

The phylogenetic placement of two enigmatic
metazoan parasites: *Polypodium hydriforme* and Myxozoa

by

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ABSTRACT

The species *Polypodium hydriforme* and the phylum Myxozoa are both intracellular fish parasites. Both parasites possess putative nematocysts, the stinging structure characteristic of all cnidarians. This morphological evidence has historically supported, with some contention, a placement of these taxa within Cnidaria. Yet, current molecular data, in the form of 18S rDNA, has challenged this placement. Repeated phylogenetic analyses of 18S rDNA data alone have revealed myxozoans and *Polypodium* to be long-branched taxa, often falling sister to one another other, but consistently grouping, with albeit weak support, as sister to Bilateria. In the following work I reevaluated the most complete available molecular data, augmenting it where possible, in an effort to drawn stronger conclusions with regards to the phylogenetic placement of these two enigmatic, parasitic, putative cnidarian taxa. Results of a more comprehensive taxon sampling of 18S rDNA support *Polypodium* as a cnidarian and myxozoans as sister to Bilateria. This suggests that previous 18S rDNA analyses of *Polypodium* suffered from artifacts of long-branch attraction. Molecular sequence variation discovered within *Polypodium* is also reported. Finally, rDNA and phylogenomic data, which support two competing molecular phylogenetic hypotheses for Myxozoa's classification, are revisited here. New analyses reveal significant conflict within phylogenomic data purported to support a cnidarian placement. Combined analyses of the most comprehensive rDNA and phylogenomic data available, weakly support the controversial placement of Myxozoa as sister to Bilateria. While this placement remains tentative, results suggest that phylogenomic studies with limited taxonomic sampling should be interpreted cautiously. Comprehensive sampling remains one of the best ways to over come artifactual placements of long-branched clades.

Chapter 1: Phylogenetic placement of the enigmatic parasite, *Polypodium hydriforme*, within the Phylum Cnidaria

INTRODUCTION

Polypodium hydriforme is an endocellular parasite whose unusual life cycle, peculiar morphology, and high rates of DNA evolution, have led to much controversy regarding its phylogenetic position within metazoans (Raikova, 1988; Siddall et al., 1995; Hanelt et al., 1996; Siddall and Whiting, 1999; Zrzavý and Hypša, 2003). *Polypodium* spends most of its life inside the oocytes of acipenseriform fishes (sturgeons and paddlefish). During this time, *Polypodium* develops from a binucleate cell into an inside-out planuliform larva and then into an elongate inside-out stolon; the epidermal cell layer is located internal to the body and the gastrodermis is located externally (Raikova, 1973; Raikova, 1980; Raikova, 1994). The embryo, larva and stolon are surrounded by a

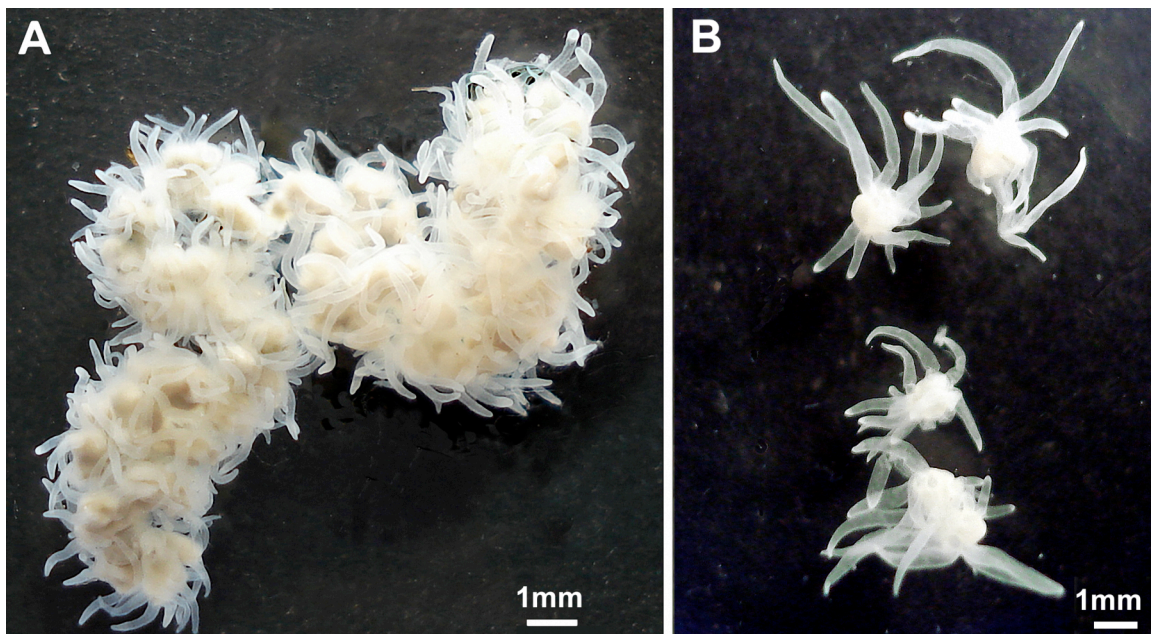


Figure 1. *Polypodium hydriforme*. A) Stolon stage just after emerging from the host oocyte. B) Four specimens of free-living *Polypodium* with 12 tentacles. Photos by E. Raikova.

protective polyploid cell, which also functions in digestion (Raikova, 1980). Just prior to host spawning, *Polypodium* everts to the normal position of cell layers, revealing tentacles scattered along the stolon. During eversion, the yolk of the host oocyte fills the gastral cavities of the parasite, supplying the future free-living stage with nutrients (Raikova, 1973; Raikova, 1980). Finally, upon emerging from the host egg in fresh water, the free-living stolon (Figure 1A) fragments into individual medusoid-like forms (Figure 1B) that go on to multiply by means of longitudinal fission, form sexual organs, and ultimately infect host fish with their gametophores (Raikova, 1973; Raikova, 1980; Raikova, 1994; Raikova et al., 1979).

Two conflicting hypotheses have been proposed regarding the phylogenetic placement of *Polypodium*. The first, more traditional, hypothesis is that *Polypodium* is a cnidarian. Some have suggested it is nested within a derived group of hydrozoans, the Narcomedusae (Hyman, 1940; Berrill, 1950; Bouillon, 1987) or the cnidarian class Scyphozoa (Lipin, 1925); while others have suggested it belongs to a separate cnidarian class, Polypodiozoa (Raikova, 1988; Bouillon et al., 2004; Bouillon et al. 2006). The assignment of *Polypodium* to Cnidaria is based primarily on morphological evidence, most notably the fact that *Polypodium* possesses nematocysts (Raikova, 1990; Ibragimov and Raikova, 2004), the stinging structures characteristic of all cnidarians. In addition, the presence of tentacles and overall body-plan organization of *Polypodium* are reminiscent of cnidarians, although it is unclear if the adult free-living stage is homologous to a polyp or medusa stage. This hypothesis is supported by a cladistic analysis of small subunit nuclear ribosomal DNA (18S rDNA) sequences in conjunction

with morphological characters (including nematocysts) (Siddall, 1995). In this study, *Polypodium* falls within the medusozoan clade of cnidarians, although the non-cnidarian placozoan, *Trichoplax* (Collins, 2002; Dellaporta et al., 2006), also fell within this clade, rendering Cnidaria paraphyletic.

The second hypothesis is that *Polypodium* is the sister taxon to Myxozoa, a diverse group of parasites in aquatic animals, and that *Polypodium* + Myxozoa is the sister group to Bilateria. This hypothesis is derived from cladistic analyses utilizing 18S rDNA sequences (Raikova, 1988, Siddall et al., 1995; Siddall and Whiting, 1999). However, because *Polypodium* and myxozoans have unusually high divergence rates in their 18S rDNA sequences, these cladistic analyses have been criticized by a number of authors that suggest that the data might be unduly affected by long-branch attraction (LBA) (Hanelt et al., 1996; Huelsenbeck, 1997; Kim et al., 1999). Despite some attempts to overcome the effects of LBA through the use of a maximum likelihood (ML) approach (Huelsenbeck, 1997; Kim et al., 1999) and pruning long branches (Hanelt et al., 1996; Kim et al., 1999), these results have been largely silent on the placement of *Polypodium*. For instance, Kim et al. (1999) applied a maximum likelihood approach to 18S rDNA sequence data and found that myxozoans and *Polypodium* did not group together. Instead, *Polypodium* was part of an unresolved polytomy that included several cnidarian lineages and *Trichoplax*, as well as myxozoans + Bilateria. Most recently, Jimenez-Guri et al. (2007) utilized multiple protein-coding gene sequences in a ML analysis and found the myxozoan, *Buddenbrockia plumatellae* nested within cnidarians. Unfortunately, this study had relatively limited sampling of cnidarians and did not include *Polypodium*.

In an attempt to resolve this controversy we greatly expanded the taxonomic sampling of cnidarian sequences for 18s rDNA and explored the influences of LBA. Using this approach, we provide evidence that *Polypodium* is nested within Cnidaria and does not group with myxozoans.

MATERIAL AND METHODS

Taxon sampling

All 132 taxa used in this study are arranged taxonomically in Table 1. 87 sequences of 18S rDNA were obtained from GenBank. 45 new cnidarian 18S rDNA sequences (including 2 from *Polypodium* taxa) were generated for this study and deposited in GenBank (see Table 1 for accession numbers). *Polypodium hydriforme* sequences were obtained from a North American and a Eurasian host (*Polyodon spathula* and *Acipenser ruthenus*, respectively). A previously published 18S *Polypodium* sequence (GenBank accession #U37526) was not included in our analyses because of concern over the quality of the sequence which included a number of ambiguities. Furthermore, while the two new *Polypodium* 18S sequences differed from each other by a total of 8 sites they differed from #U37526 by 77 and 83 sites respectively. These differences included a large number of insertions and deletions. Specific collection data for the *Polypodium* specimens used in this study are associated with each sequence submitted to GenBank (see Table 1 for accession numbers).

DNA isolation, amplification and sequencing

Genomic DNA was extracted using Qiagen DNeasy kits according to manufacturer's protocol (QIAGEN Inc., Mississauga, ON) or a standard phenol/chloroform protocol. The latter method involved tissue digestion with proteinase K (20mg/ml) in a lysis buffer (20 mM Tris-CL pH 8.0, 5 mM EDTA pH 8.0, 400 mM NaCl, 2%SDS), extraction with phenol/chloroform (1:1), precipitation with 2.5 vol. 95% EtOH, and elution in TE or H₂O.

An approximately 1.8 kb portion of the gene coding for 18S was amplified and sequenced with universal eukaryotic primers as described by Medlin et al. (1988), with the annealing temperature modified to 57°C. All gene fragments were purified and sequenced by Cogenics, Inc. (Houston, TX) and assembled and edited using Sequencher v4.5 (Gene Code Co., 2005). Sequences for each marker were aligned using the program MUSCLE (Edgar, 2004).

Phylogenetic analyses

Phylogenetic analyses were performed using both maximum likelihood (ML) and parsimony criteria. ML searches were performed using GARLI v0.951.OsX-GUI (Zwickl, 2006) under an assumed GTR model with rates estimated from the data. The assumed model of nucleotide substitution was selected by using the Akaike Information Criterion (AIC) as implemented in ModelTest (Posada and Crandall, 2000). Each run was repeated 10 times from random starting trees using default termination conditions. Each

run gave identical topologies and similar likelihood scores. 100 bootstrap replications were run in GARLI v0.951.0sX-GUI (Zwickl, 2006) under the same parameters.

To assess the effect that omitting length-variable regions has on topology, we removed these regions using the less stringent settings of Gblocks (Castresana, 2000). However, we found that removal of length-variable regions had no effect on the placement of *Polypodium* and minimal effect on overall topology in our combined ML analyses (results not shown). Therefore we performed all other analyses with the complete datasets, including the more variable regions.

Parsimony analyses were performed using TNTv.1.1 (Goloboff et al., 2003). Separate tree searches were performed with gaps coded as missing and gaps coded as a fifth state. With one exception (see results for myxozoan placement) there was no significant difference in topology. Numerous search methods available in TNT were utilized to search the tree space but the following approach was found to consistently recover trees with minimum lengths from our datasets. The implemented search was a driven new technology search with a random seed of 0 (where 0=time). Default settings for sectorial searches (RSS and CSS) and tree fusing were used (Goloboff, 1999), with 5 replicates per repetition, and a requirement that the global optimum be found 20 times. TBR branch swapping was performed on the resulting trees and a strict consensus was calculated. TNT was used to calculate standard bootstrap values (1000 replicates). Alignments and trees for 18S, 28S and combined datasets have been submitted to TreeBASE (<http://www.treebase.org/treebase/index.html>).

RESULTS

Position of Polypodium

Our 18S rDNA dataset of 132 metazoan taxa contains 3038 characters of which 1469 are parsimony informative. Both maximum likelihood (ML) and parsimony optimality criteria place *Polypodium* at the base of Bilateria (Figure 2A, 3A, 4 and 5). However, the ML topology also reflects a sister relationship between *Polypodium* and myxozoans (Figure 2A and 3A) while the parsimony topology does not (Figure 4 and 5). Moreover, under parsimony criteria the position of myxozoans is dependent upon how gaps are coded: if gaps are coded as a fifth character state, myxozoans are placed as a highly derived clade of bilaterians (Figure 4); if gaps are coded as missing, myxozoans are placed as sister to all metazoans (Figure 5). The 18S analysis showing placement of *Polypodium* with Bilateria, and more specifically as sister to myxozoans, is consistent with previously reported studies using the same marker (Siddall et al., 1995; Siddall and Whiting, 1999; Zrzavý and Hypša, 2003), but raises similar concerns of long-branch attraction (Hanelt et al., 1996).

Test of long-branch attraction

Myxozoans and *Polypodium* have unusually high rates of evolution in their 18S rDNA sequences relative to the other sampled taxa. To investigate the influence of myxozoans on the placement of *Polypodium*, we removed the myxozoans from our dataset and re-ran each analysis. Under the ML analysis of 18S rDNA, the removal of myxozoans results in

the placement of *Polypodium* nested within Cnidaria (Figure 2B and 3B). This result suggests that the placement of *Polypodium* at the base of bilaterians in the 18S analysis was indeed an artifact of LBA.

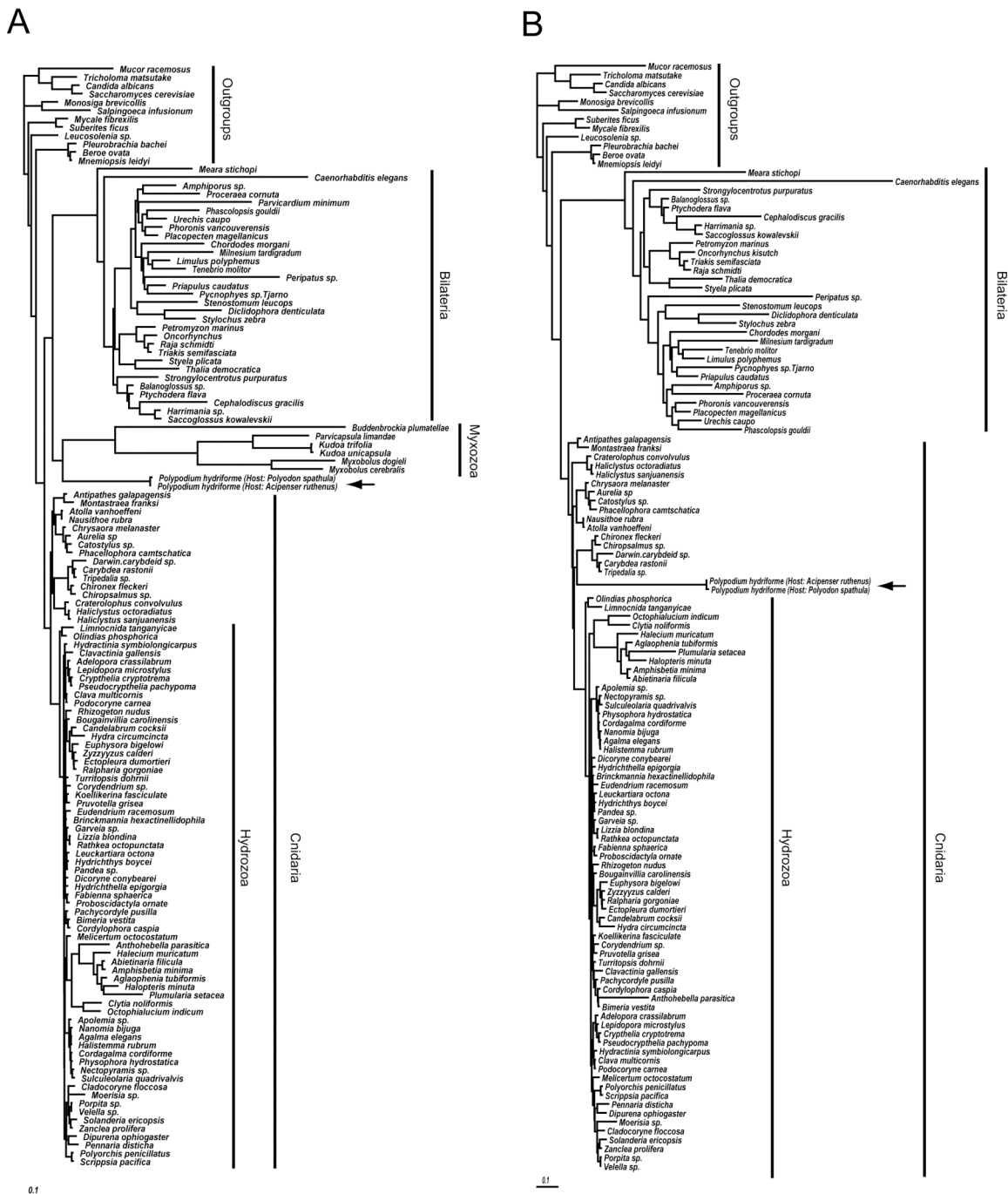


Figure 2. ML topologies of the placement of *Polypodium* within metazoan based on analyses of nearly complete 18S rDNA sequences. Arrow indicates *Polypodium* taxa. Bootstrap values for both topologies are indicated on the cladograms in Figure 3. A) 132 taxa including 6 myxozoan taxa and two *Polypodium* taxa. The assumed model (GTR+I + G) has six substitutions rates estimated from the data (A-C, 1.4071; A-G, 3.3470; A-T, 1.6901; C-G, 0.84888; C-T, 4.7638; G-T, 1.0000), an assumed proportion of invariant sites (0.1757) and a gamma shaped parameter or (0.5837). B) Same dataset as (A) but with the 6 myxozoan taxa removed. The assumed model (GTR+I + G) has six substitutions rates estimated from the data (A-C, 1.4115; A-G, 3.3559; A-T, 1.7502; C-G, 0.8342; C-T, 4.8554; G-T, 1.0000), an assumed proportion of invariant sites (0.2464) and a gamma shaped parameter or (0.6326). The length of the bar indicates 0.1 substitutions per site.



Figure 3. Cladograms, with bootstrap support values, of the ML topologies for the placement of *Polypodium* within metazoan based on analyses of nearly complete 18S rDNA sequences. Phylogram of the same analysis appears in Figure 2. Arrow indicates *Polypodium* taxa.

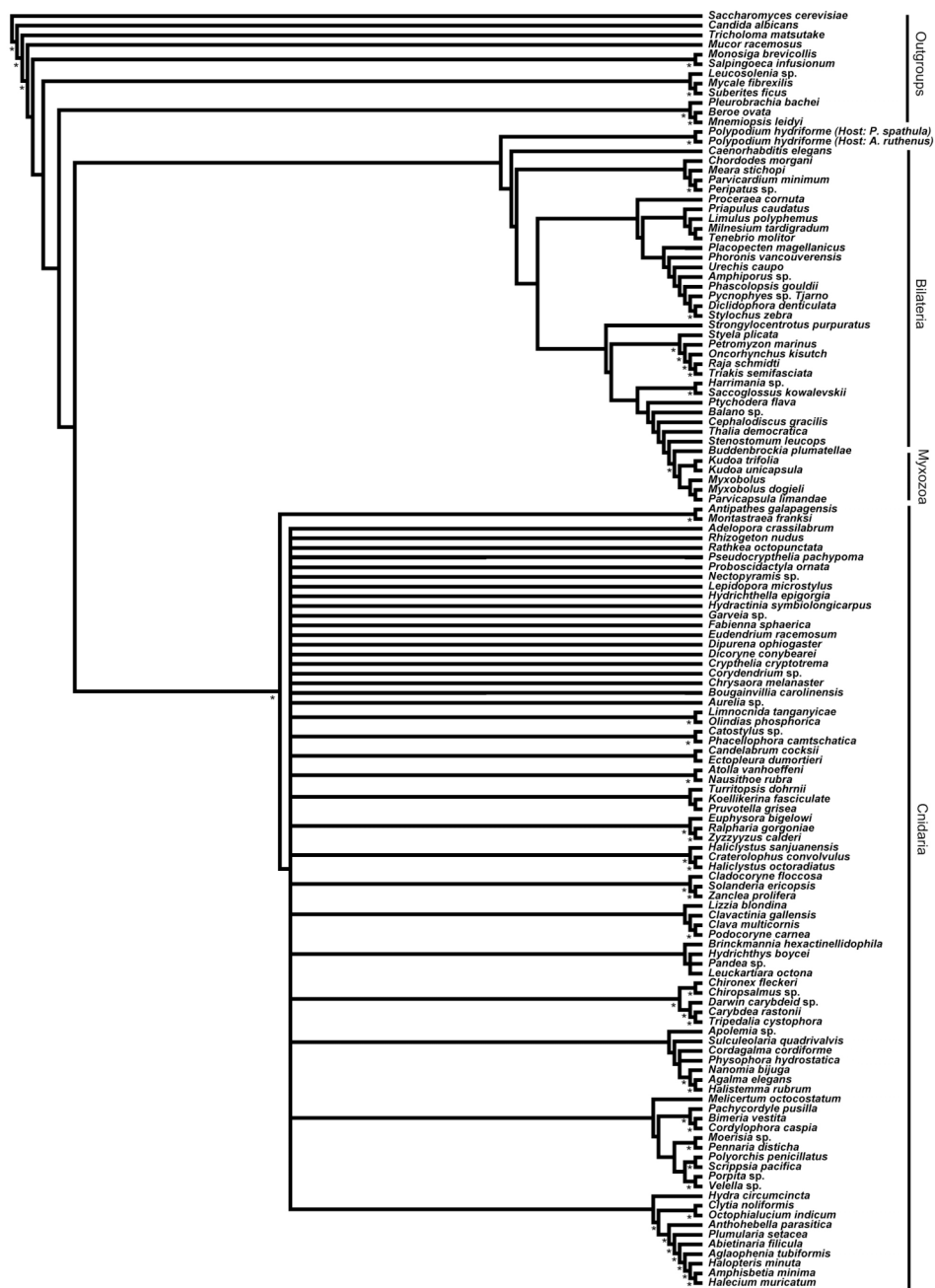


Figure 4. Parsimony topology for the placement of *Polypodium* within metazoan based on analyses of nearly complete 18S rDNA sequences, but with gaps coded as a fifth state. Figure represents a strict consensus of 120 most parsimonious trees of 17798 steps. Arrow indicates *Polypodium* taxa.

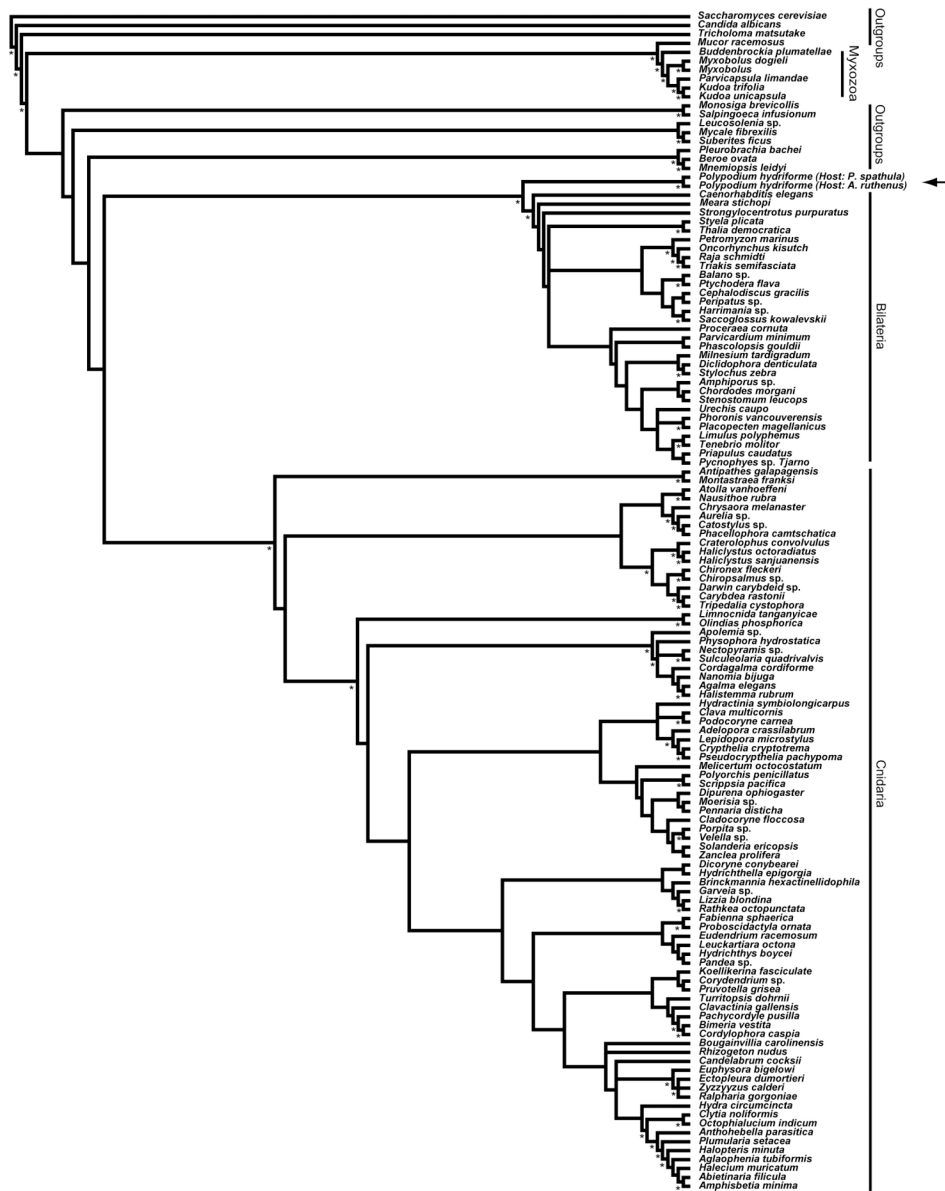


Figure 5. Parsimony topology for the placement of *Polypodium* within metazoan based on analyses of nearly complete 18S rDNA sequences, but with gaps coded as missing. . Figure represents a strict consensus of 40 most parsimonious trees of 11168 steps. Arrow indicates *Polypodium* taxa.

DISCUSSION

Effects of long-branch attraction

The well-documented effects of long-branch attraction artifacts (reviewed in Bergsten, 2005) are particularly concerning when investigating relationships amongst early-diverging metazoans, where rates between lineages vary greatly (Kim et al., 1999). Suggestions for avoiding LBA artifacts include increased taxonomic sampling to effectively break up long branches (Hillis, 1998; Zwickl, 2002), utilization of best-fit models that incorporate rate variation (Huelsenbeck, 1997; Cunningham et al., 1998; Kim et al., 1999), and identification of other long-branched taxa and exploration of their effect on the resulting topologies (Siddall and Whiting, 1999). Previous conflicting reports that show *Polypodium* and myxozoans forming a sister taxon to Bilateria (Siddall et al., 1995; Siddall and Whiting, 1999; Zrzavý and Hypša, 2003) can be explained by a combination of inadequate taxon sampling and exploration of LBA artifacts. In this study, increased taxonomic sampling of cnidarians, implementation of a model-based optimality criteria, and identification and extraction of influential long-branched taxa, myxozoans, proved critical to placing the highly divergent *Polypodium* taxon within Cnidaria.

Polypodium is a cnidarian

Through implementation of a model-based method, in the absence of the long-branched myxozoan taxa our 18S rDNA metazoan dataset, with a large taxonomic sample of cnidarians, places *Polypodium* within a monophyletic Cnidaria. This is in accord with the fact that *Polypodium* possesses nematocysts (Raikova, 1990; Ibragimov and Raikova, 2004) and a cnidarian-like body plan (Hyman, 1940; Raikova et al., 1979; Raikova, 1980; Raikova, 1994). The precise placement of *Polypodium* within Cnidaria remains less

certain, and will likely require the development of new molecular markers for this enigmatic taxon.

Evolution of Polypodium life-history characters

Although the fresh water habitat of *Polypodium* is unusual for cnidarians, it is not unheard of, especially within hydrozoans. For instance, the model organism *Hydra* and the jellyfish *Craspedacusta* are both exclusively fresh-water hydrozoans. *Hydra* and *Craspedacusta* are distantly related (Collins et al. 2006) and our analyses do not indicate a close phylogenetic affinity of *Polypodium* to either of the clades containing these taxa. Thus, it appears that in the evolution of cnidarians, invasion to fresh-water habitats has happened at least three separate times.

Although *Polypodium* is the only known intracellular cnidarian parasite, other cnidarians have adopted parasitic life-styles. For example, parasites belonging to the Narcomedusae (Hydrozoa) have been reported to live in the stomach cavities of other narcomedusae (Bouillon, 1987; Osborn, 2000; Pagès et al., 2007) and anthomedusae (Pagès et al., 2007). In addition, the sea anemone *Edwardsiella lineata* parasitizes the stomach cavity of the ctenophore *Mnemiopsis leidyi* (Bumann and Puls, 1996) and the anemone *Peachia quinquecapitata* is reported to parasitize the stomachs of hydromedusa (Spaulding, 1972).

Polypodium and Myxozoa

Our analyses are inconclusive in the placement of Myxozoa within metazoans. We found that myxozoans consistently grouped with long-branched taxa and that removal of long-

branches resulted in myxozoans being placed to the next longest branch. For example myxozoans group with *Polypodium* in the absence of Bilateria and group with Bilateria in the absence of *Polypodium* (not shown).

Myxozoans are a highly diverse group (reviewed in Kent et al., 2001) that comprise two clades, the Myxosporea and the Malacosporea (Canning et al. 2000). Jimenez-Guri et al. (2007) sampled the malacosporean, *Buddenbrockia*, and found it to fall within Cnidaria, as the sister group to two hydrozoan representatives and a single scyphozoan. Previous studies have suggested a sister group relationship between cnidarians and myxozoans (Siddall et al., 1995; Siddall and Whiting, 1999; Zrzavý and Hypša, 2003), and some morphological evidence has been used to support this view (Siddall, 1995; Raikova, 2005). Although our present study sampled both myxosporeans and the malacosporeans it does not support a relationship with Cnidaria. Future studies with a comprehensive sampling of myxozoans together with *Polypodium*, in a dataset that includes a large taxonomic sampling of cnidarians, should shed further light on the relationships between myxozoans, and *Polypodium*.

CONCLUDING REMARKS

Although previous molecular phylogenetic hypotheses conflicted with the traditional interpretation of cnidarian affinity for *Polypodium*, the molecular evidence we present, using an augmented dataset, confirms and reconciles this traditional hypothesis and suggests that *Polypodium* is indeed a cnidarian. This study also reaffirms the importance of large taxonomic sampling and thorough exploration of long-branch attraction artifacts.

Table 1. A complete list of 18S rDNA sequences used for phylogenetic analyses of *Polypodium hydriforme*. Bold numbers indicate new sequences generated for this study. KUMIP = University of Kansas Museum of Invertebrate Paleontology, KUNHM = University of Kansas Natural History Museum, MHNG = Muséum d'histoire naturelle de Genève, YPM = Yale Peabody Museum, USNM = US National Museum of Natural History.

Higher classification	Taxon ID	18S Accession numbers	Voucher
Bilateria			
Annelida	<i>Proceraea cornuta</i>	AF212179	
Annelida	<i>Urechis caupo</i>	AF342805	
Arthropoda	<i>Limulus polyphemus</i>	U91490	
Arthropoda	<i>Tenebrio sp./Tenebrio molitor</i>	X07801	
Brachiopoda	<i>Phoronis vancouverensis</i>	U12648	
Chordata	<i>Oncorhynchus sp./O. kisutch</i>	AF030250	
Chordata	<i>Petromyzon marinus</i>	M97575.1	
Chordata	<i>Raja schmidti</i>	AF278682	
Chordata	<i>Triakis semifasciata</i>	AF212180	
Echinodermata	<i>Strongylocentrotus purpuratus</i>	L28056.	
Hemichordata	<i>Cephalodiscus gracilis</i>	AF236798	
Hemichordata	<i>Harrimania sp.</i>	AF236799	
Hemichordata	<i>Ptychodera flava</i>	AF278681	
Hemichordata	<i>Ptychoderidae</i>	D14359	
Hemichordata	<i>Saccoglossus kowalevskii</i>	L28054	
Kinorhyncha	<i>Pycnophyes sp. Tjarno</i>	AY859598	
Mollusca	<i>Parvicardium minimum</i>	DQ279942	
Mollusca	<i>Placopecten magellanicus</i>	X53899	
Nematoda	<i>Caenorhabditis elegans</i>	X03680	
Nematomorpha	<i>Chordodes morgani</i>	AF036639	
Nemertea	<i>Amphiporus sp.</i>	AF119077	
Nemertodermatida	<i>Meara stichopi</i>	AF119085	
Onychophora	<i>Peripatus sp.</i>	AY210837	
Platyhelminthes	<i>Diclidophora denticulata</i>	AJ228779	
Platyhelminthes	<i>Stenostomum leucops</i>	D85095	
Platyhelminthes	<i>Stylochus zebra</i>	AF342801	
Priapulida	<i>Priapulus caudatus</i>	Z38009	
Sipuncula	<i>Phascolopsis gouldii</i>	AF342796	
Tardigrada	<i>Milnesium.sp\M. tardigradum</i>	U49909	
Urochordata	<i>Styela plicata</i>	L12444	
Urochordata	<i>Thalia democratica</i>	D14366	
Cnidaria			
Polypodiozoa	<i>Polypodium (Host: Acipenser ruthenus)</i>	EU272630	
Polypodiozoa	<i>Polypodium (Host: Polyodon spathula)</i>	EU272629	
Anthozoa, Antipatharia	<i>Antipathes galapagensis</i>	AF100943	
Anthozoa, Scleractinia	<i>Montastraea franksi</i>	AY026382	
Cubozoa, Carybdeidae	<i>Carybdea rastonii</i>	AF358108	
Cubozoa, Carybdeidae	<i>Darwin carybdeid sp.</i>	AF358105	
Cubozoa, Carybdeidae	<i>Tripedalia cystophora</i>	EU272637	
Cubozoa, Chirodropidae	<i>Chironex fleckeri</i>	AF358104	
Cubozoa, Chirodropidae	<i>Chiropsalmus sp</i>	AF358103	
Hydrozoa, Capitata	<i>Dipurena ophiogaster</i>	EU272615	KUNHM 2803
Hydrozoa, Capitata	<i>Ectopleura dumortieri</i>	EU272616	
Hydrozoa, Capitata	<i>Euphysora bigelowi</i>	EU272618	KUNHM 2829
Hydrozoa, Capitata	<i>Moerisia sp.</i>	AF358083	
Hydrozoa, Capitata	<i>Pennaria disticha</i>	AY920762	
Hydrozoa, Capitata	<i>Polyorchis penicillatus</i>	AF358090	
Hydrozoa, Capitata	<i>Porpita sp</i>	AF358086	
Hydrozoa, Capitata	<i>Ralpharia gorgoniae</i>	EU272633	KUNHM 2778
Hydrozoa, Capitata	<i>Scrippisia pacifica</i>	AF358091	
Hydrozoa, Capitata	<i>Solanderia ericopsis</i>	EU272636	MHNG INVE29593
Hydrozoa, Capitata	<i>Veella sp.</i>	AF358087	
Hydrozoa, Capitata	<i>Zanclaea prolifera</i>	EU272639	KUNHM 2793
Hydrozoa, Capitata	<i>Zyzyzus warreni</i>	EU272640	KUNHM 2777
Hydrozoa, Capitata	<i>Candelabrum cocksii</i>	AY920758	MHNG INVE29531
Hydrozoa, Capitata	<i>Cladocoryne floccosa</i>	EU272608	
Hydrozoa, Filifera	<i>Bimeria vestita</i>	EU272605	
Hydrozoa, Filifera	<i>Bougainvillia carolinensis</i>	EU272606	

Hydrozoa, Filifera	<i>Brinckmannia hexactinellidophila</i>	EU272607	MHNG INVE38148
Hydrozoa, Filifera	<i>Clava multicornis</i>	EU272609	
Hydrozoa, Filifera	<i>Clavactinia gallensis</i>	EU272610	MHNG INVE33470
Hydrozoa, Filifera	<i>Cordylophora caspia</i>	EU272612	
Hydrozoa, Filifera	<i>Corydendrium</i> sp.	EU272613	KUNHM 2764
Hydrozoa, Filifera	<i>Dicoryne conybearei</i>	EU272614	MHNG INVE32949
Hydrozoa, Filifera	<i>Eudendrium racemosum</i>	EU272617	
Hydrozoa, Filifera	<i>Fabienna sphaerica</i>	AY920767	
Hydrozoa, Filifera	<i>Garveia annulata/Garveia</i> sp.	AY920766	KUNHM 2860
Hydrozoa, Filifera	<i>Hydra circumcincta</i>	AF358080	
Hydrozoa, Filifera	<i>Hydractinia symbiolongicarpus</i>	EU272621	
Hydrozoa, Filifera	<i>Hydrichthella epigorgia</i>	EU272622	KUNHM 2665
Hydrozoa, Filifera	<i>Hydrichthys boycei</i>	EU305496	MHNG INVE37417
Hydrozoa, Filifera	<i>Koellikerina fasciculata</i>	EU272623	
Hydrozoa, Filifera	<i>Leuckartiara octona</i>	EU272624	
Hydrozoa, Filifera	<i>Lizzia blondina</i>	EU272625	
Hydrozoa, Filifera	<i>Pachycordyle pusilla</i>	EU272627	MHNG INVE32953
Hydrozoa, Filifera	<i>Pandea</i> sp.	AY920765	
Hydrozoa, Filifera	<i>Podocoryne carnea</i>	AF358092	
Hydrozoa, Filifera	<i>Proboscidactyla ornata</i>	EU272631	KUNHM 2767
Hydrozoa, Filifera	<i>Pruvotella grisea</i>	EU272632	MHNG INVE34436
Hydrozoa, Filifera	<i>Rathkea octopunctata</i>	EU272634	KUMIP 314321
Hydrozoa, Filifera	<i>Rhizogeton nudus</i>	EU272635	MHNG INVE35757
Hydrozoa, Filifera	<i>Turritopsis dohrnii</i>	EU272638	MHNG INVE29753
Hydrozoa, Leptothecata	<i>Abietinaria filicula</i>	EU272600	MHNG INVE29947
Hydrozoa, Leptothecata	<i>Aglaophenia tubiformis</i>	EU272601	MHNG INVE29967
Hydrozoa, Leptothecata	<i>Amphisbetia minima</i>	EU272602	MHNG INVE25071
Hydrozoa, Leptothecata	<i>Anthohebella parasitica</i>	EU272603	MHNG INVE29762
Hydrozoa, Leptothecata	<i>Clytia noliformis</i>	EU272611	
Hydrozoa, Leptothecata	<i>Halecium muricatum</i>	EU272619	MHNG INVE29028
Hydrozoa, Leptothecata	<i>Halopteris minuta</i>	EU272620	MHNG INVE25073
Hydrozoa, Leptothecata	<i>Melicertum octocostatum</i>	AY920757	USNM 1073342
Hydrozoa, Leptothecata	<i>Octophialucium indicum</i>	EU272626	MHNG INVE29970
Hydrozoa, Leptothecata	<i>Plumularia setacea</i>	EU272628	MHNG INVE36298
Hydrozoa, Siphonophorae	<i>Agalma elegans</i>	AY937313	YPM 35029
Hydrozoa, Siphonophorae	<i>Apolesia</i> sp.	AY937331	YPM 35090
Hydrozoa, Siphonophorae	<i>Cordagalma cordiforme</i>	AY937317	YPM 35032
Hydrozoa, Siphonophorae	<i>Halistemma rubrum</i>	AY937358	YPM 35359
Hydrozoa, Siphonophorae	<i>Nanomia bijuga</i>	AY937338	YPM 35043
Hydrozoa, Siphonophorae	<i>Nectopyramis</i> sp.	AF358068	
Hydrozoa, Siphonophorae	<i>Physophora hydrostatica</i>	AY937342	YPM 35046
Hydrozoa, Siphonophorae	<i>Sulculeolaria quadrivalvis</i>	AY937353	YPM 35357
Hydrozoa, Stylasteridae	<i>Crypthelia cryptotrema</i>	EU272641	USNM1027758
Hydrozoa, Stylasteridae	<i>Lepidopora microstylus</i>	EU272644	USNM1027724
Hydrozoa, Stylasteridae	<i>Pseudocrypthelia pachypoma</i>	EU272643	USNM1027728
Hydrozoa, Stylasteridae	<i>Adelopora crassilabrum</i>	EU272642	USNM1027760
Hydrozoa, Trachylina	<i>Limnocyclus tanganyicae</i>	AY920755	
Hydrozoa, Trachylina	<i>Olindias phosphorica</i>	AY920753	MHNG INVE29811
Scyphozoa, Coronatae	<i>Atolla vanhoeffeni</i>	AF100942	
Scyphozoa, Coronatae	<i>Nausithoe rubra</i>	AF358095	
Scyphozoa, Rhizostomea	<i>Catostylus</i> sp.	AF358100	
Scyphozoa, Semaestomeae	<i>Chrysaora melanaster</i>	AF358099	
Scyphozoa, Semaestomeae	<i>Aurelia</i> sp.	EU272604	
Scyphozoa, Semaestomeae	<i>Phacellophora camtschatica</i>	AF358096	
Staurozoa, Stauromedusae	<i>Craterolophus convolvulus</i>	AY845344	
Staurozoa, Stauromedusae	<i>Haliclystus octoradiatus</i>	AY845346	
Staurozoa, Stauromedusae	<i>Haliclystus sanjuanensis</i>	AF358102	
Myxozoa			
Malacosporea	<i>Buddenbrockia plumatellae</i>	AJ937883	
Myxosporea	<i>Kudoa trifolia</i>	AM183300	
Myxosporea	<i>Kudoa unicapsula</i>	AM490334	
Myxosporea	<i>Myxobolus cerebri</i>	EF370481	
Myxosporea	<i>Myxobolus dogieli</i>	EU003978	
Myxosporea	<i>Parvicapsula limandae</i>	EF429096	
Outgroups			
Choanoflagellida			
Codonosigidae	<i>Monosiga brevicollis</i>	AF084618	
Salpingoecidae	<i>Salpingoeca infusionum</i>	AF100941	
Ctenophora,			
Cyclocoela	<i>Beroe ovata</i>	AF293694	

Cyclocoela	<i>Mnemiopsis leidyi</i>	AF293700
Typhlocoela	<i>Pleurobrachia bachei</i>	AF293677
Fungi		
Ascomycota	<i>Candida albicans</i>	X53497
Ascomycota	<i>Saccharomyces cerevisiae</i>	M27607
Basidiomycota	<i>Tricholoma matsutake</i>	U62538.1
Mucoromycotina	<i>Mucor racemosus</i>	AJ271061
Porifera,		
Calcarea	<i>Leucosolenia sp.</i>	AF100945
Demospongia	<i>Mycale fibrexilis</i>	AF100946
Demospongia	<i>Suberites ficus</i>	AF100947

Chapter 2: Molecular variation within *Polypodium hydriforme*

INTRODUCTION

Polypodium hydriforme is currently described as a single species yet it infects the oocytes of at least 14 of 27 extant acipenseriform fishes throughout the Holarctic region (Raikova 1979, Evans et. al. 2008). In North America, *Polypodium* thus far is found to infect four separate species of sturgeon and the single species of North American paddlefish (reviewed in Raikova, 2002, Evans et al., 2008). Given that *Polypodium* is not as intensively studied in North America and Europe, as in Russia, it is likely that other species are infected but have not yet been discovered.

An excellent fossil record of Acipenseriformes supports an ancient radiation of these host fishes (Bemis and Kynard, 1997). Acipenseriformes are thought to have originated in the Triassic (ca. 200Ma) of Western Europe (Bemis and Kynard, 1997, Vadim and DeSalle, 1998). The 27 extant species of acipenseriforms are distributed throughout Europe, Asia, and North America. There are likely several major geological events that drove the diversification of this group (Bemis and Kynard, 1997, Vadim and DeSalle, 1998). In the Jurassic, during the breakup of Pangea (approx. 150 Ma), there was an initial separation of the North American and Eurasian species, and is thought to initiate the divergence of Acipenseridae (sturgeon) and Polyodontidae (paddlefish) (Bemis and Kynard, 1997, Berg, 1948). In the early Cenozoic (approx 50Ma), a faunal interchange occurred between North America and Europe that may have affected acipenseriform evolution (Bemis and Kynard, 1997, Vadim and DeSalle, 1998). In the last 5 million years, a series

of climatic changes led to glaciation events that formed the major North American river drainage systems that we have today. Pleistocene glaciation events within North American are consistent with the biographic distribution of some of the North American sturgeon species (Brown et al., 1992) and molecular studies suggest that North American sturgeon are divided into groups corresponding to their geographical ranges (Krieger et al., 2000).

The ancient divergences of the host clade, and the apparent isolation of many of these fish lineages raises a number of questions regarding the distribution of variation within *Polypodium* populations, including what it suggests about the evolution and/or spread of this parasite between acipenseriform fishes, and whether or not the genus is in fact monotypic. Raikova (1979), remains the only close comparison of *Polypodium* specimens from disparate localities and hosts, yet this study found “no significant difference in macro- or micromorphology” between North American specimens from the host *Polyodon spathula* and those from the Eurasian *Acipenser ruthenus*. Current molecular techniques provide the possibility to detect variation not reflected in morphology. With this in mind we made an effort at collecting molecular data from as many localities and hosts as possible.

The work presented below provides informative yet preliminary results. Recovering the samples necessary to test a number of evolutionary scenarios proved to be outside the scope of the time necessary to complete this degree. Instead, we present our preliminary findings in the hopes that future work will be able to draw on this effort.

MOLECULAR METHOD

Genomic DNA was extracted using Qiagen DNeasy kits according to manufacturer's protocol (QIAGEN Inc., Mississauga, ON) or a standard phenol/chloroform protocol. The latter method involved tissue digestion with proteinase K (20mg/ml) in a lysis buffer (20 mM Tris-CL pH 8.0, 5 mM EDTA pH 8.0, 400 mM NaCl, 2%SDS), extraction with phenol/chloroform (1:1), precipitation with 2.5 vol. 95% EtOH, and elution in TE or H₂O.

An approximately 1.8 kb portion of the gene coding for 18S was amplified and sequenced with universal eukaryotic primers as described by Medlin et al. (1988) with the annealing temperature modified to 57°C. All gene fragments were purified and sequenced by Cogenics, Inc. (Houston, TX) and assembled and edited using Sequencher v4.5 (Gene Code Co., 2005).

SAMPLING, RESULTS AND COMMENTS

Localities, host identity and molecular results for each of 8 sampled specimens appear listed in Figure 6. Of the 13,680 bps of 18S sequence data that overlap for all 8 samples a total of 8 sites provide variation that distinctly defines just two haplotypes (Figure 6b). We find just one of two haplotypes of 18S rDNA in each of the eight *Polypodium* samples listed below. Preliminary considerations of the distribution of these haplotypes provides no clear picture or pattern capable of strongly supporting the origin of this

variation. However, we note that samples from other biogeographic regions, particularly from hosts of Pacific regions (eastern or western) could be critical in determining whether, for example, different populations of *Polypodium* became isolated as the distribution of acipenserform fishes changed and new species arose.

Figure 6. Locality, Hosts, and 18S rDNA Haplotype Diversity for 8 *Polypodium hydriforme* Samples.

Lab ID	Host Species	Host's Common Name	Locality	18S Haplotype (based on variation at 8 of 13,680 bps)
EVR34RL	<i>Acipenser ruthenus</i>	Sterlet	Russia: North Dvina River	Haplotype A
EVR35	<i>Acipenser ruthenus</i>	Sterlet	Russia: Volga River	Haplotype A
EVR01b	<i>Acipenser gueldenstaedti</i>	Russian sturgeon	Russia: Volga River	Haplotype A
POLY023	<i>Acipenser fulvescens</i>	Lake sturgeon	North America: USA: Wisconsin: Wolf River (western shore of Lake Michigan)	Haplotype A
POLY018	<i>Scaphirhynchus platyrhynchus</i>	Shovelnose	North America: USA: Louisiana: between the Mississippi and Atchafalaya Rivers	Haplotype B
POLY019	<i>Scaphirhynchus platyrhynchus</i>	Shovelnose	North America: USA: Louisiana: between the Mississippi and Atchafalaya Rivers	Haplotype B
PF13	<i>Polydon spathula</i>	Paddlefish	North America: USA: Mississippi: Sardis Lake	Haplotype B
POLY040	<i>Polydon spathula</i>	Paddlefish	North America: USA: Oklahoma: Grand Lake	Haplotype B

Chapter 3: Phylogenetic Placement of Myxozoa: An exploration of conflict between phylogenomic and traditional molecular data

INTRODUCTION

Myxozoa is a diverse phylum comprised exclusively of microscopic obligate endoparasites, several of which can cause serious disease in a number of economically important fish (Kent et al., 2001). Yet, despite the attention myxozoans have received, higher-level classification has proven difficult due to an overall paucity of morphological characters and, more recently, the discovery of this clade's highly divergent molecular sequences. Historically, myxozoans were thought to be protists (class Myxosporia Butschli, 1881), but a number of described putative metazoan characteristics led many to question this classification (for review see Siddall et al., 1995). Included among these characteristics were multicellularity and the presence of polar capsules, structures bearing remarkable similarity to the stinging nematocysts of cnidarians (Štolc 1899; Weill 1938). Nevertheless classification of Myxozoa was not widely reconsidered until after phylogenetic analyses of 18S ribosomal DNA (rDNA) confirmed an origin derived within Metazoa (Smothers et al., 1994), with some work suggesting a cnidarian affinity (Siddall et al., 1995; Siddall and Whiting, 1999).

While debate developed around this new view of Myxozoa, analyses of 18S rDNA proved invaluable at resolving relationships within myxozoans. This included important confirmation that most myxozoans likely parasitize both vertebrate (usually fish) and invertebrate hosts, and that specimens once thought to belong to entirely different classes were in fact different stages of the same life-cycle (Andree et al., 1997).

Additionally a clear picture emerged of two divergent myxozoan classes. The more specious Myxosporea (1,350+ spp.) primarily utilizes aquatic oligochaetes as invertebrate hosts and forms two well defined clades, one of predominantly marine taxa, the other freshwater (Kent et al., 2001; Fiala, 2006). The second class, Malacosporea, was found to exclusively infect bryozoans as its invertebrate host but includes just two species, of which one, *Buddenbrockia plumatallae*, possesses a peculiar vermiform, worm-like stage complete with four longitudinal muscle blocks (Canning et al., 2002; Okamura et al., 2002; Monteiro et al., 2003; Canning and Okamura, 2004).

The very formation of this “worm” by *Buddenbrockia* has livened the debate of Myxozoa’s placement within Metazoa. Though its development clearly does not include gastrulation and speculation exists that components of its muscles are of host origin (Morris and Adams, 2007), this vermiform stage appears to some more bilaterian-like in form (Canning and Okamura, 2004). Conversely, *Buddenbrockia* clearly possesses polar capsules, which provide compelling evidence for a shared ancestry with Cnidaria. Produced by all myxozoans, polar capsules are complex intracellular structures comprised of an inverted tubule that, through eversion, is used for host attachment (Kent 2001). Parallels can easily be drawn between the development, morphology and function of polar capsules and nematocysts, the stinging structures characteristic of all Cnidaria (for a review see Siddal et al., 1995; and Cannon and Wagner, 2003). Finally, this debate elicits broader interest given that accurate phylogenetic placement of Myxozoa should not only prove critical in assessing the homology of polar capsules and nematocysts, but also provide insight for reconstructing the evolutionary history of these complex

organelles. However, given the aberrant morphology of myxozoans, it is clear that such interpretations will require a robust molecular phylogeny.

Nevertheless, numerous molecular phylogenetic analyses have so far failed to convincingly resolve this controversy. Instead, there exists two conflicting hypotheses for the relative placement of Myxozoa within Metazoa. Of these, the most recently advanced posits Myxozoa as a highly derived, long-branched, cnidarian clade, likely within Medusozoa (Myxozoa+Medusozoa). This hypothesis is supported by a phylogenomic investigation of *Buddenbrockia* (Jiménez-Guri et al., 2007). This study analyzed an amino acid matrix of 129 protein-coding genes for 60 opisthokont taxa. Though Bayesian inference results did appear robust (97% posterior probability for a *Buddenbrockia*+Medusozoa relationship), 74% of the data was missing for *Buddenbrockia*. In addition, maximum likelihood analyses recovered only 70% bootstrap support for this node and a statistical topological test failed to reject a number of alternative placements, including a basal bilaterian origin of *Buddenbrockia*. This later placement (Myxozoa+Bilateria) is supported by numerous, more traditional phylogenetic analyses that have examined increasingly taxon rich whole 18S rDNA datasets (Siddall et al., 1995; Hanelt et al., 1996; Zrzavý and Hypša, 2003; Evans et al., 2008; but see Siddall and Whiting, 1999). While both current and historic competing hypotheses were made clear by Jiménez-Guri et al. (2007), exploration of the conflict between the authors' phylogenomic results and existing 18S data was outside the purview of their study. As a result, examination of this conflict is still warranted and presents a unique opportunity not only to investigate the placement of Myxozoa, but to explore apparent discord between phylogenomic amino acid data and more traditional rDNA sequence data.

In this study we address the competing molecular hypotheses of myxozoan placement; the Myxozoa+Bilateria relationship, which is supported by studies employing comprehensive taxon sampling using a single marker, the 18S rDNA (see above) versus the Myxozoa+Medusozoa relationship, supported by a phylogenomic approach employing limited taxon sampling of numerous molecular markers (Jiménez-Guri et al., 2007). We examine both forms of molecular data to address and explore the effects of a number of potential biases and artifacts inherent to each dataset. For the 18S dataset we add an additional marker, compiling a matrix of combined 18S and 28S rDNA sequences with a comprehensive sampling of Metazoa, extensive coverage of Cnidaria, and two samples from each of the three major myxozoan clades. For the phylogenomic dataset we maximize the relative coverage for *Buddenbrockia* by selectively trimming the aligned, concatenated, amino acid matrix of Jiménez-Guri et al. (2007), which also substantially minimizes the degree of missing characters for most taxa. Finally, we combine both forms of data using both the limited sampling of Jiménez-Guri et al. (2007) and our more comprehensive metazoan sampling. We explore conflict and support for both hypotheses within and between these datasets.

MATERIAL AND METHODS

Data assembly and Taxon sampling

We assembled three separate datasets that were analyzed both individually and combined. For the first, we modified the published phylogenomic matrix of Jiménez-Guri et al. (2007) (the study of Jiménez-Guri et al., 2007 is herein referred to as JG07), which was

kindly provided by the authors. This dataset was developed for investigating the placement of *Buddenbrockia* and is comprised of 129 protein-coding genes, 60 operational taxonomic units (OTUs), and 29,773 unambiguously aligned amino acid (AA) positions. However, a number of OTUs had a substantial level of missing data, the most significant being *Buddenbrockia* with 74% of its characters missing. For our study we trimmed JG07's phylogenomic AA dataset to 7,776 aligned AA positions representing only the sequences for which near complete coverage of *Buddenbrockia* exists. This trimmed AA dataset also has significantly less missing data for well over half the taxa (Table 2).

Our second dataset is comprised of 18S and 28S rDNA sequences that mirror the taxon sampling of JG07. In an effort to maximize data coverage JG07 created chimerical associations for 24 of their 60 OTUs. By consulting the composition of these OTUs, we selected the best available representative 18S and 28S rDNA sequences making an effort to minimize any additional chimerical associations. For 6 taxa though there was no available 28S rDNA data. The resulting 60 OTUs used in our study are taxonomically arranged in Table 2. Final compositions of chimeric associations (an additional 13 were created in this study) can be found in Table 3.

Our third dataset is comprised of 389 taxa, representing a significantly more comprehensive taxonomic sampling of Metazoa than the first two datasets. Data was assembled by choosing, where possible, at least three taxa per Class from the most complete sequence data available in GenBank for 18S (> 1.6 kb) and 28S (> 2.0 kb)

rDNA (Table 4). Partial or missing data was allowed for a select number of key taxa and when it was necessary to match those taxa for which phylogenomic AA data was also examined (see above). Moreover, our dataset includes 6 myxozoan species, including the malacosporeans *Buddenbrockia plumatallae* and *Tetracapsuloides bryosalmonae*, and two species for both the marine and freshwater clades of Myxosporea. Accomplishing this required that we generate near complete 28S rDNA sequences for 5 myxozoans, as well as two near complete 18S rDNA sequences. In addition to this, we included a previously published *Buddenbrockia plumatallae* 18S rDNA sequence from a specimen both geographically and genetically distinct from that of the specimen we sequenced. Collection information for myxozoan samples examined in this study can be found associated with the proper accession numbers on GenBank (Table 4).

DNA isolation, amplification and sequencing

Phylogenomic DNA was isolated by using either a standard phenol/chloroform extraction protocol as previously reported (Evans et al., 2008) or through digestion of tissue in a DNA lysis buffer containing proteinase K incubated overnight at 37°C followed by 2 minutes at 94°C (modified from Gleason et al., 2004).

An approximately 1.8 kb portion of the gene coding for 18S was amplified and sequenced with universal eukaryotic primers and conditions as described by Medlin et al. (1988) but with an annealing temperature of 57°C. Amplification of near complete 28S sequence (~3 kb) was accomplished with an approach modified from that reported in

Table 2. Summary of 60 Operational Taxonomic Units (OTUs) and corresponding molecular data. Chimerical OTUs are listed by the lowest approximate taxonomic level representative of taxa composition within and between AA and rDNA sequence data; †designates chimerical OTUs of Jiménez-Guri et al. 2007(JG07), * designates additional chimerical OTUs created in this study with the addition of rDNA. See Supplemental Table 1 for specific chimerical compositions. Relative coverage of phylogenomic AA data for both our trimmed matrix and the original JG07 matrix appears below, with relative increases greater than 10% in bold.

OTU designations	18S rDNA GB# (seq. length - bps)	28S rDNA GB# (seq. length - bps)	% coverage in our trimmed matrix (7,776 AA)	% coverage in JG07's matrix (29,773 AA)
Fungi				
<i>Batrachochytrium dendrobatidis</i>	AH009052 (1634)	AY546693 (2943)	94.4	95
<i>Blastocladiella emersonii</i>	AY635842 (1882)	DQ273808 (3198)	97.2	83
<i>Cryptococcus*</i>	EF363152 (1862)	L14067 (3392)	99.6	98
<i>Glomus†</i>	DQ322630 (1791)	DQ273828 (3266)	46.1	32
<i>Rhizopus*</i>	AB250174 (1757)	DQ273817 (3219)	97.8	99
<i>Spizellomyces punctatus</i>	AY546684 (2620)	AY546692 (3799)	83.4	53
<i>Ustilago*</i>	DQ846895 (2111)	DQ094784 (1399)	98.2	98
Ichthyosporea				
<i>Capsaspora owczarzaki</i>	AY363957 (1777)	AY724688 (3414)	84.1	57
<i>Amoebidium parasiticum</i>	Y19155 (1797)	EU011932 (3206)	51.2	32
<i>Sphaeroforma arctica</i>	Y16260 (1728)	missing	85.8	62
Choanoflagellida				
<i>Monosiga brevicollis</i>	AF100940 (1796)	AY026374 (2981)	99.1	98
<i>Monosiga ovata</i>	AF084230 (1765)	EU011940 (3557)	93.6	76
<i>Proterospongia*</i>	AY149896 (1770)	EU011941 (3319)	52.9	34
Porifera				
Plakinidae*	AF100948 (1813)	AY561870 (1006)	48.7	49
Chalinidae*	AY734444 (1983)	AY561862 (1039)	98.1	98
<i>Suberites domuncula</i>	AJ620112 (1738)	AJ620113 (3039)	75.3	36
Cnidaria				
<i>Cyanea*</i>	AF358097 (1832)	missing	52.5	25
<i>Hydra†</i>	AF358080 (1822)	AY026371 (3297)	99.4	100
Hydractiniidae†	EU272621 (1696)	EU272568 (3250)	88.7	64
<i>Nematostella vectensis</i>	AF254382 (1723)	AY345871 (1018)	99.7	99
Scleractinia†	AY026382 (1838)	AY026375 (3162)	91.5	66
Myxozoa				
<i>Buddenbrockia plumatellae</i>	FJ981824 (1734)	FJ981817 (3393)	98.9	26
Deuterostomia				
<i>Ciona intestinalis</i>	AB013017 (1756)	AF212177 (3326)	98.3	96
<i>Ciona savignyi</i>	AF165823 (1093)	missing	99.4	99
<i>Danio rerio</i>	BX537263 (2029)	BX537263 (3990)	98.6	99
Myxinidae†	M97572 (1959)	AF061797 (4850)	97.5	76
<i>Gallus gallus</i>	FM165414 (1809)	FM165415 (1119)	90.7	89
Mammalia†	NR_003287 (1871)	NR_003287 (5035)	100	100
Stolidobranchia†	L12426 (1804)	missing	97.1	97
<i>Petromyzon marinus</i>	M97575 (1785)	AF061798 (3607)	95.6	89
<i>Xenopus†</i>	X02995 (1825)	X02995 (4082)	100	99
Ecdysozoa				
Araneae†	DQ639775 (1695)	DQ639859 (1764)	75	54
<i>Apis mellifera</i>	AB126807 (1727)	AJ302936 (2748)	96.7	97
<i>Bombyx mori</i>	Q347470 (1907)	AY038991 (1192)	99.5	97
<i>Rhipicephalus†</i>	AF018656 (1747)	AF200189 (749)	90.4	89
<i>Daphnia†</i>	AM490278 (2341)	AF346515 (3850)	99.4	99
Astacidea†	AY743945 (1758)	AY859581 (4005)	82.8	57
Eutardigrada†	EU266922 (1702)	AY210826 (3555)	77.4	46
<i>Ixodes†</i>	Z74479 (1813)	AF200190 (683)	99.1	97
Penaeidae†	DQ079766 (1781)	DQ079809 (1453)	83.8	63
Orthopteroidea†	AF370793 (1860)	EF685941 (623)	89.5	69
Chalcidoidea*	AY491051 (1889)	AY623514 (964)	93.6	93
Noctuoidea*	EU057177 (1904)	missing	95.6	76
<i>Tribolium castaneum</i>	AJ878603 (1159)	EU677678 (1028)	100	100
<i>Xiphinema*</i>	AM086673 (1753)	AY210845 (3378)	79.4	61
Lophotrochozoa				
Pectinidae†	L11265 (1815)	AY145391 (3403)	82.4	45
Gastropoda†	U65224 (1845)	AF435694 (1439)	97.7	93
<i>Capitella*</i>	U67323 (1740)	AY364863 (1885)	92.5	92
<i>Crassostrea†</i>	AB064942 (1820)	AB102757 (3761)	81.1	57
<i>Dugesia†</i>	AF050433 (1774)	DQ665968 (1575)	85.7	82
<i>Echinococcus†</i>	U27015 (2394)	AY615426 (604)	71.1	51
Sepiolidae*	AY557472 (2359)	missing	73.5	62
<i>Fasciola hepatica</i>	AJ004969 (1941)	AY222244 (1293)	38.1	23
Haementeriinae†	AY962416 (1767)	EF417050 (1003)	89	78
<i>Lottia*</i>	DQ248942 (2072)	DQ248942 (3473)	63.2	54
Lumbricidae†	AJ272183 (1813)	DQ790041 (3404)	87.6	64
Nereididae†	DQ790083 (1829)	DQ790043 (3087)	47.8	48
<i>Schistosoma japonicum</i>	Z11590 (1648)	Z46504 (3897)	93.6	88
<i>Schistosoma mansoni</i>	U65657 (1989)	AY157173 (3844)	93.6	97
<i>Schmidtea mediterranea</i>	U31084 (1794)	DQ665992 (1626)	93.3	96

Table 3. Composition and source of chimerical Operational Taxonomic Units (OTUs) used in this study.

Designation used in this study	18S & 28S rDNA taxon composition	Jiménez-Guri et. al. 2007 OTU taxon Composition
Araneae	<i>Acanthoscurria</i> sp.	<i>Acanthoscurria gomesiana</i> , <i>Dysdera erythrina</i>
Astacidea	<i>Homarus americanus</i>	<i>Homarus americanus</i> , <i>Pacifastacus leniusculus</i>
Capitella	<i>Capitella capitata</i>	<i>Capitella</i> sp.
Chalcidoidea	<i>Trichogramma minutum</i>	<i>Nasonia vitripennis</i>
Chalinidae	<i>Haliclona</i> sp.	<i>Reniera</i> sp.
Crassostrea	<i>Crassostrea gigas</i>	<i>Crassostrea virginica</i> , <i>Crassostrea gigas</i>
Cryptococcus	<i>Cryptococcus</i> sp. / <i>C. neoformans</i>	<i>Cryptococcus neoformans</i>
Cyanea	<i>Cyanea</i> sp.	<i>Cyanea capillata</i>
Daphnia	<i>Daphnia magna</i>	<i>Daphnia pulex</i> , <i>Daphnia magna</i>
Dugesia	<i>Dugesia ryukyuensis</i>	<i>Dugesia ryukyuensis</i> , <i>Dugesia japonica</i>
Echinococcus	<i>Echinococcus granulosus</i> / <i>E. multilocularis</i>	<i>Echinococcus granulosus</i> , <i>Echinococcus multilocularis</i>
Eutardigrada	<i>Milnesium</i> sp.	<i>Hypsibius dujardini</i> , <i>Macrobotus islandicus</i> , <i>Richtersius coronifer</i> , <i>tardigradum</i>
Gastropoda	<i>Biophalaria glabrata</i>	<i>Biophalaria glabrata</i> , <i>Aplysia californica</i> , <i>Lymnaea stagnalis</i>
Glomus	<i>Glomus intraradices</i>	<i>Glomus intraradices</i> , <i>Glomus versiforme</i>
Haementeriinae	<i>Helobdella stagnalis</i>	<i>Helobdella robusta</i> , <i>Helobdella stagnalis</i> , <i>Haementeria depressa</i>
Hydra	<i>Hydra circumcincta</i>	<i>Hydra magnipapillata</i> , <i>Hydra vulgaris</i>
Hydractiniidae	<i>Hydractinia symbiolongicarpus</i>	<i>Hydractinia echinata</i> , <i>Podocoryne carnea</i>
Ixodes	<i>Ixodes ricinus</i> / <i>I. scapularis</i>	<i>Ixodes scapularis</i> , <i>Ixodes pacificus</i>
Lottia	<i>Lottia digitalis</i>	<i>Lottia gigantea</i>
Lumbricidae	<i>Lumbricus terrestris</i> / <i>L. sp.</i>	<i>Lumbricus rubellus</i> , <i>Eisenia andrei</i> , <i>Eisenia fetida</i>
Mammalia	<i>Homo sapiens</i>	<i>Homo sapiens</i> , <i>Mus musculus</i> , <i>Bos taurus</i> , <i>Canis familiaris</i> , <i>Rattus</i>
Myxinidae	<i>Eptatretus stouti</i>	<i>Eptatretus burgeri</i> , <i>Myxine glutinosa</i> , <i>Eptatretus stoutii</i>
Nereididae	<i>Nereis vexillosa</i>	<i>Platynereis dumerilii</i> , <i>Nereis virens</i>
Noctuoidea	<i>Helicoverpa assulta</i> / missing	<i>Spodoptera frugiperda</i>
Orthopteroidea	<i>Locusta migratoria</i>	<i>Locusta migratoria</i> , <i>Diploptera punctata</i> , <i>Gryllus bimaculatus</i>
Pectiniidae	<i>Argopecten irradians</i>	<i>Argopecten irradians</i> , <i>Pecten maximus</i>
Penaeidae	<i>Penaeus semisulcatus</i>	<i>Litopenaeus vannamei</i> , <i>Litopenaeus setiferus</i> , <i>Penaeus monodon</i> , <i>Marsupenaeus japonicus</i>
Plakinidae	<i>Plakortis</i> sp. / <i>Plakinastrella</i> sp.	<i>Oscarella carmela</i>
Proterospongia	<i>Proterospongia choanojuncta</i> / <i>P. sp.</i>	<i>Proterospongia</i> sp.
Rhipicephalus	<i>Rhipicephalus (Boophilus) microplus</i>	<i>Rhipicephalus (Boophilus) microplus</i> , <i>Rhipicephalus appendiculatus</i>
Rhizopus	<i>Rhizopus oryzae</i> / <i>R. stolonifer</i>	<i>Rhizopus oryzae</i>
Scleractinia	<i>Montastraea franksi</i>	<i>Acropora millepora</i> , <i>Acropora palmata</i> , <i>Montastraea faveolata</i>
Sepiolidae	<i>Heteroteuthis hawaiiensis</i> / missing	<i>Euprymna scolopes</i>
Stolidobranchia	<i>Molgula manhattensis</i> / missing	<i>Molgula tectiformis</i> , <i>Halocynthia roretzi</i>
Ustilago	<i>Ustilago tritici</i>	<i>Ustilago maydis</i>
Xenopus	<i>Xenopus tropicalis</i>	<i>Xenopus tropicalis</i> , <i>Xenopus laevis</i>
Xiphinema	<i>Xiphinema rivesi</i>	<i>Xiphinema index</i>

Collins et al. (2006) but also utilizing previously published primers Myxo28S1F, 28S3R (Whipps et al., 2004) and Rev2 (Sonnenberg et al., 2007) as well as new myxozoan specific primers (for *Buddenbrockia*, Bud28s1F:CCGCATAGTTCGTAGAGATGT, Bud28s1R:ACATCTCTACGAACTATGCGG, Bud28s2F:TGAACTGGAACGTTTTGCCA, Bud28s4F:TGTCTGATTCGTGAGGTGA, Bud28s6F:CAGTTGTACCGTAAAATGGG, Bud28s7F:ACTCAACCGTATAGTCTGGCG, Bud28s9R:ACCTCATGTGCGCTCTAACA, Bud28s12R:TAACCTTAGGTTCCCTCATCGC; for *Myxobolus*, but designed also for *Henneguya*, MyxHen28sFWD: ACCAWAGAGGGTTWTAGTCCCG). Recovery of the 5' portion of 28S for both *Buddenbrockia* and *Ceratomyxa shasta* samples required an initial amplification of sequences spanning the 3' end of 18S to the universal 28S primer rev2 (Sonnenberg et al., 2007). For this we designed taxa specific 18S primers (*Ceratomyxa*, Cs18s3'F1: ATCGCTGTCGTGATTGG; *Buddenbrockia*, Bud18s3'FWD: CCGATTGAATGACTTAGCGA).

Thermal profiles employed were either (1) modifications of that reported in Evans et al. (2008), with variations in annealing temperatures between the range of 45-55°C, or (2) consisting of 4 minutes at 94°C; 5 cycles of 30 seconds at 94°C, 1 minute at 55°C, and 3 minutes at 72°C; then 3 minutes at 40°C; a ramp up from 40°C to 72°C at +0.2°C/second; followed by 30 cycles of 30 seconds at 94°C, 45 seconds at 50°C, and 3 minutes at 72°C; and finally 10 minutes at 72°C. In addition, cloning recovered two copies of 28S for *Tetracapsuloides bryosalmonae*, and was carried out using a Topo TA cloning kit (version O; Invitrogen) according to manufacture's instructions. More detailed molecular protocols for amplification of 28S rDNA from each taxa are available upon request.

All gene fragments were purified and sequenced by Cogenics, Inc. (Houston, TX) and assembled and edited using Sequencher v4.5 (Gene Code Co., 2005). Sequences for each marker were aligned in the program MAFFT (v.6) employing the E-INS-i strategy (Katoh and Toh, 2008). Malacosporea taxa displayed an approximate 300 bp insertion near the 3' end of 28S that required further refinement of the alignment. This was accomplished by identifying highly conserved flanking regions and aligning them to the existing alignment using MAFFT implemented in SeaView (v3.2). Ambiguously aligned regions in both 18S and 28S datasets were removed using Gblocks v0.91b (Castresana, 2000) under default parameters except with minimum block length set to 5 and allowing up to half the taxa to have gaps. The resulting datasets were comprised of 1,301 and 1,744 unambiguously aligned positions for 18S and 28S respectively.

The concatenated 10,821 character rDNA & AA alignment and corresponding topology can be found in TreeBASE (<http://www.treebase.org/treebase/index.html>).

Phylogenetic analysis

Phylogenetic analyses were performed on individual and combined datasets using maximum likelihood (ML) criteria implemented in RAxML (v7.0.0) (Stamatakis, 2006a). For the trimmed phylogenomic AA dataset we implemented the WAG amino acid substitution matrix with frequencies empirically estimated (+F) and a Γ model of site heterogeneity. For all analyses of rDNA data 18S and 28S were partitioned and a GTR + Γ model was used. A total of 1,000 bootstrap replicates were performed for each ML

search employing RAxML's computationally more efficient CAT approximation of rate heterogeneity (not to be confused with a similarly named amino acid substitution model) (Stamatakis, 2006b). Using both Paup* (v 4.0) and PhyUtility (v 2.2) bootstrap replicates were summarized with and without *Buddenbrockia* trimmed from each replicate topology, any differences were reported.

For the 60 taxon matrices, topologies representing alternative, competing hypotheses of *Buddenbrockia*'s placement were inferred in RAxML under the appropriate models and their significance evaluated with the Shimodaira-Hasegawa test (SH test) (Shimodaira and Hasegawa, 1999) as implemented in RAxML. For each of these topologies the negative log likelihood (-lnL) values were calculated in RAxML for every character site. The differences in per-site -lnL (Δ ps-lnL) values between competing topologies were calculated in Excel (v. 11.5.4) and plotted in Adobe Illustrator CS3 (v13.0.0).

Using the program PROCOV (v2.0; Wang et al., 2008) we evaluated the trimmed phylogenomic AA matrix under a covarion model; an approach that accounts for changes of within site rates (heterotachy) along the branches of a phylogeny. Though v2.0 of PROCOV can perform ML tree searches the AA dataset was large enough to make this computationally prohibitive. Instead, we utilized those features originally available in v.1.0 to calculate and evaluate existing competing topologies under a covarion model. Specifically, we employed a WAG substitution model and PROCOV's General covarion model which combines attributes of both Huelenbeck (2002) and Galtier (2001) models, allowing within site rate variation to turn on and off as well as allowing transitions

between different rates once a site is turned on (Wang, 2007). The General model was implemented with both traditional estimates of rates among sites (RAS) and 4 discrete gamma distributions for the covarion model (COV). Topologies were evaluated by calculating maximum $-\ln L$ values as well as the $\Delta ps-\ln L$ under both the RAS and RAS+COV models.

RESULTS

Position of Myxozoa

Our results reconfirm the existence of two competing molecular hypotheses for the placement of Myxozoa (Figure 7, 8, and 12). Individual analyses of our three datasets all produce topologies consistent with previous investigations in which phylogenomic amino acid data (AA) supports a placement of Myxozoa within Cnidaria (Figure 7A) and rDNA data, comprehensively sampled or not, support a basal bilaterian origin of Myxozoa (Figure 7B, 8 and 12).

Analysis of the 60 taxa, 7,776 AA dataset employing a WAG + F + Γ model resulted in a metazoan topology identical to that reported by JG07 (Jiménez-Guri et al., 2007) (Figure 7A). In this topology the enigmatic myxozoan *Buddenbrockia plummatella* was recovered as a long-branched taxon sister to the cnidarian clade Medusozoa (Myxozoa+Medusozoa) (Figure 7A). However, support calculated for this placement was just 54% (1000 replicates), compared to the 70% (100 replicates) recovered by JG07. Thus, while minimizing the relative amount of missing data for *Buddenbrockia* did not

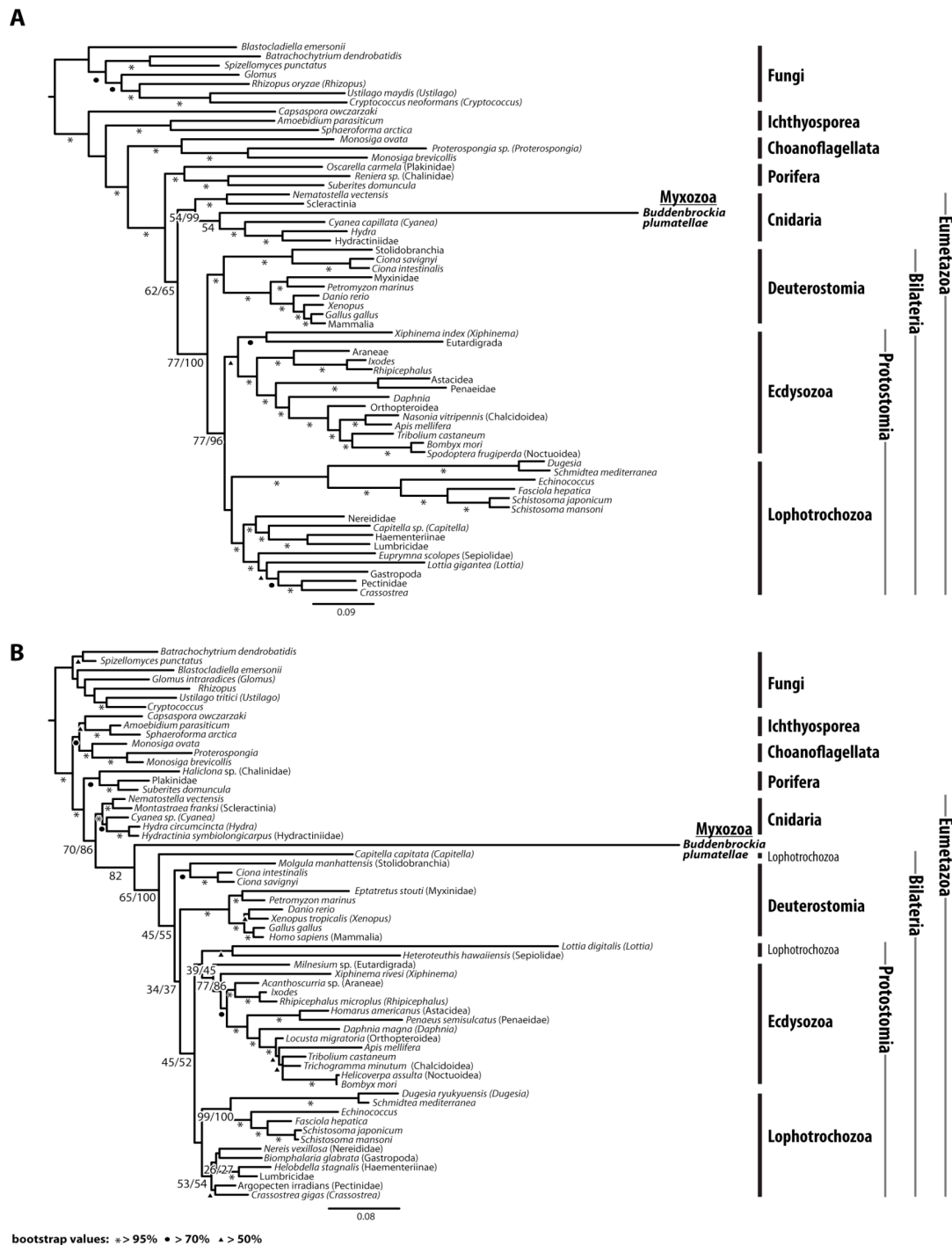


Figure 7. A) ML topology from analysis of a 60 OTU, 7,776 AA character phylogenomic matrix inferred under a WAG + F + Γ model. B) ML topology from analysis of a 60 OTU, 3,045 character combined 18S & 28S rDNA matrix inferred under a GTR + Γ model. In both topologies chimerical OTUs designations resulting from concatenation of rDNA and AA data appear in parentheses. See Table 3 for specific Chimerical OTUs compositions. Bootstrap support (based on 1000 replicates) values appear in numerical form for nodes of interest. This includes all nodes whose support is affected when *Buddenbrockia* is pruned from each replicate before summarizing bootstrap values. In these cases two bootstrap values appear, the first indicating nodal support when *Buddenbrockia*'s placement is considered, the second displaying support when this taxon is ignored.

affect its placement it appears to have affected its support. Additionally, pruning *Buddenbrockia* from each replicate before summarizing bootstrap values reveals that it compromises support for a total of four nodes which are as follows (with vs. without *Buddenbrockia*): Eumetazoa (62% vs. 65%), Cnidaria (54% vs. 99%), Bilateria (77% vs. 100%), and Protostomia (77% vs. 96%) (Figure 7A). Analysis of near complete 18S and 28S rDNA with a taxonomic sampling mirroring the 60 taxa examined in JG07 (Table 2) recovered a long-branched *Buddenbrockia* falling sister to Bilateria (Myxozoa+Bilateria), with bootstrap support of 82% (Figure 7B). In this topology, however, Bilateria does display both lower node support and a small number of unstable relationships not found in the JG07 topology. Clear artifactual placements include one polychaete worm (*Capitella capitata*) and two cephalopods falling out of Lophotrochozoa in two separate positions. Thus the data rich matrix of JG07, appears to perform better for relationships within Bilateria than the rDNA dataset with the same, albeit limited taxonomic sampling. Bootstrap values summarized with and without *Buddenbrockia* reveal a change in support for a total of 10 nodes, the most significant being Eumetazoa (70% vs. 86%) and Bilateria (65% vs. 100%) (Figure 7B). Noticeably absent in this analysis was any change in support for a monophyletic Cnidaria (97%).

Analysis of our third dataset, comprised of 18S and 28S rDNA markers with a highly comprehensive sampling of Metazoa (389 taxa), including six myxozoan species, confirms a deep divergence between Myxosporea and Malacosporea and recovers

Myxozoa + Acoela as sister to the rest of Bilateria with 100% bootstrap support for the Myxozoa/Acoela/Bilateria clade (Figure 8 and Figure 12). However, the Myxozoan + Acoela node itself does not have significant support. While previous rDNA studies support a placement of Acoela as sister to the rest of Bilateria (e.g. Wallberg et al., 2007), Acoela has also been identified to be one of a number of long-branched bilaterian clades causing topological artifacts within Bilateria. Others include Nemertodermatida, Gnathostomulida, Acanthocephala, Bryozoa, Chaetognatha, and Myzostoma (Bleidorn et al., 2007; Paps et al., 2009). To minimize potential effects imposed by these clades, we removed them from our dataset and reevaluated the placement of Myxozoa. Results revealed no change in the placement of Myxozoa, which remained sister to Bilateria (topology not shown). Given their long branches and the low support value (<50%), a Myxozoa + Acoela clade should be viewed as suspect. These results do not contradict the Myxozoa+Bilateria hypothesis recovered with our smaller dataset (Fig. 7B). Furthermore, bootstrap values summarized without Myxozoa (as was done without *Buddenbrockia* for the 60 taxa datasets) reveal no change in support for any nodes, further underscoring the stability of Myxozoa's placement at the base of Bilateria with a comprehensively sampled rDNA dataset (Figure 8 and Figure 12).

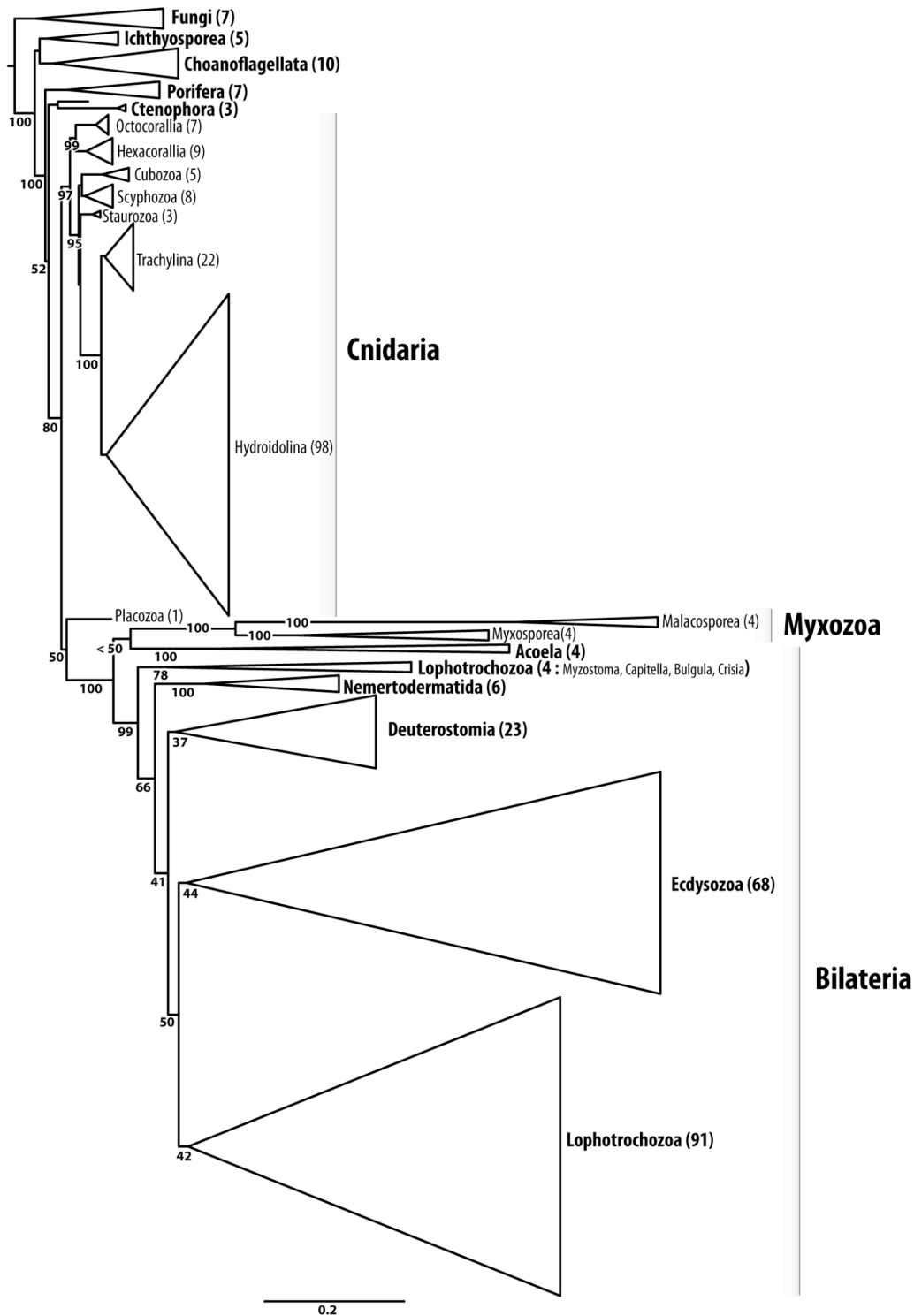


Figure 8. Condensed ML phylogeny with proportionately accurate branch lengths from analysis of a 389 metazoan taxa, 3045 character combined 18S & 28S rDNA matrix inferred under a GTR + Γ model. Bootstrap support values (based on 1000 replicates) appear in numerical form for nodes of interest. The number of taxa comprising each condensed clade appear in parentheses. Nodal support and specific lower-level topologies can be found in a cladogram in Figure 12.

Finally, we concatenated our trimmed AA matrix with each rDNA dataset (the 60 taxa and the 389 taxa), creating two alignments both consisting of 10,821 sequence characters. The latter concatenated matrix necessarily included a large amount of missing data for the AA portion. Analysis of the 60 taxon concatenated dataset recovered a placement of *Buddenbrockia* at the base of Bilateria with 83% bootstrap support (Figure 9). Artifactual bilaterian relationships appearing within our 60 taxa rDNA phylogeny (Figure 7B) were not found when analyzing this rDNA+AA matrix. Instead, with the notable exception of the different placement of *Buddenbrockia*, we find the bilaterian topology congruent with JG07 and having greater overall support than either rDNA or trimmed EST data considered alone. Moreover, pruning *Buddenbrockia* from the bootstrap replicates reveals that the 4 nodes negatively affected in the phylogenomic AA dataset alone were once again affected here. However only the cnidarian node appears to have been significantly influenced (86% vs. 100%) in this analysis (Figure 9).

Our second concatenated alignment contained 389 taxa with near complete rDNA sequences but substantial missing data for the AA portion of the matrix. Analysis of this dataset recovered a placement of Myxozoa consistent with results of the 389 taxa rDNA dataset considered alone. That is, this analysis recovered a Myxozoa+Acoela relationship at the base of Bilateria (Figure 13) and removal of acoels did not change the placement of Myxozoa (topology not shown).

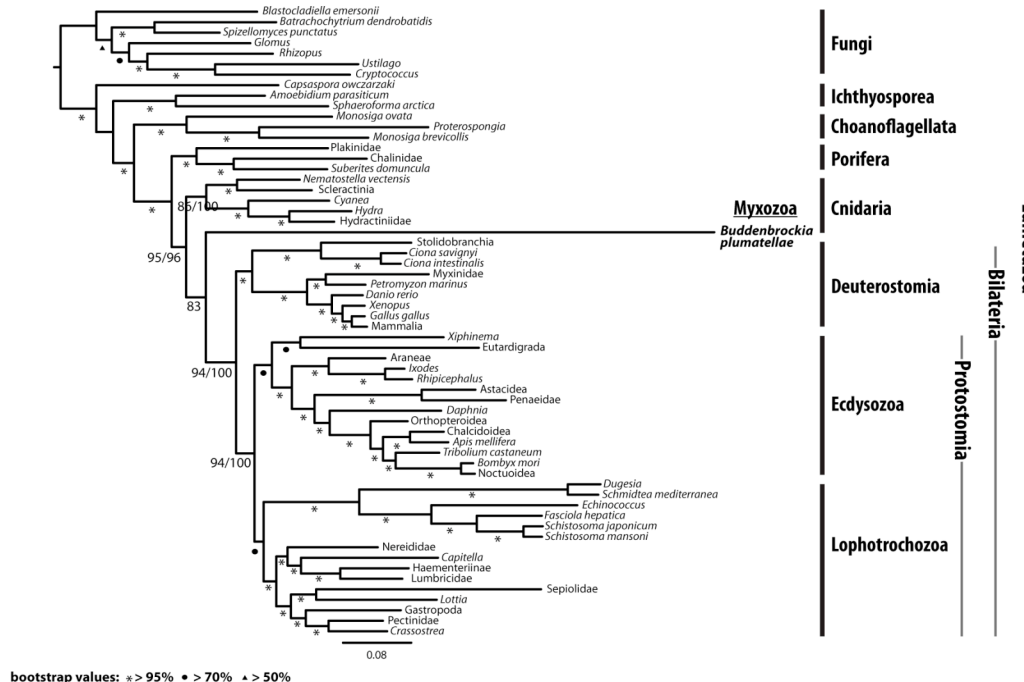


Figure 9. ML topology from analysis of a 60 OTU, 10,821 character concatenated rDNA + AA matrix inferred, respectively, under GTR + Γ and WAG + F + Γ models. See Table 3 for specific Chimerical OTUs compositions. Bootstrap support values (based on 1000 replicates) appear in numerical form for nodes of interest. This includes all nodes whose support is affected when *Buddenbrockia* is pruned from each replicate before summarizing bootstrap values. In these cases two bootstrap values appear, the first indicating nodal support when *Buddenbrockia*'s placement is considered, the second displaying support when this taxon is ignored.

Conflicting Topological Signals

Though widely accepted as a conservative measure of topological significance an SH test (Shimodaira and Hasegawa, 1999) calculated (in RAxML) from our phylogenomic AA matrix finds no significant difference in maximum $-\ln L$ values for the Myxozoa+Medusozoa topology and two alternative trees reflecting the Myxozoa+Bilateria hypothesis and an intermediate Myxozoa+Cnidaria relationship. By contrast, an SH test on the rDNA data find significant difference between its most likely topology, Myxozoa+Bilateria, and the two other alternative placements. An SH test calculated from the 60 taxa combined rDNA+AA matrix reveals that the

Myxozoa+Bilateria and Myxozoa+Medusozoa topologies do not have significantly different maximum $-\ln L$ values, but that the intermediate Myxozoa+Cnidaria topology is significantly worse. Thus, while most of this conflict appears limited to the phylogenomic AA data, addition of rDNA data limits the significance of this conflict to just the competing Myxozoa+Bilateria and Myxozoa+Medusozoa placements.

To identify and visualize those character sites in the 60 taxa rDNA+AA dataset most responsible for conflict in the placement of Myxozoa, we compared the topology shown in Figure 9 (Myxozoa+Bilateria) to an identical one generated under the same parameters with the exception that *Buddenbrockia* was constrained to be sister to Medusozoa (Myxozoa+Medusozoa). Differences in values of per-site negative log likelihood ($\Delta\text{ps-}\ln L$) were calculated for the two topologies and plotted in Figure 10. In this distribution,

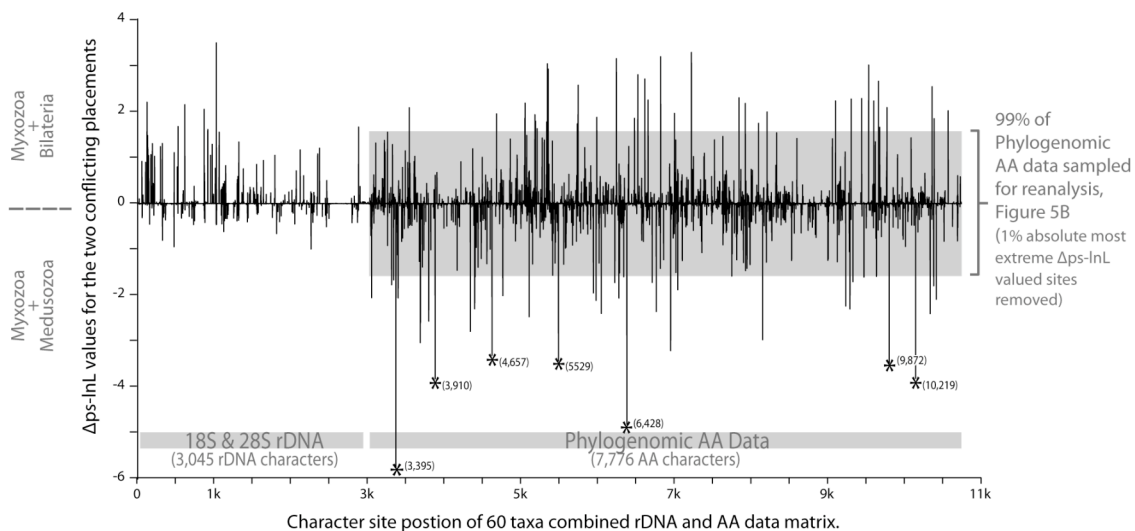


Figure 10. Differences in values of per-site negative log likelihood ($\Delta\text{ps-}\ln L$) for the two conflicting placements of *Buddenbrockia* plotted in order as they appear distributed throughout the 60 taxa combined rDNA and AA data matrix. Positive y-axis values correspond to character sites in the data matrix that display more likelihood support for the Myxozoa+Bilateria relationship and negative values correspond to those characters that display more likelihood support for the Myxozoa+Medusozoa topology. The 7 absolute most extreme $\Delta\text{ps-}\ln L$ values are identified by an asterisks with specific site number appearing in parentheses.

positive y-axis values correspond to specific characters that display more likelihood support for the Myxozoa+Bilateria relationship and negative values correspond to those characters that display more likelihood support for the Myxozoa+Medusozoa topology. Although most of the $\Delta\text{ps-lnL}$ values appear approximately around zero for both rDNA and AA matrices, there are a limited number of sites conferring significantly greater -lnL values for one placement over the other (Figure 10). For the rDNA data these outlying sites are mostly positive, appearing in support of the Myxozoa+Bilateria topology. By contrast, for the AA data, positive and negative $\Delta\text{ps-lnL}$ values can be found throughout the matrix, providing a clear depiction of the conflict within the phylogenomic data. However, there does not appear to be any particular pattern of positive or negative values along the x-axis, something that might be expected should strong conflict exist between individual markers within this AA data. Instead, the dispersion of positive and negative $\Delta\text{ps-lnL}$ values reveal that most of the conflict we measure exists both within and between individual AA markers. Last, Figure 10 also clearly displays that, among the AA characters, a small portion of negative $\Delta\text{ps-lnL}$ values (again, supporting the Myxozoa+Medusozoa placement), are more extreme than any positive or negative values present in the rDNA data.

To investigate the effects of the outlying $\Delta\text{ps-lnL}$ values within the phylogenomic AA dataset we reanalyzed this matrix in two ways. First we sequentially excluded those sites conferring the greatest absolute $\Delta\text{ps-lnL}$ values, determining that removal of as little as 7 character sites changes the placement of *Buddenbrockia* from within Cnidaria to a

slightly better bootstrap supported (60% vs. 54%) competing placement at the base of Bilateria (Myxozoa+Bilateria) (Figure 11A). These 7 sites (labeled in Figure 10) represent 0.090% of the trimmed AA matrix, and just 0.024% of that analyzed by JG07. Bootstrap replicates summarized without *Buddenbrockia* reveal that support for no other group was strongly influenced by removal of these 7 sites, specifically, Cnidaria and Bilateria remain unchanged at 99% and 100%, respectively (Figure 7A vs. Figure 11A).

Our second reanalysis of the AA matrix involved removing a total 1% (78) of those sites conferring the greatest absolute $\Delta\text{ps-lnL}$ values. Analysis of the remaining 7,698 amino acid characters (labeled in Figure 10) results in a weakly supported (<50%) placement of *Buddenbrockia* at the base of Metazoa (Figure 11B). Bootstrap values summarized with and without *Buddenbrockia* reveal that removal of these 78 sites actually changed support

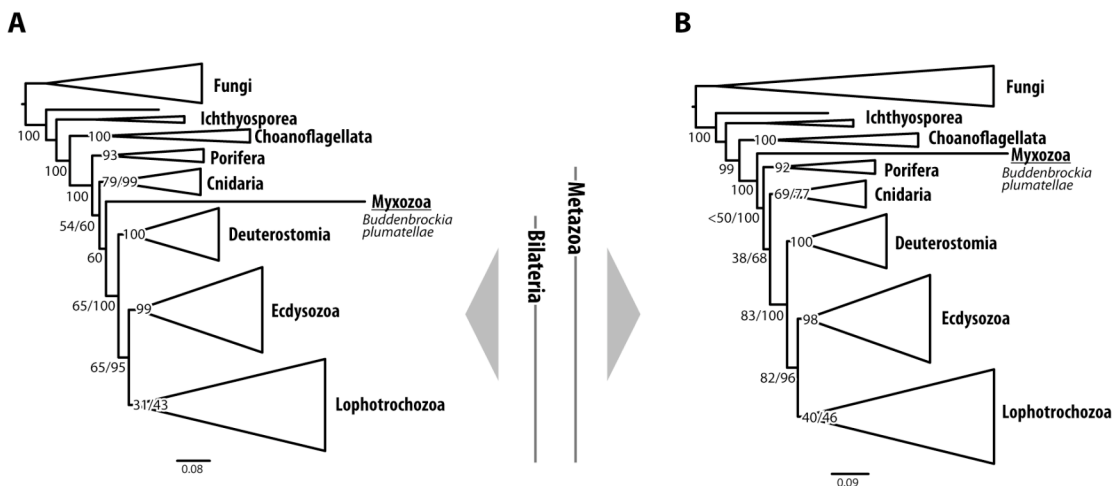


Figure 11. Condensed ML phylogenies with proportionately accurate branch lengths from analyses under a WAG + F + Γ model of the 60 OTU AA phylogenomic matrix in which the 7,776 AA matrix was modified to exclude characters conferring the greatest absolute $\Delta\text{ps-lnL}$ values (see Figure 10). In topology A) 7 such sites were removed before analysis, topology B) 78 (~1%) of such sites were removed. Only bootstrap support values of interest are shown (based on 1000 replicates). Nodes whose support is affected when *Buddenbrockia* is pruned from each replicate before summarizing bootstrap values display two values; the first indicating nodal support when *Buddenbrockia*'s placement is considered, the second displaying support when this taxon is ignored.

for a monophyletic Cnidaria in the absence of *Buddenbrockia* from 99% (Figure 7A) to 77% (Figure 11B). No such significant change was observed for any other node in this analysis. Thus, it appears that the 1% of the AA data that most influenced the Myxozoa+Medusozoa topology includes many characters that significantly contribute to the support for Cnidaria, regardless of *Buddenbrockia*'s presence.

Consideration of a covarion model for phylogenetic analyses has been demonstrated to alleviate systematic errors induced by long-branched taxa (Wang et al., 2008). However, due to the fact that accounting for variation of within site rates is computationally prohibitive, few studies employ the covarion model. Given the size of the AA matrix we too found a tree search impractical. Instead, using the program PROCOV (v2.0), we calculated the maximum $-\ln L$ values for the Myxozoa+Medusozoa, Myxozoa+Cnidaria, and Myxozoa+Bilateria topologies under both a traditional estimate of rates among sites (RAS) and PROCOV's General covarion model (COV). Implementation of the RAS+COV model reveals that the Myxozoa+Medusozoa topology still has the greatest maximum $-\ln L$ value (-279589) and that Myxozoa+Bilateria still has greater significance than the Myxozoa+Cnidaria topology (-279604 vs. -288302). The greatest relative change in maximum $-\ln L$ values between the RAS and RAS+COV model was observed for the Myxozoa+Bilateria topology. This was only slightly greater (+26.5) than that found for the Myxozoa+Medusozoa topology. However, this difference can be directly attributed to the fact that under RAS+COV model 16% more AA sites demonstrate $\Delta ps-\ln L$ values in favor of the Myxozoa+Bilateria topology than do under just the RAS model. Thus, for

the AA matrix implementation of a covarion model results in greater significance for the competing Myxozoa+Bilateria hypothesis.

DISCUSSION

Maximum Likelihood (ML) analyses of the most comprehensive molecular data available for myxozoans, consisting of 10,821 combined rDNA and phylogenomic AA characters, supports the placement of Myxozoa at the base of Bilateria (Myxozoa+Bilateria) and not within Cnidaria (Figure 9, Figure 13). This placement is consistent with analyses of rDNA data alone. Furthermore, topological results remain consistent regardless of whether data coverage is maximized by limiting taxon sampling to just 60 taxa or if a comprehensive metazoan sampling is used (389 taxa). Nevertheless, an SH test calculated for our 60 taxa rDNA+AA matrix cannot reject an alternative Myxozoa+Medusozoa hypothesis.

Additionally, we confirm the results of JG07 (Jimenez-Guri et al., 2007), specifically that phylogenomic AA data alone supports the placement of the myxozoan *Buddenbrockia* within Cnidaria (Myxozoa+Medusozoa). In fact, this conformation included first trimming JG07's matrix to increase the relative coverage of data for *Buddenbrockia* from an original 26% to 98.9%. This had the added effect of significantly increasing coverage for nearly half of the 60 taxa in this dataset (Table 2). Yet, we found that analysis of this trimmed AA matrix resulted in a significantly lower support value for *Buddenbrockia*'s placement than that of JG07 (54% vs. 70%) (Figure 7A). However,

given an overall relative increase in data coverage and our greater number of bootstrap replicates (1,000 vs. 100), we suspect that a support value of 54% is a more accurate reflection of the internal consistency of the AA data, particularly with respect to *Buddenbrockia*.

Due to having a greater number of independent loci, phylogenomic matrices arguably have more complex phylogenetic signals than that of “traditional” datasets comprised of one or few molecular markers. To an extent this was illustrated in the distribution of $\Delta\text{ps-lnL}$ (change in per-site $-\text{lnL}$) values for the conflicting placements of *Buddenbrockia* as sister to Bilateria and sister to Medusozoa, as calculated for the 60 taxa rDNA+AA matrix (Figure 10). This distribution shows little in the way of structure or pattern to suggest that many of the AA protein coding genes strongly support one competing placement over the other (Figure 10). Instead, the AA matrix appears to more or less have an even distribution of characters supporting either a Myxozoa+Bilateria or Myxozoa+Medusozoa hypothesis.

In the JG07 study, the AU test of topological significance (Shimodaira, 2002) failed to reject four alternative deep node placements for *Buddenbrockia*. These alternative placements were, sister to Metazoa, sister to Eumetazoa, sister to Cnidaria (as opposed to Medusozoa), and sister to Bilateria. We reveal similar conflict within and between our 60 taxa datasets, evidenced by the 4 topologies and bootstrap summary values (with and without *Buddenbrockia*) depicted in Figure 7 and Figure 11. However, bootstrap

summaries and SH tests also clearly demonstrate that analysis of a combined rDNA+AA matrix reduces this conflict to strictly a Medusozoa verses Bilateria placement (Figure 9).

With two dramatically different competing placements for Myxozoa, it is disconcerting that removal of just 7 AA sites from the phylogenomic matrix can produce a topology with greater support for the alternative Myxozoa+Bilateria topology (Figure 11A). In addition, implementation of a covarion model to this phylogenomic matrix improves the significance of the same alternative Myxozoa+Bilateria topology. Thus, collectively or alone, minor errors in sequencing, alignment procedures, and model specification could seemingly influence the placement of a long-branched clade, in this case *Buddenbrockia*. However, examining bootstrap summaries of rDNA only analyses reveals that increased taxon sampling, including of both malacosporeans and myxosporeans, recovers shorter branches and a more stable placement for Myxozoa (Figure 7B and Figure 8). This suggests that greater taxon sampling phylogenomic data may produce a more robust signal for the placement of Myxozoa. More broadly speaking, given the precipitous decline in costs associated with developing such data, it will be interesting to determine if existing hypotheses regarding deep metazoan relationships will continue to hold with increased taxonomic sampling of phylogenomic data (e.g. Dunn et al., 2008; Philippe et al., 2009; as well as JG07).

However, our results also suggest that the complexity of phylogenomic data necessitate considerate inclusion of as much data as possible. Our analyses demonstrate that a small number of phylogenomic characters drastically influence the support of even a stable

clade. That is, removal of 1% of those AA sites conferring the greatest conflict for the placement of *Buddenbrockia* (Figure 10) resulted in a significant loss of support for Cnidaria (99% vs. 77%) when the relative placement of *Buddenbrockia* was ignored (Figure 7A vs. Figure 11A). Thus, our work suggests that while phylogenomic data provides an enormous level of independent loci, the phylogenetic signal it provides may not be any stronger than that of traditional molecular data, yet it necessarily requires considerably larger datasets, often with significant missing data. If subsequent work finds that such datasets do indeed often possess but a small percentage of informative sites the reliability of support values must also be reconsidered. In this case calculation of both bootstrap values and posterior probabilities would be seemingly more sensitive to model specification, size of the overall dataset, and missing data.

In fact, recent empirical work suggests that significant levels of missing data can produce strongly misleading results because of its effects on the modeling of among-site rate variation (Lemmon et al., 2009). Though based on very limited taxon sampling, this study suggests that under both Bayesian and ML frameworks, significant levels of missing data can repel sister clades, attract distantly related taxa, and significantly overestimate support statistics. Confronted with the reality of Myxozoa's extremely divergent sequences, concern for such artifacts is prudent.

Last, we think it relevant to note that our study does not address the phylogenetic status of the enigmatic putative cnidarian *Polypodium hydriforme*. Evans et al. (2008) revealed that while previous analyses of 18S rDNA recovered a *Polypodium*+Myxozoa

relationship at the base of Bilateria (sometimes sister to Aceola) (Zrzavý and Hypša, 2003), analyses of *Polypodium* 18S data in the absence of Myxozoa, with increased taxon sampling of Metazoa (particularly Cnidaria), results in a placement within Cnidaria, albeit with weak support. Evans et al. (2008) did not recover the same result for myxozoans which remained, with or without *Polypodium*, at the base of Bilateria. Using the datasets presented here we found that inclusion of *Polypodium* 18S rDNA recovered results consistent with Evans et al. (2008), failing to exert any specific influence on the placement of Myxozoa yet being wholly influenced by the presence of myxozoan taxa. In addition, isolation of 28S rDNA has proven to be problematic (Evans et al., 2008). Thus, with but a single marker for such an important, long-branched clade whose placement remains both tentative and uniquely influenced by myxozoans, we feel that more molecular data (rDNA, phylogenomic, or otherwise) is required for *Polypodium* before the relationship between myxozoans and this enigmatic taxon can be properly addressed.

CONCLUDING REMARKS

Given the exceptionally long branches both leading to and within Myxozoa, the phylogenetic placement of this clade remains vulnerable to a number of artifacts including inadequate taxon sampling, insufficient levels of informative independent characters, and model misspecification. In this study we attempted to address each of these concerns by, where possible, improving data coverage, increasing taxon sampling, combining molecular data, and exploring the degree to which finite portions of the data were influencing analyses. However, thorough analyses of the most comprehensive

molecular data available support, albeit weakly, the controversial placement of Myxozoa as sister to Bilateria.

Yet, there remains merit in the assertions of Siddall and Whiting (1999) and Siddall et. al (1995) that analyses of molecular data alone cannot explain away significant morphological similarities between myxozoan polar capsules and cnidarian nematocysts. Indeed, an origin of Myxozoa outside Cnidaria is certainly more provocative, inherently begging a reassessment of the homology between cnidarian nematocysts and myxozoan polar capsules. That is, the complexity of these structures makes convergence a less viable hypothesis despite the support we find for a Myxozoa+Bilateria topology from the rDNA, rDNA+phylogenomic AA, and the AA datasets alone but with 7 extreme Δ ps-InL outlier sites removed. Instead, a non-cnidarian placement would leave us to conclude that the last common ancestor of Cnidaria and Bilateria possessed structures homologous to both nematocysts and polar capsules but were subsequently lost in bilaterians. Though our study finds little evidence to support this view, a third alternative placement of myxozoans as an early diverging cnidarian clade is compelling to consider. Specifically, this would (1) account for Myxozoa's limited cnidarian-like morphology, (2) explain the deep conflict within the phylogenomic data and (3) suggest a deep coalescent event for bilaterian and myxozoan rDNA sequences. Finally, we surmise, as many do, that given appropriate and comprehensive sampling, Myxozoa will be shown to be a cnidarian but our results clearly demonstrate that robust molecular support for this hypothesis is still lacking and that phylogenomic studies with limited taxonomic sampling should be interpreted cautiously.

Figure 12. Cladogram of ML analysis of a 389 taxa, 3045 character combined 18S & 28S rDNA matrix inferred under a GTR + Γ model. Bootstrap support values appear for each branch and are based upon 1000 replicates. Asterisks designate those taxa for which new 28S rDNA sequence was generated. Operational taxonomic units (OTUs) utilized in the 60 taxa matrices appear in bold. Chimerical OTUs designations resulting from concatenation of rDNA and AA data appear in parentheses. See Tables 3 and 4 for specific Chimerical OTUs compositions.

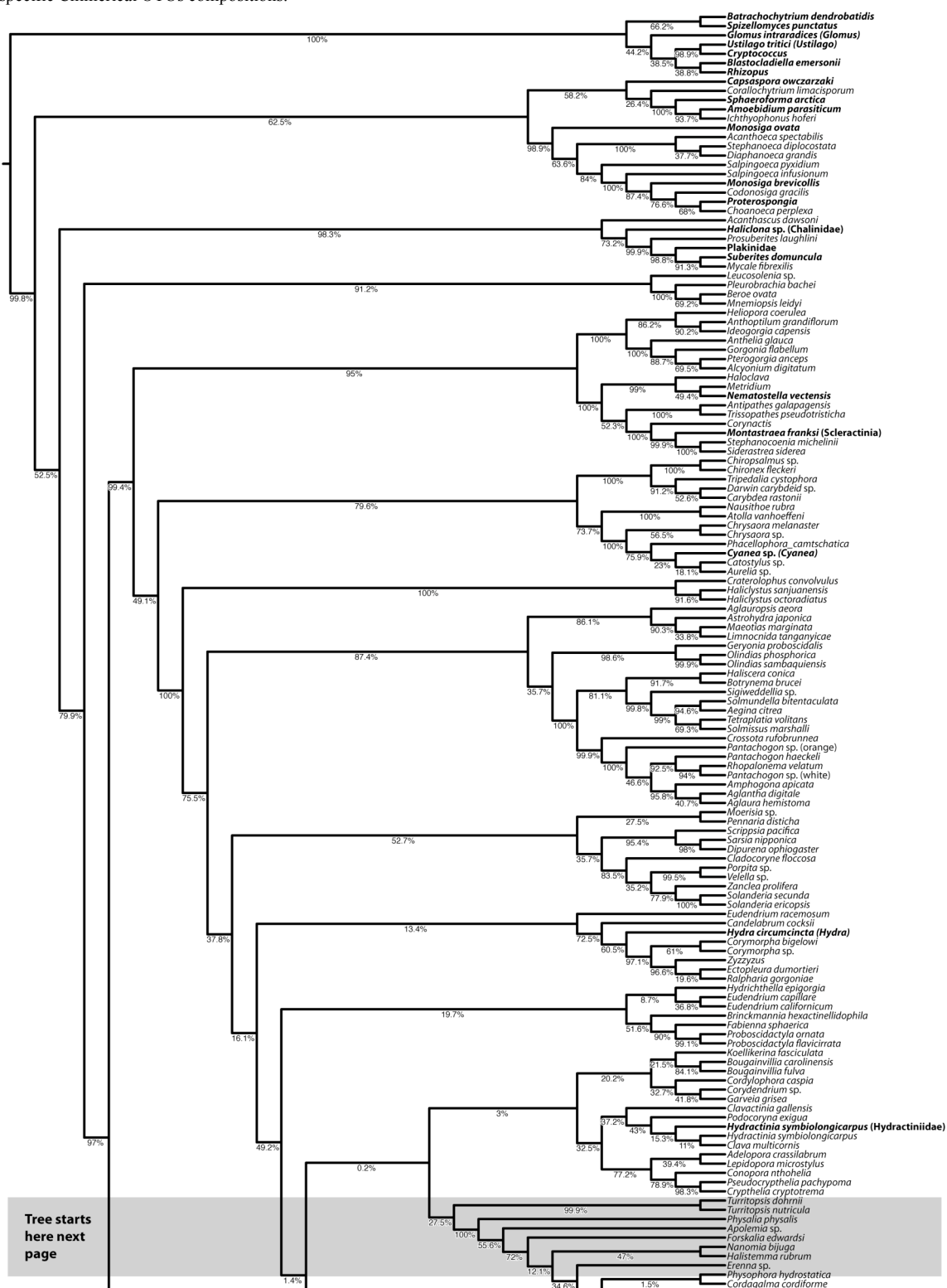


Figure 12. Continued

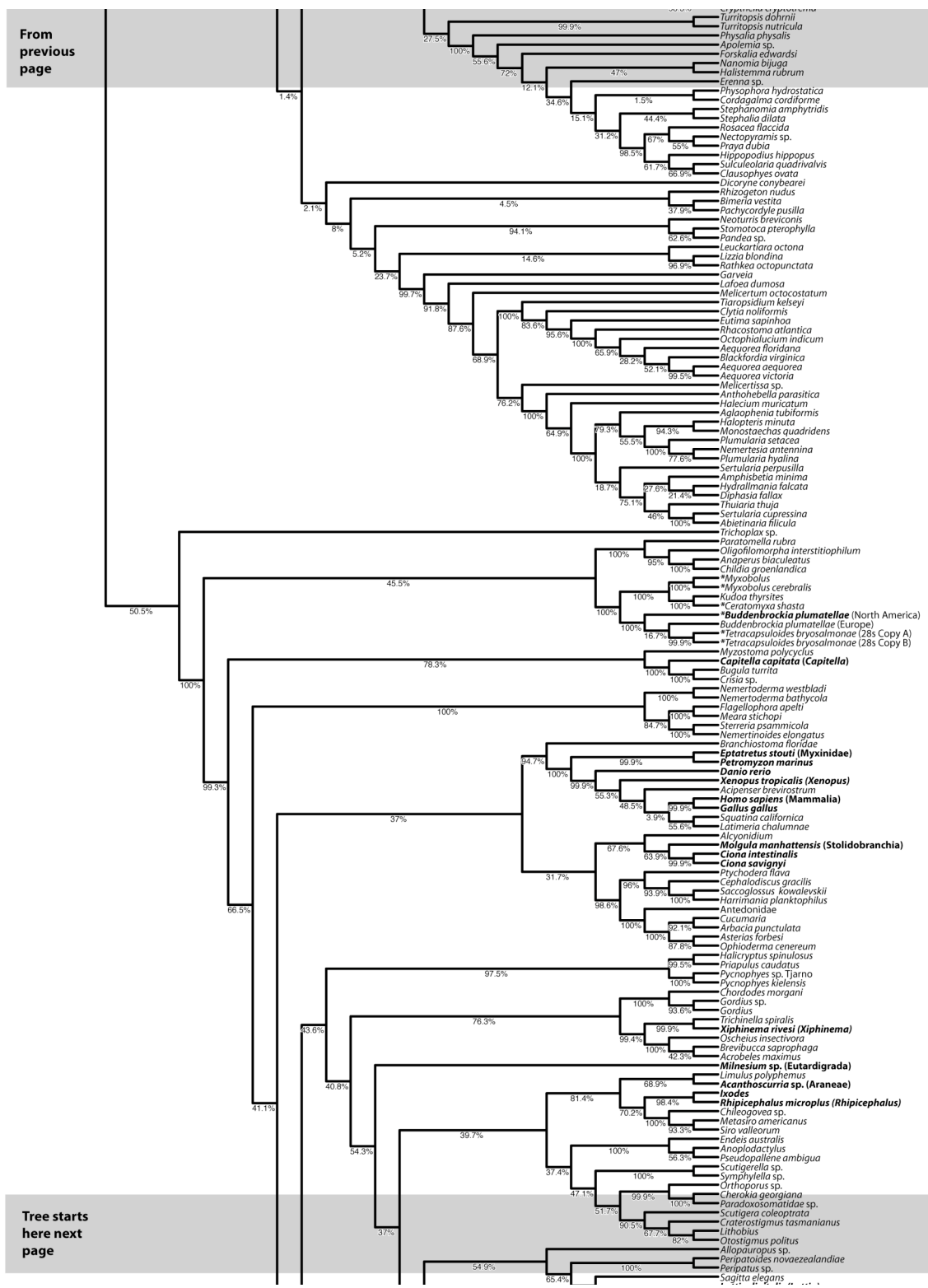


Figure 12. Continued

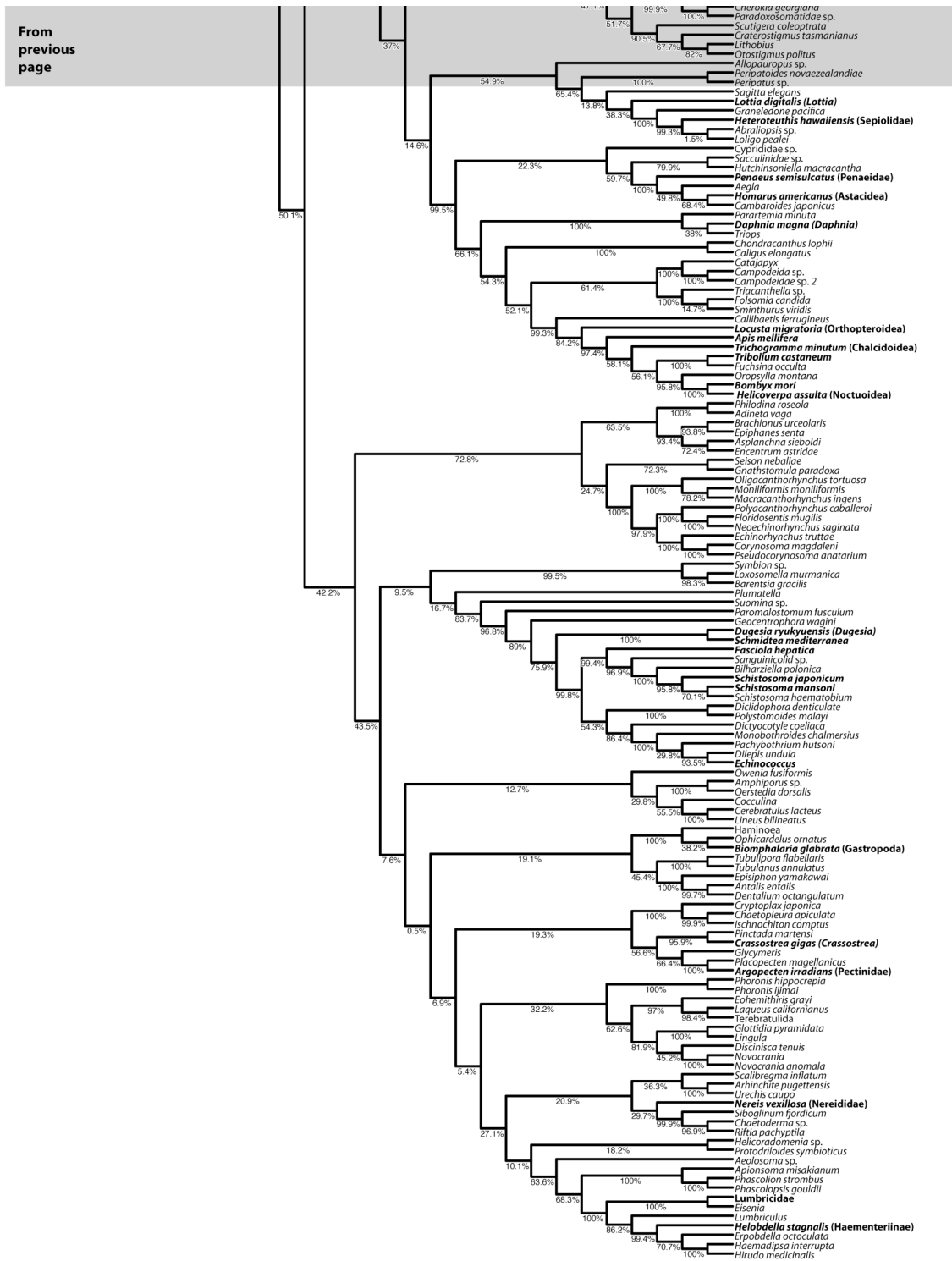


Figure 13. Cladogram of ML analysis of a 389 taxa, rDNA + AA matrix inferred, respectively, under GTR + Γ and WAG + F + Γ models. The 60 operational taxonomic units (OTUs) for which nearly all 10, 821 rDNA + AA characters exist, appear in bold. Bootstrap support values appear for each branch and are based upon 1000 replicates. Chimerical OTUs designations resulting from concatenation of rDNA and AA data appear in parentheses. Tables 3 and 4 for specific Chimerical OTUs compositions. Asterisks designate those taxa for which new 28S rDNA sequence was generated. 60 taxa matrices appear in bold.

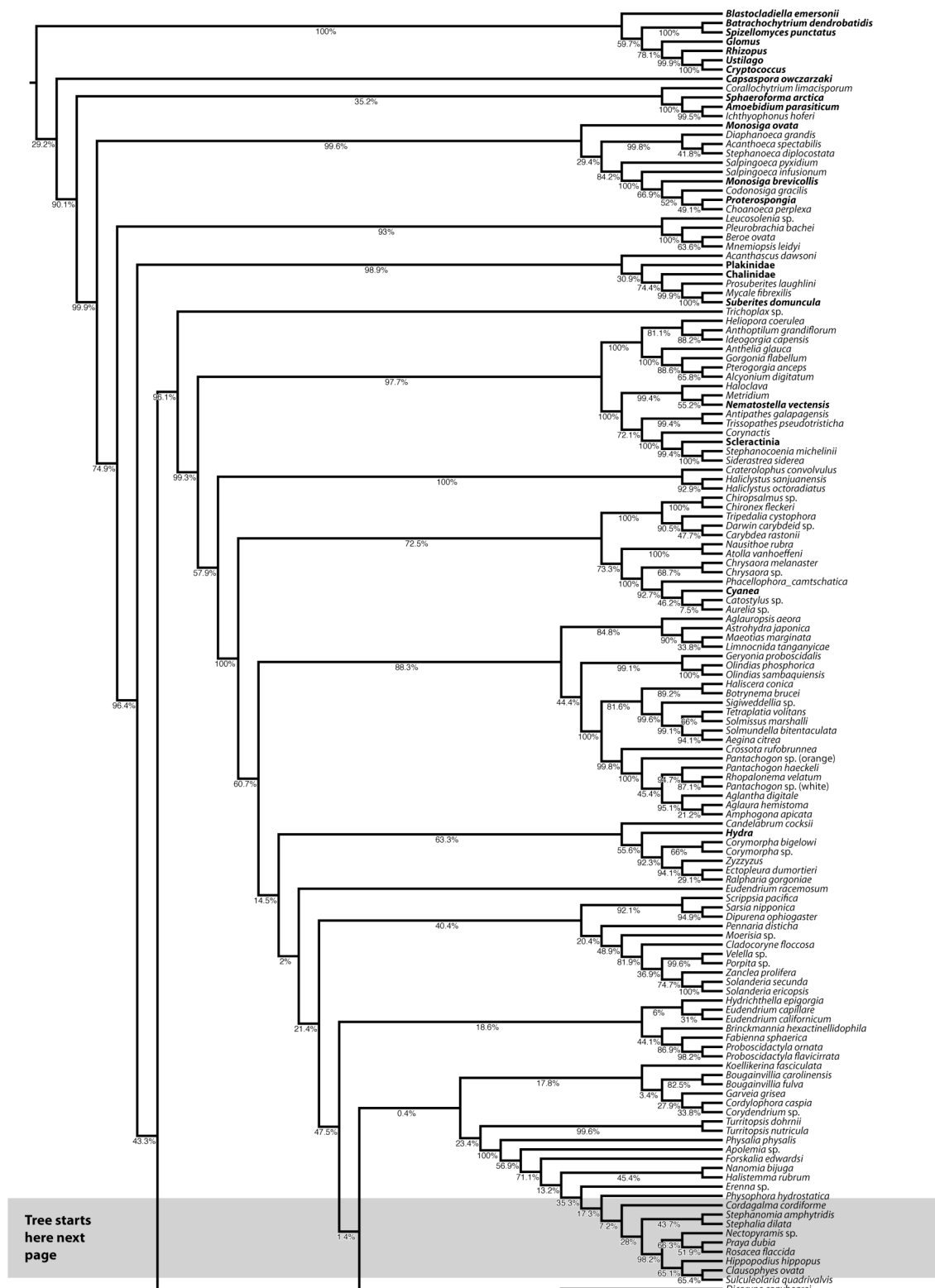


Figure 13. Continued

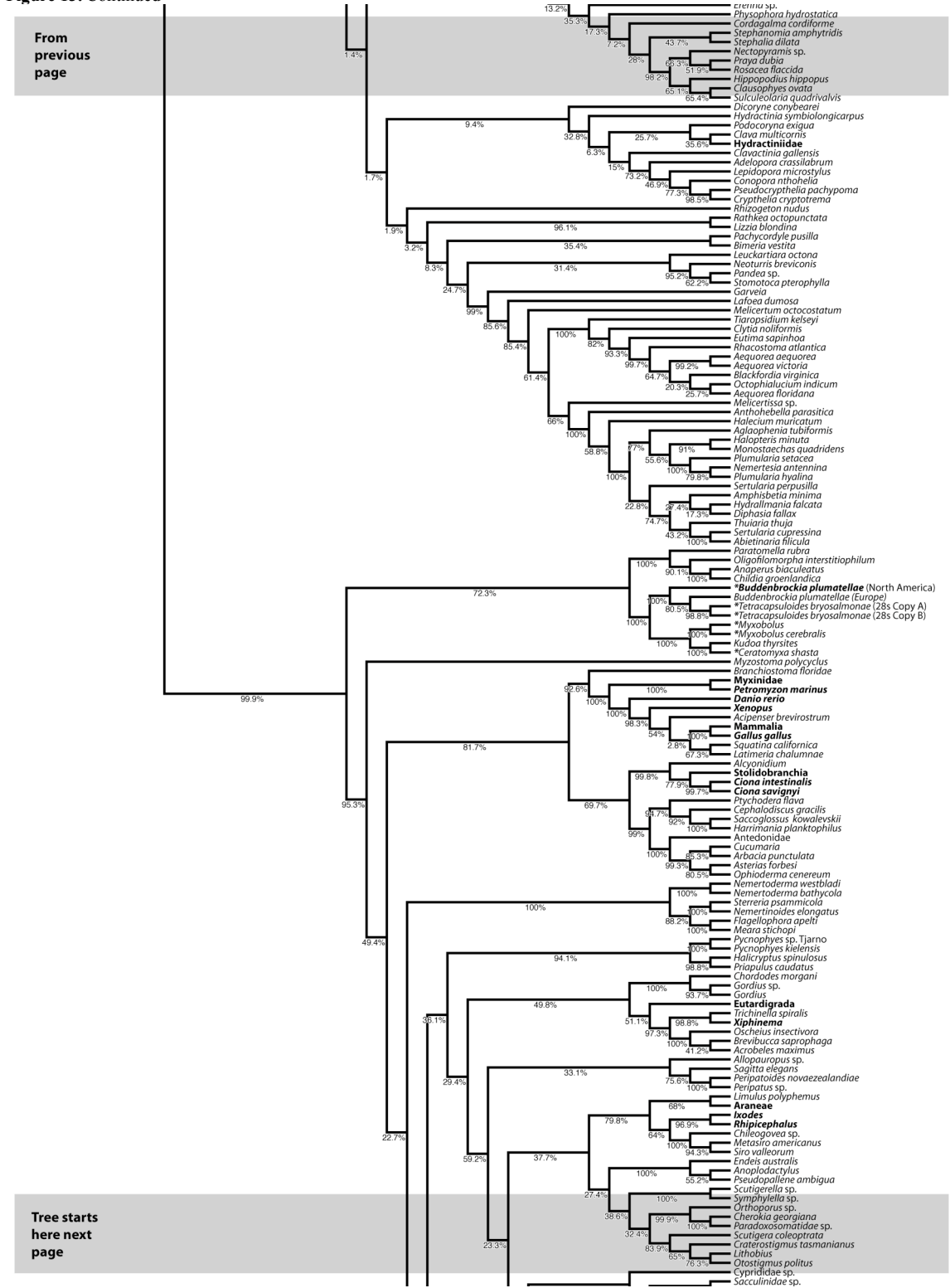


Figure 13. Continued

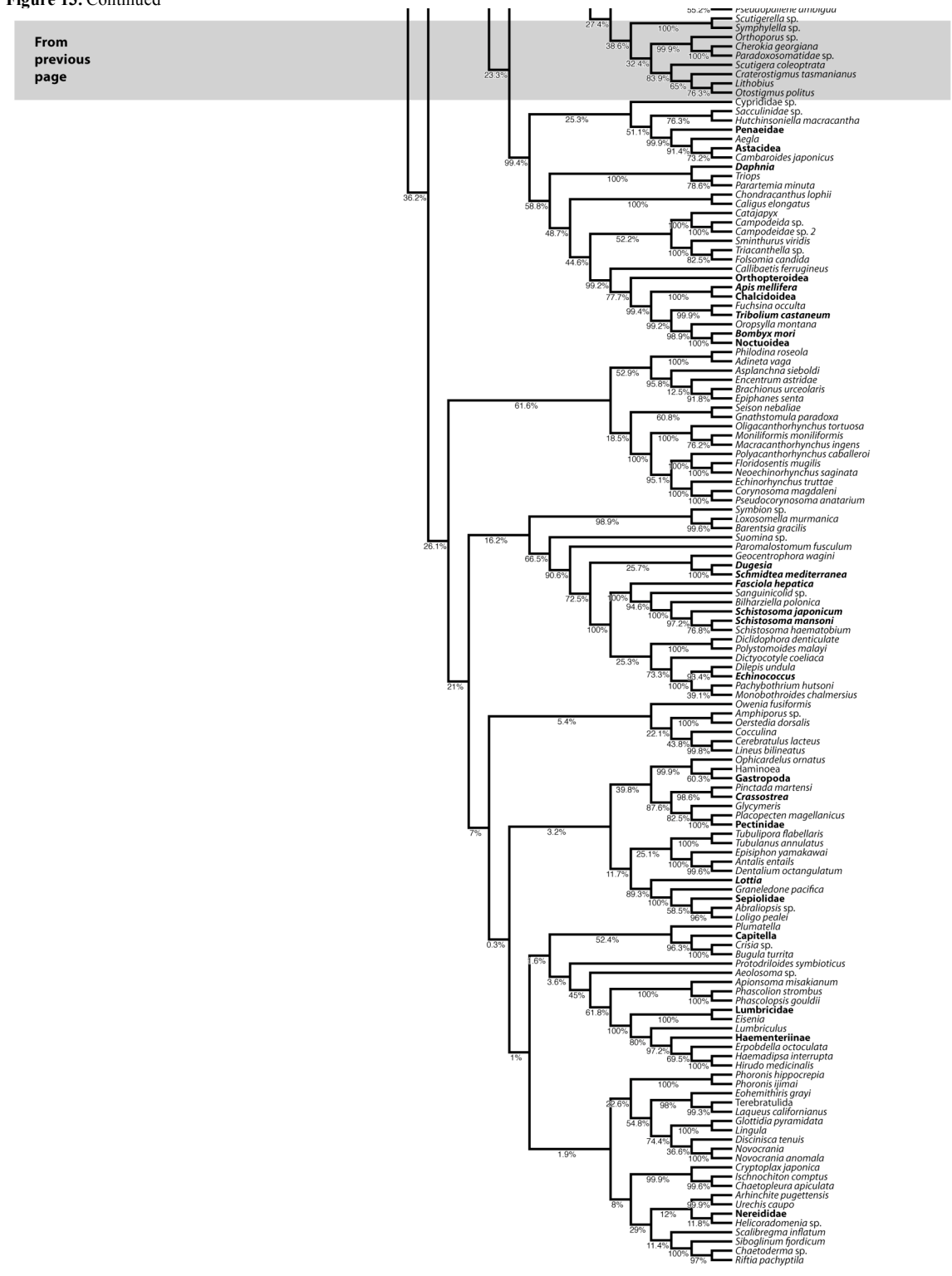


Table 4. Summary of rDNA data sampled from 389 opisthokont taxa.

Classification	Combined rDNA Taxon Designation	18S rDNA Taxa	18S GB# (seq. length - bps)	28S rDNA Taxa	28S GB# (seq. length - bps)		
Fungi	<i>Batrachochytrium dendrobatidis</i>	<i>Batrachochytrium dendrobatidis</i>	AH009052 (1634)	<i>Batrachochytrium dendrobatidis</i>	EY546693 (2943)		
	<i>Blastocladiella emersonii</i>	<i>Blastocladiella emersonii</i>	AY635842 (1882)	<i>Blastocladiella emersonii</i>	DQ273808 (3198)		
	<i>Cryptococcus</i>	<i>Cryptococcus</i> sp.	EF363152 (1862)	<i>Cryptococcus neoformanis</i>	L14067 (3392)		
	<i>Glomus intraradices</i>	<i>Glomus intraradices</i>	DQ322630 (1791)	<i>Glomus intraradices</i>	DQ273828 (3266)		
	<i>Rhizopus</i>	<i>Rhizopus oryzae</i>	AB250174 (1757)	<i>Rhizopus stolonifer</i>	DQ273817 (3219)		
	<i>Spizellomyces punctatus</i>	<i>Spizellomyces punctatus</i>	AY546684 (2620)	<i>Spizellomyces punctatus</i>	AY546692 (3791)		
	<i>Ustilago</i>	<i>Ustilago tritici</i>	DQ846895 (2111)	<i>Ustilago tritici</i>	DQ094784 (1399)		
Ichthyosporae	<i>Amoebidium parasiticum</i>	<i>Amoebidium parasiticum</i>	Y19155 (1797)	<i>Amoebidium parasiticum</i>	EU011932 (3206)		
	<i>Capsaspora owczarzakii</i>	<i>Capsaspora owczarzakii</i>	AY363957 (1777)	<i>Capsaspora owczarzakii</i>	AY274688 (3414)		
	<i>Corallochytrium limacisporum</i>	<i>Corallochytrium limacisporum</i>	L42528 (1787)	<i>Corallochytrium limacisporum</i>	EU011936 (3121)		
	<i>Ichthyophonus hoferi</i>	<i>Ichthyophonus hoferi</i>	U25637 (1808)	<i>Ichthyophonus hoferi</i>	AY026370 (3045)		
	<i>Sphaerofoma arctica</i>	<i>Sphaerofoma arctica</i>	Y16260 (1728)	-missing-	-		
Choanoflagellida	<i>Salpingoecidae</i>	<i>Choanoea perplexa</i>	AY149898 (1768)	<i>Choanoea perplexa</i>	EU011937 (3117)		
		<i>Salpingoeca infusionum</i>	AF100941 (1978)	<i>Salpingoeca infusionum</i>	EU026380 (3383)		
		<i>Salpingoeca pyxidium</i>	EU011930 (1824)	<i>Salpingoeca pyxidium</i>	EU011946 (3259)		
	<i>Acanthoecidae</i>	<i>Acanthoeca spectabilis</i>	AF084233 (1740)	<i>Acanthoeca spectabilis</i>	EU011933 (3417)		
	<i>Diaphanoeca grandis</i>	AF084234 (1794)	<i>Diaphanoeca grandis</i>	EU011939 (3245)			
	<i>Stephanoea diplocostata</i>	EU011927 (1818)	<i>Stephanoea diplocostata</i>	EU011947 (2781)			
<i>Codonosigidae</i>	<i>Monosiga brevicollis</i>	<i>Monosiga brevicollis</i>	AF100943 (1796)	<i>Monosiga brevicollis</i>	AY026374 (3261)		
	<i>Monosiga ovata</i>	<i>Monosiga ovata</i>	AF084230 (1765)	<i>Monosiga ovata</i>	EU011940 (3557)		
	<i>Proterospongia</i>	<i>Proterospongia choanojuncta</i>	AY149896 (1770)	<i>Proterospongia</i> sp.	EU011941 (3319)		
	<i>Codonosiga gracilis</i>	<i>Codonosiga gracilis</i>	AY149897 (1748)	<i>Codonosiga gracilis</i>	EU011935 (3376)		
Porifera	<i>Calcarea</i>	<i>Leucosolenia</i> sp.	AF100945 (1817)	<i>Leucosolenia</i> sp.	AY026372 (3308)		
	<i>Demospongiae</i>	<i>Haliclona</i> sp.	AY734444 (1983)	<i>Haliclona</i> sp.	AY561862 (1039)		
		<i>Mycala fibroxillii</i>	AF100946 (1831)	<i>Mycala fibroxillii</i>	AY026376 (3571)		
		<i>Plakinidae</i>	<i>Plakortis</i> sp.	AF100948 (1813)	<i>Plakinastrella</i> sp.	AY561870 (1006)	
		<i>Prosuberites laughlini</i>	EF654529 (1660)	<i>Prosuberites laughlini</i>	AY626320 (3283)		
		<i>Suberites domuncula</i>	AJ620112 (1738)	<i>Suberites domuncula</i>	AJ620113 (3039)		
	<i>Hexactinellida</i>	<i>Acanthascus dawsoni</i>	<i>Acanthascus dawsoni</i>	AF100949 (2006)	<i>Acanthascus dawsoni</i>	AY026379 (3808)	
		<i>Beroe ovata</i>	<i>Beroe ovata</i> gene	AF293694 (1801)	<i>Beroe ovata</i>	AY026369 (2966)	
		<i>Mnemopsis leidyi</i>	<i>Mnemopsis leidyi</i>	AF293700 (1803)	<i>Mnemopsis leidyi</i>	AY026373 (3093)	
		<i>Pleurobrachia bachei</i>	<i>Pleurobrachia bachei</i>	AF293677 (1801)	<i>Pleurobrachia bachei</i>	AY026378 (3084)	
	Placozoa	<i>Trichoplax</i> sp.	<i>Trichoplax</i> sp. H7	AY652579 (1775)	<i>Trichoplax</i> sp.	AY652586 (3147)	
	Cnidaria	<i>Anthozoa</i>	<i>Alcyonium digitatum</i>	<i>Alcyonium digitatum</i>	Unpublished (1745)	<i>Alcyonium digitatum</i> .ALDI SU91D5	Unpublished (3266)
			<i>Anthelia glauca</i>	<i>Anthelia glauca</i>	Unpublished (1744)	<i>Anthelia glauca</i> .ANVT133	Unpublished (3247)
			<i>Anthoplilum grandiflorum</i>	<i>Anthoplilum grandiflorum</i>	Unpublished (1721)	<i>Anthoplilum grandiflorum</i> .ANGR05	Unpublished (3251)
		<i>Antipathes galapagensis</i>	<i>Antipathes galapagensis</i>	AF100943 (1796)	<i>Antipathes galapagensis</i>	Unpublished (3157)	
		<i>Corynactis Daly</i>	<i>Corynactis Daly</i>	Unpublished (1733)	<i>Corynactis285Daly</i>	Unpublished (3356)	
		<i>Gorogonia flabellum</i>	<i>Gorogonia flabellum</i> GOR36	Unpublished (1745)	<i>Gorogonia flabellum</i> .GOR36	Unpublished (3256)	
		<i>Haloclava</i>	<i>Haloclava</i>	Unpublished (1782)	<i>Haloclava</i>	Unpublished (3305)	
		<i>Helopora coerulea</i>	<i>Helopora coerulea</i> HEL77	Unpublished (1721)	<i>Helopora coerulea</i> .HEL77	Unpublished (3249)	
		<i>Ideogorgia capensis</i>	<i>Ideogorgia capensis</i>	Unpublished (1722)	<i>Ideogorgia capensis</i> .IDEC0215	Unpublished (3258)	
		<i>Metridium</i>	<i>Metridium</i> sp.	AF052889 (1800)	<i>Metridium</i>	Unpublished (3275)	
		<i>Montastraea franki</i>	<i>Montastraea franki</i>	AY026382 (1838)	<i>Montastraea franki</i>	AY026375 (3162)	
		<i>Nematostella vectensis</i>	<i>Nematostella vectensis</i>	AF254382 (1723)	<i>Nematostella vectensis</i>	AY345871 (1018)	
		<i>Pterogorgia anceps</i>	<i>Pterogorgia anceps</i> PTAN88	Unpublished (1745)	<i>Pterogorgia anceps</i> .PTAN88	Unpublished (3252)	
		<i>Siderastrea siderea</i>	<i>Siderastrea siderea</i>	Unpublished (1754)	<i>Siderastrea siderea</i>	Unpublished (3157)	
		<i>Trissopathes pseudotristricha</i>	<i>Trissopathes pseudotristricha</i>	Unpublished (1754)	<i>Trissopathes pseudotristricha</i>	Unpublished (3265)	
		<i>Stephanococcia michelinii</i>	-missing-	-	<i>Stephanococcia michelinii</i>	Unpublished (3345)	
<i>Cubozoa</i>		<i>Carybdea rastonii</i>	<i>Carybdea rastonii</i>	AF358108 (1821)	<i>Carybdea rastonii</i>	AY920787 (3271)	
		<i>Chironex fleckeri</i>	<i>Chironex fleckeri</i>	AF358104 (1824)	<i>Chironex fleckeri</i>	AY920785 (3270)	
		<i>Chiropsalmus</i> sp.	<i>Chiropsalmus</i> sp.	AF358103 (1823)	<i>Chiropsalmus</i> sp. AGC2001	AY920786 (3270)	
		<i>Darwin carybdeid</i> sp.	<i>Darwin carybdeid</i> sp.	AF358105 (1825)	<i>Darwin carybdeid</i> sp. AGC2001	AY920788 (3248)	
<i>Staurozoa</i>		<i>Tripedalia cystophora</i>	<i>Tripedalia cystophora</i>	EU272637 (1706)	<i>Tripedalia cystophora</i>	EU272595 (3028)	
		<i>Craterolophus convolvulus</i>	<i>Craterolophus convolvulus</i>	AY845344 (1744)	<i>Craterolophus convolvulus</i>	AY920781 (3245)	
<i>Scyphozoa</i>		<i>Halicystus octoradiatus</i>	<i>Halicystus octoradiatus</i>	AY845346 (1755)	<i>Halicystus octoradiatus</i>	AH014894 (3222)	
		<i>Halicystus sanjuanensis</i>	<i>Halicystus sanjuanensis</i>	AF358102 (1820)	<i>Halicystus sanjuanensis</i>	AY920782 (3265)	
		<i>Cyanea</i>	<i>Cyanea</i> sp. AGC-2001	AF358097 (1832)	-missing-	-	
		<i>Atolla vanhoeffeni</i>	<i>Atolla vanhoeffeni</i>	AF100942 (1815)	<i>Atolla vanhoeffeni</i>	AY026368 (3270)	
		<i>Aurelia</i> sp.	<i>Aurelia</i> sp.	EU272604 (1680)	<i>Aurelia</i> sp. PC2008	EU272547 (3174)	
		<i>Catostylus</i> sp.	<i>Catostylus</i> sp.	AF358100 (1834)	<i>Catostylus</i> sp. AGC2001	AY920777 (2853)	
<i>Hydrozoa</i>		<i>Chrysaora melanaster</i>	<i>Chrysaora melanaster</i>	AF358099 (1831)	<i>Chrysaora melanaster</i>	AY920780 (3259)	
		<i>Chrysaora</i> sp.	<i>Chrysaora</i> sp.	AY920769 (1797)	<i>Chrysaora</i> sp. AGC2005	AY920779 (3260)	
		<i>Nausithoe rubra</i>	<i>Nausithoe rubra</i>	AF358095 (1826)	<i>Nausithoe rubra</i>	AY920776 (3271)	
		<i>Phacellophora camtschatica</i>	<i>Phacellophora camtschatica</i>	AF358096 (1833)	<i>Phacellophora camtschatica</i>	AY920778 (3263)	
		<i>Abietinaria filicula</i>	<i>Abietinaria filicula</i>	EU272600 (1786)	<i>Abietinaria filicula</i>	EU272540 (3154)	
		<i>Adelopora crassilabrum</i>	<i>Adelopora crassilabrum</i>	EU272642 (1698)	<i>Adelopora crassilabrum</i>	EU272541 (3222)	
		<i>Aegina citrea</i>	<i>Aegina citrea</i>	AF358058 (1820)	<i>Aegina citrea</i>	AY920789 (3256)	
<i>Aequorea aequorea</i>		<i>Aequorea aequorea</i>	AF358076 (1832)	<i>Aequorea aequorea</i>	EU305505 (3095)		
		<i>Aequorea florida</i>	-missing-	-	<i>Aequorea florida</i>	EU305506 (3175)	
		<i>Aequorea victoria</i>	<i>Aequorea victoria</i>	AF358077 (1851)	<i>Aequorea victoria</i>	AY920799 (3266)	
		<i>Aqantha diqitale</i>	<i>Aqantha diqitale</i>	EU247821 (1738)	<i>Aqantha diqitale</i>	AY920791 (3261)	
		<i>Aqlophenia tubiformis</i>	<i>Aqlophenia tubiformis</i>	EU272601 (1774)	<i>Aqlophenia tubiformis</i>	EU272543 (3234)	
		<i>Aglaura hemistoma</i>	<i>Aglaura hemistoma</i>	EU247818 (1702)	<i>Aglaura hemistoma</i>	EU247802 (3187)	
		<i>Aglauroopsis aora</i>	<i>Aglauroopsis aora</i>	AY920754 (1711)	<i>Aglauroopsis aora</i>	AY920793 (3245)	
		<i>Amphibeta minima</i>	<i>Amphibeta minima</i>	EU272602 (1688)	<i>Amphibeta minima</i>	EU272544 (3092)	
		-missing-	-	-	<i>Amphogona apicata</i>	EU247801 (3215)	
		<i>Anthobebella parasitica</i>	<i>Anthobebella parasitica</i>	EU272603 (1782)	<i>Anthobebella parasitica</i>	EU272545 (3022)	
		<i>Apolemia</i> sp.	<i>Apolemia</i> sp.	AY937331 (1755)	<i>Apolemia</i> sp.	EU272546 (3104)	
		<i>Astrohydrá iaponica</i>	-missing-	-	<i>Astrohydrá iaponica</i>	AY920794 (3270)	
		<i>Bimeria vestita</i>	<i>Bimeria vestita</i>	EU272605 (1756)	<i>Bimeria vestita</i>	EU272548 (3200)	
	<i>Blackfordia virginica</i>	<i>Blackfordia virginica</i>	AF358078 (1854)	<i>Blackfordia virginica</i>	AY920800 (3268)		
	<i>Botrynuma brucei</i>	<i>Botrynuma brucei</i>	EU247822 (1734)	<i>Botrynuma brucei</i>	EU247798 (3209)		
	<i>Bougainvillia carolinensis</i>	<i>Bougainvillia carolinensis</i>	EU272606 (1759)	<i>Bougainvillia carolinensis</i>	EU272549 (3167)		
	<i>Bougainvillia fulva</i>	<i>Bougainvillia fulva</i>	EU305490 (1718)	<i>Bougainvillia fulva</i>	EU305507 (3152)		
	<i>Brinckmannia hexactinellidophila</i>	<i>Brinckmannia hexactinellidophila</i>	EU272607 (1760)	<i>Brinckmannia hexactinellidophila</i>	EU272550 (3198)		
	<i>Candelabrum coxii</i>	<i>Candelabrum coxii</i>	AY920758 (1814)	<i>Candelabrum coxii</i>	AY920796 (3267)		
	<i>Cladocoryne floccosa</i>	<i>Cladocoryne floccosa</i>	EU272608 (1689)	<i>Cladocoryne floccosa</i>	EU272551 (3263)		
	<i>Clausophyes ovata</i>	<i>Clausophyes ovata</i>	AY937336 (1755)	<i>Clausophyes ovata</i>	EU305508 (3051)		
	<i>Clava multicornis</i>	<i>Clava multicornis</i>	EU272609 (1756)	<i>Clava multicornis</i>	EU272552 (3164)		
	<i>Clavactinia galliensis</i>	<i>Clavactinia galliensis</i>	EU272610 (1757)	<i>Clavactinia galliensis</i>	EU272553 (3015)		
	<i>Clytia noliformis</i>	<i>Clytia noliformis</i>	EU272611 (1785)	<i>Clytia noliformis</i>	EU272554 (3057)		
	<i>Conopora anthohelia</i>	<i>Conopora anthohelia</i>	EU645429 (1704)	<i>Conopora anthohelia</i>	EU305509 (3092)		
	<i>Cordaqlma cordiforme</i>	<i>Cordaqlma cordiforme</i>	AY937317 (1755)	<i>Cordaqlma cordiforme</i>	EU272555 (3171)		
	<i>Cordylphora caspia</i>	<i>Cordylphora caspia</i>	EU272612 (1757)	<i>Cordylphora caspia</i> Sch485	EU272556 (3205)		
	<i>Corydendrium</i> sp.	<i>Corydendrium</i> sp.	EU272613 (1673)	<i>Corydendrium</i> sp. PC2008	EU272557 (3200)		
	<i>Corymorpha bigelowi</i>	<i>Corymorpha bigelowi</i>	EU272618 (1678)	<i>Corymorpha bigelowi</i>	EU272553 (3108)		
	<i>Corymorpha</i> sp.	<i>Corymorpha</i> sp.	EU305494 (1711)	<i>Corymorpha</i> sp. PC2008	EU305510 (3057)		
	<i>Crossota rufo-brunnea</i>	<i>Crossota rufo-brunnea</i>	EU247824 (1737)	<i>Crossota rufo-brunnea</i>	EU247800 (3259)		
	<i>Crypthelia cryptotrema</i>	<i>Crypthelia cryptotrema</i>	EU272641 (1740)	<i>Crypthelia cryptotrema</i>	EU272558 (3143)		

Table 4. Continued

Classification	Combined rDNA Taxon Designation	18s rDNA Taxa	18S GB# (seq. length - bps)	28s rDNA Taxa	28S GB# (seq. length - bps)	
Hydrozoa (cont.)	<i>Dicoryne conybeari</i>	<i>Dicoryne conybeari</i>	EU272614 (1707)	<i>Dicoryne conybeari</i>	EU272559 (3193)	
	<i>Diphasia fallax</i>	<i>Diphasia fallax</i>	EU305491 (1818)	<i>Diphasia fallax</i>	EU305511 (3214)	
	<i>Dipurena ophiogaster</i>	<i>Dipurena ophiogaster</i>	EU272615 (1710)	<i>Dipurena ophiogaster</i>	EU272560 (3130)	
	<i>Ectopleura dumortieri</i>	<i>Ectopleura dumortieri</i>	EU272616 (1757)	<i>Ectopleura dumortieri</i>	EU272561 (3246)	
	<i>Erenna</i> sp.	<i>Erenna</i> sp.	A937361 (1755)	<i>Erenna</i> sp.	EU305512 (2527)	
	<i>Eudendrium californicum</i>	<i>Eudendrium californicum</i>	EU305492 (1702)	<i>Eudendrium californicum</i>	EU305513 (3144)	
	-missing-	-missing-	-	<i>Eudendrium capillare</i>	EU305514 (3146)	
	<i>Eudendrium racemosum</i>	<i>Eudendrium racemosum</i>	EU272617 (1700)	<i>Eudendrium racemosum</i>	EU272562 (3226)	
	<i>Eutima sapinhoa</i>	<i>Eutima sapinhoa</i>	EU305493 (1828)	<i>Eutima sapinhoa</i>	EU305515 (3181)	
	<i>Fabienna sphaerica</i>	<i>Fabienna sphaerica</i>	A920767 (1795)	<i>Fabienna sphaerica</i>	A920797 (3235)	
	<i>Forskalia edwardsi</i>	<i>Forskalia edwardsi</i>	A937320 (1755)	<i>Forskalia edwardsi</i>	EU305516 (2565)	
	<i>Garveia</i>	<i>Garveia</i> sp.	A920766 (1782)	<i>Garveia annulata</i>	EU272564 (3152)	
	<i>Garveia arisea</i>	<i>Garveia arisea</i>	EU272632 (1763)	<i>Garveia arisea</i>	EU272588 (3205)	
	<i>Geryonia proboscidalis</i>	<i>Geryonia proboscidalis</i>	EU247816 (1724)	<i>Geryonia proboscidalis</i>	EU247807 (3129)	
	<i>Halecium muricatum</i>	<i>Halecium muricatum</i>	EU272619 (1694)	<i>Halecium muricatum</i>	EU272565 (2606)	
	<i>Haliscera conica</i>	<i>Haliscera conica</i>	AF358064 (1818)	<i>Haliscera conica</i>	EU247797 (3156)	
	<i>Halistermia rubrum</i>	<i>Halistermia rubrum</i>	A937358 (1755)	<i>Halistermia rubrum</i>	EU272566 (3205)	
	<i>Halopteris minuta</i>	<i>Halopteris minuta</i>	EU272620 (1793)	<i>Halopteris minuta</i>	EU272567 (3199)	
	<i>Hippododius hippopus</i>	<i>Hippododius hippopus</i>	A937341 (1755)	<i>Hippododius hippopus</i>	EU305517 (3073)	
	<i>Hydra</i>	<i>Hydra circumcincta</i>	AF358080 (1822)	<i>Hydra circumcincta</i>	A9026371 (3297)	
	<i>Hydractinia</i> sp.	<i>Hydractinia</i> sp.	EU305495 (1778)	<i>Hydractinia</i> sp. PC2008	EU305518 (3210)	
	<i>Hydractinia symbiolonacarpus</i>	<i>Hydractinia symbiolonacarpus</i>	EU272621 (1696)	<i>Hydractinia symbiolonacarpus</i>	EU272568 (3250)	
	<i>Hydrallmania falcata</i>	-missing-	-	<i>Hydrallmania falcata</i>	EU305519 (3176)	
	<i>Hydrichthella epigorgia</i>	<i>Hydrichthella epigorgia</i>	EU272622 (1718)	<i>Hydrichthella epigorgia</i>	EU272569 (3188)	
	<i>Koellikerina fasciculata</i>	<i>Koellikerina fasciculata</i>	EU272623 (1762)	<i>Koellikerina fasciculata</i>	EU272571 (3197)	
	<i>Lafeoa dumosa</i>	-missing-	-	<i>Lafeoa dumosa</i>	EU305520 (3026)	
	<i>Lepidopora microstylus</i>	<i>Lepidopora microstylus</i>	EU272644 (1709)	<i>Lepidopora microstylus</i>	EU272572 (3072)	
	<i>Leuckartia octona</i>	<i>Leuckartia octona</i>	EU272624 (1761)	<i>Leuckartia octona</i>	EU272573 (3101)	
	<i>Limnoccidia tanganyicae</i>	<i>Limnoccidia tanganyicae</i>	A920755 (1781)	<i>Limnoccidia tanganyicae</i>	EU272574 (3175)	
	<i>Lizzia blondina</i>	<i>Lizzia blondina</i>	EU272625 (1756)	<i>Lizzia blondina</i>	EU272574 (3200)	
	<i>Maeotias marinata</i>	<i>Maeotias marinata</i>	AF358056 (1816)	<i>Maeotias marinata</i>	EU247810 (3134)	
	<i>Melicertissa</i> sp.	<i>Melicertissa</i> sp.	AF358075 (1828)	<i>Melicertissa</i> sp.	A920798 (3262)	
	<i>Melicertum octocostatum</i>	<i>Melicertum octocostatum</i>	A920757 (1815)	<i>Melicertum octocostatum</i>	EU272575 (3133)	
	<i>Moerisia</i> sp.	<i>Moerisia</i> sp.	AF358083 (1821)	<i>Moerisia</i> sp.	A920801 (3231)	
	<i>Monostachias quadridens</i>	<i>Monostachias quadridens</i>	EU305497 (1837)	<i>Monostachias quadridens</i>	EU305521 (3186)	
	<i>Nanomia bijuga</i>	<i>Nanomia bijuga</i>	A937338 (1755)	<i>Nanomia bijuga</i>	EU272576 (3193)	
	<i>Nectopyramis</i> sp.	<i>Nectopyramis</i> sp.	AF358068 (1818)	<i>Nectopyramis</i> sp.	A9026377 (3262)	
	<i>Nemertesia antennina</i>	<i>Nemertesia antennina</i>	EU305498 (1680)	<i>Nemertesia antennina</i>	EU305523 (3173)	
	<i>Neoturrus brevicornis</i>	<i>Neoturrus brevicornis</i>	EU448097 (1787)	<i>Neoturrus brevicornis</i>	EU305524 (3131)	
	<i>Octophialucium indicum</i>	<i>Octophialucium indicum</i>	EU272626 (1790)	<i>Octophialucium indicum</i>	EU272577 (3265)	
	<i>Olindias phosphorica</i>	<i>Olindias phosphorica</i>	A920753 (1763)	<i>Olindias phosphorica</i>	EU274808 (3198)	
	<i>Olindias sambaquiensis</i>	<i>Olindias sambaquiensis</i>	EU247814 (1739)	<i>Olindias sambaquiensis</i>	EU247809 (3166)	
	<i>Pachycordyle pusilla</i>	<i>Pachycordyle pusilla</i>	EU272627 (1756)	<i>Pachycordyle pusilla</i>	EU272579 (3193)	
	<i>Pandea</i> sp.	<i>Pandea</i> sp.	A920765 (1824)	<i>Pandea</i> sp.	EU272580 (3126)	
	<i>Pantachogon haeckeli</i>	<i>Pantachogon haeckeli</i>	AF358062 (1824)	<i>Pantachogon haeckeli</i>	A920792 (3261)	
	<i>Pantachogon</i> sp. orange	-missing-	-	<i>Pantachogon</i> sp. orange	EU247806 (3207)	
	<i>Pantachogon</i> sp. white	<i>Pantachogon</i> sp. white	EU247817 (1738)	<i>Pantachogon</i> sp. white	EU247805 (3158)	
	<i>Pennaria disticha</i>	<i>Pennaria disticha</i>	A920762 (1776)	<i>Pennaria disticha</i>	EU272581 (3115)	
	<i>Physalia physalis</i>	<i>Physalia physalis</i>	AF358065 (1824)	<i>Physalia physalis</i>	EU448095 (3093)	
	<i>Physophora hydrostatica</i>	<i>Physophora hydrostatica</i>	A937342 (1755)	<i>Physophora hydrostatica</i>	EU272582 (3207)	
	<i>Plumularia hyalina</i>	<i>Plumularia hyalina</i>	EU305499 (1728)	<i>Plumularia hyalina</i>	EU305525 (3062)	
	<i>Plumularia setacea</i>	<i>Plumularia setacea</i>	EU272628 (1780)	<i>Plumularia setacea</i>	EU305526 (3204)	
	<i>Podocoryna exigua</i>	<i>Podocoryna exigua</i>	AF358092 (1817)	<i>Podocoryna exigua</i>	A920802 (3247)	
	<i>Porpita</i> sp.	<i>Porpita</i> sp.	AF358086 (1823)	<i>Porpita</i> sp.	A920803 (3244)	
	<i>Praya dubia</i>	<i>Praya dubia</i>	A937326 (1755)	<i>Praya dubia</i>	EU305526 (3006)	
	<i>Proboscidiactyla flavicirrata</i>	<i>Proboscidiactyla flavicirrata</i>	EU305500 (1705)	<i>Proboscidiactyla flavicirrata</i>	EU305527 (3158)	
	<i>Proboscidiactyla ornata</i>	<i>Proboscidiactyla ornata</i>	EU272631 (1752)	<i>Proboscidiactyla ornata</i>	EU272583 (3198)	
	<i>Pseudocryptothella pachypoma</i>	<i>Pseudocryptothella pachypoma</i>	EU272643 (1726)	<i>Pseudocryptothella pachypoma</i>	EU272589 (3198)	
	<i>Ralpharia gorgoniae</i>	<i>Ralpharia gorgoniae</i>	EU272633 (1654)	<i>Ralpharia gorgoniae</i>	EU272590 (3162)	
	<i>Rathkea octopunctata</i>	<i>Rathkea octopunctata</i>	EU272634 (1684)	<i>Rathkea octopunctata</i>	EU272591 (3139)	
	<i>Rhacostoma atlantica</i>	<i>Rhacostoma atlantica</i>	EU305501 (1814)	<i>Rhacostoma atlantica</i>	EU305528 (3188)	
	<i>Rhizoetone nudus</i>	<i>Rhizoetone nudus</i>	EU272635 (1761)	<i>Rhizoetone nudus</i>	EU272592 (3208)	
	<i>Rhopalonema velatum</i>	<i>Rhopalonema velatum</i>	EU247819 (1698)	<i>Rhopalonema velatum</i>	EU247804 (3208)	
	<i>Rosacea flaccida</i>	<i>Rosacea flaccida</i>	A937328 (1755)	<i>Rosacea flaccida</i>	EU305529 (3065)	
	<i>Sarsia nipponica</i>	<i>Sarsia nipponica</i>	EU448096 (1799)	<i>Sarsia nipponica</i>	EU305530 (3131)	
	<i>Scrippsia pacifica</i>	<i>Scrippsia pacifica</i>	AF358091 (1828)	<i>Scrippsia pacifica</i>	A920804 (3265)	
	<i>Sertularia cupressina</i>	-missing-	-	<i>Sertularia cupressina</i>	EU305531 (3147)	
	<i>Sertularia perpusilla</i>	-missing-	-	<i>Sertularia perpusilla</i>	EU305532 (3183)	
	<i>Soliveddella</i> sp.	-missing-	-	<i>Soliveddella</i> sp.	EU247796 (3226)	
	<i>Solanderia ericopsis</i>	<i>Solanderia ericopsis</i>	EU272636 (1662)	<i>Solanderia ericopsis</i>	EU272593 (3083)	
	<i>Solanderia secunda</i>	<i>Solanderia secunda</i>	EU305502 (1693)	<i>Solanderia secunda</i>	EU305533 (3121)	
	<i>Solmissus marshali</i>	<i>Solmissus marshali</i>	AF358060 (1814)	<i>Solmissus marshali</i>	A920790 (3270)	
	<i>Solmundella bitentaculata</i>	<i>Solmundella bitentaculata</i>	EU247812 (1737)	<i>Solmundella bitentaculata</i>	EU247795 (3180)	
	<i>Stephalia dilatata</i>	<i>Stephalia dilatata</i>	A937357 (1755)	<i>Stephalia dilatata</i>	EU305534 (3034)	
	<i>Stephanomia amphitridis</i>	<i>Stephanomia amphitridis</i>	A937322 (1755)	-missing-	-	
	<i>Stomotoca pterophylla</i>	<i>Stomotoca pterophylla</i>	EU305496 (1790)	<i>Stomotoca pterophylla</i>	EU272570 (3199)	
	<i>Sulculeolaria quadrialvis</i>	<i>Sulculeolaria quadrialvis</i>	A937353 (1755)	<i>Sulculeolaria quadrialvis</i>	EU272594 (3176)	
	<i>Tetraplatia voltans</i>	<i>Tetraplatia voltans</i>	DQ002501 (1817)	<i>Tetraplatia voltans</i>	DQ002502 (3255)	
	<i>Thuaria thuja</i>	<i>Thuaria thuja</i>	EU305503 (1728)	<i>Thuaria thuja</i>	EU305536 (3184)	
	<i>Turritopsis dohrnii</i>	<i>Turritopsis dohrnii</i>	EU272638 (1762)	<i>Turritopsis dohrnii</i>	EU272596 (3203)	
	<i>Turritopsis nutricula</i>	<i>Turritopsis nutricula</i>	EU305504 (1793)	<i>Turritopsis nutricula</i>	EU305538 (3205)	
	<i>Velella</i> sp.	<i>Velella</i> sp.	AF358087 (1824)	<i>Velella</i> sp. PC2008	EU272597 (3135)	
	<i>Zanclea prolifer</i>	<i>Zanclea prolifer</i>	EU272639 (1672)	<i>Zanclea prolifer</i>	EU272598 (3138)	
	<i>Zyzyzus</i>	<i>Zyzyzus calderi</i>	EU272640 (1675)	<i>Zyzyzus warreni</i>	EU272599 (3154)	
	<i>Tiaropsidium kelseyi</i>	<i>Tiaropsidium kelseyi</i>	AF358079 (1838)	<i>Tiaropsidium kelseyi</i>	EU305537 (3187)	
	Myxozoa					
	Malacosporae	<i>Buddenbrockia plumatellae</i>	<i>Buddenbrockia plumatellae</i>	FJ981824 (1734)	<i>Buddenbrockia plumatellae</i>	FJ981817 (3393)
		<i>Buddenbrockia plumatellae</i> -(North America)			-missing-	-
		<i>Buddenbrockia plumatellae</i> -(Europe)		A9074914 (1793)		
		<i>Tetracapsuloides bryosalmonae</i> -(CopyA)	<i>Tetracapsuloides bryosalmonae</i>	FJ981823 (1801)	<i>T. bryosalmonae</i> (CopyA)	FJ981821 (3279)
		<i>Tetracapsuloides bryosalmonae</i> -(CopyB)	<i>Tetracapsuloides bryosalmonae</i>	FJ981823 (1801)	<i>T. bryosalmonae</i> (CopyB)	FJ981822 (3256)
	Myxosporeae	<i>Ceratomyxa shasta</i>	<i>Ceratomyxa shasta</i>	AF001579 (1643)	<i>Ceratomyxa shasta</i> NE	FJ981818 (3241)
	<i>Kudoa thyrssites</i>	<i>Kudoa thyrssites</i>	A941819 (1718)	<i>Kudoa thyrssites</i>	AY941819 (3543)	
	<i>Myxobolus</i>	<i>Myxobolus pellicides</i>	AF378339 (2027)	<i>Myxobolus notropis</i>	FJ981819 (3537)	
	<i>Myxobolus cerebralis</i>	<i>Myxobolus cerebralis</i>	U96492 (1937)	<i>Myxobolus cerebralis</i>	FJ981820 (3595)	
Acoela						
	<i>Anaperus biaculeatus</i>	<i>Anaperus biaculeatus</i>	AJ012527 (1662)	<i>Anaperus biaculeatus</i>	AY157602 (3148)	
	<i>Childia groenlandica</i>	<i>Childia groenlandica</i>	AJ012529 (1782)	<i>Childia groenlandica</i>	AY157603 (3161)	
	<i>Oligofilomorpha interstitiophilum</i>	<i>Oligofilomorpha interstitiophilum</i>	AM701823 (1745)	<i>Oligofilomorpha interstitiophilum</i>	AM701824 (3037)	
	<i>Paratomella rubra</i>	<i>Paratomella rubra</i>	AF102892 (1727)	<i>Paratomella rubra</i>	AY157604 (2979)	
Nemertodermatida						
	<i>Flaellophora apelti</i>	<i>Flaellophora apelti</i>	AM747471 (1753)	<i>Flaellophora apelti</i>	AM747472 (2472)	
	<i>Meara stichopi</i>	<i>Meara stichopi</i>	AF119085 (1768)	<i>Meara stichopi</i>	AY157605 (3350)	
	<i>Nemertinoidea elonquatus</i>	<i>Nemertinoidea elonquatus</i>	A9078381 (1724)	<i>Nemertinoidea elonquatus</i>	AM747476 (3161)	

Table 4. Continued

Classification	Combined rDNA Taxon Designation	18s rDNA Taxa	18S GB# (seq. length - bps)	28s rDNA Taxa	28S GB# (seq. length - bps)
Nemertodermatida (cont.)	<i>Nemertoderma bathycyola</i>	<i>Nemertoderma bathycyola</i>	AM747477 (1712)	<i>Nemertoderma bathycyola</i>	AM747478 (3426)
	<i>Nemertoderma westbladi</i>	<i>Nemertoderma westbladi</i>	AM747481 (1669)	<i>Nemertoderma westbladi</i>	AM747482 (2483)
	<i>Sterreria psammicola</i>	<i>Sterreria psammicola</i>	AM747479 (1752)	<i>Sterreria psammicola</i>	AM747480 (3190)
DEUTEROSTOMIA					
Chordata					
Ascidacea	<i>Ciona intestinalis</i>	<i>Ciona intestinalis</i>	AB013017 (1756)	<i>Ciona intestinalis</i>	AF212177 (3326)
	<i>Ciona saviqvivi</i>	<i>Ciona saviqvivi</i>	AF165823 (1093)	-missing-	-
	<i>Moloula manhattensis</i>	<i>Moloula manhattensis</i>	L12426 (1804)	-missing-	-
	<i>Branchiostoma floridae</i>	<i>Branchiostoma floridae</i>	M97571 (1778)	<i>Branchiostoma floridae</i>	AF061796 (3530)
	<i>Epitretus stouti</i>	<i>Epitretus stouti</i>	M97572 (1959)	<i>Epitretus stouti</i>	AF061797 (4850)
	<i>Petromyzon marinus</i>	<i>Petromyzon marinus</i>	M97575 (1785)	<i>Petromyzon marinus</i>	AF061798 (3607)
	<i>Acipenser brevirostrum</i>	<i>Acipenser brevirostrum</i>	AF188383 (1772)	<i>Acipenser brevirostrum</i>	U34340 (3418)
	<i>Danio rerio</i>	<i>Danio rerio</i>	BX537263 (2029)	<i>Danio rerio</i>	BX537263 (3990)
	<i>Squatina californica</i>	<i>Squatina californica</i>	AY049858 (1775)	<i>Squatina californica</i>	AY049857 (3657)
	<i>Latimeria chalumnae</i>	<i>Latimeria chalumnae</i>	L11288 (1779)	<i>Latimeria chalumnae</i>	U34336 (3396)
	<i>Xenopus laevis</i>	<i>Xenopus laevis</i>	X02995 (1825)	<i>Xenopus laevis</i>	X02995 (4082)
	<i>Gallus gallus</i>	<i>Gallus gallus</i>	FM55425 (1809)	<i>Gallus gallus</i>	FM165415 (3119)
<i>Homo sapiens</i>	<i>Homo sapiens</i>	NR_003287 (1871)	<i>Homo sapiens</i>	NR_003287 (5035)	
Echinodermata	<i>Asterias forbesi</i>	<i>Asterias forbesii</i>	DQ060776 (1691)	<i>Asterias forbesi</i>	AF212169 (3682)
	<i>Antedonidae</i>	<i>Dorometra aegyptica</i>	AF088803 (1737)	<i>Florometra serratissima</i>	AF212168 (3334)
	<i>Arbacia punctulata</i>	<i>Arbacia punctulata</i>	DQ073778 (1768)	<i>Arbacia punctulata</i>	AY026367 (3416)
	<i>Cucumaria</i>	<i>Cucumaria elongata</i>	AY133479 (1874)	<i>Cucumaria salma</i>	AF212170 (3723)
	<i>Ophiuroidea</i>	<i>Ophioderma cenereum</i>	AY859645 (1770)	<i>Ophioderma cenereum</i>	AY859643 (3196)
Hemichordata	<i>Harrimania planktophilus</i>	<i>Harrimania planktophilus</i>	AF236799 (1871)	<i>Harrimania planktophilus</i>	AF212173 (3992)
	<i>Ptychodera flava</i>	<i>Ptychodera flava</i>	AF278681 (1782)	<i>Ptychodera flava</i>	AF212176 (3413)
	<i>Saccoglossus kowalevskii</i>	<i>Saccoglossus kowalevskii</i>	L28054 (1818)	<i>Saccoglossus kowalevskii</i>	AF212175 (3699)
Pterobranchia	<i>Cephalodiscus aracilis</i>	<i>Cephalodiscus aracilis</i>	AF236798 (1832)	<i>Cephalodiscus aracilis</i>	AF212172 (3654)
PROTOSTOMIA					
Acanthocephala					
Archiacanthocephala	<i>Macracanthorhynchus inqens</i>	<i>Macracanthorhynchus inqens</i>	AF001844 (1765)	<i>Macracanthorhynchus inqens</i>	AY829088 (2748)
	<i>Moniliformis moniliformis</i>	<i>Moniliformis moniliformis</i>	Z19562 (1769)	<i>Moniliformis moniliformis</i>	AY829086 (2738)
Eoacanthocephala	<i>Oligacanthorhynchus tortuosa</i>	<i>Oligacanthorhynchus tortuosa</i>	AF064817 (1767)	<i>Oligacanthorhynchus tortuosa</i>	AY210466 (3317)
	<i>Floridosenis mugilis</i>	<i>Floridosenis mugilis</i>	AF064811 (1760)	<i>Floridosenis mugilis</i>	AY829111 (2802)
Palaeacanthocephala	<i>Neoechinorhynchus saginata</i>	<i>Neoechinorhynchus saginata</i>	AY830150 (1745)	<i>Neoechinorhynchus saginata</i>	AY829091 (2863)
	<i>Corynosoma magdalenae</i>	<i>Corynosoma magdalenae</i>	EU267803 (1722)	<i>Corynosoma magdalenae</i>	EU267815 (2759)
	<i>Echinorhynchus truttae</i>	<i>Echinorhynchus truttae</i>	AY830156 (1729)	<i>Echinorhynchus truttae</i>	AY829097 (2721)
	<i>Pseudocorynosoma anatarium</i>	<i>Pseudocorynosoma anatarium</i>	EU267801 (1723)	<i>Pseudocorynosoma anatarium</i>	EU267813 (2781)
Polyacanthocephala	<i>Polvacanthorhynchus caballeroi</i>	<i>Polvacanthorhynchus caballeroi</i>	AF388660 (2176)	<i>Polvacanthorhynchus caballeroi</i>	DQ089738 (3406)
Annelida					
Clitellata	<i>Lumbriculus</i>	<i>Lumbriculus varieoatvus</i>	AF209457 (1780)	<i>Lumbriculus sp.</i>	DQ790040 (3326)
	<i>Lumbriculus</i>	<i>Lumbriculus terrestris</i>	AJ272183 (1813)	<i>Lumbriculus sp.</i>	DQ790041 (3404)
Hirudinida	<i>Erpobdella octoculata</i>	<i>Erpobdella octoculata</i>	AF116001 (1777)	<i>Erpobdella octoculata</i>	AY364865 (2366)
	<i>Haemadipsa interrupta</i>	<i>Haemadipsa interrupta</i>	EU100069 (1859)	<i>Haemadipsa interrupta</i>	EU100078 (2054)
	<i>Helobdella</i>	<i>Helobdella stagnalis</i>	AY962416 (1767)	<i>Helobdella stagnalis</i>	EF417050 (1003)
	<i>Hirudo medicinalis</i>	<i>Hirudo medicinalis</i>	AY786464 (1779)	<i>Hirudo medicinalis</i>	AY364866 (2314)
Oligochaeta	<i>Aeolosoma sp.</i>	<i>Aeolosoma sp.</i>	Z83748 (1822)	<i>Aeolosoma sp.</i>	DQ790019 (3304)
	<i>Eisenia</i>	<i>Eisenia fetida</i>	AB076887 (1818)	<i>Eisenia sp.</i>	DQ790032 (3345)
Polychaeta	<i>Capitella capitata</i>	<i>Capitella capitata</i>	U67323 (1740)	<i>Capitella capitata</i>	AY364863 (1885)
	<i>Nereis vexillosa</i>	<i>Nereis vexillosa</i>	DQ790083 (1829)	<i>Nereis vexillosa</i>	DQ790043 (3087)
	<i>Owenia fusiformis</i>	<i>Owenia fusiformis</i>	AF448160 (1809)	<i>Owenia fusiformis</i>	DQ790049 (3256)
	<i>Protodriloides symbioticus</i>	<i>Protodriloides symbioticus</i>	AF508125 (1871)	<i>Protodriloides symbioticus</i>	EU118876 (3367)
	<i>Scalibregma inflatum</i>	<i>Scalibregma inflatum</i>	DQ790093 (1833)	<i>Scalibregma inflatum</i>	DQ790060 (3370)
Brachiopoda					
Craniata	<i>Novocrania</i>	<i>Neocrania huttoni</i>	U08334 (1753)	<i>Novocrania poutalei</i>	AY839246 (2036)
Lincolata	<i>Novocrania anomala</i>	<i>Novocrania anomala</i>	DQ279934 (1768)	<i>Novocrania anomala</i>	DQ279949 (2821)
	<i>Discinisca tenuis</i>	<i>Discinisca tenuis</i>	DTU08327 (1750)	<i>Discinisca tenuis</i>	AY839248 (2017)
	<i>Glottidia pyramidata</i>	<i>Glottidia pyramidata</i>	U12647 (1765)	<i>Glottidia pyramidata</i>	AY210459 (3344)
	<i>Lingula anatina</i>	<i>Lingula anatina</i>	X08331 (1749)	<i>Lingula sp.</i>	AY839250 (2017)
Phoroniformea	<i>Phoronis hippocrepia</i>	<i>Phoronis hippocrepia</i>	AF202112 (1769)	<i>Phoronis hippocrepia</i>	AY839251 (2023)
	<i>Phoronis ijimai</i>	<i>Phoronis ijimai</i>	AF202113 (1769)	<i>Phoronis ijimai</i>	AF342797 (3331)
Rhynchonellata	<i>Eohemithiris gravi</i>	<i>Eohemithiris gravi</i>	AF025936 (1768)	<i>Eohemithiris gravi</i>	AY839242 (2007)
	<i>Laqueus californianus</i>	<i>Laqueus californianus</i>	U08323 (1749)	<i>Laqueus californianus</i>	AY210460 (3288)
	<i>Terebratalia transversa</i>	<i>Terebratalia transversa</i>	AF025945 (1767)	<i>Terebratalia transversa</i>	AY839244 (2025)
Bryozoa					
Gymnolaemata	<i>Alcyonidium</i>	<i>Alcyonidium gelatinosum</i>	X91403 (1813)	<i>Alcyonidium diaphanum</i>	AY210453 (3328)
	<i>Bugula turrita</i>	<i>Bugula turrita</i>	AY210443 (1841)	<i>Bugula turrita</i>	AY210457 (3449)
Phylactolaemata	<i>Plumatella</i>	<i>Plumatella repens</i>	U12649 (1813)	<i>Plumatella sp.ZHY-2005</i>	DQ333339 (3156)
Stenolaemata	<i>Crisia sp.</i>	<i>Crisia sp.</i>	AY210444 (1830)	<i>Crisia sp.</i>	AY210458 (3018)
	<i>Tubulipora flabellaris</i>	<i>Tubulipora flabellaris</i>	UF650325 (1686)	<i>Tubulipora flabellaris</i>	DQ333340 (3311)
	<i>Sagitta elegans</i>	<i>Sagitta elegans</i>	Z19551 (1914)	<i>Sagitta elegans</i>	AF342799 (3427)
Chaetognatha	<i>Symbion sp.</i>	<i>Symbion sp.</i>	EF142085 (1770)	<i>Symbion sp.YJP-2003</i>	AY210472 (3393)
Cycliophora	<i>Arhynchite pugegensis</i>	<i>Arhynchite pugegensis</i>	AY210441 (1815)	<i>Arhynchite pugegensis</i>	AY210455 (3305)
Echiura	<i>Urechis caupo</i>	<i>Urechis caupo</i>	AF342805 (1777)	<i>Urechis caupo</i>	AF342804 (3386)
Entoprocta	<i>Barentsia gracilis</i>	<i>Barentsia gracilis</i>	AY210442 (1804)	<i>Barentsia gracilis</i>	AY210456 (3336)
	<i>Loxosomella murmanica</i>	<i>Loxosomella murmanica</i>	AY218100 (1761)	<i>Loxosomella murmanica</i>	DQ279950 (2919)
Gnathostomulida	<i>Gnathostomula paradoxa</i>	<i>Gnathostomula paradoxa</i>	DQ079925 (1717)	<i>Gnathostomula paradoxa</i>	EF151007 (1760)
Kinorhyncha	<i>Pycnophyes kielenis</i>	<i>Pycnophyes kielenis</i>	U67997 (1806)	<i>Pycnophyes kielenis</i>	AY863411 (3355)
	<i>Pycnophyes sp. Tjarno</i>	<i>Pycnophyes sp. Tjarno</i>	AY859598 (1768)	<i>Pycnophyes sp. Tjarno</i>	AY859597 (3357)
Mollusca					
Aplacophora	<i>Chaetoderma sp.</i>	<i>Chaetoderma sp.</i>	AY145369 (1739)	<i>Chaetoderma sp.</i>	AY145397 (2988)
	<i>Helicoradomenia sp.</i>	<i>Helicoradomenia sp.</i>	AY145377 (1822)	<i>Helicoradomenia sp.</i>	AY145409 (2899)
Bivalvia	<i>Argopecten irradians</i>	<i>Argopecten irradians</i>	L11265 (1815)	<i>Argopecten irradians</i>	AY145391 (3403)
	<i>Crassostrea gigas</i>	<i>Crassostrea gigas</i>	AB064942 (1820)	<i>Crassostrea gigas</i>	AB102757 (3761)
	<i>Glycymeris</i>	<i>Glycymeris sp.</i>	X91978 (1811)	<i>Glycymeris reevei</i>	AB101609 (3308)
	<i>Pinctada martensi</i>	<i>Pinctada martensi</i>	AB214464 (1824)	<i>Pinctada martensi</i>	AB214479 (3659)
Cephalopoda	<i>Placopecten maquellanicus</i>	<i>Placopecten maquellanicus</i>	X53899 (1814)	<i>Placopecten maquellanicus</i>	AF342798 (3330)
	<i>Abrallopsis sp.</i>	<i>Abrallopsis sp.</i>	AY145364 (2095)	<i>Abrallopsis sp.</i>	AY145389 (4262)
	<i>Graneledone pacifica</i>	<i>Graneledone pacifica</i>	AY145376 (2740)	<i>Graneledone pacifica</i>	AY145407 (4713)
	<i>Heteroteuthis hawaiiensis</i>	<i>Heteroteuthis hawaiiensis</i>	AY557472 (2359)	-missing-	-
	<i>Loligo pealei</i>	<i>Loligo pealei</i>	AY145383 (2326)	<i>Loligo pealei</i>	AY145415 (3412)
Gastropoda	<i>Biomphalaria glabrata</i>	<i>Biomphalaria glabrata</i>	U65224 (1845)	<i>Biomphalaria glabrata</i>	AF435694 (1439)
	<i>Cocculina</i>	<i>Cocculina messingi</i>	AF120508 (1755)	<i>Cocculina sp.</i>	DQ279973 (2157)
	<i>Haminoea</i>	<i>Haminoea hydatis</i>	AY427504 (1847)	<i>Haminoea solitaria</i>	AY145408 (3351)
	<i>Lottia digitalis</i>	<i>Lottia digitalis</i>	D0248942 (2072)	<i>Lottia digitalis</i>	DQ248942 (3472)
	<i>Ophicardelus ornatus</i>	<i>Ophicardelus ornatus</i>	DQ093442 (1795)	<i>Ophicardelus ornatus</i>	DQ256740 (2881)
Polyplacophora	<i>Chaetopleura apiculata</i>	<i>Chaetopleura apiculata</i>	AY377636 (1749)	<i>Chaetopleura apiculata</i>	AY145398 (3344)
	<i>Cryptoplax japonica</i>	<i>Cryptoplax japonica</i>	AY145371 (1809)	<i>Cryptoplax japonica</i>	AY145402 (3324)
Polyplacophora	<i>Ischnochiton comptus</i>	<i>Ischnochiton comptus</i>	AY145380 (1815)	<i>Ischnochiton comptus</i>	AY145412 (3336)
	<i>Antalis entalis</i>	<i>Antalis entalis</i>	AY145363 (1852)	<i>Antalis entalis</i>	AY145388 (3594)
Scaphopoda	<i>Dentalium octanquatulum</i>	<i>Dentalium octanquatulum</i>	AY145372 (1853)	<i>Dentalium octanquatulum</i>	AB126335 (3565)

Table 4. Continued

Classification	Combined rDNA Taxon Designation	18s rDNA Taxa	18S GB# (seq. length - bps)	28s rDNA Taxa	28S GB# (seq. length - bps)
Scaphopoda (cont.)	<i>Episiphon yamakawai</i>	-missing-	-	<i>Episiphon yamakawai</i>	AB103133 (3614)
Myzostomida	<i>Myzostoma polycyclus</i>	<i>Myzostoma polycyclus</i>	AY210446 (1817)	<i>Myzostoma polycyclus</i>	AY210462 (3065)
Nematoda					
Chromadorea	<i>Acrobeles maximus</i> <i>Brevibucca saprophaga</i> <i>Oscheius insectivora</i>	<i>Acrobeles maximus</i> <i>Brevibucca saprophaga</i> <i>Oscheius insectivora</i>	EU196016 (1700) EU196018 (1768) AF083019 (1715)	<i>Acrobeles maximus</i> <i>Brevibucca saprophaga</i> <i>Oscheius insectivora</i>	EU195987 (3242) EU195990 (3158) EU195968 (3090)
Enoplea	<i>Trichinella spiralis</i> <i>Xiphinema</i>	<i>Trichinella sp. iralis</i> <i>Xiphinema rivesi</i>	U60231 (1798) AM086673 (1753)	<i>Trichinella spiralis</i> <i>Xiphinema rivesi</i>	AF342803 (3897) AY210845 (3378)
Nematomorpha					
Gordioida	<i>Chordodes morqani</i> <i>Gordius</i> <i>Gordius sp.</i>	<i>Chordodes morqani</i> <i>Gordius paranensis</i> <i>Gordius sp.</i>	AF036639 (1783) AF421766 (1765) AB470227 (1701)	<i>Chordodes morqani</i> <i>Gordius aquaticus</i> <i>Gordius sp.</i>	AF342787 (3389) AY210817 (3395) AY863410 (3398)
Nemertea					
Anopla	<i>Cerebratulus lacteus</i> <i>Lineus bilineatus</i> <i>Tubulanus annulatus</i>	<i>Cerebratulus lacteus</i> <i>Lineus bilineatus</i> <i>Tubulanus annulatus</i>	AY145368 (1856) DQ279932 (1763) AY210452 (1881)	<i>Cerebratulus lacteus</i> <i>Lineus bilineatus</i> <i>Tubulanus annulatus</i>	AY145396 (3349) DQ279947 (2819) AY210473 (3831)
Enopla	<i>Amphiporus sp.</i> <i>Oerstedtia dorsalis</i>	<i>Amphiporus sp.</i> <i>Oerstedtia dorsalis</i>	AF119077 (1778) AY210448 (1830)	<i>Amphiporus sp.</i> <i>Oerstedtia dorsalis</i>	AF342786 (3366) AY210465 (3373)
Panarthropoda					
Arachnida	<i>Acanthoscurria sp.</i> <i>Boophilus microplis</i> <i>Chileoqoovea sp.</i> <i>Ixodes ricinus</i> <i>Metasiro americanus</i> <i>Siro valleurum</i>	<i>Acanthoscurria sp.</i> <i>Boophilus microplis</i> <i>Chileoqoovea sp.</i> <i>Ixodes ricinus</i> <i>Metasiro americanus</i> <i>Siro valleurum</i>	DQ639775 (1695) AF018656 (1747) DQ517970 (1728) Z744479 (1813) DQ825542 (1745) AY639492 (1776)	<i>Acanthoscurria sp.</i> <i>Boophilus microplis</i> <i>Chileoqoovea sp.</i> <i>Ixodes ricinus</i> <i>Metasiro americanus</i> <i>Siro valleurum</i>	DQ639859 (1764) AF200189 (749) DO518012 (2085) AF200190 (683) DQ825595 (2100) DQ513123 (2109)
Branchiopoda	<i>Daphnia magna</i> <i>Paratemia minuta</i>	<i>Daphnia magna</i> <i>Paratemia minuta</i>	AM490278 (2341) EF189631 (1760)	<i>Daphnia magna</i> <i>Paratemia minuta</i>	AF346515 (3850) EF189656 (3337)
Chilopoda	<i>Craterostigmus tasmanianus</i> <i>Lithobius</i> <i>Scutigera coleoptrata</i>	<i>Craterostigmus tasmanianus</i> <i>Lithobius variegatus</i> <i>Scutigera coleoptrata</i>	AF000774 (1854) AF000773 (1860) AF173238 (1818)	<i>Craterostigmus tasmanianus</i> <i>Lithobius sp.</i> <i>Scutigera coleoptrata</i>	DQ222133 (2897) AY210825 (2974) EF199983 (3195)
Diplopoda	<i>Cherokia georgiana</i> <i>Orthoporus sp.</i> <i>Paradoxosomatidae sp.</i>	<i>Cherokia georgiana</i> <i>Orthoporus sp.</i> <i>Paradoxosomatidae sp.</i>	AY859563 (1781) AY210829 (1791) DQ666179 (1757)	<i>Cherokia georgiana</i> <i>Orthoporus sp.</i> <i>Paradoxosomatidae sp.</i>	AY859562 (3814) AY210828 (3723) DQ666182 (3851)
Diplura	<i>Campodeidae sp.</i> <i>Campodeidae sp.</i>	<i>Campodeidae sp.</i> <i>Campodeidae sp.</i>	AY338692 (1800) AY859561 (1866)	<i>Campodeidae sp.</i> <i>Campodeidae sp.</i>	AY338691 (2149) AY859560 (3649)
Ellipura	<i>Catajapyx</i> <i>Folsomia candida</i> <i>Sminthurus viridis</i>	<i>Catajapyx</i> <i>Folsomia candida</i> <i>Sminthurus viridis</i>	AF005456 (1699) AY555515 (1761) AY859604 (1765)	<i>Catajapyx aquilonaris</i> <i>Folsomia candida</i> <i>Sminthurus viridis</i>	EF199978 (4159) EU914252 (3497) AY859603 (3480)
Eutardigrada	<i>Milnesium sp.</i>	<i>Milnesium sp.</i>	EU266922 (1702)	<i>Milnesium sp.</i>	AY210826 (3555)
Insecta	<i>Bombyx mori</i> <i>Callibaetis ferrugineus</i> <i>Chalcidoida</i> <i>Fuchsina occulta</i> <i>Helicoverpa assulta</i> <i>Locusta migratoria</i> <i>Oropsylla montana</i> <i>Tribolium castaneum</i>	<i>Bombyx mori</i> <i>Callibaetis ferrugineus</i> <i>Trichogramma minutum</i> <i>Fuchsina occulta</i> <i>Helicoverpa assulta</i> <i>Locusta migratoria</i> <i>Oropsylla montana</i> <i>Tribolium castaneum</i>	Q347470 (1907) AF370791 (1812) AY491051 (1889) EU164630 (1840) L34046 (1810) AF370793 (1860) AF336048 (1880) AJ878603 (1159)	<i>Bombyx mori</i> <i>Callibaetis ferrugineus</i> <i>Trichogramma minutum</i> <i>Fuchsina occulta</i> <i>Locusta migratoria</i> <i>Oropsylla montana</i> <i>Tribolium castaneum</i>	AY038991 (1192) AY859557 (3514) EU462314 (964) EU164667 (2084) -missing- EF685941 (623) EU336156 (2174) EU677678 (1028)
Paurodopa	<i>Apis mellifera</i>	<i>Apis mellifera</i>	AB126807 (1727)	<i>Apis mellifera</i>	AJ302936 (2748)
Pycnogonida	<i>Allopauropus sp.</i> <i>Anoplocladus portus</i> <i>Endeis australis</i>	<i>Allopauropus sp.</i> <i>Anoplocladus portus</i> <i>Endeis australis</i>	DQ399857 (2227) AY859551 (1809) DQ389892 (1772)	<i>Allopauropus sp.</i> <i>Anoplocladus evansi</i> <i>Endeis australis</i>	DQ666185 (3947) DQ390115 (2819) DQ390102 (2790)
Symphyla	<i>Pseudopallene ambigua</i> <i>Ostotignum politus</i>	<i>Pseudopallene ambigua</i> <i>Ostotignum politus</i>	DQ389930 (1767) DQ666177 (1868)	<i>Pseudopallene ambigua</i> <i>Ostotignum politus</i>	DQ390141 (2755) DQ666180 (3788)
Cephalocarida	<i>Scutigerella</i>	<i>Scutigerella</i>	DQ399856 (1902)	<i>Scutigerella</i>	DQ666184 (4112)
Malacostraca	<i>Hutchinsoniella macracantha</i> <i>Aeala</i> <i>Cambaroides japonicus</i> <i>Homarus americanus</i> <i>Penaeus semisulcatus</i>	<i>Hutchinsoniella macracantha</i> <i>Aeala liqulata</i> <i>Cambaroides japonicus</i> <i>Homarus americanus</i> <i>Penaeus semisulcatus</i>	AF370801 (1959) AY595801 (1841) DQ079742 (1755) AY743945 (1758) DQ079766 (1781)	<i>Hutchinsoniella macracantha</i> <i>Aeala septentrionalis</i> <i>Cambaroides japonicus</i> <i>Homarus americanus</i> <i>Penaeus semisulcatus</i>	EF189645 (2456) AY596076 (2882) DQ079779 (2396) AY859581 (4005) DQ079809 (1453)
Maxillopoda	<i>Caligus elongatus</i> <i>Chondracanthus lophii</i> <i>Sacculinidae sp.</i>	<i>Caligus elongatus</i> <i>Chondracanthus lophii</i> <i>Sacculinidae sp.</i>	AY857020 (1809) L34046 (1810) AY859600 (1840)	<i>Caligus elongatus</i> <i>Chondracanthus lophii</i> <i>Sacculinidae sp.</i>	DQ180337 (3166) DQ180341 (3440) AY859599 (3752)
Merostomata	<i>Limulus polyphemus</i> <i>Symphylella sp.</i>	<i>Limulus polyphemus</i> <i>Symphylella sp.</i>	L81949 (1807) DQ399855 (2057)	<i>Limulus polyphemus</i> <i>Symphylella sp.</i>	AF212167 (3424) DQ666183 (4123)
Myriapoda	<i>Peripatoides novaezealandiae</i> <i>Peripatus sp.</i>	<i>Peripatoides novaezealandiae</i> <i>Peripatus sp.</i>	AF342794 (2064) AY108337 (2476)	<i>Peripatoides novaezealandiae</i> <i>Peripatus sp.</i>	AF342793 (3916) AY210836 (3270)
Onychophora	<i>Cyprididae sp.</i>	<i>Cyprididae sp.</i>	AY210816 (1751)	<i>Cyprididae sp.</i>	AY210815 (3538)
Platyhelminthes					
Cestoda	<i>Dilepis undula</i> <i>Echinococcus</i> <i>Monobothroides chalmersius</i>	<i>Dilepis undula</i> <i>Echinococcus granulosus</i> <i>Monobothroides chalmersius</i>	AF286981 (2091) U27015 (2394) EF095244 (2198)	<i>Dilepis undula</i> <i>Echinococcus multilocularis</i> <i>Monobothroides chalmersius</i>	AF286915 (4324) AY615426 (604) EF095253 (4519)
Monogenea	<i>Pachybothrium hutsoni</i> <i>Diclidophora denticulata</i> <i>Dictyocotyle coelica</i> <i>Polystomoides malayi</i>	<i>Pachybothrium hutsoni</i> <i>Diclidophora denticulata</i> <i>Dictyocotyle coelica</i> <i>Polystomoides malayi</i>	EF095246 (1994) AJ228779 (1966) AJ228778 (2009) AJ228792 (2014)	<i>Pachybothrium hutsoni</i> <i>Diclidophora denticulata</i> <i>Dictyocotyle coelica</i> <i>Polystomoides malayi</i>	EF095260 (4071) AY157169 (3744) AY157171 (3744) AY157170 (4024)
Trematoda	<i>Bilharziella polonica</i> <i>Fasciola hepatica</i> <i>Sanquinalid sp.</i>	<i>Bilharziella polonica</i> <i>Fasciola hepatica</i> <i>Sanquinalid sp.</i>	AY157214 (1871) AJ004969 (1941) AY829250 (1709)	<i>Bilharziella polonica</i> <i>Fasciola hepatica</i> <i>Sanquinalid sp.</i>	AY157240 (3758) AY222244 (1293) AY858879 (3629)
	<i>Schistosoma haematobium</i> <i>Schistosoma japonicum</i> <i>Schistosoma mansoni</i>	<i>Schistosoma haematobium</i> <i>Schistosoma japonicum</i> <i>Schistosoma mansoni</i>	Z11590 (1648) U65657 (1989) AF050433 (1774)	<i>Schistosoma haematobium</i> <i>Schistosoma japonicum</i> <i>Schistosoma mansoni</i>	AY157263 (3778) Z46504 (3897) AY157173 (3844)
Turbellaria	<i>Dugesia ryukyuensis</i> <i>Geocentrophora wagini</i> <i>Paromalostomum fusculum</i> <i>Schmidtea mediterranea</i> <i>Suomina sp.</i>	<i>Dugesia ryukyuensis</i> <i>Geocentrophora wagini</i> <i>Paromalostomum fusculum</i> <i>Schmidtea mediterranea</i> <i>Suomina sp.</i>	AJ012509 (1767) AJ012531 (1769) U31084 (1794) AJ012532 (1651)	<i>Dugesia ryukyuensis</i> <i>Geocentrophora wagini</i> <i>Paromalostomum fusculum</i> <i>Schmidtea mediterranea</i> <i>Suomina sp.</i>	DQ665968 (1575) AY157156 (3423) AY157155 (3314) DQ665992 (1626) AY157152 (3398)
Pogonophora					
Vestimentifera	<i>Riftia pachyptila</i> <i>Siboglinum fiordicum</i>	<i>Riftia pachyptila</i> <i>Siboglinum fiordicum</i>	AF168745 (1765) X79876 (1844)	<i>Riftia pachyptila</i> <i>Siboglinum fiordicum</i>	AY210470 (3410) DQ790061 (3471)
Priapulida	<i>Halicryptus spinulosus</i> <i>Priapulus caudatus</i>	<i>Halicryptus spinulosus</i> <i>Priapulus caudatus</i>	AF342790 (1768) AF025927 (1750)	<i>Halicryptus spinulosus</i> <i>Priapulus caudatus</i>	AF342789 (3339) AY210840 (3332)
Rotifera					
Bdelloidea	<i>Adineta vaga</i> <i>Epiphanes senta</i> <i>Philodina roseola</i>	<i>Adineta vaga</i> <i>Epiphanes senta</i> <i>Philodina roseola</i>	DQ089733 (1767) DQ089735 (1767) EF154567 (1747)	<i>Adineta vaga</i> <i>Epiphanes senta</i> <i>Philodina roseola</i>	EU195987 (2868) DQ089742 (2782) AY210468 (3400)
Monoqononta	<i>Asplanchna sieboldi</i> <i>Brachionus urceolaris</i> <i>Encentrum astridae</i> <i>Seison nebaliae</i>	<i>Asplanchna sieboldi</i> <i>Brachionus urceolaris</i> <i>Encentrum astridae</i> <i>Seison nebaliae</i>	AF092434 (1728) DQ089734 (1748) DQ297695 (1759) DQ089737 (1788)	<i>Asplanchna sieboldi</i> <i>Brachionus urceolaris</i> <i>Encentrum astridae</i> <i>Seison nebaliae</i>	AY829085 (2780) DQ089740 (2777) EF151006 (2053) DQ089744 (2571)
Seisonidea	<i>Sipuncula</i>	<i>Sipuncula</i>		<i>Sipuncula</i>	
Phascolosomatidea	<i>Apionsoma misakianum</i>	<i>Apionsoma misakianum</i>	DQ299952 (1769)	<i>Apionsoma misakianum</i>	AY210454 (3373)
Sipunculidea	<i>Phascolion strombus</i> <i>Phascolopsis gouldii</i>	<i>Phascolion strombus</i> <i>Phascolopsis gouldii</i>	DQ299984 (1774) AF342796 (1770)	<i>Phascolion strombus</i> <i>Phascolopsis gouldii</i>	AY210468 (3369) AF342795 (3454)

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