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Phylogenetic Relationships of the Marine Nematode Family Comesomatidae

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Abstract: The phylogenetic relationships of the Comesomatidae have remained unresolved at the family level because they have diagnostic morphological features of both the Monhysterida and the Chromadorida. A comparison of the partial sequence of 18S rDNA from representative taxa of Comesomatidae and of morphological data, analyzed in conjunction with molecular and morphological data from monhysterids and chromadorids, suggests a closer relationship of the Comesomatidae with Monhysterida than with Chromadorida.

Key words: Comesomatidae, marine nematodes, phylogeny, ribosomal DNA, systematics.

The Comesomatidae is a cosmopolitan family of free-living marine nematodes that was first established as a subfamily by Filipjev (1918). It is represented in most benthic faunal assemblages and has been reported in many faunal surveys (e.g., Jensen, 1981; Sharma and Webster, 1983; Vanreusel et al., 1992). The phylogenetic relationships of the Comesomatidae remain unresolved at the family level because they have diagnostic morphological characteristics of both the orders Monhysterida and Chromadorida (Table 1). They have been assigned to the order Monhysterida by Filipjev (1934), Lorenzen (1981), and Jensen (1979) because, like all other members of Monhysterida, the female gonoducts are outstretched. In this case, the presence of multispiral amphids among all members of the monophyletic Comesomatidae is considered to be a derived character because it is absent among all other members of Monhysterida. However, because all chromadorids have multispiral amphids, Wieser (1954), Platt (1985), and Hope and Zhang (1995) assigned Comesomatidae to the order Chromadorida. They regarded Comesomatidae as a monophyletic family, because the females of no other members of Chromadorida have outstretched gonoducts. The Comesomatidae also have punctations and ring pores on their cuticular surface that are lacking in the Monhysterida and thus bear a superficial resemblance to the Chromadorida.

These differences in systematics are due in part to differences in the assessment of the importance of certain morphological characters and an inadequate understanding of internal morphology. Especially important has been an inability to know if similar-appearing character states among taxa are homologous. In a molecular comparison of the D3 expansion segment (26/28S ribosomal RNA gene), Litvaitis et al. (2000) concluded that the Comesomatidae comprised a sister group to the Monhysterida, yet they placed them in the Chromadorida because they considered their molecular trees to be equivocal. In a recent review of nematode systematics by De Ley and Blaxter (2002), the Comesomatids were assigned to the order Areolaimida.

The highly conserved 18S nuclear ribosomal gene (18S rRNA gene) has been used by systematists in all major groups of organisms to provide molecular data for phylogenetic analysis, particularly for specimens representing phylogenetically widely separated higher taxa. Blaxter et al. (1998) used data from 18S rDNA to derive a phylogeny for selected species that spanned the entire phylum Nematoda. In our study, we tested phylogenies of the Comesomatidae using new morphological characters, together with the characters used by previous authors and included new rDNA molecular data as well. A combination of morphological and molecular data to infer relationships of the Comesomatidae to other families, as well as relationships within the family, would help to clarify the systematics of the Comesomatidae. The mapping of morphological transformation series on trees derived from molecular data may also help to clarify whether morphological similarities across a broad span of marine nematode taxa are due to homoplasy.

Materials and Methods

Specimens were collected from Aransas Pass, Port Aransas, TX. The nematodes were extracted from the sediment by a combination of sieving and centrifugation techniques, as outlined in Montagna (2002). Specimens for molecular data were preserved in 70% or 100% ethanol, and the samples for light microscopy were fixed in 10% buffered formalin. Digital images were made of the specimens later used to obtain molecular data to confirm identifications as needed. The nematodes for light microscope study were prepared according to the method of Seinhorst (1959).

Methods for handling the nematodes and obtaining rDNA were similar to those previously described (Ferris et al., 1994, 1995, 2004). Multiple DNA preparations were made, each with one to three specimens of each isolate. The specimens were homogenized with a Radnoti glass homogenizer (Thomas Scientific, Swedes-

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TABLE 1. A summary of the assignment of Comesomatidae to order.

Author	Order assigned	Basis of classification
Filipjev, 1918; 1934	Family Monhysteridae (1918) and Order Monhysterida (1934)	Out-stretched female gonoduct
Chitwood and Chitwood, 1950	Suborder Monhysterina	Out-stretched female gonoduct
De Coninck, 1965	Order Chromadorida	Punctate cuticle; multispiral amphids
Andrássy, 1976	Order Chromadorida	Punctate cuticle; multispiral amphids
Jensen, 1979	Order Chromadorida	Punctate cuticle; multispiral amphids
Maggenti, 1981	Order Chromadorida	Punctate cuticle; multispiral amphids
Lorenzen, 1981	Order Monhysterida	Out-stretched female gonoduct
Platt, 1985	Order Chromadorida	Punctate cuticle; multispiral amphids
Hope and Zhang, 1995	Order Chromadorida	Punctate cuticle; multispiral amphids
Malakhov, 1994	Order Araeolaimida	Multispiral amphids
Litvaitis et al., 2000	Order Chromadorida	28s rDNA
De Ley and Blaxter, 2002	Order Araeolaimida	Out-stretched female gonoduct

boro, NJ) in 25 µl TE buffer, pH 7.5. Total genomic DNA was extracted using InstaGene Matrix (Bio-Rad, Hercules, CA). Primers for the approximately 600-bp fragment of the 18S gene, derived from sequence in Lumb et al. (1993), were 5′ AGGGCAAGTCTGGTGCCAGC 3′ (Forward), 5′ TAAGTTTCAGCTTTGCAACC 3′ (Reverse).

The amplified fragment was cloned in pGEM-T-vector (Promega, Madison, WI) and transformed into *Escherichia coli* strain JM109. Plasmid preparations were made using the Wizard Plus Minipreps system (Promega) from bacterial colonies containing inserts of the expected size, as assessed by PCR amplification. Products were sequenced with an automatic sequencer (ABI PRISB 3700 DNA analyzer, Applied Biosystems, Foster City, CA) at the Purdue Genomics Center. Both strands of DNA from two to four clones were sequenced for the different specimen preparations made from each nematode isolate.

In our analyses, we included data from species for which GenBank sequence data were available for the homologous piece of 18S rDNA, viz., *Stilbonema majum* (Cobb) Ott, *Desmodora ovigera* Ott (both Chromadorida), and *Adoncholaimus fuscus* (Bastian) (Enoplida). Molecular sequences were aligned using the computer program CLUSTAL X (Thompson et al., 1997). Default penalty values (gap weight and gap length) were used. Maximum Parsimony (MP) analysis was performed in PAUP*4.0b10 (Swofford, 2002) using the branch-and-bound search with gaps in the sequence data treated as missing. Support for individual branches was evaluated using the bootstrap method with heuristic search and 1,000 replicates. Trees were rooted with the outgroup taxon *Adoncholaimus fuscus*. *Adoncholaimus fuscus* grouped near the base of the molecular tree of Blaxter et al. (1998) of the phylogeny of the Nematoda, and it appears to be less derived in most morphological character states than are the ingroup species of interest to us.

TABLE 2. Morphological character states of taxa used in molecular analysis.

Genus/species/character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Adoncholaimus fuscus	0	0	0	0	0	0	na	0	0	1	0	0	0	0	na	0	0
Chromadorita pharetra	1	1	0	1	1	1	0	1	0	1	0	0	1	0	0	1	1
Desmodora ovigera	1	0	1	1	1	1	0	1	1	1	0	0	1	0	na	1	0
Dorylaimopsis metatypicus	1	1	0	1	1	1	1	0	1	0	0	1	0	0	1	0	0
Sabatieria sp.	1	1	0	1	1	1	1	0	0	0	0	1	0	0	1	0	0
Sphaerolaimus sp.	1	0	0	1	0	2	0	0	0	0	1	1	0	0	na	0	0
Stilbonema majum	1	0	1	1	1	1	0	0	0	0	0	0	1	0	na	1	0
Terschellingia longicaudata	1	0	0	1	1	2	0	0	0	0	1	1	0	1	na	1	0
Tobrilus hopei	0	0	0	0	0	0	na	0	0	1	0	0	0	0	na	0	1

Character States Used for Phylogenetic Analysis.
1. Cuticle: 0—smooth; 1—striated.
2. Cuticular punctations: 0—absent; 1—present.
3. Helmet: 0—absent; 1—present.
4. Metanemes: 0—present; 1—absent.
5. Cephalic sensilla: 0—6 + 10; 1—6 + 6 + 4.
6. Fovea (amphids): 0—cyathiform; 1—spiral; 2—circular; 3—poroid.
7. Spiral amphids with: 0—less than two turns; 1—with at least two turns.
8. Rugae: 0—absent; 1—present.
9. Odontia: 0—absent; 1—present.
10. Onchia: 0—absent; 1—present.
11. Female reproductive system: 0—didelphic; 1—monodelphic.
12. Female gonoducts: 0—reflexed; 1—outstretched.
13. Male reproductive system: 0—diorchic; 1—monorchic.
14. Caudal glands: 0—present; 1—absent.
15. Punctations laterally differentiated: 0—absent; 1—present.
16. Pharynx: 0—cylindro-clavate; 1—posterior bulb.
17. Ventro-median supplements: 0—absent; 1—present.

Morphological character states included in the phylogenetic analysis include those that have been considered by previous authors (e.g., Filipjev, 1918, 1934; Jensen, 1979; Lorenzen, 1981) in the systematics of the Comesomatidae and related families (Table 2). For a given morphological structure (e.g., the amphid), character state values were assigned based on the researchers' prior knowledge and experience plus available literature with the ancestral state = 0, a more derived state = 1. The evolution of several of the character states is, as yet, imperfectly understood, and therefore we acknowledge the assignments to be arbitrary. The available ordered character states for all structures were input into MacClade 4 (Maddison and Maddison, 2000) to create a Nexus file, which was then analyzed in PAUP to derive maximum parsimony (MP) cladograms.

Some of the branches of the best tree (fewest steps) were moved to represent alternative relationships suggested by the molecular tree using tools available in MacClade. The significance of the differences between the new topologies and the best molecular tree as well as the best tree based on morphology was examined in PAUP* using the Kishino-Hasegawa (K/H) test (Kishino and Hasegawa, 1989) under MP. When $P \geq 0.05$, the tree was considered not to be significantly different from the best tree, and the hypotheses represented by it were not rejected; when $P < 0.05$, the trees were rejected. The goal was to find a tree best supported by all the data.

Results

Morphological data and DNA sequence data were obtained for two comesomatid species, *Dorylaimopsis metatypicus* Chitwood, and *Sabatieria sp.*; two monhysterids, *Terschellingia longicaudata* de Man and *Sphaerolaimus sp.*; a chromadorid species, *Chromadorita pharetra* Ott; and the enoplid species, *Tobrilus hopei* Loof and Riemann. The comesomatid species, *D. metatypicus*, has spiral amphids typical of this family, three large teeth, and sclerotized walls of the buccal cavity, and males have biarcuate spicula. The monhysterid, *Terschellingia longicaudata*, has circular amphids, and a long cylindroconical tail. *Chromadorita pharetra* is distinguished by the presence of copulatory supplements in the male in the anterior ventral body region. All of our new sequence data have been deposited in GenBank (DQ394882–DQ394887).

Two best trees (i.e., same number of steps) were found in the analyses based on molecular data. In both of these trees, the ingroup taxa were clearly divided into two groups, with one group including the monhysterids and comesomatids (with bootstrap value 100), and the other group containing the chromadorids (with bootstrap value 77). In the latter group, *Chromadorita* is shown to be a sister group to a clade consisting of *Stilbonema* and *Desmodora*, which is supported by a 100 bootstrap frequency. *Tobrilus* grouped with the monhysterids and comesomatids. Figure 1 shows the strict consensus tree of these two trees.

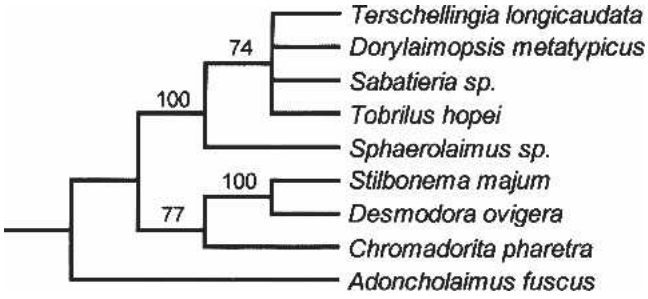

Fig. 1. Strict consensus tree (frequencies of all bipartitions are 100) of Maximum Parsimony (MP) trees based on molecular data. From bootstrap analysis, values above 50 are shown on respective branches of bipartitions.

Two best trees were also found using morphological data. Figure 2 shows a strict consensus tree of these two trees. In both of the best trees, the ingroup taxa were also divided into two groups as in the molecular trees, with one including the monhysterids and comesomatids, and the other containing the chromadorids. In the first group, the two monhysterids grouped together as did the two comesomatids, suggesting that they are sister groups. In both of the best trees based on morphology, *Tobrilus hopei* was a sister taxon to the rest of the ingroup taxa.

To find a tree of relationships among comesomatids, monhysterids and chromadorids supported by both kinds of data, branches from the molecular best trees were rearranged in MacClade with *Tobrilus hopei* allied with the comesomatidae-monhysterid group as a sister group (*Tobrilus* was part of a comesomatid-monhysterid bush in Fig. 1), and the taxa in the comesomatid/monhysterid group were arranged as indicated in the morphological tree of Figure 2. This new tree (Fig. 3) was accepted when compared with the best molecular trees using the MP K/H test ($P = 0.318$). It was also accepted when compared to the best trees based on morphology using the MP K/H test ($P = 0.104$).

Discussion

The affinity of the comesomatids with the monhysterids shown by our cladograms is in agreement with re-

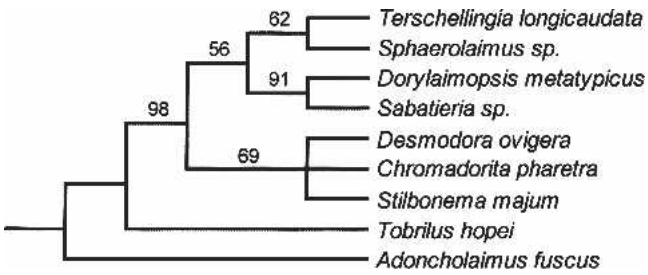

Fig. 2. Strict consensus tree (frequencies of all bipartitions are 100) of Maximum Parsimony (MP) trees based on morphological data. From bootstrap analysis, values above 50 are shown on respective branches of bipartitions.

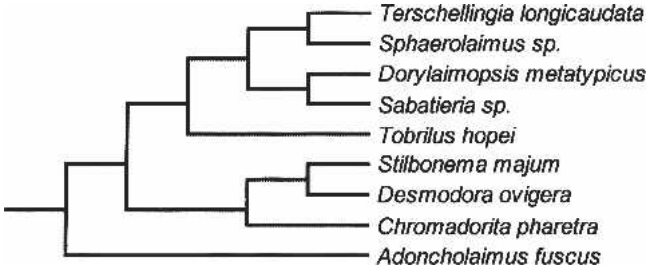

FIG. 3. Tree with combined information from Figs. 1 and 2 that was accepted when compared to the best molecular trees and the best morphology trees under the MP K/H test ($P = 0.318$ and $P = 0.104$, respectively).

lationships proposed by Filipjev, (1918, 1934) and Lorenzen (1981). The inclusion of the comesomatids in the chromadorida by many authors had been based primarily on the appearance of the punctated cuticle. A recent reinterpretation of the cuticular structure by Decraemer et al. (2003) suggests that this is a homoplasic character that has appeared independently in several taxa. The pores and punctations seen in some comesomatids that resemble those of chromadorids may be a result of convergence. Wright and Hope (1968) described rod-shaped punctations with radial spars in transmission electron microscope observations (the radial striae described by Decraemer et al., 2003) of *Acanthonchus duplicatus*, a Cyatholaimidae. Hope and Zhang (1995) described mushroom-like punctations in SEM observations for *Hopperia hexadentata*, a Comesomatidae.

The lack of ultrastructure studies on the amphid morphology of the taxa discussed raises questions about the significance of the spiral amphid for phylogenetic assessment. The ventrally spiral amphid is considered plesiomorhic by Lorenzen (1981), yet the nonspiral form appears as a secondary character loss. Our study indicates that the morphological characters used by many previous authors in the classification of the Comesomatidae must be reevaluated.

Molecular data from many more taxa are needed to validate these relationships, and additional morphological characteristics must be examined within these three families to support or refute our observations and to learn more about polarity in these structures. In particular, we hope to obtain molecular data from *Odontophora sp.* of the Axonolaimidae and *Paracanthonchus sp.* of the Cyatholaimidae.

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