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Phylogenetic Relationships of the Acanthocephala Inferred from 18S Ribosomal DNA Sequences

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Phylogenetic relationships within the Acanthocephala have remained unresolved. Past systematic efforts have focused on creating classifications with little consideration of phylogenetic methods. The Acanthocephala are currently divided into three major taxonomic groups: Archiacanthocephala, Palaeacanthocephala, and Eoacanthocephala. These groups are characterized by structural features in addition to the taxonomy and habitat of hosts parasitized. In this study the phylogenetic relationships of 11 acanthocephalan species are examined with 18S rDNA sequences. Maximum parsimony, minimum evolution, and maximum likelihood methods are used to estimate phylogenetic relationships. Within the context of sampled taxa, all phylogenetic analyses are consistent with monophyly of the major taxonomic groups of the Acanthocephala, suggesting that the current higher order classification is natural. The molecular phylogeny is used to examine patterns of character evolution for various structural and ecological characteristics of the Acanthocephala. Arthropod intermediate host distributions, when mapped on the phylogeny, are consistent with monophyletic groups of acanthocephalans. Vertebrate definitive host distributions among the Acanthocephala display independent radiations into similar hosts. Levels of uncorrected sequence divergence among acanthocephalans are high; however, relative-rate tests indicate significant departure from rate uniformity among acanthocephalans, arthropods, and vertebrates. This precludes comparison of 18S divergence levels to assess the relative age of the Acanthocephala. However, other evidence suggests an ancient origin of the acanthocephalan–arthropod parasitic association.

INTRODUCTION

Acanthocephala are helminth parasites that use arthropods and vertebrates to complete their life cycles. These helminths lack an alimentary tract and are characterized by the presence of a proboscis armed with recurved hooks, a syncytial epidermis, and a lacunar system with circulatory channels that promotes direct absorption of nutrients through the body wall. Approximately 820 species representing 125 genera have been described from the three major classes (Amin, 1985). Amphipods, isopods, ostracods, copepods, insects, and myriapods serve as intermediate hosts of acanthocephalans. Teleosts, amphibians, turtles, snakes, lizards, birds, and mammals serve as definitive hosts. Some acanthocephalan life cycles involve paratenic (transport) hosts. Paratenic hosts are usually vertebrates that ingest infected intermediate hosts and subsequently are preyed upon by the definitive host (Nickol, 1985). The diversity exhibited by the Acanthocephala in host distribution, host habitat, morphology, and life history provides a wealth of material to examine in association with a phylogenetic hypothesis.

The relationship of the Acanthocephala to other invertebrate phyla has been estimated recently by analysis of structural and molecular data. The hypothesis that the Acanthocephala and Rotifera are sister taxa has been supported in several phylogenetic studies (Backeljau *et al.*, 1993; Raff *et al.*, 1994; Schram, 1991; Winnepenninckx *et al.*, 1995). In a cladistic analysis of structural characters, Lorenzen (1985) hypothesized that the Acanthocephala share most recent common ancestry with rotifers of the class Bdelloidea. This hypothesized sister-group relationship between bdelloid rotifers and acanthocephalans was strongly supported by phylogenetic analysis of complete 18S rDNA sequences (Garey *et al.*, 1996). As a consequence of this tree topology, recognition of Rotifera in phylogenetic taxonomy necessitates the inclusion of the acanthocephalans, as was recommended by Garey *et al.* (1996).

Most investigations of acanthocephalan relationships predated the development of phylogenetic systematic methods (Hennig, 1966). Structural characters provided the basis for interpreting systematic groupings of acanthocephalans; however, using these data to

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develop phylogenetic hypotheses has been hampered by a paucity of informative characters, morphological and ecological divergence among extant acanthocephalan groups, and an inability to polarize character states (Bullock, 1969; Conway Morris and Crompton, 1982). With no known fossil record and conflicting hypotheses concerning free-living sister taxa (outgroups), determination of shared derived character states among major acanthocephalan groups has been complicated (Conway Morris and Crompton, 1982). To date, relationships among the major lineages have not been sufficiently resolved.

The currently accepted classification of the Acanthocephala is an amalgamation of certain taxonomic hypotheses of Meyer and Van Cleave as modified by others (Meyer, 1932, 1933; Van Cleave, 1948, 1952; Bullock, 1969; Amin, 1985). Three major taxonomic groups were recognized as classes in the phylum Acanthocephala: Archiacanthocephala, Palaeacanthocephala, and Eoacanthocephala (Golvan, 1959a, 1960, 1961, 1962). The three groups are distinguished by location of lacunar canals, the persistence of ligament sacs in females, number and type of cement glands in males, number and arrangement of proboscis hooks, intermediate and definitive hosts, and host ecology (Bullock, 1969; Dunagan and Miller, 1991).

In this study we examined the phylogenetic relationships of 11 acanthocephalan species as inferred from complete 18S rDNA sequences. The monophyly of traditionally recognized major taxonomic groupings was assessed, and alternative phylogenetic and taxonomic hypotheses of relationships were compared statistically using the rDNA data. Hypotheses for the evolution of various structural and life history features used traditionally in acanthocephalan taxonomy were developed by parsimony mapping on the molecular tree.

MATERIALS AND METHODS

Collection of Specimens

Acanthocephalans were collected from vertebrate definitive hosts or arthropod intermediate hosts. Specimens were stored at ultracold temperatures (−70°C) or in 95% ethanol until nucleic acids were extracted. The species used in this analysis, GenBank accession numbers for the acanthocephalan sequences, and their classification (*sensu* Amin, 1985), with source hosts (h) in parentheses, are as follows: Archiacanthocephala-*Gigantorhynchida*-Gigantorhynchidae, *Mediorhynchus grandis* AF001843 (h = *Sturnella magna,* western meadowlark); Archiacanthocephala-*Moniliformida*-Moniliformidae, *Moniliformis moniliformis* Z19562 (Telford and Holland, 1993) (h = *Rattus rattus,* rat); Archiacanthocephala-*Oligacanthorhynchida*-Oligacanthorhynchidae, *Macracanthorhynchus ingens* AF001844 (h = *Procyon lotor,* raccoon); Palaeacanthocephala-*Echinorhynchida*-Pomphorhynchidae, *Pomphorhynchus bulbocolli* AF001841 (h = *Onchorhynchus mykiss,* rainbow trout); Palaeacanthocephala-*Echinorhynchida*-Rhadinorhynchidae, *Leptorhynchoides thecatus* AF001840 (h = *Lepomis cyanellus,* green sunfish); Palaeacanthocephala-*Polymorphida*-Centrorhynchidae, *Centrorhynchus conspectus* U41399 (h = *Strix varia,* barred owl); Palaeacanthocephala-*Polymorphida*-Plagiorhynchidae, *Plagiorhynchus cylindraceus* AF001839 (h = *Armadillidum vulgare,* pillbug); Palaeacanthocephala-*Polymorphida*-Polymorphidae, *Corynosoma enhydri* AF001837 (h = *Enhydra lutris,* sea otter) and *Polymorphus altmani* AF001838 (h = *Enhydra lutris,* sea otter); Eoacanthocephala-*Neoechinorhynchida*-Neochinorhynchidae, *Neoechinorhynchus crassus* AF001842 (h = *Catostomus commersoni,* white sucker) and *Neoechinorhynchus pseudemydis* U41400 (h = *Trachemys scripta elegans,* red-eared slider).

Nucleic Acid Isolation, Polymerase Chain Reaction, and Sequencing

Total nucleic acids were extracted from individual acanthocephalan specimens. Tissues were homogenized on ice in STE buffer (10 mM Tris–HCl, pH 7.5, 10 mM NaCl, 1 mM EDTA) and digested by adding 50 µl of 20% SDS and 20 µl of proteinase K (10 mg/mL) and incubating at 50°C. The supernatant was extracted twice with buffered phenol (pH 8.0) and once with chloroform/isoamyl alcohol (24:1). The nucleic acids were precipitated overnight (−20°C) in a solution containing 50 µl of 3 M sodium acetate (pH 5.2) and 1.0 ml of absolute ethanol. The pellet was washed twice with 70% ethanol, dried, resuspended in 100 µl of TE (10 mM Tris–HCl, pH 7.5, 1 mM EDTA, pH 8.0) and stored at −20°C. The concentration of nucleic acids was estimated by spectrophotometry.

The polymerase chain reaction (PCR) was used to amplify a homologous region of the 18S ribosomal DNA (rDNA) that ranged from 1745 to 1773 bp in 10 acanthocephalan species. PCR was performed in 50-µl reactions containing 2.5 mM $MgCl_2$, 0.25 mM each deoxynucleotide, 0.5 mM each primer (forward primer 5′-AGATTAAGCCATGCATGCGTAAG-3′, reverse primer 5′-TGATCCTTCTGCAGGTTCACCTAC-3′), and 2.5 units of *Thermus aquaticus* DNA polymerase in a reaction buffer of 50 mM KCl, 10 mM Tris–HCl (pH 8.3), and 0.1% Triton X-100. Template DNA used in PCR ranged from 100 to 300 ng. Water used in the PCR was double distilled, autoclaved, and irradiated with 400 mJ/cm^2 of 254-nm light in an ultraviolet crosslinker to inactivate potential contaminating nucleic acids (Sarkar and Sommer, 1990). Thermal cycling was performed using an initial denaturation of 94°C for 4.0 min followed by 25 cycles of 94°C (30 s), 60°C (30 s.), and 72°C (1.5 min). A final incubation of 5 min (72°C) was performed to completely extend the amplified product. PCR product size was verified by electrophoresis in a 1% agarose gel using DNA size standards.

The amplified 18S product was separated from PCR reactants by ultrafiltration using a 30,000 MW cutoff polysurfone tube (Millipore Corporation), ligated into pGEM-T vector plasmid using T_4 ligase (Promega), and used to transform DH5α-*Escherichia coli*. Colonies that were positive by blue/white selection were screened using internal 18S primers and PCR to verify the identity of the insert. Plasmid DNA was isolated from individual clones and used as template for the sequencing reactions. At least two clones from each individual were sequenced.

Chain-termination cycle sequencing was performed using the Δ*Taq* Cycle Sequencing kit (Amersham United States Biochemical, Cleveland, OH) with [^{32}P] dATP as the radionuclide. Each species was sequenced for both strands using a total of 13 internal primers and 2 vector primers. Sequences of the internal forward primers with their 5′ annealing positions numbered according to the 18S rDNA of *Moniliformis moniliformis* (Acanthocephala: Archiacanthocephala, Genbank Z19562) are AACCGCGAATGGCTCATT (46), CGGAGAGGGAGCCTGAGAAACGGC (346), GCCGCGGTAATTCCAGCTC (537), CGGAAGCTGAGGTAATGATT (812), CGGGGGGAGTATGGTTGC (1073), CTTAAGCACACGAAGAGGAGC (1371), and ACACCGCCCGTCGCTACT (1600). Reverse primers used were CTCATGCTCTCTCTCCGG (363), GAATTACCGCGGCTGCTGG (549), GTTGTTCGTCTGGCGGTGATC (904), CTGGTGTGCCCCTCCGTC (1133), CCATTGTAGCGCGCGTG (1446), and TGATCCTTCTGCAGGTTCACCTAC (1766). Sequencing products were separated by electrophoresis in 6% polyacrylamide/8.3 M urea gels and visualized by autoradiography. Complete 18S sequences were assembled by overlapping individual sequence files in the ASSEMGEL program of the PC Gene package (IntelliGenetics Mountain View, CA). Ambiguities between overlapping sequence files were rechecked and resolved by sequencing additional clones as required.

Phylogenetic Analysis of Sequence Data

Sequences of 11 acanthocephalan species and 2 rotifer species (Garey *et al.*, 1996) were aligned according to a secondary structure model (Van de Peer *et al.*, 1994) using the DCSE editor (De Rijk and De Wachter, 1993). *Brachionus plicatilis* (GenBank U29235) and *Philodina acuticornis* (GenBank U41281) were used to root trees in the acanthocephalan analyses. These taxa were selected as outgroups because a more complete phylogenetic analysis of invertebrate diversity (Garey *et al.*, 1996) based on 18S sequences represented these two rotifers as most closely related to the acanthocephalans.

Maximum parsimony analyses were performed using a test version of PAUP* (4.0d54) (Swofford, 1997). In all maximum parsimony analyses, character-states inferred as gaps were treated as missing data, only minimal-length trees were retained, and zero-length branches collapsed. The branch-and-bound algorithm was utilized and bootstrap analysis (1000 replications, branch-and-bound algorithm) was used to examine the relative robustness of inferred monophyletic groups. Levels of support for groups recovered in parsimony analyses was also evaluated by decay analysis (Bremer, 1988), wherein strict consensus trees were constructed for all trees at successive steps longer than the shortest tree until the consensus tree collapsed to an unresolved bush. The decay index shows the number of substitutions that must be added to the most parsimonious hypothesis before each clade is no longer supported. The data set was assessed for phylogenetic signal by examination of the g_1 value of the tree length distribution for 10^5 randomly generated trees (Hillis and Huelsenbeck, 1992).

Phylogenetic relationships were also estimated using the minimum evolution method (Rzhetsky and Nei, 1992) as executed in PAUP* (4.0d54) with LogDet/paralinear distances (Lockhart *et al.*, 1994; Lake, 1994). Minimum evolution trees were recovered using heuristic searches with random addition of taxa (10 replicates), tree-bisection/reconnection branch-swapping, and the steepest descent option. Minimum evolution bootstrap analysis involved 1000 replications, with heuristic search options as described previously, except that random additions of taxa were not replicated.

Maximum likelihood analysis was executed using PAUP* (4.0d54) and included all sites in the aligned sequence data set. Options invoked in maximum likelihood analysis included nucleotide frequencies estimated from the data, number of substitution types set at 2, and rates assumed to follow a γ distribution with the α-shape parameter and proportion of invariable sites estimated via maximum likelihood. The γ approximation was set to four rate categories and the average rate for each category was represented by the mean. In addition, the HKY85 model (Hasegawa *et al.*, 1985) with rate heterogeneity was used, with the transition/transversion ratio estimated via maximum likelihood and starting branch lengths obtained using the Rogers–Swofford approximation. Bootstrap analysis employed 100 replications using model parameters estimated for the original dataset.

Alternative phylogenetic hypotheses (Fig. 1) were tested statistically by two different methods using the Tree Scores option in PAUP* (4.0d54). Topologies were assessed using a pairwise parsimony method proposed by Templeton (1983) and modified by Felsenstein (1993). This method uses the mean and variance of step differences between alternative topologies and is related to the test using log-likelihood differences of Kishino and Hasegawa (1989). For maximum likelihood analyses, alternative topologies were assessed using the test proposed by Kishino and Hasegawa (1989), where the mean and variance of log-likelihood differences are compared between trees. In both parsi-

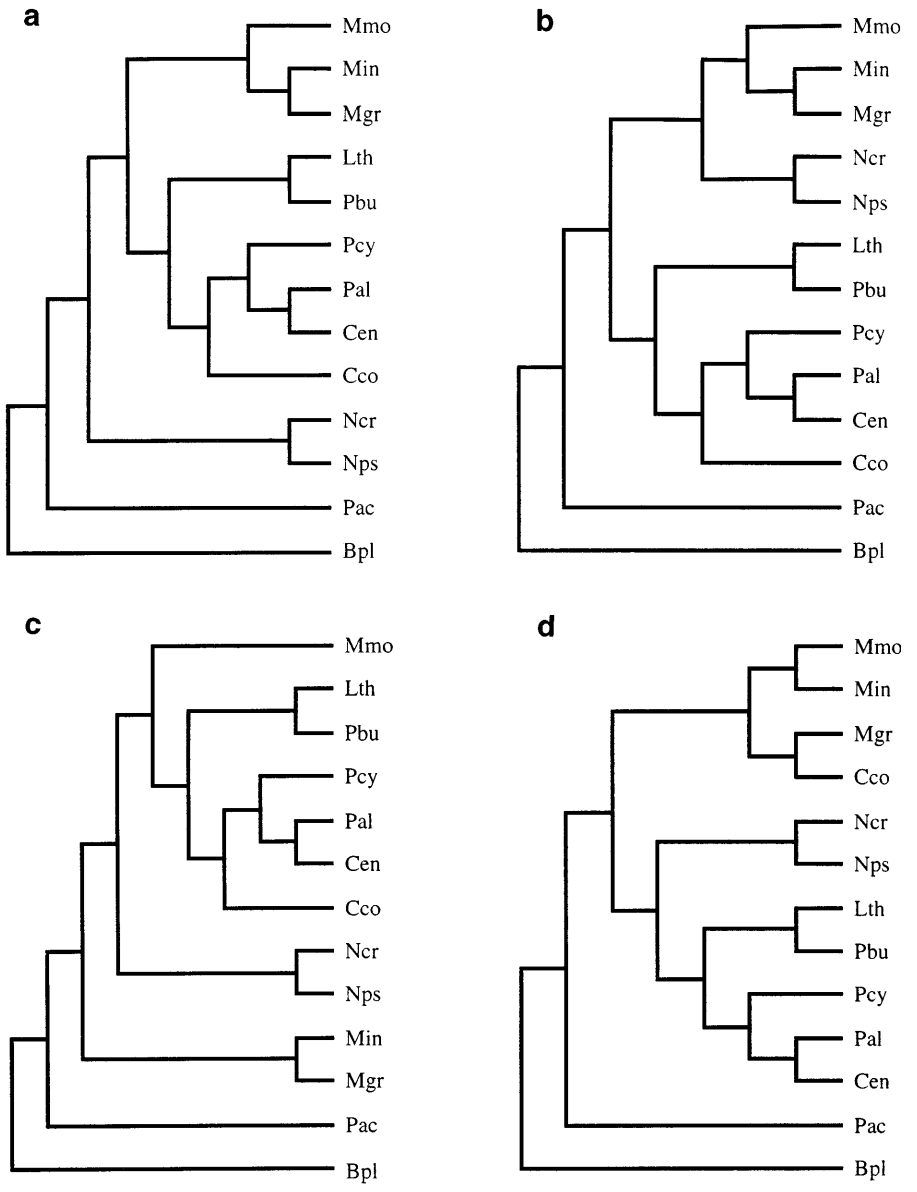

FIG. 1. Alternative topologies tested by statistical methods (see Table 3). Bpl, *Brachionus plicatilis;* Cco, *Centrorhynchus conspectus;* Cen, *Corynosoma enhydri;* Lth, *Leptorhynchoides thecatus;* Mgr, *Mediorhynchus grandis;* Min, *Macracanthorhynchus ingens;* Mmo, *Moniliformis moniliformis;* Ncr, *Neoechinorhynchus crassus;* Nps, *Neoechinorhynchus pseudemydis;* Pal, *Polymorphus altmani;* Pac, *Philodina acuticornis;* Pbu, *Pomphorhynchus bulbocolli;* Pcy, *Plagiorhynchus cylindraceus.*

mony and maximum likelihood assessments, alternative topologies were considered significantly different if the mean exceeded 1.96 standard deviations. Alternative hypotheses assessed included the inferred topologies from maximum parsimony, maximum likelihood, and minimum evolution analyses. Other topologies examined by these methods were representative of previous systematic hypotheses of acanthocephalan relationships and included the phylogenetic hypothesis of Van Cleave (1952), which depicts the Archiacanthocephala and Palaeacanthocephala as sister taxa (Metacanthocephala) (Fig. 1a), a sister-group relationship between the Archiacanthocephala and Eoacanthocephala (Fig. 1b), a hypothesis proposed by Brooks and McLennan (1993, pp. 369–373) (Fig. 1c), and the topology inferred from Petrochenko's (1956, pp. 159–162) classification of the Acanthocephala (Fig. 1d).

Analysis of Morphological and Ecological Characters

Eight morphological characters and four ecological characters were coded for analysis and treated as unordered (Table 1). All morphological characters examined were binary and three of four ecological characters were coded as multistate. Character states for indi-

TABLE 1

Matrix of Morphological and Ecological Characters for Species of Acanthocephala Used for Parsimony Mapping on Phylogenetic Hypotheses

	A	B	C	D	E	F	G	H	I	J	K	L
Moniliformis moniliformis	0	0	1	0	0	0	0	0	1	1	3	1
Macracanthorhynchus ingens	0	0	1	0	0	0	0	1	1	1	3	1
Mediorhynchus grandis	0	0	1	0	0	0	0	0	1	1	2	1
Neoechinorhynchus pseudemydis	0	0	0	1	0	1	0	0	2	0	1	0
Neoechinorhynchus crassus	0	0	0	1	0	1	0	0	2	0	0	0
Pomphorhynchus bulbocolli	1	1	1	0	1	1	1	0	0	0	0	0
Leptorhynchoides thecatus	1	1	1	0	1	1	1	0	0	0	0	0
Plagiorhynchus cylindraceus	1	1	1	0	1	1	1	0	0	1	2	1
Centrorhynchus conspectus	1	1	1	0	1	1	1	0	0	1	2	1
Polymorphus altmani	1	1	1	0	1	1	1	0	0	0	3	0
Corynosoma enhydri	1	1	1	0	1	1	1	0	0	0	3	0
Steps	1	1	1	1	1	1	1	1	2	3	5	3
Consistency index	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.33	0.60	0.33

Note. See text for description of characters and coding of character states. Number of steps and consistency index for each character, when optimized on the phylogenetic hypothesis, are indicated in the last two rows.

vidual acanthocephalan species were taken from the literature (Bullock, 1969; Meyer, 1932, 1933; Van Cleave, 1952). Characters examined and coding of character states were as follows: (A) Proboscis receptacle: 0 = single-walled; 1 = double-walled. (B) Cement gland i: 0 = giant nuclei; 1 = fragmented nuclei. (C) Cement gland ii: 0 = multiple; 1 = single. (D) Giant subcuticular nuclei: 0 = absent; 1 = present. (E) Ligament sac i: 0 = persistent; 1 = nonpersistent. (F) Ligament sac ii: 0 = double; 1 = single. (G) Lacunar system: 0 = dorsal or dorsal and ventral; 1 = lateral. (H) Protonephridia: 0 = absent; 1 = present. (I) Intermediate host: 0 = amphipod or isopod; 1 = insect or diplopod; 2 = ostracod or copepod. (J) Intermediate host habitat: 0 = aquatic; 1 = terrestrial. (K) Definitive host: 0 = fish; 1 = turtle; 2 = bird; 3 = mammal. (L) Definitive host habitat: 0 = aquatic; 1 = terrestrial. MacClade 3.0 (Maddison and Maddison, 1992) was used to map these characters by parsimony and to calculate consistency indices for these characters on the 18S gene tree and alternative topologies (Fig. 1).

18S Ribosomal DNA Sequence Divergence

Sequence divergence among acanthocephalans was compared to 18S rDNA sequence divergence among arthropods and vertebrates. Given the absence of a known fossil record for the Acanthocephala, comparative analysis of molecular data are among the only methods with the potential to provide estimates of the relative times of origin and divergence among acanthocephalan groups.

Nineteen arthropods, seven vertebrates, and a single hemichordate (Table 2) were aligned according to a secondary structure model (Van de Peer *et al.*, 1994) using the DCSE editor (De Rijk and De Wachter, 1993) and are deposited in TreeBASE (Sanderson *et al.*, 1994). Reported values for pairwise sequence divergence are based on this global alignment. Tree topologies (deposited in TreeBASE, not shown) were inferred using maximum parsimony as implemented in PAUP* 4.0. Three most-parsimonious trees were recovered and these trees were used to evaluate relative-rate variation among OTUs using the method of Wu and Li (1985) as implemented in the r8s program (Sanderson, 1997, version 0.10).

RESULTS

Phylogenetic Analysis of 18S rDNA Sequences

The aligned 18S rDNA sequence data consisted of 1848 nucleotide sites for the 11 acanthocephalan and 2 rotifer outgroup species. Eight hundred forty-one of the aligned nucleotide sites were variable and when all sites with gaps were excluded 713 sites were variable. Five hundred seventy-five of the 841 sites that varied were phylogenetically informative in maximum parsimony analysis. Unweighted maximum parsimony analysis of the aligned data set yielded a single most parsimonious tree of 1673 steps, with a consistency index (excluding uninformative characters) of 0.674 (Fig. 2). The minimum possible branch lengths supporting internal nodes within the Acanthocephala ranged from 4 to 150 apomorphies. The g_1 statistic for distribution of tree lengths from 10^5 randomly generated trees (-0.855) was significant ($P < 0.01$), indicating that the data set is more structured than are random data (Hillis and Huelsenbeck, 1992).

Minimum evolution analysis resulted in a single tree with a score of 0.9387 (Fig. 3a). This tree differed from the maximum parsimony tree (Fig. 2) with respect to relationships within the Archiacanthocephala, and the

TABLE 2

Taxa Used in 18S Sequence Divergence Comparison

Species	Classification	Accession No.
Octolasmis lowei	Arthropoda, Crustacea, Maxillopoda, Branchiura	L26518
Balanus eburneus	Arthropoda, Crustacea, Maxillopoda, Branchiura	L26510
Chelonibia patula	Arthropoda, Crustacea, Maxillopoda, Branchiura	L26514
Stenocypris major	Arthropoda, Crustacea, Maxillopoda, Ostracoda	Z22850
Berndtia purpurea	Arthropoda, Crustacea, Maxillopoda, Cirripedia	L26511
Oedignathus inermis	Arthropoda, Crustacea, Malacostraca, Decapoda	Z14062
Pugettia quadridens	Arthropoda, Crustacea, Malacostraca, Decapoda	Z22518
Daphnia galeata	Arthropoda, Crustacea, Branchiopoda	Z23111
Artemia salina	Arthropoda, Crustacea, Branchiopoda	X01723
Bosmina longirostris	Arthropoda, Crustacea, Branchiopoda	Z22731
Aeschna cyanea	Arthropoda, Uniramia, Insecta	X89481
Archaeopsylla erinacei	Arthropoda, Uniramia, Insecta	X89486
Ephemera spp.	Arthropoda, Uniramia, Insecta	X89489
Hydropsyche spp.	Arthropoda, Uniramia, Insecta	X89483
Galleria mellonella	Arthropoda, Uniramia, Insecta	X89491
Tenebrio molitor	Arthropoda, Uniramia, Insecta	X07801
Androctonus australis	Arthropoda, Chelicerata, Arachnida	X77908
Amblyomma tuberculatum	Arthropoda, Chelicerata, Arachnida	L76345
Eurypelma californica	Arthropoda, Chelicerata, Arachnida	X13457
Saccoglossus kowalevskii	Hemichordata, Enteropneusta	L28054
Amia calva	Chordata, Vertebrata, Actinopterygii	X98836
Lepisosteus osseus	Chordata, Vertebrata, Actinopterygii	X98837
Sebastolobus altivelis	Chordata, Vertebrata, Actinopterygii	M91182
Xenopus laevis	Chordata, Vertebrata, Tetrapoda, Lissamphibia	K01373
Mus musculus	Chordata, Vertebrata, Tetrapoda, Mammalia	X00686
Oryctolagus cuniculus	Chordata, Vertebrata, Tetrapoda, Mammalia	X06778
Homo sapiens	Chordata, Vertebrata, Tetrapoda, Mammalia	M10098

placement of *Plagiorhynchus cylindraceus* and *Centrorhynchus conspectus* was reversed in the Polymorphida. Groups appearing in >95% of bootstrapped minimum evolution trees included all groups recovered as >95% in maximum parsimony bootstrap analysis (Fig. 2). In addition, the clade consisting of Eoacanthocephala and Palaeacanthocephala was recovered in 87% of the bootstrap replicates (75% in maximum parsimony) and a monophyletic Palaeacanthocephala was recovered in 84% of the bootstrap replicates (78% in maximum parsimony).

Maximum likelihood analysis resulted in a single best tree with a ln likelihood score of −9810.729 (Fig. 3b). This tree is identical in topology to the minimum evolution tree, except for the relationships among the Archiacanthocephala (Fig. 3a). All branches in the maximum likelihood tree were significantly positive ($P < 0.01$) except for the node from *Macracanthorhynchus ingens* to (*Moniliformis moniliformis, Mediorhynchus grandis*), which was significantly positive at $P < 0.05$. The estimated transition/transversion ratio was 1.584 ($\kappa = 3.161$), the proportion of invariable sites was 0.172, and the estimated γ-shape parameter (α) was 0.610. Groups appearing in >95% of bootstrapped maximum likelihood trees included all groups recovered as >95% in maximum parsimony and minimum evolution bootstrap analyses except the Polymorphidae, which was supported with a bootstrap value of 81% versus 100% in both maximum parsimony and minimum evolution. Groups that appeared in >90% of bootstrapped trees which were not recovered in >90% of maximum parsimony and minimum evolution bootstrapped trees included the Eoacanthocephala–Palaeacanthocephala clade (96%) and a monophyletic Palaeacanthocephala (94%).

The examination of alternative topologies demonstrates that 18S rDNA is sufficient to discriminate statistically among most alternative hypotheses for the Acanthocephala (Table 3). Hypotheses of relationship that were determined to be significantly worse using both the modified Templeton (MT) and the Kishino–Hasegawa (K–H) tests ($P < 0.05$) include a topology depicting an Eoacanthocephala–Archiacanthocephala sister-group relationship (Fig. 1b), the grouping of Moniliformidae with the Palaeacanthocephala (Fig. 1c) (Brooks and McLennan, 1993, pp. 369–373), and the taxonomy of Petrochenko (Fig. 1d) (1956, pp. 159–162). Three alternative hypotheses were not significantly worse by MT tests: the topology recovered in minimum evolution analysis (Fig. 3a), the maximum likelihood topology (Fig. 3b), and a sister group relationship between the Archiacanthocephala and Palaeacanthocephala (Van Cleave, 1952) (Fig. 1a). Two alternative topologies were not considered significantly worse than the maximum likelihood tree using the K–H test: the maximum parsimony topology (Fig. 2) and the topology resulting from minimum evolution analysis (Fig. 3a). However, the hypothesis that the Archiacanthocephala and Palaeacanthocephala are sister taxa (Fig. 1a) (Van Cleave, 1952) was significantly worse ($P < 0.05$), as assessed by the K–H test.

Analysis of Morphological and Ecological Characters

The overall consistency index for morphological and ecological characters mapped on the maximum parsimony tree was 0.68 and the tree length was 22 steps.

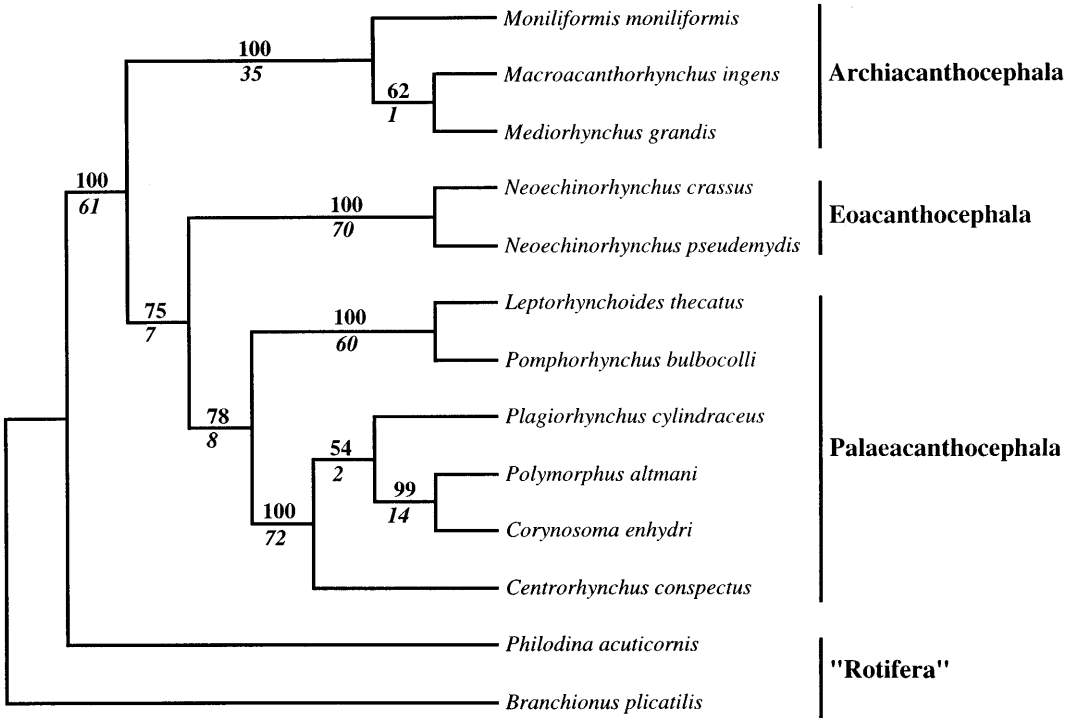

FIG. 2. Single tree resulting from maximum parsimony analysis, with a tree length equal to 1673 substitutions and a CI of 0.674. Bootstrap percentages of clades (1000 replications) are shown above nodes. Italic numbers below nodes represent decay values.

All morphological characters mapped on the phylogenetic hypothesis had a consistency index of 1.00 (Table 1). Intermediate host type had a consistency index of 1.00 and definitive host association had a consistency index of 0.60. The habitats of both the intermediate and the definitive host each had a consistency index of 0.33.

Mapping the morphological and ecological characters on the alternative topologies examined (Fig. 1) revealed character distributions that were equally parsimonious in all but two hypotheses. The hypothesis of Brooks and McLennan (1993, pp. 369–373) (Fig. 1c) had an overall consistency index of 0.63 and a tree length of 24 steps.

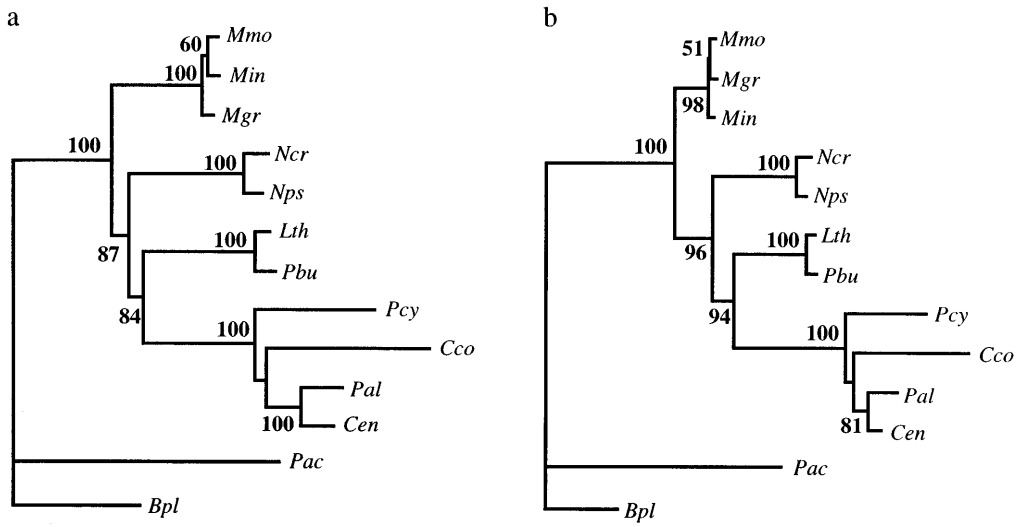

FIG. 3. (a) Single tree recovered from minimum evolution analysis (tree score = 0.9387) using LogDet/paralinear distances. Branches are drawn to scale (number of substitutions per site). Numbers indicate bootstrap percentages of clades (1000 replications). (b) Topology obtained from maximum likelihood analysis of all 1848 rDNA sites (ln likelihood −9810.729). Branches are drawn to scale (number of substitutions per site). Numbers indicate bootstrap percentages of clades (100 replications). See Fig. 1 for species abbreviations.

TABLE 3

Statistical Comparison of Alternative Topologies Using Modified Templeton Test (MT) and Kishino–Hasegawa (K–H) Test

	Maximum parsimony				Maximum likelihood		
Topology	Parsimony length (SD)	Worse by MT test?	Consistency index	Number of topologies with fewer or equal steps	*ln* L	SD of *ln* L difference	Worse by K–H test?
Fig. 2	1673	—	0.674	1	−9,812.21	3.06	No
Fig. 3a	1676 (6.56)	No	0.672	7	−9,811.34	1.26	No
Fig. 3b	1677 (6.48)	No	0.672	9	−9,810.73	—	—
Fig. 1a	1680 (6.86)	No	0.670	11	−9,836.18	9.93	Yes
Fig. 1b	1690 (6.07)	Yes	0.665	63	−9,837.97	9.30	Yes
Fig. 1c	1769 (9.54)	Yes	0.630	2,905	−9,913.01	17.35	Yes
Fig. 1d	1875 (14.77)	Yes	0.587	>34,500	−10,111.54	26.44	Yes

The taxonomic hypothesis of Petrochenko (1956) (Fig. 1d) was the least parsimonious topology examined, with a consistency index of 0.58 and a tree length of 26 steps.

DISCUSSION

Phylogenetic support for the major groups in the Meyer–Van Cleave taxonomic system has not been examined previously, and the relationship between the three major groupings of Acanthocephala has remained unresolved. Phylogenetic analysis of 18S rDNA sequence data is consistent with monophyly of previously defined taxonomic groups of the Acanthocephala. However, because of limited taxonomic sampling, conclusions regarding monophyly for certain higher taxa, such as the Eoacanthocephala, should be considered preliminary.

The Archiacanthocephala, Palaeacanthocephala, and Eoacanthocephala were each monophyletic in maximum parsimony, maximum likelihood, and minimum evolution analyses (Figs. 2, 3a, and 3b). The taxonomic hypothesis of Van Cleave (1948, 1952) (Fig. 1a) proposes a sister-group relationship between the Archiacanthocephala and Palaeacanthocephala, based on the presence of multiple cement glands in both groups. This topology could not be rejected by the modified Templeton test, but was rejected using the Kishino–Hasegawa test (Table 3). The Palaeacanthocephala–Eoacanthocephala clade was recovered in all phylogenetic analyses and received moderate support in bootstrap and decay analyses in maximum parsimony analysis (Fig. 2) and high bootstrap values in minimum evolution and maximum likelihood analyses (Figs. 3a and 3b). The ability of 18S rDNA sequences to resolve the relationships of the three major acanthocephalan groups may be enhanced with a more thorough sampling of the Echinorhynchida (Palaeacanthocephala) and Eoacanthocephala.

The 18S phylogeny contradicts the morphologically based hypotheses of relationship for the Acanthocephala proposed by Brooks and McLennan (1993, pp. 369–373), who split the Archiacanthocephala because they recognized no synapomorphy for the group. In all three of their hypotheses the Moniliformidae (Archiacanthocephala) is presented as a sister-group to the Palaeacanthocephala. This seemingly artificial grouping is based on the mistaken assignment of a double-walled proboscis receptacle to the Moniliformidae (Wanson and Nickol, 1975). In all analyses of 18S rDNA the Archiacanthocephala were monophyletic and no relationship between the Moniliformidae and Palaeacanthocephala was recovered. As an alternative hypothesis, the placement of the Moniliformidae within the Palaeacanthocephala resulted in a significantly worse topology using both the MT and K–H tests (Table 3). With respect to higher level relationships, the analysis of Brooks and McLennan yielded a polytomy.

The inferred absence of homoplasy exhibited by morphological characters when mapped on the 18S gene tree (Table 1) is not unexpected since these particular characters have traditionally been used to diagnose the major acanthocephalan groups, which were recovered as monophyletic in the 18S gene trees (Figs. 2 and 3). Other suites of morphological characters that appear to be more variable within major acanthocephalan groups are poorly characterized among some lineages, which precludes comparative analysis with respect to the 18S tree.

This 18S gene tree provides a phylogenetic framework to develop hypotheses of polarity and pattern for evolution of structural and ecological characters and also allows inferences of putative homoplasy. Development of such hypotheses of character evolution for the Acanthocephala was previously problematic because lack of identified homology of these characters in free-living rotifer outgroups prevented determination of sequence and direction of change. Hypotheses are developed herein primarily with reference to polarization inferred from the 18S rDNA gene tree.

Cement glands, which are present only in male acanthocephalans, have been used extensively in taxonomic studies. The adhesive product of the cement glands is used by the male to seal the reproductive canal of the female after insemination. Similar structures, pedal or cement glands, are present in both male and female rotifers and are located in the posterior part of the body. The pedal gland produces an adhesive substance that rotifers use to attach to substrates and as an aid to locomotion. Because bdelloid rotifers are the sister-group to the Acanthocephala (Lorenzen, 1985; Garey *et al.*, 1996), it is important to note that the bdelloid rotifers *Embata* and *Philodina* have multiple pedal glands that are uninucleate, with giant nuclei (Hyman, 1951, p. 77, Fig. 35). The morphology of these pedal glands resembles the cement glands of the Archiacanthocephala. All archiacanthocephalans have eight uninucleate cement glands with giant nuclei. In contrast, the Eoacanthocephala have a single cement gland with multiple giant nuclei, and the Palaeacanthocephala have multiple cement glands (2–8) with "fragmented" nuclei (Bullock, 1969; Conway Morris and Crompton, 1982; Van Cleave, 1952). The nesting of the Acanthocephala within the Rotifera and the similarity of morphology, anatomical position, and function of the pedal glands in rotifers and cement glands in acanthocephalans provide reasons to hypothesize that these structures are homologous. If the bdelloid rotifer condition (multiple uninucleate with giant nuclei) is used to polarize this character, then the "multiple uninucleate with giant nuclei" condition of cement glands is ancestral for the Acanthocephala. Thus, in this scenario and with reference to the 18S tree, eight uninucleate cement glands with giant nuclei is diagnostic for the Archiacanthocephala, a single cement gland with giant nuclei is an autapomorphy that is diagnostic of the Eoacanthocephala, and the condition of multiple cement glands with "fragmented" nuclei is an autapomorphy for the Palaeacanthocephala. Our hypothesis is that the pattern of evolution for the cement glands follows from multiple uninucleate with giant nuclei as the primitive condition to single cement glands with giant nuclei and multiple cement glands with "fragmented" nuclei as the derived conditions.

The presence of giant subcuticular nuclei is a putative autapomorphy in the Eoacanthocephala; the Archiacanthocephala and Palaeacanthocephala exhibit the plesiomorphic condition of fragmented or branched nuclei. The presence of a single uterine bell in females is a putative autapomorphy in the Palaeacanthocephala; the Eoacanthocephala and Archiacanthocephala have a double uterine bell. The presence of a persistent uterine bell is characteristic of the Archiacanthocephala; a nonpersistent uterine bell is found in Palaeacanthocephala and Eoacanthocephala. Unfortunately, the uterine bell is a synapomorphy which is diagnostic for the Acanthocephala and is apparently absent in the nonacanthocephalan rotifers; therefore, the plesiomorphic condition for the uterine bell cannot be unambiguously determined by reference to outgroups. The lateral lacunar system is a putative autapomorphy for the Palaeacanthocephala; the Archiacanthocephala and Eoacanthocephala exhibit the plesiomorphic dorsal or dorsal and lateral lacunar canals. The presence of protonephridia may be an autapomorphy for the archiacanthocephalan family Oligacanthorhynchidae. However, Golvan (1959b) considered protonephridia as a primitive feature that persisted from free-living ancestors of the Acanthocephala. Supporting this interpretation is the presence of protonephridia in free-living rotifers (Wallace and Snell, 1991). Therefore, the presence of protonephridia in the Oligacanthorhynchidae may represent persistence of a plesiomorphic trait that has been secondarily lost in all other acanthocephalans.

Patterns of host association in the Acanthocephala appear to be characterized by both adaptive plasticity and historical constraint. Definitive and intermediate host habitats (terrestrial vs aquatic) and vertebrate definitive host groups had low consistency index values when mapped on the parsimony tree (Table 1). This indicates that these characteristics have evolved independently in different acanthocephalan lineages. Vertebrate host plasticity is pronounced in the Acanthocephala, as demonstrated by the independent use of birds and mammals as definitive hosts in the Archiacanthocephala and Polymorphida (Palaeacanthocephala). As a result of this plasticity, the age of the Acanthocephala cannot be inferred from the age of their vertebrate hosts.

Arthropod groups utilized by acanthocephalans as intermediate hosts had a consistency index of 1.00 when mapped on the maximum parsimony tree (Table 1). This indicates that unlike their vertebrate host groups, acanthocephalans appear to have a strict pattern of historical association with regard to their intermediate hosts. The phylogenetic hypothesis for higher level groupings of arthropod intermediate hosts of the Acanthocephala resembles the topology recovered for the three major groups of acanthocephalans. The isopods and amphipods (Malacostraca) and the ostracods and copepods (Maxillopoda) are included in a monophyletic Crustacea (Wheeler *et al.*, 1993). Relationships within the Crustacea are unresolved, so it cannot be determined if the Malacostraca and Maxillopoda are sister taxa. The Crustacea are hypothesized to be the sister group to the uniramians (insects and myriapods) (Wheeler *et al.*, 1993). The Eoacanthocephala and Palaeacanthocephala, which are monophyletic in all analyses, both utilize crustaceans as intermediate hosts. The sister group to the Eoacanthocephala–Palaeacanthocephala clade is the Archiacanthocephala, which utilizes the sister group of the crustaceans, uniramians, as intermediate hosts. The phylogenetically conserved utilization of intermediate hosts (Table 1) and

the similarity in tree topologies of acanthocephalans and their arthropod hosts suggest that the Acanthocephala may have been associated with the evolution of major groups within the Mandibulata (Crustacea, Myriopoda, and Insecta), and the age of these arthropod groups may provide clues to the age of the Acanthocephala.

Diversification of acanthocephalan life cycles also involves shifts between aquatic and terrestrial systems. Previous observations have noted that significant diversification of parasite life cycles generally involve evolutionary changes in the larval stage, which then result in a shift in the definitive hosts parasitized (Brooks and McLennan, 1993). The phylogeny of the Acanthocephala supports the hypothesis that intermediate hosts (including both terrestrial and aquatic forms) have facilitated shifts between aquatic and terrestrial life histories (Fig. 4). A shift to terrestrial intermediate hosts, as exemplified by *Centrorhynchus* and *Plagiorhynchus* of the Polymorphida, allowed a radiation of these taxa into terrestrial birds that is independent of the terrestrial radiation displayed by the Archiacanthocephala. The Polymorphidae (*Polymorphus* and *Corynosoma*) are derived polymorphids that have aquatic intermediate, paratenic, and definitive hosts. The aquatic environment occupied by the Polymorphidae may have originated in a shift from a terrestrial to an aquatic habitat. Therefore the condition of utilizing aquatic hosts in the Polymorphidae and the Echinorhynchida is potentially homoplasious, since the sister group of the Polymorphidae is a terrestrial parasite (Fig. 4). Ecological shifts between aquatic and terrestrial intermediate host habitats displayed within the Palaeacanthocephala were likely promoted by the availability of malacostracans in both terrestrial and aquatic habitats. The Eoacanthocephala and their intermediate host groups (ostracods and copepods) are found only in aquatic habitats; hence, the potential for radiation of eoacanthocephalans into terrestrial vertebrates is limited by lack of terrestrial intermediate hosts. These observations suggest that associations between acanthocephalans and their intermediate hosts have been important in determining distributions among definitive hosts.

Uncorrected 18S sequence divergence among the Acanthocephala is high. The average uncorrected *p*

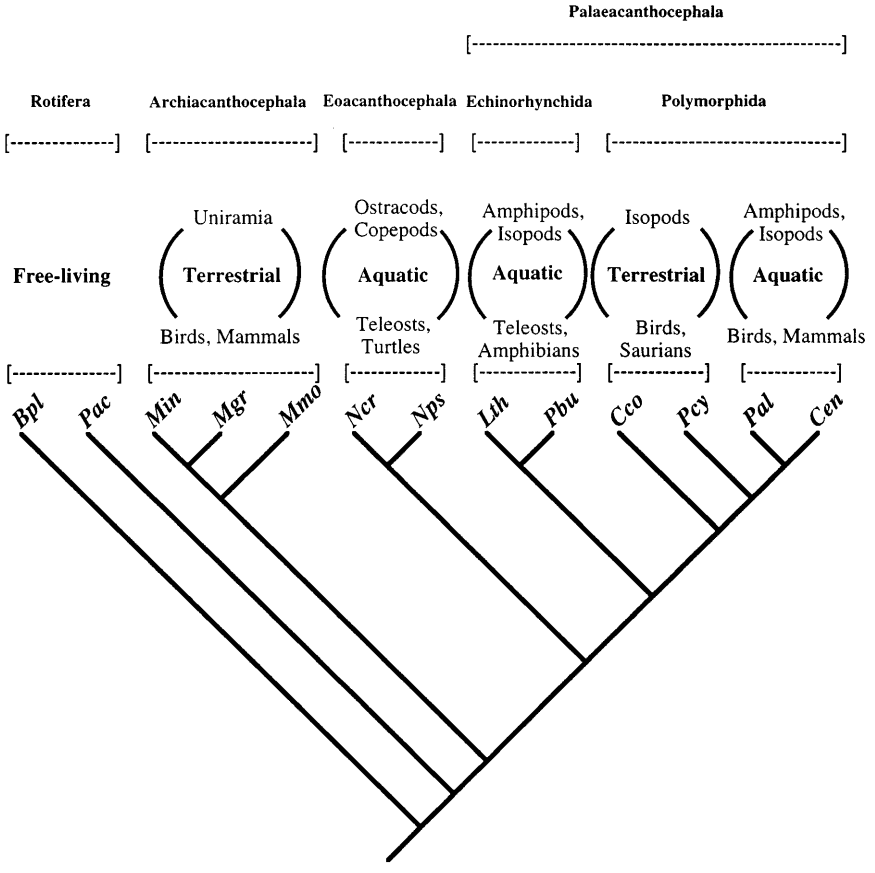

FIG. 4. Diversification in life cycle patterns for acanthocephalans illustrated on the 18S rDNA maximum parsimony tree. Taxa listed on the top (intermediate host) and the bottom (definitive host) of each circle indicates the host groups utilized by acanthocephalan taxa within brackets. The habitat of the intermediate and definitive hosts is indicated in the center of the circle. See Fig. 1 for species abbreviations.

distance among all acanthocephalans sampled (15.5%) exceeds values reported between vertebrates–invertebrates (13.1%) or among plants, animals, and fungi (14.7%) (Fernandes *et al.*, 1993). Likewise, sequence divergence among the major groups of acanthocephalans is roughly equivalent to that observed in some major groups of arthropods. However, relative rate tests revealed significant departures from rate uniformity in 53/84 three-taxon comparisons among acanthocephalans, arthropods, and vertebrates. This amount of rate variation precludes conclusions about the timing of the acanthocephalan radiation based on simple comparisons of sequence divergence.

However, one implication of the independent radiation of acanthocephalans into similar paratenic and definitive host groups, as inferred from tree topologies (Fig. 4), is that the acanthocephalan radiation may be more closely coupled to intermediate host associations. Previous workers (Conway Morris and Crompton, 1982) proposed a Cambrian origin for the Acanthocephala; the conservative nature of the arthropod intermediate host group utilized by acanthocephalans (Table 1) in combination with the similarities in trees between major clades of acanthocephalans and their arthropod hosts is consistent with such an interpretation. However, the hypothesis that acanthocephalans and priapulids form a sister group (Conway Morris and Crompton, 1982) is not supported by morphological or molecular phylogenies (Garey *et al.*, 1996; Lorenzen, 1985; Winnepenninckx *et al.*, 1995), thus invalidating the suggestion that Burgess Shale (Cambrian) fossil priapulids (e.g., *Ancalagon minor*) represent an ancestral form of acanthocephalans (Conway Morris and Crompton, 1982). Given substantial rate variation in 18S rDNA sequences among metazoan taxa, other genes will be needed to address relative times of divergence using molecular data.

The Acanthocephala have simultaneously fascinated and eluded investigators attempting to understand their evolutionary and taxonomic relationships. Unlike other parasitic helminth groups (cestodes, trematodes, and nematodes), the Acanthocephala have a relatively small number of species, a conserved two-host (arthropod–vertebrate) life cycle, and corroborated phylogenetic hypotheses showing their relationship to a free-living sister group. These features make them attractive candidates as model organisms for studying many intriguing aspects of parasite evolution. Ultimately, a molecular phylogenetic hypothesis with additional taxonomic diversity will provide a detailed framework for understanding the patterns and mechanisms of acanthocephalan evolution.

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