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

Kate F. Darling [a,b,*], Magali Schweizer [a,1], Karen Luise Knudsen [c], Katharine M. Evans [a], Clare Bird [a], Angela Roberts [b], Helena L. Filipsson [d], Jung-Hyun Kim [e,2], Gudmundur Gudmundsson [f], Christopher M. Wade [g], Martin D.J. Sayer [h], William E.N. Austin [b,i]

[a] School of Geosciences, University of Edinburgh, James Hutton Road, Edinburgh EH9 3FE, UK
[b] School of Geography and Geosciences, University of St Andrews, North Street, St Andrews KY16 9AL, UK
[c] Department of Geoscience, Aarhus University, Høegh-Guldbergs Gade 2, DK-8000 Aarhus C, Denmark
[d] Department of Geology, Lund University, Solvegatan 12, SE-223 62 Lund, Sweden
[e] NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Organic Biogeochemistry, NL-1790 AB Den Burg, The Netherlands
[f] Iceland Institute of Natural History, Urridaholtsstraeti 6-8, IS-210 Gardabaer, Iceland
[g] School of Life Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK
[h] NERC National Facility for Scientific Diving, Scottish Association for Marine Science, Dunbeg, Oban PA37 1QA, UK
[i] Scottish Association for Marine Science, Scottish Marine Institute, Oban PA37 1QA, UK

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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

* Corresponding author at: School of Geosciences, University of Edinburgh, James Hutton Road, Edinburgh EH9 3FE, UK.
E-mail address: kate.darling@ed.ac.uk (K.F. Darling).
[1] Current address: LPG-BIAF, UMR-CNRS 6112, University of Angers, 2 Bd Lavoisier, 49045 Angers Cedex, France.
[2] Current address: Korea Polar Research Institute, 26 Songdomirae-ro, Yeonsu-gu, Incheon 21990, South Korea.

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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