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Morphological distinction of molecular types in *Ammonia* – towards a taxonomic revision of the world's most commonly misidentified foraminifera

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Abstract

In this study, morphometric analysis has been performed on 178 *Ammonia* specimens belonging to 12 different molecular types, plus non-sequenced type specimens of *Ammonia beccarii* and *A. tepida*. Molecular type distinction is based on phylogenetic analysis of 267 partial LSU rDNA sequences, obtained from 202 living *Ammonia* specimens, sampled in 30 localities from 17 countries bordering the Pacific Ocean, Atlantic Ocean, Mediterranean Sea, Caribbean Sea and North Sea. Restriction fragment length polymorphism (analysis was carried out for another seven specimens). Morphometric analysis was based on measurements or assessments of 37 external test characters in spiral, umbilical, profile and close-up Scanning Electron Microscopic views. Cluster analysis, canonical variates analysis, and detrended correspondence analysis, performed on the morphological data set, suggest that each molecular type can be distinguished morphologically and can be regarded as a separate species. Primary types of *A. tepida* and topotypes of *A. beccarii* are shown to be morphologically separate from any of the molecular types so far recognised. We are aware of at least 9 more distinctive morphotypes that have not yet been sequenced, and thus we infer that the total number of genetically distinct and morphologically separable living species of *Ammonia* worldwide is likely to exceed 25–30. At this stage not all molecular types can be unequivocally assigned to formally described species. Several genetically-based species can be distinguished by the presence of one distinct character, but most are discriminated on the basis of a combination of many different characters. Morphological characters (e.g. test shape, chamber shape, porosity, prolocular diameter, folium shape, radial furrow length, umbilical diameter) are shown to be slightly more valuable in separating the molecular types than surficial ornament (beads, pustules, bosses, secondary calcite). One highly distinctive group (2–3 species – *beccarii*, *batava*, *?inflata*) is readily discriminated on the basis of its large test size, strongly beaded and grooved ornament, and the presence of fissures along the sutures on the spiral side. The results of this study imply that the widespread practice of recognising only one, two or three species of Recent *Ammonia* worldwide should be abandoned. The most commonly used name, *Ammonia beccarii*, should be restricted to a large, compressed, highly ornamented species, so far not recognised beyond its type locality in the Adriatic Sea. Other

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commonly used names, such as *A. parkinsoniana* and *A. tepida*, apply to species with far more restricted distributions than the literature would suggest.

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1. Introduction

1.1. Taxonomic confusion

Since the dawn of studies on Foraminifera, the genus *Ammonia* has been in the focus of scientific investigations. *Ammonia* Brünnich, 1772, was the first genus specifically assigned to Foraminifera (Loeblich and Tappan, 1974) and its type species, *Ammonia beccarii*, one of the first described foraminiferal species as *Nautilus beccarii* Linné, 1758.

Ammonia is one of the two most abundant foraminiferal genera worldwide (together with *Elphidium*) and mainly occurs in sheltered, shallow marine, often slightly brackish, intertidal environments (Murray, 1991). The high morphologic variability of *Ammonia* has led to considerable difficulties in species identification and more than 40 species and subspecies (or varieties) of Recent *Ammonia* have been described worldwide under the generic names *Ammonia*, *Streblus*, and *Rotalia* (Ellis and Messina, 1940 and supplements), although only a few of these are currently recognised. Today the taxonomy of *Ammonia* globally is in a confused state. Some taxonomically more rigorous works (e.g. Cimerman and Langer, 1991; Hottinger et al., 1993; Loeblich and Tappan, 1994; Colburn and Baskin, 1998; Buzas-Stephens et al., 2002) apply formal species names to each different morphological form in the traditional manner, whereas many workers have given up trying to morphologically differentiate species (e.g. Chang and Kaesler, 1974; Schnitker, 1974; Poag, 1978; Wang and Lutze, 1986; Jorissen, 1988; Walton and Sloan, 1990).

Cushman (1926) was the first to introduce the practice of lumping all *Ammonia* around the world into just three varieties (*beccarii*, *parkinsoniana*, *tepida*) of *Ammonia beccarii*. This approach was given strong support by the results of laboratory breeding experiments on specimens

from the eastern United States by Schnitker (1974). He reported that culturing of *tepida*-like forms produced offspring with a wide variety of morphotypes identifiable as all seven species previously recorded from the Atlantic coast of North America. He concluded that '*Ammonia beccarii* is, by priority, the only valid species encountered' and the other species are all ecophenotypes. Hence in many subsequent studies all specimens have been identified as one morphologically variable species – *A. beccarii* (e.g. Poag, 1978; Walton and Sloan, 1990; Yassini and Jones, 1995). This lumping approach has been aided by the extreme difficulty many workers encounter when trying to consistently discriminate more than one morphological species within a large variable population of *Ammonia* being studied for ecologic or biologic purposes. Chang and Kaesler (1974) undertook an extensive biometric morphological analysis of western North Atlantic *Ammonia*, concluding that there are two geographically separate varieties of *A. beccarii* discriminated primarily on their prolocular and umbilical diameters.

Poag (1978) and later Jorissen (1988) concluded that there are two different 'morphostocks' or species globally. They restricted *Ammonia beccarii* to large, ornamented North Atlantic and Mediterranean forms with sutural fissures on the spiral side. By priority, they applied the species name *A. parkinsoniana* to the less ornamented 'morphostock' (which includes all the specimens studied by Schnitker, 1974). Some of the formal species names are sometimes applied as form names to different morphotypes of these two 'species' (e.g. *A. beccarii* f. *inflata*, *A. parkinsoniana* f. *tepida*, *A. parkinsoniana* f. *aoteana* – Jorissen, 1988, Hayward et al., 1999). Debenay et al. (1998) showed that the *beccarii* stock were largely epiphytic and fully marine, and the other infaunal and mostly brackish.

Walton and Sloan (1990) undertook a more

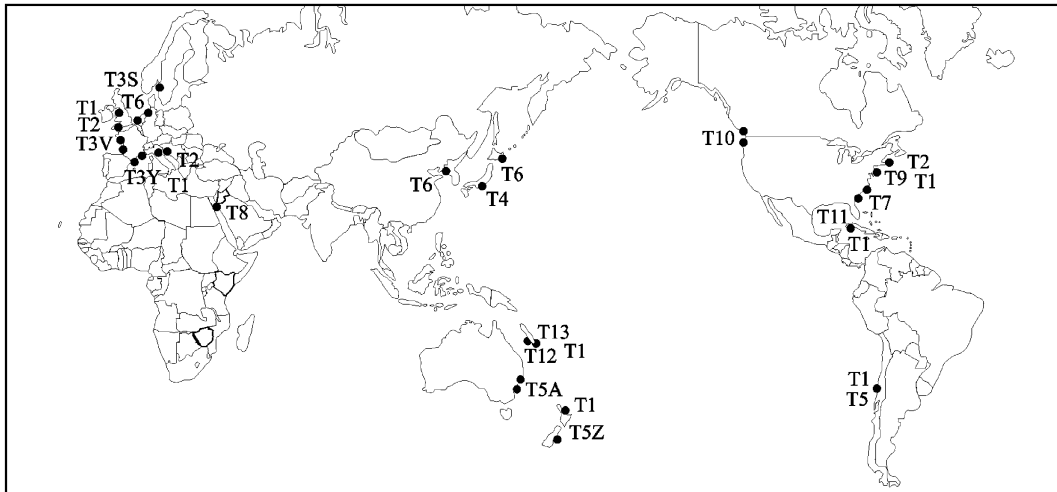


Fig. 1. Global distribution of the 13 *Ammonia* molecular types identified and morphologically analysed in this study.

comprehensive review of *Ammonia* and continued the theme of high level ecophenotypic variation, by placing all modern *Ammonia* globally into one of three forms (*f. beccarii*, *f. parkinsoniana*, *f. tepida*) of the single species, *A. beccarii*. They retained the same criteria for separating off the *beccarii* group, and recognised a gradational series from *f. parkinsoniana* with one or more umbilical bosses to *f. tepida* with no bosses.

Cifelli (1962, p. 119) noted that '*Ammonia beccarii* is imperfectly known. The figures given in the literature vary considerably, and the relationship of all the forms included in this species are not clear'. Now, forty years on, the global concept of *Ammonia beccarii* and recognition of its true identity is more chaotic than ever.

1.2. New insights using molecular techniques

The development and refinement of molecular techniques permits DNA sequencing of single foraminiferal individuals (Pawlowski et al., 1995; Holzmann et al., 1996, 1998; Holzmann and Pawlowski, 1997, 2000; Holzmann, 2000) and has opened new avenues to investigate the worldwide taxonomic status of *Ammonia* populations.

Recent studies (Holzmann, 2000; Holzmann and Pawlowski, 2000; Langer and Leppig, 2000) have already shown that eight different molecular types of *Ammonia* can be distinguished genetically

from around the world, and that in many places at least two types may occur together. Previously reported molecular studies have shown the presence of two genetically distinct types of *Ammonia* in the Lagoon of Venice, Italy (Holzmann and Pawlowski, 1997; Holzmann et al., 1998). The two molecular types could be distinguished with difficulty on the basis of their pore size, pore density and test size and appear to have slightly different ecological distribution patterns.

These previous studies suggest that further work could be beneficial in establishing a more robust taxonomic subdivision of *Ammonia* worldwide, whereby the taxa are identified genetically and subsequently characterised morphologically to allow for traditional non-genetic identification of live and dead specimens. The present study combines molecular and morphological methods in order to investigate taxonomic relationships within the genus *Ammonia*. We compare and discuss molecular and morphological data sets, which both confirm the distinction of numerous different species, contrary to the current widespread morphology-based classification which places different morphotypes into one or two species containing many ecophenotypes.

In some research areas, a more robust, genetically-based, morphologic discrimination of *Ammonia* species is likely to provide for new advances in intertidal and shallow-water ecologic,

Table 1
Collection localities and number of *Ammonia* specimens sequenced and measured

Molecular type	Locality (and abbreviations)	No. of specimens sequenced	No. partial LSUrDNA sequences	No. RFLP analysed specimens	Specimens measured (sequenced specimens in brackets)				Total measured
					Profile	Umbil	Spiral	Closeup	
<i>T1</i>	<i>Cosmopolitan: microtidal marshes, brackish estuaries</i>								
T1	UK, Aberdovey	1	2		0	1(1)	1(1)	0	1(1) ¹
T1N	New Zealand, Auckland, Waitemata Hbr	6	6		4	7(5)	7(5)	2(1)	9(5) ^{1,2}
T1	France, Camargue, Le Boucanet	11	19		0	2(2)	3(3)	2(2)	3(3) ¹
T1	France, Bretagne, Golf de Morbihan	1	2		0	1(1)	1(1)	0	1(1) ¹
T1	Netherlands, Texel, Mook Baai	2	2		0	0	0	0	0
T1	Italy, Venice Lagoon	7	10		0	2(2)	1(1)	1(1)	2(2) ¹
T1	Italy, Trieste	3	6		0	3(3)	2(2)	1(1)	3(3) ¹
T1	New Caledonia, Tieti Bch	1	1		2	0	2	2	2(0) ²
T1	Chile, La Ligua	3	4		0	0	0	0	0
T1	Cuba, Playa Bailen	9	9		3	7(7)	8(7)	0	10(7) ^{1,2}
T1	USA, Ma, Cape Cod	6	6		0	1(1)	1(1)	0	1(1) ¹
T1	USA, NY, Long Is	1	1		0	0	0	0	0
T1	Sweden, Tjaerno	1	2		0	0	0	0	0
T1	TOTAL	52	70	0	9	24(22)	26(21)	8(5)	32(23)
<i>T2</i>	<i>European and North Atlantic coasts: microtidal marshes</i>								
T2U	UK, Aberdovey, <i>A. aberdoveyensis</i> topotypes	8	16		4	4(2)	3(2)	4(2)	6(2) ^{1,2}
T2U	UK, Plymouth	2	4		0	1(1)	1(1)	0	1(1) ¹
T2U	France, Bretagne, Golf de Morbihan	2	4		0	2(2)	2(2)	0	2(2) ¹
T2	France, Camargue, Le Boucanet	9	13		0	0	0	0	0
T2M	Italy, Venice	13	16		4	7(5)	6(5)	2	9(5) ^{1,2}
T2	Italy, Trieste	2	4		0	0	0	0	0
T2	USA, Ma, Cape Cod	4	4		0	0	0	0	0
T2	TOTAL	40	61	0	8	14(10)	12(10)	6(2)	18(10)
<i>T3</i>	<i>European coasts: open marine habitats and rocky shores on algae</i>								
T3S	Sweden, Tjaerno	7	13		2	7(5)	7(5)	3	10(5) ^{1,2}
T3S	UK, Plymouth	1	2		0	1(1)	1(1)	0	1(1) ¹
T3V	France, Atlantic, Vendée	2	2		4	3(1)	3(1)	3(1)	5(1) ^{1,2}
T3Y	France, Mediter., Banyuls-sur-mer	1	1		4	3	3	2	5(0) ²
T3	TOTAL	11	18	0	10	14(7)	14(7)	8(1)	21(7)

Table 1 (Continued).

Molecular type	Locality (and abbreviations)	No. of specimens sequenced	No. partial LSUrDNA sequences	No. RFLP analysed specimens	Specimens measured (sequenced specimens in brackets)				Total measured
					Profile	Umbil	Spiral	Closeup	
<i>T4</i>	<i>Japanese coast: brackish lake</i>								
T4	Japan, Lake Hamana	3	6	0	3	3	3	2	5(0) ²
<i>T5</i>	<i>South Pacific coasts: intertidal flats and marshes, subtidal brackish estuaries and sheltered harbours</i>								
T5H	Chile, La Ligua	3	5		0	2(2)	2(2)	0	2(2) ¹
T5Z	New Zealand, Auckland, Pollen I.	12	12		0	1(1)	1(1)	0	1(1) ¹
T5Z	New Zealand, Auckland, Panmure Basin	0			3	1	2	1	3(0) ²
T5Z	NZ, Christchurch, Lyttelton Hbr	5	5		0	0	0	0	0
T5Z	NZ, Christchurch, Akaroa Hbr	15	15		0	4(4)	4(4)	0	4(4) ¹
T5Z	NZ, Dunedin, <i>aoteana</i> topotypes	0			2	0	2	2	3(0) ²
T5Z	NZ, South and Chatham Is	0			3	2	1	0	3(0) ²
T5A	Australia, NSW, Ulladulla, Burril Lake	5	5		0	3(3)	3(3)	0	3(3) ¹
T5A	Aust, NSW, Port Hacking	5	5		5	5(2)	4(2)	2	7(2) ^{1,2}
T5	TOTAL	45	47	0	13	19(12)	18(12)	5	26(12)
<i>T6</i>	<i>North Sea, Northeast China and Japanese coasts: tidal flats and marshes, brackish lake</i>								
T6E	Germany, Wilhelmshaven	9	12		0	3(1)	3(1)	0	3(1) ¹
T6E	Germany, Wilhelmshaven	9		2	0	(2)	(2)	0	(2) ¹
T6E	Denmark, Rönbjerg	0		1	0	1(1)	1(1)	0	1(1) ¹
T6E	Denmark, Vidä Sluse	0		2	0	2(2)	2(2)	0	2(2) ¹
T6E	Denmark, Store Darum	0		2	0	2(2)	2(2)	0	2(2) ¹
T6C	China, Yalu Jiang	7	7		9	9(5)	9(5)	6(3)	15(5) ^{1,2}
T6	Japan, Hokkaido, Saroma Lake	7	11		0	0	0	0	0
T6	Japan, Hamana Lake	1	1		0	0	0	0	0
T6	Netherlands, Texel, Mook Baai	5	5		0	0	0	0	0
T6	TOTAL	29	36	7	9	17(13)	17(13)	6(3)	23(13)

Table 1 (Continued).

Molecular type	Locality (and abbreviations)	No. of specimens sequenced	No. partial LSUrDNA sequences	No. RFLP analysed specimens	Specimens measured (sequenced specimens in brackets)				Total measured
					Profile	Umbil	Spiral	Closeup	
T7	<i>Atlantic coast of USA: microtidal marshes</i>								
T7	USA, Georgia, Sapelo I.	3	6		3	5(1)	4(1)	2	7(1) ^{1,2}
T7	USA, North Carolina, Beaufort	3	6		0	0	0	0	0
T7	TOTAL	6	12	0	3	5(1)	4(1)	2	7(1)
T8	<i>Red Sea: open marine habitats</i>								
T8	Israel, Taba	2	2	0	0	0	0	0	0
T9	<i>Atlantic coast of USA: microtidal marshes</i>								
T9	USA, NY, Long Is	2	3	0	3	2	2	2	4(0) ²
T10	<i>Pacific coast of USA and Canada: microtidal marshes and flats</i>								
T10	USA, Washington, Grays Harbour	7	7		3	7(5)	7(5)	2	8(5) ^{1,2}
T10	Canada, BC, Vancouver	1	1		3	2	2	2	3(0) ²
T10	TOTAL	8	8	0	6	9(5)	9(5)	4	11(5)
T11	<i>Caribbean Sea: microtidal marshes</i>								
T11	Cuba, Playa Bailen	1	1	0	7	4(1)	4(1)	3	8(1) ^{1,2}
T12	<i>Tropical Southwest Pacific: brackish estuary</i>								
T12	New Caledonia, Tieti Bch	2	2	0	3	3(1)	4(1)	2	5(1) ^{1,2}
T13	<i>Tropical Southwest Pacific: marine salinity mangroves</i>								
T13	New Caledonia, Noumea	1	1	0	6	3	3	3	6(0) ²
B	<i>Adriatic Sea: open marine beach</i>								
B	Italy, Rimini Beach, topotypes of <i>beccarii</i>	0	0	0	8	7	7	7	9(0) ²
T	<i>Caribbean Sea:</i>								
T	Puerto Rico, syntypes of <i>tepida</i>	0	0	0	3	3	3	2	3(0) ³
TOTAL SPECIMENS		202	267	7	149	127	124	60	178(79)

Specimens held in the collections of: ¹ Maria Holzmann; ² Institute of Geological and Nuclear Sciences, Lower Hutt; ³ Smithsonian Institution, Washington, DC.

biogeographic and applied palaeoenvironmental studies.

1.3. Questions

The present study addresses the following questions:

(1) Is there just one morphologically variable, cosmopolitan species of *Ammonia*, as much of the international literature would imply? Are the described species merely ecophenotypic variants of this one widespread species?

(2) Can genetically identified types of *Ammonia* be discriminated by morphological characters, and if so, what morphological characters are taxonomically the most useful?

(3) How many genetically identified types correspond to morphologically discriminated and formally described species?

(4) Do more than one genetically identified species of *Ammonia* live together, and if so can they be distinguished morphologically?

(5) What is the taxonomic meaning of the names *Ammonia beccarii* and *A. tepida*?

2. Materials and methods

2.1. Cell collection

Samples were collected from 30 different near-shore localities in the Mediterranean Sea, Irish Sea, the English Channel, North Sea, North Atlantic, Caribbean Sea, Red Sea and Pacific Ocean (Fig. 1; Table 1). Sediment samples were either taken by hand with a scraper or by means of a grab sampler (Holzmann and Pawlowski, 2000). Living *Ammonia* specimens were identified by the use of a stereomicroscope and isolated for subsequent studies.

2.2. DNA extraction

A total of 202 specimens have been used for this study, including those *Ammonia* individuals whose sequences have been published previously (Holzmann et al., 1996, 1998; Holzmann and Pawlowski, 1997, 2000). DNA was extracted one

by one from all specimens. Every specimen was ground separately in extraction buffer and incubated at 60°C for 1 h, followed by short centrifugation to remove insoluble material (Holzmann and Pawlowski, 1996). The morphology of 127 sequenced specimens was recorded by Scanning Electron Microscopy (SEM) prior to DNA extraction.

2.3. PCR amplification, cloning and sequencing

A fragment of the LSU rDNA of about 450 nucleotides was amplified by PCR in a total volume of 50 µl. The thermal cycle parameters consisted of 40 cycles of 30 s at 94°C, 30 s at 50°C and 60 s at 72°C, followed by 5 min at 72°C for final extension. Two specific foraminiferal LSU rDNA primers, Rib 2TA and Rib 7, were used for amplification (Holzmann and Pawlowski, 2000). The amplified PCR products were purified using High Pure PCR Purification Kit (Roche Diagnostics), ligated in the pGEM-T Vector system (Promega) and cloned using Supercompetent cells XL1-Blue MR (Stratagene). Sequencing reactions were prepared using ABI-PRISM Big Dye Terminator Cycle Sequencing Kit and analysed with an ABI-377 DNA sequencer (Perkin-Elmer), all according to the manufacturer's instructions.

2.4. Sequence data

A fragment of the LSU rDNA was amplified and sequenced for 202 specimens. For 48 specimens, more than one clone was sequenced (between 2 and 5 clones per specimen), resulting in a total of 267 LSU sequences (Table 1). The sequenced fragment is situated at the 5' terminal end of the LSU rRNA gene and includes the divergent domain D1 as well as two flanking regions of the conserved domains C1 and C2 (Hassouna et al., 1984). It corresponds to the positions 1–324 in the LSU rRNA gene of *Rattus norvegicus* (X01069). The length of the obtained sequences ranges from 360 basepairs (bp) to 466 bp, which is up to one and a half as much than in other eukaryotes. This is due to insertions in the divergent domain D1 that are unique to Forami-

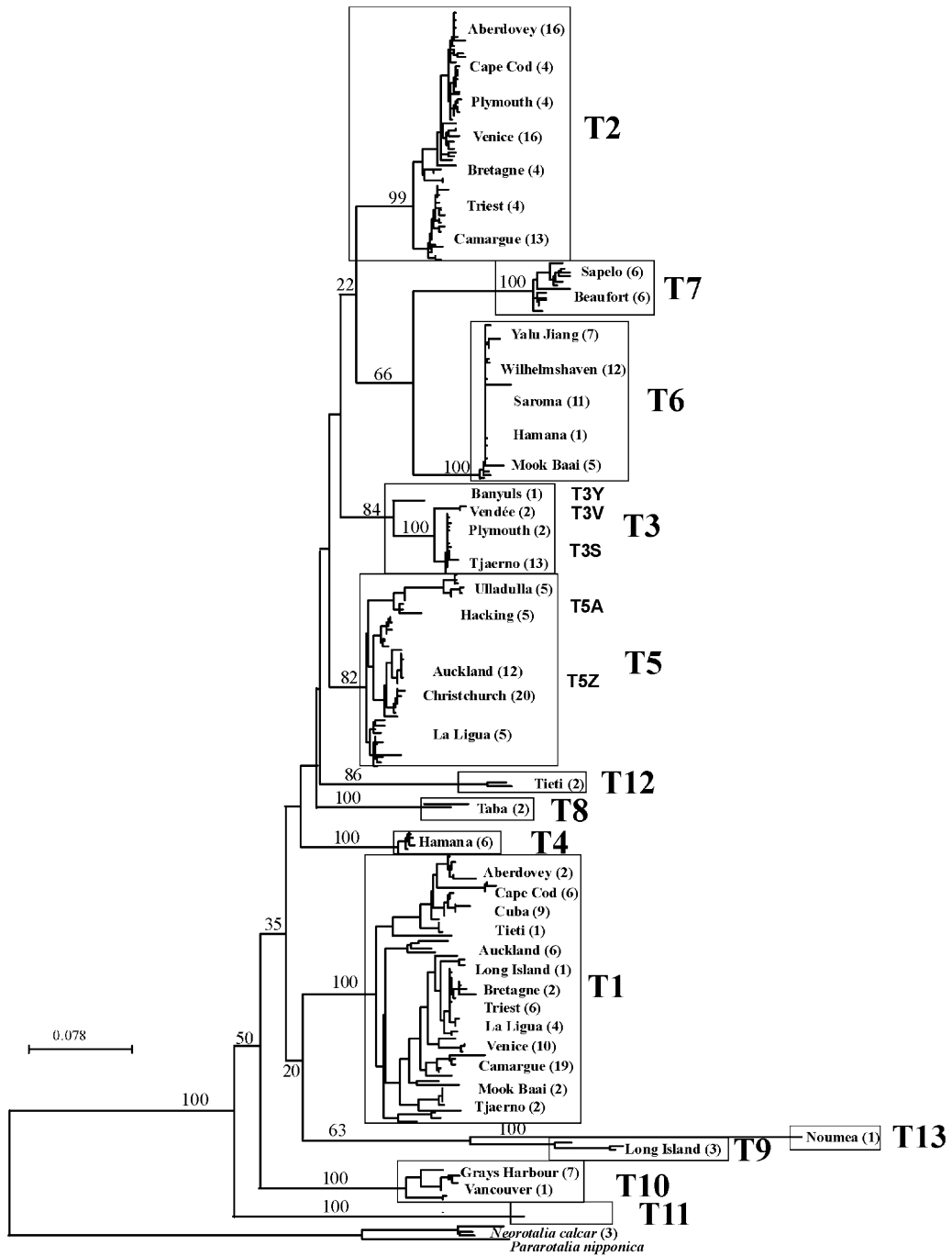


Fig. 2. Phylogenetic analysis of 267 partial LSU rDNA sequences using the NJ method. The numbers are bootstrap percent values based on 500 resamplings. The scale bar corresponds to the number of substitutions per site. The names of localities and number of sequences (in brackets) are indicated for each molecular type.

nifera (Holzmann et al., 1996). The G+C content ranges from 38.5% to 46.5%.

2.5. DNA sequence analysis

Sequences were aligned manually by using the GDE 2.2 software (Larsen et al., 1993). Phylogenetic analysis was carried out by using the Neighbor joining (NJ) method (Saitou and Nei, 1987), applied to distances corrected for multiple hits and for unequal transition and transversion rates, using Kimura's two-parameter (K2P) model (Kimura, 1980). NJ analysis was additionally tested by applying Tajima and Nei's (TN) six-parameter model (Tajima and Nei, 1984). All sites were retained for phylogenetic analysis, in order to maintain the minor intraindividual differences that occur in the sequences of some *Ammonia* specimens. The reliability of internal branches was assessed by bootstrapping (Felsenstein, 1988) with 500 resamplings. The PHYLO_WIN programme (Galatier and Gouy, 1996) was used for distance computations, NJ tree-building and bootstrapping (Fig. 2).

The new sequences presented in this study were deposited in the EMBL/GenBank Nucleotide Sequence Database under accession numbers AJ228549-AJ228551, AJ240136, AJ409926-AJ409999, AJ410000-AJ410014, AJ40112-AJ40135 and AF479676-AF479682. Earlier determined sequences used in this study are listed in Holzmann et al. (1996, 1998) and Holzmann and Pawlowski (1997, 2000).

2.6. Restriction fragment length polymorphism (RFLP) analysis

After having determined three different molecular types (T1, T2 and T6) by sequence analysis, two restriction enzymes which cut the nucleotide sequence at specific patterns were chosen for rapid distinction between these three groups (Fig. 3). PCR-product digestions were performed by first using the endonuclease ScaI (Promega) for all PCR products and then EclXI (Roche) for T2 and T6 PCR products. ScaI cuts at the palindromic sequence G/AGCTC, EclXI at C/GGCCG, according to the following protocol:

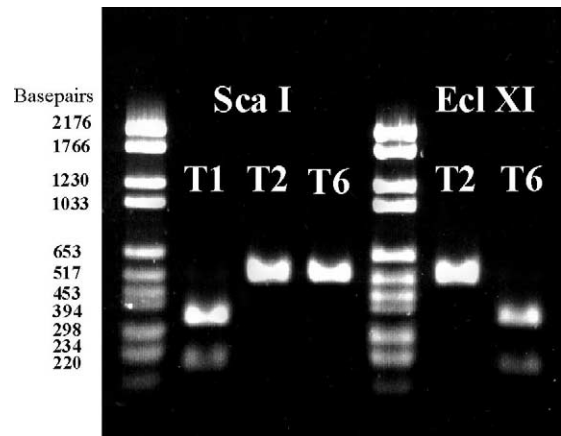


Fig. 3. Restriction patterns for three molecular types T1, T2 and T6 resulting from RFLP analysis on partial LSU rDNA PCR products, using the endonucleases ScaI (Promega) and EclXI (Roche). Each type has its specific pattern which allows recognition at first glance.

10 µl of the PCR products were directly digested for 2 h at 37°C in a total volume of 20.5 µl containing 0.5 µl enzyme (5 units), 2 µl buffer and 8 µl distilled water. Distinct patterns for each molecular type were UV detected after migration of the digested PCR products on a 1.5% agarose gel and ethidium bromide colouration. Seven specimens belonging to T6 which were analysed by RFLP method were subsequently determined morphologically (Table 1).

2.7. Morphology

A selection of digital SEM images of specimens (Table 1) from each of the identified molecular types (except T8, as no specimens were available) were measured and morphologically coded for numerical analysis. The number of specimens coded was largely determined by availability, and consisted of a mix of sequenced specimens (SEM imaged prior to destruction during DNA extraction) and non-sequenced specimens from the same localities as sequenced specimens (Table 1). Also included were three syntypes (Puerto Rico, CC12988, USNM520144-5) of *Ammonia tepida* and nine topotypes (Rimini Beach, USNM-520147, F201292) of *A. beccarii*, as these are the two most commonly identified species and

no unequivocal specimens of these had been sequenced. Juvenile specimens have less well-developed characters and thus only specimens with > 1.8 whorls were measured.

For the purpose of investigating the morphological distinctiveness and variability between and within molecular types, measured specimens were assigned to 21 molecular type groups (Table 2). These included the unsequenced type specimens of *tepida* and *beccarii*, but also geographical groupings of specimens from within molecular types T1, T2, T3, T5, and T6.

Measurements and codings were acquired from four SEM views – profile, umbilical, spiral, and close-up of penultimate chamber on the spiral side (for porosity). The 37 morphological characters used in the analyses consisted of a mix of quantitative measurements or ratios, and continuous five point qualitative assessments (Table 3; Plate I). The strength of character values in influencing discrimination between specimens or means depends on the size and range of character scores. To remove this influence, all scores for each char-

acter were standardised to range between 0 and 1 prior to their use in multivariate analyses.

Most specimens were represented by a combination of 1–3 views, with no specimens imaged from all four views. Thus the dataset contained numerous missing values where specimens lacked images from one or more views. For analyses to investigate the full morphological distinctiveness of molecular type groups the data was manipulated using two different approaches.

(a) At the individual specimen level, all missing character values were filled by estimation. For characters that were assessed as being independent of specimen maturity or size (characters 8, 10, 11, 14–18, 20–22, 28–32, 35–37; Table 3) the mean value of the character for the molecular type group was inserted. For characters that were assessed as varying with specimen age or covarying with other characters, the missing values were estimated using formulae generated based on the data that was available that linked specimen size to the values for these characters (Beale and Little, 1975).

(b) At the molecular type group level, the composite mean and two standard deviations were calculated for all characters in each molecular type group, based on the actual measurements (Table 1).

Table 2
Molecular type groups used to assess morphological differences between standardised mean values (Plate I)

Molec. type	Location
T1	UK, France, Italy, USA, Cuba, New Caledonia
T1N or N	New Zealand
T2M or M	Mediterranean coasts of France and Italy
T2U or U	UK, Atlantic coast of France
T3S or S	Sweden, United Kingdom
T3V or V	France, Atlantic Ocean, Vendee
T3Y or Y	France, Mediterranean Sea, Banyuls
T4	Japan
T5A or A	Australia
T5H or H	Chile
T5Z or Z	New Zealand
T6C or C	China
T6E or E	Germany, Denmark
T7	USA, Georgia
T9	USA, New York
T10	USA, Washington State, and Canada, British Columbia
T11	Cuba
T12	New Caledonia
T13	New Caledonia
B	Italy, Rimini Beach - <i>beccarii</i> topotypes
T	Puerto Rico - <i>tepida</i> syntypes

2.8. Computer methods

Unweighted pair-group Q-mode cluster analysis using arithmetic averages of euclidian distance matrix was used to generate a dendrogram classification of all the specimens based on the filled data set (Fig. 4). The filled dataset was also used in a Canonical Variates Analysis (CVA) of all 21 molecular type groups (Fig. 5) and also on reduced numbers of groups.

Detrended Correspondence Analysis (DCA) was used to produce 2-D ordinations (Fig. 6) of the means, and maximum and minimum two standard deviation values (based solely on actual measurements) of all 21 groups (Table 2).

2.9. Specimen repositories

All sequenced specimens were destroyed during

Table 3

Ammonia test characters measured or assessed and used in morphometric analyses

Profile, quantitative measurement:

1. gsd = greatest spiral diameter
2. gsd/h = gsd/height (thickness)
3. hs/hsu = height of spiral side/height of umbilical side

Profile, qualitative 5-point assesment:

4. umb = umbilical side (concave, flat, low convex, convex, high convex)
5. spi = spiral side (concave, flat, low convex, convex, high convex)
6. per[^] = peripheral profile/angle (keeled, angled, acutely rounded, obtuse rounded, broadly obtuse rounded)

Umbilical side, quantitative measurement:

7. du/d = relative diameter of umbilicus = gsd/largest diameter of umbilicus between ends of folia
8. rfl/w = relative length of radial sutural furrows = length of radial sutural furrow (n-1:n-2)/width (perpendicular to periphery) of chamber n-1
9. maxbos = diameter of largest umbonal boss (if present)
10. lgbos/d = relative size of single large umbonal boss (if present) = diameter of single large boss/bsd
11. nobos = number of umbonal bosses (if present)
12. fol[^] = folium angle (in degrees) of chamber n-1

Umbilical side, qualitative 5 point assesment (none, very weak, weak, medium, strong):

13. thckfol = development of thickened calcite on folia
14. folpust = coverage of folia by small pustules
15. protof = deeply notched protoforamen on chambers n... n-3
16. ragfol = blunt, ragged folium on chambers n... n-3
17. pntfol = sharp, pointed folium on chambers n... n-3
18. radbd = development of strong beads along edge of radial sutures
19. radgrv = development of grooved notches along edge of radial sutures
20. folbdgrv = folia cut into flat beads by grooves

Spiral side, quantitative measurement:

21. prol = proloculus largest diameter
22. chwh1 = number of chambers in first whorl
23. chwh2 = number of chambers in second whorl wh = number of whorls (to one decimal place); not used directly in analyses
24. d/wh = mean diameter of each whorl = gsd-prol/2 × no of whorls
25. ch/wh = mean number of chambers per whorl = number of chambers/wh
26. lc/wc = relative chamber (n-1) proportions = max length (parallel to periphery) of chamber/max width (perpendicular to periphery) of chamber
27. perout = proportion of 360° peripheral outline that is smooth, not lobular
28. spfis = length of fissure along spiral suture (when present), as proportion of a 360° circle
29. rad[^] = angle between radial (n-1:n-2) and spiral sutures

Spiral side, qualitative 5 point assesment (none, very weak, weak, medium, strong):

30. radfis = development of furrows along radial sutures (when present)
31. spsutbd = development of beads and grooves along edge of spiral suture
32. radsutcv = radial sutural curvature (suture n-1:n-2)
33. thckrad = development of raised thickened calcite along radial sutures of last whorl
34. spicac = development of raised thickened calcite over central spiral area
35. retcac = development of reticulate pattern of calcite riblets over central spiral area

Close-up view spiral side (n-1), quantitative measurement:

36. mnpor = mean diameter of 10 nearest pores to junction of n, n-1, and spiral suture. minpore = smallest diamter of 10 measured pores (as above); not used in analysis maxpore = largest diameter of 10 measured pores (as above); not used in analysis
37. poredens = pore density = number of pores/100 sq. µm near junction of n, n-1, and spiral suture

n = last chamber, n-1 = penultimate chamber

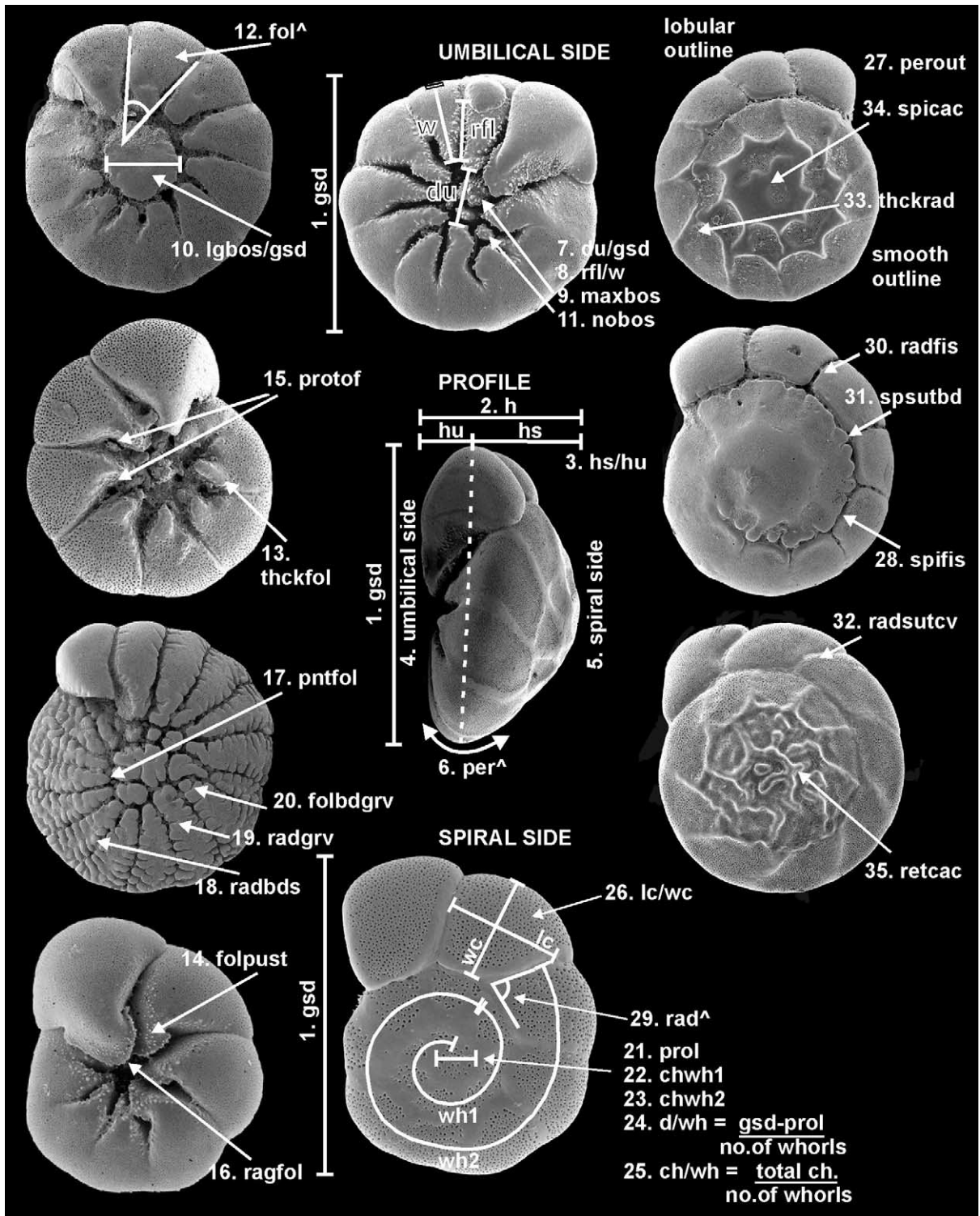


Plate I. Characters used in morphometric analysis of *Ammonia* specimens. Abbreviations are explained in Table 3. All scale bars = 100 μm .

DNA extraction, although SEM photographs were made of many prior to destruction. Non-sequenced specimens imaged by SEM for the morphometric analysis (Table 1) are either held in the collection of Maria Holzmann, the Institute of Geological and Nuclear Sciences, Lower Hutt, New Zealand (figured specimens on stubs prefixed by BWH, faunal samples prefixed by F), or the Smithsonian Institution, Washington, DC (figured and faunal specimens prefixed by CC or USNM).

3. Results

3.1. Phylogenetic analysis

NJ analysis of 262 *Ammonia* sequences is presented in Fig. 2. The sequences of *Pararotalia nipponica* and *Neorotalia calcar* were chosen as an outgroup because they represent the closest sister group to *Ammonia* available for our analysis.

The phylogenetic analysis reveals the presence of 13 distinct groups that are called here molecular types (T1–T13). Eleven of these are represented by 2–65 sequences while two types are characterised by single sequences (T11, T13). All molecular types are monophyletic and supported by high bootstrap values (82–100%). Relationships between the different groups are identical in trees based on K2P and TN models, however, they are not supported by high bootstrap values. Only two clades are moderately well supported, one consisting of T6 and T7 (66% bootstrap), the other including T9 and T13 (63% bootstrap). All investigated T5 specimens from Australia are distinguished from T5 specimens of Chile and New Zealand by two point mutations in conserved regions of the gene. The genetic differences are due to two transversions at the respective positions 191 (A → G) and 553 (A → C).

T8 has been previously assigned to a sequence showing close affinities to T6 (Holzmann, 2000; Holzmann and Pawlowski, 2000). According to our new analyses, the former molecular type T8 clusters within T6 and is thus incorporated in the latter group. T8 now designates a different molecular type.

Pairwise comparison of the sequences shows a high sequence dissimilarity between and within the investigated molecular types (Table 4). Sequence divergence between molecular types ranges from 7.5 to 43.2% and reaches up to 13.5% within a single type (T1). Intra-individual variation was checked for 8 groups (T1–T7, T9) by sequencing different clones obtained from the same individual. Polymorphic rDNA copies were found in each tested group, with the lowest values in T3 ($\leq 1\%$) and the highest values in T1 ($\leq 8.7\%$).

3.2. Morphometric analysis

3.2.1. Multivariate analyses – all molecular types

The four highest level groups (a–d) in the cluster analysis dendrogram (based on filled data set) comprise clusters of morphologically similar types (Fig. 4):

a = large, compressed, strongly ornamented tests (group B);

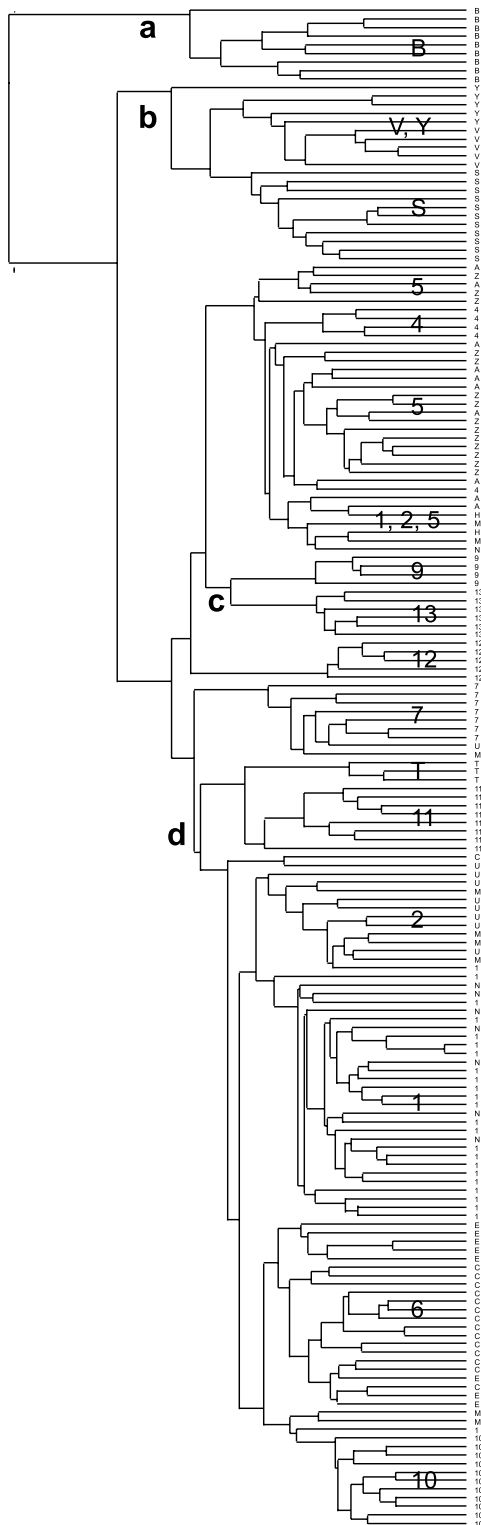
b = large, inflated, strongly ornamented tests (groups T3S, V, and Y);

c = moderate secondary calcite ornament, commonly with a boss (groups T4, T5, T9, T12, and T13);

d = small to medium-sized, least ornamented tests (groups T1, T2, T6, T7, T10, T11, and T).

The cluster analysis (Fig. 4) also suggests that nine groups (B, S, T, T4, T9, T10, T11, T12, T13) are morphologically strongly distinct from all others, with the other types exhibiting some overlap around the fringes of their variability (between T3V and Y; T1, T2, T5, T6, and T10; T2 and T7). This analysis also suggests that the geographic subgroups are morphologically similar within molecular types T1 (1, N), T2 (M, U), T5 (A, H, Z) and T6 (C, E). Only within molecular type T3 is there a morphological difference between geographic subgroups, with T3S clustering separately from T3V and T3Y.

The configuration of 95% confidence ellipses of each group on the first two CVA axes (Fig. 5), based on the filled dataset, clearly shows five distinct, non-overlapping groups (B, T3S, V, Y, T12). The remaining 16 groups are bunched together with T9 and T13 separate from the rest. These five distinct groups are also the most dis-



tinctive (highest clustering level) in the cluster analysis dendrogram (Fig. 4).

The two DCA ordinations (axes 1 v 2, 1 v 3) of the means and two standard deviations (95% confidence) of all 21 investigated groups (Table 2), based on all 37 character states (Table 3), show a clear division into two larger groups along axis 1 (Fig. 4). As shown by the ordination of characters, there is an ornamented group (B, T3S, T3V, T3Y) characterised by: their large size (gsd, d/wh); fissures along the spiral (spfis) and radial (radfis) sutures on the spiral side; and distinctly beaded and grooved ornament along the edges of the radial sutures on the umbilical side (radgrv, radbd), along the edges of the spiral suture on the spiral side (spsutbd) and over the folia on the umbilical side (folbdgrv); and a less ornamented group.

The standard deviation rectangles of eight of the molecular types (B; T3 (S,V,Y); T4; T5 (A,H,Z); T7; T9; T12; T13) are clearly separable on axes 1 and 2 of the 2D DCA plot (Fig. 4), implying that they are morphologically separable. The standard deviation rectangles of some less ornamented types overlap when plotted on the first two DCA axes. In three dimensions (upper and lower plots in Fig. 4 combined), the rectangles of variation of a further three molecular types (T1 (1,N); T6 (C,E); T10) are clearly separable from all others. This leaves only T overlapping with T11 and T2. In the cluster analysis none of these three molecular types exhibited any overlap, suggesting that they are likely to be morphologically distinguishable, too. As in the cluster analysis, the geographic subgroups within T1 (1, N), T2 (M, U), T5 (A, H, Z), and T6 (C, E) are not separable, whereas the T3S subgroup (North Sea) within T3 is again distinct from the T3V (Atlantic) and T3Y (Mediterranean) subgroups.

The major two-fold subdivision of axis 1 of the CVA and DCA ordinations (Figs. 5 and 6) and the highest level groupings (a, b) in the cluster analysis (Fig. 4) strongly overprints all analyses,

Fig. 4. Cluster analysis (based on euclidian similarity matrix) of 178 measured specimens of *Ammonia* with missing measurement values filled by estimation techniques.

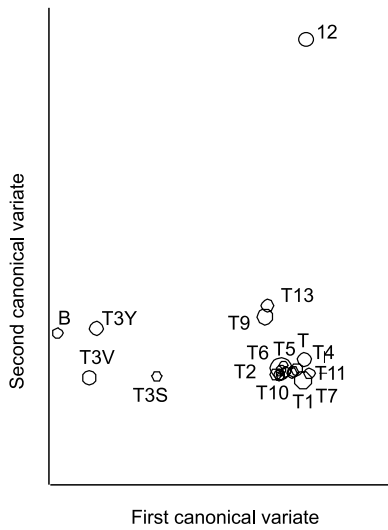


Fig. 5. 2-D CVA configuration of molecular type groups (see Table 2 for abbreviations) and their 95% confidence regions. Based on the filled data set.

so the larger, less ornamented, division was separately analysed by CVA and DCA (Fig. 7 and 8) to further investigate the inter-relationship between these groups.

3.2.2. Multivariate analyses – less ornamented molecular types

When run without the ornamented groups (B, T3S, T3V, T3Y) and the highly distinctive group T12 (Fig. 5), the CVA configuration on the first three axes (Fig. 7) portrays all the remaining less ornamented molecular type groups as morphologically discrete. There is also evidence that specimens from the geographic subgroups within T1 and T5 may be morphologically separable, although the confidence ellipses of T5H and T5Z abut. The plots suggest that geographically separate populations within T2 and T6 are morphologically indistinguishable.

DCA ordinations of the less ornamented type groups, based on subsets of the characters (Fig. 8), show that gross morphological characters (test shape, chamber shape, porosity, folium shape) are slightly more successful in separating the types than surficial ornament (beads, pustules, bosses, secondary calcite). The most distinctive group is T7 which is separable on the basis of either morphological or ornament characters. T, T1, T2, T4, T6, T10, and T11 can be discriminated using morphological characters but not or-

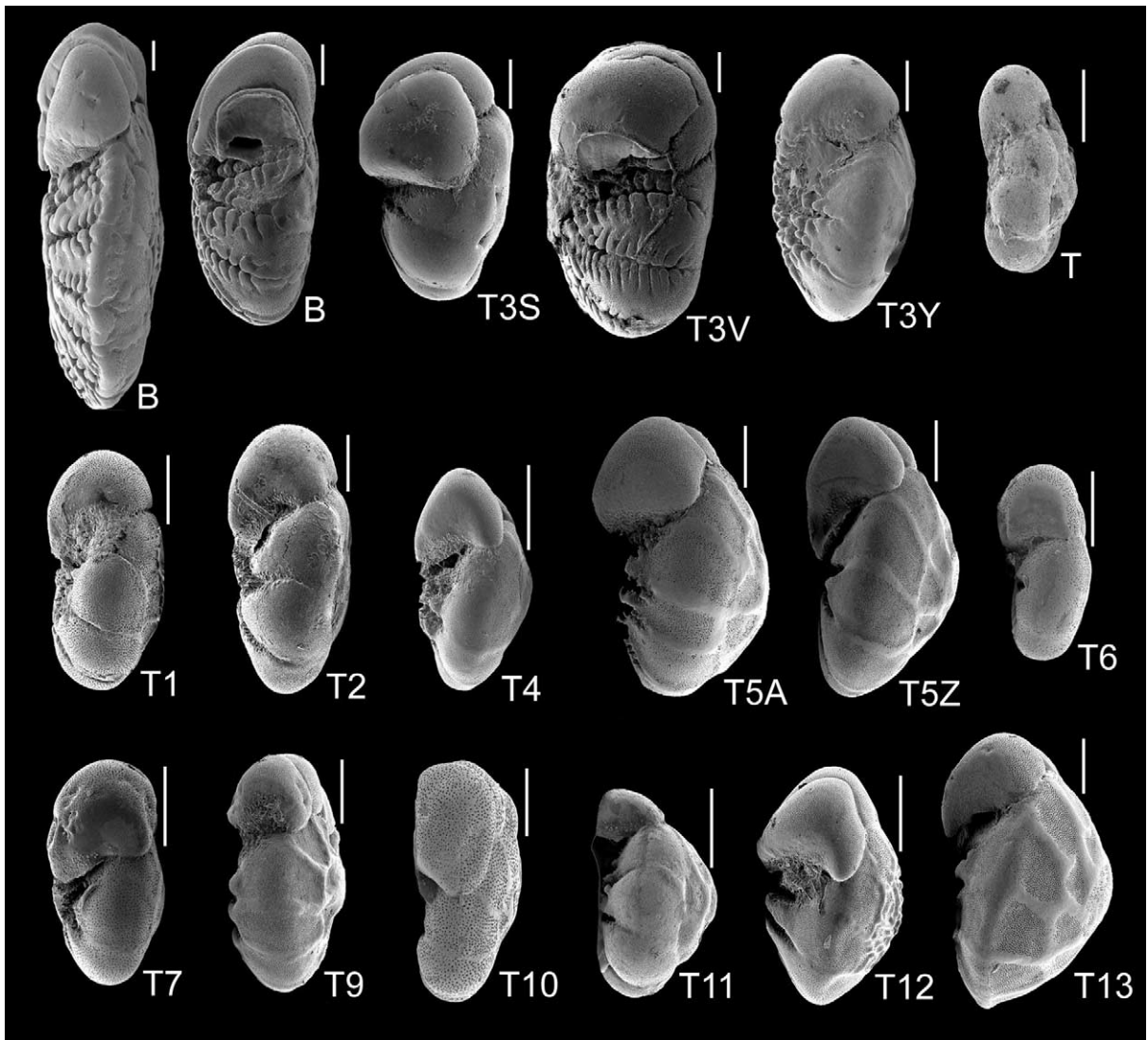
Table 4
Relative frequency of differences in partial LSU rDNA sequences of *Ammonia* spp.

	T1	T2	T3	T4	T5	T6	
T1	0–0.135						
T2	0.168–0.260	0–0.077					
T3	0.184–0.264	0.110–0.179	0.002–0.073				
T4	0.168–0.258	0.154–0.192	0.135–0.164	0.003–0.013			
T5	0.160–0.264	0.106–0.197	0.112–0.168	0.095–0.150	0–0.088		
T6	0.151–0.217	0.137–0.191	0.154–0.183	0.145–0.179	0.113–0.173	0–0.034	
T7	0.193–0.257	0.159–0.209	0.201–0.246	0.212–0.238	0.186–0.238	0.127–0.166	
T8	0.175–0.269	0.160–0.214	0.122–0.198	0.166–0.183	0.146–0.203	0.177–0.198	
T9	0.222–0.288	0.254–0.349	0.247–0.312	0.257–0.281	0.228–0.301	0.267–0.300	
T10	0.226–0.283	0.223–0.261	0.232–0.275	0.203–0.229	0.202–0.260	0.210–0.241	
T11	0.287–0.331	0.262–0.295	0.297–0.314	0.266–0.280	0.272–0.301	0.324–0.338	
T12	0.075–0.255	0.183–0.225	0.188–0.218	0.175–0.208	0.135–0.210	0.198–0.215	
T13	0.324–0.387	0.340–0.401	0.357–0.375	0.301–0.314	0.304–0.337	0.380–0.399	
	T7	T8	T9	T10	T11	T12	T13
T7	0.008–0.047						
T8	0.196–0.226	0.05					
T9	0.270–0.308	0.266–0.303	0.014–0.060				
T10	0.181–0.220	0.228–0.262	0.256–0.285	0.003–0.068			
T11	0.329–0.350	0.322–0.327	0.374–0.401	0.301–0.312	0.00		
T12	0.242–0.271	0.198–0.234	0.277–0.321	0.263–0.306	0.303–0.326	0.03	
T13	0.387–0.406	0.356–0.361	0.262–0.281	0.377–0.408	0.43	0.405–0.411	0.00

nament. T9, T12 and T13 can be discriminated using ornament, but not morphological characters. The remaining group, T5, can be seen to be distinguishable using a combination of both morphological and ornament characters, but neither on their own.

Characters with highly positive or negative DCA scores on the first two axes (Fig. 8) are therefore significant in helping discriminate these less ornamented groups include porosity (mnpor, poredens), peripheral profile (per[^], umb, spi),

number of chambers per whorl (ch/wh), presence of a large boss (maxbos, lgbos/d), ragged folium (ragfol), or weak ornament along the edge of radial sutures on the umbilical side (radgrv, radbd). In these DCA ordinations (Fig. 8) there is again no clear distinction between the geographic subgroups within molecular types T1, T2 and T5, but the European and Chinese populations of T6 are separated, possibly because of stronger developed pustules over the folia (folpust) and the smaller size (gsd) of the Chinese specimens.



3.3. Morphological discrimination

Three type groups are able to be discriminated easily from all others (Plates II–IV) on the basis of single characters and are discrete in the cluster analysis (Fig. 6) and most CVA and DCA ordinations (Figs. 4, 7 and 8). These are:

(a) T12, which has a unique reticulate raised calcite pattern (retcac) over the spire and is strongly biconvex;

(b) B, which has the largest test (gsd), which is also generally more compressed than all others;

(c) T7, which has folia that are more blunt and ragged (ragfol) than all others. It also has the smallest proloculus.

Most of the other types can be distinguished by a combination of several characters (Table 5; Appendix 1).

Among the types with strong ornament (beads and grooves) on the umbilical side, B can be distinguished from T3 by the presence of an umbilical boss and by its larger, more compressed test (Figs. 9 and 10). Meagre genetic data (two sequenced specimen from Vendée, one from Banyuls-sur-mer, and a number from Plymouth and Tjaerno), suggest that T3 may be subdivisible molecularly into three geographic subgroups – from Sweden and the UK (T3S), Vendée, Atlantic coast of France (T3V) and Banyuls-sur-mer, Mediterranean coast of France (T3Y). Our CVA and DCA ordinations (Figs. 5 and 6) suggest that these three subgroups may be able to be discriminated morphologically, although cluster analysis (Fig. 4) suggests they are closely similar, particularly T3V and T3Y. Visual examination of their morphological measurements suggests that unequivocal discrimination of these three subgroups may not be easy. T3V seems to be distinguishable from the other two subgroups by its stronger development of beads and grooves on the umbilical side which extend all the way to the periphery, but are largely restricted to the central area in T3S and T3Y. Many, but not all, T3Y specimens have a more acutely rounded periphery than in the other two subgroups. T3S may be distinguished by its slightly finer pores (mean diameter $< 1 \mu\text{m}$), greater pore density (Fig. 11), smaller angle between the radial and spiral sutures

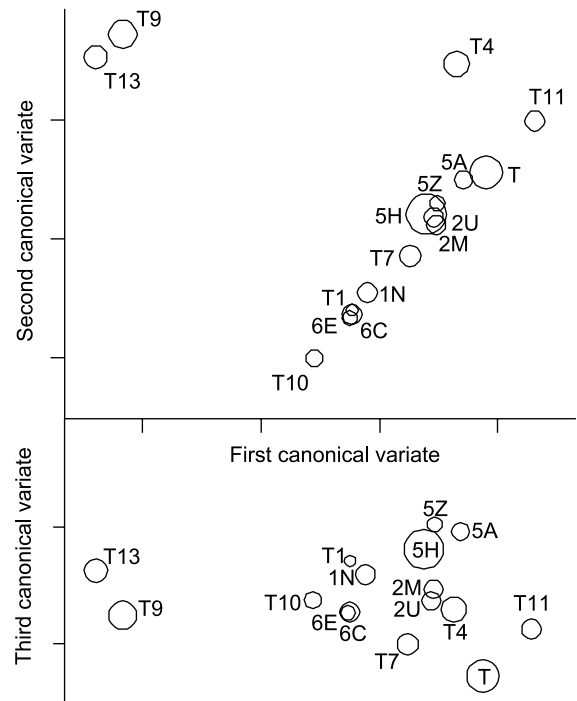


Fig. 7. 2-D CVA configuration of the 17 less ornamented molecular type groups (B, T3S, T3S, T3Y, T12 removed) and their 95% confidence regions plotted on the 1st v 2nd and 1st v 3rd canonical variate axes.

(< 90 degrees), and in having the spiral side higher than the umbilical, rather than approximately subequal as in T3V and T3Y. Many, but not all, T3S specimens also have unfilled secondary openings at the junctions of the radial sutures with the spiral suture on the spiral side (Plate IV), similar to those described in *Pseudoeponides falsobeccarii* by Rouvillois (1974).

If further sequencing of populations of T3V and T3Y individuals from France confirms a consistent distinction from T3S, it may be justified to recognise two separate species or at least geographic subspecies.

Three groups (T9, T12, T13) consistently have a large boss filling most of the umbilicus. Of these, T13 and T9 are the most similar (Fig. 5), with the former distinguished by its high convex spiral side and its longer radial sutural furrows on the umbilical side (Fig. 12).

Groups with well-developed secondary calcite in the form of thickened folia, and raised thick-

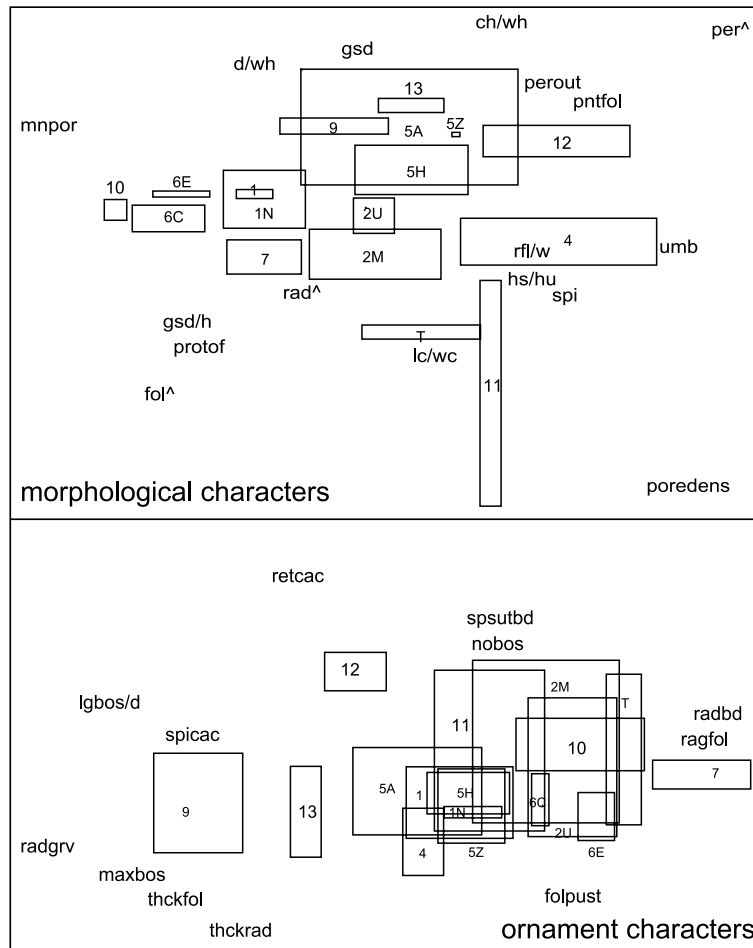


Fig. 8. 2-D DCA ordinations of the less ornamented molecular type group means, with their two standard deviation rectangles (95% confidence limits), and the most meaningful discriminant characters, based on: (a) 19 gross morphological characters (characters 1–8, 12, 15, 21–27, 29, 32 in Table 3); and (b) 13 ornament characters (characters 9–11, 13, 14, 16, 18–20, 31, 33–35).

ened calcite on the spiral side are T4, T5, T9, and T13. T4 and T5 lack large umbonal bosses and are morphologically similar, as shown by the cluster analysis (Fig. 4). They both are strongly planoconvex with acutely rounded peripheries, but T4 has smaller dimensions than T5 (e.g. gsd 0.2–0.3 mm cf. 0.3–0.6 mm; chambers per whorl 6.5–8 cf. 8–10; proloculus 0.03–0.05 mm cf. 0.05–0.08 mm, mean pore diameter 0.6–0.7 μm cf. 0.8–1.3 μm; Figs. 9, 11 and 13).

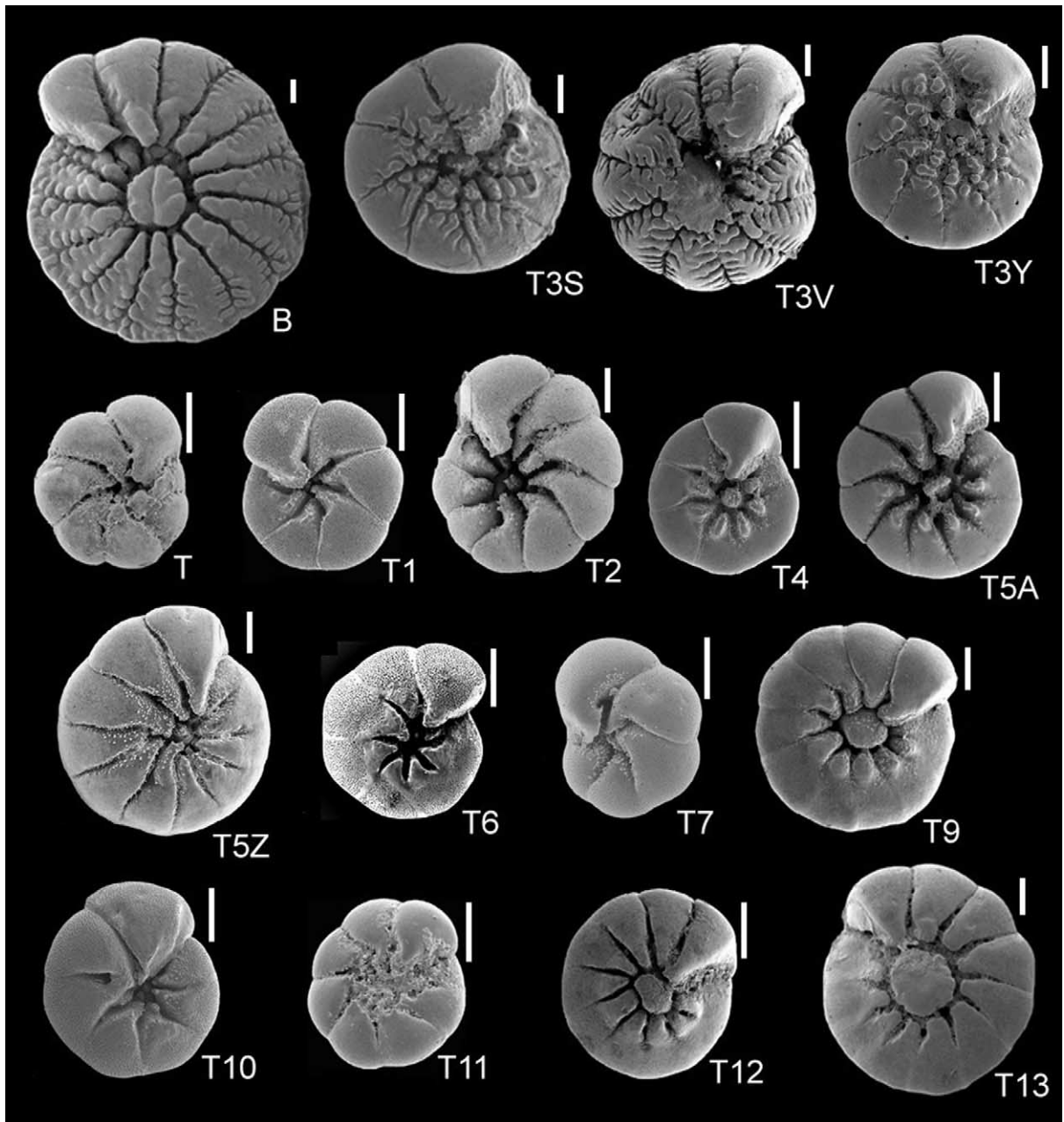
Specimens of T5 from Australia (T5A) differ slightly (Fig. 7) from those from New Zealand and Chile (T5Z, T5H) – a difference that is also apparent genetically (Fig. 2). T5A generally has

more strongly raised secondary calcite on its folia and a slightly wider umbilicus ($du/d > 2.5$) than does T5Z (Fig. 12). Four types (T4, T7, T11, T) have small tests (< 0.35 mm), small proloculi (< 0.05 mm) and narrow whorls ($d/wh < 0.11$). T4 is distinguished by its strong secondary calcite (above), and T7 by its blunt, ragged folia. T11 is distinguished from the remaining less ornamented groups by its relatively high convex spiral side (hs/hu 1.7–2.4; Fig. 10), small pores (mean diameter 0.5–0.7 μm; Fig. 11) and elongate chamber outlines on the spiral side (lc/lw 2.0–2.7). Cluster analysis, CVA and DCA (Figs. 4–6) show that the *tepida* group (T) is morphologically closest

to T11 (also from the Caribbean) with its lobular perimeter, long radial sutural furrows on the umbilical side, and small number of chambers per whorl (5.5–7.5), but differs from T11 by its less inflated profile, less convex spiral side, slightly

coarser pores and lack of thickened and raised radial sutures on the spiral side (Table 5).

Probably the most difficult groups to discriminate morphologically are those that cluster closest together in the dendrogram (Fig. 4) and have the



least secondary calcite and ornament – T1, T2, T6, and T10. All have moderate inflation, a broadly rounded periphery, 6–9 chambers per whorl and are about the same size (0.25–0.5 mm). T2 can be distinguished from the others by its small pores (mean diameter 0.7–1.0 μm ; Fig. 11) and longer radial sutural furrows on the umbilical side (rfl/w 0.6–0.8; Fig. 12). Cosmopolitan T1 is the most variable group and therefore hard to unequivocally distinguish from all others. It always possesses protoforamen (often strongly developed), and generally has more (minor) secondary calcite on the folia and spire and longer radial sutural furrows than T6 and T10 (Fig. 12). T10 can be distinguished with difficulty from T6, by its lack of imperforate secondary calcite on the folia, its lack of any umbonal bosses (sometimes present in T6), its coarser pores (Fig. 11), and by having oblique radial sutures on the spiral side, rather than perpendicular to the spiral suture.

3.4. Groups of morphologically similar species

Four high level groups recognised in the cluster analysis dendrogram (Fig. 4), based on their morphological similarities, are:

(a) B – large, compressed, highly ornamented with umbonal boss, beads, grooves, and fissured sutures;

(b) T3S, T3V, T3Y – highly ornamented with beads, grooves, and fissured sutures;

(c) T4, T5, T9, T12, T13 – moderate to strong secondary calcite, often with an umbilical boss, 7–11 chambers per adult whorl;

(d) T, T1, T2, T6, T7, T10, T11 – little to no secondary calcite, usually with no umbilical boss, 5–9 chambers per adult whorl.

Interestingly, the latter three correspond quite closely to the three global ‘ecophenotypic forms’ of *Ammonia beccarii* recognised by Walton and Sloan (1990): f. *beccarii* = cluster b; f. *parkinsoniana* = cluster c; and f. *tepida* = cluster d, and the first is type *A. beccarii*.

The various multivariate analyses indicate that within these clusters some species are consistently close together and morphologically similar:

(a) T4 and T5 (Fig. 4) – strong folia and spiral secondary calcite, Pacific Ocean temperate;

(b) T1, T2, T6, and T10 (Figs. 4 and 6) – least secondary calcite ornament, 7–9 chambers per adult whorl, dominantly Northern Hemisphere temperate–subtropical.

Plate III. Views of the umbilical sides of typical specimens from the 17 main groups used for morphometric analysis of *Ammonia* (Table 2). All scale bars = 100 μm .

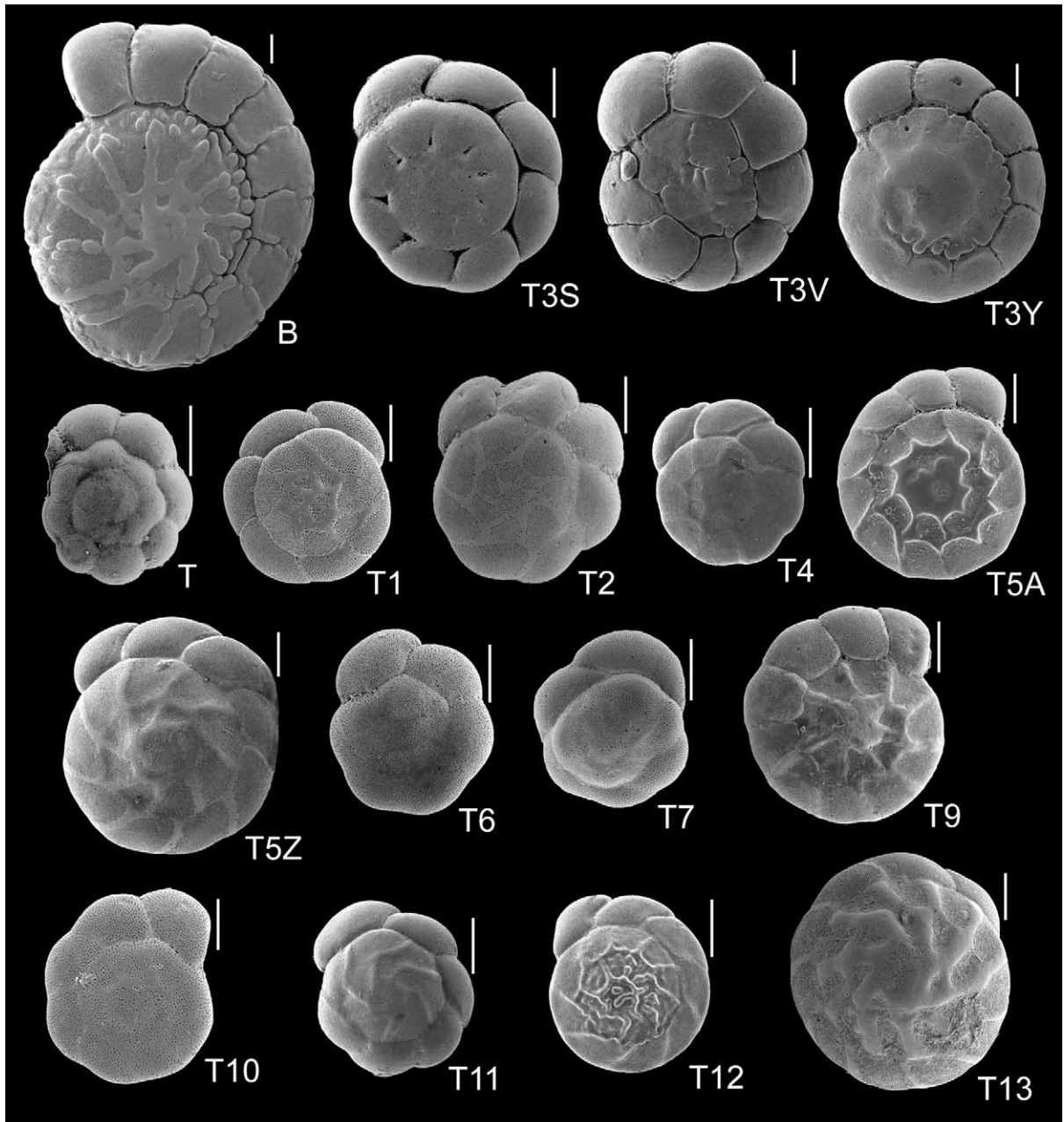
- B. *A. beccarii*, topotype, Rimini Beach, Italy (F201292, BWH151/2).
- T. *A. tepida*, syntype, Puerto Rico (CC3141).
- T1. Molecular type T1, Playa Bailén, Cuba (sequenced spec. Cuba-642).
- T2. Molecular type T2, Venice, Italy (sequenced spec. Ven-4).
- T3S. Molecular type T3, *A. batava*, Tjaerno, Sweden (BWH152/71).
- T3V. Molecular type T3, Vendée, Atlantic Ocean, France (BWH153/5).
- T3Y. Molecular type T3, ?*A. inflata*, Banyuls-sur-mer, Mediterranean Sea, France (BWH153/8).
- T4. Molecular type T4, Hamana Lake, Japan (BWH152/51).
- T5A. Molecular type T5, *A. aoteana*, Port Hacking, New South Wales, Australia (AU17644, BWH152/2).
- T5Z. Molecular type T5, *A. aoteana*, Campbells Bay, Auckland, New Zealand (AU15302, RC1/2).
- T6. Molecular type T6, Yalu Jiang, Northeast China (AU16151, BWH142/47).
- T7. Molecular type T7, Sapelo Island, Georgia, USA (Sequenced spec. Sap-2).
- T9. Molecular type T9, ?*A. parkinsoniana*, Long Island, New York, USA (BWH153/3).
- T10. Molecular type T10, Vancouver Harbour, British Columbia, Canada (AU16153, BWH151/33).
- T11. Molecular type T11, Playa Bailén, Cuba (sequenced spec. Cuba-647).
- T12. Molecular type T12, Tieti Beach estuary, New Caledonia (AU17670, BWH152/65).
- T13. Molecular type T13, Noumea, New Caledonia (AU17669, BWH151/11).

4. Discussion

4.1. Identification of molecular types

Our results show that a considerable number of molecular types exist within *Ammonia*, contrary to

the current dominant taxonomic concept for this genus that recognises only a few species with many ecophenotypes (Poag, 1978; Jorissen, 1988; Walton and Sloan, 1990). Until recently, foraminiferal taxonomy has been based exclusively on morphological characters of their tests.



The value of these characters, however, has not been reliable for the determination of polymorphic taxa such as in *Ammonia*. Recent molecular studies have shown cryptic diversity in several genera of benthic and planktic foraminifera and revealed the presence of sibling species (Darling et al., 1999; Holzmann, 2000; Tsuchiya et al., 2000; de Vargas et al., 2001).

The criteria for molecular recognition of different types vary among authors and are based on variable genetic distances. Studies on several amoeba genera have used sequence divergencies of 0.5% to more than 6%, to distinguish different types (Gast et al., 1996; Brown and de Jonckheere, 1999). In foraminifera, genetic distances of 1.1% to more than 6% have been used to distinguish different species within *Glauertella* (Tsuchiya et al., 2000) and genetic variability of more than 5% has been accepted to distinguish different molecular types within allogromiid foraminifera (Pawlowski et al., 2002). In the present study, sequence divergencies between the different molecular types of *Ammonia* range from 7.5% to more than 40% (Table 4). Given the fact that each type is monophyletic and no intermediate types have been observed and taking into consideration the elevated genetic distances, the different molecular

types (T1–T13) can be regarded as distinct species.

4.2. Morphological distinction of molecular types

The results of the multivariate analyses of the measured morphological characters show that all molecular types can be discriminated on the basis of their morphology. Some types are distinguished by one distinctive unique character (e.g. B, T12), but most require a combination of characters to be utilised to allow discrimination. Several molecular types are morphologically similar (e.g. T4 and T5; T1, T2, T6, and T10), with end-member forms hard to distinguish from each other.

Within some types, several slightly different morphologies can sometimes be discriminated and correlated with geographic separation and slight genetic difference (e.g. T3S, T3V and T3Y; T5A and T5Z; T6C and T6E).

The cosmopolitan type T1 has wide morphological variability within single populations and consistent geographic differences in morphology have not been determined so far, but more specimens and larger populations need to be measured to test this conclusion.

Plate IV. Views of the spiral sides of typical specimens of the 17 main groups used for morphometric analysis of *Ammonia* (Table 2). All scale bars = 100 μ m.

- B. *A. beccarii*, topotype, Rimini Beach, Italy (F201292, BWH151/7).
- T. *A. tepida*, syntype, Puerto Rico (CC3141).
- T1. Molecular type T1, Playa Bailén, Cuba (sequenced spec. Cuba-642).
- T2. Molecular type T2, Venice, Italy (sequenced spec. Ven-14).
- T3S. Molecular type T3, *A. batava*, Tjaerno, Sweden (BWH152/68).
- T3V. Molecular type T3, Vendée, Atlantic Ocean, France (BWH153/7).
- T3Y. Molecular type T3, ?*A. inflata*, Banyuls-sur-mer, Mediterranean Sea, France (BWH153/11).
- T4. Molecular type T4, Hamana Lake, Japan (BWH152/49).
- T5A. Molecular type T5, *A. aoteana*, Port Hacking, New South Wales, Australia (AU17644, sequenced spec. PH-660).
- T5Z. Molecular type T5, *A. aoteana*, Panmure Basin, Auckland, New Zealand (AU17638, BWH152/11).
- T6. Molecular type T6, Yalu Jiang, Northeast China (AU16151, sequenced spec. Yalu-132).
- T7. Molecular type T7, Sapelo Island, Georgia, USA (Sequenced spec. Sap-2).
- T9. Molecular type T9, ?*A. parkinsoniana*, Long Island, New York, USA (BWH153/2).
- T10. Molecular type T10, Grays Harbour, Washington State, USA (AU16155, sequenced spec. Grays-177).
- T11. Molecular type T11, Playa Bailén, Cuba (BWH152/47).
- T12. Molecular type T12, Tieti Beach estuary, New Caledonia (AU17670, BWH152/62).
- T13. Molecular type T13, Noumea, New Caledonia (AU17669, BWH151/12).

4.3. Formal species names for molecular types

Potential taxonomic identifications that match different molecular types are listed in Table 6.

T1. No topotypic material, apart from *Ammonia aberdoveyensis* (T2 below), has been sequenced and found to contain T1 specimens. Morphologically this group is not highly distinctive and thus matching sequenced specimens with type figures cannot provide a conclusive match. A number

of described species (e.g. *A. turgida*) could be based on T1 molecular type specimens, but further genetic work on topotypes is required to determine which.

T2. The only unequivocal species name applicable at present is *A. aberdoveyensis*, as a population from the type locality has been sequenced and is dominantly T2, with some T1 specimens. The holotype (Haynes, 1973, pl. 38, nos. 1–2) has a small proloculus which identifies it as T2, as this

Table 5

Range of values for the test characters measured and assessed from SEM images (see Table 3 for abbreviations) and used as the basis for morphometric analysis of 17 molecular type groups of *Ammonia* (Table 2)

Character	1 gsd	2 gsd/h	3 hs/hu	4 umb	5 spi	6 per [^]	7 du/d	8 rfl/w	9 maxbos	10 lgbos/d	11 nobos	12 fol [^]
T1	0.22–0.43	1.6–2.5	0.8–2.4	a–lx	f–x	br	0.15–0.3	0.4–0.7	0–0.03	0	0–2	30–70
T2	0.29–0.51	1.9–2.3	0.6–1.4	f–lx	f–x	br	0.15–0.3	0.6–0.8	0–0.05	0	0–4	30–60
T3S	0.45–0.72	1.6–1.8	1–1.9	f–lx	lx–x	r–br	0.15–0.3	0.9–1.0	0.02–0.07	0	0–7	35–50
T3V	0.56–0.72	1.7–1.9	0.6–1.3	f–lx	f	br	0.15–0.25	0.95–1.0	0	0	0	30–50
T3Y	0.50–0.60	1.5–1.9	0.7–1.2	lx	f–lx	r–br	0.1–0.3	0.5–1.0	0–0.04	0	0–3	30–50
T4	0.24–0.26	2.0–2.3	1.6–2.3	f	x	ar	0.2–0.25	0.7–0.85	0.02–0.05	0	1	25–50
T5A	0.40–0.57	1.8–2.0	1.4–1.8	a–lx	lx–hx	ar–r	0.25–0.35	0.75–1.0	0–0.08	0	0–6	30–50
T5Z	0.30–0.66	1.8–2.3	1.4–3.1	a–f	x–hx	ar–r	0.15–0.25	0.85–1.0	0–0.06	0	0–3	30–40
T6	0.31–0.43	2.1–2.5	0.9–1.5	a–f	f–x	br	0.2–0.35	0.35–0.5	0–0.03	0	0–5	50–70
T7	0.25–0.36	1.8–2.1	0.9–1.3	f–lx	f–lx	br	0.1–0.2	0.3–0.7	0–0.03	0	0–1	50–70
T9	0.42–0.50	1.6–2.0	0.9–2.0	f–lx	lx–x	r–br	0.25–0.3	0.35–0.6	0.1–0.11	0.2–0.3	1	35–40
T10	0.36–0.44	1.9–2.3	1.2–1.9	a–f	lx–x	br	0.15–0.25	0.4–0.6	0	0	0	50–70
T11	0.20–0.33	1.3–1.9	1.7–2.4	a–lx	x–hx	r–br	0.2–0.3	0.7–0.9	0–0.1	0	0–1	55–60
T12	0.31–0.41	1.4–1.7	1.0–1.5	hx	hx	ar	0.15–0.3	0.85–0.95	0.04–0.11	0.1–0.25	1	30–40
T13	0.47–0.68	1.3–2.0	1.6–2.2	a–lx	hx	k–ar	0.3–0.4	0.8–0.9	0.10–0.18	0.2–0.3	1	30–45
B	0.58–1.70	2.1–3.9	0.6–1.4	f–lx	f–lx	k–ar	0.2–0.3	0.85–1.0	0.07–0.25	0.1–0.2	1–8	20–40
T	0.25–0.30	2.0–2.4	1.5–2.0	a–f	x	br	0.15–0.2	0.85–1.0	0	0	0	40–50
Character	13 thckfol	14 folpust	15 protolf	16 ragfol	17 pntfol	18–20 rad, bdgrv	21 prol	22 chwh1	23 chwh2	24 d/wh	25 ch/wh	
T1	w–m	n–w	w–s	n–vw	vw–s	n	0.05–0.08	5–7	7–8	0.08–0.15	6–8	
T2	n–w	n–m	n–m	n–w	n–s	n	0.03–0.06	6–7	7–9	0.12–0.18	6.5–9	
T3S	n–w	n–vw	n	n	n	w–s	0.03–0.07	6	7–9.5	0.11–0.26	6.5–8.5	
T3V	n	n–vw	n–vw	n	w–m	s	0.06–0.10	6–7	8–8.5	0.23–0.30	7–8	
T3Y	n–vw	n	n–vw	n	w–m	w–s	0.03–0.10	7	9	0.18–0.19	7.5–8.5	
T4	m–s	n–m	w	n	m–s	n	0.03–0.05	6–7	7–9	0.08–0.10	6.5–8	
T5A	m–s	n–vw	n–vw	n	m–s	n	0.05–0.07	6–8	9–10.5	0.13–0.19	8.5–10	
T5Z	w–s	n–w	n–w	n	m–s	n	0.05–0.08	6.5–7	8–10	0.11–0.21	8–9.5	
T6	n–m	n–w	vw–w	n	n–m	n	0.03–0.06	5.5–6.5	6.5–8	0.11–0.14	6–8	
T7	n	w–m	w–s	w–s	n–w	n	0.02–0.03	5–7	7.5–9	0.07–0.11	6.5–8	
T9	s	n–w	vw	n	m	n	0.07	6–6.5	10	0.10–0.16	8–9	
T10	n	n	n–w	n–w	n	n	0.04–0.07	6	6.5–8	0.13–0.17	6–7.5	
T11	vw–m	n	n–vw	n–vw	n–m	n	0.03–0.05	5–6	5.5–7	0.07–0.11	5.5–7	
T12	w	n	n	n	s	n	0.03–0.05	6–7	8–8.5	0.10–0.12	7.5–8.5	
T13	w–s	n–vw	n–vw	n	w–s	n	0.05–0.08	6–6.5	8–10	0.11–0.17	8–10	
B	w–s	n	n–vw	m–s	n–m	m–s	0.03–0.11	7–8	9–12.5	0.24–0.55	8–11.5	
T	n	vw–m	n–vw	n	vw	n	0.03–0.05	5.5–6.5	6.5–7	0.08–0.09	6.5–7.5	

Table 5 (Continued).

Character	26 lc/wc	27 perout	28,30 sp,radfis	29 rad [^]	31 spsu tbd	32 radsutcv	33 thckrad	34 spicac	35 retcac	36 mnpore	37 poredens
T1	1.2–2.5	0–0.7	n–w	70–90	n	vw–s	n–w	w–m	n	1.3–2.2	8–12
T2	0.9–2.4	0–0.8	n	65–80	n	w–s	n–vw	n–vw	n	0.7–1.0	15–18
T3S	1.1–2.4	0–0.6	vw–s	70–80	n–w	vw–m	n–w	n–s	n	0.7–1.0	16–20
T3V	1.0–1.2	0.3–0.7	w–s	95–110	n–vw	vw–m	n	n–vw	n	1.0–1.2	8–13
T3Y	1.4–1.7	0.1–0.7	w–s	90–100	n–w	vw–m	n	w–m	n	1.0–1.2	8–11
T4	1.3–2.0	0.3–0.8	n	60–80	n	w–s	w–m	w–m	n	0.6–0.7	27
T5A	1.3–2.2	0.1–0.8	n–vw	70–90	n	vw–s	vw–s	m–s	n	1.1–1.2	15–27
T5Z	1.1–3.0	0.4–0.8	n	50–85	n	vw–s	w–m	m–s	n	0.8–1.3	12–18
T6	1.1–1.9	0–0.6	n	80–90	n	vw–w	n–w	n–vw	n	1.4–2.0	5–10
T7	1.3–2.9	0.1–0.8	n	35–75	n	w–s	n	n	n	1.1–1.5	10–15
T9	1.0–1.2	0.5–0.8	n	90	n	n–vw	vw–w	w–m	n	1.1–1.5	11–13
T10	1.1–1.8	0–0.2	n	65–80	n	vw–s	n–vw	n–w	n	2.1–2.9	5–7
T11	2.0–2.7	0–0.4	n	50–85	n	vw–s	n–w	w–m	n	0.5–0.7	27–30
T12	1.4–2.0	0.7–0.8	n	55–70	n	w–m	n–w	m–s	m–s	1.1–1.2	15–16
T13	2.0–2.8	0.6–0.8	n	60–75	n	vw–s	m	m–s	n	1.5–2.1	8–9
B	0.7–1.0	0.5–1.0	w–s	90–100	m–s	w–m	w–s	w–s	n	1.3–2.0	5–9
T	1.4–1.8	0	n	80–90	n	vw–w	n–vw	n–vw	n	0.6–1.0	23–28

Abbreviations: a, concave; ar, acutely rounded; br, broadly rounded; f, flat; hx, high convex; k, keeled; lx, low convex; m, medium; n, none; r, rounded; s, strong; vw, very weak; w, weak; x, convex

is one of the key differences between T1 and T2 (Table 5).

T3. Our T3S group from Sweden is clearly synonymous with *A. batava*, with a Netherlands type locality (Hofker, 1951). Another possible name is *A. inflata* (Seguenza, 1862), but with no types available and an Italian Pliocene type locality it will not be possible to genetically characterise topotypic material. *A. inflata* is widely used in the Mediterranean for our T3Y forms (e.g. Jorissen, 1988), although *A. beccarii* is also commonly (wrongly, see above) applied (e.g. Vénec-Peyré, 1983; Debenay et al., 1998). Further sequencing work on Mediterranean T3 specimens may confirm these as genetically distinct enough to be recognised as a taxon separate from T3S with *A. inflata* the accepted name.

T4. We have yet to find a species described from eastern Asia that is a close match for this molecular type from Japan.

T5. Specimens of this molecular type from several localities in New Zealand and Chile are morphologically a perfect match for topotypes we have examined of *A. aoteana* (Finlay, 1940), described from a subfossil Holocene sample from Otago Harbour, New Zealand.

T6. Specimens of this molecular type from Japan and northeast China appear to have the same morphology as the figured holotype of *A. aomoriensis* (Asano, 1951) from Honshu, Japan, but topotypes have not been sequenced to confirm this.

T7. Specimens of this molecular type from Georgia appear to have similar morphology to the figured holotype of *A. limnetes* (Todd and Bronnimann, 1957) from Trinidad, but topotypes would need to be sequenced to confirm this identification.

T9. This molecular type has only been recognised from Long Island, New York. It is identical morphologically to *A. sobrina* (Shupack, 1934) which was described from the same region. It has a morphology similar to *A. parkinsoniana* (d'Orbigny, 1839) described from Cuba and re-illustrated by Le Calvez (1977), which would be the senior synonym if specimens from that region are found to belong to the same molecular type.

T10. We have yet to find a species described from western North America or the North Pacific that is a close match for this molecular type from the coast of British Columbia and Washington.

T11. This molecular type has so far only been recognised from Cuba. Its morphology is similar

to that of the figured holotype of *A. irridescens* (Arnal, 1958) from the Salton Sea, California. Although similar, our morphometric analysis suggests that T11 may not be synonymous with the types of *A. tepida* (Cushman, 1926) from nearby Puerto Rico, however, topotypic specimens of *A. tepida* need to be sequenced to test this conclusion.

T12. We have yet to find a species described from anywhere that has the distinctive spiral ornament and biconvex profile of this molecular type from New Caledonia.

T13. Specimens of this molecular type from New Caledonia are morphologically a good match for the figured holotype of *A. convexa* (Collins, 1958) described from the nearby Great Barrier Reef.

4.4. What is *Ammonia beccarii*?

As the oldest available species name, and the

one that has been so widely used, it is important to be sure what the original specimens looked like. No type specimens exist and no type locality was specifically designated. The source of the original material was cited as the Adriatic and Mediterranean Seas. Unfortunately, there are a number of morphotypes and molecular types present in these seas (e.g. Jorissen, 1988; Cimerman and Langer, 1991; Holzmann, 2000).

Linné's (1758) naming of *Nautilus beccarii* is based on earlier drawings of 'Cornu Hammonis' by Plancus (1739) and 'Ammonia unita' by Gualtieri (1742). Cushman (1928) stated that the type locality for *beccarii* is one of the older classic localities at Rimini, Adriatic Sea, Italy, presumably because this is where Janus Plancus had collected the illustrated specimens referred to by Linné when establishing the name. Since then, most workers have accepted that the beach sand at Rimini is the type locality (e.g. Hofker, 1951; Cifelli, 1962, Walton and Sloan, 1990).

Table 6
Species names applicable to different *Ammonia* molecular types

Molecular type	Species name(s) available	Type locality
T1	? <i>A. veneta</i> (Schultze, 1854) <i>A. turgida</i> (Hofker, 1951)	Italy Philippines-Indonesia
T2	<i>A. aberdoveyensis</i> Haynes, 1973	Dovey Marshes, Wales
T3	<i>A. inflata</i> (Seguenza, 1862) <i>A. batava</i> (Hofker, 1951)	Pliocene, Sicily, Italy Voorne I, Netherlands
T4		
T5	<i>A. aoteana</i> (Finlay, 1940)	Dunedin, New Zealand
T6	<i>A. aomoriensis</i> (Asano, 1951)	Honshu, Japan
T7	<i>A. limnetes</i> (Todd and Bronnimann, 1957)	Gulf of Paria, Trinidad
T9	<i>A. parkinsoniana</i> (d'Orbigny, 1839) <i>A. sobrina</i> (Shupack, 1934)	Cuba New York, USA
T10		
T11	<i>A. irridescens</i> (Arnal, 1958)	Salton Sea, California, USA
T12		
T13	<i>A. convexa</i> (Collins, 1958)	Great Barrier Reef, Australia
<i>Not represented by any of the above genotypes:</i>		
B	<i>A. beccarii</i> (Linné, 1758)	Rimini Beach, Italy
T	<i>A. tepida</i> Cushman, 1926	San Juan, Puerto Rico
	<i>A. compacta</i> (Hofker, 1964)	Lesser Antilles, Caribbean
	<i>A. moroensis</i> Hofker, 1978	Philippines
	<i>A. jacksoni</i> Buzas et al., 1977	Jamaica
	<i>A. pustulosa</i> (Albani and Barbero, 1982)	Lagoon of Venice, Italy
	<i>A. paucipora</i> Zheng, 1979	South China Sea
	<i>A. decorata</i> Golik and Phleger, 1977	Gulf of Panama
	<i>A. rolshauseni</i> (Cushman and Bermudez, 1946)	Texas, USA

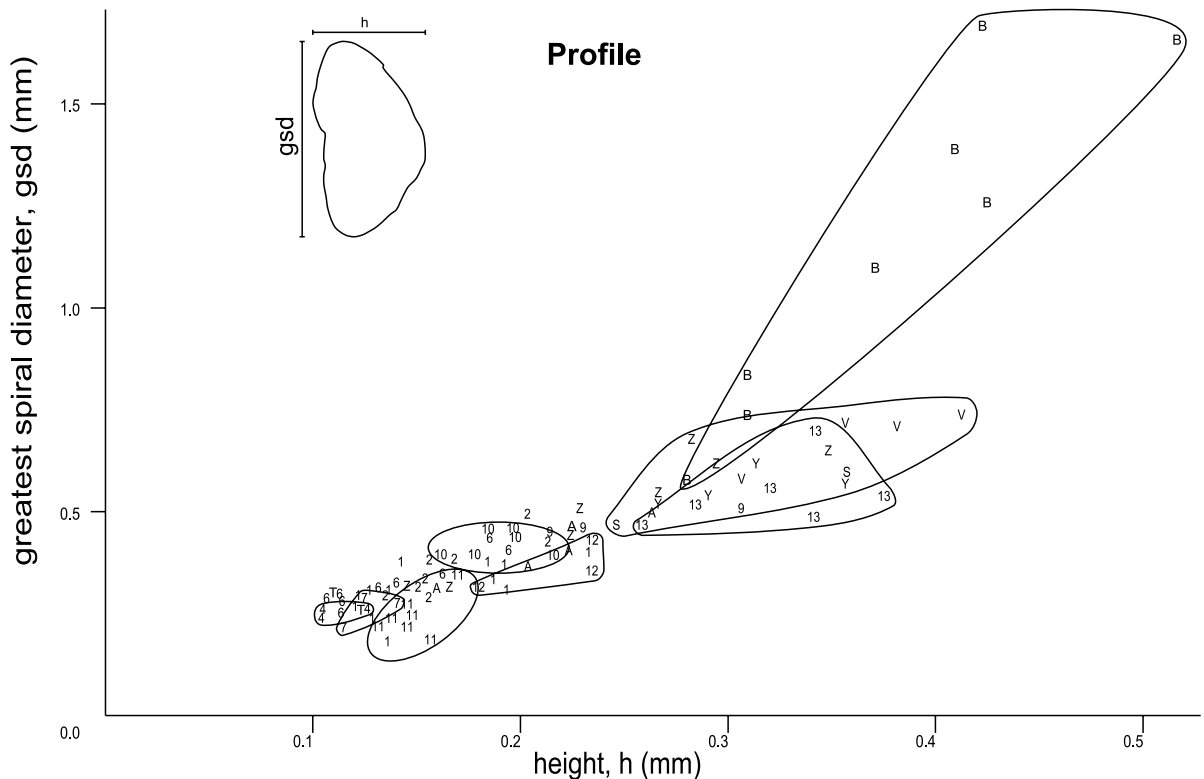


Fig. 9. Dimensions of *Ammonia* tests: test height (h) vs. greatest spiral diameter (gsd) of all measured specimens with 1.8 whorls or more. Specimen codes refer to molecular type (numeral) or molecular type and location: A, T5A Australia; B, beccarii; H, T5 Chile; S, T3S Sweden; V, T3V Vendée, France; Y, T3Y Banyuls-sur-mer, France; Z, T5Z New Zealand).

The drawings of the spiral and umbilical views by Janus Plancus are very plain and consist of a specimen with 2.3 whorls and 11 chambers in the last whorl. Three specimens (spiral side and section) are illustrated by Gualtieri having 3–3.5 whorls and 12.5–16.5 chambers in the last. The large number of chambers in the last whorl precludes all forms of *Ammonia* in the Adriatic–west Mediterranean region other than two forms from our strongly ornamented and fissured group (T3Y and B), and another, possibly unnamed, morphological group (X) which differs from the former two by its raised anastomosing costate sutural ornament on the spire and its inflated profile with an acute, keeled periphery (e.g. Loeblich and Tappan, 1987, pl. 767, figs. 1–5; Walton and Sloan, 1990, pl. 3, figs. 3a,b; Cimerman and Langer, 1991, pl. 87, figs. 3 and 4).

In our morphological analyses, a small popula-

tion of T3Y from Banyuls had 2.5–3.1 whorls with 8–9.5 chambers in the last whorl, and 9 chambers in the second whorl. Thus Plancus's and Gualtieri's specimens do not belong to this molecular type. Our population of B group from Rimini had 2.5–3 whorls with 13.5–16 chambers in the last whorl and 11–12 chambers in the second whorl. These measurements are similar to those for figured specimens in morphological group X and suggest that the specimens upon which the name *beccarii* was based belonged to either group B or X. Plancus's and Gualtieri's drawings show specimens with fairly wide whorls on the spiral side and chambers with lc/wc close to 1, which are characters of adult group B specimens but not group X.

The weight of evidence strongly supports assignment of the species name *beccarii* to the largest and dominant *Ammonia* forms in the Rimini

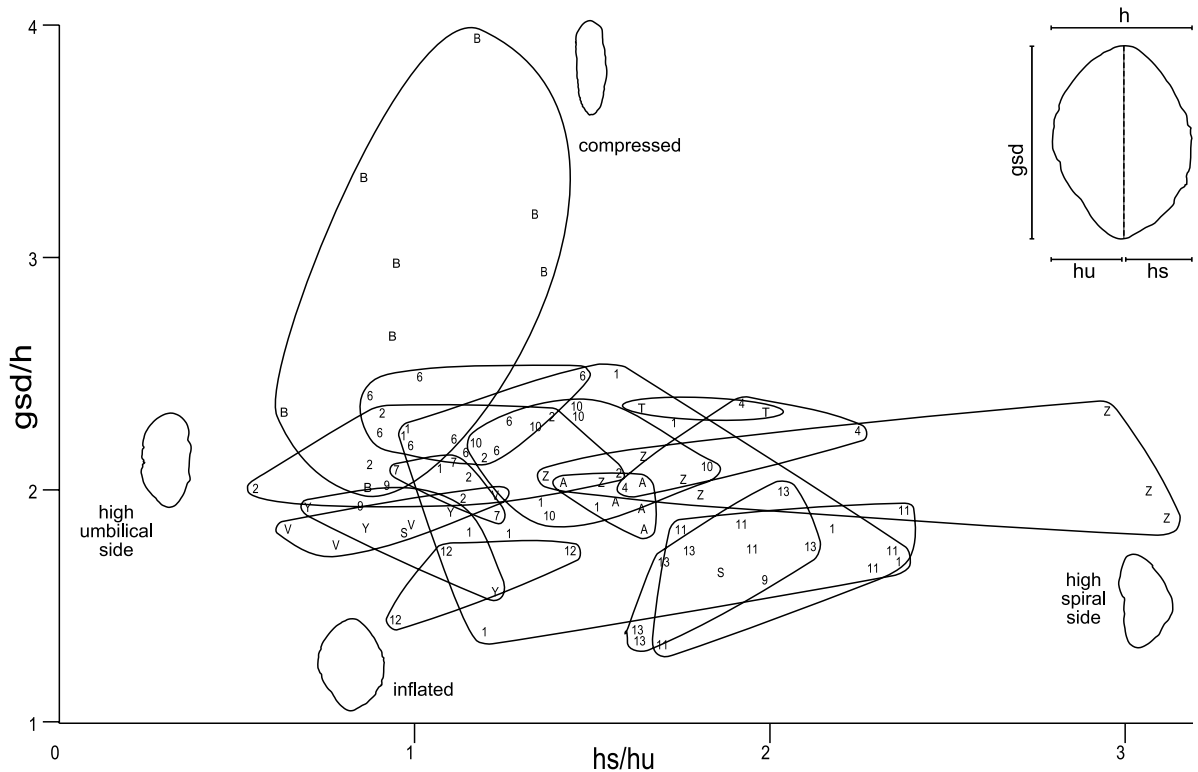


Fig. 10. Shape of *Ammonia* tests: height of the spiral side (hs) divided by the height of the umbilical side (hu) vs. greatest spiral diameter divided by test height (gsd/h) of all measured specimens. Specimen codes refer to molecular type (numeral) or molecular type and location: A, T5A Australia; B, *beccarii*; H, T5 Chile; S, T3S Sweden; V, T3V Vendée, France; Y, T3Y Banyuls-sur-mer, France; Z, T5Z New Zealand.

Beach sand – our group B (Plates II–IV). This is not surprising as *Plancus* (1739) would initially have been attracted to common, large shells (1.2–1.7 mm) rather than those of the smaller (0.3–0.7 mm), less common *Ammonia* taxa that are present. In the Rimini Beach fauna we examined (F201292, Institute of Geological and Nuclear Sciences, Lower Hutt, NZ; USNM520147, Smithsonian Institution, Washington, DC) the largest foraminifera (together with *Elphidium crispum*), and one of the most abundant, are *Ammonia* belonging to group B. Also present are smaller *Ammonia* specimens referable to our groups T1, T2, T9 and possibly T3Y. In their descriptions of ‘topotypes’ of *Ammonia beccarii* both *Cushman* (1928) and *Cifelli* (1962) appear to have included these smaller forms as juvenile stages, thus contributing to the subsequent taxonomic confusion. Previously published illustra-

tions of what we determine to be true *A. beccarii* are rare and include *Cushman* (1928, pl. 15, figs. 6 and 7), reproduced by *Loeblich and Tappan* (1964, fig. 479, 2a–c). None of the currently recognised molecular types correspond with the morphology of type *A. beccarii*.

4.5. What is *Ammonia tepida*?

Like *A. beccarii*, a wide variety of morphotypes have been ascribed to *A. tepida* (e.g. *Jorissen*, 1988; *Walton and Sloan*, 1990; *Debenay et al.*, 1998; *Hayward et al.*, 1999). Part of the reason for this was undoubtedly *Cushman’s* (1926) failure to provide a close-up illustration nor designate a holotype with his original description of *Rotalia beccarii* var. *tepida*. Although *Cushman* (1931, pl. 13, figs. 3a–c) provided excellent drawings of a syntype, these are not present in the Ellis

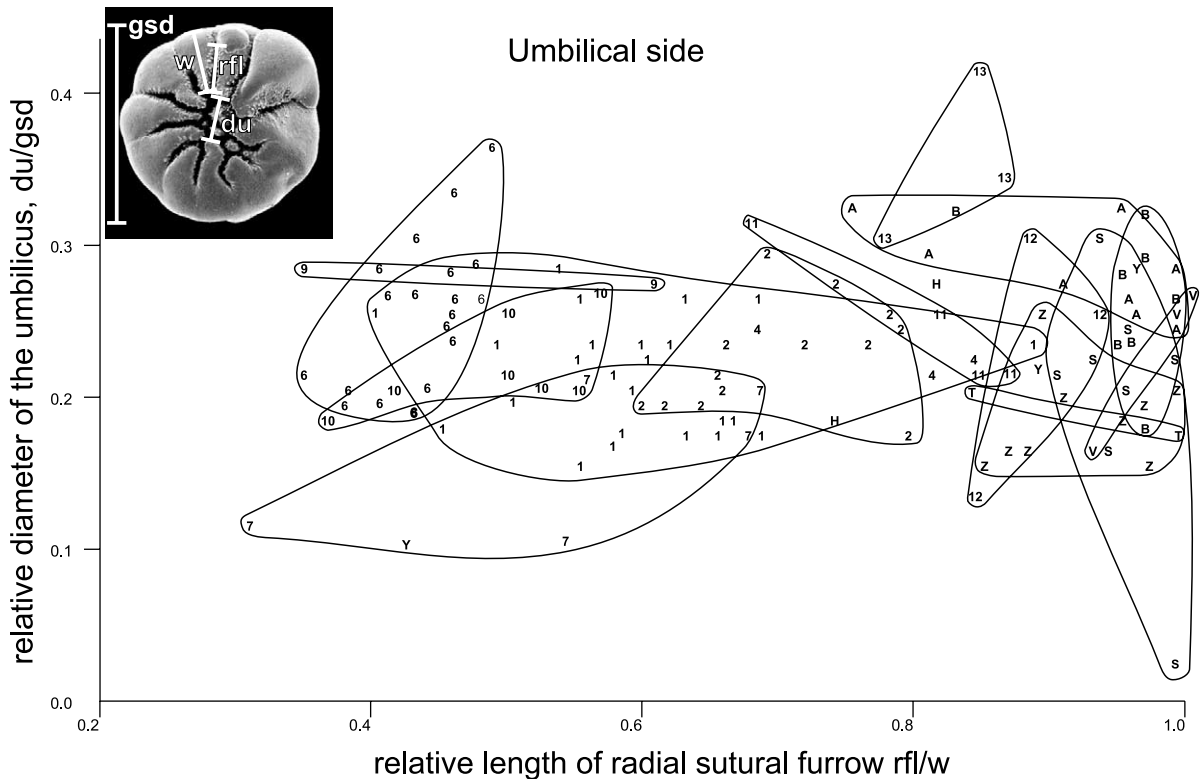


Fig. 12. Characters of the umbilical side of *Ammonia* tests: relative length of the radial sutural furrow between the penultimate ($n-1$) and antipenultimate chambers ($n-2$) and the width (folium apex to perimeter) of the penultimate chamber (rfl/w) versus the relative diameter of the umbilicus (between apices of folia) and the greatest spiral diameter of the test (du/gsd). Specimen codes refer to molecular type (numeral) or molecular type and location: A, T5A Australia; B, beccarii; H, T5 Chile; S, T3S Sweden; V, T3V Vendée, France; Y, T3Y Banyuls-sur-mer, France; Z, T5Z New Zealand.

Our results lead us to the conclusion that the number of genetically distinctive modern species could approach the number of formally named species (ca. 30–40).

5.2. Morphological basis for molecular types

Not surprisingly, there is not a one-to-one correspondence between molecular types and named species. This study suggests, however, that most (if not all) of the molecular types will be able to be recognised on the basis of a combination of detailed morphological differences, and thus species discriminated by careful morphological examination. At least 8 of our 13 molecular types can be equated to described species and several more are likely with further study. In addition,

we recognise at least another 9 described species morphotypes (*beccarii*, *tepida*, and Table 6) that have not yet been sequenced. So far, very few of the described species have been shown to be synonyms on the basis of these molecular studies, but it is still early days.

5.3. Taxonomically useful morphological characters

In this study the most useful characters for species discrimination are both structural (sutural fissures; adult test size; test profile; numbers of chambers per whorl; radial furrow length on umbilical side; whorl width; prolocular diameter), ornament (beading and grooving on sutural borders; large umbilical boss; secondary calcite over

pecially those receiving specimens transported in by wave or current reworking. The message coming out of this study, however, is that most adult specimens of these genetically identified species will be able to be discriminated morphologically, though it may be hard work. Attempts to morphological discriminate entire populations of several species which include juveniles, may be rather optimistic.

5.5. Use of the names *Ammonia beccarii* and *A. tepida*

Our study leads us to conclude that the use of the name *A. beccarii* worldwide should be abandoned and its use restricted to large, many-chambered forms, compressed and keeled in adults, with a highly ornamented umbilical side and umbilical boss, and weakly ornamented spiral side. These forms are currently known only from Rimini Beach sand. Suggestions that these may be reworked fossil specimens are not supported by their fresh, crisp preservation and abundance.

Our study also provides no support for the widespread identification of forms under the name *A. tepida*, especially in more temperate regions where most of our sequenced specimens have been collected. Specimens that morphologically resemble type *tepida* and T11 are common in tropical regions globally. Until more tropical material is sequenced, we cannot discount the possibility that *tepida* might be more widespread equatorially.

5.6. Future perspectives

This study provides just a sampling of the global diversity of the genus *Ammonia*. Many geographic regions and ecological habitats have not

been investigated to date, with gaping holes in our coverage in the Indian and South Atlantic oceans, in the tropical and east Pacific, Southeast Asia and the East Indies. This study identifies 13 molecular types, but we are aware also of 9 more morphotypes that have not been sequenced. We would predict that the present-day global diversity of *Ammonia* will be in excess of 25–30 genetically distinct species, most of which will be distinguishable on a combination of subtle morphological characters, as illustrated by this study.

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Appendix 1

Key to morphological discrimination of *Ammonia* molecular types identified in this study

- | | | |
|-----|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|
| 1a. | Possess fissures along the sutures on the spiral side; and distinctly beaded and grooved ornament along the edges of the sutures on the umbilical and/or spiral sides | 2 |
| 1b. | Lack well-developed sutural fissures or distinct grooved and beaded ornament along the edges of sutures | 5 |

2a.	Specimens large (>0.7 mm); compressed (gsd/h 3–4); numerous chambers per whorl (11–12 in second whorl); periphery acutely rounded to keeled	B (<i>beccarii</i>)
2b.	Specimens moderate size (<0.7 mm); moderately inflated (gsd/h 1.5–2); 7–10 chambers in second whorl; periphery broadly rounded	3 (T3)
3a.	Beads and grooves extending to periphery on umbilical side	T3V
3b.	Beads and grooves centrally confined, not extending to periphery on umbilical side	4
4a.	Angle between radial and spiral suture on spiral side <90°; pore density >15 per 100 sq. µm	T3S (<i>batava</i>)
4b.	Angle between radial and spiral suture on spiral side ca. 90°; pore density <15 per 100 sq. µm	T3Y (<i>?inflata</i>)
5a.	Possess raised secondary calcite thickening on spiral side; sometimes a single large umbilical boss; acutely to broadly rounded periphery	6
5b.	Secondary calcite on spiral side very weak or lacking; never has a single, large umbilical boss; broadly rounded periphery	12
6a.	Possess a single large umbilical boss (>0.1 test diameter)	7
6b.	Possess small or no umbilical bosses	9
7a.	High biconvex; reticulate pattern of secondary calcite over convex spire;	T12
7b.	Planoconvex; reticulate spiral calcite lacking	8
8a.	Long furrows along last few radial sutures on umbilical side; high convex spiral side	T13 (<i>convexa</i>)
8b.	Short furrows along all radial sutures on umbilical side; low to moderately convex spiral side	T9 (<i>parkinsoniana</i>)
9a.	Broadly rounded periphery; folia with weak secondary calcite	10
9b.	Acutely rounded periphery; elongate, pointed folia with strong secondary calcite	11
10a.	Flat to low convex spiral side; 7–8 chambers in second whorl; medium length radial sutural furrows; protoforamen always present	T1
10b.	High convex spiral side; 5.5–7 chambers in second whorl; long radial sutural furrows; protoforamen weak or lacking	T11 (<i>irridesceus</i>)
11a.	Moderate size (0.3–0.7 mm); medium proloculus (>0.05 mm); whorls medium width; 0–6 small umbilical bosses	T5 (<i>aoteana</i>)
11b.	Small size (0.2–0.3 mm); small proloculus (<0.05 mm); whorls narrow; single, small, umbilical boss	T4
12a.	Coarsely perforate (mean pore diam >1.5 µm)	13
12b.	Finely perforate (mean pore diam <1.5 µm)	14
13a.	Folium commonly elongate, pointed, with weak, imperforate, secondary calcite; often with one or more small umbilical bosses; radial sutures perpendicular to spiral suture on spiral side	T6 (<i>aomoriensis</i>)
13b.	Folium not elongate, lacking imperforate secondary calcite; no umbilical bosses; radial sutures oblique to spiral suture on spiral side	T10
14a.	Test small (<0.36 mm); whorls narrow on spiral side	15
14b.	Test medium-size (0.3–0.5 mm); whorls medium width on spiral side	T2 (<i>aberdoveyensis</i>)
15a.	Last few folia blunt and with ragged edge; weakly lobular peripheral outline; oblique radial sutures on spiral side	T7 (<i>limnetes</i>)
15b.	Last few folia lacking ragged edge; strongly lobular peripheral outline	T (<i>tepida</i>)

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