HELGOLÄNDER MEERESUNTERSUCHUNGEN
Helgoländer Meeresunters. 45, 465–485 (1991)

Ultrastructure and functional morphology of the female reproductive organs in *Protodrilus* (Polychaeta, Annelida)

Henning von Nordheim

Zoologisches Institut, Technische Universität Braunschweig; Postfach 3329, D-W-3300 Braunschweig, Federal Republic of Germany

ABSTRACT: The morphology and function of the female reproductive organs in 6 *Protodrilus* species are investigated by light- and transmission electron microscopy. Possible ways in which spermatozoa may enter the female coelom after leaving the spermatophore are discussed for species with and without special female reception organs. Only female *P. rubropharyngeus* and *P. flavocapitatus* have "dorsal organs" for spermatophore reception. The structure and function of these organs are described, as well as those of the oviduct found in 3 of the species investigated. The possible phylogenetic origin of gonoducts and different modes of oviposition within the genus are discussed. Finally, the high taxonomic significance of female traits such as dorsal organs, oviducts, cocoon glands and lateral ciliary rows in this genus is stressed.

INTRODUCTION

The gonochoric polychaete genus *Protodrilus* is relatively rich in species and has a global distribution. Since the discovery of *P. purpureus* off Helgoland by Schneider (1868), numerous further species have been described, the genus now comprising 30 species (von Nordheim, 1989a). Especially early descriptions of *Protodrilus* species were mainly based on traits such as body size, colour, ciliation, and body shape. When the inner organisation or sexual characters were also considered, this sometimes resulted in erroneous descriptions of the salivary glands, the coelomoducts, or the reproductive system.

Aiyar & Alikunhi (1944) were the first to notice the real extent of salivary glands and the position and number of sperm ducts and "lateral organs" in males. Jägersten (1952) presented a determination scheme for *Protodrilus* in which, for the first time, especially the segmental extension of salivary glands and gonads, as well as the number and position of reproductive organs, were shown to be of very high taxonomic importance, since they represent very species-specific traits. His ideas were confirmed by Jouin (1970a, b, 1971).

Despite the high taxonomic significance of the reproductive organs of *Protodrilus*, little is known about their morphology and function. Detailed light microscopical investigations were carried out by Salensky (1907) and Pierantoni (1908). These papers, however, do not contain explanations of the function of the "lateral organs", gonoducts,

"dorsal organs" or exact details about the animals' reproductive biology. The present paper aims to contribute to a better understanding of the structure and function of the reproductive system in female *Protodrilus* by light- and electron microscopical studies.

MATERIALS AND METHODS

The following *Protodrilus* species were investigated: *P. purpureus*, (Schneider 1868), from Helgoland, FRG and Kristineberg, S; *P. rubropharyngeus*, Jägersten 1940, from Kristineberg, S; from Weissenhaus, FRG; and from St. Abbs, GB; *P. ciliatus*, Jägersten 1952, from Helgoland, FRG and Kristineberg, S; *P. helgolandicus*, von Nordheim 1983, from Helgoland, FRG; *P. haurakiensis*, von Nordheim 1989, from Leigh, New Zealand; and *P. jägersteni*, von Nordheim 1989, from Leigh, New Zealand.

The animals were extracted from the sediment by using a decantation technique with 4 % aqueous magnesium chloride adjusted to ambient sea water salinity (for further methods see Higgins & Thiel, 1988). Anaesthetized animals were observed alive or were fixed for TEM in a mixture of saccharose, picric acid, formaldehyde and glutaraldehyde in a phosphate buffer (Ermak & Eakin, 1976). Best results were obtained with a saccharose content of 10 to 17 %. In *P. rubropharyngeus* from the Baltic Sea (Weissenhäuser Strand, Germany, salinity 8 ‰) no saccharose was added.

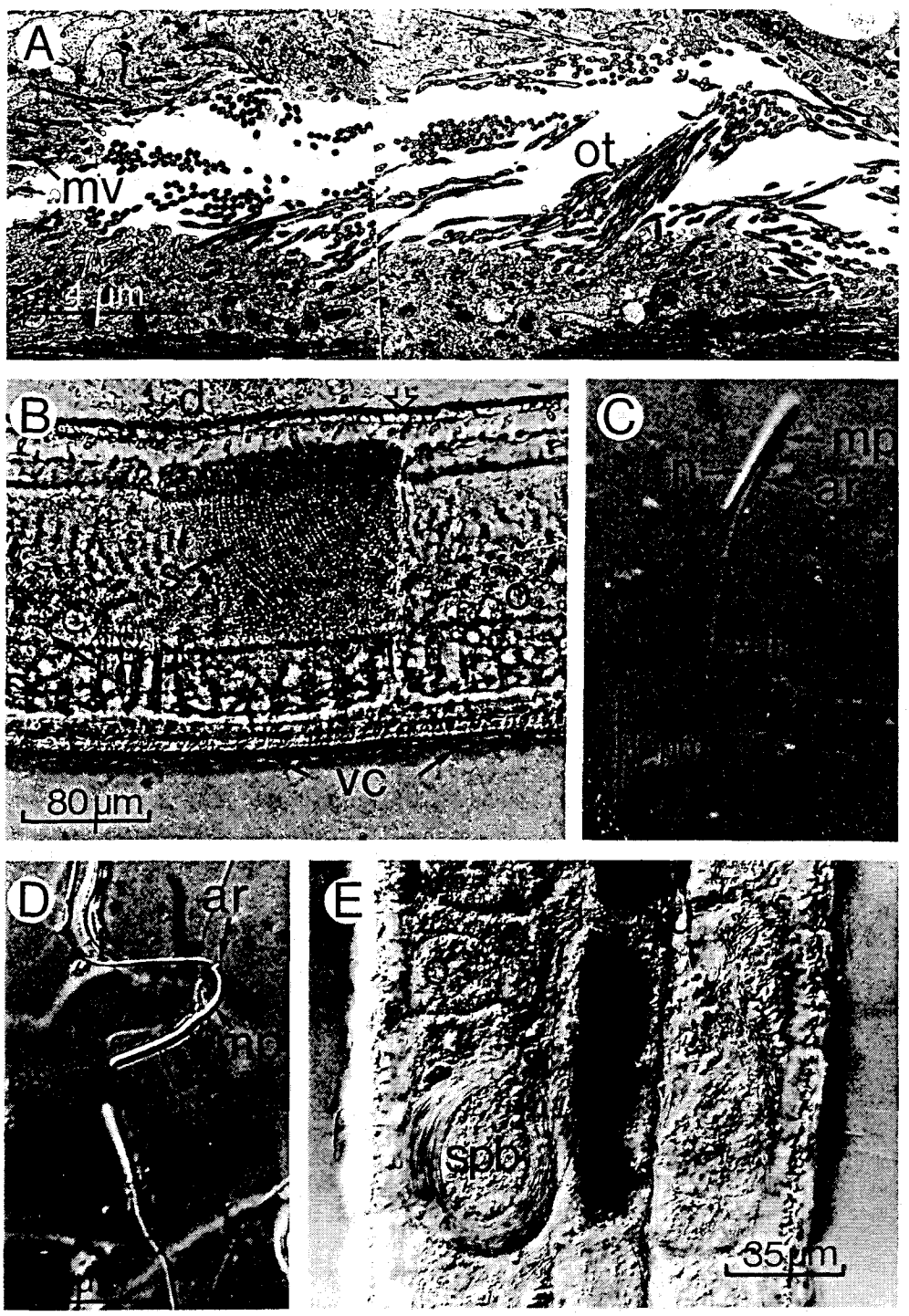
Following relaxation with a 4 % $MgCl_2$-solution for 20 min, fixation was carried out for 2 h at 4 °C with 2 to 3 renewals of fixation liquid. After rinsing for 2 to 3 h, including several renewals of 0.1 molar phosphate buffer (pH 7.3) and postfixation for 1 h at 0 °C with 1 % OsO_4-solution in phosphate buffer, the material was dehydrated in an ethanol series, transferred to propylenoxide and embedded in a five component Epon-Araldite resin.

Thin sections were made with a diamond knife on a Reichert Ultracut, stained with lead citrate and uranyl acetate in a LKB Ultrostainer, and investigated and photographed with a Zeiss EM 109.

Fig. 1. A: *Protodrilus purpureus*; female; longitudinal section of oviduct tube. B, C, D, E: Spermatozoa in females. B: *P. jägersteni*; bundle of euspermatozoa beneath penetration spot; ovaries, lateral view. C: *P. helgolandicus*; modified paraspermatozoon. D: *P. purpureus*; modified paraspermatozoa. E: *P. ciliatus*; bundle of euspermatozoa. Dorsal view

Abbreviations used in the figures

ar	anulus region	egc	external gland cell	ot	oviduct tube
bl	basal lamina	fa	formation area	ovd	oviduct
cc	coelomatic cell	ge	gut epithelium	ps	paraspermatozoon
ci	ciliary cell	gu	gut	s	sperm
coe	coelothelial cell	igc	internal gland cell	sb	secretion ball
cp	cell process	lm	longitudinal muscle	sh	sheath
cr	ciliary root	lg	lipid granule	shc	sheath cell
cu	cuticle	mes	mesenterium	sd	septate desmosome
d	dissepiment	mp	midpiece/-region	se	septum
dbv	dorsal blood vessel	mt	microtubules	spb	sperm bundle
dgc	dorsal gland cells	mv	microvilli	t	tail/-region
dia	diaphragm	n	nucleus	tm	transversal muscle
do	dorsal organ	o	ovary	vc	ventral ciliary band
dop	dorsal organ opening	of	oviduct funnel	za	zonula adhaerens
e	egg				


A
mv
ot
B
d
vc
80 µm
C
mp
ar
D
ar
no
E
spb
35 µm

RESULTS

Different methods by which spermatozoa enter the female coelom

In *Protodrilus* the spermatozoa are transferred indirectly from males to females by spermatophores (see "Discussion"). Special epidermal reception organs for spermatophores are present in only two species, *P. flavocapitatus* and *P. rubropharyngeus*, while the other species lack similar preformed epidermal structures. In females of species without reception organs, such as *P. jägersteni* and *P. ciliatus*, the spermatozoa are, after epidermal penetration, for some time in close contact with each other, forming an O-shaped or a slowly synchronized-undulating sperm bundle beneath the penetration spot (Figs 1b, e). In all species, the spermatozoa actively enter the coelom cavities after a while, and in young females can already be found in all fertile segments. Some very mobile spermatozoa enter the sterile anterior segments or even the head region.

No modification in size or structure was observed in the euspermatozoa after penetration of the female epiderm or during the resting phase in the coelom cavities. Until fertilization, euspermatozoa in male and female individuals of *P. purpureus* and *P. rubropharyngeus* do not show any structural differences, especially in terms of the acrosomal structure (von Nordheim, 1987, 1989b). In contrast, the structure of paraspermatozoa differs distinctly in males and females: The paraspermatozoa completely lose or reduce their acrosomal vesicle, probably during spermatophore formation by the lateral organs, while penetrating the epiderm or while being stored in the female coelom cavities (Figs 1c, d). The nucleus elongates and becomes cylinder-shaped, so that the length of the nucleus of paraspermatozoa in *P. helgolandicus* averages 11 µm in females and 9 µm in males, and in *P. purpureus* 8.3 µm in females compared with 7.5 µm in males. Finally, the paraspermatozoa are often bent at the base of the nucleus at an angle of about 180°, so that the midpiece region lies parallel to the nucleus (Fig. 1c). Normally, only a few paraspermatozoa can be found in females close to the penetration spot, whereas in males they form up to 20 % of all spermatozoa. They can perform only slow and undirected movements in contrast to the very mobile euspermatozoa (see also von Nordheim, 1989b).

Dorsal organs of *P. rubropharyngeus*

The dorsal organs in mature females are unpaired rosette-shaped structures extending backwards from about segments 19 to 22 on (Figs 4c, 9c). Approximately in the middle of each segment, dorsally located, one organ is seen. Under the light microscope they are rather transparent, comprising externally a round median pit with many cilia. The last 4 to 5 body segments lack dorsal organs. The organ is, in cross-section, slightly flattened and round with a maximum width of ca. 40 µm and a height of 35 µm (Fig. 2). In horizontal longisection the organ appears elliptical or oval and its edges fuse with the dorsal mesenterium which forms the longitudinal axis of the body (Fig. 3). The largest part of the dorsal organ lies directly beneath the longitudinal muscle layer and forms dorsally a narrow opening tube which extends through the muscle layer and the basal lamina of the epiderm, opening on the dorsal side in the shallow ciliated pit (Figs 2, 4b). The ventral section of the organ borders directly on the epithelium of the dorsal blood vessel (Figs 2, 4a). The organ is not surrounded by a muscle layer but by a thin epithelium

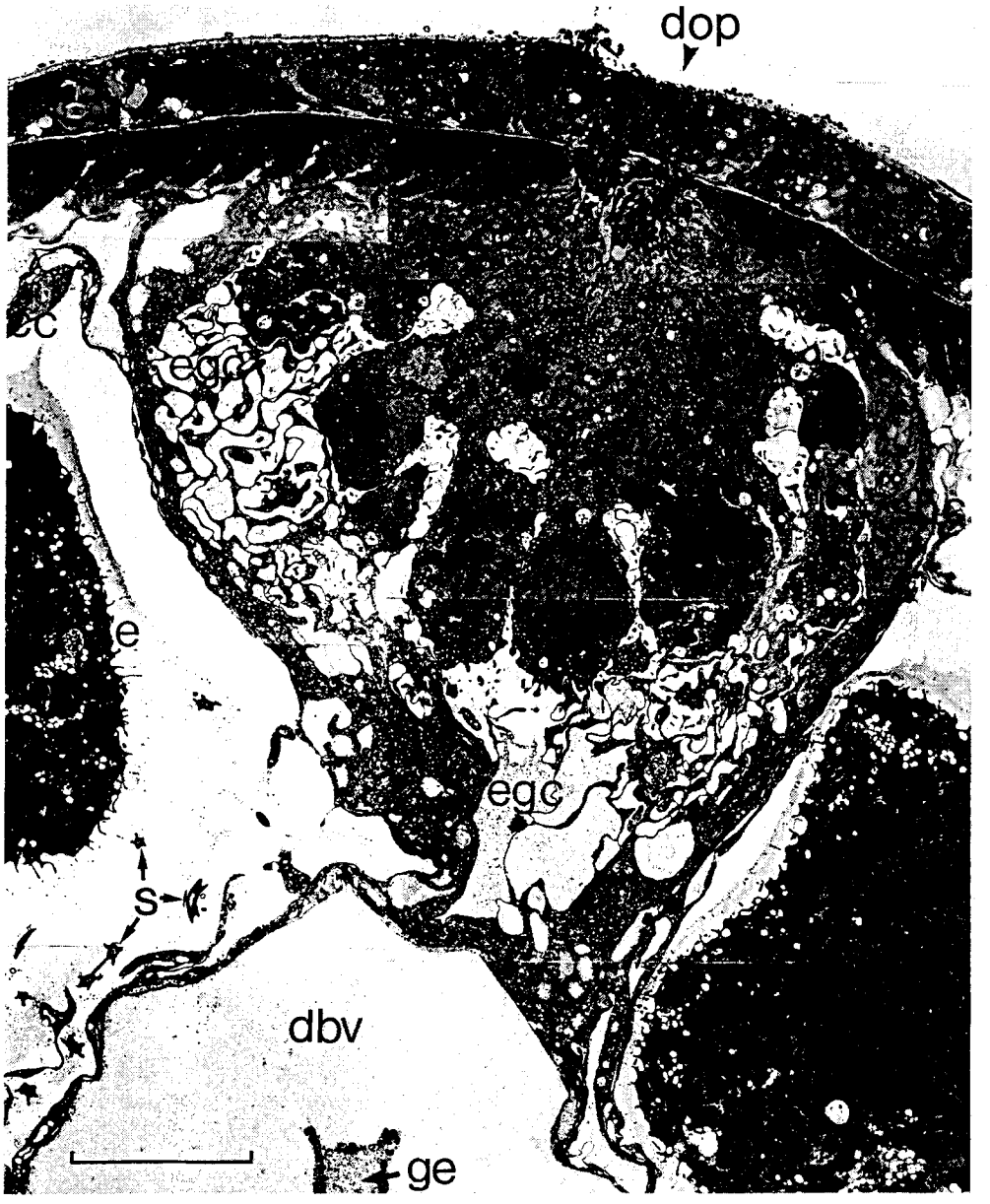


Fig. 2. *Protodrilus rubropharyngeus*; female; cross section of dorsal organ. cc – coelomatic cell, dbv – dorsal blood vessel, e – egg, egc – external gland cell, ge – gut epithelium, igc – internal gland cell, lm – longitudinal muscles, s – spermatozoa, shc – sheath cell. Scale bar: 8 μm

of coelomic cells, which extend ventrally along the sides of the dorsal blood vessel and then branch out laterally (Fig. 2). The section of the coelomic cells that contains the nucleus frequently forms a projection into the coelom.

Beneath the longitudinal muscle layer the dorsal organ consists basically of two cell types which Salensky (1907) designated as "sheath cells" and "gland cells", respectively. The sheath cells form a sheath of a single- or multi-layered epithelium with strongly interlocking cells enclosing the subepidermal section of the organ (Figs 2, 3). While in lateral regions of the dorsal organ only a thin layer of cell processes is seen (Figs 4b, 5b, c, d), in dorsal view nuclei and large cytoplasmatic areas of the sheath cells are found located mainly in posterior and anterior parts of the organ (Fig. 3). The cytoplasm of the cell processes is relatively finely granulated and dark grey in colour and contains plenty of RER, free ribosomes, dictyosomes and round to oval-shaped mitochondria. In the remaining parts the cells appear partly swollen, their cytoplasm in some areas is very light and often contains a longish nucleus, numerous large dark granules (glycogen?) and scattered large lipid droplets (diameter 1.1 to 2.8 µm) (Figs 3, 5b, c, d).

The gland cell complex of the dorsal organ consists of two types of cells each with a specific structure and position: "external gland cells" (egc) and "internal gland cells" (igc) (Figs 2, 3). The external gland cells are in close contact with the inner side of the mantle of sheath cells and are often interlocked with them. The external gland cells completely surround the internal gland cells as a relatively broad layer of tissue, thus forming – in addition to the coelomic and the sheath cell layers – a third "envelope" around the internal gland cell complex (Figs 2, 3, 4b, 5c, d).

The external gland cells contain a longish nucleus, mitochondria, dictyosomes, RER, polysomes and numerous very characteristic granules (type 1) (Figs 4b, 5b, c, d). These granules are surrounded by a membrane; they consist of fine granular material and have a roundish or horseshoe-like shape (diameter 0.15 to 0.25 µm) with a large light centre (diameter 0.08 to 0.14 µm). Some granules can be noticed in the cell processes together with free ribosomes, polysomes and RER. A second type of granule (type 2) with an oval to round shape and very variable in size (diameter 0.2 to 1.0 µm) is found only in low numbers (Figs 4b, 5b). These granules consist of a homogeneous, dark grey material and are also surrounded by a membrane.

In lateral and ventral areas of the organ the external gland cells normally possess long and ramifying cell processes, some of which reach far into the centre of the organ. They are sometimes connected to the internal glands cells by zonulae adhaerentes (Figs 3, 5b, 7a, b). These cell processes form a very complex and net-like structure with large intercellular spaces and surround like a dish the central internal glands which project into this region from the dorsal side. The processes contain only light to medium grey granular cytoplasm and many single ribosomes, so that they can be easily distinguished from the cytoplasmatic processes of the other external glands. While the processes are relatively broad at their base, they branch terminally into several long, thin lamellar structures that frequently interlock, finger-like, with each other (Figs 3, 7b). The transition zone between the cell body, with its typical components, and the cell processes is clearly visible (Figs 5b, 7b). Here one can frequently find large, type 2 granules which are often modified or partly disintegrated, as well as remnants of their membranes and much RER with clearly expanded cisterns. Most of the processes' cytoplasm is probably synthesized in this transition zone.

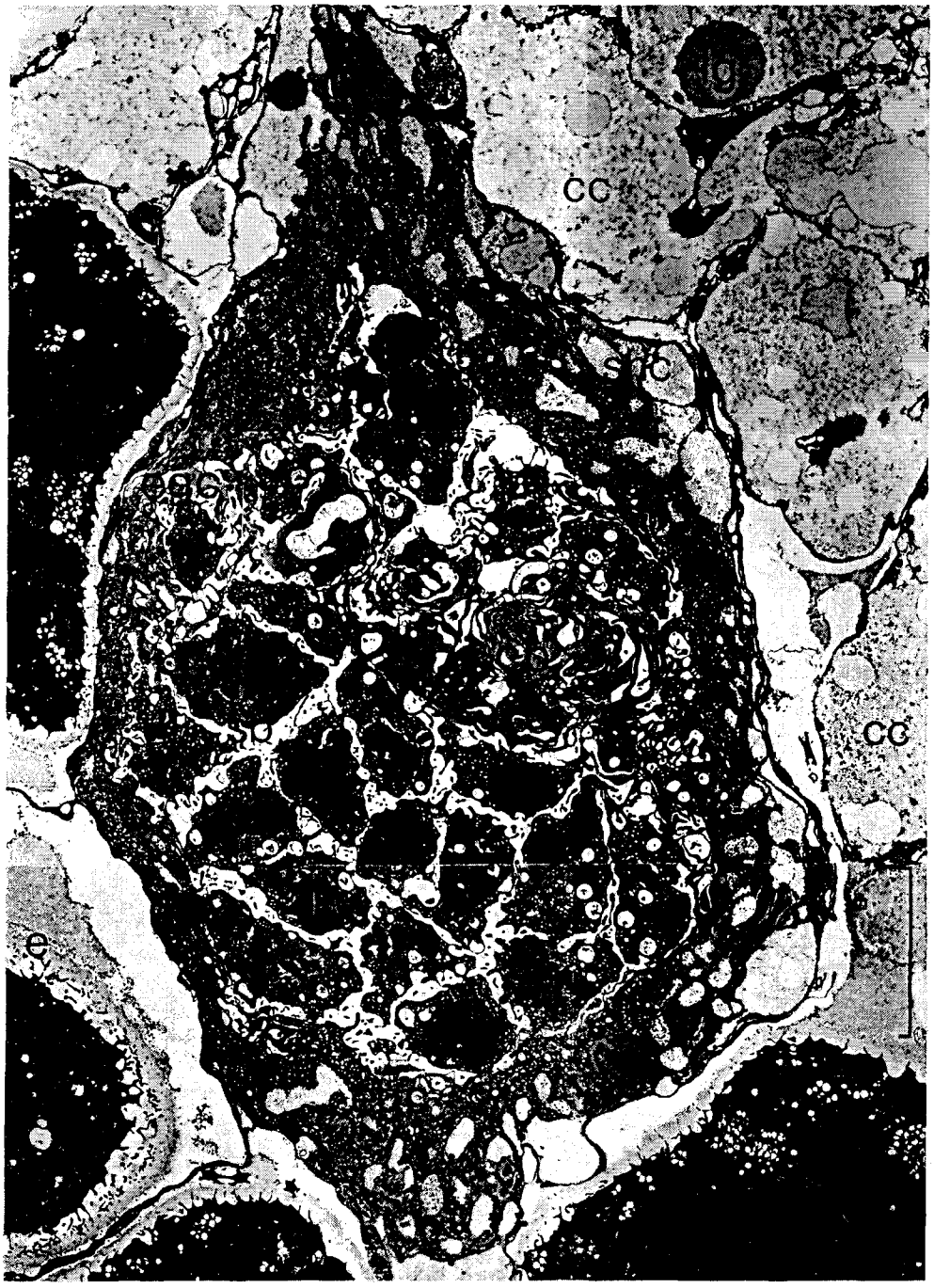


Fig. 3. *Protodrilus rubropharyngeus*; female; horizontal longitudinal section of dorsal organ. Scale bar: 8 μm

In the dorsal subepithelial section of the organ the external glands form a massive cell layer (Figs 2, 6a). Here they interlock with each other and have several connections with the dorsal parts of the internal gland cells. Further, the apical section of the cell body is split up into numerous tube-like structures that spread through the epidermal basal lamina which in this opening region of the organ is deeply invaginated and perforated (Fig. 6a).

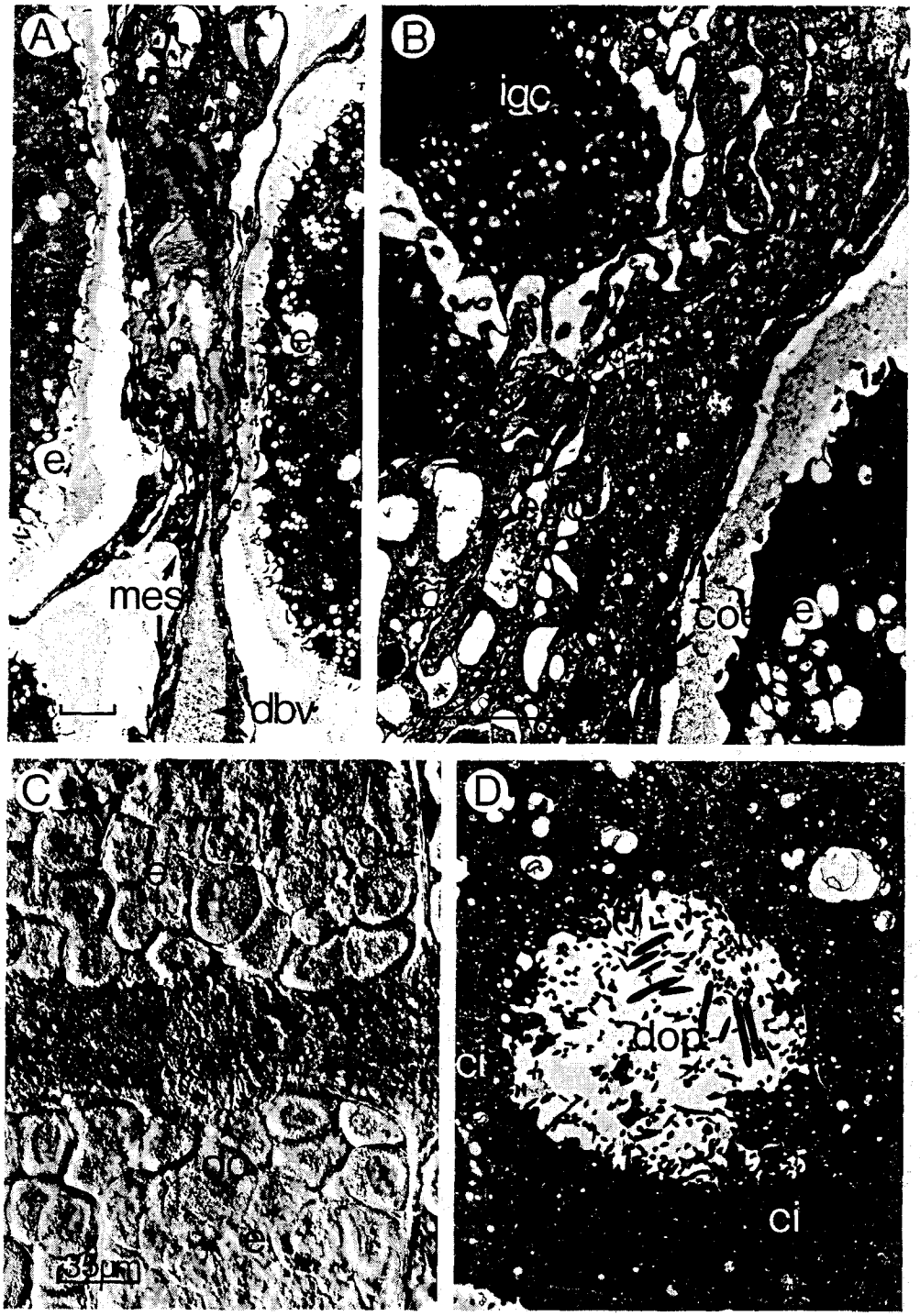
In the centre of the dorsal organ 18 to 25 internal gland cells can be found (Figs 2, 3). In horizontal longitudinal section they have an irregular round to oval shape (diameter max. 4.5 to 9 µm). In cross section, they taper dorsally bottleneck-like, while basally they are broad and round (Figs 2, 3, 6b). Between the basal portion of the cells, large intercellular spaces can be seen which are traversed by thin cytoplasmic processes of the cells. Apically, the internal glands border on the dorsal layer of external gland cells.

The basal part of the internal glands usually contains one large or several small secretory "balls" (Figs 2, 3, 4b, 6b, 7d). These are surrounded by a membrane and contain a secretion consisting of short and irregularly arranged filaments. Above this large secretion ball, the nucleus is found surrounded by much RER, polysomes and voluminous granules that contain exclusively granular substances (Fig. 7c). These granules are very likely synthesized in this region and, while moving downwards, fuse to form the larger secretion balls with the granular secretion becoming gradually arranged finally to parallel or concentric filaments. Other typical cell constituents are dark granules of type 2 (diameter 0.25 to 0.6 µm) and numerous light to medium grey granules of type 1 (diameter 0.18 to 0.28 µm) (Figs 6b, 7c, d). Both types of granules correspond structurally to the type 1 and 2 granules of the external gland cells.

The opening of the dorsal organ is formed by about 20 epidermal cells above the basal lamina. A ring (diameter ca 10 µm) of 9 ciliated cells with comparatively few cilia surround about 11 gland cells, the apical cell membranes of which have numerous microvilli (Figs 4d, 8a, b). In addition to common cell constituents, the gland cells have numerous secretory granules that are especially plentiful in the broad apical areas of these cells and consist of medium to dark grey material (Figs 8a, b). The granules have a round to oval shape (diameter 0.18 to 0.35 µm) with a surrounding membrane and their contents are secreted at the dorsal cell surface which, like the ciliated cells, is not covered by a cuticle. Further, ring-like granules are present which correspond to type 1 of the organ's internal gland cells. Special opening structures (e.g. microvilli crown) were not detected in the gland cells. The epidermal gland cells are basally split into several long root-like structures that penetrate the deeply invaginated basal lamina (Figs. 6a, 8b). Some of these tube-like roots contain numerous long microtubuli. Between the roots large dark grey cytoplasmic protuberances, probably originating from lateral epiderm cells, are found, which contain many dark, round or oval granules (neuropeptids?) (diameter 0.15 to 0.15 × 0.3 µm). Similar granules occur in other parts of the epidermal tissue only in the so-called "glia-like" cells.

In none of the 7 dorsal organs investigated were spermatozoa noticed. These were only found in the coelom cavities next to the dorsal organ (Fig. 2), between eggs, or in

Fig. 4. *Protodrilus rubropharyngeus*; female, dorsal organ. A: Basal boundary layer between dorsal organ and dorsal blood vessel; eggs. B: Cross section of the lateral area. C: Dorsal view. D: Tangential horizontal section of dorsal opening. Scale bar: 2 µm


A
e
e
mes
dbv
B
igc
e
C
e
do
e
35µm
D
dop
ci
ci

special sheath-like structures in front of or behind the dorsal organ and in close contact to the mesenterium (Figs 8c, d). The walls of these sheath structures vary in thickness and correspond exactly in their ultrastructure to the special light-grey cell processes of the external gland cells in the lateral part of the dorsal organ (Figs 3, 7a, b, 8c, d).

Oviducts

The females of most *Protodrilus* species do not possess special coelomoducts or organs to facilitate the process of oviposition (see von Nordheim, 1989a).

The paired oviducts of *P. purpureus, P. haurakiensis,* and *P. rubropharyngeus* possess a large funnel-shaped opening immediately in front of or actually in the posterior dissepiment of some hindmost body segments (Figs 9b, 10a). In *P. rubropharyngeus,* the opening is sealed by a diaphragm until oviposition (Fig. 9a). Oviduct opening and -tube are formed by multi-ciliated cells. The length of the oviducts is 80 to 90 µm in *P. haurakiensis* and *P. rubropharyngeus* and ca 120 µm in *P. purpureus.*

In the following, oviduct ultrastructure is exemplified in investigations on *P. purpureus.* In this species, the oviduct funnel has a maximum apical width of 55 × 70 µm, with an incomplete diaphragm immediately in front of the opening (Fig. 9d). The funnel region is formed by a thin layer of ciliated cells with 3 to 6 cilia per μm^2, which each have two ciliary roots, the longer one of which measures up to 5 µm (Fig. 10c). Apically, the cells have numerous mostly thin, thread-like microvilli (Figs 10a, b). Laterally, neighbouring cells interlock with each other intensively and are connected by septate desmosomes and zonulae adhaerentes.

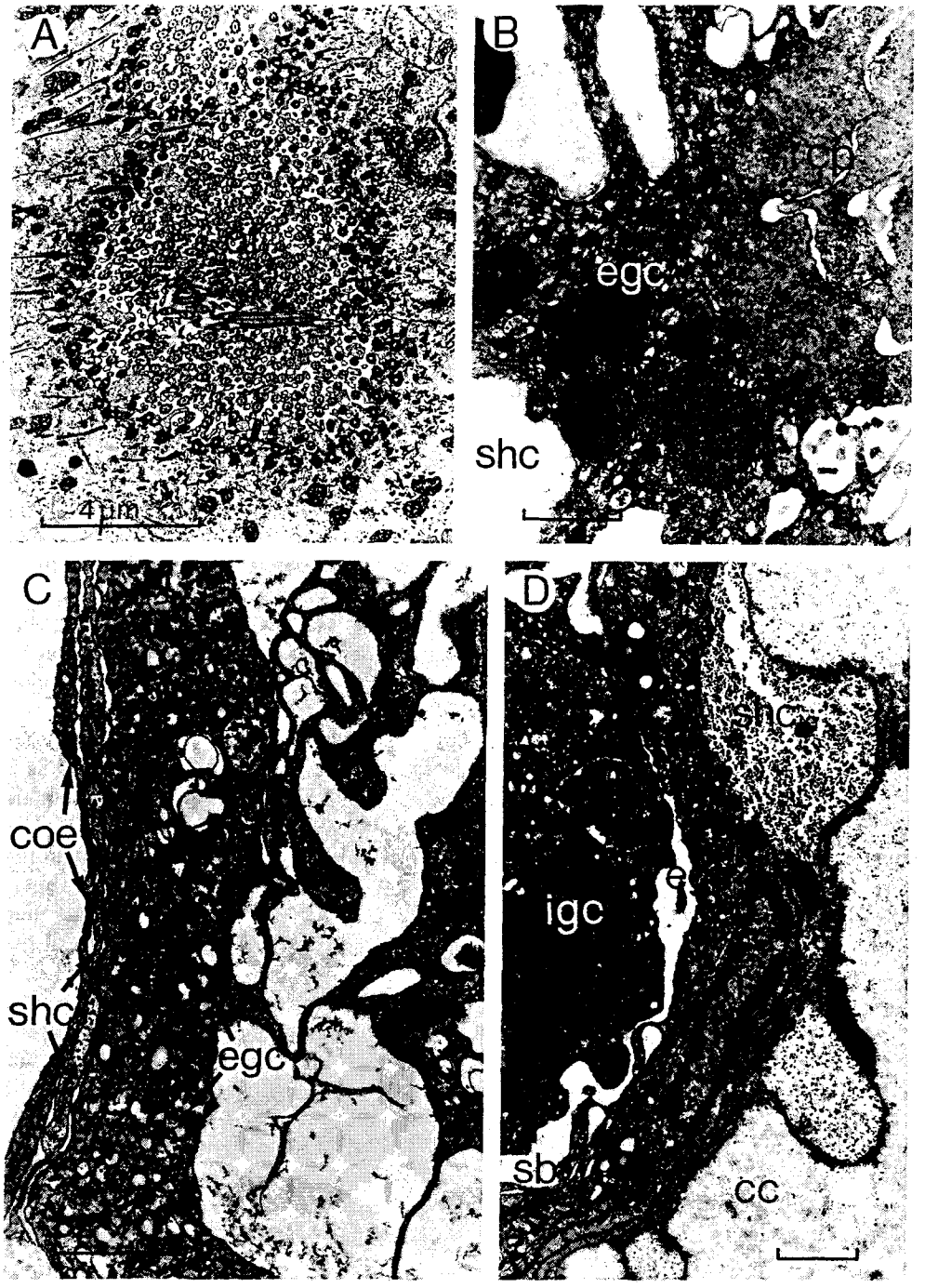
The oviduct tube (diameter of the lumen 7.5 × 8.0 µm) connects posteriorly to the oviduct funnel and in cross section is seen to be formed by 2 or 3 ciliated cells (Fig. 5a). These cells have about 3 to 5 cilia per μm^2 and many microvilli that often originate from a common broader base and have numerous constrictions (Figs 1a, 10b). Between the ciliary roots small black granules can be found, as well as round or oval mitochondria which are also present in other cell parts. The cytoplasm of the anterior tube cells is light, rich in vacuoles, and contains a large number of light grey or dark secretory granules (diameter 0.8 to 1.5 µm) (Figs 10a, b). In their posterior portion the oviduct cells contain only a few and smaller dark- to light-grey secretory granules (diameter 0.4 to 0.7 µm) (Fig. 1a). Terminally, the tube penetrates the basal lamina and the epiderm, opening onto the lateral side of the segment in a ciliated pore (Fig. 10d).

Oviduct funnel and tube are not surrounded by a special longitudinal or ring musculature. The ciliated cells are partly connected by a number of long cytoplasmic processes by means of desmosomes to the epidermal basal lamina or to the dissepiments. Special gland cells were not found in any region of the oviduct.

The females of the three species investigated show clear differences in egg size and egg number per body segment. While in *P. haurakiensis* and *P. rubropharyngeus,* egg diameter is 30 to 35 µm and egg number about 50 to 130 per fertile segment, in

---

Fig. 5. A: *Protodrilus purpureus;* female; cross section of oviduct tube. B, C, D: *P. rubropharyngeus:* female. Cross sections of dorsal organ. B: External gland cell (egc) and cell process (cp); sheath cell (shc). C: Lateral section with coelomic cell (coe), sheath cell and external gland cell. D: Sheath and internal gland cell (igc) with secretion ball (sb). Scale bar: 1 µm


A
4 µm
B
egc
shc
C
coe
shc
egc
D
shc
igc
sb
cc

P. purpureus one finds about 20 to 30 eggs per segment, each with a diameter of 50 to 60 µm. Accordingly, the oviduct funnels show a smaller diameter in *P. haurakiensis* and *P. rubropharyngeus* (diameter max. 40 to 50 µm) (Figs 9a, b).

Oviposition in the species investigated starts with wave-like constrictions of the anterior body segments. The eggs are pressed progressively through the openings in the dissepiments into posterior body regions. In *P. rubropharyngeus,* this transport is very likely faciliated by the ciliation of the dissepiments (Figs. 9c). Following rupture of the diaphragmata or similar structures, and an opening of the oviducts induced by increasing pressure, the eggs slide out through the oviduct tube, supported by the ciliation of the canal. Here they remain for a short while, connected like beads on a string, while swelling up to reach their final round shape.

DISCUSSION

How do spermatozoa enter the female body?

Probably in all *Protodrilus* species, eu- and paraspermatozoa are indirectly transferred to the female via spermatophores that are formed in the males by numerous glands of the so-called lateral organs (von Nordheim, 1987, 1991). Generally, males probably deposit their spermatophores on the sediment close to the females which then pick them up by gliding over them. Spermatophores have been observed so far in *P. rubropharyngeus, P. albicans, P. brevis* and *P. helgolandicus* (Jägersten, 1952; Jouin, 1970a, c; von Nordheim, 1983). Nevertheless, the exact mechanism of epiderm penetration by the spermatozoa is still unknown for the latter three species that lack special reception organs (von Nordheim, 1989a).

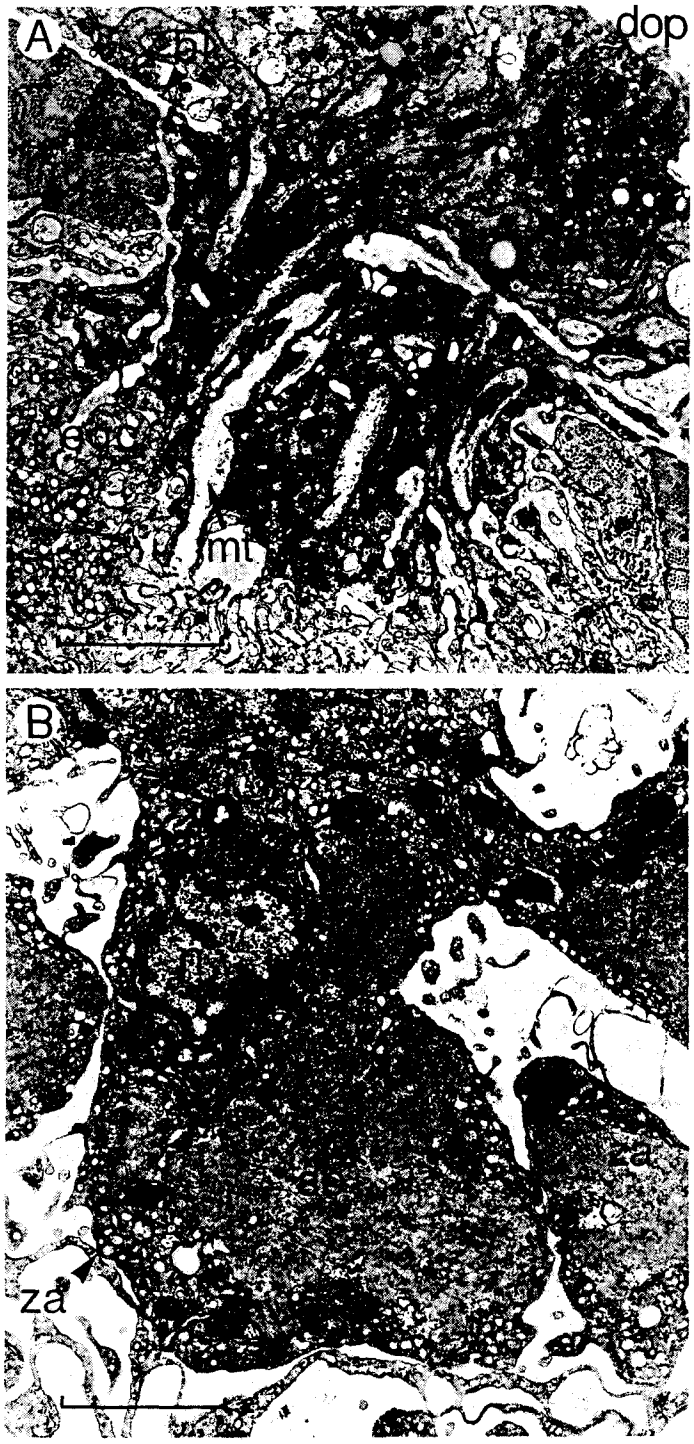
The process of spermatophore uptake was observed by Jägersten (1952) only for *P. rubropharyngeus* which as one of the only two species with dorsal reception organs is not representative of the majority of *Protodrilus* species. Jägersten noticed that a female encountering a spermatophore deposited on the sediment, bends its posterior body segments so that one dorsal organ touches the spermatophore, which then sticks to the organ. Subsequently, the balloon-shaped spermatophore empties and shrinks, and spermatozoa gather in the ventral section of the dorsal organ. "Sooner or later, however, they penetrate into the coelom and spread through the dissepiments to various parts of the body cavity" (Jägersten, 1952).

Probably, in most *Protodrilus* species the mode of spermatophore uptake by the females resembles that of *P. helgolandicus,* where spermatophores were found apparently fixed at random to various body segments which lack preformed epidermal reception areas (von Nordheim, 1983). The author supposes that in such species the female epiderm is subsequently opened histolytically by substances of the spermatophores or similar substances in the acrosome of the paraspermatozoa (von Nordheim, 1987, 1989b).

In analogy to the male "lateral organs", female *P. ciliatus* and *P. leuckarti* often show lateral ciliary rows that, however, lack accessory gland complexes. Although the function

---

Fig. 6. *Protodrilus rubropharyngeus*; female; cross section of dorsal organ. A: Dorsal opening area. B: Internal gland cells. Scale bar: 2 µm


A
dop
mt
B
za
za

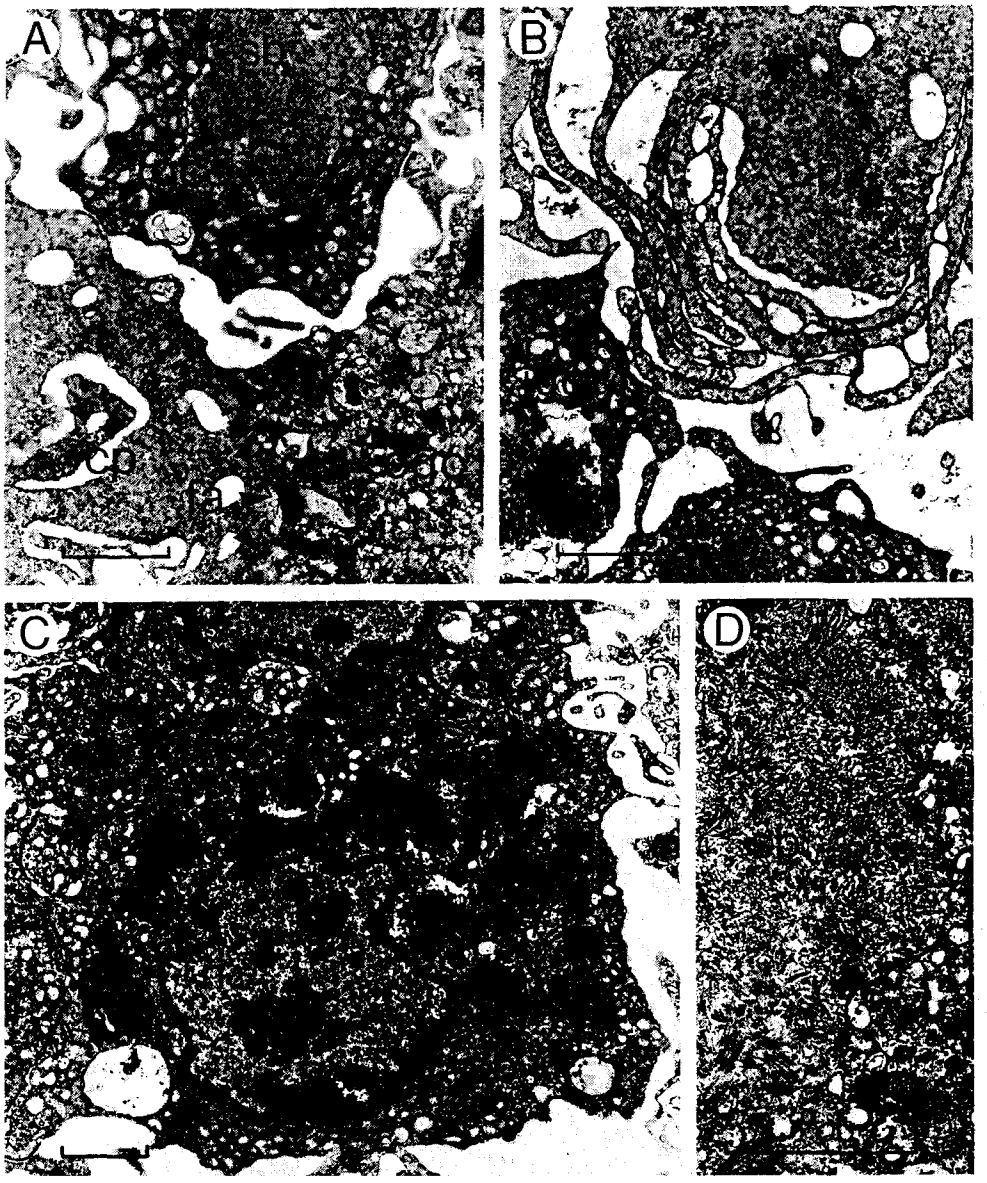


Fig. 7. *Protodrilus rubropharyngeus*; female, dorsal organ. A: Cross section of transition zone cell body/cell process of an external gland cell. B: Detail of cell process. C: Horizontal section of the formation area of secretion balls close to the nucleus; internal gland cell. D: Detail of secretion ball. Scale bar: 1 µm

of these ciliary rows is not known, they could play a role in spermatophore uptake. It may also be possible that in some *Protodrilus* species spermatozoa enter the body cavities through coelomoducts, since in ultrastructural investigations of the protonephridia (!) of *P. rubropharyngeus*, spermatozoa were found in its protonephridial ducts in spite of the presence of dorsal organs (von Nordheim, 1987).

Functional morphology of the dorsal organs

Within the genus *Protodrilus* so-called dorsal organs have been described so far only for the females of *P. flavocapitatus* and *P. rubropharyngeus* by Salensky (1907), Pierantoni (1908), Goodrich (1931) and Jägersten (1952) at the light microscopy level. Nevertheless, it is important to note that *P. rubropharyngeus* probably is a synonym for *P. flavocapitatus* (Jägersten, 1952; von Nordheim, 1989a).

Salensky (1907) gave a detailed description of the dorsal organs of *P. flavocapitatus*, but their function remained unknown to him. His results correspond essentially with the present ultrastructural findings in *P. rubropharyngeus*. Goodrich (1931) was the first to notice a conspicuous concentration of spermatozoa in and close to the dorsal organs of *P. flavocapitatus* (?), and supposed the organs to have a function in reproduction.

A synthesis of the presented results with those of Salensky, Goodrich and Jägersten leads to the following functional interpretation of the dorsal organs in *Protodrilus*. Jägersten (1952) supposed that spermatophores stick to the cilia of the organ opening. However, it is much more likely that the gland cells that seal the organ opening, dorsally secrete a sticky substance, thus guaranteeing a fixation of the spermatophore. Subsequently, the spermatozoa penetrate this layer of gland cells and invade the large subepidermal areas of the organ. There they gather in the intercellular spaces between the layers of internal and external gland cells. Like Goodrich (1931), Jägersten (1952) noticed numerous spermatozoa in this section of the organ. Nevertheless, the spermatozoa very likely stay only for a short time in the organ, since in none of the seven organs investigated by the author were spermatozoa detected. However, apart from single spermatozoa in the vicinity of the eggs or the dorsal organ, which are also mentioned by Goodrich and Jägersten, several sperm bundles enveloped by a more or less complete sheath were found in the coelom. The sheath material does not consist of spermatophore remains but corresponds structurally to the thread-like processes of the external gland cells. Probably in the intercellular spaces of the dorsal organ, the spermatozoa are enclosed in such sheath structures which are then transported in an unknown manner into the body cavities like a sort of "internal spermatophore". There the spermatozoa gradually leave the sheath. In the coelom cavity, the sheath is connected to the coelothelium of the mesenterium, which might ensure that the "internal spermatophore" with the enclosed spermatozoa is not accidentally discharged during oviposition.

Within the Annelida various types of female organs for the reception of spermatozoa are present, especially in Hirudinea and in all Oligochaeta (summarized by Lassere, 1975). All these organs are distinctly different from the unpaired epidermal dorsal organs of *Protodrilus*.

In polychaetes, however, only a few groups possess seminal receptacles (spermathecae) (Westheide, 1988). Ultrastructural investigations on polychaete receptacles

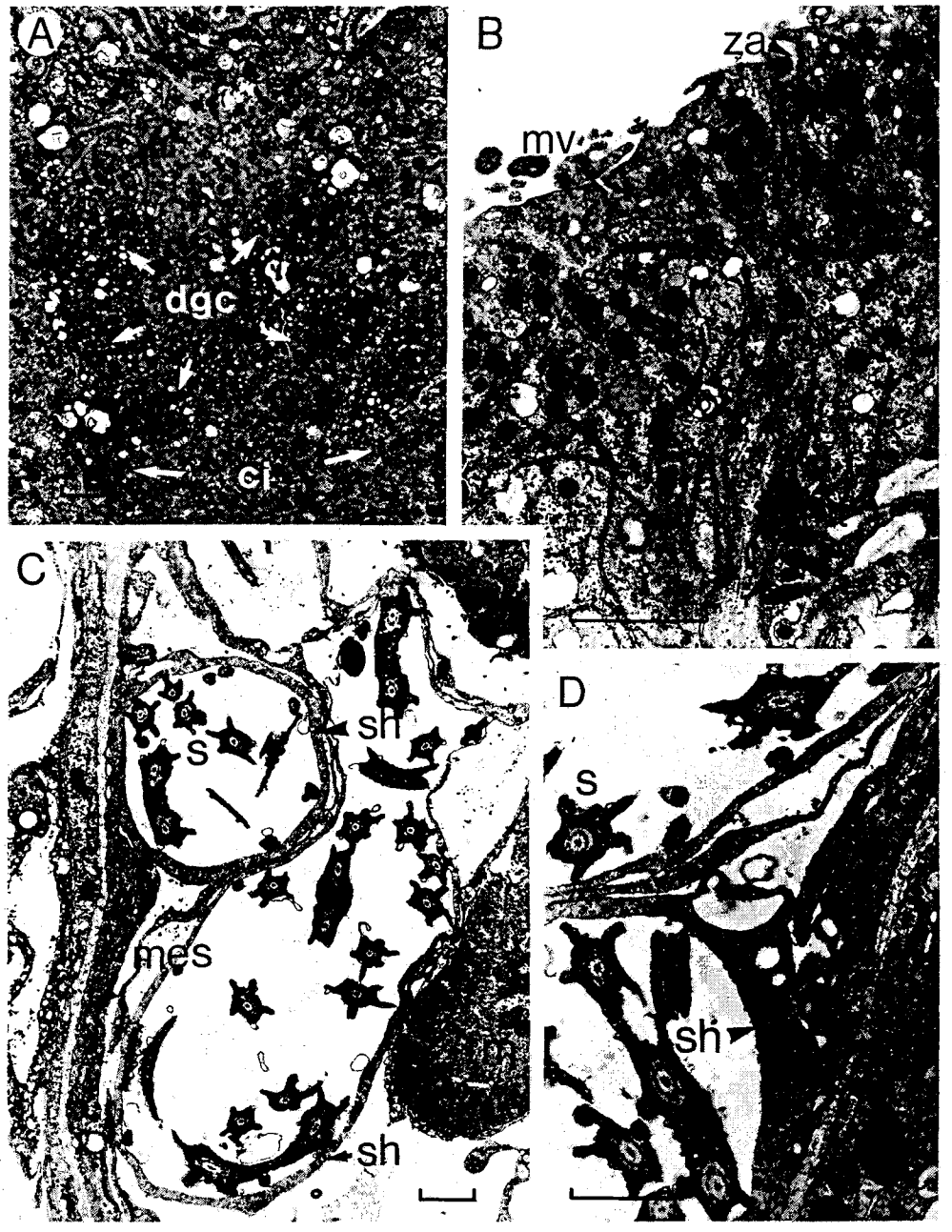


Fig. 8. *Protodrilus rubropharyngeus*; female. A, C, D: Horizontal sections. A: Gland cells that seal the dorsal opening of the dorsal organ. B: Cross section of these dorsal gland cells. C: Internal sheath structures containing spermatozoa ("internal spermatophore"). D: Detail of sheath and eusper-matozoa. Scale bar 1 µm

forming paired or unpaired epidermal blind sacs have been presented for *Ikosipodus caroliensis* (Westheide, 1982), *Apodotrocha progenerans* (Westheide & Riser, 1983) and *Spirorbis spirorbis* (Daly & Golding, 1977). Among these species the unpaired seminal receptacle of *Ikosipodus* shows some similarities to the dorsal organs of *P. rubropharyngeus*. The receptacle is located in the posterior section and an internal opening of the organ could not be detected, but the mode of transfer of the filiform spermatozoa remains unknown. In several groups of spionids, receptacles were found in different body regions. In most cases, however, these receptacles are probably epidermal invaginations without an internal opening; results of ultrastructural investigations have not been presented so far (Schroeder & Hermans 1975, Rice 1978, Mann 1984, Westheide 1988).

At light microscopy level, a surprisingly high structural similarity exists between the dorsal organs of *Protodrilus* and the unpaired epidermal gland complexes in dorsal segment areas of *Ophryotrocha labronica* and *O. notoglandulata*. These glands, however, occur only in males and in individuals in the male phase. According to the results of light microscopical investigations by Schlawny (pers. comm.), these complexes consist solely of epidermal glands that secrete their products as "mucous clods" and which are underlaid by a continuous basal lamina. He believes the secretion to have a pheromone effect on females ready for mating.

Oviducts and oviposition

In accordance with Jägersten (1952) and Jouin (1970 a, b), the oviducts of *P. purpureus, P. rubropharyngeus,* and *P. haurakiensis* were found to be simple, short, ciliated coelomoducts. Their ultrastructure corresponds essentially to that of the male sperm ducts (von Nordheim, 1987, 1991). In addition, the tube sections of the oviducts in female *P. purpureus* contain several large secretory granules that resemble the secretory granules in the oviducts of *Pisione remota* (Westheide, 1988). No glands lead into the area of the external opening. The oviduct in *P. rubropharyngeus* and *P. purpureus* is sealed until egg deposition by a diaphragm which prevents premature emergence of eggs. Such diaphragmata have so far not been reported for Annelida.

There are three possible modes of ontogenetic formation of oviducts and sperm ducts in *Protodrilus*:

(a) Gonoducts and nephridia represent a functional unit, the so-called "nephromixia" (see Goodrich, 1931);

(b) they develop from larval metanephridia (see Pierantoni, 1908);

(c) when reaching sexual maturity, gonoducts are formed as new organs, normally accompanied by simultaneous reduction of nephridia in corresponding segments.

In the species ultrastructurally investigated, no nephridia were noticed by the author in body segments with oviducts. Nephromixia which, according to Goodrich (1931), are formed from mesodermal and ectodermal cells could not be detected either. Furthermore, in the gonoduct funnels and tubes no structures were found which might indicate filtration, secretion or reabsorption occurring here. However, in female *P. brevis*, Jouin (1970a, b) found distinctly different gonoducts, termed "protonephromixia". These are present in all fertile segments, serve as nephridia and oviducts, and are considered to be a new trait of this species.

Since the females of most *Protodrilus* species do not possess special ducts or organs

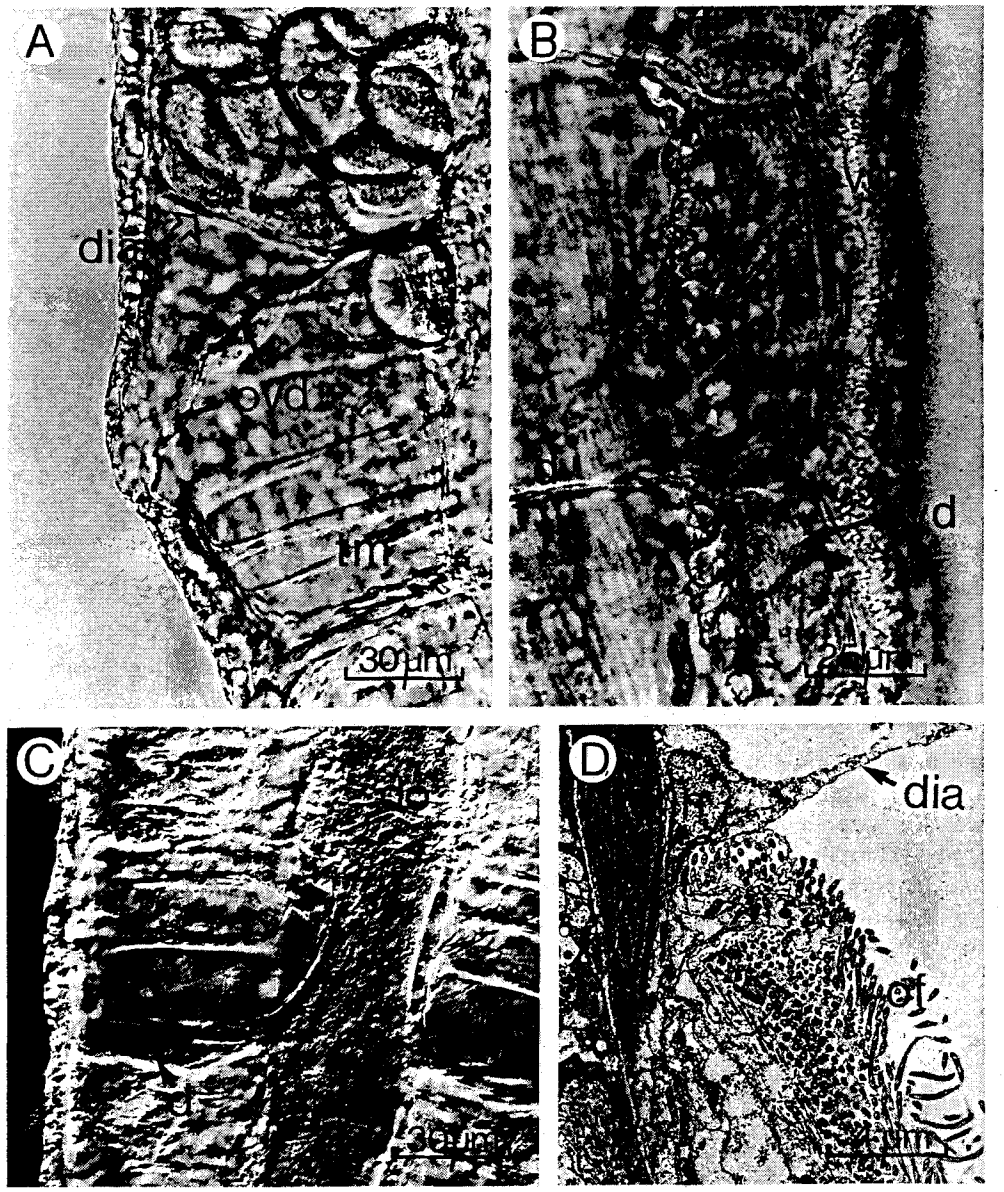
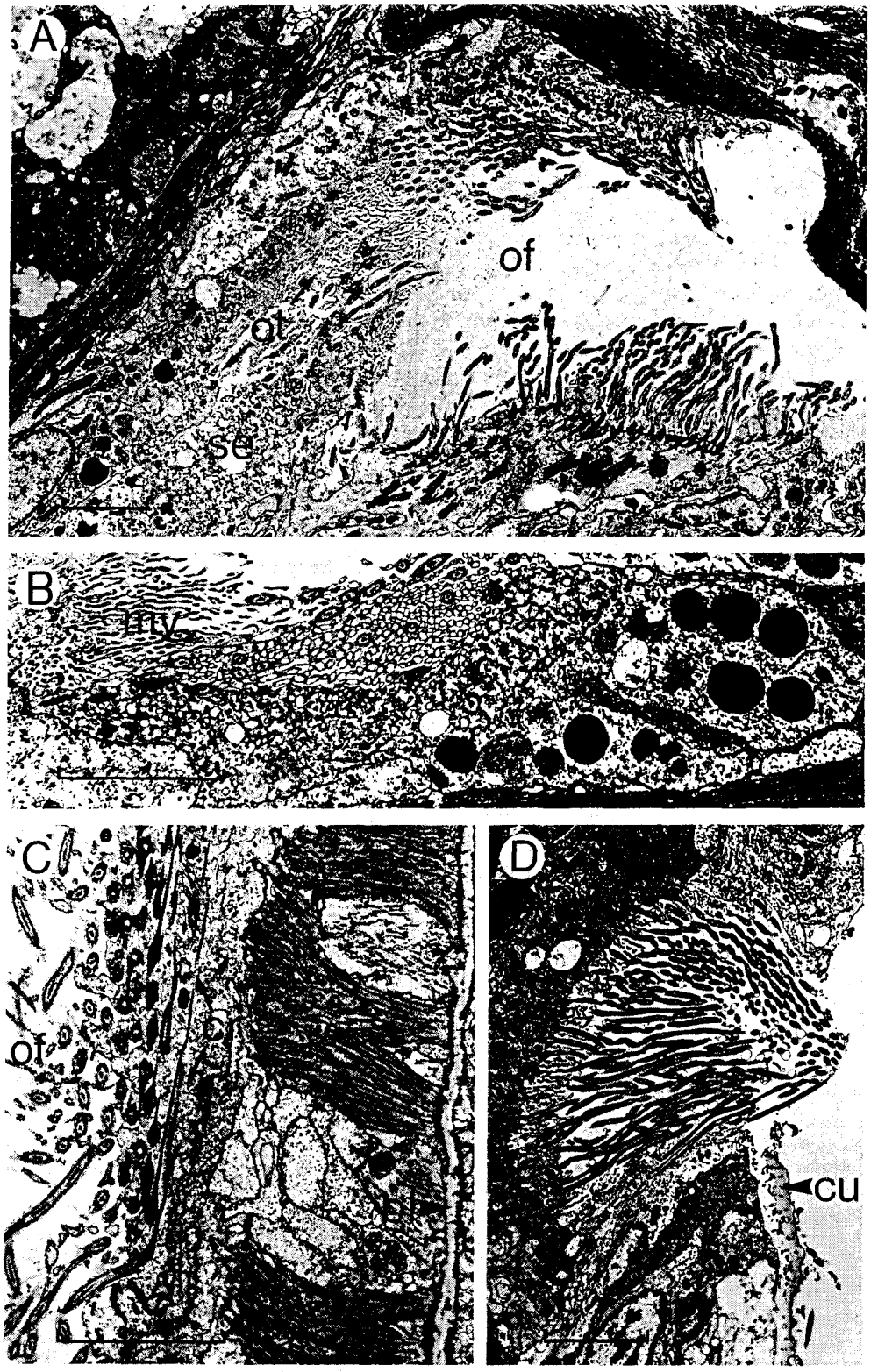


Fig. 9. Females of: A: *Protodrilus rubropharyngeus*; diaphragm, oviduct and eggs. B: *P. haurakiensis*; oviducts. C: *P. rubropharyngeus*; dorsal organ and ciliation of dissepiments. D: *P. purpureus*; longitudinal section of anterior region of the oviduct funnel and incomplete diaphragm. A, B, C: Dorsal views

---

Fig. 10. *Protodrilus purpureus*; female; logitudinal sections of oviduct. A: Oviduct funnel and basal septum. B: Tube cells with microvilli and dark granules. C: Cilia and ciliary roots of funnel cells. D: Lateral opening of the oviduct; cuticle. Scale bar: 3 µm


A
of
ot
se
B
mv
C
ot
D
cu

for oviposition, Jouin (1970b) assumes that at least in some of these species the eggs are likewise released through nephridial ducts and openings. In female *P. adhaerens*, she noticed that the nephridial tubes are thicker in fertile than in infertile body segments. Furthermore, like Jägersten (1952), she could not find any injuries of the female epiderm after oviposition.

Pierantoni (1908) supposed that oviposition in *Protodrilus* occurs through the shedding of some posterior segments, since at the end of the reproduction period he frequently found females with regenerating posterior body segments.

A further mode of oviposition is known from *P. helgolandicus*: The body wall of some posterior segments ruptures laterally, and after oviposition the wounds heal rather rapidly (von Nordheim, 1983). Oviposition combined with partial rupture of the hindmost body wall was also observed in *P. litoralis* (von Nordheim, 1989a) and very likely occurs similarly in *P. submersus*.

Thus, oviposition in *Protodrilus* occurs in a number of very different ways: (a) by special oviducts or nephromixia, (b) by nephridial ducts and/or -openings, (c) by shedding of posterior body segments, and (d) by partial rupture of the body wall.

Most *Protodrilus* species possibly deposit their eggs freely onto the sediment like many polychaete species which discharge their eggs freely into the ambient sea water (summarized in Evans, 1971, and in Schroeder & Hermans, 1975). In several cases, the author observed that following oviposition the eggs swell rapidly and stick fairly firmly to the sediment grains. In this way, perhaps, eggs are prevented from drifting away in a similar way to that reported for the genera *Trilobodrilus* and *Dinophilus* (Schmidt & Westheide, 1972; Westheide & Schmidt, 1974).

Only in mature females of *P. jouinae*, *P. gracilis* and *P. adhaerens* are there so-called "cocoon glands", the secretion of which can form a cocoon around the eggs during oviposition in a similar way to that found in many Clitellata and in some Polychaeta. Interestingly, cocoon glands are only present in *Protodrilus* species which also have special segmental adhesive organs and develop only very few but relatively large eggs per segment (von Nordheim, 1989a). These characteristics obviously represent adaptations to instable sediment conditions in their specific habitat, as is also the case in the closely related genus *Protodriloides*.

Finally, it must be stressed that special female reproductive organs, such as dorsal organs, lateral ciliary rows, oviducts and cocoon glands, represent very species-specific traits and occur only in some of the *Protodrilus* species. Thus, although the position of the organs depends to some extent on the state of maturity, these female characters are as important for taxonomic considerations in this genus as are the sperm ducts and lateral organs of the males.

Acknowledgements. My special thanks are due to my wife Dr. P. Scharnofske-von Nordheim for introducing me to the techniques of electron microscopy and for valuable discussions. I am obliged to Prof. W. Westheide for his support during my work at the Department of Zoology at the University of Osnabrück. I thank the staffs at the marine biological laboratories of the Universities of Auckland and Otago (New Zealand) and at the Biologische Anstalt Helgoland (Germany) for supplying laboratory and diving facilities. One of my visits in New Zealand was kindly financed by a grant from the DAAD (Deutscher Akademischer Austauschdienst).

LITERATURE CITED

Aiyar, R. G. & Alikunhi, K. H., 1944. On some archiannelids of the Madras coast. – Proc. natn. Inst. Sci. India *10*, 113–140.

Daly, J. M. & Golding, D. W., 1977. A description of the spermatheca of *Spirorbis spirorbis* (L.) (Polychaeta: Serpulidae) and evidence for a novel mode of sperm transmission. – J. mar. biol. Ass. U. K. *57*, 219–227.

Ermack, S. M. & Eakin, R. M., 1976. Fine structure of the cerebral and pygidial ocelli in *Chone ecaudata* (Polychaeta, Sabellidae). – J. Ultrastruct. Res. *54*, 243–260.

Evans, S. M., 1971. Behaviour in polychaetes. – Q. Rev. Biol. *46*, 379–405.

Goodrich, E. S., 1931. Notes on *Protodrilus*. – Q. Jl microsc. Sci. *74*, 303–319.

Higgins, R. P. & Thiel, H., 1988. Introduction to the study of meiofauna. Smithson. Inst. Press, Washington, 488 pp.

Jägersten, G., 1952. Studies on the morphology, larval development and biology of *Protodrilus*. – Zool. Bidr. Uppsala *29*, 425–512.

Jouin, C., 1970a. Recherches sur les protodrilidae (Archiannélides): I. Etude morphologique et systématique du genre *Protodrilus*. – Cah. Biol. mar. *11*, 367–434.

Jouin, C., 1970b. Recherches sur les archiannélides interstitielles: systématique, anatomie et developpement des Protodrilidae et des Nerillides. Ph. D. Thesis, Univ. Paris, 204 pp.

Jouin, C., 1971. Status of the knowledge of the systematics and ecology of Archiannelida. – Smithson. Contr. Zool. *76*, 47–56.

Lassere, P., 1975. Clitellata. In: Reproduction of marine invertebrates. Ed. by A. C. Giese & J. S. Pearse. Acad. Press, New York, *3*, 215–275.

Mann, T., 1984. Spermatophores. Springer, Berlin, 217 pp.

Nordheim, H. von, 1983. Systematics and ecology of *Protodrilus helgolandicus* sp.n., an interstitial polychaete (Protodrilidae) from subtidal sands off Helgoland, German Bight. – Zool. Scr. *12*, 171–177.

Nordheim, H. von, 1987. Anatomie, Ultrastruktur und Systematik der Gattung *Protodrilus* (Annelida, Polychaeta). Diss., Univ. Osnabrück, 299 pp.

Nordheim, H. von, 1989a. Six new species of *Protodrilus* (Polychaeta, Annelida) from Europe and New Zealand together with a concised presentation of the genus. – Zool. Scr. *18*, 245–268.

Nordheim, H. von, 1989b. Vergleichende Ultrastrukturuntersuchungen der Eu- und Paraspermien von 13 *Protodrilus*-Arten (Polychaeta, Annelida) und ihre taxonomische und phylogenetische Bedeutung – Helgoländer Meeresunters. *43*, 113–156.

Nordheim, H. von, 1991. Ultrastructure and functional morphology of male genital organs and spermatophore formation in *Protodrilus* (Polychaeta, Annelida). – Zoomorphology *111*, 81–94.

Pierantoni, U., 1908. *Protodrilus*. – Fauna Flora Golf. Neapel *31*, 1–226.

Rice, S. A., 1978. Spermatophores and sperm transfer in spionid polychaetes. – Trans. Am. microsc. Soc. *97*, 181–194.

Salensky, W., 1907. Morphogenetische Studien an Würmern. T. II, III und IV. – Zap. imp. Akad. Nauk *19*, 1–348.

Schmidt, P. & Westheide, W., 1972. *Dinophilus gyrociliatus* (Polychaeta). Nahrungsaufnahme und Fortpflanzung. – Encycl. cinematogr. *E 1750*, 1–16.

Schneider, A., 1868. Über Bau und Entwicklung von *Polygordius*. – Archs Anat. Phys. *56*, 51–60.

Schroeder, P. C. & Hermans, C. O., 1975. Annelida: Polychaeta. In: Reproduction of marine invertebrates. Ed. by A. C. Giese & J. S. Pearse. Acad. Press, New York, *3*, 1–213.

Westheide, W., 1982. *Ikosipodus caroliensis* gen. et sp.n., an interstitial neotenic polychaete from North Carolina. USA, and its phylogenetic relationships with the Dorvilleidae. – Zool. Scr. *11*, 117–126.

Westheide, W., 1988. Genital organs. – Microfauna mar. *4*, 263–279.

Westheide, W. & Schmidt, P., 1974. *Trilobodrilus axi*. Nahrungsaufnahme und Fortpflanzung. – Encycl. cinematogr. *E 1955*. 1–12.

Westheide, W. & Riser, N. W., 1983. Morphology and phylogenetic relationships of the neotenic interstitial polychaete *Apodotrocha progenerans* n.gen, n.sp. (Annelida). – Zoomorphology *103*, 67–87.