Comparative ultrastructure of copulatory organs having a stylet in the Proseriata (Turbellaria)

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Abstract

The ultrastructure of male copulatory organs having a stylet has been studied in some genera of the Proseriata.

Within the Monocelididae there was a variety of stylet-like hard structures. The stylet in *Monocelis fusca* was a differentiation of the basement membrane of the epithelium lining a penis-like muscular papilla. The penis papilla in *Ectocotyla* consisted of circular muscles surrounded by a thickened basement membrane and an epithelium. *Archilopsis* sp. and *Archilina* sp. with a duplex copulatory bulb, had a stylet within a spiny cirrus. The stylet in *Archilopsis* sp. was a cylindrical muscular protrusion with a thickened basement lamina that lined the cirrus lumen. The stylet structure in *Archilina* sp. was composed of four long spines which were derivatives of the basement membrane. In *Ectocotyla multitesticulata* and *Dupliminona corsicana,* the accessory prostatoid organ was provided with a hook-shaped stylet that was differentiated in the basement membrane and of which the material was continuous with the fibrous matrix between the muscles of the prostatic bulb. The stylet and needles in the *Archimonocelis* species were intracellular differentiations. The copulatory organ in *Carenscoilia biforamen* consisted of a tubiform stylet and four needles, all of which were also intracellular specializations.

I consider copulatory hard structures in the Turbellaria to be taxonomically significant in terms of structure, differentiation, and location (whether subcellular, in the basement membrane, or intracellular).

Introduction

With advancing knowledge on the ultrastructure of the Turbellaria new characteristics become available which can validly be applied in phylogenetic systematics. Previous studies on the ultrastructure of the copulatory organ have revealed that the hard parts are intracellular specializations in the Acoela (Mainitz, 1977), Macrostomida (Doe, 1982), Haplopharyngida (Doe, 1985), and Proseriata (Lanfranchi, 1978; Ehlers & Ehlers, 1980; Brüggemann, 1984; E. Martens & Schockaert, 1984), or derivatives of the basement membrane in the Proseriata (E. Martens & Schockaert, 1981; E. Martens, 1984b).

In this paper I present observations on the ultrastructure of stylet-bearing copulatory organs in several genera of the Proseriata. Such a comparative study may reveal new characteristics that clarify relationships within the Proseriata and, eventually, within the Turbellaria.

Material and methods

Specimens of *Monocelis fusca* were collected in St. Andrews (Canada) from shells of barnacles, and specimens of *Ectocotyla multitesticulata* were collected from the gills of crabs, *Chionoecetes oplio,* from Newfoundland (Canada).

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Archilina sp., *Archimonocelis* sp., and *Dupliminona corsicana* were collected in the Bay of Calvi (Corsica). *Archimonocelis oostendensis, Carenscoilia biforamen,* and *Archilopsis* sp. were found in sand samples from the low tide level of the beach of Mariakerke and in the Zwin-estuary in the North of Belgium.

The animals were extracted by decantation with a $MgCl_2$-solution isotonic to seawater, and fixed in 2% glutaraldehyde in 0.1 M phosphate buffer with 10% sucrose (pH 7.3) at 4 °C for 2–3 h. After rinsing in the same buffer they were postfixed in 1%-phosphate-buffered osmium tetroxide at 4 °C for 2 h, dehydrated in a graded acetone series, and embedded in Araldite. Semithin and ultrathin sections were cut with a Reichert OMU 3 ultramicrotome. Ultrathin sections were mounted on pioloform-coated copper grids, stained with 2% aqueous uranyl acetate and 1.2% aq. lead citrate, and examined in a Philips EM 400. The semithin sections were stained with toluidine blue and examined by light microscopy.

Observations

1. The stylet in Monocelis fusca *(Oersted, 1845) (Monocelididae)*

Under the light microscope the copulatory organ of *M. fusca* appeared to consist of a single ovoid vesicle surrounded by several muscle layers (Fig. 1). The proximal part of the vesicle appeared to function as seminal vesicle, and in its distal part prostate glands entered the vesicle from the left and the right side. A simple straight, conical stylet, 70–85 μm long, was inserted in the distal end of this vesicle.

By electron microscopy it could be seen that the proximal part of the stylet consisted of circular muscles embedded in a fine fibrous matrix and covered on both sides by an epithelium (Fig. 2). The electron dense matrix was similar to and continuous with the basement membrane. Distally, the muscles and epithelium were lacking, and only the electron dense material formed the stylet here (Figs 3–4).

The epithelia covering the luminal side and the outer side of the stylet could be followed to the edge of the muscles.

The vesicle itself was lined in its proximal part by a flattened epithelium with microvilli and cilia with a long rootlet parallel to the cell membrane. Lateral cell membranes were highly convoluted and connected to each other by a zonula adherens and a deeper septate desmosome. In the distal part of the vesicle, the epithelium was high, and its entire extent here was pierced by numerous cell necks of the prostate glands.

2. The 'penis papilla' in Ectocotyla multitesticulata *Fleming & Burt, 1978*

The copulatory organ consisted of a simple vesicle similar to that in *Monocelis fusca* but without any prostate glands. It was surrounded by a thick layer of muscles, of which the inner layer of circular muscles continued into a tubular eversible penis papilla, and a part of the longitudinal muscle layer extended to the atrial wall (Fig. 5).

In its proximal part, the seminal vesicle was lined by a flattened ciliated epithelium. Distally, the epithelial cells had long branching expansions around the inverted penis papilla. In the penis papilla, the basement membrane under this epithelium became very thick and electron-dense. It consisted of fine fibrous material and was continuous with the matrix in which the muscles were embedded. The penis papilla was covered on its luminal side by flattened epithelial cells which were connected to each other by a zonula adherens and a deeper septate desmosome and to the basement membrane by hemidesmosomes (Fig. 6). In the epithelial-cell cytoplasm several microfibrils, mitochondria, and granules of different electron densities could be seen, but no nuclei were found.

3. The stylet within a cirrus in Archilopsis *sp.*

This new species resembles *Archilopsis unipunctata* (Fabricius, 1826) in many respects, but in highly squeezed individuals a cylindrical stylet could be seen within the cirrus. A more detailed description of the species will be given later.

By electron microscopy the stylet could be seen to be double-walled. The stylet was basically a tube-shaped cirrus spine. Only in the proximal half of the stylet, was the luminal side lined with an epithelium (Fig. 12). The nuclei of this epithelium were situated far inside the copulatory bulb at the

beginning of the ejaculatory duct. The rather thin basement membrane consists of fine fibrous material and is continuous with that of the ejaculatory duct. More distally, this basement membrane formed the lining on the luminal side. The outer side of the stylet consisted only of a basement membrane, thicker here than on the inner side, and with a trilamellar structure as found in the spines (see also Martens, 1984b). The basement membranes of the inner side and of the outer side were continuous at the tip of the stylet. The outer basement membrane was also continuous with the layer that formed the spines of the surrounding cirrus (Fig. 13). The stylet wall, between the two basement membranes, consisted of processes of the muscle cells situated at the base of the stylet.

The ultrastructure of the other parts of the copulatory organ was comparable with that in *A. unipunctata* (see Martens & Schockaert, 1981). In this new species, the prostate glands discharge their contents into two 'prostatic ducts' which enter the ejaculatory duct, midway between the seminal vesicle and the cirrus.

4. The 'stylet' within a cirrus in Archilina *sp.*

This species from the Mediterranean has an anatomy very similar to that of *Archilina endostyla* (Ax, 1959). The stylet within the cirrus is, however, much shorter than in *A. endostyla,* and in a highly squeezed specimen it appeared to consist of four long spines. A description of the species will be given later.

By electron microscopy this 'stylet' could be seen to consist of four spines that had the same ultrastructure as the smaller surrounding spines of the cirrus: extensions of the sarcoplasm of underlying muscles covered by a trilamellar basement membrane without a covering epithelium (Figs. 14, 15). The four spines were closely apposed to form a tube.

In this species, the prostate glands send their necks separately through the epithelium of the seminal duct between the seminal vesicle and the cirrus. No 'prostatic ducts' have been seen.

5. The stylet of the prostatoid organ in Ectocotyla multitesticulata *Fleming & Burt, 1978 and* Dupliminona corsicana *Martens, 1984*

The accessory prostatoid organ consisted of a vesicle filled with secretory granules surrounded by a thick layer of muscles and with a stylet extending into a small atrium (Figs. 7, 10). The vesicle was lined by a basement membrane composed of fine fibrous material.

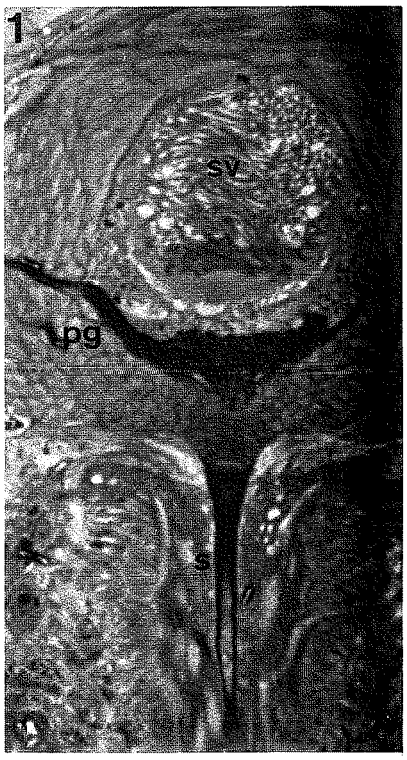
The stylet had the shape of a conical gutter, broad at its base and with a sharp, curved end (Figs. 8, 9, 11). Its lumen narrowed from the base towards the tip of the stylet where it was almost closed, the two margins nearly touching each other (Figs. 9, 11). The wall of the stylet was thinnest in its proximal part and thickened gradually towards the tip.

In *Ectocotyla* the stylet was composed of a thin outer layer of homogenous material and irregular electron-dense granules which adhered to the outer layer where their contents seemed to spread (Fig. 9). Distally, the granules became more densely packed and formed a solid electron-dense matrix in the tip of the stylet. At the base of the stylet the material was continuous with the fibrous matrix between the muscles in which strands of electron-dense granules could be seen. The outer layer of the stylet was continuous with the basement membrane surrounding the prostatoid vesicle.

In *Dupliminona,* the stylet consisted of coarse fibrillar dense material with an outer rim of homogeneous electron density (Fig. 11). Towards the tip, the fibrous matrix coalesced into a more homogeneous material. This fibrous matrix was continuous with the matrix between the muscles at the base of the stylet, while the outer rim was continuous with the basement membrane surrounding the vesicle.

6. The stylet and needles in Archimonocelis

Two species of *Archimonocelis* were studied: *A. oostendensis* Martens & Schockaert, 1981, and a new species from the Mediterranean that will be described later. In both species the copulatory organ consisted of a stylet surrounded by long needles (fourteen fine plus two thicker needles in *A. oostendensis*) and an accessory organ within the same muscular sheath (Fig. 16). This accessory organ consisted of a bundle of glands surrounded by a separate girdle of needles (eleven fine needles plus three thicker needles) in *A. oostendensis,* while in the other species the nine needles encircled the ac-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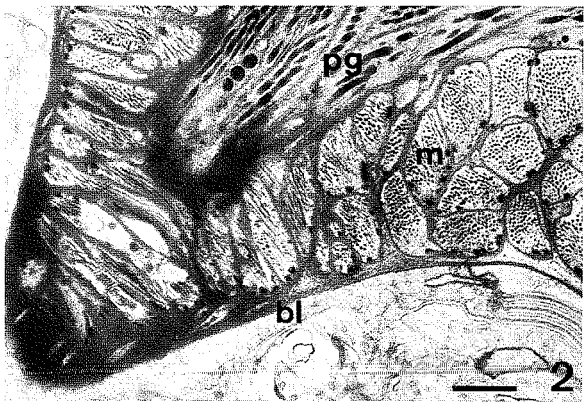
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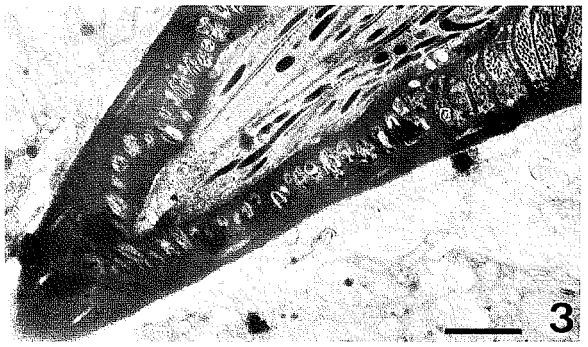
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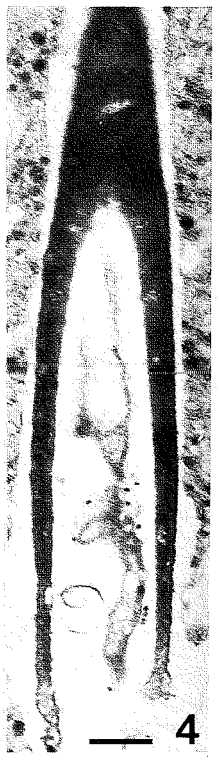
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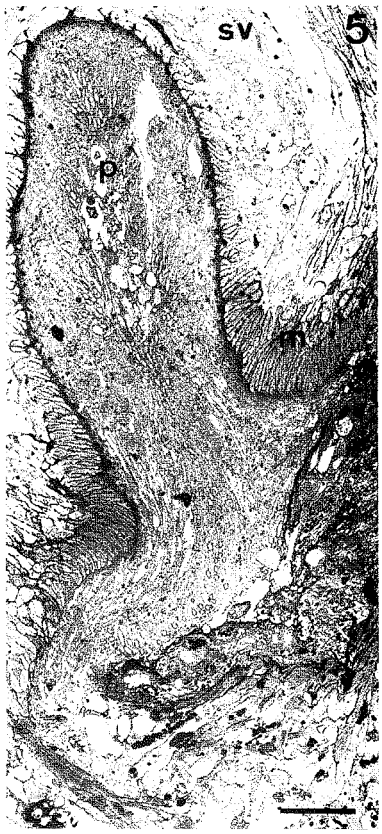
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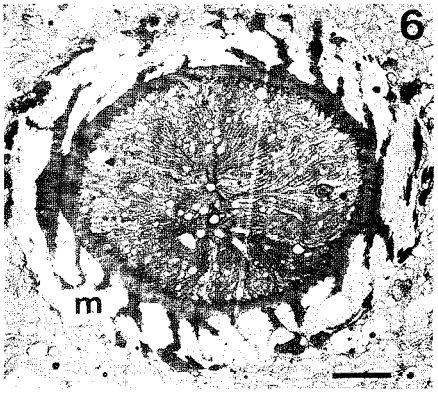
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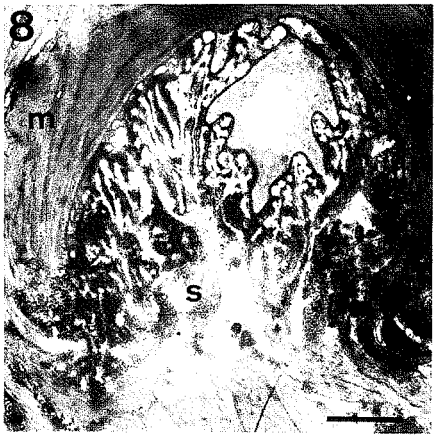
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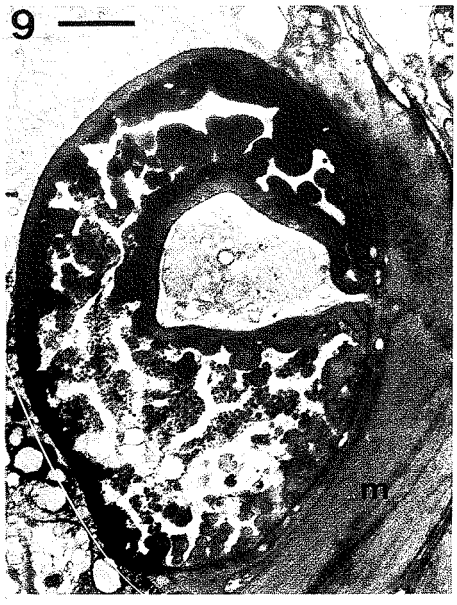
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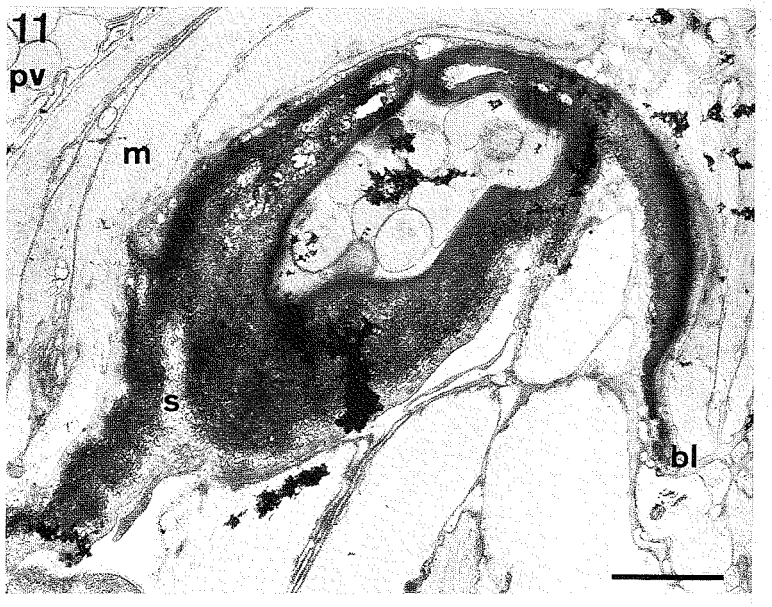
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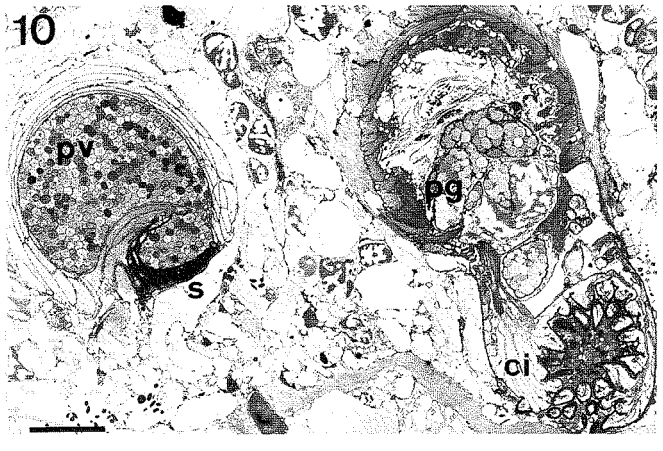
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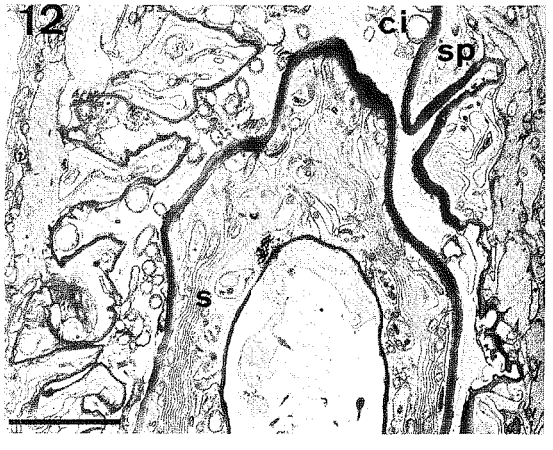
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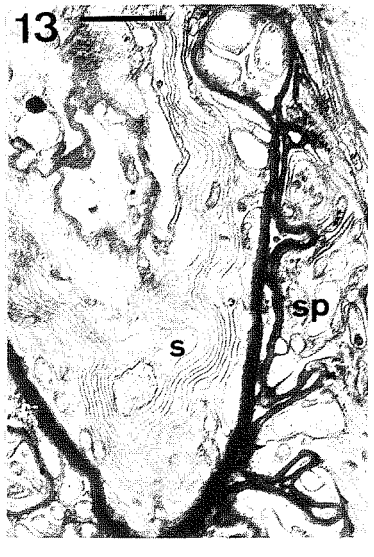
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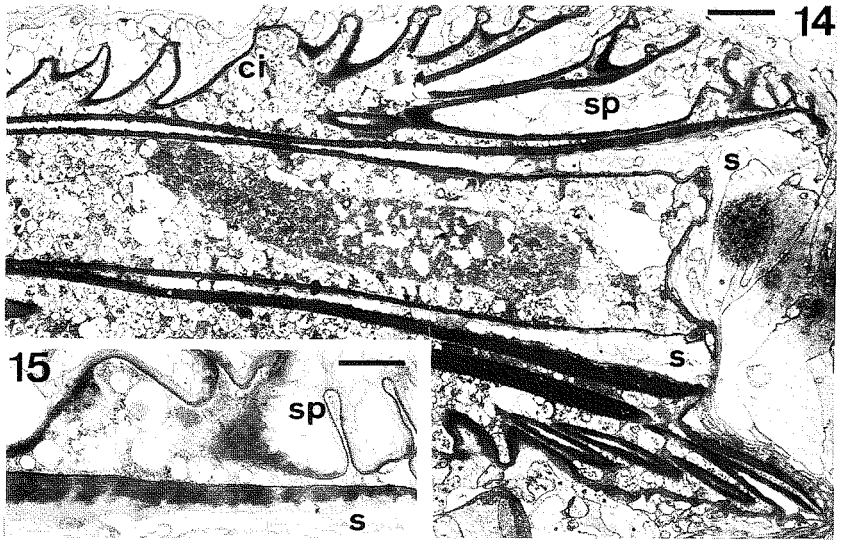
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cessory glands as well as the stylet. The stylet material consists of a coarse fibrous electron-dense matrix surrounded by a more homogeneous less-dense layer. The stylet base, however, was composed of a more flocculent material accumulated in the cytoplasm of the (probably single) matrix cell which was attached to the basement membrane by several hemidesmosomes.

The stylet had a more or less cylindrical form and a subterminal opening. Cross sections indicated that the stylet was a closed gutter-shaped structure with margins overlapping at the caudal side of the stylet. The opening was situated rostrally, so that from the opening towards the tip, the stylet consisted of two overlapping halves (Figs. 17, 18).

The needles surrounding the stylet and the accessory glands were also intracellular differentiations. Each needle was formed in its own matrix cell and consisted of a core of cross-banded fibrils embedded in a coarse fibrous material and an outer more homogeneous layer (Fig. 19). The periodicity of the cross-banding was about 73 nm. At the base, the material was more flocculent and not delimited by a membrane from the surrounding cytoplasm. The cytoplasm of a matrix cell contained a large nucleus, extensive smooth endoplasmic reticulum, ribosomes, mitochondria, Golgi complexes, and electron-dense granules.

7. The stylet apparatus in Carenscoilia biforamen *Sopott, 1972*

The stylet apparatus in *Carenscoilia bidentata* Sopott, 1972, has been studied by Ehlers & Ehlers (1980) using a subadult specimen.

Observations on adult and subadult specimens (Martens, 1984a) of *C. biforamen* confirmed the findings of Ehlers & Ehlers. As in the subadult, the stylet and needles in the adult consisted of a core of cross-banded fibrils, with a periodicity of about 73 nm, embedded in a moderately electron-dense material which was more flocculent and dense towards the base of the stylet (Figs. 20, 21, 22). Cross sections indicated that the stylet was a closed tube with an opening under the slightly curved tip.

Whether the stylet apparatus is formed in a syncytial matrix, as described by Ehlers & Ehlers (1980) or in a single cell (as is probably the case in *Archimonocelis*) could not be ascertained.

Discussion

In earlier studies, we have shown that spines in the cirrus of proseriates may have two separate origins: in the Monocelididae (see Martens & Schockaert, 1981; E. Martens, 1984b) they are differentiations of the basement membrane; in *Cirrifera* (Coelogynoporidae) they are intracellular structures (Martens & Schockaert, 1984). The present study

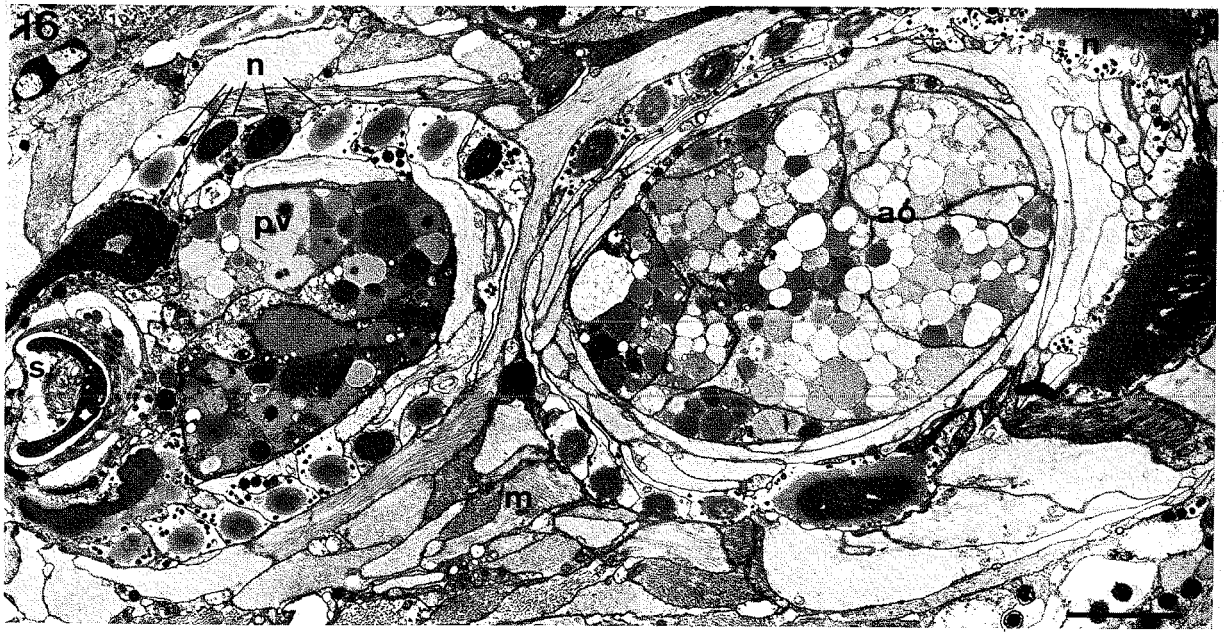
Figs 1–4. Monocelis fusca: (1) Photograph of section through copulatory bulb and stylet; (2) Electron micrograph of section through proximal part of stylet showing the epithelial lining on both sides of the stylet. Scale bar = 1 μm; (3) Section through the stylet more distally. Scale bar = 1 μm; (4) Section through the distal tip of the stylet at its opening. Scale bar = 0.5 μm.

Figs 5–9. Ectocotyla multitesticulata: (5) Sagittal section through copulatory bulb showing the penis papilla lined on both sides by an epithelium. Scale bar = 5 μm; (6) Cross section through the penis papilla. Scale bar = 2 μm; (7) Sagittal section through the accessory prostatoid vesicle and stylet. The stylet is gutter-shaped and has a subterminal opening. Scale bar = 5 μm; (8) Cross section through the stylet at its base. Scale bar = 5 μm; (9) Cross section through the stylet near its distal tip. Scale bar = 1 μm.

Figs 10–11. Duplimin̆na corsicana: (10) Sagittal section showing the copulatory organ with spiny cirrus and accessory organ with stylet. Scale bar = 5 μm; (11) Section through the stylet at its base. The stylet is gutter-shaped. Scale bar = 1 μm.

Figs 12–13. Archilopsis sp.: (12) Sagittal section showing part of the stylet within the spiny cirrus. The inner epithelium of the stylet disappears more distally. Scale bar = 2 μm; (13) Section through the stylet near its base. Scale bar = 1 μm.

Figs 14–15. Archilina sp.: (14) Sagittal section through the copulatory bulb showing stylet-spines within the spiny cirrus. Scale bar = 2 μm; (15) Higher manification of the basal lamina forming stylet and cirrus spines. Scale bar = 0.5 μm.
Abbreviations:
ao: accessory organ, bl: basement membrane, ci: cirrus, m: muscles, mc: matrix cell, n: needle, p: penis papilla, pg: prostate glands, pv: prostatic vesicle, s: stylet, sp: spines, sv: seminal vesicle, sz: spermatozoa.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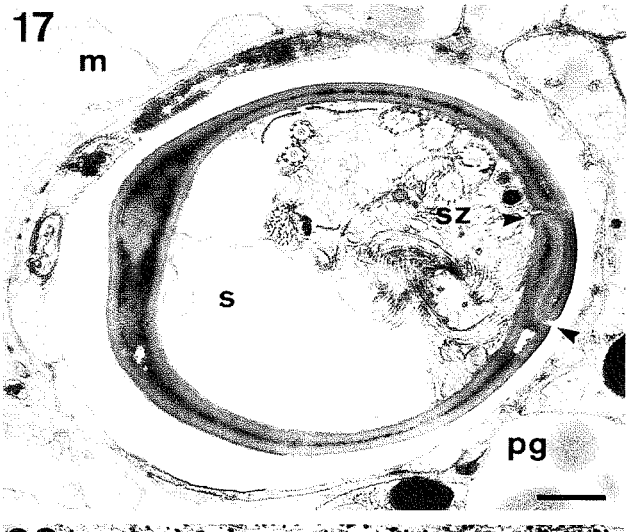
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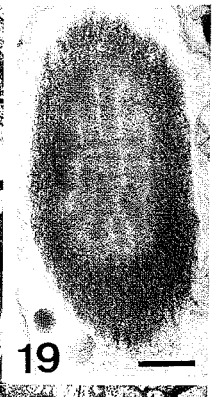
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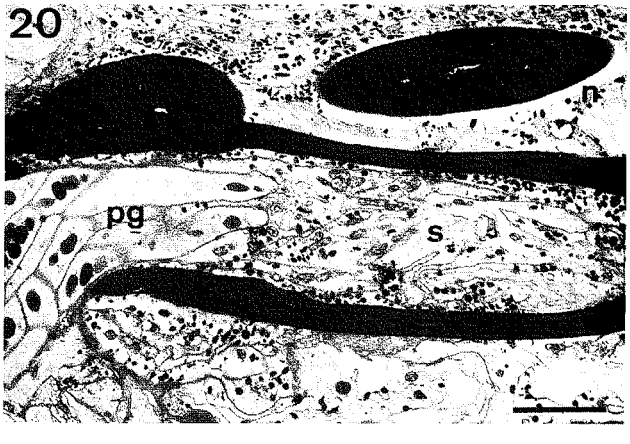
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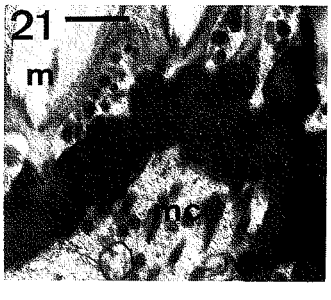
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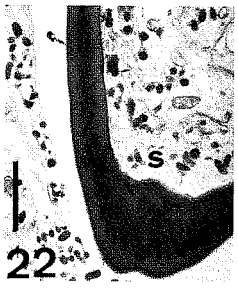
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shows that the same applies to stylet structures: again only in the Monocelididae (exclusive of *Archimonocelis*) are stylets derivatives of the basement membrane, while those in other proseriates are intracellular formations: in *Archimonocelis* (present paper), in the Coelogynoporidae (Ehlers & Ehlers, 1980; present paper), in the Otoplanidae (Lanfranchi, 1978; Ehlers & Ehlers, 1980), and in the Polystyliphoridae (Brüggeman, 1984).

As pointed out before (Martens, 1984b), the origin of hard parts in the copulatory organ of the Monocelididae from basement membrane is an autapomorphy for this family, and so *Archimonocelis* can no longer be considered as a monocelid. The intracellular origin of hard parts is the plesiomorphous situation for the Proseriata by the principle of parsimony and by out-group occurrence in Acoela (Mainitz, 1977), Macrostomida (Doe, 1982), Haplopharyngida (Doe, 1985), and in Typhloplanoida, Kalyptorhynchia, and Dalyellioidea (Brüggeman, 1984: 95).

Some other ultrastructural features of the copulatory organ may be of taxonomic importance. The fine structure of the prostatoid organ in *Ectocotyla* and in *Dupliminona* appears identical, which is strong evidence for the homology of this organ within the Minoninae – a homology that has been questioned by P. Martens (1983). This accessory organ may thus be a synapomorphy for all genera that possess it and hence is supportive of the monophyletic origin of the Minoninae.

For the time being, only for the genus *Archilopsis* is it known that the prostate glands discharge their contents into the seminal duct via a pair of 'prostatic ducts'. This character may be considered as diagnostic for this genus.

The localization and structure of the needles in *Archimonocelis* are very similar to those of the spines in the cirrus of *Cirrifera* (Martens & Schockaert, 1984). Each needle, like each cirrus spine, is enclosed (at its base) by a single cell which is characterised by a large nucleus and numerous dense granules. The stylet in *Archimonocelis, Carenscoilia,* and the Otoplanidae is basically the same. Rather surprising is the different form of the intracellular stylets: a closed tube in *Carenscoilia* and *Polystyliphora* (Brüggeman, 1984), gutter-shaped in *Archimonocelis,* and composed of two pieces fitting into each other in the Otoplanidae (Lanfranchi, 1978). Whether this represents an evolutionary line (from long spines to a stylet or the reverse) must be considered in a larger context (see also Lanfranchi, 1978).

In subadult individuals of *Carenscoilia* (Ehlers & Ehlers, 1980; E. Martens, 1984a) and Otoplanidae (E. Martens, in preparation), the core of cross-banded fibrils arises first and then becomes enveloped by electron-dense material. In differentiating spines in the cirrus of *Cirrifera* (Martens, 1984a) and stylets of the prostatoid organs in *Polystyliphora filum* (Brüggeman, 1984), the framework forming the core consists of a bundle of microtubules. The core of the cross-banded collagen-like fibrils in the needles of *Archimonocelis* and in the stylet of *Carenscoilia* may perhaps be related to the length of the hard structures. Bundles of thicker fibers give a more solid framework for long structures.

It may be of some interest to note that the stylet of *Monocelis fusca,* the stylet in the cirrus of *Archilopsis* sp., and the penis papilla in *Ectocotyla* are basically of the same construction: double-walled with an epithelial covering on the luminal side and on the outer side but not more distally and with the basement membrane (especially of the outer side) usually thickened. Though the penis papilla may be

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Figs 16–17. Archimonocelis oostendensis: (16) Cross section through copulatory bulb showing the stylet surrounded by needles and the accessory prostatoid organ with a separate circle of needles. Scale bar = 2 μm; (17) Cross section through the stylet, formed by a gutter-shaped structure with overlapping margins (arrow). Scale bar = 0.5 μm.

Figs 18–19. Archimonocelis sp.: (18) Sagittal section through the stylet at its base, which lies free in the cytoplasm of the matrix cell. Scale bar = 1 μm; (19) Section through a needle which consists of cross-banded fibrils surrounded by a layer of coarse fibrous material. Scale bar = 0.2 μm.

Figs 20–22. Carenscoilia biforamen (adult specimen): (20) Sagittal section through the stylet structure and one of the needles. Scale bar = 2 μm; (21) Cross section through the stylet wall at its base. Scale bar = 0.5 μm; (22) Cross section through the stylet wall showing the cross-banded fibrils surrounded by a more homogeneous layer. Scale bar = 1 μm. Abbreviations: see Figs 1–15.

inverted into the copulatory organ (seminal vesicle) at rest and everted when used (as we suppose) it is not comparable to a cirrus in duplex organs.

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References

Brüggeman, J., 1984. Ultrastruktur und Differenzierung der prostatoiden Organe von *Polystyliliphora filum* (Plathelminthes, Proseriata). Zoomorphology 104: 86–95.

Doe, D. A., 1982. Ultrastructure of copulatory organs in Turbellaria. I. *Macrostomum* sp. and *Microstomum* sp. (Macrostomida). Zoomorphology 101: 39–59.

Doe, D. A., 1985. Ultrastructure of the male reproductive system in *Haplopharynx quadristimulus* Ax, 1971. I. Copulatory stylet and accessory spines. This volume.

Ehlers, B. & U. Ehlers, 1980. Struktur und Differenzierung penialer Hartgebilde von *Carenscoilia bidentata* Sopott, 1972 (Turbellaria, Proseriata). Zoomorphologie 95: 159–168.

Lanfranchi, A., 1978. Morphology and taxonomy of two new otoplanids (Turbellaria, Proseriata) form the Ligurian Sea. Zool. Scr. 7: 249–254.

Mainitz, M., 1977. The fine structure of the stylet apparatus in Gnathostomulida scleroperalia and its relationship to turbellarian stylets. In T. G. Karling & M. Meinander (eds.). The A. Luther Symposium on Turbellaria. Acta zool. fenn. 154: 163–174.

Martens, E. E., 1984a. Differentation in the copulatory organ of Turbellaria (Platyhelminthes). In W. Engels (ed.). Advances in invertebrate reproduction 3. Elsevier, Amsterdam: 611.

Martens, E. E., 1984b. Ultrastructure of the spines in the copulatory organ of some Monocelididae (Turbellaria, Proseriata). Zoomorphology: 104: 261–265.

Martens, E. E. & E. R. Schockaert, 1981. Observations on the ultrastructure of the copulatory organ of *Archilopsis unipunctata* (Fabricius, 1826) (Proseriata, Monocelididae). In E. R. Schockaert & I. R. Ball (eds.). The biology of the Turbellaria. Hydrobiologia 84: 277–285.

Martens, E. E. & E. R. Schockaert, 1984. Studies on the ultrastructure of the genital organs in Proseriata (Turbellaria). I. *Cirrifera aculeata* (Ax, 1951) (Coelogynoporidae). Zool. Scr.: 14: 81–90.

Martens, P. M., 1983. Three new species of Minoninae (Turbellaria, Proseriata, Monocelididae) from the North Sea, with remarks on the taxonomy of the subfamily. Zool. Scr. 12: 153–160.