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Ultrastructure of the female reproductive system of the free-living marine nematode *Enoplus demani* (Nematoda : Enoplida)

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Summary – The ultrastructure of the female reproductive system of *Enoplus demani* was studied. The ovarian wall is made up of epithelial and myoepithelial cells. The myoepithelial cells of the ovarial sac are bipartite : they consist of the muscle parts attached to a basal lamina, and the secretory cell bodies. The latter are possibly involved in the formation of the spindle-shaped extracellular bodies that fill the space between the terminal oocyte and the perikarya of myoepithelial cells. The oviduct is lined with glandular cells that surround the narrow lumen. The non-muscular sphincter is composed of a central cellular core enveloped by an external syncytial epithelium. The central cellular mass is the continuation of the glandular epithelium of the oviduct. The external epithelium of the sphincter has a very plicated outer wall and a cytoplasm rich in bundles of filaments. It is the external epithelium that grades into the uterine wall.

Résumé – *Ultrastructure du système reproducteur femelle chez le nématode libre marin* Enoplus demani *(Nematoda : Enoplida)* – Étude a été réalisée du système reproducteur femelle d'*Enoplus demani*. La paroi ovarienne est constituée de cellules épithéliales et myoépithéliales. Les cellules myoépithéliales du sac ovarien sont bipartites : elles sont divisées en une partie musculaire attachée à la lame basale et en corpuscules sécréteurs cellulaires. Ces derniers pourraient être impliqués dans la formation des corpuscules extracellulaires qui remplissent l'espace situé entre l'oocyte terminal et les périkaryons des cellules myoépithéliales. L'oviducte est bordé par des cellules glandulaires entourant une lumière étroite. Le sphincter, non musculaire, est composé d'une partie cellulaire centrale entourée par un épithélium syncitial. Cette partie cellulaire centrale est la continuation de l'épithélium glandulaire de l'oviducte. L'épithélium externe du sphincter montre une paroi très plissée et un cytoplasme riche en faisceaux de filaments. C'est cet épithélium qui se prolonge pour constituer la paroi utérine.

Key-words : *Enoplus demani,* gonoduct, myoepithelial cells, nematode, ovary, ultrastructure.

The general anatomy of the female reproductive system is widely used as an important diagnostic character in the systematics of nematodes (Coomans, 1965; Lorenzen, 1978; Geraert, 1983). The principles of the structure of female genitalia were elucidated in a number of reviews (Hope, 1974; Chitwood & Chitwood, 1977; Lorenzen, 1981; Bird & Bird, 1991; Turlygina & Chizhov, 1991). However, new approaches to the phylogeny of nematodes based on the structure of the reproductive system require more than investigations of only wholemount specimens.

Until now the ultrastructure of the ovaries and oviducts has been examined only in few taxa of parasitic nematodes (Yuen, 1971; Adamson, 1983; Foor, 1983; Preston & Jenkins, 1983; MacKinnon, 1987; Van de Velde *et al.*, 1990 *a;* Van de Velde & Coomans, 1988). However, little attention has been paid to free-living marine nematodes. The review of Hope (1974) includes only one detailed light microscopical investigation of the histology of the female reproductive system in a primitive enoplid nematode, *Deontostoma californicum* (Enoplida, Leptosomatidae).

The goal of the present study was to elucidate both the histology and the ultrastructure of the female reproductive system of another primitive marine nematode *Enoplus demani* Galtsova, 1976 (Enoplida, Enoplidae) with special emphasis on the structure of ovary, oviduct and sphincter. The ultrastructure of the oocytes and uterine wall remained outside the scope of this paper.

Materials and methods

The adult females of *Enoplus demani* were obtained from sand collected in the intertidal zone at the White Sea Biological Station of Moscow State University (Kandalakshskiy Bay, the White Sea). The animals were cut into halves at the vulva. As a result both branches of the reproductive system were extruded slowly from the body by internal pressure. Extracted female genitalia were fixed for TEM in 2.5 % glutaraldehyde in 0.05 M cacodylate buffer containing 12.8 mg/ml NaCl and then postfixed in 2 % osmium tetroxide in the same buffer containing 9.2 mg/ml NaCl. The postfixation was followed by *en bloc* staining for 12 h in 1 % uranyl acetate,

then the specimens were dehydrated in ethanol and acetone series and embedded in Araldite. Semithin and ultrathin sections were cut with a Reichert Ultracut E ultratome. The semithin sections were stained with methylene blue, mounted in Araldite and then examined with a Reichert Polyvar light microscope. The ultrathin sections were stained with lead citrate and examined with a JEOL JEM 100 B electron microscope.

Results

General anatomy and histology as revealed by light microscopy

Enoplus demani has an amphidelphic female reproductive system that consists of two branches with the same structure. Each branch is subdivided into three main parts : *i)* the antidromous reflexed ovary; *ii)* the oviduct and *iii)* the uterus (Fig. 1). In turn, three zones may be seen in the ovary : the germinal, growth and ripening zones. The ovarian wall in the germinal zone is made up of flattened or pyramidal epithelial cells. Several of these cells surround the distal tip of the ovary. The ovarian wall in the growth zone consists of spindle-shaped myoepithelial cells. These cells stretch along the ovary and have a centrally located flattened nucleus. The ripening zone is represented by an ovarial sac, where the oocytes terminate their growth. The wall of the ovarial sac is also made up of myoepithelial cells, but there, these cells are clearly subdivided into a flattened part attached to basal lamina and a perikaryon that protrudes into the cavity of the ovarial sac (Fig. 2 A). The space between the perikarya and the terminal oocyte is filled with extracellular spindle-shaped bodies.

The oviduct is connected to the middle part of the ovary at the growth zone and is followed by a short narrow sphincter, which is connected to the distal part of the uterus (Fig. 1). Most of the cells of the oviduct are stained strongly with methylene blue (Fig. 2 B). There, the oviduct has a narrow central lumen filled with a dark-stained substance. Half of the perimeter of the transverse section through the oviduct is lined with the processes of the myoepithelial cells of the ovarial sac (Fig. 2 B). No processes appear near the boundary between oviduct and sphincter.

The sphincter is distinctly bilayered (Fig. 2 C). Dark stained cells form a central core without an apparent lumen. Occasional spermatozoa were observed between these cells. The external pale-stained layer of the cells in the transverse semithin sections exhibit a tripartite structure. Three bulges, each containing a large nucleus, alternates with thin cytoplasmic bridges. It is the external layer of the sphincter that is continuous with the uterine wall. The internal cellular core is interrupted distal to the mass of spermatozoa that fill the lumen of the distal part of the uterus.

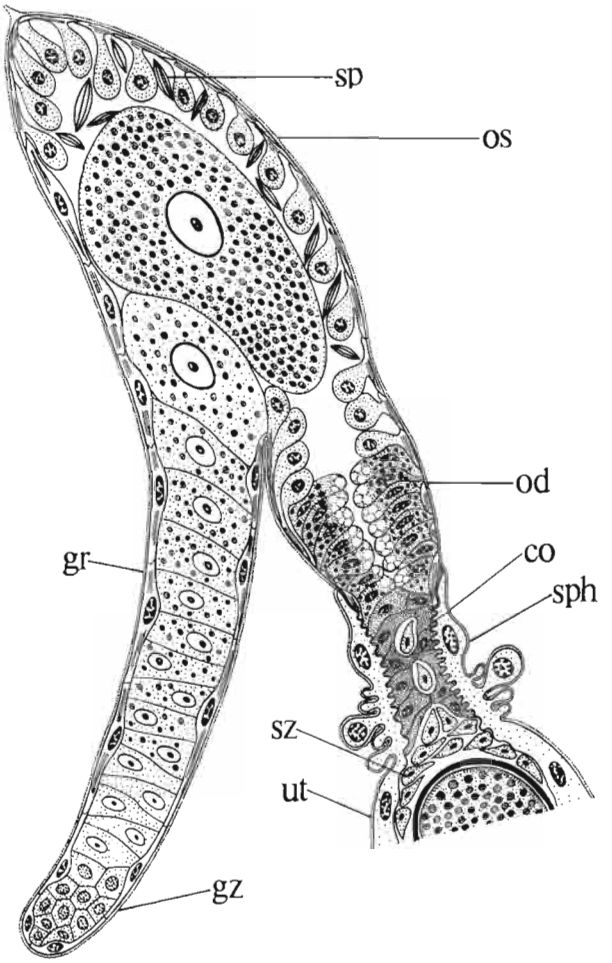

Fig. 1. *Schematic representation of a longitudinal section through one branch of the female reproductive system of* Enoplus demani.

List of abbreviations : *bf : bundles of filamens; ch : condensed chromatine; co : central cellular core of sphincter; cp : cytoplasm of muscle process; ex : external epihelium of sphincter; F : filamentous layer; gb : Golgi body; gc : glandular cell of oviduct; HD : homogeneous droplet; gr : growth zone of ovary; gz : germinal zone of ovary; lu : lumen of oviduct; mf : bundle of myofilaments; mi : mitochondrion; mp : muscle process; N : nucleus; NP : nucleoplasm; oc : oocyte; od : oviduct; of : cytoplasmic outfoldings; os : ovarial sac; P : perikaryon of myoepithelial cell; pg : basal process of glandular cell; rer : endoplasmic reticulum; sp : spindle-shaped body; sph : sphincter; sz : spermatozoa; ut : uterus; v : vesicles.*

Ultrastructure

Germinal zone

All the cells of the gonadal wall are epithelial in nature and are attached to a basal lamina. The most distal region of the ovarian wall (about 10 µm long) is lined by simple epithelial cells of uniform ultrastructure. Each of these cells contains a large irregular or pyramidal nucleus with condensed chromatin scattered throughout

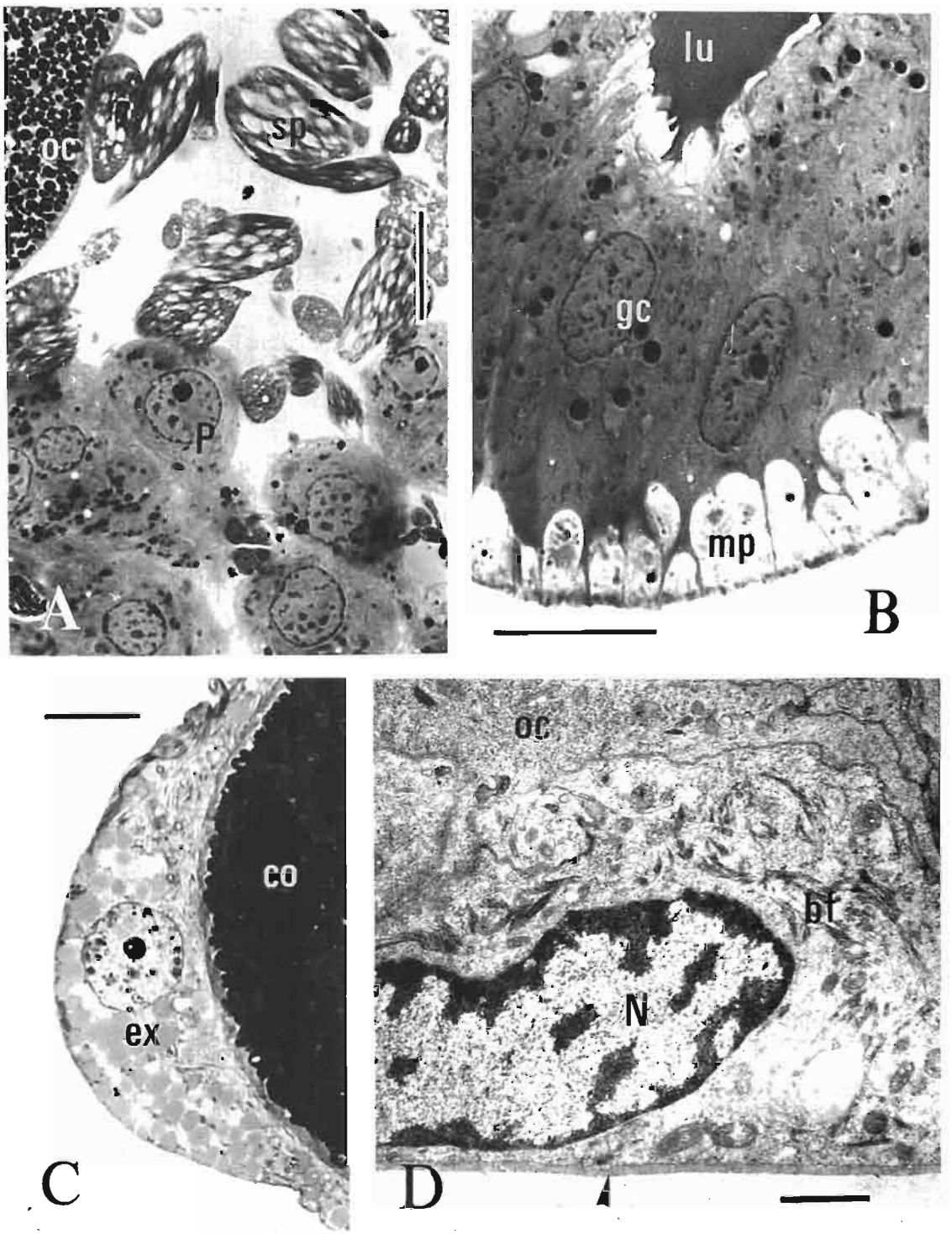

Fig. 2. *A : Longitudinal semithin section through the ovarial sac; B : Transverse semithin section through the oviduct; C : Transverse semithin section through the sphincter; D : Ovarian wall at the germinal zone, transverse thin section. The* arrowhead *indicates the basal lamina.* (Bar : A-C = 10 µm; D = 1 µm.)

For abbreviations see Fig. 1.

the nucleoplasm and attached to the nuclear envelope (Figs 2 D; 7 A). The cytoplasm contains many small mitochondria and numerous randomly distributed bundles of filaments. The periphery of the epithelial cells is very thin and contains no organelles. The epithelial cells are in contact with neighbouring cells, but there are no cytoplasmic bridges.

Several epithelial cells, each with a large nucleus and very small amounts of cytoplasm, envelop the very distal tip of the ovary. Thus, there is no separate single " cap cell " in the ovary of *E. demani.*

Growth zone

The growing oocytes form a straight chain surrounded by a sheath of longitudinally oriented, spindle-shaped, myoepithelial cells. Each of these cells contains a central flattened nucleus with condensed chromatin scattered throughout the nucleoplasm and attached to the nuclear envelope (Figs 3 A; 7 B). The main feature of the basal region of the myoepithelial cells are the bundles of the myofilaments running parallel to the longitudinal axes of the cells. The narrow terminal processes of the myoepithelial cells appear in transverse sections as cytoplasmic islets (Fig. 3 B). Each islet contains a bundle of the myofilaments and small amount of cytoplasm with mitochondria.

Dense longitudinally oriented bundles of tonofilaments attach to the hemidesmosomes that anchor the myoepithelial cells to the basal lamina of the epithelial sheath. The cytoplasm of the myoepithelial cell contains numerous small mitochondria, occasional Golgi bodies, and lipid droplets.

Ovarial sac

The wall of the ovarial sac also consists of myoepithelial cells, but they are bipartite. The wall proper is made up of a basal lamina and a sheath that is composed of numerous long, narrow extensions of the contractile regions of these cells. These extensions are devoid of nuclei but contain bundles of myofilaments and small mitochondria (Figs 3 C, D; 7 C). The contractile part of the myoepithelial cell is relatively narrow in comparison to the non-contractile perikaryon (Fig. 4 A). Numerous cell bodies fill the voluminous space between the cap of the ovarial sac and the terminal ripening oocyte. The cell bodies each contains an irregular nucleus with condensed chromatin particles scattered throughout the nucleoplasm and attached to the nuclear envelope (Fig. 4 B). One or two round homogeneous nucleoli occur in the nucleoplasm. An extensive rough endoplasmic reticulum (RER), which occurs frequently as stacks and whorls, numerous Golgi bodies, mitochondria, and numerous small vesicles with dense content, suggest that perikarya are of a secretory nature (Figs 3 D; 4 A, B). The cell bodies can fit tightly against the surface of the oocyte.

Very characteristic of the ovarial sac are the groups of extracellular spindle-shaped bodies usually situated between the perikarya of the myoepithelial cells and the ripening oocyte (Fig. 2 A). In the thin sections, these bodies look like a round grid when cut transversely and have a regular multilayered structure in longitudinal sections (Fig. 4 C).

The oviduct

The wall of the oviduct is lined with a high columnar cells with dense cytoplasm (Figs 2 B; 7 D). A large ovoid or flattened nucleus with condensed chromatin and dark stained nucleoplasm occupies the middle region of the cell (Fig. 5 A). The secretory nature of these cells is evidenced by their dense cytoplasm containing extensive RER, numerous darkly stained Golgi bodies, mitochondria, and vesicles of various sizes and contents. The apical cytoplasm facing the lumen is electronlight and contains Golgi bodies, vesicles, and sparse flocculent material. The lumen of the oviduct is filled with homogeneous dark-stained substance.

Another component of the oviduct wall are numerous longitudinal muscle processes running off the myoepithelial cells of the ovarial sac (Figs 2 B; 4 D; 7 D). These processes are concentrated at the side of the oviduct next to the body wall. The basal parts of the glandular cells of the oviduct are organized here into long cytoplasmic processes which penetrate in between the muscle processes and anchor to the basal lamina by hemidesmosomes (Fig. 4 D). The ultrastructure of the muscle processes is the same as in the ovarial sac (Fig. 4 D).

The sphincter

The central cellular core of the sphincter appears to be an expansion of the secretory epithelium of the oviduct. At a distinct transverse section between the oviduct and sphincter the basal lamina overlying the epithelium of the oviduct intrudes into the external epithelial tube of the sphincter, which in turn has its own basal lamina (Fig. 5 B). As a result, the basal lamina appears to be two-layered in longitudinal sections.

The cells constituting the central core of the sphincter show no distinct polarity and have an ultrastructure similar to the secretory cells of the oviduct. The core cells have irregular nuclei and are heavily interdigitated. Occasional spermatozoa are squeezed between these cells. Small intercellular cavities filled with darkly stained material occur in the central core, but there is no distinct lumen.

It is very likely that the external epithelial layer of the sphincter is of a syncytial nature : we failed to find membrane boundaries between the compartments of the epithelial cytoplasm containing the nuclei. We traced the cytoplasm continuity in both transverse and longitudinal sections of the sphincter. In transverse sections the external layer consists of three bulging regions each containing a single nucleus (Figs 5 C; 6 A; 7 E). Each large round nucleus contains a single homogeneous nucleolus. Condensed chromatin appears as osmiophilic glo-

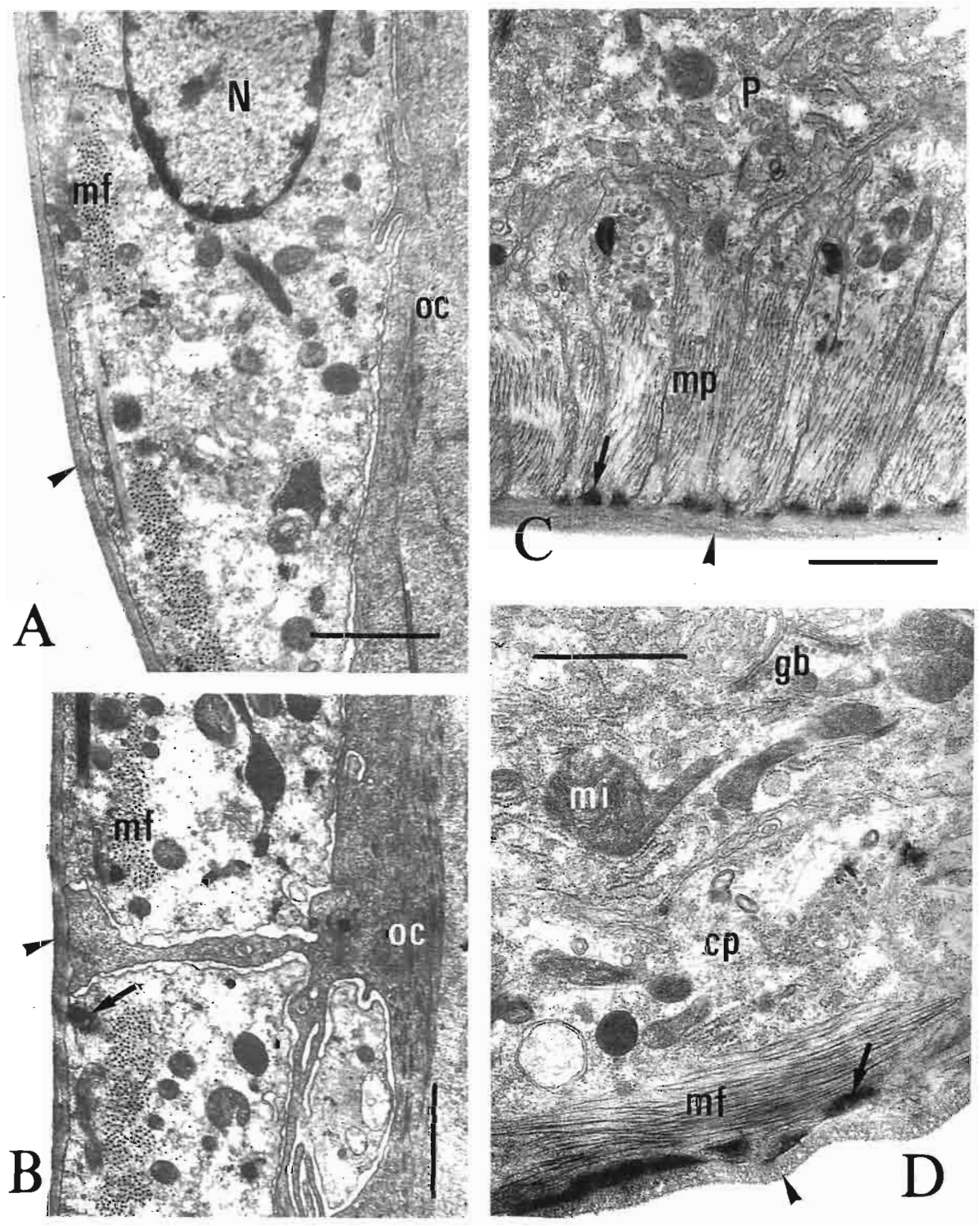

Fig. 3. *A : Myoepithelial cell of the ovarian wall at the growth zone, transverse section; B : Processes of the myoepithelial cells at the growth zone, transverse section; C, D : Myoepithelial sheath of the ovarial sac in transverse (C) and longitudinal (D) sections. The* arrowheads *indicate the basal lamina, and the* arrows *point to the place of attachment of hemidesmosomes.* (Bar : 1 µm.)

For abbreviations see Fig. 1.

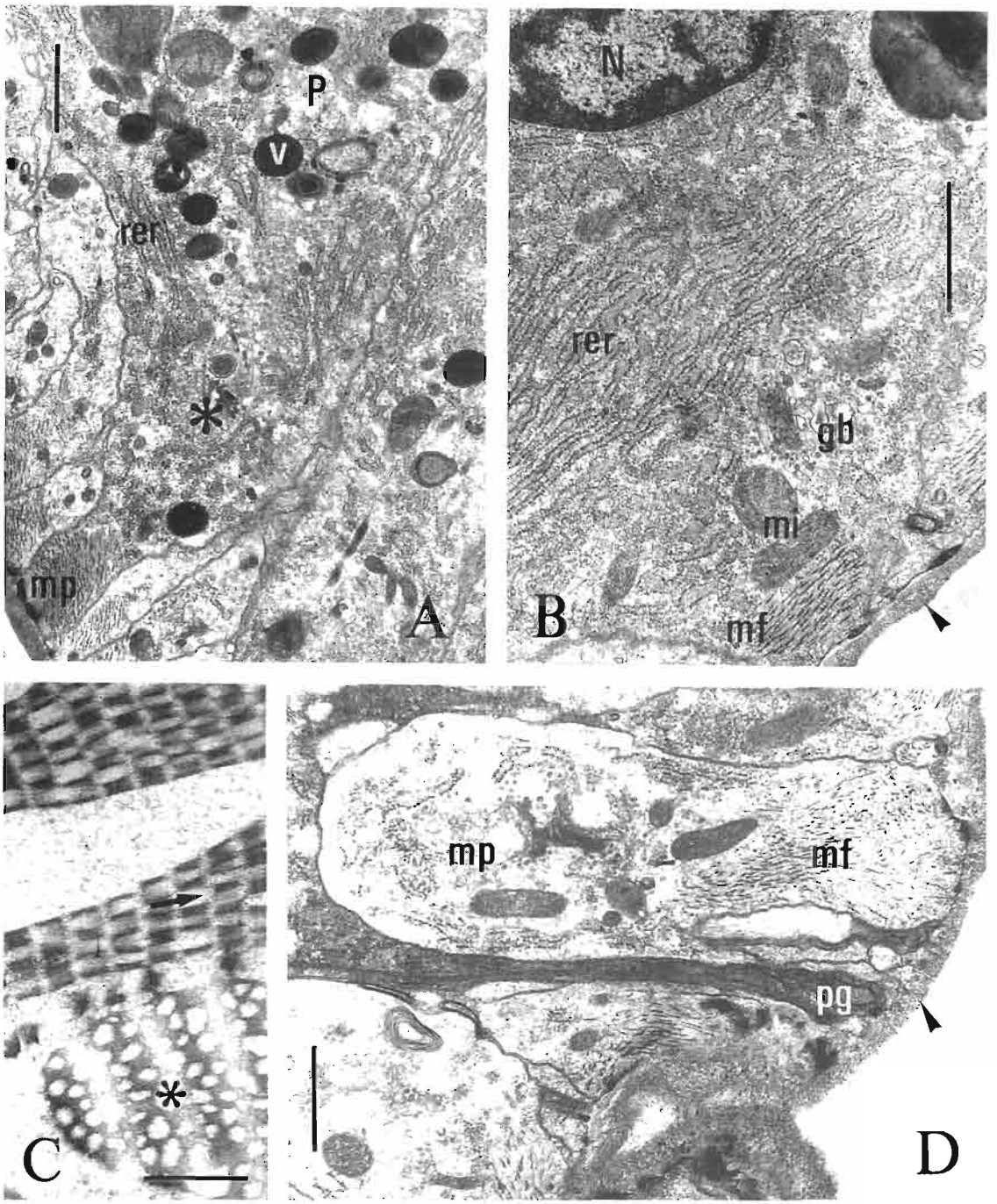

Fig. 4. *A : Cytoplasmic bridge* (asterisk) *connecting the muscle process (mp) to the perikaryon (P) of the myoepithelial cell of the ovarial sac; B : Section through the myoepithelial cell of the ovarial sac, showing the extensive rough endoplasmic reticulum (rer). The* arrowhead *indicates the basal lamina; C : Longitudinal* (arrow) *and transverse* (asterisk) *sections through the extracellular spindle-shaped bodies of the ovarial sac; D : Transverse section through the oviduct, the processes of the glandular cell (pg) anchoring to the basal lamina* (arrowhead). (Bar : A, B, D = 1 µm; C = 0.5 µm.)

For abbreviations see Fig. 1.

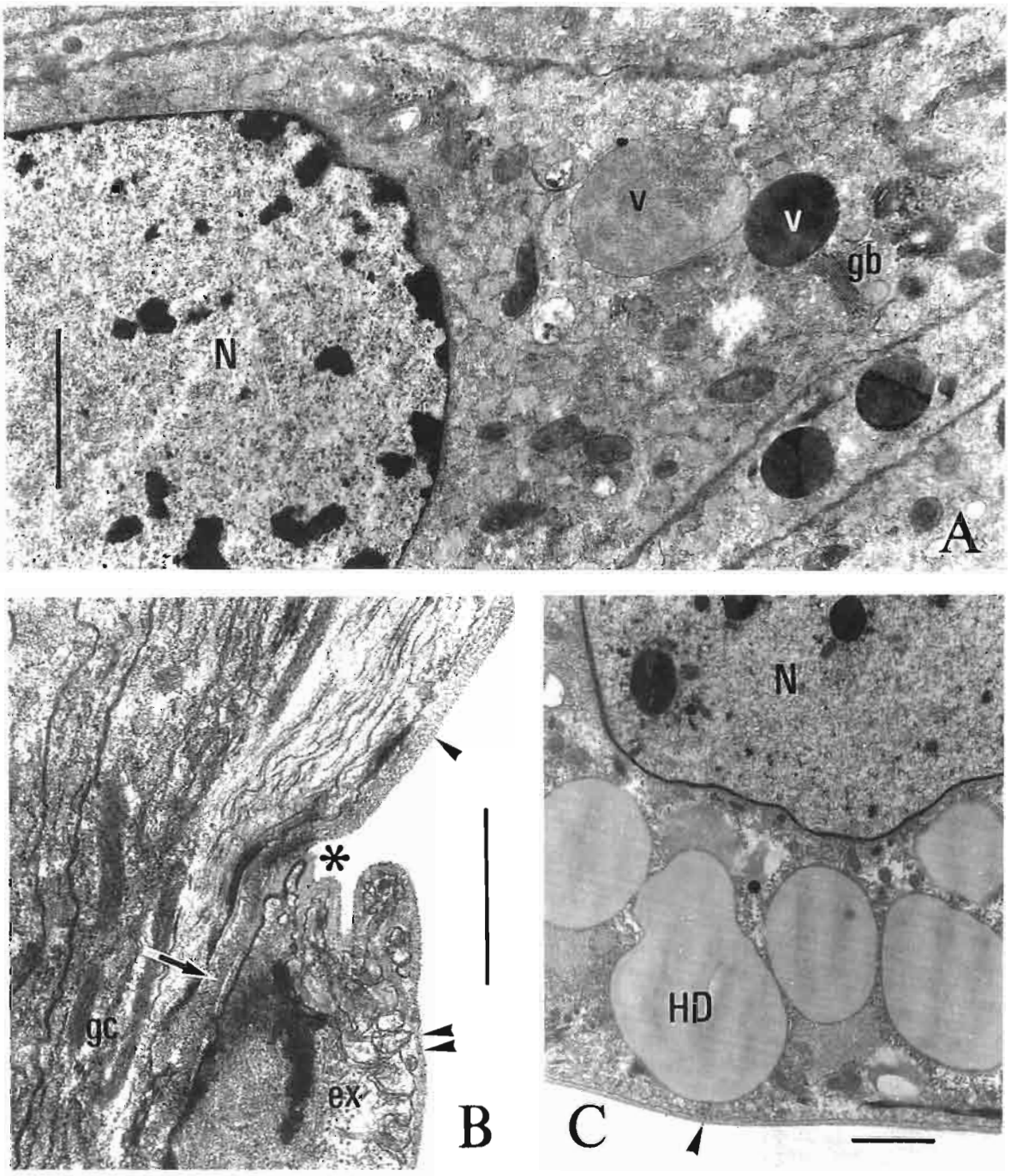

Fig. 5. *A : Transverse section through the oviduct, glandular cell (left to right = basal to apical direction); B : The boundary between the oviduct and sphincter in longitudinal section. The* asterisk *indicates the place of doubling of the basal lamina* (arrowhead) *of the oviduct into the basal lamina* (double arrowhead) *of the sphincter and basal lamina* (arrow) *dividing central glandular cells from the external epithelium of the sphincter; C : Fragment of a transversal section through the sphincter, showing the large homogeneous droplets in the external epithelium. The* arrowhead *indicates the basal lamina.* (Bar : A = 2 μm; B, C = 1 μm.)

For abbreviations see Fig. 1.

bules dispersed throughout the electron-translucent nucleoplasm (Figs 6 A; 7 E). The perinuclear space is filled with darkly stained substance.

Numerous homogeneous droplets are the most evident cytoplasmic feature of the bulge (Fig. 5 C). Small mitochondria, Golgi bodies, small vesicles, randomly distributed bundles of filaments, and occasional multivesicular bodies are also present in the cytoplasm (Fig. 6 A).

The apical cytoplasm is filled with filamentous material and bundles of filaments oriented circumferentially encircling the central core (Figs. 6 B; 7 E). These bundles of filaments are the main cytoplasmic component of the thin cytoplasmic regions connecting the bulges (Fig. 6 C). There the basal lamina is highly folded. The filaments of the external epithelial tube of the sphincter do not have the regular structure which is characteristic of muscle filaments.

Apically the external epithelium is interdigitated with the cells of the central cellular core (Fig. 6 B). There the cells are separated by a thin, darkly stained substance organized into a layer of uniform thickness.

There is no distinct boundary between external epithelia of the sphincter and uterus. There the basal lamina forms large outfoldings and small nuclei are placed in the individual cytoplasmic pockets. Proximally, the epithelial sheath grades into uterine epithelium. The lumen at the distal end of the uterus, blocked by the proximal end of the core of the sphincter, is filled with sperm.

Discussion

The female reproductive system of *Enoplus demani* is a relatively simple structure. There are two epithelial tubes each subdivided into three principal zones : ovary, oviduct, and uterus. Each ovary has a well developed ovarial sac where the oocytes terminate their growth.

The wall of the ovaries was described for many nematodes as a sheath of simple squamous epithelial or myoepithelial cells stretched along the ovary (Harada *et al.*, 1970; Lee & Lešťan, 1971; Yuen, 1971; Adamson, 1983; Foor, 1983; Franz & Büttner, 1983; Strome, 1986; MacKinnon, 1987; Van de Velde & Coomans, 1988). The ovarian wall of *E. demani* comprises both types of cells. Epithelial cells were found at the germinal end of the ovary, where these cells enclose a cluster of early oocytes. The remainder of the ovary is lined by myoepithelial cells of two types : *i)* flattened spindle-shaped myoepithelial cells enclosing a single file of growing oocytes; *ii)* bipartite cells of the ovarial sac, where the flattened contractile parts of the cells are connected with perikarya protruding into the cavity of the ovarial sac. The latter type of cells differs from those previously described in the ovary of nematodes thus far studied.

The ovarian myoepithelial cells with cell bodies that fill the ovarial sac have been described earlier in only one nematode species, *Xiphinema meridianum* (Van de Velde *et al.*, 1990 *a*) belonging to the order Dorylaimida, which is closely related to the order Enoplida. However, in *E. demani*, the cell bodies of the myoepithelial cells evidently reveal features very characteristic of exosecretory cells (extensive RER, Golgi bodies, numerous vesicles). Thus, the bipartite cells of the ovarial sac of *E. demani* appear to be bifunctional in having both contractile and secretory capabilities. Similar type of cells was found in the pharynx of nematodes (Grotaert & Coomans, 1980) and gastrotrichs (" myoglanduloepithelial cells "; Ruppert, 1982). However, these are the cross-striated myoepithelial cells which secrete the cuticle lining of the pharynx.

The epithelial sheath of the ovary, presumably connected with the nutrition of the oocytes, supposedly forms the ovarian wall of several parasitic nematodes (Harada *et al.*, 1970; Lee & Lešťan, 1971; Yuen, 1971; Franz & Büttner, 1983; MacKinnon, 1987). Unfortunately, there is no clear evidence of the nutritive function of ovarian cells in nematodes (Foor, 1983). Thus, there are no reasons to assign the cells of the ovarial sac of *E. demani* to cells of the follicle type. It is more likely that the secretory part of the myoepithelium is responsible for the development of the extracellular spindle-shaped bodies that fill the cavity between the ovarian wall and terminal oocyte. Such bodies have never been found earlier in the ovaries of nematodes and their function is obscure.

The oviduct of *E. demani* is an epithelial tube formed of high columnar cells of secretory nature. The same cells are believed to form the central cellular core of the sphincter. The glandular structure of the oviduct is not an unusual feature. It was described in several ultrastructural studies of nematode gonoducts (Lee & Lešťan, 1971; Yuen, 1971; Adamson, 1983; Foor, 1983). The absence of special glands in the gonoducts is considered to be a primitive feature characteristic of all nematode taxa (Fitzgerald & Foor, 1988).

The bilayered sphincter of *E. demani* is somewhat similar in structure to the bilayered oviduct of another enoplid, *Deontostoma californicum* (Hope, 1974). In both cases there is an internal epithelial tube of a glandular nature overlapped by an external epithelial layer continuous with the uterine wall. The bilayered structural pattern of the oviduct was indicated earlier for other enoplid nematodes : *Enoplus communis* and *Adoncholaimus fuscus* (Hope, 1974). However, in *D. californicum* the external epithelium envelops the whole glandular tube of the oviduct (Hope, 1974). In *E. demani*, a separate bilayered region of the gonoduct connects the oviduct to the uterus and is designated here as a sphincter.

The circumferential filaments in the external epithelium of the sphincter may be referred to : *i)* filaments providing the non-muscular contraction after the passing of oocytes or *ii)* tonofilaments providing elastic

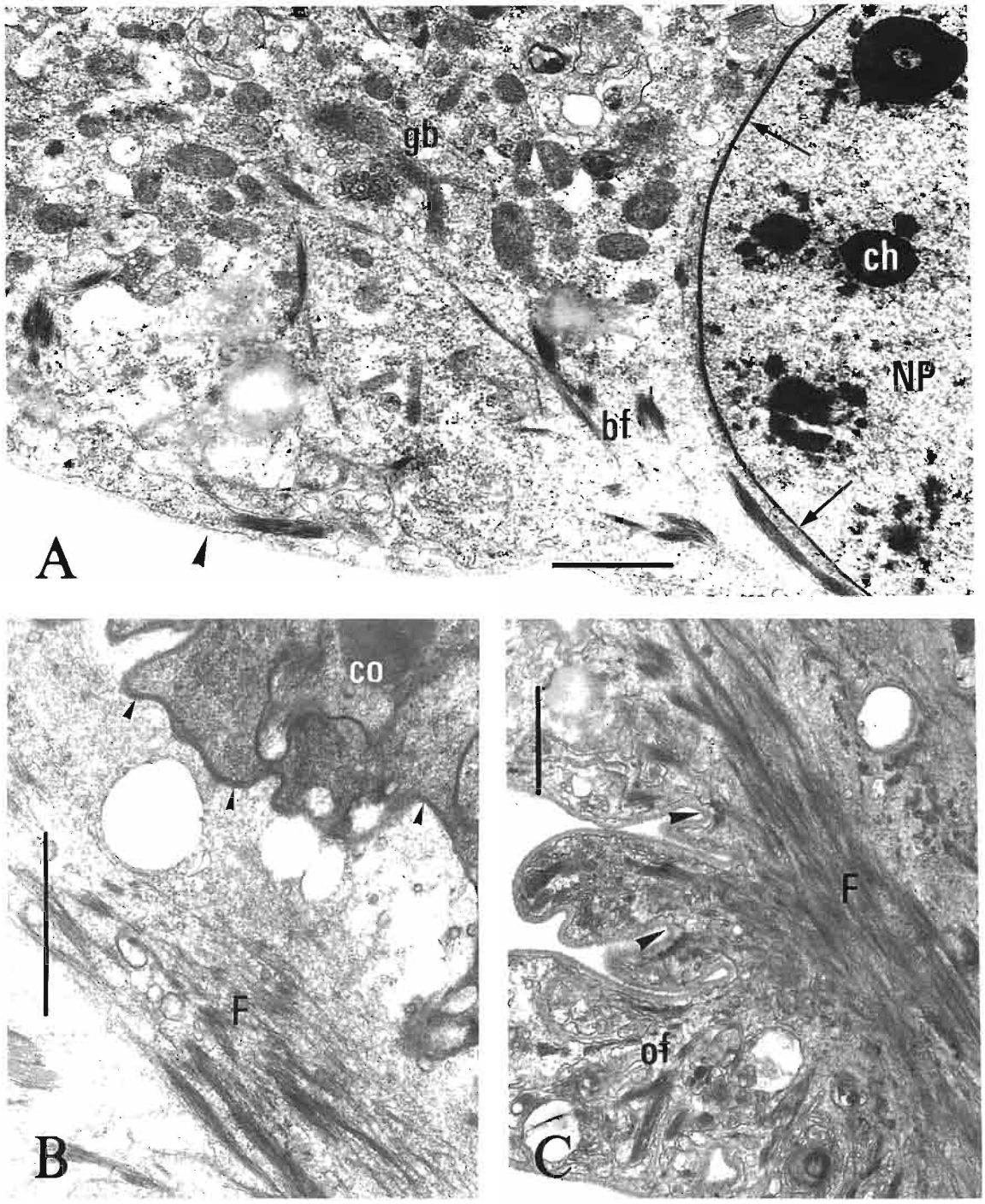

Fig. 6. *A : Transverse section through the external epithelial tube of the sphincter. The* arrowhead *indicates the basal lamina, the* arrow *indicates the nuclear envelope; B : Transverse section through the sphincter, showing the boundary* (arrowheads) *between the apical filamentous part of the external epithelium and central cellular core; C : Transverse section through the sphincter, the region of the filamentous cytoplasmic bridge between bulge compartments. The* arrowheads *indicate the infoldings of the basal lamina.* (Bar : A-C = 1 µm.)

For abbreviations see Fig. 1.

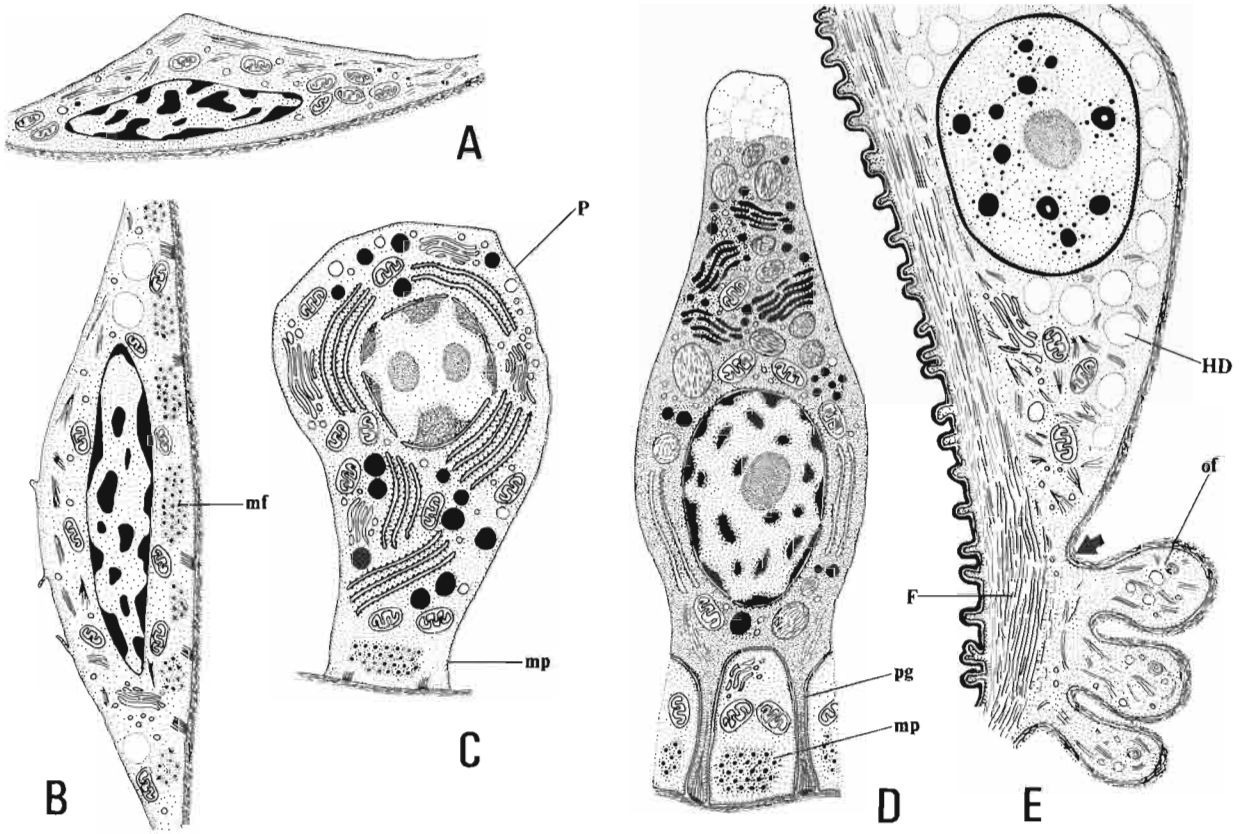

Fig. 7. *A-E : Schematic representations of the ultrastructure of cells that constitute the different parts of a gonadal wall of* Enoplus demani, *transverse sections. A : Epithelial cell of the ovarian germinal zone; B : Flattened myoepithelial cell of the ovarian growth zone; C : Bipartite myoepithelial cell of the ovarial sac with muscle process (mp) connected with perikaryon (P); D : Glandular cell of the oviduct attached to basal lamina by processes (pg); E : Part of the external epithelium of the sphincter, the* arrow *indicates the place where the cytoplasm of the bulge grades into the cytoplasmic bridge.*

For abbreviations see Fig. 1.

properties to the sphincter tube. In any case, the sphincter of *E. demani* has a non-muscular nature.

The bilayered structure is not unique for the enoplid gonoduct. Such a character has been described for oviducts, sphincters, and uteri in other taxa of nematodes (Yuen, 1971; Bleve-Zacheo *et al.*, 1976; Geraert *et al.*, 1980; Franz & Büttner,1983; Geraert, 1983; Van de Velde *et al.*, 1990 *a, b*). The external muscular layer that envelops the internal epithelial tube of the oviduct was also found in a variety of zooparasitic species (Bogoyavlenskij *et al.*, 1982).

The highly folded basal lamina as well as the syncytial structure of the external epithelium of the sphincter of *E. demani* should be considered as an adaptation for drastic expansion of the cellular tube. To date, syncytia in the female reproductive system have been observed only in several secernentean species (Harada *et al.*, 1970; McLaren, 173; Bogoyavlenskij *et al.*, 1982; Foor, 1983; Strome, 1986). Our ultrastructural observations on *E. demani* show that such syncytial tissues occur also in the primitive marine nematodes.

Finally we would like to outline the ultrastructural peculiarities of the female reproductive system of *E. demani;* (1) several epithelial cells at the distal tip of the ovary; (2) well-developed myoepithelium in the ovarian wall; (3) bipartite glandulomuscular cells in the wall of the ovarial sac; (4) spindle-shaped extracellular bodies in the cavity of the ovarial sac; (5) well-developed columnar, glandular epithelium in the oviduct; (6) bilayered structure of the sphincter; (7) syncytial nature of the external non-muscular sheath of the sphincter. These features may be interesting in comparative analysis of the nematode gonoducts.

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