Acta Zoologica

Published for The Royal Swedish Academy of Sciences and The Royal Danish Academy of Sciences and Letters

Acta Zoologica (Stockholm) **91**: 199–211 (April 2010) doi: 10.1111/j.1463-6395.2008.00399.x

The evolution of the protonephridial terminal organ across Rotifera with particular emphasis on *Dicranophorus forcipatus*, *Encentrum mucronatum* and *Erignatha clastopis* (Rotifera: Dicranophoridae)

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Keywords:
transmission electron microscopy, protonephridia, terminal organ, rotifera, phylogeny

Accepted for publication:
1 December 2008

Abstract

Riemann, O. and Ahlrichs, W. H. 2010. The evolution of the protonephridial terminal organ across Rotifera with particular emphasis on *Dicranophorus forcipatus*, *Encentrum mucronatum* and *Erignatha clastopis* (Rotifera: Dicranophoridae). — *Acta Zoologica* (Stockholm) **91**: 199–211

We report on the ultrastructure of the protonephridial terminal organ in three species of dicranophorid rotifers (*Dicranophorus forcipatus*, *Encentrum mucronatum* and *Erignatha clastopis*). Differences between the three species relate to shape and size, the morphology of the filter region and the number of microvilli and cilia inside the terminal organ. A comparison across Rotifera indicates that the terminal organs in *D. forcipatus* display a number of plesiomorphic characters, but are modified in *E. mucronatum* and *Er. clastopis*. This is in accordance with the results of phylogenetic analyses suggesting a basal position of *D. forcipatus* compared with the more derived species *E. mucronatum* and *Er. clastopis*. Moreover, we survey available data on the terminal organ in Rotifera and discuss its evolutionary transformations. The protonephridial terminal organ in the common ancestor of Rotifera consisted of a cytoplasmic cylinder with cilia united into a vibratile flame and a single circle of circumciliary microvilli. Depending on the topology on which characters are optimized, the site of ultrafiltration was formed by longitudinal cytoplasmic columns spanned by a fine filter diaphragm or by pores in the wall of the terminal organ. In several taxa of Rotifera, the terminal organ – probably independently – lost its circumciliary microvilli.

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Introduction

Rotifers are a morphologically diverse group of aquatic micrometazoans inhabiting both freshwater and marine environments (Fontaneto *et al.* 2006; Wallace *et al.* 2006). About 2000 valid species are currently recognized (Segers 2007). Like many acoelomate bilaterians and the larvae of some coelomate taxa, rotifers possess a system of protonephridia involved in excretion and osmoregulation (Ruppert and Smith 1988; Bartolomaus and Ax 1992). The paired protonephridial system in ploimid rotifers generally consists of: (1) few to several terminal organs that collect fluids from the body cavity through ultrafiltration, (2) a system of collecting tubules through which the primary urine passes and is modified by reabsorption processes, and (3) a protonephridial bladder that empties its contents into a cloaca through contraction of its muscular wall (Clément and Wurdak 1991). The protonephridial system in Bdelloidea

and Gnesiotrocha slightly differs from this general pattern: a protonephridial bladder is absent and the collecting tubules directly discharge into the contractile cloaca (Clément and Wurdak 1991).

Over the last decades, the protonephridial system in rotifers has been studied repeatedly (see Brakenhoff 1937 and Pontin 1963 for results of light microscopic investigations; for studies relying on transmission electron microscopy (TEM) see Braun *et al*. 1966, Mattern and Daniel 1966, Clément 1968, Warner 1969, Schramm 1978, Clément and Fournier 1981, Clément 1985, Bartolomaus and Ax 1992, Ahlrichs 1993a,b, 1995). Of the different components of the protonephridial system, the multiciliated terminal organs ('flame bulbs') have been explored most intensely. As a consequence, the largest dataset is available for this region of the protonephridial system. In the present study, we focus on the terminal organs in *Dicranophorus forcipatus* (Müller 1786), *Encentrum mucronatum* Wulfert 1936 and *Erignatha clastopis* (Gosse 1886) as members of the species rich taxon Dicranophoridae, for which no data have been published. We provide reconstructions of their ultrastructure based on complete ultrathin serial sections and evaluate our findings against the background of a recent phylogenetic analysis of Dicranophoridae (Riemann *et al*. 2009). We also place the results of our investigation in a comparative context with previous studies on other rotifer species and draw on recently published phylogenetic analyses (Sørensen 2002; Sørensen and Giribet 2006) for a discussion of the evolutionary transformation of the protonephridial terminal organ across Rotifera.

Materials and Methods

Specimens of *D. forcipatus* (O. F. Müller 1786) and *Er. clastopis* (Gosse 1886) were sampled in shallow ditches covered with *Lemna* sp. near Oldenburg, northwest Germany. *Encentrum mucronatum* Wulfert 1936 was obtained from wet moss cushions squeezed out into Petri dishes. For TEM studies, specimens were anaesthetized for 5 min in an aqueous solution of 0.25% bupivacaine (Bucain®) and subsequently fixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer at 4 °C. After fixation, specimens were dehydrated in an increasing acetone series, subsequently embedded in Araldite hardened at 60 °C for 72 h and ultrasectioned (70 nm) on a Reichert ultracut followed by automatic staining with uranyl acetate and lead citrate (Leica EM Stain). The resulting TEM preparations were observed on a Zeiss 902 TEM at 80 kV. Photographs of the sections were taken with a Dual Scan CCD camera and subsequently assembled digitally using the multiple image alignment (MIA) function of ITEM® software (Soft Imaging System, Olympus, Münster, Germany). The chief advantage of composite images is that imaging of larger structures at higher magnifications and better resolution is possible. Such digitally assembled images provided the basis for the reconstructions of the protonephridial terminal organs. In total, four specimens were investigated (two specimens of *D. forcipatus*, and one specimen each of *E. mucronatum* and *Er. clastopis*). The reconstructions are based on complete serial sections of a single protonephridial terminal organ in each specimen. However, to check for potential variation within single specimens and to verify the reconstructions obtained, those terminal organs not used for the reconstructions were also investigated. Observations of living specimens under a Leica DM-LB light microscope were carried out using both bright field and differential interference contrast. Digital images were taken with an Olympus colour view I digital camera.

Remark on terminology

The terms 'proximal' and 'distal' referring to positions in the terminal organ are used in accordance with Bartolomaus and Ax (1992). They are defined in relation to the direction of urine flow. The movement of cilia that are positioned proximally in the terminal organ produces negative filtration pressure. In the distal section of the terminal organ, this negative filtration pressure causes an inflow of fluids from the body cavity into the lumen of the terminal organ via a filtration structure.

Results

The terminal organ: general construction

The protonephridial terminal organs in *D. forcipatus* (Fig. 1A), *E. mucronatum* (Fig. 1B) and *Er. clastopis* (Fig. 1C) all follow the same general organization: proximally, they begin with a cytoplasmic cap characterized by the presence of a high number of mitochondria and a considerable amount of endoplasmic reticulum. In the proximal cytoplasmic cap, the basal bodies of the cilia of the vibratile flame are situated. An accessory centriole is absent as are ciliary rootlets. More distally, the cytoplasmic cap is continued by the filter region of the terminal organ, the site of ultrafiltration. In this section, the terminal organ constitutes a hollow cylinder whose walls are composed of a single circle of longitudinal cytoplasmic columns separated from each other by narrow clefts. The cytoplasmic columns are interconnected by a fine diaphragm that constitutes the ultrafiltration barrier. Inside the lumen of the terminal organ, cilia and microvilli are present. The cilia are closely adjacent to each other with their axonemata aligned; the microvilli are positioned external to the cilia. Continuing distally, the cytoplasmic columns separated by longitudinal clefts cease and are replaced by an unbroken cytoplasmic wall enveloping a lumen into which cilia and microvilli project. In this section of the terminal organ, a nucleus is present, positioned in a cytoplasmic pocket shifted sideways. More distally, the cilia and microvilli terminate and the lumen of the terminal organ is continued by a capillary canal into which the primary urine is discharged. There is no

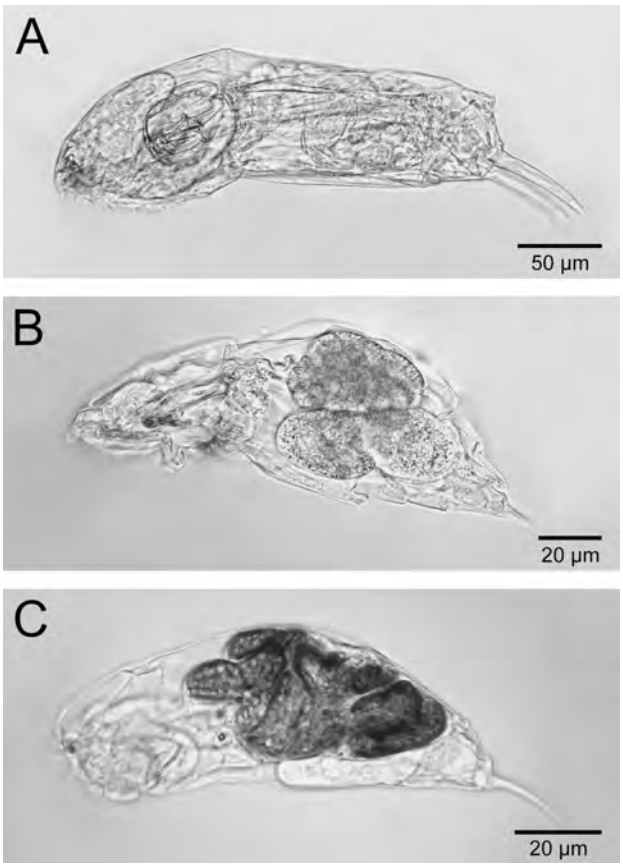

Fig. 1—Living specimens in lateral view. Light microscopic images (bright field). —**A**. *Dicranophorus forcipatus*. —**B**. *Encentrum mucronatum*. —**C**. *Erignatha clastopis.*

cell border between the terminal organ and the discharging capillary canal. The whole terminal organ is surrounded by a fine layer of extracellular matrix.

The terminal organ in detail: D. forcipatus

In *D. forcipatus*, the terminal organ is conspicuously flattened and in the filter region it is approximately 1.2 µm across and 5 µm wide. Distally, its width decreases continuously to about 3 µm. The total length of the terminal organ from the proximal cytoplasmic cap to the junction of terminal organ and capillary canal amounts to about 9 µm; the region of the filter is about 4 µm long (Fig. 3). Inside the hollow cytoplasmic cylinder, about 45 cilia are present (diameter about 0.2 µm). They are arranged in transverse rows of two to four cilia (Figs 2C,D and 3). The arrangement of the ciliary basal bodies reflects the convex shape (Fig. 3) of the cytoplasmic cap of the terminal organ: the cilia that – as a functional unit – form the vibratile flame do not all begin at exactly the same level. Their basal bodies are arranged such that the basal bodies in the lateral corners of the terminal organ are positioned more distal than those situated in the middle. Consequently, in a single section the cilia are sectioned at different levels (Fig. 2C). All cilia are in close contact and, in the region of the filter, immediately adjacent to each other (Fig. 2D). Electron-dark material enveloping all cilia together is absent. External to the cilia, about 20 microvilli are positioned (Figs 2A,C,D,F and 3). Their diameter reaches about 0.25 µm. In the filter region, the microvilli are evenly spaced at a distance of about 0.5 µm from each other (Fig. 2C,D). Compared with the cilia, the microvilli end more proximally in the lumen of the terminal organ. Their distal tips are in close contact with the cell membrane of the cytoplasmic cylinder without, however, complete fusion (Fig. 2F). More peripherally in the construction of the terminal organ, the microvilli in the filter region are followed by cytoplasmic columns separated from each other by longitudinal clefts. In cross-section, the individual cytoplasmic columns are sickle-shaped with rounded edges (Fig. 2C–E). A pattern in the distribution of narrow and wider cytoplasmic columns is apparent: for every three narrow columns (about 0.1 µm), a wider column (about 0.2 µm) is interspersed (Fig. 2C,D). The wider cytoplasmic columns are regularly distributed between two microvilli (Fig. 2C,D). The cross-sections of the cytoplasmic columns taken together are arranged on an undulating line with regular bulging facing the microvilli and indentations between two microvilli (Fig. 2A,C,D). Conspicuous arc-shaped structures are produced. The cleft between the cytoplasmic columns (about 40 nm) is bridged by a fine, electron-dark diaphragm. Ciliary rootlets supporting the filtration structure are absent. The wall of the cytoplasmic cylinder distal to the filter region is characterized by the presence of considerable amounts of endoplasmic reticulum (Fig. 2F,G). At the distal end of the terminal organ, a nucleus (about 2 µm across) is positioned in a cytoplasmic pocket (Fig. 2H).

The terminal organ in detail: E. mucronatum

The terminal organ of *E. mucronatum* is rounded to oval in cross-section and about 1.5 µm wide and 0.75 µm across. Its total length from the cytoplasmic cap to the beginning of the capillary canal reaches 2.5 µm; the length of the filter region is about 1.5 µm (Fig. 5). Inside the lumen of the cytoplasmic cylinder, 4 cilia are present (diameter about 0.2 µm). All cilia are in close contact along their length and, as a functional unit, are surrounded by a fine layer of electron-dark material (Fig. 4B–E). The number of cilia is reflected in the number of microvilli: four microvilli are present that are conspicuously positioned in the corners of the elongate terminal organ (Fig. 4B–E). Their diameter is about 0.25 µm and slightly exceeds that of the cilia. Distally, the microvilli decrease in diameter and fill out indentations in the wall of the cytoplasmic cylinder (Fig. 4E,F). However, as in *D. forcipatus*, no fusion between microvilli and cell membrane of the cytoplasmic cylinder is present; two immediately adjacent membranes

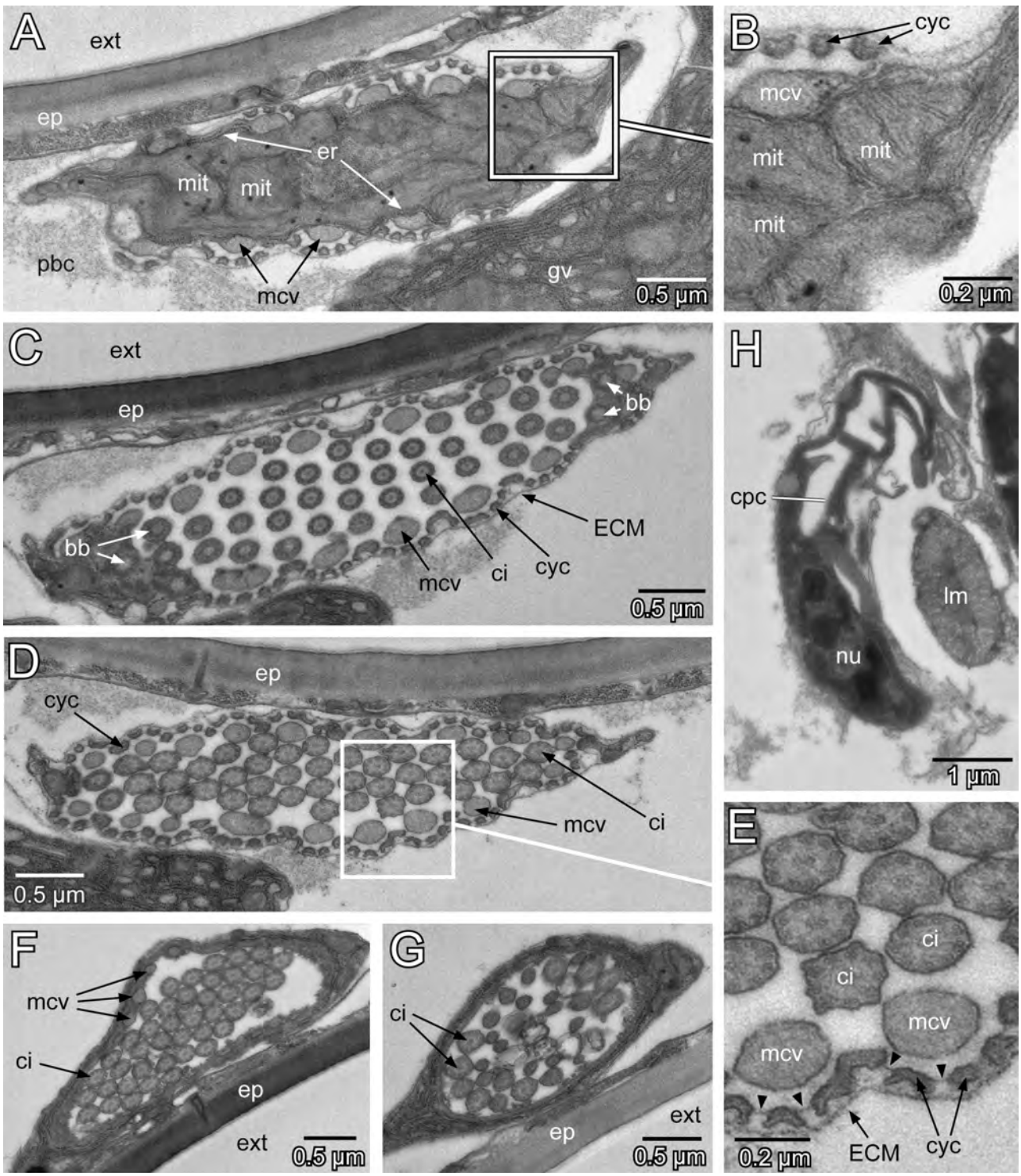

Fig. 2—Cross-sections through different parts of the protonephridial terminal organ in *Dicranophorus forcipatus*. Transmission electron micrographs. —**A**. Section through cytoplasmic cap of terminal organ. —**B**. Close-up of A. Note mitochondria with internal membranes. —**C**. Level of section more distal to that of A. Beginning of filter region. —**D**. Section through filter region. —**E**. Close-up of D. Note construction of filter. Arrowheads indicate filter diaphragm. —**F.** Section through terminal organ distal to filter. —**G**. Level of section slightly below junction of terminal organ and discharging capillary canal. —**H**. Section through nucleus and discharging capillary canal. bb, cilia basal bodies; ci, cilium; cpc, capillary canal; cyc, cytoplasmic column; ECM, extracellular matrix; ep, epidermis; er, endoplasmic reticulum; ext, exterior; gv, germovitellarium; lm, longitudinal muscle; mcv, microvilli; mit, mitochondrion; nu, nucleus; pbc, primary body cavity.

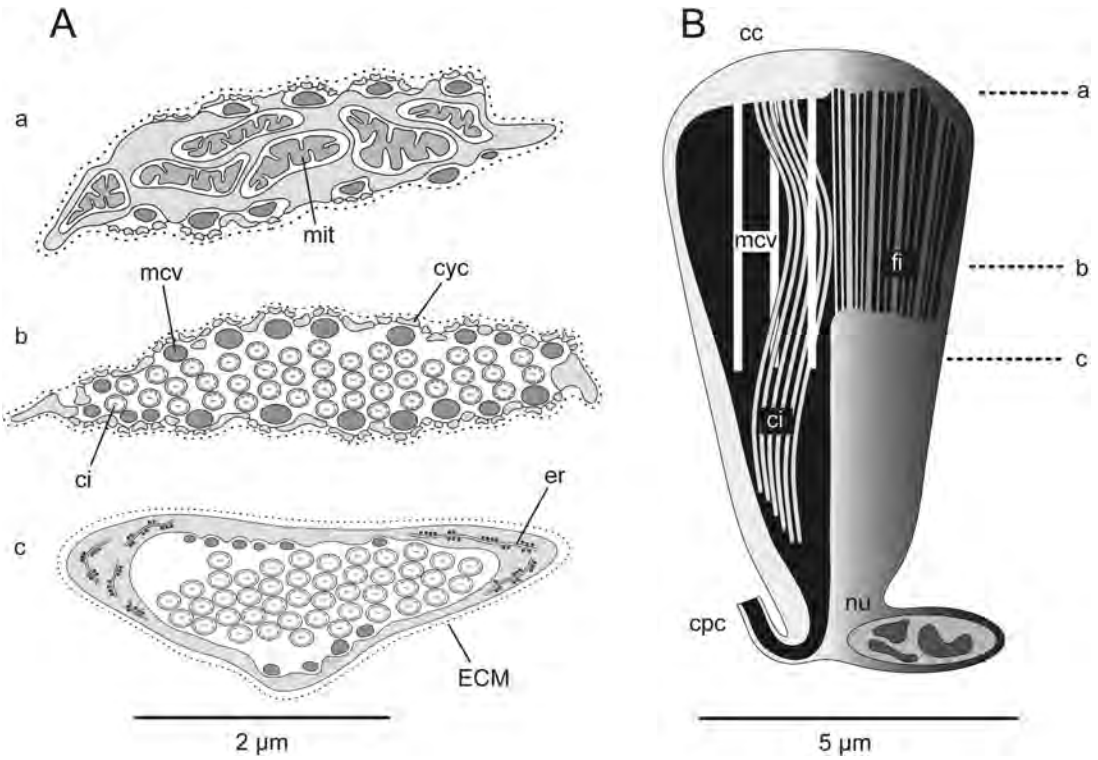

Fig. 3—Schematic reconstruction of the protonephridial terminal organ in *Dicranophorus forcipatus* based on ultrathin serial sections. Terminal organ is partly opened demonstrating internal organization. Note that for clarity, only a small number of cilia and microvilli have been included in the drawing from lateral. Broken lines indicate levels of section for schematic cross-sections in A–C. cc, cytoplasmic cap; ci, cilia; cpc, capillary canal; cyc, cytoplasmic column; ECM, extracellular matrix; er, endoplasmic reticulum; fi, filter; mcv, microvilli; mit, mitochondrion; nu, nucleus.

can be discerned (Fig. 4E). The sections of the cytoplasmic columns are from 50 to 100 nm across (Fig. 4B,C). An alternating pattern of wider and narrower cytoplasmic columns as in *D. forcipatus* is absent. The cleft between the cytoplasmic columns is about 40 nm wide and is bridged by a fine, electron-dark diaphragm (Fig. 4B,C). Ciliary rootlets supporting the filtration structure are absent. The wall of the cytoplasmic cylinder distal to the filter region is conspicuous for the presence of large amounts of endoplasmic reticulum (Fig. 4E). At the distal end of the terminal organ, an irregularly shaped nucleus (about 2 µm across) is positioned in a cytoplasmic pocket (Fig. 4F).

The terminal organ in detail: Er. clastopis

The terminal organ of *Er. clastopis* is rounded in cross-section and in the filter region has a diameter of about 1.2 µm. From the proximal section of the cytoplasmic cap to the beginning of the capillary canal, the total length of the terminal organ measures about 2.9 µm (Fig. 7). The filter region has a proximo-distal extension of about 1.3 µm. Inside the hollow cytoplasmic cylinder of the terminal organ, four closely adjacent cilia surrounded by electron-dense material and forming a functional unit, the vibratile flame, are present (Figs 6A–F and 7). The diameter of the cilia is about 0.25 µm. Peripheral to the cilia are four microvilli with a maximum diameter of 0.15 µm, positioned at approximately equal distances from each other (Fig. 6B–G). Distally, they taper considerably to about 75 nm and run along the length of the terminal organ in shallow indentations of the luminal membrane (Fig. 6E). Cilia and microvilli distally terminate at the same level at the junction of the terminal organ and capillary canal (Fig. 6G). The cytoplasmic columns of the filter region are evenly spaced and measure from 50 to 100 nm in cross-section (Fig. 6A–D). The narrow cleft between two adjacent cytoplasmic columns is about 40 nm wide and is bridged by a very fine electron-dark diaphragm (Fig. 6D). There are no ciliary rootlets stabilizing the filter structure. The cytoplasm of the terminal organ distal to the filter region and also that of the proximal section of the capillary canal is characterized by large amounts of endoplasmic reticulum (Fig. 6E,G). An irregularly shaped nucleus (about 1.5 µm across) is positioned in a cytoplasmic pocket distal to the filter region and at a considerable distance of about 4 µm from the lumen of the cytoplasmic cylinder (Fig. 6F).

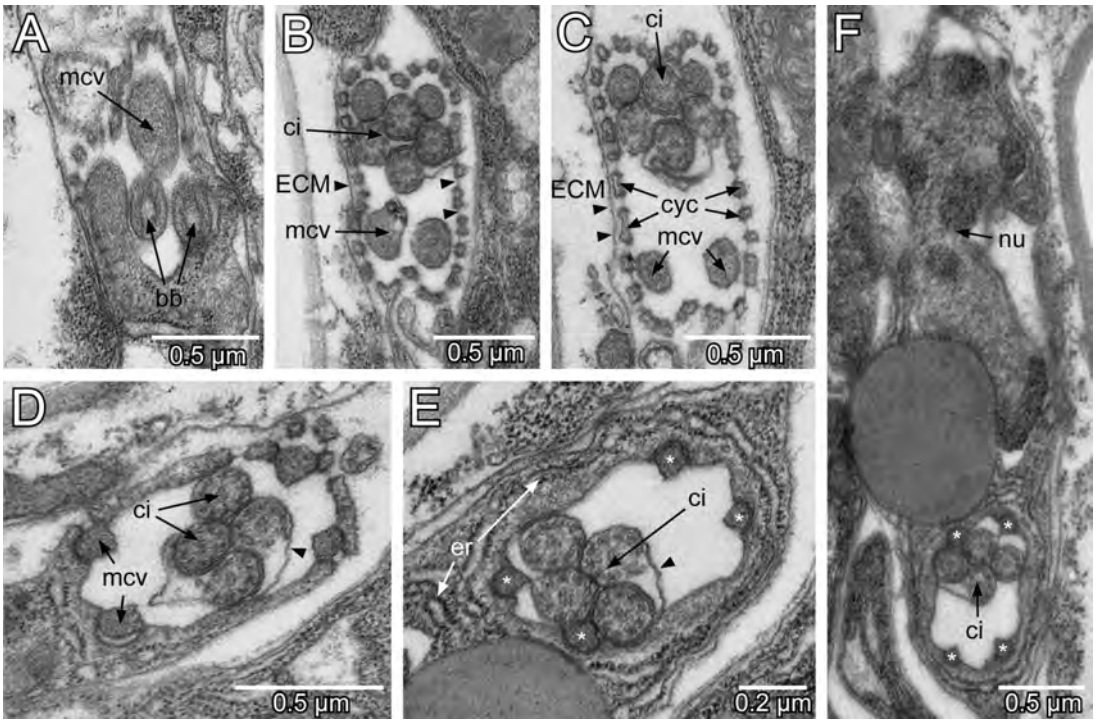

Fig. 4—Cross-sections through different parts of the protonephridial terminal organ in *Encentrum mucronatum*. Transmission electron micrographs. —**A**. Section through proximal part of terminal organ. Beginning of filter —**B**. Section through filter region. Note position of microvilli (mcv) in rounded corners of terminal organ. Arrowheads indicate fine diaphragm between cytoplasmic columns. —**C**. Level of section more distal to that of B. —**D**. Level of section at distal end of filter region. Arrowhead indicates electron-dark material enveloping cilia. —**E**. Level of section distal to filter region. Note that microvilli (*) are immediately adjacent to luminal cell membrane. Arrowhead indicates electron-dark material enveloping cilia. —**F**. Section through terminal organ at level of nucleus. Microvilli indicated by asterisks (*). bb, cilia basal bodies; ci, cilia; cyc, cytoplasmic column; ECM, extracellular matrix; er, endoplasmic reticulum; mcv, microvilli; nu, nucleus.

Discussion

The terminal organ in Dicranophoridae

Although generally following the same organization, the terminal organs in *D. forcipatus*, *E. mucronatum* and *Er. clastopis* differ considerably in structural detail (for the following comparison, see also Table 1). These differences relate to (1) their size and shape, (2) the morphology of the filter region and (3) the number and specific arrangement of microvilli and cilia inside the hollow cytoplasmic cylinder of the terminal organ.

As regards size, the terminal organ in *D. forcipatus* in its proximodistal extension is almost four times the size of that in *E. mucronatum* and *Er. clastopis*. In *D. forcipatus* the terminal organ is fan-shaped and conspicuously flattened, while in *E. mucronatum* and *Er. clastopis* it is almost round in cross-section with only very slight flattening in *E. mucronatum*. Another difference between *D. forcipatus* on the one hand and *E. mucronatum* and *Er. clastopis* on the other concerns the morphology of the filter region. In cross-sections the cytoplasmic columns in *D. forcipatus* are arranged in an undulating manner with regular bulging and indentations (see above), which results in a pattern of regular arcs. This contrasts with the simpler arrangement of the cytoplasmic columns in *E. mucronatum* and *Er. clastopis*, where no arcs occur. Moreover, there are considerable differences in the number of both cilia (40–45 in *D. forcipatus*, four in *E. mucronatum* and *Er. clastopis*) and microvilli (20 in *D. forcipatus*, four in *E. mucronatum* and *Er. clastopis*). In all three species the ciliary axonemata in the terminal organ are aligned, so one can assume that the cilia in all three species act in concert and form a vibratile flame. However, electron-dark material surrounding all cilia is only present in *E. mucronatum* and *Er. clastopis*.

Considering outgroup taxa, we find several rotifer species that, as far as their terminal organs are concerned, correspond to the situation in *D. forcipatus* (see also Table 1). Let us first consider external features: terminal organs noticeably flattened have also been recorded in *Asplanchna priodonta* (see Braun *et al.* 1966), *Asplanchna brightwelli* (see Warner 1969), *Notommata copeus* (see Clément 1968), *Taphrocampa*

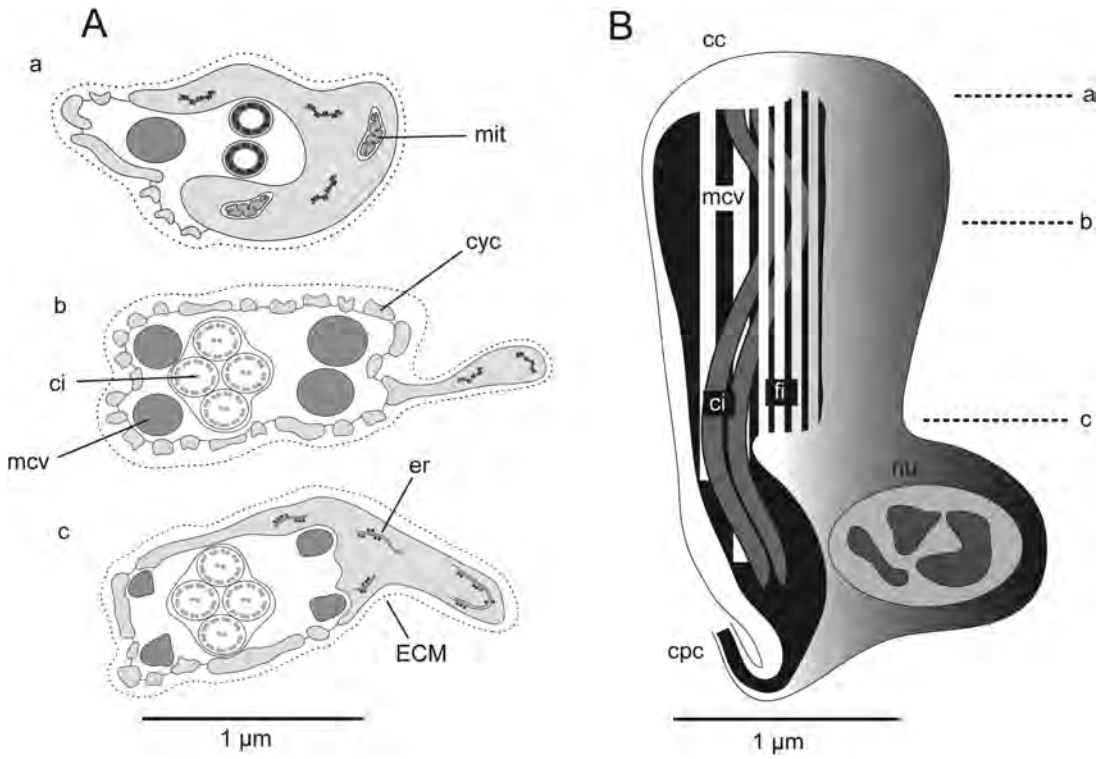

Fig. 5—Schematic reconstruction of the protonephridial terminal organ in *Encentrum mucronatum* based on ultrathin serial sections. Terminal organ is partly opened demonstrating internal organization. Broken lines indicate level of section for schematic cross-sections in A–C. Note that for clarity, in lateral view (B) only two cilia and two microvilli have been drawn. cc, cytoplasmic cap; ci, cilia; cpc, capillary canal; cyc, cytoplasmic column; ECM, extracellular matrix; er, endoplasmic reticulum; fi, filter; mcv, microvilli; mit, mitochondrion; nu, nucleus.

selenura (see Ahlrichs 1995) and *Monommata longiseta* (see Ahlrichs 1995). This shape apparently coincides with the specific arrangement of cyptoplasmic columns as specified above. As far as internal features of the terminal organ in these species are concerned, *D. forcipatus*, *A. priodonta*, *A. brightwelli*, *N. copeus*, *T. selenura* and *M. longiseta* are all characterized by a high number of cilia (ranging from 21 in *T. selenura* to about 65 in *N. copeus*) and many microvilli (from 12 in *T. selenura* to about 38 in *A. priodonta*).

These findings can be taken to indicate that, for Dicranophoridae, the terminal organ in *D. forcipatus* displays a number of plesiomorphic features (flattened shape, high number of cilia and microvilli, specific arrangement of cytoplasmic columns) also present in several outgroup taxa (Notommatidae: *N. copeus*, *T. selenura*, *M. longiseta*; Asplanchnidae: *A. priodonta*, *A. brightwell*, see Sørensen 2002; compare also Sørensen and Giribet 2006). The terminal organ in *E. mucronatum* and *Er. clastopis*, by contrast, can be assumed to be derived in several respects (rounded to elongate in cross-section, only four cilia and four microvilli probably resulting from secondary reduction). These conclusions are in accordance with phylogenetic analyses that suggest a basal position of *D. forcipatus* compared with the more derived taxa *E. mucronatum* and *Er. clastopis* (see Riemann *et al*. 2009).

The terminal organ in Monogononta

Apart from differences in size and shape of the terminal organs and the arrangement of the cytoplasmic columns, the most obvious difference between the individual species of Monogononta so far investigated lies in the presence or absence of microvilli (see also Table 1). The presence of microvilli inside the cytoplasmic cylinder of the terminal organ, positioned peripheral to the cilia of the vibratile flame, has been confirmed for all monogonont species with the exception of *Proales reinhardti* (see Ahlrichs 1993a) and *Colurella colurus* (see Ahlrichs 1995). Terminal organs without microvilli have also been found in all bdelloid rotifers studied so far (*Habrotrocha rosa*, see Schramm 1978; *Philodina roseola*, see Clément and Wurdak 1991; *Rotaria rotatoria*, see Bartolomaus and Ax 1992; *Zelinkiella synaptae*, see Ahlrichs 1995) as well as in *Seison annulatus* (see Ahlrichs 1993b). However, phylogenetic analyses (Sørensen 2002; Sørensen and Giribet 2006) neither suggest that *Proales reinhardti* and *Colurella colurus* are closely related to Bdelloidea and *Seison* nor is there any indication that they are closely allied themselves (i.e. that the absence of microvilli in the terminal organs is a shared derived character). At present, it seems more plausible to assume that in *Proales reinhardti*

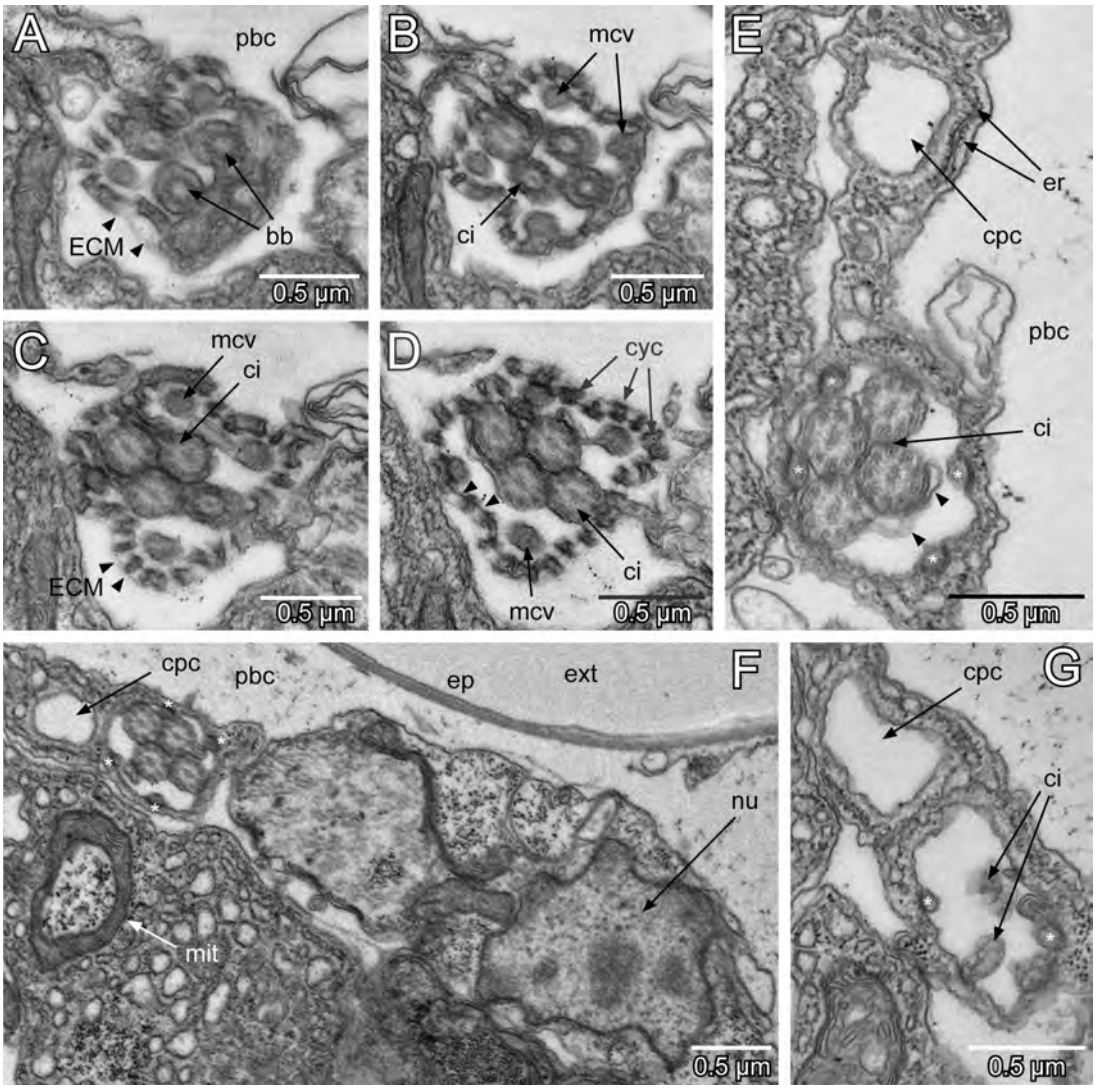

Fig. 6—Cross sections through different parts of the protonephridial terminal organ in *Erignatha clastopis*. Transmission electron micrographs. —**A**. Section through proximal part of terminal organ. Beginning of filter. —**B**. Section through filter region. Note position of microvilli (mcv) in rounded corners of terminal organ. —**C**. Level of section more distal to that of B. —**D**. Level of section at distal end of filter region. Arrowheads indicate electron-dark filter diaphragm. —**E**. Level of section distal to filter region. Note that microvilli (*) are immediately adjacent to luminal cell membrane. Arrowhead indicates electron-dark material enveloping cilia. Capillary canal is also sectioned. —**F**. Section through terminal organ at level of nucleus. Microvilli indicated by asterisks (*). —**G**. Section through most distal part of terminal organ. Note that cilia and microvilli terminate at about the same level. Asterisks (*) indicate microvilli. bb, cilia basal bodies; ci, cilia; cpc, capillary canal; cyc, cytoplasmic column; ECM, extracellular matrix; ep, epidermis; er, endoplasmic reticulum; ext, exterior; mcv, microvilli; mit, mitochondrium; nu, nucleus; pbc, primary body cavity.

and *Colurella colurus* (and probably other monogonont taxa not investigated so far) the terminal organs lost their microvilli independently.

Another difference in the structure of the terminal organs in Monogononta relates to the relative diameters of microvilli and cilia (see also Table 1). In some taxa the microvilli exceed the cilia in diameter (*Notommata copeus*, see Clément 1968; *D. forcipatus*, this study), in others the microvilli and cilia are more or less identical in diameter (*A. priodonta*, see Braun *et al.* 1966; *A. brightwelli*, see Warner 1969; *Taphrocampa selenura*, *Monommata longiseta*, see Ahlrichs 1995; *E. mucronatum*, this study). There are also some species, in which the cilia exceed the microvilli in diameter (*Trichocerca rattus*, see Clément and Wurdak 1991; *Notholca bipalium*, see Ahlrichs 1995; *Er. clastopis*, this study). A survey of outgroup taxa indicates that in *Catenula* sp.

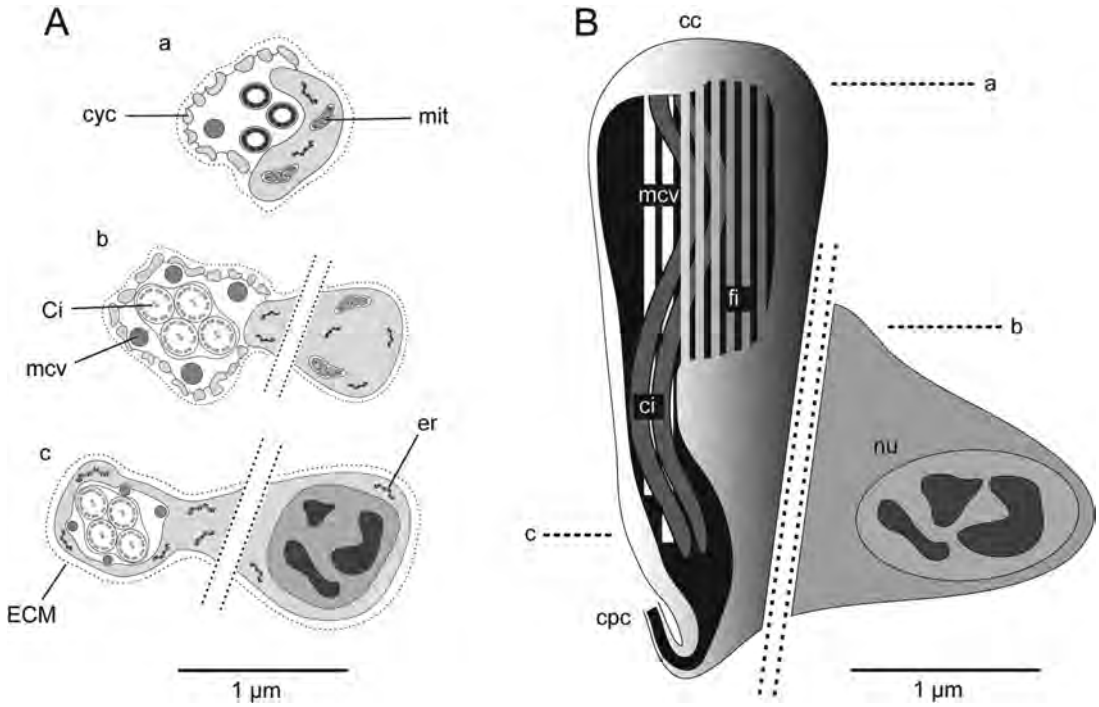

Fig. 7—Schematic reconstruction of the protonephridial terminal organ in *Erignatha clastopis* based on ultrathin serial sections. Terminal organ is partly opened demonstrating internal organization. Broken lines indicate level of section for schematic cross-sections in A–C. Parallel dashed lines symbolize that cytoplasmic pocket in its relative dimension is larger than depicted. Note that for clarity, in lateral view (B) only two cilia and two microvilli are drawn. cc, cytoplasmic cap; ci, cilia; cpc, capillary canal; cyc, cytoplasmic column; ECM, extracellular matrix; er, endoplasmic reticulum; fi, filter; mcv, microvilli; mit, mitochondrion; nu, nucleus.

(Plathelminthes, see Rohde and Watson 1994), *Gnathostomula paradoxa* (Gnathostomulida, see Lammert 1985) and *Limnognathia maerski* (see Kristensen and Funch 2000), the cilia are always conspicuously larger in diameter than the microvilli. Such a state apparently represents the ancestral condition, possibly already inherited from the bilaterian common ancestor (compare Bartolomaus and Ax 1992). However, given the data available today it is very difficult to identify a pattern in this character across Monogononta: even in narrowly circumscribed taxa such as Dicranophoridae, all three conditions (cilia < microvilli, cilia = microvilli, cilia > microvilli) are present. Possibly, the diameter of the microvilli is functionally related to different sizes of the terminal organs. It appears to be correlated with the proximo-distal extension of the terminal organs (see Table 1). Continuing along these lines, it is conceivable to assume that the larger the terminal organ (and the stronger the negative pressure created by the vibratile flame inside the cytoplasmic cylinder), the larger the diameter of microvilli required to stabilize the architecture of the filter region and prevent its collapse during filtration (for structural adaptations to negative filtration pressure in protonephridia, see Ruppert and Smith 1988).

The terminal organ in Rotifera

The protonephridial terminal organs in all monogonont rotifers investigated so far share the absence of ciliary rootlets (see Table 1). Only for *Filinia longiseta* (see Bartolomaus and Ax 1992, based on unpublished material by H.-U. Taeschner), ciliary rootlets are included in a schematic drawing of the protonephridial terminal organ. This contrasts with the situation in *Seison annulatus* (see Ahlrichs 1993b), bdelloid rotifers (*Habrotrocha rosa*, see Schramm 1978; *Philodina roseola*, see Clément and Wurdak 1991; *Rotaria rotatoria*, see Bartolomaus and Ax 1992) and Plathelminthes (*Catenula* sp., see Rohde and Watson 1994), where long ciliary rootlets extend along the whole length of the filter region and probably serve as a means to stabilize the filter architecture. For Acanthocephala, the situation as to the absence or presence of ciliary rootlets is unclear (*Macracanthorhynchus hirudinaceus*, see Dunagun and Miller 1986). Short ciliary rootlets have been reported in Gnathostomulida (*Gnathostomula paradoxa*, see Lammert 1985) and *Limnognathia maerski* (see Kristensen and Funch 2000). Such a distribution indicates that long ciliary rootlets extending into the filter region were probably lost in the stem lineage of Monogononta (Figs 8 and 9).

Table 1 Characters of the protonephridial terminal organ in Rotifera and related taxa

	Proximodistal extension	Terminal organ flattened	Microvilli number	Cilia number	Relative diameter mic. / cil.	Ciliary rootlets	Filter morphology	Arcs of cytoplasmic columns	Position nucleus	Literature
Monogononta										
Dicranophorus forcipatus	9 μm	+	20	40–45	micr > cil	–	longit. cl.	+	dist.	present study
Encentrum mucronatum	2.5 μm	–	4	4	micr = cil	–	longit. cl.	–	dist.	present study
Erignatha clastopis	2.9 μm	–	4	4	micr < cil	–	longit. cl.	–	dist.	present study
Proales reinhardti	5–8 μm	–	–	about 15	–	–	longit. cl.	–	dist.	Ahlrichs 1993a
Asplanchna priodonta	10–14 μm	+	about 38	about 60	micr = cil	–	longit. cl.	+	?	Braun *et al.* 1966
Asplanchna brightwelli	10–12 μm	+	about 20	30–36	micr = cil	–	longit. cl.	+	dist.	Warner 1969
Notommata copeus	6–8 μm	+	about 30	about 65	micr > cil	–	longit. cl.	+	dist.	Clément 1968
Colurella colurus	?	–	–	7	–	–	longit. cl.	–	dist.	Ahlrichs 1995, unpublished
Taphrocampa selenura	?	+	12	21	micr = cil	–	longit. cl.	+	?	Ahlrichs 1995
Notholca bipalium	?	–	30	about 30	micr < cil	–	longit. cl.	–	?	Ahlrichs 1995
Tricherca rattus	?	–	28	13	micr < cil	–	longit. cl.	–	?	Clément and Wurdak 1991
Monommata longiseta	?	+	30	45	micr = cil	–	longit. cl.	+	?	Ahlrichs 1995
Rhinoglena frontalis	?	?	present, number ?	?	micr = cil	–	?	?	?	Clément 1985
Filinia longiseta	?	–	19	9	micr = cil	+	longit. cl.	–	dist	Bartolomaus and Ax 1992
Bdelloidea										
Habrotrocha rosa	?	–	–	10–12	–	+	longit. cl.	–	dist.	Schramm 1978
Philodina roseola	?	–	–	12	–	+	longit. cl.	–	?	Clément and Wurdak 1991
Zelinkiella synaptae	?	–	–	13	–	?	longit. cl.	–	?	Ahlrichs 1995
Rotaria rotatoria	?	–	–	10	–	+	longit. cl.	–	dist.	Bartolomaus and Ax 1992
Seison										
Seison annulatus	about 6 μm	–	–	29	–	+	reg. pores	–	dist.	Ahlrichs 1993b
Acanthocephala										
Macracanthorhynchus hirudinaceus	> 15 μm	–	–	about 250	–	–	irreg. pores?	–	?	Dunagun and Miller 1986
Micrognathozoa										
Limnognathia maerski	3–4 μm	–	9 to 10	1	micr < cil	+	weir*	–	lat.	Kristensen and Funch 2000
Gnathostomulida										
Gnathostomula paradoxa	4.0–4.8 μm	–	8	1	micr < cil	+	irreg. pores?	–	prox.	Lammert 1985
Plathelminthes										
Catenula sp.	5–6 μm	–	4–5	2	micr < cil	+	interdigit.†	–	prox.	Rohde and Watson 1994

Abbreviations used: ci, cilia; dist, distal; interdigit, interdigitating; irreg. pores, irregular pores; lat, lateral; longit. cl, longitudinal cleft; mic, microvilli; prox, proximal; reg. pores, regular pores.

*The morphology of the filter region in *Limnognathia maerski* is not completely clear. The central cilium is surrounded by 9 to 10 microvilli that constitute the inner rods of a weir apparatus. Apparently, the canal cell is also involved in the formation of the weir and contributes the outer rods of the weir (for details, see Kristensen and Funch 2000).

†In *Catenula* sp. the filter region is formed by finger-shaped, horizontal cytoplasmic processes of the terminal cell that interdigitate and leave a meandering cleft between them (for details, see Rohde and Watson 1994).

As far as the position of the nucleus of the terminal organ is concerned (see Table 1), it is obvious that in all monogonont rotifers, bdelloids (*Habrotrocha rosa*, see Schramm 1978) and *Seison* (see Ahlrichs 1993b) the nucleus is situated distal to the filter region. This is in contrast to *Limnognathia maerski* (nucleus positioned lateral to filter region, Kristensen and Funch 2000), *Gnathostomula paradoxa* (nucleus positioned proximal to filter region, Lammert 1985) and *Catenula* sp. (nucleus positioned proximal to filter region, Rohde and Watson 1994). Hence, one can assume that in the rotiferan stem lineage the nucleus was shifted to a position distal to the filter region (Figs 8 and 9).

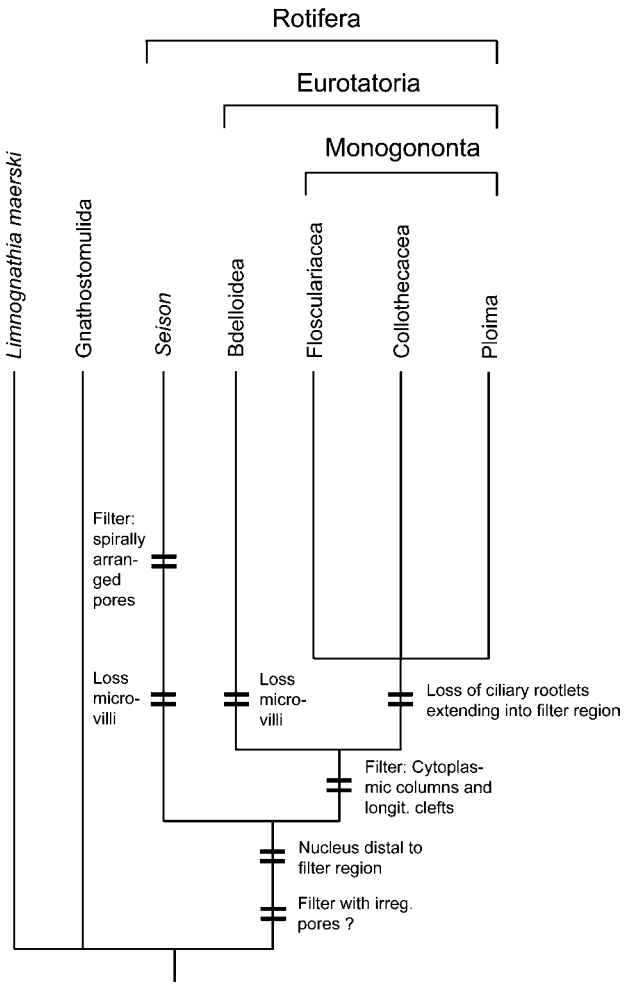

Fig. 8—Phylogenetic relationships of Rotifera and outgroup taxa (simplified after Sørensen 2002). Selected protonephridial characters mapped onto stem lineages (see Discussion for details).

While the protonephridial terminal organs of most monogonont rotifer species investigated so far bear microvilli (see Table 1), they are absent in *Seison (*see Ahlrichs 1993a), bdelloid rotifers (Schramm 1978; Clément and Wurdak 1991; Bartolomaus and Ax 1992; Ahlrichs 1995) and acanthocephalans (Dunagun and Miller 1986). Terminal cells with circumciliary microvilli have been recorded from potential outgroup representatives of Rotifera (Plathelminthes: *Catenula* sp., see Rohde and Watson 1994; Gnathostomulida: *Gnathostomula paradoxa*, see Lammert 1985; *Limnognathia maerski*, see Kristensen and Funch 2000) and, moreover, are suggested to have already been present in the common ancestor of Bilateria (compare Bartolomaus and Ax 1992), so their absence in *Seison*, bdelloid rotifers and acanthocephalans is certainly secondary. Whether microvilli were lost only once or twice independently depends on the underlying topology (Fig. 8: convergent loss in Bdelloidea and *Seison*; Fig. 9: single loss in Hemirotifera). A single loss of microvilli in *Seison*, bdelloid rotifers and acanthocephalans coincides with the conclusion of phylogenetic analyses that find evidence for a clade of *Seison*, acanthocephalans and bdelloid rotifers ('Hemirotifera') as sister taxon of Monogononta (Sørensen and Giribet 2006).

As regards the evolutionary transformation of the filter region across Rotifera, different conclusions have to be drawn depending on the underlying system of phylogenetic relationships. According to the topology provided by Sørensen 2002 (Fig. 8), it is unproblematic to assume for the common ancestor of Eurotatoria a filter region constituted by cytoplasmic columns and longitudinal clefts. This character is certainly derived and evolved in the stem lineage of Eurotatoria. This conclusion rests on the presence of such a filter region in all members of monogonont and bdelloid rotifers investigated so far (see Table 1). Pores in the cytoplasmic cylinder of the terminal organ as have been documented in *Seison annulatus* (see Ahlrichs 1993b) can be assumed to be an ancestral character present also in the common ancestor of Rotifera, because a filter region constituted by pores is probably also present in Gnathostomulida (compare with *Gnathostomula paradoxa*, see Lammert 1985). However, the arrangement of such pores in *Seison* on a spiral line is certainly apomorphic for *Seison* and only evolved in its stem lineage. Based on the system of phylogenetic relationships provided by Sørensen and Giribet 2006 (Fig. 9), a filter region of cytoplasmic columns and longitudinal clefts must already have been present in the common ancestor of Rotifera. Pores in the cytoplasmic cylinder of the terminal organ as in *Seison annulatus* (see Ahlrichs 1993b) and *Macracanthorhynchus hirudinaceus* (see Dunagun and Miller 1986) can be assumed to have evolved secondarily and would then be synapomorphic for *Seison* and acanthocephalans. While in acanthocephalans the distribution of pores remained irregular, in *Seison* a conspicuous arrangement of pores positioned on spiral lines evolved.

In summary, for the topology provided by Sørensen (2002) the following characters may be assumed for the common ancestor of Rotifera, Eurotatoria and Monogononta (compare with Fig. 8).

- **Rotifera**: Terminal organ as cytoplasmic cylinder, single circle of circumciliary microvilli surrounding central cilia, ciliary rootlets extending into filter region present, nucleus distal to filter region, filter region with pores.
- **Eurotatoria**: Terminal organ as cytoplasmic cylinder, single circle of circumciliary microvilli surrounding central cilia, ciliary rootlets extending into filter region present, nucleus distal to filter region, filter region consisting of cytoplasmic columns and longitudinal clefts.
- **Monogononta**: Terminal organ as cytoplasmic cylinder, single circle of circumciliary microvilli surrounding central cilia, ciliary rootlets extending into filter region absent,

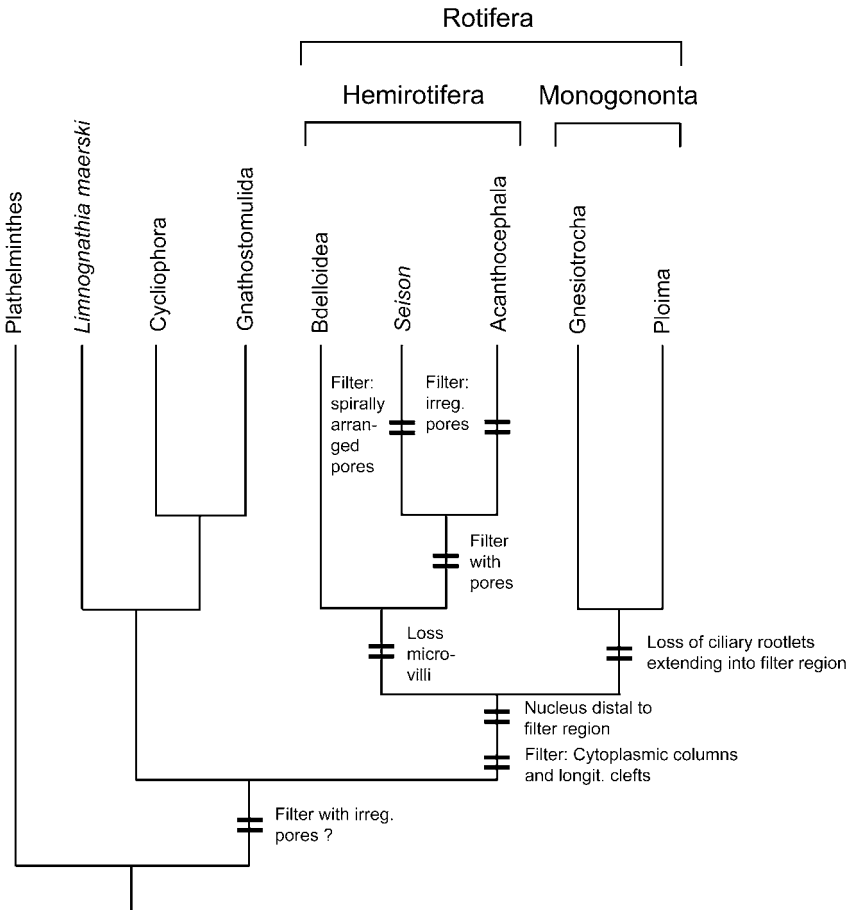

Fig. 9—Phylogenetic relationships of Rotifera and outgroup taxa (simplified after Sørensen and Giribet 2006). Selected protonephridial characters mapped onto stem lineages (see Discussion for details).

nucleus distal to filter region, filter region consisting of cytoplasmic columns and longitudinal clefts.

Based on the topology of Sørensen and Giribet (2006), the following characters were probably present in the common ancestor of Rotifera, Hemirotifera and Monogononta (compare with Fig. 9).

- **Rotifera**: Terminal organ as cytoplasmic cylinder, single circle of circumciliary microvilli surrounding central cilia, ciliary rootlets extending into filter region present, nucleus distal to filter region, filter region consisting of cytoplasmic columns and longitudinal clefts
- **Hemirotifera**: Terminal organ as cytoplasmic cylinder, microvilli absent, central cilia, ciliary rootlets extending into filter region present, nucleus distal to filter region, filter region consisting of cytoplasmic columns and longitudinal clefts
- **Monogononta**: Terminal organ as cytoplasmic cylinder, single circle of circumciliary microvilli surrounding central cilia, ciliary rootlets extending into filter region absent, nucleus distal to filter region, filter region consisting of cytoplasmic columns and longitudinal clefts

With regard to the evolution of the protonephridial terminal organ, the two topologies yield almost identical results. The only major difference relates to the morphology of the filter region in the common ancestor of Rotifera ('filter region with pores' reconstructed on topology of Sørensen 2002, see Fig. 8; 'filter region consisting of cytoplasmic columns and longitudinal clefts' reconstructed on topology of Sørensen and Giribet 2006, see Fig. 9). This difference essentially depends on the position of *Seison* and bdelloid rotifers. When *Seison* is considered the sister taxon of all other rotifers (Eurotatoria *sensu* Sørensen 2002, see Fig. 8), a filter region consisting of cytoplasmic columns and longitudinal clefts arose only in the stem lineage of Eurotatoria. However, when *Seison* (+ acanthocephalans) and bdelloid rotifers together (Hemirotifera *sensu* Sørensen and Giribet 2006, see Fig. 9) constitute the sister taxon of Monogononta, a filter region consisting of cytoplasmic columns and longitudinal clefts can be assumed to have already been present in the common ancestor of Rotifera.

Conclusion

To substantiate our knowledge of the terminal organ in Monogononta, it would be interesting to investigate representatives of the species-rich taxon Gnesiotrocha

(Floscullariacea and Collothecacea in Fig. 8), the sister group of Ploima. Apart from a simplified drawing of the protonephridial terminal organ in *Filinia longiseta* (see Bartolomaus and Ax 1992, based on unpublished data by H.-U. Taeschner), detailed investigations exist only for ploimid rotifer species. Future studies should also address the question of microvillus absence in Monogononta. Is this character more widely distributed or only restricted to some species of *Proales* and *Colurella*? If yes, might the absence of microvilli in Monogononta result from a single loss and could it indicate closer relationship of such taxa or is the absence of microvilli scattered across the phylogenetic tree of Monogononta so that multiple instances of secondary loss are more plausible? Moreover, it would be helpful to re-examine the filter region in Gnathostomulida and Acanthocephala as taxa closely related to Rotifera. Such examinations will provide additional evidence for reconstructing the evolution of the protonephridial terminal organ in Rotifera.

Acknowledgements

We gratefully acknowledge financial support granted to O.R. by Evangelisches Studienwerk Villigst. Support was also provided by Deutsche Forschungsgemeinschaft DFG. Heike Ötting's skilled and patient assistance with the TEM is greatly appreciated.

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