

Gnathostomulida—An Enigmatic Metazoan Phylum from both Morphological and Molecular Perspectives¹

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On the basis of few and contentious morphological characters Gnathostomulids have been thought to be the sister-group of either the Platyhelminthes or the Syndermata (Rotifera + Acanthocephala). We provide a full 18S rDNA sequence for a species of *Gnathostomula* and attempt to resolve its position among the Metazoa, on the basis of molecular evidence. Sixty sequences, representing 30 nominal phyla and including new entoproct and gastrotrich sequences, were used to reconstruct phylogenies using maximum-parsimony, neighbor-joining, and minimum evolution models. We were unable to support either of the morphological hypotheses outright and, moreover, our data supported more strongly a third possible relationship with the gnathostomulids as a member of the Nematoda + Chaetognatha clade. Superficially, as active benthic, vermiform creatures with sclerotized cuticular jaws, they fit a predicted ancestral form of the Nematoda + Chaetognatha clade and, as such, would arguably be members of the Ecdysozoa. The molecular data at least call for a reevaluation of the morphological data and a denser sampling of the lesser phyla. Data from morphology and molecules act synergistically in estimating phylogeny; morphology alone provided limited phylogenetic signal and alternative phylogenetic hypotheses, whereas the molecular solution suggested an alternative topology which, when interpreted in the light of comparative anatomy, may suggest previously unconsidered possibilities. © 1998

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INTRODUCTION

The phylum Gnathostomulida has long been considered an enigmatic taxon. Described first by Ax (1956),

gnathostomulids have attracted interest as they represent a unique body plan among the Bilateria and one that is judged by some to be among the earliest divergent or “most primitive” of the spiralian metazoa (e.g., Haszprunar, 1996a). On the basis of four supposed synapomorphies, Ax (1985, 1987, 1989, 1996) placed the Platyhelminthes and Gnathostomulida as sister taxa, and hence, in his terms, they belong to perhaps the earliest divergent bilaterian taxon surviving today. The autapomorphies uniting the Plathelminthomorpha (Platyhelminthes plus Gnathostomulida; Ax, 1984) are hermaphroditism, direct transfer of sperm, internal fertilization of the egg cells, thread-like sperm, and lack of mitosis in somatic cells (Ax, 1996). However, not all investigators are convinced of this association or the homology of these features. Nielsen (1995) has recently regarded the gnathostomulids as specialized annelids on the basis of the jaw apparatus and cross-striated muscles (Kristensen and Nørrevang, 1977), and his morphological cladistic analysis (Nielsen *et al.*, 1996) ignores the group entirely. Sterrer *et al.* (1985) have questioned the link between the flatworms and the gnathostomulids on the basis of the presence of an anal pore, the bilateral symmetry of the pharynx, and the cross-striation of all musculature, which are found widely among the Aschelminthes (see papers in Harrison and Ruppert, 1991). Some workers have alluded to the similarity between the gnathostomulids and the Rotifera based on jaw ultrastructure (e.g., Rieger and Rieger, 1977, 1980) and also to the Gastrotricha based on a shared monociliary epithelium and similar protonephridial morphology (Sterrer *et al.*, 1985; see also Rieger and Mainitz, 1977). Most recently Rieger and Tyler (1995) gave convincing arguments as to the homology between the trophi of rotifers belonging to the genus *Seison* and the sclerotized jaws of gnathostomulids, placing the Gnathostomulida as the sister-group to the aschelminth clade containing Rotifera plus Acanthocephala, thereby invalidating the “Plathelminthomorpha” and supporting earlier work by Reisinger (1961). Wallace *et al.* (1996) took these data further and

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with maximum parsimony reanalyzed a suite of 45 morphological characters from the aschelminths. Although a number of clades were well resolved and supported, they concluded that one of the chief issues remaining to be resolved was the placement of the Gastrotricha and the Gnathostomulida. Haszprunar (1996b) independently criticized the monophyly of the Platyhelminthes and Plathelminthomorpha and argued for a sister-group relationship between the Gnathostomulida and the "Syndermata" (=Acanthocephala + Rotifera clade) on the basis of 4 characters; this new clade, the Gnathifera, was placed as the sister-group to the higher Spiralia with the paraphyletic platyhelminths occupying a basal position on the metazoan tree.

Gnathostomulids are minute, with an average length of 1.5 mm and an average diameter of 45–65 μm , and to some degree this explains the difficulty in studying them and finding sufficient morphological evidence to reach a consensus view on their position among the metazoa. As part of our quest to find the sister-group to the Platyhelminthes (or at least the Rhabditophora *sensu* Ax, 1996) we wished to test independently the relative position of the gnathostomulids among the wider metazoa using a molecular systematic approach. In the tradition of modern molecular phylogenetic approaches we have turned to the 18S rRNA gene. Until now, all known phyla have been sampled for this gene except the Gnathostomulida, the Loricifera, and the recently described Cyclophora (Funch and Kristensen, 1995). Thus, sequencing the same gene for *Gnathostomula* gives us the best opportunity to determine the phylogenetic position of the gnathostomulids, while adding significantly to a growing metazoan database of a phylogenetically useful gene. Recently, the position of the phylum Orthonectida (Hanelt *et al.*, 1996) and the phylum Myxozoa (Siddall *et al.*, 1995) were placed with some confidence among the metazoa in a similar way. Raff (1996, p. 116) bemoaned the lack of molecular data for the gnathostomulids and here we put this to rights. We present 18S rRNA gene sequence data for *Gnathostomula paradoxa* and additional sequence data for one gastrotrich (*Chaetonotus* sp.) and a newly described kamptozoan entoproct (*Barentsia hildegardae*). These data complement a recent molecular study testing the monophyly of the Aschelminthes (Winnepenninckx *et al.*, 1995) and provide the densest sampling of pertinent metazoan taxa currently available for placing the gnathostomulids in a molecular phylogeny. We further test our observations based on molecular data by comparing and contrasting with previously published morphological matrices incorporating the Gnathostomulida. We are not concerned here with the paraphyly of the Platyhelminthes, independently demonstrated on the basis of morphological (Haszprunar, 1996b) and molecular evidence (Katayama *et al.*, 1996; Carranza *et al.*, 1997; Littlewood *et*

al., unpublished data) and so have chosen only published sequences of the Rhabditophora to represent the flatworm clade. Of the published platyhelminth sequences available, the Rhabditophora (*sensu* Ax, 1996) are confirmed as monophyletic. Sequences from other, early divergent flatworm taxa (acoels and catenulids) are also confirmed as long-branch taxa (Carranza *et al.*, 1997) and do not appear as members of a monophyletic platyhelminth clade. Subsequently these taxa were omitted from the analyses. If gnathostomulids are most closely related to the Platyhelminthes (*sensu strictu*) they should at least appear as sister-taxon to the Rhabditophora.

MATERIALS AND METHODS

Specimen Collection

A sample of five individuals of one species of gnathostomulid were collected from the Island of Sylt off the coast of Germany and were kindly identified as *G. paradoxa* by Anno Faubel. Twenty specimens of *Chaetonotus* sp. (Gastrotricha) were collected from Lake Windermere, North England. The entoproct *B. hildegardae* was supplied by Kerstin Wasson and is a newly described species (Wasson, 1997). All specimens were fixed and stored in >95% ethanol prior to DNA extraction.

DNA Extraction, Gene Amplification, and Sequencing

Specimens of each species were washed twice in TE, ground in 150 μl TE (pH 8.0), 0.5% SDS, and digested for 3–4 h with the addition of 6 μl proteinase K (10 mg/ml) at 37°C. Genomic DNA was phenol–chloroform extracted and precipitated over 15 min at –20°C in the presence of 0.1 vol sodium acetate, pH 4.5–6.0, and 2.5 vol 100% ethanol. After washing in 70% ethanol DNA pellets were dried and redissolved in TE (pH 8.0).

Complete 18S rDNA was amplified from each extract with PCR (Saiki *et al.*, 1988) using primers A and B of Medlin *et al.* (1988) but without the polylinkers attached. Standard 50- μl PCRs were set up (final concentrations: 200 μM each dNTP, 2 mM MgCl_2 , 1 \times reaction buffer (Perkin–Elmer), 1 U *Taq* polymerase (AmpliTaq, Perkin–Elmer) and cycling conditions were: hot start (95°C for 5 min) followed by 30 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min. Successful primary amplification was achieved with *Barentsia*, but a secondary nested PCR was required to amplify the gene from *Gnathostomula* and *Chaetonotus*. The nested PCR involved a secondary amplification using the purified (Wizard, Promega) products of the primary PCR (1 μl of primary reaction) in two subsequent PCRs; one tube contained the 5'-primer A plus an internal 3'-primer, another tube contained an internal 5'-primer and the 3'-primer B. The amplified products overlapped one another by approximately 1000 bp. At least two reactions were performed for each template. Amplified

products were run on a 1% TAE agarose gel, cut out, pooled, and purified with Wizard Preps (Promega).

Gene fragments were directly sequenced using standard reaction mixes and procedures on an ABI 373 automated sequencer with the PRISM dye terminator cycle sequencing ready reaction kit (ABI, Perkin-Elmer). The 18S rDNA fragment was sequenced using primers A and B and 11 other standard (eukaryote-specific) internal primers. Both strands of the DNA were sequenced and contigs were assembled with Sequencher v.3.0 (Gene Codes Corp., MI).

Full 18S rRNA gene sequences for *G. paradoxa*, *Chaetonotus* sp., and *B. hildegardae* have been deposited with EMBL/GenBank under Accession Numbers Z81325, AJ001735, and AJ001734, respectively.

Taxa Used

Table 1 indicates the taxa and sequences used in estimating a metazoan phylogeny and the placement of *Gnathostomula*. Where possible we have chosen early divergent members of major clades based on previous molecular and morphologically based phylogenetic hypotheses.

Sequence Alignment

Initially, we took aligned reference sequences from the SSU rRNA database at the WWW rRNA server (URL <http://rrna.uia.ac.be>; Van de Peer *et al.*, 1997) and added sequences with ClustalW (Thompson *et al.*, 1994) using default weighting and gap penalties and the profile alignment option. Bases which could not be aligned unambiguously by eye and regions of the alignment involving autapomorphic insertions greater than two bases were removed prior to phylogenetic analysis. Wherever possible we selected regions of the alignment which either began and ended with invariant bases or were identical in terms of purine (A/T) or pyrimidine (G/C). Alignments were handled using GDE for a SUN workstation and exported to a Macintosh for phylogenetic analysis. Ambiguously aligned regions of our alignment were discarded prior to phylogenetic analysis. The full sequence alignment used in these analyses has been deposited with EMBL (Accession No. DS31733) and is available via anonymous FTP from <ftp.ebi.ac.uk> under the directory `pub/databases/embl/align` or from the corresponding author.

Molecular Phylogenetic Analysis

With a full suite of representative metazoan 18S-like small subunit rRNA gene sequences available, we inferred phylogenies using three methods: maximum-parsimony, neighbor-joining, and minimum evolution distance methods with the distance matrix calculated using a maximum-likelihood model (PAUP v.4.0.52; Swofford, in press). We performed these analyses with and without the sequence for *Gnathostomula*, as it became clear that in any given solution that the branch leading to *Gnathostomula* was relatively long and

would therefore, in turn, possibly lead to problems associated with long-branch attraction (see Swofford *et al.*, 1996; p. 427). Our first aim was to construct a reliable phylogeny for the metazoa before including *Gnathostomula*. With maximum parsimony we conducted heuristic searches (10 random addition replicates) and weighted all characters equally. In all analyses gaps were treated as a fifth character state. All trees involving the full 60 taxa were rooted on an outgroup consisting of the Placozoa, Porifera, Ctenophora, and Coelenterata, i.e., the non-bilaterian taxa.

For methods using distance matrices (NJ and minimum evolution) the distance matrix was calculated using the maximum-likelihood option in PAUP* (Swofford, in press). The transition:transversion ratio (Ti:Tv), the gamma statistic, and the proportion of invariable sites were calculated using an initial NJ tree constructed using distance matrix calculated using the Log Det option. Each of these values was calculated separately; its value was then entered into the model while the next parameter value was calculated. This procedure was repeated iteratively until the parameter values (and the log likelihood) did not change.

Our optimal topology, found by both distance methods and parsimony, was compared to phylogenies found in the literature based on morphological data. Using MacClade (Maddison and Maddison, 1992) we generated trees reflecting the alternative hypotheses of a Plathelminthomorpha (Ax, 1996) or a Gnathifera (Haszprunar, 1996) clade and determined distance matrices and tree lengths for these topologies. Also, using both parsimony and the paralinear/LogDet distance method (Lake, 1991; Lockhart *et al.*, 1994), we reconstructed molecular phylogenies using backbone constraints holding the Plathelminthomorpha and Gnathifera as monophyletic taxa and compared tree statistics between these and the unconstrained solutions to determine the statistical significance of support (Kishino and Hasegawa, 1989) provided by the molecular data for the morphological hypotheses.

RESULTS

All neighbor-joining trees, constructed using the full list of taxa, yielded a phylogenetic solution with the topology shown in Fig. 1 regardless of which model of molecular evolution was applied. We present the results of a minimum evolution model (Hasegawa *et al.*, 1985) as determined with PAUP*. Multiple iterative remodeling resulted in a log likelihood of 15746.4, a gamma shape parameter of 0.731, Ti:Tv = 1.69, and a minimum evolution score of 2.31. The topology of the trees and the interrelationships of the phyla remained the same regardless of whether or not we included the *Gnathostomula* sequence. Bootstrap support using the same model parameters and 1000 replicates was low throughout; values $\geq 50\%$ are shown in Fig. 1.

TABLE 1
List of Taxa Used in This Study

Species	Phylum	EMBL/GenBank Accession No.
<i>Xenopus laevis</i>	Chordata	X04025
<i>Lampetra aegyptera</i>	Chordata	M97573
<i>Branchiostoma floridae</i>	Chordata	M97571
<i>Saccoglossus kowalevskii</i>	Hemichordata	L28054
<i>Balanoglossus carnosus</i>	Hemichordata	D14359
<i>Antedon serrata</i>	Echinodermata	D14357
<i>Ophioplocus japonicus</i>	Echinodermata	D14361
<i>Scolopendra cingulata</i>	Arthropoda	U29493
<i>Berndtia purpurea</i>	Arthropoda	L26511
<i>Macrobotus hufelandi</i>	Arthropoda	X81442
<i>Artemia salina</i>	Arthropoda	X01723
<i>Tenebrio molitor</i>	Arthropoda	X07801
<i>Lingula lingua</i>	Brachiopoda	X81631
<i>Sagitta elegans</i>	Chaetognatha	Z19551
<i>Paraspadella gotoi</i>	Chaetognatha	D14362
<i>Pedicellina cernua</i>	Bryozoa	U36273
<i>Nereis limbata</i>	Annelida	U36270
<i>Eisenia fetida</i>	Annelida	X79872
<i>Lanice conchilega</i>	Annelida	X79873
<i>Haemopsis sanguisuga</i>	Annelida	X91401
<i>Glycera americana</i>	Annelida	U19519
<i>Lineus</i> sp.	Nemertini	X79878
<i>Prostoma eilharidi</i>	Nemertini	U29494
<i>Ochetostoma erythrogrammon</i>	Echiura	X79875
<i>Phascolosoma granulatum</i>	Sipuncula	X79874
<i>Ridgeia piscesae</i>	Vestimentifera	X79877
<i>Siboglinum fiordicum</i>	Pogonophora	X79876
<i>Glottidia pyramidata</i>	Brachiopoda	U12647
<i>Plumatella repens</i>	Ectoprocta	U12649
<i>Barentsia hildegaerde</i>	Entoprocta	AJ001734
<i>Phoronis vancouverensis</i>	Phoronida	U12648
<i>Acanthopleura japonica</i>	Mollusca	X70210
<i>Lepidochitona corrugata</i>	Mollusca	X91975
<i>Priapulidus caudatus</i>	Priapulida	X87984, Z38009
<i>Moliniformis moliniformis</i>	Acanthocephala	Z19562
<i>Neoechinorhynchus pseudemydis</i>	Acanthocephala	U41400
<i>Gnathostomula paradoxa</i>	Gnathostomulida	Z81325
<i>Chaetonotus</i> sp.	Gastrotricha	AJ001735
<i>Lepidodermella squamata</i>	Gastrotricha	U29198
<i>Philodina acuticornis</i>	Rotifera	U41281
<i>Brachionus plicatilis</i>	Rotifera	U29235
<i>Gordius aquaticus</i>	Nematomorpha	X80233
<i>Pycnophyes kielensis</i>	Kinorhyncha	U67997
<i>Nematodirus battus</i>	Nematoda	U01230
<i>Haemonchus similis</i>	Nematoda	L04152
<i>Meloidogyne arenaria</i>	Nematoda	U42342
<i>Planocera multitentaculata</i>	Plathelminthes	D83382
<i>Lobatostoma manteri</i>	Plathelminthes	L16911
<i>Fasciolopsis buski</i>	Plathelminthes	L06668
<i>Gyliauchen</i> sp.	Plathelminthes	L06669
<i>Trichoplax adhaerens</i>	Placozoa	L10828
<i>Mnemiopsis leidyi</i>	Ctenophora	L10826
<i>Beroe cucumis</i>	Ctenophora	D15068
<i>Anemonia sulcata</i>	Cnidaria	X53498
<i>Tripedalia cystophora</i>	Cnidaria	L10829
<i>Anthopleura kurogane</i>	Cnidaria	Z21671
<i>Scypha ciliata</i>	Porifera	L10827
<i>Microciona prolifera</i>	Porifera	L10825

Note. All diploblasts (Placozoa, Porifera, Ctenophora, and Cnidaria) were held as outgroup taxa; the phylum Plathelminthes is represented by rhabditophoran flatworms. EMBL/GenBank accession numbers are given for corresponding small subunit 18S rRNA or rDNA sequences.

Parsimony analysis, using maximum parsimony and 10 replicate heuristic searches, found 60 equally parsimonious solutions (length = 3081 steps; CI = 0.352; RI = 0.542) with *Gnathostomula*, priapulids, and *Pycnophyes* (Kinorhynch) as the earliest divergent taxa. However, we found that by constraining these three taxa to lie among the clade including arthropods, nematodes, chaetognaths, priapulids, nematomorphs, and Eutrochozoa (Ghiselin, 1988; Eernisse *et al.*, 1992; Eernisse, 1997), as suggested by the minimum evolution solution, the most parsimonious solution was only one step longer (Table 2; equivalent to Ecdysozoa constraint). All constraint analyses were repeated using the minimum evolution model and multiple reiterative remodeling as detailed above. As with the neighboring solution, few nodes were well supported on the maximum-parsimony solution but the parsimony solution was so unstable that trees up to three steps shorter were already completely different from the most parsimonious solution.

Regardless of the method of reconstruction, in none of the unconstrained solutions did *Gnathostomula* group with either the platyhelminths or the Rotifera + Acanthocephala. Indeed, forcing *Gnathostomula* as sister-group to the flatworms (Plathelminthomorpha; Ax, 1996) or to the Rotifera + Acanthocephala (Gnathifera; Haszprunar, 1996b; Wallace *et al.*, 1996) clade, required a further six and two steps, respectively (Table 2) with parsimony. A Templeton's (Wilcoxon's signed rank) test indicated that none of these solutions was statistically significant from one another under the parsimony model.

DISCUSSION

In analyses using all 60 sequences the interrelationships of the major metazoan groups follow those of Eernisse (1997) although we rooted our trees differently. Certain phyla (e.g., the Annelida) appear anomalously as paraphyletic in both maximum-parsimony and minimum evolution solutions. This has been noted previously (e.g., Mackey *et al.*, 1996) and is undoubtedly due to the rapid radiation of the Eutrochozoa and the perceived inability, or at least inconsistency, of 18S rRNA genes to resolve deep divergences (Philippe *et al.*, 1994; Raff *et al.*, 1994). Nevertheless, the clades that support the morphological scenarios being tested are well supported with 18S rDNA (e.g., Rhabditophora; Carranza *et al.*, 1997; Syndermata; Winneppenninckx *et al.*, 1995) and when testing the affinities of the Gnathostomulida a gene sampled for all metazoan phyla is appropriate.

From a morphological perspective the phylum Gnathostomulida has been thought to have affinities with the Platyhelminthes and the Syndermata (Acanthocephala + Rotifera). Neither relationship is supported by more than four morphological characters and the homology of all of these is still subject to debate and

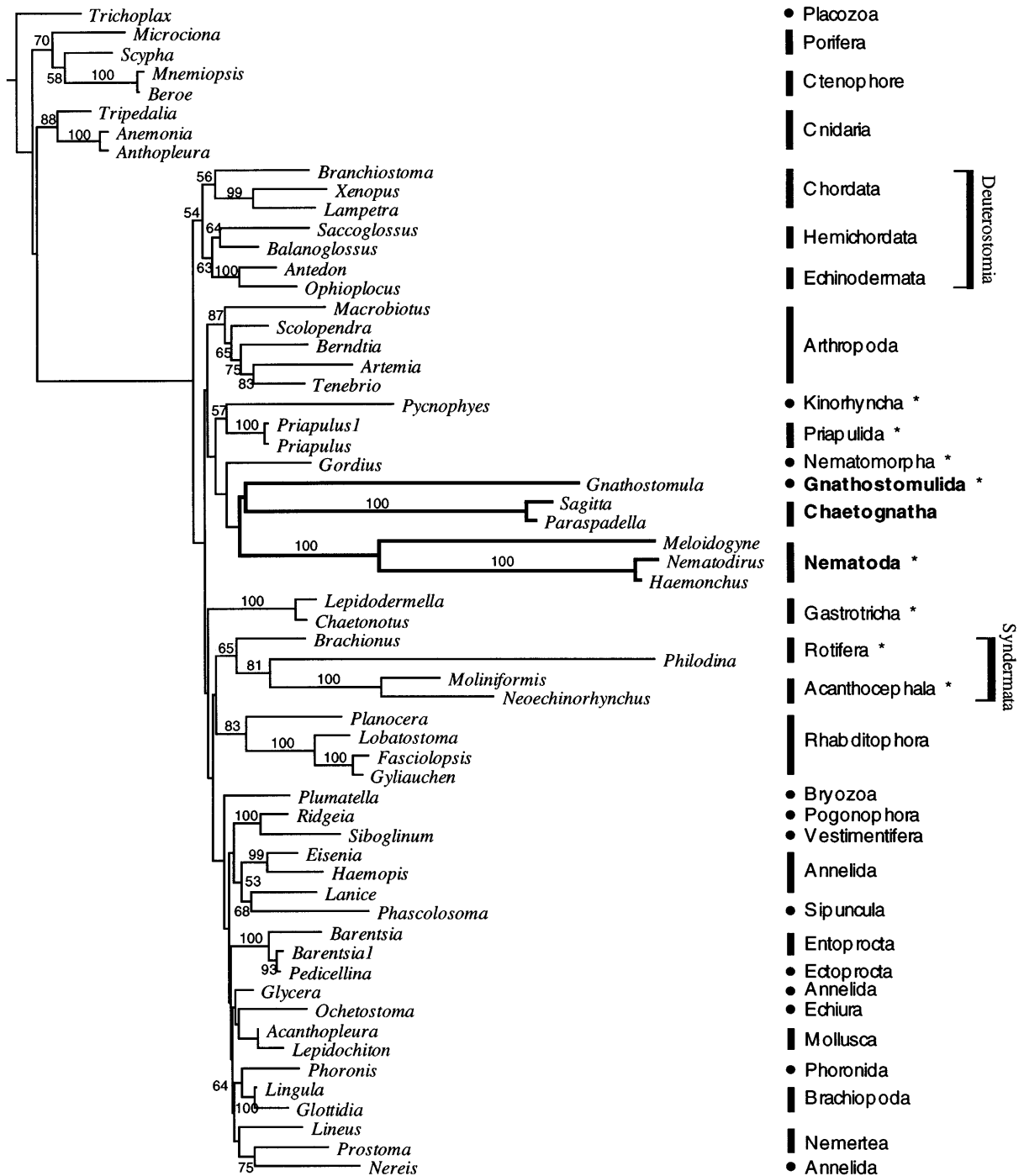


FIG. 1. NJ tree using the minimum evolution HKY85 model in PAUP* (Swofford, in press); transition/transversion ratio = 1.69; $-\ln$ likelihood = 15746.4; gamma rate distribution with shape parameter = 0.731. Numbers on branches represent bootstrap resampling percentages ($n = 1000$); values below 50% are omitted. Aschelminthes are marked with an asterisk.

interpretation (e.g., Haszprunar, 1996b; Ax, 1996; Wallace *et al.*, 1996, and references therein). Our molecular analyses based on 18S rRNA gene reject neither of these hypotheses outright but our data suggest a third, previously unconsidered, affiliation with the chaetognaths and nematodes, a clade which itself is subject to speculation on morphological grounds. Halanych (1996)

recently investigated the evolutionary relationships and origins of the chaetognaths and also found strong evidence for a nematode + chaetognath clade based on molecular data. His extensive analyses indicated that this relationship was robust, in spite of a potential problem with long-branch attraction. Eernisse (1997) has also demonstrated the nematode + chaetognath

TABLE 2

Tree Statistics for Unconstrained and Constrained Phylogenetic Solutions

Phylogenetic Solution	Maximum Parsimony				Minimum evolution	
	No. trees	Length	CI	RI	-ln	ME
Unconstrained	60	3081	0.352	0.542	15,746.4	2.256
Ecdysozoa	4	3082	0.352	0.542	15,746.4	2.256
Gnathifera	8	3083	0.352	0.542	15,746.0	2.269
Plathelminthomorpha	86	3087	0.351	0.541	15,776.7	2.267

Note. Constraints forced monophyletic groupings of *Gnathostomula* + Platyhelminthes (Plathelminthomorpha, *sensu* Ax 1996), *Gnathostomula* + Acanthocephala + Rotifera (Gnathifera, *sensu* Haszprunar 1996) and, as suggested by the minimum evolution model, *Gnathostomula* + Arthropoda + Chaetognatha + Nematoda + Kinorhyncha + Nematomorpha (Ecdysozoa, *sensu* Aguinaldo *et al.* 1997; see also Eernisse, 1997). For the distance method NJ was used employing the LogDet/paralinear model followed by multiple iterative remodeling under a maximum-likelihood model. CI, consistency index excluding uninformative sites; RI, retention index; -ln, log likelihood; ME, minimum evolution score.

relationship in a wider metazoan context, and each author has provided some morphological interpretation of the association, which in turn may serve to warrant a reevaluation of the gnathostomulids. We believe the notion of gnathostomulids as members of the Ecdysozoa is not outlandish and without recourse to molecular systematics, such an affinity may have been overlooked.

As early as 1886, Schneider recognized a chaetognath–nematode association based on a perceived homology between the arrangement of their muscular bands, and Metschnikov (1867) drew attention to the affinities of certain marine free-living nematodes and the chaetognaths (cited in Ghirardelli, 1968, p. 356). Halanych (1996, p. 232) speculated that “the node defined by the last common ancestor of chaetognaths and nematodes may include other metazoan taxa that were not examined in this study (e.g., Nematomorpha and Gastrotricha).” Our analyses have included these taxa and although the gastrotrichs were resolved as sister-group to the flatworms (Rhabditophoran platyhelminths), the nematomorphs (*Gordius*) did appear to share a common ancestor with the nematodes and chaetognaths. Is the position of the gnathostomulids, as a member of the nematodes + chaetognaths clade, an artifact?

In all of our analyses, the branches leading to the nematodes, chaetognaths, and *Gnathostomula* are long and the competing morphologically based phylogenies, although less well supported, were not statistically different from our solutions based on maximum-parsimony or neighbor-joining methods. Rather than analyze the data further it seems clear that these three phyla need to be sampled more densely in an attempt to find representative taxa with shorter branches. However, as Telford and Holland (1997) recently noted, extant chaetognaths probably underwent a recent rapid radiation and it is unlikely that denser sampling will split these long branches. Similarly, until more (free-living and parasitic) nematodes are sampled we are

unable to avoid the long-branch problem with nematodes (Aguinaldo *et al.*, 1997).

Until such time that the long branch problem can be addressed, our data suggest that certain morphological features might benefit from reevaluation. Halanych (1996) provided an evolutionary scenario for the origin of the chaetognath + nematode lineage and some striking similarities appear between the putative ancestor and the gnathostomulids. It was argued that the ancestor would possibly be vermiform and benthic, with enlarged or hardened modified feeding structures to subdue prey quickly. Gnathostomulids are active interstitial, marine grazers and possess sclerotized, cuticular jaws and a basal plate against which the jaws work. These features alone do not persuade an acceptance of the new hypothesis and, like all molecular systematic studies, the strength of interpretation relies on comparative morphological data to make biological sense.

In the early days of molecular systematics 18S rDNA was hailed as the one gene which would provide an answer to metazoan interrelationships. It is unlikely that any single gene will yield a robust or biologically plausible metazoan phylogeny when all major clades have been sampled sufficiently. While it is unable to provide complete resolution, 18S rDNA does still have the power to allow to us assess and reconsider some of the more tenuous morphologically based scenarios.

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