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Research paper

The genetic diversity, phylogeography and morphology of Elphidiidae (Foraminifera) in the Northeast Atlantic

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ABSTRACT

Genetic characterisation (SSU rRNA genotyping) and Scanning Electron Microscope (SEM) imaging of individual tests were used in tandem to determine the modern species richness of the foraminiferal family Elphidiidae (*Elphidium*, *Haynesina* and related genera) across the Northeast Atlantic shelf biomes. Specimens were collected at 25 locations from the High Arctic to Iberia, and a total of 1013 individual specimens were successfully SEM imaged and genotyped. Phylogenetic analyses were carried out in combination with 28 other elphidiid sequences from GenBank and seventeen distinct elphidiid genetic types were identified within the sample set, seven being sequenced for the first time. Genetic types cluster into seven main clades which largely represent their general morphological character. Differences between genetic types at the genetic, morphological and biogeographic levels are indicative of species level distinction. Their biogeographic distributions, in combination with elphidiid SSU sequences from GenBank and high resolution images from the literature show that each of them exhibits species-specific rather than clade-specific biogeographies. Due to taxonomic uncertainty and divergent taxonomic concepts between schools, we believe that morphospecies names should not be placed onto molecular phylogenies unless both the morphology and genetic type have been linked to the formally named holotype, or equivalent. Based on strict morphological criteria, we advocate using only a three-stage approach to taxonomy for practical application in micropalaeontological studies. It comprises genotyping, the production of a formal morphological description of the SEM images associated with the genetic type and then the allocation of the most appropriate taxonomic name by comparison with the formal type description. Using this approach, we were able to apply taxonomic names to fifteen genetic types. One of the remaining two may be potentially cryptic, and one is undescribed in the literature. In general, the phylogeographic distribution is in agreement with our knowledge of the ecology and biogeographical distribution of the corresponding morphospecies, highlighting the generally robust taxonomic framework of the Elphidiidae in time and space.

1. Introduction

Elphidiidae are found largely in the coastal and shelf sediments throughout the world's oceans. They are among the most common and widespread groups of benthic foraminifera in the neritic zone (Murray, 1991). Off the west coast of South France for example, elphidiids were found to occur mostly on the inner shelf (0–50 m; Pujos, 1976). However, although elphidiids are generally shallower shelf forms, they may extend to deeper environments (several

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hundreds of meters) in the Arctic, sometimes in connection with freshwater outflow from rivers (e.g., Bergsten, 1994; Polyak et al., 2002).

As for all calcareous foraminifera, elphidiid tests preserve readily and are important in reconstructing past marine environments. They have a well-known fossil record that extends as far back as the Eocene (Cushman, 1939) and have particular utility in stratigraphy, the reconstruction of Quaternary climate and sea-level cycles (e.g., Haslett, 2002; Murray, 2006). This utility largely derives from their widespread occurrence from the high to low latitudes and presence from the high-intertidal to continental slope environments. Currently, palaeoclimate reconstructions utilise morphological criteria of benthic foraminifera based on the species concept to constrain numerical and geochemical palaeoproxies (e.g., Buzas and Gibson, 1969; Jansen, 1989; Hayek and Buzas, 1997; Lear et al., 2002; Elderfield et al., 2006; Groeneveld and Filipsson, 2013). However, the morphospecies concept can vary between different taxonomic schools (e.g., Jones, 2013), where different morphological criteria are used to define the taxon and/or different formal name are adopted to define the same taxon (i.e., a synonym), resulting in highly complex synonymies for many elphidiid morphospecies (Miller et al., 1982). Additionally, the lack of carefully illustrated specimens in the literature also makes it impossible to track the taxonomic concepts of these schools and their modifications, causing confusion for palaeoenvironmental studies.

This situation makes it extremely difficult to construct biogeographical distributions of the key elphidiid morphospecies and hence to understand their ecological ranges, upon which palaeoclimate reconstructions ultimately depend. For example, benthic foraminifera transfer function methods which reconstruct temperature and salinity (Sejrup et al., 2004) or sea-level (e.g., Horton and Edwards, 2006) all fundamentally depend on the stability of the taxonomic unit (i.e., morphospecies). In addition, the use of taxon-specific biogeochemical proxies is highly dependent upon the taxonomic stability and hence ecological knowledge of the taxon. It has been shown that biogeochemical proxy calibrations are often species-specific (e.g., Rosenthal et al., 1997; Elderfield et al., 2006), and it is of crucial importance to establish the consistent application of each morphospecies concept.

In the last few years, attempts have been made to integrate the morphological concept of the benthic foraminiferal taxon unit with molecular characterisation (e.g., Hayward et al., 2004; Schweizer et al., 2005, 2009, 2012; Pillet et al., 2013). However, despite recent progress combining Elphidiidae molecular and morphological data collected from a range of sites within the North Atlantic (Pillet et al., 2013; Voltski et al., 2015), their genetic diversity and biogeographic distribution still requires much further investigation for the enhancement of palaeoenvironmental reconstructions. Molecular studies have shown evidence of previously unrecognised genetic diversity (cryptic diversity) within some foraminiferal morphospecies (i.e., Darling and Wade, 2008; Pawlowski and Holzmann, 2008). Conversely, there are instances where morphological variants are recognised as distinct species, despite there being no underlying genetic differences (Schweizer et al., 2009; Pillet et al., 2013; André et al., 2014).

The aims of this study were first, to gain a more comprehensive understanding of the genetic diversity and biogeography of elphidiids within the Northeast Atlantic shelf seas. We then used an integrated approach, employing both genotyping and morphological examination using Scanning Electron Microscope (SEM) imaging, to link each genetic type to the specific morphological characteristics of their tests in order to generate a morphological profile for each genetic type. To achieve this aim, we have provided the first comprehensive description of each genetic type (morphological profile) based on the SEM images of individual genetically characterised specimens. Using selected high-quality SEM images/illustrations from published literature, we then discuss the link between our genetic type morphological profiles and morphospecies concepts (i.e., formal descriptions) to establish a taxonomically stable and widely applicable biogeography for the Northeast Atlantic.

2. Methods

2.1. Sampling

The sampling strategy included a wide range of shelf provinces and biomes found within the middle to high latitude regions of the Northeast Atlantic. The biogeographic classification of the shelf and upper continental slope is shown in Fig. 1, which follows the most recent biogeographic classification produced for the Oslo and Paris Conventions (OSPAR) Maritime Area (Dinter, 2001). There were 25 major sampling sites in the study, which expands to 51 sampled stations when counting multiple sampling sites (Table 1, Supplementary Table S1). They range from north of Svalbard to as far south as Portugal. To maximize our biogeographic sampling range, we have incorporated sampling sites from the literature, where genetic characterisation was carried out by other scientists. The majority of samples originated from the intertidal zone, although several were obtained from deeper waters by SCUBA divers or by deployment of coring devices. Sampling locations and site descriptions are shown in Fig. 1, Table 1, Supplementary Table S1. The sampled sediments and seaweeds were maintained in sea water at a constant temperature of 4 °C prior to processing.

2.2. Detection of live specimens for SEM imaging

Sediments were sieved (63 μm) using sea water from the same location, wherever possible. Samples were examined microscopically and individual specimens were picked using a fine brush. For the Icelandic material, paper labels placed in the sediment sample bottles attracted many live elphidiids, which were then brushed off into Petri dishes for picking. Picked specimens were washed in filtered sea water and observed to determine whether they were alive. This was carried out either by observing individual activity overnight in a Petri dish containing fine sediment or by "foram racing", which involved their departure from lines drawn onto the base of a Petri dish. The latter method proved particularly useful for the rapid detection of live intertidal elphidiids. Live specimens were then placed onto micropalaeontological slides and allowed to dry at room temperature. They could be kept for several weeks at room temperature (Holzmann and Pawlowski, 1996) before being mounted on stubs for gold coating and imaging using the SEM (Philips XL30CP). During this step, each individual test was given a unique identification number which was used at each progressive stage of the DNA extraction, amplification and sequencing process. The obtained SEM images were corrected with the XL-Streach software (Philips) to transform rectangular pixels in square ones.

2.3. DNA extraction and amplification

Following SEM imaging, individual tests were transferred to a 0.5 ml microfuge tube and crushed into 60 μl of 1 × DOC buffer (Pawlowski, 2000). An ~1000 bp region at the terminal 3′ end of the small subunit (SSU) rRNA gene was amplified in two rounds of PCR using a thermocycler (Techne TC-412, Bibby Scientific Ltd). The primer pairs s14F3 (5′-acgcaagtgtgaaacttg-3′) and sB (Pawlowski, 2000) were used for the primary amplification and primer pairs s14F1 (Pawlowski, 2000) and J2 (5′-aggttcacctacggatgcctt-3′) for the secondary amplification. PCR conditions were 2 min at 94 °C followed by 40 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 2 min and ending with 72 °C for 10 min. The secondary amplification was duplicated apart from a slight increase in annealing temperature (52 °C) and cycle number (42). Where specimens were proving difficult to amplify, a shorter fragment (~500 bp) was generated using primer pairs s14F1 and N6 (White et al., 1990) in the secondary PCR. Amplification products were run on 1.2% agarose gels stained with Ethidium Bromide and purified using a Montage Gel Extraction Kit (Merck Millipore) or a High Pure PCR Purification Kit (Roche Diagnostics). Where there was evidence of multiple gene copies within an individual (intra-individual variation), PCR products were

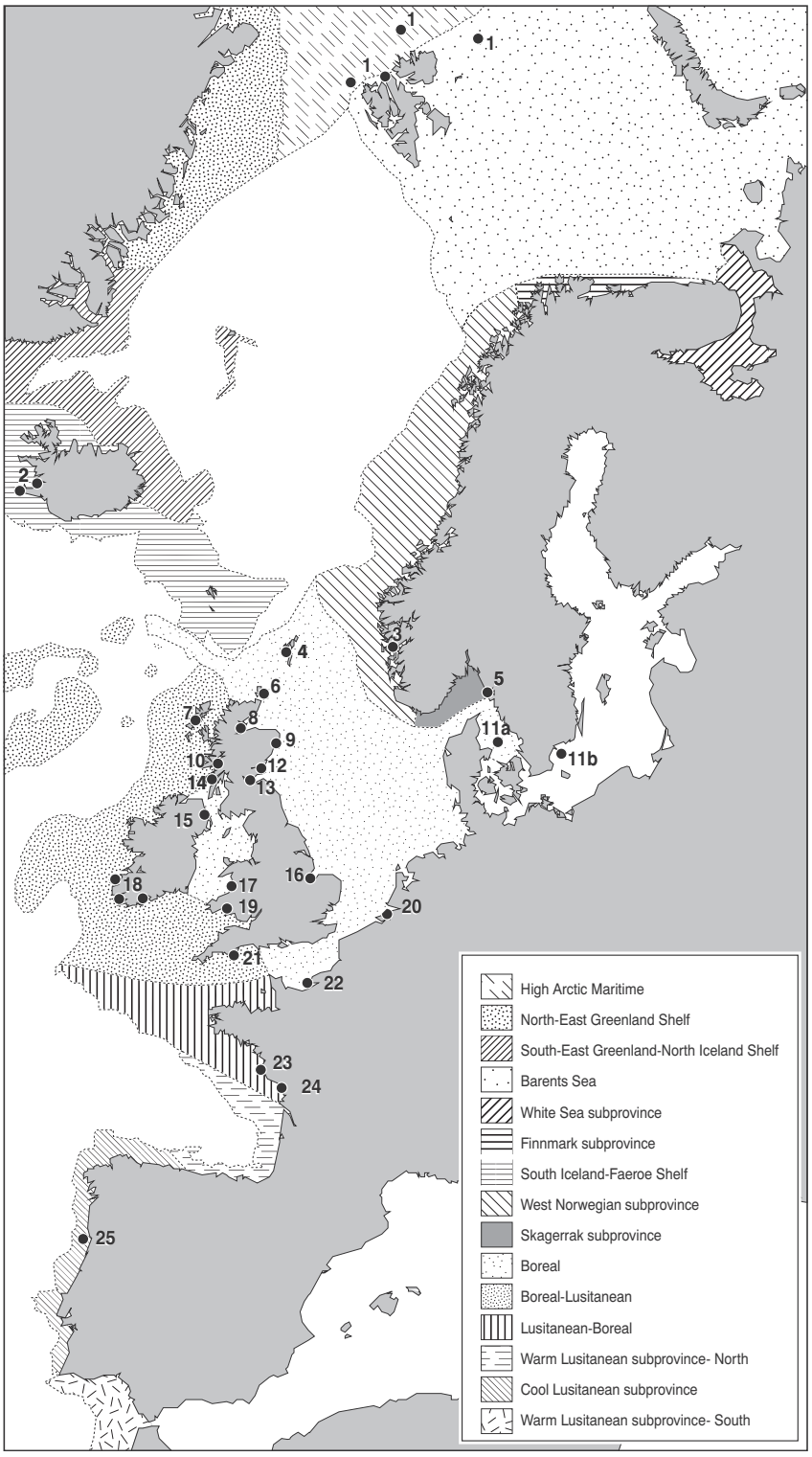

Fig. 1. Location map showing sampling sites (numbered north to south) for the present study in the Northeast Atlantic (Table 1). The map also shows the biogeographic classification of the benthic, nerito-pelagic and ice-cover biomes of the shelf and upper continental slope (Dinter, 2001: Fig. 105).

cloned using either pGEM®-T Easy Vector (Promega) or the pCR®-TOPO® Vector (Invitrogen). Between two and 15 clones were sequenced per specimen to ensure accurate designation of genetic type. Intra-individual variation was found to be common in elphidiid genetic types.

2.4. Genetic characterisation using sequencing and screening

Sequencing was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and an ABI 3730 DNA sequencer (Applied Biosystems) according to manufacturer's instructions. All

Table 1
Location of sampling sites with location description and genetic types identified. See Supplementary Table S1 for multiple sampling site coordinates and descriptions.

Location number (see map Fig.1)	Location name	Coordinates	Location description	Genetic types identified genetically	Genetic types identified by morphology alone
1	Svalbard (Sv)	Supplementary Table S1	Supplementary Table S1	S4, S8, S15	S7
2	Iceland (Is)	Supplementary Table S1	Supplementary Table S1	S1, S4	S7
3	Bergen (Bg)	60°15′38.28″N 5°13′ 11.4″E	Fjord sediment, 39 m	S10	
4	Shetland (SH)	Supplementary Table S1	Supplementary Table S1	S1, S2, S4, S9, S10,S16	S5, S7, S15
5	Skagerrak (Sk)	58°19′24″N 11°32′49.2″E	Fjord sediment, 119 m	S4	
6	Orkney (OK)	58°56′31.35″N 3°5′ 22.15″W	Intertidal sediment	S1	
7	North Uist (NU)	Supplementary Table S1	Supplementary Table S1	S1, S2, S4, S6, S9, S10, S14, S16, S17	
8	Cromarty (CR)	57°40′35.17″N 04°02′ 45.19″W	Intertidal sediment	S1, S16	S7
9	Ythan (YN)	57°20′N, 01°57′W	Intertidal sediment	S1, S5, S7, S16	
10	Dunstaffnage (DF)	56°27′40″N 05°26′61″W	Subtidal sediment, 31.6 m	S10	S1, S4, S5, S9, S14
11	Baltic (BA)	Supplementary Table S1	Supplementary Table S1	S4, S7	S5
12	Eden (ED/SA)	56°22′00.00″N 02°50′.00 W	Intertidal sediment	S1, S16	
13	Cramond (Cd)	55°59′22.92″N 03°17′ 53.16″W	Intertidal sediment	S1, S5, S6, S16	S14
14	Loch na Cille (LK)	55°57′36.00″N 05°41′ 24.00″W	Intertidal sediment	S1, S14, S16	
15	Whiterock Bay (WR)	54°29′05.42″N 05°39′ 12.58″W	Intertidal sediment	S1, S2, S3, S16	
16	Norfolk (NF)	52°49′02.41″N 00°21′ 46.16″E	Intertidal sediment	S1, S16	
17	Aberdovey Bay (AB)	52°31′45.01″N 04°00′ 07.06″W	Intertidal sediment	S1	
18	Cork (CK)	Supplementary Table S1	Supplementary Table S1	S1, S3, S9, S16	
19	Laugharne Castle (LC)	51°46′12.00″N 04°27′ 00.00″W	Intertidal sediment	S16	S5
20	Grevelingenmeer (Gv)	51°44′50.04″N 3°53′ 24.06″E	Brackish lake, 34 m	S5	
21	Dartmouth (DM)	50°21′04.84″N 03°34′ 11.33″W	Intertidal sediment	S1, S2, S3, S5, S9, S13, S16, S17	
22	Baie de Seine (BS)	Supplementary Table S1	Supplementary Table S1	S5	
23	Ile d'Yeu (Ye)	46°43′12.35″N 2°20′13″ W	Intertidal sediment with seaweeds	S12	
24	Baie de l'Aiguillon (Ai)	46°15′17.00″N 01°08′ 27.00″W	Intertidal sediment	S16	
25	Portugal (Po)	41°09′01.24″N 8° 52′ 00.90″W	Sand, 50 m	S11	

genetic types were characterised using the sequence of the full ~ 1000 bp 3′ fragment. Once genetic type boundaries were confirmed by sequencing and cloning, two further approaches were adopted to speed up genetic characterisation. The first was to use a short sequence incorporating the first variable region only, providing that it defined the genetic type. The second was to use a genetic type specific screening method to confirm the identity of the most common encountered genetic types S1 and S16. These genetic types are morphologically identifiable and can be picked out of an assemblage with reasonable confidence (see Fig. 3). Primary PCR amplifications were carried out as described above. Potential S1 specimens were screened in a secondary PCR containing a 0.5 μM mix of the two forward S1-specific primers EW1 (5′-gacccacgtttacgcgtg-3′) and EW2 (5′-ctactatactgcacattatgtgta-3′), together with the reverse primer J2 to give two products of 650 bp and 419 bp, respectively. Potential S16 specimens were screened in a secondary PCR reaction containing a 0.5 μM mix of the three forward S16-specific primers HG1a (5′-gcgtatgtgcatcacatatattt-3′), HG1b (5′-gcgtatgtgcatcacaatatt-3′) and HG1c (5′-gcgtatgtgcaccatatattt-3′), together with the reverse primer J2. The three forward primers produced a single 445 bp product by annealing to one of three different intra-individual variant sequences. Positive reactions were identified by visualisation of the correct number and size of bands on an agarose gel and by the initial sequencing of products. The specificity of all primers was confirmed by negative PCR results for specimens belonging to other genetic types and to other foraminiferal genera. Any specimens producing negative results following screening were sequenced.

2.5. Phylogenetic analysis

Sequences were edited in ChromasPro v1.5 (Technelysium Pty Ltd) and manually aligned in BioEdit v7.0.9.0 (Hall, 1999). All elphidiid sequences currently in the GenBank database (up to July 2015) were also included in the alignment to bring additional diversity to our dataset (Table 2, Supplementary Table S2). Up to six sequences (but no consensus sequence) of each genetic type were selected for inclusion in the alignment for phylogenetic analyses, the number depending on the degree of intra-individual variation found. Of the 1210 nucleotide sites in the alignment, 601 unambiguously aligned sites were utilised in phylogenetic analysis.

Phylogenetic trees were constructed using three different methods. A Bio Neighbor-Joining (BioNJ) tree (Gascuel, 1997) was constructed using Seaview 4 (Gouy et al., 2010) with 1000 bootstrap (BS) replicates (Felsenstein, 1985). Maximum likelihood (ML) analysis was performed with 2000 BS replicates using PhyML (Guindon and Gascuel, 2003) implemented in Seaview 4. Finally, Bayesian analysis (BA) was performed with MrBayes 3.2 (Ronquist et al., 2012). Two independent analyses were carried out at the same time with four simultaneous chains (one cold and three heated) run for 10,000,000 generations, and sampled

Table 2
SSU rDNA sequences used for phylogenetic analyses (Fig. 2) including both genetic types from this study (S1–S17) and the literature (S18–S22, Patagonia and Canada). Accession numbers are shown with previously published sequences in italic and new ones in bold.

Genetic type	Accession number	DNA isolate	Location name	Location number (Fig. 1, Table 1)	Reference
S1	**KP347002**	Cd273_A	Cramond, Scotland, UK	13	This study
S1	**KP347003**	CK78_A	Timoleague, County Cork, Ireland	18	This study
S1	**KP347005**	ED182_B	Eden Estuary, Scotland, UK	12	This study
S1	**KP347004**	WR64_C	Whiterock Bay, Northern Ireland, UK	15	This study
S1	*AY359162*		Fromentine, France		Ertan et al., 2004
S1	*HM213839*		Chezzetcook Inlet, Canada		Pillet et al., 2011
S2	**KP347016**	DM41_C	Dartmouth, England, UK	21	This study
S2	**KP347017**	DM66_D	Dartmouth, England, UK	21	This study
S2	**KP347018**	WR15_A	Whiterock Bay, Northern Ireland, UK	15	This study
S3	**KP346990**	CK108	Timoleague, County Cork, Ireland	18	This study
S3	**KP346991**	DM21	Dartmouth, England, UK	21	This study
S3	**KP346992**	WR46_B	Whiterock Bay, Northern Ireland, UK	15	This study
S3	*EF534073*		Den Oever, Netherlands		Schweizer et al., 2008
S4	**KP346996**	Is267	Ellidavogur, Reykjanes Peninsula, Iceland	2	This study
S4	**KP346998**	Sk232	Gullmar Fjord, Skagerrak, Sweden	5	This study
S4	**KP346997**	Sv665	Sv11-HH11-16A, Svalbard	1	This study
S4	*GQ853566*		Kiel Fjord, Germany		Schweizer et al., 2011
S4	*KF042561*		White Sea, Russia		Pillet et al., 2013
S5	**KP346999**	DM127_A	Dartmouth, England, UK	21	This study
S5	**KP347000**	YN02_A	Ythan Estuary, Scotland, UK	9	This study
S5	**KP347001**	YN28_C	Ythan Estuary, Scotland, UK	9	This study
S5	*AY465845*		Port Pleasance, France		Ertan et al., 2004
S5	*GQ853558*		Mokbaai, Netherlands		Schweizer et al., 2011
S5	*HM213829*		Chezzetcook Inlet, Canada		Pillet et al., 2011
S6	**KP347019**	Cd146_C	Cramond, Scotland, UK	13	This study
S6	**KP347021**	Cd146_N	Cramond, Scotland, UK	13	This study
S6	**KP347020**	Cd146-R	Cramond, Scotland, UK	13	This study
S7	**KP347028**	YN03_A	Ythan Estuary, Scotland, UK	9	This study
S7	**KP347029**	YN16_D	Ythan Estuary, Scotland, UK	9	This study
S7	**KP347030**	YN37_C	Ythan Estuary, Scotland, UK	9	This study
S7	*HM213832*		White Sea, Russia		Pillet et al., 2011
S8	**KP347031**	Sv250_2	JM10-03-BC, Svalbard	1	This study
S8	**KP347034**	Sv253_1	JM10-03-BC, Svalbard	1	This study
S8	**KP347033**	Sv384_19	JM10-02-BC, Svalbard	1	This study
S8	**KP347032**	Sv386_1	JM10-02-BC, Svalbard	1	This study
S8	*KF042553*		White Sea, Russia		Pillet et al., 2013
S9	**KP347006**	CK97_B	Ring, County Cork, Ireland	18	This study
S9	**KP347007**	CK97_C	Ring, County Cork, Ireland	18	This study
S9	*HM213824*		Trebeurden, France		Pillet et al., 2011
S10	**KP347008**	DF149_A	Dunstaffnage, Scotland, UK	10	This study
S10	**KP347009**	DF193_B	Dunstaffnage, Scotland, UK	10	This study
S10	*HM213834*		Porquerolles, France		Pillet et al., 2011
S11	**KP347010**	Po83_4	Portugal	25	This study
S11	**KP347011**	Po84_6	Portugal	25	This study
S11	**KP347012**	Po85_2	Portugal	25	This study
S12	**KP347022**	Ye45	Ile d'Yeu, France	23	This study
S12	**KP347023**	Ye53	Ile d'Yeu, France	23	This study
S12	*Z69618*		St Cyr, France		Pawlowski et al., 1997
S13	**KP346994**	DM103_A	Dartmouth, England, UK	21	This study
S13	**KP346995**	DM103_E	Dartmouth, England, UK	21	This study
S13	**KP346993**	DM151_L	Dartmouth, England, UK	21	This study
S14	**KP347027**	LK51	Loch Na Cille, Scotland, UK	14	This study
S14	**KP347024**	NU313	Bagh a Chaise, North Uist, Scotland, UK	7	This study
S14	**KP347025**	NU327	North Uist, Scotland, UK	7	This study
S14	**KP347026**	NU354	North Uist, Scotland, UK	7	This study
S15	**KP347035**	Sv661_1	Sv11-HH11-10A, Svalbard	1	This study
S15	**KP347036**	Sv661_2	Sv11-HH11-10A Svalbard	1	This study
S16	**KP347038**	ED25_A	Eden Estuary, Scotland, UK	12	This study
S16	**KP347037**	ED29_A	Eden Estuary, Scotland, UK	12	This study
S16	*Z69615*		Golfe du Morbihan, France		Pawlowski et al., 1997
S16	*EF534074*		Den Oever, Netherlands		Schweizer et al., 2008
S17	**KP347039**	DM178	Dartmouth, England, UK	21	This study
S17	**KP347041**	DM344_D	Dartmouth, England, UK	21	This study
S17	**KP347042**	DM344_E	Dartmouth, England, UK	21	This study
S17	**KP347040**	NU287	North Uist, Scotland, UK	7	This study
S18	*HM213825*		Roscoff, France		Pillet et al., 2011
S18	*HM213826*		Roscoff, France		Pillet et al., 2011
S19	*KF042546*		White Sea, Russia		Pillet et al., 2013
S19	*KF042549*		White Sea, Russia		Pillet et al., 2013
S20	*KF042580*		White Sea, Russia		Pillet et al., 2013
S20	*KF042584*		White Sea, Russia		Pillet et al., 2013
S21	*KF042554*		White Sea, Russia		Pillet et al., 2013
S21	*KF042587*		White Sea, Russia		Pillet et al., 2013

(continued on next page)

Table 2 (*continued*)

Genetic type	Accession number	DNA isolate	Location name	Location number (Fig. 1, Table 1)	Reference
S22	*KF042557*		Kara Sea, Russia		Pillet et al., 2013
S22	*KF042590*		Kara Sea, Russia		Pillet et al., 2013
Patagonia	**KP347013**	Be06	Beagle Canal, Argentina		This study
Patagonia	**KP347014**	Be07	Beagle Canal, Argentina		This study
Patagonia	**KP347015**	Be11	Beagle Canal, Argentina		This study
Patagonia	*JN655700*		Seno Otway, Chile		Pillet et al., 2012
Canada	*HM213840*		Chezzetcook Inlet, Canada		Pillet et al., 2011
Canada	*HM213841*		Chezzetcook Inlet, Canada		Pillet et al., 2011
Ammonia	*Z69617*		Camargue, France		Pawlowski et al., 1997
Ammonia	*EF534072*		Not known		Schweizer et al., 2008
Ammonia	*GQ853567*		Lizard Island, Australia		Schweizer et al., 2011
Ammonia	*GQ853575*		Kiel Fjord, Germany		Schweizer et al., 2011

every 1000 generations with 2500 initial trees discarded as burn-in after convergence was reached. The posterior probabilities (PP), calculated during the BA, estimated the reliability of internal branches. The evolutionary models selected are General Time Reversible or GTR (Tavaré, 1986) for ML and Kimura 2 parameters or K2P (Kimura, 1980) for BioNJ. A mixed model was used for BA which sampled across the GTR model space (Huelsenbeck et al., 2004). To correct for among-site variations, the alpha parameter of gamma distribution (G), with four rate categories, was calculated by Seaview and MrBayes.

The choice of outgroup for the elphidiids is problematic due to their high evolution rates compared to the other rotaliid clades (Schweizer et al., 2008). Although the genera *Elphidium*, *Haynesina* and *Ammonia* fall as sister groups in the complete SSU rDNA phylogeny, their true evolutionary relationships remain unclear due to the possible long-branch attraction artefacts, high heterogeneity of sequences and uncertain position of the root of elphidiids. However, multigene analysis suggests that *Elphidium* and *Ammonia* may be less closely related than indicated by SSU phylogenies (Sierra et al., 2013). This is also consistent with their

Table 3
The number of SSU rRNA genetic types (S1–17) genetically characterised within the study area are shown together with the total number of specimens of each genetic type sequenced/screened (bold) or morphologically identified at each location. The seven elphidiids genetically characterised for the first time are highlighted (**new**).

Genetic type		S1	**S2**	S3	S4	S5	**S6**	S7	S8	S9	S10	**S11**	S12	**S13**	**S14**	**S15**	S16	**S17**	Total/region
New genetic types			**new**				**new**					**new**		**new**	**new**	**new**		**new**	
Map location																			
Svalbard (Sv)	1				**2**/2			2	**10**/54							**1**			**13**/58
Iceland (Is)	2	**23**/30			**6**/14			4											**29**/48
Bergen (Bg)	3										**1**								**1**
Shetland (SH)	4	**26**/5	**1**/1		**4**/4	4		1		**8**/50	**1**					1	**13**		**53**/66
Skagerrak (Sk)	5				**9**/6														**9**/6
Orkney (OK)	6	**23**/7																	**23**/7
North Uist (NU)	7	**36**	**15**/2		**7**/1		**2**/1			**23**/21	**1**/1				**12**/9		**18**	**1**	**115**/35
Cromarty (CR)	8	**6**						1									**10**		**16**/1
Ythan (YN)	9	**20**/6				**20**/7		**10**									**7**		**57**/13
Dunstaffnage (DF)	10	1			5	2				5	**3**/19				1				**3**/33
Baltic (BA)	11				**79**/8	3		**8**											**87**/11
Eden (ED/SA)	12	**103**															**87**		**190**
Cramond (Cd)	13	**4**/4				**7**/5	**2**/4								1		**26**		**39**/14
Loch na Cille (LK)	14	**14**/6													**3**/1		**3**		**20**/7
Whiterock (WR)	15	**16**	**1**	**3**													**19**		**39**
Norfolk (NF)	16	**16**															**46**		**62**
Aberdovey Bay (AB)	17	**19**																	**19**
Cork (CK)	18	**49**/13		**24**/3						**1**							**33**		**107**/16
Laugharne Castle (LC)	19					3											**23**		**23**/3
Grevelingen (Gv)	20					**4**													**4**
Dartmouth (DM)	21	**28**/3	**5**	**24**/5		**10**/1				**1**				**2**			**20**	**3**	**93**/9
Baie de Seine (BS)	22					**2**/5													**2**/5
Ile d'Yeu (Ye)	23												**3**/16						**3**/16
Baie de l'Aiguillon (Ai)	24																**3**		**3**
Portugal (Po)	25											**3**/2							**3**/2
Loch Sunart, Scotland (SU)	Table S1	1	1	1	5					2	3								13
Oslofjord, Norway (Os)	Table S1				2	1													3
Den Oever, Netherlands	Table S1	1		1															2
Porto Columbu, Sardinia, Italy	Table S1									2									2
Groomsport, Northern Ireland	Table S1	1																	1
Guadiana River, Portugal	Table S1													5					5
Genetic type		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	
Total sequenced		**131**	**22**	**51**	**107**	**43**	**4**	**18**	**10**	**33**	**6**	**3**	**3**	**2**	**15**	**1**	**81**	**4**	**534**
Total genetically screened		**252**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**227**	–	**479**
Total genetically identified																			**1013**
Total morphologically identified		78	4	10	47	29	5	8	54	80	23	2	16	5	12	1	–	–	376
Sequences (including clones)		181	31	68	112	56	19	51	48	36	15	18	5	18	15	3	168	5	849

morphology, since *Elphidium* and *Haynesina* are both planispiral and *Ammonia* trochospiral. We have therefore used *Ammonia* as an outgroup in this study, following Pillet et al. (2013) and Voltski et al. (2015).

2.6. Genetic type and morphospecies names

We strongly recommend that morphospecies names should not be placed on molecular phylogenies, unless both the morphology and genetic type have been linked to the formally named holotype (Roberts et al., 2016). Otherwise, doing so inevitably introduces taxonomic bias, being entirely dependent on the views of the individual taxonomists using potentially different taxonomic schemes and criteria. However, to aid the practical application of an elphidiid taxonomy in this publication, we have produced morphological profiles for each of the 17 individual genetic types from the SEM images of the genetically characterised tests (1013 images, Table 3), and used them as the basis for taxonomic designations.

3. Results

3.1. Genetic characterisation and molecular phylogeny

In total, 1013 individual specimens of elphidiids were successfully SEM imaged and genetically characterised using the partial SSU rRNA

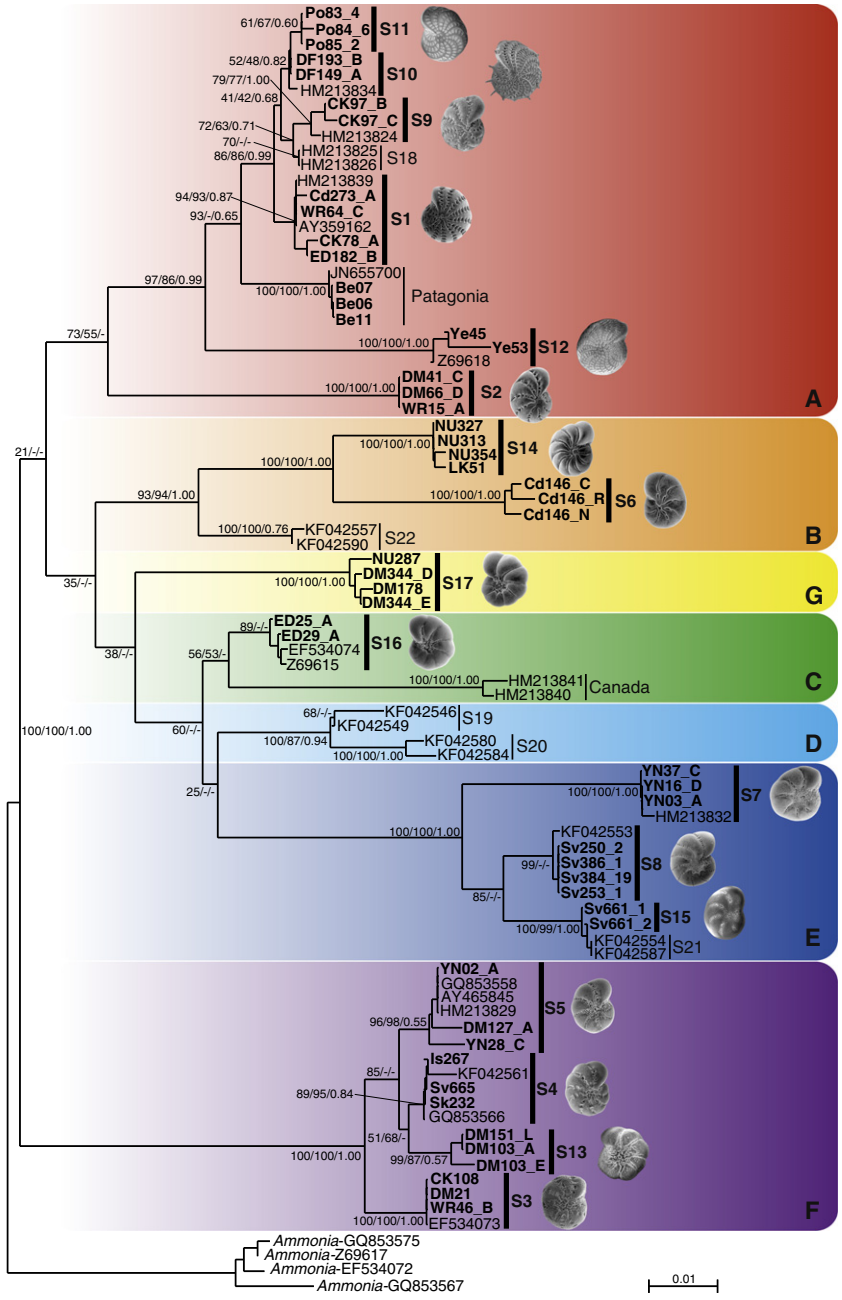

Fig. 2. Molecular phylogeny of elphidiids based on partial SSU rDNA sequences inferred using the BioNJ method with the K2P model. The tree is rooted on *Ammonia* and support values for BioNJ/ML/BA are indicated at the main nodes.

gene (Table 3). Of these, 534 were directly sequenced or cloned (see Methods), producing 849 DNA sequences for deposition in GenBank (accession numbers KP346990-KP347042 and KX962638-KX963335) and the molecular database of foraminifera "foramBARCODING" (http://forambarcoding.unige.ch) once our series of publications based on them are in press. The remaining 479 specimens were fast screened with SSU genetic type-specific primers (see Methods). For comparative analysis, the sequences were manually aligned (1210 nt) together with 125 elphidiid SSU rDNA sequences from GenBank (Camancho et al., unpublished; Pawlowski et al., 1997; Langer, 2000; Ertan et al., 2004; Habura et al., 2008; Schweizer et al., 2008, 2011; Pillet et al., 2011, 2013; Grimm et al., unpublished; Langer and Langer, unpublished). The sequences within the alignment separate into 24 discrete genetic types (Table 2), of which 22 were identified within the Northeast Atlantic study area (Fig. 1, Tables 1, 3, Supplementary Table S1). The remaining two occur outside the study area (Patagonia and Canada). Each genetic type was assigned an "S" number, designating it as an SSU genetic type. Of the 22 genetic types identified within the study area, seven have been sequenced for the first time (S2, S6, S11, S13, S14, S15, S17).

The phylogeny includes all the Northeast Atlantic genetic types identified in this study together with representative elphidiid sequences available in GenBank. Morphospecies names are excluded from the tree to avoid taxonomic bias (see Methods). A total of 85 SSU rDNA sequences were used for phylogenetic analyses (Table 2); 32 sequences were from GenBank and 53 sequences are new (this study). The evolutionary relationships among the elphidiids are shown in a BioNJ tree, rooted on *Ammonia* (Fig. 2; see Methods). The general topologies retrieved using ML and BA were slightly different (Supplementary Figs. S1, S2; see Methods). This discrepancy can be explained by the low phylogenetic signal resulting from the relatively limited number of informative sites in the dataset. We selected the BioNJ tree for the main figure (Fig. 2) in this study because its general topology was most similar to the phylogeny published by Pillet et al. (2013), which was based on the complete SSU rRNA gene to maximize the phylogenetic signal. The statistical support for all three analyses is shown on the common branches of the BioNJ tree (Fig. 2).

Seven main clades of elphidiids are recognised in the BioNJ analysis (Fig. 2). Six of them were already described by Pillet et al. (2013) and retain the same names here. These are Clade A (S1, S2, S9–S12, S18 and Patagonia), Clade B (S6, S14 and S22), Clade C (S16 and Canada), Clade D (S19 and S20), Clade E (S7, S8, S15 and S21) and Clade F (S3, S4, S5 and S13). Clade G is newly described here and contains only one genetic type, S17. Clade B (BioNJ: 93%, ML: 94%, BA: 1.00), Clade D (BioNJ: 100%, ML: 87%, BA: 0.94), Clade E (BioNJ: 100%, ML: 100%, BA: 1.00), Clade F (BioNJ: 100%, ML: 100%, BA: 1.00) and Clade G (BioNJ: 100%, ML: 100%, BA: 1.00) are well supported in the analyses, whereas Clade A (BioNJ: 73%, ML: 55%, BA: -) and Clade C (BioNJ: 56%, ML: 53%, BA: -) are not so firmly supported.

Most of the 24 genetic types recognised in the alignment form clearly individualised clades with long branches in the phylogenetic analyses. However, because of the degree of relatedness between genetic types in combination with the restricted amount of information from the partial SSU fragment (only 601 sites), some genetic types do not form well separated distinct clusters. The differences observed within the most variable regions of the SSU partial fragment become excluded in the 601 site analysis. For example, the closely related genetic types S10 and S11 or S15 and S21 do not resolve well in either BioNJ, ML or BA analyses (Fig. 2, Supplementary Figs. S1, S2). In order to investigate these issues in more detail, sub-trees of Clade A and Clades B, C, D, E and G were generated (Supplementary Figs. S3, S4). By rooting the sub-trees on the basal genetic type of each sub-dataset, an increased number of potentially informative sites could be recruited into the analysis. The Clade A BioNJ sub-tree (650 sites; Supplementary Fig. S3) varies slightly from the main BioNJ tree in topology but better resolves the individual genetic types S10 and S11 (89/70/-). Similarly, the BioNJ sub-tree for Clades B, C, D, E and G (656 sites; Supplementary Fig. S4) also varies slightly in topology but fully resolves the genetic types S15 and S21 (100/97/0.99).

3.2. Morphological characterisation of molecular clades

Representative specimens typical of each genetic type are grouped according to clade and shown in Fig. 3. All seven clades share the common characteristics of elphidiids, namely having a planispiral test, sutural canal systems and interio-marginal or areal aperture openings, but can be further subdivided according to additional morphological features. A similar approach linking genetic type to morphology was used by Pillet et al. (2013) for the additional genetic types S18–S22 and those from Patagonia and Canada. These genetic types were absent in our Northeast Atlantic dataset (Table 2). Morphological features of each clade include some of the following:

Clade A: Well-defined sutural bridges, small test pores, often with numerous and narrow chambers, periphery often acute and sometimes keeled (including S18: Pillet et al. (2013), Pl. 3, Figs. I-L and Patagonia: Pillet et al. (2013), Pl. 3, Figs. A–D).
Clade B: Small test pores, rounded to sub-acute periphery, depressed sutures with septal bridges absent or very few (including S22: Pillet et al. (2013), Pl. 1, Figs. Q–S). However, S22 differs morphologically from S14 and S6 by having a double row of septal pores along its sutures.
Clades C, *D*, *E* and *G*: Distinct umbilical papillae, often extending into the sutures, small test pores, rounded periphery (including S19: Pillet et al. (2013), Pl. 2, Figs. Q–R; S20: Pillet et al. (2013), Pl. 2, Figs. M–P; S21: Pillet et al. (2013), Pl. 2, Figs. I–L and Canada: Pillet et al. (2013), Pl. 1, Figs. E–H).
Clade F: Rounded, often lobate periphery, wide and coarsely perforate chambers, sutures with irregular septal bridges.

3.3. Morphological profiles of genetic types

In order to aid the future practical application of the results of this study, we have sought to build a morphologically stable profile description of each genetic type. The following morphological diagnoses of genetic types S1–S17 are based on the full SEM dataset of specimens genotyped in the study (n = 1013, Table 3). However, we accept that for the genetic types where morphological evidence is limited (<5 specimens), the morphological descriptions may require revision when further specimens become available. Representative images of genetic types S1–S17 are shown in Fig. 3. For SEM illustrations which relate to genetic types S18–S22, Patagonia and Canada, see Pillet et al., 2013.

Genetic type S1 (n = 383). Test inflated with rounded periphery, very small densely scattered test pores, and generally between 8 and 12 chambers in the final whorl. Sutures are only slightly backwards curving, generally flush with the surface and with regular, well-defined, relatively long sutural bridges. The test is smooth and only the septal pits are covered with papillae. The umbilical region is small or totally absent.
Genetic type S2 (n = 22). Test relatively small, compressed with rounded periphery and very small densely scattered test pores. Generally, between 9 and 11 chambers in the final whorl, sutures backwards curving and with regular, well-defined sutural bridges. The test is smooth and only the septal pits and apertural area are covered with papillae. A flat and smooth central plug is often present in the umbilical region, but sometimes it is not well-developed or even absent.
Genetic type S3 (n = 51). Test relatively small, inflated with rounded periphery and very coarse test pores. Generally between 9 and 11,

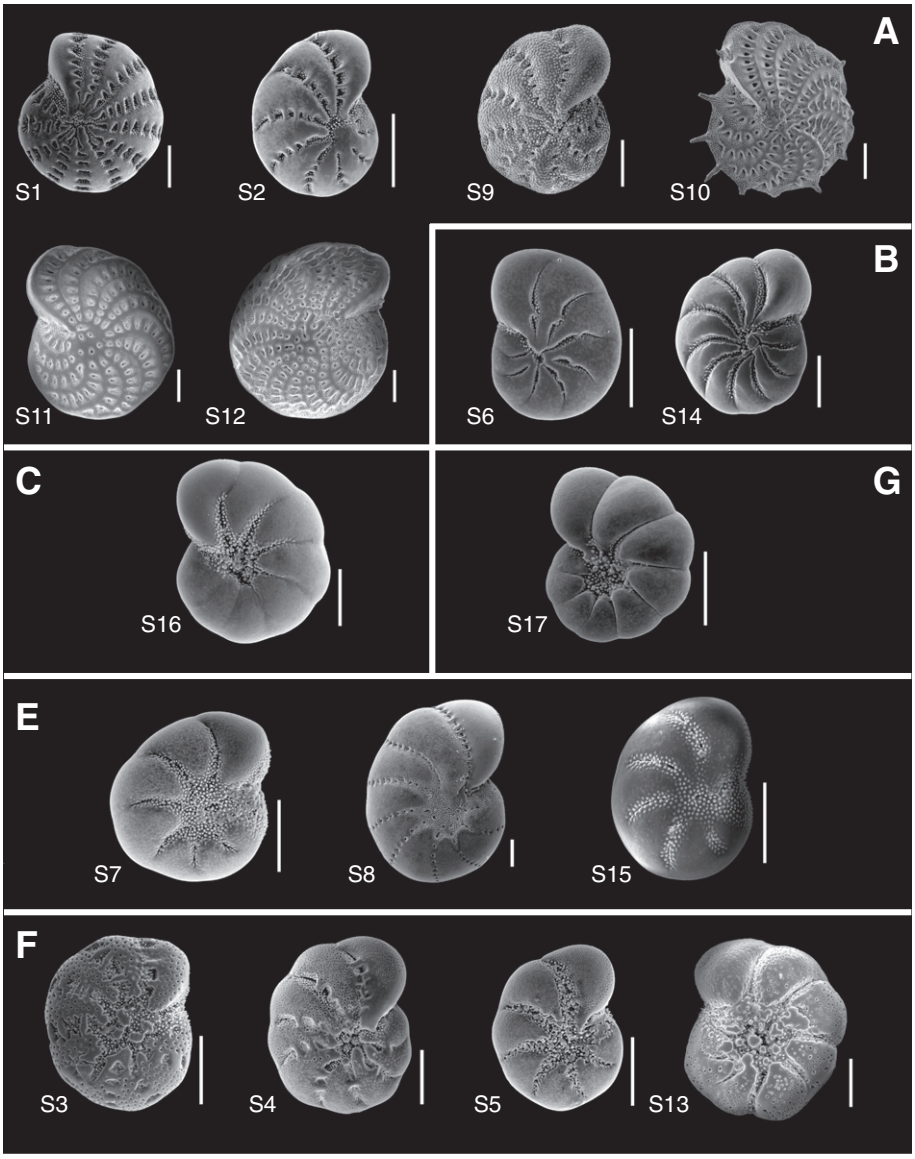

Fig. 3. SEM image plate showing representative specimens typical of each elphidiid genetic type. The genetic types are grouped according to the clade subdivisions shown in Fig. 2.

often indistinct, chambers in the final whorl. It has long and irregular sutural bridges, and the sutures are widely open towards a large umbilical area, which is covered by irregular bosses and papillae.

Genetic type S4 (n = 107). Test inflated with rounded, moderately lobulate periphery, 7–10 chambers in the final whorl, and with relatively coarse scattered test pores. Sutures depressed, backwards curving and with a few (usually 2–7), short sutural bridges. The sutures are usually closed or constricted before reaching the umbilical area. A clear central knob is often present in the umbilical region, but it may be only partly developed or even absent.

Genetic type S5 (n = 43). Test inflated with rounded moderately lobate periphery, 7–10 chambers in the final whorl, and with relatively coarse and densely scattered test pores. Sutures depressed, backwards curving and with a few (usually 2–7), short and often poorly developed sutural bridges. The sutures are usually broad and widely open towards the umbilical region, which is covered by irregular papillae and often also with a few clear umbilical knobs.

Genetic type S6 (n = 4). Test with rounded relatively smooth periphery, 9–10 chambers in the final whorl, and with very small and densely scattered test pores. Sutures only slightly depressed, backwards curving and with very few (usually 1–3), short and often poorly developed sutural bridges, which leave distinct longitudinal depressed slits along the sutures. The sutures typically merge towards a very small umbilical region.

Genetic type S7 (n = 18). Test relatively small with rounded, only slightly lobate periphery, 6–9 chambers in the final whorl, and with relatively small and densely scattered test pores. Distinct broad backwards curving sutures, without sutural bridges. The sutures are tapering towards the periphery but are widely open towards a large umbilical region. The sutures, the apertural face, and the umbilical region are covered by a large number of papillae, giving a star-like appearance. The papillae are sometimes fused into a few central knobs in the umbilical region. The initial 1–2 chambers of the final winding are also covered by papillae.

Genetic type S8 (n = 10). Test with rounded, only slightly lobate periphery, 8–11 chambers in the final whorl, and with very small and densely scattered test pores. Narrow backwards curving sutures with a number of short, regular sutural bridges, leaving distinct round pores along the sutures, continuing across the periphery. The central umbilical region is covered by papillae, which also cover the innermost part of the sutures, as well as the entire apertural face and the initial 1–2 chambers of the final whorl.

Genetic type S9 (n = 33). Test with acute to keeled, only slightly lobate periphery, 8–10 relatively narrow chambers in the final whorl. The entire test is covered by coarse, short papillae, which obscure the test pores. Broad backwards curving sutures with long, sometimes irregular and indistinct sutural bridges, also covered by knobs. In some specimens, more or less irregular, thickened radial ridges without knobs are developed along the sutures.

Genetic type S10 (n = 6). Test with acute to keeled periphery and numerous narrow chambers (12–17). Exhibits a few more or less distinct radial spines along the periphery, mostly along the initial part of the final whorl. Very long, well-defined sutural bridges cover most of the test, so that the chambers appear as narrow and smooth elevated ridges. The sutural pores and part of the chambers are covered by papillae.

Genetic type S11 (n = 3). Test with acute to keeled, smooth periphery and numerous narrow chambers (around 14). Very long, well-defined sutural bridges cover most of the test, leaving the chambers as narrow and smooth elevated ridges. Only the septal pits are covered by papillae. A distinct, smooth and elevated, relatively large umbilical region is typically penetrated by distinct rounded or irregular holes with papillae on the inner side.

Genetic type S12 (n = 3). Test with acute to keeled, smooth periphery and numerous narrow chambers (18–20). Very long, well-defined sutural bridges cover most of the test, leaving most of the chambers as narrow and smooth elevated ridges. The septal pits, and sometimes part of the chambers, are covered by papillae. The relatively large umbilical region is covered by irregular ridges and knobs, surrounded by papillae.

Genetic type S13 (n = 2). Test inflated with rounded, rather lobate periphery, 8–9 chambers in the final whorl and with very coarse test pores. Sutures depressed, backwards curving, usually without sutural bridges but sometimes with a single poorly developed bridge. The sutures taper towards the periphery, but open widely towards a large umbilical region, which is covered by a large number of irregular papillae and often several central knobs.

Genetic type S14 (n = 15). Test inflated, slightly lobate with rounded to sub-acute periphery, 8–10 chambers in the final whorl with very small, densely scattered test pores. The relatively broad distinct sutures are deeply depressed, backwards curving and tapering towards the periphery. There are usually no sutural bridges, but sometimes a single poorly developed bridge is present. Towards the umbilical area, the sutures are often restricted to a narrow passage, occasionally even closed and terminate in a relatively small umbilical area. Both the sutures and the umbilical area are covered by relatively coarse papillae, and a single more or less well-developed, often irregular umbilical knob occurs in some specimens.

Genetic type S15 (n = 1). Test with rounded, only slightly lobate periphery, 6 chambers in the final whorl, and with relatively small test pores. Distinct broad backwards curving sutures, generally without sutural bridges, are covered by a large number of papillae, which stop abruptly before reaching the periphery. The sutures continue, with similar width, into a relatively small umbilical area covered by papillae. The apertural face and the initial 1–2 chambers of the final whorl are also covered by papillae.

Genetic type S16 (n = 308). Test relatively small with rounded, slightly lobate periphery, around 8–10 chambers in the final whorl and with very small, densely scattered test pores. Sutures only slightly depressed, backwards curving and without sutural bridges. A relatively small umbilical area is covered by irregular papillae, which continue into the innermost part of the sutures, tapering about half way to the periphery. Only the basal part of the apertural face is covered by a narrow rim of papillae.

Genetic type S17 (n = 4). Test relatively small, compressed with rounded, rather lobate periphery, around 8–10 chambers in the final whorl and with very small, densely scattered test pores. Sutures depressed, backwards curving and without sutural bridges. A distinct rather large depressed umbilical area is covered by irregular papillae, which also continue as very narrow bands into the innermost part of the deeply curved sutures.

3.4. The biogeography of elphidiid genetic types in the Northeast Atlantic

A description of the biogeographical distribution of each genetic type identified in this study, presented in Table 4, is accompanied by an individual genetic type distribution map (Figs. 4A–Q). The biogeographic provinces and subprovinces are based on the OSPAR Maritime Area classification of the benthic, nerito-pelagic and ice-cover biomes of the shelf and upper continental slope (Fig. 1, see Methods). Distribution maps include sampling sites where genetic types were genetically characterised in this study. In addition, it includes the sampling sites with genetic types deposited by others in GenBank (see Table S2 for details). Once the morphological profile of each genetic type was established (see above), it was possible to assign genetic type identity to the specimens for which genotyping had failed, but for which SEM images existed. A total of 376 of these SEM images were morphologically characterised, and the individual numbers for each associated genetic type are shown in Table 3 and included in Figs. 4A–Q. In addition, to gain further information about the biogeography of elphidiids, the same strict morphological profiles were used to screen the published literature on the distribution of the Elphidiidae in the Northeast Atlantic. We used only those publications which specified a collection locality and also included high-quality SEM or light microscope images. Results of our screening for these morphotypes in published literature are listed in Supplementary Table S3, including reference to the published illustrations and the collection site for each of these specimens.

4. Discussion

4.1. Genetic characterisation and molecular phylogeny

Elphidiid genetic types were characterised by direct comparison of SSU rDNA sequences within the 1210 nucleotide site alignment. Only half of the sites could be unambiguously aligned for use in the phylogenetic analyses (Fig. 2), demonstrating the high levels of variation that exists between the different elphidiid genetic types. Variation can occur within the variable units of a single genetic type or even between the cloned sequences within an individual specimen (intra-individual variation). Such sequence variation was found within the majority of the elphidiid genetic types. Individual genetic type boundaries can be recognised even when the sequence variation only occurs within the variable regions. Although very few of these sites would be available for phylogenetic analysis in a conservative alignment such as in this study, the variation is characterised by a set of fixed units typical for each variable region and which are unique to the genetic type (e.g., Supplementary Fig. S5). The cross commonality of units within the clones of all individuals therefore defines the genetic type. In foraminifera, intra-

Table 4
List of genetic types and combined number of specimens genetically and morphologically identified within the study area together with a description of their biogeographical range as shown in maps Figs. 4A–Q, based on the OSPAR Maritime Areas (Dinter, 2001).

Genetic type	Number of specimens	Map	Phylogeographic distribution
S1	461	Fig. 4A	Widespread throughout NW Europe and is reported as extending from the White Sea subprovince to the Warm Lusitanean subprovince-South, with the Gulf of Cádiz as the southern-most confirmed record. There are no reported occurrences of this genotype in the Barents Sea or High Arctic-Greenland provinces
S2	26	Fig. 4B	Distribution extends from the South Iceland–Faeroe Shelf province to the Warm Lusitanian subprovince and into the Mediterranean Sea. There are no reported occurrences of this genotype in the West Norwegian subprovince or northwards, suggesting a southerly and westerly distribution from the Boreal to Lusitanean provinces
S3	61	Fig. 4C	Geographically restricted to the Boreal and Boreal-Lusitanean provinces, extending into the Warm Lusitanean subprovince in the Bay of Biscay
S4	154	Fig. 4D	Extends southwards from the High Arctic Maritime province to the Boreal-Lusitanean province, including known occurrences in the Baltic Sea and the South East Greenland–North Iceland Shelf province
S5	72	Fig. 4E	Distribution is constrained to the Boreal, West Norwegian subprovince in the north to Lusitanean-Boreal province in the south, including additional occurrences in the Baltic Sea
S6	9	Fig. 4F	Rare, restricted to the Boreal and Boreal-Lusitanean provinces, with an additional occurrence in the Baltic Sea
S7	26	Fig. 4G	Distribution extends from the Boreal province to the High Arctic Maritime province and extends into the White Sea subprovince, South Iceland-Faeroe Shelf province and the Baltic Sea
S8	64	Fig. 4H	Characterises the northern provinces, including occurrences in the High Arctic Maritime, Barents Sea and the White Sea subprovince
S9	113	Fig. 4I	Ranges from Lusitanean-Boreal, Boreal-Lusitanean and Boreal provinces and the Skagerrak and West Norwegian subprovinces into the White Sea subprovince, with occurrences in the Mediterranean Sea as well
S10	29	Fig. 4J	Range extends from the Mediterranean Sea, via the Lusitanean-Boreal, Boreal-Lusitanean, Boreal and West Norwegian subprovince
S11	5	Fig. 4K	Southern genotype, extending from the Cool to Warm Lusitanean subprovinces into the Mediterranean Sea
S12	19	Fig. 4L	Range from the Boreal province to the Mediterranean Sea, with an additional occurrence in the Lusitanean-Boreal province
S13	7	Fig. 4M	Rare, extends from the Boreal and Boreal-Lusitanean provinces to the Warm Lusitanian subprovince
S14	27	Fig. 4N	Rare, restricted to the Boreal-Lusitanean province on the west coast of Scotland. Additional, morphologically similar specimens also occur in the Boreal province on the east coast of Scotland and in the Mediterranean
S15	2	Fig. 4O	Rare, occurring only in the High Arctic Maritime province; morphologically characterised specimens also occur in the Boreal province off the Shetland Islands
S16	308	Fig. 4P	Extends from the Cool Lusitanean subprovince, to the Lusitanean-Boreal, Boreal-Lusitanean and Boreal provinces and into the West Norwegian subprovince
S17	4	Fig. 4Q	Rare, extending from the Warm Lusitanean subprovince, via the Lusitanean-Boreal, Boreal-Lusitanean and Boreal provinces, northwards into the West Norwegian subprovince

individual variation is common in various benthic groups (Pillet et al., 2012; Weber and Pawlowski, 2014) and also in a limited number of planktonic groups (Darling and Wade, 2008).

The phylogenetic analysis performed by Pillet et al. (2013) on the complete SSU rRNA gene included more nucleotide sites (1687 versus 601) but fewer genetic types than ours (15 versus 24). Having almost three times more sites to analyse improves the stability of their tree topology, resulting in better statistical support and greater correspondence between their ML and BA trees (Fig. 1 in Pillet et al., 2013). Nevertheless, their trees are largely congruent with our BioNJ analysis based on 601 sites (Fig. 2). An examination of genetic types common to both analyses (Fig. 1 in Pillet et al., 2013, and our Fig. 2) shows that the tree topologies are similar, except for S1 and S10 and for S7 and S21, respectively, which swap positions but remain in the same clades. Therefore, although far fewer sites were analysed and the statistical support was much lower, a very similar topology was obtained with the partial SSU BioNJ analysis (Fig. 2) compared to the complete SSU ML analysis (Pillet et al., 2013). Once the molecular phylogeny of a family or a genus is established with complete SSU rDNA sequences, it is possible to perform phylogenetic analyses based on partial SSU sequences and use the complete gene phylogenetic analysis as a guide to choose the most comparable topology in phylogeny based on partial gene sequences.

4.2. Taxonomic ranks

The clustering of genetic types into seven main clades (A–G) in this study (Fig. 2) is consistent with the elphidiid phylogeny of Pillet et al. (2013): Fig. 1, Clades A–F). The present Clades A–F correspond to those of Pillet et al. (2013). Clade G is newly defined here and comprises only one genetic type, S17, which was not sequenced by Pillet et al. (2013). To examine intermediate taxonomic ranks such as families and genera and grouping genetic types into different clades can be a rather empirical and subjective exercise, due to variable evolution rates and low statistical support (elphidiids: Pillet et al., 2013, this study; cibicidids: Schweizer et al., 2009; uvigerinids: Schweizer et al., 2005). However, phylogenetic analyses clearly demonstrate the morphological heterogeneity of the elphidiid clades and the potential for further morphologically based groupings (Pillet et al., 2013, Voltski et al., 2015; this study). A combination of automated recognition of clades such as the ones tested for planktonic foraminiferal genetic types (André et al., 2014) and other organisms (Barraclough, 2010) and morphometric multivariate analyses (e.g., Roberts et al., 2016) can provide independent evidence for the elucidation of molecular phylogenetic clustering. Currently, the genetic clustering challenges the morphology-based classification of Loeblich and Tappan (1987) and Sen Gupta (2002), who include the genera *Elphidium* and *Haynesina* into two different morphologically-based taxonomic families (Elphidiidae and Nonionidae). This issue of the taxonomic affinity of these two genera as belonging to the family Elphidiidae is discussed in detail by Pillet et al. (2013) and confirms a previous study where Nonionidae were identified as a polyphyletic family (Schweizer et al., 2008). The taxonomic confusion of the generic distinction of *Haynesina* and *Protelphidium* has also been discussed recently by Voltski et al. (2015).

4.3. Linking genetic type morphology to taxonomy

As mentioned in the methods (Section 2.6.), we believe that morphospecies names should not be placed onto molecular phylogenies, unless both the morphology and genetic type have been linked to the formally named holotype (Roberts et al., 2016). The uniqueness of this study however, is that all specimens of each genetic type can be directly

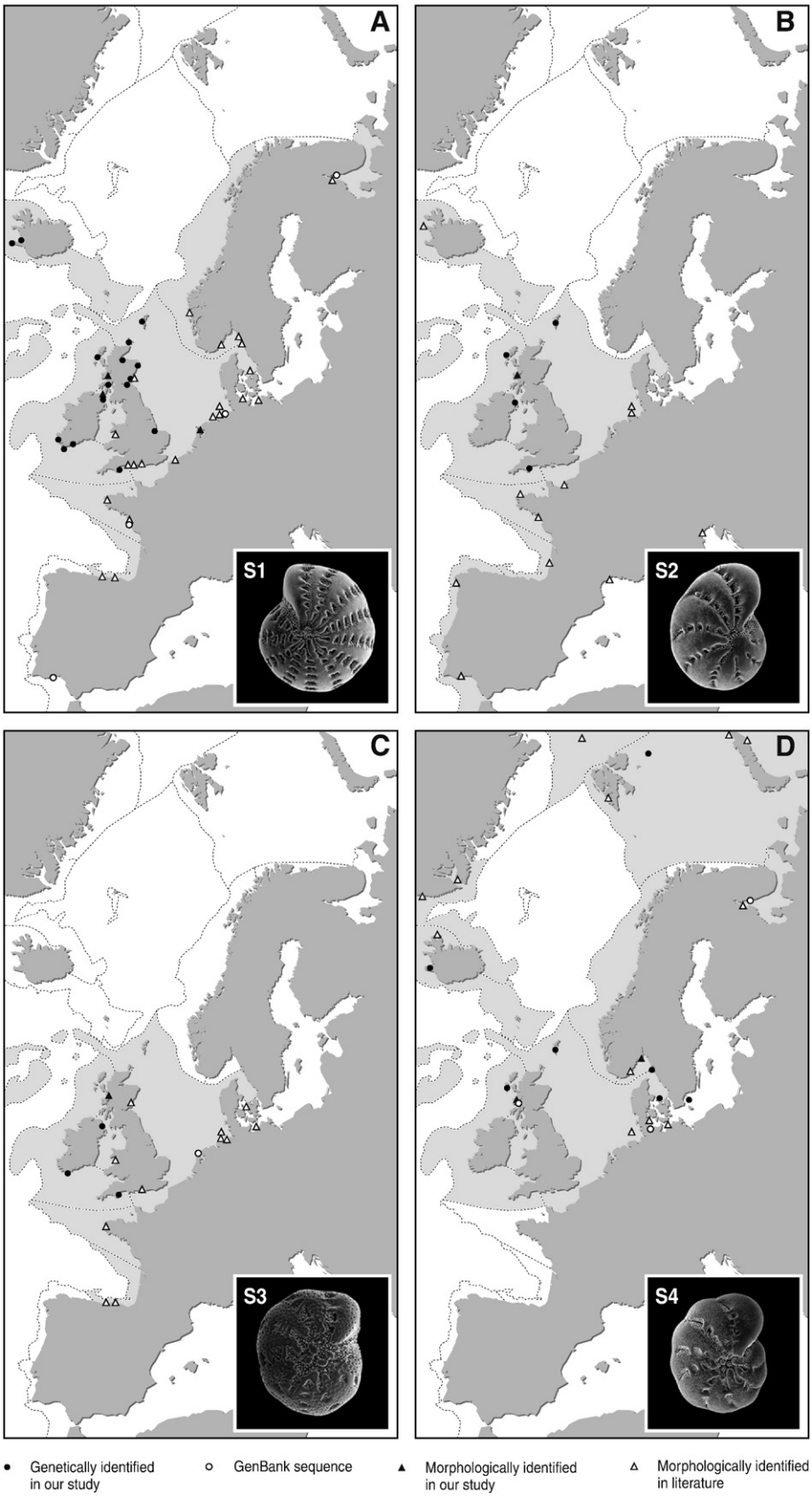

Fig. 4. Biogeographical distribution maps for each of the different genetic types S1-S17 (maps A–Q). (●) Closed circles represent specimens genetically identified in this study; (○) open circles represent sequences already in GenBank. Using strict morphological criteria based on the individual genetic type morphological profiles, (▲) closed triangles represent a genetic type morphologically identified in our study for which DNA amplification failed. The same strict morphological profiles were used to screen the published literature using only those publications which specified a collection locality and also included high-quality SEM or light microscope images (Supplementary Table S3); (△) Open triangles represent a genetic type morphologically identified in the literature. See Table 5 for taxonomic links.

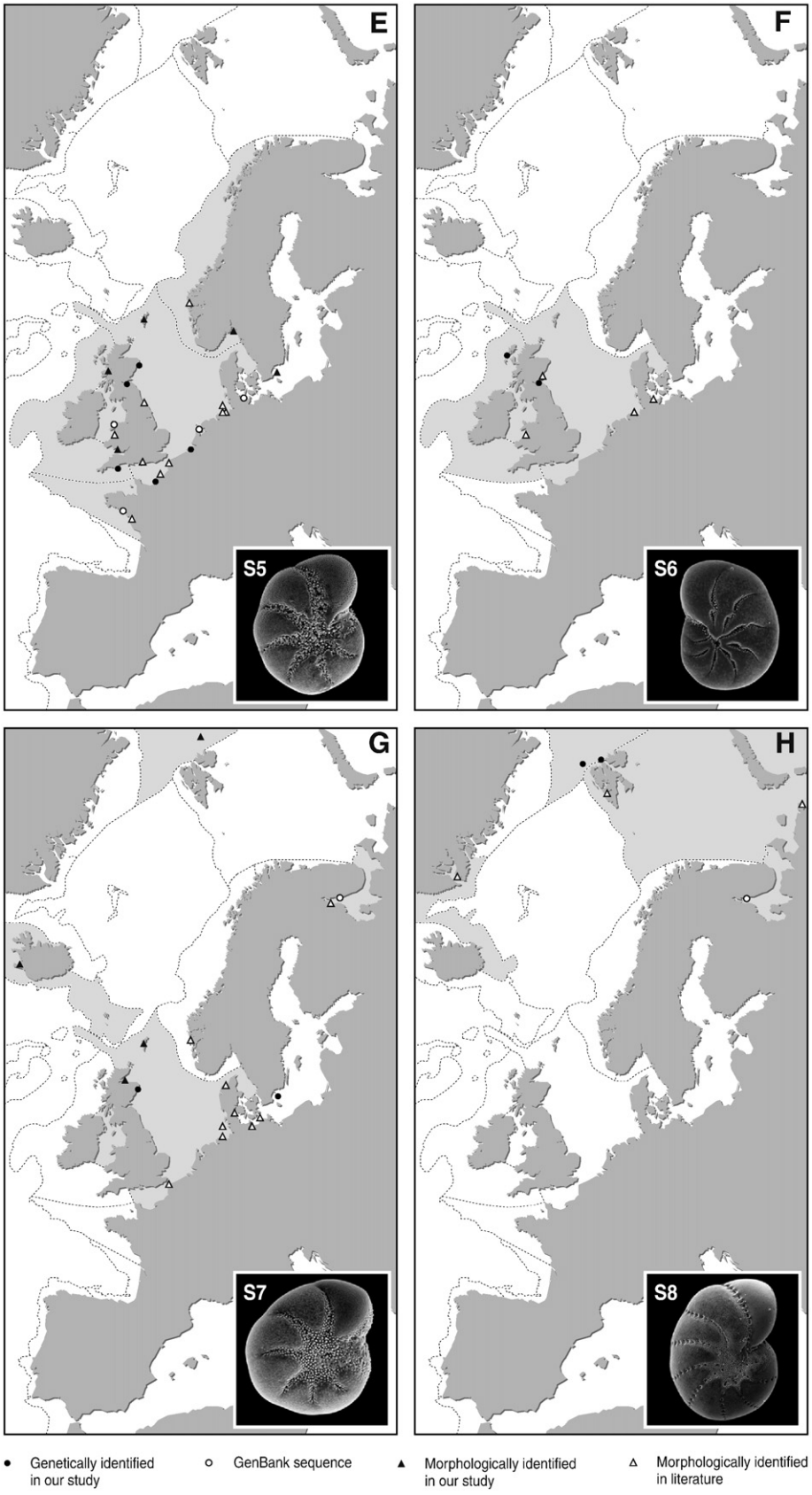

Fig. 4 (*continued*).

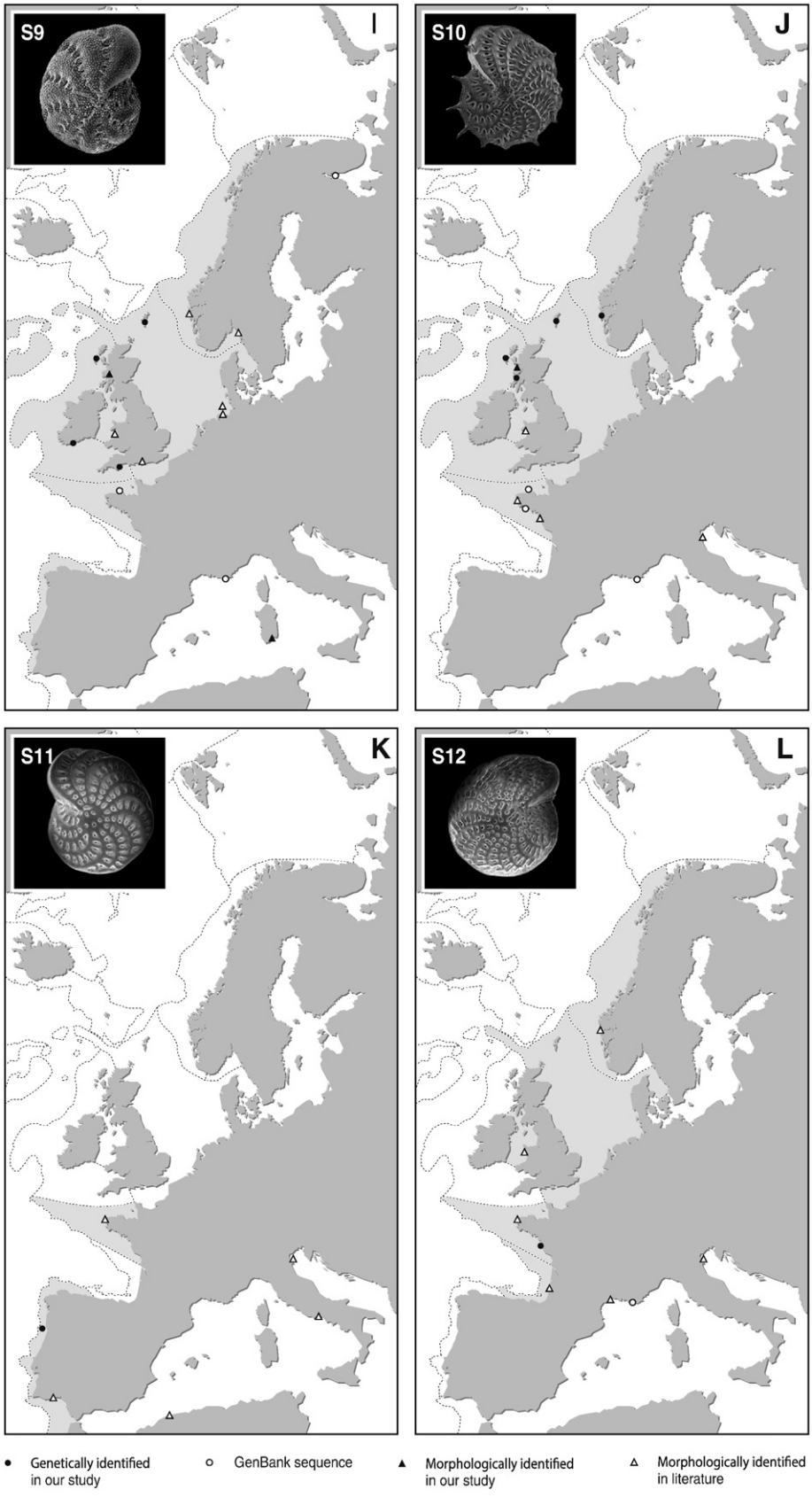

Fig. 4 (*continued*).

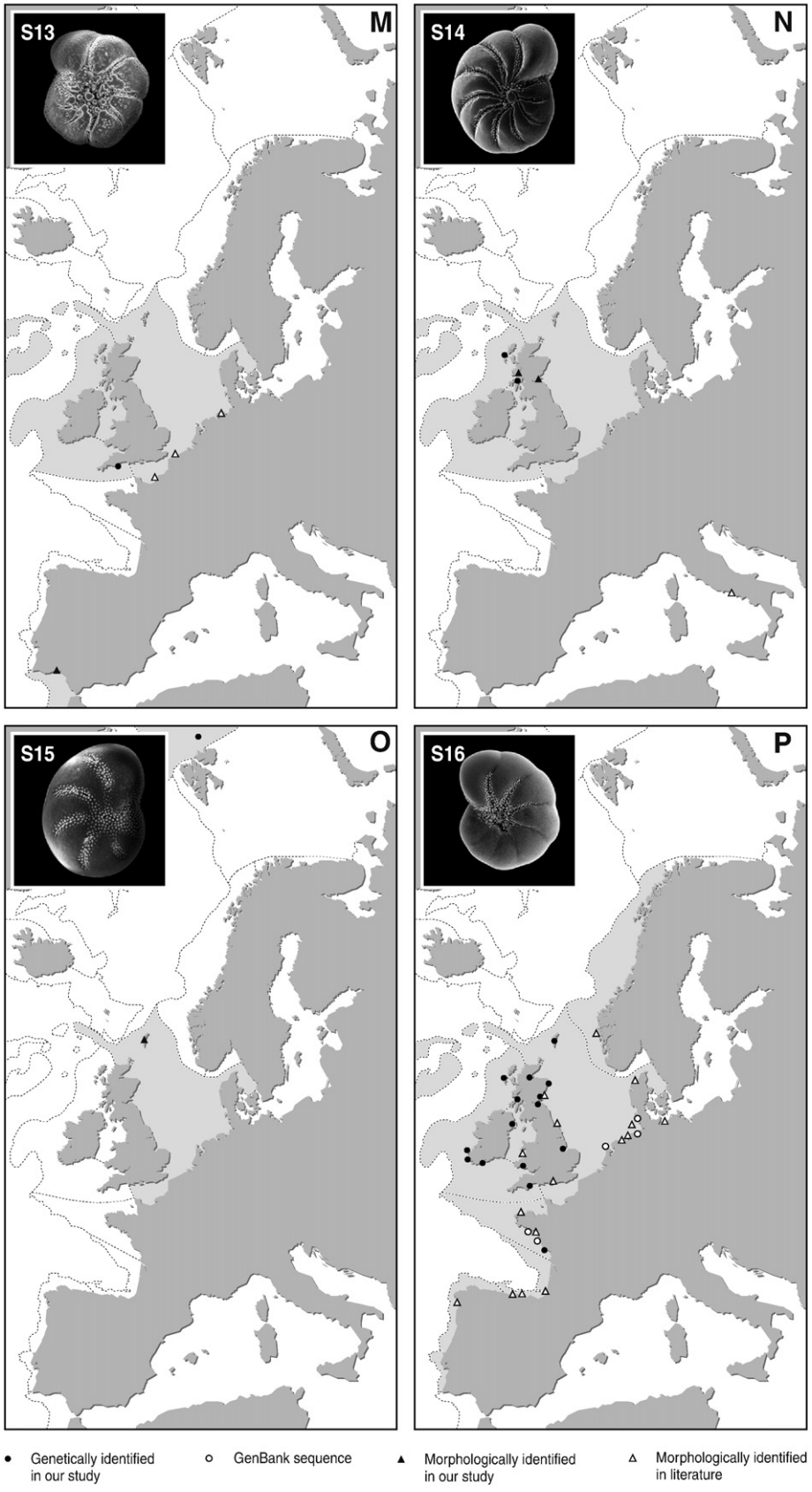

Fig. 4 (*continued*).

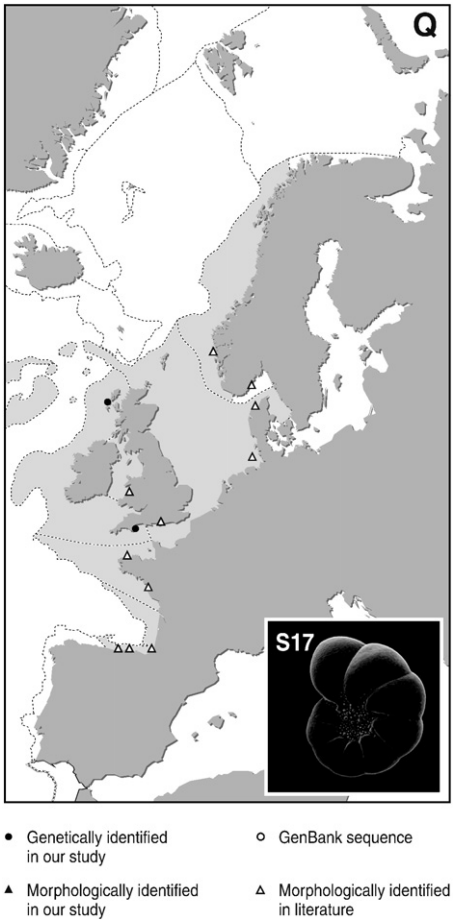

Fig. 4 (*continued*).

linked to a distinct morphological profile (see Results) because of the high resolution SEM image obtained before DNA extraction. Over 1000 individual specimens have been genetically and morphologically linked in this way, making this the first study of the Elphidiidae where morphological profiles have been produced for each individual genetic type. These morphological profiles can also be quantified and potentially used to objectively discriminate individual genetic types (Hayward et al., 2004). Each genetic type was found to represent a distinct morphological profile (results Section 3.3.) and to aid the practical application of an elphidiid taxonomy, we have used the profiles as the basis for taxonomic designations. In Table 5, we list the taxonomic assignment we have applied to each of the 17 genetic types found within the present study. Species assignments based on low specimen numbers (<5) are marked with an asterisk in Table 5 to highlight where the morphological evidence is limited. Our assignments were made based on the original description of each taxon, according to the Catalogue of Foraminifera of Ellis and Messina (1949), supplements up to and including 2009; Supplementary Table S4) with generic names applied according to the concept of Haynes (1981). Seven of these genetic types have been sequenced for the first time and we believe that five of them can be linked to the known taxa *Elphidium gerthi* (S2), *Elphidium incertum* (S6), *Elphidium crispum* (S11), *Elphidium lidoense* (S13) and *Haynesina depressula* (S17). The remaining two genetic types (S14 and S15) have previously unrecognised morphologies which we believe to be currently undescribed. Table 5 also includes a complete cross-reference to the genetic types identified by Pillet et al. (2013), together with a note of their taxonomic assignments. This highlights the problem of linking genetic type morphology to taxonomy, since our assignment of taxonomic names does not always correspond to those assigned by Pillet et al. (2013) to the same genetic type (e.g., S10, Table 5). Where particular genetic types showed a high degree of morphological variation or where taxonomic synonymy (i.e., multiple names for the same morphospecies concept) occurs in the available literature, we provide the following explanations as supplementary to Table 5. There is also a problem about the generic attribution to these different morphospecies, which could differ between traditional morphologically based taxonomies and the clustering within molecular phylogeny (see Discussion 4.2).

A morphometric study by Roberts (PhD thesis, 2016) indicates that there is a minor morphological overlap between the genetic types S1 and S2, which are linked to the morphospecies *E. williamsoni* and *E. gerthi* (Table 5), as well as between genetic types S16 and S17, which are linked to the morphospecies *H. germanica* and *H. depressula*, indicating a pseudo-cryptic problem.

The genetic types S4 and S5, correspond to two taxa traditionally named *Elphidium excavatum* forma *clavata* and *E. excavatum* forma *selseyensis*, which have been interpreted as ecophenotypes, i.e., two forms or phenotypical variations of the same morphospecies *E. excavatum* (cf. Feyling-Hanssen, 1972). The *clavata* (S4) form is generally found in the Arctic while the *selseyensis* (S5) form is generally distributed further south; this led Feyling-Hanssen (1972) to conclude that they were ecophenotypes. However, the present molecular study clearly shows (Fig. 2) that they should be considered as two quite distinct species as previously shown by Schweizer et al. (2011) and Pillet et al. (2013). In these studies, our genetic types S4 and S5 are identified as *E. excavatum clavatum* and *E. excavatum excavatum* or *E. excavatum* respectively (Table 5). While both these nomenclatural concepts are consistent with Feyling-Hanssen's (1972) original ecophenotypes, the

Table 5
List of the applied species names for each of the genetic types S1–17 (this study) and those applied in Pillet et al. (2013; S18–S22, Patagonia and Canada). The original morphospecies description references are listed in Supplementary Table S4. To highlight where the morphological evidence is limited (<5 specimens), an asterisk has been placed against the applied species name.

Genetic type	Species names (this study)	Species names (Pillet et al., 2013)
S1	*Elphidium williamsoni* Haynes, 1973	*Elphidium williamsoni*
S2	*Elphidium gerthi* van Voorthuysen, 1951	Not sequenced by Pillet et al., 2013
S3	*Elphidium oceanense* (d'Orbigny, 1826)	Not sequenced by Pillet et al., 2013
S4	*Elphidium clavatum* Cushman, 1930	*Elphidium excavatum clavata*
S5	*Elphidium selseyense* (Heron-Allen and Earland, 1911)	*Elphidium excavatum*
S6	**Elphidium incertum* (Williamson, 1858)	Not sequenced by Pillet et al., 2013
S7	*Elphidium albiumbilicatum* (Weiss, 1954)	*Cribroelphidium albiumbilicatum*
S8	*Elphidium bartletti* Cushman, 1933	*Elphidium bartletti*
S9	*Elphidium margaritaceum* Cushman, 1930	*Elphidium margaritaceum* 1
S10	*Elphidium aculeatum* Silvestri, 1900	*Elphidium aculeatum-crispum*
S11	**Elphidium crispum* (Linné, 1958)	Not sequenced by Pillet et al., 2013
S12	**Elphidium macellum* (Fichtel and Moll, 1798)	Not sequenced by Pillet et al., 2013
S13	**Elphidium lidoense* Cushman, 1936	Not sequenced by Pillet et al., 2013
S14	Elphidium – new and unnamed	Not sequenced by Pillet et al., 2013
S15	*Elphidium – new and unnamed	Not sequenced by Pillet et al., 2013
S16	*Haynesina germanica* (Ehrenberg, 1840)	*Haynesina germanica*
S17	**Haynesina depressula* (Walker and Jacob, 1798)	Not sequenced by Pillet et al., 2013
(S18)	Not sequenced in this study	*Elphidium margaritaceum* 2
(S19)	Not sequenced in this study	*Elphidium asklundi* Brotzen, 1943
(S20)	Not sequenced in this study	*Haynesina nivea* (Lafrenz, 1963)
(S21)	Not sequenced in this study	*Elphidium frigidum* Cushmen, 1933
(S22)	Not sequenced in this study	*Elphidiella groenlandica* (Cushman, 1933)
Patagonia	Sequenced in this study but outside the study area	*Elphidium macellum* (Fichtel and Moll, 1798)
Canada	Not sequenced in this study	*Haynesina orbiculare* (Brady, 1881)

taxonomic naming of these forms should now be revisited in light of this new molecular evidence and renamed according to the rules of the ICZN (1999). In this case, we recommend that the name *E. clavatum* should be applied to genetic type S4 and that the name *E. selseyense* should be applied to S5, rather than the subspecies names used by Pillet et al. (2013) (Table 5).

Elphidium clavatum was originally described by Cushman (1930) as *E. incertum* var. *clavatum* from Maine on the east coast of America. Loeblich and Tappan (1953) raised this form to specific rank (*E. clavatum*) in an emendation, which is based on a restudy of the holotype, as well as the Cushman collection and the United States National Museum collections. *Elphidium selseyense* was originally described by Heron-Allen and Earland (1911) as *Polystomella striatopunctata* var. *selseyensis* from shore sands in Selsey Bill, UK. It was referred to the genus *Elphidium* by Cushman (1939), who also raised the form to specific rank (*E. selseyense*). The taxonomy of this species is discussed in detail by Haynes (1973) on the basis of Heron-Allen and Earland's 'Students Collection' in the NHM, London (the holotype depository is not given), as well as topotype material from Selsey shore sands. Lutze (1965) and Lévy et al. (1969) regarded *E. selseyense* to be a junior synonym of *E. excavatum*. However, the type specimen of *E. excavatum* Terquem is lost, and the re-description of a topotype by Lévy et al. (1969) states that *E. excavatum* is without granules in the umbilical area, a diagnosis which appears to exclude *E. selseyense* (see further discussion by Haynes, 1973).

In this study, we link genetic type S6 to the taxon *E. incertum* (Williamson, 1858). We also note the close morphological similarity of this form to *E. voorthuyseni*, described by Haake (1962) from the intertidal areas off NW Germany. Our examination of Williamson's original syntypic material of *E. incertum* (Williamson, 1858) in the Natural History Museum, London, has confirmed the presence of the morphology of the S6 genetic type. This same morphology was illustrated and named as *E. incertum* by Haynes (1973). One of Williamson's syntypes is also illustrated by Horton and Edwards (2006: Plate 4 Fig. 18). Our opinion is that *E. voorthuyseni* has the same morphology as both of the images of Haynes (1973) and Horton and Edwards (2006), leading us to the conclusion that the two species names are synonyms. Since *E. incertum* has priority as the senior synonym, we have a priori opted to use that name in this study. However, in the literature, the name *E. incertum* has been used to describe a much wider morphology, which remains an issue to be resolved in future studies and highlights the growing need for well-illustrated images to support taxonomic assignments.

Genetic types S7 and S15 can both be related to the morphotype *E. albiumbilicatum* (Weiss, 1954). However, our study shows that S7 and S15 are genetically highly distinct and should therefore be considered as cryptic species. Unfortunately, the S15 genetic type is rare in our sample set (2 specimens), but these specimens do exhibit morphological features (Fig. 3) that may eventually allow their discrimination as separate morphotypes. Both S7 and S15 have curved sutural depressions filled with papillae. However, in S15 the sutural area is narrower towards the umbilical region than in S7 (Fig. 3). In addition, the papillae in the S7 genetic type form a star-like structure over the umbilical area and taper along the sutures towards the periphery; the sutural papillae in S15 form an even band. If further specimens become available that allow S7 and S15 to be securely discriminated on their morphology, then we suggest that S7 is the most similar to the specimen described and named as *E. albiumbilicatum* by Weiss (1954) and that S15 would require a new taxonomic name and description.

Genetic type S9 is provisionally linked to *Elphidium margaritaceum* in our study and to *E. margaritaceum* 1 by Pillet et al. (2013). A closely related genetic type S18 (GenBank sequence, this study), called *E. margaritaceum* 2 by Pillet et al. (2013), is morphologically very similar to our *E. margaritaceum* (*E. margaritaceum* 1). However, while Pillet et al. (2013) did describe characters to distinguish these two forms, further work on Cushman's type material will be required to determine which of these genetic types should be formally named *E. margaritaceum*. Pillet et al. (2013) suggested that genetic type S9 (*E. margaritaceum* 1) is closer to Cushman's concept and this means that genetic type S18 (*E. margaritaceum* 2) will require a new species name.

Genetic types S10 and S11 are attributed to *E. aculeatum* and *E. crispum*, respectively, in this study with reference to original illustrations of these species (see Supplementary Table S4). Pillet et al. (2013) did not sequence S11 and assigned the S10 genetic type to *E. aculeatum-crispum*, yet our study shows that these two names can be attributed to two distinct genetic types with different morphologies on the basis of the original description. Although the phylogenetic divergence between these two genetic types is relatively small (Fig. 2), the

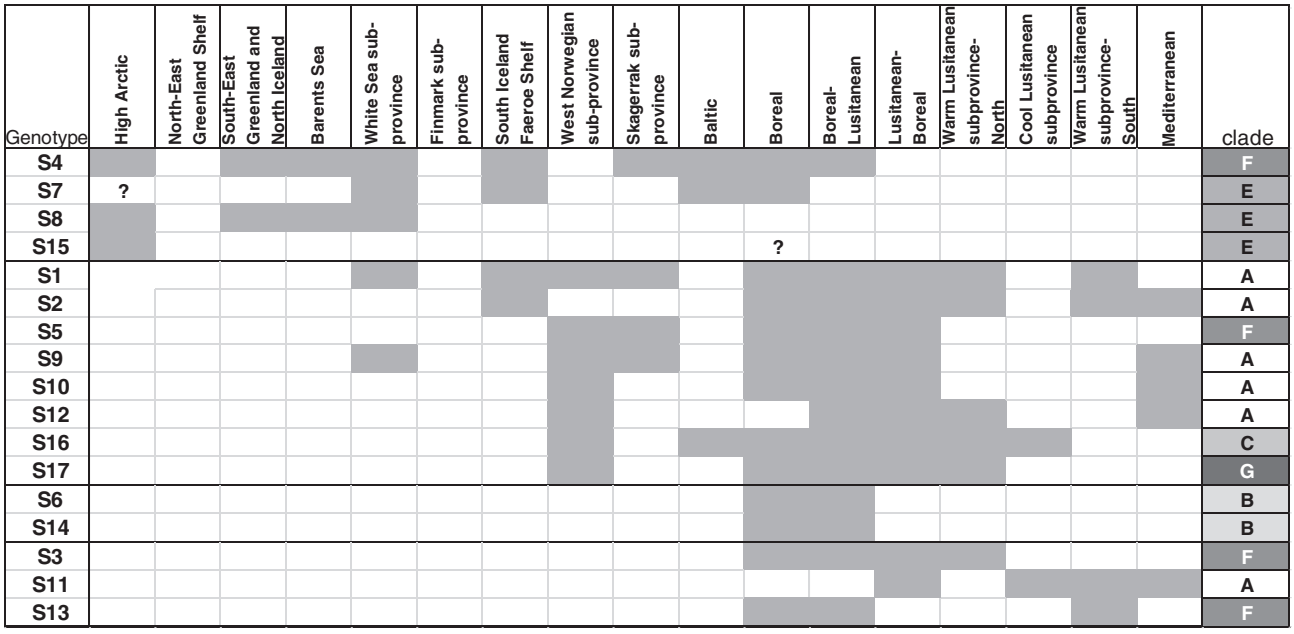

Fig. 5. Latitudinal biome distribution of genetic types. Biogeographic distribution of genetic types within the latitudinal biomes of Dinter (2001). The question marks denote the possible presence/absence of genotypes S7 and S15, highlighting their cryptic nature. The Mediterranean is included to feature the Southern genetic types identified there from the literature.

intra-individual variation shown in Supplementary Fig. S5 highlights their genetic distinction as two separate genetic types. For adult specimens, the spines can be used as a morphological character that separates *E. aculeatum* from *E. macellum*. It should be noted however, that unornamented forms of *E. macellum* may reveal spinose juvenile chambers (Adams, 1963; Haynes, 1973).

The genetic type S12 has been attributed to *E. macellum* in this study with reference to original illustrations of this species (see Supplementary Table S4; Rögl and Hansen, 1984). However, Pillet et al. (2013) did not have representatives of S12 in their phylogenetic analysis and assigned the name *E. macellum* to a highly distinct genetic type found in Patagonia, following the taxonomy traditionally used in that region (Pillet et al., 2012). While morphologically similar, the South American form is a different species, as shown by our phylogenetic analysis (Fig. 2, genetic type "Patagonia").

The genetic type S13 is linked to the species *E. lidoense* in this study (Table 5). It is well known that Northern and Southern taxonomic schools in Europe have, in some cases, adopted different formal names for identical morphologies. This problem was noted by Feyling-Hanssen (1972), when he considered that *E. lidoense* may be synonymous with *Elphidium granosum* (d'Orbigny), a common species in the Mediterranean. A genetic study of this Mediterranean species is needed in order to solve this taxonomic issue.

Genetic type S14 is a potentially new species of *Elphidium*, which to our knowledge has yet to be formally described. A review of the literature from the Northwest European area has revealed a few illustrated specimens which may represent genetic type S14. One of these is an illustration by Sgarella and Moncharmont Zei (1993): Pl. 21, Figs. 8, 9, as *Elphidium* sp. A), which appears to be morphologically identical to genetic type S14. They reported it as an abundant species in the Gulf of Naples (Mediterranean) which is the only modern occurrence we have found in the literature. Other illustrations of fossil occurrences that may represent morphotypes of S14 are published by Poignant et al. (2000): Pl. 1, Fig. 2, as *Haynesina germanica*), in Miocene deposits (Aquitain Basin, France) and by Cearreta et al. (2007: Pl 1, Fig. 11, as *Haynesina depressula*) in Holocene deposits (Melides Lagoon, SW Portugal).

4.4. Regional genetic type biogeography and diversity patterns

The observed occurrences of the genetic types suggest that they tend to exhibit species-specific, rather than clade-specific biogeographies, with the exception of Clade E (Fig. 5). Groups of genetic types show latitudinal preferences, often transitioning in their ranges around the Boreal-Lusitanean provinces. Four of the observed genetic types (S4, S7, S8 and S15) are adapted to live in the High Arctic and Barents Sea provinces. Of these, the members of Clade E (S7, S8, S15), including S21 sequenced by Pillet et al. (2013), appear to be higher latitude specialists, with S8 (Fig. 4H) and the rare genetic type S15 (Fig. 4O) possibly endemic to the High Arctic. However, we note (see Section 4.3) that S15 is cryptic with S7 (Fig. 4G). Therefore, it is possible that the two specimens we have morphologically identified as genetic type S7 in the High Arctic biome (Table 3; Fig. 5) are in reality genetic type S15. However, it must be noted that S7 does occur in the higher latitudes, since it has been genetically identified in the subprovince of the White Sea (Pillet et al., 2012). Equally, we have morphologically identified a single specimen as being genetic type S15 in the Boreal province (Fig. 4O), but this may well be genetic type S7 which has a much wider distribution. This highlights the problems arising when two genetic types are found to be cryptic, which is fortunately a rare event in our study. The remaining elphidiid genetic types exhibit their highest diversity around the Boreal and Boreal-Lusitanean provinces. This Northeast Atlantic "diversity hub" represents a region of biogeographic overlap between (i) two genetic types (S4 and S7) which extend their biogeographic ranges northwards to the High Arctic, (ii) a group of widely distributed genetic types, which extend both to the north and south (S1, S2, S5, S9, S10, S12, S16, S17), (iii) two potential endemics (S6, S14) within the "hub" centre and (iv) a group of genetic types (S3, S11, S13) which are distributed only to the south.

Given that Northeast Atlantic shelf environments were repeatedly glaciated as far south as the present day Boreal-Lusitanian province throughout the late Pleistocene, we know that the current marine fauna of the Arctic continental shelves must have either (i) occupied glacial refugia within the Arctic (e.g., Clarke and Crame, 2010), or (ii) have been seeded from beyond the glacially grounded ice sheet limits

to the south. These Southern glacial ice sheet grounding limits of the Northwest European shelf seas are well known (e.g., Scourse et al., 2009) and occurred within the modern Boreal-Lusitanian provinces. We speculate that the high number of elphidiid genetic types observed today within this Boreal-Lusitanian "diversity hub" represents the combined presence of eurythermal (tolerating a wide range of temperatures) genetic types which have since radiated northwards from the grounding limits of the last glacial maximum (LGM) and warm-water genetic types which have spread northwards from their LGM refugia during the current interglacial period. We consider that these warm-water genetic types are most likely close to their lower temperature limit. On the overall regional geographic scale, our data are consistent with the observation that temperature alone can be used to predict up to 99% of the present-day biogeography of shallow marine benthic faunas (Belanger et al., 2012). However, environmental variables such as salinity, dissolved oxygen concentration and productivity will control more local and seasonal distributions of benthic foraminifera (Murray, 1991; Jorissen et al., 1995).

Palaeontological evidence from the Quaternary deposits of Northwest Europe demonstrate the widespread occurrence of High Arctic

Table 6
List of genetic types S1–17 (this study), their applied morphospecies names and known ecology with ecological references.

Genetic type	Applied species name	General ecology	Ecology references
S1	*Elphidium williamsoni* Haynes, 1973	Shallow intertidal to subtidal species. Tolerant to large variability in temperature and salinity. It is common in Lusitanean and Boreal waters, and it occasionally occurs in the Arctic in restricted shallow pools, which are warmed up during summers. The taxon is particularly common and widespread in the intertidal to subtidal environments.	Haake, 1962; Murray, 1971, 1991; Haynes, 1973; Alve and Murray, 1999; Horton and Edwards, 2006; Korsun et al., 2014
S2	*Elphidium gerthi* van Voorthuysen, 1951	Shallow subtidal to intertidal species, which is distributed in normal marine salinity of Lusitanean and Boreal waters along the western European coasts.	Haake, 1962; Lutze, 1965, 1974; Murray, 1971, 1991; Jennings et al., 2004; Mendes et al., 2012
S3	*Elphidium oceanense* (d'Orbigny, 1826)	Shallow intertidal to subtidal, marginal marine species, which tolerates relatively large variability in temperature and salinity, (brackish to fully marine), and it is often found connected to high organic contents of the sediment. It is distributed in Lusitanean and Boreal waters along the northwest European coasts.	Haake, 1962; Murray, 1971, 1991; Haynes, 1973; Alve and Murray, 1999
S4	*Elphidium clavatum* Cushman, 1930	An opportunistic, very widespread taxon, which has its main distributions in the Arctic. It is particularly frequent in glacier-proximal environments, being tolerant to sediment loaded waters. It is found living down to several hundreds of meters depths in the Arctic. In addition, it is common in restricted environments in Boreal areas, for instance in the Baltic, where it inhabits deeper part of the basins which are often oxygen depleted.	Madsen and Knudsen, 1994; Steinsund, 1994; Wollenburg, 1995; Hald and Korsun, 1997; Alve and Murray, 1999; Polyak et al., 2002; Jennings et al., 2004; Murray, 2006; Korsun et al., 2014
S5	*Elphidium selseyense* (Heron-Allen and Earland, 1911)	An opportunistic, very widespread intertidal to subtidal taxon, which has its main distributions in Boreal and Lusitanean waters. It is tolerant to relatively large variations in temperature and salinity.	Haake, 1962; Richter, 1964; Murray, 1971, 1991; Haynes, 1973; Austin and Sejrup, 1994; Horton and Edwards, 2006
S6	*Elphidium incertum* (Williamson, 1858)	*E. incertum* is an intertidal to subtidal species, found commonly in brackish, inner shelf water areas (salinity >25) of Lusitanean and Boreal waters, where it is particularly frequent just below the halocline in stratified waters. It also occurs in Arctic estuaries.	Lutze, 1974; Murray, 1991; Wollenburg, 1995; Polyak et al., 2002
S7	*Elphidium albiumbilicatum* (Weiss, 1954)	This species has its main distribution in shallow, intertidal to subtidal, low-salinity Boreal and Lusitanean waters, but is also found in the Arctic. It tolerates extremely low salinity, found down to salinities as low as 3.	Lutze, 1965; Wollenburg, 1995; Alve and Murray, 1999; Murray, 2006; Korsun et al., 2014
S8	*Elphidium bartletti* Cushman, 1933	An Arctic shallow-water species, which is common in brackish, river-proximal environments.	Loeblich and Tappan, 1953; Steinsund, 1994; Wollenburg, 1995; Hald and Korsun, 1997; Polyak et al., 2002
S9	*Elphidium margaritaceum* Cushman, 1930	This species occurs in shallow intertidal to subtidal, Boreal to Lusitanean waters. An open marine, relatively stenohaline species, which tolerates only slightly lowered salinity (>25).	Haake, 1962; Haynes, 1973; Alve and Murray, 1999
S10	*Elphidium aculeatum* Silvestri, 1900	A Boreal to Lusitanean shallow-water species, which requires normal marine salinity. Particularly common in the Mediterranean Sea and along the Lusitanean coasts of western Europe.	Haynes, 1973; Albani and Barbero, 1990
S11	*Elphidium crispum* (Linné, 1958)	A Lusitanean shallow-water species, which requires normal marine salinity. Particularly common in the Mediterranean Sea and along the Lusitanean coasts of western Europe.	Rosset-Moulinier, 1972; Albani and Barbero, 1990; Murray, 1991; Sgarella and Moncharmont Zei, 1993
S12	*Elphidium macellum* (Fichtel and Moll, 1798)	A Lusitanean to low-boreal shallow-water species, which requires normal marine salinity. Particularly common in the Mediterranean Sea and along the Lusitanean coasts of western Europe	Haynes, 1973; Pujos, 1976; Albani and Barbero, 1990; Murray, 1991
S13	*Elphidium lidoense* Cushman, 1936	A Lusitanean to low-boreal shallow-water species, which also commonly occurs in the Mediterranean. It requires normal marine salinity in subtidal to upper shelf areas.	Haake, 1962; Lévy et al., 1969; Rosset-Moulinier, 1972; Murray, 1991
S14	*Elphidium* - unnamed		
S15	*Elphidium* - unnamed		
S16	*Haynesina germanica* (Ehrenberg, 1840)	Shallow intertidal to subtidal brackish-water species, which is common in Lusitanean and Boreal waters. Tolerant to relatively large variability in temperature and salinity.	Haynes, 1973; Banner and Culver, 1978; Murray, 1991; Alve and Murray, 1999
S17	*Haynesina depressula* (Walker and Jacob, 1798)	An open marine subtidal species, which is relatively stenohaline but tolerates slightly lowered salinity (>24). It is distributed in Lusitanean and Boreal waters along the Northwest European coasts.	Haynes, 1973; Banner and Culver, 1978; Murray, 1991; Alve and Murray, 1999

faunas at lower latitudes during cold intervals, strongly suggesting that elphidiid biogeographical ranges shifted southwards at these times. For example, the high latitude genetic type S8 is morphologically linked to *Elphidium bartletti*. This morphospecies was found in a late glacial sediment record from the Hebridean shelf, Northwest Scotland (e.g., Austin and Kroon, 1996), showing that its biogeographical distribution shifted southwards during the last glacial period. We cannot, however, discount the Arctic glacial refugium hypothesis using palaeontological evidence and note that elphidiid genetic types such as *Elphidium clavatum* (linked to genetic type S4), are known to extend to relatively deep waters in the Arctic, where they are found living down to 600–700 m depth (Bergsten, 1994).

Such off-shelf refugia in deep waters would allow populations of genetic type S4 to remain in the Arctic throughout the glacial period, leading to allopatric isolation and potential changes in their SSU gene sequences. It is uncertain whether such short term isolation within Arctic refugia would be reflected in the SSU rRNA gene sequences of benthic foraminifera, but molecular evidence for allopatric isolation in planktonic foraminiferal SSU sequences may provide some clues. Populations of the planktonic foraminifera *Neogloboquadrina pachyderma* became isolated within the Benguela upwelling system from those of the southern Ocean in the later Quaternary (Darling et al., 2004). The relict SSU Benguela genetic types are subtly distinct, being defined mainly by differences within the variable regions of their SSU sequences. Since the evolution rates within the *Ammonia* and *Elphidium* lineages are more comparable to those of the planktonic foraminifera than to other benthic groups (Pawlowski et al., 1997), isolation within glacial refugia would most likely lead to similar detectable differences in the SSU sequences of the high Arctic elphidiid S4. However, there is complete sequence identity between all the S4 SSU sequences throughout its range, suggesting that S4 populations are unlikely to have been subjected to recent allopatric isolation.

4.5. Comparative distributions of genetic type and morphospecies

Completing the link between genetic type, morphotype and taxonomic identity allows the distribution and ecology of the elphidiids to be discussed in greater detail. In general, the phylogeographic distribution (Fig. 4A–Q) is in agreement with our knowledge of the ecology and biogeographical distribution of the corresponding morphospecies (Tables 4, 6). However, there are some notable absences and unexpected geographical occurrences. The absences partly arise from our literature search being limited to using only high resolution images and not morphospecies taxonomic lists, since this was the only rigorous way to link the distribution to the morphology of the genetic type. In addition, the geographical distributions described are inevitably subject to taxonomic uncertainty, some of which are mentioned above. We have also encountered problems when trying to relate the taxonomic concepts of the northern to those of the southern European taxonomic schools, due to the lack of availability of carefully illustrated specimens in the literature. Using northern school taxonomic names inevitably means that we will miss a proportion of the southern school morphospecies distribution. To address this problem, we used strict morphological criteria and applied them to high resolution images within the literature where possible, irrespective of the originally applied taxonomic designation.

Furthermore, it was not possible during sampling to consistently collect specimens from the deeper habitats across the whole of the Northeast Atlantic shelf seas, or across the seasonal range. However, we have accumulated an enormous genetic type dataset from the inner shelf and intertidal ecosystems that the elphidiids largely inhabit and we believe that the depth distribution issue in our sampling is counterbalanced by the more representative dataset of the morphologically identified specimens from the literature. We discuss the similarities and differences in distribution of each genetic type with the known distribution and ecology of their corresponding morphospecies (Table 6) below.

The distribution of genetic type S1 shows it to be a widespread Lusitanean and Boreal species which is consistent with the morphospecies distribution of *Elphidium williamsoni* from the literature. The surprising occurrence of the genetic type in the White Sea (Pillet et al., 2013; Fig. 4A), is in agreement with the results of Korsun et al. (2014) who found this morphospecies in shallow Arctic waters. S1 was also identified on the east USA coast (Table S2, Habura et al., 2008), making it a potentially cosmopolitan genetic type.

Both genotyping and literature confirm that S2 (*Elphidium gerthi*) is restricted to the Boreal and Lusitanean provinces. However, in this study it was only encountered on the west coast of the British Isles and not on the east coast (Fig. 4B). This is most likely to be a result of too shallow sampling sites on the east coast, as the species is more common in subtidal rather than in intertidal environments. It is known from the literature that it is distributed throughout the North Sea coastal regions.

The genotyping results of the biogeographical distributions of S3 (*E. oceanense*) correspond to the established knowledge of their occurrences in Boreal and Boreal-Lusitanean waters. This species is widespread in intertidal and subtidal marginal marine areas of the Northwest European coasts (Fig. 4C). However, it was absent in our molecular data from the east coast of Scotland, possibly due to its strong seasonality. Specimens collected for genetic characterisation were sampled during the spring and summer, while *E. oceanense* blooms during September to January on the east coast of Scotland (Austin, 2003).

The literature shows that *Elphidium clavatum*, genetic type S4, is an opportunistic species, known to be mainly restricted to Arctic regions, often dominant in glacier-proximal environments. Surprisingly, the present study shows that this genetic type is also rather common further south in the Boreal-Lusitanian and Boreal provinces, extending into the Baltic Sea (Fig. 4D). This distribution pattern indicates that temperature is not necessarily the only constraint on its distribution and that its opportunistic behaviour may also be an important controlling feature.

Genetic type S5 (*E. selseyense*) has now been shown to be a separate morphospecies from *E. clavatum* and not an ecophenotype of *E. excavatum* (see above). *Elphidium selseyense* clearly has a more southerly distribution than *E. clavatum*, being restricted to Boreal and Lusitanian waters in this study (Fig. 4E). The literature suggests that this taxon is actually distributed even further south, but this cannot be confirmed in this study due to the lack of good quality SEM images.

In this study S6 (*E. incertum*) was found in Lusitanean and Boreal waters (Fig. 4F). However, if the wider morphology attributed to *E. incertum* in the literature is found to be associated with S6, the distribution of this morphospecies ranges as far as the Arctic (Polyak et al., 2002) and thus not endemic to the "hub" (see above).

The genetic type S7 (*E. albiumbilicatum*) was found in Boreal and Arctic waters including the low-salinity Baltic Sea in this study (Fig. 4G). It was not found south of the Boreal province and appears to be absent from the western coast of the UK. This is consistent with the established knowledge of its occurrence, with the exception of one occurrence in Loch Etive, West Scotland, which is the only known record from the west coast of the UK (Murray et al., 2003).

In this study the genetic type S8 (*E. bartletti*) was found endemic to the Arctic (Fig. 4H). This is in accordance with the literature, which shows the modern distribution being restricted to the high-Arctic region.

The genetic type S9 (*E. margaritaceum*) was found in Boreal and Lusitanean waters (Fig. 4I), consistent with the literature which records it as common in intertidal to subtidal areas. Genetic type S9 is linked to *E. margaritaceum* in our study and to *E. margaritaceum* 1 in Pillet et al. (2013; Plate 3, E–H). The genetically close and morphologically similar genetic type S18, which was denoted *E. margaritaceum* 2 by Pillet et al. (2013; Plate 3, I–L) was not recovered in our material. In the palaeoenvironmental literature, these two genetic types would have been grouped together, due to their morphological similarity. Because

their biogeographic distributions appear to be similar (Pillet et al., 2013), such grouping is unlikely to have caused any problems for previous palaeoenvironmental interpretation.

The genetic types S10 (*E. aculeatum*), S11 (*E. crispum*) and S12 (*E. macellum*) are all widespread in the Boreal to Lusitanean provinces, extending into the Mediterranean (Fig. 4J–L), in accordance with the literature, which indicates that they are common in southern regions. However, within these provinces, both our study and the literature confirm that S10 (*E. aculeatum*) and S12 (*E. macellum*) are found as far north as the west Norwegian subprovince, while S11 (*E. crispum*) has a more southern distribution.

The distribution of the genetic type S13 (*E. lidoense*) in Boreal to Lusitanean provinces (Fig. 4M) is in accordance with the literature. If this genetic type turns out to be synonymous with the morphospecies *E. granosum* (see above), its biogeographical distribution would expand to include the Mediterranean.

The distribution of the very rare and unnamed *Elphidium* genetic type S14 is limited in our sample set to the northern UK. In the literature, a very similar unnamed form has been reported by Sgarella and Moncharmont Zei (1993) to be an abundant species in the Gulf of Naples (Mediterranean; Fig. 4N), which is the only modern occurrence we found.

The distribution of the unnamed *Elphidium* genetic type S15 is completely unknown, due to its previous inclusion into the species concept of *E. albiumbilicatum* (S7). We have genetically identified one specimen from the High Arctic Maritime province. However, we have tentatively also morphologically identified S15 in the Boreal province off the Shetland Isles (Fig. 4O). This genetic type is morphologically very similar to S7 (see above) which is common in the Boreal province but also present in the Arctic province, leading to potential taxonomic confusion. The morphologically identified S15 collected in Shetland may therefore in reality belong to genetic type S7. If this is the case, then S15 could be an Arctic endemic. Palaeoenvironmental interpretations may therefore be currently confused as a result of the taxonomic uncertainty surrounding the biogeographical distribution of S7 (*E. albiumbilicatum*) and S15, particularly if S15 is relatively common in the Arctic.

The genetic types S16 (*H. germanica*) and S17 (*H. depressula*) are both widespread in Lusitanean and Boreal waters along the Northwest European coasts as far north as Bergen (Fig. 4P–Q). Their genetic and morphologically identified biogeographical distribution corresponds to the established knowledge of their occurrences from the literature, though S16 is the most common of the two and they are known to have different ecological preferences.

4.6. Morphologically distinct, not-sequenced elphidiids

Although the majority of elphidiid morphospecies have now been genetically characterised in the Northeast Atlantic and Arctic Ocean, several well-known elphidiid morphospecies were missed during sampling in the present study and also in Pillet et al. (2013). The taxonomy of elphidiids is extremely complicated since the literature contains many synonyms and homonyms and it is difficult to assess the number of genetic types remaining to be sequenced. However, we are aware of the following highly distinctive morphospecies: *Elphidium hallandense* Brotzen 1943 (synonym *E. subarcticum* Cushman, 1944), *E. tumidum* Natland, 1938 and *E. oregonense* Cushman and Grant, 1927 which occur in shallow High Arctic waters of the Northeast Atlantic and Arctic Ocean (Murray, 1991; Steinsund, 1994; Polyak et al., 2002). In the North Atlantic, the Arctic morphotype *Elphidiella hannai* (Cushman and Grant, 1927) has been recorded living in shallow waters of the Scoresby Sund Fjord, East Greenland (Madsen and Knudsen, 1994). Also, some important southern morphospecies have eluded sampling such as *Elphidium translucens* Natland, 1938, *E. magellanicum* (Heron-Allen and Earland, 1932), living in shallow Boreal to Lusitanean waters, and *E. advenum* Cushman, 1922, *E. poeyanum* (d'Orbigny, 1826) and *E. granosum* (d'Orbigny, 1839), which are common in shallow Lusitanean and Mediterranean waters (Murray, 1991). Further genetic studies will therefore be needed to comprehensively understand the relationship between morphospecies and genetic types within the Elphidiidae.

5. Summary and conclusions

This study represents the first major biogeographic investigation carried out on North Atlantic benthic foraminifera which combines both genetic characterisation and high resolution imaging of individual tests. Specimens of Elphidiidae were collected from 25 locations across the Northeast Atlantic from the Arctic to the Mediterranean, and 1013 were successfully SEM imaged, genetically characterised and their distribution mapped. Seventeen distinct elphidiid genetic types were identified within the study area, seven being sequenced for the first time. Five further elphidiid genetic types were also identified within the region by Pillet et al. (2013), providing a total of 22 for inclusion in phylogenetic analyses. Genetic types cluster into seven main clades characterised by general morphological characters. Differences between genetic types at the genetic, biogeographic and morphological levels support their species distinction. Their comparative biogeographic distributions show that they predominantly exhibit species-specific rather than clade-specific biogeographies, with the exception of the high latitude specialists in Clade E.

Our results show that high numbers of elphidiid genetic types occur today within a Boreal-Lusitanian "diversity hub", which we suggest represents the combined presence of eurythermal and warm-water genetic types; the latter appear to be close to their lower temperature limit. On a regional geographic scale, our results are consistent with the observation that temperature alone can be used to predict up to 99% of the present-day biogeography of shallow marine benthic faunas (Belanger et al., 2012).

Genetic characterisation of SEM imaged tests was used to question the reality of ecophenotypy and potential cryptic diversity among the Elphidiidae. As already discussed by Pillet et al. (2013), molecular analysis confirms that genotypes S4 and S5, traditionally regarded as ecophenotypes of the same species (*E. excavatum* forma *clavata* and *E. excavatum* forma *selseyensis*), are two quite distinct species. We recommend that the taxonomic species names *E. clavatum* and *E. selseyense* are now applied to these forms. We also recognise the presence of cryptic diversity (e.g. between genetic types S7 and S15); such findings have significant implications for the interpretation of palaeoenvironmental records, as they potentially reduce the precision in faunal/geochemical reconstructions.

Due to taxonomic uncertainty and divergent taxonomic concepts between schools, we believe that morphospecies names should not be placed onto molecular phylogenies, unless both the morphology and genetic type have been linked to the formally named holotype, or equivalent. We advocate a new, three-stage approach to taxonomy for practical application in micropalaeontological studies: These are: (i) genetic characterisation with high resolution imaging of the test, (ii) genetic type delineation by generating a morphotype description produced only from the range of test morphologies associated with the genetic type and (iii) allocation of the most appropriate taxonomic name by linking the genetic type morphotype description to a taxonomic morphospecies description, using only strict morphological criteria.

A taxonomic understanding, supported by genetic studies of benthic foraminifera has proved to be an excellent approach for the documentation of the true diversity and biogeographical distribution patterns for each species. On the whole, we conclude that the existing morphologically-based taxonomy of the elphidiids is relatively robust but will greatly benefit from this type of integrated approach whereby well-illustrated material is linked to a specific genetic type. Where the genetic characterisation of material is not possible or impractical, we strongly urge the inclusion of well-illustrated material to support the taxonomy adopted. We conclude that a new, globally robust taxonomic

framework for benthic foraminifera is now within our grasp and would argue that significant gains in palaeoecological and palaeoclimatic research lie ahead.

Supplementary data to this article, including the *Elphidium* Game, can be found online at http://dx.doi.org/10.1016/j.marmicro.2016.09.001.

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