

Morphologic and molecular diversity of the foraminiferal genus *Globocassidulina* in Admiralty Bay, King George Island

WOJCIECH MAJEWSKI^{1*} and JAN PAWLOWSKI²

¹*Institute of Paleobiology, Polish Academy of Sciences, Twarda 51/55, 00-818 Warszawa, Poland*

²*Department of Zoology and Animal Biology, University of Geneva, Sciences III, 30 Quai Ernest Ansermet, CH 1211 Genève 4, Switzerland*

*wmaj@twarda.pan.pl

Abstract: Four distinctive morphological types can be found among living *Globocassidulina* in surface sediments of Admiralty Bay (King George Island, South Shetland Islands). The molecular analysis of the SSU and ITS rDNA indicates that they are monospecific and belong to *Globocassidulina biora*, except for minute forms from deeper than 200 m water depth which probably represent *G. subglobosa*. The morphological types of *G. biora* that show doubled or branched apertures, varied test size and shape as well as colour of cytoplasm, represent populations at different stages of ontogenetic development. However, the variability among large *G. biora* from the same locations is difficult to comprehend. It seems probable that *G. biora* is the only recent, large, shallow water *Globocassidulina* represented throughout the Antarctica, while *G. crassa* is typical for the Magellan region.

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Introduction

The genus *Globocassidulina* belongs to the most common benthic calcareous foraminifera in the western Antarctic Peninsula region inner bay settings (Ishman 1990, Li *et al.* 2000, Majewski & Anderson 2009) as well as on eastern side of the Peninsula (Milam & Anderson 1981). The species include *G. crassa*, *G. crassa rossensis*, (considered by some as an autonomous species *G. rossensis*), as well as *G. biora*, and *G. subglobosa*. The first three are larger and more inflated in shape than *G. subglobosa*. The last species tends to be rather small in test size, compact, globular, and with a single comma-shaped aperture perpendicular to the basal suture of the final chamber. *Globocassidulina crassa*, *G. rossensis*, and *G. biora* are all closely related and differ primarily by apertural shape. *Globocassidulina crassa* has a single aperture that is a part of the basal suture of the ultimate chamber, or parallel to it. *Globocassidulina rossensis* shows a bifurcated aperture, with its branch perpendicular to the basal suture, while *G. biora* has two apertures parallel to the basal suture of the ultimate chamber (the two apertures while roughly parallel, may join at one end (Quilty 2003)).

There is a considerable degree of inter-gradation between these three types (Finger & Lipps 1981), with significant ontogenetic changes in morphology of particular species (Nomura 1984), and different understandings of their taxonomic and evolutionary relationships. According to Quilty (2003), the three morphologically close foraminifera are subspecies of *G. crassa*. Fillon (1974) discussed in detail the close relation between *G. crassa* and *G. biora* and

suggested that the latter is ancestral to the former, as he has not found *G. biora* among “living” fauna in the Ross Sea. However, *G. biora* was recorded from Recent sediments around Antarctica by Finger & Lipps (1981), Violanti (1996), Igarashi *et al.* (2001), and Majewski (2005). Fillon (1974) also suggested that specimens of *G. crassa* from the Vestfold Hills described by Crespin (1960) are immature specimens of *G. biora* that form a continuous range of ontogenetic stages between the immature and mature types. On the other hand, Ward & Webb (1986) suggested that some *G. biora*-like specimens of Fillon (1974, pl. 1, fig. 8) may belong rather to *G. crassa* and do not show characteristics of immature specimens.

As indicated above, it is rather difficult in practice to unequivocally assign specimens to particular *Globocassidulina* species, which gives rise to important taxonomic discrepancies between reports from the same regions that apparently discussed very similar fauna. Comparison of different studies on modern foraminifera from bays of King George Island provides an excellent example. Li *et al.* (2000) and Yoo *et al.* (2006), who analysed Holocene cores from Maxwell Bay, noted the presence of *G. biora* and *G. crassa rossensis*. On the other hand, Mayer (2000) who investigated shallow water assemblages, (down to 30 m), from surface sediment of Potter Cove (part of Maxwell Bay) acknowledged the presence of *G. crassa* and *G. subglobosa*. Chang & Yoon (1995) noted *G. biora*, *G. crassa*, and *G. subglobosa* in roughly similar numbers from Marian Cove (another part of Maxwell Bay) and water depths less than 100 m. All specimens of *Globocassidulina* collected by Majewski (2005) from surface sediments of nearby Admiralty Bay were assigned to *G. biora*.

Table I. List of sampling stations indicating site location, its water depth, and method of sampling.

Station	Water depth (m)	GPS location	Method
KG1	17.5–25	62°10.998'S, 058°37.306'W	tube-sampler
KG2	17.5	62°11.216'S, 058°37.492'W	tube-sampler
KG4	17	62°10.688'S, 058°32.115'W	tube-sampler
KG5	35	62°06.247'S, 058°19.777'W	tube-sampler
KG6	47.5	62°04.416'S, 058°19.932'W	tube-sampler
KG7	30	62°06.220'S, 058°19.676'W	tube-sampler
KG8	119	62°09.650'S, 58°34.774'W	Van Veen
KG9	114	62°09.623'S, 58°34.510'W	Van Veen
KG10	107	62°09.584'S, 58°34.287'W	Van Veen
KG13	108	62°09.461'S, 58°29.737'W	Van Veen
KG14	100	62°09.290'S, 58°29.439'W	Van Veen
KG15	16.3	62°05.123'S, 058°23.471'W	diving
KG16	29–31.4	62°04.760'S, 058°21.495'W	diving
KG17	20.8	62°05.319'S, 058°23.777'W	diving
KG18	8.0–12.0	Goulden Cove, ~150 m from ice edge	diving
KG19	40	South slopes of Napier Rock	diving
KG20	249	62°09.053'S, 058°30.435'W	Van Veen
KG21	254	62°07.488'S, 058°27.545'W	Van Veen
KG22	233	62°05.610'S, 058°22.944'W	Van Veen
KG23	223	62°13.361'S, 058°22.892'W	Van Veen
KG24	179	62°12.057'S, 058°23.666'W	Van Veen

Globocassidulina is an important Antarctic genus of foraminifera. According to Ishman (1990), *G. subglobosa* and *G. biora* both dominate biofacies from western Antarctic Peninsula inlets. Li *et al.* (2000) noted the association of *G. biora* with coarse sediment. They speculated that high abundance of this foraminifer may imply a high-energy environment and considered *G. biora* to be the most important species for palaeoenvironmental reconstructions in shallow water inlet settings due to its high abundance and robustness. This foraminifer was also critical for some palaeoenvironmental reconstructions (e.g. Khim *et al.* 2001, Yoo *et al.* 2006, Majewski & Anderson 2009). All those facts highlight the need for a better understanding of morphological and molecular variability within the Antarctic *Globocassidulina*.

Material and methods

Surface sediment samples for population and morphological study of *Globocassidulina* were taken at 21 locations in Admiralty Bay from water depths between 8 and 254 m in

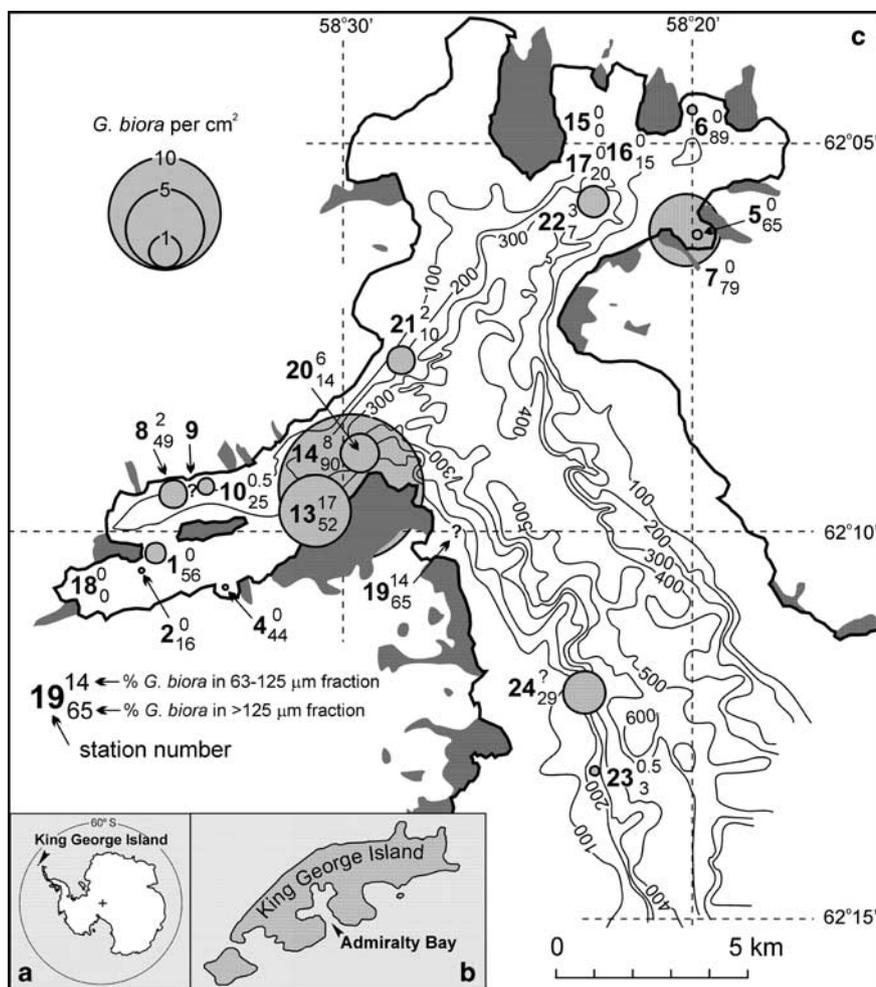


Fig. 1. The location of a. King George Island, b. Admiralty Bay, and c. sampling stations (KG1 through KG24) with *G. biora* abundances shown by circles expressing number of specimens per cm², and percentages in total Rose Bengal stained assemblage. Stations with the percentages only were sampled by scuba divers. Dark gray indicates inland glacier free areas (Battke 1990). The bathymetric contour lines in metres after Straten (unpublished).

Table II. List of DNA samples used in this study.

# of DNA extraction	Location	SSU	ITS	Morphological type	Original description
7796	KG8	+	+	3	medium
7797	KG8	+		3	medium
7800	KG8	+	+	2	large
7836	KG9	+	+	3	medium
7839	KG9		+	2	large
7900	KG7	+	+	1	large flat
7901	KG7	+	+	1	large flat
7902	KG7	+	+	1	large flat
7907	KG7	+	+	3	medium, green
7909	KG7	+	+	3	medium, green
7962	KG5	+		2	large globular
7963	KG5	+	+	2	large globular
7964	KG5		+	2	large globular
7967	KG5	+	+	3	medium, green
7995	KG13	+		4	<i>G. subglobosa</i> -like, greenish, stick shape
8087	KG14	+	+	4	grey, biora/subglobosa
8088	KG14	+	+	4	grey, biora/subglobosa
8026	KG13	+	+	4	<i>G. subglobosa</i> typical aperture, stick shape
8234	KG20	+	+	4	<i>G. subglobosa</i> green/yellow,

early 2007 (Table I, Fig. 1). They were wet sieved through 63 μm and 125 μm sieves and stained with Rose Bengal (1g l^{-1}) and 70% ethanol diluted in seawater. The stained residues were washed in tap water and dried. In most samples, all “living” (stained) foraminifera were picked from the 63–125 μm and >125 μm fractions. Few faunally-rich samples were divided using a dry microsplitter. The maximum test diameter of all stained *Globocassidulina* from these samples was measured optically at 63x magnification with $\pm 16\ \mu\text{m}$ precision. Apertural shapes of selected specimens were analysed under SEM.

Nineteen specimens of *Globocassidulina* representing different morphological types were fixed for DNA examination (Table II). They were isolated from the upper 2–3 cm of surface sediments collected at seven locations. As soon as possible after collection, the sediment was washed with cold seawater and wet sieved through 125 μm sieves. The residues were stored in the refrigerator at about 2°C, for periods of several days. The foraminifera 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 buffers.

A 3' fragment of the SSU rRNA gene was successfully amplified for 17 DNA isolates (specimens). Foraminiferal

specific primer s14F3 and universal eukaryotic primer sB were used for the first amplification. Foraminiferal specific primer s14F1 and primer sB were used for reamplification. PCR conditions and primer sequences are described in Pawlowski (2000).

The ITS region (ITS1 + 5.8S + ITS2) was successfully amplified for 18 DNA isolates. The PCR amplification was performed using the foraminiferal-specific primer pair s14F3 and 2TAIC and re-amplified with the universal eukaryotic primer sBr and foraminiferal specific primer 2TAIC. The compatibility of the SSU and ITS sequences was tested by amplifying a longer fragment, using primers s14F1 and For2 situated in the central part of ITS region (Fig. 2). Primers used for amplification of ITS region are described in Pawlowski *et al.* (2007).

The amplified PCR products were purified using High Pure PCR Purification Kit (Roche Diagnostics). They were ligated in the Topo Cloning vector (Invitrogen), 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 ABI-PRISM 3100 sequencer (Applied Biosystems), all according to the manufacturer's instructions.

In the SSU analysis, 31 sequences obtained after cloning of 17 PCR products were manually aligned using Seaview

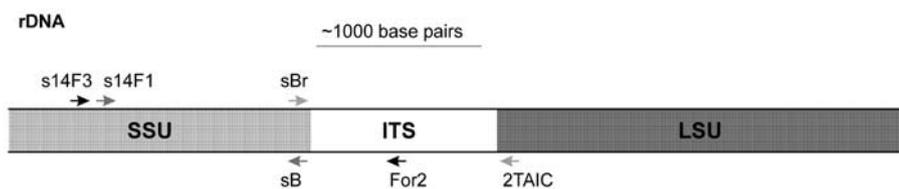


Fig. 2. Schema of rDNA showing the position of primers used for amplification of SSU and ITS rDNA (dark and bright gray arrows, respectively) and for testing the compatibility of the SSU and ITS sequences (black arrows).

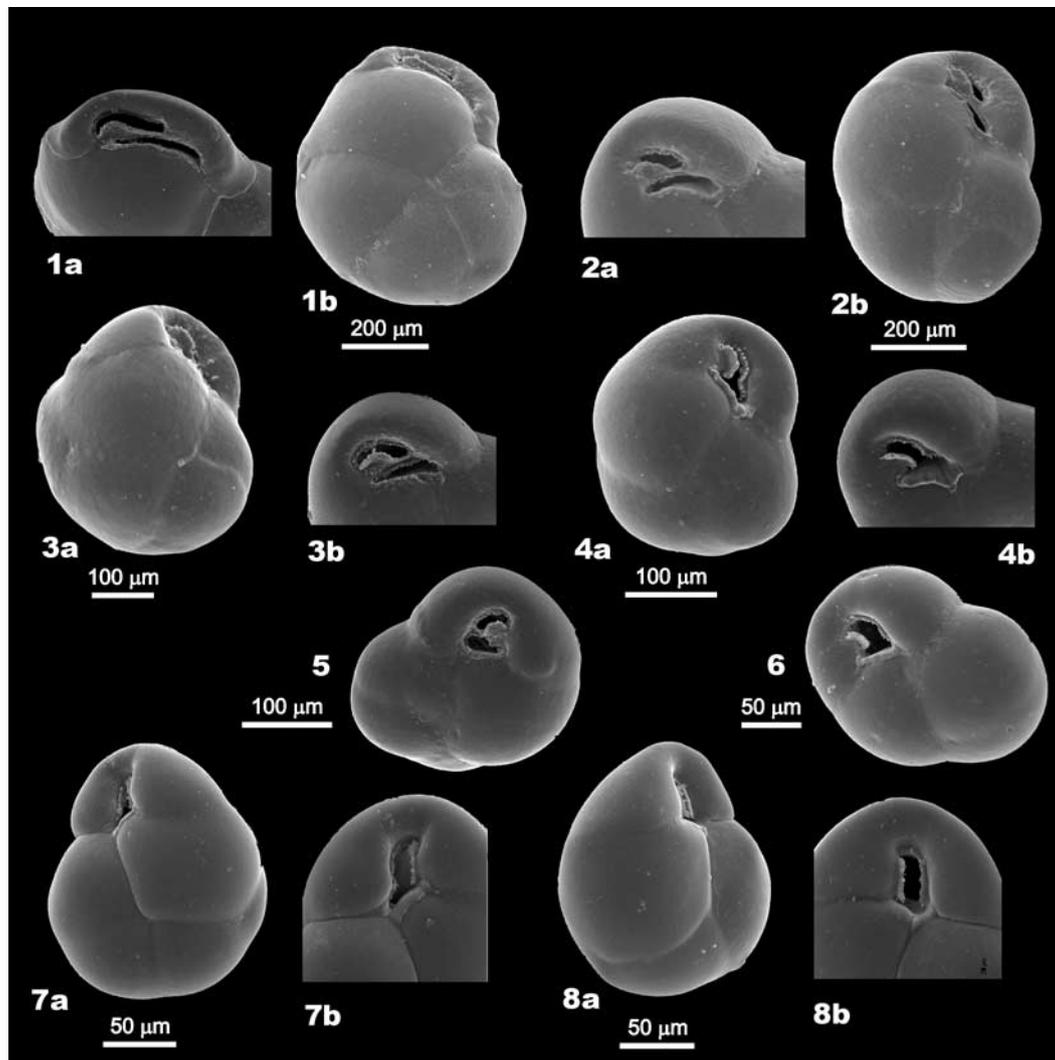


Fig. 3. SEM images of *Globocassulina* from Admiralty Bay, belonging to the four morphologic types. Type 1 (large flat): **1.** KG7; type 2 (large globular): **2.** KG8; type 3 (middle in size): **3.** KG7, **4–6.** KG8; type 4 (*G. subglobosa*-like): **7.** KG20, **8.** KG23.

software (Galtier *et al.* 1996) to 21 sequences of foraminifera from *Cassidulinidae* family, obtained mostly from the same region. Highly variable regions of the alignment were removed, leaving 1163 sites for phylogenetic analysis. The phylogenetic tree was constructed using maximum likelihood method with GTR + GI model, using Treefinder (Jobb *et al.* 2004). The same programme was used to analyse 34 sequences of the ITS region.

Results

Morphologic diversity of Globocassidulina in Admiralty Bay

Among the stained samples from the recent survey in Admiralty Bay, *Globocassidulina* was found everywhere except at station KG15 located within Martel Inlet, next to the Keller Peninsula (Table I, Fig. 1). In eight samples, substantial populations (>50 specimens) of stained

Table III. Morphological types in the population of *Globocassidulina* from in Admiralty Bay, based on living specimens from sites KG1 through KG24 (Table I, Fig. 1).

Morphological type	Colour of cytoplasm	Apertural shape	Test size	Bathymetric range (mwd)
1 large flat	yellow, pink	doubled aperture parallel to the basal suture	500–800 μm	30–179
2 large globular	pink to green	doubled aperture parallel to the basal suture	500–800 μm	30–179
3 medium in size	green	doubled or bifurcated aperture	100–600 μm	30–254
4 small, <i>G. subglobosa</i> -like	grey, yellow, pink	single aperture, perpendicular or oblique to basal suture of the last chamber	> 200 μm	223–254

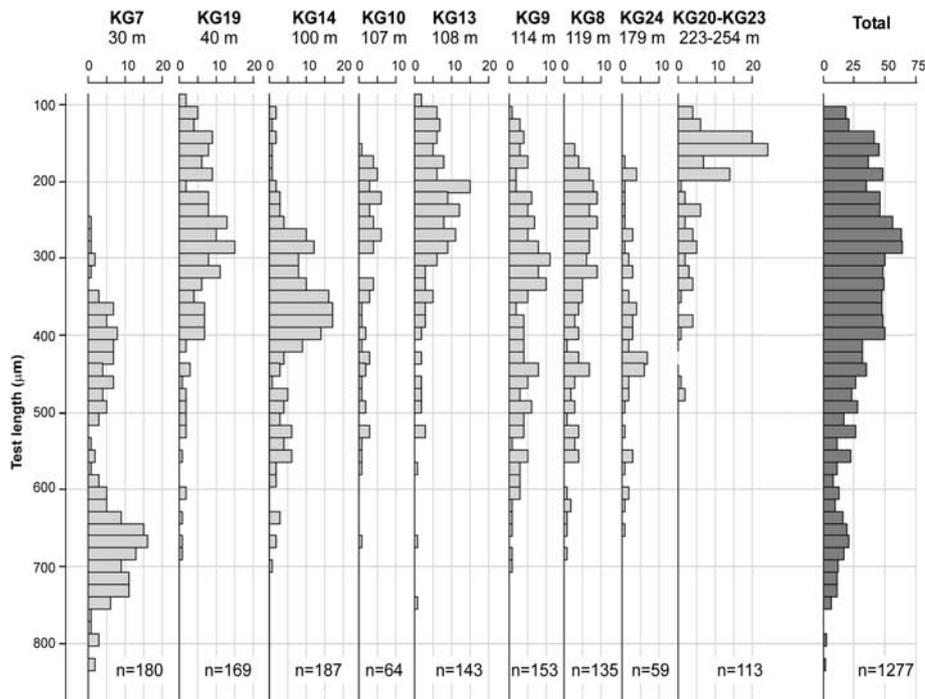


Fig. 4. Test size (maximum length) measurements from 12 locations, arranged according to increasing water depth. Note different scale on the composite, most right graph.

Globocassidulina were encountered. Among truly living specimens, recognized by coloured cytoplasm rather than by Rose Bengal staining, four morphological types were identified. They include 1) large inflated forms with broad apertural area, doubled aperture and pink to yellow cytoplasm, 2) large forms with more globular outline, doubled aperture and pink to green cytoplasm, 3) medium in size with bifurcated or double aperture and greenish cytoplasm, and 4) small forms with single aperture and greenish or yellowish cytoplasm. The presence of four morphological types among *Globocassidulina* in Admiralty Bay was supported by SEM inspections of stained specimens (Fig. 3). Their characteristic features are summarized in Table III.

Test size measurements (Fig. 4) on the total of 1277 specimens confirmed the presence of considerable morphological heterogeneity in *Globocassidulina* population. In the shallowest sample with substantial population of stained specimens (KG7, 30 m depth), 180 specimens show bimodal test size distribution. Most specimens have tests of 600–750 µm, while the rest are smaller than 500 µm. The group of samples from water depths between 40 and 179 m (KG8, KG9, KG10, KG13, KG14, KG19, and KG24) show a more homogenous test size distribution with only a few specimens larger than 600 µm and the overwhelming majority within a broad range between 100 and 600 µm, but rather smaller than 400 µm. In contrast, the deepest water samples from 223 to 254 m (KG20, KG21, KG22, KG23) show the greatest numbers of specimens between 100 and 200 µm, and significantly fewer specimens that are up to 500 µm in test size. To summarize, the presence of at least three morphological types within *Globocassidulina*

population in Admiralty Bay, is generally confirmed by the test size results.

Genetic diversity of Globocassidulina in Admiralty Bay

To verify whether observed morphological differences correspond to genetic differentiation between the morphological types, we amplified and sequenced a 3' fragment of the SSU and the complete ITS region of the rDNA of specimens representing all morphotypes. The analysis of SSU rDNA (Fig. 5) shows considerable homogeneity between specimens, except for the isolate 8234, which may represent *G. subglobosa*. All morphologically distinctive types of *Globocassidulina* from Admiralty Bay merge into one group clearly distinctive from other *Cassidulinidae*. Because of the presence of a doubled aperture in the largest, most characteristic specimens, we consider this group to represent *G. biora*. Moreover, four isolates suspected of belonging to *G. subglobosa* (7995, 8026, 8087, 8088) blend within the *G. biora* cluster, suggesting that they may be in fact immature *G. biora*.

The test whether the genetic homogeneity of the SSU rDNA sequences is not due to the relatively low mutation rate in this gene (Bowser *et al.* 2006), we also sequenced more rapidly evolving ITS rDNA region. Phylogenetic analysis of ITS sequences gives a tree similar to the one obtained using SSU rDNA, with no clear differentiation between different morphotypes, except for the DNA isolate 8234, probably representing *G. subglobosa* (Fig. 6). Within the *G. biora* group, the distances between sequences obtained from

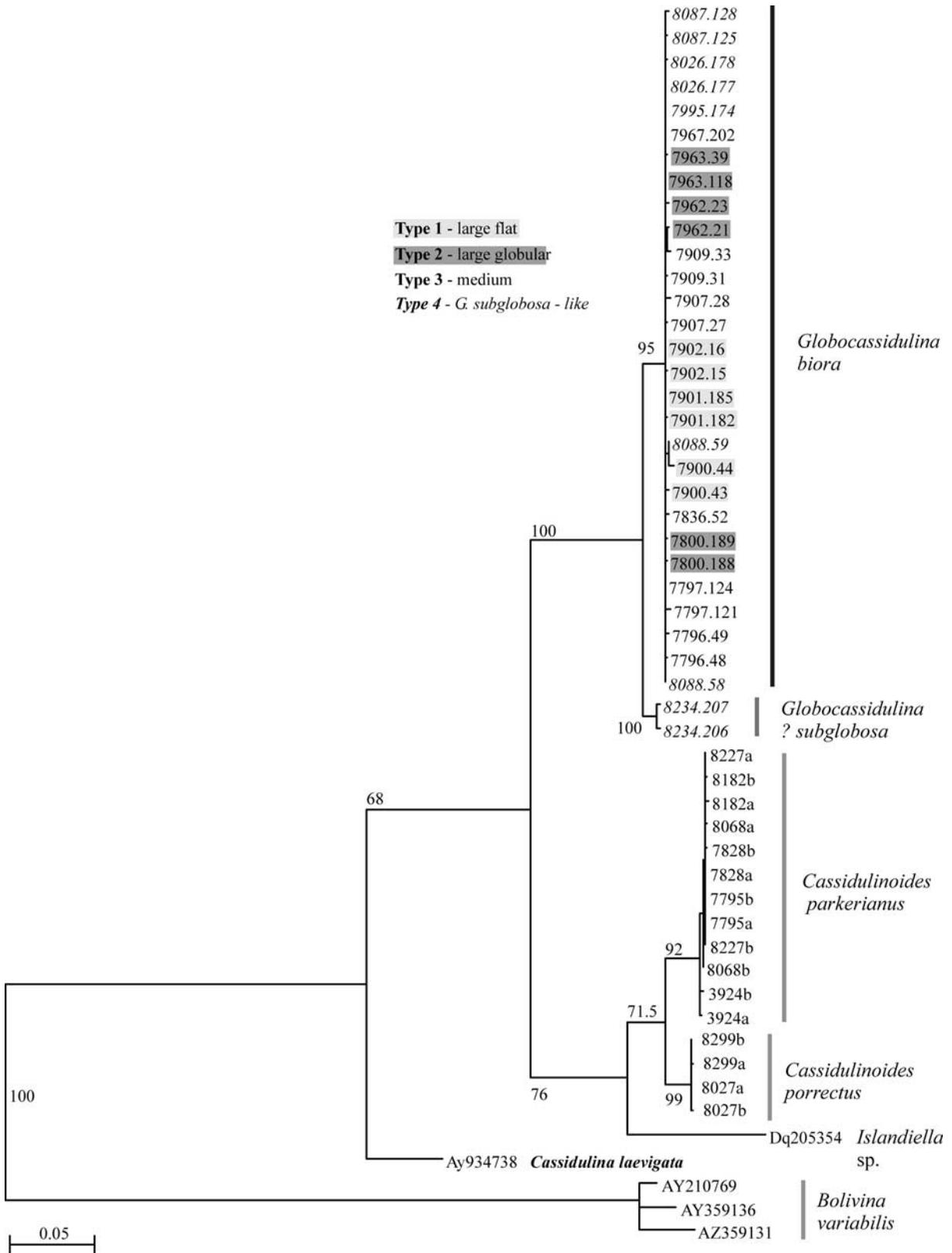


Fig. 5. SSU rDNA tree showing relations between selected *Cassidulinidae* from various high-latitude locations.

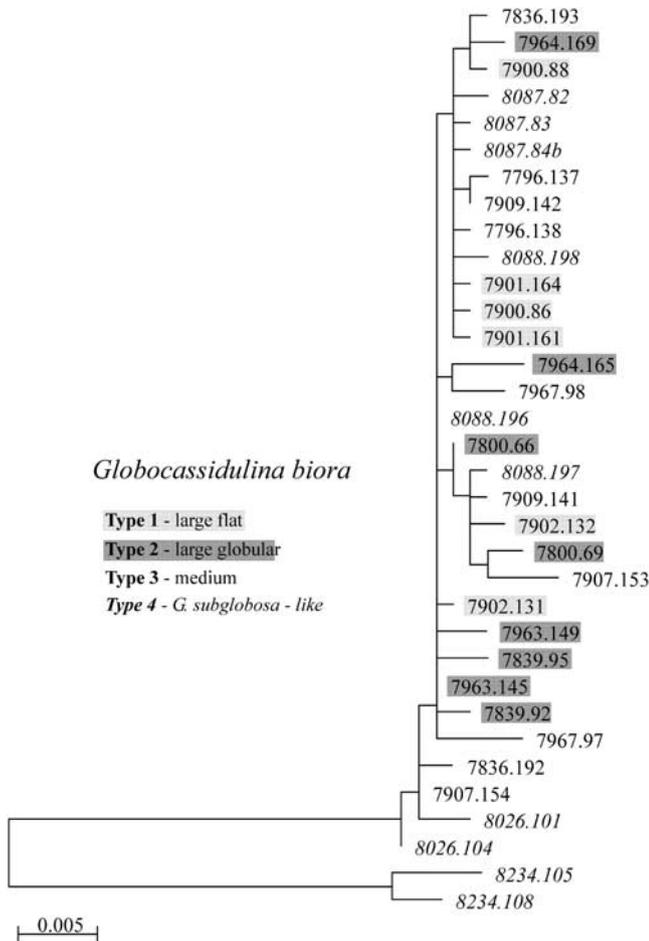


Fig. 6. ITS rDNA tree showing sequences obtained from different morphotypes of *G. biora*.

different isolates (inter-isolate variation) averaged 0.49%, with the range between 0 and 1.21, while between the two sequences obtained from the isolate 8234 and *G. biora* the distances were almost an order of magnitude larger and averaged 3.9% with the range between 3.59 and 4.40%. The intra-isolate variation was an average of 0.60%, actually exceeding the inter-isolate variation within the *G. biora* cluster. To conclude, comparison of the ITS sequences obtained from the four morphological types within *G. biora* falls within 0.38–0.60% range and reveals no significant differences between intra- and inter-morphological variability

Table IV. Average sequence divergence (in percentage) between different morphological types of *G. biora* from Admiralty Bay, based on the analysis of the ITS fragment of the rRNA gene.

	Type 1	Type 2	Type 3	Type 4
Type 1	0.38	0.48	0.50	0.44
Type 2	0.48	0.55	0.6	
Type 3	0.46	0.52		
Type 4	0.34			

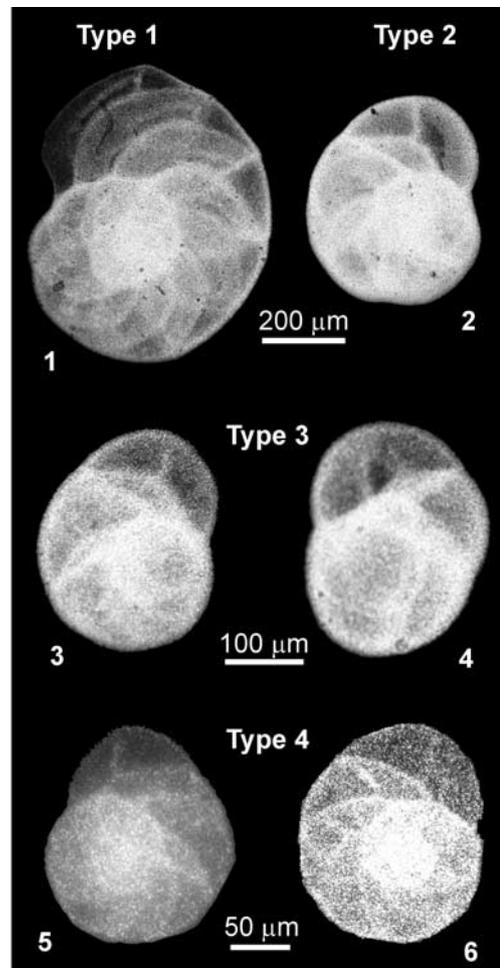


Fig. 7. X-ray images of four morphological types of *Globocassidulina* from Admiralty Bay. **1.** Type 1, KG7, **2.** Type 2, KG8, **3 & 4.** Type 3, KG8, KG14, **5 & 6.** Type 4, KG20, KG20.

(Table IV), confirming the monospecific character of the *G. biora* cluster.

Measurement of the proloculus

For analysing sizes of proloculi, 32 specimens of *Globocassidulina* from Admiralty Bay were X-rayed (Fig. 7). In the case of 28 micrographs, it was possible to measure the sizes of proloculi. In the larger specimens (type 1 and 2) a degree of uncertainty in identification of proloculi existed. However, dissection of 30 specimens from KG7 and KG8 confirmed presence of large proloculi (~120 μm) in majority of specimens. Only three dissected specimens were found to be microspheric. The plot of the proloculi sizes against test diameters (Fig. 8) shows no significant difference in the size of proloculus between types 1 and 2. Their average diameter of ~140 μm exceeds values measured on dissected specimens, due to rather obscured X-ray view of inner whorls in the larger and thicker-walled specimens. Type 3 shows proloculi at

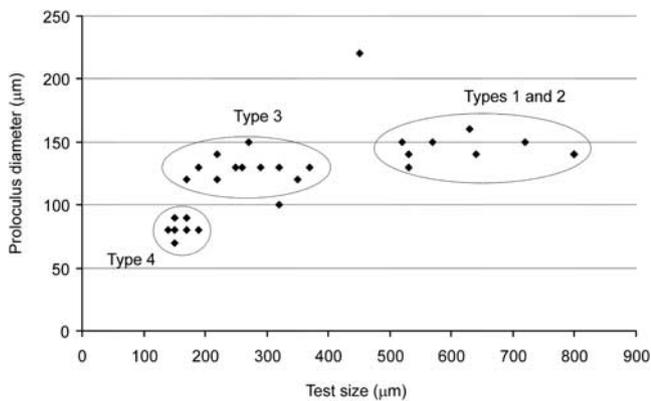


Fig. 8. Test versus proloculus diameter among morphologic types of Rose Bengal stained *Globocassidulina* in Admiralty Bay. Note that average proloculus diameter measured on dissected specimens from type 1 and 2 was on average $\sim 120 \mu\text{m}$. The difference is due to obscured X-ray views of inner whorls in the larger and thicker-walled specimens.

$\sim 125 \mu\text{m}$ in average, which stays in agreement with the values measured on the dissected specimens of types 1 and 2. On contrary, the smallest specimens, represented by type 4, show relatively small proloculi ($\sim 80 \mu\text{m}$ on average or less). All specimens of type 4, that were X-rayed, were from samples 20 and 23 (249 and 223 m) and they may represent *G. subglobosa*, similar to the isolate 8234 that was the only one from below 200 m analysed genetically in this study and which SSU and ITS sequences clearly differed from remaining *Globocassidulina* isolates (Figs 5 & 6).

Discussion

What is the reason for morphological diversity of G. biora?

Despite the presence of considerable morphological variability within *Globocassidulina* in Admiralty Bay (Table III, Fig. 3), the molecular analysis of the SSU and ITS fragments of rRNA gene indicates that except for minute forms from deeper than 200 m probably representing *G. subglobosa*, the rest of *Globocassidulina* from Admiralty Bay is monospecific and belongs to *G. biora* (Table IV, Figs 5 & 6). What could be the reason for the significant morphologic variability within *G. biora*? There appears to be a few possibilities: 1) development of distinctive micro and megalospheric generations, 2) a presence of distinct cohorts that at the time of collection were at different growth stages, and/or 3) it could be due to environmental variability in benthic habitats throughout Admiralty Bay that resulted in development of different morphologies.

The first possibility was tested by analysing inner-test morphology of the four types of *G. biora*. Although the life cycle is more varied in foraminifera than in any other group of protists (Goldstein 1999) and no specific information on life cycle in *Cassidulinidae* is available, in general, the

foraminiferal life cycle is characterized by alternation of sexual and asexual generations. As a result, megalospheric gamonts with a large proloculus and relatively small overall test diameter, and microspheric agamonts with tiny proloculus but relatively large overall test may develop (Goldstein 1999). Nomura (1984) reported the presence of megalospheric and microspheric forms in *G. biora* from raised beaches and modern deposits of the Lützow–Holm Bay, East Antarctica. He reported the presence of two types of tests having a proloculus of $72 \mu\text{m}$ in average and of less than $10 \mu\text{m}$ in diameter.

It appears that among *G. biora*, morphologic dimorphism, i.e. a distinction between micro and megalospheric generations, is not responsible for the morphological variability observed. However, clear difference in the size of proloculus is observed between *G. biora* and *G. subglobosa* ($\sim 120 \mu\text{m}$ vs $\sim 80 \mu\text{m}$; Fig. 8). Moreover, megalospheric proloculi of *G. biora* from Admiralty Bay ($\sim 120 \mu\text{m}$) are on average larger than those reported from Lützow–Holm by Nomura (1984) ($72 \mu\text{m}$).

The second possibility of significant morphological changes throughout ontogeny among *Globocassidulina* was noted by Finger & Lipps (1981). They suggested that smaller specimens of *G. crassa rossensis* and *G. biora* are similar to *G. subglobosa*, but grade into larger and more compressed forms with branched or doubled apertures. In the same study, they reported dissection revealing that earlier chambers of adult *G. crassa rossensis* and *G. biora* display fully developed apertures without any indications of apertural growth stages. Thus, their final conclusion was not unequivocal. Our data suggest that a large part of morphological variability observed among *G. biora* in Admiralty Bay may be a result of observing specimens at different growth stages. The presence of single, bifurcated, and doubled apertures among the four morphological types that, according to the molecular analysis all fell into the *G. biora* cluster, suggests that throughout ontogeny the aperture becomes first bifurcated and then matures into doubled. Similarly, the test shape becomes more inflated in the largest specimens, as observed in type 1 (Fig. 3). All these findings are in agreement with the conclusions of Nomura (1984) who presented analogous morphological variability through ontogenetic development of *G. biora* from the Lützow–Holm Bay.

On the other hand, the ontogenetic changes cannot explain morphological differences between type 1 and type 2, both representing relatively large specimens (Table III, Fig. 3) and falling within a single group on the proloculus vs test size plot (Fig. 8). Dissection of 30 specimens revealed that in both types the megalospheric forms dominate; however, a few microspheric forms are also present. Significant intraspecific morphologic variability is not unknown among benthic foraminifera. Study of *Ammonia* showed wide morphologic diversity in natural habitats and cultured populations. Up to six different “morphospecies” were found in their natural habitats and in clonal cultures that

were suggested to be ecotypes of *Ammonia beccarii* developed due to environmental factors (Schnitker 1974).

A review of ecological parameters that may have impact on benthic foraminiferal tests morphology was presented by Boltovskoy *et al.* (1991). It appears that the differences in test shape between types 1 and 2 of *G. biora* may be also a consequence of ecological variability in benthic habitats throughout Admiralty Bay. A problem with this interpretation however, is that the large globular specimens of morphological type 2, that are encountered at several locations (KG5, KG8, KG9, KG13, KG19, KG24), at all locations except KG5 and KG24 blend with the inflated specimens of type 1. Therefore it is difficult to propose any hydrographic (e.g. temperature, salinity, calcium carbonate solubility etc.) or substrate-related factor that could lead to development of two different morphologies in *G. biora* at the same locations. Among the ecological parameters, reviewed by Boltovskoy *et al.* (1991), only the nutrition, or more precisely food type could be a candidate for the development of the two different types of large *G. biora*. There are numerous studies showing morphological and cytological response of benthic foraminifera to heavy metal pollution (e.g. Alve 1991, Le Cadre & Debenay 2006) and it was suggested that the pollutants may penetrate the foraminiferal cell together with food (Yanko *et al.* 1998). However, so far no data show that different types of food could actually lead to development of different test morphologies in benthic foraminifera. In the case of *G. biora* from Admiralty Bay, the difference in cytoplasm colouration between types 1 and 2 (Table III) may give slight support for such possibility.

Is there only one large Globocassidulina (G. biora) in the South Shetland Islands and Antarctica?

The first detailed study on Recent benthic foraminiferal distribution in Admiralty Bay in the 2002/03 summer season used only the > 125 µm fraction (Majewski 2005). Specimens of *Globocassidulina* were encountered at all locations, between 8 and 520 m, except for a single site in Hervé Cove (8 m), where only a few *Quinqueloculina* were found. All relatively large specimens of *Globocassidulina* were reported as *G. biora*. However, a considerable variability in their apertural shapes was noted. It was also clear that although *G. biora* inhabits practically the entire Admiralty Bay down to at least 520 m, it dominates shallow water assemblages at less than 200 m (Majewski 2005). However, other studies in South Shetland Islands (Finger & Lipps 1981, Chang & Yoon 1995, Mayer 2000, Li *et al.* 2000, Gray *et al.* 2003, Yoo *et al.* 2006), have reported the presence of more than one representatives/species of *Globocassidulina*. Keeping this fact in mind, along with an investigation of living monothalamous foraminiferal assemblages in Admiralty Bay (Majewski *et al.* 2007), particular attention was paid also to morphological diversity among living *Globocassidulina*.

The molecular homogeneity, despite considerable morphological variation suggests that *G. crassa* reported from modern sediments at King George Island by earlier studies (e.g. Chang & Yoon 1995, Mayer 2000) may be in fact *G. biora* as might *G. crassa rossensis* reported from Holocene sections by Li *et al.* (2000) and Yoo *et al.* (2006). In studies of recent Antarctic benthic foraminifera, Finger & Lipps (1981) have already suggested a common over-reporting of the true *G. crassa* type of d'Orbigny (1839). They suggested that many workers have ignored the diagnostic apertural branches that differentiate other taxa from *G. crassa*, having only a single aperture. Interestingly, despite the comment of Finger & Lipps (1981), Gray *et al.* (2003) who subsequently studied recent deposits at the same locations, listed *G. crassa* along with *G. biora* as present at Deception Island, even though their specimen illustrated as *G. crassa* seems to have a doubled aperture. They did, however, point out difficulties in classifying *Globocassidulina* in cases of poor aperture preservation.

Finger & Lipps (1981) acknowledged the presence of two large *Globocassidulina* taxa in Deception Island (~150 km south-west from King George Island). They dissected some large specimens of *G. biora* and what they regarded as *G. crassa rossensis* and noted that their inner chambers displayed fully developed apertures without any indication of apertural growth stages. Their observation was questioned by Nomura (1984), who observed changes in apertural shape from single, through L-shaped into double, parallel in mature stage of *G. biora*, in agreement with another suggestion of Finger & Lipps (1981) about potential difficulties in discriminating between immature specimens of *G. biora* and *G. crassa rossensis*. In the view of the present findings, it seems to be likely that all specimens reported from Deception Island may in fact belong to *G. biora*.

It appears that at least some of the morphological types of *G. biora* distinguished in Admiralty Bay may have broad pan-Antarctic distribution. Crespin (1960) who described *G. biora* from Vestfold Hills illustrated large, globular specimens that could correspond to type 2 from this study. Similarly, large specimens from raised beaches of Lützow–Holm Bay (Nomura 1983, 1984) and from the Firth of Tay Holocene section (Majewski & Anderson 2009) resemble type 2. On the other hand, in the Ross Sea, Violanti (1996) reported forms that may correspond to type 1 in this study. She noted *G. subglobosa*, *G. crassa*, *G. rossensis*, and *G. biora* in a single nearshore sample (IB 3) from near Terra Nova Station (Ross Sea) and noted a more compressed shape of the largest *G. biora* as compared with *G. crassa*. A large and inflated specimen of *G. biora* was illustrated by Ward & Webb (1986) from materials deposited onshore Ross Island by the ice shelf. It seems to correspond to the type 1 from Admiralty Bay as well. The living specimens of type 1 morphotype were also observed by one of us (JP) at shallow depths in McMurdo Sound.

According to Quilty (2003), the three morphologically close species (i.e. *G. crassa*, *G. rossensis*, and *G. biora*) are in fact subspecies of *G. crassa*. Their very close phylogenetic relationship is in concordance with the ontogenetic development of the aperture in *G. biora* (Nomura 1984) and also reflected in the molecular homogeneity among forms with differently developed apertures in Admiralty Bay. The absence of the true *G. crassa* in Deception Island and possibly at other Antarctic locations, as suggested by Finger & Lipps (1981), may imply that *G. crassa*, originally reported north of the Polar Front, and *G. biora*, common in recent Antarctic settings, could represent two distinct species with populations restricted to the Magellan region and the Antarctic, respectively. It seems probable that large, typical specimens of *G. biora* are more representative for shallow waters (Bernhard 1987, Li *et al.* 2000, Majewski 2005) than for greater water depths (Fillon 1974, Osterman & Kellogg 1979, Kellogg & Kellogg 1987), mainly due to control by water mass properties with respect to CaCO₃ dissolution (e.g. Anderson 1975, Ishman & Szymcek 2003). This bathymetric constraint could limit *Globocassidulina* gene flow between the Antarctic and the Magellan region.

Conclusions

Four distinctive morphological types found among living *Globocassidulina* in Admiralty Bay include 1) large inflated forms with a broad apertural area, doubled aperture and pink to yellow cytoplasm, 2) large forms with a more globular outline, doubled aperture and pink to green cytoplasm, 3) medium forms with a bifurcated or double aperture and greenish cytoplasm, and 4) small forms with a single aperture with greenish or yellowish cytoplasm. The molecular analysis of the SSU and ITS rDNA indicates that they are monospecific and belong to *G. biora*. Among the smallest forms, only a single isolate, the only one from deeper than 200 m, stands out in the molecular analyses. It may represent the true *G. subglobosa*. The morphological types of *G. biora* seem to represent populations at different stages of ontogenetic development. However, the variability among large *G. biora* collected at the same locations is difficult to interpret. It seems that different types of food may be the most likely factor that could lead to development of different test morphologies in adult *G. biora*. It also seems probable that *G. biora* is the only recent, large, shallow water *Globocassidulina* represented throughout South Shetland Islands and possibly throughout the Antarctica, while *G. crassa* is typical of the Magellan region north of the Polar Front.

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