Journal of Micropalaeontology, **19:** 85–95. 0262-821X/00 $15.00 © 2000 British Micropalaeontological Society

Taxonomic relationships in the genus *Ammonia* (Foraminifera) based on ribosomal DNA sequences

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ABSTRACT - The genus *Ammonia* is a common benthic foraminifer which is widely distributed in nearshore marine environments. Its large morphological variability causes considerable difficulties in species identification. In the present study, we investigated taxonomic relationships in *Ammonia* by using a molecular approach based on ribosomal DNA sequences. We obtained 149 partial large subunit ribosomal DNA (LSU rDNA) sequences and 23 small subunit ribosomal DNA (SSU rDNA) sequences from 88 living *Ammonia* specimens which were collected from free-living populations in 14 localities. Sequence analysis revealed the presence of eight distinct genotypic groups (T1–T7, T9) and one distinct genotype that is represented by one specimen (T8). Examination of morphological characters shows that only one genotypic group can be clearly distinguished by its morphology. Biogeographical and ecological features are used for an additional characterization and it seems that the different groups live in relatively well defined environmental conditions and that only one genotypic group is cosmopolitan, while the others have a rather restricted geographical distribution. According to our study, three of the genotypic groups can be regarded as distinct species. *J. Micropalaeontol.* **19**(1): 85–95, May 2000.

INTRODUCTION

Foraminifera are classified exclusively on the basis of the morphological characters of their tests. The value of these characters, however, is questionable for the determination of polymorphic taxa. Molecular techniques, especially the analysis of DNA sequences, provide a data set that is independent of morphological characters. This allows a revision of controversial taxonomic issues that cannot be solved with morphological data alone, as is the case for *Ammonia*.

The genus *Ammonia*, Brünnich, 1772, is widely distributed in nearshore and marginal marine environments. Extensive studies on its test structure and morphology have been published by Cifelli (1962), Banner & Williams (1973) and Höttinger (1980). Vénec-Peyré (1980), investigated the chemical and mineralogical composition of extant and fossil *Ammonia* tests. The high morphologic variability of *Ammonia* leads to difficulties in the identification of species and has resulted in numerous discussions. Two conflicting theories prevail over the taxonomy of this genus. Some authors ('lumpers') consider that most *Ammonia* morphotypes are ecophenotypes of the same species while others ('splitters') claim that these morphotypes belong to different species (Schnitker, 1974; Poag, 1978; Wang & Lutze, 1986; Walton & Sloan, 1990; Haynes, 1992).

Recently, the analysis of partial ribosomal DNA sequences has been used to investigate inter- and intraspecific relationships in *Ammonia* (Pawlowski *et al.*, 1995; Holzmann *et al.*, 1996; Holzmann & Pawlowski, 1997; Holzmann *et al.*, 1998). Based on the analysis of LSU rDNA sequences, different genotypic groups could be distinguished for several morphotypes of *Ammonia*, thus evidently denying the hypothesis of one species represented by different ecophenotypes. Two genetically, morphologically and ecologically different types of *Ammonia* have been found in living foraminiferal assemblages from the Mediterranean Sea, North Atlantic and South Pacific, which were called *Ammonia* sp. 1 and *Ammonia* sp. 2 (Holzmann *et al.*, 1996; Holzmann & Pawlowski, 1997; Holzmann *et al.*, 1998).

In the present study, we obtained partial rDNA sequences from 88 *Ammonia* specimens, collected in 14 different localities. The morphology of most specimens from which DNA was extracted was examined by scanning electron microscopy (SEM). Molecular data allowed us to distinguish eight different genotypic groups (T1–T7, T9) and one genotype comprising one sequence (T8). Among the genotypic groups, T1 and T2 correspond to the already previously described types of *Ammonia* sp. 1 and *Ammonia* sp. 2 respectively. Morphological, biogeographical and ecological data were used for an additional characterization of the different genetic groups.

MATERIAL AND METHODS

Cell collection

The specimens of *Ammonia* were sampled from different coastal regions of the Mediterranean Sea, Irish Sea, the English Channel, North Sea, North Atlantic, and Pacific (Fig. 1). Sediment samples from marginal marine environments were collected by hand with a scraper, as described by Holzmann *et al.* (1998). *Ammonia* specimens from rocky shores were collected from algae, attached to the rocks. *Ammonia* individuals from open marine habitats were collected by means of a grab sampler. The collected *Ammonia* specimens were maintained in laboratory cultures (Holzmann & Pawlowski, 1996) and living individuals were isolated using a dissecting microscope.

DNA extraction

A total of 88 specimens, including those *Ammonia* individuals whose sequences are already published (Holzmann *et al.*, 1996; Holzmann & Pawlowski, 1997; Holzmann *et al.*, 1998) have been used for this study. DNA was extracted one by one from all specimens. Every specimen was ground separately in extraction buffer, then incubated for 1 hour at 60°C, followed by short centrifugation to remove the insoluble material (Holzmann & Pawlowski, 1996). The morphology of 64 specimens was examined by SEM prior to DNA extraction.

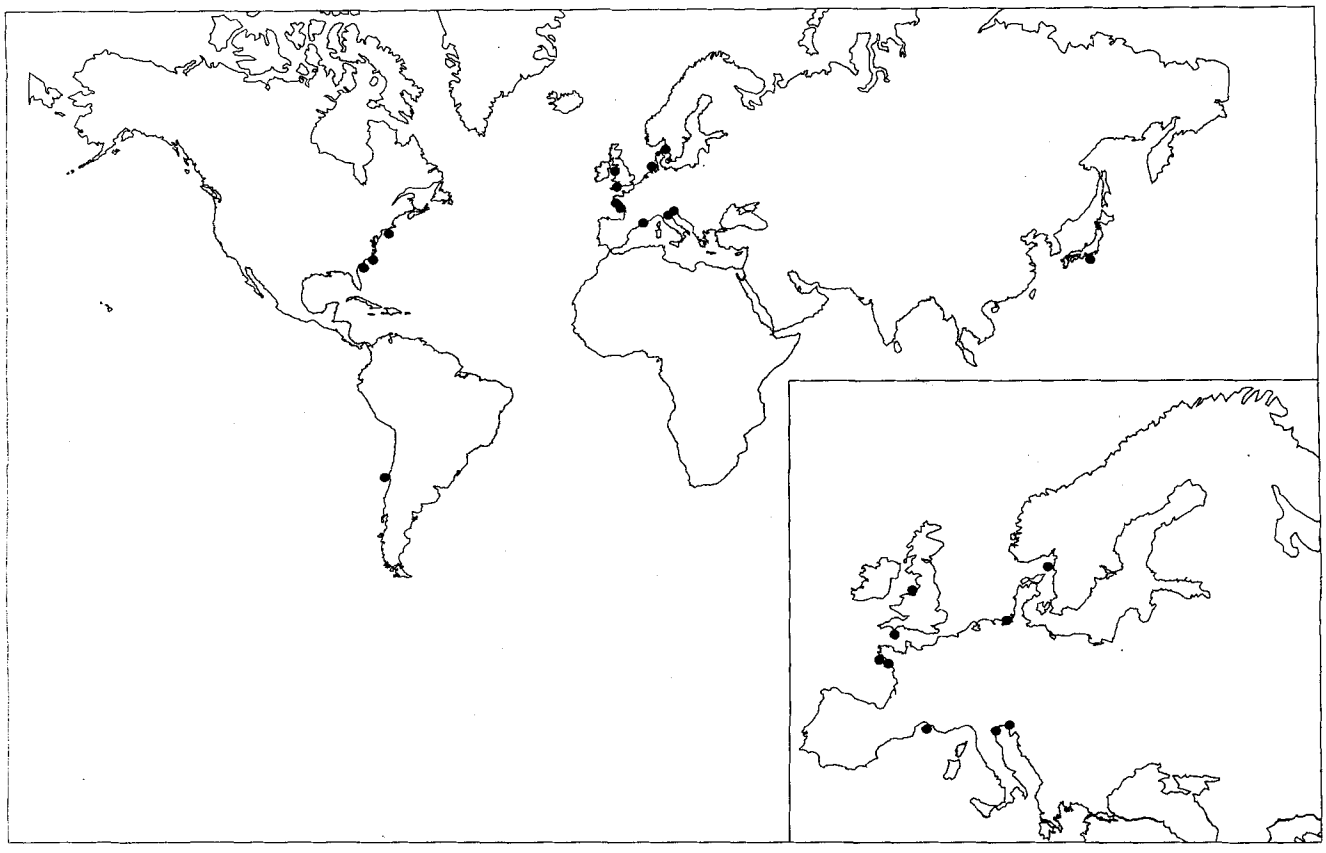


Fig. 1. Location map showing the collection sites.

PCR amplification, cloning and sequencing

A fragment of the LSU rDNA of about 650 nucleotides was amplified by PCR. Two specific foraminiferal LSU rDNA primers, Rib 2TA (5'CACATCAGCTCGAGTGAG; position 1–18 in rat) and Rib 1F (5'ACTCTCTCTTTCACTCC; position 385–402 in rat) were used for amplification (Holzmann & Pawlowski, 1996). The amplified PCR products were purified using Spin-Bind DNA extraction Units (FMC) and cloned in the pGEM-T Vector system (Promega) using Supercompetent cells XL1-Blue MR (Stratagene). Both strands of a fragment of about 450 nucleotides were sequenced by using the specific foraminiferal primers Rib 2TA and Rib 7 (5'GATG(AT)GTCATTACCACC; position 309–324 in rat), (Holzmann & Pawlowski, 1996). For 48 specimens, more than one clone was sequenced (between two and five clones per specimen).

Additionally, a fragment of the SSU rDNA of about 800 nucleotides was amplified for 23 specimens. Two specific foraminiferal SSU rDNA primers, s12 (5'CTACCAAAAGCGAAAGC; position 998–1002 in rat) and s14rf (5'CCTTCAAGTTTCACACTTGC; position 1183–1202 in rat) were used (Pawlowski *et al.*, 1996). The PCR products were cloned and sequenced as described above, using the same primers for amplification and sequencing. One clone was sequenced per specimen.

DNA sequence analysis

Gel sequences were assembled using PC/Gene (Bairoch, 1989) and aligned manually, using the Genetic Data Environment (GDE), version 2.2 (Larsen *et al.*, 1993). Evolutionary distances were computed according to Kimura's (1980) method of correction for multiple hits and unequal rates of transitions and transversions. All sites were retained for phylogenetic analysis, in order to maintain the minor intraindividual differences that occur in the sequences of some *Ammonia* specimens. Phylogenetic trees were built using the neighbor joining (NJ) (Saitou & Nei, 1987) and maximum likelihood (ML) method (Olsen *et al.*, 1994).

The reliability of internal branches in the NJ tree was assessed using the bootstrap method (Felsenstein, 1988) with 500 replicates for the NJ trees and 50 replicates for the ML trees. The phylo_win program (Galtier & Gouy, 1996) was used for distance computations, inference of NJ and ML trees and bootstrapping. The phylogenetic trees were plotted using the njplot program (Perrière & Gouy, 1996).

The new sequences presented in this study were deposited in the EMBL/GenBank Nucleotide Sequence Database under accession numbers Z77773–Z77777, Z77779, Z77781, Z77783–Z77787, Z77789, Z77791, Z77793, Z77795, Z77797, Z77799–Z77825, X99811–X99824, AJ228517–AJ228548, AJ228552–

AJ228559. Accession numbers for the LSU rDNA and SSU rDNA sequence of rat (*Rattus norvegicus*) used as references for primer positions are X01069 and K01593 respectively.

RESULTS AND DISCUSSION

Sequence analysis

LSU rDNA fragment

A fragment of the LSU rDNA was amplified and sequenced for 88 specimens. For 48 specimens, more than one clone was sequenced, resulting in a total of 149 LSU sequences (Table 1). The fragment is situated at the 5'terminal end of the LSU rRNA gene and includes the divergent domain D1 and flanking regions of the conserved domains C1 and C2 (Hassouna *et al.*, 1984). Its length ranges from 364 to 421 nucleotides. The base composition of the analysed fragment is characterized by a relatively high proportion of A + T, which extends from 54% to 60.9%. This is mostly due to serial repeats of A and T either as single nucleotides or as doublets.

Table 1. Number of investigated Ammonia specimens and of sequences obtained for the LSU and SSU rDNA fragments

Genetic types	Number of specimens	Number of LSU sequences	Number of SSU sequences
T1	26	44	7
T2	36	57	6
T3	9	16	3
T4	3	6	1
T5	2	4	1
T6	3	6	1
T7	6	12	2
T8	1	1	1
T9	2	3	1

A phylogenetic tree, generated by the NJ method, reveals the presence of eight distinct genotypic groups of *Ammonia* (T1–T7, T9) and one genotype represented by one sequence (T8) (Fig. 2). All genotypic groups are monophyletic and supported by high bootstrap values (97%–100%). Two monophyletic clades can be distinguished: One clade is composed of the genotypic groups T2, T3, T4 and T5 and supported by a bootstrap value of 94%. Another clade is formed by the genotypic groups T6 and T7, but its monophyly is supported by a much lower bootstrap value (61%). The genotype T8 branches between these two monophyletic clades, but its separation from the clade containing T6 and T7 is supported by a very low bootstrap value (33%). T1 and T9 branch separately, with T1 as a sister group to T2–T8 and T9 as a sister group to all other genotypic groups.

Pairwise comparison of the sequences shows a high sequence dissimilarity between and within the investigated genotypic groups (Table 2). Sequence divergence between genotypic groups ranges from 5.5% to 28.6%. and reaches up to 11.6% within a single genotypic group (T1). The comparison of different clones, obtained from the same individual shows that the examined fragment of the rDNA is polymorphic (Holzmann *et al.*, 1996). The intra-individual variation was found in each group, with the lowest values in T3 ($\leq$0.7%) and the highest values in T1 ($\leq$7.7%).

SSU rDNA fragment

A fragment of the SSU rDNA was obtained for each genotypic group and the genotype, by investigating one representative from each sampling locality (Table 1). The amplified fragment is located in the middle part of the SSU rDNA gene and its length varies from 481 to 573 nucleotides. It includes the variable area V5 and flanking helices 24 and 25 as well as parts of the flanking helices 21, 22, 26 and 28 (Neefs & Wachter, 1990). The A + T content ranges from 54.3% to 57.9%.

Phylogenetic trees were inferred by NJ and ML methods. The NJ tree of the SSU rDNA sequences (Fig. 3) is nearly identical to the LSU rDNA tree and differs only in two points from the latter one. First, the genotype T8, which branches separately in the LSU rDNA tree, forms a a well supported (100%) clade with the genotypic group T6. The second difference concerns the branching order in the clade comprising the genotypic groups T2, T3, T4 and T5. While T3 branches as a sister group to the others in the LSU rDNA tree, T4 and T5 form a sister group to the others in the SSU rDNA tree.

ML analysis of the SSU rDNA fragment results in a tree that is very similar to the NJ tree in Figure 3 (data not shown). Two monophyletic clades were observed, one consisting of the genotypic groups T2, T3, T4 and T5 and the other comprising the genotypic groups T1, T6 and T7. The main difference between the ML and the NJ SSU rDNA trees consists in the position of T1 which does not form a sister group to all others in the ML tree.

Table 2. Relative frequence of differences in partial LSU rDNA sequences of *Ammonia* spp.

	T1	T2	T3	T4	T5	T6	T7	T8	T9
T1	0–0.116								
T2	0.176–0.250	0–0.077							
T3	0.198–0.255	0.125–0.177	0–0.012						
T4	0.161–0.234	0.111–0.149	0.119–0.156	0.005–0.052					
T5	0.173–0.229	0.114–0.140	0.124–0.149	0.093–0.112	0.003–0.013				
T6	0.140–0.213	0.162–0.211	0.207–0.244	0.163–0.208	0.158–0.199	0.004–0.039			
T7	0.173–0.244	0.216–0.283	0.240–0.286	0.206–0.278	0.233–0.271	0.119–0.188	0.008–0.054		
T8	0.167–0.230	0.063–0.073	0.051–0.057	0.055–0.058	0.059–0.066	0.098–0.144	0.201–0.253	0	
T9	0.211–0.255	0.240–0.317	0.242–0.304	0.244–0.284	0.231–0.263	0.217–0.272	0.242–0.271	0.228–0.275	0.014–0.066

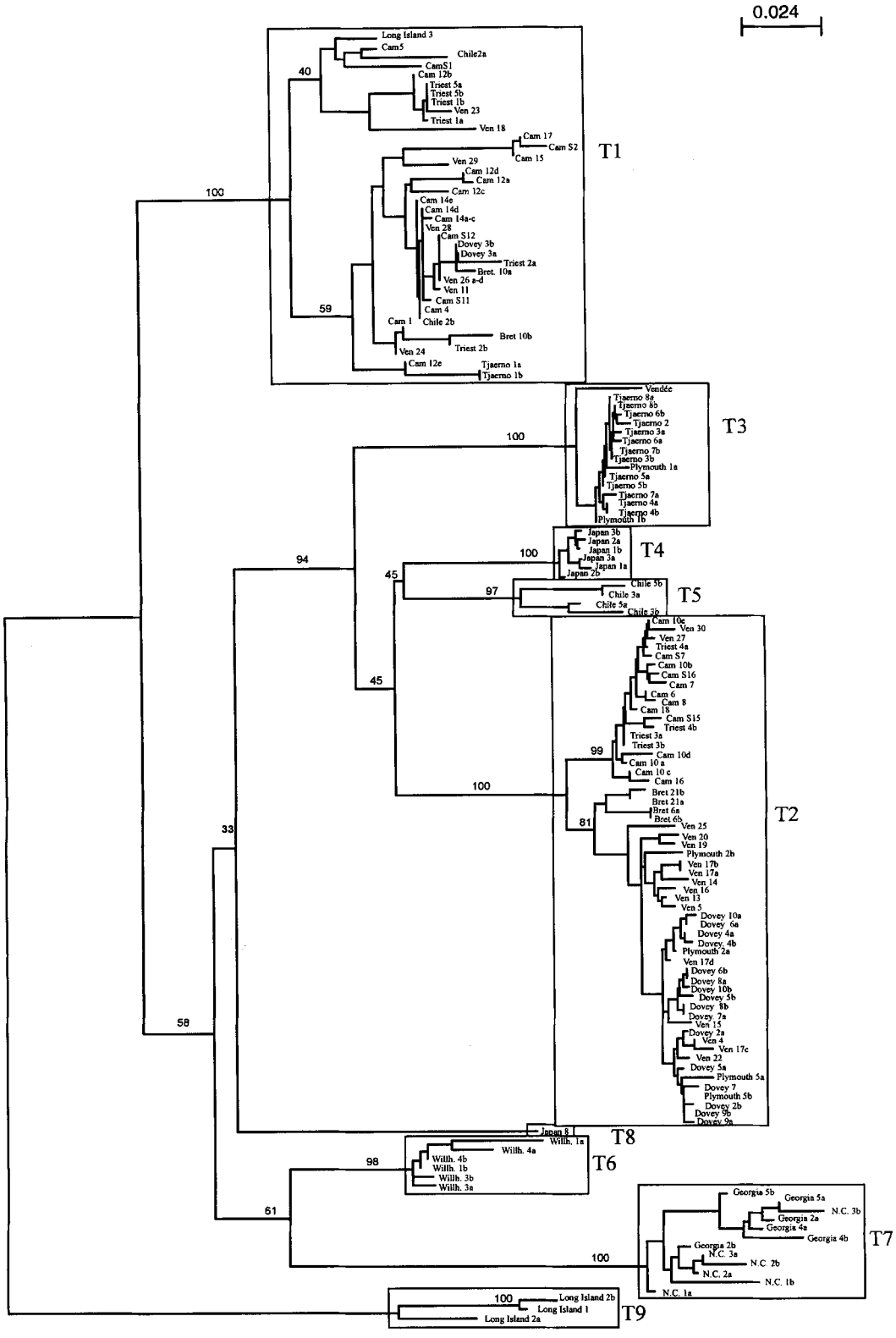


Fig. 2. Phylogenetic analysis of 144 partial LSU rDNA sequences using the NJ method. The numbers are bootstrap percent values based on 500 replicates. The sequences of different clones originated from the same specimen are indicated by letters a–e.
List of used abbreviations: Bret, Bretagne; Cam, Camargue; N.C, North Carolina; Wilh., Wilhelmshaven; Ven, Venice.

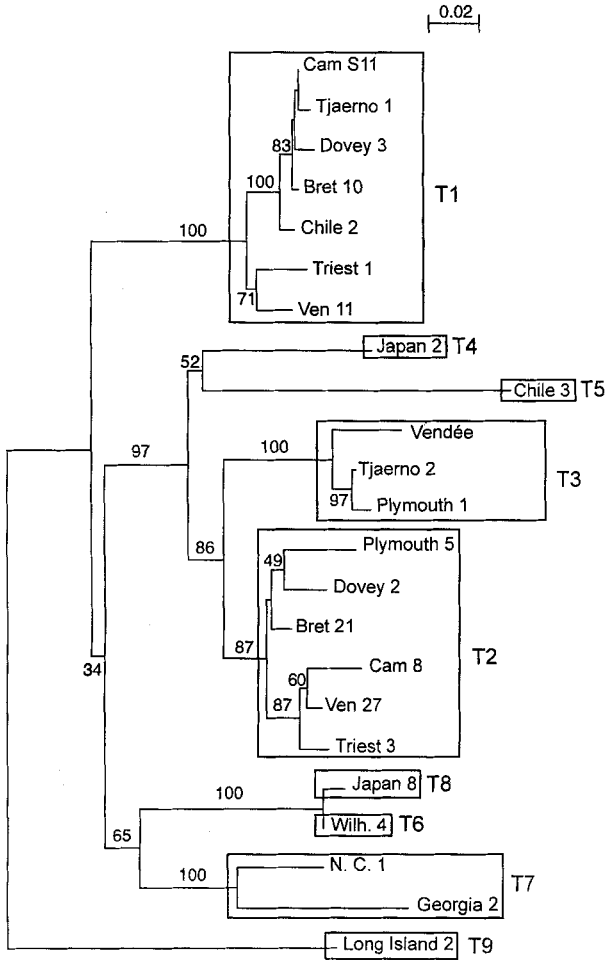


Fig. 3. Phylogenetic analysis of 23 partial SSU rDNA sequences using the NJ method. The numbers indicate bootstrap percent values based on 500 replicates.

Combined analysis of the LSU and SSU rDNA fragment

The corresponding sequences of the LSU rDNA were joined to the SSU rDNA data set and analysed as one fragment. The NJ tree (not shown) shows nearly the same branching pattern as the tree in Figure 2. The only difference to the latter concerns the position of T8, which forms a cluster with T6, as in the NJ tree of the SSU rDNA fragment. ML analysis of the combined fragments results in a tree (not shown) that is identical to the tree in Figure 3.

MORPHOLOGY

Typical specimens representing each genotypic group and the single genotype of *Ammonia* are illustrated on Plates 1 and 2. Among the eight genotypic groups and the one genotype differentiated by DNA sequence analysis, only T3 (Plate 1, fig. 1a-b) can be clearly distinguished by its morphology. It is the largest among all *Ammonia* examined in this study. Its test has a mean diameter of 0.62 mm, while the diameter of other *Ammonia* types does not exceed 0.4 mm. It is characterized by a thick walled, low trochospiral test. The spiral sutures are distinct and deeply incised in the last whorl. The sutural areas on the umbonal side are stud with numerous irregular knobs. The beading, fluting and/or furrowing along the umbonal sutures is typical for these strongly ornamented forms. The umbonal cavity is filled up with numerous calcareous plugs of different size.

In other groups, some distinct features have been observed. For example, Holzmann & Pawlowski (1997) and Holzmann *et al.* (1998) have shown that T1 specimens are characterized by very distinct spiral sutures and that they can be distinguished from T2 specimens by their test size and pore diameter. The genotypic groups T4, T5 and T6, however are quite similar to T2 in their external appearance and although they are genetically clearly distinct, a separation on the basis of morphological features is not possible.

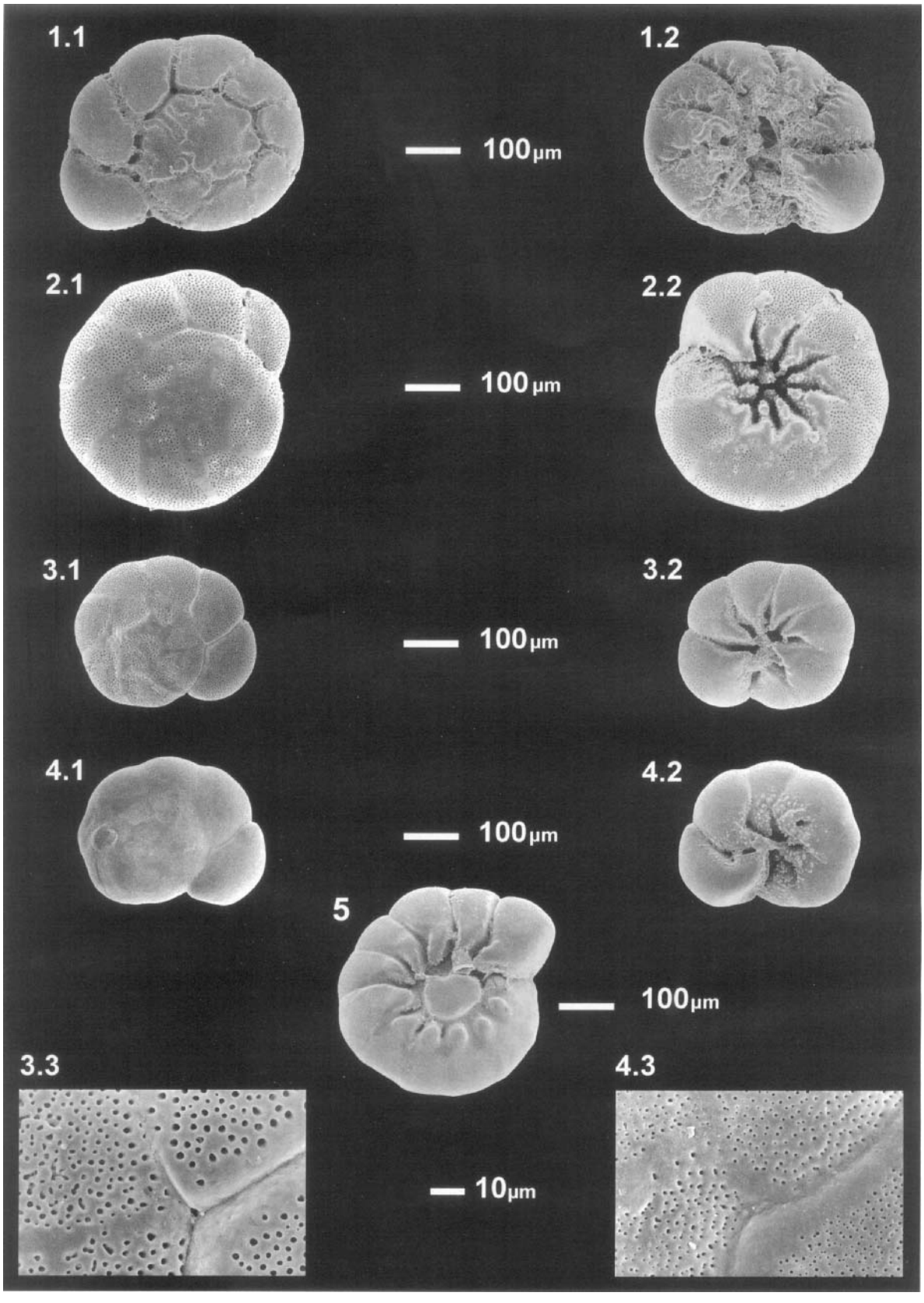
Specimens belonging to T7 also display some morphological differences: their chambers are rather elongated and not rounded like in other *Ammonia* and the last chamber is considerably inflated in most individuals. The genotypic group T9 differs from other investigated *Ammonia* in having a large umbilical plug and that the umbilical space between the plug and the chamber flaps is completely closed by smooth, calcareous fillings. The single *Ammonia* specimen representing T8 is characterized by extremely fine, elongated and pointed chamber flaps.

With the exception of the genotypic groups T1–T3, only a few specimens could be investigated for each other group (T4 - T7 and T9) and one specimen could be examined for T8. It is therefore not possible to draw any conclusions about the general morphology for most types of *Ammonia*. A closer investigation on a population level will be necessary to see if the genetically distinct types of *Ammonia* can also be distinguished morphologically from each other.

BIOGEOGRAPHY AND ECOLOGY

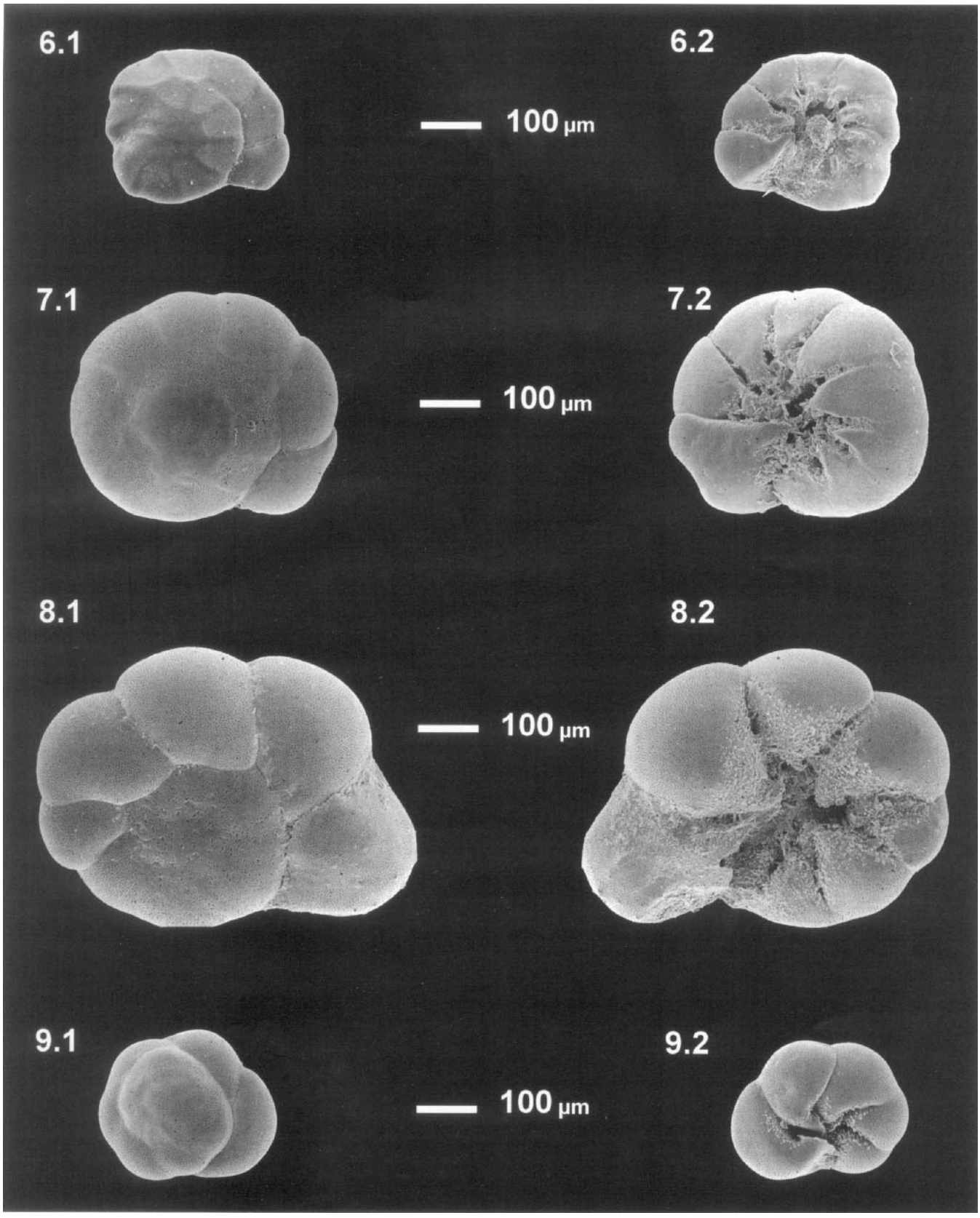
An overview of the habitats and geographic distribution of each genotypic group is given in Table 3. The majority of samples were collected from microtidal salt marshes. Few samples were also taken from meso- to macrotidal flats, a brackish water lake, rocky shores and open marine habitats. Although the number of sampling localities is limited, it seems that at least some of these groups have a rather restricted geographical distribution and live in relatively well defined environmental conditions.

Only one genotypic group, T1 seems to be cosmopolitan. It is present in all samples from microtidal marshes in Europe, on the northeast coast of the United States and in Chile. It was not found however, in samples from similar habitats on the southeast coast of the USA and in Japan. In European salt marshes, the genotypic groups T1 and T2 commonly occur together. T1 was also found in Long Island (N.Y.) and La Ligua (Chile), where it occurs together with other genotypic groups, respectively T9 and T5. Although T1 usually seems to share its habitat with a second genotypic group of *Ammonia*, both may not have the same ecological preferences. A former study, based on specimens sampled from the Lagoon of Venice shows some ecological differences in the distribution of T1 and T2 (Holzmann & Pawlowski, 1997). T1 is mainly confined to the internal, silty part of the Lagoon which is rich in nutrients, whereas T2 is dominant in the outer, sandy part of the Lagoon



Explanation of Plate 1.

figs 1.1–4.1: spiral view, **figs 1.2–4.2, 5:** umbilical view; **figs 3.3–4.3:** enlargement of an area between the last and the penultimate chamber and the spiral sutures in the last whorl; magnifications: ×1400; **figs 1.1, 1.2:** *Ammonia* specimen/T3 (Tjaerno); **figs 2.1, 2.2:** *Ammonia* specimen/T8 (Hamana Lake); **figs 3.1, 3.2, 3.3:** *Ammonia* specimen/T1 (Dovey Estuary); **figs 4.1, 4.2, 4.3:** *Ammonia* specimen/T2 (Dovey Estuary); **fig 5:** *Ammonia* specimen/T9 (Long Island).



Explanation of Plate 2.

figs 6.1–9.1: spiral view, **figs 6.2–9.2:** umbilical view; **figs 6.1, 6.2:** *Ammonia* specimen/T4 (Hamana Lake); **figs 7.1, 7.2:** *Ammonia* specimen/T5 (La Ligua); **figs 8.1, 8.2:** *Ammonia* specimen/T6 (Wilhelmshaven); **figs 9.1, 9.2:** *Ammonia* specimen/T7 (Georgia).

Table 3. Distribution and habitat of investigated *Ammonia* specimens

Genetic type	Number of specimens	Distribution	Collection localities	Habitat
T1	26	cosmopolitan	Lagoon of Venice, Italy Triest, Italy Camargue, France Bretagne, France Dovey Estuary, England Tjaerno, Sweden Long Island, New York La Ligua, Chile	microtidal marshes
T2	36	European coasts	Lagoon of Venice, Italy Triest, Italy Camargue, France Bretagne, France Dovey Estuary, England Plymouth, England	microtidal marshes
T3	9	European coasts	Vendée, France Plymouth, England Tjaerno, Sweden	open marine habitats rocky shores
T4	3	Japanese coast	Hamana Lake, Japan	brackish water lake
T5	2	Chilean coast	La Ligua, Chile	microtidal marshes
T6	3	Wadden Sea, Germany	Wilhelmshaven, Germany	meso/macrotidal flats
T7	6	Southern Atlantic coast, USA	Sapelo Island, Georgia, USA Beaufort, North Carolina, USA	microtidal marshes
T8	1	Japanese coast	Hamana Lake, Japan	brackish water lake
T9	2	Northern Atlantic coast, USA	Long Island, New York	microtidal marshes

which contains less organic detritus, but has higher oxygen levels than the inner part.

With the exception of T1, all other marsh-inhabiting *Ammonia* have a relatively restricted geographic distribution. A distinct genotypic group T5 was found in salt marshes from Chile. Two other genetically different types (T4, T8) have been sampled from the brackish water Hamana Lake, in Japan. One genotypic group (T4) might represent a local form, while the other type (T8) shows some genetic affinities to T6.

Another genotypic group, T6, was identified in the Wadden Sea, which is part of the Southern North Sea. The Wadden Sea is the largest continous tide dominated depositional environment in the world (Reineck, 1978; Janke & Kraemer, 1990). It is a meso- to macrotidal environment and the sediment surface is widely covered with a diatom carpet. Living *Ammonia* specimens can be easily gained from the surface sediment layers. More samples from different stations of the the North Sea will be needed to clarify the question if T6 specimens are unique to the Wadden Sea or if they also occur in other parts of the North Sea.

The genotypic group T7 seems to be characteristic for southern salt marshes in the USA. *Ammonia* specimens of T7 occur south of Cape Hatteras, along the Atlantic coast of the USA. Warm Gulf stream water characterizes this coastal region and the foraminiferal assemblages are generally dominated by warm water species (Schnitker, 1971). T7 specimens differ genetically and morphologically from *Ammonia* spp. sampled in Long Island (T1 and T9). In the latter case, the coastal water is influenced by the cold Labrador Current Extension, the different *Ammonia* types might thus be adapted to different climatic regions.

The genotypic group T3 represents the only *Ammonia* group that inhabits rocky shores and open marine habitats. T3 representatives were only collected from European coasts. Given the limited number of samples, however, the distribution of this group might not be restricted to this area. According to Jorissen (1988) who investigated *Ammonia* specimens that correspond morphologically to T3, this form seems to be not so much dependent from a special substrate type, but has a rather specialized food uptake, which includes epi-benthic deposit feeders in areas without vegetation as well as forms that collect food from the vegetation cover.

TAXONOMIC REMARKS

The substantial question is whether the different genotypic groups of *Ammonia* can be considered as distinct species or not. It has been shown for planktonic foraminifera, that morphospecies can include different genotypes which are in some cases highly divergent and represent examples of cryptic speciation (Huber *et al.*, 1997; Darling *et al.*, 1999; De Vargas *et al.*, 1999). The investigated *Ammonia* specimens cluster in different, well defined genotypic groups and by comparing the genetic distances between these groups to the level of divergence in cryptic species of planktonic foraminifera, the former could be regarded as distinct species of *Ammonia*. Most of the genotypic groups of *Ammonia*, however contain only a few individuals from one sampling site (T4–T7, T9). Because of the limited sample size these genotypic groups cannot be regarded with certainty as distinct species and further investigations on a population level have still to be carried for each of these groups as well as for the genotype T8. For three of the genotypic groups (T1–T3), however there are some arguments in favor for their taxonomic status as distinct species.

T1 and T2 have been investigated on a population level. They

are genetically clearly distinguished and may be adapted to different ecological conditions (Holzmann & Pawlowski, 1997; Holzmann *et al.*, 1998). T1 and T2 show a partly overlapping biogeographical distribution and, where they occur in sympatry in the same habitat, they seem to be reproductively isolated, as no hybrids have been identified until now. Specimens belonging to the genotypic group T3 are characterized by very distinct morphological features, are genetically well defined and occur in different habitats, compared to the other *Ammonia* types.

The problem that remains to be solved, however, is whether existing specific names can be used to describe the different genotypic groups of *Ammonia* or not. Given the 'nomenclatural chaos' that reigns over the taxonomy of *Ammonia,* this is not an easy task. Table 4 presents a list of some taxonomic identifications that match different genetic types of *Ammonia.* Among the eight groups, there are only two (T3, T9) whose taxonomic identification is relatively easy.

Table 4. Some taxonomic identifications for different Ammonia types

Genetic type	Taxonomic identification	References
T1	*A. tepida*	Daniels (1970)
		Zaninetti (1984)
		Cimerman & Langer (1991)
		Hohenegger *et al.* (1993)
	A. beccarii forma rigonfa*	Albani & Serandrei-Barbero (1990)
	Rotalia veneta	Schultze (1854)
	Rotalia inca	Cushman & Kellett (1929)
T2	*A. aberdoveyensis*	Haynes (1973)
	A. parkinsoniana forma parkinsoniana	Jorissen (1988)
T3	*A. beccarii*	*Jorissen (1988)*
		Debenay *et al.* (1998)
T4	*A. beccarii* forma 2	Matoba (1970)
T5	*Rotalia inca*	Boltovskoy (1976)
T6	*Rotalia beccarii* var. *flevensis*	Hofker (1930)
	Streblus beccarii tepida	Richter (1967)
	Ammonia beccarii var.	Phleger (1970)
T7	*A. beccarii*	Akers (1970)
	A. beccarii forma tepida	Goldstein & Moodley (1993)
	A. parkinsoniana	Goldstein & Frey (1986)
T8	*A. beccarii* forma 1	Matoba (1970)
T9	*A. parkinsoniana*	Cushman & Cole (1930)
		Pawlowski *et al.* (1995)
	Rotalia beccarii var. *sobrina*	Shupack (1934)

The description of these species also fits T2 specimens

The genotypic group T3 can be most probably assigned to *Ammonia beccarii,* to which it corresponds in its external morphological features. *A. beccarii* was first described as *Nautilus beccarii* by Linné (1758), but a type specimen does not exist. Later investigations on material from the type locality (Rimini, Adriatic Sea, Italy) have been carried out by d'Orbigny (1826), Cushman (1928) and Cifelli (1962). All *A. beccarii* so far described from the type locality resemble in its external features the genotype T3.

The T3 specimens used in our study however, were sampled from the Western Atlantic, North Sea and the English Channel. Hofker (1951) described strongly ornamented *Ammonia* individuals from the North Sea as *Streblus batavus* and considered these forms being different from Adriatic *A. beccarii,* although he states that the two forms 'suggest much resemblance' (Hofker, 1951, p. 501). According to his description, *A. batavus* is characterized by having secondary chambers while he could never observe such a feature in Adriatic *A. beccarii.* Vénec-Peyré (1983) however, described *A. beccarii* from the French Mediterranean coast and reported variation along a morphological cline between 'batavus' forms, having interlocular spaces that correspond to Hofker's secondary chambers and 'beccarii' forms, lacking them. Considering the existence of these transitional stages, *A. batavus* might be a junior synonym of *A. beccarii,* but to date we do not have any sequences of *A. beccarii* specimens from the Adriatic Sea that could be used for a molecular comparison with T3 specimens.

T9 specimens resemble in their morphology *Ammonia parkinsoniana.* The species was first described by d'Orbigny (1839a) from recent sediments of Cuba, but a type specimen does not exist. A neotype was determined by Le Calvez (1977) and deposited in the collections of the Museum d'Histoire Naturelle in Paris, but it got lost (pers. comm., Dr Marie-Thérèse Vénec-Peyré). Therefore our determination is based solely on a comparison of our specimens with illustrations of the neotype presented by Le Calvez (1977, pl. 11, fig. 1–3). *Ammonia* specimens which are akin to the illustrated neotype of *A. parkinsoniana* and which were sampled from the same locality (Long Island) as our T9 specimens have been described as *Rotalia beccarii* var. *sobrina* by Shupack (1934). The specimens illustrated by Shupack (1934) (fig. 4a–c) also correspond in their external morphology to our T9 specimens and considering the morphological resemblance of the latter with the neotype of *A. parkinsoniana, Rotalia beccarii* var. *sobrina* might be a junior synonym of *A. parkinsoniana.*

It is much more difficult to attribute specific names to the remaining genotypic groups. Most of the *Ammonia* morphotypes found in our samples are usually described as *A. beccarii* or *A. tepida.* The latter name is often used for morphotypes similar to T1 (Zaninetti, 1984; Cimerman & Langer, 1991; Debenay *et al.*, 1998). However, in a previous study (Holzmann & Pawlowski, 1997), we examined the test morphology of 14 syntypes of *A. tepida* from the Cushman collection of the Smithsonian Institution, Washington D.C. (courtesy of Dr Martin Buzas) and found their test morphology different, compared to T1 specimens. But as no living specimens of *A. tepida* from the type locality (San Juan Harbour, Puerto Rico) have been genetically analysed so far, a molecular comparison between *A. tepida* and T1 specimens is not possible.

One possible solution to resolve the problem of taxonomic determination would be the use of names that have been given to some local populations of *Ammonia.* For example, the genotypic group T2 could be most probably assigned to *Ammonia aberdoveyensis* (Haynes, 1973). This species is characterized by a low conical test with rounded periphery and a lack of ventral ornament, test diameter averaging 0.40 mm, which is comparable to mean test size of T2. By sequencing nine living specimens from the type locality (Dovey Estuary, England), it turned out that eight of them are indeed branching within T2. However,

one specimen of the type locality clusters among T1 specimens. The *Ammonia* assemblage from the type locality consists thus of two different genotypic groups. But as T1 specimens do not correspond in their external features to the type description of *A. aberdoveyensis*, the name could be used for T2.

If such names are used, however, their validity has to be verified. For example, the *Ammonia* specimens from Chile have been assigned to *Rotalia inca* by Cushman & Kellett (1929) and have been considered as an endemic South American species (Boltovskoy, 1976). The species was first described by d'Orbigny (1839b) as *Rosalina inca* from Lima, Peru, and its type specimen is preserved in the Museum d'Histoire Naturelle in Paris. We have examined the type specimen specimen and found out that it is definitely not an *Ammonia*.

Given the complexity of the problem of taxonomic determination of *Ammonia*, we prefer to use an open nomenclature (Holzmann & Pawlowski, 1997; Holzmann *et al.*, 1998). In this way we avoid the creation of the new specific names, which would only further contribute to the confusing taxonomy of *Ammonia*.

Acknowledgements

Special thanks are extended to W. Piller and L. Zaninetti. We would further like to express our gratefulness towards all people that contributed to our work either by sending us samples or by helping us collecting, especially J. Bernhard, J. P. Debenay, S. Goldstein, J. Haynes, H. Kitazato, C. Manley and J. Montoya-Burgos. We would also like to thank J. Fahrni for technical assistance. Previously unpublished aspects of work reported here were funded by grants from the Austrian 'Fonds zur Foerderung der wissenschaftlichen Forschung', projects PO9817-BIO and P12105-BIO (M. H.) and by the Swiss Natural Science Foundation grant 3100-49513.96 (J. P.).

Manuscript received 25 February 1999
Manuscript accepted 5 December 1999

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