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Molecular data reveal high taxonomic diversity of allogromiid Foraminifera in Explorers Cove (McMurdo Sound, Antarctica)

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Abstract Allogromiids are organic-walled or agglutinated, single-chambered Foraminifera, common in deep-sea and polar benthic communities. The simple forms and paucity of distinctive features make allogromiid identification difficult by traditional means. Molecular phylogenetic methods offer alternative tools for species identification and are used here to investigate allogromiid diversity. We obtained 135 partial small-subunit ribosomal DNA sequences of allogromiids collected in Explorers Cove, McMurdo Sound, Antarctica. In contrast to the 27 morphotypes identified, phylogenetic analysis revealed 49 molecular types (considered separate species) that differ by more than 5% of sequence divergence. The 49 genetic types form 28 molecular supra-groups that differ by more than 20% and probably represent distinct genera or families. Large genetic distances separating the molecular types indicate unexpectedly high taxonomic diversity. Comparison of our data with sequences of non-Antarctic allogromiids suggests that Explorers Cove species might be endemic and only distantly related to comparable northern hemisphere fauna.

Introduction

Foraminifera are an important component of benthic marine communities. Organic-walled and large agglutinated monothalamous (i.e. single-chambered) species constitute a major proportion of foraminiferal assemblages in the deep sea and polar settings (Gooday 1990; Korsun and Hald 1998; Schewe and Soltwedel 1998). Unfortunately, their diversity is poorly known because their simple tests possess few distinctive morphological features, required for proper species identification. Moreover, because of their poor preservation in sediment samples and lack of fossilized remains, they are easily overlooked in micropaleontology-oriented studies. Many organic-walled taxa are also neglected because of their tiny size (Pawlowski et al. 1993; Gooday et al. 1995).

Traditionally, organic-walled and large agglutinated single-chambered Foraminifera are classified in the suborders Allogromiina and Astrorhizina, respectively (Loeblich and Tappan 1988, 1989). Recent detailed morphological, cytological and behavioral studies, however, suggested a common origin of these two suborders (Bowser et al. 1995). Their close relationship was later confirmed by molecular data, which further show that some naked, freshwater foraminifers branch closely to these groups (Pawlowski et al. 1999; Pawlowski 2000). Following this morphological and molecular evidence, the definition of allogromiids was recently enlarged to include all monothalamous (single-chambered) Foraminifera that are naked or possess organic or agglutinated tests. This definition, elaborated during the First International Workshop on Diversity of Allogromiid Foraminifers (Tjärno, Sweden, August 2000) is accepted here.

The allogromiids sensu lato (i.e. including the organic-walled and agglutinated single-chambered species) are the dominant group of Foraminifera living in Explorers Cove. This locality, situated on the western side of McMurdo Sound, possesses specific characteristics that resemble oligotrophic deep-sea conditions and is characterized by low macrofaunal densities comparable to those found in the bathyal deep sea (Dayton and Oliver 1977). Similarities between Explorers Cove and the deep sea include low temperature, physical tranquility and seasonal food input (Gooday et al.

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1996). For several years, Explorers Cove has been the focus of a series of investigations on the taxonomy, ecology and cell biology of Foraminifera (DeLaca 1985; Bernhard 1987, 1993; Bowser et al. 1991). These studies led to the description of two new species (*Notodendrodes antarctikos*; DeLaca et al. 1980, and *Crithionina delacai*; Gooday et al. 1995), and the revision of another species (*Astrammina rara*; DeLaca 1986). Detailed taxonomic studies of Explorers Cove Foraminifera report 38 morphotypes of allogromiids including 7 species, 10 genera and 21 undetermined forms (Gooday et al. 1996).

In this paper, the diversity of Explorers Cove allogromiids is examined, using phylogenetic analysis of partial SSU rDNA sequences. Application of molecular phylogenetic methods to investigate eukaryotic diversity in natural habitats led to the discovery of unexpected numbers of new protistan taxa in marine nano- and picoplankton (Lim 1996; Lopez-Garcia et al. 2001; Moon-van der Staay et al. 2001). The present study is one of the first attempts to use molecular methods to evaluate the diversity of benthic protists in natural environments.

Materials and methods

Specimens were collected in Explorers Cove, McMurdo Sound, Antarctica during two field missions in 1998 and 1999 (for an accurate site description, see DeLaca et al. 2001). Additional specimens were collected at Cape Armitage, Cape Evans and Little Razorback Island (eastern McMurdo Sound). Allogromiids were isolated from surface sediment samples, obtained either by an airlift sampler (detailed in Pollock and Bowser 1995) or from cores collected by divers. Sediment samples were sieved through stacked 0.125-mm, 0.400-mm and 1-mm meshes, and individual allogromiids were picked from residues using a dissecting microscope. Some attached allogromiids were obtained from scallop shells and pycnogonid legs. Following isolation, each specimen was thoroughly cleaned and washed to eliminate possible contaminants and maintained under low temperature until DNA extraction. The tests of monothalamous agglutinated allogromiids were broken and, where possible, the cell bodies (sarcodes) were isolated. The sarcodes are encased in an organic theca and resemble free-living, organic-walled allogromiids. In total, 423 DNA isolates of allogromiids were obtained.

DNA was extracted from single or several cells using either the DNeasy Plant Mini Kit (Qiagen) or the guanidine method as described in 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 High Pure PCR Purification Kit (Boehringer), 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.

Two fragments situated in the 3′ region of the SSU rDNA (Fig. 1) were amplified and sequenced. A short fragment of 350–600 base pairs (bp) was amplified using the foraminiferal-specific primers s14F3 [5′ ACG CA(AC) GTG TGA AA CTT G] and s17 (5′ CGG TCA CGT TCG TTG C). A specific primer s14F1 (5′ AAG GGC ACC ACA AGA ACG C) was used for the direct sequencing of PCR products. A longer fragment of about 1,000 bp, which includes the shorter fragment (Fig. 1), was amplified using the primers s14F3 and sB (5′ TGA TCC TTC TGC AGG TTC ACC TAC). The region s14F1–sB corresponds to positions 1212–1871 in *Rattus norvegicus* (K01593); the primer s17 is situated in position 1395 in this sequence. The new sequences reported in this paper have been deposited in the EMBL/GenBank database (acession numbers AJ307774–AJ307877).

The sequences were aligned manually using SEAVIEW software (Galtier and Gouy 1996), following the SSU rRNA gene secondary structure-based model (Van de Peer et al. 2000). Sequence alignment is available at the website of our laboratory http://www.unige.ch/sciences/biologie/biani/msg/dnadata/.

Neighbor-joining (NJ) sequence analysis (Saitou and Nei 1987) was applied to distances corrected for multiple hits, and for unequal transition and transversion rates, using the K2 model (Kimura 1980). Maximum likelihood (ML) analysis was performed using the fast DNAm1 program including F84 model (Olsen et al. 1994). The NJ analysis was performed using pairwise distances for the s14F3-s17 fragment, and using total gap removal option for the s14F3-sB fragment. The reliability of internal branches in the NJ and ML trees was assessed using the bootstrap method (Felsenstein 1988), with 1,000 and 100 replicates respectively. The PHYLO_WIN program (Galtier and Gouy 1996) was used for distance computation, tree building and bootstrapping.

Results

Identification of morphotypes

Twenty-seven different morphotypes of allogromiids, including those with organic (Allogromiina) and agglutinated (Astrorhizina) tests walls, were identified in this study (Table 1). The majority of them were isolated from Explorers Cove samples. Four agglutinated morphotypes and some previously unidentified organic-walled species were found in three other localities on the eastern side of McMurdo Sound. All of these, except *Hippocrepinella* spp., were rare and usually represented by single specimens.

A total of 5 organic-walled Allogromiina morphotypes and 22 agglutinated Astrorhizina morphotypes were distinguished. Most of the organic-walled allogromiids were small and occurred predominantly in the 125- to 400-µm size fraction, whereas astrorhizids were usually larger (often >1 mm). The taxonomic determination of Explorers Cove species was based on a previous morphological study carried out by Gooday et al. (1996). All agglutinated and 3 out of 5 organic-walled genera presented in that study were also identified in our samples. Of the remaining two genera, *Micrometula* and *Cylindrogullmia* were not identified. However, we distinguished two new morphotypes of Allogromiidae:

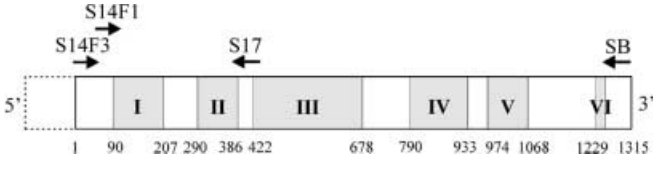

Fig. 1 Schema of the amplified fragment of the SSU rDNA showing the position of amplification primers. The variable regions (expansion segments) are designated by Roman numerals *I–VI*. The *numbers* below correspond to the length of different regions in the sequence from *Notodendrodes antarctikos*

Table 1 List of McMurdo Sound allogromiid species and morphotypes including number of DNA extractions

Higher taxon[a]	Species/morphotype	Code[b]	Reference	Locality[c]	Size[d] (μm)	DNA[e] extracts
Allogromiina						
Allogromiidae	*Gloiogullmia* sp. (ovoid, green)	GL	Gooday et al. (1996)	EC	400–1000	25
	Tinogullmia sp. (sausage-shaped)	TI	Gooday et al. (1996)	EC	125–400	6
	Nemogullmia sp. (very long, thread-like)	NE	Gooday et al. (1996)	EC,LR	125–400	14
	Allogromiid sp. 1 (tiny, brown-red, bean-like)	BL		EC	125–400	6
	Allogromiid sp. 2 (elongate or ovoid, creamy-pink)	CP		EC	125–400	38
	Miscellaneous	AL		EC,LR,CE	> 125	73
Astrorhizina						
Astrorhizidae	*Astrammina triangularis*	AT	Gooday et al. (1996)	EC	> 1000	8
	Pelosinella fusiformis	PF	Gooday et al. (1996)	EC	> 1000	1
	Pelosina sp.	PE	Gooday et al. (1996)	EC	> 400	6
Rhabdamminidae	*Rhabdammina cornuta*	RB	Gooday et al. (1996)	EC	> 1000	10
Psammosphaeridae	*Psammosphaera* spp.	PS	Gooday et al. (1996)	EC	> 400	10
Saccamminidae	*Astrammina rara*	AR	DeLaca (1986)	EC,CA	> 1000	31
	Psammophaga sp.	PP	Gooday et al. (1996)	EC	125–400	3
	Saccamminid sp. 1 (silver sphere)	S1	Gooday et al. (1996)	EC	> 400	9
	Saccamminid sp. 2 (silver elongate)	S2		EC	125–400	5
	Saccamminid sp. 3 (ovoid, flask)	S3		EC	125–400	3
	Saccamminid sp. 4 (long neck)	S4		EC	125–400	2
	Saccamminid sp. 5 (lemon-shaped)	S5	Gooday et al. (1996)	EC	125–400	11
	Saccamminid sp. 6 (tiny sphere)	S6		EC	125–400	11
Hemisphaeramminidae	*Crithionina delacai*	CD	Gooday et al. (1995)	EC,CA	> 1000	6
	Crithionina mamilla	CM	Gooday et al. (1996)	EC	> 400	5
	Crithionina sp. (free form)	C1		EC,CA	> 400	9
	Crithionina sp. (attached form)	C2		EC		25
Notodendrodidae	*Notodendrodes antarctikos*	NA	DeLaca et al. (1980)	EC	> 1000	5
	Flower notodendrodid – quartzball	QB	DeLaca et al. (2001)	EC	> 400	50
Hippocrepinellidae	*Hippocrepinella* spp.	HP		EC,CA,LR	> 125	43
Komokiidae	Mudballs (Komoki?)	MB	Gooday et al. (1996)	EC	> 1000	4
Hippocrepinidae	*Hyperammina*?	HY	Gooday et al. (1996)			4
Total						423

[a]Higher taxa according to Loeblich and Tappan (1988, 1989)
[b]Symbols used to indicate the morphotypes in Table 2 and Fig. 3
[c]Localities (*EC* Explorers Cove, *CA* Cape Armitage, *LR* Little Razorback Island, *CE* Cape Evans)
[d]Sediment size fraction in which the specimens were found
[e]Number of DNA isolates for each species or morphotype

Allogromia sp. 1 and *Allogromia* sp. 2. The first species, *Allogromia* sp. 1, is characterized by its small test (about 100 μm diameter), with rounded or bean-like outline, a single aperture, and red-brown coloured cytoplasm. The morphotype *Allogromia* sp. 2 includes a number of elongate or ovoid specimens, having a creamy-pink colour and usually lacking a distinctive aperture. Some of these specimens look similar to the sarcodes of *Rhabdammina cornuta* or *Notodendrodes* spp. Numerous organic-walled specimens that did not correspond to any recognized morphotype were included in a ''miscellaneous'' category.

Among the morphotypes of Astrorhizina, we distinguished six morphotypes of Saccamminids. Saccamminids spp. 1, 3 and 5 correspond respectively to the Silver Saccamminid, Saccamminid sp. 3 and Saccamminid sp. 4, described by Gooday et al. (1996). Three other saccamminids (spp. 2, 4, 6) are new. Saccamminid sp. 2 has

a tiny, elongate test with two apertures and a finely agglutinated wall with a distinctive silver appearance. Saccamminid sp. 4 is characterized by its tiny, coarsely agglutinated test, rounded in outline, with a single aperture on a long neck. Saccamminid sp. 6 possesses a spherical (diameter 60–100 mm), finely agglutinated, whitish test, lacking a distinct aperture. We also separated the *Crithionina*-like morphotypes into free and attached forms, the latter being similar to the genus *Hemisphaerammina*.

Gromia and *Gromia*-related morphotypes were not included in the present work. Despite morphological similarities to organic-walled allogromiids, *Gromia* is not related to foraminiferans and belongs to the separate phylum, Filosea (Bovee 1985). A study concerning the genetic diversity of *Gromia* collected in Explorers Cove and other localities of McMurdo Sound is in progress, and will be published separately (F. Burki, unpublished work).

Phylogenetic relationships

To evaluate phylogenetic relationships between Explorers Cove allogromiids, a longer fragment of the SSU rDNA (s14F3–sB) was sequenced. The examined fragment covers the entire 3′ region of the gene, including several conserved and variable regions. It contains almost 600 homologous sites that are incorporated in the analysis, i.e. about 3 times more than in the shorter fragment of SSU rDNA used for the distinction of molecular types.

Sequences of the larger fragment were obtained for 31 molecular types from Explorers Cove and compared to 13 sequences of allogromiids from other localities. Four sequences of multi-chambered agglutinated species (Textulariina) and two sequences of porcelaneous Foraminifera (Miliolina) were included in our analyses. Miliolina were used as an outgroup following a previous molecular study (Pawlowski 2000). Phylogenetic analysis shows that the Antarctic allogromiids group into 14 distinctive clades, each one supported by bootstrap values higher than 85% in the NJ tree (Fig. 2). The first group to diverge is the clade containing *Allogromia* spp. and *Crithionina* spp. This group is followed by a simultaneous divergence of several lineages, for which phylogenetic relationships are not well resolved. Three clades, including *Psammosphaera*, *Gloiogullmia*, *Cylindrogullmia*, and several undetermined allogromiids, form a monophyletic group together with Textulariina (bootstrap value of 80%). The 14 clades also appear in ML analysis, but their branching order is different. The phylogenetic groupings found in both types of analyses are the group including three allogromiid clades and Textulariina, and the group including *Nemogullmia* and *Tinogullmia*.

Five out of 14 clades contain exclusively Antarctic species, while the remaining 9 include either both Antarctic and non-Antarctic species or a single sequence of the non-Antarctic athalamiid, *Reticulomyxa filosa*. Some non-Antarctic species (e.g. *Gloiogullmia eurystoma*, *Crithionina granum*, *Hemisphaerammina bradyi* and *Psammophaga simplora*) appear to be closely related to Explorers Cove types. Their close relationship, however, is due to a lack of variation in the conserved regions selected for the phylogenetic analysis. A separate analysis of the variable regions in the shorter SSU fragment (s14F3–s17) shows that sequences of Antarctic and non-Antarctic species differ by more than 10% from each other (data not shown).

Distinction of molecular types

To assess the genetic diversity of Explorers Cove allogromiids, a short fragment of the SSU rDNA was examined. The length of the fragment averages 350–400 bp in most allogromiids, except in *Crithionina* spp. where it reaches up to 600 bp. The examined fragment includes two variable and three conserved regions (Fig. 1). Although the fragment is too short to resolve phylogenetic relationships at a higher taxonomic level, such as those described in the previous section, its variable regions contain sufficient phylogenetic information to distinguish clusters of closely related sequences.

A total of 135 sequences was obtained. Among these, 103 were sequenced directly and 32 were cloned before sequencing. Intra-individual variability was checked by sequencing several clones for each of the 32 DNA isolates. The majority of cloned sequences were either identical or differed by only a few (1–4) base substitutions.

A phylogenetic tree obtained by NJ analysis of 135 sequences is presented in Fig. 3. Percentage divergence was calculated for each pair of sequences to determine closely related clusters. Such closely related clusters consist of sequences that differ by less than 5% from each other. Each cluster is regarded as a distinct molecular type. Following this rule, 26 molecular types could be distinguished. Another 23 molecular types are represented by single sequences only. Among the 26 types, only 8 show intra-type sequence variations higher than 1% (Table 2). The sequence A406, which differs from types 1 and 2 by less than 5% (4.6 and 4.8%, respectively), was included in type 2 because it lacks a test. In total, 49 molecular types are distinguished. The 49 types can be arranged in 28 higher groups that differ from each other by more than 20%. Among them, 18 are represented by single molecular types.

Molecular data were obtained for 24 of the 27 morphotypes distinguished in the present work. PCR amplification of 3 morphotypes (Saccamminid sp. 5, Saccamminid sp. 6 and *Hyperammina*) did not yield positive results. Fifteen morphotypes were represented by single molecular types. The remaining 9 morphotypes split into 34 molecular types. Among them, six molecular types were found in quartzball-like tests. Two of these types (nos. 15 and 16) were closely related to the genus *Notodendrodes*, while the others represented independent lineages. Seven molecular types (nos. 18, 21, 32, 33, 46, 48, 49) branching in 3 different clades were

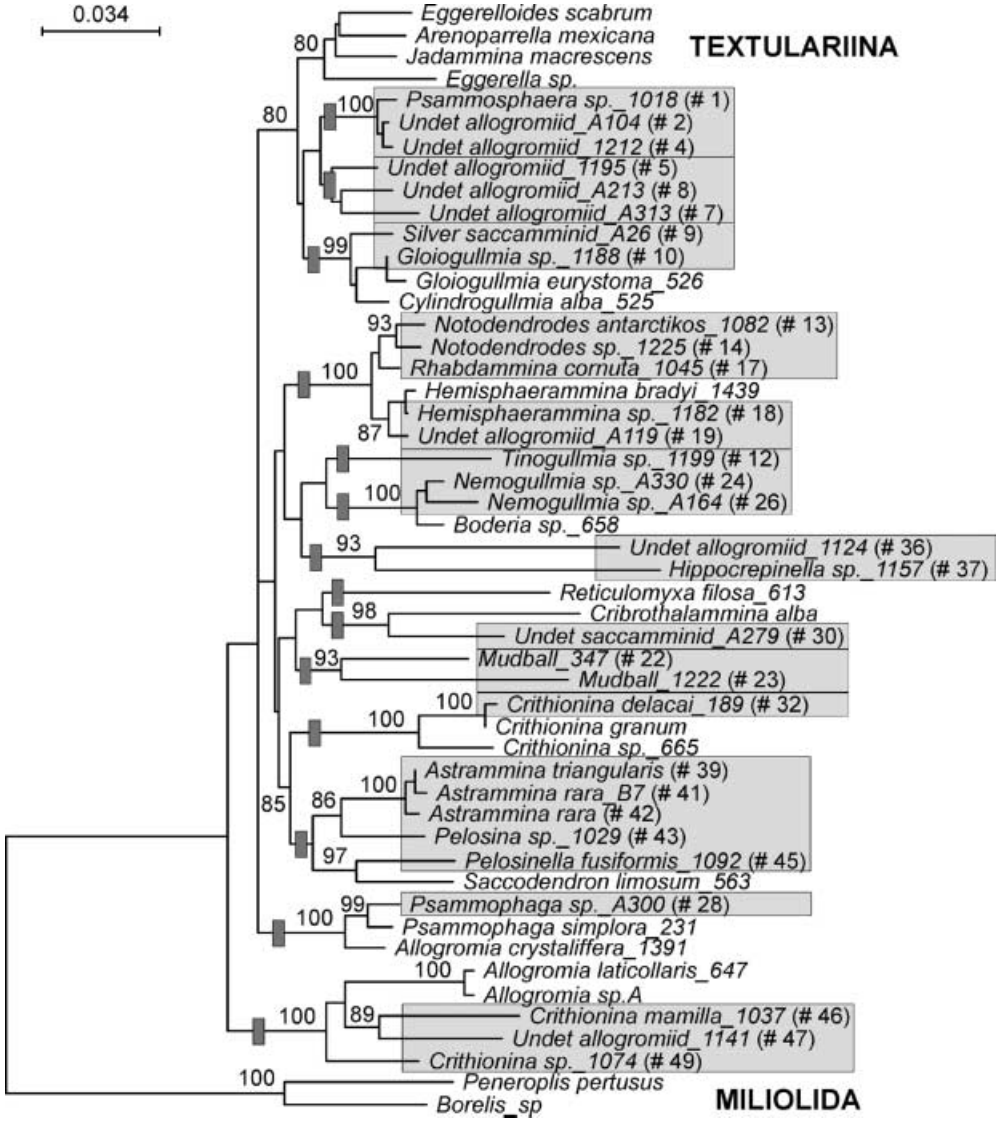

Fig. 2 Neighbor-joining tree of Antarctic and non-Antarctic allogromiids inferred from the s14F3-sB fragment of the SSU rDNA. *Small rectangles* denote distinct clades; Antarctic sequences are in *shaded boxes*. Sequence names include the morphospecies name, if determined, and the Forams DNA Collection identification number. *Numbers* above branches are bootstrap support values; only values higher than 70% are indicated

identified within *Crithionina*-like morphotypes that contained both attached and free forms. Distinct molecular types were also found among *A. rara* (nos. 8, 40, 41, 42, 47), *Nemogullmia* spp. (nos. 24, 25, 26), *Hippocrepinella* spp. (nos. 11, 37) and in the group of miscellaneous Allogromiina (nos. 6, 7, 20, 31, 38).

The majority of the 49 distinct molecular types appeared restricted to the western side of McMurdo Sound. Three types (nos. 34, 37, 47) were exclusively found on the eastern side of McMurdo Sound, and 5 types (nos. 1, 2, 8, 11, 32) occurred on both sides (Table 2). Among the latter, only 1 type (no. 11), corresponding to *Hippocrepinella* spp., seemed to be abundant on both sides. The other allogromiids were rare in the East Sound. They were represented either by single sequences (nos. 34, 37, 47) or belonged to the types found in most Explorers Cove samples (nos. 1, 2, 32). One type (no. 8) was found inside agglutinated *Astrammina*-like tests in Cape Armitage, but lacked a test in Explorers Cove samples.

Discussion

Molecular versus morphological data

The high taxonomic diversity of Explorers Cove allogromiids revealed by our molecular data contrasts with diversity assessments based on morphology. Specifically, the number of molecular types far outnumbers

►

Fig. 3 Neighbor-joining tree of 135 Antarctic allogromiids inferred from the s14F3-s17 fragment of the SSU rDNA. The tree was rooted at *Crithionina mamilla* (CM) clade following the analysis of the larger SSU fragment (Fig. 2). Each sequence is denoted by a Forams DNA Collection identification number. The molecular types are numbered from 1 to 49. For each molecular type, the corresponding morphotype is indicated by the *two-letter symbol* referred to in Table 1. Morphotypes represented by a single molecular type are labelled with an *asterisk*. *Numbers* above branches are bootstrap support values; only values higher than 70% are indicated

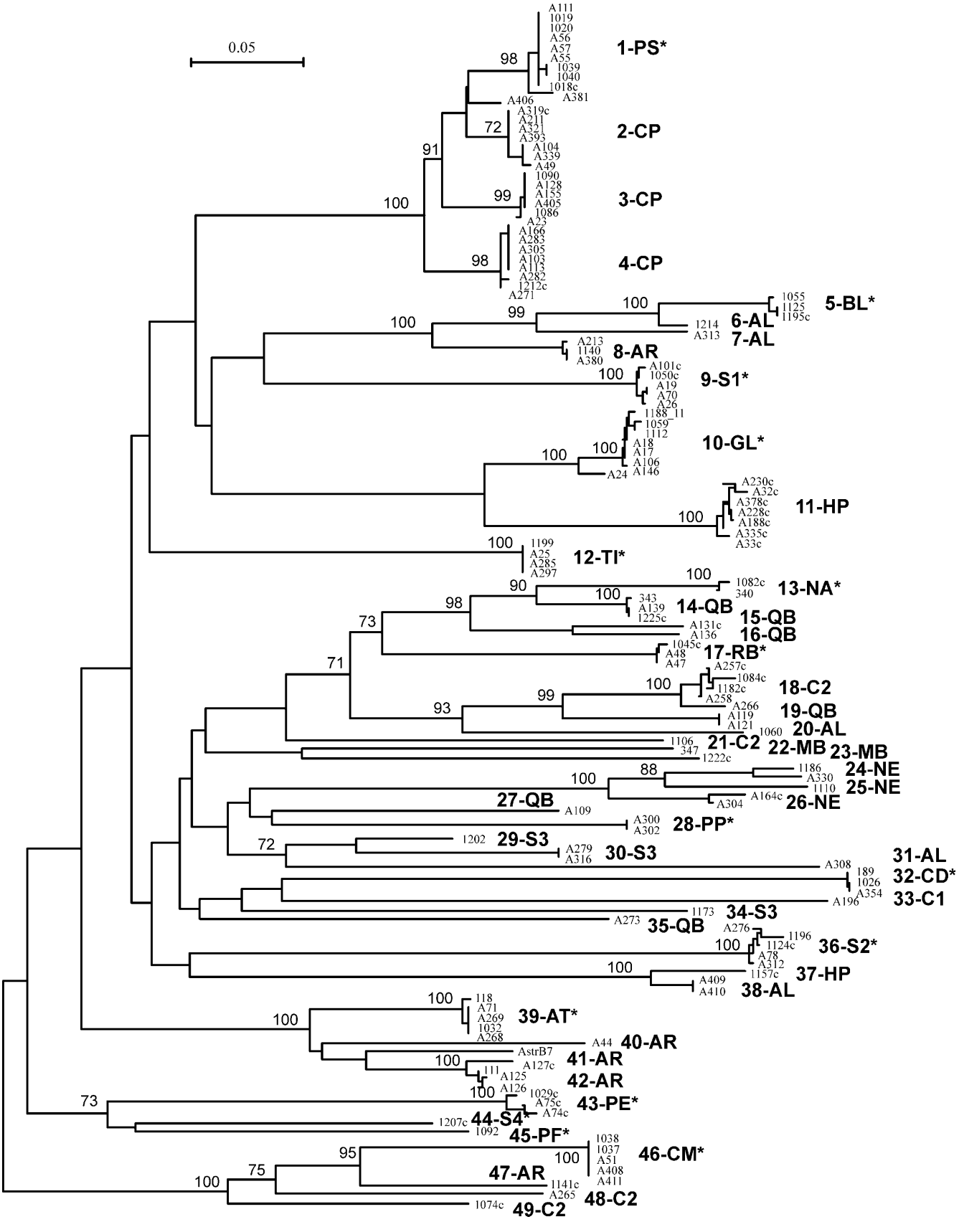
0.05
1-PS*
2-CP
3-CP
4-CP
5-BL*
6-AL
7-AL
8-AR
9-S1*
10-GL*
11-HP
12-TI*
13-NA*
14-QB
15-QB
16-QB
17-RB*
18-C2
19-QB
20-AL
21-C2
22-MB
23-MB
24-NE
25-NE
26-NE
27-QB
28-PP*
29-S3
30-S3
31-AL
32-CD*
33-C1
34-S3
35-QB
36-S2*
37-HP
38-AL
39-AT*
40-AR
41-AR
42-AR
43-PE*
44-S4*
45-PF*
46-CM*
47-AR
48-C2
49-C2

Table 2 List of molecular types and their principal morphological features

Type no.	Code	Number of sequences	Max % divergence	Species/morphotype	Locality
1	PS	10	1.4	*Psammosphaera* sp. with typical agglutinated test	EC, CA
2	CP	8	4.5	Large, ovoid or elongate, creamy-pink sarcode	EC,CA,LR
3	CP	6	0.0	Large, ovoid, creamy-pink sarcode	EC
4	CP	8	0.8	Large, ovoid or elongate, creamy-pink sarcode	EC
5	BL	3	0.4	Small, red-brown, bean-like sarcode	EC
6	AL	1	0.0	Small, ovoid sarcode	EC
7	AL	1	0.0	Large, pear shaped, membraneous test	EC
8	AR	3	0.5	*Astrammina*-like sarcode with or without aggl. test	EC,CA
9	S1	5	1.0	Silver saccamminid, spherical (*Pilulina argentea*?)	EC
10	GL	8	3.8	*Gloiogullmia* sp.	EC
11	HP	7	1.8	*Hippocrepinella* sp.	EC,CA,LR
12	TI	4	0.0	*Tinogullmia* sp., sausage shaped, 2 apertures	EC
13	NA	2	0.3	*Notodendrodes antarctikos*	EC
14	QB	3	0.3	Flower notodendrodid – quartzballs	EC
15	QB	1	0.0	Quartzball-like	EC
16	QB	1	0.0	Quartzball-like	EC
17	RB	3	0.3	*Rhabdammina cornuta*	EC
18	C2	5	3.8	*Crithionina*-like attached to pecten shell	EC
19	QB	2	0.0	Silver saccamina-like quartzballs	EC
20	AL	1	0.0	Tiny, naked, white sarcode	EC
21	C2	1	0.0	*Crithionina*-like attached to pycnogonid leg	EC
22	MB	1	0.0	*Komoki*-like mudball	EC
23	MB	1	0.0	*Komoki*-like mudball	EC
24	NE	2	4.0	*Nemogullmia*-like	EC
25	NE	1	0.0	*Nemogullmia*-like, very long	EC
26	NE	2	?	*Nemogullmia*-like, very long, with 2 apertures	EC
27	QB	1	0.0	Small sarcode inside quartzball-like test	EC
28	PP	2	0.0	*Psammophaga* sp.	EC
29	S3	1	0.0	Large, flask, white, finely agglutinated test	EC
30	S3	2	0.0	Flask-shaped, white, finely agglutinated test	EC
31	AL	1	0.0	Tiny, naked sarcode	EC
32	CD	3	0.2	*Crithionina delacai*	EC,CA
33	C1	1	0.0	*Crithionina*-like sarcode in spherical agglutinated test	EC
34	S3	1	0.0	Small, ovoid flat, finely agglutinated test	CE
35	QB	1	0.0	Yellow, ovoid sarcode in quartball-like test	EC
36	S2	5	1.0	Silver saccamminid, elongate, 2 apertures	EC
37	HP	1	0.0	Bottle-shaped, white, finely agglutinated test	CA
38	AL	2	0.0	Small, grey sarcodes with black inclusions	EC
39	AT	5	0.5	*Astrammina triangularis*	EC
40	AR	1	0.0	*Astrammina rara*	EC
41	AR	1	0.0	*Astrammina rara*	EC
42	AR	4	2.6	*Astrammina rara*	EC
43	PE	3	1.8	*Pelosina* sp.	EC
44	S4	1	0.0	Tiny, spherical saccamminid with long neck	EC
45	PF	1	0.0	*Pelosinella fusiformis*	EC
46	CM	5	0.0	*Crithionina mamilla*	EC
47	AR	1	0.0	*Astrammina*-like	CA
48	C2	1	0.0	*Crithionina*-like attached to pecten shell	EC
49	C2	1	0.0	*Crithionina*-like attached to pecten shell	EC

the morphotypes identified in this and previous studies (Bernhard 1987; Gooday et al. 1996). This hidden diversity is particularly evident in those morphotypes that lack clearly distinctive features. It is surprising to see how many different molecular types could be found in morphotypes such as quartzballs, crithioninids, mudballs or small naked sarcodes. Gooday et al. (1996) suggested that some of these undetermined spheres and domes may include more than one species, which cannot be identified using morphological characters alone. The most unexpected result of our analyses, however, is the fact that the similar morphologies evolved several times in very distantly related species.

Furthermore, cryptic diversity may also exist in morphologically well-defined allogromiids. *A. rara*, for example, which represents the dominant astrorhizid in Explorers Cove, includes at least 3 different but closely related molecular types (nos. 40, 41, 42). However, *Astrammina*-like specimens found in eastern McMurdo Sound samples (nos. 8, 47) are genetically very different from those collected in Explorers Cove, despite their morphological similarity (DeLaca 1986). The genetic diversity of *Astrammina*, however, seems to be exceptional. Many well-defined morphospecies, such as *Astrorhiza triangularis*, *N. antarctikos* or *C. delacai*, are represented in Explorers Cove by a single genotype.

Good congruence between molecular and morphological types was also found for all those morphotypes that are characterized by very specific morphological features, e.g. the presence of mineral grains inside the tests of *Psammophaga*. It is probable that more detailed morphological studies may detect such distinctive features in other molecular types.

The relationship between morphological and molecular types is additionally complicated by the "squatting" behaviour of some naked or organic-walled allogromiids. Gooday et al. (1996) described the case of vermiform allogromiid *Nemogullmia* specimens that were found inside the empty tests of some large agglutinated species such as *Psammosphaera fusca* in Explorers Cove. "Squatting" behaviour was also observed in some deep-sea and shelf Foraminifera (Gooday 1986; Moodley 1990). We have found *Nemogullmia* specimens inside quartzballs tests, but in these cases the "squatter" was relatively easy to identify because of its obvious morphological difference to that of the host cell. It may be much more difficult to identify tiny naked "squatters" in allogromiids that possess large agglutinated tests, such as the crithioninids and mudballs.

Taxonomic status of molecular types

The large number of molecular types detected within Explorers Cove allogromiids, and their great morphological variability, raise the question of the taxonomic status of these types. Until now, foraminiferal species were described exclusively based on morphological characters of their tests (Loeblich and Tappan 1988). Many authors argued, however, that morphological features may be taxonomically unreliable because of ecophenotypic and life-cycle related variability (Boltovskoy and Wright 1976; Haynes 1992). The inconsistent taxonomic value of morphological characters was also demonstrated by molecular systematic studies, which revealed the presence of sibling species in benthic (Holzmann 2000) and planktonic (Huber et al. 1997; de Vargas et al. 1999) Foraminifera.

Molecular criteria used for species distinction vary among authors. Holzmann (2000) used sequence divergences larger than 10% in the hypervariable region of LSU rDNA to distinguish different molecular types in the calcareous foraminiferal genus *Ammonia*. The genetic distances between *Glabratella* species (also calcareous) range from 1.1 to 6.4% in the LSU and from 3.8 to 6.7% in the SSU (Tsuchiya et al. 2000). In a study of the genus *Acanthamoeba* based on SSU rDNA, distinct sequence types are defined as single sequences or groups of sequences that differ from each other by more than 6% (Gast et al. 1996). Much smaller genetic distances were accepted to distinguish species in the genus *Naegleria*. The sequences of some well-recognized *Naegleria* species differ by only 0.5–1.2% in the 800-bp region of the SSU rDNA (De Jonckheere and Brown 1997; Brown and De Jonckheere 1999). The description of the new species *Naegleria robinsoni* was based on an SSU sequence difference of 2.4% compared to its closest relative (De Jonckheere and Brown 1999). In ciliates, the genetic species separated by breeding experiments ("syngens") were found to differ by only a few changes in their ribosomal genes (Nanney et al. 1998).

Distinctions between molecular types in our study are based on sequence divergences that are higher than 5 percent, which correspond to the upper limit of genetic variability we have allowed within a molecular type. The 5% level was arbitrarily chosen based on observed sequence variations in the examined SSU fragment. In fact, most of the types are characterized by sequence variations lower than 1% and differ from each other by more than 10%. For example, the sequences of well-defined morphospecies *Notodendrodes antarctikos* and *Notodendrodes* sp. (flower notodendrodid), which correspond to type nos. 13 and 14 in the present study, differ by 12%. Detailed analysis of their intraspecific variation (Pawlowski et al. 2001) shows that the sequences of different specimens are almost identical ($<0.1\%$). In this case, the equivalence between molecular type and species seems certain.

The taxonomic status of the molecular types that differ by less than 10% is more controversial. For example, sequence divergence between the 4 different types that branch within the *Psammosphaera* clade averages 5%. One of these types (no. 1) possesses a typical *Psammosphaera*-like agglutinated test, whereas the three other types (nos. 2, 3, 4) are naked or, in exceptional cases, include specimens found within the tests of other species. Given the equal genetic distance between the four types, it seems justifiable to consider them separate species. In the absence of any morphological evidence, however, the situation is less clear. This is the case in type nos. 5 and 6, nos. 37 and 38, and nos. 15 and 16, which differ by 6%, 6% and 8% respectively. The taxonomic status of different molecular types within *Astrammina rara* (nos. 40, 41 and 42) is also controversial. In these cases, further molecular studies will have to be carried out before one can decide whether they should be considered different species.

To conclude, our molecular data seem to give a good approximation of species diversity in allogromiid foraminiferans. Most of the molecular types distinguished probably equate to different species. Those that differ by more than 20% may even represent different genera and families. Using sequence divergence values for taxonomic identification, however, requires special caution. Sequence variations depend on the choice of the molecule and the examined fragment. The two species of *Notodendrodes*, for example, differ by 12% or 3.4% in our analysis depending on which fragment of the SSU rDNA (14F3–17 or 14F3–B) was selected. Even closely related species may differ significantly in their evolutionary rates, as has been shown in the planktonic foraminiferal genus *Globorotaliella* (de Vargas and Pawlowski 1998). Therefore, it is important to corroborate the SSU rDNA data by analysis of other genes,

as well as by morphological or physiological studies, whenever possible.

Origin of Explorers Cove allogromiids

Based on comparative morphological studies, it has been proposed that the Antarctic shelf fauna originated from adjacent bathyal regions (Brey et al. 1996). However, foraminiferal assemblages of Explorers Cove and adjacent McMurdo Sound deep water differ considerably in their species composition (Ward et al. 1987). The foraminiferal fauna from Explorers Cove resembles that found in deep sublittoral areas in the northern hemisphere (Gooday et al. 1996). Indeed, several Explorers Cove allogromiids (*C. mamilla*, *C. delacai*, *Psammosphaera fusca*, *Astrammina rara*, *Astrorhiza triangularis*) have been reported from, or have morphologically similar counterparts in, the northern hemisphere (Kaminski 1985; Gooday et al. 1996). In particular, certain genera of organic-walled allogromiids identified in the present study (*Gloiogullmia*, *Tinogullmia* and *Nemogullmia*) were originally described in Scandinavian fjords (Nyholm 1953, 1954, 1974). Only a few allogromiids, such as the genus *Notodendrodes*, seem to have a much more restricted distribution and have not yet been reported from other parts of Antarctica (Violanti 1996).

Contrary to morphological studies, our molecular data highlight the endemic character of the allogromiid fauna from Explorers Cove. Our analyses show that none of the sequences of non-Antarctic species cluster within Antarctic molecular types. Among the northern hemisphere species, close relationships are observed between *C. granum* and *C. delacai*, as well as between *Gloiogullmia eurystoma* and *Gloiogullmia* sp. In both cases, however, the sequences differ by more than 10% when the whole sequenced fragment is compared (data not shown), suggesting that they may belong to the same genera but certainly represent different species. Moreover, in view of our data, the northern hemisphere cold-water taxa do not always appear as the closest relatives of Explorers Cove species. The clade *Psammophaga*, for example, is represented in our analyses by 3 species; among them, Explorers Cove *Psammophaga* sp. (no. 28) is more closely related to *Psammophaga simplora* collected in the western Atlantic (Sapello Island, USA) than to *Allogromia crystallifera* from the Gullmar Fjord. Similarly, the *Nemogullmia* types nos. 24 and 26 from Explorers Cove are more closely related to *Boderia* sp. from Lizard Island than to *Nemogullmia longivariabilis* from Kösterfjord (data not shown). These examples show that the gross morphology used to distinguish allogromiid genera is often insufficient for precise species identification.

The endemism of Explorers Cove allogromiids is in agreement with a biogeographic hypothesis regarding the relict autochthonous character of the Antarctic fauna (Dayton 1990). If this hypothesis is correct, then the morphological resemblance between foraminiferal taxa from northern and southern hemispheres is due to convergence rather than their common origin. Given the limited number of sequence data from other Antarctic and non-Antarctic settings, however, alternative hypotheses about the origin of Explorers Cove allogromiids, e.g. by emergence from deep-sea waters or by migration from the Antarctic Peninsula, cannot be excluded. Further genetic studies of deep-sea and high-latitude Foraminifera are necessary to test these hypotheses.

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