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Phylogenetic analyses of the polycystine Radiolaria based on the 18s rDNA sequences of the Spumellarida and the Nassellarida

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Abstract

Acantharea, Polycystinea, and Phaeodarea have members that are widely distributed in the marine plankton. Many biologists use the conventional term "Radiolaria" to include these three classes. However, on the basis of an 18S rDNA study, Polet et al. (2004, Protist 155, 53–63) recently suggested that the Phaeodarea should be moved into the Phylum Cercozoa. In the present paper, the phylogenetic relationships of the Acantharea and the Polycystinea, especially the phylogenetic positions of Nassellarida and Spumellarida, were inferred from 18S rDNA sequences including those we have determined from the Family Spongodiscidae (Class Polycystinea, Order Spumellarida) and the Family Pterocorythidae (Class Polycystinea, Order Nassellarida). Among major eukaryotic lineages, the Polycystinea were shown to constitute a paraphyletic group: in the phylogenetic trees for the relationships among polycystines, the collosphaerid, sphaerozoid, and thalassicollid spumellarians and the pterocorycid nassellarians constantly formed a monophyletic group, from which the spongodiscid spumellarians were excluded. This conclusion is not consistent with the current taxonomy of the "Radiolaria," and leads us to consider that the collosphaerid, sphaerozoid, and thalassicollid spumellarians and the pterocorycid nassellarians may have evolved from an ancestor with triradiate branched spicules.
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Introduction

Members of Acantharea, Polycystinea, and Phaeodarea are planktonic protists that are widely distributed in tropical, subtropical, and even polar marine environments. They have roughly spherical–subspherical cells and thread-like pseudopodia extending radially over the endoskeleton that facilitate a floating existence (e.g. Anderson 1983). Currently, many researchers use the term "Radiolaria" as a conventional name that comprehensively includes all Acantharea, Polycystinea, and Phaeodarea.

Müller (1858) first assigned the term "Rhizopoda radiolaria" to all planktonic organisms with a central capsule and a radiating skeleton. He included the polycystine and acantharian species in this group. Thereafter, Haeckel (1887) used the term "Radiolaria"

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to designate the following four taxa: Acantharia, Spumellaria, Nassellaria, and Phaeodaria. This use of the term was partially attributed to similarities in cytoplasmic morphology, including a perforated capsular wall with fusules and their radial skeletons. However, in a modern taxonomic system, Levine et al. (1980) did not recognize the "Radiolaria" as a taxonomic group, apparently on the basis of the substantial differences among the species in the classes, and they excluded the term "Radiolaria" from their system. This was principally because within the Actinopoda the chemical composition of the skeletons differs, being of strontium sulfate ($SrSO_4$) in the Class Acantharea, opaline silica (SiO_2) in the Class Polycystinea, and opaline silica with organic matter in the Class Phaeodarea. As with other organisms, radiolarian classification systems mainly based on morphological studies may have problems due to convergent or parallel evolution in similar environments.

Over several decades, much research based on molecular analyses, which is independent of morphology, has shown that DNA sequences are useful markers of evolutionary relationships (e.g. Sogin et al. 1989), and the effectiveness of analyses combining the methods of morphology and molecular biology has been shown for many taxonomic groups (e.g. Kühn et al. 2000). In particular, the comparison of ribosomal DNA (rDNA) sequences has been shown to be a useful tool for clarifying the molecular evolution of both eukaryotes and prokaryotes (e.g. Van de Peer et al. 2000).

Amaral Zettler et al. (1997) first examined 18S rDNA sequences of two acantharian and four polycystine species (including three colonial forms and one solitary skeletonless form) in order to explore the diversity of the Superclass Actinopoda. They suggested that among the so-called "crown" (Knoll 1992) eukaryotes, the colonial and solitary skeletonless Polycystinea had emerged before the Acantharea, and they concluded that the Polycystinea and the Acantharea do not form a monophyletic group (Amaral Zettler et al. 1997). On the other hand, López-García et al. (2002) examined the 18S rDNA sequences of polycystine- and acantharian-related genomic fragments from Antarctic deep waters and revealed a diversity of pico-size protists in the deep Antarctic Ocean. They showed that the Polycystinea and the Acantharea formed a monophyletic group at the apical region of the "crown" group in their phylogenetic trees. Their data indicated a close relationship between the Polycystinea and the Acantharea that was different from the result of Amaral Zettler et al. (1997). López-García et al. (2002) referred to long-branch attraction (LBA) artifacts (Felsenstein 1978) to explain the differences in the topologies, because all known polycystine branches at that time (e.g. *Siphonosphaera cyathina*) were recognized to be fast-evolving lineages (Amaral Zettler et al. 1999).

Recently, Polet et al. (2004) and Nikolaev et al. (2004) reported 18S rDNA and actin sequence data for the radiolarians. In their phylogenetic trees, the acantharian and polycystine clade constantly included *Sticholonche zanclea* Hertwig (taxopodid Heliozoa), and interestingly the Phaeodarea appeared in the Phylum Cercozoa. They therefore insisted upon the polyphyly of "Radiolaria" and supported the close relationships among the Radiolaria, the Foraminifera, and the Cercozoa in the group "Rhizaria" (Cavalier-Smith 2002).

We have already reported 18S rDNA sequences of three solitary shell-bearing species of the spongodiscid spumellarian polycystines (Takahashi et al. 2004). In our report, we also referred to the possibility that the Polycystinea and the Acantharea form a monophyletic group among eukaryotes. In that study, however, there were insufficient taxa to resolve the detailed taxonomic affiliations of the Acantharea and especially the Polycystinea. On the basis of 18S rDNA sequences of three further species of the Family Spongodiscidae (spumellarian Polycystinea) and two species of the Family Pterocorythidae (nassellarian Polycystinea) that we have determined, we here attempt to obtain a better understanding of the phylogenetic relationship between the Polycystinea and the Acantharea and also to determine the phylogenetic position of the Nassellarida among them.

Materials and methods

Sample collection

Polycystine samples were collected from surface seawater in spring 2002 using a plankton net (60 cm diameter opening with 37 μm mesh net) at Site 990528 (26°37′N, 127°47′E), located approximately 5 km northwest of Okinawa Island, Japan. The collected samples were stored at about 5 °C and immediately brought to the laboratory at the Tropical Biosphere Research Center, University of the Ryukyus. In the laboratory, polycystine species were transferred from the samples to six-well culturing dishes containing filtered seawater and stored until 18S rDNA region amplifications were processed.

The spumellarian polycystines that were subjected to molecular analysis are *Euchitonia elegans* (Ehrenberg) (Fig. 1a), *Dicranastrum furcatum* Haeckel (Fig. 1b), and *Triastrum aurivillii* Cleve (Fig. 1c), all from the Family Spongodiscidae. The nassellarian polycystines that were examined were two species belonging to Family Pterocorythidae: *Eucyrtidium hexagonatum* Haeckel (Fig. 1d) and *Pterocorys zancleus* (Müller) (Fig. 1e).

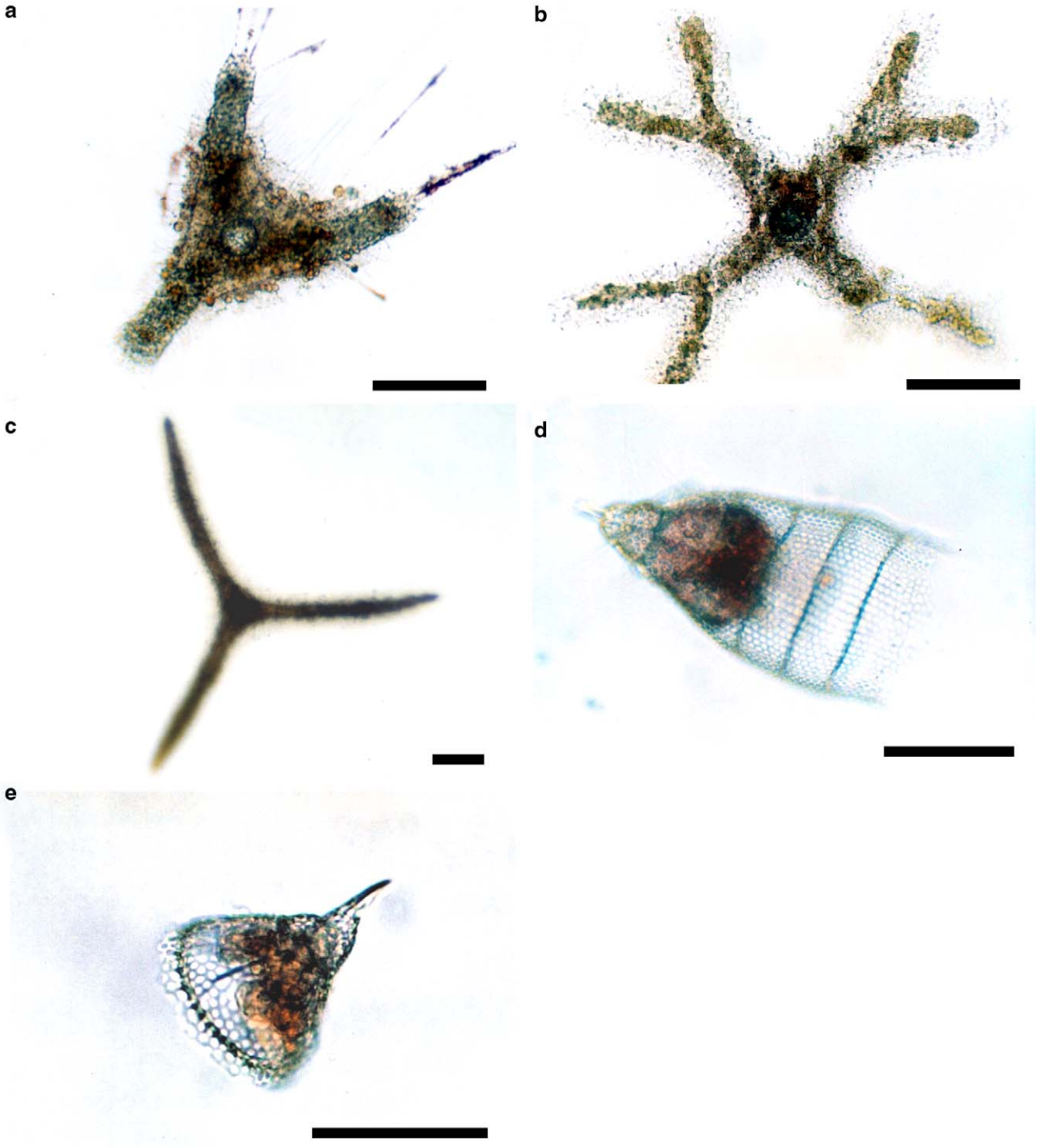

Fig. 1. Light micrographs (LM) of the Polycystinea used in this study. Scale bars indicate 100 μm. (a) *Euchitonia elegans* (Ehrenberg). (b) *Dicranastrum furcatum* Haeckel. (c) *Triastrum aurivillii* Cleve. (d) *Eucyrtidium hexagonatum* Haeckel. (e) *Pterocorys zancleus* (Müller).

DNA extraction and amplification

A single cell of each polycystine was rinsed twice in filtered seawater, and the central capsule was physically separated with a sterilized razor blade from the ectocytoplasm, which contained endosymbiotic algae. The central capsule was rinsed twice more in distilled water, and then incubated for 30 min at 37 °C in 0.2 μg/μl Proteinase K solution. This sample was used as a template for the amplification of 18S rDNA regions.

Polymerase chain reaction (PCR) amplification was accomplished using the eukaryotic specific forward

primer 90F (Hendriks et al. 1989): 5′- GAAACTGC-GAATGGCTCATT-3′ and the reverse primer B (Medlin et al. 1988): 5′-CCTTCTGCAGGTTCACCTAC-3′. The PCR amplification was performed using PCR Thermal Cycler TP3000 (TaKaRa, Gifu, Japan) with an initial denaturation step of 95 °C for 3 min, followed by 35 amplification cycles each consisting of 95 °C for 1 min, 55 °C for 2 min, and 72 °C for 3 min. The PCR products were purified by the GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences, Piscataway, USA) and then cloned in the pGEM-T Easy Vector System (Promega, Madison, USA) using *Escherichia coli* JM109 Competent cells (Promega). After plating, 10–20 positive transformants per library were screened by PCR amplification of inserts using flanking vector primers, namely pGEM-Forward: 5′-ACTCAC-TATAGGGCGAATTGG-3′ and pGEM-Reverse: 5′-TCAAGCTATGCATCCAACG-3′.

Sequencing

Five clones of each of the amplified fragments from the inserts were sequenced using FITC-labeled primers and reagents from the Thermo Sequences Fluorescent Labeled Primer Cycle Sequencing Kit with 7-deaza-dGTP (Amersham Biosciences) along with the sequencing protocol developed for DSQ2000L (Shimadzu, Kyoto, Japan). The protocol consisted of an initial denaturation at 95 °C for 3 min, prior to 20 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min, and 20 cycles of 95 °C for 30 s and 72 °C for 1 min. Sequences of *Euchitonia elegans*, *D. furcatum*, *T. aurivillii, Eucyrtidium hexagonatum,* and *Pterocorys zancleus* have been submitted to DDBJ under accession numbers AB179732 to AB179736.

Alignment

Alignment of all 18S rDNA sequences was based on the SSU rRNA alignment of Nikolaev et al. (2004) with our sequences added. The alignment was imported into the ARB program (Ludwig et al. 2004) and aligned using the Fast Aligner option, subsequently checked and adjusted by eye. The accession numbers of the 18S rDNA sequences used in this study are given in Tables 1 and 2. The complete sequence alignment file in this study is available from the authors.

Phylogenetic analysis

Molecular phylogenetic relationships were inferred from Bayesian analysis with MrBayes ver. 3.0b4 (Huelsenbeck and Ronquist 2001) for both large and small data sets (Tables 1 and 2) and the maximum-likelihood (ML) method (Felsenstein 1981) with PAUP*

Table 1. Accession numbers of Eukaryotic 18S rDNA sequences (excluding Acantharea, Phaeodarea, Polycystinea and environmental samples) used in this study

	Taxon	Accession number
Alveolata	*Blepharisma americanum*	M97909
	Crypthecodinium cohnii	M64245
	Cryptosporidium parvum	L16996
	Loxophyllum uticulariae	L26448
	Noctiluca scintillans	AF022200
	Oxytricha nova	X03948
	Toxoplasma gondii	U12138
Amoebozoa	*Acanthamoeba castellanii*	M13435
	Amoeba proteus	AJ314604
	Dermamoeba algensis	AY294148
	Dictyostelium discoideum	X00134
	Echinamoeba exundans	AF293895
	Endolimax nana	AF149916
	Entamoeba histolytica	X65163
	Filamoeba nolandi	AF293896
	Gephyramoeba sp. ATCC50654	AF293897
	Glaeseria mira	AY294146
	Hartmannella cantabrigiensis	AY294147
	Leptomyxa reticulata	AF293898
	Mastigamoeba balamuthi	L23799
	Mastigamoeba invertens	AF153206
	Mastigamomeba sp. ATCC50617	AF421220
	Mayorella sp. JJP-2003	AY294143
	Pelomyxa palustris	AF320348
	Physarum polycephalum	X13160
	Platyamoeba stenopodia	AY294144
	Vannella anglica	AF099101
	Vexillifera minutissima	AY294149
Apusozoa	*Amastigomonas mutabilis*	AY050182
	Ancyromonas sigmoides	AF174363
	Apusomonas proboscidea	L37037
Choanoflagellata	*Monosiga brevicollis*	AF100940
Cercozoa	*Bigelowiella natans*	AF054832
	Cercomonas sp. ATCC50317	U42449
	Euglypha rotunda	X77692
	Gymnophrys cometa	AJ514866
	Lecythium sp.	AJ514867
	Lotharella amoeboformis	AF076170
	Massisteria marina	AF174370

Table 1. (*continued*)

	Taxon	Accession number
	Nucleraria-like filose amoeba N-Por	AF289081
	Paulinella chromatophora	X81811
	Pseudodifflugia cf. *gracilis*	AJ418794
Cryptophyta	*Cryptomonas ovata*	AF508270
	Goniomonas truncata	U03072
	Guillardia theta	X57162
Excavates	*Diplonema ambulator*	AF380996
	Euglena gracilis var. *bacillaris*	AY029409
	Jakoba incarcerata	AY117419
	Jakoba libera	AY117418
	Malawimonas jakobiformis	AY117420
	Naegleria gruberi	M18732
	Reclinomonas americana	AY117417
	Streblomastix strix	AY188885
	Trimastix marina	AF244905
	Trypanosoma cruzi	X53917
Foraminifera	*Allogromia* sp.	X86093
	Ammonia beccarii	X86094
	Reticulomyxa filosa	AJ132367
Fungi	*Cryptococcus neoformans* var. *neoformans*	D12804
	Coprinopsis cinerea	M92991
	Saccharomyces cerevisiae	Z75578
	Schizosaccharomyces pombe	X58056
Glaucophyta	*Cyanophora paradoxa*	X68483
	Cyanoptyche gloeocystis	AJ007275
Gromiidae	*Gromia oviformis*	AJ457811
Haplosporidia	*Haplosporidium nelsoni*	X74131
	Urosporidium crescens	U47852
Haptophyta	*Emiliania huxleyi*	M87327
	Pavlova salina	L34669
	Pleurochrysis carterae	AJ246263
Heliozoa	*Actinosphaerium eichhornii*	AY305011
	Chlamydaster sterni	AY268042
	Clathrulina elegans	AY305009
	Hedriocystis reticulata	AY305010
	Heterophrys marina	AF534710
	marine microheliozoan TCS-2002	AF534711
	Pterocystis sp. JJP-2003	AY268043
	Raphidiophrys ambigua	AY305008
	Sticholonche zanclea	AY268045
	Undetermined centrohelid JJP-2003	AY268041
Metazoa	*Branchiostoma floridae*	M97571
	Limulus polyphemus	L81949
	Lumbricus rubellus	Z83753
	Podocoryne carnea	AF358092
Nucleariidae	*Nucleria simplex*	AF349566
Phytomyxea	*Phagomyxa odontellae*	AF310904
	Plasmodiophora brassicae	U18981
Rhodophyta	*Compsopogon coeruleus*	AF087128
	Glaucosphaera vacuolata	AB045583
	Palmaria palmata	X53500
Stramenopiles	*Achlya bisexualis*	M32705
	Cafeteria roenbergensis	AF174364
	Ciliophrys infusionum	L37205
	Fucus distichus	M97959
	Labyrinthuloides minuta	L27634
	Pseudobodo tremulans	AF315604
	Schizochytrium minutum	AB022108
Viridiplantae	*Helianthus annuus*	AF107577
	Mesostigma viride	AJ250108
	Psilotum nudum	X81963
	Scherffelia dubia	X68484
	Volvox carteri f. *nagariensis*	X53904

ver. 4.0b10 (Swofford 2003) for the small data set. Totals of 144 taxa (959 bp) and 137 taxa (1316 bp) were used in the phylogenetic analyses for the large data set. The analysis of the 137 data set was run omitting the partial sequences for environmental samples to determine what effect their absence had on the tree topology.

Bayesian analyses were performed by using the GTR model (Lanave et al. 1984) of substitution among sites. Two million and five million generations were run, 20000 and 50000 trees were sampled, 4000 and 10000 of which were discarded as burn-in, respectively. For the small data set, to decide on the evolutionary model that provided the best to fit our data, we used the Modeltest program (Posada and Crandall 1998) to conduct a likelihood ratio test (LRT). The resulting model was used in the ML analyses. The evolutionary distance model employed the Tamura-Nei (TrN) model (Tamura and Nei 1993) with Gamma distributed site-to-site rate variation (*G*) and an allowance for invariant sites (*I*).

Table 2. Accession numbers of 18S rDNA sequences of Acantharea, Phaeodarea, Polycystinea and environmental samples used in this study

	Taxon	Accession number
Polycystinea	*Acrosphaera* sp. CR6A	AF091148
	Collosphaera globularis-huxleyi	AF018163
	Collozoum inerme	AY266295
	Collozoum pelagicum	AF091146
	Collozoum serpentinum	AF018162
	Dicranastrum furcatum	AB179733
	Dictyocoryne profunda	AB101540
	Dictyocoryne truncatum	AB101541
	Euchitonia elegans	AB179732
	Eucyrtidium hexagonatum	AB179735
	Pterocorys zancleus	AB179736
	Rhaphidozoum acuferum	AF091147
	Siphonosphaera cyathina	AF091145
	Sphaerozoum punctatum	AF018161
	Spongaster tetras	AB101542
	Thalassicolla nucleata	AY266297
	Thalassicolla pellucida	AY266297
	Triastrum aurivillii	AB179734
Acantharea	*Acanthometra* sp.	AF063240
	Arthracanthid 206	AF063239
	Chaunacanthid 217	AF063241
	Chaunacanthid sp. 218	AF018158
	Haliommatidium sp.	AF018159
	Symphyacanthid 211	AF063242
Phaeodarea	*Aulacantha scolymantha*	AY266294
	Aulosphaera trigonopa	AY266292
	Coelodendrum ramosissimum	AY266293
Environmental samples	A2_E002	AY046714
	AT4_94	AF530525
	AT8_54	AF530524
	C1_E045	AY046642
	C3_E010	AY046840
	C3_E013	AY046843
	C3_E029	AY046858
	CS_E020	AY046647
	CS_E043	AY046664
	DH145-HA2	AF382824
	DH145-KW16	AF382825
	DH147-EKD17	AF290072
	OLI11015	AJ02332
	OLI11016	AJ402333
	OLI11032	AJ402342

The relative levels of support for nodes were assessed by using the posterior probabilities (PP) and/or calculating full heuristic bootstrap proportion values (BV) (Felsenstein 1985) based on 100 replicates in the ML analyses.

Results

The length in base pairs and the G+C % (G+C content) of the 18S rDNA sequences used in this study were as follows: *E. elegans*, 1728 bp, 50.0% GC; *D. furcatum*, 1727 bp, 49.6% GC; *T. aurivillii*, 1726 bp, 50.1% GC; *E. hexagonatum*, 1711 bp, 47.0% GC; *Pterocorys zancleus*, 1725 bp, 46.8% GC. Molecular phylogenetic trees including our determined sequences are shown in Figs. 2a, 2b, and 3. These were inferred from both the large and small data sets (Tables 1 and 2) using the Bayesian analysis (Figs. 2a and b) and the maximum likelihood (ML) method (Fig. 3). The PP and/or the BV at the nodes are given for all analyses.

Figs. 2a and b show the Bayesian trees for the large data set. The Bayesian analyses based on both the 144 and 137 data sets led to a strong support (PP of 0.98 and 0.93, respectively) for the clade including the Acantharea and the Polycystinea. This clade shown in Fig. 2a contained the Foraminifera and the Heliozoa, while in Fig. 2b includes only the Heliozoa and the Foraminifera branched near the base of the Cercozoa. Therefore, the phylogenic placement of the Foraminifera was not stable. On the other hand, Phaeodarea, which had been generally included in ''Radiolaria'' together with the Polycystinea and the Acantharea (e.g. Anderson 1983), branched in our analyses among the Cercozoa, as reported by Polet et al. (2004).

Our large trees (Figs. 2a and b) strongly supported paraphyly of the polycystines. Figs. 2a and b both show that the collosphaerid, sphaerozoid, and thalassicollid spumellarians and the pterocorycid nassellarians formed a monophyletic clade (PP of 1.00 and 0.94, respectively). This clade did not include the spongodiscid spumellarians, which had been generally classified into the same taxonomic group with the collosphaerid, sphaerozoid, and thalassicollid spumellarians as the Order Spumellarida. This result contradicts phylogenetic schemes based on the morphological data (e.g. Campbell 1954; Riedel 1967), in which close relationships among the spumellarians and the nassellarians are assumed in the Polycystinea.

Fig. 3 shows the ML tree with the corresponding PP and BV labeled at the nodes for the small data set. A total of 44 taxa (959 bp) was used in the phylogenetic analyses of Fig. 3. The small data set contained all available 18S rDNA sequences from taxa belonging to the Polycystinea and the Acantharea. This data set was used to infer the position of the nassellarians and the spumellarians among the polycystine and acantharian group. In the phylogenetic tree for the small data set, the collosphaerid, sphaerozoid, and thalassicollid spumellarians and the pterocorycid nassellarians formed a monophyletic group with PP of 1.00 and BV of 100%, whereas the spongodiscid spumellarians branched at the base of the polycystine, acantharian, and taxopodid

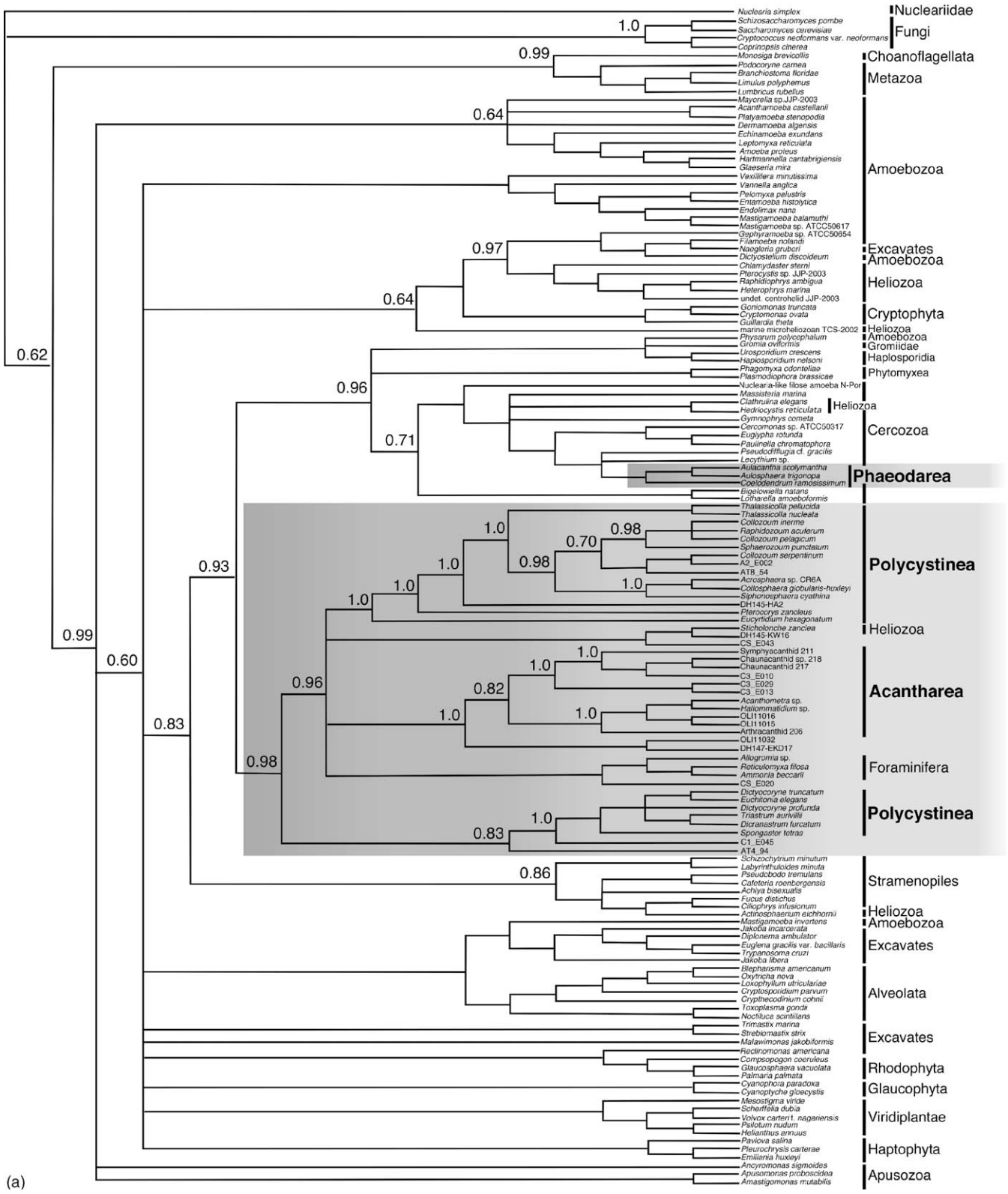

Fig. 2. 18S rDNA phylogenetic trees based on Bayesian inference. Other information is already mentioned in the methods section. Posterior Probabilities (PP) over 0.50 are given at respective nodes. (a) 18S rDNA phylogenetic tree of 144 operational taxonomic units (OTUs) and 959 nucleotide sites. (b) 18S rDNA phylogenetic tree of 137 OTUs and 1316 nucleotide sites.

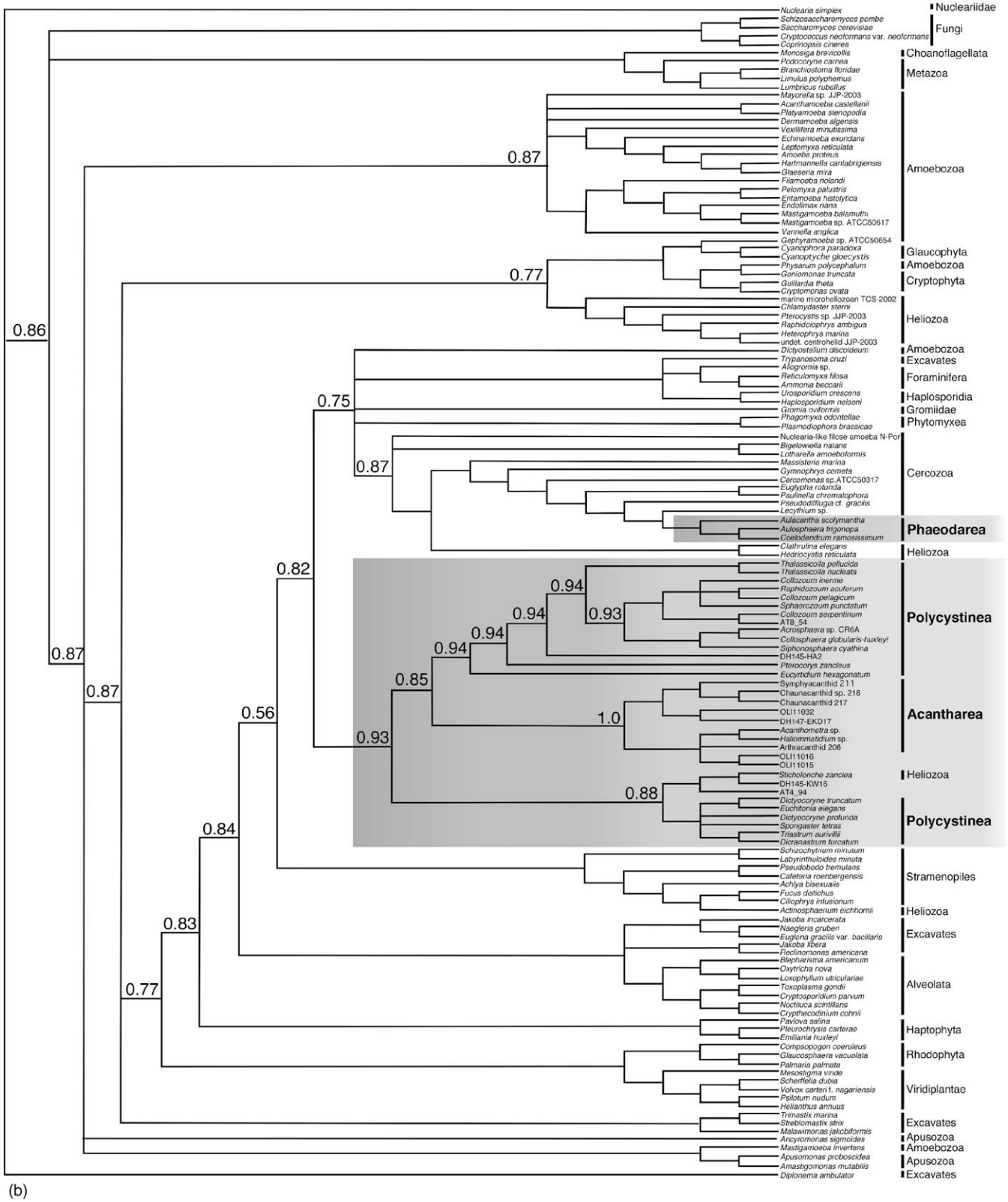

Fig. 2. (*Continued*)

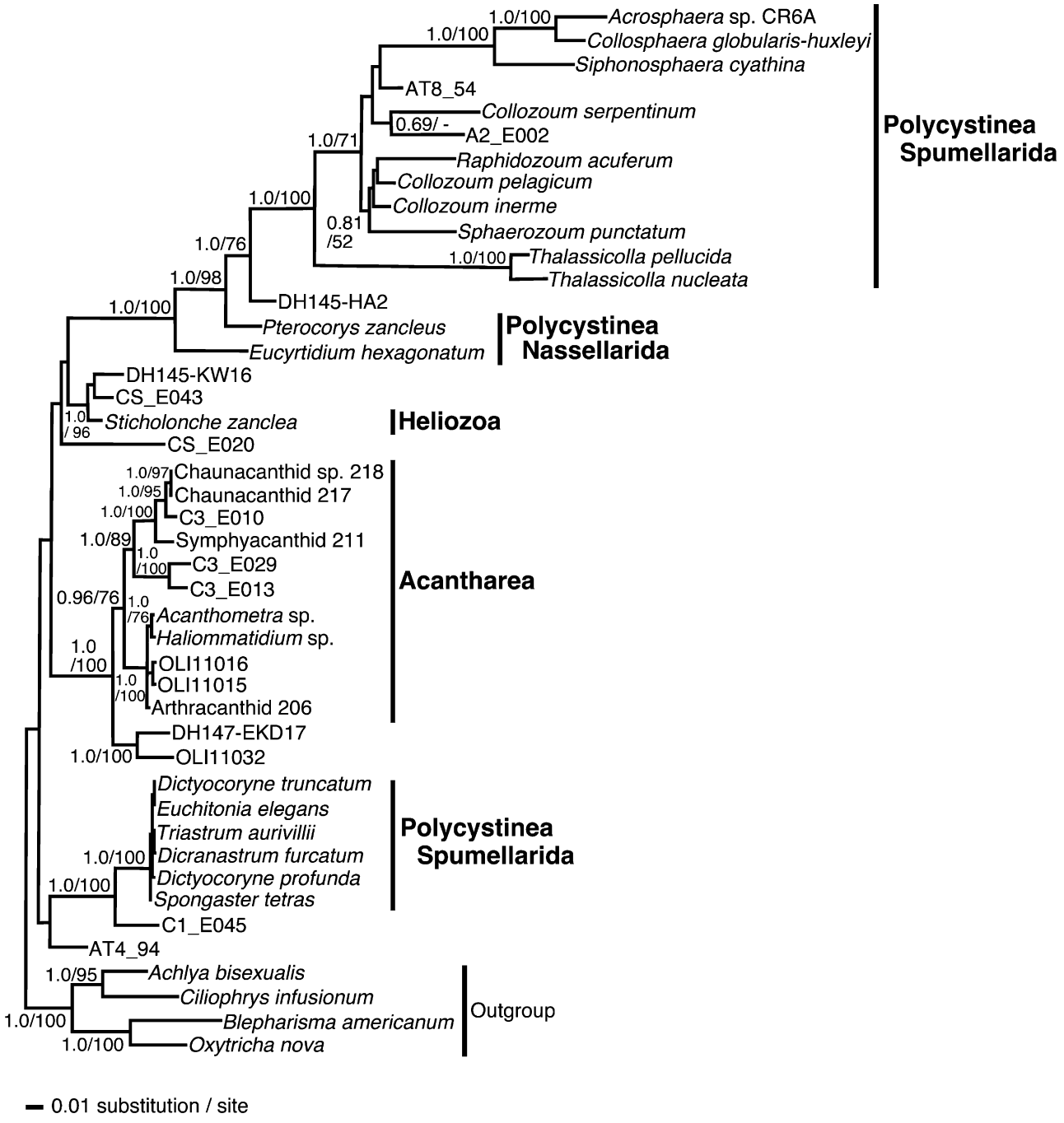

Fig. 3. 18S rDNA phylogenetic tree of smaller data set (44 OTUs and 959 nucleotide sites), based on sequence comparisons inferred with the maximum-likelihood (ML). Posterior Probabilities (PP: left number) over 0.50 and Bootstrap values (BV: right number) over 50% are given at respective nodes.

heliozoan lineage. These clades were also inferred by the Bayesian analyses for the large data set as indicated in the Figs. 2a and b. Therefore, all our trees suggested that the polycystines formed a paraphyletic group.

In both the large and small data sets, *Stichoionche zanclea* Hertwig (Taxopodida, Heliozoa) constantly branched among the polycystine and acantharian clade, as reported by Nikolaev et al. (2004). *S. zanclea* grouped with the collosphaerid, sphaerozoid, and thalassicollid spumellarians, pterocorycid nassellarians, and acantharians in Fig. 3, and in Fig. 2a it is in the same clade containing foraminifera. The clade was supported with PP of 0.96 for Fig. 2a, but it was not well supported with PP <0.50 and BV $<50\%$ for Fig. 3. On the other hand, Fig. 2b showed that *S. zanclea* grouped with the spongodiscid spumellarians (PP of 0.88). Hence, the

branching pattern of *S. zanclea* relative to the polycystine and acantharian clades also varied depending on the choice of the phylogenetic method and the taxa used in the analyses.

Discussion

As already pointed out by López-García et al. (2002), analyses including members of the Polycystinea and the Acantharea may be affected by LBA artifacts (Felsenstein 1978) due to the long, unbroken branches of the colonial and solitary skeletonless spumellarians (Sphaerozoidae, Collosphaeridae, Thalassicollidae), and also due to the small numbers of polycystine operational taxonomic units (OTUs) available for the phylogenetic analyses. In this phylogenetic reconstruction, we included eight more sequences of polycystines than any other study, including the three spongodiscid spumellarian and two pterocorythid nassellarian sequences determined by us and reported here. The spongodiscid spumellarians and the pterocorythid nassellarians that we used showed shorter branches than those of the sphaerozoid, collosphaerid, and thalassicollid spumellarians (see also Figs. 2a, 2b, and 3); in particular, the nassellarians were useful to break the long branches of the sphaerozoid, collosphaerid, and thalassicollid spumellarians. Therefore, the spongodiscid spumellarians and the pterocorythid nassellarians were potentially useful for avoiding phylogenetic reconstruction problems such as the LBA artifact.

Another interesting finding concerning the acantharian and polycystine clade in our Bayesian trees is that the Foraminifera sometimes branched among the Acantharea and the Polycystinea, and they sometimes moved into the Cercozoa. This phenomenon was already discussed by Cavalier-Smith and Chao (2003) and Nikolaev et al. (2004). Even though in our analyses we added the short-branched nassellarian polycystines and more spumellarian OTUs, the molecular phylogenetic position of the Foraminifera was not stable. Thus, in work with 18S rDNA sequences, the phylogenetic position of the Foraminifera among the Cercozoa, the Polycystinea, and the Acantharea remains ambiguous. However, it is clear that the Foraminifera are very closely related to the Cercozoa, the Polycystinea, the Acantharea, and the taxopodid Heliozoa.

Close relationship between the Nassellarida and the colonial and skeletonless Spumellarida

In the phylogenetic trees for both the large and small data sets (Figs. 2a, 2b, and 3), the collosphaerid, sphaerozoid, and thalassicollid spumellarians and the pterocorythid nassellarians consistently formed a monophyletic group that excluded the spongodiscid spumellarians. It suggested paraphyly of the spumellarian polycystines among the Polycystinea. This result is not consistent with the current taxonomy of ''Radiolaria.''

In the current scheme of taxonomy, the Class Polycystinea is divided into two orders: the Spumellarida and the Nassellarida. The taxonomy of these orders has been constructed on the basis of their cytoplasmic features, especially characteristics of the central capsule (e.g. Anderson 1983). The Order Spumellarida is characterized by a concentrically perforated central capsule. In contrast, the Order Nassellarida has a central capsule mainly perforated in the direction of one of its extremities (e.g. Anderson 1983).

Sugiyama and Anderson (1998) examined the fine structures of *Acanthodesmia vinculata* (Müller) and *Lithocircus reticulatus* (Ehrenberg) belonging to the Family Spyroidea in the Nassellarida. According to their conclusions, while *A. vinculata* has a typical nassellarian arrangement of fusules, the fusules of *L. reticulatus* are distributed radially from all around the central capsule, and this characteristic has been described as a typical feature of spumellarians. Sugiyama and Anderson (1998) suggested that these species should be separated at a rank above the Family Spyroidea, and that it is necessary to reconstruct the classification of nassellarians to avoid contradiction. The results of our molecular phylogenetic study combined with the conclusions of Sugiyama and Anderson (1998) indicate that the arrangement of fusules may not be useful for the classification of the Polycystinea.

An important question is raised concerning the nature of the common ancestral feature among the pterocorythid nassellarians and the collosphaerid, sphaerozoid, and thalassicollid spumellarians. The Sphaerozoidae and the Thalassicollidae have triradiate branched or simple unbranched spicules in their ectocytoplasmic bodies that resemble the nassellarian internal (cephalic) spicules around the central capsule. Consequently, the present results suggest that the collosphaerid, sphaerozoid, thalassicollid spumellarians and the pterocorythid nassellarians may have evolved from an ancestor with triradiate branched spicules.

Our analyses of the nassellarian 18S rDNA sequences revealed the close relationship between the Nassellarida and the colonial, skeletonless Spumellarida. In the absence of a through understanding of their biology, we have often accepted easy but arbitrary and inappropriate criteria for classifying the ''Radiolaria''. It is also necessary to accumulate information from cytology and skeletal morphology, as well as stratigraphic data from continuous fossil records, in order to further elaborate the phylogeny reconstructed by molecular analyses. Ultimately, making comparisons across these various types of information will reveal the true evolutional history of ''Radiolaria''. Furthermore, such

study of "Radiolaria", whose early emergence has been identified in the evolution of eukaryotes, will also provide useful information for the analysis of the phylogeny of diverse protists.

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