[1]*Institute of Biological and Environmental Sciences, Department of Systematics and Evolutionary Biology, University of Oldenburg, Oldenburg, Germany;* [2]*German Centre for Marine Biodiversity Research (DZMB), Senckenberg Research Institute, Wilhelmshaven, Germany*

Phylogeny of Dicranophoridae (Rotifera: Monogononta) – a maximum parsimony analysis based on morphological characters

O. Riemann[1], A. Kieneke[1,2] and W. H. Ahlrichs[1]

Abstract

This study presents the first phylogenetic analysis of Dicranophoridae (Rotifera: Monogononta), a species rich rotifer family of about 230 species currently recognized. It is based on a maximum parsimony analysis including 77 selected ingroup and three outgroup taxa and a total of 59 phylogenetically informative morphological characters. Character coding is based on personal investigation of material collected by the authors and an extensive survey of the literature. Apart from covering general body organization, character coding primarily relies on scanning electron microscopic preparations of the mastax jaw elements. Our study suggests monophyly of Dicranophoridae with a clade of *Dicranophorus* and *Dorria* as the sister taxon of all other dicranophorid species. Monophyly of *Encentrum*, the most species rich genus within Dicranophoridae, cannot be demonstrated. Within Dicranophoridae our study identifies the monophyletic taxa Caudosubbasifenestrata, Intramalleata, Praeuncinata and Proventriculata, each based on unambiguous character transformations evolved in their stem lineages. However, resolution within Praeuncinata and Proventriculata is very limited. Although some terminal clades within Praeuncinata and Proventriculata are recognized, basal splits remain obscure. Probably, other characters such as DNA sequence data are needed to further our understanding of phylogenetic relationships within these poorly resolved taxa.

Key words: Phylogeny – mastax jaw elements – scanning electron microscopy – key character transformations – monophyletic subtaxa

Introduction

The family Dicranophoridae comprises a species rich taxon of monogonont rotifers. About 230 species are formally recognized as valid in the most recent monographic treatment (De Smet 1997; for checklist of valid species names, see Segers 2007). Dicranophorid rotifers are characterized by a grasping mastax that can be protruded through the mouth opening to seize various items of prey (forcipate mastax type in the traditional terminology that identifies different mastax types). Originally treated as a subfamily Dicranophorinae within Notommatidae (Harring 1913), the possession of a forcipate mastax shared by all dicranophorid rotifers provided the basis for establishing the family Dicranophoridae (Remane 1929–1933). The majority of dicranophorid rotifer species are a component of the freshwater meiofauna inhabiting the periphyton and benthic region of inland water bodies (for data on ecological range, see Pejler and Berzins 1993). Some species regularly occur in the interstices between sand grains of lakes, ponds and riverbeds. Others inhabit wet cushions of moss and lichens. In marine environments, the diversity of dicranophorid rotifers is limited to lower numbers of species compared to freshwater habitats. Some dicranophorid rotifers occur periphytically among seaweeds; others are a component of the interstitial meiofauna. Finally, there is a small number of parasitic species that live both endo- and ectoparasitically on oligochaetes and slugs. In their natural habitats, dicranophorid rotifers rarely ever appear in large numbers; population densities throughout the life cycle are generally low.

An early comprehensive account of Dicranophoridae was contributed by Harring and Myers (1928), who brought together previous information and, moreover, described dozens of unknown species. Voigt (1957) was the first to provide a large identification key to dicranophorid rotifers of central Europe, including also data on ecology and distribution. De Smet's (1997) monographic treatment of Dicranophoridae marks the most recent landmark in systematic research on dicranophorids. The merits of his work are twofold: firstly, a comprehensive list of literature on dicranophorid rotifers is compiled and a critical assessment of the validity of all morphospecies described in the literature is given. Thus, this work provides an invaluable starting point for phylogenetic analyses. Secondly, for a large number of dicranophorid rotifer species, excellent scanning electron microscopic (SEM) preparations of the jaw elements (trophi) are given (for details of preparation see De Smet 1998). Such preparations reveal a host of structural details that can be used for phylogenetic studies of this otherwise morphologically fairly uniform group of rotifers.

The phylogenetic affinities of Dicranophoridae to other monongonont rotifers are anything but certain. Formerly, notommatid and dicranophorid rotifers with their creeping lifestyle, soft integument and homogeneously ciliated rotatory apparatus were considered ancestral (De Beauchamp 1909; Harring and Myers 1928). Remane (1929–1933) disagreed and placed rotifers with a malleate mastax such as *Epiphanes* at the base of Ploima. More recent analyses (Markevich and Kutikova 1989; Sørensen 2002; Sørensen and Giribet 2006) suggest a more strongly derived position of Dicranophoridae within Monogononta. As with most other rotifer families, no studies of dicranophorid rotifers have been attempted in the framework of contemporary cladistics. Phylogenetic analyses on family level have so far only been put forward for Ituridae (Segers et al. 1994), Conochilidae (Segers and Wallace 2001) and Asplanchnidae (Walsh et al. 2005). The present analysis based on selected species covering all dicranophorid genera is intended to be a first step towards a phylogenetic system of Dicranophoridae. The overall aim of this study is not only to provide an outline of a phylogenetic system, but also to elucidate key character transformations that occurred in the evolution of this group and that may have sparked the enormous species richness of dicranophorid rotifers.

Materials and Methods

Material used for coding

Two different sources of material were used in this study on which coding is based: first, selected dicranophorid rotifer specimens collected by the authors near Oldenburg, North-West Germany, were investigated by light microscopy and SEM. Second, the characters for the morphological character matrix were obtained from the literature on Dicranophoridae, especially from De Smet's guide to the Dicranophoridae (De Smet 1997) and from the original descriptions of individual species. For detailed information on the literature used for coding the species in this analysis, the reader is referred to Table S1 accompanying the online version of this study.

Living specimens were studied using bright field and differential interference contrast microscopy on a Leica DMLB compound microscope. Images were taken with an Olympus colour view I digital camera. For the analysis of the mastax jaw elements and for a more detailed examination of the external morphology, SEM was used. Preparations of the trophi were carried out by dissolving the tissue of the specimen following the protocol given by Kleinow et al. (1990). The dissolving agent was obtained by first preparing a stock solution (5.2 g SDS + 0.24 g NH_4HCO_3 in 100 ml aqua dest). Before applying the dissolving agent to the specimen, 0.1 g DTT (AppliChem, Darmstadt, Germany) was added to 5 ml of the stock solution and vigorously stirred. Individual specimens were subsequently selected under the stereomicroscope and transferred into a droplet of the dissolving agent. The resulting isolated trophi were repeatedly rinsed with distilled water and air-dried on a round coverslip. Subsequently, they were coated with gold and examined on a Zeiss DSM 940 SEM. Light microscopic images of the trophi were obtained either by carefully squashing specimens or by adding a droplet of the dissolving agent (see Kleinow et al. 1990) onto the slide with the specimen and subsequently drawing the droplet under the coverslip by means of a dry slip of tissue paper. After dissolution of the mastax tissue, the trophi, if necessary, were manipulated in their position by carefully touching the edges of the coverslip. For SEM observations of complete specimens, individual rotifers were isolated under a dissecting microscope and anaesthetized either with carbonated water or a 0.25% aqueous solution of bupivacaine (Bucain®; Curasan, Kleinostheim, Germany). When relaxed and fully extended, they were killed off with a droplet of 1% OsO_4 buffered in 0.1 M NaCaCodylate buffer. For fixation, picric acid–formaldehyde (239 mOsm) was used (Melone and Ricci 1995). After dehydration in a graded ethanol series and critical-point drying, specimens were coated with gold and examined on a Zeiss DSM 940 SEM.

Taxa in the analysis

Of the total number of dicranophorid rotifers, 77 species were selected. Given the total number of well over 230 dicranophorid species described so far, a selection had to be made for practical reasons. Our choice of 77 ingroup taxa was made based on the following two principles: (i) inclusion of representatives of all dicranophorid genera and (ii) inclusion of all species for which SEM preparations of their trophi elements are available. It needs to be stressed, however, that SEM preparations do not exist for representatives of all genera. Unfortunately, no SEM preparations are available for the rare, monotypic genera such as *Dorria*, *Inflatana*, *Pedipartia*, *Streptognatha* and *Glaciera*. As a consequence, coding for these taxa is incomplete and restricted to characters that are given in the original species descriptions and that are based on light microscopy only.

Three species (*Notommata glyphura* Wulfert, 1935; *Itura myersi* Wulfert, 1935 and *Lindia tecusa* Harring and Myers, 1922) were chosen as outgroup representatives. *Itura myersi* is assumed to be closely related to *Dicranophorus* (Segers et al. 1994; Pourriot 1997). This assumption has been corroborated by recent phylogenetic analyses relying on both morphological and molecular data (Sørensen 2002; Sørensen and Giribet 2006). The mastax of *I. myersi* is considered intermediate between the forcipate (grasping) and virgate (sucking) type. With some species of Dicranophoridae, *I. myersi* has in common the ventrally positioned, caudally extended buccal field. *L. tecusa* is probably also closely related to dicranophorid rotifers (Sørensen 2002;

Sørensen and Giribet 2006). The mastax jaw elements of *L. tecusa* have a similar shape to those of species of the genus *Dicranophorus*, but they cannot be thrust out of the mouth opening. Like *I. myersi* and some dicranophorid rotifers, *L. tecusa* is characterized by the presence of a caudally extended buccal field, creeping movements and a flexible integument. In cladistic analyses, species of the genus *Notommata* cluster with dicranophorid rotifers (Sørensen 2002; Sørensen and Giribet 2006). The mastax of *N. glyphura* is of the virgate type and cannot be protruded through the mouth opening.

The taxonomic placement of *Dorria dalecarlica* Myers, 1933 is not completely certain. First recorded by Myers (1933), this species was originally placed within notommatid rotifers. Remane stressed its similarities to dicranophorids, leaving open, however, the question of whether the similarities evolved convergently or are based on close phylogenetic relationship (Remane 1929–1933). Koste (1978) provisionally moved *D. dalecarlica* to the Dicranophoridae. De Smet (1997) followed this suggestion in his monographic treatment of Dicranophoridae, pending more information on the structure of the trophi. Judging from Myers' (1933) original description, the trophi of *D. dalecarlica* appear to be characterized by dorsally-bent rami that deviate from the rami in all other dicranophorid rotifers and show clear similarities to the shape of the rami in species with a virgate mastax and a pumping action. Moreover, the uncus is suggested by Myers to be composed of two teeth ('[...] a single powerful tooth, somewhat clubbed at the tip, followed by a much weaker accessory tooth'). However, recent rediscoveries of *D. dalecarlica* indicate that Myers' original observations were mistaken (personal communication provided by anonymous reviewer): the rami apparently are *not* dorsally-bent but dorso-ventrally flattened and, moreover, what was considered by Myers to be an accessory uncus tooth is actually an uncus apophysis as typically observed in species of the genus *Dicranophorus*. Following these more recent observations, we have decided to code *D. dalecarlica* accordingly.

The character matrix

All data included in the character matrix (see Appendix S1) were assembled using the Nexus format (Maddison et al. 1997) and the Nexus data editor (NDE, version 0.5.0, Page 2001a). The character matrix contains 80 taxa and 59 characters. Forty-nine characters were coded in binary mode; for 10 characters multistate coding was used. Character states in multistate characters were considered unordered. All characters were treated with equal weight and no *a priori* assumptions on character polarization were made. Coding '0' merely denotes a character state among others and does not necessarily represent the plesiomorphic condition. All 59 characters are parsimony informative. The problem of inapplicable data in characters logically dependent on each other (e.g. existence of a certain structure and specific shape of this structure) was approached following Strong and Lipscomb (1999) by introducing inapplicable ('–') character states in characters where, in the absence of a certain structure, specifications of this structure are logically impossible. We consider 'inapplicable coding' the best method for adequately representing in a two dimensional data matrix the hierarchical nature of both characters and taxa nested within each other. Coding generally was done cautiously. In case of uncertainty as to the existence and shape of structures, coding as missing data ('?') was preferred. Details regarding the coding of individual characters are given in the character descriptions.

Description of characters

The coding of characters for the individual species is either based on personal observations or on the literature sources given in Table S1 (in the online version). For an illustration of the different states of a character, individual species are sometimes given ('character state X, type *species name*'). These references to individual species are intended to demonstrate what is meant by a certain character state. Wherever possible, a reference to the figure plates is made to complement the purely linguistic description of characters and states. Since many of the following characters are based on the fine structure of the jaw elements, a consistent terminology is needed that unambiguously identifies relative positions and directions of the jaws. The following
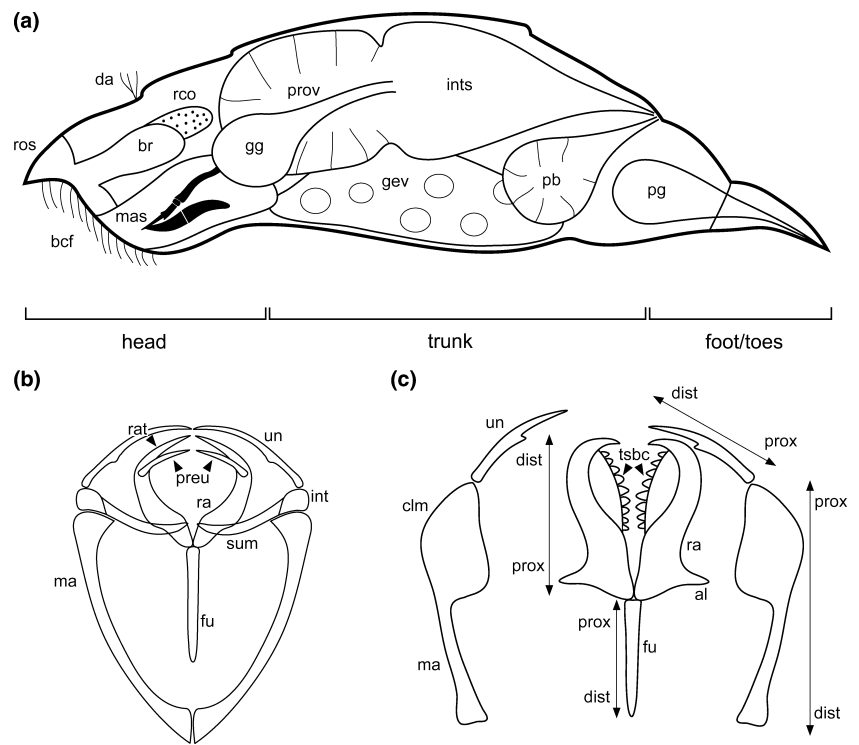
Fig. 1. Body organization, internal anatomy (a) and cuticular jaw elements (b, c) of dicranophorid rotifers, diagrammatic. al, alula; bcf, buccal field; br, brain; clm, manubrial clava; da, dorsal antenna; dist, distal; fu, fulcrum; gev, germovitellarium; gg, gastric glands; int, intramalleus; ints, intestine; ma, manubrium; mas, mastax; pb, protonephridial bladder; pg, pedal glands; preu, pre-uncinal teeth; prov, proventriculus; prox, proximal; ra, ramus; rat, ramus apical tooth; rco, retrocerebral organ; ros, rostrum; tsbc, teeth of ramus sub-basal chamber; un, uncus

terms are used throughout: both the elements of the incus (unpaired fulcrum, paired rami) and the elements of the malleus (paired manubria and paired unci) are at one point connected. This connection serves as a point of reference for the terms proximal and distal referring to relative positions (see Fig. 1c). To identify relative positions in the complete specimen, the terms frontal and caudal are used.

(1) Buccal field extension: 0 = evenly ciliated, caudally extending, ventral buccal field (Fig. 3a, d, e), type *Dicranophorus forcipatus*, 1 = evenly ciliated, oblique buccal field, caudal extension limited (Fig. 2d, e), type *Encentrum mustela*, 2 = ciliation and extension of buccal field very much reduced, type *Albertia naidis*.

This character refers to the structure of the ciliated buccal field, the part of the rotatory organ most strongly developed in dicranophorid rotifers (for the classical description of the elements of the rotatory organ, see De Beauchamp 1907; see also Wallace et al. 2006). Dicranophorid species differ in the caudal extension and orientation of the buccal field. These differences are possibly related to different modes of locomotion.

(2) Rotatory organ with lateral auricles: 0 = absent, 1 = present (Fig. 3e), type *Itura myersi*.

In the rotatory organ of some rotifer species, conspicuous lateral ciliary tufts called auricles are present. These lateral auricles probably represent parts of the circumapical band shifted onto lateral protuberances of the trunk.

(3) Trunk dorso-ventral flattening: 0 = absent, 1 = present, type *Wigrella depressa*.

(4) Trunk integument with lateral sulci: 0 = absent, 1 = present (Fig. 3a, b).

In some species of dicranophorid rotifers, the integument of the trunk is characterized by dorso-laterally and ventro-laterally positioned longitudinal infoldings of the integument traditionally called sulci. Similarly termed infoldings are also present in other taxa across monogonont rotifers as, for example, in *Euchlanis*, *Mytilina* (see Koste 1978) and *Cephalodella* (see Nogrady and Pourriot 1995). However, at present it is very difficult to say whether the sulci are homologous in all taxa.

(5) Trunk with distinct integumentary plates: 0 = absent, 1 = present (Fig. 3a), type *D. forcipatus*.

This character refers to integumentary plates in the trunk that confer a certain amount of rigidity to the integument of species of the genus *Dicranophorus*. Although it is very difficult to quantify stiffness,

it is possible to judge from observations of living specimens whether or not the trunk integument changes its shape when the animals move about and contract. Such observations reveal marked differences between species of the genus *Dicranophorus* and, for example, *Encentrum*.

(6) Trunk with distinct longitudinal ridges: 0 = absent, 1 = present (Fig. 3b), type *Dicranophoroides caudatus*.

Species of the genus *Dicranophoroides* are conspicuous for the numerous longitudinal ridges of the trunk integument visible in the light microscope and, even more apparent, under SEM.

(7) Integument with narrow annulation: 0 = absent, 1 = present (Fig. 2a), type *Parencentrum plicatum*.

Some species of the genera *Encentrum* and *Parencentrum* are characterized by a narrow annulation of the trunk integument. In the past, the presence of transverse (and longitudinal) folds served to assign certain species to the subgenus *Parencentrum* (see De Smet 1997 for details).

(8) Integument with numerous wrinkled folds: 0 = absent, 1 = present, type *Paradicranophorus sordidus*.

(9) Trunk integument with adhering mineral and/or detrital particles: 0 = absent, 1 = present, type *P. sordidus*.

Some species of the genus *Paradicranophorus* are coated with a layer of detrital and/or mineral particles, partly masking the specimen. This is probably due to a sticky substance secreted by the epidermis (Donner 1968).

(10) Rostrum: 0 = present (Fig. 3c), 1 = absent.

In the majority of dicranophorid rotifers, the dorsal epidermis of the rotatory organ is frontally continued by a hyaline, unciliated projection. Morphologically similar projections are present in species of Lepadellidae as well (Koste 1978). In Lepadellidae, the rotatory organ can be retracted into the rigid lorica with the triangular rostrum shielding the rotatory organ from harmful influence from the outside. Whether or not a similar function holds true for the rostrum in dicranophorid rotifers and, moreover, whether or not the rostrum in the two otherwise only distantly related taxa (Sørensen 2002; Sørensen and Giribet 2006) is homologous is unclear.

(11) Rostrum shape: 0 = hook-shaped, curved ventrally (Fig. 3c), type *D. forcipatus*, 1 = blunt, short, type *Myersinella longiforceps*.

This character is coded 'inapplicable' ('–') for species without a rostrum.

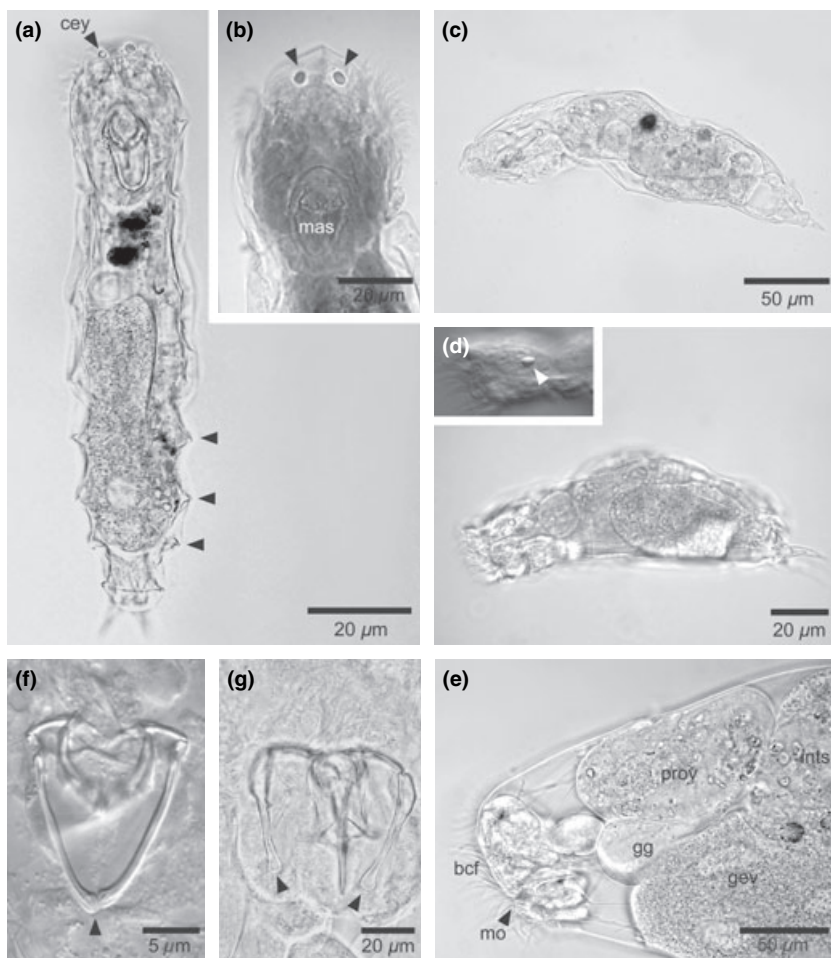(12) Large colourless eyespots at base of rostrum: 0 = absent, 1 = present (Fig. 2a, b), type *P. plicatum*.

Fig. 2. Dicranophorid rotifers and selected details of their organization, light micrographs. (a) *Encentrum saundersiae*, habitus. Arrowheads indicate narrow annulation of trunk integument; (b) *Parencentrum plicatum*, detail of head and mastax. Note colourless eyespots highlighted by arrowheads; (c) *Encentrum lutra*, lateral view; (d) *Encentrum marinum*, lateral view. Inset: *Encentrum marinum*, light refracting body in subcerebral gland indicated; (e) *Encentrum putorius*, detail of frontal section of specimen; (f) *Encentrum rapax*, trophi. Arrowhead points out close connection of distal ends of manubria; (g) *Dicranophorus forcipatus*, trophi. Note that distal ends of manubria are not in close contact. bcf, buccal field; cey, colourless eyespots; gev, germovitellarium; gg, gastric glands; ints, intestine; mas, mastax; mo, mouth opening; prov, proventriculus

In a small number of dicranophorid species of the genera *Encentrum* and *Parencentrum*, there are paired, unpigmented light refracting bodies at the base of the rostrum. Given their position, these structures are apparently distinct from superficially similar looking light refracting structures on either side of the fulcrum (char. 23) or in the subcerebral glands (char. 55).

(13) Cerebral eyespots: 0 = absent, 1 = present, type *Itura myersi*.

(14) Aggregation of red pigment granules caudal to brain: 0 = absent, 1 = present, type *Encentrum diglandula*.

Certain species of the genus *Encentrum* are characterized by red pigment granules caudal to the brain. It is not entirely clear whether they are positioned in the retrocerebral sac or in an appendix to the brain (De Smet 1997). Although their function is unclear, they may be involved in light detection.

(15) Apical palps: 0 = absent, 1 = present, type *Dicranophoroides claviger*.

(16) Jaws protrusible with dorso-ventrally flattened, pincer-shaped rami: 0 = absent, 1 = present.

This character refers to the characteristic grasping movements brought about by the jaws of the forcipate mastax. The jaws can be thrust out of the mouth opening and actively seize the prey. A functionally similar movement is carried out by the jaws of the incudate mastax in species of the genus *Asplanchna* (Koste 1978). However, in the case of the incudate mastax the trophi in their resting position are oriented perpendicular to the fronto-caudal axis of the specimen and are tilted by an angle of 90° when they are extruded. This is in contrast to the grasping movement in dicranophorid rotifers, where the trophi upon extrusion are thrust forward in a simple manner without any tilting.

(17) Jaws overall symmetry: 0 = present, 1 = absent (Figs 4a and 5d, e), type *Aspelta circinator*, 2 = absent, type *Pedipartia gracilis*.

In the majority of dicranophorid species, indeed of all rotifers, the jaw elements are perfectly bilaterally symmetrical. Species of the genus *Aspelta*, however, are remarkable for their asymmetrical trophi. The degree of asymmetry is usually highest in the unci, but also the rami and the manubrial clavae are asymmetrical. *Pedipartia gracilis* is also characterized by strongly asymmetrical jaws and a single, large square alula. However, a character state distinct from *Aspelta* was chosen. Since the original species description of *P. gracilis* is restricted to data obtained from light microscopic observations, it is very difficult to code for many characters and, consequently, the systematic placement of *P. gracilis* is very uncertain.

(18) Fulcrum shape lateral view: 0 = broad, parallel-sided, type *D. forcipatus*, 1 = gradually tapering, elongate triangular, type *A. circinator*, 2 = short, trapezoidal, type *D. caudatus*, 3 = triangular, curved ventrally, type *Encentrum mucronatum*, 4 = base broad, abruptly tapering distally, type *Encentrum marinum*, 5 = almost parallel-sided, short, type *Encentrum villosum*, 6 = long, gradually tapering to blunt distal end, type *Encentrum astridae*, 7 = equilateral triangle, type *P. plicatum*, 8 = short, parallel-sided, type *Paradicranophorus sinus*, 9 = narrow, parallel-sided, type *Inflatana pomazkovae*, 10 = rounded, triangular, type *Myersinella uncodonta*, 11 = narrow, parallel-sided, type *Erignatha clastopis*, 12 = elongate, triangular, type *Albertia vermiculus*, 13 = very narrow, long, type *Glaciera schabetsbergeri*, 14 = long and only very slightly curved ventrally, type *Wierzejskiella velox*.

This character identifies different shapes of the fulcrum seen in lateral view. Although the differences in shape are sometimes small, it is possible to identify distinct states each realized in a limited number of species. A very characteristic and easily recognizable shape is coded as state 3 (Fig. 6e) present in a number of *Encentrum* species.

(19) Fulcrum in dorsal view triangularly expanded: 0 = absent, 1 = present (Fig. 5f), type *D. caudatus*.

(20) Fulcrum distal end knobbed: 0 = absent, 1 = present (Fig. 4c, f), type *Encentrum algente*.

In a number of species of the genus *Encentrum*, the distal end of the fulcrum in dorsal and ventral view is conspicuously widened. Unlike in
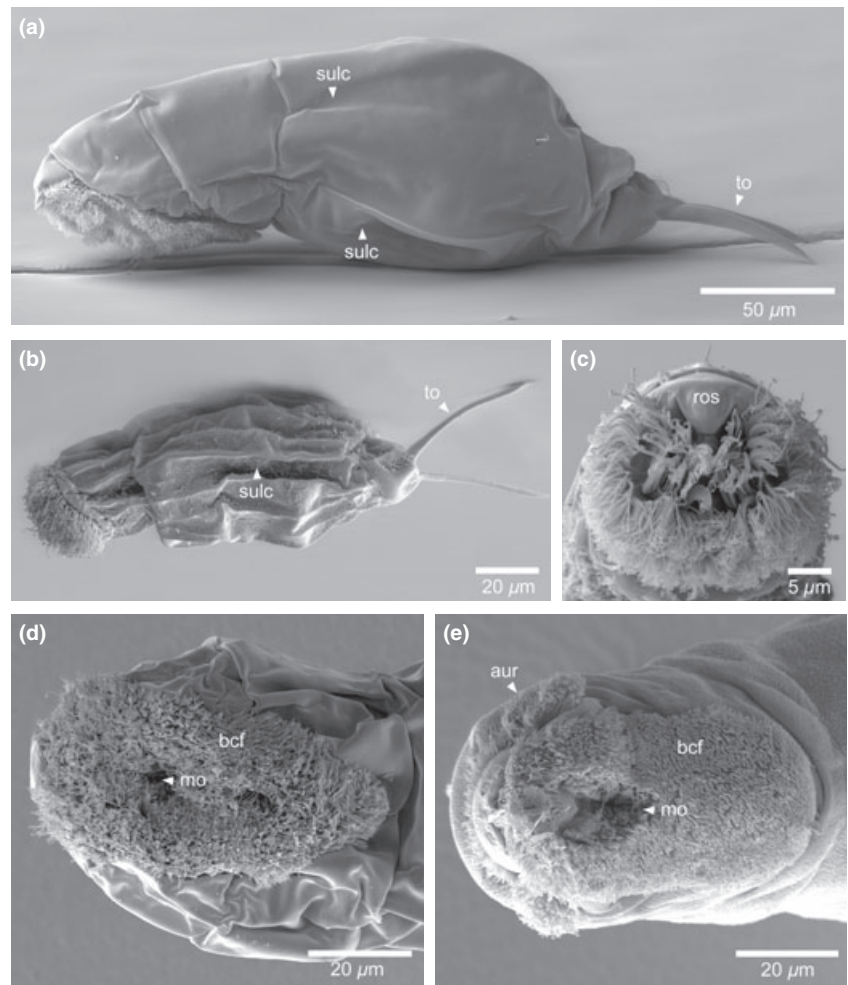
Fig. 3. Dicranophorid rotifers (a-d) and selected details of their external organization, scanning electron micrographs. (a) *Dicranophorus forcipatus*, lateral view; (b) *Dicranophoroides caudatus*, lateral view; (c) *Parencentrum plicatum*, frontal view; (d) *Dicranophorus forcipatus*, ventral view of buccal field; (e) *Notommata glyphura*, ventral view of buccal field. aur, auricle; bcf, buccal field; mo, mouth opening; ros, rostrum; sulc, sulcus; to, toes

Dicranophoroides (char. 19), however, where the fulcrum is triangularly expanded across its whole length, the expansion of the fulcrum to which character 20 refers is limited to the distal end.

(21) Fulcrum distally split into dorsal and ventral part: 0 = absent, 1 = present, type *Aspelta clydona*.

In SEM images of the fulcrum in some *Aspelta* species, the fulcrum appears to be split lengthwise into a stronger ventral and a smaller dorsal part (De Smet 1997).

(22) Fulcrum very short relative to ramus: 0 = absent, 1 = present, type *Dicranophorus halbachi*.

(23) Light refracting bodies on either side of fulcrum: 0 = absent, 1 = present, type *Encentrum martes*.

In some species of the genus *Encentrum*, conspicuous light refracting bodies are present on either side of the fulcrum, the nature of which is uncertain. Unfortunately, there seems to be intraspecific variability in the presence or absence of this character (De Smet 1997). As a consequence, some species may falsely be coded absent for this character when, in fact, light refracting bodies do exist in some individuals of these species. However, to maximize the information content of the data matrix, this character has been included all the same.

(24) Unci teeth number: 0 = one major and one to several accessory teeth (Fig. 7e), type *N. glyphura*, 1 = a single tooth, type *D. forcipatus*, 2 = two delicate teeth, type *G. schabetsbergeri*.

While in the outgroup representatives one major and one to several smaller unci teeth are present, there is usually only one uncus tooth in dicranophorid rotifers. *Glaciera schabetsbergeri* is characterized by two long, delicate and slightly curved teeth. They differ considerably from the firmly fused unci teeth in *N. glyphura* (Fig. 7e) and *I. myersi* and are probably autapomorphic for *G. schabetsbergeri* (see Jersabek 1999).

(25) Uncus with vestigial jugal lines: 0 = present (Fig. 5c), type *D. forcipatus*, 1 = absent.

In SEM images of the uncus in rotifers, the exact number of teeth united in a functional unit can very often be deduced from the number of jugal lines running as fine depressions along the length of the uncus. Multiple jugal lines corresponding to multiple uncus teeth are particularly obvious in rotifers with a malleate and virgate mastax (Sørensen 2002). In species of the genera *Dicranophorus* and *Aspelta* with only one uncus tooth, there is one major and one to several vestigial jugal lines in the uncus, indicating that the single-toothed uncus common in dicranophorid rotifers possibly resulted from a reduction in the number of uncus teeth compared to the outgroup (*N. glyphura*: Fig. 7e, *L. tecusa*: De Smet 2005; see also Markevich and Kutikova 1989 for conceivable transformation series).

(26) Manubrium shape clava: 0 = elongate chamber with median opening, accessory chambers with conspicuous lamellae (Fig. 5a, b), type *D. forcipatus*, 1 = lamellae minute triangular processes, type *Dicranophorus robustus*, 2 = accessory chambers triangular lamellae with acute projections (Fig. 5f), type *D. caudatus*, 3 = elongate chamber with median opening, accessory chambers reduced, clava club-shaped, (Fig. 5d, e), type *A. circinator*, 4 = strongly elongate clava without accessory chambers, type *A. vermiculus*, 5 = accessory chambers inconspicuous triangular lamellae (Fig. 6b,c), type *E. mucronatum*, 6 = with hook-shaped, distal process (Fig. 7f), type *L. tecusa*.

This character identifies different shapes in the manubrial clavae. While the basic organization of the manubrium is similar in all dicranophorid rotifers (cauda represented by the distally elongate median manubrial chamber, accessory manubrial chambers reduced to flattened lamellae, see De Smet 1997; Sørensen 2002), there are different degrees of reduction of the accessory chambers.
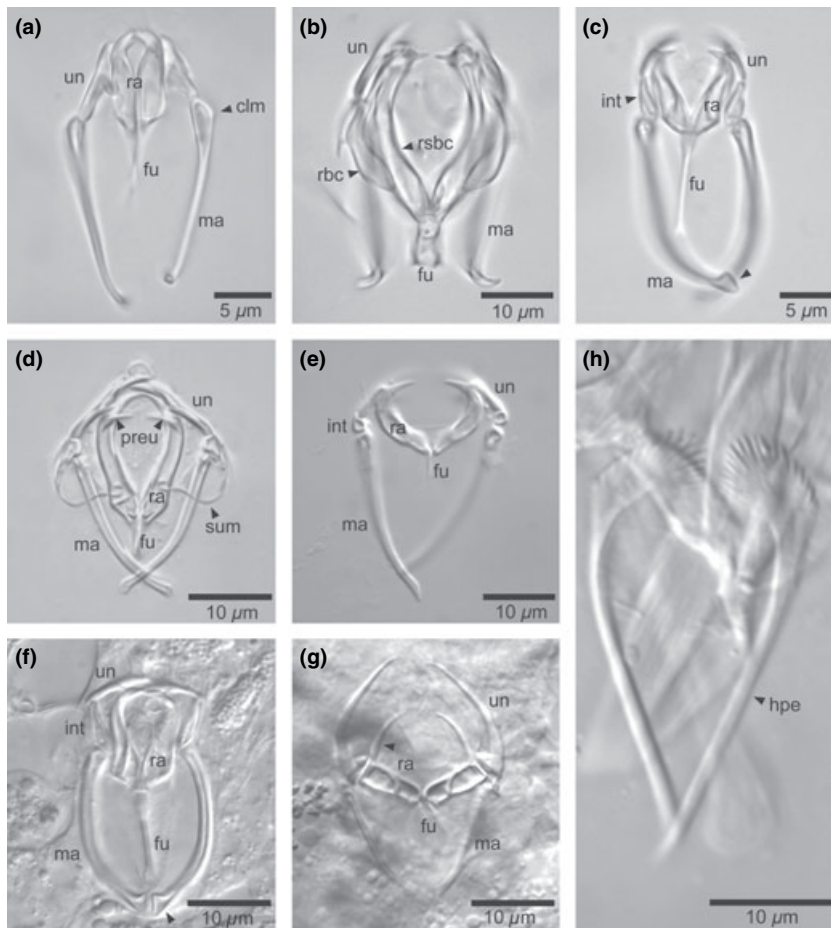
Fig. 4. Cuticularized jaw elements (trophi) of dicranophorid rotifers, light micrographs. (a) *Aspelta circinator*; (b) *Dicranophoroides caudatus*; (c) *Encentrum marinum*. Note the crutched distal end of the manubrium indicated by an arrowhead; (d) *Encentrum uncinatum*; (e) *Encentrum martes*; (f) *Encentrum algente*. Crutched distal end of manubrium indicated by arrowhead; (g) *Erignatha clastopis*. Arrowhead indicates distal end of ramus set at right angle to proximal ramus section; (h) *Dicranophorus forcipatus*, detail of jaw apparatus with hypopharyngeal elements. clm, manubrial clava; fu, fulcrum; hpe, hypopharyngeal element; int, intramalleus; ma, manubrium; preu, preuncinal teeth; sum, supramanubrium; ra, ramus; rbc, ramus basal chamber; rsbc, ramus subbasal chamber; un, uncus

(27) Manubrial distal end spatulate: 0 = absent, 1 = present (Fig. 5b), type *D. forcipatus*.

In species of the genus *Dicranophorus* the distal end of the manubrium is very often expanded forming a spatulate structure.

(28) Manubrial distal end unidirectionally crutched: 0 = absent, 1 = present (Fig. 6g, e), type *E. lutra*.

(29) Manubrial distal end bidirectionally crutched: 0 = absent, 1 = present (Figs 4c, f and 6c), type *E. marinum*.

Characters 28 and 29 distinguish two different shapes of the distal end of the manubrium. While in, for example, *E. lutra* (Fig. 6g) the distal end of the manubrium is expanded only in the caudal direction, it is drawn out in both frontal and caudal direction in *E. marinum* (Fig. 6c).

(30) Relative position of distal manubria ends: 0 = not in close contact (Fig. 2g), 1 = in close contact (Figs 2f and 4f), type *E. algente*.

This character refers to the relative position of the distal ends of the manubria. Two markedly different states can be distinguished: (i) a situation where the distal ends of the manubria are at some distance from each other and (ii) a situation with the distal ends of the manubria kept in close contact, even during a forward thrust of the whole jaw apparatus. These differences probably reflect different degrees of relative independence of the manubria and hint at differences in the exact mode of prey seizure (see Discussion).

(31) Frontal section of rami curved dorsally: 0 = absent, 1 = present, type *N. glyphura*.

While in dicranophorid rotifers with a grasping (forcipate) mastax the rami are pincer-shaped and dorso-ventrally flattened, they are characteristically arched dorsally in rotifer species whose mastax works as a sucking device (virgate mastax).

(32) Lateral margins of rami slightly concave, apical teeth strongly incurved: 0 = absent, 1 = present (Fig. 5a), type *D. forcipatus*.

(33) Ramus basal chambers displaced laterally: 0 = absent, 1 = present (Figs 4b and 5f), type *D. caudatus*.

This character highlights the conspicuous arrangement of the ramus subbasal and the ramus basal chamber in species of the genus *Dicranophoroides*. Unlike in all other dicranophorid rotifers, the ramus basal chambers in *Dicranophoroides* are strongly shifted fronto-laterally and the longitudinal axis of the rami is exclusively formed by the ramus subbasal chambers.

(34) Rami with narrow, frontally displaced median space and hook-shaped apical teeth: 0 = absent, 1 = present (Figs 4a and 5d, e), type *A. circinator*.

(35) Rami outer margin more or less convex, space between rami wide: 0 = absent, 1 = present (e. g. Fig. 4e), type *E. mustela*.

The inner margins of the rami in species of the genus *Aspelta* are in close contact over much of their length. Only very frontally do they leave a space between them. This contrasts markedly with the situation in most species of the genus *Encentrum*, where the rami are curved laterally with their outer margins convex and a wide space between the rami.

(36) Distal half of rami bent inwards at right angle: 0 = absent, 1 = present (Figs 4g and 5g), type *E. clastopis*.

(37) Rami basal chamber distally elongated: 0 = absent, 1 = present, type *P. sinus*.

This character refers to the conspicuously elongated ramus basal chambers relative to the ramus subbasal chambers in species of the genus *Paradicranophorus*.

(38) Rami straight with uncurved blunt tip: 0 = absent, 1 = present, type *Streptognatha lepta*.

While the rami in almost all dicranophorids distally terminate in a more or less pronounced and incurved tooth, they are straight and with a blunt, completely uncurved distal end in *S. lepta* and *G. schabetsbergeri*.

(39) Rami attached to unci by cuticular bridge: 0 = absent, 1 = present, type *A. vermiculus*.

Scanning electron microscopic preparations demonstrate the presence of a fine cuticular bridge connecting the rami and unci in species of the parasitic genus *Albertia* (De Smet 1997).
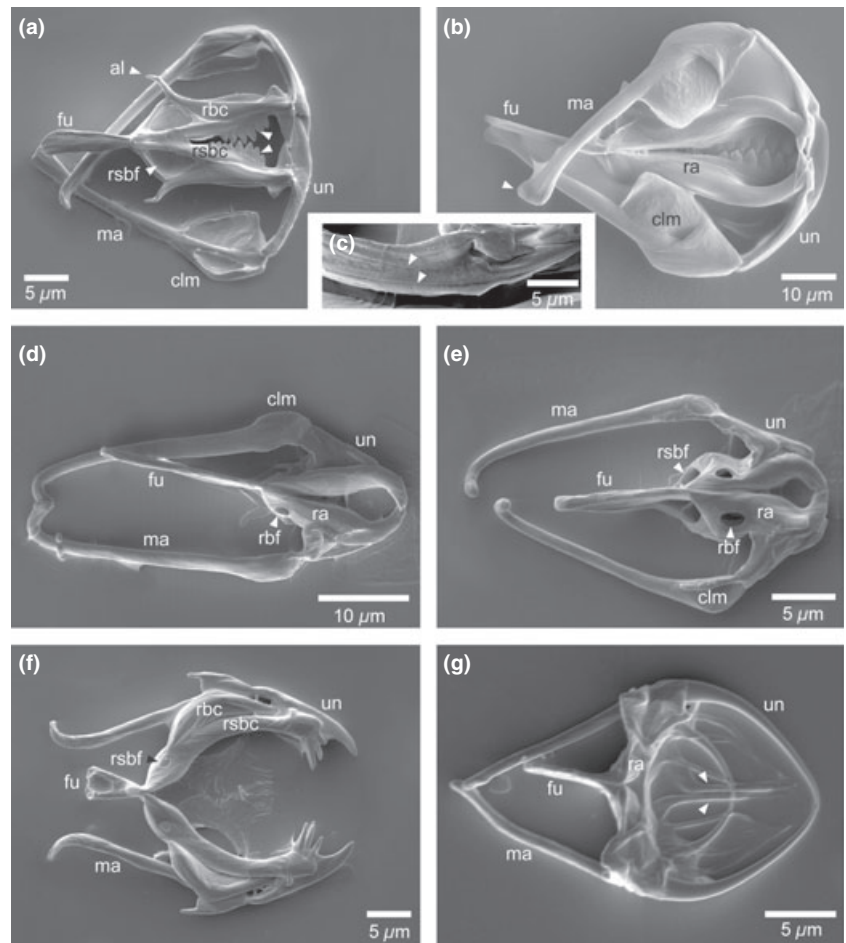
Fig. 5. Cuticularized jaw elements (trophi) of dicranophorid rotifers, scanning electron micrographs. (a) *Dicranophorus luetkeni*, vental view. Note teeth on inner margin of ramus subbasal chamber indicated by arrowheads; (b) *Dicranophorus forcipatus*, dorsal view. Arrowhead indicates distally spatulate manubrium; (c) *Dicranophorus forcipatus*, frontal view of uncus tooth. Arrowheads point out jugal lines; (d) *Aspelta angusta*, dorsal view; (e) *Aspelta circinator*, dorsal view; (f) *Dicranophoroides caudatus*, ventral view; (g) *Erignatha clastopis*, dorsal view. Note the narrow supramanubria projecting frontally. al, alula; clm, manubrial clava; fu, fulcrum; ma, manubrium; ra, ramus; rbc, ramus basal chamber; rbf, ramus basifenestra; rsbc, ramus subbasal chamber; rsbf, ramus subbasifenestra; un, uncus

(40) Ramus subbasifenestra: 0 = pointing ventro-laterally, type *D. forcipatus* (Figs 5a, f), 1 = pointing caudally (Figs 5e and 6d), type *E. mustela*.

(41) Ramus basifenestra: 0 = pointing laterally (Fig. 5a), type *D. forcipatus*, 1 = pointing dorsally (Figs 5d, e and 6b), type *E. mucronatum*, 2 = pointing dorsally, positioned at base of alulae (Fig. 7d), type *I. myersi*.

The ramus subbasal chamber and the ramus basal chamber have distinct openings visible under SEM. These openings are called subbasifenestra (pl. subbasifenestrae) and basifenestra (pl. basifenestrae) respectively (Markevich 1989; Sørensen 2002). Characters 40 and 41 distinguish different orientations of these openings.

(42) Ramus subbasal chamber teeth: 0 = present (Fig. 5a), 1 = absent.

In many taxa of monogont rotifers, the inner margin of the ramus subbasal chamber is studded with interlocking teeth (Koste 1978; Sørensen 2002). Such teeth are only present in some dicranophorid rotifer species.

(43) Ramus subbasal chamber teeth shape: 0 = strong shearing teeth (Fig. 5a, b), type *D. forcipatus*, 1 = distally displaced compound projection of few teeth, type *D. kostei*, 2 = single tooth on one, few teeth on other subbasal chamber, type *D. haueri*, 3 = fine, sharp teeth in most distal section of subbasal chamber (Fig. 5f), type *D. caudatus*, 4 = coarsely denticulate (Fig. 7e), type *N. glyphura*.

This character is coded 'inapplicable' ('–') for species where ramus subbasal chamber teeth are absent.

(44) Alulae: 0 = present, evenly curved, type *D. forcipatus* (Figs 5a and 7d, f), 1 = present, large, asymmetrical, on one ramus only, type *P. gracilis*, 2 = asymmetrical, on only one ramus, type *A. clydona*, 3 = absent.

In the jaws of many rotifer species ventro-lateral appendages of the rami are present. They serve as sites for muscle attachment of the jaw musculature (De Smet 1997; Riemann and Ahlrichs 2008).

Closer examination reveals that what is uniformly called 'alula' is structurally different. In species of the genus *Dicranophorus* as well as in the outgroup representatives *I. myersi*, *N. glyphura* and *L. tecusa*, the alulae are curved projections of the ramus basal chamber. Available data on *Aspelta* indicates that the alulae, where present, derive from the ramus subbasal chamber (De Smet 1997). The single alula in *P. gracilis* considerably deviates in size and position from all other alulae reported. However, SEM data is needed for clarification.

(45) Rod-shaped hypopharyngeal elements: 0 = absent, 1 = present (Fig. 4h), type *D. forcipatus*.

In some species of the genus *Dicranophorus*, more or less rod-shaped hypopharyngeal elements are present ventral to the rami as accessory elements of the mastax. Their homology to other cuticular formations, collectively called epipharynges (Sørensen 2002), is uncertain (see Riemann and Ahlrichs 2008).

(46) Intramalleus: 0 = absent, 1 = present (Figs 4c, f and 6c).

In a large number of dicranophorid rotifers, this additional element of the malleus, inserted between the manubrial clava and the uncus, is present.

(47) Supramanubrium: 0 = absent, 1 = present (Figs 4d and 6g).

In many species with an intramalleus, the intramalleus bears a variously shaped, medially projecting process called supramanubrium.

(48) Supramanubrium shape: 0 = narrow and distally hooked (Fig. 7b), type *W. velox*, 1 = evenly curved and tapering (Fig. 6g), type *E. mucronatum*, 2 = short process of intramalleus (Fig. 6c), type *E. marinum*, 3 = long, projecting frontally (Fig. 5g), type *E. clastopis*, 4 = rounded (Fig. 4d), type *P. sinus*, 5 = spiniform, type *Kostea wockei*, 6 = triangular, type *E. saundersiae*, 7 = short, triangular, type *Inflatana pomazkovae*.

This character is coded 'inapplicable' ('–') for species without a supramanubrium.

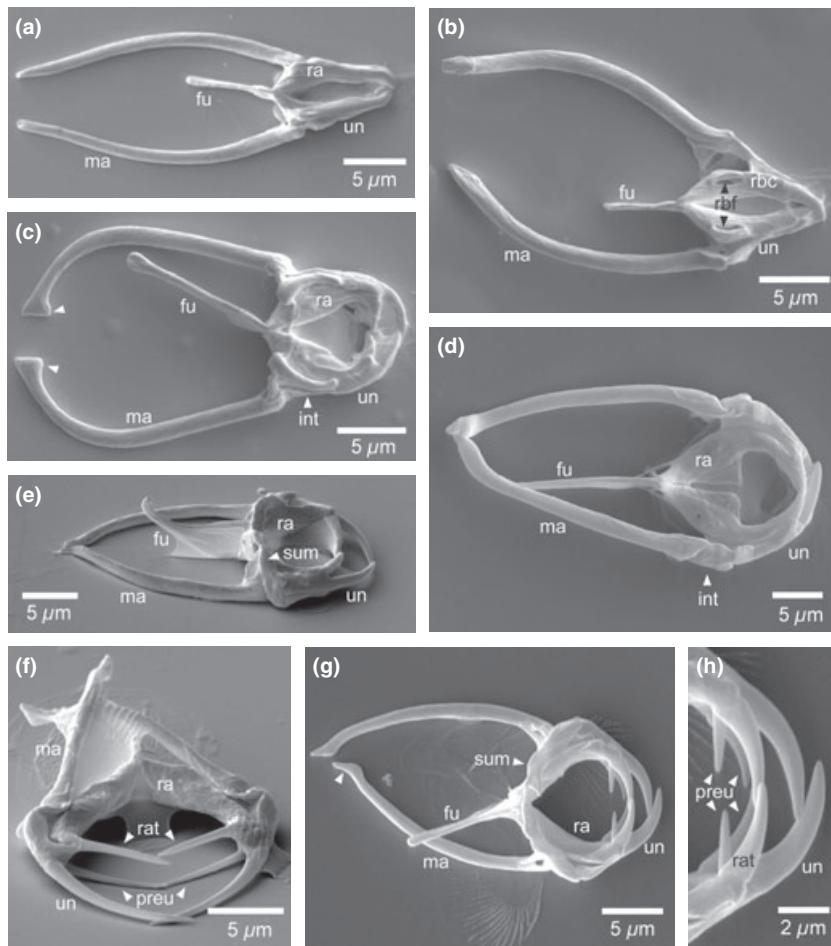(49) Preuncinal teeth: 0 = absent, 1 = present (Figs 4d and 6f, h).

Fig. 6. Cuticularized jaw elements (trophi) of dicranophorid rotifers, scanning electron micrographs. (a) *Encentrum diglandula*, dorsal view; (b) *Encentrum villosum*, dorsal view; (c) *Encentrum marinum*, ventral view. Note bidirectionally crutched distal end of manubrium indicated by arrowheads; (d) *Encentrum mucronatum*, dorsal view; (e) *Encentrum martes*, ventro-lateral view. Note ventrally curved fulcrum; (f) *Encentrum mustela*, frontal view; (g) *Encentrum lutra*, ventral view. Note unidirectionally crutched distal end of manubrium indicated by arrowheads; (h) *Encentrum lutra*, close up of (g) showing teeth involved in grasping of prey. fu, fulcrum; int, intramalleus; ma, manubrium; preu, preuncinal teeth; ra, ramus; rat, ramus apical tooth; rbc, ramus basal chamber; rbf, ramus basifenestra; sum, supramanubrium; un, uncus

The rami of a fairly large number of dicranophorid rotifers bear one or two pairs of accessory teeth at their tips, called preuncinal teeth. Judging from SEM preparations, the preuncinal teeth seem to be derivatives of the ramus basal chamber.

(50) Preuncinal teeth shape: 0 = accessory ramus teeth without caudo-lateral process (Fig. 6h), type *E. mucronatum*, 1 = accessory ramus teeth with conspicuous process pointing caudo-laterally, type *Myersinella longiforceps*.

This character is coded 'inapplicable' ('–') for species without preuncinal teeth.

In *M. uncodonta* and *M. longiforceps*, the rami are remarkable for their caudo-lateral processes. However, according to De Smet (2007), these processes can be presumed to be extensions of modified preuncinal teeth.

(51) Proventriculus: 0 = absent, 1 = present (Figs 1a and 2e), type *E. mustela*.

In a number of dicranophorid rotifers, the gastric tract is modified by the presence of a dilatable, hyaline section between the oesophagus and the stomach called proventriculus. At the junction of the proventriculus and stomach, the gastric glands discharge their contents into the gastric lumen.

(52) Gastric glands stalked: 0 = absent, 1 = present (Figs 1a and 2e), type *E. mustela*.

(53) Massive gastric glands distorting shape of trunk: 0 = absent, 1 = present, type *A. vermiculus*.

In species of the parasitic genera *Albertia* and *Balatro*, the gastric glands are very strongly developed and inflate the lateral sides of the trunk.

(54) Connection gastric glands and mastax: 0 = absent, 1 = present, type *E. mustela*.

In some species of the genus *Encentrum*, the gastric glands are apparently connected to the mastax by fine ligaments.

(55) Light refracting bodies in subcerebral glands: 0 = absent, 1 = present (Fig. 2d), type *E. marinum*.

(56) Position of foot: 0 = in continuation of trunk, 1 = shifted ventrally, type *P. sinus*.

(57) Distal segments of foot telescopically retractable: 0 = absent, 1 = present, *W. velox*.

In some species of the genus *Wierzejskiella*, the distal segments of the foot can be telescoped into each other, in effect strongly reducing the length of the foot.

(58) Toes: 0 = present, 1 = strongly reduced, type *A. vermiculus*.

(59) Toes shape: 0 = sword–shaped, long (Fig. 3a, b), type *D. forcipatus,* 1 = slender, needle–shaped, type *A. circinator,* 2 = long and strongly curved ventrally, type *W. depressa*, 3 = very slender, acutely pointed, type *K. wockei*, 4 = long and slender, slightly curved, tapering distally, type *E. clastopis*, 5 = laterally compressed, type *D. dalecarlica,* 6 = short and more or less conical (Fig. 2c, d), type *E. mucronatum*, 7 = papilliform, short, type *L. tecusa*.

Cladistic analysis

The maximum parsimony analysis of the data set was carried out in PAUP* 4.0 b10 (Swofford 2002). Given the dimensions of the data matrix, a heuristic search strategy was adopted with the branch swapping algorithm tree bisection reconnection and 1000 replicates with random addition sequence of taxa. No assumptions on outgroup status were defined in PAUP. The resulting topologies were rooted against *N. glyphura*. For convenient visualization of the trees, the program TREE VIEW (Page 2001b) was used. For the analysis of character evolution, we drew on MACCLADE 4.0 (Maddison and Maddison 2000) and the 'trace character' function for individual characters. To obtain a full list of character transformations, an

Fig. 7. Cuticularized jaw elements (trophi) of dicranophorid rotifers (a, b) and outgroup representatives (c–f), scanning electron micrographs. (a) *Parencentrum plicatum*, ventral view; (b) *Wierzejskiella velox*, ventral view; (c) *Notommata glyphura*, caudo-lateral view. Note the lamellar appearance of the manubrial clava and the slit-shaped openings of the manubrial accessory chambers; (d) *Itura myersi*, dorso-lateral view; (e) *Notommata glyphura*, frontal view. Note that there is one major and several minor, oblique uncus teeth. Arrowheads indicate jugal lines of minor uncus teeth; (f) *Lindia tecusa*, dorsal view. Arrowheads point out hook-shaped processes of manubrium. al, alula; clm, manubrial clava; fu, fulcrum; int, intramalleus; ma, manubrium; ra, ramus; rbf, ramus basifenestra; rsbf, ramus subbasifenestra; tsbc, teeth of ramus subbasal chamber; un, uncus
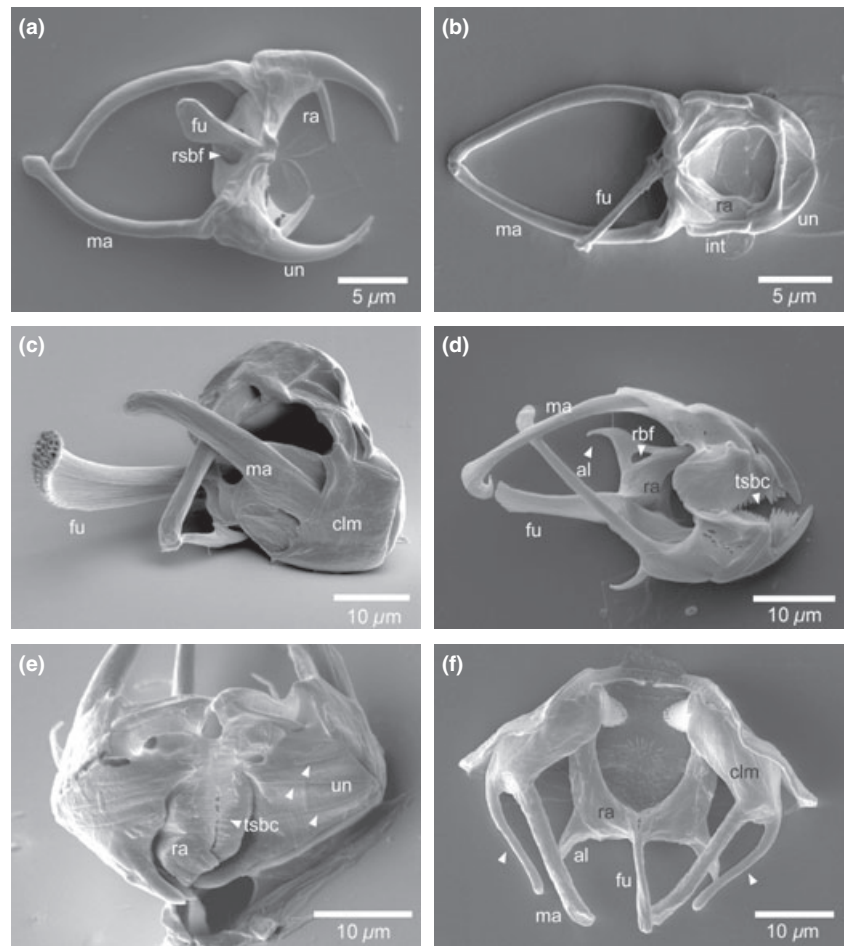
apomorphy list was generated in PAUP (based on 'ACC–TRAN' character optimization). To assess the node robustness of the resulting topology, a bootstrap analysis (Felsenstein 1985) with 1000 replicates was run. Additionally, we calculated Bremer support indices (Bremer 1988) for the individual nodes using a parsimony ratchet approach based on the program PRAP (Müller 2004).

Remark on nomenclature

Our study intends to reconstruct the evolutionary pathways within the monogonont rotifer taxon Dicranophoridae. It identifies character transformations and monophyletic subtaxa of Dicranophoridae. Names introduced in this study and assigned to these subtaxa derive from characteristic apomorphic features that evolved in their respective stem lineages.

Results

The PAUP search with the above search parameters yielded 1371 equally parsimonious trees with a tree length of 111 steps, consistency index of 0.8829 and retention index of 0.9749. The 50% majority rule consensus tree of all equally parsimonious trees is given in Fig. 8, the strict consensus tree in Fig. 9. In the 50% majority rule consensus tree (Fig. 8), bootstrap support values above 50% are indicated above the black numbered squares representing apomorphy boxes for the stem lineages of individual monophyletic clades. Bremer support values are given below the numbered squares. In the strict consensus tree (Fig. 9), the support statistics are given above (bootstrap) and below (Bremer) the branches of the corresponding nodes. The following description of phylogenetic relationships is based on

the 50% majority rule consensus tree (Fig. 8). Not all of the resulting sister group relationships, however, are reflected in the strict consensus tree (Fig. 9).

The monophyly of Dicranophoridae is supported by two unambiguous character transformations (#16, $0 \rightarrow 1$; #24, $0 \rightarrow 1$). The statistical support values for a monophyletic taxon Dicranophoridae are a bootstrap support of 96% and a Bremer support index of 3. The monophyletic taxon *Dicranophorus* + monotypic *Dorria* is the sister group of all other dicranophorid rotifers. Within Dicranophoridae, monophyletic *Dicranophoroides* is the sister group of the large taxon Caudosubbasifenestrata. Caudosubbasifenestrata is supported by two unambiguous character transformations (#40, $0 \rightarrow 1$; #42, $0 \rightarrow 1$). The support statistics for Caudosubbasifenestrata is a bootstrap support of 67%. The most basal clade within Caudosubbasifenestrata is the monophyletic taxon *Aspelta* supported by two unambiguous character transformations (#17, $0 \rightarrow 1$; #34, $0 \rightarrow 1$) and statistical support of a bootstrap of 94% and a Bremer support index of 2. Within Caudosubbasifenestrata, *P. gracilis* is the sister taxon of the large monophylum Intramalleata supported by two unambiguous character transformations (#46, $0 \rightarrow 1$; #30, $0 \rightarrow 1$), a bootstrap support of 79% and a Bremer support index of 2. Praeuncinata is a large monophyletic taxon nested within Intramalleata. It is supported by one unambiguous character transformation (#49, $0 \rightarrow 1$), a bootstrap support of 63% and a Bremer support index of 1. Its sister group is a monophyletic taxon comprising *E. clastopis* + *Erignatha longidentata* and *G. schabetsbergeri* + *S. lepta*. Internal resolution within

Praeuncinata, however, is only very poor. The largest clade within Praeuncinata is the monophyletic taxon Proventriculata supported by two unambiguous character transformations (#51, 0 → 1; #52, 0 → 1) in its stem lineage.

Discussion

Monophyly of Dicranophoridae

In the most recent phylogenetic analysis of Rotifera (Sørensen and Giribet 2006) employing morphological characters and

DNA sequence data, the monophyly of Dicranophoridae is supported based on morphological characters. *Dicranophorus forcipatus* is the basal sister taxon of a clade comprising *E. astridae* and *E. tectipes*. Although only three species are included, such a scenario corresponds well to the results of our analysis. When sequence data are used, either as the only data source or in a combined analysis including also morphological characters, monophyly of Dicranophoridae turns out to be questionable (Sørensen and Giribet 2006). However, branch support in this combined analysis is limited and with only three

dicranophorid species included, the taxon sampling is very severely restricted. Thus, dismissal of dicranophorid monophyly based on molecular data would certainly as yet be premature.

From a morphological perspective, dicranophorid rotifers are convincingly supported as a monophyletic taxon based on the two unambiguous apomorphies (i) protrusible jaw apparatus with dorso-ventrally flattened rami and (ii) presence of a single uncus tooth (see Fig. 8: apomorphy box 1). Dorso-ventrally flattened rami are present in the outgroup representatives *L. tecusa* (see De Smet 2005) and *I. myersi* (see Pourriot 1997) as well, but the jaw apparatus cannot be extruded in these taxa. Dorso-ventrally flattened rami are also present in members of the planktonic genus *Asplanchna*, where they can be thrust out of the mouth opening to capture prey (Koste 1978; Walsh et al. 2005). However, closer inspection reveals that the modes of jaw protrusion in Dicranophoridae and Asplanchnidae are considerably different. While in the incudate mastax of Asplanchnidae the trophi in their resting position are oriented perpendicular to the fronto-caudal axis of the animal and tilted by an angle of 90° when they are thrust out of the mouth opening, they are positioned parallel to the body axis in the forcipate mastax of Dicranophoridae and are protruded without any tilting movement. These differences suggest that the two superficially similar modes of prey capture evolved convergently in the omnivorous, planktonic Asplanchnidae and the omnivorous or carnivorous, benthic Dicranophoridae. Such a conclusion is also supported by the fact that previous phylogenetic analyses do not reveal a closer phylogenetic relationship of Asplanchnidae and Dicranophoridae (Sørensen 2002).

Apart from a protrusible, dorso-ventrally flattened jaw apparatus, the monophyly of Dicranophoridae rests on the presence of a single uncus tooth evolved in their stem lineage. Within Dicranophoridae, the number of uncus teeth has apparently undergone secondary modifications in taxa such as, for example, the isolated *G. schabetsbergeri*, where two evenly curved, extremely slender uncus teeth are present (Jersabek 1999). A comparable process of secondary multiplication in the number of uncus teeth may also have occurred in some insufficiently known species provisionally placed in the genus *Dicranophorus*, for which, unfortunately, no SEM preparations of the trophi exist (e. g. *Dicranophorus biastis* and *D. grypus*, see De Smet 1997). In the outgroup representatives chosen for our analysis (*N. glyphura*, *L. tecusa* and *I. myersi*), two or several uncus teeth are present. *Notommata glyphura* is characterized by a virgate (sucking) mastax with dorsally arched rami and one major and several accessory uncus teeth whose presence is indicated in SEM preparations by oblique jugal lines dorsal to the major uncus tooth (see also Sørensen 2002). The jaws of *L. tecusa* bear an uncus plate with the individual teeth tightly bound together and acting as a

Fig. 8. Phylogenetic relationships of Dicranophoridae (77 taxa) and three outgroup representatives based on 59 parsimony informative characters. Fifty per cent majority rule consensus tree of 1371 equally parsimonious trees with a length of 111 steps. Numbers in black squares refer to apomorphy blocks specified below. Numbers above black squares indicate bootstrap support values, numbers below squares Bremer support indices. In the following, character transformations selected from an apomorphy list generated in PAUP are given. Whenever possible, unambiguous transformations have been chosen. Ambiguous transformations are indicated in italics. Numbers () refer to characters introduced in the character description. **(1)** Jaws protrusible with dorso-ventrally flattened, pincer-shaped rami (#16): present; Unci teeth number (#24): a single tooth, type *Dicranophorus forcipatus*. **(2)** Ramus alulae (#44): absent; *Ramus basifenestra (#41): pointing dorsally, type Encentrum mucronatum.* **(3)** Ramus subbasifenestra (#40): pointing caudally, type *Encentrum mustela*; Ramus subbasal chamber teeth (#42): absent. **(4)** Rami outer margin more or less convex, space between rami wide (#35): present, type *Encentrum mustela*. **(5)** *Manubrium clava shape (#26): accessory chambers inconspicuous triangular lamellae, type Encentrum mucronatum.* **(6)** Relative position of distal manubria ends (#30): in close contact, type *Encentrum algente*; Intramalleus (#46) present. **(7)** Buccal field extension (#1): evenly ciliated, oblique buccal field, caudal extension limited, type *E. mustela*. **(8)** Supramanubrium (#47): present **(9)** Preuncinal teeth (#49): present. **(10)** Proventriculus (#51): present, type *E. mustela*; Gastric glands stalked (#52): present, type *E. mustela*. **(11)** Connection gastric glands and mastax (#54): present, type *E. mustela*. **(12)** Manubrial distal end unidirectionally crutched (#28): present, type *E. lutra*. **(13)** Light refracting bodies on either side of fulcrum (#23): present, type *Encentrum martes*. **(14)** *Lateral margins of rami slightly concave, apical teeth strongly incurved (#32): present, type D. forcipatus; Manubrial distal end spatulate (#27): present, type D. forcipatus.* **(15)** Trunk integument with lateral sulci (#4): present; Trunk with distinct integumentary plates (#5): present, type *D. forcipatus*. **(16)** Rod-shaped hypopharyngeal elements (#45): present, type *D. forcipatus*. **(17)** Fulcrum very short relative to ramus (#22): present, type *Dicranophorus halbachi*; Ramus subbasal chamber teeth shape (#43): frontally displaced compound projection of few teeth, type *Dicranophorus kostei*. **(18)** Ramus subbasal chamber teeth shape (#43): single tooth on one, few teeth on other subbasal chamber, type *Dicranophorus haueri*. **(19)** Trunk with distinct longitudinal ridges (#6): present, type *Dicranophoroides caudatus*; Apical palps (#15): present, type *Dicranophoroides claviger*; Fulcrum in dorsal view triangularly expanded (#19): present, type *D. caudatus*; Ramus basal chambers displaced laterally (#33): present, type *D. caudatus*. **(20)** Jaws overall symmetry (#17): absent, type *Aspelta circinator*; Rami with narrow, frontally displaced median space and hook-shaped apical teeth (#34): present, type *A. circinator*. **(21)** Fulcrum distally split into dorsal and ventral part (#21): present, type *Aspelta clydona*; Alulae (#44): asymmetrical, on only one ramus, type *A. clydona*. **(22)** Buccal field extension (#1): ciliation and extension of buccal field very much reduced, type *Albertia naidis*; Massive gastric glands distorting shape of trunk (#53): present, type *Albertia vermiculus*; Toes (#58): strongly reduced, type *A. vermiculus*. **(23)** Rami attached to unci by cuticular bridge (#39): present, type *A. vermiculus*. **(24)** Trunk dorso-ventral flattening (#3): present, type *Wigrella depressa*. **(25)** Aggregation of red pigment granules caudal to brain (#14): present, type *E. diglandula*. **(26)** Toes shape (#59): very long and slender, slightly curved, tapering distally, type *Erignatha clastopis*. **(27)** Distal half of rami bent inwards at right angle (#36): present, type *E. clastopis*. **(28)** Rami straight with uncurved blunt tip (#38): present, type *Streptognatha lepta*. **(29)** Supramanubrium shape (#48): narrow and distally hooked, type *Wierzejskiella velox*; Distal segments of foot telescopically retractable (#57): present, *W. velox*. **(30)** Integument with narrow annulation (#7): present, type *Parencentrum plicatum*; Large colourless eyespots at base of rostrum (#12): present, type *P. plicatum*; Fulcrum shape lateral view (#18): equilateral triangle, type *P. plicatum*. **(31)** Fulcrum shape lateral view (#18): short, parallel sided, type Paradicranophorus sinus; *Supramanubrium shape (#48): rounded, type P. sinus.* **(32)** Integument with numerous wrinkled folds (#8): present, type *Paradicranophorus sordidus*; Position of foot (#56): shifted ventrally, type *P. sinus*. **(33)** Trunk integument with adhering mineral and/or detrital particles (#9): present, type *P. sordidus*; Rami basal chamber distally elongated (#37): present, type *P. sinus*. **(34)** Fulcrum shape lateral view (#18): base broad, abruptly tapering distally, type *E. marinum*; Fulcrum distal end knobbed (#20): present, type *Encentrum algente*; Manubrial distal end bidirectionally crutched (#29): present, type *E. marinum*; Supramanubrium shape (#48): short process of intramalleus, reduced, type *E. marinum*. **(35)** Light refracting bodies in subcerebral glands (#55): present, type *E. marinum*. **(36)** Rostrum shape (#11): blunt, short, type *Myersinella longiforceps*; Preuncinal teeth shape (#50): accessory ramus teeth with conspicuous process pointing caudolaterally, type *M. longiforceps*.
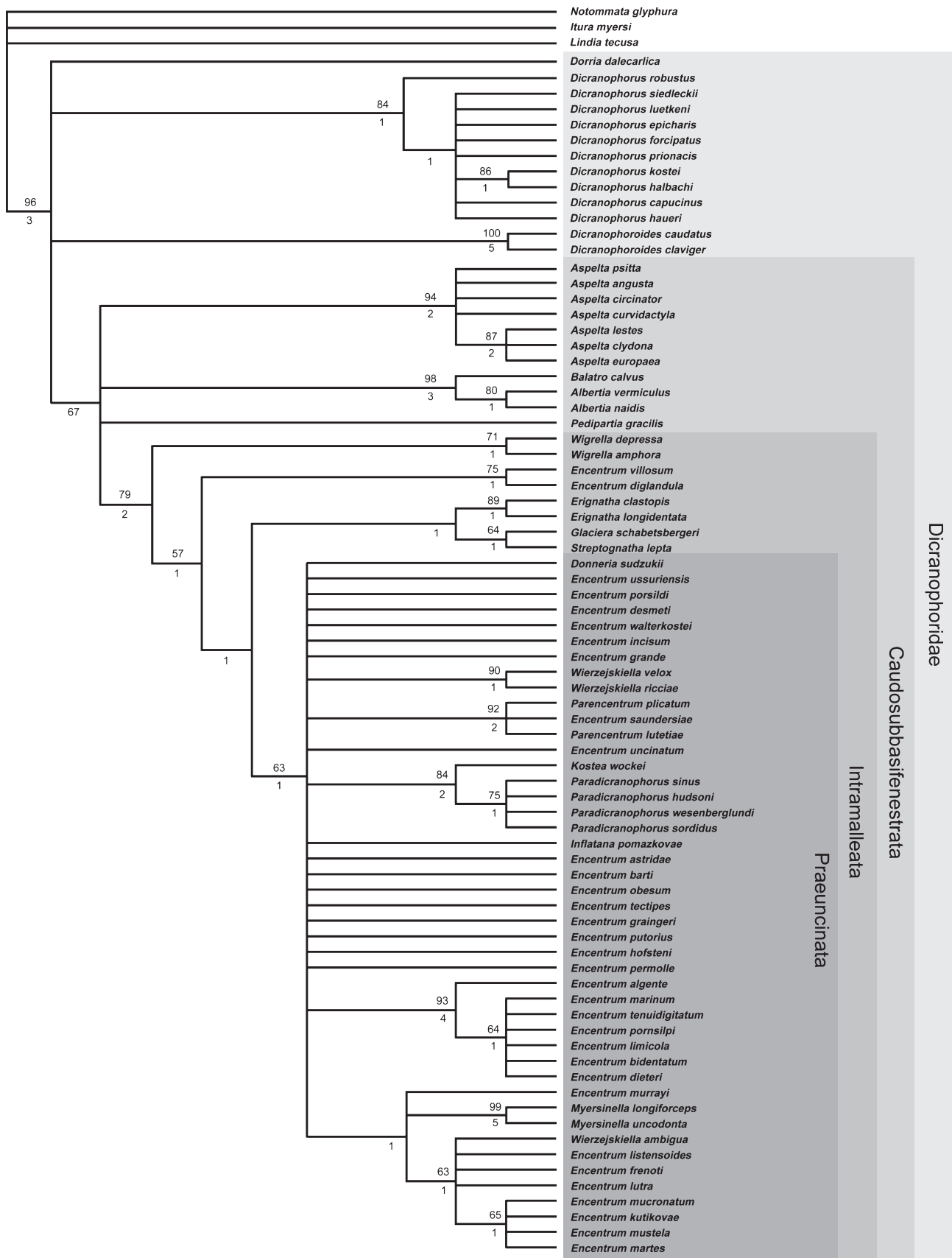
Notommata glyphura
Itura myersi
Lindia tecusa
Dorria dalecarlica
Dicranophorus robustus
Dicranophorus siedleckii
Dicranophorus luetkeni
Dicranophorus epicharis
Dicranophorus forcipatus
Dicranophorus prionacis
Dicranophorus kostei
Dicranophorus halbachi
Dicranophorus capucinus
Dicranophorus haueri
Dicranophoroides caudatus
Dicranophoroides claviger
Aspelta psitta
Aspelta angusta
Aspelta circinator
Aspelta curvidactyla
Aspelta lestes
Aspelta clydona
Aspelta europaea
Balatro calvus
Albertia vermiculus
Albertia naidis
Pedipartia gracilis
Wigrella depressa
Wigrella amphora
Encentrum villosum
Encentrum diglandula
Erignatha clastopis
Erignatha longidentata
Glaciera schabetsbergeri
Streptognatha lepta
Donneria sudzukii
Encentrum ussuriensis
Encentrum porsildi
Encentrum desmeti
Encentrum walterkostei
Encentrum incisum
Encentrum grande
Wierzejskiella velox
Wierzejskiella ricciae
Parencentrum plicatum
Encentrum saundersiae
Parencentrum lutetiae
Encentrum uncinatum
Kostea wockei
Paradicranophorus sinus
Paradicranophorus hudsoni
Paradicranophorus wesenberglundi
Paradicranophorus sordidus
Inflatana pomazkovae
Encentrum astridae
Encentrum barti
Encentrum obesum
Encentrum tectipes
Encentrum graingeri
Encentrum putorius
Encentrum hofsteni
Encentrum permolle
Encentrum algente
Encentrum marinum
Encentrum tenuidigitatum
Encentrum pornsilpi
Encentrum limicola
Encentrum bidentatum
Encentrum dieteri
Encentrum murrayi
Myersinella longiforceps
Myersinella uncodonta
Wierzejskiella ambigua
Encentrum listensoides
Encentrum frenoti
Encentrum lutra
Encentrum mucronatum
Encentrum kutikovae
Encentrum mustela
Encentrum martes

Dicranophoridae
Caudosubbasifenestrata
Intramalleata
Praeuncinata

Fig. 9. Phylogenetic relationships of Dicranophoridae (77 taxa) and three outgroup representatives based on 59 parsimony informative characters. Strict consensus tree of 1371 equally parsimonious trees with a length of 111 steps. Numbers above branches indicate bootstrap support values, numbers below branches Bremer support indices

functional unit (De Smet 2005). *Itura myersi* (see Pourriot 1997) is a borderline case; there is only one uncus shaft from which distally two teeth project.

What can we make of all this? Maybe, in the evolution of a ploimid subtaxon, a reduction in the number of uncus teeth occurred. This process eventually resulted in the retention of only one uncus tooth in the stem lineage of Dicranophoridae, functionally correlated with an increasing emphasis on grasping movements of the jaw apparatus. Besides providing a possible scenario accounting for the reduction in the number of uncus teeth, such reasoning also suggests that the forcipate mastax of dicranophorid rotifers evolved from a functionally less specialized mastax of virgate or even virgo-malleate function. Against such a background, the accessory jugal lines running along the length of the single-toothed uncus in species of the genera *Dicranophorus* and *Aspelta* may be interpreted as vestigial accessory uncus teeth that were reduced in the evolution of the forcipate mastax.

Monophyly of dicranophorid genera

Our analysis suggests that the genera *Dicranophorus* (Fig. 8: Apomorphy box 15), *Dicranophoroides* (Fig. 8: Apomorphy box 19), *Aspelta* (Fig. 8: Apomorphy box 20), *Wigrella* (Fig. 8: Apomorphy box 24), *Erignatha* (Fig. 8: Apomorphy box 27), *Paradicranophorus* (Fig. 8: Apomorphy box 33), *Myersinella* (Fig. 8: Apomorphy box 36) and a clade of the parasitic genera *Balatro* + *Albertia* (Fig. 8: Apomorphy box 22) are monophyletic. For the species rich *Encentrum* group, monophyly cannot be demonstrated. Monophyly of the genus *Wierzejskiella* is equally doubtful. It needs to be stressed, however, that not all dicranophorid species have been included in the analysis. Considering also those species for which no SEM preparations of the trophi are available might result in a different picture with regard to the monophyly of the genera presently recognized. However, a comprehensive taxonomic revision of the whole taxon Dicranophoridae based on phylogenetic principles is clearly beyond the scope of this study. The fact that our analysis does not support *Encentrum* and *Wierzejskiella* as monophyletic groups is not surprising. Their monophyly has already been called into doubt (*Encentrum*: De Smet 1997; *Wierzejskiella*: Tzschaschel 1979). Based on SEM preparations, De Smet (1997) distinguishes three subgenera within *Encentrum*: *Euencentrum*, *Isoencentrum* and *Pseudencentrum*. A monophyletic taxon of our analysis comprising *E. algente* + *E. marinum* + *E. tenuidigitatum* + *E. pornsilpi* + *E. limicola* + *E. bidentatum* and *E. dieteri* (Fig. 8: Apomorphy box 34) corresponds very well to the subgenus *Euencentrum* sensu De Smet (1997). The two species of the subgenus *Pseudencentrum* sensu De Smet (1997) included in our analysis (*E. villosum* and *E. diglandula*, Fig. 8: Apomorphy box 25) also come out monophyletic. According to our analysis, however, monophyly of the subgenus *Isoencentrum* sensu De Smet (1997) is questionable.

Supraspecific monophyletic groups within Dicranophoridae

Caudosubbasifenestrata

Both the ramus subbasal and the ramus basal chamber are cavities with distinct openings under SEM (*fossae* in Koehler and Hayes 1969; *subbasifenestrae* and *basifenestrae* in Marke-

vich 1989 and in Sørensen 2002). The cavities are partly filled with epithelial cells that line the cuticular walls and communicate with epithelial cells outside the ramus chambers (see Riemann and Ahlrichs in press). Across monogonont rotifers, the subbasifenestrae and the basifenestrae project into different directions. In species of the genera *Dicranophorus* and *Dicranophoroides*, the subbasifenestrae are oriented ventro-laterally (De Smet 1997; Sørensen 2002; this study). Such a state is also realized in the outgroup representatives *I. myersi* (Sørensen 2002; this study) and *N. glyphura* (Sørensen 2002; this study). Within Dicranophoridae, a shift in the orientation of the subbasifenestrae seems to have taken place (Fig. 8: Apomorphy box 3). From a ventro-lateral orientation, the subbasifenestrae in the stem lineage of Caudosubbasifenestrata moved closer to the fulcrum, eventually projecting caudally. Such a state is present in all species of the taxon Caudosubbasifenestrata for which SEM preparations of the trophi are available.

Intramalleata

In the jaw apparatus of a large number of dicranophorid rotifers the intramalleus, an accessory trophus element inserted between uncus and manbrium, is present (Koste 1978; De Smet 1997). Very often, a variously shaped, elongate supramanubrium oriented medially is attached to the intramalleus. According to our analysis, overall parsimony suggests that the intramalleus evolved only once in the stem lineage of a large monophylum for which we suggest the name Intramalleata (Fig. 8: Apomorphy box 6). For those species of Intramalleata without an intramalleus (*M. uncodonta*, *M. longiforceps* and *Paradicranophorus hudsoni*), secondary character loss has to be assumed (for a discussion of the role of secondary character loss in phylogenetic analyses relying on morphological characters, see Bleidorn 2007).

Apparently, the evolution of the intramalleus was accompanied by a process of the distal ends of the manubria moving closer together and, ultimately, lying immediately next to each other (char. 30). Our own observations of living specimens indicate that throughout the cycle of jaw extrusion, grasping of prey and withdrawal of the jaws, the distal ends of the manubria always remain in close contact. Such a mode of action differs considerably from how the jaw elements in dicranophorid rotifers without an intramalleus operate (e.g. in the genus *Dicranophorus*, see Riemann and Ahlrichs 2008). While in species without an intramalleus where the left and the right manubrium and the attached unci have a certain degree of independence, in species of Intramalleata the distally connected manubria and unci act as a functional unit. Possibly, such a movement represents a more advanced and efficient, but also more narrowly specialized mode of the grasping (forcipate) mastax type. Continuing along these lines, the evolution of the intramalleus may be seen in correlation with a notable shift in diet. Although data on feeding habits across dicranophorid rotifers is sparse (see De Smet 1997), what we do know suggests that from primary carnivory in *Dicranophorus* and *Aspelta* (see Fig. 10a, b), secondary omnivory or even herbivory evolved (well documented in diatom-feeding, marine species of the genus *Encentrum*, see, for example, Tzschaschel 1979; *E. clastopis* ingesting the phytoflagellate *Cryptomonas*, Fig. 10c). Remarkably, these differences coincide with the absence or presence of an intramalleus. We may speculate that, by conferring a more efficiently
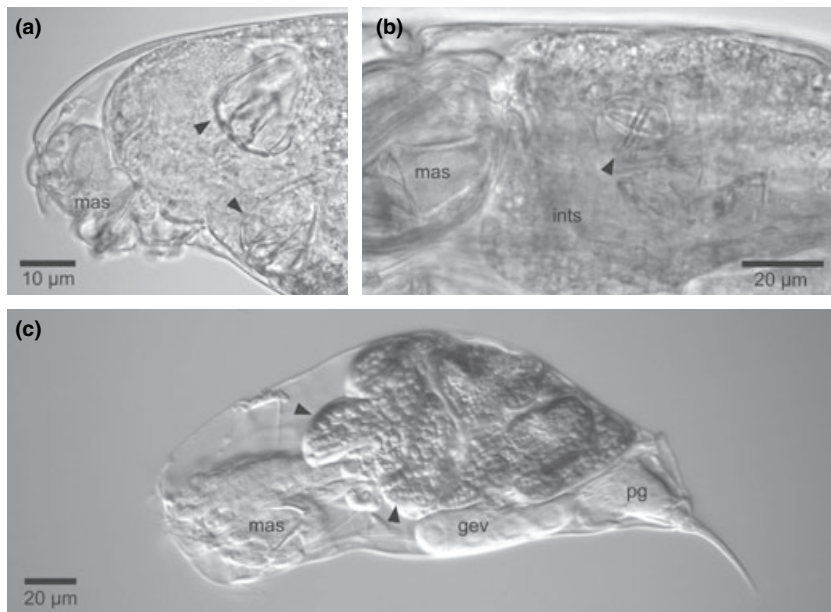
Fig. 10. Different food sources in Dicranophoridae, light micrographs. (a) *Aspelta circinator*, a carnivorous predator. Arrowheads indicate trophi of ingested specimens of *Dicranophorus luetkeni*, itself a voracious predator; (b) *Dicranophorus forcipatus* with trophi of ingested bdelloid prey; (c) *Erignatha clastopis*. Arrowheads highlight ingested specimens of the phytoflagellate *Cryptomonas*

specialized way of feeding that allowed species to utilize single celled algae, the evolution of the intramalleus triggered a process of adaptive radiation and diversification resulting in the high number of species within Intramalleata.

Praeuncinata

Within a monophyletic subtaxon of Intramalleata, paired accessory teeth caudal to the ramus apical teeth have evolved (Fig. 8: Apomorphy box 9). There are either one or two pairs of teeth. SEM images suggest that they are derivates of the ramus basal chamber (De Smet 1997; this study). Traditionally, such teeth are called preuncinal teeth, although there is no indication that they are associated with the uncus. Given the single evolution of preuncinal teeth in the stem lineage of a subtaxon within Intramalleata, we call this group Praeuncinata. Although very important for exact species identification, the preuncinal teeth are sometimes difficult to observe light microscopically, since they are at the resolution limit of light microscopy and can clearly be detected by SEM only (De Smet 1997). Hence, many species descriptions in the literature relying on light microscopy only are probably very often not fully accurate. The presence of preuncinal teeth may be assumed to be an advanced adaptation to the grasping feeding mode. By securing an extra hold on the prey, seizing and ingestion is considerably facilitated.

Proventriculata

A fairly large number of dicranophorid rotifers within Praeuncinata are characterized by a hyaline, dilatable portion of the gastric tract at the junction of oesophagus and stomach called proventriculus (Harring and Myers 1928; Koste 1978; De Smet 1997). The borderline separating proventriculus and stomach is marked by the site of attachment of the gastric glands to the stomach. In species in which a proventriculus is present, the gastric glands are differentiated into a rounded proximal section and a distal stalk. Overall parsimony suggests that such a proventriculus evolved only once and is autapomorphic for the taxon Proventriculata (see Fig. 8: Apomorphy

box 10). In the stem lineage of a clade within Proventriculata, a secondary attachment of the proximal section of the gastric glands to the mastax via a fine filament seems to have occurred (see Fig. 8: Apomorphy block 11, De Smet 1997, 2000, 2002). However, it is very difficult to ascertain in living material, whether such a fine connection really is present in a given specimen and, moreover, where such a fine filament attaches. As in the case of preuncinal teeth, it is very hard to tell whether all species descriptions are fully accurate in this respect. Assumptions regarding the function of the proventriculus are purely speculative at present, but given its ability to contract and dilate, it may serve for holding large pieces of food drawn in by the jaws.

Suggestions for future research

The present analysis of phylogenetic relationships within Dicranophoridae largely relies on SEM preparations of the highly species specific mastax jaw elements. Their usefulness for phylogenetic analyses has repeatedly been demonstrated (Segers and Wallace 2001; Sørensen 2002; Sørensen and Giribet 2006) and is particularly important for Dicranophoridae, where the mastax hard parts are considered the most important characters for phylogenetic analyses and taxonomy (De Smet 1997). Unfortunately, SEM preparations of the jaw elements are lacking for quite a large number of dicranophorid rotifers. This is particularly the case in monotypic genera or genera with only very few species that have either been found only once or very rarely since their first record (*S. lepta*, *G. schabetsbergeri*, *Wigrella* spp., *I. pomazkovae*, *P. gracilis*, *D. dalecarlica*). In all these taxa, the jaw morphology, as obtained by light microscopic examinations, considerably deviates from other dicranophorid rotifers. However, only SEM preparations can clarify whether the descriptions in the literature are fully accurate and, moreover, they are needed for a more convincing assessment of the phylogenetic affinities of the species involved (De Smet 1997).

Within the large monophyletic group Praeuncinata and its subtaxon Proventriculata, many species are similar in most or all diagnostic characters such as the possession of a

proventriculus and stalked gastric glands, the number of ramus preuncinal teeth and the shape of intramallei and supramanubria. Differences only exist in the shape and relative sizes of the jaw elements and slight differences in characters of the internal and external organization. It is very difficult in these cases to decide from the literature, whether a given species is a valid morphospecies or, in fact, synonymous with another species only poorly described (see De Smet 1997). Conversely, we may also expect hidden genetic diversity in apparently uniform morphospecies (see Gilbert and Walsh 2005, 'Brachionus calyciflorus complex'; Schröder and Walsh 2007, 'Epiphanes senta complex').

The overall poor resolution within Praeuncinata and Proventriculata indicates that morphology alone does not provide parsimony informative characters on which to base kinship hypotheses. In many cases individual morphospecies can be identified by autapomorphic features but potential synapomorphies suggesting a sister group relationship are apparently absent. We therefore may expect that either (i) many species within Praeuncinata and Proventriculata are in fact variations of a single complex species with different morphotypes or (ii) given that all morphospecies described are valid, other characters such as DNA sequence data or biochemical data need to be applied in order to identify phylogenetic relationships in clades where our study provides only poor resolution.

Acknowledgements

Zusammenfassung

Zur Phylogenie der Dicranophoridae (Rotifera: Monogononta) – Eine Maximum Parsimonie Analyse basierend auf morphologischen Merkmalen

Die hier vorgelegte Studie stellt die erste phylogenetische Analyse des Taxons Dicranophoridae (Rotifera: Monogononta) dar, einer artenreichen Familie der Rotiferen mit zurzeit etwa 230 validen Arten. Die resultierenden phylogenetischen Verwandtschaftsbeziehungen fußen auf einer Maximum Parsimonie Analyse mit 77 ausgewählten Vertretern der Innen– und 3 Vertretern der Außengruppe insgesamt 59 Parsimonie–informativen Merkmalen. Die Kodierung der Merkmale basiert einerseits auf Material, das von den Autoren selbst gesammelt und bestimmt wurde und andererseits auf einem ausgedehnten Studium der relevanten Literatur. Neben der Erfassung von Merkmalen zur allgemeinen Körperorganisation stützt sich die Merkmalskodierung vor allem auf rasterelektronenmikroskopische Präparationen der Hartelemente des Mastax. Das Ergebnis der Analyse stützt die Monophylie der Dicranophoridae. Innerhalb der Dicranophoridae stellt ein monophyletisches Taxon, das die Gattungen *Dicranophorus* und *Dorria* umfasst, die Schwestergruppe aller übrigen Dicranophoridae dar. Die bei weitem artenreichste Gattung *Encentrum* lässt sich nicht als Monophylum begründen. Als monophyletische Teilgruppen innerhalb der Dicranophoridae identifiziert unsere Analyse die Taxa Caudosubbasifenestrata, Intramalleata, Praeuncinata und Proventriculata, die jeweils durch mindestens eine unzweideutige Merkmalstransformation in ihren Stammlinien begründet werden. Innerhalb der Taxa Praeuncinata und Proventriculata bietet unsere Analyse nur sehr begrenzte Auflösung. Obgleich sich einzelne Teilgruppen über unzweideutige Merkmalstransformationen als Monophyla begründen lassen, fehlen Merkmale für die Auflösung der basalen Verzweigungen innerhalb der Praeuncinata und Proventriculata. Es ist zu erwarten, dass andere Merkmalssysteme, wie zum Beispiel DNA Sequenzdaten, bei der Aufklärung der Verwandtschaftsbeziehungen innerhalb dieser Teilgruppen Klärung erbringen.

References[1]

*Bergendal D (1892) Beiträge zur Fauna Grönlands. Ergebnisse einer im Jahre 1890 in Grönland vorgenommenen Forschungsreise. Acta Univ Lundensis 28 (2), no **4:**1–180.

Bleidorn C (2007) The role of character loss in phylogenetic reconstruction as exemplified for the Annelida. J Zool Syst Evol Res **45:**299–307.

Bremer K (1988) The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution **42:**795–803.

De Beauchamp P (1907) Morphologie et variations de l' appareil rotateur dans la série des Rotifères. Arch zool exptl et gen ser **4, 6:**1–29.

De Beauchamp P (1909) Recherches sur les Rotifères: Les formations tégumentaires et l'appareil digestif. Arch Zool Exp gén 4e sér **X:**1–410.

*De Smet WH (1995) Description of *Encentrum dieteri* sp. nov. (Rotifera, Dicranophoridae) from the High Arctic, with redescription of E. bidentatum (Lie–Pettersen, 1906) and E. murrayi Bryce, 1922. Belg J Zool **125:**349–361.

*De Smet WH (1997) Rotifera 5: The Dicranophoridae. In: Dumont HJ, Nogrady T (eds), Guides to the Identification of the Microinvertebrates of the Continental Waters of the World 12. SPB Academic Publishing BV, The Hague, The Netherlands, pp 1–325.

De Smet WH (1998) Preparation of rotifer trophi for light and scanning electron microscopy. Hydrobiol **387∕388:**117–121.

De Smet WH (2000) Three new species of the genus *Encentrum* (Rotifera, Monogononta, Dicranophoridae). Sarsia **85:**77–86.

De Smet WH (2002) Marine Rotifera from the Crozet and Kerguelen Islands (Subantarctica), with the description of a new *Encentrum* (Monogononta: Dicranophoridae). Internat Rev Hydrobiol **87:**411–422.

*De Smet WH (2003) *Paradicranophorus sinus* sp. nov. (Dicranophoridae, Monogononta) a new rotifer from Belgium, with remarks on some other species of the genus Paradicranophorus Wiszniewski, 1929 and description of Donneria gen. nov. Belg J Zool **133:**181–188.

De Smet WH (2005) Redescription of *Lindia gravitata* with comments on *L. tecusa* (Rotifera: Monogononta: Lindiidae). J Mar Biol Ass UK **85:**1467–1473.

De Smet WH (2007) Description of two new species of *Myersinella* (Rotifera: Monogononta: Dicranophoridae) from the Mediterranean. J Mar Biol Ass UK **87:**1105–1110.

*De Smet WH, Chernyshev AV (2006) Two new species of Dicranophoridae (Rotifera: Monogononta) from Peter the Great Bay, Sea of Japan. J Mar Biol Ass UK **86:**657–663.

Donner J (1968) Zwei neue Schlamm–Rotatorien aus dem Neusiedler See, *Paradicranophorus sudzukii* und *Paradicranophorus sordidus*. Anz Öst Akad Wiss **105:**224–232.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39:**783–791.

Gilbert JJ, Walsh EJ (2005) *Brachionus calyciflorus* is a species complex: mating behaviour and genetic differentiation among four geographically isolated strains. Hydrobiol **546:**257–265.

Harring HK (1913) Synopsis of the Rotatoria. Bull US Nat Mus Wash **81:**1–226.

Harring HK, Myers FJ (1928) The rotifer fauna of Wisconsin. IV. The Dicranophorinae. Trans Wis Acad Sci, Arts Lett **23:**667–808.

*Hauer J (1965) Zur Rotatorienfauna des Amazonasgebietes. Int Revue ges Hydrobiol **50:**341–389.

*Jersabek CD (1994) *Encentrum (Parencentrum) walterkostei* n. sp., a new dicranophorid rotifer (Rotatoria: Monogononta) from the high alpine zone of the Central Alps (Austria). Hydrobiol **281:**51–56.

[1]References marked with an asterisk '*' regard only the authorship of taxa inTable S1.

Jersabek CD (1999) New dicranophorids (Rotifera, Monogononta) from the Austrian Alps, including a new genus. J Nat Hist **33:**177–192.

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). Zoomorphol **109:**329–336.

Koehler JK, Hayes TL (1969) The rotifer jaw: a scanning and transmission electron microscopic study. II. The trophi of *Asplanchna sieboldi*. J Ultrastruct Res **27:**419–434.

*Koste W (1961) *Paradicranophorus wockei* nov. spec., ein Rädertier aus dem Psammon eines norddeutschen Niederungsbaches. Zool Anz **167:**138–141.

Koste W (1978) Rotatoria. Die Rädertiere Mitteleuropas. Ein Bestimmungswerk, begründet von Max Voigt. Ueberordnung Monogononta, 2nd edn. I. Textband, 673 p., II. Tafelband, 234 Taf., Gebr. Borntraeger, Berlin, Stuttgart.

*Kutikova LA (1985) New species of rotifers (Rotatoria) from coastal shallows of Lake Baikal. In: Kutikova LA (ed), Rotifera. Material from the 2nd. All–Union Symposium on Rotifers. Nauka, Leningrad pp 54–66.

Maddison DR, Maddison WP (2000) MacClade: Analysis of Phylogeny and Character Evolution. Version 4.0. Sinauer Associates, Sunderland, MA.

Maddison DR, Swofford D, Maddison WP (1997) NEXUS: an extensible file format for systematic information. Syst Biol **46:**590–621.

Markevich GI (1989) Morphology and the principle organisation of the sclerite system of the mastax in rotifers. In: Shilova AI (ed), Biological and Functional Morphology of Freshwater Animals Vol. 56. Proceedings of the Institute of the Biology of Inland Waters, Leningrad pp 27–82.

Markevich GI, Kutikova LA (1989) Mastax morphology under SEM and its usefulness in reconstructing rotifer phylogeny & systematics. Hydrobiol **186/187:**285–289.

Melone G, Ricci C (1995) Rotatory apparatus in Bdelloids. Hydrobiol **313/314:**91–98.

Müller K (2004) PRAP–computation of Bremer support for large data sets. Mol Phyl Evol **31:**780–782.

Myers FJ (1933) A new genus of rotifers (*Dorria*). With observations on *Cephalodella crassipes* (Lord): *Cephalodella crassipes* (Lord) and *Dorria dalecarlica*. Gen. n., Sp. n. J Roy Microsc Soc 53, ser. **3:**118–121.

*Myers FJ (1936) Psammolittoral rotifers of Lenape and Union lakes, New Jersey. Am Mus Nov **830:**1–22.

Nogrady T, Pourriot R (1995) Rotifera 3: The Notommatidae. In: (Dumont HJ, Nogrady T eds), Guides to the Identification of the Microinvertebrates of the Continental Waters of the World 8. SPB Academic Publishing BV., The Hague, The Netherlands, pp 1–229.

Page RDM (2001a) Nexus Data Editor for Windows (NDE). Version 0.5.0. University of Glasgow, Available at: http://taxonomy.zoology.gla.ac.uk/rod/rod.html (Accessed at August 2008).

Page RDM (2001b) Tree View. Version 1.6.5. University of Glasgow, Available at: http://taxonomy.zoology.gla.ac.uk/rod/rod.html (Accessed at August 2008).

Pejler B, Berzins B (1993) On the ecology of Dicranophoridae (Rotifera). Hydrobiol **259:**129–131.

Pourriot R (1997) Rotifera 5: the Ituridae. In: Dumont HJ, Nogrady T (eds), Guides to the Identification of the Microinvertebrates of the Continental Waters of the World 12. SPB Academic Publishing BV., The Hague, The Netherlands, pp 329–344.

Remane A (1929–1933) Rotatoria. In: Bronn's Klassen und Ordnungen des Tier–Reichs Bd. 4, Abt. Akademische Verlagsgesellschaft, Leipzig, I/1:pp1–577.

Riemann O, Ahlrichs WH (2008) Ultrastructure and function of the Mastax in *Dicranophorus forcipatus* (Rotifera: Monogononta). J Morph **269:**698–712.

Schröder T, Walsh EJ (2007) Cryptic speciation in the cosmopolitan *Epiphanes senta* complex (Monogononta, Rotifera) with the description of new species. Hydrobiol **593:**129–140.

Segers H (2007) Annotated checklist of the rotifers (Phylum Rotifera), with notes on nomenclature, taxonomy and distribution. Zootaxa **1564:**1–104.

*Segers H, Chittapun S (2001) The interstitial Rotifera of a tropical freshwater peat swamp on Phuket Island, Thailand. Belg J Zool **131:**65–71.

Segers H, Wallace RL (2001) Phylogeny and classification of the Conochilidae (Rotifera, Monogononta, Flosculariacea). Zool Scr **30:**37–48.

Segers H, Mbogo DK, Dumont HJ (1994) New Rotifera from Kenya, with a revision of the Ituridae. Zool J Linn Soc **110:**193–206.

*Sørensen MV (1998) Marine Rotifera from a sandy beach at Disko Island, West Greenland, with the description of *Encentrum porsildi* n. sp. and *Notholca angakkoq* n. sp. Hydrobiol **386:**153–165.

*Sørensen MV (2001a) Two new species of the family Dicranophoridae (Rotifera, Ploima) from the littoral psammon, with notes on other brackish water rotifers in Denmark. Hydrobiol **452:**121–128.

*Sørensen MV (2001b) On the rotifer fauna of Bermuda, including notes on the associated meiofauna and the description of a new species of *Encentrum* (Rotifera: Ploima: Dicranophoridae). Proc Biol Soc Washington **114:**725–735.

Sørensen MV (2002) On the evolution and morphology of the rotiferan trophi, with a cladistic analysis of Rotifera. J Zool Syst Evol Res **40:**129–154.

Sørensen MV, Giribet G (2006) A modern approach to rotiferan phylogeny: combining morphological and molecular data. Mol Phyl Evol **40:**585–608.

Strong EE, Lipscomb D (1999) Character coding and inapplicable data. Cladistics **15:**363–371.

Swofford DL (2002) PAUP* – Phylogenetic Analysis Using Parsimony. Version 4.0b10. Sinauer Associates, Sunderland, MA.

Tzschaschel G (1979) Marine Rotatoria aus dem Interstitial der Nordseeinsel Sylt. Mikrofauna Meeresboden **71:**1–64.

Voigt M (1957) Rotatoria. Die Rädertiere Mitteleuropas. I. Textband, 508 p.; II. Tafelband, 115 Tafeln. Gebrüder Bornträger, Berlin.

Wallace RL, Snell TW, Ricci C (2006) Rotifera 1: Biology, ecology and systematics, 2nd edition. In: Segers H, Dumont HJ (eds), Guides to the Identification of the Microinvertebrates of the Continental Waters of the World 23. Kenobi productions, Ghent, Belgium and Backhuys Academic Publishing bv, The Hague, The Netherlands, pp 299.

Walsh EJ, Wallace RL, Shiel RJ (2005) Toward a better understanding of the phylogeny of the Asplanchnidae (Rotifera). Hydrobiol **546:**71–80.

*Wiszniewski J (1932) Les rotifères des rives sablonneuses du lac Wigry. Note préliminaire. Wrotki piaszczystych brzegów jeziora Wigry. Doniesienie tymczasowe. Arch Hydrobiol Rybactwa **6:**86–100.

*Wiszniewski J (1934) Wrotki psammonowe. Les rotifères psammiques. Ann Mus Zool Polon **10:**339–399.

Wulfert K (1935) Beiträge zur Kenntnis der Rädertierfauna Deutschlands. Teil I. Arch Hydrobiol **28:**583–602.

*Wulfert K (1936) Beiträge zur Kenntnis der Rädertierfauna Deutschlands. Teil II. Arch Hydrobiol **30:**401–437.

Authors' addresses: Ole Riemann (for correspondence), Alexander Kieneke, Wilko H. Ahlrichs, Institut für Biologie und Umweltwissenschaften (IBU), AG Systematik und Evolutionsbiologie, Carl von Ossietzky Universität Oldenburg, 26111 Oldenburg, Germany. E-mail: ole.riemann@uni-oldenburg.de, akieneke@senckenberg.de, wilko.ahlrichs@uni-oldenburg.de

Supporting information

Additional supporting information may be found in the online version of this article:

  Appendix S1. Morphological character matrix

  Table S1. Taxa included in the analysis

  Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.