

Bipolar gene flow in deep-sea benthic foraminifera

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Abstract

Despite its often featureless appearance, the deep-ocean floor includes some of the most diverse habitats on Earth. However, the accurate assessment of global deep-sea diversity is impeded by a paucity of data on the geographical ranges of bottom-dwelling species, particularly at the genetic level. Here, we present molecular evidence for exceptionally wide distribution of benthic foraminifera, which constitute the major part of deep-sea meiofauna. Our analyses of nuclear ribosomal RNA genes revealed high genetic similarity between Arctic and Antarctic populations of three common deep-sea foraminiferal species (*Epistominella exigua*, *Cibicides wuellerstorfi* and *Oridorsalis umbonatus*), separated by distances of up to 17 000 km. Our results contrast with the substantial level of cryptic diversity usually revealed by molecular studies, of shallow-water benthic and planktonic marine organisms. The very broad ranges of the deep-sea foraminifera that we examined support the hypothesis of global distribution of small eukaryotes and suggest that deep-sea biodiversity may be more modest at global scales than present estimates suggest.

Keywords: deep-sea diversity, geographical distribution, ITS rDNA, meiofauna, protists

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Introduction

Recent molecular phylogenetic studies unveiled an extraordinarily rich hidden diversity of marine single-cell eukaryotes (Moreira & López-García 2002). However, most of these studies focused on shallow-water and pelagic environments, while relatively little is known on diversity of eukaryotes living at the bottom of the deep ocean (Edgcomb *et al.* 2002; López-García *et al.* 2003). It has been proposed that the deep-sea diversity is astronomically large, based on local scale studies of bathyal macrofauna (Grassle & Maciolek 1992). However, the extent to which high local biodiversity can be extrapolated to larger spatial scales is hotly disputed (Lambhead & Boucher 2003). There is a general lack of genetic data on geographical ranges of bottom-dwelling deep-sea species (Etter *et al.* 1999). A few genetic studies of deep-sea hydrothermal vent animals have, however, provided evidence for dispersal barriers and isolation of deep-sea populations related to

deep-oceanic currents and topographical features (Won *et al.* 2003; Hurtado *et al.* 2004). Whether such genetic differentiation can also be observed in deep-sea micro- and meiofauna is unknown.

Benthic foraminifera constitute the major component of deep-sea meiofauna and include several cosmopolitan species that occur in almost all regions of the global ocean (Murray 1991; Gooday *et al.* 2004b). Some have an excellent Cenozoic and Quaternary fossil record and are widely used as palaeo-oceanographic and palaeoclimatic proxies (Thomas 1992; Zachos *et al.* 2001). Their identification is based entirely on test morphology. Recently, however, the accuracy of such identifications has been challenged by molecular studies that revealed extraordinarily rich cryptic diversity among many groups of foraminifera (Darling *et al.* 1999; Holzmann 2000; Pawlowski *et al.* 2002). Most of these studies used the SSU and LSU ribosomal RNA (rRNA) genes that evolve at different rates in different taxonomic groups (Pawlowski *et al.* 1997). The fast evolving SSU rDNA sequences have been used to show that some planktonic morphospecies comprise a number of cryptic species, usually with different geographical distributions and ecological preferences (Darling *et al.* 1999;

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de Vargas *et al.* 1999). The SSU rDNA sequences were also used for studying the diversity of benthic monothalamous foraminifera (Pawlowski *et al.* 2002), while the diversity of the shallow-water rotallid genus *Ammonia* was inferred from partial LSU rDNA sequences (Holzmann & Pawlowski 2000). The most rapidly evolving ITS rDNA region was used rather exceptionally in the case of some planktonic (de Vargas *et al.* 2001) and benthic (Tsuchiya *et al.* 2003; Schweizer *et al.* 2005; Grimm *et al.* 2007) species. The foraminiferal mitochondrial genes are not available yet (work is in progress).

Most molecular studies focused on planktonic and shallow-water foraminifera. Until now, there have been few genetic analyses of deep-sea benthic foraminifera (Gooday *et al.* 2004a; Gooday & Pawlowski 2004). One of these studies revealed cryptic diversity in a foraminiferal genus *Chilostomella* collected at depths between 600 and 1500 meters (Grimm *et al.* 2007). Bearing in mind the growing evidence for genetic differentiation among metazoan populations on bathyal continental margins (Etter *et al.* 1999), we expected that cryptic diversity would be prevalent among benthic foraminiferal species in the deep sea.

To test this hypothesis we analysed 223 sequences of the SSU and ITS ribosomal genes from three well-known calcareous, foraminiferal morphospecies (*Epistominella exigua*, *Cibicides wuellerstorfi*, *Oridorsalis umbonatus*), all of which are widely distributed on the ocean floor (Murray 1991). Our results show that Arctic and Antarctic populations of these species are very similar genetically, suggesting that molecular diversity of deep-sea meiofauna may be lower than expected.

Materials and methods

Sampling

The samples were taken from 30 sites in the Arctic Ocean and the Southern Ocean with distances between sampling sites ranging from 15 487 to 16 981 km and water depths ranging from 572 to 4975 m (Fig. S1, Table S1 in Supplementary material). In total, we examined 37 samples from Arctic Ocean, 24 samples from Weddell Sea, two samples from Norwegian Sea and two samples from NorthEast Atlantic. Foraminifera were collected during RV *Polarstern* cruises ANT-XIV/4 (ANDEEP project cruise II), ANT-XXII/3 (ANDEEP project cruise III) and ARK XXI-1b. Additional specimens (2) from northeast Atlantic were collected during the RRS *Charles Darwin* cruise 158 and Eurostrataform mission 64PE218. Coordinates and depths of sampling sites are given in Table S1 (Supplementary material). The foraminifera were picked individually on board from sediment samples sieved through serial 1-mm, 0.5-mm and 0.125-mm meshes. Some specimens were immediately transferred to microtubes containing 60 µL of

guanidine buffer while others were frozen. Most of the extractions have been done with a single specimen, except eight extractions of *Epistominella exigua* and four extractions of *Oridorsalis umbonatus*, in which up to six specimens were extracted together. Because some extractions contained more than one specimen, we used a term 'isolate' rather than 'specimen'.

DNA extraction, PCR, cloning and sequencing

DNA was isolated from single cells following the guanidine DNA extraction protocol (Pawlowski 2000). A fragment of the SSU rRNA gene was amplified by polymerase chain reaction (PCR) with the foraminiferal specific primer s14F3 [5'-ACG CA(AC) GTG TGA AAC TTG], starting at position 1807 in *Ammonia beccarii* sequence X86094 and universal eukaryotic primer sB (5'-TGA TCC TTC TGC AGG TTC ACC TAC), starting at position 2853 in *A. beccarii* sequence X86094. It has been re-amplified using nested foraminiferal specific primer s14F1 (5'-AAG GGC ACC ACA AGA ACC C), starting at position 1838 in *A. beccarii* sequence X86094, and universal primer sB. The complete ITS rDNA region was amplified using universal eukaryotic primer sBr (5'-GTA GGT GAA CCT GCA GAA GG) situated at the 3' end of the SSU rDNA (position 2853 in *A. beccarii* sequence X86094) and the foraminiferal specific primer 2TAIC (5'-CTC ACT CGA GCT GAT GTG) situated at the 5' end of the LSU rDNA. PCR products were purified using the High Pure PCR Purification Kit (Roche Diagnostics), ligated into pCR2.1 TOPO TA vector (Invitrogen) and cloned in XL-2 Blue Ultracompetent Cells (Stratagene). Clones were screened by amplification with the OriMaster kit (Eppendorf). Products were sequenced without previous purification using the ABI-PRISM Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems) and analysed with an ABI-3130xl DNA sequencer (Applied Biosystems), all according to the manufacturer's instructions. The new sequences were deposited in the EMBL/GenBank Nucleotide Sequence Database (accession numbers EF 653455–EF 653572).

Phylogenetic analyses

Twenty-one SSU and 202 ITS rDNA sequences were obtained from 29 isolates of *E. exigua*, 21 isolates of *Cibicides wuellerstorfi* and 16 isolates of *O. umbonatus*. For the majority of specimens, 3–5 clones were sequenced. The sequences were aligned using SEAVIEW (Galtier *et al.* 1996) and sequence divergence was calculated using PHYLOWIN (Galtier *et al.* 1996). Phylogenetic analysis of SSU rDNA sequences, including 23 sequences of other rotallid foraminifera, were performed using PHYML version 2.4 (Guindon & Gascuel 2003), with a GTR + Γ model (six categories, $\alpha = 0.107$). The ITS rDNA sequences were

Table 1 SSU rDNA and ITS rDNA sequence data for *Epistominella exigua*, *Cibicides wuellerstorfi* and *Oridorsalis umbonatus*

	<i>E. exigua</i>		<i>C. wuellerstorfi</i>		<i>O. umbonatus</i>	
	SSU	ITS	SSU	ITS	SSU	ITS
Number of analysed sequences	14	92	10	53	23	54
Total length of sequence alignment*	961	977	1023	1136	989	865
Percentage of variable sites	0.9%	12.1%	0.0%	9.2%	4.4%	5.1%
Percentage of informative sites	0.2%	1.9%	0.0%	1.8%	3.6%	0.9%
Maximum sequence divergence†	0.4%	1.4%	0.0%	1.1%	2.9%	1.1%
Maximum intraindividual sequence divergence‡	0.4%	1.2%	0.0%	1.0%	2.3%	0.9%

*Without gaps and ambiguous characters (estimated by PhyloWin, Galtier *et al.* 1996).

†Excluding the ITS of *C. wuellerstorfi* (5247) and *O. umbonatus* (5156, 5832), which sequences differed by 2.15% and 3.97%, respectively.

aligned and analysed separately for each species. Consensus ITS sequences for each individual specimen were obtained using SEAVIEW (Galtier *et al.* 1996). Haplotype ITS networks were established using TCS, with gaps considered as 5th state (Clement *et al.* 2000). The TCS program implements a statistical parsimony approach for estimating genealogical relationships among sequences using the method of Templeton *et al.* (1992). The program collapses identical sequences into haplotypes, calculates the frequencies of the haplotypes in the sample and connects them into a network. By taking into account the presence of ancestral haplotypes, low level of variation and possibility of recombination, the TCS method provides more accurate estimates of gene genealogy at the population level than traditional phylogenetic methods.

Statistical analyses

The extent of differentiation between populations was assessed by performing an Analysis of Molecular Variance (AMOVA, Excoffier *et al.* 1992), taking the genetic diversity between clones within individuals into account. For this analysis, sites with indels were discarded. For *E. exigua*, individuals sampled below 4650 m were assigned to a 'deep' Antarctic population, which was contrasted to the other samples of the south population and individuals from the north population. All significance levels were obtained by 10 000 permutations.

Results

SSU and ITS rDNA phylogeny

Initially, we analysed a fragment of the SSU rDNA that is commonly used to study the genetic diversity in foraminifera (Pawlowski 2000). The total length of this fragment varies between 961 and 1023 nucleotides (Table 1). We found no differences in the SSU sequences of all examined *Cibicides wuellerstorfi* and only a single

transition in two Antarctic *Epistominella exigua* belonging to a 'deep' abyssal population. The SSU sequences of *Oridorsalis umbonatus* were much more variable. This species shows an unusually high level of intraindividual polymorphism, with some copies of SSU rDNA diverging by up to 2.3% within the same individual (Table 1); however, most of these variations are present in a single variable region of the SSU. As shown in Fig. 1, the three examined species belong to the same clade of rotaliid foraminifera. This clade includes also species from different localities in northern hemisphere (*Stainforthia fusiformis*, *Bulimina marginata*, *Cibicides lobatulus*) but none of them are as genetically homogenous as the Arctic and Antarctic populations of *E. exigua* and *C. wuellerstorfi*.

To ensure that our first results were not due to an artefact caused by exceptionally slow evolutionary rates of the SSU rDNA of examined species, we analysed the ITS rDNA which is the most rapidly evolving region of a nuclear genome available for foraminifera. The total length of the ITS alignment ranges from 865 nucleotides in *O. umbonatus* to 1136 in *C. wuellerstorfi*, with most of the length variations observed in the ITS1 region. The number of variable sites was relatively elevated; however, only few of these sites were phylogenetically informative (Table 1). For each of the three species, the majority of ITS sequences were almost identical, with mean sequence divergence less than 1% and the maximum values of intraindividual polymorphism as high as the variability between individuals (Table 1). We found only three specimens (*Cibicides* isolate 5247, and *Oridorsalis* isolates 5156 and 5832) that were significantly different from all other representatives of their respective species. Excluding these three isolates, the specimens of each species, represented by ITS consensus sequences, formed a network in statistical parsimony analysis (Fig. 2). Genetic differentiation between Arctic and Antarctic populations was visible only in *O. umbonatus*, in which two populations differed by one transition and four indels. However, since the Southern Ocean population of this

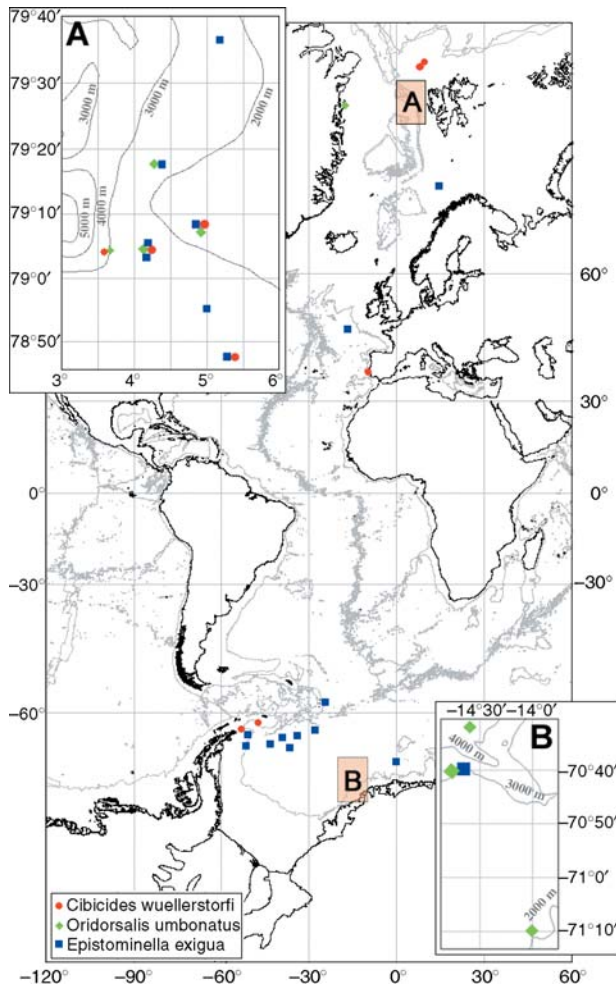


Fig. 1 Map indicating sampling localities of deep-sea foraminifera from the Arctic, Antarctic and North Atlantic.

species was represented by two specimens only, it is highly probable that more diverse ITS copies will be found later. No phylogenetic signal clearly distinguishes the Arctic and Antarctic populations in other two species.

Population genetics

A population-genetics approach was used to study the historical relationship between the polar populations of the three foraminifera species. The analysis of the genetic structure was performed by considering three populations of *E. exigua*: deep abyssal Antarctic *E. exigua* (4650–4975 m, $n = 28$ sequences); abyssal Antarctic *E. exigua* (2600–4600 m; $n = 13$) and bathyal northern *E. exigua* (1352–2784 m; $n = 40$). The AMOVA analysis revealed that *E. exigua* was significantly differentiated, since 28% of the genetic diversity ($P < 0.001$; 90% CI: 5.0–45.0) was due to differences between populations. The analysis of pairwise

distances, however, showed that this large differentiation was mainly due the divergence of the deep abyssal Antarctic population. Indeed, F_{CT} values between the deep abyssal population and the remaining southern and northern populations were equal to 0.28 and 0.17, respectively, while the abyssal Antarctic population differed much less from the northern bathyal population only ($F_{CT} = 0.12$, $P = 0.002$). For *O. umbonatus*, the northern and southern populations were significantly differentiated ($P = 0.04$) with an F_{CT} value of 0.33 (90% CI: 0.24–0.43), while the northern and southern populations of *C. wuellerstorfi* were interestingly not statistically differentiated, with a very low F_{CT} value of 0.03 ($P = 0.078$, 90% CI: 0.02–0.04).

Discussion

In contrast to the high degree of molecular diversity exhibited by some coastal benthic (Pawlowski *et al.* 2002) and oceanic planktonic (Darling *et al.* 2004) polar foraminifera, we found very limited genetic differentiation among two out of three deep-sea species that we examined. Each probably represents a huge metapopulation extending from pole to pole, living wherever a suitable habitat exists. A few sequences of *Epistominella exigua* and *Cibicides wuellerstorfi* obtained from the Porcupine Abyssal Plain, Setubal Canyon and Norwegian Sea (in red in Fig. 2) indicate that genetically similar individuals are present in the North Atlantic Ocean. Long-distance dispersal capabilities and high levels of gene flow have been observed in deep-sea hydrothermal vent macroinvertebrates with planktonic larval stages (Van Dover *et al.* 2002). Although planktotrophic larvae can disperse widely, various geographical and hydrological barriers seem to limit their distribution at larger spatial scales (Won *et al.* 2003; Hurtado *et al.* 2004). Among microbial eukaryotes, global dispersal has been suggested for hydrothermal vent flagellates (Atkins *et al.* 2000), marine picoeukaryotic algae (Slapeta *et al.* 2006) and some planktonic foraminifera (Darling *et al.* 2000). However, only bacteria and archaeobacteria have so far been reported to have genetically similar species in Arctic and Antarctic oceans (Hollibaugh *et al.* 2002; Brinckmeyer *et al.* 2003; Bano *et al.* 2004). Our study provides the first evidence for bipolar, and possibly global, distributions among benthic organisms of meiofaunal size.

How do we explain the high degree of genetic similarity of deep-sea populations? Size may be a critical factor. Some macrofaunal (animal) morphospecies appear to be confined to particular regions of the deep ocean (Grassle & Maciolek 1992; Allen & Sanders 1996). For microbial eukaryotes and small metazoans (< 1 mm), on the other hand, it has been proposed that the huge population sizes ensure ubiquitous distributions (Finlay 2002; Finlay *et al.* 2004). Genetic analyses of freshwater and marine

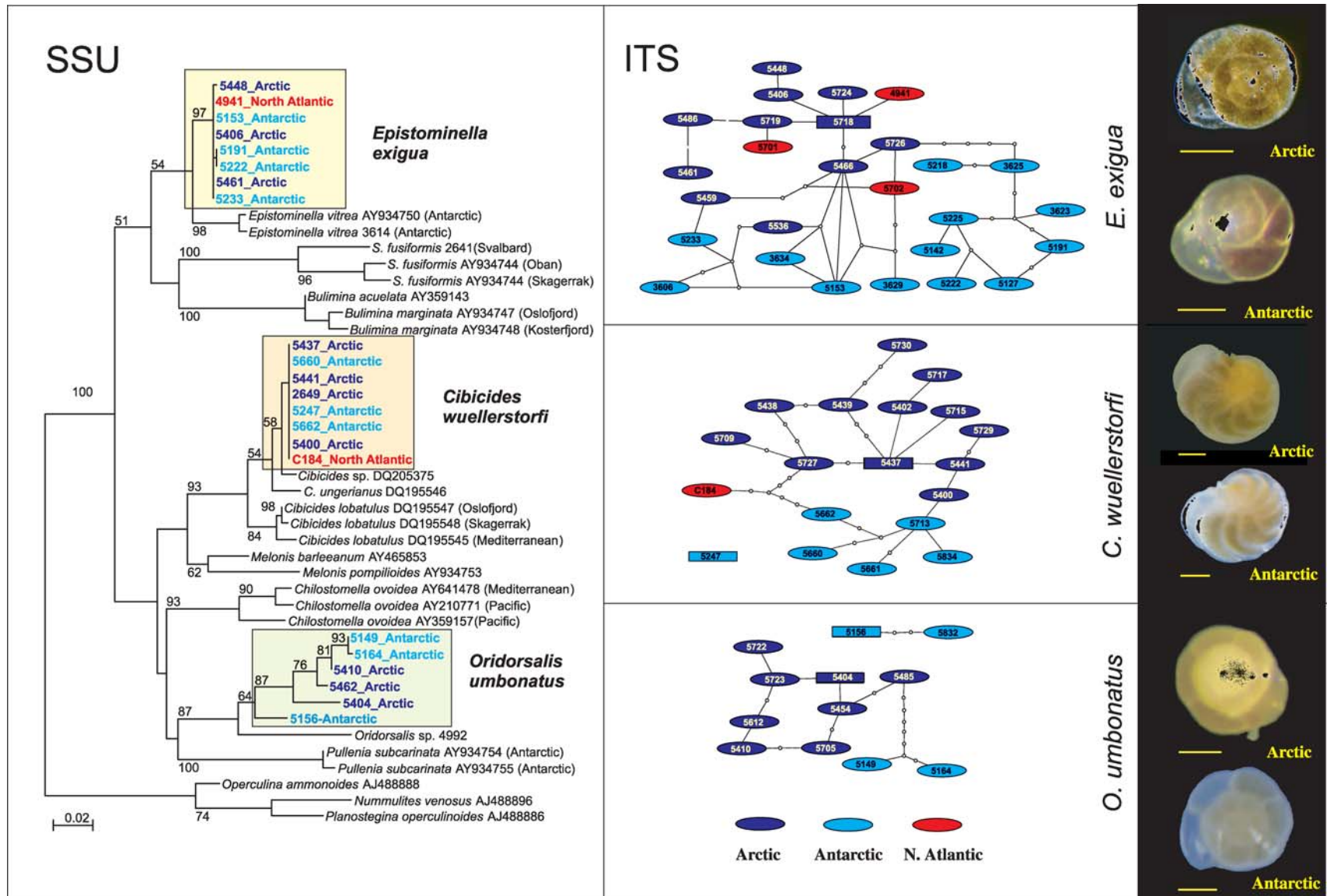


Fig. 2 Relationships between Arctic, Antarctic and North Atlantic deep-sea foraminifera (*Epistominella exigua*, *Cibicides wuellerstorfi*, *Oridorsalis umbonatus*) illustrated by the SSU rDNA-based maximum likelihood (ML) tree and the ITS rDNA-based haplotype networks obtained by rcs. Networks within each clade were delineated with 95% certainty, the gaps being treated as a fifth state. Each sequence in the ML tree and each haplotype in the rcs network are marked with DNA isolate number referred to the Table 1. The numbers at internal nodes of ML tree represent the bootstrap support values based on 100 replicates. The representative specimens of each species from Arctic and Antarctic are illustrated. Scale bar = 0.1 mm.

picoeukaryotes, averaging 1–2 microns in size (Atkins *et al.* 2000; Richards *et al.* 2005; Slapeta *et al.* 2006) have confirmed this observation for the very small organisms. The tests of the foraminiferal species that we examined are one to two orders of magnitude larger, in the size range of 100–500 microns. Nevertheless, the survival of microbe-sized foraminiferal propagules in habitats that are unfavourable to adults (Alve & Goldstein 2003) implies an ability to disperse widely in marine environments, and even small adult tests can be entrained by currents (Alve 1999). Thus, gene flow between Antarctic and Arctic populations via the Atlantic Ocean may be facilitated by the transport of juvenile or possibly small adult foraminifera by thermohaline circulation. The Weddell Sea is the source of Antarctic Bottom Water (AABW) which provides much of the deep water of the World Ocean. AABW flows into the southwest Atlantic, penetrates into the northwest Atlantic through the Vema Channel and finally into the northeast Atlantic via the Romanche Fracture Zone. The influence of AABW persists in a diluted form at least as far north as 56°N in the Rockall Trough (New & Smythe-Wright 2001). The bathymetric ranges of the species that we examined (*C. wuellerstofi* 1106–3485 m; *E. exigua* 1351–4975 m; *Oridorsalis umbonatus* 573–4407 m) are wide enough to enable them to cross most bathymetric obstacles.

The extremely low rates of evolution in some deep-sea foraminiferal species could be another factor explaining our results. The substitution rates in foraminiferal ribosomal genes can vary between taxonomic groups, and it has been shown that some planktonic species evolve more than 50 times faster than the benthic ones (Pawlowski *et al.* 1997; de Vargas & Pawlowski 1998). Similarly, it cannot be excluded that the deep-bottom species evolve much slower than the shallow-water ones. This sounds particularly plausible in view of the exceptional geological longevity of deep-sea foraminiferal species compared to coastal ones. Modern deep-sea assemblages emerged 15 million years in the Middle Miocene and many species have fossil records extending back to that period (Miller *et al.* 1992). One of our bipolar species, *E. exigua*, first appeared in the Oligocene, 35 million years ago (Thomas & Gooday 1996); yet the recent specimens of *E. exigua* are morphologically undistinguishable from their fossil ancestors. Is this morphological stasis related to a lower level of genetic variations in this species? If further molecular studies should confirm this hypothesis, then we could suggest that the observed homogeneity of Antarctic and Arctic populations reflects a combination of dispersal by thermohaline circulation and genetic slowdown.

Establishing the geographical ranges of deep-sea benthic species is a crucial step in extrapolating from local species diversity on the ocean floor to regional and global diversity. Yet even at the morphospecies level, ranges are poorly known for many taxa (Thistle 2003). Existing estimates

assume a relatively high rate of species turnover leading to a very diverse ocean (Grassle & Maciolek 1992). However, if wide species ranges are common (i.e. species turnover rates are low), and cryptic species uncommon, then the global benthic diversity may be much more modest than these estimates suggest. Our study clearly shows that genetic diversity in some important, typically abyssal foraminiferal species is minimal on a global scale. We predict that other deep-sea foraminifera with cosmopolitan distributions (Murray 1991; Gooday *et al.* 2004b) will also prove to be genetically homogeneous. This prediction implies slow rates of species turnover which would place constraints on the magnitude of regional and global foraminiferal diversity. Recent evidence suggests that even nematodes, a group which lacks dispersive larval stages, have wide morphospecies ranges in the abyssal deep sea (Lamshead & Boucher 2003), and so these results may apply to other meiofaunal taxa as well.

Our study has further important implications in palaeoceanography. The demonstration of gene flow across large distances among well-known, deep-sea calcareous foraminifera suggests that test morphology provides a sound basis for discriminating species. This conclusion reinforces the widespread use of the fossil shells of these protists to reconstruct the productivity and circulation of ancient oceans (Gooday 2003).

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References

- Allen JA, Sanders HL (1996) The zoogeography, diversity and origin of deep-sea protobranch bivalves of the Atlantic: the epilogue. *Progress in Oceanography*, **38**, 95–153.
- Alve E (1999) Colonization of new habitats by benthic foraminifera: a review. *Earth-Science Reviews*, **46**, 167–185.
- Alve E, Goldstein ST (2003) Propagule transport as a key method of dispersal in benthic foraminifera (Protista). *Limnology and Oceanography*, **48**, 2163–2170.
- Atkins MS, Teske AP, Anderson DR (2000) A survey of flagellate diversity at four deep-sea hydrothermal vents in Eastern Pacific ocean using structural and molecular approaches. *Journal of Eukaryotic Microbiology*, **47**, 400–411.
- Bano N, Ruffin S, Ransom B, Hollibaugh JT (2004) Phylogenetic composition of Arctic Ocean Archaeal assemblages and

- comparison with Antarctic assemblages. *Applied Environmental Microbiology*, **70**, 781–789.
- Brinckmeyer R, Knittel K, Jürgens J, Weyland H, Amann R, Helmke E (2003) Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. *Applied Environmental Microbiology*, **69**, 6610–6619.
- Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.
- Darling KF, Wade CM, Kroon D, Leigh Brown AJ, Bijma J (1999) The diversity and distribution of modern planktic foraminiferal small subunit ribosomal RNA genotypes and their potential as tracers of present and past ocean circulations. *Paleoceanography*, **14**, 3–12.
- Darling KF, Wade CM, Stewart IA, Kroon D, Dingle R, Leigh Brown AJ (2000) Molecular evidence for genetic mixing of Arctic and Antarctic subpolar populations of planktonic foraminifers. *Nature*, **405**, 43–47.
- Darling KF, Kucera M, Pudsey CJ, Wade CM (2004) Molecular evidence links cryptic diversification in polar planktonic protists to Quaternary climate dynamics. *Proceedings of the National Academy of Sciences, USA*, **101**, 7657–7662.
- Edgcomb VP, Kysela DT, Teske A, de Vera Gomez A, Sogin ML (2002) Benthic eukaryotic diversity in the Guaymas Basin hydrothermal vent environment. *Proceedings of the National Academy of Sciences, USA*, **99**, 7658–7662.
- Etter RJ, Rex MA, Chase MC, Quattro JM (1999) A genetic dimension to deep-sea biodiversity. *Deep-Sea Research*, **46**, 1095–1099.
- Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.
- Finlay BJ (2002) Global dispersal of free-living microbial eukaryote species. *Science*, **296**, 1061–1063.
- Finlay BJ, Esteban GF, Fenchel T (2004) Protist diversity is different? *Protist*, **155**, 15–22.
- Galtier N, Gouy M, Gautier C (1996) SEAVIEW and PHYLO_WIN, two graphic tools for sequence alignment and molecular phylogeny. *CABIOS*, **12**, 543–548.
- Gooday AJ (2003) Benthic foraminifera (Protista) as tools in deep-water palaeoceanography: environmental influences on faunal characteristics. *Advances in Marine Biology*, **46**, 1–90.
- Gooday AJ, Pawlowski J (2004) *Conqueria laevis* General and sp. nov., a new soft-walled, monothalamous foraminiferan genus from the deep Weddell Sea. *Journal of the Marine Biological Association of the UK*, **84**, 919–924.
- Gooday AJ, Holzmann M, Guiard J, Cornelius N, Pawlowski J (2004a) A new monothalamous foraminiferan from 100 to 6300 m water depth in the Weddell Sea: morphological and molecular characterization. *Deep-Sea Res.*, **II** (51), 1603–1616.
- Gooday AJ, Hori S, Todo T, Okamoto T, Kitazato H, Sabbatini A (2004b) Soft-walled, monothalamous benthic foraminiferans in the Pacific, Indian and Atlantic Oceans: aspects of biodiversity and biogeography. *Deep-Sea Research*, **I** (51), 33–53.
- Grassle JF, Macirolek J (1992) Deep-sea species richness: regional and local diversity estimates from quantitative bottom samples. *American Naturalist*, **139**, 313–341.
- Grimm GW, Stögerer K, Ertan KT *et al.* (2007) Diversity of rDNA in *Chilostomella*: Molecular differentiation patterns and putative hermit types. *Marine Micropaleontology*, **62**, 75–90.
- Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, **52**, 696–704.
- Hollibaugh JT, Bano N, Ducklow HW (2002) Widespread distribution in polar oceans of a 16S rRNA gene sequence with affinity to Nitrospira-like ammonia-oxidizing bacteria. *Applied Environmental Microbiology*, **68**, 1478–1484.
- Holzmann M (2000) Species concept in foraminifera: Ammonia as a case study. *Micropaleontology*, **46**, 21–37.
- Holzmann M, Pawlowski J (2000) Taxonomic relationships in the genus *Ammonia* (Foraminifera) based on ribosomal DNA sequences. *Journal of Micropaleontology*, **19**, 85–95.
- Hurtado LA, Lutz RA, Vrijenhoek RC (2004) Distinct patterns of genetic differentiation among annelids of eastern Pacific hydrothermal vents. *Molecular Ecology*, **13**, 2603–2615.
- Lambshhead JDP, Boucher G (2003) Marine nematode deep-sea biodiversity – hyperdiverse or hype? *Journal of Biogeography*, **30**, 475–485.
- López-García P, Philippe H, Gail F, Moreira D (2003) Autochthonous eukaryotic diversity in hydrothermal sediment and experimental microcolonizers at the Mid-Atlantic ridge. *Proceedings of the National Academy of Sciences, USA*, **100**, 697–702.
- Miller KG, Katz ME, Berggren WA (1992) Cenozoic deep-sea benthic foraminifera; a tale of three turnovers. In: *BENTHOS 90*, pp. 67–75. Tokai University Press, Sendai.
- Moreira D, López-García P (2002) The molecular ecology of microbial Eukaryotes unveils a hidden world. *Trends in Microbiology*, **10**, 31–38.
- Murray JW (1991) *Ecology and Paleocology of Benthic Foraminifera*. Longman Scientific and Technical, Essex.
- New AL, Smythe-Wright D (2001) Aspects of the circulation in the Rockall Trough. *Continental Shelf Research*, **21**, 777–810.
- Pawlowski J (2000) Introduction to the molecular systematics of foraminifera. *Micropaleontology*, **46** (Suppl. 1), 1–12.
- Pawlowski J, Bolivar I, Fahrni JF, de Vargas C, Gouy M (1997) Extreme differences in rates of molecular evolution of foraminifera revealed by comparison of ribosomal DNA sequences and the fossil record. *Molecular Biology and Evolution*, **14**, 498–505.
- Pawlowski J, Fahrni JF, Brykczynska U, Habura A, Bowser SS (2002) Molecular data reveal high taxonomic diversity of allogromiid Foraminifera in Explorers Cove (McMurdo Sound, Antarctica). *Polar Biology*, **25**, 96–105.
- Richards TA, Veprikitskiy AA, Gouliamova DE, Nierzwicki-Bauer SA (2005) The molecular diversity of freshwater picoeukaryotes from an oligotrophic lake reveals diverse, distinctive and globally dispersed lineages. *Environmental Microbiology*, 1–13.
- Schweizer M, Pawlowski J, Duijnste IAP, Kouwenhoven TJ, van der Zwaan GJ (2005) Molecular phylogeny of the foraminiferan genus *Uvigerina* based on ribosomal DNA sequences. *Marine Micropaleontology*, **57**, 51–67.
- Slapeta J, Lopez-Garcia P, Moreira D (2006) Dispersal and ancient cryptic species in the smallest marine eukaryotes. *Molecular Biology and Evolution*, **23**, 23–29.
- Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, **132**, 619–633.
- Thistle D (2003) In: *The Deep Seafloor. Ecosystems of the World* (ed. Tyler PA). Elsevier, Amsterdam.
- Thomas E (1992) Cenozoic deep-sea circulation: evidence from deep-sea benthic foraminifera. *Antarctic Research Series*, **56**, 141–165.

- Thomas E, Gooday AJ (1996) Cenozoic deep-sea benthic foraminifera: tracers for changes in oceanic productivity. *Geology*, **24**, 355–358.
- Tsuchiya M, Kitazato H, Pawlowski J (2003) Analysis of internal transcribed spacer of ribosomal DNA reveals cryptic speciation in *Planoglabratella opercularis*. *Journal of Foraminiferal Research*, **33**, 285–293.
- Van Dover CL, German CR, Speer KG, Parson LM, Vrijenhoek RC (2002) Evolution and biogeography of deep-sea vent and seep invertebrates. *Science*, **295**, 1253–1257.
- de Vargas C, Pawlowski J (1998) Molecular versus taxonomic rates of evolution in planktonic foraminifera. *Molecular Phylogenetics and Evolution*, **9**, 463–469.
- de Vargas C, Norris R, Zaninetti L, Gibb SW, Pawlowski J (1999) Molecular evidence of cryptic speciation in planktonic foraminifers and their relation to oceanic provinces. *Proceedings of the National Academy of Sciences, USA*, **96** (101), 2864–2868.
- de Vargas C, Renaud S, Hilbrecht H, Pawlowski J (2001) Pleistocene adaptive radiation in *Globorotalia truncatulinoides*: genetic, morphologic, and environmental evidence. *Paleobiology*, **27**, 104–125.
- Won Y, Young CR, Lutz RA, Vrijenhoek RC (2003) Dispersal barriers and isolation among deep-sea mussel populations (Mytilidae: Bathymodiolus) from eastern Pacific hydrothermal vents. *Molecular Ecology*, **12**, 169–184.
- Zachos J, Pagani M, Sloan L, Thomas E, Billups K (2001) Trends, rhythms and aberrations in global climate 65 Ma to present. *Science*, **292**, 686–693.

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Supplementary material

The following supplementary material is available for this article:

Table S1 List of isolates of the three examined foraminiferal species with detailed description of sampling sites.

This material is available as part of the online article from:
<http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-294X.2007.03465.x>
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