Molecular evidence for genetic mixing of Arctic and Antarctic subpolar populations of planktonic foraminifers

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Bipolarity, the presence of a species in the high latitudes separated by a gap in distribution across the tropics, is a well-known pattern of global species distribution. But the question of whether bipolar species have evolved independently at the poles since the establishment of the cold-water provinces 16–8 million years ago, or if genes have been transferred across the tropics since that time, has not been addressed. Here we examine genetic variation in the small subunit ribosomal RNA gene of three bipolar planktonic foraminiferal morphospecies. We identify at least one identical genotype in all three morphospecies in both the Arctic and Antarctic subpolar provinces, indicating that trans-tropical gene flow must have occurred. Our genetic analysis also reveals that foraminiferal morphospecies can consist of a complex of genetic types. Such occurrences of genetically distinct populations within one morphospecies may affect the use of planktonic foraminifers as a palaeoceanographic proxy for climate change and necessitate a reassessment of the species concept for the group.

Patterns of bipolar (anti-tropical) distributions are found in a diverse range of terrestrial and marine groups[1,2]. Bipolar organisms were first observed by biologists during the mid-nineteenth century[3–5], and are now known to be recurrent phenomena through considerable spans of geological time[1]. Within the marine environment, bipolar species are observed in many planktonic groups. It remains unclear whether they are isolated within their respective high-latitude water masses, or whether trans-tropical transit occurs; such transit would allow intermixing and consequent gene flow between the populations from the northern and the southern hemispheres. Morphology alone can provide few clues, as morphological identity may be due to convergent or parallel evolution in similar environments. Molecular data enable us to investigate this issue directly. If high-latitude populations of bipolar taxa were isolated from one another, different mutations would be expected to accumulate over time, leading to genetic divergence between the two populations. Conversely, if trans-tropical mixing occurs resulting in genetic exchange, then the two populations would be expected to be genetically homogeneous.

Advances in planktonic foraminiferal molecular genetic analysis[6,7] provide a tool with which to investigate genetic interchange in marine planktonic organisms exhibiting a bipolar distribution[8]. The planktonic foraminifera have an outstanding fossil record, and their calcitic shells (tests) form one of the most widely used microfossil assemblages for the reconstruction of past oceanic environments. They are globally distributed, and their component taxa are found within distinct faunal provinces that broadly correspond to the main hydrographic features of the ocean[8] (Fig. 1). Planktonic foraminiferal nucleotide sequence data now provide a new dimension for the investigation of oceanic gene flow[9] which will enable us to answer the key question of whether genetic exchange occurs between high-latitude populations.

Molecular evolution of planktonic foraminifera

Small subunit ribosomal DNA (SSU rDNA) sequences are highly variable in planktonic foraminifera, and phylogenetic analysis has revealed that many morphologically defined species (morphospecies) of planktonic foraminifera in fact represent complexes of different and often highly divergent genetic types (genotypes)[9]. Some of these genotypes are now considered to be cryptic sibling species[6,10,11], a commonly observed phenomenon amongst marine taxa[12]. We have analysed SSU rDNA sequences of cool-water representatives of three planktonic foraminiferal morphospecies—*Globigerina bulloides* d'Orbigny, *Turborotalita quinqueloba* (Natland) and *Neogloboquadrina pachyderma* (Ehrenberg)—that exhibit a predominantly disjunct, bipolar distribution[8]. Arctic specimens were collected from the south of Iceland to the southeast Greenland margin along two transects, and Antarctic specimens were collected from eight sites along a transect between the Falkland Islands and the Antarctic Peninsula (Fig. 1; Methods).

A foraminiferal SSU rDNA phylogeny incorporating these species is shown in Fig. 2. The phylogeny is based on a restricted data set comprising only those nucleotide positions that could be aligned across all 22 genera (see Fig. 2 legend for details). The placement of *G. bulloides* and *T. quinqueloba* within the spinose cluster in the molecular phylogeny is consistent with the evolutionary relationships inferred from palaeontological data[13,14]. The location of right- and left-coiling *N. pachyderma* within the benthic region of the tree is, however, inconsistent with palaeontological data, providing further evidence that the extant planktonic foraminifera are not monophyletic in origin[6,7].

Each of the *G. bulloides*, *T. quinqueloba* and *N. pachyderma* morphospecies consist of a complex of distinct genotypes (Fig. 2 and Table 1; individual genotypes have identical SSU rDNA sequences). Both left- and right-coiling *N. pachyderma* genotypes cluster with *Neogloboquadrina dutertrei* which forms the warm-water member of the morphological complex[15] (Fig. 2). However, the relationships observed within this cluster are surprising. Unexpectedly, *N. pachyderma* left-coiling genotypes cluster together with

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N. dutertrei, which is right-coiling, and away from the *N. pachyderma* right-coiling genotype. Thus the genetic data we report here confirm that coiling direction in *N. pachyderma* is associated with genetic divergence[16] and not with temperature during development.

Bipolar SSU rDNA genotypes in foraminiferal morphospecies

When considering identity between genotypes, we utilize not only the conserved regions used for the phylogenetic analysis but also the remaining ~500 nucleotide sites from the highly informative variable regions (Fig. 3). Such comparisons of the entire SSU rDNA fragment revealed that all three morphospecies examined had at least one identical SSU rRNA genotype in both the Arctic and Antarctic subpolar provinces (Figs 2 and 3, and Table 1). There were two separate bipolar genotypes (types IIa and IIb) in *G. bulloides*, one bipolar genotype (type IIa) in *T. quinqueloba* and one bipolar genotype (type R) in *N. pachyderma* right-coiling.

The *G. bulloides* genotypes, types IIa and IIb, differed from one another at 36 of 980 nucleotide sites (variable and conserved regions; Fig. 3 and data not shown). There were also two other closely related genotypes in the *G. bulloides* cluster. Type IIc was found only in the subantarctic province, and differed from the type IIa bipolar genotype at 31 sites and from the type IIb bipolar genotype at 49 sites (Fig. 3 and data not shown). Type IId was found in the transitional zone[9],and differed from the type IIa bipolar genotype at 43 sites and from the type IIb bipolar genotype at 18 sites (Fig. 3 and data not shown). Two further genotypes were also found in *T. quinqueloba* that were closely related to the bipolar type IIa: type IIb in the subarctic and type IIc in the subantarctic provinces. Type IIb differs from type IIc in only 8 of 1,168 nucleotide sites (variable and conserved) and these differ from the bipolar Type IIa genotype at 83 and 82 nucleotide sites, respectively. The Antarctic type III and type IV genotypes of *N. pachyderma* (left-coiling) differ from one another by as many as 145 nucleotide sites (Fig. 3 and data not shown). Thus, where detailed comparisons may be made across both conserved and variable regions, there is a continuum of genetic divergence in the cool-water 'genotype clusters' extending from identical bipolar genotypes through very closely related variants to others which differ at more than 10% of sites in the region sequenced.

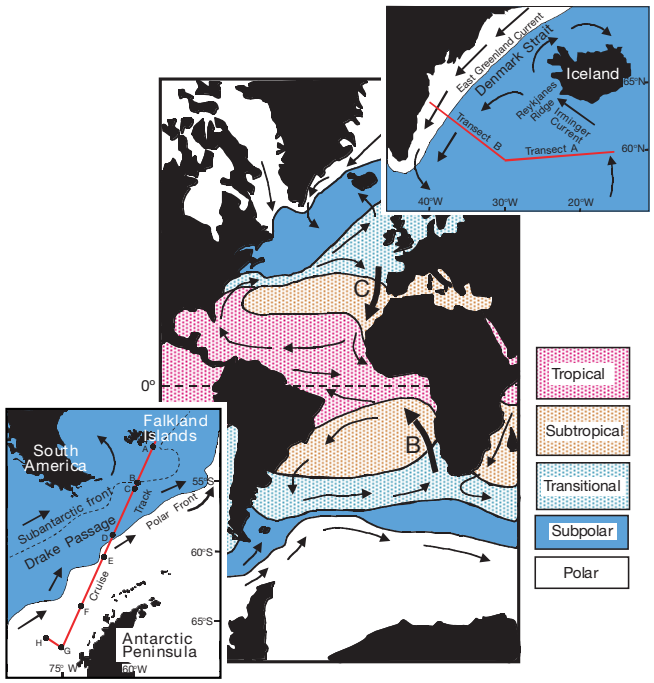

Figure 1 Sampling localities and distribution of the high-latitude morphospecies. The pictorial map (main figure) shows the five zoogeographical planktonic foraminiferal faunal provinces[8]— polar, subpolar, transitional, subtropical and tropical—of the North and South Atlantic Ocean. The three planktonic foraminiferal morphospecies *G. bulloides*, *T. quinqueloba* and *N. pachyderma* (right- and left-coiling) used in this study are found predominantly in the polar, subpolar and transitional provinces, and consequently exhibit a bipolar (anti-tropical) distribution[8]. The cool-water eastern boundary cold currents, the Canary and Benguela (C and B, large arrows), strengthen seasonally and flow equatorwards, bringing cool water and transitional morphospecies into the subtropical province where they bloom in the transient cool seasonal upwelling systems of the subtropical/tropical eastern Atlantic. However, there is always a clear belt of warm tropical water moving seasonally north and south as the seasons build and decay. An outline of the present-day Atlantic Ocean surface circulation patterns (small arrows) are shown. The expanded maps (insets) highlight the sampling transect, sample sites (see Methods) and surface currents within the Arctic and Antarctic subpolar/polar provinces.

Genetic exchange between high-latitude populations

Foraminiferal sequence data have allowed us to address the fundamental question of whether gene flow occurs between bipolar-distributed planktonic marine organisms. The presence of identical foraminiferal sequences in the high latitudes of each hemisphere, coupled with their extensive fossil record, has allowed us to establish that trans-tropical genetic exchange has been recent. The well-calibrated foraminiferal biostratigraphic record permits the estimation of the expected level of divergence between two sequences over time in the absence of gene flow. Rates of evolution for foraminiferal SSU rRNA genes have been estimated by both our group[9] and others[17,18]. These estimates have given approximate rates of substitution of $(2.6–4.6) \times 10^{-9}$ substitutions per site per year for the spinose planktonic group[9,18]. Taking an average, we would expect 1.82 substitutions per million years in the conserved 505-base-pair (505-b.p.) region alone, or 29–15 substitutions since the cold-water provinces were established 16–8 million years ago in the Middle to Late Miocene epoch[19].

In addition to the conserved regions, intra-specific comparisons can be made on the variable regions that cannot be utilized for between-morphospecies analysis (Fig. 2). On the basis of our data, the rate of substitution over the variable regions is estimated to be at least 10 times greater than the rate for the conserved regions: thus we expect between 160 and 320 substitutions over the entire sequence since the establishment of the polar provinces. These calculations establish that a substantial degree of divergence would be expected if sequences were isolated for between 8 and 16 million years, yet we have identified examples of complete sequence identity between the high-latitude morphospecies. This strongly suggests that genetic exchange has occurred recently relative to the establishment of the cold-water provinces. This conclusion is reinforced by the fact that it was observed independently in three separate lineages.

Trans-tropical gene flow

Genetic exchange between the high latitudes can only be explained by trans-equatorial transit. The tropical ocean is, however, a formidable barrier for cool-water genotypes to cross (Fig. 1). Foraminiferans do not encyst—a process that provides resistance to inhospitable environments in many other planktonic groups. It is therefore unlikely that the subpolar genotypes would tolerate the high surface water temperatures of the western tropical regions. Yet for complete bipolar genetic mixing to occur, foraminifers crossing the tropics must also return to the high latitudes within the warm tropical ocean gyral surface currents (Fig. 1).

How then can gene flow occur between the cooler-water populations across the tropical Atlantic? The present-day oceanographic setting provides a perspective in which to investigate this issue. The eastern boundaries of the subtropical Atlantic Ocean off west Africa are associated with cool boundary currents (shown as B and C in Fig. 1) which act as corridors for the introduction of cool-water

genotypes into the cool seasonal upwelling zones of the subtropical region, where they may bloom in significant numbers. The upwelling cells could then provide a 'stepping stone' for the transit of the cool-water genotypes into the permanent equatorial upwelling zone (which is 2–9 °C cooler than surrounding surface water). Yet genetic exchange between populations within these waters alone cannot account for the genetic homogeneity observed between the subpolar populations. For bipolar genetic exchange to occur, the foraminifera would have to negotiate the high tropical sea surface temperatures of the western equatorial region into which they are passively carried. This region is most unlikely to be conducive to the survival of the cool-water genotypes, either as a direct result of temperature

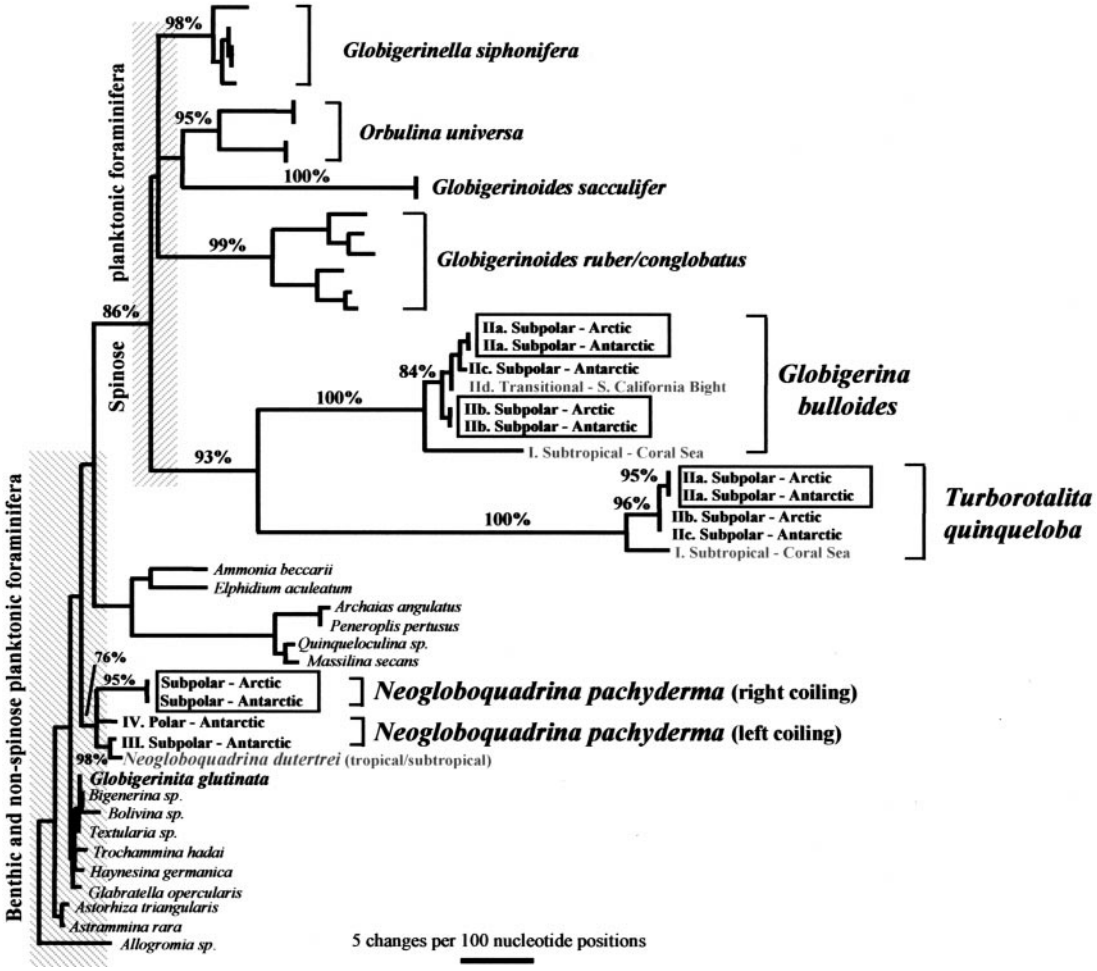

Figure 2 Foraminiferal SSU rDNA phylogeny, highlighting the evolutionary placement of the planktonic foraminiferal high-latitude morphospecies. The phylogeny shows the genotypic variants identified for *G. bulloides* d'Orbigny ($n = 55$), *T. quinqueloba* (Natland) ($n = 24$) and *N. pachyderma* (Ehrenberg) right-coiling ($n = 26$) and left-coiling ($n = 31$). *N. pachyderma* falls within the benthic and non-spinose planktonic foraminiferal region of the tree, and *G. bulloides* and *T. quinqueloba* fall within the spinose planktonic foraminiferal group (shaded boxes). Genotypes of *G. bulloides* and *T. quinqueloba* isolated from the transitional and subtropical regions are shown in grey. *N. dutertrei*, the warm-water member of the *Neogloboquadrina* morphological complex is also shown in grey. High-latitude genotypes of *G. bulloides*, *T. quinqueloba* and *N. pachyderma* (right- and left-coiling) are shown in black. Genotypes which are identical in both the Arctic and Antarctic (bipolar genotypes) are boxed. Bootstrap values (expressed as a percentage) are shown for the main branches when supported in >70% of replicates. Interrelationships within the transitional and subpolar *G. bulloides* cluster are unresolved. The number of specimens obtained for each genotype and their location (Fig. 1) are shown in Table 1.

Table 1 Number and location of specimens obtained for each genotype.

Morphospecies	Location	Genotype	Specimens	Sample Sites
G. bulloides	Subpolar–Arctic ($n = 44$)	IIa	32	Subarctic transect B
		IIb	12	Subarctic transect A
	Subpolar–Antarctic ($n = 11$)	IIa	5	Site A + B + C + D
		IIb	4	Site D
		IIc	2	Site A
T. quinqueloba	Subpolar–Arctic ($n = 17$)	IIa	8	Subarctic transect A + B
		IIb	9	Subarctic transect A + B
	Subpolar–Antarctic ($n = 7$)	IIa	5	Site C + D
		IIc	2	Site A
N. pachyderma	Subpolar–Arctic ($n = 24$)	Right	24	Subarctic transect A + B
	Subpolar–Antarctic ($n = 14$)	Right	2	Site A
		Left III	12	Site B + C + D
	Polar–Antarctic ($n = 19$)	Left III	3	Site E + F
		Left IV	16	Site E + F + G + H

Locations identified in Fig. 1.

or as a consequence of other physical/biological requirements being limiting within the ecosystem. It is possible that transit occurs through these regions by tropical submergence into cooler levels of the thermocline. The route and timing of transit is likely to be morphospecies-specific due to differences in their capacity to survive transit across the tropics. Whether gene flow is uni-directional or bi-directional remains to be determined.

Trans-equatorial transit and subsequent genetic exchange is likely to increase substantially during cooling periods associated with glacial cycling in the Quaternary period (the past 1.8 million years), and indeed the low-latitude sedimentary record shows the frequent occurrence of planktonic foraminiferal subpolar assemblages within the equatorial zone during these cooling cycles[20]. Revised estimates of tropical cooling during the Last Glacial Maximum (18,000 ^{14}C years before present)[21–24] indicate a substantial drop in temperature of 3–5 °C in the tropical Atlantic, accompanied by an equatorward extension and intensification of the cool-water boundary currents[25–27] (B and C in Fig. 1). This would have increased the potential for genetic exchange between the bipolar populations. It is, however, possible that genetic exchange could be continuous and may be occurring at present. The available data are unable to resolve timescales of this magnitude, and the issue can only be addressed by sampling living planktonic foraminifers in the subtropical/tropical regions to establish the distribution pattern, proportion and seasonality of cool-water genotypes in the present day.

Speciation in planktonic foraminifers

Whereas molecular studies have shown that many planktonic foraminiferal morphospecies comprise more than one genetically distinct population which may warrant 'cryptic' species status[6,9,10,11], we have also shown that planktonic foraminiferal populations intermix on a global scale (Arctic–Antarctic, this study; Pacific–Atlantic, refs 6 and 9). This makes any simple speciation model based on geographical isolation (allopatric speciation) difficult to sustain. Yet allopatric isolation may occur in the marine environment, and there are many examples of population subdivision in marine species despite a high dispersal potential[28]. Subdivision may result from the sporadic and discontinuous seeding of seasonal upwelling cells or alternating ocean circulation patterns associated with past climate change. Such isolation could give rise to genetically distinct populations.

In order for speciation to occur, genetic divergence must be accompanied by reproductive isolation[29]. In marine organisms with broadcast release of gametes such as the planktonic foraminifera, this may be biological, involving, for example, synchrony of gametogenesis[10,28,30]. Alternatively, isolation may be molecular and there is evidence that this may be achieved through high rates of evolution in proteins involved in gamete recognition[31,32]. Recent developments in population genetic theory of sympatric speciation[33,34] suggest further possibilities for speciation in the marine planktonic environment without the necessary initial requirement for allopatry. We conclude that there are several currently recognized mechanisms that could be involved in the speciation of planktonic foraminifera, but the main problem with interpretation of these processes is lack of knowledge of the structuring of their environment in either biotic or abiotic terms. The discovery of genotype complexes in the planktonic foraminifera does, however, necessitate the urgent reassessment of species concepts for the group.

Distribution of planktonic foraminiferal cool-water genotypes

The high-latitude genotypes are not ubiquitous in the surface waters throughout the cool-water provinces. For example, in the Arctic subpolar region, *G. bulloides* type IIa was largely confined to transect B and type IIb to transect A (Fig. 1, Table 1). Given that sampling density was relatively low in the Antarctic subpolar region (due to adverse weather conditions during sampling), *G. bulloides* type IIa was distributed from sites A–D—from the Falkland Islands to the polar front—but type IIb was found only at the polar front (site D, Fig. 1, Table 1). Similarly, *N. pachyderma* left-coiling type III was mostly found between the Falkland Islands and the polar front (sites B–D, Table 1) and type IV was isolated in the true polar waters (sites E–H, Table 1). Although we have no direct evidence at present, such distribution patterns may indicate that individual genotypes are adapted to specific hydrographic or trophic environments; we note that de Vargas *et al.*[11] found a close correlation between the distribution of sibling species and trophic regimes in the spinose species *Orbulina universa.*

Implications for palaeoceanography and palaeoclimate

The morphological, chemical and stable-isotope differences associated with planktonic foraminiferal calcitic shells are used extensively by palaeoceanographers for climate reconstruction. For such studies, the assumption is made that each morphospecies represents a genetically continuous species with a single environmental/habitat preference. If this is not the case—as indicated by this study and others[6,9,10,11]—stable-isotope and geochemical analyses of planktonic foraminiferal shells, and census-based transfer-function techniques derived from such pooled data, must include significant

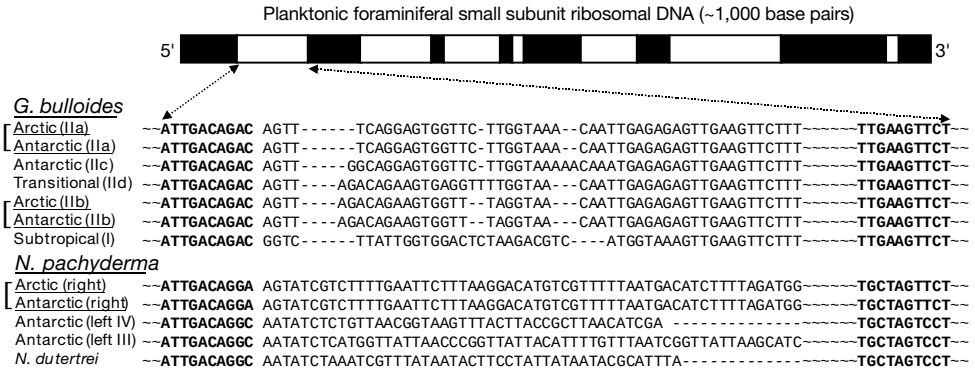

Figure 3 Sequence variability in the SSU rRNA gene. Shown is a schematic representation of the ~1,000-b.p. region of the SSU rRNA gene sequenced for the planktonic foraminifers. Conserved regions alignable across all foraminiferal taxa (505 b.p.) and used in phylogenetic tree construction (see Fig. 2) are highlighted in black. Variable regions, not used in phylogenetic tree construction because they could not be aligned across all taxa, are highlighted in white. Examples of the sequence variation observed in one of the variable regions are given for *G. bulloides* and *N. pachyderma/dutertrei.* Considerable variability is observed within the variable region, even among closely related cool-water genotypes. The bipolar genotypes (marked with a bracket and underlined) are identical across the entire sequenced region. (~ denotes sequences not displayed; – denotes gaps introduced in aligning sequences).

noise, if not error. If genetic differences can be correlated with specific environment and habitat preferences, and the genotypes differentiated in the fossil record, a new level of precision for climate modelling could be achieved. □

Methods

Sampling localities

Arctic subpolar specimens of *G. bulloides*, *T. quinqueloba* and *N. pachyderma* right-coiling were collected on board RV *Professor Logachev* along two transects (A and B, Fig. 1). Transect A ran from a point 59° 58′ N, 11° 34′ W (south of Iceland) to 58° 56′ N, 30° 24′ W (above the Reykjanes ridge). Transect B ran from the Reykjanes ridge to the southeast Greenland margin at 63° 48′ N, 40° 20′ W across the Denmark Strait. Specimens were obtained by pumping continually from 4.5 m depth through a plankton net suspended on deck. Antarctic subpolar specimens of *G. bulloides*, *T. quinqueloba* and *N. pachyderma* left- and right-coiling were collected from eight sites (A–H) between the Falkland Islands (53° 21′ S, 58° 20′ W) and west of the Antarctic Peninsula (65° 36′ S, 77° 39′ W) (inset map, Fig. 1). Specimens were obtained by pumping from 6 m depth through a 63-μm filter on board RRS *James Clark Ross* (JR19). Transitional specimens of *G. bulloides* were collected ~2 km NNE of the Santa Catalina Marine Science Centre, Santa Catalina Island, California (33° N, 118° W), and subtropical specimens of *G. bulloides* and *T. quinqueloba* were collected off the Great Barrier Reef , Australia (0.8 nautical miles due east of Ribbon Reef 10).

Isolation and sequencing of SSU genes

DNA extraction, amplification by polymerase chain reaction and direct automated sequencing of an ~1,000-b.p. region of the terminal 3′ end of the foraminiferal SSU rRNA gene was as described previously[6,9,35]. The SSU rRNA genes form a large multigene family in all organisms where data are available. Within the gene family, as mutations accumulate in individual copies, a level of homogeneity is maintained between copies by the process of concerted evolution. All data presented here have been derived from a consensus sequence amplified from the gene family of a single individual using a direct sequencing approach. This has the advantage of eliminating the risk of sampling non-orthologous copies, as different copies would be detected as ambiguities in the consensus sequence. Using this approach we have detected no evidence of ambiguity in any of the genotypes presented here, apart from very minor variability between copies within individuals of left-coiling *N. pachyderma*.

Sequence analysis

Partial SSU rDNA sequences were aligned manually within version 2.2 of the Genetic Data Environment (GDE) package[36]. 505 unambiguously aligned nucleotide sites were employed in phylogeny reconstruction. The regions included are indicated schematically in Fig. 3 ("conserved regions"). A neighbour-joining[37] phylogeny was generated using γ rate corrected F84 distances within PAUP* version 4.0d64 (kindly provided by D. L. Swofford[38]). α values for rate correction were estimated within PAUP*. Bootstrap resampling[39] (2,000 replicates) was employed to assign support to the neighbour-joining tree. Cool-water planktonic foraminiferal taxa are placed within a background of additional planktonic[9] and benthic[17] foraminifers. The tree was rooted on *Allogromia*, a membranous-walled benthic foraminifer which is thought to have diverged early in the history of the clade[40].

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