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Phylogeny of Catenulida and support for Platyhelminthes

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Abstract

Molecular studies have shown that Platyhelminthes is polyphyletic, placing Rhabditophora within Lophotrochozoa, whereas Acoela and Nemertodermatida are separate early bilaterian branches. However, there has been little evidence to support the position of Catenulida, a group that was traditionally classified within Platyhelminthes. In Ehlers' pioneering cladistic system of the Platyhelminthes they were placed as the earliest clade. Other morphologists have considered the Catenulida as an early bilaterian clade separate from Rhabditophora, a position that was supported in an early molecular study. Subsequent molecular phylogenetic studies, which placed Catenulida as the sister group of Rhabditophora with no or low branch support, included 18S rDNA data from only one or two catenulid species. The aims of the present study were (1) to test the putative sister-group relationship of Catenulida and Rhabditophora by improving the taxon sampling of molecular data spanning a larger part of catenulid taxonomic diversity and (2) to provide a phylogenetic framework for the systematization of Catenulida. Twelve catenulid species were sampled around Sweden. Both the 18S rDNA gene and the 28S rDNA gene were sequenced and analysed in a Metazoa-wide data set within parsimony and Bayesian frameworks. The results unambiguously support Catenulida as the sister group of Rhabditophora within Lophotrochozoa. Parsimony-based inferences about the common ancestor of Catenulida and Rhabditophora are presented. A definition of the name Platyhelminthes is suggested.

Keywords: Metazoa; Phylogeny; Bilateria; Ribosomal DNA

Introduction

Catenulida is a group of small worms comprising about 100 species worldwide. Most live in freshwater habitats such as mires, ponds, streams and moist terrestrial habitats where they often are very abundant, whereas the members of the marine Retronectidae are very rare. Catenulids have a simple anatomy and lack sclerotized parts such as copulatory stylets, which makes species identification problematic. Many currently recognized species are regarded as cosmopolitan, perhaps due to the paucity of distinguishing morphological features.

The monophyly of Catenulida is undisputed, with an unpaired, dorsomedially located protonephridium, anterodorsal testes and male genital pore, and aciliary nonmobile sperm as proposed synapomorphies (Ehlers 1985). On the other hand, the phylogenetic position of Catenulida within Bilateria is more controversial. Conventionally the group was classified as a basal clade within the Platyhelminthes (Ehlers 1985). However, Smith et al. (1986) pointed out that there are no known

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morphological synapomorphies uniting the main platyhelminth clades Catenulida, Acoela, Nemertodermatida and Rhabditophora. In a study of bilaterian phylogeny based on morphological characters, Haszprunar (1996) considered Platyhelminthes as paraphyletic, with Acoela, Nemertodermatida and Rhabditophora as the most basal bilaterian clades, followed by Catenulida as sister to the remaining bilaterians.

Attempts to determine the phylogenetic position of Catenulida using rDNA were based on no more than two catenulid species. In the first study using ribosomal 18S rDNA data (Carranza et al. 1997), the single catenulid species *Stenostomum leucops* (Dugs) branched first within Bilateria, separately from Rhabditophora. Zrzavy et al. (1998) proposed a new phylum Catenulida based on parsimony analysis of 18S rDNA and morphological characters (branch support was not evaluated), again involving a single *S. leucops* sequence. The internal phylogeny of Platyhelminthes was analysed by Littlewood et al. (1999a), based on 82 platyhelminth and 13 non-platyhelminth bilaterian 18S rDNA sequences. In their study the four sequences derived from *S. leucops* formed a monophyletic sister group to the Rhabditophora in the most parsimonious tree, but this relationship received no bootstrap support greater than 50%. Subsequent studies, including one *S. leucops* sequence (Peterson and Eernisse 2001) or one *S. leucops* plus one sequence identified as derived from a *Suomina* sp. (Jondelius et al. 2002), also reported no support for a sister-group relationship between Catenulida and Rhabditophora. Partial 28S rDNA sequences from two catenulid species did support such a relationship (Littlewood et al. 1999b), but the Catenulida + Rhabditophora grouping was again not supported by the 18S rDNA data partition in the same study. Telford et al. (2003) found low bootstrap support for a sister-group relationship between Catenulida and Rhabditophora when using the 18S rDNA sequences from *S. leucops* and *Suomina* sp. in combination with new 28S rDNA sequences in a model-based analysis. These conflicting hypotheses are summarized in Fig. 1. The results placing Catenulida and Rhabditophora as sister groups (Peterson and Eernisse 2001; Jondelius et al. 2002; Telford et al. 2003) have been cited as strongly supported by "denser sampling" in a review of the phylogeny of Platyhelminthes (Baguñà and Riutort, 2004). It should be clear from the above that the claim of a strongly supported monophylum consisting of Catenulida and Rhabditophora is a grave distortion of our current understanding of catenulid phylogeny. Low or non-existent bootstrap support based on one or two terminals is not an example of strong support derived from dense taxon sampling. On the contrary, the clade Catenulida + Rhabditophora is highly tentative and needs further testing through acquisition and analysis of more data from a wider diversity of catenulids, so that truly dense taxon sampling can be obtained. New data (from new catenulid taxa) may improve consistency of the tree topology in parsimony analyses (Rydin and Källersjö 2002), whereas inadequate sampling may lead to statistical support for erroneous groupings (Wallberg et al. 2004). Denser taxon sampling of catenulid sequences is clearly desirable.

In the present study we analyse 18S rDNA from a minimum of 12 catenulid species represented by 21 terminals, and 28S rDNA from 10 catenulid species. In order to reconstruct the position of Catenulida we compile a data set spanning as many higher bilaterian groups as possible. Compared to previous studies, the Bilateria-wide combined 18S/28S rDNA data set represents a substantial increase in number of catenulid taxa as well as number of characters. Our aim is to test whether the tentatively preferred hypothesis of a Catenulida + Rhabditophora clade will withstand falsification attempts with more than five times as many catenulid terminals as previously available. In other words: are Catenulida the sister group of Rhabditophora or a high-ranking bilaterian clade? We

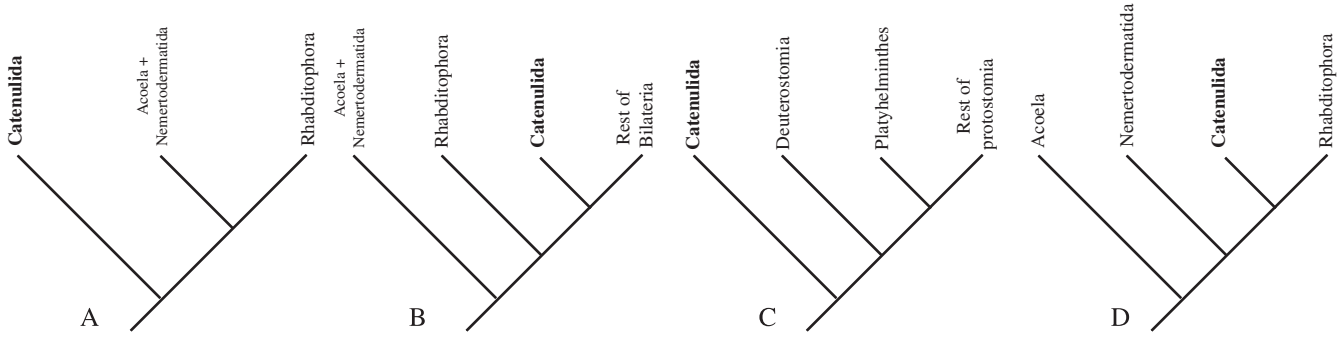

Fig. 1. Schematic illustration of conflicting hypotheses regarding the phylogenetic position of Catenulida. (A) Sister group of all other Platyhelminthes, including Acoela and Nemertodermatida, according to Ehlers (1985). (B) Sister group to all Bilateria except Acoela, Nemertodermatida and Rhabditophora, according to Haszprunar (1996). (C) Sister group to Bilateria according to Carranza et al. (1997). (D) Sister group to Rhabditophora, according to Peterson and Eernisse (2001), Jondelius et al. (2002) and Telford et al. (2003).

also aim at providing a first molecular phylogenetic framework for the development of a classification of the Catenulida. Finally, we use our phylogenetic hypothesis to test whether the marine Retronectidae form the sister group of the freshwater Catenulida, as was proposed by Ehlers (1994).

Material and methods

Collection and identification of species

Catenulids were sampled during 2003–2004 from various locations in Sweden. The specimens were collected by searching the samples with a stereo microscope, then identified live under a microscope equipped with differential interference contrast optics. Photos and drawings were made to document the specimens prior to preservation in 95% ethanol. Specimens that could be assigned to a nominal species are identified in Fig. 2 and Table 2. Two catenulid specimens that could not be assigned to any currently known species are referred to with the provisional names *Stenostomum* 'smallpit' and *S.* 'bigmouth'. The circumscriptions of these and other new species, as well as their phylogeny based on 4 molecular markers, are presented in Larsson et al. (2008).

DNA extraction, PCR amplification and sequencing

DNA was extracted from ethanol-preserved specimens using the DNeasy Tissue Kit (Qiagen) following the manufacturer's protocol. Amplifications were performed with 2 µl DNA extract and 1 µl of each primer, using Ready-To-Go PCR beads (Amersham Biosciences) each containing 2.5 U of PuReTaq DNA Polymerase, 10 mM Tris–HCl, 50 mM KCl and 1.5 mM $MgCl_2$, and 200 µM each of dNTP and stabilizers including bovine serum albumin; the final volume was 25 µl. 18S rDNA was amplified in two overlapping fragments using the primer combination 4fb + 1806R (1200 base pairs) and 5fk + S30 (900 base pairs). 28S rDNA was amplified with the primers LSU5 + L1642R (1450 base pairs). See Table 1 for primer sequences and references.

The PCR conditions for both genes were: 30 s of denaturation at 94 °C; annealing at 45–55 °C for 30 s; extension at 72 °C for 30 s; final extension at 72 °C for 5 min, 35–40 cycles. Products were purified with the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's protocol. The PCR products were sequenced by Macrogene Inc. (Seoul, Korea), using the additional internal primers listed in Table 1. Sequences were assembled and edited using the software STADEN (Judge et al. 2001).

Taxon sampling

We sequenced 18S rDNA from 12 species of Catenulida and 28S rDNA from 10 species. We then searched GenBank for relevant 28S rDNA sequences of at least 1500 nucleotides length. The search yielded 100 28S rDNA sequences, including one additional catenulid sequence. Subsequently, 106 18S rDNA sequences from the same species were downloaded from GenBank, together with three additional 18S rDNA sequences of catenulids. 18S rDNA from the nemertodermatid *Meara stichopi* was also sequenced. The combined data set comprised 125 terminals, out of which 15 lacked 28S rDNA data. Table 2 lists the sequences used and their accession numbers.

Data set for phylogenetic reconstruction

125 18S rDNA sequences and 110 28S rDNA sequences were aligned separately using the software package Hmmer v. 2.3.2 (Eddy 1998). A set of metazoan sequences aligned according to secondary structure was downloaded from the European ribosomal RNA database (Wuyts et al. 2004). This data set was used to create a model of sequence evolution with Hmmer for each gene. The models were then used to align our separate data sets in Hmmer using default parameters (for the models used with Hmmer, see Supplementary material 1 in the online edition of this paper).

Hypervariable sites were detected by frequency of gaps, >15% for the 18S rDNA data and >50% for the 28S rDNA data. The final aligned data sets were 1662 bp (18S rDNA) and 2035 bp (28S rDNA); these were subsequently concatenated.

Phylogenetic reconstruction

A Bayesian inference (BI) analysis was conducted on the combined data set, using the software MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003) and a GTR + I + Γ model with four MCMC chains running for 2 million generations that were sampled every 100 trees. The result of the BI analysis was summarized in a 95% majority rule consensus tree, excluding a burn-in of 850,000 generations. This type of consensus was chosen, since Bayesian posterior probabilities (BPP) above 0.95 had at least 95% probability of recovering true clades in simulation studies (Erixon et al. 2003).

Parsimony jackknifing was performed on the combined data set, using the software TNT (Goloboff et al. 2003) and the following parameters: 1000 jackknife replicates each with 50 random additions and TBR branch swapping, with a deletion frequency of 36%. Such analyses were also performed on the separate 18S rDNA and 28S rDNA data sets. Finally, to test for

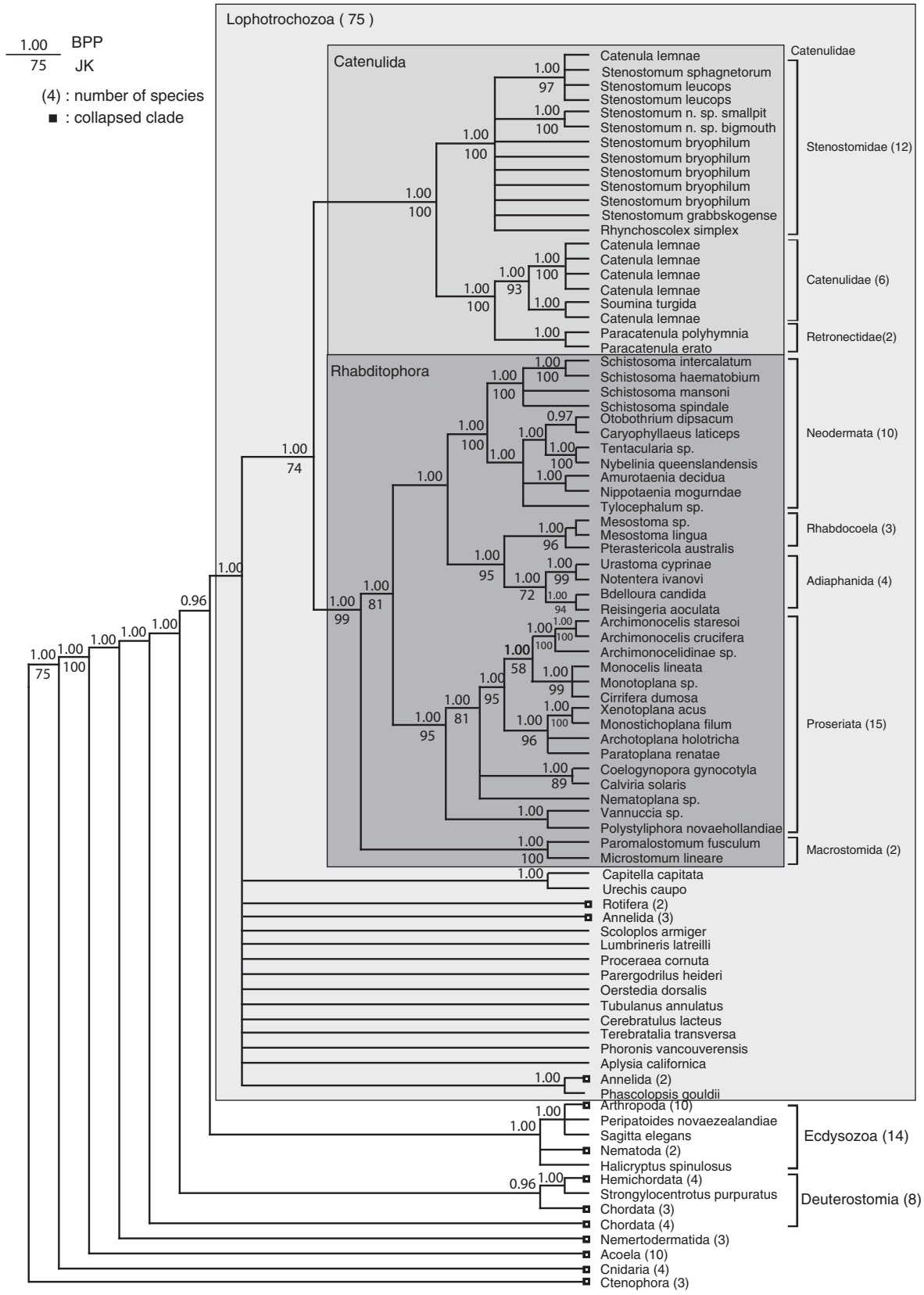

Fig. 2. 95% majority rule consensus tree summarizing Bayesian analysis and parsimony jackknifing of a combined 18S rDNA and 28S rDNA data set. Bayesian posterior probabilities (BPP) given above branches, jackknife frequencies (JK) higher than 50% below branches. Bayesian analyses used a GTR + I + Γ model with four MCMC chains running for 2 million generations, and a burn-in of 850,000 generations. Parsimony jackknifing used 1000 jackknife replicates with 50 random additions and TBR branch swapping.

Table 1. Primers used for amplification and sequencing

Primer	Gene	Used for	Primer sequence 5′-3′	Reference
S30	18S	PCR	GCTTGTCTCAAAGATTAAGCC	Norén and Jondelius (1999)
5fk	18S	PCR	TTCTTGGCAAATGCTTTCGC	Norén and Jondelius (1999)
4fb	18S	PCR	CCAGCAGCCGCGGTAATTCCAG	Norén and Jondelius (1999)
1806R	18S	PCR	CCTTGTTACGACTTTTACTTCCTC	Norén and Jondelius (1999)
7fk	18S	Sequencing	GCATCACAGACCTGTTATTGC	Norén and Jondelius (1999)
4fbk	18S	Sequencing	CTGGAATTACCGCGGCTGCTGG	Norén and Jondelius (1999)
7f	18S	Sequencing	GCAATAACAGGTCTGTGATGC	Norén and Jondelius (1999)
5f	18S	Sequencing	GCGAAAGCATTTGCCAAGAA	Norén and Jondelius (1999)
L300F	28S	PCR/sequ.	CAAGTACCGTGAGGGAAAGTTG	Littlewood et al. (2000)
LSU5	28S	PCR/sequ.	TAGGTCGACCCGCTGAAYTTAAGCA	Littlewood et al. (2000)
L1642R	28S	PCR/sequ.	CCAGCGCCATCCATTTTCA	Lockyer et al. (2003)

potential long-branch attraction between Catenulida and Rhabditophora, an additional parsimony jackknifing analysis of the combined data set excluding the Rhabditophora species was performed in TNT with the same parameters as above.

Results

Fig. 2 shows the 95% majority rule consensus tree from the Bayesian analysis of the combined data set, with parsimony jackknife frequencies indicated where >50%. The parsimony analysis resulted in 669 most parsimonius trees with a length of 29,854 and a C.I. of 0.2015. A phylogram showing inferred branch lengths in the Bayesian analysis is given in Fig. 3.

A monophyletic Catenulida (1.00 BPP/100% jackknife) is the sister group (1.00/74) of the monophyletic Rhabditophora (1.00/99). Acoela (1.00/100) and Nemertodermatida (1.00/74) are basal bilaterian clades separate from the rhabditophorans. Within Catenulida, the Stenostomidae (1.00/100), Catenulidae (1.00/93) and Retronectidae (1.00/100) are monophyletic, with the exception of one of the *Catenula lemnae* specimens, which is positioned among the Stenostomidae. The marine Retronectidae are the sister group of Catenulidae (1.00/100). Catenulidae + Retronectidae form the sister group of Stenostomidae (1.00/100). Within the Rhabditophora the monophyletic Neodermata (1.00/100), Rhabdocoela (1.00/96), Adiaphanida (1.00/72), and Proseriata (1.00/95) are congruent with the results of Norén and Jondelius (2002), and Willems et al. (2006).

The parsimony jackknifing analysis of the 18S rDNA data set resulted in a tree (see Supplementary material 2) similar to the one from the combined data set (Fig. 2). The Rhabditophora–Catenulida clade had 83% jackknife frequency, higher than that in the combined analysis. The rhabditophoran groups Adiaphanida and Rhabdocoela were not supported. Acoela and Nemertodermatida were separate from the Rhabditophora but not positioned basally in Bilateria. The separate 28S rDNA parsimony jackknife analysis resulted in a topology with less resolution than the 18S rDNA data set. The parsimony analysis of the combined data set with the Rhabditophora species excluded did not alter the position of Catenulida among the Lophotrochzoa (Supplementary material 3).

Discussion

The results presented here are congruent with previous analyses of 18S rDNA (Peterson and Eernisse 2001; Jondelius et al. 2002; Telford et al. 2003) but support the Rhabditophora–Catenulida sister-group relationship more strongly. The position of one of our *Catenula lemnae* terminals among the Stenostomidae is most probably due to a contaminated sample or a confusion between samples, since the morphological differences between *Catenula* species and *Stenostomum* species are so distinct that a misidentification is unlikely. Unfortunately, the available material of the erroneously placed *C. lemnae* specimen did not allow resequencing. This underscores the importance of adequate taxon sampling, so that misidentifications can be detected. An example of inadequate taxon sampling can be studied in Carranza et al. (1997), where the single sequence representing the Nemertodermatida (“Nemertodermate” in their figures) is actually derived from a misidentified proseriate, thus groups within Rhabditophora (Jondelius et al. 2002).

Controlling for long-branch attraction

Rate heterogeneity among taxa could lead to incorrect topologies being supported in phylogenetic analyses, so-called long-branch attraction (LBA; Felsenstein 1978). Parsimony is considered more sensitive to this artifact

Table 2. Sequences used and their GenBank accession numbers

Taxa	18S rRNA	28S rRNA
Acoela		
Actinoposthia beklemischevi	ABE012522	AJ849491
Anaperus biaculeatus	ABI012527	AY157602
Childia groenlandica	AY078365	AY157603
Childiidae sp.	AY297954	AJ849498
Mecynostomum auritum	AJ845244	AJ849493
Paraphanostoma brachypostium	AY297952	AJ849499
Paraphanostoma cyclopostium	AF329178	AJ849494
Paraphanostoma macroposthium	AY297951	AJ849500
Paraphanostoma submaculatum	AY297953	AJ849496
Paratomella rubra	AF102892	AY157604
Annelida		
Capitella capitata	AF508118	AY364863
Ctenodrilus serratus	AY340426	AY364864
Eisenia fetida	AB076887	AF212166
Eurythoe complanata	AY040685	AY364849
Hirudo medicinalis	AF116011	AY364866
Hrabeiella periglandulata	HPE310501	AY364867
Lumbrineris latreilli	AB106247	AY366512
Ophelia rathkei	AF448157	AY366513
Parergodrilus heideri	PHE31050	AY366514
Procera cornuta	AF474312	AF212165
Scoloplos armiger	AY53267	AY366515
Arthropoda		
Aponomma concolor	AF018643	AF199116
Baculume tradentatum	AY121173	AY125313
Catomerus polymerus	AY520648	AY520614
Haemaphysalis humerosa	AF018646	AF199115
Limulus polyphemus	LPU91490	AF212167
Nasutitermes sp.	AY491151	AY125280
Pollicipes pollicipes	AY52065	AY52065
Semibalanus balanoides	AY520626	AY520592
Triops longicaudatus	AF144219	AY157606
Verruca stroemia	AY520649	AY520615
Brachiopoda		
Phoronis vancouverensis	AY210450	AF342797
Terebratalia transversa	AF025945	AF342802
Catenulida		
Catenula lemnae[a]	FJ196318	FJ196336
Catenula lemnae[a]	FJ196322	—
Catenula lemnae[a]	FJ196323	—
Catenula lemnae[a]	FJ196324	—
Catenula lemnae[a]	FJ196325	—
Catenula lemnae[a]	FJ196321	—
Paracatenula cf. *erato*	AY218103	—
Paracatenula cf. *polyhymnia*	AY218104	—
Rynchoscolex simplex[a]	FJ196328	FJ196340
Suomina turgida[a]	FJ196329	FJ196339
Stenostomum 'bigmouth'[a]	FJ196330	FJ196341
Stenostomum bryophilum[a]	FJ196319	
Stenostomum bryophilum[a]	FJ196320	
Stenostomum bryophilum[a]	FJ196326	FJ196337
Stenostomum bryophilum[a]	FJ196333	FJ196343
Stenostomum bryophilum[a]	FJ196334	FJ196344
Stenostomum grabbskogense[a]	FJ196327	FJ196338
Stenostomum leucops	LE012519	AY157151
Stenostomum leucops[a]	FJ196332	FJ196342
Stenostomum 'smallpit'[a]	FJ196331	—
Stenostomum sphagnetorum[a]	FJ183793	FJ196335
Chaetognatha		
Sagitta elegans	Z19551	AF34279
Chordata		
Branchiostoma floridae	M97571	AF061796
Oikopleura sp.	AB013015	AF158726
Petromyzon marinus	M97575	AF061798
Raja schmidti	AF278682	AF278683
Styela plicata	M97577	AF158724
Thalia democratica	TDE18SJ	AF158725
Triakis semifasciata	AF212180	AF212182
Cnidaria		
Atolla vanhoeffeni	AF100942	AY026368
Hydra circumcincta	AF358080	AY026371
Montastrea franksi	AY026382	AY026375
Nectopyramis sp.	AF358068	AY026377
Ctenophora		
Pleurobrachia bachei	AF293677	AY026378
Beroe ovata	AF293694	AY026369
Mnemiopsis leidyi	L10826	AY026373
Echinodermata		
Strongylocentrotus purpuratus	L28056	AF212171
Echiura		
Urechis caupo	F342805	AF342804
Hemichordata		
Cephalodiscus gracilis	AF236798	AF212172
Harrimania planktophilus	AF236799	AF212173
Ptychodera flava	AF278681	AF212176
Saccoglossus kowaleskii	L28054	AF212175
Mollusca		
Aplysia californica	AY039804	AY026366
Nematoda		
Chordodes morgani	AF036639	AF342787
Trichinella spiralis	AY497012	AF342803
Nemertinea		
Cerebratulus lacteus	AY145368	AY145396
Oerstedia dorsalis	AY928353	AY210465
Tubulanus annulatus	AY210452	AY210473
Nemertodermatida		
Meara stichopi[a]	AF119085	
Meara stichopi		AY157605
Nemertoderma bathycola	AF327725	
Nemertoderma westbladi	AF327726	
Onychophora		
Peripatoides novazealandiae	AF342794	AF342791
Priapulida		
Halicryptus spinulosus	AF342790	AF342789

Table 2. (*continued*)

Taxa	18S rRNA	28S rRNA
Rhabditophora		
Amurotaenia decidua	AF124474	AF286932
Archimonocelidinae sp.	ASP27015	AJ270164
Archimonocelis crucifera	ACR27015	AJ270163
Archimonocelis staresoi	AST27015	AJ270166
Archotoplana holotricha	AEL24367	AJ270165
Bdelloura candida	BCZ99947	AJ270167
Calviria solaris	CSO27015	AJ270168
Caryophyllaeus laticeps	CLA28748	AF286911
Cirrifera dumosa	CDU27015	AJ270169
Coelogynopora gynocotyla	CGY24367	AJ270170
Mesostoma lingua	MLI27015	AJ270171
Mesostoma sp.	MLI24368	
Microstomum lineare	MLU70082	AJ270172
Monocelis lineata	MLU45961	AY157159
Monostichoplana filum	MFI27015	AJ270173
Monotoplana sp.	MCF27015	AJ270174
Nematoplana sp.	NSP27016	AJ270175
Nibelinia queenslandensis	AF287005	AF286975
Nippotaenia mogurndae	NMO28754	AF286934
Notentera ivanovi	NIV28754	AY157167
Otobothrium dipsacum	ODI28755	AF286972
Paramalostomum fusculum	PFU01253	AY157155
Paratoplana renatae	PRE01251	AJ270176
Polystyliphora novahollandiae	PNO27016	AJ270177
Pterastericola australis	PAU01251	AY157161
Reisingeria aoculata	AF065426	AY157157
Schistosoma haematobium	Z11976	AJ223838
Schistosoma intercalatum	AY157235	AJ223841
Schistosoma mansoni	SMU65657	
Schistosoma spindale	Z11979	Z46505
Tentacularia sp.	AF124461	AF286976
Tylocephalum sp.	TSP28758	AF286929
Urastoma cyprinae	AF167422	AY157165
Vannuccia sp.	VSP27016	AJ270180
Xenotoplana acus	XAC27015	AJ270181
Rotifera		
Philodina roseola	AF154567	AY210469
Sinanthera socialis	AY210451	AY210471
Sipunculida		
Phascolopsis gouldii	AF342796	AF342795

[a]Sequenced for present study.

than some model-based inference methods that compensate for branch-length differences as part of the substitution models, at least when the model used is not violated. A number of different strategies to control for LBA have been suggested: denser taxon sampling to break up long branches, exclusion of potential long-branch attractors such as outgroup taxa, and method concordance between parsimony and model-based approaches (for a recent review, see Bergsten 2005). Is the Rhabditophora–Catenulida clade found in our analyses an effect of LBA? We controlled for long-branch attraction by using a large number of catenulid terminals in our analyses, by using both parsimony and Bayesian phylogenetic reconstruction, and by excluding the hypothesized sister group of the Catenulida (Rhabditophora) from one of the parsimony analyses. Parsimony and Bayesian analyses both supported a Rhabditophora–Catenulida clade. Exclusion of the Rhabditophora did not alter the position of Catenulida within the Lophotrochozoa. Furthermore, we examined assigned branch lengths in one of the 669 most parsimonious trees from the combined 18S + 28S rDNA data set (Supplementary material 4). The three longest branches belong to the nematode *Trichinella* (482 steps), the chaetognath *Sagitta* (480 steps) and the rotiferan *Philodina* (415 steps), respectively. None of these group within or as sister group to Catenulida or Rhabditophora. The average assigned branch length in the tree is 121 steps. The two branches connecting Catenulida and Rhabditophora, respectively, to the rest of the tree are 134 and 184 steps long. As can be seen in Fig. 3, the longest inferred branches in the Bayesian analysis do not belong to Catenulida or Rhabditophora, but are nested within Deuterostomia, Lophotrochozoa and Ecdysozoa.

The parsimony jackknife support for the monophyletic Rhabditophora–Catenulida group is 74. The Bayesian posterior probabilities for Catenulida–Rhabditophora are at the maximum value of 1.00. Based on these results we regard our hypothesis as a current best estimate of the catenulid phylogenetic position, but more sequence data, e.g. from protein coding genes, and developmental data, lacking for the Catenulida, are highly desirable.

Sister-group relationship of Catenulida and Rhabditophora

The position of Catenulida as the sister group of Rhabditophora differs from Ehlers' (1985) pioneering hypothesis, in which Catenulida was regarded as the sister group of Rhabditophora + Acoela and Nemertodermatida. The latter two taxa are now considered basal within the Bilateria separate from the Rhabditophora (Ruiz-Trillo et al. 1999, 2002; Jondelius et al. 2002; Telford et al. 2003; Wallberg et al. 2007). Based on morphology, Ehlers (1994) regarded Catenulida as a monophyletic group and positioned the marine Retronectidae as the sister group to all other catenulids. This is not in congruence with our results, in which Retronectidae + Catenulidae forms the sister group of Stenostomidae. It should be noted that Ehlers, when framing his hypothesis, assumed monophyly of Catenulida, Acoela, Nemertodermatida and Rhabditophora, i.e. Platyhelminthes sensu lato, which necessitated ad hoc

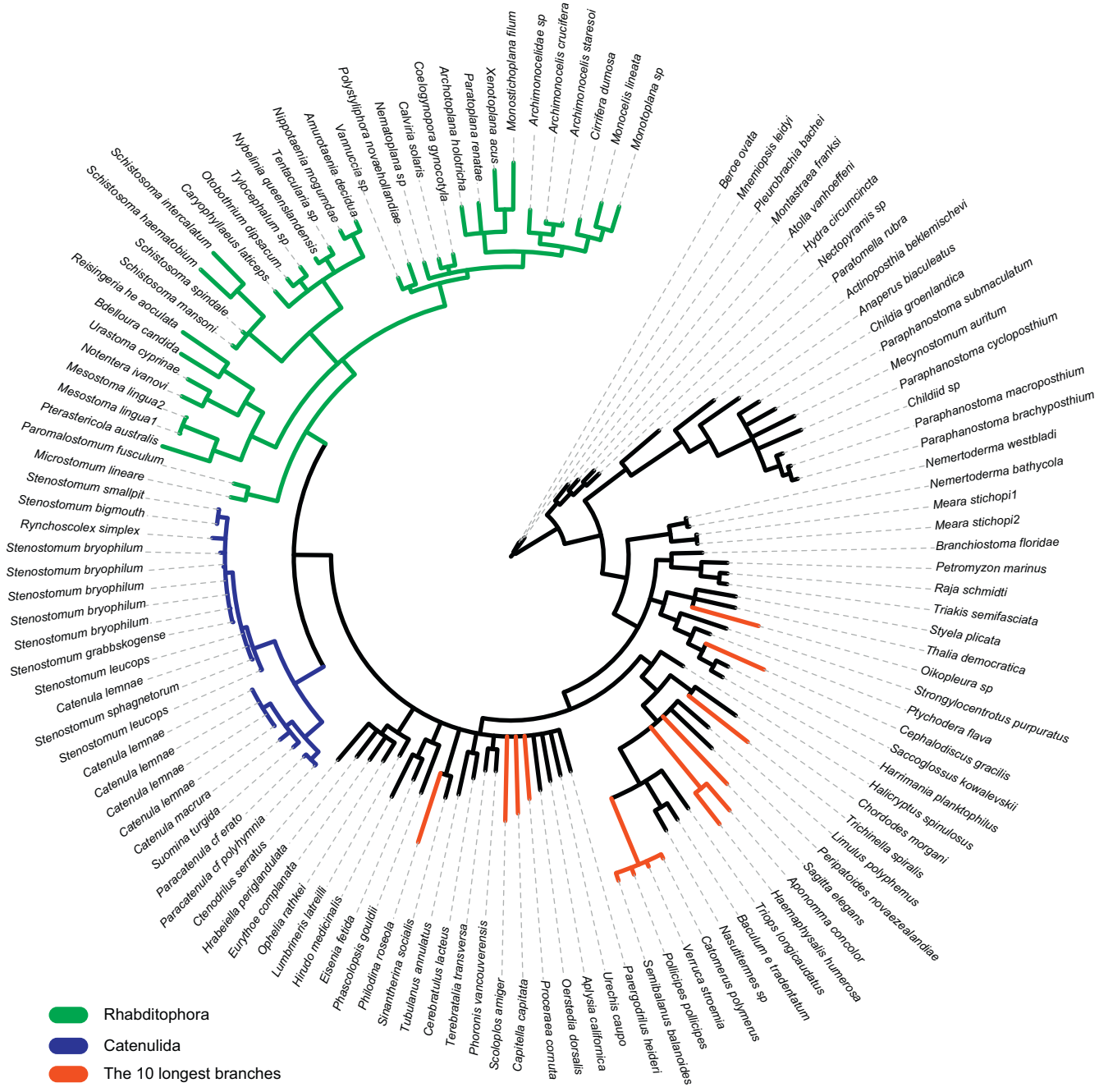

Fig. 3. Phylogram of 95% majority rule consensus tree from Bayesian analysis of combined data set. The 10 longest inferred branches are all outside Catenulida and Rhabditophora.

explanations of the reduction of protonephridia in Acoela and Nemertodermatida. A sister-group relationship between Catenulida and Rhabditophora was discussed by Smith et al. (1986), but the evidence from morphology was considered inconclusive. The sister-group relationship between Catenulida and "eubilaterians" tentatively proposed by Haszprunar (1996) is incompatible with our analyses. There is no real character conflict here, as Haszprunar, too, considered the morphological evidence for the phylogenetic position of the Catenulida as "open to debate". Moreover, it appears that there still are no known morphological synapomorphies uniting Catenulida and Rhabditophora (Baguñà and Riutort 2004).

The ancestor of Catenulida and Rhabditophora

Here, we offer some parsimony-based inferences about the common ancestor of Catenulida and Rhabditophora,

i.e. Platyhelminthes as defined above. That ancestor likely was a hermaphrodite with internal fertilization. Depending on the phylogenetic position of Platyhelminthes within the protostomes, which is yet unclear, these two traits may be apomorphies. Furthermore, the ancestor was a benthic small worm using ciliary locomotion. Direct development, no anus, and anterior brain are likely plesiomorphies present in the most recent ancestor of the Platyhelminthes. Statocysts occur in some taxa within both Catenulida and Rhabditophora, but their morphology differs widely, and independent evolution is likely (Ehlers 1991). The single biflagellate protonephridium of catenulids surely is an autapomorphy. All catenulids have an anterior mouth opening, a condition that is relatively uncommon within the Rhabditophora, even though it occurs both within the Macrostomida and the Neoophora. Haszprunar (1996) suggested that the anterior mouth could be an apomorphy grouping Catenulida with non-rhabditophoran Bilateria, which generally do have an anterior mouth. However, under the Catenulida–Rhabditophora hypothesis an anterior mouth is the plesiomorphic condition, as it is the norm in other lophotrochozoans. A mid-body or posterior location of the mouth probably evolved several times within the Rhabditophora, but reconstruction of the exact sequence requires a fully resolved phylogenetic hypothesis of rhabditophoran phylogeny. A noteworthy apomorphy for Rhabditophora, not shared with Catenulida, is the modification of the mitochondrial genetic code in this taxon (Telford et al. 2000).

The name 'Platyhelminthes'

As a consequence of the results presented here and, more importantly, of the substantial evidence for different phylogenetic positions of Acoela, Nemertodermatida and Catenulida + Rhabditophora (e.g. Ruiz-Trillo et al. 1999, 2002; Jondelius et al. 2002; Telford et al. 2003; Wallberg et al. 2007), the name Platyhelminthes can no longer be used to refer to a clade comprising Acoela, Nemertodermatida, Catenulida and Rhabditophora. Platyhelminthes should only be used to refer to the clade composed of Catenulida and Rhabditophora. A definition of the name Platyhelminthes could be worded as follows: Platyhelminthes is defined as the least inclusive clade containing *Stenostomum leucops* Dugés, 1828 and *Microstomum lineare* (Müller, 1774).

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Appendix A. Supplementary Information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ode.2008.09.002.

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