ORIGINAL ARTICLE

Molecular identification of *Ammonia* and *Elphidium* species (Foraminifera, Rotaliida) from the Kiel Fjord (SW Baltic Sea) with rDNA sequences

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Abstract *Ammonia* and *Elphidium* collected in the Kiel Fjord for the present study were first identified on morphological bases as *Ammonia beccarii* (Linné, 1758) and *Elphidium excavatum* (Terquem, 1876). Phylogenetic analyses based on partial SSU rDNA and LSU rDNA sequences show that *Ammonia* specimens sampled in the Kiel Fjord belong to the phylotype T6, which has a disjunct distribution (Wadden and Baltic Seas/China and Japan) and has been identified as *Ammonia aomoriensis* (Asano, 1951). Partial SSU rDNA sequence analyses indicate that *Elphidium* specimens from the Kiel Fjord belong to the clade *E. excavatum,* confirming the morphological identification. This clade can be further divided in three subclades. Kiel Fjord *Elphidium* belong to two of these subclades and were identified morphologically as the subspecies *E. excavatum excavatum* (Terquem, 1876) and *E. e. clavatum* Cushman, 1930.

Introduction

Ammonia and *Elphidium* are the two most abundant benthic foraminiferal genera worldwide (Murray 1991). *Elphidium* species occur from tropical to polar regions and from the intertidal zones to the continental slope (Murray 2006). *Ammonia* species are found from the subtidal to the outer continental shelves. They avoid the high latitudes, having therefore a more restricted distribution (Walton and Sloan 1990; Murray 2006). *Ammonia* and *Elphidium* species are important constituents of the benthic foraminiferal faunas from the European marginal seas. In the shallow Mediterranean, English Channel, North Sea and western Baltic Sea, these genera constitute a high proportion of the living foraminiferal assemblages (Murray 1991). Their tests are readily preserved in the fossil record and serve as proxies for paleoenvironmental analyses (e.g. Kubisch and Schönfeld 1985; Poignant et al. 2000; Xiang et al. 2008).

Taxonomy of *Ammonia* and *Elphidium*

Despite the widespread occurrence and ecological relevance of *Ammonia* and *Elphidium*, the taxonomy of these genera has been the subject of long-standing controversies. More than 40 species, subspecies or varieties of recent *Ammonia* have been described (Ellis and Messina 1940 and supplements). A culture study of *Ammonia* (Schnitker 1974) revealed that the offspring of a single specimen

Communicated by H.-D. Franke.

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showed seven different morphotypes, resembling different species and therefore leading to the conclusion that there was only one valid species: *Ammonia beccarii* (Linné, 1758). Consequently, *Ammonia* from the European marginal seas were commonly determined as *A. beccarii*, even though they showed morphological variability (Haake 1962; Lutze 1965; Murray 1991). Morphometric analyses of *Ammonia* populations globally inferred two discernable 'morphostocks' of large, ornamented *A. beccarii* and less ornamented *Ammonia parkinsoniana* (d'Orbigny, 1839) (Poag 1978; Jorissen 1988). Other varieties were assigned as form names to these two morphospecies (Jorissen 1988; Hayward et al. 1999). This concept was corroborated by the observation that the 'morphostocks' showed consistent differences in their ecological preferences (Debenay et al. 1998). In the North Sea and western Baltic Sea, the species names *Ammonia batava* (Hofker, 1951)*, A. beccarii, A. catesbyana* (d'Orbigny 1839)*, A. flevensis* (Hofker 1930) and *A. tepida* Cushman 1926 were used (Haake 1962; Lutze 1965; Brodniewicz 1965; Hofker 1977; Langer et al. 1989; Langer and Leppig 2000; de Nooijer 2007). The name *A. tepida* has also been used for populations from other European marginal seas (e.g. Bouchet et al. 2007; Pascal et al. 2008). However, the lectotypes of *A. tepida* from the West Indies show a different morphology, and this name should be avoided for populations from temperate regions (Hayward et al. 2003). A re-assessment of the taxonomic status of *Ammonia* populations based on phylogenetic analysis of variable regions D1 and D2 of the Large Subunit of ribosomal DNA (LSU rDNA) revealed thirteen different molecular types of *Ammonia* worldwide (Pawlowski et al. 1995; Holzmann 2000; Holzmann and Pawlowski 1997, 2000; Hayward et al. 2004; Pawlowski and Holzmann 2008). Eight of these phylotypes can be assigned to described species on the basis of morphological features, whereas the extensive use of the name *A. beccarii* should be abandoned (Hayward et al. 2004). Furthermore, the phylotype T6 has a disjunct distribution, in the Wadden Sea and marginal seas off Japan and northern China, where the names *Ammonia aomoriensis* (Asano 1951) and *Ammonia "beccarii"* forma 1 have been used (Nomura and Seto 1992; Xiang et al. 2008; Takata et al. 2006) The name *A. aomoriensis* was therefore proposed for the phylotype T6 (Hayward et al. 2004).

More than 60 morphospecies of *Elphidium* were reported in studies of recent foraminifers worldwide (Murray 1991). Several of them were identified in the North Sea and western Baltic Sea: *Elphidium albiumbilicatum* (Weiss, 1954), *E. excavatum clavatum* Cushman 1930, *E. excavatum excavatum* (Terquem 1876), *E. gerthi* van Voorthuysen, 1957, *E. guntheri* Cole, 1931, *E. incertum* (Williamson, 1858) and *E. williamsoni* Haynes, 1973 (Lutze 1965; Frenzel et al. 2005; Schönfeld and Numberger 2007a;

Nikulina et al. 2008; Polovodova and Schönfeld 2008). These species are easily distinguishable by their morphology (Lutze 1965). Nevertheless, the most confused taxonomic situation has always been associated with *E. excavatum* because of its high intraspecific variability (Feyling-Hanssen 1972; Miller et al. 1982). A total of eleven morphotypes, considered as either species, subspecies or ecophenotypes, have been described for *E. excavatum* (Goubert 1997). In the western Baltic Sea, the two subspecies *E. e. excavatum* and *E. e. clavatum* seem to co-occur, even though they show different depth preferences (Lutze 1965). For the time being, *Elphidium* has been studied much less than *Ammonia* with molecular phylogeny, and only a few sequences are deposited in the EMBL/GenBank database.

Ecology of *Ammonia* and *Elphidium* from the Kiel Fjord

The Kiel Fjord is a narrow, N–S extending, 10–20 m deep inlet of the Kiel Bight in the western Baltic Sea. Its hydrography is characterised by a low-salinity surface water (10–19 psu), which is separated by a halocline at 16–18 m depth from the more saline Kattegat Water (17–21 psu). The prevailing *Ammonia* species, first identified as *A. beccarii,* is a shallow-water species living in muddy sands at a water depth of 4–14 m with brackish conditions of 15–23 psu (Rottgardt 1952; Lutze 1965; Nikulina et al. 2008). The sediments in the Kiel Fjord are enriched in organic matter (C_{org}: 3–5% and biogenic silica: 3–7%) when compared to the Baltic Sea (Balzer 1984; Emelyanov 1988). They mirror a high bioproductivity of more than 200 g C m^{-2} $year^{-1}$ providing a sufficient amount of food for benthic foraminifera (Schönfeld and Numberger 2007b). Similar nutritional conditions were described by Irion (1994) in the tidal flats from Wilhelmshaven (North Sea) where *Ammonia* is frequent as well. In other fjords of the Kiel Bight, *A. beccarii* was found above the picnocline in sandy sediments with the lowest food concentration (Exon 1972; Polovodova et al. 2009), which confirms its opportunistic behaviour when compared to other foraminiferal species (Nikulina et al. 2008).

Among the *Elphidium* taxa considered in this study, *E. gerthi* occupies muddy sediments of the near-shore zones, and *E. excavatum* inhabits sandy-muds enriched in organic matter (C_{org}: 2–5% and biogenic silica 1–6%) of the Kiel Bight (Polovodova et al. 2009). According to Lutze (1965), *E. excavatum* is a deep-infaunal species, which is found down to 6 cm depth and occurs therefore mainly in well-oxygenated sediments with active water exchange. Two subspecies of *E. excavatum* are distinguished: *E. e. excavatum* is limited to the shallow areas of the Kiel Bight, whereas the cold-water-adapted *E. e. clavatum*

Fig. 1 SEM pictures of *Ammonia* (**a**) and *Elphidium* (**b**) specimens collected in the Kiel Fjord with their DNA identification numbers. *Scale bar* = 100 μm
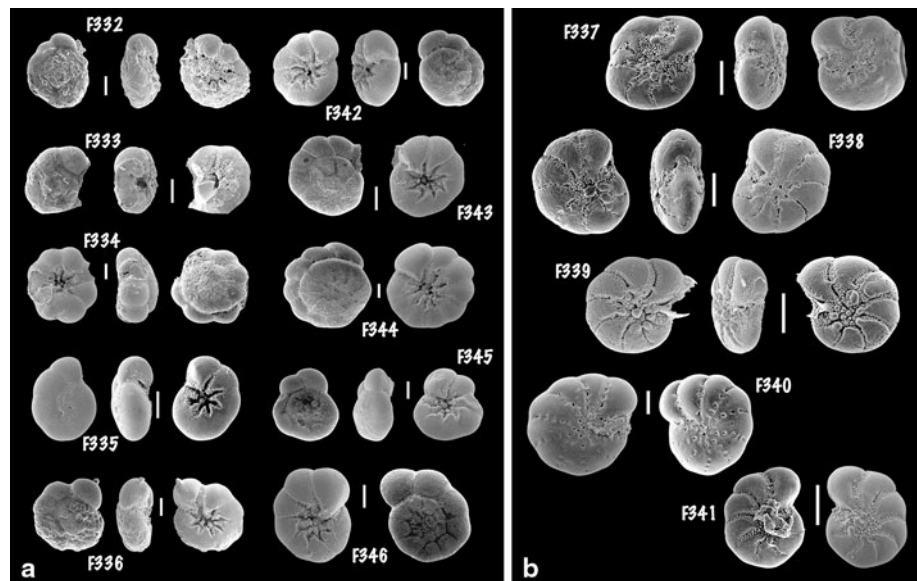
prefers the deeper areas in and below the halocline (Lutze 1965, 1974; Nikulina et al. 2008).

Materials and methods

Sampling and SEM imaging

Surface sediment samples were collected in the inner Kiel Fjord under the net cages of the "Kieler Lachsforelle" fish farm by SCUBA divers in September 2007. This site was chosen because the high food input and occurrence of different substrates promoted the co-occurrence of different species, which otherwise inhabited separated distribution areas in the fjord. Live foraminifers were distinguished from dead ones by natural colouration of the protoplasm and pseudopodial activity under a dissection microscope. Alive specimens were picked out, cleaned in filtered seawater with a paint brush, transferred to Eppendorf tubes and dried at room temperature. Ten *Ammonia* and five *Elphidium* (Fig. 1) specimens were imaged with a scanning electron microscope (SEM) at ETH Zurich for taxonomical documentation.

DNA extraction, amplification and sequencing

After SEM imaging, the 15 dried foraminiferal tests were crushed to extract DNA from each of them individually by the DOC method (Pawlowski 2000). The two regions of rDNA examined here correspond to 3' fragment of the SSU rRNA and 5' fragment of the LSU rRNA genes (Fig. 2). The SSU fragment (primers s14F3-sB for the amplification and s14F1-sB for the reamplification) is about 1,000 nucleotides (nt) and is widely used in foraminiferal studies. The LSU fragment (primers 2TA-L1F for the amplification and 2TA- L7 for the reamplification) was specifically used to recognise the different genotypes of *Ammonia* (e.g. Pawlowski et al. 1995) and represents a more variable and shorter fragment (about 400 nt). All positive amplifications were purified with the High Pure PCR Purification Kit (Roche Diagnostics) and sequenced directly using a Big-Dye Terminator v3.1 Cycle Sequencing Kit and an ABI-Prism 3100 DNA sequencer (both Applied Biosystems), all according to the manufacturer's instructions.

Phylogenetic analysis

The new sequences (19 partial SSU and 11 partial LSU ones) have been deposited in the EMBL/GenBank database (accession numbers given in Tables 1, 2). The new sequences were aligned manually with related sequences available from GenBank in two separate datasets (SSU and LSU) using Seaview (Galtier et al. 1996). The sequences of *Ammonia* available from the EMBL/GenBank database are mainly LSU sequences (Pawlowski et al. 1994, 1995;
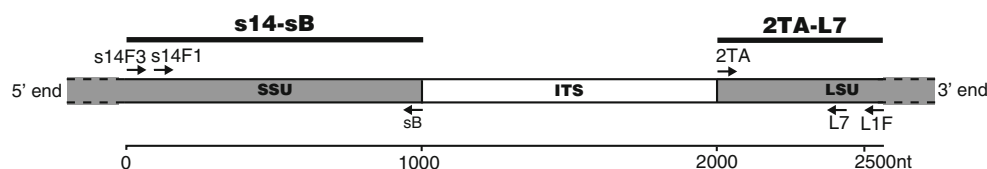
Fig. 2 Location of the primers used to amplify the SSU and LSU rDNA fragments

Table 1 List of specimens from the Kiel Fjord with their DNA numbers and the EMBL/GenBank accession numbers

Species	DNA number	SSU sequence	LSU sequence
Ammonia aomoriensis	F332	GQ853570	GQ853578
Ammonia aomoriensis	F333	GQ853571	GQ853579
Ammonia aomoriensis	F334	GQ853572	GQ853580
Ammonia aomoriensis	F335	GQ853573	GQ853581
Ammonia aomoriensis	F336	Negative	Negative
Elphidium excavatum	F337	GQ853563	Negative
Elphidium excavatum	F338	GQ853564	Negative
Elphidium excavatum	F339	GQ853565	Negative
Elphidium excavatum	F340	GQ853566	Negative
Elphidium gerthi	F341	Negative	Negative
Ammonia aomoriensis	F342	Not done	GQ853582
Ammonia aomoriensis	F343	GQ853574	GQ853583
Ammonia aomoriensis	F344	Not done	GQ853584
Ammonia aomoriensis	F345	GQ853575	GQ853585
Ammonia aomoriensis	F346	Not done	GQ853586

Holzmann et al. 1996; Holzmann and Pawlowski 2000; Hayward et al. 2004), but there are also some SSU sequences attributed to *Ammonia* sp., *A. beccarii*, *A. catesbyana* and *A. tepida* (Pawlowski et al. 1996, 1997; Langer and Leppig 2000; Ertan et al. 2004; Schweizer et al. 2008). Two environmental sequences (Habura et al. 2008) branching inside the *Ammonia* clade were also added. SSU sequences belonging to three species of *Elphidium* (*E. aculeatum* (d'Orbigny, 1846), *E. excavatum* and *E. williamsoni*) are available from the EMBL/GenBank database (Pawlowski et al. 1997; Langer 2000; Ertan et al. 2004; Schweizer et al. 2008). One environmental sequence (Habura et al. 2008) branching in the clade of *E. williamsoni* was also added. The genus *Haynesina* was chosen as the out-group taxon, because it is the closest known relative of *Ammonia* and *Elphidium* (Schweizer et al. 2008).

The regions that were impossible to align properly were removed to obtain two final alignments of 820 sites for the

SSU and 286 sites for the LSU, from which 750 and 270 sites were, respectively, used for analyses (numbers of sites calculated with Phylo_Win, Galtier and others 1996).

Maximum likelihood (ML) analyses were performed with 100 bootstrap (BS) replicates by PhyML 2.4.4 (Guindon and Gascuel 2003) under the HKY (Hasegawa, Kishino, Yano) model (Hasegawa et al. 1985) for the SSU (Fig. 3) and the LSU (Fig. 4) alignments. In order to correct for among-site rate variations, the proportion of invariable sites (I) and the alpha parameter of gamma distribution (Γ), with six rate categories, were estimated by PhyML (HKY + I + Γ). In addition, BIONJ phylogenetic trees (Gascuel 1997) were inferred with Phylo_Win under the K2P (Kimura's two parameter) evolution model (Kimura 1980) with non-parametric bootstrapping (1,000 replicates).

Results

Out of ten Kiel Fjord *Ammonia* extracted for DNA, all but one (F336) gave positive results for PCR. SSU sequences were obtained from six *Ammonia* specimens and LSU sequences from nine specimens (Table 1). Among the five specimens of *Elphidium* from the Kiel Fjord, which were extracted for DNA, all but F341 (*E. gerthi*) gave SSU sequences (Table 1). However, the results were negative for the LSU fragment. Because *Elphidium* has one of the fastest evolutionary rates among benthic foraminifers (Schweizer et al. 2008), these negative results could be due to the fact that the 5′ end of the LSU gene had slightly diverged in this genus, causing a mismatch error with LSU primers and preventing the DNA amplification.

SSU rDNA analysis

The topologies of ML (Fig. 3) and BIONJ (data not shown) trees were rather similar. Only the position of the Australian

Table 2 List of other new rDNA sequences with their DNA numbers, locality of sampling and EMBL/GenBank accession numbers. Samples 475, 476, 1244 and 3978 belong to the Forams DNA Collection in Geneva

Species	DNA number	Locality	Sequence name
Haynesina germanica	F323	Den Oever (NL)	GQ853557 (SSU)
			GQ853576 (LSU)
Elphidium sp.	1244	Mok Baai (NL)	GQ853558 (SSU)
			GQ853559 (SSU)
Elphidium sp.	3978	Dunstaffnage (UK)	GQ853560 (SSU)
			GQ853561 (SSU)
			GQ853562 (SSU)
Ammonia sp.	475	Lizard Island (AUS)	GQ853567 (SSU)
Ammonia sp.	476	Lizard Island (AUS)	GQ853568 (SSU)
Ammonia aomoriensis	F583	Den Oever (NL)	GQ853569 (SSU)
Ammonia aomoriensis	A23	Mok Baai (NL)	GQ853577 (LSU)

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Fig. 3 Molecular phylogeny of *Ammonia* and *Elphidium* based on partial SSU rDNA sequences inferred using the ML method (HKY + I + Γ). Tree is rooted on *H. germanica*, and bootstrap values for ML and BIONJ analyses are indicated at the nodes
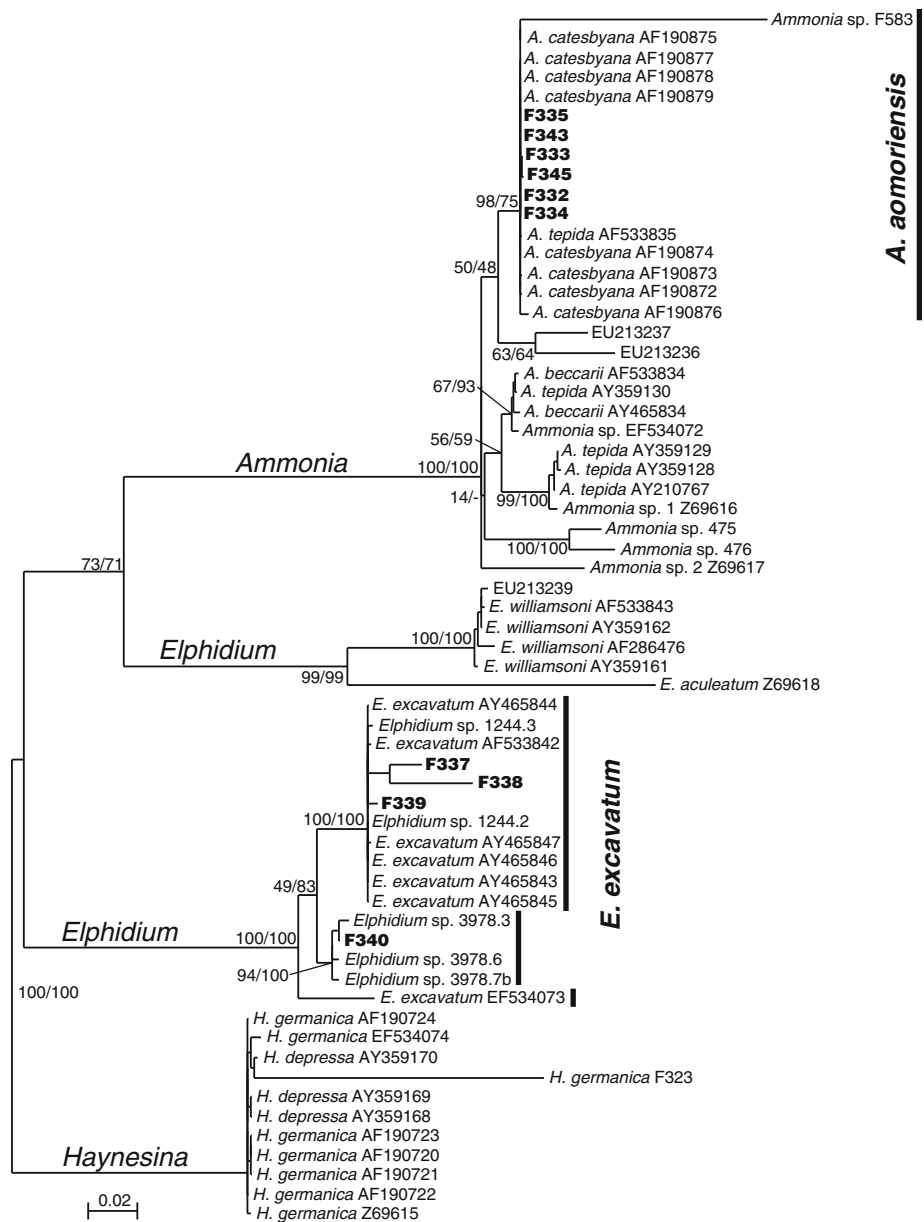
clade (475 and 476) inside the *Ammonia* clade moved slightly between both analyses. This difference in topology and the low statistical supports of the intermediate nodes were signs that the phylogenetic signal was too weak to infer robust relationships between clades due to the shortness of the studied region. However, the terminal clades were stable, with rather high BS (usually above 60%).

The *Ammonia* sequences formed a monophyletic group (100% BS), which was composed of six clades (Fig. 3). One of these clades included the six *Ammonia* sequences from the Kiel Fjord, the sequences of specimens named *A. catesbyana* from Crildumersiel in the Wadden Sea (Langer and Leppig 2000) and another sequence attributed to *A. tepida* from the North Frisian Islands (Ertan et al. 2004).

This clade had a high statistical support (98/75% BS), and it was sister group to the two environmental sequences from USA.

The *Elphidium* sequences formed a paraphyletic group composed of two clades at the basis of the *Ammonia* clade (Fig. 3). The sister clade of *Ammonia* was composed of the sequences of *E. williamsoni* and *E. aculeatum*, and the most basal clade included the *E. excavatum* sequences. The *E. excavatum* clade subsumed three subclades: one with a sequence from Den Oever (EF534073, Schweizer et al. 2008), one with F340 and clones of a Scottish *Elphidium* (3978) and one with F337, F338, F339, six sequences from the Bay of Biscay (Ertan et al. 2004) and two from the Wadden Sea (1244).

LSU rDNA analysis

The 13 phylotypes of *Ammonia*, which had been previously defined by Hayward et al. (2004), were also retrieved in this analysis (Fig. 4). The general topology of the tree was similar to the one published by Pawlowski and Holzmann (2008). The statistical supports of the 13 phylotypes were high; above 70% BS for most of them, but the other nodes had variable supports. This can be explained by the shortness of the fragment giving a weak phylogenetic signal of the deeper nodes. The nine LSU sequences of *Ammonia* from the Kiel Fjord branched in the phylotype T6 with a support of 72/97% BS, together with other European sequences from the Wadden Sea and Asian sequences from China and Japan. All the sequences were very homogeneous with less than 1.5% divergence.

Discussion

Molecular identification of *Ammonia* specimens

Comparisons between SSU (Fig. 3) and LSU (Fig. 4) rDNA analyses are difficult because, except for the Kiel Fjord samples, different specimens were sequenced for both genes. Moreover, species names were attributed to SSU-analysed specimens (except *Ammonia* sp. 1 and 2 corresponding to T1 and T2, respectively) based on their

Fig. 4 Molecular phylogeny of *Ammonia* based on partial LSU rDNA sequences inferred using the ML method (HKY + I + Γ). Tree is rooted on *N. calcar*, *P. nipponica* and *H. germanica*, and bootstrap values for ML and BIONJ analyses are indicated at the nodes
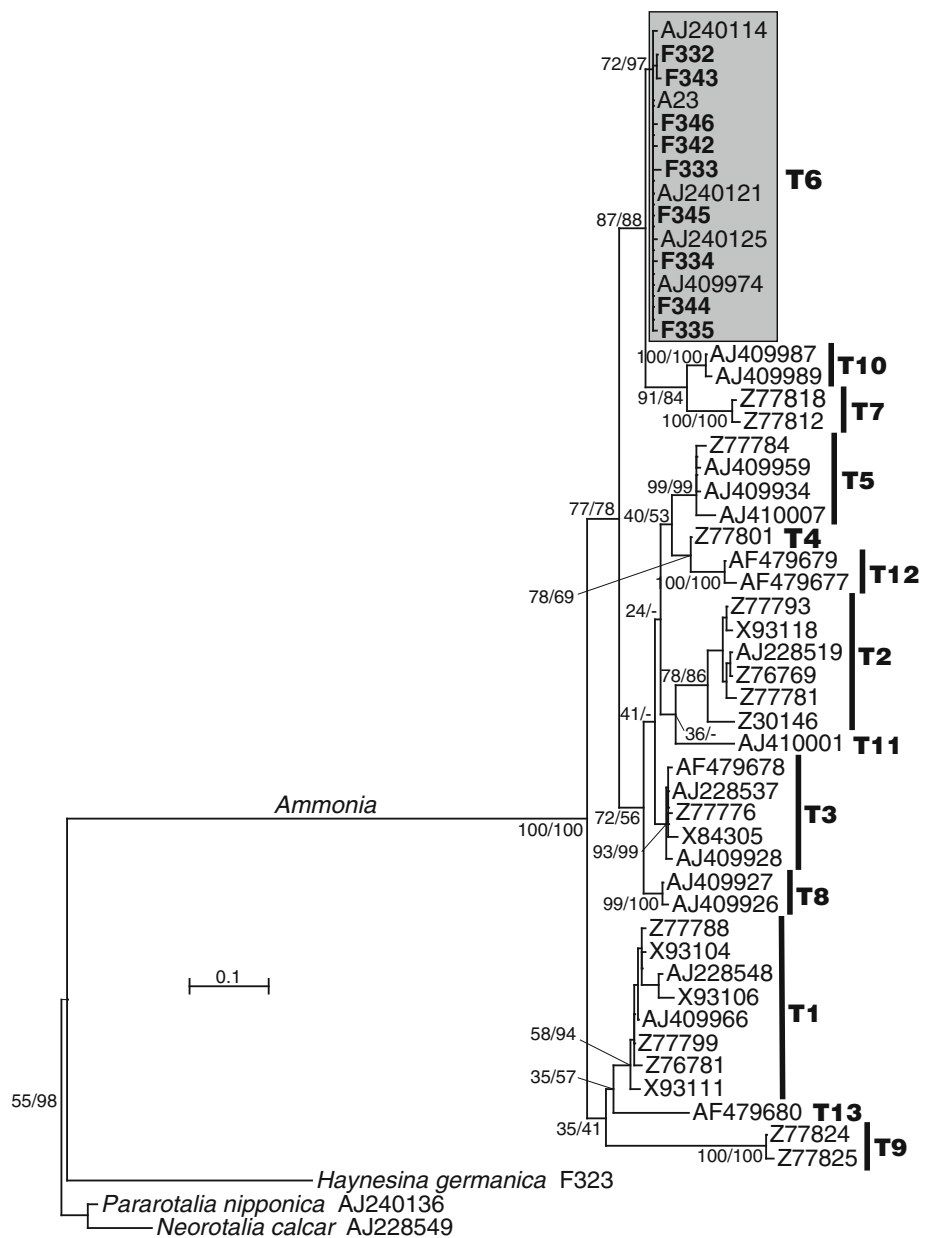
Fig. 5 Distribution of the phylotype T6 worldwide (**a**) and in the region of Wadden and West Baltic Seas (**b**). Sampled sites are indicated by *circles* for Asia and *stars* for Europe. Data from Hayward et al. ([2004](#)), Langer and Leppig ([2000](#)), Ertan et al. ([2004](#)) and the present study
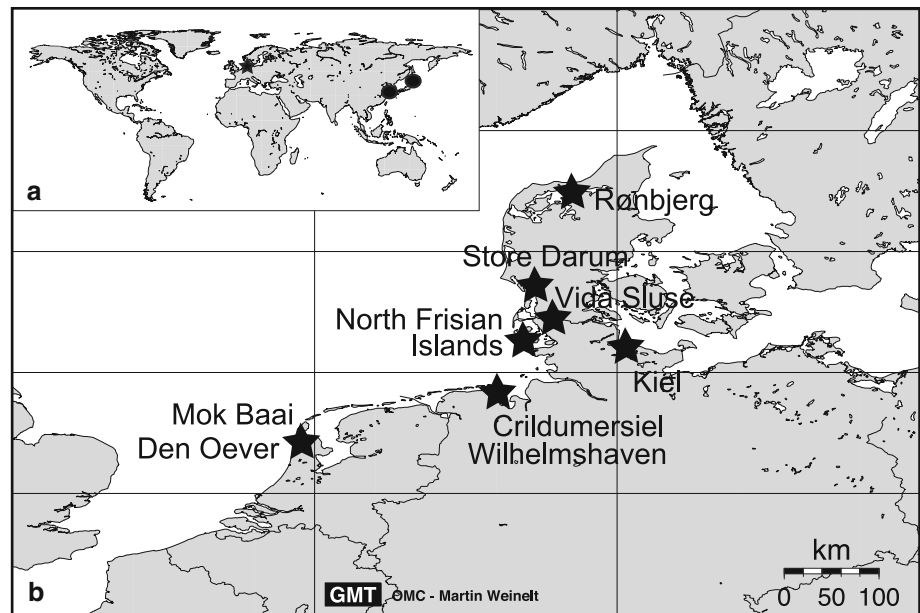
morphology, whereas LSU-analysed specimens were first defined as phylotypes and subsequently attributed to morphospecies (Hayward et al. [2004](#)).

In the LSU analysis, the Kiel Fjord *Ammonia* branch within the phylotype T6. Other members of T6 have been collected in Japan (lakes in Hokkaido and Honshu) and China (Yalu Jiang), as well as in the Dutch (Mok Baai, Den Oever) and German (Wilhelmshaven) Wadden Sea and in Denmark (Fig. [5](#)). In the SSU analysis, Kiel Fjord *Ammonia* group with specimens collected in Crildumersiel (near Wilhelmshaven) and identified as *A. catesbyana* (Langer and Leppig [2000](#)) and with one sequence attributed to *A. tepida* and collected in the North Frisian Islands (Ertan et al. [2004](#)). In view of the LSU results, these specimens can also be attributed to T6.

In Mok Baai, the phylotype T6 co-occurs with T1, the only phylotype with a cosmopolitan distribution (Pawlowski and Holzmann [2008](#)). Moreover, it is difficult to discriminate morphologically T6 from T1, T2 and T10 because of the lack of salient characteristics (Hayward et al. [2004](#)). Therefore, further studies with a wider sampling are needed to check whether T6 is the only phylotype present in the Kiel Fjord.

If we assume that the *Ammonia* populations from the Wadden and Baltic Seas represent mainly one genotype, which also occurs in eastern Asia, a consistent species name is required. The morphospecies *A. beccarii*, *A. tepida* and *A. catesbyana* have been described from very distant localities: Cuba and Martinique for *A. catesbyana* (d'Orbigny [1839](#)), Rimini, Italy, for topotypic specimens of *A. beccarii* (Cushman [1928](#)) and Puerto Rico for *A. tepida* (Cushman [1926](#)). Moreover, there is currently no recognised phylotype corresponding to the morphology of these

species (Hayward et al. [2004](#)). Phylotype T6 specimens resemble morphologically the holotype of *A. aomoriensis* from the Pliocene Hamada Formation from Honshu (Hayward et al. [2004](#)), a species also reported from unspecified recent deposits of northern Japan (Asano [1951](#)). This phylotype was also related to *A. beccarii* forma 1 (Toyofuku et al. [2005](#)), a morphotype often identified in Japanese studies (Nomura and Seto [1992](#); Takata et al. [2006](#)). However, for the time being and on the basis of the molecular analyses, we propose to follow Hayward et al. ([2004](#)) by using the denomination of *A. aomoriensis* for the phylotype T6, including the populations from the European Wadden and Baltic Seas.

The most likely explanation for this disjunct distribution is the transport of Asian individuals through water used for ships' ballast (Pawlowski and Holzmann [2008](#)). This hypothesis is strengthened by the fact that most of the sampled localities in Europe are close to harbours and that *Ammonia* is apparently a newcomer in the Kiel Fjord. A core taken from the outer Kiel Fjord, with a record approximately going back to 1950, showed that *Ammonia* was always absent or rare until 2000 when it became abundant (Polovodova and Nikulina, unpublished data). So, we could speculate that local *Ammonia* species were transported regularly with bottom water inflows from the North Sea to the western Baltic Sea but perished due to inadequate salinity conditions as suggested for species that occur sporadically or as single specimens (Brodniewicz [1965](#)). The abundance of *Ammonia* observed since the year 2000 could therefore be explained by the arrival of the newcomer *A. aomoriensis*, brought to the North Sea with ships' ballast and then following the same way than former indigenous *Ammonia* species, but settling successfully in

the western Baltic Sea because it was better adapted to this brackish environment.

Molecular identification of *Elphidium* specimens

Seven different *Elphidium* taxa were reported from the Kiel Bight, and they were attributed to the genera *Elphidium, Cribrononion* or *Cribroelphidium* (Lutze 1965, 1974; Frenzel et al. 2005; Nikulina et al. 2008). Two taxa were considered as subspecies of *Elphidium excavatum,* namely *E. e. excavatum* (forma *selseyensis* of Frenzel et al. 2005) and *E. e. clavatum*. The distinction between both taxa was initiated by Lutze (1965), who had topotypic specimens of *Elphidium excavatum* from Dunkerque, France and samples from the Arctic with *Elphidium clavatum* available, and he compared them with his specimens from the Baltic Sea for species determination. The discernation concept was later corroborated by morphological, biogeographical and evolutionary studies (Feyling-Hanssen 1972; Miller et al. 1982; Goubert 1997; Poignant et al. 2000). *Elphidium e. excavatum* was originally described from beach sands off Dunkerque, France (Terquem 1876). Tests of *E. e. excavatum* exhibit numerous papillae of different sizes in the umbilical area. They are surrounded by thickened extensions of the chamber walls pointing into the umbilicus. The sutural bridges are generally indistinct. The test of *Elphidium e. excavatum* is slightly compressed with a width * 10/diameter ratio of 4–5 (Lutze 1965). The other subspecies, *Elphidium e. clavatum*, was described from Frenchmans Bay, Maine, USA (Cushman 1930). Tests of *E. e. clavatum* show a central knob in the umbilical area. The sutural bridges are broad and distinct, and the sutures close before reaching the umbilicus. *Elphidium e. clavatum* is with a width * 10/diameter ratio of 5–6 markedly thicker than *E. e. excavatum*. Our specimens F337-F339 were morphologically attributed to *E. e. excavatum*, because their tests have wide sutural and umbilical areas, which harbour pustules. The test of F340 is stouter (higher width/diameter ratio of the profile) and shows clear sutural bridges crossing simple sutures that radiate from a closed umbilicum; therefore, it was identified as *E. e. clavatum* (Fig. 1). The DNA results have confirmed the attribution to *E. excavatum* as well as the distinction in two subgroups (Fig. 3). The three subclades subsumed in *E. excavatum* (Fig. 3) may correspond to some of the different morphotypes previously described (e.g. Goubert 1997). These subclades cannot be considered as ecophenotypes (e.g. Miller et al. 1982) because of their genetic differentiation, but they represent different subspecies or species.

Elphidium excavatum excavatum and *E. e. clavatum* usually inhabit different depth intervals in the western Baltic Sea with different hydrographic and sedimentary environments (Lutze 1965, 1974). To consider them as different subspecies or species implies spatially or temporally isolated populations with rather limited gene exchanges. In the western Baltic Sea, both taxa were occasionally found co-occurring in the living assemblage or exchanging each other in shallow-water areas over the year (Lutze 1965; Haake 1967). The present study brings supplementary genetic evidence of the co-occurrence of these taxa.

Conclusion

Molecular identification with rDNA sequences gives clear answers, which can help untangling a complex taxonomy when coupled with a morphological study. The present results are particularly good for a genus already well studied phylogenetically such as *Ammonia*, but answers are also obtained for a genus with fewer sequences published such as *Elphidium*.

The *Ammonia* specimens collected in the Kiel Fjord belong to clade T6 (Figs. 3, 4), a phylotype reported from China and Japan, which has been also found in the Wadden and Baltic Seas and the Danish Skagerrak (Fig. 5). According to Hayward et al. (2004), the *Ammonia* belonging to the phylotype T6 are referable on morphological characters to the Japanese species *A. aomoriensis*. However, this species has been described from the Pliocene, and whether it is extant or not has to be investigated. Likewise, further sampling is needed in the western Baltic Sea to verify whether T6 is the only *Ammonia* phylotype present in this area.

The *Elphidium* specimens from the Kiel Fjord belong to the species *E. excavatum*. Moreover, the phyloclade *E. excavatum* is composed of at least three subclades (Fig. 3), which mirrors the high morphological diversity translated in the description of different ecophenotypes, subspecies or species (Feyling-Hanssen 1972). The large genetic differences between specimens determined as *E. e. excavatum* and *E. e. clavatum* corroborate the evidence for their discrimination as subspecies rather than ecophenotypes. Further investigations are needed to understand the relationships between these different types of *E. excavatum*, their taxonomic status, the connection between genetic and morphological variability and their ecology.

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