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Ultrastructural Study of Sensory Cells of the Proboscidial Glandular Epithelium of *Riseriellus occultus* (Nemertea, Heteronemertea)

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ABSTRACT Only one sensory cell type has been observed within the glandular epithelium of the proboscis in the heteronemertine *Riseriellus occultus*. These bipolar cells are abundant and scattered singly throughout the proboscis length. The apical surface of each dendrite bears a single cilium enclosed by a ring of six to eight prominent microvilli. The cilium has the typical 9 × 2 + 2 axoneme arrangement and is equipped with a cross-striated vertical rootlet extending from the basal body. No accessory centriole or horizontal rootlet was observed. Large, modified microvilli (stereovilli) surrounding the cilium are joined together by a system of fine filaments derived from the glycocalyx. Each microvillus contains a bundle of actin-like filaments which anchor on the indented inner surface of a dense, apical ring situated beneath the level of the ciliary basal body. The tip of the cilium is expanded and modified to form a bulb-like structure which lies above the level where the surrounding microvilli terminate. In the region where the cilium emerges from the microvillar cone, the membrane of the microvillar apices makes contact with a corresponding portion of the ciliary membrane. At this level microvilli and cilium are apparently firmly linked by junctional systems resembling adherens junctions. The results suggest that these sensory cells may be mechanoreceptors.

The most prominent and distinctive feature of the nemertines is a tubular proboscis that can be everted from a fluid-filled rhynchocoel. The proboscis has been described as serving many functions: prey capture and perhaps defense, locomotion, burrowing, and sensory probing (exploratory tactile organ) (Dakin and Fordham, '36; Pantin, '50; Hyman, '51).

Compared to most other invertebrate phyla, few ultrastructural studies on nemertines have been undertaken; although members of the Nemertea have been subjects of electron microscopic investigation, our knowledge of their ultrastructure remains incomplete (for review see Turbeville and Ruppert, '85; Turbeville, '91). Their most conspicuous organ, the proboscis, has scarcely been studied at the ultrastructural level; the only electron microscopic studies of the proboscis are those of Ling ('71), Anadon ('76), Stricker and Cloney ('81, '83), Turbeville and Ruppert ('85), and Turbeville ('91). This is rather surprising, since this organ is of crucial importance to nemertine biology and an important character for phylogenetic considerations.

Because the everted proboscis is involved in the functions mentioned above, the epithelial layer exposed to the external environment should possess sensory receptors. These receptors have been shown with light microscopy (Gibson, '83; Norenburg, '86), which provided evidence for such an assumption. To date, detailed published accounts on the fine structure of proboscis sensory receptors have been restricted to the heteronemertines *Lineus ruber* by Ling ('71) and *L. viridis* (=*gesserensis*) by Anadon ('76), and the paleonemertine *Tubulanus pellucidus* by Turbeville ('91). Sensory cells of the proboscis of *T. pellucidus* characteristically display modified microvilli surrounding one cilium, and a com-

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plex, branched rootlet fiber arising from the ciliary basal body (Turbeville, '91). Sensory cells with a similar organization occur in the proboscis of *L. ruber* and *L. viridis*.

In the suboral and marginal ciliary bands of an unidentified pilidium larva, similar collar cells were observed associated with neurons (Lacalli and West, '85). In the cerebral organ of the hoplonemertines *Tetrastemma candidum, Paranemertes peregrina,* and *Amphiporus lactifloreus,* there are sensory cells possessing one (or rarely two) cilium and a ring of microvilli (Amerongen and Chia, '87); these authors suggested that such receptor cells are chemosensory.

Collared sensory cells described in nemertines have two structures in common with many epithelial mechanoreceptor cells in other animals, namely stereovilli (modified microvilli) and one associated, modified or unmodified cilium (Flock, '71; Teuchert, '76; Ehlers, '77; Ehlers and Ehlers, '77; Bone and Ryan, '78). This type of receptor has been the subject of much interest and speculation. Notwithstanding the general interest in nemertines and the desirability of ultrastructural studies for detailed comparison with those in other groups, turbellarians and annelids, with which they are said to be related (Turbeville and Ruppert, '85), detailed information of their structure is lacking. Moreover, only a comparative examination of the sensory cells of the nemertine proboscis would allow one to clarify their structural characteristics common to other receptors of known function.

The present work focuses on the ultrastructure of the proboscis sensory cells of the heteronemertine *Riseriellus occultus* with the aim of 1) filling the gap that still exists in our knowledge about the ultrastructure of such receptors in nemertines and 2) relating their structure and function. While our data concerning the gross morphology of these sensory cells coincide with those obtained by Ling ('71), Anadon ('76), and Turbeville ('91), we obtained different information about the architecture of their dendrite which is presented in this paper. We discuss the putative function of these receptors in relation to their structure and, briefly, their phylogenetic implications.

MATERIALS AND METHODS

Specimens of the heteronemertine *R. occultus* (Rogers et al., '93) were collected by hand at low tide from the Foz Estuary, northwestern Spain, during October 1993. The individuals were found on the upper shore in consolidated mud among roots of *Spartina* sp. and in muddy sands with *Zostera noltii*. The worms were kept in an aquarium with muddy sand on the bottom and supplied with running seawater at about 16°C.

The specimens were processed 1–2 days after collection. Prior to fixation for transmission electron microscopy, the animals were relaxed by immersion in cold (2–4°C) seawater for about 10 min, until motionless, to prevent fixation-induced autotomy and contractions. Slow and complete proboscis eversion occurred spontaneously when the worms were transferred into the cold fixative fluid. Subsequently, proboscides from 10 animals were quickly cut off with razor blades, and portions of the anterior, middle, and posterior regions were cut from those with the aid of a stereomicroscope. The fixation of these small pieces, representing different regions of the proboscis, was completed in the same fixative fluid.

The samples were fixed in a variety of solutions in an effort to find the optimal fixation. The most satisfactory techniques were 1) immersion in 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.4) at 4°C for 2 h; the fixed samples were rinsed at 4°C with the same buffer containing 0.5% sodium chloride, and then postfixed in 1% osmium tetroxide in 0.2 M sodium cacodylate buffer (pH 7.4) at 4°C for 2 h; and 2) immersion in 2.5% glutaraldehyde, 0.5% sodium chloride in 2.5 M Millonig's ('64) phosphate buffer (pH 7.4) at 4°C for 2 h; the fixed portions were rinsed at 4°C with the same buffer containing 1.07% sodium chloride, and subsequently postfixed in 1% osmium tetroxide, 0.5% sodium chloride in 0.2 M Millonig's phosphate buffer (pH 7.4) at 4°C for 2 h.

Thereafter, each group of samples was stained en bloc with 2% uranyl acetate in 70% ethanol for 2 h during ethanol-propylene oxide dehydration and routinely embedded in Araldite resin.

Ultrathin serial sections were cut with a diamond knife on a Reichert-Jung Ultracut E ultramicrotome, and ribbons of sections were mounted on Formvar-coated copper grids, poststained with lead citrate, and then examined in a Zeiss E 10C transmission electron microscope operating at 80 kV.

RESULTS

The general organization of the proboscis of *R. occultus* (for a detailed description of the morphology of this nemertine see Rogers

et al., '93) closely agrees with the generalized heteronemertine form described by Ling ('71).

When the proboscis is everted, its tissue layer exposed to the external environment is a columnar epithelium composed of interdigitated supportive cells with microvilli, numerous gland cells of different types, and sensory cells distributed among them (Fig. 1). The apical cytoplasmic extensions of this epithelium are hidden under a mucous layer. Because the fixation procedure often causes loss of this layer, such structures frequently appear uncovered. Stricker and Cloney ('83) refer to this epithelium as the "glandular epithelium" (GE) of the proboscis.

As indicated above, the proboscidial GE of *R. occultus* is equipped with sensory cells (Fig. 2) intermingled with the other cell types. These sensory cells are relatively abundant and are scattered singly all over the proboscis surface throughout its length. Their distributional pattern, whether ordered or random, is not apparent in the GE.

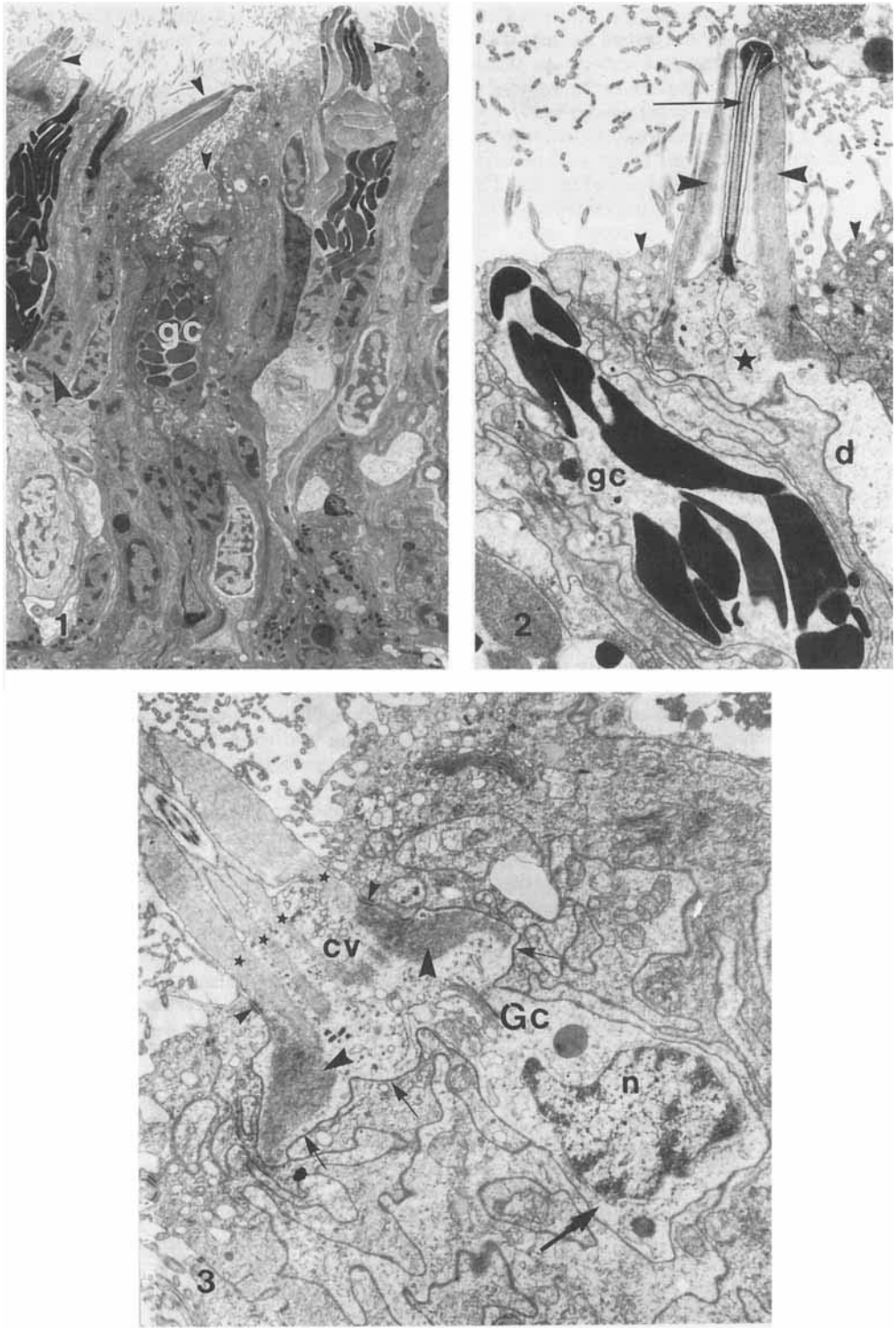
Only one sensory cell type has been observed. The bodies of these cells are situated entirely within the GE of the proboscis (Fig. 3). They are primary sensory cells; their relatively small perikaryon, generally located in the middle region of the GE, bears the nucleus and a thin layer of surrounding cytoplasm that is largely free of organelles and other structures. From each perikaryon a single dendrite extends distally between supportive cells and gland cells and terminates at the epithelium surface to form the receptor process (Figs. 2, 3), while proximally a single axon leaves its base to join the basiepithelial nerve plexus. The dendrite is a slender process with relatively clear cytoplasm; its apical portion expands forming a bulb beneath the level of the epithelium surface (Fig. 3), and is connected to the adjacent epithelial cells by apicolateral zonula adherens and septate junctions (Fig. 4). The apical surface of each dendrite bears a single cilium enclosed by a ring of prominent microvilli (Fig. 2). In transverse sections these microvilli appear regularly spaced, forming a circular collar surrounding the central cilium (Fig. 5). This collar and ciliary apparatus is the most striking ultrastructural feature of these bipolar, monociliary sensory cells.

The cilium (3.5–5.5 μm long) protrudes from the middle of the apical dendrite surface being level with the cell membrane and the epithelial cell surface, and extending beyond the microvillar tips (Fig. 2). The cilium arises from a typical basal body from which a tapering, cross-striated rootlet extends up to 1.7 μm into the dendrite (Fig. 6). The basal body is embedded in a moderately electron-dense material and delicate strands appear to link its distal end to the cell membrane (Figs. 6, 7). No accessory centriole has been observed.

The cilium narrows for a distance of about 0.23 μm from the apical surface of the dendrite (Fig. 6) so that its diameter at this level is only 0.22 μm. Beyond this short region the diameter of the cilium increases to approximately 0.4 μm.

Longitudinal sections through the narrow basal part of the ciliary shaft show the typical appearance of the transition zone in Metazoa (Pitelka, '74). The centriolar triplet microtubules change to doublets at this level, and transverse sections through this part show the ciliary membrane tightly constricted around a crown of nine microtubular doublets devoid of arms and embedded in a layer of dense material (Fig. 8). The cilium has a basal plate approximately 0.23 μm from the surface of the cell; this ciliary basal plate is located just at the upper level of the shaft constriction (Fig. 9). The cilium is smooth and cylindrical (Fig. 10); its ciliary axoneme has a $9 \times 2 + 2$ arrangement of microtubules. The two central microtubules appear to originate about 0.1 μm above the basal plate; the peripheral doublets of microtubules are electron dense but appear very likely to have the normal complement of dynein arms (Figs. 5, 11). Faint spokes run radially from the central microtubules to the microtubular doublets.

The tip of the cilium is expanded and modified to form a bulb-like structure approximately 0.7 μm in diameter, which lies above the level where the surrounding microvilli terminate (Fig. 10). The axonemal microtubules terminate inside this globular tip (Fig. 12) in a cluster of coarse, electron-dense granules (approximately 36 nm in diameter). These granules appear arranged forming a cap-like structure (Figs. 12, 13). Furthermore, within the ciliary tip a "swallow's nest"-shaped structure is formed of electron-dense material in which the ends of the axonemal microtubules and the granules surrounding them are embedded (Figs. 12–14). In sections of samples fixed using Millonig's ('64) phosphate buffer, such material had a granular appearance. At the level of the ciliary tip there is a cylinder of electron-dense material



Figures 1–3

located beneath the peripheral axonemal microtubules (Figs. 14, 15).

The cilium is surrounded by a circle of usually seven, but sometimes six or eight, prominent closely apposed microvilli (Figs. 5, 11). These microvilli extend from the apical surface of the dendrite and taper distally displaying a cone-like arrangement (Fig. 10). The microvilli are between 3 and 4 μm in length. At their base they are polygonal or bean-like in appearance in cross section, 0.5–0.7 μm in diameter. Proceeding toward the microvillar tip the section becomes circular, tapering to approximately 0.16 μm in diameter.

Each microvillus contains a bundle of orderly actin-like filaments, approximately 6 nm in diameter, arranged longitudinally and closely packed in a dense, paracrystalline pattern (Figs. 9, 11, 16). The filaments terminate at the microvillar tip in an apical cap of electron-dense material (Fig. 12). The filaments of each microvillus extend proximally as a bundle for about 2.5 μm into the dendrite, just under each microvillus, forming a rod-like structure that anchors in the apical cytoplasm like the fibrillar rootlets of stereovilli (Figs. 10, 17). An electron-dense, granular material forms a prominent, characteristic apical cylinder beneath the level of the ciliary basal body, enclosing the striated ciliary rootlet, mitochondria, a Golgi complex, and several vesicles (Figs. 3, 4, 18, 19). This dense sheath is in close contact with the cellular membrane associated with the adherens junctions at the lateroapical cell membrane connecting the receptor ending to neighboring supportive epithelial cells (Figs. 3, 4, 19). The filament bundles of the microvilli are anchored on the indented inner surface of this dense ring.

The microvillus collar has an inner diameter of approximately 0.5 μm for most of its length, but narrows somewhat near its end. The microvilli keep a distance of 50 nm from the central cilium in their basal two-thirds but they converge on the cilium distally. The apices of the microvilli approach and come into contact with the cilium beneath its globular tip (Fig. 12). Adjacent microvilli are connected laterally over their entire length by a system of fine strands of glycocalyx material. In cross sections, this fibrillar material extends from microvillus to microvillus in a series of concentric rings (Figs. 8, 9). Apparently, the filaments link the microvilli of the collar together, forcing them into a closed cylindrical arrangement, the microvillar cone. Furthermore, a layer of fibrous material is attached to the inner edge of the microvilli in each collar (Figs. 9, 20) and forms a connecting cylindrical membrane. The same material interconnects the microvilli and the cilium (Fig. 11). The microvillar cone and cilium are held in a concentric position by these fibrous components of their membrane coats. The organized structure of this linking system is best revealed in tissue that has been overstained en bloc, although this procedure obscures other details.

In the region where the cilium emerges from the microvillar cone, the membrane of the microvillar apices makes contact with a corresponding portion of the ciliary membrane (Figs. 12, 14, 21) within a short region of up to 0.28 μm in height. In the contact region the distance between both membranes is reduced to approximately 15 nm. At this level microvilli and cilium are apparently linked to a firm structural unit by fibrillar material with moderate electron density completely filling the intermembrane space between both components. In the contact region the membrane of each microvillus is supported additionally by an electron-dense coat on its cytoplasmic surface in which the inner microvillar filaments terminate (Figs. 12, 21). This junctional system resembles an adherens junction. As viewed in longitudinal and transverse sections through the junctional region, an electron-dense plate is located subjacent and attached to the ciliary membrane. Each plate appears as an aggre-

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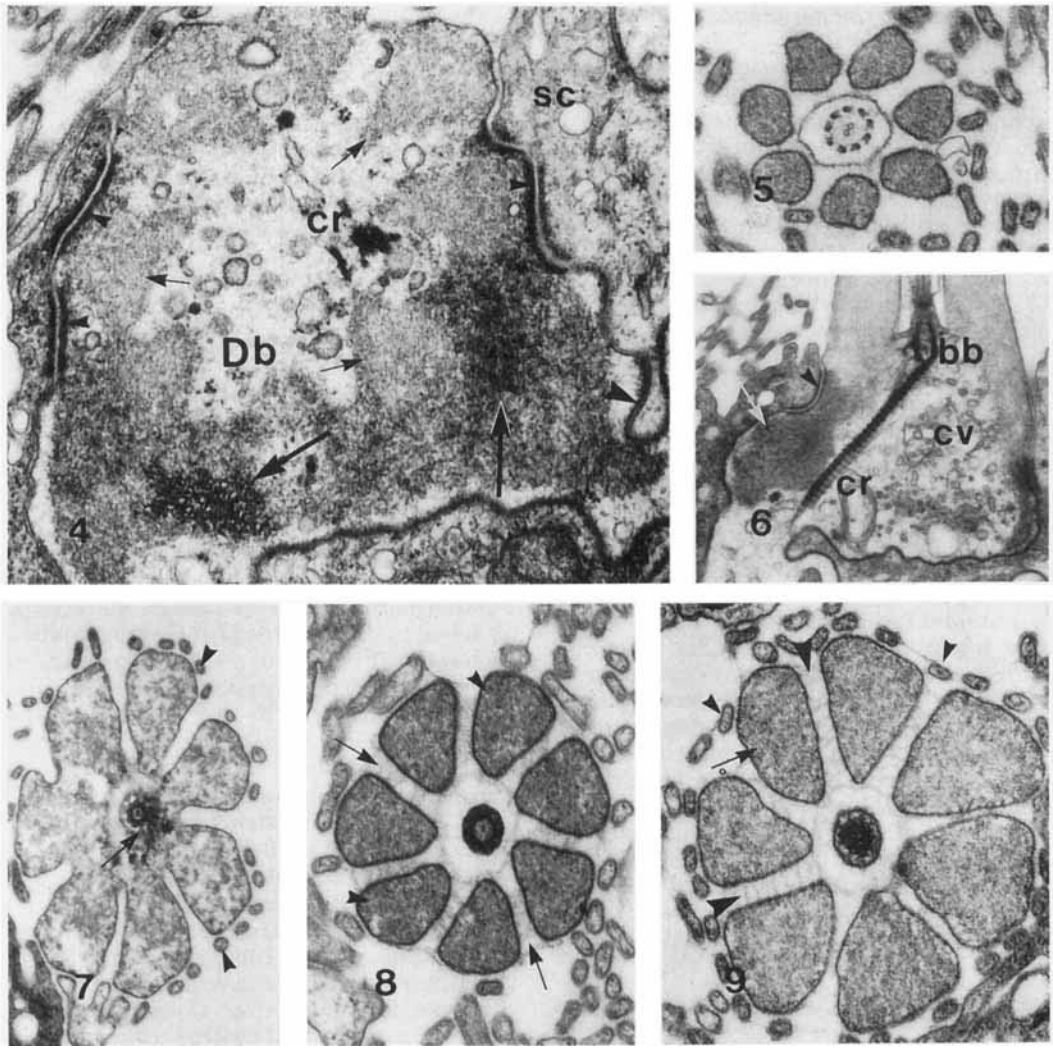
Figs. 1–3. Everted proboscides of *Riseriellus occultus*. Glandular epithelium, transmission electron microscopy (TEM).

Fig. 1. Panoramic view showing gland cells (gc), supportive cell (large arrowhead), and receptor processes (small arrowheads). ×7,800.

Fig. 2. Apical half of the epithelium. Longitudinal section of a receptor process showing the single cilium (arrow) and microvilli (large arrowheads) protruding from the apical surface of the dendrite bulb (star). Small arrowheads point to supportive cells. gc, glandular cell; d, dendrite. ×10,000.

Fig. 3. General appearance of a sensory cell. The large arrow is directed to the perikaryon containing the nucleus (n) and small arrows point to the dendritic bulb. Small arrowheads indicate adherens junctions; large arrowheads show the electron-dense apical cylinder. Stars show the filament bundles of the microvilli extending into the dendritic bulb. cv, clear vesicles; Gc, Golgi complex. ×10,400.



Figs. 4–9. Everted proboscides of *Riseriellus occultus*. Sensory cells of the glandular epithelium. TEM.

Fig. 4. Oblique section of a dendritic bulb (Db) joined with an adjacent supportive cell (sc) by adherens (small arrowheads) and septate junctions (large arrowhead). Small arrows indicate the filament bundles of the microvilli extending into the bulb. Large arrows show portions of the electron-dense apical cylinder. cr, cross-sectioned ciliary rootlet. ×34,500.

Fig. 5. Transverse section through the middle region of a microvillar cone and associated cilium showing their general appearance. ×38,500.

Fig. 6. Longitudinal section of a dendritic bulb and its microvillar cone showing the ciliary basal body (bb), the cross-striated rootlet (cr), clear vesicles (cv), and the electron-dense apical cylinder (arrow). Arrowhead indicates adherens junction. ×14,500.

Fig. 7. Cross section through the basis of a microvillar cone. Delicate strands extend obliquely from the ciliary basal body (arrow) toward the cell membrane. The microvillar cone is enclosed by a thin collar of finger-like, cytoplasmic processes (arrowheads) of the adjacent supportive cells. ×5,200.

Fig. 8. Transverse section through the ciliary transition zone. Note the absence of the two central microtubules of the axoneme at this level, and cross-sectioned filaments filling the microvilli (arrows). Arrowheads point to fibrillar material joining microvilli in concentric rings. ×30,500.

Fig. 9. Transverse section through the ciliary basal plate. Note the cross-sectioned filaments filling the microvilli (arrow). Large arrowheads indicate the fibrillar material connecting adjacent microvilli. Small arrowheads show finger-like, cytoplasmic processes of supportive cells enclosing the microvillar cone. ×31,600.

gate of finely granular, electron-dense material (Figs. 12, 21). Furthermore, very fine strands of material of moderate electron density extend between the plates and the electron-dense material in which the tips of axonemal microtubules are embedded.

The microvillar cones are generally surrounded by a thin collar of long, finger-like protrusions of the apical surface of the supportive epithelial cells surrounding the corresponding dendritic process (Figs. 7, 9, 11). Both dendrite and adjacent epithelial cells are joined distally by zonulae adhaerentes and septate junctions (Figs. 4, 6, 19). In some instances we observed microvillar cones covered by a mucous layer up to 6.1 μm thick (Fig. 22). As a result of their abundance, these microvillar cones show a close spatial relationship in the uneverted proboscis (Fig. 23).

The cytoplasm of the dendrite process also contains a few mitochondria clustered near the ciliary rootlet, as well as short segments of rough endoplasmic reticulum. A single Golgi body lies immediately below the ciliary apparatus (Fig. 3). Multivesicular bodies and a relatively large number of vesicles of varying diameter and electron density occur scattered in the region surrounding this Golgi body. Many clear vesicles of varying size are typically concentrated in the apical region of the cytoplasm among the filament bundles of the microvilli (Figs. 3, 6, 17). They have not been seen to discharge externally. No microtubules were observed inside the dendritic process.

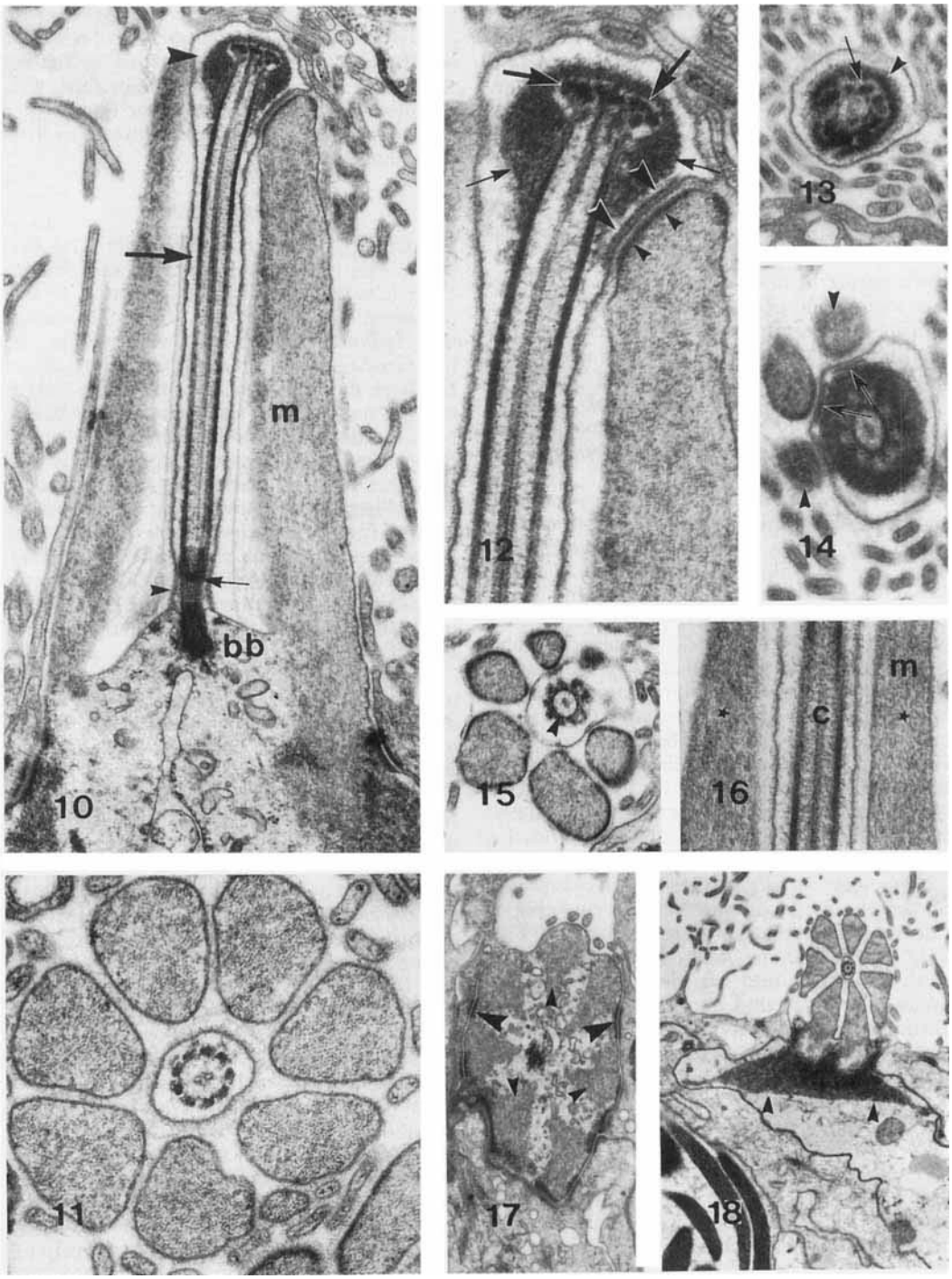
The clear, moderately granular cytoplasm of the sensory cell perikaryon contains a nucleus of an irregular ellipsoidal shape (Fig. 24). The nucleus is situated near the center of the cell body and has clearly demarcated areas of hetero- and euchromatin. The euchromatin is in contact with the nuclear envelope and often extends toward the center of the nucleus. A few mitochondria and profiles of rough endoplasmic reticulum occur in the narrow ring of cytoplasm that surrounds the nucleus.

A single cytoplasmic process extends proximally from the base of the perikaryon and becomes a slender axon. The thin axonal profile, seldom exceeding 1 μm in diameter, extends between other elongated processes of supportive and gland cells toward the basiepithelial nerve plexus. The axon contains a few profiles of rough endoplasmic reticulum and small mitochondria near its origin. It has not been possible to follow the course of the axon, as a result of its irregular outline, or to identify it in the nerve plexus, because this axon is indistinguishable from other nerve fibers of the plexus; its synapses have not been identified either.

DISCUSSION

Although collared sensory cells have been described in the proboscis of the heteronemertines *Lineus ruber* (Ling, '71) and *L. viridis* (Anadon, '76), and in that of the paleonemertine *Tubulanus pellucidus* (Turbeville, '91) by transmission electron microscopy, this is the first detailed description of their structure in nemertines. Our studies on the proboscis sensory cells of the heteronemertine *R. occultus* extend results obtained by such previous ultrastructural research and reveal new observations supporting a mechanoreceptive function of those cells. Earlier ultrastructural investigations on the collared sensory cells of the proboscis of the nemertine species mentioned above and the present data indicate that all of them represent the same type of sensory cell.

While the gross ultrastructural appearance of these cells is similar in the species studied, there are some morphological differences among them, although the shape and dimension of the microvillar cone and its cilium vary with stretching of the proboscis. Some of the differences, e.g., the number of microvilli, are obviously species-specific. The assumption of Anadon ('76), however, that the collared sensory cells in the proboscis of *L. viridis* possess one cilium or, more frequently, two cilia is based on electron microscopic investigations on uneverted proboscides that had been fixed with a relatively rough fixation technique. Furthermore, the abundance of collared sensory cells makes their cilium-microvilli complexes appear in close spatial relationship in uneverted proboscides. Considering our ultrastructural data and similar findings (Ling, '71; Turbeville, '91), Anadon's ('76) reconstruction of the apical cell surface of this sensory cell type in *L. viridis* should be modified. In *R. occultus*, *L. ruber*, and *T. pellucidus*, and surely also in *L. viridis*, these sensory cells bear only one cilium and a ring of microvilli. It is important to note, however, that no similar receptors have been reported in the epidermis of any nemertine.



Figures 10–18

The collared sensory cells in the proboscis of *R. occultus* do not have a diplosomal ciliary basal body, one of the features that characterize the "choanocyte-like" cells or "collar" cells (Rieger, '76). The cilium extends a short distance above the microvilli tips, but the mucous coating that is prominent in the nemertine proboscis would conceivably also cover portions of the cilium. The microvilli of these sensory cells are characterized by a stout bundle of densely packed, actin-like filaments which could serve as a stabilizing structure. A similar core of actin filaments occurs in stereovilli of several sensory cells of other metazoans (Welsch and Storch, '76; Hudspeth and Jacobs, '79; Tilney et al., '80; Phillips and Friesen, '82; Kinnamon and Westfall, '84; Wolfrum, '90; Golz and Thurm, '91; Kass-Simon and Hufnagel, '92; Golz, '94). The fact that the filamentous cores of the sensory cell stereovilli in *R. occultus* extend into the dendrite without a basal constriction indicates a particular rigid organization of such stereovilli. As in *L. ruber* (Ling, '71) and *L. viridis* (Anadon, '76), an apical electron-dense cylinder exists in the dendrite of these sensory cells in *R. occultus* and represents a specialized part of the cytoskeleton upon which the bundles of stereovillar filaments anchor.

One new morphological detail revealed by our study is the spaced bridges of filamentous material between adjacent stereovilli of the collar. The regularity of the tubular arrangement of the stereovilli around the cilium may be related to the bridges found between them. This would indicate that the structural integrity of each stereovillar cone depends not only on the core of filaments of its stereovilli and its anchorage within the dendrite but also on the presence of such filamentous bridges. Adjacent stereovilli of other sensory cells are also known to be interconnected by similar cross bridges (Phillips and Friesen, '82; Neugebauer and Thurm, '84; Schmidt and Thurm, '84; Takumida et al., '88; Golz and Thurm, '91; Nagel et al., '91; Pickles et al., '91; Golz, '94). We suppose that the stereovillar cone—anchored at the intracellular cytoskeleton and stabilized by the stereovillar filamentous cores and the interstereovillus bridges—surrounds the cilium as a solid mechanical support. This presumption is substantiated by our observation that stereovillar cones always show the same straight outline. In *R. occultus,* the tip of each stereovillus is linked to the cilium by a structure that has been considered a specialized junction. These junctions within the stereovilli-cilium complex are exclusively located within the contact region indicating a specific function. Obviously the modified stereovilli of these collared sensory cells in *R. occultus* have a stabilizing function for the cilium and the firm connections of the stereovillar tips to the cilium suggest that this cilium is nonmotile.

The proboscis of *R. occultus* is the first part of the body to be exposed to the medium when exploring; therefore, the GE of the

Figs. 10–18. Everted proboscides of *Riseriellus occultus.* Sensory cells of the glandular epithelium. TEM.

Fig. 10. Sagittal section of a microvillar cone showing the ciliary basal body (bb), transition zone (small arrowhead), basal plate (small arrow), shaft (large arrow), and globular tip (large arrowhead). m, microvillus. ×27,700.

Fig. 11. Cross section through the middle region of the ciliary shaft. The ciliary axoneme has a 9 × 2 + 2 arrangement of microtubules. Note the actin-like filaments filling the microvilli. ×52,300.

Fig. 12. Detail of the contact region between the tips of the cilium and a microvillus. Note the electron-dense coat (small arrowheads) on the cytoplasmic surface of the microvillus and the electron-dense plate (large arrowheads) in the ciliary cytoplasm. Large arrows point to the cluster of electron-dense granules in which axonemal microtubules terminate. Small arrows indicate electron-dense material enclosing the granule cluster. ×56,700.

Fig. 13. Cross section through the globular ciliary tip showing electron-dense granules (arrow) enclosed by electron-dense material (arrowhead). ×28,400.

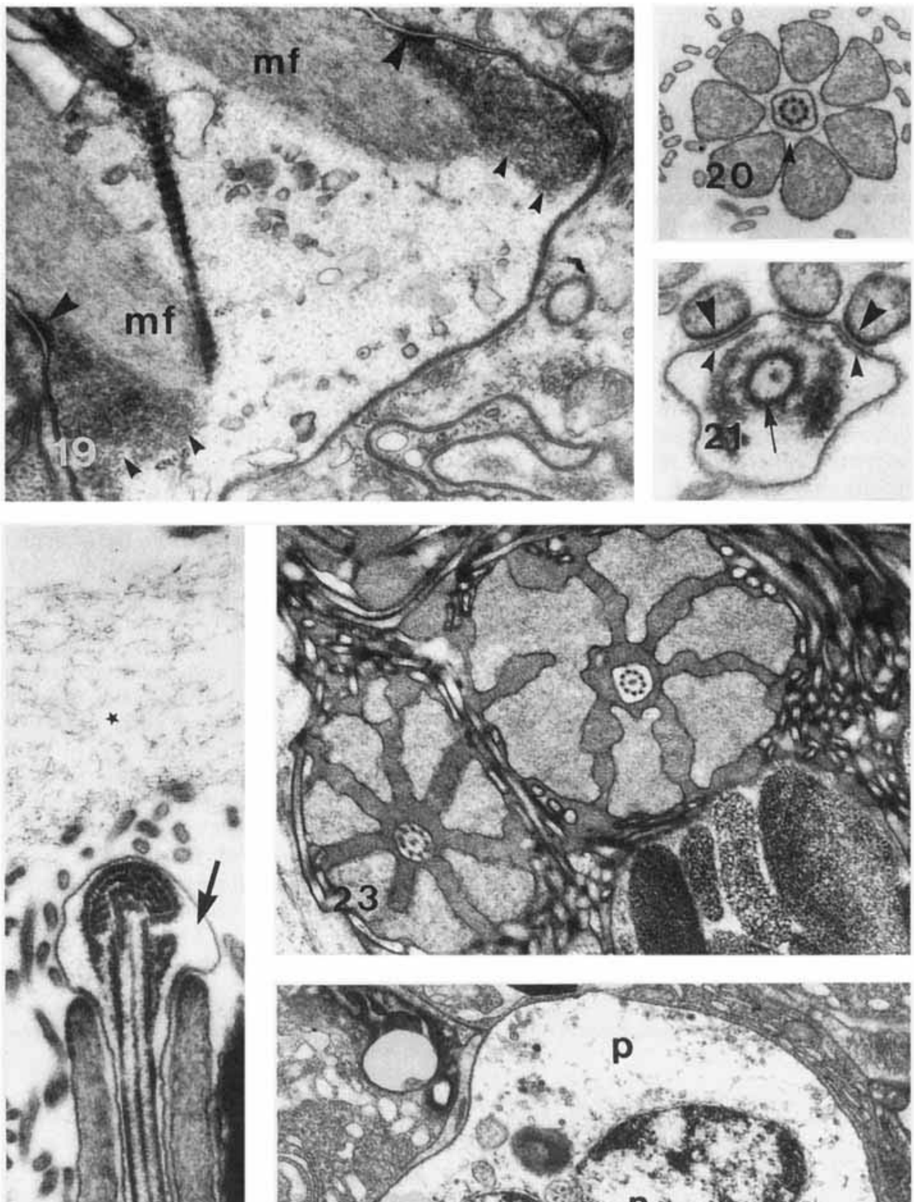
Fig. 14. Cross section through the microvillar tips (arrowheads) showing contact regions (arrows) between them and the globular ciliary tip. ×25,200.

Fig. 15. Cross section through the base of the globular ciliary tip showing the electron-dense cylinder (arrowhead) beneath the peripheral axonemal microtubules. ×25,600.

Fig. 16. Longitudinal section of the middle region of a microvillar cone. The microvilli (m) contain filaments (stars) arranged longitudinally and closely packed in a paracrystalline pattern. c, cilium. ×42,300.

Fig. 17. Transverse section through the apical region of a dendrite bulb showing seven cross-sectioned filament bundles (small arrowheads) of the microvilli, adherens junctions (large arrowheads), and clear vesicles. ×14,300.

Fig. 18. Oblique section of a dendritic bulb showing the electron-dense apical cylinder (arrowheads). ×9,800.



Figures 19–24

proboscis must be a sensory surface of major importance for the animal. The ultrastructural features of its collared cells (presence of a single, modified cilium with its specialized associated structures) and their distribution all over the proboscis surface throughout its length are strong arguments suggesting these are possible sites of sensory reception, and consequently their sensory function.

The cilium-stereovilli complex is a common component of a great number of different types of epithelial receptors that are widespread in metazoans (Flock, '71; Teuchert, '76; Ehlers and Ehlers, '77; Bone and Ryan, '78; Holstein and Hausmann, '88; Budelmann, '89; Kass-Simon and Hufnagel, '92; Golz and Thurm, '94), but distinctions between them can be observed. It can be assumed that the different types of receptive endings have different functions. It is difficult to distinguish the sensory nature of collared cells solely on the basis of their structure; there is no reliable ultrastructural criterion whereby the modality of these kinds of receptors can be determined. The most important functions discussed for such cells are chemo- and mechanoreception; however, the morphology of their dendrite gives no definitive clue to their modality, since both mechano- and chemoreceptors are commonly ciliated. Although there is no physiological evidence for the sensory modality of most of these collared receptors, experimental findings verify a mechanoreceptive function for cnidarian nematocytes (Golz and Thurm, '91; Golz, '94). Thus, collar receptors in invertebrates are usually interpreted as mechanoreceptors. Although any statement of function for the collared sensory cells of the nemertine proboscis is speculative in the absence of neurophysiological data, a hypothesis of function can be advanced based on their morphological similarity to known receptors. The ultrastructure of the stereovilli-cilium complex of collared sensory cells in *R. occultus* resembles that of collar cells (Budelmann, '89) with putative or ascertained mechanosensitivity; this similarity suggests mechanoreception rather than a chemosensory function for those receptors of *R. occultus*.

As a mechanoreceptive structure the stereovilli-cilium complex of collared sensory cells in the proboscis of *R. occultus* should comply with at least two requirements (Thurm and Lewonn, '90): it must be constructed in a manner 1) to withstand structural disruption by strong mechanical forces and 2) to transmit adequate mechanical stimuli to the site of mechanoelectric transduction. Stabilization of the entire stereovilli-cilium complex against mechanical forces is achieved by the formation of a firm cytoskeletal cylinder beneath the apical surface of the dendrite, in close contact with the cellular membrane associated with the adherens junctions. The proximal parts of all the stereovillar cores of actin-like filaments are directly connected to that somatic cytoskeleton, thus anchoring the complete stereovillar cone in the dendrite. Furthermore, the system of filaments connecting adjacent microvilli contributes to this stabilization of the cone. Like the stereovilli and cnidocil of hydrozoan nematocytes (Golz and Thurm, '91), the stereovilli and cilium of the collared sensory cells in *R. occultus* are interconnected by a fibrillar material. The association of the sensory cilium with the stereovilli containing stiff filamentous cores indicates that such a cilium may function as a mechanoreceptive transducer sensitive to displacement by contact with the stereovilli. Thus, mechanical energy could be transferred to the membrane areas of the cilium and stereovilli connected within

Figs. 19–22; 23,24. Everted proboscides of *Riserellus occultus*. Fig. 23. Uneverted proboscis of *R. occultus*. TEMs of sensory cells of the GE.

Fig. 19. Longitudinal section a dendritic bulb. Note adherens junctions (large arrowheads) in close relationship with the electron-dense apical cylinder (small arrowheads) on whose inner surface the filament bundles (mf) of the microvilli are anchored. ×33,500.

Fig. 20. Transverse section through the middle region of a microvillar cone showing fibrous material (arrowhead) attached to the inner edge of the microvilli. ×12,100.

Fig. 21. Oblique section of the globular ciliary tip, showing contact regions between it and three microvilli. Small arrowheads point to the electron-dense plates in the cilium cytoplasm and large arrowheads indicate the electron-dense coat on the cytoplasmic surface of the microvilli. Arrow is directed at the electron-dense cylinder beneath the peripheral axonemal microtubules. ×43,900.

Fig. 22. Longitudinal section of the apical region of a microvillar cone. The globular ciliary tip (arrow) is covered by a mucous layer (asterisk). ×28,400.

Fig. 23. Transverse section of two microvillar cones showing their close spatial relationship. ×18,900.

Fig. 24. Cross section through the perikaryon (p) of a sensory cell, showing the clear, moderately granular cytoplasm. n, nucleus. ×14,300.

the contact regions within which the mechanical stimuli could be transduced.

The ciliary membrane domain to which the mechanical force is transmitted is not directly associated with the axonemal microtubules but is connected to the electron-dense plates located in the ciliary cytoplasm beneath the contact regions; moreover, these plates are associated with the electron-dense material in which the distal ends of the axonemal microtubules are embedded. Because these structures are restricted to the contact regions, we suggest that this part of the cilium is the site of mechanoelectric transduction. If we accept the possibility that the stereovilli-cilium complex could be a morphological feature correlated with functional activity, we may assume that mechanical stimuli acting on the complex could cause mechanical deformation and stress between the cilium and its stereovillar support producing a depolarization of the cell membrane. Due to the circular arrangement of stereovilli and cilium within the contact regions, mechanical stimuli applied transversely to the longitudinal axis of the cilium could produce receptor potentials of similar size independent of the direction of the stimulus to the symmetry axis of the stereovillar cone, as Golz ('94) pointed out for hydrozoan nematocytes. However, as noted above, at the present time there is no physiological evidence to support these views for the collared sensory cells in *R. occultus*.

Cells bearing a single cilium, surrounded by a collar of fiber-linked microvilli, are common among numerous animal phyla (for reviews see Kümmel and Brandenburg, '61; Nørrevang and Wingstrand, '70; Lyons, '73; Rieger, '76; Ehlers and Ehlers, '77). Such cells, generally referred to as collar or choanocyte-like cells, are frequently encountered in the epidermis, where they occur as sensory structures. However, collared cells also function in various aspects of nutrition and excretion among several phyla. A full discussion of the possible taxonomic and phylogenetic implications of such cells is not within the scope of the present paper, but it may be useful to draw attention to some of the gaps in our knowledge of the groups concerned.

The collared sensory cells of *R. occultus* proboscis can be regarded as an example of this cell type. Collar receptors are also known from representatives of several groups within the invertebrates: cnidarians (Holstein and Hausmann, '88), turbellarians (Ehlers, '77; Ehlers and Ehlers, '77; Sopott-Ehlers, '84), nemertines (Ling, '71; Anadon, '76; Lacalli and West, '85; Norenburg, '85; Turbeville, '91), oligochaetes (Knapp and Mill, '71; Storch, '72; Phillips and Friesen, '82), priapulids (Moritz and Storch, '71), chaetognaths (Reisinger, '70), and echinoderms (Nørrevang and Wingstrand, '70). These receptors, however, differ from each other with regard to their position relative to the epidermal cells, the construction of their more or less modified stereovilli and cilium, and their complement of organelles (Moritz and Storch, '71; Ehlers and Ehlers, '77).

Known monociliary receptors in turbellarians show extensive similarity in their main organization (McRae, '67; Storch and Abraham, '72; Bedini et al., '73, '75; Reuter, '75; Ehlers, '77; Ehlers and Ehlers, '77; Sopott-Ehlers, '84; Rohde and Watson, '93). The perikaryon of these receptors is always subepidermal. Ehlers and Ehlers ('77) showed that the collared sensory cells in several groups of the Turbellaria appear to be homologous to each other, and pointed out that this fact does not imply that the embedded receptors in the Turbellaria are homologous to collared sensory cells whose perikaryon is situated entirely within the epidermal epithelium above the subjacent, extracellular matrix, as it occurs in nemertines. In conclusion, the ultrastructure and position of both the stereovilli-cilium complex and the perikaryon of the collared sensory cells in the proboscis of *R. occultus* suggest that they are not homologous to those in turbellarians. Many different types of collared sensory cells have been found in other metazoan phyla. These receptors differ in the number, arrangement, and structure of their microvilli, and in the morphology of cilium, basal body, and ciliary rootlets, as well as in the absence or presence of electron-dense cuffs around the ciliary basis, and in the location of the perikaryon. There are two different possibilities to explain the many different types and the widespread occurrence of collar cells in the Metazoa. The collar cells may be derived, as a result of specializations, from a primitive cell type, which has been retained in several phyla (Nørrevang and Wingstrand, '70; Lyons, '73), or they have arisen more than once within the metazoans, evolving as a result of convergence in response to several functions (Hibberd, '75; Ehlers and Ehlers, '77; Cantell et al., '82).

Sensory cells have, it seems, been evolutionarily conservative, and have utilized two available classes of proteins, tubulin and actin, to construct two classes of cell surface extensions—cilia and stereovilli—which present specialized "transducer" regions of the cell membrane for optimal exposure to environmental stimuli (Moran and Rowley, '83). In accordance with Hibberd ('75), Ehlers and Ehlers ('77), and Cantell et al. ('82), we think the morphological differences between the different types of collar cells are so clearly distinct that it is impossible to assume that all of these are derived from an ancestral choanocyte-like cell. Contrary to the opinion of Nørrevang and Wingstrand ('70) and Lyons ('73), the different types of collar cells do not appear to have a common origin; they most likely have arisen by convergence. Collared sensory cells by themselves are of only limited significance and are by no means reliable criteria for the clarification of the phylogenetic relationships of metazoans. A phylogenetic interpretation within Nemertea of the fine structure of collared sensory cells of the proboscis of the heteronemertine *R. occultus* awaits completion of comparative studies with nemertines of the other classes. The few papers published on this subject (Ling, '71; Anadon, '76; Turbeville, '91) and our study of only one species do not contribute much to such a question.

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