

Monophyly of the Family Desmoscolecidae (Nematoda, Desmoscolecida) and Its Phylogenetic Position Inferred from 18S rDNA Sequences

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To infer the monophyletic origin and phylogenetic relationships of the order Desmoscolecida, a unique and puzzling group of mainly free-living marine nematodes, we newly determined nearly complete 18S rDNA sequences for six marine desmoscolecoid nematodes belonging to four genera (*Desmoscolex*, *Greeffiella*, *Tricoma* and *Paratricoma*). Based on the present data and those of 72 nematode species previously reported, the first molecular phylogenetic analysis focusing on Desmoscolecida was done by using neighbor joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) methods. All four resultant trees consistently and strongly supported that the family Desmoscolecidae forms a monophyletic group with very high node confidence values. The monophyletic clade of desmoscolecoid nematodes was placed as a sister group of the clade including some members of Monhysterida and Araeolaimida, *Cyartonema elegans* (Cyartonematidae) and *Terschellingia longicaudata* (Linhomoeidae) in all the analyses. However, the present phylogenetic trees do not show any direct attraction between the families Desmoscolecidae and Cyartonematidae. Within the monophyletic clade of the family Desmoscolecidae in all of the present phylogenetic trees, there were consistently observed two distinct subgroups which correspond to the subfamilies Desmoscolecinae [*Greeffiella* sp. + *Desmoscolex* sp.] and Tricominae [*Paratricoma* sp. + *Tricoma* sp.].

INTRODUCTION

Free-living marine nematodes are enormously diverse and abundant, forming a major component of marine benthic communities. Desmoscolecids are marine nematodes with a unique appearance, possessing an annulated body cuticle with additional rings of secreted and foreign material (Decraemer, 1996; Timm, 1970). Currently the order Desmoscolecida comprises about 280 described species, of which 255 are recorded from marine habitats (Decraemer and Smol, 2006; Rho et al., 2007).

The classification and phylogenetic relationships of the order Desmoscolecida based on traditional morphological characters have long been debated. Desmoscolecida was initially regarded as an isolated group due to its uncertain systematic position (Filipjev, 1918; 1921), and later considered as having affinities either with Araeolaimida (Decraemer, 1977), Monhysterida (Lorenzen, 1981) or Chromadorida (Lorenzen, 1981; 1994; Platt and Warwick, 1983). Desmoscolecida has been raised to the ordinal level by Filipjev (1929), but some researchers considered them as a taxon of the subordinal level (Chitwood, 1933; Lorenzen, 1981; 1994). Recently, De Ley and Blaxter (2002; 2004) and Decraemer and Smol (2006) placed the family Cyartonematidae (Tchesunov, 1994; type genus: *Cyartonema*) in Desmoscolecida partially following Tchesunov who pointed to their close relationship with the Meyliidae. The genus *Cyartonema* had been once placed within the family Siphonolaimidae (Monhysterida) on the basis of ovary shape or within the family Aegialolaimidae (Araeolaimida) by the pharynx structure. The overall debates on the desmoscolecoid classification scheme are summarized in Table 1.

Several molecular phylogenetic studies on the basis of 18S rDNA and/or 28S rDNA sequences have been carried out at various categorical levels within the phylum Nematoda (Aleshin et al., 1998; Blaxter et al., 1998; De Ley and Blaxter, 2002; 2004; De Ley et al., 2006; Holterman et al., 2006; 2008; Kampfer et al., 1998; Litvaitis et al., 2000; Meldal et al., 2007) in order to overcome the limits of morphology-based phylogeny. Unfortunately, only a few of these studies have included members of Desmoscolecida (De Ley and Blaxter, 2002; 2004; De Ley et al., 2006; Holterman et al., 2008), which were mainly inferred from combining data of morphological characters and DNA sequences. According to the results, Desmoscolecida appeared as a separate order within the class Chromadorea.

The exact phylogenetic relationships of Desmoscolecida are still ambiguous because of lack of DNA sequence data from desmoscolecoid species - only one 18S rDNA sequence for *Desmoscolex* sp. (EF591342, Holterman et al., 2008) and only two 28S rRNA gene sequences for *Tricoma* sp. (DQ077785,

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Table 1. Overview of changes of the classification scheme of Desmoscolecida

Chitwood and Chitwood (1950)	De Coninck (1965)	Gerlach and Riemann (1973; 1974)	Andrássy (1976)	Maggenti (1983)
Chromadorida	Desmoscolecida	Desmoscolecida	Desmoscolecida	Desmoscolecida
Chromadorina	Desmoscolecoidae	Desmoscolecidae	Desmoscolecina	Desmoscolecina
Desmoscolecoidae	Desmoscolecidae	Desmoscolecinae	Desmoscolecoidae	Desmoscolecoidae
	Desmoscolecinae	Greeffellinae	Desmoscolecidae	Desmoscolecidae
	Meyliidae	Meyliidae	Desmoscolecinae	Greeffelloidea
	Meyliinae		Tricominae	Greeffellidae
	Greeffelloidea		Greeffelloidea	
	Greeffellidae		Greeffellidae	
			Calligyridae	
			Meyliina	
			Meyloidea	
			Meyliidae	
Lorenzen (1981)	Decraemer (1985)	Lorenzen (1994)	Tchesunov (1994)	De Ley and Blaxter (2002; 2004) Decraemer and Smol (2006)
Monhysterida	Desmoscolecida	Chromadorida	Desmoscolecida	Desmoscolecida
Desmoscolecina	Desmoscolecidae	Desmoscolecina	Desmoscolecina	Desmoscolecina
Desmoscolecoidae	Tricominae	Desmoscolecoidae	Desmoscolecoidae	Desmoscolecoidae
Desmoscolecidae	Desmoscolecinae	Meyliidae	Desmoscolecidae	Desmoscolecidae
Desmoscolecinae	Meyliidae	Tricominae	Chromadorida	Desmoscolecinae
Greeffellinae		Meyliinae	Leptolaimina	Tricominae
Meyliidae		Desmoscolecidae	Meyloidea	Meyliidae
Tricominae		Desmoscolecinae	Meyliidae	Cyartonematidae
Meyliinae		Greeffellinae	Cyartonematidae	

De Ley et al., 2005; AF210426, Litvaitis et al., 2000) are available. The members of Desmoscolecida are small (usually around 300–500 µm long) and form only 1–2% of the nematofauna; therefore, they are rarely studied morphologically, and few phylogenetic analyses have been conducted on the basis of their DNA sequences. In the present study, we determined the nearly complete nucleotide sequences of 18S rDNAs from six desmoscolecoid specimens belonging to four genera (*Desmoscolex*, *Greeffella*, *Tricoma*, and *Paratricoma*) of the family Desmoscolecidae (order Desmoscolecida). The purpose of this study is to clarify currently debated classification schemes within the order Desmoscolecida and to explore monophyly of the family Desmoscolecidae and its relationships.

MATERIALS AND METHODS

Sampling of taxa

The six marine desmoscolecoid nematodes (*Desmoscolex* sp. 1, *Desmoscolex* sp. 2, *Greeffella* sp., *Tricoma* sp. 1, *Tricoma* sp. 2, and *Paratricoma* sp.) examined in this study were obtained from the washings of sublittoral or deep-sea sediments, calcareous algae, and various invertebrates such as sponges (*Callispongia elegans* and *Cliona celata*), tube-dwelling polychaetes (*Pomatoleios krausii* and *Serpula vermicularis*) and bryozoans (*Adenonella platalea* and *Thalamoporella lioticha*). Table 2 shows localities and habitats of the marine desmoscolecoid nematodes examined in this study. Samples were filtered through a 67 µm mesh sieve in the field after rinsing with freshwater for less than a minute to reduce osmotic shock (Kristensen, 1989), and then were fixed in 99% ethanol. Extraction from coarse sediments was performed by decantation and sieving method using Ludox[®] (DuPont) HS 40 (Burgess, 2001).

All specimens used in this study were identified only to the generic level due to the small numbers of specimens available for both morphological and molecular analyses. For identification to generic level, the identification keys in Decraemer and Smol (2006) were used.

DNA extraction, PCR amplification, purification, and sequencing

The samples were rinsed several times with distilled water to remove detritus particles. Next, total genomic DNA was extracted from a single individual of each species using a QIAamp tissue kit (QIAGEN Inc.) according to the manufacturer's instruction, and then used as a template for the following polymerase chain reactions (PCR).

The 18S rDNA of the desmoscolecoid nematodes was amplified by PCR using *Taq* DNA polymerase (Promega, USA). The PCR reaction was as follows: an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 54°C for 1 min and elongation at 72°C for 2 min. A final extension step at 72°C for 10 min was then performed. All species were amplified with the primer set 328/329 designed on the basis of sequences of the conserved regions at the 5'- and 3'- ends of eukaryotic homologs (Nelles et al., 1984). Detailed information on PCR and the sequencing primer sets used in this study is listed in Table 3. The PCR products were identified on 1% agarose gel, and then were purified using a GeneClean (Bio101) Nal/glass-powder Kit.

The purified PCR products were directly sequenced using universal internal primers on an ABI PRISM[®] 3100 automated DNA Analyzer using a Big Dye Terminator Cycle Sequencing Ready Kit (Applied Biosystems). All desmoscolecoid 18S rDNA sequences obtained from the present study have been depos-

Table 2. The collection sites of the marine desmoscolecoid nematodes examined by this study

Subfamily	Species	Locality and habitat
Desmoscolecinae	<i>Desmoscolex</i> sp. 1	Pohang, Gyeongsangbukdo, Korea (N 36°03'30", E 129°32'72"); subtidal various Invertebrates
	<i>Desmoscolex</i> sp. 2	Anin, Gangwondo, Korea (N 37°44'12", E 128°59'21"); subtidal various invertebrates
	<i>Greeffiella</i> sp.	Gijang, Gyeongsangnamdo, Korea (N 35°14'32", E 129°14'46"); intertidal calcareous algae
Tricominae	<i>Tricoma</i> sp. 1	Anin, Gangwondo, Korea (N 37°44'12", E 128°59'21"); subtidal various invertebrates
	<i>Tricoma</i> sp. 2	Guryongpo, Gyeongsangbukdo, Korea (N 35°59'24", E 129°33'35"); intertidal fine sediments
	<i>Paratricoma</i> sp. 1	East Sea, Korea (N 35°35'83", E 130°15'83"); deep-sea sediments (200 m deep)

Table 3. Oligonucleotide primers for PCR and sequencing used in this study

Primer name	Sequence (5'-3')	Position ^a
328 (forward)	TAC CTG GTT GAT CCT GCC AG	1-20
R (forward)	AAA GAT TAA GCC ATG CAT GT	38-57
A- (forward)	TGG AGG GCA AGT CTG GTG	556-570
A (reverse)	CTG GCA CCA GAC TTG CCC T	570-553
G- (forward)	AGA GGT GAA ATT CTT GGA	909-926
G (reverse)	TCC AAG AAT TTC ACC TCT	926-909
I- (forward)	AAA CTT AAA GGA ATT GAC GGA	1142-1162
I (reverse)	TCC GTC AAT TCC TTT AAG TTT	1162-1142
D- (forward)	TGT GAT GCC CTT AGA	1441-1455
D (reverse)	TCT AAG GGC ATC ACA	1455-1441
T (reverse)	ACC TTG TTA CGA CTT TTA	1762-1779
329 (reverse)	TAA TGA TCC TTC CGC AGG TT	1791-1810

^aNucleotide position in the *Artemia salina* 18S rDNA sequence of Nelles et al. (1984)

ited to GenBank under the accession numbers FJ460252 to FJ460257.

Sequence alignment and phylogenetic analyses of 18S rDNA sequences

The species list, related references, and GenBank accession numbers for the 78 nematode species employed in this study are summarized in Table 4. With the present data (six desmoscolecids), we retrieved 18S rDNA sequences for the 70 major nematode species from GenBank, covering all the major marine nematode orders (Araeolaimida, Chromadorida, Desmodorida, Desmoscolecida, Enoplida, and Monhysterida). *Longidorus elongatus* and *Xiphinema rivesi* (Dorylamida) were used as two outgroup taxa. In a recent study by Holterman et al. (2008), the 18S rDNA sequence of *Desmoscolex* sp. (EF591342) was excluded from their analysis because it appeared to induce a strong destabilizing effect on the tree and placed an aberrant phylogenetic position within the Tricominae clade, apart from the other *Desmoscolex* species. This sequence was also excluded in the present analysis because it could produce misleading artifacts in the phylogenetic trees. In total, 78 nearly complete 18S rDNA sequences of nematodes were initially aligned using Muscle ver. 3.7 multiple alignment program (Edgar, 2004). The alignment was further adjusted by eye using Se-Al ver.2.0a11 (Rambaut, 1996). Only well aligned, conserved alignment sites were extracted from the revised alignment using the Gblock program with the default option (Castre-

sana, 2000). The extracted alignment finally used in this study has a total of 1,177 nucleotide positions.

The neighbor-joining (NJ), maximum parsimony (MP), maximum-likelihood (ML), and Bayesian inference (BI) methods were used for the present phylogenetic analyses. An optimal sequence evolution model was selected using the MODELTEST 3.7 (Posada and Crandall, 1998): TrN + I + G was selected as an optimal model for the aligned data set under the likelihood ratio test. The detail parameters related to the selected model were as follows: Base frequencies, A (0.313317), C (0.175462), G (0.235877) and T (0.275344); substitution rates, A and C (1.00000), A and G (2.26532), A and T (1.00000), C and G (1.00000), C and T (5.00177), G and T (1.00000); proportion of invariable sites, 0.311811; shape parameter of Gamma distribution, 0.592043. The selected model and parameters were applied to the subsequent phylogenetic analyses. The NJ and MP analyses were performed with PAUP* 4.0b10 (Phylogenetic Analysis Using Parsimony) (Swofford, 2002), ML with PhyML, and BI with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Node confidence values of NJ and MP trees were assessed by bootstrap procedures with 1,000 replicates, and those of ML were obtained with 100 bootstrap replicates (BP, bootstrapping values in percent). For the BI analysis, the following options were implemented: the number of generation, 1,000,000; sample frequency, 100; the number of chains, 4; burnin generation, 10,000. To estimate the node reliability, Bayesian posterior probability values (BPP) were shown on the BI tree.

Table 4. List of species names, their classification, references, and GenBank accession numbers used in the present study. The classification scheme tentatively refers to De Ley and Blaxter (2002; 2004), De Ley et al. (2006), and Decraemer and Smol (2006).

Taxon	Reference	Accession number
Ingroups		
Phylum Nematoda		
Class Enoplea		
Subclass Enoplia		
Order Enoplida		
Family Enopliidae		
<i>Enoplus brevis</i>	Aleshin et al. (1998)	U88336
<i>Adoncholaimus fuscus</i>	Cook et al. (2005)	AY854195
Family Thoracostomopsidae		
<i>Enoploides brunettii</i>	Cook et al. (2005)	AY854193
Family Enchelidiidae		
<i>Calytronema maxweberi</i>	Cook et al. (2005)	AY854199
Family Oncholaimidae		
<i>Pontonema vulgare</i>	Aleshin et al. (1998)	AF047890
<i>Oncholaimus</i> sp.	Cook et al. (2005)	AY854196
<i>Viscosia</i> sp.	Cook et al. (2005)	AY854197
<i>Viscosia viscosia</i>	Cook et al. (2005)	AY854198
Family Anoplostomatidae		
<i>Anoplostoma</i> sp.	Cook et al. (2005)	AY854194
Family Ironidae		
<i>Syringolaimus striatocaudatus</i>	Cook et al. (2005)	AY854200
Family Tripyloididae		
<i>Tripyloides</i> sp.	Cook et al. (2005)	AY854202
<i>Bathylaimus</i> sp.	Cook et al. (2005)	AY854201
<i>Bathylaimus assimilis</i>	Meldal et al. (2007)	AJ966476
Class Chromadorea		
Subclass Chromadoria		
Order Desmoscolecida		
Family Desmoscolecidae		
Subfamily Desmoscolecinae		
Tribe Desmoscolecini		
<i>Desmoscolex</i> sp. 1	Present study	FJ460252
<i>Desmoscolex</i> sp. 2	Present study	FJ460253
Tribe Greeffiellini		
<i>Greeffiella</i> sp.	Present study	FJ460254
Subfamily Tricominae		
<i>Tricoma</i> sp.1	Present study	FJ460256
<i>Tricoma</i> sp. 2	Present study	FJ460257
<i>Paratricoma</i> sp.	Present study	FJ460255
Family Cyartonematidae		
<i>Cyartonema elegans</i>	Cook et al. (2005)	AY854203
Order Chromadorida		
Family Chromadoridae		
<i>Atrochromadora microlaima</i>	Cook et al. (2005)	AY854204
<i>Dichromadora</i> sp.	Cook et al. (2005)	AY854209
<i>Chromadora nudicapitata</i>	Cook et al. (2005)	AY854205
<i>Chromadorina germanica</i>	Cook et al. (2005)	AY854207
<i>Chromadorita tentabundum</i>	Cook et al. (2005)	AY854208
<i>Spilophorella paradoxa</i>	Cook et al. (2005)	AY854211
Family Cyatholaimidae		
<i>Cyatholaimus</i> sp.	Cook et al. (2005)	AY854213
<i>Paracanthonchus caecus</i>	Aleshin et al. (1998)	AF047888
<i>Paracyatholaimus intermedius</i>	Meldal et al. (2007)	AJ966495
<i>Praecanthonchus punctatus</i>	Cook et al. (2005)	AY854214

(continued)

Taxon	Reference	Accession number
Order Desmodorida		
Family Desmodoridae		
<i>Desmodora ovigera</i>	Kampfer et al. (1998)	Y16913
<i>Desmodora communis</i>	Cook et al. (2005)	AY854215
<i>Leptonemella</i> sp.	Kampfer et al. (1998)	Y16920
<i>Stilbonema majum</i>	Kampfer et al. (1998)	Y16922
<i>Catanema</i> sp.	Kampfer et al. (1998)	Y16912
<i>Laxus cosmopolitus</i>	Kampfer et al. (1998)	Y16918
<i>Eubostrichus dianeae</i>	Kampfer et al. (1998)	Y16915
<i>Acanthopharynx micans</i>	Kampfer et al. (1998)	Y16911
<i>Chromadoropsis vivipara</i>	Aleshin et al. (1998)	AF047891
<i>Xyzzors</i> sp.	Kampfer et al. (1998)	Y16923
<i>Metachromadora remanei</i>	Cook et al. (2005)	AY854216
<i>Robbea hypermnestra</i>	Kampfer et al. (1998)	Y16921
<i>Spirinia parasitifera</i>	Cook et al. (2005)	AY854217
Family Microlaimidae		
<i>Calomicrolaimus parahonestus</i>	Cook et al. (2005)	AY854218
<i>Calomicrolaimus</i> sp.	Cook et al. (2005)	AY854219
<i>Molgolaimus demani</i>	Cook et al. (2005)	AY854220
Family Monoposthiidae		
<i>Monoposthia costata</i>	Cook et al. (2005)	AY854221
<i>Nudora bipapillata</i>	Cook et al. (2005)	AY854222
Order Monhysterida		
Family Monhysteridae		
<i>Diplolaimella dievengatensis</i>	Meldal et al. (2007)	AJ966482
<i>Diplolaimelloides meylli</i>	Blaxter et al. (1998)	AF036611
Family Xyalidae		
<i>Daptonema setosum</i>	Meldal et al. (2007)	AY854226
<i>Daptonema hirsutum</i>	Meldal et al. (2007)	AY854223
<i>Daptonema oxycera</i>	Meldal et al. (2007)	AY854225
<i>Daptonema procerus</i>	Aleshin et al. (1998)	AF047889
<i>Daptonema normandicum</i>	Meldal et al. (2007)	AY854224
<i>Theristus acer</i>	Meldal et al. (2007)	AJ966505
Family Sphaerolaimidae		
<i>Sphaerolaimus hirsutus</i>	Cook et al. (2005)	AY854228
Family Linhomoeidae		
<i>Terschellingia longicaudata</i>	Cook et al. (2005)	AY854230
<i>Desmolaimus</i> sp. 1	Unpublished data	EF591332
<i>Desmolaimus</i> sp. 2	Unpublished data	EF591333
Order Araeolaimida		
Family Axonolaimidae		
<i>Ascolaimus elongatus</i>	Cook et al. (2005)	AY854231
<i>Axonolaimus helgolandicus</i>	Cook et al. (2005)	AY854232
<i>Odontophora rectangula</i>	Cook et al. (2005)	AY854233
Family Cyindrolaimidae		
<i>Cylindrolaimus</i> sp.	Unpublished data	AF202149
Family Comesomatidae		
<i>Sabatieria celtica</i>	Cook et al. (2005)	AY854234
<i>Sabatieria punctata</i> 343	Cook et al. (2005)	AY854237
<i>Sabatieria punctata</i> 200	Cook et al. (2005)	AY854235
<i>Sabatieria punctata</i> 223	Cook et al. (2005)	AY854236
<i>Setosabatieria hilarula</i>	Cook et al. (2005)	AY854240

(continued)

Taxon	Reference	Accession number
Order Plectida		
Family Plectidae		
<i>Plectus acuminatis</i>	Unpublished data	AF037628
<i>Anaplectus</i> sp.	Meldal et al. (2007)	AJ966473
<i>Chiloplectus</i> sp.	Meldal et al. (2007)	AJ966508
<i>Tylocephalus auriculatus</i>	Unpublished data	AF202155
<i>Wilsonema schuurmansstekhoveni</i>	Meldal et al. (2007)	AJ966513
Order Rhabditida		
Family Rhabditidae		
<i>Rhabditoides inermiformis</i>	Unpublished data	AF083017
<i>Poikilolaimus regenfussi</i>	Unpublished data	AF083022
Outgroups		
Subclass Dorylaimia		
Order Dorylaimida		
Family Longidoridae		
<i>Longidorus elongatus</i>	Blaxter et al. (1998)	AF036594
<i>Xiphinema rivesi</i>	Blaxter et al. (1998)	AF036610

RESULTS AND DISCUSSION

Phylogenetic position of Desmoscolecida inferred from 18S rDNA sequences

Nearly complete 18S rDNA sequences were determined for the six marine desmoscolecoid nematode species (*Desmoscolex* sp. 1, *Desmoscolex* sp. 2, *Greeffiella* sp., *Tricoma* sp. 1, *Tricoma* sp. 2, and *Paratricoma* sp.) in the present study. With the present data plus the 18S rDNA sequences of 72 nematodes retrieved from GenBank shown in Table 4, phylogenetic analyses were performed using four different tree reconstruction methods: NJ, MP, ML, and BI. In the present analyses, we focused on determining whether the family Desmoscolecidae is a monophyletic group or not, and exploring the phylogenetic position of the family Desmoscolecidae and the phylogenetic relationships among major nematode taxa.

All four phylogenetic trees reconstructed using the four different algorithms generally showed the same topology with minor discrepancies (Figs. 1 and 2). The resultant trees supported that the monophyly of the family Desmoscolecidae with very high node confidence values (BP 100% in NJ, MP, and ML; BPP 0.99 in BI). The monophyletic Desmoscolecidae was grouped with a clade including some members of Monhysterida and Araeolaimida in all the analyses (BP 75% in NJ, BP 58% in MP, BP 81% in ML, BPP 0.99 in BI). *Cyartonema* formed a separate group with one of the monhysterid species, *Terschellingia longicaudata* (Linhomoeidae). The orders Monhysterida and Araeolaimida appear to be paraphyletic.

Phylogenetic relationships among desmoscolecoid members inferred from 18S rDNA sequences

Within the monophyletic Desmoscolecidae, two distinct subgroups were coincidentally observed: *Greeffiella* sp. + *Desmoscolex* sp. (BP 99% in NJ, MP, and ML; BPP 0.99 in BI) and *Paratricoma* sp. + *Tricoma* sp. (BP 75–84% in NJ, MP, and ML; BPP 0.97 in BI); the two groups correspond to the subfamilies Desmoscolecinae and Tricominae, respectively (Figs. 1 and 2). This coincides with Lorenzen (1969)'s recognition of two subfamilies within the Desmoscolecidae, Desmoscolecinae and Tricominae. Decraemer (1985) and De Ley and Blaxter (2002; 2004) also adopted that both subfamilies belong to the family

Desmoscolecidae within the order Desmoscolecida as monophyletic groups.

Recently, De Ley and Blaxter (2002; 2004) designated *Cyartonema elegans* (family Cyartonematidae) as a member of Desmoscolecida due to its close relationship with the Meyliidae, a family belonging to the Desmoscolecida, as described by Tchesunov (1994). Unfortunately, it is not possible to examine if their classification is supported or not because the Meyliidae were not included in the present study. In the phylogenetic trees, *Cyartonema* was consistently placed as a sister group of *Terschellingia longicaudata* (Linhomoeidae, Monhysterida) with very strong supports (BP 99% in NJ, MP and ML, BPP 1.0 in BI) within a clade including also other members of Monhysterida and members of the Araeolaimida. This indicates that the present results do not show any attraction between the families Desmoscolecidae and Cyartonematidae. These results are in agreement with the previous molecular phylogenetic investigations of Holterman et al. (2008) and Meldal et al. (2007), and indicate that *Cyartonema* (Cyartonematidae) could be a member of the Monhysterida because it has always clustered with *Terschellingia*, a monhysterid member. However, as Decraemer and Smol (2006) mentioned, the higher classification scheme within Desmoscolecida is not fully settled down and still open for further discussion, especially pending more detailed morphological studies on the Cyartonematidae with further molecular researches on the various taxa of it. Further, the questionable position of the family Meyliidae, either more closely related to the Cyartonematidae or to the Desmoscolecidae, also needs to be investigated based on molecular data.

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A Maximum parsimony

B Neighbor joining

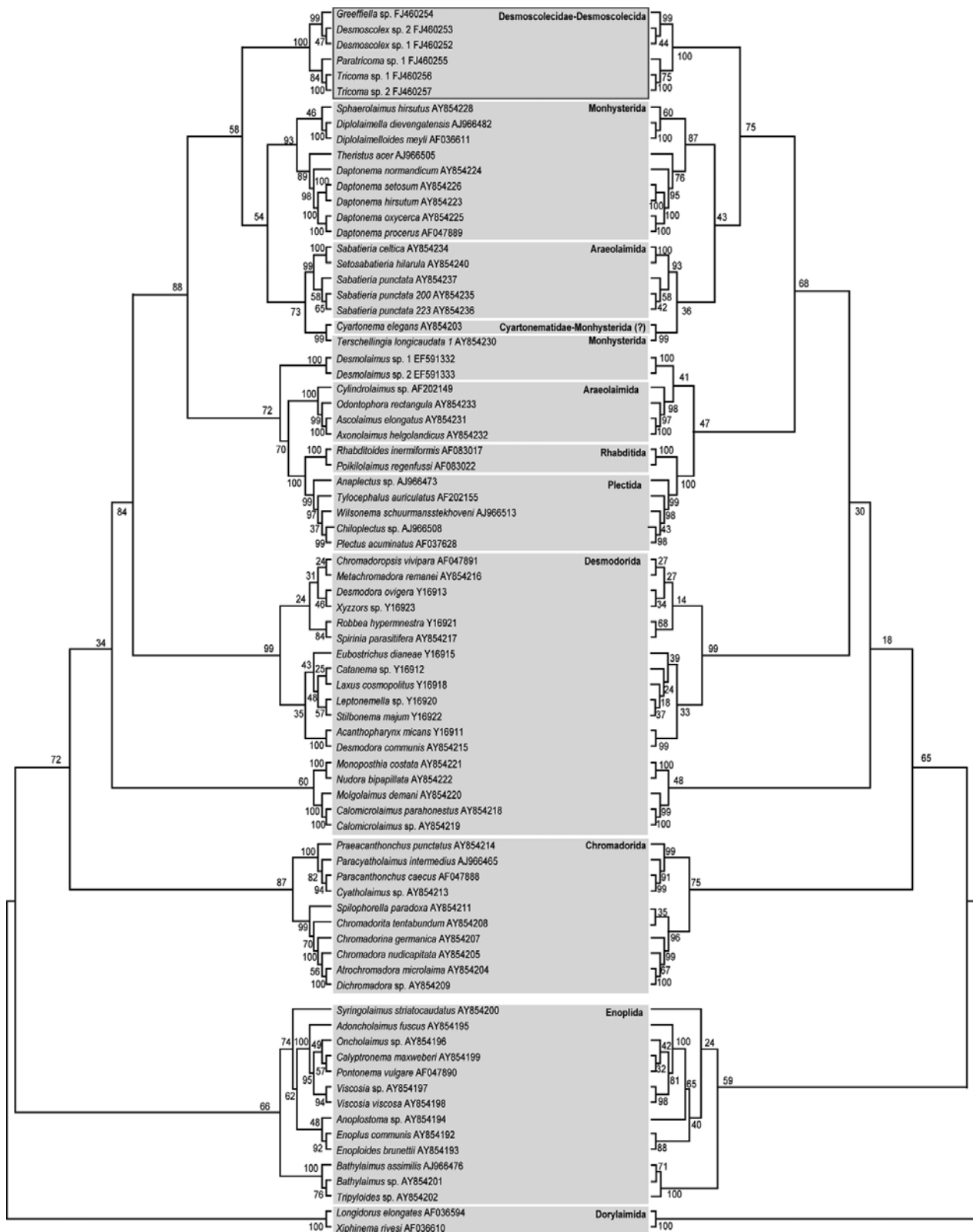


Fig. 1. Maximum parsimony and neighbor joining trees showing phylogenetic relationships among desmoscolecoid members. The numbers above or below the branches indicate bootstrapping values in percent (BP) which show node confidence values.

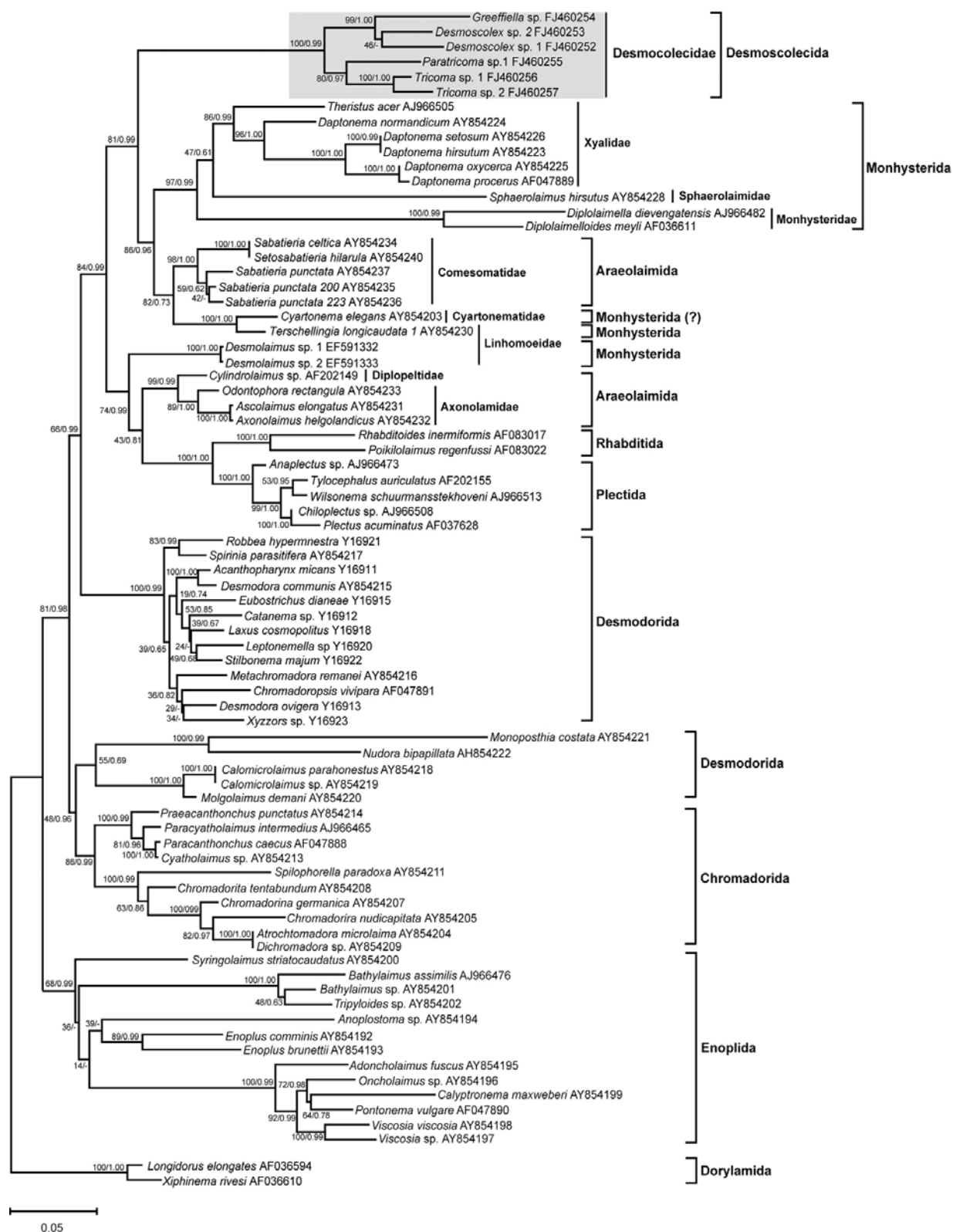


Fig. 2. Maximum likelihood tree showing phylogenetic relationships among desmoscolecoid members. The numbers above or below the branches indicate bootstrapping values in percent (BP) and Bayesian posterior probabilities (BPP) in order, which show node confidence values. Because the topology of the BI tree was very similar to that of the ML tree, only ML tree is presented here and BPP values of the BI tree are shown with BP values on each node of the ML tree. A gray box indicates the members of the family Desmoscolecidae.

writing the manuscript.

REFERENCES

- Aleshin, V.V., Kedrova, O.S., Milyutina, I.A., Vladychenskaya, N.S., and Petrov, N.B. (1998). Relationships among nematodes based on the analysis of 18S rRNA gene sequences: molecular evidence for monophyly of chromadorian and secernentian nematodes. *Russ. J. Nematol.* 6, 175-184.
- Andrássy, I. (1976). Evolution as a Basis for the Systematization of Nematodes (London: Pitman Publishing).
- Blaxter, M.L., De Ley, P., Garey, J.R., Liu, L.X., Scheldemann, P., Vierstraete, A., Vanfleteren, J.R., Mackey, L.Y., Dorris, M., Frisse, L.M., et al. (1998). A molecular evolutionary framework for the phylum Nematoda. *Nature* 392, 71-75.
- Burgess, R. (2001). An improve protocol for separating meiofauna from sediments using colloidal silica sols. *Mar. Ecol. Prog. Ser.* 214, 161-165.
- Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.* 17, 540-552.
- Chitwood, B.G. (1933). A revised classification of the Nematoda. *J. Parasitol.* 20, 131.
- Chitwood, B.G., and Chitwood, M.B. (1950). An Introduction to Nematology, 2nd ed. (Baltimore: Monumental Printing Company).
- Cobb, N.A. (1920). One hundred new nemas. *Contrib. Sci. Nematol.*, IX. (Baltimore, USA), pp. 217-343.
- Cook, A.A., Bhadury, P., Debenham, N.J., Meldal, B.H.M., Blaxter, M.L., Smerdon, G., Austen, M.C., Lamshead, P.J.D., and Rogers, A.D. (2005). Denaturing gradient gel electrophoresis (DGGE) as a tool for the identification of marine nematodes. *Mar. Ecol. Prog. Ser.* 291, 103-113.
- De Coninck, L.A.P. (1965). Systématique des Nématodes. Grassé, P.-P. Ed., *Traite de Zoologie* 4, 1-731.
- Decraemer, W. (1977). Origin and evolution of the Desmoscolecida, an aberrant group of nematodes. *J. Zool. Syst. Evol. Res.* 15, 232-236.
- Decraemer, W. (1985). Revision and phylogenetic systematics of the Desmoscolecida (Nematoda). *Hydrobiologia* 120, 259-283.
- Decraemer, W. (1996). New information on the ultrastructure of the lip region in the genus *Desmoscolex* and description of *Desmoscolex* (*Desmoscolex*) *parvospiculatus* sp. n. (Nemata: Desmoscolecida) from Papua New Guinea. *Nematologica* 42, 9-23.
- Decraemer, W., and Smol, N. (2006). Orders Chromadorida, Desmodorida and Desmoscolecida. In *Freshwater Nematodes*, E. Abebe, I. Andrássy, and W. Trautspurger, eds. (CABI Publishing), pp. 555-573.
- De Ley, P., and Blaxter, M.L. (2002). Systematic position and phylogeny. In *The Biology of Nematodes*, D. Lee, ed. (Harwood Academic Publishers), pp. 1-30.
- De Ley, P., and Blaxter, M.L. (2004). A new system for Nematoda: combining morphological characters with molecular trees, and translating clades into ranks and taxa. In *Nematology Monographs and Perspectives* 2, R. Cook, and D.J. Hunt, eds. (Brill), pp. 633-653.
- De Ley, P., De Ley, I.T., Morris, K., Abebe, E., Mundo-Ocampo, M., Yoder, M., Heras, J., Waumann, D., Rocha-Olivares, A., Jay Burr, A.H., et al. (2005). An integrated approach to fast and informative morphological voucher of nematodes for applications in molecular barcoding. *Philos. Trans. R. Soc. Lond., B Biol. Sci.* 360, 1945-1958.
- De Ley, P., Decraemer, W., and Abebe E. (2006). Introduction: summary of present knowledge and research addressing the ecology and taxonomy of freshwater nematodes. In *Freshwater Nematodes*, E. Abebe, I. Andrássy, and W. Trautspurger, eds. (CABI Publishing), pp. 3-30.
- Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32, 1792-1797.
- Filipjev, I.N. (1918). Free-living marine nematodes of the Sevastopol area. *Transactions of the Zoological Laboratory and the Sevastopol Biological Station of the Russian Academy of Sciences Series II No. 4* (Issue I and II) (Translated from Russian).
- Filipjev, I.N. (1921). Free-living marine nematodes of the Sevastopol area. *Trudy Osob. Zool. Lab. Sebastop. Biol. Sta. Series II* 4, 351-614.
- Filipjev, I.N. (1929). Les nematodes libres de la baie de la neva et de l'extrémité orientale de Golfe de Finlande. Première partie. *Arch. Hydrobiol.* 20, 637-699.
- Freudenhammer, I. (1975). Desmoscolecida aus der Iberischen Tiefsee, zugleich eine Revision dieser Nematodenordnung. "Meteor" Forschungsschiffe-Ergebnisse 20, 1-65.
- Gerlach, S.A., and Riemann, F. (1973; 1974). The Bremerhaven checklist of aquatic nematodes. A catalogue of Nematoda - Adenophorea excluding the Dorylaimida. Veröffentlichungen des Institutes für Meeresforschung in Bremerhaven, Sonderdruck 4, 1-404 (Part I., 1973), 405-734 (Part II, 1974).
- Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., Bakker, J., and Helder, J. (2006). Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Mol. Biol. Evol.* 23, 1792-1800.
- Holterman, M., Holovachov, O., van den Elsen, S., van Megen, H., Bongers, T., Bakker, J., and Helder, J. (2008). Small subunit ribosomal DNA-based phylogeny of basal Chromadoria (Nematoda) suggests that transitions from marine to terrestrial habitats (and vice versa) require relatively simple adaptations. *Mol. Phylogenet. Evol.* 48, 758-763.
- Kamper, 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.
- Kristensen, R.M. (1989). Marine Tardigrada from the southeastern United States coastal waters I. *Paradoxipus orzeliscoides* n. gen., n. sp. (Arthrotardigrada: Halechiniscidae). *Trans. Am. Micros. Soc.* 108, 262-282.
- Litvaitis, M.K., Bates, J.W., Hope, W.D., and Moens, T. (2000). Inferring a classification of the Adenophorea (Nematoda) from nucleotide sequences of the D3 expansion segment (26/28S rDNA). *Can. J. Zool.* 78, 911-922.
- Lorenzen, S. (1969). Desmoscoleciden (eine Gruppe freilebender Meeresnematoden) aus Küstensalzwiesen. *Veröff. Inst. Meeresforsch. Bremerh.* 12, 231-265.
- Lorenzen, S. (1981). Entwurf eines phylogenetischen systems der freilebenden nematoden. Veröffentlichungen des Institut für Meeresforschungen Bremerhaven, Sonderdruck 7, 1-472.
- Lorenzen, S. (1994). The phylogenetic systematics of freelifing nematodes (London: The Ray Society).
- Maggenti, A.R. (1983). Nematode higher classification as influenced by species and family concepts. In *Concepts in Nematode Systematics*. The systematics association special volume no. 22, A.R. Stone, H.M. Platt, and L.F. Khalil, eds. (London, UK: Academic Press), pp. 25-40.
- Meldal, B.H.M., Debenham, N.J., De Ley, P., De Ley, I.T., Vanfleteren, J.R., Vierstraete, A.R., Bert, W., Borgonie, G., Moens, T., Tyler, P.A., et al. (2007). An improved molecular phylogeny of the Nematoda with special emphasis on marine taxa. *Mol. Phylogenet. Evol.* 42, 622-636.
- Nelles, L., Fang, B.L., Volckaert, G., Vandenbergh, A., and De Wachter, R. (1984). Nucleotide sequence of a crustacean 18S ribosomal RNA gene and secondary structure of eukaryotic small subunit ribosomal RNAs. *Nucleic Acids Res.* 12, 8749-8768.
- Platt, H.M., and Warwick, R.M. (1983). Freelifing marine nematodes: Part I British enoplids. (Cambridge University Press).
- Posada, D., and Crandall, K.A. (1998). Modeltest: testing the model of DNA substitution. *Bioinform. Appl. Notes* 14, 817-818.
- Rambaut, A. (1996). Se-Al: Sequence Alignment Editor. Available at <http://evolve.zoo.ox.ac.uk/>.
- Rho, H.S., Kim, W., and Chang, C.Y. (2007). Description of two new free-living marine nematode species of the subgenus *Desmolorenzia* (Desmoscolecida, genus *Desmoscolex*) from Korea. *J. Nat. Hist.* 41, 313-326.
- Ronquist, F., and Huelsenbeck, J.P. (2003). MrBayes 2: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572-1574.
- Swofford, D.L. (2002). PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods). Version 4, Sinauer Associates, Sunderland, MA, USA (software program).
- Tchesunov, A.V. (1994). On the morphology and systematic position of the family Meyliidae (Nematoda: Chromadoria). *Nematologica* 40, 369-378.
- Timm, R.W. (1970). A revision of the nematode order Desmoscolecida Filipjev. (1929). *Univ. Cali. Public. Zool.* 93, 1-99.