Phylogeny of Some Ascaridoid Nematodes, Inferred from Comparison of 18S and 28S rRNA Sequences

Steven A. Nadler
Department of Biological Sciences, Northern Illinois University

Reverse transcription of cellular RNA was used to obtain sequences from regions of 18S and 26/28S ribosomal RNA for eight species of ascaridoid nematodes. Phylogenetic relationships among these species were inferred from the aligned sequences by maximum-parsimony and maximum-likelihood methods. Seventy-nine of the 168 sites that varied were phylogenetically informative in parsimony analysis. Phylogenetic inference based on maximum-likelihood analysis of all sequence sites yielded a tree of topology similar to that of the parsimony result. Monophyletic groups that were strongly supported by bootstrap resampling of these data included species constituting the Ascaridinae, as well as those representing the Toxocarinae. Alternative topologies that included a member of the Ascaridinae with the Toxocarinae were rejected statistically on the basis of analysis of the mean and variance of parsimony step differences between trees. The conformance of these sequence data to a molecular-clock model of evolution was evaluated statistically by a maximum-likelihood approach. The inferred rate of rRNA sequence change along the branch leading to Parascaris equorum is not consistent with a clocklike model of evolution.

Introduction

Endoparasites of the superfamily Ascaridoidea (Nematoda: Secernentea) are classified among four families in the order Ascaridida and include species that are of veterinary, medical, and economic importance. Adults of most ascaridoid species parasitize the alimentary tract of a specific vertebrate host, and many taxa have a heteroxenous life-history pattern involving development of larval stages in one or more intermediate hosts (Sprent 1983; Adamson 1986). The subfamilies Ascaridinae and Toxocarinae [family Ascarididae sensu Gibson (1983)] contain species, such as the common pig roundworm (Ascaris suum), routinely infecting terrestrial mammals.

Analysis of phylogenetic relationships among certain parasitic organisms, including ascaridoid nematodes, is confounded by potential convergence/parallelism and a lack of defined morphological characters. As stated by Gibson (1983, p. 331), "When considering the evolutionary relationships among ascaridoid groups, we must analyze a mass of confusing and incomplete data." Because of these difficulties, the evolutionary history of ascaridoid nematodes has remained a subject of debate among helminthologists (Osche 1958, 1963; Sprent 1962, 1982, 1983; Inglis 1965; Gibson 1983; Fagerholm 1991). Most recent evolutionary hypotheses for the Ascaridoidea have been limited to proposals of relationships at the suprageneric level (Gibson 1983; Sprent 1983; Anderson 1984) and are based on individual author's interpretations of

1. Key words: rRNA sequencing, phylogeny, Nematoda, Ascaridoidea.

Address for correspondence and reprints: Steven A. Nadler, Department of Biological Sciences, Northern Illinois University, Dekalb, Illinois 60115-2861.

© 1992 by The University of Chicago. All rights reserved.
0737-4038/92/0905-0013$02.00

932
relatively few characters, including structural ones (excretory system, esophago-intestinal complex, lip features, and male secondary sexual characteristics), life-cycle patterns, distribution among host groups, and presumed host primitiveness. These evolutionary hypotheses conflict with respect to the genealogy of higher taxa. At intergeneric and generic levels, phylogenetic studies are generally lacking; proposals of evolutionary relatedness at these levels are limited to considerations of overall similarities of a few morphological or life-history characters.

Nucleotide sequence data hold great potential for phylogenetic investigations of helminths (Qu et al. 1986; Gill et al. 1988; Nadler 1990; Zarlenga and Barta 1990; Ali et al. 1991; Baverstock et al. 1991). Molecular techniques such as allozyme electrophoresis have proved to be of limited utility even for comparisons within a genus, because helminth congener frequently have such high levels of genetic differentiation that allomorphs of the same mobility are rare (Bullini et al. 1978; Baverstock et al. 1985; Nadler 1987; Woodruff et al. 1987; Andrews et al. 1989). Investigations of zooparasitic-nematode phylogeny that are based on nucleic acid sequence data are uncommon; however, two studies of small-subunit ribosomal RNA (rRNA) have revealed the applicability of this approach for resolving questions at both congeneric and higher taxonomic levels (Qu et al. 1986; Gill et al. 1988).

In the present study, phylogenetic relationships among ascaridoid species representing six genera, two families (Anisakidae and Ascarididae), and three of the four subfamilies within the Ascarididae (Ascaridinae, Toxocarinae, and Heterocheilinae) were inferred using nucleotide sequence data from regions of the 18S and 26/28S rRNA. These phylogenetic estimates are compared with previous evolutionary hypotheses and the current classification of the group. Trees inferred from character-state analyses are tested statistically against alternative phylogenetic hypotheses by using methods based on parsimony (Templeton 1983) and maximum likelihood (ML; Kishino and Hasegawa 1989).

Material and Methods

Adult specimens of eight ascaridoid species were collected and stored at ultracold temperature until prepared for nucleic acid extraction. The species and their classification (sensu Gibson 1983) (final vertebrate host in parentheses) are as follows: Ascarididae-Ascaridinae—Ascaris suum (Sus scrofa, domestic pig), Parascaris equorum (Equus caballus, domestic horse), Baylisascarisa procynis (Procyon lotor, raccoon), and Baylisascarisa transfuga (Ursus americanus, black bear); Ascarididae-Toxocarinae—Toxocara canis (Canis familiaris, domestic dog) and Toxocara cati (Felis catus, domestic cat); Ascarididae-Heterocheilinae—Heterocheilus tunicatus (Trichechus manatus, West Indian manatee); and Anisakidae-Anisakinae—Terranova caballeroi (Coluber constrictor, blacksnake). Although these taxa are representative of significant diversity within the family Ascarididae, they represent only a small part of the diversity within the superfamily Ascaridoidea, which contains >50 described genera.

Total nucleic acids were extracted from ~0.25g of muscle tissue or, in the case of small individuals, from whole worms from which the intestine had been removed. The tissue was homogenized on ice in a mixture containing 500 µl buffer (10 mM Tris hydroxymethyl amino methane–HCl pH 8.0, 1 mM ethylenediaminetetraacetate pH 8.0), 50 µl 20% sodium dodecyl sulfate, and 500 µl buffered phenol. The aqueous layer was extracted once with phenol/chloroform and once with chloroform, followed by precipitation in a solution containing 0.1 vol. 3 M sodium acetate (pH 5.8) and ~2.5 vol. cold absolute ethanol. The resulting pellet was rinsed once with 80% ethanol,
dried, and resuspended in 10 mM Tris-HCl pH 8.0. All aqueous solutions used for extraction were made with diethylpyrocarbonate (DEPC)-treated water.

Sequences were obtained directly from RNA templates by extension of oligonucleotide primers by using avian myeloblastosis virus reverse transcriptase in the presence of dideoxynucleotide triphosphates, deoxynucleotide triphosphates (Lane et al. 1985), and one alpha-32P-labeled deoxynucleotide triphosphate (Youvan and Hearst 1981). Each reverse transcription was "double loaded" onto a sequencing gel to maximize the number of nucleotides resolved. For each species and primer, a minimum of two sequencing reactions was performed. To identify potentially informative regions of rRNA sequence, four species were sequenced using five nuclear 26/28S primers and two nuclear 18S primers. Sequences of the 26/28S primers used and their corresponding annealing positions in *Caenorhabditis elegans* (Nematoda: Secernentea) rRNA (Ellis et al. 1986) are 28B, GGTCCGTGTTCGACACGG (4359–4378); 28C, GCTATCCTGAGGAAACTTCGG (4697–4718); 28D, CTTGGAGACCTGCGG (5630–5647); 28E, CCTTATCCCGAAGTTACG (5705–5722); and 28F, CAGACACTGGGCAGAAATCAC (5982–6003). Sequences of the 18S primers used and their annealing positions in *C. elegans* rRNA are 18G, TGGCACCAGACTTGGCTC (1465–1482); and 18J, TCTAAGGGCATCACAGCTGTTATTG (2315–2341). Results of this survey revealed that primers 28B and 18J yielded sequences with potentially informative substitutions at positions inferred to be homologous. These primers were used to sequence rRNA from all eight taxa. Multiple bands at the same site on a sequencing gel were scored with one of the following IUB ambiguity codes (where T = U): R = A/G; Y = C/T; S = C/G; M = A/C; W = A/T; K = G/T; and N = A/C/G/T.

Alignments were performed initially with the programs GAP and LINEUP from the University of Wisconsin Genetic Computer Group software package (Devereux et al. 1984). Final alignments were improved manually by preferring gaps to transition differences and transition differences to transversion differences. The published 18S sequence of *C. elegans* (Ellis et al. 1986) was aligned with the region sequenced by primer 18J, but the *C. elegans* region corresponding to 28B sequences is not shown, because of multiple uncertainties of alignment. The absence of complete 18S and 26/28S sequence data for ascaridoid nematodes (or other closely related species) precluded analysis of rRNA secondary structure. Therefore, the effects of potential compensatory substitutions (Wheeler and Honeycutt 1988) on the phylogenetic analysis could not be investigated.

The aligned sequence data were analyzed by two character-state methods: maximum parsimony (MP) using PAUP (*phylogenetic analysis using parsimony*, version 3.0; Swofford 1989), and ML (Felsenstein 1981; programs DNAML and DNAMLK) using PHYLIP (*phylogeny inference package*, version 3.3; Felsenstein 1990). Gaps were treated as missing data in all parsimony analyses. Reported values for the consistency index (CI) exclude uninformative characters. For analysis of the eight ascaridoid species (18S plus 28S data), MP and DNAML trees were rooted using *H. tunica* as the outgroup. This rooting of the ingroup taxa was supported by the results of the MP analysis of the 18S data alone, with *C. elegans* as the outgroup. Bootstrapped parsimony analysis (1,000 iterations) was performed using the branch-and-bound MP option of PAUP as a resampling approach to estimate how reliably these data support inferred monophyletic groups (sensu Sanderson 1989). Reported bootstrap values for clades should not be interpreted as confidence limits, since a multiple-tests correction was not performed (Felsenstein 1988), and the potential impact of compensatory
substitutions on the assumption of character (site) independence was not tested. ML analysis included all sequence sites (not only "phylogenetically informative" ones) and was performed using program options in PHYLIP to (a) reconsider the position of every species (Global search), (b) use the empirical frequencies of bases from the sequences (Frequencies), and (c) root the output tree (DNAML only). Felsenstein's (1990) program uses the transition probability formulas of Kishino and Hasegawa (1989). The transition/transversion-ratio option (the expected ratio of transitions to transversions) was set to its default value of 2.0.

Alternative phylogenetic hypotheses were tested statistically by two independent methods. Templeton's (1983) pairwise parsimony method (as modified by J. Felsenstein and executed in PHYLIP and DNAPARS) tests the mean and variance of step differences between trees, taken across characters. The ML method (as executed in DNAML) of Kishino and Hasegawa (1989) involves comparison of the mean and variance of log-likelihood differences between trees; this test is nonparametric and more robust than the standard likelihood-ratio test and gives results similar to those of bootstrap resampling of ML estimates (Hasegawa and Kishino 1989; Kishino and Hasegawa 1989).

Results

One hundred-sixty-eight of the 395 sites in the aligned sequences of the eight ascaridoid species varied (not counting gaps) in at least one species (fig. 1). One hundred thirty-five of the sites that varied occurred in the 26/28S sequence region. Seventy-nine of the 168 sites that varied were phylogenetically informative in MP analysis (sites that varied in a single species, as well as those that do not discriminate among possible trees, are uninformative in MP analysis). Of these 79 sites in eight species, 35 (44%) require transitions and 44 (56%) require transversions. Among all sites that varied (both informative and uninformative sites), 70 (42%) require transitions and 98 (58%) require transversions.

MP analysis of the aligned sequences performed using the branch-and-bound algorithm of PAUP yielded a single most parsimonious tree with 226 steps and a CI (excluding uninformative sites) of 0.723 (fig. 2). The minimum possible branch lengths supporting internal nodes of this tree ranged from 2 to 12 apomorphies (fig. 2). Bootstrap resampling and MP analysis (fig. 2) revealed how reliably these sequence data support the inferred monophyletic groups. These bootstrap values should not be interpreted as confidence limits, since a multiple-tests correction was not performed. Groups appearing in >95% of the bootstrapped trees include (Ascaris suum, Parascaris equorum) and [(A. suum, P. equorum), (Baylisascaris transfiga, B. procyonis)]—all of which are from the Ascaridinae—and (Toxocara canis, T. cati)—both of which are from the Toxocarinae. MP analysis of the 26/28S data alone (73 informative sites) also yielded a single most parsimonious tree, which had the same topology as seen in figure 2 (200 steps; CI 0.720), and the bootstrapped MP consensus tree of these 26/28S data resulted in the same three groups of taxa (cited previously for analysis of the complete data set) appearing in >95% of the bootstrapped trees.

ML analysis with the constraint of a molecular clock resulted in a tree with topology identical to that of the MP tree (fig. 2). Without the molecular-clock constraint (program DNAML), the topology of the resulting tree changed with respect to the sister-taxon relationship of B. procyonis and B. transfiga (fig. 3). The parsimony tree with the same topology as seen in the DNAML (without-clock) tree shown in figure 3 requires one more step (i.e., length 227) than does the most parsimonious solution.
FIG. 1.—Aligned 18S and 26/28S sequences of Secernentean nematodes. Position numbers correspond to those in Caenorhabditis elegans (Ellis et al. 1986; GenBank accession no. X03680) and are marked by the first digit of the number. The 26S C. elegans sequence is not shown, because of uncertainties of alignment. Gaps (*) have been inserted to increase sequence similarity. IUB ambiguity codes are given in Material and Methods. Ascaridoid sequences reported herein have been deposited in GenBank under accession numbers M90441–M90456.
Molecular Phylogeny of Ascaridoid Nematodes

7 + Ascaris suum

Baylisascaris procyonis

Baylisascaris transfuga

Toxocara cati

Toxocara canis

Parascaris

Terranova caballeroi

Heterocheilus tunicatus

FIG. 2.—MP tree obtained from analysis of all 395 rRNA sites. A single most parsimonious tree of 226 steps, with a CI of 0.723, was obtained. Bootstrap percentages of clades (1,000 iterations) are indicated (in parentheses) below internal nodes. Apomorphies assigned by MP are shown above each branch; this may be followed by a slash (/) and the minimum possible branch length, if different. ML analysis under the constraint of a molecular clock (by using DNAMLK) also resulted in this tree (In likelihood −1,560.38). The tree was rooted on the basis of parsimony analysis of 18S data, with C. elegans as outgroup (see text).

All six tested taxa arrangements that place a single member of the Ascaridinae with the Toxocarinae (figs. 4b-g) are significantly worse on the basis of parsimony comparison (table 1). Parsimony trees corresponding to figures 4b–g range in length from 239–244 steps (table 1). Five of these six trees (figs. 4b–f) are also significantly worse on the basis of likelihood comparison [i.e., the difference between the mean likelihood values of the trees is >1.96 times the standard deviation (SD) of the ln likelihood differences at individual sites]. The exception among the six trees is that shown in figure 4g, which cannot be rejected statistically, on the basis of likelihood comparison, when compared with figure 3. Two radical topologies that separate con-geners are also significantly worse: { (T. canis, P. equorum), (T. cati, A. suum), [(B. procyonis, Terranova caballeroi), (B. transfuga, Heterocheilus tunicatus)]; In likelihood −1,670.54 (288 parsimony steps)} and that shown in figure 4h (table 1).

MP analysis of the 18S data alone, with Caenorhabditis elegans as the outgroup (12 informative sites), resulted in a single tree (45 steps; CI 0.857); however, relationships among members of the Ascarididae were unresolved. Only one monophyletic group was supported reliably in bootstrap resampling and MP analysis of these 18S data. The group consisting of (T. cati, T. canis, Te. caballeroi, P. equorum, B. procyonis, B. transfuga, A. suum) occurred in 95% of the bootstrap replicates and was supported by six apomorphies; five of these apomorphies were found in all most parsimonious reconstructions. ML analysis of these 18S data also supported this rooting. Two alternative topologies that placed Te. caballeroi as ancestral to the other ascaridoids each required six additional steps for these 18S data and were significantly worse on the basis of Templeton’s (1983) parsimony test.

Conformance of these rRNA data to a molecular clock was examined by using
the likelihood-ratio test to compare the log likelihoods obtained from DNAML and DNAMLK (Felsenstein 1990) for a seven-taxon data set (*B. procyonis* was excluded because this test requires that DNAML and DNAMLK each recover the same unrooted topology). The statistic of comparison is the $\chi^2$ distribution of two times the difference in the log likelihoods (2difML) of the DNAML results versus the DNAMLK results. For the seven-taxon test, the results were significant ($P < 0.01$) and not consistent with clocklike evolution for these rRNA regions [2difML = 31.6; degrees of freedom (df) 5]. Seven iterative DNAML/DNAMLK comparisons were performed by removing one species at a time and repeating the test on the remaining six species (df 4). Six of these tests could be evaluated statistically (the same topology was recovered by DNAML and DNAMLK); the results were significant ($P < 0.01$; i.e., rate constancy was rejected) in all tests except when *P. equorum* was removed from the data set (without *P. equorum*, 2difML = 2.3; without *B. transfuga*, 2difML = 15.0; without *A. suum*, 2difML = 21.5; without *H. tunicatus*, 2difML = 35.8; without *Te. caballeroi*, 2difML = 35.4; and, without *T. cati*, 2difML = 23.8). In the seven-taxon test, the length of the terminal node leading to *P. equorum*, estimated over all 395 sites in DNAML (84 substitutions/1,000 sites), is 2.3 times longer than when it is constrained by equal rates in DNAMLK (36 substitutions/1,000 sites), indicating a higher rate of substitution for this lineage. The different relative rates of substitution among these species (e.g., *A. suum* and *P. equorum*) is also shown by the number of autapomorphies recovered by MP (fig. 2) and by the branch lengths inferred by ML (fig. 3).

**Discussion**

The 26/28S rRNA region corresponding to *Caenorhabditis elegans* positions 4163–4357 provided 73 phylogenetically informative sites in MP analysis of eight ascaridoid nematodes, for comparisons spanning congeneric to between-family levels. The more conserved 18S region corresponding to *C. elegans* positions 2132–2308
Table 1

Statistical Comparisons of Alternative Trees

<table>
<thead>
<tr>
<th>Tree</th>
<th>Ln Likelihood</th>
<th>SD of Ln Likelihood Difference</th>
<th>Worse by Maximum Likelihood</th>
<th>No. of Parsimony Steps (SD)</th>
<th>Worse by Parsimony</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 2</td>
<td>-1,541.26</td>
<td>2.94</td>
<td>No</td>
<td>226</td>
<td>...</td>
</tr>
<tr>
<td>Figure 3</td>
<td>-1,541.12</td>
<td>...</td>
<td>...</td>
<td>227 (1.73)</td>
<td>No</td>
</tr>
<tr>
<td>Figure 4a</td>
<td>-1,554.91</td>
<td>8.12</td>
<td>No</td>
<td>232 (2.83)</td>
<td>Yes</td>
</tr>
<tr>
<td>Figure 4b</td>
<td>-1,567.81</td>
<td>9.70</td>
<td>Yes</td>
<td>239 (3.61)</td>
<td>Yes</td>
</tr>
<tr>
<td>Figure 4c</td>
<td>-1,582.63</td>
<td>12.42</td>
<td>Yes</td>
<td>243 (4.80)</td>
<td>Yes</td>
</tr>
<tr>
<td>Figure 4d</td>
<td>-1,582.63</td>
<td>12.42</td>
<td>Yes</td>
<td>243 (4.80)</td>
<td>Yes</td>
</tr>
<tr>
<td>Figure 4e</td>
<td>-1,581.98</td>
<td>13.08</td>
<td>Yes</td>
<td>243 (4.80)</td>
<td>Yes</td>
</tr>
<tr>
<td>Figure 4f</td>
<td>-1,582.25</td>
<td>13.01</td>
<td>Yes</td>
<td>244 (4.70)</td>
<td>Yes</td>
</tr>
<tr>
<td>Figure 4g</td>
<td>-1,560.44</td>
<td>10.84</td>
<td>No</td>
<td>235 (4.13)</td>
<td>Yes</td>
</tr>
<tr>
<td>Figure 4h</td>
<td>-1,672.52</td>
<td>26.01</td>
<td>Yes</td>
<td>287 (11.01)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* The optimal DNAML tree topology (fig. 3) from analysis of all sequence sites was compared statistically against alternative trees, by the method of Kishino and Hasegawa (1989). In likelihood values and standard deviations are from DNAML (no molecular-clock assumption).

b The shortest parsimony tree (fig. 2) was compared statistically against alternative trees, by the method of Templeton (1983) as modified, by J. Felsenstein, in PHYLIP.

provided only six phylogenetically informative sites in MP analysis of the eight-species data set. The relative conservation of this 18S region is also indicated by its observed similarity to the corresponding region in *C. elegans*. In contrast, differences between the *C. elegans* 26/28S sequence and this region in ascaridoid nematodes made inference of positional homology tenuous, and this prevented the inclusion of *C. elegans* as an outgroup in the analysis of the complete data set. For four of the six ascaridoid genera (*Baylisascaris*, *Ascaris*, *Parascaris*, and *Heterocheilus*), the surveyed species are likely to serve as exemplars for their respective genus. In each case, the genus is composed of six or fewer described species, and members possess marked and diagnosable features (Sprent 1968; Fagerholm 1991). In contrast, the genera *Toxocara* and *Terranova* are more speciose and diverse; the study of additional taxa is warranted to assess whether each genus is monophyletic.

In bootstrap MP analysis of the ascaridoid rRNA data, four species representing three genera from the Ascaridinae were members of a single clade in 98% of the trees, and two congener representing the Toxocarinae were members of a single clade in 99% of the trees (fig. 2). Bootstrap MP analysis of the 26/28S rRNA data alone also revealed reliable support for groups studied at the within-family level, and this region may provide a sufficient number of phylogenetically informative sites for analysis of other ascaridoid taxa. In contrast, the 18S region that was sequenced provided few phylogenetically informative sites at this taxonomic level, but, because of its relative conservation, it may prove useful in higher-level studies within the Ascaridida.

rRNA is often considered to have a relatively constant rate of change over time (Hasegawa et al. 1985; Woese 1987; Baverstock et al. 1989), and this property has been used to justify its use as a molecular clock (Woese 1987). The conformance of nucleotide sequence data to a molecular-clock model of evolution can be evaluated statistically by comparing the log likelihoods of the same tree topology obtained using the PHYLIP programs DNAML (without the clock) and DNAMLK (with the clock constraint) in a likelihood-ratio test (Felsenstein 1990). These ascaridoid sequence data fit a clocklike model of change only when *Par ascars equorum* is excluded from
FIG. 4.—Alternative topologies tested by statistical methods (see Table 1). A. s = *Ascaris suum*; P. c = *Parascaris equorum*; B. p = *Baylisascaris procyonis*; B. t = *Baylisascaris transfuga*; T. ct = *Toxocara cati*; T. cn = *Toxocara canis*; Te. c = *Terranova caballeroi*; and H. t = *Heterocheilus tunicatus*.

the analysis; in comparison with the other ascaridoid species, this species has a greater estimated rate of substitution for these sequence regions. This observation is also supported by the number of autapomorphies distributed along the terminal nodes leading to the sister taxa *A. suum* (four apomorphies) and *P. equorum* (28 apomorphies). Methods of tree inference that assume rate constancy (i.e., methods such as DNAMLK and UPGMA of distance measures) may result in erroneous trees, at least for analysis of RNA sequence data among ascaridoid species. This finding may also account for a previous report of nematode paraphyly based on a phenogram of *K*_\text{nu}^*_e_ distances derived from rRNA sequence data that included *Parascaris* (Gill et al. 1988); the monophyly of nematodes was supported by an MP reanalysis of those data (Nadler 1990). Phylogenetic hypotheses based on parsimony methods may also be subject to systematic errors when evolutionary rates in different lineages are sufficiently unequal (Felsenstein 1978; Hasegawa and Yano 1984; Kishino and Hasegawa 1989). In such
cases, rate-independent methods of inferring phylogeny and estimating confidence limits for tree topologies (e.g., maximum likelihood) may be preferable.

In contrast to MP methods, ML tree-inference procedures utilize a probabilistic model of base substitution to calculate (and compare) likelihood functions for alternative topologies. Felsenstein's (1981) method is robust to rate differences among lineages (Hasegawa and Yano 1984), and, by invoking the global search option of DNAML, this program can be used to search for the topology with the highest likelihood. The optimal ML tree for ascaridoid nematodes (fig. 3) is slightly less resolved than the MP tree (fig. 2), the difference involving loss of monophyly of the Baylisascaris species. In view of the resolving power of these sequence data, the two topologies (figs. 2 and 3) are not significantly different on the basis of statistical comparison by parsimony and ML methods. The observed MP tree lengths are consistent with the inability to discriminate statistically between these two trees (i.e., the ML tree is only one step longer than the most parsimonious solution). Thus, in view of both the probabilistic base-substitution model used in ML and its statistical interpretation, these data are not sufficient to allow one to choose between these two competing phylogenetic hypotheses. Similarly, the likelihood of the tree in figure 3 is not preferred statistically over that of a more traditional arrangement of taxa within the Ascaridinae (fig. 4a), an arrangement that groups Ascaris and Baylisascaris species as members of a clade, on the basis of structural similarities of their postcloacal papillae and other external morphological features (Sprent 1968). In contrast, the tree in figure 4a is significantly worse than the optimal MP tree, on the basis of Templeton's parsimony test. Thus, this analysis (and the MP bootstrap analysis) provide support for monophyly of the morphologically divergent taxa A. suum and P. equorum. The contradiction between some of the statistical outcomes based on ML and MP methods (table 1) most likely results from differences in how these inference procedures treat distinct classes of substitution events. An ML inference method involves an explicit probabilistic model of (parametric) base substitution (Felsenstein 1988; Kishino and Hasegawa 1989). Felsenstein's (1981) method allows for both (1) different expected ratios of transitions to transversions and (2) unequal frequencies of the nucleotides. In contrast, unweighted MP analysis of unordered nucleotide data (Fitch 1971) assigns equal weight to substitutions of different classes (transitions and transversions).

Fortunately, these rRNA data are sufficient to discriminate statistically (by both ML and MP methods) among most alternative phylogenetic hypotheses for these ascaridoid nematodes. For example, the MP tree (fig. 2) was preferred statistically (a) over six tested trees that included a single member of the Ascaridinae with the Toxocarinae (figs. 4b–g) and (b) in cases of radical topological rearrangements that separate congeners (e.g., fig. 4h). The longest alternative tree not rejected as significantly worse on the basis of MP analysis was 227 steps (fig. 3), or 0.4% longer than the most parsimonious solution. In contrast, the longest alternative tree not rejected on the basis of ML comparison was 235 steps (fig. 4g), or 4.0% longer than the MP tree. Some investigators employing sequence data in phylogenetic analysis have suggested that trees within 1% of the most parsimonious result cannot be rejected (Bremer and Bremer 1989; Smith 1989); these results indicate that consideration of a larger percentage of near-shortest trees may be warranted.

MP analysis of the 18S data including C. elegans yielded virtually no information about ascaridoid relationships at the congeneric and intergeneric levels; however, it did provide an outgroup by which to root the ingroup species. Six apomorphies separated H. tunicatus from the remaining ascaridoid species, and this node was recovered
in 95% of the bootstrap replicates. The topology of the 18S ML tree was also consistent with this rooting of the ingroup taxa.

The phylogeny inferred from these sequence data is not entirely consistent with the most generally accepted classification of ascaridoid taxa. This classification (Hartwich 1974; Gibson 1983; Jones and Gibson 1987) is not based on a cladistic analysis of morphological characters but, instead, rests on evaluations of unique combinations of morphological features (i.e., similarities) that define genera or higher groups. For example, members of the Ascaridinae have rounded hexagonal lips with lateral indentations and lack a gubernaculum and a ventriculus. Studied taxa from this subfamily formed a monophyletic group in both the MP and ML analyses of the complete rRNA data and appeared as a clade in 98% of the bootstrap MP replicates. Statistical comparison of alternative topologies by Templeton’s parsimony method supported trees that preserved the monophyly of the Ascaridinae. Likewise, bootstrap MP and ML analyses supported the monophyly of the Toxocarinae, which are characterized morphologically by both the presence of a short ventriculus and the absence of a gubernaculum. The bootstrap MP tree also reveals reasonable support (79%) for the Toxocarinae-plus-Ascaridinae clade, which contains studied parasite species from terrestrial mammalian hosts.

The discrepancy between the rRNA-based phylogeny and the most commonly accepted classification of ascaridoids involves apparent paraphyly of the Ascarididae. In contrast, certain alternative classifications and evolutionary scenarios (e.g., see Sprent 1983) are consistent with this sequence-based phylogeny. For example, Sprent (1980) formally redefined and expanded the Heterocheilinae and suggested that heterocheilines represent the most ancestral ascaridoids (Sprent 1983). Clearly, a much wider spectrum of ascaridoid diversity must be studied phylogenetically before taxonomic conclusions are justified. rRNA sequence data should prove to be of great utility in providing characters for phylogenetic analysis of this diverse group of endoparasitic nematodes.

Acknowledgments

I am grateful to R. Lawson and C. Beck for collecting nematode specimens. I thank D. Pashley for providing laboratory facilities during the initial phase of this study. I also thank T. Friedlander, M. Hafner, J. Martin, B. McPherson, M. Parrish, D. Pashley, E. Zimmer, and R. Zink for helpful discussions concerning methodology and data analysis. I am very grateful to J. Felsenstein for providing the PHYLIP computer package and to W. Ellis for the Macintosh-executable version. B. Hall, W. Fitch, and two anonymous reviewers provided valuable comments on previous drafts of this paper. This research was supported in part by NSF grant BSR-8817329 and by a Graduate Council Research Award from Northern Illinois University.

LITERATURE CITED


BARRY G. HALL, reviewing editor

Received August 2, 1991; revision received March 31, 1992

Accepted April 6, 1992