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**S. Montalvo · C. Roldán · J. Junoy**
**P. García-Corrales**

# Ultrastructural study of two glandular systems in the proboscidial glandular epithelium of *Riseriellus occultus* (Nemertea, Heteronemertea)

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**Abstract** Two different types of glandular system in the proboscidial epithelium of *Riseriellus occultus* have been investigated by transmission electron microscopy. As expected, most of the epithelial cells are glandular in nature. With regard to differences in the ultrastructure of these gland cells and in the formation and morphology of their secretory granules, we have categorized and described four types of gland cell, indicated as $G_1$, $G_2$, $G_3$, and $G_4$. Each gland cell has a completely intraepithelial body characterized by a prominent nucleus, developed rough endoplasmic reticulum, Golgi complexes, and numerous secretory granules at different stages of maturation. These four types of gland cell appear associated in pairs forming numerous glandular systems of two types (A, B). These glandular systems are restricted to the ventral surface of the proboscis and are scattered irregularly throughout its length. Each glandular system consists of two gland cells of different types. The gland cell necks in each glandular system extend together to the epithelial surface; they protrude onto this and form a papilla where they open in a common area. The epithelial supportive cells adjacent to the glandular systems have long, stout microvilli which have a core of tonofilaments. These tonofilaments gather into dense bundles which pass vertically through the supportive cells and attach to the extracellular matrix underlaying the cells by hemidesmosomes. Moreover, a single sensory process stands close to each papilla. The ultrastructural morphology of the type A glandular systems suggests that they have an adhesive function operating in a similar way to that of the duo-gland adhesive systems in other invertebrate groups, although they are not homologous with these. The spatial arrangement of the secreted products of the type B glandular systems suggests that these may contribute to increasing the grip of the proboscis on the prey. The secretory granules (=pseudocnids) of the type $G_3$ gland cells are very likely an autapomorphy of the Anopla, providing a character by which the relationships within the Nemertea can be evaluated.

S. Montalvo · J. Junoy · P. García-Corrales (✉)
Departamento de Biología Animal, Universidad de Alcalá,
E-28871 Alcalá de Henares, Spain
Fax: (+34) 18855080

C. Roldán
Departamento de Biología Animal I, Facultad de Biología,
Universidad-Complutense, E-28040 Madrid, Spain

## A. Introduction

Members of the Nemertea possess an eversible proboscis which has been described as a food-catching apparatus (for review see McDermott and Roe 1985). The central lumen of the proboscis is surrounded by a glandular epithelium that becomes the outer layer of the everted part of the organ. Gland cells in the epithelium produce a sticky, venomous mucus composed of various types of secretion products that may serve to increase the adhesion of the proboscis to the prey and causes paralysis or death of the captured organism (Jennings and Gibson 1969; Stricker and Cloney 1983; Kem 1988). The proboscis may also help in locomotion (Pantin 1950; Moore and Gibson 1985; Sundberg 1989) and in burrowing (Wilson 1900; Dakin and Fordham 1936). On the other hand, some morphological features and functions of the proboscis have been used as characters in the systematic of the Nemertea (Sundberg 1989; Gibson 1990; Iwata 1993, Moore and Gibson 1993; Norenburg 1993; Sundberg and Hylbom 1994). For these reasons the proboscis of a number of species of the Nemertea has been studied from different aspects.

The glandular nature of the proboscidial epithelium is frequently mentioned in literature on the Nemertea, but the description of the gland cells is usually neglected (e.g., Jennings and Gibson 1969; Turbeville and Ruppert 1985; Riser 1990; Iwata 1993; Norenburg 1993; Moore et al. 1995). Most of what is known about the glandular epithelium of the proboscis is based on light microscopic studies (e.g., Hylbom 1957; Iwata 1967, 1970; Gerner 1969; Friedrich 1979; Gibson 1979, 1983), but observations at this level offer limited information on the gland cells in this epithelium whose com-

plex construction, even with electron microscopy, is difficult to unravel. However, few reports are available on any aspect of the proboscis, based on transmission electron microscopy (Gontcharoff 1957; Ling 1971; Anadón 1976; Stricker and Cloney 1981, 1982, 1983; Stricker 1984, 1985; Turbeville and Ruppert 1985; Turbeville 1991; Montalvo et al. 1996). There are remarkably few data on the ultrastructure and function of the gland cells in the proboscidial epithelium and most of them are fragmentary. In the Anopla, Ling (1971) described as many as three types of gland cell in the epithelium of the middle region of the proboscis in *Lineus ruber* (Müller, 1744) (Heteronemertea); one forming 'rhabdites' (=pseudocnids), another secreting mucus, and another producing acidophilic granules. Anadón (1976) found four types of proboscidial gland cell in *Lineus viridis* [=*gesserensis*] (Müller, 1744) (Heteronemertea): the first one secreting electron-dense granules, the second one forming less electron-dense granules, the third one secreting mucus, and the fourth one producing rhabdites (=pseudocnids). Gland cells producing rhabdoids (=pseudocnids) have also been observed by Turbeville (1991) in the proboscidial epithelium in *Zygeupolia rubens* (Coe, 1895) (Heteronemertea) and *Tubulanus* cf. *pellucidus* (Coe, 1895) (Palaeonemertea). As for the Enopla, only one species has been studied. Stricker and Cloney (1983) observed four types of proboscidial gland cell in *Paranemertes peregrina* Coe, 1901 (Hoplonemertea). Type I and type II cells form 'fusiform rhabdoids' and 'coalescent rhabdoids', respectively, type III cells contain acidophilic granules and type IV cells produce flocculent material.

The pseudocnids (terminology after Martin 1914) are a special type of rhabdoid only found in the proboscidial epithelium of representatives of the Anopla. Their morphology has been extensively examined by light microscopy (e.g., Jennings and Gibson 1969; Gibson 1979, 1981; Riser 1990; Norenburg 1993), but relatively little is known about their ultrastructure (Gontcharoff 1957; Ling 1971; Anadón 1976; Turbeville 1991). Turbeville (1991) pointed out that the presence of unique pseudocnids in the Palaeonemertea and the Heteronemertea could be provisionally interpreted as a synapomorphy of these groups. Nevertheless, as Norenburg (1993) indicated, attempts to use pseudocnids for the systematic of the Nemertea are perhaps premature because little information is available and it only refers to a few species.

The present investigation was undertaken to study the ultrastructural features of the proboscidial epithelium in *Riseriellus occultus* Rogers, Junoy, Gibson, Thorpe, 1993 (Heteronemertea) and, thus, contribute to the fund of information available on this organ in the Nemertea. This paper deals with the fine structure of four proboscidial gland cells which appear associated in pairs forming two different kinds of glandular system that have not been previously observed in the Nemertea. These glandular systems are compared with adhesive systems of other invertebrate groups and their probable roles are discussed. The ultrastructure of the pseudocnids in *R. occultus* is described, and the sequence of their formation is delineated. This allows us to compare them with those previously described in other species with the aim of determining if all pseudocnids are homologous and can be used in the elucidation of phylogenetic relationships within the Nemertea.

## B. Materials and methods

Specimens of *R. occultus* were collected by hand at low tide from the Foz Estuary, northwestern Spain. The individuals were found on the upper shore in consolidated mud among roots of *Spartina* sp. and in muddy sands with *Zostera noltii* Hornemann, 1832. The worms were kept in an aquarium with muddy sand on the bottom and supplied with running seawater at about 16° C. Small pieces of the anterior, middle, and posterior regions of both everted and uneverted proboscides were obtained, fixed, and prepared for transmission electron microscopy (TEM) according to the methods described in a previous report (Montalvo et al. 1996).

## C. Results

The proboscis of *R. occultus* is an adhesive organ which can stick to substrates tenaciously. In an extraction dish, the animal moves over the bottom and walls; when it is exposed to strong water currents from a pipette, it will attach itself to the bottom of the dish by the proboscis. Once the disturbance has passed, it releases slowly. The outer tissue layer of the everted proboscis is a columnar epithelium (about 15 μm thick) resting on the extracellular matrix (Fig. 1). The height of the epithelial cells varies along the proboscis as a result of different stages of stretching at the time of fixation. This epithelium is composed of three intermingled types of cell. Most of the epithelial cells are glandular in nature, while a few (supportive cells) have bundles of tonofilaments and others are sensory cells (Montalvo et al. 1996). All of the gland cells are intraepithelial. With regard to differences in the ultrastructure of these cells and in the morphology and

**Figs. 1–5** Everted proboscis of *Riseriellus occultus.* Transmission electron micrographs (TEM) of the glandular epithelium ▶

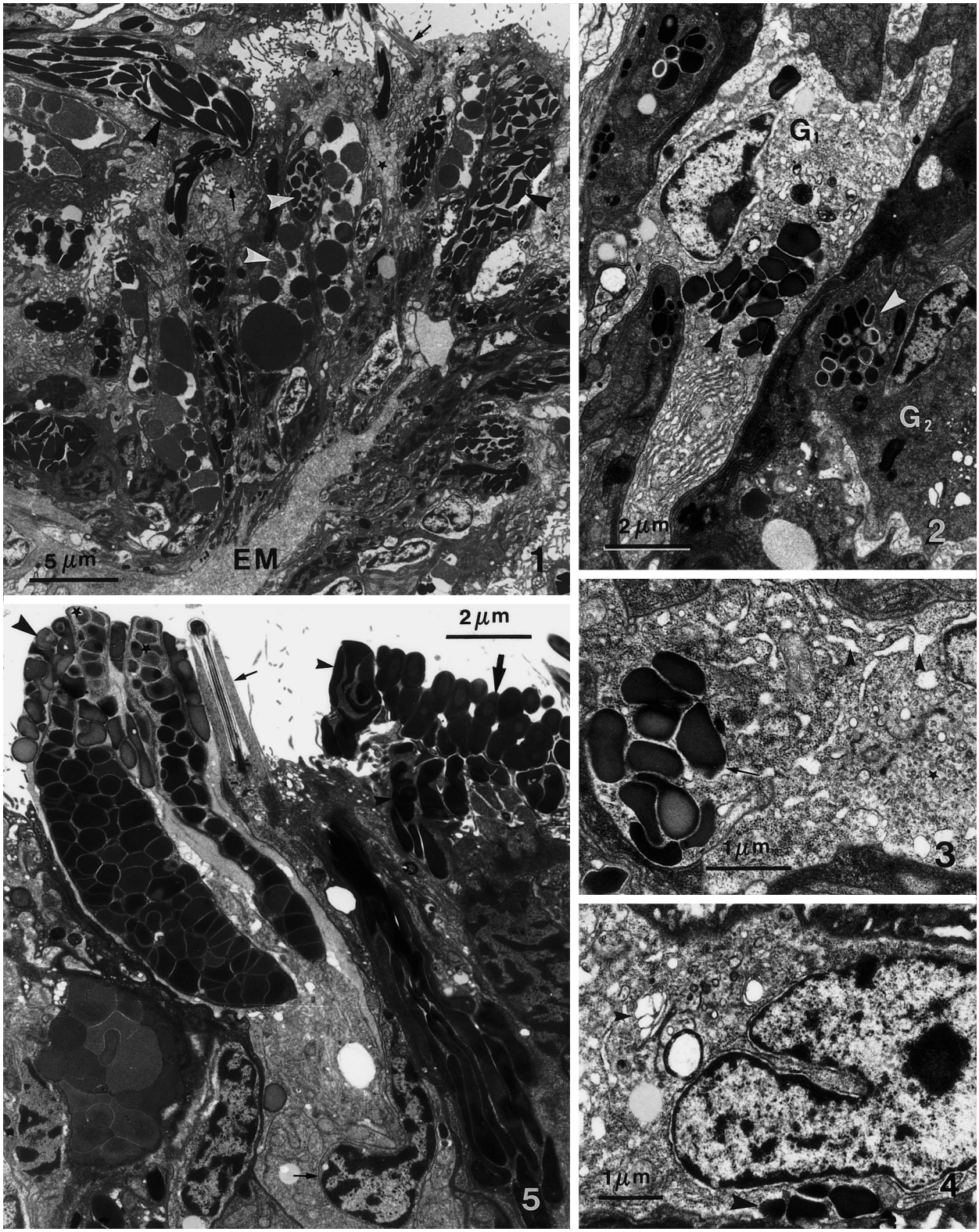
**Fig. 1** Panoramic view showing gland cells (*arrowheads*), supportive cells (*asterisks*), and receptor processes (*arrows*). *EM* Extracellular matrix

**Fig. 2** Low magnification image illustrating the general cell body organization of the type $G_1$ ($G_1$) and $G_2$ ($G_2$) gland cells of a type A glandular system. *Arrowheads* Secretory granules

**Fig. 3** Detail of the type $G_1$ gland cell body. *Arrow* Cluster of secretory granules, *arrowheads* RER cisternae, *asterisk* Golgi-derived vesicles

**Fig. 4** Perinuclear cytoplasm of a type $G_1$ gland cell. *Large arrowhead* Secretory granules, *small arrowhead* Golgi complex and associated condensing vacuoles

**Fig. 5** Longitudinal section of the proboscidial glandular epithelium through a type A glandular system papilla (*asterisks* type $G_2$ gland cell neck, *large arrowhead* type $G_1$ gland cell neck) and a type B glandular system papilla (*large arrow* type $G_3$ gland cell secretory granules, *small arrowheads* type $G_4$ gland cell necks). *Small arrows* Sensory cell




formation of their secretory granules, we have categorized various types of gland cell.

The present work focuses on four types of epithelial gland cell which appear associated in pairs forming numerous glandular systems of two types which are easily recognizable. Transmission electron microscopy reveals that each glandular system is composed of two types of gland cell. The bodies of these gland cells lie adjacent to one another in a more or less discrete group at the base of the epithelium. Processes arising from them extend to-

gether to the epithelial surface where they open in a common area. The glandular systems are abundant, but they are concentrated on the ventral surface of the proboscis and are scattered irregularly throughout its length.

## I. Type A glandular systems

Each glandular system consists of two gland cells of different types, named $G_1$ and $G_2$. These gland cells are easily distinguished as they exhibit differences in cytoplasmic electron density and in the morphology of the secretory granules (Fig. 2).

Both gland cells typically have a proximal, broad body against the subjacent extracellular matrix. The cell bodies have irregular outlines due to their close packing against each other and against other epithelial cells; each one tapers toward a narrower projection, the neck, which extends distally from the cell body to the surface of the epithelium between the other epithelial cells.

The nucleus of the type $G_1$ gland cell shows an irregular profile, lies in the basal half of its cellular body, and is eccentrically positioned (Fig. 2). The overall cytoplasmic electron density of the type $G_1$ gland cell body is clear in appearance, due to the dilated and separate cisternae of the rough endoplasmic reticulum (RER) and to the fact that the cisternae have electron-lucent contents. These RER cisternae fill the central region of the cell body, where they are irregularly arranged. A few Golgi complexes and associated condensing vacuoles are scattered between the RER cisternae in the inner body cytoplasm (Figs. 3, 4). Clusters of secretory granules being formed are observed in the vicinity of the condensing vacuoles which are peripherally located. The secretory granules vary in shape from spherical to polymorphic and in size (0.7–1 μm in average diameter); they contain a homogeneous material of moderate electron density (Fig. 3). Most of them are uniformly dense, but some have a thin, slightly more dense, peripheral layer; this difference may be due to fixation.

The neck of the type $G_1$ gland cell protrudes (4.5–5.5 μm) onto the epithelial surface forming the bulk of a papilla, without any specialized microville surrounding it (Figs. 5, 6). The papillae vary in shape with most being tubiform or cone-shaped. Their outline is approximately circular in cross section, 6–8 μm in diameter. At the papillary level the neck is longitudinally folded showing one deep groove (Fig. 7). Mature secretory granules move within the cell neck where they pack tightly together losing their shape. They make contact with the plasma membrane of the neck so that there is little ground cytoplasm in this area. No typical tubular lining of longitudinal microtubules has been found in the neck (Figs. 7, 8).

The ovoid nucleus of the type $G_2$ gland cell occupies the basal half of its cellular body where it is laterally located. The type $G_2$ gland cell body stands out from the type $G_1$ one because of its greater overall electron density (Fig. 2). It is characterized by an extensively developed RER whose tubular cisternae contain a homogeneous matrix of amorphous, electron-lucent material (Fig. 9). A large number of free ribosomes and short profiles of smooth tubular cisternae lie among those of the RER. The smooth cisternae form buds which become progressively larger and are finally detached and released. The newly formed buds contain granular material that undergoes changes during the course of bud release. At the end of this process, the rounded granules contain a large area of faintly granular material of moderate electron density surrounded by a thin layer of light material (Fig. 9). The granules accumulate forming several clusters peripherally distributed within the cell body.

A developed Golgi complex lies in the vicinity of each granule cluster. The vesicles newly released from the Golgi complex contain amorphous material of moderate electron density which condenses to form solid dense granules. These granules fuse to form larger ones filled with a homogeneous electron-opaque material (Fig. 10). They are closely intermingled with those arising from the smooth cisternae, all of them displaying a marked variation in shape and size. The majority of them are near-spherical to ovoid; however, they are sometimes notched to accommodate one another. Granules of both types (Figs. 9, 11) fuse with each other to form the secretory granules. Secretory granules that are apparently newly formed (judging by the morphology of the granules at the distal end of the type $G_2$ gland cell neck), exhibit a spherical, homogeneous, darkly stained nucleoid. This is enclosed in an area of faintly granular material of moderate electron density that fills the granules (Fig. 9). As these mature, a thin peripheral ring of electron-lucent material is differentiated. Later on, concomitant with the increase in thickness of this light layer, a decrease in the amount of the granular material occurs, suggesting that both events are correlated (Figs. 6, 12). At the end of the maturation process, the faintly granular material becomes more coarse and less dense. As they move along the neck, the granules adopt a near-spherical shape, 0.6–1.2 μm in diameter (Fig. 5), but in the branches of

**Figs. 6–12** Everted proboscis of *R. occultus.* TEM of type A glandular systems ▶

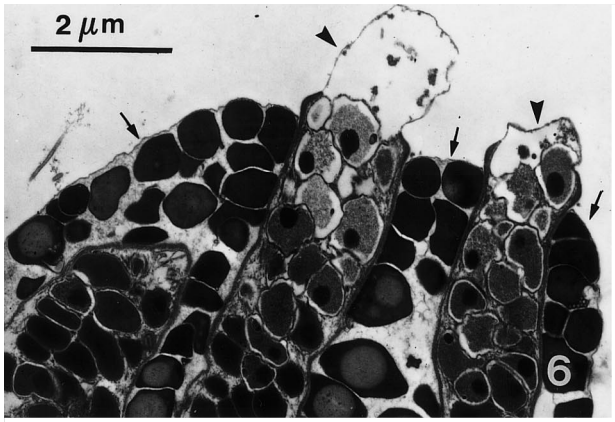
**Fig. 6** Longitudinal section through a papilla showing openings and necks of type $G_1$ (*arrows*) and $G_2$ (*arrowheads*) gland cells

**Fig. 7** Cross section of a papilla. *Arrows* Type $G_1$ gland cell neck, *asterisk* receptor process, *large arrowheads* type $G_2$ gland cell necks, *small arrowheads* plasma membrane lining the groove of the type $G_1$ gland cell neck

**Fig. 8** Longitudinal section through the necks of type $G_1$ gland cells ($G_1$) and type $G_2$ gland cells ($G_2$). *Arrowheads* Lining of tonofilaments within the type $G_2$ gland cell necks

**Figs. 9–11** Details from the cytoplasm of type $G_2$gland cell bodies showing the spatial relationships among Golgi complexes and associated condensing vacuoles (*arrowheads*), RER tubular cisternae (*asterisks*), and secretory granules at different stages of coalescence (*arrow*) and maturation (*1–4*)

**Fig. 12** Cross section through a papilla showing the lining of tonofilaments (*small arrowheads*) within type $G_2$ gland cell necks ($G_2$). *Large arrowheads* Plasma membrane lining the infolding of the type $G_1$ gland cell neck ($G_1$)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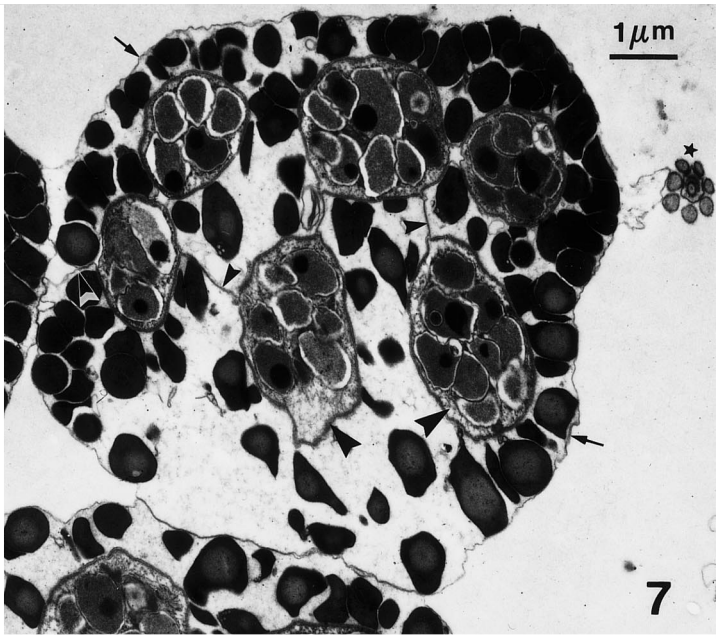
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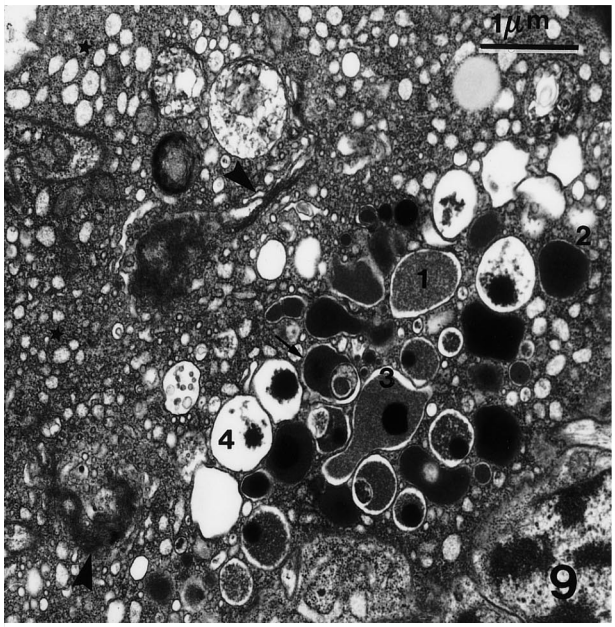
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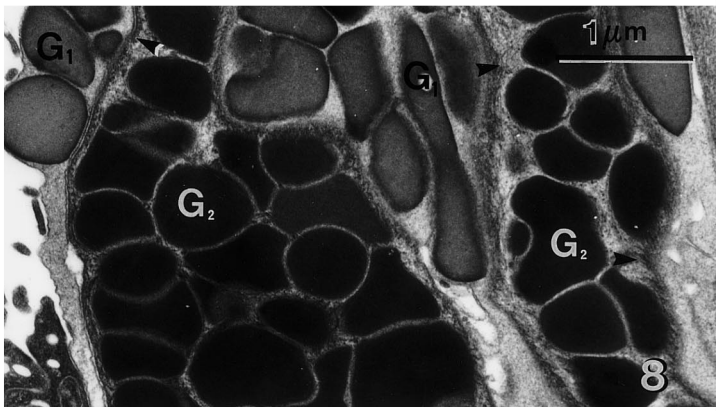
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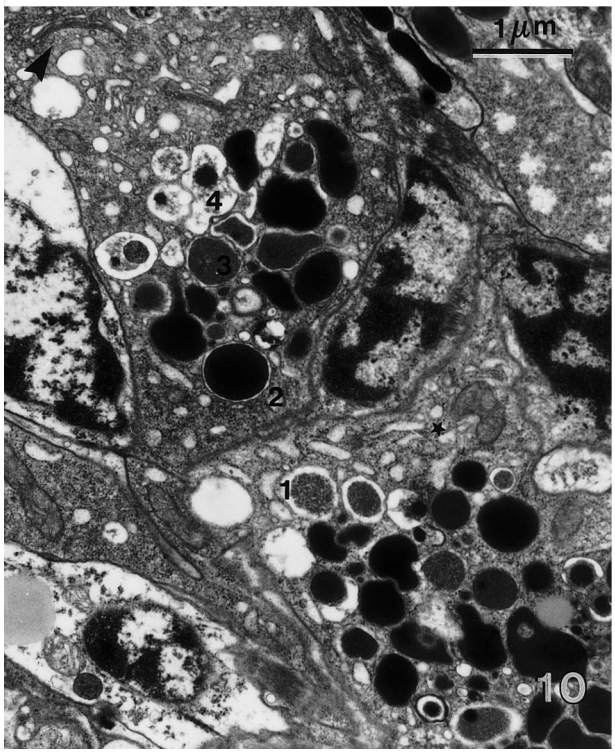
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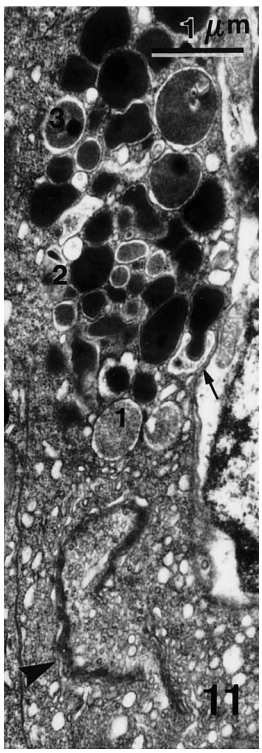
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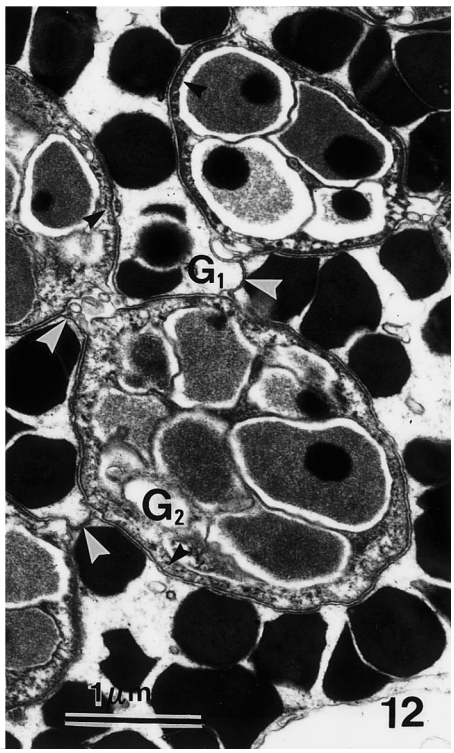
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the neck, at the level of the papilla, they pack tightly together and lose their shape (Fig. 6).

The neck of the type $G_2$ gland cell branches before reaching the epithelial surface (Figs. 5, 6) and it sends four to seven narrow finger-like extensions along the deep groove of the type $G_1$ gland cell neck (Fig. 7). Thus, the termination of this neck enwraps the branches of the type $G_2$ gland cell neck completely in the papilla. The branches protrude slightly (about 0.5 μm) onto the papillary apex where each one opens through its own separate pore (Figs. 5, 6). They are circular in cross section (2–3 μm in diameter) and have a thin layer of electron-dense cytoplasm between the secretory granules and the plasma membrane (Fig. 7, 12). Numerous tonofilaments with a helicoidal arrangement are present in this cytoplasmic layer (Figs. 8, 12). The tips of the branches show blebbing of their plasma membrane.

## II. Type B glandular systems

As in the A type, each type B glandular system is composed of two distinguishable gland cells, named $G_3$ and $G_4$, and their cell bodies lie in the basal region of the epithelium. One process projects from each cell body and these processes extend together to the epithelial surface.

The nucleus of the type $G_3$ gland cell is eccentrically located in the basal half of the cell body and it has an ovoid to spherical shape (Fig. 13). The bulk of the cell body is occupied by numerous secretory granules in various stages of maturation which form conspicuous clusters. In addition to granules, the cell body has an extensive RER and free ribosomes (Fig. 14).

The rod-shaped, immature secretory granules (up to 2.9 μm in length and up to 0.6 μm in diameter) contain a homogeneous, darkly stained matrix (Fig. 13). In many cases, Golgi complexes are visible in the vicinity of incompletely formed granules and small vesicles of the Golgi complex can be seen apparently in the process of contributing to the matrix (Fig. 15). As maturation of the granules proceeds, their length diminishes (1.8–2.2 μm) as their diameter increases (0.7–1 μm); at the same time the granule contents become gradually differentiated into concentrically arranged layers of different electron densities (Figs. 13, 14). When fully differentiated, the content of a mature granule displays a characteristic banding pattern in longitudinal sections (Fig. 16). It presents a lightly stained thread-like core parallel to the longitudinal axis of the granule. The core is enclosed in a cup-shaped layer of very dense material and surrounding this there is a cortical layer of moderate electron density in which a thin, peripheral ring of less-stained material can be discerned. Concomitant with the granule maturation process, the RER cisternae dilate and their contents become electron lucent (Figs. 15, 17).

The mature secretory granules move into the cell neck and fill it. The distal portion of the neck reaches the epithelial surface and protrudes from this no more than 4.5 μm, forming a cushion-shaped papilla. The granules tend to accumulate within the papilla forming a monolayered cluster and they are always positioned with the bottom of their cup-shaped layer facing proximally (Figs. 18, 19).

The nucleus in the type $G_4$ gland cell is basally situated as are other organelles, including a few short, dilated, rough endoplasmic cisternae and a Golgi complex. Most of this gland cell is occupied by elongate secretory granules packed tightly together. These have a uniform, faintly granular-fibrous substructure of variable electron density (Fig. 20). Newly formed secretory granules have contents of moderate electron density, but later on these become gradually more electron dense and homogeneous (Fig. 21).

The termination of the type $G_4$ gland cell neck is expanded and lobed. It protrudes only slightly onto the epithelial surface, emerging independently of that of the type $G_3$ gland cell (Fig. 22). Its secretory granules are released onto the epithelial surface, beneath and alongside the papilla of the type $G_3$ gland cell. The material arising from the granules is mucus-like in appearance and forms a sheet of material in which the discharged secretory granules of the type $G_3$ gland cell are embedded.

All of the supportive cells of the proboscidial epithelium have microvilli, but those adjacent to gland cells of the type B glandular systems possess longer and stouter microvilli with a prominent fibrous core (Fig. 24). These microvilli are rather irregularly arranged around the protruding necks of the type $G_3$ and $G_4$ gland cells. Tonofilaments from the cores of the microvilli are gathered into dense bundles (Fig. 25) which pass vertically through the supportive cells (Figs. 26, 27). They extend basally to be anchored by hemidesmosomes onto the extracellular matrix underlaying the cells (Fig. 28).

**Figs. 13–19** Everted proboscis of *R. occultus.* TEM of type B glandular systems ▶

**Fig. 13** Low magnification image showing the general organization of the type $G_3$ gland cell body. *Asterisk* RER cisternae, *1* immature secretory granules, *2* mature secretory granules

**Fig. 14** Detail from the cytoplasm of two type $G_3$ gland cell bodies. *Large arrowhead* Golgi complex and associated vesicles, *small arrowheads* RER flattened cisternae, *1* immature secretory granules, *2* mature secretory granules

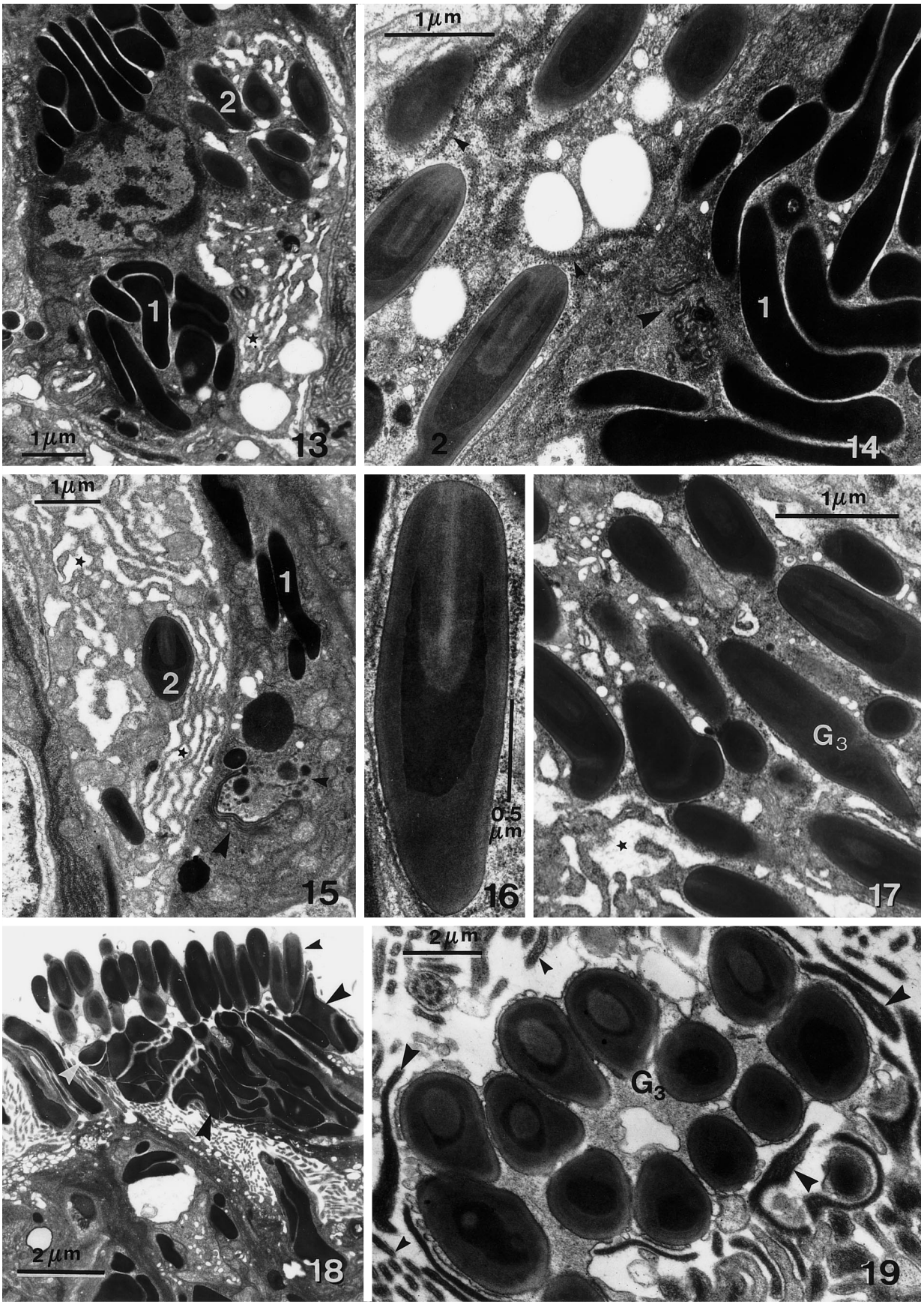
**Fig. 15** Detail from two type $G_3$ gland cell bodies. Note the spatial relationships among Golgi complex (*large arrowhead*), associated condensing vacuoles (*small arrowhead*), and immature secretory granules (*1*). *Asterisks* RER dilated cisternae, *2* mature granules

**Fig. 16** Longitudinal section through a mature secretory granule of a type $G_3$ gland cell

**Fig. 17** Distal region of a type $G_3$ gland cell body showing mature secretory granules ($G_3$) and RER dilated cisternae (*asterisk*).

**Fig. 18** Longitudinal section from the distal region of the glandular epithelium illustrating a papilla. *Large arrowheads* Type $G_4$ gland cell secretory granules, *small arrowhead* type $G_3$ gland cell secretory granules

**Fig. 19** Cross section of a papilla. $G_3$ Type $G_3$ gland cell secretory granules, *large arrowheads* type $G_4$ gland cell secretory granules, *small arrowheads* supportive cell microvilli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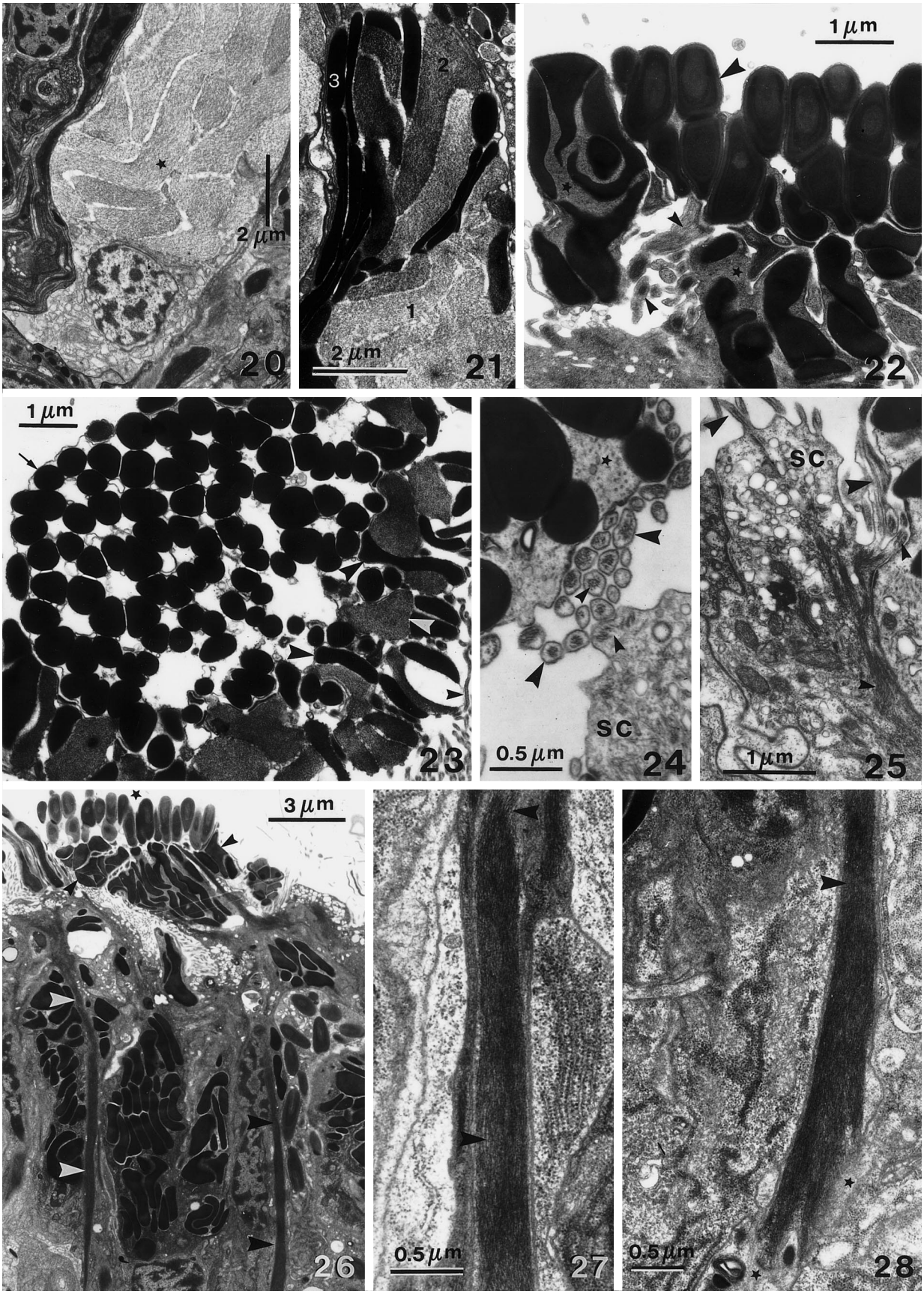
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28

In most glandular systems, a single sensory process bearing a cilium–stereovilli complex (Montalvo et al. 1996) stands close to the protruding necks (Fig. 5). We have not seen morphological evidence of synaptic contact between the gland cells and neural processes.

## D. Discussion

The feeding biology of the Nemertea has been reviewed by McDermott and Roe (1985). According to them, the proboscis forms the basis of the feeding mechanism, playing an important role in the catching of food material. The proboscis in the Heteronemertea lacks a calcified stylet and it must be firmly attached to the prey to prevent the prey being lost during its capture. Although specimens of *R. occultus* could not be induced to feed, we suppose that they use the proboscis to catch food material, as this occurs in most other Heteronemertea (see McDermott and Roe 1985). The proboscis has been also described as serving locomotion in several species of the Nemertea (see Pantin 1950; Moore and Gibson 1985; Sundberg 1989). Whether *R. occultus* uses the proboscis for locomotion cannot be determined in its natural environment. Because the proboscis is involved in the functions mentioned above, the tissue layer exposed to the external environment when it is everted should possess adhesive systems which help the proboscis in such functions.

◀ **Figs. 20–28** Everted proboscis of *R. occultus*. TEM of type B glandular systems

**Fig. 20** Low magnification image showing the general organization of the type $G_4$ gland cell body. *Asterisk* Immature secretory granules

**Fig. 21** Proximal region of a type $G_4$ gland cell neck. *1–3* Secretory granules at different maturation stages

**Fig. 22** Tangential section of a papilla showing the spatial relationships between a type $G_3$ gland cell neck, filled with secretory granules (*large arrowhead*) packed tightly together, and a type $G_4$ gland cell neck (*asterisks*). *Small arrowheads* Distal region and microvilli of supportive cell

**Fig. 23** Cross section of a papilla illustrating the spatial relationships among the necks and secretory granules of type $G_3$ (*arrow*) and type $G_4$ (*large arrowheads*) gland cells. *Small arrowhead* Supportive cell microvilli

**Fig. 24** Longitudinal section through the apical region of a supportive cell (*sc*). *Asterisk* Type $G_3$ gland cell neck, *large arrowheads* supportive cell microvilli, *small arrowheads* tonofilaments within microvilli and supportive cell

**Fig. 25** Supportive cell (*sc*) apical region. *Large arrowheads* Supportive cell microvilli, *small arrowheads* tonofilaments

**Fig. 26** Panoramic view of the proboscidial glandular epithelium illustrating tonofilament bundles (*large arrowheads*). *Asterisk* Type $G_3$ gland cell secretory granules, *small arrowheads* type $G_4$ gland cell secretory granules

**Fig. 27** High magnification image of the supportive cell middle region showing a tonofilament bundle (*arrowheads*)

**Fig. 28** Supportive cell basal region illustrating a tonofilament bundle (*arrowhead*) anchoring onto the extracellular matrix (*asterisks*)

We have found up to ten different types of gland cell in the proposcidial epithelium in *R. occultus* (Heteronemertea) (unpublished observations). The most significant new finding is that four of them appear associated in pairs forming the two types of glandular system described in this paper. These glandular systems are restricted to the ventral surface of the proboscis; this surface becomes in contact with the prey during its capture, and adheres to surfaces. Although essentially no differences were observed between mechanically stimulated secretions and the normal exocytosis of secretion products of the proboscidial gland cells that is seen during prey attack (Stricker and Cloney 1983), it is often difficult to determine the exact role of such gland cells in the dynamic food-catching process or in substrate adhesion when relying solely on reconstruction of stages observed in static electron micrographs.

### I. Type A glandular systems

The general appearance of the type A glandular systems in *R. occultus* is quite similar to that of duo-gland adhesive systems found among a wide diversity of systematic groups (for review see Rieger and Tyler 1979; Tyler and Rieger 1980; Tyler 1988). They are distinguishable, however, from these by differences both in the ultrastructural morphology of their secretion granules and in the tension-bearing elements. It could be that both gland cell types of the type A glandular system contribute materials that combine to form a sticky substance which facilitates the adhesive function of the proboscis. But it is more likely that each type of gland cell, $G_1$ and $G_2$, have a different function. This is especially suggested by the marked differences in the morphology of their granules, and in the arrangement of their respective necks. Their actual, functional roles are not evident from our morphological studies. Evidence for such functional roles is only indirect, coming from comparative studies of Plathelminthes and Gastrotricha.

The pattern of neck arrangement in the papilla of the type A glandular systems in *R. occultus* is very similar to that observed in the duo-gland adhesive systems in the Dolichomacrostomidae (see Tyler 1976). Although there is some variation among both glandular systems in the size and density of the secretion granules, we consider that the type $G_1$ gland cell could be the viscid gland. The type A glandular system is unlike the duo-gland adhesive systems of representatives of the Dolichomacrostomida and other Plathelminthes, however, in having completely intraepithelial gland cell bodies and in lacking specialized anchor cells. Considering this lack of differentiated anchor cells and the large number of type A glandular systems in the proboscis of *R. occultus*, we suppose that the bundles of tonofilaments parallel to the apical–basal axis of the epithelium, found in the majority of the supportive cells, could provide structural support to bear the tension of adhesion.

At present, duo-gland adhesive systems morphologically similar to the type A glandular systems in *R. occul-*

*tus* have not been observed in any species of Nemertea so far studied and, therefore, there is no basis for comparison of these structures within the Nemertea. The only ultrastructural data relating to adhesion functions in the Nemertea known to us are those from researches carried out in *Paranemertes peregrina* (Hoplonemertea) (see Stricker and Cloney 1981, 1983). These authors described one type of proboscidial gland cell surrounded by anchor cells packed with tonofilaments. Moreover, they found other type of gland cell distributed along the entire proboscis secreting acidophilic granules. The authors assigned them an adhesive function. In any case, the single gland adhesive systems observed in *P. peregrina* are morphologically different from the type A glandular system in *R. occultus.* All these considerations preclude, at the moment, a discussion of the phylogenetic importance of the glandular adhesive systems in the Nemertea, and of their value as systematic characters.

II. Type B glandular systems

The ultrastructural morphology of pseudocnids in *R. occultus* is similar to that of 'rhabdites' described in *Lineus ruber* by Gontcharoff (1957) and Ling (1971), in *L. viridis* by Anadón (1976), and to that of pseudocnids in *Zygeupolia rubens* and *Tubulanus* cf. *pelucidus* by Turbeville (1991). The pseudocnids all share the same basic substructure: a filament-like core of variable electron density, an electron-dense medulla, and an outer somewhat electron-lucent cortex. There are some secondary differences, however, in this basic banding pattern between the pseudocnids studied. These differences might be attributable to interspecific variation. Alternatively, different fixation techniques may also have led to different views of the pseudocnids. It is possible that methods used by Ling (1971) and Anadón (1976) produced structures which were artifacts.

Norenburg (1993) described the pseudocnid of *Riserius pugetensis* Norenburg, 1993 (Heteronemertea), appearing as a slender, clavate capsule enclosing an 'eversible', thread-like tubule, and Ling (1971) and Anadón (1976) pointed out that the tubular core of the pseudocnids of *L. ruber* and *L. viridis*, respectively, can be ejected. Nevertheless, our study fails to support such views. In *R. occultus*, the pseudocnids are liberated in clusters and neatly bundled into packets with the long axes directed perpendicular to the epithelial surface, as it has been observed in other species of the Anopla (see Jennings and Gibson 1969; Ling 1971; Riser 1990; Iwata 1993), Jennings and Gibson (1969) indicated that pseudocnids in four species of the Anopla are completely ejected from the formative cell, and Iwata (1993) noted that extruded pseudocnids frequently remained attached to the cell which produced them. Our observations in *R. occultus* agree with those of the authors mentioned above and suggest that the pseudocnids appear to be attached to the epithelial surface by means of the secretion products of the type $G_4$ gland cells, conferring a file-like appearance to the ventral epithelial surface of the proboscis. Since neither *Lineus sanguineus* (Rathke, 1799) nor *L. ruber* paralyze or kill the prey during capture (Jennings and Gibson 1969; Ling 1971), the function of pseudocnids in the Heteronemertea simply appears to be to increase the grip of the proboscis on the prey. We suppose that this is the function of pseudocnids in *R. occultus.* Ling (1971) observed on some occasions that the contents of discharged pseudocnids of *L. ruber* pass into the surrounding medium. This suggests that secretion products of the type $G_3$ and $G_4$ gland cells could also mix to form an adhesive substance.

Current systems of the Nemertea are almost exclusively based on characters discernible at the light microscopic level (Moore and Gibson 1985, 1993; Norenburg 1985; Sundberg 1989, 1993; Sundberg and Hylbom 1994; Ax 1996). Pseudocnids provide a character derived from electron microscopy that is useful in understanding the phylogeny of the Nemertea. Pseudocnids are unique to Palaeonemertea and Heteronemertea; so far, they have not been reported in any species of the Enopla. There are strong similarities in the morphology of known pseudocnids, and the gland cells producing them lie exclusively in the proboscidial epithelium. Therefore, the pseudocnids are an autapomorphy of the Anopla which, consequently, must be considered as a monophyletic taxon. This view agrees with that pointed out by Turbeville (1991), Gibson (1994), Sundberg and Hylbom (1994), and Ax (1996).

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