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Zoomorphology

Ultrastructure of Copulatory Organs in Turbellaria

I. *Macrostomum* sp. and *Microstomum* sp. (Macrostomida)

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Summary. The copulatory organs in *Macrostomum* sp. and *Microstomum* sp. contain simple tubular stylets which are intracellular specializations. The stylet in *Macrostomum* sp. is produced in a syncytium covering part of the prostatic vesicle. The proximal region of the stylet surrounds the vesicle which contains six prostatic gland ducts and six accessory (sensory) cells containing ciliary rootlets. The stylet in *Microstomum* sp. is produced in an extension of a syncytium which lines the combined seminal-prostatic vesicle. The stylet is connected to the combined vesicle by a narrow bridge of matrix syncytium through which sperm, prostatic gland products and sensory cilia pass from the vesicle to the stylet lumen. In both species the matrix syncytium can be interpreted as a specialized terminal end of the male canal epithelium. Stylets of Turbellaria and other lower Metazoa are discussed in regards to structure (one or several pieces) and location (in separate cells, in a syncytium, or extracellular).

A. Introduction

Recently, several so called "cuticular" structures in the Turbellaria have been shown to be intracellular or basement lamina specializations, and not extracellular products as previously thought. The proboscis hooks and teeth in the Kalyptorhynchia-Schizorhynchia are basement lamina derivatives while the proboscis hooks in the Kalyptorhynchia-Eukalyptorhynchia are intracellular specializations (Doe 1976). Studies of "cuticular" copulatory hard structures have demonstrated their intracellular nature in the orders Acoela (Mainitz 1977), Macrostomida (Doe 1977) and Proseriata (Lanfranchi 1978; Ehlers and Ehlers 1980; Martens and Schockaert 1981).

In this paper I present observations of the fine structure of the copulatory organs of two species of Macrostomida. This includes not only the ultra-

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structure of each stylet but also its relationship and connection to the other components of the copulatory organ and the male canal. Relatively little is known about the fine structure of these organs in the Turbellaria (Rieger 1981) and a comparative study will most certainly provide valuable information about phylogenetic relationships.

B. Materials and Methods

Specimens of *Macrostomum* sp. (small *Macrostomum* sp. of Rieger, 1977), were collected from the upper 2 cm of sand at the MTL of the White Oak River inlet, Swansboro, North Carolina. A specimen of *Microstomum* sp. was collected in a meiofauna dispersal apparatus set up in the water column at the pier of the Marine Science Institute of the University of North Carolina in Morehead City, N.C. All specimens were extracted with the magnesium chloride technique. Two of the *Macrostomum* sp. specimens (one for transverse sections and one for sagittal sections) and the *Microstomum* sp. specimen were relaxed in a $MgCl_2$ solution isotonic to sea water for 10 minutes and fixed in 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer (pH 7.3 with 10% sucrose and traces of $CaCl_2$) at 4° C for 2 hours; rinsed overnight in buffer; and postfixed for $1^1/_2$ hours in phosphate-buffered osmium tetroxide at 4° C. The animals were dehydrated in an ethanol series and embedded in an Epon-Araldite mixture (Mollenhauer # 2). A third specimen of *Macrostomum* sp. (for transverse sections) was treated similarly except for being fixed in 10% formalin and sea water for $3^1/_2$ hours instead of phosphate-buffered glutaraldehyde.

Sections were cut with a diamond knife on a Reichert OMU2 ultramicrotome, stained with 5% aqueous uranyl acetate (20–25 min) and instant lead citrate (8–10 min), and examined in a Zeiss EM 9S2 electron microscope.

C. Observations

1. Macrostomum sp.

The copulatory organ of *Macrostomum* sp. is basically a prostatic vesicle containing a stylet that is funnel shaped with a curved end (Fig. 1A). The proximal (top of funnel) part of the stylet is approximately 9–11 μm in diameter across the top and 2.5 μm in diameter at the level where the curve begins. The stylet then narrows gradually to a point at the distal end. The stylet is 17–19 μm long from the proximal end to the beginning of the curvature and approximately 13 μm long from the curvature to the distal end. The opening is subterminal on the lower surface. The copulatory organ connects proximally to the seminal vesicle.

The stylet is an intracellular specialization within the matrix syncytium (Figs. 1C, 4). This matrix syncytium is most prominent near the proximal end of the copulatory organ where it reaches a thickness of 2 μm (Figs. 1C, 2A, 4). It quickly narrows to a thin (0.5 μm thick) tube which extends 10–12 μm toward the narrow part of the stylet. The matrix syncytium then enlarges slightly to a thickness of 1 μm before ending (Fig. 3C). Where the matrix syncytium ends, the outer and inner syncytial membranes close toward the stylet and adhere tightly to the outer and inner surfaces of the stylet along the rest of its length. The syncytium is surrounded by a 10–50 nm thick intercellular matrix. There are gaps in the intercellular

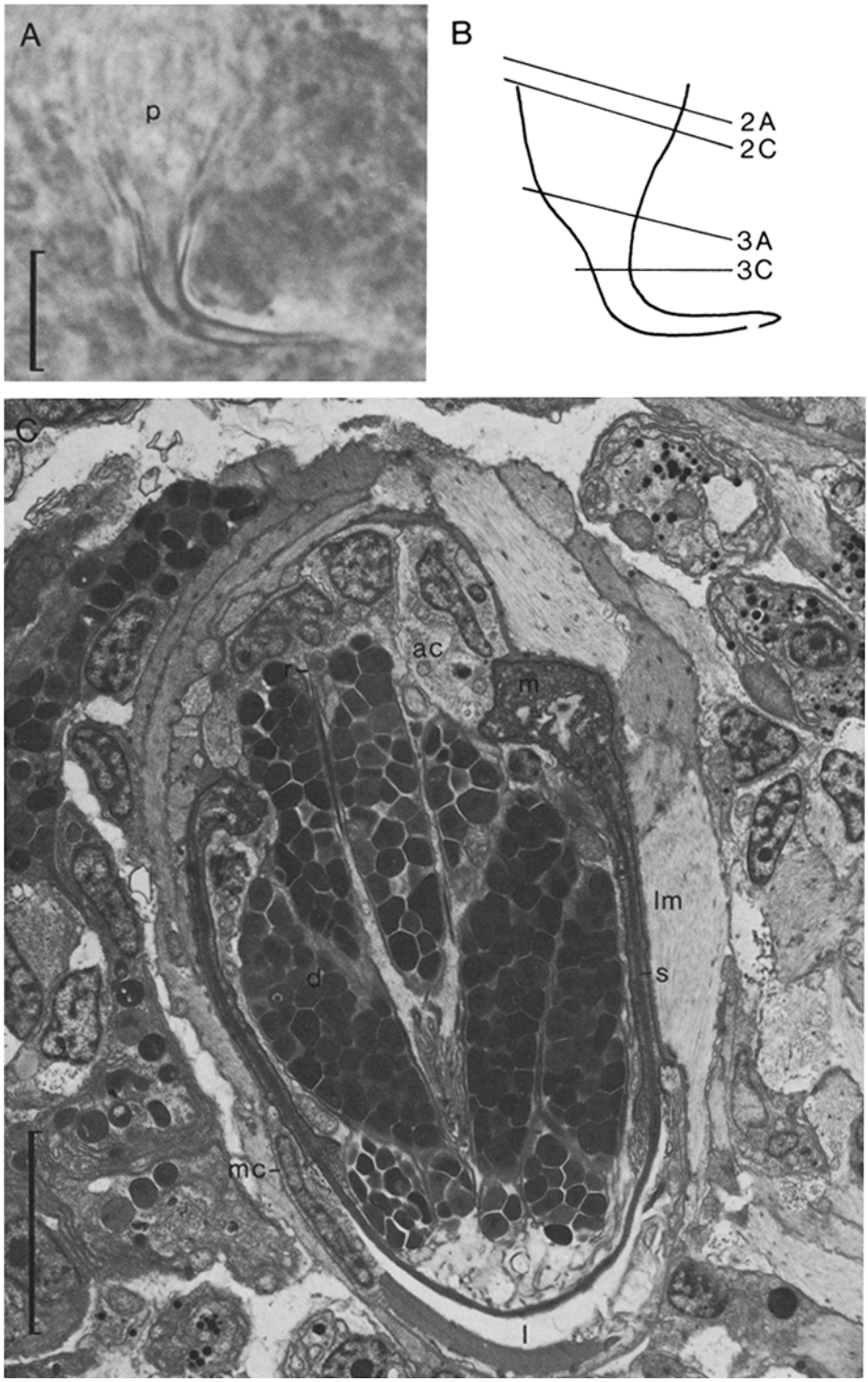
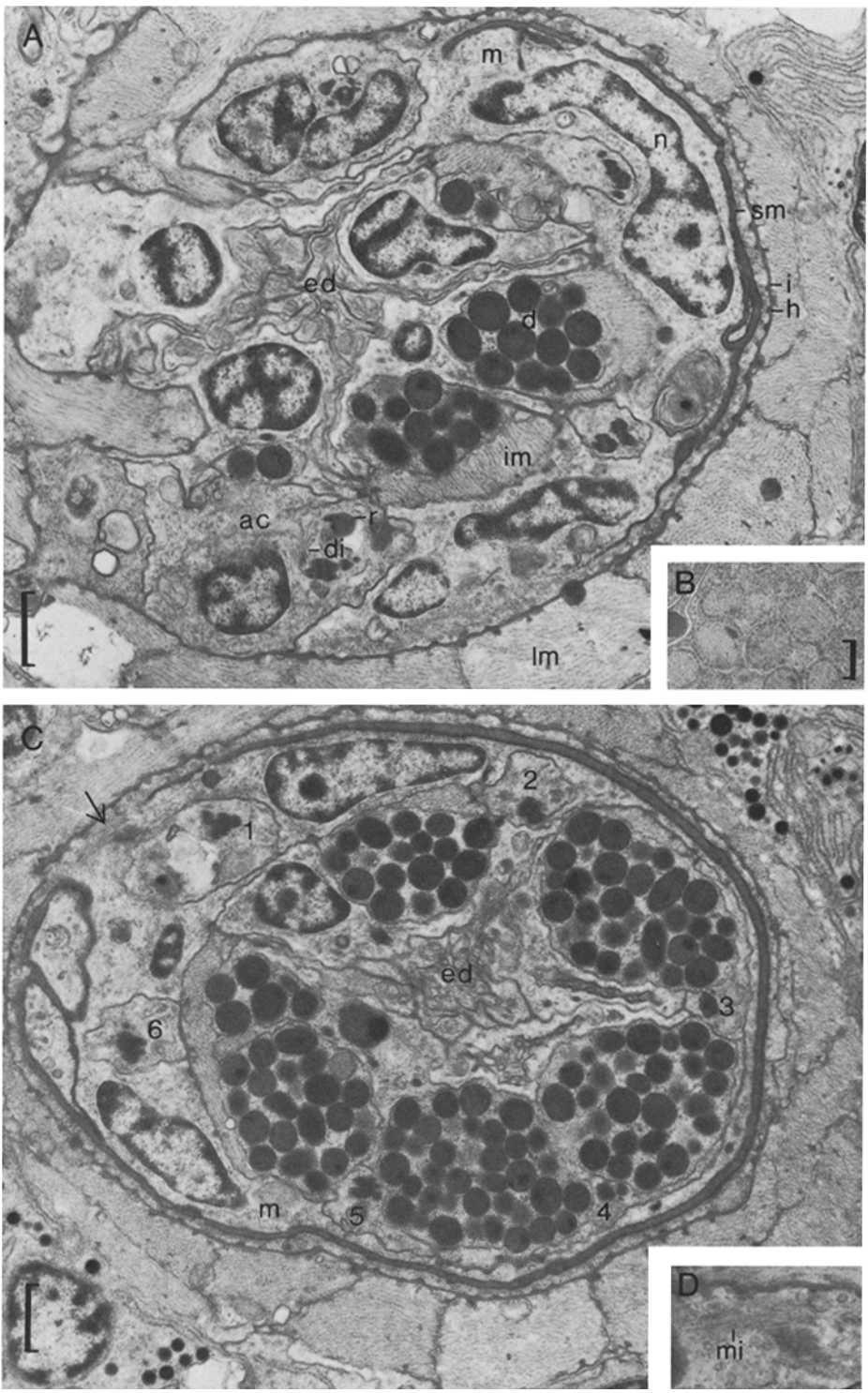


Fig. 1A–C. *Macrostomum* sp. **A** Male copulatory organ. Scale: 10 μm. **B** Drawing of stylet in (**A**). Lines represent planes of section in Figs. 2 and 3. **C** Oblique sagittal section through copulatory organ. Note stylet matrix syncytium, accessory cells with rootlets, gland ducts, and ejaculatory duct. Scale: 5 μm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matrix but they are not as prevalent as those in the matrix below the epidermis (see Doe 1981). The matrix syncytium is attached to the intercellular matrix by hemidesmosomes (Figs. 2A and C, 3A).

The stylet consists mainly of electron-dense material with a thin rim of denser material. The stylet is thinnest in the proximal part of the matrix syncytium where small 25–50 nm thick pieces of electron-dense material occur individually (Fig. 2A, C, D). Distally, the stylet material coalesces into a solid unit that forms a closed circle when observed in transverse section (Fig. 3A). The stylet wall thickens to 150–200 nm at the level where the matrix syncytium cytoplasm ends and then tapers to 100–150 nm distally toward the tip (Figs. 3C, 4).

The stylet wall, as seen in several micrographs, has a 40–50 nm thick central region which appears to contain filamentous or tubular subunits (Fig. 3C). Similar material is present in the proximal part of the matrix syncytium between pieces of stylet wall material (Fig. 2C and D).

In addition to the stylet material, the proximal region of the matrix syncytium also contains several nuclei and other organelles (Figs. 1C, 2A and C). A few mitochondria and dictyosomes are present, as well as vacuoles filled with concentric rings of membranous material. Vesicles of various sizes and densities are also present. The cytoplasm appears darker and more granular in the formalin fixed specimen, but this may be due to the loss of material during fixation. Distally, in the narrow region of the matrix syncytium, most organelles are excluded except for some small vesicles (Figs. 3A, 4).

The distal region of the matrix syncytium is also the site of the junction between the syncytium and the male canal epithelium (Figs. 1C, 3C). Here the matrix syncytium bears numerous microvilli. Most of them are present on the membrane inside the stylet (Fig. 3C). The male canal epithelium overlaps the outer part of the matrix of the matrix syncytium for 1–2 μm and then courses toward the male opening with the stylet inside its lumen (Figs. 1C, 4). The epithelium is thin and partially enclosed by circular muscles. The stylet presses against the epithelium filling almost all of the canal lumen. The canal was not investigated beyond the region containing the stylet.

The copulatory organ contains several other cell types in addition to the matrix syncytium. Most prominent are the six ducts of prostatic gland cells whose cell bodies lie outside the prostatic vesicle (Figs. 1C, 2A and C, 3A, 4). Although the significance of having this number of ducts is unclear, both copulatory organs which were sectioned transversely contained

Fig. 2A–D. *Macrostomum* sp. **A** Oblique transverse section through proximal region of copulatory organ. Note intracellular position of stylet material, accessory cell bodies with rootlets, and close association of gland cell ducts and muscle cells. Scale: 1 μm. **B** Prostatic duct granules fixed with 10% Formalin and sea water. Note loss of homogeneous material and appearance of tubular filaments. Scale: 0.5 μm. **C** Oblique transverse section through proximal region of copulatory organ but distal to (**A**). Note groupings of six accessory cells (*No. 1–6*) and six gland cell ducts. **D** Enlargement of area at arrow on 2C showing microfilaments between sections of stylet material.

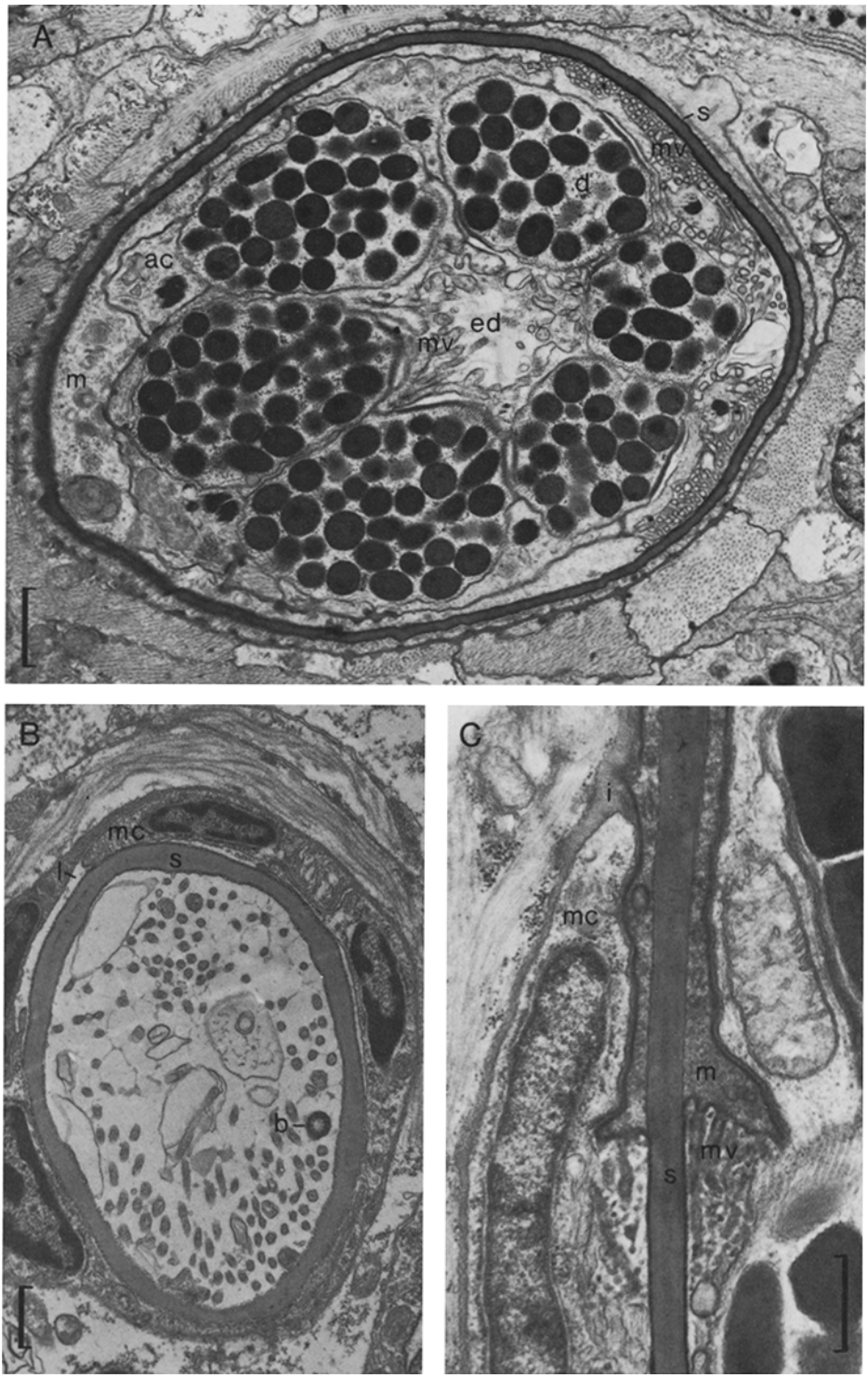


Fig. 3A–C. *Macrostomum* sp. **A** oblique transverse section through copulatory organ in region of stylet – male canal junction. Note microvilli inside stylet lumen and inside ejaculatory duct. Also, note stylet is a complete circle in transverse section. Scale: 1 μm. **B** Transverse section through stylet distal to prostatic vesicle. Note male canal epithelium, cell membranes on both surfaces of stylet, and basal body of cilium of accessory cell. Scale: 0.5 μm. **C** Oblique sagittal section through copulatory organ. Distal region of matrix syncytium showing apposition of membranes on stylet, microvilli, and male canal epithelium. Scale: 0.5 μm

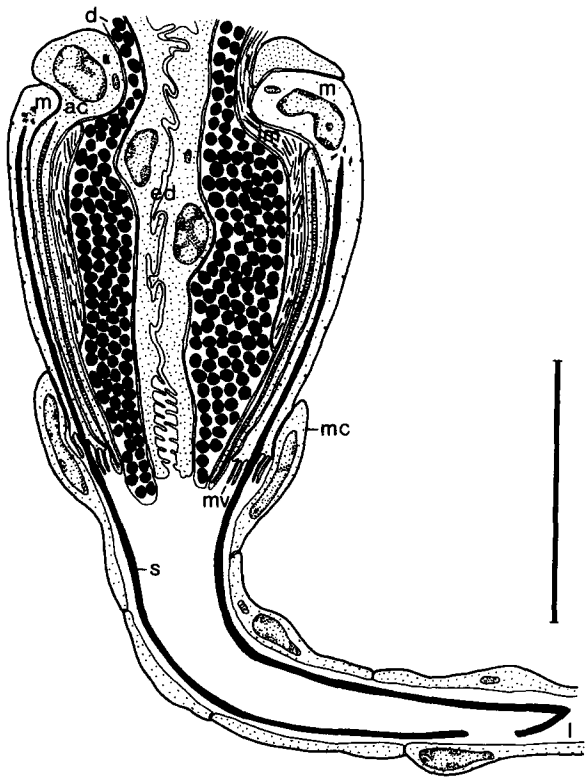


Fig. 4. *Macrostomum* sp. Sagittal reconstruction of copulatory organ. Slightly schematic. Scale: 10 μm

six. The large ducts occupy most of the space within the stylet proximal to the curve. A single row of microtubules lines the periphery of each duct just beneath the cell membrane. The gland cell ducts continue distally beyond the level of the end of the matrix syncytium cytoplasm. However, then the duct membranes lose their integrity (possibly due to merocrine secretion) and, as a result, it is difficult to differentiate individual ducts in the stylet lumen (Fig. 3B).

The ducts contain a single type of membrane-bound secretory granule which is basically spherical to elliptical in shape (up to 0.6 μm in diameter and 1.2 μm long), but may take on different shapes (i.e. polyhedral) when closely packed together. Granule morphology appear different when comparing glutaraldehyde and formalin fixed material. With the former fixative the granules are electron dense and homogeneous. However, with the formalin fixation each granule has a central crystalloid matrix and a periphery consisting of a single circle of tubular filaments lying just beneath the membrane (Fig. 2B). The appearance of the granule substructure in the formalin-fixed material may be due to the extraction of some protein material during washing after fixation.

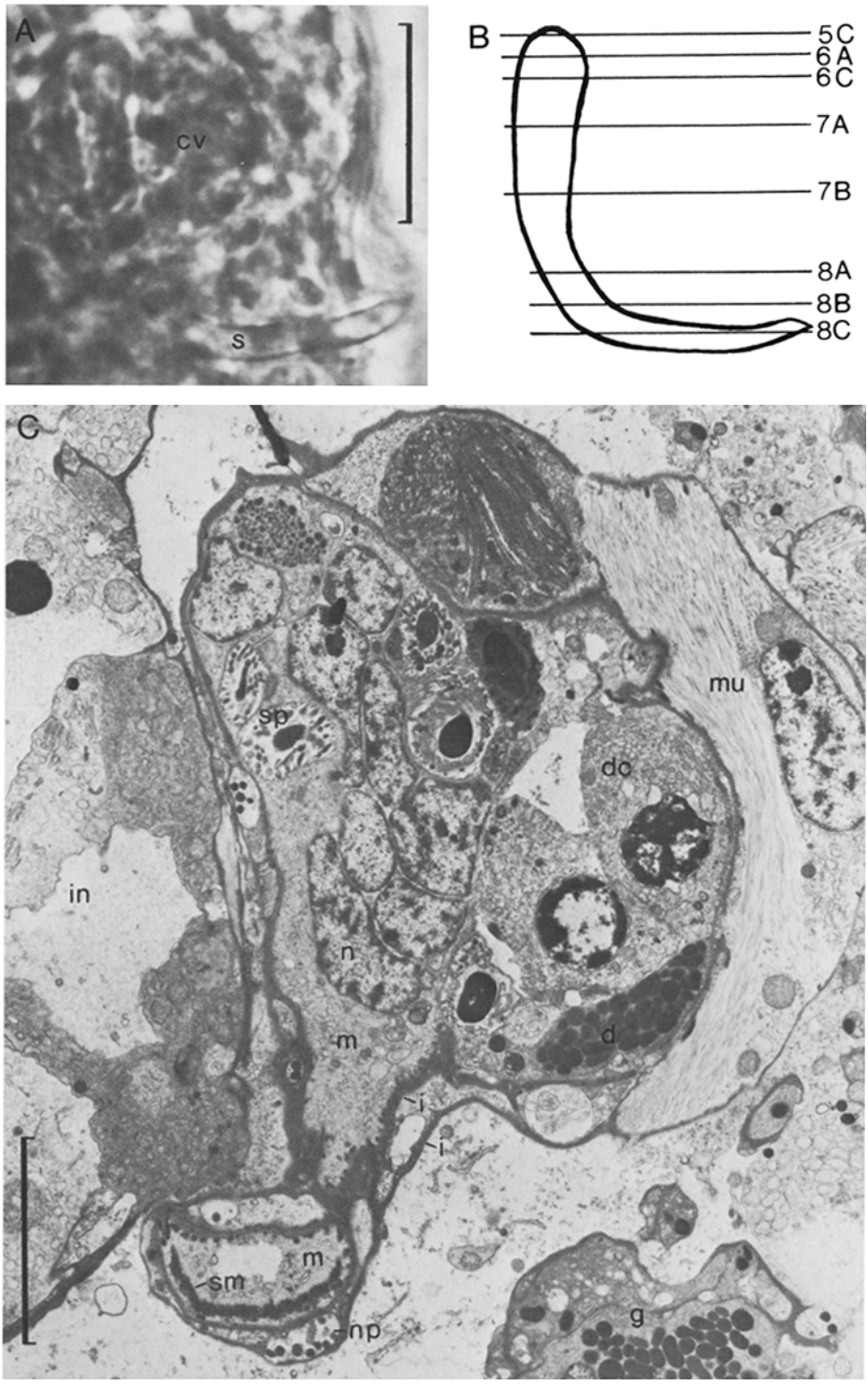


Fig. 5A–C. *Microstomum* sp. **A** Male copulatory organ. Scale: 5 μm. **B** Drawing of stylet in (**A**). Lines represent planes of section in Figs. 5, 6, 7, 8. **C** Transverse section through combined vesicle and stylet posterior to vesicle-stylet connection. Note matrix syncytium nuclei and sperm in matrix syncytium cytoplasm. Note also prostatic gland ducts and degenerative cells with aberrant nuclei and multiple intracellular axonemal configurations. Scale: 5 μm

The stylet of *Macrostomum* sp. also surrounds six elongated cells which extend from the proximal part of the copulatory organ distally beyond the end of the matrix syncytium cytoplasm into the stylet lumen (Figs. 1C, 2A and C, 3A, 4). As with the matrix syncytium, most of the cytoplasm of this accessory cell type lies in the proximal region of the copulatory organ along with the nucleus (Fig. 2A). This region of the cells, which ranges up to 3 μm across when observed in transverse section, also contains other typical organelles including dictyosomes. The accessory cell nuclei usually lie proximal to the matrix syncytium nuclei.

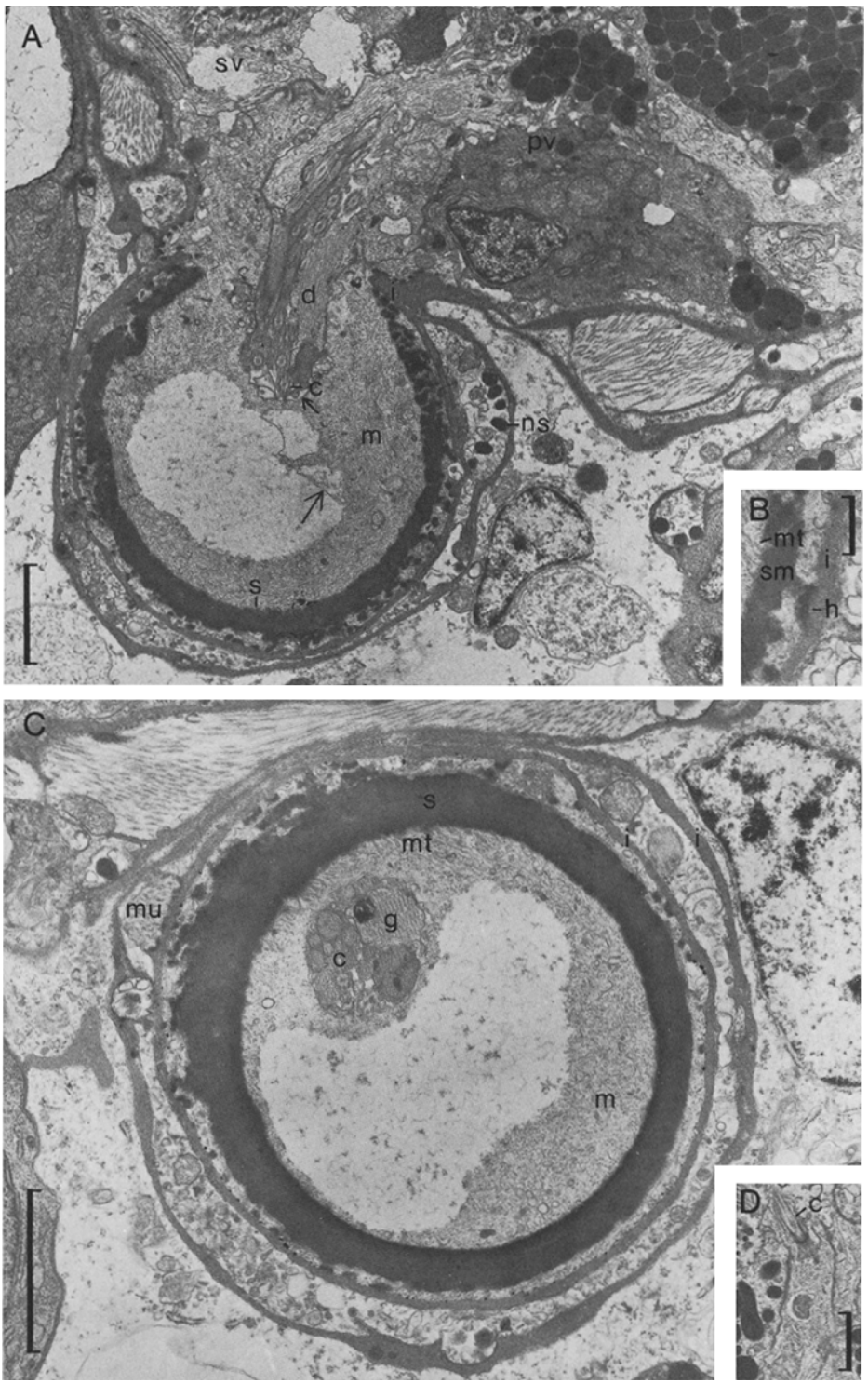
At the level of the matrix syncytium nuclei, each accessory cell becomes a thin ribbon between the matrix syncytium and the prostatic gland cell ducts, or between adjacent gland cell ducts (Figs. 2A and C, 3A, 4). At the level of the copulatory organ where the matrix syncytium cytoplasm ends, the accessory cells extend into the stylet lumen as narrow cylinders (Fig. 3B).

The most conspicuous feature of each accessory cell is the long ciliary rootlet (or rootlets) which occupy the entire length of the cell (Figs. 1C, 2A and C, 3A, 4). Secondary rootlets, if present, are not as long as the principal rootet. Although the rootlets reach the distal end of each cell, only one (of the more than twelve observed in the two transverse sectioned stylets) was attached to a basal body and cilum (Fig. 3B). This cilium extended distally to the stylet opening. The other rootlets ended in the cytoplasm of the narrow accessory cells as the cells protruded into the stylet lumen. The function of the accessory cells is unclear but they appear to be sensory structures.

The ejaculatory duct, which runs down the center of the copulatory duct is lined by at least four epithelial cells. At the level of the proximal end of the copulatory organ the cell membranes at the duct lumen exhibit extensive outfolding and interdigitation (Figs. 2A and C, 4). Distally, the outfoldings disappear and microvilli extend into the duct lumen (Figs. 3A, 4). The duct cannot be followed into the distal portion of the stylet lumen (Fig. 3B).

The musculature of the copulatory organ consists of both external and internal muscles. Externally, a sheath of longitudinally and spirally oriented muscles surrounds the stylet matrix syncytium (Figs. 1C, 2A and C, 3A). The muscle cells contain thick and thin filaments with scattered dense bodies, a small number of sarcoplasmic reticulum tubules and cisternae. The muscles are attached to the intercellular matrix supporting the stylet matrix syncytium by hemidesmosomes (Figs. 2A and C, 3A). Some muscles of the sheath continue distally as a layer surrounding the male canal while others continue as the stylet protractor muscles (Figs. 1C, 3B). Internally, muscle cells are closely associated with the prostatic gland cell ducts in the proximal region of the copulatory organ (Figs. 1C, 2A and C, 4). These isolated muscles, which lie adjacent to, but not surrounding the ducts, end before the level of the junction of the matrix syncytium and male canal.

The copulatory organ is innervated by nerve processes penetrate singly into the organ musculature but synapses were not observed.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II. Microstomum sp.

The copulatory organ of *Microstomum* sp. is a combined seminal-prostatic vesicle (=combined vesicle) connected to an approximately 51 μm long stylet that bends at a 90° angle near the halfway point along its length (Fig. 5A). The cylindrical stylet tapers from a diameter of 7 μm at its proximal end to 4 μm just before the bend. After the bend it tapers slowly to a point with the opening occuring subterminally and laterally.

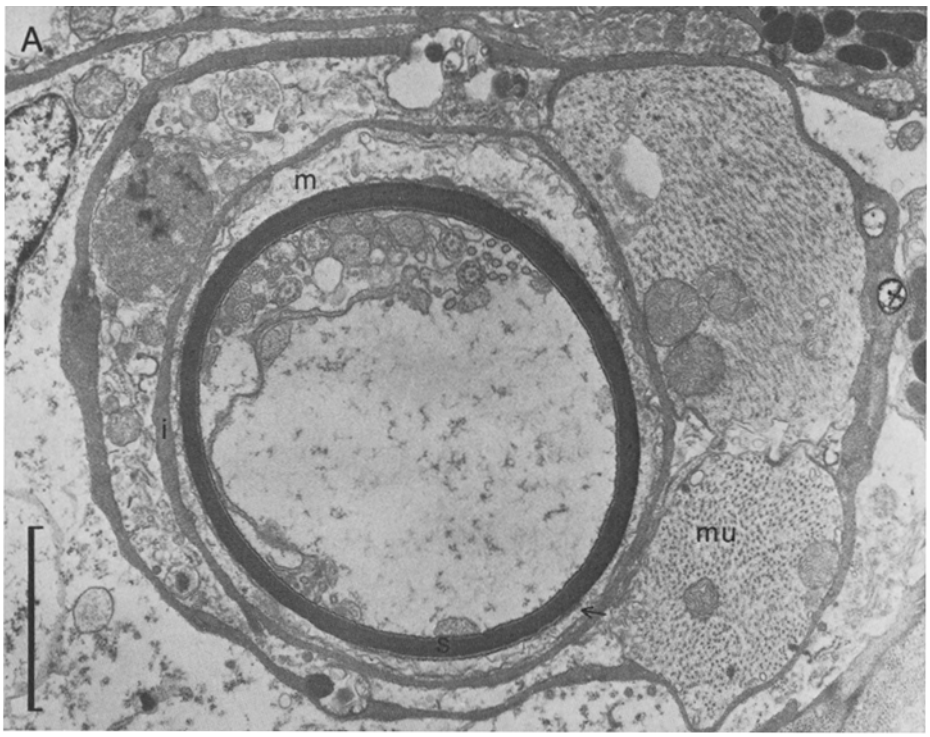
The combined seminal-prostatic vesicle is lined by a matrix syncytium (Fig. 5C). The syncytium contains a large group of closely arranged nuclei located on the seminal side of the vesicle. The stylet is an intracellular specialization within an extension of the matrix syncytium connected to the vesicle by only a thin cytoplasmic bridge (Figs. 6A, 9). This cytoplasmic connection also acts as a conduit for the passage of sperm, prostatic gland secretions and sensory structures from the vesicle to the stylet. The connection between the combined vesicle and stylet occurs subterminally on the stylet.

At the proximal end of the stylet, small pieces of stylet wall material lie close together (Figs. 6A and B). The 100–150 nm wide amorphous electron dense pieces also extend across the cytoplasmic connection into the vesicular part of the syncytium. In addition to the pieces free in the cytoplasm, there are also a large number that are attached to small tuft-like proliferations of the underlying intercellular matrix. These hemidesmosome-like structures may serve to anchor the stylet to the well developed 100–200 nm thick intercellular matrix. The structures also extend along the intercellular matrix lining the connection to the vesicle. They are present for only a few micrometers distal to the vesicle-stylet connection and disappear as the stylet wall becomes a complete circle (Fig. 6C).

The stylet wall consists of homogeneous electron-dense material which does not exhibit any substructure (Fig. 6C). The wall is 500 nm thick just distal to the vesicle-stylet connection and then narrows to a thickness of 200 nm toward the stylet tip.

The stylet lies within the syncytial cytoplasm for a short distance distal to the vesicle-stylet connection (Fig. 6C). The syncytial membranes then extend toward the stylet wall and adhere to its outer and inner surfaces.

Fig. 6A–D. *Microstomum* sp. **A** Transverse section through connection between vesicle and stylet. Note large area of reduced density with adjacent vesicles (*large arrow*), and passage of prostatic gland ducts and sensory cilia from prostatic region into stylet (*small arrow*). Observe nerve process between rings of intercellular matrix. Scale: 2 μm. **B** Enlargement of stylet wall. Note electron-dense material in cytoplasm and also attached to intercellular matrix. Note also microtubules in cytoplasm and attached to stylet material. Scale: 5 μm. **C** Transverse section through stylet distal to vesicle-stylet junction. Note stylet wall forms a complete circle inside syncytial cytoplasm and surrounds the large less dense area, prostatic gland ducts and sensory cilia. A syncytial membrane separates the prostatic gland cell ducts and sensory cilia from the syncytial cytoplasm. Scale: 2 μm. **D** Sensory process in prostatic region of vesicle. Scale: 1 μm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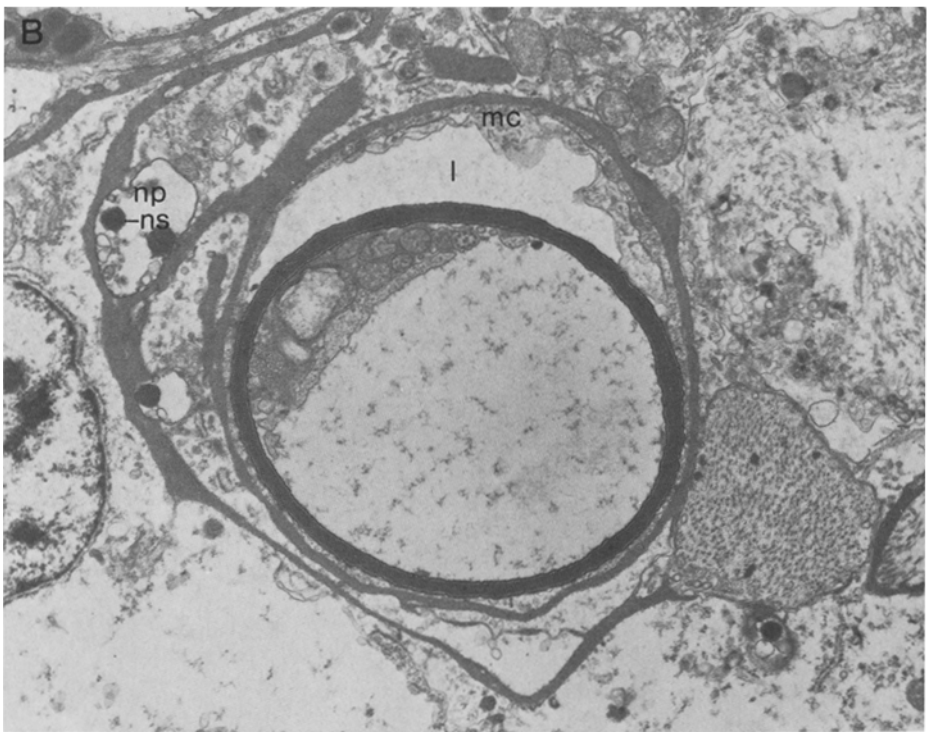
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This occurs more proximally on the inner surface of the stylet wall. Approximately 1 μm distally, the outer syncytial membrane pressed inward against the stylet outer surface (Fig. 7). This is also the position along the stylet where the male canal epithelium joins to the matrix syncytium on the outer surface of the stylet (Fig. 7A).

The male canal epithelium is very narrow (Fig. 7A). The canal lumen is almost entirely filled by the stylet (Figs. 7B, 8). The nuclei of the epithelium are located near the bend in the stylet. The stylet in the specimen investigated protrudes through the body wall (most probably a fixation artifact) (Figs. 5A, 8C, 9). However, the stylet broke through regular epidermal cells and not through the male pore so that this region of the canal was damaged and the connection with the pore was not observed.

The matrix syncytium of the combined vesicle contains several nuclei with scattered areas of chromatin and heterochromatin (Fig. 5C). The cytoplasm is rich with numerous vesicles and microtubules. The vesicles exhibit a wide range of sizes (100–600 nm in diameter) and contain electron-dense or translucent material. Microtubules are present across the connection to the stylet and some are attached to the stylet wall (Fig. 6A and B). The cytoplasm also contains free ribosomes and a few mitochondria.

The matrix syncytium also contains a large less dense area containing sparce flocculent material (Figs. 5C, 6A and C, 9). This area does not appear to be surrounded by a membrane but, in several micrographs, large vesicles are present at the boundary between it and the darker cytoplasm (Fig. 6A). The vesicles contain material similar to that observed in the area and they could be discharging their products into it. Distally, this area ends as the syncytial cytoplasm protrudes into stylet lumen and the membrane presses against the inner wall of the stylet. *Microstomum* sp. lacks an epithelial ejaculatory duct and this region of the matrix syncytium may serve as a conduit for sperm between the combined vesicle and the stylet lumen. Sperm were observed in the syncytium of the combined vesicle but not in the stylet (Fig. 5C).

Prostatic gland cell ducts and sensory cilia from the prostatic region of the combined vesicle extend into the stylet (Fig. 6A and B). The gland cell ducts and cilia are surrounded by the matrix syncytium; even though they appear to be intracellular in a transverse section, their position is actually extracellular. As the gland cell ducts and cilia pass through the matrix syncytium of the vesicle-stylet junction, they are compressed into a tight

Fig. 7A–B. *Microstomum* sp. **A** Transverse section through stylet-male canal junction. Note membrane lining inside of stylet. Note also prostatic gland ducts and cilia with reduced numbers of microtubules. Transition from syncytium to male canal epithelium is not complete as shown by lack of membrane around entire stylet wall. Areas of transition revealed by folded membrane (arrow) on surface of stylet. Scale: 2 μm. **B** Transverse section distal to (**A**) showing stylet completely surrounded by membranes and inside male canal lumen. Note prostatic gland ducts and sensory cilia remain pressed against stylet wall. Observe breakdown of outer ring of intercellular matrix and presence of muscle cells. Same magnification as (**A**)

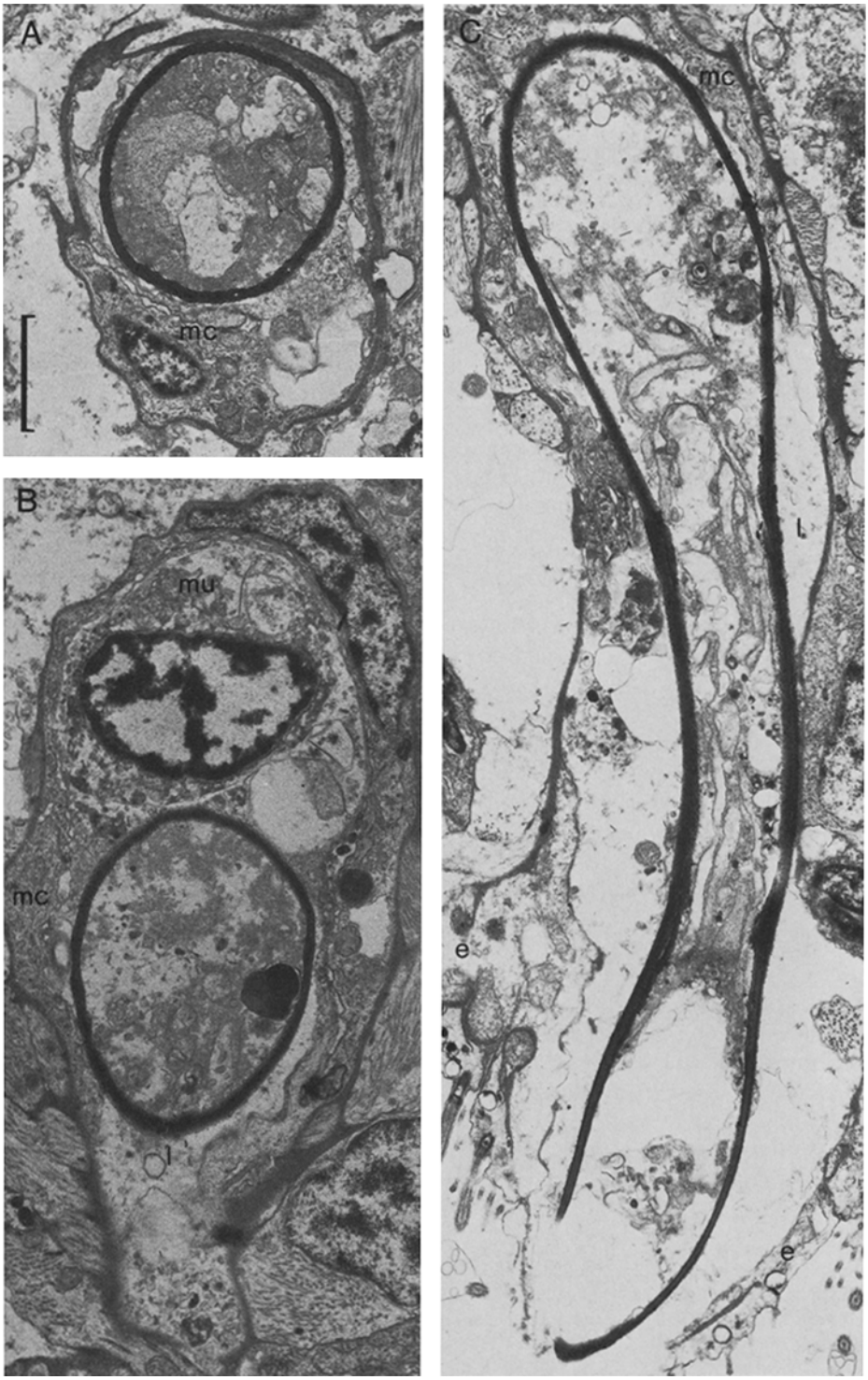

Fig. 8A–C. *Microstomum* sp. **A** Transverse section through stylet proximal to bend. Note glandular (?) material in stylet lumen and nucleus of male canal epithelial cell. Scale: 2 μm. **B** Transverse section through stylet closer to bend. Note sarcoplasmic portion of muscle cell protruding through male canal epithelial cell and muscles attached to intercellular matrix. **C** Sagittal section through stylet from bend to tip. Stylet opening is subterminal. Protrusion of stylet through epidermis is probably an artifact of fixation. All at same magnification

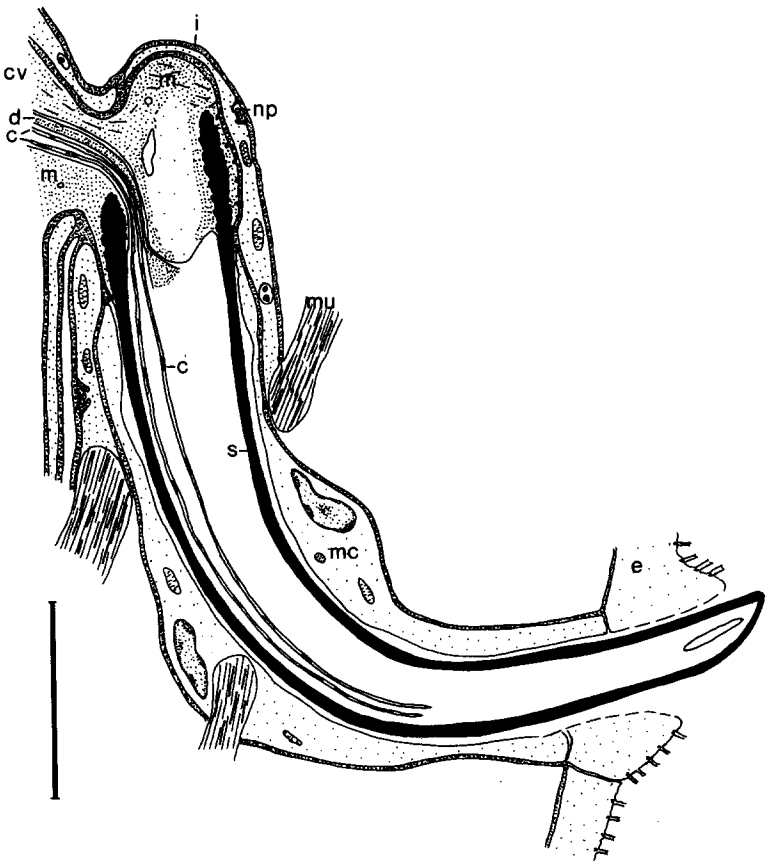

Fig. 9. *Microstomum* sp. Sagittal reconstruction of stylet, matrix syncytial connection to combined vesicle and male canal. The combined vesicle lies to the left of the connection. Slightly schematic, especially the male canal where the stylet has broken through the epidermis. Scale: 10 μm

bundle (Fig. 6A and C). Distally, where the syncytial cytoplasm ends, they spread out along one side of the stylet and lie free within the lumen (Fig. 7).

The prostatic gland cell bodies lie outside of the combined vesicle with granules being stored in large areas in the vesicle that also contain a granular cytoplasm (Figs. 5C, 6A). A single gland cell type is present. It produces spherical to elliptical electron-dense granules with a length up to 1 μm and a diameter up to 0.6 μm. The gland cell ducts in the stylet do not contain secretory granules but they do contain numerous microtubules. The microtubules often filled the entire lumen of the ducts and were not restricted to the periphery (Fig. 6C).

In the specimen investigated, nine cilia emerged from the prostatic part of the vesicle and entered the stylet lumen (Fig. 6A and C). The cilia arise from thin cytoplasmic processes (Fig. 6D). The base of each cilium is sunken in a cuplike structure without a rootlet below the basal body. Most of the cilia exhibit a reduction in axonemal microtubular number from the typical 9 + 2 doublet configuration as they extend through the stylet (Fig. 7).

The number drops to 10–14 single microtubules scattered within the cilium. As the number of microtubules decreases, so does the diameter of each cilium. In addition, most of the cilia end before the angle in the stylet; only two extending beyond the angle.

The prostatic side of the combined vesicle also contains a few unusual cells which appear to be degenerating epithelial cells, possibly from the vas deferens (Fig. 5C). The cells contain remnants of ciliary axonemes and basal bodies. The cytoplasm, apart from numerous vesicles, appears disorganized as shown by the lack of discrete organelles. The nuclei of these unusual cells are pycnotic.

The stylet, as well as the combined vesicle, is surrounded by two concentric sheaths of well developed intercellular matrix (Figs. 5C, 6A and C, 9). The 100–200 nm thick inner sheath around the stylet supports the matrix syncytium and then, distally, the male canal epithelium. The space between the inner sheath and the 100–250 nm thick outer sheath contains stylet protractor muscles and nerve processes. The same space around the combined vesicle contains muscles and nerve processes.

At least four muscles are found between the sheaths of intercellular matrix along the stylet proximal to the angle. One is attached to the intercellular matrix supporting the matrix syncytium by hemidesmosome-like structures (Fig. 6C). Three others, located along the male canal epithelium, do not appear to form similar attachment structures (Fig. 7A). Just proximal to the angle in the stylet the outer sheath of intercellular matrix breaks down as many additional muscles are attached to the male canal (Figs. 7B, 8).

Nerve processes are found in the area between the two sheaths of intercellular matrix around the combined vesicle and the stylet (Figs. 5C, 6A, 7). The processes range from 0.2 μm to 1 μm in diameter. Many are conspicuous by the presence of 150–500 nm diameter electron-dense granules in addition to the more typical smaller clear vesicles (Figs. 6A and C, 7A). The processes containing the granules (neurosecretory?) appear similar to processes observed in the periphery of the longitudinal nerve cords lying lateral within the organism. Neuronal processes in the pharyngeal nerve ring also contain similar granules (Doe, unpublished observation). The majority of the processes along the stylet lie adjacent to or near muscle cells. However, synapses were not observed.

D. Discussion

The fine structural analysis of the copulatory stylets in *Macrostomum* sp. and *Microstomum* sp. identifies these structures as intracellular specializations, and not true cuticularizations or basement lamina derivatives as previously suggested (see Luther 1905). These results are consistent with the intracellular localization of stylets in the Acoela (see Mainitz 1977) and Proseriata (see Ehlers and Ehlers 1980; Lanfranchi 1978). It is unclear whether the stylet and stylet sheath in *Gyratrix hermaphroditus* (Rhabdocoela) are intracellular (see Reuter 1977, Fig. 1).

The results support the view that, in the Platyhelminthes, supportive and other hard structures are developed as intracellular specializations of epithelia or basement lamina derivatives. Beside copulatory stylets, other examples include kalyptorhynch proboscis hooks and teeth, trematode spines and cestode scolex hooks (see also Rieger and Doe 1975; Doe 1976; Ehlers and Ehlers 1980). The only possible hard structures which appear to be derived from true cuticularization are the epidermal marginal spines in *Enantia spinifera* Graff 1889 (Rieger 1981).

A comparison of the copulatory organs of *Macrostomum* sp. and *Microstomum* sp. reveals differences in the relative positions of the seminal vesicle, prostatic vesicle and stylet. In the former species, the stylet is produced in the matrix syncytium enveloping the prostatic vesicle. In the latter species, the two vesicles are combined into a single structure. The stylet is formed in an extension of the posterior region of the combined vesicle matrix syncytium and is attached to the combined vesicle by a narrow bridge of matrix syncytium. In both species the matrix syncytium can be interpreted as a specialized terminal end of the male canal epithelium.

The sytlets of both species consist of amorphous electron-dense material forming a basically tubular shape with a continuous wall. The use of adult specimens for investigation generally precludes any information about stylet formation and components. However, the presence of what appear to be microfilaments or microtubules in some micrographs of the stylet in *Macrostomum* sp., may indicate a structure similar to that described by Ehlers and Ehlers (1980) for a sexually immature specimen of *Carenscoilia bidentata*. The cross-striated microfibrils in the proximal region of the stylet in *C. bidentata* are more visible than those in the distal end. This is probably a result of the microfibrils being enveloped in electron-dense material which obscures their presence. Thus, it would be expected that the microfibrils would become almost completely hidden in the stylet of a sexually mature specimen of *C. bidentata*.

The proximal region of the matrix syncytium in both species in this study contains small amounts of amorphous electron-dense material used in stylet formation. Although the nature of this material was not determined cytochemically, the presence of free ribosomes and a few dictyosomes indicates that the material is proteinaceous. Again, the role of these organelles could also be demonstrated more successfully with sexually immature specimens, as in the case of *C. bidentata*, where there are many large dictyosomes in this stylet region (see Ehlers and Ehlers 1980).

The position of sensory or possible sensory elements in the prostatic vesicle also varies between the species. The unusual accessory cells in *Macrostomum* sp. may have a sensory function, although almost all do not have cilia. Rieger (1971, Fig. 14C) observed possibly similar cells with long non-ciliated extensions between the stylet-forming cells and the developing ejaculatory duct in *Myozonaria bistylifera* at the light microscopic level. Cilia have been reported in the prostatic vesicle of *Macrostomum hystricinum* (Luther 1905), which is in the same species group as *Macrostomum* sp. (see Rieger 1977). Although sensory cilia characterized by a decrease in

cilia length or changes in axonemal configuration have been reported (i.e. *Microstomum* sp. in this study; see also Reuter 1975), it is difficult to attribute a sensory function to a rootlet without a cilium attached. It is also possible that the accessory cells lose and reform their cilia.

The ciliated cells in the vesicle of *Microstomum* sp. appear to be more similar to sensory cells reported from epithelia of other species. However, these cells lack the microvillar collar found on some of the receptors in other species (see Ehlers 1977). In both *Macrostomum* sp. and *Microstomum* sp. the sensory (?) cells lie close to the gland cell ducts. In the latter species the cilia must pass through the matrix syncytium along with the gland cell ducts to reach the stylet lumen.

The cytology of the stylet muscle cells in the two species investigated is similar to that in their body wall and pharynx musculature (see Doe 1981). Differences occur in the position of the muscles relative to the vesicle and stylet. In *Macrostomum* sp. the prostatic vesicles and stylet, as well as the seminal vesicle (unpublished observation), are completely encased in a muscular sheath. However, in *Microstomum* sp., only a few muscle cells are present around the outer edge of the combined vesicle. Also, only a few muscles are attached to the stylet matrix syntytium. The musculature arrangement in *Macrostomum* sp. is more similar to that described for *Gyratrix hermaphroditus* by Reuter (1977), but the muscle cells in *G. hermaphroditus* contain a greater elaboration of sarcoplasmic reticulum. Reuter (1977) states that the amount of sarcoplasmic reticulum is related to the speed of muscle contraction, which would infer that the stylet in *G. hermaphroditus* is moved at a faster speed than the macrostomid stylets. Also, see Reuter (1977) for a discussion of the roles of desmosome and hemidesmosome-like structures in anchoring muscle cells to the stylet.

The present study reveals differences in the fine structure of nerve processes innervating the stylet apparatus in the species investigated. The processes in *Macrostomum* sp. contain secretory vesicles similar to those observed in other turbellarians (see Reuter and Lindross 1979, for a discussion of the turbellarian peripheral nervous system). The processes in *Microstomum* sp. contain large electron-dense granules observed in only a few of the turbellarian species investigated so far. Reuter et al. (1980) found similar granules in the stomatogastric nervous system of *Microstomum lineare* and called them peptidergic neurosecretory granules. This author has observed similar nerve processes in the pharyngeal nerve ring and peripheral nerve bundles of *Microstomum* sp. (unpublished observation). However, typical nerve processes are more prevalent in these nerve cords.

The profound differences in the amount of musculature and the types of nerve processes associated with the stylets in the two species investigated may indicate differences in the control and action of the stylets. These structures in *Macrostomum* sp. are more similar to the fast-acting musculature and simple nerve processes described for *G. hermaphroditus* by Reuter (1977). The sparce musculature and numerous neurosecretory processes in *Microstomum* sp. may indicate slower initiation and speed of contractions during copulation. They may also be linked to the use of asexual reproduc-

tion by this genus. However, the specimen investigated contained an egg and was not part of a zooid chain.

The formation of the stylet in a matrix syncytium instead of in separate matrix cells is directly related to the structure of the stylet. The stylet is formed in a matrix syncytium if it is a simple tube, or has accessory structures such as needles and teeth connected to it as in *C. bidentata* (see Ehlers and Ehlers 1980). A tubular stylet could also be formed in a single large cell. The presence of a syncytium, therefore, may be due to size limitations for turbellarian cells. If the stylet parts are not connected, as in *Paratomella rubra* (see Mainitz 1977) or *Parotoplanina geminoducta* (see Ehlers and Ehlers 1980), then they are formed in separate matrix cells. However, they could also be formed in different regions of a syncytium. A solid-walled stylet, or one with connected parts, cannot be formed intracellularly in separate cells because the cell membranes would prevent structural continuity. Thus, the cell membranes between adjacent cells have disappeared to form the matrix syncytium. A solid-walled stylet could, however, be formed by a cellular epithelium if it was produced extracellularly (i.e. a cuticular derivative, see below).

The question of which condition is primitive in the Turbellaria can not be completely answered until more stylets, as well as the male canal epithelium, in more species are investigated. However, the cellular nature of the male canal epithelium in *Macrostomum* sp. suggests that the matrix syncytium is the derived character.

Intracellular copulatory stylets have also been reported in groups with true cuticles. The stylet in *Microphthalmus* c.f. *similis* (Polychaeta) consists of eight intracellular rods composed of electron-dense amorphous material surrounding a central striated cylinder (Westheide 1979). However, the stylet of *Microphthalmus* c.f. *listensis* is a true cuticular structure formed in the shape of a hook-like cylinder with a solid wall (Westheide 1978). The presence of both intracellular and extracellular stylets in the same genus reflects again the dichotomy between solid one-piece stylets and stylets with separate parts. Within *Microphthalmus*, the first type of stylet is formed from the cuticle overlying a cellular epithelium. The stylets with separate parts, as previously reported in the Turbellaria, are intracellular specializations.

This dichotomy is also present in the Gnathostomulida. Mainitz (1977) demonstrated that the stylets in several species contain intracellular and extracellular components. The stylets contain an inner core of separate intracellular rods and a continuous outer sheath of cuticular material.

Ehlers and Ehlers (1980) believe the similarities between the stylet striations in *C. bidentata* and *M.* c.f. *similis* are due to convergence. Mainitz (1977) feels that possible structural similarities between stylets in gnathostomulids and turbellarians are probably due to homoiolog situations due to similar simple construction of the ancestral forms. The presence of intracellular rods in all three phyla probably reflect the limitations of the construction of a stylet made of several separate parts. If the parts are not connected, then they are produced in separate cells, not a syncytium. Thus, the similarity is due to analogy and not homology (see also Rieger and Tyler, 1979).

The reserve stylets in the proboscis armature of the nemertine *Paranemertes peregrina* are also intracellular specializations (Stricker and Cloney 1981). These solid stylets are formed in membrane-bound vacuoles and, thus, are separated from the cytoplasm of the cell. In contrast, the stylet material in the turbellarians is not separated form the cytoplasm.

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Abbreviations used in figures. *ac* accessory cell; *b* basal body; *c* cilium; *cv* combined vesicle; *d* prostatic gland duct; *dc* degenerative cell; *di* dictyosome; *e* epidermis; *ed* ejaculatory duct; *g* prostatic gland cell; *h* hemidesmosome; *i* intercellular matrix; *im* internal muscle; *in* intestine; *l* lumen of male canal; *lm* longitudinal muscle; *m* matrix syncytium; *mc* male canal epithelial cell; *mi* microfilaments; *mt* microtubules; *mu* muscle cell; *mv* microvilli; *n* nucleus; *np* nerve process; *ns* neurosecretory (?) granule; *p* prostatic vesicle; *pv* prostatic part of combined vesicle; *r* rootlet; *s* stylet; *sm* stylet material; *sp* sperm; *sv* seminal part of combined vesicle