Zootaxa 2130: 1–20 (2009)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Comparison of the predatory rotifers *Pleurotrocha petromyzon* (Ehrenberg, 1830) and *Pleurotrocha sigmoidea* Skorikov, 1896 (Rotifera: Monogononta: Notommatidae) based on light and electron microscopic observations

EIKE F. WILTS, OLAF R.P. BININDA-EMONDS & WILKO H. AHLRICHS

AG Systematics and Evolutionary Biology, Department of Biology and Environmental Sciences, Carl von Ossietzky University Oldenburg, 26111 Oldenburg, Germany. E-mail: eike.f.wilts@mail.uni-oldenburg.de

Abstract

In the course of phylogenetic investigations across Rotifera, we reinvestigated *Proales sigmoidea* (Skorikov, 1896) and found significant similarities with respect to its morphology, ecology and behaviour to *Pleurotrocha petromyzon* (Ehrenberg, 1830). Both species feed on stalked ciliates and show a similar habitus as well as similar virgate trophi. We here present new morphological details for both species based on light and electron micrographs. In light of our results, we suggest the reassignment of *Proales sigmoidea* to *Pleurotrocha* Ehrenberg, 1830. To further support our case, we provide additional comparisons with other species of the latter genus, including *Pleurotrocha robusta* (Glasscott, 1893), for which we present additional information.

Key words: Monogononta, Proalidae, SEM, trophi

Introduction

Within Rotifera, the monophyletic status of families Notommatidae Remane, 1933 and Proalidae Bartos, 1959 is known to be highly problematic and that both families are in need of revision by modern, phylogenetic approaches (Sørensen 2005). Both taxa represent taxonomically unsatisfactory assemblages of numerous, diverse and mostly insufficiently described taxa that often have not been found again since their initial description (Nogrady *et al.* 1995; De Smet 1996). The monophyly of each is questionable because some species within each family seem to be more closely related to species of other rotifer families.

Compounding the taxonomic assessment of these families is the fact that type material of proalid and notommatid species is generally no longer available and the preserved material that is available is often in bad condition and unsuitable for comparative identification purposes; instead, living specimens show much more definitive morphological detail and can be determined more accurately. Furthermore, original descriptions of many species are short and lack both good drawings and adequate microscopic pictures (the latter partly because of the age of the studies). Owing to this general lack of information, robust phylogenetic studies based on morphological traits are hardly possible for most species of Notommatidae or Proalidae at present. Hence, a clear need for these two families is the comprehensive examination of more rotifer species to compile large, quality data-sets on their morphology and ecology as a prerequisite to future phylogenetic analyses. To meet this goal, electron-microscope investigations present an important and promising method to confirm or reject previous light-microscope observations (Fontaneto & Melone 2003) and contribute new morphological data as demanded by Wallace (2002). Yet, despite scanning electron microscopy (SEM) accompanied by careful preparation techniques, as used by Ricci *et al.* (2001) or Fontaneto & Melone (2003), being indispensible and of high value for the elucidation of complex morphological structures such as the corona and the trophi, they have not been applied consequently in species descriptions to date.

Accepted by D. Gordon: 11 May 2009; published: 11 Jun 2009

In investigating Proalidae and Notommatidae, the former represents a more manageable problem and thus a starting point in light of its more restricted number of taxa and diversity. Within Proalidae, *Proales* Gosse is the most problematic genus from a systematic point of view. *Proales* undoubtedly represents an assemblage of diverse species from which several have been moved in and out of the genus in the past. Therefore, as a first step towards addressing the taxonomic problems of the Proalidae and Notommatidae, we reinvestigated several species of *Proales*, among them *Proales sigmoidea* (Skorikov, 1896), with particular focus on internal and external morphology as elucidated using light and electron microscopy. In so doing, we follow the new standard in describing rotifers suggested overall by Fischer & Ahlrichs (2006) and Riemann *et al.* (in press), and display both the specimens and their trophi (individual mastax jaw elements) from different angles.

This study focuses primarily on *P. sigmoidea* and the notommatid *Pleurotrocha petromyzon* (Ehrenberg, 1830), which it strongly resembles morphologically. Initially, *P. sigmoidea* was described as *Pleurotrocha sigmoidea* by Skorikov (1896). Subsequently, Fadeew (1927) assigned this species to *Proales*. The new, additional information we present serves both to expand and correct previous descriptions and also to contribute urgently needed information for rotiferan morphology and taxonomy. In particular, we argue on the basis of our data for the reassignment of *P. sigmoidea* within *Pleurotrocha* and refer to it from this point onwards as *Pleurotrocha sigmoidea*.

Material and methods

Submerged plant material was collected during November 2006 from the littoral zone of the Haaren River, crossing Oldenburg in northwest Germany (53°08`55.43” N, 8°10`03.34” E), returned to the laboratory and subsequently cultured with water sampled from the collection locality in aquaria for two weeks. Well-developed stalked ciliate colonies (*Carchesium* and *Vorticella*) were isolated and searched for *P. petromyzon* and *P. sigmoidea* under a stereomicroscope. Additional specimens of *P. sigmoidea* were cultured on colonies of *Carchesium* in embryo dishes with water from the sampling location.

Pleurotrocha robusta was found in September 2008 in a pond near Leer, northwest Germany (53°15′30″ N, 7°31′12″ E). Whole ciliate colonies and single rotifer specimens were studied by differential interference light microscopy (Leica DMLB) as well as by scanning electron microscopy (Zeiss DSM 940). *Pleurotrocha robusta* was examined solely using light microscopy, because only a few specimens were found. Light-microscope pictures were taken with a digital camera (ColorView). Isolated rotifer specimens and ciliate colonies were narcotized with bupivacaine or bicarbonated water and fixed with a 4% OsO_4 solution and picric acid-formaldehyde at 240 mOsm after Melone (1998). Dehydration was performed using a graded ethanol series followed by critical-point drying. Specimens and colonies were mounted on stubs and coated with gold. Trophi were prepared following the procedure of De Smet (1998) with SDS/DTT (modified after Kleinow *et al.* 1990) as the dissolving agent. For transmission electron microscopy (TEM), specimens were anaesthetized for five minutes in an aqueous solution of 0.25% bupivacaine (Bucain®) and subsequently fixed with 1% OsO4 in 0.1m sodium cacodylate buffer at 8° C. After fixation, specimens were dehydrated in an increasing acetone series, embedded in Araldite hardened at 60° C for 72 hours and ultrasectioned (75 nm) on a Reichert ultracut followed by automatic staining with uranyl acetate and lead citrate (Leica EM Stain). The ultrathin sections were viewed with a Zeiss 902A TEM at 80 kv.

Our descriptions of the individual mastax jaw elements (trophi) and their relative positions use the terminologies introduced previously by Riemann & Ahlrichs (2008) and Riemann *et al.* (in press).

Results

Pleurotrocha sigmoidea Skorikov, 1896 (Notommatidae)

Pleurotrocha macropoda Zavadowsky, 1926
Proales sigmoidea Fadeew, 1927

Diagnosis. Species with bulbous body; toes short and conical; cerebral ganglion with small retrocerebral organ (less than half of head length), apical field of corona with palp-like ciliary tufts; pedal glands as long as foot; virgate trophi; triangular rami with pointed alulae; manubria with a small, lateral, knob-like protuberance; crescentic unci with 2 large and several minor uncinal teeth; fulcrum dorsoventrally expanded and terminally broadening.

General body organization of parthenogenetic female

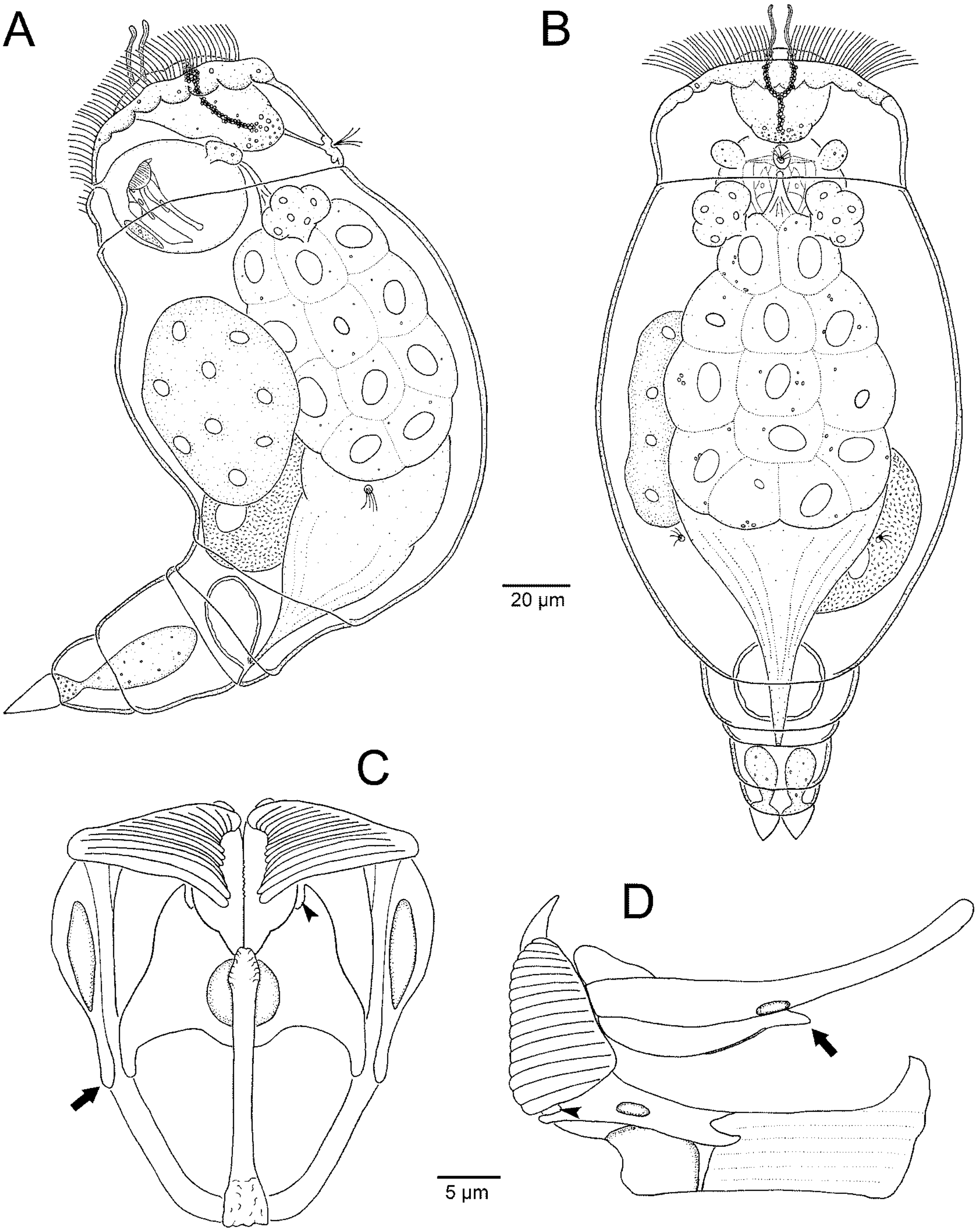

FIGURE 1. *Pleurotrocha sigmoidea.* A, habitus in lateral view. B, habitus in dorsal view. C, trophi in ventral view. D, trophi in lateral view. Arrow (projection of ventral manubrial chamber), arrow head (gap of ramus).

Habitus (Figs 1A, B; 2A–C, 3A–E)**.** Body bulbous and hyaline, divided into 3 distinct regions: head, trunk and foot with toes. Epidermis hardly stiffened, with smooth surface. Head short, sloped, contractile and offset from trunk by neck fold. Corona coursing from apical to ventral head region, characterized by a dense pseudotrochus composed of locomotory cilia. Pseudotrochus interrupted dorsally (Fig. 3E), merging ventrally with cilia field leading to mouth opening. A pair of lobe-like epidermal projections (Fig. 3C) limits mouth opening post-orally. Locomotory cilia enclosing an apical field that bears a pair of long, palp-like tufts composed of fused cilia, with a pair of shorter ciliary tufts above openings of retrocerebral organ (Fig. 3E). Center of apical field shows additional short cilia arranged along an inverted, U-shaped fold (Fig. 3E). Trunk bulbous, arched dorsally, composed of 3 pseudosegments and forms ovate outline in dorsal view. First pseudosegment comprises most of trunk; it is followed by a narrower, shorter lumbar pseudosegment and finally by a preanal pseudosegment (Figs 1A, B). Foot massive, tapering gradually, divided into 2 almost equal-sized pseudosegments with 2 short, pointed, conical toes distally. During swimming, foot is caudally directed. Foot and toes are retractile.

Digestive system (Figs 1A, B; 2A, C)**.** Mouth opening in ventral part of corona leads to short, slender, ciliated buccal tube that discharges ventrally to spherical mastax. Oesophagus diverges dorsally from first third of mastax (Fig. 1A). Stomach multicellular, with large, yellow-brownish, egg-shaped droplets, and occupies one-third of trunk (Fig. 1A, 2A). Ciliated intestine offset from the stomach (Fig. 2C). Anus discharges below preanal pseudosegment.

Trophi (Figs 1C, D, 4A–D)**.** Virgate type, bilaterally symmetrical with fulcrum residing in the longitudinal axis of the body. Trophi elements appear as follows:

Rami. Stout and triangular in ventral view, curved upwards anteriorly in a sickle-shaped bend, inner margins provided with small denticles (Fig. 1C). Ramus subbasal and basal chambers exhibit distinct openings with the large, oval ramus foramen subbasalis directed ventrally (Figs 1C, D, 4A) and circular ramus foramen basalis pointing caudodorsally (Figs. 1D, 4B). Basal rami chambers provided with slender, acuminate alulae distally; space between rami forms a convex bowl.

Fulcrum. Long, rod-shaped and slender in ventral view (Fig. 4A). In lateral view high, with longitudinal striae, expanding distally into a stamp-like plate characterized by a bunchy surface, margins arched upwards and a diagonal orientation to the longitudinal fulcrum axis (Fig. 4C).

Unci. Bent, crescent-shaped plates, with 2 distinct ventral teeth and 3 very small median teeth. Some additional reduced teeth recognizable only by series of denticles and jugal lines (Fig. 4D).

Manubria. Taper gradually from broad basal clava to distal end of long, rod-shaped cauda. Clava composed of 3 chambers that are not clearly distinguishable in detail but show distinct openings. Ventral manubrial chamber shows elongate, ventrally directed opening. It features a small, knob-like projection (Figs 1C, D, 4A, B) situated ventrolateral to longitudinal middle of manubrium. Median manubrial chamber constitutes largest part of clava and also forms long cauda. Its opening lies above projection of ventral manubrial chamber and is ventrolaterally directed (Figs 1D, 4C). Small dorsal manubrial chamber opens dorsolaterally (Fig. 4B).

Nervous system and sensory organs. Cerebral ganglion rounded, very small (Figs 1A, 2A). Dorsal antenna consists of several cilia encircled by flat, rounded collar; located on head in front of neck fold (Fig. 3B). Lateral antennae lying dorsolaterally in posterior third of trunk; cilia surrounded by flat, rounded collar. Two pairs of ciliary tufts with possible sensory function arranged apically on head (Fig. 3E).

Glandular system (Figs 1A, B, 2A). Retrocerebral organ a dark Y-shaped duct located posterior to brain (Fig. 1B); in some specimens, only the duct is visible with light microscopy. A pair of large, kidney-shaped salivary glands resides in mastax complex. Both gastric glands possess 5 nuclei, are cauliflower-shaped and diverge anteriorly from stomach with short stalks. Pedal glands as long as foot, with terminally constricted mucus reservoir above toes.

Protonephridial system. Paired protonephridia with distinct terminal organs (exact number and position not documented), opening into contractile bladder (Figs 1A, B, 2C) that in turn empties into terminal part of the intestine (cloaca).

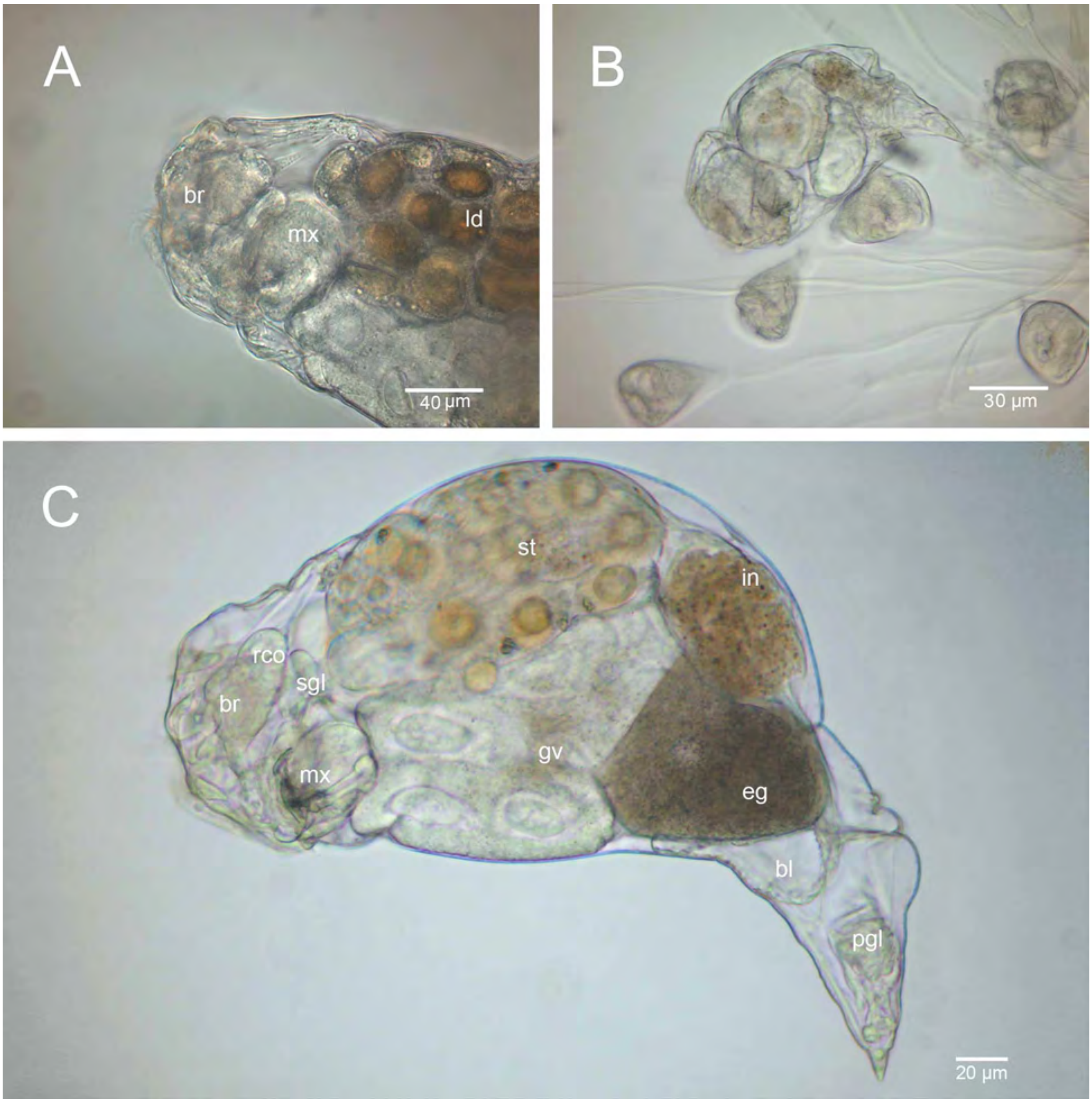

FIGURE 2. Light-microscope images of *Pleurotrocha sigmoidea*. A, head of adult female. B, juvenile among *Vorticella* aggregation. C, adult female. **bl** bladder, **br** brain, **eg** egg, **gv** germovitellarium, **in** intestine, **ld** lipid droplets, **mx** mastax, **pgl** pedal glands, **rco** rectrocerebral organ, **st** stomach, **sgl** salivary gland.

Reproductive system (Figs 1A, B, 2A, B): Germovitellarium unpaired, eight-nucleated, in ventral region of trunk; a large, dark egg often present.

Measurements. Total length 300–310 µm, maximum dorsoventral extent 108–121 µm, maximum width 124–132 µm, foot length 60 µm, toe length 20 µm, trophi length 35 µm, ramus length 22 µm, manubrium length 32 µm, manubrium width 8 µm, fulcrum length 19–23 µm.

Ecology. The species is limnosaprobic and uncommon, occurring in stagnant or running fresh waters in colder seasons. It has been reported from Germany (Koste 1968), France (De Beauchamp 1948), Russia (Skorikov 1896), Canada (De Smet 1996) and England (Reiss & Schmid-Araya 2008). It lives solitarily among degraded macrophytes or between *Lemna* on colonies of large sessile ciliates (especially *Vorticella*) (Fig. 3F) that it feeds on. The ciliates are relatively large in comparison to the small mouth opening of the rotifers (compare Figs 3E and 3F) and are eaten in parts. De Beauchamp (1948) also reported the species living on *Campanella umbellaria*. It appears that *P. sigmoidea* has a preference for larger ciliates and does not switch to other prey. Because of its rare appearance, however, and uncommon field observations, *P. sigmoidea* may have a broader prey spectrum.

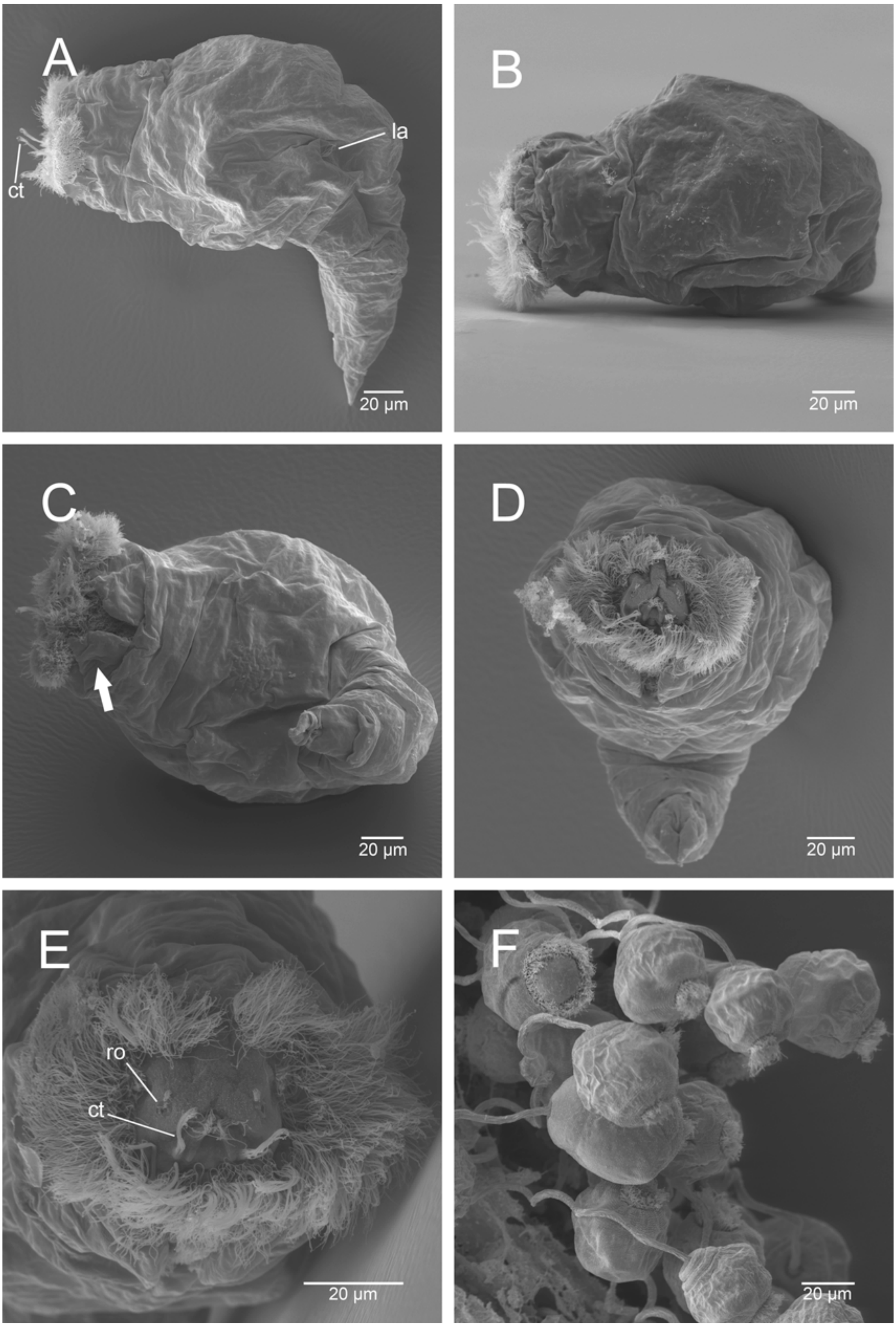

FIGURE 3. SEM images of *Pleurotrocha sigmoidea* and its prey. A, lateral view. B, dorsal view. C, ventral view. D. frontal view. E. corona in frontal view. F. prey (*Vorticella campanula*). **ct** ciliary tuft, **la** lateral antenna, **ro** retrocerebral organ opening. Arrow (epidermal projection shielding the mouth region)

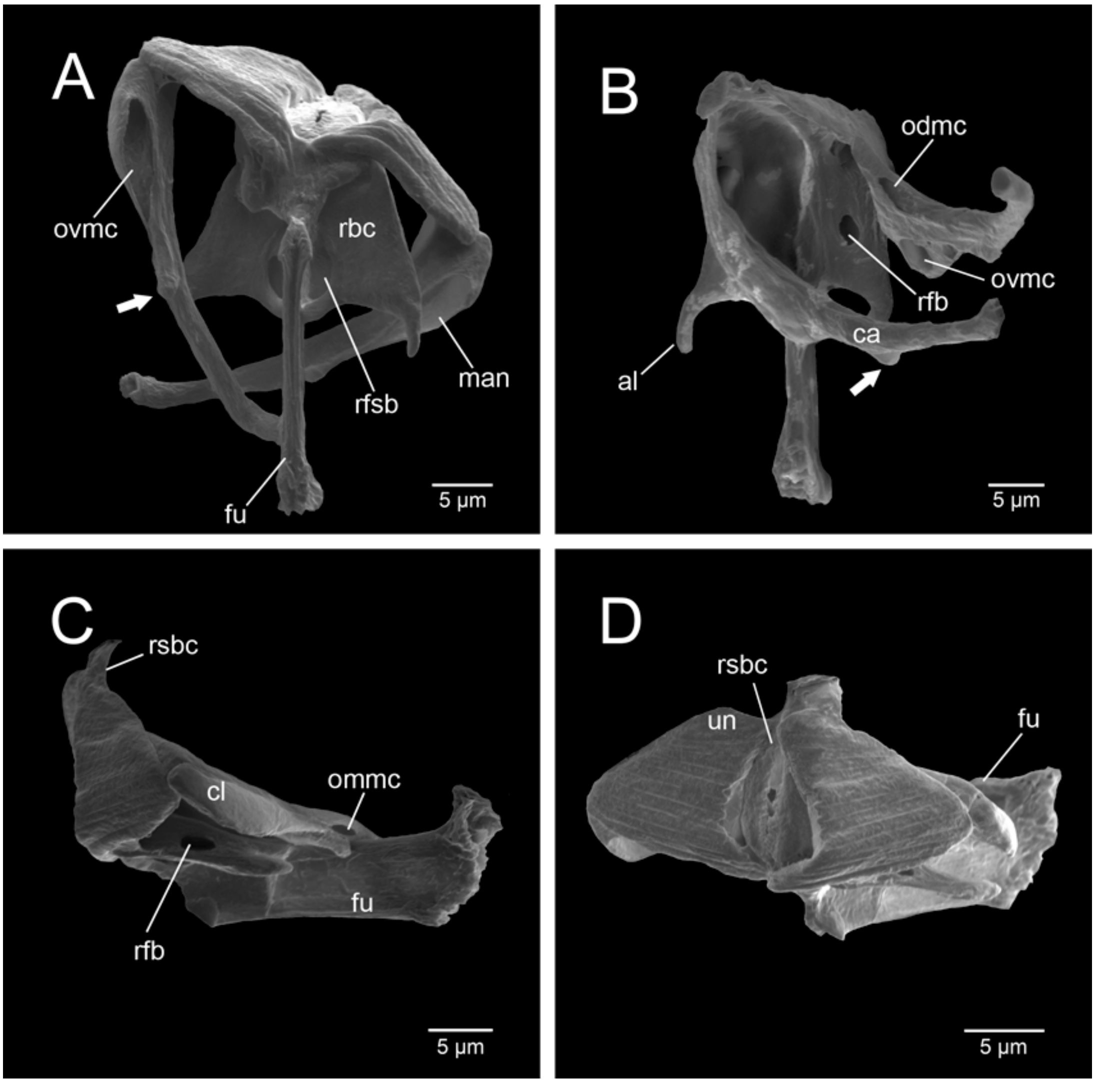

FIGURE 4. SEM images of the trophi of *Pleurotrocha sigmoidea*. A, ventral view. B, dorsal view. C, lateral view. D, laterofrontal view. **al** alula, **ca** cauda, **cl** clava, **fu** fulcrum, **man** manubrium, **odmc** opening of dorsal manubrial chamber, **ommc** opening of median manubrial chamber, **ovmc** opening of ventral manubrial chamber, **rbc** ramus basal chamber, **rfb** ramus foramen basalis, **rfsb** ramus foramen subbasalis, **rsbc** ramus subbasal chamber, **un** uncus. Arrow (projection of ventral manubrial chamber).

Pleurotrocha petromyzon (Ehrenberg, 1830) (Notommatidae)

Notommata petromyzon Ehrenberg, 1830
Notommata gibba Ehrenberg, 1832
Notommata petromyzon: Ehrenberg 1838
Proales petromyzon: Hudson & Gosse 1886
Notops laurentinus Jennings, 1894
Proales laurentius Jennings, 1896
Pleurotrocha laurentina: Harring 1913

Diagnosis. Species with fusiform, stout body; toes short and conical; retrocerebral organ with posterior sphere followed by dark red eye; large gastric glands with 9 nuclei; large pedal glands extending into trunk; triangular rami with rounded alulae; manubrium with large, lateral, knob-like protuberance; crescent-shaped unci with 2 large and 3 minor uncinal teeth; end of distal fulcrum flattened and arched dorsally.

General body organization of parthenogenetic females

Habitus (Figs 5A, B, 6A–C, 7A–E)**.** Body stout, fusiform, hyaline, divided into 3 distinct regions: head, trunk and foot with toes. Epidermis hardly stiffened, with smooth surface. Head sloped, contractile and offset from trunk by weakly developed neck fold. Corona extending between apical and ventral head region, characterized by dense pseudotrochus of locomotory cilia. Pseudotrochus merges ventrally with cilia field running to mouth opening. This restricted posteriorly by paired lobe-like epidermal projection (Fig. 7E). Locomotory cilia enclosing apical field on head; middle of apical field with 2 openings of retrocerebral organ. Trunk arched dorsally, much wider than head (Figs 5B, 7B), and comprising 3 pseudosegments. First pseudosegment large, followed by narrower, shorter lumbar pseudosegment and preanal pseudosegment. Foot retractile, tapering conically, divided into 2 pseudosegments and 2 relatively short, pointed, conical toes (Figs 5A, 7B), directed caudally during swimming process. Terminal foot pseudosegment twice length of first one.

TABLE 1. Comparison of the morphology and ecology of *Pleurotrocha sigmoidea*, *P. petromyzon*, *P. robusta* and *Proales* sensu stricto based on eleven key characters. **vmc** ventral manubrial chamber, **rbc** ramus basal chamber, **rsbc** ramus subbasal chamber.

Character traits	*Pleurotrocha petromyzon*	*Pleurotrocha sigmoidea*	*Pleurotrocha robusta*	*Proales* sensu stricto
habitus	bulbous	bulbous	bulbous	spindle-shaped
toes	short, conical	short, conical	short, conical	long, slender with fine distinct tips
eyes	not observed, single cerebral eye after Koste (1976, 1978)	single cerebral eye	single cerebral eye	two apical eyes
giant morphs	known	known	unknown	unknown
habitat	limnic	limnic, rarely brackish	limnic	marine, *P. reinhardti* also brakish
diet	predatory on stalked ciliates	predatory on stalked ciliates, necrophagic on dead crustaceans	unknown	grazing on diatoms and other algae
trophi type	virgate	virgate	virgate	modified malleate
rami	bent upwards, sickle-shaped, corrugated inner margins, symmetrical, alulae	bent upwards, sickle-shaped, corrugated inner margins, symmetrical, alulae	bent upwards, sickle-shaped, corrugated inner margins, symmetrical, alulae	dorsoventrally flattened, molar surface and basal apophyses, asymmetrical, no alulae, rbc and rsbc more or less separated
fulcrum	long, directed straight caudally	long, directed straight caudally	long, directed straight caudally	short, directed ventrocaudally
manubria	long, slender, cauda slightly bent, highly reduced clava, with projection of vmc	long, slender, cauda slightly bent highly reduced clava, with projection of vmc	long, slender, cauda slightly bent, highly reduced clava	long, slender, cauda strongly bent, cauda usually reduced
unci	crescent-shaped, 2 distinct ventral and 3 small median teeth plus several reduced teeth	crescent-shaped, 2 distinct ventral and 3 distinct dorsal teeth	crescent-shaped, single ventral uncus tooth	stout, more or less quadrangular, 4-7 gradually sized teeth

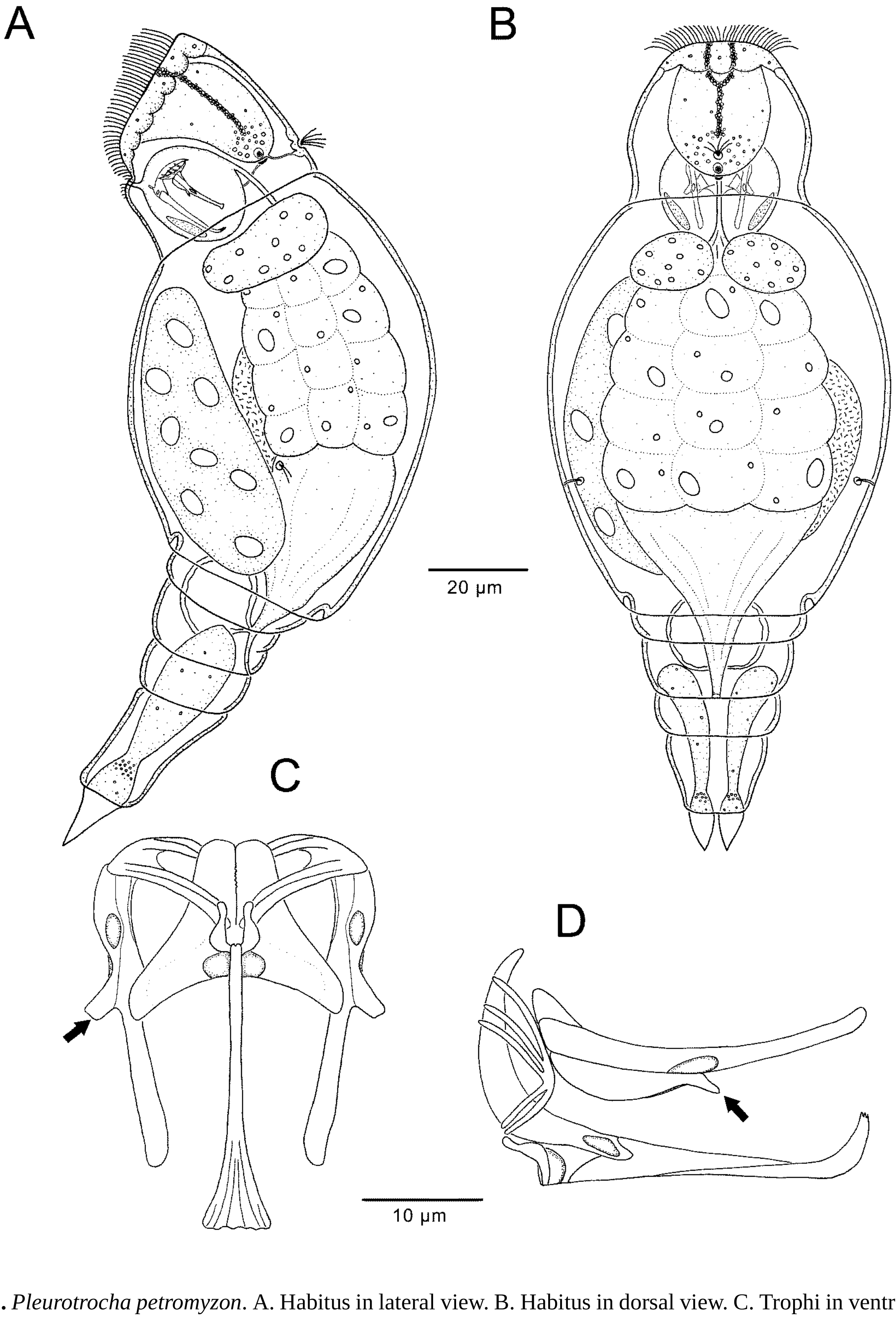

FIGURE 5. *Pleurotrocha petromyzon*. A. Habitus in lateral view. B. Habitus in dorsal view. C. Trophi in ventral view. D. Trophi in lateral view. Arrow (projection of ventral manubrial chamber).

Digestive system (Figs 5A, B)**.** Mouth opening in ventral part of corona leads to short, slender, ciliated buccal tube that discharges ventrally to spherical mastax. Oesophagus diverges from anterior third of mastax dorsally. Stomach multicellular, ciliated, with yellow-brownish, small and large egg-shaped droplets, occupying one-third of trunk (Fig. 6A). Ciliated intestine offset from stomach. Anus discharges below preanal pseudosegment.

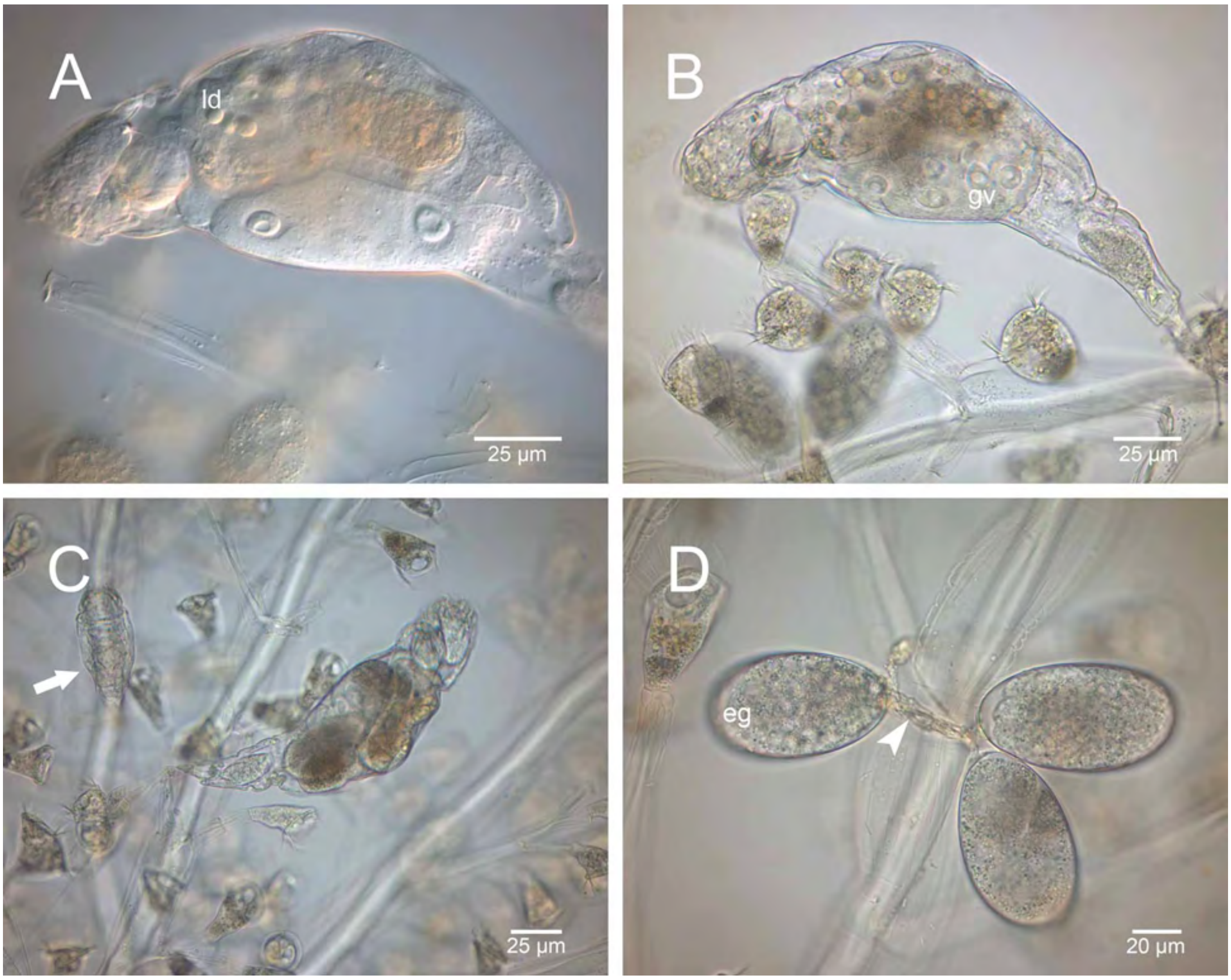

FIGURE 6. Light microscopic images of *Pleurotrocha petromyzon* in a *Carchesium* -colony. A. Adult female with remaining ciliate stalk. B. Adult female stretching to eat. C. Adult female and juvenile (arrow). D. Deposited eggs with stalk (arrow head) formed by the adhesive secretion from the pedal glands. **eg** egg, **gv** germovitellarium, **ld** lipid droplets.

Trophi (Figs 5C, D, 9A–D)**.** Trophi virgate, bilaterally symmetrical with fulcrum residing almost in longitudinal axis of body.

Rami. Stout, in ventral view appearing triangular, arched upward anteriorly in sickle-shaped bend (Fig. 10), inner margins with fine denticles (Fig. 5C). Ramus foramen subbasalis small, somewhat circular (Figs 5C, 9C), directed ventrally. Ramus foramen basalis large, ovate, directed dorsally. Rami with broad, acuminate alulae distally (Figs 5C, 9A).

Fulcrum. Long, rod-shaped, longitudinally striate; in ventral view slender, expanding distally; in lateral view flattened and curved upward distally.

Unci. Slightly curved plates, with 2 ventral and 3 more dorsal principal teeth; ventral and dorsal tooth group separated by triangular gap (Fig. 5D, 9D).

Manubria. Slightly curved, tapering gradually from broad basal clava to distal end of long, rod-shaped cauda. Clava composed of 3 chambers that are not clearly distinguishable but show distinct openings. Ventral manubrial chamber with small, rounded, ventrally directed opening and features a small, knob-like protuberance (Figs 5C, D, 9B) ventrolateral to median chamber of manubrium. Median manubrial chamber forms elongated cauda and shows small, oval, ventrolaterally directed opening (Fig. 9B). Small dorsal manubrial chamber opens dorsolaterally, with ovate opening.

Hypopharynx. a fork-like structure at the proximal end of the fulcrum (Fig. 9A).

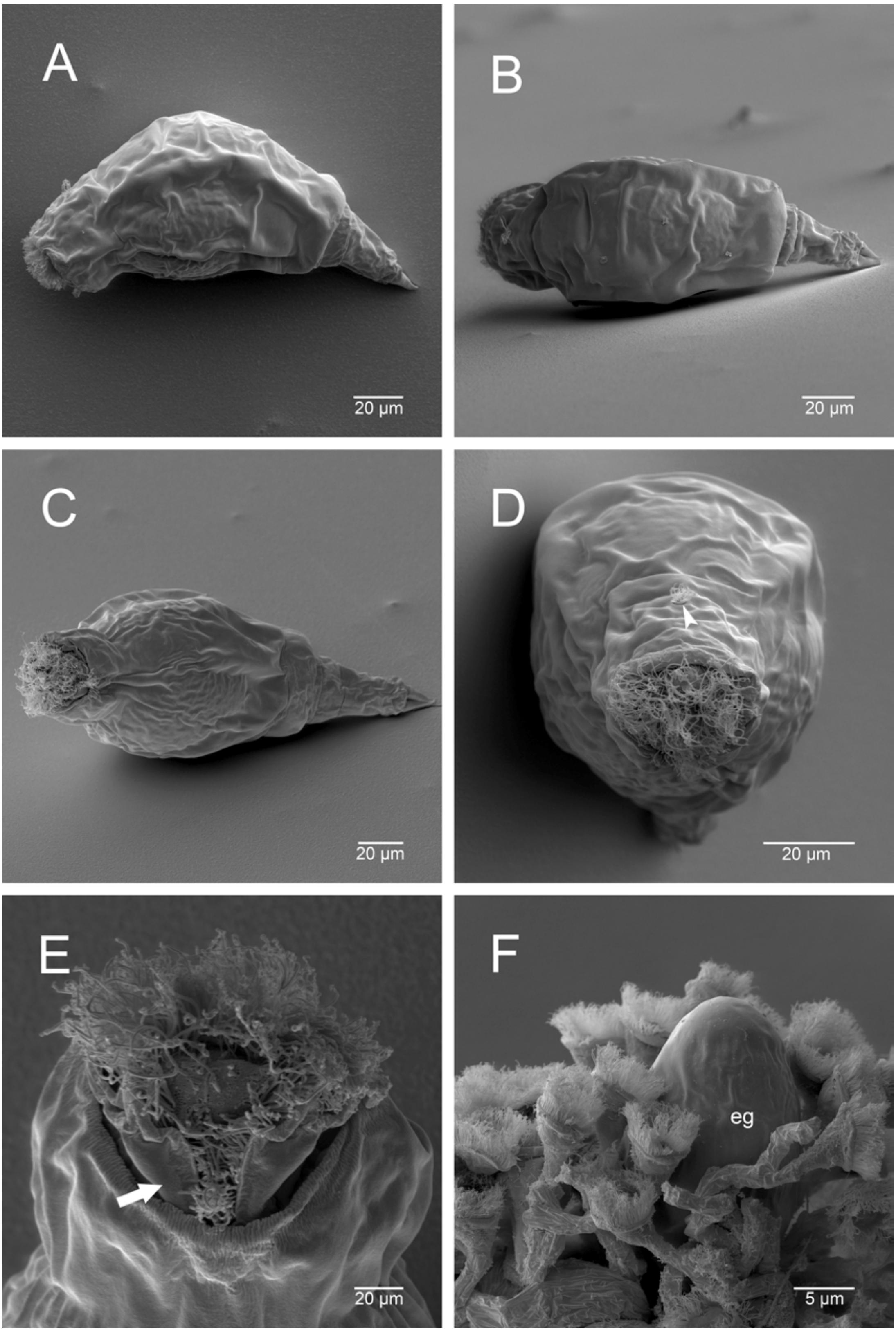

FIGURE 7. SEM images of *Pleurotrocha petromyzon*. A. Lateral view. B. Dorsal view. C. Ventral view. D. Frontal view. E. Head in ventral view with epidermal projection covering the mouth opening (arrow). F. Egg in *Carchesium* -colony. **eg** egg. Arrow head (dorsal antenna)

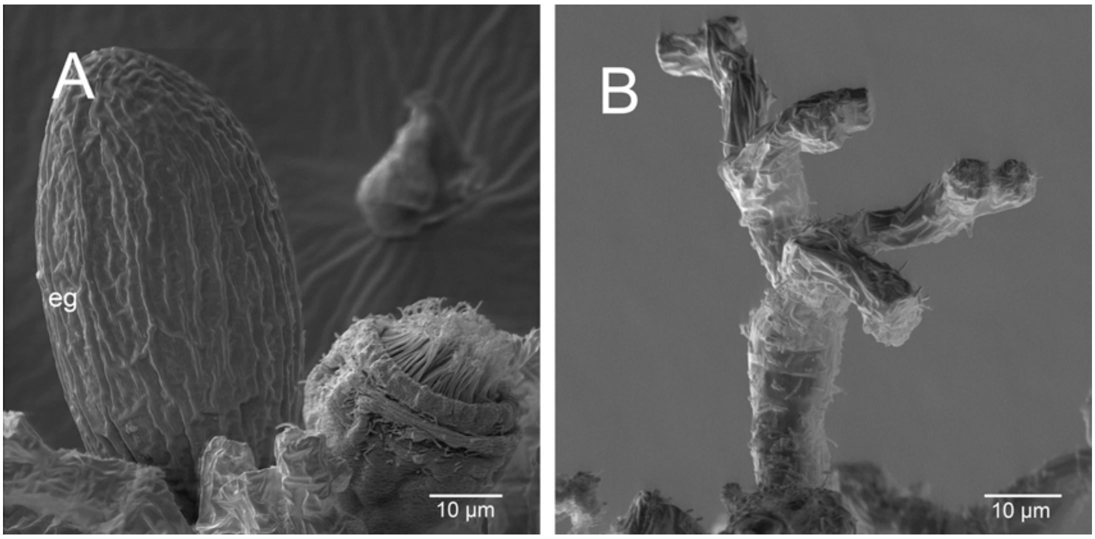

FIGURE 8. SEM images. A. Sculptured egg of *Pleurotrocha petromyzon in Carchesium* -colony. B. Remaining stalks of a *Carchaesium* -colony. **eg** egg.

Nervous system and sensory organs. Cerebral ganglion rounded, extending to centre of head, followed by large, reasonably hyaline retrocerebral organ that cannot be discriminated from brain by light microscopy. A dark-red kidney-shaped eye present at posterior end of retrocerebral organ (Fig. 10). A fine connection inserts close to eye and courses down to mastax (Figs 5A, 6A). Dorsal antenna consists of several cilia encircled by flat, rounded collar located in front of head fold (Fig. 7D). Lateral antennae comprising a few cilia, with a flat, rounded collar, located just beyond midpoint of trunk.

Glandular system (Figs 5A–B, 6B)**.** Two lateral salivary glands visible, integrated into mastax complex. Both gastric glands large, ball-shaped, unstalked, with 9 nuclei each, diverging anteriorly from stomach. Retrocerebral organ lies behind cerebral ganglion and opens with 2 anterior ducts on apical field of corona (Fig. 10). In posteriormost region of retrocerebral organ, and immediately in front of the eye, is a vesicle-filled sphere. Pedal glands extend into preanal pseudosegment, with terminally constricted mucus reservoir above toes.

Protonephridial system (Figs 5A, B)**.** Protonephridia paired, with distinct terminal organs (exact number and position not determined). Collecting tubules open into contractile bladder that in turn empties into terminal part of intestine (cloaca).

Reproductive system (Figs 5A, B, 6A, B)**.** Germovitellarium unpaired, with 8 nuclei, located in the ventral region of trunk, often associated with a large, dark egg.

Measurements. Total length 150–255 µm, maximum dorsoventral extent 70 µm, maximum width 60–80 µm, foot length 28 µm, toe length 12–26 µm, trophi length 35 µm, ramus length 16 µm, manubrium length 21 µm, manubrium width 7 µm and fulcrum length 24 µm.

Ecology. The species is limnosaprobic and free-living, occurring in running, acidic, brackish water and is present in both colder and warmer seasons. Distribution is cosmopolitan (Nogrady *et al.* 1995). The species is necrophagic and includes smaller sessile peritrich ciliates in its diet, especially *Carchesium*. Several generations, including juveniles and eggs, often live on a single ciliate colony (Figs 6C, D). In one instance, six specimens were observed living on a single *Carchesium* colony. Specimens are well protected in the colony, which encloses the rotifers following disturbances. Feeding specimens attach with their pedal glands, stretch and approach the body of an individual ciliate. The ciliate is eaten completely, leaving only its stalk (Figs 6A, 8B). Usually 2–3 eggs are deposited and fixed to the substratum, with a stalk formed by an adhesive secretion from the pedal glands (Fig. 6D). Smooth (Fig. 7F) as well as sculptured eggs (Fig. 8A) were found. Specimens were also found in the middle of stalked ciliates growing on *Macrocyclops*. Ehrenberg (1838) reported the species on *Epistylis digitalis*, *Carchesium polipinum*, *Zoothamnium* and piercing *Volvox globator*.

Budde (1925) also found specimens in *Volvox* and in the shells of dying and dead crustaceans (*Daphnia*, *Chydorus*, Entomostraca), where they always feed towards the head, deposit 2–3 eggs and then leave the individual. The species was also found on trichopteran eggs, on *Hydra* (De Beauchamp 1905) and on snail eggs (Wulfert 1935).

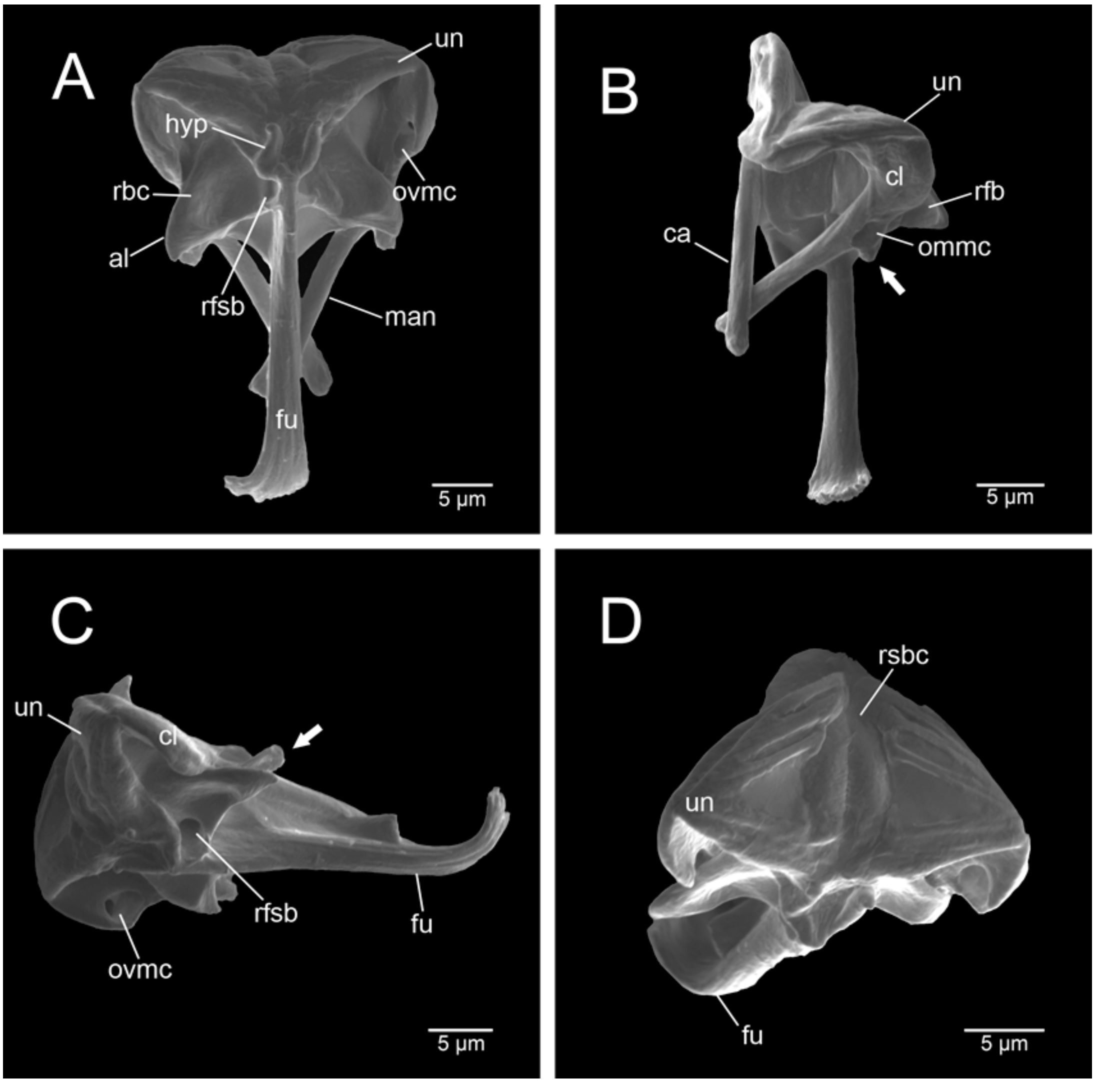

FIGURE 9. SEM images of the trophi of *Pleurotrocha petromyzon*. A. Ventral view. B. Dorsal view. C. Ventro-lateral view. D. Frontal view. **al** alula, **ca** cauda, **cl** clava, **fu** fulcrum, **hyp** hypopharynx, **man** manubrium, **ommc** opening of median manubrial chamber, **ovmc** opening of ventral manubrial chamber, **rfb** ramus foramen basalis, **rfsb** ramus foramen subbasalis, **rbc** ramus basal chamber, **rsbc** ramus subbasal chamber, **un** uncus. Arrow (projection of ventral manubrial chamber).

Discussion

Comparison with other species of *Pleurotrocha* and *Proales*

Our light- and electron-microscope studies reveal significant similarities between *P. sigmoidea* and *P. petromyzon* in terms of their habitus, structure of corona and trophi, and even their ecology and behavior. The lack of sufficient good-quality comparative data means that we cannot exclude that these similarities represent shared primitive features or convergent adaptations to a similar lifestyle. The same lack of information prohibits a robust phylogenetic evaluation of the character traits among the relevant species at this time. Instead, such analyses are possible only by means of further detailed examinations of additional *Pleurotrocha* and other notommatid species and this study represents an initial step toward achieving this goal. We would argue, however, that these similarities are so all-encompassing, especially when other species of *Pleurotrocha* are taken into account, and more significant than with other Proalidae, that they are unlikely to be symplesiomorphic or the result of convergent evolution and instead argue for the re-inclusion of *P. sigmoidea* in *Pleurotrocha* to which it was initially assigned.

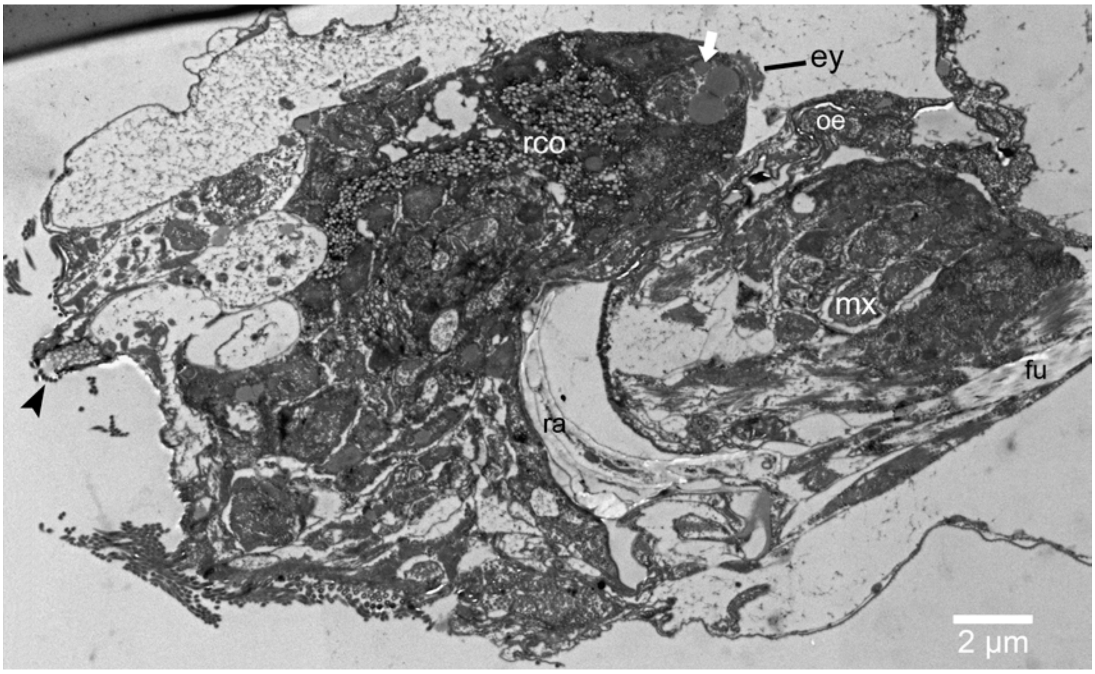

FIGURE 10. TEM image of longitudinal section through head of *Pleurotrocha petromyzon*. **ey** eye, **fu** fulcrum, **mx** mastax, **oe** oesophagus, **ra** ramus, **rco** retrocerebral organ. Arrow (vesicle-filled sphere). Arrow head (opening of the retrocerebral organ).

However, in so doing, it needs to be pointed out that the taxonomic placement of both *P. sigmoidea* and *P. petromyzon* has been unstable historically. Both were assigned initially to different genera than at present (*Pleurotrocha* and *Notommata*, respectively) and have been relegated variously to either Notommatidae or Proalidae (Ehrenberg 1838; Hudson & Gosse 1886; Skorikov 1896; Fadeew 1927).

In building our case, we compare *P. sigmoidea* with other *Pleurotrocha* species to highlight similarities and differences between them. We focus largely on *P. sigmoidea*, *P. petromyzon* and *Pleurotrocha robusta* (Glascott) because the data currently available for these species are the best. The data for *P. robusta* (Figs 11A–C, 12A–C) are based on our own observations, whereas information for *Pleurotrocha hyalina* Wulfert, *P. atlantica* Myers and *P. altila* Myers is based on the descriptions and illustrations of Koste (1978) and Nogrady *et al.* (1995). Furthermore, we highlight character traits arguing against a relationship between *P. sigmoidea* and *Proales* sensu stricto (taken here to be the type species *Proales reinhardti* (Ehrenberg) and putatively closely related species like *Proales littorales* De Smet, *Proales christinae* De Smet, *Proales theodora* (Gosse), *Proales oculata* Tzschaschel, *Proales halophila* (Remane), *Proales syltensis* Tzschaschel and *Proales germanica* Tzschaschel. A short summary is listed in Table 1.

Both *P. sigmoidea* and *P. petromyzon* possess a similar habitus, identical numbers of pseudosegments, virgate trophi with upwardly bent rami, a long caudally directed fulcrum, crescent-shaped unci, and slender manubria with a rod-shaped cauda. Regarding their general appearance, both species resemble *P. atlantica* and *P. robusta*, although *P. atlantica* carries its dorsal antenna on the trunk behind the neckfold and *P. robusta* (Fig. 11) presents only one toe. Species of *Proales* sensu stricto are generally more spindle-shaped and have more slender toes with fine, distinct tips.

FIGURE 11. Light microscopic images of *Pleurotrocha robusta*. A. Adult female. B. Juvenile specimen. C. Slightly compressed adult specimen. **br** brain, **eg** egg, **gg** gastric glands, **gv** germovitellarium, **mx** mastax, **rco** retrocerebral organ, **st** stomach. Arrow head (cerebral eye with low position).

Based on our data, *P. sigmoidea* (310 μm) compared to *P. petromyzon* (255 μm) and *P. robusta* (220 μm), is the largest species, with the clumsiest body. Giant forms, which probably represent different feeding morphs, have been described for both *P. sigmoidea* (550 μm; Koste 1968) and for *P. petromyzon* (480 μm; Wulfert 1935). The common occurrence of a giant morph in these two species represents another argument in favour of their close relatedness, although it might be caused by polyploidy. The gastric glands in *P. sigmoidea* are somewhat cauliflower-shaped, whereas those of *P. petromyzon* are ball-shaped. However, the giant forms of the latter species, as documented by Wulfert (1935), also clearly show trilobed glands, again hinting at a close relationship of the two species.

The position of the cerebral eye in *P. petromyzon* agrees with that in *P. atlantica*, whereas *P. hyalina* and *P. robusta* have a cerebral eye located anteriorly beneath the brain (Figs 11A, B). By contrast, our specimens of *P. sigmoidea* lack eyes entirely, although Koste (1978) described a cerebral eye in the species, which, if true (see below), would argue against a close relationship with *Proales* sensu stricto, whose species typically have apical eyes.

The corona in both *P. petromyzon* and *P. sigmoidea* is characterized by a pseudotrochus that is particularly strongly developed in the latter. The latter also shows a dorsal interruption between the ciliary bands similar to that found in *P. atlantica*, whereas *P. petromyzon* exhibits an uninterrupted pattern like *P. robusta*. On the apical field of both *P. petromyzon* and *P. sigmoidea*, small openings of the retrocerebral organ and few cilia are present. In addition, *P. sigmoidea* also possesses two palp-like ciliary tufts. The epidermal projection shielding the mouth opening, present in both *P. sigmoidea* and *P. petromyzon*, is also visible in *P. robusta*. Owing to the limited information about the detailed morphology of the rotatory organ in the literature, further comparison with other species, and those of Proales sensu stricto in particular, is not possible.

Both *P. sigmoidea* and *P. petromyzon* live in limnosaprobic waters among sessile colonial peritrich ciliates upon which they prey. The yellow-brownish stomach inclusions (lipid droplets), which have not been reported for other species of the family, may be associated with this diet. This mode of feeding is not reported for other species of *Pleurotrocha* or species of *Proales*.

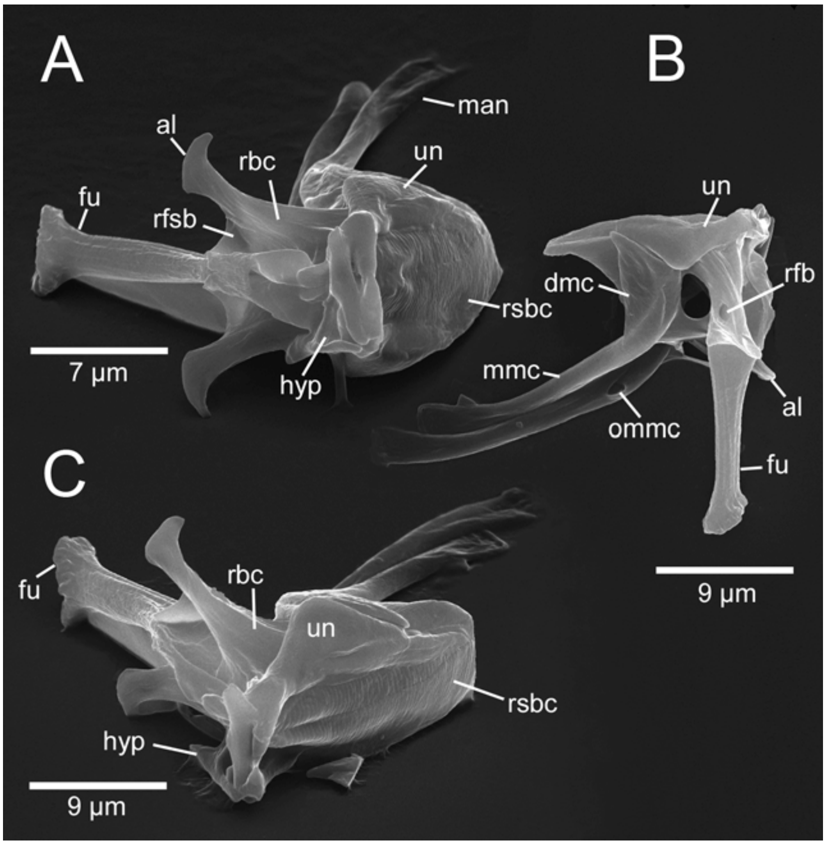

FIGURE 12. SEM images of the trophi of *Pleurotrocha robusta*. A. Ventral view. B. Lateral view. C. Latero-frontal view. **al** alula, **dmc** dorsal manubrial chamber, **fu** fulcrum, **hyp** hypopharynx, **man** manubrium, **mmc** median manubrial chammber, **ommc** opening of median manubrial chamber, **rbc** ramus basal chamber, **rfb** ramus foramen basalis, **rfsb** ramus foramen subbasalis, **rsbc** ramus subbasal chamber, **un** uncus.

Perhaps the strongest argument supporting the placement of *P. sigmoidea* in *Pleurotrocha* involves the extreme similarity of the trophi in these taxa and their distinctiveness from those of *Proales* sensu stricto. The form of the trophi is highly diagnostic within Rotifera, both at the species and at higher levels. Although *P. sigmoidea* and *Pleurotrocha* both possess virgate trophi, this does not suffice to explain the high morphological similarity. Species within Synchaetidae, Trichocercidae and other notommatid genera for example also possess virgate trophi, yet the trophi are recognizably different from those of *Pleurotrocha*. The same is true of the virgate trophi of *Proales* sensu lato, arguing strongly against convergent evolution between *P. sigmoidea* and *Pleurotrocha.*

In particular, the symmetrical trophi of *P. sigmoidea* and *P. petromyzon* are highly similar. In both species, the rami are bent dorsally in a sickle-shaped manner. This conformity can also be found in other notommatid species (such as *Notommata prodota* Myers and *Eosphora anthadis* Harring & Myers), whereas species of *Proales* sensu stricto possess more or less flattened rami. Furthermore, the rami in *Proales* sensu stricto are mostly asymmetrical and present a molar surface with tubercles and basal apophyses. By contrast, the rami of *P. sigmoidea* and *P. petromyzon* show corrugated inner margins as found in *P. robusta* (Fig. 12C) and *P. atlantica*. These corrugated inner margins can only be found within Notommatidae, but not within Proalidae.

The distinct alulae of the trophi in *P. sigmoidea* and *P. petromyzon* both point caudally. In contrast, the alulae of *P. robusta* are directed more ventrally and have hook-like tips (Fig. 12A), whereas those in *P. sigmoidea* are somewhat acuminate. Species of *Proales* sensu stricto usually lack distinct alulae.

Pleurotrocha robusta, *P. sigmoidea* and *P. petromyzon* possess a long, rod-shaped, caudally directed fulcrum that is typical for most notommatid species. By contrast, species within Proalidae possess a short fulcrum that is often directed more ventrally and especially so in *Proales* sensu stricto.

The unci of *P. hyalina*, *P. robusta*, *P. petromyzon* and *P. sigmoidea* are crescent-shaped and generally have highly reduced teeth. Whereas *P. petromyzon* and *P. hyalina* show several larger unci teeth, *P. sigmoidea* displays only two larger ventral unci teeth in addition to several small denticles and *P. robusta* possesses only a single uncus tooth (Fig. 12C). The species of *Proales*, in contrast, possess more-or-less quadrangular, plate-like unci with several equally developed teeth.

Finally, the manubria in most species of *Pleurotrocha* are long, more or less rod-shaped, and possess a reduced cauda; only the manubria of *P. atlantica* are shorter with a broad cauda. The ventral manubrial chamber in *P. sigmoidea* and *P. petromyzon* uniquely shows a ventral knob-like projection medially (Figs 4B, 8A) that is larger in the latter species. This feature possibly represents a synapomorphic character trait linking the two as sister species or part of a clade with other as yet undescribed species of *Pleurotrocha*.

Comparison with earlier descriptions

In comparison with previous descriptions of *Pleurotrocha sigmoidea* and *P. petromyzon*, we noted several discrepancies and also obtained many new observations, both of which we now discuss briefly:

Pleurotrocha sigmoidea

All the specimens that we investigated had two foot pseudosegments — a preanal pseudosegment and a lumbar pseudosegment. The former two sets of pseudosegments are displayed correctly in the drawings of Koste (1968) for *P. sigmoidea*, but a lumbar pseudosegment is not mentioned. Drawings in the landmark study of De Smet (1996), like those of Skorikov (1896), show three foot pseudosegments and a preanal pseudosegment; a lumbar pseudosegment ("tail", De Smet 1996) is mentioned in the description of the species, but does not appear in the drawings. The differing data with respect to pseudosegment identity and number undoubtedly originate from the difficulties associated with observations of living animals using light microscopy. Indeed, this represents a serious problem for all rotifer species, with data on the number of pseudosegments in the literature often being incorrect (or at least dubious) and with descriptions and drawings often being in conflict. Accurate data in this case can be obtained only by careful observations of living animals using light microscopy in combination with SEM analyses of well-stretched and -fixed individuals, all supported by precise descriptions and drawings.

Koste (1978) stated that a cerebral eye is present in the species; however, evidence of a cerebral eye in his drawings is recognizable only in Koste (1976), not Koste (1968, 1978). Like Skorikov (1896) and Zavadovsky (1926), we also failed to observe any eyes even though we investigated both juveniles and adults. Therefore, ontogenetic changes like those in *Proales reinhardti*, in which eyes apparently disappear in adults, can be ruled out. It might be, however, that the species is polymorphic for this trait, perhaps on a population level. For instance, although most specimens of *Lophocharis* Ehrenberg lack eyes, some individuals with eyes have apparently been found on occasion (Bilfinger 1894).

De Smet (1996) stated the absence of a constriction between the stomach and intestine, but our specimens clearly show such a constriction (Fig. 2C).

To date, no detailed information on the rotatory organ of *P. sigmoidea* is present in the literature. We noticed two openings of the retrocerebral organ, two palp-like cilia tufts and a small, inverted U-shaped row of cilia in the apical field. The ciliary tufts are extended anteriorly in the living animal and appear to have an undetermined sensory function. The paired epidermal projection shielding the mouth region caudally has not been described before in *P. sigmoidea* (or in *P. petromyzon*), probably because of its relative transparency and therefore difficulty in observing it using only light microscopy. We consider that these projections might function to avoid the loss of food during ingestion.

Pleurotrocha petromyzon

Although Sørensen (2002) presents good quality SEM images of well-prepared trophi for this species, a detailed description was not included in this more broadly comparative study. We therefore build on his work both to identify the different chambers of the trophi as well as to present SEM pictures from different angles.

Both Harring (1913) and Nogrady *et al.* (1995) state that the uncus of *P. petromyzon* has two ventral teeth. This is confirmed by both our SEM images and those of Sørensen (2002), which also reveal three additional teeth. We did not observe the pleural rods mentioned by Nogrady *et al.* (1995), either by light or by electron microscopy. However, we do document a previously unmentioned hypopharynx that is also not visible on the SEM images of Sørensen (2002), possibly as a result of the more aggressive sodium hypochlorite treatment that he used to prepare the trophi.

To date, demonstration of the presence of a retrocerebral organ in *P. petromyzon* has been neglected (Nogrady *et al.* 1995) and the brain was described as reaching to the posterior end of the mastax. Although we were also unable to distinguish the retrocerebral organ from the brain by light microscopy, TEM observations show clearly that the cerebral ganglion extends to the centre of the head (Fig. 10) only to be followed by a retrocerebral organ that reaches the level of the dorsal antenna. No openings of the retrocerebral organ could be observed in our SEM observations because of the disadvantageous positioning of the cilia. However, ultrathin sections clearly show a widespread retrocerebral glandular system pervading the brain of *P. petromyzon* (Fig. 10). The glandular system diverges in the front region of the brain into two ducts that open on the apical field of the corona. Our own conflicting observations therefore demonstrate that the invisibility of this gland system in light-microscopic observations does not automatically imply its absolute absence. Thus, its absence in other rotifer species, when inferred on basis of light-microscopic observations, has to be appraised with caution. We could not verify the presence of any subcerebral glands in our specimens, such as have been found in the giant forms of *P. petromyzon* observed by Wulfert (1935) and Koste (1976) as small, dark structures ventral to the cerebral ganglion.

The large, vesicle-filled sphere described by Koste (1978) as a crystal body is embedded in the retrocerebral organ and associated with the cerebral eye. The eye in *P. petromyzon* is not located at the end of the brain as described by several authors, but instead follows the retrocerebral organ posteriorly. (This, however, is to some degree a semantic issue, given that the distinction between anterior brain and posterior retrocerebral organ was not recognized previously.) We therefore hypothesize that the vesicle-filled sphere might somehow be related to the reception of light, possibly through shadowing the eye. To confirm this hypothesis, further studies are necessary.

Finally, although Ehrenberg (1838) observed *P. petromyzon* in ciliate colonies and documented both the species and its eggs, it was not known that the eggs present stalks like those of *P. reinhardti*. We could identify both smooth and sculptured eggs, but it remains unclear whether or not this might represent a preparation artifact.

Future perspectives

The example of *Pleurotrocha sigmoidea* demonstrates clearly that there are still difficulties with the classification of single species within Proalidae and Notommatidae and that further morphological investigations as well as comprehensive and precise species descriptions are desperately needed as a first step to resolve existing taxonomic problems. The morphological data thus obtained will play a crucial role in clarifying phylogenetic relationships within both rotifer families and in retracing the evolution of specific characters. We are hopeful that our study both highlights the general problem of data deficiency in these groups and presents a systematic protocol towards its solution. The subsequent phylogenetic analyses that these data will enable will also permit a robust test of our reassignment of *P. sigmoidea* to *Pleurotrocha*.

Eosphora anthadis Harring & Myers also shares significant morphological character traits with species of *Pleurotrocha* with respect to habitus and especially trophi, but future investigations, especially SEM images of the trophi, are necessary for any further conclusions. Though, *E. anthadis* does not fit the other species of *Eosphora* very well regarding to both morphology and ecology (Nogrady *et al.* 1995), it is necessary to include this species in a future phylogenetic analyses.

Acknowledgements

We gratefully acknowledge the financial support provided by the Deutsche Forschungsgemeinschaft and the Evangelisches Studienwerk Villigst for this study. We also wish two thank two anonymous reviewers for their helpful comments and criticism.

References

Bilfinger, L. (1894) Zur Rotatorien fauna Württembergs. 2. Beitrag. *Jahreshefte des Vereins für vaterländische Naturkunde in Württemberg*, 50, 35–65.

Budde, E. (1925) Die parasitischen Rädertiere mit besonderer Berücksichtigung der in der Umgebung von Minden I. W. beobachteten Arten. *Zeitschrift für Morphologie und Ökologie der Tiere*, 3, 706−784.

De Beauchamp, P. (1905) Remarques sur deux Rotifères parasites. *Bulletin de la Société zoologique de France*, 30, 117−124.

De Beauchamp, P. (1948) Sur deux espèces de *Proales* (Rotifères). *Bulletin de la Société zoologique de France*, 73, 136−140.

De Smet, W.H. (1996) Rotifera. Volume 4. The Proalidae (Monogononta). *In:* Nogrady, T. & Dumont, H.J. (Eds), *Guides to the Identification of the Microinvertebrates of the Continental Waters of the World, 9*. SBP Academic Publishing b.v. Amsterdam, New York, 102 pp.

De Smet, W.H. (1998) Preparation of rotifer trophi for light and scanning electron microscopy. *Hydrobiologia*, 387/388, 117−121.

Ehrenberg, C.G. (1830) Organisation, Systematik und geographisches Verhältnis der Infusionsthierchen. *Abhandlungen der königlichen Akademie der Wissenschaften zu Berlin* gehalten in den Jahren 1828 u. 1830, 1−108.

Ehrenberg, C.G. (1832) Über die Entwicklung und Lebensdauer der Infusionsthiere, nebst ferneren Beiträgen zu einer Vergleichung ihrer organischen Systeme. *Abhandlungen der königlichen Akademie der Wissenschaften zu Berlin*, 1−154, pl. 1−4.

Ehrenberg, C.G. (1838) *Die Infusionsthierchen als vollkommende Organismen. Ein Blick in das tiefere organische Leben der Natur.* Leopold Voss, Leipzig, 548 pp.

Fadeew, N.N. (1927) Matériaux pour la connaissance de la faune des rotifères del´ U.R.S.S. *Travaux de la Société des Naturalistes de Charkov*, 50, 141−155.

Fischer, C. & Ahlrichs, W.H. (2006) *Cephalodella ungulata* n. sp. (Monogononta: Notommatidae), a new rotifer species from North-West Germany, with notes on *C. tenuiseta* (Burn, 1890). *Zootaxa* 1378, 49−59.

Fontaneto, D. & Melone, G. (2003) Redescription of *Pleuretra hystrix*, an endemic alpine bdelloid rotifer. *Hydrobiologia*, 497, 153−160.

Glasscott, L.S. (1893) A list of some of the Rotifera of Ireland. *Scientific Proceedings of the Royal Dublin Society new series*, 8, 29–86.

Harring, H.K. (1913) Synopsis of the Rotatoria. *Bulletin of the United States National Museum, Washington* 81, 7−226.

Hudson, C.T. & Gosse, P.H. (1886) *The Rotifera or Wheel-Animalcules, both British and foreign*. London. Vol. 1, vi, 128 pp., Vol. 2, 144 pp.

Jennings, H.S. (1894) A list of the Rotatoria of the Great Lakes and some of the inland lakes of Michigan. *Bulletin of the Michigan Fish Commission*, 3, 1–34.

Jennings, H.S. (1896) Report on the Rotatoria with description of a new species. *Bulletin of the Michigan Fish Commission*, 1896, 1–9.

Kleinow, W., Klusemann, J. & Wratil, H. (1990) A gentle method for the preparation of hard parts (trophi) of the mastax of rotifers and scanning electron microscopy of the trophi of *Brachionus plicatilis* (Rotifera). *Zoomorphology*, 109, 329−336.

Koste, W. (1968) Über *Proales sigmoidea* (Skorikow, 1896) (eine für Mitteleuropa neue Art) und *Proales daphnicola* (Thompson, 1892). *Archiv für Hydrobi ologie*, 65, 240−245.

Koste, W. (1976) Über die Rädertierbestände (Rotatoria) der oberen und unteren mittleren Hase in den Jahren 1966−1969. *Osnabrücker naturwissenschaftliche Mitteilungen*, 4, 191−263.

Koste, W. (1978) *Rotatoria. Die Rädertiere Mitteleuropas. Ein Bestimmungswerk, begründet von Max Voigt. Ueberordnung Monogononta, 2nd edn. I. Textband*. Gebrüder Borntraeger, Berlin, Stuttgart, 673 pp.

Melone, G. (1998) The rotifer corona by SEM. *Hydrobiologia*, 387/388, 131−134.

Nogrady, T., Pourriot, R. & Segers, H. (1995) Rotifera 3: Notommatidae and Scaridae. *In:* T. Nogrady & H.J. Dumont (Eds.), *Guides to the Identification of the Microinvertebrates of the Continental Waters of the World, 8*. SBP Academic Publishing b.v., Amsterdam, New York, 248 pp.

Reiss, J. & Schmid-Araya, J.M. (2008) Existing in plenty: abundance, biomass and diversity of ciliates and meiofauna in small streams. *Freshwater Biology*, 53, 652−668.

Ricci, C., Melone, G. & Walsh, E. J. (2001) A carnivorous bdelloid rotifer, *Abrochta carnivora* n. sp. *Invertebrate Biology*, 120, 136−141.

Riemann, O. & Ahlrichs, W. H. 2008. Ultrastructure and function of the mastax in *Dicranophorus forcipatus* (Rotifera: Monogononta). *Journal of Morphology* 269: 698–712.

Riemann, O., Wilts, E.F., Ahlrichs, W.H. & Kieneke, A. (In press). Body musculature of *Beauchampiella eudactylota* (Gosse, 1886) (Rotifera: Euchlanidae) with additional new data on its trophi and overall morphology. *Acta Zoologica*.

Skorikov, A.S. (1896) Rotateurs des environs de Kharkow. *Travaux de la Societe de Naturalistes des Charkov,* 30, 207−774.

Sørensen, M. V. (2002) On the evolution and morphology of the rotiferan trophi, with a cladistic analysis of Rotifera. *Journal of Zoological Systematics and Evolutionary Research*, 40, 129–154.

Sørensen, M.V. (2005) Musculature in three species of *Proales* (Monogononta, Rotifera) stained with phalloidin-labeled fluorescent dye. *Zoomorphology*, 124, 47−55.

Wallace, R. L. (2002) Rotifers: Exquisite metazoans. *The Society for Integrative and Comparative Biology*, 42, 660−667.

Wulfert, K. (1935) Beiträge zur Kenntnis der Rädertierfauna Deutschlands. I. Teil. *Archiv für Hydrobiologie*, 2, 583−602.

Zavadovsky, M.M. (1926) Rotifers of the family Notommatidae from the area of the hydrobiological station Zwenigorodskoj. *Trudy Laboratorii éksperimental'noĭ biologii Moskovskogo zooparka*, 2, 261–295.