



Phylogenetic study on *Proales daphnicola* Thompson, 1892 (Proalidae) and its relocation to *Epiphanes* (Rotifera: Epiphaniidae)

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ABSTRACT

Within Rotifera, many morphological, taxonomic and phylogenetic questions still remain unanswered. Many families and genera have only poor phylogenetic support and classification is doubtful in a large number of species. To address these problems, a comprehensive reinvestigation of various species is necessary, especially for families such as Proalidae, in which several species have frequently been moved in and out. Here, we reinvestigated the species *Proales daphnicola* using light and electron microscopical techniques as well as morphological and molecular phylogenetic analyses in order to evaluate its phylogenetic position. Based on our results, we reassign *P. daphnicola* to *Epiphanes* and give a redescription of *Epiphanes daphnicola* (Thompson, 1892) n. comb. As a consequence of this conclusion, we also reassign *Proales kostei* to *Epiphanes*.

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1. Introduction

Within the past decade, our understanding of rotifer phylogeny has improved by using modern morphological methods (e.g. Sørensen, 2002; Segers and Wallace, 2008; Riemann et al., 2009), molecular methods (e.g. Herlyn et al., 2003; Yoshinaga et al., 2004; Garcia-Varela and Nadler, 2006) and a combination of both (Sørensen and Giribet, 2006). Admittedly, phylogenetic investigations do not exist for many taxa of this group of small microinvertebrates. In particular, morphological cladistic analyses are difficult to perform without time-consuming reinvestigations, because detailed morphological descriptions are lacking for most rotifers. In an ongoing investigation, we study the inner and outer morphology of different rotifer species mainly belonging to Proalidae, in order to clarify the phylogeny and existing taxonomical problems of this monogonont taxon as stressed by Sørensen (2005), Wilts et al. (2009a) and Wilts and Ahlrichs (2010). The genus *Proales* Gosse, 1886, currently accommodating 44 species (Segers, 2007), is one of the most problematic genera from a systematic point of

view, because it represents a taxonomically unsatisfactory assemblage of diverse species. The genus was subdivided into different groups very early (see Remane, 1929–33; Voigt, 1956–57). Koste (1978) subdivided the genus into two main groups: freshwater species resembling the type species of *Proales*, *Proales decipiens* (Ehrenberg, 1832), group A, and the predominantly marine species resembling *Proales reinhardti* (Ehrenberg, 1834), group B. However, several species of *Proales* do not fit into any of the two categories. Two of these species, *Proales sigmaidea* (Skorikov, 1896) and *Proales werneckii* (Ehrenberg, 1834), have recently been reassigned to *Pleurotrocha* (Notommatidae) and *Pourriotia* (Notommatidae) by Wilts et al. (2009a) and De Smet (2009), and possibly further reassignments will be required when more detailed data are acquired for additional species. In the present study, we reinvestigated *Proales daphnicola* Thompson, 1892, a limnic species which clearly differs from *Proales* species belonging to the subgroups A and B. In fact, our morphological investigation reveals that *P. daphnicola* rather resembles the genus *Epiphanes* in respect of habitus, corona, and trophi morphology and also biology. Phylogenetic analyses of morphological data support this hypothesis; moreover, also molecular phylogeny is in agreement with a closer relationship of *P. daphnicola* to *Epiphanes* than to other species of *Proales*. The species *Proales kostei* Nogrady and Smol, 1989 has close resemblance to *P. daphnicola*, and it should likewise be closely related to *Epiphanes* according to results of our morphological analyses.

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2. Material and methods

2.1. Morphological preparations and species identification

P. daphnicola was found living in large numbers on *Daphnia* sp. collected with a plankton sieve (64 µm mesh size) from a pond near Leer, Germany (53°25′03.50″N, 7°52′48.25″E) in October 2007 and 2008 and from a ditch in Oldenburg, Germany (53°15′27.41″N, 8°16′60.12″E) in October 2008 and 2009. Under a stereomicroscope (LEICA MZ12s), the single *Daphnia* specimens were tested for attached rotifer specimens. The crustaceans were isolated in a drop of water on a glass slide, where the rotifers left the crustaceans separately. *Epiphanes senta* was found in a ditch in Oldenburg, Germany (53°15′31.31″N, 8°16′87.53″E) in March 2010. Individuals of both rotifer species were studied by differential interference light microscopy (LEICA DMLB) as well as by scanning electron microscopy (ZEISS DSM 940). Isolated rotifer specimens were narcotized with bupivacaine and fixed with 4% OsO₄ solution and picric acid formaldehyde at 240 mOsm (after Melone, 1998), and preserved using a 4% paraformaldehyde solution. Dehydration was carried out in a graded ethanol series followed by critical point drying. Then, specimens were mounted on stubs and coated with gold and platinum, respectively. Trophi were generally prepared applying the procedure described by De Smet (1998) but dissolving tissues surrounding the trophi with a mixture of SDS/DTT (modified after Kleinow et al., 1990). Line drawings were created with Adobe Illustrator® CS2 and Adobe Photoshop® CS2.

Although the initial description of *P. daphnicola* by Thompson (1892) does not contain information on or drawings of its trophi, different authors (e.g. Harring and Myers, 1924) gave a sufficient description of the species, also including drawings of general habitus and trophi. In our determination, we refer to Harring and Myers who described a rounded postero-dorsal ramus margin for the species (see Plate XVIII, Fig. 5 in Harring and Myers, 1924 and Figs. 7B and 8B in this study). In his great work on the Proalidae, De Smet (1996a) presented modified drawings and SEM pictures of trophi featuring a distinct spine on the postero-dorsal ramus margin (see Figs. 333, 334 and Plate 13, Fig. 3 in De Smet, 1996a) for the same species. Admittedly, this posterior-dorsal spine was not present in any specimen of *P. daphnicola* collected in Oldenburg and Leer (a location 60 km from Oldenburg), whereas *Proales pejeri* (Fig. 1B and C in De Smet et al., 1993) shows this character. The species was synonymized with *P. daphnicola* by De Smet (1996a). De Smet (personal communication) noticed a length variation of the dorsal spine depending on the studied population, however, according to our data and the description given by Harring and Myers (1924), it is also conceivable that two different species exist, one without a spine and one with a spine varying in length, making a revalidation of *P. pejeri* necessary. In future studies, a detailed comparison of our specimens with those featuring a dorsal spine on the rami would help to address this problem.

2.2. Morphological character matrix and cladistic analysis

For phylogenetic analyses, 20 species were included in a data matrix and opposed to 43 morphological characters (see Table 1 and list of characters in Appendix A) compiled from direct personal observations and from the following literature sources: Thompson (1892), Harring and Myers (1924), Voigt (1956–57), Koste (1978), Koste and Terlutter (2001), Kleinow et al. (1990), Nogrady and Smol (1989), De Smet (1996a, 1996b), Melone (2001), Sørensen (2002), De Smet and Gibson (2008). The character matrix was constructed in Nexus Data Editor (NDE, version 0.5.0, Page, 2001) containing 27 characters of binary and 16 characters of multistate coding. Six characters are parsimony uninformative, but have been retained in the analysis, since they are illustrative

synapomorphies for the ingroup taxa. All characters, except for character no. 35, were treated as unordered and all characters were equally weighted without any *a priori* assumptions on character polarization. Therefore, the coding 0 does not necessarily represent a plesiomorphic condition. The taxon sampling for the ingroup included *P. daphnicola*, *P. kostei*, and several other rotifers of the subtaxon Ploima with malleate or modified malleate trophi (Transversiramida, see Sørensen, 2002). At least one species from each transversiramida family (Brachionidae, Euchlanidae, Lepadellidae, Lecanidae, Mytilinidae, Proalidae, Trichotriidae) was included in the matrix. For Epiphaniidae, several representatives from all genera (i.e. *Epiphanes*, *Rhinoglena*, *Mikrocodides* and *Cyrtonia*) were included in order to obtain a better resolution of the family. Due to our presumption *P. daphnicola* may be related to them. *P. pejeri* De Smet, van Rompu and Beyens 1993 was not included because of its incomplete habitus description. Furthermore, species of *Proales* group A (namely *P. fallaciosa* and *P. tillyensis*) and of *Proales* group B (namely *P. reinhardti* and *P. theodora*) were included in the matrix, in order to test if *P. daphnicola* and *P. kostei* are related to one or the other *Proales* group. The notommatid species *Pleurotrocha petromyzon* (Ehrenberg, 1830) was selected as outgroup, because this ploimid taxon is expected to stand apart from other ploimid species with malleate trophi (see Sørensen, 2002). This species has virgate trophi and adequate data are available (see Wilts et al., 2009a). While compiling the data matrix, we considered further characters to be included, such as the ultrastructure of protonephridia, the detailed construction of the corona or the morphology of the somatic musculature, but such information is currently restricted to a very limited number of species.

The maximum parsimony analysis of the matrix was carried out with PAUP* 4.0 b10 (Swofford, 2002) using the branch-and-bound search method. To calculate the node robustness of the obtained tree, a bootstrap analysis with 1000 replicates was run. Additionally, the Bremer support (Bremer 1988) (decay index) was calculated in TreeRot 3 (Sorenson and Franzosa, 2007). The program FigTree 1.3.1 (Rambaut, 2006–2009) was used to visualize the trees. The character evolution was retraced with MESQUITE 2.74 (Maddison and Maddison, 2010) using ACCTRAN (accelerated transformation) character optimisation.

2.3. Molecular data and phylogenetic analysis

In order to provide support for the hypothesis of *P. daphnicola* being more similar to *Epiphanes* than to *Proales*, we performed a phylogenetic analysis for a taxon set very similar to the one used for morphological analysis. We focused on the mitochondrial gene cytochrome oxidase c subunit I (COI). It is known that COI is not very reliable to resolve relationships between families; nevertheless, using the translated amino acid alignment can improve its usefulness (Fontaneto and Jondelius, 2011). Moreover, a preliminary screening of genetic distances between and within *Proales* and other genera of Ploima from loci already available in GenBank (COI, 18S and 28S) did not provide evidence for ribosomal loci being better than COI in solving relationships.

To obtain new COI sequences, single clean individual rotifers were isolated, put in a 0.5 ml tube and dehydrated. DNA was then extracted by adding 35 µl of Chelex (Bio-Rad Instagene Matrix) to each sample processed at 56 °C for 20 min and at 100 °C for 10 min. A fragment of COI was then amplified using the primers COI-F (AGTTCTAATCATAARGATATYGG) and COI-R (TAAACTTCAGGTGACCAAAAATCA). Cycle conditions were 94 °C for 5 min, 40 cycles of 94 °C for 30 s, 50 °C for 40 s, 72 °C for 40 s, and a final extension step of 72 °C for 7 min. Purification and sequencing were performed by Macrogen Korea. Sequences were checked and aligned by eye and no gaps had to be inserted. Phylogenetic reconstructions were performed (1) on the nucleotide alignment using the GRT+G+I

Table 1
Cytochrome oxidase c subunit I sequences used for the phylogenetic reconstructions.

Species	GenBank number	Origin	Source
<i>Brachionus plicatilis</i>	EF524555	Australia	Mills et al. (2007)
<i>Brachionus quadridentatus</i>	EU499779	UK	Swanstrom et al. (2011)
<i>Cyrtonia tuba</i>	HQ873047	Italy	New
<i>Epiphanes daphnicola</i>	HQ873040	Germany	New
<i>Epiphanes senta</i> ¹	JF714413	Svalbard	New
<i>Epiphanes senta</i> ²	JF714414	Germany	New
<i>Epiphanes senta</i> ³	DQ089728	Mexico	García-Varela and Nadler (2006)
<i>Floscularia melicerta</i>	EU499880	UK	Swanstrom et al. (2011)
<i>Pleurotrocha petromyzon</i>	EU499803	UK	Swanstrom et al. (2011)
<i>Proales doliaris</i>	DQ297790	USA	Sørensen and Giribet (2006)
<i>Proales fallaciosa</i>	HQ873041	Germany	New
<i>Proales similis</i>	DQ297791	Bermuda	Sørensen and Giribet (2006)
<i>Proales theodora</i>	HQ873045	Svalbard	New
<i>Testudinella patina</i>	EU499826	UK	Swanstrom et al. (2011)

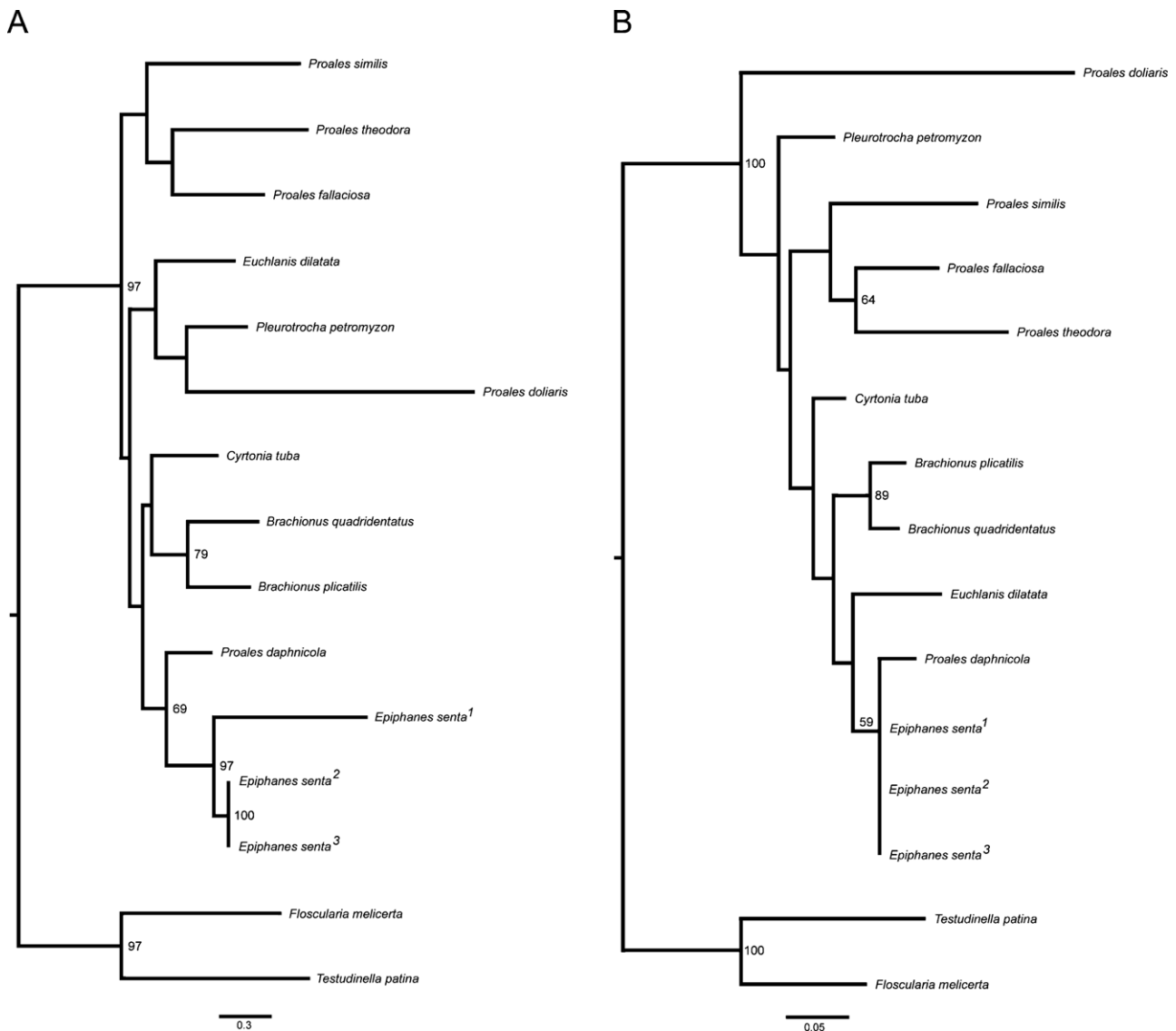


Fig. 1. Maximum likelihood phylogenetic reconstruction of the COI dataset performed with PhyML, using (A) GTR + G + I model on the nucleotide alignment and (B) MtART + G model on the amino acid alignment. Branch lengths are proportional to numbers of substitutions per site according to the selected models. Bootstrap support values in proximity of the nodes were obtained from 100 replicates. Support values are not shown for very short branches and for values under 50%. *Epiphanes senta*¹ = specimen from Svalbard, *Epiphanes senta*² = specimen from Germany, *Epiphanes senta*³ = specimen from Mexico.

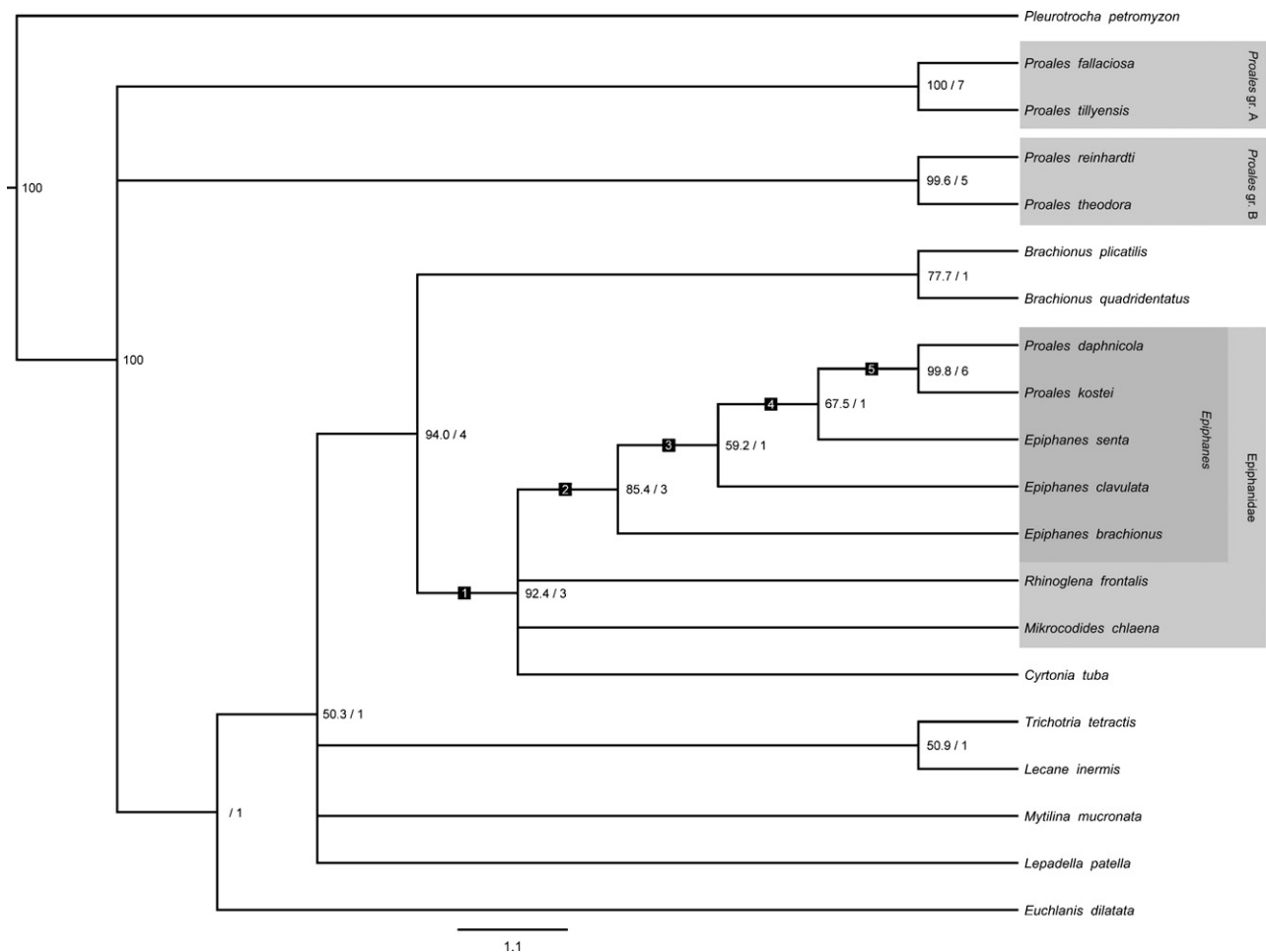


Fig. 2. Strict consensus tree of 36 equally parsimonious trees at 108 steps, generated by PAUP*. Numbers behind nodes indicate bootstrap support values, numbers following after/Bremer support indices. Numbers in black squares refer to selected synapomorphic character states: (1) epidermis stiffened to lorica (character 1): absent; habitus shape (character 2): bulbous saccate, type *Epiphanes senta*; direction of ramus subbasalis in relative to the fulcrum (character 27): directing inferio-posteriorly, type *Epiphanes senta*; ventral manubrial chamber enlarged (character 35): tendency present, type *Mikrocodides chlaena*; epipharynx (character 38): present, type *Mikrocodides chlaena*; hypopharynx (character 39): cuticular strands, type *P. daphnicola*; sexual dimorphism (character 40): males well developed, little smaller than females, type *Epiphanes senta*. (2) Position of dorsal antenna (character 11): displaced posteriorly, type *Epiphanes senta*; tips of ramus basal apophyses (character 24): with hairs, type *Epiphanes senta*; ventral manubrial chamber enlarged (character 35): well developed, type *Epiphanes senta*. (3) Position of lateral antennae (character 12): middle of the trunk, type *P. daphnicola*. (4) Eye position (character 19): ventrally on brain, type *Epiphanes senta*; manubrium foramen dorsalis (character 36): opened, chamber reduced to flat plane, type *Epiphanes daphnicola*. (5) Number of eyes (character 18): no eyes; incus y-shaped (character 21): present, type *Epiphanes daphnicola*; ramus basal apophyses enlarged (character 23): present, type *Epiphanes daphnicola*; shape of ramus foramen subbasalis (character 26): 0 = very large, oval, type *Epiphanes daphnicola*; shape of uncus (character 32): curved plate with curved teeth, type *Epiphanes daphnicola*; subuncus shape (character 34): basing on largest uncus tooth, lamellar, type *Epiphanes daphnicola*; epipharynx (character 38): absent; deposition of eggs on exterior substrate (character 43): present, type *Proales reinhardti*.

model, which was suggested as the best model by ModelGenerator (Keane et al., 2006), and (2) on the translated amino acid alignment with the MtART + G model suggested by ProtTest 2.4 (Abascal et al., 2005). Maximum Likelihood reconstructions for both nucleotide and amino acid datasets were performed with PhyML 3.0 (Guindon and Gascuel, 2003) with 100 bootstrap replicates.

As an additional test, we used the matrix of pairwise genetic distances to support the hypothesis that *P. daphnicola* is more closely related to *Epiphanes* than to *Proales*. We obtained pair-wise distances between *P. daphnicola* and the species in the two genera in two ways: (1) by calculating the uncorrected genetic distances between sequences in the nucleotide alignment, and (2) by calculating the patristic distances between terminals in the two trees. Thus, we had three datasets to perform the test: the first on raw distances not assuming any evolutionary model, the other two incorporating different evolutionary models from phylogenetic reconstructions based on nucleotide and amino acid alignments. Pair-wise genetic distances and linear models (LM) to perform the tests were computed in R 2.10.0 (R Development Core Team, 2010).

3. Results

3.1. Phylogeny

3.1.1. Molecular analysis

The molecular phylogenetic reconstruction using COI sequences from both the nucleotide (Fig. 1A) and the amino acid alignments (Fig. 1B) support our two hypotheses: (1) that *Proales* is a potentially polyphyletic genus, and (2) that *P. daphnicola* clusters with *Epiphanes* and not with *Proales* (Fig. 1). Unfortunately, bootstrap support values for the monophyly of *Epiphanes* + *P. daphnicola* are never very high, ranging between 59 and 69.

Uncorrected genetic distances between *P. daphnicola* and *Epiphanes* are 16.3–18.8%, whereas they are 26.1–32.2% between *P. daphnicola* and *Proales*. These differences are statistically significant (LM: $p=0.002$). The uncorrected genetic distances between *P. daphnicola* and Epiphaniidae (*Epiphanes* and *Cyrtonia*) are all below 20%, distances to other Ploima, including Proalidae are between 20 and 33% and distances to Gnesiotrocha (*Testudinella* and *Floscularia*) are above 30%. Thus, a strong phylogenetic

signal in COI supports a close relationship to Epiphaniidae, mostly to *Epiphanes*.

Patristic distances, including evolutionary models, are also significantly lower between *P. daphnicola* and *Epiphanes* than between *P. daphnicola* and *Proales* for both the tree from the nucleotide (LM: $p = 0.012$) and the tree from the amino acid alignment (LM: $p = 0.003$). On the amino acid tree, patristic distances from *P. daphnicola* are below 3% to *Epiphanes*, 10.7% to *Cyrtonia*, between 11% and 40% to other Ploima, and above 40% to *Gnesiotrocha*.

3.1.2. Morphological analyses

The PAUP* analyses yielded 36 equally parsimonious trees with a tree length of 108 steps. The strict consensus tree of all equally parsimonious trees (Fig. 2) supports monophyly for Epiphaniidae with a bootstrap value of 92.4% and a Bremer support index of 4. Likewise, the monophyly of *Epiphanes* (including *P. daphnicola* and *P. kostei*) is statistically supported with a bootstrap value of 85.4% and a Bremer support index of 3. *P. daphnicola* and *P. kostei* are supported as sister taxa with a bootstrap value of 99.8% and a Bremer index of 6. Epiphaniidae cluster with *Brachionus* showing a relationship closer than that with other transversiramid species with a bootstrap value of 94.0% and a Bremer index of 4. While *P. daphnicola* and *P. kostei* cluster with *Epiphanes*, other species of *Proales* show a different position, outside a complex of Epiphaniidae and *Brachionus*. In conclusion, the tree shows that *P. daphnicola*

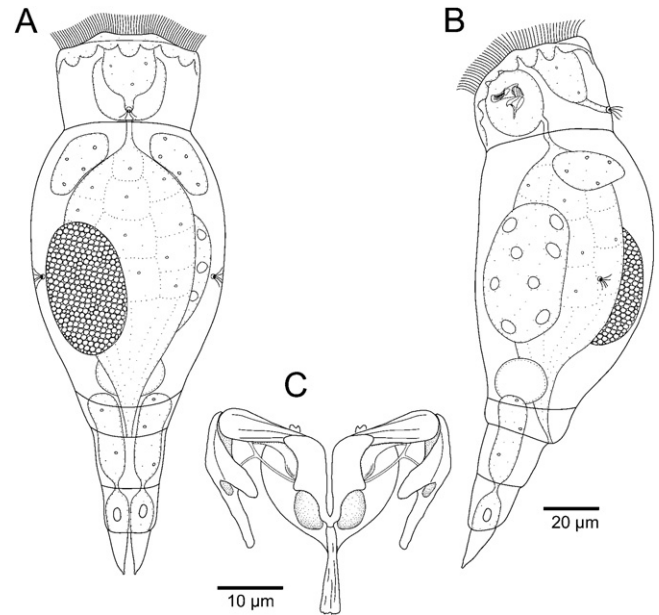


Fig. 3. General body organization of *Epiphanes daphnicola*. (A) Habitus in dorsal view; (B) habitus in lateral view; (C) trophi in frontal view.

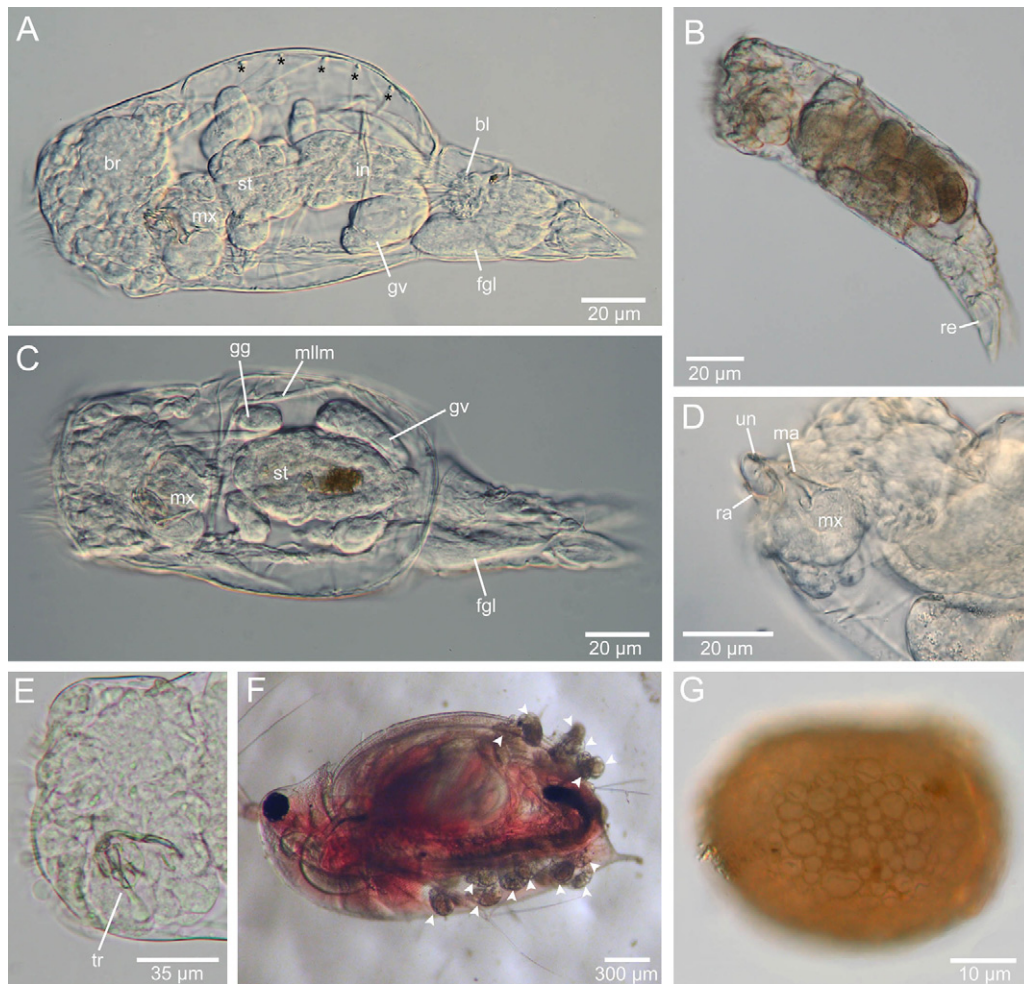


Fig. 4. Light microscopic images of *Epiphanes daphnicola*. (A) Specimen in lateral view (asterisks indicate circular muscles); (B) specimen in lateral view; (C) specimen in dorsal view; (D) head with protruding trophi; (E) head with trophi in relaxed position; (F) infested *Daphnia* specimen (arrow heads indicate rotifer specimens); (G) resting egg. bl, bladder; br, brain; fgl, foot gland; gg, gastric gland; gv, germovitellarium; in, intestine; ma, manubrium; mllm, musculus longitudinalis lateralis medius, mx, mastax; ra, ramus; re, reservoir; st, stomach; tr, trophi; un, uncus.

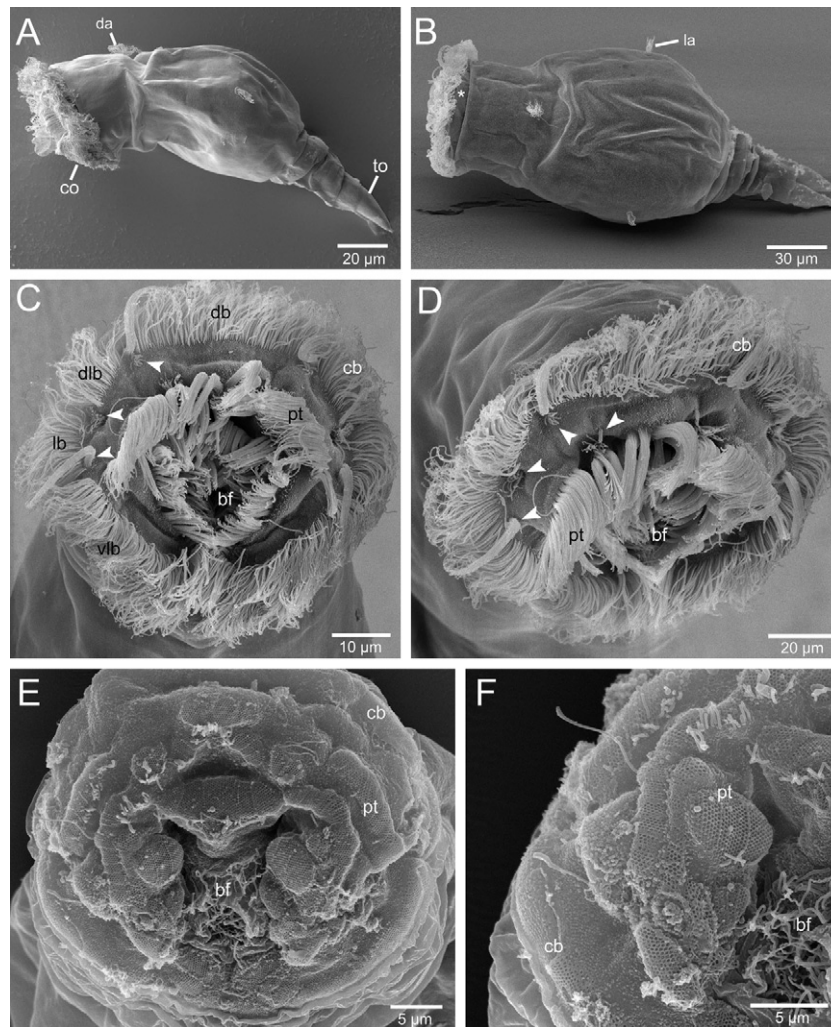


Fig. 5. Scanning electron microscopic (SEM) images of the habitus of *Epiphanes daphnicola*. (A) Specimen in lateral view; (B) specimen in dorsal view; (C) corona in ventrofrontal view (arrow heads indicate sensory cilia tufts); (D) corona in frontal view (arrow heads indicate sensory cilia tufts); (E) deciliated corona in frontal view; (F) detail of left part of deciliated corona. bf, buccal field; cb, circumpapical band; co, corona; da, dorsal antenna; db, dorsal cilia band; dlb, dorsolateral cilia band; la, lateral antenna; lb, lateral cilia band; pt, pseudotrochus; to, toe; vlb, ventrolateral cilia band.

and *P. kostei* have a much closer relation to Epiphaniidae, especially *Epiphanes*, than to other species of *Proales* or to species belonging to Transversiramida. Furthermore, both *Proales* groups are supported as monophyletic with high bootstrap values (100% and 99.6%) and Bremer support indices (7 and 5) which leave *Proales* as polyphyletic.

Following our morphological analyses and the hints that we got from the molecular analysis we argue for relocating *P. daphnicola* and *P. kostei* to *Epiphanes* and, from this point refer to them as *E. daphnicola* (Thompson, 1892) n. comb and *Epiphanes kostei* (Nogrady and Smol, 1989) n. comb.

3.2. Morphological description

E. daphnicola (Thompson, 1892) n. comb.

Syn. *P. daphnicola* (Thompson, 1892), *Proales nova* Wlastow, 1953

3.2.1. Type material

Neotype: a parthenogenetic female in a permanent glycerin glass slide mount deposited at Museum für Naturkunde, Germany, Berlin, (ZMB) Generalkatalog freilebende Würmer ZMB Vermes 11214.

Additional material: three parthenogenetic females in permanent glycerin glass slide mounts (ZMB Vermes 11215-1, -2 and -3), 17 parthenogenetic females mounted on a SEM stub (ZMB Vermes 11215-4, -5, -6, -7, -8, -9, 10, -11, -12, -13, -14, -15, -16, -17, -18, -19 and -20) and a trophi preparation with three trophi mounted on a SEM stub (ZMB Vermes 11215-21, -22 and -23).

Material location: a ditch in Oldenburg, Germany (53°15'27.41"N, 8°16'60.12"E), October 2009.

3.2.2. Diagnosis

Illoricate, plump body; head with a small rostrum-like element anteriorly; corona with diagonal orientation, circular circumpapical band, pseudotrochus well developed with elevated fields of compound cilia; foot with two pseudosegments and two stout conical toes; trophi derived from malleate type but without molar surfaces; incus Y-shaped; basal apophyses very large; ramus without alulae and projections; fine cuticular strands connecting unci and rami; short fulcrum with curved dorsal and ventral margins; manubrium broad, with ventral hook-like projection; uncus with three well-developed curved teeth; subuncus with deeply digitated margin.

3.2.3. Body organization of parthenogenetic females

The hyaline species has an illoricate, flexible and plump body (Figs. 3A and B, 4A–C and 5A and B) that is divided into three

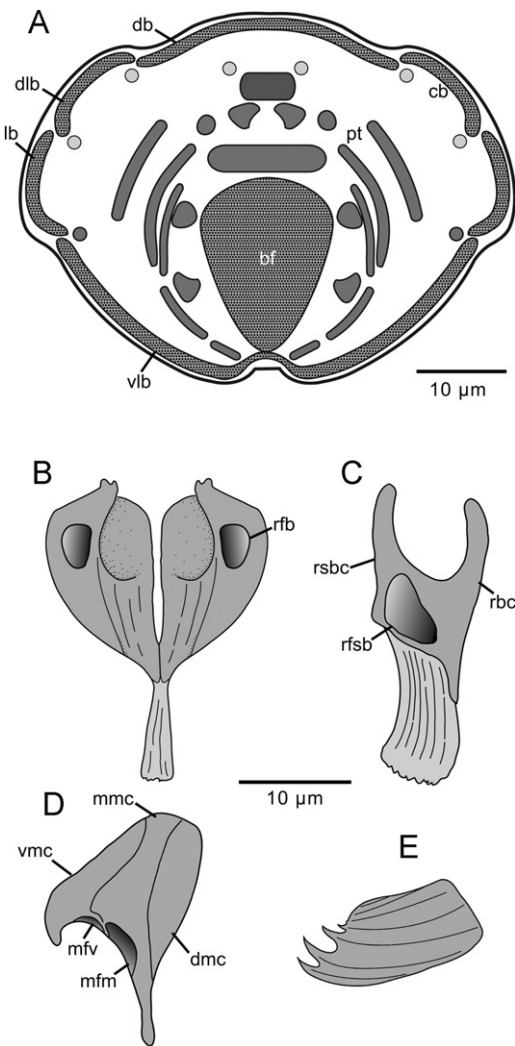


Fig. 6. Schematic drawings of *Epiphanes daphnicola*, (A) rotatory organ, (B–E) trophi. (A) Corona with pseudotrochal cilia fields (dark grey), and sensory cilia (light grey); (B) incus in caudal view; (C) incus in lateral view; (D) manubrium; (E) uncus. bf, buccal field; cb, circumapical band; db, dorsal cilia band; dlb, dorsolateral cilia band; dmc, dorsal manubrial chamber; lb, lateral cilia band; mfm, manubrium foramen medius; mf, manubrium foramen ventralis; mmc, median manubrial chamber; pt, pseudotrochus; rbc, ramus basal chamber; rfb, ramus foramen basalis; rfsb, ramus foramen subbasalis; rsbc, ramus subbasal chamber; vib, ventrolateral cilia band; vmc, ventral manubrial chamber.

distinct regions: head, trunk and foot with toes. The integument is hardly stiffened and presents a smooth surface (Fig. 5A and B). The head can be retracted into the trunk and shows a small, frontal fold demarcating a small rostrum-like structure (Figs. 3A and B and 5B). The head has a dorsal antenna in direct proximity to the neck fold, which separates the head from the trunk. The corona is oriented diagonal to the longitudinal body axis (Figs. 4A and B and 5A) and is mainly restricted to the apical head region. It consists of an external circumapical band, a pseudotrochus and a funnel-shaped buccal field (Figs. 5C–F and 6A). The densely ciliated circumapical band appears to form a complete ring of cilia engirding the head, but closer examination reveals that it can be subdivided into a dorsal band, a paired dorsolateral band, a paired lateral band and an unpaired ventrolateral band (Figs. 5C and 6A). The ends of these bands are somewhat displaced to each other and the particular interspaces are exiguous (Fig. 6A). A ciliary tuft displaying either long or short cilia is found on the level of each interspace (Fig. 5C and D; arrow heads). The pseudotrochus is represented by different ciliary fields surrounding the mouth opening dorsally and laterally.

These fields lie on petite elevations and show regular arrangements of compound cilia (cirri) (Fig. 5D–F). The stronger the cirri the more externally they are located. Mediodorsally to the buccal field is a broad, unpaired ciliary field followed ventrally by two smaller fields and another unpaired, broad field with a transversal direction (Fig. 6A). A paired ciliary field with diagonal direction is located dorsolaterally. It is followed internally by two long and one circular ciliary fields (Fig. 6). Two paired, rod-shaped fields are located ventrolaterally and are followed internally by a paired, rounded field (Figs. 5E and F and 6A). The uniformly ciliated buccal field is located centrally on the head and forms a funnel that leads to the mouth opening (Figs. 5C–F and 6A). The bulbous trunk is wider than the head, dorsally arched and subdivided into two pseudosegments. The large anterior pseudosegment has two lateral antennae (Fig. 5B) and is followed by a short preanal pseudosegment. The foot comprises two pseudosegments and two stout, conical toes with fine, tubular tips (Fig. 3A and B, 4A and B and 5A). The toes are retractable into the last foot pseudosegment. Two pedal glands with distinct reservoirs extend from the posterior part of the trunk to the base of the toes (Fig. 4B).

3.2.4. Digestive system

The digestive tract of *E. daphnicola* consists of the mouth opening, the spheroidal mastax, a narrow oesophagus, the stomach and intestine (Figs. 3A and 4A and C). The mouth opening is located medially in the corona and leads to the cavity of the mastax that resides in the anterior third of the trunk (Figs. 3A and 4A and B). The narrow oesophagus is attached to the dorsal part of mastax and connects it with the stomach. A pair of large, bulbous gastric glands attach anteriorly on the stomach (Fig. 4C). The stomach consists of distinctly recognizable stomach cells and connects with the ciliated intestine, which is clearly distinguished by a transverse constriction. The intestine leads to the cloaca, which opens dorsally below the preanal pseudosegment (Fig. 3A).

3.2.5. Mastax hard parts (trophi)

The compact, modified malleate trophi system is bilateral symmetrical (Figs. 3C, 6B–E and 7A–F). The individual trophi elements are embedded in epithelial tissue, from which cells extend into the cuticular cavities of the rami (ramus basal and ramus subbasal chambers) and manubria (dorsal, median and ventral manubrial chambers). The trophi can be extruded with only the fulcrum and caudae remaining in the mastax tissue (Fig. 4D).

The rami (ra) appear bifurcate in lateral view (Fig. 6C) and are almost oval in caudal view (Figs. 6B and 7B). Both the ramus basal (rbc) and the ramus subbasal chambers (rsbc) display distinct openings with the oval ramus foramen subbasalis (rfsb) directing ventrofrontally (Figs. 6C and 7A) and the circular ramus foramen basalis (rfb) facing caudally (Figs. 6B and 7B). The anterior part of each ramus consists of an enlarged, broad basal apophysis (ba) built on the ramus subbasal chamber (Fig. 6C). The basal apophyses have densely arranged scleropilar structures on their inner margins, but a molar surface with tubercles is absent. The posterior part of each ramus is built on the ramus basal chamber (Fig. 6C). The basal chambers terminate apically into a fine, less cuticularized structure. The lateral margins are rounded and alulae are lacking. Fine cuticular strands or ligaments (li), probably leftovers of a reduced hypopharynx, lie on the rami, connecting them with manubria (ma) and unci (un) (Figs. 3C and 7A).

The short, unpaired fulcrum (fu) is usually oriented in a dorsoventral (vertical) direction (Figs. 3B and 4E). The base of the short fulcrum attaches obliquely to the rami. In caudal view, the fulcrum appears slender and broadens gradually from the base towards the distal, irregularly sculptured end. In lateral view, the fulcrum is high and displays curved margins and longitudinal striae laterally (Figs. 6C and 7C).

Table 2

Morphological character matrix used in the cladistic analyses. Character numbers refer to the characters listed in Appendix A.

Taxa (20)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<i>P. petromyzon</i>	0	5	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0
<i>P. fallaciosa</i>	0	0	1	1	1	1	1	0	0	1	0	0	?	0	1	0	0	1	0	0
<i>P. tillyensis</i>	0	0	1	1	1	1	0	0	0	1	0	0	?	0	1	0	0	1	0	0
<i>P. reinhardti</i>	0	4	0	1	0	0	0	0	0	0	0	2	0	0	0	0	1	2	3	1
<i>P. theodora</i>	0	4	0	1	0	0	0	0	0	0	0	2	?	0	0	0	1	2	3	0
<i>B. plicatilis</i>	1	2	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	?	0
<i>B. quadridentatus</i>	1	1	0	0	0	0	0	0	0	0	0	3	0	1	0	1	0	1	0	0
<i>E. dilatata</i>	1	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0
<i>P. daphnicola</i>	0	3	0	0	0	0	0	0	0	4	2	1	0	1	0	1	0	0	–	0
<i>P. kostei</i>	0	3	0	0	0	0	0	0	0	0	2	?	0	1	0	1	0	0	–	0
<i>E. senta</i>	0	3	0	0	0	0	0	0	0	0	2	1	0	1	0	1	0	1	1	0
<i>E. brachionus</i>	0	3	0	0	0	0	0	0	0	0	2	0	0	1	0	1	0	1	0	0
<i>E. clavulata</i>	0	6	0	0	0	0	0	0	0	0	2	1	0	1	0	1	0	1	0	0
<i>R. frontalis</i>	0	3	0	0	0	0	0	0	1	0	1	0	0	1	0	1	0	2	2	0
<i>M. chlaena</i>	0	3	0	0	0	0	0	1	0	0	0	0	0	1	0	?	0	1	1	0
<i>C. tuba</i>	0	3	0	0	0	0	0	0	0	0	0	0	0	1	0	?	0	1	1	0
<i>T. tetractis</i>	1	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0
<i>M. mucronata</i>	1	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
<i>L. patella</i>	1	9	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1	2	4	0
<i>L. inermis</i>	1	A	1	0	0	0	0	0	0	3	0	0	0	0	0	0	1	0	–	0

Taxa (20)	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
<i>P. petromyzon</i>	0	0	0	–	2	4	1	0	3	1	1	2	0	–	0	0	0	4	?	0	0	0	1
<i>P. fallaciosa</i>	0	0	0	2	0	5	1	0	1	1	1	0	1	2	0	0	0	3	0	0	0	0	1
<i>P. tillyensis</i>	0	0	0	2	0	5	1	0	1	1	1	0	1	2	0	0	0	3	0	0	0	0	1
<i>P. reinhardti</i>	0	1	1	3	2	5	0	0	0	1	1	0	1	0	0	0	0	2	1	0	1	1	1
<i>P. theodora</i>	0	1	1	4	2	6	2	0	0	1	1	0	1	0	0	0	0	?	?	0	0+1	1	1
<i>B. plicatilis</i>	0	0	0	1	0	2	0	1	0	0	1	0	1	0	0	1	1	0	0	0	1	0	0
<i>B. quadridentatus</i>	0	0	0	1	0	?	0	1	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0
<i>E. dilatata</i>	0	0	0	1	2	5	1	0	0	1	1	0	?	?	0	1	0	1	?	0	0	0	0
<i>P. daphnicola</i>	1	0	1	0	0	2	1	0	0	1	1	1	1	2	2	0	0	1	1	0	0	1	1
<i>P. kostei</i>	1	0	1	0	2	1	2	1	0	0	1	1	1	1	2	2	0	0	1	1	0	0	?
<i>E. senta</i>	0	0	0	0	2	2	1	0	0	1	0	1	0	2	2	0	1	1	1	1	0	0	0
<i>E. brachionus</i>	0	0	0	0	2	2	1	0	0	1	0	1	0	2	1	0	1	1	1	0	0	0	0
<i>E. clavulata</i>	0	0	0	0	3	2	1	0	0	1	0	1	0	2	1	0	1	1	1	1	1	0	0
<i>R. frontalis</i>	0	0	0	1	1	2	2	1	0	0	0	0	1	0	0	1	0	1	1	1	0	0	0
<i>M. chlaena</i>	0	0	0	1	0	2	2	1	2	0	0	0	1	0	1	1	0	1	?	?	0	0	0
<i>C. tuba</i>	0	0	0	1	0	2	2	?	0	0	0	0	1	0	1	1	0	?	1	?	0	0	0
<i>T. tetractis</i>	0	0	0	?	3	?	?	0	1	1	1	0	?	?	0	0	0	?	?	?	0	0	0
<i>M. mucronata</i>	0	0	0	?	1	2	1	0	1	1	1	0	1	3	0	0	0	2	0	0	0	0	0
<i>L. patella</i>	0	0	0	1	1	?	?	0	0	1	1	0	1	?	0	1	0	?	?	0	0	0	0
<i>L. inermis</i>	0	0	0	?	3	7	0	0	1	1	1	0	?	?	0	?	0	?	?	0	0	0	0

The paired unci are formed by curved plates, carrying one small and three large, bent unci teeth that are more or less well developed (Figs. 6E and 7D and F). Three additional reduced unci teeth are recognizable by their straight jugal lines (Figs. 6E and 7F). The anterior parts of the unci are situated in the space between the ramus basal and ramus subbasal chambers (Figs. 6C and 7A and D). A large subuncus with several lamellar teeth at its inner margin is located ventral to each unci. In live specimens, the lamellar teeth of the subuncus (su) are in close contact with the inner surface of the basal apophyses (Fig. 7B and F).

The manubria attach proximally to the unci by fine ligaments and are divided into a broad, proximal clava (cl) and a short, slender cauda (ca) (Fig. 7A). The ventral manubrial chamber (vmc) extends into a hook-shaped protrusion (Figs. 6D and 7A, C and E). The manubrium foramen ventralis (mfv) is clearly visible and points ventrally. The median manubrial chamber (mmc) constitutes the largest part of the manubrium and forms the clava. The manubrium foramen medius (mfm) is located in direct proximity of the manubrium foramen ventralis facing ventrally also. A manubrium foramen dorsalis is lacking due to the reduction of the dorsal manubrial chamber (dmc) (Figs. 6D and 7C).

3.2.6. Nervous system and sensory organs

The small cerebral ganglion is positioned in the anterior part of the head above the mastax, with a caudal enlargement attaching to the dorsal antenna (Figs. 3A and B and 4A). Independently of the season, eyes were never found either in juvenile or adult specimens. A retrocerebral organ is also lacking. The dorsal antenna composed of numerous cilia encircled by a ring-shaped collar, is located posteriorly on the head near the neck fold (Fig. 5A). The lateral antennae are positioned about halfway down the trunk upon the dorsolateral surfaces and also consist of several cilia encircled by a flat collar (Fig. 5B). Small ciliary tufts that may have a sensory function are positioned on the apical head region below the circumapical band (arrow heads in Fig. 5C and D and light grey circles in Fig. 6A).

3.2.7. Excretory system

The protonephridial system presents at least three distinct terminal organs distributed laterally in the body cavity. The collecting tubules open into a contractile bladder that is positioned ventrocaudally in the trunk. The fluid of the bladder is emptied into the terminal part of the intestine (cloaca) (Fig. 3A).

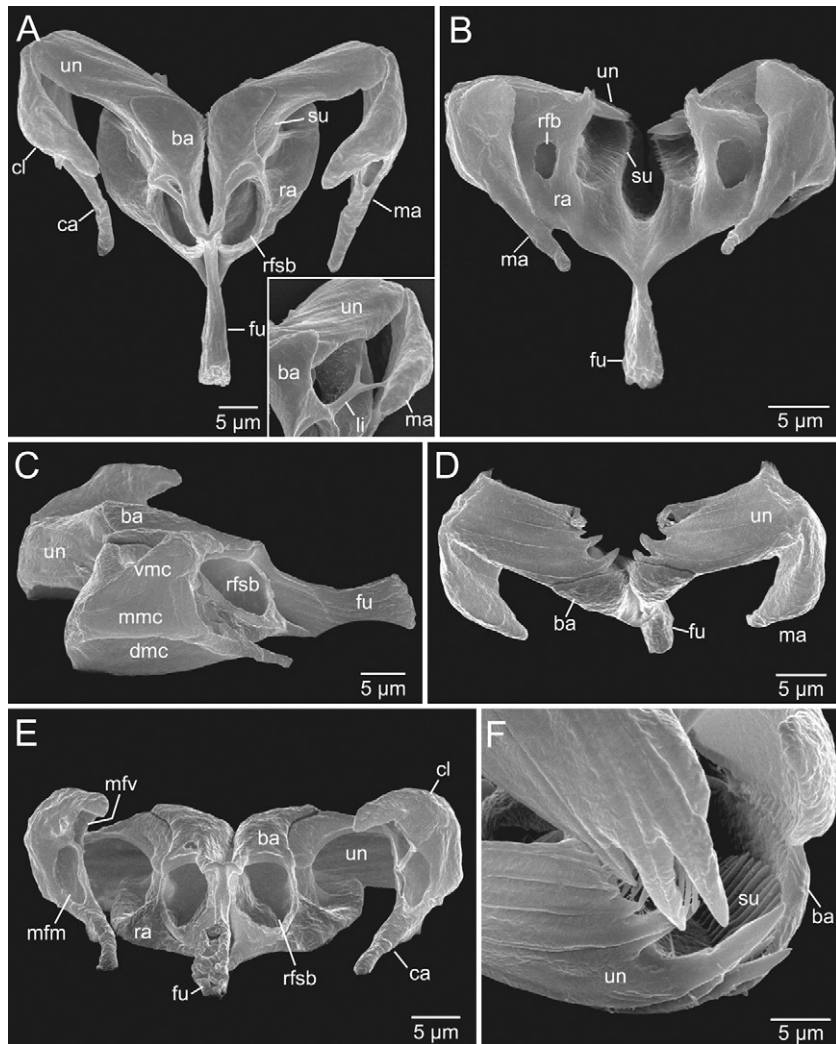


Fig. 7. SEM images of the mastax hard parts (trophi) of *Epiphanes daphnicola*. (A) Trophi in apical view (detail shows ligament connecting ramus and manubrium); (B) trophi in caudal view; (C) trophi in lateral view; (D) trophi in dorsal view; (E) trophi in ventral view; (F) uncus and inner view of basal apophyses. ba, basal apophysis; ca, cauda; cl, clava; dmc, dorsal manubrial chamber; fu, fulcrum; li, ligament; ma, manubrium; mfm, manubrium foramen medius; mfv, manubrium foramen ventralis; mmc, median manubrial chamber; ra, ramus; rfb, ramus foramen basal; rfsb, ramus foramen subbasalis; su, subuncus; un, uncus; vmc, ventral manubrial chamber.

3.2.8. Reproductive organs

E. daphnicola is an oviparous species. The parthenogenetic females have a syncytial germovitellarium with eight nuclei situated dorsolaterally in the posterior part of the trunk (Figs. 3A and B and 4A). Most observed amictic females contained one or two dark pigmented, ovoid eggs (Fig. 4B).

3.2.9. Ecology and behaviour

E. daphnicola lives epibiontic on *Daphnia* spp. The rotifers are attached to the crustaceans by a mucus strand produced by their pedal glands. Sometimes more than 20 rotifers are attached to a single *Daphnia* specimen (Fig. 4F). Eggs are mostly deposited on the inside or outside of the carapace. Males occur in large numbers and are well-developed. They resemble the females but are somewhat smaller. Sperms are easily visible. Resting eggs are brown-ochre and possess an ornamented surface with flattened granules (Fig. 4G). Females feed on small Euglenacea and sessile ciliates (Koste, 1978).

3.2.10. Measurements

Total length 175–220 µm (juveniles smaller), greatest dorso-ventral depth 70–85 µm, greatest width 65–75 µm, foot length 35–45 µm, toe length 18–20 µm, trophi length 30 µm, trophi width

30–35 µm, manubria length 20–23 µm, cauda width 13 µm and fulcrum length 12 µm, egg width 35 µm, egg length 45 µm.

4. Discussion

4.1. Classification of *E. daphnicola*

The designation of *E. daphnicola* to *Proales* by Thompson (1892) was plausible, because at that time *Proales* was generally presumed to be a taxon mainly composed of parasitic species. Thompson's classification was motivated on (1) the assumption that the species parasitizes cladocerans and (2) ecological similarities with *P. petromyzon*, a species also assigned to *Proales* by that time and likewise occurring on *Daphnia* and attaching its eggs on a substrate. Some authors, including Harring (1913) and Wulfert (1959) even categorized the species as *Pleurotrocha daphnicola*. However, these superficial similarities do not rely on a phylogenetic relationship, which becomes obvious by comparing the trophi of both species. Moreover, neither *P. petromyzon* and *E. daphnicola* nor most other *Proales* species known today are parasitic. Comparing *E. daphnicola* and its sister taxon *E. kostei* with representatives of the two subgroups of the genus *Proales* reveals only little resemblance regarding the habitus, but rather obvious

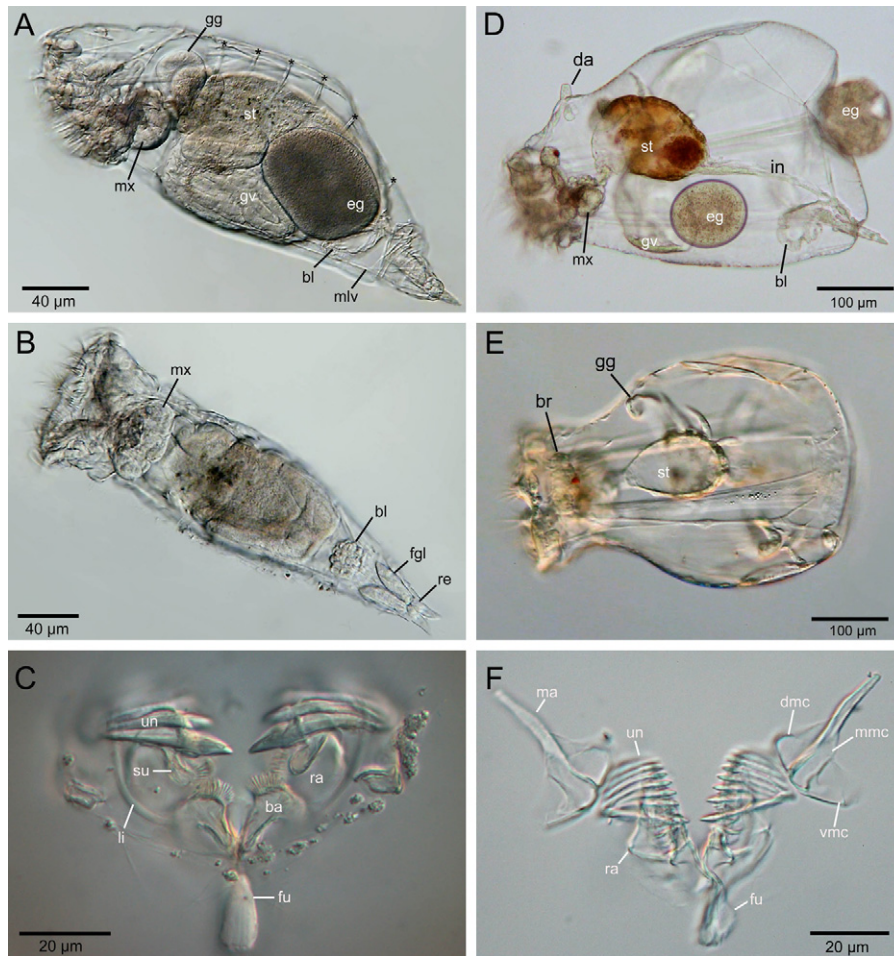


Fig. 8. Light microscopic images of *Epiphanes senta* (A–C) and *Epiphanes clavulata* (D–F). (A) Specimen in lateral view (asterisks indicate circular muscles); (B) specimen in ventral view; (C) trophi in apical view; (D) specimen in lateral view; (E) specimen in dorsal view; (E) trophi in apical view. ba, basal apophysis; bl, bladder; br, brain; da, dorsal antenna; dmc, dorsal manubrial chamber; eg, egg; fgl, foot gland; fu, fulcrum; gg, gastric gland; gv, germovitellarium; in, intestine; ma, manubrium; mmc, median manubrial chamber; mlv, musculus longitudinalis ventralis; mx, mastax; ra, ramus; re, reservoir; st, stomach; su, subuncus; un, uncus; vmc, ventral manubrial chamber.

differences in trophi morphology, overall morphology and ecology. Additionally, COI sequences support a close relationship with *Epiphanes*. The uncorrected genetic distances between *E. daphnicola* and *E. senta* (16.3–18.8%) were within the usual ranges observed between species within the same genus or family in rotifers (see e.g. for Brachionidae: Reyna-Fabian et al., 2010; for Bdelloidea: Fontaneto et al., 2011), whereas the distances of *E. daphnicola* to species of *Proales* were always much higher, and well above 25%. Contrary to general assumptions, COI is a good candidate for phylogenetic hypotheses in rotifers. Also apomorphic morphological characters argue for a close relationship with *Epiphanes*. Some morphological characters regarding general habitus morphology (Figs. 8A, B, D, E and 9A–C), corona morphology (Figs. 9D–F and 10A) and trophi morphology (Figs. 8C and F, 10B–D and 11A–F) as well as some ecological characters are discussed in the following. The given numbers “()” refer to the characters of the morphological character matrix listed in Table 2 and explained in Appendix A.

4.2. Habitus comparison of *E. daphnicola* and other species

The habitus of *E. daphnicola* is very similar to that of *E. senta* and other Epiphanidae (#2). It is somewhat bulbous or saccate, usually showing a large head, an oblique corona and a well-developed but short foot (Figs. 8A, B and D and 9A, B, D and E). The foot glands

have large reservoirs in front of the bases of the stubby toes (Figs. 4B and 8B). On the contrary, species of the subgroups of *Proales* have either a straight, telescopic, tubular habitus with a hardly offset foot carrying two conical toes (*Proales* group A) or a spindle-shaped, bent habitus with a large, highly moveable foot carrying two long, gradually tapering toes with needle-shaped tips (*Proales* group B). In *E. daphnicola*, the dorsal antenna is displaced to a position near the neck fold (Figs. 5A and B and 9C), which is quite an uncommon character turning out to be an autapomorphic character for *Epiphanes* (#11). In species of *Proales*, *Cyrtonia tuba* (Ehrenberg, 1834), *Mikrocodides chlaena* (Ehrenberg, 1886) and species of *Rhinoglena* possess a dorsal antenna located more anteriorly on the head (see Pl. 4 Fig. 2C in Koste, 1978 and Fig. 2B in De Smet and Gibson, 2008).

4.3. Comparison of coronal characters in *Epiphanes* and some outgroup taxa

The rotatory apparatus of *E. daphnicola* also differs distinctly from those in species of *Proales*. *E. daphnicola* has no ventral extended buccal field (#15), as present in representatives of *Proales* subgroup A (e.g. *Proales tillyensis*, see Fig. 4C in Wilts and Ahlrichs, 2010), or a paired epidermal structure covering the mouth opening (#17) like in representatives of *Proales* subgroup B (e.g. *Proales litoralis*, see Fig. 3 in De Smet, 1996b; *P. reinhardti* and *P. oculata*,

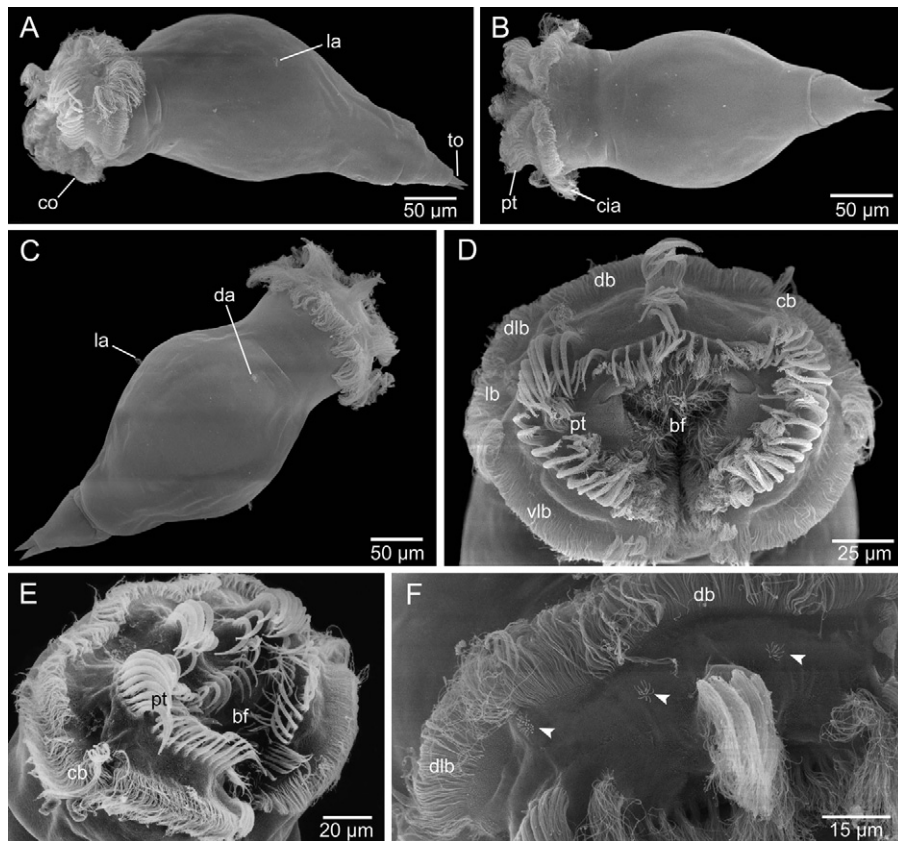


Fig. 9. Scanning electron microscopic (SEM) images of the habitus of *Epiphanes senta*. (A) Specimen in lateral view; (B) specimen in ventral view; (C) specimen in dorsal view. (D) Corona in frontal view; (E) corona in frontolateral view; (F) detail of upper corona region (arrow heads indicate sensory cilia tufts). bf, buccal field; cb, circumapical band; co, corona; da, dorsal antenna; db, dorsal cilia band; dlb, dorsolateral cilia band; la, lateral antenna; lb, lateral cilia band; pt, pseudotrochus; to, toe; vlb, ventrolateral cilia band.

E.F. Wilts personal observation). Moreover, species of *Proales* do not possess a corona with a pseudotrochus subdivided into different cilia fields, as far as it can be evaluated on the basis of the available data. The corona of *E. daphnicola* rather shows a morphology resembling that of other Epiphanidae and Brachionidae. A distinct buccal funnel (#14) in combination with ciliary fields of compound cilia lying on small elevations (#16) seem to have evolved in the stem lineage of a clade that includes *Brachionus* and Epiphanidae. Unfortunately, more phylogenetic conclusions regarding the corona cannot be drawn as long as detailed information on the corona of other rotifer species is lacking. Anyhow, morphological similarities of the corona in *E. senta* and *E. daphnicola* are striking (compare Figs. 5C–F and 6A with Figs. 9D–F and 10A). The corona of both species is oriented diagonal to the body axis and features an external circumapical band composed of an unpaired dorsal, a paired dorsolateral, a paired lateral and an unpaired ventrolateral band (compare Figs. 6A and 10A in this paper with Fig. 9 in Melone, 1998). Small ciliary tufts reside beneath the circumapical band at identical positions. Furthermore, the pseudotrochus in both species is well developed with compound cilia on small elevations, but different in shape. The reduction of the inner pseudotrochal cilia field mediodorsally to the buccal field and the reduction of the outer ventrolateral pseudotrochal cilia field in *E. daphnicola* are noteworthy (compare Figs. 6A and 10A). Melone (2001) observed a very similar corona morphology in *Rhinoglena frontalis* Ehrenberg 1853 and a modified pattern can also be recognized in *Brachionus plicatilis* (see Fig. 62 in Stoßberg, 1932 and Figs. 1 and 2 in Melone, 1998) whose pseudotrochus displays only three main cilia fields lying on elevations that are much more developed than in *Epiphanes* and *Rhinoglena*.

4.4. Comparison of trophi characters in *Epiphanes* and some outgroup taxa

The trophi of *E. daphnicola* and *E. kostei* show several differences from the modified malleate trophi of the subgroups A (species resembling *P. decipiens*) and B (species resembling *P. reinhardtii*) of *Proales*, however, a remarkable structural accordance with the trophi of Epiphanidae (especially *E. senta*). Both subgroups of *Proales* usually present a distally broadened and almost caudally directed fulcrum, a well-developed hypopharynx and, in most cases, distinct alulae. Moreover, representatives of the subgroup B possess long manubria with distally curved caudae and rami which show a more or less large separation of basal and subbasal chambers (e.g. *Proales theodora*) (#22). Like other Epiphanidae and *Brachionus*, *E. daphnicola* and *E. kostei* show trophi with a short, slender ventrally orientated fulcrum (#28). They lack alulae as well as a well-developed, planar hypopharynx (#39) and display short, manubria with straight caudae. Although Thompson (1892) designated the trophi of *E. daphnicola* as notommatous, they are in fact malleate (Harring and Myers, 1924; De Smet, 1996a) or derived from a malleate type. However, the rami in *E. daphnicola* and *E. kostei* have undergone some modifications: (1) the basal apophyses are strongly enlarged (#23); (2) the subbasal chambers have undergone some changes as well (the molar surface of the rami comprising tubercles and scleropili typical of species with malleate trophi, is completely reduced), making the incus appear Y-shaped (#21); (3) the uncus forms a curved triangular plate carrying a few stout and several reduced, converging teeth (#32); and (4) the subuncus is represented by a membranous structure attaching mainly to the largest uncus tooth displaying a deeply digitated margin

(#34). The basal apophyses feature scleropilar hairs on their anterior margins (#24) like those of *E. senta* (Fig. 9D). Following the ramus modification, the uncus of *E. daphnicola* and *E. kostei* interdigitates as a whole with the rami instead of a broad uncus with well developed, parallel oriented unci teeth interdigitating with the molar surface. Both species possess a paired cuticular strand or ligament that connects rami, manubria and unci (#39) (see Figs. 3C and 7A in this study and Fig. 14 in De Smet, 1996a). This cuticular ligament (ligamentum incudiuncicum, see Martini, 1912) is also observable in other species of Epiphanidae (*E. senta*, see Figs. 8C and 11D in this study; *E. clavulata*, see Fig. 11E in this study; *E. brachionus*, see <http://users.unimi.it/melone/trophi/index.html>, *Rhinoglena tokioensis*, see Fig. 6D in De Smet and Gibson, 2008; *R. frontalis*, see Fig. 15 in Melone, 2001; *C. tuba*, see Fig. 2 in Fontaneto and Melone, 2003). This character could be a potential autapomorphy for Epiphanidae (including *E. daphnicola* and *E. kostei*) and seems to represent remains of a hypopharynx, because these cuticular ligaments can be recognized connecting the paired membranous hypopharynx with manubria and unci in Brachionidae (e.g. *B. plicatilis*, see Figs. 1B and 4 in Kleinow et al., 1990; *Brachionus manjavacas*, see Fontaneto et al., 2007; *Keratella serrulata* and *Platyonus patulus*, see Sørensen, 2002; *Platyas quadricornis* see <http://users.unimi.it/melone/trophi/index.html>). Like in *E. senta*, *M. chlaena*, *C. tuba*, *R. frontalis*, and *Brachionus*, rami and fulcrum in *E. daphnicola* and *E. kostei* display an angle of 180° (#30) with the fulcrum directing ventrally. This specific character normally cannot be found elsewhere in monogonont species with malleate or modified malleate trophi and seems to be synapomorphic for Brachionidae and Epiphanidae. But the most conspicuous structural similarity shared by *E. daphnicola*, *E. kostei* and other Epiphanidae is the shape of the manubrium being somewhat triangular to club-shaped with a broad clava and a slender cauda. In *E. daphnicola* (Figs. 6D and 7A and E), *E. kostei* (see Pl. 14 Fig. 1 in De Smet, 1996a), *E. senta* (Fig. 11A and C), *E. clavulata* (Figs. 8F and 11E), and *E. brachionus* (see <http://users.unimi.it/melone/trophi/index.html>) the ventral manubrial chamber is elongated and widened, while the cauda is formed by the median chamber exclusively. This specific enlargement of the ventral manubrial chamber (#35) makes the manubrium appear broader than long and is an autapomorphy for Epiphanes. Only a tendency towards this character is present in *C. tuba* (Fontaneto and Melone, 2003) and *M. chlaena* (see Sørensen, 2006). Moreover, the foramen of the dorsal manubrial chamber seems to enlarge stepwise in the evolution of Epiphanidae (#36), as the different species show different stages of enlargement of the manubrium foramen dorsalis. In *E. senta* (Fig. 11A and C), *E. daphnicola* and *E. kostei*, it is even so large that walls of the foramen are lacking and the former presence of the dorsal manubrial chamber can only be recognized by a flat plane. This character seems to be synapomorphic for these three taxa, since *E. brachionus* and *E. clavulata* (Fig. 12E) still possess a dorsal manubrial chamber being only partly opened. Indeed, a completely opened dorsal manubrial chamber is also present in *R. fertoeensis* (see Fig. 4 in De Smet and Gibson, 2008), however, since most *Rhinoglena* species possess partly closed dorsal manubrial chambers, this character seems to rely on a convergent evolution. This scenario is more plausible than to assume that the complete dissolution of the outer wall of the dorsal manubrial chamber is a plesiomorphic character for *E. senta*, *E. daphnicola*, *E. kostei*, and *R. fertoeensis* and that the wall was secondarily reconstructed in *E. brachionus*, *E. clavulata* and other *Rhinoglena* species.

Regarding the species' biology, it is conspicuous that the males of *E. daphnicola* are well developed, like those of *Epiphanes* and *Rhinoglena*. The sexual dimorphism in respect of difference in size between the sexes is less distinct than in other rotifer species. Another interesting ecological feature is the fact that females of *P. daphnicola* can be attached to the substrate (mostly *Daphnia*) via

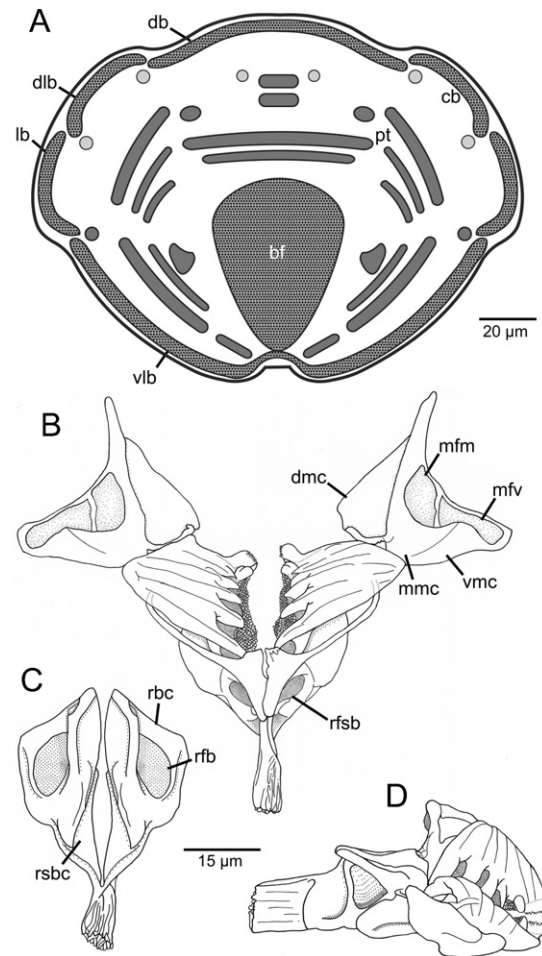


Fig. 10. Schematic drawings of *Epiphanes senta*, (A) rotatory organ, (B–D) trophi. (A) Corona with pseudotrochal cilia fields (dark grey), and sensory cilia (light grey). (B) Trophi in apical view; (C) trophi in caudal view; (D) trophi in lateral view. bf, buccal field; cb, circumapical band; db, dorsal cilia band; dlb, dorsolateral cilia band; dmc, dorsal manubrial chamber; lb, lateral cilia band; mfm, manubrium foramen medius; mfv, manubrium foramen ventralis; mmc, median manubrial chamber; pt, pseudotrochus; rbc, ramus basal chamber; rsbc, ramus subbasal chamber; rfb, ramus foramen basalis; rfsb, ramus foramen subbasalis; vlb, ventrolateral cilia band; vmc, ventral manubrial chamber.

a long mucus strand produced by their foot glands. We observed the same behaviour in *E. senta* and *C. tuba* circling around their own body axis, feeding on algae or ciliates as it is known for *Brachionus*, too.

4.5. Comparison of the somatic musculature of *E. senta* and *E. daphnicola*

Further support to the close relationship between *E. daphnicola* and *E. senta* can be found in the somatic muscle system. The musculature of *E. daphnicola* was analyzed by Sørensen (2005), who compared it with the musculature of two other *Proales* species. There are obvious differences, especially with respect to the musculature of *P. fallaciosa* (representative of *Proales* subgroup A) in which most muscles are doubled (see Wilts et al. 2010 for details). Comparing Sørensen's results with those of the study of musculature in *E. senta* performed by Martini (1912), Remane (1929–22), and Leasi et al. (2010) and applying the terminology of Riemann et al. (2008) and Wilts et al. (2009b) reveals similarities of the musculature in both species (Fig. 10A and B). Both species possess a pars coronalis (pc), which is composed of two strains in *Epiphanes* (Fig. 10B), a broad coronal sphincter (cs) and several

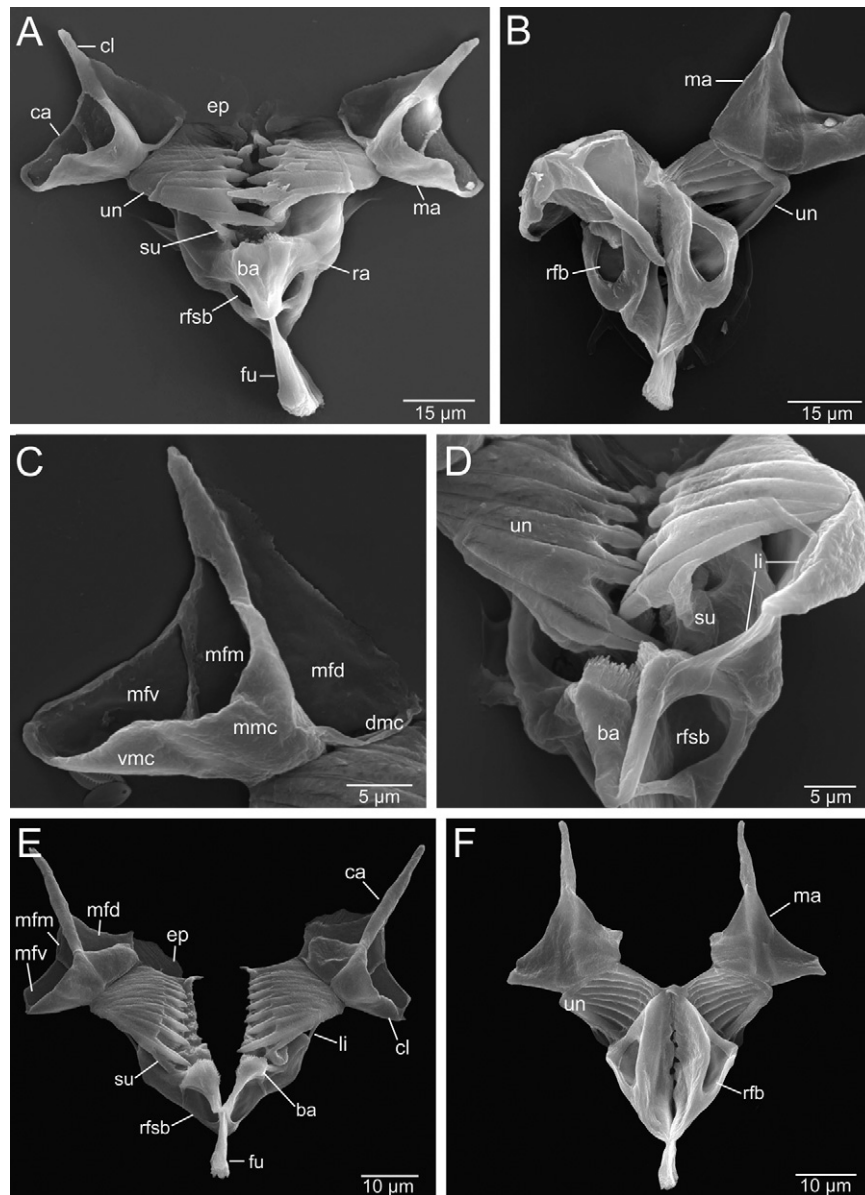


Fig. 11. SEM images of the mastax hard parts (trophi) of *Epiphanes senta* (A–D) and *Epiphanes clavulata*. (A) Trophi in apical view; (B) trophi in caudal view; (C) manubrium; (D) closer view of ramus and uncus; (E) trophi in apical view; (F) trophi in caudal view. ba, basal apophysis; ca, cauda; cl, clava; dmc, dorsal manubrial chamber; fu, fulcrum; li, ligament; ma, manubrium; mfd, manubrium foramen dorsalis; mfm, manubrium foramen medius; mfv, manubrium foramen ventralis; mmc, median manubrial chamber; ra, ramus; rfb, ramus foramen basalitis; rfsb, ramus foramen subbasalis; su, subuncus; un, uncus; vmc, ventral manubrial chamber.

ventrally interrupted circular muscles in the trunk. *E. daphnicola* and *E. senta* fairly agree in their number of circular muscles (mc) in the trunk, the former possessing five and the latter possessing six muscles that show a narrow dorsal interruption (Fig. 10A and B). A musculus circumpedalis (mcp) was not observed in *E. daphnicola* by Sørensen (2005), but is reported for *E. senta* by Leasi et al. (2010). Both *E. daphnicola* and *E. senta* show a musculus longitudinalis retractor tentaculatus dorsalis (mlrtd) (*sensu* Remane, 1929–33). This V-shaped muscle was reported for *E. senta* by Martini (1912) and Leasi et al. (2010) and for *E. daphnicola* by Sørensen (2005). Although Sørensen termed this muscle dorsal head retractor, it anchors on the level of the dorsal antenna in both species. A comparison with the musculature of several other rotifer species investigated so far (e.g. *R. frontalis*, *Euchlanis* sp. and *Brachionus calyciflorus*, Stoßberg, 1932; *Dicranophorus forcipatus*, Riemann et al., 2008; *Bryceella stylata*, Wilts et al., 2009b) shows that retraction of the head is done by contracting the musculus longitudinalis capitis (mlc) *sensu* Riemann et al. (2008) (musculus

retractor centralis, see Remane, 1929–33) instead, which is also present in both species and usually the most distinct longitudinal muscle in monogonont rotifers stretching dorsally from the midbody to the head. The musculus longitudinalis dorsalis (mld) in *E. daphnicola* (dorsolateral head retractor, see Sørensen, 2005) stretches from the head to the posterior third of the trunk, whereas it continues into the foot in *E. senta* (musculus retractor dorsalis, see Remane, 1929–33; lateral longitudinal muscle, see Leasi et al., 2010). The musculus longitudinalis lateralis superior (mls) in *E. daphnicola* (lateral head retractor, see Sørensen, 2005) and *E. senta* (musculus retractor lateralis medius, see Remane, 1929–33) is relatively short. The musculus longitudinalis lateralis medius (mlm) in *E. senta* (musculus retractor lateralis medius, see Remane, 1929–33) stretches laterally from the head to the posterior part of the trunk, where it splits into several endings, whereas it can be assumed that the muscle shows a median interruption (see Remane, 1929–33 for muscle development scenarios) in *E. daphnicola* (longitudinal trunk retractor and foot extensor, see Sørensen, 2005). The

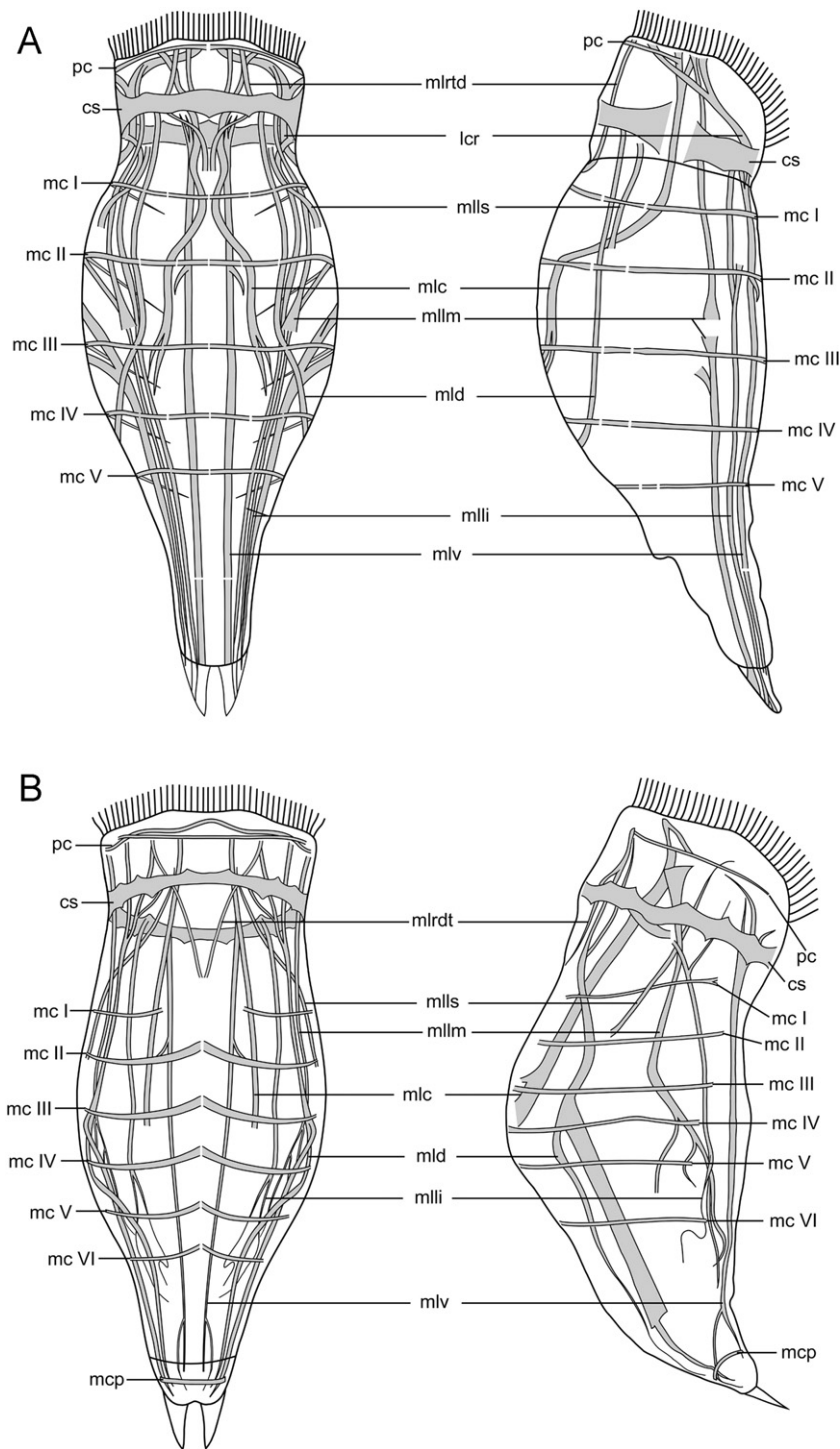


Fig. 12. Schematic drawing of the somatic musculature of (A) *Epiphanes daphnicola* (after Sørensen, 2005) and (B) *Epiphanes senta* (after Martini, 1912). cs, coronal sphincter; lcr, lateral coronal retractor; mc I–VI; musculus circularis I–VI; mcp, musculus circumpedalis; mlc, musculus longitudinalis capitis; mld, musculus longitudinalis dorsalis; mlli, musculus longitudinalis lateralis inferior; mllm, musculus longitudinalis lateralis medius; mlls, musculus longitudinalis lateralis superior; mlrdt, musculus longitudinalis lateralis retractor dorsalis tentaculatus; mlv, musculus longitudinalis ventralis; pc, pars coronalis.

musculus longitudinalis lateralis inferior (mlli) in *E. senta* (musculus retractor lateralis inferior, see Remane, 1929–33; ventral longitudinal muscle, see Leasi et al., 2010) stretches from the corona to the posterior part of the trunk. In *E. daphnicola* (ventral foot retractor, see Sørensen, 2005), the muscle is doubled with one part terminating near the midbody region and the other strand terminating in the head region. Both species feature a musculus

longitudinalis ventralis (mlv) (*E. senta*: musculus retractor ventralis, see Remane, 1929–33 and ventral longitudinal muscle, see Leasi et al., 2010; *E. daphnicola*: ventral trunk retractor, see Sørensen, 2005) extending from the corona to the base of the foot, presenting medially a short hook-like branch. Additionally, *E. daphnicola* shows a lateral coronal retractor (lcr) (see Sørensen, 2005) in the head with diagonal direction. Maybe, this muscle represents

only a secondarily separated anterior part of the musculus longitudinalis ventralis, because it is more developed showing anteriorly a similar, diagonal direction in *E. senta*.

5. Conclusion

Our assumption that *P. daphnicola* is more closely related to species of Epiphanidae than to other *Proales* species was tested and supported via morphological and molecular data analyses. By demonstrating apomorphies, retracing character evolution and presenting further morphological and ecological evidence, we have justified a relocation of *P. daphnicola* and its assumed sister taxon *P. kostei* to Epiphanes. Unfortunately, there are no further sequences of other Epiphanidae and Proalidae available at present so that the phylogenetic relationships within Epiphanidae remain to be solved by future molecular analyses using more sequences of further species. This study may encourage taxonomists to reinvestigate other rotifer species that have already been described in detail, especially those which do not well fit the taxa they are classified in, in order to discover existing para- and polyphylies and to contribute to their elimination.

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Appendix A. Appendix

Description of characters

- (1) Epidermis stiffened to lorica: 0 = absent, 1 = present, type *Brachionus quadridentatus*.
- (2) Habitus shape: 0 = tubular, type *Proales fallaciosus*, 1 = rectangular, type *B. quadridentatus*, 2 = oval, type *B. plicatilis*, 3 = bulbous saccate, type *E. senta* (Figs. 9A and B and 10A–C), 4 = spindle-shaped, bent, type *P. reinhardti*, 5 = inflated elongate, type *P. petromyzon*, 6 = giant, saccate, type *Epiphanes clavulata*, 7 = quadrangular, type *Trichotria tetractis*, 8 = elongated, oval, type *Mytilina mucronata*, 9 = flattened, oval, type *Lepadella ovalis*, A = flattened, elongated-oval, type *Lecane inermis*.
- (3) Neck pseudosegment: 0 = absent, 1 = present, type *P. fallaciosus*. This character refers to the presence of a short trunk pseudosegment following the head (see De Smet, 1996a).
- (4) Lumbal pseudosegment: 0 = absent, 1 = present, type *P. fallaciosus*. This character refers to the presence of a posterior trunk pseudosegment located in front of the preanal pseudosegment.
- (5) Several dorsal and longitudinal trunk folds: 0 = absent, 1 = present, type *P. fallaciosus*.

Some *Proales* species display a number of deep, longitudinal infoldings running across the dorsal and lateral sides of the trunk (see De Smet, 1996a).

- (6) Foot: 0 = distinctly offset from trunk, type *P. reinhardti*, 1 = foot and trunk gradually tapering, type *P. fallaciosus*.
- (7) Knob-like appendage above toes: 0 = absent, 1 = present, type *P. fallaciosus*.
- (8) Direction of toes: 0 = both toes in same direction, 1 = one toe abducted, type *M. chlaena*.
- (9) Head with proboscis: 0 = absent, 1 = present, type *R. frontalis*. In *Rhinoglena* the head features an apical elongation called proboscis (see Melone, 2001).
- (10) Head: 0 = anteriorly rounded, without structure, 1 = with offset, short rostrum, type *P. fallaciosus*, 2 = with offset, cap-like rostrum, type *Lepadella*, 3 = with semicircular rostrum, type *L. inermis*, 4 = with offset, small element, type *E. daphnicola*. In some proalid rotifers, the dorsal epidermis of the rotatory organ frontally runs out into a hyaline projection, which varies in the different taxa in form.
- (11) Position of dorsal antenna: 0 = centrally on head, type *P. fallaciosus*, 1 = displaced anteriorly, type *R. frontalis*, 2 = displaced posteriorly, type *E. senta* (Figs. 6A and B, 9D and 10C).
- (12) Position of lateral antenna: 0 = posterior third of trunk, type *P. petromyzon*, 1 = middle of the trunk, type *E. daphnicola* (Figs. 3A and 10A and C), 2 = close to preanal pseudosegment, type *P. reinhardti*, 3 = displaced on spines, type *B. quadridentatus*.
- (13) Openings of retrocerebral organ: 0 = absent, 1 = present, type *P. petromyzon*.

The retrocerebral organ is usually associated with a paired duct that leads near the center of the corona (see Wilts et al., 2009a).

- (14) Distinct buccal funnel: 0 = absent, 1 = present, type *E. senta* (Figs. 6C and 10D).
- (15) Corona with caudally elongated buccal field: 0 = absent, 1 = present, type *P. fallaciosus*. Like most species of Dicranophoridae, Notommatidae and Lindiidae, species of *Proales* group A possess an elongated postoral, ciliated field in the ventral head region.
- (16) Corona with compound cilia arranged on elevations: 0 = absent, 1 = present, type *E. senta*. This character refers to the elevated pseudotrochal cilia fields in Epiphanidae that display compound cilia or membranellae (Figs. 6C–F and 10D–F).
- (17) Corona caudally with labial projections: 0 = absent, 1 = present, type *P. reinhardti*. This character refers to the presence of epidermal projections that border the corona caudally. It can be found e.g. in species of *Proales* group B, *Pleurotrocha* (Wilts et al., 2009a), *Bryceella* (Wilts et al., 2009b) and Lepadellidae (Wilts et al., in press).
- (18) Number of eyes: 0 = no eyes, 1 = one eye present, 2 = two eyes present.
- (19) Eye position: 0 = posterior on brain, type *P. petromyzon*, 1 = ventrally on brain, type *E. senta*, 2 = displaced on proboscis, type *R. frontalis*, 3 = apically, type *P. reinhardti*, 4 = laterally in corona, type *L. ovalis*.
- (20) Rami asymmetry: 0 = absent, 1 = present, type *P. reinhardti*.
- (21) Incus Y-shaped: 0 = absent, 1 = present, type *E. daphnicola*.

This character refers to the unique shape of the incus of *E. daphnicola* (Fig. 7C) and *E. kostei* (see De Smet, 1996a).

- (22) Ramus chambers separating from each other: 0 = absent, 1 = present, type *P. theodora*.

This character refers to the fact that in species of *Proales* group B the cuticular chambers building the rami show a sep-

aration being incomplete in *P. reinhardti* and complete in *P. theodora* (see De Smet, 1996a).

- (23) Ramus basal apophyses enlarged: 0 = absent, 1 = present, type *E. daphnicola*.
- (24) Tips of ramus basal apophyses: 0 = with hairs, type *E. senta*, 1 = with small, regular teeth, type *R. frontalis*, 2 = with spines, type *P. fallaciosa*, 3 = with irregular margin, type *P. reinhardti*, 4 = with blunt gradually decreasing teeth, type *P. theodora*, 5 = blunt, without teeth or hairs, type *B. plicatilis*.
- In several taxa the basal apophyses display varying structures distally from regular teeth in *Rhinoglena* (see De Smet and Gibson, 2008) to fine hairs in *Epiphanes* (Figs. 8A and D and 12D and E).
- (25) Lateral margin of ramus: 0 = rounded, type *E. daphnicola*, 1 = crenated, type *R. frontalis*, 2 = with alula, type *P. petromyzon*, 3 = with rounded alula, type *T. tetractis*.
- (26) Shape of ramus foramen subbasalis: 0 = very large, oval, type *E. daphnicola*, 1 = very large, elongated oval, type *E. kostei*, 2 = medium-sized rounded, type *E. senta*, 3 = large rounded, type *E. clavulata*, 4 = small rounded, type *P. petromyzon*, 5 = very small, type *P. reinhardti*, 6 = very large, circular, type *P. Theodora*, 7 = medium-sized, circular, type *L. inermis*.
- (27) Direction of ramus foramen subbasalis in relative to the fulcrum: 0 = directing posteriorly, type *P. tillyensis*, 1 = directing inferiorly, type *P. petromyzon*, 2 = directing inferioposteriorly, type *E. senta* (Fig. 12A, D and E).

This character refers to the orientation of the ramus foramen subbasalis which varies among the different rotifer taxa. In Lepadellidae e.g. they face like the ramus basal chambers superiorly.

- (28) Fulcrum orientation: 0 = \pm caudally, type *P. petromyzon*, 1 = \pm ventrally, type *E. senta*.
- This character refers to the position the fulcrum takes up in rest position in the animal. In most monogonont rotifer species the fulcrum lies in the longitudinal axis of the rotifer (horizontally). In *Brachionus* and *Epiphanes* (Figs. 4B and 5E) the fulcrum is oriented vertically.
- (29) Fulcrum length: 0 = short, maximal half the length of the ramus, type *E. senta*, 1 = as long as ramus, type *P. tillyensis*, 2 = medium long, maximal two-third of the ramus length, type *M. chlaena*, 3 longer than ramus length, type *P. petromyzon*.
- (30) Angle of ramus and fulcrum: 0 = ramus and fulcrum planar forming an angle of $\pm 180^\circ$, type *E. senta*, 1 = ramus and fulcrum angled, type *P. petromyzon*.

This character refers to the angle that is formed by ramus and fulcrum. In Epiphaniidae fulcrum and ramus lie in a more or less planar level whereas in other monogonont rotifers ramus and fulcrum are angled.

- (31) Number of major uncus teeth: 0 = 7–10 teeth, 1 = 0–6 teeth.
- (32) Uncus shape: 0 = rectangular plate with parallel orientated teeth, type *E. senta*, 1 = curved plate with curved teeth, type *E. daphnicola* (Figs. 7E and 8D), 2 = crescentic, type *P. petromyzon*.
- (33) Subuncus: 0 = absent, 1 = present.
- (34) Subuncus shape: 0 = basing on complete uncus, evenly brush-like, type *B. plicatilis*, 1 = basing on largest uncus tooth, lamellar, type *E. daphnicola*, 2 = brush-like elements basing on single uncus teeth, type *P. fallaciosa*, 3 = scleropilar element on ramus, type *M. mucronata*.

This character refers to the development of the subuncus. The subuncus is a structure residing below the uncus that can be brush-like with sclereopili or lamellar with finger- or teeth-like indentations. In *E. senta* and *E. clavulata* (Fig. 12A, D and E) and *B. plicatilis* (Kleinow et al., 1990) it stretches below the complete uncus, in *E. daphnicola* and *E. kostei* it is lamellar and only attaches the principal uncus tooth (*E. daphnicola*) (Fig. 8A, B and F). In species of *Proales*-group A it consists of

some large brush-like elements that originate mainly from the largest uncus teeth and see *P. tillyensis* (see Wilts and Ahlrichs, 2010; *P. fallaciosa*, E.F. Wilts personal observation).

- (35) Ventral manubrial chamber enlarged: 0 = absent, 1 = tendency present, type *M. chlaena*, 2 = well developed, type *E. senta* [character ordered 0-1-2].
- (36) Manubrium foramen dorsalis: 0 = small, type *P. tillyensis*, 1 = large, type *Epiphanes brachionus*, 2 = opened, chamber reduced to a flat plane, type *E. daphnicola*.
- (37) Manubria with twisted shape: 0 = absent, 1 = present, type *B. plicatilis*. This character refers to the wringled form of the cauda in *Brachionus* species.
- (38) Epipharynx: 0 = absent, 1 = present, type *M. chlaena*.
- (39) Hypopharynx: 0 = membranous structures with cuticular strands, type *B. plicatilis*, 1 = cuticular strands, type *E. daphnicola*, 2 = large element with fine-denticulated platelets, type *P. reinhardti*, 3 = large element with two-teethed platelets, type *P. tillyensis*, 4 = small fork-like structure, type *P. petromyzon*.
- (40) Sexual dimorphism: 0 = dwarf males, type *B. plicatilis*, 1 = males well developed, little smaller than females, type *E. senta*.
- (41) Germovitellarium band-like: 0 = absent, 1 = 1 present, type *E. clavulata*.
- (42) Macro habitat: 0 = fresh water, 1 = marine environment.
This character refers to the habitat. It is coded \pm for *P. theodora* because the species occurs in fresh- and marine water.
- (43) Deposition of eggs on exterior substrate: 0 = absent, 1 = present, type *P. reinhardti*.

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