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Review paper

The evolutionary relationships of rotifers and acanthocephalans

James R. Garey[1,*], Andreas Schmidt-Rhaesa[1], Thomas J. Near[2] & Steven A. Nadler[3]
[1]*Department of Biology, University of South Florida, 4202 East Fowler Avenue, SCA 110 Tampa, FL 33620-5150, U.S.A. (*author for correspondence)*
[2]*Center for Biodiversity, Illinois Natural History Survey, Champaign, IL 61820, U.S.A.*
[3]*Department of Nematology, University of California, Davis, CA 95616-8668, U.S.A.*

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Abstract

Advances in morphological and molecular studies of metazoan evolution have led to a better understanding of the relationships among Rotifera (Monogononta, Bdelloidea, Seisonidea) and Acanthocephala, and their relationships to other bilateral animals. The most accepted morphological analysis places Acanthocephala as a sister group to Rotifera, although other studies have placed Acanthocephala as a sister taxon to Bdellodea or Seisonidea. Molecular analyses using nuclear 18S rRNA and mitochondrial 16S rRNA genes support Acanthocephala as a sister taxon to Bdelloidea, although no molecular data is available for Seisonidea. Combining molecular and morphological analyses of Bilateria leads to a tree with Platyhelminthes, Rotifera, Acanthocephala and Gnathostomulida (and probably Gastrotricha) as a sister group to the annelid-mollusc lineage of the Spiralia (Lophotrochozoa).

Introduction

The phylogenetic position of rotifers and acanthocephalans among metazoans has been a major problem in evolutionary studies for many years. Traditionally, both rotifers and acanthocephalans have been included within the Aschelminthes, and a close association between the two groups has been suspected since Haffner (1950), although not generally accepted until recently. The purpose of this article is to (1) review the morphological and molecular evidence for the relationships among the three major rotifer groups (Bdelloidea, Monogonontea, Seisonidea) and acanthocephalans, and their evolutionary relationships to other metazoans, and (2) to suggest areas of future studies. Two important advances since the last Rotifer Symposium have been new ultrastructural studies of *Seison* and the use of molecular phylogenetic analyses.

Morphological evidence for evolutionary relationships among the Rotifera

Phylum Rotifera consists of three groups, the classes Bdelloidea, Monogononta, and Seisonidea. A three taxon tree has only three rooted solutions, and each has been proposed at various times for the rotifers. These are illustrated in Figure 1. Tree A (Figure 1) is probably the most accepted, because it unites Bdelloidea and Monongononta with a number of characters that are most certainly synapomorphic for the two taxa such as clefts but no pores in the terminal organ of the protonephridia, unpaired retrocerebral glands, salivary glands integrated into the mastax (Ahlrichs, 1995, 1997) and the presence of a vitellarium (Wallace & Colburn, 1989). In this tree, Seisonidea is the most basal group. Wallace and Colburn (1989) suggested that Bdelloidea + Monogononta be united as the Eurotatoria, and that all three classes make up the phylum Rotifera, while Ahlrichs (1997) only applies the name Rotifera to Bdelloidea + Monogononta. Tree B (Figure 1) has been suggested by Pennak (1989) with Seisonidea and Bdelloidea united as the digonont rotifers (paired female gonads), forming a sister group to Monogononta (unpaired female gonads). Paired gonads are most likely the plesiomorphic condition within Bilateria, and would not unite Seisonidea with Bdelloidea. Tree C (Figure 1) has Bdelloidea as the most basal rotifer with Seisonidea and Monogononta united

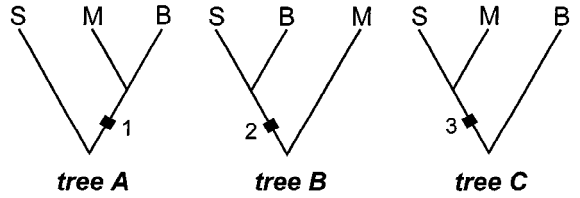

Figure 1. Possible relationships between Seisonidea (S), Monogononta (M) and Bdelloidea (B). 1: Clefts but no pores in terminal organ of the protonephridia; rotatory organ; unpaired retrocerebral glands; salivary glands integrated into the mastax (Ahlrichs, 1997); vitellarium (Wallace & Colburn, 1989). 2: Paired ovaries, ramate mastax, absence of secreted tube (Pennak, 1989). 3: Males present, no bladder, cellular stomach with microvilli (Ricci et al., 1993), similarities of internal layer in their syncytial integument (Clement, 1993).

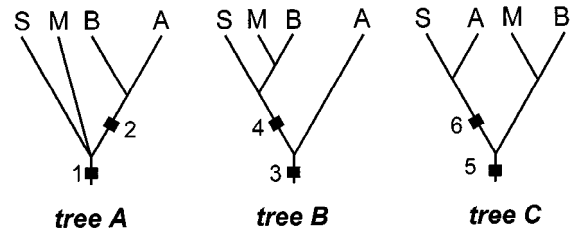

Figure 2. Proposed relationships between Seisonidea (S), Monogononta (M), Bdelloidea (B) and Acanthocephala (A). 1: Internal layer of syncytial epidermis. 2: Lemnisci and proboscis present (Lorenzen, 1985). 3: Pseudocoel present, syncytial epidermis, monociliated pit absent, hermaphorditism absent, acrosome present, anteriorly inserting flagellum on sperm (Wallace et al., 1996), internal layer in the syncytial epidermis (Nielsen, 1995). 4: Parthenogenesis, hypodermic impregnation, collagen absent (Wallace et al., 1996), toes with adhesive glands (Nielsen 1995). 5: Internal layer in the syncytial epidermis, anteriorly inserted flagellum on sperm cell, outer epidermal cell membrane intrusions with bulbs. 6: Dense bodies within spermatozoa, epidermis with filament bundles (Ahlrichs, 1997).

based on males being present, no bladder, and cellular stomach with microvilli (Ricci et al., 1993). However, these characters are most likely plesiomorphic because they are found in outgroup taxa such as Platyhelminthes. Another character, similarities of the internal layer in the syncytial integument (Clement, 1993) has been discussed by Ahlrichs (1997).

Morphological evidence for the evolutionary relationship between Rotifera and Acanthocephala

Although rotifers and acanthocephalans have historically been included among the Aschelminthes (Ruppert & Barnes, 1994), it is clear that Aschelminthes is a polyphyletic (Lorenzen, 1985; Malakhov, 1994; Neuhaus, 1994; Winnepenninckx et al., 1995; Ehlers et al., 1996; Wallaceet al., 1996) or paraphyletic (Nielsen, 1996) assemblage and that the pseudocoelom evolved independently in several aschelminth phyla (Remane, 1963; Ruppert, 1991; Nielsen, 1995). Despite this, a close affinity between Rotifera and Acanthocephala was suspected by Haffner (1950) based on common characters such as a cloaca, protonephridia, egg segmentation, and muscles that retract the anterior region of the body (Remane, 1963). Figure 2 shows three trees that have been proposed for the relationship between Rotifera and Acanthocephala based on morphological data.

Lorenzen (1985) suggested that rotifers and acanthocephalans can be united based on the internal layer of the syncytial epidermis found in both (Storch & Welsch, 1969) and the testis attached to a reduced intestine in monogononts comparable to the ligament cord found in acanthocephalans (Haffner, 1950). Lorenzen's analysis did not resolve the relationship between seisonid and monogonont rotifers, but he united Bdelloidea + Acanthocephala based on the presence of lemnisci and similarities of the proboscis in both taxa (Figure 2, tree A). These two characters have been rejected by Clement (1993) and Nielsen (1995) as synapomorphies for Bdelloidea + Acanthocephala because the 'proboscis' of acanthocephalans develops from different regions in the embryo than the comparable structure in bdelloid rotifers. The lemnisci are sac-like structures with a high number of lacunes and a still not completely understood function (Miller & Dunagan, 1985; Dunagan & Miller, 1991), while the structures in bdelloids are most likely thickened regions of the epidermis that carry the rotatory organ (Ahlrichs, pers. comm.). However, ultrastructural investigations of this region are still lacking.

Nielsen (1995) and Wallace et al. (1996) have both proposed a sister relationship between Rotifera and Acanthocephala, leaving each phylum monophyletic (Figure 2, tree B). The characters used to group all three classes of rotifers separately from acanthocephalans are parthenogenesis, hypodermic impregnation, absence of collagen (Wallaceet al., 1996) and toes with adhesive glands (Nielsen, 1995). However, many of those characters may not be autapomorphies for Rotifera. Seisonidea reproduce exclusively by sexual reproduction (Clement & Wurdak, 1991), so parthenogenesis is not an autapomorphy for Rotifera. Apparently, copulation has never been observed in *Seison*, which, unlike other rotifers, lacks a penis but has a spermatophore-like structure (Ricci et al., 1993; Ahlrichs, 1995). Free sperm cells have been ob-

served only in the reproductive tract of female *Seison*, and it is likely that sperm enter through the cloaca, so hypodermic impregnation is not likely to be an autoapomorphy for Rotifera. We are not aware of any studies that conclusively demonstrate that collagen is absent from *Seison*. The presence of toes with adhesive glands as an autoapomorphy of Rotifera has come under question because the cement glands of acanthocephalans may be homologous to the adhesive glands of rotifers (Near et al., 1998).

A novel scheme has recently been proposed (Ahlrichs, 1997) that most closely relates Acanthocephala with Seisonidea (Figure 2, tree C) using dense bodies within the spermatozoa and bundles of filaments within the epidermis as synapomorphies. These characters have not before been used for phylogenetic studies and so their significance remains to be confirmed. Ahlrichs retains a monophyletic Rotifera as Bdelloidea + Monogononta, and uses the taxon name Syndermata for Rotifera + Seisonidea + Acanthocephala based on the presence of a syncytial epidermis with an internal layer, outer epidermal cell membrane intrusions with bulbs and an anterior insertion of the flagellum on sperm cells.

Molecular studies of rotifers and acanthocephalans

Molecular studies of phylogeny are based on aligning the DNA sequences of orthologous genes, and deducing trees by one of three common methods (reviewed in Li, 1997). In distance methods, a matrix of evolutionary distances between all pairs of sequences are calculated, and a tree is deduced from the distance matrix most commonly by the Neighbor-Joining (NJ) method. A number of algorithms can be used to calculate distances from the alignment which correct for multiple substitutions at the same site and/or correct for different nucleotide substitution rates at different sites (site to site variation). NJ trees can be calculated very quickly and their polarity is determined by an outgroup. In Maximum Parsimony (MP) trees, the alignment is used to choose the tree with the shortest path that accounts for the nucleotide changes. Considering that there are over 34 million possible topologies for even a 10 taxon tree, MP trees can take a lot of computation time. MP analysis generally does not correct for multiple substitutions at the same site or site to site variation. In Maximum Likelihood (ML) trees, a maximum likelihood value for character state configurations among the sequences are calculated for each possible tree and the tree with the largest value chosen. This method can accommodate corrections for multiple substitutions at the same site and for site to site variation. The ML method is usually the slowest of the three kinds of analyses.

Confidence in molecular trees is most often determined by bootstrap analysis (Felsenstein, 1988; Hillis & Bull, 1993) in which new datasets are constructed from the original alignment by selecting sites from the original alignment randomly with replacement. Trees are made from each bootstrapped dataset and the percent of bootstrapped trees that support each branch is reported when greater than 50%, and the closer a value is to 100%, the more confidence one has in that region of the tree. Bootstrap analysis can be carried out on any type of tree, although ML bootstrap analysis is usually impractical because of long computation times. Other statistical methods include Confidence Probability (CP) values for NJ trees in which the confidence that a given branch is greater than zero is calculated (Kumar et al., 1994). The closer a CP value for a given branch is to 100, the higher the confidence one has in that branch of the tree. Decay analysis is used in MP trees, and refers to the number of steps that a tree can be lengthened and still retain a particular clade (Donoghue et al., 1992). The higher the number, the more probable the clade, although computation time often limits the number of steps that can be tried. Although various statistical analyses are the most widely used determinant of confidence in a tree, it is possible to have statistical support for an incorrect tree (Hillis et al., 1994).

Molecular studies have contributed to the evidence that rotifers and acanthocephalans are closely related. The complete 18S rRNA gene of the archiacanthocephalan *Moniliformis moniliformis* was published in 1993 (Telford & Holland) in a study of chaetognath affinites, but no rotifer sequence was included. The first mention of an association between rotifers and acanthocephalans (Raff et al., 1994) was a reference to an unpublished study in a review article on animal phylogeny, but no statistical support for the association was given, and a subsequent paper describing the analysis was not published. The first rigorous molecular study of aschelminth phylogeny (Winnepenninckx et al., 1995) included nearly complete 18S rRNA gene sequences from the acanthocephalan *M. moniliformis*, the monogonont rotifer *Brachionus plicatilis*, numerous nematodes, a gastrotrich, a nematomorph, and a priapulid. The study showed that aschelminths are

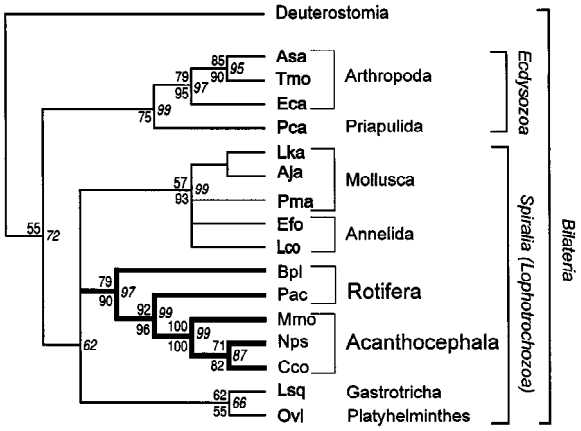

Figure 3. Molecular phylogeny of Bilateria based on the 18S rRNA gene. The tree shown is a strict consensus of NJ, MP, and ML analyses from Garey et al. (1996a). Numbers above and below each fork represent the percentage of 1,000 bootstrap replicates that support the branch in the MP and NJ trees, respectively. Numbers to the right of each fork are CP values from the NJ tree. Values are shown only when greater than 50. The Rotifera + Acanthocephala clade, Bdellodea + Acanthocephala clade, and the Acanthocephala clade were all supported by decay indices greater than 20. Taxon abbreviations: *Artemia salina*, **Asa**; *Tenebrio molitor*, **Tmo**; *Eurypelma californica*, **Eca**; *Priapulus caudatus*, **Pca**; *Limicolaria kambeul*, **Lka**; *Acanthopleura japonica*, **Aja**;*Placopecten magellanicus*, **Pma**; *Eisenia foetida*, **Efo**; *Lanice conchilega*, **Lco**; *Brachionus plicatilis*, **Bpl**;*Philodina acuticornis*, **Pac**;*Moniliformis moniliformis*, **Mmo**; *Neoechinorhynchus pseudemydis*, **Nps**; *Centrorhynchus conspectus*, **Cco**; *Lepidodermella squammata*, **Lsq**; Platyhelminthes: *Opisthorchis viverrini*, **Ovi**. See Garey et al. (1996a) for Genbank accession numbers and other details of the analysis.

polyphyletic, but supported a rotifer + acanthocephalan clade with a weak bootstrap value of 52%, and a CP value of 86 in an NJ tree. The MP tree revealed the rotifer + acanthocephalan clade but with a bootstrap value below 50%.

A more recent study (Garey et al., 1996a) contributed new 18S rRNA gene sequences from the bdelloid rotifer *Philodina acuticornis*, the palaeacanthocephalan *Centrorhynchus conspectus* and the eoacanthocephalan *Neoechinorhynchus pseudemydis*. To date, the monogonont *B. plicatilis* and the bdelloid *P. acuticornis* are the only rotifers for which 18S rRNA sequences have been published. Therefore, the presently available molecular data cannot discriminate between any of the trees in Figure 1 concerning the relationships among the three rotifer classes.

The presently available molecular evidence (Garey et al., 1996a) overwhelmingly supports a sister relationship between Bdelloidea and Acanthocephala (Figure 3), favoring tree A in Figure 2, based on the hypothesis of Lorenzen (1985), contradicting the idea of Acanthocephala as a sister taxon to monophyletic Rotifera (tree B, Figure 2), or a monophyletic Bdelloidea + Monogononta (tree C, Figure 2). In the analyses, NJ, ML, and MP trees were found to be congruent in regard to the relationship between rotifers and acanthocephalans with remarkably strong statistical support. Bootstrap support for Rotifera + Acanthocephala ranged from 79 to 90%, and was 92 to 96% for Bdelloidea + Acanthocephala. CP values were similarly high, and decay analyses (not shown) indicated that even 20 steps were insufficient to decay the two clades.

One problem with the study is that the rotifer *P. acuticornis* and the acanthocephalan *C. conspectus* have 18S rRNA genes that evolve much more rapidly than other metazoans, and it is possible that unequal rate effects could cause an incorrect tree. It has been shown that unequal rates and other problems can cause all tree making methods (NJ, MP and ML) to produce identical but incorrect trees (Hillis et al., 1994). In Figure 4, the NJ tree from Garey et al. (1996a) is shown with branches drawn to scale. It can be seen that the long branches leading to *C. conspectus* and *P. acuticornis* are not directly adjacent to one another and they are not in a basal part of the tree, both which would be expected if unequal rate effects were a factor (see Figure 1 in Aguinaldo et al., 1997). The tree in Figure 4 is only a portion of the tree produced by the analysis, which also included nematode 18S rRNA genes with very long branches that appeared incorrectly as basal to the bilateria. If unequal rate effects were a factor, one would expect that the *C. conspectus* and *P. acuticornis* branches would have been attracted toward the long branches of the nematode genes and appeared more basal. In additional analyses, the sequences from *C. conspectus* and *P. acuticornis* were removed from the tree separately and together, with no change in topology of the tree (Garey et al., 1996a), further evidence that unequal rates are not a factor.

The mitochondrial genome contains a number of highly conserved genes that are useful for phylogenetic analysis. One of the most conserved is that of the mitochondrial 16S rRNA gene which is inherited independently from the nuclear rRNA genes. We sequenced a 600 bp fragment of the 16S rRNA gene from *B. plicatilis*, *P. acuticornis*, and *M. moniliformis* and present the analysis in Figure 5. Although the 16S rRNA gene is less conserved that the nuclear 18S rRNA, there is sufficient signal to indicate relationships between closely related taxa. For example, the

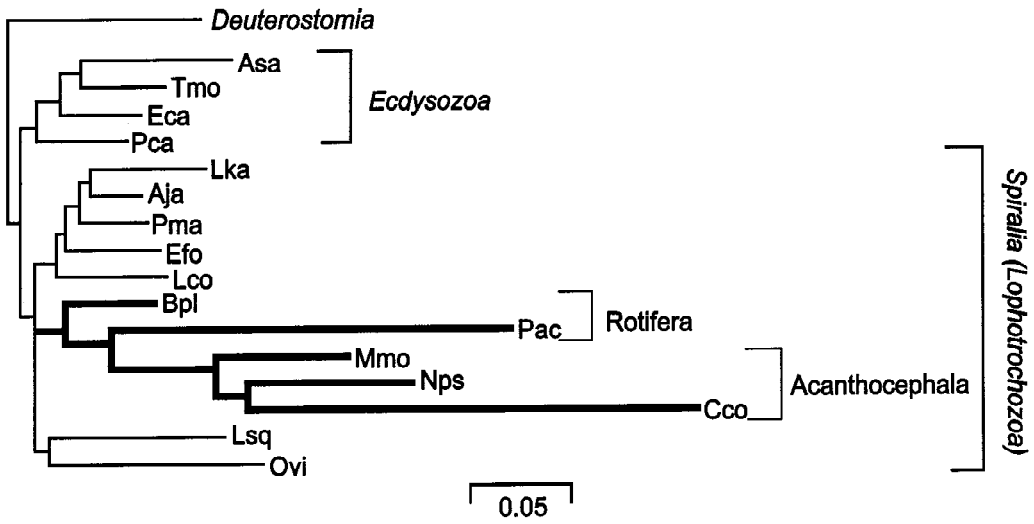

Figure 4. The tree from Figure 3 drawn with branch lengths proportional to evolutionary distance to illustrate the unequal evolutionary rates of rotifers and acanthocephalans. The rotifer *P. acuticornis* and the acanthocephalan *C. conspectus* are evolving at a rate approximately 5 times as fast as most other taxa in the tree. When the fastest evolving rotifer sequence (*P. acuticornis*) was removed from the analysis, the acanthocephalans remained as a sister taxon of the rotifers. When the fastest acanthocephalan sequence (*C. conspectus*) was removed, the other acanthocephalans remained within the rotifer clade, demonstrating that the position of acanthocephalans as a sister taxon to bdelloid rotifers is not likely to be an artifact due to unequal rate effects (from Garey et al., 1996a). Taxon labels are defined in Figure 3.

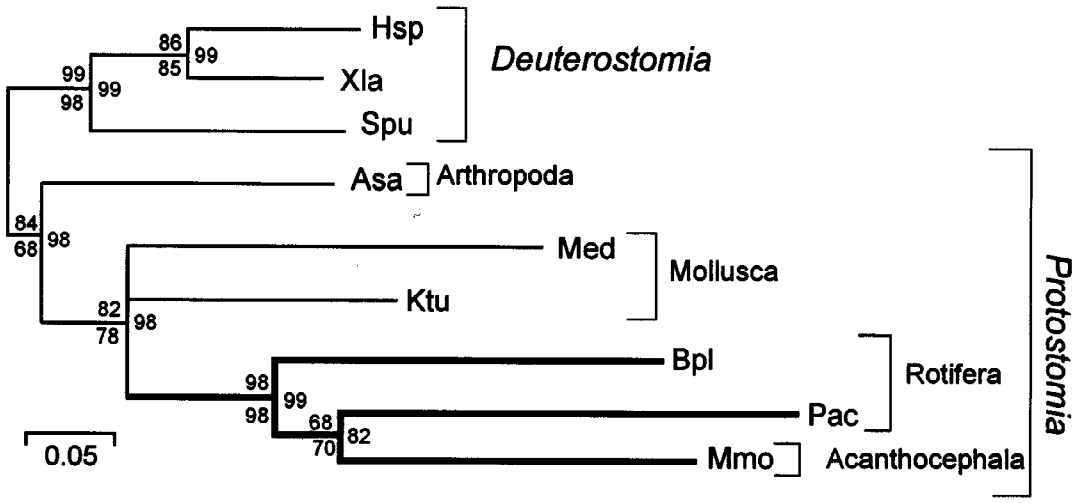

Figure 5. Molecular phylogeny of Bilateria based on a 600 bp alignment of the mitochondrial 16S rRNA gene. The tree shown is a NJ tree. Bootstrap values for Kimura distances with gamma correction (a = 0.72) are shown above the forks, values for Tamura & Nei distances are below and numbers to the right are CP values for Kimura distances. See Kumar et al. (1995) for details. The same topology was recovered with all NJ analyses and with ML analysis with multiple rate categories but not with MP or ML analysis without multiple rate categories (see text). Taxon abbreviations and Genbank accession numbers: *Artemia salina*, **Asa**, M21833; *Brachionus plicatilis*, **Bpl**, AF108106; *Homo sapiens*, **Hsa**, D38112; *Katherina tunicata*, **Ktu**, U09810; *Moniliformis moniliformis*, **Mmo**, AF108107; *Mytilus edulis*, **Med**, M83756; *Philodina acuticornis*, **Pac**, AF108108; *Strongylocentrotus purpuratus*, **Spu**, X12631; *Xenopus laevis*, **Xla**, X01601. Portions of mitochondrial 16S rRNA genes corresponding to a sea urchin 16S rRNA gene (Genbank Accession 12825) from nucleotides 814–833 and 1406–1425 were PCR amplified from cellular DNA isolated from *P. acuticornis*, *B. plicatilis*, and *M. moniliformis*, and resulted in a fragment about 450 nucleotides in length. Primers were 16S-RNA1:16S-RNA1 CCGGAATTCCGCCTGTTTATCAAAAACAT, and 16S-RNA2: CCCAAGCTTCTCCGGTTTGAACTCAGATC, which have EcoRI and HindIII site tails, respectively. PCR products were cloned into M13 and sequenced in both directions. All sequences were aligned according to a secondary structure model (De Rijk & De Wachter, 1993) and trees produced using MEGA (Kumar et al., 1994) for NJ trees and Phylip (Felsenstein 1993) for ML and MP trees. Sites with gaps were not used in the analyses.

tree in Figure 5 properly groups deuterostomes together, and indicates the same relationship between Rotifera and Acanthocephala as the 18S rRNA study in Figures 3 and 4. The NJ tree in Figure 5 was calculated using a variety of distance-correction methods which correct for multiple substitutions at the same site and site to site rate variation, all resulting in the topology shown in Figure 5 with similar bootstrap values. It can be seen that branch lengths are more consistent among taxa than in the 18S rRNA gene analyses, so rate effects are unlikely to be significant.

The importance of correcting for site to site variation and multiple substitutions at the same site is important for the fast evolving mitochondrial 16S gene. MP analysis does not carry out those corrections, and produced a tree similar to tree B in Figure 2, with Acanthocephala as a sister taxon to Rotifera. Similarly, when ML analysis was carried out without correcting for site to site variation, Acanthocephala appeared as the sister taxon to Rotifera, but when the analysis was repeated with multiple rate categories (four categories: 10% of sites with no variation, 20% each with rates of 1, 5, and 20) the topology shown in Figure 5 (Bdelloida and Acanthocephala as sister taxa) was recovered with a higher likelihood (In likelihood = −2651) than without the correction (In likelihood = −2754). It is well established that rRNA genes demonstrate site to site variation of evolutionary rates as some sites are completely conserved and others are not (Hillis & Dixon, 1991).

Morphological evidence for the position of Rotifera-Acanthocephala within the Bilateria

In most textbooks, rotifers are placed among the aschelminths (e.g. Ruppert & Barnes, 1994) or loosely grouped with other pseudocoelomate taxa (e.g. Hyman, 1951; Brusca & Brusca, 1990). Other views such as a relationship to derived platyhelminth groups (Markevich, 1993) are rare. Some cladistic studies of the entire Metazoa consider the pseudocoelom an important character which can result in a monophyletic aschelminth clade (e.g. Schram, 1991; Eernisse et al., 1992), but the pseudocoelom has been shown to be a doubtful phylogenetic character (Ruppert, 1991). It is clear that the aschelminths are polyphyletic, but the more rigorous treatments of aschelminth taxa often fail to include mainstream metazoan phyla such as arthropods, annelids and molluscs (e.g. Lorenzen, 1985; Wallace et al., 1996), although a few recent studies have (Ehlers et al., 1996; Nielsen et al., 1996). Recent morphological analyses from a number of laboratories seem to be converging on the concept of two ‘aschelminth’ clades, one (Nemathelminthes) containing Priapulida + Kinorhyncha + Loricifera + Nematoda + Nematomorpha + Gastrotricha (Nebelsick, 1993; Neuhaus, 1994; Ehlers et al., 1996; Nielsen et al., 1996, Wallace et al., 1996), the other clade (Syndermata) containing Acanthocephala + Rotifera (Nielsen et al., 1996; Wallace et al., 1996) and possibly including Gnathostomulida (Ahlrichs, 1997). While the Nemathelminthes are most probably the sister group of Spiralia (Lophotrochozoa) within Protostomia (Ehlers et al., 1996), Syndermata + Gnathostomulida (named Gnathifera) have been hypothesized as the sister taxon of Platyhelminthes within Spiralia (Ahlrichs, 1995).

Molecular evidence for the position of Rotifera-Acanthocephala within the Bilateria

A major difficulty in relating minute animals like rotifers to larger animals such as annelids, molluscs, and arthropods has been the scarcity of uniting characters. Molecular phylogenetic studies are ideal for relating more distant taxa that have few uniting characters. Several 18S rRNA gene based metazoan phylogenies (Winnepenninckx et al., 1995; Garey et al., 1996b; Aguinaldo et al., 1997) placed Rotifera + Acanthocephala within a clade loosely including Platyhelminthes and sometimes Gastrotricha which together formed a sister group to Annelida + Mollusca although these relationships were only weakly supported by statistical testing. More recent studies based on the 18S rRNA gene have extended the entire clade to also include lophophorates and entoprocts (Halanych et al., 1995; Mackey et al., 1996) with better statistical support and Halanych et al. (1995) named the clade Lophotrochozoa. Our mitochondrial 16S rRNA gene analysis is consistent with the 18S rRNA gene findings (Figure 5).

In most 18S rRNA gene studies, Priapulida appeared in another protostome clade as a sister group to Arthropoda. Nematoda + Nematomorpha usually appeared basal to the bilateria, an artifact now recognized as caused by unequal rate effects (Aguinaldo et al., 1997). Another 18S rRNA study (Garey et al., 1996b) extended the clade of Arthropoda + Priapulida to include Tardigrada. Recently, Aguinaldo et al. (1997) solved the problem of the placement

of Nematoda within Bilateria by finding a nematode (*Trichinella spiralis*) with a slow evolving 18s rRNA gene. With careful attention to unequal rate effects, they provided evidence that the protostomes consist of two clades: the Ecdysozoa includes all molting animals (e.g. nematodes and arthropods), and is the sister taxon to Spiralia (Lophotrochozoa). Since ecdysozoans generally lack spiral cleavage which is present in spiralians, we prefer to use the term Spiralia instead of Lophotrochozoa (see Malakhov, 1994 and Nielsen, 1995 for descriptions of cleavage in nematodes). The Ecdysozoa/Spiralia (Lophotrochozoa) structure of protostomes appears consistent with morphological characters. For example, the developmental pattern of growth by molting under the control of the steroid hormone ecdysone has been confirmed among Arthropoda, Nematoda, and Tardigrada (see Gupta, 1990; Davies & Fisher, 1994).

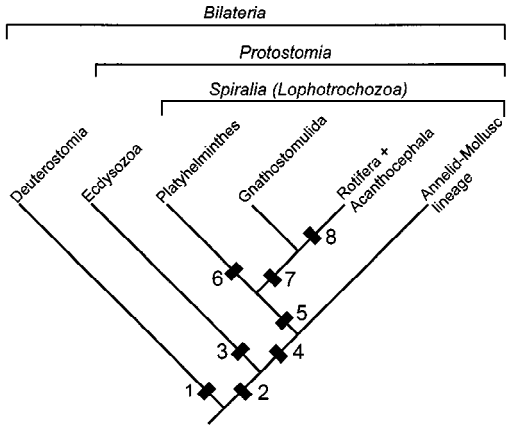

Figure 6. Proposed position of Rotifera within the Bilateria based on morphological and molecular data. The annelid-mollusc lineage refers to the bulk of the non-ecdysozoan protostomes, but not necessarily all of them. Only a few key characters are given. 1: Blastopore becomes the anus. 2: Ventral lateral nerve chord (Ahlrichs, 1995). 3: Molting by ecdysis (Aguinaldo et al., 1997). 4: Spiral cleavage. 5: Filiform sperm without accessory centriole (Ahlrichs, 1995). 6. Biciliary terminal cell in the protonephridia (Ax, 1996). 7: Jaws composed of rods imbedded in a cuticular matrix (Ahlrichs, 1997). 8: Internal layer in the syncytial epidermis (Storch & Welsch, 1969).

Conclusions and future directions

The molecular and morphological evidence is overwhelmingly in favor of a close relationship between Rotifera and Acanthocephala. Analyses of nuclear 18S rRNA and mitochondrial 16S rRNA genes strongly favor a sister relationship between Bdelloidea and Acanthocephala, one of three possible relationships argued by morphological studies, but the morphological support for the sister relationship of Bdelloidea and Acanthocephala appears weak and is very controversial. In this regard, the time is ripe for a series of rigorous ultrastructural comparisons of the epidermis underlying the rotatory organ of bdelloid rotifers to the lemnisci of acanthocephalans. Similarly, ultrastructural studies should be carried out to compare rotifer adhesive glands and acanthocephalan cement glands.

The molecular data supporting the sister group relationship between Bdelloidea and Acanthocephala appears very strong, but it is based on only two genes from two species of rotifers, and artifacts due to unequal rate effects cannot be completely ruled out. The sequences of more genes from more rotifer taxa should be analyzed, particularly from *Seison*. Studies of rotifer 18S rRNA genes from a large number of rotifer taxa are underway (Walsh, pers. comm.). Other suitable genes would include those of elongation factor-1α, heat shock proteins, triose phosphate isomerase, and some of the more conserved mitochondrial protein genes such as those from cytochrome b and cytochrome oxidase subunit I. We are currently sequencing the complete mitochondrial genome of *B. plicatilis* (Li and Garey, unpublished) to make it easier to PCR amplify the mitochondrial genes of other rotifers. It is important for new molecular analyses to be carried out rigorously, with special attention paid to alignments, unequal rate effects, site to site variation, and multiple substitutions at the same site.

The position of Rotifera is becoming clearer as morphological and molecular evidence are considered together. We propose a scheme (Figure 6) that places Rotifera among the Bilateria and appears to be consistent with many of the more recent molecular and morphological studies. In this phylogeny, Rotifera + Acanthocephala is considered a sister group to Gnathostomulida because they all have jaws composed of rods imbedded in a cuticular matrix (Rieger & Tyler, 1995; Ahlrichs, 1997). Rotifera + Acanthocephala + Gnathostomulida are then placed as a sister group to Platyhelminthes with filiform sperm cells without accessory centrioles as a possible synapomorphy (Ahlrichs, 1995). Rotifera + Acanthocephala + Gnathostomulida + Platyhelminthes are considered a sister group to the annelid-mollusc lineage of Spiralia, which in turn is the sister group to Ecdysozoa.

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