Zoomorphologie 91, 101–118 (1978)

Zoomorphologie

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Ultrastructure of the Genital Organs in Interstitial Polychaetes

I. Structure, Development, and Function of the Copulatory Stylets in *Microphthalmus* cf. *listensis*

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Summary. The paired copulatory stylets of the hermaphroditic *Microphthalmus* cf. *listensis* are hard cone-shaped tubes with a syringe-like distal opening and a cuff-like lower edge and surround the external openings of the two ejaculatory ducts. They each lie in a deeply invaginated epidermal fold and are attached basally to an elongated muscle bulb, which is composed of a number of disc-like muscle cells. A prominent gland is situated behind the stylets. Transfer of sperm into the partner occurs probably by mechanical hypodermal injection. Hereby, the epidermal folds are protruded as small sacks, pulling out the stylets. The development of the entire male genital apparatus occurs in autumn when the animals have about 16 setigerous segments. During this differentiation, two elongated papillae arise. They consist of various well defined cells, some of which border a central ciliated lumen. The stylet tubes arise by transformation of the at first normal cuticle of these papillae into a hard electron-dense wall.

Zusammenfassung. Die beiden paarigen Kopulationsstilette des zwittrigen *Microphthalmus* cf. *listensis* sind feste, tütenförmige Röhren mit einer spitzen distalen Öffnung und einer manschettenförmigen unteren Kante. Sie liegen in je einer tief in den Körper hineinziehenden Epidermisfalte und umgeben die Endabschnitte der beiden Ductus ejaculatorii. Basal sind sie an je einem länglichen Muskelkörper befestigt, der aus scheibenförmigen Elementen zusammengesetzt ist. Hinter dem Stilett befindet sich eine Drüse. Das Sperma wird wahrscheinlich mechanisch hypodermal in den Geschlechtspartner injiziert. Die Epidermisfalten sind hierbei sackförmig ausgestülpt und die Stilette dabei nach außen gezogen. Die Entwicklung des gesamten männlichen Geschlechtsapparates erfolgt im Herbst, wenn die Tiere ungefähr 16 Borstensegmente besitzen. Hierbei bilden sich im 3. Borstensegment zwei längliche Papillen. Sie bestehen aus verschiedenen, gut zu unterscheidenden Zellen, von denen einige um ein zentrales bewimpertes Lumen angeordnet sind. Durch Umwandlung der zunächst normalen Kutikula dieser Papille zu einer elektronendichten festen Wand entsteht die Stilettröhre.

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A. Introduction

Hard stylets that serve as transfer organs during copulation are widely distributed in soft-bodied animals practicing a direct sperm transfer. In polychaetes, where fertilization is generally external, these stylets are rare, even in those genera having internal insemination. Fine structure and development of the stylets are completely unknown. An ultrastructural investigation, as well as a comparison with similar hard structures in other invertebrate groups, is thus considered desirable. A better understanding of sperm transfer in these species may also be achieved by this structural analysis.

B. Material and Methods

A *Microphthalmus* species (length about 1.5 mm) from North Carolina, USA (exposed beach near low tide level on the SW end of Bogue Banks) was investigated. On the basis of fixed immature material this species was at first thought to be identical with, or a subspecies of, the European *Microphthalmus listensis* Westheide (Westheide, 1977). In addition to slight differences in size, pigmentation, and ciliation between the European and North American forms, differences in the size and form of the copulatory stylet are also present (see also Rieger and Ruppert, 1978). This character has, for example in the Turbellaria, proven to be very constant, even in geographically distant populations of a species (Rieger, 1977) and is considered a very useful taxonomic feature of this group. Nevertheless, the polychaete species investigated here will, for the present, be referred to as *Microphthalmus* cf. *listensis*, with regard to a future taxonomic revision. This report will then contain an extensive comparison of the two amphiatlantic populations, including biological, ecological, and ultrastructural investigations.

Two fixatives were used in this study. For both procedures the animals were first relaxed for 10–15 min in a $MgCl_2$ solution isotonic to sea water. Fixation 1 started with 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer (pH 7.3, 10% sucrose and traces of $CaCl_2$) at 4° C for 2 h. Specimens were rinsed overnight in buffer, which was changed 5 times prior to postfixation in 1% phosphate-buffered osmium tetroxide at 0° C.

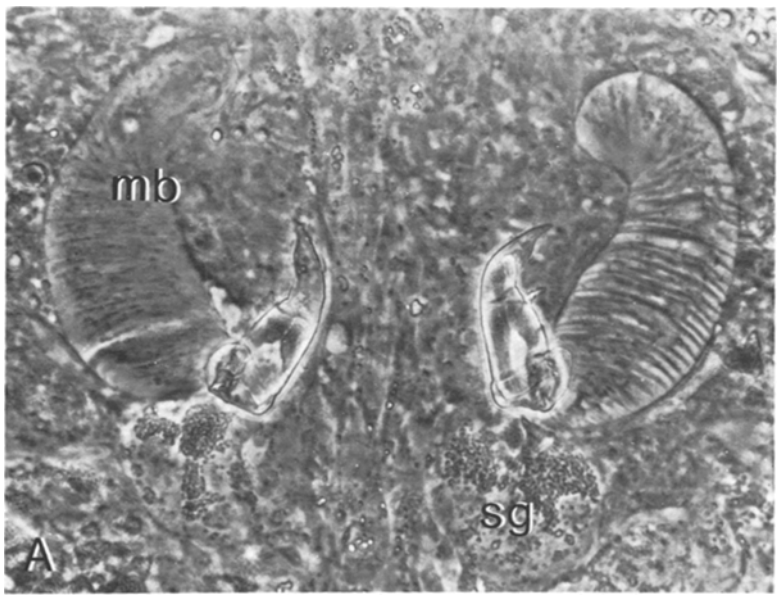
Fixation 2 followed the procedure used by Ermak and Eakin (1976) in a sucrose-picric acid-formaldehyde-glutaraldehyde solution at room temperature. Specimens were then rinsed for 2 h in phosphate buffer (buffer changed 5 times) and postfixed in phosphate-buffered 1% osmium tetroxide (0° C, for 1 h).

The animals were dehydrated in an ethanol series, embedded in an Epon-Araldite mixture, sectioned with a diamond knife on a Reichert OMU2 ultramicrotome and examined with a Zeiss EM9S2 electron microscope. Sections were stained with uranyl acetate and lead citrate. A part of the material originates from resin embedments of quantitative meiofauna samples, which were made available to me by Dr. R.M. Rieger (see Rieger and Ruppert, 1978).

C. Results

I. Light Microscopical Investigations

Microphthalmus cf. *listensis* is hermaphroditic, as are all *Microphthalmus*-species. The eight anterior setigerous segments are differentiated as male, the following ones as female. The complex male copulatory apparatus consists of a paired duct system extending from the second to the fourth or fifth setigerous segments. In structure they correspond to the organs of the European *M. listensis* (Westheide, 1967). The external openings of the two ducts are each surrounded



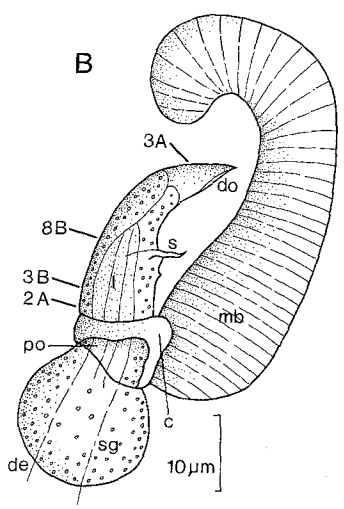


Fig. 1 A and B. Copulatory stylets and muscle bulbs.
A Phasecontrast photo of a living specimen.
B Drawing after squash preparation of a living specimen. The bars marked *2A, 3A, 3B, 8B* give the approximate levels of the cross-sections in the corresponding figures. *c* cuff, *de* ductus ejaculatorius, *do* distal opening, *l* lumen, *mb* muscle bulb, *po* proximal opening, *s* spur-like process, *sg* stylet gland

by a hard stylet (Fig. 1). They each lie in the third setiger in an epidermal fold that extends far into the body. In squash preparations the stylets resemble cone-shaped tubes. Their distal opening (*do*) is curved to the side of the body and resembles the tip of a syringe. The basal edge of the organ is turned up as a cuff (*c*) of unequal height. A small irregular spur (*s*) is found on the side of the tube. The stylets measure 30–35 µm from the base to the tip and are up to 13 µm wide at the base. Short ear-shaped extensions of the

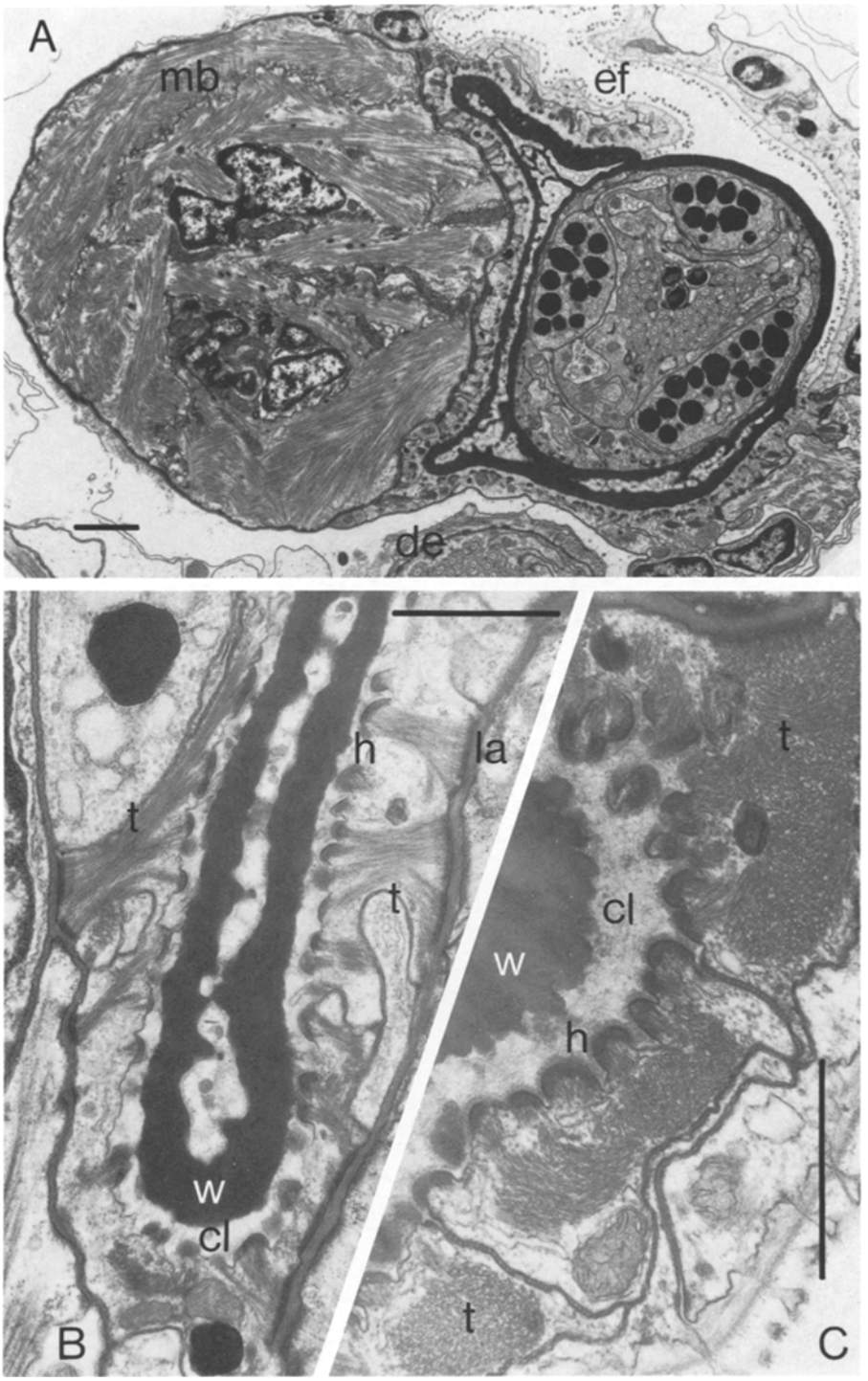


Fig. 2A–C. Basal part of the stylet. **A** Cross-section, stylet tube with cuff, ear-shaped extensions of which encompass the muscle bulb. Scale = 2 μm. **B** Fold of the cuff. Scale = 1 μm. **C** Lower edge of the stylet. Scale = 1 μm. **A**, **B**, **C** = Fixation 2. *cl* cuticle layer, *de* ductus ejaculatorius, *ef* epidermal fold, *h* hemidesmosome, *la* lamina surrounding the muscle bulb, *mb* muscle bulb, *t* tonofibrils *w* electron-dense wall of the cuff and the stylet

cuff encompass the caudal area of a long muscular organ, referred to from now on as the muscle bulb (*mb*). Its anterior area is bent more or less inwardly; the length varies from 58 to 78 µm, depending on the degree of contraction. The width also varies (13–25 µm), it is even thinner frontally. Processes from a prominent gland (*sg*) enter the stylet.

II. Fine Structure Investigations

Longitudinal and cross-sections verify that the stylet is a cylindrical hard structure composed of extracellular material (Figs. 2 and 3). The wall is electron-dense and, with the exception of the cuff, smooth (0.2–0.3 µm thick). It appears two-layered due to a fine median line (Fig. 3).

The edge of the cuff is fused at the side and top with the stylet tube. At the level of the cuff the tube builds folds which enter the ear-shaped extensions. The latter also prove to be folds, increasing the surface area with which the base of the stylet is in contact with the muscle bulb. A cuticle layer mostly free of fibrious material (about 0.1–0.3 µm thick) and an epidermal layer are situated between a basal lamina surrounding this muscle organ and the stylet wall. The epidermis is with 0.2 µm partly very thin (Fig. 2A and B). It is transversed here by numerous tonofibril bundles (*t*) which anchor the stylet to the muscle body. They originate at special cell junctions on the lamina of the muscle bulb and terminate in rounded hemidesmosomes which extend into that cuticle layer (Fig. 2). The hemidesmosomes often surround a microvillus arising from the epidermis. At the level of the lower edge of the stylet the tonofibril bundles are especially well developed and build an almost uniform fibrillar layer up to 0.4 µm in thickness (Fig. 2C). The spur-like extension halfway up the stylet (length about 2.5 µm) is an irregular slightly arched fold of the stylet wall protruding outward from the tube like a fingernail (Fig. 8B).

The inside of the stylet tube is filled with elongated cells whose nuclei lie outside or at its base. (Details will be given in the section on the development of the stylet.) The ejaculatory duct enters the center of the tube. Its lumen (1.5–3.5 µm) is surrounded by microvillus-like cell extensions and is filled with cilia (*c*) (Figs. 2A and 3). Cross-sections of about 38 cilia are found at the base, distally fewer are seen. Characteristic for the fully differentiated stylet are the processes of three (in one case four) gland cells (*cg*) that compress and deform the remaining cellular elements (Figs. 2A and 3). Their apical areas completely fill the distal part of the stylet and protrude out of the stylet opening into the epidermal fold (Fig. 3A). Fixation enhances this process, but it is generally not a fixation artefact since it was also observed in living individuals. As a result, parts of the cells originally lying within the stylet are pushed to the exterior and destroyed. Their remains (*sc*) fill part of the epidermal fold together with the gland cell cytoplasm.

The proximal parts of the three gland cells lie close to each other and form the pouch-like stylet gland (*sg*). It is surrounded by a lamella and hangs into the body cavity which is filled with mesenchyme. It is about 14 µm wide (Fig. 4A). A small muscle inserts at the caudal end of the gland. The nuclei

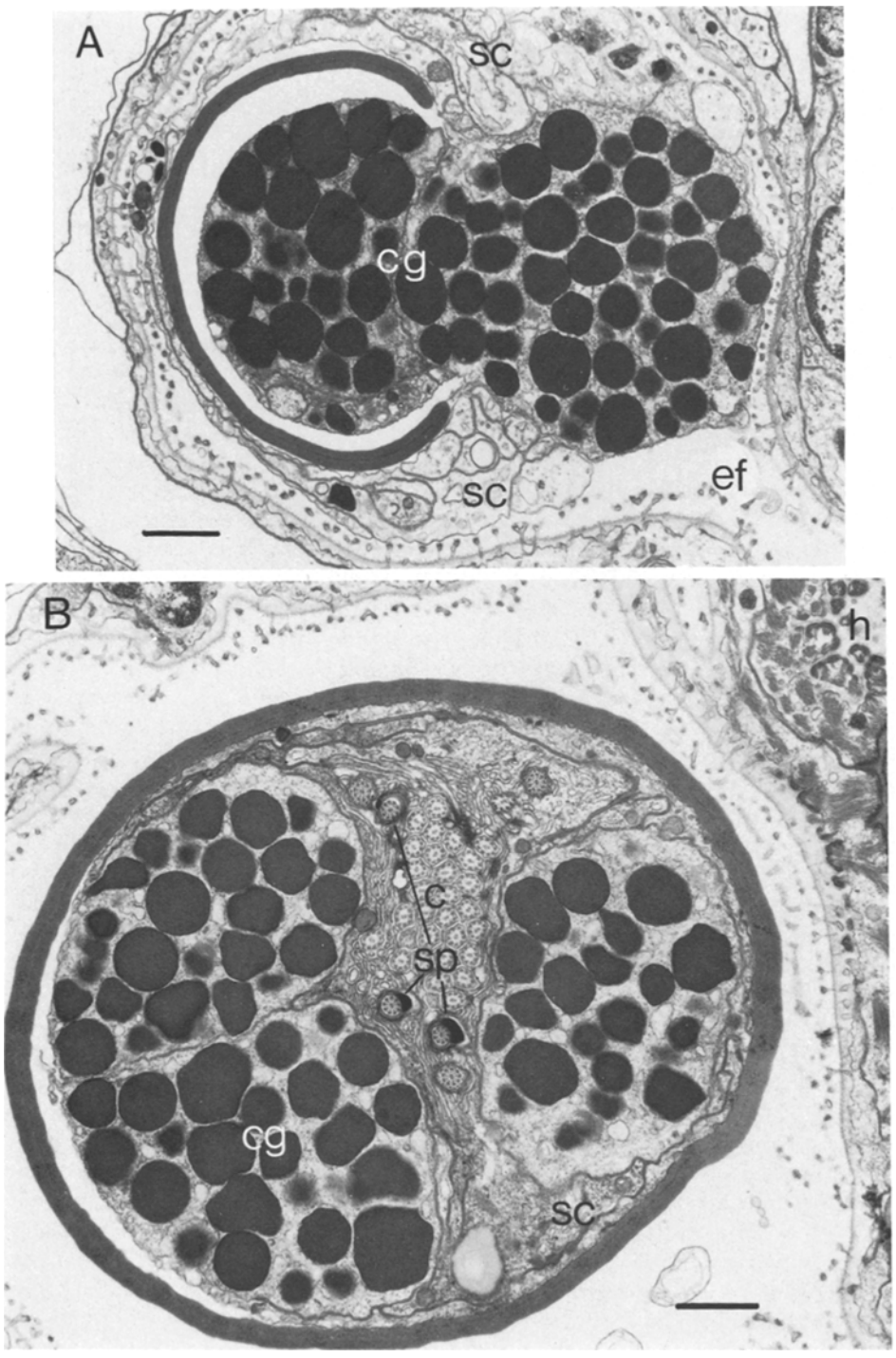


Fig. 3A and B. Stylet, cross-sections. **A** Level of the distal opening. **B** Level just above the cuff. Scale for **A**, **B** = 1 μm. **A**, **B** = Fixation 2. *c* cilia of the lumen, *cg* cytoplasm with secretory granules of the stylet gland cell processes, *ef* epidermal fold, *h* hemidesmosomes, *sc* stylet cells and parts of the cells originally lying within the stylet, which are pushed out by the gland cell processes, *sp* sperm in the ciliated lumen

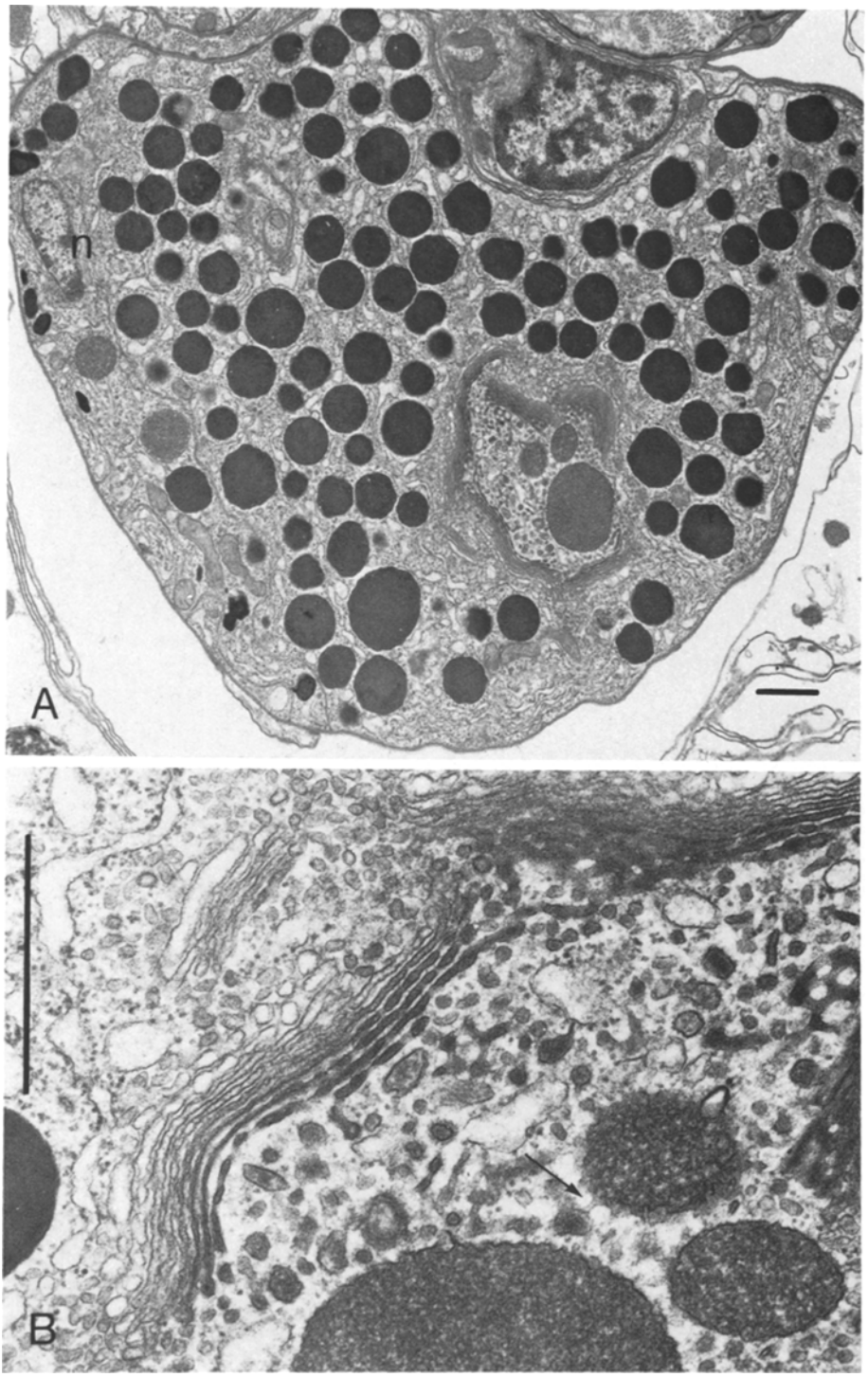


Fig. 4A and B. Stylet gland. **A** Cross-section of the entire gland. **B** Part of the Golgi complex. Scale for **A**, **B** = 1 μm. **A**, **B** = Fixation 2. *n* nucleus. *Arrow* indicates a connection between vesicle and centrally located spherical mass

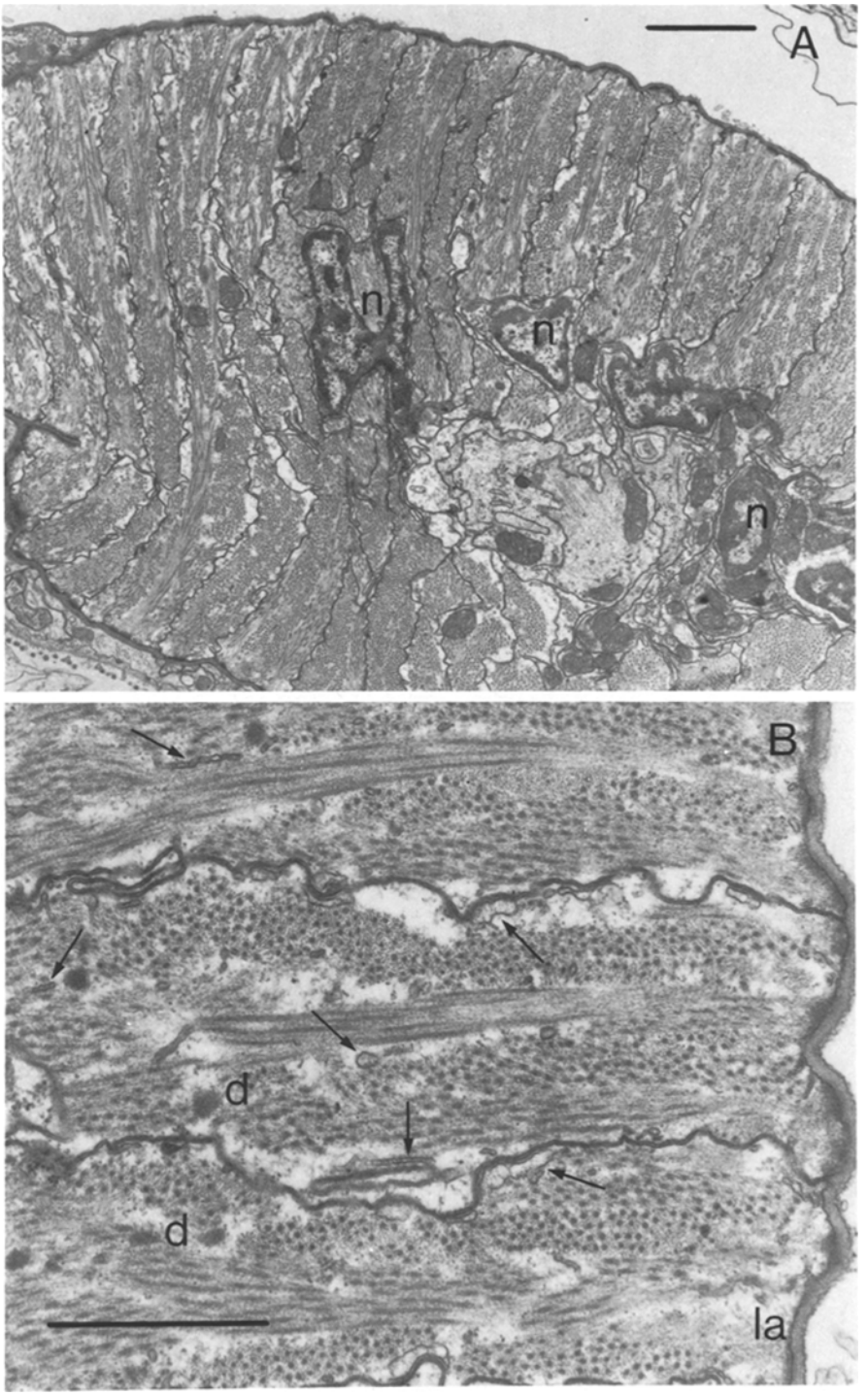


Fig. 5 A and B. Muscle bulb, sagittal sections. Scale for **A** = 2 μm, for **B** = 1 μm. **A**, **B** = Fixation 2. *d* dot, *la* outer lamina, *n* nucleus. *Arrows* indicate tubules and vesicles of the sarcoplasmic reticulum

of the individual gland cells are at least 4.5 μm in diameter and irregularly lobed. Granular endoplasmic reticulum lies mainly in the periphery. Mitochondria of the cristae type are scattered. Dictyosomes are grouped into a globe- or egg-shaped Golgi complex in the center of the cell with a diameter of up to 5 μm. A network of globular and tubular vesicles originates from the cisternae (Fig. 4B). They concentrate their contents into several large centrally located spherical masses with a spongy gray structure. The largest formations of this type are surrounded by a membrane and most probably give rise to the numerous secretory granules which are situated throughout the cytoplasm, especially in the direction of the stylet. Their contents are, however, completely electron-dense (diameter 0.4 to 0.75 μm).

The muscle bulbs lie somewhat oblique to the longitudinal axis of the body trunk. They consist of vertically arranged, round, disc-shaped elements (Figs. 2A, 5, and 6). At least 33 of them are present, lying behind one another like a roll of coins. The height of each disc is about 1 μm. Those lying frontally are thicker and formed somewhat differently. The diameter after fixation varies between 9 and 18 μm. The whole organ is surrounded by a 50–100 nm thick lamina (*la*) which has already been mentioned. The myofilaments do not traverse the whole width of the cell or run in the same direction; rather, they form groups of crossing chords in the cell discs. Thus cross-sections distantly resemble turbine wheels (Fig. 6A). Such sections cut all filaments longitudinally. In sagittal sections, however, groups of filaments are cut transversely or, over short distances, longitudinally (Fig. 5). Each disc contains several such groups lying next to each other. Their number is probably not constant in the individual discs. Cross-sectioned groups show thick filaments surrounded by thinner ones; possibly only the thin filaments are attached to the inside of the outer lamina of the muscle bulb. It cannot yet be determined, if each disc possesses one nucleus (*n*) and if each disc is a single cell. The nuclei generally lie in the center of the muscle bulb. They are irregularly lobed and broader than the height of the discs at the periphery. So the discs bulge in the middle, meshing here with the neighboring ones (Fig. 5A). The mitochondria also are usually situated in the center. Only a few electron-dense dots (*d*) can be found between the filaments (Fig. 5B). Possibly they correspond to the dots that Rieger and Rieger (1975) found in the muscle bulbus of *Trilobodrilus* and which they believed to be homologous with the bars of the Z-system. No regularity in the arrangement of these dots can yet be determined. A sarcoplasmic reticulum system consisting of very narrow tubules and small vesicles is present. The latter are limited to the periphery of the discs, whereas the tubules occur next to the basal lamina as well as between groups of filaments (Fig. 5B). Nerve terminals reach the surrounding basal lamina of the muscle bulb at various places. A muscle anchors the organ frontally to the outer muscle layer of the body trunk (Fig. 6B).

III. Development of the Stylet

The anlagen of the entire duct system including the stylets are formed in autumn.

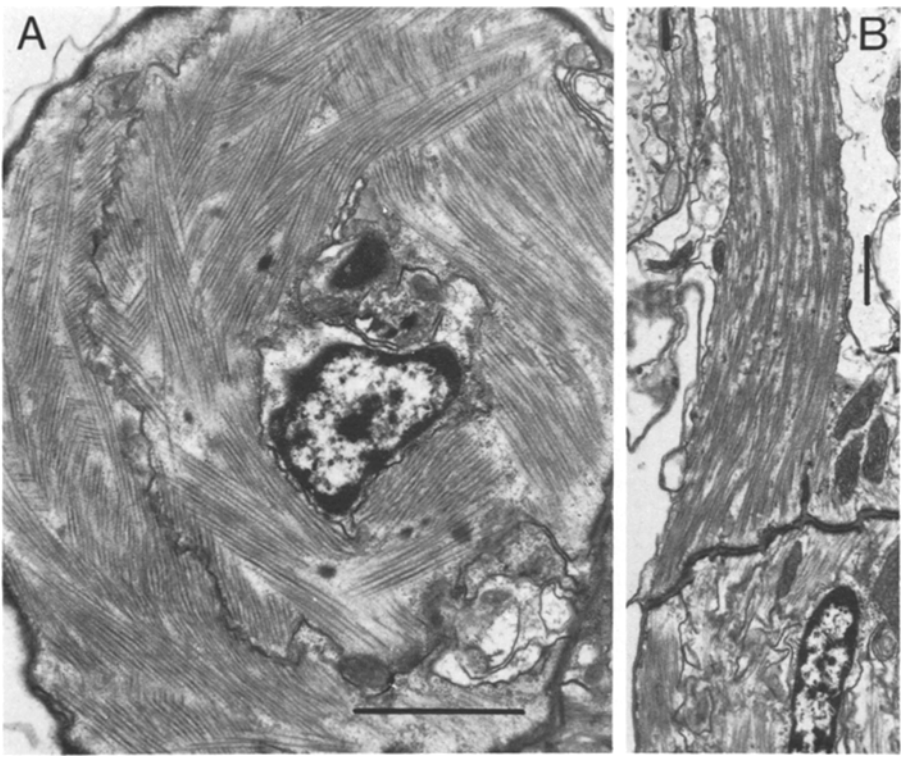


Fig. 6A and B. Muscle body. **A** Transverse section with two disc-like cells. Scale = 1 µm. **B** Frontal part with muscle that anchors the organ. Scale = 2 µm. **A** = Fixation 2, **B** = Fixation 1

The time at which differentiation occurs is probably dependent at first on the number of setigers, i.e., on the age of the animals. A population of *M.* cf. *listensis* from Bogue Banks had an average number of setigerous segments of $\bar{x} = 16.2$ (SD = 1.3, $n = 44$) on November 6, 1976. At this time only animals with 16 or more fully developed setigers possessed fully differentiated copulatory organs with stylets and gland ducts. In animals with 15 or less segments only the anlagen of the copulatory organs, at most, could be recognized. Already at the beginning of December some individuals showed a distinct regression of their male organs, especially in the reduction of the glands ($\bar{x} = 16.4$ setigers, SD = 2.3, $n = 29$). On January 3, 1977 ($\bar{x} = 17.0$, SD = 0.9, $n = 23$), and on January 25, 1977 ($\bar{x} = 16.6$, SD = 0.8, $n = 14$), a third of the individuals had already passed their male maturation phase, but the stylets were still clearly recognizable. At this time even those animals with less than 16 segments had reached sexual maturity. In the following months the population consisted of adult organisms with completely reduced male organs, including the stylets, as well as an increasing number of young animals from a new generation.

Rieger and Ruppert (1978) showed a developing stylet of this species in a sac-like invagination of the epidermis. Our ultrastructural investigations confirm this. A local proliferation of epidermal cells is situated in the dorso-lateral

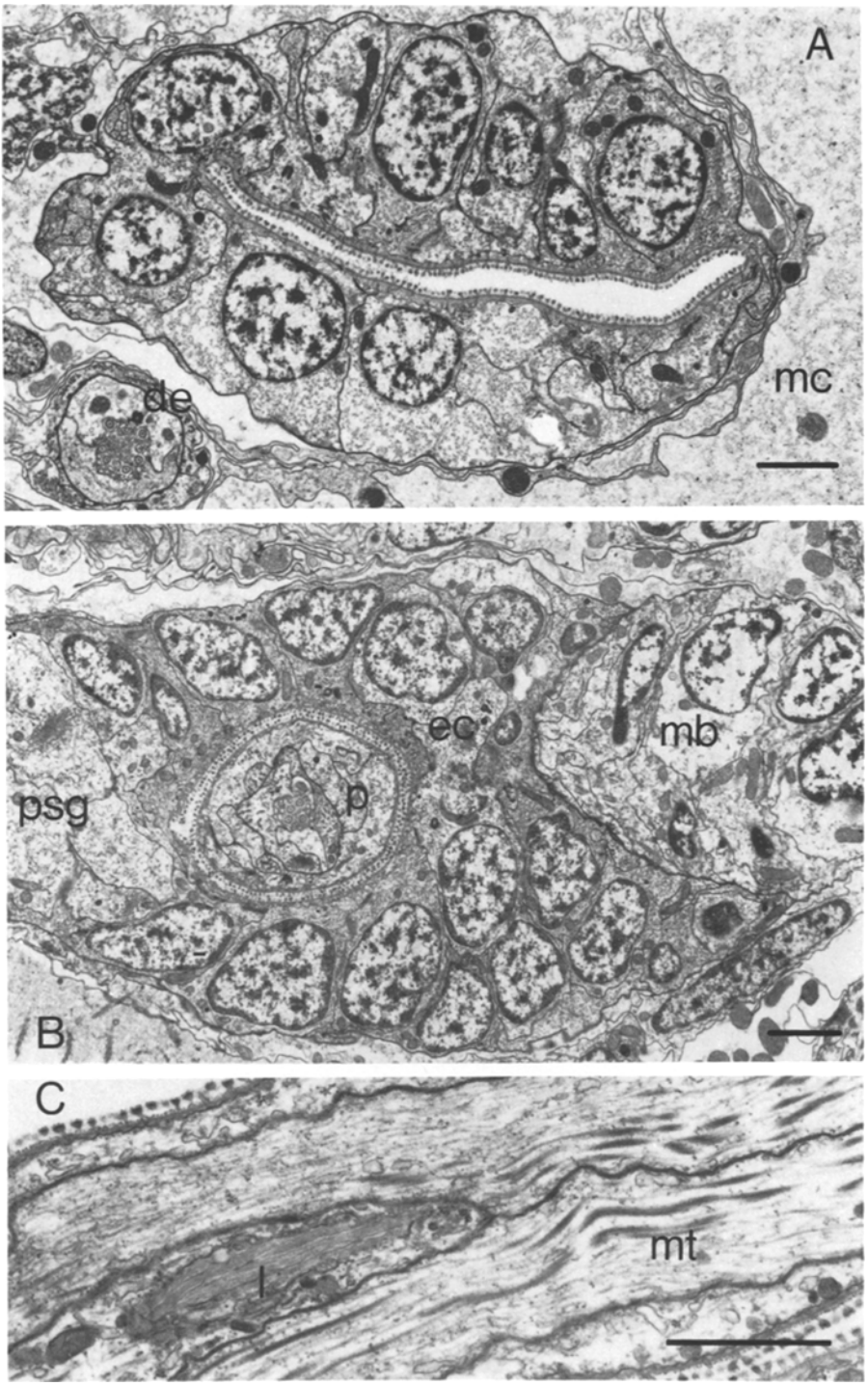


Fig. 7A–C. Development of the stylet. **A** Cross-section of the epidermal fold in the third setigerous segment, early stage. **B** Later stage, cross-section of the papilla. **C** Distal part of the papilla, tangential-section. Scale for **A**, **B**, **C** = 2 μm. **A** = specimen that has been cut out from a quantitative meiofauna sample slide (see Rieger and Ruppert, 1978), **B**, **C** = Fixation 1. *de* ductus ejaculatorius, not fully differentiated, *ec* cells of the epidermal fold, epidermal layer, *l* central lumen, *mb* anlagen of the muscle bulb, *mc* mesemchyme cell, *mt* microtubules in the presumptive gland cells, *p* papilla, *psg* presumptive cells of the stylet gland

area, anterior to the parapodia of the third setigerous segment. From here a deep narrow fold sinks into the body, running ventrally at first, then caudally parallel to the longitudinal axis of the body (Fig. 7A). A round elongate papilla, bent slightly outward at the apex, arises from the floor of this fold at a later stage of differentiation. It almost completely fills the lumen in the basal part of the fold (Figs. 7B and 8A). This epidermal fold is surrounded by an epithelial layer whose dark cytoplasm contrasts with that ot the much larger mesenchymal cells occupying the space between intestine and body wall. Furthermore, a thin basal lamina separates the mesenchyme from the epidermis. Ventral to the papilla the cytoplasm of several epidermal cells (*psg*) is much lighter (Fig. 7B). These cells also possess a centrally located Golgi complex. According to their position, they are most likely the future cells of the stylet gland. A group of larger cells (Fig. 7B, *mb*) lies dorso-laterally to the fold. They are separated distally from the epidermal cells and surrounded by a prominent basal lamina. The muscle bulb develops from these larger cells which probably arise from the mesenchyme, rather than the epidermis.

The papilla itself contains elongate, more or less arranged in parallel and interdigitated cell bodies (Figs. 7B, C, 8A, and 10). Only processes from the presumptive stylet gland cells and from two epidermal cells (*ap*) extend to the tip of the papilla. The latter form the peripheral cover of its distal part. At this time the apical areas of these cell processes contain numerous microtubules. In the presumptive gland cells they lie close together and are oriented parallel to the longitudinal axis of the papilla (diameter about 200 Å) (Fig. 7C). The occurrence of microtubules in cells undergoing differentiation is well documented. In general it is assumed that these organelles are involved in the production and maintenance of cell form (Tilney, 1971). Perhaps they also play a role here in the migration of the granules to the cell apex. The papilla does not possess a distal opening; the central lumen ends distally beneath the gland processes. Here it is surrounded by two elongate cells (*cc*). Toward the base of the papilla two additional cells push between the apical cells and the lumen, so that here it is also bordered by only two cells (Fig. 10A). At the base of the papilla the outer layer is also composed of two or three additional epidermal cell processes. Thus, cross-sections at this level show four or five peripheral cells, four central cells (usually only two of them enclose the ciliated lumen) and, between the outer and the inner cells, the three or four spindle-shaped sections of the future gland cells. The ductus ejaculatorius proper (*de*) is not fully differentiated at this time; it joins the base of the papilla and consists only of small short cells surrounded by muscle cells.

In the early stages the cuticle of the papilla does not differ substantially from that of the fold. In both areas the epicuticle and the fibrous layer of the basal cuticle are only slightly differentiated (Figs. 7B, C and 8A). The numerous microvilli are distally branched and somewhat shorter than on other areas of the body wall (Westheide and Rieger, 1978). Slightly older developmental stages, however, already show clear deviations (Fig. 8C). The fibrous layer of the papillary cuticle has become more dense and will give rise to the smooth completely elctrondense wall of the stylet. Microvilli no longer penetrate the electrondense layer, but at first their distal swellings still lie outside the wall

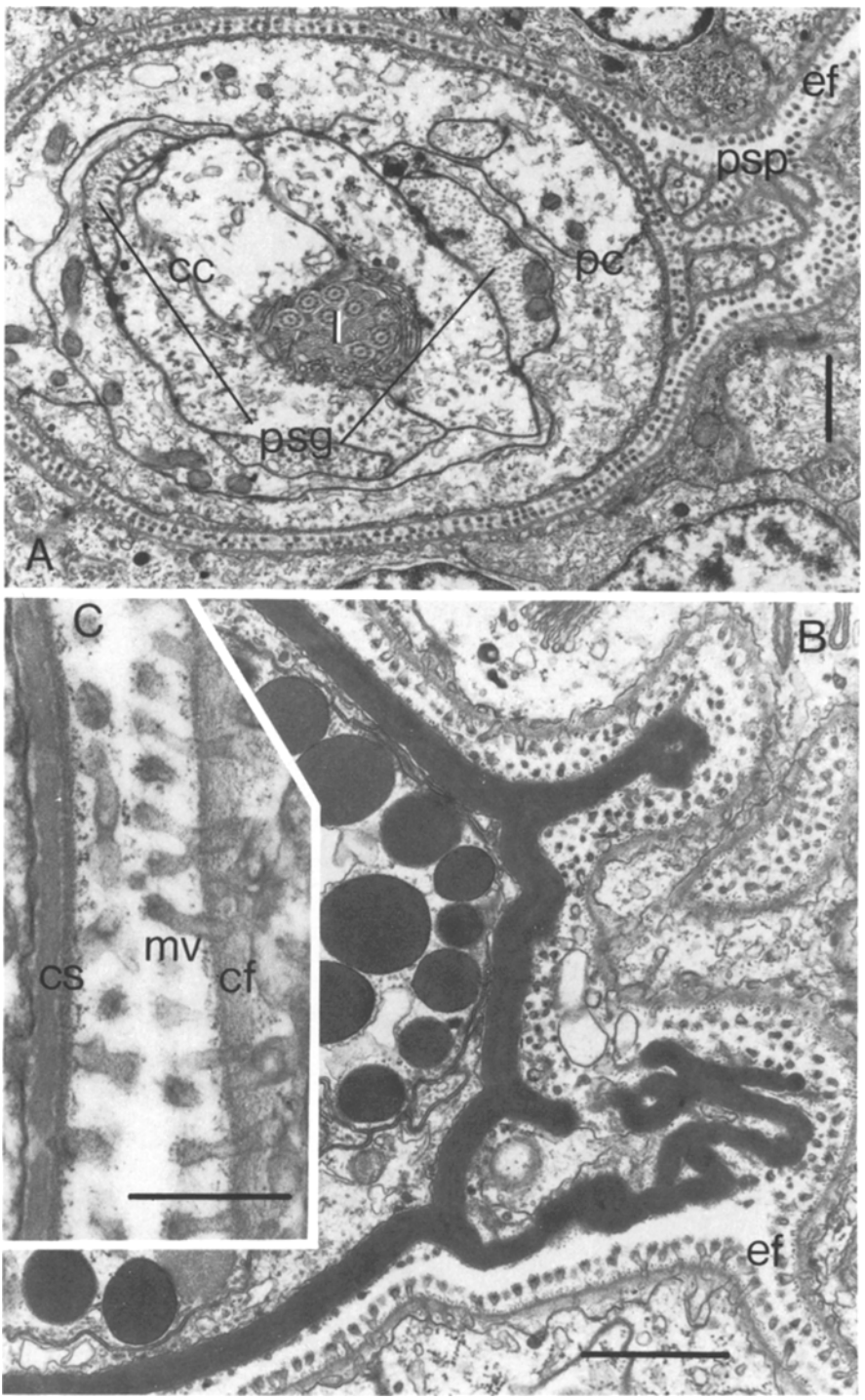


Fig. 8A–C. Development of the stylet. **A** Papilla, cross-section at mid-level. **B** Stylet, cross-section at the level of the spur-like process. Wall completely differentiated, but with microvilli swellings still on the outside. **C** Transformation stage of the papilla cuticle. Scale for **A**, **B**, **C** = 1 μm. **A**, **B** = Fixation 1, **C** = specimen from a quantitative meiofauna sample slide (see Rieger and Ruppert, 1978). *cc* central stylet cells, *cf* cuticle of the fold, *cs* cuticle of the stylet, *ef* epidermal fold. *l* ciliated lumen, *mv* microvillus, *pc* peripheral stylet cells, *psg* presumptive stylet gland cells, *psp* presumptive spur-like process (fold of the papilla cuticle)

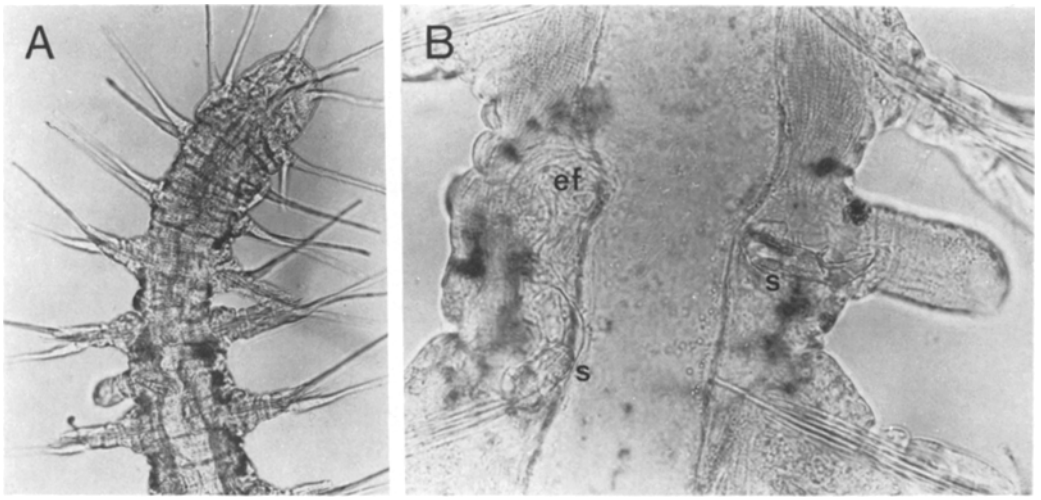


Fig. 9A and B. Protruding epidermal fold after sperm transfer. Note the different positions of the stylets in **B**. On the right side it is pulled to the front, turning to the outside at about 90°; the tip of the stylet is pointed forward. *ef* epidermal fold, *s* stylet

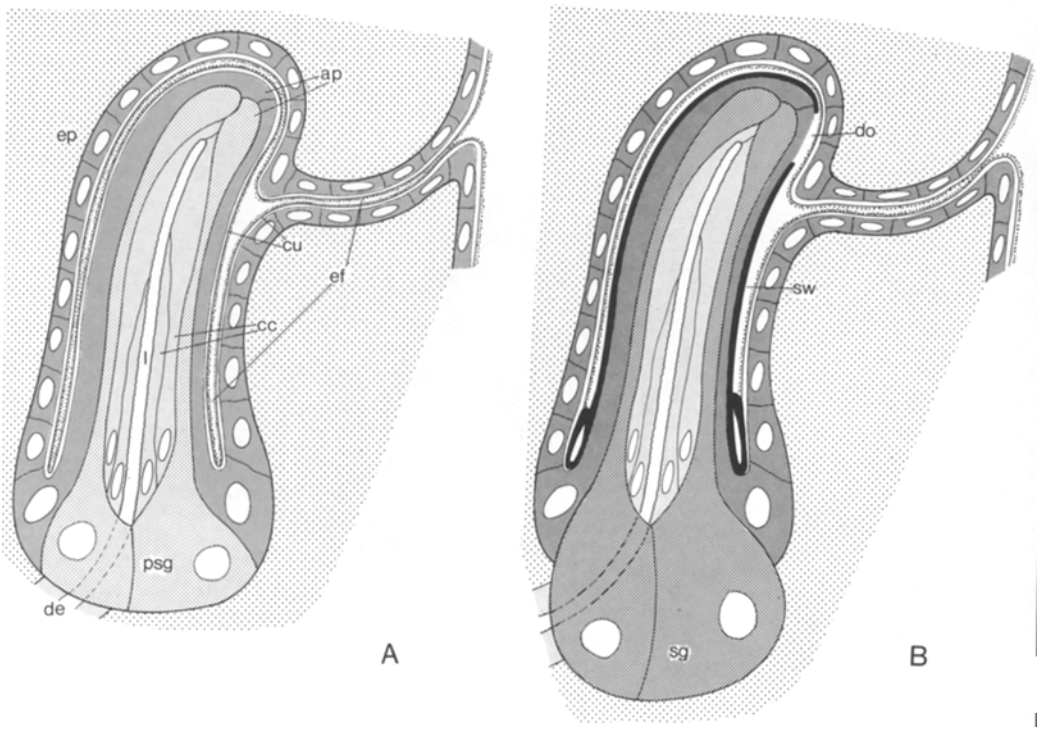


Fig. 10. A Reconstruction of the epidermal fold with papilla. **B** Transformation of the papilla cuticle into the stylet wall. Very schematic. *ap* cell apex of the peripheral stylet cells and the stylet gland cells, *cc* central stylet cells, *cu* cuticle, *ef* epidermal fold, *ep* epidermal layer, *de* ductus ejaculatorius, *do* distal opening, *psg* presumptive stylet gland cells, *sg* stylet gland, *sw* stylet wall

(Fig. 8B). They are totally absent from the completely differentiated stylet (Fig. 3). Only in the region of the subdistal opening of the stylet, where the cuticle does not harden, is the developmental process different. Perhaps the two layers of the stylet wall originate from different zones of the normal cuticle: epicuticle – fibrous layer of the basal cuticle or fibrous layer – zone of the basal cuticle free of fibrous material (see Westheide and Rieger, 1978). The cuff arises from the transformation of an area of cuticle of the epidermal fold near the base of the papilla into an electron-dense layer (Fig. 10). In addition to the outer roughness mentioned previously, this part of the stylet shows two other peculiarities. The wall is not two-layered and the cuticle is not fully transformed, i.e., a basal layer remains and is firmly bound to the electron-dense wall (Fig. 2B). In contrast, in the interior of the stylet tube, the cell walls directly border the hard structure and are not fused with it. Thus these cells can be easily deformed and pressed out of the tube, as mentioned earlier.

IV. Function

Sexually mature animals were observed during copulation in culture dishes. Two, or in a few cases several, animals entwine closely. Thus, the use of the stylets cannot be observed. Following such mating activity, the animals occasionally can be seen with a protruding genital fold on one or both sides of the body (Fig. 9). These protuberances hang like small sacks between the second and third setigers. Figure 9B, for example, suggests that the genital fold functions in pulling the stylet out of the body. The entire structure of the stylet indicates that it penetrates the partner during mating. The sperm are transported into the stylet by contractions of the muscles surrounding the ejaculatory duct and the numerous cilia of the duct lumen. (Entry of sperm into the stylet was observed on a suitable squash preparation.) The injection of a part of the glandular cytoplasm containing the secretory granules (see Fig. 3A) or the release of the secretion probably occurs at the moment of transfer. Injected sperm were observed beneath the epidermis, in the mesenchyme, and directly on the oocytes in the female region of two animals (see also Rieger and Ruppert, 1978; Fig. 4H and J). All that has been observed about the function of the muscle bulbs is that they sometimes contract in squash preparations. This results in a flap-like horizontal movement of the stylet. During mating a similar process may occur; the muscle bulbs, which are pulled out with the protrusion of the epidermal folds, contract and press the stylets against the epidermis of the partner.

D. Discussion

Female genital pores that function in the uptake of the sperm from the sexual partner have not been found in *Microphthalmus* cf. *listensis* (see also Westheide, 1967, for the European *M. listensis*). All available observations on the reproductive biology of the species indicate that sperm transfer occurs by hypodermal

injection. Only very few, though well-documented, examples of this reproductive mechanism are found within the polychaetes (Schroeder and Hermans, 1975). For example, the epidermis is opened histolytically at nearly any location in the dinophilids *Dinophilus* and *Trilobodrilus* (Jägersten, 1944; Traut, 1966; Ax, 1968; Schmidt and Westheide, 1972; Westheide and Schmidt, 1974); a mechanical perforation of the epidermis probably occurs in the histriobdellids (Shearer, 1910). In the species under investigation here, a mechanical penetration using the syringe-like stylet is most probable. It is not possible to exclude completely the additional participation of histolytically active secretions (stylet gland?). However, various accessory sex glands with different functions are also found in species with normal sperm transfer through female genital pores (Adiyodi and Adiyodi, 1975).

Copulatory stylets are rare within the polychaetes. Stiff penes were described by Bobretzky (1880) for *Microphthalmus similis* and *M. fragilis*. A species close to *M. similis* from North Carolina has each male genital papilla stiffened by 8 hard rods. However, fine structure and development (intracellular!) differ totally from those of the species described in this report (Westheide, in preparation). The two partially protrusible penes in *M. urofimbriatus* are supported by '4–5 small cuticular rods resembling setae' (Alikunhi, 1948). Genital papillae with a type of hard narrow tube were found in a Brazilian *Microphthalmus* (Westheide, 1974). The corresponding organs in the Histriobdellidae greatly resemble the *listensis* stylet. Haswell (1900) and Lang (1950) described and figured the unpaired penis stylet in *Stratiodrilus tasmanicus* Haswell and *S. platensis* Cordero as a hollow, black, 'chitinous' thorn-like structure lying in a fold of the integument. The corresponding organ in *Histriobdella* is a firm, semi-solid, pear-shaped body composed of two lateral blades with a median canal between them (Shearer, 1910). Finally, several species within the Saccocirridae have penis papillae which are stiffened by differing types of stylet rods: *Saccocirrus major* (Hempelmann, 1912), *S. minor* (Aiyar and Alikunhi, 1944), *S. pussicus* (Du Bois-Reymond Marcus, 1948), *S. heterochaetus* (Jouin, 1975).

Stylets are frequently found in aquatic oligochaetes of the family Tubificidae (Brinkhurst and Jamieson, 1971). They are described as tube-like 'chitinous' penis sheaths in the genera *Tubifex, Limnodrilus, Psammoryctides,* and *Peloscolex*. In form and position they resemble the stylet described in this paper.

Certain similarities exist between the stylets of *M.* cf. *listensis* and the unpaired copulatory organ of the Gnathostomulida. Mainitz (1977) described the stylet sheath in the latter group as most likely an extracellular secretion product that might be called a 'cuticular structure'. She states that it may act as an injection tube during sperm transfer. How the stylet develops in Turbellaria is known only for a few species, despite its being characteristic of the group. In *Paratomella rubra* Rieger and Ott (Acoela) the stylet is composed of 11 rod-like intracellular hard structures (Mainitz, 1977) (8 to 12 according to Crezée, 1978). The copulatory organ in *Macrostomum*—a tubular funnel-shaped 'hard' stylet—has also been identified as an intracellular structure (Doe, 1977). So far, hard structures within the Platyhelminthes have been identified as derivatives of the basement lamina or as elaborations of the outer part of an epithelium or epidermis (Rieger and Doe, 1975; Doe, 1976). Evidence for true cuticular

structures is still lacking in this phylum. The scalids of kinorhynchs, the stomal armature of nematodes, gnathostomulids and rotifers and the spines and hooks in gastrotrichs are hard structures derived from a true cuticle (see discussion in Rieger and Doe, 1975). Thus they best correspond to the polychaete stylet herein described.

Neither the structure nor the function of the muscle bulb, which is intimately associated with the stylet, has been completely elucidated. Muscular organs associated with the male genital openings in *Microphthalmus sczelkowii* and in *M. arenarius* were considered to be suctorial organs (Westheide, 1967, 1973). A large bulbus at the base of the genital papillae in *M.* cf. *similis* shows similarities to the muscle bulb described here. Perhaps contraction is not the only function of the muscle organ. Its specific construction of muscle discs packed one behind the other somewhat resembles the structure of the chorda dorsalis in *Branchiostoma* (Welsch, 1968), even though the direction and arrangement of the filaments differ greatly. Therefore, it also seems plausible to ascribe a supportive function to the muscle bulb of *M.* cf. *listensis,* possibly serving as a resistance when the stylet penetrates the epidermis of the mate. A similar structure consisting of muscle plates has been found in the dinophilid pharynx bulbus (Rieger and Rieger, 1975). It can be everted as a type of tongue in order to scrape food from sand grains (Jennings and Gelder, 1969). The principle of construction involved in assembling disc-like muscle cells one behind the other is also utilized here in the formation of an organ requiring both elasticity and firmness.

Acknowledgements. I am indebted to the hospitality of the Department of Zoology, University of North Carolina, Chapel Hill, USA, where this work has been carried out. In particular I am grateful to my friends Drs. Reinhard M. Rieger and Gunde Rieger, who encouraged this study with continuous support and valuable discussions. I am also indebted to Dr. David A. Doe, Wilma Hanton, and Dorothea Bürger for technical assistance. Assistance in translation by Dr. Christel Marschall and Stephen L. Gardiner is gratefully acknowledged.

Financial support was provided by the Stiftung Volkswagenwerk (Akademie-Stipendium) and the National Science Foundation (Grant GB 42221, R.M. Rieger, principal investigator).

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Received April 17, 1978