Inferring a classification of the Adenophorea (Nematoda) from nucleotide sequences of the D3 expansion segment (26/28S rDNA)

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Abstract: Nucleotide sequences of the D3 expansion segment of the 28S rDNA gene were used to reconstruct evolutionary relationships within the Adenophorea. Neighbor-joining and parsimony analyses of representatives of most major taxa revealed a paraphyletic Adenophorea (p = 0.0005). Within Adenophorea, the Enoplia, Enoplida, and Enoplina were paraphyletic (p = 0.0024, 0.0014, and 0.0120, respectively). A major division was evident within the Enoplida, with one lineage consisting of a basal Thoracostomopsidae and Enoplidae, and a second lineage consisting of Oncholaimidae and Encheliididae. Tripyloidina clustered close to the basal enoplid branch and formed a monophyletic taxon. Although appearing as paraphyletic in the maximum-parsimony and neighbor-joining trees, constraining Chromadoria and Chromadorida into monophyletic groups did not result in a longer tree. Within the Chromadoria, the order Desmodorida sensu Malakhov (1994) was paraphyletic. However, Desmodorida sensu Lorenzen (1994), which does not include Ceramonematidae, was monophyletic. Monhysterida formed a monophyletic order within Chromadoria, equivalent to Chromadorida and Desmodorida. The position of the Comesomatidae was tentatively identified among the Chromadorida, however, the possibility of their placement among the Monhysterida cannot be excluded completely.

Résumé : Les séquences de nucléotides du segment d'expansion D3 du gène de l'ADNr 28S ont été utilisées pour reconstituer la relation évolutive qui prévaut au sein des Adenophorea. L'analyse des liens avec les voisins et une analyse basée sur la parcimonie des représentants des taxons principaux ont révélé que les Adenophorea sont un groupe paraphylétique (p = 0,0005). Parmi les Adenophorea, les Enoplia, Enoplida et Enoplina sont paraphylétiques (p = 0,0024, 0,0014 et 0,0120 respectivement). Une division importante est devenue apparente au sein des Enoplida, une lignée formée à la base des Thoracostomopsidae et des Enoplidae, l'autre lignée constituée des Oncholaimidae et des Encheliididae. Les Tripyloidina sont regroupés près de la branche de base des Enoplidae, et forment un taxon monophylétique. Bien que l'analyse basée sur la parcimonie maximale et l'arbre qui relie les voisins en fassent des groupes paraphylétiques, contraindre les Chromadoria et les Chromadorida sensu Malakhov (1994) en groupes monophylétiques ne génère pas un arbre plus long. Chez les Chromadoria, l'ordre des Desmodorida sensu Malakhov (1994) est paraphylétique. Cependant, les Desmodorida sensu Lorenzen (1994) qui excluent les Ceramonematidae, sont monophylétiques. Les Monhysterida forment un ordre monophylétique au sein des Chromadoria, qui équivalent aux Chromdorida et aux Desmodorida. Les Comeomatidae ont été placés avec prudence parmi les Chromadorida, mais la possibilité qu'ils appartiennent aux Monhysterida ne peut être rejetée entièrement.

[Traduit par la Rédaction]

Introduction

Despite their numerical abundance and biological importance, nematodes represent a unique challenge to systematic biologists. While their relationship to other aschelminth phyla has received a fair amount of attention (Remane 1963; Lorenzen 1985; Winnepenninckx et al. 1995; Wallace et al. 1996; Aguinaldo et al. 1997; Aleshin et al. 1998*b*), relationships within the phylum are highly controversial (Andrassy 1976; Lorenzen 1981; Platt and Warwick 1983*a*, 1983*b*; Malakhov 1994). Depending on the author's perception, Nematoda has been assigned either independent phylum status (Chitwood 1933; Chitwood and Chitwood 1950) or class-level rank among aschelminth taxa (Malakhov 1994). In this study, we consider Nematoda at the phylum level, and lower hierarchical rankings will reflect a corresponding change. Based on the presence of phasmids (i.e., caudal sensory organs), the class Secernentea (also called Phasmidia, Rhabditea) is recognized by many nematode taxonomists (Linstow 1905; Chitwood 1933; Chitwood and Chitwood 1933, 1950; De Coninck 1965). Nematodes lacking phasmids are grouped into the class Adenophorea (originally termed Aphasmidia by Chitwood 1933) and are subdivided into Chromadorida and Enoplida (Chitwood 1933; Chitwood and Chitwood 1950)

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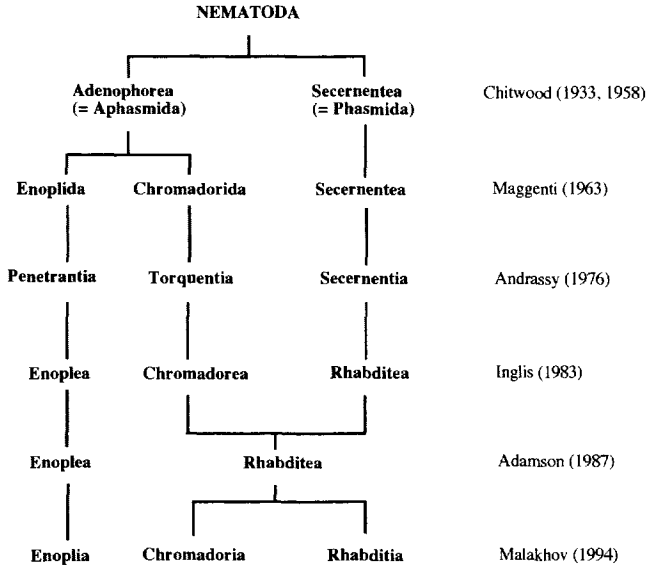
Fig. 1. Major taxonomic divisions of the Nematoda as recognized by various authors.



(Fig. 1). Maggenti (1963) maintains a Secernentea–Adenophorea division, but also recognizes a closer relationship of the Chromadorida with the Secernentea than with the Enoplida. Lorenzen (1981) postulates monophyly of the Secernentea based on the absence of caudal glands and the presence of a single testis; however, no apomorphies for the Adenophorea are identified.

Since the initial division, the monophyly of the Adenophorea has been questioned several times. Both Andrassy (1976) and Inglis (1983) split Nematoda into three main lineages (Fig. 1). In Andrassy's (1976) scheme, the Secernentea are renamed Secernentia, and two new groups with ranks equivalent to the class rank of the Secernentia, namely the Torquentia (corresponding to the subclass Chromadoria) and the Penetrantia (corresponding to the subclass Enoplida) are recognized (Fig. 1). Andrassy (1976) characterizes Secernentea as having a buccal capsule derived from five basic elements (cheilostom, protostom, mesostom, metastom, and telostom), pore-like amphids situated on the lips of the buccal orifice, the presence of a H-shaped excretory organ, and the presence of phasmids. Torquentia on the other hand, are characterized by either round, looped, spiral, or slit-like external amphids, and the Penetrantia by pocket-shaped recessed amphids (Andrassy 1976; Malakhov 1994). Inglis (1983) divided the Nematoda into the classes Rhabditia, Enoplea, and Chromadorea, corresponding roughly to the Secernentea, Enoplida, and Chromadorida, respectively.

Paraphyly of the Adenophorea is again reflected in Adamson's (1987) analysis, with the grouping of Chromadorea with Rhabditea and postulating that Enoplea is the sister group of this clade (Fig. 1). Finally, the most recent morphology-based classification scheme (Malakhov 1994) upholds three classes: the Enoplia, the Chromadoria, and the Rhabditia, the last of which includes all nematodes grouped among the Secernentea (Fig. 1).

Whereas several molecular studies of nematode relationships have tended to focus on secernentean taxa (Nadler 1992; Vanfleteren et al. 1994; Fitch et al. 1995; Al-Banna et al. 1997), three recent studies have incorporated specimens from the Adenophorea as well (Aleshin et al. 1998*a*; Blaxter et al. 1998; Kampfer et al. 1998). And, while all three studies use 18s rDNA sequences for the reconstruction of molecular phylogenies, Kampfer et al. (1998) also include a 17-character morphological data set, which allows for a comparison of the resulting morphological and molecular trees. Despite the use of the same gene, the molecular phylogenies of these studies are not congruent, and the conclusions drawn by the respective authors vary. Most notably, Kampfer et al. (1998) find strong support for the monophyly of the Adenophorea and of the Secernentea. Aleshin et al. (1998*a*) and Blaxter et al. (1998), however, conclude that the adenophoreans are a paraphyletic group, because they include the ancestors of the Secernentea. In part, this discrepancy may be due to different sampling strategies. The three studies overlap to some extent in their secernentean specimens; however, Kampfer et al. (1998) concentrate mostly on adenophoreans belonging to the Desmodoridae, especially the subfamily Stilbonematinae, and one representative of the Enoplida. The study by Blaxter et al. (1998), on the other hand, includes a more varied representation of Adenophorea (e.g., Enoplida, Trichocephalida, Chromadorida, Mononchida, Triplonchida, Mermithida, Dorylaimida, and Monhysterida). The adenophoreans used by Aleshin et al. (1998*a*) belong to the Enoplida, Chromadorida, Desmodorida, and Monhysterida.

Disagreement at the class-level ranking is further reflected to lower taxonomic levels, for example, relationships in the subclass Chromadoria are also quite unclear. Lorenzen (1981) grouped the order Monhysterida separately, because its members possess outstretched ovaries (as opposed to reflexed ovaries). According to Malakhov (1994), monhysterids, characterized by finely striated cuticles, cephalic sensory organs arranged into two or three circles, circular amphids representing a spiral prototype, and a single cylindrical pharynx, can be considered the most primitive order. It is thought that primitive Monhysterida gave rise to the forms in the orders Plectida and Desmoscolecida. Desmodorida and Chromadorida, with their annulated cuticles, are considered more derived (Malakhov 1994).

Even at the familial level, nematode classification is controversial. The family Comesomatidae has been assigned to the order Monhysterida by Lorenzen (1994) on the basis of the outstretched female reproductive system, a diagnostic character for the order. However, based on amphid and cuticle structures, Platt (1985) places the Comesomatidae in the Chromadorida, and Hope and Zhang (1995), in their new species descriptions of the comesomatids *Hopperia hexadendata* and *Cervonema deltensis*, also place the family in the Chromadorida.

Thus, an ever-changing classification system and no consensus phylogeny are the imminent proofs of the unresolved evolutionary relationships among nematodes. The issues are further confounded by parallel and convergent evolution, resulting in homogeneous morphologies throughout the group, regardless of taxonomic status. Additionally, ecological constraints add to parallelisms in buccal morphology that are quite unrelated to actual systematics (Wieser 1953).

The main objective of the present study was to revise the taxonomy of the Adenophorea using a set of characters

independent of morphology, life histories, or ecology. Specifically, we wanted to determine (*i*) the monophyly of the Adenophorea or, alternatively, the validity of splitting the group into separate clades of rank equivalent to the Secernentea, (*ii*) the monophyly of the Enoplida and the Chromadorida, (*iii*) the relationship between the orders Chromadorida and Monhysterida within the Chromadoria, and (*iv*) the placement of the Comesomatidae, which have been assigned to both the Monhysterida and the Chromadorida.

We used the D3 expansion segment of the 26/28S rDNA gene and its adjoining conserved flanking sequences to address our objectives. Nunn (1992) found that the generally rapidly evolving expansion segments (especially D3) exhibited an unusual homogeneity at the generic level and were useful in reconstructing evolutionary relationships to class-level. Additionally, a comparative evaluation of evolutionary rates of the 28S rRNA gene and the mitochondrial gene coding for cytochrome oxidase II, showed that the D3 expansion segment was especially suited for nematode systematics (Nunn 1992). Since then, this particular segment has not only been used to resolve relationships among pratylenchid nematodes (Al-Banna et al. 1997), but also among isopods (Nunn et al. 1996) and platyhelminths (Litvaitis et al. 1996; Litvaitis and Rohde 1999).

Materials and methods

Specimens were collected in New Hampshire, Massachusetts, Florida, and the Schelde estuary in the Netherlands (for collection localities and taxa see Table 1). In fine silty sediments, samples were obtained using a trowel scrape, to a depth of either 4–6 cm for U.S. specimens or no more than 2 cm for Dutch specimens, and stored at 5°C. Total meiofauna of U.S. samples was extracted using the $MgCl_2$–anesthization/decantation technique (Hulings and Gray 1971). In coarse sandy sediment, an in-field decantation method was employed, whereby sediment was placed in a large bucket with excess seawater. Vigorous stirring suspended the lighter meiofauna, which were then easily trapped in a cone-shaped filter of 50 μm when the suspension was passed through the sieve, while the heavier sediment remained in the bucket. Schelde estuary samples were extracted using centrifugation–flotation with the nontoxic silica gel Cecasol 40C (Sobrep). In some cases, nematodes were obtained from monospecific laboratory cultures on agar, set up from small sediment inoculations as described by Moens and Vincx (1998).

Nematodes were identified under a compound microscope equipped with differential interference contrast optics. Identifications were based on available keys (Hope and Murphy 1972; Tarjan 1980; Hope 1982; Platt and Warwick 1983*a*, 1983*b*, 1994; Bussau 1990*a*, 1990*b*, 1991*a*, 1991*b*). United States specimens were verified by one of the authors (W.D.H.), who is curator of the nematode collection at the Smithsonian Institution, Washington, D.C.; Schelde estuary specimens were verified by Dr. M. Vincx. Voucher specimens were fixed in TAF (triethanolamine formaldehyde: 7% formalin – 2% triethanolamine – 91% distilled H_2O). Shrinkage due to fixation is negligible in marine nematodes. Following fixation, the specimens were suspended in pure anhydrous glycerine via a slow evaporation process, mounted on glass slides with an appropriate glass bead size to avoid crushing (Riemann 1988), and sealed with Glyceel (Bates 1997).

Specimens to be used for DNA sequencing were placed individually in microfuge tubes using an insect pin. This ensured transfer of minimal amounts of seawater into the microfuge tube. Specimens were covered with 1 mL of 95% ethanol until processed. Prior to DNA extraction, the ethanol was vacuum-evaporated in a Savant Speed-Vac. DNA extractions followed the protocol of Litvaitis et al. (1994, 1996). The D3 expansion segment with flanking regions was amplified using the conditions and primers described in Litvaitis et al. (1994). All specimens were sequenced in both directions. DNA purification and sequencing protocols were described in Litvaitis et al. (1994, 1996). All secernentean sequences, and those of the adenophoreans *Plectus* sp. and *Xiphinema index*, were obtained from GenBank. Sequences generated during this study for specimens identified to at least the generic level have been deposited in GenBank under the accession numbers AF210398–AF210427.

The initial editing of sequences was done using the SeqEd program (version 1.0.3; ABI). Sequences were aligned using the multiple sequence alignment program CLUSTAL (Higgins et al. 1992) and improvements to the alignment were made by eye. Highly variable sites of ambiguous alignment were eliminated from the analysis. Alignments are available from the first author upon request. Phylogenetic trees were constructed using distance- and parsimony-based algorithms (beta version 4.0 of PAUP* written by David L. Swofford (1999)). Log determinant (LogDet) distance measure transformations (Lockhart et al. 1994) were used in the construction of neighbor-joining (NJ) trees (Saitou and Nei 1987), and maximum-parsimony (MP) trees were generated using the heuristic search option (10 random addition replicates). Transition: transversion ratios of 7:10 were determined after pairwise comparisons of many taxa. Gaps were treated as missing data, and the tree bisection–reconnection (TBR) branch-swapping algorithm with collapsing zero branch length option was employed. Reliability of internal nodes of trees was ascertained by 2000 bootstrap replications.

Whereas some authors have suggested that nematomorphs are the immediate sister group of the Nematoda (Wallace et al. 1996), others contend that nematodes are closer to Gastrotricha (Malakhov 1994) or to Kinorhyncha and Priapulida (Malakhov 1980; Malakhov and Adrianov 1995). In an analysis of aschelminth 18S rRNA, Winnepenninckx et al. (1995) arrive at contradictory conclusions as to the sister group of the nematodes. Depending on the algorithm employed during phylogenetic analysis, nematomorphs and nematodes are sister groups (distance methods) or nematomorphs are grouped with the arthropods (parismony methods) (Winnepenninckx et al. 1995).

We used the platyhelminth *Calicophoron calicophorum* as an outgroup for two reasons. First, we wanted to be able to compare our trees with the trees of Kampfer et al. (1998). Secondly, outgroups do not need to be the immediate sister taxon of the ingroup (Nixon and Carpenter 1994), but character states of the outgroup must share enough historical similarities to infer plesiomorphic states of the ingroup (Wheeler 1990). Platyhelminthes have been considered as an early clade forming the sister group of all other Bilateria (Hyman 1951), as an early branch within the protostomes (Ax 1987), or as a clade derived from a coelomate protostome ancestor (Siewing 1980).

Because our aim was to distinguish among competing phylogenetic hypotheses, we compared trees constrained in various ways with the consensus MP tree (Table 2). To determine if tree lengths of constraint trees were significantly different from the MP tree, the nonparametric ranked-sign test of Templeton (Larson 1994) was applied ($\alpha = 0.05$).

Results

Amplification with the D3 primers resulted in products of about 300 bp (for fragment lengths of individual specimens see Table 1). Of the 328 total characters, almost 59% were parsimony informative, and a heuristic search with 10 random addition replicates found two equally parsimonious trees of

Table 1. Collection localities and fragment lengths for specimens used in this study.

Taxon	Collection locality	Fragment length (bp)
Class: Adenophorea		
Subclass: Chromadoria		
Order: Chromadorida		
Family: Chromadoridae		
Chromadoridae	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	320
Chromadora nudicapitata	Paulina salt marsh, Schelde Estuary, the Netherlands, N51 21 E3 44	326
Family: Comesomatidae		
Setosabatieria sp.-1	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	326
Setosabatieria sp.-2	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	278
Comesomatidae-1	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	330
Comesoma sp.	Portsmouth, New Hampshire, N43 04 28.6 W070 44 36.8	316
Sabatieria sp.	Portsmouth, New Hampshire, N43 04 28.6 W070 44 36.8	315
Family: Cyatholaimidae		
Cyatholaimidae-1	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	282
Cyatholaimidae-2	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	276
Paracanthonchus sp.	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	273
Praeacanthonchus punctatus	Paulina salt marsh, Schelde Estuary, the Netherlands, N51 21 E3 44	305
Family: Plectidae		
Plectus sp.	GenBank Accession U61758	300
Family: Selachinematidae		
Choanolaimus sp.	Fort Pierce, Florida, 50′ Range Day Mark N27 27 59.0 W080 18 49.4	294
Order: Desmodorida (sensu Malakhov 1994)		
Family: Desmodoridae		
Metachromadora sp.	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	343
Desmodora sp.	Portsmouth, New Hampshire, N43 04 28.6 W070 44 36.8	319
Family: Ceramonematidae		
Pselionema sp.	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	313
Family: Monoposthiidae		
Monoposthia sp.-1	Fort Pierce, Florida, 50′ Range Day Mark N27 27 59.0 W080 18 49.4	298
Monoposthia sp.-2	Fort Pierce, Florida, 50′ Range Day Mark N27 27 59.0 W080 18 49.4	295
Order: Desmoscolecida		
Family: Desmoscolecidae		
Tricoma sp.	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	274
Order: Monhysterida		
Family: Xyalidae		
Theristus sp.	Fort Pierce, Florida, 50′ Range Day Mark N27 27 59.0 W080 18 49.4	284
Theristus acer	Paulina salt marsh, Schelde Estuary, the Netherlands, N51 21 E03 44	317
Family: Sphaerolaimidae		
Sphaerolaimus sp.	Paulina salt marsh, Schelde Estuary, the Netherlands, N51 21 E03 44	336
Family: Monhysteridae		
Geomonhysteria disjuncta	Paulina salt marsh, Schelde Estuary, the Netherlands, N51 21 E03 44	329
Subclass: Enoplia		
Order: Enoplida		
Family: Enchelidiidae		
Calyptronema maxweberi	Paulina salt marsh, Schelde Estuary, the Netherlands, N51 21 E03 44	300
Family: Enoplidae		
Enoplus sp.	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	324
Family: Oncholaimidae		
Adoncholaimus fuscus	Paulina salt marsh, Schelde Estuary, the Netherlands, N51 21 E03 44	300
Oncholaimidae-1	Portsmouth, New Hampshire, N43 04 28.6 W070 44 36.8	319
Oncholaimidae-2	Portsmouth, New Hampshire, N43 04 28.6 W070 44 36.8	283
Oncholaimus sp.	Fort Pierce, Florida, 50′ Range Day Mark N27 27 59.0 W080 18 49.4	317
Viscosia sp.	Molenplaat, Schelde Estuary, the Netherlands, N51 26 E03 58	309
Family: Rhabdodemaniidae		
Rhabdodemania sp.-1	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	335
Rhabdodemania sp.-2	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	335

Table 1 (*concluded*).

Taxon	Collection locality	Fragment length (bp)
Family: Thoracostomopsidae		
Enoplolaimus sp.	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	329
Mesacanthion sp.	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	322
Epacanthion sp.	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	318
Enoploides sp.	Fort Pierce, Florida, 6 mile station N27 29 03.6 W080 11 07.3	322
Enoploides longispiculosus	Molenplaat, Schelde Estuary, the Netherlands, N51 26 E03 58	302
Family: Tripyloididae		
Tripyloides sp.	Paulina salt marsh, Schelde Estuary, the Netherlands, N51 21 E03 44	240
Order: Dorylaimida		
Family: Longidoridae		
Xiphinema index	GenBank Accession U47561	302
Class: Secernentea		
Subclass: Rhabdita		
Order: Rhabditoidea		
Family: Cephalobidae		
Zeldia punctata	GenBank Accession U61757	297
Family: Heterorhabditiae		
Heterorhabditis bacteriophora	GenBank Accession U47560	294
Family: Rhabditidae		
Caenorhabditis elegans	GenBank Accession X03680	304
Cruznema tripartitum	GenBank Accession U73450	287
Pellioditis marina	Walsoorden, Schelde Estuary, the Netherlands, N51 22 60 E04 02 55	297
Subclass: Diplogastria		
Order: Tylenchida		
Family: Heteroderidae		
Meloidogyne javanica	GenBank Accession U47559	289
Family: Pratylenchidae		
Hirschmanniella belli	GenBank Accession U47556	301
Pratylenchus penetrans	GenBank Accession U47546	300
Radopholus similis	GenBank Accession U47558	302
Nacobbus aberrans	GenBank Accession U47557	302

10 501 steps each (consistency index = 0.3363; rescaled consistency index = 0.1873). Except for small differences restricted to branch lengths among Cyatholaimidae, Oncholaimidae, and Thoracostomopsidae, the two trees were congruent in their topology.

Regardless of the tree-building algorithm employed, extensive paraphyly among the Adenophorea was revealed (Figs. 2 and 3). This paraphyly was further confirmed by constraint analysis, resulting in a tree that was 261 steps longer (p = 0.0005; Table 2). The Secernentea were not resolved into a monophyletic clade but instead formed an unresolved clade with some of the adenophoreans (Figs. 2 and 3). However, when constraining their monophyly during parsimony, the resulting tree was only 16 steps longer, which was not significant at α = 0.05 (Table 2). Among the Secernentea, the Tylenchida formed a clade that also contained, as a basal branch, the cephalobid rhabditoid *Zeldia punctata*, thus making the Rhabditoidea paraphyletic (Figs. 2 and 3). Even though this clade was supported in 84% of the bootstrap replications in the NJ tree (less than 50% support in parsimony analysis), a constraint analysis of a monophyletic Rhabditoidea (subclass Rhabdita) proved not significant (64 additional steps; Table 2).

Paraphyly of Enoplia was evident in both trees and was further supported by constraint analysis (241 additional steps; p = 0.0024; Table 2). Among the Enoplia, two major clades were recognizable: one containing the Oncholaimidae and one specimen of the Enchelidiidae (*Calyptronema maxweberi*) and the other consisting of the Thoracostomopsidae and the only individual of the Enoplidae (*Enoplus* sp.) available for the study (Figs. 2 and 3). In the NJ tree, the two clades were supported by 99 and 100% of the bootstrap replications, respectively (Fig. 2), while in the consensus MP tree, they were supported by 94 and 98% of the bootstrap replications, respectively (Fig. 3). In both trees, the clade Thoracostomopsidae–Enoplidae formed a basal linage and clustered closely with another enoplid family, the Rhabdodemaniidae (Figs. 2 and 3). In morphology-based phylogenies, Rhabdodemaniidae are grouped with Tripyloididae into the suborder Tripyloidina. The NJ analysis resolved such a relationship (Fig. 2), whereas in the MP tree, *Tripyloides* sp. clustered with the Monhysterida (Fig. 3). To resolve this inconsistency, a monophyletic Tripyloidina was constrained, resulting in a tree that required an additional 10 steps (p not significant at α = 0.05; Table 2). Furthermore, when constraining a monophyletic clade of Thoracostomopsidae–Enoplidae and Tripyloidina, the resulting tree was 40 steps longer, which also was not significant at α = 0.05 (Table 2).

The second clade, containing Oncholaimidae and Enchelidiidae, formed part of an unresolved trichotomy in the NJ

Table 2. The results of constraint analysis.

	No. of additional steps required	*p*
Monophyly of:		
Secernentea	16	—
Secernentea + *Plectus* sp.	4	—
Rhabditoidea	64	—
Adenophorea	261	0.0005
Enoplia	241	0.0024
Enoplida	185	0.0014
Enoplina	192	0.0120
Tripyloidina	10	—
[Thoracostomoposidae + Enoplidae] and Tripyloidina	40	—
X. index + Enoplia (excluding Oncholaimidae + Enchelidiidae)	54	—
Chromadoria	96	—
Chromadorida (sensu Platt and Warwick 1983; Lorenzen 1994)[a]	127	—
Chromadorida (sensu Malakhov 1994)[b]	48	—
Desmodorida (sensu Malakhov 1994)[c]	414	<0.0001
Desmodorida (sensu Lorenzen 1994)[d]	68	—
Monhysterida	19	—
Sister-group relationship of:		
Comesomatidae and Monhysterida	50	—
Oncholaimidae–Encheliidae and Monhysterida	58	—
Comesomatidae and *Plectus* sp.	34	—
Secernentea and [Oncholaimidae + Desmodoridae]	203	0.0008
Chromadorida (sensu Platt and Warwick 1983)[a] and Monhysterida	119	—
Chromadorida (sensu Malakhov 1994)[b] and Monhysterida	132	—

Note: PAUP heuristic search (transition/transversion ratio 7:10) was employed. *Calicophoron calicophorum* (Platyhelminthes) was used as the outgroup. Constraint trees were compared with the consensus MP tree (tree length 10 501 steps) at $\alpha = 0.05$; "–," not significant.

[a]Includes Chromadorina, Leptolaimina, and Desmoscolecina.

[b]Includes Chromadorina (Chromadoridae) and Cyatholaimina (Cyatholaimidae and Selachinematidae).

[c]Includes Desmodoridae, Ceramonematidae, and Monoposthiidae.

[d]Includes Desmodoridae and Monoposthiidae.

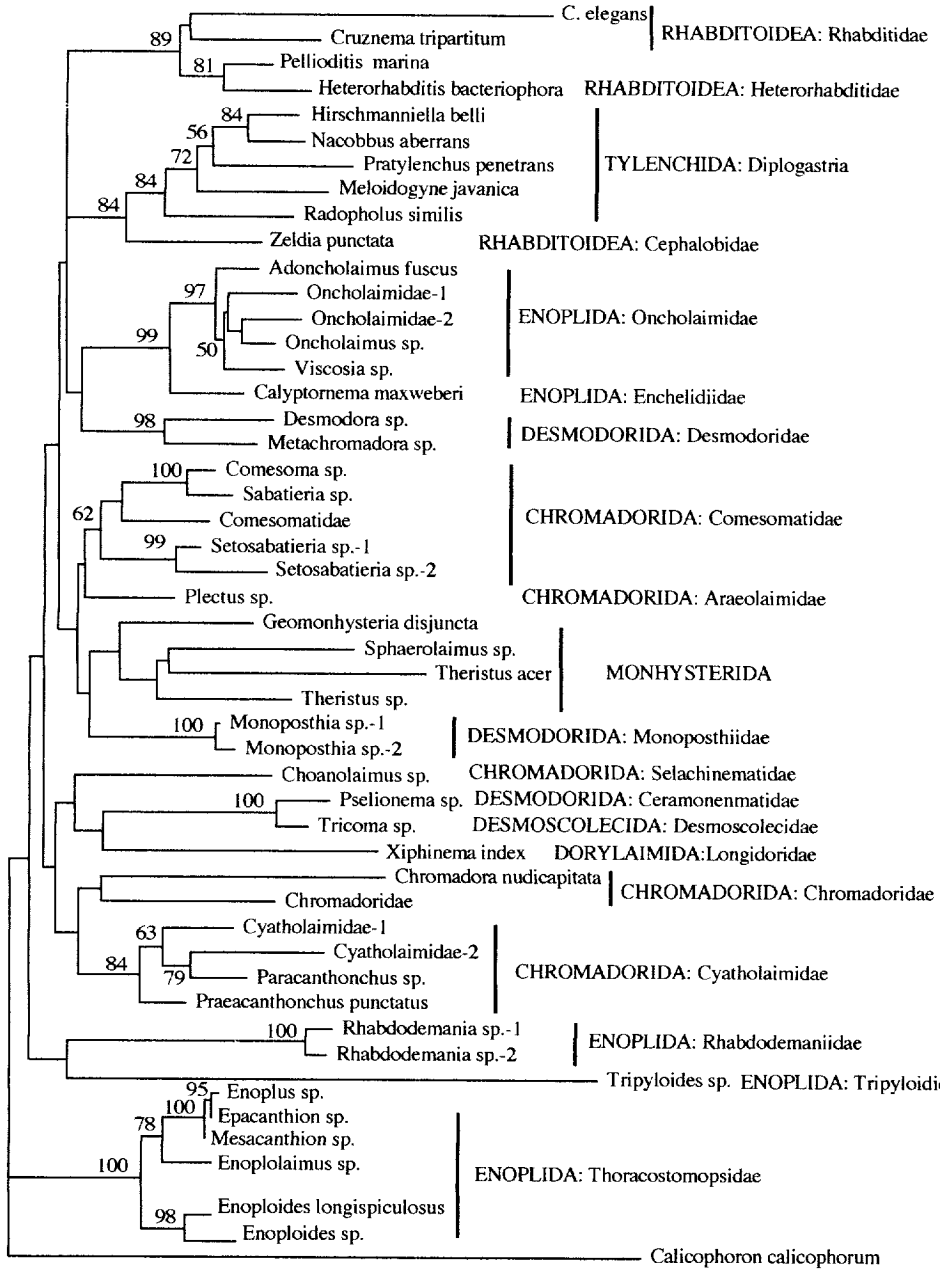
tree (Fig. 2) and, in the MP tree, was part of a large clade consisting of chromadorids and monhysterids (Fig. 3). A constraint analysis of a possible sister-group relationship of Oncholaimidae–Enchelidiidae and Secernentea, as suggested by the NJ analysis, resulted in a significantly longer tree (203 additional steps, $p = 0.0008$; Table 2) and is, therefore, rejected. Because of the variable placement of the Oncholaimidae, the paraphyly of Enoplia is carried through to lower taxonomic levels, rendering the order Enoplida and the suborder Enoplina (sensu Platt and Warwick 1983*a*) invalid ($p = 0.0014$ and $p = 0.0120$, respectively; Table 2).

Although the Chromadoria appear paraphyletic in both trees (Figs. 2 and 3), constraint analyses revealed no paraphyly (96 additional steps; Table 2). Furthermore, constraining a monophyletic Chromadorida, sensu Platt and Warwick (1983*b*) (i.e., inclusive of Chromadorina, Leptolaimina, and Desmoscolecina) or according to Malakhov (1994) (i.e., inclusive of Chromadorina and Cyatholaimina), did not result in longer trees (127 and 48 additional steps, respectively; Table 2). Among the Chromadoria, well-defined families (e.g., Comesomatidae and Cyatholaimidae) were recognizable, although the relationships of these families with each other were quite variable. Cyatholaimidae, supported by 84% (NJ tree) and 86% (MP tree) of the bootstrap replications, formed the most basal chromadorid lineage (Figs. 2 and 3). In the distance-based tree, this family formed the sister group of a clade of various other chromadorid families (i.e., Selachinematidae, Ceramonematidae, and Desmoscolecidae) and of *Xiphinema index*, an enoplid belonging to the Dorylaimida (Fig. 2). In the MP analysis, the Cyatholaimidae formed the sister group of a clade containing most chromadorids, the monhysterids, the enoplids (with the exception of the Enoplidae–Thoracostomopsidae), and all Secernentea (Fig. 3).

The Comesomatidae, supported by 62% of the bootstrap replications in the NJ tree, formed the sister group of a clade containing the Monhysterida plus Monoposthiidae (Fig. 2) and, in the MP analysis, formed the immediate sister group of the Rhabditidae and Heterorhabditidae (Fig. 3). In addition, *Plectus* sp. was part of the comesomatid group in the distance-based tree (Fig. 2). However, in the MP tree, *Plectus* sp. formed the most basal lineage of the Tylenchida–Cephalobidae clade (Fig. 3).

In the NJ analysis, the Monhysterida and Monoposthiidae formed a sister group of the Comesomatidae (including *Plectus* sp.) (Fig. 2), whereas in the parsimony analysis, the Monhysterida (plus *Tripyloides* sp.) formed a sister-group relationship with Oncholaimidae and Monoposthiidae (Fig. 3). However, except for the grouping of the two *Theristus* species, neither clade was supported by bootstrap percentages. Constraining a monophyletic Monhysterida did not result in

Fig. 2. Neighbor-joining tree of 49 nematode specimens, constructed using partial sequences of the 28S rDNA gene. Numbers at nodes represent percentages of 2000 bootstrap replications; only values above 50% are reported.



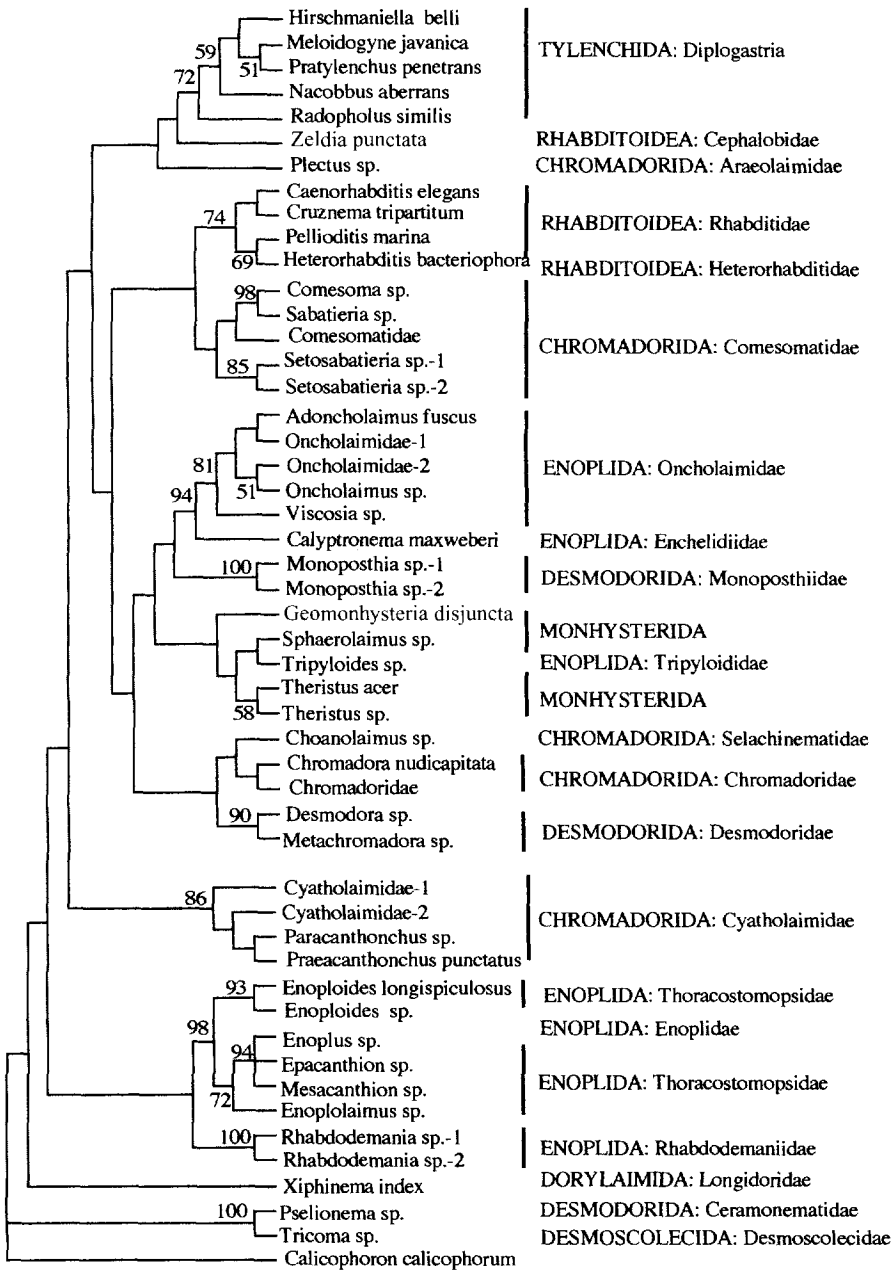
a significantly longer tree (19 additional steps; Table 2). Furthermore, constraint analysis also revealed that the Monhysterida represented the sister group of the Chromadorida (p not significant; Table 2).

Desmodorida (sensu Malakhov 1994), consisting of representatives of the Desmodoridae, Ceramonematidae, and Monoposthiidae, formed a paraphyletic clade (Figs. 2 and 3). When constraining a monophyletic Desmodorida, the resulting tree was significantly longer than the MP tree (414 additional steps, $p < 0.0001$; Table 2). This desmodorid paraphyly may be responsible, in part, for the odd placement of the dorylaimid *X. index* in the NJ tree and the basal positioning of *Pselionema* sp. and *Tricoma* sp. in the MP tree. Lorenzen (1994) though, does not include the Ceramonematidae among the Desmodorida and, when constraining a tree accordingly, the resulting tree is not different from the MP tree (Table 2).

Discussion

Because of a paucity of reliable taxonomic characters and difficulties in assigning polarity to existing characters, nematode systematics is highly controversial and phylogenetic classification schemes may not be representative of actual evolutionary relationships (Maggenti 1963; Andrassy 1976; Lorenzen 1981; Inglis 1983; Platt and Warwick 1983*a*, 1983*b*; Adamson 1987; Malakhov 1994). With the exception of three studies (Blaxter et al. 1998; Kampfer et al. 1998; Aleshin et

Fig. 3. Maximum parsimony tree of 49 nematode specimens, constructed using partial sequences of the 28S rDNA gene. Numbers at nodes represent percentages of 2000 bootstrap replications; only values above 50% are reported.



al. 1998*a*), molecular analyses of nematode systematics have concentrated mostly on relationships among and within secernentean families (Butler et al. 1981; Nadler 1992, 1995; Vanfleteren et al. 1994; Fitch et al. 1995; Al-Banna et al. 1997). The present study included many adenophorean specimens in a phylogenetic analysis based on the D3 expansion segment of the gene coding for the large ribosomal subunit.

Adenophorea and Secernentea

Consistent with Blaxter et al. (1998) and Aleshin et al. (1998*a*), we found the Adenophorea to be a paraphyletic clade (Figs. 2 and 3; Table 2). Using morphological characters, Andrassy (1976) and Adamson (1987) also concluded that the Adenophorea do not represent a natural taxon. The characters commonly used to justify the group (i.e., shape and position of amphids, paired testes, and single-celled excretory organs) are, in fact, plesiomorphies and can, therefore, not be used to infer relationships. The Secernentea formed a natural clade within the Adenophorea. Constraining them into a monophyletic taxon exclusive of the adenophoreans resulted in a tree that was not significantly longer than the MP tree (Table 2). It appears that the closest free-living sister group of the Secernentea is to be found either among the Plectidae or the Comesomatidae (Fig. 3; Table 2). Chitwood and Chitwood (1950) claimed that the plectids shared certain features of the pharynx with secernentean nematodes and considered that these similarities were plesiomorphies of all nematodes. However, a careful examination of pharyngeal structures and their functioning and postembryonic develop-

ment led Maggenti (1963) to reject this hypothesis; Maggenti (1963) explained the similarities as analogies, and proposed an undifferentiated cylindrical pharynx as the plesiomorphic nematode pharynx. Dereids (dorsolateral sense organs), known only from the Secernentea that are found in *Plectus*, led Lorenzen (1994) to propose that Plectidae may be closer to the Secernentea than to the remaining Adenophorea. In accordance with Blaxter et al. (1998), our parsimony analysis also suggested a link between *Plectus* sp. and the Secernentea (Fig. 3) and, when we constrained a monophyletic Secernentea to include *Plectus* sp., the resulting tree was as acceptable as the MP consensus tree (p not significant; Table 2).

Similarly to Blaxter et al. (1998), we found a paraphyletic Rhabditoidea using MP analysis. However, resolving this paraphyly required only an additional 64 steps, resulting in a tree not significantly longer (Table 2). Therefore, we suggest that trees be constrained to assess paraphyly, and that trees found more concordant with morphological phylogenies be favoured, even if they involve a few extra steps, as long as the resulting tree is not significantly longer than the MP tree (as determined by the nonparametric ranked-sign test of Templeton). Such a compromise will allow for a meaningful integration of morphological and molecular data.

Enoplida and Dorylaimida

Earlier phylogenetic schemes have suggested a deep division within the adenophoreans, separating the Enoplia from the remaining nematodes (Maggenti 1963; Andrassy 1976; Inglis 1983). While we agree with a closer relationship of chromadorids with secernenteans, we found an additional division within the enoplids. Thoracostomopsidae and one specimen of Enoplidae (*Enoplus* sp.) formed the most basal branch of our trees. A second enoplid clade, containing the Oncholaimidae and Enchelidiidae, clustered with the chromadorids and monhysterids (Figs. 2 and 3). According to Platt and Warwick (1983*a*), the Enoplidae, Thoracostomopsidae, Enchelidiidae, and Oncholaimidae are all members of the suborder Enoplina. However, we found no evidence of such a grouping, using either parsimony- or distance-based analysis. In addition, constraining a suborder Enoplina resulted in a significantly longer tree (p = 0.0120; Table 2). An early emergence for the Thoracostomopsidae–Enoplidae is supported by a single-celled excretory organ that opens ventrally, a nonstriated integument, a uniform pharynx, and paired testes (Maggenti 1963; Malakhov 1994). The distinctiveness of these two families (Thoracostomopsidae and Enoplidae) is also reflected in Lorenzen's (1994) classification, in which they are grouped in the taxon Enoploidea, together with the Anoplostomatidae, Phanodermatidae, and Anticomidae. In addition, Kampfer et al. (1998), using a combination of morphological and 18S rDNA data, also found that the enoplid *Enoplus meridionalis* was consistently the most basal branch of their analyses.

While maintaining the Oncholaimidae and Enchelidiidae within the Enoplina, Lorenzen (1994) recognized the need to separate these two families from other Enoplina and, thus, he erected the Oncholaimoidea. According to this author, Oncholaimoidea are characterized by the location of the cervical gland. In specimens where a cervical gland is present, the gland is always located on the right side of the intestine (Lorenzen 1994). This character then becomes the defining synapomorphy of the Oncholaimoidea. We recommend that the term "Enoplina" be redefined to not include the Oncholaimidae and Enchelidiidae, so that this superfamilial taxon will represent a true natural group. Additionally, a final decision requires that representatives from other families currently included in the Enoplina be sampled to assess warranting their inclusion.

An early emerging lineage within the Enoplida is represented by the Tripyloidina (Tripyloididae and Rhabdodemaniidae). The Tripyloidina clearly represented a monophyletic clade (Fig. 2; Table 2), the clustering of *Tripyloides* sp. with the monhysterids in the MP tree being an artefact of long-branch attraction. We conclude that the Tripyloidina form a valid taxon (based on our NJ and constraint analyses) and that, together with Thoracostomopsidae–Enoplidae, are part of the Enoplida. This conclusion is also supported by constraint analysis (40 additional steps, p not significant; Table 2).

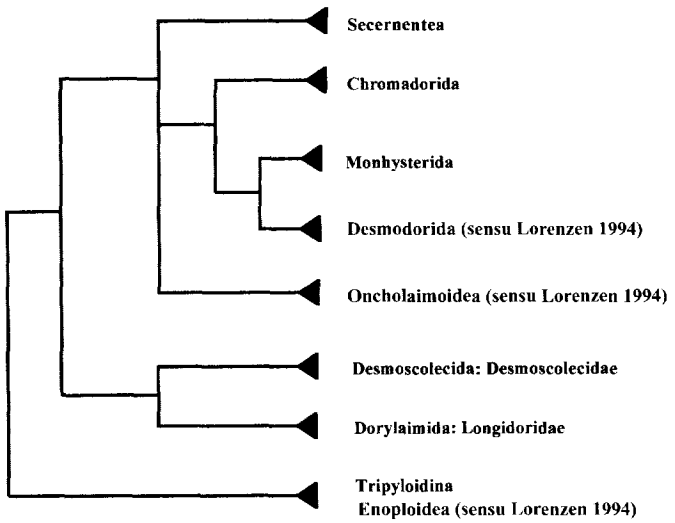
Our analysis included only one specimen of the Dorylaimida, namely *X. index*. Parsimony analysis resolved its position as a basal enoplid (Fig. 3), a positioning that has been proposed by Maggenti (1963), Chitwood and Chitwood (1950), and Lorenzen (1994). NJ analysis, though, grouped *X. index* with representatives of two chromadorid orders (*Pselionema* sp. and *Tricoma* sp.) and, while *Pselionema* sp. and *Tricoma* sp. are supported by 100% of the bootstrap replicates, their clustering with *X. index* is supported at less than 50%. Additionally, from the branch lengths of these three taxa, we infer that they evolve more rapidly than some of the other lineages. Thus, their unnatural clustering may be due to long-branch attraction and (or) the fact that only individual dorylaimid and desmoscolid specimens were available. Their exact placment among adenophorean nematodes awaits improved rate-correction models and increased taxonomic sampling.

Chromadorida

Although in the phylogenetic trees of the current study, Chromadorida appeared to be paraphyletic (Figs. 2 and 3), this paraphyly is not significant as determined by constraint analysis (Table 2). Whether defining Chromodorida sensu Platt and Warwick (1983*b*) or as a narrower clade according to Malakhov (1994), the constraint trees were not significantly longer. Paraphyly of Chromadorida has been suggested previously on the basis of morphological characters (review in Lorenzen 1994), and has recently been confirmed using 18S rDNA data (Blaxter et al. 1998). However, care needs to be taken when referring to chromadorids. It appears that Blaxter et al. (1998) use Chromadorida and Chromadoria interchangably. In fact, in their study, a member of the Desmodorida (*Metachromadora* sp.) clustered with the cyatholaimid *Praeacanthonchus* sp., and the leptolaimids *Plectus aquatilis*, *Plectus acuminatus*, and *Teratocephalus lirellus* formed a second cluster. Thus, these authors based their conclusion of paraphyly of Chromadorida on five specimens, four of which (*Metachromadora* sp., the two plectids, and *T. lirellus*) are placed in orders other than the Chromadorida by Malakhov (1994).

Although our sampling among chromadorids is not all-inclusive, it does represent the most extensive molecular representation to date. Based on our findings, we conclude that the order Chromadorida is a valid monophyletic taxonomic unit that includes the Chromadoridae, Comesomatidae, Cyatho-

Fig. 4. Diagrammatic representation of adenophorean relationships based on the partial 28S rDNA gene. Note that the positions of Desmoscolecida and Dorylaimida are tentative and need to be confirmed using additional samples.



laimidae, Plectidae, and Selachinematidae. Additionally, other families that were not available for this study (e.g., Epsilonematidae, Draconematidae, Leptolaimidae, and Microlaimidae) may possibly be included in this order.

Desmodorida and Desmoscolecida

As mentioned earlier, only one specimen of the Desmoscolecida was available. Regardless of the tree-building algorithm employed, *Tricoma* sp. clustered with the desmodorid *Pselionema* sp. (Figs. 2 and 3). This grouping was always supported in 100% of the bootstrap replications. The Desmodorida, consisting of representatives of the Desmodoridae, Ceramonematidae, and Monoposthiidae (i.e., sensu Malakhov 1994), were paraphyletic and, when constraining them into a monophyletic taxon, the tree was significantly longer (Table 2). Most authors, though, do not include the Ceramonematidae in the Desmodorida (Platt and Warwick 1983*a*, 1983*b*; Lorenzen 1994), in which case, the group is monophyletic in a constrained tree (Table 2).

Monhysterida

Systematically, the Monhysterida (an order of Chromadoria) are separated from the Chromadorida on the basis of ovarian structure; chromadorid ovaries are reflexed, whereas monhysterid ovaries are outstretched (Filipjev 1934; Chitwood and Chitwood 1950). In fact, Lorenzen (1994) defines the character outstretched ovaries as the monhysterid synapomorphy. Our NJ analysis clearly resolved the Monhysterida as a monophyletic taxon, with the Monoposthiidae forming the immediate sister group and the Comesomatidae plus *Plectus* sp. forming a more distant sister-group relationship (Fig. 2). This is in accordance with findings by Aleshin et al. (1998*a*). These authors propose a close relationship among Chromadorida, Plectida, Secernentea, and Monhysterida.

While Monhysterida was not supported by bootstrap percentages, the constrained tree was not significantly longer than the MP consensus tree (Table 2). The unexpected grouping of *Tripyloides* sp. with the monhysterids in the MP tree (Fig. 3) is almost certainly due to long-branch attraction (Felsenstein 1978). As is evident from the distance-based tree, *Tripyloides* sp. is indeed characterized by being the fastest evolving nematode among the sampled individuals. Despite this, the NJ analysis resolved its position correctly, with members of the Rhabdodemaniidae forming the suborder Tripyloidina (Fig. 2). Based on our analysis, Monhysterida certainly represents a valid taxon and forms a distinct, separate order within the Chromadoria. This is in accordance with a view proposed by Aleshin et al. (1998*a*).

Finally, Lorenzen (1994) groups the Xyalidae and Sphaerolaimidae more closely together within the monhysterids, using characters associated with cuticle striation and number of tail setae. We find a similar clustering of these two families to the exclusion of the Monhysteridae. While this initial finding is encouraging, we recommend that a more extensive sampling be done to confirm these results.

Comesomatidae

The position of the Comesomatidae within the Chromadoria has been controversial. Filipjev (1934) placed the family in the Monhysterida on the basis of the character outstretched ovaries, a placing that was repeated in Lorenzen's (1994) outline classification. Chitwood and Chitwood (1950) included the family in the Chromadorida on the basis of the presence of pharyngeal tubes. Using punctuated cuticle, multispiral amphids, and the presence of precloacal supplements as characters, Platt (1985) and Hope and Zhang (1995) grouped the Comesomatidae in the Chromadorida. The absence of a punctuated cuticle in a few comesomatid species is explained as secondary loss. Contrary to the claim that *Cervonema*, *Setosabatieria*, and *Leptolaimella* do not have a punctuated cuticle (Platt 1985; Lorenzen 1994), Hope and Zhang (1995) showed that *Cervonema deltensis* and a specimen of *Setosabatieria* sp. do indeed have punctuated cuticles.

Our analysis found a well-supported monophyletic Comesomatidae (Figs. 2 and 3). However, the placement of the comesomatids within the phylogenetic tree differed depending on the tree-building algorithm employed. Furthermore, constraining either a monophyletic clade of Monhysterida and Comesomatidae or a sister-group relationship of the two, did not result in a significantly longer tree (Table 2). Although the Comesomatidae were not found interspersed among the Monhysterida, we conclude that the present analysis is not able to resolve the position of the comesomatids unequivocally. Our tentative conclusion, though, places the taxon as the immediate sister group of the Monhysterida (Fig. 2; Table 2).

In conclusion, this study is an attempt to resolve issues among adenophorean relationships using molecular data. This study contains the widest sampling among the Adenophorea to date, and even though representatives of some families were not available, most major groups have been included. Based on our analysis, we conclude that current dichotomous and trichotomous classification schemes of the Nematoda do not represent true phylogentic relationships (Fig. 4). We recommend that the term Adenophorea be used in a purely historical sense. This would then allow for the establishment of the classes Enoplia and Chromadoria. The Enoplia may need to be divided into two taxa of equivalent rank. We propose to follow Lorenzen (1994), and elevate his Enoplacea and Oncholaimacea to at least the level of order (Fig. 4).

Although this adds to the increase in taxonomic divisions among adenophoreans, the deep split within the Enoplia has now been recognized by many different authors (Lorenzen 1994; Kampfer et al. 1998; Blaxter et al. 1998) and needs to be recognized by appropriate terminology.

Further, we prefer to elevate the Chromadoria to class level. Within this class, the orders Chromadorida, Desmodorida (excluding Ceramonematidae), Desmoscolecida, and Monhysterida are recognized. A possible order, Leptolaimida, will need further investigation. Also, to maintain natural groups, the position of the Plectidae needs closer examination, with a possible placement in the Secernentea. While this seems controversial, a close association of plectids and Rhabditia has been suggested previously (Chitwood and Chitwood 1950; Lorenzen 1994; Malakhov 1994; Blaxter et al. 1998).

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References

Adamson, M.L. 1987. Phylogenetic analysis of the higher classification of Nematoda. Can. J. Zool. **65**: 1478–1482.

Aguinaldo, A.M.A., Turbeville, J.M., Linford, L.S., Rivera, M.C., Garey, J.R., Raff, R.A., and Lake, J.A. 1997. Evidence for a clade of nematodes, arthropods and other moulting animals. Nature (London), **387**: 489–493.

Al-Banna, L., Williamson, V., and Gardner, S.L. 1997. Phylogenetic analysis of the genus *Pratylenchus* using 26S rDNA. Mol. Phylogenet. Evol. **7**: 94–102.

Aleshin, V.V., Kedrova, O.S., Milyutina, I.A., Vladychenskaya, N.S., and Petrov, N.B. 1998*a*. Relationships among nematodes based on the analysis of 18S rRNA gene sequences: molecular evidence for monophyly of chromadorian and secernentian nematodes. Russian Journal of Nematology, **6**: 175–184.

Aleshin, V.V., Milyutina, I.A., Kedrova, O.S., Vladychenskaya, N.S., and Petrov, N.B. 1998*b*. Phylogeny of Nematoda and Cephalorhyncha derived from 18S rDNA. J. Mol. Evol. **47**: 597–605.

Andrassy, I.A. 1976. Evolution as a basis for the systematization of nematodes. Pitman Publishing, London, San Francisco, Melbourne.

Ax, P. 1987. The phylogenetic system. John Wiley & Sons, Chichester.

Bates, J.W. 1997. The slide-sealing compound "Glyceel." J. Nematol. **29**: 565–566.

Blaxter, M.L., De Ley, P., Garey, J.R., Liu, L.X., Scheldeman, P., Vierstraete, A., Vanfleteren, J.R., Mackey, L.Y., Dorris, M., Frisse, L.M., Vida, J.T., and Thomas, W.K. 1998. A molecular evolutionary framework for the phylum Nematoda. Nature (London), **392**: 71–75.

Bussau, C. 1990*a*. Free-living nematodes from the coastal dunes and adjoining biotopes of the German and Danish coasts. I. General part and redescription of some Chromadoria (Nematoda). Zool. Anz. **225**: 161–188.

Bussau, C. 1990*b*. Free-living nematodes from the coastal dunes and adjoining biotopes of the German and Danish coasts. II. Monhysterida, Enoplida and Trefusiida (Nematoda). Zool. Anz. **225**: 189–209.

Bussau, C. 1991*a*. Free-living nematodes from the coastal dunes and adjoining biotopes of German and Danish coasts. III. Dorylaimida. Zool. Anz. **226**: 33–63.

Bussau, C. 1991*b*. Free-living nematodes from the coastal dunes and adjoining biotopes of German and Danish coasts. IV. Rhabditida and Tylenchida (Nematoda). Zool. Anz. **226**: 114–148.

Butler, M.H., Wall, S.M., Luehrsen, K.R., Fox, G.E., and Hecht, R.M. 1981. Molecular relationships between closely related strains and species of nematodes. J. Mol. Evol. **18**: 18–23.

Chitwood, B.G. 1933. A revised classification of the Nematoda. J. Parasitol. **20**: 131.

Chitwood, B.G. 1958. The designation of official names for higher taxa of invertebrates. Bull. Zool. Nomencl. **15**: 860–895.

Chitwood, B.G., and Chitwood, M.B. 1933. The characters of a protonematode. J. Parasitol. **20**: 130.

Chitwood, B.G., and Chitwood, M.B. 1950. Introduction to nematology. University Park Press, Baltimore.

De Coninck, L.A.P. 1965. Classe des nematodes—systematique des nematodes et sous-classe des Adenophorea. *In* Traité de zoologie. Vol 4. Part 2. *Edited by* P.P. Grasse. Masson et Cie, Paris. pp. 3–217.

Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Syst. Zool. **40**: 366–375.

Filipjev, I. 1934. The classification of free-living nematodes and their relation to the parasitic nematodes. Smithson. Misc. Collect. **89**: 1–63.

Fitch, D.H.A., Bugaj-Gaweda, B., and Emmons, S.W. 1995. 18S ribosomal RNA gene phylogeny for some Rhabditidae related to *Caenorhabditis*. Mol. Biol. Evol. **12**: 346–358.

Higgins, D.G., Bleasby, A.J., and Fuchs, R. 1992. CLUSTAL V: improved software for multiple sequence alignment. Comp. Appl. Biosci. **8**: 189–191.

Hope, W.D. 1982. Structure of head and stoma in the marine nematode genus *Deontostoma* (Enoplida: Leptosomatidae). Smithson. Contrib. Zool. No. 353. pp. 1–22.

Hope, W.D., and Murphy, D.G. 1972. A taxonomic hierarchy and checklist of the genera and higher taxa of marine nematodes. Smithson. Contrib. Zool. No. 137. pp. 1–101.

Hope, W.D., and Zhang, Z. 1995. New nematodes from the Yellow Sea, *Hopperia hexadendata* n.sp., and *Cervonema deltensis* n.sp. (Chromadorida: Comesomatidae), with observations on morphology and systematics. Invertebr. Biol. **114**: 119–138.

Hulings, N., and Gray, J.S. 1971. A manual for the study of meiofauna. Smithson. Contrib. Zool. No. 78. pp. 1–83.

Hyman, L.H. 1951. The invertebrates. Vol. 2. Platyhelminthes and Rhynchocoela: the acoelomate Bilateria. McGraw Hill, New York.

Inglis, W.G. 1983. An outline classification of the phylum Nematoda. Aust. J. Zool. **31**: 243–255.

Kampfer, S., Sturmbauer, C., and Ott, J. 1998. Phylogenetic analysis of rDNA sequences from adenophorean nematodes and implications for the Adenophorea–Secernentea controversy. Invertebr. Biol. **117**: 29–36.

Larson, A. 1994. The comparison of morphological and molecular data in phylogenetic systematics. *In* Molecular ecology and evolution: approaches and applications. *Edited by* B. Schierwater, B. Streit, G.B. Wagner, and R. DeSalle. Birkhäuser Verlag, Basel. pp. 371–390.

Linstow, O. 1905. Neue Helminthen. Arch. Naturgesch. **71**: 267–276.

Litvaitis, M.K., and Rohde, K. 1999. A molecular test of platyhelminth phylogeny: inferences from partial 28S rDNA sequences. Invertebr. Biol. **118**: 42–56.

Litvaitis, M.K., Nunn, G., Thomas, W.K., and Kocher, T.D. 1994. A molecular approach for the identification of meiofaunal turbellarians. Mar. Biol. (Berlin), **120**: 437–442.

Litvaitis, M.K., Curini-Galletti, M.C., Martens, P.M., and Kocher, T.D. 1996. A reappraisal of the systematics of the Monocelididae (Platyhelminthes, Proseriata): inferences from molecular data. Mol. Phylogenet. Evol. **6**: 150–156.

Lockhart, P.J., Steele, M.A., Hendy, M.D., and Penny, D. 1994. Recovering evolutionary trees under a more realistic model of sequence evolution. Mol. Biol. Evol. **11**: 605–621.

Lorenzen, S. 1981. Entwurf eines phylogenetischen Systems der freilebenden Nematoden. Veroeff. Inst. Meeresforsch. Bremerhav. Suppl. **7**: 1–472.

Lorenzen, S. 1985. Phylogenetic aspects of pseudocoelomate evolution. *In* The origins and relationships of lower invertebrates. *Edited by* S. Conway Morris, J.D. George, R. Gibson, and H.M. Platt. Clarendon Press, Oxford. pp. 210–223.

Lorenzen, S. 1994. The phylogenetic systematics of free-living nematodes. The Ray Society, London.

Maggenti, A. 1963. Comparative morphology in nemic phylogeny. *In* The lower Metazoa: comparative biology and phylogeny. *Edited by* E.C. Dougherty. University of California Press, Berkeley. pp. 273–282.

Malakhov, V.V. 1980. Cephalorhyncha, a new type of animal kingdom uniting Priapulida, Kinorhyncha, Gordiacea and a system of Aschelminthes worms. Zool. Zh. **59**: 485–499.

Malakhov, V.V. 1994. Nematodes: structure, development, classification and phylogeny. *Edited by* W.D. Hope. Smithsonian Institution Press, Washington, D.C.

Malakhov, V.V., and Adrianov, A.V. 1995. Cephalorhyncha—a new phylum of the animal kingdom. K.M.K. Scientific Press Ltd., Moscow.

Moens, T., and Vincx, M. 1998. On the cultivation of free-living marine and estuarine nematodes. Helgol. Meeresunters. **52**: 115–139.

Nadler, S.A. 1992. Phylogeny of some ascaridoid nematodes, inferred from comparison of 18S and 28S rRNA sequences. Mol. Biol. Evol. **9**: 932–944.

Nadler, S.A. 1995. Advantages and disadvantages of molecular phylogenetics: a case study of ascaridoid nematodes. J. Nematol. **27**: 423–432.

Nixon, K.C., and Carpenter, J.M. 1994. On outgroups. Cladistics, **9**: 413–426.

Nunn, G.B. 1992. Nematode molecular evolution. An investigation of evolutionary patterns among nematodes based upon DNA sequences. Ph.D. thesis, University of Nottingham, U.K.

Nunn, G.B., Theisen, B.F., Christensen, T.B., and Arctander, P. 1996. Simplicity correlated with size growth of the D3 ribosomal RNA expansion segment in the crustacean order Isopoda. J. Mol. Evol. **42**: 211–223.

Platt, H.M. 1985. The freeliving marine nematode genus Sabatieria (Nematoda: Comesomatidae). Taxonomic revision and pictoral keys. Zool. J. Linn. Soc. **83**: 27–78.

Platt, H.M., and Warwick, R.M. 1983*a*. Free-living marine nematodes, part I. British enoplids. *In* Synopsis of the British fauna, No. 28. *Edited by* D.M. Kermack and R.S.K. Barnes. E.J. Brill/Dr. W. Backhuys Publishing, Leiden.

Platt, H.M., and Warwick, R.M. 1983*b*. Free-living marine nematodes, part II. British chromadorids. *In* Synopsis of the British fauna, No. 28. *Edited by* D.M. Kermack and R.S.K. Barnes. E.J. Brill/Dr. W. Backhuys Publishing, Leiden.

Remane, A. 1963. The systematic position and phylogeny of Pseudocoelomata. *In* The lower Metazoa. *Edited by* E.C. Dougherty. University of California Press, Berkeley. pp. 247–255.

Riemann, F. 1988. Nematoda. *In* Introduction to the study of meiofauna. *Edited by* R.P. Higgins and H. Thiel. Smithsonian Institution Press, Washington, D.C. pp. 293–299.

Saitou, N., and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**: 406–425.

Siewing, R. 1980. Das Archicoelomatenkonzept. Zool. Jahrb. Syst. **103**: 439–482.

Swofford, D. 1999. PAUP*: phylogenetic analysis using parsimony (and other methods). Version 4.0. Sinauer Associates, Sunderland, Mass.

Tarjan, A.C. 1980. An illustrated guide to the marine nematodes. Institute of Food and Agricultural Sciences, University of Florida, Gainsville.

Vanfleteren, J.R., Van de Peer, Y., Blaxter, M.L., Tweedie, S.A.R., Trotman, C., Lu, L., Van Hauwaert, M.-L., and Moens, L. 1994. Molecular genealogy of some nematode taxa as based on cytochrome c and globin amino acid sequences. Mol. Phylogenet. Evol. **3**: 92–101.

Wallace, R.L., Ricci, C., and Melone, G. 1996. A cladistic analysis of pseudocoelomate (aschelminth) morphology. Invertebr. Biol. **115**: 104–112.

Wheeler, W.C. 1990. Nucleic acid sequence phylogeny and random outgroups. Cladistics, **6**: 363–368.

Wieser, W. 1953. Die Beziehung zwischen Mundhöhlengestalt, Ernährungsweise, und Vorkommen bei freilebenden marinen Nematoden. Ark. Zool. **2**: 439–484.

Winnepenninckx, B., Backeljau, T., Mackey, L.Y., Brooks, J.M., DeWachter, R., Kumar, S., and Garey, J.R. 1995. 18S rRNA data indicate that Aschelminthes are polyphyletic in origin and consist of at least three distinct clades. Mol. Biol. Evol. **12**: 1132–1137.