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Myzostomida: A review of the phylogeny and ultrastructure

Igor Eeckhaut* & Déborah Lanterbecq
Marine Biology Laboratory, Natural Sciences Building, University of Mons-Hainaut, Mons, Belgium
*(*Author for correspondence: Tel.: +32-65-373439; Fax: +32-65-373434; E-mail: Igor.Eeckhaut@umh.ac.be)*

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Abstract

Myzostomids are minute, soft-bodied, marine worms associated with echinoderms since the Carboniferous. Due to their long history as host-specific symbionts, they have acquired a highly derived body plan that obscures their phylogenetic affinities to other metazoans. Because certain organs are serially arranged a closer relationship between polychaetes and myzostomids has repeatedly been discussion. We presented here a review on the ultrastructure of myzostomids with the most recent analyses that concern their phylogenetic position. The ultrastructure of the integument, digestive system, excretory system and nervous system are summarized. Unpublished information on the gametogenesis and reproductive systems of myzostomids are also exposed with a view on their reproductive process.

Introduction

Myzostomids, or myzostomes, are minute, soft-bodied, marine worms that are all associated with echinoderms (but see Grygier, 2000 for possible exceptions). They are found in all oceans from subtidal to a depth of over 3000 m. Most of them are ectocommensals of crinoids but some species that are parasites of crinoids, asteroids, or ophiuroids infest the gonads, coelom, integument or digestive system. The association between myzostomids and echinoderms is very old: signs of parasitic activities, similar to those induced by extant gallicolous myzostomids (i.e., those deforming echinoderm stereom), are found on fossilized crinoid skeletons dating back to the Carboniferous (Warn, 1974; Meyer & Ausich, 1983; Eeckhaut, 1998). Some pits found on Ordovician crinoid fossils could also have been induced by parasitic myzostomids (Eeckhaut, 1998). Due to their long history as host-specific symbionts, myzostomids have acquired a unique, highly derived anatomy that obscures their phylogenetic affinities to other metazoans (see Fig. 1). The body plan of most myzostomids is indeed singular inasmuch as they are incompletely segmented, parenchymous, acoelomate organisms with chaetae (see Grygier, 2000 for a review of the myzostomid body plans).

For most myzostomids, the body consists of an anterior cylindrical introvert (also called proboscis) and a flat, oval or disk-like trunk (Fig. 1). The introvert is extended when the individuals feed but it is retracted into an antero-ventral pouch of the trunk most of the time. The trunk ranges from a few millimeters to three centimeters long for the largest species. Five pairs of parapodia are located latero-ventrally in two rows, each parapodium containing a protrusible hook, some replacement hooks, and a support rod (or acicula). Most species have four pairs of slit- or disk-like latero-ventral sense organs, commonly named lateral organs, and the trunk margin often bears flexible needle-like cirri (more than one hundred in some species). Hump-like or pointed cirri also occur at the base of each parapodium of ca. 20 species. Two male gonopores are located at the level of the third pair of parapodia and the female gonopore opens close to the anus, postero-ventrally.

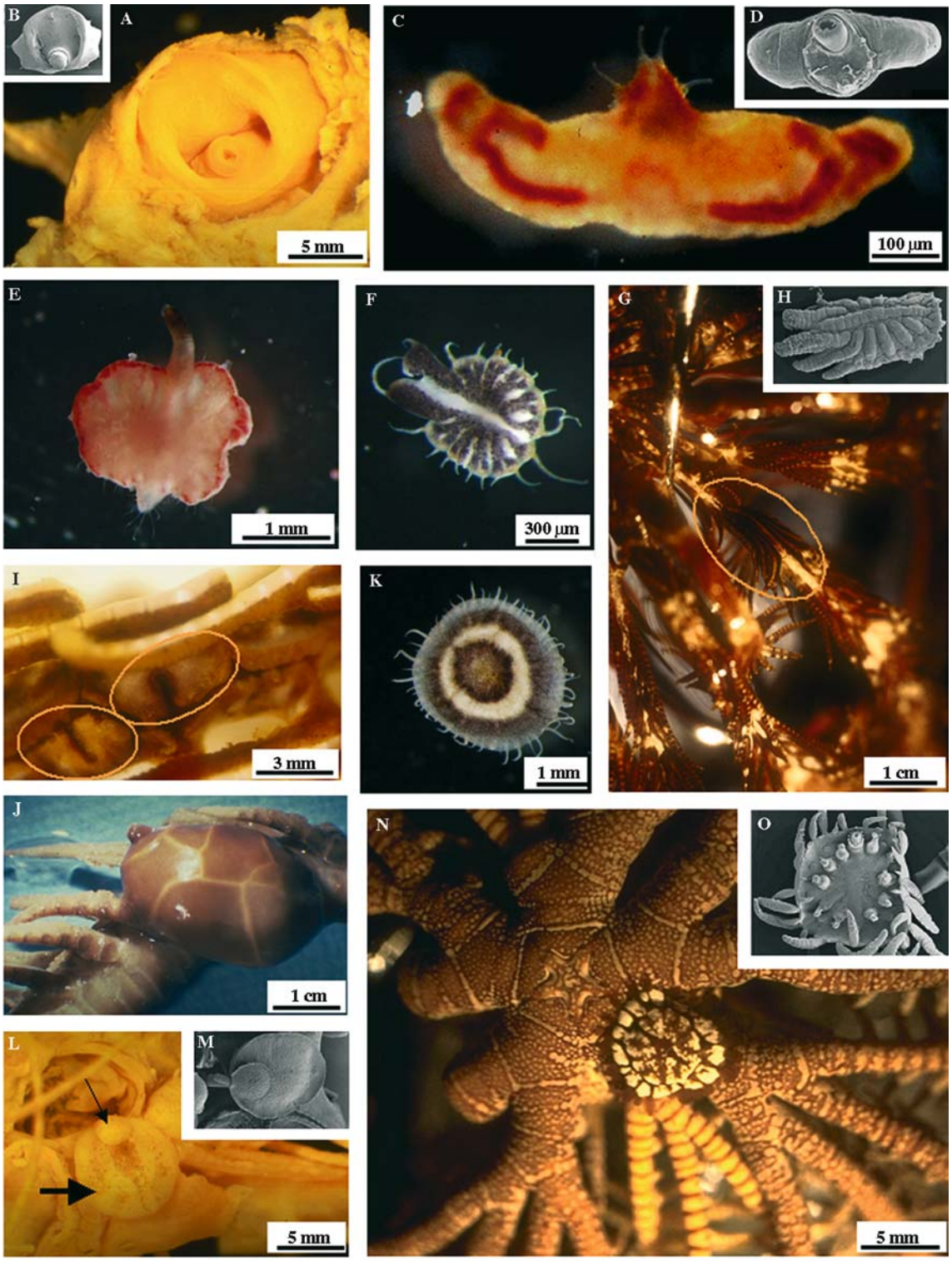
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Figure 1. Illustrations of different species of myzostomids (OM and SEM views) and of the deformities they induce on crinoids. (A, B) The female of *Pulvinomyzostomum pulvinar* in the anterior part of the digestive system of its Mediterranean crinoid host, *Leptometra phalangium*; the myzostomid is seen after partial dissection of the host (OM) and after total extraction from the host (SEM). (C, D) *Contramyzostoma bialatum* (OM view of the lateral side and SEM view of the dorsal side), a parasite of the integument of the Singaporean crinoid *Comaster gracilis*. (E) A parasite of the integument, *Myzostoma toliarense*, dorsal view, with its introvert everted. (F) A young ectocommensal, *Myzostoma pseudocuniculus* (characterized by one pair of caudal appendages), observed dorsally. The two species in E and F live on crinoids from Madagascar. (G, H) *Myzostoma fissum*, a species infesting preferentially crinoids of the family Mariametridae; the individual is seen in life on its host (within the oval) and with SEM; the body of the individual observed is very similar in shape and coloration to some parts of the crinoid arms. (I, J) Two cysts induced by *Contramyzostoma sphaera* and a gall created by *Endomyzostoma tenuispinum*; all are induced on crinoid arms; the cysts are made of soft tissue and the wall of the gall is made of deformed original ossicles and newly formed ossicles induced by the presence of the parasite. (K) Dorsal side of *Myzostoma polycyclus*, a common Indo-West Pacific species mainly observed on *Comanthus* crinoids. (L, M) A small male of *Myzostoma alatum* (small arrow) attached on the dorsal side of a big female (large arrow), both located close to the mouth of their Mediterranean crinoid host, *Leptometra phalangium*; they are observed in life and with SEM. (N, O) *Myzostoma mortenseni*, a large ectocommensal observed on the aboral side of a *Clarkcomanthus albinotus* from Papua New Guinea, seen in life and with SEM; the SEM view of the ventral side shows the everted introvert, parapodia, and lateral organs.

The body of parasites is often highly modified (Fig. 1). The introvert, external appendages and sensory organs are usually reduced or have disappeared. According to the location of myzostomids in the host, their trunk will be folded up dorsally (e.g., *Pulvinomyzostomum pulvinar*; Fig. 1A and B), very much longer than wide (e.g., Protomyzostomatidae and Mesomyzostomatidae), very much wider than long (*Contramyzostoma* species; Fig. 1C and D), mushroom-shaped (e.g., *Mycomyzostoma calcidicola*), or totally irregular (unnamed species described by Heinzeller et al., 1995).

Leuckart (1827, 1830, 1836) was the first to observe myzostomids and he made the first diagnosis on a new genus of unknown organisms that he named *Myzostoma* (Leuckart, 1836). At present, ca. 170 species are known from the scientific literature, with 80% of them being ectocommensals of crinoids. Most of the ultrastructural analyses of myzostomids have been made on a single species, the European myzostomid *Myzostoma cirriferum*. Phylogenetic analyses are extremely recent. They are based on morphological data (Haszprunar, 1996; Rouse & Fauchald, 1997), molecular data (Eeckhaut et al., 2000), or both (Zrzavỳ et al., 2001). This work is a review of myzostomid ultrastructure and phylogeny. Only analyses of adult ultrastructure are mentioned, the fine structure of myzostomid larvae having been investigated recently (Eeckhaut et al., 2003). Some unpublished data from Master and Ph.D. theses are included. An overview of the anatomy, taxonomy, and ecology of myzostomids can be found in Grygier (2000).

Phylogenetic position within the Metazoa

The phylogenetic position of myzostomids within metazoa has been a subject of controversy since their discovery. Myzostomids have been first considered as Trematoda (Leuckart, 1827), Crustacea (Semper, 1858) or Stelechopoda (i.e., a taxon grouping myzostomids with Tardigrada and Pentastomida; Graff, 1877). Benham (1896) was the first to suggest them as a separate class of annelids (rather than derived polychaete annelids), a position supported later by other investigators (e.g., Fedotov, 1929; Kato, 1952). Jägersten (1940) grouped the Myzostomida and the Annelida (as two separate classes) into a coelomate protostome clade called Chaetophora. More recently, Mattei & Marchand (1987), based on ultrastructural similarities between the spermatozoa of Myzostomida and Acanthocephala, considered these two taxa as sister groups defining a phylum they called Procoelomata. Because myzostomids exhibit characters such as parapodia with chaetae and acicula, a trochophora-type larva, and a segmentation (though incomplete), they are classified in all textbooks and encyclopaedias as a family or an order of Polychaeta or as a class of Annelida. This suggestion is also based on the very intuitive idea that the body plan of ectocommensal myzostomids evolved from that of an errant polychaete ancestor

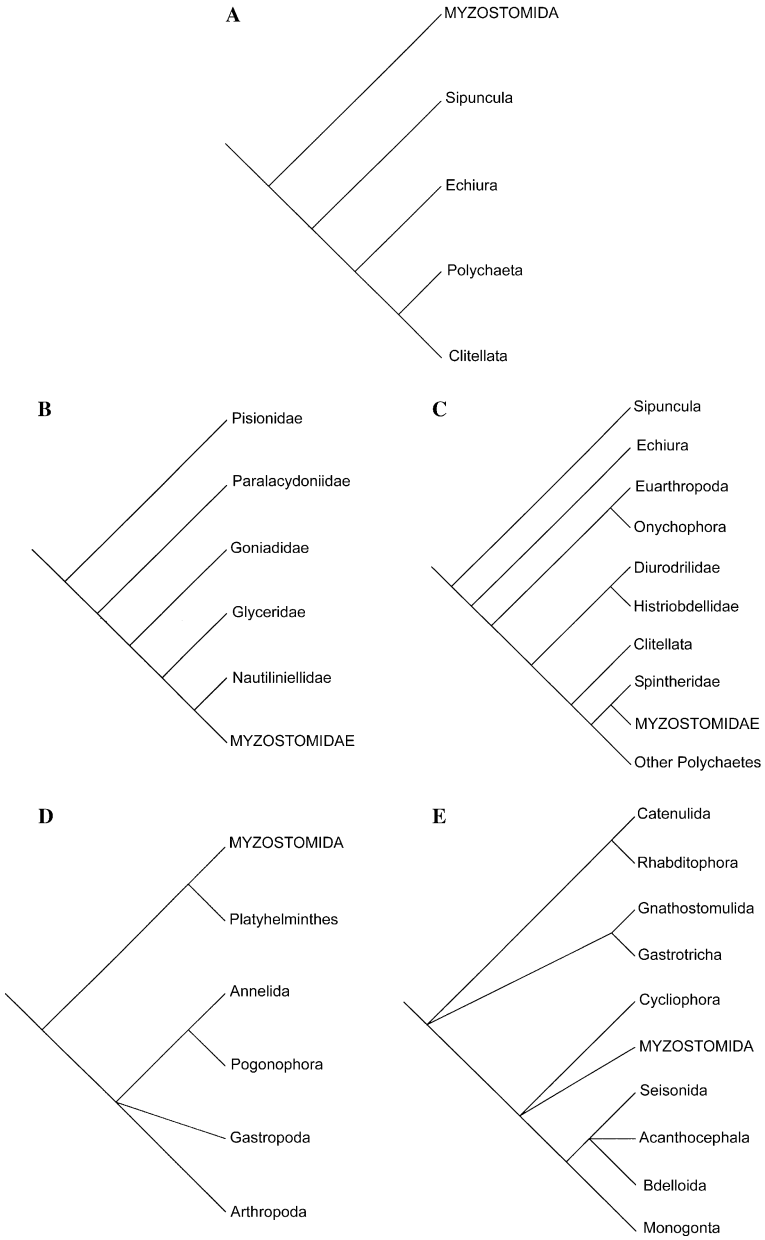

Figure 2. Dendrograms illustrating the results of the analyses concerning the phylogenetic position of the Myzostomida within the Metazoa. The analyses are those of Haszprunar (1996) (A), Rouse and Fauchald (1997) (B and C), Eeckhaut et al. (2000) (D) and Zrzavý et al. (2001) (E).

that lost the notopodial chaetal system, with a migration of the neuropodia onto the ventral side of the body. The overdevelopment of the ventral side of myzostomids and the regression of locomotory and sense organs could have happened once or several times and led to the body plan(s) of parasitic myzostomids.

During the last seven years, eight phylogenetic analyses based on phenotypical and/or molecular characters have included data from the Myzostomida. The first two analyses were based on morphological data. Haszprunar (1996) made a cladistic analysis to point out molluscan affinities with other Bilateria. His analyses suggested that

the Myzostomida are the sister group of a clade including the Sipuncula, Echiura, and Annelida (Fig. 2A). Rouse & Fauchald (1997) investigated polychaete systematics by means of cladistic analyses including myzostomids as a family (i.e., the Myzostomatidae). The different analyses supported the placement of the myzostomids as a family nested within the Polychaeta; the Myzostomatidae were either associated to the Spintheridae (a family including ectoparasitic species of sponges) or they clustered with polychaetes with a hypertrophied axial pharynx (Fig. 2B and C).

Chenuil et al. (1997) were the first to obtain a DNA sequence from a myzostomid: they sequenced a small part of the large ribosomal subunit RNA gene (LSU hereafter) of *Myzostoma* sp. They compared the secondary structure of this segment with that of other metazoans including polychaetes and oligochaetes and pointed out the difference existing between myzostomids and annelids. Zrzavỳ et al. (1998) used phenotypical (morphological, ultrastructural, developmental and ecological characters) and molecular data (DNA sequences coding for the RNA included in the small ribosomal subunit, SSU hereafter) in their cladistic analyses. No SSU sequences were available for myzostomids and the analyses comprised only phenotypical characters for this group. The analyses suggested that the Myzostomida are the sister group of a clade including Echiura, Pogonophora, and annelids.

Eeckhaut et al. (2000) inferred the phylogenetic position of the Myzostomida within the Metazoa by analysing the DNA sequences of two slowly-evolving nuclear genes: the SSU and the elongation factor-1α (EF-1α hereafter). Eeckhaut et al. (2000) sequenced five species of myzostomids (*Endomyzostoma clarki*, *Myzostoma cirriferum*, *M. fissum*, *Notopharyngoides aruense*, and *Contramyzostoma sphaera*) for SSU sequences and two species (*Myzostoma alatum* and *Pulvinomyzostomum pulvinar*) for EF-1α sequences. All analyses yielded best trees with the Myzostomida not nested within the Annelida and suggested that myzostomids are closer to flatworms than they are to annelids (Fig. 2D).

Zrzavỳ et al.'s (2001) analyses comprised SSU and LSU sequences of *Myzostoma glabrum*, together with phenotypical characters. The analyses showed myzostomids as the sister group of Cycliophora, closely related to the rotifer-acanthocephalan clade (=Syndermata) (Fig. 2E). The myzostomid-cycliophoran-syndermate clade, accommodated within the Platyzoa, was strongly supported by most analyses. Zrzavỳ et al. (2001) proposed the new name Prosomastigozoa for this group, due to the presence of highly derived spermatozoa with an anteriorly directed flagellum, at least present in myzostomids and syndermates (cycliophoran sperm ultrastructure was insufficiently known).

Recently, two works inferred the phylogenetic position of non-myzostomidan clades but used myzostomidan sequences in the ingroup. Littlewood et al. (2001) used EF-1α amino-acid sequences to estimate the position of the Acoela within the Metazoa. Arthropods, vertebrates, molluscs, myzostomids, and annelids plus pogonophorans were each represented as monophyletic groups. Platyhelminthes appeared to be a polyphyletic group and Myzostomida were found outside the Annelida. Rota et al. (2001) inferred the phylogenetic position of the only two truly terrestrial non-clitellate annelids, *Parergodrilus heideri* and *Hrabeiella periglandulata*, using a data set of new 18S rDNA sequences as well as homologous sequences already available for 18 polychaetes including one species of Myzostomatidae, *Myzostoma* sp. The results included Mollusca, Sipuncula, Echiura, and Pogonophora, as well as *Myzostomum* sp. within the Polychaeta in all of the most parsimonious trees but without bootstrap support for the position of the myzostomids.

In summary, three possibilities exist concerning the phylogenetic position of the Myzostomida within the Metazoa: (1) phylogenetic analyses considering the Myzostomida as a group outside the Annelida are wrong and similarities between the two groups are true synapomorphies; these analyses are right and the similarities between the two groups are either (2) convergences or (3) plesiomorphies.

Integument

The ultrastructure of the regular (i.e., non-sensory) integument of myzostomids is known in four species: *Myzostoma cirriferum* (Eeckhaut & Jangoux, 1993), *Contramyzostoma bialatum* (Eeckhaut

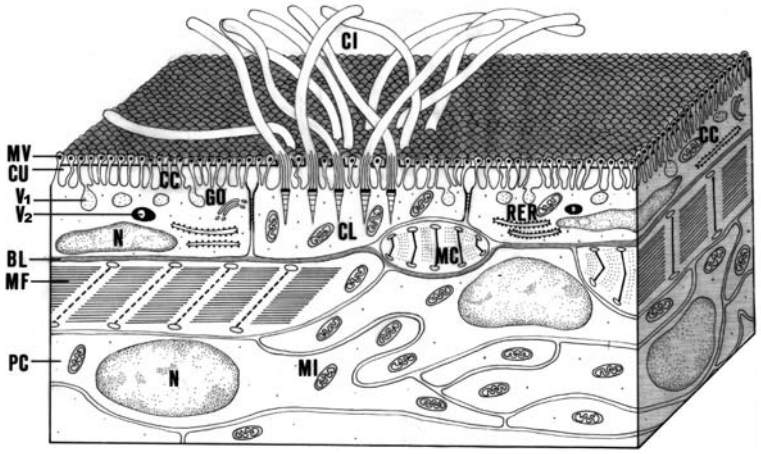

Figure 3. Myzostoma cirriferum. Schematic drawing of a portion of a longitudinal section through a non-sensory area of the integument (Eeckhaut & Jangoux, 1993). Abbreviations: BL – basal lamina; CC – covering cell; CI – cilium; CL – ciliated cell; CU – cuticle; GO – Golgi apparatus; MC – myoepithelial cell; MI – mitochondrion; MF – muscular fibre; MV – microvillus; N – nucleus; PC – parenchymal cell; RER – rough endoplasmic reticulum; V1 and 2 – vesicles of 1st and 2nd types.

& Jangoux, 1995), *Pulvinomyzostomum pulvinar* (Kronenberg, 1997), and *Myzostoma alatum* (Kronenberg, 1997). In all of them, the integument consists of an epidermis with cuticle and a parenchymo-muscular layer that extends between the internal systems (i.e., the digestive, nervous, excretory and genital systems). A mesothelium lining a coelom is not evident in myzostomids, although the female genital tract and ovaries are sometimes considered as coeloms. There is no mineralized skeleton and the only hard parts are the chitinous hooks and aciculae of parapodia that have not yet been studied ultrastructurally except in larvae (Eeckhaut et al., 2003).

Non-ciliated and ciliated cells form most of the myzostomid epidermis (Fig. 3). They have been observed in the four species cited here above and in *Contramyzostoma sphaera*, *Myzostoma cuniculus*, *M. laingense*, *M. horologium*, and *Notopharyngoides aruense* (unpublished observations). The cuticle of all these species is ca.1 μm thick and consists of several superposed fibrillar layers, the outer ones being more electron-dense. The cuticle is crossed by numerous microvilli that end in bulges (Fig. 3). Non-ciliated cells, also called covering cells, are flattened to cylindrical, and mainly characterised by having two types of vesicles (Fig. 3). Vesicles of the first type are spherical to ovoid in shape and contain electron-dense material that forms a dark margin. Vesicles of the second type are generally ovoid and are located in the most apical part of the cytoplasm, just under the cuticle. They are either empty or full of a granular material and are thought to participate in the formation of the cuticle, while the role of the vesicles of the first type is unknown. Ciliated cells are of similar shape and size to covering cells and bear from 20 to 50 cilia (Fig. 3). The cilia are 10–20 μm long, have the classical microtubular arrangement ($9 \times 2 + 2$ microtubules), and are each prolonged by a ciliary rootlet. Most species have usual, tube-like cilia but paddle-like cilia have been observed in *M. jagersteni* (Eeckhaut et al., 1994), *M. fissum*, and *M. ambiguum* (Lanterbecq, 2000). The proportions of covering and ciliated cells vary according to the myzostomid species considered and this seems to be related to the type of association existing between the myzostomid and the host: in ectocommensals, ciliated cells are sparse with a ratio of ciliated cells to non-ciliated cells of about 1:5. At the opposite extreme, the trunk of intradigestive myzostomids such as *P. pulvinar* and *N. aruense* is almost totally covered by cilia (CC/NC ratio of about 1:1). Ciliary beating induces a water current at the surface of the individuals and surely facilitates the intake of both oxygen and dissolved nutrients through the integument. The overdevelopment of epidermal cilia in intradigestive myzostomids could be an adaptation to their symbiotic way of life.

Two types of gland cell have been observed in the myzostomid epidermis. The first type is mainly found in parapodia, in cirri, and in the folds that surround lateral organs. It has been observed in *M. cirriferum* (Eeckhaut & Jangoux, 1993), *P. pulvinar* (Kronenberg, 1997), *M. alatum* (Kronenberg, 1997), and in *N. aruense*, *M. laingense*, and *M. horologium* (unpublished observations). These cells are cylindrical in shape and their cytoplasm is full of ovoid vesicles including Alcian Blue-positive granular material. These gland cells most likely release mucous for protecting places where contact with the substratum or the host is frequent (Eeckhaut & Jangoux, 1993). The second type of gland cell has been observed in the villous and ciliated central part of the lateral organs of *M. cirriferum* (Fig. 5). This type has a large cell body resting under the other epidermal cells, and an elongated apical process running to the apex of the epidermis. The cytoplasm is full of spherical vesicles filled with a homogenous, finely granular, Alcian Blue-positive material. The vesicle content has various electron densities and some vesicles appear empty.

Muscle fibre cells, called myoepithelial cells by Eeckhaut & Jangoux (1993), were observed in the epidermis of *M. cirriferum* (Eeckhaut & Jangoux, 1993) and *C. bialatum* (Eeckhaut & Jangoux, 1995), but they were not found in *M. alatum* nor in *P. pulvinar* (Kronenberg, 1997). They are thin, elongated muscle cells lying under the covering and ciliated cells (they never contact the cuticle) in such a way that their longitudinal axis is perpendicular to the antero-posterior axis of the myzostomid (Fig. 3). They are particularly abundant at the level of the introvert (Eeckhaut & Jangoux, 1993), where their main action is to reduce the introvert diameter and its length while inducing deep pleats when they contract.

The parenchymo-muscular layer includes muscle cells and parenchymal cells. Dermal muscles are conspicious ventrally as well as in the introvert and parapodia; they are less developed dorsally. They consist of longitudinal, circular, and transverse muscle cells of the double-obliquely striated type. In addition, dorso-ventral muscles, which form septa, occur in the trunk. The thickness of the parenchyma varies according to the development of the gonads (it is the thinnest when gonads are mature). The parenchyma is particularly well developed in the dorsal part of the myzostomid trunk where parenchymal cells occur in contact with developing female gametes. In the dorsal side of the trunk of *Myzostoma gopalai*, the parenchyma is completed by a wall of collagen fibres that has only been analysed by histochemical methods (Rao & Sowbhagyavathi, 1972). Parenchymal cells have a highly variable size and shape. Their cytoplasm includes very few organelles. The external matrix that surrounds parenchymal cells is generally poorly developed and is made of a granular material.

Nervous system and epidermal sensory regions

The anatomy of the nervous system has been described most thoroughly for several species of

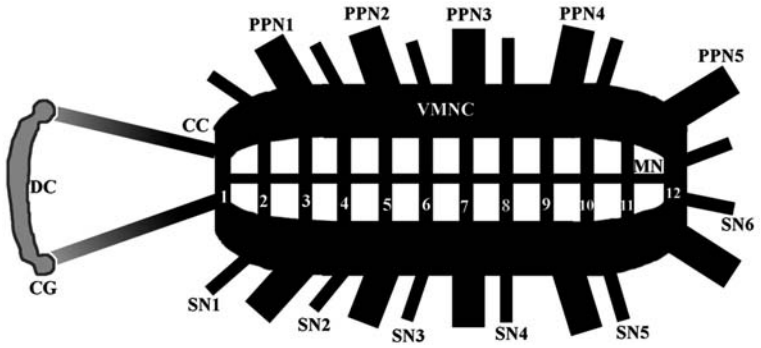

Figure 4. Myzostoma cirriferum. Schematic drawing of the central nervous system observed in the trunk of individuals (drawn from the pictures of Müller & Westheide, 2000). Abbreviations: CC – circumpharyngeal connective; CG – cerebral ganglia; DC – dorsal commissure; MN – median nerve; PPN 1–5 – parapodial nerves one to five; SN 1–6 – side nerves one to six; VMNC – ventral main nerve cord. The numbers 1–12 indicate the twelve commissures that connect the two ventral main nerve cords. Black and grey structures are ventral and dorsal, respectively.

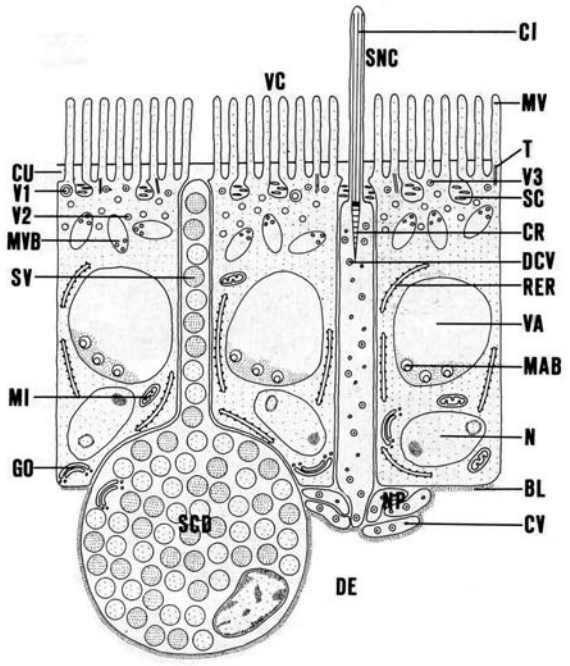

Figure 5. *Myzostoma cirriferum*. Schematic drawing of a section through the epidermis of the lateral organ dome (not to scale) (Eeckhaut & Jangoux, 1993). Abbreviations: BL – basal lamina; CI – cilium; CR – ciliary rootlet; CU – cuticle; CV – clear vesicle; DCV – dense cored vesicle; DE – dermis; GO – golgi apparatus; MAB – multiannular body; MI – mitochondrion; MV – microvillus; MVB – multivesicular body; N – nucleus; NP – nerve process; RER – rough endoplasmic reticulum; SC – subcuticular chamber; SCD – secretory cell of the dome; SNC – sensory cell; SV – secretory vesicle; T – tonofilaments; V1, V2 and V3 – vesicles of the 1st, 2nd and 3rd type; VA – vacuole; VC – vacuolar cell.

Myzostoma (see Grygier 2000 for review). Its ultrastructure has only been studied in *M*. *cirriferum* (Müller & Westheide, 2000), by means of confocal laser microscopy. In this species, the nervous system consists of two small cerebral ganglia connected by a dorsal commissure, a ventral nerve mass, and a pair of circumpharyngeal connectives joining the former to the latter (Fig. 4). The two neuropil cords within the ventral nerve mass curve outward and are joined to one another anteriorly and posteriorly. They are connected by twelve commissures forming a ladder-like system (Fig. 4). A single median nerve runs along the midventral axis (Fig. 4). In addition to the circumpharyngeal connectives, eleven peripheral nerves arise from each cord (Fig. 4). The first innervates the anterior body region. The others form five groups of two nerves each, the first and thicker nerve of which is the parapodial nerve, innervating the parapodium and two corresponding cirri (Fig. 4). Except for those of the most posterior group, the second nerves innervate the lateral organs and the trunk margin. One pair of dorsolateral longitudinal nerves was visualized by tubulin staining. The arrangement of the peripheral nerves and commissures strongly suggests that the myzostomid body is made of six segments (Müller & Westheide, 2000).

Sensory regions of the epidermis either show small structural variations from the regular epidermis or markedly differ from it (Eeckhaut & Jangoux, 1993). Small variations occur in the cirri, buccal papillae that surround the mouth of some species, body margin and parapodia, where ciliated sensory cells insinuate between epidermal cells. These are supposed to be mechanoreceptor sites that give information on the structural variations of the host integument; they could also participate in self-recognition of individuals (Eeckhaut & Jangoux, 1993). Ciliated sensory cells are dendritic processes of nervous cells, the cell bodies of which lie mostly in the ventral nerve cord. They usually run either singly or in pairs from bundles of basiepidermal nerve processes and reach the apex of the epidermis. Each sensory process bears up to five small cilia that cross the cuticle and have the usual microtubular arrangement ($9 \times 2 + 2$). Their basal body is prolonged by a ciliary rootlet. The sensory epidermis in the four pairs of lateral organs differs markedly from the regular epidermis. In *M*. *cirriferum* (Eeckhaut & Jangoux, 1993) and other myzostomids, there are almost always four pairs of ventral lateral organs alternating with the parapodia. A lateral organ consists of a villous and ciliated, dome-like central part that is surrounded or covered by a peripheral fold. The dome-like central part is the sensitive region of the organ and consists in ciliated sensory cells, secretory cells, and complex vacuolar cells that have numerous long microvilli and multivesicular bodies (Fig. 5). Lateral organs are presumed to allow the myzostomids to recognize the host integument and prevent them from becoming displaced onto the surrounding inhospitable substratum (Eeckhaut & Jangoux, 1993).

Digestive system

Ectocommensals of crinoids and many parasitic myzostomids that induce cysts or galls on crinoids feed on particles that they divert thanks to their introvert (i.e., most species of the genus *Myzostoma*; all the species of *Contramyzostoma* and *Endomyzostoma*). *Mesomyzostoma*, *Mycomyzostoma,* and *Protomyzostoma* species feed on host tissues, which is probably also the case for the *Asteromyzostomum* species. *Pulvinomyzostomum pulvinar*, *Asteriomyzostomum asteriae*, and the *Notopharyngoides* species live in the digestive lumen of their hosts where they ingest particles. No indication about the feeding behaviour of *Asteriomyzostomum fisheri* and *Stelechopus hyocrini* exists.

The anatomy of the digestive system of the Myzostomida is very similar in almost all species. It consists of a pharynx which is included in the introvert, a stomach with usually two or three pairs of blind, branching caeca, and an intestine. The only exceptions to this general scheme are: (1) there are no digestive diverticula in the female of *Mycomyzostoma calcidicola* and in *Stelechopus hyocrini*, (2) there are one right and one left U-shaped digestive diverticula in *Contramyzostoma bialatum*, (3) the male of *Mycomyzostoma calcidicola* has no digestive system; (4) the introvert is absent in the representatives of the Pharyngidea. The ultrastructure of the myzostomid digestive system is only known for *Myzostoma cirriferum* (Eeckhaut et al., 1995). All the cell types described in the digestive system of this species have, however, also been observed in *Contramyzostoma sphaera*, *Myzostoma capitocutis*, *Myzostoma cuniculus*, and *M. horologium* (unpublished observations).

The myzostomid pharyngeal epithelium is covered by a cuticle similar to the one that covers the epidermis. The structure of the epithelium differs on the lip (i.e., the part that surrounds the mouth) and in the pharynx *sensu stricto* (Fig. 6A and B). The lip is made of ciliated sensory cells, supporting cells, and salivary gland cells (Fig. 6A). Supporting cells are goblet-shaped cells made of an upper part, contacting the cuticle, and an inner part where the nucleus lies, both being connected by a thin cell process. The upper part of the cell is filled with Alcian blue-positive, ovoid vesicles. Salivary gland cells are common in myzostomids; they have been described in representatives of all genera except in *Mycomyzostoma calcidicola* (Eeckhaut, 1998) and *Pulvinomyzostomum pulvinar* (Jägersten, 1940). They lie in the parenchyma at the junction between the pharynx and the stomach. In *M. cirriferum*, they are about 20 in number, each being made of a large cell body from which starts a long, narrow cell process that ends on the lip. The cytoplasm is full of vesicles filled with electron-dense material that is assumed to be released in the pharyngeal lumen to digest food particles (Eeckhaut et al., 1995). Behind the lip, the pharyngeal lumen is only bordered by secretory cells that look very similar to epidermal covering cells but have many more apical, Alcian blue-positive, electron-dense vesicles (Fig. 6B). Myoepithelial cells, similar to those of the epidermis, have been observed in the pharynx of *M. cirriferum* (Eeckhaut & Jangoux, 1993).

Eeckhaut et al. (1995) proposed a model explaining how food particles are swallowed and carried into the stomach in ectocommensal species. When in search of food, the introvert continuously retracts and protrudes until it is applied at an appropriate place. Retraction results from the contraction of the longitudinal muscle cells that extend through the parenchyma of the introvert. The mechanism for protrusion appears to be more indirect because the introvert does not contain any antagonistic muscular or fibrillar (e.g., collagen fibre) system. The introvert of myzostomids has no internal cavity and changes in volume are due to the fact that muscles and parenchymal cells move from the introvert to the trunk and *vice versa*. The initiator of protrusion is probably located outside the introvert and could be the dorso-ventral muscle cells of the trunk; protrusion would depend on the forcing action of these muscle cells, which would push the pharynx and parenchyma into the relaxed introvert. Once the introvert is extended into an ambulacral groove of the host, food particles are swallowed due to the action of the muscle cells of the lip first, then those of the pharynx. The contraction of radial muscle cells of the lip increases the mouth diameter, thus sucking up food particles from the host's groove. The muscle sheet of the pharynx, made mainly of alternating radial and circular muscle cells, then starts to work. The

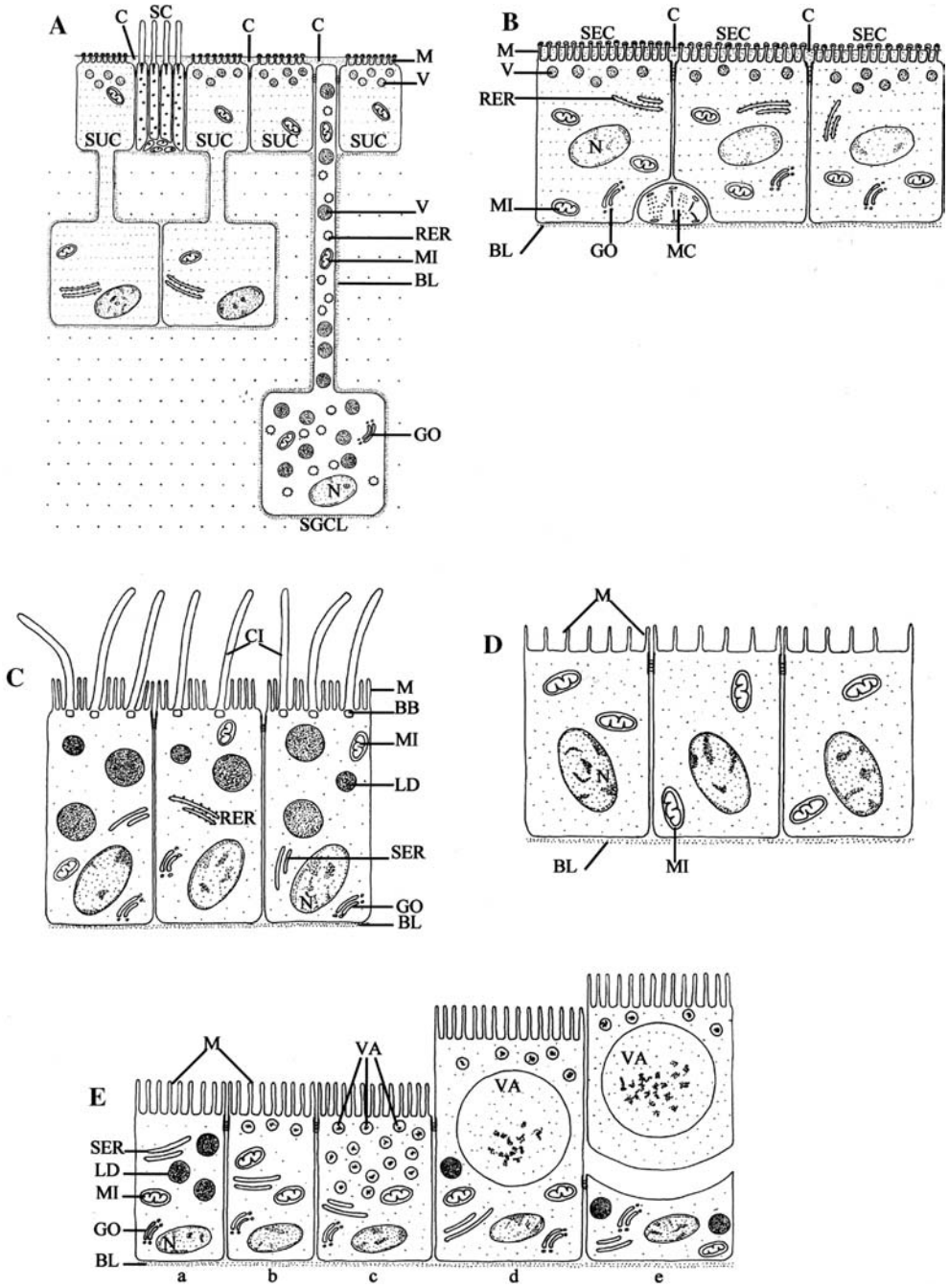

Figure 6. *Myzostoma cirriferum*. Schematic drawings of the epithelia (A) of the lip, (B) of the pharynx, (C) of the stomach, (D) of the intestine and (E) of the digestive caeca (a,b,c,d,e refer to successive metabolic stages of a caecal cell observed during the intradigestive process). Abbreviations: BB – basal body; BL – basal lamina; C – cuticle; CI – cilium; GO – Golgi apparatus; LD – lipidic droplet; M – microvillus; MC – myoepithelial cell; MI – mitochondria; N – nucleus; RER – rough endoplasmic reticulum; SC – sensory cell; SEC – secretory cell; SER – smooth endoplasmic reticulum; SGCL – salivary gland cells of the lip; SUC – supporting cell; V – vesicle; VA – vacuole.

contraction of radial cells enlarges the diameter of the pharyngeal lumen (sucking up the food particles) while the contraction of circular muscle cells reduces the diameter of the pharyngeal lumen (pushing down the food particles). The resulting peristaltic wave carries food particles into the inner organs of the digestive system, namely the stomach, the digestive caeca, and the intestine, each possessing a non-cuticularized epithelium surrounded by circular muscle cells.

The epithelium of the myzostomid stomach is made of a single cell type that is ciliated and cylindrical (Fig. 6C). In *M. cirriferum*, these cells bear many microvilli and several cilia that have the usual $9 \times 2 + 2$ microtubular arrangement and no rootlet below their basal body. Their cytoplasm includes numerous lipidic droplets that disappear when individuals are starving. Food particles carried by the pharyngeal peristalsis are conveyed towards the digestive caeca due to the action of the stomachal ciliature. Once in the caeca, particles are endocyted by caecal cells, the single cell type occurring in the caecal epithelium, and transferred into a single large vacuole – *viz*. a phagolysosome – in which they are digested. That vacuole regularly increases in size due to its fusion with additional phagosomes (Fig. 6E). When it has reached a size roughly corresponding to half the caecal cell volume, the vacuole, together with a fringe of cytoplasm that surrounds it, is expelled into the caecal lumen by an apocrine process. Detached cell fragments are forced out of the caecal lumen to the stomachal lumen due to a contraction of the caecal musculature. The cell fragments progressively gather together in the stomachal lumen, being embedded in an alcian blue-positive agglutinating matrix that is supposedly produced as a secretion of the pharyngeal secretory cells. A spindle-shaped faecal mass is finally formed, transferred to the intestine, and expelled to the outside by the contraction of the stomachal and intestinal musculatures. The intestinal epithelium is made of a single flattened cell type that harbours many microvilli but lacks cilia (Fig. 6D).

Excretory system

The excretory system of myzostomids consists of protonephridia whose ultrastructure has been described in *M. cirriferum* for both adults (Pietsch & Westheide, 1987) and larvae (Eeckhaut et al., 2003). The presence of protonephridia has not yet been investigated in any other species. In adults of *M. cirriferum*, five pairs of protonephridia were first described in the trunk (Pietsch & Westheide, 1987), but a closer examination using confocal laser scanning microscopy revealed six pairs of 90 μm long, S-shaped protonephridia in this species (Müller & Westheide, 2000). Nephridiopores are located ventrally in two rows of six that are parallel to the sagittal plane. Each protonephridium comprises three terminal cells and one duct cell (Fig. 7). Each terminal cell has six to nine flagella (without rootlets and with a $9 \times 2 + 2$ microtubular arrangement), which are each surrounded by rod-like cell processes. The duct cell bears microvilli and cilia. Weir-like fenestrations in the peripheral wall of the terminal cells make up the connection between the central lumina and the parenchymal extracellular space.

Usually, a pair of ducts connecting the uterus to the intestine occur in myzostomids and these have long and erroneously been named metanephridia. Spermatozoa have been observed in the lumen of these ducts and the only obvious function that can actually be assigned to them is to expel the excess of free spermatozoa, thus possibly preventing polyspermy during fertilization.

Oogenesis and female genital system

Most myzostomids are hermaphrodites. They often are functional simultaneous hermaphrodites even though the male genital system develops a bit earlier than the female genital system during organogenesis. In some other species, a male and a female are found and are often interpreted as the two stages of a protandrous hermaphroditic species, the dwarf male being supposed to transform into a female once it lives alone. Only *Mycomyzostoma calcidicola* is considered to be dioecious (Eeckhaut, 1998). The breeding period is only known for the European species *M. cirriferum* in which it extends throughout the year (Eeckhaut & Jangoux, 1997).

The female genital system of adult myzostomids consists of a branched duct and a diffuse

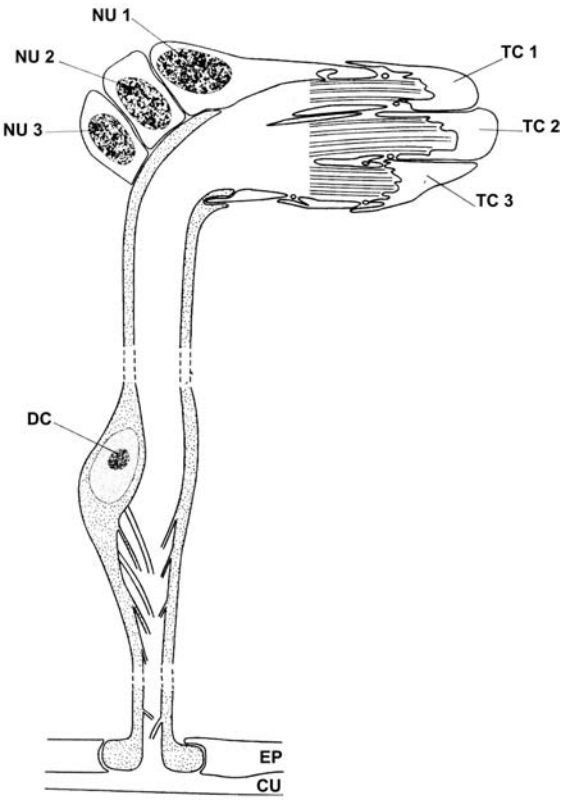

Figure 7. Myzostoma cirriferum. Schematic drawing of a protonephridium (Pietsch & Westheide, 1987). Abbreviations: CU – cuticle; DC – duct cell; EP – epidermal cell; NU 1–3 – nuclei of terminal cells; TC 1–3 – terminal cells.

ovary (i.e., female germinal cells growing in between parenchymal cells), both located in the dorsal part of the trunk in most species. The female genital system is found ventrally or has an ovary developed both ventrally and dorsally in some other species, however, usually, the branched genital duct follows the course of the digestive organs of the trunk (i.e., intestine, stomach, and digestive caeca). The ovary lies above it in such a way that a section through the dorsal part of most individuals will show the epidermis, then the parenchyma with developing female germinal cells, below that the genital duct and finally the digestive system. In fully mature specimens, germinal cells often invade the whole dorsal part of the trunk, with parenchymal cells scattered between them. The ultrastructure of the developing germinal cells and that of the genital duct have only been described for *Myzotoma cirriferum* (Eeckhaut, 1995). In this species, the genital duct consists of a uterus and uterine diverticula. The uterus is a sagittal cavity that lies above the stomach and intestine and opens to the outside through a postero-ventral gonopore. The uterine diverticula branch off from the anterior part of the uterus, dichotomise and end at the body margin. According to the state of maturity of individuals, the uterus and uterine diverticula may be flat and empty or thick and full of fertilised eggs. The lumen of both organs is bordered by an epithelium surrounded by a fine basal lamina 50 nm thick. The epithelium consists of ciliated cells and microvillous cells (Figs 8 and 9). The former cell type forms the dorsal wall of the uterine diverticula and the latter is observed in the ventral wall of the uterine diverticula and borders the uterus lumen both ventrally and dorsally. The ciliated cells are flattened, ca. 10 μm long and 3 μm thick at most, their periphery being extremely thin (Figs 8 and 9A). They are joined together by septate junctions and zonula adhaerentes and bear numerous cilia that have a $9 \times 2 + 2$ microtubular arrangement. Each cilium has a basal body and one ciliary rootlet with two branches that form an angle of ca. 150° (Fig. 9A). As a result of the current created by the ciliary beating, the ciliated cells drive fertilised eggs towards the uterus. The microvillous cells are ca. 10 μm long and 3 μm thick (Fig. 9A–C). In the uterine diverticula, they are separated from the caecal cells of the digestive system by the basal lamina; in the uterus, they are underlain by circular muscle cells whose contractions expel the fertilised eggs through the gonopore. They have numerous microvilli and may be storage cells; their cytoplasm sometimes includes lipidic droplets and possesses a well developed smooth endoplasmic reticulum (Fig. 9B).

The developing germinal cells are not separated by a basal lamina from the surrounding parenchymal cells, nor are they enclosed by an epithelium. Oogonia, previtellogenic oocytes, and vitellogenic oocytes occur in the ovary of adult myzostomids (Figs 8 and 10A–C). The older they are, the closer to the uterine diverticula they come, and vitellogenic oocytes pierce the epithelium of the diverticula and fall into the lumen where they are fertilised (Fig. 8). The oogonia are ovoid cells of 5–10 μm in diameter (Figs 8 and 10A). They have a voluminous, nucleolated nucleus where heterochromatin is condensed into

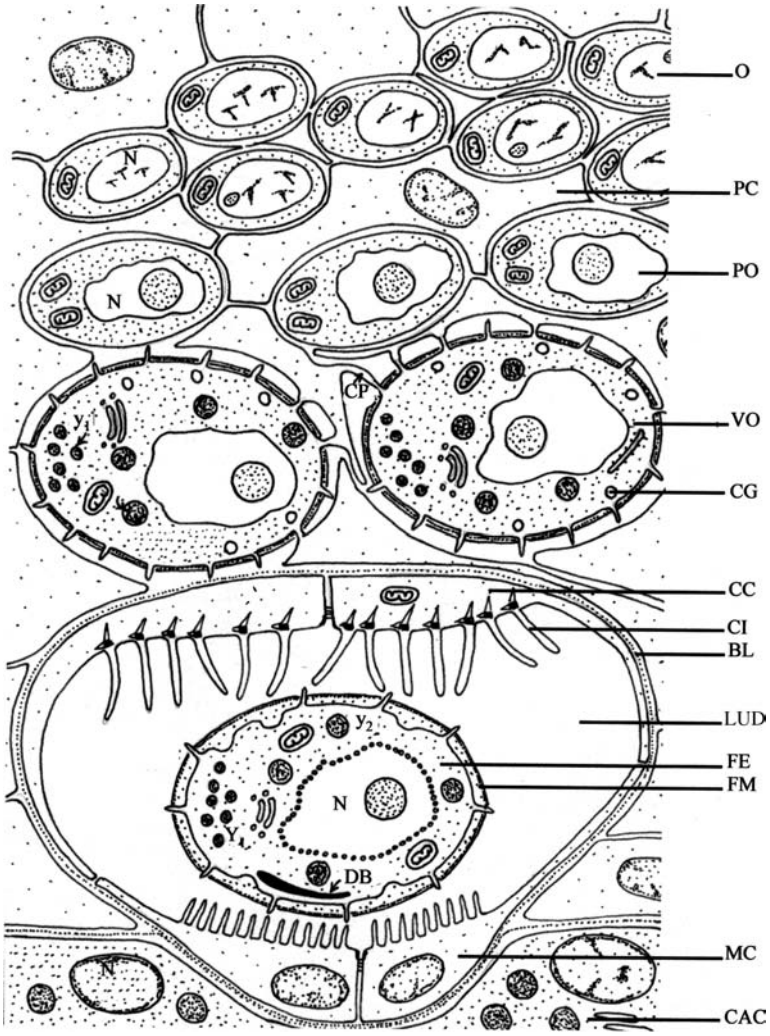

Figure 8. *Myzostoma cirriferum*. Schematic drawing of a portion of the ovary and of a uterine diverticulum (sectioned transversally). Abbreviations: BL – basal lamina; CAC – caecal cell; CC – ciliated cell; CG – cortical granule; CI – cilium; CP – cytoplasmic process; DB – dense body; FE – fertilised egg; FM – fertilising membrane; LUD – lumen of an uterine diverticulum; MC – microvillous cell; N – nucleus; PC – parenchymal cell; PO – previtellogenic oocyte; O – oogonium; VO – vitellogenic oocyte;$Y_{1,2}$ – yolk granules of types 1 and 2.

chromosomes. The cytoplasm includes some scattered mitochondria. Previtellogenic oocytes are cells of 10–20 μm in diameter with an irregular-shaped nucleus (Figs 8 and 10B). The cytoplasm includes mitochondria often concentrated at one side of the cell (Fig. 10B), a well developed rough endoplasmic reticulum and Golgi apparati. The cell membranes of both the oogonia and previtellogenic oocytes are smooth and deprived of microvilli. The vitellogenic oocytes are cells of 20–30 μm in diameter, with a nucleus similar in shape to that of the previtellogenic oocytes (Figs 8 and 10C). Mitochondria are scattered throughout the cytoplasm, which includes a highly developed rough endoplasmic reticulum (Fig. 10E). Two types of yolk granules occur within the cytoplasm: some are small granules of 100–500 nm in diameter, concentrated at the vegetal pole of the cell (Fig. 10F); the others consist of large granules of 1–3 μm in diameter scattered in the cytoplasm (Fig. 10G). Cortical granules of 0.5–1 μm in diameter are found at the

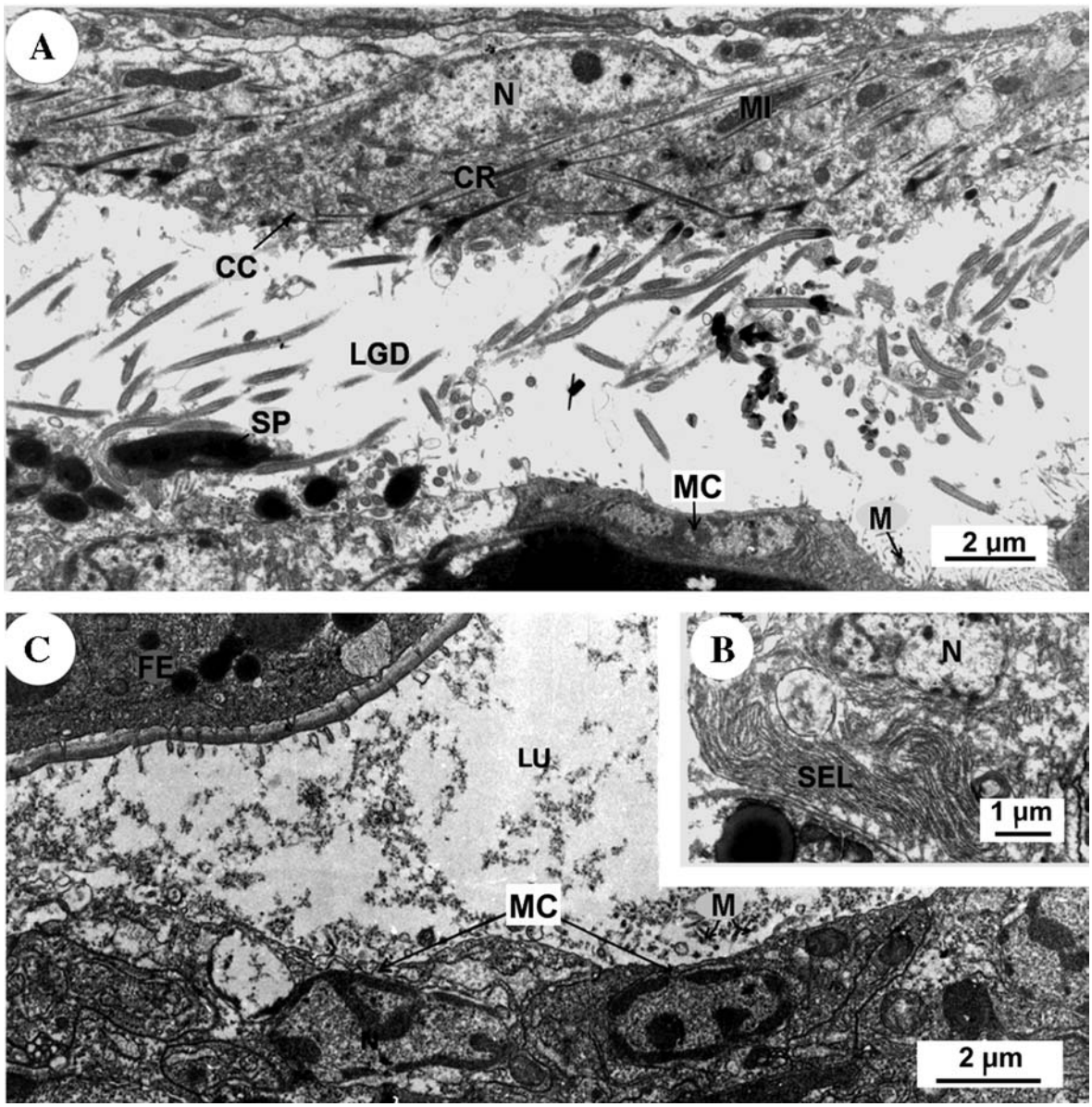

Figure 9. Myzostoma cirriferum. Female genital duct (TEM views). (A) transversal section of an uterine diverticula, (B) basal part of a microvillous cell and (C) sagittal section in the mid ventral part of the uterus. Abbreviations: CC – ciliated cell; CR – ciliary root; FE – fertilised egg; LGD – lumen of a genital duct; LU – lumen of the uterus; M – microvillus; MC – microvillous cell; MI – mitochondria; N – nucleus; SEL – smooth endoplasmic reticulum; SP – spermatozoon.

periphery of the cell just under the cell membrane, which is outlined by a thin vitelline envelope 100 nm thick (Fig. 10H and I). The vitelline envelope is crossed by microvilli, some of which forming cytoplasmic bridges between germinal cells and follicle cells (Fig. 10I and J). Cytoplasmic bridges are small cell processes that are sometimes filled with small vesicles (Fig. 10I). Except for the presence of these intercellular bridges, the ultrastructure of follicle cells does not differ from that of parenchymal cells: they do not show any sign of a particular metabolic activity. Nurse cells have been observed in some Myzostomatidae (Eckelbarger, 1992). They are abortive

germ cells that maintain cytoplasmic continuity with the developing oocytes through intercellular bridges. According to Wheeler (1896), nurse cells are absorbed by the developing oocytes, but Jägersten (1939) suggested that nurse cells transform into follicle cells and eventually are disposed in several layers around the oocytes. Eeckhaut (1995) did not observe such nurse cells in *M. cirriferum*.

Fertilised eggs only occur in the lumen of the uterine diverticula and uterus (Figs 8 and 10D). They are 30 μm in diameter and have both types of yolk granules, mitochondria scattered throughout the cytoplasm, and cisternae of a highly developed rough endoplasmic reticulum and Golgi apparatus. The cytoplasm includes an electron-dense body that is supposed to come from the fertilising spermatozoon (Fig. 10D and N). Cortical granules are empty, their membrane having fused with the oolema (Fig. 10L). The fertilising envelope, 200 nm thick, is formed of the upper, old vitelline envelope and of a new, inner layer made of the material secreted by cortical granules (Fig. 10D and M). Microvilli cross the fertilising envelope. The nuclear membrane is fragmented into numerous small vesicles of 10 nm in diameter (Fig. 10K). In some eggs where the fragmentation is advanced, the vesicles are scattered into the nucleo-cytoplasm (Fig. 10O).

Spermatogenesis and male genital system

The male genital system of myzostomids is basically paired: there are usually two male genital apparati that are ventral and each separated from the other by the nerve cord. Each male genital apparatus consists of one or two diffuse testes (according to the species) and one genital duct, the latter consisting of a penis, a seminal vesicle, one or two vasa deferentia, and numerous vasa efferentia. Except for the vasa efferentia that are lined by a matrix, the lumen of all male organs is bordered by an epithelium, itself surrounded by muscles. Epithelial cells are joined together by zonula adhaerentes and septate junctions. Muscles form a continuous (at the level of the seminal vesicles) or discontinuous (at the level of the penises and vasa deferentia) sheath of circular muscle cells. The wall of the vasa efferentia is a thick 300 nm basal lamina (also named the tunica propria) that is continuous with the 50 nm thick basal lamina of the vasa deferentia.

Structural variability in the male genital system of myzostomids includes the presence of an epithelium lining at least part of the vasa efferentia in some species and the absence of seminal vesicles or their division into a narrow proximal duct and a sac-like distal portion in some others (Jägersten, 1939). Compact rather than diffuse testes occur in many Endomyzostomatidae and in *Stelechopus hyocrini* (Jägersten, 1939, see also Grygier, 2000).

The ultrastructure of myzostomidan spermatozoa and spermatogenesis has been extensively studied (Bargalló, 1977; Afzelius, 1983, 1984; Mattei & Marchand, 1987, 1988) but the fine structure of male genital ducts is only known for *M. cirriferum* (Eeckhaut, 1995). In this species, the lumen of the penis is bordered by non-ciliated and ciliated cells similar to those found in the epidermis (Fig. 11A–C). Their shape varies according to whether the penis is retracted or extended and will be cylindrical or flat, respectively. These cells are covered by a cuticle while all the other epithelial cells of the male genital system are not. Vacuolar and spumous gland cells form the epithelium of the seminal vesicles (Fig. 12A, C and E). The first are located at the base of each seminal vesicles, close to the penis (Fig. 12A and C). They are cylindrical cells, 20 μm high with a basal nucleus and cytoplasm full of large vacuoles of 5–10 μm in diameter. They include an electron-lucent fibrillar material. These vacuoles are expelled with the spermatophores that are formed in the seminal vesicles. They will form the base of it and will participate in the lysis of the integument of the receiver individual during the intradermic penetration of the germinal cells (described below). The rest of the seminal vesicle is lined by spumous gland cells that are flat cells 7 μm high (Fig. 12E). They contain vacuoles whose content is assumed to be secreted to form the matrix that coats the spermatophore contents. The vasa deferentia (Fig. 12B) are lined by vesicular gland cells. These are cylindrical cells 15 μm high with a basal nucleus; they have well-developed smooth endoplasmic reticulum and possess rod-shaped, electron-dense vesicles apically (Fig. 12F and G). These rod-shaped vesicles are secreted during the

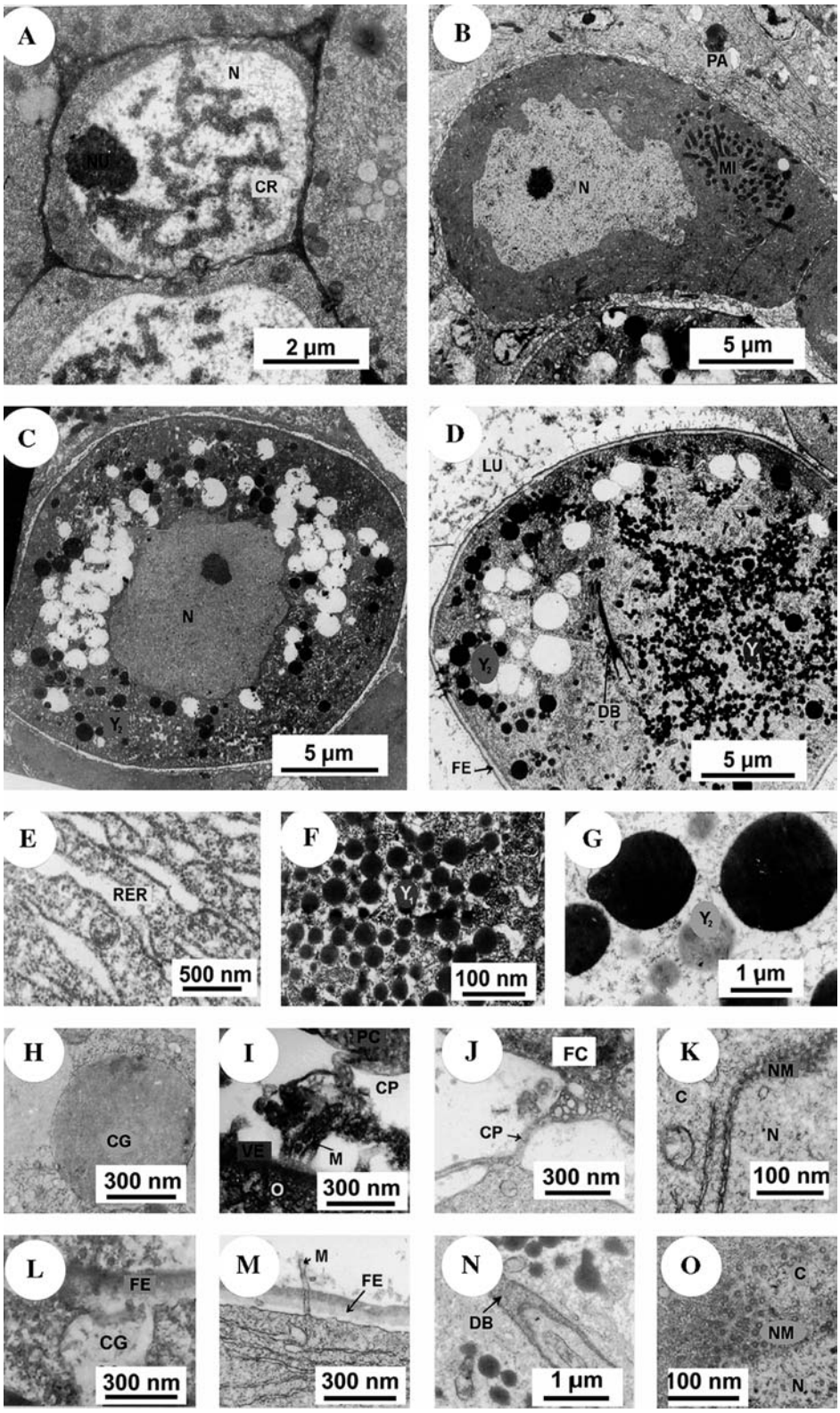
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Figure 10. *Myzostoma cirriferum*. Female germinal cells (TEM views). General views of (A) an oogonia, (B) of a previtellogenic oocyte, (C) of a vitellogenic oocyte and (D) of a fertilised egg. Details of a vitellogenic oocyte illustrating (E) the rough endoplasmic reticulum, (F) yolk granule of first and (G) second type, (H) a cortical granule, (I) vitelline envelope and microvilli, and (J) a cytoplasmic process between an oocyte and a parenchymal cell. Details of a fertilised egg illustrating (L) a cortical granule, (M) the fertilising envelope and microvilli, (N) the intracytoplasmic dense body and fragmentation of the nuclear membrane into numerous small vesicles (beginning and advanced stages of fragmentation: K and O, respectively). Abbreviations: C – cytoplasm; CG – cortical granule; CP – cytoplasmic process; CR – chromatin; DB – dense body; FC – follicle cell; FE – fertilised egg; LU – lumen of uterus; M – microvillus; MI – mitochondria; N – nucleus; NM – nuclear membrane; NU – nucleole; O – oocyte; PA – parenchyma; RER – rough endoplasmic reticulum; VE – vitelline envelope; $Y_{1,2}$ – yolk granules of types 1 and 2.

advance of the germinal cells to the seminal vesicles. They are also found in the lumen of the seminal vesicles, and at the base of the spermatophores between the vacuolar gland cells and the male germinal cells that came from testes. The vasa efferentia are dichotomously branched ducts connected to the testes; they convey the germinal cells to the vasa deferentia. The testes comprise many follicles scattered in the parenchyma. The follicles include many cysts, also called

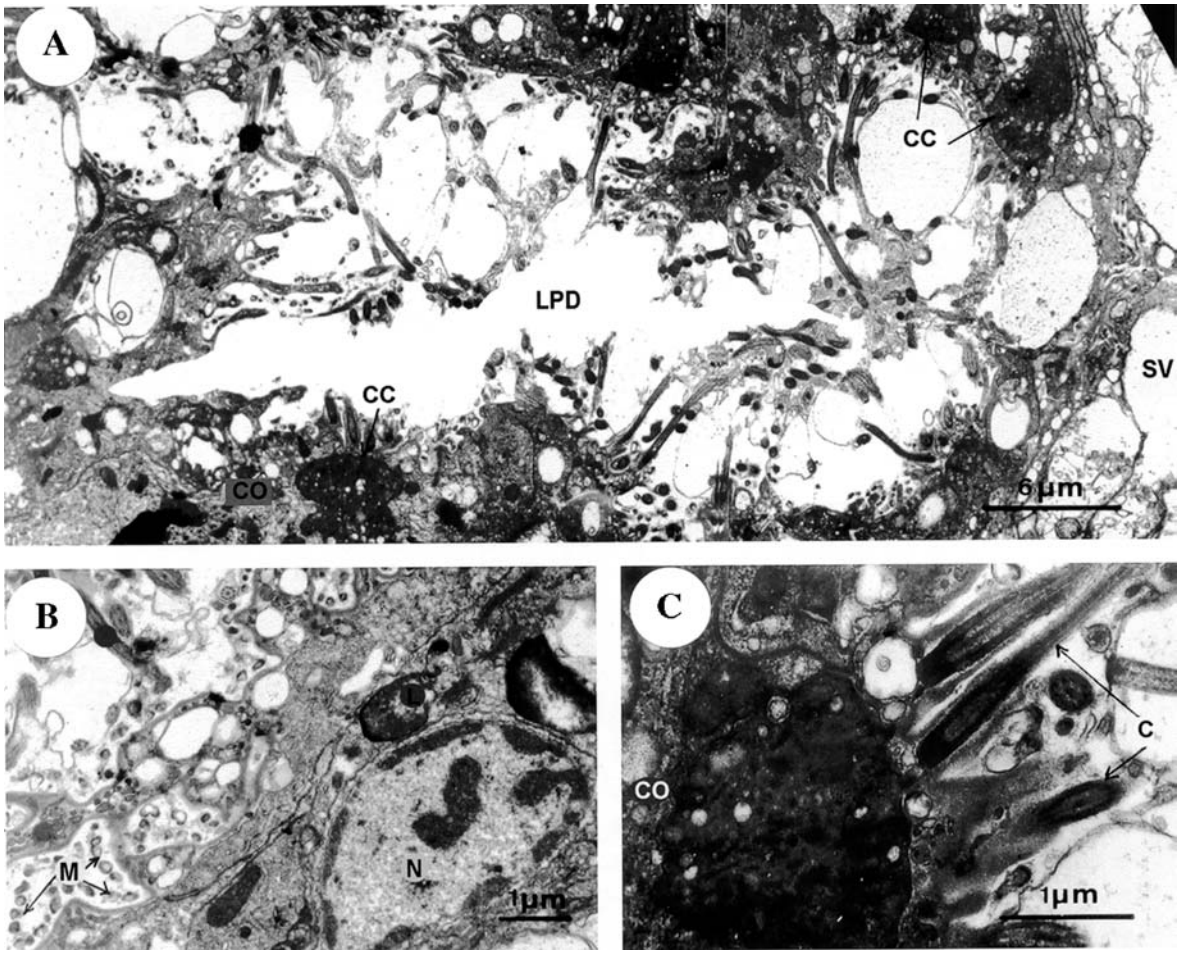

Figure 11. *Myzostoma cirriferum*. Male genital duct (TEM views). (A) General view of the epithelium bordering the retracted penial duct and details of (B) covering and (C) ciliated cells. Abbreviations: C – cilium; CC – ciliated cell; CO – covering cell; LPD – lumen of penial duct; M – microvillus; N – nucleus; SV – seminal vesicle.

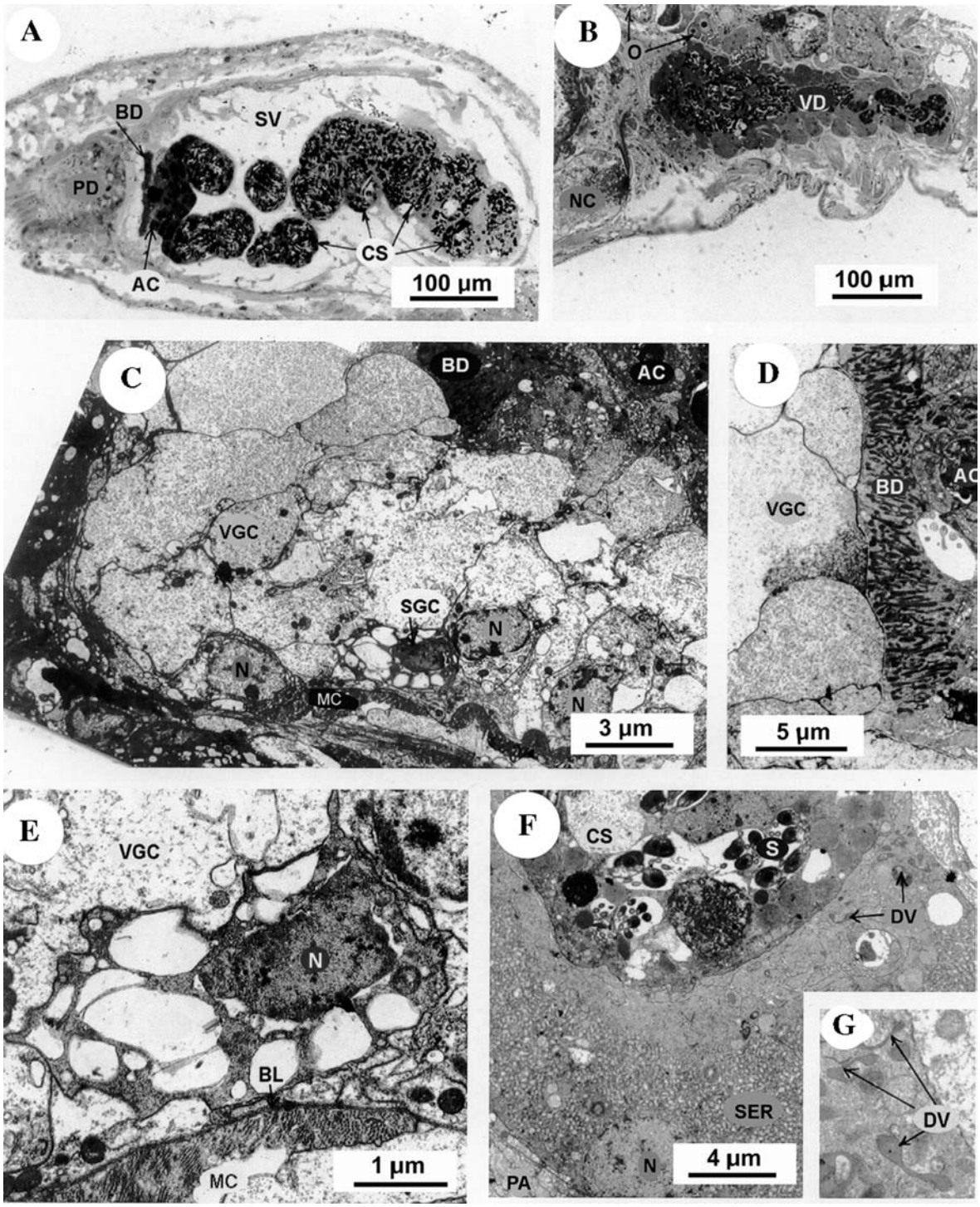

Figure 12. Myzostoma cirriferum. Male genital duct (OM and TEM views). (A) Sagittal sections through a penis and a seminal vesicle; (B) longitudinal section of a vas deferens; (C) aspect of the basal part of a seminal vesicle (near the penis); (D) details of electron-dense vesicles lying on the apex of vacuolar gland cells; (E) details of a spumous cell; (F) transverse section through a portion of a vas deferens with the lumen filled with spermatocysts and (G) details of their electron-dense, apical vesicles. Abbreviations: AC – abortive cyst; BD – basal disc with electron-dense vesicles; BL – basal lamina; CS – cyst containing spermatozoa; DV – dense vesicle; N – nucleus; NC – nervous chain; MC – muscle cell; O – ovary ; PA – parenchyma; PD – penial duct; S – spermatozoon; SER – smooth endoplasmic reticulum; SGC – spumous gland cell; SV – seminal vesicle; VD – vasa deferentia; VGC – vacuolar gland cell.

spermatocysts, each consisting of one cyst cell that encloses developing germinal cells. Cyst cells are ovoid cells of 20–40 μm in diameter. They contain many mitochondria, well-developed rough endoplasmic reticulum and numerous polysaccharide-rich vesicles. The number of germinal cells surrounded by a cyst cell varies according to the stage of development of the former (from 1 to 64 in *M. cirriferum*). Germinal cells that are surrounded by the same cyst cell are all at the same stage. Nothing is known about the cyst structure in young myzostomids but it is probable that, initially, a single germinal cell becomes surrounded by a cyst cell and divides later. The youngest cyst cells observed in mature myzostomids are those containing spermatogonia, which are ovoid cells of 5 μm in diameter (Fig. 13A). Spermatogonia have a large, nucleolated nucleus where the chromatin condenses into chromosomes. Young spermatids are still ovoid cells of 5 μm in diameter (Fig. 13B). In the periphery of the nucleus, intranuclear, electron-dense spheres form. These have been considered either as heterochromatin (Afzelius, 1984; Eeckhaut & Jangoux, 1991) or protein granules (Mattéi & Marchand, 1988). Spermatids gradually lengthen and acquire a flagellum with a $9 \times 2 + 0$ axoneme (Fig. 13C). At the end of spermiogenesis, the spermatids separate from the cytoplasmic remnants and transform into spermatozoa (Fig. 13D and E). Myzostomid spermatozoa are elongated cells about 30 μm long and 1 μm in diameter. They are provided with a long flagellum whose length is almost twice that of the spermatozoon body. The flagellum arises from one extremity of the cell; it bends as soon as it leaves the cell body and borders the latter along its whole length, being attached to the cell membrane through extracellular processes. It ends in a 40 μm long free portion that extends out opposite to the flagellar pole of the spermatozoon and that is supposed to beat forwards according to Mattei & Marchand (1988) . The spermatozoon nucleus is highly elongated and typically includes one row of 40–50 dense spheres. According to Mattei & Marchand (1987), the nuclear membrane is open in the spermatozoa which allows the nucleoplasm and cytoplasm to come into contact. One or two enlarged mitochondria and a manchette of 16–22 microtubules extend from one pole of the spermatozoon to the other. The microtubules are located between the nucleus and the cell membrane in the cytoplasm facing the attached part of the flagellum. On the opposite side, a myelin-like sheath presumably derived from the Golgi apparatus, caps the nucleus over its whole length. An acrosome is not obvious in *Myzostoma* spermatozoon.

Cysts that contain spermatogonia are located in the outermost ends of the follicles, close to the myzostomid body margin. With the division of spermatogonia, cysts occupy more space and push the rear cysts into the vasa efferentia first, then into the vasa deferentia. Only cysts that include spermatozoa enter vasa deferentia. There, contractions of circular muscle cells force the cysts into the seminal vesicles, where spermatophores form.

Spermatophores and reproduction

Reproduction in myzostomids is realised by the emission of spermatophores followed by the intradermic penetration of sperm cells. Emissions of spermatophores have been observed in *M. ambiguum* (Kato, 1952), *M. cirriferum* (Eeckhaut & Jangoux, 1991), *M. alatum* (Eeckhaut & Jangoux, 1992), and *M. capitocutis, M. nigromaculatum*, *M. polycyclus*, and *Contramyzostoma sphaer*a (unpublished observations). All are ectocommensal species except the last one, whose individuals live singly in cysts that they induce on the arms of their crinoid hosts. In ectocommensals, matings involve two mature individuals that contact each other, one of them ejecting one spermatophore that attaches to the integument of the other individual. The spermatophore in the other seminal vesicle does not participate in this process. Contacts between the two myzostomids are very brief and the two separate after mating. Spermatophores are generally attached to the back of the receiver but they can be emitted successfully to any part of the receiver's body. In most cases, receiver individuals have one attached spermatophore, sometimes two. In *C. sphaera*, gamete exchange occurs when an individual extends a very long penis to contact another individual lying in an adjacent cyst (unpublished observation).

Emitted spermatophore are white V-shaped, club-shaped, or ball-shaped baskets according to

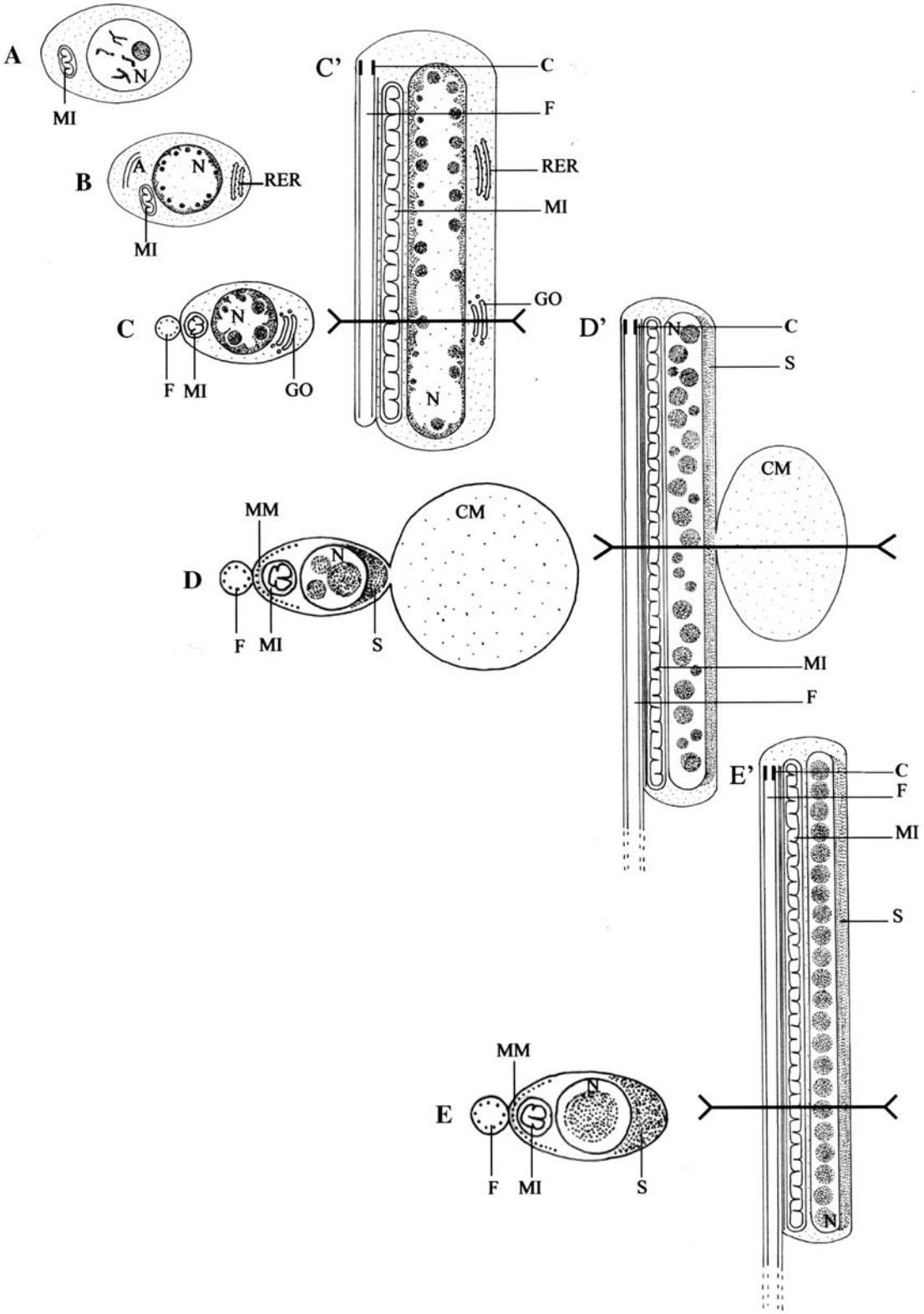
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Figure 13. Myzostoma cirriferum. Schematic drawings of male germinal cells undergoing spermatogenesis. (A) spermatogony; (B) young spermatid; (C) a more advanced spermatid sectioned transversely and (C′) sagitally; (D and D′) old spermatid; (E and E′) spermatozoon. Abbreviations: A – axoneme; C – centriole; CM – cytoplasmic mass; F – flagellum; GO – Golgi apparatus; MI – mitochondria; MM – manchette of microtubules; N – nucleus; RER – rough endoplasmic reticulum; S – myelin-like sheet.

the species considered. They are 50–500 μm long and are formed by a translucent extracellular matrix containing cysts. The cysts are packed close together and tend to form numerous sinuous chains which interlace each other. In *M. cirriferum*, spermatophores consist of three regions: the body with the curved horns, the foot, and the basal disc (Fig. 14A). The body-horns region forms the upper two third of the spermatophore and includes spermiocysts each of which contains ca. 64 spermatozoa (Fig. 14B). The foot extends below the body-horns region and includes cysts with abortive germinal cells (Fig. 14C). These cysts are assumed to prevent the spermiocysts from being attacked by the lytic enzymes that create a hole in the receiver's integument. The basal disc of the spermatophore attaches to the receiver's cuticle. It is composed of upper,

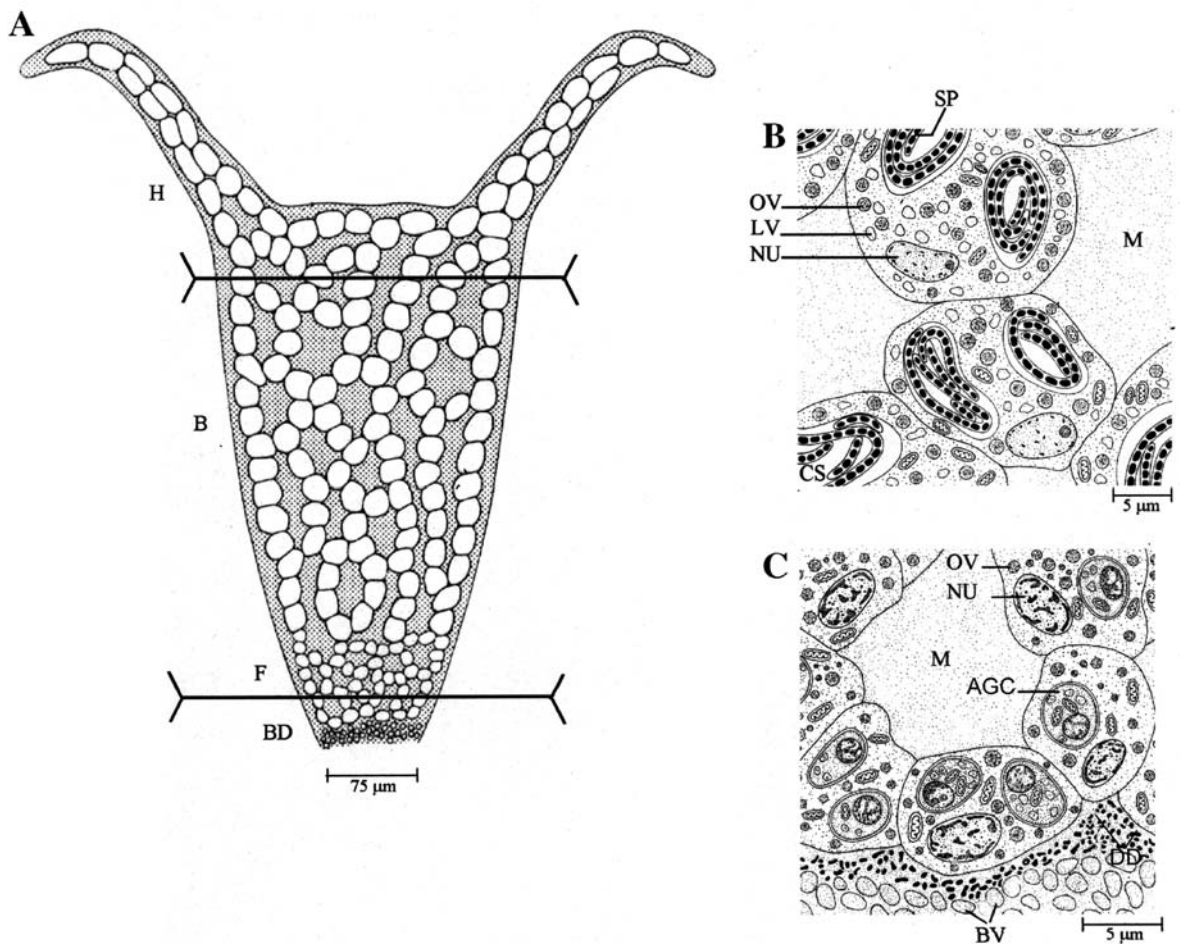

Figure 14. Myzostoma cirriferum. (A) schematic drawing of a spermatophore just after emission (*in vivo* observation); (B) structure of cysts containing spermatozoa (at the level of the body and horns of the spermatophore); (C) structure of the basal part of the spermatophore with abortive cysts (at the level of the foot) and the dense vesicles and lucent vacuoles found in the basal disc (Eeckhaut & Jangoux, 1991). Abbreviations: AGC – abortive germinal cell; B – body; BD – basal disc; BV – basal vesicle; DD – dense droplet; F – foot; H – horn; LV – lucent vesicle; M – matrix; NU – nucleus; OV – osmiophilic vesicle; SP – spermatozoon.

electron-dense, rod-shaped vesicles of unknown function and lower vacuoles filled with a fibrillar material that is responsible of the degradation of the integument (Fig. 14C). The two kinds of vesicles are respectively synthesised by the vesicular gland cells of the vasa deferentia and the vacuolar gland cells of the seminal vesicles.

After attachment, the spermatophores pierce the integument and release all the cysts through it. Intradermic penetration has been observed in *M. ambiguum* (Kato, 1952), *M. cirriferum* (Eeckhaut & Jangoux, 1991) and *M. alatum* (Eeckhaut & Jangoux, 1992). Penetration can be observed *in vivo* thanks to the presence of white trails representing the spermatophore contents that are introduced into the translucent body of the receiver. These trails appear 10–30 min after attachment and after 1–5 h, the spermatophores are reduced to matrix only. In *M. cirriferum*, four phases have been distinguished during the intradermic penetration process: fixation, degradation, penetration, and expansion (Eeckhaut & Jangoux, 1991). Fixation occurs between the basal disc of a spermatophore and the cuticle of the receiver individual. The membranes of the basalmost spermatophoral vacuoles (those with fibrillar content) disappear and, consequently, the lower part of the basal disc appears as a single large fibrillar mass that sends digitations through the cuticle. During the degradation phase, the digitations flow through both the cuticle and epidermis. The cuticle is strongly altered and epidermal cell membranes become degraded, allowing the lytic material to penetrate the cells. Invasion of the parenchymal layer by the digitations has not been observed. Penetration starts when the cysts pass into the integument. At the very beginning of this phase, the cytoplasmic membranes of all cyst-cells fuse together, leading to the formation of an extremely large syncytium. The whole syncytium encloses both the spermatozoa and the abortive germinal cells. At the point of penetration, both the cuticle and epidermis disappear. Epidermal and parenchymal cells in contact with the penetrating syncytium degenerate: their nuclei become nearly entirely heterochromatic and their cytoplasm becomes highly reduced and deprived of organelles. The expansion phase corresponds to the extension of the whole syncytium into the parenchyma of the receiver. Once at the level of the uterine diverticula, the syncytium breaks up and the spermatozoa are released free into the uterine lumen where they fertilise the vitellogenic oocytes.

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