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Ultrastructural study of the bacillary, granular and mucoid proboscidial gland cells of *Riseriellus occultus* (Nemertini, Heteronemertini)

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Abstract

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The ultrastructure of six types (G_5-G_{10}) of proboscidial gland cells whose cell necks emerge independently on the epithelium surface is analysed and compared with data from other nemerteans. These types differ in cytological features, as well as in the morphology of their respective secretory granules. Secretory granules of the types G_5 and G_6 have a bacillary shape, and differ from each other based on their contents and dimensions. Secretory granules of the types G_7 and G_8 are spherical to ovoid; type G_8 gland cells are monociliated, and their secretory granules contain a paracrystalline material. Types G_9 and G_{10} gland cells are typically goblet-shaped; secretory granules in the type G_9 have a spherical shape, contain a homogeneous electron dense material and maintain their individuality, whereas those of the G_{10} type are elongate and have fibrillar contents, showing a tendency to fuse before they are extruded. The mucus sheet of the proboscis is responsible for lubrication of its epithelial surface. Secretion products of type G_{10} gland cells form the background substance of this mucus, and those of the G_5 type confer stickiness to it. Type G_9 gland cells could provide the toxic component to the mucus, and type G_7 and G_8 gland cells could be concerned with the production of enzymatic secretions.

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Introduction

Our present understanding of proboscis functions in the Nemertini indicate that the major roles of this organ are prey capture and, perhaps, defence; it may also help in locomotion, burrowing, adhesion and sensory probing (e.g. Gibson 1972; Stricker and Cloney 1983; McDermott and Roe 1985; Montalvo *et al.* 1996, 1998). When the proboscis is everted, the epithelium exposed to the external environment is made up mainly of gland cells of several types. In addition, there are supportive cells and sensory cells intermingled with those ones (see literature in Montalvo *et al.* 1998). Examination of the literature published on functions of the proboscidial gland cell secretions indicates that these may increase the adhesion of the proboscis to the prey and/or substratum, and may also cause the paralysis or death of a captured organism (for review see Turbeville 1991). In some cases, functional evidence is drawn mainly from behavioural observations, but structural and physiological data are still lacking. Ultrastructural investigations of the proboscidial gland cells may provide useful data for this purpose.

The glandular nature of the proboscidial epithelium is frequently reported in light microscopic studies describing the anatomy of new species of the Nemertini. However, those do not include either detailed descriptions or illustrations on the proboscidial gland cells (e.g. Stricker 1982; Gibson 1986; Riser 1990; Iwata 1993; Norenburg 1993;

Rogers *et al.* 1993; Moore *et al.* 1995). In such studies, several types of proboscidial gland cells have been distinguished based on either staining properties or shape of their secretory products. Thus, these gland cells have been referred to in a general way as acidophilic, basophilic, producing mucus or forming rhabdoids.

The proboscidial gland cells have been subject of review by Turbeville (1991). A few ultrastructural studies on the proboscidial gland cells have been performed in only four species of the Nemertini. Gontcharoff (1957) studied the 'rhabdites' of *Lineus ruber* (Müller, 1774) (Heteronemertini). In the same species, Ling (1971) described three different types of proboscidial gland cells, and Anadón (1976) found four ones in *Lineus viridis* (Müller, 1774) [= *gesserensis*] (Müller, 1788) (Heteronemertini), three of which correspond morphologically to those observed by Ling. The gland cells are restricted to the proboscis middle region in both heteronemerteans. Stricker and Cloney (1983) reported the occurrence of four types of proboscidial gland cells located in the proboscis anterior part in *Paranemertes peregrina* Coe, 1901 (Hoplonemertini). In a previous paper (Montalvo *et al.* 1998) we postulated the existence of 10 types of proboscidial gland cells in *Riseriellus occultus* (Rogers, Junoy, Gibson, Thorpe, 1993) (Heteronemertini). In that paper we concentrated on four types (G_1-G_4) which appeared in pairs. Such cells formed two kinds of adhesive glandular systems that occurred throughout the length of the proboscis but only on the ventral surface. Despite the functional importance of the proboscis, our knowledge about its gland cells is still incomplete, and there are few comparative data to elucidate the occurrence and distribution of the different types of proboscidial gland cells in the Nemertini. Our initial observation that the proboscidial epithelium in *R. occultus* is more complex than that of the other nemerteans, tends to justify a new study of such gland cells. The present study completes a series of investigations designed to clarify our knowledge about the structure and functions of the proboscis in the Nemertini. In this paper, the ultrastructure of the other six types of gland cells (G_5-G_{10}) with necks that emerge independently from the epithelial surface of the proboscis in *R. occultus* is analysed and compared with data from the other nemerteans; the functional significance of our observations is also discussed.

Materials and Methods

Specimens of *R. occultus* were collected in October 1996 by hand at low tide from the Foz Estuary, north-western 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 animals were kept in an aquarium with muddy sand on the bottom and supplied with running seawater at about 16 °C. Small pieces from different regions of the proboscis were fixed in buffered 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide. For details regarding the fixation and TEM protocol, see Montalvo *et al.* (1996).

For light microscopy, samples of the proboscis were fixed in cold Zenker's fluid for 24 h. Fixed samples were washed, dehydrated in an ethanol series followed by xylene, and then embedded in paraffin (melting point, 52 °C). Transverse and longitudinal serial sections were cut at 5–7 μm and stained with the Heidenhain's azan method (Gabe 1968).

Results

The periphery of the everted proboscis of *Riseriellus occultus* is lined by a columnar epithelium that is of variable thickness, rather convoluted, and rich in intraepithelial gland cells distributed among supportive cells and sensory cells (Fig. 1A). The present study focuses on the six types of gland cells distributed throughout the lateral and dorsal region of the proboscis, and whose cell necks emerge independently on the epithelium surface. Semithin sections stained with toluidine blue and white methylene blue, as well as paraffin sections stained with the Heidenhain's azan method reveal the basophilic nature of the secretory granules of these six types of gland cells. For the purposes of this paper, three broad categories of proboscidial gland cells have been distinguished on the basis of the overall shape of their secretory granules: bacillary, granular and mucoid gland cells. Two types of gland cells are included in each category.

Bacillary gland cells

These proboscidial gland cells are characterized by the bacillary shape of their secretory granules (Fig. 1D,G). The cells are spindle shaped, tapering towards the cell neck distally and tapering proximally in an extension which reaches the extracellular matrix subjacent to the proboscidial epithelium. As a rule, their perikarya are located at the middle and/or basal third of the epithelium. Besides a flattened, elongate nucleus, each perikaryon has the cytoplasmic features common to typical secretory cells, and large numbers of secretory granules in various stages of maturation occupy most of the cell body (Fig. 1C,F). Golgi vesicles can be seen apparently in process of contributing material to matrix of developing secretory granules that surround the Golgi zone.

Each gland cell has a single neck that reaches to the epithelium surface. Necks are difficult to follow because their large numbers and tortuous courses; they are often densely packed into the epithelium intertwining with one another as they course between the supportive cells (Fig. 1B). As secretory granules are synthesized they accumulate at the proximal region of the cell necks, where they range in size and shape, and their contents become more defined (Fig. 1C,F). Mature secretory granules move within the

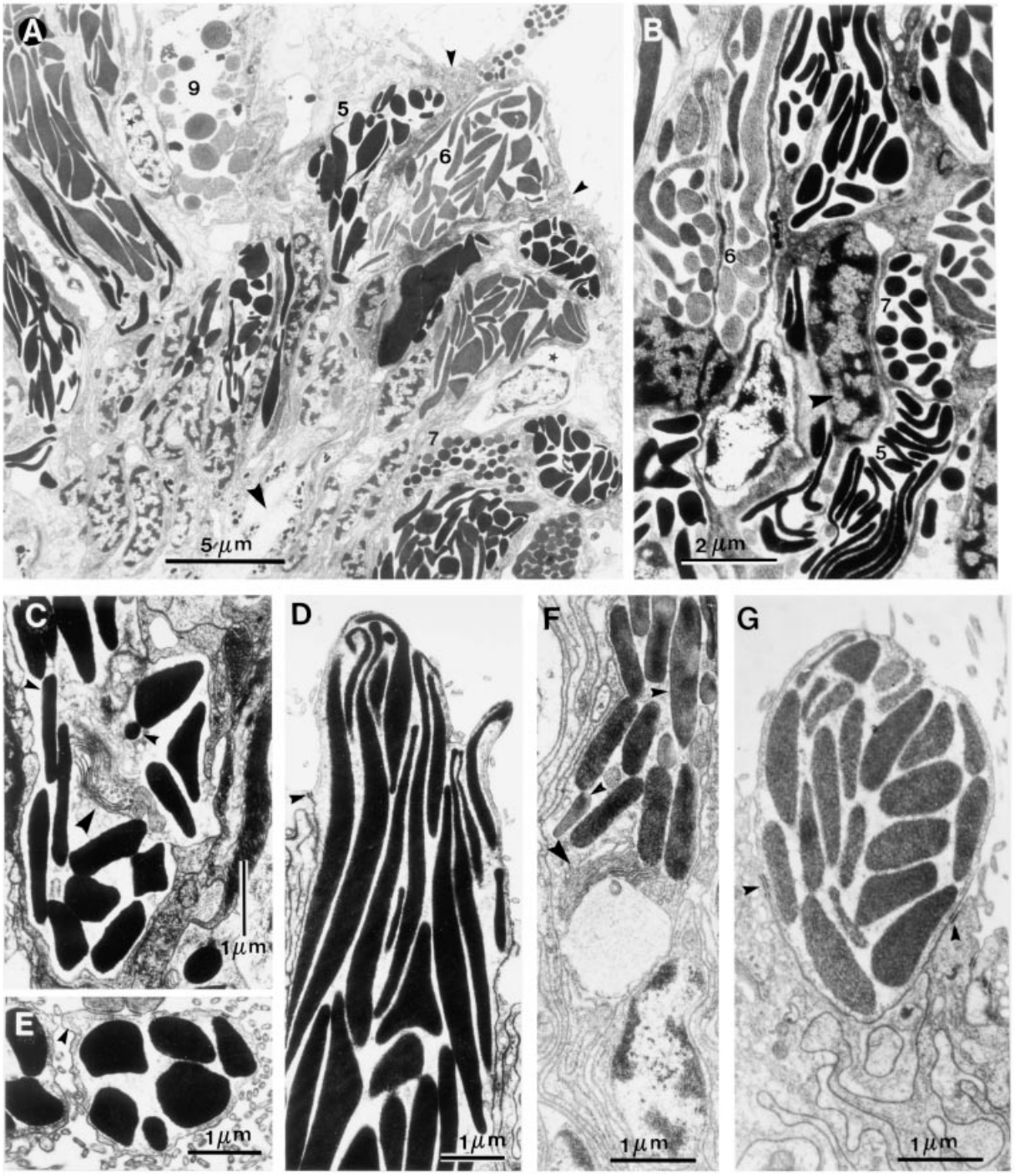

Fig. 1—Everted proboscis of *Riseriellus occultus*. Transmission electron micrographs of the glandular epithelium. —**A**. Panoramic view showing sensory cells (star), supportive cells (small arrowheads), and type G_5 (5), G_6 (6), G_7 (7) and G_9 (9) gland cells. Large arrowhead, extracellular matrix. —**B**. Longitudinal section through middle region of the glandular epithelium showing type G_5 (5), G_6 (6) and G_7 (7) gland cell necks densely packed. Arrowhead, supportive cell. —**C-E**. Type G_5 gland cell. —**C**. Longitudinal section of cell body illustrating the close spatial relationship among Golgi complex (large arrowhead) and secretory granules (small arrowheads). —**D**. Longitudinal section through the distal region of cell neck. Arrowhead, zonula adhaerens. —**E**. Cross-section of papilla. Arrowhead, peripheral microtubules. —**F-G**. Type G_6 gland cell. —**F**. Longitudinal section of cell body. Large arrowhead, Golgi complex; small arrowheads, secretory granules. —**G**. Longitudinal section through the distal region of cell neck. Arrowheads, zonulae adhaerentes.

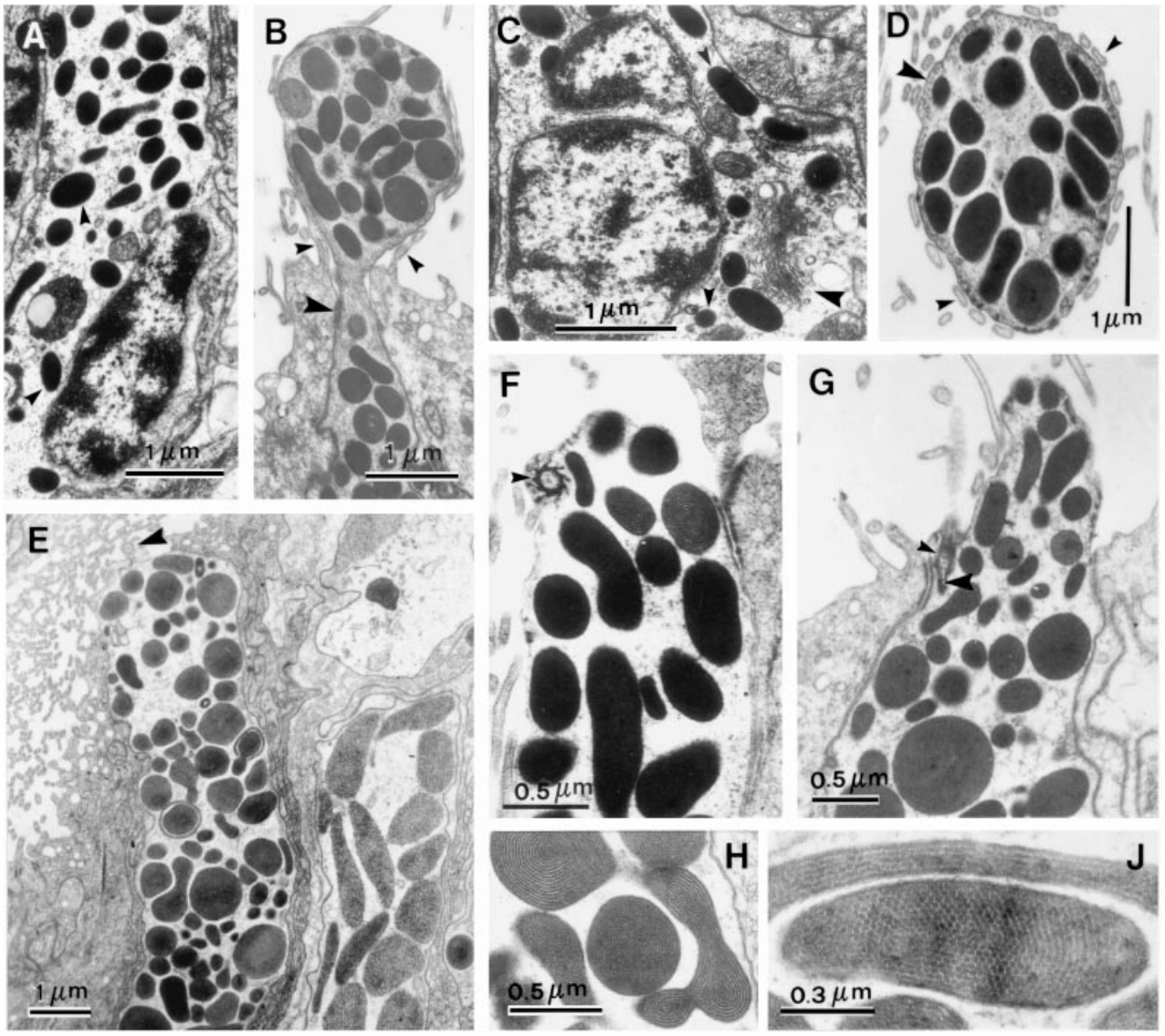

Fig. 2—Everted proboscis of *Riseriellus occultus*. Transmission electron micrographs of the glandular epithelium. —**A-D**. Type G_7 gland cell. —**A**. Longitudinal section of cell body. Arrowheads, secretory granules. —**B**. Longitudinal section through the distal region of cell neck. Large arrowhead, zonula adhaerens; small arrowheads, microvilli of adjacent supportive cells. —**C**. Detail from cell body. Large arrowhead, Golgi complex; small arrowheads, secretory granules. —**D**. Cross-section of papilla. Large arrowhead, peripheral microtubules; small arrowheads, microvilli of adjacent supportive cells. —**E-J**. Type G_8 gland cell. —**E**. Longitudinal section through the distal region of cell neck. Arrowhead, cilium. —**F-G**. Details from the distal region of cell neck showing ciliary basal body (small arrowhead) and vestigial ciliary rootlet (large arrowhead). —**H-J**. Secretory granules showing their substructure at different magnifications.

necks where they are packed tightly together (Fig. 1B). Necks are narrow along most of their course, but at isolated points they are swollen with the accumulation of secretory granules, especially just beneath the exterior surface of the epithelium (Fig. 1A). Neck walls are thin, and the only inclusion besides secretory granules to be seen in the distal part of necks is a system of microtubules that lies parallel to the neck longitudinal axis and close to the cell membrane (Fig. 1E). The necks are joined distally to adjacent supportive cells by zonulae adhaerentes, and protrude (about 3.5 μm) onto the epithelium surface forming cone-shaped papillae without any specialized microvilli surrounding them (Fig. 1D,E,G); they open independently of one another through their own separate pore.

On the basis of differences in formation and substructure of their secretory granules, two types of bacillary gland

cells can be distinguished: G_5 and G_6. Type G_5 gland cells have a prominent Golgi area consisting of a stack of parallel saccules and small vesicles associated with condensing vacuoles which contain a uniform, darkly stained material (Fig. 1C). Their secretory granules (3–8 μm in length and 0.4–0.8 μm in diameter) retain their high electron density and homogeneous appearance in all regions of the cell, i.e. perikaryon, conducting neck and neck apex. However, those aggregate within necks possess narrower ends (Fig. 1C,D). Type G_6 gland cells have Golgi complex typically associated with a large condensing vacuole containing a faintly fibrillar material of moderate electron density (Fig. 1F). This material eventually condenses into the more electron dense fibrils seen in secretory granules in various maturation stages that surround the Golgi zone. Their mature secretory granules (3–5 μm in length and 0.4–1.3 μm in diameter) possess rounded ends (Fig. 1G), and their contents exhibit a fibrillar appearance.

Granular gland cells

Characteristically, the secretory granules of the proboscidial gland cells included in this category have a more or less electron dense content and are spherical to ovoid, although other shapes also occur (Fig. 2B,E). These cells possess a pyriform shape, and their cell bodies lie within the base of the epithelium. They have an eccentric nucleus, well developed Golgi complex, short profiles of rough endoplasmic reticulum, free ribosomes, few mitochondria, and numerous secretory granules (Fig. 2A,C). One process from each cell body extends to the epithelial surface and is almost filled with mature secretory granules (Fig. 2A,B,E). The shape, structure and location of the cell necks in the granular gland cells are similar to those described above for the bacillary ones (Fig. 2B,D,G).

Two types of granular gland cells, G_7 and G_8, are easily distinguishable. Most of the secretory granules (up to 0.8 μm in diameter) of type G_7 gland cells have a homogeneous, electron dense content (Fig. 2B,D). The distal part of the type G_7 necks protrudes from the epithelial surface and expands to form a globular papilla (Fig. 2B). These papillae vary irregularly in shape and in size; most of them are approximately circular in transverse section (3–4 μm in diameter) and stand about 2 μm high above the epithelium surface. Each papilla is encircled by a collar of microvilli from adjacent supportive cells (Fig. 2B,D).

The most striking feature of the type G_8 gland cells is that the apical surface of their necks bears a single cilium. This has a standard $9 \times 2 + 2$ axoneme, and arises from a typical basal body from which a vestigial ciliary rootlet extends slightly into the neck (Fig. 2E,F,G). At low magnifications, the contents of the secretory granules (up to 1.2 μm in diameter) of type G_8 gland cells appear homogeneous and densely stained (Fig. 2E). When viewed at high magnifications in favourable sections, the granules have a substructure consisting of thin profiles of electron dense material concentrically arranged and closely packed (Fig. 2H). At higher magnifications, they exhibit a paracrystalline composition (Fig. 2 J), each one of their concentric lines being made up of densely packed granules (about 17 nm in diameter). Occasionally, some secretory granules appear flattened and curved forming a ring around other ones to which seems to fuse (Fig. 2E).

Mucoid gland cells

These gland cells are typically goblet-shaped; their distal two thirds are filled with large numbers of mucoid secretory granules. Such granules are densely crowded, and their contents display a variable substructure and electron density. The nucleus and synthetic organelles are situated beneath the mass of secretory granules. As in the other categories of proboscidial gland cells, there are also two types of mucoid gland cells: G_9 and G_{10}.

Type G_9 gland cells possess several kinds of secretory granules whose size and appearance change inside the same cell (Fig. 3A,C); these differences represent various stages of their maturation process. When newly formed, the secretory granules have a spherical shape (up to 2.5 μm in diameter) and contain a homogeneous electron dense material (Fig. 3B). As granules form, they apparently move towards the cell apical region where they aggregate. Concurrently, they alter their morphology in that their contents become coarsely granular and they acquire more angular profiles due to compression (Fig. 3C). At the apical end of the cell, the contents of the mature granules undergoing secretion often lose their condensation showing an uniformly dense core enclosed within a light area (Fig. 3A,C). In some instances, these granules seem to fuse just before their contents are extruded through breaks in the apical plasma membrane of cell.

Newly formed secretory granules of type G_{10} gland cells are spherical and contain a fine granular material of homogeneous, moderate electron density (Fig. 3D). Mature granules have an elongate rather than spherical shape. Their contents show a very fine even-texture which eventually condenses into diffuse fibrils; the electron density of granule contents varies from low to high even within an individual gland cell (Fig. 3E,F). These variations could represent different stages of the secretory phase, or they could be due to vagaries in fixation. The mass of tightly packed secretory granules extends as a continuum from the middle to the apical region of the cell. These granules are membrane bounded and maintain their individuality (Fig. 3D). However, they show a tendency to lose their intervening membranes and to fuse to each other forming a continuous mass of secretory material. Such masses contain a few profiles of membranes, and the disrupted outlines of the original granules may still be identifiable (Fig. 3E,F).

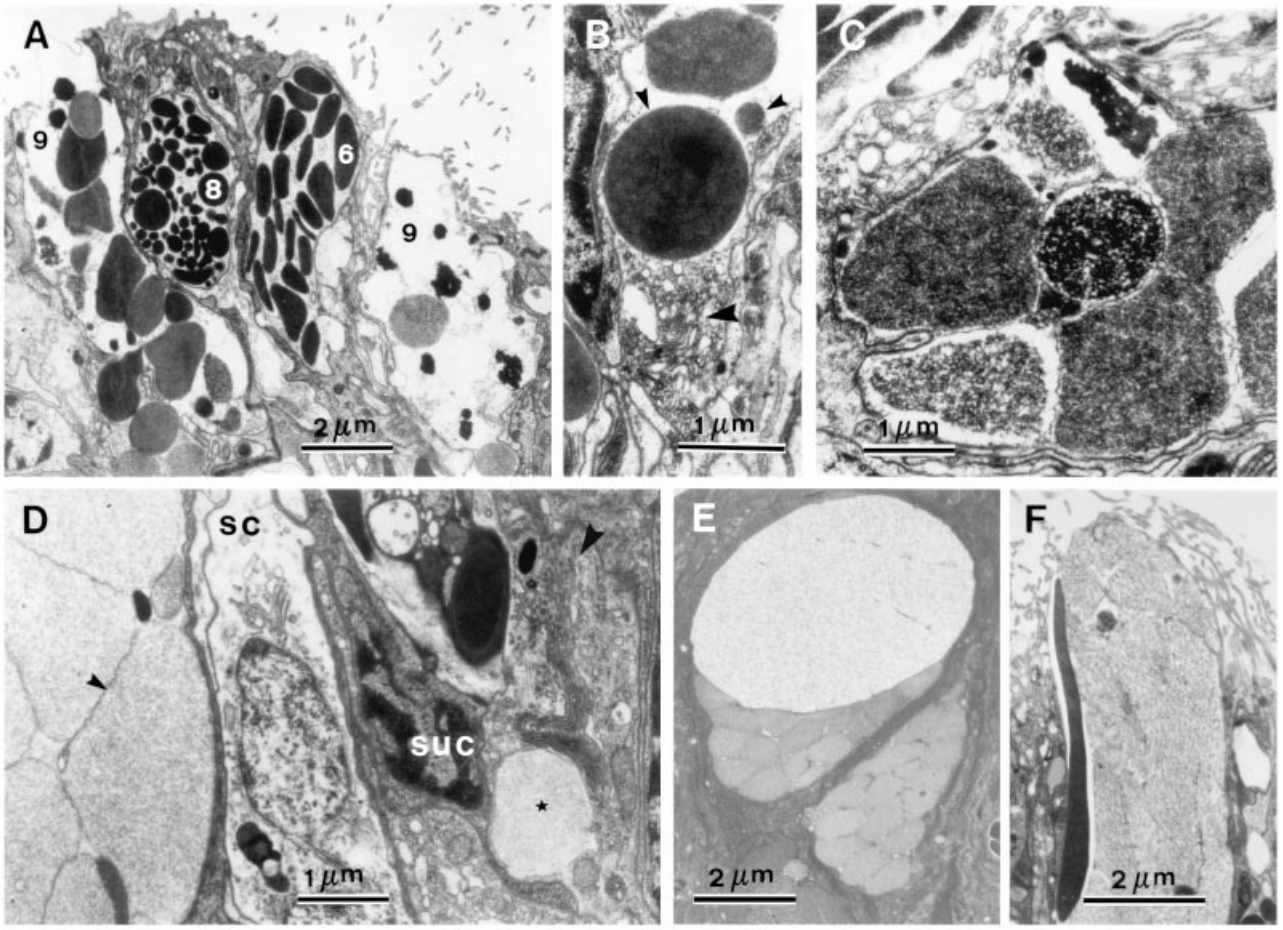

Fig. 3—Everted proboscis of *Riseriellus occultus*. Transmission electron micrographs of the glandular epithelium. —**A**. Low magnification image showing the distal region of two type G_9 gland cell necks (9) at different stages of secretory phase, and type G_6 (6) and G_8 (8) gland cell necks. —**B-C**. Type G_9 gland cell. —**B**, Detail from cell body showing Golgi zone (large arrowhead) and secretory granules (small arrowheads). —**C**. Oblique longitudinal section through the distal region of cell neck filled with secretory granules. —**D-F**. Type G_{10} gland cell. —**D**. Detail from cell body showing Golgi complex (large arrowhead), newly formed secretory granule (star), and mature secretory granules (small arrowhead). sc, sensory cell; suc, supportive cell. —**E**. Oblique longitudinal section through the distal half of cell neck filled with tightly packed secretory granules. —**F**. Longitudinal section of the distal region of cell neck.

Discussion

The proboscidial gland cells in the Nemertini have been repeatedly studied at the light microscopical level (e.g. Jennings and Gibson 1969; Stricker 1982; Gibson 1986; Riser 1990; Norenburg 1993; Rogers *et al.* 1993; Moore *et al.* 1995). However, the examination of such gland cells in the light microscope offers only limited information on their structure. Therefore, it is difficult to correlate the proboscidial gland cells recognized by conventional light microscopy in several species of the Nemertini to some of the six types in *Riseriellus occultus* described at ultrastructural level in this paper.

There are few data about the ultrastructure of the proboscidial gland cells in the Nemertini; the only studies of these cells using electron microscopy have been done by Gontcharoff (1957), Ling (1971), Anadón (1976), Stricker and Cloney (1981, 1982, 1983), and Montalvo *et al.* (1998). Our findings on the distribution of proboscidial gland cells in *R. occultus* disagree with those for the other studied species of the Nemertini. In two species of the Heteronemertini, *Lineus ruber* (see Ling 1971) and *L. viridis* (see Anadón 1976) the gland cells are restricted to the middle region of the proboscis, whereas they are distributed throughout the length of the proboscis in *R. occultus*. On the other hand, most of such gland cells are located at the anterior chamber of the proboscis in *Paranemertes peregrina* (Hoplonemertini) (see Stricker and Cloney 1983). As Norenburg (1985) hypothesized, the differences in the distribution of proboscidial gland cells may be the result of an evolutionary link among the

endowment of these, and the habits and feeding mechanism of the several nemertean species (see Jennings and Gibson 1969; McDermott and Roe 1985; Turbeville 1991).

The proboscidial epithelium of *R. occultus* is rich in intraepithelial gland cells. Ten types of gland cells can be distinguished; they differ in cytological features, as well as in the morphology of their respective secretory granules. There is only little variation in these characters and there is no overlap, so that they distinctly separate the 10 types. In a previous paper (Montalvo *et al.* 1998) we described the four types (G_1-G_4) which appeared in pairs. The secretory granules of the type G_5 and G_6 gland cells have bacillary shape. However, those of the type G_5 show narrower ends and contain a homogenous material of high electron density whereas those of the type G_6 are shorter and wider and possess rounded ends, exhibiting contents of less electron density and fibrillar in appearance. The secretory granules of the type G_7 and G_8 are spherical to ovoid. The cells of the type G_8 are monociliated, and their secretory granules exhibit paracrystalline contents, whereas those of the type G_7 are smaller and have homogeneous electron dense contents. The types G_9 and G_{10} are typically goblet-shaped. The secretory granules in the G_9 are spherical in shape, contain a homogeneous electron dense material and maintain their individuality whereas those of the G_{10} are elongated and have fibrillar contents of moderate density, showing a tendency to fuse before they are extruded.

Type G_{10} gland cells of *R. occultus* correspond morphologically to the mucus-secreting cells of *Lineus ruber* (Ling 1971; fig. 23) and to the type II gland cells containing coalescent rhabdoids in *Paranemertes peregrina* (Stricker and Cloney 1983; figs 18, 19). Likewise, the type I gland cells containing fusiform rhabdoids in *P. peregrina* (Stricker and Cloney 1983; figs 16, 17, 18) have a morphology similar to the type G_5 gland cells of *R. occultus*. During laboratory test, alive, dead or damaged annelids, molluscs or crustaceans supplied to specimens of *R. occultus* failed to cause a feeding response; the same result was obtained with liver fragments. In the absence of behavioural, physiological and cytochemical data, a hypothesis of the functions of proboscidial gland cells in *R. occultus* can only be advanced based on morphological similarities to others known systems. Thus, the secretion products of the type G_{10} and G_5 gland cells may contribute to the formation of the mucus, playing the same role than that postulated for the coalescent and fusiform rhabdoids of *P. peregrina* (Stricker and Cloney 1983). Type G_9 gland cells of *R. occultus*, morphologically similar to those of the type IV of *P. peregrina*, could provide the toxic component of the venom (Stricker and Cloney 1983). Type G_7 and G_8 gland cells of the proboscis in *R. occultus* could be concerned in the production of some enzymatic secretion, although any direct evidence is available. Jennings and Gibson (1969) found non-specific esterase and exopeptidase activities in the proboscidial epithelium of several species of the Nemertini. They suggested that both enzymes were concerned with extracorporal digestion. Therefore, it is possible that the proboscis in *R. occultus* may be also involved in a nonintestinal nutritional function.

Nevertheless, the comparison of proboscidial gland cells in different species of the Nemertini could be problematic because morphologically similar gland cells may have evolved independently in species belonging to different taxa. On the other hand, species of the same taxon could possess dissimilar gland cells resulting from adaptation to different ways of life.

The various types of granular and mucoid gland cells observed in the proboscidial epithelium of *R. occultus* may be morphologically assimilated to the types of bacillary and mucous, respectively, cells stated by Norenburg (1985) for the integumentary gland cells in the Nemertini. However, in the Heteronemertini, the organization of the proboscidial epithelium differs from that of the epidermis (Norenburg 1985; Turbeville 1991) because of all proboscidial gland cells are intraepithelial whereas the integumentary bacillary and mucous gland cells are mainly subepidermal. This fact supports the Norenburg's (1985) view, according to which the dermal gland cells in the Heteronemertini are epidermally derived.

Although we can give no experimental data to confirm some of the views mentioned above, it can be safely assumed that secretions of the various proboscidial gland cells in *R. occultus* contribute to at least some of the functions referred to. However, further cytochemical and physiological studies, as well as behavioural observations are required for a definitive elucidation of the nature of the secretory granules and the actual function of each type of proboscidial gland cells in the Nemertini.

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