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ABSTRACT

We reconstructed kinorhynch phylogeny using maximum-likelihood and Bayesian analyses of nuclear 18S and 28S rRNA gene sequences from 30 species in 13 genera (18S) and 23 species in 12 genera (28S), representing eight families and both orders (Cyclorhagida and Homalorhagida) currently recognized in the phylum. We analyzed the two genes individually (18S and 28S datasets) and in combination (18S+28S dataset). We detected four main clades (I–IV). Clade I consisted of family Echinoderidae. Clade II contained representatives of Zelinkaderidae, Antygomonidae, Semnoderidae, *Centroderes*, and *Condyloderes*, the latter two currently classified in Centroderidae; within Clade II, Zelinkaderidae, Antygomonidae, and Semnoderidae comprised a clade with strong nodal support. Clade III contained only two species in *Campyloderes*, also currently classified in the Centroderidae, indicating polyphyly for this family. Clades I–III, containing all representatives of Cyclorhagida included in the analysis except for *Dracoderes abei*, formed a clade with high nodal support in the 28S and 18S+28S trees. Clade IV, resolved in the 18S and 18S+28S trees with high nodal support, contained only species in order Homalorhagida, with the exception of the cyclorhagid *Dracoderes abei*. Order Cyclorhagida as it currently stands is thus polyphyletic, and order Homalorhagida paraphyletic. Our results indicate that Dracoderidae has been misplaced in Cyclorhagida based on homoplasious characters. Our analyses did not resolve the relationships among Clades I–III within Cyclorhagida. Neither gene alone nor the combined dataset resolved all nodes in trees, indicating that additional markers will be needed to reconstruct kinorhynch phylogeny.

Keywords:

Kinorhyncha; Cyclorhagida; Homalorhagida; maximum likelihood; Bayesian inference

1. Introduction

Phylum Kinorhyncha consists of microscopic (up to about 1.1 mm long) benthic marine animals primarily inhabiting the upper layer of sediment or interstices among sessile organisms such as colonial ascidians, barnacles, and algae. Kinorhynchs are distributed worldwide from equatorial to polar regions and comprise approximately 190 species (Higgins, 1988; Sørensen and Pardos, 2008; Neuhaus, 2012). The body consists of a retractable head with numerous spinous appendages (scalids), a neck with closing plates (placids), and a trunk of 11 segments (e.g., Neuhaus and Higgins, 2002; Sørensen and Pardos, 2008). Because of this unique suite of morphological features, the monophyly of the phylum has generally been accepted, and a molecular phylogenetic analysis that included eight kinorhynch species (Sørensen et al., 2008) supported monophyly. Kinorhynchs are ecdysozoans, with Priapulida being their likely sister taxon (Nebelsick, 1993; Neuhaus, 1994; Lemburg, 1995; Giribet and Ribera, 1998; Zrzavý et al., 1998; Giribet et al., 2000; Mallatt and Giribet, 2006; Dunn et al., 2008; Sørensen et al. 2008; Paps et al., 2009; Hejnol et al., 2009).

In the currently accepted classification, Kinorhyncha comprises 21 genera and nine families distributed between two orders, Cyclorhagida and Homalorhagida (Table 1). Relationships within the phylum remain incompletely understood, with specific problems including the suggestion by Sørensen et al. (2012) that Dracoderidae, currently placed in order Cyclorhagida, might actually be nested within Homalorhagida, and uncertain familial affiliations for two genera (Sørensen et al., 2007; Sørensen and Thormar, 2010). Some attempts have been made to resolve relationships at lower taxonomic levels.

G[a]Ordóñez et al. (2008) proposed a hypothesis for morphological evolution within the speciose genus *Echinoderes*, although this was not based on rigorous cladistic methodology. Sørensen (2008) conducted a cladistic analysis of 36 morphological characters to reconstruct phylogeny within Echinoderidae.

The kinorhynch classification as a whole has not been tested with a comprehensive molecular phylogenetic analysis. Sørensen et al. (2008) conducted virtually the only molecular study to date with information on intra-phylum relationships. The primary goal of that study, however, was to assess the phylogenetic position of Loricifera among other ecdysozoans, and it included only eight kinorhynch species representing three genera, three families, and both orders. In this limited taxon sampling, orders Cyclorhagida and Homalorhagida were each monophyletic, and *Centroderes* nested within a paraphyletic *Echinoderes* clade. In the present study, we used nearly full-length nuclear 18S and 28S rRNA gene sequences and a much broader taxon sampling (23–30 species representing 12 or 13 genera, eight families, and both orders) with the goals of evaluating the monophyly of higher taxa and reconstructing the relationships among them. Ultimately, a reliable, comprehensive reconstruction of kinorhynch phylogeny will be necessary to understand character-state transitions and potential homoplasies in the phylum, and eventually to understand macroevolutionary patterns. It will also be useful in classifying genera whose familal affiliation is unclear.

2. Material and Methods

2.1. Sampling and DNA sequencing

Kinorhynch specimens were collected in Japan from 2008 to 2012; Table 2 summarizes collecting data and other information for each specimen in the study, including locality, depth, sampling device, GenBank (NCBI) accession numbers, and the catalog number of the morphological voucher. Kinorhynchs were extracted from sediment samples by using the bubbling and blot method (Sørensen and Pardos, 2008) and were preserved in 99% EtOH until DNA extraction. Specimens were tentatively identified to the genus or species level by a light microscope (Olympus BX51) prior to DNA extraction. Total genomic DNA was extracted from single individuals with the DNeasy Tissue Kit (Qiagen, Tokyo), following the protocol of Cruickshank et al. (2001), modified so that specimens were incubated in lysis buffer solution for one night rather than two nights. The exoskeleton from each specimen was recovered from the lysis buffer by centrifugation and mounted on a glass slide in Hoyer's medium, as a morphological voucher. Voucher specimens were later examined in more detail by a light microscope or scanning electron microscope (SEM; Hitachi S-3000N). Eight vouchers failed to be recovered during DNA extraction (indicated by "lost" in Table 2); in these cases, other specimens from the same locality were examined to confirm the identity of the individual from which DNA had been extracted. There were no ambiguities because only one species or one species per genus occurred at the any sampling locality, and in any case lost vouchers left only one family without a physical record. The morphological vouchers have been deposited in the invertebrate collection of the Hokkaido University Museum (formerly the Zoological Institute), Hokkaido University, Sapporo, Japan (ZIHU) under catalog numbers ZIHU 4284–4296 (Table 2).

Nearly full-length sequences of the nuclear 18S (1725–1806 bp) and 28S (3227–3387 bp) rRNA genes were amplified from each specimen by PCR with primers 18S-F1 and 18S-R9 for 18S, and 28S-01 and 28Sr for the 3′ part of 28S, 28Sf and 28S-3KR for the middle part, and 28S-2KF and 28jj-3′ for the 5′ part; see Table 3 for primer references and sequences. PCR conditions were 95°C for 1 min; 35 cycles of 95°C for 30 sec, 45°C for 1 min 30 sec, and 72°C for 3 min; and 72°C for 7 min. All nucleotide sequences were determined by direct sequencing with the BigDye Terminator Kit ver. 3.1 (Life Technologies, Co., USA) and a 3730 DNA Analyzer (Life Technologies, Co., USA).

2.2. Phylogenetic analyses

Sequence fragments were assembled by using the Phred/Phrap/Consed software package (Ewing and Green, 1998; Ewing et al., 1998; Gordon et al., 1998). Assembled sequences (1725–1806 bp long for 18S; 3227–3387 bp long for 28S) were deposited in GenBank. In addition, 18S and/or 28S sequences were obtained from GenBank for the following 13 species (see Table 2 for GenBank numbers and source references): *Centroderes* sp.; *Echinoderes horni*, *E. lanceolatus*, *E. spinifurca*, *E. truncatus*; *Paracanthonchus caecus*; *Priapulus caudatus*; *Pycnophyes greenlandicus*, *Py. kielensis*, *Py.* sp. Tjärnö; *Trichinella spiralis*; *Xiphinema rivesi*; and *Zelinkaderes* sp. Among these, *Pr. caudatus* (Priapulida), and *Pa. caecus*, *T. spiralis*, and *X. rivesi* (Nematoda) were included as outgroup taxa. Construction of secondary structures and pre-alignments of sequences were performed with RNAsalsa ver. 1.4.2 (Stocsits et al., 2009). As structural constraints for RNAsalsa, the secondary structures of *Daphnia pulex* (18S;

Crease and Colbourne, 1998) and *Apis mellifera* (28S; Gillespie et al., 2006) were used.

After alignment, all sites that included gaps were deleted from the data, except for the gaps due to the shorter sequence of *Centroderes* sp. MVS 2008 (length of the sequence for *Centroderes* sp. MVS 2008 was ca. 77% of those for the others). Three datasets were prepared for phylogenetic analyses: (1) 18S sequences (18S dataset; 1547 bp long after gap removal), (2) 28S sequences (28S dataset; 2842 bp long), and (3) both 18S and 28S sequences (18S+28S dataset; 4427 bp long). Although an incongruence length difference test (ILD test) in PAUP 4.0 beta 10 (Swofford, 2002) indicated that the 18S and 28S datasets were not congruent ($P < 0.05$), the combined dataset was analyzed nonetheless, because both genes belong to the same family, are known to evolve similarly, and produce similar trees (Mallatt and Winchell, 2007). Furthermore, MrBayes (Ronquist and Huelsenbeck, 2003) allows 'separate' analyses in which a different set of model parameters is assigned to each gene partition. Homogeneity of base frequencies for each dataset was tested with a chi-square test in Kakusan4 (Tanabe, 2007). All of the tests indicated $p > 0.05$, i.e. the base composition of each dataset was a significantly homogeneity (Table 4).

The optimal substitution models for each gene were selected with Kakusan4. Table 4 lists characteristics of the datasets and the substitution models used in the analyses. Phylogenetic trees were constructed by using maximum likelihood (ML) implemented in raxmlGUI 1.2 (Stamatakis, 2006; Silvestro and Michalak, 2012), and Bayesian inference (BI) implemented in MrBayes 3.2.1 (Ronquist and Huelsenbeck, 2003). For ML trees, nodal support was assessed through analyses of 1000 bootstrap replicates. For BI, Markov-chain Monte-Carlo searches were performed with four chains, each of which

was run for 1,000,000 generations, with trees sampled every 100 generations. Stationarity was evaluated by monitoring likelihood values graphically. The initial 20% of trees from each run was discarded as burn-in, and the remaining trees were used to construct majority-rule consensus trees and determine the Bayesian posterior probability for each clade (Huelsenbeck and Ronquist, 2001). Nodal support values from the ML and BI analyses are expressed in percent in the form (ML bootstrap value / BI posterior probability), with bootstrap values less than 60% and posterior probabilities less than 95% considered nonsignificant and indicated by dashes.

3. Results and Discussion

3.1. Overall topology

The BI and ML analyses produced trees of very similar topology; hence, we show and discuss only the ML tree for each dataset, but include support values for both ML and BI near the nodes (Figs. 1–3). Minor differences between the ML and BI trees do not affect any of the conclusions presented herein. Four major kinorhynch clades (Clade I–IV) were detected, with universally high nodal support except for Clade IV. This group was not supported in the 28S ML analyses, but had high nodal support in the 18S (90/100) and 18S+28S analyses (94/100). Clades I–III correspond to order Cyclorhagida, and Clade IV to order Homalorhagida, except that the cyclorhagid *Dracoderes abei* appears in Clade IV in the 18S (Fig. 1) and 18S+28S (Fig. 3) trees. Clades I–III comprise a monophyletic group in the 28S (Fig. 2) and 18S+28S ML (Fig. 3) trees, but form an unresolved polytomy with Clade IV in the 18S (Fig. 1) tree. The 18S and

28S genes supported different aspects of the phylogeny. Among the single-gene datasets, only 18S supported Clade IV, and only 28S grouped Clades I–III. The combined 18S+28S dataset supported both Clade IV and Clade (I+II+III).

3.2. Clade I

Clade I, with high support values in all trees, contained exclusively echinoderid species. This corroborates the monophyly of Echinoderidae in Sørensen's (2008) morphology-based cladistic analysis. In that study, however, Echinoderidae appeared more closely related to Dracoderidae than to *Zelinkaderes*, while our rRNA study placed Dracoderidae far away (see above). All trees strongly supported the monophyly of *Cephalorhyncha*, whereas representatives of genus *Echinoderes* formed an unresolved polytomy in all trees. In our results, Clade I corresponded to Echinoderidae, which is currently diagnosed as having, in combination, (1) the absence of midterminal spine and (2) the neck comprised 16 placids (e.g., Sørensen and Pardos, 2008). These character states, however, are also found in (1) Clade IV and (2) Clades II and III, respectively. As far as we are aware, there is no morphological synapomorphy for Clade I.

3.3. Clades II and III

Clade II included species of Antygomonidae, Semnoderidae, Zelinkaderidae, and the centroderids *Centroderes* and *Condyloderes*, with this clade showing high nodal support in all trees. Within Clade II, Antygomonidae, Semnoderidae, and Zelinkaderidae formed a clade of their own, also with high

nodal support in all trees. None of our trees shows *Antygomonas* as monophyletic; this genus remains of uncertain status in Figs. 1, 2, due to unresolved polytomies, and it is "significantly" polyphyletic in combined-gene tree in Fig. 3. Our analyses clearly indicate polyphyly for Centroderidae, which classically was said to contain *Centroderes*, *Condyloderes*, and *Campyloderes*. In our trees (Figs. 1–3), representatives of the former two genera appear in Clade II, whereas the two species representing *Campyloderes* comprise Clade III, with strong nodal support.

Neuhaus and Sørensen (2012) summarized several morphological characters unique to *Campyloderes*, such as fused outer oral styles, internal septa in the primary scalids, and an elongated lateroventral acicular spine on segment 1. Our results indicate that these are synapomorphies for Clade III (i.e., *Campyloderes*). As to Clade II, we have no idea what can be the morphological synapomorphy for it. Representatives of Clade II share, in combination, non-fused outer oral styles and a midterminal spine. These characters are, however, also found in members of Clades I and III, respectively.

3.4. Clade IV

Clade IV, supported only in the 18S (Fig. 1) and 18S+28S (Fig. 4) trees, contained species of Dracoderidae (Cyclorhagida), Neocentrophyidae and Pycnophyidae (Homalorhagida), and an undescribed genus, with nodal support values (ML/BI) of 90/100 for 18S and 94/100 for 18S+28S. All analyses strongly supported the monophyly of Pycnophyidae. Within Pycnophyidae, *Pycnophyes* emerged as monophyletic only in the 28S (Fig. 2) tree, with low nodal support, whereas it was formed unresolved

polytomies with *Kinorhynchus yushini* in the 18S (Fig. 1) and 18S+28S (Fig. 3) trees. Unexpectedly, Clade IV also contained Dracoderidae, classically placed in order Cyclorhagida, whereas Neocentrophyidae and Pycnophyidae alone were traditionally said to comprise Homalorhagida. Sørensen et al. (2012) have previously noted, however, that species of Dracoderidae share some morphological characters with homalorhagidans, such as the scalid arrangement and alternating sizes of the outer oral styles (also found in the undisputed homalorhagidans, *Neocentrophyes* and *Paracentrophyes*).

4. Conclusions

This study, which reconstructed the phylogeny of kinorhynchs using nearly full-length 18S and 28S rRNA gene sequences, largely corroborated major features of the current classification, with some informative differences and some uncertainties due to unresolved nodes. The 28S and 18S+28S trees supported monophyly for Cyclorhagida (including Clades I–III), and the 18S and 18S+28S trees supported monophyly for Homalorhagida (Clade IV), if one accepts that Dracoderidae was formerly misplaced in Cyclorhagida due to homoplasious characters, and actually belongs in Homalorhagida. Most significantly, nodal support for the two monophyletic orders was high in the combined 18S+28S tree based on the largest number of nucleotide characters. The 28S and 18S+28S trees also showed monophyletic Cyclorhagida subdivided into three main clades (I-III), though relationships among these clades were not resolved in any of our trees: Clade I consisted of Echinoderidae; Clade II contained Zelinkaderidae, Antygomonidae, Semnoderidae, *Centroderes*, and *Condyloderes*; and Clade III comprised only *Campyloderes*.

Neither 18S, 28S, nor the combined dataset resolved all nodes at all levels in trees, suggesting that additional markers and more taxa will be necessary to fully resolve kinorhynch phylogeny. Questions highlighted by low resolution in this study that need to be addressed in the future include relationships among the three clades in Cyclorhagida; the exact phylogenetic position of Dracoderidae; and whether *Pycnophyes* and *Antygomonas* are monophyletic. Furthermore, the phylogenetic positions of taxa not included in this study, such as Cateriidae and genera of unknown affinity, await resolution.

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Figure captions

Fig. 1. Maximum-likelihood tree for kinorhynchs, based on the 18S dataset. Numbers near nodes are the bootstrap value for ML and the posterior probability for BI, respectively, in percent; support values less than 60% (ML) and 95% (BI) are indicated by a hyphen. The scale bar indicates the number of substitutions per site.

Labeling of values is as in Fig. 1.

Fig. 2. Maximum-likelihood tree for kinorhynchs, based on the 28S dataset. Labeling of values is as in Fig. 1.

Fig. 3. Maximum-likelihood tree for kinorhynchs, based on the 18S+28S dataset. Labeling of values is as in Fig. 1.

Table 1

Current classification of Kinorhyncha, based on morphological characters (Sørensen and Pardos, 2008). Asterisks indicate genera with representative species included in this study.

Order	Family	Genus
Cyclorhagida	Antygomonidae	*Antygomonas**
	Cateriidae	*Cateria*
	Centroderidae	*Campyloderes**
		*Centroderes**
		*Condyloderes**
	Dracoderidae	*Dracoderes**
	Echinoderidae	*Cephalorhyncha**
		*Echinoderes**
		Fissuroderes
		Meristoderes
		Polacanthoderes
	Semnoderidae	*Semnoderes*
		*Sphenoderes**
	Zelinkaderidae	*Triodontoderes*
		*Zelinkaderes**
	incertae sedis	*Tubulideres*
		Wollunquaderes
Homalorhagida	Neocentrophyidae	*Neocentrophyes*
		*Paracentrophyes**
	Pycnophyidae	*Kinorhynchus**
		*Pycnophyes**

Table 2

Taxa included in this study. Asterisks indicate sequences obtained from GenBank.

Taxa	Sampling site or reference		Depth (m)	Collecting device	Accession number		Catalogue number of the voucher
	Latitude	Longitude			18S	28S	
Order Cyclorhagida							
Family Antygomonidae							
Antygomonas sp. 1	32°23′17″N	129°56′1″E	77.5	SM grab	AB738338	AB738339	ZIHU 4284
Antygomonas sp. 2	32°12′31″N	128°56′45″E	385	Beam trawl	AB738340	AB738341	ZIHU 4285
Antygomonas sp. 3	26°14′10″N	127°32′22″E	47	Dredge	AB738342	AB738343	ZIHU 4286
Family Centroderidae							
Campyloderes sp. 1	26°40′31″N	127°45′20″E	124	SM grab	AB738344	AB738345	ZIHU 4287
Campyloderes sp. 2	28°29′30″N	127°59′54″E	1079	Benthos net	AB738346	AB738347	ZIHU 4288
Centroderes sp. MVS 2008	Sørensen et al. (2008)				EU669452*	—	—
Condyloderes sp.	42°56′42″N	140°02′18″E	1006	Benthos net	AB738348	AB738349	ZIHU 4289
Family Dracoderidae							
Dracoderes abei	33°54′14″N	132°09′16″E	17.2	SM grab	AB738350	AB738351	ZIHU 4290
Family Echinoderidae							
Cephalorhyncha sp. 1	43°22′39″N	145°32′9″E	15	SM grab	AB738352	AB738353	lost
Cephalorhyncha sp. 2	42°28′19″N	141°49′13″E	35	SM grab	AB738354	AB738355	lost
Echinoderes horni	Sørensen et al. (2008)				EU669453*	—	—
Echinoderes lanceolatus	—				GQ229038*	—	—
Echinoderes sp. 1	26°40′17″N	127°45′26″E	138	SM grab	AB738356	AB738357	ZIHU 4291
Echinoderes sp. 2	26°12′5″N	127°22′21″E	Intertidal	Washing sediment	AB738358	AB738359	lost

			zone				
Echinoderes sp. 3	43°00′51″N	145°19′40″E	46	SM grab	AB738360	AB738361	ZIHU 4292
Echinoderes sp. 4	33°54′14″N	132°09′16″E	17.2	SM grab	AB738362	AB738363	ZIHU 4293
Echinoderes spinifurca	Sørensen et al. (2008)				EU669455*	—	—
Echinoderes truncatus	Sørensen et al. (2008)				EU669456*	—	—
Family Semnoderidae							
Sphenoderes poseidon	26°40′17″N	127°45′26″E	138	SM grab	AB738364	AB738365	ZIHU 4294
Family Zelinkaderidae							
Zelinkaderes sp. 1	30°53′19″N	131°02′33″E	157	Dredge	AB738366	AB738367	lost
Zelinkaderes sp. JKP 2005	Park et al. (2006)				AY746985*	—	—
Order Homalorhagida							
Family Neocentrophyidae							
Paracentrophyes anurus	28°31′27″N	126°57′43″E	339	Beam trawl	AB738368	AB738369	ZIHU 4295
Family Pycnophyidae							
Kinorhynchus yushini	43°12′42″N	140°51′29″E	8	SM grab	AB738370	AB738371	lost
Pycnophyes greenlandicus	Giribet et al. (2004)				AY428820*	—	—
Pycnophyes kielensis	Aleshin et al. (1998b)				U67997*	—	—
	Petrov and Vladychenskaya (2005)				—	AY863411*	—
Pycnophyes oshoroensis	43°12′42″N	140°51′29″E	8	SM grab	AB738372	AB738373	lost
Pycnophyes sp. 1	26°40′17″N	127°45′26″E	138	SM grab	AB738374	AB738375	ZIHU 4296
Pycnophyes sp. 2	44°28′18″N	144°04′6″E	204	Benthos net	AB738376	AB738377	lost
Pycnophyes sp. Tjärnö	Mallatt and Giribet (2006)				AY859598*	AY859597*	—
Family *incertae sedis*							

Undescribed genus	30°34′12″N	130°51′54″E	55	Dredge	AB738378	AB738379	lost
OUTGROUP TAXA							
Phylum Priapulida							
Priapulus caudatus	Winnepenninckx et al. (1995)				X87984*	—	—
	Mallat et al. (2004)				—	AY210840*	—
Phylum Nematoda							
Trichinella spiralis	Aguinaldo et al. (1997)				U60231*	—	—
Paracanthonchus caecus	Aleshin et al. (1998a)				AF047888*	—	—
Xiphinema rivesi	Gutiérrez-Gutiérrez et al. (2011)				HM921344*		
	Mallatt et al. (2004)					AY210845*	

Table 3

List of PCR and cycle sequencing (CS) primers used in this study.

Marker	Primer name	Reaction	Primer sequence (in 5′-3′ direction)	Direction	Source
18S rRNA	F1	PCR & CS	TACCTGGTTGATCCTGCCAG	Forward	Yamaguchi and Endo (2003)
	R9	PCR & CS	GATCCTTCCGCAGGTTCACCTAC	Reverse	Yamaguchi and Endo (2003)
	F2	CS	CCTGAGAAACGGCTRCCACAT	Forward	Yamaguchi and Endo (2003)
	F3	CS	GYGRTCAGATACCRCCSTAGTT	Forward	Yamaguchi and Endo (2003)
	F4	CS	GGTCTGTGATGCCCTYAGATGT	Forward	Yamaguchi and Endo (2003)
	R6	CS	TYTCTCRKGCTBCCTCTCC	Reverse	Yamaguchi and Endo (2003)
	R7	CS	GYYARAACTAGGGCGGTATCTG	Reverse	Yamaguchi and Endo (2003)
	R8	CS	ACATCTRAGGGCATCACAGACC	Reverse	Yamaguchi and Endo (2003)
28S rRNA	28S-01	PCR & CS	GACTACCCCCTGAATTTAAGCAT	Forward	Kim et al. (2000)
	28Sr	PCR & CS	ACACACTCCTTAGCGGA	Reverse	Luan et al. (2005)
	28Sf	PCR & CS	TGGGACCCGAAAGATGGTG	Forward	Luan et al. (2005)
	28S-3KR	PCR & CS	CCAATCCTTTTCCCGAAGTT	Reverse	This study
	28S-2KF	PCR & CS	TTGGAATCCGCTAAGGAGTG	Forward	This study
	28jj-3′	PCR & CS	AGTAGGGTAAAACTAACCT	Reverse	Palumbi (1996)
	28S-n05R	CS	CTCACGGTACTTGTTCGCTAT	Reverse	This study
	28SR-01	CS	GACTCCTTGGTCCGTGTTTCAAG	Reverse	Kim et al. (2000)
	28S-15R	CS	CGATTAGTCTTTCGCCCCTA	Reverse	This study
	28S-3KF	CS	AGGTGAACAGCCTCTAGTCG	Forward	This study
	28v-5′	CS	AAGGTAGCCAAATGCCTCATC	Forward	Palumbi (1996)
	28S-42F	CS	GAGTTTGACTGGGGCGGTA	Forward	This study

Table 4

Characteristics of and model parameters for the 18S and 28S datasets. The numbers of variable and informative sites, and the nucleotide frequencies.

Abbreviations: NS, number of sites; NT, number of taxa; PIS, number of parsimony informative sites; VS, number of variable sites.

Dataset	NT	NS	VS	PIS	Nucleotide frequencies				Chi-square value	Analysis	Model
					A	T	G	C			
18S	34	1547	593	386	0.255	0.247	0.276	0.222	24.57	ML	GTR+G
									(*p*=1.00)	BI	SYM+G
28S	25	2842	1075	702	0.244	0.209	0.312	0.236	65.64	ML	GTR+G
									(*p*=0.69)	BI	GTR+G
18S+28S	25	4427	1608	1042	0.247	0.222	0.299	0.231	68.00	ML	GTR+G
									(*p*=0.61)	BI	SYM+G, GTR+G

Figure 1

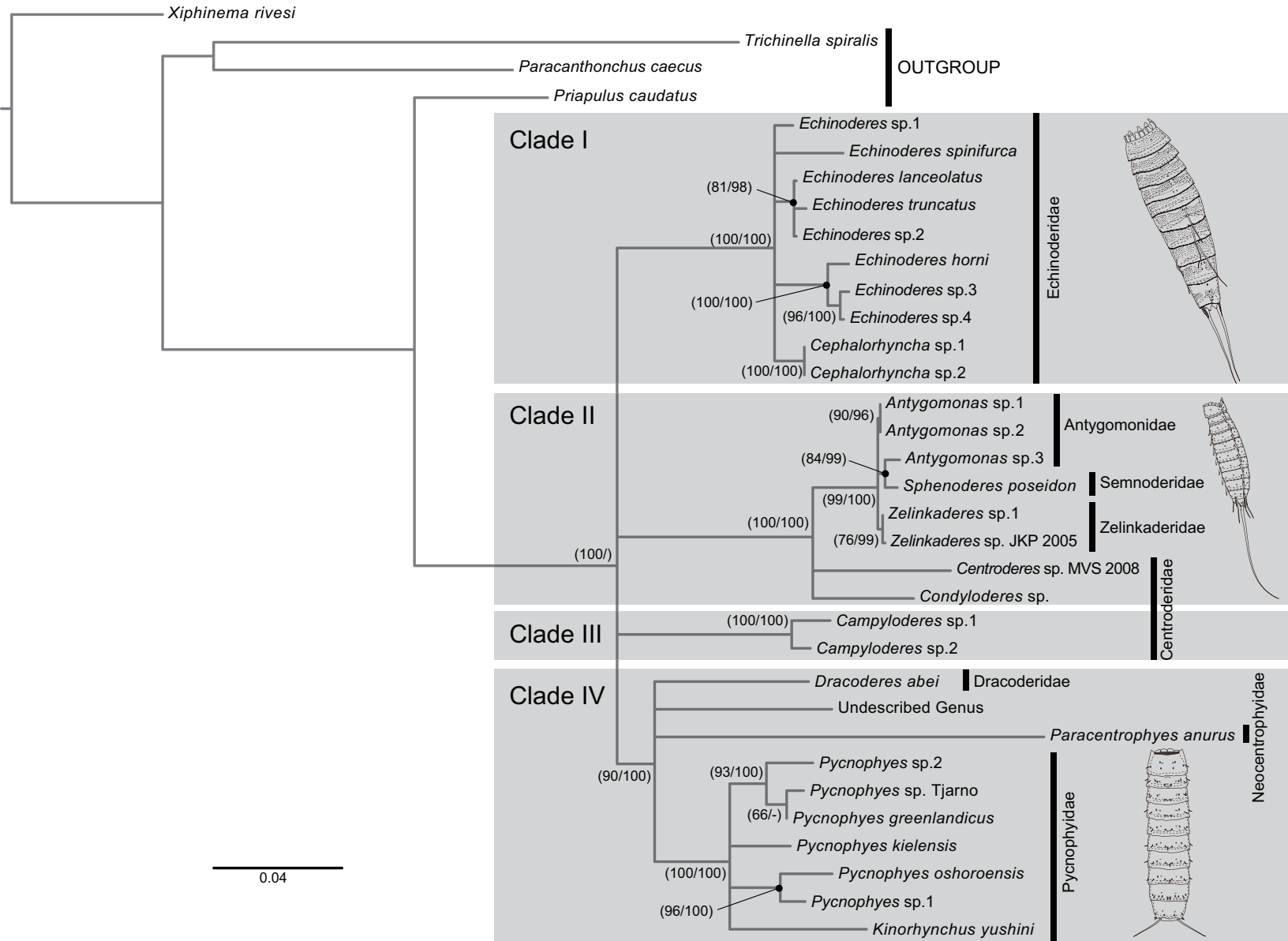

Xiphinema rivesi
Trichinella spiralis
Paracanthonchus caecus
OUTGROUP
Priapulus caudatus
Clade I
Echinoderes sp.1
Echinoderes spinifurca
Echinoderes lanceolatus
(81/98)
Echinoderes truncatus
Echinoderes sp.2
(100/100)
Echinoderes horni
Echinoderes sp.3
(100/100)
(96/100)
Echinoderes sp.4
Cephalorhyncha sp.1
(100/100)
Cephalorhyncha sp.2
Echinoderidae
Clade II
(90/96)
Antygomonas sp.1
Antygomonas sp.2
Antygomonidae
(84/99)
Antygomonas sp.3
Sphenoderes poseidon
Semnoderidae
(99/100)
Zelinkaderes sp.1
(100/100)
(76/99)
Zelinkaderes sp. JKP 2005
Zelinkaderidae
(100/)
Centroderes sp. MVS 2008
Condyloderes sp.
Centroderidae
Clade III
(100/100)
Campyloderes sp.1
Campyloderes sp.2
Clade IV
Dracoderes abei
Dracoderidae
Undescribed Genus
Paracentrophyes anurus
Neocentrophyidae
(90/100)
Pycnophyes sp.2
(93/100)
Pycnophyes sp. Tjarno
(66/-)
Pycnophyes greenlandicus
Pycnophyes kielensis
(100/100)
Pycnophyes oshoroensis
(96/100)
Pycnophyes sp.1
Kinorhynchus yushini
Pycnophyidae
0.04

Figure 2

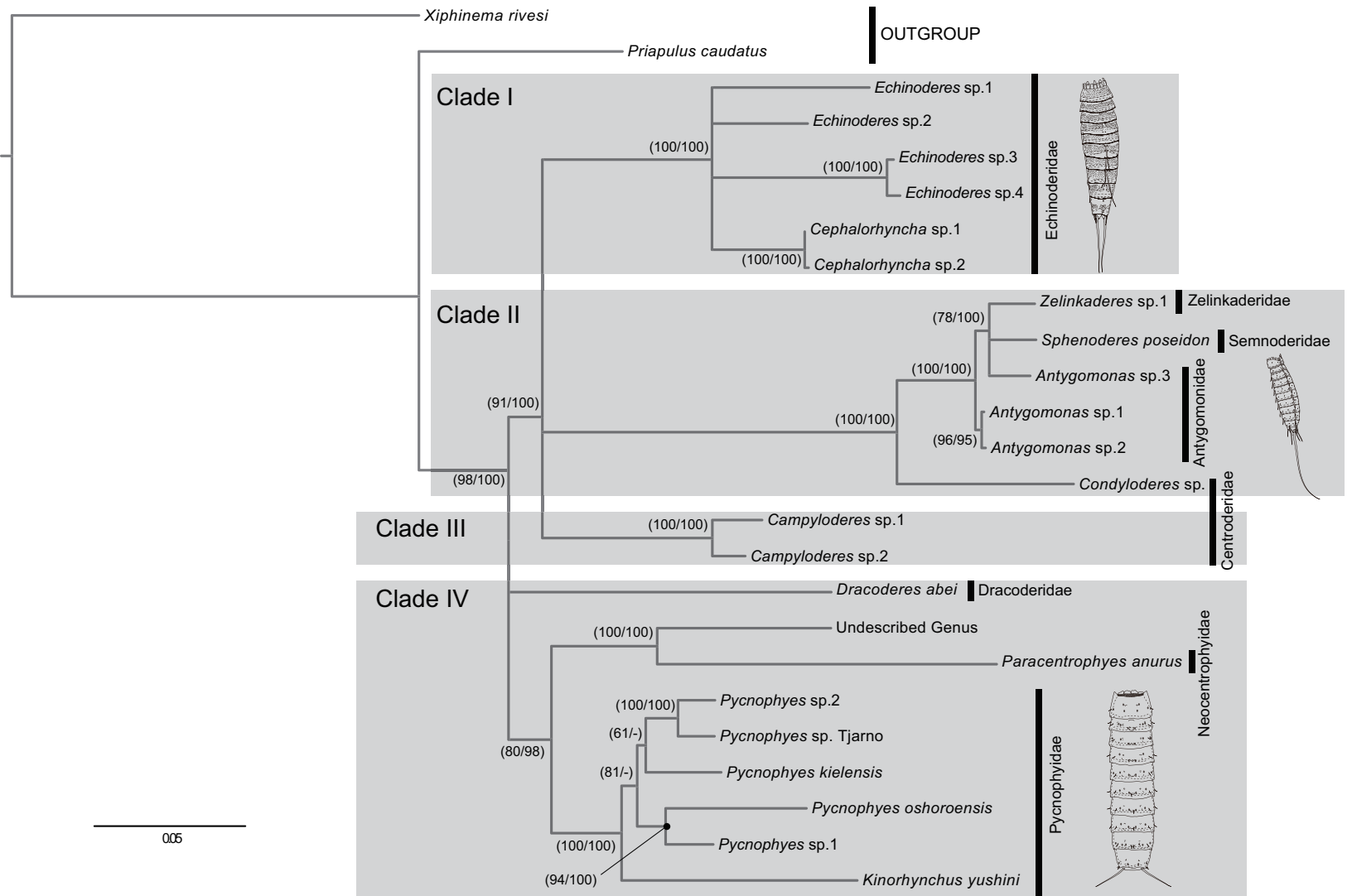
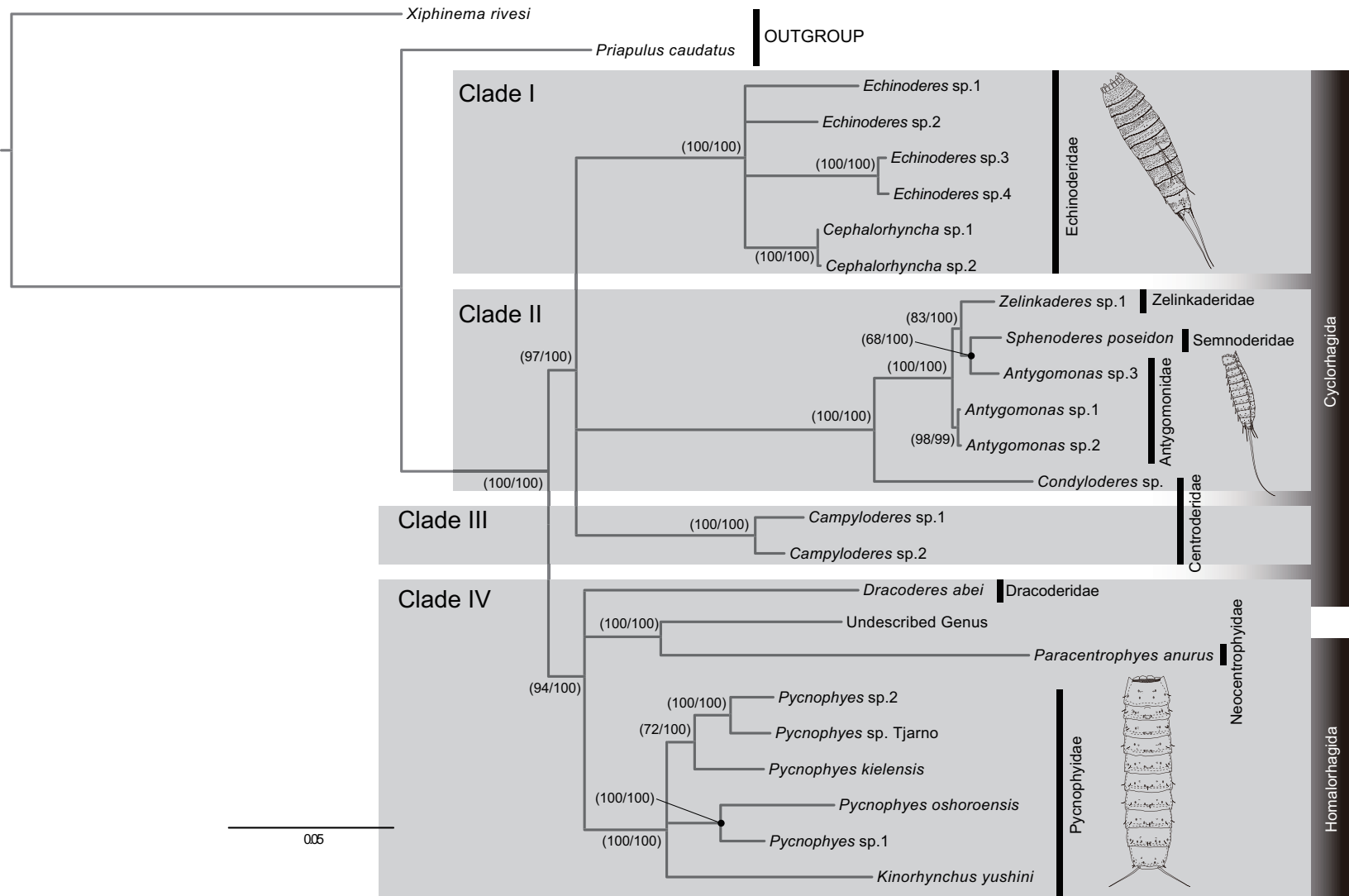

Xiphinema rivesi
Priapulus caudatus
OUTGROUP
Clade I
Echinoderes sp.1
Echinoderes sp.2
(100/100)
Echinoderes sp.3
(100/100)
Echinoderes sp.4
Cephalorhyncha sp.1
(100/100)
Cephalorhyncha sp.2
Echinoderidae
Clade II
Zelinkaderes sp.1
Zelinkaderidae
(78/100)
Sphenoderes poseidon
Semnoderidae
(100/100)
Antygomonas sp.3
(91/100)
(100/100)
Antygomonas sp.1
(96/95)
Antygomonas sp.2
Antygomonidae
(98/100)
Condyloderes sp.
Centroderidae
Clade III
(100/100)
Campyloderes sp.1
Campyloderes sp.2
Clade IV
Dracoderes abei
Dracoderidae
(100/100)
Undescribed Genus
Paracentrophyes anurus
Neocentrophyidae
(100/100)
Pycnophyes sp.2
(61/-)
Pycnophyes sp. Tjarno
(80/98)
(81/-)
Pycnophyes kielensis
Pycnophyes oshoroensis
(100/100)
Pycnophyes sp.1
(94/100)
Kinorhynchus yushini
Pycnophyidae
0.05

Figure 3


Xiphinema rivesi
Priapulus caudatus
OUTGROUP
Clade I
Echinoderes sp.1
Echinoderes sp.2
(100/100)
Echinoderes sp.3
(100/100)
Echinoderes sp.4
Cephalorhyncha sp.1
(100/100)
Cephalorhyncha sp.2
Echinoderidae
Clade II
Zelinkaderes sp.1
Zelinkaderidae
(83/100)
(68/100)
Sphenoderes poseidon
Semnoderidae
(97/100)
(100/100)
Antygomonas sp.3
(100/100)
Antygomonas sp.1
(98/99)
Antygomonas sp.2
Antygomonidae
(100/100)
Condyloderes sp.
Centroderidae
Cyclorhagida
Clade III
(100/100)
Campyloderes sp.1
Campyloderes sp.2
Clade IV
Dracoderes abei
Dracoderidae
(100/100)
Undescribed Genus
Paracentrophyes anurus
Neocentrophyidae
(94/100)
(100/100)
Pycnophyes sp.2
(72/100)
Pycnophyes sp. Tjarno
Pycnophyes kielensis
(100/100)
Pycnophyes oshoroensis
(100/100)
Pycnophyes sp.1
Kinorhynchus yushini
Pycnophyidae
Homalorhagida
0.05