J. Eukaryot. Microbiol., 51(2), 2004 pp. 173–179

Unexpected Foraminiferal Diversity Revealed by Small-subunit rDNA Analysis of Antarctic Sediment

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ABSTRACT. Studies of benthic Foraminifera typically rely on the morphological identification of dried specimens. This approach can introduce sampling bias against small, delicate, or morphologically ambiguous forms. To overcome this limitation, we extracted total DNA from sediment followed by PCR using group- and species-specific primers. Phylogenetic analyses revealed that approximately ninety percent of the PCR products represented previously undescribed sequence types that group with undersampled members of the allogromiid Foraminifera. We also used a modification of this technique to track individual species in sediment fractions too fine for normal morphological identification, and to confirm species placement of morphologically ambiguous foraminiferans. We were able to identify the DNA of several large foraminiferal species in fine fractions in a seasonally-dependent manner, indicating that in some seasons the majority of the standing stock of these species exists as gametes/juveniles. The approach outlined here represents a powerful strategy for exploring the total diversity of benthic foraminiferal communities.

Key Words. Antarctica, biodiversity, environmental DNA, molecular species, morphospecies, ribosomal RNA.

FORAMINIFERAN protists are an important component of modern marine ecosystems (Lee and Anderson 1991). The shells (tests) of foraminiferans, particularly multi-chambered calcareous species, produce vast sedimentary deposits, and their diversity is well documented over geological time (Haynes 1981; Murray 1991; Sen Gupta 1999). By comparison, the diversity of single-chambered, soft-bodied (allogromiid) and agglutinated (astrorhizid) foraminiferans, which often dominate deep-sea and high latitude settings (Gooday 2002a, b), is poorly known, due in part to the simple morphology of their organic or agglutinated tests (e.g. Bowser et al. 1995; Pawlowski et al. 2002a, b; for a discussion of the morphology and classification of Foraminifera, see Lee 1993).

Explorers Cove (western McMurdo Sound, Antarctica) is a low-productivity, shallow marine setting that shares certain physical and biological characteristics with the bathyal deep sea (Dayton and Oliver 1977). Early assessments of the foraminiferal assemblage at this site (Bernhard, 1987; Ward, Barrett, and Vella 1987), which employed classic micropaleontologic techniques such as floating of dried tests, reported low species diversity. Re-analysis using wet picking of preserved sediments (Gooday, Bowser, and Bernhard 1996) revealed a surprising diversity of single-chambered agglutinated species that were not apparent during the initial surveys. Many of the newly identified morphotypes comprised a bewildering array of ''quartz spheres'' and ''mudballs'' that were difficult to identify beyond the genus or, in some cases, the family level. More recent efforts (Bowser et al. 2002; Pawlowski et al. 2002a, b) adopted molecular methods to characterize these problematic members of the assemblage. The results of these investigations exposed an even richer diversity of single-chambered species, which phylogenetic analyses showed to reflect an explosive radiation of early Foraminifera (Pawlowski et al. 2002a, b). Despite these advances, the true diversity in Explorers Cove likely remains underestimated because soft-shelled and friable agglutinated foraminiferans are vulnerable to loss or mechanical disruption during the process of sieving and picking (Murray and Bowser 2000).

Previous studies (e.g. Lopez-Garcia et al. 2001) showed that PCR analysis of bulk DNA extracts from environmental samples often reveals the presence of previously undescribed meiofauna, including Foraminifera (Holzmann et al. 2003). Individual known taxa can also be identified in environmental DNA using group-specific PCR primers (e.g. David et al. 1996). We describe here the development of methods and initial results from a molecular survey of benthic Foraminifera from Explorers Cove and elsewhere in McMurdo Sound based on environmental DNA samples. We also use an extension of these techniques to assess the robustness of morphological characters used in the identification of agglutinated allogromiids.

MATERIALS AND METHODS

Sample collection. Antarctic sediment samples were collected by divers near the McMurdo station jetty (23.6 m water depth, S 77° 91.714′ E 166° 65.903′) in January 2001, at the Explorers Cove ''Ice Hut'' site (26.6 m depth, S 77° 34.574′ E 163° 31.640′) in November and January 2001 and November 2002, and at ''Ice Cliff'' (26.6 m water depth, S 77° 34.302′ E 163° 30.702′) and ''Tile Hole'' (26.6 m water depth, S 77° 34.429′ E 163° 30.299′) in November 2002. Further site details can be found at http://www.bowserlab.org/datastorelaunch.htm. In all cases, the top 1 cm. of sediment cores was harvested, and ''whole-sediment'' samples were not processed further. Size-fractionated samples were obtained by wet-sieving through serial 1-mm, 0.5-mm, 250-μm, 125-μm, and 63-μm sieves; the $<$ 63-μm fraction was left undisturbed for several hours after which the excess water was decanted from the settled fines. Samples were then preserved by freezing: McMurdo samples were frozen at − 20 °C in 15-ml aliquots, while Explorers Cove samples were snap-frozen in liquid nitrogen in 50-ml aliquots. In all cases, the samples were shipped to our lab in Albany and stored at − 70 °C. Foraminifera isolated from 1-mm sieve fractions and dried Antarctic sediment were collected at Explorers Cove in 1999 and 1997, respectively. Live specimens in seawater were transported on ice and maintained in the lab at − 1.8 °C (the mean ambient temperature at Explorers Cove); bulk sediment was dried at 80 °C and stored at room temperature.

DNA extraction from sediment and individual organisms. Total DNA was extracted from sediment samples as previously described (Holzmann et al. 2003). The DNA equivalent to the extract from 1.5 ml of sediment was further purified for species-specific panels with the DNeasy kit (QIAGEN Inc., Valencia, CA). The DNA from isolated organisms was purified either by a small-scale version of the sediment prep or with the DNeasy kit. New sequences from the species *Pyrgo peruviana*, *Glandulina antarctica*, and *Cornuspira antarctica* have been deposited as AY179176, AY179177, and AY435097, respectively.

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Table **1**. Position of the 5′ end of the primer is given relative to the foraminiferal SSU sequence whose accession number is listed in parentheses.

	Species name	Position of 5′ end	Primer sequence
Forward primer			
s10	All Foraminifera (Pawlowski 2000)	849 (AJ132368)	CAC TGT GAA CAA ATC AG
s14F1	All Foraminifera (Pawlowski 2000)	1511 (AJ132368) 1 (AY179177)	AAG GGC ACC ACA AGA ACG C
s14F3A	All Foraminifera	1480 (AJ132368)	ACG CAA GTG TGA AAC TTG
Alloolig	*Allogromia* sp.	271 (Z69607)	TAA AGT TGC TGC ATT GTT TTT TAA CTT TGC
Ara	*Astrammina rara*	100 (AF411218)	TGT AAT AAT TTA TAT ATA ATT TAT TTT
Astrolig	*Astrammina rara* and *Astrammina triangularis*	247 (AF411218) 199 (AJ307811)	CCT TAT AGA CTT TTC TAT TGA TAT CAG CCT T
Cde5	*Crithionina delacai*	46 (AJ307868)	GCG TCA TTT CAC TTT GGT TT
Glanolig	*Glandulina antarctica*	253 (AY179177)	ATA ATT ACG TAT GCT GTT AAC GCT TTG ACC
Glo5	*Gloiogullmia* sp.	100 (AJ307751)	TGA ATC GTT TTT ATC TTT TA
Gloiolig	*Gloiogullmia* sp.	262 (AJ307751)	CGG TTA TGT TT($^{C}_{T}$) AAT GCA TTT TGT TAC CCC
McM11	Unidentified foraminiferan	221 (AY179178)	ATA CTG CGA GGT GTA TCG TGA CTT GTT TAC
Nhy5	*Notodendrodes hyalinosphaira*	104 (AJ311214)	ATA TGG TAT TGT ATC AAT GC
Nhyaolig	*Notodendrodes hyalinosphaira*	256 (AJ311214)	GCG TTT CAC ATA TGG CTC GTA CG
Pfu5	*Psammosphaera fusca*	61 (AJ307789)	GCT TTT ATT TTT ATA ACT AAG CA
Psamolig	*Psammosphaera lithocollis*	239 (AJ307745)	AAA ACG TAC GTT GCG GCG TTT CGA TCC
Ppe5	*Pyrgo peruviana*	91 (AY179176)	GTG ATC GCA TAA TAG AAT TT
Pyrolig	*Pyrgo peruviana*	253 (AY179176)	GTT CTG CCT TCA CAG GAT TCT GAA CTT G
QFA	All Foraminifera	186 (AF190879)	GAT CTG TCT GCT TAA TTG CGT TTC
Rco5	*Rhabdammina* cf. *cornuta*	107 (AJ311215)	TAG TTC TTA CAT ACG TTG GG
Rhabolig	*Rhabdammina* cf. *cornuta*	303 (AJ311215)	GTC CAC TCA TGT TTT TAT TGC TCT GTG AC
Reverse primer			
B	Univeral (Pawlowski 2000)	948 (AY179176)	TGA TCC TTC TGC AGG TTC ACC TAC
QFR	All Foraminifera	497 (AF190879)	AAT CAT TGT AGC ACG TGT GCA GCC CG
s14rf	All Foraminifera (Pawlowski 2000)	1482 (AJ132368)	CCT TCA AGT TTC ACA CTT GC
s17R	All Foraminifera (Pawlowski 2000)	322 (AF190879) 1862 (AJ132368)	CGG TCA CGT TCG TTG C
s20R	Universal (Pawlowski 2000)	855 (AF190879)	GAC GGG CGG TGT GTA CAA

The sequences of the environmental clones have been deposited as AY452783-AY452799.

PCR and primers. Sediment extracts were diluted 1:50 to serve as PCR templates. Sediment samples used for species-specific panels were adjusted so that each reaction contained template representing total yield from 30 mm^3 of sediment. Group-specific amplification of foraminiferal SSU rDNA was performed using the primers s10, s14rf, s14F3A, s14F1, s17, s20R, and B (Pawlowski 2000) and QFA and QFR (Table 1). Novel species-specific primers were designed using published sequences, except those for *Pyrgo peruviana* and *Glandulina antarctica*, which were sequenced for this work. Additional sequence from the environmental clone McM11 (AY179178) was also used for primer design. Sequences for the new genus- and species-specific primers are listed in Table 1.

PCR reactions were performed using ExTaq proofreading *Taq* polymerase premix (TaKaRa Bio Inc., Madison, WI) in a Techne Genius thermocycler (Techne Inc., Princeton, NJ). Cycling parameters for species discovery were: 1 cycle (94 °C 2 min, 48 °C 1 min, 70 °C 2 min); 35 cycles (94 °C 30 s, 48 °C 1 min, 70 °C 2 min); 1 cycle (94 °C 30 s, 48 °C 1 min, 70 °C 15 min). The parameters for species-specific panels were: 10 min 94 °C, followed by 35 cycles (94 °C 30 s, 58 °C 1 min, 70 °C 2 min). If nested PCR was required, the second set of reactions was done with 20 cycles instead of 37, with the annealing temperature set at 51 °C.

Sequencing and phylogeny. Products of species-specific PCR reactions were sequenced directly using the primer s17. The products of reactions using oligos of general specificity for all Foraminifera were cloned into the sequencing vector pGEM®-T Easy (Promega Corp., Madison, WI) and replicated in *E. coli* strain JM109. Individual clones were purified using Promega's Wizard Midipreps kit or QIAGEN's SpinPrep. Clones were grouped into classes (''riboprinted'') based on the pattern of digestion with the restriction endonuleases *Ava*II, *Bsi*HKAI, *Hin*fI, and *Ssp*I, and multiple representative clones from each group (if available) were used for sequencing. Clones used for phylogenetic studies were sequenced in both directions using primers M13 and M13 reverse with a PE-Biosystems ABI PRISM 377XL automated DNA sequencer. Sequences were compared with the BLAST utility (NCBI) to known sequences in GenBank, which includes 537 SSU rDNA sequences (as of September 2003) from foraminiferans, including 162 from Antarctic taxa. Novel sequences and close GenBank matches were aligned using CLUSTAL W, and the alignment was then manually edited in SEAVIEW to correspond to secondary structure predictions. The alignment can be viewed at http://www.bowserlab.org/supplementary.htm. Sequences were then checked for chimerism by comparing six variable regions of the SSU to the equivalent regions in other sequences (see Pawlowski 2002a, Fig. 1). Environmental clones that showed evidence of chimerism were discarded.

Four hundred and thirty-two reliably-aligned sites were retained for phylogenetic analysis. Evolutionary relationships were inferred by maximum-likelihood, neighbor-joining, and maximum-parsimony methods using the PHYLIP 3.6a (Felsenstein 1989; 2003 release) utilities DNAML (for ML analysis), DNAPARS (MP analysis), DNADIST and NEIGHBOR. The reliability of internal branches was determined by bootstrap analysis with SEQBOOT, using 100 replicates for ML, 1000 for NJ, and 500 for MP. For ML analyses, seven rate categories were used, based on the rate assignments in Wuyts et al. (2001). Distances for NJ (Saitou and Nei 1987) were corrected using

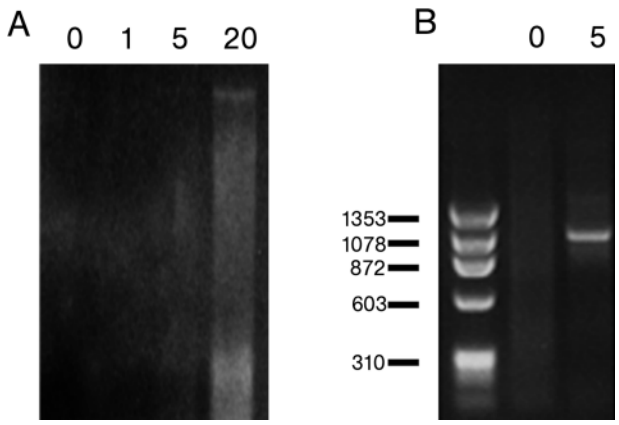

Fig. **1.** Foraminiferal DNA can be recovered from Antarctic sediment. **A:** Recovery from artificial sediment. One milliliter of dried sediment was ''spiked'' with 0, 1, 5, or 20 live specimens of *Astrammina rara*. DNA was recovered in a quantitative fashion. **B:** Ecologically significant numbers of specimens were detectable. DNA from two samples with 0 and 5 specimens of *A. rara* was amplified using the foraminiferal-specific PCR primers s10 and s14rf. The primers do not recognize the control template, but amplify a band of appropriate size from the template containing DNA from 5 cells.

the F84 model (Felsenstein 1989), assuming a gamma-distributed rate of variation (Jin and Nei 1990).

RESULTS

Recoverability of DNA from sediment. As assessed by gel electrophoresis, DNA was recovered from mock Explorers Cove sediment in quantities that were proportional to the number of foraminiferans added (Fig. 1A). When the purified DNA was used as the template for PCR using the foraminiferal-specific primers s10 and s14rf (Pawlowski 2000), a band of appropriate size was amplified from the spiked sediment, but not from a control sample lacking added specimens (Fig. 1B). This finding demonstrates that ecologically relevant numbers of foraminiferans can be readily detected by this method.

Species discovery. Nineteen distinct molecular types were identified from the 136 clones analyzed (Table 2). All sequences were clearly foraminiferal in origin, and all but two were from single-chambered taxa. The sequences of the analyzed region of foraminiferal SSU rDNA can vary from 0.1% to 5% intraspecifically, and species within the same genus may show sequence divergence up to 7% (Pawlowski et al. 2002b; Tsuchiya, Kitazato, and Pawlowski, 2000). Using these criteria, only six of the nineteen sequence groups could be confidently assigned to the same molecular type as any sequence deposited in GenBank (Table 2). Of these, all were species that were previously identified in Explorers Cove (Pawlowski et al. 2002a), namely Allogromiid 347, *Cibicides refulgens*, *Hippocrepinella* 1157, *Nemogullmia* A330, and *Psammosphaera* A104. Two different sequence types cluster with *Hippocrepinella* 1157, one being 99% identical and the other only 96% identical. It is likely that these sequence groups represent two distinct but closely-related species. A seventh sequence appears to be related to *Cornuspira antarctica* but is unlikely to be a conspecific.

The twelve remaining sequence groups may represent cryptic members of the McMurdo Sound foraminiferal assemblage. In order to gain insight into the possible morphology and affinities of these new forms, we performed a phylogenetic analysis on the environmental clones (Fig. 2), using known Antarctic foraminiferans and close GenBank matches for reference. Several sequence types represented new members of established foraminiferal clades (Pawlowski 2002a, b). Sequence group 6 was placed with good support in Clade E, which contains several species of allogromiids that take crystals into their cell bodies. Group 8 fell within Clade L, a group of small allogromiids with finely agglutinated tests. Group 19 further expanded a group of poorly described ''mudball'' foraminiferans that, to date, appear restricted to Explorers Cove.

The most abundant signal derived from a group of sequence types allied with the foraminiferans *Hippocrepinella hirudinea* and *Phainogullmia aurata*. One of the two sequences that grouped with *Hippocrepinella* was close enough to be congeneric; the other was identical to *Hippocrepinella* 1157, an Antarctic foraminiferan that is closely related to *H. hirudinea*. The sequences that grouped with the Arctic Sea allogromiid *Phainogullmia*, however, were more phylogenetically distant. *Phainogullmia* has not been detected in morphological surveys of

Table **2**. Amplification products derived from sediment DNA extracts. Individual clones are designated by site: IC = Ice Cliff; IH13 = Ice Hut, >1 mm fraction; TH = Tile Hole; McM = McMurdo Station Jetty.

Sequence group	Closet known taxon (≥93% identity)	Sequenced clones represented
1	*Psammosphaera* A104 (99.0% identical)	McM 3, 16, 17, 18, 20
2	*Cibicides refulgens* (95.2% identical)	McM 13
3	*Cornuspira antarctica* (94% identical)	IC 15
4	*Allogromiina* 347 (99.6% identical)	McM 4
5		McM 1, 19
6		McM 2
7	*Nemogullmia* A330 (99.7% identical)	McM 5
8		McM 11
9	*Hippocrepinella* 1157 (96.0% identical)	TH 3-6, 15, 22
10	*Hippocrepinella* 1157 (99.7% identical)	IC 13; IH13 5, 6, 11, 16
11		TH 7, 8; IC 17; IH13 3
12		TH 2, 11, 16, 18, 21, 25; IH13 1
13		IC 12
14		IC 20, 23, 24
15		TH 12-14, 17, 19, 20
16		IC 16, 19, 21, 22
17		IC 14, 30, 31, 36, 38, 39, 45
18		IC 28
19		IC 42, 48, 49

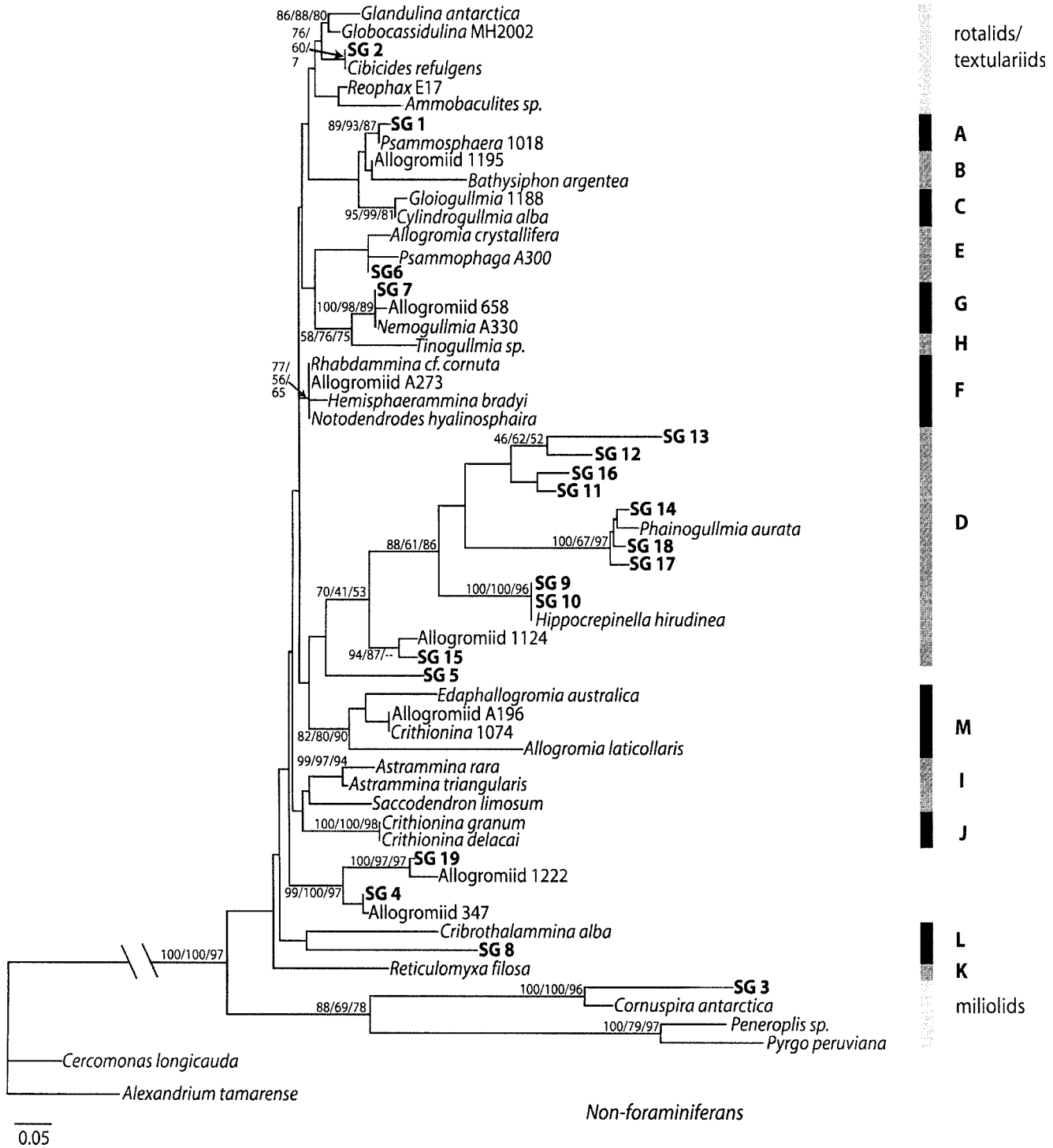

Fig. 2. Phylogenetic analysis of novel clones of Foraminifera illustrated by a neighbor-joining tree. Nineteen environmental clones, representing the sequence groups derived from McMurdo Sound sediment (see Table 2), were compared to the SSU sequences of 38 foraminiferal species, representing all major lineages of allogromiids as well as multilocular groups. Twelve sequences are clearly foraminiferal but cannot be assigned to any known taxon; of the rest, most are nearly identical to a single species common to Antarctic sediment. Clades of foraminifera defined by Pawlowski et al. (2002a, b) are labeled. Bootstrap values are given in the order Neighbor-Joining/Maximum Likelihood/Maximum Parsimony and are listed for any node where one or more of these values is > 60.

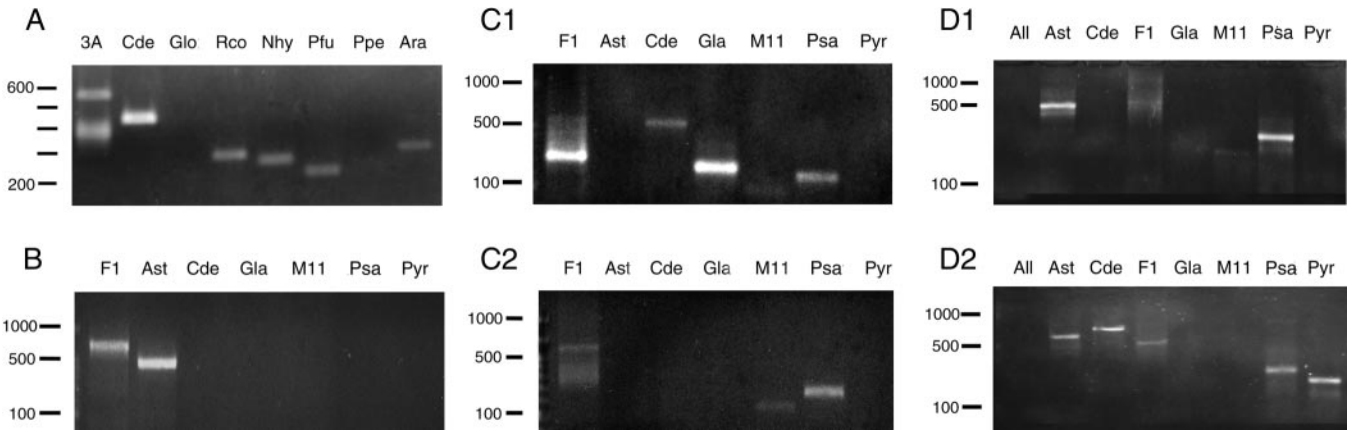

Fig. **3.** Individual species of Foraminifera can be detected in sediments using molecular tools. **A:** Detection of individual species in a mixed template. A foram-rich fraction of Explorers Cove sediment was extracted and used as a template for primers specific for individual species of forams (Ara—*Astrammina rara*; Cde—*Crithionina delacai*; Glo—*Gloiogullmia* sp.; Nhy—*Notodendrodes hyalinosphaira*; Pfu—*Psammosphaera fusca*; Ppe—*Pyrgo peruviana*; Rco—*Rhabdammina cornuta*). Amplification with a forward primer specific for all Foraminifera (3A) yields a heterogeneous mixture of products, but species-specific primers either produce a single band of appropriate size, or no band at all. Predicted sizes for the bands are: Ara: 274 bp; Cde: 465 bp; Glo: 276 bp; Nhy: 269 bp; Pfu: 228 bp; Ppe: 212 bp; Rco: 270 bp. Reverse primer is s17. **B:** Primers identified individual species. A cell extract from a single species (*A. rara*) is detected only by a universal-foram primer (F1) and its corresponding species-specific primer (Ast). Reverse primer is QFR. **C:** Assemblage diversity in different sediments. A primer panel was used to challenge extracts from Explorers Cove sediment (C1) and McMurdo Station sediment (C2). Assemblages are distinct, although some species are found in both sediments (e.g. M11, Psa). Reverse primer is QFR. **D:** Size fractionation of foram assemblages. An early-season sample of Explorers Cove sediment was processed by sieving, and $>$ 1 mm (D1) and $<$ 63 μm (D2) fractions were screened. Screens for large species of Foraminifera showed signal not only in the coarse fraction (presumably adults) but also in the fine fraction (e.g. Ast, F1, Psa). Some species were found only in the fine fraction (e.g. Cde, Pyr), and may be present predominantly as juveniles during this season. Reverse primer is QFR.

Antarctic habitats, so these new sequences may represent a related foraminiferan with a different morphology. Another sequence type appeared to be related to an Antarctic saccaminid (Allogromiid 1124) that was identified in a previous morphological screen. Finally, four sequence types do not seem to be closely related to any known foraminiferan, although they still grouped in allogromiid Clade D, which contains both *Hippocrepinella* and *Phainogullmia*.

Detecting and measuring individual species with molecular tools. Species-specific forward primers (Table 1) were used with reverse primers specific for the SSU rDNA of all foraminiferans. A bulk extract from a $>$ 1-mm sieve fraction, which contains the larger foraminiferans, macrofaunal invertebrates, and other organisms from approximately 15 ml of Explorers Cove sediment, was screened with a panel of the primers. PCR from this template using primers specific for all Foraminifera produced an obviously heterogeneous mixture of products, which is consistent with the presence of many different species in the sample (Fig. 3A, lane ''3A''). PCR using species-specific primers yielded either a single discrete band of appropriate size or no product at all (Fig. 3A). Sequencing of these PCR products confirmed that the amplified fragment was from the target species. When a panel was used to challenge a purified extract from a single foraminiferan, only the matching primer gave signal (Fig. 3B). These results show that the primers can be used to identify a single foraminiferal species from a mixed template with good fidelity. Sediment harvested from Explorers Cove typically contained signal from many different previously identified foraminiferans, but samples harvested elsewhere in McMurdo Sound frequently revealed only one or a few identified species (Fig. 3C). Some taxa, such as *Psammosphaera* type A104, were nearly ubiquitous in these samples. Other species appeared to be considerably more restricted in their distribution (data not shown).

Comparison between a group-specific and species-specific PCR reaction provided a first-order estimate of the number of cryptic foraminiferans present in a sample. The group-specific reaction for a McMurdo Station sediment sample (Fig. 3C) contained an assortment of product sizes, suggesting that the foraminiferal assemblage at that site was diverse. Relatively few of the species-specific primers targeting members of the Explorers Cove assemblage yielded product, which is consistent with morphological assessments showing that the McMurdo Station assemblage is dominated by different foraminiferal taxa (e.g. Ward, Barrett, and Vella 1987).

Size fractionation of sediment detected presumptive reproduction events in larger, robust foraminferans. For example, analysis of an early-season (October 2001) Explorers Cove sample showed that while some species (*Astrammina rara* and *Astrammina triangularis*, *Psammosphaera* sp.) were already present as large adults, a number of additional species were identified in the finest fractions (Fig. 3D). Because DNA from dead foraminiferans is not persistent in sediment (AH, unpubl. results), the signal was most likely from gametes or from very small juveniles of these species. In some cases, signal from larger species (e.g. *Crithionina delacai*) was detected in fine fractions but not in the largest, indicating that adults were rare in the assemblage at that time. A similar screen of a late-season fine fraction from the same locality revealed much less signal from these large foraminiferans (data not shown), which suggests that juveniles had either matured or died by the height of the Austral summer.

Correlation between morphology and SSU rDNA. Identification of species in the field is by necessity based on morphology. In order to determine whether the described morphological characters of a common Antarctic psammosphaerid were sufficient for accurate identification, two researchers independently picked specimens based on these criteria. Twelve cells were processed separately for DNA extraction; nine of these specimens yielded amplifiable DNA. All nine gave the same molecular signal based on SSU rDNA sequence, which corresponded to that of *Psammosphaera* sp. type A104 (GenBank

accession number AJ307745). Its SSU sequence is distinct from all other agglutinated foraminiferans derived from Explorers Cove, including those that could be mistaken for this species. The morphological and molecular characters of this foraminiferan together define a distinct and well characterized taxon.

DISCUSSION

Foraminifera are among the most abundant eukaryotes in the marine benthos, and determining foraminiferal community structure is crucial to understanding marine trophic dynamics. We show here that conventional ''picking'' methods, while informative, may not yield complete information about a foraminiferal assemblage, even when carefully applied. The use of DNA isolation and analysis techniques is shown here to be an effective and rapid complementary approach. Our initial DNA-based survey was sufficient to identify almost a dozen novel types of foraminiferans in perhaps the best-characterized polar setting (Gooday et al. 1996).

Further, it appears that most of the abundant foraminiferans in these sediments, which had been exhaustively searched by morphological methods, had gone undetected. In screens of Antarctic sediment samples, we have found that up to 90% of all clones generated are from apparently novel taxa. This result is especially surprising because the Foraminifera, which have been studied for more than 300 yrs, are one of the most thoroughly described of all protist groups, with more than 4,000 identified living species. The discovery of substantial cryptic diversity in even this population would suggest that recent assessments of global cryptic diversity among protists (e.g. Blaxter 2003) may be underestimates.

All of the significantly divergent sequences identified in our screen group with allogromiids in phylogenetic analyses. This is probably due to an inherent sampling bias in harvesting: calcareous and relatively hard-walled multilocular agglutinated foraminiferans are more impervious to rough handling and are also easier to identify, thanks to the details of chamber formation and patterning. Some allogromiids and athalamids, on the other hand, are easily broken up even during careful handling, and would be expected to be preferentially lost during sieving. Their relative lack of identifiable characters also may have caused them to have been overlooked in the samples analyzed for the previous morphological and molecular studies (Bernhard 1987; Gooday, Bowser, and Bernhard, 1996). Indeed, the significant expansion of Clade D in this survey reinforces this idea, since the foraminiferans in this group tend to be flexible, elongate cylinders with simple morphology.

We have also shown that species-specific primers can be used to identify rapidly and unambiguously a particular species in a mixed pool of DNA. This method reveals that foraminiferal assemblages vary temporally and spatially within McMurdo Sound. The potential speed advantage of the methods described here allows more detailed investigation of fluctuations in the distribution of individual foraminiferal species. Combined with quantitative techniques, such as real-time PCR, this approach will allow rapid, highly specific tracking of seasonal population changes.

The widespread McMurdo Sound foraminiferan *Psammosphaera* sp. (Pl. 3, Fig. A in Gooday, Bowser, and Bernhard, 1996) possesses only a few distinctive characters, such as test size (1.40–2 mm diam.), fine-grained mortar, and rough, oval outline, which, unfortunately, it can share with other species (especially juveniles of *Astrammina rara*). This situation typifies many of the ''quartz sphere'' and ''mudball'' specimens encountered in the Cove. However, preferential selection of certain materials or grain sizes probably indicates underlying genetic differences, which are important in understanding the biomechanics of test formation in agglutinating foraminiferans. We have shown that molecular techniques are useful in determining whether a given character is a genuine and reliable taxonomic marker, as we demonstrated here for psammosphaerid A104. A formal taxonomic description of this new species will appear elsewhere.

Comparisons between molecular and morphological data derived from size-fractionated sediments can also help determine how much of a particular molecular signal is due to small, hard-to-assign juveniles, as compared to the larger, morphologically distinctive adults. It has recently been demonstrated that foraminiferans can remain in sediment for several months as propagules and/or juveniles before becoming large enough to detect using standard morphological methods (Alve and Goldstein 2002). The techniques we described can be used to identify these propagules before they become apparent. This method will also help clarify the life cycles of many species of foraminiferans that have yet to be cultured in the laboratory.

Studies of protistan ecology have been hampered by difficulties in isolating and identifying all of the organisms in a community, even in the case of relatively large and conspicuous protists, such as foraminiferans. We have shown that molecular techniques are powerful tools for identifying previously undescribed foraminiferans directly from sediment. This approach has the advantage of greatly reducing selection bias in harvesting and identification (Caron et al. 1999). In addition, because assignment to a specific taxon is accomplished by molecular criteria, morphologically unusual members of a group will not be rejected. This feature of the method is highly relevant to studies of systematics as well as the ecological studies addressed here. Species-specific oligonucleotides can also be coupled to a fluorophore or radioisotope and used as a probe for in situ hybridization (Wray et al. 1995). By this method, a DNA sequence can be used to identify a morphological type of foraminiferan that corresponds to a particular molecular type. We expect that these methods will greatly expand the known diversity of basal foraminiferans.

ACKNOWLEDGMENTS

This manuscript is dedicated to the memory of our late colleague, T. Moran, who managed the Wadsworth Center's Molecular Genetics Core Facility; his service, and the Core, are gratefully acknowledged. We thank D. Rosen for helpful discussions and comments on the manuscript, C. English for technical assistance, and S. P. Alexander, J. M. Bernhard, D. Coons, P. Forte, G. Gwardschaladse, N.W. Pollock, and J. Tyson for field assistance. This work was supported by grants from the Swiss National Science Foundation (31-964073.00 awarded to J.P.) and the National Science Foundation (OPP-9725830 and OPP-0003639 awarded to S.S.B.).

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Received 06/13/03; accepted 10/30/03