Ultrastructure and phylogenetic significance of the head kidneys in *Thalassema thalassemum* (Echiura, Thalassematinae)

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Abstract

Recent molecular analyses consistently resolve the "spoon worms" (Echiura) as a subgroup of the Annelida, but their closest relatives among annelids still remain unclear. Since the adult morphology of echiurans yields limited insight about their ancestry, we focused on characters of their larval anatomy to contribute to this discussion. Electron microscopical studies of the larval protonephridia (so called head kidneys) of the echiuran species *Thalassema thalassemum* revealed distinct correspondences to character states in serpulid polychaetes although a close relationship of Echiura and Serpulidae is not supported by any phylogenetic analysis. The larval head kidneys of *T. thalassemum* consist of only two cells, a terminal cell and a duct cell. The terminal cell forms a tuft of six cilia projecting

into the lumen of the terminal cell. The cilia are devoid of circumciliary microvilli. A filter structure is formed by two to three layers of elongate microvilli which surround the lumen of the terminal cell in a tubular manner. A thin layer of extracellular matrix (ECM) encloses the outer microvilli of the tubular structure. The tips of the microvilli project into the lumen of the adjacent duct cell but are not directly connected to it. However, mechanic coupling is faciliated by the surrounding ECM and abundant hemidesmosomes. The distal end of the multiciliary duct cell forms the external opening of the nephridium; a specialized nephropore cell is absent. Apart from the multiciliarity of the duct cell, details of the head kidneys in *T. thalassemum* reveal no support for the current assumption that Echiura is closely related to Capitellida and/or Terebelliformia. Available data for other echiuran species, however, suggest that the head kidneys of *T. thalassemum* show a derived state within Echiura.

Introduction

Echiura share numerous developmental and morphological characters with Annelida, like the ultrastructure of chaetae and cuticle, the structure and position of the blood vessels, and the development of the trunk mesoderm (Orrhage 1971; Ax 1999; Ruppert et al. 2004). In addition, serially repeated ganglia in echiuran larvae correspond to typical metamerical ganglia in annelids (Hessling and Westheide 2002; Hessling 2002; 2003). On the other hand characters like the unsegmented trunk with a single secondary body cavity, the extensible elongate muscular proboscis and the presence of anal sacs (anal vesicles sensu Newby 1940) clearly distinguishes echiurans from typical annelidan taxa (Ax 1999; Ruppert et al. 2004). Therefore, based on morphological data, Echiura were considered either as a highly derived subtaxon of the Annelida (Eibye-Jacobsen and Nielsen 1996; Ax 1999; Nielsen 2001) or as separate taxon with close a relationship to annelids (Newby 1940; Korn 1960; Rouse and Fauchald 1995; Edmonds 2000). The former hypothesis implies that Echiura have secondarily lost characteristic features of annelids like trunk

segmentation, parapodia, and a metamerical nervous system in adults (Purschke et al. 2000; Bleidorn 2007).

Recent molecular and combined morphological and molecular analyses support a systematic positioning of the Echiura within a clade comprising the annelid families Terebellidae, Arenicolidae, Maldanidae and Capitellidae (Struck et al. 2007, 2008; Zrzavy et al. 2009). Most of these analyses revealed a sister group relationship between Echiura and Capitellidae but some multigene analyses also support a close relationship to Terebellidae (Colgan et al. 2006) or Pectinariidae (Rousset et al. 2007, “restricted” dataset). Morphological support for any of these sister group relationships is still wanting. This is due to the problem that morphological characters traditionally used in echiurid systematics are mostly lacking in other annelid taxa or show derived states within Echiura (see Purschke et al. 2000; Ruppert et al. 2004).

Due to striking similarities in cleavage and early ontogenetic patterns it appears promising to focus on morphological features of larval stages for phylogenetic inferences. Like most polychaetous annelids, Echiura show a biphasic life cycle with a planktonic trochophore larva (Baltzer 1931). It is generally accepted that this larva already evolved in the common ancestor of the Trochozoa, which comprise at least Annelida, Entoprocta and Mollusca (Rouse and Fauchald 1995; Ax 1999; Nielsen 2004, 2005). Trochophore larvae are characterized by a specialized circumlarval ciliary belt (the prototroch), a sensory apical organ, and one pair of transitory protonephridia (Rouse 1999; Nielsen 2004). These protonephridia are located in the periphery of the larval blastocoel anteriorly to the anlagen of the trunk mesoderm (Hatschek 1880; Goodrich 1945). Due to their position in the presumptive head region they have been named head kidneys (“Kopfnieren”) by Hatschek (1878, 1880). During metamorphosis the head kidneys disintegrate and become functionally replaced by segmentally arranged nephridia in annelids or by the anal sacs in most echiurans (Baltzer 1931; Goodrich 1945; Bartolomaeus and Ax 1992).

Within Annelida the head kidneys of about 17 species have been investigated ultrastructurally (see Bartolomaeus 1995; Bartolomaeus and Quast 2005). The structural data of the head kidneys in these species provide a number of discrete characters, like their composition (number of cells), the construction of the filtration area, and the ciliation in the distinct nephridial parts and cells, respectively. Some of these features are characteristic for high ranking subtaxa within the Annelida (Bartolomaeus 1995, 1998; Quast 2007) and thus are useful for unraveling annelidan phylogeny.

A comparison of the head kidney morphology in Echiura could accordingly contribute further insight on their phylogenetic position within Annelida. For this purpose, we examined the head kidneys of *Thalassema thalassemum* (Pallas, 1766) (Echiuridae, Thalassematinae) by means of transmission electron microscopy, providing the first ultrastructural data on head kidneys in echiurans. The main goal of our study is to search for structural correspondences that support a close relationship to the traditional Capitellida (i.e. Capitellidae, Arenicolidae, and Maldanidae) as suggested by the above mentioned phylogenetic analyses (Struck et al. 2007; Zrzavy et al. 2009).

Material and Methods

Reproductive adults of *Thalassema thalassemum* (Pallas, 1766) were collected in April and May 2008 in Le Cabellou, Concarneau, France. Animals were taken from rock crevices in the mid-intertidal zone. Adults were kept in small aquaria at the Freie Universität Berlin with running artificial seawater (13–15°C).

Gametes where obtained by dissecting gonoducts containing ripe ova or sperm respectively. Artificial fertilisation was conducted in glass bowls with ultrafiltrated cooled seawater (9°C) from the Atlantic coast in Concarneau. The larvae were reared in a large 2 l beaker at 15–18°C. 98h old larvae were fixed for 1hin cold (4°C) 1.25% glutaraldehyde buffered in filtered (0.2 µm) PBS (0.05 M sodium phosphate with 0.3M NaCl, pH 7.2) containing traces of ruthenium red. After fixation, the larvae were washed

three times in PBSand postfixed in 1% OsO_4 in PBS for 1 h at 4° C. Subsequently, they were dehydrated in a graded acetone series and embedded in araldite M (FLUKA). Complete series of silver interference colored ultrathin sections (60–70 nm) were cut with a diamond knife on a Leica Ultracut S microtome. The sections were mounted on formvar-covered single-slot copper grids, automatically stained with uranyl-acetate and lead-citrate with a Nanofilm TEM Stainer and examined with a Philips CM120 Bio-TWIN electron microscope. Images were digitally recorded on imaging plates (DITABIS). The obtained 16 bit images were enhanced in contrast and converted to 8 bit gray levels with AnalySIS (SIS Münster) or the the ImageJ software package (http://rsbweb.nih.gov/ij/). An aligned series of selected micrographs of one head kidney is available at: https://www.morphdbase.de?B_Quast_20110303-S-2.1. The 3D reconstruction of the protonephridium was conducted using Adobe Illustrator CS 3 for segmentation of the cell contours and Blender (http://www.blender.org) with the MorphMesh Plugin (http://www.q-terra.de/biowelt/3drekon/index.html) for generating a 3D surface model. From the 3D reconstruction virtual sections where imported into Adobe Illustrator and used as draft for a schematic illustration of the protonephridium.

Results

The 98h old larva of *T. thalassemum* possesses one pair of protonephridia (Fig. 1). Each protonephridium is tubular in shape and measures about 44 µm in length (Fig. 2). Both nephridia are composed of two cells only, a terminal cell and a duct cell. They extend straightly from the forgut anlage toward the anus and open to the exterior by piercing the epidermis close to the anus. The external opening is situated ventrolaterally close to the gastrotroch and ca. 40 µm anteriorly to the anus (Fig. 1). A specialized nephridiopore cell that is embedded with most of its cell body into the epidermis is lacking.

Terminal cell

The terminal cell is situated laterally to the anlage of the foregut and the mouth opening. The cell is elongate and measures about 16 µm in length (Fig. 2). The proximal end of the cell tapers off into a small apex. At this apex the terminal cell is connected to a muscle cell via dense plaques (Fig. 3A, B). The nucleus is located within the proximal part of the terminal cell, in a distance of about 2 µm to the apex. It contains a nucleolus and heterochromatin. The nucleus has a diameter of about 3 µm.

With its distal most part the terminal cell forms a hollow, cylinder like compartment (Fig 2). The extracellular space represents the lumen of the terminal cell and is continuous with the lumen of the adjacent duct cell. The outer wall of the cylinder is formed by numerous elongate microvilli. The microvilli are about 3µm in length and emanate from the margin of a small flattened area that has a diameter of about 1.5µm (Fig 3C). Actin filaments are present within the microvilli (Fig 3D, E). In sections of their basal most part some microvilli appear to be interconnected by cytoplasm (Fig 3D), but this is due to the level of sectioning. No anastomoses or interconnections are found between the microvilli. The microvilli are arranged in a ring-like area of about 0.3 µm thickness. Usually two to three microvilli are orientated one behind the other suggesting an arrangement of at least two irregular circles (Fig. 3D, 3F). The outer circle of microvilli is surrounded by a thin layer of electron dense extracellular matrix. Occasionally, hemidesmosomes connect the extracellular matrix to the underlying microvilli (Fig. 3D). The matrix is continuous with the extracellular matrix surrounding the entire terminal cell. Any additional extracellular membrane or diaphragm on or in between the microvilli seems to be absent.

From the flattened cytoplasmic area between the bases of the microvilli six cilia protrude into the lumen of the terminal cell. Each cilium is anchored by a basal body with a short rootlet and a lateral basal foot (Fig. 3C). The ciliary rootlet measures about 0.4 µm in length. The rootlets of the six cilia are interconnected via microtubuli. None of the ciliary basal structures possesses an accessory centriol.

The cytoplasm of the terminal cell contains several mitochondria (Fig. 3A), smooth and rough endoplasmatic reticula and golgi complexes. Several lipid droplets are located in the perikaryon. In the region of the proximal apex and near the origin of the cilia, the cytoplasm contains numerous coated and uncoated vesicles (Fig. 3C). Membrane pits occur at the abluminal membran in the middle and distal part of the terminal cell.

Duct cell

The duct cell forms a stretched tubule with a length of approximately 30 µm (Fig. 2). The nucleus is located in a lateral bulge in the middle part of the duct cell (Fig. 4D). It contains heterochromatin and two nucleoli. Several golgi complexes, a dense system of endoplasmatic reticulum and numerous mitochondria occur in the cytoplasm of the lateral bulge. In the proximal part of the duct cell enlarged cisternae of the endoplasmatic reticulum run almost parallel to the adluminal membrane and thus indicate a layer-like construction of the adluminal cytoplasm (Fig 4A, B). Vesicles of various sizes are densely distributed within the cytoplasm of the middle and distal sections of the duct cell (Fig 4D, E). The adluminal and abluminal membranes of the duct cell possess numerous uncoated membrane pits (Fig. 4B, C, D, E).

A thin electron dense layer of extracellular matrix surrounds the entire duct cell (Fig. 4A, B). This basal membrane is continuous with the basal membrane of the terminal cell and the adjacent epidermis cells. Hemidesmosomes connect the basal membrane to the duct cell. The extracellular membrane in addition with hemidesmosomes solely provides the mechanical connection between terminal and duct cell.

The lumen of the duct cell is percellular, i. e. the cytoplasm encompassing the lumen is interconnected by an adluminal zonula adherentes and septate junctions (Fig. 4A, B, E). In the proximal part of the duct cell the adluminal membrane forms neither cilia nor microvilli. Only the cilia and microvilli of the terminal cell extend into this part of the duct lumen (Fig. 4A, B). Because of their different length, the microvilli end successively at

different levels in the proximalmost part of about 1 µm of the duct lumen. A direct connection between the microvilli and the duct cell was not detected at any site of the adluminal membrane. The six cilia of the terminal cell project about a length of 15 µm into the lumen of the duct cell and thus extend to the level of its perikaryon (Fig. 4D). The duct lumen of the proximal part of the cell has a diameter of about 0.5 µm (Fig 4B).

In the region of the perikaryon, the duct lumen widens to a diameter of 2.5 µm (Fig. 4D, E). Here, about 15 cilia project from the adluminal membrane into the lumen. Each cilium is anchored to the cytoplasm by a basal body with a lateral basal foot and a single rootlet. The cilia insert separately and their basal structures are distributed over the whole adluminal membrane in the middle part of the duct cell (Fig. 4D, E).

From the region of the nucleus on, there also protrude numerous finger-like cytoplasmic processes into the nephridial lumen (Fig. 4D, E). This cytoplasmic protrusions occassionally contain electron dense material, but no actin filaments were found within them.

No cilia are originating from the distal part of the duct cell, but similar finger like cell processes are observed as in the region of the perikaryon. The distalmost part of the duct cell passes through the epidermis and forms the external opening of the nephridium (Fig 4F). In this region, the thickness of the cytoplasm encompassing the lumen thins out and measures less than 0.2 µm. At its distal margin, the duct cell is connected to the adjacent epidermis cells by adherens junctions. The nephridiopore is about 1 µm in diameter. Fingerlike cell processes protrude from the duct cell at the margin of the nephridiopore and partly overlap it (Fig. 4F). Some of the cilia of the middle part of the duct cell extend through the nephridiopore into the external medium. A cuticular lining is absent both on the exterior surface of the duct cell, as well as on the adjacent epidermis cells.

Discussion

Based on a comparison of protonephridial systems in bilaterian animals, Bartolomaeus and Ax (1992) hypothesized one pair of protonephridia for the ground pattern of the Bilateria. In Annelida this pair of protonephridia exists as head kidneys exclusively in early developmental stages and is replaced by serially repeated nephridia in the trunk segments during postembryonic or postlarval development. The segmentally arranged nephridia are either protonephridia or metanephridial systems (Goodrich 1945; Ruppert and Smith 1988; Bartolomaeus 1999). Head kidneys and segmental protonephridia of Annelida often exhibit an increase of the number of circumciliary microvilli in the terminal cell to at least ten (Bartolomaeus and Ax 1992; Bartolomaeus 1995). According to their comparative survey of protonephridia in Bilateria Bartolomaeus and Ax (1992) assume that protonephridia composed of three cells only, one terminal cell, one duct cell and one nephropore cell belong to the ground pattern of Bilateria.

Structural variability of head kidneys within Annelida

Within Annelida head kidneys consisting of three cells have hitherto only been found in *Chaetopterus variopedatus* (Renier, 1804), *Spirorbis spirorbis* (Linnaeus, 1758), *Magelona mirabilis* Müller, 1858, and *Scoloplos* cf. *armiger* (Bonch-Bruevich and Malakhov 1987; Bartolomaeus 1993a, 1995, 1998). In terebelliform species investigated thus far the head kidneys are composed of two terminal and duct cells each, while "oligochaetous" clitellates show a reduction of the nephridiopore cell (Bartolomaeus 1995; Quast 2007). Due to the lack of ultrastructural data currently no further apomorphy hypotheses can be drawn from the cell numbers in annelidan head kidneys (Bartolomaeus 1995; Bartolomaeus and Quast 2005, see below). In contrast ultrastructural data of the terminal cells and their filtration sites provide additional character states with phylogenetic significance. In species of the Phyllodocidae and Syllidae it was shown that the cytoplasmic cylinder of the terminal cell is reduced; the supporting structure of the filter is

solely formed by strengthened circumciliary microvilli (Bartolomaeus 1989, 1993b). The same structural peculiarity proved to be shared by taxa closely related to Syllidae and Phyllodocidae and was accordingly considered as an autapomorphy of a larger subtaxon within the Phyllodocida comprising at least Phyllodocidae, Syllidae, Alciopidae, Pisionidae, Nephtyidae Glyceridae, Tomopteridae, Hesionidae and Pisionidae (Bartolomaeus 1995, 1997). A supporting structure of the filtration matrix formed by cylindrically arranged microvilli, as now revealed for the echiuran *T. thalassemum*, has never been described for any head kidneys in annelids. The filter-forming terminal cell of the head kidneys of this echiuran, however, corresponds to the state in two serpulid species, *Serpula vermicularis* Linnaeus, 1767 and *Spirorbis spirorbis*, in bearing a tuft of several cilia (Pemerl 1965; Bartolomaeus 1993a). In the serpulid species, eachcilium of the tuft is devoid of circumciliary microvilli, but a huge number of microvilli surround the entire ciliary tuft as in *T thalassemum*. Serpulids differ, however, from *T. thalassemum* since a slashed outer cytoplasmic cylinder encloses the microvilli (Table 1).

Further correspondences with these two serpulid species unfortunately remain unclear as no detailed data are available for the number of cells forming the duct and the nephropore. Among polychaetes, however, the lack of a nephropore cell as revealed for *T. thalassemum* is also only known thus far from a serpulid species, *Pomatoceros triqueter* (Linnaeus, 1767) (Wessing and Polenz 1974) (Table 1). This correspondence is furthermore shared by the "oligochaete" taxa *Tubifex* sp. and *Dendrobaena veneta* (Rosa, 1886) among clitellates (Quast 2007). These two “oligochaete” clitellates differ from *T. thalassemum* in that the single terminal cell of the head kidneys is transformed into a nephrostome that opens into a secondary body cavity. Among polychaetes, head kidneys connected to a secondary body cavity by a proximally opened nephrostome only occur in spionid species (Schlötzer-Schrehardt 1992; Bartolomaeus and Quast 2005). This transformation of the terminal cell

has accordingly been considered as potential synapomorphy of Spionida and Clitellata (Quast 2007).

The head kidneys of *T. thalassemum* presently reveal no distinct morphological support for a close affinity of Echiura to a clade of Capitellidae, Maldanidae, Arenicolidae, and terebelliform taxa, as has been retrieved in recent molecular analyses (e.g. Bleidorn 2003a, 2003b; Hall et al. 2004; Colgan et al. 2006; Struck et al. 2007; Zrzavy et al. 2009). Ultrastructural data on the head kidneys in these polychaetes are only available for the terebelliform species *Lanice conchilega* (Pallas, 1766) (Terebellidae) and *Pectinaria auricoma* Müller, 1776 (Pectinariidae), as well as some preliminary personal observations for the capitellid species *Capitella teleta* Blake, Grassle and Eckelbarger, 2009. In these species, the head kidneys are composed of two or more terminal and duct cells and are equipped with a nephropore cell (Heimler 1981, 1983; Bartolomaeus 1995). The terminal cells variably differ in the number and arrangement of cilia, which possess circumciliary microvilli, as well as in their filtration structures that are basically formed by clefts in the terminal cell's cytoplasm. The apparent absence of filtration structures in *L. conchilega* caused Heimler (1988) to assume that the head kidneys are nonfunctional in this species, but it cannot be excluded that in the developmental stages studied by him they already started to disintegrate (Bartolomaeus 1995). The head kidneys of *T. thalassemum* and the terebelliform and capitellid polychaetes studied thus far only seem to correspond in the multiciliarity of the duct cells. Multiciliary duct cells are widespread in annelids but the ancestral state in annelids (multi- versus monociliary duct cells) still remains unclear (Bartolomaeus, pers. communication).

Stuctural variability of head kidneys within Echiura

For echiuran species other than *T. thalassemum* only light microscopic data on the head kidneys are available. In the Bonelliidae, their structure basically seems to correspond to the state in *T. thalassemum*. Baltzer (1914) described the head kidneys in late larvae of

Bonellia sp. as unbranched tubes with a blind terminal end bearing solenocytes (i.e. terminal cells with a single cilium surrounded by an elongate tubular filtration structure; see Goodrich 1898, p. 442). Dawydoff's (1959) illustration of a head kidney instead suggests that the terminal end forms a multiciliary tuft. More detailed information on the terminal structure and on the number of cells composing the head kidneys in bonellids is still missing.

Head kidneys in the Echiurinae, in contrast, seem to show surprising similarities to the state in *C. teleta* (Capitellidae). In the larva of *Echiurus* sp. the head kidneys were described as short branched tubular ducts with several monociliary terminal cells connected to the ducts via a thin pipe-like tubule (Hatschek 1880; Goodrich 1910; Baltzer 1917; Korn 1960). Strikingly, the definitive protonephridia in the dwarf males of *B. viridis* (Bonellidae) show a similarity to to the head kidneys in *Echiurus* larvae since they possess several terminal cells. Based on ultrastructural studies, Schuchert (1990) found protonephridia in males of *B. viridis* composed of five terminal cells that jointly attach to the terminal end of an unbranched multicellular duct. As in *T. thalassemum*, the terminal cells form ciliary tufts surrounded by numerous microvilli-like cell protrusions, but they differ from the state in *T. thalassemum* in the higher number of about 20 cilia and in the presence of anastomotic interconnections between the microvilli-like cell protrusions. Because these protonephridia arise in postlarval stages and are differently positioned at the posterior end of the trunk, the protonephridia in males of *B. viridis* are not homologue to the larval head kidneys in other species and hence could be unsuitable for any phylogenetic inference. The structural similarities of the head kidneys and segmental protonephridia in polychaetes like *Polygordius* sp., *Glycera dibranchiata* Ehlers, 1868, and *Phyllodoce* ("*Anaitides*") *mucosa* Oersted, 1843, however, support the view that they are serially homologous and thus probably based on the same genetic information (Smith and Ruppert 1988; Bartolomaeus 1989). Since the interrelationships among the echiuran high-ranking

subtaxa still remain unclear (see, e.g., Lehrke and Bartolomaeus 2009), the correspondences of the protonephridia in *Echiurus* larvae (Echiurinae), the dwarf males of *B. viridis* (Bonellidae), and larvae of *C. teleta* (Capitellidae) may accordingly indicate that the head kidneys in larvae of *T. thalassemum* (Thalassematinae) and Bonellidae are derived from the state shown by *Echiurus* larvae.*Conclusions*

Recent molecular insight on a close relationship between Echiura and the polychaete subgroups Capitellida and Terebelliformia receives no corroboration by the structure of the larval head kidneys in *T. thalassemum*. In this echiuran species, the head kidneys rather correspond to states in serpulid polychaetes. Available light microscopical data on *Echiurus* and preliminary data on *Capitella* larvae, however, indicate that this resemblance in *Thalassema* and Serpulidae might rather be based on convergent transformations. Further comparative ultrastructural studies of the head kidneys based on a denser taxon sampling are promising to clarify their evolution.

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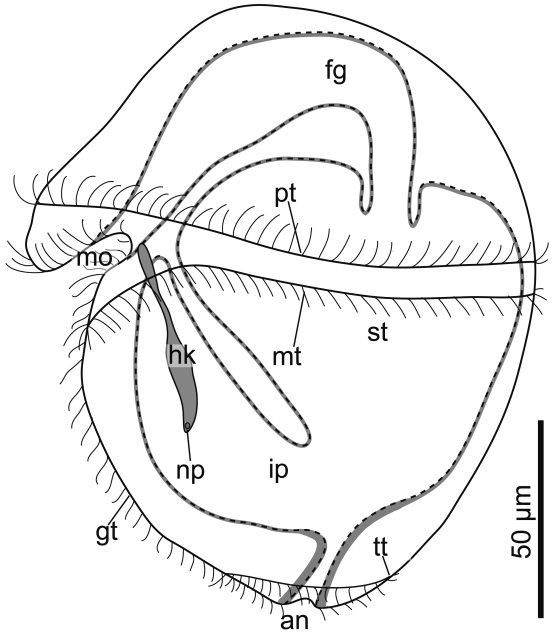

Fig. 1 Schematic drawing of the 98h old larva of *Thalassema thalassemum*, lateral view. The head kidneys (*hk*) are located ventrolaterally beside the stomach (*st*) and the intestinal pouch (*ip*) (only left head kidney is shown). Each head kidney extends from the level of the mouth opening (*mo*) towards the posterior third of the larva, where the nephropore (*np*) is located. *an* anus; *fg* foregut anlage; *gt* gastrotroch; *mt* metatroch; *pt* prototroch; *tt* telotroch.

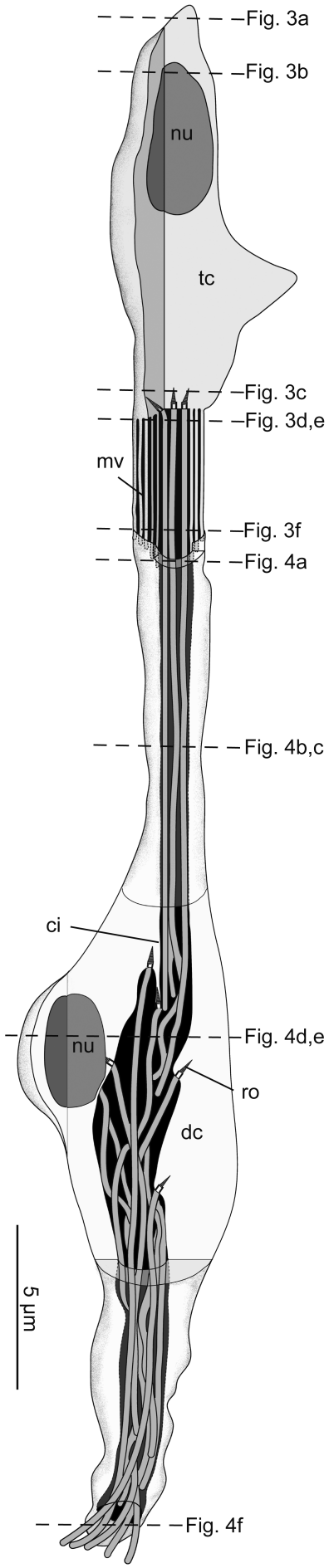

Fig. 2 Schematic representation of the head kidney in the 98 h old larva. The head kidney is composed of two cells only. The filtration structure is formed by elongated microvilli (*mv*) emerging from the terminal cell (*tc*). *ci* cilium; *dc* duct cell; *mc* muscle cell, *nu* nucleus; *ro* ciliary rootlet; *tc* terminal cell.

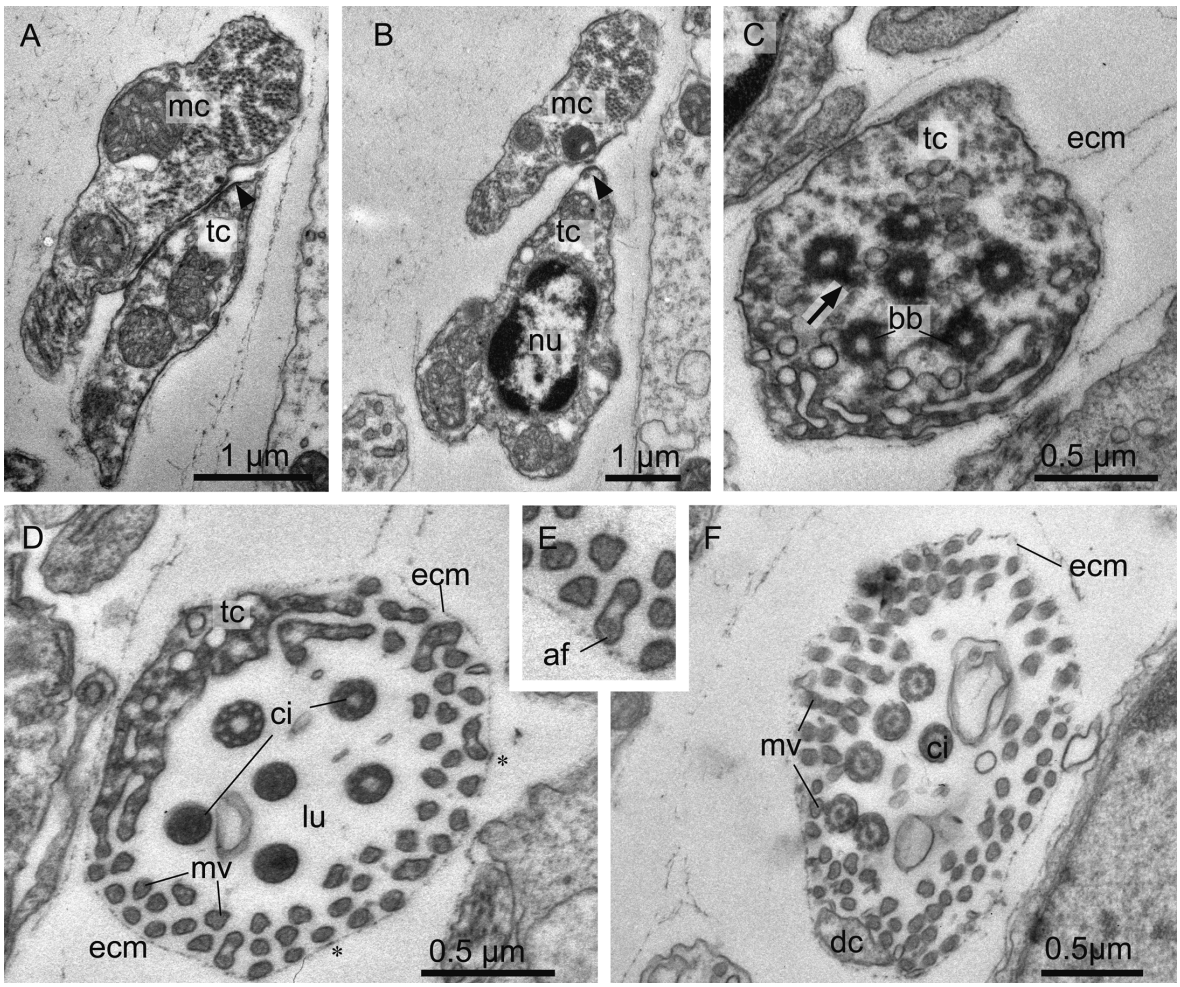

Fig. 3 TEM, cross sections of the terminal cell **a** The apex of the cell (*tc*) is connected to a muscle cell (*mc*) via dense plaques (*arrowhead*). **b** The nucleus (*nu*) is located in the proximal part of the terminal cell (*tc*). The perikayon shows further connections to the muscle cell (*mc*) by dense plaques (*arrowhead*). **c** The distal end of the cell forms a flattened area from which six cilia protrude into the nephridial lumen. Basal bodies (*bb*) with a short rootlet and a basal footlet (*arrow*) anchor the cilia within this area. **d** Distally circularly arranged microvilli (*mv*) enclose the lumen (*lu*) of the terminal cell. The outermost microvilli are covered by a thin layer of extracellular matrix (*ecm*) to which they are connected by hemidesmosomes (*asterisk*). **e** Actin filaments (*af*) are present within the microvilli. **f** Transition from the terminal cell to the duct cell (*dc*). The thin layer of extracellular matrix (*ecm*) that surrounds the outermost microvilli (*mv*) is continuous with the matrix enclosing the duct cell (*dc*). *ci* cilium

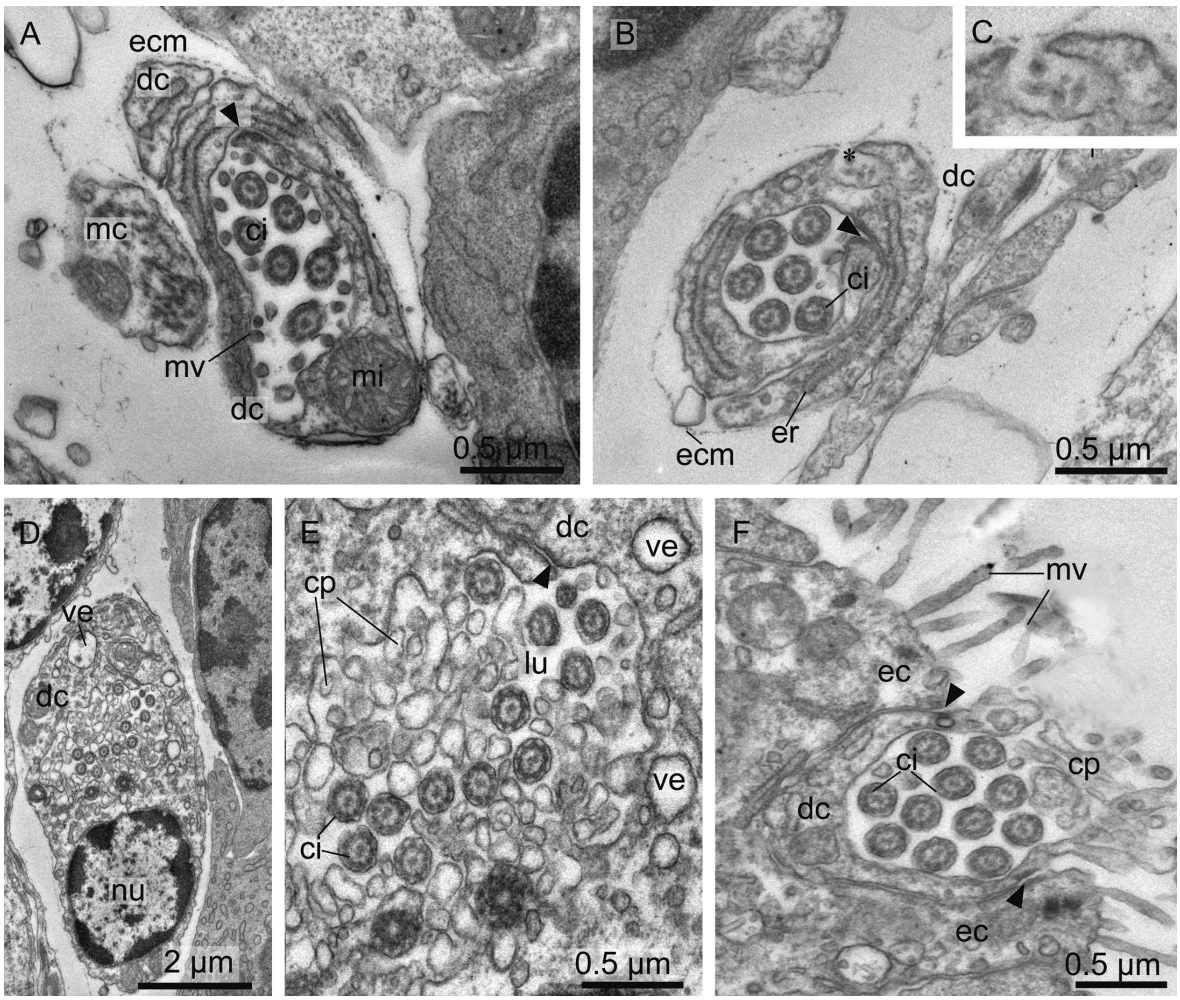

Figure 4 TEM, cross sections of the duct cell **a** Proximal part of the duct cell (*dc*). In this section the duct cell shows a small lateral bulge (*bl*), that fuses with the rest of the cell in the subsequent sections. Cilia (*ci*) and microvilli (*mv*) of the terminal cell extend into the duct lumen. *arrowhead* indicates adluminal adherens junction. **b** Cross section of duct cell proximally to the perikaryon.The lumen contains cilia (*ci*) of terminal cell. The duct cell enfolds the percellular lumen. An adluminal adherens junction (*arrowhead*) forms a longitudinal seal along its whole length. The adluminal membranes possess pits indicating transcytotic processes (*asterisk* and inset **c**). **d** Middle part of the duct cell (*dc*) with nucleus (*nu*). Lumen contains cilia (*ci*) and cytoplasmic protrusions originating from the adluminal membrane of the duct cell. **e** Detail from **d**, showing finger-like cytoplasmatic protrusions (*cp*) and pits at the adluminal membrane. The cytoplasm of the duct cell

contains numerous vesicles (*ve*). **f** The distal part of the duct cell pierces the epidermis (*ec*) and opens to the exterior via a nephropore. Some cilia (*ci*) extend through the nephopore to the outside.

510 *ecm* electron dense extracellular matrix;*er* endoplasmatisches reticulum; *lu* lumen of duct; *mc* muscle cell, *mi* mitochondrium.

Table 1: Character states of head kidneys in Echiura and outgroup taxa supposed to be closely related to Echiura by Struck et al. (2007) and Zrzavy et al. (2009)

Species	Terminal structure				Duct			Nephridiopore		References
	# cells	# cilia / cell	*cmv*	filter	# cells	# cilia / cell	*mv*	# cells	# cilia / cell	
Echiura										
Thalassema thalassemum	1	mc (6)	-	mv ring	1	~15	-	-	n.a.	this paper
Echiurus sp.[1]	several	1	?	?, *	?	?	?	?	?	Hatschek 1880; Goodrich 1910
Bonellia sp.[1]	?	mc	-	?	?	?	?	?	?	Baltzer 1931
Bonellia viridis[2] dwarf male posterior protonephridium	5	mc	-	perforated cytoplasm	many	mc	-	?	?	Schuchert 1990
Capitellida										
Capitella teleta	several	1-2	10-11	perforated cytoplasm	>2	mc	?	1	?	pers. observation
Terbelliformia										
Lanice conchilega	2	mc (~15)	?	?	2	?	?	1	?	Heimler 1981, 1983, 1988
Pectinaria auricoma	2	mc (~30)	8-12	perforated cytoplasm	2	mc	∞	1	-	Bartolomaeus 1995
Clitellata										
Dendrobaena veneta	1	mc (∞)	-	nephrostome	1	mc	∞	-	n.a.	Quast 2007
Tubifex sp.	1	mc (∞)	-	nephrostome	1	mc	∞	-	n.a.	Quast 2007
Serpulida										
Pomatoceros triqueter	1	mc	-	“Weir”		mc	?	-	n.a.	Wessing & Polenz 1974
Serpula vermiculosus	1	mc	-	perforated cytoplasm	?	mc	?	?	?	Pemerl 1965
Spirorbis spirorbis	1	mc	-	perforated cytoplasm	1	mc	∞	1	mc	Bartolomaeus 1995; Bartolomaeus & Quast 2005

All data refer to ultrastructural investigations except for taxa marked with[1] (light microscopic data); [2] data from definitive nephridium; * “Solenocyte” according to Goodrich 1910; *cmv* circumciliary microvilli; *mc* multiciliated; *mv* microvilli; *n.a.* not applicable