Ultrastructure and functional morphology of male genital organs and spermatophore formation in *Protodrilus* (Polychaeta, Annelida)

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Summary. The structure and functional morphology of lateral organs and sperm ducts, as well as the mechanisms of spermatophore formation and transfer, are investigated by means of light and electron microscopy in the genus *Protodrilus*. The sperm ducts are simple, ciliated, intercellular gonoducts with a funnel section surrounded by a thin muscle layer and a tube section opening externally in the anterior region of the lateral organs. No glands are present in the sperm ducts. The lateral organs are formed by long epidermal invaginations enclosing an elongate lumen into which numerous cilia project and a large number of glands open. Five to ten different gland types with strikingly distinctive secretory granules are found in the different species. In addition, special supporting cells, the so-called "sponge cells," sensory cells and an underlying nervous tissue are developed in the lateral organs. It is stated that apart from some similarities to the ventral atrium of *Nerilla antennata* no corresponding organs are known within the Annelida. It is argued that in *Protodrilus* the spermatophores are formed by the lateral organs as there are a high number of glands opening into the lumen of the organ. The possible origin and genesis of the male gonoducts as well as the mode of spermatophore transfer in *Protodrilus* is discussed.

A. Introduction

The genus *Protodrilus* is comprised of approximately 30 species of small interstitial polychaetes the first of which, *P. purpureus*, was described in 1868 by Schneider from Helgoland, North Sea. At the beginning of this century Salensky (1907) and Pierantoni (1908) presented two detailed works on the morphology and systematics of *Protodrilus*. After a purely structural study on the nephridia, gonoducts and dorsal organs by Goodrich (1931), intensive light microscopical investigations of the systematics and morphology of this genus were conducted by Jäger-

sten (1952) and Jouin (1970a, b). Jägersten discovered the taxonomic importance of the traits of the male and female reproductive system and developed a taxonomy based on these characters which was later extended by Jouin (1970a, b).

Sperm ducts and the so-called lateral organs, the existence of which was discovered first by Pierantoni (1908), were noticed in the male individuals in all *Protodrilus* species, which were thoroughly described up to now (see von Nordheim 1989a). Despite the high taxonomic value of the reproductive organs, comparatively little is known about their morphology and function. Whereas in a separate publication the ultrastructure of the female reproductive system of *Protodrilus* is presented (von Nordheim 1991), further detailed information about the exact structure and function of the lateral organs, the sperm ducts and the mode of spermatophore formation and transfer in the males is needed. In the present paper a combined analysis of light and electron microscopy is used to present a functional interpretation of the morphology of the respective male organs of *Protodrilus*.

B. Material and methods

The following *Protodrilus* species were investigated: *P. purpureus* (Schneider, 1868), *P. oculifer* Pierantoni, 1908, *P. rubropharyngeus* Jägersten, 1940, *P. ciliatus* Jägersten, 1952, *P. adhaerens* Jägersten, 1952, *P. helgolandicus* von Nordheim, 1983, *P. jaegersteni* von Nordheim, 1989, *P. gracilis* von Nordheim, 1989, *P. submersus* von Nordheim, 1989.

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

For TEM studies, following narcotization with 4% MgCl₂ solution (see above) for 20 min, fixation was carried out for 2 h at

4° C with 2 or 3 renewals of fixation liquid. After rinsing for 2–3 h with several renewals of 0.1 M phosphate buffer (pH 7.3) and post-fixation for 1 h at 0° C with 1% OsO₄ solution in phosphate buffer, the material was dehydrated in an ethanol series, transferred in 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.

Specimens for SEM studies were fixed in cacodylate buffered 2.5% glutaraldehyde (pH 7.2) and postfixed in 1% osmium tetroxide. After dehydration and critical point drying, the material was coated with gold. Photographs were taken with a Zeiss Novascan 30.

C. Results

I. Sperm ducts

The males of all *Protodrilus* species investigated possess a species-specific number and position of sperm ducts in anterior body segments that are always paired, and simply constructed coelomoducts. Their funnel-shaped opening sections are located in the coelom cavities above the transverse muscles lateral to the gut (Fig. 1 A, B). They can be found in all species in the posterior third of the body segments or immediately in front of the posterior dissepiment. In the region of each dissepiment, the sperm duct narrows, extends through the dissepiment, and opens laterally in the anterior section of the lateral organ of the following body segment (Figs. 1 A, 2 B–D, 3 C, 6). Having basically the same function, the morphology of the sperm ducts is very similar in all species investigated. Thus the structure will be exemplified in the following in detail for *P. helgolandicus*.

This species has three pairs of sperm ducts (the total length of each is approximately 80 µm) in body segments 6, 7 and 8, with external openings in the lateral organs of segments 7, 8 and 9 (Figs. 2 B, C, 3 C, 5 A, 6 A). The large internal opening funnel has a maximum external diameter of 18 × 28 µm and a length of about 20 µm (Figs. 1 B, 2 A–C). Its lumen is 14 × 24 µm in diameter and is surrounded by five or six multiciliated cells ("ciliated cells") the membranes of which are connected by septate desmosomes and zonulae adhaerentes. Each ciliated cell has a round or shapeless basal nucleus, dictyosomes, a few cisternae of rough ER, numerous spherical mitochondria and many electron-dense granules, 80–120 nm in diameter, beneath the apical cell membrane. Approximately six cilia can be found per µm² cell surface area and the basal sections of the cilia are surrounded by thin microvilli (Figs. 1 B, 2 A). The cytoplasm of the ciliated cells forms, in the anterior section of the sperm ducts, a thin but extensive layer around the lumen and contains mainly mitochondria and granules (Fig. 2 A). More posteriorly the ciliated cell cytoplasm is distinctly thicker and contains the nucleus (Fig. 1 B). In this section the diameter of the lumen is only about 9 × 11 µm. The funnel part of the sperm ducts is completely surrounded by a thin layer of circular and longitudinal muscles, without a basal lamina separating these layers.

Directly behind the funnel section, the diameter of the sperm duct is abruptly reduced to a tube that penetrates the posterior septum and runs beneath the transverse muscles to the lateral organs (Figs. 2 C, 5 A). The lumen of the tube is about 7.5 × 8.5 µm in diameter and walled by three or four ciliated cells bearing approximately nine cilia per µm² and numerous, rod-shaped microvilli (Fig. 3 D). As in *P. rubropharyngeus* and *P. gracilis*, the many cilia fill the lumen (Fig. 3). In *P. helgolandicus* the ciliated cells of the tube are connected to the epidermal basal lamina by cytoplasmic processes. The tube is not surrounded by muscle tissue (Fig. 5 A).

In the funnel section of the sperm ducts some "paraspermatozoa" (von Nordheim 1989 b) are normal, as are the acrosomes of numerous "euspermatozoa" (Fig. 2 A). At the same time in the tube section, only a few paraspermatozoa can be found. The same results were obtained from *P. gracilis* (Fig. 3 A), *P. rubropharyngeus* (Fig. 3 B), *P. purpureus*, *P. ciliatus* and *P. oculifer*.

Terminally the diameter of the sperm duct tube diminishes to 3 × 3 µm, the tube penetrates the epidermal basal lamina a little below the lateral organ and opens into the anterior area of the organ. Here, in cross-section, the tube is formed by two or three ciliated cells, the basal processes of which are connected to the basal lamina by hemidesmosomes.

Ciliary movement is normally not observed in the inactive sperm ducts of live animals. During the transport of spermatozoa through the duct into the lateral organ, however, slow ciliary movement can be observed which seems to facilitate the gliding of the spermatozoa. Before deposition the spermatozoa lie parallel to each other in large bundles in the coelomic cavities of those segments possessing sperm ducts (Figs. 2 B, C). At this stage in *P. gracilis* and *P. adhaerens*, the spaces between spermatozoa are filled by a grey, granular substance which is possibly formed by coelomic cells and might guarantee, as it is a viscous substance, the cohesion of the sperm bundle (von Nordheim 1989 b).

Spermatophores or constituents of spermatophores were not found in the coelom cavities or sperm ducts in any of the species investigated. Further, no glands are present that could secrete their contents into the sperm ducts. Sperm ducts and nephridia were never noticed in the same segment in *P. helgolandicus*, whereas in *P. ciliatus* in one male a presumptive rudimentary nephridal canal was found close to the sperm duct which was typically developed.

II. Lateral organs

In all *Protodrilus* species with detailed species descriptions the authors mention the presence of so-called "lateral organs" in the male individuals. These structures were determined to be lateral organs by their position on the sides of a limited number of body segments. These paired organs develop in their typical, species-specific structure, number and body region only in fully mature males. Normally the first and last pair of organs are morphologically different from the others (Fig. 4). The first organ is often rosette-shaped with a median pore
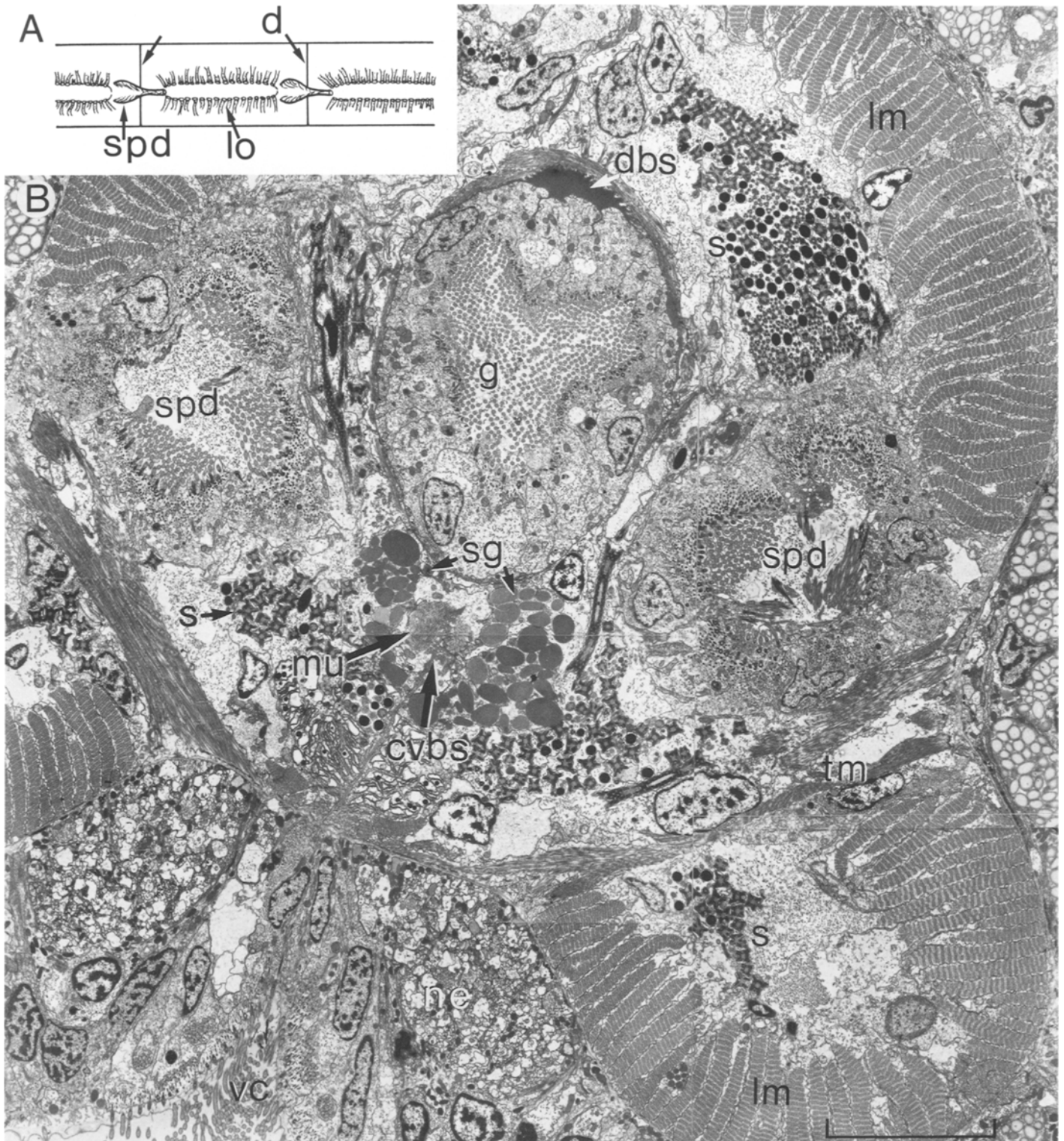
Fig. 1A, B. *P. helgolandicus*: **A** Schematic presentation of the position of the sperm ducts (*spd*) and lateral organs (*lo*); lateral view. **B** Cross-section of sperm duct region in body segment 8. Gut (*g*), dorsal and contractile ventral blood sinus (*dbs, cvbs*), salivary glands (*sg*), spermatozoa (*s*), muscles (*tm, lm*) and ventral ciliary band (*vc*). All scale bars: 10 μm

that is surrounded by one single gland type (Fig. 4A, F). The last organ, however, usually, possesses a particularly strong and dense ciliation and several sensory cilia (Fig. 4B, D). In some species it extends from the lateral to the dorsal side, where it even can meet, as in *P. leuck-* *arti*, the organ of the opposite side (Jouin 1970a). In a species-specific number the sperm ducts open only into the anterior section of the hindmost lateral organs (Fig. 4D, 6). In all species more pairs of lateral organs than pairs of sperm ducts are developed.
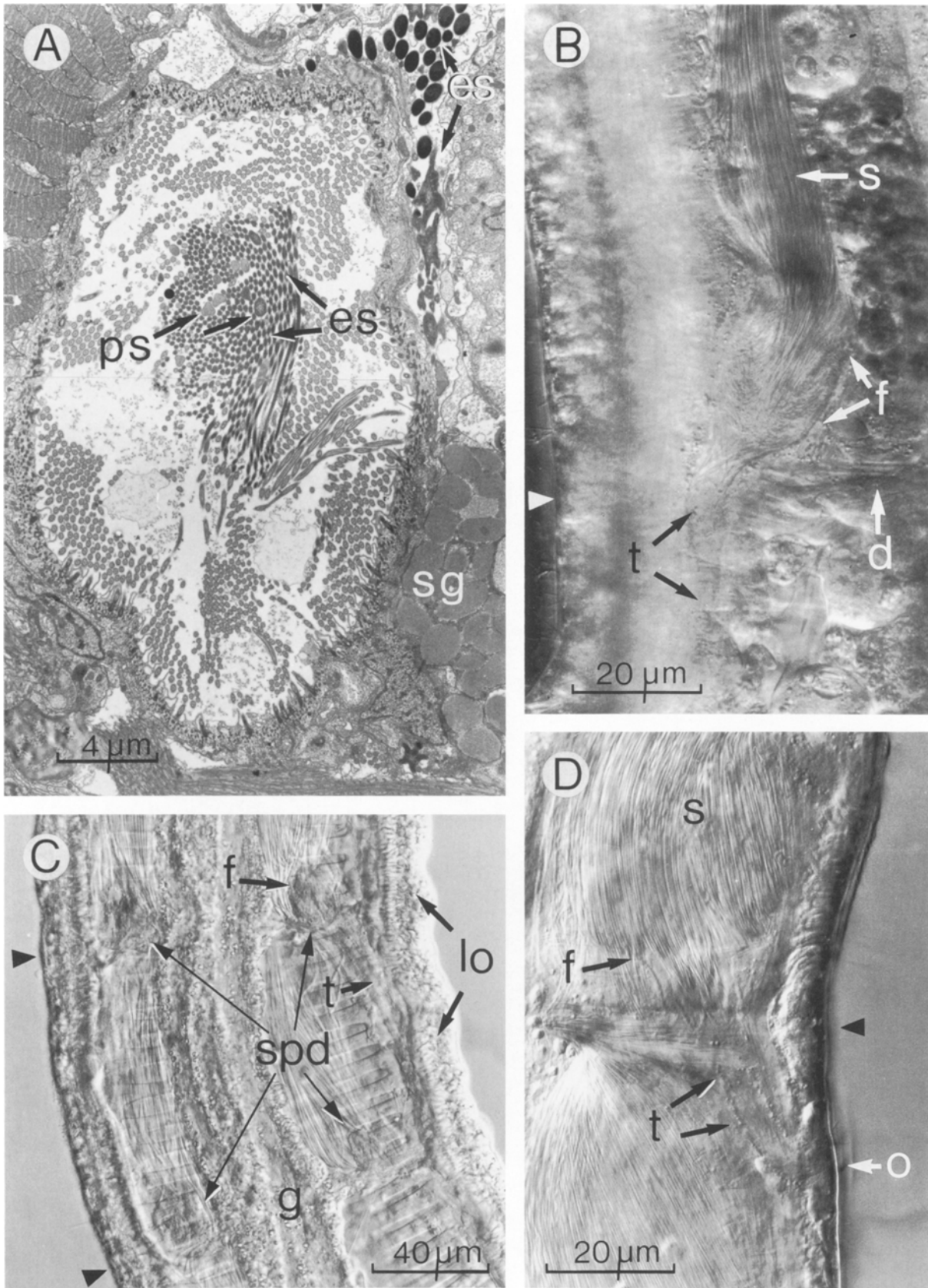
Fig. 2. A–C *P. helgolandicus*: **A** Cross-section through sperm duct funnel and different sections of eu- and paraspermatozoa (*es*, *ps*); salivary glands (*sg*). **B** Sperm bundle and different regions of the sperm duct, dorsal view. **C** Dorsal view of two pairs of lateral organs and sperm ducts in segments 8 and 9. **D** *P. ciliatus*: Dorsal view of sperm bundle, sperm duct and opening, lateral organ. *Lateral arrows* indicate segment borders
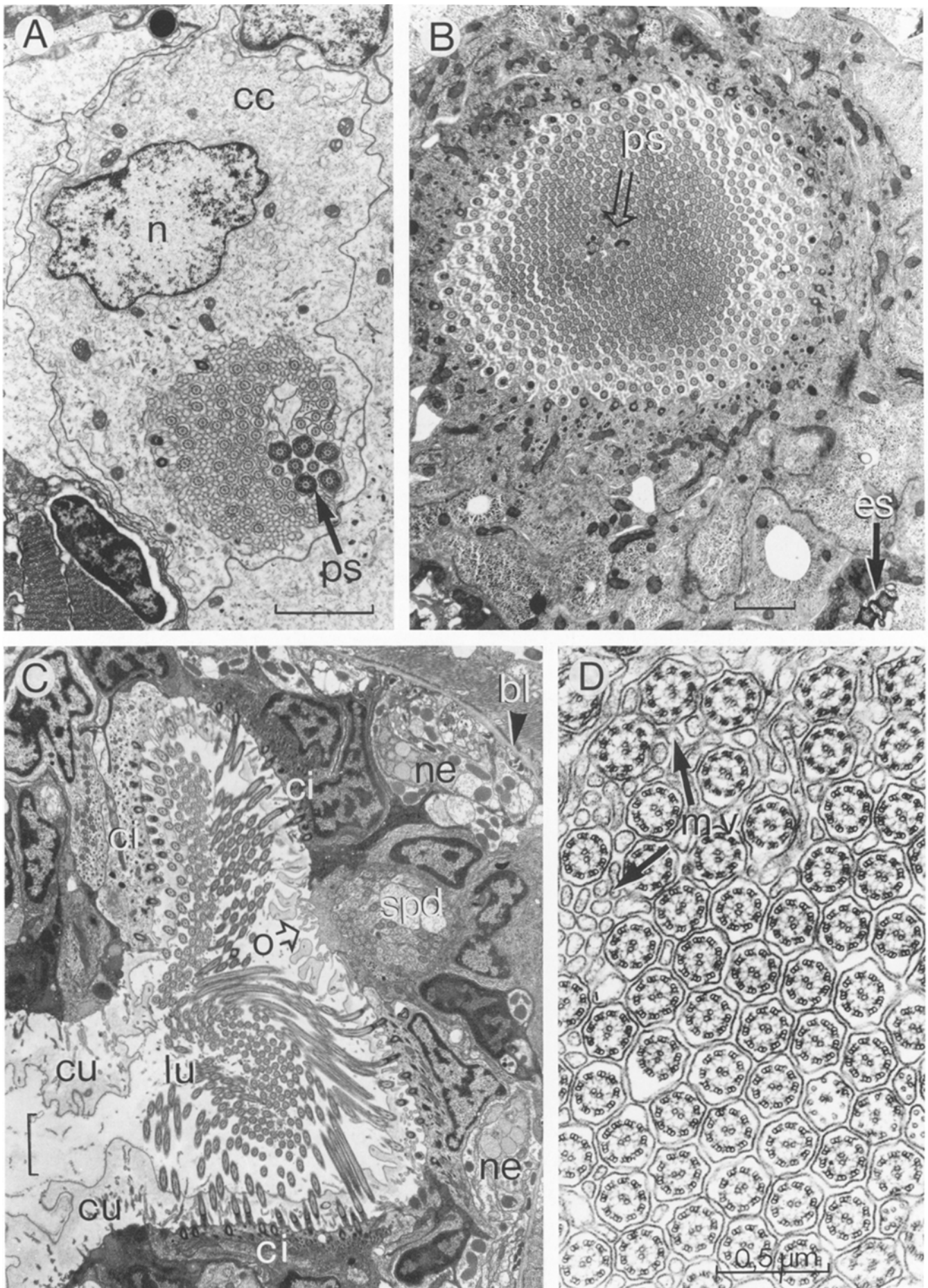
Fig. 3. A, B Cross-section of sperm duct tubes with paraspermatozoa (*ps*): **A** *P. gracilis*. **B** *P. rubropharyngeus*. **C, D** *P. helgolandicus*, cross-sections: **C** Lateral organ and sperm duct opening. **D** Sperm duct tube; showing cilia and microvilli. **A–C** Scale bar: 2 μm
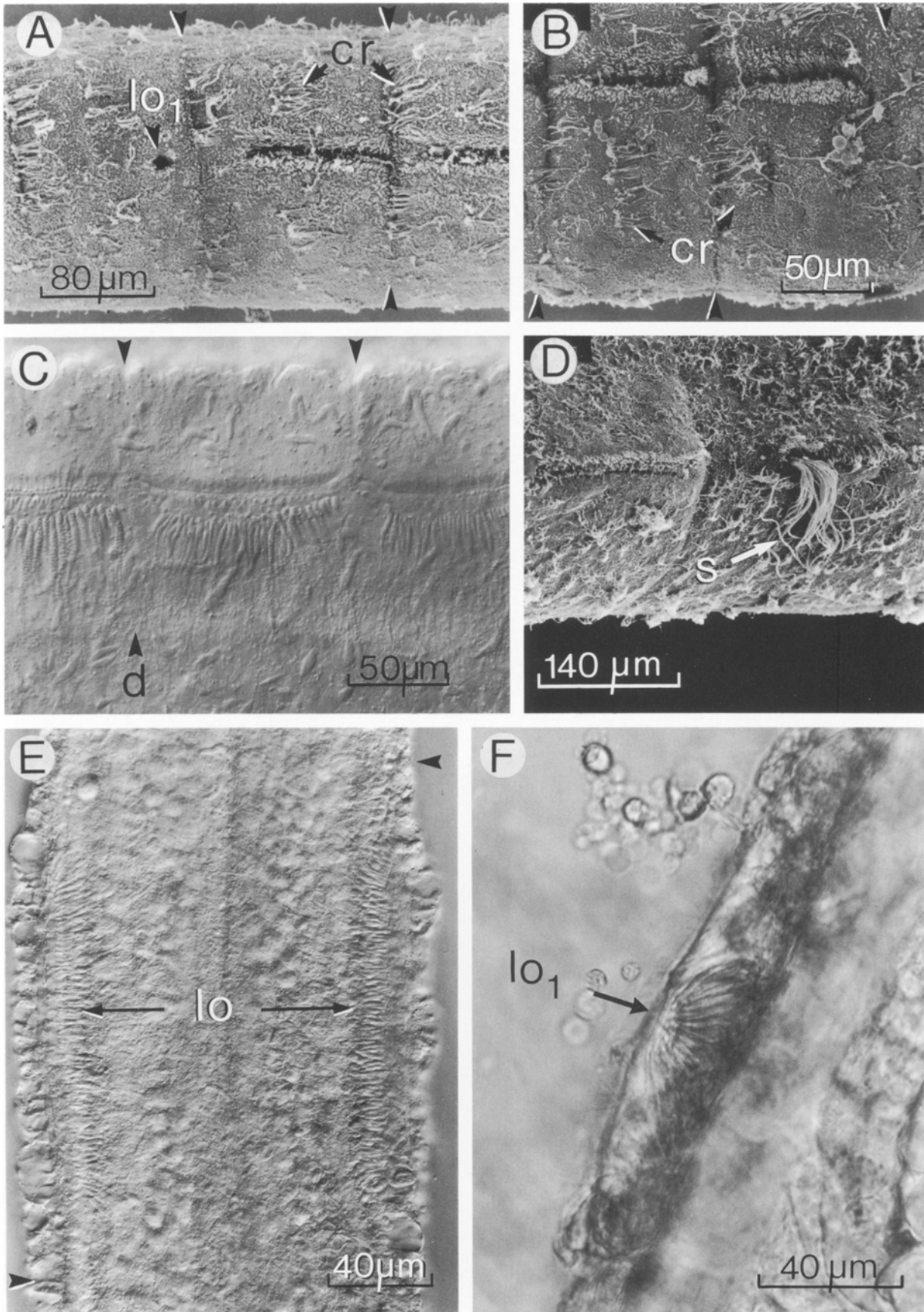
Fig. 4A–F. Lateral organs. **A–D** Lateral views. **E, F** Dorsal views. **A** *P. ciliatus*: First, second and third organ. **B** *P. ciliatus*: Last but one and last organ. **C** *P. ciliatus*: Middle organs showing the typical arrangement of glands. **D** *P. purpureus*: Last but one and last organ with sperm duct opening and with emerging spermatozoa caused by the fixation process. **E** *P. helgolandicus*: Last organ; glands. **F** *P. submersus*: First organ; glands. *Lateral black arrows* indicate segment borders

The morphology of the lateral organs is basically similar in all species investigated. Therefore these structures in *P. helgolandicus* will exemplify the details for all species. Further, since the first organs in most species show a slightly different morphology (as is mentioned above) and probably only play a secondary role in reproduction, only the structure of the lateral organs in which sperm ducts open externally will be described. Structural differences between lateral organs in different species mainly involve the number and character of the different gland types. So, for example, in a lateral organ of *P. helgolandicus* eight different gland types can be identified, in *P. adhaerens* ten and in *P. gracilis*, five, each with a species-specific ultrastructure (for more details concerning the latter two species, see von Nordheim 1987).

P. helgolandicus has four pairs of lateral organs in the segments 6, 7, 8 and 9. Under the light microscope they can be noticed as continuous lateral grooves bordered by many glands and cilia (Figs. 2C, 4E). Whereas the organs of segments 6 and 9 extend over only two thirds of the length of segment, the organs of segments 7 and 8 reach from the anterior to the posterior dissepiments and are approximately 120 µm long.

Lateral organs are exclusively epidermal formations basally defined by a basal lamina. The epidermis in this region is deeply invaginated in *P. helgolandicus* thus forming a broad, long lumen into which all lateral organ glands open and numerous cilia project (Figs. 3C, 5A, 6A). The lumen is completely covered by a more or less thin cuticle, that is interrupted only by the openings of glands (i.e. "microvillar crowns") and by the sperm duct opening (Figs. 3C, 5A, 7A, D, E, 8C). As in *P. purpureus* (Fig. 4D), *P. adhaerens*, *P. jaegersteni*, *P. ciliatus* or *P. rubropharyngeus* the sperm duct opening is in the anterior section immediately in front of the area of the different gland openings (Figs. 3C, 6).

The different cells of the lateral organ in *P. helgolandicus* are comprised of numerous gland cells, some supporting cells and few sensory cells. Further, large vacuolized cells (determined as "sponge cells") and several multiciliate cells ("ciliated cells") develop that correspond ultrastructurally to similar cells of the ventral ciliary band. An irregular layer of glia-like cells and nervous tissue containing vesicles of neuropeptides lies just above the basal lamina (Figs. 3C, 5A).

The ciliated cells have very broad apices and possess four or five cilia per µm² of cell surface area (Fig. 5B, C). Two ciliary rootlets each make angles of almost 90° with the longitudinal axis of the cilia; the longer root can be up to 4.5 µm long (Fig. 5C). Normally each long rootlet is paralleled by a long, rod-shaped mitochondrion. ER, Golgi apparatuses, diverse granules and a broad, flattened nucleus can be found directly below the roots. The cells in many cases taper basally and connect to the basal lamina by stalk-like processes (Figs. 3C, 5A).

Mono- or multiciliate sensory cells occur sporadically on the exterior parts of the lateral organ. No sensory cells can be found in the lumen. Typical supporting cells develop mainly in the exterior sections of the organs and in the region of the sperm duct opening, whereas they are clearly outnumbered by gland cells in the lumen. Often their cell bodies taper basally and apically contain the nucleus (Figs. 3C, 5A).

The sponge cells probably represent a special type of supporting cells. They are often cup-shaped, slightly tapering apically with comparatively broad bases (Figs. 5A, 7A). They are connected by hemidesmosomes to the basal lamina by a narrow, basal, cytoplasmic process. The nuclei are always found basally together with some mitochondria, Golgi, and RER (Fig. 7A, C). The largest part of the cell body consists of a large vacuolized area ("lumen") that is surrounded by a thin cytoplasmic layer. This layer contains all typical cell constituents of apical sections of "normal" supporting cells, such as light vesicles of dictyosomes, larger dark and light granules, and terminally numerous, irregular microvilli with internal filaments (Fig. 7A, B, C). The lumen of each sponge cell is completely filled by a labyrinth of cisternae, vacuoles and vesicles of varying shape and size. These structures originate from numerous foldings and ligatures of the cell membrane above the basal nucleus and of the inner cell membrane of the lateral "cytoplasmic walls" (Fig. 7C). In contrast to the gland cells, the sponge cells have no internal, apical ring of microtubules or a microvillar crown and no distinct opening into the lumen of the lateral organ.

All glands of the lateral organ are typical, exocrine, merocrine gland cells. The basal synthetic areas are normally not found in the lateral organ proper but are positioned more dorsally or ventrally. They contain the nucleus, large cisternae and tubes of RER and dictyosomes. The more-or-less long excretory ducts of the glands are always surrounded internally by rings of microtubules that are parallel to the longitudinal axis (Figs. 7D, 8D). Apically the microtubules in cross-section are arranged in semi-circular groups (Fig. 8D). The excretory canal is filled with secretion granules which, due to hydration or dehydration processes, partly change their size and structure until they are shed into the lumen of the organ. The openings of the excretory tubes penetrate the cuticle and are surrounded by a microvillar crown (Figs. 6A, 7D, E). In *P. helgolandicus* eight different types of glands (*gl* 1–8) secrete their products into the lumen of the lateral organ. Especially in the middle region of the organ, the secretory areas are clearly divided by three rows of large sponge cells into a lower, a median and an upper section (Fig. 5A). All secretion granules are surrounded by membranes.

Gland type 1 has homogeneous, dark-grey granules with peripheral light areas (Figs. 7A, D, 8B, D). The granules are egg-shaped or long and oval to ellipsoid (size 0.4×0.25 to 1.0×0.35 µm). The synthetic areas are usually ventral to the lateral organ while the excretory canal opens into the lumen between the lower and middle rows of sponge cells (Figs. 5A, 7A, 8B).

The synthetic area of the second gland type is dorsal as well as ventral to the lateral organ, but the glands always open between the middle and upper sponge cells. The granules are round to ellipsoid ($\emptyset$ = diameter approximately $0.6–1.2 \times 0.7$ µm) and have large light centres with an occasional dark spot in the middle. The
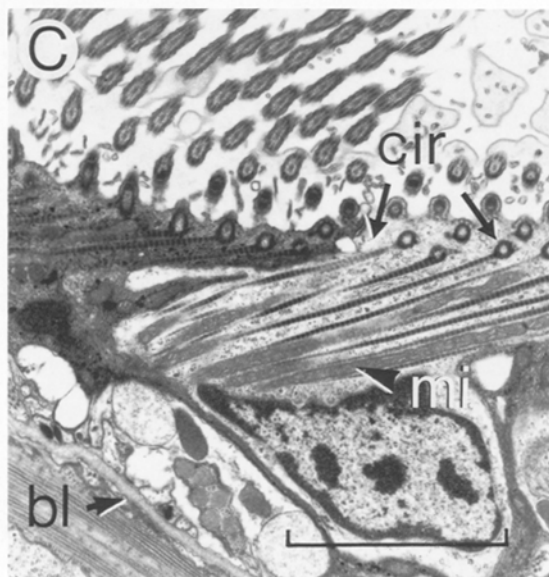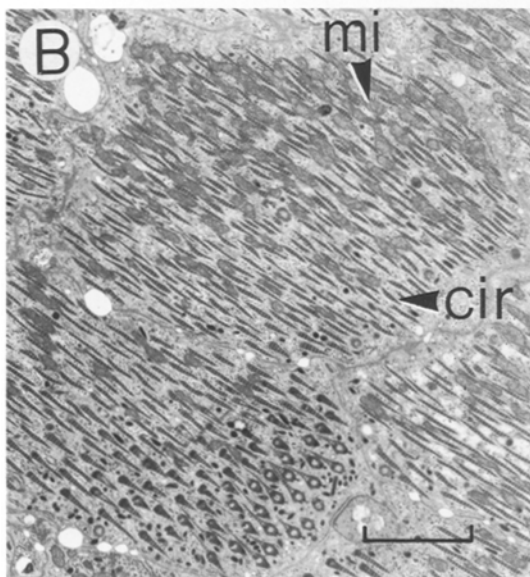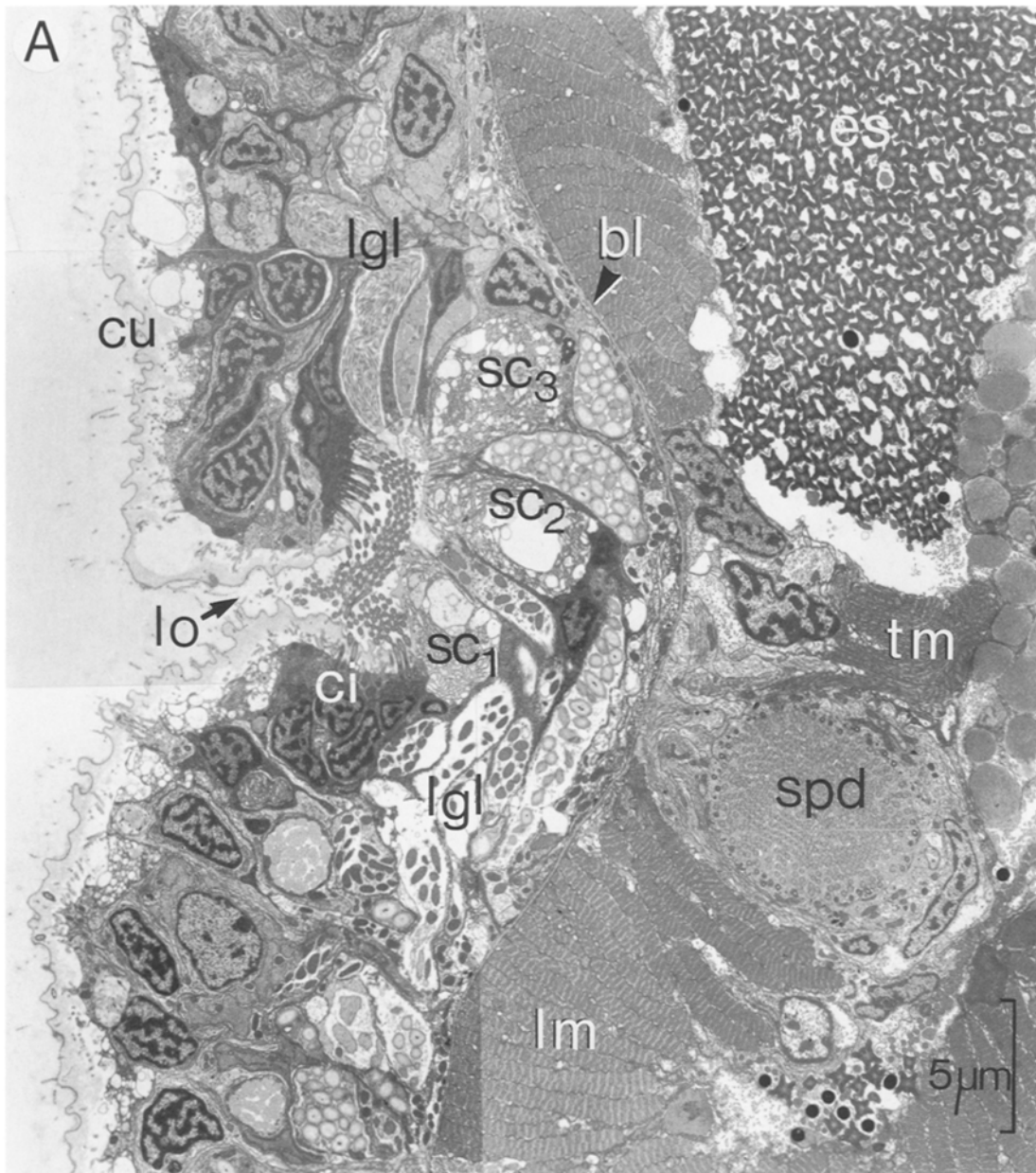
Fig. 5A–C. *P. helgolandicus.* **A** Cross-section of lateral organ and sperm duct. **B** Ciliate cells with ciliary roots and mitochondria. Tangential section beneath the cell surface. **C** Cross-section of ciliate cell showing ciliary roots. All scale bars: 5 μm
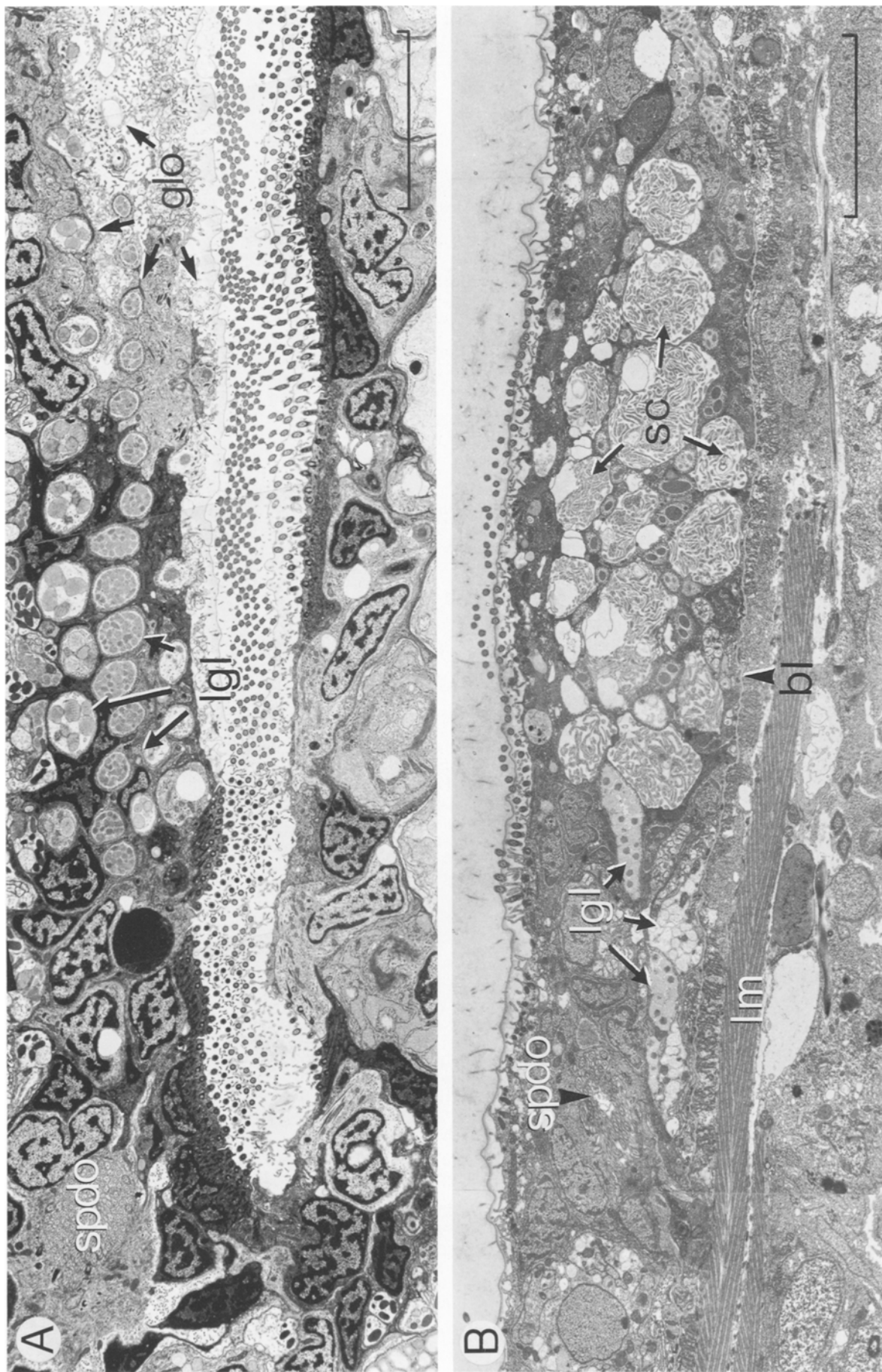
Fig. 6A, B. Longitudinal sections of lateral organs and sperm duct openings: **A** *P. helgolandicus*; tangential vertical section. **B** *P. adhaerens*; horizontal section. All scale bars: 5 μm
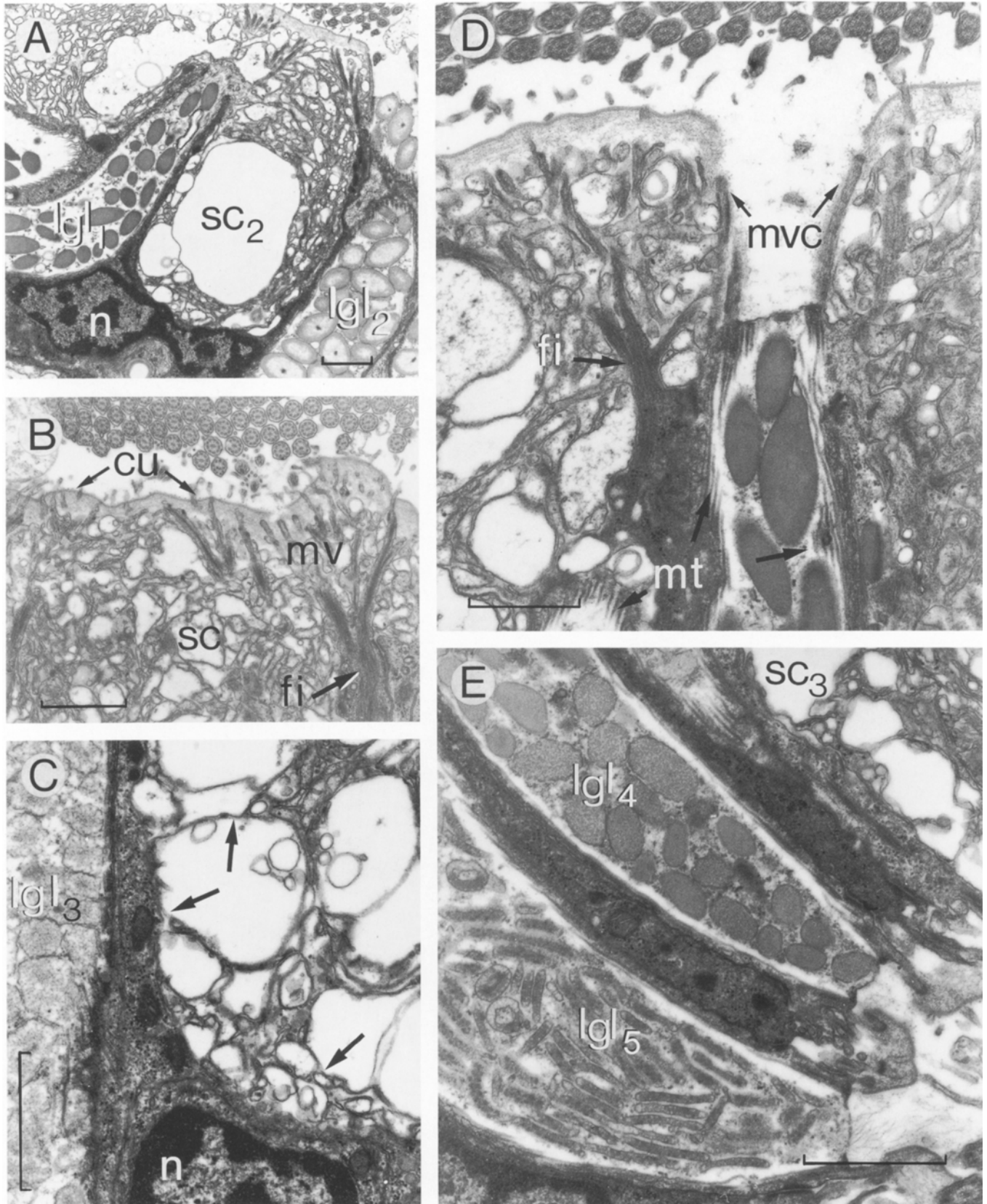
Fig. 7A–E. *P. helgolandicus*, cross-section: **A** Sponge cells (*sc*) and lateral organ glands (*lgl*). **B** Apical area of sponge cell; cuticle. **C** Basal area of sponge cell with cytoplasmic "walls" (*arrows*). **D** Sponge cells; microtubules (*mt*) and microvilli crown (*mvc*) of gland type 1. **E** Apical areas of gland cells type 4 and 5; sponge cell. All scale bars: 1 μm
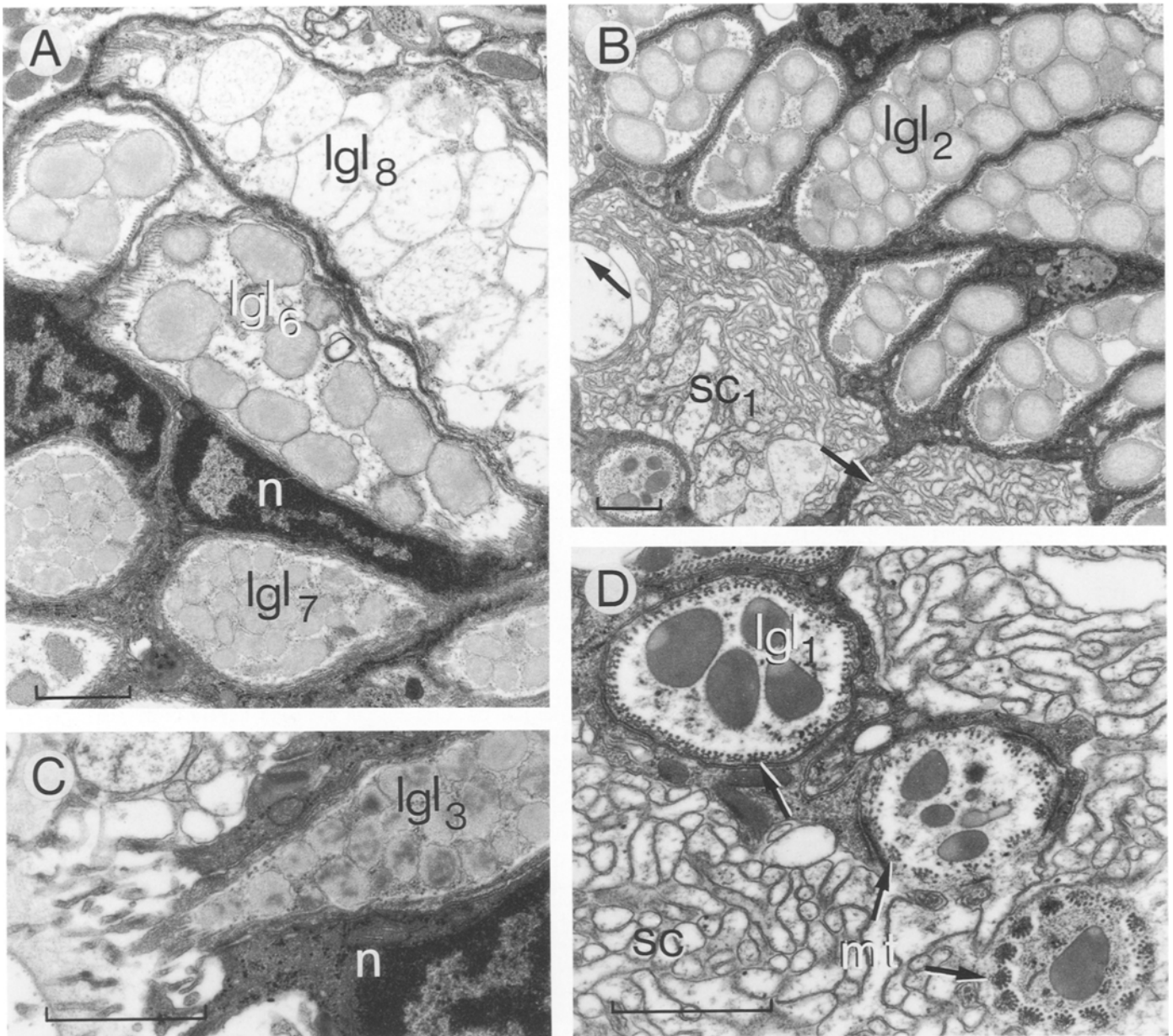
Fig. 8 A–D. *P. helgolandicus*, sponge cells, different types of lateral organ glands and gland cell openings: **A**, **B**, **D** Tangential longitudinal section. **C** Cross-section. All scale bars: 1 μm

centre is surrounded by a grey mantle layer that is approximately 80–100 nm thick (Figs. 5A, 7A, 8B). The granules are transversed by numerous thin, irregular filaments that may meet in the centre.

Gland types 3, 4, 5 and 6 open exclusively in the upper region of the lumen (Figs. 5A, 7E, 8B, C, D), the synthetic area of each lies dorsal to the lateral organ. The granules of gland type 3 are round to oval approximately 0.3–0.5 μm in diameter with a very loose, filamentous substructure and a slightly darker central area (Figs. 7C, 8C). Gland type 4 contains oval to round, dark-grey secretion granules (∅ 0.25–0.3 × 0.6 μm). They show two different substructures that are very likely due to different states of hydration. While most granules with comparatively larger volume have a regular, coarse-granular structure, the smaller granules consist

of very homogeneous, fine-granular material (Fig. 7E). A special morphological diversity is found in the secretory products of gland type 5 (Fig. 7E). Normally they are rod-shaped (length 0.55 μm, width 0.06 μm), but some also form rings or discs (∅ approximately 0.25–0.3 μm). The light-grey secretory bodies contain thin longitudinal or transversely arranged filaments as well as dark granules (∅ 25 nm). Gland type 6 contains large round or oval secretory granules (∅ 0.7–0.6 × 0.8 μm) surrounded by a wavy membrane and containing numerous concentrically arranged filaments. The centre of each granule is slightly more dense than the periphery (Fig. 8A).

In the anterior region of the lateral organ just behind the opening of the sperm duct two further types of lateral organ glands can be found (types 7 and 8). The granules

of gland type 7 consist of a heterogeneous, light-grey, granular substance and are almost round or oval ($\emptyset$ 0.18–0.6 × 4.5 µm). Immediately before shedding their contents into the lumen the fine membrane of the granules dissolves (Fig. 8A). Gland type 8 secretes its content as large, round, spongy granules ($\emptyset$ 1.5–2.0 µm) the membranes of which also dissolve shortly before or at secretion into the lumen (Fig. 8A). In the synthetic area the light secretory product has finely branching filamentous substructures that condense in the excretory tube to a few small granules ($\emptyset$ 30–45 nm).

D. Discussion

I. Morphology and development of the sperm ducts of Protodrilus

The morphology of the sperm ducts in *Protodrilus* is similar to that described for the polychaetes in general by Westheide (1988). The present results correspond to the light microscopical findings of Jägersten (1952) and Jouin (1970a, b). These authors described the sperm ducts of this genus as always being paired organs in a species-specific number of certain anterior or median body segments which structurally resemble simple gonoducts. Each organ consists of a funnel-shaped opening section that is surrounded by a thin muscle layer and which projects into the coelomic cavity. A subsequent short excretory tube penetrates the posterior dissepiments and opens externally in the anterior region of the lateral organ of the following body segment. Goodrich (1931) supposed that the opening section of the lateral organ in *P. flavocapitatus* can be evaginated like a penis. Since a similar mechanism could not be demonstrated for any of the *Protodrilus* species investigated here, including *P. rubropharyngeus*, Goodrich's assumption must be generally denied for the genus. *P. rubropharyngeus* is very likely a synonym for *P. flavocapitatus* (see Jägersten 1952; Jouin 1970a, b; von Nordheim 1989a).

Among all species investigated, only in mature males of *P. ciliatus* were rudimentary nephridial organs (protonephridia) found in body segments containing sperm ducts. Similarly, the females of *P. purpureus*, *P. haurakiensis* and *P. rubropharyngeus* do not have nephridia in segments possessing oviducts (von Nordheim 1991). Also, no nephromixia are developed in the males or females of the species investigated and in the different gonoduct sections no structures were found which indicate processes of filtration, secretion or reabsorption (see also von Nordheim 1991). Only in females of *P. brevis* have large complex "protonephromixia" in all fertile body segments been described by Jouin (1970a, b), which she believes to be a new character of this species.

Since the male sperm ducts resemble the female oviducts to a large extent (von Nordheim 1991), an identical origin must be considered. It seems most likely that the gonoducts are formed as new organs with the onset of sexual maturity. Jouin (1970b) concluded that for males this process is probably generally accompanied by simul-

taneous reduction of the protonephridia in the respective segments. This conclusion is supported by my observations of a mature male of *P. ciliatus*, in which there was a well-developed sperm duct next to a presumptive persistent nephridial tube. In addition, ultrastructural investigations of the large protonephridia of *P. rubropharyngeus* (Schrader and von Nordheim, unpublished data) revealed the sperm ducts of this species to be distinctly different, especially the tube sections. Westheide (1988) also observed in *Microphthalmus carolinensis* certain ultrastructural differences between the ejaculatory ducts and the nephridial duct.

II. Lateral organs and formation of spermatophores

Ripe spermatozoa formed in fertile body segments gather in those segments containing the sperm ducts where they form distinct sperm bundles of eu- and paraspermatozoa (von Nordheim 1989b). When they are discharged and pass through the sperm duct opening into the lateral organ, the spermatozoa are immediately enclosed in a round (*P. rubropharyngeus*) or an elongate (*P. helgolandicus*) spermatophore with a short stalk. As already concluded by Jouin (1970b) the spermatophore sheath is formed by secretions from some of the lateral organ glands. Formation of the spermatophores in the sperm ducts as presumed by Jägersten (1952) must be denied, because neither the tube cells nor any other gland cell secretes into the sperm duct.

Spermatophores in *Protodrilus* were first described by Jägersten (1952) for *P. rubropharyngeus*. Others were reported in *P. brevis* and *P. albicans* (see Jouin 1970a, b), and *P. helgolandicus* (see von Nordheim 1983). Thus it seems likely that all *Protodrilus* species form spermatophores. Since no direct spermatophore transfer has been observed so far in this genus, spermatophores are probably generally transferred indirectly to the females. Jägersten (1952) noticed that spermatophores deposited by the male *P. rubropharyngeus* onto sediment grains were subsequently picked up by females that glided over them. Because of this indirect transfer mode, apart from forming the spermatophore sheath, some additional functions of the 5 to 10 (depending on the species) different gland secretion types must be postulated. In the leech *Glossiphonia complanata*, for example, Damas (1968a, b) could establish 11 different gland types, the secretions of which form either the spermatophore sheath or its stalk or are enclosed together with the spermatozoa in the spermatophore.

In *Protodrilus* the secretory products of some glands may possibly serve as a nutrient for the spermatozoa in the spermatophore, others could have a species-specific pheromone function to attract the females. The stalk of the spermatophore, as in *G. complanata*, might contain histolytic secretions to dissolve the female epidermis at contact, because most *Protodrilus* species lack any special epidermal structures for spermatophore reception. Such organs ("dorsal organs") are described only for *P. flavocapitatus* and *P. rubropharyngeus* (see Salensky 1907; Jägersten 1952; von Nordheim 1987, 1991).

Fertilization by means of spermatophores is quite common among different taxa and is described in invertebrates, for example, for different groups of Plathelminthes, Nemathelminthes, Arthropoda, Mollusca, Pogonophora and Annelida (summarized by Mann 1984). Although Westheide and Ax (1965) considered this mode of sperm transfer to be "a common life form character of the interstitial fauna of marine sands", among true interstitial polychaetes indirect sperm transfer by spermatophores was described, apart from *Protodrilus*, only for *Hesionides arenaria* (Westheide and Ax 1965; Westheide 1982, 1984) and *Microphthalmus aberrans* (Westheide 1967). In contrast to *Protodrilus*, in these two hesionids the spermatophores are formed by secretory products of glands in the genital duct and are directly attached to the females by special copulatory structures.

In the semi-interstitial polychaete *Nerilla antennata* long stalked, egg-shaped spermatophores, similar to *Protodrilus*, are not directly transferred to the females but deposited at random on the sediment (Magagnini 1982). Subsequently the females lay clutches of eggs next to the spermatophores which open up and release the spermatozoa. Magagnini concluded that certain substances of the spermatophore sheath stimulate the females to deposit their eggs at those spots. In the males of *Nerilla antennata* six sperm ducts converge, together with the exretory canals of large gland cells, in an unpaired, median genital atrium on the ventral side that opens externally by a single pore (Goodrich 1912). It is likely that the spermatozoa are enclosed in the atrium in a sheath formed by the secretory products of the glands. In this case a surprisingly high similarity would exist between the function of the strongly ciliated atrium of *Nerilla* and the lateral organs of *Protodrilus*. Also in both genera the mode of spermatophore deposition is very similar, although in *Nerilla antennata* the fertilization of the eggs is external.

Among non-interstitial polychaete families, for which also indirect sperm transfer by means of spermatophores onto or close to the sexual partner is described (summarized by Schroeder and Hermans 1975; Mann 1984), the comparison of *Protodrilus* with different Spionidae is especially interesting. The formation of spermatophores was observed in eight species of different genera of Spionidae (Rice 1978). Like *Protodrilus*, most of these species transfer the spermatophores indirectly but shed their balloon-shaped, ellipsoid or thread-like spermatophore freely in the ambient sea water. Finally these planktonic spermatophores are picked up by the females (Greve 1974; Mann 1984). Only *Microspio mecznikowianus* and *Scolelepis squamata* place their spermatophores in an unknown manner directly onto the females (Cerruti 1908; Richards 1970; Rice 1978).

In males of *Pygospio elegans* the spermatophores are probably formed by large gland cell complexes in the terminal sections of modified nephridia in a various number of median body segments (Schlötzer-Schrehardt 1987). The openings of these paired nephromixia are on the dorsal sides of segments 3 to 35 or 40 lateral to relatively short transverse ciliary bands (so-called "dorsal organs"). These organs show no closer similarity to the lateral organs of *Protodrilus*, because, in addition to their transverse orientation, they consist exclusively of ciliate cells and do not possess any accessory glands (Schlötzer-Schrehardt 1987). She concludes that in male *Pygospio elegans* the spermatophores are only transported by means of these "dorsal organs" to special arterior "dorsal appendages" of the second setiger, by which they can directly be transferred to the females.

The ultrastructure of the formation of spermatophores in spionid polychaetes was described by Rice (1980) for *Polydora ligni* and *P. websteri*. In these species paired nephromixia in sperm-producing segments serve as gonoducts. The distal halves of these ducts are distinctly swollen, as in distal sections the spermatozoa gather shortly before discharge and are enclosed in long thin or only terminally enlarged spermatophores (Rice 1980). Although the mode of spermatophore formation and spermatophore shape is clearly different in *Protodrilus* and the Spionidae, most species of both groups appear to have an indirect mode of sperm transfer and internal fertilization.

Abbreviations used in the figures

bl	basal lamina	*mt*	microtubules
cc	coelomic cell	*mu*	muscle
ci	ciliated cell	*mv*	microvilli
cir	ciliary root	*mvc*	microvillar crown
cr	ciliary ring	*n*	nucleus
cu	cuticle	*ne*	nervous tissue
cv bs	contractile ventral blood sinus		
d	dissepiment/septum	*o*	opening
dbs	dorsal blood sinus	*ps*	paraspermatozoa
es	euspermatozoa	*rer*	rough endoplasmatic reticulum
f	funnel	*s*	spermatozoa
fi	filament	*sc*	sponge cell
g	gut	*sg*	salivary gland
glo	gland openings	*spd*	sperm duct
lgl	lateral organ gland	*spdo*	sperm duct opening
lm	longitudinal muscle	*t*	tube
lo	lateral organ	*tm*	transverse muscle
lu	lumen	*vc*	ventral ciliary band
mi	mitochondrion		

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