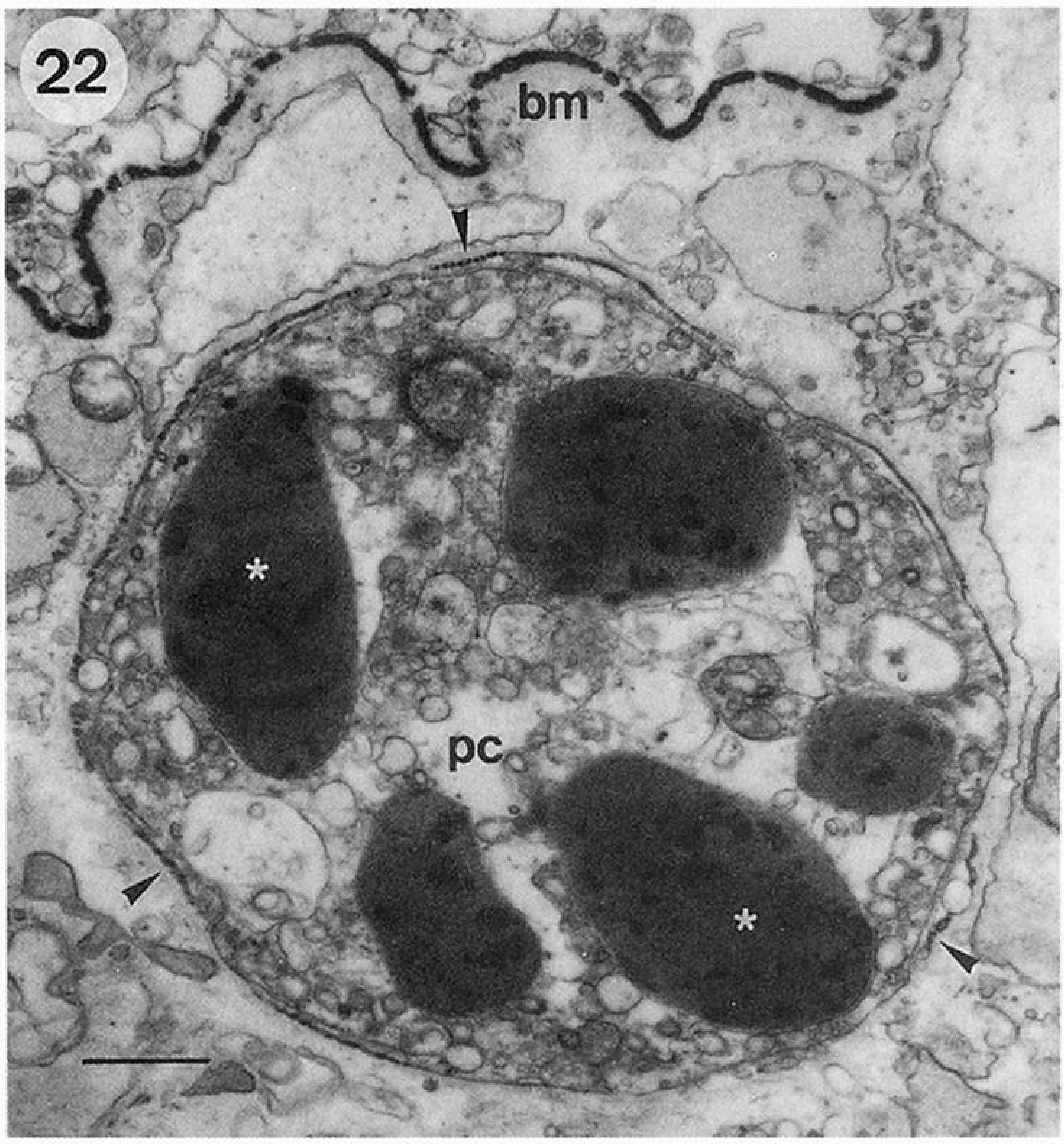
Figure 16. Two multivesicular bodies of a sensory cell. Scale bar 0.2 μm.

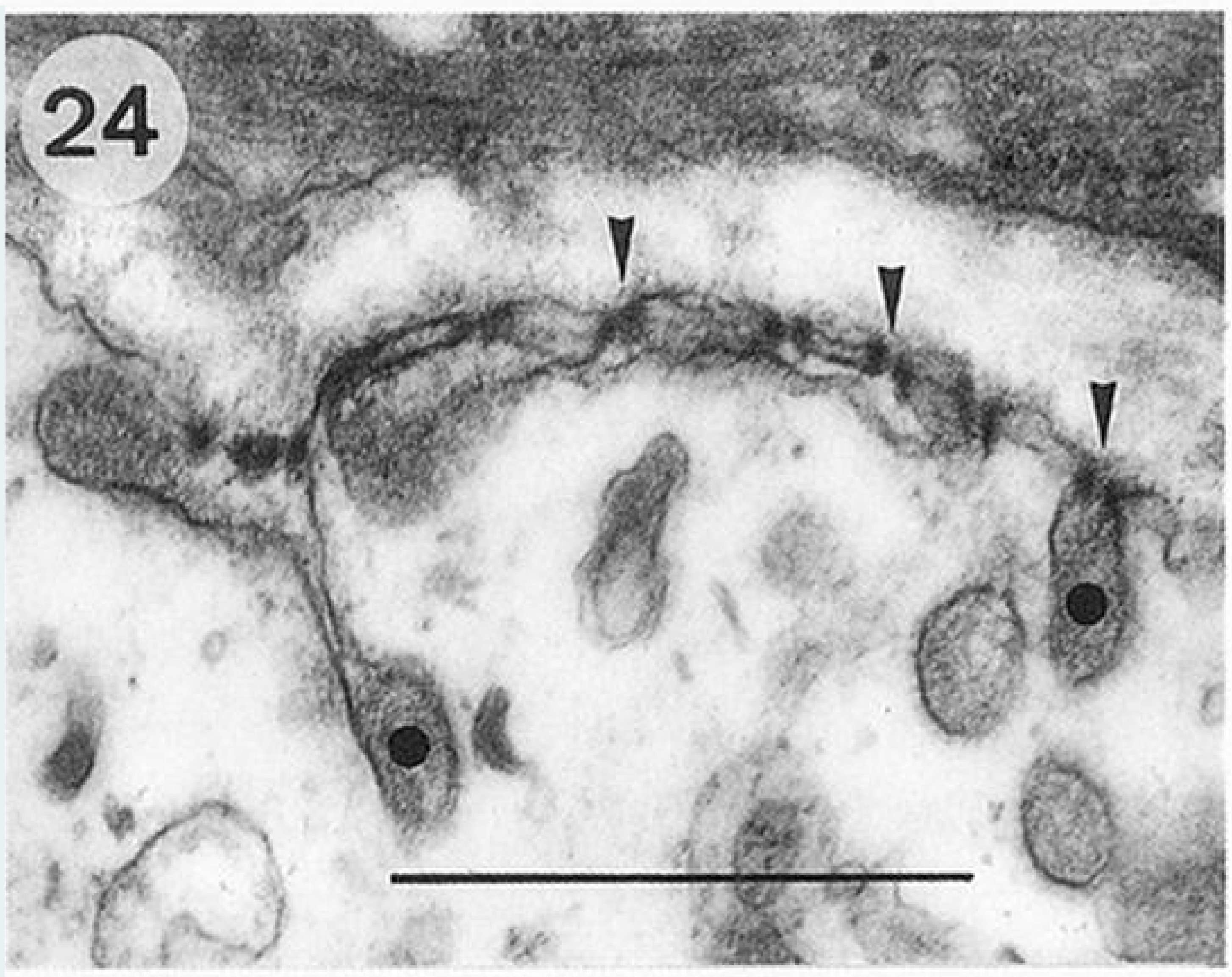
Figure 17. Longitudinal section of a ciliary cell showing two roots (r) and mitochondria (m). Scale bar 1 μm.

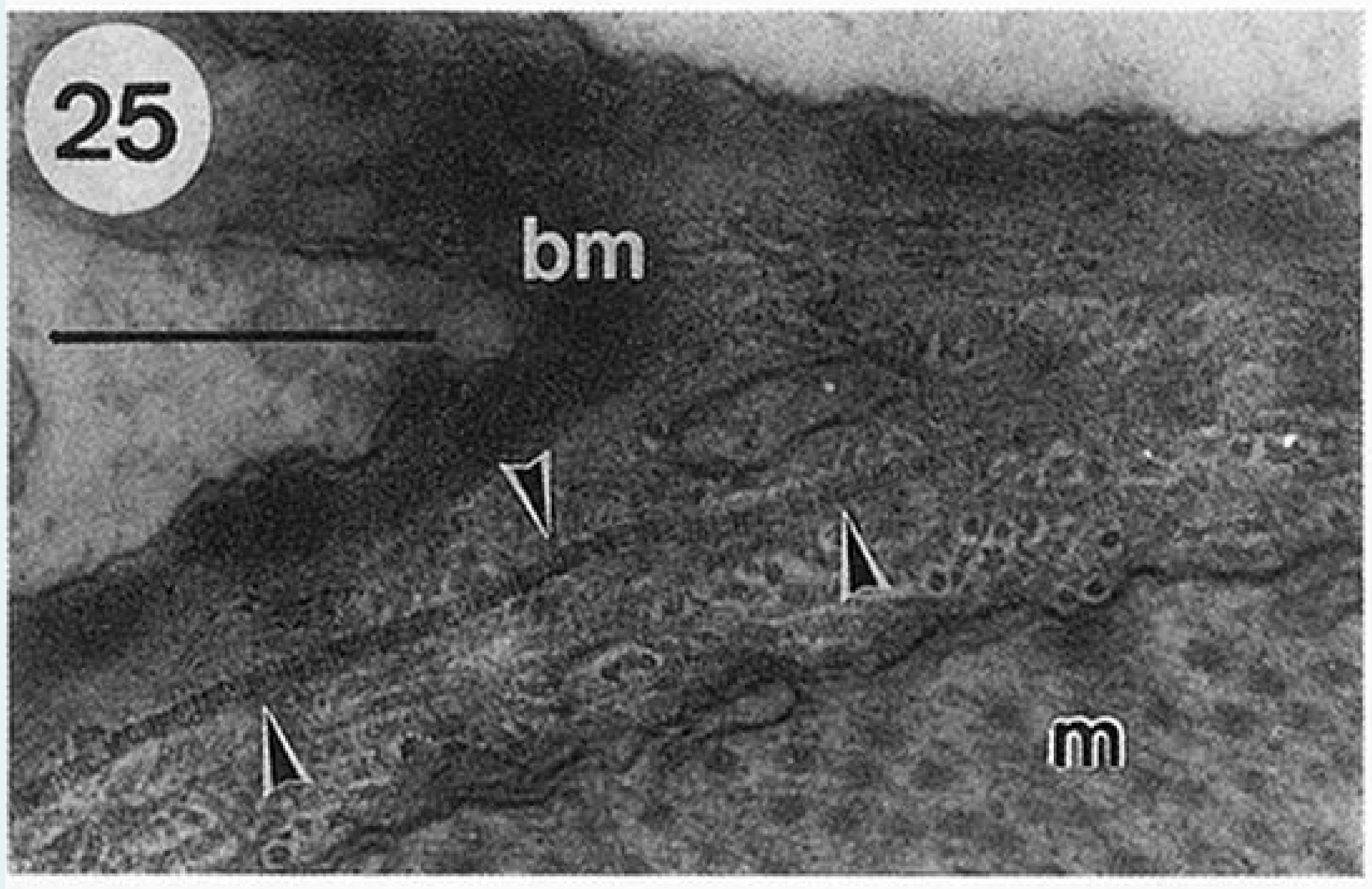
Figure 18. Transverse section of a ciliary tuft showing ciliary shafts arranged in parallel, the direction of main beat is to 'two o'clock'. At a particular height electron dense bulges (arrows) and 'wings' (arrowheads) occur. Microvilli covered with glycocalyx are interspersed basally. Scale bar as in figure 17.

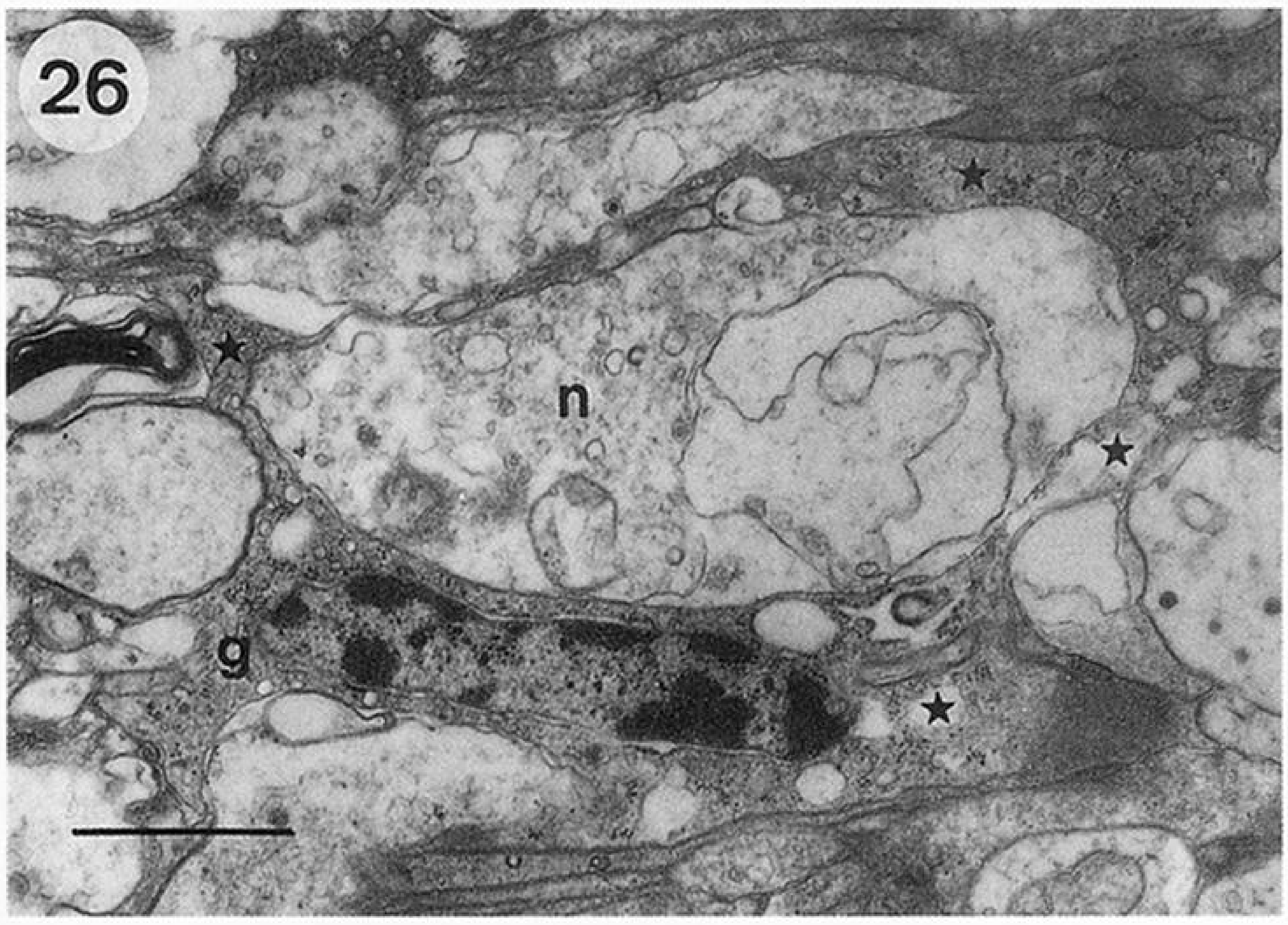












Figures 19–26. For description see opposite