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PHYLOGENY OF ALLOGROMIID FORAMINIFERA INFERRED FROM SSU rRNA GENE SEQUENCES

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ABSTRACT

Allogromiids are classically defined as a group of monothalamous, soft-walled foraminiferans. Recent morphological, cytological, and molecular studies, however, challenge this view, showing that the soft-walled allogromiids are closely related to naked athalamids and unilocular agglutinated foraminiferans. To establish the phylogenetic relationships among these three groups we obtained partial small-subunit ribosomal DNA sequences of 50 species and undetermined morphotypes, and compared them to other foraminiferal taxa. Phylogenetic analyses of our data show that allogromiids, athalamids and astrorhizids comprise an assemblage of 13 lineages branching together at the base of the foraminiferal tree. Among these lineages, two are represented by a single species and four comprise similar genera, while the remaining seven are heterogeneous groups composed of several species having different types of wall structure and different test morphologies. All lineages are relatively well supported, yet the relationships among them are not resolved. In view of our data, we propose to revise the definition of allogromiids to include all naked and testate unilocular granuloreticuloseans that diverged early in the evolution of Foraminifera.

INTRODUCTION

Traditionally, allogromiids are defined as a group of monothalamous, soft-walled foraminiferans possessing a membranous or proteinaceous test, sometimes adorned with small quantities of agglutinated material (Loeblich and Tappan, 1987). It is widely accepted that allogromiids represent the most primitive group from which all other foraminiferal lineages originated (for historical reviews of foraminiferal classification, see Loeblich and Tappan, 1964; Cifelli and Richardson, 1990; Sen Gupta, 1999). As such, extant allogromiids are thought to hold clues to understanding the early evolution of Foraminifera (Cushman, 1950). Unfortunately, the origin and phylogeny of the group remain largely unknown, because the tests of these organisms are poorly preserved in the fossil record.

In recent morphology-based taxonomic schemes of the Foraminifera, the allogromiids are classified in the suborder Allogromiina, composed of four families and 56 genera (Loeblich and Tappan, 1988; Sen Gupta, 1999). The majority of genera (53) belong to the families Lagynidae and Allogromiidae, with two minor families represented by a single genus (*Phthanotrochus*) and a fossil group (Maylisoriidae). Loeblich and Tappan (1987) defined the major allogromiid families by the type of test wall (membranous in Lagynidae and proteinaceous in Allogromiidae) as well as the type of gametes (biflagellate and amoeboid, respectively). Galloway (1933) used pseudopodial morphology to distinguish the filose Lagyninae and Amphitreminae from the reticulose Myxothecinae, Allogrominae, and Rhynchogrominae. Cushman (1950) excluded filose species from the Foraminifera and separated the family Allogromiidae into two subfamilies, Myxothecinae and Allogrominae, based on the absence or presence of a distinct aperture. Grassé (1953) placed some allogromiid genera (*Allogromia*, *Lieberkuehnia*) in the order Thalamia, a sister group to the Foraminifera within the class Granuloreticulosa, and retained only some soft-walled genera (*Boderia*, *Myxotheca*, *Shepheardella*). A similar system that divides allogromiids between the orders Monothalamida and Foraminifera was presented by Bovee (1985), but the criteria for this division were not well defined. All authors recognized the artificial character of these classifications and emphasized the need for further taxonomic revision.

The first molecular phylogenetic studies of foraminiferans brought new insight on the systematics of allogromiids. The analysis of small-subunit ribosomal DNA (SSU rDNA) sequence of the naked freshwater granuloreticulosean *Reticulomyxa filosa*, traditionally classified as an athalamid (Bovee, 1985), showed that this species branches within the Foraminifera, close to some allogromiids (Pawlowski and others, 1999a, 1999b). More recent molecular studies of environmental DNA samples using specific foraminiferal probes demonstrated the presence of several groups of inconspicuous freshwater foraminiferans that are closely related to allogromiids (Holzmann and others, in preparation). Finally, a new species of soft-walled allogromiid was isolated from an Australian tropical rainforest, demonstrating that foraminiferans also colonized a soil habitat (Meisterfeld and others, 2002).

Molecular studies (Pawlowski, 2000; Pawlowski and others, 2002) also show the close relationships between soft-walled allogromiids and some astrorhizids, which are traditionally classified with other agglutinated foraminiferans in the order Textulariina (Loeblich and Tappan, 1987) or in a separate suborder Astrorhizina (Loeblich and Tappan, 1989). Close affinities between certain members of both groups were previously suggested based on morphological, cytological, and behavioral observations (Bowser and others, 1995). Comparisons of allogromiid and astrorhizid SSU rDNA sequences confirm these observations and show that both groups branch together in the middle part of the foraminiferal phylogenetic tree (Pawlowski, 2000).

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In the present study, we have analyzed complete and partial SSU rDNA sequences from 50 species and undetermined morphotypes of naked, soft-walled, and agglutinated unilocular foraminiferans. Our objective was to establish the phylogenetic position of these groups within the Foraminifera and to examine their phylogenetic relationships. These data prompt us to redefine the assemblage of examined foraminiferans and to propose the division of this assemblage into new phylogenetic groups.

MATERIALS AND METHODS

The majority of foraminiferans examined in this study were collected either in Explorers Cove (McMurdo Sound, Antarctica) or in Kosterfjorden and Gullmarfjorden (Sweden) during several field trips in 1998–2000. The Antarctic foraminiferans were isolated from surface sediment samples obtained either by an airlift sampler (detailed in Pollock and Bowser, 1995) or from cores individually collected by divers. The Scandinavian foraminiferans were isolated from sediment samples collected by sledge. Sediment samples were sieved either on a 0.125, 0.400, or 1 mm mesh, and individuals were isolated using a dissecting microscope. Following isolation, each specimen was thoroughly cleaned and washed to eliminate contaminants and was maintained at ambient low temperatures until DNA extraction. Astrorhizid tests were opened and cell bodies (sarcodes) were isolated whenever possible. Species were identified using traditional morphological criteria. A list of examined species and undetermined morphotypes, their taxonomic classification, locality and date of their collection, the number of DNA isolates analyzed, and sequence accession numbers is given in Table 1.

DNA was extracted from a single or several cells by using either a DNeasy Plant Mini Kit (Qiagen) or the guanidine method (Tkach and Pawlowski, 1999). PCR amplifications were performed in a total volume of 50 μl, with an amplification profile consisting of 40 cycles of 30 s at 94°C, 30 s at 50°C, and 120 s at 72°C, followed by 5 min at 72°C for final extension. The amplified PCR products were purified using a High Pure PCR Purification Kit (Roche), and either sequenced directly or ligated into the pGEM-T Vector System (Promega) and subsequently cloned in XL-2 Ultracompetent Cells (Stratagene). Sequences were obtained using an ABI PRISM Big Dye Terminator Cycle Sequencing Kit and an ABI 377 DNA sequencer (Perkin-Elmer), all according to the manufacturer's instructions.

A fragment of about 1000 nucleotides (nt) situated near the 3′ end of the SSU rDNA was amplified by using the foraminiferal-specific primers s14F3 (5′ ACG CA(AC) GTG TGA AA CTT G) and sB (5′ TGA TCC TTC TGC AGG TTC ACC TAC). Another foraminiferal-specific primer s14F1 (5′ AAG GGC ACC ACA AGA ACG C) was used for reamplification of PCR products, if necessary. Complete SSU sequences were obtained as described previously (Pawlowski and others, 1999). Additionally, short SSU sequences obtained by PCR amplification using foraminiferal-specific primer s17 (as described in Pawlowski and others, 2002) were analyzed. The sequences reported in this paper have been deposited in the EMBL database.

Sequences were aligned manually using the SEAVIEW program (Galtier and others, 1996) and analyzed using the neighbor-joining (NJ) method (Saitou and Nei, 1987), applied to distances corrected for multiple hits, and for unequal transition and transversion rates, using the K2P model (Kimura, 1980). The maximum likelihood (ML) method (Felsenstein, 1981) was also used, as implemented in the fast DNAm1 program (Olsen and others, 1994). The reliability of internal branches in the NJ and ML trees was assessed using the bootstrap method, with 1000 and 100 replicates, respectively. The PHYLO_WIN program (Galtier and others, 1996) was used for distance computation, and tree building and bootstrapping.

RESULTS

Sequence Data

Complete SSU rDNA sequences were obtained for 14 foraminiferans, including one athalamid (*Reticulomyxa filosa*), one soft-walled allogromiid (*Allogromia* sp.A) and three astrorhizids (*Astrammina rara, Astrammina triangularis, Cribrothalammina alba*). The lengths and GC content of these SSU rRNA sequences are given in Table 2. The two *Astrammina* species possess the longest (4140 and 4066 nt respectively) sequences among the Foraminifera. The mean length of foraminiferal SSU sequences is about 3000 nt, which is much above the usual length of eukaryotic SSU (averaging 1800 nt). This unusual length is due to several long insertions present in variable and conserved regions, some of them unique to foraminiferans. The foraminiferal SSU is also characterized by a relatively low GC content, which averages 34.4%. The lowest GC values are found in miliolids (27.0% to 29.6%) and two saccamminids, *A. rara* (28.4%) and *A. triangularis* (28.9%). GC content is also below the mean foraminiferal value in *R. filosa* and *Allogromia* sp. A.

Partial SSU rDNA sequences were obtained for 50 species. The sequenced fragment (s14F3—sB) is located in the 3′ terminal region of the molecule and includes seven conserved segments. The length of the fragment ranges from 881 nt in *Cribrothalammina alba* to 1444 nt in *Astrammina rara.* The GC content in this SSU fragment ranges from 30.2% in *A. rara* to 56.3% in *Nemogullmia* sp. Intra-specimen variability, assayed by sequencing several clones for selected single-cell isolates, is lower than 0.1%. The majority of cloned sequences for each isolate were either identical or differed only by a few nucleotide substitutions that can be attributed to PCR or sequencing errors. Intraspecific variations in this fragment, measured by comparing the genetic distance between different isolates of the same morphospecies, are lower than 1%.

For 33 of 50 species, 106 additional sequences of a shorter SSU rDNA fragment (s14F3-s17) were obtained (data published in Pawlowski and others, 2002). The number of additional sequences per species varies from 1 to 19 (Table 1). The divergence between sequences of different isolates of the same morphospecies was again usually lower than 1%; higher divergence values were only observed in *Nemogullmia* sp. (4.0%), *Gloiogullmia* sp. (3.8%), *Hemisphaerammina* sp. (3.8%), *Astrammina rara* (2.6), and *Pelosina* sp. (1.8%). Isolates having sequence divergence below 5%

TABLE 1. List of species/morphotypes examined in this study, including their taxonomic position (according to Loeblich and Tappan, 1988), site of collection, number of DNA isolates and DNA sequences. Number of additional short SSU sequences obtained for some DNA isolates are indicated in parentheses.

Species	Family	Locality	DNA isolate #
Allogromia crystallifera	Allogromiidae	Kosterfjorden	1391 (2)
Allogromia laticolaris	Allogromiidae	culture	647 (2)
Allogromia sp. A	Allogromiidae	Antalya	AL_A (3)
Allogromia sp. J	Allogromiidae	Jamaica	AL_J
Armorella sphaerica	Saccamminidae	Kosterfjorden	547
Astrammina-like	Saccamminidae	Explorers Cove	1141
Astrammina rara	Saccamminidae	Explorers Cove	111 (6)
Astrammina triangularis	Saccamminidae	Explorers Cove	118 (5)
Boderia sp.	Allogromiidae	Lizard Island	658
Cribrothalammina alba	Saccamminidae	Sapelo	226
Crithionina delacai	Hemisphaeramminidae	Explorers Cove	189 (3)
Crithionina granum	Hemisphaeramminidae	Kosterfjorden	156
Crithionina mammila	Hemisphaeramminidae	Explorers Cove	1037 (5)
Crithionina sp.	Hemisphaeramminidae	Maldives	665
Crithionina sp.	Hemisphaeramminidae	Explorers Cove	1074
Cylindrogullmia alba	Allogromiidae	Kosterfjorden	525 (3)
Edaphoallogromia australica	Allogromiidae	Queensland	2266
Gloiogullmia eurystoma	Allogromiidae	Kosterfjorden	526
Gloiogullmia sp.	Allogromiidae	Explorers Cove	1188 (7)
Hemisphaerammina bradyi	Hemisphaeramminidae	Banyuls s/mer	1439 (2)
Hemisphaerammina sp.	Hemisphaeramminidae	Explorers Cove	1182 (5)
Hippocrepinella hirudinea	Hippocrepinellidae	Kosterfjorden	530
Hippocrepinella sp.	Hippocrepinellidae	Explorers Cove	1157
Nemogullmia sp.	Allogromiidae	Explorers Cove	A330 (2)
Nemogullmia sp.	Allogromiidae	Explorers Cove	A164 (2)
Notodendrodes antarctikos	Notodendrodidae	Explorers Cove	1082 (6)
Notodendrodes hyalinosphaira	Notodendrodidae	Explorers Cove	1225 (19)
Ovammina opaca	Saccamminidae	Barents Sea	2485 (2)
Pelosina sp.	Astrorhizidae	Explorers Cove	1029 (3)
Pelosinella fusiformis	Astrorhizidae	Explorers Cove	1092
Toxisarcon alba		Scotland	wc18h (2)
Psammophaga simplora	Saccamminidae	Sapelo	231
Psammophaga sp.	Saccamminidae	Southampton	2359 (2)
Psammophaga sp.	Saccamminidae	Explorers Cove	A300 (2)
Psammosphaera sp.	Psammosphaeridae	Explorers Cove	1018 (10)
Reticulomyxa filosa		culture	613 (2)
Rhabdammina cornuta	Rhabdamminidae	Explorers Cove	1045 (3)
Saccodendron limosum	Astrorhizidae	Kosterfjorden	563
Silver saccamminid	Saccamminidae	Explorers Cove	A26 (5)
Tinogullmia sp.	Allogromiidae	Explorers Cove	1199 (4)
Undetermined allogromiid		Lizard Island	664
Undetermined allogromiid		Explorers Cove	A104 (7)
Undetermined allogromiid		Explorers Cove	1212 (8)
Undetermined allogromiid		Explorers Cove	1195 (3)
Undetermined allogromiid		Explorers Cove	A313
Undetermined allogromiid		Explorers Cove	A213 (3)
Undetermined allogromiid		Explorers Cove	1124 (5)
Undetermined quartzball		Explorers Cove	A119 (2)
Undetermined saccamminid		Explorers Cove	A279 (2)
Undetermined saccamminid		Nissum B.	2399 (2)

were considered to belong to the same molecular type (Pawlowski and others, 2002).

PHYLOGENETIC ANALYSIS

Maximum likelihood (ML) analysis of complete SSU sequences (Fig. 1) shows Foraminifera branching with Cercozoa, as a sister-group to the filosean *Gromia oviformis*. The topology of the tree shown in Fig. 1 is typical for SSU-based phylogenies of organisms belonging to the so-called crown of eukaryotes. Because the position of the root of the eukaryotic phylogeny is still subject to debate, the tree is presented in an unrooted format, with a basal trichotomy separating amoebae, opisthokonts, and the rest of eukaryotes. The topology of the neighbor joining (NJ) tree only differs from that of the ML tree in the branching order between some of the eukaryotic groups (Fig. 1).

The position of major foraminiferal groups varies considerably depending on the method of analysis. In the ML tree (Fig. 1), the two *Astrammina* species branch at the base of the tree, followed by *Cribrothalammina alba* and *Reticulomyxa filosa. Allogromia* sp. appears as a sister taxon to the Miliolida. Textulariida and Rotaliida form the crown of the tree. In the NJ tree, the Miliolida branch as the earliest foraminiferal lineage, followed by *C. alba* and *R. filosa*,

TABLE 2. List of species for which the complete SSU rDNA sequences were obtained, including their total length, GC content, and GenBank accession numbers. Taxonomic position according to Loeblich and Tappan (1988), except *R. filosa* (order Athalamida, following Bovee, 1985).

Species	Family	SSU length	GC content	Accession #
Allogromia sp. *A*	Allogromiidae	3043	32.7	X86093
Astrammina rara	Saccamminidae	4140	28.4	AJ318223
Astrammina triangularis	Saccamminidae	4066	28.9	AJ318224
Cribrothalammina alba	Saccamminidae	2709	37.4	AJ318225
Reticulomyxa filosa	Athalamida	3347	32.6	AJ132367
Peneroplis pertusus	Peneroplidae	2464	29.4	AJ132368
Sorites orbiculus	Soritidae	2264	29.6	AJ132369
Borelis schlumbergeri	Alveolinidae	2866	27.0	AJ404295
Eggereloides scabrum	Eggerellidae	3553	36.4	AJ318228
Trochammina sp.	Trochamminidae	3341	36.8	X86095
Nummulites venosus	Nummulitidae	3366	42.0	AJ318226
Heterostegina depressa	Nummulitidae	3413	42.0	AJ508453
Bolivina spathulata	Bolivinidae	3111	40.9	AJ318227
Pararotalia nipponica	Rotaliidae	3168	41.0	AJ508454

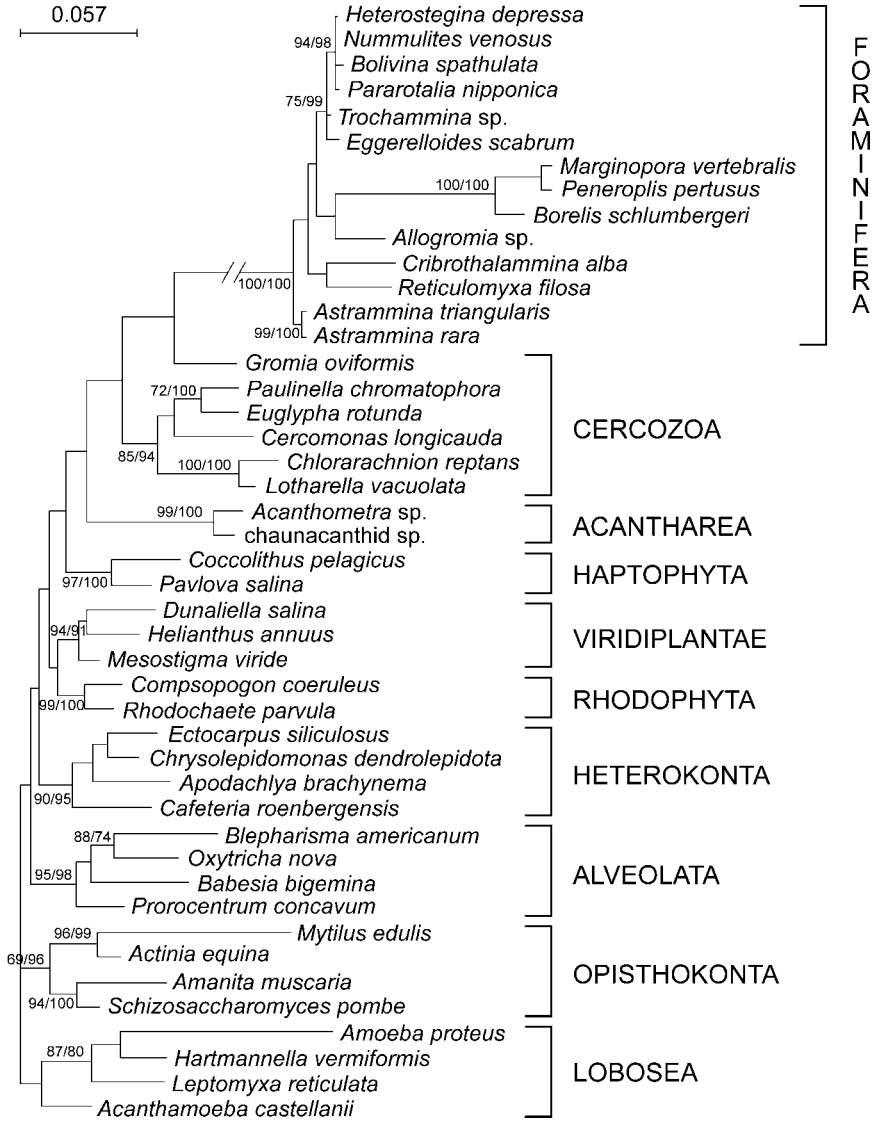

FIGURE 1. Maximum likelihood tree of eukaryotes based on complete (1109 sites) SSU rDNA sequences, showing the close relationship between Foraminifera and Cercozoa. All branches are drawn to scale, except the stem branch leading to the foraminiferan clade, which was reduced to a fourth of its actual size. The numbers of internal branches are bootstrap values of maximum likelihood and neighbor joining analyses, based on 100 and 1000 replicates, respectively.

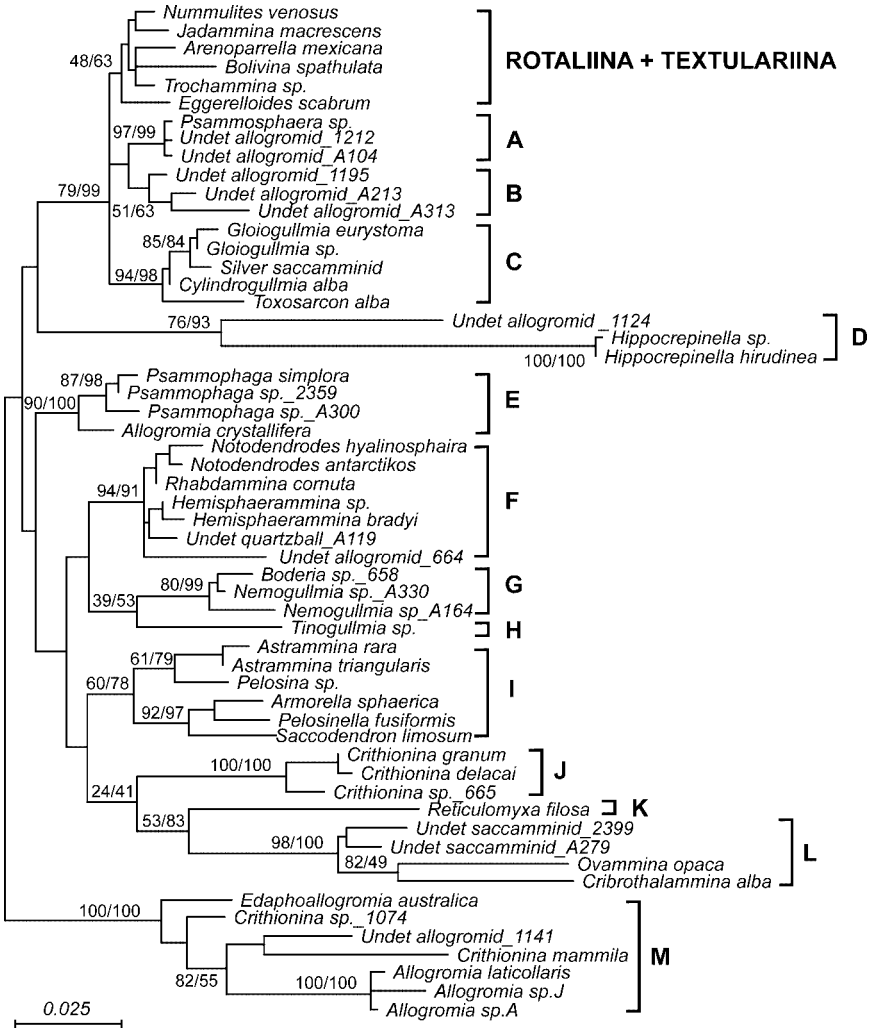

FIGURE 2. Phylogenetic relationships among Foraminifera as inferred from partial (541 sites) SSU rDNA sequences using the maximum likelihood method. The numbers above internal branches are bootstrap values of maximum likelihood and neighbor joining analyses, based on 100 and 1000 replicates, respectively. Only values higher than 50% are noted. All branches are drawn to scale.

Allogromia sp., the two *Astrammina* species, and the Textulariida and Rotaliida, which again form the crown of the tree.

To examine the relationships among Allogromiida, Astrorhizida, and other foraminiferans, partial SSU sequences (s14F3-sB) were analyzed (Fig. 2). Because the exact branching order among foraminiferal lineages differ in ML and NJ trees, the clade of *Allogromia* was arbitrarily chosen as the outgroup. The miliolids were excluded because of their ambiguous position. Preliminary NJ analyses were done with a large number of rotaliids and textulariids, but because they all grouped together, their number was later reduced to perform the more time consuming ML analyses. In total, the analyses presented here were performed with 56 species, including one athalamid, 19 soft-walled allogromiids, 30 astrorhizids, and six polythalamous (four textulariids and two rotalids) species.

The sequences of athalamids and monothalamids form 13 lineages (Fig. 2). We defined as lineages all groups that were supported by bootstrap values higher than 50% in both types of analyses. The relationships among lineages were not well resolved, and their branching order changed depending on the type of analysis. Among the lineages that do not change their respective position, the best support exists for the grouping of lineages A, B, and C, which branch as sister groups to Textulariina and Rotaliina. Lineages J, K, and L also group together in both NJ and ML trees, but their association is only supported by 41 and 24% bootstrap values, respectively. In both analyses, *Tinogullmia* is a sister to the *Nemogullmia* lineage (G), but the support for their relationship ranges from 39 to 53 % (Fig. 2).

LINEAGE DESCRIPTION

The species composition of the 13 lineages identified in this study and their general morphological features are presented in Table 3. Among these lineages, two are represented by single species (H, K), while the 11 others comprise between two to eight species (molecular types). Four of these lineages include relatively homogenous morphotypes (E, G, J, L). The remaining seven lineages form heterogeneous assemblages of different morphospecies, and

TABLE 3. List of molecular lineages of unilocular foraminifera and their morphological characteristics.

Clade	Species	Morphological characteristics
A	*Psammosphaera* sp. + 2 undetermined allogromiids	Test agglutinated in *Psammosphaera*; membranous, elongate or ovoid in the other two morphotypes
B	3 undetermined allogromiids	Test membranous, ovoid or bean-shaped
C	*Gloiogullmia* + *Cylindrogullmia* + silver saccaminid + *Toxisarcon*	Test membranous, elongate or ovoid with single aperture; may be enclosed by delicate agglutinated test or finely agglutinated, irregular form, without aperture
D	*Hippocrepinella*	Test finely agglutinated, elongate, with terminal aperture
E	*Psammophaga* + *A. crystallifera*	Test finely agglutinated with single terminal aperture and small mineral inclusions within cytoplasm
F	*Notodendrodes* + *Hemisphaerammina*	Test agglutinated; free, erect or branching in *Notodendrodes*; dome-like and attached to substratum in *Hemisphaerammina*
G	*Nemogullmia*	Test membranous, thread-like, 2 apertures
H	*Tinogullmia*	Test membranous, sausage-like, 2 apertures
I	*Astrammina* + *Armorella* + *Pelosina* sp. + *Saccodendron*	Test agglutinated enclosing spherical monothalamous sarcode
J	*Crithionina*	Test agglutinated, irregularly-shaped cell body
K	*Reticulomyxa*	Naked, irregular plasmodium
L	*Ovammina* + *Cribrothalammina*	Test finely agglutinated, ovoid, single aperture
M	*Allogromia* + *Crithionina* sp.	Monothalamous theca, ovoid *Crithionina* finely agglutinated with irregularly-shaped cell body

some of them include a variety of soft-walled and agglutinated species. A detailed description of each lineage is presented below.

Lineage A

This lineage includes an agglutinated species (*Psammosphaera* sp.) and two undetermined allogromiids (# 1212, # A104). Two other types of allogromiids belonging to this lineage were identified by SSU fragment analysis (data not shown). Except for *Psammosphaera,* the molecular types included here are characterized by an elongate white or pink cell body, similar to those inside the agglutinated tests of *Rhabdammina* sp. or *Notodendrodes antarctikos,* with few mineral grains attached to their rather sticky walls.

Lineage B

Three undetermined soft-walled allogromiids belong to this lineage. One type (# 1195) has a very distinctive, tiny, bean-shaped test and orange-red cytoplasm. The two other types possess ovoid, white cell bodies without any distinctive features, and occur either free or enclosed in an *Astrammina*-like test. All representatives of lineages A and B were collected in Explorers Cove, Antarctica.

Lineage C

Lineage C is one of the most heterogeneous lineages identified in this study. The four species grouping here represent three very different morphotypes. Two species (*Gloiogullmia eurystoma* and *Cylindrogullmia alba*) are characterized by a membranous, ovoid to elongate test with a single terminal aperture. *Gloiogullmia* possesses a particularly sticky wall (*gloio* = sticky). Our specimens *G. eurystoma* and *C. alba* were isolated in Kosterfjorden, near the locality of their original description (Nyholm, 1974). Moreover, the genus *Gloiogullmia* was identified in Explorers Cove by Gooday and others (1996). Several specimens of this species were isolated and sequenced (Pawlowski and others, 2002).

The second morphotype is dubbed ''silver saccamminid'' and is known only from Explorers Cove (Gooday and others, 1996). It is characterized by a large, rounded test vested by ''plate-like grains which impart distinctive silver, diffusely reflective appearance'' (Gooday and others, 1996). The silver saccamminids occur either free or enclosed in delicate and friable agglutinated tests. The species resembles *Pilulina argentea* (Höglund, 1947), from which it differs by the shape of the aperture.

The third morphotype is represented by the genus *Toxisarcon.* Its morphology is very different from the other foraminiferans belonging to this group. *Toxisarcon alba,* described as a new species (T. Wilding, 2002, this volume), has a large reticulate cell body covered by a loosely agglutinated test that can be readily abandoned. Another new species of the same genus, *Toxisarcon synsuicidica* (T. Cedhagen, 2002, this volume), also likely belongs to this group. Its sequence was not included here, because only a short SSU fragment is currently available.

Lineage D

The three molecular types that form this clade represent the genus *Hippocrepinella* (*H. hirudinea* from Kosterfjorden and *Hippocrepinella* sp. from Explorers Cove) and an undetermined morphotype, dubbed ''silver *Tinogullmia.*'' The two *Hippocrepinella* are morphologically similar and their sequences differ by only 0.1% in our set of selected sites. Silver *Tinogullmia* represents a very different morphotype. Its short, elongate test with the apertures at both ends resembles those of the genus *Tinogullmia,* but its wall is characterized by a distinctive silver color similar to that observed for the silver saccamminid of Lineage C. Despite the relatively high bootstrap value (76–93%), the grouping of these two morphotypes seems doubtful.

Lineage E

This lineage includes sequences of *Psammophaga simplora* (Arnold, 1982) and *Allogromia crystallifera* (Dahl-

gren, 1962), as well as two undetermined *Psammophaga*-like morphotypes. All of these have a soft-walled, ovoid test and single aperture. Their characteristic feature is the presence of large mineral crystals, readily visible in the cytoplasm near the apertural region. The lineage seems to have a wide geographic distribution, with the examined isolates originating from the Atlantic coast of the US, Scandinavian fjords, and Antarctic shelf. The Antarctic specimens were described previously as *Psammophaga* sp. by Gooday and others (1996).

Lineage F

This lineage includes two distinct morphogroups. One group, collected from Explorers Cove, includes *Rhabdammina cornuta* and two species of the genus *Notodendrodes*. All three species are characterized by an allogromiid-like cell body enclosed within a characteristic agglutinated test, which can be tubular (*R. cornuta*) or spherical with arborescent structures (*Notodendrodes*). The second group includes *Hemisphaerammina bradyi* (the type species of the genus *Hemisphaerammina*, collected along the Mediterranean coast at Banyuls, France) and similar morphotypes found attached to the scallop *Adamussium colbecki* in Explorers Cove (# 1182). *Hemisphaerammina* is characterized by a domed agglutinated test covering a large, irregular cell body; it is typically attached to a solid substratum. Two undetermined forms—# A119 from Explorers Cove and # 664 from Lizard Island, Australia—also belong to this lineage. The Explorers Cove member was isolated from an agglutinated quartz sphere resembling the silver saccamminid, whereas the Australian specimen was isolated from a laboratory culture.

Lineage G

All members of this lineage are characterized by a very long, thread-like cell body that usually exceeds 1 mm in length. Terminal apertures in the organic test are present but not readily observed. The Antarctic specimens (# A330, # A164) resemble *Nemogullmia longevariabilis*, described by Nyholm (1953). In fact, our analysis of a SSU fragment of *N. longevariabilis* specimens from the type locality (Gullmarfjorden) places this species as the sister group to two Explorers Cove isolates (data not shown). The third sequence was obtained from cultured material originating from Lizard Island and was tentatively identified as *Boderia* sp., although the morphological differences between *Nemogullmia* and *Boderia* are not clear. Despite the morphological similarities of specimens from this lineage, their sequences are quite divergent compared to the other morphologically homogenous groups.

Lineage H

The single sequence included here represents the genus *Tinogullmia*, a sausage-shaped, soft-walled allogromiid with apertures located at both ends of the test. Our specimens resemble *Tinogullmia* sp. identified in Explorers Cove by Gooday and others (1996). Although the genus seems to be widely distributed (Gooday, 1990), we have not yet found it outside Antarctica.

Lineage I

This lineage includes six species that possess a spherical cell body enclosed in an agglutinated test. Some species, such as *Astrammina rara* (DeLaca, 1986) and *Astrammina triangularis* (Bowser and others, this volume), are characterized by a coarsely agglutinated wall. The tests of the other species are finely agglutinated. The genera *Pelosina* and *Saccodendron* possess long tubular branching arms. This lineage also seems to include two other species, *Pelosina variabilis spheriloculum* and *Radicula limosa*, but only short SSU fragments are presently available for these organisms.

Lineage J

This lineage represents the genus *Crithionina*, including *Crithionina granum* and *Crithionina delacai* and one *Crithionina*-like morphotype isolated from the Maldives. Both *C. granum* and *C. delacai* have large, finely agglutinated tests, usually without obvious apertures, and differ mostly in the composition and arrangement of particles within the test (Gooday and others, 1995). They have been described from Skagerrak (Göes, 1894) and Explorers Cove (Gooday and others, 1995).

Lineage K

Reticulomyxa filosa (Nauss, 1949) is the only described species of the genus *Reticulomyxa*, which represents the order Athalamida (Bovee, 1985). This genus lacks a test and occurs as an irregular plasmodium. The biology of this commonly cultured freshwater species is extensively studied (e.g., Hauser and others, 1989; Koonce and others, 1986; Gothe and others, 1997; Orokos and others, 1997).

Lineage L

This lineage includes two sequences of specimens identified as *Ovammina opaca* (Dahlgren, 1962) from the Barents Sea and *Cribrothalammina alba* from Sapelo, US. Both species are thought to be closely related based on test morphology (Goldstein and Barker, 1990). Two other isolates belonging to this lineage were not identified; however, their general morphology (ovoid form, terminal aperture on short neck, and finely agglutinated wall) suggests their close relationship to the genus *Ovammina*.

Lineage M

This is a morphologically heterogenous group, which includes the soft-walled genera *Allogromia* and *Edaphoallogromia*, two representatives of the genus *Crithionina*, and one unidentified isolate. All examined species of the genus *Allogromia* (*A. laticollaris*, *Allogromia* sp. A, and *Allogromia* sp. J) originated from our cultures. The other three sequences derive from specimens collected in Explorers Cove and represent *Crithionina mamilla*, a *Crithionina*-like morphotype (# 1074), and an unidentified allogromiid (# 1141). *Crithionina mamilla* from Explorers Cove differs from *Crithionina delacai* by its smaller size, more rounded outline, and presence of projecting sponge spicules (Gooday and others, 1995). The group also includes the newly de-

scribed species *Edaphoallogromia australica* isolated from Australian tropical rain forest soil (Meisterfeld and others, 2002).

DISCUSSION

Phylogenetic Position

Phylogenetic analysis of complete SSU rDNA sequences shows that allogromiids, athalamids, and astrorhizids diverged early in the evolution of Foraminifera. This is in agreement with traditional (morphology-based) classification schemes that place these groups either as a sister group to Foraminifera or as their most primitive representatives (Cushman, 1950; Loeblich and Tappan, 1974; Bovee, 1985; Tappan and Loeblich, 1987). Our data differ from these classifications in two major ways. First, the athalamid *Reticulomyxa filosa* branches among the monothalamous foraminiferans, rather than as their ancestor—a finding that is in agreement with previous analyses of this species (Pawlowski and others, 1999). Second, the soft-walled and agglutinated species branch together as a common assemblage—a finding that is in total disagreement with morphology-based classifications of these groups (e.g., Loeblich and Tappan, 1989; Sen Gupta, 1999).

Our present results differ significantly from previous analyses based on partial SSU rDNA sequence data, which showed allogromiids and astrorhizids branching in the middle part of the foraminiferal tree, after the divergence of Miliolida (Pawlowski and others, 1997; Pawlowski 2000). The early origin of miliolids was hypothesized based on the distinctive structure of their rRNA genes (Pawlowski, 2000), and on different actin isoforms demonstrated by immunoblotting with monoclonal antibodies (Fahrni and Pawlowski, 1995). However, recent analysis of miliolid actin gene sequences shows that the Miliolida branch among other foraminiferans rather than at the base of foraminiferal clade (Pawlowski and others, unpublished data). Moreover, a reexamination of miliolid position using phylogenetic methods less prone to long-branch attraction (LBA) artifacts caused by unequal base-pair composition (Philippe and Germot, 2000) indicates that this group branches within other calcareous taxa (Pawlowski and others, unpublished data). Thus, the phylogenetic position of Miliolida should be considered as unresolved.

Our data raise questions about the origin of the Foraminifera and their possible ancestors. According to previous rRNA-based phylogenies, the Granuloreticulosa diverged relatively early in the evolution of eukaryotes (Pawlowski and others, 1994; 1996). However, the position of Foraminifera in these rRNA trees was biased by the rapid acceleration of the evolution of their rRNA genes. Analyses of protein-coding genes (Keeling, 2001; Archibald and others, in press; Longet and others, unpublished data) and reanalysis of rRNA data (Berney and Pawlowski, unpublished data; see Fig. 1) show that Foraminifera are related to the Cercozoa. If this is the case, then the divergence of Foraminifera would have occurred sometime within the radiation of eukaryotes, probably less than one billion years ago, but still earlier than is suggested by their first appearance in Early Cambrian strata (Culver, 1991). Given our present analyses, the foraminiferan ancestors were most probably membranous, filosean protists resembling *Gromia oviformis*. The position of the athalamid species examined in this study among the monothalamous Foraminifera suggests that they have secondarily lost their tests in adaptation to freshwater environments. Clearly, the search for extant representatives of the basal foraminiferans requires a more thorough study of marine athalamids and filoseans.

Molecular Diversity

The identification of 13 molecular lineages of allogromiid foraminiferans indicates a relatively high diversity in this group. Although the taxonomic status of these lineages is not yet established, the fact that some of them include several different genera suggests that they may correspond to a family level in traditional taxonomy. The high diversity of allogromiids revealed by our molecular data contrasts with the relatively low number of higher taxa (20 families) distinguished by morphological classifications (Loeblich and Tappan, 1987). This difference is particularly visible for the soft-walled allogromiids which, although present in seven molecular lineages, are traditionally grouped into only two families. The fact that the number of higher-level taxa in the Foraminifera is significantly underestimated compared to other protists, as discussed by Loeblich and Tappan (1964), culminated in the establishment of three new subfamilies of Allogromiina (Loeblich and Tappan, 1987). This is, however, still far less than the number of allogromiid lineages revealed by our molecular analyses.

Our molecular data also revealed high species diversity in allogromiids. The majority of allogromiid genera are represented by single species descriptions (Nyholm, 1974; Cedhagen and Mattson, 1991, 1992). Because of the paucity of morphological characters, species distinctions in this group are particularly difficult. The use of molecular techniques circumvents these difficulties and leads to more precise species identifications. The application of these techniques to the study of foraminiferal diversity revealed the presence of cryptic species in various planktonic (Huber and others, 1997; de Vargas and others, 1999; 2001) and benthic (Holzmann and Pawlowski, 1997; Holzmann, 2000) foraminiferans. A parallel study of molecular diversity in Antarctic allogromiids (Pawlowski and others, 2002) revealed over 50 molecular types in the foraminiferal assemblage composed of 20 distinct morphospecies.

The discrepancy between the molecular and morphological diversities of allogromiids seems to be partly due to a lack of relationships between morphotypes and molecular types. Among the lineages identified in this study are groups that are morphologically and molecularly homogenous, e.g., lineages of *Ovammina* and *Nemogullmia* or the group *Astrammina* + *Pelosina* + *Saccodendron*. In some lineages, it was possible to identify distinct characters shared by all members, such as the mineral crystals in the *Psammophaga* lineage. There are, however, lineages composed of very different morphotypes with little in common. Some of these, e.g., the *Crithionina*-like morphotype in lineages J and M, appear in more than one lineage, indicating that a given suite of structural characters may have developed independently, i.e., by convergent evolution. We suggest that these lineages are less specialized than those exclusively com-

posed of well-determined morphotypes. In the light of our data, the evolution of allogromiids appears as a spectrum of attempts by monothalamous foraminiferans to develop various types of agglutinated test.

Taxonomic Implications

In view of our data, the present classification of monothalamous soft-walled and agglutinated foraminiferans requires a profound revision. Our study clearly shows that both types of test evolved independently in several lineages. The phylogenetic relationships among soft-walled and agglutinated species seem to be much more complex than suggested by morphology-based classifications. Therefore, the traditional taxonomic distinction of Allogromiina and Astrorhizina, based on wall structure, cannot be retained.

The preliminary character of the present study does not yet allow us to propose any alternative taxonomic scheme. Even the recent attempt to revise the definition of these groups was a difficult task, as was shown by animated discussions during the Tjärno workshop. The main difficulty is that the allogromiid foraminiferans do not form a monophyletic group and share few derived characters. Rather, they appear as a paraphyletic assemblage composed of several independent lineages, some of which give rise to more highly evolved polythalamous foraminifera. The only character that seems to unite all these lineages is the presence of a unilocular test. This character may also be shared with freshwater athalamids, if we assume that the latter lost their tests secondarily. The origin of multiple chambers can therefore be viewed as a fundamental advance in the evolution of Foraminifera. Determining the molecular phylogeny of polythalamous soft-bodied foraminiferans (e.g., *Nodellum moniliniforme*) represents one test of this hypothesis.

Conclusion

Based on the available molecular data, we propose that allogromiid foraminiferans be defined as an assemblage of monothalamous granuloreticulosans that diverged early in the evolution of Foraminifera and developed different types of organic or agglutinated wall structures. This definition, however, should be considered a working hypothesis that must be tested by further molecular, morphological, and ultrastructural studies.

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