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## High diversity of deep-sea *Gromia* from the Arabian Sea revealed by small subunit rDNA sequence analysis

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**Abstract** *Gromia* is a large marine protist with filose pseudopodia and ovoid test, common in coastal intertidal and sublittoral waters. Although deep-water *Gromia*-like morphospecies were discovered in the 1990s, their relations to the shallow water species have never been established. Moreover, very little is known about the diversity within *Gromia*, reflecting the fact that these morphologically relatively simple protists have few characters useful for species identification. Consequently, we have analysed the SSU rDNA and ITS rDNA genes to examine gromiid diversity in two different areas located on the Oman and Pakistan margins of the Arabian Sea (water depths 1,000–2,000 m). In total, 27 deep-sea gromiid sequences of the SSU rDNA gene and six sequences of the ITS rDNA region were obtained. Our data confirm that *Gromia*-like protists from the bathyal deep sea are related to the shallow-water gromiids. Within Arabian Sea *Gromia*, we identified seven distinctive lineages, five of which form a monophyletic group branching as a sister group to shallow-water species. Six lineages of Arabian Sea *Gromia* can be defined morphologically, while one lineage includes specimens that look identical to specimens from two other lineages. This indicates that each *Gromia* lineage represents probably a separate species and suggests that deep-sea gromiid diversity is higher than indicated by their simple morphology.

**Abbreviations** bp: (Base pair) · SSU: rDNA (small subunit ribosomal DNA) · LSU: rDNA (large subunit ribosomal DNA) · ML: (Maximum likelihood) · NJ: (Neighbour-joining)

### Introduction

Gromiids are large benthic protozoa with filose pseudopodia and an organic, proteinaceous test (Hedley 1960), which includes an inner layer of “honeycomb membranes”, a feature unique to this genus (Hedley and Bertaud 1962). One species, *Gromia oviformis*, is the best-known representative of this group. It is a cosmopolitan species that inhabits coastal intertidal and sublittoral waters and is found on the weed of coralline pools, on *Cladophora*, on the walls of rock crevices, undersurfaces of stones, holdfasts of kelp and the surface layer of sandy and muddy sediments (Jepps 1926; Hedley 1958; Hedley and Bertaud 1962; Arnold 1972, 1982; Bowser et al. 1996). Although this group is well known from shallow water, it was unknown in the deep sea until the first species was discovered at bathyal depths (between 1,200 and 1,700 m water depth) on the Oman margin of the Arabian Sea (Gooday et al. 2000). This species, characterized by a spherical test and multiple apertures, was identified as a gromiid on the basis of its wall structure and described as *G. sphaerica* (Gooday et al. 2000). Since then, a variety of undescribed gromiid-like protists have been found at other localities on the Oman and Pakistan margins. One of these, a small species that typically lives on elevated substrates, has recently been described as *G. pyriformis* on the basis of morphological criteria (Gooday and Bowser 2005). An elongate object with attached foraminifera collected from the Santa Catalina Basin of California, identified by Jumars (1976) as a “faecal pellet”, closely resembles one of the elongate gromiid morphotypes found in the Arabian Sea. This one probable record suggests that gromiids may be quite common in bathyal organically enriched, mildly dysoxic

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environments. Moreover, *Gromia*-like species have been frequently observed in bathyal polar waters (Pawlowski et al. 2005; Gooday et al. 2005) but their relationship to shallow water *G. oviformis* have not been established.

The taxonomic position of *Gromia* has been debated for many years. Nineteenth century authors confused *G. oviformis* with a foraminiferan *Allogromia ovoidea* (Hedley 1958). Rhumbler (1904) was the first to show that gromiids were distinct from allogromiid foraminifera in having filose rather than reticulose pseudopodia. He therefore assigned them to the Filosea, a group of testate amoebae with filose pseudopodia, an assignment followed later by Bovee (1985). Patterson et al. (2000) regarded *Gromia* as an “amoeba of uncertain affinities”. The first molecular data available for *Gromia* was based on a partial sequence of the gene coding for the LSU rDNA; this showed that *Gromia* branches within the eukaryotic “crown” (Pawlowski et al. 1994). More recently, Burki et al. (2002), based on the SSU rDNA gene sequences, showed that *Gromia* is closely related to the Cercozoa, a group of various amoeboid and flagellated protists, defined solely on the basis of molecular criteria (Bhattacharya et al. 1995; Cavalier-Smith and Chao 1997). As defined by Cavalier-Smith and Chao (2003), in addition to gromiids, the Cercozoa include cercomonads, chlorarachniophytes, euglyphids, plasmodiophorids, and haplosporids. The recent studies, based on SSU rDNA (Berney and Pawlowski 2003; Nikolaev et al. 2003) and protein sequences (Longet et al. 2003, 2004), place *Gromia* as a sister group to the Foraminifera and/or Haplosporidia, in the newly created supergroup Rhizaria (Nikolaev et al. 2004).

The studies reviewed above were based on shallow-water *G. oviformis*. Here, we report the first molecular data on species of *Gromia* from the bathyal deep sea. Our new molecular results are consistent with earlier morphological and ultrastructural evidence (Gooday et al. 2000) and clearly establish that gromiids inhabit deep-water environments.

## Materials and methods

### Study area

The Arabian Sea is characterised by one of the most extensive oxygen minimum zones (OMZ—defined as zones where bottom-water oxygen concentrations fall below 0.5 ml/l) in the world (Helly and Levin 2004). On both the Oman and Pakistan margins of the Arabian Sea, the OMZ intersects the seafloor between about 200 and 1,000 m water depth. The Oman margin is strongly dissected by submarine canyons while the Pakistan margin has a much smoother slope. Conductivity temperature depth (CTD) profiles indicate that water temperatures range from 26°C at the surface to 2°C near the seafloor at 3,000 m water depth on both margins. On the Oman margin, sediments where gromiids were found were muddy at 1,000 m and consisted of *Globigerina*

sand at 1,250 m (Levin et al. 1997). On the Pakistan margin, at 1,200 m water depth, sediments showed three consecutive layers: a top thick phytodetritus layer, followed by a brown layer with numerous fine burrows on top of a third layer of compacted sediment. At 1,850 m water depth, light grey clays were covered by orange/brown flocculent material. In general, both margins were organically enriched, in particular at the sediment surface between 1,000 and 2,000 m water depth, with high nutrient concentrations of phosphate, silicate and nitrate (Bett 2003).

### Sampling

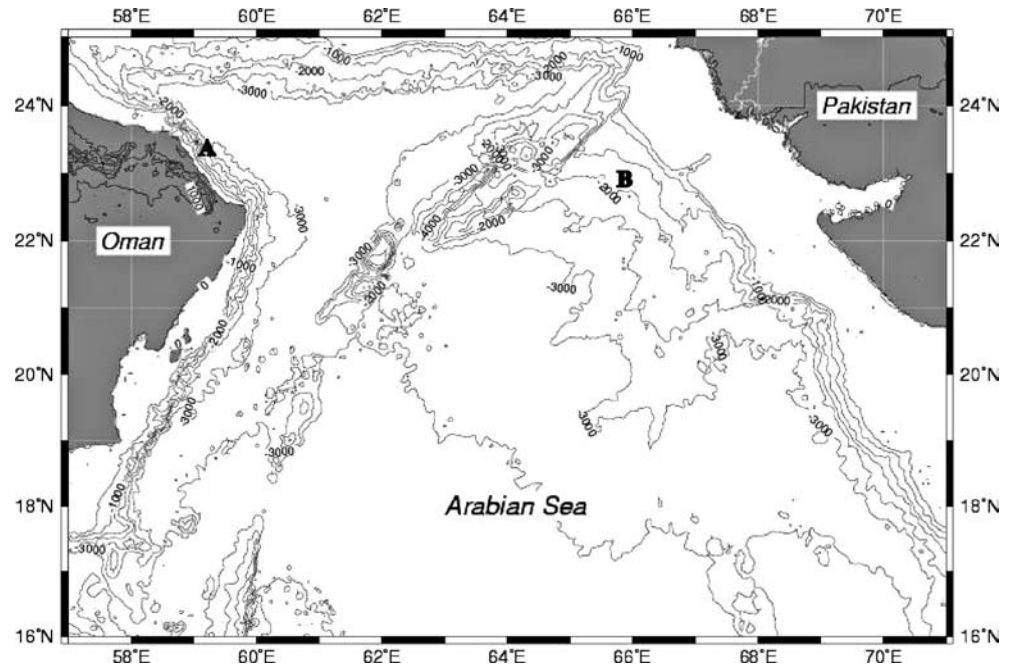
Gromiid specimens were collected at depths below the OMZ (~1,000–2,000 m) on both the Oman and Pakistan margins during a series of cruises in 2002 and 2003 by the British research ship R.R.S *Charles Darwin* (CD). They were obtained using either a megacorer equipped with 100 mm id plastic core tubes, or a multicorer equipped with 57 mm id core tubes (Barnett et al. 1984). Both devices obtain samples in which the sediment-water interface is virtually undisturbed, maintaining gromiids and other organisms in their life positions. At some depths, numerous gromiids were also collected using an Agassiz Trawl. Off Oman, the samples were obtained during CD cruise 143 in December 2002 (Fig. 1). On the Pakistan margin, samples were collected during CD cruises 145 (March–April 2003), 146 (April–May 2003), 150 (August–September 2003) and 151 (September–October 2003) (Fig. 1). Station details are summarised in Table 1.

As soon as possible after recovery, samples were taken to a shipboard temperature-controlled laboratory adjusted to the appropriate bottom water temperature. All gromiids found on the core surfaces were removed using a plastic pipette or forceps and frozen at either –20°C or –80°C for molecular analysis. Cores were then sliced into 1 cm thick layers down to 3 cm sediment depth. The sediment slices were examined for additional gromiids under a binocular microscope with ice packs used to keep the samples cool. All gromiids found were frozen as above.

### DNA extraction, amplification, cloning and sequencing

Frozen gromiids were first photographed (Fig. 2) and then cut into four to eight pieces, which were extracted one by one using the DNeasy Plant Mini Kit (Qiagen, Basel, Switzerland). Fragmentation of *Gromia* cells was necessary to avoid the inhibition of PCR amplifications, which occurred regularly when complete cells were extracted. As the amplifications were successful for only a few extracts from each cell, we can speculate that the majority of examined *Gromia* contained only one nucleus. However, because of a very dense cytoplasm,

**Fig. 1** Map showing cruise sampling locations on the Oman margin (A) and Pakistan margins (B)



**Table 1** Details of stations on the Oman and Pakistan margins of the Arabian Sea where gromiids were collected from molecular genetic analysis

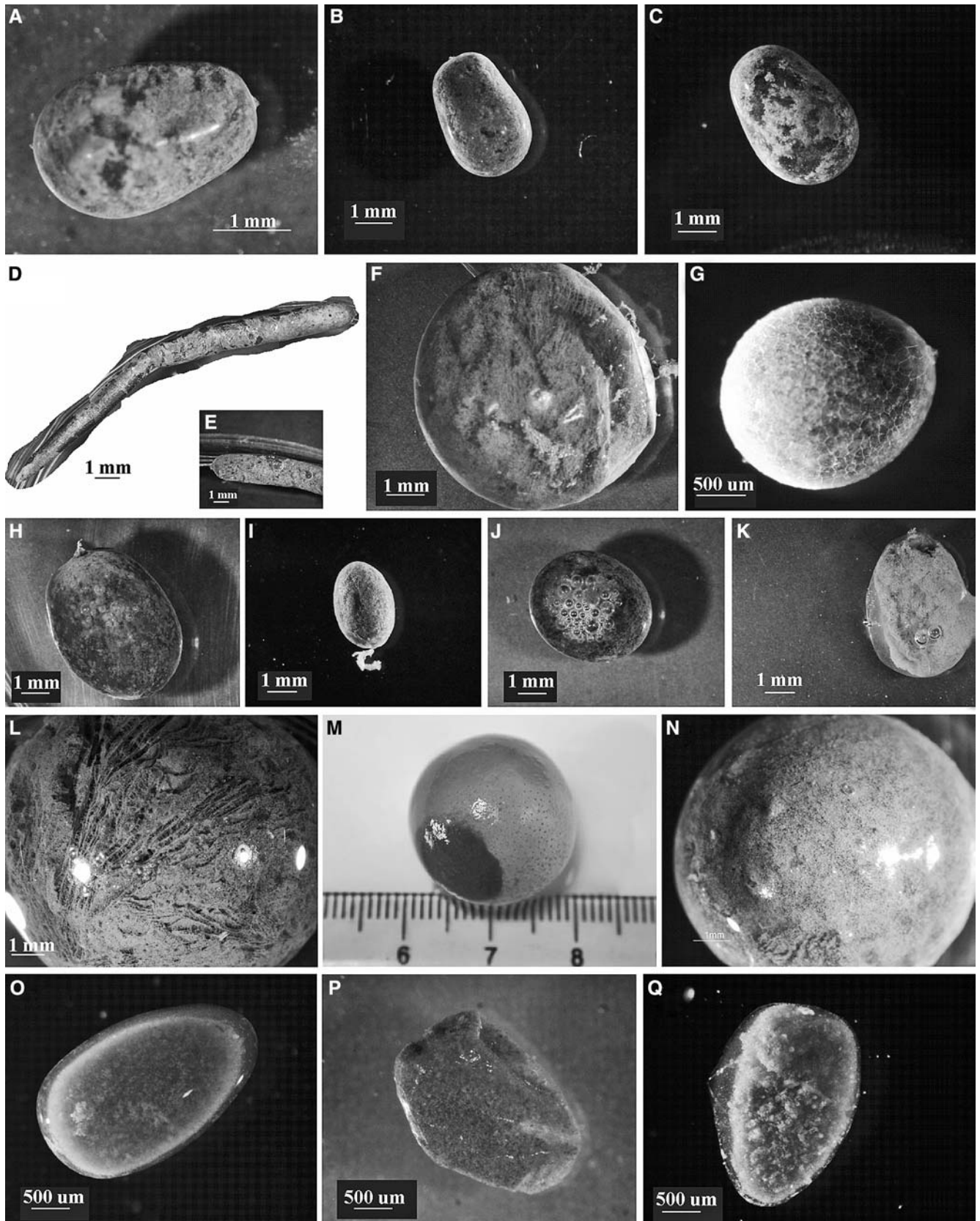
Station and series	Latitude	Longitude	Water depth (m)	Specimens
Oman				
55710#1	23°21.83'N	59°06.03'E	1,422	1, 2, 3, 4, 15 and 17
55725#2	23°20.31'N	59°09.85'E	2,010	11
55759#1	23°23.37'N	59°03.67'E	1,260	8
55767#1	23°22.10'N	59°05.60'E	1,390	16
Pakistan				
55910#3	22°52.24'N	66°00.09'E	1,875	5, 6, 12 and 13
56137#12	22°52.39'N	66°00.00'E	1,852	7, 9 and 14
56140#2	22°52.42'N	66°59.97'E	1,859	10

the observation of *Gromia* nuclei would require sectioning of specimens, which was not possible with the material examined here.

PCR reactions were performed in a total volume of 52 µl including 2 µl of DNA extract. The PCR amplification profile consisted of 40 cycles, with 30 s at 94°C, 30 s at 50°C and 2 min at 72°C, followed by 5 min at 72°C. The first PCR was followed by nested PCR, the profile of which consisted of 25 cycles, with 30 s at 94°C, 30 s at 52°C and 2 min at 72°C, followed by 5 min at 72°C for the final extension. The re-amplified PCR products were then purified using High Pure PCR purification kit (Roche, Rotkreuz, Switzerland). Whenever possible, products were sequenced directly using the Big Dye Terminator Cycle sequencing Kit and analysed with an ABI-3100 DNA sequencer (Applied Biosystems, Rotkreuz, Switzerland). When direct sequencing failed, purified PCR products were ligated into pGEM Vector system (Promega, Wallisellen, Switzerland), cloned in XL-2 Ultracompetent Cells (Stratagene, Basel, Switzerland) and then sequenced as above, all according to the manufacturer's instructions.

First, a short fragment of the SSU rDNA (~700 bp) was amplified for all possible specimens using the

universal primer pair S12.2 (5'GAT YAG ATA CCG TCG TAG TC 3') and SB (5'TGA TCC TTC TGC AGG TTC ACC TAC 3'). Then, the PCR products were re-amplified using *Gromia* specific primer S13r\_Gr (5'CTG TGG ATA GGA CTC GYT CCAG 3') and universal primer SB. A longer fragment of the SSU rDNA was amplified for selected species, using *Gromia* specific primer S6Gr (5'GGG CAA GTC TGG TGC 3') and SB, and re-amplified using combination of primers: S6Gr and GRSSU1 (5' TCC AAA GTT TTC ACC GGA TC 3'). The amplification of ITS fragment was performed for selected isolates, using primers S13r\_Gr and GRLSU1 (5'TGA CAT CAC ATT CCA ATG AA 3') for the first PCR and primers S20Gr\_F: (5' CTA CCG ATG GAA CGA TCC 3') and GRLSU1 for the re-amplification. Specific *Gromia* primers S13r\_Gr, S6Gr, GRSSU1, GRLSU1, and S20Gr\_F were designed by Burki et al. (2002). Using specific primers was necessary because *Gromia* isolates contained DNA of some other eukaryotes (for example, organisms related to *Crythecomonas longipes*, *Labyrinthuloides haliotidis*, *Gymnophrys cometa*, *Trinema enchelys*, *Massisteria marina* and uncultured eukaryote isolates C1\_E023, C2\_E025, C5\_E006, E170 and clones Bola471 and



**Fig. 2** Light photographs of extracted specimens for phylogenetic analysis. Specimens [DNA number]: A 17 [4319, 4320], B 16 [4433], C 15 [4390], D 5 [4331], E 6 [4440], F 10 [4421], G 4 [4399], H 12 [4338], I 11 [4363, 4364 and 4366], J 13 [4333], K 14 [4298], L 9 [4353, 4357, 4358], M 8 [4375], N 7 [4466], O 2 [4396], P 3 [4411] and Q 1 [4385, 4386]

Sey017), as indicated by BLAST comparison of sequenced PCR products obtained with universal primers.

### Phylogenetic analyses

Evolutionary trees were inferred using the neighbour-joining (NJ) (Saitou and Nei 1987) and maximum likelihood (ML) (Felsenstein 1981) methods. *Gromia* SSU rDNA sequences and their eukaryotic homologs were aligned using Clustal X (Thompson et al. 1994), and further adjusted by eye with Seaview (Galtier et al. 1996). To infer the phylogenetic position of *Gromia* among eukaryotes, we used an alignment of 32 SSU rDNA sequences (comprising 8 *Gromia* sequences) and 799 unambiguously aligned sites. The alignment of *Gromia* sequences consisted of 27 sequences and 445 sites. We used PHYLO\_WIN program to obtain the NJ trees, with distances corrected using K2, HKY and LogDet models (Galtier et al. 1996). The maximum likelihood approach was achieved with PhyML v2.1b1, using GTR model (Guindon and Gascuel 2003). The proportion of invariable sites and the shape of the gamma distribution were adjusted to maximize the likelihood of the phylogeny. The reliability of internal branches was assessed using the bootstrap method (Felsenstein 1985) with 1,000 bootstrap replicates for NJ tree and 100 bootstrap replicates for ML trees.

## Results and discussion

### Monophyly of Gromiida

We obtained the sequences of about 2/3 of the whole length of the SSU rDNA for three different isolates, representing *G. sphaerica* [4466] from Pakistan, *Gromia* sp. 6 [4319] from Oman and *Gromia* sp. 3 [4440] from Pakistan. The length of the sequences ranged from 1,215 to 1,286 and the base composition was 46.73–47.42% GC. These sequences were compared to five previously published *Gromia* sequences (Burki et al. 2002), 11 sequences of Cercozoa, 3 plasmodiophorids, 4 haplosporidians and 7 Foraminifera (Table 2).

The ML and NJ analyses give congruent results showing that all *Gromia* sequences form a monophyletic group, branching between Plasmodiophora and Haplosporidia (Fig. 3). The monophyly of Gromiida is supported by 100% bootstrap values in both analyses. The genetic divergence between *Gromia* sequences is remarkably low and ranges from 0.6 to 6.2%, with mean value of 3.06%. This low level of genetic variations corresponds in some sense to the morphological homogeneity of the genus, which is composed mainly of similar large grape or sausage-shaped morphotypes. However, it is surprising to find such low sequence divergence given the rather broad ecological and geographical settings from which the examined *Gromia* were

**Table 2** List of previously published sequences used in our analysis

Taxonomic position	Species name	Accession number
Gromiida	<i>Gromia oviformis</i> (Madeira)	AJ457811
	<i>G. oviformis</i> (Reunion)	AJ457812
	<i>G. oviformis</i> (McMurdo)	AJ457813
	<i>G. oviformis</i> (Guam)	AJ457814
	<i>G. oviformis</i> (Tunisia)	AJ457815
Cercozoa	<i>Euglypha rotunda</i>	X77692
	<i>Paulinella chromatophora</i>	X81811
	<i>Cercomonas</i> sp.	U42448
	<i>Thaumatomonas</i> sp.	U42446
	<i>Heteromita globosa</i>	U42447
	<i>Pseudodiffugia</i> sp.	AJ418794
	<i>Lecythium</i>	AJ514867
	<i>Nuclearia</i> -like	AF289081
	<i>Gymnophrys cometa</i>	AJ514866
	<i>Massisteria marina</i>	AF174369
	<i>Chlorarachnion reptans</i>	X70809
Plasmodiophora	<i>Plasmodiophora brassicae</i>	U18981
	<i>Spongopora subterranea</i>	AF310899
	<i>Phagomyxa odontellae</i>	AF310904
Haplosporidia	<i>Bonamia ostreae</i>	AF262995
	<i>Minchinia teredinis</i>	U20319
	<i>Haplosporidium nelsoni</i>	X74131
Foraminifera	<i>Astrammia rara</i>	AJ318223
	<i>Allogromia</i> sp.	X86093
	<i>Nummulites venosus</i>	AJ318226
	<i>Trochammina</i> sp.	X86095
	<i>Syringammina corbicula</i>	AJ514856
	<i>Cribrothalammina alba</i>	AJ318225
	<i>Reticulomyxa filosa</i>	AJ132367

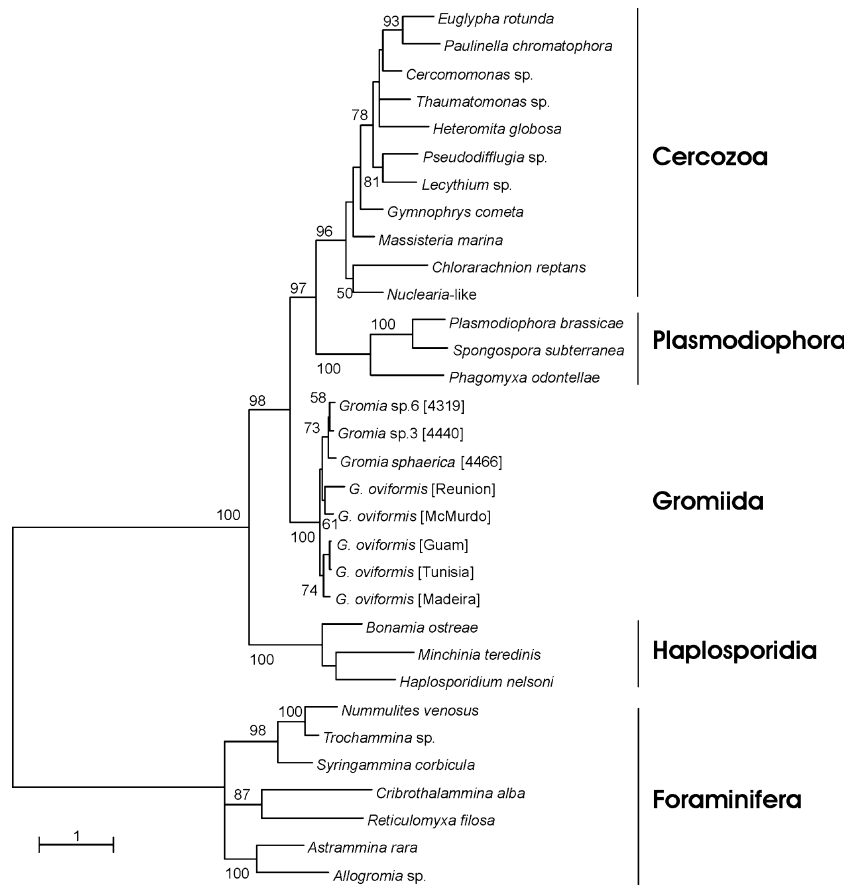
obtained. This result could suggest that the Gromiida is a relatively recently evolving group, which was very successful in colonizing practically all marine habitats. Alternatively, it is possible that *Gromia* is characterized by unusually slow rates of SSU rDNA evolution. This idea can be examined by further testing of their protein coding genes.

The phylogenetic position of Gromiida, branching between Haplosporidia and Plasmodiophora, differs slightly from the initial study of *Gromia* (Burki et al. 2002) in which Haplosporidia were not included. It differs also from some analyses of Cercozoa phylogeny (Cavalier-Smith and Chao 2003), in which *Gromia* branches within the clade formed by Plasmodiophora and Haplosporidia. However, given that our analyses are based on partial SSU rDNA sequences, with relatively limited taxon sampling, the phylogenetic position of Gromiida cannot be firmly established and we cannot exclude the possibility that *Gromia* is a sister group to Foraminifera or Haplosporidia as indicated in some other studies (Berney and Pawlowski 2003; Longet et al. 2003, 2004).

### Molecular and morphological diversity of Arabian Sea *Gromia*

A fragment of the SSU rDNA of the length averaging 760 nucleotides was sequenced for 23 *Gromia* isolates

**Fig. 3** Phylogenetic position of *Gromia* among Rhizaria inferred from partial SSU rDNA sequences, using the maximum likelihood method. The numbers at the nodes represent percentage of bootstrap support greater than 50% following ML and NJ data re-sampling



from Arabian Sea. The phylogenetic analysis of these sequences revealed seven distinctive clades or lineages (Fig. 4). One of them (clade D) corresponds to previously described species *G. sphaerica* (Gooday et al. 2000). Five other clades can be defined morphologically on the basis of their test morphology and wall structure (Aranda da Silva, Gooday and Bowser, unpublished data). Molecular and morphological descriptions of each clade are given below.

Clade A is composed of four sequences obtained from grape-shaped gromiids from Oman (Fig. 4). Their tests are very similar to shallow-water *G. oviformis* except that they have a thin translucent organic wall (Fig. 2). The four sequences are almost identical and form a clade supported by 100% bootstrap values. The position of this clade at the base of the tree and its distance from all other gromiids, strongly suggests that this is a distinctive species, called here *Gromia* sp. 1.

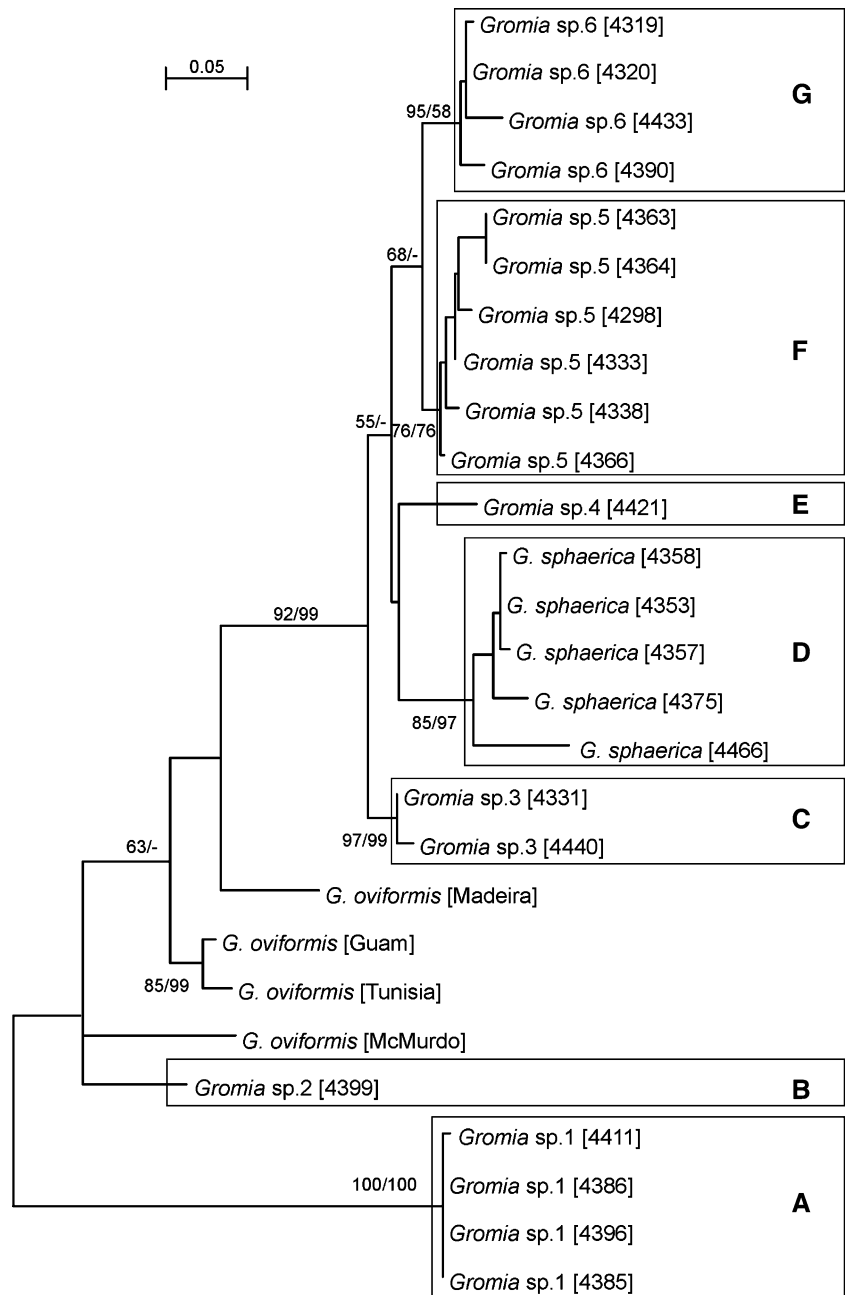
Clade B is represented in our tree by a single sequence of *Gromia* from Oman, but more partial sequences are available (data not shown). At first sight, this species, designated here as *Gromia* sp. 2, closely resembles *Gromia* sp. 1. However, the aperture end of the test is more pointed and a honeycomb-shaped structure (not to be confused with the honeycomb membrane) is visible under a binocular microscope within the thickness of the wall. This structure may confer some rigidity to the test, perhaps as a defence against predators or possible

adverse environmental conditions. According to our analyses, *Gromia* sp. 2 branches independently between *Gromia* sp. 1 and the shallow water *G. oviformis*. It seems weakly related to *Gromia* from coastal waters in McMurdo Sound, Antarctica. The position of *Gromia* sp. 2 close to the shallow-water gromiids may suggest that this species colonized the bathyal deep sea independently from other Arabian Sea gromiids.

Clade C is represented by a sausage-shaped form with a thick and opaque wall. This elongate morphotype, which we designate here as *Gromia* sp. 3, is unique among Arabian Sea gromiids. Similar but smaller morphotypes have been observed in fjords in Western Svalbard (Arctic Ocean) (Gooday et al. 2005) and in the samples from under the Ross Ice Shelf (Pawlowski et al. 2005), but none of these species has been sequenced yet. Our study is therefore the first report of molecular data from an elongate gromiid morphotype. Its position as a sister group to the grape-shaped and spherical species is not stable and in some NJ analyses, we found it branching between clades F and G. However, in all analyses, the clades C to G together form a strongly supported (92–99% bootstrap values) group, composed exclusively of Arabian Sea gromiids.

Clade D corresponds to *G. sphaerica*, a large protozoan (average test diameter of 15 mm) with characteristic multiple test apertures. This species has been described previously from the Oman margin (Gooday

**Fig. 4** Maximum likelihood tree of Arabian Sea gromiids based on partial SSU rDNA sequences. The analysis includes 23 deep sea *Gromia* and four shallow water *Gromia* (from Burki et al. 2002). The numbers at the nodes represent percentage of bootstrap support greater than 50% following ML and NJ data re-sampling



et al. 2000). Here we report it for the first time from the Pakistan margin. The sequence divergence within this clade ranges from 0.2 to 0.8% between sequences 4,358, 4,353, 4,357 and 4,375, with one fast evolving sequence 4,466, which differs from others by 5.7%. Almost all differences are in the SSU region V7 following the nomenclature of Neefs et al. (1993). Among five sequences representing this species, four originate from Pakistan and one originates from Oman. However, the Oman sequence (4,375) is almost identical to two of the Pakistan sequences (4,353, 4,357), suggesting that there are no barriers between the two regions (see below).

Clade E is represented by a single sequence obtained from small (average test diameter 2.6 mm), spherical

specimens, which we designated as *Gromia* sp. 4. This species resembles *G. sphaerica*, in being characterized by the perfect spherical shape; the main differences are that *G. sphaerica* has a large test with multiple apertures while *Gromia* sp. 4 is smaller and with a single aperture. We were able to obtain SSU rDNA sequence from only one specimen, which forms a sister group to *G. sphaerica*, but its position is not supported.

Clade F is the most diverse group and contains six grape-shaped forms from the Oman and Pakistan margins. In contrast to all other clades, this group cannot be defined by any specific morphological features. The test wall is either thin and translucent or thick and opaque and the test morphologies span those of the other grape-

shaped species described here. We consider it as a separate species, *Gromia* sp. 5, because the clade is relatively well supported (76% bootstrap values) and the divergence between six sequences is relatively low.

Clade G is represented by a grape-shaped gromiid similar to *Gromia* sp. 1 but with a much thicker wall. This species, called here *Gromia* sp. 6, is morphologically distinctive. Its sequences form a strongly supported clade in ML analysis (95% bootstrap values), but it is supported only by 58% in NJ analysis, probably due to the presence of the fast evolving sequence 4433, which differs from others by 1.5–2.0%.

Prior to this study, three gromiid morphotypes were described, the oval- or spherical-shaped *G. oviformis* (and some other related shallow-water species), the spherical *G. sphaerica*, and the pear-shaped *G. pyriformis*. Excluding *G. pyriformis*, for which molecular data are not available, the current study has expanded the number of deep-sea gromiid species to at least seven, comprising at least five basic morphotypes. Within *Gromia*, we identified seven clades, six of which (A, B, C, D, F, and G) are well defined morphologically. The spherical (clades C and D) and sausage-shaped (clade F) gromiids are relatively well-defined on molecular criteria and have good morphological support. On the other hand, grape-like species are more difficult to distinguish morphologically and there is some confusion between specimens belonging to clades A, E and G. Indeed, the clade E is defined purely on molecular characteristics and includes specimens that look identical to individuals from clades A, and G. The presence of clade E indicates the likely existence of cryptic species and suggests that species diversity is higher than indicated by test morphology. Nevertheless, the fact that most of the molecular clades correspond to test morphotypes gives our molecular results credibility.

#### Biogeography of Arabian Sea *Gromia*

Gromiids occurred in core and trawl samples from the Oman and Pakistan margins of the Arabian Sea. They were more abundant in the Oman margin (Aranda da Silva and Gooday, unpublished data) than on the Pakistan margin. Figure 4 shows seven clades, representing at least seven groups of Arabian Sea gromiids. Three of these seven clades (A, B, and G) were confined to the Oman margin; two (clades E and C) were only found in the Pakistan margin and two (clades D and F) occurred on both margins. However, although an extensive collection of material was obtained for molecular work, not all the specimens could be sequenced and therefore the data are incomplete. For example, we were unable to obtain sequences from morphological representatives of clades E and C on the Oman margin. Although, gromiid abundance is higher on the Oman than on the Pakistan margin, there is no evidence that diversity (species numbers) is different between the two margins.

In order to compare species that are common to both margins, a fragment of the ITS rDNA, the most variable region of rDNA genes, was amplified for *Gromia* sp. 5 from the Oman and Pakistan margins. We cloned *Gromia* sp. 5 isolates 4,364 from Pakistan and 4,338 from Oman and we obtained sequences from at least three clones from each of the PCR products. The length of the sequences varied between 1,056 and 1,080 nucleotides and the base composition was 40.96% GC. All sequences were similar (< 1.2%), showing few differences between and within the clones from the two specimens. We did not find a clear difference between specimens from the opposite margins, suggesting that there is probably a gene flow between the two regions. However, this should be repeated with a larger number of specimens from different clades in order to ensure that this result is not confined to one gromiid species. If the lack of difference between Oman and Pakistan populations is confirmed, we can predict that the same gromiids will be found along the Markan margin, which forms the northern border of the Arabian Sea between Oman and Pakistan.

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#### Conclusions

The current study has enhanced considerably our knowledge of gromiids in general and deep-sea gromiids in particular. Our study confirmed that the shallow and deep-water gromiids are closely related and form a monophyletic group within the Rhizaria. As shown by our analyses, several species of *Gromia* inhabit bathyal depths on the Oman and Pakistan margins. The Oman margin assemblages are more abundant, but not necessarily more diverse than those from the Pakistan margin. Some *Gromia* species inhabit both margins. To a large extent, the diversity of the Arabian Sea gromiids is expressed in test morphology. However, our results also reveal evidence for cryptic speciation, suggesting morphology alone will underestimate species numbers, and that molecular data is essential when evaluating diversity within this group.

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