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A modern approach to rotiferan phylogeny: Combining morphological and molecular data

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Abstract

The phylogeny of selected members of the phylum Rotifera is examined based on analyses under parsimony direct optimization and Bayesian inference of phylogeny. Species of the higher metazoan lineages Acanthocephala, Micrognathozoa, Cycliophora, and potential outgroups are included to test rotiferan monophyly. The data include 74 morphological characters combined with DNA sequence data from four molecular loci, including the nuclear 18S rRNA, 28S rRNA, histone H3, and the mitochondrial cytochrome *c* oxidase subunit I. The combined molecular and total evidence analyses support the inclusion of Acanthocephala as a rotiferan ingroup, but do not support the inclusion of Micrognathozoa and Cycliophora. Within Rotifera, the monophyletic Monogononta is sister group to a clade consisting of Acanthocephala, Seisonidea, and Bdelloidea—for which we propose the name Hemirotifera. We also formally propose the inclusion of Acanthocephala within Rotifera, but maintaining the name Rotifera for the new expanded phylum. Within Monogononta, Gnesiotrocha and Ploima are also supported by the data. The relationships within Ploima remain unstable to parameter variation or to the method of phylogeny reconstruction and poorly supported, and the analyses showed that monophyly was questionable for the families Dicranophoridae, Notommatidae, and Brachionidae, and for the genus *Proales*. Otherwise, monophyly was generally supported for the represented ploimid families and genera.
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1. Introduction

Rotifera is a group of mostly microscopic, aquatic invertebrates with about 1900 described species (Segers, 2002a). They are generally characterized by the presence of a corona (or wheel organ) formed of ciliary bands in the cephalic region. They also have a complex pharyngeal apparatus, the mastax, composed of hard parts (trophi), connective musculature, and ligaments (Nogrady et al., 1993). In a phylogenetic and taxonomic context, the morphology of the trophi is among the most significant charac-

teristics. A total of nine different types of trophi (Fig. 1), plus some intermediate forms, are recognized.

Three major clades are usually recognized within Rotifera: Seisonidea, Bdelloidea, and Monogononta (Wallace and Colburn, 1989; Wallace and Snell, 1991; Melone et al., 1998a; Sørensen, 2002). Seisonidea comprises only three species in the genera *Seison* and *Paraseison* (Sørensen et al., 2005). They are epizoic symbionts on the members of the leptostracan crustacean genus *Nebalia*, and are characterized by possessing a special fulcrate trophus type (Fig. 1E), and gamogenetic reproduction (Ricci et al., 1993; Ahlrichs, 1995a; Segers and Melone, 1998). Bdelloidea comprises ca. 400 species with a rather conserved body plan. The body consists of a trunk and telescopic retractable head and foot regions. The head carries a retractable rostrum with adhesive glands used for crawling, and the trophi are of the ramate type (Fig. 1A). Furthermore, all bdelloids reproduced
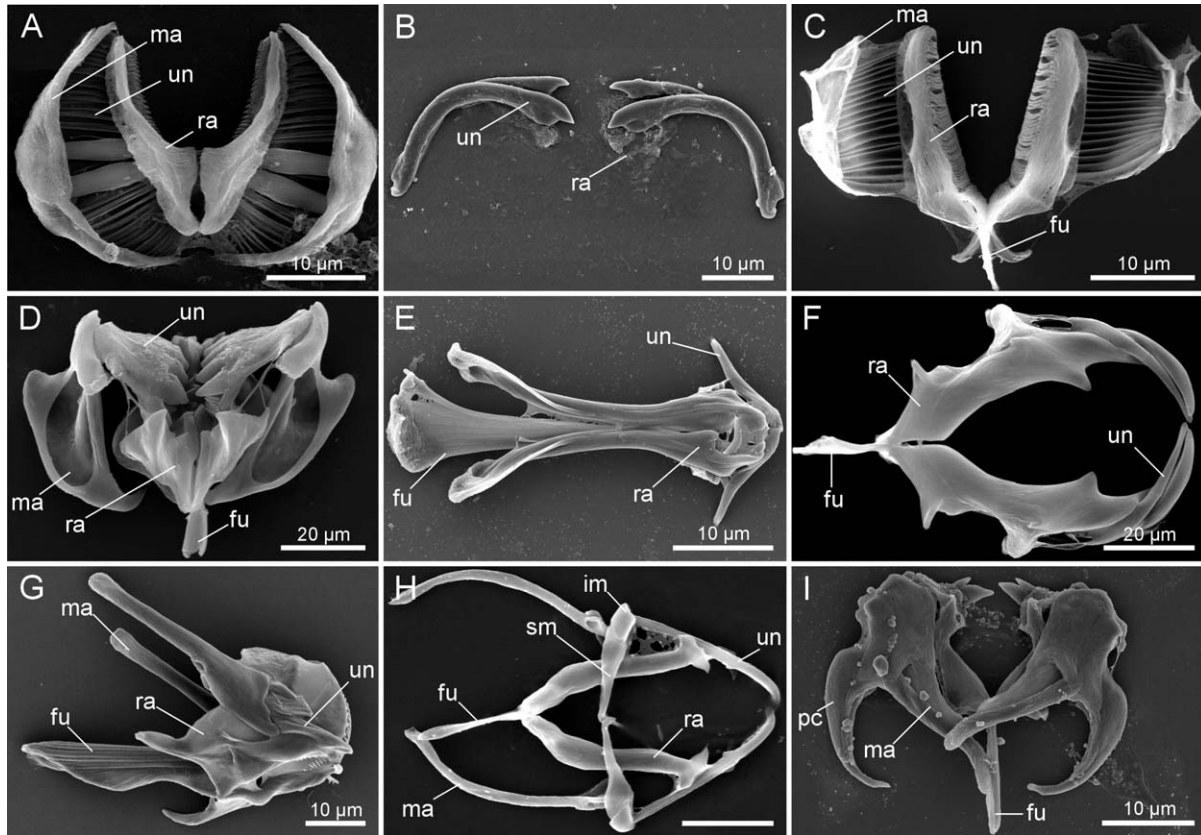
Fig. 1. Scanning electron micrographs showing the nine rotiferan trophus types. (A) Ramate trophi of *Rotaria neptunia* (Bdelloidea), caudal view. (B) Uncinate trophi of *Stephanoceros fimbriatus* (Collothecacea), ventral view. (C) Malleoramate trophi of *Filinia longiseta* (Flosculariacea), dorsal view. (D) Malleate trophi of *Brachionus plicatilis* (Ploima: Brachionidae), ventral view. (E) Fulcrate trophi of *Seison nebaliae* (Seisonidea), ventral view. (F) Incudate trophi of *Asplanchnopus dahlgreni* (Ploima: Asplanchnidae), ventral view. (G) Virgate trophi of *Eothinia elongata* (Ploima: Notommatidae), right lateral view. (H) Forcipate trophi of *Encentrum astridae* (Ploima: Dicranophoridae), ventral view. (I) Cardate trophi of *Lindia torulosa* (Ploima: Lindiidae). Abbreviations: fu, fulcrum; ma, manubrium; im, intramalleus; pc, posterior manubrial chamber; ra, ramus; sm, supramanubrium; un, uncus.

solely by parthenogenesis and most species have cryptobiotic capabilities (Donner, 1965; Ricci, 1987, 1998a; Mark Welch and Meselson, 2000; Ricci and Melone, 2000). The third clade, Monogononta, with ca. 1500 described species, is by far the most diverse rotifer group and it also displays the greatest morphological disparity. Monogononts are mostly free-swimming, even though sessile taxa are represented as well. The remaining seven trophus types are found in this clade. Contrary to the exclusively parthenogenetic bdelloids, many monogonont rotifers follow a complex cycle composed of a parthenogenetic amictic phase and a mictic phase that include sexual reproduction and the presence of haploid dwarf males (Nogrady et al., 1993; Melone and Ferraguti, 1999).

Also related to rotifers are the members of a parasitic clade of metazoans, Acanthocephala. While still considered a phylum in their own, recent molecular data derived from nuclear ribosomal genes suggest that acanthocephalans are related to rotifers (Herlyn et al., 2003), perhaps nesting within them as the sister group to bdelloids (García-Varela et al., 2000; Giribet et al., 2004) or sister to Seisonidea (Zrzavý, 2001a; Herlyn et al., 2003). As such, we cannot attempt to evaluate rotiferan relationships without considering acanthocephalans.

Early microscope pioneers such as van Leeuwenhoek, Linnaeus, and Müller originally classified rotifers among the diffuse group "Vermes" or the protozoan Infusoria. Later, Rotifera were assigned to the polyphyletic Aschelminthes, but more recently an increasing amount of evidence supports a close relationship between Rotifera and Gnathostomulida, and together with Acanthocephala and Micrognathozoa they are united in the clade Gnathifera, supported by the presence of jaws with identical ultrastructure (Ahlrichs, 1995a,b; Rieger and Tyler, 1995; Haszprunar, 1996; Giribet et al., 2000; Sørensen et al., 2000; Kristensen and Funch, 2000; Giribet, 2002a; Sørensen, 2003; Funch et al., 2005). The jaws have been lost secondarily in the highly specialized, endoparasitic acanthocephalans, but the presence of a syncytial epidermis with an intraskeletal lamina supports a close relationship with Rotifera (Haffner, 1950; Koehler, 1966; Storch and Welsch, 1969, 1970). The two groups have traditionally been considered sister taxa, but recently the rotifer monophyly with respect to Acanthocephala has been questioned. Morphological data have suggested various combinations of sister-group relationships between Acanthocephala and the three main rotifer clades, Seisonidea, Bdelloidea, and Monogononta

(Lorenzen, 1985; Ahlrichs, 1995a, 1997, 1998; Ferraguti and Melone, 1999; Sørensen et al., 2000), but all these have been based on dubious homology statements or contradictory details in sperm anatomy (Melone et al., 1998a; Ricci, 1998b; Funch et al., 2005). Hence, morphological data cannot at present provide critical support for Acanthocephala as a rotifer ingroup, although a steadily growing amount of molecular data strongly support its inclusion (Garey et al., 1996, 1998; Near et al., 1998; Giribet et al., 2000, 2004; Mark Welch, 2000; Miquelis et al., 2000; Near, 2002).

The discovery of the intriguing Micrognathozoa has placed a question mark to rotiferan monophyly. Kristensen and Funch (2000) originally described it as the sister group to Rotifera and Acanthocephala, whereas De Smet (2002) interpreted the single micrognathozoan species *Limnognathia maerski* as an aberrant monogonont rotifer; 18S rRNA and 28S rRNA sequence data suggested a position closer to Cycliophora and Gnathostomulida (Giribet et al., 2004).

Different morphological studies have dealt with the internal phylogeny of Rotifera and most recent work supposes a sister-group relationship between Bdelloidea and Monogononta, united in the clade Eurotatoria, and place Seisonidea as their sister group (Wallace and Colburn, 1989; Wallace and Snell, 1991; Melone et al., 1998a; Sørensen, 2002). However, surprisingly few studies have attempted to resolve the phylogeny within Eurotatoria. Since 1970, only three studies, Markevich (1989), Melone et al. (1998a), and Sørensen (2002), have dealt with the relationships of bdelloid and/or monogonont rotifers, and only the two latter were based on modern numerical methods. Melone et al. (1998a) focused solely on bdelloid interrelationships, whereas Markevich (1989) and Sørensen (2002) included most families of rotifers. Both studies produced some congruent results, especially concerning the relationships within the diverse and species-rich monogonont clade Ploima. But they also differed fundamentally as Markevich (1989) based his conclusions on the assumption that bdelloids present the ancestral character traits, whereas Sørensen (2002) polarized characters based on outgroup comparison, which placed bdelloids as the sister group to monogononts. Both studies coincided in the difficulties in comparing the morphology of the various rotifer taxa and revealed how the obtained results would be highly dependent on interpretations and character transformations.

In a recent review that summarizes our current knowledge on rotifer systematics, ecology and phylogeny (Wallace, 2002) a call is placed for future studies on rotifer phylogeny that focus on (1) inclusion of more rotifer taxa in the analyses, (2) new morphological characters, and (3) addition of more molecular sequences. In the present study, we comply with all three demands, as we combine a new morphological data set with sequences from four molecular loci representing 53 rotifer species. Our primary goal is to analyze the relationships between the rotifer main clades and within Monogononta, but also to test rotifer monophyly with respect to Acanthocephala and Micrognathozoa.

2. Materials and methods

2.1. Taxon sampling

The ingroup comprises 48 monogononts, four bdelloids, four acanthocephalans, *Seison nebaliae* (Seisonidea), and *L. maerski* (Micrognathozoa), spanning most of the diversity within the phylum Rotifera and potential ingroups. Outgroup taxa include representatives of other gnathiferan and platyzoan phyla, including five Gnathostomulida, four Platyhelminthes, and the two described species of Cycliophora. Most of the ingroup taxa were collected and sequenced specifically for the present study, while most outgroup sequences were obtained by the authors for two related studies (Giribet et al., 2004; Sørensen et al., 2006). Details on collecting localities and GenBank accession numbers are reported in Table 1.

2.2. DNA sequencing

New DNA sequences were extracted from fresh tissues using the Qiagen DNeasy® Tissue Kit immediately after the animals were sorted out. Preferably 10–15 specimens were used for each extraction, but from samples with a more restricted number of individuals extractions could turn out successfully with only 1–3 specimens.

Four molecular loci were chosen for the study. Ribosomal sequence data of complete 18S rRNA and a fragment of 28S rRNA were selected to resolve the deeper nodes in the trees, whereas the nuclear protein-coding gene histone H3 (H3 hereafter) and the mitochondrial protein-coding gene cytochrome *c* oxidase subunit I (COI hereafter) were included to resolve the more recent evolutionary events. The complete 18S rRNA loci were amplified into three overlapping fragments using the following primer pairs: 1F–4R, 3F–18sbi, and 18Sa2.0–9R (Giribet et al., 1996; Whiting et al., 1997). A fragment of 28S rRNA was obtained with the primer pairs 28S D1F–28Sb or alternatively 28S D1F–28Sr D4b, which produce fragments of ca. 1200 and 860 bp, respectively (Park and Ó Foighil, 2000; Whiting et al., 1997; Crandall et al., 2000). Histone H3 was amplified with H3aF–H3aR (Colgan et al., 1998), and COI with LCO1490–HCO2198 (Folmer et al., 1994) or alternatively with COI-7–COI-D (Kojima et al., 1997).

The amplified samples were purified using QIAquick® PCR Purification Kit. The purified fragments were labeled using BigDye® Terminator v3.0, and sequenced with an ABI 3730 genetic analyzer.

Chromatograms obtained from the automated sequencer were read and "contig sequences" (assembled sequences) were assembled using the sequence editing software Sequencher™ 4.0. For the non-protein-coding genes (18S rRNA and 28S rRNA), sequences were edited in MacGDE (Linton, 2005) and compared with secondary structure models and then split into accordant fragments using internal primers and some visualized secondary structure features (Giribet and Wheeler, 2001; Giribet, 2002b).

Table 1
Species represented in the phylogenetic analyses with collecting localities and GenBank accession numbers for each sampled molecular locus

	Family	Species	Collecting locality	18S rRNA	28S rRNA	Histone H3	COI
Platyhelminthes	Haplopharyngidae	*Haplopharynx rostratus**	—	AJ012511	AF022746	—	AJ405977
	Macrostomidae	*Macrostomum hystricinum**	—	AF051329	—	—	—
	Microstomidae	*Microstomum lineare**	—	D85092	AJ270172	—	AJ405979
	Notoplanidae	*Notoplana australis**	—	AJ228786	—	—	—
Cycliophora	Symbiidae	*Symbion americanus*	Maine and Nova Scotia	AY218107	AY218134	AY218154	AY218085
		Symbion pandora	Sweden	AY218106	AY218133	AY218153	AY218084
Gnathostomulida	Austrognathiidae	*Austrognatharia strunki*	Belize	AY218110	AY218137	—	—
	Gnathostomulidae	*Gnathostomula armata*	Maine, USA	AY218112	AY218139	AY218158	AY218088
	Haplognathiidae	*Haplognathia ruberrima*	Bermuda	DQ079930	DQ079954	AY218156	DQ079968
	Mesognathariidae	*Labidognathia longicollis*	Belize	AY218111	AY218138	AY218087	AY218157
	Rastrognathiidae	*Rastrognathia macrostoma*	Denmark	DQ079935	DQ07959	DQ079988	DQ079970
Micrognathozoa	Limnognathiidae	*Limnognathia maerski*	Disko Isl., Greenland	AY218108	AY218135	AY218155	AY218086
Acanthocephala	Echinorhynchidae	*Echinorhynchus gadi*	From cod; Disko Isl., Greenland	AY218123	AY218146	AY218163	AY218095
	Oligacanthorhynchidae	*Macracanthorhynchus ingens**	—	AF001844	—	—	AF416997
	Moniliformidae	*Moniliformis monoliformis**	—	Z19562	—	—	AF416998
	Pomphorhynchidae	*Pomphorhynchus laevis*	From flounder; Denmark	AY218124	—	AY218164	AY218096
Rotifera	*Seisonidae*	*Seison nebaliae*	Roscoff, France	DQ297761	DQ297762	—	DQ297765
	Adinetidae	*Adineta vaga*	Cultured by Dr. M. Meselson	DQ079913	DQ079940	DQ079974	DQ079961
	Philodinidae	*Philodina acuticornis**		U41281	—	—	—
		Rotaria neptunia	Denmark	AY218122	—	—	AY218094
		Rotaria rotatoria	Denmark	AY218121	—	—	AY218093
	Collothecidae	*Collotheca campanulata*	Mount Desert Isl., MA, USA	DQ297686	DQ297725	—	DQ297766
	Conochilidae	*Conochilus hippocrepis*	White Mountains, NH, USA	DQ297687	DQ297726	DQ297797	DQ297767
		Conochilus unicornis	White Mountains, NH, USA	DQ297688	DQ297727	DQ297798	—
	Filiniidae	*Filinia longiseta*	Fort Pierce, FL, USA	DQ079914	DQ079941	—	—
	Flosculariidae	*Ptygura libera*	Lake Okeechobee, FL, USA	DQ297689	DQ297728	—	DQ297768
		Sinantherina ariprepes	White Mountains, NH, USA	DQ297690	DQ297729	—	—
	Testudinellidae	*Testudinella* sp.	Mount Desert Isl., MA, USA	AY218113	AY218140	AY218159	—
	Gastropodidae	*Ascomorpha ovalis*	Mount Desert Isl., MA, USA	DQ297691	DQ297730	DQ297799	DQ297769
	Asplanchnidae	*Asplanchnopus dahlgreni*	Mount Desert Isl., MA, USA	DQ079916	DQ079943	DQ079976	DQ079963
	Brachionidae	*Brachionus calyciflorus*	Cultured by Dr. M. Meselson	DQ297692	DQ297731	—	DQ297770
		Brachionus plicatilis	—	AY218118	AY218144	—	AY218090
	Notommatidae	*Cephalodella forficula*	Fort Pierce, FL, USA	DQ297693	—	—	—
		Cephalodella gibba	Mount Desert Isl., MA, USA	AY218114	AY218141	AY218160	AY218089
	Dicranophoridae	*Dicranophorus forcipatus*	Fort Pierce, FL, USA	DQ297694	DQ297732	—	DQ297771
		Encentrum astridae	Bermuda	DQ297695	DQ297733	DQ297800	DQ297772
		Encentrum tectipes	Bermuda	DQ297696	DQ297734	DQ297801	—
	Notommatidae	*Eothinia elongata*	Everglades, FL, USA	DQ079917	DQ079944	DQ079977	DQ079964
	Euchlanidae	*Euchlanis alata*	New Hampshire, USA	DQ079915	DQ079942	DQ079975	DQ079962
		Euchlanis dilatata	Mount Desert Isl., MA, USA	AY218116	AY218143	—	DQ297773
	Brachionidae	*Keratella quadrata*	Lake Okeechobee, FL, USA	DQ297697	DQ297735	DQ297802	DQ297774
	Lecanidae	*Lecane bulla*	Fort Pierce, FL, USA	DQ297698	DQ297736	DQ297803	DQ297775
		Lecane elsa	Denmark	DQ297699	DQ297737	DQ297804	DQ297776
		Lecane leontina	Everglades, FL, USA	DQ297700	DQ297738	—	DQ297777
	Lepadellidae	*Lepadella patella*	Fort Pierce, FL, USA	DQ297701	DQ297739	DQ297805	DQ297778
		Lepadella rhomboides	Fort Pierce, FL, USA	DQ297702	DQ297740	DQ297806	DQ297779
	Lindiidae	*Lindia tecusa*	Denmark	DQ297703	DQ297741	—	—
		Lindia torulosa	Fort Pierce, FL, USA	DQ297704	DQ297742	—	—
	Trichotriidae	*Macrochaetus collinsi*	Pocono Mountains, PA, USA	DQ297705	DQ297743	—	DQ297780
	Microcodidae	*Microcodon clavus*	Mount Desert Isl., MA, USA	DQ297706	DQ297744	DQ297807	DQ297781
	Notommatidae	*Monommata maculata*	White Mountains, NH, USA	DQ297707	DQ297745	DQ297808	—
	Mytilinidae	*Mytilina mucronata*	Denmark	DQ297708	DQ297746	DQ297809	DQ297782
		Mytilina ventralis	Fort Pierce, FL, USA	DQ297709	DQ297747	DQ297810	DQ297783
	Brachionidae	*Notholca acuminata*	Winter Harbor, MA, UAS	AY218115	AY218142	AY218161	—
	Notommatidae	*Notommata alantois*	Lake Okeechobee, FL, USA	DQ297710	DQ297748	DQ297811	DQ297784
		Notommata cordonella	Fort Pierce, FL, USA	DQ297711	DQ297749	—	DQ297785
	Brachionidae	*Plationus patulus*	White Mountains, NH, USA	DQ297712	DQ297750	DQ297812	DQ297786
		Platyias quadricornis	Lake Okeechobee, FL, USA	DQ297713	—	—	—
	Synchaetidae	*Ploesoma hudsoni*	Mount Desert Isl., MA, USA	DQ297714	—	DQ297813	DQ297787
		Ploesoma truncatus	Pocono Mountains, PA, USA	DQ297715	DQ297751	—	DQ297788
		Polyarthra remata	Fort Pierce, FL, USA	DQ297716	DQ297752	DQ297814	DQ297789

Table 1 (*continued*)

Family	Species	Collecting locality	18S rRNA	28S rRNA	Histone H3	COI
Proalidae	*Proales doliaris*	Mount Desert Isl., MA, USA	DQ297717	DQ297753	DQ297815	DQ297790
	Proales reinhardti	Woods Hole, MA, USA	DQ297718	DQ297754	DQ297816	—
	Proales similis	Bermuda	DQ297719	DQ297755	—	DQ297791
Scaridiidae	*Scaridium longicaudum*	Fort Pierce, FL, USA	DQ297720	DQ297756	DQ297817	DQ297792
Trichocercidae	*Trichocerca elongata*	Mount Desert Isl., MA, USA	DQ297721	DQ297757	DQ297818	DQ297793
	Trichocerca rattus	Mount Desert Isl., MA, USA	DQ297722	DQ297758	DQ297819	DQ297794
	Trichocerca tenuior	Fort Pierce, FL, USA	DQ297723	DQ297759	—	DQ297795
Trichotriidae	*Trichotria tetractis*	Pocono Mountains, PA, USA	DQ297724	DQ297760	DQ297820	DQ297796

All sequences (except those marked with an asterisk) have been generated by the authors either for this or for previously published studies.

The protein-coding gene histone H3 showed no length variation. COI showed some minor length variation and was analyzed as a single fragment. All new sequences have been deposited in GenBank under the Accession Nos. DQ297686–DQ297762 and DQ297765–DQ297820 (Table 1).

2.3. Molecular data

The 18S rRNA fragment was divided into 47 fragments according to primer regions and secondary structure features, as in our previous analyses (e.g., Giribet, 2002b); all 47 fragments were used in the analyses. Sequence length for the 18S rRNA fragment varied between 1747 nt in *Echinorhynchus gadi* to 1760 nt in *Philodina acuticornis*, a rather low sequence length variation. Variation within the outgroups was larger, between 1717 nt in the gnathostomulid *Gnathostomula armata* and 1781 nt in another gnathostomulid, *Austrognatharia strunki.*

The 28S rRNA fragment of ca. 1 kb also showed 73 nt of length variation within the ingroup but ranged between 830 and 1119 nt when including outgroups. This gene fragment was divided into nine regions; the last region measuring 34 bp was excluded from the analysis because it was missing for more than half of the taxa. Eleven terminals are missing for the 28S rRNA fragment.

The COI fragment was amplified for a total of 53 terminals and due to the presence of length variation (between 651 and 660 nt), it was divided into six regions, aided by the amino acid translation.

The histone H3 fragment did not amplified well for several terminals studied and amplification was possible for 40 terminals. The fragment, of 327 nt, showed no length variation and was analyzed as a single pre-aligned region.

2.4. Morphological matrix

A morphological matrix containing 74 characters was compiled from literature sources and from direct observations of specimens and in particular trophi mounted for SEM. Preparation of trophi for SEM followed the standard procedure given by De Smet (1998) and Sørensen (2003). All terminals are coded in accordance with their actual morphology, whereas coding based on proposed ground patterns have been avoided. During the construction and coding of the matrix the inclusion of a snumber of charac-

ters (generally concerning ultrastructure of sperm and integument) were considered. These characters could have resolved some of the basal syndermate splits, but were in general omitted—either because their character states were known only for a very restricted number of terminals (three or less) or because the homology of the characters was considered uncertain. Further comments to these characters are given in the following discussion. The list of characters and their explanations are given in Appendix A and the morphological matrix is given in Appendix B.

2.5. Data analyses

The morphological data matrix was analyzed with TNT (Tree analysis using New Technology) (Goloboff et al., 2003) under the new technology search using a driven search aimed to stabilize a consensus up to five times after finding trees of minimal length multiple times (Goloboff and Farris, 2001; Goloboff, 2002). This search strategy was compared with a standard heuristic search strategy consisting of 1000 Wagner addition trees with subtree pruning and regrafting (SPR) and tree bisection and reconnection (TBR) branch swapping, retaining up to 10 trees per replicate.

The molecular data were analyzed using a "one-step phylogenetics" approach (Giribet, 2005) in POY v. 3.0.12 (Wheeler et al., 2004) using the direct optimization method (Wheeler, 1996; Wheeler et al., 2006) with parsimony as the optimality criterion. The data for all the genes were analyzed independently and in combination. In addition, the molecular data were analyzed in combination with the morphological data matrix in POY. Tree searches were conducted in parallel (using PVM—Parallel Virtual Machine) on a cluster of 30 dual-processor nodes (between 1 and 2.4 GHz) assembled at Harvard University (darwin.oeb.harvard.edu). Commands for load balancing of spawned jobs were in effect to optimize parallelization procedures (−parallel −dpm −jobspernode 2). Trees were built through a random addition sequence procedure (20 replicates) followed by a combination of SPR and TBR branch-swapping, and continuing with tree fusing (TF; Goloboff, 1999, 2002) in order to further improve tree length. While SPR and TBR allow branch rearrangement within a given tree, tree fusing allows exchanging of branches of identical composition among different trees, as in other simulated evolutionary algorithms (Moilanen, 1999, 2001). Discrepancies

between heuristic and actual tree length calculations were addressed by adjusting slop values (−checkslop 10). While doing tree refinements using TBR, −checkslop *n* accept all trees that are within *n*-tenths of a percent of the current minimum value. For example −checkslop 10 accepts all trees up to 1% above the current minimum length while doing TBR.

POY facilitates efficient sensitivity analysis (Wheeler, 1995; Giribet, 2003). All data sets (individual genes and combinations) were analyzed under 12 parameter sets, for a range of indel-to-transversion ratios and transversion-to-transition ratios (see Table 2). Implied alignments—a topological-unique "alignment" or synapomorphy scheme (Wheeler, 2003; Giribet, 2005)—can be easily generated for each tree.

After the initial round of 20 replicates with SPR + TBR + TF was executed for the 12 parameter sets in the combined analysis of all data, the trees obtained were pooled and given to POY for a subsequent round of tree fusing, the so-called sensitivity analysis tree fusing (SATF; Boyer et al., 2005).

To identify the optimal parameter set, we employed a character-congruence technique which is a modification of the incongruence length difference (ILD) metric developed by Mickevich and Farris (1981) (see also Farris et al., 1995), as proposed by Wheeler (1995) (Table 2). The value is calculated for each parameter set by subtracting the sum of the scores of all partitions from the score of the combined analysis of all partitions, and normalizing it for the score of the combined length. Although the reliability of the ILD measure employed here has been questioned because it may

show a trivial minimum in circumstances in which partitions are given disproportionate weights (Aagesen et al., 2005), this is not the case here. The modified ILD technique has been interpreted as a meta-optimality criterion for choosing the parameter set that best explains all partitions in combination, the one that maximizes overall congruence and minimizes character conflict among all the data (Giribet, 2003). This parameter set was given special consideration in the analysis of data from each individual gene and is referred to throughout this paper as the "optimal parameter set." Additionally, we discuss results from the strict consensus of all parameter sets explored, which has been interpreted as a measure of stability to parameter choice, as applied in statistical sensitivity analyses (Wheeler, 1995; Giribet, 2003). Nodal support for all topologies was measured by parsimony jackknifing (Farris et al., 1996; Farris, 1997).

In order to evaluate the potential effect of treating each gap as an independent character (e.g., see Giribet and Wheeler, 1999; Simmons and Ochoterena, 2000), we run the combined analysis of all the data under the optimal parameter set using a nonlinear (affine) gap function, where the gap opening value was higher than that of the gap extension. This was done by generating the implied alignment for one of the trees obtained under the optimal parameter set and using different opening and extension gap costs (2 and 1, respectively). This implied alignment was further used to perform a Bayesian analysis (Huelsenbeck et al., 2001) in MrBayes v 3.1.1 (Ronquist and Huelsenbeck, 2005). The best-fit model for such implied alignment was calculated using the Akaike information criterion (AIC) in Modeltest v. 3.7 (Posada and Crandall, 1998; Posada and Buckley, 2004; Posada, 2005). After selecting the appropriate model in MrBayes, four chains of 500,000 generations were run monitoring the potential scale reduction factor (PSRF) value for convergence. A 95% majority rule consensus tree was generated with the trees retained after discarding the burnin.

3. Results

3.1. Morphological data analysis

The driven search strategy performed in TNT generated a stable consensus containing 41 nodes resulting from a total of 95 retained trees of 128 steps (Fig. 2). An identical consensus was obtained after performing 1000 replicates of the traditional heuristic search with SPR and TBR branch swapping, although this strategy generated 4530 trees and examined 5.8×10^8 rearrangements as opposed to the 3.4×10^7 rearrangements examined with the first strategy (execution times were 1 and 66 s, respectively, on a Pentium 4 CPU at 2 GHz, 1GB of RAM). When the same heuristic strategy was implemented in PAUP* (Swofford, 2002) in a PowerPC G4 at 1.33 GHz, 1 GB of RAM, the analysis took 751 s (for 9.1×10^8 rearrangements).

Table 2
Tree lengths for the different partitions analyzed (18S, 18S rRNA; 28S rRNA; H3, histone H3; COI, cytochrome *c* oxidase subunit I; MOR, morphological data; MOL, four loci combined; TOT, morphology + four loci combined) and congruence value (ILD) for the combined analysis of morphology + four molecular loci combined at different parameter sets (left column)

	18S	28S	H3	COI	MOR	MOL	TOT	ILD
111	3736	4232	1277	5148	128	14899	15088	0.037580
121	5600	6413	1890	7990	256	22623	23005	**0.037209**
141	9146	10483	3058	13461	512	37350	38128	0.038502
181	16226	18474	5344	24341	1024	66664	68159	0.040347
211	4161	4818	1277	5249	256	16039	16425	0.040426
221	6387	7404	1891	8152	512	24688	25414	0.042024
241	10660	12458	3057	13814	1024	41366	42814	0.042066
281	19168	22256	5344	24907	2048	74736	77663	0.050732
411	4767	5589	1277	5305	512	17539	18291	0.045979
421	7550	8868	1890	8269	1024	27652	29111	0.051870
441	12955	15291	3057	14051	2048	47233	50061	0.053115
481	23789	28109	5348	25470	4096	86392	91934	0.055714

The first numeral used in the parameter set column corresponds to the ratio between indel/transversion and the following two numbers correspond to the ratio between transversion/transition; e.g., 111 is equal weights; 121 corresponds to a indel/transversion ratio of 1 and a transversion/transition ratio of 2:1—so indels have a cost of 2, transversions have a cost of 2, and transitions have a cost of 1. (For a list of the specific step matrices that this involves, see Giribet et al., 2002, Appendix 4). Optimal ILD value is indicated in bold.
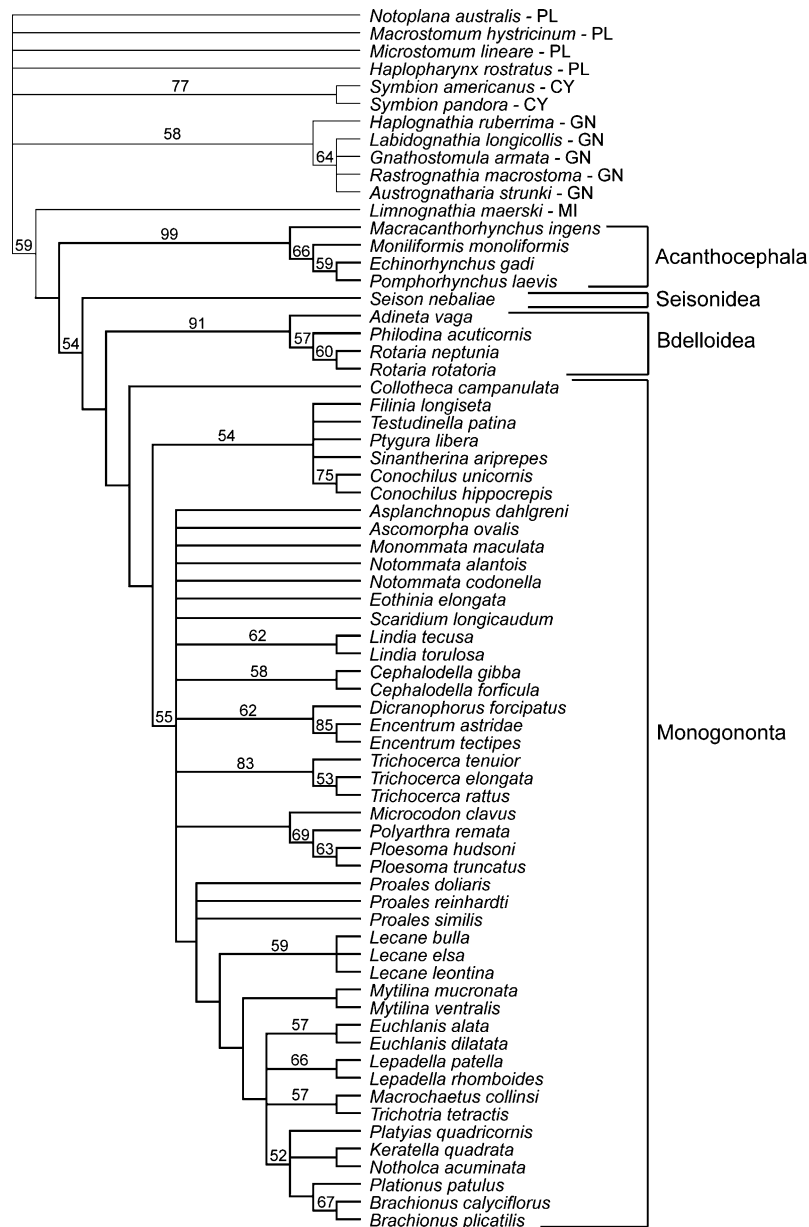
Fig. 2. Strict consensus of 95 trees at 128 steps obtained after analysis of the morphological data. Numbers on branches indicate jackknife proportions above 50%. Thicker branches indicate ingroup taxa. Abbreviations for outgroups: PL for Platyhelminthes, CY for Cycliophora, GN for Gnathostomulida, and MI for Micrognathozoa.

All trees show monophyly for Syndermata [jackknife frequency (JF hereafter) <50%] and support a sister-group relationship between Acanthocephala (99% JF) and Rotifera (54% JF). Within Rotifera, Seisonidea is sister group to Eurotatoria (<50% JF), the latter consisting of Bdelloidea (91% JF) and Monogononta (<50% JF). Within Monogononta, *Collotheca campanulata* (viz., the only representative for Collothecaceae in the analysis) branches off as the most basal taxon and forms the sister taxon to a clade consisting of monophyletic Flosculariacea and Ploima. Hence, monophyly of Gnesiotrocha (consisting of Collothecaceae and Flosculariaceae) is not supported. The morphological analysis could not resolve the overall relationships within Ploima (55% JF). However, the strict consensus tree shows monophyly for all taxa with malleate trophi, which form a clade that corresponds to the Transversiramida of Markevich (1989) (see also Sørensen, 2002). Furthermore, monophyly is supported for several clades at the generic and/or family level, i.e., Lindiidae, Dicranophoridae, Trichocercidae, Lecanidae, Mytilinidae, Euchlanidae, Lepadellidae, Trichotriidae, and Brachionidae.

3.2. Molecular and total evidence analyses

The combined molecular data were analyzed under parsimony direct optimization and Bayesian inference. Subsequently, the combined molecular and morphological data were analyzed under direct optimization. For the
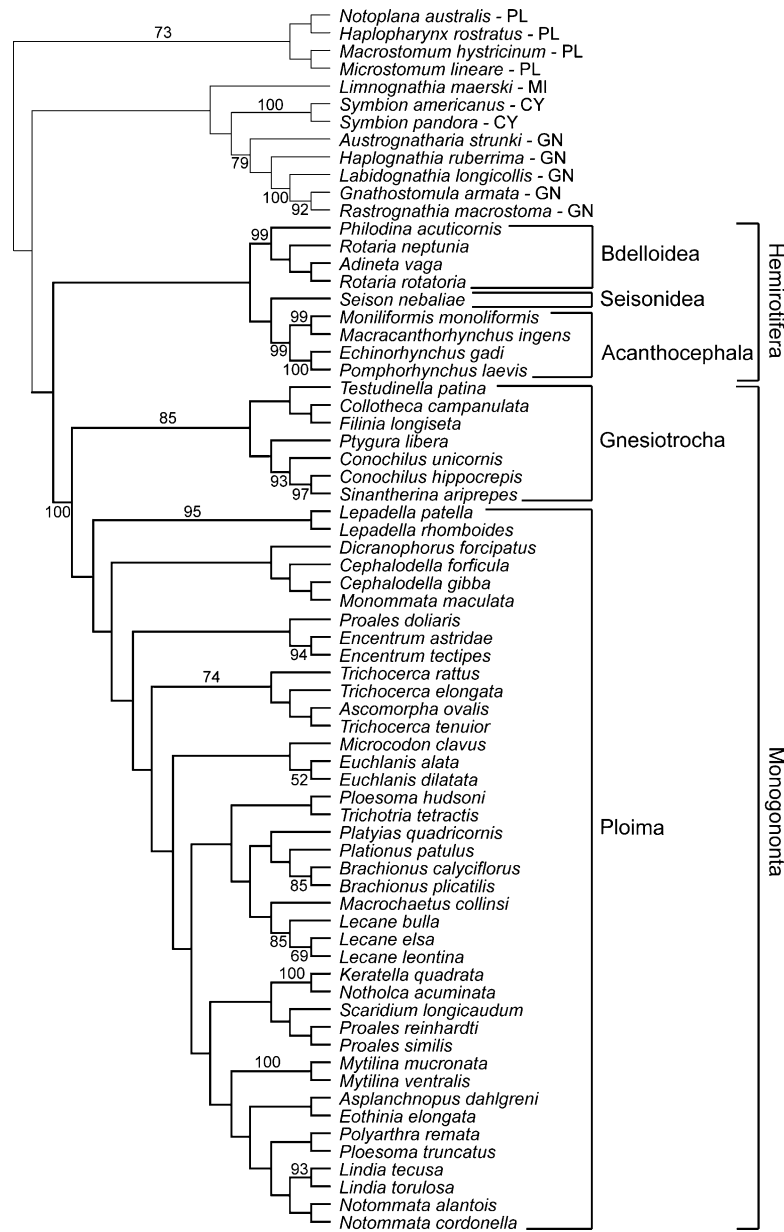
Fig. 3. Single shortest trees at 22,623 weighted steps for the combined analysis of all molecular data analyzed under direct optimization for parameter set 121. Symbols, legends, and abbreviations for outgroups as in Fig. 2.

direct optimization analyses, the congruence measure identified parameter set 121 (indel cost = 2; transversion cost = 2; transition cost = 1) as the "optimal" parameter set (see Table 2). The combined analysis of all molecular data under this parameter set after SATF resulted in a single tree of 22,623 weighted steps (Fig. 3). The tree was almost identical to the resulting trees of the combined molecular and morphological data analyzed under direct optimization, hence both results will be discussed together below.

The AIC implemented in Modeltest chose a general time-reversible (GTR) model with corrections for the proportion of invariant sites and among-site rate variation as the best-fit model for the implied alignment obtained in POY. Under this model specification, MrBayes was exe-

cuted with two runs of 500,000 generations each until PSRF approached 1. We discarded 40% of the trees as burnin, retaining 60,000 trees (30,000 per run). The tree (Fig. 4) shows syndermate monophyly [posterior probability (pp hereafter) of 1.00] and divides it into two main clades consisting of (1) Monogononta (1.00 pp) and (2) Bdelloidea, Seisonidea, and Acanthocephala (1.00 pp). In the latter, Seisonidea and Bdelloidea are sister groups (1.00 pp). Monogononta is divided into Gnesiotrocha (1.00 pp) and Ploima (1.00 pp). *Collotheca campanulata* branches off inside Flosculariaceae, as sister taxon to *Filinia longiseta*.

Within Ploima, the two species representing *Lepadella* branch off as the most basal clade. The analysis generally supports monophyly for all genera, except *Trichocerca*,
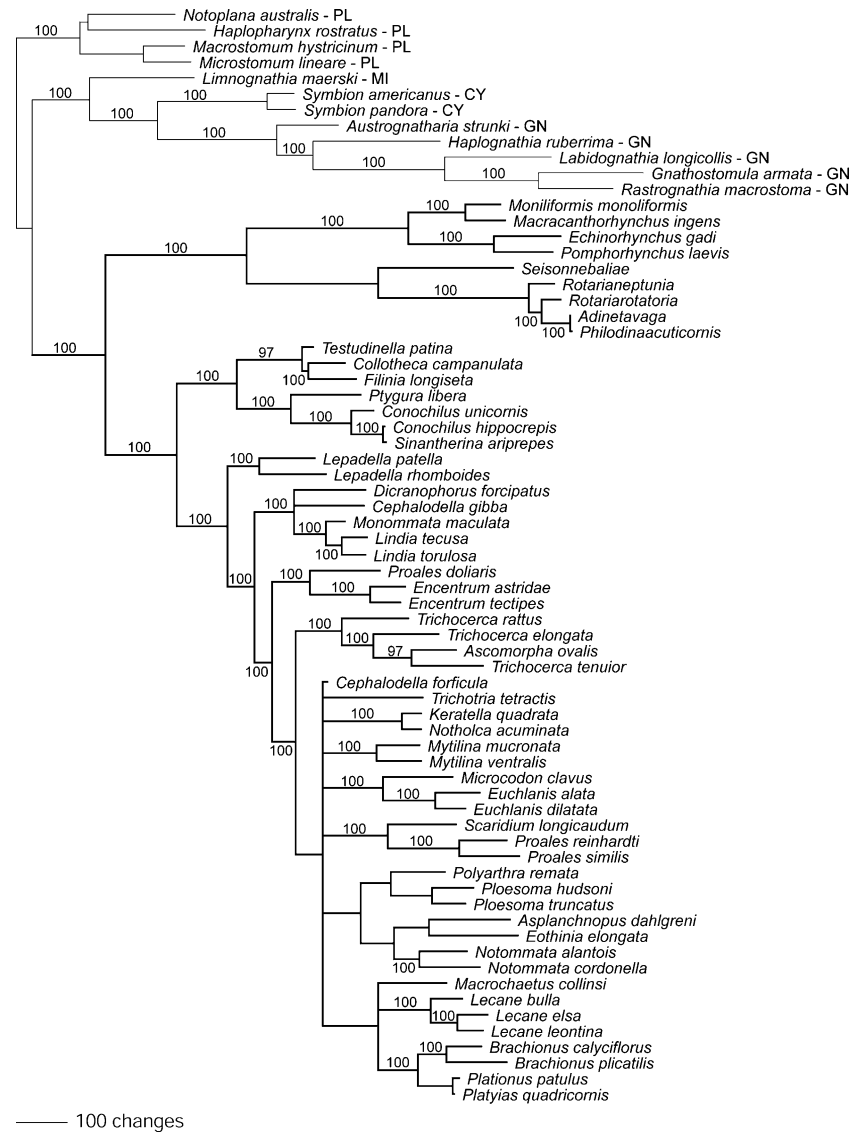
Fig. 4. Fifty percent majority rule consensus of the 60,000 trees retained after discarding the burnin for the Bayesian analysis of phylogeny under a GTR + G + I model of sequence evolution. Numbers on branches indicate posterior probabilities multiplied by 100. Thicker branches indicate ingroup taxa. Abbreviations for outgroups as in Fig. 2.

Proales, and *Cephalodella*. Monophyly is supported for Synchaetidae, but not for Dicranophoridae, Notommatidae, Brachionidae, or Trichotriidae (Fig. 4).

The combined analysis of morphological and molecular data for the optimal parameter set resulted in three trees of 23,005 weighted steps which consensus is presented in Fig. 5. Monophyly for Syndermata is supported under most of the tested parameter sets (Fig. 6), but the jackknife support for parameter set 121 is less than 50%. In all trees with monophyletic Syndermata, Rotifera is paraphyletic with respect to Acanthocephala. Micrognathozoa appears within Rotifera only under parameter set 481 (Fig. 6). In this tree, both Syndermata and Rotifera are paraphyletic with respect to Gnathostomulida, and Micrognathozoa is sister group to Bdelloidea (tree not shown). Congruence plots (= Navajo rugs) for selected relationships involving the major syndermate

clades (Fig. 6) and within Ploima (Fig. 7) are presented to illustrate the analyses' stability to parameter choice.

Under parameter set 121, Syndermata is divided into two clades consisting of (1) Monogononta (100% JF) and (2) Bdelloidea, Seisonidea, and Acanthocephala although this clade has JF below 50%. Within the latter clade, Bdelloidea is sister group to a clade containing Acanthocephala and Seisonidea. Both Acanthocephala and Bdelloidea receive jackknife support values near 100%, whereas JF for Seisonidea + Acanthocephala is below 50% (Figs. 3 and 5).

Monogononta consists of the clades Gnesiotrocha (85% JF with molecular data; 90% JF with combined molecular and morphological data) and Ploima (<50% JF). *Collotheca campanulata* is nested within Flosculariaceae, as sister taxon to *F. longiseta*, hence Flosculariaceae is paraphyletic. Ploima is monophyletic under all tested
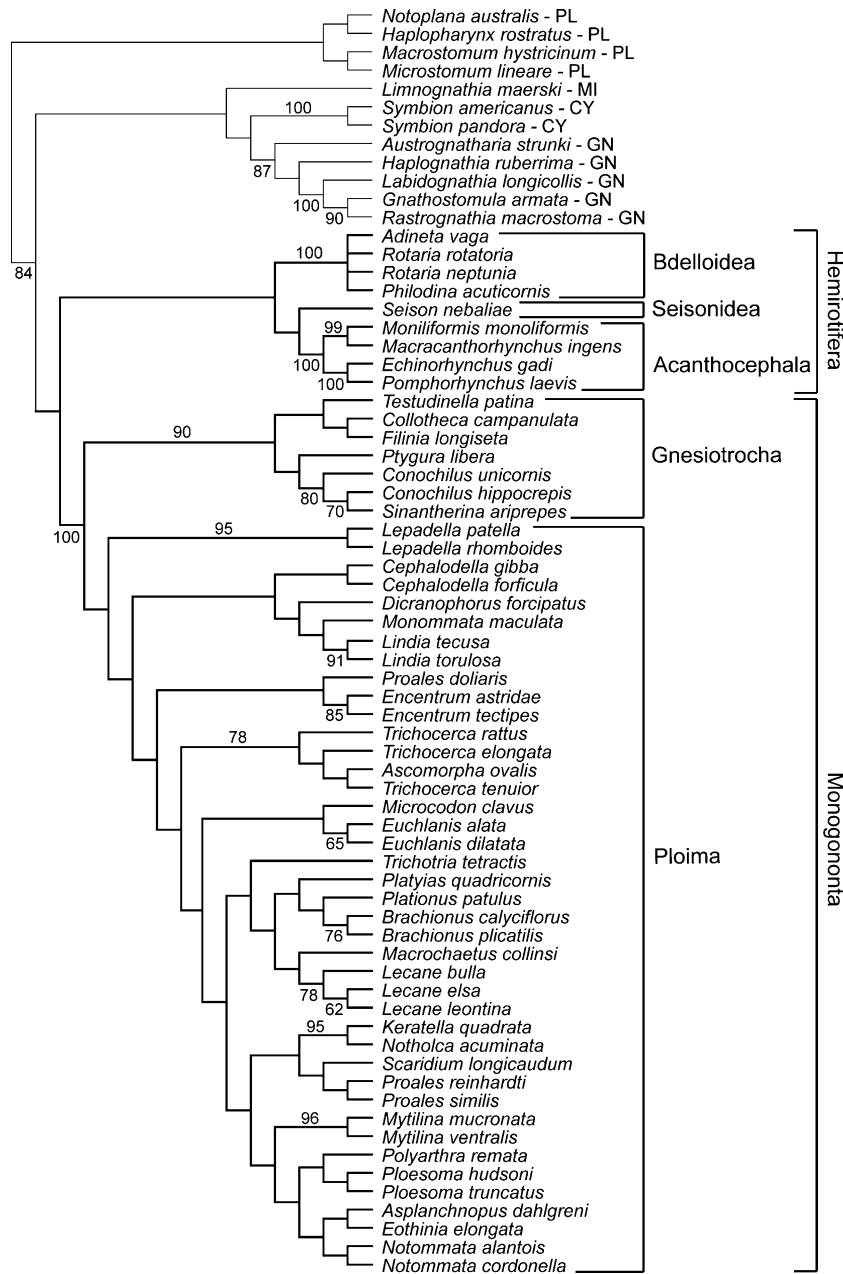
Fig. 5. Strict consensus of three shortest trees at 23,005 weighted steps for the combined analysis of morphology and molecular data analyzed under direct optimization for parameter set 121. Symbols, legends, and abbreviations for outgroups as in Fig. 2.

parameter sets, and the two species of *Lepadella* branch off as the most basal clade under all parameter sets except 481. Under the optimal parameter set (121), the ploimid clades branch off in a ladder-like appearance. However, only few of these clades obtain JF greater than 50%. Monophyly is supported for most genera represented by multiple species. Exceptions are *Proales* (polyphyletic under all parameter sets) and *Trichocerca* (monophyletic under most parameter sets). Five families, Dicranophoridae, Brachionidae, Notommatidae, Trichotriidae, and Synchaetidae, are represented by more than one genus, but monophyly is only unambiguously confirmed for the latter.

4. Discussion

4.1. Acanthocephalans and their implications for rotifer monophyly

Analyses of the combined molecular and morphological data supported syndermate monophyly under most tested parameter sets for the direct optimization analyses (Fig. 6), despite showing jackknife values below 50% (Fig. 5). This is also the case in other deep nodes such as the one uniting the non-monogonont forms, or Seisonidea + Acanthocephala. The possible disconnection between nodal support and nodal stability has been explicitly discussed (Giribet, 2003),
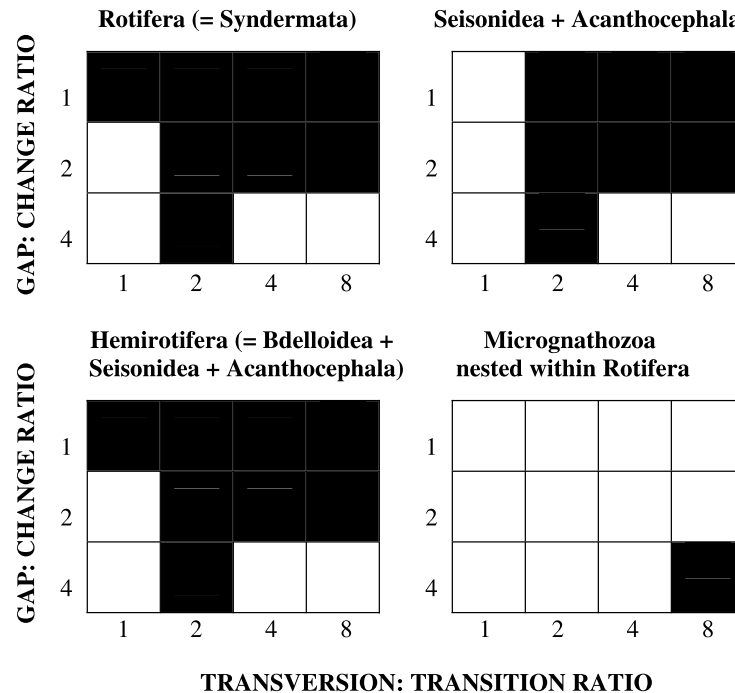
Fig. 6. Congruence plots (Navajo rugs) for selected relationships involving major rotiferan clades (at different gap:change ratios and transversion:transition ratios) based on the parsimony analyses of the combined morphological and molecular data. Black squares indicate monophyly in all trees found under the given parameter set. White squares indicate non-monophyly.

and stability may be preferred over support by some systematists. Syndermate monophyly is also supported in the molecular analyses under direct optimization (Fig. 3) as well as with Bayesian inference of phylogeny (Fig. 4). On the contrary, monophyly of rotifers, excluding acanthocephalans, was only obtained when the morphological data were analyzed alone (Fig. 2), whereas the molecular data and combined data set supported acanthocephalans as being a rotifer ingroup (Figs. 3–6)—i.e., the "syndermatan hypothesis." Rotifer paraphyly with respect to Acanthocephala has been debated for years, and nearly all possible relationships between Acanthocephala and the three rotiferan main groups—Bdelloidea, Monogononta, and Seisonidea—have been proposed (e.g., Wallace, 2002; Herlyn et al., 2003; Funch et al., 2005).

Scrutiny of morphological characters has not provided an unambiguous solution to this issue, due to the scarcity of obviously comparable character traits in the highly specialized, endoparasitic acanthocephalans and the morphologically disparate rotifers. Ahlrichs (1995a,b, 1997) suggested a sister-group relationship between Acanthocephala and Seisonidea based on the presence of bundles of fibers in the mid-zone of the epidermis and dense bodies (probably mitochondrial derivatives) in the spermatozoa. The latter character is problematic because studies on sperm ultrastructure in monogonont rotifers are restricted to species of the genera *Epiphanes*, *Asplanchna*, and *Brachionus* (Melone and Ferraguti, 1999), of which only *Brachionus plicatilis* is included in the present character matrix. Ahlrichs (1995a) furthermore admits that aspects of the acanthocephalan sperm morphology and in particular their mitochondrial

modifications remain unclear, which adds more uncertainty to the suggested character. Based on the current availability of data, we preferred not to include the character in our data matrix for the time being. The similarities between the fine structure of integumental details in Seisonidea and Acanthocephala are certainly a better candidate for a reliable morphological character. However, we hesitate to introduce such a character as it deals with very special ultrastructural details not necessarily reported in studies that precede Ahlrichs (1995a) publication. Interpreting a character trait as "absent," solely because it has not been mentioned in the description, should always be done with caution. We furthermore have trouble accepting the similarity between the arrangement of the fibers in Acanthocephala and Seisonidea [compare Figs. 6 and 7 in Dunagan and Miller (1991) with Fig. 2 in Ahlrichs (1997)], hence we prefer to treat the homology between fibers in acanthocephalans and seisonids as uncertain until more data become available.

In the study of Sørensen et al. (2000), the absence of a sperm acrosome was considered synapomorphic for a clade consisting of Eurotatoria (= Bdelloidea + Monogononta) and Acanthocephala. However, as pointed out above, information about monogonont sperm morphology is scarce and in our data set restricted to *B. plicatilis*, hence we decided not to include the character.

The potential problems with the placement of highly specialized endoparasites in phylogenetic analyses have been discussed by Jenner (2004), who pointed out how extreme morphologies acquired by the parasitic groups may mislead morphological cladistic analyses. In our case,
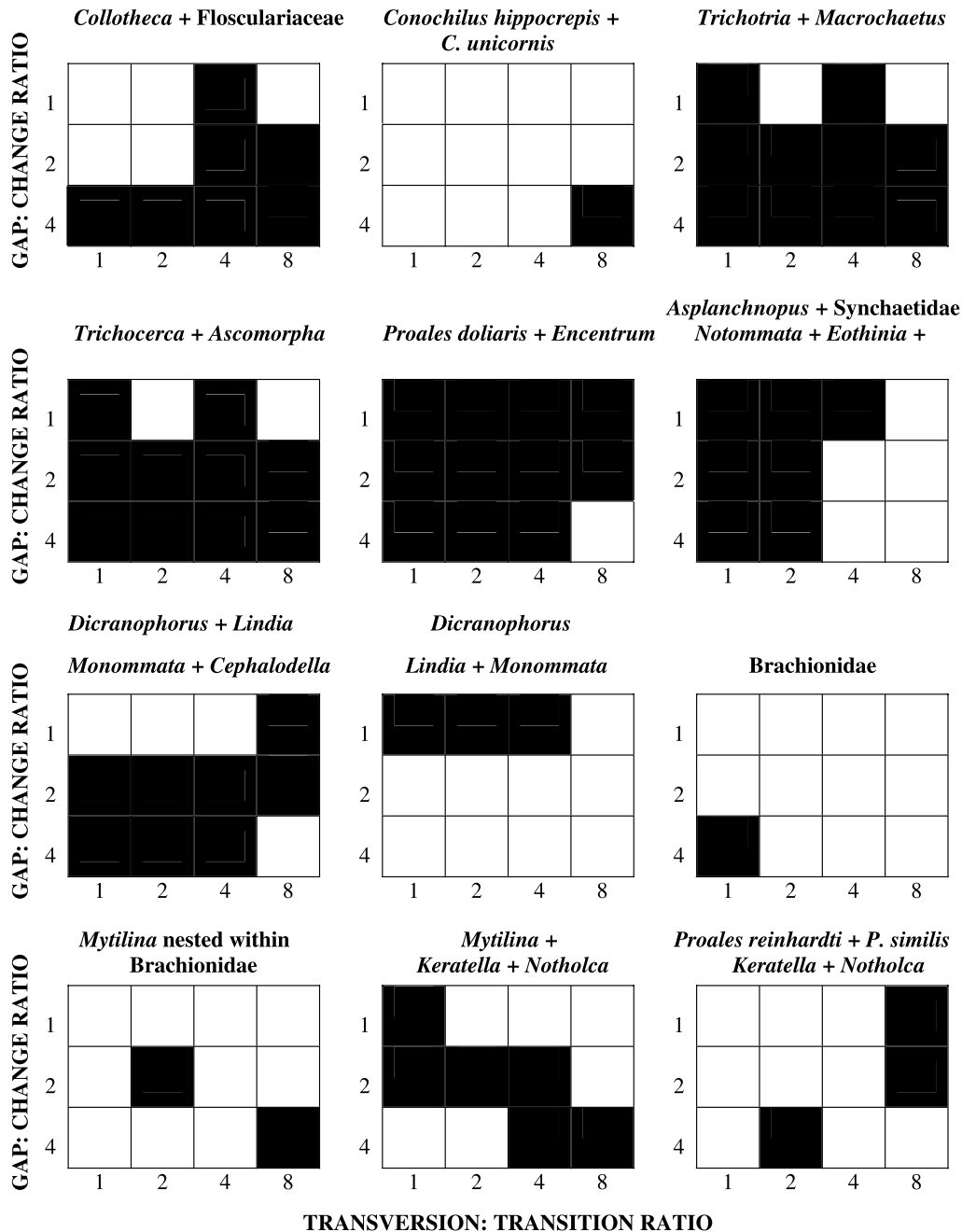
Fig. 7. Congruence plots (Navajo rugs) for selected relationships involving monogonont taxa based on the parsimony analyses of the combined morphological and molecular data. Legends and symbols as in Fig. 6.

we are explicitly choosing to omit the suggested characters due to doubtful homology statements or to inapplicability for most of our terminal species.

The lack of reliable resolution based on morphological characters has prompted a wealth of molecular evidence to attempt to resolve rotiferan/acanthocephalan interrelationships. However, these studies have contributed further to the confusion and proposed acanthocephalans as the sister group to either Seisonidea (Zrzavý, 2001a; Herlyn et al., 2003), Bdelloidea (Garey et al., 1996, 1998; García-Varela et al., 2000; Giribet et al., 2000, 2004), or Eurotatoria (Mark

Welch, 2000; Miquelis et al., 2000). In spite of these incongruities, it is, however, noteworthy that all studies that involve molecular evidences unambiguously support Acanthocephala as a rotifer ingroup (but see Near, 2002). In the present contribution, we corroborate previous results, testing them by using a variety of analytical conditions and different phylogenetic approaches and employing the most comprehensive rotifer taxon sampling so far. Hence, we find it justifiable to formally include Acanthocephala as a class within the phylum Rotifera. This inclusion makes the names Rotifera and Syndermata synonyms, Syndermata

being the junior synonym. For the sake of stability, it is our recommendation to use the phylum name Rotifera instead of its junior synonym Syndermata. It is in this sense that we use the term Rotifera in this discussion, unless it is otherwise indicated.

The position of Acanthocephala within Rotifera remains unsolved. The parsimony analyses supported a sister-group relationship between Seisonidea and Acanthocephala under a majority of the tested parameter sets (Figs. 3, 5, and 6). In this context, it is noteworthy that those parameter sets that reject Seisonidea–Acanthocephala monophyly mostly coincide with those that do not recognize rotiferan monophyly and some of those parameters show the highest amounts of incongruence among data. Thus, the clade appears to be relatively stable to parameter variation although jackknife support for a clade of Seisonidea + Acanthocephala is low, and the analysis under the optimal parameter has a jackknife support lower than 50% (Fig. 5). Furthermore, the combined data sets produced a different result when analyzed with Bayesian inference, and showed an alternative clade consisting of Bdelloidea + Seisonidea as the sister group to acanthocephalans (Fig. 4), but with low posterior probability. In summary, our data appear to favor a Seisonidea–Acanthocephala relationship, but the result requires further corroboration.

In spite of the incongruity between the parsimony and Bayesian analyses, the present results still allow us to evaluate the proposed synapomorphies for both Seisonidea + Acanthocephala (Ahlrichs, 1995a, 1997) and Eurotatoria + Acanthocephala (Sørensen et al., 2000), as discussed above. In all analyses that included molecular data, the reduction of a sperm acrosome would have been homoplastic, which supports the questionable nature of the character. The presence of dense spermatozoon bodies in Acanthocephala and Seisonidea would, on the other hand, have appeared as a non-homoplastic character, but the lack of males (and spermatozoa) in bdelloid rotifers would render this character as ambiguously optimized. The presence of filamental bundles in the epidermis of Seisonidea and Acanthocephala is supported as synapomorphic by the parsimony analyses only (Figs. 3 and 5), as the Bayesian analysis favors a sister-group relationship between Seisonidea and Bdelloidea (Fig. 4). However, ultrastructural data from species of Bdelloidea are extremely scarce, and we still prefer to treat this character with caution until further data from bdelloid species have been obtained.

4.2. The rotiferan main clades

The parsimony analysis under the optimal parameter set and the Bayesian analysis, both supported the division of Rotifera into two major clades consisting of (1) Monogononta and (2) a clade containing Bdelloidea, Seisonidea, and Acanthocephala. While *L. maerski* (Micrognathozoa) has been suggested to belong to Monogononta (De Smet, 2002), only the analysis under parameter set 481 showed Micrognathozoa as nested within Rotifera (Fig. 6). This

cladogram also showed Rotifera and Syndermata paraphyletic with respect to Gnathostomulida and results in the highest incongruence of all parameter sets (Table 2). Instead, based on the congruent results from all other analyses, we find it reasonable to reject *L. maerski* as part of Rotifera. This result has been supported in several recent studies (Sørensen, 2003; Giribet et al., 2004; Funch et al., 2005).

The clade consisting of Bdelloidea, Seisonidea, and Acanthocephala was recognized under most parameter sets (Figs. 5 and 6) and obtains a posterior probability of 1.00 (Fig. 4), hence it can be considered as well supported. Zrzavý (2001b) suggested the name Lemniscea for this clade, although the name was originally proposed for a clade consisting only of Bdelloidea and Acanthocephala (Garey et al., 1996). We prefer to avoid this name, as the presence of lemnisci in Bdelloidea and Seisonidea is doubtful (Ricci, 1998b; see discussion for character 8 in Appendix A), and introduce the new name Hemirotifera instead.

All analyses of morphological, molecular, and combined data supported monogonont monophyly, which agrees with nearly all previous studies (Wallace and Colburn, 1989; Nogrady et al., 1993; Melone et al., 1998a; Sørensen, 2002). The clade is well supported morphologically, and obtains the highest possible jackknife support and posterior probability in the combined analyses of all data (Figs. –3–5).

4.3. Flosculariaceae and Collothecaceae

The class Monogononta is traditionally divided into the superorders Pseudotrocha (with the single-order Ploima) and Gnesiotrocha (Kutikova, 1970; Nogrady et al., 1993; Segers, 2002a). The latter consists of the two orders Flosculariacea and Collothecacea, and is characterized as a group of mostly sessile animals with either malleoramate (Fig. 1C) or uncinate (Fig. 1B) trophi, respectively.

In the present study, Flosculariaceae was represented with seven terminals and Collothecaceae with a single representative, *C. campanulata*. Whereas all analyses supported monophyly of Gnesiotrocha, the parsimony analyses of the combined data only supported the sister-group relationship between Flosculariacea and Collothecacea under certain parameter sets (Fig. 7). Alternatively, *C. campanulata* was positioned as sister group to *F. longiseta* (Figs. 3–5). It is, however, likely that this uncertainty is due to the rather restricted taxon sampling for Collothecacea, and our data do not seem robust or stable enough for rejecting the prevalent hypothesis about a sister-group relationship between the two orders.

Relationships between the included species of Flosculariaceae varied with parameter choice and obtained low jackknife support under the optimal parameter set (Fig. 5). This, together with the obvious problems recovering the position of *C. campanulata*, shows that the proposed relationships within the clade are rather unstable, and probably

not a reflection of the actual relationships. Hence, we do not find that the illustrated paraphyly of the genus *Conochilus* (Figs. 3–5) is likely, as a recent study by Segers and Wallace (2001) shows monophyly of genus.

4.4. Ploima

With ca. 1300 described species, the order Ploima contains more than two-thirds of the total rotifer species. The order consists of 87 genera, 85 according to Segers (2002a) plus two described subsequently by De Smet (2003a,b), and 21 families. Generic monophyly is generally considered well supported within the order, whereas the monophyly is more questionable for some families, i.e., Proalidae and Notommatidae (De Smet, 1996; Sørensen, 2002). Analyses of the ploimid interrelationships have only been attempted once using modern numerical methods (Sørensen, 2002), and our knowledge of this part of the rotifer tree is still extremely limited.

In all analyses of combined molecular and morphological data with the exception of parameter set 481, Lepadellidae, represented with two species of *Lepadella*, was the sister group to all remaining Ploima, whose relationships are extremely parameter-dependant. In all examined trees, the families Notommatidae and Dicranophoridae come out as not monophyletic. All parsimony analyses of combined molecular and morphological data (except under parameter set 481) supported a clade consisting of *Lindia*, *Monommata*, and *Dicranophorus* (Fig. 5) and under most parameter sets a sister-group relationship between this clade and *Cephalodella* was supported (Fig. 7). These affinities were also indicated by the Bayesian analysis, even though the position of *Cephalodella* could not be fully resolved (Fig. 4). The remaining representatives of Dicranophoridae, viz., two species of *Encentrum*, show strong affinities to *Proales doliaris* in most analyses, whereas *Notommata* and *Eothinia* (both Notommatidae) often come out together with *Asplanchnopus* and Synchaetidae (Figs. 4, 5, and 7). The obvious polyphyly of Notommatidae is not surprising, as the family has served to include groups that would not fit in anywhere else. Hence, its status as non-monophyletic has already been noted by several authors (e.g., see Koste and Shiel, 1991; Nogrady, 1995; Segers, 1995; Sørensen, 2002). Under some of the tested parameter sets, the clade consisting of *Cephalodella*, *Lindia*, *Monommata*, and *Dicranophorus* branches off early within Ploima, whereas other analyses place it as the sister group to a clade consisting of the remaining Notommatidae and *Asplanchnopus*. The restricted taxon sampling of the present analyses does not allow us to determine whether the notommatid genera are distantly related and evolved from different positions within Ploima, or whether they are closely related and constitute part of a clade that also contains taxa such as *Asplanchnopus*, *Lindia*, and Synchaetidae. Interesting taxa that should be added to solve this question would be the species of the remaining notommatid genera, but probably also representatives of the genera *Tetrasiphon* and *Itura*.

Contrary to the somehow expected notommatid polyphyly, it was more surprising to find non-monophyly of Dicranophoridae. The family appears to be well supported morphologically (Fig. 2), e.g., by its unique forcipate trophus type (Fig. 1H) (see also De Smet, 1997). It is relatively easy to imagine the transitional series from the robust trophi in *Dicranophorus* to the simpler trophi in *Encentrum* and closely related genera. However, the trophi in *Dicranophorus* and other dicranophorid genera, i.e., *Dorria*, also share some basic similarities with the virgate trophus type (Fig. 1G) and the rami in the cardate trophi present in *Lindia* (Fig. 1I). In previous studies, *Dicranophorus* has been suggested to be one of the most basal ploimid genera, displaying several plesiomorphic traits, i.e., the ventral corona (see Remane, 1929–1933). If the forcipate trophus type likewise is a plesiomorphic trait for Ploima, it could be considered a precursor for the virgate trophus type, which would explain the polyphyly of Dicranophoridae.

Brachionidae is the third large ploimid family with questionable monophyly. The parsimony analysis of the combined molecular and morphological found support for its monophyly only under parameter set 411 (Fig. 7), and in these trees the clade is only supported because of the inclusion of morphological data. The family is represented by species of several different genera in the data set, and in all analyses the genera form two clades consisting of (1) *Brachionidae*, *Platyias*, and *Plationus*, and (2) *Notholca* and *Keratella*. Under some parameter sets, the latter forms a clade with *Proales reinhardti* and *P. similis*, whereas other parameter sets support a sister-group relationship with Mytilinidae (Fig. 7). Interestingly, the two parameter sets support a monophyletic group consisting of all the brachionid species and Mytilinidae together. Based on the present results, we find that it is too premature to reject the possibility of monophyly for Brachionidae, but the position of Mytilinidae and species of *Proales* should probably be taken into account in future studies of the family.

Three species of *Trichocerca* and one species of *Ascomorpha* were included in the data set. These species represent two well-supported families, Trichocercidae and Gastropodidae. Previous studies have already demonstrated some affinities between the families based on morphological data (Sørensen, 2002). This affinity was not confirmed by the morphological analysis in the present study (Fig. 2), but all analyses of the combined molecular and morphological data sets supported a close relationship between these two families. Under most parameter sets, *Ascomorpha* and *Trichocerca* came out as sister groups (Fig. 7), whereas two parameter sets (121 and 181; see Fig. 5) and the parsimony and Bayesian analyses of combined molecular data (Figs. 3 and 4) placed *Ascomorpha* as an ingroup in *Trichocerca*. We consider that the suggested paraphyly of *Trichocerca* is unlikely, because a sister-group relationship between Trichocercidae and Gastropodidae appear quite stable to parameter choice.

The family Lecanidae is represented by three species of *Lecane*, and all analyses confirmed the monophyly of this group. Likewise, monophyly was found for Lindiidae, Mytilinidae, Euchlanidae, and Synchaetidae.

4.5. Future perspectives

Even though the present study represents the most comprehensive molecular data set on rotifers assembled so far and the first combined analysis of molecular and morphological data, many questions await to be addressed. The data set corroborates former evidence for the inclusion of Acanthocephala within the former Rotifera, in the clade Hemirotifera also containing Bdelloidea and Seisonidea, but the exact relationship between them still needs further examination. Future studies should focus on new morphological characters that could be compared among the free-living rotifers and the highly modified acanthocephalans and seisonideans. Furthermore, more comprehensive molecular data, e.g., complete sequences of 28S rRNA (see Mallatt and Winchell, 2002) and the inclusion of more species from these groups, might produce more conclusive results.

Within Ploima, information about the overall relationships between the families is still limited. However, an important step forward is to test whether the ploimid families represent well-supported monophyletic entities. The results of the present study do not resolve many questions concerning the ploimid relationships. Instead, they propose some possible relationships, candidates for further scrutiny, and point out some possible non-monophyletic clades at generic and family levels that should be used with caution in future studies.

5. Conclusions

Analyses of combined molecular and morphological data support monophyly of Syndermata and show that Micrognathozoa is not a member of this clade. Monophyly of Rotifera sensu stricto is not supported by any of the analyses. Instead, most analyses support the clade Hemirotifera—consisting of Acanthocephala, Bdelloidea, and Seisonidea—as sister group to Monogononta. Based on this evidence and previous congruent results, we formally propose the inclusion of Acanthocephala within Rotifera.

All analyses support monophyly of Monogononta, Gnesiotrocha, and Ploima. The sister-group relationship between the gnesiotrochan orders Flosculariaceae and Collothecaceae is only supported in a few trees, but this is most likely an artifact due to insufficient taxon sampling for Collothecaceae. The relationships within Ploima are not resolved, but the analyses indicate that monophyly should be questioned for the families Dicranophoridae, Notommatidae, and Brachionidae. The analyses furthermore support a close relationship between Trichocercidae and Gastropodidae, and confirmed the monophyly for most of the included genera and for the families Lepadel-

lidae, Lindiidae, Euchlanidae, Lecanidae, Synchaetidae, and Mytilinidae.

Appendix A. Morphological character description

All characters were coded as non-additive, unless otherwise indicated.

A.1. General morphology and biology

(1) *Epidermis with intraskeletal lamina: 0 = absent, 1 = present.*

This character refers to the presence of a proteinaceous lamina located in the cytoplasm close to the apical membrane of the epidermal cells. It is absent in gnathostomulids, but present in all rotifers and acanthocephalans, as well as in the dorsal side of *L. maerski*.

(2) *Epidermis structure: 0 = cellular, 1 = syncytial.*

In most animals, the integument consists of a cellular epidermis, but in all rotifer and acanthocephalan species the epidermis is syncytial. This special condition is reflected in the name Syndermata Ahlrichs, 1995.

(3) *Digestive system: 0 = absent, 1 = present.*

The majority of taxa in this analysis possess a fully developed digestive system, including mouth, pharynx, and differentiated gut. However, in the endoparasitic Acanthocephala, the digestive system has been totally reduced.

(4) *Animals sessile or mobile: 0 = all stages mobile; 1 = adults sessile (or attached to other individuals in colony); 2 = adults semi-sessile.*

The character refers to the locomotory capabilities and strategies, as these appear to follow some evolutionary trends. The majority of the taxa in the analysis are mobile throughout their life history, however, the feeding stages in Cycliophora are always sessile (Funch and Kristensen, 1995, 1997). This is also true for most rotifers belonging to Collothecacea and Flosculariacea, as they often reside in tubes made of pellets or gelatinous material, or live attached to other individuals in large colonies. Among the few exceptions are the flosculariid families Testudinellidae and Filiniidae, in the analysis represented by *Testudinella patina* and *F. longiseta*, that are both free-swimming. Species of Seisonidae stay attached most of their life history, even though they are capable of detaching and crawl over for short distances, hence they are coded as semi-sessile.

The character does not apply to the endoparasitic acantho-cephalans, hence these are coded as inapplicable.

(5) *Animals live in large, free-swimming colony: 0 = absent, 1 = present.*

The character refers to the special colonial rotifers, in this analysis represented by *Conochilus hippocrepis* and *C. unicornis.*

(6) *Endoparasites with vertebrate host and intermediate arthropod host: 0 = absent, 1 = present.*

This character refers to a common trait for all acantho-cephalans, namely their special life cycle that always involves a vertebrate main host and an arthropod interme-diate host.

(7) *Endoparasite host choice: 0 = fish host, 1 = mammalian host.*

The acanthocephalan choice of main host may very likely contain phylogenetically relevant information (Herlyn et al., 2003), even though former analysis of acanthocephalan rela-tionships are incongruent (Near et al., 1998; Monks, 2001; Garcia-Varela et al., 2002; Herlyn et al., 2003).

(8) *Lemnisci: 0 = absent, 1 = present.*

Lemnisci are paired syncytial, fluid-filled sacks, present in all acanthocephalans (Hyman, 1951; Ruppert et al., 2004). Their function is unknown, but they might be involved in nutrition uptake and fluid transport. Lorenzen (1985) suggested a homology between the acanthocephalan lemnisci and some hypodermic cushions found in certain bdelloid rotifers. However, Ricci (1998b) demonstrated that these cushions are present in all rotifers with a well-devel-oped corona and serve to accommodate the striated ciliary rootlets. Besides their ectodermal origin, there are no struc-tural similarities between the lemnisci and the rotifer cush-ions, hence we follow the view of Ricci (1998b) and code lemnisci as absent in all rotifers.

(9) *Epidermal lacunar system: 0 = absent, 1 = present.*

The lacunar system is a special acanthocephalan trait that consists of a closed channel system in the epidermis (Hyman, 1951; Ruppert et al., 2004).

(10) *With retractile, ciliated rostrum: 0 = absent, 1 = present.*

The character refers to the ciliated rostrum present in most species of Bdelloidea.

(11) *Protonephridia: 0 = absent, 1 = present.*

Protonephridia are present in all taxa included in the present study, except three acanthocephalan species.

A.2. Reproductive system

(12) *Mode of reproduction: 0 = obligate gamogenetic reproduction, 1 = obligate parthenogenesis, 2 = heterogamic reproduction.*

This character refers to the reproductive strategy. *Lim-nognathia maerski* is coded as either state 1 or 2, because males have not yet been recorded. On the other hand, it cannot be rejected that males might occur during a very short period. All platyhelminths and gnathostomulids are hermaphrodites, and their reproduction is gamogenetic.

Cycliophora is coded as gamogenetic as well because dur-ing a part of their life cycle they reproduce sexually with separate sexes, whereas neither the inner budding nor the formation of pandora larvae correspond to the parthenoge-netic reproduction present in the amictic part of the heter-ogamic cycle in monogonont rotifers.

(13) *Female organs with germovitellarium: 0 = absent, 1 = present.*

A syncytial germovitellarium is present in all rotifers except Seisonidea. The germovitellarium present in some platyhelminths is not considered homologous with the roti-fer germovitellarium.

(14) *Condition of germovitellarium: 0 = unpaired, 1 = paired.*

The rotifer germovitellarium can either be paired, as present in species of Bdelloidea, or unpaired as present in monogononts. Taxa without a germovitellarium are coded an inapplicable for this character.

(15) *Life cycle: 0 = dioecious, 1 = hermaphrodite.*

The characters distinguish between hermaphroditism and having separate sexes. *Limnognathia maerski* is coded with a question mark, since it is yet uncertain whether dwarf males are present during a short period of the life cycle.

(16) *Mode of hatching: 0 = oviparous, 1 = viviparous.*

Most taxa in the analysis are oviparous, but the bdelloid species *Rotaria neptunia* and *R. rotatoria* carry the embryos in a brood chamber until they are fully developed (Donner, 1965).

(17) *Reproductive organs contained inside collagenous lig-ament sack(s): 0 = absent, 1 = present.*

The reproductive organs in acanthocephalans are con-tained inside one or more ligament sacks. Such sacks are not found in any taxa outside Acanthocephala.

(18) *Number of ligament sacks: 0 = one, 1 = two; paired ligament sacks.*

Taxa without ligament sacks are coded an inapplicable for this character.

(19) *Female organs with differentiated bursa and pre-bursa: 0 = absent, 1 = present.*

The female organs in bursovaginoid gnathostomulids consist of a sack-shaped organ, which is differentiated into a bursa and a pre-bursa. The bursal system is not found in taxa outside Bursovaginoidea.

(20) *Self-perpetuation by inner budding: 0 = absent, 1 = present.*

The character refers to the inner budding found in feeding stages of Cycliophora. Similar types of budding are found in certain species of Entoprocta and Ectopr-octa, but their homology is doubtful (Funch and Kristen-sen, 1997). Inner budding does not occur in any other taxa included in this analysis.

A.3. Trophi

(21) *Jaws composed of translucent rods with an electron-dense core: 0 = absent, 1 = present.*

The character refers to the presence of pharyngeal hard parts composed of longitudinally arranged rods with a conspicuous ultrastructure (see Ahlrichs, 1995a; Rieger and Tyler, 1995; Herlyn and Ehlers, 1997; Kristensen and Funch, 2000). Such jaws are present only in Gnathostomulida, Micrognathozoa, and Rotifera (but not in Acanthocephala).

(22) *Fulcrum and rami connected by large hypopharyngeal muscle working as a piston: 0 = absent, 1 = present.*

Most rotifers have a strong hypopharyngeal muscle that attaches anteriorly to the posterior part of the rami and posteriorly to the fulcrum or pharyngeal wall. In some taxa, this muscle is enlarged and pumps like a piston, creating a vacuum in the pharynx.

(23) *Structure of central forceps in jaws: 0 = forceps solely composed of one layer of parallel rods, 1 = homogenous mass with tubes inside.*

The jaws in Gnathostomulida, Micrognathozoa, and the non-parasitic Rotifera contain longitudinally arranged rods, but in the two latter taxa the rods are contained inside a homogenously appearing material and are thus visible only in sections. In Gnathostomulida, however, the central forceps of the jaws, referred to as the articularium (see Riedl and Rieger, 1972), are composed solely of these rods, and the rods are therefore easily visualized with SEM on isolated hard parts (Sørensen, 2000; Sørensen and Sterrer, 2002).

(24) *General appearance of rami: 0 = rami fan-shaped composed of small shaft and curved, close-set teeth, 1 = rami short and bulbous, with long projecting alulae (fulcrate; Fig. 1E), 2 = rami narrow, ribbon-shaped (ramate; Fig. 1A), 3 = rami extremely thin and delicate (uncinate; Fig. 1B), 4 = rami elongate, broadly flattened with fully opened chambers (malleoramate; Fig. 1C), 5 = rami forceps-shaped with inner margins extended into 1–5 tooth-like processes (incudate; Fig. 1F), 6 = rami stout, appearing triangular in cross-section (malleate; Fig. 1D), 7 = rami with apical parts bent characteristically towards dorsal side (virgate; Fig. 1G), 8 = rami elongate, flattened, with large basal chamber filling the apical and basal rami parts (forcipate; Fig. 1H).*

The character refers to the general appearance of the rotifer rami and micrognathozoan main jaws. The rotifer trophi are traditionally divided into nine types, namely fulcrate, ramate, uncinate, malleoramate, incudate, malleate, virgate, cardate, and forcipate. However, since several intermediate types of trophi occur as well, this character is restricted to the general appearance of the rami. The cardate trophus type (Fig. 1I) is mostly defined by the specialized manubria (see character 49), whereas the rami resemble those of the virgate type.

(25) *Rami with basal ramus chambers extremely delicate, forming lateral wing-shaped extensions: 0 = absent, 1 = present.*

Rotifer rami are usually thick and solid structures. This character refers to the extremely delicate rami present in some taxa, i.e., *Microcodon*, *Ploesoma*, *Polyarthra*, and *Synchaeta* (latter not represented in analysis).

(26) *Rami with paired ventral ramus lamellae near midline of rami: 0 = absent, 1 = present.*

The character refers to the paired ventral lamellae, present on the rami of *Ploesoma*, *Polyarthra*, and *Synchaeta*.

(27) *Anterior rami parts containing basal ramus chambers isolated, forming antero-dorsal extending projection: 0 = absent, 1 = present.*

In species of *Cephalodella*, the basal ramus chambers tend to be isolated from the rami, forming a dorsal projection.

(28) *Inner margins of rami densely covered with long scleropili: 0 = absent, 1 = present.*

Dense set digitiform or needle-shaped structures named scleropili may occur in various positions in the rotifer trophi. A special feature for species of Flosculariacea is that the rami inner margins always are densely covered by well-developed scleropili (Fig. 1C).

(29) *Apical parts of rami equipped with strong, curved band of cristae that fit with uncus teeth: 0 = absent, 1 = present.*

A conspicuous curved band of cristae shaped so that it interlocks with the unci teeth in the relaxed trophi, is present in several taxa.

(30) *Ventral sides of rami with anterior processes: 0 = absent, 1 = present.*

Anterior processes are isolated sclerites that attach to the ventral side of the rami through ligaments. The structures are present in different species of Brachionidae.

(31) *Basal apophyses of rami with scleropili: 0 = absent, 1 = present.*

The character refers to the presence or absence of scleropili on the basal ramus apophyses, located basally on the ventral side of the rami.

(32) *Each ramus terminates into prominent terminal tooth: 0 = absent, 1 = present.*

In most species of Dicranophoridae, each ramus terminates into a large, well-defined terminal tooth (Fig. 1H). Other taxa, i.e., *Asplanchnopus* (Fig. 1F), may have rami terminating into a tooth-like process, but the character only codes present for species with a well-defined terminal tooth.

(33) *Rami with slender alulae projecting caudally: 0 = absent, 1 = present.*

The character refers to the presence of caudally or laterocaudally projecting alulae (Figs. 1C and E–G). Alulae are present in various clades within Rotifera (Sanoamuang, 1993; Segers and Wallace, 2001; Sørensen, 2002).

(34) *Rami with cardal apophyses interlocking with unci: 0 = absent, 1 = present.*

In species of Dicranophoridae and Ituridae (latter not represented in analysis), the apical parts of the rami have crests or knobs that interlock with the unci.

(35) *Trophi with unci: 0 = absent, 1 = present.*

This character refers to the presence of unci (Fig. 1), i.e., paired sclerites, often with teeth, located ventroapical to the rami and proximally articulating with the manubria, forming the functional units named mallei. Unci are absent in Gnathostomulida, but present in *L. maerski* (Kristensen and Funch, 2000; De Smet, 2002; Sørensen, 2003), and all jawed

rotifers included in the analysis, inclusive *S. nebaliae* (see Segers and Melone, 1998). In the following characters, the coding related to the micrognathozoan uncus morphology follows the interpretation of Kristensen and Funch (2000) and Sørensen (2003) that suggested a homology between the rotifer unci and micrognathozoan pseudophalangids.

(36) *Apical minor uncus teeth: 0 = absent, 1 = present.*

The unci in species of Bdelloidea and Flosculariacea carry long arrow-like teeth that are differentiated into a median group of broad teeth and groups of apical and/or sub-basal teeth that are thinner (Sanoamuang, 1993; Segers, 1997a; Melone et al., 1998b; Segers and Wallace, 2001; Fontaneto et al., 2003). This character refers to the presence of the apical group of teeth, present in both Bdelloidea and Flosculariacea.

(37) *Sub-basal minor uncus teeth: 0 = absent, 1 = present.*

This character refers to the presence of sub-basal minor uncus teeth, present in Bdelloidea only (Melone et al., 1998b).

(38) *Uncus with scleropilar subuncus: 0 = absent, 1 = present.*

This character refers to the presence of an area densely covered with scleropili on the internal surface of the uncus. A scleropilar subuncus is present in various rotifer species.

(39) *Surface of uncus teeth (non-additive): 0 = no surface structures, 1 = pore located between uncus head and shaft, 2 = uncus tooth with conspicuous jugal line.*

In most species, the uncus teeth have either a distinct pore or a deep cleft, named jugal line after Markevich (1989), located on the external surface of each uncus tooth. However, these structures are absent in *S. nebaliae* and *L. maerski* (Segers and Melone, 1998; Sørensen, 2003). All bdelloids have a pore in the distal part of each uncus tooth (Melone et al., 1998b), whereas monogononts have jugal lines (Markevich, 1989; Sørensen, 2002).

(40) *Appearance of proximal part of uncus teeth: 0 = uncus teeth not connected proximally, 1 = uncus teeth proximally connected by a thin membrane or embedded in more solid uncus shaft.*

The character refers to the difference in uncus morphology that is observed in species of Bdelloidea and Monogononta. In Bdelloidea, the uncus teeth are not connected proximally. Instead, each single tooth articulates directly with the manubria via ligaments (Melone et al., 1998b). In Gnesiotrocha, the uncus teeth fuse with a thin proximal uncus membrane, whereas species of Ploima have a well-developed proximal uncus shaft.

(41) *Size of major uncus teeth with jugal lines or pore: 0 = teeth equally sized, 1 = size gradually increasing from anterior to posterior-most tooth, 2 = one main tooth, others considerably smaller or reduced to a small plate.*

In Bdelloidea and Gnesiotrocha, the major uncus teeth are equally sized, whereas several species of Ploima have a large posterior tooth followed by teeth that gradually decrease in size. Certain ploimid species only have one prominent uncus tooth next to a plate or a group of considerably smaller teeth.

(42) *Trophi with manubria: 0 = absent, 1 = present.*

Manubria articulate with the unci and are located laterally in the trophi (Fig. 1). They are present in bdelloids, monogononts, and in *L. maerski* but absent in Seisonidea. In the following characters, the coding related to the micrognathozoan manubrium morphology follows the interpretation of Kristensen and Funch (2000) and Sørensen (2003) that suggested a homology between the rotifer manubria and micrognathozoan accessory sclerites.

(43) *Manubria compartmentalized: 0 = absent, 1 = present.*

The character refers to the presence of chambers in the proximal manubrium heads of monogonont species.

(44) *Manubria crescentic with posterior extension: 0 = absent, 1 = present.*

The character refers to the morphology of the manubria in Flosculariacea as coded and described by Segers and Wallace (2001).

(45) *Distal cauda present in manubria: 0 = absent, 1 = present.*

The character refers to the presence of a distal rod-shaped cauda, present in all ploimid species.

(46) *Shape of manubrial cauda: 0 = simple rod-shaped, 1 = broadly sickle-shaped.*

The character refers to the special sickle-shaped manubrial cauda present in species of *Brachionus* (see also Segers et al., 1993).

(47) *Intramalleus between manubrium and uncus: 0 = absent, 1 = present.*

The character refers the presence of a small square- or droplet-shaped sclerite, the intramalleus, inserted between the uncus and manubrium (Fig. 1H). The intramalleus occurs in various species of Dicranophoridae (De Smet, 1997; Sørensen, 2001).

(48) *Supramanubrium between manubrium and uncus: 0 = absent, 1 = present.*

The character refers the presence of a larger sclerite, the supramanubrium, between the uncus and manubrium and, which is characterized as a large projection that points towards the main axis of the trophi (Fig. 1H). The supramanubrium occurs in various species of Dicranophoridae (De Smet, 1997; Sørensen, 2001).

(49) *Walls of posterior manubrial chamber distally isolated, forming conspicuous appendage on manubrium: 0 = absent, 1 = present.*

The character refers to the special manubria that are present in *Lindia* (Fig. 1I). In species of *Lindia*, the walls of the posterior manubrial chamber form an isolated appendix that only joins the manubrium proximally (e.g., Segers, 2002b).

(50) *Right manubrium always strongly reduced; left manubrium well developed: 0 = absent, 1 = present.*

The character refers to the condition in Trichocercidae where the right manubrium always is strongly reduced (e.g., see Sanoamuang and Stout, 1993; Segers, 1997b).

(51) *Unpaired fulcrum caudal to rami/main jaws: 0 = absent, 1 = present.*

The character refers to the presence of an unpaired median structure that extends caudally from the articulation point of the rami/main jaws (Fig. 1). A fulcrum is present in Gnathostomulida, Micrognathozoa, Seisonidea, and Monogononta, but not in Bdelloidea (Melone et al., 1998b; Segers and Melone, 1998; Sørensen and Sterrer, 2002; Sørensen, 2002, 2003).

(52) *Fulcrum very short and strongly expanded laterally at distal end: 0 = absent, 1 = present.*

The character refers to the peculiar fulcrum present in species of Lepadellidae. This fulcrum is very short and strongly expanded laterally at its distal end, giving it a triangular appearance from a dorsal view.

A.4. Ciliation

(53) *Ciliation of epidermis: 0 = monociliated, 1 = multiciliated, 2 = non-ciliated.*

The character refers to the number of cilia in the epidermal cells. Acanthocephalans lack epidermal ciliation, whereas gnathostomulids have monociliated epidermal cells. All other taxa in the analysis have multiciliated epidermis.

(54) *Location of cilia from multiciliated cells: 0 = cilia cover entire animal, 1 = ciliation restricted to head region, forming a corona consisting of an undivided circumapical band and a ciliated buccal field.*

In rotifers, the epidermal ciliation is restricted to the cephalic region, where cilia are arranged in conspicuous bands, forming a wheel-organ or corona. The bands may be modified, reduced, or further differentiated but generally the corona is made up by an undivided circumapical band, apically delimited by the trochus and caudally by the cingulum. The non-ciliated area apical to the trochus is called the apical field, and the often densely ciliated area around the mouth, inside the circumapical band, is referred to as the buccal field (Remane, 1929–1933; Nogrady et al., 1993). *Limnognathia maerski* has cephalic ciliation as well, but the cilia do not form a corona consisting of an undivided circumapical band and buccal field. Taxa without multiciliated epidermal cells are coded as inapplicable for this character.

(55) *Corona type: 0 = Seison type, 1 = Philodina/Adineta type, 2 = Collotheca type, 3 = Conochilus type, 4 = Hexarthra type, 5 = Euchlanis type, 6 = Notommata/Dicranophorus type, 7 = Asplanchna type.*

The rotifer corona has traditionally been divided into certain morphological types (Remane, 1929–1933; Ruttner-Kolisko, 1974). The *Seison* type is strongly reduced and only present in the etcoparasitic/commensal Seisonidea. The corona consists of a small ciliated buccal field with two lateral tufts of cilia. Remane (1929–1933) considered it close to the *Asplanchna* type, whereas Ricci et al. (1993) saw it as closer to the *Euchlanis* type. Due to these uncertainties and the special appearance of *Seison* in general, we prefer to code the *Seison* corona in a separate state. The *Philodina* type is present in species of the bdelloid order Philodinida

(Melone and Ricci, 1995) and consists of a well-developed ciliary field and paired trochi forming ciliary disks. Another type, the *Adineta* type, is also present among species of Bdelloida. This type is much more reduced, but yet closely related to the *Philodina* type (Remane, 1929–1933), hence we chose to code both types in the same state. The *Collotheca* type is present only in the Collothecacea, and is characterized by a completely reduced circumapical band and buccal field ciliation modified into a number of stiff tentacle like bristles. The *Conochilus* type is formed by a narrow circumapical band, delimited by well-developed and U-shaped trochus and cingulum. This type is present in species of the flosculariid family Conochilidae. The *Hexarthra* type, in older literature referred to as the *Pedalia* type, is close to the *Conochilus* type. It also consists of a narrow circumapical band, a very well-developed trochus, and a slightly less-developed cingulum. The apical field is large and the rim of the trochus may form large lobes. The *Hexarthra* type is present in all Flosculariacea, exclusive Conochilidae. The *Euchlanis* type is dominated by the large buccal field, and parts of it may be modified into tufts of cirri called pseudotrochi. The circumapical band is a simple band and the rims are not differentiated into trochus and cingulum. The *Euchlanis* type is present in various ploimid clades, including Brachionidae, Euchlanidae, and Lepadellidae. The *Notommata* type also consists of a large buccal field, and the circumapical band forms two tufts of large cilia that may be located on large auricles. The *Dicranophorus* type is very close to the *Notommata* type and is here treated under the same state. The *Asplanchna* type consists of a simple circumapical band without differentiated trochus and cingulum. A buccal field is absent. This type is usually found in planktonic ploimids.

(56) *Corona with auricles: 0 = absent, 1 = present.*

The character refers to the presence of auricles, which are large, paired rostral extensions densely covered with long cilia. Auricles are usually present in the *Notommata* corona type.

(57) *Pseudotroch form tufts with stiff cirri: 0 = absent, 1 = present.*

The character refers to the presence of a pseudotroch with stiff cirri formed by modification of cilia in the buccal field. The pseudotroch is usually present in the *Euchlanis* corona type.

(58) *Corona with trochal discs on pedicles, separated by broad upper lip: 0 = absent, 1 = present.*

In some species of Bdelloidea, two conspicuous pedicles, separated by a broad upper lip, extend rostrally from the head. The pedicles serve as support for the trochal discs (Melone and Ricci, 1995).

A.5. Lorica

(59) *Thickening of integument: 0 = Body illoricate, 1 = Lorica composed of a dorsal and a ventral plate. 2 = Lorica composed of two lateral and a ventral plate, 3 = Lorica fusiform with dorsal ridge(s) on anterior part,*

4 = Lorica disk-shaped without sulci, 5 = Lorica composed of one plate with ventral sulcus.

All rotifers possess an integument with an intracellular filamentous layer, called the intraskeletal lamina. This lamina may be extremely thin in some species, whereas in other species it is extraordinary thick, forming heavy plates that together form a body armor or a so-called lorica. The developmental grade of the lorica is extremely variable, and nothing indicates that the rotiferan lorica evolved only once. However, it is likely that the different lorica shapes and compositions display some phylogenetic signal. The present character defines five different lorica types, mainly based on the number and location of lorica plates, and the presence or absence of sulci between the plates.

(60) *Appearance of lorica composed of dorsal and ventral plates: anterior margins on both plates with conspicuous spines: 0 = absent, 1 = present.*

The character refers to the presence or absence of spines on the anterior margins of the plates in loricas composed of a dorsal plate and a ventral plate. Taxa coded other than state 1 in character 59 are coded as inapplicable for this character.

(61) *Number of spines on dorsal lorica plate: 0 = two spines, 1 = four spines, 2 = six spines.*

The character refers to the number of spines at the anterior margin of the dorsal lorica plate in animals with a lorica composed of a dorsal plate and a ventral plate. Taxa coded other than state 1 in character 60 are coded as inapplicable for this character.

(62) *Dorsal lorica plate with conspicuous ornamentation forming ridges and notches: 0 = absent, 1 = present.*

The character refers to the presence of a conspicuous ornamentation, forming ridges and lines on the dorsal lorica plate.

(63) *Dorsal and ventral lorica plates connected by broad and deep sulcus: 0 = absent, 1 = present.*

The character refers to the conspicuous deep sulcus between the dorsal and ventral plates found in species of *Euchlanis.*

(64) *Ventral lorica plate with distinct transverse fold: 0 = absent, 1 = present.*

The character refers to the distinct transverse fold on the ventral lorica plate, found in species of *Lecane.*

A.6. Other characters

(65) *Caudal foot: 0 = absent, 1 = present.*

Most rotifers have a movable foot that extends from the posterior or in some cases the ventral part of the animal. A foot may be present in sessile as well as mobile animals.

(66) *Appearance of foot: 0 = pseudosegment, 1 = annulated.*

The rotifer foot usually consists of one to a few easily recognizable pseudosegments. In *Ploesoma* and some species of Brachionidae, however, the foot consists of numerous rings and appears distinctively annulated.

(67) *Proximal foot pseudosegment differentiated into pre-pedal fold: 0 = absent, 1 = present.*

In species of Lecanidae, the most proximal foot, pseudosegment is modified into a pre-pedal fold (see Segers, 1997c).

(68) *Foot pseudosegments covered with heavy, lorica: 0 = absent, 1 = present.*

The character refers to the heavily armored foot, present in species of Trichotriidae.

(69) *Foot equipped with toes: 0 = absent, 1 = present.*

In most rotifers, the foot terminates into one or usually two distal toes. In species of Bdelloidea, the number of toes can be even higher. However, toes are absent in sessile species and in furthermore in a few free-swimming species as well. In the latter, the foot terminates into a ciliated area.

(70) *Foot with conspicuous spurs next to toes: 0 = absent, 1 = present.*

The character refers to the flexible paired spurs near the toes, present in species in species of Trichocercidae.

(71) *Toes lengths differ considerably (ratio > 10:1): 0 = absent, 1 = present.*

Rotifer toes are usually equally sized, but in certain species of *Trichocerca* the toes differ in lengths in a ratio greater than 10:1.

(72) *Toes widest medially: 0 = absent, 1 = present.*

Rotifer toes are usually conical or parallel sided, but in some species of *Euchlanis* the toes are clearly widest medially, rather than distally or proximally.

(73) *Pedal glands: 0 = absent, 1 = present.*

This character refers to the presence of pedal glands, which are present in all rotifers. *Limnognathia maerski* has adhesive glands associated with the ventral ciliophores. However, these do not resemble rotiferan pedal glands, but are more similar to the adhesive glands found the polychaete *Diurodrilus* (Kristensen and Funch, 2000).

(74) *Number of pedal glands: 0 = one pair, 1 = multiple glands.*

The character refers to the number of pedal glands that always are fixed to two in species of Ploima, whereas it can be much higher in other rotifers.

Appendix B. Morphological data matrix representing the 74 characters described in Appendix A and coded for the relationships of selected rotiferan species plus outgroups

Question marks (?) indicate missing data, dashes (-) indicate inapplicable character states, and A indicates a polymorphism for 1 or 2

	1	11	21	31	41	51	61	71
Notoplana australis	001000 - 000	100 - 100 - 00	0 - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - 10 - - - - - -	- - - - 0 - - - - -	- - 0 -
Macrostomum hystricinum	001000 - 000	100 - 100 - 00	0 - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - 10 - - - - - -	- - - - 0 - - - - -	- - 0 -
Microstomum lineare	001000 - 000	100 - 100 - 00	0 - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - 10 - - - - - -	- - - - 0 - - - - -	- - 0 -
Haplopharynx rostratus	001000 - 000	100 - 100 - 00	0 - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - 10 - - - - - -	- - - - 0 - - - - -	- - 0 -
Symbion americanus	001100 - 000	100 - 0 - 0 - 01	0 - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - 10 - - - - - -	- - - - 0 - - - - -	- - 0 -
Symbion Pandora	001100 - 000	100 - 0 - 0 - 01	0 - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - 10 - - - - - -	- - - - 0 - - - - -	- - 0 -
Limnognathia maerski	101000 - 000	1A0 - ?00 - 00	1010 - 0 - 000	000010000 -	- 100000000	1010 - - - - 0 -	- - - - 0 - - - - -	- - 0 -
Haplognathia ruberrima	001000 - 000	100 - 100 - 00	100 - - 0 - - - -	0 - 0 - 0 - - - - -	- 0 - - - - - - -	100 - - - - - - -	- - - - 0 - - - - -	- - 0 -
Labidognathia longicollis	001000 - 000	100 - 100 - 10	100 - - 0 - - - -	0 - 0 - 0 - - - - -	- 0 - - - - - - -	100 - - - - - - -	- - - - 0 - - - - -	- - 0 -
Gnathostomula armata	001000 - 000	100 - 100 - 10	100 - - 0 - - - -	0 - 0 - 0 - - - - -	- 0 - - - - - - -	100 - - - - - - -	- - - - 0 - - - - -	- - 0 -
Rastrognathia macrostoma	001000 - 000	100 - 100 - 10	100 - - 0 - - - -	0 - 0 - 0 - - - - -	- 0 - - - - - - -	100 - - - - - - -	- - - - 0 - - - - -	- - 0 -
Austrognatharia strunki	001000 - 000	100 - 100 - 10	100 - - 0 - - - -	0 - 0 - 0 - - - - -	- 0 - - - - - - -	100 - - - - - - -	- - - - 0 - - - - -	- - 0 -
Moniliformis monoliformis	110 - - 11110	000 - 001100	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - 2 - - - - - 0 -	- - - - 0 - - - - -	- - 0 -
Echinorhynchus gadi	110 - - 10110	000 - 001000	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - 2 - - - - - 0 -	- - - - 0 - - - - -	- - 0 -
Pomphorhynchus laevis	110 - - 10110	000 - 001000	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - 2 - - - - - 0 -	- - - - 0 - - - - -	- - 0 -
Macracanthorhynchus ingens	110 - - 11110	100 - 001100	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - 2 - - - - - 0 -	- - - - 0 - - - - -	- - 0 -
Seison nebaliae	111200 - 000	100 - 000 - 00	1111 - 0 - 000	001010000 -	- 0 - - - - - - -	101100000 -	- - - - 10000 -	- - 11
Adineta vaga	111000 - 000	1111000 - 00	1012 - 0 - 000	0000111010	01000 - 0000	0 - 1110000 -	- - - - 100010	0011
Philodina acuticornis	111000 - 000	1111000 - 00	1012 - 0 - 000	0000111010	01000 - 0000	0 - 1110010 -	- - - - 100010	0011
Rotaria neptunia	111000 - 000	1111010 - 00	1012 - 0 - 000	0000111010	01000 - 0000	0 - 1110010 -	- - - - 100010	0011
Rotaria rotatoria	111000 - 000	1111010 - 00	1012 - 0 - 000	0000111010	01000 - 0000	0 - 1110010 -	- - - - 100010	0011
Collotheca campanulata	111100 - 000	1210000 - 00	1013 - 0 - 000	00001000?1	011?0 - 0000	101120000 -	- - - - 10000-	–11
Filinia longiseta	111000 - 001	1210000 - 00	1014000100	0010110021	01110 - 0000	101140000 -	- - - - 0 - - - 0 -	- - 11
Testudinella patina	111000 - 001	1210000 - 00	1014000100	000110021	01110 - 0000	101140004 -	- - - - 10000 -	- - 11
Conochilus unicornis	111110 - 001	1210000 - 00	1014000100	0010110021	01110 - 0000	101130000 -	- - - - 10000 -	- - 11
Conochilus hippocrepis	111110 - 001	1210000 - 00	1014000100	0010110021	01110 - 0000	101130000 -	- - - - 10000 -	- - 11
Ptygura libera	111100 - 001	1210000 - 00	1014000100	0000110021	01110 - 0000	101140000 -	- - - - 10000 -	- - 11
Sinantherina ariprepes	111100 - 001	1210000 - 00	1014000100	0000110021	01110 - 0000	101140000 -	- - - - 10000 -	- - 11
Asplanchnopus dahlgreni	111000 - 001	1210000 - 00	1015000000	0010100021	21?0100000	101170000 -	- - - - 100010	0010
Brachionus calyciflorus	111000 - 001	1210000 - 00	1016000011	1000100121	1110110000	1011501011	2000110010	0010
Brachionus plicatilis	111000 - 001	1210000 - 00	1016000011	1000100121	1110110000	1011501011	2000110010	0010
Keratella quadrata	111000 - 001	1210000 - 00	1016000010	1000100121	1110100000	1011501011	21000 - - - - -	- - 10
Notholca acuminata	111000 - 001	1210000 - 00	1016000010	1000100121	1110100000	1011501011	30000 - - - - -	- - 10
Plationus patulus	111000 - 001	1210000 - 00	1016000011	1000100121	1110100000	1011501011	3100110010	0010
Platyias quadricornis	111000 - 001	1210000-00	1016000010	1000100121	1110100000	1011501011	1100100010	0010
Dicranophorus forcipatus	111000 - 001	1210000 - 00	1018000000	0111100021	2110100000	101160000 -	- - - - 100010	0010
Encentrum astridae	111000 - 001	1210000 - 00	1018000000	010110002 -	- 110101100	101160000 -	- - - - 100010	0010
Encentrum tectipes	111000 - 001	1210000 - 00	1018000000	010110002 -	- 110101100	101160000 -	- - - - 100010	0010
Euchlanis alata	111000 - 001	1210000 - 00	1016000010	1000100121	1110100000	1011500010	0010100010	0110
Euchlanis dilatata	111000 - 001	1210000 - 00	1016000010	1000100121	1110100000	1011500010	0010100010	0110
Ascomorpha ovalis	111000 - 001	1210000 - 00	1117000000	0010100021	2110100000	101170000 -	- - - - 0 - - - - -	- - 10
Lecane bulla	111000 - 001	1210000 - 00	1016000000	0000100?21	1110100000	1011500010	0001101010	0010
Lecane elsa	111000 - 001	1210000 - 00	1016000000	0000100?21	1110100000	1011500010	0001101010	0010
Lecane leontina	111000 - 001	1210000 - 00	1016000000	0000100?21	1110100000	1011500010	0001101010	0010
Lepadella patella	111000 - 001	1210000-00	1016000010	1000100?21	1110100000	111150004 -	0 - - - 100010	0010
Lepadella rhomboides	111000 - 001	1210000 - 00	1016000010	1000100?21	1110100000	111150004 -	0 - - - 100010	0010
Lindia tecusa	111000 - 001	1210000 - 00	1018000000	0010100021	2110100010	101161000 -	- - - - 100010	0010
Lindia torulosa	111000 - 001	1210000 - 00	1018000000	0010100021	2110100010	101161000 -	- - - - 100010	0010
Microcodon clavus	111000 - 001	1210000 - 00	1117100000	0000100021	?110100000	1011?0000 -	- - - - 100010	0010
Mytilina mucronata	111000 - 001	1210000 - 00	1016000010	0000100?21	1110100000	101150002 -	- - - 0100010	0010
Mytilina ventralis	111000 - 001	1210000 - 00	1016000010	0000100?21	1110100000	101150002 -	- - - 0100010	0010
Cephalodella gibba	111000 - 001	1210000 - 00	1117001000	0000100021	2110100000	101160000 -	- - - - 100010	0010
Cephalodella forficula	111000 - 001	1210000 - 00	1117001000	0000100021	2110100000	101160000 -	- - - - 100010	0010
Monommata maculata	111000 - 001	1210000 - 00	1117000000	0010100021	2110100000	101160000 -	- - - - 100010	0010
Notommata alantois	111000 - 001	1210000 - 00	1117000000	0010100021	2110100000	101161000 -	- - - - 100010	0010
Notommata codonella	111000 - 001	1210000 - 00	1117000000	0010100021	2110100000	101161000 -	- - - - 100010	0010
Eothinia elongata	111000 - 001	1210000 - 00	1117000000	0010100021	2110100000	101160000 -	- - - - 100010	0010
Proales doliaris	111000 - 001	1210000 - 00	1016000100	0000100121	1110100000	101160000 -	- - - - 100010	0010
Proales reinhardti	111000 - 001	1210000 - 00	1016000000	1000100121	1110100000	101160000 -	- - - - 100010	0010
Proales similes	111000 - 001	1210000 - 00	1016000100	1010100121	1110100000	101160000 -	- - - - 100010	0010

Appendix B (*continued*)

	1	11	21	31	41	51	61	71
Scaridium longicaudum	111000 - 001	1210000 - 00	1117000000	0010100021	2110100000	101170000-	- - - - 100010	0010
Polyarthra remata	111000 - 001	1210000 - 00	1117110000	0000100001	2110100000	101170000 -	- - - - 0 - - - - -	- - 10
Ploesoma hudsoni	111000 - 001	1210000 - 00	1117110000	0000100001	2110100000	101170005 -	0 - - - 110010	0010
Ploesoma truncatus	111000 - 001	1210000 - 00	1117110000	0000100001	2110100000	101170005 -	0 - - - 110010	0010
Trichocerca elongata	111000 - 001	1210000 - 00	1117000000	0010100021	2110100001	101170003 -	0 - - - 100011	1010
Trichocerca rattus	111000 - 001	1210000 - 00	1117000000	0010100021	2110100001	101170003 -	0 - - - 100011	1010
Trichocerca tenuior	111000 - 001	1210000 - 00	1117000000	0010100021	2110100001	101170003 -	0 - - - 100011	0010
Macrochaetus collinsi	111000 - 001	1210000 - 00	1016000010	?000100?21	1110100000	1011500010	0100100110	0010
Trichotria tetractis	111000 - 001	1210000 - 00	1016000010	1000100?21	1110100000	1011500010	0100100110	0010

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