Acanthocephalan Phylogeny and the Evolution of Parasitism[1]

THOMAS J. NEAR[2]

Center for Population Biology, University of California, One Shields Ave., Davis, California 95616

SYNOPSIS.   The study of parasite evolution relies on the identification of free-living sister taxa of parasitic lineages. Most lineages of parasitic helminths are characterized by an amazing diversity of species that complicates the resolution of phylogenetic relationships. Acanthocephalans offer a potential model system to test various long-standing hypotheses and generalizations regarding the evolution of parasitism in metazoans. The entirely parasitic Acanthocephala have a diversity of species that is manageable with regards to constructing global phylogenetic hypotheses, exhibit variation in hosts and habitats, and are hypothesized to have close phylogenetic affinities to the predominately free-living Rotifera. In this paper, I review and test previous hypotheses of acanthocephalan phylogenetic relationships with analyses of the available 18S rRNA sequence database. Maximum-parsimony and maximum-likelihood inferred trees differ significantly with regard to relationships among acanthocephalans and rotifers. Maximum-parsimony analysis results in a paraphyletic Rotifera, placing a long-branched bdelloid rotifer as the sister taxon of Acanthocephala. Maximum-likelihood analysis results in a monophyletic Rotifera. The difference between the two optimality criteria is attributed to long-branch attraction. The two analyses are congruent in terms of relationships within Acanthocephala. The three sampled classes are monophyletic, and the Archiacanthocephala is the sister taxon of a Palaeacanthocephala + Eoacanthocephala clade. The phylogenetic hypothesis is used to assess the evolution of host and habitat preferences. Acanthocephalan lineages have exhibited multiple radiations into terrestrial habitats and bird and mammal definitive hosts from ancestral aquatic habitats and fish definitive hosts, while exhibiting phylogenetic conservatism in the type of arthropod intermediate host utilized.

INTRODUCTION

Acanthocephala is a small group of obligate parasites that utilize arthropods and vertebrates in a conserved two-host life cycle. The name of the phylum refers to the thorny retractable proboscis that anchors the adult worm to the intestine of the vertebrate host. In addition to the thorny proboscis, acanthocephalans are distinguished morphologically as cylindrical and unsegmented worms. The trunk is a hollow structure that contains the excretory, reproductive, and nervous systems and is filled with psuedocoelomic fluid (Dunagan and Miller, 1991). An interesting feature of acanthocephalans is the absence of an alimentary tract. The absorption of nutrients occurs entirely through the body wall and is facilitated by a syncytial epidermis and a lacunar system of circulatory channels (Starling, 1985). Other unique morphological features of acanthocephalans include a proboscis receptacle at the anterior end of the trunk and the paired lemnisci that extend into the trunk, from an attachment on the neck.

Acanthocephalans are gonochoristic and invariably utilize arthropods as intermediate hosts and vertebrates as definitive hosts. Occasionally, vertebrates serve as paratenic hosts harboring larval acanthocephalans that do not develop to adults unless ingested by the appropriate vertebrate definitive hosts (Nickol, 1985). As in many helminth parasites, acanthocephalan life cycles exploit trophic interactions between arthropods and vertebrates, with the initial stages of the life cycle involving ingestion of viable shelled embryos by the arthropod intermediate host. Completion of the life cycle, including reproduction, occurs when an appropriate vertebrate definitive host ingests an infected arthropod intermediate host. For some species, transport or paratenic hosts are required to complete the life cycle.

Traditionally, the taxonomic groups in Acanthocephala have been identified based on morphological features and host characteristics (Bullock, 1969). In general, there is a large degree of congruence between morphological and molecular phylogenetic hypotheses of Acanthocephala, as well as congruence between phylogenetic hypotheses and traditional taxonomic classifications (Near *et al.*, 1998; Garcia-Varela *et al.*, 2000; Monks, 2001). Phylogenetic investigations of acanthocephalans have used 18S ribosomal RNA (rRNA) sequence data (Near *et al.*, 1998; Garcia-Varela *et al.*, 2000) and morphological characters (Monks, 2001). The molecular phylogenetic analyses result in monophyly for all three sampled acanthocephalan classes (Archiacanthocephala, Palaeacanthocephala, and Eoacanthocephala). However, phylogenetic analysis of 138 morphological characters does not result in monophyly of Archiacanthocephala (Monks, 2001). Both molecular and morphological analyses support a sister taxon relationship between Palaeacanthocephala and Eoacanthocephala (Near *et al.*, 1998; Garcia-Varela *et al.*, 2000; Monks, 2001). The 18S rRNA phylogenetic analysis of Garcia-Varela *et al.* (2000) and cladistic analysis of morphology in Monks (2001) does not recover the palaeacanthocephalan order Echinorhynchida as monophyletic; however, this result was not com-

[2] E-mail: tjnear@ucdavis.edu

pared to the null hypothesis of echinorhynchid monophyly.

The phylogenetic hypothesis based on 18S rRNA sequence data has been used to examine evolutionary trends of ecological diversification within Acanthocephala. The type of arthropod intermediate hosts used by each acanthocephalan class is conservative and hence diagnostic: Archiacanthocephala utilize myriapods and insects, Palaeacanthocephala utilize malacostracans, and Eoacanthocephala utilize maxillopods (Bullock, 1969; Schmidt, 1985; Near et al., 1998). In contrast, the types of vertebrate definitive hosts utilized by acanthocephalans are not diagnostic for the monophyletic classes. For instance, there have been at least two independent radiations of acanthocephalans into mammals and birds in Archiacanthocephala and Palaeacanthocephala, with an unambiguous ancestral state of fishes as definitive hosts in Palaeacanthocephala (Near et al., 1998). Eoacanthocephalans primarily use fishes as definitive hosts; however, there is a radiation of *Neoechinorhynchus* in North American freshwater turtles. Also, within Acanthocephala there have been multiple shifts between aquatic and terrestrial intermediate and definitive hosts (Near et al., 1998).

The evolution of parasitism is a tantalizing question in evolutionary biology, and in many respects acanthocephalans provide a potential model system to investigate adaptive processes associated with the evolution of parasitism. First, the diversity of acanthocephalans is limited to approximately 1,150 described species (Amin, 1985). Second, the basic life cycle is highly conserved among all acanthocephalans. Third, substantial phylogenetic evidence from both morphology and molecular data indicates that acanthocephalans have a close evolutionary relationship with Rotifera (Clement, 1985; Lorenzen, 1985; Winnepenninckx et al., 1995; Garey et al., 1998; Mark Welch, 2000). The identification of a free-living sister taxon to the entirely parasitic Acanthocephala offers an unprecedented opportunity to study the evolution of obligate parasitism in terms of character innovations *versus* character loss, evolutionary trends in host and habitat specificity, and adaptive radiation with regard to morphological and ecological diversification (Brooks and McLennan, 1993).

Phylogenetic hypotheses relating parasitic lineages to a free-living sister taxon allow direct comparisons of characters and features regarded as important in the origin and diversification of parasites. For example, both the Nematoda and Platyhelminthes contain obligate parasites as well as free-living species. Comparison of parasitic and free-living species in these groups is complicated by the very large diversity of species in each of these lineages and the difficulties associated with developing phylogenetic hypotheses for such large groups of organisms. Undaunted by the extensive diversity of species, investigations have attempted to examine the evolution of parasitism in both nematodes (Blaxter et al., 1998) and platyhelminths (Littlewood et al., 1999) using phylogenetic hypotheses inferred from rRNA. These studies indicate that animal parasitism has at least four independent origins in the nematodes and a single origin in the platyhelminths. Based on sister taxon comparisons, parasitic lineages within the nematodes seem to be associated with free-living lineages that are bacteriovores (Blaxter et al., 1998). These conclusions are preliminary, however, as much of the diversity of nematodes (both parasitic and free-living) is absent from the rRNA database, and recovered relationships do not receive appreciable bootstrap pseudoreplicate support. The situation is somewhat different for platyhelminths, where parasitic lineages comprise a monophyletic Neodermata. Identification of the sister group to the Neodermata is complicated by lack of resolution in morphological and rRNA characters. Based on an extensive examination of the available character sets, Littlewood et al. (1999) conclude that a Fecampiida + Urastomidae clade, or the neoophorans, represent the best candidate lineages that are the sister taxa of the parasitic Neodermata; however, other hypotheses could not be rejected.

Unlike the situation in nematodes and platyhelminths, substantial evidence supports a close phylogenetic affinity of acanthocephalans to the predominately free-living rotifers. Traditionally acanthocephalans were considered to be related to a number of pseudocoelomate animal phyla in the Aschelminthes (Hyman, 1951; Brusca and Brusca, 1990). Phylogenetic analysis of 18S rRNA sequences demonstrate, however, that the Aschelminthes is not monophyletic, and among sampled sequences Acanthocephala and Rotifera were recovered as a monophyletic group with substantial branch support (Winnepenninckx, 1995). Four morphological features have been presented as apomorphies for a monophyletic Rotifera + Acanthocephala clade: (1) syncytial epidermis, (2) intracytoplasmic lamina, (3) sperm cells with flagellum inserted anteriorly, and (4) epidermal cells with apical crypts (Clement, 1985; Lorenzen, 1985; Nielsen et al., 1996; Wallace et al., 1996; Ahlrichs, 1997; Garey et al., 1998; Kristensen and Funch, 2000). Alrichs (1997) suggested the name Syndermata for this clade.

An earlier study using morphological evidence hypothesized that Acanthocephala is the sister taxon of the rotifer class Bdelloidea, making Rotifera paraphyletic (Lorenzen, 1985). This relationship was also recovered in a preliminary analysis of 18S rRNA sequences sampled from two rotifers, one monogonont and one bdelloid, and three acanthocephalans (Garey et al., 1996). Garey et al. (1996) proposed the name Lemniscea for this clade. Despite the strong initial support for this relationship from rRNA sequence data, the hypothesis of Lorenzen (1985) is not popular among rotifer taxonomists, and the validity of the homology assessment of characters designated as apomorphies for a Bdelloidea + Acanthocephala clade has been questioned (Melone et al., 1998; Ricci, 1998). In addition to critical examination of the morphological evidence, the monophyly of the Lemniscea was re-

jected, and a monophyletic Rotifera was recovered in a phylogenetic analysis with expanded taxon sampling of 18S rRNA (Garcia-Varela *et al.*, 2000). The preliminary analysis of Garey *et al.* (1996) used an alignment of 18S rRNA sequences based on the inferred secondary structure model of the molecule. The use of secondary structure models to assess positional homology in rRNA genes is preferred over methods of pairwise similarity-based methods of alignment (Kjer, 1995). The conclusions of Garcia-Varela *et al.* (2000) regarding the monophyly of Rotifera were derived from analyses using dendogram-based similarity alignments that were adjusted by eye. No biologically meaningful criterion (*i.e.,* secondary structure) was presented to justify the 18S rRNA alignments used by Garcia-Varela *et al.* (2000). Additional evidence of non-monophyly of Rotifera has come from phylogenetic analysis of the heat shock protein, hsp82 (Mark Welch, 2000). Phylogenetic relationships inferred from hsp82 depicted the rotifer class Seisonidea as the sister taxon of a monophyletic trichotomy that included Acanthocephala, Bdelloidea, and Monogononta. Bdelloidea was not recovered as the sister taxon of Acanthocephala (Mark Welch, 2000). Alternative hypotheses of relationship were not statistically compared to optimal trees using the hsp82 dataset.

The purposes of this study are to reassess relationships among acanthocephalans using an expanded 18S rRNA dataset presented in Garcia-Varela (2000), examine alternative phylogenetic hypotheses of acanthocephalan relationships, and use the phylogenetic tree of acanthocephalans to develop hypotheses of evolutionary diversification addressing host and habitat use. In addition, I reexamine the controversy regarding the relationships of acanthocephalans and rotifers using all available 18S rRNA sequences. Specifically, I investigate the power of maximum-parsimony (MP) and maximum-likelihood (ML) analyses of 18S rRNA gene sequences to discriminate among alternative phylogenetic hypotheses.

MATERIALS AND METHODS

Sequences of the 18S rRNA gene of 21 acanthocephalans, 6 rotifers, and several other invertebrate phyla were downloaded from Genbank (Table 1) and added to the secondary structure alignment of Garey *et al.* (1996) and Near *et al.* (1998) using the DCSE editor (De Rijk and Wachter, 1993). Pairwise genetic distances, transition:transversion ratios, and base composition values were calculated using PAUP* (Swofford, 2000). Saturation of nucleotide substitutions was estimated by plotting observed numbers of transitions *versus* observed transversions. A linear relationship between transitions and transversions with the slope equal to the transition:transversion ratio is expected of data that are not saturated with multiple nucleotide substitutions (Moritz *et al.*, 1992).

MP and ML analyses were executed using PAUP*. For MP analyses, heuristic tree searches were used with tree bisection-reconnection (TBR) branch swap-

ping with 10 random sequence additions. Character states inferred as gaps were treated as missing data. Bootstrap (2,000 pseudoreplications) and decay analyses (Bremer, 1988) were used to assess the relative support for recovered nodes in the most-parsimonious topology. For ML analysis the preferred model of sequence evolution for the 18S rRNA dataset was determined using a best-fit criterion (Frati *et al.*, 1997; Cunningham *et al.*, 1998), assessed using hierarchical likelihood ratio tests with a chi-square distribution (Huelsenbeck and Crandall, 1997). The least complex model that resulted in a significant increase in the likelihood score was chosen. The computer program Modeltest ver. 2.0 (Posada and Crandall, 1998) was used to calculate likelihood scores and execute likelihood ratio tests. PAUP* was used to execute heuristic tree searches with subtree pruning-regrafting (SPR) branch swapping with ten replicates using random addition of taxa. Bootstrap analysis in maximum-likelihood was executed with 100 pseudoreplicates using the preferred model of sequence evolution.

Alternative hypotheses of phylogenetic relationships between acanthocephalans and rotifers, and among major lineages of acanthocephalans (Fig. 1) were statistically compared to those recovered from parsimony analyses using the modified Templeton (MT) test (Templeton, 1983; Felsenstein, 1993), and maximum-likelihood analysis using the Kishino-Hasegawa (K-H) test (Kishino and Hasegawa, 1989), as executed in PAUP* (Swofford, 2000). Separate constraint tree searches were used to find the optimal MP and ML trees that depicted the alternative hypotheses in Figure 1.

The evolutionary diversification of host utilization and host habitats were investigated by optimizing these features onto the MP and ML trees using both ACTRAN and DELTRAN parsimony as executed using MacClade 4.0 (Maddison and Maddison, 2000). Character states for hosts and host habitats were taken from the literature (Van Cleave, 1953; Golvan, 1959, 1960, 1961, 1962; Bullock, 1969).

RESULTS

Secondary structure-based alignment of the 18S rRNA sequences (Table 1) contained 2,163 nucleotide positions, with 1,029 phylogenetically informative sites for parsimony. Plotting transitions *versus* transversions did not indicate the presence of multiple substitution (not shown). MP analysis resulted in a single most-parsimonious tree (Fig. 2). Rotifera and Acanthocephala comprise a monophyletic group with high bootstrap pseudoreplicate recovery and decay score (Fig. 2). Similar to the earlier phylogenetic studies using 18S rRNA (Garey *et al.*, 1996), the rotifers are paraphyletic because *Philodina acuticornis* is placed as the sister taxon of a monophyletic Acanthocephala with high bootstrap and decay support (Fig. 2). The bdelloid genus *Philodina* is not monophyletic, and the remaining species of rotifers form a monophyletic clade (Fig. 2). Within Acanthocephala, hypothesized relationships are identical to those in studies by Near

TABLE 1.   *18S rRNA sequences examined.**

Species	Accession no.	Class-order
Acanthocephala		
Macracanthorhynchus ingens	AF001844	Archiacanthocephala - Oligacanthorhynchida
Oligacanthorhynchus tortuosa	AF064817	Archiacanthocephala - Oligacanthorhynchida
Oncicola sp.	AF064819	Archiacanthocephala - Oligacanthorhynchida
Mediorhynchus grandis	AF001843	Archiacanthocephala - Gigantorhynchida
Mediorhynchus sp.	AF064813	Archiacanthocephala - Gigantorhynchida
Moniliformis moniliformis	Z19562	Archiacanthocephala - Moniliformida
Floridosentis mugilis	AF064811	Eoacanthocephala - Neoechinorhynchida
Neoechinorhynchus crassus	U41400	Eoacanthocephala - Neoechinorhynchida
Neoechinorhynchus pseudemydis	AF001842	Eoacanthocephala - Neoechinorhynchida
Echinorhynchus gadi	U88335	Palaeacanthocephala - Echinorhynchida
Filisoma bucerium	AF064814	Palaeacanthocephala - Echinorhynchida
Leptorhynchoides thecatus	AF001840	Palaeacanthocephala - Echinorhynchida
Koracantha pectinaria	AF092433	Palaeacanthocephala - Echinorhynchida
Pomphorhynchus bulbocolli	AF001841	Palaeacanthocephala - Echinorhynchida
Arhythmorhynchus brevis	AF064812	Palaeacanthocephala - Polymorphida
Centrorhynchus conspectus	U41399	Palaeacanthocephala - Polymorphida
Centrorhynchus microcephalus	AF064813	Palaeacanthocephala - Polymorphida
Corynosoma enhydri	AF001837	Palaeacanthocephala - Polymorphida
Plagiorhynchus cylindraceus	AF001839	Palaeacanthocephala - Polymorphida
Polymorphus altmani	AF001838	Palaeacanthocephala - Polymorphida
Polymorphus sp.	AF064815	Palaeacanthocephala - Polymorphida
Rotifera		
Asplanchia sieboldi	AF092434	Monogononta
Brachionus platus	AF154568	Monogononta
Brachionus plicatilis	U29235	Monogononta
Lecane bulba	AF154566	Monogononta
Philodina acuticornis	U41281	Bdelloidea
Philodina roseola	AF154567	Bdelloidea
Nematomorpha		
Gordius aquaticus	X87984	
Arthropoda		
Artemia salina	X01723	
Priapulida		
Priapulus caudatus	X87984	
Kinorhyncha		
Pycnophyes kielensis	U67997	
Gastrotricha		
Lepidodermella squamata	U29198	
Chaetonotus sp.	AJ001735	
Platyhelminthes		
Opisthorchis viverrini	X55357	
Nematoda		
Haemonchus placei	L04154	
Nematodirus battus	U01230	
Gnathostomulida		
Gnathostomula paradoxa	Z81325	
Gnathostomula sp.	AF119083	
Haplognatha sp.	AF119084	
Annelida		
Lanice conchilega	X79873	
Cycliophora		
Symbion pandora	Y14811	

* Class and order are given for acanthocephalans and class for rotifers.

et al. (1998) and Garcia-Varela *et al.* (2000). Each of the three classes of Acanthocephala sampled (Archiacanthocephala, Palaeacanthocephala, and Eoacanthocephala) are monophyletic (Fig. 2). Archiacanthocephala is recovered as the sister taxon of a Palaeacan- thocephala + Eoacanthocephala clade (Fig. 2). These nodes were all supported with high bootstrap pseudoreplicate recovery and decay scores. Two subtaxa are recognized within the Palaeacanthocephala, the Echinorhynchida and Polymorphida. The Echinorhyn-
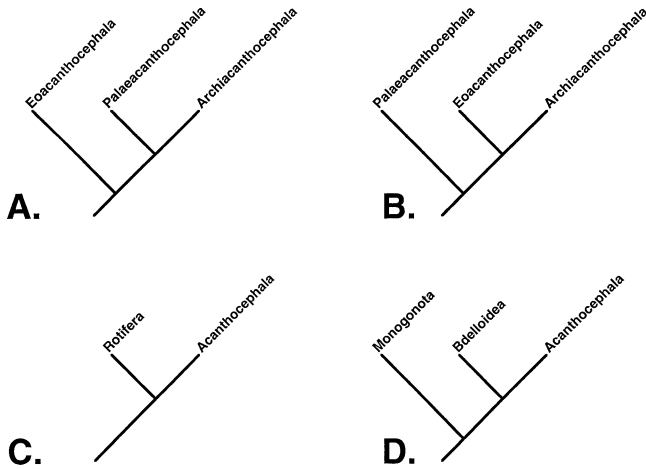
FIG. 1. Phylogenetic hypotheses tested using MP and ML methods (see Tables 2 and 3).

chida is paraphyletic, as the *Filisoma bucerium* + *Echinorhynchus gadi* clade is more closely related to the Polymorphida (Fig. 2).

Most alternative phylogenetic topologies (Fig. 1) are rejected using the MT test (Table 2). Neither of the two alternative topologies relating the three acanthocephalan classes (Fig. 1A and B) are rejected (Table 2). The best trees that depict a monophyletic Echinorhynchida are significantly less parsimonious than the MP topology. The constraint tree search of a monophyletic Rotifera recovers two trees, and neither is significantly different from the MP tree (Table 2). The constraint search of Lemniscea monophyly (Fig. 1D) recovers seven trees, and only one of these trees is significantly different from the MP tree (Table 2).

The ML tree (Fig. 3) is generally congruent with the MP tree; however, there are important differences (Fig. 2). The inferred phylogenetic relationships between acanthocephalans and rotifers differ appreciably. In the ML analysis, both Rotifera and the two bdelloid species of *Philodina* are recovered as monophyletic, but both of these clades receive low bootstrap pseudore-
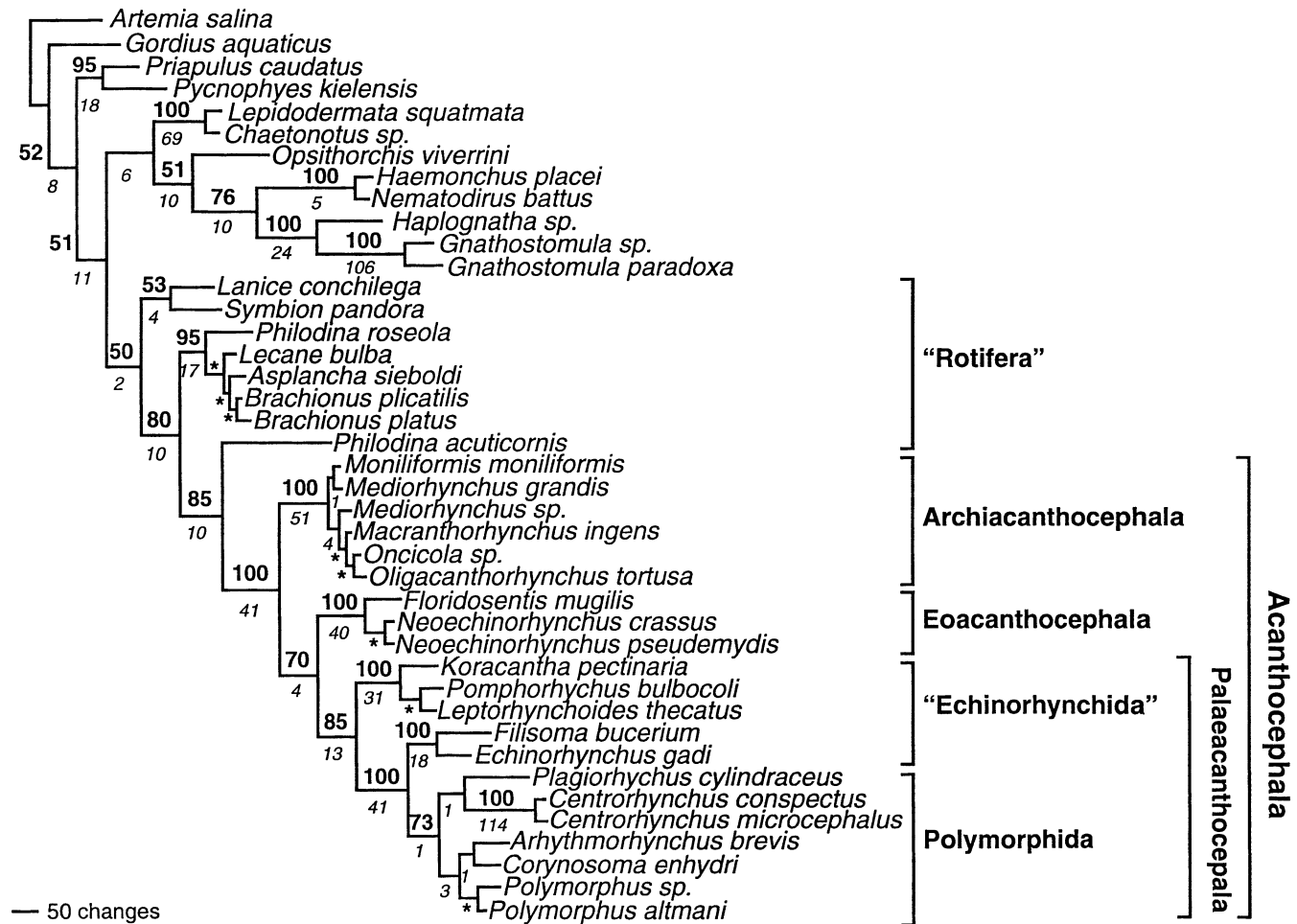

FIG. 2. Single tree recovered in maximum-parsimony analysis. Tree length = 5,459 steps, C.I. (excluding uninformative characters) = 0.405. Numbers in bold represent percent recovery in bootstrap analysis (2,000 pseudoreplicates), and numbers in italics are decay scores. Asterisks indicate nodes recovered in at least 50% of bootstrap pseudoreplicates and a decay score >3. Quotation marks indicate nonmonophyletic taxa.

TABLE 2. *Summary of modified Templeton (MT) Tests of alternative topologies using maximum parsimony.†*

Topology	Number of trees	Tree length	Length difference	P^b
MP (Fig. 2)	1	5,459	—	—
Metacanthocephala[a] (Fig. 1a)	1	5,463	4	0.465
Archiacanthocephala-Eoacanthocephala[a] (Fig. 1b)	2	5,468	9	0.072–0.170
Rotifera monophyletic[a] (Fig. 1c)	2	5,475	16	0.144–0.176
Lemniscea monophyletic[a] (Fig. 1d)	7	5,479	20	0.048*[c]–0.127
Echinorhynchida monophyletic	2	5,502	43	<0.001*

† A significant difference between topologies is indicated with an asterisk.
[a] Most-parsimonious tree(s) from constraint search.
[b] Probability of getting a more extreme T-value under the null hypothesis of no difference between the two trees (two-tailed test).
[c] One of seven trees yield $P < 0.05$.

plicate scores. Within Acanthocephala the differences between ML and MP in tree topology are restricted to relationships among species in the Archiacanthocephala and Polymorphida. These relationships are not strongly supported, as measured by bootstrap and decay scores, in either analysis (Figs. 2 and 3). All other recovered nodes in the ML tree are congruent with the MP tree, and nodes that were recovered in a high number of bootstrap pseudoreplicates in MP (Fig. 2) were also characterized by high bootstrap scores in the ML analysis (Fig. 3).

Comparison of alternative hypotheses to the ML tree using the K-H test revealed that all alternative topologies examined (Fig. 1) differed significantly
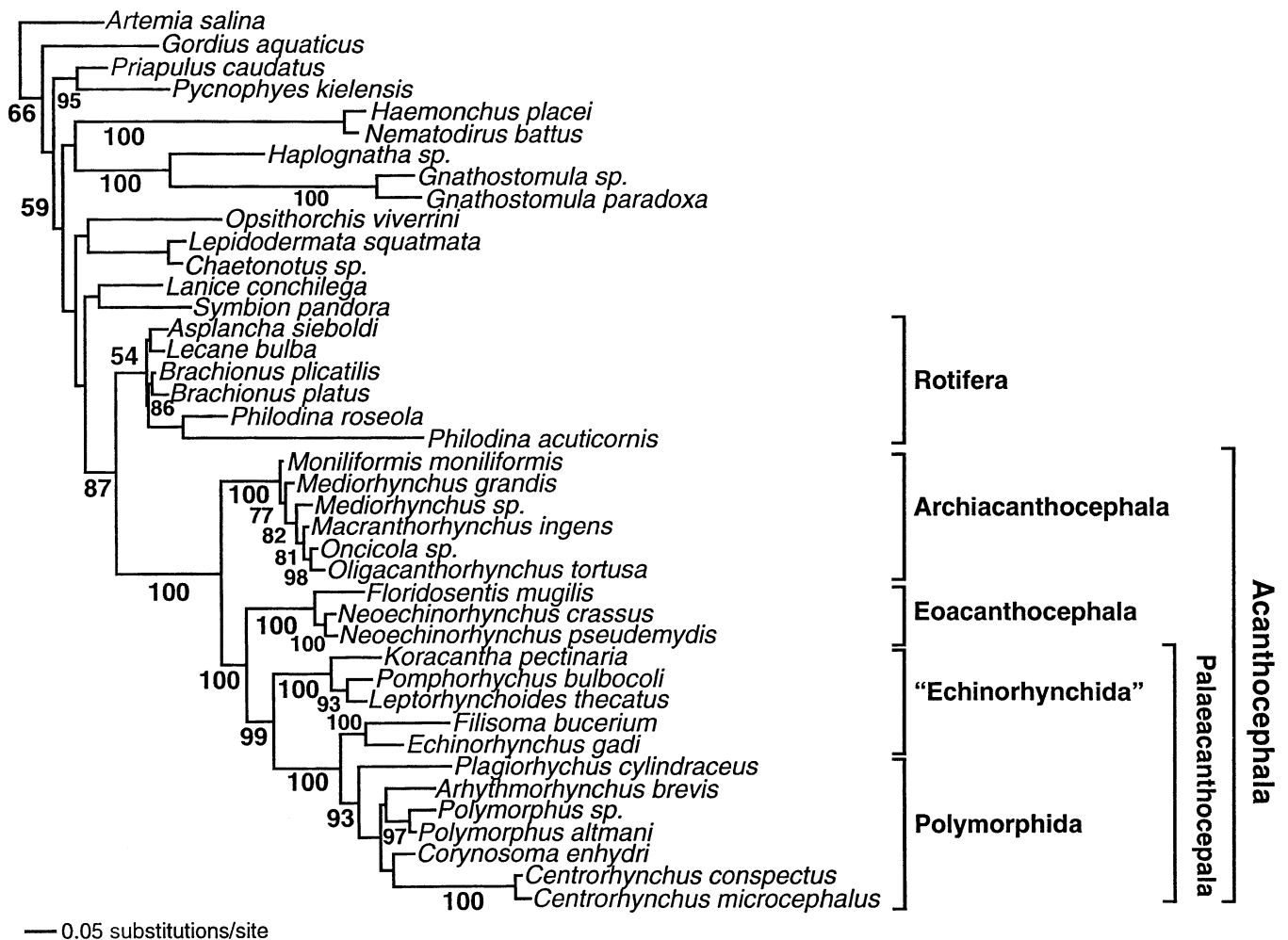

FIG. 3. Single tree recovered in maximum-likelihood analysis using GTR + Γ + I model of sequence evolution. *ln* L = −26,224.40, α = 0.5969, I = 0.1322. Numbers in bold represent percent recovery in bootstrap analysis (100 pseudoreplicates). Quotation marks indicate nonmonophyletic taxa.

TABLE 3. *Summary of Kishino-Hasegawa (K-H) Tests of alternative topologies using maximum likelihood.*†

Topology	ln L	In difference	P[b]
ML (Fig. 3)	−26,224.40	—	—
Metacanthocephala[a] (Fig. 1a)	−26,243.51	19.11	0.015*
Archiacanthocephala-Eoacanthocephala[a] (Fig. 1b)	−26,247.62	23.22	0.008*
Lemniscea monophyletic[a] (Fig. 1d)	−26,245.07	20.67	0.025*
Echinorhynchida monophyletic[a]	−26,352.25	127.85	<0.001*

† A significant difference between topologies is indicated with an asterisk.
[a] ML tree from constrain search.
[b] Probability of getting a more extreme T-value under the null hypothesis of no difference between the two trees (two-tailed test).

from the ML tree (Table 3). Perhaps most significant, the hypothesis that represents the Lemniscea as monophyletic (Fig. 1D) was rejected (Table 3). The two alternative hypotheses of relationships among the three acanthocephalan classes (Fig. 1A and B) were each significantly less likely than the ML tree (Table 3).

DISCUSSION

Phylogenetic analyses of 18S rRNA sequences demonstrate significant incongruence between parsimony and likelihood optimality criteria with regard to the relationships between acanthocephalans and rotifers. Parsimony analyses recover a paraphyletic Rotifera, where a bdelloid rotifer and acanthocephalans form a monophyletic clade (Fig. 2). However, incorporation of a model of molecular evolution in likelihood analysis results in a monophyletic Rotifera (Fig. 3). Garcia-Varela *et al.* (2000) present a hypothesis that the recovery of a monophyletic Rotifera in their analyses were the result of alignment choice, taxon sampling, and utilization of ML optimality criteria. The use of a secondary structure-based alignment in ML analysis results in a monophyletic Rotifera (Fig. 3). Therefore, the difference in alignment choice, at least with respect to the relationships of Rotifera as inferred from ML, appears not to influence tree topology. There is little doubt that taxon sampling can affect phylogenetic analyses (Lecointre, 1993; Graybeal, 1998). However, in this case the difference in taxon sampling presented in Garey *et al.* (1996) *versus* that in Garcia-Varela (2000) seems to have little effect, as the sister taxon relationship of *P. acuticornis* + Acanthocephala is consistently recovered using MP (Fig. 2) with the expanded taxon sample.

The recovery of Rotifera as paraphyletic in MP analysis may result from the phenomena of long-branch attraction. Long-branch attraction is especially problematic when more than one lineage exhibits an accelerated rate of evolution and the internal nodes connecting the lineages are relatively short (Felsenstein, 1978). In both the MP and ML phylograms, *P. acuticornis* has a much longer terminal branch than the other rotifer species (Figs. 2 and 3). Methods such as ML account for both observed and unobserved changes. The inclusion of taxa that exhibit long-branches does not lead to inconsistency, defined as failure to recover the true tree, because ML uses

branch lengths to calculate the probabilities of nucleotide substitution (Hillis *et al.*, 1994; Swofford *et al.*, 1996). Long branches may confound MP analyses, ML considers changes along long branches to be more likely than those observed on shorter ones. As a result, ML is often consistent when conditions exist in which MP is expected to be inconsistent (Hillis *et al.*, 1994; Swofford *et al.*, 1996).

The 18S rRNA inferred phylogeny provides a framework to investigate the evolutionary diversification of features associated with parasitism in Acanthocephala (Fig. 4). Utilization of arthropod and vertebrate hosts is universal throughout acanthocephalans; however, in a phylogenetic context there are varying degrees of constraint and plasticity of host use. The class of arthropod used as intermediate host is very conserved on the phylogeny. The malacostracans used by palaeacanthocephalans and the maxillopods used by eoacanthocephalans are both included in a monophyletic Crustacea. Relationships among crustaceans are unresolved, but the hypothesized sister taxon of Crustacea is the Uniramia (Wheeler *et al.*, 1993; Giribet *et al.*, 2001), which serve as intermediate hosts of the Archiacanthocephala. The general congruence of phylogenetic topologies of acanthocephalans and their arthropod hosts suggests an association of acanthocephalans with the diversification of the major lineages of the Mandibulata (Crustacea, Myriopoda, and Insecta).

Unlike the conserved use of particular arthropod groups as intermediate hosts, acanthocephalans exhibit wide variation of vertebrate definitive hosts at the species level, as well as when comparing the major lineages. Many acanthocephalan species use a wide variety of vertebrate definitive host species, but some major lineages are confined to specific groups of vertebrate hosts. Mapping definitive hosts on the phylogeny reveals that acanthocephalans have independently invaded bird and mammal hosts in the Archiacanthocephala and the Polymorphida (Fig. 4).

Physiologically, the relationship between early life stage acanthocephalans and their arthropod intermediate hosts is much more invasive than that of later life stages of acanthocephalans and their vertebrate definitive hosts. Early life stages of acanthocephalans actually penetrate the intestinal wall of the arthropod intermediate host and continue development in the body cavity of the intermediate host (Nickol, 1985), while
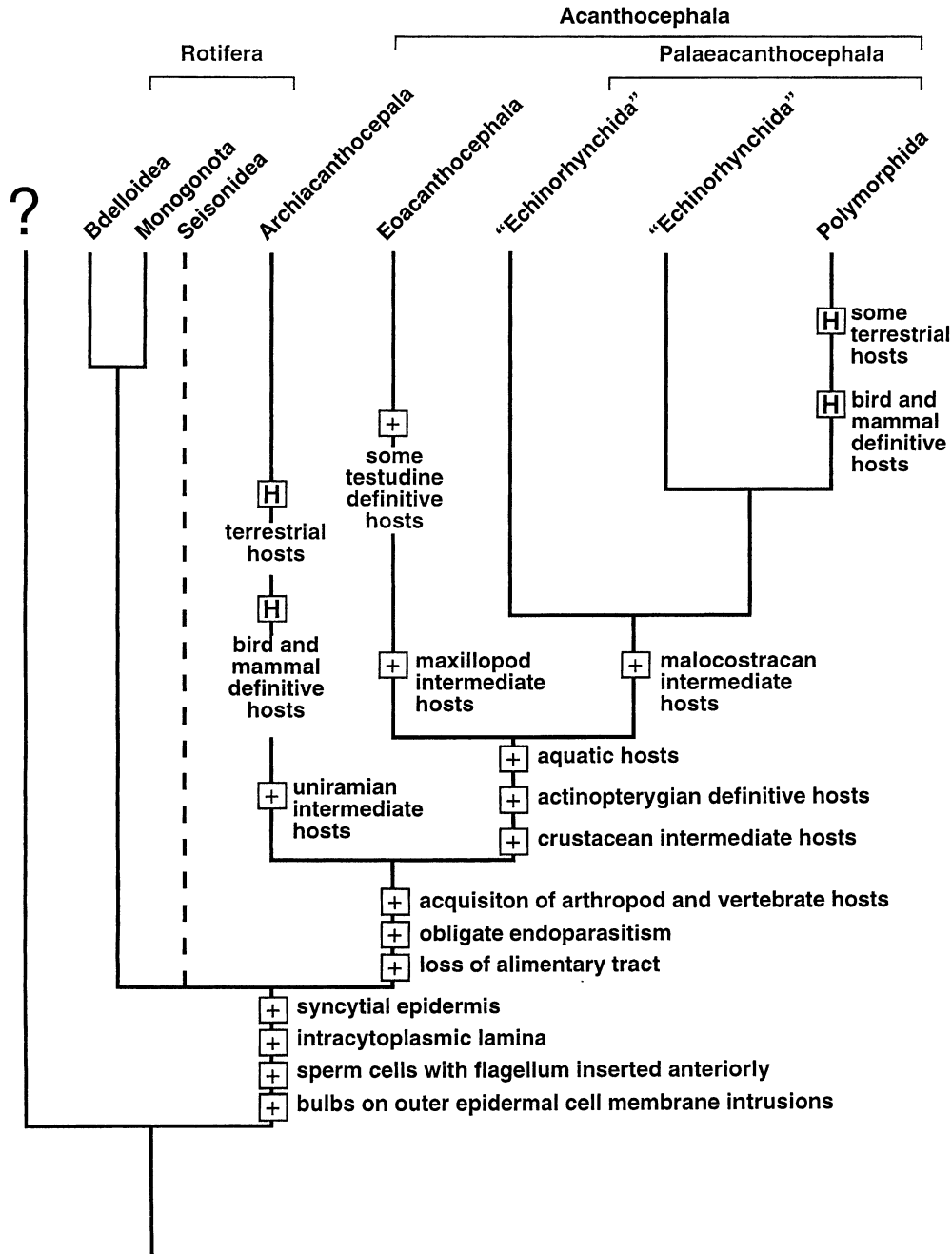
FIG. 4.   Optimization of host and habitat characteristics on the 18S rRNA inferred phylogeny of Acanthocephala. + indicates unique characters; H indicates homoplasious characters.

the later life stages are found in the intestinal lumen of vertebrate definitive hosts and normally do not enter the body cavity. Because of the more intimate relationship between acanthocephalans and their arthropod intermediate hosts, early life stages may be more constrained to the physiological environment of arthropod hosts than later life stages are to the environment of vertebrate hosts. This constraint may limit acanthocephalan lineages to particular arthropod hosts, whereas the use of particular vertebrate hosts may be more free to vary. This model of relative host constraint

would be expected to produce the phylogenetic pattern observed when host types are optimized on the acanthocephalan phylogeny (Fig. 4). Conservation of intermediate hosts may be driven by physiological constraint, and independent evolution to similar definitive hosts may be more influenced by trophic interactions among intermediate and definitive hosts.

The evolution of acanthocephalan life cycles is also characterized by shifts between aquatic and terrestrial environments. Unfortunately, the reconstruction of the ancestral state as aquatic or terrestrial for acantho-

cephalans is equivocal (Fig. 4). The optimization of the ancestral node leading to the Palaeacanthocephala + Eoacanthocephala clade is aquatic (Fig. 4). Within Palaeacanthocephala there is a shift to terrestrial hosts in the polymorphids (Fig. 4). The shift between aquatic and terrestrial hosts in Acanthocephala is probably driven by availability of appropriate arthropod intermediate hosts in the particular habitat. For example, the monophyletic Polymorphida includes hosts that are both aquatic and terrestrial (Fig. 4). Shifts between aquatic and terrestrial habitats were likely promoted by the availability of malacostracans in both terrestrial and aquatic habitats. In contrast to the diversity of habitat utilization in Palaeacanthocephala, all eoacanthocephalans and their intermediate hosts (maxillopod crustaceans) are entirely aquatic. Therefore, the radiation of eoacanthocephalans to terrestrial hosts is prevented by the lack of appropriate intermediate hosts.

The phylogenetic relationships of the Syndermata (Rotifera + Acanthocephala) are not completely resolved. Absent from the 18S rRNA database is a complete sequence for *Seison*, representing the rotifer class Seisonidea. Sampling this taxon is critical, as morphological evidence has been used to hypothesize that *Seison* and Acanthocephala are sister taxa (Ahlrichs, 1997), and that Eurotatoria (Bdelloidea + Monogononta) and Acanthocephala are sister taxa (Kristensen and Funch, 2000). Both hypotheses result in a paraphyletic Rotifera, but with different rotifer lineages as the sister taxon of Acanthocephala. If Rotifera (Bdelloidea + Monogononta + Seisonidea) is monophyletic and sister to Acanthocephala, then tests of character change associated with the evolution of parasitism rely on an unambiguous identification of the sister taxon to Syndermata.

Resolution of the phylogenetic relationships within the Syndermata, identification of the sister taxon of the Syndermata, increased taxon sampling of the acanthocephalan 18S rRNA database, and optimization of morphological and ecological characters on the phylogeny will provide a critical test of several longstanding hypotheses and generalizations regarding the evolution of parasitism in metazoans. For example, the hypothesis that the ability of parasites to colonize new hosts is dependent on the degree of host specialization potentially explains the observed disparity in phylogenetic conservation in Acanthocephala with regard to the diversity of arthropod and vertebrate hosts parasitized (Fig. 4). The generalization that parasites are simple and degenerative when compared to closely related free-living taxa can only be tested by comparing the amount and type of character changes within parasitic lineages and their free-living sister groups (Brooks and McLennan, 1993). Significant amounts of character loss *versus* character innovation is expected if the evolution of parasitism in Acanthocephala is truly ''degenerative.'' Similarly, the hypothesis that parasitic lineages exhibit elevated levels of adaptive plasticity in morphology can be tested by detecting significantly higher levels of morphological homoplasy in Acantho-

cephala *versus* its free-living sister taxon (Brooks and McLennan, 1993). Future phylogenetic investigations of Acanthocephala will provide a historical framework to examine these hypotheses and possibly illuminate evolutionary biology to the processes involved with the origin and evolution of obligate parasitism.

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