HELGOLÄNDER MEERESUNTERSUCHUNGEN
Helgoländer Meeresunters. 48, 467–485 (1994)

Ultrastructure and functional morphology of the protonephridia and segmental fenestrated lacunae in *Protodrilus rubropharyngeus* (Polychaeta, Protodrilidae)

H. von Nordheim & A. Schrader

Federal Agency for Nature Conservation, Dept. for Coastal and Marine Nature Conservation; Isle of Vilm, D-18581 Lauterbach/Rügen, Germany

ABSTRACT: The protonephridia of *Protodrilus rubropharyngeus* are described. They consist of a terminal cell, one nephridiopore cell, and different types of duct cells (proximal, medial, distal) with the duct running intracellularly. Reabsorption takes place in the duct by means of very unique lamellar foldings. An interesting characteristic of the nephridial system in *P. rubropharyngeus* is the presumed double filtration of the primary urine that occurs in the walls of both the lateral blood lacunae and the terminal cell. The structure of excretory organs in relation to the particular coelomatic conditions found in different groups of polychaetes is discussed.

INTRODUCTION

The nephridia of *Protodrilus flavocapitatus* from the Black Sea were studied for the first time by Uljanin (1877) and mentioned in his description of this new species. The excretory organs were then examined in detail by Salensky (1907) who designated them as metanephridia. Pierantoni (1908) and later Goodrich (1931) – in a more detailed and comprehensive study – also referred to these organs as metanephridia.

When Jägersten (1940) first described a new species of *Protodrilus* from the Baltic Sea, *P. rubropharyngeus*, he pointed out that the "new" species is possibly a synonym for *P. flavocapitatus* (Jägersten, 1940, 1952); however, as there was and still is a lack of type material of *P. flavocapitatus*, he decided to continue describing *Protodrilus rubropharyngeus* as a "new" species. The assumption of synonymity of the two "species" was later supported by investigations on *P. rubropharyngeus* by Jouin (1970) and Von Nordheim (1987, 1989), but this synonymity still remains to be proven by comparison with material from the type locality of *P. flavocapitatus* in the Bay of Sebastopol, Ukraine. Because our material for this investigation originates from the type locality of *P. rubropharyngeus* and other Baltic Sea areas and from the United Kingdom, and due to perfect conformity with Jägersten's detailed species description, it was considered to be *P. rubropharyngeus* Jägersten.

The light microscopical examinations of the nephridia of *P. flavocapitatus* from the Black Sea (Salensky, 1907; Pierantoni, 1908; Goodrich, 1931) and *P. rubropharyngeus*

from the Baltic Sea (Jägersten, 1952; Jouin, 1970) did not lead to an exact evaluation of these organs. Jouin (1970) pointed out that she could not determine whether the "initial ampulla" of the nephridium in *P. rubropharyngeus* was open or closed and thus could not clarify whether meta- or protonephridia were present. Von Nordheim (1987) clearly noted for the first time the presence of protonephridia in adult *P. rubropharyngeus* in his ultrastructural investigations on the systematics of the genus *Protodrilus.*

In this paper, the ultrastructure and the functional morphology of these protonephridia are described. Ultrastructural investigations of the nephridial system often contribute to a more substantiated hypothesis of the phylogenetic position of a taxon in comparison to existing hypotheses (see e.g. Ax, 1984; Westheide, 1986; Smith & Ruppert, 1988; Rohde et al., 1988).

MATERIAL AND METHODS

Individuals of *Protodrilus rubropharyngeus* (Jägersten) from the following localities were investigated:

(a) Kristineberg (type locality) (Sweden/Skagerrak; salinity 20 ‰); (b) Weissenhäuser Strand (Germany/Baltic Sea; salinity 8–10 ‰); (c) St. Abbs Head (Scottish east coast/ North Sea; salinity 30–33 ‰).

The specimens were extracted from the sediment using a decantation technique with 2 % magnesium chloride adjusted to ambient sea-water salinity. Anaesthetized animals were observed alive or were fixed for transmission electronmicroscopy (TEM) in a mixture of sucrose, picric acid, formaldehyde and glutaraldehyde in a phosphate buffer (Ermak & Eakin, 1976). The best results were obtained with a sucrose content of 10 to 17 %. In *P. rubropharyngeus* from Weissenhäuser Strand, no sucrose was added.

Following narcotization for 20 min, fixation was carried out for 2 h at 4 °C with 2 to 3 replacements of fixation liquid. After rinsing several times with 0.1 molar phosphate buffer solution (pH 7.3) and postfixation in 1 % OsO_4 solution in phosphate buffer for 1 h at 0 °C, the material was dehydrated in an ethanol series, transferred to propylene oxide, and embedded in an Epon-Araldite mixture.

Thin sections were made with a diamond knife on a Reichert Ultracut, stained with lead citrate and uranyl acetate in a LKB Ultrostainer, examined and photographed with a Philips EM 300.

RESULTS

General morphology of the nephridia

In *Protodrilus rubropharyngeus*, from the first septum backwards, one pair of protonephridia is found in every segment. These organs are especially well developed in the more anterior segments. In certain segments of the fertile region both sexes have gonoducts instead of nephridia.

In the posterior section of a segment, in front of the septum, the following terminal elements of the protonephridium are found: a terminal cell, the tip of which lies closer to the gut than its caudal part, and three nephridioduct cells (Figs 1 A, B; 2 A). The 4th cell of the nephridioduct penetrates the septum and continues into the next segment. Subsequently, the nephridioduct cells are almost round or oval and their typical arrangement

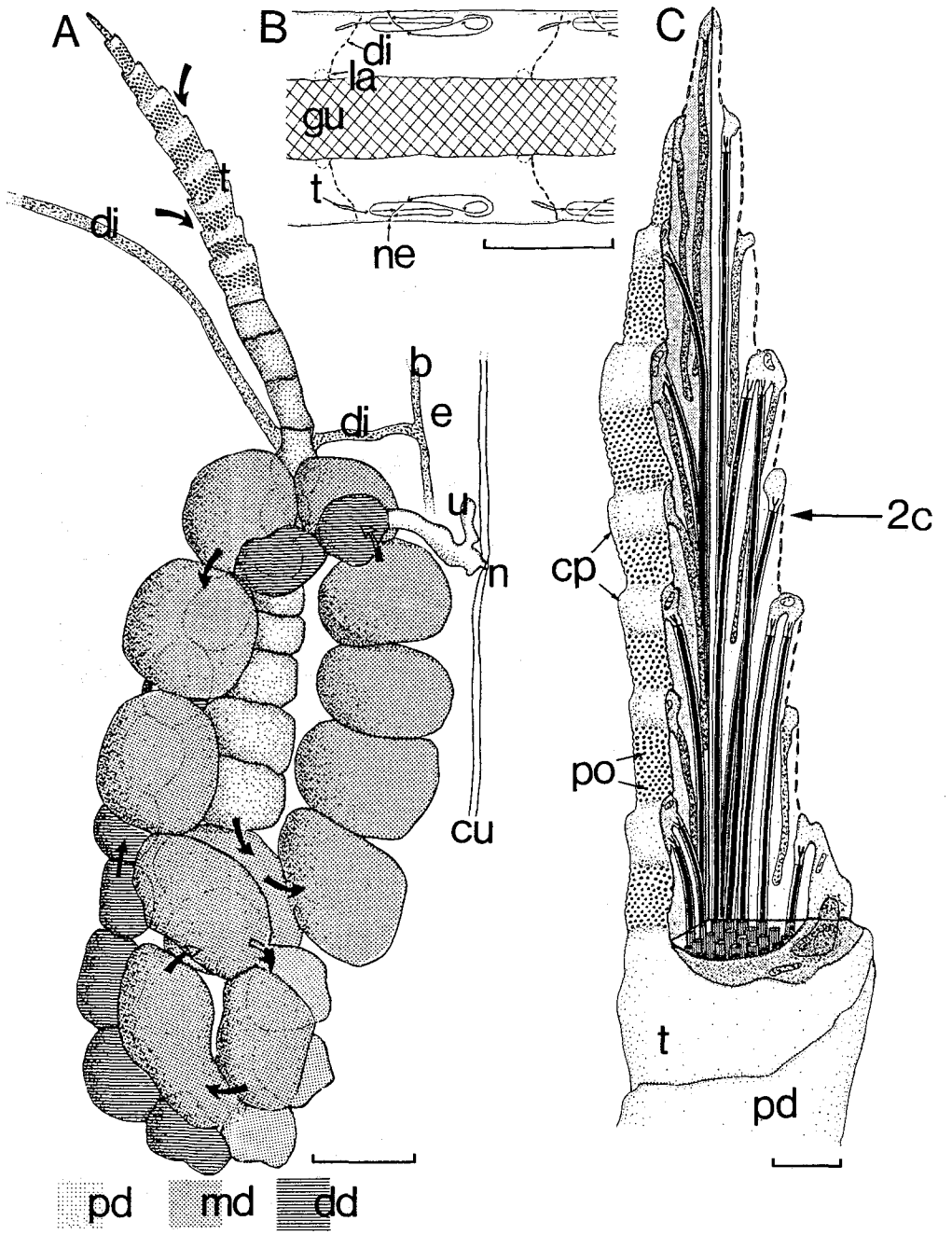

Fig. 1. *Protodrilus rubropharyngeus*. A: Diagram of complete protonephridium. Dorsal view. Arrows indicate the direction of liquid flow. Legend: *pd* – proximal duct section, *md* – medial duct section, *dd* – distal duct section. B: General, dorsal view of the position of the nephridia in a segment. C: Diagram of a terminal cell. Large arrow indicates position of cross section shown in Fig. 2 C. Scale bar: A = 10 μm, B = 100 μm, C = 2 μm

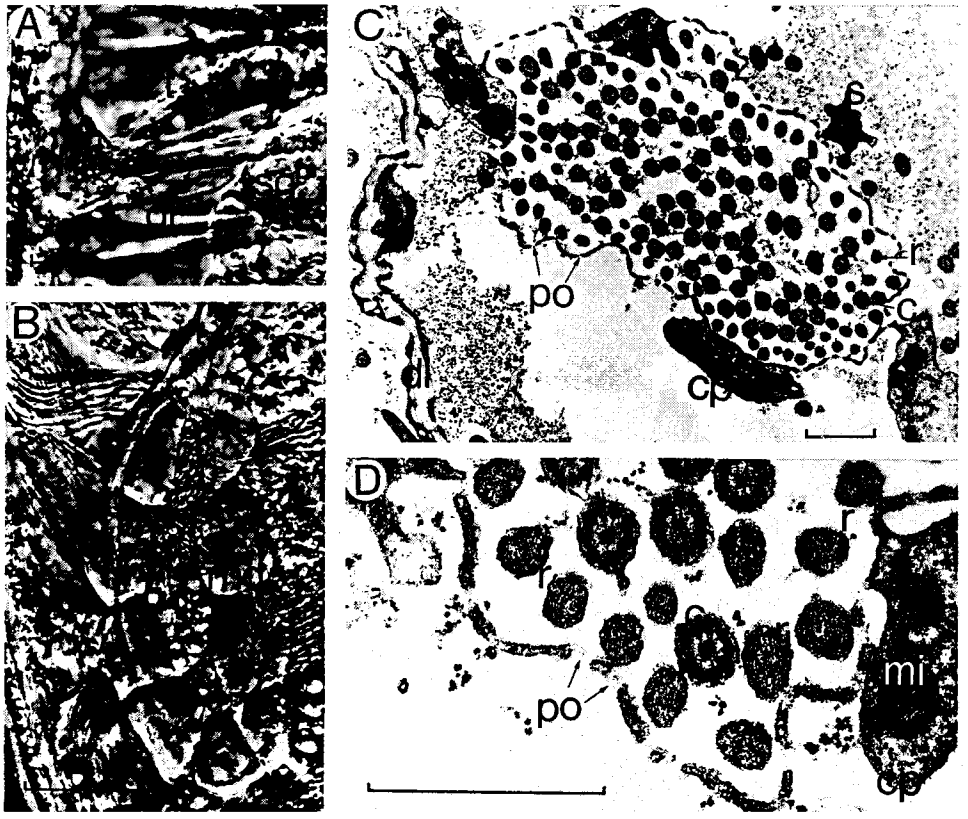

Fig. 2. *Protodrilus rubropharyngeus.* A, B: Light microscopical view of the protonephridial terminal cell (A) and part of the medial duct (B). C, D: TEM micrographs; C: Cross section of terminal cell. Note its relative position to neighbouring septum (for position of section see Fig. 1 C). D: Detail of cross section of terminal cell. Scale bar: A, B = 20 μm, C, D = 1 μm

makes this part of the nephridioduct look like a string of pearls (Figs 1 A, 2 B). The medial region of the nephridioduct forms 2 loops. The distal part terminates with a nephridiopore cell which opens ventrolaterally through the cuticle in the first third of the segment (Figs 1 A, B). The protonephridium consists of about 37 to 39 cells. In the immediate vicinity of the lumen of the excretory canal, one cell is always linked to the next by desmosomes (Figs 3 C, 4 A) and, occasionally, in addition by septate junctions. Some data on *P. rubropharyngeus* and the size of its nephridia are given in Table 1.

Terminal cell

The terminal cell is long and slender; its diameter increases by cascade-like widenings step by step towards the base (Figs 1 C, 2 A). The number of these protrusions in the different protonephridia varies from 7 to 9, each of them consisting of cytoplasm in which cilia and microvillar rods are anchored. In cross section, the basal part of the cell reveals up to 100 cilia, surrounded by about 100 rods. Their numbers decrease stepwise towards

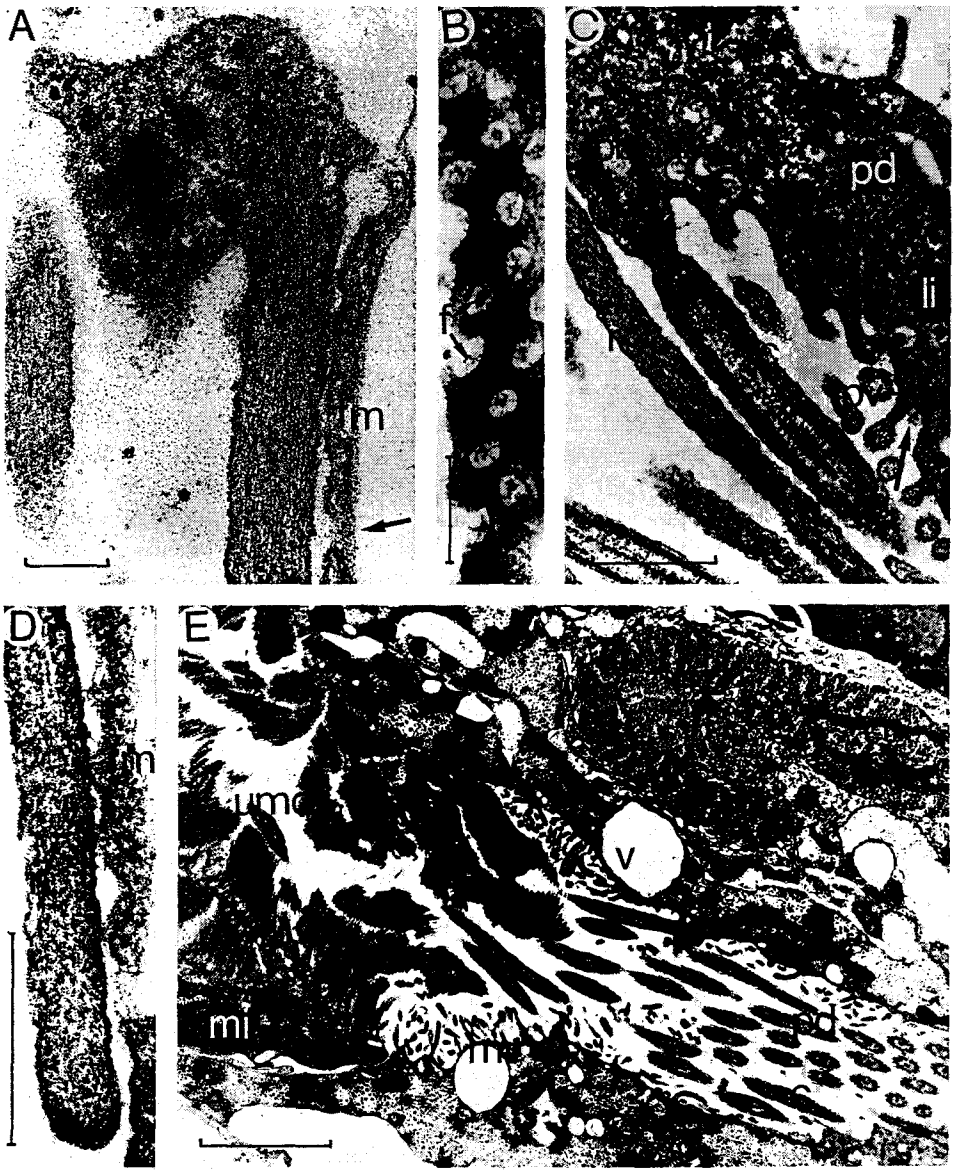

Fig. 3. *Protodrilus rubropharyngeus*. A: Microvillar rod anchored by fibrils in a cytoplasmic protrusion of the terminal cell. Note ECM covering the pore (arrow). B: Filtration layer with pores in tangential section. C: Transition of the terminal cell (t) to the first proximal duct cell (pd). Large arrow points to genesis of pinocytotic vesicle in proximal duct cell. Longitudinal section. D: A microvillar rod. E: Transition from proximal duct to upper medial duct (umd) in longitudinal section. Scale bars: A = 0.2 μm, B = 0.3 μm, C and E = 2.5 μm, D = 0.5 μm

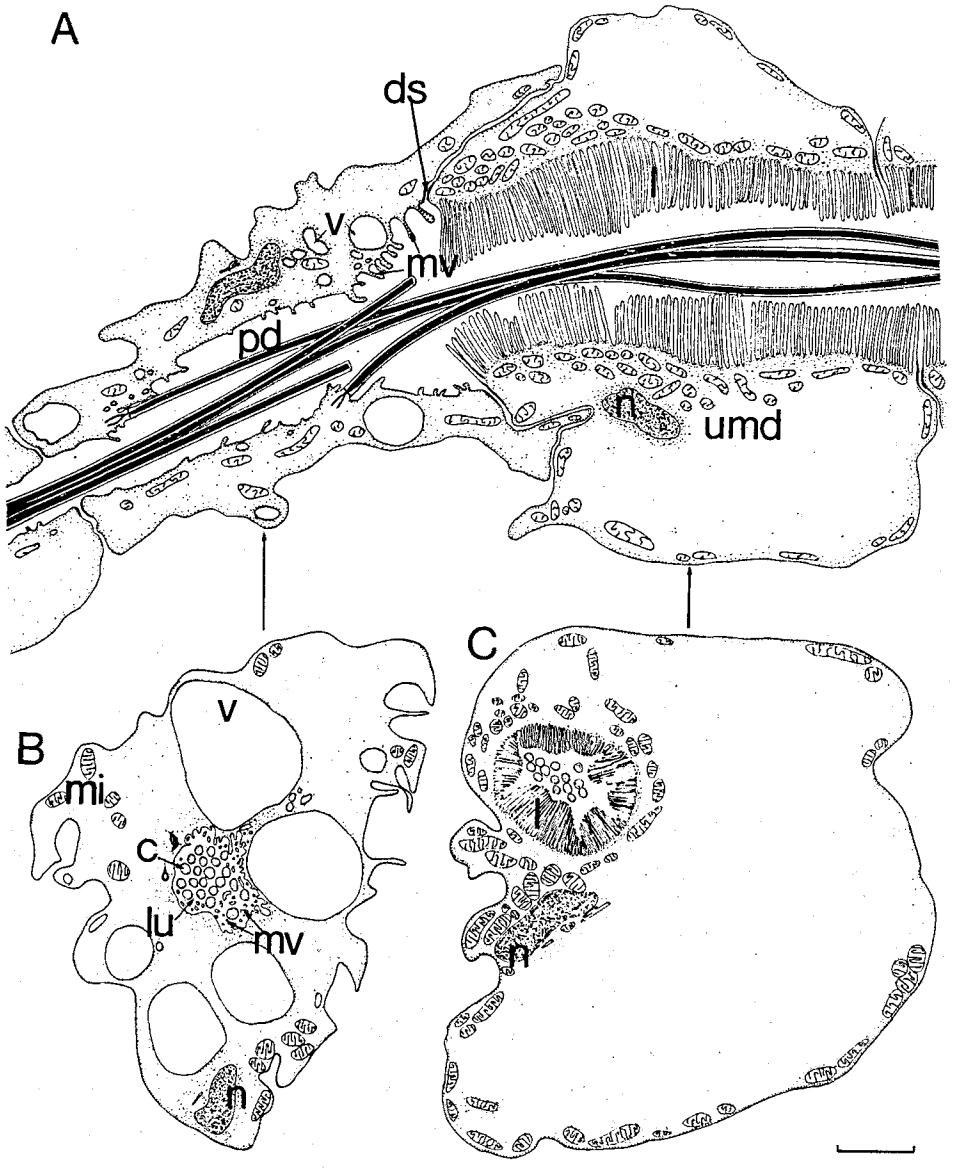

Fig. 4. *Protodrilus rubropharyngeus.* A: Diagram of the transition of the proximal duct to the upper medial duct. B: Cross section through proximal duct. C: Cross section through medial duct. Positions of sections B and C are indicated in A by arrows. Scale bar: 2 μm

the tip of the terminal cell. The major portion of the cytoplasm, containing the nucleus, rough endoplasmic reticulum (RER), mitochondria, as well as a few vesicles and granules, forms the cell base.

Beginning with the tip, and below each cytoplasmic protrusion of the "cascade", a

Table 1. Sizes of adult individuals of *Protodrilus rubropharyngeus* (mean values from Sweden and Germany)

Body size (n = approx. 100 individuals)	
Total body length	6–12 mm
Number of segments	20–50
Length of a middle segment	150 µm
Width of a middle segment	160 µm
Protonephridium size (n = 20–50)	
Length	110 µm
Total length of nephridioduct	320 µm
Length of terminal cell	31 µm
Width of terminal cell	5 µm (maximum)

filtration layer with many pores stretches to the lower protrusion and covers the numerous internal microvillar rods and cilia (Figs 1 A; 2 C, D; 3 A, C, D). The filtration layer consists of cytoplasmic bridges, 50–75 nm thick, that surround pores of 70–100 nm in diameter. Only in the pores is an extracellular matrix (ECM) found that is strengthened by crossed fibrils in the centre (Figs 2 C, D; 3 A, B). About two thirds of the terminal cell's total surface consist of this filtration layer, and the pores (about 7000–8000 per terminal cell) make up about 20 % of the surface area of the layer. The terminal cell as a whole does not seem to be completely enveloped by ECM or a derivative of the basal lamina of the septum.

Microvillar rods are only found in a peripheral position within the terminal cell closely below the filtration layer, and they surround the cilia in the centre of the cell like a weir (Figs 2 C, D). The rods can be described as large microvilli (diameter 200 nm; maximum length up to 10 µm) containing numerous, long filaments arranged parallel to the longitudinal axis. They are fixed in the cytoplasm of a protrusion by filaments and project into the peripheral lumen of the terminal cells (Figs 3 A, C, D).

The cilia of the terminal cell are also anchored in the protrusions by two rootlets heading in the opposite direction perpendicular to the cilium itself. In most cases, one rootlet is about twice as long as the other (1.2 µm and 0.5 µm respectively). The basal body of the cilium, designated by Pitelka (1974) as a "type II basal body", and the basal plate project a little over the normal level of the cell membrane (Fig. 3 C). Occasionally, an accessory centriole can be seen lying perpendicularly to the basal body (see Fig. 5 B), and several round mitochondria are found regularly in the cytoplasm of the protrusions (Figs 2 C, D). The longest section of a cilium that was measured in a longitudinal section was 16 µm; thus, the cilia are certainly always much longer than the microvillar rods.

Duct cells

Proximal section. In the proximal section, the intracellular duct is formed by a single row of 11 cells. In cross section, most of them are more or less spherical (Figs 4 B, C). Cells 1 to 3 are found between the terminal cell and the septum, while cells 4 to 11 lie behind the septum (Fig. 1 A).

In addition to a few peripheral mitochondria, a large nucleus and endoplasmic reticulum are found in each duct cell. Near the lumen of the duct, dictyosomes lie in an

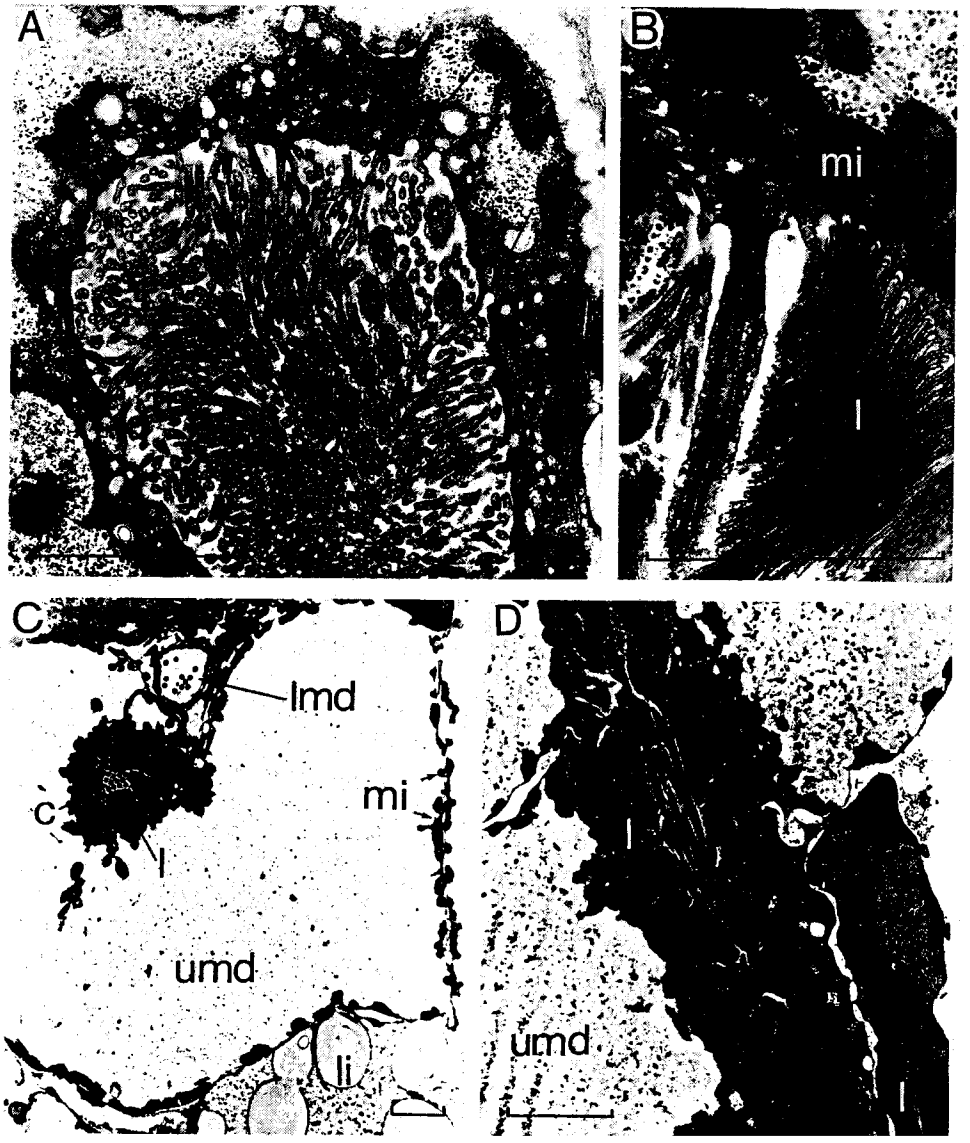

Fig. 5. *Protodrilus rubropharyngeus*. A: Proximal duct cell in tangential section. Note genesis of pinocytotic vesicles (large arrows). B: Accessory centriole near basal body of a cilium in the medial duct, lamellae (l). C: Cross section through the median region and the lower terminal region of the medial duct. D: Medial duct in longitudinal section showing groups of lamellae. Scale bar: 2 µm

electron-dense layer of cytoplasm. The remaining less-electron-dense cytoplasm contains numerous coarse granules and a few lipid droplets, as well as several large vacuoles built by confluent pinocytotic vesicles (Fig. 3 E). Hence, the luminal cell surface becomes irregular because of membrane invaginations and some short microvilli. (Fig. 5 A; N. B.:

Because of the tangential section in this figure, many microvilli can be seen.) The diameter of the lumen decreases distally.

In the intracellular lumen, 10 to 25 cilia with type II basal bodies (Pitelka, 1974) effect the fluid transport. They are anchored in different parts of the duct by just one rootlet with a length of about 2.5 µm and by a short basal foot.

Medial section. This part, consisting of about 15 to 16 cells with an intracellular lumen, can be differentiated into an upper medial section (about 10 cells) and a lower medial section (about 5 cells). Since there is a gradual transition between these two sections, a typical cell of each part is described.

In the upper medial section, the large round cells have a smooth outer surface and a maximum diameter of 10–15 µm (Figs 4 A, C; 5 C). The cytoplasm is less-electron-dense compared to that of the preceding section because of the high fluid content of the cell. The cytoplasm also contains the nucleus, many small coarse granules, RER and even some dictyosomes, which are always found close to the base of a cilium. Many mitochondria can be seen just below the cell surface or around the duct (Fig. 5 C). The most striking characteristic of these cells is the great enlargement of the cell surface by numerous foldings in the duct. These foldings of the inner cell membrane form large numbers of lamellae that are arranged in closely packed groups and that are normally not interdigitating (Figs 4 A, C; 5 B, C, D; 7 D; see also Fig. 7 B). The lamellae extend far into the lumen where their ends are a little thickened, or sometimes appear to be even bloated. Mitochondria are always found at the base of a lamella (Fig. 7 D). About 12 to 20 cilia propel the fluid inside the duct. They are anchored in the same way as in the proximal section, although their rootlets seem to be a little shorter (only 0.7 µm). An accessory centriole is found near the basal body (Fig. 5 B).

In the lower medial section, the diameter of the cells decreases continuously to about 3–4 µm. The cytoplasm surrounds the intracellular duct like a thin mantle and contains the nucleus, RER and mitochondria (Figs 6 A, B; 7 B). Groups of comparatively short lamellae almost fill the lumen completely, and there is only a little space for the 8–12 cilia and the excretion fluid (Fig. 7 B). The number of mitochondria is clearly reduced compared to the upper medial section. As there are only few dictyosomes but many granules (Fig. 7 B), the cytoplasm of these cells has a darker appearance than that of the cells of the upper medial section.

Distal section. The last section before the nephridiopore consists of about 10 cells forming again an intracellular duct (Figs 6 C, 7 A). The diameter of these cells is about 3–6 µm or even less. The cytoplasm contains the nucleus, RER, granules and a few dictyosomes and mitochondria. Around the duct the cytoplasm has a spongy appearance because of many small pinocytotic vesicles. The cell surface surrounding the duct lumen bears only a few irregularly arranged microvilli (Figs 6 C, 7 A). At the end of this section, the number of cilia decreases, so that only about 6 cilia reach into the nephridiopore cell.

Nephridiopore

At the end of the excretory duct, the nephridiopore is formed by just one epidermal cell, and the duct penetrates the basal lamina before passing through this cell in several windings (Figs 6 D, 7 C). In the epidermal region, a lateral extension forming a large lumen may be described as a "urinary bladder" (Figs 6 D, 7 C). The cytoplasm of the cells

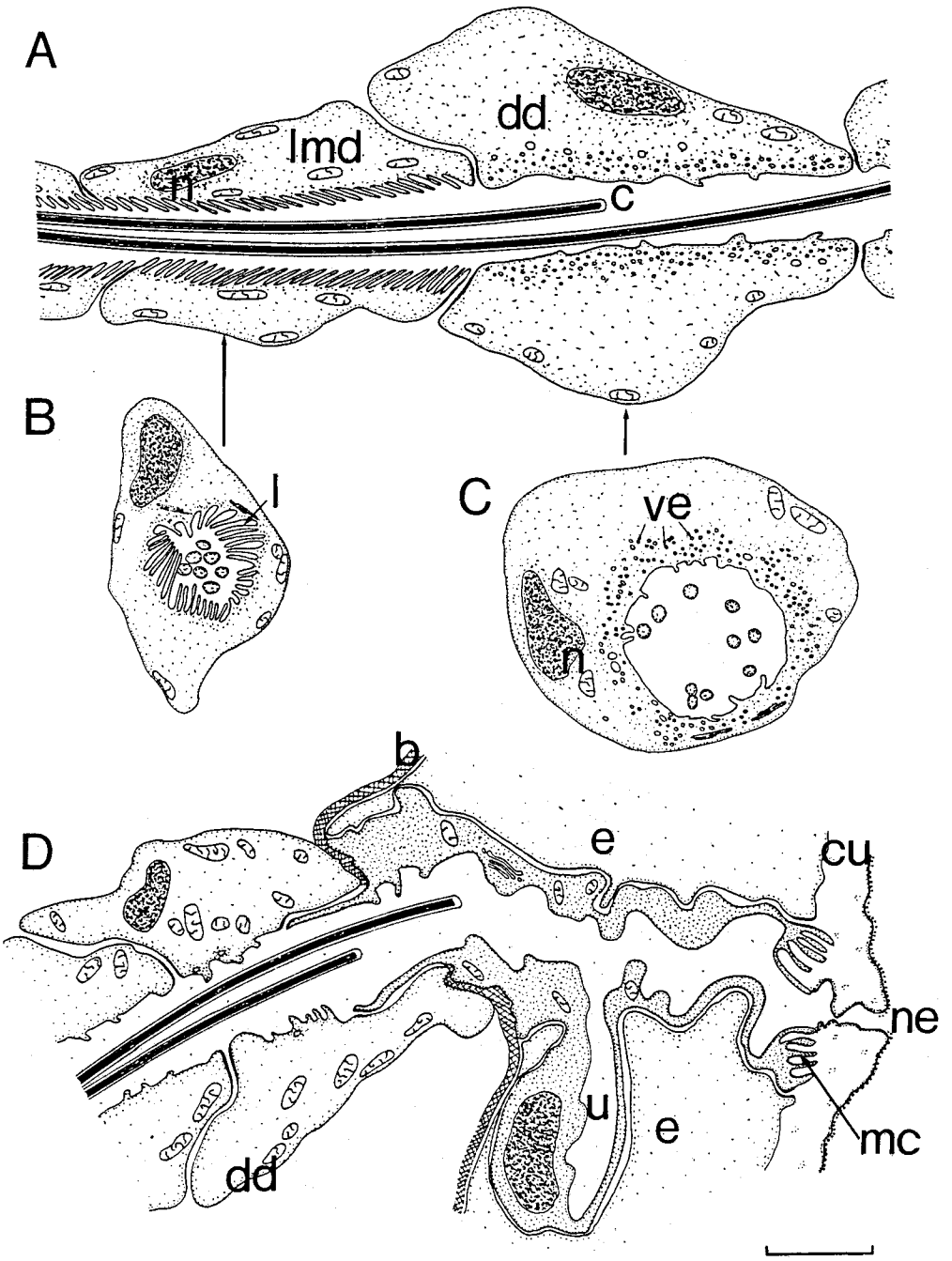

Fig. 6. *Protodrilus rubropharyngeus*. A: Diagram of the transition between lower medial duct and distal duct. B: Cross section through lower medial duct. C: Cross section through distal duct. Positions of sections B and C are indicated in A by arrows. D: Diagram of region of nephridiopore (main pore). Scale bar: 2 μm

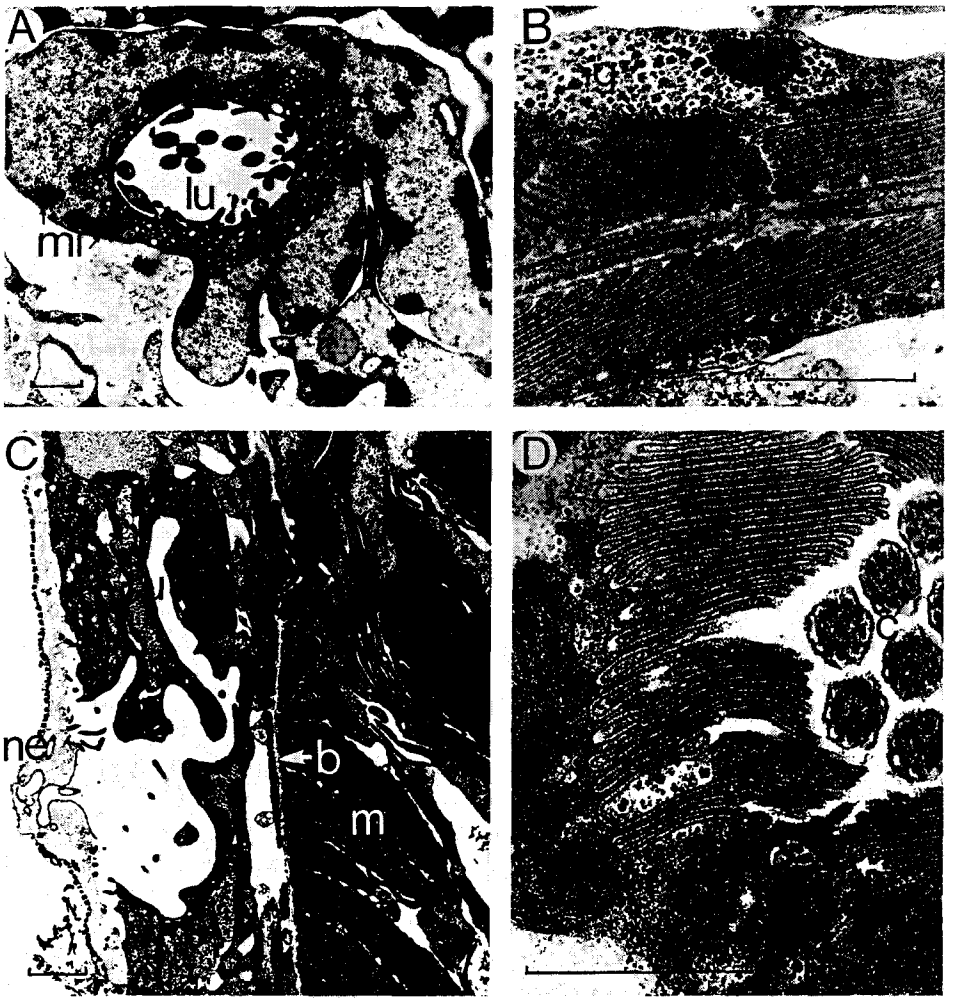

Fig. 7. *Protodrilus rubropharyngeus*. A: Cross section through distal duct. B: Lamellae of the lower medial duct in longitudinal section. C: Longitudinal section of the nephridiopore region showing the "urinary bladder", the main excretion pore and some smaller pores. D: Lamellae of the upper medial duct area in cross section. Scale bar: 1 μm

forms a thin layer around the lumen of the duct and contains the nucleus, a few mitochondria, dictyosomes and coarse granules. The true pore is stabilized by a microvillar crown (Fig. 6 D). Here the cuticle forms a projection and the epicuticle extends from the external layer to the tips of the microvillar crown. In addition, a number of smaller pores can be found in the cuticle next to the main excretory pore.

Special structures of the segmental lateral lacunae of the blood lacunae system

In *Protodrilus rubropharyngeus*, the septa consist of two thin coelothelial layers separated by a basal lamina (Figs 8 C, D). Close to the gut, the coelothelial layers form segmentally arranged lateral haemolymph lacunae. These lacunae are always found anterior to the septa close to the terminal cell (Fig. 8 C), but only in segments where terminal cells are present. The anterior coelothelial layer of the lacuna forms numerous podocyte-like structures. Between these "pedicels" there are slots about 25–35 nm wide covered by a thin extracellular matrix (ECM). See tips of triangles in Figures 8 A and B. Below the pedicels, the inner surface of a lacuna is also lined by extracellular material (Fig. 8 A), as is also the case in the ventral and dorsal haemolymph lacunae of this species. Furthermore, the anterior coelothelial layer of the septum facing the terminal cell also shows openings with a diameter of approx. 50–80 nm covered with thin ECM (indicated by tips of triangles in Fig. 8 D). These openings are always found close to the terminal cell. Below the coelothel, as in the lacunae, there is a second slightly thicker ECM (Fig. 8 D).

DISCUSSION

General morphology of the nephridia

Some results of the present study correspond to those of Salensky (1907), Pierantoni (1908) and Goodrich (1931), obtained from *Protodrilus flavocapitatus*, and to those of Jouin (1970), obtained from *P. rubropharyngeus*, which is very likely a synonym for *P. flavocapitatus* (see Jägersten, 1940; Jouin, 1970; Von Nordheim, 1987, 1989; Westheide, 1990). For example, Jouin, who did not examine the nephridia of *P. rubropharyngeus* histologically, stated on the basis of light microscopical observations that "the intracellular duct is coiled two times" and that it starts anterior to the septum with an "initial ampulla" about 30 µm in length. Jouin could not determine whether this ampulla was a closed or an open structure, and therefore could not decide if proto- or metanephridia were present. Salensky (1907) and Goodrich (1931) noted in *P. flavocapitatus* that the nephridial duct is formed by big cells arranged like a string of pearls.

The fact that each excretory organ occupies two segments, the proximal end with its terminal organ or nephrostome located in one segment and the duct and nephridiopore located in the next, is considered to be typical for annelids (Penzlin, 1980). As in oligochaetes (Boveri-Boner, 1920) and in other polychaete species, no nephridia are found in segments containing gonoducts (Goodrich, 1931; Jägersten, 1952; Jouin, 1970; Von Nordheim, 1991a, b).

Terminal cell

Basically, three types of construction of the "blind" proximal end of the duct are discussed in the literature (Wilson & Webster, 1974, p. 127; but see also Smith & Ruppert, 1988): (1) flame cells, (2) flame bulbs and (3) solenocytes. The present results reveal that the terminal cell of *Protodrilus rubropharyngeus* is of the "flame bulb" type, since a tuft of flagella is found in a blind-end tube of cytoplasm, and most of its cytoplasm, containing the nucleus, is located at the cell base. Furthermore, it can be described as a monocellular filter (see Lammert, 1985).

In some polychaetes, the terminal organ is entirely covered with a "basal lamina" on

Fig. 8. *Protodrilus rubropharyngeus*. A, B, C, D: Horizontal longitudinal sections. A: Lateral lacuna of the blood lacuna system. B: Pedicel of lacuna. C: Gut, lacuna, septum, transversal muscle and (cross section of) terminal cell. D: Septum with openings and filtration barriers (tips of triangles) in the anterior wall. Scale bar: A, B, D = 0.5 μm, C = 5 μm

the outside, for example in *Serpula vermicularis* (Pemerl, 1965), or with "extracellular material", as in *Glycera dibranchiata* (Smith & Ruppert, 1986); these structures serve as the only filtration barrier in those species. In *P. rubropharyngeus*, the extracellular material (ECM) is restricted to the region of the filtration pores of the terminal cell; in addition, there are fibrils in the pores. Kümmel (1962), however, pointed out that more important than the absolute filtration surface area is the relative filtration surface area, i.e. the proportion of slots or pores relative to the total surface area of the weir. This ranges from 3.7 %, in the miracidium of *Fasciola hepatica* (Trematoda), to 40 % in *Stenostomum* (Turbellaria). The relative filtration surface area of, for example, the solenocytes in *Glycera* (Polychaeta) with 33 % is clearly higher than that of the terminal cell of *P. rubropharyngeus* with about 20 %.

The prototype of the terminal cell of protonephridia in Bilateria has just one cilium ("solenocyte"), a filter (ECM), an accessory centriole and eight microvillar rods (Ax, 1984). From this prototype, protonephridia evolved that show a common pattern: i.e. a multiplication of cilia, a loss of accessory centrioles and a size reduction of microvillar rods (see also Bartolomaeus & Ax, 1992). In *P. rubropharyngeus*, there are about 100 cilia and 100 microvillar rods per terminal cell, and some accessory centrioles can still be found in both terminal cell and nephridioduct. Thus, in this species the protonephridia represent a highly derived type. As in other protonephridia, the microvillar rods in *P. rubropharyngeus* prevent the terminal cell from collapsing (see Brandenburg, 1975).

In polychaete protonephridial systems, a primary urine is extracted from the haemolymph by ultrafiltration into the terminal cell. By tracer experiments with *Glycera dibranchiata* (Polychaeta), Smith & Ruppert (1986, 1988) discovered that the real filtration barrier is only permeable for molecules with less than about 36 nm in diameter. In *P. rubropharyngeus*, the diameter of a terminal cell pore is about 2 to 3 times larger (70 to 100 nm), but is reduced by fibrillar material. The lateral haemolymph lacunae of the septa, however, possess clefts in their walls which are 25 to 35 nm wide. The particular size and shape of the clefts in the lacunae are determined by prodocytes with small protrusions, also known as pedicels. This kind of filtration structure is described by Kümmel (1977) for several excretion systems in different taxa: metanephridia, antennal glands and even mammalian kidneys. Fenestrated capillaries close to the nephrostome are well known in polychaetes possessing metanephridia (Jones, 1957; Dales & Cummings, 1987; Fransen, 1988; Ruppert & Smith, 1988). Nevertheless, the very peculiar situation found in *P. rubropharyngeus*, i.e. fenestrated capillaries (lacuna "walls") combined with protonephridia, has only been documented once within the Polychaeta: the trochophora larvae of *Sabellaria* possess protonephridia (Smith & Ruppert, 1988) and "discontinuities" in the walls of the capillaries (Fransen, 1988).

Excretory duct and nephridiopore

Normally, the primary urine is considered to be modified by reabsorption and secretion within the nephridioduct in three cytologically different sections of the duct. The sections are the proximal, medial and distal parts of the duct (Smith & Ruppert, 1988). In *Protodrilus rubropharyngeus*, there are also three different sections that can be distinguished in the excretory duct. Proximally, very few irregularly shaped microvilli project into the lumen. Many vesicles indicate reabsorption by pinocytosis. They fuse to form

larger vacuoles. In the medial section, the cell surface within the duct is greatly enlarged by numerous unique lamellae. This enables an intensified exchange of substances between duct and cell lumen. Pfannenstiel & Grünig (1982) described the situation in *Ophryotrocha puerilis* (Polychaeta), where the surface of the metanephridial duct is enlarged by "brushborders of microvilli". The surface enlargement resembles the basal labyrinth in the distal and proximal tubule cells of nephrons found in vertebrates (Ude & Koch, 1982). In these taxa, the enlarged cell surface facilitates active ion transport against a concentration gradient, which causes water to follow passively. In *P. rubropharyngeus,* the bloated condition of the cells of the medial duct region can be considered as further evidence for an intensive flow of liquid into the cytoplasm. The large number of mitochondria at the base of the lamellae indicates that active, energy-consuming metabolic processes, such as active ion transport, take place here. Also Wessing & Polenz (1974) noticed many mitochondria in the duct cells of the trochophora of *Pomatoceros triqueter.*

In the cytoplasm of the distal duct cells in *P. rubropharyngeus,* many granules can be found which fill these cells almost completely, and between the granules and the luminal cell surface, rough ER and some dictyosomes are present. McKanna (1968) considered such granules to be products of the Golgi-complexes and to be made up of reabsorbed material. However, Brüggemann (1986) took the presence of dictyosomes as evidence of a secretory function of these cells. Since an indication for a secretion process could not be found in the duct cells of *P. rubropharyngeus,* the authors suppose that the granules are synthesized from reabsorbed material.

In *P. rubropharyngeus,* the number and size of the membrane structures and respective organelles decrease from the proximal to the distal section of the duct. A very similar situation is noted by Brüggemann (1986) for *Paromalostomum proceracauda* (Plathyelminthes) where it is believed to be correlated with the proximally-to-distally decreasing concentration of substances which could be reabsorbed from the urine.

The lumen of the duct always runs intracellularly in *P. rubropharyngeus.* The only other species for which an intracellular lumen in the nephridial system is known are the annelids *Apodotrocha progenerans* (Westheide, 1985) and *Myzostoma cirriferum* (Pietsch & Westheide, 1987). A partially intracellular lumen has been described for *Hesionides arenaria* (Westheide, 1986) and *Glycera dibranchiata* (Smith & Ruppert, 1986).

The nephridiopore cell does not open externally by only one main pore; but a number of smaller openings surrounding the main pore exist that may also serve as excretion pores in the cuticle. Kristensen & Hay-Schmidt (1989) described similar observations in the kinorhynch *Echinoderes aquilonius* where a sieve-like pore plate with 20–30 pores can be found that penetrate the cuticle.

Functional morphological interpretation of the excretory process and some phylogenetic considerations

In *P. rubropharyngeus,* the definitive urine is probably generated in the following way: certain constituents of the haemolymph circulating in the blood lacunae system enter the coelom through the clefts in the coelothel of the lacuna and the septa. In these clefts, an initial filtration takes place by the thin ECM covering the clefts and by the underlying ECM. Due to metabolic processes in the coelomic cavities, this 1st-step-primary-urine is very likely slightly modified, and it is filtered again when entering the

terminal cell in order to prevent metabolic products from being excreted. After passing through this second filter structure that consists of the ECM in the cytoplasmic pores of the terminal cell, the 2nd-step-primary-urine produced by double filtration is propelled through different duct sections while its composition is modified again. Pinocytosis takes place in the proximal and distal sections, while in cells of the medial section the "coarse granules" are synthesized, and active ion reabsorption causes a passive flow of water into the medial duct cells. At the end of the duct system, the final urine is excreted through the nephridiopore consisting of a main pore surrounded by smaller pores.

Since one filtration barrier should be sufficient, especially as intensive reabsorption in the nephridioduct follows, this double filtration system might represent a transitional state in the course of evolution. In spite of the numerous neotenic traits in this polychaete genus, we suppose that the blood lacuna system and septa with podocytes, in combination with protonephridia, might represent a transitional stage in the course of development towards a true metanephridial system, the functioning of which requires the presence of a vascular system (see Smith & Ruppert, 1988; Bartolomaeus & Ax, 1992). So far no evidence could be found for the alternative hypothesis that the blood lacuna system in *P. rubropharyngeus* is a relict of a former metanephridial system, and the blood lacunae have not been completely reduced, even though protonephridia have evolved, the functioning of which does not need a blood lacuna system.

As to which hypothesis applies to the course of evolution of the excretory system in this species (and maybe for the genus *Protodrilus*), a decision can be made only after a thorough ultrastructural investigation of different larval stages. Because the development of nephridia of *P. rubropharyngeus* could not be studied due to lack of suitable material, we refer to Jägersten's (1940, 1952) light microscopical investigations of different larval stages. He stated (1952, p. 459) that the immature larva of *P. rubropharyngeus* possesses only two pairs of "larval nephridia" in the head segment that "disappear during maturity and are therefore missing in the fully mature stage, where the definitive nephridia... are found instead". Jägersten did not determine the larval nephridia as protonephridia but stressed their structural differences in comparison to what he considered to be "metanephridia" in the mature larva. Judging by the figures of Jägersten (1952, Figs 20 A–D), however, the larval nephridia are very likely protonephridia, the hindmost pair of which undergoes some morphological changes and becomes the first definitive pair of protonephridia in the first septum of the mature individual. If this is correct, then all developmental stages of *P. rubropharyngeus* possess protonephridia, and this is a neotenic and a plesiomorphic trait of the polychaetes (see Bartolomaeus & Ax, 1992).

Recently, Bartolomaeus & Ax (1992) discussed the relation of proto- and metanephridia within the Bilateria. They hypothesize that protonephridia were evolved in a monophasic acoelomate organism in the stem lineage of the Bilateria. Furthermore, "according to the differences between the metanephridia of phoronids and annelids", they emphasized "that there is no possibility to trace back all bilaterian taxa with a coelom to a common stem species". Hence, protonephridia are considered to be homologous organs throughout the Bilateria, whereas metanephridia must have evolved independently, at least twice. Bartolomaeus & Ax (1992) could find "no arguments for the hypothesis that segmental metanephridia belong to the ground pattern of annelids".

On the other hand, Smith & Ruppert (1988) considered proto- and metanephridia to be equivalent homologous organs, and stated that the type of excretory organ is not

dependent on the phylogenetic position of a species, but on its specific body plan and its transformation during ontogeny. Hence, certain species can have protonephridia in the larval stage and metanephridia in the adult stage. Smith & Ruppert did not assume one type of nephridia to be more primitive than another. Such an example for the correlation between type of excretory organ and body size was noted by Schuchert (1990) in *Bonellia viridis* (Echiurida). The larva of *B. viridis* has no nephridia. During metamorphosis, a pair of protonephridia develops in both sexes, but is reduced later. Finally, metanephridia form in the female, while protonephridia develop in the much smaller male.

In *Protodrilus hypoleucus*, Jouin (1970) noted the presence of metanephridia, but was not absolutely sure about her observation. In some other protodrilids, she described several different types of protonephridia with a comparatively simple structure. On the basis of light microscopical observations, Von Nordheim (1989) observed protonephridia in *P. haurakiensis* from New Zealand; he supposed that these have a similarly complex structure as those of *P. rubropharyngeus*. Therefore, it is possible that in general the retention of protonephridia in adult *Protodrilus*, as in other interstitial polychaetes (see Smith & Ruppert, 1988), is an adaption to comparatively small body size.

Although the blood vascular system of *Protodrilus* is, to some extent, reduced in comparison to larger species, for example *Nereis*, it is well developed with regard to the small size of these animals. Like other protodrilids investigated, adult *P. rubropharyngeus* has well developed coelomic cavities, lined by a coelothel, and a complex blood lacuna system. Messenger et al. (1985) describe similar conditions in *Nephtys* (Polychaeta), where a blood vascular system and segmental protonephridia are found.

Whereas the nephridia of most protodrilids have a short excretory duct (Jouin, 1970), the nephridial duct of *P. rubropharyngeus* is long with intensive reabsorption structures and its own typical windings. Similar structures were only observed in *P. haurakiensis* (von Nordheim, 1989). In addition, these structures are very identical in populations of *P. rubropharyngeus* from the North Sea, the British east coast (salinity 30 to 33 ‰) and from different places on the Baltic Sea coast (salinity from 20 to 8 ‰). Since *P. rubropharyngeus* is the only species of this genus so far known that enters brackish-water habitats (apart from perhaps *P. spongoides*, according to a very uncertain description of this species by Pierantoni [1908]), this fact among other adaptions might be due to the specific structure of its very complex excretory system.

Abbreviations used in the figures

a	accessory centriole	g	granule	ne	nephridiopore
b	basal lamina	gu	gut	p	pedicel
bb	basal body	gue	gut epithelium	pd	proximal duct cell
bf	basal foot	l	lamellae	po	pore
c	cilium	la	lacuna	pv	pinocytotic vesicle
cp	cytoplasmic protrusion	li	lipid drop	r	microvillar rod
cu	cuticle	lmd	lower medial duct	s	spermatozoa
dd	distal duct cell	lu	lumen	sg	salivary gland
di	septum	m	muscle	t	terminal cell
ds	desmosome	mc	microvillar crown	u	urinary bladder
e	epiderm	md	medial duct cell	umd	upper medial duct
er	RER	mi	mitochondria	v	vacuole
f	fibrils	mv	microvilli	ve	vesicle
fm	filtration matrix	n	nucleus		

Acknowledgements. We wish to thank Prof. C. O. Hermans, Rohnert Park, CA/USA, and Dipl. Biol. P. Siemann, Braunschweig, Germany for supplying *Protodrilus* material from the British east coast (North Sea).

LITERATURE CITED

Ax, P., 1984. Das phylogenetische System. Fischer, Stuttgart, 349 pp.

Bartolomaeus, T. & Ax, P., 1992. Protonephridia and metanephridia – their relation within the Bilateria. – Z. zool. Syst. Evolutionsforsch. *30*, 21–45.

Boveri-Boner, Y., 1920. Beiträge zur vergleichenden Anatomie der Nephridien niederer Oligochaeten. Fischer, Jena, 50 pp.

Brandenburg, J., 1975. The morphology of the protonephridia. – Fortschr. Zool. *23* (2–3), 1–17.

Brüggemann, J., 1986. Feinstruktur der Protonephridien von *Paromalostomum proceracauda* (Plathelminthes, Macrostomida). – Zoomorphology *106*, 147–154.

Dales, R. P. & Cummings, M. O., 1987. Role of the nephridia in the elimination of foreign cells from the coelomic fluid of two polychaete annelids, with observations on the structure of the nephridia. – Tissue Cell *19*, 387–397.

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

Fransen, M. E., 1988. Blood vascular system. – Microfauna mar. *4*, 210–213.

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

Jägersten, G., 1940. Zur Kenntnis der äußeren Morphologie, Entwicklung und Ökologie von *Protodrilus rubropharyngeus* n.sp. – Ark. Zool. *32*A (16), 1–19.

Jägersten, G., 1952. Studies on the morphology, larval development and biology of *Protodrilus*. – Zool. Bidr. Upps. *29*, 427–515.

Jones, M. L., 1957. On the morphology of the nephridium of *Nereis vexillosa* Grube. – Biol. Bull. mar. biol. Lab., Woods Hole *113*, 407–413.

Jouin, C., 1970. Recherches sur les archiannélides interstitielles: systématique, anatomie et développement des Protodrilidae et des Nerillidae. Diss., Univ. Paris, 204 pp.

Kristensen, R. M. & Hay-Schmidt, A., 1989. The protonephridia of the arctic Kinorhynch *Echinoderes aquilonius* (Cyclorhagida, Echinoderidae). – Acta zool., Stockh. *70*(1), 13–28.

Kümmel, G., 1962. Zwei neue Formen von Cyrtocyten. Vergleich der bisher bekannten Cyrtocyten und Erörterung des Begriffs „Zelltyp". – Z. Zellforsch. mikrosk. Anat. *57*, 172–201.

Kümmel, G., 1977. Der gegenwärtige Stand der Forschung zur Funktionsmorphologie exkretorischer Systeme. Versuch einer vergleichenden Darstellung. – Verh. dt. zool. Ges. *1977*, 154–174.

Lammert, V., 1985. The fine structure of protonephridia in Gnathostomulida and their comparison within Bilateria. – Zoomorphology *105*, 308–316.

McKanna, J. A., 1968. Fine structure of the protonephridial system in Planeria. II: Ductules, collecting ducts and osmoregulatory cells. – Z. Zellforsch. mikrosk. Anat. *92*, 524–535.

Messenger, E. M., Smith, P. R. & Ruppert, E. E., 1985. Ultrastructure and function of the protonephridium of *Nephtys* (Polychaeta). – Am. Zool. *25*, 41A.

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

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

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

Nordheim, H. von, 1991b. Ultrastructure and funcional morphology of the female reproductive organs in *Protodrilus* (Polychaeta, Annelida). – Helgoländer Meeresunters. *45*, 465–485.

Pemerl, J., 1965. Ultrastructure of the protonephridium of the trochophore larva of *Serpula vermicularis* (Annelida, Polychaeta). – Am. Zool. *5*, 666–667.

Penzlin, H., 1980. Lehrbuch der Tierphysiologie. Fischer, Stuttgart, 566 pp.

Pfannenstiel, H. D. & Grünig, C., 1982. Structure of the nephridium in *Ophryotrocha puerilis* (Polychaeta, Dorvilleidae). – Zoomorphology *101*, 187–195.

Pierantoni, U., 1908. *Protodrilus*. – Fauna Flora Golf Neapel *31*, 1–230.

Pietsch, A. & Westheide, W., 1987. Protonephridial organs in *Myzostoma cirriferum* (Myzostomida). – Acta zool. Stockh. *68*, 195–203.

Pitelka, D. R., 1974. Basal bodies and root structures. In: Cilia and flagella. Ed. by M. A. Sleigh. Acad. Press, London, 437–469.

Rohde, K., Cannon, L. R. G. & Watson, N., 1988. Ultrastructure of the protonephridia of *Monocelis* (Proseriata, Monocelididae). – J. Submicrosc. Cytol. Pathol. *20*, 425–436.

Ruppert, E. E. & Smith, P. R., 1988. The functional organization of filtration nephridia. – Biol. Rev. *63*, 213–258.

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

Schuchert, P., 1990. The nephridium of the *Bonellia viridis* male (Echiura). – Acta zool., Stockh. *71*, 1–4.

Smith, P. R. & Ruppert, E. E., 1986. Filtration and reabsorption of macromolecules by protonephridial excretory organs. – J. Cell Biol. *103*, 59.

Smith, P. R. & Ruppert, E. E., 1988. Nephridia. – Microfauna mar. *4*, 231–262.

Ude, J. & Koch, M., 1982. Die Zelle. Fischer, Jena, 269 pp.

Uljanin, W. N., 1877. Observations on the genus *Polygordius* of the Bay of Sebastopol. – Byull. mosk. Obshch. Ispyt. Prir. *52*, 53–96. (In Russian)

Wessing, A. & Polenz, A., 1974. Structure, development and function of the protonephridia in trochophores of *Pomatoceros triqueter* (Annelida, Polychaeta, Sedentaria). – Cell Tissue Res. *156*, 21–33.

Westheide, W., 1985. Ultrastructure of the protonephridia in the dorvilleid polychaete *Apodotrocha progenerans* (Annelida). – Zool. Scr. *14*, 273–278.

Westheide, W., 1986. The nephridia of the interstitial polychaete *Hesionides arenaria* and their phylogenetic significance (Polychaeta, Hesionidae). – Zoomorphology *106*, 35–43.

Westheide, W., 1990. Polychaetes: Interstitial families. – Backhuys, Oegstgeest, 152 pp. (Synopses of the British Fauna Nr 44.)

Wilson, R. A. & Webster, L., 1974. Protonephridia. – Biol. Rev. *49*, 127–160.