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Bowseria arctowskii gen. et sp. nov., new monothalamous foraminiferan from the Southern Ocean

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Abstract: *Bowseria arctowskii* gen. et sp. nov., a new organic-walled monothalamous (single-chamber) foraminiferan is described from samples collected in Admiralty Bay (King George Island, West Antarctica) at 100–200 m water-depth (mwd). The species is characterized by a large (1–2 mm) elongate theca with a single terminal aperture. Molecular phylogenetic analyses, based on partial small subunit rDNA sequences, indicate that the new species belong to a clade of single-chambered foraminifers that branch as a sister group to the multi-chambered textulariids and rotaliids. The most closely related to the new species is an undetermined allogromiid from under the Ross Ice Shelf.

Key words: Antarctica, South Shetlands, Foraminifera, Recent.

Introduction

The monothalamous foraminiferans constitute a highly diverse group of early evolved, single chambered species, having organic or agglutinated test (theca). In the traditional morphology-based classification, the organic-walled and agglutinated monothalamids were included in the orders Allogromiida and Astrorhizida, respectively (Loeblich and Tappan 1987; Sen Gupta 1999). However, the distinction of both orders was seriously invalidated by molecular phylogenetic studies, which show that both types of the wall can be found in closely related species (Pawlowski *et al*. 2002c, 2003).

Although the monothalamous foraminifers are an important component of high-latitude and deep-sea meiobenthic communities, their diversity remains largely unknown (Gooday 2002). The monothalamid species are rarely included in the description of modern or fossil foraminiferal assemblages because their theca or tests are poorly preserved in routinely dried sediment samples. Moreover, the species identification and taxonomy in this group are hindered by the simple morphology of their tests and limited number of distinctive morphological features.

Recently, the monothalamous foraminifers have been object of several studies focusing particularly on the diversity of polar species. A long-term survey of foraminiferal fauna from Explorers Cove (McMurdo Sound, Ross Sea) provided a unique insight into the diversity of Antarctic monothalamous foraminifers based on morphological study (Gooday *et al.* 1996), molecular analyses of isolated specimens (Pawlowski *et al.* 2002a, b) and a survey of environmental DNA samples (Habura *et al.* 2004). Several new monothalamous species have been described from this area (DeLaca *et al*. 2002; Bowser *et al.* 2002; Sabbatini *et al.* 2004). Many of these species resemble morphologically the monothalamids from the Arctic Ocean (Gooday *et al.* 2005; Majewski *et al.* 2005). The genetic analyses, however, show clearly that they represent distinctive species (Pawlowski *et al.* in press).

Here, we describe a new monothalamous genus and species from Admiralty Bay, King George Island. The description is based on morphological criteria and molecular phylogenetic data. Similar morphotypes with identical DNA sequences have been observed in samples collected from under the Ross Ice Shelf (Pawlowski *et al.* 2005), suggesting that the species may have a panantarctic distribution.

Material and methods

Sampling sites. — The samples were collected during the authors' stay at the Polish *Arctowski* Station on King George Island, in March–April 2007. The specimens of the new species were found in sea-floor sediment samples from six locations throughout Admiralty Bay (Fig. 1) at depths ranging from 107 to 254 m (Table 1). All samples were collected using a *Van Veen* sampler operated from the M/S *Polar Pioneer*.

Material isolation and fixation. — The specimens were isolated from the upper 2–3 cm of surface sediments. As soon as possible after collection, the sediment was washed with cold sea-water and wet-sieved through 500 and 125 mm sieves. The residues were stored in the refrigerator at about 2°C, for periods of several days. The foraminiferans were sorted under a binocular microscope from the residues kept cool on a tray of ice. Specimens for molecular study were transferred one by one into guanidine or AP1 (DNAEasy, Qiagen) extraction buffer, while those for morphological study were fixed in formalin.

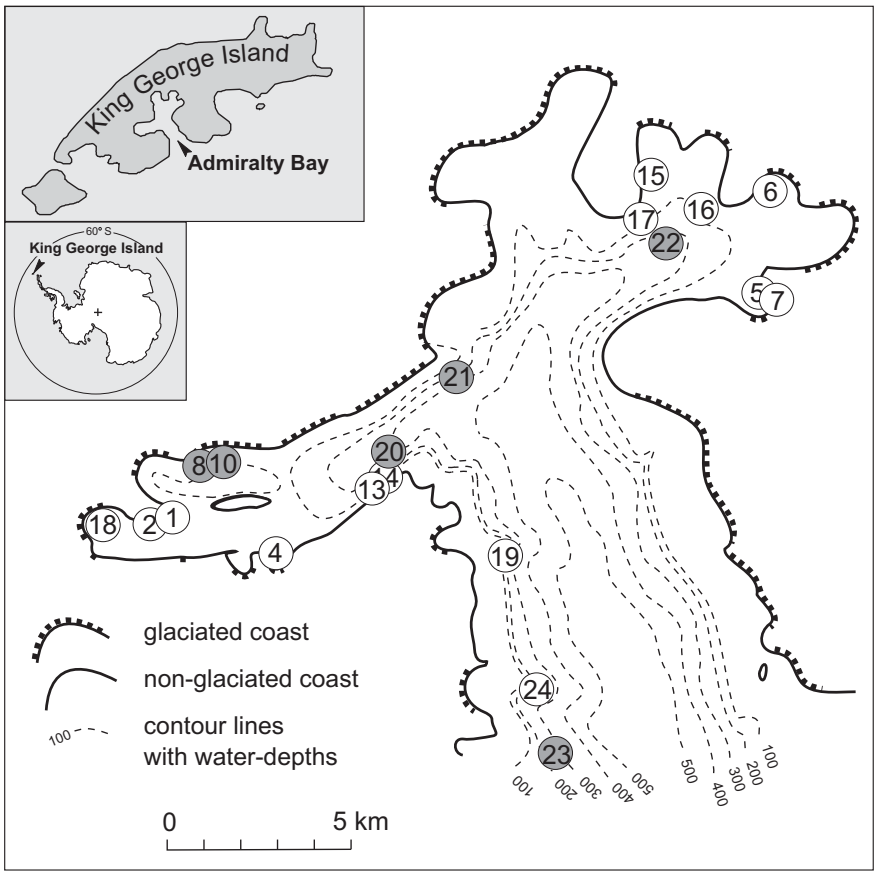

Fig. 1. Location of sampling stations throughout Admiralty Bay, King George Island. The gray circles mark samples bearing *Bowseria arctowskii* gen. et sp. nov. specimens.

Morphological study. — The living specimens were photographed immediately after isolation using a Leitz stereomicroscope with reflected light. The fixed specimens were photographed in the laboratory using an Olympus photomicroscope with transmitted light. The SEM pictures were taken using Zeiss DSM 940 A, after critical point drying of the specimens.

Molecular analysis. — A fragment of the SSU rRNA gene was amplified by PCR with the primer pair s14F3 (5'ACG CA(AC) GTG TGA AAC TTG) and sB (5' TGA TCC TTC TGC AGG TTC ACC TAC), and re-amplified using nested primer s14F1 (5' AAG GGC ACC ACA AGA ACG C). The amplified PCR products were purified using High Pure PCR Purification Kit (Roche Diagnostics) and sequenced directly or after cloning. In the latter case, the purified products were ligated in the Topo Cloning vector (Invitro Gene), and cloned using ultracompetent cells XL2-Blue MRF' (Stratagene). Sequencing reactions were prepared using an ABI-PRISM Big Dye Terminator Cycle Sequencing Kit and analysed with an

Table 1. List of sampling stations where *B. arctowskii* occurred and number of specimens preserved in guanidine (GUA), formaline (FOR) and alcohol (EtOH)

Station	GPS location	Depth (mwd)	Number of specimens		
			GUA	FOR	EtOH
KG8	S 62°09.650' W 58°34.774'	119	1		
KG10	S 62°09.584' W 58°34.287'	107	7	5	
KG20	S 62°09.053' W 58°30.435'	249	2		
KG21	S 62°07.488' W 58°27.545'	254			1
KG22	S 62°05.610' W 58°22.944'	233	1		2
KG23	S 62°13.361' W 58°22.892'	223	1	2	

ABI-377 DNA sequencer or an ABI-PRISM 3100 (Applied Biosystems), all according to the manufacturer's instructions.

Sequences were compared to 49 other foraminiferan sequences and manually aligned using the Seaview software (Galtier *et al.* 1996). Highly variable regions of the alignment were removed, leaving 697 sites for phylogenetic analysis. The phylogenetic tree was constructed using maximum likelihood method with GTR + GI model, using Treefinder (Jobb *et al.* 2004).

Systematics

Following the traditional classification, based on the organic character of the wall (Loeblich and Tappan 1987; Sen Gupta 1999), the new genus should be placed in the order Allogromiida. However, because molecular data suggests that this order is polyphyletic in nature, we prefer not to attribute it to any higher taxonomic rank, waiting until the revised higher-level systematics of foraminifera will be developed (Pawlowski in preparation).

Phylum FORAMINIFERA Cavalier-Smith, 1998

Genus *Bowseria* gen. nov.

Type species: *Bowseria arctowskii* sp. nov.

Etymology: The genus was named in honor of Dr. Sam Bowser (USA), a protistologist and polar explorer, who has spent many years studying Antarctic monothalamous foraminifera and contributed immensely to our knowledge of their biology, ecology and diversity.

Diagnosis: Test free, monothalamous, elongate, tubular, up to 2 mm in length and 0.5 mm in width; single terminal aperture without gromiids-like oral capsule; wall transparent, organic, with smooth outer surface; cytoplasm granular, white or yellow in color, without stercomata (Figs 2, 3).

Remarks: At first sight, the new genus resembles morphologically some undescribed elongate gromiids found in meiobenthic samples from polar regions (Gooday *et al.* 2005). Although gromiids are only distantly related to foraminifers,

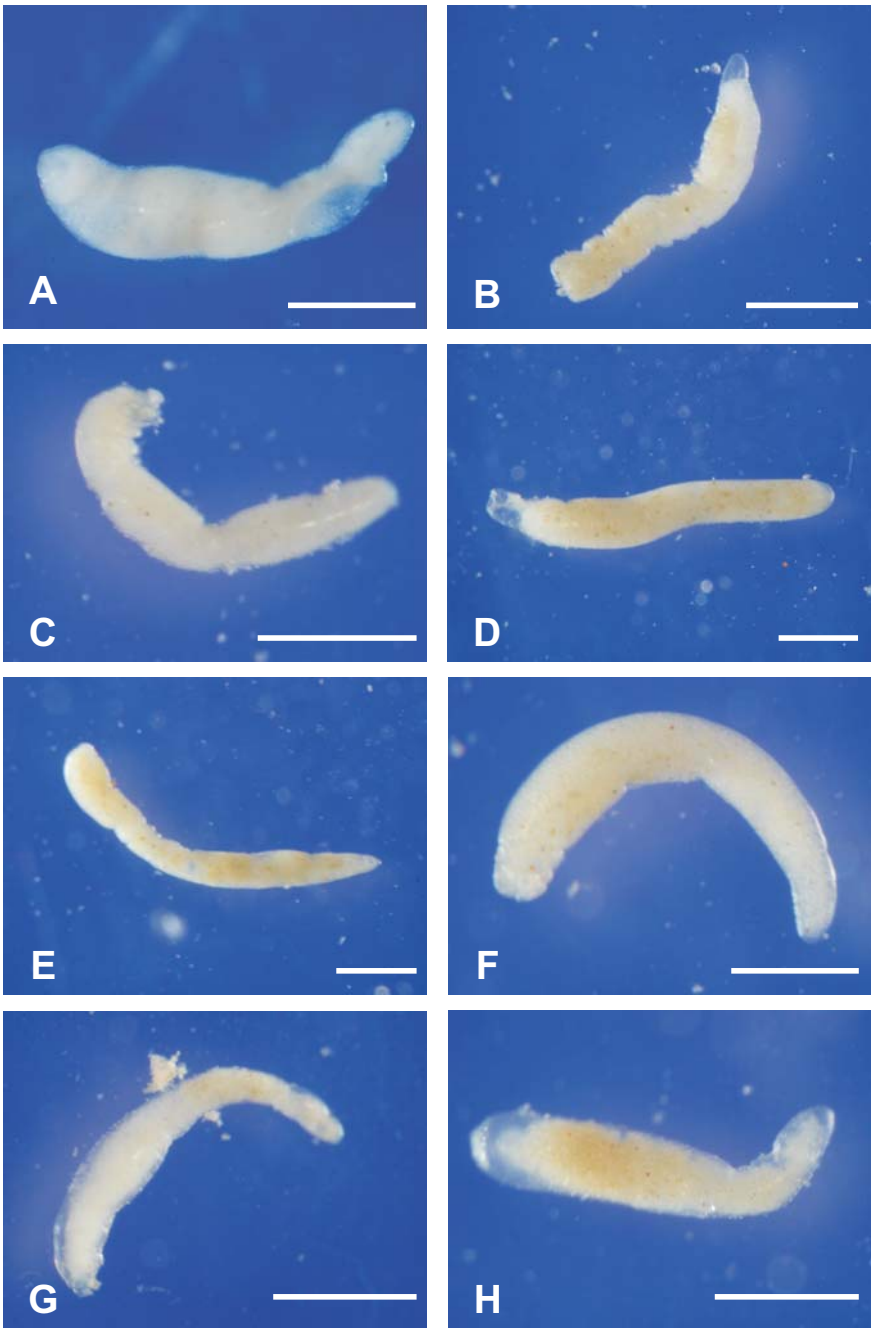

Fig. 2. *Bowseria arctowskii* gen. et sp. nov. Micrographs of holotype (A) and specimens, from which DNA was extracted (B–H). **A**. Holotype, KG 10. **B**. # 7855, KG10. **C**. # 7856, KG10. **D**. # 7864, KG10. **E**. # 7865, KG10. **F**. # 7867, KG10. **G**. # 8241, KG23. **H**. # 8219, KG20. Specimens B–F have been sequenced and included in the phylogenetic tree (Fig. 4). All specimens are illustrated with the proximal (apertural) end at the left side. Scale bar = 0.5 mm.

they share similar morphological characteristics and their distinction is not always easy. Principal features characterizing gromiids, the filose pseudopodia and honeycomb membrane, can hardly be observed under a standard stereomicroscope. The genus *Bowseria* also differs from gromiids in having a very thin, often curved and wrinkled theca, less prominent aperture, finely granular white to yellowish cytoplasm, and the absence of stercomata (Figs 2, 3).

On the other hand, *Bowseria* resembles morphologically the undetermined allogromiid sp. 5 identified by Gooday *et al.* (1996) in the samples from Explorers Cove, Antarctica, and some allogromiids from under Ross Ice Shelf (Pawlowski *et al.* 2005).

Bowseria arctowskii sp. nov.

(Fig. 2A)

Holotype: Figured on Fig. 2A.

Type material: A specimen collected at station KG10 (S 62°09.584', W 58°34.287', 107 mwd) and preserved in formalin was selected as holotype. It is deposited in the Museum of Natural History in Geneva.

Other material examined: A total of 22 additional specimens (12 from the type locality) were either extracted in guanidine, fixed in formalin or preserved in alcohol (Table 1).

Etymology: The species was named in honor of Professor Henryk Arctowski (1871–1958), Polish geologist-geophysicist and oceanographer, scientific leader of the famous *Belgica* Expedition (1897–1899). His name was given to the Polish Antarctic Station, where the authors first investigated the new species.

Diagnosis: As for genus.

Description: The theca is elongate, tubular, often slightly curved and irregularly shaped. The length ranges from 1 to 2 mm, while its width may vary between 0.125 and 0.350 mm in the same specimen. The apertural (proximal) end is usually wider and more broadly rounded compared to the narrower apical (distal) end (Fig. 3). The wall is very thin (~1 μm), transparent, with a distinctively shiny, reflective surface (Fig. 2). The theca is often wrinkled and becomes easily distorted due to shrinkage, particularly in specimens preserved in alcohol.

The aperture is single, terminal, small and rounded. It may project slightly but is sometimes retracted and invisible. It seems to be prolonged by an internal structure but the details were not easily visible in examined specimens (Fig. 3).

The cytoplasm is white to yellowish, dense and fluffy with fine granules. It fills the theca almost entirely, sometimes leaving small empty spaces at both ends (Fig. 2).

Molecular characterization: Seven DNA isolates, each containing a single specimen collected at station KG10 were examined (Fig. 2B–F). PCR amplification of the SSU rDNA fragment produced positive results for all seven isolates. PCR products were sequenced directly with primers S14F1 and S18F. The obtained se-

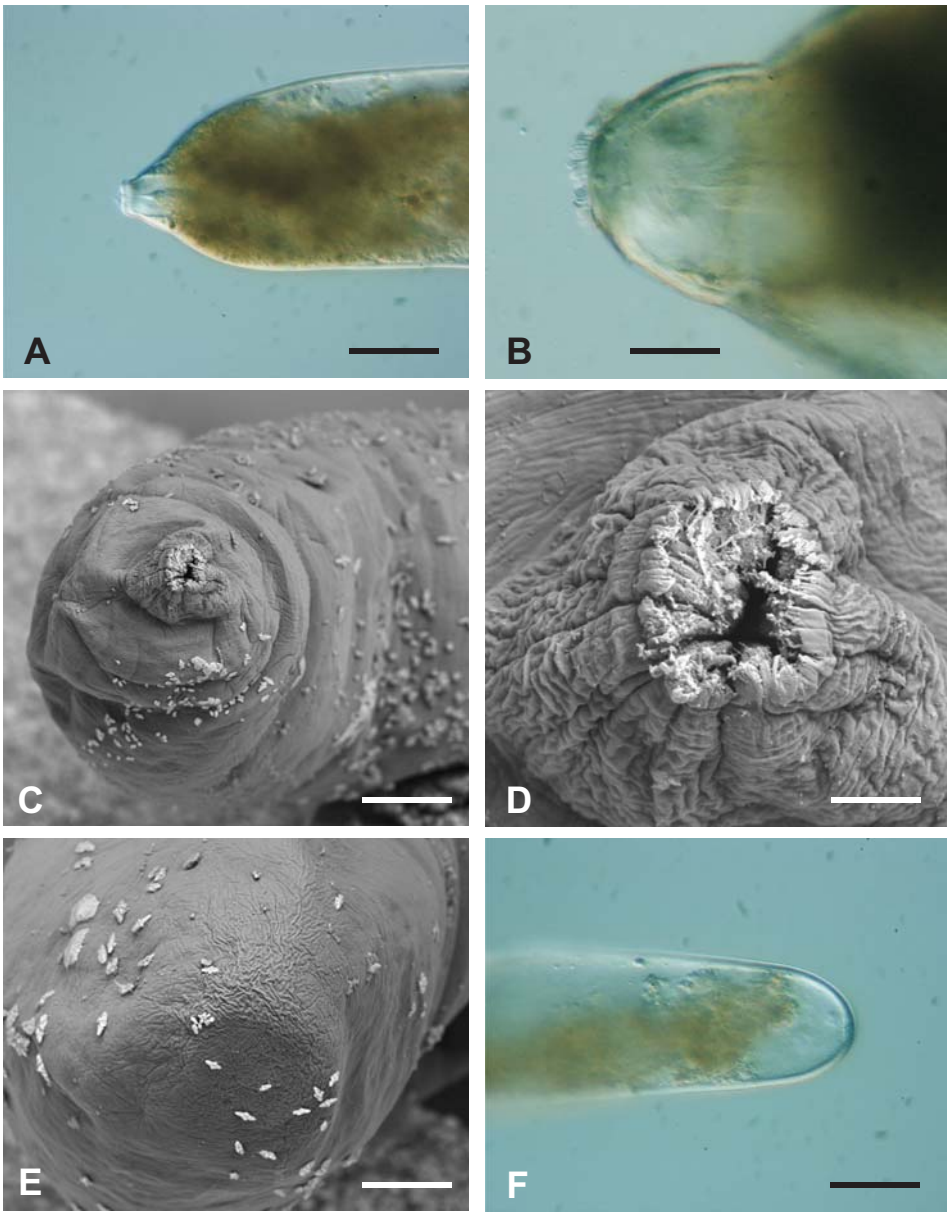

Fig. 3. *Bowseria arctowskii* gen. et sp. nov.; micrographs using transmitted light, showing the apertural (**A**, **B**) and distal ends (**F**); SEM micrographs showing apertural end (**C**), detail of aperture (**D**) and distal end (**E**). All specimens from KG10. Scale bars = 0.1 mm (A, B, F), 0.05 mm (C), 0.01 mm (D), 0.04 mm (E).

quences were of a good quality, except for a variable region corresponding to foraminiferal specific insertion II (Habura *et al.* 2004) or insert F5 (Schweizer *et al.* 2008), situated between helices 39 and 41. Reading of the sequences in this region

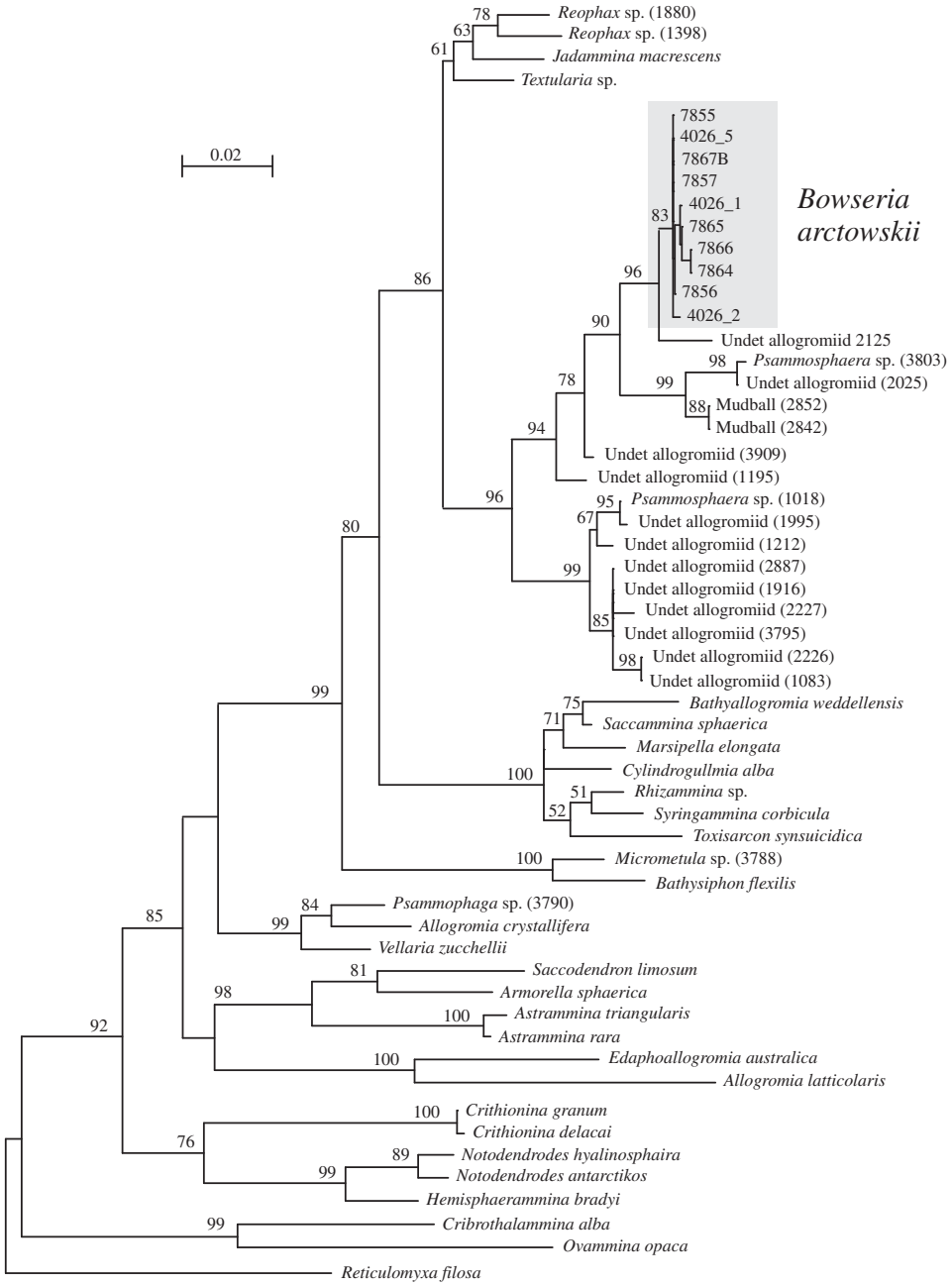

Fig. 4. Phylogenetic tree showing the position of *Bowseria arctowskii* gen. et sp. nov. based on the partial SSU rDNA sequences. The tree was obtained using maximum likelihood method with GTR + I + G model of evolution. The numbers at internal nodes indicate the bootstrap values based on 1000 replicates; only values higher than 50% are shown.

was impeded by a long poly T fragment. To obtain a complete sequence, the PCR product of the DNA isolate 7867 was cloned and sequenced entirely. In other sequences, the bases in unreadable region were replaced by N.

Seven sequences obtained in this study were aligned to 49 sequences of other foraminiferans and analysed using ML method (Fig. 4). Our analysis shows that the seven sequences group together with three clones of an indeterminate isolate 4026 from the sediment samples collected under Ross Ice Shelf (Pawlowski *et al.* 2005). All ten sequences are practically identical, with small heterogeneity due to a few ambiguities.

Bowseria arctowski groups strongly (96% BV) with the indeterminate isolate 2125 collected in 1999 in Explorers Cove (McMurdo Sound). The isolate 2125 resembles the new species in its irregularly shaped shiny theca and white-grey cytoplasm; however, both species differ in about 8% of the sequence, mainly in the variable regions of the sequenced fragment. Together with 15 other mainly indeterminate allogromiids, they form a clade, which corresponds to the lineages A and B, identified by Pawlowski *et al.* (2002c).

Distribution: *Bowseria arctowskii* gen. et sp. nov. was found at six locations in Admiralty Bay, below 100 mwd (Table 1). The new species was common only in station KG10, where it constituted 6.5% of the total assemblage of monothalamous foraminifers (Majewski *et al.* 2007). The occurrence of the species, designed as "undet sausage-like", ranges from 0.6% (KG21) to 4.2% (KG22) in other samples (Majewski *et al.* 2007). After re-examination, two specimens from station KG14, characterized by a creamy, probably finely agglutinated wall, were excluded from this species.

Discussion

This study is a new contribution to a long-lasting effort to describe Antarctic monothalamous foraminifers. It may appear surprising that such large (>1 mm) and relatively common species still remained undescribed. However, this is mainly due to the particular status of organic-walled foraminifers, which are ignored by most marine biologists as well as by micropaleontologically-oriented specialists of foraminiferans. Despite a sustained effort of a small group of "allogromiologists" (Cedhagen *et al.* 2002), there is still an enormous gap in our knowledge of this group compared to other foraminifers. The majority of monothalamous species found in Admiralty Bay are either undescribed, often at higher taxon level, or classified in genera that are known to contain several genetically distinctive and yet undescribed species (Majewski *et al.* 2007).

There are two specific questions raised by our study. The first one concerns the distribution of the new species. Its genetic similarity to the isolate 4026 from under the Ross Ice Shelf as well as its morphological similarity to the allogromiid sp. 5

(Gooday *et al.* 1996) provides new evidence for the wide distribution of Antarctic benthic foraminiferans and their large bathymetric ranges. It is in agreement with a previous study showing genetic similarity of *Epistominella vitrea* collected at shallow depths in McMurdo Sound and at a 1000 mwd site in the Weddell Sea (Pawlowski *et al.* 2007). Our work in progress suggests than many species from Admiralty Bay have identical rDNA sequences to those from McMurdo Sound. However, sequencing of a more variable rDNA region than the SSU would be necessary to confirm the panantarctic distribution of *B. arctowskii*.

The second question concerns the phylogenetic position of the new species. Interestingly, *B. arctowskii* belongs to a clade that branches as a sister group to the large radiation of the multi-chambered textulariids and rotaliids. This clade is composed of two subclades, called lineages A and B in Pawlowski *et al.* (2002c). Both lineages contain *Psammosphaera*-like species as well as a number of indeterminate, organic-walled allogromiids. Some (2125, 1212) resemble *B. arctowskii*, but many have very different morphologies. In some cases, they live as squatters in other foraminiferal tests (1995, 1083). At the moment, *B. arctowskii* is the only described species in this clade. Further investigations of this species, focusing on its ultrastructure and genomics, may be essential for understanding the evolutionary transition between monothalamous and polythalamous foraminiferal lineages.

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