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Original article

Molecular phylogeny of solitary shell-bearing Polycystinea (Radiolaria)

Phylogénie moléculaire des Radiolaires polycystines solitaires à squelette

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Abstract

The phylogeny of the Family Spongodiscidae (polycystine Radiolaria), which includes *Dictyocoryne profunda* Ehrenberg, *Dictyocoryne truncatum* (Ehrenberg) and *Spongaster tetras* Ehrenberg, was examined using 18S ribosomal DNA (small-subunit ribosomal DNA) sequence analysis. Three types of tree construction methods, the neighbor joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) methods, were used to infer the phylogenetic relationships of the polycystine and acantharian Radiolaria among eukaryotes. The obtained 18S rDNA molecular phylogenetic tree argues for the monophyly of the two groups. Furthermore, the Polycystinea is divided into at least two distinct lineages consisting of: (1) colonial and skeletonless Polycystinea, including Thalassicollidae, Collospaeridae, and Sphaerozoidae; and (2) shell-bearing solitary Polycystinea, including Spongodiscidae. The Polycystinea thus show a paraphyly among Radiolaria. Moreover, the monophyly of the clade including the acantharians and the spongodiscid polycystines was supported by bootstrap values, which were 94%, 53%, and 59% in the NJ, MP, and ML analyses, respectively. This lineage is characterized by having latticed or spongy skeletons of different chemical composition, namely SiO_2 (Class Polycystinea) or $SrSO_4$ (Class Acantharea). According to the present taxonomic scheme, the Acantharea and the Polycystinea have not been placed in different classes, but the results of our molecular study show the opposite. We therefore suggest, based on the monophyly of the two clades, that a new taxonomic group of Radiolaria can be established. Our molecular data also suggest that the currently used radiolarian taxonomic system may need serious revisions.

Résumé

La phylogénie de la famille Spongodiscidae (Radiolaires Polycystines), qui comprend les espèces *Dictyocoryne profunda* Ehrenberg, *D. truncatum* (Ehrenberg) et *Spongaster tetras* Ehrenberg, a été examinée sur la base de leur séquence d'ADN ribosomique 18S (petite sous-unité ribosomique ADN). Trois types de construction d'arbre, « neighbor joining » (NJ), « maximum parsimony » (MP) et « maximum likelihood » (ML) ont été utilisés afin d'établir les relations phylogénétiques des Radiolaires Polycystines et Acanthaires parmi les eucaryotes. Les arbres phylogénétiques moléculaires obtenus mettent en évidence la monophylie des deux groupes. En outre, les Polycystines sont divisés en au moins deux lignées distinctes : 1) les Polycystinea coloniaux sans squelette, tels que les Thalassicollidae, Collospaeridae et Sphaerozoidae ; et 2) les Polycystinea solitaires à squelette, y compris les Spongodiscidae. Les Polycystinea montrent ainsi une paraphylie parmi les Radiolaires. En outre, la monophylie du clade qui comprend les Acanthaires et les Polycystines spongodiscid a été favorisée par les valeurs « bootstrap », qui étaient à 94 %, 53 et 59 % dans les analyses NJ, MP et ML, respectivement. Cette lignée est caractérisée par des squelettes perforés ou spongieux de compositions chimiques diverses, notamment SiO_2 au sein de la Classe Polycystinea ou $SrSO_4$ au sein de la Classe Acantharea. Selon les schémas taxonomiques actuels, les Acantharea et les Polycystinea ne sont pas placés dans des classes différentes, mais les résultats de notre étude moléculaire indique le contraire. Par conséquent nous suggérons, sur la base de la monophylie des deux clades, qu'un nouveau groupe taxonomique soit établi chez les Radiolaires. Nos données moléculaires suggèrent aussi que le système taxonomique des radiolaires en cours a probablement besoin de révisions importantes.

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Mots-clés : Radiolaires ; Polycystines ; Acanthaires ; Spongodiscidae ; Spumellaires ; 18S rDNA ; Phylogénie moléculaire

1. Introduction

Radiolaria sensu lato (Class Acantharea, Class Polycystinea, and Class Phaeodarea: Haeckel, 1887) are holoplanktonic protists. They have roughly spherical cells and thread-like pseudopodia extending radially over the endoskeleton that facilitate a floating existence (e.g. Anderson, 1983). They are largely nonmotile organisms that occur in modern open ocean and neritic environments, ranging from arctic to tropical waters and occurring throughout the water column from the surface to the greatest depths (Casey, 1971).

With the exception of some species or genera, most Radiolaria bear a skeleton. The chemical composition of the skeletons differs within the radiolarian group, being of strontium sulfate ($SrSO_4$) in Class Acantharea, opaline silica (SiO_2) in Class Polycystinea, and opaline silica with organic matter in Class Phaeodarea (e.g. Anderson, 1983). Since the skeletons of both Acantharea and Phaeodarea are very easily dissolved in seawater, the Acantharea have not been found and the Phaeodarea have been rarely found as fossils (e.g. Reshetnjak, 1971; Takahashi et al., 1983). On the other hand, the Polycystinea have rich fossil records due to the presence of skeletal material composed of solid opaline silica. They are commonly preserved in marine sediments.

Since Meyen's (1834) first report on polycystine Radiolaria, many taxonomic studies of Radiolaria have been published (e.g. Campbell, 1954; Sanfilippo and Riedel, 1985). Almost all of these studies were performed on fossil records from marine sediments and sedimentary rocks. Most taxonomic systems have, therefore, been devised on the basis of radiolarian skeletal morphology (e.g. Haeckel, 1887), and the evolutionary histories of various radiolarian lineages have been accumulated from detailed stratigraphic records (e.g. Sanfilippo and Riedel, 1985). There have been very few studies comparing these data with taxonomic information obtained from studies of living radiolarians.

The application of molecular analysis to radiolarians has been limited because of the difficulty of culturing radiolarians in a laboratory. The first determination of 18S ribosomal DNA (small subunit ribosomal DNA) sequences in the Acantharea and the colonial Polycystinea was reported by Zettler et al. (1997). They used the genomes of the swarmer cells that endogenously formed in individual vegetative cells as templates for polymerase chain reaction (PCR) amplification. Zettler et al. (1997) suggested that the Polycystinea emerged earlier than the other eukaryotes including the Acantharea, and that Radiolaria do not represent a monophyletic evolutionary assemblage. Subsequently, the variety of the genotype in the Genus *Thalassicolla* (solitary skeletonless Polycystinea), the phylogenetic relationships of the Collosphaeridae and Sphaerozoidae (colonial Polycystinea), and the phylogeny of some acantharians were revealed by Zettler et al. (1998, 1999) and Zettler and Caron (2000).

Recently, López-García et al. (2002) insisted that the Polycystinea and the Acantharea may have emerged as sister groups, as suggested by the phylogenetic analyses (with 18S rDNA sequences) of polycystine- and acantharian-related genomic fragments from Antarctic deep waters. This conclusion, however, contradicts the results of Zettler et al. (1997).

In this paper, we will address this question and attempt to clarify the phylogenetic relationship among the Polycystinea and Acantharea based on the 18S rDNA sequences. In particular, we will focus on the phylogenetic relationships of Haeckel's "solitary shell-bearing" Polycystinea. The majority of the polycystine species is solitary and has siliceous skeletons and tests. So far, they have not been the subjects of any molecular studies, in spite of their important contributions to the paleontological record.

2. Materials and methods

2.1. Materials

Radiolarians were collected from surface seawater (up to 3 m depth) on May 29th and September 17th in 2001, using a plankton-net (60 cm net opening, mesh size 37 μm) at Site 990528 (26°37′N, 127°47′E) located approximately 5 km northwest of Okinawa Island, Japan. The concentrated plankton samples were diluted with seawater from the same site and brought to the laboratory at the Tropical Biosphere Research Center of the University of the Ryukyus. Using an inverted microscope or a binocular stereoscopic microscope, Radiolarians were isolated as soon as possible and transferred to culture dishes containing filtered seawater. The radiolarians studied were *Dictyocoryne truncatum* (Ehrenberg), *Dictyocoryne profunda* Ehrenberg, and *Spongaster tetras* Ehrenberg (Family Spongodiscidae, Order Spumellaria) as follows; they are all solitary and have spongy, siliceous tests, which are about 200 μm in diameters (Figs. 1–3).

D. truncatum (Ehrenberg) (Fig. 1): a light microscopic (LM) image of living *D. truncatum* shows a triangular, flattened test with rounded apices, with the maximum length of the test ranging from about 200 to 300 μm. The surface of the test has a spongy meshwork without ornamentation, enclosing light greenish-yellow to orange-colored cytoplasm and radiating pseudopodia.

D. profunda Ehrenberg (Fig. 2): morphologic features of living *D. profunda* using LM observation closely resemble those of *D. truncatum. D. profunda* has also a triangular, flattened test with rounded apices ranging in length from about 200 to 300 μm. The surface of the test has a spongy

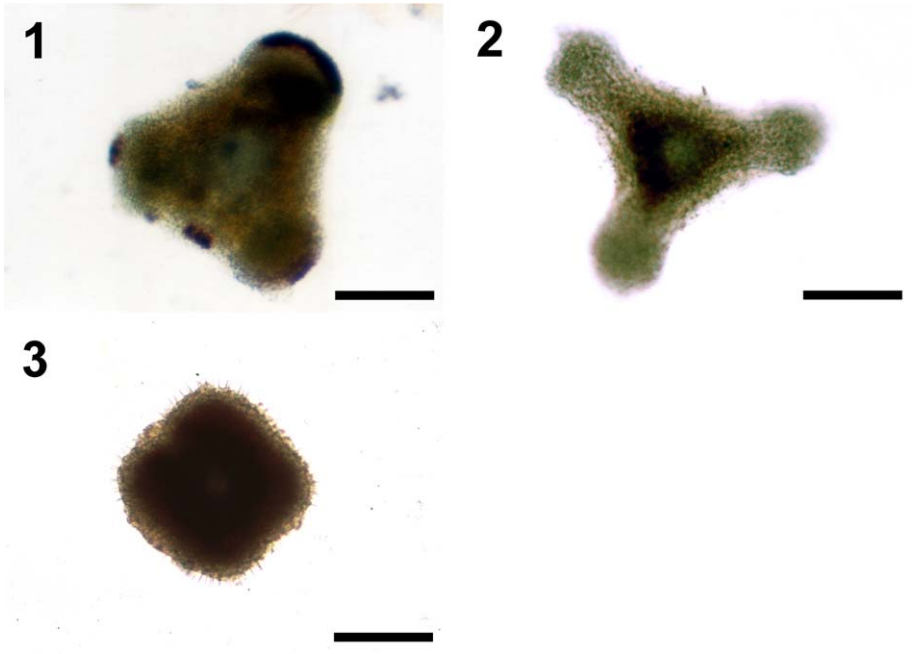

Fig. 1–3. Light micrographs (LM) of radiolarians used in this study. Scale bars indicate 100 μm. **1**: LM photograph of *D. truncatum* (Ehrenberg). **2**: LM photograph of *D. profunda* Ehrenberg. **3**: LM photograph of *S. tetras* Ehrenberg.
Figs. 1–3.Microphotographies des radiolaires utilisés dans cette étude. L'échelle indique 100 μm. **1** : *D. truncatum* (Ehrenberg). **2** : *D. profunda* Ehrenberg. **3** : *S. tetras* Ehrenberg.

meshwork, enclosing brownish-orange to red-colored cytoplasm with radiating pseudopodia. *D. profunda* is distinguished from *D. truncatum* in having rather dark colored (brownish-orange to red) cytoplasm and rather rounded apices of the test.

S. tetras Ehrenberg (Fig. 3): *S. tetras* has a spongy, flattened tetragonal test ranging in length from about 150 to 200 μm, with rounded corners. The surface of the test has a spongy meshwork with numerous minute spines, enclosing light greenish-yellow to orange-colored cytoplasm and radiating pseudopodia. The color of the cytoplasm under LM closely resembles those of *D. truncatum*.

2.2. DNA extraction and PCR amplification

The protoplasm of each radiolarian cell is composed of two parts, an ectocytoplasm and an endocytoplasm, bounded by a perforate organic membrane called the central capsule (e.g. Anderson, 1983). Each radiolarian cell was microdissected on a glass slide with a sterilized razor blade in distilled water (DW) and the central capsule was physically separated from the ectocytoplasm, which contained endosymbiotic algae. The central capsule of each specimen was rinsed twice in DW by pipetting and was immediately ground, then incubated with 0.2–μg/μl proteinase K at 37 °C for 30 min.

Each sample containing radiolarian genomic DNA was used as a target template in PCR amplification. Each 18S rDNA fragment of about 1700 base pairs (bp) was amplified by PCR using eukaryotic universal primers for 18S rRNA coding regions: forward primer 90F (Hendriks et al., 1989): 5′-GAAACTGCGAATGGCTCATT-3′; reverse primer B (Medlin et al., 1988): 5′-CCTTCTGCAGGTTCACCTAC-3′. PCR amplification consisted of 3.5 min of denaturation at 94 °C followed by 35 amplification cycles, each consisting of 94 °C for 1 min, 55 °C for 2 min, and 74 °C for 3 min using a Program Temperature Control System PC-701 (ASTEC) thermal cycler. The amplified PCR products were purified using GFX PCR DNA and the Gel Band Purification Kit (Amaersham Pharmacia Biotech) and cloned in the pGEM-T Easy Vector system (Promega) using *E. coli* JM109 Competent cells (Promega). Purified plasmids containing the clones were subjected to DNA sequencing.

2.3. Sequencing

Cycle sequencing of the cloned products (plasmid DNAs) was accomplished using FITC-labeled primers and reagents from the Thermo Sequence Fluorescent Labeled Primer Cycle Sequencing Kit with 7-deaza-dGTP (Amaersham Pharmacia Biotech), which consisted of 3 min of denaturation at 95 °C, prior to 20 cycles of 30 s at 95 °C, 30 s at 50 °C and 1 min at 72 °C, and 20 cycles of 30 s at 95 °C and 1 min at 72 °C using a PTC-150 (MJ Research) thermal cycler. This was followed by Double-stranded sequencing of the entire forward and reverse strands of the rRNA coding regions was conducted for cloned products using the DSQ2000L (SHIMADZU) automated sequencer. In each radiolarian species, the 18S rDNA sequence was determined from the sequencing of five-pooled clones, which were originally amplified from the genome of a single radiolarian cell. Sequences have been submitted to GenBank under accession numbers AB101540–AB101542

2.4. Alignment

Seventeen sequences of the Acantharea and the Polycystinea, and other eukaryotic sequences were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html) and the European ribosomal RNA database at the University of Gent (BELGIUM) (http://www.oberon.fvms.ugent.be:8080/rRNA/index.html) and aligned. Our determined sequences were individually added to the above-aligned data set through a profile alignment process of Clustal W ver. 1.81 (Thompson et al., 1994). Subsequently, the alignment was manually refined using the nucleotide sequence editor Se-Al ver. 1.0a1 (Andrew, 1995), and the positions with gaps were removed for subsequent phylogenetic analyses, resulting in an alignment with 1329 positions. The complete sequence alignment file in this study is available from the authors upon request.

2.5. Phylogenetic reconstruction

Phylogenetic trees were constructed with PAUP* ver. 4.0b10 (Swofford, 2002) on the basis of the neighbor joining (NJ) method (Saitou and Nei, 1987), the maximum parsimony (MP) method (Eck and Dayhoff, 1966), and the maximum likelihood (ML) method (Felsenstein, 1981). The NJ analyses were applied to distances corrected for unequal transition and transversion rates using the Hasegawa, Kishino, and Yano (HKY) model (Hasegawa et al., 1985). The MP analyses were conducted using heuristic analyses (100 replicates), the tree bisection-reconnection (TBR) algorithm and random orders of sequence addition. The ML analyses were performed using the HKY85 two-parameter model of nucleotide substitution (Hasegawa et al., 1985). We initially obtained a topology by the NJ method as the starting tree and ran heuristic searches with TBR branch swapping for the more detailed searching of the ML topology. Relative levels of support for nodes of the NJ, MP, and ML trees were assessed by calculating full heuristic bootstrap proportion (BP) values (Felsenstein, 1985) based on 1000, 100, and 100 replicates in the NJ, MP, and ML analyses, respectively.

3. Results

The length in base pairs and G% + C% (G + C content) obtained in each species were as follows: *D. profunda* (accession No. AB101540), 1726 bp, 50.1% GC; *D. truncatum* (accession No. AB101541), 1727 bp, 49.8% GC; and *S. tetras* (accession No. AB101542), 1726 bp, 49.9% GC. These lengths range from 1726 to 1727 bp. The G + C content of the partial or entire 18S rDNA of our investigated species ranged from 35.0% (*Acrosphaera* sp. CR6A and *Collosphaera globularis-huxleyi*) to 50.1% (*D. profunda*).

Fig. 4(a, b) show the NJ and ML reconstructed trees of major eukaryotic groups, respectively, including our determined sequences (for a total of 39 sequences) (Table 1). The bootstrap values at the nodes are given by all analyses. Concerning the relationship between the Acantharea and the Polycystinea, the MP tree (not shown) and the ML tree (Fig. 4(b)) are consistent with trees previously reported by López-García et al. (2002); namely, the Acantharea and the Polycystinea emerge as sister groups and this is supported by bootstrap values of 71% and 49% in the MP and ML methods, respectively. On the other hand, the monophyly of both groups is not supported by the NJ method (Fig. 4(a)). In the NJ tree, the colonial and skeletonless Polycystinea branch off just before the divergence of the so-called crown group (Knoll, 1992).

The Kishino–Hasegawa (KH) test (Kishino and Hasegawa, 1989) and the Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa, 1999), which are implemented in PAUP* ver. 4.0b10 (Swofford, 2002), were used to compare the NJ, MP, and ML topologies using the ML method. The KH and SH tests estimated a best tree and the probability *(P)* of branching for each lineage at alternative positions in the NJ, MP, and ML trees. The log likelihood differences between the best (ML: lowest log likelihood) and the NJ and MP topologies suggest that the MP and ML topologies could not be rejected by the KH and SH tests, whereas the NJ topology was rejected ($P \leq 0.05$) (Table 1).

Fig. 4(a, b) show the remarkable finding that the Acantharea and our determined sequences of the Polycystinea are constantly monophyletic, and are supported by bootstrap values of 98%, 42%, and 32% in the NJ, MP, and ML methods, respectively. To address the relationship of the Acantharea and the Polycystinea among the Radiolaria, the NJ, MP, and ML reconstructed trees for all available radiolarian sequences, including our determined sequences, are shown in Fig. 5(a–c), respectively.

The topologies of the three trees are quite similar to each other. Both the monophyly of the Acantharea and the monophyly of the colonial and skeletonless Polycystinea are strongly supported by 100% bootstrap values in every analysis, and they have already been reported by Zettler et al. (1997,1999) and López-García et al. (2002) as the same major clades. Our determined sequences of spongodiscids, *D. profunda, D. truncatum*, and *S. tetras*, also form a monophyletic group with 100% bootstrap values in each analysis (Fig. 5(a–c), respectively). Moreover, these shell-bearing species are more closely related to the Acantharea rather than the colonial and skeletonless Polycystinea, as was also indicated in the larger data set (Fig. 4(a, b)). The monophyly of our determined sequences and the Acantharea is weakly relative, with 53% and 59% bootstrap values in the MP and ML trees, respectively, whereas the monophyly is strongly supported by a 94% bootstrap value in the NJ analysis.

This result contradicts the modern morphological data, which propose a close relationship between the shell-bearing Polycystinea and the colonial and skeletonless Polycystinea. The Polycystinea is divided into at least two distinct lineages consisting of: (1) the Polycystinea including the Col-

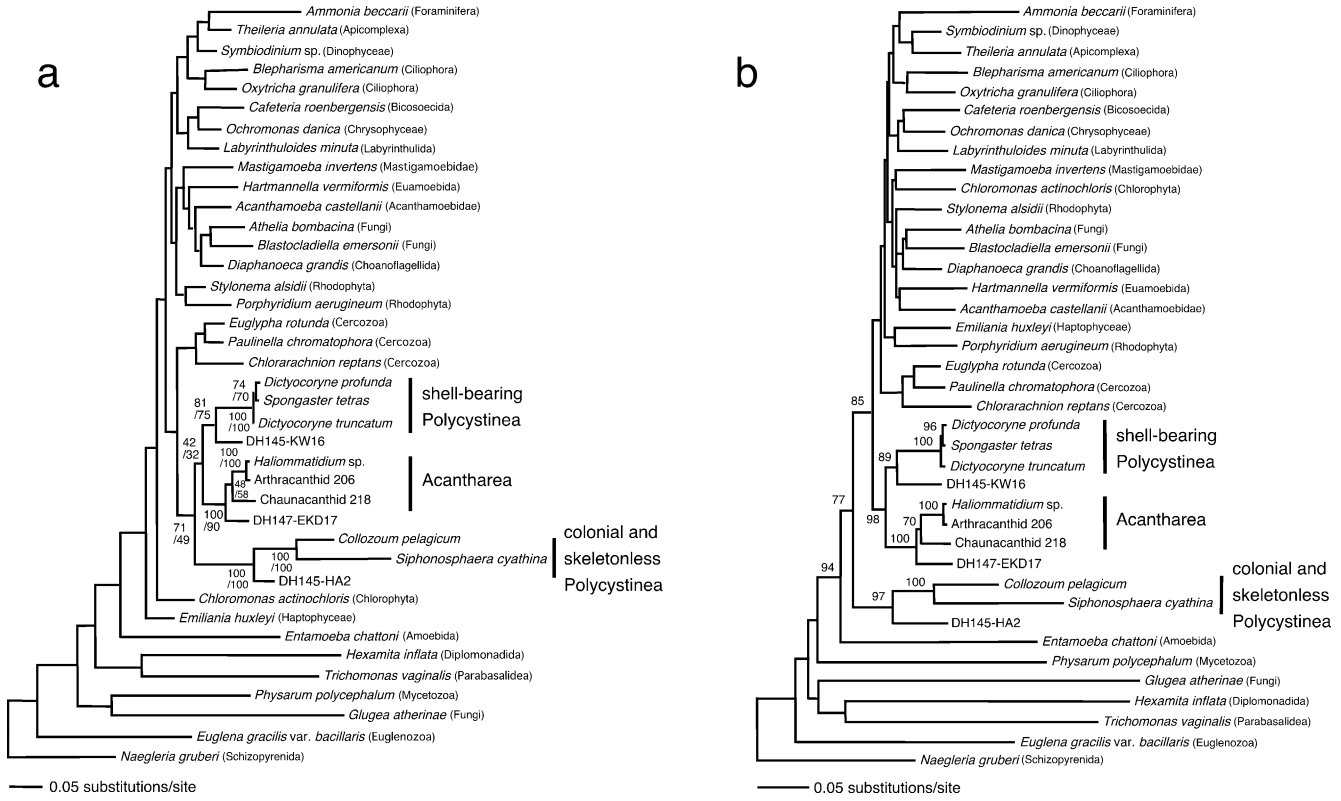

Fig. 4. Phylogenetic trees of 18S rDNA sequences obtained from 11 radiolarian taxa (1329 nucleotide sites) and 28 major eukaryotes. Sequences of the Spongodiscidae (solitary shell-bearing Polycystinea) were obtained from the current study. Bootstrap values were calculated on the basis of 1000, 100, and 100 replicates in the NJ, the MP, and the ML analyses, respectively; bootstrap values greater than 50% are shown (bar = 0.05 substitutions per site). **a**: NJ tree with the bootstrap values of the NJ topology. **b**: ML tree with the bootstrap values of the MP (left number) and ML (right number) topologies.

Fig. 4.Arbres phylogénétiques des gênes de rADN 18S obtenus à partir de 11 taxa de radiolaires (1329 sites nucléotiques) et 28 groupes majeurs d'eucaryotes. Les séquences de Spongodiscidae (Polycystines solitaires à squelette) ont été obtenues lors de cette étude. Les valeurs « bootstrap » ont été calculées sur la base de 1000, 100 et 100 répliques des analyses par « neighbor joining » (NJ), parcimonie maximale (MP) et « maximum likelihood » (ML), respectivement ; les valeurs « bootstrap » supérieures à 50 % sont indiquées (barre = 0,05 substitutions par site). **a** : arbre NJ avec les valeurs « bootstrap » de la topologie NJ. **b** : arbre ML avec les valeurs « bootstrap » des topologies MP (chiffres gauches) et ML (chiffres droits).

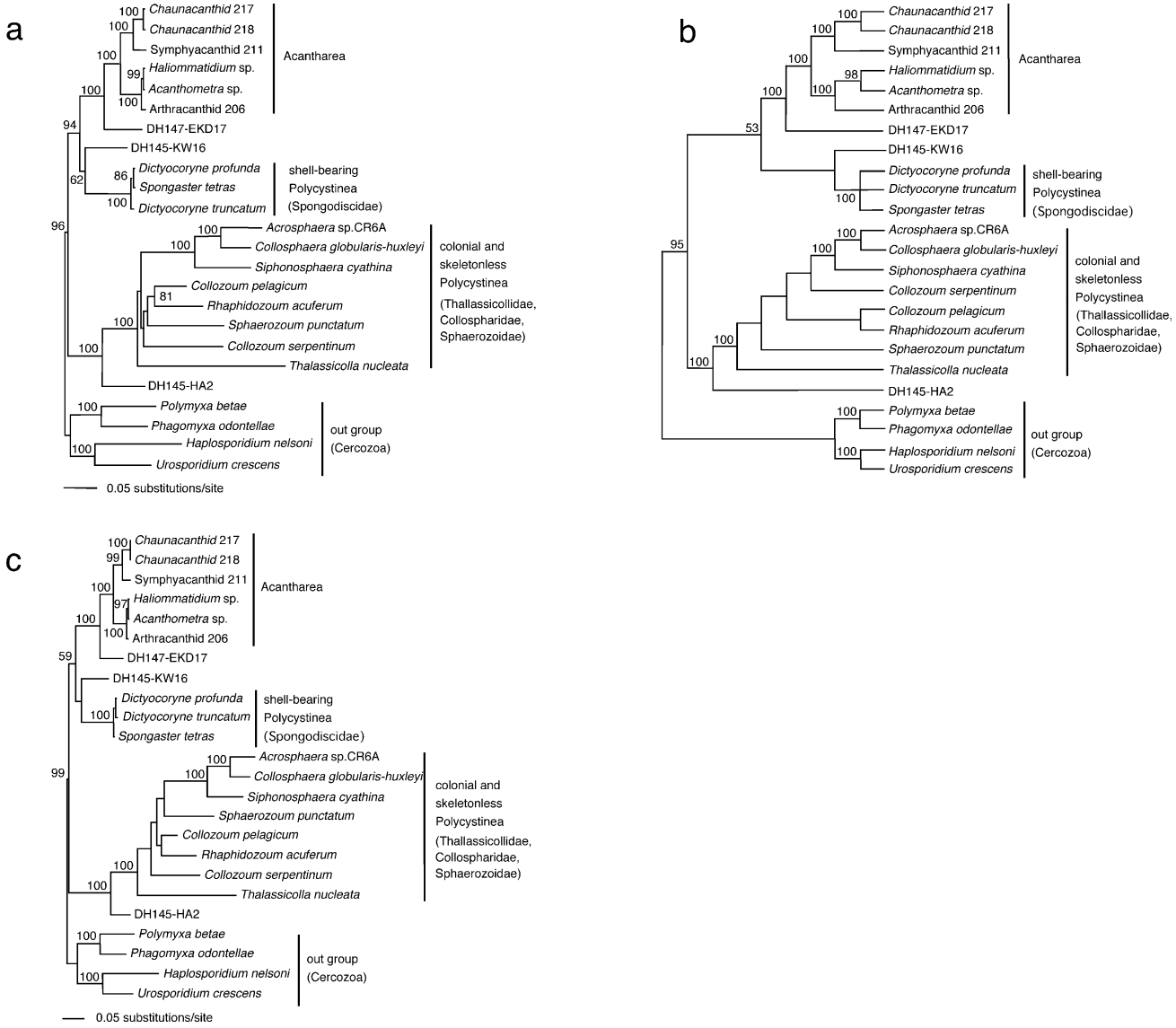

Fig. 5. Phylogenetic trees of 18S rDNA sequences obtained from 21 radiolarian taxa (1329 nucleotide sites). Sequences of the Spongodiscidae (solitary shell-bearing Polycystinea) were obtained from the current study. Bootstrap values were calculated on the basis of 1000, 100, and 100 replicates in the NJ, the MP, and the ML analyses, respectively; bootstrap values greater than 50% are shown (bar = 0.05 substitutions per site). **a**: NJ tree with the bootstrap values of the NJ topology. **b**: MP tree (consensus of two trees) with the bootstrap values of the MP topology. **c**: ML tree with the bootstrap values of the ML topology.

Fig. 5.Arbres phylogénétiques des gênes de rADN 18S obtenus à partir de 21 taxa de radiolaires (1329 sites nucléotiques). Les séquences de Spongodiscidae (Polycystines solitaires à squelette) ont été obtenues lors de cette étude. Les valeurs « bootstrap » ont été calculées sur la base de 1000, 100 et 100 répliques des analyses par « neighbor joining » (NJ), parcimonie maximale (MP) et « maximum likelihood » (ML), respectivement ; les valeurs « bootstrap » supérieures à 50 % sont indiquées (barre = 0,05 substitutions par site). **a** : arbre NJ avec les valeurs « bootstrap » de la topologie NJ. **b** : arbre MP (consensus de deux arbres) avec les valeurs « bootstrap » de la topologie MP. **c** : arbre ML avec les valeurs « bootstrap » de la topologie ML.

Table 1
KH and SH tests for the NJ, MP1, MP2, and the MP consensus, and ML topologies of major eukaryotic groups, including our determined radiolarian sequences—ln *L*: log likelihood. Diff. ln *L*: difference of log likelihood. *P*: probability
Tests KH et SH pour les groupes majeurs d'eucaryotes (y compris les séquences de radiolaires que nous avons déterminées) selon les topologies d'arbres établies par les méthodes de « neighbor-joining » (NJ), parcimonie maximale (MP1, MP2 et MP de consensus) et « maximum likelihood » (ML)

Tree	ln *L*	Diff. ln *L*	KH-test (*P*)	SH-test (*P*)
NJ	17 898.07985	76.50155	0.025 *	0.045 *
MP1	17 860.83386	39.25556	0.287	0.222
MP2	17 863.41375	41.83546	0.262	0.214
Mpcons	17 850.08470	28.50641	0.450	0.402
ML	17 821.57829	(best)		

* $P < 0.05$.

lospaeridae, Sphaerozoidae, and Thalassicollidae; and (2) the shell-bearing solitary Polycystinea including the Spongodiscidae.

4. Discussion

Early reports of the Acantharea (e.g. Müller, 1858; Haeckel, 1887) grouped the Acantharea and the Polycystinea within Radiolaria based on the radial appearance of their pseudopodia, as well as their skeletal features. On the other hand, as explained earlier, the modern higher rank taxonomy of the classes within Radiolaria has largely been based on differences in the chemical composition and morphology of their skeletons; therefore, the Acantharea have in many modern taxonomic systems not been grouped together with the Polycystinea and the Phaeodarea as Radiolaria (e.g. Riedel, 1967; Anderson, 1983). The important question of whether the Acantharea and the Polycystinea are monophyletic or not thus remains. The present study again raises the possibility that the monophyly of the two groups of Radiolaria exists (Fig. 4(b)), as already proposed by López-García et al. (2002). The phylogenetic relationship between the Acantharea and the Polycystinea is a subject of continuing debate.

We do, however, suggest at least the two following higher rank taxonomic possibilities: (1) the Polycystinea is divided into two distinct lineages; and (2) the Acantharea and the Spongodiscidae may be closely related, but this lineage is characterized by two groups that have latticed or spongy skeletons with different chemical compositions, SiO_2 and $SrSO_4$, respectively. The monophyly of the clade including the Acantharea and the Spongodiscidae suggests that the chemical compositions of the skeletons are less important for determining the radiolarian taxonomy than are the textural/morphological features.

Also Zettler et al. (1997) acknowledge that the skeletal material seems to be of lesser importance for the higher rank taxa. At later stages of swarmer production in the Collospaeridae and the Thalassicollidae, some researchers have observed a crystalline inclusion in a vacuole of the swarmer cell (Anderson, 1976). The chemical compositions of the crystals include a high strontium content (Hollande and Martoja, 1974; Anderson, 1981), and strontium is the same substance that is present in the acantharean skeletons. Although it is unknown whether strontium sulfate inclusions are present within the swarmers of the Spongodiscidae, our results indicate that the Polycystinea and the Acantharea have a common ancestor.

The fossil record of radiolarians documents their evolutionary history. However, as mentioned above, the acantharians and the polycystine skeletonless forms are not preserved as fossils. Owing to the lack of paleontological evidence, it is not possible to evaluate how well systematic schemes derived on the basis of morphology reflect phylogenetic relationships. Classical morphological approaches combined with molecular genetic analyses hold promise for a better understanding of the radiolarian systematics and evolution; in particular, sequencing and phylogenetic analyses based on a larger number of radiolarian species are needed. Moreover, to elucidate the whole radiolarian lineage, analyses of representatives of the Order Nassellarida and the Class Phaeodarea are also required. On the other hand, although 18S ribosomal DNA analyses are the most widely used molecular marker for phylogenetic studies we are fully aware of the limitations of a single gene analysis. Future use of a broader range of nuclear genes will provide better constraints to fully test phylogenetic affinities.

5. Concluding remarks

Our present results indicate that the Spongodiscidae have a close phylogenetic relationship with the Acantharea. Both groups have a common textural feature in that they have latticed or spongy skeletons of different chemical compositions. If this one characteristic, the mineralized latticed or spongy skeleton, is common between the two groups, the monophyly of the two clades, the Acantharea and the Spongodiscidae, would require a new taxonomic group. This shared characteristic may lead us to examine other taxonomic criteria that could be latent in these organisms.

Radiolarian science in various fields, e.g. morphology, ecology, cytology, reproduction, and physiology, is still in an early stage. For a better understanding of the radiolarian phylogeny, we may come closer to a natural taxonomic system by using the molecular tools that are now available. A natural consequence of this could very well be a total revision of the present radiolarian taxonomic scheme.

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References

Anderson, O.R., 1976. Fine structure of a collodarian radiolarian (*Sphaerozoum punctatum* Müller, 1858) and cytoplasmic changes during reproduction. Marine Micropaleontology 2, 251–264.

Anderson, O.R., 1981. Radiolarian fine structure and silica deposition. In: Simpson T., Volcani B.E. (Eds.), Silicon and Siliceous Structures in Biological Systems. Springer, New York, pp. 347–380.

Anderson, O.R., 1983. Radiolaria. Springer, New York.

Andrew, R., 1995. Sequence Alignment Editor, Ver 1.0a1. http://www.evolve.zoo.ox.ac.uk/.

Campbell, A.S., 1954. Radiolaria. In: Moore R.C. (Ed.), Treatise on Invertebrate Paleontology, Part D, Protista 3. Geological Society of America and University Kansas Press, Lawrence, pp. D11–D195.

Casey, R.E., 1971. Distribution of polycystine Radiolaria in the oceans in relation to physical and chemical conditions. In: Funnell B.M., Riedel W.R. (Eds.), The Micropalaeontology of Oceans. Cambridge University Press, Cambridge, pp. 151–159.

Eck, R.V., Dayhoff, M.O., 1966. Atlas of Protein Sequence and Structure. National Biomedical Research Foundation, Silver Spring, Maryland.

Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal of Molecular Evolution 17, 368–376.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Haeckel, E., 1887. Report on the Radiolaria collected by HMS Challenger during the years 1873–1876. In: Thompson C.W., Murray J. (Eds.), Report on the Scientific Results of the Voyage of the HMS Challenger, Zoology 18, 2 parts. Her Majesty's Stationery Office, London.

Hasegawa, M., Kishino, H., Yano, T., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22, 160–174.

Hendriks, L., Goris, A., Neefs, J., Van de Peer, Y., Hennebert, G., De Wachter, R., 1989. The nucleotide sequence of the small ribosomal subunit RNA of the yeast *Candida albicans* and the evolutionary position of the fungi among the eukaryotes. Systematic and Applied Microbiology 12, 223–229.

Hollande, A., Martoja, R., 1974. Identification du cristalloïde des isospopres de Radiolaires à un cristal de célestite ($SrSO_4$). Protistologica 10, 603–609.

Kishino, H., Hasegawa, M., 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. Journal of Molecular Evolution 29, 170–179.

Knoll, A.H., 1992. The early evolution of eukaryotes: a geological perspective. Science 256, 622–627.

López-García, P., Rodríguez-Valera, F., Moreira, D., 2002. Toward the monophyly of Haeckel's Radiolaria: 18S rRNA environmental data support the sisterhood of Polycystinea and Acantharea. Molecular Biology and Evolution 19, 118–121.

Medlin, L., Hille, J.E., Shawn, S., Sogin, M.L., 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene 71, 491–499.

Meyen, F.J.F., 1834. Über das Leuchten des Meeres und Beschreibung einiger Polypen und anderer niederer. Thiere. Verh. Kaiserl. Leopoldin.-Carolin. Akad. Naturf. 16, 125–216.

Müller, H., 1858. Über die Thalassicollen, Polycystinen und Acanthometren des Mittelmeeres. Abhandlung Königliche Akademie der Wissenschaft Berlin 1–61.

Reshetnjak, V.V., 1971. Occurrence of phaeodarian Radiolaria in recent sediments and tertiary deposits. In: Funnell B.M., Riedel W.R. (Eds.), The Micropalaeontology of Oceans. Cambridge University Press, pp. 343–349.

Riedel, W.R., 1967. Subclass Radiolaria. In: Harland W.B. et al. (Eds.), The Fossil Record. Geological Society of London, London, pp. 291–299.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4, 406–425.

Sanfilippo, A., Riedel, W.R., 1985. Cenozoic Radiolaria. In: Bolli H.M., Saunders J.B., Perch-Nielsen K. (Eds.), Plankton Stratigraphy. Cambridge University Press, Cambridge, pp. 631–712.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Molecular Biology and Evolution 16, 1114–1116.

Swofford, D.L., 2002. Phylogenetic Analysis Using Parsimony (PAUP*), ver 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Takahashi, K., Hurd, D.C., Honjo, S., 1983. Phaeodarian skeletons: their role in silica transport to the deep sea. Science 222, 616–618.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research 22, 4673–4680.

Zettler, L.A., Caron, D.A., 2000. New insight into the phylogeny of the Acantharea based on SSU rRNA gene sequencing. European Journal of Protistology 36, 34–39.

Zettler, L.A., Sogin, M.L., Caron, D.A., 1997. Phylogenetic relationships between the Acantharea and the Polycystinea: a molecular perspective on Haeckel's Radiolaria. Proceedings of the National Academy of Sciences of the United States of America 94, 11411–11416.

Zettler, L.A., Anderson, O.R., Caron, D.A., 1998. Insights on the diversity within a "species" of *Thalassicolla* (Spumellaria) based on 16S-like ribosomal RNA gene sequence. Journal of Eukaryotic Microbiology 45, 488–496.

Zettler, L.A., Anderson, O.R., Caron, D.A., 1999. Towards a molecular phylogeny of colonial spumellarian radiolaria. Marine Micropaleontology 36, 67–79.