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Diversity and geographic distribution of benthic foraminifera: a molecular perspective

Jan Pawlowski · Maria Holzmann

Abstract The diversity and distribution of modern benthic foraminifera has been extensively studied in order to aid the paleoecological interpretation of their fossil record. Traditionally, foraminiferal species are identified based on morphological characters of their organic, agglutinated or calcareous tests. Recently, however, new molecular techniques based on analysis of DNA sequences have been introduced to study the genetic variation in foraminifera. Although the number of species for which DNA sequence data exist is still very limited, it appears that morphology-based studies largely underestimated foraminiferal diversity. Here, we present two examples of the use of DNA sequences to examine the diversity of benthic foraminifera. The first case deals with molecular and morphological variations in the well-known and common calcareous genus *Ammonia*. The second case presents molecular diversity in the poorly documented group of monothalamous (single-chambered) foraminifera. Both examples perfectly illustrate high cryptic diversity revealed in almost all molecular studies. Molecular results also confirm that the majority of foraminiferal species have a restricted geographic distribution and that globally distributed species are rare. This is in opposition to the theory that biogeography has no impact on the diversity of small-sized eukaryotes. At least in the case of foraminifera, size does not seem to have a main impact on dispersal capacities. However, the factors responsible for the dispersal of foraminiferal species and the extension of their geographic ranges remain largely unknown.

Keywords Benthic foraminifera · Molecular and morphological variation · Geographic distribution

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Introduction

There are about 5,000 species of modern (living) foraminifera and more than 50,000 fossil species (Debenay et al. 1996). Almost all these species have been described based on morphological characters of their test. Compared to many other protists, biological features such as cell structures or life cycles are usually not considered in foraminiferal systematics (Pawlowski and Lee 1992). Amazingly, the majority of foraminiferal species has never been observed alive. It is a common practice to sort and identify hard-shelled foraminifera from dried sediment samples while organic-walled allogromiids are preserved in formalin or alcohol fixed samples. To recognize living foraminiferal specimens, sediment samples are regularly stained with Rose Bengal, but the effectiveness of this method is quite disputed.

During 1930 and 1950, the number of newly described foraminiferal species was rapidly increasing at an average rate of one species per day (Thalmann 1952). This was due to the extraordinary development of applied micropaleontological research and a general tendency for "splitting", i.e., describing species on the base of very subtle morphological differences, often ignoring intraspecific variations. The result was a widespread increase of synonymy in many foraminiferan taxa, creating chaos in foraminiferan nomenclature (Boltovskoy and Wright 1976). This tendency became reversed in the 1970s when experimental laboratory studies demonstrated large ecophenotypic variation in cultivated foraminifera (Schnitker 1974). Despite some critical remarks concerning ecophenotypy in foraminifera (Haynes 1992), "lumping", i.e., including a wide range of morphotypes from various geographic regions in the same morphospecies, became a dominant tendency in foraminiferal research. As a consequence, the tendency to describe new species dropped drastically. For example, only seven new recent species have been described in the Journal of Foraminiferal Research during the past 10 years.

The wide use of benthic foraminifera in palaeoecological reconstructions largely contributed to the development of ecological studies in modern benthic foraminifera (Culver and Buzas 1998). It is generally accepted that most species have distinctive depth ranges, even if these ranges are broad and change from one area to another. Biogeography was used to define the foraminiferal associations typical for particular habitats in different geographic regions (Murray 1991). Among 938 common morphospecies analysed in Murray's study, more than half show a restricted distribution from 1 to 10 biogeographic regions established by the author. Among the 25 most widely distributed species, only 20 were found in more than five regions (Table 1). Remarkably, the three most ubiquitous species (*Epistominella exigua*, *Bulimina marginata* and *Globocassidulina subglobosa*) are characteristic of bathyal and abyssal environments. Whether these species are truly ubiquitous or represent a variety of indiscriminately lumped species, as suggested by some authors (Haynes 1992), is one of the main challenges of molecular studies in benthic foraminifera.

Molecular diversity of benthic foraminifera

One of the main controversial issues in conventional morphology-based taxonomy of foraminifera is the identification of species. The limited number of morphological characters of foraminiferal tests and their pronounced variations make the distinction of some species quite arbitrary. Studies about foraminiferal diversity can sometimes be strongly influenced by the authors' tendency for lumping or splitting. The situation is particularly

Table 1 Twenty-five of the most globally distributed species of benthic forminifera (adapted from Murray 1991). Species absence shown by dark colored area

Species	Depth (m)	NE Atl	Mex	SE Atl	W Atl	Medit	Indian	W Pac	E Pac	South	Arctic
Epistominella exigua	500-7500										
Bulimina marginata	100-3800										
Globocassidulina subglobosa	50-4100										
Ammonia beccarii	0-60										
Miliammina fusca	intertidal										
Quinqueloculina seminula	0-120										
Uvigerina peregrina	30-3800										
Adercotryma glomerata	20-6200										
Cibicides lobatulus	0-2700										
Trifarina angulosa	60-1900										
Bulimina aculeata	100-1900										
Cassidulina laevigata	30-2500										
Hoeglundina elegans	2000-3800										
Nuttalides umboniferus	2900-5500										
Trochammina inflata	intertidal										
Cibicides wuellerstorfi	1300-5500										
Elphidium crispum	0-25										
Hanzawaia concentrica	20-90										
Saccammina atlantica	30-200										
Bucella frigida	0-400										
Elphidium clavatum	0-200										
Haynesina germanica	intertidal										
Islandiella islandica	50-500										
Melonis barleanum	280-3800										
Rosalina globularis	0-60										

difficult for some common species, described a long time ago, where early descriptions are uninformative and the holotypes have either been lost or have never been deposited.

During the past 10 years, molecular techniques based on analysis of DNA sequences offered new tools for the identification of foraminiferal species and studies of their intraspecific variation (Holzmann 2000; Pawlowski 2000). All these studies are based on sequences of nuclear ribosomal RNA genes. These genes bear the advantage of being easily amplified even from single-cell DNA extractions. Three rDNA regions are commonly used in foraminiferal research: the 3′ fragment of the small subunit (SSU), the internal transcribed region (ITS) and the 5′ fragment of the large subunit (LSU). Each of these fragments has its own particular rate of evolution, which may differ from one taxonomic group to another. The ITS region is the fastest evolving one and seems most appropriate for species distinction, but its use until now was rather limited (Tsuchiya et al. 2003; Schweizer et al. 2005).

Using ribosomal genes as a tool for species identification bears certain inconveniences. Foraminiferal rDNA is extremely variable in length, difficult to align and often evolves at very different rates even between closely related groups. Moreover, in some species a strong intraindividual polymorphism of rDNA copies adds a supplementary difficulty to the determination of species-specific sequences (Holzmann and Pawlowski 1996). Nuclear genes coding for actin, tubulin and ubiquitin, which have been sequenced recently for some foraminifera are too conserved to be useful for analysis at species level (Flakowski et al. 2005). We expect that more variable molecular markers will be found in the mitochondrial genome whose sequencing is still in progress.

Here, we present two examples of rDNA-based studies of benthic foraminifers' diversity. The first one describes the comparison of morphologic and molecular variations in the well-known, common shallow-water genus *Ammonia*, while the second presents the

molecular diversity of a poorly known group of monothalamous foraminifera. These two examples offer abundant material for the discussion of diversity and geographic distribution in benthic foraminifera.

Molecular versus morphologic variability in Ammonia

Ammonia is widely distributed in marshes and near-shore environments around the world. The great variety of morphotypes and the lack of easily recognizable morphological characters causes difficulties in the identification (Holzmann 2000). Thirty-seven modern *Ammonia* species are listed in Ellis and Messina (1940) and supplements, the first species description dating from 1758 and the last from 1979. Of these 37 species, 26 type specimens are represented by drawings (three species including drawings of thin sections), two species are without type figures, two species were examined by scanning electron microscopy, and seven are represented by photographs. Measurements of external morphological characters are in most cases only given for the type specimens and only in one case these measurements are based on more than 100 individuals. A total of four different morphological characters had been measured in these 37 recent *Ammonia* species. Most measurements concentrate on the diameter of the test (21 species), in two species the height of the test was calculated. The diameter of the proloculus was assessed in three species and the thickness in 10 species. Two up to three of these morphological characters have been measured per species. Given the scarcity of data combined with the morphological variability in this genus it is no wonder that discussions arose about the identification of species in *Ammonia*.

Molecular studies have shown the presence of several genetically distinct types of *Ammonia* in the Mediterranean Sea, the North Atlantic and the South Pacific (Pawlowski et al. 1995; Holzmann et al. 1996; Holzmann and Pawlowski 1997; Holzmann et al. 1998; Holzmann 2000; Holzmann and Pawlowski 2000). In many places at least two different phylotypes occur together. In a comprehensive work (Hayward et al. 2004), molecular and morphological methods were combined to establish a more robust taxonomic subdivision of *Ammonia* worldwide. Thirteen phylotypes (T1–T13) could be distinguished and discriminated on the basis of morphometric analyses (Figs. 1, 2). The distinction of phylotypes is based on phylogenetic analysis of 267 partial LSU rDNA sequences, obtained from 202 living *Ammonia* specimens sampled at 30 localities from the Pacific Ocean, the Atlantic Ocean, Mediterranean Sea, Red Sea, Caribbean Sea and North Sea (Table 2, Fig. 2). The morphology of 127 sequenced specimens was recorded by SEM prior to DNA extraction and the images were utilized for morphometric analysis. Measurements or assessments of 37 external test characters were used to perform different types of analysis, suggesting that each phylotype can also be distinguished morphologically. At least 8 of the 13 phylotypes can be equated to described species. Morphometric analysis can therefore be successfully used to distinguish species in highly variable taxa if a sufficient number of specimens and morphological characters are taken into consideration.

Each phylotype (T1–T13) is monophyletic and separated by elevated genetic distances from other types. Furthermore, as no intermediate types have been observed, the different phylotypes can be regarded as distinct species (Hayward et al. 2004). This is in contrast to the popular taxonomic concept on the genus *Ammonia* that only recognizes a limited number of species with many ecophenotypes (Poag 1978; Walton and Sloan 1990). The recognition that *Ammonia* represents only a single or very few species worldwide should therefore be abandoned as a theory lacking a genetic basis. Analysis of the biogeographical patterns shows that most *Ammonia* phylotypes are characterized by a restricted distribution
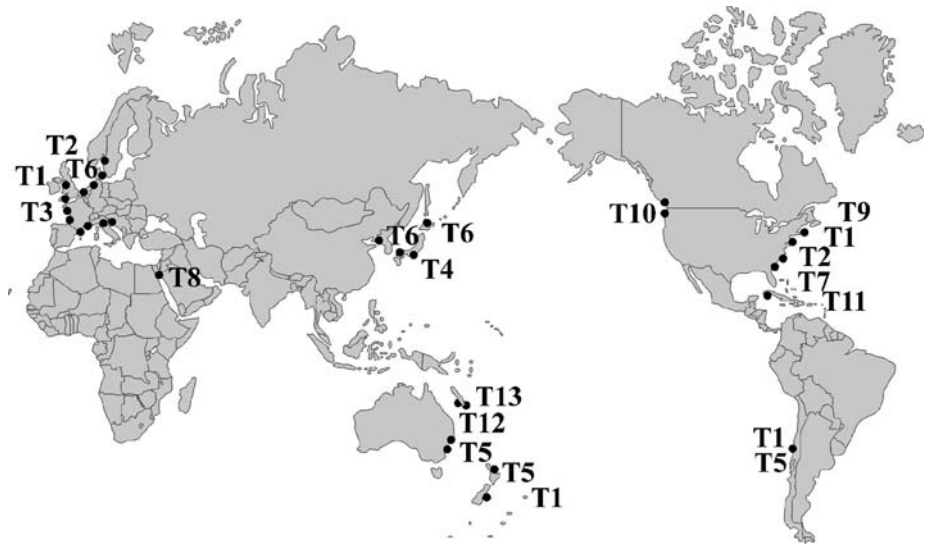
Fig. 1 Global distribution of the 13 *Ammonia* phylotypes (T1–T13) genetically identified and morphologically analysed

(Fig. 1, Table 2). Only one phylotype (T1) features a cosmopolitan distribution. Several other types are dispersed only in the northern or southern hemisphere (T2, T3, T5), some of them showing a transoceanic distribution (T2, T5). Many phylotypes are regionally restricted (T4, T7, T8, T9, T10, T11, T12, T13) while one phylotype (T6) shows a disjunct area. While transoceanic dispersal (T2, T5) could be aided by surface currents and/or transport via seabirds (Hayward and Hollis 1994), this would be difficult to accept with the disjunct distribution of T6. Human-assisted dispersal is the most likely explanation in the latter case. The genotype T6 is distributed around the coasts of China and Japan (Fig. 1) which is congruent with the habitat of *Eriocheir sinensis*, a decapod that has been introduced in the Wadden Sea at the end of the 19th century by shipping (Nehring and Leuchs 2000). Some of the ballast tank water that included *E. sinensis* could also have contained *Ammonia* individuals of the genotype T6 which since then spread out in the Wadden Sea and the adjacent Baltic Sea. Human-induced introduction of foraminifera is not an unknown phenomenon and has also played a role in the agglutinated foraminifer *Trochammina hadai* from Japan that has invaded the bay of San Francisco in the mid 1980s, most likely from ballast waters and sediments discharged from ships (Mc Gann and Sloan 1996).

The results of our studies provide just a sampling on the global diversity of the genus *Ammonia*. There are extended geographical regions that have not yet been investigated (Indian and South Atlantic Oceans, tropical and east Pacific, Southeast Asia and the East Indies). It is highly likely that the number of genetically distinct species could approach the number of formally named species (about 40; Ellis and Messina 1940 and supplements), most of which will be also distinguishable by a combination of subtle morphological characters.

Cryptic diversity in monothalamous foraminifera

In contrast to *Ammonia*, monothalamous foraminifera are a poorly known group, widely ignored by micropaleontologists. They are characterized by single-chambered organic-walled
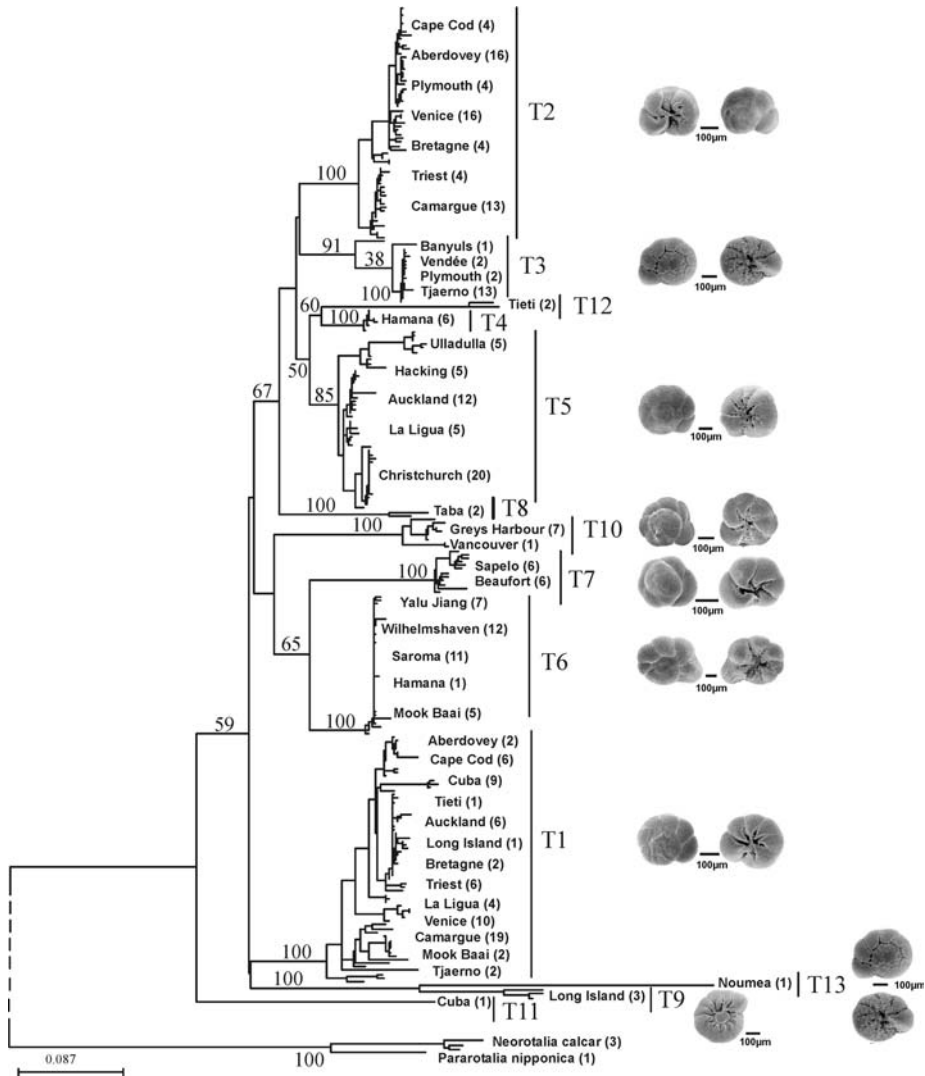
Fig. 2 Phylogenetic analysis of 267 partial LSU rDNA sequences using the Neighbour Joining method. The numbers are bootstrap percent values based on 500 resamplings. The scale bar corresponds to the number of substitutions per site. The names of sampling localities and number of sequences (in brackets) are indicated for each molecular type

or agglutinated tests that are rarely preserved in the fossil record. The fact that this group is particularly abundant in widely undersampled deep-sea and high latitude waters (Gooday et al. 2004) is also contributing to our lack of knowledge concerning its diversity. Monothalamous foraminifera are traditionally classified in the orders Allogromiida and Astrorhizida (Debenay et al. 1996). Morphological distinction of the latter orders is based on wall structure but is not confirmed by molecular studies (Pawlowski et al. 2003). SSU rDNA sequences have been used to resolve higher-level phylogenetic relationships (Pawlowski et al. 2002a, b; Pawlowski and Holzmann 2002), yet relations at lower taxonomic level remain unexplored.

Table 2 Collection localities, distribution and habitat of investigated *Ammonia* specimens

Phylotype	Number of investigated specimens	Number of partial LSU rDNA sequences	Distribution	Collection localities	Habitat
T1	52	65	Cosmopolitian	France: Camargue, Bretagne, Vendée; Italy: Lagoon of Venice, Triest; The Netherlands: Mook Bai; GBR: Dovey Estuary; Sweden: Tjaerno; USA: Cape Cod, Long Island; New Caledonia: Tieti Beach; Cuba: Playa Bailén; Chile: La Ligua; New Zealand: Waitemata Harbour	Microtidal marshes; Brackish water estuary
T2	40	61	European coasts; Northern Atlantic coast, USA	France: Camargue, Bretagne, Vendée; Italy: Lagoon of Venice, Triest; GBR: Dovey Estuary; Sweden: Tjaerno; USA: Cape Cod	Microtidal marshes
T3	11	18	European coasts	France: Banyuls-sur-mer, Vendée; Sweden: Tjaerno	Open marine habitats, rocky shores
T4	3	6	Japanese coast	Japan: Hamana Lake	Brackish water lake
T5	45	47	Chilean coast, New Zealand, Australia	Chile: La Ligua; New Zealand: Akaroa, Pollen Island, Governors Bay; Australia: Ulladulla-Burril Lake, Port Hacking-Grays Point	Microtidal marshes
T6	29	36	Wadden Sea, Baltic Sea; NE China, Japanese coast	The Netherlands: Mook Bai; Germany: Wilhelmshavan; Japan: Lake Saroma, Hamana Lake; China: Yalu Jiang	Meso/macrotidal flats; Microtidal marshes, rocky shores
T7	6	12	Northern Atlantic coast, USA	USA: Georgia-Sapelo Island, Beaufort-North Carolina	Microtidal marshes
T8	2	2	Red Sea	Israel: Taba	Open marine habitats
T9	2	3	Northern Atlantic coast, USA	USA: Long Island	Microtidal marshes
T10	8	8	Northern Pacific coast, USA, CA	USA: Washington State, Grays Harbour; Canada: Vancouver	Microtidal marshes
T11	1	1	Caribbean Sea	Cuba: Playa Balién	Microtidal marshes
T12	2	2	Southern Pacific	New Caledonia: Tieti Beach	Brackish water estuary
T13	1	1	Southern Pacific	New Caledonia: Noumea Tjibaou	Mangroves

Our long-term survey of monothalamous foraminifera has revealed some unexpected results. Monothalamous lineages play a key role in the early evolution of foraminifera (Pawlowski et al. 2003). Their genetic diversity at different taxonomic levels by far exceeds what could be expected from morphological studies. Molecular data from material collected in Antarctica revealed an extraordinarily rich assemblage of monothalamous species. Allogromiids, athalamids and astrorhizids comprise an assemblage of more than a dozen lineages branching together at the base of the foraminiferal tree. Molecular data also show high species diversity in allogromiids (Pawlowski et al. 2002a, b, 2005). Because of the paucity of morphological characters, species distinction is particularly difficult and the majority of allogromiid genera are represented by single species descriptions (Nyholm 1974). Molecular analyses also confirmed the presence of allogromiids in freshwater and terrestrial environments (Meisterfeld et al. 2001; Holzmann and Pawlowski 2002; Holzmann et al. 2003). Very few of the genetically distinctive monothalamous taxa have been characterized morphologically and formally described or revised (Bowser et al. 2002; Gooday et al. 2004; Gooday and Pawlowski 2004; Sabbatini et al. 2004). Furthermore, a few lineages are only identified from environmental DNA extractions (Holzmann et al. 2003; Habura et al. 2004).

One of our research projects concerning monothalamous foraminifera focuses on the geographic distribution of this group, and in particular on the genetic comparison of similar morphotypes found in polar and subpolar waters of the northern and southern hemisphere. Some results of this yet unpublished study are reported here. We have compared SSU rDNA sequences of species belonging to four genera (*Micrometula*, *Psammophaga*, *Gloiogullmia* and *Hipocrepinella*) from western Svalbard (Arctic) and McMurdo Sound (Antarctic), including their representatives from the deep southern Ocean (Weddell Sea) and Arctic Ocean (Fram Strait) as well as from northern European fjords (Sweden, Scotland) wherever it was possible.

Phylogenetic analysis of our data show that within the four examined morphotypes, Arctic and Antarctic species form clearly distinctive sister clades (Fig. 3). The clades are separated by relatively large genetic distances (>5%), except in *Psammophaga* (<1%), due to either relatively rapid radiation or to an unusual slowdown of evolutionary rates in this genus. The isolates from Svalbard are closely related to those from other northern European settings. The Weddell deep-sea isolates of *Gloiogullmia* and *Micrometula* form sister groups to coastal Antarctic isolates and the Arctic deep-sea isolate of *Micrometula*, branches as sister group to the respective Antarctic clade. Interestingly, the specimens from Dunstaffnage (Scotland) either form a sister group to other northern hemisphere isolates (*Psammophaga*, *Gloiogullmia*) or to both polar clades (*Micrometula*).

Our data not only show the genetic differentiation between northern and southern populations of the examined taxa but also reveal several genetic lineages that considerably differ from each other. At present, three of the four examined genera are represented by only one described species (*Micrometula hyalostriata*, *Gloiogullmia eurystoma*, *Psammophaga simplora*). Each of these species is represented in our analyses by sequences from the area close to the type locality (Skagerrak for *M. hyalostriata*; Oslofjord *for G. eurystoma*; and Sappelo, Georgia, US for *P. simplora*). The fourth species, *Hippocrepinella hirudinea*, has been described from the Southern Ocean, and we consider our McMurdo sequences as closely related to the original type. Additionally, 12 genetically distinctive phylotypes have been revealed in our study. Remarkably, each of these types has a restricted geographic distribution. Given their apparent isolation and genetic differentiation, we may consider them as new, yet undescribed species.
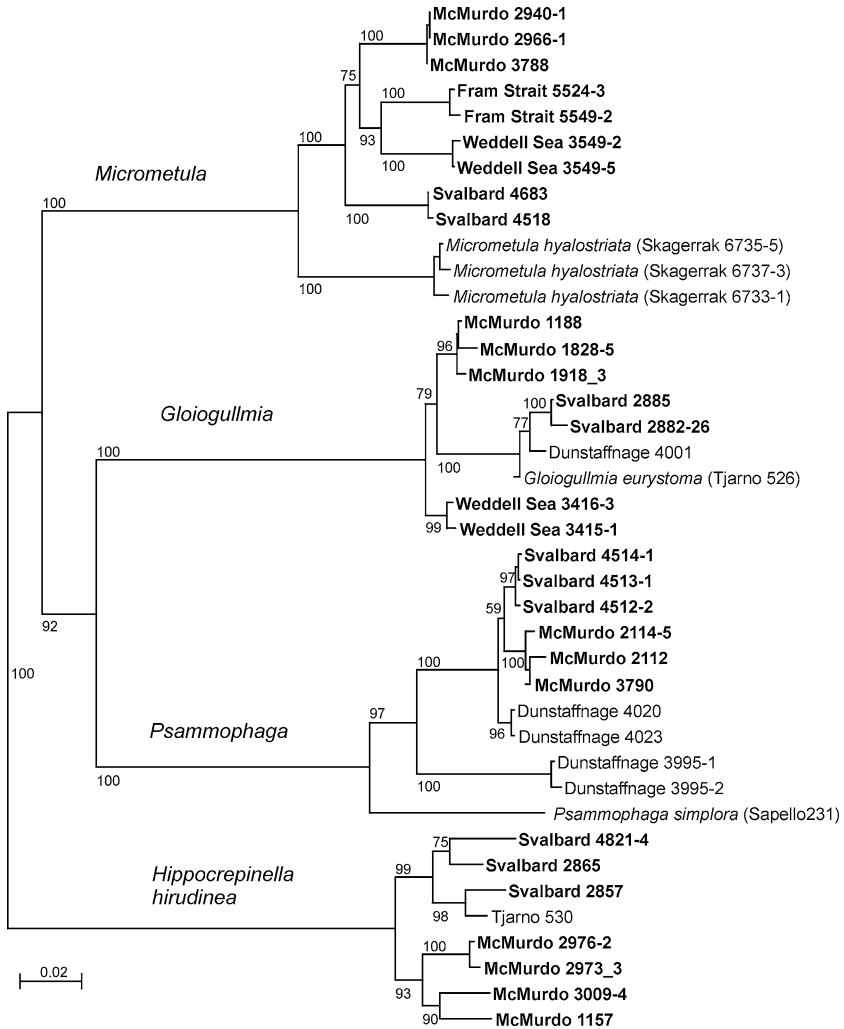
Fig. 3 Phylogenetic relations between Arctic and Antarctic monothalamous foraminifera. Sequence names indicate the locality and isolate number. Polar isolates are in bold. Species names are given to isolates from the area close to the locality of original description. The tree was obtained by the neighbour joining method with pairwise distances and 1000 bootstrap replicates

Is foraminiferan diversity different?

It has been proposed that the diversity of free-living protists is different from the diversity of larger organisms because small-sized organisms can be dispersed everywhere, and therefore the rates of allopatric speciation is low (Finlay et al. 2004). The authors assumed that small-sized organisms are generally ubiquitous and that the same species can be found wherever its preferred habitat is present (Finlay 1998). These arguments were based mostly on the study of ciliates morphospecies and have only recently been confirmed by molecular data for different ecotypes within the ciliate species *Cyclidium glaucoma* (Finlay et al. 2006).

Other results, however, point to the fact that the geographic distribution of many protist species is limited and about one third of species might be endemic in a morphological and/or genetic way (Chao et al. 2006; Foissner 2006).

Morphological and molecular studies suggest that most but not all foraminifera seem to have restricted geographic distribution. The data presented in Table 1 are based on more than 1000 studies (Murray 1991) and clearly show that globally distributed foraminiferal morphospecies represent a small proportion out of the 25 selected species. For some of them, such as *Ammonia beccarii*, molecular studies have shown that this morphospecies actually comprises an assemblage of genetically distinctive lineages. However, this does not mean that there are no ubiquitous foraminifera. As shown by molecular analyses, at least one lineage of *Ammonia* (type 1) has a global distribution. A recent molecular study shows very weak genetic differentiation between Arctic and Antarctic populations of three common species of deep-sea foraminifera (Pawlowski et al. 2007). We certainly need more molecular data to test how widely dispersed deep-sea species are. In the case of shallow-water foraminifera, however, our data indicate that most species have a limited geographic distribution.

Does size has something to do with the restricted distribution of most foraminifera? Compared to other protists, foraminifera are often larger in size and some of them particularly agglutinated polar and deep-sea species or calcareous tropical species can reach up to several centimetres in size (Haynes 1981). Yet, the majority of foraminiferal species measures from 50 to 500 μm, which is within the range of typical meiofaunal size. Undoubtedly, this is still much larger than some marine picoplanktonic algae (~ 2 μm), whose global distribution was demonstrated recently (Slapeta et al. 2006). The dispersal of such small organisms could be greatly facilitated by water currents. However, the example of *Ammonia* type 1 cited above shows that size might not be the main factor responsible for the dispersal of foraminiferal species. *Ammonia* specimens belonging to type 1 are within the same size range than representatives of other *Ammonia* types, and yet they are widely distributed while the others are not. What makes that particular *Ammonia* type ubiquitous is an intriguing question. Perhaps this type is the only one capable to produce dispersal forms such as the propagules observed by Alve and Goldstein (2003). Or, there are other physiological or ecological mechanisms that facilitate the dispersal of some foraminiferal species, independently of their size.

The examples presented here not only show evidence for geographic distribution of species but also confirm the importance of molecular studies for estimating the diversity of foraminifera. In both case studies, the analysis of DNA sequences revealed an extraordinarily high diversity of phylotypes at different taxonomic levels. Such high molecular diversity was found also in other foraminifera, including Soritinae (Garcia-Cuetos et al. 2006) and Glabratellidae (Tsuchiya et al. 2000, 2003). We can expect that if each of molecular types would be formally described, the number of foraminiferal species would increase at least by one factor of magnitude. The most spectacular rise of diversity is expected in the group of monothalamous foraminifera. In the much better known rotaliid genera, such as *Ammonia* or *Elphidium*, whose taxonomy is overloaded with synonyms, the number of phylotypes revealed by molecular data may approach that of described morphospecies.

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