RECONCILING MOLECULES AND MORPHOLOGY IN BENTHIC FORAMINIFERA: A MORPHOMETRIC STUDY OF *AMMONIA* AND ELPHIDIIDAE IN THE NE ATLANTIC

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Reconciling molecules and morphology in benthic foraminifera: A morphometric study of *Ammonia* and Elphidiidae in the NE Atlantic

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This thesis is submitted in partial fulfilment for the degree of PhD

at the

University of St Andrews

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Abstract

A robust and consistent taxonomy underpins the use of fossil material in palaeoenvironmental research and long-term assessments of biodiversity. However, the successful identification of benthic foraminiferal species is often challenged by enigmatic morphological species boundaries, nomenclatural uncertainty and the recent advent of molecular techniques and the identification of cryptic species. This thesis sought to reconcile molecules and morphology in two scientifically important yet taxonomically challenging groups of benthic foraminifera, the genus *Ammonia* and the family Elphidiidae, in samples obtained widely across the NE Atlantic shelf seas. Through the production of detailed quantitative morphometric analysis of over 750 genetically sequenced specimens, coupled with assessments of their biogeographical distributions, this thesis provides the most comprehensive re-evaluation of these taxa conducted to date. The integration of these new lines of taxonomic evidence has refined interspecific boundaries, clarified key diagnostic morphological features and has unveiled the presence of a number of enigmatic species boundaries (particularly within the genus *Ammonia*). The results highlight that classical morphospecies concepts may not always accurately reflect the genetic diversity currently found within this region, which could have important repercussions for applied taxonomic investigations. The extensive sampling across the NE Atlantic has also enabled one of the first re-evaluations of intraspecific morphological variability, revealing that many specimens taken from distinct sampling localities can be morphologically delineated. In a first step to addressing temporal dynamics of previously unrecognised cryptic species, a time series study was conducted in the NW Scottish shelf seas, which unveiled subtle seasonal partitioning between two sympatric species of *Ammonia.* Finally, a new taxonomic framework was developed which bridges the current discontinuity between molecular and morphological lines of evidence. This new framework, applied to *Elphidium williamsoni* (Haynes, 1973), provides the first clear link between morphologically characterised type material and a unique genotype.

For Mum and Dad

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Firstly I would like to thank my supervisors Professors William Austin and Kate Darling for their invaluable advice and guidance during my PhD. They have supported me throughout the course of my research and have shared so much of their knowledge and enthusiasm with me.

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Collaborations statement

This thesis benefits from data provided by several project collaborators. Where additional data has been used, this has clearly been acknowledged at the beginning of each chapter, and also within the main text itself. Contributions from collaborators are also briefly acknowledged below.

This thesis is situated within a larger NERC project entitled "Combined genetic, morphological and ecological approaches to reduce uncertainty in palaeoclimate reconstructions using benthic foraminifers". The PhD project itself was funded through NERC award NE4/G018502/1 and supervised by Professors William Austin and Kate Darling. The sampling of live foraminifera specimens across the NE Atlantic used in Chapters 3, 5 and 6 was a combined effort between this researcher and the projector collaborators. For example, samples were also collected by Professor Kate Darling, Professor Bill Austin, Dr Magali Schweizer, Dr Clare Bird and Dr Kath Evans, the RSPB, the NFSD, Julia Dougherty and Dr Nikki Khanna. A full breakdown of which researchers collected the samples is detailed in the Appendix.

The picking of live foraminiferal specimens from surface sediment samples was a collaborative effort between Professor Kate Darling, Professor Bill Austin, Dr Magali Schweizer, Dr Clare Bird and Dr Kath Evans, Dr David McCarthy and this researcher. The majority of the SEM images utilised within this thesis (Chapters 3, 5 and 6) were provided by project collaborators from the University of Edinburgh including Professor Kate Darling, Dr Magali Schweizer, Dr Clare Bird and Dr Kath Evans.

The unpublished phylogenetic data (including phylogenetic trees) used in Chapters 3, 5 and 6 was provided by research collaborators, Professor Kate Darling, Dr Magali Schweizer, Dr Clare Bird and Dr Kath Evans at University of Edinburgh. The biogeographic maps presented in Chapters 3 and 5 were produced in a combined effort between Professor Kate Darling, Professor Bill Austin, Professor Karen Luise Knudsen, Dr Magali Schweizer, Dr Clare Bird and this researcher (Bird et al., in prep. and Darling et al., in prep.)

The surface sediment samples and bottom water samples used in Chapter 4 from Dunstaffnage were collected by divers from the NERC National Facility for Scientific Diving at SAMS. Temperature data associated with the seasonal assemblage samples (Chapter 4) was provided by Dr Martin Sayer.

Abbreviations

BWT- Bottom water temperatures

CART- Classification and Regression Tree

DFA- Discriminant function analysis

ESEM- Environmental scanning electron microscopy

H'-Shannon Weiner diversity index

ICZN- International Commission on Zoological nomenclature

LSU- Large subunit

K-NN- K- neighbour joining analysis

LGM- Last glacial maximum

NE- North East

NERC- Natural Environment Research Council

NFSD - National Facility for Scientific Diving

NHM- Natural History Museum (London)

nm- Nanometres

PC- Principal coordinate

PCO- Principal coordinate ordination

µm- Micrometres

UPGMA - Unweighted Pair Group Method with Arithmetic Mean

RGB- Red Green Blue colour space

rRNA- Ribsomal RNA

SAMS- Scottish Association for Marine Science

SEM- Scanning electron microscopy

SST- Sea surface temperature

SSU- Small subunit

δ18O- Ratio of stable isotopes 18O:16O

Table of Contents

List of Figures

List of Tables

Chapter 1

Introduction

Chapter 1: Introduction

The impacts of anthropogenic climate change are multifaceted, resulting in changes to the ocean's properties encompassing an increase in sea surface temperatures, ocean acidification, deoxygenation and increased inputs of freshwater supplies (IPCC, 2014). These climatic changes are altering marine ecosystems by affecting biodiversity, community structures, species ranges and biogeochemical cycles (Beaugrand et al., 2015; Deutsch et al., 2015; Gattuso et al., 2015). It is estimated that future environmental change will be similar or even greater than the abruptness and magnitude of climate change experienced in the recent past (Hönisch et al., 2012; IPCC, 2014; Clarkson et al., 2015; Moffitt et al., 2015). A prerequisite of projecting future climate is to understand and compare the magnitudes and timing of past climate change (Lea, 2015). It is of crucial importance to assess the nature of past climate change and how biota respond (composition and biodiversity) to different environmental conditions, particularly during periods of abrupt environmental change (e.g. glacial to interglacial transitions and Dansgaard-Oeschger events). Therefore, assessments of these responses will lead to a better understanding of the natural climatic variability and the long-term response of biota; in turn, this could shed light on the driving mechanisms of environmental change and may enable the recognition of precursor warnings for future climate change (Belanger et al., 2012). However, as quantitative instrumental time series records of climate change are largely temporally constrained after 1800 and are often spatially incomplete, it is necessary to rely on climatic information derived from palaeoenvironmental proxies (Stein, 2008). These palaeoenvironmental proxies can provide environmental records that depict the full spectrum of climatic variability and provide insights into biodiversity changes over time, thus ultimately providing vital information to facilitate the calibration of future climate models (Brannoct et al., 2012).

Foraminifera provide robust palaeoenvironmental proxies, which can be utilised to reconstruct past climates owing to their exceptional fossil record and their sensitivity to environmental perturbations. Foraminifera are a diverse group of marine protists that are abundant and ubiquitous in both planktic and benthic habitats of the oceans. Foraminifera are unicellular eukaryotic organisms that possess a cytoplasmic body, which are most commonly enclosed in a test (shell) of one or more interconnected chambers (Loeblich and Tappan, 1987). The climatic signal is preserved in foraminiferal microfossils by two pathways, firstly in their geochemical test composition and secondly by their faunal responses to different environmental conditions (Murray, 1991). These environmental calibrations are derived from the knowledge of extant

foraminiferal species occurrence and ecological preferences, which can then be used as an analogue to infer palaeoenvironmental conditions (e.g. Schaefer, 2000; Holbourn and Henderson, 2002; Brückner, 2007; Hillaire-Marcel and de Vernal, 2007; Cage and Austin, 2010). Therefore, foraminifera provide unparalleled archives of faunal characteristics and habitat changes over time. In addition, owing to their sensitivity to short-term changes in environmental conditions, foraminifera are also increasingly being used as bioindicators for ecological monitoring (Alve, 1995; Hallock et al., 2003; Nigam et al., 2006; Bouchet et al., 2012).

A prerequisite for the accurate identification of foraminifera in applied taxonomic investigations is a robust taxonomic framework with stable nomenclature. This is crucial, as an underlying assumption within palaeoenvironmental reconstructions is that there is morphological and genetic consistency between extant and fossil species, and that each species has a distinct ecology (Kucera and Darling, 2002). However, over time the taxonomy and systematics of benthic foraminifera has been a subject of controversy. Traditionally foraminifera are classified into taxonomic groups based on their morphological characteristics. However, confusion arises over the high morphological plasticity exhibited by foraminifera, which has led to enigmatic species boundaries and countless emendations. The advent of molecular systematics has aided the clarification of these taxonomic uncertainties, and exposed discrepancies within classical morphospecies concepts. Notably, new molecular evidence has revealed previously unrecognised cryptic diversity, thereby indicating that classical morphospecies concepts may not be as robust as previously identified (e.g. Holzmann 2000; Tsuchiya et al., 2003; Hayward et al., 2004; Darling and Wade, 2008). The recognition of previously unrecognised cryptic species challenges the accuracy of identification of foraminiferal taxa based on morphology. This in turn could lead to a misrepresentation of a species' biogeographic distribution and ecological preferences, which can consequently have significant impacts on applied sciences, particularly affecting the robustness of palaeoenvironmental reconstructions.

Whilst molecular evidence is a powerful tool for species discrimination and has provided much needed insights into species relationships within benthic foraminiferal taxonomy, this approach is not without limitations. Notably, to date it is not possible to routinely extract molecular sequences (aDNA) from individual fossil specimens (Lejzerowicz et al., 2013; Pawlowski et al., 2014). Thus in applied taxonomic situations fossil and extant foraminifera can only be delineated based upon their morphological features. This highlights the continued importance of elucidating interspecific boundaries by their morphological characteristics. Over recent years,

there have been advancements in the synthesis of molecular and morphological data in elucidating taxonomic boundaries (e.g. Hayward et al., 2004). However, these integrated taxonomic approaches are still in their infancy, and are limited to a few species and in their geographic coverage.

The overarching objective of this thesis is to reconcile molecules and morphology in a number of key benthic foraminiferal taxa, placing emphasis on quantifying interspecific and intraspecific morphological boundaries. Ultimately, this investigation presents an opportunity to create a more stable taxonomic framework, which in turn will improve the value of foraminifera in applied taxonomic investigations.

1.1 Foraminiferal biology

1.1.1 Growth and form of foraminifera

> *Form of an organism is determined by the rate of growth in various directions* (Thompson, 1942. p.60)

Foraminifera are typically composed of an organic, agglutinated or calcareous test (Goldstein, 1991). Foraminiferal test growth is accomplished by two pathways. Firstly, test growth can be accomplished by increasing the size of a single chamber (Sen Gupta, 2002). Whilst the most common form of growth in foraminifera is through the accretion and secretion of chambers at regular intervals onto pre-existing chambers, increasing in size each time (Scott 1974; Murray and Alve, 2002). A review of foraminiferal test growth is provided by Goldstein (1999).

The growth and form of the foraminiferal test varies at different stages of ontogenetic development; this change in shape due to size is often termed allometry (Gould, 1977). Primarily the overall shape and size of the foraminiferal test is dependent on the chamber arrangement and growth during ontogeny (Scott, 1974). It is important to note that ontogenetic changes in morphology are often species-specific (Langer and Schmidt-Sins, 2006). For example, a number of species are polymorphic in shape whereby they change from biserial to uniserial forms during different stages of their ontogeny (Hottinger, 2006). Ultimately, test size and form are affected by the life strategies of an organism (Tyszka, 2004). Foraminiferal growth rates are also controlled by the state of their physical environment, e.g. increasing ocean acidification has affected growth rates (Prazeres et al., 2015).

1.1.2 Life cycles and reproduction

Foraminifera have complex reproductive cycles, as most species alternate between asexual and sexual reproduction (Lee et al., 1991; Goldstein, 1999; Stouff et al., 1999; Goldstein, 2002). This alteration of generations is thought to create two distinctive size groups (dimorphism), the asexual generation exhibit a small proloculus (microspheric), whilst the sexual generation exhibits a larger initial chamber (macrospheric) (Lee et al., 1991; Gooday and Alve, 1991). Asexual reproduction is thought be predominant in stable environments (Murray, 1991), whilst sexual reproduction is thought to be advantageous in fluctuating environmental conditions (Hallock, 1985). Reproduction provides a mechanism for mutation and evolution (Jones, 2013).

Current estimations of foraminiferal life spans range from three to four months for smaller foraminifera e.g. *Nonion depressulus,* to three months to two years for larger foraminifera (Hallock, 1985). The life span of foraminifera is largely species specific and the average life span

of foraminifera is estimated to be around a year (Lee et al., 1991). Owing to the difficulties in the direct observation of foraminiferal life cycles in either *in situ* investigations or culturing experiments, the exact triggers of reproduction are currently poorly understood.

1.1.3 Feeding and nutrition

Benthic foraminifera utilise a broad range of feeding strategies, and obtain food from a number of nutritional resources. The staple diet of foraminifera is thought to be algae and diatoms (Murray, 1973), but food sources can also include fungal fragments, yeasts and metazoans (Lipps, 1983; Hohenegger et al., 1989). Foraminifera can exhibit a selective preference for certain food types, as illustrated in experimental studies (Lee et al., 1966; Lee, 1980; Suhr et al., 2003; Ernst and van der Zwaan, 2004; Pillet et al., 2011).

Foraminifera operate at different trophic levels ranging from unselective deposit feeding, suspension feeding, the active selection of food (Lee et al., 1966; Lee, 1980; Lipps, 1983; Goldstein and Corliss, 1994; Goldstein, 1999), carnivory (Bowser et al., 1986, 1992) and parasitism (Cedhagen, 1994). Pseudopodia have strong functional significance for feeding, as they gather in food and subdue prey (Bowser et al., 1992). Often the feeding strategies of benthic foraminifera switch dependent upon the availability of food (Lipps 1983; Cedhagen, 1988), the age of the food organism or the age of the foraminifera (Lee et al., 1966). The seasonal influx of phytodetritus to the seafloor is thought to be an important food source for deeper water taxa (Goldstein, 1999), whilst diatoms and algae are considered to be the primary source of food for marsh dwellers, inter-tidal and shallow-water foraminifera (Hallock et al., 1985; Murray, 2006). The versatility of feeding strategies adopted by foraminifera could explain their ubiquitous occurrence across a range of environments, as their varied test morphologies enable their adaption to different environmental conditions (Haynes, 1981, 2001).

1.1.3.1 Symbionts

Foraminifera were also identified to have symbiotic relationships with algae (as reviewed by Hallock, 2003). These symbiotic relationships are thought to benefit the foraminiferal host by providing a source of energy from photosynthesis, as well as enhancing the calcification of the test (Lopez, 1979; Cedhagen, 1991; Lee and Anderson, 1991; Bernhard and Bowser, 1999; Hallock, 2000; Bernhard, 2003).

1.2 Morphological variation within benthic foraminifera

Foraminifera display great diversity in their test morphology and form. The test reflects the phylogenetic history, the life habits and habitats of a species, population or an individual. Understanding the controls of intraspecific and interspecific morphological variability in benthic foraminifera is crucial to provide insights into evolution, ecology and interpretations of palaeoenvironments. The morphological makeup (phenotype) of foraminifera is the result of both the genetic composition and other non-genetic factors e.g. environment. Morphological similarity between species and higher taxonomic levels (e.g. genera) may be controlled by common ancestry and genetic inheritance (homology) (Gould, 1970). In addition, independent evolution could result in genetically distinct yet morphologically similar tests. This convergent (parallel) evolution occurs when species evolve separately, but converge in function and form owing to the selective pressures of the morphospace. For example, if the taxa are occupying similar ecological niches, there may only be a limited number of biologically viable pathways for adaptation (Gould, 1970; Schulter, 2000; Gould, 2002).

Equally, morphological divergence within a single species could arise from phenotypic plasticity due to other non-genetic factors including reproduction e.g. alteration of generation (Lee et al., 1991), ontogeny (Hottinger, 2006) and different environmental conditions including temperature, salinity, depth and dissolved oxygen content (Boltovskoy and Wright, 1976; Haynes, 1981; Boltovskoy et al., 1991; Murray, 1991, 2006). In the absence of independent genetic information, classical taxonomic investigations have often interpreted the expression of morphological variability within benthic foraminifera to be a product of ecophenotypy (e.g. Schnitker et al., 1974; Rathburn and Corliss, 1994). An ecophenotype can be defined as "A phenotype exhibiting non-genetic adaptation to a given habitat, or an environmental factor" (Haynes, 1992, p.62). The principle of ecophenotypy took root within the foraminifera literature based on the conclusions of an experimental study of *Ammonia tepida* conducted by Schnitker (1974). The study reported significant morphological variability within *A. tepida* in response to different environmental conditions. Based on the findings of Schnitker's (1974) culturing experiment, subsequent studies discarded the idea that a genetic component controls morphological variation, instead the ubiquity of ecophenotypes was asserted (e.g. Poag, 1978; Miller et al., 1982; Walton and Sloan, 1990; Colburn and Baskin, 1998). This provided the impetus for the recognition of broad species concepts, with many morphological variants (Haynes, 1992).

1.2.1 Functional morphology

Functional morphology is the relationship of function and form. Functional traits are driven by different directional selection processes imposed by different environments or through evolution (Hottinger, 2000; Dubicka et al., 2015). Elucidating the functional significance not only provides insights into the controls on intraspecific morphological variability, but functional traits are increasingly utilised to assess the spatial distribution of traits and phylogenetic relationships (Corbelli et al., 2015). Over recent years, ecologists in the broader biological community recognised that a trait-based approach might be more meaningful to understand species responses to the environment, as functional diversity represents the diversity of species niches or functions (McGill et al., 2006; Petchley et al., 2006; Corbelli et al., 2015).

Functional traits of foraminifera are primarily expressed through changes in the size and shape of the test, which is the only permanent part of the foraminifera (Hottinger, 2000). Life positions and feeding habits are reflected by test morphology (Corliss, 1985; Hottinger, 2000). The foraminiferal test is thought to provide shelter, provide protection from predators, to be a receptacle for excreted matter, to assist growth of the cell and to provide buoyancy control (Marszalek et al., 1969; Murray, 1991; Hallock et al., 1991; Hottinger, 2000). Subtle differences in the morphological test characteristics also provide avenues for different functions. For example, the presence of tubercules 'teeth' in the sutures and in the apertural region are functionally significant as they can break up aggregates such as food and detritus (Haynes, 1982; Bernhard and Bowser, 1999; Austin et al., 2005; Khanna et al., 2013). Moreover, the presence of a canal system (commonly found within rotaliids) is thought to replace primary and secondary apertures, as it enables the pseudopodia to be extruded at any point across the test (Röttger et al., 1984). Foraminiferal spines are thought to support pseudopodia, thereby reducing the risk of foraminifera sinking into soft substrates (Murray, 2006). Additionally, pores are thought to provide organic outgrowths and test stabilisation in sedimentary environments (Dubicka et al., 2015). Untangling the form and function of the foraminiferal test is crucial in understanding both their ecology and palaeoecology, and the functional diversity of ecosystems. However, this is currently under-researched and poorly understood.

1.3 Classification and taxonomy of benthic foraminifera

1.3.1 What is a species?

One of the prominent themes which runs through this thesis is what comprises a species and how can one robustly delineate between species. Species are fundamental natural units and their proper circumscription is an essential requirement for our understanding of changes in biodiversity, ecology, biogeography, evolution and mechanisms of speciation (Hilterman, 1956; Coyne and Orr, 2004; Bickford et al., 2007). Despite their importance, the definition of a species has been an area of controversy in science for more than 300 years. Darwin summarised the problem as "No-one definition has yet satisfied all naturalists, but every naturalist knows vaguely what he means by species" (Darwin, 1859, p. 44). To date there are approximately 20-30 definitions of a species (Mayr, 1942; Coyne and Orr, 2004; Zapata and Jiménez, 2012; Edwards and Knowles, 2014). Therefore, prior to any attempt to unravel the taxonomic relationships of benthic foraminifera, the concept of a species needs to be qualified.

Two key philosophical species concepts emerged from the debate over what is a species, the morphological species concept and the biological species concept. A species is recognised by the morphospecies concept when a homogenous group of individuals is considered representative of a population, and that these individuals all have similar morphology (Mayr, 1942). Whilst the morphological variability exhibited within a species may be extensive, it is often gradational in nature and all the specimens are easily distinguishable from other species units (Bickford et al., 2007).

In contrast, the biological species concept is defined by taxa that are naturally interbreeding populations, which have a distinctive range of form, and that are reproductively isolated and occupy a distinct ecological niche (Mayr, 1942, 1954, 1964). Populations are considered distinct species only when they are unable to interbreed (Mallet, 1995). However, as previously discussed, the paucity in knowledge of foraminiferal reproduction and life processes makes it difficult in practice to classify foraminifera using the biological species concept. Moreover, the biological species concept cannot be applied to fossil specimens or populations that asexually reproduce (Mayr and Ashlock, 1991).

Owing to the preeminent utility of foraminifera in micropalaeontology, foraminifera were historically classified using the morphospecies concept as it enabled the study and classification of extinct organisms (fossils). In recent years, the advent of molecular systematics has helped to refine the understanding of the interrelationships between foraminiferal taxa and evolutionary

mechanisms (as discussed in Section 1.6). The development of molecular techniques has brought to the fore the phylogenetic species concept which primarily regards a species to be a unit of natural selection and descent which is monophyletic (belonging to one clade), and that forms a genetically coherent cluster of individual organisms (Hennig, 1966; Mayden, 1997).

However, this new evidence still does not fully resolve the paucity of knowledge surrounding foraminiferal biology. For example, whilst genetic exchange has been identified in foraminifera between Arctic and Antarctic oceans (Darling et al., 2004; Pawlowski et al., 2007), there remains no consensus on whether a genotype represents a breeding population (Tsuchiya et al., 2014).

This thesis advocates the species concept of De Quieroz (2007) which reconciles the numerous species concepts previously proposed, whilst acknowledging that these traditional species concepts share a common element i.e. criteria which can be utilised in synergy to assess species boundaries. De Quieroz (2007) suggests that a unified species concept can be achieved by "treating existence as a separately evolving metapopulation lineage as the only necessary property of species and the former secondary species criteria as different lines of evidence (operational criteria) relevant to assessing lineage separation" (De Quieroz, 2007, p. 879). This unified species concept was adopted as it enables the integration of information provided by different species delimitation methods in empirical applications (De Quieroz, 2007). Furthermore, this unified species concept assigns equal weight to all lines of evidence for species delimitation (such as molecules and morphology) and the more lines of taxonomic evidence (operational criteria) available, the higher degree of corroboration for species delimitation (De Quieroz, 2007). Thus this unified species concept provides a holistic approach to species delimitation, which can be applied to both extinct specimens and asexually reproducing populations.

1.3.2 Classification of foraminifera

Foraminifera exhibit numerous forms with extensive morphological variability. Therefore, it is unsurprising that the compilation of a single, informative classification scheme has eluded foraminiferal taxonomists. Some of the earliest descriptions of foraminifera include those by Herodotus (484-425 BC) and Strabo (63 BC to 20 AD), who noted the accumulation of lens-shaped objects in the limestone blocks of the pyramids (Cifelli, 1990). However, foraminifera were only entered into the scientific literature relatively recently through the publication of Linneaus' *Systema Naturae* in 1758. Foraminifera are primarily delineated using their

morphological characteristics, and were only unequivocally treated as a distinct group from other protists in 1826 by D'Orbingy who distinguished foraminifera by their growth patterns.

Since D'Orbigny's (1826) first classification system, a diverse range of classification schemas and diagnostic features have been used to classify foraminifera across time and space. Some of the first foraminiferal classifications were based on delineating differences in form and chamber structure e.g. single chambered vs multi-chambered foraminifera (Schultze, 1854). In addition, the presence or absence of pores has also been ascribed taxonomic weight by early classification schemes (Reuss, 1861; Carpenter et al., 1862). Historically, differences in the foraminiferal test (wall) composition were deemed to be one of the most diagnostically important morphological features (Williamson, 1858; Pokorny, 1963; Loeblich and Tappan, 1964). Foraminifera can be divided into three main groups based on their test composition: an organic test, an agglutinated test or a calcareous test (Cushman, 1948). Within the calcareous taxa, wall ultrastructure can be used to further delineate taxa into porcellaneous foraminifera (e.g. Miliolids), microgranular foraminfera (e.g. Fusulinina) and hyaline foraminifera (e.g. Rotaliids) (Lee, 1990). To this day, foraminiferal test composition and ultrastructure are some of the primary diagnostic criteria used to delineate foraminifera in applied taxonomy.

Over the course of the 20th century, there was a significant increase in the number compilations of genera with accompanying systematic classification, which separated foraminifera using a range of qualitative test characters. These included the number and mode of chamber addition, crystallography, shape of apertural structures and lamellar structures (Chapman, 1902; Galloway, 1933; Cushman, 1948; Hoftker, 1951; Towe and Cifelli, 1967; Leutenegger and Hansen, 1979; Haynes, 1981; Mikhalevich and Debenay, 2001). For a detailed history of foraminiferal classification see Cifelli (1990).

The seminal work of Loeblich and Tappan (1964, 1987, 1992) which delinated genera into 12 suborders, 74 superfamilies, 296 families and 302 subfamilies has been one of the most commonly used classification schemes in the literature. This scheme utilised the wall composition and the microstructure of the test to distinguish foraminifera into an order with several sub-orders. Subsequent investigations modified Loeblich and Tappan's (1992) classification scheme, retaining the core structural foundation but with the addition of discrete modifications, e.g. the number of orders/classes and subclasses recognised increased to 16 (Sen Gupta, 1999; Mikahalevich, 2004; Kaminski, 2005) as depicted in Table 1.1.

Despite extensive taxonomic investigation over the past 250 years by numerous researchers, uncertainty still surrounds which morphological test traits are relevant at different taxonomic levels, as different classification schemes have identified different diagnostic criteria (Towe & Cifelli, 1967; Cifelli & Richardson, 1990; Haynes, 1990; Sen Gupta, 1999). Additionally, the advancements in the elucidation of higher-level classifications (suprageneric) within foraminifera have been hindered by a paucity of knowledge surrounding their evolutionary relationships (which are difficult to infer from morphology). The advent of molecular systematics has enabled a re-evaluation of the traditional classification schemas; this new evidence has shed light on evolutionary relationships. Pawlowski et al. (2013) proposed that there are two main classes of foraminifera: Globothalamea and Tubothalamea, and a paraphyletic assemblage of Monothalamids based on new molecular evidence (Table 1.1). Pawlowski et al. (2013) identified that basic chamber shapes, the prevailing mode of coiling and the distance between successive apertures, correspond better to the evolutionary traits than traditional characters of wall composition and structure. Whilst the re-evaluation of benthic foraminiferal taxonomic relationships using molecular evidence is still in its infancy, this clarification highlights the importance of reassessing benthic foraminiferal taxonomy in light of this new molecular perspective. However, the taxonomic framework of benthic foraminifera at both suprageneric, genus and species levels will only stabilise with the incorporation of further genetic evidence (Pawlowski et al., 2013).

Loeblich and Tappan (1988)	**Sen Gupta (1999)**	**Mikhalevich (2004)**	**Kaminski (2005)**	**Pawlowski et al. (2013)**
Order	***Class***	***Phylum***	***Class***	***Phylum***
Foraminiferida	Foraminifera	Foraminifera	Foraminifera	Foraminifera
Suborders	***Orders***	***Classes***	***Orders***	***Classes and orders***
Allogromiina	Allogromiida	Astrorhizata	Allogromiida	Monothalamids
Textulariina	Astrorhizida	Lagynana	Astrorhizida	Class *Tubothalamea classis nov*
Fusulinina	Lituolida	Astrorhizana	Lituolida	
Involutinina	Trochamminida	Spirillinata	Loftusiida	Order *Miliolida*
Spirillinina	Textulariida	Ammodiscana	Textulariida	Order *Spirillinida*
Carterinina	Fusulinida	Spirillinana	Fusulinida	Class *Globothalamea classis nov*
Miliolina	Miliolida	Miliolata	Miliolida	
Silicoloculinina	Carterinida	Miliamminana	Silicoloculinida	
Lagenina	Spirillinida	Miliolana	Involutinida	Order *Rotaliida*
Robertinina	Lagenida	Nodosariata	Robertinida	Order *Robertinida*
Globigerinina	Rotaliida	Hormosinana	Favusellida	Order "*Textulariida*"
Rotaliina	Buliminida	Nodosariana	Spirillinida	Order *Carterinida*
	Globigerinida	Rotaliata	Lagenida	*Incertae sedis* orders
	Involutinida	Textulariana	Buliminida	
	Robertinida	Rotaliana	Rotaliida	Order *Lagenida*
	Silicoloculinida	Globigerinana	Globigerinida	Order *Fusulinida*
				Order *Involutinida*

Table 1.1 Recent high-rank classification schemas of foraminifera by Loeblich and Tappan (1988), Sen Gupta (1999), Mikhalevich (2004), Kaminski (2005) and Pawlowski et al. (2013). This table is reproduced with permission from Elsevier from Pawlowski et al. (2013).

1.3.3 Foraminiferal taxonomy and nomenclature

Taxonomic uncertainty is not only confined to suprageneric and genus levels, as unravelling foraminiferal taxonomy at a species level is just as, if not more complex. Foraminiferal species are almost exclusively delineated based on their morphological characteristics. Each morphospecies is appointed a type specimen, which exhibits the key diagnostic morphological characters. This type specimen anchors the meaning of the name and the species concept, providing a standard point of reference. Historically, foraminiferal species were delineated by the application of predefined rules based on morphological characters, which reveal traits and patterns that are common to all individuals. These rules reduce reality into qualifiable and quantifiable traits, using a language of specialised terms to designate species of similar morphologies into the same group (Hottinger, 2000).

However, one of the principal taxonomic problems is that significant morphological plasticity is exhibited by the foraminiferal test. Uncertainty at species level has arisen due to a lack of scientific consensus on the interpretation of this morphological variability, which in turn led to the development of two parallel taxonomic schools. The first taxonomic school (practice) is often referred to as 'lumping', whereby few cosmopolitan species, which possess extensive morphological variability are recognised. The second taxonomic school focuses upon delineating foraminiferal species based on subtle morphological differences, whereby numerous species concepts with restricted biogeographical distributions are recognised. This taxonomic practice is commonly referred to as 'splitting'. The assignment of individual specimens into a particular species is often a compromise; hence, species identification is often down to the personal and provincial bias of the researcher. Notably, the researcher's access to the primary literature/ reference material often dictates which taxonomic practice (school) was employed (Buzas, 1966).

The traditional method of distributing taxonomic knowledge within the academic community has also compounded taxonomic uncertainty. For example, a fundamental limitation in the documentation of foraminiferal taxonomy is that many of the early descriptions of the most common species are often uninformative and the accessibility and quality of many of the type specimens is varied. For example, many of the holotypes have been lost or were never deposited (Fossiner and Hawksworth, 2009). Moreover, many of the original line drawings used to illustrate test morphology are often simplistic and neglect many of the key morphological features (Holbourn and Henderson, 2002). Additionally, the species descriptions and illustrations of foraminifera are dispersed amongst a wide range of publications (Holbourn and

Henderson, 2002). There is also a scarcity of studies which are devoted to quantitatively analysing the interspecific morphological boundaries of foraminifera, e.g. Brooks, (1967), Buzas (1966), Buzas et al. (1985), Debenay et al. (1998), Burgess and Schnitker, (1990) and Gooday et al. (2001). These studies have primarily focused upon quantifying a handful of line measurements (e.g. maximum test diameter) and presence/absence features (e.g. presence of umbilical boss) to assess structural features. Consequently, limited emphasis has been placed upon quantifying the full range of morphological variability exhibited by each species. Recent advancements in imaging technology such as the development of X-ray tomography and transmission electron microscopy (TEM) have presented opportunities to assess and quantify morphological variability within foraminifera (Spejjer et al., 2008; Briguglio et al., 2011; Prazeres et al., 2015). However, many of the recent studies utilising these new imaging techniques have primarily focused on quantification of growth rates (biometry), placing limited emphasis on using these new insights as an additional line of taxonomic evidence to elucidate interspecific boundaries. In contrast, the majority of studies within the foraminiferal literature have focused on delineating taxa based on qualitative morphological traits. However, no standardised terminology or consistent morphological features were analysed; this hinders the degree of comparability of species classifications between studies. Consequently, these factors made it difficult to compare taxa across time and space. Moreover, these classical morphological studies are primarily limited to a single line of taxonomic evidence. Thus, the value of the diagnostic features identified is hampered by the uncertainty surrounding whether the species boundaries identified correspond to genetically distinct species. As species delineation within classical taxonomy is often based on the judgement of an individual observer rather than unambiguous criteria, it is unsurprising that historically the identification of foraminifera at the species level is tentative at best (Murray, 2007).

Owing to the complex history of foraminifera, the determination of a reliable estimate of foraminiferal diversity is challenging. One of the original estimates of the total number of foraminifera recorded by Brady (1884) in the Challenger report estimated that there are 875 species. Whilst other authors have estimated foraminiferal diversity to be between 10,000 to 12,000 species (Boltovskoy and Wright, 1976), 10,000 species (Vickerman, 1992), 8000 species (Minelli, 1993), 6000 extant species (Jones, 1994) and 5,000 species were recognised by Debenay et al. (1996). One of the main challenges associated with compiling an estimate of foraminiferal biodiversity is the extensive synonymy found within the literature. Murray (2007) suggests that the number of living benthic foraminiferal species ranges from ∼3959 to ∼4280

species at 10% synonymy, and from ~3210 to ~3531 species at 25% synonymy. The latest global estimate lists 16,207 taxa, and 11,457 accepted species (combined extant and fossil estimates) in the world foraminiferal database (Hayward et al., 2015). This uncertainty of foraminiferal diversity and high levels of synonymy complicates the transfer of knowledge between researchers. Robust and consistent taxonomic names are required to create and provide the taxonomic placement of a species, without which the empirical data would have no meaning (Valdecasas et al., 2013). Thus nomenclatural instability may hamper the value of foraminifera in applied taxonomic investigations. Consequently, the clarification of species concepts and nomenclature is called for, to attain a more stable taxonomy.

1.4 Foraminiferal biogeography

Foraminiferal biogeography is governed by a combination of evolutionary history, biotic interactions and environmental conditions. An understanding of the environmental and biogeographical controls of extant benthic foraminifera is crucial, as these underpin the interpretation of fossil benthic foraminifera and their derived palaeoenvironments (Murray, 1991). Moreover, an understanding of the biogeographical distribution of extant foraminifera can help to inform our understanding of species range limits and the biotic response of taxa to subtle environmental change (Wiens et al., 2011). This is important because if current rates of anthropogenic climate change remain unabated, this will result in range shifts and alterations of ecosystem structure and function (as identified in other marine organisms e.g. Pörtner et al., 2014; Beaugrand et al., 2013; Burrows et al., 2014).

The processes governing foraminiferal occurrence and distribution include speciation, range expansion and contraction, habitat and mobility (including migration by propagules or gamete transportation) (Alve and Goldstein, 2010; Gooday and Jorrisen, 2012; Murray, 2013). The dominant biotic factors which control foraminiferal distribution include the availability of food (quantity and type), sediment type, temperature, salinity, light intensity, oxygen availability (in water and substrate) and water turbulence (e.g. winds and tides) (Nyholm, 1961; Reiss and Hottinger, 1984; Jorissen, 1995; Wollenburg and Mackensen, 1998; Van der Zwann, 1999; Murray, 2006).

In order to determine the biodiversity and distribution of foraminifera it is essential to have a strong taxonomic foundation from which specimens can be easily and consistently identified. As taxa are primarily identified based upon their morphological characters, classical morphospecies taxonomy has shaped the current understanding of foraminiferal biogeography and ecology.

However, as discussed previously, the taxonomy and nomenclature of benthic foraminifera is complex, owing to uncertain species boundaries, which has resulted in extensive synonymy. For example, Murray (2007) estimated that 10-25% of modern species are actually synonyms. This taxonomic uncertainty has profound implications on our understanding of the occurrence and biodiversity of foraminifera. This uncertainty may have led to an artificial inflation of species distributions, or conversely could provide the impression of higher levels of endemism than actually exist (Gooday and Jorrisen, 2012; Murray, 2013). This could seriously impede species-specific proxies which are reliant on an understanding of the distributional controls and ecological preferences of extant foraminifera (Murray, 2001).

The current understanding of foraminiferal distribution and occurrence has also been hampered by the paucity of knowledge surrounding the temporal and spatial variability of extant foraminifera, as the majority of studies have only analysed foraminiferal occurrence at a specific point in space and time. Buzas (1968, p. 11) notes that deriving an assemblage composition from a single sample is "analogous to observing a single frame of a motion picture". Therefore, unless detailed replicate sampling was conducted both spatially and temporally, it is unclear whether the assemblage identified is in equilibrium with the environment and therefore representative of the area (Buzas, 1968). Additionally, spatial heterogeneity (patchiness) has also been identified at both centimetre to metre scales and could have significant impacts on the assemblage compositions recorded (Boltovskoy and Lena, 1969; Morvan et al., 2006; Lejzerowicz et al., 2014). Therefore, the paucity of knowledge surrounding the spatial and temporal variability of foraminiferal occurrence, as well as a dearth of knowledge of genetic diversity may have impeded the detection of foraminiferal occurrence and biodiversity.

1.5 The use of benthic foraminifera in applied taxonomic investigations

The exceptional preservation potential of foraminiferal tests within marine sediments enables the provision of palaeoenvironmental proxies, high-resolution biostratigraphic dating, as well as informing our understanding of evolution (Murray, 2006). The palaeoecological interpretation of fossil foraminifera depends on a thorough understanding of the ecology, distribution and taphonomic processes operating in the present (Murray, 2013). Using the principle of 'the present is key to the past', one can utilise modern day assemblages from a known environment as an analogue for past climates (e.g. Sejrup et al., 2004; Hillaire-Marcel, 2007; Kemp et al., 2012). These modern assemblages can be used as 'training sets' to determine the abundance of a species in relation to current ambient conditions. The value and quality of these proxies are

derived from an understanding of extant species ecological preferences and distributions (Van der Zwann, 1999).

Palaeoenvironmental data can be extracted using two approaches: Firstly, using a biological approach in which faunal characteristics including foraminiferal abundance, diversity and taxonomic assemblage composition are utilised to infer palaeoenvironments, e.g. covariance of species (Bernhard, 1986; Fontainer et al., 2002). Secondly, a geochemical approach analyses the elemental composition of the test, as a means of constraining physio-chemical parameters of the marine palaeoenvironment. For example, the carbon (δ13C) and oxygen (δ18O) isotopic compositions of foraminifera were found to contain information pertaining to the physicochemical environment (Emiliani, 1955; Shackleton and Opdyke, 1973; Shackleton, 1974, 1977; Imbrie et al., 1984; reviewed in Ravelo and Hillaire-Marcel, 2007). Additionally, foraminifera have been successfully used to determine other environmental properties such as water depth, water mass characteristics, bottom water oxygen content, seasonal variability of organic fluxes to the ocean floor, primary productivity and past sea levels (Imbrie and Kipp, 1971; Horton and Edwards, 2005; Hilliare-Marcel, 2007; James and Austin, 2008; Kemp et al., 2011). Finally foraminiferal abundance in both modern and fossil environments provides the opportunity to assess adaptive radiation and speciation through time (Neige et al., 2013).

A stable taxonomy is a precursor to the consideration of foraminifera in applied taxonomic situations, as these rely upon taxonomic consistency to derive precise assessments of species biogeographic distributions, ecological preferences and geochemical properties. This is crucial, as the majority of proxies derived from foraminifera are species-specific (Sen Gupta, 2002). For example, the foraminiferal test records properties of ambient seawater in its test at the time of calcification (Allison and Austin, 2003). As different species exhibit distinct ecological adaptations and often calcify at different times of the year, this can create differences in the geochemical test composition between species (McCorkle et al., 1990; McCorkle et al., 2008). This indicates why a stable taxonomic framework is required. However as previously discussed, the taxonomic history of foraminifera is complex, owing to the uncertain taxonomic (morphological) boundaries.

In order to attain taxonomic consistency within applied taxonomic situations, there is a tendency to amalgamate different species under broad species concepts. For example, in order to attain taxonomic consistency Sejrup et al. (2004) grouped all species of *Lagena* together under a single species concept and grouped all *Discorbinella* species together. Additionally, Sejrup et al. (2004)

grouped all *Elphidium* variants including morphospecies that were historically thought to occur in warmer environments (i.e. *E.E* forma *selseyensis*), with cold-water *Elphidium* morphospecies, (i.e. *E.E* forma *clavata)* (Feyling-Hanssen, 1974). The use of broad morphospecies concepts could add significant noise/ error into the estimations of past climate. Moreover, an uncertain taxonomic foundation could have profound implications beyond taxonomic investigations. For example, their misidentification can affect the science, such as that included in the IPCC reports, which underpins policy decisions. Therefore, this highlights the importance of resolving some of the taxonomic challenges faced by researchers in applied taxonomic investigations, in order to provide a more stable taxonomic framework.

1.6 Molecular systematics

The advent of molecular approaches in foraminiferal systematics over the past 20 years has provided new insights in elucidating species relationships and resolving taxonomic uncertainty. Phylogenetic analysis is a powerful approach that provides new lines of taxonomic evidence for species identifications and assessments of biodiversity. These new insights are crucial, as historically species relationships were inferred from differences in the test morphology (as discussed in Section 1.3.1). The molecular approach uses genetic divergence to infer species concepts and evolution (Bowser et al., 2006). Molecular studies have also helped to resolve the phylogenetic position of foraminifera in relation to other distant taxonomic groups, e.g. eukaryotic and prokaryotic protists (Pawlowski et al., 1997; Pawlowski and Holzmann 2002; Pawlowski and Burki, 2009). Moreover, these new lines of taxonomic evidence have also revealed the occurrence of foraminifera in freshwater and terrestrial environments (Holzmann et al., 2003; Lejzerowicz et al., 2010).

Ribosomal RNA genes (rRNA) are the most frequently used genes to resolve phylogenetic relationships between and within taxonomic groups (e.g. Pawlowski and Holzmann, 2002; Schweizer et al., 2008). Although a limited number of other proteins including actin (Fahrni et al., 1997; Flakowski et al., 2005), RNA polymerase (RPB1) (Longet et al., 2003; Flakowski et al., 2005, 2006), ubiquitin (Archibald et al., 2003) and tubulin (Tuschiya, 2003; Habura et al., 2005) have been successfully amplified to elucidate taxonomic relationships. Nevertheless, the ribosomal genes are most commonly employed in molecular systematics, as they possess the advantage of being abundant with several hundreds of copies in each cell, thus providing the opportunity to amplify the rRNA from a single foraminiferal specimen (Bowser et al., 2006). Whilst the small subunit (SSU) rRNA is the most commonly used gene within molecular

systematics (Pawlowski et al., 2013), sequences of the large ribosomal subunit (LSU) have also been used to great effect to elucidate the taxonomic positions of a number of benthic foraminiferal species (e.g. Hayward et al., 2004). The SSU rRNA is considered the optimal genetic marker in phylogenetic approaches, as it exhibits more divergence between species than the LSU rRNA (Bowser et al., 2006).

1.6.1 Cryptic diversity

The new lines of taxonomic evidence provided by molecular systematics have brought into question the criteria by which foraminiferal species are classified, as this approach has revealed previously unrecognised genetic diversity within many of the classical morphospecies concepts. Cryptic species can be defined as "Two or more species that have been classified as a single nominal species and are superficially morphologically indistinguishable" (Bickford, 2007, p.149). Thus, cryptic species are thought to be the product of the inability of classic morphology to resolve species divergence at the evolutionary level (Amato et al., 2007). Cryptic species are a common phenomenon within plantkic foraminifera. For example, out of 26 morphospecies concepts of planktic foraminifera, 66 genetically distinct species were identified (Darling et al., 1999; de Vargas, 1999; Darling et al., 2000, Darling et al., 2004, Darling et al., 2007; Pawlowski and Lecroq, 2010; as reviewed in Darling and Wade, 2008). In contrast, cryptic speciation is seemingly less prevalent within benthic foraminifera and to date cryptic species have only been identified within a limited number of taxa. Cryptic species were found within *Ammonia* (Pawlowski et al., 1995; Holzmann et al., 1996; Holzmann and Pawlowski 1997, 2000; Hayward et al., 2004; Pawlowski et al., 2008; Schweizer et al., 2008; Schweizer et al., 2011), *Planoglabratella* (Tsuchiya et al., 2000; 2003), *Chilostomella* (Grimm et al., 2007) and in some monothalous foraminifera (Gooday et al., 2004; Gooday and Pawlowski, 2004; Pawlowski and Holzmann, 2008). The new lines of taxonomic evidence have also revealed the presence of 'reverse' cryptic diversity, whereby there is high morphological plasticity but low genetic diversity (Schweizer et al., 2005; André et al., 2012).

The potential misidentification of species using classical morphology based taxonomy has significant ramifications for the interpretations of past, current and future estimates of biodiversity and the understanding of foraminiferal biogeographical distributions. For example, hidden species richness affects the interpretation of ecosystem properties including its stability, identification of the key (foundation) species and trophic levels (Bickford et al., 2007). In addition, the emergence of previously unrecognised cryptic species, could lead to the redefinition of species ecological ranges and preferences. This can have significant implications

for palaeoenvironmental reconstructions, as these investigations are underpinned by an understanding of the biogeography and ecological preferences of extant species e.g. species-specific geochemical calibrations (Murray, 2006). The clarification of cryptic species with molecular systematics within planktic foraminifera has identified the potential to reduce errors in palaeoenvironmental reconstructions (Malgrem et al., 2001; Kucera and Darling, 2002; Darling and Wade, 2008). This highlights the importance of resolving taxonomic relationships within benthic foraminiferal taxonomy in order to improve and constrain climate models.

Whilst the advent of molecular systematics has helped to resolve taxonomic relationships within foraminiferal taxonomy, it is important to highlight that this approach is not without its own set of limitations. For example, the robustness of genetic delineations is dependent upon access to large numbers of sequences to establish divergence and to contextualise the delineations (Bowser, 2006). A recent plea by Pawlowski and Holzmann (2014) highlighted that the main limiting factor in elucidating taxonomic relationships using a molecular approach, is that there is currently a dearth of molecular data and there is insufficient sampling at both intraspecific and interspecific levels. The robustness of the genetic delineations produced can also be affected by the choice of genetic out-group (Pawlowski et al., 1997) and potential contamination problems (Pawlowski and Holzmann, 2002). Uncertainties can also arise when rRNA of variable lengths is used to construct phylogenetic trees. This variability in the sequence length arises when only a fragment of the rRNA is successfully amplified, which makes aligning the sequences difficult (Pawlowski et al., 1997; Pawlowski and Holzmann, 2002; Foissner and Hawksworth, 2009; Tsuchiya et al., 2009; Groussin et al,. 2011).

Moreover, to date fossil specimens can only be robustly delineated based upon their test morphology. Although there have been significant advancements in recent years with the extraction of aDNA from fossil specimens (Pawlowska et al., 2014), which has provided a new avenue for assessing and understanding genetic diversity. However, this molecular approach is not currently applicable in everyday taxonomic situations and uncertainty remains about how these new lines of taxonomic evidence can be reconciled with classical morphospecies concepts and nomenclature. Hence, morphology remains invaluable for species delineation, though the need to re-examine classical morphospecies concepts and boundaries in light of new taxonomic (molecular) evidence is recognised.

1.7 Integrated taxonomy

The potential pitfalls of both classical morphology-based taxonomy and molecular taxonomy indicate that a single approach may not accurately reflect species boundaries. As a result, the synthesis of multiple lines of taxonomic evidence including molecules, morphology and biogeography is required to test and corroborate species boundaries. Over recent years, integrated taxonomic approaches have been advocated and employed with great effect by the broader biological community, to overcome the potential caveats when constructing species boundaries based on a single line of taxonomic evidence (Lipscomb et al., 2003; Dayrat et al., 2005; De Salle et al., 2005; Will et al., 2005; Padial et al., 2010; Edwards et al., 2014).

The application of integrated taxonomic approaches to foraminiferal systematics is in its infancy. Although the integrated approach is becoming much more common within planktic foraminiferal taxonomy (e.g. Morard et al., 2009; Aurahs et al., 2011; Quillévéré et al., 2013; Weiner et al., 2015), to date it has not been widely adopted within benthic foraminiferal taxonomy. Over the past 20 years, the majority of benthic foraminiferal taxonomic studies have primarily focused upon elucidating the genetic delineations with limited emphasis placed on developing secondary lines of taxonomic evidence such as morphometrics and biogeography. For example, recent investigations such as those conducted by Tsuchiya et al. (2000), Kitazota et al. (2003); Etran et al. (2004), Grimm et al. (2007), Pawlowski et al. (2007), Schweizer et al. (2008), Schweizer et al. (2009), Pillet et al. (2011), Schweizer et al. (2011), Pillet et al. (2012), Pawlowski et al. (2013) and Pillet et al. (2013) provided phylogenetic sequences, yet only characterise morphological variability using classical morphospecies descriptors. Moreover, there is a tendency in many of these aforementioned studies to directly reattach classical taxonomic names without consulting original type material or species descriptions. This practice has not helped to resolve the taxonomic confusion, as these studies reintroduce the error and ambiguity associated with classical taxonomy, as the majority of species names are laden with uncertain taxonomic history.

Despite the considerable advancements in molecular systematics over the past 20 years, the development of quantitative morphological approaches to delineate between species has been limited. To date there are only a handful of studies that have successfully synthesised morphometrics with new lines of molecular evidence to validate interspecific boundaries (Tsuchiya et al., 2003; Hayward et al., 2004; Schweizer et al., 2005; Tsuchiya et al., 2008, 2009; Tsuchiya et al., 2014).

One of the most comprehensive integrated taxonomic studies of benthic foraminifera conducted to date was undertaken by Hayward et al. (2004) whereby a combination of LSU rRNA sequences, 37 external test character assessments (morphometric and categorical) and biogeographical distributions were used to elucidate taxonomic relationships within the genus *Ammonia*. Another recent noteworthy example of an integrated taxonomic approach was conducted by Tsuchiya et al. (2014) on *Planoglabratella opercularis*. Although this study did not provide detailed morphometric assessments, it represents the first investigation to combine molecular evidence, classical morphological descriptions, as well as providing an understanding of their life cycles, growth rates and ecological preferences, to clarify the taxonomic and ecological position of this species.

Whilst these integrated taxonomic approaches represent a significant advancement in elucidating interspecific and intraspecific boundaries, it should be noted that these studies only represent the preliminary stages of re-examining classical benthic foraminiferal taxonomy. Notably only a limited number of taxa, often from geographically restricted areas have been analysed. Additionally, uncertainty remains with regards to how the new lines of taxonomic evidence can be reconciled with classical taxonomic concepts. A striking example of how the new lines of taxonomic evidence remain disconnected from the broader literature is evidenced by the continued use of open nomenclature such as *Ammonia beccarii* and *Ammonia tepida,* despite molecular evidence that has illustrated that the use of these 'bucket' species concepts masks underlying genetic diversity (Hayward et al., 2004). This problem brings to the fore the importance of conducting further integrated taxonomic investigations in order to resolve this taxonomic confusion. In turn, this would strengthen the value of biogeographic and ecological evidence derived from extant foraminiferal species in applied taxonomic investigations.

1.8 Summary of research aims and objectives of the thesis

Underpinning the use of foraminifera in any applied taxonomic situation is the requirement of a robust taxonomic foundation. However, foraminiferal taxonomy is often challenging and interspecific boundaries are enigmatic. The overarching aim of this thesis is to conduct a detailed re-evaluation of the taxonomic boundaries of a number of key benthic foraminiferal taxa and to address some of the uncertainties faced by researchers in applied taxonomic situations.

Principally, this thesis aims to:

1) Address the utility of morphology in delineating between genetically distinct species of benthic foraminifera
2) Further the understanding of the biogeographic range and occurrence of benthic foraminifera
3) Explore patterns of intraspecific morphological variation within benthic foraminifera
4) Investigate whether classical morphospecies concepts and nomenclature can be reconciled with new lines of taxonomic evidence

Ultimately, the primary aim of this thesis is to help to constrain species boundaries, enhance morphological characterisation and identification of foraminifera in applied taxonomic situations. The creation of a stable taxonomic framework would enhance the value of foraminifera in palaeoenvironmental and ecological investigations.

In order to address these aims two key taxonomic groups, the genus *Ammonia* and the Elphidiidae family, were selected for investigation. These were chosen because these taxa are some of the most ubiquitous and abundant species globally (Murray, 1991). Owing in part to their ubiquitous nature and extensive fossil record, species within these two taxonomic groups are important tools for understanding Quaternary climate and sea level cycles (e.g. Haslett, 2002; Murray, 2006). For example, numerous biological and geochemical proxies have been produced using these taxa (Sejrup et al., 2004; Horton and Edwards, 2005; Cage and Austin, 2010).However, these groups also represent two of the most taxonomically challenging and morphologically variable taxa within benthic foraminiferal taxonomy. Whilst recent advancements in molecular systematics have helped to elucidate some of the taxonomic relationships within these two groups (e.g. Hayward et al., 2004; Pillet et al., 2013), there remains a paucity of data on the genetic and morphological variability within these two taxonomic groups. Previous studies have been constrained due to the limited numbers of specimens analysed, or geographically constrained sampling. Given the ecological and palaeoenvironmental significance of these taxa, this necessitates a re-evaluation of morphological limits and a re-examination of their biodiversity and biogeography. The extensive sampling across the NE Atlantic shelf seas employed by this thesis presents an opportunity for a comprehensive assessment of the diversity (morphological and genetic) and biogeographical distributions of Elphidiidae and *Ammonia* across a range of environmental conditions.

This thesis is composed of seven chapters as follows:

Chapter 1 has presented a general introduction to foraminifera, taxonomy, classification, and a range of other topics that are addressed in this thesis. **Chapter 2** introduces and provides an overview of the materials and methods, which were employed throughout this thesis.

Chapter 3 focuses upon the cryptic genus *Ammonia* which has a turbulent taxonomic history, owing in part to uncertain morphological boundaries and the prevalence of the taxonomic practice of 'lumping'. This chapter examines the interspecific morphological relationships of seven distinct genotypes of *Ammonia* to establish if morphology, molecules and biogeography can be reconciled to provide a stable taxonomic framework for the identification of *Ammonia* in the NE Atlantic. This chapter also presents a case study conducted on *Ammonia* genotype S1 which assessed if any intraspecific morphological patterns could be identified across a large geographic spatial scale.

Chapter 4 examines the temporal dynamics of benthic foraminiferal assemblages from the NW Scottish shelf seas. The focus of this chapter is to assess whether any ecological/ seasonal partitioning of *Ammonia* species occurs. This chapter thereby provided an opportunity to assess the applicability of the taxonomic framework of *Ammonia* developed in Chapter 3 in an applied taxonomic situation. Additionally, the time-series study recorded changes in foraminiferal abundance and composition over the course of a year, thereby providing insights into the importance of having an understanding of the temporal variability when estimating biodiversity.

Chapter 5 undertakes the first comprehensive effort to quantify the interspecific morphological boundaries within the Elphidiidae family in light of the new genetic evidence. This chapter comprises two components. The first component examines the interspecific morphological relationships and the biogeographic distributions of 17 genetically distinct genotypes identified within the Elphidiidae group by Darling et al. (in prep). The second component of this chapter employs two case studies to examine intraspecific morphological variability across a large geographic spatial scale.

In response to the taxonomic uncertainty encountered while trying to synthesise new lines of taxonomic evidence with classical taxonomy, **Chapter 6** examines how traditional taxonomic species concepts and nomenclature can be reconciled with quantitative morphological boundaries and genetic sequences. This chapter focuses on the case study of *Elphidium williamsoni* (Haynes, 1972) to assess how these lines of taxonomic evidence can be reconciled

in practice. This chapter concludes by establishing a framework which aims to provide a new protocol to try to bridge the gap between classical taxonomy and new lines of taxonomic evidence.

Finally, **Chapter 7** provides a synthesis of the thesis and conclusions. This chapter focuses on presenting the general conclusions and providing an outlook for future work.

Chapter 2

Materials and methods

Chapter 2: Materials and methods

2.1 Study area

The study area of this thesis focuses upon the North East Atlantic Shelf seas, extending from the high Arctic to the Portuguese margins. This region is spatially complex and consists of diverse coastal and offshore marine landscapes including intertidal areas, fjordic environments and open bays. Bathymetry, ocean circulation and temperature have pronounced effects on the ecosystems and habitats within this region (OSPAR 2000; 2010) (Figure 2.1). The North East Atlantic Shelf seas are an important area of scientific interest, as historically this region has been exposed to both short term and long term environmental change (Scourse and Austin, 2002; Moffa-Sánchez et al., 2014). Additionally, this region is currently undergoing substantial anthropogenic driven climate change (Pörtner et al., 2010; IPCC, 2014). The current rate of sea surface temperature (SST) increase is unprecedented in recorded (instrumental) history. The SST of North Atlantic and UK coastal waters have warmed 0.2–0.6 °C over the past 30 years (MacKenzie and Schiedek, 2007). These temperature and other associated biogeochemical (i.e. ocean acidification) changes have affected this region's ecology. Notably, shifts in the distribution of populations and communities have taken place, providing opportunities for the encroachment of southern warm-water 'invasive' species pole-wards and the retreat of cold-water species into higher latitudes (Edwards et al., 2004; Beaugrand et al., 2013; Burrows et al., 2014).

The circulation of Atlantic waters plays a strong role in controlling the water characteristics of the NE Atlantic shelf seas, which in turn affects the biology and ecology of this region. Notably, the northeastern extension of the Gulf Stream, the North Atlantic Current (NAC) transports relatively warm, nutrient and oxygen rich water from the NW Atlantic to the European shelf seas (Käse and Krauss, 1996; Rossby, 1996; Inall et al., 2009; Roessler et al., 2015) (Figure 2.1). Local variations in temperature and circulation are often controlled by different meteorological conditions (e.g. winds and tides), freshwater discharge and seasonal stratification; these are also important controls on the ecological conditions of an area (Holt and Proctor, 2008; Huthnance et al., 2009).

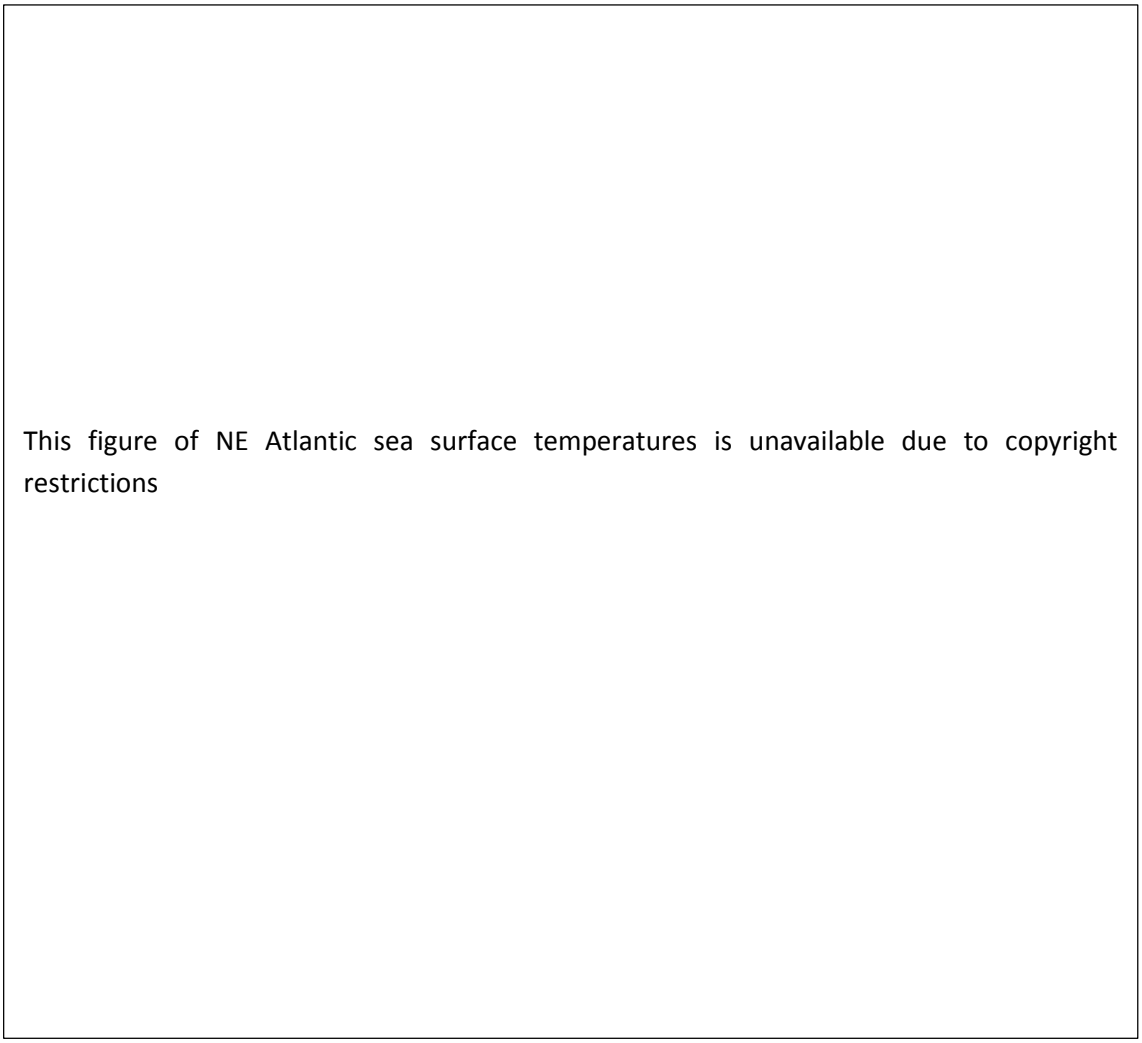


Figure 2.1 Sea surface temperature map for October 2009 (derived from the high resolution Mercator ocean forecasting system for 13 October 2009) and a schematic representation of the ocean circulation patterns within the NE Atlantic. Figure taken from OSPAR (2010).

The complex patterns of biology/ ecology exhibited within the NE Atlantic shelf seas are governed by a combination of oceanographic and meteorological processes at local, regional and global scales. These processes create distinctive biogeographic zones within this region that possess specific oceanography and characteristic biological communities. These distinctive biogeographic provinces were characterised by Dinter et al. (2001) (Figure 2.2). This thesis employs a sampling regime which aims to capture the diversity of benthic foraminifera across as many of these distinct biogeographic zones as possible. Sampling was inevitably opportunistic and for this reason some provinces are more heavily sampled than others.

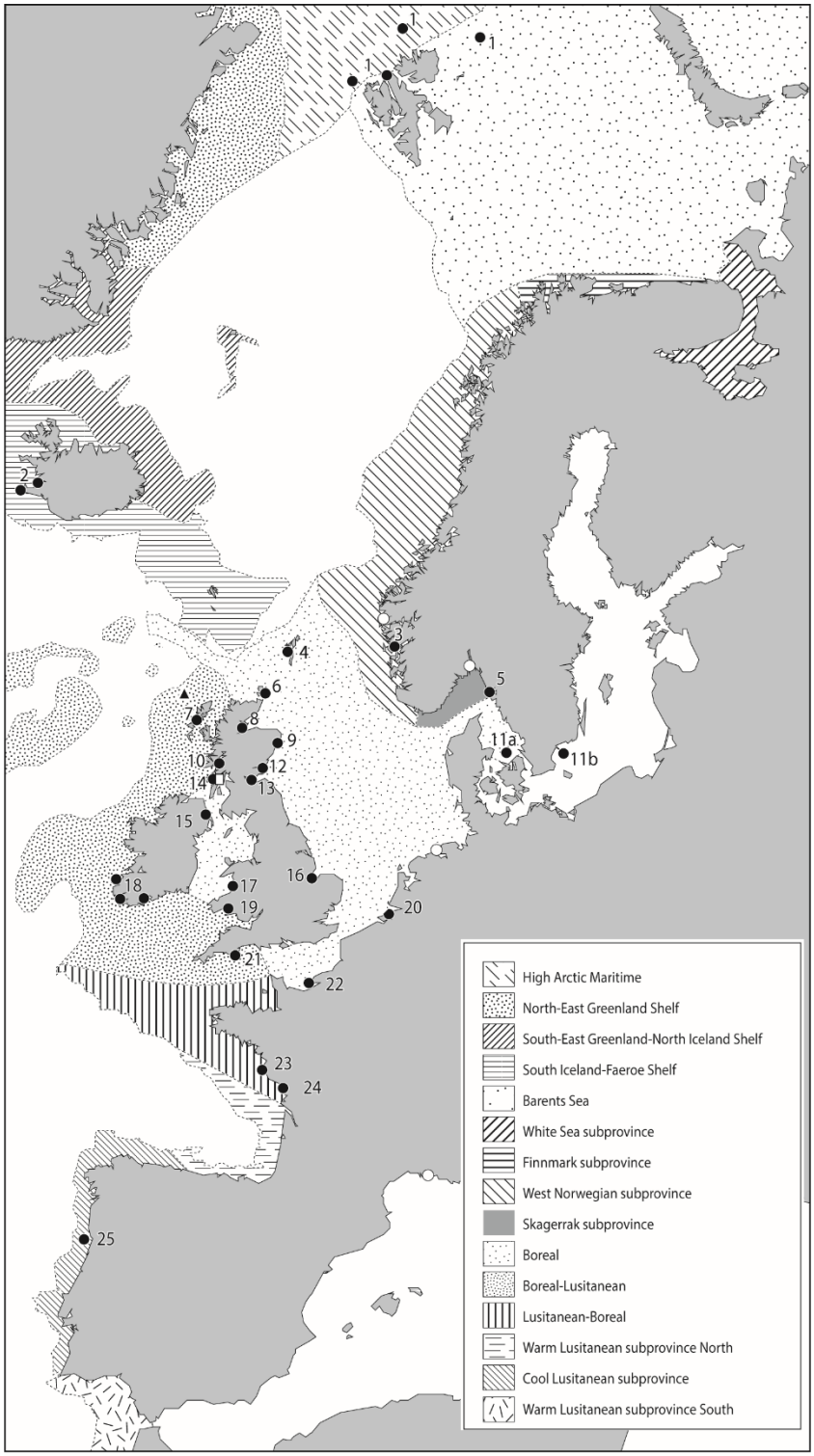


Figure 2.2 Map of the NE Atlantic Shelf seas illustrating the sampling localities at which benthic foraminifera were collected for studies included in the present thesis. The site localities where live foraminifera specimens were obtained for morphological and genetic analysis (circles) are detailed. In addition, the site locality in the NW Scottish shelf seas from which a seasonal study of benthic foraminiferal assemblages was conducted (square) is illustrated. The biogeographic zones depicted correspond to the distinct biogeographic provinces identified in Dinter et al. (2001).

2.2 Sampling

The thesis is comprised of two taxonomic research components. The first component focuses on re-evaluating classical morphospecies concepts from two key foraminiferal taxonomic groups by integrating multiple lines of evidence obtained from contemporary live foraminifera collected from across the NE Atlantic. The second component examines the temporal dynamics of benthic foraminifera from a fjordic environment in the NW Scottish shelf seas through an investigation of relative and absolute abundances of 'live' Rose Bengal stained specimens. The two taxonomic investigations conducted in this thesis employ different materials and methods, which are outlined below.

2.2.1 Contemporary sample collection for the integrated taxonomic investigation

In order to reassess the taxonomic diversity of benthic foraminifera within this region, 29 sites were sampled across the NE Atlantic from 2010-2013 (Figure 2.2). Where possible, sub-site samples were also collected. This sampling regime was employed as part of a larger NERC funded collaboration to re-evaluate benthic foraminiferal taxonomy in the NE Atlantic (see the Appendix for breakdown of which researcher collected the samples).

The majority of samples collected in this sampling regime were retrieved from intertidal areas, through a combination of surface sediment scrapes and seaweed collection. In order to isolate the living specimens from the intertidal surface sediment scrapes, the top 1 cm of sediment was collected using a spoon/ trowel and was placed into sampling pots. In order to isolate the live specimens from the seaweeds, the plants were vigorously washed in ambient seawater in buckets to collect the residue sediments. This suspension was stirred and put aside to enable the residue to settle. These residues were then decanted into sediment bottles.

A limited number of samples were also obtained from the subtidal region. These samples were collected using a range of retrieval techniques including the deployment of a Veen grab corer, a box corer, a multi-corer and a piston corer. Additionally, a limited number of surface samples were collected by scuba divers from the NERC National Facility for Scientific Diving (NFSD). The most appropriate sampling technique was chosen for each site based on considerations of the depth of the sediment, substrate type and the availability of sampling equipment at the time of collection. Again, the top 1-2 cm of the surface sediment was obtained by each technique employed. Prior to processing, the samples collected were stored in a cold room or a fridge at a constant temperature of 4^{o}C.

2.2.2 Sediment sample collection for the seasonal assemblage study

The sediment samples used in Chapter 4 were retrieved from Dunstaffnage by scuba divers from the NFSD. Two replicate samples were collected by hand at a depth of 32m, at 2-4 week intervals between August 2007-2008. The top 1cm of sediment was collected into a plastic bag at the seafloor. In addition, two replicate bottom water samples were also obtained. Immediately after sampling, the surface sediments collected were preserved in sample bottles in a Rose Bengal ethanol solution (1g Rose Bengal per 1 litre of ethanol) (Walton, 1952). These samples were then stored in a cold room at 4^{o}C until they were processed. The samples utilised within this study are part of a long-term sediment archive collected by the NFSD at this site, from 2007 to the present day. The archive is held at the University of St Andrews.

2.3 Foraminiferal analysis

2.3.1 Extraction and identification of live foraminifera by pseudopodial activity

The live foraminiferal specimens utilised in Chapters 3, 5 and 6 were identified by pseudopodial activity. The identification of live specimens from these samples was a collaborative effort between this researcher and others from the larger NERC project. Initially, surface sediment scrapes were sieved at 63 µm in ambient seawater (where available) in order to concentrate the foraminifera and to remove smaller particulate matter. The sieved residues were then stored in seawater in the fridge or a cold room at a constant temperature of 4^{o}C.

In order to extract potential live specimens from the samples, a small amount of the sediment was transferred into a small seawater filled petri dish using a pipette. The petri dish was placed onto an insulated ice block to keep the samples at a constant temperature. The ice block was insulated with bubble wrap where available or paper towelling to prevent the samples from super cooling (i.e. becoming too cold). Initially, potentially live foraminifera were differentiated from the dead (empty) tests within the sediments based on their cytoplasm test colouration. Live foraminifera commonly exhibit green, orange or pink test colouration (Murray, 2006); an example of cytoplasm colouration is exhibited in Figure 2.3.

These potentially living foraminifera were extracted from the sediment using a 0000-gauge paintbrush and were placed into a separate seawater filled petri dish, which was kept cold by an insulated ice block. These picked specimens were then observed for any signs of pseudopodial activity to determine if they were live. The first protocol employed by this thesis, investigated pseudopodial activity by foraminiferal 'racing'. This technique is effective for foraminifera taken from dynamic environments e.g. from intertidal areas. Specimens were organised into lines

drawn on the base of the petri dish and were left for a couple of hours. Specimens were determined live if they departed from their predefined position on the lines. If no movement was observed, the second protocol for recognising pseudopodial activity was employed, which is outlined below.

Live foraminiferal specimens can also be identified by detecting the overnight formation of a sediment cocoon. Potential live specimens were thoroughly cleaned and placed into a petri dish filled with seawater and fine sediment. These petri dishes were then carefully placed into the fridge or cold store overnight. Live foraminifera were identified by the presence of a sediment cocoon, which was formed by pseudopodial activity which draws in and sifts through fine sediment in search of food. Examples of sediment cocoons are illustrated in Figure 2.3.

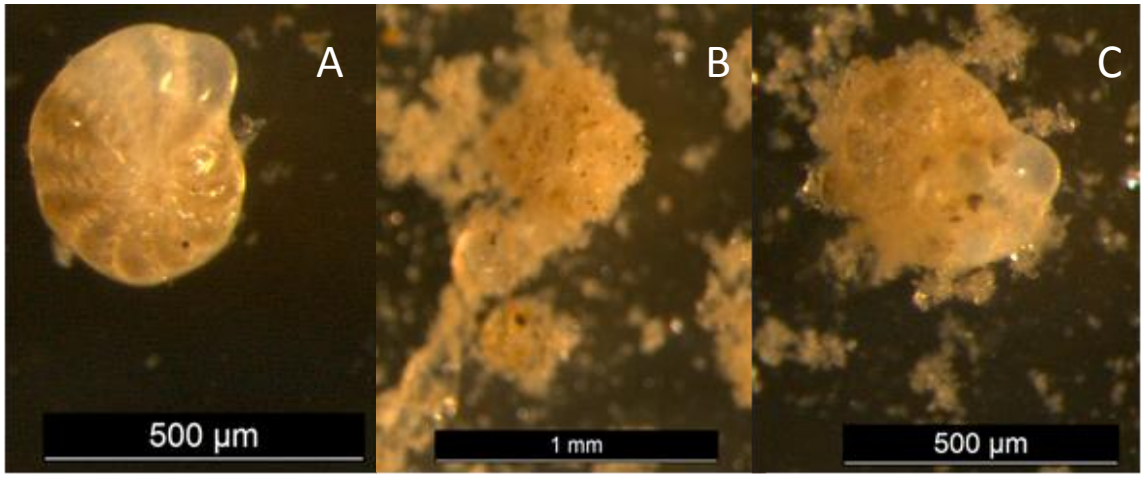


Figure 2.3 Light microscope images illustrating the overnight formation of a sediment cocoon. A) Distinctive cytoplasm colouration B) An example of a fully formed sediment cocoon, C) An example of a half-formed sediment cocoon.

Once the live specimens were identified from the surface sediment samples, these specimens were cleaned and placed onto micropalaeontological slides to dry, so that they were ready for SEM imaging prior to rRNA extraction. If the specimens did not exhibit any pseudopodial activity overnight, the petri dishes were returned to a fridge or a cold room and were checked daily for signs of pseudopodial activity over a course of 3-5 days.

2.3.2 Extraction and identification of 'live' Rose Bengal stained foraminifera

Prior to processing the samples collected by the scuba divers (NFSD) used in Chapter 4, the volume of the sediment in the sample pot was calculated. This enabled the standardisation of foraminiferal abundances per 100ml in later proceedings. The sediment surface in the sample bottle was marked with a water resistant permanent marker. The sediments were then wet

sieved at 63µm using a fine water spray until the effluent water ran clear. These samples were dried at 40^{o}c degrees and the dry weight of each sediment sample was calculated. Between sieving each sediment sample, the sieves were washed and then submerged in methyl blue solution. This solution stains calcium carbonate blue identifying any residual contaminant specimens within the sample. Following the sieving, the marked sample containers were refilled with water up to the mark to detect the settled sample volume.

Small proportions of the sediment were then evenly distributed across a gridded brass-picking tray and 'live' stained individuals were identified based on their Rose Bengal staining (distinctive pink/red colouration). Rose Bengal staining of foraminifera is an important technique as it is an inexpensive and easy method for identifying live foraminifera, as it stains the protoplasm within the test (Walton, 1952). However, this staining technique has in recent years came under scrutiny, because it can also stain specimens which have died weeks or even months prior to sampling but still retain undecayed protoplasm (Bernhard, 1988; Murray and Bowser, 2002). Therefore, in order to minimise errors assigning 'live' stained specimens a strict protocol was enforced, whereby only individuals that were stained pink/ red across all of the test were considered alive. In situations where foraminifera exhibited pale or inconsistent staining, these specimens were considered dead at the time of collection (Goldstein and Harben, 1993). However, it should be noted that the degree of staining in foraminifera is often species-specific. For example, the porcellanous wall structure of some species obscures the colour of staining; therefore, a small amount of water was applied to these specimens in order to help identify the presence of staining within these taxa (as per recommendations of Schönfeld et al., 2012).

Once specimens were identified as 'live', these specimens were then extracted from the sediments using a 0000-gauge paintbrush. In order to avoid bias of preferential picking towards more visible individuals, every foraminiferal specimen was picked from each square before moving onto the next square. Where possible, 300 specimens were extracted from each sample. This is because the target number of specimens needed for a reliable estimation of foraminiferal abundance is 300 specimens (Patterson and Fishbein, 1989). In samples with low abundances, a minimum target of 100 specimens was made (when possible). This provides a 99% confidence interval that species making up >5% of the assemblage are captured (Fatela and Taborda, 2002) and that species as rare as 3% of the assemblage are captured at a 95% confidence interval (Dennison and Hay, 1967).

2.3.2.1 Presentation of foraminiferal assemblage data

The foraminiferal assemblage counts presented in Chapter 4 were expressed as different measures of the community structure outlined below.

Relative abundance: percentage of each foraminiferal species in relation to all other counted foraminiferal specimens in a sample.

Absolute abundance (or standing crop): the number of live (Rose Bengal stained) specimens per standardised volume. Historically benthic foraminiferal standing crop is normally expressed as foraminifera per cm^3 (Murray, 2006). However, as the area and depth of sediment at time of sampling is unknown, the absolute abundance (standing crop) in this thesis is expressed as 'live' foraminifera per 100ml.

Diversity indices: Measures of taxonomic diversity and community structure are outlined below. These measures of diversity were calculated using the PAST software v.2.17 (Hammer et al., 2001).

Species richness was calculated as the total number of species identified per sample.

Measures of *Species diversity* were calculated using both the Shannon Weiner diversity index (H') and the Fisher's alpha diversity index (Fisher et al., 1943). The Shannon Weiner diversity index (H') provides a measure of species abundance and relative richness (Shannon, 1948). The Shannon Weiner index takes into account the number of individuals and the number of taxa; consequently, this index does not place much significance upon the chance occurrence of rare species (Hammer, 2006). The Fisher's alpha species index (Fisher et al., 1943) was included as it is one of the most common measures of foraminiferal taxonomic diversity employed within the NE Atlantic, particularly in the NW Scottish shelf seas (Hannah and Rogerson, 1997; Murray, 2002; Austin and Cage, 2010). Fisher's alpha diversity index (Fisher et al., 1943) attempts to understand patterns of relative abundance of species in a community. This statistic is calculated using a log series model to predict the number of different species at different levels of abundance.

Species Evenness was calculated using the Pielou species Evenness index, whereby the distribution of individual densities between different species was analysed (Pielou, 1966). A high Evenness index number equates to a relatively diverse sample.

2.4 Foraminiferal Imaging

2.4.1 SEM

The SEM images provided in this thesis have been imaged in two localities. Specimens analysed within Chapters 3 and 5 were imaged at the University of Edinburgh by project collaborators on a Phillips XL30CP SEM. Specimens from Chapter 6 were imaged at the University of St Andrews by this researcher and were taken on a Joel JSM-35CF SEM.

Prior to imaging, foraminiferal specimens were mounted onto an SEM stub covered with double-sided adhesive tabs. These stubs were then coated using a sputter coater (Emscope SC 500 X) which coated the specimens with a 15-20 nm layer of gold. Each individual test image was given a unique identification number, which was used at each stage of the RNA sequencing and morphometric analysis.

2.4.2 Alternative imaging techniques to SEM

Not all of the specimens analysed in this thesis were suitable for imaging using Scanning Electron Microscopy techniques. The taxonomic significance of the type material of *Elphidium williamsoni* (Haynes, 1976) and *Polystomella umbilicata* (Williamson, 1858) utilised in Chapter 6, meant that these specimens were not allowed to be removed from the original slides or gold coated. Thus, alternative imaging approaches were investigated to identify an imaging technique that yields mutually comparable images to those taken by traditional SEM imaging. A specimen of *Elphidium crispum* (genotype S10) obtained from the seasonal assemblage study in Chapter 4 was used as a trial specimen.

2.4.2.1 SEM imaging of uncoated specimens

An uncoated foraminiferal specimen was subjected to SEM imaging at a low beam voltage at the School of Chemistry, University of St Andrews on a JSM 5600 SEM. The images produced by this imaging technique were not suitable for morphometric analysis because these images were of an insufficient resolution and they exhibited poor contrast (Figure 2.4 A).

2.4.2.2 Imaging using a high resolution light microscope

The same uncoated specimen was then imaged using a VHX-2000 'super-resolution' digital microscope. Two images were taken, one of the overall profile of the foraminifera (Figure 2.4B), as well as a close up image (Figure 2.4 C) in order to assess whether fine resolution features could be robustly identified. Although this imaging technique produced much clearer images than the uncoated SEM images (Figure 2.4 A), they were not mutually comparable to the SEM images, as certain morphological traits including sutural ornamentation were difficult to identify (Figure 2.4 B, C).

Figure 2.4 A) An uncoated specimen imaged using a JSM 5600 SEM at a low voltage, B) Light microscope image of the uncoated specimen imaged using a VHX-2000 'super-resolution' digital microscope, C) A close up image of the uncoated specimen taken by the VHX-2000 microscope.

However, the development of this imaging technique in the future presents interesting possibilities, as this imaging technique provides a more 'representative' and realistic image of foraminifera which is akin to what is seen under a normal light microscope in an applied taxonomic situation. An additional benefit of this imaging technique is that specimens do not require any preparation prior to imaging.

2.4.2.3 ESEM imaging

Ultimately, Environmental Scanning Microscopy (ESEM) was used to image the type material, as it provided the highest quality images. The ESEM enables the imaging of the microstructural features of the foraminifera within an SEM chamber in their uncoated natural state. A Quanta FEG 650 at Herriot Watt University was utilised to image the type specimens used within Chapter 6. This imaging approach provided good quality images, which were directly comparable to the SEM images analysed throughout this thesis.

2.5 Morphometrics and morphological character assessments

The morphometric measurements and character assessments analysed in this thesis were derived from SEM and ESEM images. Morphometrics is the quantitative analysis of biological form (Rohlf, 1990). The morphometric features analysed are constructed from a combination of linear measurements, angles, ratios, binary and multistate (categorical) variables. Thus, this thesis employs a 'traditional morphometric' approach to assess morphological variance within benthic foraminifera (Sneath and Sokal, 1973; Marcus, 1990). In recent years, the advent of geometric morphometric techniques has helped to elucidate taxonomic relationships through the development of landmark-based approaches (Rohlf and Marcus, 1993; Klingenberg, 2011). However, it was not feasible to implement these techniques within this thesis primarily because of the unsuitability of the SEM images, which were of variable quality, orientation and scale. Moreover, the employment of geometric morphometric approaches does not enable the re-evaluation of the efficacy of classical morphospecies concepts and species descriptors in discriminating between newly delineated genotypes. It should be noted that the application of geometric morphometric techniques could be a potential avenue for future research, as it would provide another independent line of taxonomic evidence to augment species delineations.

2.5.1 Image pre-processing procedures

A series of image pre-processing procedures were undertaken prior to the acquisition of morphometric measurements and the employment multivariate statistical analyses.

2.5.1.1 Screening of SEM/ ESEM images

The SEM and ESEM images were screened to assess whether they were of sufficient quality to provide robust morphological measurements. In order to be included within the morphometric analysis, specimens should be suitably orientated in the SEM image and more than 90% of the morphological test should be visible. In situations where the SEM images exhibit some test damage or obscuration (i.e. due to debris), the missing variable from the individual specimen was estimated. This estimation procedure followed the protocol set out by Hayward et al. (2004) whereby the mean value of the character for the genotype was utilised. This accounted for 0.83% of the measurements.

2.5.1.2 Calibrating measurements to a known distance

The morphometric measurements derived from the images were measured using Image Pro Express and ImageJ v1.48 software. Prior to morphometric analysis, each individual SEM image was calibrated to the SEM's original dimensions. This process allowed the image software to recognise the size of the specimen based on a known number of pixels. In order to calibrate the SEM images in ImageJ software, the length of the known distance (scale bar) was measured by fitting a line over the scale bar using the straight-line selection tool. The select scale option was then employed (Analyse> Set Scale), in which the known distance and unit length is entered. Once this function was applied, the scale of each new feature was then automatically calculated from the registered distance. A new line was drawn over the scale bar to check that the image was calibrated successfully. A similar procedure was undertaken in the Image Pro Express software; however, this software does not automatically set the scale. Instead, all of the morphological traits were measured in pixels and exported into an excel spreadsheet. Next, a straight-line feature was drawn over the known distance (scale bar) to calculate the length of this feature in pixels. All of the features measured were manually converted using the known unit per pixel ratio (derived from the known length of the scale bar).

2.5.1.3 Foraminiferal test outline

In order to calculate the foraminiferal test roundness and total foraminiferal area, a series of pre-processing steps were undertaken. Initially, the unprocessed images were imported into the Image Pro Express software and a trace measurement tool was employed to create a line feature, which defines the periphery of the foraminiferal test. A snapshot image of this outline feature (depicted by the green line) was taken (as illustrated in Figure 2.5A). This snapshot image was imported to the ImageJ software. The threshold function (Image> Adjust> Threshold) was utilised to segment the feature of interest (test outline) and the background. The threshold limit was manually chosen to capture the outline feature based on its distinctive green colour. Once the feature of interest was within the threshold limits (i.e. feature was outlined in red) the image was transformed from a RGB image to an 8-bit greyscale image mask. In this image mask, the black pixels represent pixels whose values were within the threshold limit (test outline), and the white pixels represent the background features which were outside the threshold limit (Figure 2.5 B). Prior to the calculation of the foraminiferal test area, the test foraminiferal outline was in-filled using the flood fill tool in ImageJ (as illustrated in Figure 2.5 C). This enabled the calculation of the area of the entire feature, not just the area of the line surrounding the periphery of the foraminifera.

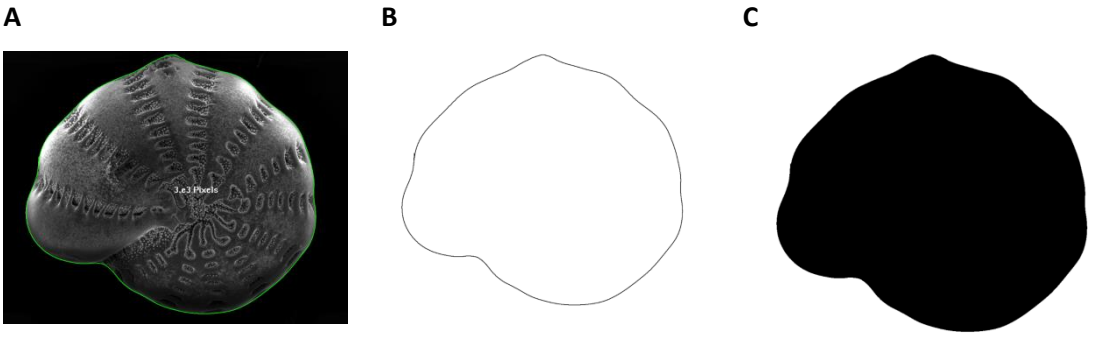


Figure 2.5 Image processing steps for calculating foraminifera peripheral test outlines, A) The test periphery of the foraminifera is outlined in green by the automatic tracing tool software in Image Pro Express, B) Image mask produced by the ImageJ threshold analysis, C) Peripheral outline of the foraminiferal test with flood fill.

Owing to the fact that this pre-processing procedure was often labour/ time intensive, a macro was developed in ImageJ to increase the efficiency of processing and extracting the measurements of the test peripheral outline (see Macro 1, Appendix).

2.5.2 Image pre-processing for species- specific morphological measurements

In order to obtain a number of morphological features which were specific to the Elphidiidae taxonomic group (Chapters 5 and 6), an additional series of imaging pre-processing procedures were undertaken.

2.5.2.1 Quantification of septal pit morphological traits

In classical taxonomy the number, size and shape of septal pits have been ascribed significant diagnostic weight for the classification of elphidiids (Haynes, 1973). To quantify these test traits a series of image pre-processing steps were employed. Initially, the unprocessed SEM images (Figure 2.6 A) were imported into Adobe Illustrator CS6 software. Each individual septal pit was manually digitised using a graphics tablet and the septal pit outlines were exported as Tiff grayscale images (as shown in Figure 2.6 B).

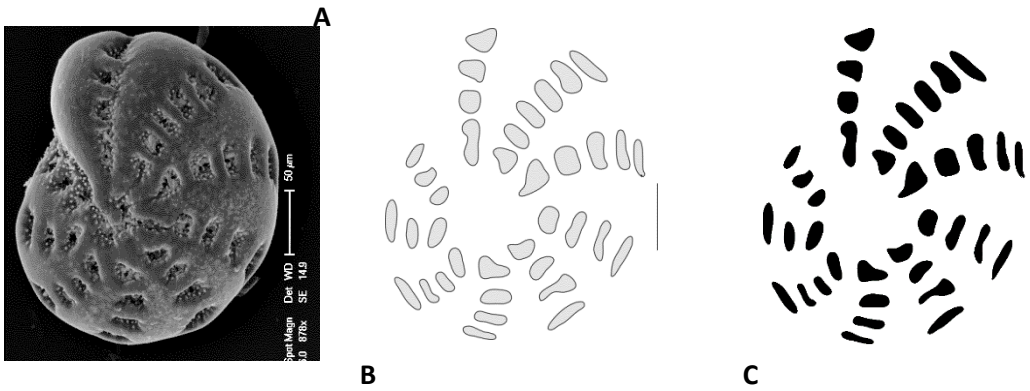


Figure 2.6 Image processing steps for the calculation of septal pit characteristics, A) un-processed image, B) image of manually digitised septal pits, C) binary image mask of septal pits after the thresholding procedure.

The greyscale images of the septal pits were then imported into the ImageJ software and an automatic threshold procedure was applied (Image> Adjust>Threshold). This process converts the greyscale image to a binary image, where features in black are the particles analysed and the white pixels represent the background (Figure 2.6 C). This binary image was then analysed using the 'analyse particles' tool in ImageJ (Analyse>Analyse particles) which calculates the size and shape characteristics of the septal pits. A macro was also developed in ImageJ to increase the efficiency of processing and calculating these shape measurements (Macro 2, Appendix).

2.6 Morphometric measurements and character assessments

In order to quantify and delineate between genetically distinct species, a series of morphometric measurements and morphological character assessments were conducted. These include a combination of count measurements (e.g. total number of chambers), presence/absence features (e.g. presence of a boss), multistate qualitative assessments (e.g. degree of ornamentation) and biometric, point-to-point measurements (e.g. maximum test diameter). Many of the morphological characters analysed were constructed and modified from classical morphospecies descriptors or were derived from previous integrated taxonomic studies such as Hayward et al. (2004). Some of the key morphological features measured in this thesis are depicted in Figure 2.7. For detailed descriptions of the morphological traits examined within the two taxonomic groups, refer to Chapters 3 and 5.

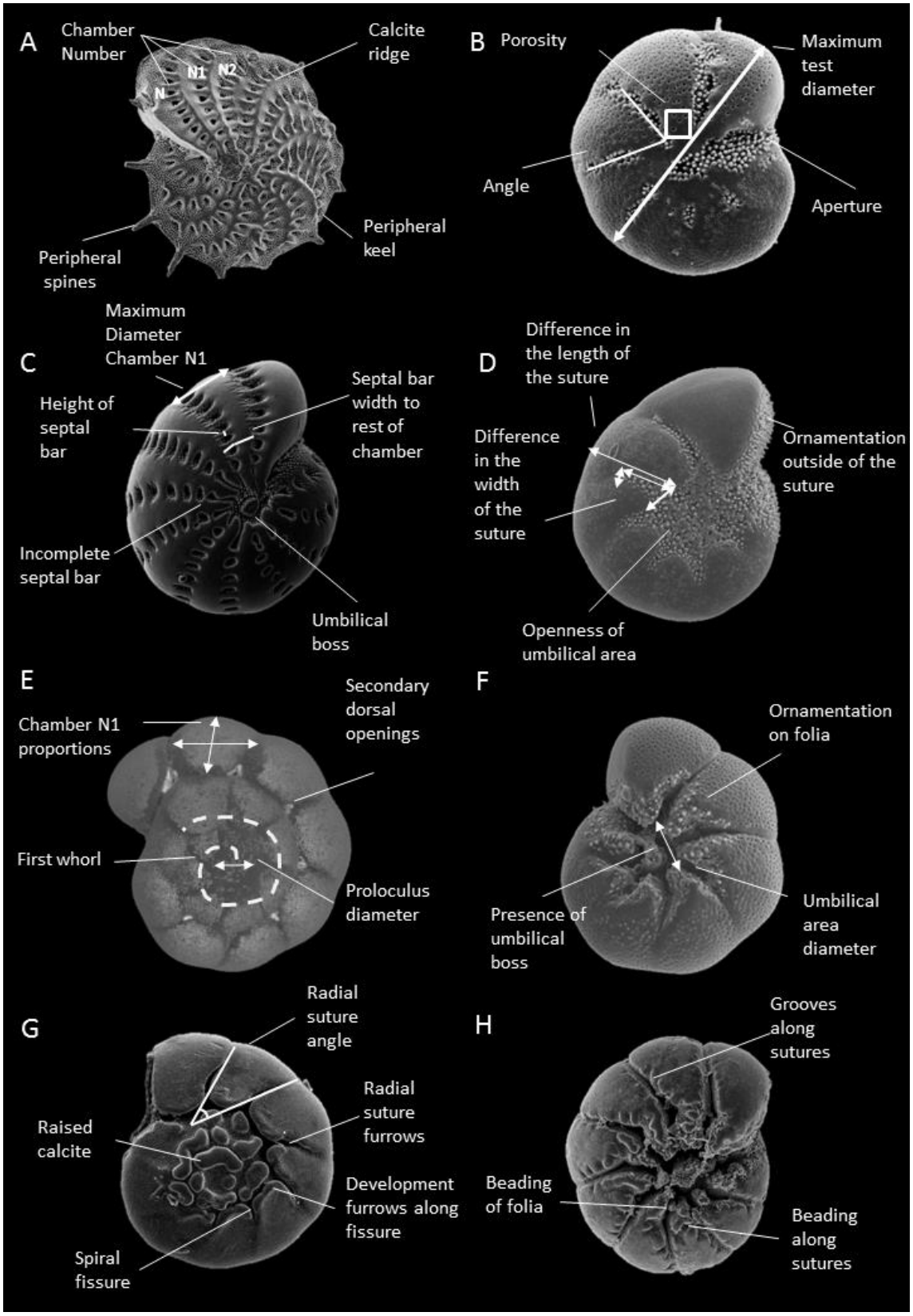


Figure 2.7 Schematic representation of some of the main morphological traits measured from elphidiid specimens (A-D); see Chapter 5 and *Ammonia* specimens (E-H); see Chapter 3 in this thesis.

2.7 Data accuracy and standardisation techniques

Prior to multivariate statistical analysis, the morphometric data is standardised in order to remove the influence of ontogeny. This is crucial as previously ontogeny was identified to exert a strong control on foraminiferal size and shape (Tyszka, 2004). In order to limit the influence of size, most of the morphometric measurements utilised within this thesis were calculated as ratios relative to the maximum test diameter; this standardisation technique follows the methodology set out by Hayward et al. (2004). The potential effects of the curvature of foraminifera affecting the measurements were also taken into consideration. This potential bias was minimized as the majority of the features measured were taken from the junction of penultimate (chamber N1) and antepenultimate chamber (chamber N2) towards the umbilical area. This focus on the penultimate chamber also avoids the potential effects of unusual terminal morphologies.

In addition, further data transformation was required in order to remove the effects of the variable range of scales of the morphological data, which may affect the discriminatory strength of the traits in the multivariate analysis. In order to eliminate this potential bias, the morphometric data was standardised between 0 to 1 following methods set out by Hayward et al. (2004) and Schweizer et al. (2005). Data transformation was only employed when appropriate, as the decision tree analysis (CHAID and CART) does not require any a priori data transformation (Breiman et al., 1984).

In order to test for consistency and repeatability of measurement a proportion of the features were randomly re-examined. In addition, all unusual measurements within the dataset were confirmed by repeated measurement to test for reproducibility. Overall, as all specimens are treated the same and the measurements were undertaken by a single researcher, this created a database of mutually comparable results.

2.8 Statistical analysis

2.8.1 Exploratory morphological statistical analysis

Two exploratory data analysis techniques (i.e. not necessarily inferential) were used to assess the utility of morphology in delineating between species without any *a priori* knowledge of their genetic groupings.

2.8.1.1 Principal coordinate analysis

Principal coordinate analysis (PCO) provides a geometric representation of the distances and dissimilarities between specimens and extracts principal coordinates to describe the major trends in multidimensional data (Legendre and Legendre, 1983). PCO analysis looks for patterns of morphological structure between and within taxonomic groups (Davis, 2001). This statistical approach is commonly used in plant systematics (Loo et al., 2001; Henderson 2006). PCO analysis was employed over principal component analysis (PCA), as PCO analysis robustly handles mixed qualitative and quantitative datasets (Legendre and Legendre, 1983). However, a disadvantage of PCO analysis is that it does not provide a breakdown of the component scores associated with each variable. The PCO statistic was calculated using PAST statistical software v.2.17.

2.8.1.2 UPGMA cluster analysis

Cluster analysis is an exploratory tool for classifying objects, whereby the association between specimens is assessed (Legendre and Legendre, 1998). In cluster analysis, no statistical assumptions are made about the data. An UPGMA (unweighted pair-group method using arithmetic averages) was employed in this thesis, as this algorithm is the standard cluster analysis approach employed in systematics (e.g. Hayward et al., 2004; Henderson, 2006). This statistic was calculated using dendroUPGMA programme (Garcia-Vallve et al., 2010) because its output can be saved in the Newick format, which can be subsequently imported into the Phylowidget software (Jordan et al., 2008) to create a circular dendrogram. It should be noted that the output of the UPGMA cluster analysis in PAST software and the dendroUPGMA programme are mutually comparable. However, UPGMA analysis was not calculated in the PAST software because it did not yield an optimal visual presentation of the clustering patterns in the large datasets used in this thesis i.e. the UPGMA cluster analysis dendrograms created in the PAST software extended across multiple pages.

2.8.2 Classification techniques

Three distinct multivariate classification analyses were employed by this thesis to assess the relative importance of morphological traits for discriminating between genotypes. In addition, the use of these classification approaches provides an opportunity to compare the efficacy of each of the techniques to each other and to the two exploratory statistical approaches (PCO analysis and UPGMA cluster analysis). The three classification techniques were conducted in SPSS v22.

2.8.2.1 Discriminant function analysis

Discriminant function analysis is one of the most widely employed statistical approaches used in systematics to investigate taxonomic differences and to delineate between morphologically similar specimens (Fisher, 1936). This statistical approach is widely utilised in foraminiferal systematics (Quillévéré et al., 2013; Weiner et al., 2015). DFA discriminates amongst pre-defined groups of individuals based on a combination of variables, which are used to create classification functions, which are themselves used to determine group membership of the specimens (Henderson, 2006).

It is important to recognise that DFA requires the control of several assumptions including multivariate normality (as discussed at greater depth in Tabachnik and Fidell, 2007). However, this classification technique is relatively insensitive to violations of its internal assumptions (Tabacknick and Fidell, 2007; Hammer and Harper, 2008). This is crucial, as ecological/ taxonomic datasets almost never fulfil all these assumptions (Williams, 1983; Sarawasti and Sabnis, 2006). The classification performance of this procedure was cross-validated by a leave one out approach. This approach omits one individual from the dataset, then recalculates the discriminant function and assigns this specimen to a group using the new discriminate function (Klecka, 1980).

2.8.2.2 Decision tree analysis

A non-parametric decision tree approach to classification was also employed in studies in this thesis. This approach can robustly handle complex ecological data, address non-linear relationships and can handle missing data (Breiman et al 1984; De'ath and Fabricius, 2000; Feldesman, 2002). Presently, decision trees are seldom used in foraminiferal taxonomy (Saraswati and Sabnis, 2006) but have been used to great effect in public health (e.g.; Robledo et al., 2007) and aquaculture research (e.g. Elliot and Owens, 2015). Two decision tree algorithms were employed by this thesis. The first decision tree CART (Classification and regression tree) is built upon a binary recursive partitioning and tree development (Feldesman, 2002). In contrast, CHAID analysis (Chi-square Adjusted Interaction Detection) (Kass, 1980) uses recursive partitioning and tree development which classifies based on a dependant measure and a large series of possible predictors. The difference between CART and CHAID analysis is that the CHAID tree is not restricted to binary decisions, i.e. CHAID allows for more branching than CART if there are significant differences (Rokach and Maimon, 2007). Additionally, CHAID analysis has been identified as the optimal technique for handling large and unequal datasets (Breiman et al., 1984).

A ten V-fold cross validation approach was employed for both CHAID and CART analysis, whereby the data is split into ten random subsamples which were taken from the dataset (Rockach, 2007). A tree was computed ten times, each time one of the subsamples was omitted from the computation. The cross validation estimates were computed for each of the ten test samples and the results were averaged to give a cross-validation error.

2.8.2.3 K-nearest neighbour analysis

K-nearest neighbour analysis (K-NN) is a non-parametric approach which can discriminate between genotypes by assessing the similarity of a specimen to its nearest neighbour (Dudan, 1976). This procedure predicts the test category based on the K training sample and classifies the specimen into the category with the highest probability (Kim et al., 2011). This statistical approach is rarely employed within taxonomy, but is commonly utilised in ecology (Mäkelä and Pekkarinen, 2004) and medical research (Polat, 2012; Belekar et al., 2015).

2.9 Additional parameters

2.9.1 Phylogenetic framework

The aim of this thesis was to evaluate quantitative interspecific morphological boundaries against new independent lines of genetic evidence. The phylogenetic framework employed by this thesis was produced as part of a larger NERC project (NE4/G018502), results from which are allied to this thesis (Bird et al., in prep.; Darling et al., in prep). This framework was provided by collaborators at the University of Edinburgh foraminiferal genetic laboratory. With the exception of the Rose Bengal stained specimens analysed in Chapter 4 and 75 topotypic specimens of *Elphidium williamsoni* from the Aberdovey marshes analysed in Chapter 6, all specimens morphologically analysed in this study had an allied genetic sequence. These genetic sequences were identified by SSU rRNA sequences which were amplified and extracted following methods outlined by Schweizer et al. (2008).

2.9.2 Environmental parameters

Environmental parameters including bottom water temperature and salinity were analysed for Chapter 4. A full account of the environmental variables included in the seasonal study are provided in Chapter 4.

Chapter 3

Elucidating taxonomic relationships within the cryptic genus *Ammonia* across the North East Atlantic shelf seas

The sampling of live Ammonia specimens across the NE Atlantic was a collaborative effort between this researcher and researchers from the allied NERC funded project (NE4/G018502/1). The SEM images used in the morphometric analysis were provided by project collaborators, Dr Clare Bird, Dr Magali Schweizer, Dr Kath Evans and Professor Kate Darling. The unpublished genetic framework used in this chapter was provided by Dr Clare Bird, Dr Magali Schweizer, Dr Kath Evans and Professor Kate Darling (Bird et al., in prep.). The biogeographic maps were constructed as a combined effort between this researcher and Dr Clare Bird.

Chapter 3: Elucidating taxonomic relationships within the cryptic genus *Ammonia* across the North East Atlantic shelf seas

3.1 Introduction

Species within the genus *Ammonia* are some of the most ubiquitous and abundant foraminifera within the marine benthos (Murray, 2014). Their occurrence in numerous environmental settings, coupled with their high test preservation potential and strong sensitivity to environmental factors, has enabled species within this genus to be widely used as palaeoenvironmental proxies (Allison and Austin, 2003; Cage and Austin, 2010). A strong taxonomic framework is needed for the genus, to enable robust identification of *Ammonia* species in both contemporary and palaeoenvironmental settings. This is crucial because palaeoenvironmental reconstructions are derived from species-specific calibrations of environmental conditions of extant species (Murray, 1991).

Despite the genus *Ammonia* being one of the most studied taxa, with a taxonomic history spanning over the past 250 years, species within this genus are some of the world's most commonly misidentified foraminifera (Hayward et al., 2004). To date, 51 species/subspecies have been identified within the literature (Foissner and Pawlowski, 2009; Hayward et al., 2015). The crux of the taxonomic confusion is the ambiguity surrounding the species concepts and their boundaries, which have changed from worker to worker. Taxonomic uncertainty has also arisen from the prevalence of the theory of ecophenotypy, where few species concepts are formally recognised and phenotypic plasticity is thought to be a product of different environmental conditions (Schnitker, 1974; Poag, 1978). Ecophenotypy has had significant taxonomic repercussions, as it inherently promotes the idea of taxonomic conservatism in which only three main species concepts are consistently recognised within the literature: *Ammonia beccarii* (Linnaeus, 1758), *Ammonia tepida* (Cushman, 1926) and *Ammonia parkinsoniana* (d'Orbigny, 1839).

The advancements in molecular techniques over recent decades have enabled the provision of new lines of independent taxonomic evidence to complement and test the traditional morphospecies concepts. This new molecular evidence has unveiled previously unrecognised cryptic diversity, highlighting that traditional morphology-based taxonomy may have underestimated the true extent of genetic diversity found within this genus (Holzmann, 2000; Hayward et al., 2004). Despite the elucidation of taxonomic boundaries through the introduction of molecular systematics, considerable uncertainty surrounds how these new lines of taxonomic

evidence can be reconciled with traditional nomenclature and species concepts. There is currently a dearth of knowledge of the biogeographic distribution and ecological preferences of these newly identified genotypes. As a consequence, considerable uncertainty surrounds the "value" of delineating between the genetically distinct species in applied taxonomic situations; as a result, many authors continue to use broad species concepts and open nomenclature in order to uphold taxonomic consistency.

Given the continued proliferation of open nomenclature and recognition of broad *Ammonia* species concepts in the literature, it is critical to readdress and clarify the interspecific taxonomic boundaries found within this genus. This chapter re-examines the degree of morphological and genetic diversity of *Ammonia* within the NE Atlantic in order to clarify taxonomic relationships between the species and to identify any biogeographical patterns of species distribution. The degree of genetic and morphological diversity exhibited within this region is then compared to previous integrated taxonomic frameworks, to validate the robustness of the current taxonomic frameworks in the literature.

3.2 Taxonomic history of the genus *Ammonia*

Ammonia (Brunnich, 1772) was the first foraminiferal genus to be formally recognised in the scientific literature, and its type specimen *Ammonia beccarii*, was one of the first foraminiferal species to be identified (*Nautilus beccarii,* Linné, 1758). Despite the long history of taxonomic inquiry, this genus has a turbulent taxonomic history. Traditionally, species within the genus *Ammonia* have been delineated exclusively on morphological characteristics of the test. The high degree of morphological variability exhibited within this genus has led to considerable uncertainty surrounding the interspecific boundaries. Numerous taxonomic investigations have attempted to elucidate the taxonomic relationships within the genus *Ammonia,* including extensive investigation of test morphology such as those conducted by Cifelli (1962), Banner and Williams (1973), Chang and Kaesler (1974), Poag (1978), Hottinger (1980), Wang and Lutze (1986), Jorissen (1988), Debenay et al. (1998) and Colburn and Baskin (1998). The subjective interpretation of highly variable morphological characters, the lack of scientific consensus surrounding species concepts and their associated interspecific boundaries has led to considerable nomenclatural and taxonomic uncertainty within this genus (Cifelli, 1962; Chang and Kaesler, 1974).

Some of the entrenched taxonomic confusion surrounding this genus has arisen from the two different key taxonomic practices used to delineate between species, informally referred to as 'splitters' and 'lumpers'. Splitters identify visible morphological differences in the test characteristics, and consider these differences to be significant enough to represent a new species. These subtly different morphological forms are then ascribed new formal taxonomic names (e.g. Cinnerman and Langer, 1991; Haynes, 1992; Hottinger et al., 1993; Loeblich and Tappan, 1994; Colburn and Baskin, 1998; Buzas-Stephens et al., 2002). In contrast, lumpers identify all morphological variability as being intraspecific in nature, therefore leading to the creation of a limited number of broad species concepts (e.g. Cushman, 1926; Walton and Sloan, 1990). The emergence of two co-existing taxonomic practices has led to the development of different diagnostic morphological criteria and conflicting species concepts. This has often cumulated in the assignment of different formal taxonomic names to the same species.

Owing in part to the uncertainty surrounding interspecific boundaries, the taxonomic practice of lumping is predominately used within the literature, as it promotes a conservative taxonomic framework. Ultimately, as mentioned above only three species concepts of *Ammonia* have

received lasting taxonomic recognition within the literature as detailed below (Walton and Sloan, 1990):

1) *Ammonia beccarii* (Linné, 1758)- An ornamented morphotype exhibiting distinct beading, fluting and furrowing along the sutures.
2) *Ammonia beccarii* forma *parkinsoniana* (d'Orbigny, 1839)- An unornamented morphotype with one or more umbilical bosses, this form lacks any beading and fluting along sutures and typically has 9 –11 compact chambers in the final whorl.
3) *Ammonia beccarii* forma *tepida* (Cushman, 1926). This morphotype is typically smaller, more lobate, and lacks an umbilical boss or any ornamentation.

The taxonomic practice of lumping and the use of open nomenclature within the literature was supported by the results of an experimental breeding study of *Ammonia* conducted by (Schnitker, 1974). This experiment illustrated that from culturing '*Ammonia tepida*-like specimens' that seven distinct morphotypes could be identified from its offspring. This led to the conclusion that *Ammonia beccarii* (Linné, 1758) is the only "valid" species concept and that all other morphological variants, were a product of ecophenotypy (Schnitker, 1974). The conclusions from this experiment has had significant ramifications on the taxonomic history of *Ammonia* as it provided the impetus for subsequent studies to only formally recognise a limited number of *Ammonia* species (Chang and Kaesler, 1974; Poag, 1978; Venec-Peyre, 1983; Malmgren, 1984; Wang and Lutze, 1986, Jorissen, 1988; Colburn and Baskin, 1998; Walton and Sloan, 1990; Yasssini and Jones, 1995; Buzas-Stephens et al., 2002). Whilst many of these studies have acknowledged a gradational series of intermediate morphologies, this test variability was perceived to be the product of different environmental conditions including: temperature, salinity, food availability, feeding strategies, oxygen availability, sediment type, substrate or biogeography (Poag, 1978; Venec- Peyre, 1983; Malgrem, 1984; Wang and Lutze, 1986; Jorissen, 1988; Walton and Sloan, 1990).

The conservative taxonomic framework and the proliferation of open nomenclature, especially the predominant use of the 'bucket' species concept of *Ammonia beccarii* is not without its criticisms (Billmann, 1980; Haynes, 1992). For example, Haynes (1992) suggests that the species concept of *Ammonia beccarii* has been misused, as many of the representations of *A. beccarii* within the literature bear little resemblance to the type description of *Nautilus beccarii* (Linné, 1758). In addition, Haynes (1992) highlights that the taxonomic practice of "lumping" does not

take into account the potential genetic diversity, therefore the understanding of distribution and ecological preferences may not be as robust as previously identified.

Considerable taxonomic uncertainty also surrounds *Ammonia* at the genus level, as species of *Ammonia* have also been assigned to different genera including: *Streblus* (Fischer de waldheim, 1817), *Rotalina* (Blainville, 1828), *Rotalia* (Lamarck, 1804), *Pseudoeponides* (Uchio, 1950) amongst others. As a consequence of the complex taxonomic history of *Ammonia* at both the species and genus level, calculating the total number of extant species of *Ammonia* is very difficult. Previous estimates of the total number of *Ammonia* species has ranged from 25 species (Walton and Sloan, 1990), to 46 species (Ellis and Messina, 1940), whilst currently 51 species are recognised within the world foraminiferal database (Hayward et al., 2015).

3.2.1 Molecular techniques provide new taxonomic perspectives

The introduction of molecular systematics has helped to clarify many of the taxonomic uncertainties surrounding *Ammonia*. The re-assessment of taxonomic relationships utilising molecular evidence based on LSU rRNA (large subunit of ribosomal DNA) and SSU rRNA (small subunit of ribosomal DNA) sequences has revealed previously unidentified diversity within *Ammonia* across the globe (Holzmann, 1996; Holzmann and Pawlowski, 1997; Holzmann et al., 1998; Holzmann, 2000; Holzmann and Pawlowski, 2000; Langer and Leppig, 2000; Ertran et al.,2004; Hayward et al., 2004; Toyofuku et al., 2005; Pawlowski and Holzmann, 2009; Schweizer et al., 2011). On the basis of this new molecular evidence, it is estimated that there are likely to be as many as 40 genetically distinct species of *Ammonia* globally (Foissner and Pawlowski 2009). This highlights that the recognition of broad species concepts and many ecophenotypes within the literature lacks a genetic basis (Hayward et al., 2004).

Phylogenetics has also paved the way for the re-evaluation of classical morphospecies concepts and their associated biogeographic distributions; these interspecific boundaries can be tested through the integration of multiple lines of taxonomic evidence. To date the most comprehensive integrated study of *Ammonia* was conducted by Hayward et al. (2004). This taxonomic investigation revealed that 13 genetically distinct species of *Ammonia* were identified globally (Figure 3.1). The subsequent morphological analysis of these newly identified genotypes revealed that after extended morphological analysis subtle morphological test characters could be used to discriminate between the genotypes.

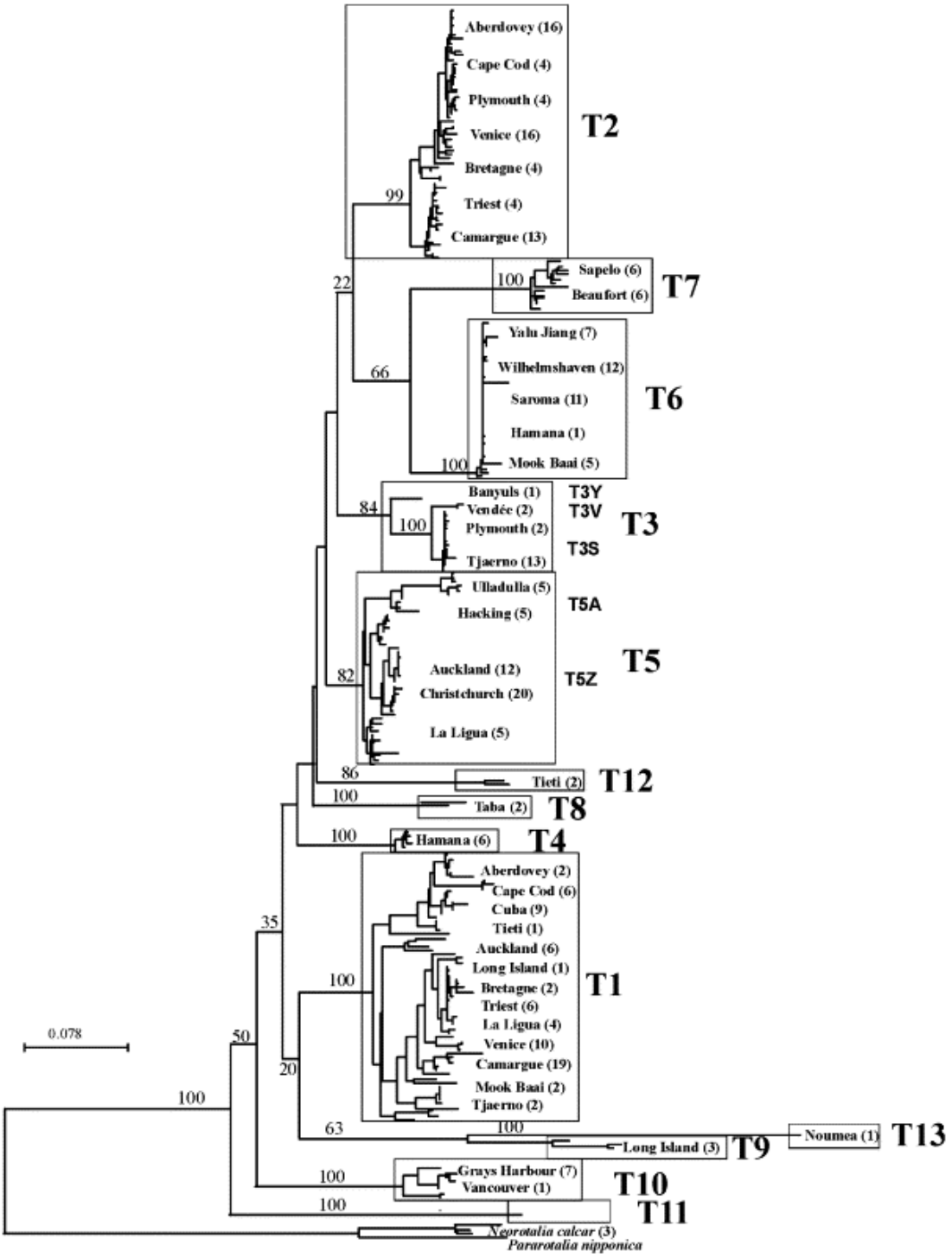


Figure 3.1 Phylogenetic tree of the 13 *Ammonia* genotypes (T1-T13) identified by the LSU rRNA collected worldwide by Hayward et al. (2004). The phylogeny is based on analysis of 267 partial LSU rRNA sequences using the Neighbour Joining method; the numbers shown on each of the branches illustrate the bootstrap percentage values based on 500 resamplings. Reproduced with the permission of the rights holder Elsevier from Hayward et al. (2004).

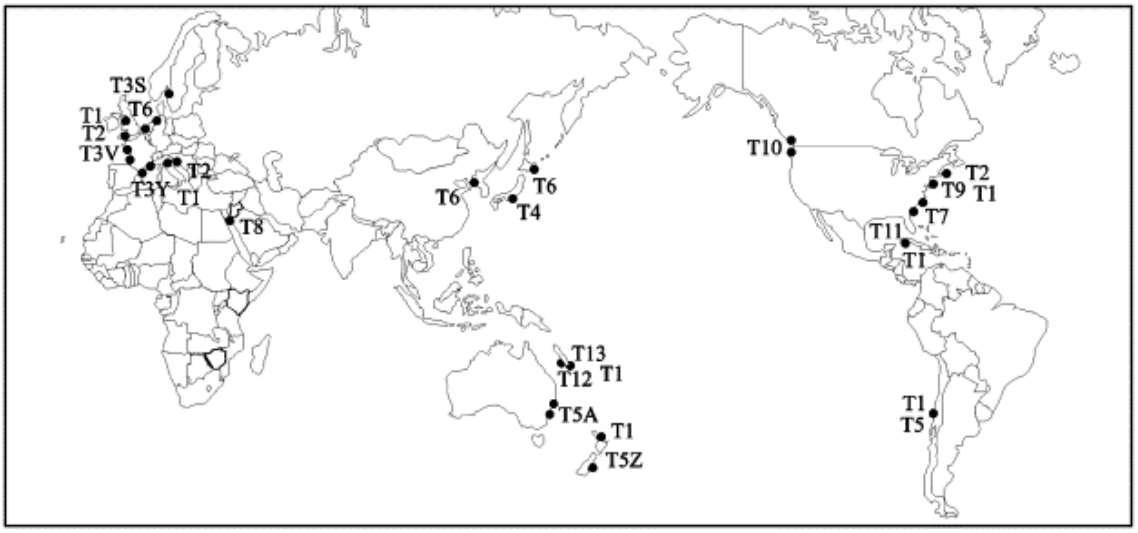


Figure 3.2 Global biogeographic distribution of *Ammonia* genotypes previously identified in Hayward et al. (2004). Reproduced with the permission of the rights holder Elsevier, taken from Hayward et al. (2004).

The analysis of the biogeographic distribution of *Ammonia* genotypes identified by Hayward et al. (2004) reveals that the majority of species exhibit a narrow biogeographic distribution (Figure 3.2). Only one *Ammonia* genotype (T1) has been identified as exhibiting a cosmopolitan distribution. Moreover, Hayward et al. (2004) revealed that up to two genetically distinct species of *Ammonia* can co-exist within a single site locality. This highlights that previous taxonomic investigations which delineate between *Ammonia* using classical morphospecies concepts may have overestimated the biogeographic distributions and/or have underestimated biodiversity and ecological preferences of *Ammonia.*

The integrated taxonomic investigation conducted by Hayward et al. (2004) has laid down the foundations for future taxonomic assignments and it provides a global overview of the genetic and morphological diversity found within the genus *Ammonia.* Nevertheless, whilst the study conducted by Hayward et al. (2004) represents an important step in the taxonomic re-evaluation of *Ammonia*, their study was not exhaustive. There are still some significant gaps in the taxonomic sampling regime, particularly within the NE Atlantic. Additionally, only a limited number of specimens of *Ammonia* have been jointly morphologically and genetically analysed at each site by Hayward et al. (2004). For example, although 178 specimens were morphometrically analysed in Hayward et al. (2004), only 79 of these specimens had an allied genetic sequence. Therefore it is unlikely that Hayward et al. (2004) have captured the entire range of interspecific and intraspecific morphological and genetic diversity within the genus *Ammonia.* Thus, the taxonomic re-evaluation of this genus is still within its relative infancy and

further taxonomic investigations are needed to assess the validity of the current genetic and morphological species boundaries identified in Hayward et al. (2004). Moreover, despite the significant taxonomic effort by Hayward et al. (2004), the taxonomic status of *Ammonia* within the literature remains controversial, as the classical morphological conservative framework with broad species concepts and open nomenclature continues to prevail. In part, this could be attributed to the uncertain nomenclatural placement of the newly delineated genotypes, and the current dearth of knowledge of the ecological preferences associated with each genotype. A concerted effort should therefore be given to improving our understanding of foraminiferal ecology, biology, morphological variability and biogeographic distribution of each of the extant genotypes of *Ammonia*; so that there is an improved understanding of the taxonomic 'value' of delineating between these genotypes in applied taxonomic situations.

In order to address some of these uncertainties and to clarify the interspecific boundaries, this chapter begins by examining the efficacy of morphology as a tool for discriminating between the seven genetically distinct species of *Ammonia* present in the NE Atlantic (these seven species were identified in Bird et al., in prep.). The detailed sampling regime employed within this study has provided an opportunity to develop a more comprehensive understanding of the biodiversity and biogeography of *Ammonia* within the NE Atlantic shelf seas. Additionally, it has enabled the collection of specimens from biogeographic regions not previously captured by Hayward et al. (2004). Moreover, the morphometric analysis of over 156 genetically sequenced specimens presented in this chapter provides an opportunity to analyse nearly double the number of genetically sequenced *Ammonia* specimens than has been previously analysed by Hayward et al. (2004). These new integrated lines of taxonomic evidence presented in this chapter are placed into a broader context, through examining the congruence of the interspecific morphological diversity and biogeographical distributions found in the NE Atlantic against the species boundaries identified in previous taxonomic investigations (e.g. Hayward et al., 2004; Schweizer et al., 2011). Overall, the integration of new lines of taxonomic evidence of *Ammonia* provided in this chapter presents an opportunity to elucidate taxonomic relationships and to develop a stable taxonomic platform from which extant and fossil specimens can be robustly and consistently identified.

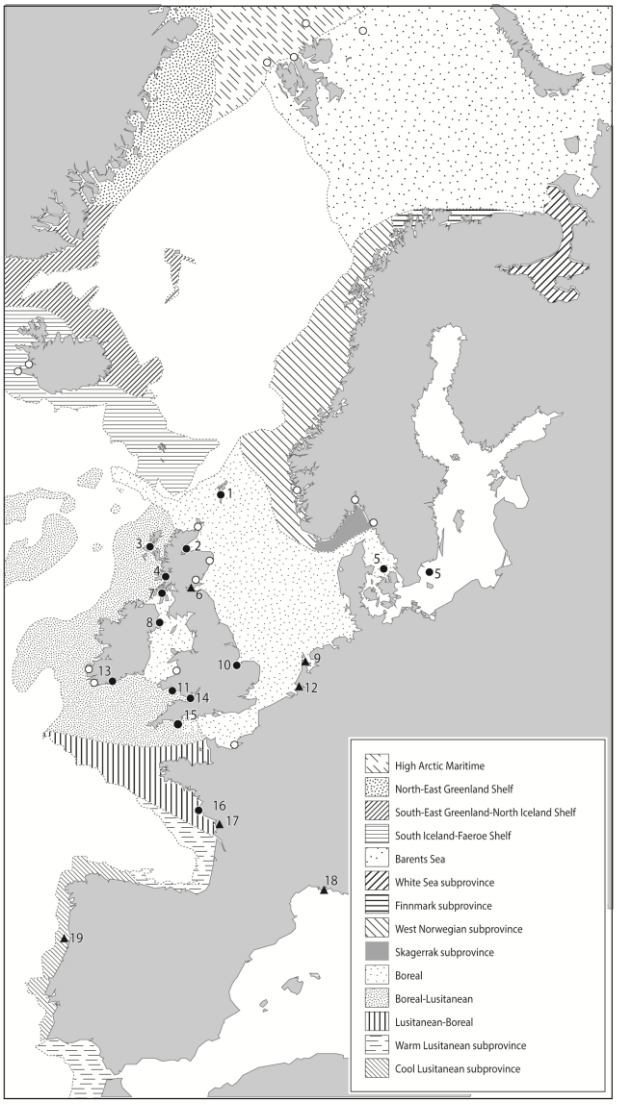
Aims

1. To test whether the seven genetically distinct genotypes of *Ammonia* identified in the North East Atlantic by Bird et al. (in prep.) can be robustly distinguished based upon their morphological characteristics.
2. To characterise in detail the interspecific and intraspecific morphological species boundaries of the seven genotypes and to identify any key diagnostic morphological features.
3. To evaluate the accuracy and utility of different numerical taxonomic approaches in accurately delineating specimens into their genetic groups.

3.3 Methods

3.3.1 Sample site localities

Live specimens of *Ammonia* were successfully identified from 19 site locations in the North Atlantic shelf seas (Figure 3.3, Table 3.1). A detailed description of the range of sampling techniques used to extract live specimens from intertidal and sub-tidal areas are described in Chapter 2, Section 2.2.1.



● Sample site locations where *Ammonia* specimens have been successfully morphologically and genetically characterised.

▲ Sample site locations where only genetic sequences of *Ammonia* are available for analysis.

○ Sample locations where specimens of *Ammonia* specimens were absent

Figure 3.3 Location map of sample site localities from which *Ammonia* specimens were collected across the NE Atlantic. The different symbols indicate sites where specimens of *Ammonia* have only been genetically analysed (filled triangles), site locations where genetic sequences and morphological data were available for analysis (filled circles) and site localities where *Ammonia* specimens were absent or genetic sequencing was unsuccessful (open cirlces). The biogeographic provinces depicted correspond to those identified by Dinter et al. (2001).

Table 3.1 Total number of *Ammonia* specimens genetically and morphologically characterised at each site and sub site locality. A brief site description of each sample locality is provided. Site location numbers correspond to the sites illustrated in Figure 3.3.

Location number	Location name and abbreviations	Sampling site	Site description	Total number of specimens genotyped in each site locality	Total number of specimens morphologically analysed in each site locality
1	Shetland (SH)		Sub-tidal sediment 12 metres	S5a (n=12)	S5a (n=1)
2	Cromarty (CR)		Inter-tidal sediment	S2 (n=1)	S2 (n=1)
3	North Uist (NU)	Bagh a Chaise, Sound of Harris IT5SW	Inter-tidal seaweed	S5a (n=13)	S5a (n=0)
		LPSW2	Seaweeds	S5a (n=3)	S5a (n=1)
		LM1B	Seaweeds	S5a (n=14)	S5a (n=0)
4	Loch Sunart (SU)		Sub-tidal sediment	S4 (n=1) S5a (n=2) S6 (n=3)	S4 (n=0) S5a (n=0) S6 (n=2)
4	Dunstaffnage (DF)		Sub-tidal sediment	S4 (n=1) S5a (n=11) S6 (n=8)	S4-(n=0) S5a (n=2) S6 (n=6)
5	Baltic (BA)	C-Ha-1-low salinity Hanö Bay	Sediment, 15-65m, 7-13 psu	S1 (n=18)	S1 (n=14)
		C-An-1-normal salinity Anholt Kattegat	Sediment, 12-30m, 18-32 psu	S5a (n=1)	S5a (n=0)
6	Torry Bay (TB)		Inter-tidal sediment	S1 (n=8)	S1 (n=0)
6	Cramond (Cd)		Inter-tidal sediment	S1 (n=52)	S1 (n=0)
7	Loch na Cille (LK)		Inter-tidal sediment	S2 (n=13) S5a (n=8)	S2-(n=12) S5a-(n=2)
8	White Rock (WR)		Inter-tidal sediment	S2 (n=18)	S2-(n=16)
9	Den Oever (F)			S1 (n=2)	S1 (n=0)
10	Norfolk (NF)		Inter-tidal sediment	S1 (n=30) S2 (n=2)	S1-(n=20) S2- (n=1)
11	Laugharne Castle (LC)		Inter-tidal sediment	S1 (n=2)	S1-(n=2)
12	Grevelingen (Gv)		Brackish lake, 34m	S1 (n=2)	S1 (n=0)
13	Cork (CK)		Estuarine inter-tidal sediment	S3 (n=28) S4 (n=2)	S3-(n=16) S4 (n=0)
14	Cardiff (CF)		Inter-tidal sediment	S1 (n=20)	S1 (n=14)

Location number	Location name and abbreviations	Sampling site	Site description	Total number of specimens genotyped in each site locality	Total number of specimens morphologically analysed in each site locality
15	Dartmouth (DM)	Upper shore	Inter-tidal sediment	S2 (n=6)	S2 (n=6)
		Mid shore	Inter-tidal sediment	S2 (n=12) S4 (n=2) S5a (n=1)	S2 (n=6) S4 (n=2) S5a (n=0)
		Lower shore	Inter-tidal sediment	S2 (n=49) S4 (n=2) S5a (n=14)	S2 (n=28) S4 (n=2) S5a (n=2)
16	Ile d'Yeu (Ye)		Inter-tidal sediment with seaweeds	S5b (n=10)	S5b (n=2)
17	Baie d'Aiguillon (Ai)		Inter-tidal sediment	S1 (n=2)	S1 (n=0)
18	Mediterranean (Rh/F)			S5a (n=4) S6 (n=3)	S5a n=0 S6-n=0
19	Portugal (Po)		Sand, 50m	S5a (n=17) S6 (n=2)	S5a-n=0 S6 (n=0)
Total numbers of specimens analysed				186	158

3.3.2 Foraminiferal preparation and molecular analysis

Live specimens of *Ammonia* were identified and extracted from the sediment samples following the methods laid out in Chapter 2, Section 2.3.1. The picked specimens were mounted onto SEM stubs; these were gold coated using a splutter coater to approximately 20nm thickness. The specimens were imaged using Scanning Electron Microscopy from both the spiral and umbilical views at the University of Edinburgh by collaborators from the NERC-funded project. Following SEM imaging, a 1000 base pair of the SSU rRNA from each specimen of *Ammonia* was extracted. This molecular analysis was conducted by the University of Edinburgh foraminiferal genetics laboratory. The molecular techniques employed followed those outlined by Schweizer et al. (2011). A total of 397 *Ammonia* specimens were genotyped with the partial SSU rRNA and these were aligned against 87 *Ammonia* partial SSU sequences present in the GenBank database to exhaustively define the genotypes and to compare against genotypes previously identified (Figure 3.4). The phylogeny was primarily constructed using a Bio Neighbor-Joining (BioNJ) tree with 1000 bootstrap (BS) replicates (Figure 3.4). Additionally, a Maximum likelihood (ML) analysis with 1000 BS replicates and Bayesian analysis (BA) were also conducted by Bird et al. (in prep.). The statistical support of all three of these phylogenetic analyses are shown in Figure 3.4.

The phylogenetic analysis revealed that seven genetically distinct species of *Ammonia* could be identified in the North East Atlantic shelf seas (Figure 3.4, Bird et al., in prep.).

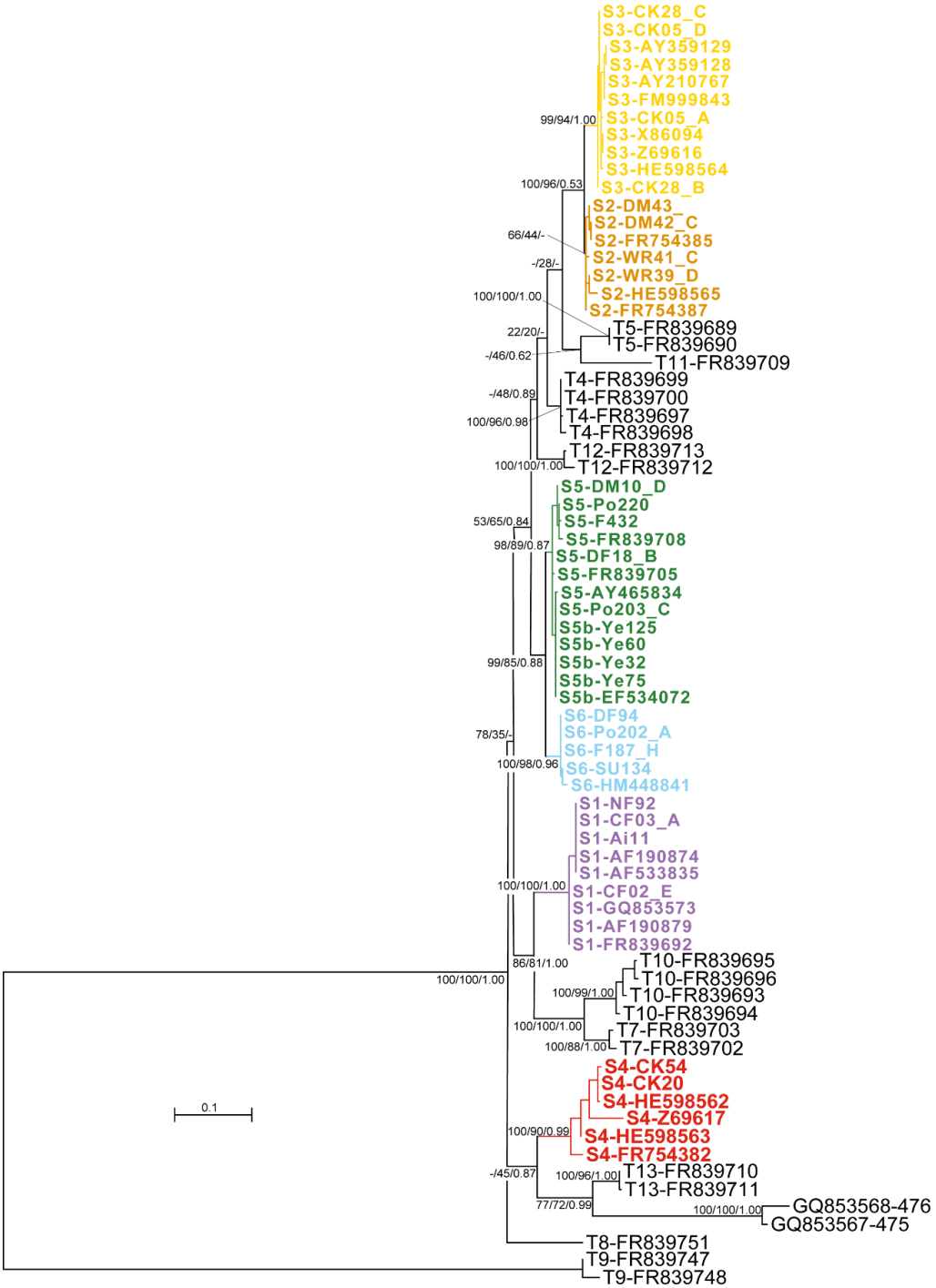
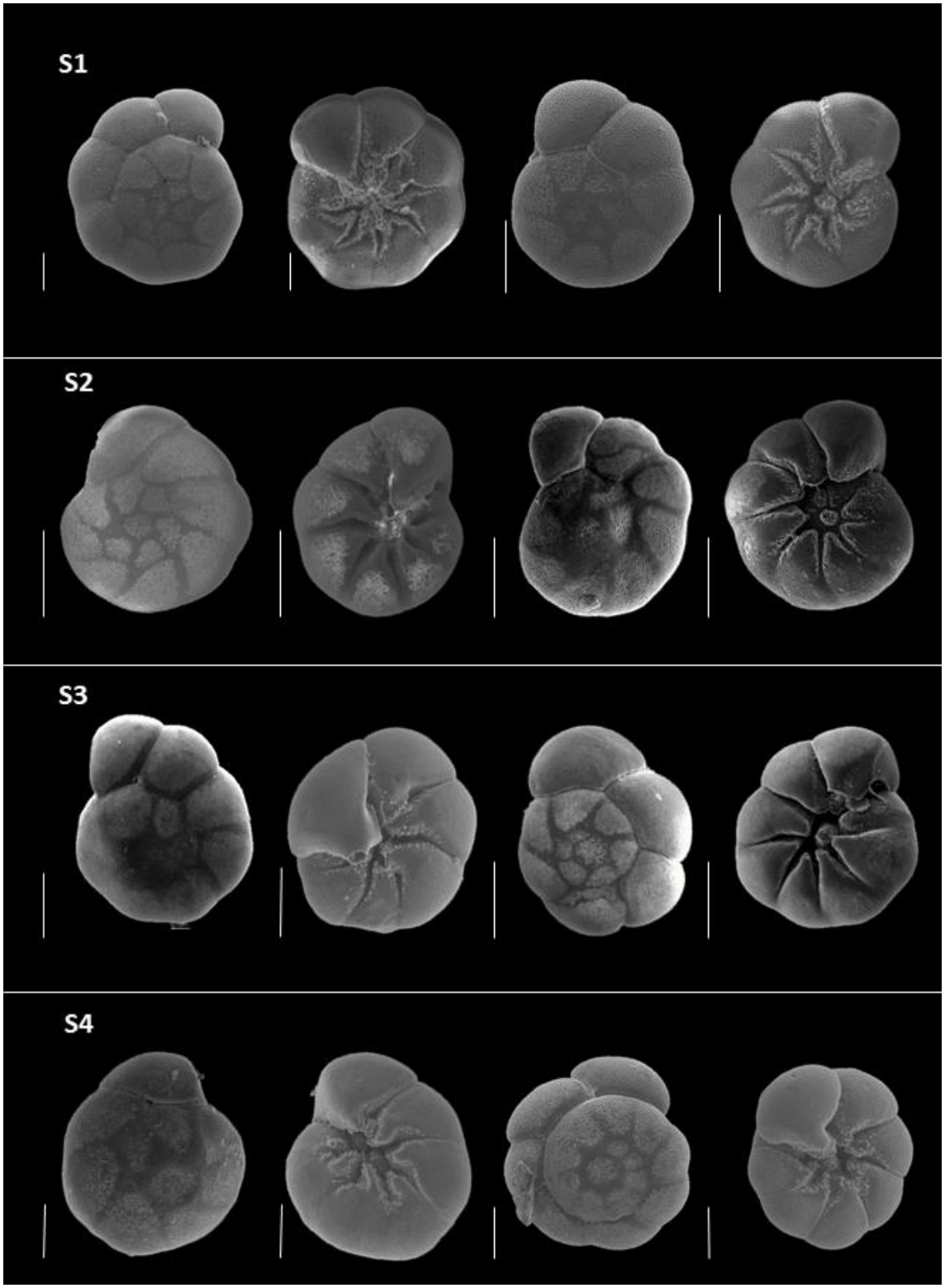


Figure 3.4 Phylogenetic tree of *Ammonia* based upon the SSU rRNA sequences, revealing seven distinct *Ammonia* genotypes S1-S7 (depicted in colour) in the NE Atlantic (Bird et al., in prep.). Numbers at the branches indicate the statistical support/bootstrap values of the BioNJ, Maximum Likelihood and Bayesian analyses. Phylogeny includes all the NE Atlantic genotypes together with 87 representative *Ammonia* sequences available in GenBank (shown in black).


S1
S2
S3
S4

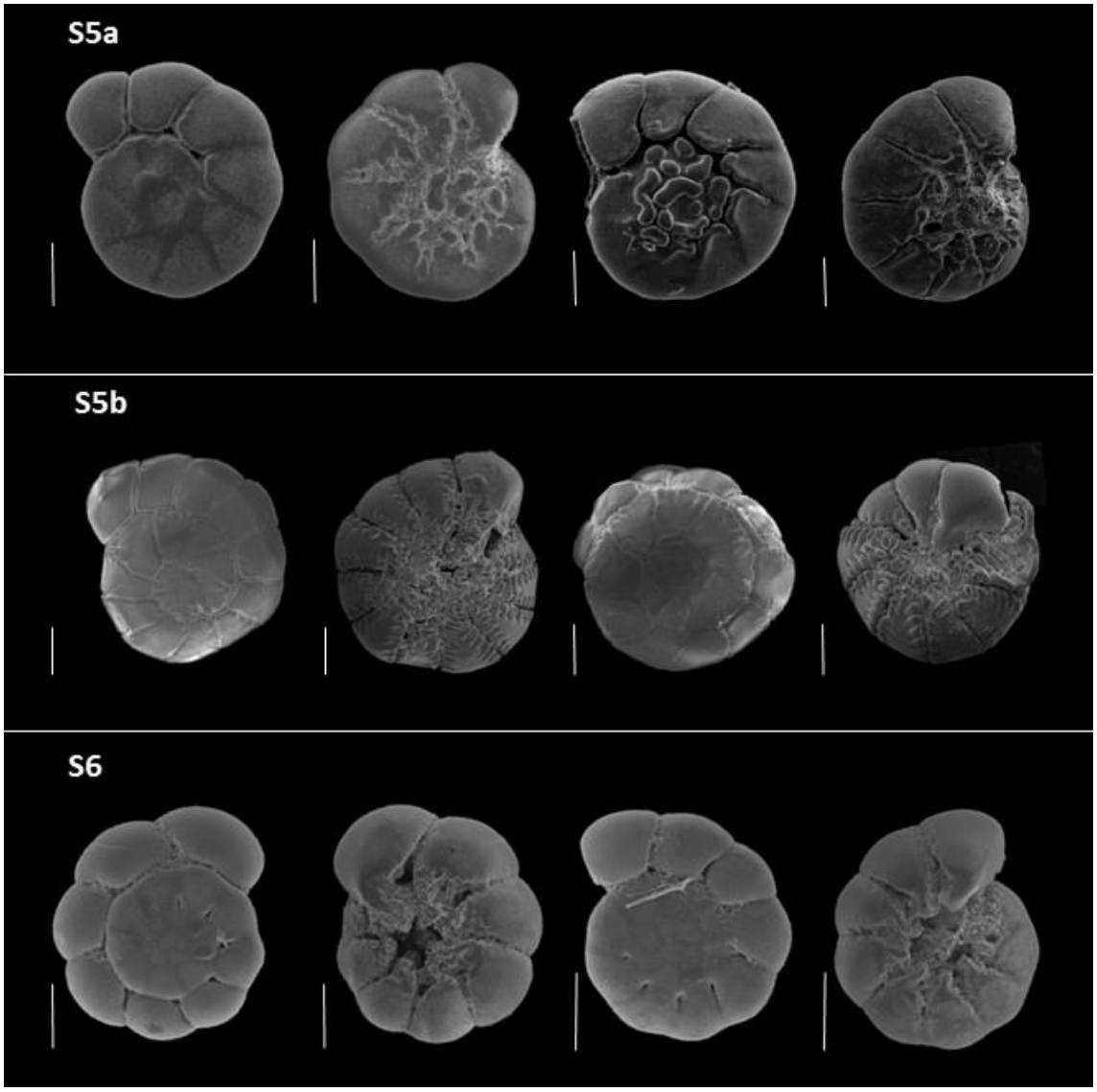


Figure 3.5 Representative umbilical and spiral SEM images of the seven *Ammonia* genotypes identified in Bird et al. (in prep.). The scale bar illustrated is 100 µm.

Figure 3.5 illustrates the typical morphology exhibited by each genotype on both the spiral and umbilical sides of the test.

3.3.3 Morphometric analysis

Prior to morphological analysis a series of image pre-processing procedures were conducted following the methods set out in Chapter 2, Section 2.5. The initial step in the image pre-processing procedures is an assessment of the suitability of the available SEM images for morphological analysis. Specimens were omitted from the morphometric analysis, if the specimen had not been SEM imaged from both the umbilical and spiral views. Specimens were also excluded if a significant proportion of the test was obscured or damaged. In situations where only minor obscuration or damage is exhibited, an infilling procedure was conducted following the methods set out in Hayward et al. (2004). In total 158 out of 186 genetically sequenced specimens were deemed suitable for analysis.

A total of 25 morphological test characteristics were measured and assessed from the combination of umbilical and spiral SEM views of each specimen (Table 3.2). The majority of morphological characters measured and assessed have been derived from Hayward et al. (2004, Table 2, Plate I, p. 247-248) with some minor modifications and omissions.

Table 3.2 Test characteristics measured or assessed from the umbilical and spiral SEM profiles of the *Ammonia* specimens. These morphological characteristics measured have been derived from Hayward et al. (2004) with some minor modifications. The qualitative five-point assessment utilised in this study includes: 1 -None, 2- Very weak, 3- Weak, 4 -Medium, and 5- Strong. The three-point scale utilised in this investigation includes: 1- Absent, 2- Moderately developed and 3 -Strongly developed. Chamber N is equivalent to the final chamber, whilst N1 is the penultimate chamber etc., as depicted in Figure 2.7.

Type of character	Variable Number	Corresponding variable in Hayward et al. (2004)	Morphological feature name	Method of measurement	Unit/ Category/ Type
			Umbilical Side		
Quantitative	1	7	Relative diameter of the umbilical area	Largest diameter of umbilicus between the ends of the folia/ maximum test diameter	Ratio
Quantitative	2	10	Relative maximum boss diameter	Maximum diameter of the largest umbilical boss (if present)/ maximum diameter	Ratio
Quantitative	3	11	Total number of umbilical bosses	Number of umbilical bosses (if present)	Count
Quantitative	4	n/a	Radial curvature of suture N1	Curvature of suture between chambers N1 and N2. This feature was calculated using the arc tool in Image Pro express	Angle
Quantitative	5	n/a	Mean pore diameter	Mean pore diameter of the ten pores nearest the junction between chamber N1 and chamber N2	Micrometres

Table 3.2 continued.

Type of character	Variable Number	Corresponding variable in Hayward et al. (2004)	Morphological feature name	Method of measurement	Unit/ Category/ Type
Quantitative	6	n/a	Total number of chambers visible	Total number of chambers visible/ maximum test diameter	Ratio
Quantitative	7	n/a	Relative width of radial suture	Maximum width of suture nearest to the umbilical area/ maximum width of suture at the test periphery	Ratio
Quantitative	-	n/a	Maximum test diameter	Maximum distance between two peripheral edges of the test	This feature was not directly used in the morphometric analysis.
Categorical	8	13	Degree of development of thickened calcite on folia	Development of thickened calcite on chambers N-N3	1 to 3 scale
Categorical	9	16	Degree of blunt, ragged folium	Blunt, ragged folium on chambers N- N3	1 to 5 Scale
Categorical	-	17	Sharp, pointed folia on chambers N-N3	Sharp, pointed folia on chambers N-N3. This feature was omitted from analysis due to strong similarity to variable number 11.	This feature was not directly used in the morphometric analysis.

Table 3.2 continued.

Type of character	Variable Number	Corresponding variable in Hayward et al. (2004)	Morphological feature name	Method of measurement	Unit/ Category/ Type
Categorical	10	20	Development of beading on the folia	Folia cut into flat beads by grooves on chambers N-N3	Presence/ absence
Categorical	11	18	Development of beading along the radial sutures	Development of strong beads along edge of radial sutures on chambers N-N3	1 to 5 scale
Categorical	12	14	Degree of ornamentation on folia	Coverage of folia by small pustules on chambers N-N3	1 to 5 scale
Categorical	13	19	Development of grooved notches	Development of grooved notches along radial edge of sutures	1 to 5 scale
Categorical	14	n/a	Development of small pustules radial edge of sutures	Development of small pustules along the radial edge of sutures in chambers N-N3	1 to 5 scale
			Spiral view		
Quantitative	15	22	Number of chambers in the first whorl	Total number of chambers in the first whorl	Count

Table 3.2 continued.

Type of character	Variable Number	Corresponding variable in Hayward et al. (2004)	Morphological feature name	Method of measurement	Unit/ Category/ Type
Quantitative	-	23	Total number of chambers in the second whorl	Total number of chambers in the second whorl	This feature was not directly used in the morphometric analysis.
Quantitative	16	26	Relative chamber proportions	Maximum length (parallel to the periphery) of chamber N1/ maximum width (perpendicular to periphery) of the chamber	Ratio
Quantitative	17	Modified variable 27	Test roundness	Test roundness has been calculated using the Image outline analysis tool in ImageJ software. To obtain this measurement a series pre-processing steps were conducted (see Chapter 2, Section 2.5.1 for further details)	0 to 1
Quantitative	18	29	Angle between radial and spiral sutures	Angle between radial and spiral sutures in Chamber N1	Angle

Table 3.2 continued.

Type of character	Variable Number	Corresponding variable in Hayward et al. (2004)	Morphological feature name	Method of measurement	Unit/ Category/ Type
Quantitative	19	28	Relative length of fissure	Length of fissure along the spiral suture (when present)/ maximum test diameter	Ratio
Quantitative	20	36	Mean pore diameter	Mean pore diameter of the 10 nearest pores to the junction between chamber N1 and chamber N2	Micrometres
Quantitative	21	37	Pore density	Pore density was calculated from total number of pores/ 100 sq. µm	Count
Quantitative	22	21	Proloculus diameter	Maximum diameter of the proloculus	Micrometres
Categorical	23	30	Development of radial suture furrows	Development of furrows along radial sutures (when present)	1 to 5 scale
Categorical	24	33	Development of thickened calcite	Development of raised thickened calcite over central spiral area	1 to 5 scale
Categorical	25	n/a	Development of secondary dorsal openings	Development of discrete non-continuous secondary dorsal openings	Presence (1) / Absence (2)

3.3.4 Multivariate statistical analysis

Prior to multivariate statistical analysis, the morphological characters were standardised between 0 to 1 to reduce the impact of variable scales; this was achieved by following the methodology outlined in Hayward et al. (2004). These standardised morphological traits were then used to assess whether the seven distinct genotypes of *Ammonia* in the North East Atlantic can be delineated based upon their test morphology.

Principal coordinate ordination analysis (PCO) and UPGMA cluster analysis were first used to assess the utility of morphological characters in delineating the genotypes, without *a priori* knowledge of genetic groupings. The PCO analysis was conducted in PAST version 2.17 (Hammer et al., 2001), and the dendroUPGMA software (Garcia-Vallve et al., 2010) was used to conduct the UPGMA cluster analysis.

Discriminant function analysis (DFA), a K-neighbour joining analysis (k-NN) and a classification and regression tree (CART) were also employed to establish the best combination of morphological traits, which can be utilised to distinguish these genotypes. The three multivariate classification techniques outlined above were performed in SPSS v.22. Initially, a DFA analysis with a leave one out cross validation procedure was conducted on the morphometric data using *a priori* knowledge of the genetic groupings. This classification procedure was employed despite some minor violations of the test's internal assumptions (e.g. departures from normal distribution), because previous investigations have identified that DFA is robust to violations of assumptions (Hammer and Harper, 2006).

A k-NN classifier technique was then employed. This nonparametric approach can discriminate between variables by assessing the similarity of a specimen to the nearest neighbour. The optimum K value was tested by cross validation analysis. The optimal model (K=3) which exhibited the smallest error is retained for analysis. Next, a classification and regression tree analysis (CART) was employed with a ten V-fold cross validation procedure. This is a non-parametric classification technique separates data into groups in a series of binary splits (Breiman et al., 1984). The Gini index was chosen as the measure for impurity (splitting criteria) and the optimal combination for node structure was a minimum of ten cases in the parent node and two cases in the child node.

3.4 Results

3.4.1 Interspecific morphological variation

A PCO analysis was employed to assess the utility of morphology as a tool for *Ammonia* classification without *a priori* knowledge of genetic groupings.

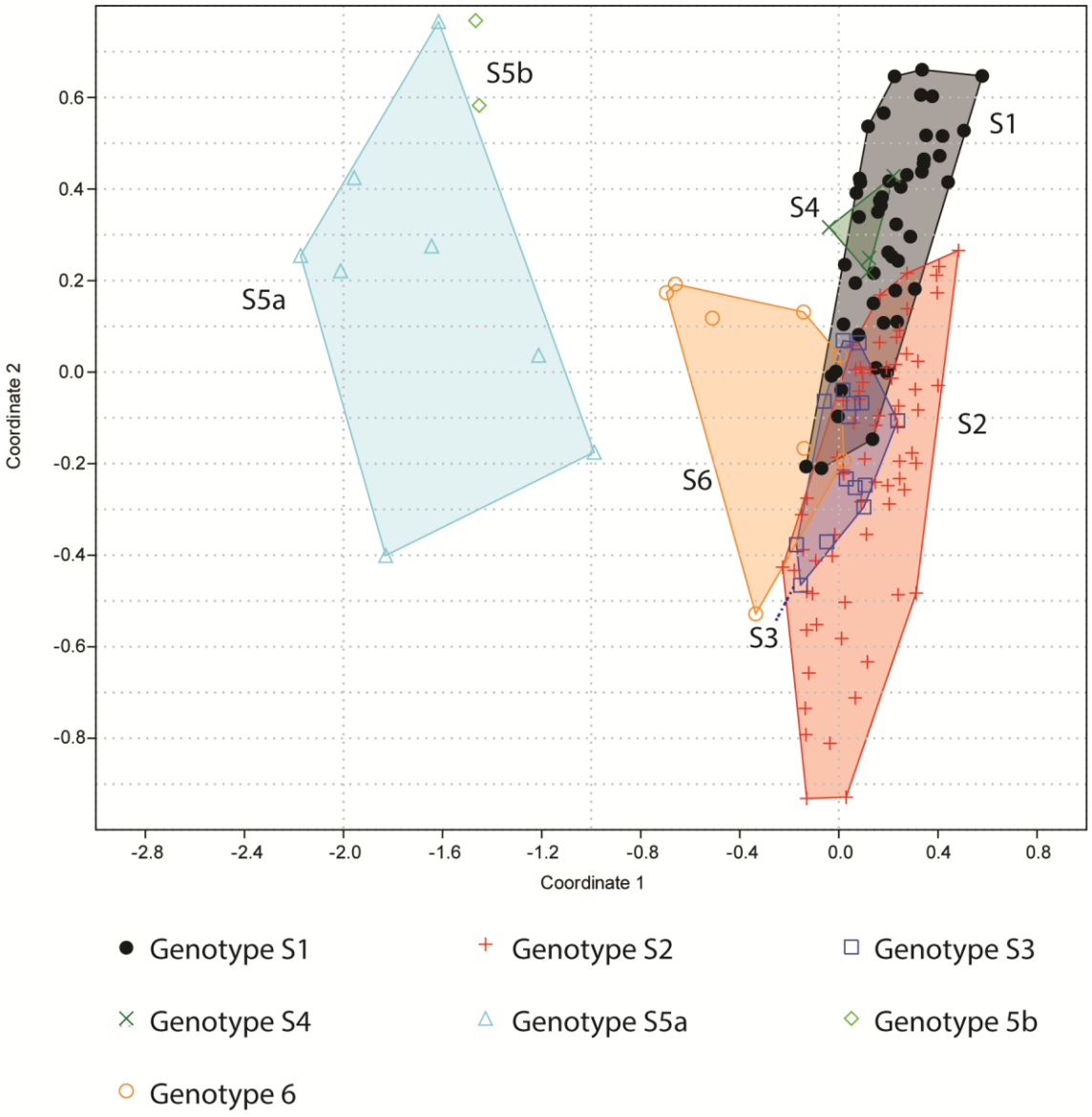


Figure 3.6 PCO analysis of morphometric data of the seven distinct *Ammonia* genotypes found across North East Atlantic. Each genetic group is bounded by a convex hull. The first two principal coordinates account for 35.6% of the total variation.

The PCO Illustrated that genotypes S5a and S5b can clearly be distinguished from genotypes S1, S2, S3, S4 and S6 in the PCO morphospace (Figure 3.6). In addition, despite low numbers of specimens these two forms can also be separated from each other. Unfortunately, no further

morphological differentiation can be detected, as extensive overlap is exhibited by the convex hulls of genotypes S1, S2, S3, S4 and S6 in the PCO morphospace (Figure 3.6).

A visual inspection of the SEM images reveals that genotypes S5a and S5b can be delineated based upon morphological character traits including the development of beading and fluting (as illustrated in Figure 3.7). In contrast, specimens of genotypes S1-S4 and S6 exhibit simpler morphologies with considerably less test ornamentation and morphological variation.

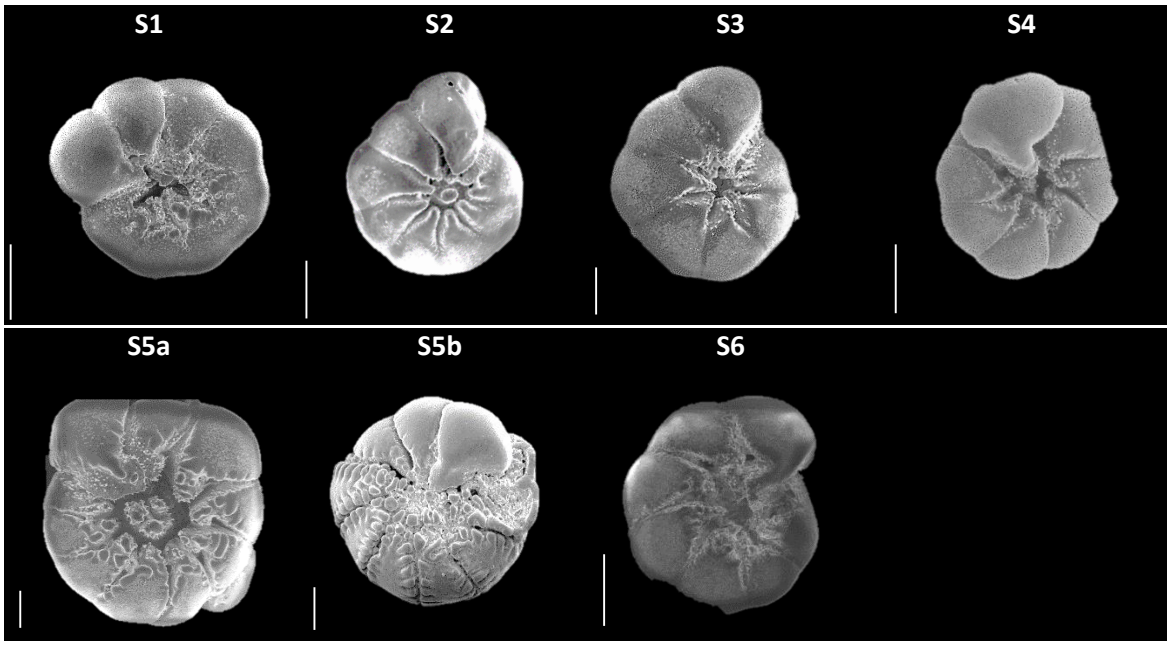


Figure 3.7 SEM images of representative specimens of seven *Ammonia* genotypes from the umbilical side. It is evident that genotypes S1-S4 and S6 can be separated from genotypes S5a and S5b by their ornamental features. The scale bars illustrated are 100 µm.

The UPGMA cluster analysis revealed that genotypes S5a, S5b and S6 are morphologically distinct from genotypes S1-S4 as they form discrete clusters within the cluster dendrogram (Figure 3.8). In comparison, no clear clustering patterns have been identified between the less ornamented genotypes S1-S4; this suggests extensive morphological overlap between these specimens.

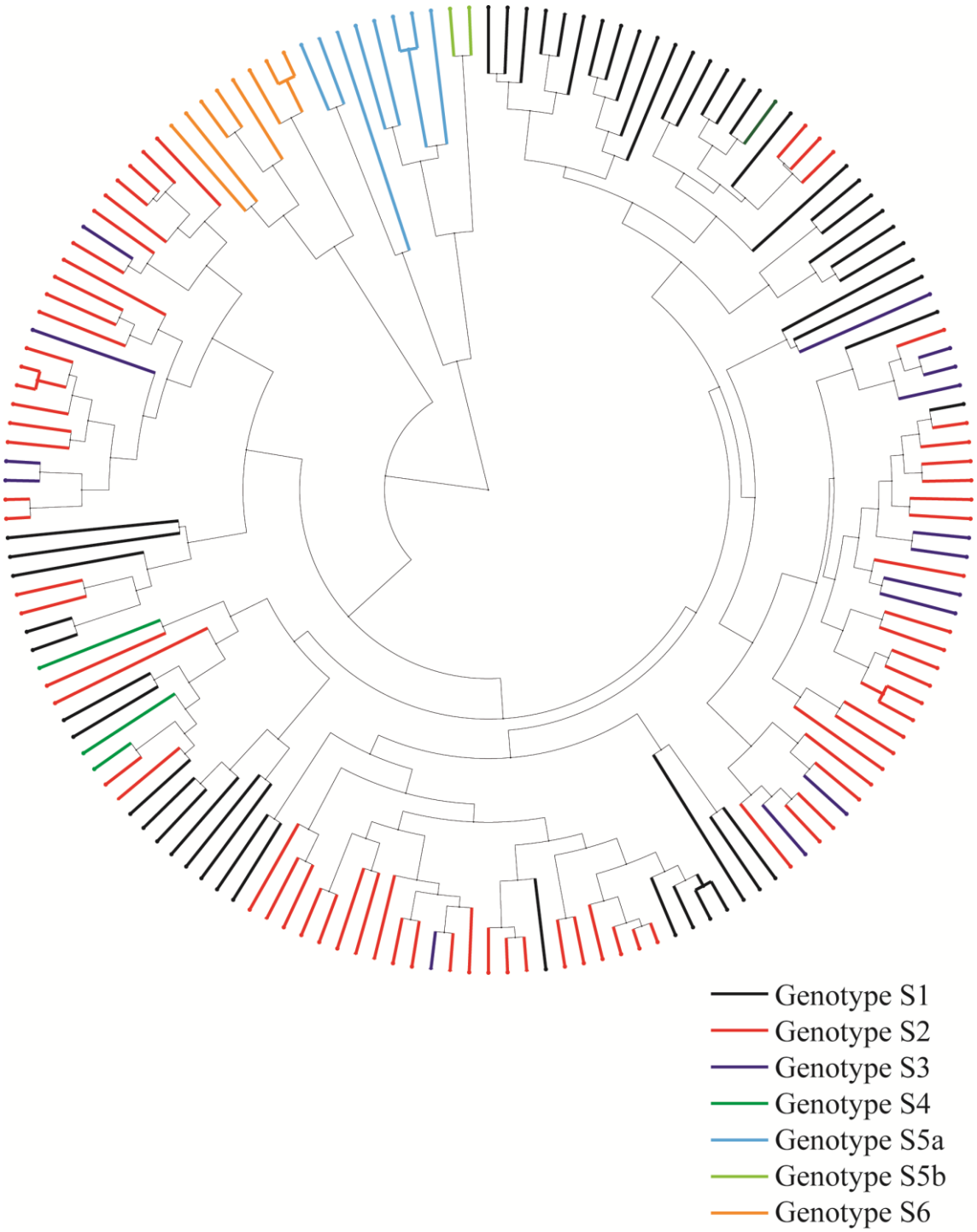


Figure 3.8 UPGMA cluster dendrogram based on the morphological characteristics of the seven *Ammonia* genotypes identified across the NE Atlantic (n=158).

The results of the UPGMA analysis (Figure 3.8) corroborates the findings of the PCO analysis, revealing that genotype S5a and S5b can be discretely separated based upon their morphological traits. The UPGMA analysis has also further elucidated the taxonomic relationship

between the less ornamented genotypes. For example, genotype S6 is distinctly separated within the cluster analysis tree.

In order to clarify the validity of the morphological separation of genotype S6 within the UPGMA analysis (Figure 3.8) a refined PCO analysis was conducted. This analysis omitted specimens from genotypes S5a and S5b because they were shown to be morphologically distinct (Figure 3.6).

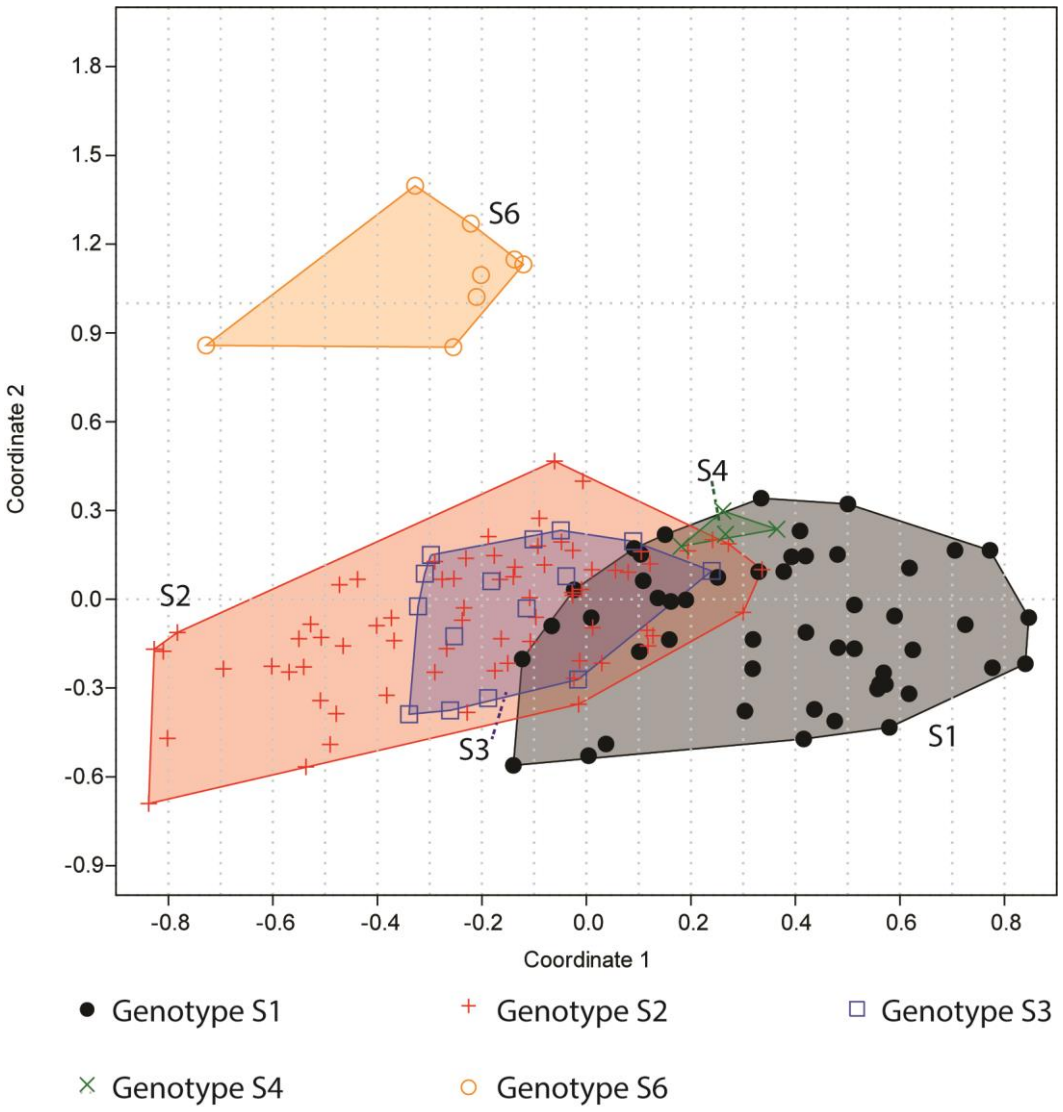


Figure 3.9 PCO analysis of morphological characters of *Ammonia* genotypes S1, S2, S3, S4 and S6. Each of the genotypes identified in Bird et al. (in prep.) is bounded by a convex hull. The two principal coordinates account for 28.8% of the total variation.

The refined PCO analysis illustrates that specimens from *Ammonia* genotype S6 form a discrete non-overlapping cluster, which is clearly distinct from the PCO morphospace occupied by genotypes S1-S4 (Figure 3.9). This revised delineation of genotype S6 within the PCO

morphospace (Figure 3.9) corroborates the results from the UPGMA cluster analysis (Figure 3.8). These results show that specimens of genotype S6 can be differentiated from genotypes S1-S4 based on morphology. This extended multivariate morphological analysis also reveals that no other genotype can be clearly delineated, as substantial morphological overlap is observed between genotypes S1-S4 within the PCO morphospace (Figures 3.6 and 3.9). Although it should be noted that whilst specimens of genotypes S3 and S4 are completely encompassed by the convex hulls of genotypes S1 and S2 in the PCO morphospace, these two genotypes do not exhibit any overlap with each other (Figure 3.9). It is also possible to identify a large number of specimens from *Ammonia* genotypes S1 and S2, which again do not exhibit any morphological overlap with any other genotype within the PCO morphospace; this illustrates that they exhibit potentially distinctive morphologies (Figure 3.9). Examples of the non-overlapping end member specimens of the two genotypes (S1 and S2) are illustrated in Figure 3.10. Some of the morphological features that can be used to delineate between these two genotypes include porosity, pore density, total number of chambers visible and the development of thickened calcite on the folia.

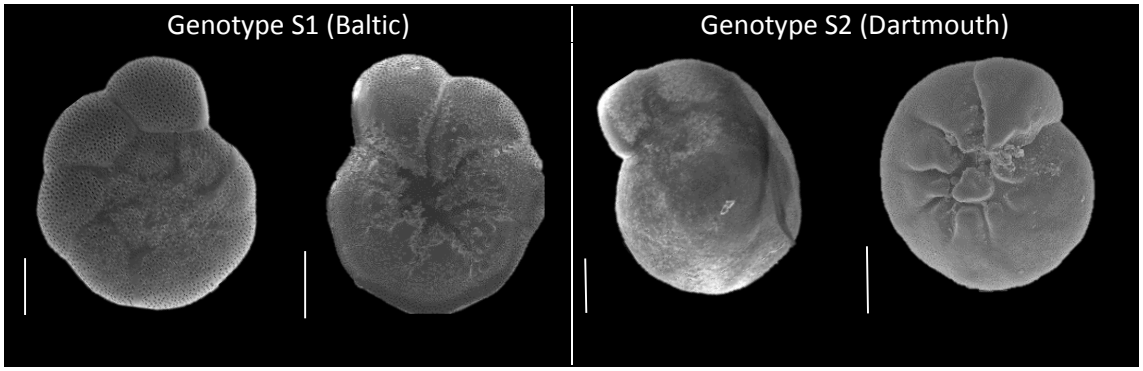


Figure 3.10 SEM images of the representative morphologies of the non-overlapping specimens of *Ammonia* genotypes S1 and S2 within the PCO morphospace (Figure 3.9). The scale bars illustrated are 100 µm.

3.4.2 Multivariate classification

A series of multivariate classification techniques including DFA, k-NN and CART analysis were conducted to assess the effectiveness of morphology in predicting group membership and to identify the diagnostic value of the morphological features analysed. Genotype S5b was excluded from the three multivariate classification procedures, because only two specimens were available for morphological analysis within this genotype.

3.4.2.1 Discriminant function analysis

The discriminant function analysis revealed that the six *Ammonia* genotypes could be delineated by their morphological attributes (Wilks: 0.001, significance p: <0.001). Overall, the DFA reveals

that 94.3% of *Ammonia* specimens were correctly classified into their genotype based upon their morphological test characteristics. The cross validation procedure correctly classified 89.2% of specimens based upon their morphological characteristics. From a total of 158 *Ammonia* specimens, seven specimens were misclassified in the DFA, and 24 specimens were misclassified in the cross validation analysis (Table 3.4).

Genotype	Percentage correctly classified	Percentage correctly classified after cross- validation
S1	98	94
S2	98.7	97.2
S3	80	66.7
S4	100	25
S5a	100	100
S6	100	50

Table 3.3 Percentage of *Ammonia* specimens correctly classified into their genotypes based upon their morphological characters in the discriminant function analysis and cross validation procedure.

Genotype S5a exhibits the highest assignment success based upon morphology, as all of the specimens were correctly classified in both the DFA and cross validation procedures (Table 3.3). Specimens from genotype S6 also exhibits perfect discrimination in the DFA based upon their test morphology. However, the cross validation procedure illustrates that four specimens of genotype S6 were incorrectly classified into other genotypes. This misclassification could be explained as a key discriminatory variable (presence of secondary dorsal openings) was omitted from the DFA, as it did not display enough variance between the groups. Thus, this indicates that even with the exclusion of a key morphological trait, this genotype can be successfully discriminated from other *Ammonia* genotypes based on its test morphology.

In contrast, the results of the DFA and cross validation procedure indicate that morphological separation between the less ornamented genotypes S1-S4 might be more challenging. Although the DFA illustrates that 80-100% of specimens from these four genotypes can be correctly classified, only 25-97.2% of specimens were classified into their correct groups in the cross validation procedure. The misclassification of specimens is evenly distributed between the four genotypes. This indicates that the interspecific morphological boundaries between these genotypes are not discrete and are gradational in nature. However, no morphological overlap

was observed between genotypes S3 and S4 (Table 3.4) suggesting that it may be possible to separate these genotypes from one another based on morphology.

	Discriminant function analysis					
	Predicted genotype					
Observed genotype	S1	S2	S3	S4	S5	S6
S1	**49**	1	-	-	-	-
S2	-	**70**	1	-	-	-
S3	-	3	**12**	-	-	-
S4	-	-	-	**4**	-	-
S5a	-	-	-	-	**8**	-
S6	-	-	-	-	-	**8**
	Cross validation procedure					
	Predicted genotype					
Observed genotype	S1	S2	S3	S4	S5a	S6
S1	**47**	2	1	-	-	-
S2	-	**69**	1	1	-	-
S3	-	5	**10**	-	-	-
S4	2	1	-	**1**	-	-
S5a	-	-	-	-	**8**	-
S6	-	2	1	1	-	**4**

Table 3.4 Confusion matrix of the number of *Ammonia* specimens correctly classified into each genotype in the DFA and the cross validation procedure.

The key diagnostic morphological variables identified by the DFA include a combination of ornamentation and structural features such as: development of thickened calcite on the spiral side (24), development of beads and grooves along the edge of the suture (10, 11), porosity features including pore density and pore diameter (5, 20, 21), degree of thickened calcite on folia (8), the development of radial sutural furrows (23) and test roundness (17). The morphological traits in brackets correspond to the variables described in Table 3.2 and presented in Table 3.7.

3.4.2.2 K-nearest neighbours classification (k-NN)

A k-NN classification analysis was used as a pattern recognition technique for the identification of morphological differences in the six *Ammonia* genotypes. The k-NN classification analysis yielded an overall correct classification rate of 84%. Specimens of genotypes S5a and S6 were perfectly discriminated based on their morphological attributes (Table 3.5).

Observed genotype	Predicted genotype						Percentage correctly classified
	S1	S2	S3	S4	S5	S6	
S1	**39**	8	3	-	-	-	78
S2	1	**68**	2	-	-	-	95.83
S3	-	8	**7**	-	-	-	46.7
S4	2	2	-	-	-	-	0
S5a	-	-	-	-	**8**	-	100
S6	-	-	-	-	-	**8**	100

Table 3.5 Confusion matrix obtained from classification by the k-NN analysis of the different *Ammonia* genotypes. Depicted are the number of *Ammonia* specimens correctly and incorrectly classified in the k-NN analysis.

In contrast, delineating between specimens using morphology within the less ornamented genotypes (S1-S4) proved challenging. For example, k-NN analysis indicates that all of the specimens within *Ammonia* genotype S4 were misclassified. Of the less ornamented forms, genotype S2 exhibits the highest correct classification assignment (95.83%). However, as specimens of genotypes S1, S3 and S4 were also misclassified into genotype S2, this indicates that the specimens within these genotypes exhibit gradational morphological features.

The key diagnostic morphological features identified by the k-NN analysis include porosity (average pore width and pore density), development of furrows along radial suture (23), presence of secondary openings (25), development of thickened calcite on the spiral side (24) and development of pustules on the folia (12). Less diagnostic weight has been placed upon: the total number of bosses (3), test roundness (17), relative chamber proportions (16), proloculus diameter (22), relative size of umbilical boss (2), development of pustules along suture (14) and the angle between radial and spiral sutures on the spiral side (18).

Overall the results from the k-NN classification analysis indicate that genotypes S5a and S6 can be robustly discriminated using their morphology, whilst considerable uncertainty remains around the interspecific morphological boundaries of the less ornamented genotype.

3.4.2.3 Classification and regression tree

Finally, a CART analysis was employed to assess the utility of morphology in delineating between the genotypes. In total 95.5% of the *Ammonia* specimens were correctly classified into their

respective genotypes based upon their morphology and 16.7% of specimens were misclassified in cross validation analysis.

Specimens from genotypes S5 and S6 can be robustly delineated by their morphological traits as evidenced by their 100% correct classification assignment (Table 3.6). The CART analysis illustrates that all cases of misclassification observed occurred between specimens within the less ornamented genotypes (75%-98% correctly assigned). The distribution of misclassified specimens is spread evenly across all of these genotypes, with the exception that no misidentifications were observed between genotypes S3 and S4.

Observed genotype	Predicted genotype						Percentage correctly classified
	S1	S2	S3	S4	S5a	S6	
S1	**49**	-	1	-	-	-	98
S2	-	**68**	2	1	-	-	95.8
S3	-	2	**13**	-	-	-	86.7
S4	1	-	-	**3**	-	-	75
S5a	-	-	-	-	**8**	-	100
S6	-	-	-	-	-	**8**	100

Table 3.6 Confusion matrix of CART analysis depicting the number of *Ammonia* specimens correctly classified into their respective genotypes based on their morphological characters.

The CART analysis identified a complex combination of structural and ornamental test characteristics that can be used to delineate between the *Ammonia* genotypes. These morphological traits include: porosity features such as pore diameter and pore density (5, 20, 21), development of furrows along radial edge (23), development of beading along the sutures (11), development of thickened calcite on the spiral side (24), number of chambers visible in final chamber (6), relative proportions of chamber N1 (16), relative diameter of umbilical area (1), and the presence of discrete secondary openings (25).

3.4.3 Morphological discrimination of the *Ammonia* genotypes

Overall, the results reveal that three *Ammonia* genotypes S5a, S5b and S6, can be perfectly delineated based on test morphology (Figures 3.6 and 3.8 and Tables 3.3, 3.5 and 3.6). From these three genotypes, only genotype S6 could be discriminated by a single discrete morphological test trait, the presence of secondary dorsal openings (as illustrated in Figure 3.5). The remaining genotypes were discriminated using a combination of structural and ornamental test characteristics (Table 3.7).

Table 3.7 Range of morphological character values measured and assessed for each of the *Ammonia* genotypes. The character numbers correspond to those listed in Table 3.2

Character	Diameter of umbilical area	Relative maximum boss diameter	Total number of bosses	Relative curvature of suture	Mean pore diameter	Total number of chambers visible	Relative width of suture
	1	2	3	4	5	6	7
S1	0.13-0.63	0-0.108	0-3	0-70.38	1.39-8.64	0.01-0.04	0.44-14.61
S2	0.16-0.44	0-0.197	0-3	0.39-84.78	0.33-2.02	0.02-0.05	1.09-12.13
S3	0.21-0.32	0-0.134	0-1	13.6-72.48	0.36-0.87	0.02-0.04	1.16-4.02
S4	0.20-0.39	0-0.103	0-1	9.8-45.67	0.79-1.85	0.02-0.03	4.06-9.90
S5a	0.18-0.36	0-0.208	0-3	24.5-55.46	0.84-1.36	0.02-0.03	1.61-4.01
S5b	0.34-0.482	0-0.126	0-0	2.45-61.57	0.38-0.7	0.02-0.04	2.08-11.38
S6	0.13-0.30	0-0.107	0-1	37.19-58.5	1.28-1.68	0.02-0.02	0.62-1.15
Character	Degree of calcium on folia	Degree of rugged folia	Degree of beading folia	Degree of beading along radial sutures	Degree of small pustules on folia	Degree of grooved notches	Degree of small pustules along radial edge
	8	9	10	11	12	13	14
S1	1-3	2-5	1-1	1-1	1-5	1-1	1-5
S2	1-2	1-5	1-1	1-1	1-5	1-1	1-5
S3	1-3	2-4	1-1	1-1	1-2	1-1	2-2
S4	1-2	2-3	1-1	1-1	2-3	1-1	2-3
S5a	4-5	1-3	1-2	2-4	1-3	1-4	1-2
S5b	2-3	1-3	1-2	5-5	2-3	5-5	1-2
S6	2-2	2-3	2-2	1-1	2-2	1-2	1-1
	Number of chambers in the first whorl	Relative chamber proportion	Test roundness	Angle between radial and spiral sutures	Relative length of fissure	Mean pore diameter	Pore density
	15	16	17	18	19	20	21
S1	6-11	3872.2-41929.9	0.76-0.98	61.88-205.24	0-1.39	1.00-4.23	0.54-11.35
S2	7-12	2015.7-20171.2	0.76-0.95	38.43-149.93	0-3.32	0.51-1.26	4.76-29.40
S3	8-9	3565.9-10088.6	0.76-0.87	56.66-100.40	0-0.447	0.78-1.29	6.06-17.35
S4	6-8	8195.9-12375.6	0.83-0.96	79.55-94.68	0.534-1.15	1.40-1.69	6.24-8
S5a	8-12	5388.5-18923.6	0.79-0.87	70.90-96.21	0.574-1.74	1.00-1.58	7.32-14.09
S5b	10-10	13996.6-20107.6	0.83-0.93	72.29-102.42	0-1.275	0.67-1.07	13.02-20.88
S6	5-10	4073.3-10359	0.77-0.84	38.88-84.99	1.326-2.197	1.07-1.19	9.17-11.42

Table 3.7 continued.

Character	Proloculus diameter	Degree of radial suture furrows	Degree of thickened calcite central area	Presence secondary dorsal openings
	22	23	24	25
S1	7.76-59.67	1-2	1-1	1-1
S2	17.51-71.34	1-2	1-1	1-1
S3	12.55-32.07	1-2	1-1	1-1
S4	44.65-66.11	2-2	1-2	1-1
S5a	38.42-86.71	4-5	1-3	1-1
S5b	20.26-52.97	2-3	1-1	2-2
S6	44.29-59.58	3-3	1-1	1-1

Genotypes S5a and S5b can be robustly distinguished from genotypes S1-S4 as these genotypes exhibit strong secondary calcite formation and test ornamentation. Genotype S5a can be morphologically distinguished based on a combination of morphological characters including the development of thickened calcite over the spiral central area (as depicted in Figure 3.5). This species also typically exhibits a more pronounced development of the radial sutural furrows than specimens from genotype S5b (Table 3.7). In addition, genotype S5a commonly possesses a number of umbilical bosses (0-3). In contrast, genotype S5b lacks a distinctive umbilical boss. Instead, genotype S5b seems to be distinguishable from genotype S5a by its stronger development of beads and grooved notches on the umbilical side, which sometimes extend all the way to the periphery of the test (as depicted in Figure 3.5, Table 3.7).

As developed previously the most difficult genotypes to discriminate morphologically are genotypes S1-S4, as they overlap in the PCO morphospace, UPGMA cluster analysis tree and in the classification procedures (Figures 3.6, 3.8 and 3.9 and Tables 3.3, 3.5 and 3.6) and exhibit gradational test characteristics (Figures 3.5 and Table 3.7). Notably these genotypes exhibit the least test ornamentation, possess a broadly rounded periphery and have a similar number of visible test chambers per whorl (Table 3.7). Genotype S1 can be distinguished from the others by its large pores (mean diameter 1.0-4.23 µm on the spiral side and 1.39-8.69 µm on the umbilical side, Table 3.7). In contrast, genotype S2 generally has smaller pores (mean diameter 0.51-1.26 µm on the spiral side and 0.33-2.02 µm on the umbilical side) and higher pore density (4-28 pores per 100 sq. µm). Both genotypes S1 and S2 also sometimes display the development of small pustules along the edges of umbilical sutures (often extending to the periphery) and

ornamentation on the folia, which can help to distinguish these species from genotypes S3 and S4. Genotype S3 commonly has the smallest pore diameters out of the least ornamented genotypes (e.g. mean pore diameter 0.39-0.87 µm on the umbilical side) and rarely exhibits the development of small pustules along the umbilical sutures. Genotype S4 typically exhibits slightly lower pore density in contrast to other three *Ammonia* genotypes (6-8 pores per 100sq. µm) and commonly has larger pores than genotypes S2 and S3 (mean pore diameter 1.40-1.69 µm). In addition, genotype S4 always possesses a fissure on the spiral side, although this feature it is not always strongly developed. Finally, genotype S4 also rarely possesses an umbilical boss, and when a specimen does present this feature, the bosses are small and are often depressed in the umbilical region. This genotype also sometimes exhibits very weak to weak secondary calcite on the spiral area (Table 3.7).

3.4.4 Intraspecific morphological variability

The integration of molecular techniques into the taxonomic investigations of *Ammonia* has also increased the capacity to identify intraspecific taxonomic relationships within this genus. By performing extended quantitative morphological analysis on genetically sequenced specimens of *Ammonia* collected from across distinct biogeographical provinces in the NE Atlantic, this study has provided a unique opportunity to reassess the relationship between intraspecific morphological variability and environment. Specimens of *Ammonia* genotype S1 were employed as a case study to identify whether any morphological differences could be elucidated across the species biogeographic range. Two multivariate statistical analyses were employed to assess this relationship. Firstly, a PCO analysis was conducted to assess the degree of intraspecific morphological variability of *Ammonia* genotype S1 specimens without any *a priori* knowledge of their sample site locations.

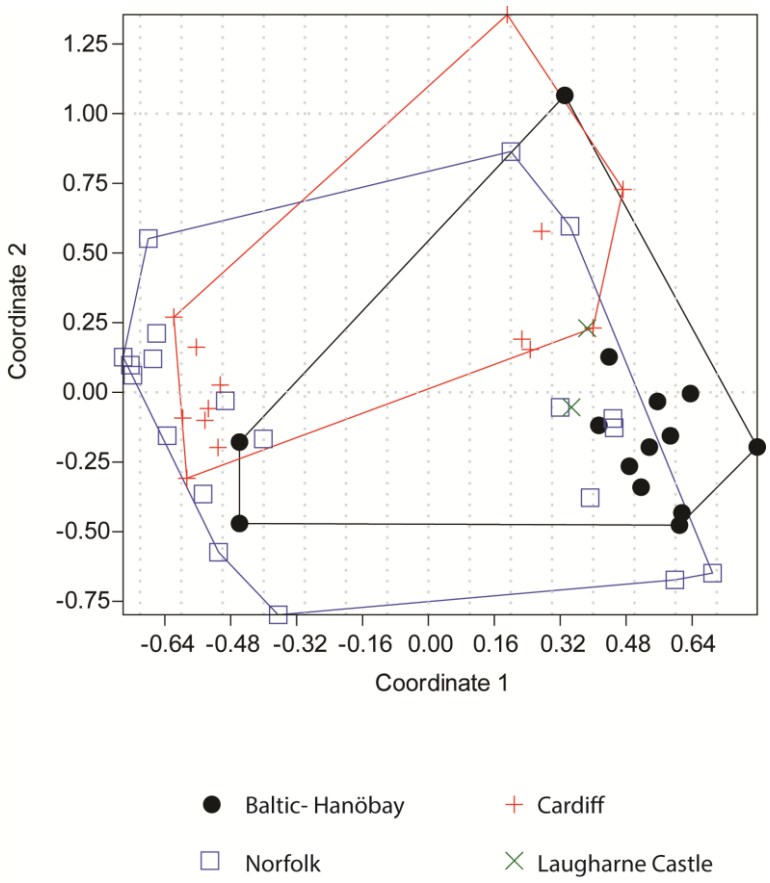


Figure 3.11 PCO analysis of the morphological characters measured and assessed from specimens of *Ammonia* genotype S1 taken from four distinct sampling localities. The first two principal coordinates account for 35.6% of total variation.

The PCO analysis identified that the specimens clustered into two distinctive groups of *Ammonia* genotype S1 within the PCO morphospace, these groupings are seemingly unrelated to site locality (Figure 3.11). A visual examination of the morphological features of specimens within each cluster revealed that they separated based on the occurrence of the presence/absence of a very weak spiral fissure (Figure 3.12). Although no discernible morphological differences were identified between the site locations, it is important to note that the separation of specimens based on the presence of weak spiral fissure could be an ecophenotypic trait.

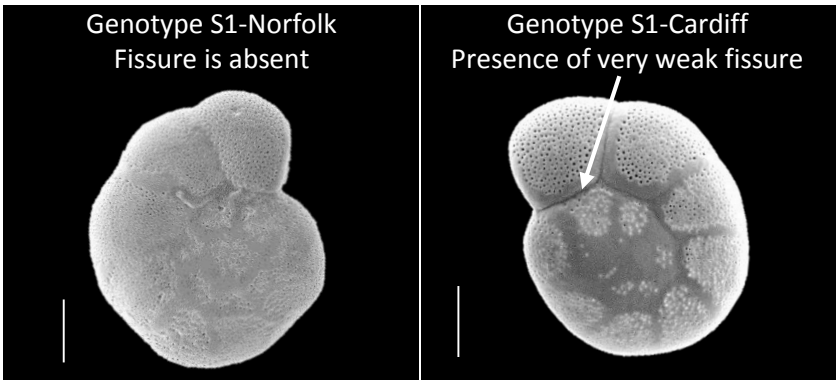


Figure 3.12 Examples of specimens of *Ammonia* genotype S1, which can be delineated by the morphological feature of development of fissure on the spiral side (as separated in the PCO morphospace, Figure 3.11). A) Specimen exhibits no fissure, B) Specimen exhibits a very weak fissure on the spiral side. Scale bar depicted is 100 µm.

A CART analysis was also conducted to explore whether the PCO analysis placed too much diagnostic weight on a singular morphological trait, therefore obscuring any morphological differences between the site localities. The CART analysis classified specimens using the site locality as the *a priori* groupings.

The CART analysis reveals that 98% of specimens can be correctly classified into their site locality using their morphology. Specimens from the Baltic, Norfolk and Laughcarne Castle site localities exhibit perfect classification (Table 3.8). In addition, the majority of specimens from Cardiff could be robustly delineated (92.9% correctly classified). One specimen from Norfolk was misclassified into the Cardiff site locality (Table 3.8). Examples of the some of the intraspecific morphological variability observed by specimens collected from different sampling localities are illustrated in Figure 3.13.

Observed site locality	Predicted site locality				
	Baltic	Cardiff	Norfolk	Laughcarne Castle	Percentage correctly classified
Baltic	**14**	-	-	-	100
Cardiff	-	**13**	1	-	92.9
Norfolk	-	-	**20**	-	100
Laughcarne Castle	-	-	-	**2**	100

Table 3.8 Confusion matrix of the total number of *Ammonia* genotype S1 specimens correctly predicted into their site locality based upon their morphological characteristics in the CART analysis.

It is important to note that the predictive accuracy of the final tree is poor, as the risk estimate in the 10 V-fold cross validation procedure is 0.46; thereby indicating that on average 46% of specimens were misclassified across the sub-samples (classification trees) generated. This highlights that the patterns of intraspecific morphological variability identified in this study may not be completely robust, owing to the low sample sizes.

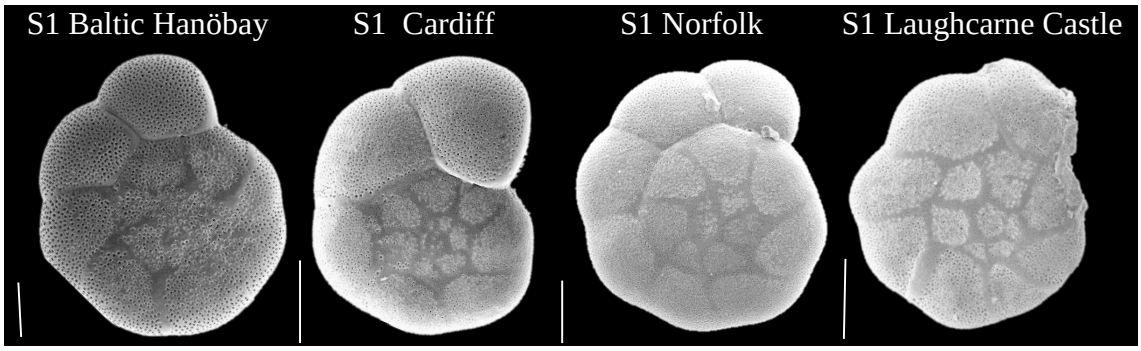


Figure 3.13 Spiral SEM images of *Ammonia* genotype S1 specimens from different site localities. The scale bar shown is 100 µm.

The CART analysis places strong diagnostic weight on morphological features including porosity including average pore diameter (5, 20), pore density (21), relative chamber proportions (16), total number chambers visible (6), test roundness (17) and angle between radial and spiral suture (18) to delineate between specimens taken from different site localities. Additionally, the relationship between pore characteristics and site locality was further explored using a Kruskal Wallis test. The results revealed that there are statistically significant differences (p: <0.001) between the pore characteristics of *Ammonia* genotype S1 specimens taken from different site localities. As illustrated in Figure 3.14 specimens from the Baltic site locality exhibit considerably larger pores in contrast to specimens from other site localities. In addition, it should be acknowledged that a large proportion of specimens from the Baltic also exhibit test deformations (e.g. significant signs of etching), but these morphological traits were not quantitatively measured in this study.

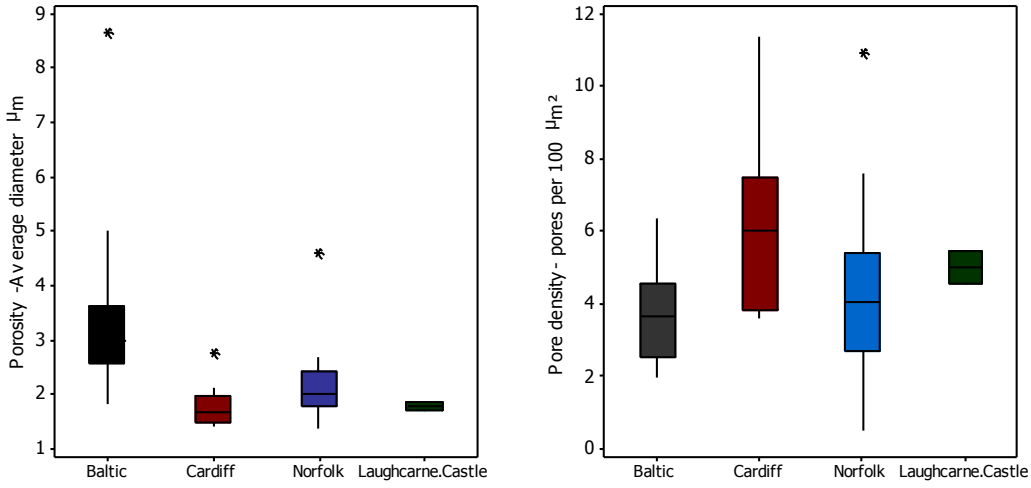


Figure 3.14 Box plots of the range of pore characteristics of *Ammonia* genotype S1 between the four different site localities, A) average pore diameter (µm), (B) pore density (pores per 100 µm²)

3.5 Discussion

3.5.1 Reconciling morphology and molecules within the genus *Ammonia* in the NE Atlantic

Despite extensive taxonomic investigation conducted on the genus *Ammonia* over the past 250 years, the efficacy of morphology as a tool for species delineation remains poorly understood. To clarify the effectiveness of morphology as a tool for species delineation, this chapter has directly compared the interspecific taxonomic boundaries identified by quantitative morphological analysis against the seven distinct genotypes identified across the NE Atlantic by Bird et al. (in prep.). The results reveal that there is good congruence between morphological and molecular lines of taxonomic evidence in three out of the seven *Ammonia* genotypes; genotypes S5a, S5b and S6 exhibit almost perfect discrimination (Figure 3.7, Tables 3.3, 3.5, 3.6). These genotypes can be robustly distinguished based upon their morphological traits.

In contrast, it is harder to delineate between the less ornamented genotypes (S1-S4) based upon morphology. Although a significant proportion of specimens from these genotypes can be successfully delineated by morphology, the morphological boundaries between these genotypes are not always discrete (Figure 3.9). This is evidenced by the incorrect classification of a number of specimens from each of the less ornamented genotypes, even after extended morphological analysis (Tables 3.3, 3.5 and 3.6). Genotypes S3 and S4 have been identified as some of the most challenging specimens to delineate based upon morphology, as these two genotypes exhibit high degrees of morphological overlap with genotypes S1 and S2 (Figure 3.9). This mismatch between molecules and morphology, even after extended morphological analysis highlights that some of the specimens within genotypes S1-S4 are in practical terms morphologically cryptic. Thus, in an applied taxonomic situation it would be difficult to delineate robustly between these genotypes based upon their morphological traits. The results reveal that there is no clear relationship between genetic divergence and morphological variability within this genus. Figure 3.4 highlights that genotypes S3 and S4 are clearly genetically distinct from genotypes S1 and S4, yet these genotypes exhibit overlapping morphological test characteristics. Overall, the results reveal that morphology can be an effective tool for delineating species of *Ammonia.* However, the results underscore the necessity for employing multiple lines of taxonomic evidence for re-evaluating taxonomic boundaries within this genus, because morphology on its own is insufficient for elucidating diversity. This contrasts the previous taxonomic investigations, which identified that all of the newly delineated genotypes can be morphologically discriminated (Hayward et al., 2004; Schweizer et al., 2011), as discussed in Section 3.5.4.

3.5.2 The relationship of molecules and morphology between genotypes S5a and S5b

Limited genetic divergence was identified between genotypes S5a and S5b (Bird et al., in prep); however, these two genotypes exhibit clearly distinctive morphologies (Figures 3.5, 3.8 and 3.9). For example, genotype S5b has more extensive test ornamentation including: beading, pustules, ribbing on both the spiral and umbilical sides, compared to the test ornamentation exhibited by genotype S5a (Table 3.7, Figure 3.5). The strong genetic similarity yet high morphological diversity raises the question of what mechanisms are driving this divergence?

It could be asserted that these genotypes diversified as an adaptation to localised environmental conditions, as they were identified at adjacent site localities (Figure 3.15). The differences between the two genotypes may have adaptive or functional significance. For example, test ornamentation including beading and pustules is important for feeding strategies in other foraminiferal species (e.g. Bernhard and Bowser, 1999; Austin et al., 2005; Khanna et al., 2013). It could be speculated that the different morphologies exhibited by these genotypes could be functional adaptations to different selective environments. However, the functional significance of test morphology of these two genotypes remains unresolved and further ecological investigation is needed.

Moreover, it is crucial that morphological variability of these two species is analysed at a population level in order to elucidate the morphological range exhibited by each genotype. This is important, as only two specimens of genotype S5b were available for the quantification of the interspecific morphological boundary. Therefore, the diagnostic criteria in this study may need to be revised in the future with the addition of new specimens. Intensive sampling coupled with environmental surveys and/or foraminiferal culturing experiments are required in order to ascertain the potential niche differentiation of these two genotypes.

3.5.3 Comparison of multivariate statistical analysis for morphological classification of *Ammonia* genotypes

In this chapter a series of multivariate statistics were applied to the quantitative morphological data, in order to examine their efficacy as tools for robust species classification. The three classification procedures (CART, DFA and k-NN) employed in this study were identified as the most efficient statistical tools for species delineation over the exploratory statistical techniques (PCO and UPGMA analyses). This is because these classification techniques successfully delineated a greater number of specimens into their genotypes based on morphology. The greater efficacy of classification techniques over exploratory statistical techniques is not

unprecedented, as these approaches often fail to identify subtle morphological differences, as they place greater significance on gross morphological features (Metrani, 2005). However, the value of the exploratory statistical techniques comes to the fore when no *a priori* knowledge of the genetic groupings is available (Jaiswara et al., 2013). Thus this technique is useful for classifying fossil specimens based upon their morphological test characteristics.

In this study the optimal classification technique for species delineation was identified as the CART analysis; this technique correctly assigned the highest number of *Ammonia* specimens (95.3%, correctly assigned). This non-parametric procedure can be applied to a wide range of morphological datasets, because it does not assume any specific data distributions and it is also not strongly affected by outliers or missing data (Breiman et al., 1984; Vayssieres, 2000). This is important as morphometric/ ecological data rarely satisfies the inherent assumptions of many of the classification procedures used in systematics e.g. such as those found within DFA (McGarigal et al., 2000; Tabachnick and Fidell, 2007). However, the high fidelity between species delineations identified by the DFA and the CART analysis reveals that despite a number of minor violations to the DFA's internal assumptions such as multivariate normality, this statistical approach can successfully handle these violations. This supports the findings of previous investigations (Lachenbruch, 1975; Klecka, 1980; Marcus, 1990; McGarigal et al., 2000; Karels et al., 2004; Tabachnick and Fidell, 2007; Kovarovic et al., 2011). The least efficient multivariate statistical technique for species delineation was identified as the k-NN classification analysis, as only 83% of the specimens were correctly classified (Table 3.6). This non-parametric statistic could be viewed as a more conservative approach to species delineation, perhaps because it is not as robust at delineating between specimens with small sample sizes (as previously identified by Fukunaga, 2013).

3.5.4 Congruence of the interspecific morphological boundaries of *Ammonia* in the NE Atlantic to the existing taxonomic framework

Taxonomy is an iterative process; species boundaries are revised and refined with the addition of new taxonomic evidence. Prior to the taxonomic investigation presented in this chapter, the most comprehensive integrated taxonomic study produced to date was conducted by Hayward et al. (2004). Hayward et al.'s (2004) study revealed that 13 distinct genotypes of *Ammonia* can be delineated based upon an examination of 37 quantitative morphological characters. The re-evaluation of interspecific morphological boundaries presented in this chapter illustrates that whilst the majority of specimens can be robustly delineated; there are some partially

morphologically cryptic specimens. This study contrasts the findings of Hayward et al. (2004), as their study notes that "all molecular types can be discriminated based upon their morphology" (p.259).

Previously unrecognised genetic diversity could account for the differences in the morphological boundaries between this study and those of Hayward et al. (2004) (Table 3.9). New molecular sequencing of *Ammonia* specimens across the NE Atlantic has uncovered the presence of seven genetically distinct species (Bird et al., in prep.). In contrast, Hayward et al. (2004) previously identified four genetically distinct genotypes of *Ammonia* from within this region. The higher levels of genetic variability identified by recent molecular analyses could be attributed to differences in examining SSU rRNA (Schweizer et al., 2008; Bird et al., in prep.) in contrast to LSU rRNA sequences (Hayward et al., 2004). For example, Bird et al. (in prep.) have identified two distinct genotypes from within Hayward et al.'s (2004) *Ammonia* T2 genotype (Table 3.9). Moreover, Bird et al. (in prep.) have identified an additional three genetically distinct species of *Ammonia* from within the T3 genotype of Hayward et al. (2004). This genetic delineation corroborates the phylogenetic results of Schweizer et al. (2011) who identified the presence of more than one genotype from within Hayward et al.'s (2004) T3 genotype.

Genotypes identified in Bird et al. (in prep.) (SSU rRNA)	Genotypes identified in Hayward et al. (2004) (LSU rRNA)
SSU S1	LSU T6
SSU S2	LSU T2
SSU S3	LSU T2
SSU S4	LSU T1
SSU S5a	LSU T3
SSU S5b	LSU T3
SSU S6	LSU T3

Table 3.9 A comparison of the SSU *Ammonia* genotypes identified in the NE Atlantic by Bird et al. (in prep.) in contrast to the corresponding LSU genotypes identified by Hayward et al. (2004).

The interspecific morphological boundaries identified by Hayward et al. (2004) may be an aggregation of diagnostic morphological characters of more than one genotype. In addition, while Hayward et al. (2004) analysed the morphology of 179 specimens, only 79 of these specimens were genetically sequenced. Therefore, the interspecific boundaries presented by

Hayward et al. (2004) may have unknowingly included morphological traits of more than one genotype in the key diagnostic features of each genotype. In contrast, this study delineated interspecific boundaries from 156 *Ammonia* specimens, all of which were genetically and morphologically analysed. Thus, it could be asserted that the morphological relationships presented in this study may be more representative of the interspecific taxonomic boundaries within this genus.

The different morphological boundaries identified between this study and those of Hayward et al. (2004) could also be the product of the different sampling regimes employed and the different morphological characteristics analysed. For example, this study measured 23 out of the 37 morphological characters originally assessed by Hayward et al. (2004). Nine of Hayward et al.'s (2004) morphological features were omitted in this study due to the unavailability of SEM images taken from the profile aspect of the foraminifera. Therefore, the taxonomic re-evaluation of the morphological boundaries of *Ammonia* presented in this study might not have captured all the key diagnostic traits. For example, Hayward et al. (2004) identified that the profile diameter is a strong diagnostic character, thus the inclusion of this feature in future investigations may help to elucidate between partially morphologically cryptic specimens. Nevertheless, the additional morphological features analysed within this study and the minor modifications to some of Hayward et al.'s (2004) original variables might have helped to elucidate the taxonomic relationships within this genus. Additionally, this study utilised computer-aided techniques to standardise the measurements of a number of morphological characteristics, thereby reducing human subjectivity. For example, foraminiferal test roundness was calculated using an image outline analysis tool in the ImageJ software. In contrast, Hayward et al. (2004) originally measured the peripheral test outline by analysing the proportion of the 360^{o} peripheral outline that it smooth rather than lobular (Hayward et al., 2004, table 3, p.247).

3.5.5 Diagnostically important morphological test characteristics of *Ammonia*

The key diagnostic morphological criteria used to delineate species of *Ammonia* have changed across time and space due to the variable nature of test characteristics, and the lack of scientific consensus regarding the species concepts within this genus (Chang and Kaesler, 1974). Traditionally, strong diagnostic weight has been placed upon test ornamentation characteristics including presence of beading, fluting and the presence of an umbilical boss (Poag, 1978; Jorissen, 1988; Wang and Lutze, 1986; Walton and Sloan, 1990). In addition, over the years, structural test features including maximum test diameter, proloculus diameter and umbilical

area diameter have also been ascribed significant taxonomic weight (Chang and Kaesler, 1974). As discussed previously, the recent advancements in molecular techniques have elucidated taxonomic boundaries in this genus revealing the presence of previously unrecognised diversity (e.g. Holzmann, 2000; Hayward et al., 2004). This has brought into question the validity of the morphological criteria used for species delineation by classical morphology-based taxonomy.

Prior to the study conducted by Hayward et al. (2004), the preliminary integrated taxonomic investigations identified that test characters including pore size, pore density and maximum test diameter are diagnostically important (Holzmann et al., 1998; Holzmann, 2000). However, these initial studies only analysed a limited number of test characters and these were not always sufficient for robust delineation (Holzmann et al., 1996, 1998; Holzmann, 2000). It was not until the seminal study conducted by Hayward et al. (2004), that the morphological boundaries within the genus were comprehensively delineated.

This study presented in this chapter builds on the taxonomic delineations presented by Hayward et al. (2004) and supports their findings that a combination of structural and ornamentation features of the test are required for *Ammonia* species delineation. The taxonomic weightings ascribed to the key diagnostic features analysed in this study (Section 3.5.3) are broadly congruent with those identified by Hayward et al. (2004). The successful delineation of *Ammonia* genotypes in the NE Atlantic requires a combination of structural and ornamentation features including: measures of porosity (pore density and mean pore diameter), development of thickened calcite on the folia and on the spiral side, development of beading and grooving along sutural borders, the number of chambers in the first whorl and the radial angle between chambers N1 and N2 (spiral side) (Table 3.7). The presence of secondary dorsal openings (exhibited by genotype S6) is the only morphological character that is diagnostically important by itself.

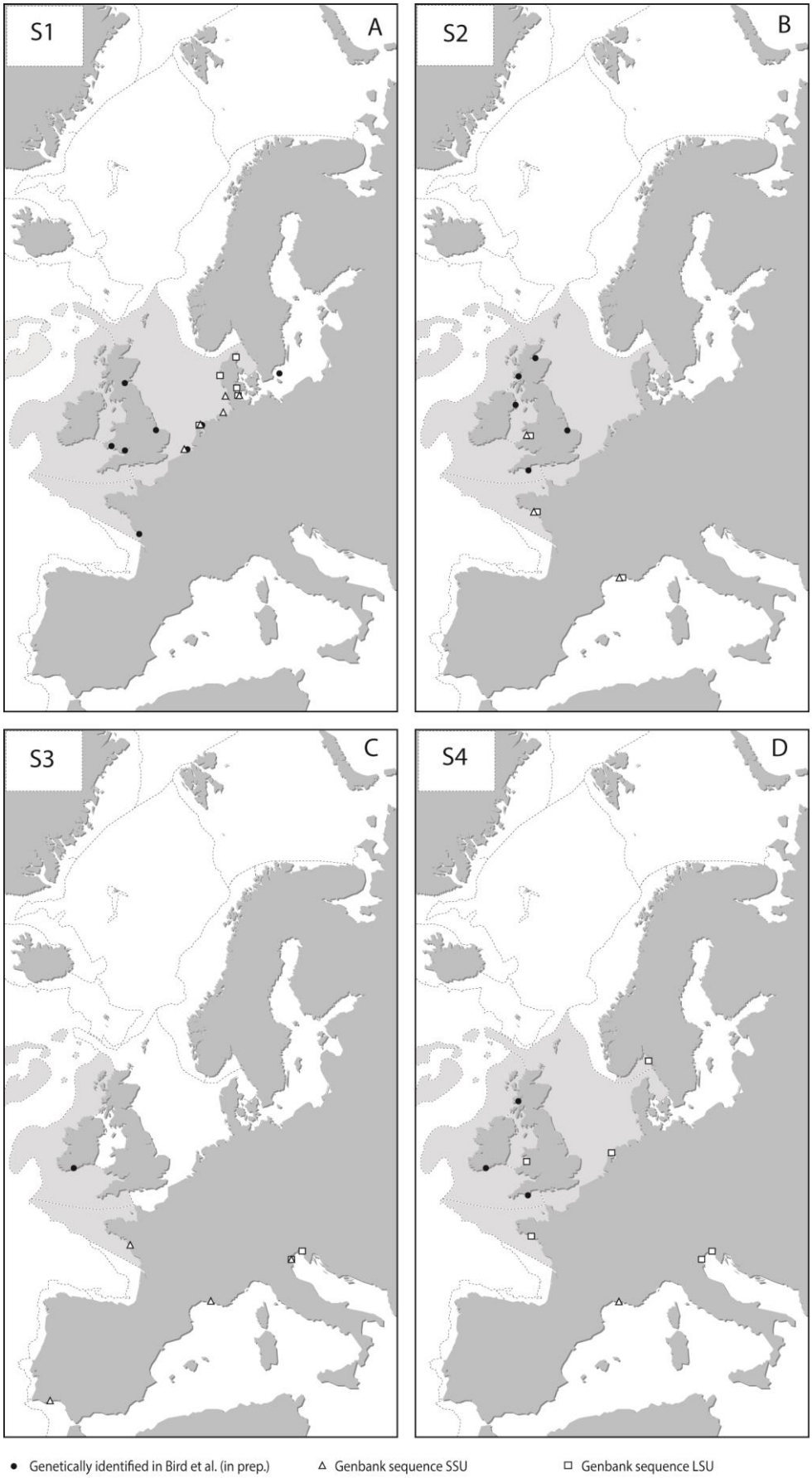
Notably, this study reveals that some specimens of genotypes S1-S4 were identified as partially morphologically cryptic. It is perhaps unsurprising that identifying key diagnostic criteria for less ornamented genotypes is difficult. A combination of mainly gross (structural) morphological features are required for species delineation (Table 3.7). These features are broadly congruent to the diagnostically important morphological characters identified by Hayward et al. (2004) who have previously recognised that foraminiferal roundness, porosity and chamber shape can help to delineate between the less ornamented genotypes. The morphological results presented in this study also reveal that features including relative width and length of sutures and the total

number of chambers visible are diagnostically important. With the exception of the total number of chambers visible, there are very few discrete diagnostic features that can be used to delineate between specimens from genotypes S1-S4. This therefore indicates that these genotypes would be practically ('visually') cryptic in an applied taxonomic situation.

Overall, the taxonomic re-evaluation of *Ammonia* genotypes illustrates that many of the diagnostic features identified by classical taxonomy are no longer useful for species delineation. Most notably, the presence and relative width of umbilical boss were previously ascribed significant taxonomic weight (Walton and Sloan, 1990). However, the results from this study illustrate these test features are no longer taxonomically informative, as extensive overlap is exhibited between all the genotypes (Table 3.7). Despite these limitations, it is important to note that a number of classically diagnostic features remain taxonomically useful (e.g. beading and fluting along the sutures), but typically only when analysed in combination with other morphological traits.

3.5.6 Biogeographical distribution of *Ammonia* species in the NE Atlantic

The discovery of partially and 'visually' cryptic species of *Ammonia* in the NE Atlantic illustrates the necessity for re-examining the biogeography of *Ammonia* genotypes; as it is likely that previous distributions have been over-estimated. The sampling of live *Ammonia* specimens in this study from across a range of distinctive biogeographical provinces has enabled a detailed regional analysis of their occurrence. Overall, the seven *Ammonia* genotypes exhibit a mid-latitude and southerly biogeographical distribution, which extends across three central biogeographic provinces in the NE Atlantic (Boreal, Boreal-Lusitanian and Baltic). The biogeographic pattern of *Ammonia* species identified in this region displays good congruence to the biogeographical affinities of *Ammonia* genotypes previously identified in Genbank (Figure 3.15). This analysis has also extended the known biogeographic distributions and species ranges of many *Ammonia* genotypes.


S1
A
S2
B
S3
C
S4
D
Genetically identified in Bird et al. (in prep.)
Genbank sequence SSU
Genbank sequence LSU

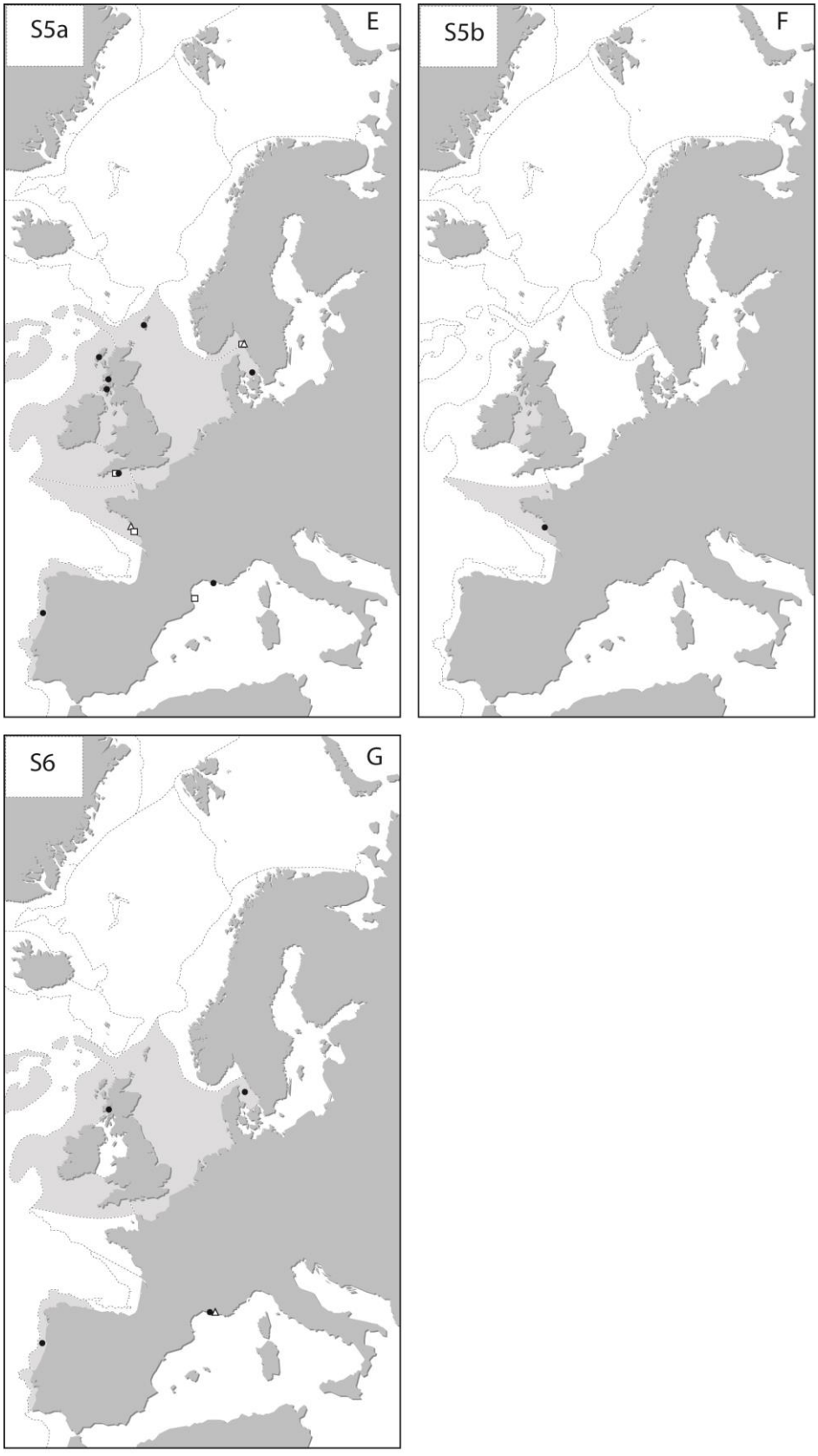


Figure 3.15 Biogeographic distributions of the seven *Ammonia* genotypes that were identified from across the North East Atlantic. The shaded areas correspond to the distinctive biogeographic provinces identified by Dinter et al. (2001). The key for these biogeographic provinces is illustrated in Figure 3.1. Figure taken from Bird et al. (in prep.).

The majority of *Ammonia* genotypes exhibit complex biogeographies in the NE Atlantic. It is evident that species within the genus *Ammonia* are mid to low latitude species because they are absent from the high latitudes. Only genotype S5b exhibits a restricted distribution, and it has only been sequenced from a single site locality at Ile d'Yeu (Figure 3.15 E). In contrast, the rest of the *Ammonia* genotypes exhibit broad and often overlapping biogeographic distributions. However, it is important to note that subtle differences in the biogeographic patterns of each genotype can be identified. For example, *Ammonia* genotype S3 exhibits an overall southerly distribution (Figure 3.15 C), whilst genotype S1 exhibits a central and easterly distribution (Figure 3.15 A). It is therefore unsurprising that extensive sympatry was identified in the NE Atlantic because the *Ammonia* species exhibit similar biogeographical ranges. For example, up to four genotypes have been found co-existing at a single site locality (Table 3.10). The recognition of high levels of sympatry in the NE Atlantic contrasts previous taxonomic investigations that only identified two co-existing genotypes at a single site locality (Hayward et al., 2004).

Genotype S5a is one of the most ubiquitous species of *Ammonia* identified in the NE Atlantic, with its biogeography extending from Shetland to the Portuguese margin (Figure 3.15 E). This species was identified as the most likely species to be living in sympatry with other *Ammonia* genotypes, typically with genotypes S4 and S6 (Table 3.10). The co-occurrence of these genotypes does not pose too many taxonomic problems for species identification in an applied taxonomic investigation because they can be morphologically delineated under a light microscope. In contrast, the co-existence of genotypes S1, S2 in Norfolk, genotypes S3 and S4 in Cork and genotypes S1, S2 and S4 in Dartmouth (Table 3.10) could complicate species delineation, as these less ornamented specimens are practically cryptic under a light microscope. Moreover, even after extended morphological analysis, there may be some partially cryptic specimens between these species. Thus in an applied taxonomic situation it would be difficult to delineate between the species. The overlapping biogeographic ranges, and extensive sympatry of the *Ammonia* species illustrates that biogeographic distribution of each individual genotype cannot be used as a secondary line of taxonomic evidence for species discrimination.

Genotype / Locality	S1	S2	S3	S4	S5a	S5b	S6
Loch Sunart							
Dunstaffnage							
Loch na Kille							
Norfolk							
Cork							
Dartmouth							
Mediterranean							
Portuguese margin							

Table 3.10 Site localities where more than one *Ammonia* genotype has been identified. The shaded areas demarcates the presence of the *Ammonia* genotypes at each site locality.

The co-occurrence of *Ammonia* at sub-site level, however, reveals potential localised partitioning between some of the genotypes. For example, a potential partitioning across the vertical sampling profile of the intertidal sediments at Dartmouth was identified. Genotype S1 was only identified in samples taken from the upper marsh, but is absent from samples taken from the mid and lower salt marsh. In contrast genotypes S2, S4 and S5a are absent from samples taken from the upper salt marsh, but have been found in samples taken from the mid and lower salt marsh. The distribution of these genotypes could be attributed to different ecological conditions across the salt marsh. Foraminiferal occurrence within this habitat has been traditionally attributed to changes in the availability of food, differences in substrates and frequency of tidal inundation (Gehrels, 2000; Gehrels et al., 2011).

The identification of genotype S1 only in the upper salt marsh could also help to elucidate the global biogeographical pattern of this genotype. Previously, this genotype has been identified as part of two disjunct (isolated) populations, one in China and the other in the Wadden seas (Hayward et al., 2004; Schweizer et al., 2008). It is currently hypothesised that this genotype was dispersed through ship ballast waters (Hayward et al., 2004; Toyofuku et al., 2005). This idea was supported because this genotype was identified at site locations near to major harbours (Hayward et al., 2004; Schweizer et al., 2008; Pawlowski and Holzmann, 2008). However, the re-examination of this genotype's biogeographical occurrence in the NE Atlantic brings into question its global biogeographic pattern and hypothesised dispersal mechanism. This genotype was identified across three biogeographic provinces in the NE Atlantic which has significantly expanded its known biogeographical range (Figure 3.15). This indicates that this genotype could

exhibit a more ubiquitous (global) distribution than previously recognised, or that it has rapidly expanded from its location of introduction in this region. In addition, the potential fine scale depth partitioning of this genotype (as identified in Dartmouth), may highlight that previous taxonomic sampling regimes have not captured the full biogeographical species range of this genotype. As a consequence further taxonomic scrutiny of its geographic limits and ecological preferences is warranted in order to clarify the *Ammonia* species range overlap and degree of habitat segregation of sympatric genotypes. Chapter 4 presents a preliminary investigation, which attempts to assess whether any seasonal partitioning can be identified between sympatric *Ammonia* genotypes in a NW Scottish shelf seas.

Whilst the taxonomic sampling regime employed in this thesis provides the most extensive taxonomic re-evaluation of *Ammonia* biodiversity and distribution conducted to date in the NE Atlantic, it is important to recognise it is not exhaustive. For example, owing to the opportunistic nature of the sampling some of the biogeographic provinces identified in Dinter et al. (2001) have not been sampled e.g. the White Sea, while other biogeographic provinces have only been marginally sampled. In addition, there is a potential sampling bias towards inter-tidal areas; as a consequence it is unlikely that the full genetic and morphological diversity of *Ammonia* species in the sub-tidal areas has been captured.

3.5.7 Intraspecific morphological variability within the NE Atlantic

The question of how much morphological variability is attributable to genetic or phenotypic controls within the genus *Ammonia* has been a subject of substantial discussion (Haynes, 1992; Holzmann, 2000). Traditionally, morphological test variability within the genus *Ammonia* was attributed to being a phenotypic response to different environmental conditions (Schnitker, 1974). However, the new insights provided by molecular techniques, illustrate that a large proportion of the morphological plasticity exhibited, is genetically controlled (e.g. Holzmann, 2000; Hayward et al., 2004; Schweizer et al., 2008). Despite extensive re-evaluation of interspecific boundaries, considerably less attention has been focused on understanding the relationship of intraspecific morphological variability with environment. Hayward et al. (2004) present one of the few integrated taxonomic investigations which has evaluated the potential phenotypic expression of test morphology in different geographic locations. For example, Hayward et al. (2004) identified three morphologically and geographically distinct populations of the LSU rRNA T3 *Ammonia* genotype. However, as discussed previously (Section 3.5.4), new molecular evidence has identified greater genetic variability in Hayward et al.'s (2004) LSU rRNA

T3 genotype (Schweizer et al., 2011; Bird et al., in prep.). It could therefore be hypothesised that the morphological differences identified between the three populations of the *Ammonia* T3 genotype are the product of genetic divergence as species adapt to different environmental conditions.

The re-examination of the interspecific taxonomic boundaries of *Ammonia* within the NE Atlantic presented in this chapter has also provided an opportunity to re-evaluate the relationship between morphological plasticity and biogeography within this genus. *Ammonia* genotype S1 was chosen as a case study due to the availability of a relatively large number of morphologically and genetically analysed specimens. In addition, this genotype exhibits a broad biogeographic distribution, which extends across three distinct biogeographic provinces in the NE Atlantic (Figure 3.15). *Ammonia* genotype S1 was chosen as a case study because this genotype occupies a large proportion of the PCO morphospace (Figure 3.6), thus indicating that specimens within this species exhibit morphological plasticity.

Subtle morphological differences were observed between specimens of *Ammonia* genotype S1 obtained from different sampling localities in the NE Atlantic. Most notably, the results illustrate that statistically significant differences can be identified between the pore characters of the *Ammonia* specimens from different sites (Figure 3.14). For example, *Ammonia* genotype S1 specimens from the Baltic exhibited larger pores in comparison to the other *Ammonia* genotype S1 specimens. The recognition of large pores at this site is interesting because these specimens were taken from a low salinity environment (7-13 psu). Previous investigations have identified that pore characters are responsive to different environmental conditions (Corliss, 1985; Gooday, 2003; Bernhard et al., 2010; Glock et al., 2012; Dubicka et al., 2015). For example, Moodley and Hess (1992) illustrated in a culturing experiment of '*Ammonia beccarii*' that specimens found within low oxygenated conditions exhibit larger pores. This finding is supported by numerous studies conducted on other foraminiferal species (e.g. Kuhnt et al., 2014). However, as only limited environmental data is available at each sampling locality, it is only possible to speculate that the morphological test variability exhibited is a product of ecophenotypy.

It is important to recognise that the intraspecific morphological differences between site localities identified within this study may not be completely robust. Firstly, the intraspecific morphological variability observed at each site locality was only derived from a limited number of specimens. Consequently, this study has not captured the full range of intraspecific

morphological variability within each 'population' examined. Although the CART analysis has correctly classified 98% of specimens into their site localities based on their morphology, the cross validation error attained by the CART analysis is poor (46% specimens misclassified). This illustrates that the morphological boundaries identified may not be supported by additional morphological evidence. The poor cross validation results could be the product of the small and unbalanced number of specimens available for analysis at each site location. For example, only two specimens were available for analysis from Laughcarne castle, and this may be insufficient to provide robust delineations in the cross validation procedure. Despite these uncertainties, this study outlines the general intraspecific morphological trends of *Ammonia* genotype S1 across a large geographic spatial scale; most notably highlighting the differences in porosity features between *Ammonia* genotype S1 specimens obtained from different site localities.

It should also be noted that ontogeny could also drive intraspecific morphological test variability. To negate potential ontogenetic effects, size standardisation techniques were employed in the measurement of morphological test features. However, the ontogenetic morphological development of the *Ammonia* species remains poorly defined. Until the full range of morphological variability across all stages of ontogenetic development is quantified, the role of ontogeny in controlling intraspecific morphological variability cannot be ruled out. Thus, in order to understand the relationship between intraspecific variability and environment, detailed *in situ* investigations of live *Ammonia* genotypes and extensive environmental surveys of their natural habitats are required.

3.5.8 The nomenclatural implications of the new taxonomic framework

This study outlines a new taxonomic framework for the genus *Ammonia*, which rigorously documents the degree of morphological variability exhibited between the newly delineated genotypes. However, trying to reconcile the new taxonomic evidence with classical taxonomy is difficult. In taxonomy, it is fundamental that a species can be referred to an unambiguous taxonomic name. This is because taxonomic names are the unit through which taxonomic affinities, biogeography and ecological preferences are communicated (Waterton et al., 2013). However, situating the newly delineated genotypes into classical taxonomy is complicated by the 'chaotic' taxonomic and nomenclatural history of this genus, which features extensive synonymy. This is coupled with the fact that many traditional morphospecies concepts could have significantly underestimated genetic diversity.

To date, little effort has been made to reconcile traditional taxonomic concepts to newly delineated species. Numerous recent molecular investigations have assigned formal taxonomic names to a genotype often without linking this to the original type description and type material (as commonly implemented in the *Elphidium* taxonomy, e.g. Pillet et al., 2013). The extensive integrated study conducted by Hayward et al. (2004) is one of a limited number of taxonomic investigations, which has attempted to attach formal species names to molecular types. However, out of the 13 *Ammonia* genotypes successfully delineated by Hayward et al. (2004) only genotype *Ammonia* T2 could be successfully reconciled to the formal species name of *Ammonia aberdoveyensis*, Haynes, 1973. Hayward et al. (2004) also ascribed potential taxonomic names to seven genotypes based on similarities to the type descriptions. However, greater genetic divergence has been identified within Hayward et al.'s original LSU rRNA species concept (Schweizer et al., 2008; Bird et al., in prep.) brings into question the validity of these proposed taxonomic names (Table 3.11).

Genotype identified in Hayward et al. (2004)	Genotype identified in Bird et al. (in prep.)	Proposed species names by Hayward et al. (2004)
T1	S4	*Ammonia veneta*, Schultze, 1854
T2	S2,S3	*Ammonia aberdoveyensis*, Haynes, 1973
T3	S5a, S5b, S6	*Ammonia inflata*, Seguenza 1862 *Ammonia batava,* Hofker, 1951
T6	S1	*Ammonia aomoriensis* Asano, 1951

Table 3.11 Proposed taxonomic names assigned to each genotype from Hayward et al. (2004) and the equivalent genotype of the specimens in Bird et al. (in prep.).

The new taxonomic evidence highlights that the ascription of formal taxonomic names to these genotypes may not be as straightforward as previously suggested. For example, two genetically distinct yet partially morphologically cryptic species were identified from within Hayward et al.'s (2004) T2 genotype. The T2 genotype was previously reconciled with the formal name of *Ammonia aberdoveyensis* (Table 3.11). Uncertainty still surrounds how new lines of taxonomic evidence can be integrated with classical taxonomy. Reconciling newly delineated species of *Ammonia* with formal taxonomic names is complex and requires extensive time and resources; as a consequence it is outside the remit of this study.

Overall, it is evident that the prevalent use of broad species concepts and open nomenclature of *Ammonia* within the academic literature needs to be avoided wherever possible. This is because the continued proliferation of this taxonomic practice can create incorrect classifications and as a consequence may lead to misleading interpretations in applied taxonomic investigations; particularly when undertaking palaeoenvironmental reconstructions (as discussed in Chapter 4). Until the interspecific boundaries are refined and reconciled with classical taxonomy, an interim taxonomic practice should be employed where specimens of *Ammonia* (both fossil and extant) are assigned to potential genotypes, based upon their morphological characteristics. Where this is not possible, it is recommended that specimens be assigned to *Ammonia* sp.

3.6 Conclusions

This study presents the most comprehensive re-evaluation of interspecific morphological relationships in the genus *Ammonia* in the NE Atlantic conducted to date. Three *Ammonia* genotypes S5a, S5b and S6 can be perfectly discriminated based on a suite of structural and ornamental test characteristics. In contrast, the morphological species boundaries between the less ornamented genotypes S1-S4 are more enigmatic, as these species exhibit gradational morphological features. The inability to reconcile perfectly between molecules and morphology between some of the less ornamented genotypes, even after extended morphological analysis, highlights that some specimens within these genotypes are partially morphologically cryptic. In addition, the absence of discrete diagnostic features indicates that these species are likely to be 'visually' cryptic in an applied taxonomic situation. This is in contrast to the current taxonomic framework which asserted that all molecular types can be morphologically discriminated (Hayward et al., 2004). The existence of several genetically distinct specimens which exhibit ambiguous morphological boundaries has significant ramifications for applied taxonomic situations, particularly as these genotypes exhibit overlapping species ranges and have often been identified living in sympatry with each other. These findings highlight that classical taxonomy is unlikely to reflect the overall genetic diversity found within the NE Atlantic. In future, an understanding of fine scale habitat segregation may provide another line of taxonomic evidence to help elucidate these *Ammonia* species. For example, the results revealed that genotype S1 potentially exhibits localised partitioning from other sympatric species based on depth. Thus the localised ecological partitioning of co-existing taxa warrants further study.

This study has also presented the first detailed examination of intraspecific morphological variability in the genus *Ammonia* across a large geographic spatial scale. Notably, specimens of *Ammonia* genotype S1 taken from different site localities could be morphologically discriminated based on a range of test traits including their pore characters. However, this elucidation of intraspecific variability in relation to biogeography remains a work in progress, as each site was only represented by a small number of specimens. Additionally, owing to the paucity of knowledge on the environmental conditions at each site, it is only possible to speculate that the intraspecific variability exhibited is a product of ecophenotypy. Detailed *in situ* investigations of live *Ammonia* genotypes and extensive environmental surveys are required to elucidate the relationship between morphology and environment.

Overall, it is only when morphology and molecules are interpreted in concert that the taxonomic relationships within this genus can be fully resolved. In order to stabilise the taxonomic framework of this genus in the future and to maximise the value of *Ammonia* in applied taxonomic situations, additional investigation is required. Notably, emphasis should be placed in the future upon quantifying both the intraspecific and interspecific limits, as well as clarifying the species ecological preferences through experimental culturing investigations and field-based surveys.

Chapter 4

Temporal dynamics of benthic foraminiferal assemblages in the NW Scottish shelf seas: assessing the effect of seasonality upon co-existing cryptic species of *Ammonia*

The bottom water and sediment samples utilised in this study were collected by divers at the NERC National Facility of Scientific Diving with support from the allied NERC funded project (NE4/G018502/1). The unpublished bottom water temperature data associated with the foraminiferal time series study was provided by Dr Martin Sayer.

Chapter 4: Temporal dynamics of benthic foraminiferal assemblages in the NW Scottish shelf seas: assessing the effect of seasonality upon co-existing cryptic species of *Ammonia*

4.1 Introduction

The Scottish shelf seas are an area of specific interest for scientific research, as these marginal marine environments are exposed not only to short term (seasonal) changes in physical and chemical regimes; but also long term climatic variability (Scourse and Austin, 2002). In addition, these regions provide an important link between the deep ocean and terrestrial records, thereby helping to capture land-ocean interactions (Backhaus, 1996; Scourse and Austin, 2002; Cage and Austin, 2008). Within the shelf seas, Scottish fjords (sea lochs) are important as these sheltered locations often exhibit high sedimentation rates of 1cm yr^{-1}(Cage and Austin, 2010). The fjords provide the opportunity for high resolution palaeoclimate studies at both centennial and millennial timescales (Mikalsen et al., 2001; Kristensen et al., 2004; Eiriksson et al., 2006; Austin et al., 2006; Cage and Austin, 2008; Reynolds et al., 2013).

Benthic foraminifera provide key palaeonvironmental proxies in this region due to their high test preservation potential in the sediments. The calcium carbonate tests of benthic foraminifera provide two key proxy pathways for palaeoenvironmental reconstructions, firstly by the co-variance of species abundances with environment (e.g. Jorrisen et al., 2007) and secondly by the incorporation of elements within the test (e.g. Shackleton, 1987). Specimens of *Ammonia* are commonly used to derive species-specific geochemical proxies (e g. stable carbon and oxygen isotopes) within this region due to their ubiquitous nature and high natural abundance within the sediments (Allison and Austin, 2003; Cage and Austin, 2010). The successful interpretation of the fossil foraminifera from the sediment archives in this region requires a thorough knowledge of the ecology and biogeography of their counterpart extant species (Murray, 1991). The occurrence and distribution of foraminifera in the modern environment is a combination of spatial and temporal dynamics.

Extensive taxonomic investigations have been conducted to assess the spatial variability of modern benthic foraminifera within the Scottish shelf seas, e.g. Heron-Allen and Earland (1916) collected samples in Loch Sunart; Edwards (1982) from the North Minch; Hannah and Rogerson (1997) sampled in the Clyde sea, Murray (2003a) sampled in the Hebridean shelf seas, Murray et al. (2003) sampled in Loch Etive, and Austin and Cage (2010) in the Clyde seas To date, limited emphasis has been placed upon assessing the temporal variability of the living benthic

foraminiferal assemblages in these environments. Instead, the majority of studies only provide a 'snapshot' of the assemblage structure at the time of collection. Therefore, it is unlikely that previous investigations have captured the full range of the natural variability of the foraminiferal assemblage composition in this region; particularly as the foraminiferal assemblage structure is extremely responsive to short-term changes in environmental conditions (Murray and Alve, 2000; Sen Gupta, 2002). Therefore, our current interpretations of biodiversity in this region may be misleading.

The convention of using modern foraminifera as analogues for reconstructing palaeoenvironments is based on the assumption that it is possible to consistently classify specimens in the faunal assemblages and that the species identified are morphologically and genetically homogenous (Kucera and Darling, 2002). However, the recent integration of new lines of molecular evidence in benthic foraminiferal taxonomy has revealed greater genetic diversity within many of the classical morphospecies concepts than has been previously identified (Holzmann, 2000; Hayward et al., 2004; Schweizer et al., 2008). For example, as presented in Chapter 3**,** seven genetically distinct genotypes of *Ammonia* were identified in the NE Atlantic; three of which co-exist in the NW Scottish shelf seas (Bird et al., in prep.). This contradicts the conservative taxonomic framework that has proliferated in Scottish shelf seas research in which specimens of *Ammonia* have been classified into *Ammonia batavus,* Hofker, 1951 (Murray, 2003a; Cage and Austin, 2008; Cage and Austin, 2010; Reynolds et al., 2013) or were lumped into the broad species concepts of *Ammonia beccarii*, Linné, 1758 (Cage and Austin, 2010; Mokeddem et al., 2010).

The discovery of cryptic diversity within this genus brings into question prior biodiversity estimates, biogeographic occurrences and the robustness of the species-specific environmental proxies. For example, changes in the geochemical composition of the test have been derived from a single species concept of *Ammonia* in this region, which was thought to calcify in spring and summer (Austin and Scourse, 1997; Scourse et al., 2004; Cage and Austin, 2008). However, the new taxonomic framework presented in Chapter 3 reveals that the traditional broad morphospecies concepts may represent aggregates of genetically distinct species, which may have distinct ecological preferences. This could introduce significant noise/errors into palaeoenvironmental reconstructions. Therefore, it is important to re-evaluate classical benthic foraminiferal taxonomy in order to constrain palaeoenvironmental reconstructions and to

understand these relationships in the context of seasonally variable environmental conditions (e.g. Austin et al., 2006).

4.1.1 Aims

This present study characterises the seasonal composition and structure of a benthic foraminiferal assemblage in the NW Scottish shelf seas. Additionally, this study provides an opportunity to evaluate the utility of the integrated taxonomic framework of *Ammonia* (presented in Chapter 3) in an applied taxonomic situation. The aims of this chapter are:

- To examine the temporal dynamics of benthic foraminiferal assemblages found within the NW Scottish shelf seas.
- To depict (if present) any seasonal patterns or partitioning of different *Ammonia* species found within this site.
- To investigate potential periods of reproduction within the *Ammonia* species found at this site.

4.2 Materials and methods

4.2.1 Study site location

The site location for this study is situated within a fjordic environment on the North West coast of Scotland near Oban (56° 27.403' N, 005° 26.614' W) (Figure 4.1). The samples were retrieved from a depth of 32m. For a detailed account of the regional oceanographic setting typical of this site locality, the reader is referred to Austin and Inall (2002) or Cage and Austin (2010).

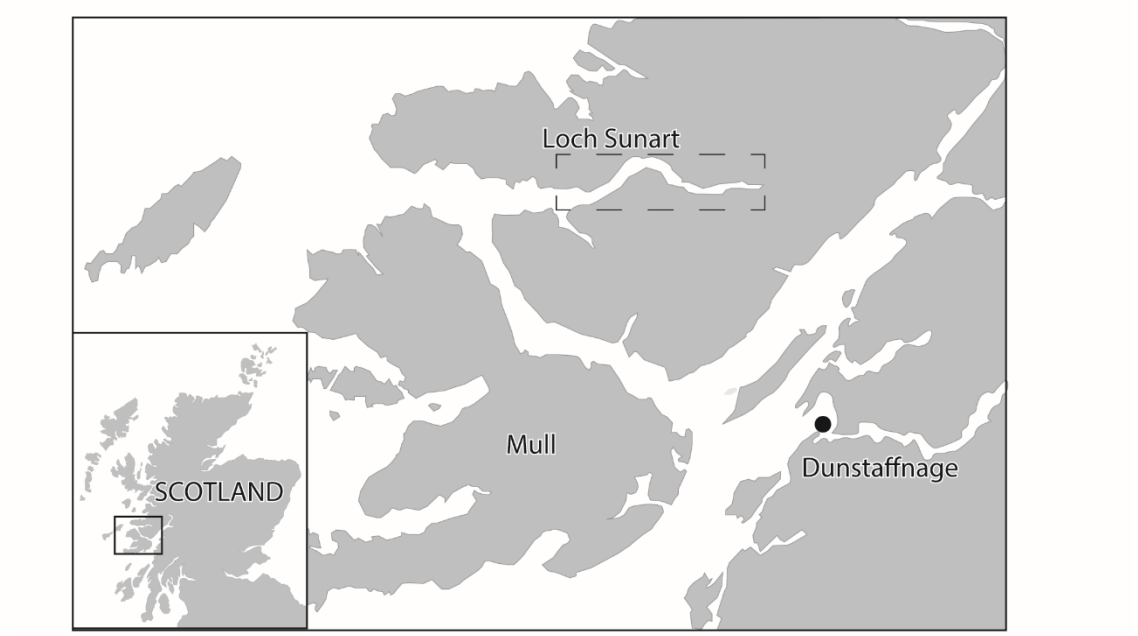


Figure 4.1 Location of Dunstaffnage sampling site and its regional context within the Scottish shelf seas.

Figure 4.1 illustrates the proximity of the site location to Loch Sunart (outlined by the dashed line). Loch Sunart is an important Scottish fjord because high resolution palaeoclimate archives derived from this area has been used to reconstruct Holocene climate change (Cage and Austin 2010; Mokeddem et al., 2010; Reynolds et al., 2013)

4.2.2 Sample collection

To examine the temporal variations in benthic foraminiferal assemblage structure, bottom water and surface sediment samples were collected on a monthly basis by SCUBA divers at the NERC National Facility for Scientific Diving facility (NFSD) from September 2008 to August 2009. During each dive, two replicate samples of the surface sediment and bottom water were collected. The top 1 cm of the soft sediment was collected into plastic bags at the seabed. Following sample collection, the two replicate sediment samples were preserved and stored in 95% ethanol made up as 1 g L^{-1} Rose Bengal solution. The bottom water samples and the sediment samples collected were then stored in a cold room at 4^{o}C.

The samples analysed within this study were obtained from a longer-term time series sediment archive held at the University of St Andrews, which collates the sediment samples collected by the NFSD at the site locality (Dunstaffnage) from 2007 up to the present day. The period of investigation from August 2008 to September 2009 was chosen for analysis because the samples comprised of a series of previously undisturbed and unprocessed sediment samples. In total 24 sediment samples were analysed (two replicate samples for each month). For a detailed account of the sampling employed and the general site conditions, refer to the Appendix.

4.2.3 Foraminiferal analysis

The sediment volume of each replicate was calculated; following methods set out in Chapter 2.3.2. The sediment samples were then wet sieved at 63 µm using a fine water spray. The sieved samples were left to oven dry at 40°C. The total weight of the dry residues was then calculated.

From each of the samples, a target of 300 Rose Bengal stained specimens were picked using a 0000 paintbrush. In samples with low foraminiferal density, at least 100 Rose Bengal stained specimens were picked where possible. A minimum number of 100 specimens have been deemed sufficient for detecting up to 99% of species that make up ≥ 5 % of an assemblage (Fatela and Taborda, 2002) and 95% species making up to 3% of an assemblage (Dennison and Hay, 1967). In order to minimise the over-estimation of 'live' specimens by Rose Bengal staining (Bernhard 1988, 2000), a strict staining protocol was adopted. Specimens were considered 'live' when individuals were stained bright pink or red. In situations where the degree of staining of a specimen is unclear, the specimen was directly compared to examples of perfectly stained specimens within each individual sample. Special attention was given to assessing the degree of staining of agglutinates and miliolids, as it was difficult to recognise colouration within these taxa. Consequently, specimens within these taxonomic groups were evaluated following protocols set out in Schönfeld et al. (2012); whereby dry specimens were wetted to assess the degree of staining.

4.2.3.1 Foraminiferal species identification

Specimens were then classified into species based on their morphological characteristics primarily using taxonomy descriptions from Loeblich and Tappan (1987, 1992), Haynes (1973), Austin (1991) and Murray (2003a). This study also utilises the new integrated taxonomic framework of *Ammonia* presented in Chapter 3, to re-evaluate the diversity of *Ammonia* species found at the sampling location. This taxonomic framework revealed the presence of three genetically distinct sympatric species of *Ammonia* in the Scottish shelf seas (Chapter 3, Table

3.10). As previously discussed in Chapter 3 (Table 3.7), these sympatric genotypes can be clearly delineated based on key diagnostic morphological test characteristics, which are visible under a light microscope. Specimens of *Ammonia* identified in this study are provisionally ascribed to a genotype based upon their morphological features.

4.2.3.2 Foraminiferal community structure

The foraminiferal community structure was derived from counting live (stained) specimens from each month. Initially the assemblage structure for each of the replicate samples was individually analysed. However, this yielded insufficient numbers for the robust determination of the foraminiferal community structure. As a consequence, in order to obtain statistically significant census counts, the counts from the two replicates were pooled together and then analysed. The absolute abundance of stained individuals (total standing stock) calculated for each month was standardised to a sediment volume of 100 ml. To provide an assessment of the degree of spatial heterogeneity at this site, the standard error of the standing stock of the two replicate samples was calculated. In addition, the percentage relative abundance of each species was calculated for each month (the two replicate samples were aggregated).

For each month diversity indices including: species richness (the total number of species), Pileou species evenness index, Shannon Weiner diversity index (H'), and Fisher Alpha diversity index were calculated. The foraminiferal diversity measures were calculated using PAST statistics v.2.17.

4.2.3.3 *Population dynamics of Ammonia* species

To evaluate the population dynamics of *Ammonia* species identified at this site locality, the maximum test diameter of each *Ammonia* specimen was measured under a binocular microscope using a calibrated eyepiece graticule. The *Ammonia* specimens were then grouped into size classes based upon the range of maximum test diameters identified. The population dynamics of each *Ammonia* species were calculated as a percentage of the relative abundance of each size fraction. This allows for the identification of periods of peak reproduction, which is commonly expressed by a shift towards a dominance of smaller test sizes in the population structure (Murray, 1982).

4.2.4 Environmental variables

Bottom water salinity

Salinity was calculated using a Guildline Portasal salinometer at the Marine Scotland Laboratories in Aberdeen. In total 90 bottom water samples, which were collected by the NFSD from 2007 to 2011, were analysed in order to provide the wider context of the inter-annual variability in salinity at this site. The Portasal salinometer was calibrated using IAPSO standards at the start and end of each batch of samples. To correct for potential drift in salinity values the Portasal salinometer was calibrated after every five samples within each batch. Salinity was reported with a precision of +-0.02 salinity units.

Temperature

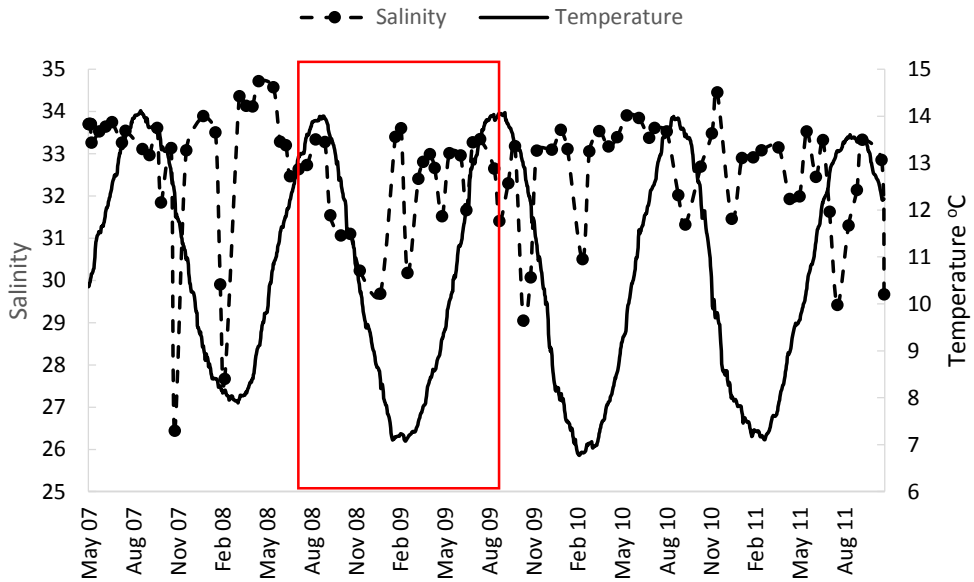
The bottom water temperature was collected by two temperature loggers from a depth 30m. This data was provided by Dr Martin Sayer from the NERC diving facility. The temperature loggers recorded data at 10-minute intervals and the average daily temperature was utilised in this study.

4.3 Results

4.3.1 Environmental conditions

The long-term trend in bottom water temperature (August 2007-2011) at this site illustrates that there is a strong seasonal cycle of up to 7°C each year (Figure 4.2A). The long-term trend in salinity illustrates that there are intervals with lowered salinity, which potentially suggest periods of prolonged stratification. These periods may be in response to high freshwater runoff and/or weakened wind-driven mixing (Gillibrand et al., 2005).

A)



B)

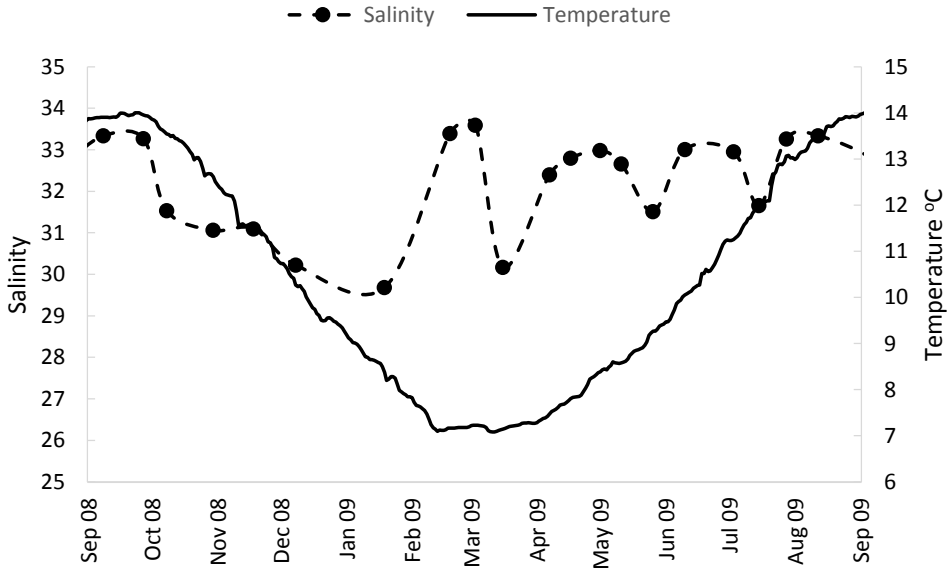


Figure 4.2 Temperature and salinity of the bottom water at Dunstaffnage. A) Bottom water temperatures and salinity from May 2007 to November 2011. The area highlighted in red demarks the period of study under investigation. B) Bottom water temperature and salinity values from September 2008 to September 2009.

A seasonal cycle of bottom water temperature is evident across the period of investigation (September 2008-August 2009), in which there is a large temperature change of nearly 7°C (Figure 4.2B). The maximal bottom water temperature of 14°C was reached during September 2008, whilst the minimum temperature occurred during early March 2009. The salinity values at this site ranged from 29 (January 2009) to 33 (March 2009) (Figure 4.2B). A noticeable decline in salinity is observed from September 2008 to January 2009, which could indicate a period of prolonged stratification of the water column. This period of prolonged lower salinity values, is proceeded by a sudden increase in salinity, which could indicate the presence of a renewal event (Gillibrand et al., 2005). Overall, it is evident that the range of temperature and salinity values experienced during the period of investigation (Figure 4.2B) are representative of the normal seasonal variability found at this site location (Figure 4.2A).

4.3.2 Seasonal variations in foraminiferal standing crop

The standing crop of benthic foraminifera at this site illustrates significant fluctuations in live specimens numbers over the period of investigation (Figure 4.3). The standing crop reached a maximum of 454 ± 42 specimens per 100ml during May 2009, whilst the minimum standing crop occurred in November 2008 with 113 ± 10 specimens per 100ml. The main peak in the standing crop occurs in the spring, and the lowest density of live foraminiferal numbers was identified during late autumn/ early winter. An additional peak in absolute abundance was also observed during January, reaching 403 ± 20 specimens per 100ml.

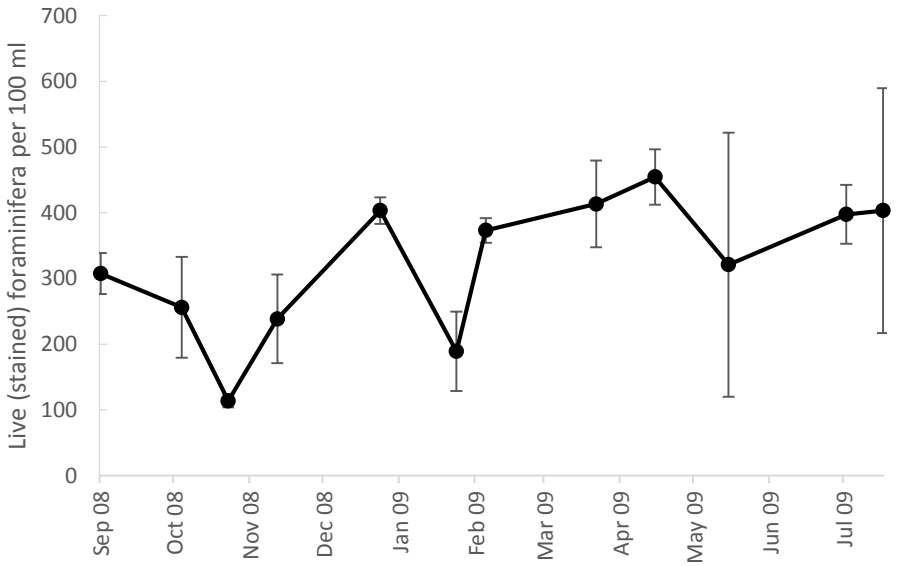


Figure 4.3 Total number of live (stained) foraminifera per 100ml over the sampling period from September 2008 to August 2009. Data shown is the aggregate of the two replicate samples collected each month. The standard error of the two replicate samples is also illustrated.

Potential spatial heterogeneity (patchiness) has also been identified at this site; the large standard error illustrates the significant difference between the standing crop of the two replicate samples. This difference is most notable during June and August 2009, when a standard error of ± 201 and ± 187 live individuals per 100ml were identified, respectively.

Finally, simple linear regressions were conducted to establish if the total live standing crop was directly correlated to temperature and salinity recorded at the site. However, the results revealed that no statistically significant relationships were identified between the total standing crop and both salinity (r: 0.284, p: 0.371) and temperature (r: 0.119 p: 0.713). An additional series of linear regressions were also conducted to assess whether the foraminiferal assemblage exhibit a lagged response to the two environmental variables. The results revealed that no statistically significant relationships were identified between these variables and the total standing stock (Table 6 in the Appendix).

4.3.3 Foraminiferal taxonomic composition and community structure

4.3.3.1 Classification of Ammonia species

Prior to the calculation of the foraminiferal assemblage structure, the species diversity of *Ammonia* was evaluated using the integrated taxonomic framework outlined in Chapter 3. Two morphologically distinct species of *Ammonia,* genotypes S5a and S6, were clearly identified from the samples by their discrete diagnostic morphological features including: the presence of a secondary dorsal openings and the development of extensive ornamentation, such as beading and fluting along the sutures. For a detailed outline of the key diagnostic morphological criteria of each of these species, see Chapter 3 (Table 3.7). The two species of *Ammonia* that were morphologically discriminated in this study are referred to herein by their proposed genotype identity.

4.3.3.2 Species diversity

In total, 52 live (stained) foraminiferal species were identified at this site. The total number of species identified each month ranged from a maximum of 29 species in September and August to a minimum number of 18 species in October and May (Table 4.1). The highest level of diversity was identified in November and December 2008 (H' 2.12-2.26, Fisher alpha 4.80-6.20 and Pileou evenness index 0.44-0.46) (Table 4.1). An additional peak in diversity was identified in August (H' 2.33, Fisher alpha 7.34 and Evenness 0.33). The lowest level of species diversity was observed during May and June 2009 (H'-1.22-1.45, Fisher Alpha 3.85-4.05, Evenness 0.17-0.24) (Table 4.1).

Month	Sep 08	Oct 08	Nov 08	Dec 08	Jan 09	Feb 09	Mar 09	Apr 09	May 09	Jun 09	Jul 09	Aug 09
Shannon-Weiner (H')	1.85	1.58	2.26	2.12	1.57	1.67	1.74	1.92	1.45	1.22	1.79	2.33
Fisher alpha	7.03	4.20	6.20	4.80	5.14	4.47	5.69	4.68	3.85	4.05	5.62	7.34
Evenness	0.22	0.27	0.46	0.44	0.20	0.28	0.22	0.31	0.24	0.17	0.24	0.36
Species richness	29	18	21	19	24	19	26	22	18	20	25	29

Table 4.1 Species diversity, Species richness and Evenness identified across the year.

No clear seasonal pattern in species diversity was identified during the period of investigation. Instead, the overall species diversity at this site could be related to the abundance (dominance) of *Nonionella turgida.* For example, the lowest level of biodiversity coincides with a peak in the abundance of *N. turgida* within the assemblage composition (Figures 4.4 and 4.5).

4.3.3.3 Foraminiferal species composition

The foraminiferal assemblage structure is dominated by three calcareous species: *Nonionella turgida, Ammonia* genotype S5a, *Ammonia* genotype S6 and one agglutinated species *Eggerelloides scaber* (Figure 4.4). Overall, *N. turgida* is the most dominant species, and constitutes up to 71% of the total (live) assemblage. This species exhibits clear temporal fluctuations across the period of investigation, notably the abundance (absolute and relative) of *N. turgida* rapidly decreases during the winter months (Figure 4.5 and Figure 4.6A). For example, during December *N. turgida* only accounts for 7% of the total assemblage structure (absolute abundance 13± 6 specimens per 100ml). The rapid decrease in *N. turgida* during the winter months has led to an overall assemblage composition shift towards a more 'equilibrated' assemblage structure e.g. greater species evenness (Table 4.1 and Figures 4.5 and 4.6A). During December, *Ammonia* genotype S5a is the most dominant species and this species accounts for 36% of the total assemblage structure (the temporal dynamics of this species is discussed in detail in Section 4.3.4).

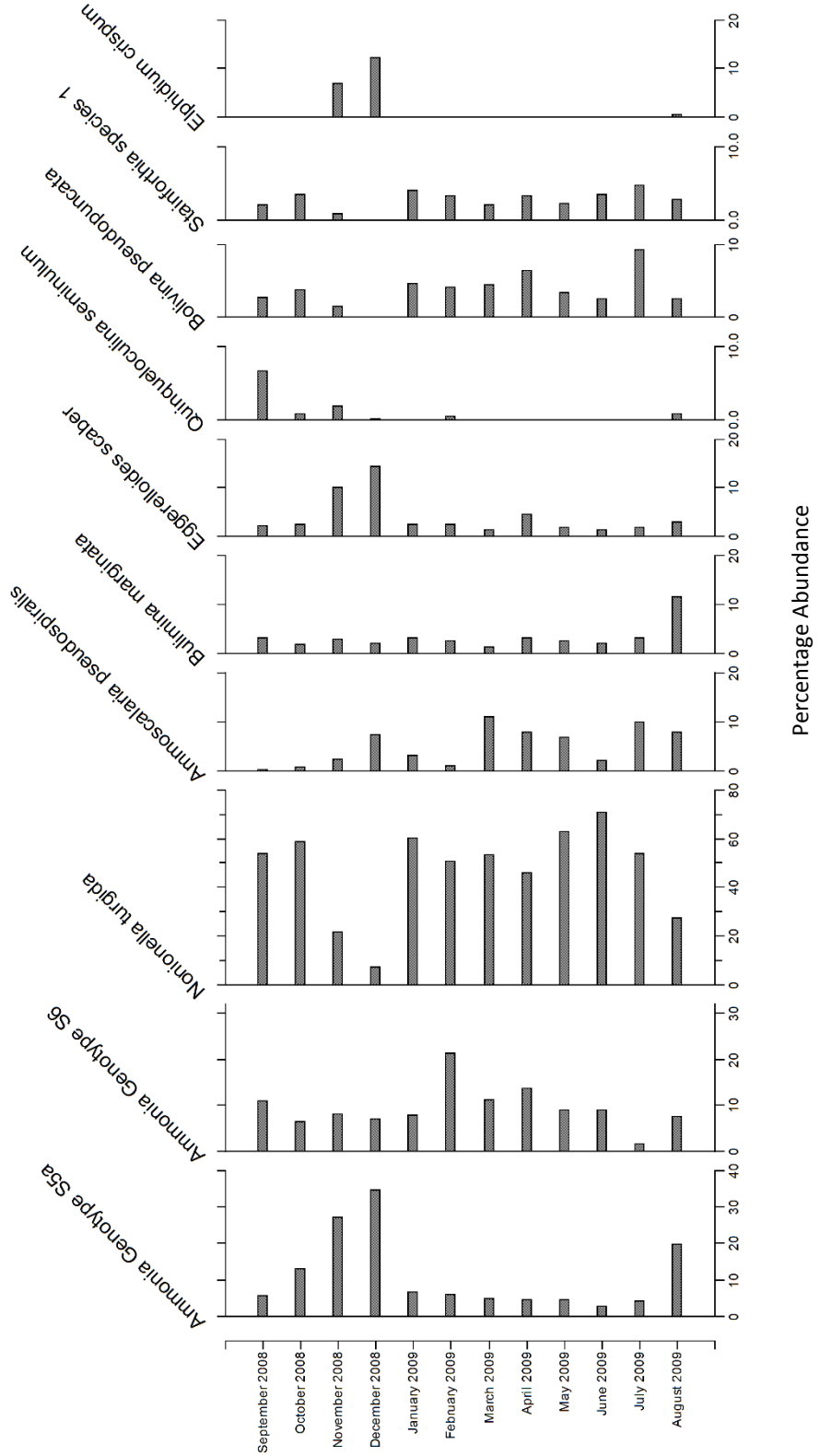


Figure 4.4 Summary of foraminiferal assemblage composition at the Dunstaffnage site locality. Foraminiferal frequencies are illustrated as the relative abundance of the total assemblage composition. Only species which exhibit a relative abundance >5% are illustrated.

One of the most notable changes in the assemblage composition in the winter months is the influx of *E. cripsum* and *E. scaber*. The increase in the absolute abundance of *E. crispum*, to its peak of 22 ± 4. specimens per 100ml (14.5% relative abundance) is striking, because this species is predominately absent for the rest of the year, with the exceptions of very low occurrences during August e.g. 2± 1 specimens per 100ml (0.80% relative abundance) (Figures 4.5 and 4.6C). *E. scaber* also exhibits an analogous increase in abundance during November and December, though its seasonal cycle is not as apparent as the temporal trend exhibited by *E. crispum* because this species retains low-level occurrences throughout the year (Figures 4.5 and 4.6B).

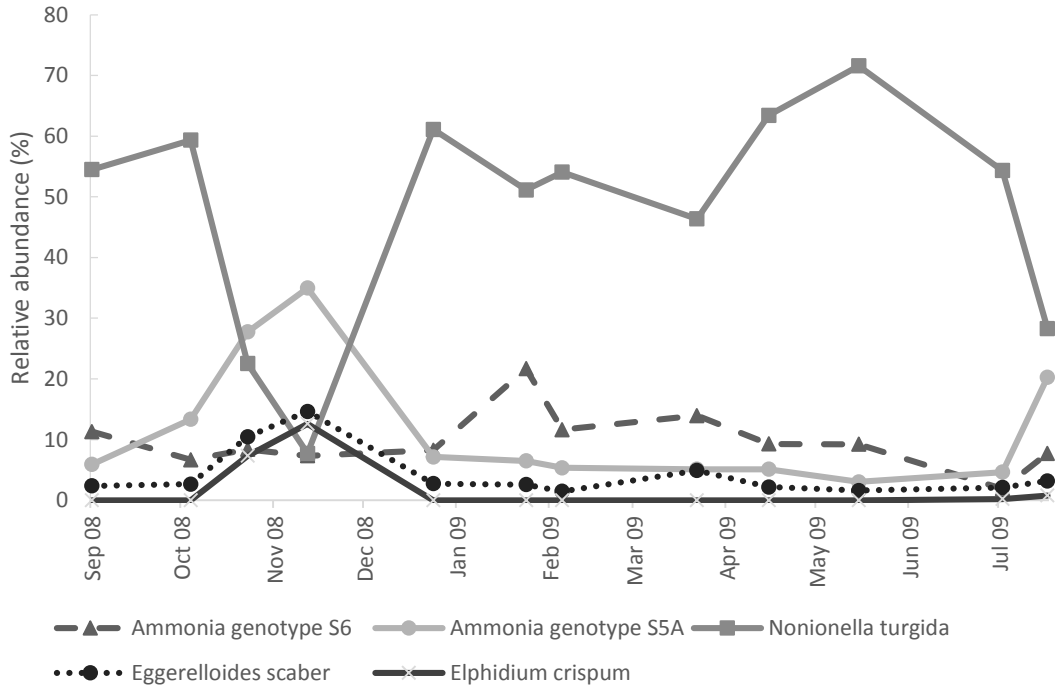


Figure 4.5 Relative abundance of the five most dominant taxa across the period of investigation at the Dunstaffnage site locality.

A shift in the assemblage composition was also identified during January, where the community structure is once again dominated by *N. turgida* (61% relative abundance, 113 ± 26 specimens per 100ml). As a consequence, this results in a parallel decline in the abundance of foraminiferal species including *Ammonia* genotype S5a, *E. crispum* and *E. scaber* (Figure 4.6A-C). *Nonionella turgida* remains the most dominant species in the assemblage from January to August. However, during August the relative abundance of this species declines to 41 ± 5 specimens per 100ml (28% relative abundance) (Figures 4.5A and 4.6). This could potentially illustrate the beginning of a shift in assemblage composition, as this decline coincides with a marked increase of *Ammonia* genotype S5a that accounts for 20.3% of the relative abundance.

Simple linear regressions were conducted to assess if there was a relationship between the absolute abundance of the five dominant species and the bottom water temperature and salinity measured at this site. An additional series of linear regressions were also conducted to assess whether the foraminiferal assemblage exhibit a lagged response to the two environmental variables. The results revealed that no statistically significant relationships were identified between these variables (Appendix, Table 6).

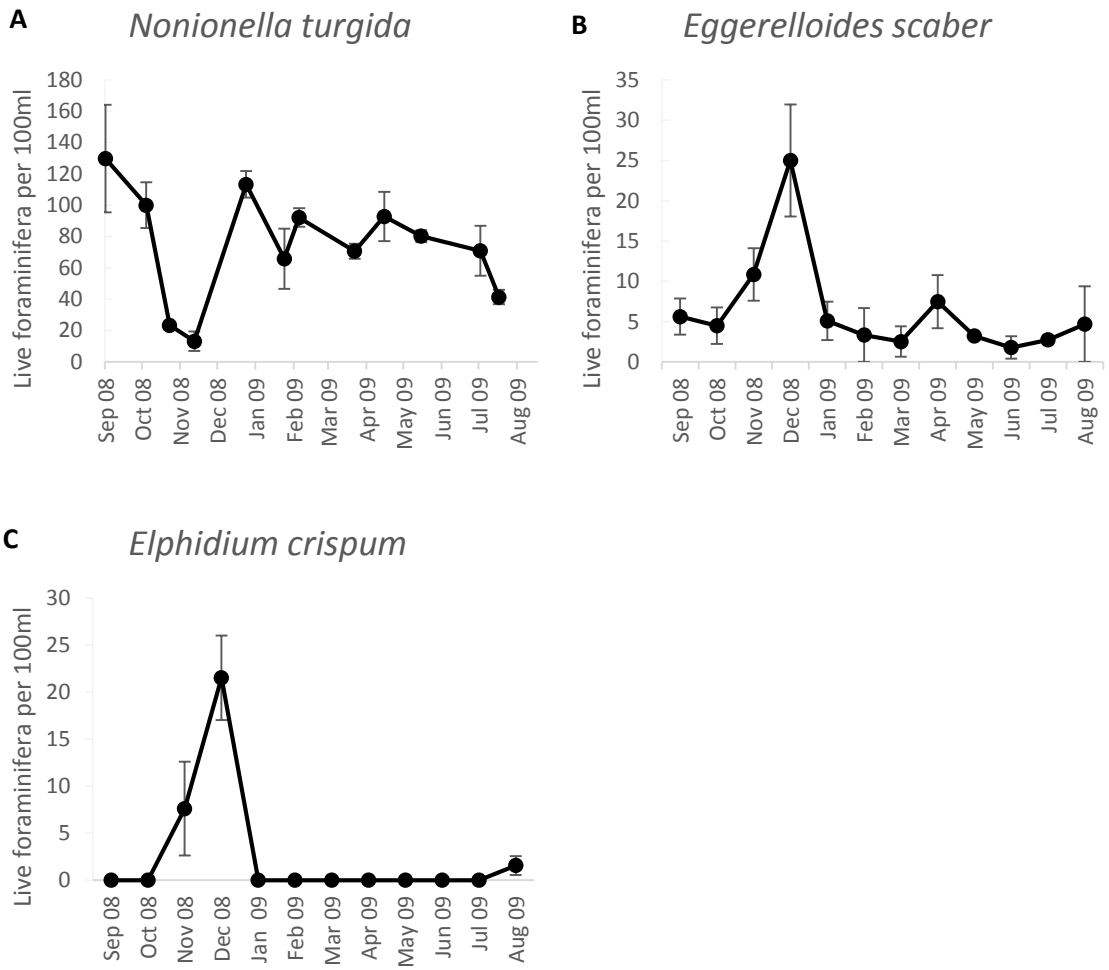


Figure 4.6 Standing crop (Live stained foraminifera per 100ml) of three of the most dominant taxa in the seasonal study, A) *Nonionella turgida,* B) *Eggerelloides scaber,* C) *Elphidium crispum.* Please note the different scale bars employed by each graph. The standard error of the replicate samples is illustrated to provide context of the spatial patchiness at this site.

4.3.4 Temporal variability of *Ammonia* species

An examination of the temporal dynamics of the two morphologically distinct species of *Ammonia* reveals that genotype S5a exhibits a distinctive peak in its absolute abundance in December 80 ± 10 specimens per 100 ml (Figure 4.7). In addition, another peak in absolute abundance was exhibited for this species during August, reaching 61 ± 59 specimens per 100ml (Figure 4.7). In contrast, no distinctive peak in absolute abundance was identified for *Ammonia* genotype S6. Instead, this species exhibits a gradual increase in standing crop from January to May. The maximal standing crop of *Ammonia* genotype S6 was achieved in February (36 ± 8 specimens per 100 ml) and the lowest standing crop was identified in November (9 ± 1 specimens per 100 ml). It is evident that *Ammonia* genotype S6 is more prevalent than *Ammonia* genotype S5a from January to June. In contrast, *Ammonia* genotype S5a is more dominant than *Ammonia* genotype S6 from October to December.

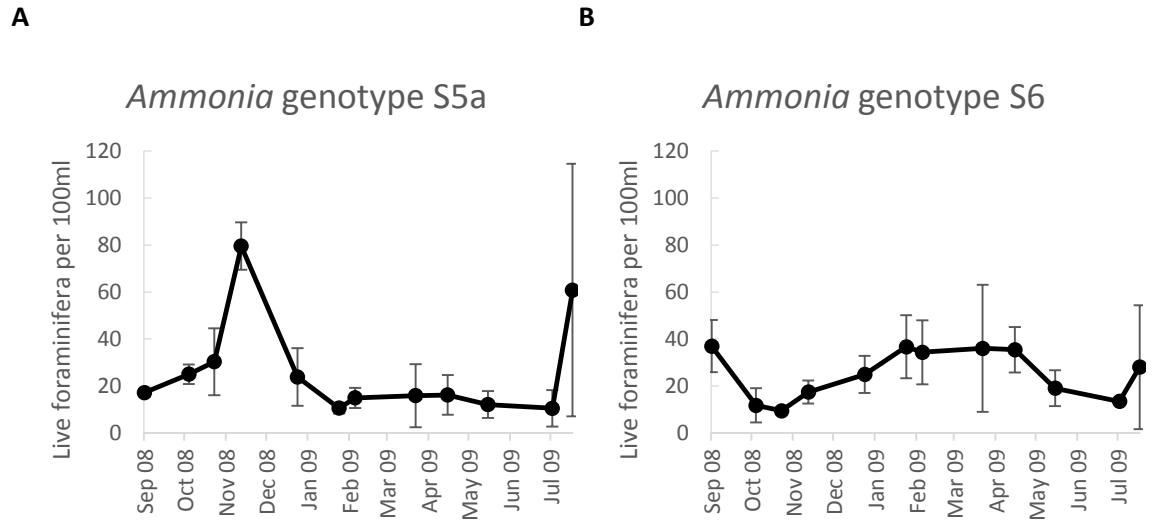


Figure 4.7 Standing stock (total live foraminifera per 100ml) of the two *Ammonia* genotypes. A) Genotype S5a, B) Genotype S6. The standard error of the mean of the two replicate samples is illustrated.

Whilst it is evident that both species of *Ammonia* co-exist throughout the year, subtle temporal partitioning can also be identified between these two species. For example, the relative abundance of these two species illustrates that *Ammonia* genotype S5a exhibits an autumnal/early winter preference (relative abundance 28-35%) (Figure 4.8). In comparison, during this time period, *Ammonia* genotype S6 exhibits its lowest levels of relative abundance (6.6-8.8%). Instead, *Ammonia* S6 exhibits a late winter to early summer preference as the relative abundance of this species exceeds that of *Ammonia* genotype S5a from January to June (Figure 4.8).

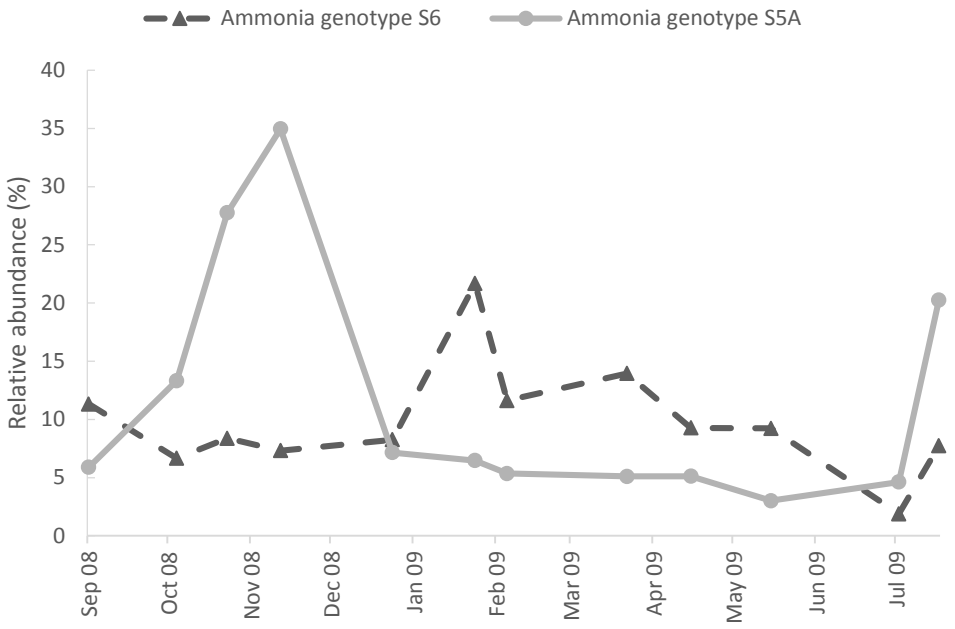


Figure 4.8 Relative abundance of the two *Ammonia* genotypes (based on aggregates of the two replicate samples).

4.3.5 Population dynamics of the *Ammonia* species

In order to examine the population dynamics of the two *Ammonia* species, the maximum test diameter of each specimen was measured. This data was plotted as the percentage relative frequency of each size class for each month (Figure 4.9). The results illustrate that only a limited number of juvenile specimens of *Ammonia* (<100 µm) were identified in the samples. The highest occurrence of juvenile specimens for genotype S5a was found in July (5.4% relative frequency), whilst the highest occurrence of juvenile specimens for genotype S6 was identified during November (4.9% relative frequency). A total of 17 *Ammonia* juvenile specimens were omitted from this analysis, because it was not possible to classify these specimens into a potential genotype due to the lack of visible diagnostic test characteristics at this early stage in ontogenetic development.

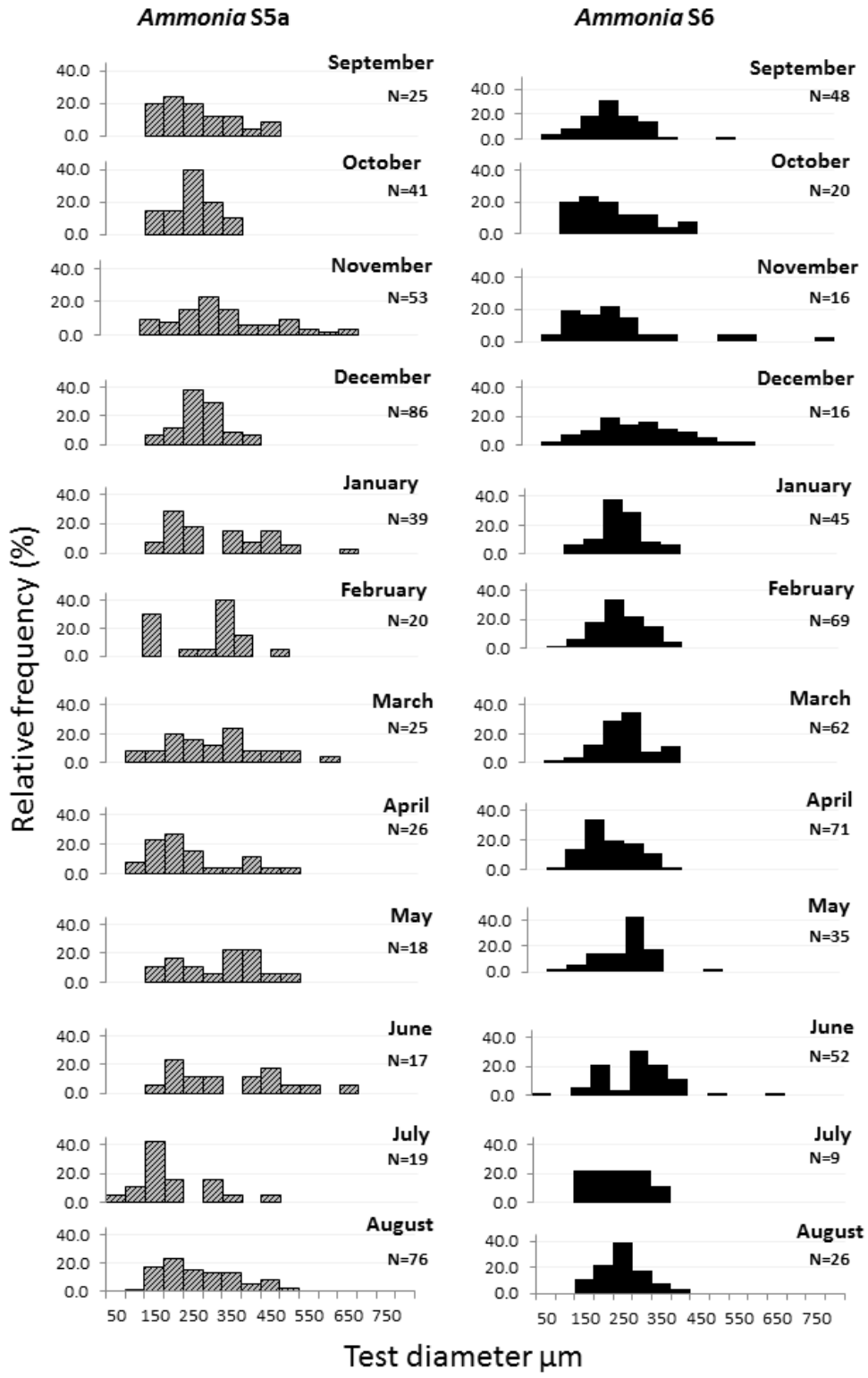


Figure 4.9 Population size structure (maximum test diameter) of *Ammonia* genotype S5a and S6 from September 2008 to August 2009. Results are displayed as relative frequency of each of the size classes for the two species.

The size frequency data of *Ammonia* genotype S5a reveals that this species continuously reproduces throughout the period of investigation (Figure 4.9). This is evidenced by the presence of several cohorts of different sized specimens, which are living simultaneously. For example, the size frequency data exhibits a bi-modal distribution during January, February, June and July, which indicates the presence of two distinct cohorts (Figure 4.9). Two phases of increased reproduction were potentially identified for *Ammonia* genotype S5a during September and October, and again in July. These phases of increased reproduction were recognised by the shift in the population size distribution towards smaller specimens, which is then proceeded by a shift towards larger specimens in the following months.

Additionally, the results reveal that *Ammonia* genotype S6 also continuously reproduces over the period of investigation (Figure 4.9). This is illustrated by the continuous presence of smaller specimens throughout the year and the fact that the distribution of the size frequency data is predominantly unimodal. A potential period of increased reproduction was noted in April, as the size distribution subtly shifts towards smaller specimens. It should also be noted that during November, December and June there is a prevalence of larger specimens, this is striking because during these months this species exhibits its lowest absolute and relative abundance (Figures 4.7 and 4.8).

4.4 Discussion

Understanding the temporal variability of foraminiferal assemblage structure is crucial as modern analogues of assemblages are commonly used to provide quantitative estimates of palaeoenvironments (Murray, 1991). To date, limited temporal analysis of foraminiferal assemblages has been conducted in NE Atlantic marginal marine environments (Hannah and Rogerson, 1997). Previous research conducted in this area only provides a 'snapshot' of the assemblage composition at the time of sampling (e.g. Edwards, 1982; Murray, 2003a; Scott et al., 2003; Austin and Cage, 2010). As a consequence, very few studies provide an understanding of how diversity changes with the seasonal variability of environmental conditions exhibited within this region. This study presents the first temporal analysis of changes in the foraminiferal assemblage structure and species diversity in a NW Scottish fjordic environment. This study has also re-evaluated *Ammonia* taxonomy within this region utilising the new lines of taxonomic evidence, as presented in Chapter 3. The results have identified the co-existence of two species of *Ammonia,* which exhibit subtle seasonal partitioning during the period of investigation.

4.4.1 Features of the foraminiferal assemblage structure

4.4.1.1 Species diversity

The diversity indices identified in this study are consistent with previously published investigations within this region. For example, the Fisher alpha diversity index calculated at this site ranges from 3.85 to 7.34. This is typical for this region as the Fisher alpha index can range from 2 to 4 in sheltered localities (Murray, 1992; Murray et al., 2003) to greater than 5 in open shelf sea environments (Murray, 1970, 1991). The total number of live species identified in this study (51 species) is also characteristic of this region. For example, Hannah and Rogerson (1997) identified 52 foraminiferal species from a comparative single site location in the Clyde Sea.

Despite the fluctuations in the diversity indices during the period of investigation, there is no obvious trend in species diversity over time. Instead, the diversity measures appear to be related to the abundance of *N. turgida.* For example, the lowest level of diversity exhibited in May and June coincides with the dominance of *N. turgida* in the assemblage (standing crop: 80-92 specimens per 100ml, 63-71% relative abundance, Figures 4.5 and 4.6). Previous studies identified that *N. turgida* is extremely reactive to phytodetritus inputs (Goineau et al., 2012), as discussed in depth in Section 4.4.1.3. This supports previous investigations, which have associated periods of low species richness and the dominance of a single species with the organic enrichment of the sediments (Gooday, 1999). Hence diversity at this site could be seasonally

controlled, as species dominance varies throughout the year with the changes in response to the availability of food.

4.4.1.2 Standing crop

The standing crop of live foraminifera exhibited at this site locality is low (e.g. 110-450 live specimens per 100ml), in comparison to the open Scottish shelf seas where > 100 live individuals per $10cm^3$ are commonly identified (Hannah and Rogerson, 1997). For example, in Stanton Deep in the Scottish shelf seas, a standing crop has of 817 specimens per $10cm^3$ was (Murray, 2003b). However, the low level of foraminiferal abundance exhibited in this study is not unprecedented as Murray et al. (2003) identified a standing crop of <73 specimens per $10cm^3$ from the inner basin of Loch Etive.

There is no obvious seasonal pattern in the foraminiferal standing crop; instead the total number of live foraminifera fluctuates across the year (Figure 4.3). No clear causal link was identified between the foraminiferal standing stock and temperature or salinity at this site. Thus it is possible that the fluctuations in the standing crop might be affected by other biotic and abiotic factors that were not quantified in this investigation. For example, previous investigations revealed that live foraminiferal density is extremely responsive to seasonal phytoplankton blooms that often occur during spring and autumn in UK waters (Murray, 1983; Scott et al., 2003). In addition, Murray (2002) hypothesised that the low foraminiferal density in Loch Etive was associated with low levels of organic flux. However, it is not possible to completely rule out the role that salinity and temperature may play in controlling foraminiferal abundance at this site, as foraminiferal density is often controlled by the complex combination of different environmental conditions (Murray and Alve, 2000).

4.4.1.3 Dominant species

The assemblage structure at this site locality was dominated by three main species: *N. turgida, Ammonia* genotype S5a and *Ammonia* genotype S6. The temporal dynamics of the two *Ammonia* species are discussed in detail in Section 4.4.2. One of the most striking features of the assemblage structure is the variability in abundance of the dominant species *N. turgida* during the year. In this study, no clear relationship was identified between the abundance of *N. turgida* and temperature or salinity. As developed previously, traditionally *N. turgida* has been identified as an opportunistic infaunal species (Murray, 2006). This species proliferates in localities with high phytodetritus inputs (Hohenegger et al., 1989; Barmawidjaja et al., 1995; Gustafsson and Nordberg, 2001; Diz and Frances, 2008; Goineau et al., 2011; Goineau et al.,

2012). Therefore, the temporal variability exhibited by *N. turgida* within this study could be driven by changes in the availability of food/phytoplankton blooms. A similar relationship was recognised by Alve (2010) whereby populations of *Nonionella iridea* were dependent upon the seasonal supply of fresh detritus.

The temporal variability of *N. turgida* also influences the overall assemblage structure. The decline in *N. turgida* abundance encouraged the growth of opportunistic taxa and resulted in a shift towards a relatively diverse and equilibrated fauna (Figure 4.4, Table 4.1). For example *E. crispum* (genotype S10) and *E. scaber* reached their maximum relative abundance during December, though are largely absent for the rest of the year (Figure 4.5). The peak abundance of *E. crispum* during winter is striking because traditionally this species was thought to have an ecological preference for warm water, with temperatures ranging from 8 to 18^{o}C (Murray, 2014). The occurrence of *E. crispum* towards its lower limit of known temperature tolerance (9^{o}C in this study, Figure 4.2) brings into question why this species is absent during periods of warmer, more optimal bottom water temperatures. It could be hypothesised that unlike *N. turgida* this species is not dependent upon a regular supply of fresh detritus for population maintenance, but could survive on alternative food supplies including refractory organic material and degradation products (Murray, 1963; Alve, 2010). This hypothesis is supported by evidence that *E. crispum* can survive on a range of food sources including photosynthetic products from its algal chloroplasts (Murray, 1963; Lee and Lanners, 1988, Lee and Anderson, 1991). However, until extended environmental surveys are conducted at this site locality, it is only possible to hypothesise the environmental controls on foraminiferal assemblage structure.

4.4.2 Temporal dynamics of *Ammonia* species

4.4.2.1 Classification of Ammonia species

The integrated taxonomic framework presented in Chapter 3 was employed to classify specimens of *Ammonia.* On the basis of morphological characters exhibited by *Ammonia* specimens, two species of *Ammonia,* genotypes S5a and S6 were identified in this study. This highlights that the current taxonomic practice of recognising a single species of *Ammonia* in this area has potentially underestimated species diversity in this region.

However, new molecular analysis conducted in the NW Scottish shelf seas has discovered the presence of three co-existing genotypes of *Ammonia* S4, S5a and S6 at both Dunstaffnage and also in Loch Sunart (Bird et al., in prep.). The absence of *Ammonia* genotype S4 in this study is interesting and could indicate that the ecological (microhabitat) requirements of *Ammonia*

genotype S4 are not met at this site locality. Bird et al. (in prep.) hypothesised that the occurrence of *Ammonia* genotype S4 may be controlled by water depth, as this genotype is abundant in intertidal areas but is rare in sub-tidal localities. For example, only a single specimen of *Ammonia* genotype S4 was identified at Dunstaffnage and Loch Sunart. The absence of *Ammonia* genotype S4 at this site could therefore be attributed to the sampling depth of 32m, which may be beyond the depth limit of this species. Further investigation is needed to clarify the ecological preferences (including depth partitioning) of these *Ammonia* species.

In practice it was sometimes difficult to classify juvenile specimens of *Ammonia* (<100 µm) using the key diagnostic morphological features identified in Chapter 3. This illustrates one of the major caveats of the integrated taxonomic framework presented in Chapter 3, as there is currently a paucity of knowledge of the morphological variability of *Ammonia* species at a population level.

4.4.2.2 Partitioning of Ammonia species

Currently there are a dearth of investigations which evaluate the influence of environmental controls on *Ammonia* abundance. Previously, temperature (Bradshaw 1957, 1961), salinity (Bradshaw 1957, 1961; Pascal et al., 2008), oxygen availability (Moodley and Hess, 1992; Martins et al., 2015) and the availability of organic matter (Martins et al., 2015) have been identified as important environmental controls on *Ammonia* abundance. However, these investigations predominantly classified specimens of *Ammonia* into broad morphospecies concepts including *Ammonia beccarii* and *Ammonia tepida.* As a consequence, the validity of previously identified *Ammonia* species-specific responses to environmental conditions are brought into question, as the broad morphospecies concepts previously recognised may represent an amalgamation of genetically distinct species.

This study reveals the presence of two species of *Ammonia* which co-exist in all the samples, whilst also exhibiting some subtle temporal partitioning (Figures 4.6, 4.7 and 4.8). However, the results revealed that no causal relationship between absolute abundance and temperature or salinity was identified. Alternatively, the partitioning exhibited between the two *Ammonia* species could be attributed to other abiotic and biotic factors that were not quantified in this study. For example, A*mmonia* genotype S6 exhibits a similar temporal pattern to *N. turgida,* albeit at a lower population density with a minor lag in response (Figures 4.6 and 4.7). The difference in response rates could be attributed to the species-specific reproduction cycles. Guffaston and Nordberg (2001) identified that thinner shelled taxa (*like N. turgida*) can rapidly

reproduce and grow to adulthood within less than a month, whilst taxa that possess thicker tests (e.g. *Ammonia*) lag behind because their reproduction cycles are longer. Furthermore, it could be hypothesised that abundance of *Ammonia* genotype S6 is controlled by the same environmental conditions as *N. turgida* such as food availability (Goineau et al., 2012). In contrast, the peak abundance of *Ammonia* genotype S5a coincides with the lowest relative abundance of *N. turgida* (Figure 4.5). Thus it could be asserted that *Ammonia* S5a is responsive to decreased competition and may be tolerant to low levels of phytodetritus or that it can survive on a range of food sources.

The hypothesised ecological preference of *Ammonia* genotype S6 for high organic matter is supported by prior investigations which identified that *Ammonia falsobeccarii* is prevalent in high abundance in organically enriched sediments (Fontainer et al., 2002; Pucci et al., 2009; Schweizer et al., 2011). *Ammonia* genotype S6 was previously ascribed to the species concept *Ammonia falsobeccarii* by Schweizer et al. (2011). This species concept of *Ammonia falsobeccarii* is one of the few taxa within the *Ammonia* genus which has been consistently classified throughout time, and its morphospecies concept holds up against new lines of taxonomic evidence (Schweizer et al., 2011; Bird et al., in prep). As illustrated in Chapter 3, *Ammonia* genotype S6 exhibits the diagnostic feature of discrete secondary dorsal openings which was originally identified as a key diagnostic feature in the type description of *A. falsobeccarii* (originally *Pseudoeponides falsobeccarii* Rouvillois, 1974). Therefore, it could be considered that the previously identified ecological preferences of this taxon previously are robust. In contrast, little is known about the ecological preferences of *Ammonia* genotype S5a, owing to the historical taxonomic and nomenclatural confusion and the absence of a consistently recognised discrete diagnostic test identified diagnostic test features (as discussed in greater depth in Chapter 3).

4.4.2.3 Recognition of reproductive phases of Ammonia

Foraminiferal growth is often rapid and episodic in nature, with foraminiferal species frequently exhibiting clear seasonality in growth and reproduction (Murray, 1991; Lee et al., 1991). During foraminiferal calcification (growth), a layer of calcite is directly precipitated on both the inside and outside of the test from the ambient seawater (Bé et al., 1979; Debenay et al., 2000). As such, foraminifera have significant utility as biogeochemical recorders of seawater conditions (James and Austin, 2008). For example, the test provides a record of the $\delta^{18}O$ of the seawater, water temperature and salinity at the time of calcification (Allison and Austin, 2003; Cage and Austin, 2008; Pearson, 2012). It is crucial to be able to identify phases of growth and reproduction in *Ammonia* in order to both refine our understanding of foraminiferal biology and to improve our understanding of the stable isotopic composition of extant and fossil foraminifera for palaeoenvironmental interpretations (as discussed in detail In Section 4.4.3).

Presently, there is a dearth in the understanding of *Ammonia* reproduction. Previous population dynamic studies have identified that *Ammonia beccarii* continuously reproduces throughout the year (Haake, 1967; Basson and Murray, 1995). Venec-Peyre (1983) by contrast, suggested that *A. beccarii* undergoes four distinctive phases of reproduction. Species of *Ammonia* in the NW Scottish shelf seas (classified under *A. batavus or A. beccarii)* have previously been found to exhibit seasonal phases in growth and reproduction. For example, individuals of *Ammonia* from stratified localities were found to grow (calcify) in late summer (Austin and Scourse, 1997; Scourse et al., 2004), whilst specimens from mixed localities grow (calcify) during spring or early summer (Scourse et al., 2004; Cage and Austin, 2008). However, all the studies outlined above use broad morphospecies concepts of *Ammonia.* As a consequence, our current understanding of *Ammonia* biology and reproductive stages may not be reliable, as current understanding may represent the calcification and reproduction behaviours of a mix of genetically distinct species.

By taking into account previously unrecognised cryptic diversity, this study presents one of the most accurate pictures of the population dynamics of *Ammonia* species yet undertaken. The results reveal the presence of co-existing populations of different size classes throughout the year for both *Ammonia* genotypes S5a and S6. This indicates that both species exhibit continuous reproduction over the period of investigation (Figure 4.9) and supports the findings of previous investigations into the population dynamics of *Ammonia* (Haake, 1967; Basson and Murray, 1983). The results also reveal potential phases of increased reproduction for both *Ammonia* species at different times of the year, these phases often coincide with the species'

respective peak absolute abundances. Therefore, it could be hypothesised that these species maintain a background population throughout the year and only exhibit significant growth (calcification) during favourable environmental conditions.

This study could be strengthened by sampling the site at a higher temporal resolution in order to further elucidate the phases of increased reproduction for the two species. This is crucial, as it has been previously identified that monthly sampling may not always successfully capture recruitment (Murray, 1983). In addition, specimens of *Ammonia* can take as little as 20 days to reach full maturity during growth (Bradshaw, 1957, 1961). Further investigation is needed to elucidate the growth, life cycles and reproductive behaviours of these species. In addition, extensive environmental surveys, and culturing investigations will be essential in helping to elucidate the biological behaviours and ecological preferences of these species.

4.4.3 Implications for palaeoenvironmental proxies

Species specific biological and geochemical proxies have been derived from *Ammonia* in the NW Scottish shelf seas in order to reconstruct past climates of the past 1000 years (Cage and Austin, 2010; Mokeddem et al., 2010; Reynolds et al., 2013). As previously discussed, *Ammonia* was utilised to provide a proxy for summer temperatures, as it primarily calcified during late spring and early summer (Cage and Austin, 2008; Cage and Austin, 2010). This earlier work only recognised a single morphospecies of *Ammonia* from the NW Scottish shelf seas commonly classified as either *A. batavus or A. beccarii*. However, this study identified two co-existing species of *Ammonia* which exhibits subtle seasonal partitioning and this poses significant implications for previous reconstructions. For example, the results from this study suggest that the two species exhibit subtly different reproductive (hence calcification behaviours) across the period of investigation (Figures 4.7, 4.8 and 4.9). These different reproductive behaviours would affect the isotopic signals which are incorporated into the test as a function of marked seasonality in the temperature of the bottom waters.

Thus species specific proxies utilised by previous investigations may not be as reliable as previously considered, because it is likely that the previous calibrations of geochemical proxies from *Ammonia* are likely to be based on a mix of populations of genetically distinct species. Seasonal differences in calcification behaviours can have significant implications for the interpretation of isotopic signals. For example, a temperature difference of 2^{o}C was identified from the offset of δ^{18}O from specimens of *A. batavus* and *Q. seminulum,* which calcify in different seasons (Austin and Scourse, 1997, Scourse et al., 2004). In addition, Cage and Austin (2008)

identified an offset in $\delta^{18}O_{foram}$ of *Ammonia beccarii* equating to a temperature difference of 1°C when mixed season populations were analysed. However, the robustness of the previously identified isotopic offsets is further questioned, as these studies did not take into account the potential genetic diversity of *Ammonia,* which previous taxonomic studies failed to identify. Nevertheless, previous studies demonstrate the importance of quantifying both the interspecific and intraspecific $\delta 18O_{foram}$ throughout the year. Further work is required to elucidate the calcification behaviours of these two *Ammonia* species at this site and to establish the seasonal variability of the $\delta^{18}O$ test composition. If both the population dynamics and the seasonal isotopic effects on these two species could be untangled, this would refine the accuracy and reliability of *Ammonia* proxies in palaeoenvironmental reconstructions potentially by up to 2°C (Scourse et al., 2004; Cage and Austin, 2008).

4.4.4 Data limitations and constraints of sampling techniques

Spatial heterogeneity (patchiness) in foraminiferal distribution can occur at centimetre to metre scales (e.g. Boltovskoy and Lena, 1969; Fontanier et al., 2003; Morvan et al., 2006). Currently the causes of patchiness are poorly understood but have previously been attributed to grazing and predation (Murray and Alve, 2000), or the uneven distribution of organic matter (Murray, 1991; Swallow, 2000; Morvan et al., 2006). Spatial heterogeneity can introduce bias to the calculation of foraminiferal density (absolute abundance) obscuring the understanding of temporal variability (Buzas, 1968, 1970; Schafer, 1971; Buzas et al., 2002; Morvan et al., 2006). In order to quantify the spatial heterogeneity at a site, it is recommended that replicate samples are taken and that these are independently analysed (Schönfeld et al., 2012). Although this study analysed replicate samples, in order to attain statistically significant census counts the replicates were aggregated. Consequently, it was not possible to undertake a full assessment of the degree of spatial heterogeneity in this study. However, the standard error of the absolute abundance of each pair of replicates was calculated (Figure 4.3). The difference between the absolute abundance of the pairs of replicates was most noticeable during June and July (Figure 4.3); this suggests that patchiness occurs at this site and it may be seasonally variable. However, the aggregation of replicate pairs in this study minimises the potential bias caused by patchiness and should therefore provide a good representation of the temporal variability in assemblage structure at this site (Murray, 1991; Schönfeld and Numberger, 2007).

The spatial heterogeneity exhibited between the standing crops of the two replicate samples may also be attributed to the sampling techniques employed in this study. Although significant

care was taken to collect samples from the top 1-2 cm of the sediment, no standardised volume of sediment was taken. Consequently, there is a possibility that this technique may have captured samples at different depths in the sediment. Samples taken below the optimal depth of 1-2 cm may dilute the number of live foraminifera per 100ml due to the inclusion of an increased number of empty (dead) tests from deeper layers (Fontainer et al., 2003; Schönfeld et al., 2012). The aggregation of the paired replicate samples also helps to reduce any sampling bias. This study could be strengthened by quantifying the impact of the potential sampling bias and spatial heterogeneity by analysing the foraminiferal assemblage structure from a series of push cores (circa 5) which have known volumes and sediment depths.

Another potential limitation of this study is that it uses Rose Bengal staining to identify live foraminiferal species. However, the efficacy of Rose Bengal staining was previously questioned as protoplasm can persist days or even weeks after death (Walker et al., 1974; Bernhard, 1988, 2000; Corliss and Emerson, 1990; Murray and Bowser, 2000). To address this, a stringent protocol of assessing the degree of Rose Bengal staining was implemented.

Finally, whilst this study provides a detailed account of the inter-annual variability of the foraminiferal assemblage structure, it is unclear whether the same temporal trends are replicated on an inter-annual basis. For example, previous time series studies have recognised that seasonal patterns are not always reproduced on a year to year basis, and that analysis of a single year may not reveal the underlying cyclicity in species diversity and assemblage compositions (Boltovskoy and Lena, 1969; Scott and Medioli, 1980; Basson and Murray, 1995; Alve and Murray, 2000; Swallow, 2000; Morvan et al., 2006). This highlights the necessity of further taxonomic investigation at this site to verify the reoccurrence of these seasonal trends.

4.5 Conclusions

This study provides the first time-series analysis of a coastal environment in NW Scotland in which the temporal dynamics of benthic foraminiferal diversity and assemblage composition are investigated. Notably this is the first applied taxonomic investigation which incorporates new lines of taxonomic evidence to document the seasonal variability of two previously cryptic species of *Ammonia*. Two species of *Ammonia* were identified co-existing throughout the year, with some evidence to suggest that they exhibit subtle seasonal partitioning. *Ammonia* genotype S5a is dominant during November-January, whilst *Ammonia* genotype S6 is dominant during February-June. This subtle seasonal partitioning could indicate that these *Ammonia* species occupy distinct ecological niches.

Additionally, this study has provided baseline information on how foraminiferal diversity and overall assemblage composition changes seasonally. This understanding is crucial, as it is a prerequisite for downstream studies that assess how biodiversity and species ranges change in response to abrupt climate change. In total 52 species were identified at this site and the assemblage was dominated by three species: *Nonionella turgida, Ammonia* genotype S5a and *Ammonia* genotype S6. No clear seasonal trends in biodiversity nor standing crops were identified over the period of investigation. However, the results have identified a clear temporal trend in changes to the overall assemblage composition. For example, the abundance of *Nonionella turgida* shifts over the course of the year, i.e. it is dominant in spring-summer, whilst less prevalent in winter. However, no clear causal relationship was identified between the abundance of the five dominant taxa and temperature and salinity measured at this site. Interpreting the controls on this temporal variability is difficult due the paucity of environmental data available. However, the results reveal that species occurrences may be driven by the source and input of food supply at this site (e.g. seasonal phytoplankton blooms, Murray, 1983; Scott et al., 2003).

Future investigation should focus on conducting extended sampling, coupled with detailed environmental surveys to analyse if these temporal trends are repeated inter-annually and to elucidate the environmental controls on the assemblage structure. Additionally, further investigation is needed to clarify the ecology, biology and geochemistry of the newly delineated *Ammonia* genotypes so that species-specific proxies can be refined, this in turn can help to inform our understanding of past and future climate change.

Chapter 5

Morphological distinction of genotypes from the Elphidiidae family across the NE Atlantic

The sampling of elphidiid specimens across the NE Atlantic for this chapter was a collaborative effort between this researcher and researchers from within a larger NERC- funded project (NE4/G018502/1). The phylogenetic framework and the SEM images used and presented in this chapter were produced by Kate Darling, Magali Schweizer, Clare Bird, Kath Evans at the University of Edinburgh (Darling et al., in prep). This chapter also benefits from unpublished biogeographic maps and a list of ascribed classical taxonomic names which have been attached to newly delineated genotypes this data has been provided by Kate Darling, William Austin, Karen Luise Knudsen, Magali Schweizer, Clare Bird, Kath Evans (Darling et al., in prep.).

Chapter 5: Morphological distinction of genotypes from the Elphidiidae family across the NE Atlantic

5.1 Introduction

Species within the Elphidiidae family (Galloway, 1993) are some of the most common and ubiquitous foraminifera within the marine benthos (Murray, 1991). Members of Elphidiidae are present from tropical to polar regions and occur across intertidal zones and extend subtidally onto the continental slopes (Murray, 2006). Their strong sensitivity to environmental conditions coupled with their exceptional fossil record dating back to the Eocene (Cushman, 1939) has enabled species within this family to be widely used as palaeoenvironmental proxies (Murray, 1991). A strong taxonomic framework is required to enable the robust morphological identification of species within this family in both contemporary and palaeoenvironmental settings. This taxonomic consistency is crucial because elphidiid species are frequently used to reconstruct Quaternary climates (Sejrup et al., 2004) and past sea levels (Gehrels, 2000) based on species-specific biological and geochemical proxies.

Despite over 250 years of taxonomic investigation, the classification of elphidiids is complex and continues to challenge taxonomists. Traditionally, species within this taxonomic group have been exclusively classified by their morphological test characteristics. Some of the entrenched taxonomic confusion has arisen from the uncertainty surrounding the morphological species boundaries, owing to the vast range of test morphology exhibited within this family and exacerbated by poorly illustrated (type) specimens (Buzas, 1966). The crux of the problem which has been known for some time is “there are either many species or else a great amount of variation of one species” Cushman (1944, p.25). Confusion arises when different taxonomic schools employ different conceptual approaches to species delineation and ascribe different morphological characteristics different taxonomic weight. For example, ‘lumpers’ emphasise the morphological similarities between specimens, recognising few species concepts; thereby allowing a considerable breadth of morphological variability in the species concepts (Cushman, 1930; Feyling-Hanssen, 1972; Poag et al., 1978; Miller et al., 1982). In contrast, ‘splitters’ emphasise the morphological differences between species and split taxa based on minute morphological variations (Weiss, 1954; Brodniewicz, 1965). The parallel development of these two taxonomic schools has led to the emergence of different taxonomic descriptors and species concepts between researchers across time and space (Cushman, 1944; Buzas, 1966; Haynes, 1973; Buzas et al., 1985).

Not surprisingly, the nomenclatural history of the Elphidiidae is extremely complex and synonymy is rife within the literature. For example, at least 17 synonyms have been identified at a genus level including: *Elphidium* (de Montfort, 1808), *Cribroelphidium* (Cushman and Brönnimann, 1948), *Elphidiella* (Cushman, 1936), *Pellatispirella* (Hanzawa, 1937) , *Polystomella* (Lamarck, 1822), *Rectoelphidiella* (He et al., 1965), *Stomoloculina* (He et al., 1965), *Cribrononion* (Thalmann, 1946), *Cellanthus* (de Montfort, 1808), *Elphidiononion* (Hofker, 1951), *Helicoza* (Moebius, 1880), *Ozawaia* (Cushman, 1931), *Porosononion* (Putrya in Voloshinova, 1958), *Nonionina* (Brady, 1881), *Nonion* (Cushman 1948) and *Pseudononion* (Cushman, 1931). Most morphospecies have been classified into different genera by different researchers at least once (Buzas et al., 1985). Pillet et al. (2013) has nicely summarised and reviewed the taxonomic complexities of this group at the genus level.

Taxonomic uncertainty of Elphidiidae is not only confined to the genus level, unravelling the taxonomic history of elphidiids at the species level proves to be just as complex. Culver and Buzas (1980) estimated that there are approximately 40 morphospecies of *Elphidium*, whilst Murray (1991) estimated that more than 60 species have been recorded. The most recent estimate in the world foraminiferal database suggests that there are over 120 species concepts recognised in the literature just for the genus *Elphidium* (Hayward et al., 2015). The nomenclatural confusion in the Elphidiidae family is typified by the uncertainty surrounding the species boundaries of *Elphidium excavatum*. Despite being one of the first foraminiferal species identified (*Polystomella excavata*, Terquem, 1875), this species is one of the world's most commonly misclassified foraminiferal species (Buzas et al., 1985). When trying to clarify the extreme morphological diversity exhibited by this species, different researchers over the years have associated this variability to specific effects (Weiss, 1954; Brodniewicz, 1965), subspecific effects (Wilkinson, 1979) or ecophenotypy (Feyling-Hanssen, 1972; Poag et al., 1980; Miller et al., 1982; Rodriguez and Hooper, 1982; Goubert et al., 1997). Therefore, it is unsurprising that this has created considerable nomenclatural confusion with a myriad of synonyms, mistakes and misidentifications in the academic literature. This confusion is clearly illustrated in Figure 5.1 (Miller et al., 1982).

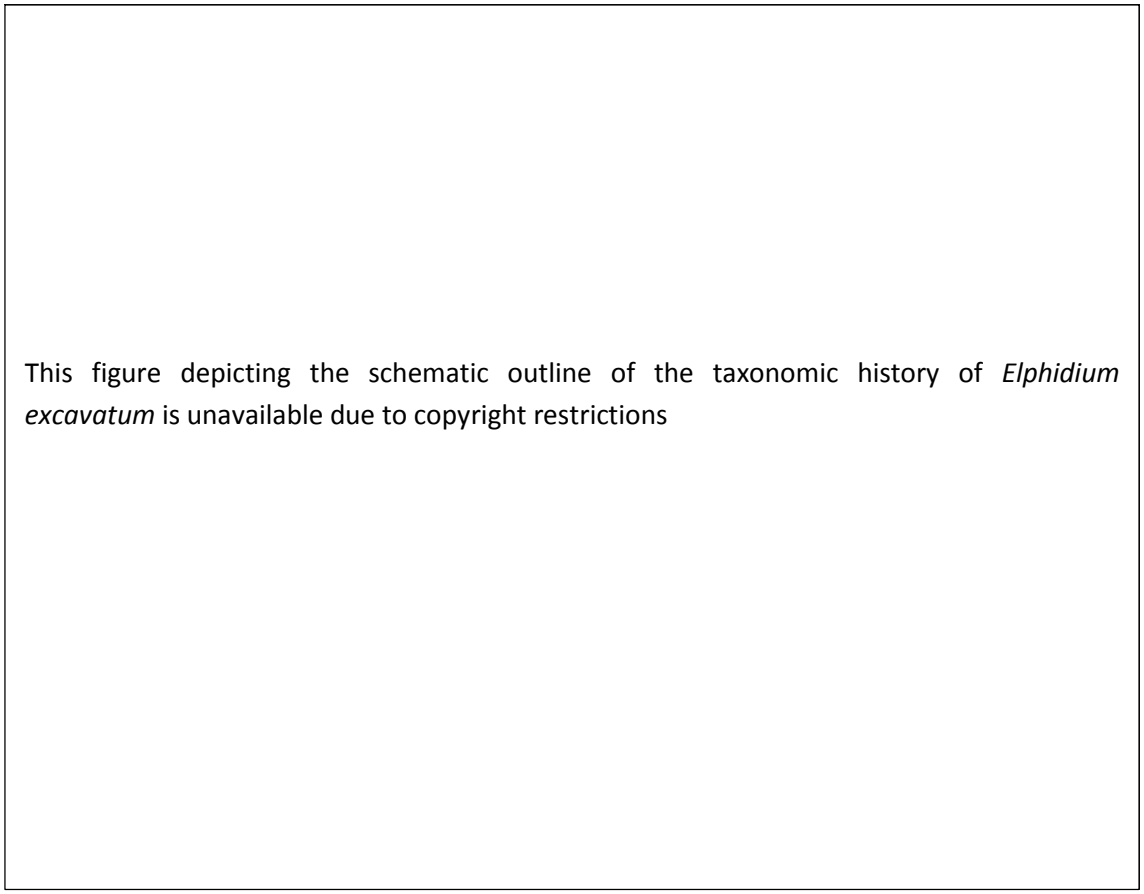


Figure 5.1 Schematic outline of the taxonomic history of *Elphidium excavatum* detailing the synonymy, uncertain affinities and nomenclatural errors within this complex. This figure has been redrawn from Miller et al. (1982).

Historically, the intraspecific morphological variability exhibited within *Elphidium excavatum* was attributed to ecophenotypy. The concept of ecophenotypy was first applied to the genus *Elphidium* by Feyling-Hanssen (1972) who associated the different test morphologies exhibited by *Elphidium excavatum* to be a product of different environments and/ or biogeographic localities (as detailed in Table 5.1). The identification of phenotypic plasticity as a response to different environmental conditions was supported by an experimental breeding study of *Ammonia* conducted by Schnitker (1974). Schnitker (1974) identified seven ecophenotypic variants from a single species concept of *Ammonia* (*Ammonia tepida*). This study provided the impetus for subsequent taxonomic investigations of *Elphidium* to recognise a limited number of species concepts and numerous ecophenotypic variants. In total 11 *Elphidium excavatum* ecophenotypes have been described in the literature (Poag et al., 1980; Miller et al., 1982; Goubert et al., 1997). The five most common ecophenotypes and their biogeographical and ecological preferences are detailed in Table 5.1.

Elphidium	Environment	Distribution
Clavatum	Normal marine to brackish, cold	Arctic
Excavatum	Intertidal	-
Selseyensis	Estuarine, temperature to polar (1 to 16^{o}C)	Boreal
Lidonesis	Estuarine, warm to temperature	Lusitanian
Magna	Near shore, turbulent zone	-

Table 5.1 Environmental preferences and distributions (if known) of ecophenotypes of *Elphidium excavatum* from Feyling-Hanssen (1972) and Miller et al. (1982). Reproduced with permissions of Cambridge University Press from Murray (2014).

Despite extensive taxonomic investigation on Elphidiidae specimens, the uncertainty surrounding the diagnostic characteristics, high levels of synonymy and the paucity of carefully illustrated specimens, has made keeping a track of different species concepts across time and space an onerous task. Consequently, it is difficult to compare species concepts from different sources (Miller et al., 1982). Ambiguous taxonomy could have significant implications in applied taxonomic investigations, as these studies require a strong taxonomic platform, which assumes that the species recognised are genetically homogenous, and have distinct ecological preferences across time (Murray, 2014). Erroneous species identification can have significant implications, potentially leading to a misrepresentation of biodiversity, species' natural biogeographical ranges and ecological preferences (Scott and Medioli, 1978; Murray, 1991).

5.1.1 New taxonomic insights provided by molecular systematics

Molecular systematics has shed light on the taxonomic relationships within the Elphidiidae taxonomic group (Pawlowski et al., 1997; Langer, 2001; Habura et al., 2008; Schweizer et al., 2008; Schweizer et al., 2011; Pillet et al., 2011; Pillet et al.,2012; Pillet et al., 2013). The new molecular evidence has identified that no ecophenotypic variants could be found within the *E. excavatum* species complex, instead morphotypes should be attributed to different subspecies or species (Schweizer et al., 2011; Pillet et al., 2013). The most comprehensive molecular analysis of the Elphidiidae taxonomic group to date was conducted by Pillet et al. (2013). This study identified 15 genetically and morphologically distinct species of Elphidiidae globally (Figure 5.2).

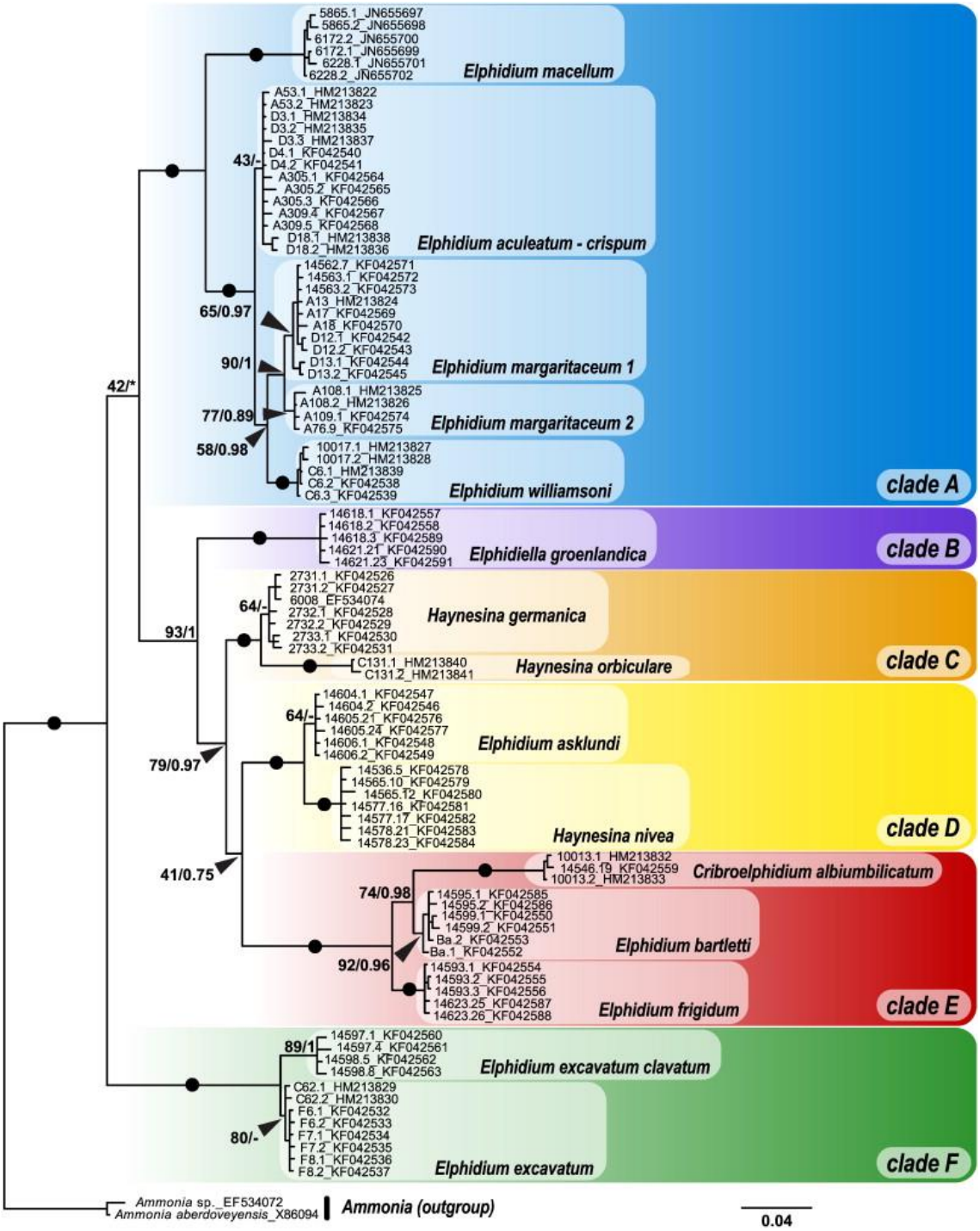


Figure 5.2 Phylogenetic tree of the SSU rRNA of 16 Elphidiid genotypes, which are separated into six highly supported clades. Figure reproduced with Elsevier from Pillet et al. (2013).

Whilst recent advancements in molecular techniques have provided a strong taxonomic foundation for the Elphidiidae family, it is important to recognise that these studies are not exhaustive. For example, Pillet et al. (2013) only inferred the taxonomic relationships within this family on the basis of 66 SSU rRNA sequences and 26 partial SSU rRNA sequences. Moreover, whilst their sampling regime has provided a global overview, it is not exhaustive. For example, Pillet et al. (2013) only sampled 17 site locations globally. Consequently, Pillet et al. (2013) may not have captured the complete diversity within Elphidiidae, as there are discrete sampling gaps. Previous molecular investigations have suggested that further interrogation of genetic relationships and more extensive sampling is required to provide a more stable taxonomic framework (Pillet et al., 2011; Pawlowski et al., 2014).

Although the recent molecular investigations have better characterised the phylogenetic relationships within the Elphidiidae family, this new taxonomic framework still lacks a satisfactory resolution for use in a practical morphological application. For example, instead of quantifying the interspecific morphological boundaries of the genotypes, qualitative classical morphological descriptors were used to describe the morphological variability (Pillet et al., 2011; Schweizer et al., 2011; Pillet et al., 2013). Additionally, the majority of studies only illustrate end member test morphology (Schweizer et al., 2011; Pillet et al., 2013). Therefore, these studies fail to resolve the uncertainty surrounding interspecific morphological boundaries. This understanding is crucial as at present fossil foraminifera can only be delineated using their morphological characteristics. This highlights that the re-evaluation of the Elphidiidae is a work in progress and further taxonomic investigation is required to quantify the taxonomic relationships within this family.

This chapter aims to create a more stable and practical taxonomic framework of the Elphidiidae family by integrating new lines of taxonomic evidence from elphidiids in the NE Atlantic. The taxonomic boundaries of Elphidiidae are revisited in this study through exploring the morphometric and biogeographical limits of genetically defined species (after Darling et al., in prep.). This study represents the first detailed assessment of the quantitative interspecific morphological boundaries in the Elphidiidae family conducted to date. Additionally, the extensive sampling regime employed in this study across the distinctive biogeographic provinces in the NE Atlantic has provided an opportunity to gain a more comprehensive understanding of the genetic diversity and biogeography of elphidiids within the Northeast Atlantic shelf seas.

The key aims of this chapter include:

- To examine the value of test morphology in delineating between the 17 Elphidiidae genotypes identified within the North East Atlantic by Darling et al. (in prep.).
- To characterise the interspecific morphological boundaries of each genotype and to identify (if present) the key diagnostic morphological traits.
- To examine the intraspecific morphological variability across spatial scales in the NE Atlantic.

5.2 Methods

5.2.1 Sample collection

Surface sediment samples were collected from 25 sites across the North East Atlantic shelf seas (Figure 5.3, Table 5.2). A combination of sediment scrapes, intertidal seaweeds (shallow sites), box coring, multi-coring and SCUBA divers (deeper sites) were utilised to collect the top 1-2 centimetres of surface sediment. For a detailed outline of the sediment sampling techniques refer to Chapter 2, Section 2.2.1. The surface sediments were then sieved and stored in seawater at a temperature of 4^{o}C in a cold room or in a refrigerator prior to analysis.

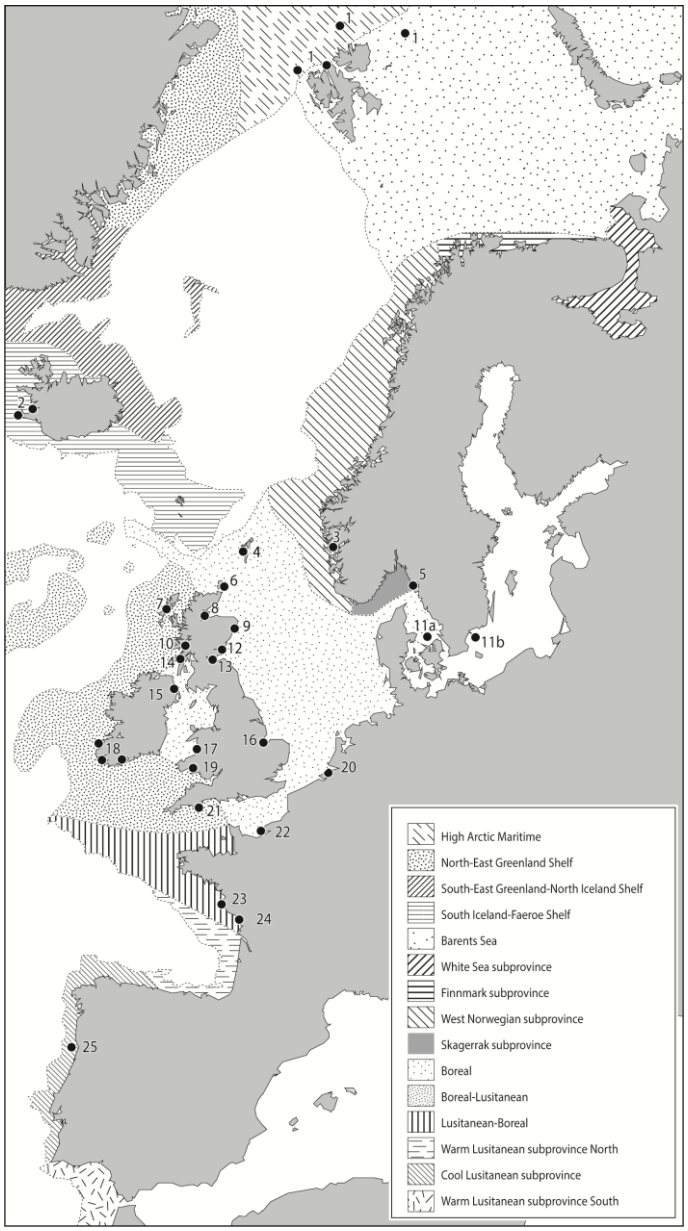


Figure 5.3 Sample site location map. The fourteen biogeographic provinces identified by Dinter et al. (2001) across the NE Atlantic are also depicted.

Table 5.2 Number of Elphidiidae specimens morphometrically and genetically analysed at each site. The numbers shown in bold are the total number of specimens both morphologically and genetically analysed. Numbers in the standard font represent the total number of specimens genetically sequenced. Six sites are shown without any data as the elphidiid specimens collected were either not successfully sequenced or no live elphidiid specimens were identified at this site.

Genotype	Svalbard (SV)	Iceland (IS)	Bergen (BE)	Shetland (SH)	Skagerrak (SK)	Orkney (OK)	North Uist (NU)	Cromarty (CR)	Ythan (YN)	Dunstaffnage (DF)	Baltic (BA)	Eden (ED/SA)	Cramond (CD)	Loch na Cille (LK)	White Rock (WR)	Norfolk (NF)	Aberdovey Bay (AB)	Cork (CK)	Laugharne (LC)	Gevelingen (GE)	Dartmouth (DM)	Baie de Seine (BS)	Ile d'Yeu (YE)	Baie d'Aiguillon (AI)	Portugal (PO)	Sunart	Oslo Fjord	Den Oever Holland	Sardinia Mediterranean	Groomsport Ireland	Portugal Guadina River
Number on map	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25						
S1		**17**/ 33		0/ 26		**23**	**36**	**6**	**9**/ 26			**88**	**3**/4	0/ 14	**16**	**14**	**20**	**22**/ 49			**26**/ 28										
S2				**1**			**15**								**1**						**5**										
S3															**3**			**22**/ 24			**24**										
S4	**2**	**4**		**4**	**8**/9		**7**				**10**																				
S5									**20**				**5**/7							0/4	**10**	**2**									
S6							**2**						**2**																		
S7									**10**																						
S8	**10**																														
S9				**8**			**23**											**1**			**1**										
S10			0/1	**1**			**1**			**3**																					
Sthe11																									**3**						
S12																							**2**/3								
S13																					**2**										
S14							**12**							**3**																	
S15	**1**																														
S16				**6**			**13**/ 18	**6**/ 10	**6**/9			**14**/ 72	**6**/ 26	**1**/3	**14**/ 21	**13**/ 44		**15**/ 34	**13**/ 23		**12**/ 20			0/4							
S17							1														**3**										
Total	**13**	**21**/ 37	0/1	**20**/ 46	**8**/9	**23**	**84**/ 115	**6**/ 16	**45**/ 65	**3**	**10**	**102** / 160	**16**/ 35	**18**/ 20	**34**/ 41	**13**/ 58	**20**	**60**/ 108	**13**/ 23	0/4	**83**/ 93	**2**	**2**/3	0/4	**3**						

5.2.2 Preparation of foraminiferal specimens

Live foraminiferal specimens were identified and picked from the surface sediment samples following the methods outlined in Chapter 2, Section 2.3.1. Live specimens were identified by their natural protoplasm colouration and pseudopodial activity (e.g. sediment cocoons and 'foram racing'). Once pseudopodial activity was identified, the live specimens were thoroughly cleaned. The picked foraminiferal specimens were then transferred onto SEM stubs and were subsequently gold coated using a splutter coater to approximately 20nm thickness. The specimens were imaged using an SEM (Phillips XL30CP) from the umbilical (side) profile by project collaborators at the University of Edinburgh.

5.2.3 Phylogenetic analysis

The molecular sequences of the Elphidiidae specimens collected from across the NE Atlantic were provided by the University of Edinburgh foraminiferal genetics laboratory as part of a larger NERC-funded project. The SSU rRNA was extracted from live specimens following the methods of Schweizer et al. (2011). In total 1,013 individual specimens of elphidiids were successfully characterised using the partial SSU rRNA gene. For comparative analysis, the sequences were manually aligned together with 125 elphidiid SSU rRNA sequences from GenBank. Phylogenetic trees were constructed using three different methods. A Bio Neighbor-Joining (BioNJ) tree with 1,000 bootstrap replicates was constructed (Figure 5.4) as well as Maximum Likelihood (ML) analysis, with 2,000 bootstrap replicates and Bayesian analysis (BA) (Darling et al., in prep.). The BioNJ tree was chosen because the general topology is most similar to the phylogeny produced by Pillet et al. (2013). The statistical support of the ML and BA trees are shown on the common branches of the BioNJ tree (Figure 5.4). In total 17 genetically distinct elphidiid species were identified in the NE Atlantic, falling into four distinctive clades as illustrated in Figure 5.4 (Darling et al., in prep.).

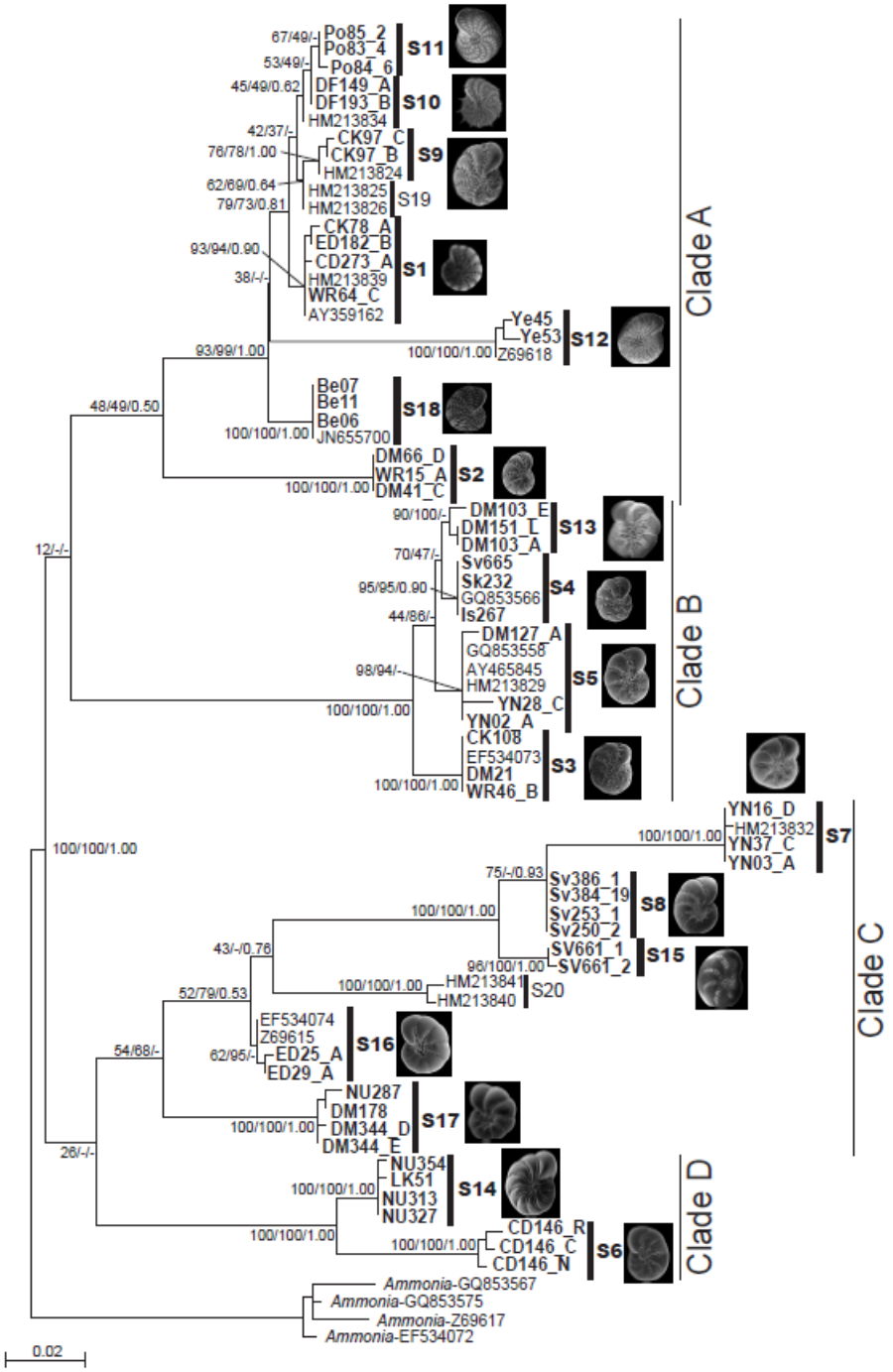


Figure 5.4 SSU Phylogenetic tree of Elphidiidae specimens collected across the North East Atlantic shelf seas indicating the 17 distinct genotypes, and four genetic clades. The phylogeny also includes representative elphidiid sequences available in GenBank. This phylogeny is produced using a Bio Neighbor-Joining (BioNJ) tree using 1,000 bootstrap replicates. The numbers at the branches illustrate the statistical support of the BioNJ tree as well as the Maximum likelihood (ML) analysis with 2,000 BS replicates and a Bayesian analysis. Figure taken from Darling et al. (in prep.).

Figure 5.5 illustrates the typical morphology exhibited by each of the 17 Elphidiidae genotypes identified by Darling et al. (in prep.)

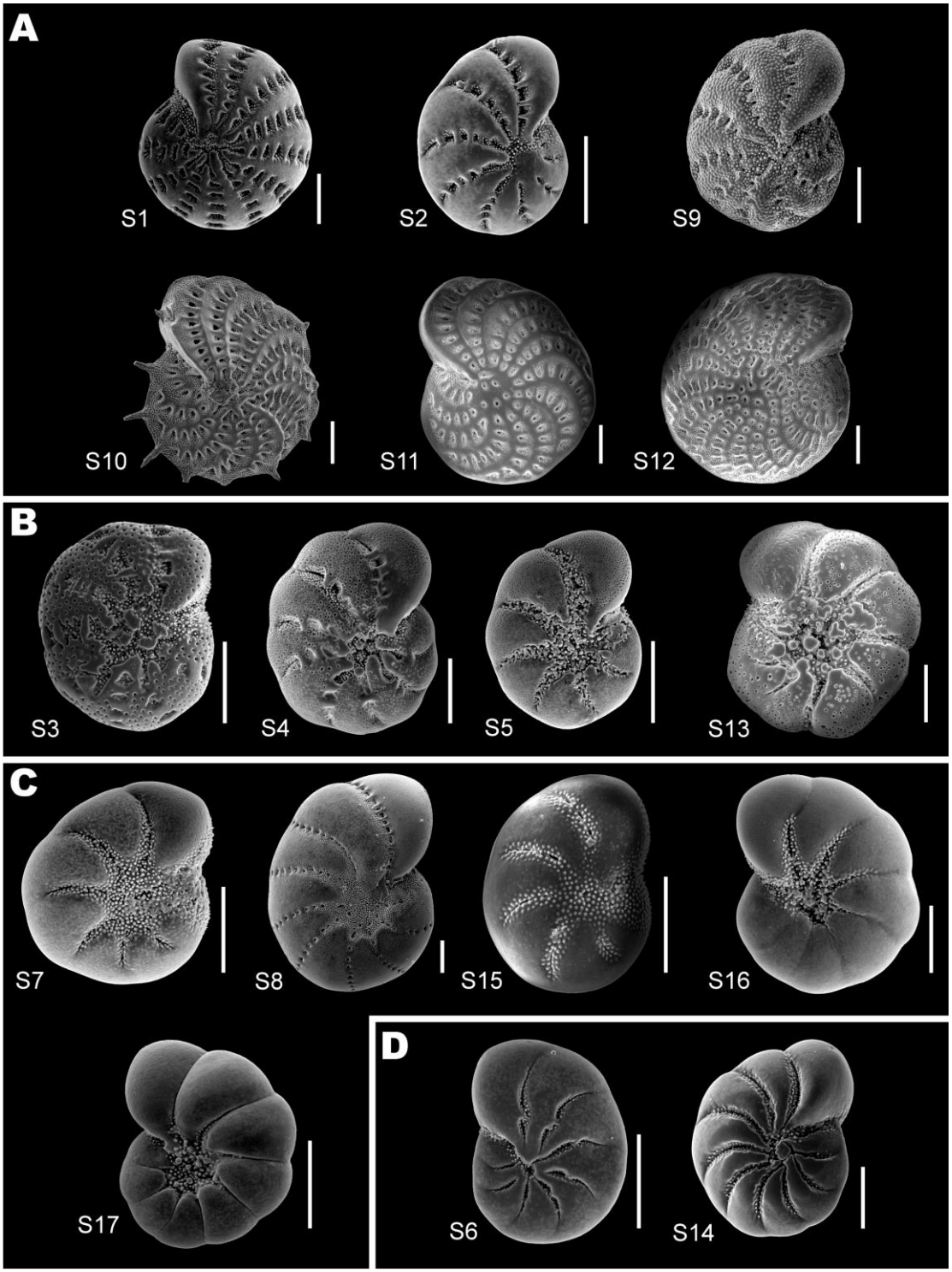


Figure 5.5 Umbilical SEM views of 17 Elphidiidae genotypes identified across the NE Atlantic based on SSU rRNA as identified by Darling et al. (in prep.). These genotypes are separated into four distinctive clades (A-D). The scale bar illustrated is 100 µm. Figure taken from Darling et al. (in prep.).

5.2.4 Morphometric analysis

Prior to morphometric analysis, a series of image pre-processing procedures were conducted on each SEM image (as described in detail in Chapter 2, Section 2.5.1). Initially, an assessment of the suitability of the SEM images for morphological analysis was conducted. Specimens were omitted from the analysis if their morphological features were obscured or destroyed due to debris cover or poor preservation. In situations where only one or two morphological features were obscured, an infilling estimation technique was performed using the methods set out in Hayward et al. (2004). In total 599 specimens (images) were suitable for morphological analysis (Table 5.2). The screened SEM images were calibrated to the known length of the SEM scale bar (in micrometres) prior to morphological analysis. Additional image pre-processing procedures were conducted as set out in Chapter 2 (Section 2.5.1) in order to calculate traits such as test roundness and septal pit features.

A total of 27 morphological measurements and assessments were taken from each individual SEM image (Table 5.3). The morphological traits were quantified using a combination of ImageJ v1.48 and Image Pro Express software. In order to reduce the impact of ontogeny, the morphological traits quantified were standardised against maximum test diameter, following a similar standardisation procedure outlined in Hayward et al. (2004). In addition, to avoid the effect of unusual terminal morphologies, the majority of the morphological measurements were taken from the junction of the penultimate chamber (N1) and the antepenultimate chamber.

Table 5.3 Morphometric characters assessed or measured from the SEM images of Elphidiidae specimens. Chamber N is equivalent to the final chamber, chamber N1 is the penultimate chamber etc. For a schematic diagram of some of the features analysed refer to Figure 2.7 in Chapter 2.

Type of character	Name	Variable Number	Method of measurement	Unit/ Category/ Type
Morphometric	Maximum test diameter	-	Maximum diameter of test parallel to axis of coiling (Not used directly in analysis)	Micrometres
Morphometric	Maximum width of chambers in the chamber N1	1	Maximum diameter of penultimate chamber calculated by the boundaries of the sutures i.e. from the end of one suture to the end of the next suture/ maximum test diameter	Ratio
Morphometric	Average septal bar height in the suture between chamber N1 and chamber N2	2	Average diameter of the first three septal bars (if present) from the umbilical area towards the periphery edge of the foraminifera	Micrometres
Morphometric	Relative difference in the width of the septal bar to the width of the rest of the chamber	3	Difference between the width of septal bar (if present) in comparison to width of rest of the chamber. The average is calculated from the first three septal pits closest to the umbilical area	Ratio
Morphometric	Relative length of sutural furrow in the penultimate chamber (N1)	4	Length of the sutural furrow in the penultimate chamber/ length of suture at the test periphery	Ratio
Morphometric	Sutural angle along the interseptal space between the chamber N1 and chamber N2.	5	The curvature of the suture between the final and penultimate chamber is calculated using the arc measure tool in Image Pro Express	Degree (Angle)

Table 5.3 continued.

Type of character	Name	Variable Number	Method of measurement	Unit/ Category/ Type
Morphometric	Total number of chambers	6	Number of chambers visible in the final whorl/ maximum diameter	Ratio
Morphometric	Total number of septal pits of the penultimate chamber	7	Number of complete septal pits, defined and bounded by two septal bars	Count
Morphometric	Roundness of the foraminiferal test	8	As calculated from the outline of the entire shape: 4*area/ (π*major_axis^2). This feature requires an image pre-processing procedure	0-1
Morphometric	Average roundness of the septal pit	9	Mean roundness of the pit averaged across the foraminifera: As calculated from the outline: 4*area/ (π*major_axis^2). This feature requires an image pre-processing procedure	0-1
Morphometric	Mean septal pit area	10	Mean septal pit area averaged across the entire foraminifera. This feature requires an image pre-processing procedure.	Area
Morphometric	Relative proportion of septal pit area to rest of the chamber.	11	Total area of the septal pits/ total foraminifera area This feature requires an image pre-processing procedure.	Ratio
Morphometric	Ratio of width of suture on chamber N1	12	Width of suture from the umbilical area/ width of the sutural furrow at the periphery of the test	Ratio
Morphometric	Relative proportion of apertural ornamentation covering the first visible chamber	13	Percentage coverage of apertural ornamentation on the first visible chamber	Percentage

Table 5.3 continued.

Type of character	Name	Variable Number	Method of measurement	Unit/ Category/ Type
Morphometric	Incomplete sutural bridge development	14	Number of septal bars which do not completely cross the suture	Count
Morphometric	Number of umbilical bosses	15	Number of bosses in the umbilical area/ maximum diameter	Ratio
Morphometric	Maximum boss diameter	16	Maximum boss diameter/ maximum test diameter	Ratio
Morphometric	Total number of septal pits	17	Total number of septal pits/ maximum test diameter	Ratio
Categorical	Porosity (Strength)	18	Strength of width of pores. Ten pores were analysed at junction between chambers N1 and N2. The average width of these pore measurements was then calculated and this average was then grouped into one of three categories: fine pores < 1 µm, medium pores 1-2 µm or large pores > 2 µm	Fine-1 Medium-2 Large – 3
Categorical	Presence of spines	19	Presence/ absence of peripheral spines	Absence - 1 Presence -2
Categorical	Presence of keel	20	Presence/ absence of peripheral keel	Absence-1 Presence-2
Categorical	Presence of calcite ridges	21	Presence/ absence of raised calcite ridges	Absence - 1 Presence - 2
Categorical	Degree of apertural ornamentation	22	Angularity of tubercles around the aperture	None -1 Very weak -2 Weak -3 Medium -4 Strong-5

Table 5.3 continued.

Type of character	Name	Variable Number	Method of measurement	Unit/ Category/ Type
Categorical	Openness of the umbilical area	23	Openness of umbilical area	None-1 Very weak-2 Weak-3 Medium -4 Strong-5
Categorical	Degree of ornamentation within umbilical area	24	Angularity and regularity of tubercules within sutures	None-1 Very weak-2 Weak-3 Medium -4 Strong-5
Categorical	Degree of ornamentation within the sutures (including pits)	25	Angularity and regularity of tubercules within sutures	None-1 Very weak-2 Weak-3 Medium -4 Strong-5
Categorical	Degree of ornamentation along radial edge of sutures	26	Angularity and regularity of tubercules along the radial edge of the sutures	None-1 Very weak-2 Weak-3 Medium -4 Strong-5
Categorical	Degree of ornamentation outside the sutures	27	Regularity and angularity of ornamentation on test surface outside the sutures (excluding apertural ornamentation)	None- 1 Weak -2 Medium - 3 Strong – 4

5.2.5 Multivariate statistics

5.2.5.1 Interspecific morphological variability

Prior to multivariate analysis, in order to create scale free, dimensionless variables the morphological characters analysed were standardised between 0 and 1, following methods described by Hayward et al. (2004). In order to evaluate the efficacy of morphology in delineating the 17 Elphidiidae genotypes in the NE Atlantic, two multivariate ordination techniques, PCO and UPGMA cluster analysis were employed without *a priori* knowledge of the genetic groupings.

Two multivariate classification techniques, DFA and CHAID analysis, were also conducted to identify the most diagnostic morphological characters which can be used to discriminate between the genotypes. These classification techniques utilise *a priori* knowledge of the genetic groupings to calculate the percentage of specimens that have been correctly classified into their genetic group based upon their morphological traits. A leave one out cross-validation procedure was also conducted in the DFA. The DFA was employed despite some minor violations to its internal assumptions, as previous studies have identified that this analysis is robust even with violations of its internal assumptions (Klecka, 1980).

A non-parametric classification technique, CHAID analysis was also employed in order to assess the robustness of the DFA procedure. CHAID analysis was chosen over the alternative decision tree CART analysis, as it is the optimal classification technique for handling both the large morphometric dataset and the unequal sampling design (Breiman, 1984). A comparison of these two techniques (not illustrated) highlighted that CART is suboptimal, as it failed to correctly classify genotypes with low numbers of specimens. The CHAID analysis employed a 10 V-fold cross validation procedure. The optimal combination for node structure was a minimum of five cases in the parent node and one case in the child node.

5.2.5.2 Intraspecific morphological variation

The large number of genetically sequenced and morphologically analysed specimens collected across distinctive biogeographic zones in the NE Atlantic provides an unprecedented opportunity to assess intraspecific morphological variability across a wide geographical area Two case studies utilising specimens of genotype S1 and genotype S4 were chosen to examine the relationship of intraspecific morphological variability between specimens taken from different sampling locations. Within each case study a series of multivariate statistical

techniques were employed including PCO analysis, DFA and CART analysis, to assess whether any phenotypic differences could be identified between the different site localities.

5.3 Results

5.3.1 Interspecific morphological variability

A PCO analysis was conducted to assess the effectiveness of morphology in effectively delineating between Elphidiidae without any *a priori* knowledge of genetic groupings.

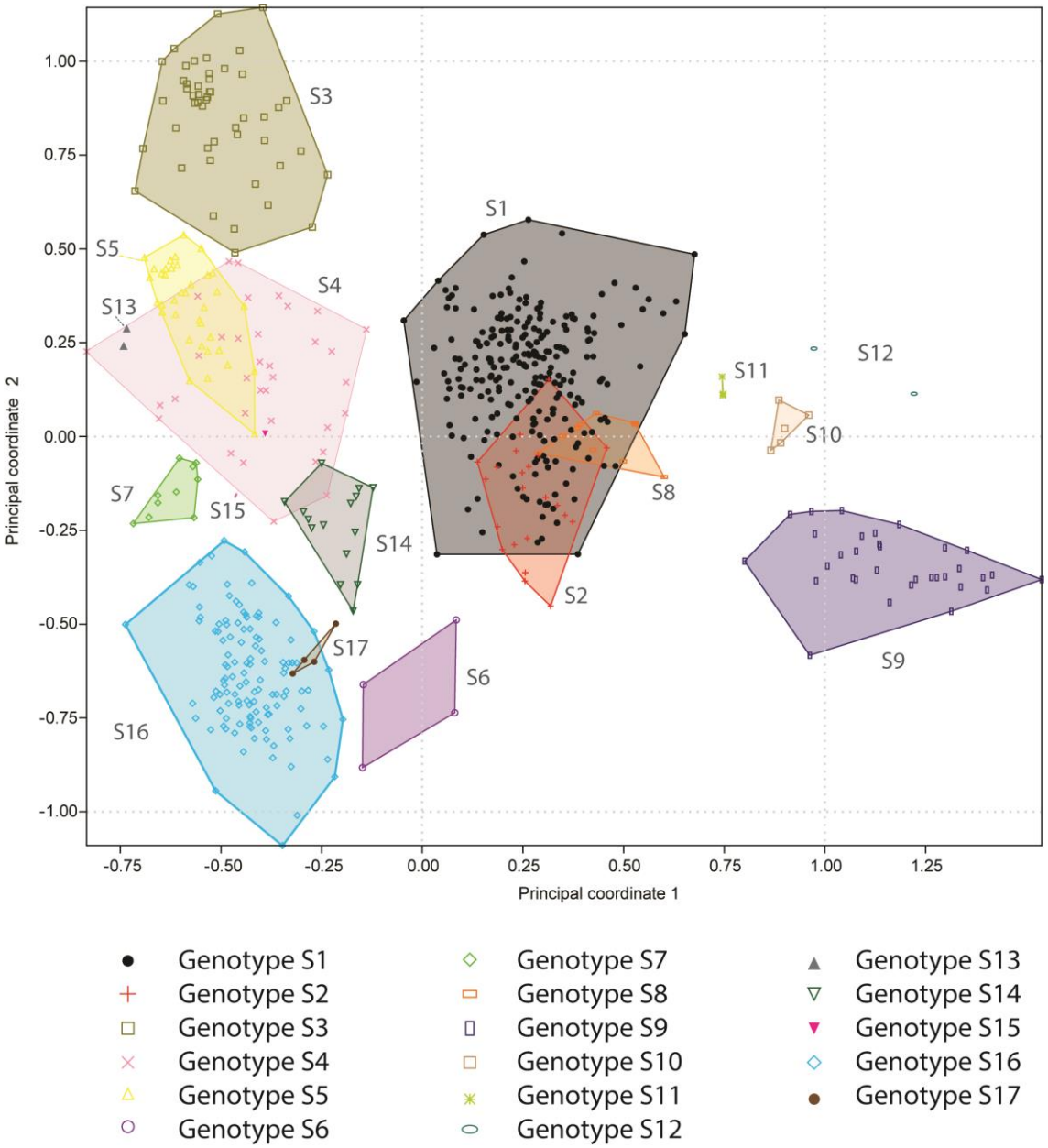


Figure 5.6 A PCO bi-plot depicting the morphospace of the 17 distinct Elphidiiae genotypes. Each genotype is bounded by a convex hull. The first two principal coordinates account for 38.3% of the total variance (PC1 21.7% and PC2 19.5%).

The PCO analysis clearly delineated seven genotypes within the PCO morphospace: genotypes S3, S6, S7, S9, S10, S11 and S12 (Figure 5.6). The remaining ten genotypes exhibit partial or

extensive morphological overlap within the PCO morphospace, as exhibited by the overlapping convex hulls.

A closer visual inspection of some of the SEM images that exhibit overlap in the PCO morphospace (Figure 5.6) reveals that these specimens share similar structural (gross) morphological test characteristics. For example, extensive morphological overlap is exhibited amongst specimens of genotypes S1, S2 and S8. However, these three genotypes share many morphological traits including the presence of septal pits, similar test shape and size (as illustrated in Figure 5.7). Despite these similarities, these specimens can be visually delineated based on subtle morphological test characters including degree of test ornamentation outside the suture and the total number of septal pits.

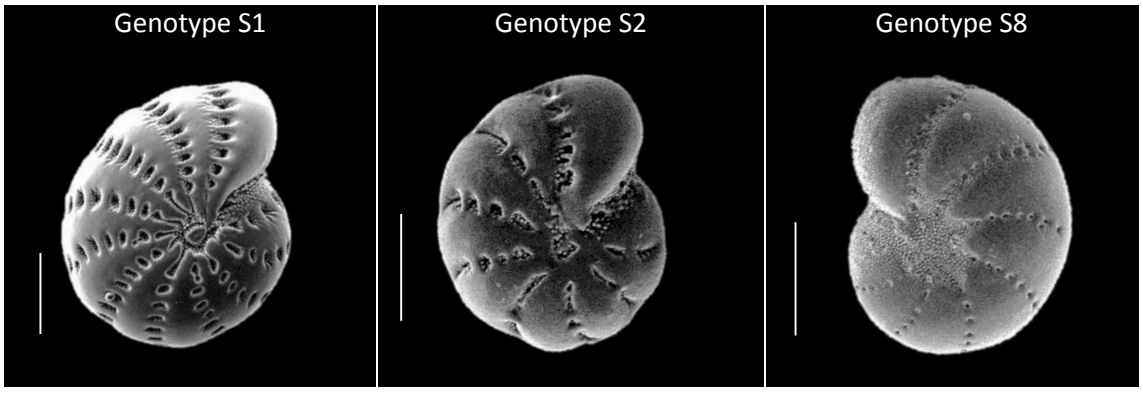


Figure 5.7 Representative SEM images of *Elphidium* genotypes S1, S2 and S8 which overlap in the PCO morphospace (Figure 5.6). The scale bar illustrated is 100 µm.

The UPGMA cluster analysis reveals that seven genotypes S3, S5, S8, S9, S10, S11 and S12 are morphologically distinct as they form discrete clusters within the UPGMA cluster analysis tree (Figure 5.8). The remaining ten genotypes in the UPGMA cluster analysis tree exhibit partial or extensive overlap within the UPGMA dendrogram. For example, extensive morphological overlap is exhibited between specimens of genotypes S1 and S2 and two specimens from genotype S6. Additionally, the UPGMA cluster analysis revealed that some specimens of genotypes S6, S14, S16 and S17 are clustered together.

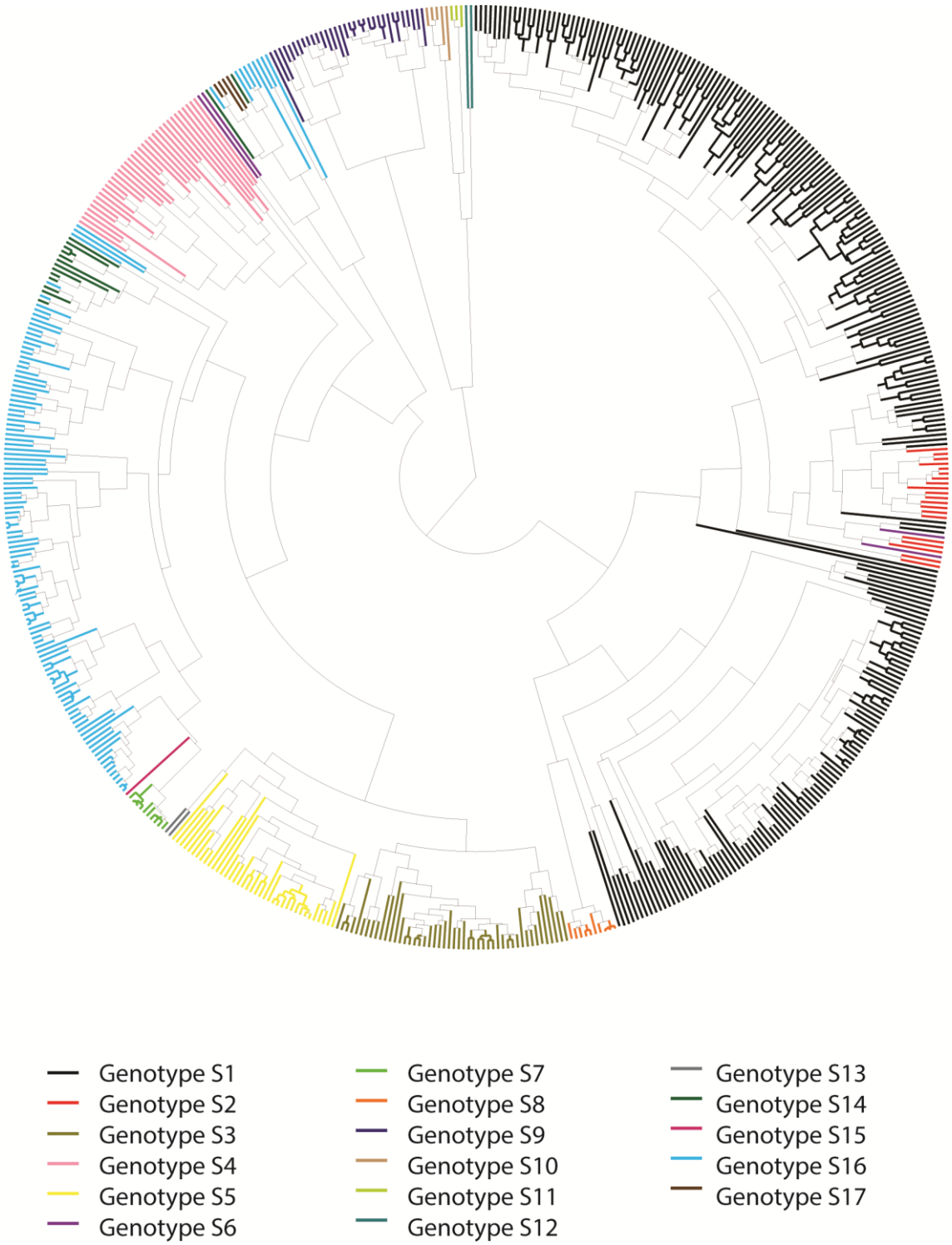


Figure 5.8 UPGMA cluster analysis tree of 17 Elphidiidae genotypes identified across the NE Atlantic based on their morphological characteristics (n=599).

Specimens that cluster together in the UPGMA cluster analysis tree exhibit similar structural test features. For example, genotypes S6, S14, S16 and S17 all exhibit thin curved sutures, limited test ornamentation and predominately lack septal pits, as illustrated in Figure 5.9.

Additionally, these specimens can be visually delineated by the development of ornamentation in the sutures and the umbilical area, as well as the shape (length and width) of their sutures.

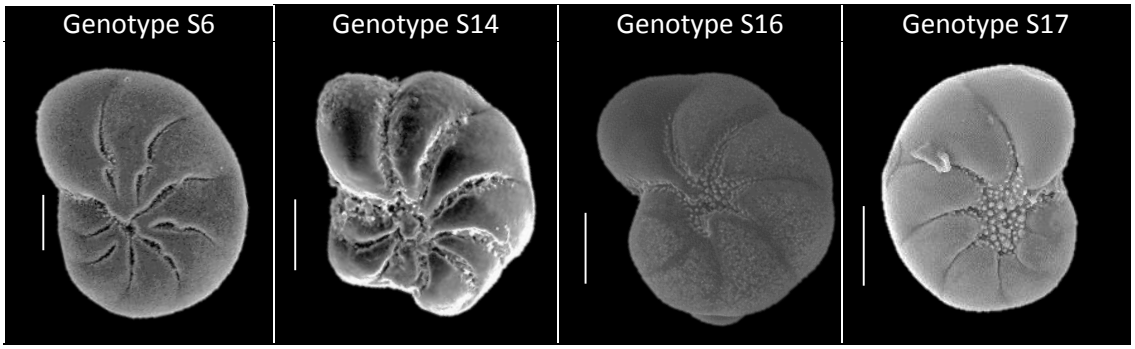


Figure 5.9 Specimens of Elphidiidae genotypes S6, S14, S16 and S17 which cluster together within the UPGMA cluster analysis tree (Figure 5.8). The scale bar illustrated is 100 µm.

5.3.1.1 Multivariate analysis of morphologically similar specimens

The results from the PCO analysis and UPGMA cluster analysis (Figures 5.6 and 5.8) reveal that only five genotypes: S3, S9, S10, S11 and S12 can be consistently delineated based on their morphological test features. The remaining twelve genotypes exhibit partial or extensive morphological overlap in either one or both statistical analyses. A visual inspection of the SEM images of the overlapping specimens reveals that subtle morphological characters can be used to delineate between these specimens (Figures 5.7 and 5.9). Therefore, to clarify the relationships between these overlapping taxa, a series of refined multivariate (PCO) analyses were employed on a reduced morphometric dataset. Specimens that exhibit extensive or partial morphological overlap in either of the multivariate analyses were grouped and analysed in isolation from the morphologically distinct genotypes.

5.3.1.1.1 Delineating between genotypes in subgroup I: S3, S4, S5, S13, S14 and S15

A refined PCO analysis was performed upon the morphological test characteristics of genotypes S3, S4, S5, S7, S13, S14 and S15 (herein referred to as subgroup I) to evaluate their morphological distinctiveness.

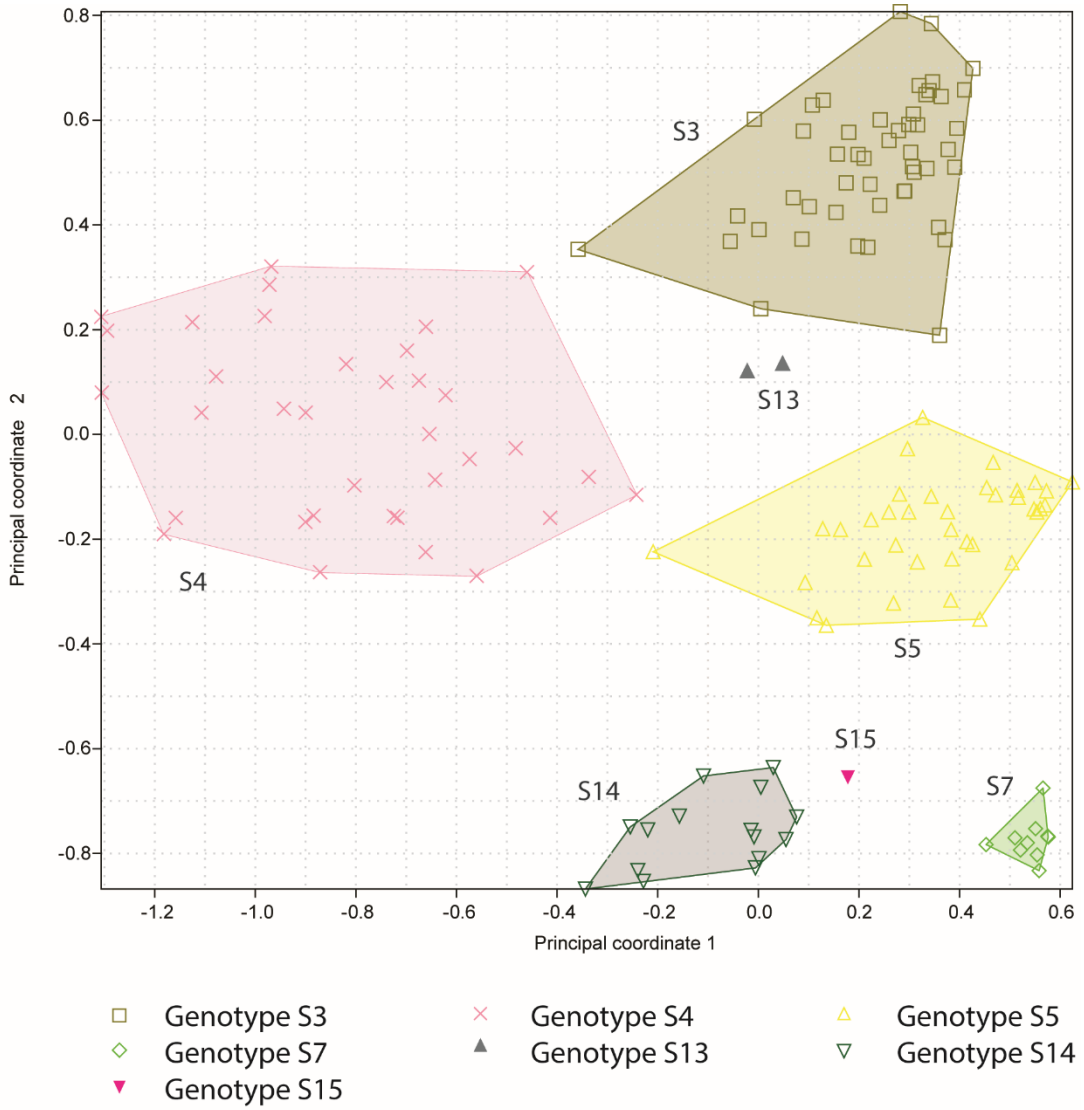


Figure 5.10 PCO analysis of morphological traits of Elphidiidae genotypes S3, S4, S5, S7, S13, S14 and S15 (subgroup I). The genotypes were mapped onto the PCO morphospace and are bounded by convex hulls. The first two principal coordinates account for 56.21% of the total variation.

The refined PCO analysis reveals that each genotype within subgroup I forms a discrete non-overlapping cluster within the PCO morphospace (Figure 5.10). These results illustrate that each genotype is clearly differentiated by their test morphology.

5.3.1.1.2 Delineating between genotypes in subgroup II: S6, S14, S16 and S17

Previous multivariate analysis conducted on the morphological characters of all 17 Elphidiidae genotypes has revealed extensive morphological overlap amongst specimens of genotypes S6, S14, S15 and S17 (subgroup II) (Figures 5.6 and 5.8). Therefore, to clarify the relationships within subgroup II, a separate refined PCO analysis was conducted.

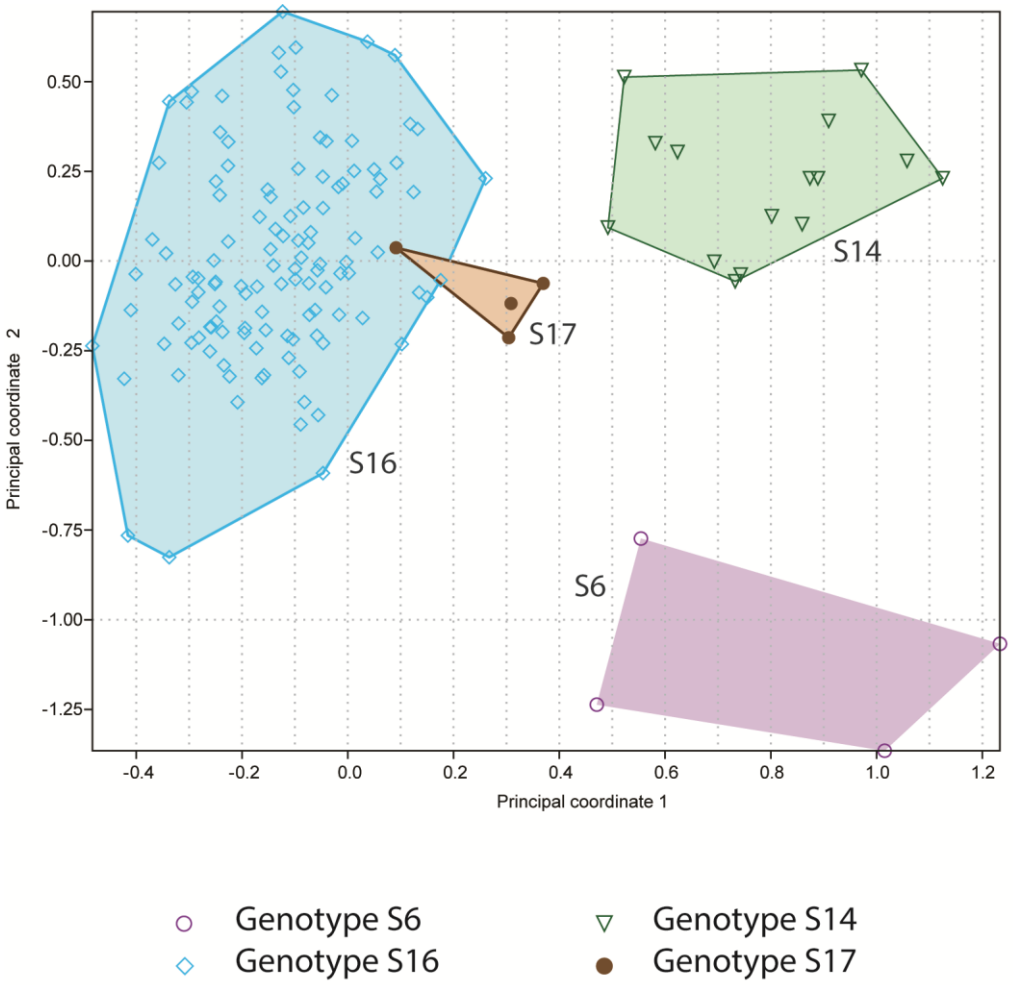


Figure 5.11 A PCO analysis of the morphological characters of the Elphidiidae genotypes S6, S14, S16 and S17 (subgroup II). The first two principal coordinates account for 40.79% of the total variance. Each genotype is bounded by a convex hull.

The refined PCO analysis reveals that two genotypes S6 and S14 are morphologically distinct as they exhibit discrete (non-overlapping) clusters within the PCO morphospace (Figure 5.11). With the exception of a single specimen of genotype S17 and a single specimen of genotype S16 (as illustrated in Figure 5.12), the remaining specimens from genotypes S16 and S17 can be morphological delineated, as they exhibit limited overlap within the PCO morphospace.

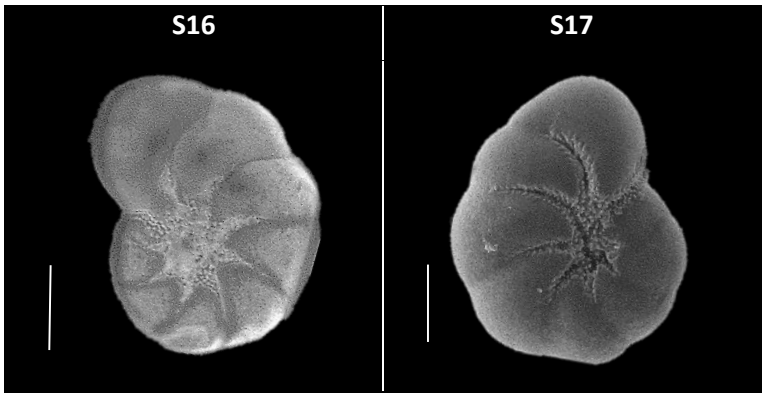


Figure 5.12 Images of specimens of elphidiid genotypes S16 and S17 that exhibit overlap in the PCO morphospace (Figure 5.11). The scale bar illustrated is 100 µm.

5.3.1.1.3 Delineating between genotypes in subgroup III: S1, S2, S6 and S8

Extensive morphological overlap was also previously identified between specimens from genotypes S1, S2, S6 and S8 (subgroup III) (Figures 5.6 and 5.8). Notably, significant morphological overlap was observed between specimens from genotypes S1 and S2. Therefore, to elucidate the taxonomic boundaries of these four genotypes, a refined PCO analysis was conducted.

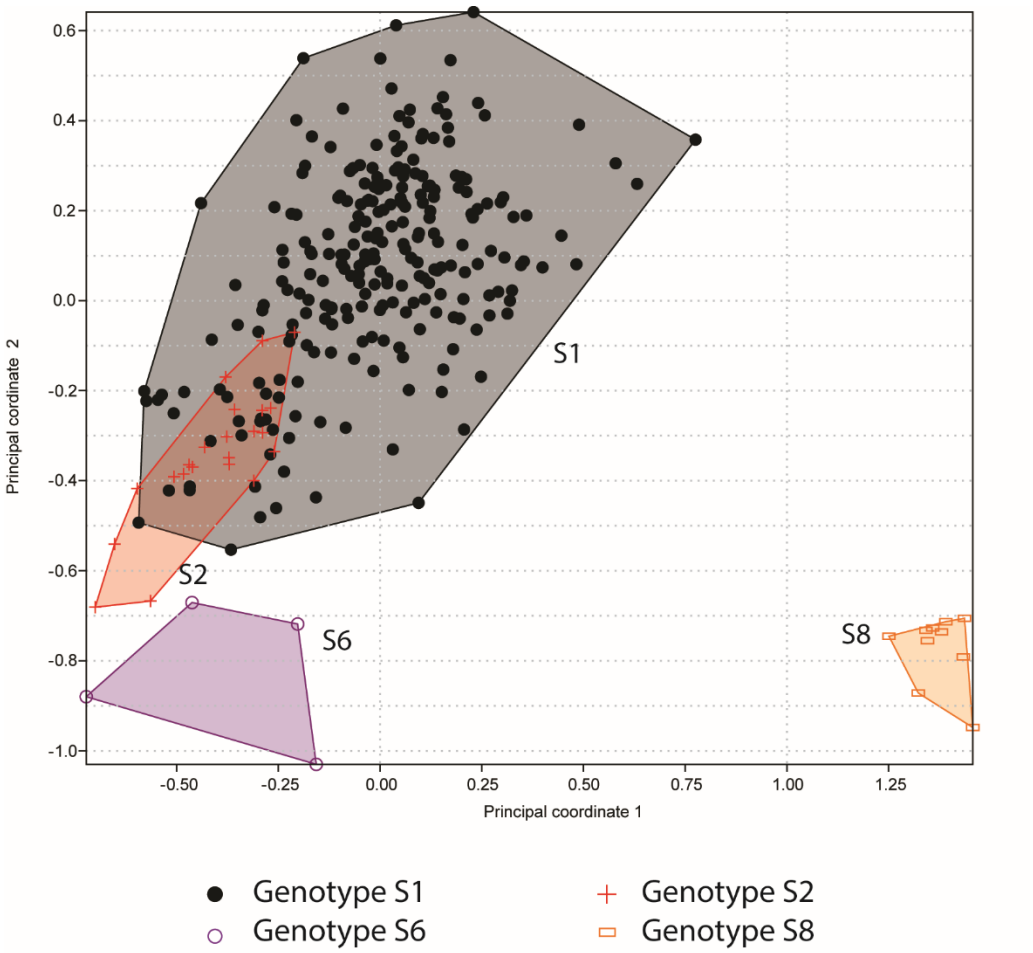


Figure 5.13 PCO bi-plot of the morphological characteristics of Elphidiidae genotypes S1, S2, S6 and S8. The PCO morphospace boundaries of each genotype are bounded by a convex hull. The first two principal coordinates account for 35.2% of the total variation.

The refined PCO analysis reveals that genotypes S6 and S8 form discrete clusters within the PCO morphospace (Figure 5.13). This indicates that these taxa can be clearly morphologically delineated. However, the PCO analysis has also revealed that no further morphological separation could be identified between specimens from genotypes S1 and S2, as these two species showed extensive morphological overlap within the PCO morphospace.

5.3.1.1.4 Delineating between genotypes S1 and S2

The failure of the refined multivariate analysis in delineating between specimens of genotypes S1 and S2 (Figure 5.13), brings into question whether these two genotypes are morphologically cryptic. In order to elucidate the taxonomic (morphological) relationships between these two genotypes, an additional refined PCO analysis was conducted exclusively on the morphological test characters of genotypes S1 and S2.

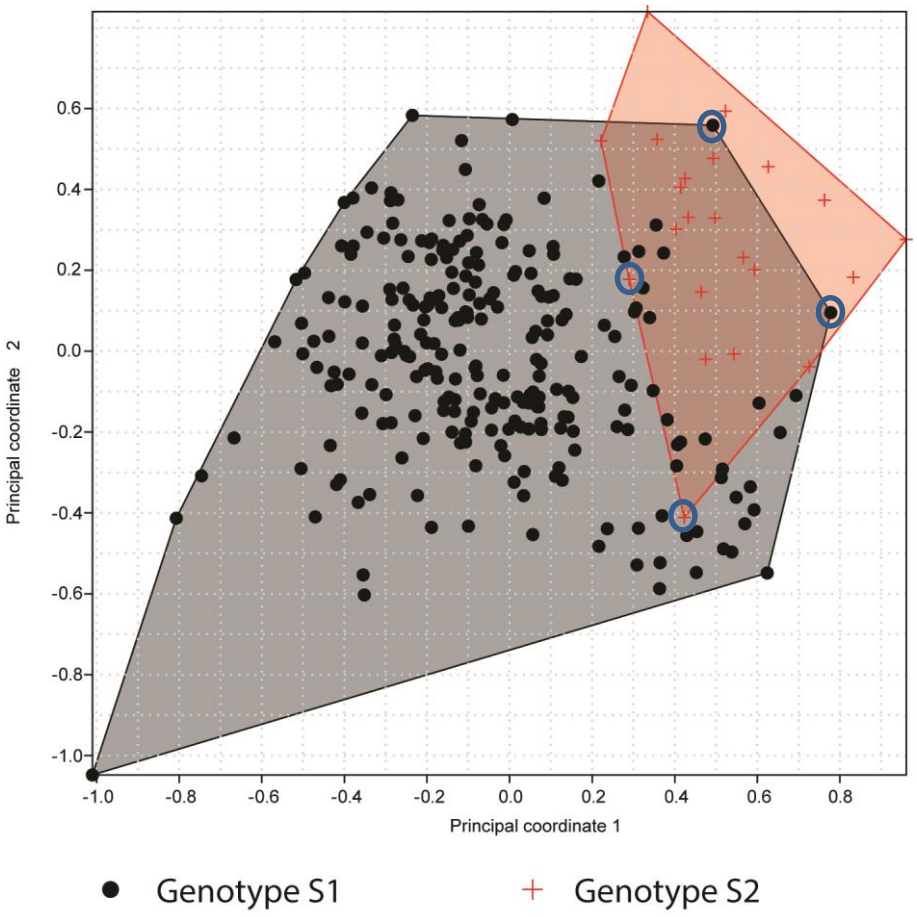


Figure 5.14 PCO analysis of the morphological attributes of *Elphidium* genotypes S1 and S2. Each genotype is bounded by a convex hull. The first two principal coordinates account for 35.39% of the total variance. Four outlier specimens which exhibit the greatest degree of overlap are highlighted by the blue circles, and are illustrated in Figure 5.15.

The refined PCO analysis illustrates an overlap between the convex hulls of genotypes S1 and S2 in the PCO morphospace (Figure 5.14). However, the majority of genotype S1 specimens (n=263) do not occupy the overlapping region of the PCO morphospace. Only 17 specimens of genotype S1 and 15 specimens of genotype S2 are situated within the overlapping region in the PCO morphospace. This indicates that the majority of genotype S1 specimens are morphologically distinct from genotype S2. A considerable proportion of the overlap exhibited by the convex hulls can be attributed to the four outlier specimens (two specimens from genotype S1 and two specimens from genotype S2), which clearly cluster outwith their respective group (Figure 5.14).

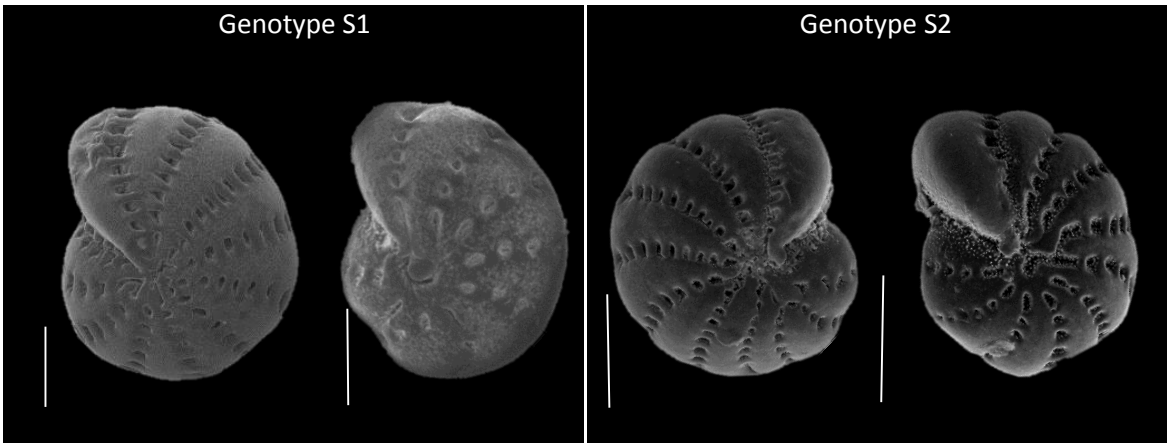


Figure 5.15 SEM images of four outlier specimens of *Elphidium* genotypes S1 and S2 as identified within PCO analysis (Figure 5.14). The scale bar illustrated is 100 µm.

A visual inspection of these four outlier specimens reveals they exhibit anomalous morphologies and gradational morphological features (Figure 5.15). Notably the two specimens of genotype S2 exhibit a large number of septal pits, a lobate test periphery and exhibit a large total number of chambers. In contrast, the specimens of genotype S1 exhibit limited sutural and apertural ornamentation, relatively closed umbilical regions and a very round test periphery. Consequently, it could be asserted that these specimens are morphologically cryptic, based on the visual assessment of their test characteristics.

5.3.2 Classification of Elphidiidae genotypes

Two multivariate classification procedures, a DFA and a CHAID analysis were employed to evaluate the accuracy of morphological attributes in predicting genotype membership and to identify the key diagnostic morphological characters. The morphological characters of 16 Elphidiidae genotypes were used in the multivariate classification analyses. Genotype S15 was omitted from the DFA and CHAID analysis due to an insufficient number of morphologically analysed specimens within this genotype (genotype S15 is represented by a single specimen).

5.3.2.1 Discriminant function analysis

The discriminant function analysis correctly identified 99% of specimens into their genotype based upon their morphological attributes and these delineations were statistically significant (Wilks Lambda: 0.022, P value: < 0.01) (Table 5.4). The discriminant function analysis retains high accuracy classification rates even with cross-validation, as 98.3% of the specimens were correctly classified (Table 5.4). The DFA obtained 100% classification success for 11 out of the 16 Elphidiidae genotypes based on test morphology.

Genotype	DFA % correct classification	Cross validation % correct classification
S1	98.8	97.6
S2	95.5	95.5
S3	100	100
S4	100	100
S5	97.3	97.3
S6	100	50.0
S7	100	100
S8	100	100
S9	100	100
S10	100	100
S11	100	100
S12	100	100
S13	100	100
S14	100	100
S16	99.2	99.2
S17	100	100
Total	99.0	98.3

Table 5.4 Percentage of specimens correctly classified into 16 Elphidiidae genotypes in the DFA and the cross validation procedure.

Almost all the misclassifications occurred between specimens of genotypes S1 and S2 in both the DFA and the cross-validation procedure (Table 5.5). In total four specimens, one specimen from genotype S1 and three specimens from genotype S2 were misclassified in the DFA.

The cross validation procedure misclassified one specimen of genotype S1 and four specimens of genotype S2 (Table 5.5). This identification of ambiguous morphological boundaries between these two genotypes is concordant with the findings of the previous multivariate analyses (Figure 5.14). Additionally, the DFA revealed that a single specimen of genotype S5 was misidentified as genotype S13 and that two of genotype S5 were misclassified into genotype S13 by the cross validation procedure (Table 5.5). Finally, the DFA reveals that a

single specimen of S16 was misclassified into genotype S17 by the cross validation procedure (Table 5.5).

The discriminant function analysis reveals that the optimal combination of morphological traits for the discrimination of the Elphidiidae genotypes include: formation of an ornamental calcite ridge (21), difference in the length of suture (4), total number of septal pits (6), degree of ornamentation along radial edge of suture (26), mean septal pit area (10), openness of umbilical area (23), degree of apertural ornamentation (22), ratio of septal pit width to the rest of chamber (11), number of umbilical bosses (15), relative width of the suture (12) and mean septal pit roundness (9). The morphological trait numbers correspond to the variables described in Table 5.3.

Table 5.5 Confusion matrix of the specimens from the 16 Elphidiidae genotypes misclassified in the DFA and the cross validation procedure based upon their morphological characteristics. Elphidiidae genotypes which exhibited perfect classification are not illustrated.

	Discriminant function analysis																
	Predicted genotype																
Observed genotype	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17
S1	**244**	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S2	1	**21**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S5	-	-	-	-	**37**	-	-	-	-	-	-	-	1	-	-	-	-
S16	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	**119**	-
	Cross validation (DFA)																
	Predicted genotype																
Observed genotype	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17
S1	**243**	4	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
S2	1	**21**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S5	-	-	-	-	**35**	-	-	-	-	-	-	-	2	-	-	-	-
S6	-	-	-	-	-	**2**	-	-	-	-	-	-	-	1	-	1	-
S16	-	-	-	-	-	1	-	-	-	-	-	-	-	2	-	**119**	-

5.3.2.2 Classification tree analysis

The CHAID analysis correctly classified 94.5% of the 16 Elphidiidae genotypes based upon morphology. The cross validation procedure employed in the CHAID analysis indicates a misclassification rate of 10.4%. The CHAID reveals that eight genotypes can be perfectly discriminated based upon their morphological characteristics, genotypes S4, S5, S6, S7, S8, S9, 11, 12, and S13 (Table 5.6). The remaining genotypes exhibit partial or complete morphological overlap. For example, three specimens of genotype S3 were misidentified as genotype S1 based on morphology (Table 5.6). Partial morphological overlap was also exhibited in the CHAID analysis as two specimens of genotype S6 were misclassified into genotype S14 and three specimens of genotype S10 were misclassified into genotype S11. Additionally, two specimens of genotype S17 were misclassified into genotype S16.

Table 5.6 Classification matrix obtained by CHAID analysis with a 10 V-fold cross validation procedure. The numbers of genotypes correctly and incorrectly classified and the percentage of specimens correctly classified are illustrated.

Observed genotype	Predicted genotype																Percent correctly classified
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S16	S17	
S1	**247**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100%
S2	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
S3	3	-	**45**	-	-	-	-	-	-	-	-	-	1	-	-	-	91.8%
S4	-	-	-	**35**	-	-	-	-	-	-	-	-	-	-	-	-	100%
S5	-	-	-	-	**37**	-	-	-	-	-	-	-	-	-	-	-	100%
S6	-	-	-	-	-	**2**	-	-	-	-	-	-	-	2	-	-	50%
S7	-	-	-	-	-	-	**10**	-	-	-	-	-	-	-	-	-	100%
S8	-	-	-	-	-	-	-	**10**	-	-	-	-	-	-	-	-	100%
S9	-	-	-	-	-	-	-	-	**33**	-	-	-	-	-	-	-	100%
S10	-	-	-	-	-	-	-	-	-	**5**	-	-	-	-	-	-	100%
S11	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	0%
S12	-	-	-	-	-	-	-	-	-	-	-	**2**	-	-	-	-	100%
S13	-	-	-	-	-	-	-	-	-	-	-	-	**2**	-	-	-	100%
S14	-	-	-	-	-	-	-	-	-	-	-	-	-	**15**	-	-	100%
S16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**120**	-	100%
S17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	**2**	50%

The highest misclassification rates were observed between specimens of genotypes S1 and S2, as all specimens of genotype S2 were misidentified as genotype S1 (Table 5.6). The poor classification assignment success of genotype S2 contradicts the previous multivariate analyses, which had previously identified that the majority of specimens can be morphologically delineated (Figure 5.14 and Table 5.5). For example, only five specimens of genotype S2 were misclassified in the DFA (Table 5.5). A refined non-parametric CART analysis was conducted on a reduced dataset comprising the morphological characters of genotypes S1 and S2 to clarify the morphological boundaries between these two genotypes and to test the validity of previous multivariate analyses. Due to the smaller dataset CART analysis was the optimal classification procedure over CHAID analysis. The CART analysis revealed that 99.6% of specimens were classified into their genetic group based upon morphology (99.6% correct classification into genotype S1 and 95.5% correct classification into genotype S2). The results revealed an overall misclassification rate of 4.5% in the cross validation procedure. In total, only two specimens were misclassified, one from each genotype. The CART analysis revealed that morphological test characters including: mean septal pit area (10), average bar height (2), relative width of sutures (14), maximum length of chamber N1 (1) and ratio of septal pit area to the rest of the chamber (11) were diagnostic for delineating between genotypes S1 and S2.

Overall, the CHAID analysis highlights the efficacy of morphology in delineating between the majority of Elphidiidae genotypes and the results are concordant with the results from previous multivariate analyses (PCO and DFA, Figure 5.6 and Table 5.4). The CHAID analysis has revealed a combination of morphological test characteristics including total number of pits (17), the degree of umbilical ornamentation (24), porosity (18), difference in the length of suture (4), ratio of septal bar to the rest of the chamber (3), number of umbilical bosses (15), apertural ornamentation (22), formation of a calcite ridge (21), openness of umbilical area (23), degree of ornamentation outside of the suture (27) and average septal bar height (2), as all being diagnostically important for the discrimination of the 16 Elphidiidae genotypes. However, it should be noted that some of the partial morphological overlap (misclassification) exhibited in the CHAID analysis (Table 5.6) could be resolved by visually analysing the specimens. For example, the three specimens of genotype S3 which were misclassified into genotype S1 can be discriminated by their coarsely perforate tests. In addition, the CHAID analysis failed to take into account the presence of peripheral spines, which is a discrete test feature that can be used to delineate specimens of genotype S11 from genotype S10.

5.3.2.3 Morphological discrimination of Elphidiidae genotypes

The morphometric results previously presented indicates that the majority of elphidiid genotypes could be morphologically discriminated by their morphological traits. However, unequivocal morphological discrimination may be difficult between specimens of genotypes S1 and S2, and genotypes S16 and S17 (Figures 5.11 and 5.14, Tables 5.5 and 5.6). Overall, the elphidiid specimens possess similar gross morphological characters including a planispiral test, sutural canal systems and interio-marginal or areal aperture openings. The results revealed that only genotype S10 can be unambiguously delineated based on a single discrete morphological character, the presence of ornamental peripheral spines (Table 5.7, an example of which is illustrated in Figure 5.5). Instead, a combination of test characteristics including structural and ornamental traits allows for the unambiguous classification of the majority of the genotypes. Notably, the results indicate that nearly all of the morphological features are gradational amongst the genotypes (Table 5.7).

Species within the Elphidiidae family can be primarily delineated based on the development of septal bridges and pits. For example, species that possess numerous, well-defined septal pits include genotypes: S1, S2, S3, S8, S9, S10, S11 and S12. Amongst these genotypes, genotype S1 has exhibited enigmatic morphology (partially cryptic) with some genotype S2 specimens (Figure 5.15). Genotype S1 can predominantly be differentiated from genotype S2 based on a combination of morphological traits, as it generally possesses more septal pits, larger septal bars (5.37-44.26 µm) and larger septal pit areas (99.64-721.9 µm). Additionally, genotype S1 can sometimes have a small umbilical boss (Table 5.7). Genotype S2 can be distinguished from genotype S1 as it typically exhibits a more 'closed' umbilical area (umbilical openness 1-3) and lacks an umbilical boss (Table 5.7). Genotype S8 also possesses numerous septal pits, but this genotype can be distinguished from the others based on the size and shape of the septal pits (mean septal pit area: 17.98-173.51 µm, mean pit roundness: 0.69-0.73). Additionally, genotype S8 has distinctive ornamentation which extends from the umbilical area, along the radial edge of many of the sutures and across the apertural face (as illustrated in Figure 5.5). Genotype S9 also exhibits well-defined septal pits and extensive ornamentation outside the sutures. This genotype is distinctive as ornamental papillae cover the entire surface of test (Figure 5.5). Moreover, the presence of a keeled test and raised calcite ridges can be used to discriminate specimens of genotype S9 from the other genotypes (Figure 5.5, Table 5.7). In contrast, genotype S3 is less ornamented, with ornamentation primarily restricted to the sutures, with the exception of the development of slight ornamentation along the radial edge

of the sutures in a handful of specimens. Genotype S3 can primarily be distinguished from the other genotypes as it is coarsely perforate (> 2 µm) and often possesses numerous umbilical bosses.

Genotypes S10, S11 and S12 share many similar test morphologies which can be used to differentiate these genotypes from other elphidiids, as these species possess large tests with numerous narrow chambers, well defined-septal pits and bridges and a peripheral keel (as depicted in Figure 5.5). Therefore, it is unsurprising that these genotypes were situated together within the PCO morphospace (Figure 5.6) and the UPGMA cluster analysis tree (Figure 5.8). Amongst these taxa, genotype S10 can be distinguished based on the presence of peripheral ornamental spines. Moreover, this genotype exhibits ornamentation within the septal pits, along the radial edge of the sutures and on the apertural face. This genotype (S10) sometimes also possesses an umbilical boss (Table 5.7). In contrast, genotype S12 exhibits ornamentation (papillae) over the majority of the test. Genotype S12 also commonly has less curved sutures (sutural angle: 50.3-80.9°) and has a smaller first chamber (maximum width of chamber N1: 0.16-0.17) than genotypes S10 and S11. Genotype S11 can be distinguished from genotypes S10 and S12 as this genotype's ornamentation is primarily restricted to within the sutures, with the exception of the development of weak ornamentation around the apertural area. Additionally, this genotype has a larger ratio of septal pit area to the rest of the chamber (0.28-0.32) than genotypes S10 and S12.

Four genotypes within the Elphidiidae family, S4, S5, S6 and S15, possess irregular septal pits. Amongst these species, genotype S4 typically has the largest number of septal pits (total number of septal pits: 0.03-0.15) and is moderately to coarsely perforate. In contrast, the septal pits within genotypes S5, S6 and S15 are much more irregular (Table 5.7). Genotype S5 is distinctive as it commonly exhibits extensive umbilical and sutural ornamentation and has medium sized pores (1-2 µm). The sutures of this genotype often extend to the periphery of the test (difference in the length of the suture: 0.80-1.00). Genotype S15 also displays an open umbilical area with distinctive umbilical and sutural ornamentation; however, a distinctive trait of this genotype is that ornamentation extends along the radial edges of the sutures. This genotype exhibits fine pores (< 1 µm) and the sutures do not extend to the periphery of the test (difference in the length of the suture 0.66). Finally, genotype S6 can be differentiated from the other genotypes with irregular septal pits, as this genotype commonly has a closed

umbilical area, possesses fine pores (< 1 µm) and has thin sutures, which often do not extend to the periphery of the test (0.42-0.85).

Lastly, a number of genotypes in the Elphidiidae family do not possess any septal pits, this includes genotypes S7, S13, S14, S16 and S17. Among these taxa, genotype S7 can be differentiated based on its extensive umbilical ornamentation, which extends into the sutures. This genotype also illustrates small pores (< 1 µm), tapered sutures and an open umbilical area (Figure 5.5, Table 5.7). In contrast, genotype S13 also typically exhibits an open umbilical area, but this genotype also exhibits numerous umbilical bosses. Genotype S13 can also be distinguished from the other taxa, as it is coarsely perforate (> 2 µm). In contrast, genotype S14 has fine pores (< 1 µm), deeply incised sutures and sometimes possesses a singular umbilical boss (Figure 5.5 and Table 5.7). The sutures in this genotype also commonly extend to the periphery of the test (difference in length of suture: 0.75-1.0). Amongst the taxa which display no septal pits, specimens of genotypes S16 and S17 were the most difficult to discriminate morphologically, perhaps owing in part to the variable nature of the test morphology of genotype S16 (Figures 5.11 and 5.12). Both of these genotypes possess fine pores (< 1 µm) and display similar test roundness. These two genotypes can be discriminated based on a complex combination of morphological traits. Specimens within genotype S16 generally exhibit more tapered sutures than those of genotype S17 (ratio of width of suture: 0.08-0.60) which often do not extend to the periphery of the test (difference in the length of suture: 0.21-0.82, as depicted in Figure 5.5). Whilst genotype S17 typically has thin, even sutures (ratio width of suture: 0.11) which often extend towards the periphery of the test (0.67-0.96). Additionally, most of the specimens within genotype S16 possess an open umbilical area (1-5), whilst all specimens within genotype S17 exhibit an open umbilical area (5-5) (Table 5.7). Finally, specimens of genotypes S16 and S17 can also be differentiated upon an assessment of their test ornamentation. Typically ornamentation is more extensive in genotype S16 often extending along the radial edge of the suture and this genotype displays moderate apertural ornamentation. In contrast, the ornamentation in genotype S17 is restricted to within the sutures and this genotype only possesses weak apertural ornamentation (Table 5.7).

Table 5.7 Range of values of morphological characters measured and assessed from the Elphidiidae genotypes. See Table 5.3 for details of the abbreviations.

Character	Maximum width chamber N1	Average bar height	Average width of septal bar	Difference length of suture	Angle of Suture N1	Total number of chambers	Number of septal pits chamber N1	Test roundness	Septal pit roundness
	1	2	3	4	5	6	7	8	9
S1	0.12-0.93	5.37-44.26	0.19-1.85	1.00-1.00	0-102	0.01-0.09	0.05-0.63	0.71-0.96	0.25-0.61
S2	0.31-0.61	4.61-12.17	0.18-0.56	1.00-1.00	7.51-106	0.03-0.06	0.01-0.04	0.73-0.90	0.45-0.61
S3	0.29-0.57	6.72-18.12	0.13-1.13	1.00-1.00	0.45-98.4	0.02-0.06	0.01-0.03	0.69-0.96	0.39-0.68
S4	0.21-0.60	0.00-22.70	0.00-0.61	0.38-1.00	11.5-93.8	0.03-0.05	0.00-0.04	0.70-0.90	0.00-0.64
S5	0.36-0.62	0.00-13.34	0.00-0.67	0.80-1.00	8.86-113	0.02-0.06	0-0.0254	0.72-0.89	0.00-0.85
S6	0.28-0.39	0.00-8.421	0.00-0.34	0.42-0.85	63-70.3	0.04-0.05	0.00-0.02	0.75-0.83	0.41-0.69
S7	0.27-0.51	0.00-0.00	0.00-0.00	0.34-0.71	10.7-71.4	0.03-0.03	0.00-0.00	0.72-0.86	0.00-0.00
S8	0.28-0.42	11.08-18.68	0.15-0.38	1.00-1.00	31.9-74.2	0.01-0.02	0.01-0.02	0.74-0.82	0.69-0.74
S9	0.37-0.83	7.54-16.99	0.22-0.70	1.00-1.00	37.8-152	0.02-0.05	0.01-0.03	0.71-0.87	0.34-0.68
S10	0.19-0.32	7.94-14.38	0.73-0.97	1.00-1.00	111-164	0.02-0.03	0.02-0.03	0.77-0.84	0.49-0.65
S11	0.20-0.29	10.72-19.66	0.84-1.25	1.00-1.00	141-151	0.02-0.03	0.02-0.03	0.80-0.86	0.53-0.58
S12	0.16-0.17	10.06-12.38	1.12-1.75	1.00-1.00	50.3-80.9	0.03-0.04	0.02-0.03	0.80-0.83	0.49-0.45
S13	0.32-0.38	0.00-0.00	0.00-0.00	0.80-1.00	35.1-37.5	0.02-0.028	0.00-0.00	0.75-0.86	0.00-0.00
S14	0.23-0.47	0.00-0.00	0.00-0.00	0.75-1.00	23.3-118	0.02-0.05	0.00-0.00	0.71-0.89	0.00-0.00
S15	0.40-0.40	10.39-10.39	0.18-0.18	0.66-0.66	75-75	0.03-0.03	0.01-0.01	0.75-0.75	0.39-0.39
S16	0.25-0.56	0.00-0.00	0.00-0.00	0.21-0.82	1.08-92.8	0.02-0.05	0.00-0.00	0.69-0.87	0.00-0.00
S17	0.37-0.68	0.00-0.00	0.00-0.00	0.67-0.96	41.2-78.1	0.04-0.06	0.00-0.00	0.81-0.84	0.00-0.00

Table 5.7 continued.

Character	Mean septal pit area	Ratio of septal pit area to rest of chamber	Ratio of width of suture	Apertural ornament	Incomplete septal bridge	Number of bosses	Maximum boss diameter	Total number of septal pits	Porosity
	10	11	12	13	14	15	16	17	18
S1	99.64-721.9	0.06-0.67	0.01-11.82	0-2	0-64	0-0.12	0-0.15	0.04-0.53	1-1
S2	38.55-130.29	0.04-0.13	0.658-3.72	0-1.2	0-17	0-0	0-0	0.17-0.28	1-1
S3	73.32-590.51	0.02-0.52	0.28-3.59	0.5-1	0-10	0-0.24	0-0.26	0.03-0.17	3-3
S4	0.00-779.64	0.00-0.383	0.25-2.64	0-0.5	0-3	0-0.09	0-0.18	0.03-0.15	2-3
S5	0.00-645.67	0.00-0.102	0.17-1.07	0-1	0-11	0-0.10	0-0.11	0-0.07	2-2
S6	60.74-184.26	0.004-0.04	0.56-1.04	0.1-0.1	0-2	0-0	0-0	0.05-0.12	1-1
S7	0.00-0.00	0.00-0.00	0.134-0.33	1-1	0-0	0-0	0-0	0-0	1-1
S8	17.98-173.51	0.005-0.04	0.14-0.69	1.5-2	0-0	0-0	0-0	0.08-0.16	1-1
S9	28.92-182.84	0.01-0.17	0.00-4.57	0-0	0-0	0-0	0-0	0.34-0.71	1-1
S10	133.38-219.4	0.09-0.16	2.34-3.46	1-1	0-0	0-0.12	0-0.11	0.17-0.27	1-1
S11	526.5-641.6	0.28-0.32	0.90-1.07	1-1	0-0	0-0	0-0	0.20-0.27	1-1
S12	1108.57-1554.5	0.22-0.27	3.07-3.66	1-1	0-3	0-0	0-0	0.25-0.33	1-1
S13	0.00-0.00	0.00-0.00	0.18-0.24	0-0	0-0	0.52-0.79	0.08-0.11	0.02-0.02	3-3
S14	0.00-0.00	0.00-0.00	0.28-2.22	0-1	0-0	0-0.06	0-0.11	0.01-0.05	1-1
S15	14.27-14.27	0.004-0.004	0.94-0.94	1-1	0-0	0-0	0-0	0.04-0.04	1-1
S16	0.00-0.00	0.00-0.00	0.09-0.60	0-0.2	0-0	0-0	0-0	0-0	1-1
S17	0.00-0.00	0.00-0.00	0.12-0.12	0.1	0-0	0-0	0-0	0-0	1-1

Table 5.7 continued.

Character	Peripheral spines	Presence of keel	Formation of a ridge	Apertural ornament	Openness of umbilical area	Umbilical ornament	Sutural ornament	Ornament along radial edge	Ornament outside of suture
	19	20	21	22	23	24	25	26	27
S1	0-0	0-0	0-0	0-5	2-5	1-4	1-5	1-3	0-0
S2	0-0	0-0	0-0	0-3	1-3	1-2	1-3	1-2	0-0
S3	0-0	0-0	0-0	2-4	3-5	1-4	3-5	1-2	0-0
S4	0-0	0-0	0-1	1-4	2-5	1-4	1-5	1-2	0-0
S5	0-0	0-0	0-0	2-5	3-5	2-4	3-5	1-2	0-0
S6	0-0	0-0	0-0	0-2	1-3	1-4	1-2	1-1	0-0
S7	0-0	0-0	0-0	4-4	5-5	5-5	4-5	1-1	0-0
S8	0-0	0-0	0-0	5-5	5-5	5-5	1-1	3-5	0-1
S9	0-0	0-1	1-1	0-4	4-4	3-4	1-3	3-5	1-2
S10	1-2	1-1	1-1	3-3	3-3	1-2	3-3	4-4	0-0
S11	0-0	1-1	1-1	3-3	2-2	1-2	3-3	1-1	0-0
S12	0-0	1-1	1-1	2-3	2-2	2-2	4-4	2-5	1-1
S13	0-0	0-0	0-0	3-3	5-5	2-3	3-3	1-1	0-0
S14	0-0	0-0	0-0	1-4	3-5	1-3	1-4	1-2	0-0
S15	0-0	0-0	0-0	4-4	5-5	2-2	5-5	1-1	0-0
S16	0-0	0-0	0-0	1-4	1-5	1-4	1-5	1-4	0-0
S17	0-0	0-0	0-0	1-2	5-5	3-4	1-1	1-1	0-0

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5.3.3 Intraspecific morphological variability

The question of to what extent ecophenotypy controls intraspecific morphological variability within the Elphidiidae family has been the subject of considerable debate (Feyling-Hanssen, 1972; Miller et al., 1982). New molecular evidence has revealed that a considerable proportion of the morphological variation within Elphidiidae is the product of genetics (Pillet et al., 2013). Despite the recent elucidation of interspecific taxonomic boundaries, there is currently a paucity of knowledge of how intraspecific test morphological changes with different environmental conditions. The sampling regime and the detailed quantitative morphological analysis conducted during this study allows for an exploration of intraspecific morphological variation within the Elphidiidae family. This study employed two case studies to assess the intraspecific morphological variability of *Elphidium* genotypes S1 and S4 between geographically distinct site locations. Genotype S1 was chosen as a case study because this genotype has the largest number of morphologically and genetically analysed specimens (n=248) collected across many distinctive biogeographic zones the NE Atlantic. Genotype S4 was chosen as a case study because it exhibits a large biogeographic distribution extending from the high Arctic to the Baltic. In addition, this genotype has been previously thought to be an ecophenotypic variant of the *Elphidium excavatum* complex (Miller et al., 1982).

In each case study, a PCO analysis was conducted to assess whether morphology alone can delineate between specimens from different site locations. In addition, DFA and CART analyses were conducted to assess the robustness of morphology in classifying each specimen into its respective site location and to identify (if present) any diagnostic features between the site localities. The PCO analysis revealed the absence of morphologically discrete locally sampled 'populations' within the PCO morphospace (Figure 5.16). Instead, a continuum of morphological forms with extensive overlap was identified between the different site localities. However, the PCO results revealed that no morphological overlap was exhibited between specimens from the four Icelandic sub-sites and Dartmouth, or between Aberdovey and Dartmouth, or between Cramond and Dartmouth. This suggests that at the extremes of the continuum of morphological variability it may be possible to discriminate some of the specimens morphologically from different site localities.

5.3.3.1 Case Study 1- Elphidium genotype S1

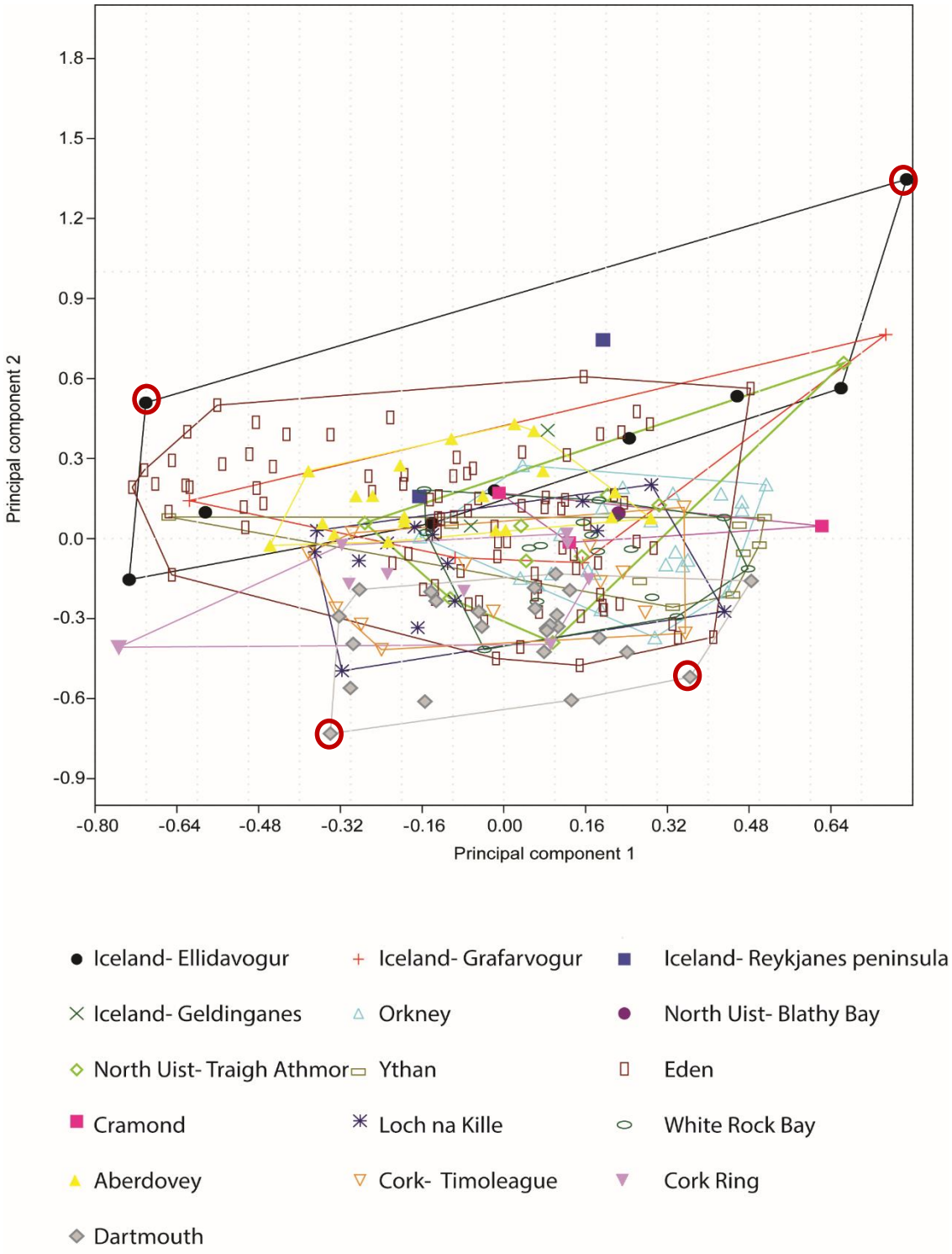


Figure 5.16 PCO analysis of the morphological traits of specimens of *Elphidium* genotype S1 from 16 distinct sample site locations. A convex hull bounds the specimens from each site locality. The first two principal coordinates account for 28.29% of the total variance. Specimens highlighted in red circles depict some of the extremes of morphological variability within this genotype (as illustrated in Figure 5.17).

A visual inspection of four end member specimens within the PCO morphospace (Figure 5.17) illustrates that these specimens can be delineated based on their morphological characters including the total number of septal pits and the degree of sutural and apertural ornamentation (Figure 5.17). It is important to note that these specimens can be visually discriminated based on their test size. This could indicate that despite the allometric correction applied in this study, ontogeny may shape some of the intraspecific morphological patterns identified in this analysis.

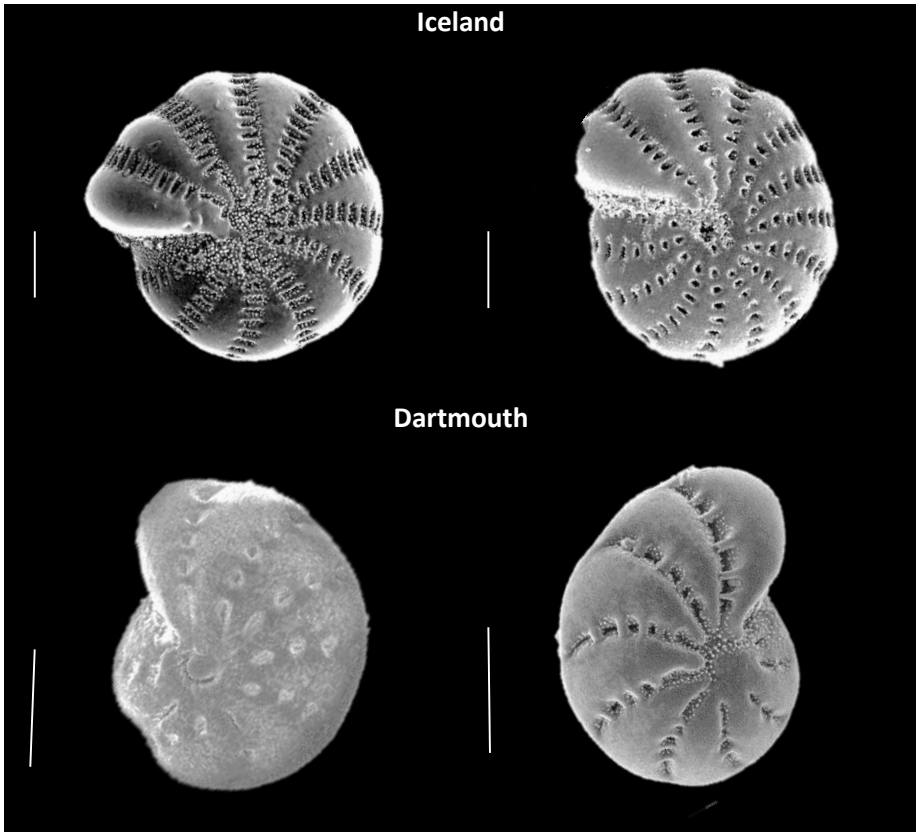


Figure 5.17 Examples of the extremes of intraspecific morphological variability exhibited within the *Elphidium* genotype S1 as highlighted in the PCO morphospace (Figure 5.16). The scale bar illustrated is 100 µm.

A discriminant function analysis was also conducted on the test morphology of genotype S1 specimens obtained from different sampling locations and this yielded an overall correct classification rate of 65.6% (Wilks Lambda: 0.27, P value: 0.00). The cross validation procedure successfully classified 47.4% of the specimens into their site locations; this may indicate the poor discriminatory power of morphology in discriminating between specimens taken from different site localities (Table 5.8).

Site and sub-site locations	Percentage correctly classified in DFA	Percentage correctly classified in DFA cross validation
Iceland -Ellidavogur (IS-EL)	56.6	0
Iceland –Grafarvogur (IS-GR)	50	0
Iceland- Reykjanes peninsula (IS-RP)	100	0
Iceland - Geldinganes (IS-GE)	50	50
Orkney (OK)	78.3	60.9
North Uist--Blathy Bay (NU-BB)	100	0
North Uist- Traigh Athmor (NU-TA)	22.2	0
Ythan (YN)	55.6	22.2
Eden (ED)	76.1	68.2
Cramond (CD)	66.7	0
Loch na Kille (LK)	50	42.9
White Rock Bay (WR)	37.5	18.8
Aberdovey (AB)	85.0	70
Cork - Timoleague (CK-TM)	23.1	0
Cork- Ring (CK-RG)	22.2	0
Dartmouth (DM)	88	68.0

Table 5.8 Overall percentage of *Elphidium* genotype S1 specimens correctly classified into their site location in the DFA. The predictive power of the discriminant analysis is shown by the cross-validation results.

The correct classification of individuals into their original site locations using morphology ranged from 0-100% (Table 5.8). However, there were much lower correct classification rates in the cross validation procedure (0-68% correct classification), indicating gradational morphological features between specimens taken from different site localities. The highest classification assignment success was identified for specimens sampled from the Orkney (78.3%, cross validation 65%), Dartmouth (88%, cross validation 68%) and Eden (76.1%, cross validation 70.5%) site localities (Table 5.8). For the most part the misclassified specimens were evenly distributed across all the different site localities (Tables 5.9 and 5.10). Specimens from the Eden site locality had the most ambiguous morphological boundaries, as 37 out of 88 Eden specimens were misclassified into other site localities and 16 specimens from the other site locations misclassified into the Eden site locality.

Observed site locality	Discriminant Function analysis Predicted site locality															
	IS-EL	IS-GR	IS-RP	IS-GE	OK	NU-BB	NU-TA	YN	ED	CD	LK	WR	AB	CK-TM	CK-RG	DM
IS-EL	**5**	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-
IS-GR	-	**2**	-	-	-	1	-	-	1	-	-	-	-	-	-	-
IS-RP	-	-	**2**	-	-	-	-	-	-	-	-	-	-	-	-	-
IS-GE	-	-	-	**1**	-	-	-	-	1	-	-	-	-	-	-	-
OK	-	1	-	-	**18**	-	-	2	1	-	-	-	-	-	-	1
NU-BB	-	-	-	-	-	**1**	-	-	-	-	-	-	-	-	-	-
NU-TA	-	-	-	-	-	-	**2**	-	5	-	-	-	-	-	-	-
YN	-	-	-	-	2	-	-	**5**	-	-	-	1	1	1	-	-
ED	1	1	-	3	2	-	1	2	**67**	-	-	1	2	2	1	5
CD	-	-	-	-	-	-	-	-	1	**2**	-	-	-	-	-	-
LK	-	-	-	-	-	-	-	-	3	-	**7**	2	-	-	-	2
WR	-	-	-	-	-	-	1	1	5	-	-	**6**	-	3	-	-
AB	-	-	-	-	-	-	-	-	3	-	-	-	**17**	-	-	-
CK-TM	-	-	-	-	1	-	-	-	5	-	1	1	-	**3**	-	2
CK-RG	-	-	-	-	-	-	-	-	7	-	-	-	-	-	**2**	-
DM	-	-	-	-	-	-	-	1	-	-	-	-	-	2	-	**22**

Table 5.9 Confusion matrix of the *Elphidium* genotype S1 specimens classified into each site locality in the DFA based on morphology. Site and sub-site location codes are listed Table 5.8.

	Cross validation analysis (DFA) Predicted site locality															
Observed site locality	IS-EL	IS-GR	IS-RP	IS-GE	OK	NU-BB	NU-TA	YN	ED	CD	LK	WR	AB	CK-TM	CK-RG	DM
IS-EL	-	-	-	1	-	-	-	-	5	2	-	-	1	-	-	-
IS-GR	-	-	-	-	1	1	-	-	1	-	-	-	-	-	1	-
IS-RP	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-
IS-GE	-	1	-	**1**	-	-	-	-	1	-	-	-	-	-	-	-
OK	-	-	-	-	**14**	-	1	3	3	-	-	-	-	-	-	1
NU-BB	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
NU-TA	-	-	-	1	-	-	-	-	6	-	-	-	-	-	-	2
YN	-	-	-	-	2	-	-	**2**	2	-	-	2	-	1	-	-
ED	3	2	-	3	2	-	1	1	**60**	1	1	2	-	2	1	7
CD	-	-	-	-	1	-	-	-	2	-	-	-	-	-	-	-
LK	-	-	-	-	-	-	-	-	3	-	**6**	3	-	-	-	2
WR	-	-	-	-	-	-	2	2	6	-	-	**3**	-	-	3	-
AB	-	-	-	-	-	-	-	-	6	-	-	-	**14**	-	-	-
CK-TM	-	-	-	-	1	-	-	1	6	-	1	2	-	-	-	2
CK-RG	-	-	-	-	-	-	-	-	9	-	-	-	-	-	-	-
DM	-	-	-	-	-	-	-	1	2	-	-	-	-	4	1	**17**

Table 5.10 Confusion matrix of the number of *Elphidium* genotype S1 specimens correctly and incorrectly classified into each site locality in the DFA cross validation procedure. Site and sub-site location codes are listed Table 5.8.

It is important to note that no morphological overlap (misclassification) occurred between specimens from the four Icelandic site localities and Dartmouth, nor between specimens from Aberdovey and Dartmouth, and Cramond and Dartmouth site localities in the DFA and the cross validation procedure (Tables 5.9 and 5.10). This is congruent to the morphological patterns identified in the PCO analysis (Figure 5.16) suggesting that it may be possible to distinguish the specimens taken from these different site localities. The discriminant function analysis places strong diagnostic weight on morphological test characteristics including maximum number of chambers (6), the ratio septal pit area to rest of the chamber (11), degree of apertural ornamentation (22) and the ratio of width of the sutures (12) to delineate specimens from different site localities.

The CART analysis conducted on the morphological characters of the *Elphidium* genotype S1 corroborated and extended the results of the DFA analysis (Tables 5.9 and 5.10). In total, 60.3% of genotype S1 specimens were correctly classified into their respective site locality based on morphology. The CART analysis yielded a 60.4% classification error within the cross validation analysis. The site localities with the highest assignment success include Dartmouth (78%), Aberdovey (80%), Eden (88.6%) and Orkney (78.3%) (Table 5.11). Again, the misclassified specimens are evenly distributed across all the sampling localities.

The CART analysis identifies that morphological test features including: openness of umbilical area (13), maximum chamber diameter (1), ratio of septal pit area to rest of the chamber (11), degree of sutural ornamentation (25) and maximum number of chambers (6) are diagnostically important in the delineation of *Elphidium* genotype S1 specimens from different sampling locations.

Overall, the two multivariate classification analyses (DFA and CART) conducted in this case study are congruent and have revealed that there is significant morphological overlap between *Elphidium* genotype S1 specimens from different site localities. Additionally, the results indicate that a proportion of the intraspecific test variability exhibited by the specimens could be the product of different test size (ontogeny).

Observed site locality	Predicted site locality																Total % correctly classified
	IS-EL	IS-GR	IS-RP	IS-GE	OK	NU-BB	NU-TA	YN	ED	CD	LK	WR	AB	CK-TM	CK-RG	DM	
IS-EL	-	-	-	-	1	-	-	-	6	-	-	-	1	-	-	1	0
IS-GR	-	**1**	-	-	-	-	-	-	2	-	-	-	1	-	-	-	25.5
IS-RP	-	1	-	-	-	-	-	-	1	-	-	-	1	-	-	-	0
IS-GE	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	0
OK	-	-	-	-	**18**	-	-	-	2	-	-	-	2	-	-	1	73.9
NU-BB	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	0
NU-TA	-	-	-	-	1	-	-	-	4	-	-	-	2	-	-	2	11.1
YN	-	-	-	-	1	-	-	**4**	1	-	-	-	1	-	-	2	44.4
ED	-	-	-	-	2	-	-	1	**78**	-	-	-	5	-	-	2	88.6
CD	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	2	0
LK	-	-	-	-	-	-	-	-	7	-	**6**	-	-	-	-	1	42.9
WR	-	-	-	-	3	-	-	-	9	-	-	**1**	3	-	-	-	6.3
AB	-	-	-	-	-	-	-	-	4	-	-	-	**16**	-	-	-	80
CK-TM	-	-	-	-	3	-	-	-	1	-	-	1	-	**4**	-	4	30.8
CK-RG	-	-	-	-	-	-	-	-	8	-	-	-	-	-	**1**	-	11.1
DM	-	-	-	-	4	-	-	-	2	-	-	-	-	-	-	**19**	76.0

Table 5.11 Classification matrix of the CART analysis, depicting the number of specimens correctly and incorrectly classified into each site locality and the total percentage of specimens correctly classified. Site and sub-site location codes are listed Table 5.8.

5.3.3.2 Case study 2- *Elphidium* genotype S4

The extensive biogeographical distribution of *Elphidium* genotype S4 extending from the Skagerrak to the Baltic seas presented an opportunity to assess intraspecific morphological variability of this genotype across a wide range of biogeographic provinces.

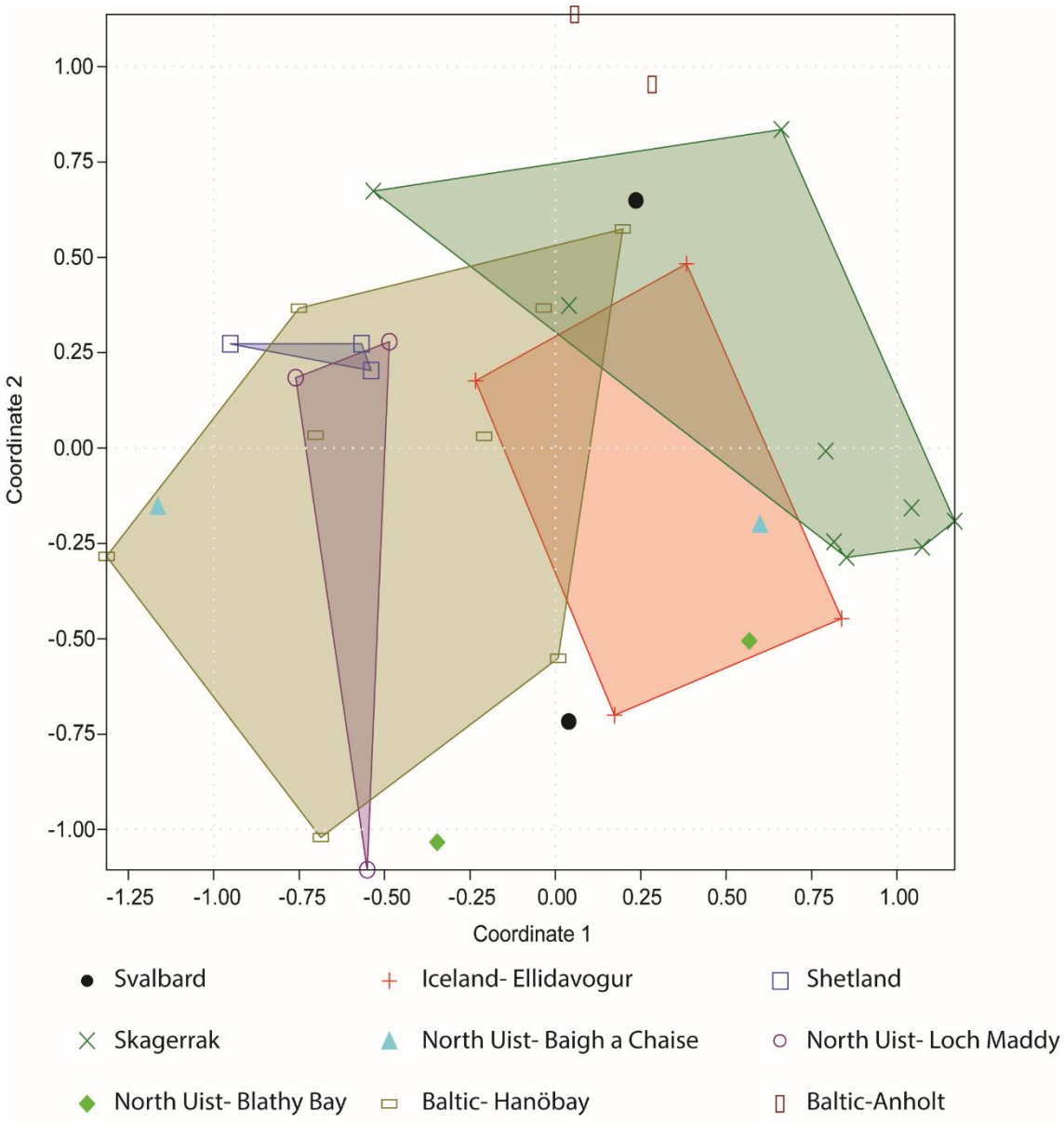


Figure 5.18 PCO analysis of the morphological characters of *Elphidium* genotype S4 sampled from different site localities. The two principal coordinates account for 38.8 % of the total variation. Each site locality is bounded by a convex hull.

The PCO analysis reveals that the two specimens from the Baltic- Anholt sub-site locality can clearly be distinguished from the other genotype S4 specimens from the other sampling locations, as these specimens exhibit no overlap within the PCO morphospace (Figure 5.18).

The remaining specimens exhibit partial to extensive morphological overlap, which is illustrated by the overlapping convex hulls. It should be noted that the specimens from North Uist (Loch Maddy), Iceland (Ellidavogur) and Shetland site localities do not exhibit any morphological overlap with specimens from Svalbard, Skagerrak or from North Uist (Blathy Bay). This suggests that these specimens are morphologically distinct. Additionally, specimens from North Uist (Blathy Bay) do not exhibit any morphological overlap with the specimens from Skagerrak; this indicates that it may be possible to delineate these specimens from one another.

The DFA conducted on the morphological characters of specimens of genotype S4 reveals that the different locally sampled 'populations' can be perfectly discriminated (Wilks: 0.009 P value: <0.001). However, only 17.1% of specimens were correctly classified by the cross validation procedure. For example, specimens from Svalbard, Shetland and North Uist (Blathy Bay) were all misclassified (Tables 5.12 and 5.13). This highlights that the discrete morphological discontinuities between site localities identified in the DFA may not be robust. The poor model performance in the cross validation procedure may be due to the small sample sizes.

Site Locality	Percentage correctly classified in the DFA	Percentage correctly classified in the cross validation analysis
Svalbard (SV)	100	0
Iceland-Ellidavogur (IS-E)	100	25
Shetland (SH)	100	0
Skagerrak (SK)	100	12.5
North Uist -Baigh a Chaise (NU-BAC)	100	50
North Uist- Loch Maddy-(NU-LM)	100	33.3
North Uist- Blathy Bay (NU-BB)	100	0
Baltic –Hanöbay (BA-HA)	100	12.5
Baltic –Anholt (BA-AN)	100	50

Table 5.12 Percentage of specimens of *Elphidium* genotype S4 correctly classified in the DFA and the cross validation procedure.

Observed site locality	Predicted site locality								
	SV	IS-E	SH	SK	NU-BAC	NU-LM	NU-BB	BA-HA	BA-AN
SV	-	-	--	--	--	1	1	--	-
IS-E	-	**1**	-	-	-	1	1	-	-
SH	-	-	-	-	-	-	2	1	-
SK	-	3	-	**1**	1	1	-	-	-
NU-BAC	1	-	-	-	**1**	-	-	-	-
NU-LM	-	1	-	-	-	**1**	-	1	-
NU-BB	-	1	1	-	-	-	-	-	-
BA-HA	-	3	-	1	1	-	-	**1**	2
BA-AN	-	-	-	1	-	-	-	1	-

Table 5.13 Confusion matrix of *Elphidium* genotype S4 specimens correctly and incorrectly classified into their sampling locality based on their test morphology in the cross validation (DFA) procedure.

The key diagnostic features identified for the delineation of different *Elphidium* genotype S4 specimens at different site localities by the DFA analysis include maximum boss diameter (16), openness of the umbilical area (23) and the degree of ornamentation in the sutures (25).

The CART analysis yielded an overall classification rate of 88.6%. However, 62.9% of specimens were misclassified in the cross validation analysis. Specimens from the Svalbard, Shetland, Skagerrak, North Uist (Loch Maddy) and North Uist (Blathy Bay) sampling localities exhibit perfect classification in the CART analysis based on morphology (Table 5.14). The remaining specimens taken from the different site localities exhibit partial morphological overlap, as one specimen from each site locality was misidentified (Table 5.14).

Observed site locality	Predicted site locality									Percent correctly classified
	SV	IS-E	SH	SK	NU-BAC	NU-LM	NU-BB	BA-HA	BA-AN	
SV	**2**	-	-	-	-	-	-	-	-	100
IS-E	-	**3**	-	1	-	-	-	-	-	75
SH	-	-	**4**	-	-	-	-	-	-	100
SK	-	-	-	**8**	-	-	-	-	-	100
NU-BAC	1	-	-	-	**1**	-	-	-	-	50
NU-LM	-	-	-	-	-	**3**	-	-	-	100
NU-BB	-	-	-	-	-	-	**2**	-	-	100
BA-HA	1	-	-	-	-	-	-	**7**	-	87.5
BA-AN	-	-	-	-	1	-	-	-	**1**	50

Table 5.14 Confusion matrix of specimens of *Elphidium* genotype S4 from the 10 V-fold cross validation CART analysis.

The CART analysis reveals that key diagnostic features that can be used to delineate specimens between the different site localities include mean septal pit area (10), test roundness (8), ratio of septal pit to the rest of the chamber (11) and openness of the umbilical area (23).

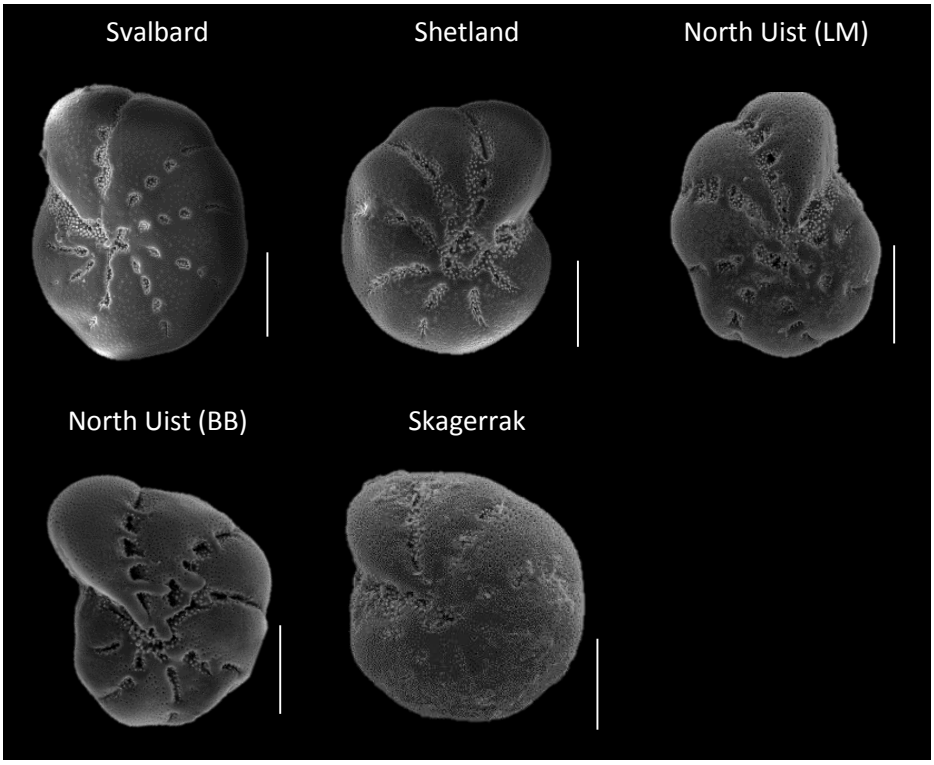


Figure 5.19 SEM images of the representative *Elphidium* genotype S4 specimens from five distinct site localities that exhibit perfect classification in the DFA and CART analysis. Scale bar illustrated is 100 µm.

Overall, there is good concordance between the results of the CART and DFA analyses, indicating that there are subtle morphological differences between specimens of genotype S4 collected from different site localities (as illustrated in Figure 5.19).

5.4 Discussion

5.4.1 Integrating molecules and morphology in the Elphidiidae family

The Elphidiidae family is often considered a particularly challenging taxonomic puzzle owing to its extensive morphological plasticity, the paucity of knowledge surrounding the interspecific (morphological and genetic) boundaries, coupled with nomenclatural uncertainty resulting from extensive synonymy (Feyling-Hanssen, 1972; Miller et al., 1982; Haynes, 1992). To elucidate the interspecific morphological relationships within Elphidiidae, this study analysed the efficacy of morphology in delineating 17 genotypes identified across the NE Atlantic (Darling et al., in prep.). The results revealed strong congruence between morphology and molecules as 13 out of 17 genotypes: S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14 and S15, exhibit almost perfect discrimination based upon their morphological characteristics (Figures 5.6 and 5.8, Tables 5.4 and 5.6). Minor discrepancies were identified between the CHAID and DFA classification results, as a limited number of different specimens (genotypes) were misidentified by each statistical procedure (Tables 5.4 and 5.6). However, as all of these genotypes can be successfully discriminated by refined PCO analyses (conducted without *a priori* knowledge of genetic groupings) (Figures 5.10, 5.11 and 5.13); this indicates that these genotypes exhibit unambiguous morphological boundaries. The divergence between the results produced by the statistical analyses could be a product of how each procedure handles the unbalanced sampling design, as certain genotypes had comparatively smaller numbers of specimens. Previous studies identified that unequal sampling design in DFA and CHAID analyses can significantly influence the classification results, particularly in the cross validation procedures (Braga-Neto, 2004; Fu et al., 2005).

The most challenging genotypes to classify using morphology were genotypes, S1, S2, S16 and S17. Even after extended analysis, a small number of specimens (n=5) were consistently misclassified and exhibited morphological overlap within the PCO morphospace and in the classification procedures (Figures 5.14 and 5.15 and Tables 5.4 and 5.6). This mismatch between molecules and morphology indicates the presence of specimens with gradational morphological features that masks the genetic diversity (partial cryptic diversity). Despite the recognition of cryptic specimens, it should be noted that the majority of elphidiids could be successfully unambiguously discriminated. This illustrates the utility of morphology in discriminating between genotypes within the Elphidiidae family.

Additionally it is important to note that the genotypes cluster into four main clades, which largely represent their general morphological character. Genotypes within Clade A exhibit well-defined sutural bridges, small test pores and often have numerous and narrow chambers. Genotypes within Clade B exhibit small test pores, distinct umbilical papillae (ornamentation) which often extends into the sutures and have a rounded periphery. Genotypes within Clade C have small test pores, rounded to sub-acute periphery, depressed sutures and very few septal pits. Finally, genotypes within Clade D have a rounded, often lobate periphery, wide and coarsely perforate chambers, and irregular septal bridges. As each clade exhibits distinctive morphological features, this highlights that there is a strong relationship between the degree of morphological variation observed and genetic divergence.

5.4.2 Congruence of the interspecific boundaries of Elphidiidae to the current taxonomic framework

Taxonomy is a dynamic process that continuously incorporates new lines of evidence to revise and refine species boundaries. Prior to the integrated taxonomic approach presented in this study, only a limited number of integrated taxonomic investigations were conducted on the Elphidiidae family (Langer, 2001; Schweizer et al., 2011; Pillet et al., 2011; Pillet et al., 2012 and Pillet et al., 2013). In addition, many of these studies took either a regional focus or a species-specific focus. For example, Schweizer et al. (2011) examines *Elphidium* diversity in the Kiel fjord, whilst Langer (2001) focused on *Elphidium williamsoni* and Pillet et al. (2012) focused upon *Elphidium macellum*. To date the most comprehensive re-evaluation of taxonomic relationships within the Elphidiidae family was conducted by Pillet et al. (2013) whereby the SSU rRNA sequences were used to identify 17 genotypes globally (15 of which were identified in the study area) (Table 5.15).

This study expands on the current taxonomic framework providing a detailed re-evaluation of the taxonomic relationships within Elphidiidae based on 895 genetic sequences (Darling et al., in prep.) and the quantitative morphological analysis of 599 specimens (Table 5.2). The molecular phylogeny of Elphidiidae (Darling et al., in prep) is congruent with the molecular phylogenies of previous taxonomic investigations (Schweizer et al., 2011; Pillet et al., 2012; Pillet et al., 2013). For example, the overall structure of the SSU rRNA phylogeny is largely unchanged.

Genotype identified in Darling et al. (in prep.)	Genotype identified in Pillet et al. (2013)
S1	*Elphidium williamsoni*
S2	Not sequenced by Pillet et al. (2013)
S3	Not sequenced by Pillet et al. (2013)
S4	*Elphidium excavatum clavata*
S5	*Elphidium excavatum*
S6	Not sequenced by Pillet et al. (2013)
S7	*Cribroelphidium albiumbilicatum*
S8	*Elphidium bartletti*
S9	*Elphidium margaritaceum* 1
S10	*Elphidium aculeatum-crispum*
S11	Not sequenced by Pillet et al. (2013)
S12	Not sequenced by Pillet et al. (2013)
S13	Not sequenced by Pillet et al. (2013)
S14	Not sequenced by Pillet et al. (2013)
S15	Not sequenced by Pillet et al. (2013)
S16	*Haynesina germanica*
S17	Not sequenced by Pillet et al. (2013)
Not sequenced by Darling et al. (in prep.)	*Elphidium margaritaceum* 2
Not sequenced by Darling et al. (in prep.)	*Elphidium asklundi* (Brotzen, 1943)
Not sequenced by Darling et al. (in prep.)	*Haynesina nivea* (Lafrenz, 1963)
Not sequenced by Darling et al. (in prep.)	*Elphidium frigidum* (Cushman, 1933)
Not sequenced by Darling et al. (in prep.)	*Elphidiella groenlandica* (Cushman, 1933)
Patagonia genotype (Not sequenced)	*E. macellum* (Fichtel and Moll, 1798)
Canada genotype (Not sequenced)	*Haynesina orbiculare* (Brady, 1881)

Table 5.15 List of elphidiid genotypes identified by Darling et al. (in prep.) and their corresponding genotype from Pillet et al. (2013). Pillet et al. (2013) ascribed classical taxonomic names directly onto each genotype. Two elphidiid genotypes identified outside of the study area (Patagonia and Canada) were included to provide insight into the global diversity of the Elphidiidae family. Table taken from Darling et al. (in prep.)

From the 22 Elphidiidae genotypes identified in the NE Atlantic, Darling et al. (in prep.) identified 17 genotypes (Tables 5.15, Figure 5.4). Seven of these genotypes (S2, S6, S11, S13, S14, S15 and S17) were identified for the first time. Notably, Pillet et al. (2013) identified five elphidiid genotypes in the NE Atlantic which were not found by Darling et al. (in prep.).

5.4.3 Congruence of the morphological boundaries to the current taxonomic framework

Prior to the detailed quantitative morphological analysis presented in this study, the interspecific morphological boundaries had been under-researched and poorly understood. Historically, a limited number of quantitative morphological studies were conducted on Elphidiidae species; these studies identified discrete (quantifiable) morphological characteristics that were used to delineate between Elphidiidae species (Buzas, 1966; Buzas et al., 1985). However, the taxonomic value of these diagnostic traits is questionable, as the species boundaries recognised were not supported by molecular evidence. Whilst recent molecular investigations have helped to clarify the morphological relationships among Elphidiidae species, there remains a paucity of knowledge surrounding the interspecific morphological limits. Recent studies have only analysed morphology using classical descriptive taxonomic practices (Pillet et al., 2011; Pillet et al., 2013). Moreover, a number of taxonomic studies have provided genetic sequences without any supporting morphological data (Langer, 2001; Etran et al., 2004; Habura et al., 2008).

In addition, even the most extensive re-evaluation of the interspecific morphological relationships within the Elphidiidae family conducted by Pillet et al. (2013) is not exhaustive. Pillet et al. (2013) primarily focused on summarising the morphological traits of each molecular clade. Thus little emphasis was placed on clarifying interspecific morphological boundaries and quantifying the key morphological traits. Moreover, whilst Pillet et al. (2013) tried to assess morphological traits using only genotyped specimens, this was not always possible. When sequenced specimens were not available for morphological analysis, Pillet et al. (2013) analysed specimens taken from the same population. The inherent and potentially erroneous assumption in Pillet et al.'s (2013) study is that specimens were conspecific (i.e. no cryptic species), or that if sympatric species occur these specimens could be robustly morphologically discriminated. This study's identification of partial cryptic diversity within the Elphidiidae family reveals that the putative assumption held in recent taxonomic investigation that each elphidiid genotype exhibits discrete diagnostic morphological features might not be valid. Although it is important to highlight two of the elphidiid genotypes (S2 and S17) that exhibit gradational morphological

features were only sequenced for the first time by Darling et al. (in prep.) (Table 5.13). Thus, their morphological attributes and their similarities to other genotypes were not previously captured.

5.4.4 Diagnostic test characteristics of Elphidiidae species

The diagnostic weight ascribed to the different morphological features has changed across time and space, owing in part to the complex taxonomic history of the Elphidiidae family. This coupled with the changeable and subjective terminology used for species delineation, has limited the effectiveness of interpreting and comparing species concepts (Miller et al., 1982). Traditionally, morphological test characters including the radial wall structures were deemed taxonomically significant in the Elphidiidae family (Loeblich and Tappan, 1964; Buzas, 1966; Miller et al., 1982). Additionally, test characteristics including the presence of an umbilical boss (Cushman, 1930) and the number of septal pits were deemed diagnostically important for species delineation within the genus *Elphidium*. As discussed previously, few studies quantified the key morphological characteristics of Elphidiidae species (Buzas, 1966; Buzas et al., 1985), though without knowledge of their genetic diversity, it was unclear if these characters are phylogenetically informative.

This study has re-evaluated the taxonomic significance of classical qualitative traits by quantifying many of the classical morphospecies descriptors. The results revealed that a complex combination of both structural and ornamentation features were necessary to delineate between species; these include: formation of a calcite ridge (21), length and relative width of sutures (3,12), total number of septal pits (17), degree of ornamentation along radial edge (26), degree of apertural ornamentation (22), mean septal pit roundness (9), porosity (18), average septal bar height (2) and openness of umbilical area (23) (Table 5.7). The results revealed that there are very few discrete species-specific morphological characters which were sufficient on their own to unambiguously delineate Elphidiidae species. Instead, there was an extensive overlap in the ranges of morphological traits between genotypes (Table 5.7). Overall, the results reveal that the majority of the classical morphological descriptors retain the capacity for species delineation but only when a combination of characteristics are analysed, as many of the interspecific morphological differences are subtle in nature.

The presence of ornamental spines as a species-specific morphological trait (characteristic of genotype S10) within this study is notable, as this finding contradicts previous investigations that have identified the presence of spines as an ontogenetic feature (Haynes, 1973; Pillet et al.,

2012; Pillet et al., 2013). For example, the presence of a spinose keel was identified as an ontogenetic feature in *Elphidium aculeutum-crispum* (Pillet et al., 2013, genotype S10 in this study) and in *Elphidium macellum* (Pillet et al., 2012). As spinose forms are found in other genotypes, this calls into question the validity of the presence of spines as a species-specific diagnostic trait in this study. It could be speculated that as only a limited number of specimens of genotypes S10, S11 and S12 were analysed in this study (n=10), it is unlikely that the full range of morphological variability at a population level (across all ontogenetic stages) was captured.

The identification of sutural and apertural ornamentation as key diagnostic morphological traits for species delineation is also interesting, as ornamentation has been ascribed functional significance in the Elphidiidae family (Pillet et al., 2011). For example, numerous studies identified that ornamentation such as 'teeth like' tubercules have functional significance whereby they can disassemble diatom frustals and comb/sieve and isolate chloroplasts (Banner and Culver, 1978; Banner, 1984; Bernhard and Bowser, 1999; Austin et al., 2005). Moreover, Pillet et al. (2011) identified that Elphidiidae species which exhibit accentuated septal bridges and ornamentation display an increased ability to feed on a wider range of diatoms. To date, the full functional significance of morphology within the Elphidiidae family remains unresolved and further ecological investigation is required.

Whilst significant emphasis was placed upon documenting and quantifying the full range of morphological variability in each of the elphidiid genotypes, the morphological database created is not exhaustive. Notably, the lack of SEM images taken from the apertural (peripheral) profile meant it was not possible to assess the morphological traits from this perspective. Historically, morphological features taken from this peripheral profile such as foraminiferal width and aperture characteristics were identified as being diagnostically important (Banner and Culver, 1978; Haynes, 1973). Thus this taxonomic re-evaluation of Elphidiidae might not have captured all the key diagnostic traits. Future investigations should aim to provide SEM images of the apertural profile, as these might help to resolve ambiguous interspecific boundaries identified between some of the partially cryptic specimens in this study. Additional close up SEM images would also be beneficial, as these would provide the opportunity for detailed quantitative measurements of porosity such as pore density and maximum pore diameter or measurements of ornamentation including height, width, and density of tubercules (teeth). Despite some potential caveats, the results highlight the practical utility of morphology as a robust line of taxonomic evidence for the clarification of taxonomic relationships within the Elphidiidae family.

5.4.5 Biogeographical distribution of Elphidiidae across the NE Atlantic

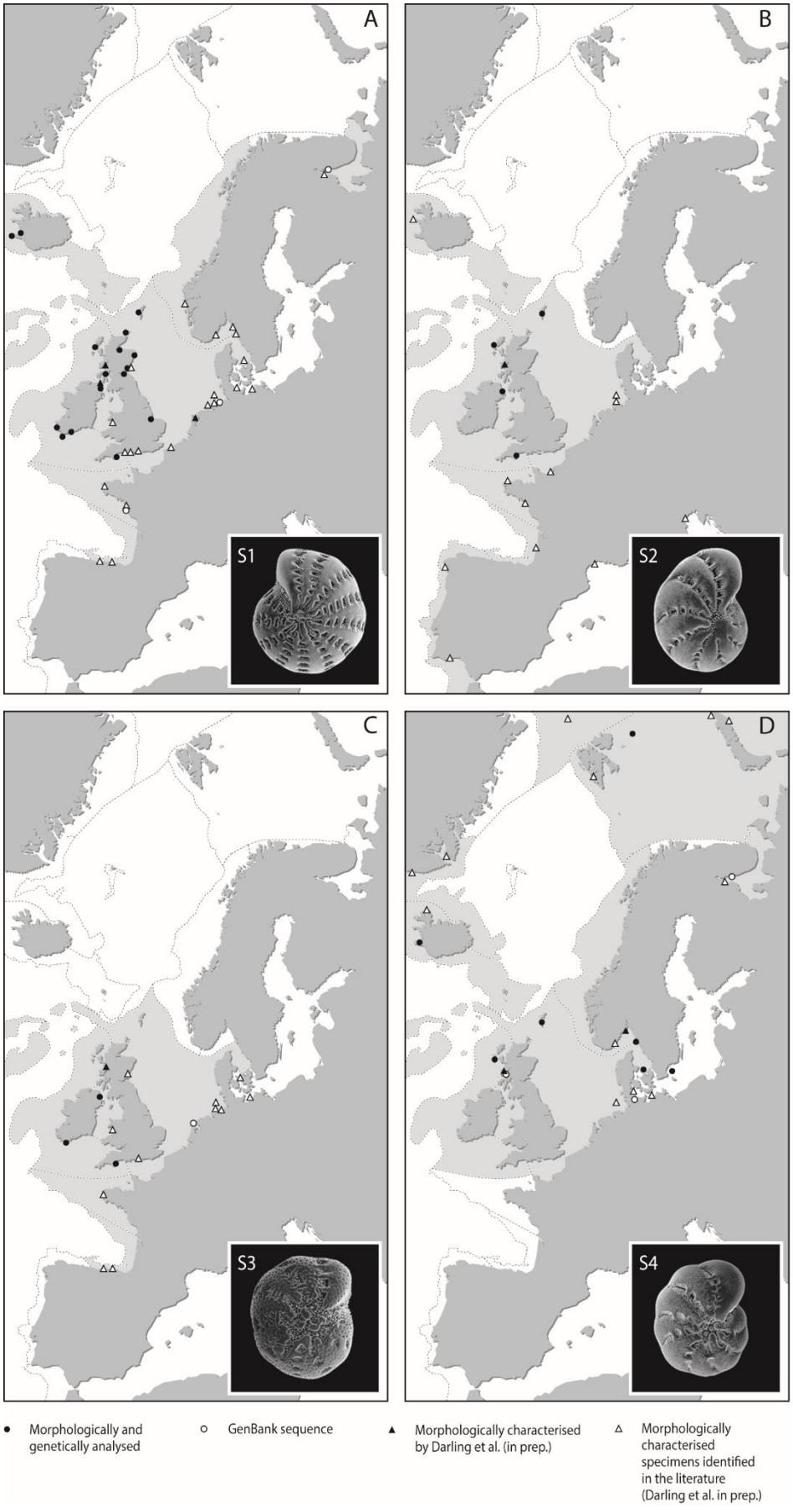
Biogeographic distributions were historically derived from the occurrences of species, which were identified using classical morphology based taxonomy. Understanding the biogeography of Elphidiidae species is crucial as this helps to define foraminiferal associations with habitats and regions, which is vital for palaeoenvironmental reconstructions (Murray, 1991). However, owing to the complex taxonomic history of the Elphidiidae family, the current understanding of geographical ranges within the family may be strongly influenced by the discrepancies between the taxonomic practices employed by different researchers across time and space. The absence of a consistent taxonomic framework, coupled with high levels of synonymy within the Elphidiidae could impede the understanding of species distributions. It is likely, for example, that species natural biogeographical ranges were previously incorrectly identified; i.e. either underestimated or overestimated. Although previous molecular investigations explored the diversity of Elphidiidae in the NE Atlantic, the sampling regimes employed by these studies were not exhaustive. For example, Pillet et al. (2013) only sampled 10 site localities within the NE Atlantic. Therefore, the re-evaluation of biogeographic distributions of Elphidiidae species in light of new molecular evidence have thus far received limited attention. This study's sampling of live Elphidiidae specimens from 26 site localities across distinctive biogeographical provinces (Figure 5.3 and Table 5.2) presents the most comprehensive documentation of diversity and biogeography of Elphidiidae in the NE Atlantic conducted to date (Figure 5.20). The biogeographic analysis of these specimens extends the known species ranges and occurrences of Elphidiidae genotypes previously identified in Genbank (as detailed in Figure 5.20).

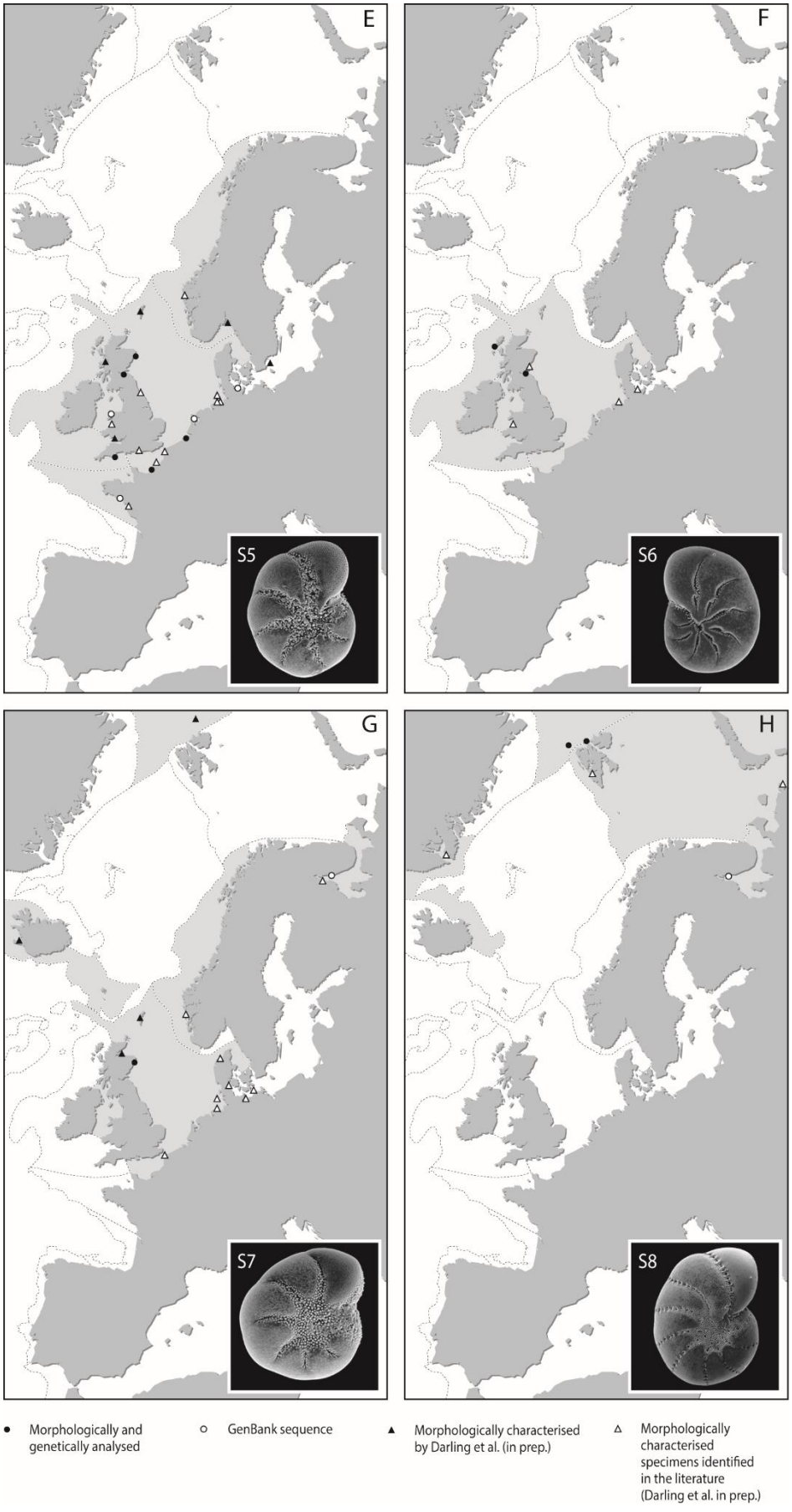
The biogeographical maps (Figure 5.20 A-Q) also depict the distributions of morphologically characterised specimens from across the NE Atlantic, which do not have an allied genetic sequence (i.e. specimens that were unsuccessfully sequenced by Darling et al., in prep.). These specimens were ascribed to a genotype based on a qualitative assessment of their morphological similarities to the diagnostic test characteristics of the genotyped specimens (Darling et al., in prep.). In addition, the maps illustrate the distributions of Elphidiidae specimens identified from the open literature, which were re-examined in light of the new taxonomic evidence (Darling et al., in prep.). Darling et al. (in prep.) screened the open literature for studies which included Elphidiidae specimens with a known site locality and that had a high quality SEM or light microscope image. Specimens were ascribed to a potential genotype, based on their morphological similarity to the diagnostic morphological characteristics of each genotype (designation irrespective of applied taxonomic name). However, Darling et al. (in

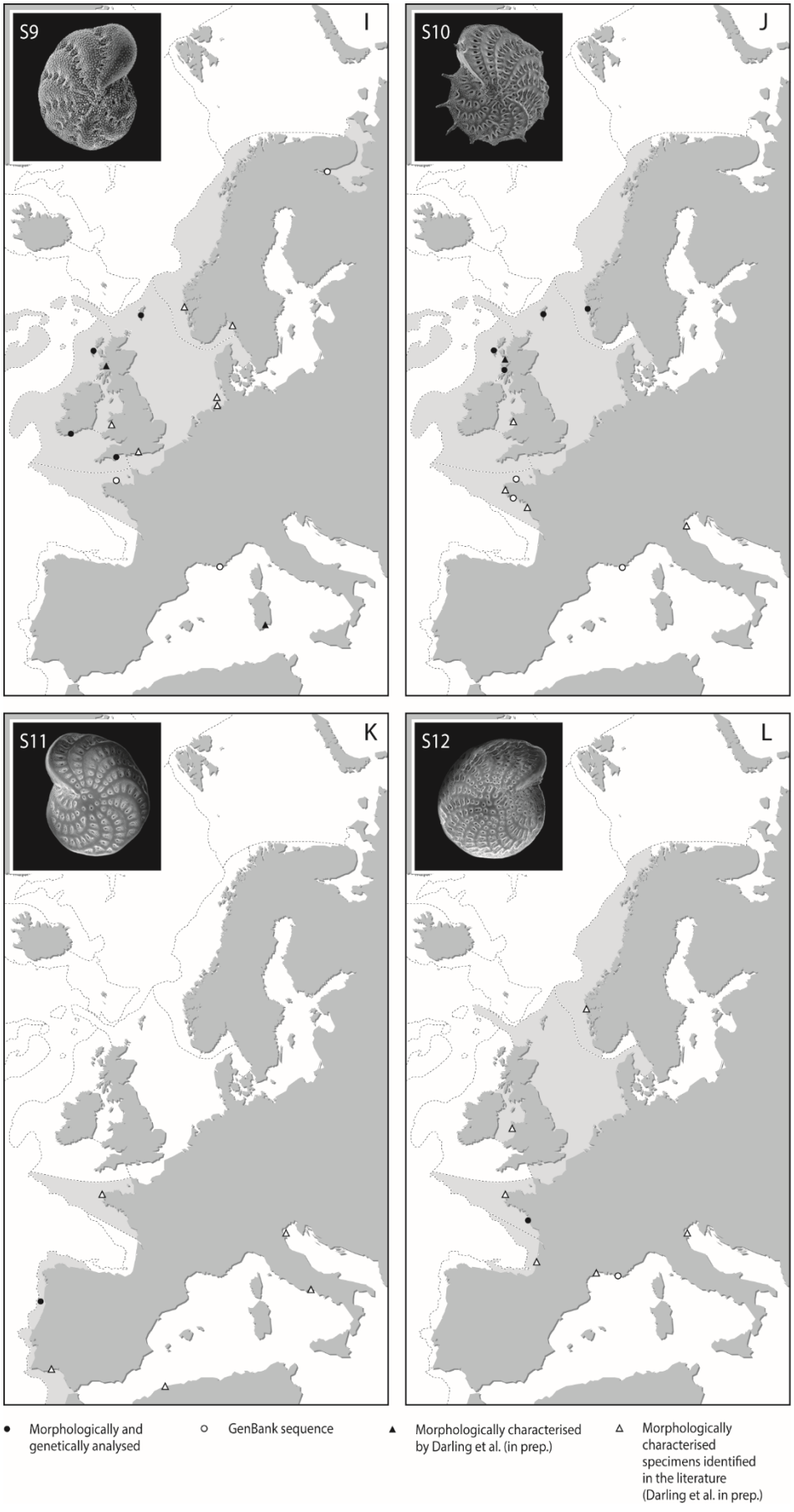
prep.) only analysed morphological similarities based upon qualitative descriptors. However, the clarification of the quantitative interspecific morphological boundaries and recognition of partially cryptic species within this study brings into question the validity of the biogeographic distributions of the morphologically characterised specimens (Figure 5.20 A-Q).

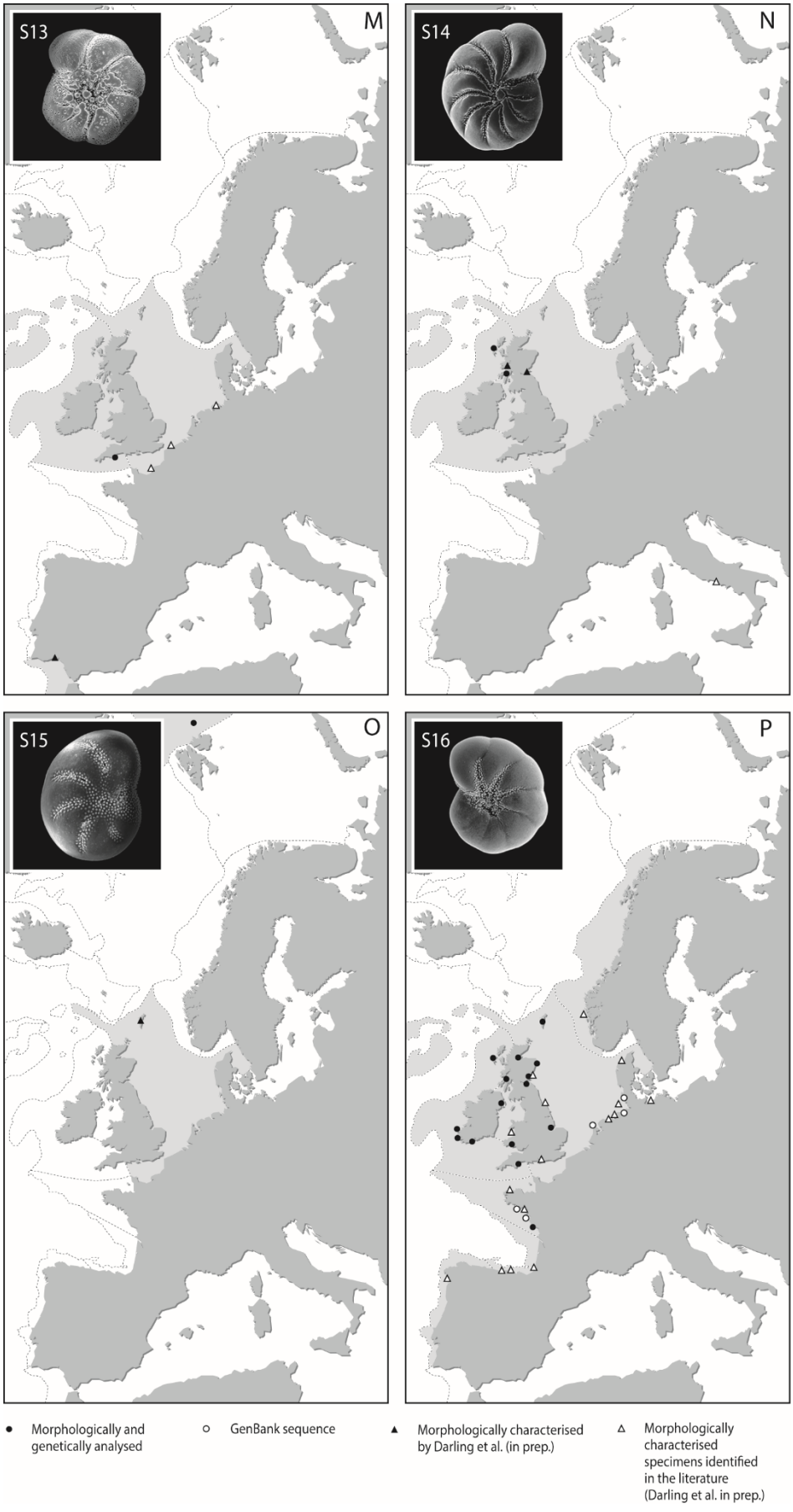
However, the ramifications of this previously unrecognised cryptic diversity are unlikely to significantly affect the biogeographic ranges of morphologically characterised specimens because only a small number of specimens exhibited gradational morphological features. Moreover, the majority of taxonomic studies within the academic literature only illustrate 'representative' end member morphologies in their SEM plates/ light microscope images. Therefore, it is unlikely that specimens with gradational morphological features would be illustrated. Consequently, the inclusion of morphologically characterised specimens into the re-evaluation of species biogeographical ranges will have a limited impact.

Overall, the 17 Elphidiidae genotypes identified in the NE Atlantic exhibit complex, often overlapping biogeographic distributions. It is evident that certain species within the Elphidiidae family exhibit widespread biogeographic distributions, whilst other species exhibit restricted distributions. The geographical segregation of some of the Elphidiidae species in the NE Atlantic is likely to be influenced by the ecological requirements of the genotype.


A
S1
B
S2
C
S3
D
S4
Morphologically and genetically analysed
GenBank sequence
Morphologically characterised by Darling et al. (in prep.)
Morphologically characterised specimens identified in the literature (Darling et al. in prep.)


E
S5
F
S6
G
S7
H
S8
Morphologically and genetically analysed
GenBank sequence
Morphologically characterised by Darling et al. (in prep.)
Morphologically characterised specimens identified in the literature (Darling et al. in prep.)


S9
I
S10
J
S11
K
S12
L
Morphologically and genetically analysed
GenBank sequence
Morphologically characterised by Darling et al. (in prep.)
Morphologically characterised specimens identified in the literature (Darling et al. in prep.)


S13
M
S14
N
S15
O
S16
P
Morphologically and genetically analysed
GenBank sequence
Morphologically characterised by Darling et al. (in prep.)
Morphologically characterised specimens identified in the literature (Darling et al. in prep.)

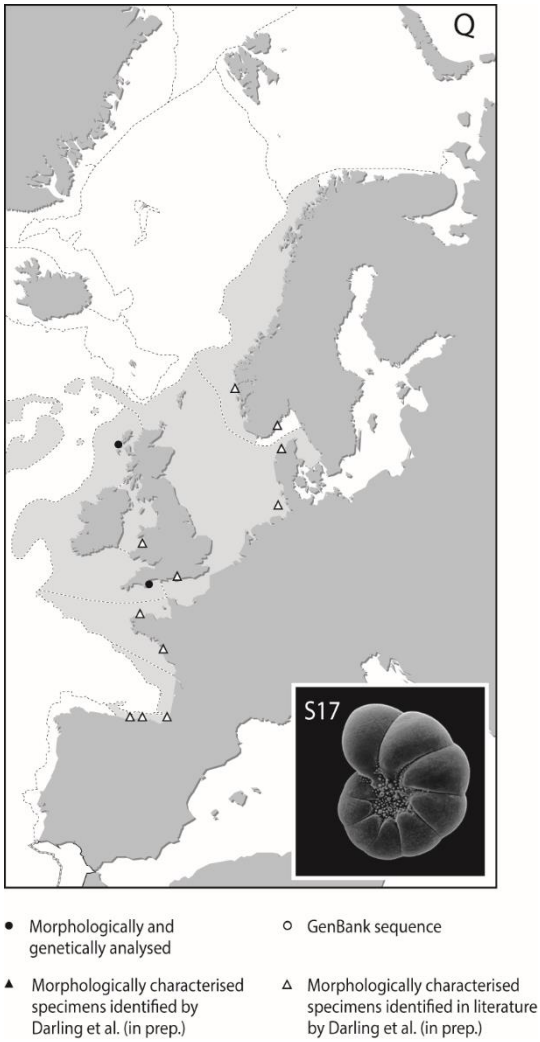


Figure 5.20 Biogeographic distributions of 17 Elphidiidae genotypes across the NE Atlantic. The shaded areas demarcate the presence of a species within a biogeographic province. The list of biogeographic provinces is illustrated in Figure 5.3 (Dinter et al., 2001). The biogeographic maps are modified from Darling et al. (in prep.).

The two most cosmopolitan species within the Elphidiidae family are genotypes S1 and S16 (Figure 5.20, A and P). Both these species are highly prevalent in the Boreal and Boreal-Lusitanean biogeographic provinces. These are predominately mid to low latitude species, as they are absent from high Arctic regions including the Barents Sea and the Greenland shelf. The distribution of genotype S1 extends from the White Sea biogeographic province to the Bay of Biscay (Figure 5.20, A). This ubiquitous species was identified in eight distinctive biogeographical provinces. As genotype S1 exhibited partial cryptic diversity with genotype S2 (Figure 5.14, Table 5.6), it is important to note that based on molecular sequences alone, the biogeography of genotype S1 is more constrained. Notably, the biogeographical occurrence of genotype S1 based on molecular evidence alone extends from Iceland to Dartmouth. Genotype S16 also exhibits a ubiquitous distribution, extending across six biogeographic provinces from the West Norwegian

biogeographic province to the Portuguese margins (Figure 5.20, P). As partially cryptic species were identified within genotypes S16 and S17 (Figure 5.11, Table 5.6), it is important to highlight that the biogeographic range of genotype S16 based solely on molecular data is restricted to just three biogeographic provinces.

Although other elphidiid species exhibit relatively widespread distributions, different species are more prominent in different areas. For example, while the biogeographic distribution of genotype S9 extends from the Mediterranean up to the White Sea (Figure 5.20, I), it is prominent in Boreal and Boreal- Lusitanean provinces and has yet to be identified in the warm and cool Lusitanean biogeographic provinces (Figure 5.20, I). Additionally, genotype S2 exhibits a broad species range extending from Iceland to the Mediterranean (Figure 5.20, B). This genotype (S2) exhibits a predominantly south-westerly distribution within the NE Atlantic, with the exception of two morphologically characterised occurrences in the east of the Boreal-Lusitanean province. Genotypes S10 and S12 exhibit similar cosmopolitan distributions, which do not extend further north than the western Norwegian biogeographic province (Figure 5.20, J and L). It is interesting that both these genotypes are predominantly distributed in western regions of the NE Atlantic (exceptions in the Mediterranean). However, it should be noted that the biogeography of genotype S12 is primarily constructed from the occurrences of morphologically characterised specimens, as this genotype was only sequenced from two site localities (Figure 5.20, C). This highlights that further clarification of interspecific boundaries are required to test the validity of the biogeographical ranges identified from purely morphologically characterised specimens.

In contrast, other Elphidiidae genotypes display restricted biogeographic distributions in the NE Atlantic. Notably, genotypes S8 and S15 exhibit northerly distributions, as they are only found in the northern Arctic biogeographic provinces (Figure 5.20, H and O). Genotype S4 also exhibits a predominately northern distribution which extends southwards from the high arctic into the Boreal, Boreal-Lusitanean biogeographic provinces, and the Baltic seas (Figure 5.20, D). Additionally, genotype S7 also exhibits an overall northerly distribution ranging from the High Arctic maritime to the Boreal biogeographic provinces (Figure 5.20, G). However, it should be noted that the occurrence of this genotype in the high Arctic was based solely on a morphologically characterised specimen.

Genotype S6 exhibits a mid-latitude biogeographic distribution as it is found within the Boreal, Boreal-Lusitanean and Baltic biogeographic provinces (Figure 5.20, F). In addition, genotype S14 also exhibits a mid-latitude distribution, predominately occurring around the Scottish coast

(Figure 5.20, N). Additionally Darling et al. (in prep.) potentially identified this genotype (S14) from within the Bay of Naples based on a morphologically characterised specimen from the literature. Genotype S3 also exhibits a central distribution as this species is common within Boreal and Boreal-Lusitanean provinces, although its distribution does extend into the Baltic seas and down into the northern Warm Lusitanean biogeographic province (Figure 5.20, C). In addition, genotype S5 also exhibits a predominately-central biogeographical distribution (Figure 5.20, E). Although its species range does extend up to the West Norwegian biogeographic province, this genotype is most common within the Boreal, Boreal-Lusitanean biogeographic provinces. Genotype S17 also exhibits a relatively broad biogeographic distribution extending from West Norwegian biogeographic province extending down into the northern Lusitanean-warm biogeographic province (Figure 5.20, Q). However, when analysing the biogeographic distribution of genotype S17 based on molecular evidence this species exhibits a much more restricted distribution, only being identified along the western coast of the UK. Finally, genotype S13 exhibits a mostly central biogeographical distribution, prevalent within the Boreal and Boreal-Lusitanean provinces (Figure 5.20, M). This genotype exhibits a disjunct distribution as it is also found in the warm Lusitanean biogeographic province but there are noticeable absences in other southern biogeographic provinces. This disjunct distribution could indicate this genotypes is rare.

Finally, genotype S11 exhibits a restricted southerly distribution in the NE Atlantic where it is found extending from the Mediterranean up to the Lusitanean-Boreal province (Figure 5.20, K). Again, it should be noted that this genotype was only sequenced at a single locality in the Lusitanean cool biogeographic province, thus the species range identified is reliant upon the accurate identification of morphologically characterised specimens by Darling et al. (in prep.).

5.4.5.1 Sympatry

Identifying the presence of co-existing species has great significance for applied taxonomic investigations, it can both highlight biodiversity patterns and help to elucidate the ecological preferences of species by revealing localised partitioning. Extensive sympatry of Elphidiidae species was identified within the NE Atlantic (Table 5.16) as at least two genotypes were observed co-existing in 12 out of 25 localities (Table 5.16). This is unsurprising, as extensive overlap in the species biogeographic distributions were previously observed (Figure 5.20 A-Q). The highest levels of sympatry were identified at Dartmouth, as eight genotypes were found to co-exist at this single site locality (Table 5.16).

Unsurprisingly, the two most cosmopolitan genotypes S1 and S16 were also identified as the two most likely genotypes to be found living in sympatry with other elphidiid genotypes (Table 5.16). In contrast, only two genotypes, S11 and S12 were not found co-existing with other Elphidiidae genotypes in the NE Atlantic (Table 5.16). Additionally, whilst genotype S4 and S5 are often found in close proximity and exhibit overlap in their biogeographical ranges (Figure 5.20, D and E); these two genotypes were not found living in sympatry by this study (Table 5.16). This potential partitioning has taxonomic significance because in classical morphology based taxonomy these genotypes are thought to exhibit distinct biogeographic and environmental preferences, as they are often identified as ecophenotypes, or subspecies within the *Elphidium excavatum* species complex (Feyling-Hanssen, 1972; Miller et al., 1982). Thus, it could be speculated that these genotypes may have discrete ecological preferences. However, further investigation is required to identify the species ranges and elucidate potential ecological partitioning between these genotypes.

Table 5.16 Distribution and co-occurrence of Elphidiidae genotypes across the NE Atlantic site localities. Shaded areas demarcate the presence of a genotype at a site locality. Site location codes are listed in Table 5.2.

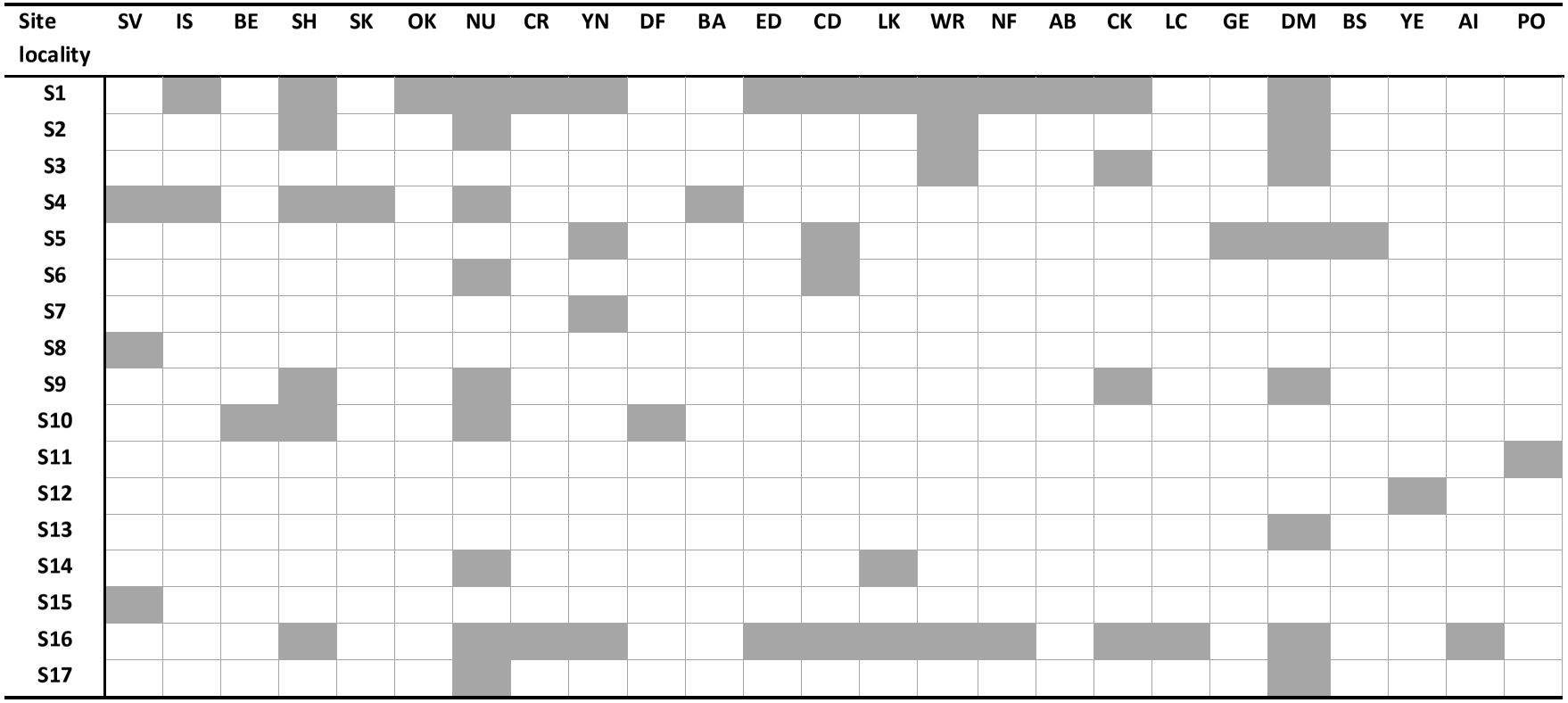
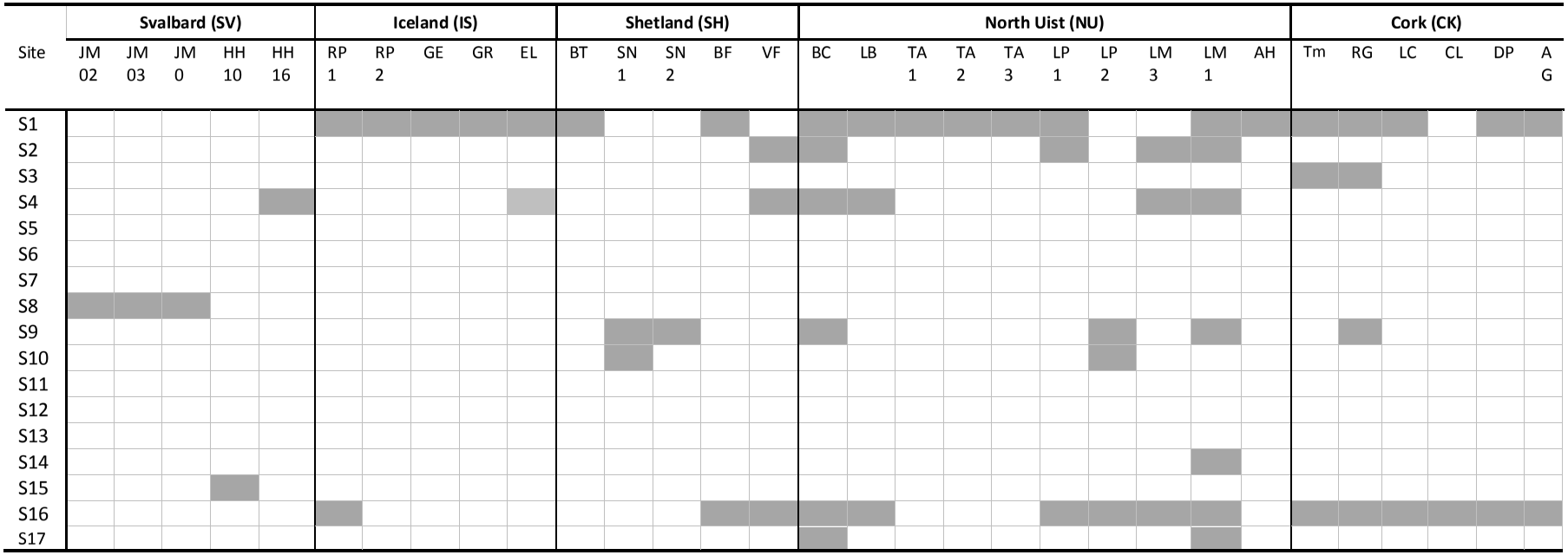


Figure 5.17 Sympatry of Elphidiidae genotypes at sub-site locations. Shaded areas demarcate the presence of a genotype at a sub-site location. Sub site location codes are depicted in Table 2 in the Appendix



Consideration of sub-site location information (where available) revealed additional insights into the co-occurrence and localised partitioning of the genotypes (Table 5.17). Notably high levels of elphidiid biodiversity were identified at two sub-site locations in North Uist. For example, seven genotypes were found in sympatry at Loch Maddy (LM1), whilst six genotypes were found in sympatry at Bagh a Chaise (BC) (Table 5.17). The high biodiversity at these locations is interesting, as both of these samples were retrieved from seaweeds. Historically seaweeds were relatively under-sampled within the NE Atlantic, as classical taxonomic studies have predominately focused upon sediment sampling; therefore, classical taxonomic studies may have underestimated biodiversity across the NE Atlantic. Analysis of the co-occurrence of elphidiid genotypes in sub-site locations in Svalbard also reveals that genotypes S4, S8 and S15 were not found in sympatry with each other (Table 5.17). This partitioning revealed that genotype S15 was not living in sympatry with any other genotypes in the NE Atlantic. To date the high levels of sympatry observed within this study are currently unprecedented, as previous studies have only identified the presence of three elphidiid genotypes living in sympatry at a single site locality (Pillet et al., 2011, in the Chezzetook Inlet, Canada). Although it should be noted that historically Miller et al. (1982) identified the presence of five ecophenotypes at one location.

The complex patterns of biodiversity and distribution of Elphidiidae in the NE Atlantic probably reflect the intricate evolutionary and biogeographic history of these genotypes. It is clear that the central biogeographic regions such as Boreal and Boreal-Lusitanean provinces exhibit the highest levels of biodiversity within the NE Atlantic (Tables 5.16 and 5.17, and Figure 5.20 A-Q). In contrast, the lowest levels of biodiversity were observed in the high Arctic provinces (Tables 5.16 and 5.17, and Figure 5.20). The prevalence of the highest levels of biodiversity within the central biogeographic provinces in the NE Atlantic could be a product of their historical biogeography. Previously it was acknowledged that abrupt shifts in climate (glacial-interglacial cycles) have a strong effect upon the distribution and the levels of biodiversity of taxa in the NE Atlantic (Yashura et al., 2014). Notably, during the last glacial maximum (LGM) c 20ka, the sea surface temperatures decreased by 10^{o}c, and there was an expansion of ice sheets extending into mainland Europe and the British Isles (Clark et al., 2001; Scourse et al., 2009). This drove the southwards migration of many species in the NE Atlantic (Hewitt, 1999; Provan and Bennett, 2008). As the glaciers receded in the Holocene and the temperatures increased, this provided the potential for populations to recolonize newly available habitats (Davis and Shaw, 2001). The prevalence of genotypes which exhibit predominately westerly distribution in the NE Atlantic

(such as genotypes S2, S10 and S11) extending up from the south could represent the recolonisation of populations by propagule dispersal (Alve et al., 2003; Alve and Goldstein, 2010), following water currents such as North Atlantic current. Additionally, the extant northern species may represent populations that survived in isolated refugia in the arctic during the LGM (Dahl, 1998).

The highest levels of biodiversity observed within the Boreal and Boreal-Lusitanean regions could also be the product of a potential sampling bias present in this study and in the academic literature. Owing in part to accessibility, relatively limited sampling was conducted in the Arctic provinces, in contrast to the extensive sampling of temperate regions (Table 5.2). Although this study presents the most comprehensive re-evaluation of biodiversity and biogeographic distributions to date, the sampling regime employed is not exhaustive. Notably, this study's sampling regime is biased towards intertidal areas. Therefore, the sampling regime may be inadequate to evaluate fully the diversity of Elphidiidae in deeper habitats. In addition there were sampling gaps, notably within the high Arctic biogeographic provinces. Further scrutiny of the biogeographic limits of Elphidiidae species is therefore warranted. Attention should be given to clarifying the species range overlap and the degree of habitat segregation where species are found in sympatry. Valuable insights into species ecological partitioning could also be obtained in the future by conducting detailed *in situ* investigations or laboratory culturing experiments. Moreover, it is crucial to expand the taxonomic re-evaluation of this family outwidth of the NE Atlantic to gain a global perspective on the diversity of Elphidiidae.

5.4.6 Intraspecific morphological variability

Quantifying the range of morphological variation within a species is doubtlessly important for taxonomy. In addition, untangling the environmental controls on morphological characters could help to improve the precision of palaeoenvironmental reconstructions (Murray, 1991). Historically, the extent to which intraspecific morphological variation within Elphidiidae is controlled by either genetics or environment has been the subject of considerable debate (Wilkinson, 1979; Miller et al., 1982; Haynes, 1992). As previously discussed, intraspecific morphological differences in the Elphidiidae family, particularly within the *Elphidium excavatum* species complex, were associated with different environmental conditions and/or biogeographic distributions (Feyling-Hanssen, 1972; Poag et al., 1980; Miller et al., 1982; Goubert, 1997, as highlighted in Table 5.1). However, recent molecular analysis revealed that

test morphological variability traditionally associated with ecophenotypy is actually the product of genetics (Pillet et al., 2013; Darling et al., in prep.).

To date, Pillet et al. (2012) is the only study which has re-evaluated the intraspecific morphological boundaries in light of new taxonomic (molecular) evidence within the Elphidiidae family. Pillet et al. (2012) examined '*Elphidium macellum*' at seven distinct site localities within the Patagonian fjords. Pillet et al. (2012) hypothesised that the intraspecific morphological variability exhibited by this genotype is a product of interspecific hybridisation. This interpretation was favoured because high morphological variation was observed within each population. Moreover, the test traits that exhibited variation were typically associated with 'species characteristics' rather than environmentally variable test characteristics (Pillet et al., 2013). However, the underlying forces on intraspecific morphological variability within this genotype remain unclear, as Pillet et al. (2013) could not rule out the potential role of ecophenotypy due the paucity of (detailed) environmental data. Additionally, Pillet et al. (2013) only examined the morphological differences between the populations based on qualitative morphological descriptors. There may be subtle quantitative characters that could distinguish between these populations, which were not taken into account by Pillet et al. (2013). Furthermore, Darling et al. (in prep.) calls into question the validity of Pillet et al.'s (2013) hypothesis of interspecific hybridisation within *'E. macellum*', as they failed to take intra-individual genetic variability into account. This highlights the paucity of knowledge surrounding the degree and controls of intraspecific morphological variability within the Elphidiidae family. This study presents the most comprehensive re-examination of intraspecific morphological variability within Elphidiidae conducted to date. Two case studies were employed to assess the quantitative intraspecific morphological variations across a spatial scale for two genotypes, S1 and S4. Unfortunately, this study could not quantify the degree of morphological plasticity between different environmental conditions because detailed environmental surveys were not conducted in this investigation.

5.4.6.1 Case study 1

Genotype S1 was chosen to assess intraspecific variability across a spatial scale for several reasons. This genotype has the largest number of morphologically and genetically analysed specimens (n=248) in this study (Table 5.2). In addition, this genotype exhibits widespread distribution across the NE Atlantic; therefore it is likely to encounter a wide range of

environmental conditions (Figure 5.20). Finally, this genotype exhibits extensive intraspecific morphological variability in the multivariate statistical analyses (Figure 5.6).

This case study identified that no pronounced morphological variation associated with spatial scales was observed within genotype S1 across the NE Atlantic, as extensive morphological overlap was identified between the specimens at different site localities (Figure 5.16 and Table 5.7). Instead, the results revealed that specimens at the extremes of morphological test variability (as observed in Figure 5.17), can be visually discriminated based on their overall test size. Moreover, the multivariate statistical analysis has identified that test characteristics such as the total number of chambers and the diameter of the penultimate chamber (N1) can be used to differentiate specimens of genotype S1 at the extremes of the morphological variation in the PCO morphospace. Historically these gross (structural) morphological features were associated with ontogeny (Murray and Alve, 2002; Sen Gupta, 2002). This could indicate that to some degree the intraspecific morphological variability was subtly influenced by ontogeny. However, it could also be speculated that the morphological characters associated with test size could be an adaptive response to environmental conditions (Boltovskoy et al., 1991; Hottinger, 2000).

5.4.6.2 Case study 2

Genotype S4 was identified as an important genotype for intraspecific morphological analysis across spatial scales because it exhibits a large biogeographic distribution from the high Arctic to the Baltic (Figure 5.20 D). In addition, Pillet et al. (2013) and Darling et al. (in prep.) have associated this genotype to the species complex *Elphidium excavatum*. As previously discussed, species within the *Elphidium excavatum* complex were historically considered to exhibit limited genetic diversity and high intraspecific morphological variability as a product of their biogeography (Miller et al. 1982). New lines of molecular evidence have since refuted this theory (Pillet et al., 2013; Darling et al., in prep.). However, the degree to which intraspecific morphological variability in relation to environment/ biogeography is yet to be examined for the newly delineated genotypes associated with this species complex.

In this case study, subtle differences in test morphology were identified between genotype S4 specimens collected from nine different site localities (Figure 5.18, Tables 5.12 and 5.14). Notably specimens from Svalbard, Skagerrak, North Uist Loch Maddy and Blathy Bay, and Shetland exhibited discrete morphological characteristics (Tables 5.12 and 5.14). These morphological delineations were constructed from a combination of structural and ornamental features, including maximum boss diameter, degree of sutural ornamentation, openness of

umbilical area, total roundness and mean septal pit area. The recognition of morphological discontinuities between different site localities is interesting because it could indicate that morphological plasticity is a product of different biogeographical or even environmental controls. However, as limited environmental data was available, the potential controls of morphological variability can only be speculated. In addition, it is uncertain if the morphological differences observed between the locally sampled 'populations' play a functional role, as previously discussed ornamentation in the Elphidiidae family was previously associated with a functional feeding role (e.g. Austin et al., 2005). However, the functional roles of morphology within this genotype remain unclear and require further investigation.

Moreover, it should also be noted that the intraspecific morphological differences identified between the different site localities might not be robust with the inclusion of additional specimens in future investigations. Although the multivariate analysis reveal that 86.8-100% specimens can be correctly classified into their species, the cross validation error attained was poor, as 100% of specimens were misclassified in the DFA and 62.9% were misclassified in the CART analysis (Tables 5.12 and 5.14). The poor cross validation results could be the product of the low numbers of specimens analysed (n=35) and the unbalanced sampling design in this study (number of specimens analysed ranged from one to six specimens per site locality). As a consequence of the relatively low numbers of specimens analysed, it is unlikely this study has captured the full range of intraspecific (population-level) variability at each site. This makes uncoupling the controls of intraspecific morphological variability within this genotype difficult; highlighting the need for further investigation.

5.4.7 Utility of new taxonomic framework of Elphidiidae

This study presents a new taxonomic framework for Elphidiidae, integrating molecules, morphology and biogeography. However, uncertainty still surrounds how these newly delineated genotypes can be reconciled with classical taxonomy and nomenclature. Recent taxonomic studies have helped to clarify interspecific boundaries through the provision of independent lines of taxonomic evidence. These studies may perpetuate taxonomic confusion by directly ascribing classical species names to newly delineated genotypes, without returning to original type material (Langer, 2001; Etran et al., 2004; Pillet et al., 2011; Schweizer et al., 2011; Pillet et al., 2012; Pillet et al., 2013). This can result in taxonomic difficulties, as the nomenclatural history of the Elphidiidae family is so complex that discrepancies in the taxonomic names ascribed to newly delineated genotypes have arisen between the different taxonomic

investigations (Table 5.18). Notably, differences in the taxonomic names assigned to genotype S5 were identified. Pillet et al. (2013) ascribes the taxonomic name *E. excavatum* onto genotype S5, whilst Darling et al. (in prep.) follows the taxonomic designation of Feyling-Hanssen (1972). However, until there is formal taxonomic re-evaluation of both these original species concepts and type material, nomenclatural uncertainty remains.

It is also noteworthy that Pillet et al. (2013) did not come to a consensus on a single taxonomic name for the genotype *E. crispum – aculeatum* (genotype S10 in this study), as they recognised that some specimens within this genotype exhibited morphological traits resembling *E. crispum*, whilst other specimens resembled *E. aculeatum.* In contrast, Darling et al. (in prep.) assigned these two taxonomic names to two distinct genotypes (one of these genotypes was not sequenced by Pillet et al., 2013). Additionally, whilst Darling et al. (in prep.) ascribed the name *E. macellum* to genotype S12, Pillet et al. (2013) rather unusually assigned this name to a new Patagonian genotype.

These discrepancies in the taxonomic names assigned to different genotypes by recent taxonomic investigations highlight the necessity to reconcile new taxonomic evidence with original type descriptions and material (an example of which is illustrated in Chapter 6). Until formal taxonomic re-assessments can be conducted, a taxonomic protocol should be implemented to avoid reintroducing historical taxonomic confusion onto newly delineated genotypes. These genotypes should first be individually numbered and then a potential taxonomic name should be ascribed. These protocols were employed by Hayward et al. (2004), Darling et al. (in prep.) and Bird et al. (in prep.). This taxonomic protocol allows for the nomenclature of a genotype to be easily untangled if emendation was required in light of new taxonomic evidence.

Table 5.18 Proposed taxonomic names ascribed to each genotype in Darling et al. (in prep.) and their corresponding taxonomic name in Pillet et al. (2013). This table also includes two genotypes that were sequenced by Pillet et al. (2013) from outwidth of the study area. Table taken from Darling et al. (in prep.)

Genotype identified in Darling et al. (in prep).	Proposed species names (Darling et al., in prep).	Proposed 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 excavatum* (Terquem) forma *clavata* (Cushman, 1930)	*Elphidium excavatum clavata*
S5	*Elphidium excavatum* (Terquem) forma *selseyensis* (Heron-Allen and Earland, 1932)	*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	*Elphidium margaritaceum* 2
S19	Not sequenced	*Elphidium asklundi* (Brotzen, 1943)
S20	Not sequenced	*Haynesina nivea* (Lafrenz, 1963)
S21	Not sequenced	*Elphidium frigidum* (Cushman, 1933)
S22	Not sequenced	*Elphidiella groenlandica* (Cushman, 1933)
Patagonia	Elphidium (unnamed)	*E. macellum* (Fichtel and Moll, 1798)
Canada	Not sequenced	*Haynesina orbiculare* (Brady, 1881)

Not all of the Elphidiidae genotypes identified in the NE Atlantic can be provisionally ascribed taxonomic names e.g. genotypes S14 and S15 remain unassigned (Darling et al., in prep., Table 5.18). Whilst Darling et al. (in prep.) identified a live specimen from the Bay of Naples which exhibited similar key morphological traits to that of genotype S14 (Sgarrella and Zei, 1993), this specimen was classified under the blanket term *Elphidium* sp. Thus, genotype S14 has yet to be formally described in the literature. Moreover, trying to assign a taxonomic name to genotype S15 was more complex. Originally, Darling et al. (in prep.) associated both genotypes S7 and S15 to the classical taxonomic species concept (and name) *Elphidium albiumbilicatum* (Weiss, 1954). However, the taxonomic name was ascribed to genotype S7, as this genotype displayed characteristic star shaped ornamentation, which is a key diagnostic criterion of this species. As Darling et al. (in prep.) could not assign a taxonomic name to either genotypes S14 or S15, this indicates that both of these genotypes are probably new species and requires formal description. A recent debate in the broader taxonomic community calls into question whether new species concepts can be constructed from singletons (Dayrat et al., 2005). This in turn calls into question the validity of erecting a formal taxonomic description from a single specimen of S15. However, as rarity is part of nature, it has been argued that singletons should be recognised in order to encapsulate diversity (Lim et al., 2012). Further analysis of the genetic and morphological boundaries of genotype S15 would be beneficial to enable better delineation of this genotype and to clarify that there are no gradational morphological boundaries/ cryptic species.

5.4.8 Implications for palaeoenvironmental reconstructions.

Palaeoenvironmental reconstructions ultimately depend on the stability of the taxonomic framework employed. For example, the computation of palaeoenvironments in transfer functions is underpinned by an understanding of the relative abundance of morphospecies and their spatial correlation to modern environmental conditions (Gehrels, 2000). However, owing to the nomenclatural chaos surrounding the Elphidiidae family, in order to retain taxonomic consistency species were often lumped together. For example, Sejrup et al. (2004) amalgamated all morphospecies of *Elphidium excavatum* including historically cold water morphospecies (*E. excavatum* forma *clavata*) with warm water morphospecies (*E. excavatum* forma *selysensis*) under the blanket name of *Elphidium excavatum*.

This study provides valuable insights into interspecific morphological boundaries and biogeographic distributions within the Elphidiidae family, therefore providing a strong

taxonomic platform for use in applied taxonomic situations. The clarification of taxonomic relationships within the Elphidiidae family is unlikely to have a significant effect on current transfer functions (Sejrup et al., 2004; Horton and Edwards, 2005) because the majority of specimens can be unambiguously delineated using their morphological traits. Additionally, the Elphidiidae genotypes exhibit extensive and overlapping species ranges (Figure 5.20). However, it should be acknowledged that future elucidation of interspecific boundaries as well as an understanding of their ecological preferences could enable the refinement of species-specific geochemical proxies in the future. Further investigation is needed to clarify species-specific responses (both geochemical and biological) to different environmental conditions, which would help to improve and refine palaeoenvironmental reconstructions.

5.5 Conclusions

This study represents the first investigation to reconcile molecular evidence with quantitative morphological assessments in order to untangle the historically complex taxonomy of the Elphidiidae family. The results suggest that for the most part molecules and morphology can be successfully reconciled to create a new taxonomic framework from which to test and validate species boundaries. However, a limited number (n=5) of partially cryptic specimens were also identified between the end members of genotypes S1 and S2, and genotypes S16 and S17. The broad biogeographic sampling regime employed by this study has also enhanced the understanding of the biogeographical occurrences of Elphidiidae genotypes through significantly increasing the known biogeographic distributions and species ranges. It is evident that these species exhibit overlapping ranges, and extensive sympatry.

The extensive sampling regime across the NE Atlantic also provided an unprecedented opportunity to analyse morphological variability across a range of distinctive biogeographical zones. The two case studies conducted on *Elphidium* genotypes S1 and S4 represent the first quantitative analysis of intraspecific morphological variation with this taxonomic family. No clear intraspecific trends related to site locality were identified within genotype S1. In contrast, subtle morphological differences were identified between specimens of genotype S4 from different site localities. However, these data are constrained by the low numbers of specimens analysed at each site locality. Further investigation is warranted to elucidate the controls of phenotypic variability and particular emphasis should be placed on clarifying how ontogeny and different ecological conditions shape the intraspecific morphological patterns observed. Additionally, future investigations should also focus upon trying to reconcile the new lines of

taxonomic evidence presented in this chapter to the classical taxonomic framework so that these discoveries can be easily communicated, thereby maximising the value of elphidiids in applied taxonomic investigations.

Chapter 6

A new integrated approach to benthic foraminiferal taxonomy: the fusion of morphological and molecular systematics with type material

The sampling of elphidiid specimens across the NE Atlantic for this chapter was a collaborative effort between this researcher and researchers from within a larger NERC- funded project (NE4/G018502/1). The unpublished genetic framework used in this chapter was provided by Dr Clare Bird, Dr Magali Schweizer, Dr Kath Evans and Professor Kate Darling (Darling et al., in prep.)

Chapter 6: A new integrated approach to benthic foraminiferal taxonomy: the fusion of morphological and molecular systematics with type material

6.1 Introduction

The first formal classification system of the foraminifera was proposed in 1826 by D'Orbigny, and since then their identification and delineation as distinct species has been the subject of continued and active enquiry. Despite, or perhaps because of, numerous taxonomic studies spanning nearly 200 years, the current status of benthic foraminiferal taxonomy is one of near chaos. For example, an estimated 10-25% of modern benthic foraminiferal names have been suggested to be synonyms (Murray, 2007). Traditionally, specimens of benthic foraminifera have been classified based on a comparative assessment of differences in morphological characteristics. These morphological species concepts are formally situated and constructed around name-bearing type specimens. Type specimens allow an objective application of the species name and provide a standard of reference by which the application of that name can be determined (ICZN, 1999). Therefore, this approach offers representative examples of a morphological species concept which allows users a set of objective reference points when analysing specimens of unknown taxonomic affinity (Scott, 2011). In practice, one of the principal taxonomic problems faced is the significant level of morphological plasticity exhibited in certain taxonomically important features of the foraminiferal test (as evidenced in Chapters 3 and 5). This has led to erroneous and inconsistent species identifications, particularly between closely related species where these problematic morphological boundaries are often poorly defined (Miller et al., 1982, Holzmann, 2000, Hayward et al., 2004).

There are currently very few established quantitative morphological frameworks from which one can consistently identify and place a specimen into a well-defined species concept (Buzas, 1966, Patra, 2000). This has led to the prevalent use of an open nomenclature (i.e. 'lumping'), leading to the potential merging of species based upon broad morphological features. This is particularly problematic with the assignation of juveniles (Murray, 2007), where their morphologies differ from those of the adult form. The occurrence of numerous polymorphic species incorporating a range of gradational diagnostic features, inevitably leads to erroneous species identification. This in turn introduces error into foraminiferal-based environmental reconstructions (Sejrup et al., 2004, Horton and Edwards, 2005), some of which underpin the physical science basis for our current understanding of climate change (Stocker et al., 2013).

In order to exploit their impressive and exceptionally long fossil record, it is vital that both extant and fossil foraminifera can be unambiguously attributed to an established and stable taxonomic nomenclature. Only within the confines of such a system can the true taxonomic affinities and biogeochemical, genetic and morphological properties of a valid species be communicated within the academic literature (Bowker, 2000; Waterton et al., 2013). Palaeoenvironmental research in particular requires a strong taxonomic platform, since the corner stone of most studies relies on a comparative analysis of modern and fossil species compositions. Our understanding of the ecological niches and biogeographical distributions of modern species can then be applied in time and space (Murray, 2001; Gooday and Jorissen, 2012). Erroneous species identifications have the potential to undermine the credibility of research, leading to flawed current and future research agendas (Bortolus, 2008, Dayrat, 2005, Ebach, 2011). Such problems lead us to question the degree of stability and reliability in the current, morphology-based species concepts practiced in foraminiferal research. It is imperative that a more robust and stable morphology-based taxonomy is developed and adopted.

Over the past 20 years, the focus of taxonomic endeavour has been moving away from classical morphology-based taxonomy to concentrate on molecular systematics. Molecular approaches using typically a fragment of the SSU ribosomal RNA gene have enabled the genetic characterisation of single specimens of foraminifera (Pawlowski and Holzmann, 2014). The extensive genetic data now available highlights the limitations of a taxonomy built purely upon the descriptions of test morphology. For example, genetic analysis has enabled the delineation of many phylogenetically separate species which cannot currently be morphologically discriminated e.g. cryptic species e.g. Holzmann, 2000; Darling and Wade, 2008. The potential presence of cryptic diversity has significant implications for the interpretation of palaeoenvironmental records, because faunal analyses which comprise an amalgamation of cryptic genotypes, will compromise the degree of precision in faunal reconstructions (Darling and Wade, 2008).

Whilst molecular systematics is widely acknowledged as an important tool for re-examining species level relationships in the living assemblage, it does not provide sufficient evidence alone for its application to the fossil assemblages. Individual fossil specimens cannot be directly tested using molecular techniques and can only be practically delineated based on their test morphology. Prior to the development of molecular systematics, the morphological approach to taxonomy in the fossil record, though largely robust, could not resolve many of the practical

taxonomic problems faced by the benthic foraminiferal community. Over-reliance upon these singular methods of delineation, be it molecular or morphometric, comes with significant limitations. The tools are now available to combine these different lines of taxonomic evidence to provide an integrated approach to taxonomy in the fossil record.

An integrated foraminiferal taxonomic framework offers the potential to test species boundaries, allowing the development of a framework which can be consistently applied. A recent suite of papers have successfully utilised an integrated molecular and morphological approach to delineate between species to revise and redefine many benthic foraminiferal taxonomic positions (Holzmann, 2000, Hayward et al., 2004, Schweizer et al., 2005, Schweizer et al., 2011b, Pillet et al., 2012, Pillet et al., 2013, Holzmann et al., 1998, Tsuchiya et al., 2008). Despite considerable technological advancements in imaging techniques over the past 20 years, there has been limited progress in quantitatively delineating species based upon their morphology, since many of the aforementioned studies placed their emphasis on genetic delineations with qualitative morphological descriptions. Many of the recent combined taxonomic studies, regardless of the current evidence for taxonomic confusion, continue to attach classical taxonomic names to newly delineated genotypes, nearly always without reference to the original type material. However, this approach carries the inherent danger of reasserting the cumulative taxonomic confusion associated with the historical, sometimes tortuous, synonymy of a morphology-based taxon concept to the newly delineated genotype.

Owing to their fossil record, it is imperative that there is consistency within the nomenclature that is currently applied to the morphological concepts of foraminifera. In order to connect the present to the past, it also essential that taxonomic delineations based upon molecular systematics are situated within the same taxonomic framework. However attractive this latter approach might seem, there is no context from which to communicate effectively these delineations and any attempts to name these genotypes without reference to a morphology-based classification scheme would likely compromise the rules of nomenclature set out by the International Commission on Zoological Nomenclature (ICZN, 1999).

This chapter sets-out a new taxonomic framework from which the recent developments in molecular systematics can be reconciled with traditional morphology-based taxonomy. The aim is to test the classical descriptive taxonomic species concept with quantitative morphological measurements and an independent DNA-based component, utilising both museum type specimens and topotypic specimens, e.g. specimen originating from the type locality of the

species or subspecies to which it is thought to belong (ICZN, 1999). This chapter will establish for the first time, a secure method whereby the formal taxonomic nomenclature of the type material can be mapped onto morphologically characterised topotypic specimens whose contemporary genotype is established.

In order to achieve these goals, the morphology-based taxonomic concept of *Elphidium williamsoni*, Haynes, 1973 and *Polystomella umbilicatula,* Williamson, 1858 type specimens and descriptions were compared with the morphometric and allied molecular identity of contemporary topotype specimens. In addition, the type and topotypic material were compared against the contemporary specimens of the same genetic type collected from across the NE Atlantic sites (as analysed in Chapter 5). The aim is also to establish whether or not a common molecular signature exists within the morphometric concept of *E. williamsoni*. At the same time, this study defines the quantitative morphological boundary of *E. williamsoni*, in comparison to other *Elphidium* species which have previously been associated, or even confused with the original *E. williamsoni* species concept.

The overall aim is to allow an objective assessment of morphology which can be statistically evaluated to determine if any given specimen be it fossil or contemporary conforms to the original morphological concept of *E. williamsoni*.

6.2 Materials and Methods

6.2.1 Material collection

6.2.1.1 Topotype specimens

Contemporary live topotypic specimens were collected from Haynes' original *E. williamsoni* type site location along the Clettwr transect, Dovey Marshes, Wales (Site locality 17, Figure 5.3) (Haynes and Dobson, 1969; Haynes, 1973; Haynes, per. comms. 20th February 2013). Surface sediment samples (upper 1 cm) were collected by hand with a scraper during a low tide on 28th March 2013. These samples were processed as follows: specimens were examined under a stereomicroscope and potential living specimens were distinguished by the natural colouration of the protoplasm and were extracted from seawater using a fine paintbrush. These pre-screened specimens were placed in clean seawater and subsequently examined to establish if there was any pseudopodial activity, such as the overnight formation of sediment cocoons around the test or the movement of specimens from a predefined position (as detailed in Chapter 2, Section 2.3.1). Once the live specimens were identified, they were picked, dried and

mounted prior to scanning electron microscopy (SEM) imaging. In total 75 live elphidiid specimens were SEM imaged at the University of St Andrews by this researcher, whilst 20 specimens were sent to the University of Edinburgh foraminiferal genetics laboratory to be imaged by project collaborators.

At the University of Edinburgh, following SEM imaging, the 20 specimens set aside for molecular analysis were individually crushed for DNA extraction and genetic characterisation using the small subunit ribosomal RNA gene (Darling et al., in prep). In total, 20 genetically characterised topotypic specimens were examined and 75 specimens were represented only by SEM images. All the contemporary topotypic specimens collected from the Dovey Marshes were genetically characterised as belonging to the distinct genotype *Elphidium* S1 (Darling et al., in prep.), which has also been widely identified across three biogeographic provinces in the North East Atlantic (Chapter 5, Figure 5.20). This genetic type has previously been deposited in GenBank by Langer (2001), Ertan et al. (2004), Pillet et al. (2011), Grimm (unpublished) and Pillet et al. (2013).

6.2.1.2 Type Material

The type material was obtained on loan from the Natural History Museum London (NHM) in March 2013, these specimens consisted of *Elphidium williamsoni,* Haynes, 1973 (NHM Reference Number: Slide 1970: II: 26:431-42 (10 paratypes) and Stub 1970: II: 26:597 (holotype)) and *Polystomella umbilicatula*, Williamson, 1858 (NHM reference number: 96.8.13.16 (n=25)). These valuable reference materials were unavailable for normal SEM analysis, as this would have required gold coating of the specimens. Therefore, these specimens were imaged using an environmental SEM (ESEM) at Herriot Watt University (April 2013).

6.2.1.3 Contemporary specimens collected from across the North East Atlantic

Contemporary specimens collected from across the North East Atlantic (Chapter 5, Figure 5.3 detailed in Table 5.2) were also included within this study for the comparison of the morphological attributes holotype, and topotypic material to the natural variability of *Elphidium* specimens found within the North East Atlantic.

6.2.2 Morphological analysis

6.2.2.1 Quantitative morphological analysis

Morphological analysis was conducted on the contemporary topotype material and type material. To investigate the morphological similarity between specimens, a combination of 20 morphometric and categorical variables were acquired from the SEM images available (Table 6.1). This reduced subset of morphological features assessed (in contrast to Table 5.3) were chosen because the morphological characters measured were derived from and are intended to quantify the key diagnostic features which were included in the original species description and diagnosis of *Elphidium williamsoni* by Haynes (1973, p. 207):

Diagnosis: "A rotund species of *Elphidium* with rounded periphery and slight, rather flat umbilicus on each side filled with irregular ends of the chambers. Fossettes and septal bars well developed, reaching about eight or nine in number on each side and covering about half of the chambers. Up to 14 chambers visible. Wall smooth with relatively sparse tubercules within the septal pits and at the base of the apertural face".

Description: "Test semi-inflated, slightly umbilicate with rounded periphery, entire becoming semi-lobate at the last few chambers- chambers arranged in an involute planispire, 13 visible, slowly increasing in size with marked septal pits (fossettes) increasing from six to eight or nine on each side (ten on third chamber from the last), strong, narrow septal bars almost equal in length to rest of each chamber, in one case (on the last chamber) with a proximal opening, pits lozenge shaped, tuberculate within; septal sutures flush- not visible; wall radial, finely perforate, pores rather less than 1 micron in diameter, tuberculate below the apertural face; aperture a series of irregular openings along the basal suture of the last chamber, linking with pits of the first exposed chamber".

In order to standardise the measurements, the morphometric measurements were taken from SEM side views of the test. A selection of SEM images including the holotype and paratype specimens of *E. williamsoni,* type material of *P. umbilicatula* and contemporary sequenced specimens were chosen to highlight and encapsulate the range of interspecific and intraspecific morphological variability that has been captured in this study as shown in Figure 6.1.

Table 6.1 *Elphidium* test characters measured or assessed and used in morphometric analysis. N= last chamber, N1= penultimate chamber etc. as depicted in Figure 2.7

Type of character	Name	Variable Number	Method of measurement	Unit/ Category/ Type
Morphometric	Maximum test diameter	1	Maximum diameter of test parallel to axis of coiling	Not directly analysed in this study
Morphometric	Maximum width of chambers in the penultimate chamber (N1)	2	Maximum diameter of penultimate chamber calculated by the boundaries of the sutures (i.e. from the end of one suture to the end of the next suture/maximum test diameter	Ratio
Morphometric	Average septal bar height in the suture between chambers N1 and N2	3	Average diameter of the first three septal bars (if present) from the umbilical area towards the periphery edge of the foraminifera	Micrometres
Morphometric	Relative difference in the width of the septal bar to the rest of the chamber	4	Difference between the width of septal bar in comparison to rest of the chamber	Ratio
Morphometric	Relative width of sutural furrow in the penultimate chamber (N1)	5	Width of the sutural furrow in the penultimate chamber at the umbilical region/ width of the sutural furrow at the periphery of the test	Ratio
Morphometric	Sutural angle along the interseptal space between the chambers N1 and N2	6	The curvature of the suture between the final and penultimate chamber is calculated using the arc measure tool in Image Pro Express	Degree
Morphometric	Total number of chambers	7	Number of chambers visible in the final whorl/ maximum test diameter	Ratio

Table 6.1 continued.

Type of character	Name	Variable Number	Method of measurement	Unit/ Category/ Type
Morphometric	Total number of septal pits	8	Number of complete septal pits (defined and bounded by two septal bars) / maximum test diameter	Ratio
Morphometric	Roundness of the foraminiferal test	9	As calculated from the outline of the entire shape:4*area/(π*major_axis^2)	0-1
Morphometric	Average roundness of the septal pit	10	Mean roundness of the pit averaged across the foraminifer :As calculated from the outline :4*area/(π*major_axis^2)	0 -1
Morphometric	Relative proportion of septal pit area to rest of the chamber	11	The relative proportion is calculated by total foraminiferal area/ total area of the septal pits	Ratio
Morphometric	Ratio of unconstricted sutures to constricted sutures	12	Number of unconstricted sutures/ number of constricted sutures	Ratio
Morphometric	Ratio of width of suture on the penultimate chamber measured at the umbilical area against width of suture at the periphery of the test	13	Width of suture from the umbilical area/ width of the sutural furrow at the periphery of the test.	Ratio
Morphometric	Relative proportion of apertural ornamentation covering the first visible chamber	14	Percentage coverage of the first visible chamber by apertural ornamentation	Percentage
Morphometric	Incomplete sutural bridge development	15	Number of septal bars which do not completely cross the suture	Count

Table 6.1 continued.

Type of character	Name	Variable Number	Method of measurement	Unit/ Category/ Type
Morphometric	Number of umbilical bosses	16	Number of bosses in the umbilical area/maximum diameter	Count
Categorical	Porosity (strength)	17	Strength of width of pores. Ten pores were analysed at junction between chambers N1 and N2. The average width of these pore measurements was then calculated and this average was then grouped into one of three categories: fine pores< 1 µm, medium pores 1-2 µm or large pores > 2 µm	Fine-1 Medium-2 Coarse-3
Categorical	Degree of apertural ornamentation	18	Angularity of tubercles around the aperture	None-1 Very weak-2 Weak-3 Medium- 4 Strong-5
Categorical	Openness of the umbilical area	19	Openness of umbilical area	None-1 Very weak-2 Weak-3 Medium- 4 Strong-5
Categorical	Degree of ornamentation within the sutures (including pits)	20	Angularity and regularity of tubercules within sutures	None-1 Very weak-2 Weak-3 Medium- 4 Strong-5

All morphological measurements from a standard side view. A combination of the Image Pro Express and ImageJ v.1.47 software (Abràmoff et al., 2004) were used to collect the morphometric measurements. Infilling procedures following the methodology of Hayward et al. (2004) were utilised when morphological characters were obscured by debris or presence of a broken test, this accounted for 0.21% of the total features measured. The morphological matrix was standardised by ranging the variation between each character from 0 to 1, following the methods set out by Hayward et al. (2004).

For the purpose of investigating the morphological distinctiveness and interspecies variability, two morphologically similar yet genetically distinct outlier groups were utilised in the morphological analysis (*Elphidium* genotypes S4 and S5 from Darling et al., in prep.). These groups were chosen because based on traditional taxonomic concepts, their morphological characteristics have previously been confused with the *Elphidium excavatum* (Terquem) complex, as a result *E. williamsoni* has been previously named *E. excavatum* (Feyling-Hanssen, 1964; Haake, 1962; Haynes and Dobson, 1969; Cushman, 1930; Cushman, 1939; Cushman, 1949; Todd and Low, 1961; Brodniewicz, 1965; Adams and Frampton, 1965; Murray, 1965). This morphospecies has also been considered a subspecies of *E. excavatum*, under the name of *E. excavatum williamsoni* (Hayward et al., 1997; Gross, 2001).

To explore the potential range of morphological variation captured by the museum type material and the contemporary topotype specimens, a further 213 specimens of the same genotype collected from across the NE Atlantic shelf seas were also morphologically examined (Figure 6.1).

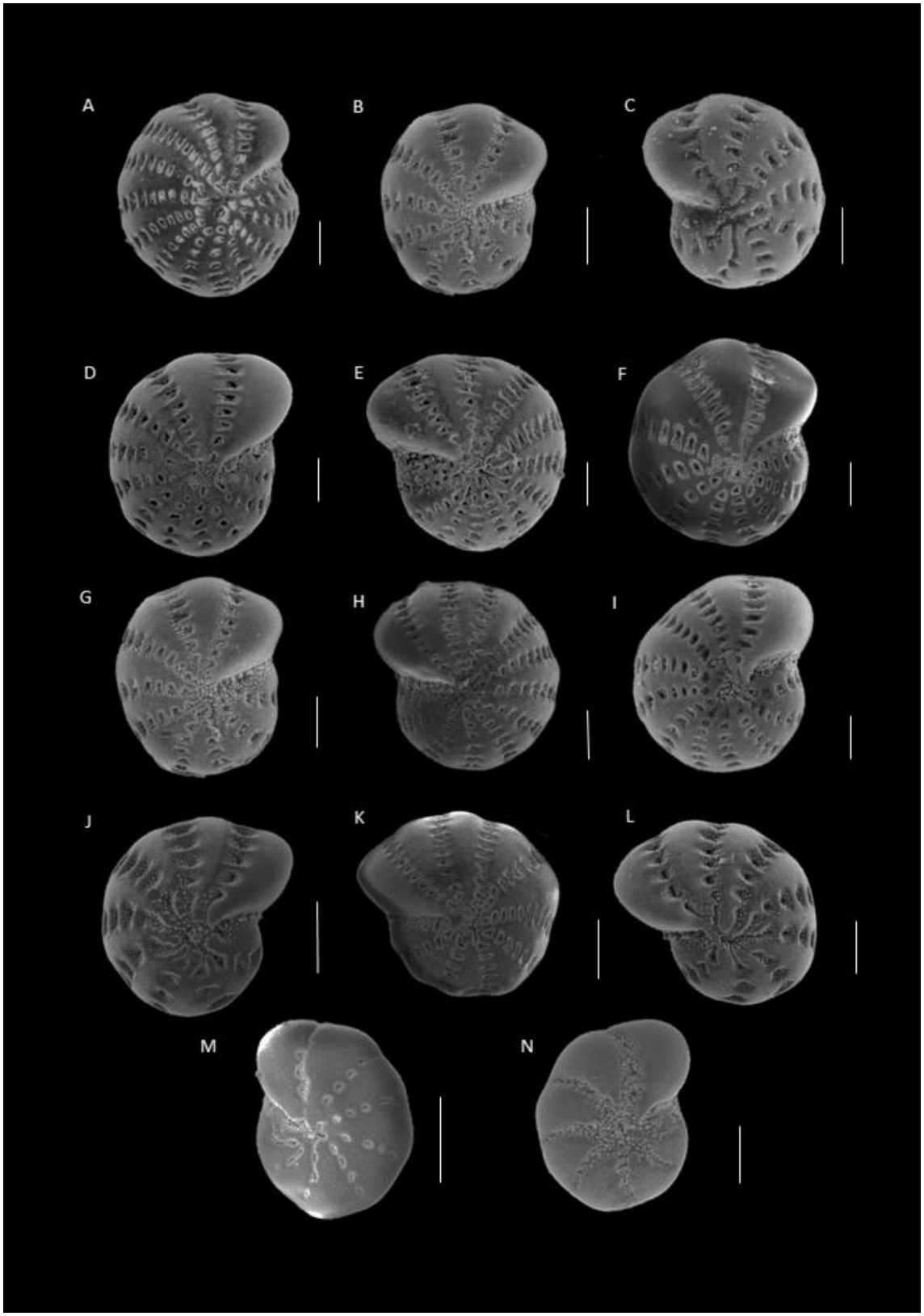


Figure 6.1 SEM and ESEM pictures of (A) *Elphidium williamsoni* Haynes, 1973 (Holotype specimen), (B-C) *Elphidium williamsoni* (paratypes), (D-F) Contemporary topotypic sequenced specimens, (G-I) *Polystomella umbilicatula,* Williamson, 1858, *(J-L) Elphidium* genotype S1 specimens collected from across NE Atlantic, (M) *Elphidium* genotype S4 *and (N) Elphidium* genotype S5. Scale bars correspond to 100 µm.

6.2.3 Data analysis

The morphological data was analysed using a Principal coordinate analysis (PCO), UPGMA cluster analysis, discriminant function analysis (DFA) and CART analysis which were performed using a combination of PAST v.13 (Hammer and Harper, 2006), SPSS v.22 and dendroUPGMA software (Garcia-Vallvé and Puigbo, 2010). To reduce the dimensionality of the dataset, a PCO was performed upon all the morphometric characters collated from the contemporary topotypic material and the NHM type series collections. In addition, a second PCO analysis was performed, whereby an additional 213 genotyped specimens from the NE Atlantic were added into the analysis.

An unweighted pair-group Q mode cluster analysis (UPGMA) using arithmetic averages of Euclidean distance was used to generate a cluster diagram of the morphological relationships between the topotypic material, NHM type material, the additional N. Atlantic specimens of the same genotype and the genetically distinct *Elphidium* outliers.

Finally, a discriminant function analyses was derived from the results of the standardised dataset to establish the key diagnostic criteria which can be used to reconcile molecules and classical type concepts in order to aid classification of specimens into each genetically distinct groups. The robustness of the assignment is assessed through a resampling cross-validation procedure in SPSS v.22.

6.3 Results

6.3.1 Morphological analysis

6.3.1.1 Morphological differentiation between type and topotypic material

The results from the UPGMA cluster analysis and the PCO analysis, shown in Figures 6.2 and 6.3 illustrate that Haynes' original type description and species concept can be reconciled with the contemporary topotypic material.

A PCO of the assessed morphological characters was utilised to determine the relationship between the morphology of the topotypic material from Aberdovey Wales and the morphology of the type material from the NHM. The results of the PCO indicate that there is morphological congruence between the type and topotypic material. Most of the variation common to all of these forms is described by the first two principal coordinates (PC) which account for 63.4% of the total variance. The results illustrate that there are three morphologically distinct clusters of specimens and that the type and topotypic material are strongly segregated from the genetically distinct *Elphidium* S4 and S5 outlier specimens. Moreover, it can be demonstrated from the 95% confidence envelopes that Haynes' 1973 type material, including the holotype is situated within the centre of the morphospace occupied by the contemporary topotypic specimens sampled in 2013. However, it should be noted that there is some morphological overlap between the genetic outlier groups as evidenced by the 95% confidence envelopes; this is due to seven outlier specimens, which do not cluster with the majority of the *Elphidium* genotype S4 specimens in the PCO morphospace.

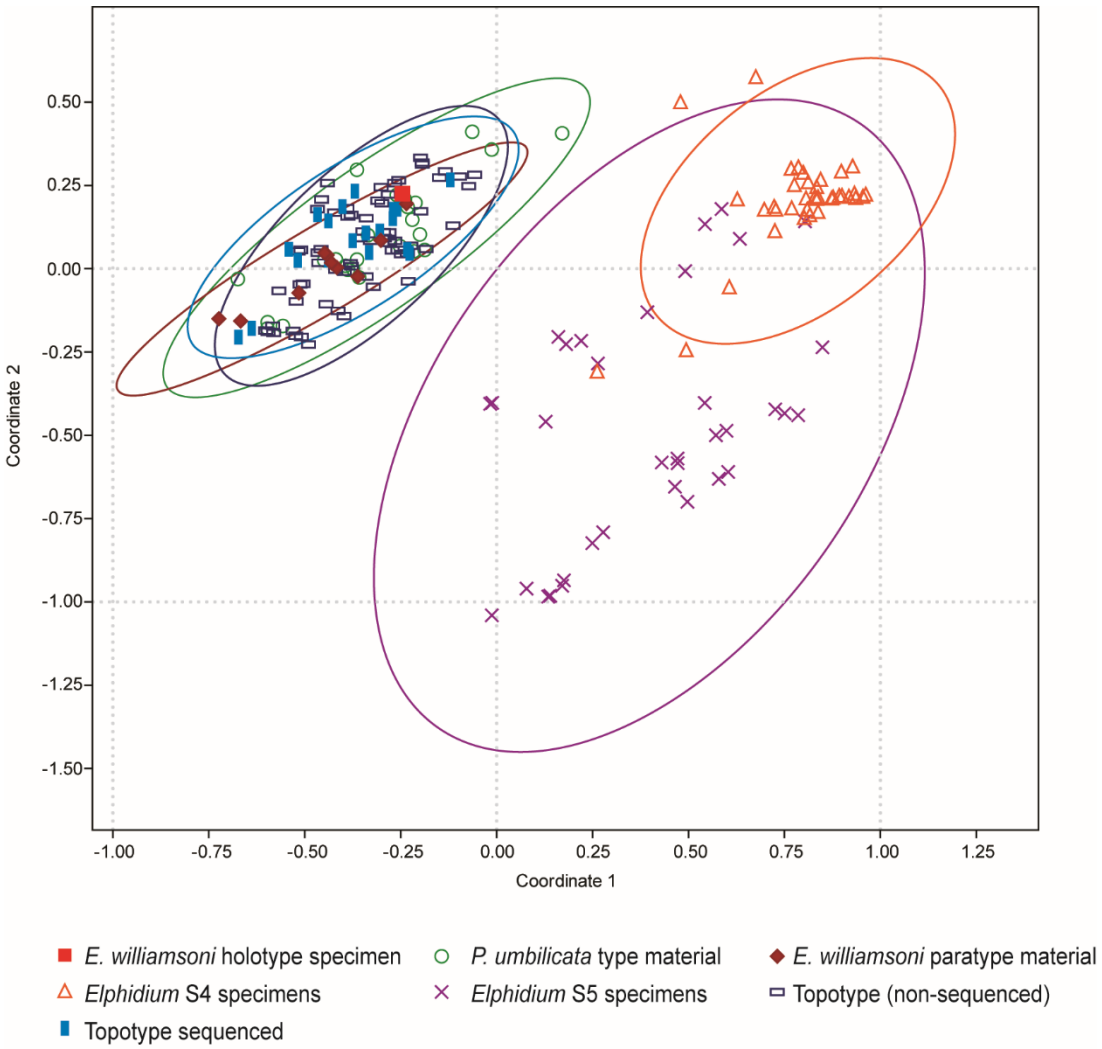


Figure 6.2 Bi-plot of the PCO analysis based on the morphological characters of *Elphidium williamsoni* Haynes, 1973 Type specimens, contemporary topotypic material, *Polystomella umbilicatula* Williamson, 1858 type material and the two outlier species *Elphidium* S4 and *Elphidium* S5. These groups are bounded by 95% confidence intervals. The first two principal coordinates account for 63.4% of the total variance.

The results from the UPGMA cluster analysis (Figure 6.3) confirm the results from the PCO analysis, that the type and contemporary topotypic specimens are morphologically distinct from the genetic outliers. Overall, the UPGMA cluster analysis highlights that three main morphological groups can be determined, despite some morphological overlap of four incorrectly clustered specimens between the two genetically distinct outlier groups (Figure 6.3).

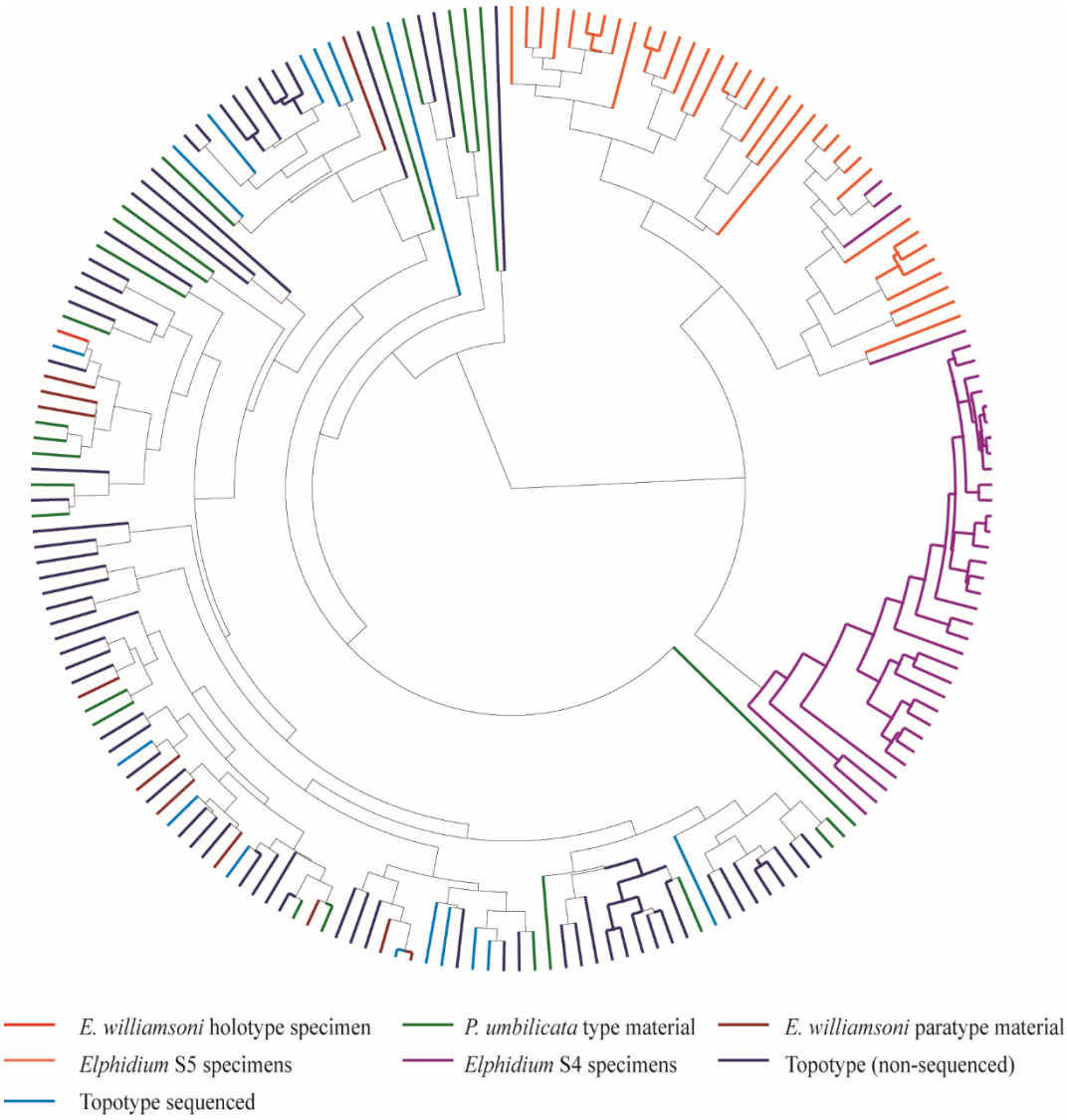


Figure 6.3 UPGMA cluster analysis tree based on the morphological characters of *Elphidium williamsoni*, Haynes, 1973, contemporary topotypic material, *Polystomella umbilicatula*, Williamson, 1858 type specimens and the two genetic outlier specimens *Elphidium* genotypes S4 and *Elphidium* S5.

6.3.1.2 Multivariate analysis between topotype, type material and other specimens across the North East Atlantic

In order to determine whether the full extent of morphological variability of *E. williamsoni* has been captured from the type material of Haynes' 1973, the morphological attributes of the topotypic and type material were compared and analysed against the morphology of 213 *Elphidium* genotype S1 specimens, collected from across the North East Atlantic (as detailed in Chapter 5, Table 5.2).

Haynes' 1973 type specimens of *E. williamsoni* falls within the morphological variability of all the genotyped *Elphidium* S1 material collected from across the North East Atlantic as illustrated

from the 95% confidence envelopes (Figure 6.4). The results indicate that the genetically distinct outlier groups clearly separate themselves from the type and topotypic material.

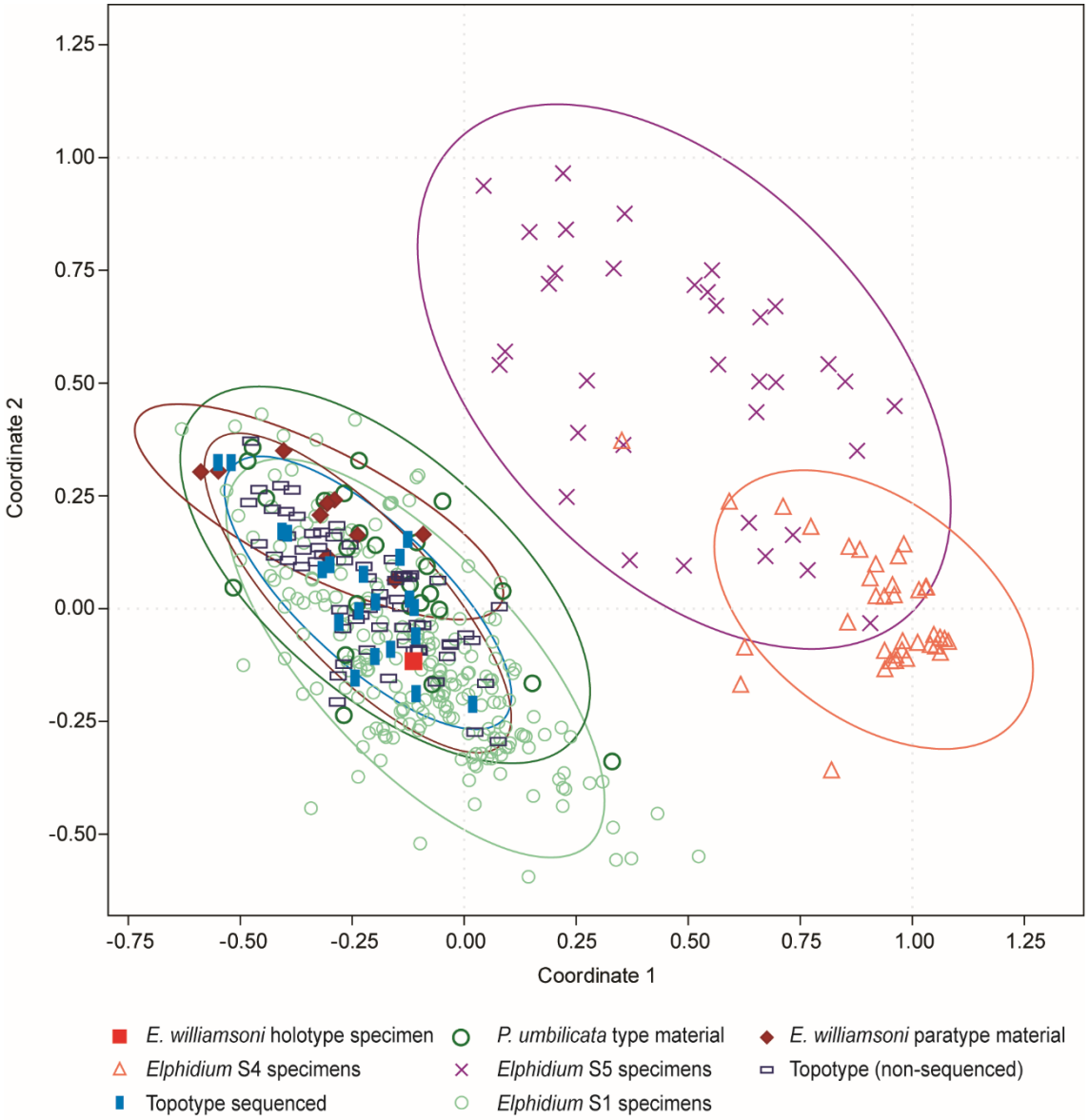


Figure 6.4 Bi-plot of the PCO analysis based on the morphological characters of *E. williamsoni* Haynes, 1973 type specimens, *Polystomella umbilicatula*, Williamson, 1858 type specimens, contemporary *Elphidium* genotype S1 and the two outlier species *Elphidium* genotypes S4 and *Elphidium* S5. These groups are bounded by 95% confidence intervals. The first two principal coordinates account for 52.0% of the total variance.

The results from the UPGMA cluster analysis (Figure 6.5) highlights that three genetically distinct forms can be separated based upon their morphology. Although it also evidenced that there is some morphological overlap amongst the *Elphidium* genetic outlier specimens. Figure 6.5 also indicates that the topotypic specimens are situated across multiple clusters, suggesting that this material has captured a significant proportion of the morphological variability exhibited by *E. williamsoni* from across the North East Atlantic.

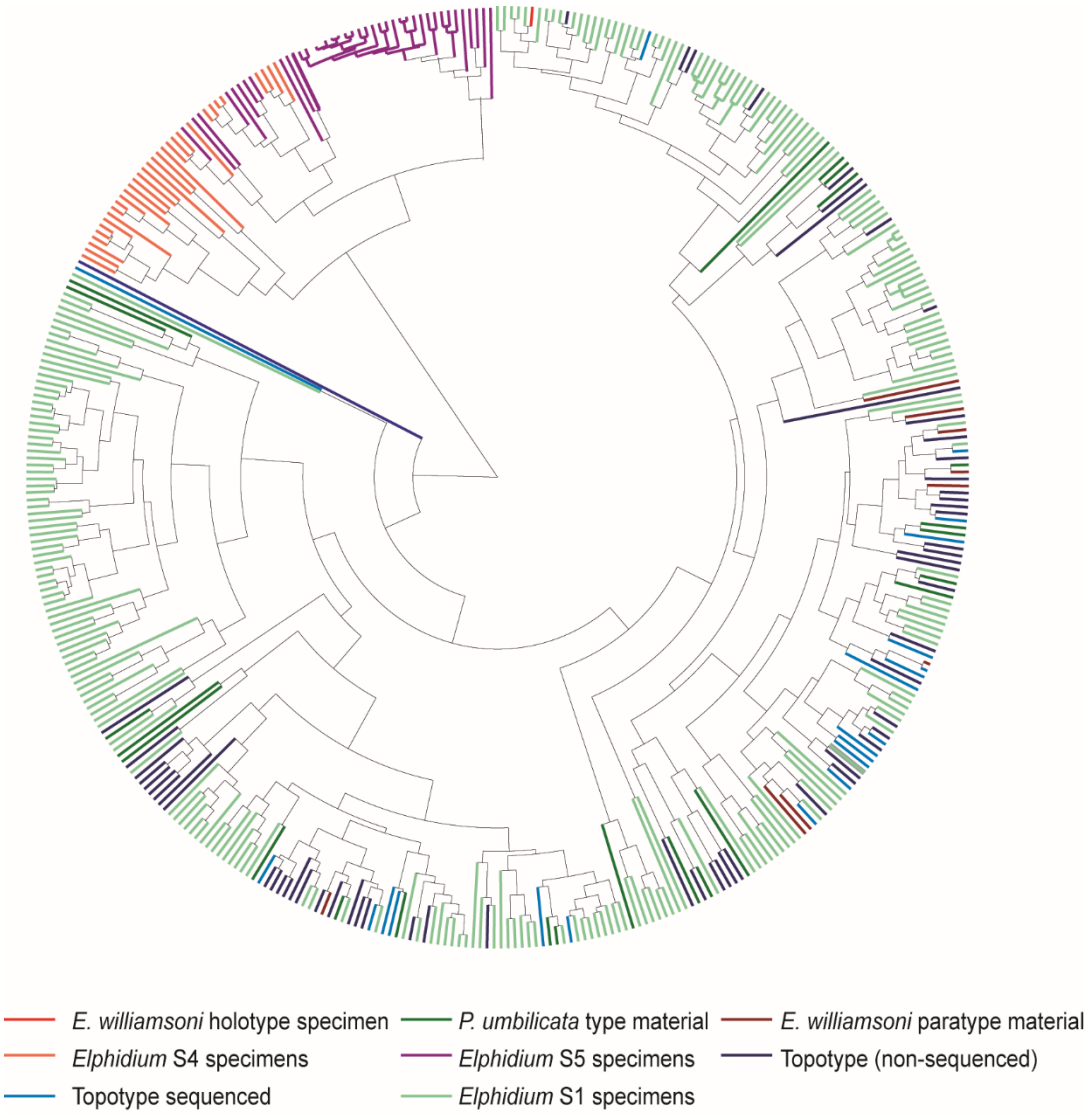


Figure 6.5. UPGMA cluster analysis tree based on the morphological characters of *Elphidium williamsoni* Haynes, 1973 type, specimens, *Polystomella umbilicatula* Williamson, 1858 type specimens, contemporary *Elphidium* S1 specimens collected from across the North East Atlantic shelf seas and the two genetic outlier groups *Elphidium* S4 and *Elphidium* S5.

6.3.2 Morphological discrimination of *Elphidium williamsoni*

A DFA was performed on the dataset to identify key characters to aid classification of specimens into the genetically and morphologically assigned concept of *E. williamsoni.* To optimise the morphological interspecific discrimination of *E. williamsoni*, the DFA was performed utilising the genetic groups as the *a priori* groupings (type material is combined with the genotyped topotype material based on the results of the PCO and cluster analyses, Figures 6.4 and 6.5 respectively). The DFA showed good membership within the three genotypes, and the percentage of the total overall correct classification of specimens accurately assigned into the genetic groups is 99.8% and 99.5**%** after the cross validation procedure (Wilks: 0.21, p: <0.05). The results illustrate that

the specimens within *Elphidium* genotype S1 (including type and topotypic material) are distinct morphological entities from the genetic outlier groups (Table 6.2).

			DFA confusion matrix		
Genotype	DFA % correctly classified	CV % correctly classified	S1	S4	S5
S1	100	100	**347**	-	-
S4	97.1	94.3	-	**34** (33)	1 (2)
S5	100	100	-	-	**37**

Table 6.2 Percentage of specimens correctly classified into their respective genotype based on their morphological characteristics in the DFA and cross validation analysis. Also illustrated is the confusion matrix of the number of specimens correctly and incorrectly classified in the DFA and cross validation procedures. Numbers shown in brackets depict the results from the cross validation analysis.

Overall, the results of the DFA indicate that each genotype exhibits discrete interspecific diagnostic morphological characters (Table 6.2). These key morphological characters which delineate between these genetically distinct species include: Number of septal pits in the penultimate chamber (2) (S1: 0.0057-0.0600, S4: 0.0038-0.0403, S5: 0-0.025), openness of the umbilical area (19) (S1: 2-5, S4: 2-5, S5: 3-5), mean septal pit roundness (10) (S1: 0.25-0.61, S4: 0-0.64, S5: 0-0.85), ratio of septal pit area to the rest of the chamber (11) (S1: 0.06-0.667, S4: 0.00-0.383, S5: 0.00-0.102), average ratio of the septal bar to the rest of the chamber (4) (S1: 0.19-2.51, S4: 0.00-0.61; S5: 0.00-0.67), apertural ornamentation (18) (S1: 0-5, S4; 1-4 S5: 2-5) and porosity (17) (S1: 1-1; S4: 2-3, S5: 2-2). These quantitative morphological boundaries identified can be employed in the future as a model for the morphological recognition of *E. williamsoni.*

An additional CART analysis with 10 V-fold cross validation analysis was conducted and it illustrates that the three genotypes can be perfectly discriminated based upon morphology, and that this model is 99.95% accurate after 10 V-Fold cross validation analysis. CART analysis identified that porosity (17) (S1: 1-1; S4: 2-3, S5: 2-2), total number of septal pits (8) (S1: 0.04-0.53, S4: 0.03- 0.15, S5: 0-0.07), openness of umbilical area (19) (S1: 2-5, S4: 2-5, S5: 3-5), mean septal pit roundness (10) (S1: 0.25-0.61, S4: 0-0.64, S5: 0-0.85), sutural ornamentation (20) (S1: 1-5, S4: 1-5, S5: 3-5), maximum width of chamber N1 (2) (S1: 0.12-0.93, S4: 0.21-0.60, S5: 0.36-0.62), sutural angle between chambers N1 and N2 (6) (S1: 1.46-102, S4: 11.5-93.8, S5: 8.86-113)

and total number of chambers (7) (S1: 0.01-0.09, S4: 0.03-0.05, S5: 0.02-0.06) are key diagnostic characters.

6.4 Discussion

This study provides a new taxonomic framework (outlined in Figure 6.6) that integrates partial SSU rRNA sequences of contemporary topotypic specimens and quantitative morphometric analysis of type and contemporary topotypic material, to reconcile the morphological species concept to a distinct genotype. This study utilises Haynes' 1973 *Elphidium williamsoni* type material to implement this framework.

6.4.1 *Elphidium williamsoni* and the *Elphidium excavatum* complex

Elphidium williamsoni was chosen as the first benthic foraminiferal taxon for applying the integrated analytical approach for several reasons. It is used extensively in palaeoenvironmental studies(Horton and Murray, 2007; Kemp et al., 2011), particularly in proxy-based relative sea level (RSL) reconstructions due to its strong and quantifiable relationship within inter-tidal zones. Understanding the true intraspecific morphological variation within *Elphidium williamsoni* would enable comparative high-resolution environmental studies to be carried out throughout its biogeographic range. This has only recently become possible due to the large number of genotyped specimens with corresponding SEM images which were produced during an extensive biogeographical study in the North East Atlantic (presented in Chapter 5 and Darling et al., in prep.) which were made available for morphometric analysis. To complement this, Haynes' original type material of *E. williamsoni* was available for analysis from the NHM London and Haynes' original type site location was also recorded in detail and could be easily accessed Haynes, 1973; Haynes, pers. comm. 2013).

Resolving the taxonomic identity of *E. williamsoni* has always proved challenging because this taxon is situated within one of the largest and most morphologically diverse genera of benthic foraminifera. Delineating species within the *Elphidium* genus has posed a significant challenge to taxonomists due to the considerable amounts of intraspecific and interspecific variation exhibited in the key morphological characteristics. Thus, considerable taxonomic uncertainty has been conferred upon the species and as a consequence, its species concept has been subject to continued emendation. *Elphidium williamsoni* was originally described by Williamson (Williamson, 1858, p. 43) as *Polystomella umbilicatula* and was then later reclassified into the genus *Elphidium*. It was renamed *E. williamsoni* in Williamson's honour by Haynes in 1973. However, this species also has phenotypic similarities with other *Elphidium* species, which has

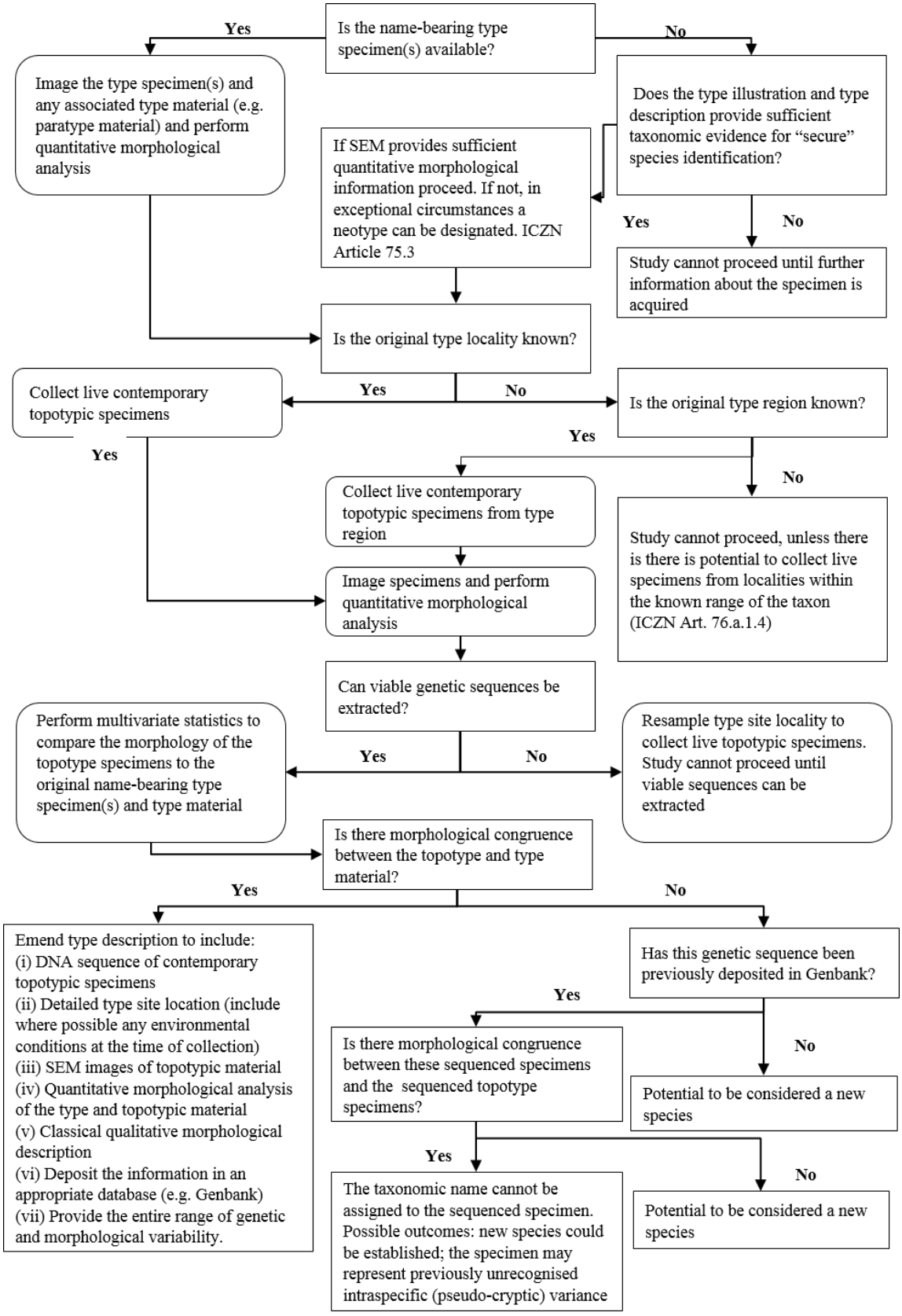


Figure 6.6 Taxonomic framework. The initial protocol requires (i) a candidate specimen with distinctive test morphology, (ii) the potential for DNA extraction (including fossil aDNA), (iii) a comprehensive and detailed literature review, which includes qualitative morphological comparison of the candidate specimen against the type descriptions and illustrations.

led to it being confused with *Cribrononion cf alvarezianum* (Lutze, 1965), *Polystomella striatopunctata* (Wilkinson, 1979), *Elphidium umbilicatulum* (Williamson, 1858), *Elphidium articulatum* Lévy et al., 1969) and as E. excavatum (Murray, 1979).

Elphidium williamsoni has also been considered as belonging to the *Elphidium excavatum* complex, and has consequently been previously named *E. excavatum* Cushman, 1930, 1939, 1949; Todd and Low, 1961; Haake, 1962; Feyling-Hanssen, 1964; Adams and Frampton, 1965; Murray, 1965; Brodniewicz, 1965; Haynes and Dobson, 1969). Furthermore, it has been considered a possible subspecies of *E. excavatum,* under the name of *E. excavatum williamsoni* (Hayward et al., 1997; Gross, 2001). The two genetically distinct outliers utilised/used for comparative morphological analysis in this chapter (*Elphidium* genotypes S4 and S5), represent two different morphotypes that have previously been regarded as members of the *Elphidium excavatum* complex. They were therefore specifically selected for inclusion within the analysis to definitively unravel the taxonomic confusion associated with the *Elphidium excavatum* complex discussed above.

6.4.2 Morphometric analysis

The integrated taxonomic, genetic and morphometric framework adopted here has enabled the verification of the robustness of Haynes' 1973 original taxonomic description and type material of *E. williamsoni* against the contemporary topotypic material. The results indicate that there is mutually supporting evidence for three genetically and morphologically distinct groups. It can also be demonstrated that there is also strong morphological congruence between the *E. williamsoni* type specimens and contemporary topotypic material, as they distinctly group together. Therefore, the results presented here strongly support the results presented by Pillet et al. (2013) that *Elphidium williamsoni* is a genetically distinct species, and consequently should not be considered as a subspecies of the *E. excavatum* complex.

It is important to note that whilst the genetic outlier specimens (*Elphidium* genotypes S4 and S5) are always distinct from the *E. williamsoni* genotype, a few specimens do not always cluster within their respective genotype as notably shown in Figures 6.2 and 6.4. This could be due to the key diagnostic features chosen for analysis which have been specifically derived from Haynes' 1973 type description. However, as presented in Chapter 5, when additional key morphological characters are added to the analysis the morphological overlap between the two genetic outlier groups is resolved (Table 5.10). Therefore, it is important to highlight that within any morphometric study, the key morphological character combinations that help to delineate species will change with the choice of genetic outlier selection.

Nevertheless, the morphometric characters used in this analysis are optimal for determining the morphological congruence between the type and contemporary topotypic material. The results illustrate that the morphological characters of Haynes' 1973 type specimens have captured a significant proportion of the intraspecific morphological variation, as these specimens fall within the morphological range exhibited by the contemporary topotypic material (Figures 6.2 and 6.4). It can also be illustrated that the morphological attributes of the type specimens are situated across a large proportion of morphological variability of the *Elphidium* genotype S1 specimens collected from across three biogeographically distinct zones in the North East Atlantic. However, it is important to acknowledge that these specimens do not encompass the entire breadth of intraspecific morphological variability within this species (Figures 6.4 and 6.5). As there are only 11 type specimens available for the comparative analysis, unsurprisingly these specimens may not have captured the entire range of intraspecific morphological variation within this species. An example of range of the morphological characters within these specimens is highlighted in Figure 6.1 (specimens J-L) and also in Chapter 5 (Figure 5.17), where extensive intraspecific morphological variability is observed within this genotype.

6.4.3 Key diagnostic features of *Elphidium williamsoni* following morphometric analysis

Haynes' 1973 type description of *E. williamsoni* emphasises certain key diagnostic morphological test features to aid future classification of this taxon. The results from the DFA and CART analysis illustrate that many of these key diagnostic features, including : the openness of the umbilical area, number of septal pits in each chamber, apertural ornamentation, mean septal pit roundness and porosity are important test features in determining interspecific relationships between the *Elphidium williamsoni* type specimens and the genetically distinct outliers. The important diagnostic features highlighted in this study correspond to Haynes' original description and diagnosis.

However, the results also demonstrate that other diagnostically important features recognised by Haynes, such as septal pit ornamentation (tubercules), test peripheral roundness and the total number of chambers, were not significant in this study's comparative analysis of *E. williamsoni* against the *Elphidium* S4 and S5 genetically distinct outlier groups. Nonetheless, these seemingly less important characteristics could in the future be considered fundamental in determining interspecific relationships against other *Elphidium* species or may become crucial for improving the understanding of intraspecific variation (due to ontogeny or environmental conditions). The results highlight that there is not a single morphological character which can be utilised to delineate between the genotypes; instead a combination of morphological

characteristics are required for successful discrimination. This conclusion not only supports the value of Haynes' original type description and diagnosis, but also attests to his taxonomic skill in choosing type material which is representative of morphological variability within the species concept of *E. williamsoni*.

6.4.4 Taxonomic challenges to a fully integrated approach

Previous studies have encountered significant difficulties in reconciling classical taxonomic names to molecular genotypes Holzmann, 2000; Hayward et al., 2004; Bird et al., in prep.; Darling et al., in prep.). Hayward et al. (2004) presented a comprehensive integrated taxonomic approach to reinvestigate species boundaries within the genus *Ammonia.* This study concluded that 13 distinct genotypes can currently be phylogenetically delineated globally, and only one topotypic genotype (*Ammonia* genotype T2) could be "unequivocally" attached to the taxonomic name of *Ammonia aberdoveyensis.* Two genetically distinct types (*Ammonia* genotypes T1 and T2) were collected from contemporary topotypic material at the type site locality of *A. aberdoveyensis* on the Aberdovey Marshes, Wales. Hayward et al. (2004) determined that a key discriminating feature between these two genetic types is the presence of a small umbilical boss (Hayward et al., 2004, Table 5, p.260). The morphological characters of these sympatric genotypes were then qualitatively compared to an SEM image of an *A. aberdoveyensis* specimen (Haynes, 1973, Pl.18, fig 15) and from the basis of the presence of a small umbilical boss illustrated by the SEM image, the taxonomic name of *A. aberdoveyensis* was attached to the *Ammonia* T2 genotype.

However, on closer analysis of Haynes' original type description, the "holotype" SEM that Hayward et al. (2004) (Hayward et al., 2004) bases the reconciliation of a taxonomic name to a genetic type is actually a paratype specimen, and the morphology of the holotype specimen is only illustrated by a line drawing (Haynes, 1973, Text-Fig. 38, no 1-3). This is important because there is no umbilical boss visible or described in Haynes' original type description or holotype illustration. In fact, the morphological features of the genotype *Ammonia* genotype T1 are more similar to the key diagnostic features described and illustrated in Haynes' original type description and line illustration of *Ammonia aberdoveyenesis.* This is coupled with the fact that Bird et al. (in prep.) identified an additional genotype from within Hayward et al.'s original T2 genotype (as discussed in Chapter 3, Section 3.5.4). This example reinforces the necessity of quantitatively reanalysing the type specimen, and highlights the difficulties working with morphologically similar (pseudo-cryptic) sympatric species.

Further taxonomic challenges may be encountered when implementing a fully integrated approach as resampling of contemporary live topotypic material from the original type locations may be problematic, especially if the type specimens were collected hundreds of years ago (Holzmann, 2000). Over this period of time there may have been substantial environmental changes, e.g. IPCC. It is unknown to what degree these changes in environmental conditions will have upon the test morphology of the species in question. It is also unclear whether the biogeographic distribution of the type species in question have changed over time due to varying environmental conditions (Burrows et al., 2014). This is especially important if the original type specimens were collected at the edge of its biogeographic range, therefore this species may have expatriated, as it may be more vulnerable to changes in environmental conditions. In addition, there is also potential for new species to occupy the type site after the type specimens were collected. These scenarios reinforce the importance of quantitative comparison of the original type series material against genetically sequenced contemporary topotypic material.

Another potential challenge faced when implementing the taxonomic framework includes the possible misplacement or loss of the original type material (Scott, 2011). The ICZN (1999) Article 73.14 states the absence of a type specimen does not always invalidate the designation. However, many of the original type specimens have been poorly illustrated with simplistic line illustrations that often neglect many of the important key morphological features (Holbourn and Henderson, 2002). Therefore, in exceptional circumstances where the original material is lost or the type illustrations and SEM images provide insufficient detail for robust species delineation, a neotype can be designated. The designation of this neotype should follow the requirements set out by ICZN Article 75 (ICZN, 1999).

Caution should be exercised when determining and designating a new species. It is crucial to establish if the candidate specimen has any morphological and/or genetic similarity to previously described type material, type descriptions or illustrations. In addition, its genetic and morphological identity should be compared against any previously genotyped specimens. Identifying this information is critical, as this specimen may be part of previously unrecognised (pseudo-cryptic) intraspecific variability. In addition, it is important to note that a candidate specimen may not always have morphological congruence to type material. Whilst name bearing type specimens are vital reference points for the assignment of a taxonomic name, these specimens are typically chosen in order to portray the exaggerated morphological features of the species in question (Forey et al., 2004, Scott, 2011). As a direct consequence, in an applied taxonomic situation a user often only has a few catalogued morphological end members from

which they can choose and apply a taxonomic name and species concept to a specimen. There is therefore the potential to encounter a greater degree of morphological variation within a genetic distinct species which has not been encapsulated by the type material. Thus, there should be a concerted effort to analyse, archive, image and quantify the entire range of morphological and genetic variability exhibited by a species, so that in the future the process of designating a new species is more transparent and robust.

The proposed taxonomic framework in this chapter (Figure 6.6) consolidates the progressive integrated benthic foraminiferal taxonomic studies such as those provided by Holzmann (2000), Hayward et al. (2004), Tsuchiya et al. (2008) and the studies presented in Chapters 3 and 5 by adding another level of analysis in which the morphology of the name bearing type specimens are quantitatively analysed. It is hoped that these approaches presented in this study will therefore reduce the over-reliance upon the individual taxonomist's judgement for species delineation of other extant and fossil foraminifera in the future.

6.4.5 Scientific communication of species concepts

The fusion of the morphometric and molecular taxonomic evidence provided through the proposed taxonomic framework implemented in this study (Figure 6.6) is only useful if there is a taxonomic setting from which we can communicate these delineations within the academic literature. Traditionally, the distribution of taxonomic knowledge within the academic community has tended to compound the complexity of foraminiferal taxonomy Holbourn and Henderson, 2002). Some of this confusion can be associated with the fact that few studies provide accompanying SEM/ light microscope images. In addition, there is widespread use of different terminologies and morphological characters used to describe and define a species. This raises the question of how can a reliable comparative assessment of taxa occur across time and space, and how can one implement the proposed taxonomic protocol to reduce this confusion in the future?

The development of the new, less expensive imaging techniques and the formation of online digital molecular and taxonomic databases such as GenBank, World Foraminiferal Database, foramBARCODING and www.foraminifera.eu offer the potential for open access communication of taxonomic knowledge. In particular, they provide a platform to distribute and debate images associated with taxonomic names. However, whilst these resources are becoming more and more valuable for applied taxonomic studies, they need to be properly curated and managed to ensure that a consistent taxonomy is applied across all these resources. This is particularly important as many of these resources, whilst providing independent genetic data, continue to

reattach a taxonomic name to a genetic type without returning to the original type material descriptions and species concepts. Thus it should be emphasised that these online databases should complement and not replace the curation of original type material. Nevertheless, these online databases provide a platform from which new species constructs can be developed and openly shared, while also allowing traditional species constructs to be critiqued.

6.5 Conclusions

It is evident that the current approach to benthic foraminiferal taxonomy needs to be reformed in light of recent technological advancements and the uncertain historical taxonomic setting of many key foraminiferal species. One of the major challenges for benthic foraminiferal taxonomy is how should future descriptions of taxa be handled? Wherever possible, taxonomic studies should try to clarify historical synonymies and aim to attach the original taxonomic name to a genetic type. Whilst revisiting and clarifying the past historical nomenclature may seem a daunting task in light of the long history of foraminiferal research, its successful achievement will help to resolve some of the taxonomic uncertainties faced by the benthic foraminiferal community today. It will only be through the integration of historical nomenclature, reliable type material and the integrated taxonomic approach described here, that a stable platform from which species concepts and delineations can be communicated.

It is therefore pivotal that future taxonomic studies should focus upon creating combined and discrete multisource variables, to delineate species. Each foraminiferal taxonomic species description should ideally include: (i) a genetic sequence, (ii) detailed quantitative morphological measurements, (iii) traditional morphological descriptions, (iv) detailed type locality information, (v) SEM image of the holotype specimen and should follow the taxonomic and nomenclatural guidelines set-out by the ICZN (ICZN, 1999). It is imperative that empirical evidence of the full range of morphological and genetic variability is reliably recorded within the type descriptions. A more objective way would be to have a representative series of type series specimens (paratypes) which encapsulate the range of morphological variability within a population or across different biogeographical ranges. It is also essential that this type material is properly archived for future reference, for example through its deposition in a National Museum.

The case study of *Elphidium williamsoni* highlights the importance of an integrated taxonomic approach to resolving taxonomic complexity faced by the benthic foraminiferal community today. Since Williamson's first description of *Polystomella umbilicatula* in 1858, this study now presents the first clear link between morphologically characterised type material (to which the

formal name *E. williamsoni* is directly attributable) and the unique genotype of *E. williamsoni.* The taxonomic framework proposed here provides a bridge between molecular and morphological evidence and its implementation could provide increased rigour for species identification and discovery. It also has the potential to be robust enough for new character definitions, new species, and new lines of taxonomic evidence to be added in the future. If other key taxa are systematically redefined this would provide a foundation for a transcontinental and trans-generational benthic foraminiferal taxonomy to evolve.

Chapter 7

Synthesis, conclusions and future perspectives

Chapter 7: Synthesis, conclusions and future perspectives

With increasing public concern and political emphasis placed on understanding the impacts of anthropogenic climate change on the marine ecosystem, it is crucial to strengthen the understanding of the magnitude and timings of past climate change and the responses of biota to these changes. Benthic foraminifera provide key biological and geochemical proxies for assessing both current and past climatic change. In order to achieve high-resolution paleoenvironmental reconstructions, a precise taxonomy and an exact knowledge of species ecological preferences are required (Murray, 1991). However, as underscored in Chapter 1, benthic foraminiferal taxonomy is plagued by uncertainty, particularly with the recent recognition of cryptic species (e.g. Hayward et al., 2004). The advent of molecular systematics underlines the need to re-evaluate the use of morphology in species delineation. However, the synthesis of the molecular and morphological taxonomic approaches has thus far been limited and requires further investigation to realise its full potential.

Accordingly, this thesis sought to reconcile molecules and morphology in two taxonomically challenging, yet scientifically important benthic foraminiferal taxonomic groups in the NE Atlantic shelf seas; the Elphidiidae family and the genus *Ammonia*. A major contribution of this thesis was the morphometric analysis of over 750 genetically sequenced specimens. This detailed morphometric analysis (outlined in Chapters 3, 5 and 6), coupled with molecular insights provided from a parallel NERC investigation into the molecular diversity of these groups, entails the most comprehensive taxonomic re-evaluation of these taxa in the NE Atlantic conducted to date. Additionally, the research documented herein has yielded new insights into the biodiversity, biogeographical occurrences and ecological preferences of benthic foraminiferal taxa in the NE Atlantic (Chapters 3, 4 and 5). In doing so, this thesis aimed to overcome some of the taxonomic challenges encountered by applied investigations through stabilising nomenclature and species concepts, therefore ultimately improving the value of foraminiferal proxies in applied taxonomic situations. This chapter seeks to address the research objectives and identify future challenges and perspectives.

7.1 Research objective 1: To address the utility of morphology in delineating between genetically distinct species of benthic foraminifera

In recent years, foraminiferal systematics has faced the fundamental question as to whether morphological characters alone are sufficiently robust to classify both fossil and extant species. Despite recent methodological advances, such as the use of molecular sequencing, the value of

morphology as a line of taxonomic evidence for species delineation, while fundamental, is still poorly understood. In order to assess the utility of morphology in delineating between benthic foraminiferal species, two morphometric studies were conducted to compare the interspecific boundaries identified by morphology and molecules (Chapters 3 and 5).

The morphometric study conducted on the enigmatic Elphidiidae family (Chapter 5) is significant as it is the first detailed assessment of quantitative interspecific morphological boundaries in this family. Prior to this, morphological boundaries of Elphidiidae genotypes have only been assessed using qualitative descriptors (e.g. Schweizer et al., 2008; Pillet et al., 2012; Pillet et al., 2013). In seeking to assess the utility of morphology in delineating genetically distinct species within this family, a combination of quantitative and qualitative morphological characters of 17 Elphidiidae genotypes (sequenced by Darling et al., in prep.) were analysed. The results revealed that morphology could be successfully reconciled with molecules for the majority of specimens. Notably, 13 out of 17 genotypes identified in the NE Atlantic could be perfectly discriminated based on their morphological test characters. However, there was not always a direct correspondence between species boundaries identified by the morphometric and molecular approaches, as ambiguous interspecific morphological boundaries were identified between a handful of specimens (n=5) within genotypes S1 and S2, and genotypes S16 and S17. This highlights that the morphological limits identified in previous studies (e.g. Schweizer et al., 2011; Pillet et al., 2013) may not be as well defined as previously inferred.

In contrast to the Elphidiidae family, where morphology remains a powerful tool for species delineation, the interspecific morphological boundaries of genotypes within the genus *Ammonia* are more enigmatic (Chapter 3). Whilst the results revealed that three genotypes (genotypes S5a, S5b and S6) could be perfectly discriminated using morphological traits, uncertainty clouds the morphological species boundaries between the less ornamented forms (genotypes S1-S4). Although the majority of specimens within these four species can be delineated after extended morphological analysis using *a priori* knowledge of their genetic groupings, there remains uncertainty at the interspecific morphological limits, because they appear to exhibit gradational morphological traits (Table 3.7). The identification of partial cryptic diversity within this genus diverges from previous research, which identified that all *Ammonia* genotypes could be morphologically discriminated (Hayward et al., 2004). The elucidation of previously unrecognised cryptic diversity in *Ammonia* may be due to the extensive sampling conducted by this study. In addition, nearly double the number of genetically sequenced *Ammonia* specimens

were morphologically examined, in comparison to previous research. Additionally, this study sampled across the biogeographic provinces of the NE Atlantic (Dinter et al., 2001), many of which were not previously captured by Hayward et al. (2004). However, the future challenge will be to differentiate between these partially cryptic species using additional lines of taxonomic evidence to minimise their potential misidentification in applied taxonomic investigations.

Nevertheless, the further elucidation of interspecific morphological boundaries of *Ammonia* and elphidiids presented in this thesis are crucial because to date, fossil foraminifera in applied taxonomic situations can only be robustly classified based on assessments of their morphological test characteristics. Traditionally classical taxonomy discriminated between taxa based on a limited number of discrete morphological traits described by qualitative descriptors (as discussed in Chapter 1). However, the studies conducted in this thesis (Chapters 3, 5 and 6) indicate that only a limited number of discrete diagnostic criteria were useful for discriminating taxa in applied taxonomic situations. For example, the presence of secondary dorsal openings can be used to delineate specimens of *Ammonia* genotype S6 (Table 3.7) and the presence of peripheral spines can be used to delineate specimens of *Elphidium* genotype S10 (Table 5.7). Instead, the morphometric analyses revealed that genotype delineations require a complex combination of quantitative and qualitative morphological features, including structural traits and ornamental characters. It is noteworthy that some of these morphological distinctions between the genotypes are so subtle that in an applied taxonomic situation it may be difficult for workers to differentiate specimens correctly. For example, *Ammonia* genotypes S1-S4 were deemed 'visually cryptic' in an applied taxonomic investigation, owing to the paucity of easily identifiable test traits under a light microscope (Chapter 3). Thus, this indicates species delineation based on classical morphospecies descriptions may be insufficient for the identification of all *Ammonia* and Elphidiidae taxa. This illustrates the important contribution of this thesis, which provides the potential for increased rigour in species delineations in applied taxonomic settings, through the quantification of the interspecific boundaries and identification of key diagnostic criteria.

Moreover, the insights provided by the new lines of taxonomic evidence (morphological and molecular) reveal potentially overlooked diversity within classical taxonomy where species have only been delineated based on qualitative morphological descriptors, without any knowledge of their underlying genetic diversity. For example, although *Elphidium* genotypes S7 and S15 could be successfully morphometrically delineated from each other, it is important to highlight that it

is unlikely these taxa would be discriminated successfully using classical taxonomy. This is evidenced as Darling et al. (in prep.) originally associated the name *Elphidium albiumbilicatum* (Weiss, 1954) to both genotypes S7 and S15, but eventually deemed that genotype S15 most likely represents a new, previously undescribed species (Chapter 5, Table 5.18).

Overall, the morphometric techniques presented in this thesis yield useful information for species delineation of benthic foraminifera, as the results have helped to clarify the key diagnostic features and the interspecific morphological limits. In doing so, it has ultimately provided taxonomic frameworks to improve future species identification in applied investigations. Morphology has a distinct advantage over molecules for practical applications, as it bridges the gaps in the classification and placement of taxa when no molecular data is available (e.g. fossil specimens). However, new insights from molecular analysis (Bird et al., in prep.; Darling et al., in prep.) reveal that morphology might not be sufficient on its own to elucidate species boundaries. Notably, the identification of ambiguous interspecific morphological boundaries between *Ammonia* genotypes S1-S4 (Chapter 3) reveals the shortcomings of purely relying on a traditional morphological approach to species delineation; this clearly demonstrates the necessity for an integrated taxonomic approach to benthic foraminiferal species delineation.

7.2 Research objective 2: To further the understanding of the biogeographic ranges and occurrences of benthic foraminifera in the NE Atlantic

An understanding of foraminiferal biodiversity and occurrence is crucial as it refines knowledge of the species ecological ranges, providing baseline data which underpins paleoenvironmental reconstructions (Murray, 1991), as well as providing the potential to identify any changes in their biogeography in response to anthropogenic climate change (e.g. species range shifts). However, as acknowledged in Chapter 1 the current understanding of foraminiferal biodiversity and distribution has been hampered in part by classical morphospecies taxonomy, whereby confusion arises as species identification is determined by the personal and provincial biases of an individual researcher (Murray, 2013). The sampling regime employed in this thesis provided new insights into the patterns of benthic foraminiferal diversity and distribution in the NE Atlantic across a wide range of ecological and oceanographic conditions (Chapters 3 and 5). New insights were also obtained through the employment of a time series analysis (Chapter 4) which helped to elucidate both the ecological and seasonal preferences of benthic foraminifera in the NW Scottish shelf seas.

The analysis of the biogeographical patterns of species within the Elphidiidae family presented in Chapter 5 has strengthened the understanding of the distributions and ecological ranges of these taxa in the NE Atlantic. The Elphidiidae genotypes exhibited complex patterns of biogeographical distribution, often with overlapping species ranges (Figure 5.20). Surprisingly extensive sympatry was identified, with up to eight elphidiid genotypes co-existing at a single site locality (Table 5.16). Only three genotypes (S11, S12 and S15) were found not to co-exist with another genotype. These new insights shed light into the validity of the current understanding of the key elphidiid morphospecies ranges. Notably, the findings contradict prior research that identified that many *Elphidium* genotypes exhibited restricted distributions and distinct ecological preferences (Feyling-Hanssen, 1972; Miller et al., 1982, as depicted in Table 5.1). Although the morphometric results revealed a strong concordance between molecules and morphology in the Elphidiidae family, the complex synonymies and different approaches to species delineation (e.g. 'lumpers' vs 'splitters') bring into the question the validity of previous biogeographical distributions and ecological ranges inferred from classical morphospecies concepts. However, as the results in Chapter 5 revealed that only a handful of specimens within this family were morphologically cryptic, this also highlights that the biogeographical understanding gained from classical morphospecies concepts in the published literature should not be completely discarded. This study affirms that Darling et al.'s (in prep.) reassessment of published SEM images using an understanding of the genotypes' morphological characters, provides a strong avenue for re-evaluation of previously published species' biogeographical distributions and ecological preferences. It also highlights the value of well-illustrated taxonomic work, so that species identification can be verified.

The revelation of partially cryptic species, high levels of sympatry and the absence of discrete diagnostic features between some of the *Ammonia* genotypes (Chapter 3), has illustrated that the current understanding of *Ammonia* biogeography and ecological preferences in the NE Atlantic is poor. It is likely that previous studies have underestimated the complexity of biodiversity in this region. Therefore, this calls into question the validity of the ecological preferences and biogeographical distributions of *Ammonia* species inferred from classical morphospecies concepts. Unlike the Elphidiidae, the results revealed that it is not feasible to re-analyse the distributions of *Ammonia* from the published literature, owing to the prevalence of broad species concepts, open nomenclature and the absence of discrete morphological characters for discrimination.

Additionally, this thesis has not only extended the understanding of the biogeographical distributions of elphidiids and *Ammonia* in the NE Atlantic, but it has also provided insights into the global biogeography of these taxa. For example, previous research considered that *Ammonia* genotype S1 was thought to be part of two disjunct populations, one in China and one in the Wadden seas (Hayward et al., 2004; Schweizer et al., 2008). However, the new biogeographic data presented in Chapter 3 illustrates that this genotype (S1) could be more cosmopolitan than previously identified, or it may represent dispersal from a point of origin. This highlights the requirement for increased global sampling coverage to strengthen the understanding of the global diversity and to help to elucidate some of the mechanisms that shape their distribution.

Historically, the understanding of biodiversity and distribution of benthic foraminifera has been potentially hindered by a paucity of knowledge surrounding the seasonal changes in biodiversity, as the majority of studies only sample an assemblage at a single 'snapshot' in time (e.g. Hannah and Rogerson, 1997; Murray et al., 2003). Seeking to explore the importance of understanding the temporal dynamics of the biodiversity and composition of an assemblage, a time series study was conducted in the NW Scottish shelf seas (Chapter 4). Whilst this region has long been an important area of scientific interest, this was the first time that an investigation assessed the temporal dynamics of benthic foraminiferal assemblages in this region. Crucially, this study utilised the new lines of taxonomic evidence (outlined in Chapters 3 and 5) to dispel any uncertainty surrounding species identified from classical taxonomy in this region. For example, traditionally only a limited number of broad *Ammonia* morphospecies concepts including *Ammonia batavus* and *Ammonia beccarii,* were identified in the NW Scottish shelf seas (e.g. Cage and Austin, 2008; Mokeddem et al., 2010). Chapter 4 outlined the dynamic changes in species diversity and the overall assemblage composition across the course of a year. It is noteworthy that two species of *Ammonia* (genotypes S5a and S6) were identified living in sympatry throughout the year, as well as the observation that subtle seasonal partitioning can be observed between these two species. For example, *Ammonia* genotype S5a is prevalent from November to January, whilst *Ammonia* genotype S6 is prevalent from February to June (Figures 4.7 and 4.8). These new insights reveal that *Ammonia* diversity within this region has previously been underestimated, and the prevailing practice of 'lumping' may have obscured potentially significant ecological relationships. Further investigation is required to assess if patterns are replicated over longer time scales, to elucidate the environmental controls on these assemblage changes and to identify the potential effects of spatial patchiness. Overall, the results from Chapter 4 reveal that the paucity of studies that examine seasonal variability in foraminiferal

assemblages has potentially prevented a finer understanding of biodiversity changes and ecological partitioning of species.

In providing an in-depth account of biogeographic and distribution patterns of a number of benthic foraminiferal taxa in light of new taxonomic evidence, this thesis has brought into question the robustness of the biogeography and ecological preferences inferred from classical morphospecies taxonomy. Additionally, the studies present a baseline understanding of the range distributions of species (Chapters 3 and 5), as well as new insights into seasonal occurrences of benthic foraminifera (Chapter 4). In future, this information will be vital to ascertain the mobility of species in response to anthropogenic climate change. Further sampling is required at a range of scales (macro-scale, meso-scale and micro-scale levels), in order to discover broad biogeographical patterns of distributions, as well uncovering ecological adaptations and localised partitioning of species.

7.3 Research objective 3: To explore intraspecific morphological variation in benthic foraminifera

The extraordinary morphological variability in the test form and shape of foraminifera has long represented a considerable puzzle in benthic foraminiferal taxonomy; there is for example, an ongoing debate as to whether the morphological plasticity observed is indicative of genetic variability or ecophenotypy (Haynes, 1992). Historically, in the absence of molecular evidence, the theory of ecophenotypy was pervasive in the literature, particularly when identifying morphospecies of *Ammonia* (Schnitker, 1974; Walton and Sloan, 1990) or morphospecies related to the *Elphidium excavatum* complex (Feyling-Hanssen, 1972; Miller et al., 1982). In recent years, molecular systematics has provided new insights into factors controlling phenotypic plasticity, revealing that many of the classical ecophenotypes recognised are in fact genetically distinct species (e.g. Hayward et al., 2004; Pillet et al., 2013). Despite these advancements, currently there is a paucity of knowledge surrounding whether ecophenotypic variability can be identified within benthic foraminifera in light of this new taxonomic evidence.

The unique combination of detailed morphometric, biogeographical and genetic data presented in this thesis allowed for an exploration of the controls on morphological test variability. Three case studies were conducted to examine intraspecific morphological variability across a spatial scale within two genotypes of *Elphidium* (genotypes S1 and S4) and one genotype of *Ammonia* (genotype S1). The first study conducted on *Ammonia* genotype S1 (Chapter 3) revealed that subtle morphological differences could be identified between specimens from different site

localities. Notably, these locally sampled 'populations' could be differentiated by their pore characteristics (Figure 3.14). In contrast, although subtle morphological differences were identified between the specimens of *Elphidium* genotype S4 from different site localities (Chapter 5), no discrete morphological test traits could be identified to clearly aid this discrimination. Instead, specimens were distinguished based on a combination of subtle structural and ornamental features (Chapter 5, Section 5.3.3). Finally, the case study conducted on *Elphidium* genotype S1 revealed no clear morphological trends in relation to site locality and that gradational morphological features were equally observed between and within site localities (Chapter 5). However, a proportion of the intraspecific morphological variability is in part controlled by differences in the overall test size (i.e. ontogenetic development).

Ultimately, it is not possible to resolve to what extent ecophenotypy controls the intraspecific morphological variability found within these genotypes, as efforts are impeded by the paucity of understanding of intraspecific variability at a population level, the unbalanced and small datasets available at each site locality and the lack of detailed environmental surveys. Nevertheless, whilst the understanding of the controls of intraspecific morphological variability are far from being resolved, the case studies presented in this thesis provide interesting insights into some of the patterns of intraspecific morphological variability within these two taxonomic groups.

7.4 Research objective 4: To investigate whether classical morphospecies concepts and nomenclature can be reconciled with new lines of taxonomic evidence

As discussed throughout this thesis a consistent and robust taxonomy is central to the correct interpretation of foraminiferal species within modern and fossil assemblages. The unit through which the taxonomic affinities, biogeochemical, genetic and morphological properties are communicated is a taxonomic name (Bowker and Star, 2000; Waterton et al., 2013). Therefore, it is pivotal that one can associate taxonomic names unequivocally to species, so that a reliable reference system is in place for modern and paleoenvironmental reconstructions. However, as evidenced in Chapter 3 (Section 3.5.8) and Chapter 5 (Section 5.4.7) significant taxonomic challenges are encountered when trying to reconcile classical taxonomic names to newly delineated genotypes. For example, the same taxonomic name can be ascribed to different genotypes by different studies (as evidenced in Table 5.18). Moreover, the advancement and development of new lines of evidence can call into question the validity of taxonomic names previously assigned to a genotype. For example, two genetically distinct yet partially

morphologically cryptic species were identified from within Hayward et al.'s (2004) T2 *Ammonia* genotype (Chapter 3).

Seeking to address some of these taxonomic challenges, Chapter 6 presents a case study using the benthic foraminifera *Elphidium williamsoni* to illustrate how it is possible to unequivocally link a morphological and molecular species concept to its formal taxonomic name. Using a combination of quantitative morphometric analysis on topotypic, type and contemporary material, this study represents the first reconciliation of a classical benthic foraminiferal species concept to a molecular type. Chapter 6 also outlines a protocol for future research which enables the reconciliation of type material to new lines of taxonomic evidence (Figure 6.6). However, as discussed in Chapter 6, implementing this taxonomic protocol is likely to be challenging and will not happen overnight. Therefore, until a species name can be reconciled with a genotype, the interim taxonomic protocol as outlined in Chapters 3 and 5, should be employed whereby specimens are assigned to a potential genotype (where possible), and specimens are referred to by both the potential genotype number and its ascribed classical morphospecies name. This will help to foster continuity and nomenclatural stability in applied taxonomic investigations.

7.5 Future research perspectives and challenges

This thesis provides several new insights into foraminiferal diversity, interspecific morphological limits and ecological preferences in a number of benthic foraminiferal taxa in the NE Atlantic. In doing so, it also raises many questions for future investigation.

Although the interspecific morphological boundaries of *Ammonia* (Chapter 3) and Elphidiidae (Chapter 5) identified in this thesis represent the most comprehensive assessments of these taxa conducted to date, it should be noted that these studies are not without limitations. Notably, as discussed in Chapters 3 and 5, a notable caveat of this thesis is that no morphological assessments of test traits were analysed from the apertural (peripheral) view, owing to the lack of SEM images taken from this profile. Thus, there may be additional important diagnostic morphological characters, which were not assessed in these studies that have the potential to resolve some of the ambiguity observed between a number of interspecific morphological species boundaries. Future studies would also benefit from additional SEM imaging of both the apertural (peripheral) view, as well as close up imaging of test features such as pore density and the detail and degree of ornamentation. This would likely improve the understanding of both interspecific and intraspecific morphological variability within these taxa.

Further morphometric analysis is also required to test the consistency of the patterns observed. Although a large number of specimens were included in the analysis of interspecific and intraspecific variation in Chapters 3, 5 and 6, the number of specimens analysed were not evenly distributed between the genotypes and/or site localities, owing to availability of genetically sequenced specimens. Thus many genotypes and site localities were under-represented, which made the fixation of diagnostic morphological characteristics for a number of genotypes and site localities difficult. Notably, the interspecific boundary of *Elphidium* genotype S15 is based upon a single specimen (Chapter 5). Therefore, consideration of additional material, coupled with further replication of the morphometric assessments are required to validate the interspecific morphological boundaries and to test the patterns of intraspecific morphological variability identified in this thesis. This is crucial, as there may be more discordance between molecules and morphology than has currently been observed, as the studies within this thesis may not have fully captured diversity at a population level.

Moreover, although this thesis has greatly enhanced the depth and breadth of sampling of *Ammonia* and Elphidiidae within the NE Atlantic, the sampling regime employed was not exhaustive. Notably, the sampling region is biased towards samples collected from intertidal regions. Additionally, there are also some gaps in the coverage of sampling, particularly in the arctic biogeographic provinces. Thus, the next critical step is to advance the sampling coverage of these two taxa both within the NE Atlantic and globally. Additionally, this thesis (Chapters 3 and 5) has uncovered high levels of foraminiferal biodiversity in seaweed sampling, particularly from those collected from North Uist. Thus far, seaweeds have rarely been sampled in the NE Atlantic; this indicates that this biodiversity has been potentially overlooked in the literature. This illustrates that in the future more targeted sampling across a range of habitats is required.

Future study should also aim to examine both the ontogenetic and ecophenotypic controls on intraspecific morphological plasticity. Although the case studies employed in this thesis provided much needed insights into intraspecific morphological variability across the NE Atlantic, these pilot studies (Chapters 3 and 5) were limited because it was only possible to infer that the morphological trends observed were controlled by ecophenotypy, owing to the general paucity of environmental data. As discussed in Chapters 3 and 5, the development of experimental culturing studies and or detailed *in situ* investigations could help to provide much needed insights into the environmental controls of morphological variability. This understanding would ultimately aid ecological interpretation in applied taxonomic situations, particularly if it is

possible to identify characters which can be associated with specific environmental conditions. The clarification of a species ecological niche could also aid in the identification of a species and/or provide insights into functional (adaptive) significance of morphological traits.

An additional area that needs to be addressed is how these new lines of taxonomic evidence will affect species identification in applied taxonomic investigations such as paleoenvironmental reconstructions. For example, considering that a stable taxonomy is a prerequisite for the use of benthic foraminifera in applied taxonomic situations, the existence of ambiguous interspecific boundaries between *Ammonia* genotypes S1-S4 (Chapter 3), could have significant ramifications for paleoenvironmental research. One of the next critical steps is to re-examine previous studies which employed species-specific proxies to assess the impacts of this previously hidden diversity on the strength and robustness of the paleoclimatic reconstructions produced. Moreover, further research is needed to clarify if sympatric species exhibit ecological partitioning, which could be reflected in their test isotopic composition, which may not have been accounted for in previous species-specific calibrations. Refining our understanding of a species' ecological preference, biology, interspecific morphological boundaries and test geochemistry could help to constrain future climate models that depend on paleoenvironmental reconstructions for their testing. Additionally, a refined understanding offers the potential to identify small climatic shifts over time, which may have been previously obscured when delineating specimens using a classical morphospecies taxonomic approach.

A potential avenue for future research which may yield new information for elucidating taxonomic relationships within benthic foraminifera is the employment of geometric morphometric techniques. As discussed in Chapter 2, this approach has been increasingly used within the broader biological community to study the shape and form of taxa (e.g. Smith and Hendricks, 2013). This technique utilises the relative positions of anatomical landmarks and sets of points to approximate outlines and surfaces to quantify size and shape (Rohlf and Marcus, 1993; Klingenberg et al., 2011). To date, this approach has not been employed in foraminiferal taxonomy, yet offers exciting new insights which can complement traditional morphometric analyses.

The taxonomic framework outlined in Chapter 6 provides a new approach to reconcile new lines of taxonomic evidence with classical morphospecies concepts in order to stabilise foraminiferal taxonomy. However, this approach has only been successfully implemented for a single species concept (*Elphidium williamsoni*) owing to numerous taxonomic challenges, e.g. access to type

material. Nevertheless in the future, a concerted effort should be made to clarify historical synonymies and to attach the original taxonomic name to a genetic type in order to resolve some of the taxonomic uncertainties faced by the benthic foraminiferal community today.

In order to make the process of delineating benthic foraminiferal specimens more transparent and robust, it is crucial that these new insights are properly analysed, documented and archived. As advocated in Chapter 6, emphasis should be placed on developing online databases, as this medium connects both taxonomists and applied foraminiferal practitioners, facilitating the easy access to a large amount of data. This counters some of the challenges faced in classical taxonomy, whereby access to resources has hampered the communication of knowledge (Holbourn and Henderson, 2002). Finally, the next steps for benthic foraminiferal systematics should focus upon conducting integrated taxonomic research and utilising the taxonomic approaches employed in this thesis to re-examine other benthic foraminiferal taxa. This is crucial as the re-evaluations of the taxonomic boundaries within the genus *Ammonia* and the Elphidiidae family represent only a small proportion of species recognised by classical taxonomy; thereby highlighting the importance for increased taxonomic coverage.

7.6 Conclusions

This thesis provides the most comprehensive re-evaluation of Elphidiidae and *Ammonia* in the NE Atlantic conducted to date, through the production of detailed quantitative morphometric analysis of over 750 specimens, coupled with assessments of their biogeographic distributions. The detailed morphometric study conducted on the enigmatic genus *Ammonia* (Chapter 3) is the most comprehensive re-evaluation of interspecific morphological relationships of the genus in the NE Atlantic. From the morphological analysis of 25 variables, it is identified that three *Ammonia* genotypes S5a, S5b and S6 can be perfectly discriminated based on a suite of structural and ornamental test characteristics. The finding that the morphological species boundaries between the less ornamented genotypes S1-S4 exhibits gradational morphological features and are therefore partially morphologically cryptic is surprising, as this contradicts previous morphometric research. In addition, the absence of discrete diagnostic features indicates that these species are likely to be 'visually' cryptic in an applied taxonomic situation. These findings highlight that classical taxonomy is unlikely to reflect the overall genetic diversity found within the NE Atlantic. Thus, this indicates species delineations based on classical morphospecies descriptions may be insufficient for the identification of all *Ammonia* in the NE Atlantic. This study has also presented the first detailed examination of intraspecific

morphological variability in the genus *Ammonia* across a large geographic spatial scale. The results revealed that *Ammonia* genotype S1 specimens could be morphologically discriminated based on a range of test traits including their pore characters. However, owing to the small dataset and lack of environmental data the relationship between morphology and environment is not fully resolved.

The seasonal assemblage study conducted in Chapter 4 presents the first time-series analysis of a coastal environment in NW Scotland in which the temporal dynamics of benthic foraminiferal diversity and assemblage composition have been investigated, alongside new lines of taxonomic evidence, to document the seasonal variability of two previously cryptic species of *Ammonia*. In total 52 species were identified at the Dunstaffnage site and the assemblage was dominated by three species: *Nonionella turgida, Ammonia* genotype S5a and *Ammonia* genotype S6. These two previously cryptic species of *Ammonia* were identified to be co-existing throughout the year, with some evidence to suggest that they exhibit subtle seasonal partitioning. For example, *Ammonia* genotype S5a is prevalent from November to January, whilst *Ammonia* genotype S6 is prevalent from February to June. This subtle seasonal partitioning could indicate that these *Ammonia* species occupy distinct ecological niches. A clear temporal trend in changes to the overall assemblage composition is also observed across the period of investigation. For example, the abundance of *Nonionella turgida* shifts over the course of the year, it is dominant in spring-summer, whilst less prevalent in winter. The primary controls of shifts in the assemblage composition are still uncertain as no clear causal relationship was identified between the abundance of the five dominant taxa, and temperature and salinity measured at this site. However, the results reveal that species occurrences may be driven by the source and input of food supply at the site.

The detailed morphometric study conducted on the Elphidiidae family (Chapter 5) suggests that for the most part molecules and morphology can be successfully reconciled to create a new taxonomic framework from which to test and validate species boundaries. In total 13 out of 17 Elphidiidae genotypes identified in the NE Atlantic could be perfectly discriminated based on their morphological test characters. Although a limited number (n=5) of partially cryptic specimens were identified between the end members of genotypes S1 and S2, and genotypes S16 and S17. The broad biogeographic sampling regime employed by this study has also enhanced the understanding of the biogeographical occurrences of Elphidiidae genotypes through significantly increasing the known biogeographic distributions and species ranges. It is

evident that these species exhibit overlapping ranges and extensive sympatry. Finally, the two case studies conducted on *Elphidium* genotypes S1 and S4 represent the first quantitative analysis of intraspecific morphological variation with this taxonomic family. No clear intraspecific trends related to site locality were identified within genotype S1. In contrast, subtle morphological differences were identified between specimens of genotype S4 from different site localities. However, this data is constrained by the low numbers of specimens analysed at each site locality.

Finally, the taxonomic protocol outlined in Chapter 6 provides a bridge between molecular and morphological evidence and its implementation could provide increased rigour for species identification and discovery in the future. The case study of *Elphidium williamsoni* provides a practical example of this framework and reveals that the morphologically characterised type material (to which the formal name *E. williamsoni* is directly attributable) and the unique genotype of *E. williamsoni* can be successfully reconciled.

7.7 Closing remarks

Overall, this thesis provides a framework for reconciling molecules, morphology and classical taxonomy which serves as a model for future research on other taxonomically challenging benthic foraminiferal species. This thesis exemplifies how different lines of taxonomic evidence are powerful tools to solve long-standing taxonomic problems, uncover hidden cryptic species diversity and to test and refine taxonomic boundaries. Notably, the results presented demonstrate that morphology remains a crucial component for foraminiferal systematics, as it underpins molecular species identification and provides a link to which these new lines of evidence can be reconciled with known taxa (both extinct and extant). However, it should be noted that there is no shortcut to good taxonomy. Neither genetics nor morphological approaches to species delineations are without limitations. Consequently, this thesis advocates that taxonomy should not be restricted to a single line of taxonomic evidence, but should be a holistic approach combining multiple lines of taxonomic evidence including biogeography, phylogeny, ecology and morphology. The taxonomic resolution of benthic foraminifera is an iterative process, and is likely to take a long time to resolve. In the meantime, the focus should be on consolidating and augmenting the new lines of taxonomic evidence, whilst aiming to reconcile these new findings with classical taxonomy and nomenclature.

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Appendices

Table 1

Sampling locations across the NE Atlantic (Chapters 3, 5 and 6)

Table 1. Main sampling locations for Elphidiidae genotypes (Chapters 5 and 6).

Location number (Figure 2.2)	Location name	Co-ordinates	Location description	Sampled by
1	Svalbard (Sv)	See Table 2 for co-ordinates of multiple sampling sites	See Table 2 for co-ordinates of multiple sampling sites	MS/WA
2	Iceland (Is)	See Table 2 for co-ordinates of multiple sampling sites	See Table 2 for co-ordinates of multiple sampling sites	MS
3	Bergen (Bg)	60°15'38.28"N 5°13'11.4"E	Fjord sediment, 39m	AR
4	Shetland (SH)	See Table 2 for co-ordinates of multiple sampling sites	See Table 2 for co-ordinates of multiple sampling sites	AR/KD
5	Skagerrak (Sk)	58° 19' 24" N 11° 32' 49.2" E	Fjord sediment, 119m	MS
6	Orkney (OK)	58° 56' 31.35"N 3° 5' 22.15"W	Inter-tidal sediment	RSPB
7	North Uist (NU)	See Table 2 for co-ordinates of multiple sampling sites	See Table 2 for co-ordinates of multiple sampling sites	AR/KD/CB/WA/MS
8	Cromarty (CR)	57° 40' 45.59"N 04° 02' 28.12"W	Inter-tidal sediment	KD
9	Ythan (YN)	57°20'N, 01°57'W	Inter-tidal sediment	JD/NK
10	Dunstaffnage (DF)	56°28'48"N 05°25'48"W	Sub-tidal sediment	CB/MS
11	Baltic (BA)	See Table 2 for co-ordinates of multiple sampling sites	See Table 2 for co-ordinates of multiple sampling sites	WA
12	Eden (ED/SA)	56°22' 00.00"N 02°50'.00W	Inter-tidal sediment	KD
13	Cramond (Cd)	55° 59' 22.92''N 03° 17' 53.16''W	Inter-tidal sediment	KD/MS
14	Loch na Cille (LK)	55° 57' 36.00"N 05° 41' 24.00"W	Inter-tidal sediment	KD
15	White Rock (WR)	54° 29' 05.42"N 05° 39' 12.58"W	Inter-tidal sediment	KE
16	Norfolk (NF)	52° 49' 02.41"N 00°21' 46.16"E	Inter-tidal sediment	KD
17	Aberdovey Bay (AB)	52° 31' 45.01'' N 04° 00' 07.06'' W	Inter-tidal sediment	AR
18	Cork (CK)	See Table 2 for co-ordinates of multiple sampling sites	See Table 2 for descriptions of multiple sampling sites	KD/CB/MS
19	Laugharne Castle (LC)	51° 46' 12.00"N 04° 27' 00.00"W	Inter-tidal sediment	KD
20	Grevelingen (Gv)	51° 44' 50.04" N 3° 53' 24.06" E	Brackish lake, 34m	MS
21	Dartmouth (DM)	50° 21' 04.84"N 03° 34' 11.33"W	Inter-tidal sediment	CB

Location number (Figure 2.2)	Location name	Co-ordinates	Location description	Sampled by
22	Baie de Seine (Bs)	See Table 2 for co-ordinates of multiple sampling sites	See Table 2 for descriptions of multiple sampling sites	MS
23	Ile d'Yeu (Ye)	46°43'12.35"N 2° 20' 13" W	Inter-tidal sediment with seaweeds	MS
24	Baie d'Aiguillon (Ai)	46° 15' 17.00''N 01°08'27.00''W	Inter-tidal sediment	MS
25	Portugal (Po)	41°09'01.24"N 8° 52'00.90"W	Sand, 50m	MS

AR-Angela Roberts, MS-Magali Schweizer, CB-Clare Bird, KD-Kate Darling, KE-Kath Evans, RSPB-Royal Society for Protection of Birds, JD-Julia Dougherty, WA-Bill Austin, NK-Nikki Khanna

Table 2

Sub-site sampling locations across the NE Atlantic (Chapters 3, 5 and 6)

Table 2 List of sub-site locations collected across the NE Atlantic with general site descriptions. Location numbers correspond to those found in Figure 2.2

Location number	Location name	Site	Sub-site code	Co-ordinates	Site description
1	Svalbard	JM10-02-BC	JM02	80° 04’ 26.88”N 08° 39’ 39.90”E	Sediment, 497m
		JM10-03-BC	JM03	80° 02' 34.26''N 10° 00' 01.80''E	Sediment, 501m
		JM10-04-BC	JM04	79° 38' 25.62''N 15° 27' 13.74''E	Sediment, 138m
		SV11-HH11-10A-BC	HH10	81° 14' 52.80''N 25° 24' 15.00''E	Sediment, 236m
		SV11-HH11-16A-BC	HH16	79° 41’ 15.06”N 34° 34’ 04.62”E	Sediment, 234m
2	Iceland	Is10-Osar1, Reykjanes Peninsula	IS-RP1	63° 56' 28.00”N 22°38'55.00”W	Inter-tidal sediment
		Is10-Osar5, Reykjanes Peninsula	IS-RP2	63° 56' 39.00”N 22° 38' 61.00”W	Inter-tidal sediment
		Is 10 Geldinganes, Reykjanes Peninsula	IS-GE	64° 09 '31.00”N 21° 47' 15.00”W	Inter-tidal sediment
		Is10 Grafarvogur , Reykjanes Peninsula	IS-GR	64° 07' 57.00”N 21° 48' 23.00”W	Inter-tidal sediment
		Is10 Ellidavogur, Reykjanes Peninsula	IS-EL	64° 07' 50.00”N 21° 50' 43.00”W	Inter-tidal sediment
4	Shetland	Site 1 Bridge of Twatt	SH-BT	OS grid: HU323526	Inter-tidal sediment
		Site 2 Snaraness	SH-SN1	60° 17' 43.04"N 01° 34' 09.28"W	Inter-tidal seaweeds
		NE Snaraness	SH-SN2	60° 17' 43.04"N 01° 34' 09.28"W	Inter-tidal sedment
		Site 3 East Burra Firth	SH-BF	60° 18' 14.99"N 01° 20' 50.69"W	Inter-tidal sediment
		Voe of Firth	SH-VF	60^{o} 14’ 31.20”N 1^{o} 22’ 40.68”W	Sediment, 12m

Location number	Location name	Site	Sub-site code	Co-ordinates	Site description
7	North Uist	Bagh a Chaise, Sound of Harris IT5SW	NU-BC	57° 38' 47.81"N 07° 04' 42.29"W	Inter-tidal seaweed
		Loch Blathaisbhal 1	NU-LB	57° 37' 19.33"N 07° 11' 48.23"W	Sub-tidal sediment
		Traigh Athmor IT1	NU-TA1	57° 38' 28.20"N 07° 12' 59.28"W	Inter-tidal sediment
		Traigh Athmor IT2	NU-TA2	57° 38' 58. 80"N 07° 15' 50.82"W	Front salt marsh sediment
		Traigh Athmor IT3	NU-TA3	57° 38' 58.86"N 07° 15' 48.30"W	Salt marsh
		Loch Portain 1	NU-LP1	57° 37' 54.93"N 07° 06' 55.07"W	Sub-tidal sediment
		Loch Portain SW2	NU-LP2	57° 37' 18.72"N 07° 09' 02.80"W	Seaweeds
		Loch Maddy Harbour Core 3	NU-LM	57° 35' 52.43'' N 07° 09' 05.01'' W	Sediment, 8m
		Loch Maddy 1B	NU-LM	57° 36' 17.75"N 07° 09' 43.50"W	Seaweeds
		Aird Heisgeir	NU-AH	57° 36' 17.75"N 07° 09' 40.50"W	
11	Baltic	C-Ha-1-low salinity, Hanoby	BA-HA	55° 38' 00.00”N 14° 50’ 00.00”E	Sediment, 15-65m, 7-13 ppt
		C-An-1-normal salinity, Anholt	BA-AN	56° 26’ 02.88”N 11° 50’ 02.58”E	Sediment, 12-30m, 18-32 ppt
18	Cork	Timoleague, County Cork	CK-TM	51° 38' 29.40''N 08° 45' 44.50''W	Estuarine inter-tidal sediment
		Ring, County Cork	CK-RG	51° 36’ 39.50”N 08° 51’ 14.00”W	Estuarine inter-tidal sediment
		Lisseycrimeen, County Cork	CK-LC	51° 35’ 47.49”N 08° 45’ 56.52”W	Estuarine inter-tidal sediment

Location number	Location name	Site	Sub-site code	Co-ordinates	Site description
		Clonakilty, County Cork	CK-CL	51° 36’ 17.20”N 08° 52’ 29.59”W	Estuarine inter-tidal sediment
		Dingle Peninsula, SW Ireland	CK-DP	52° 08' 13.83''N 10°17'11.89''W	Inter-tidal sediment
		Adrigole, Beara Peninsula, SW Ireland	CK-AG	51° 41’ 27.72”N 09° 43' 38.08''W	Estuarine inter-tidal sediment
22	Baie de Seine	Col 7d	BA-1	49° 31’ 50.4”N 0° 1’ 6.18”E	Sediment, 18.2m
		Col 28a	BA-2	49° 20’ 3.96”N 0° 6’ 1.5”W	Sediment, 12.5m

Table 3

Sampling information for the time series study (Chapter 4)

Table 3: Sampling from the time series study (Chapter 4) at the Dunstaffnage site locality detailing the collection date, position and water depth and general comments such as diving conditions and marine benthos. Data provided by Dr Martin Sayer.

Sample pot no.	Collection date	Collection time	Position		depth (m)	General comments
			Lat	Long		
30A	27-Sep-08	15:10	056° 27.403' N	005° 26.614' W	34	Soft mud with some weed on the sea bottom. 1-2m visibility
30B	27-Sep-08	15:10	056° 27.403' N	005° 26.614' W	34	Soft mud with some weed on the sea bottom. 1-2m visibility
32A	30-Oct-08	12:38	056° 27.403' N	005° 26.614' W	30.5	Soft mud with some weed on the sea bottom. 1-2m visibility
32B	30-Oct-08	12:38	056° 27.403' N	005° 26.614' W	30.5	Soft mud with some weed on the sea bottom. 1-2m visibility
33 A	18-Nov-08	09:58	056° 27.403' N	005° 26.614' W	33.1	Soft mud with some weed on the sea bottom. 1-2m visibility
33B	18-Nov-08	09:58	056° 27.403' N	005° 26.614' W	33.1	Soft mud with some weed on the sea bottom. 1-2m visibility
34A	08-Dec-08	14:48	056° 27.403' N	005° 26.614' W	33.2	Soft mud with some weed on the sea bottom. 1-2m visibility
34B	08-Dec-08	14:48	056° 27.403' N	005° 26.614' W	33.2	Soft mud with some weed on the sea bottom. 1-2m visibility
35A	19-Jan-09	12:38	056° 27.403' N	005° 26.614' W	33.3	Soft mud with some weed on the sea bottom. 1-2m visibility
35B	19-Jan-09	12:38	056° 27.403' N	005° 26.614' W	33.3	Soft mud with some weed on the sea bottom. 1-2m visibility
36A	19-Feb-09	12:35	056° 27.403' N	005° 26.614' W	32	Soft mud with some weed on the sea bottom. 3-4m visibility
36B	19-Feb-09	12:35	056° 27.403' N	005° 26.614' W	32	Soft mud with some weed on the sea bottom. 3-4m visibility

Sample pot no.	Collection date	Collection time	Position Lat	Long	depth (m)	General comments
37A	03-Mar-09	15:18	056° 27.403' N	005° 26.614' W	32	Soft mud with some weed on the sea bottom. 1-2m visibility
37B	03-Mar-09	15:18	056° 27.403' N	005° 26.614' W	32	Soft mud with some weed on the sea bottom. 1-2m visibility
38A	16-Mar-09	15:47	056° 27.403' N	005° 26.614' W	32	Soft mud with some weed on the sea bottom. 1-2m visibility
38B	16-Mar-09	15:47	056° 27.403' N	005° 26.614' W	32	Soft mud with some weed on the sea bottom. 1-2m visibility
40A	17-Apr-09	11:35	056° 27.403' N	005° 26.614' W	34	Soft mud with some weed on the sea bottom. 1-2m visibility
40B	17-Apr-09	11:35	056° 27.403' N	005° 26.614' W	34	Soft mud with some weed on the sea bottom. 1-2m visibility
42 A	11-May-09	14:33	056° 27.403' N	005° 26.614' W	30.6	Soft mud with some weed on the sea bottom. 3-4m visibility
42B	11-May-09	14:33	056° 27.403' N	005° 26.614' W	30.6	Soft mud with some weed on the sea bottom. 3-4m visibility
44A	10-Jun-09	15:06	056° 27.403' N	005° 26.614' W	31	Soft mud with some weed on the sea bottom. 3-4m visibility
44B	10-Jun-09	15:06	056° 27.403' N	005° 26.614' W	31	Soft mud with some weed on the sea bottom. 3-4m visibility
47B	28-Jul-09	11:22	056° 27.403' N	005° 26.614' W	33.9	Soft mud with some weed on the sea bottom. 1-2m visibility. Heavy plankton in upper 10-15m of water column.
48A	12-Aug-09	10:14	056° 27.403' N	005° 26.614' W	33	Soft mud with some weed on the sea bottom. 1-2m visibility. Heavy plankton in upper 10-15m of water column.

Sample pot no.	Collection date	Collection time	Position		depth (m)	General comments
			Lat	Long		
48B	12-Aug-09	10:14	056° 27.403' N	005° 26.614' W	33	Soft mud with some weed on the sea bottom. 1-2m visibility. Heavy plankton in upper 10-15m of water column.

Table 4

Foraminiferal taxonomic list

Table 4 Taxonomic lists of species identified in the time series study (Chapter 4).

Adercotryma glomeratum (Brady, 1878)
Ammonia genotype S5a
Ammonia genotype S6
Ammoscalaria pseudospiralis (Williamson, 1858)
Amphicoryna scalaris (Batsch, 1791)
Astrononion gallowayi (Loeblich and Tappan,1953)
*Bolivina pseudoplicata (*Heron-Allen & Earland, 1930)
Bolivina pseudopunctata (Höglund, 1947)
Bolivina spathulata (Williamson, 1858)
Bolivina variabilis (Williamson, 1858)
B*ulimina marginata* (d'Orbigny, 1826)
*Cibicides lobatulu*s (Walker and Jacob, 1798)
Cibicides sp.
Cornuspira involvens (Reuss, 1850)
Dentalina sp. 1
Dentalina sp.2
Eggerelloides scaber (Williamson, 1858)
Elphidium sp.
Elphidium genotype S10 (*E. crispum*, Linnaeus, 1758)
Fissurina lucida (Willamson, 1858)
Guttulina sp.
Fissurina orbignyana (Seguenza, 1862)
Lagena clavata (d'Orbigny, 1846)
Lagena semistriata (Williamson 1858)
Lagena striata (d'Orbigny, 1839)
Lenticulina sp.
Miliolid sp1
Miliolid sp.2
Miliolinella subrotunda (Montagu, 1803)
Nonionella turgida (Williamson, 1858)
Pyrgo williamsoni (Silvestri, 1923)
Quinqueloculina seminulum (Linnaeus, 1758)
Reophax fusiformis (Williamson, 1858)
*Reophax scotti (*Chaster, 1892)
Reophax sp.
Rosalina anomala (Terquem, 1875)
Rosalina bradyi (Cushman 1915)
Rosalina praegeri (Heron-Allen & Earland, 1913)
Spiroplectammina earlandi (Parker, 1952)
Stainforthia fusiformis (Williamson, 1848)
Stainforthia sp.1
*Textularia bocki (*Höglund, 1947)
*Textularia sp1. (*cf *T. earlandi,* Parker, 1952)
Textularia sp2.
Trifarina fluens (Todd, 1948)
Trochammina bradyi (Robertson, 1891)
Trochammina sp.

Table 5

Foraminiferal species counts (Chapter 4)

Table 5 Species counts of live Rose Bengal stained foraminifera for the seasonal study conducted in Chapter 4. The counts illustrated are the combination of the two replicate samples.

	Sep-08	Oct-08	Nov-08	Dec-08	Jan-09	Feb-09	Mar-09	Apr-08	May-08	Jun-08	Jul-09	Aug-08
Adercotryma glomeratum	0	2	0	1	0	2	0	3	1	2	6	1
***Ammonia* juvenile**	0	0	1	1	0	0	1	1	0	0	5	8
***Ammonia* S5a**	25	41	53	86	39	20	25	26	18	17	19	76
***Ammonia* S6**	48	20	16	16	45	69	62	71	35	52	9	26
Ammoscalaria pseudospiralis	2	3	5	19	18	4	62	42	29	13	49	31
Amphicoryna scalaris	0	1	0	0	1	3	1	1	2	2	0	1
Astrononion gallowayi	5	4	1	0	6	3	5	9	6	1	2	0
Bolivina pseudoplicata	0	4	1	3	1	2	2	0	1	0	0	1
Bolivina pseudopunctata	12	12	3	0	26	13	25	34	15	15	45	10
Bolivina spathulata	5	4	5	1	6	2	8	10	0	0	1	4
Bolivina variabilis	0	0	0	0	0	0	0	1	0	0	0	0
Bulimina marginata	15	6	6	6	19	9	9	18	12	14	16	44
Cibicides lobatulus	0	0	7	6	1	0	1	0	0	1	0	0
***Cibicides* sp. 1**	0	0	0	1	0	0	0	0	0	0	0	0
Cornuspira involvens	1	0	0	0	0	0	0	0	0	0	0	1
***Dentalina* sp. 1**	1	0	1	0	1	0	1	1	1	1	1	0
***Dentalina* sp.2**	0	0	0	0	0	0	0	0	1	0	0	0
Eggerelloides scaber	10	8	20	36	15	8	8	25	9	9	10	12
***Elphidium crispum* (genotype S10)**	0	0	14	31	0	0	0	0	0	0	1	3
***Elphidium* juvenile**	0	0	0	0	0	0	1	0	0	1	0	0
***Elphidium* sp. 1**	0	0	0	0	1	0	0	0	0	1	0	0
Fissurina lucida	0	0	0	0	0	0	1	0	0	0	0	1
Fissurina orbignyana	0	0	0	0	0	1	0	0	0	0	0	0
***Guttulina* sp.1**	0	0	0	0	0	0	1	0	0	0	0	0

	Sep-08	Oct-08	Nov-08	Dec-08	Jan-09	Feb-09	Mar-09	Apr-08	May-08	Jun-08	Jul-09	Aug-08
Indeterminate	1	0	0	0	0	0	0	0	0	0	0	0
Lagena clavata	2	0	0	0	0	1	2	3	0	0	2	0
Lagena semistriata	1	0	0	0	0	0	0	0	0	0	1	0
Lagena striata	2	0	0	1	1	1	2	0	0	3	0	3
***Lenticulina* sp.**	0	0	0	0	0	0	1	0	0	0	0	0
***Miliolid* sp.2**	1	1	0	0	1	0	1	3	0	1	1	0
***Miliolid* sp.1**	5	0	0	0	0	0	0	0	0	0	5	10
Miliolinella subrotunda	1	0	0	0	0	0	0	0	0	0	0	0
Nonionella cf iridea	0	0	0	0	0	1	0	0	0	0	0	2
Nonionella turgida	231	178	43	19	333	158	293	236	260	403	258	106
Pyrgo williamsoni	0	1	0	0	0	0	0	1	1	0	0	0
Quinqueloculina seminulum	29	3	4	1	1	2	0	0	0	0	0	4
Reophax fusiformis	1	0	0	0	1	0	6	0	1	0	4	0
Reophax scotti	0	1	4	0	0	0	0	0	0	0	0	1
***Reophax* sp. 1**	4	1	0	0	2	0	2	2	1	0	3	1
Rosalina anomala	2	0	2	6	1	1	3	0	0	2	0	1
Rosalina bradyi	0	0	0	0	0	0	0	0	0	0	1	0
Rosalina praegeri	3	0	0	7	0	0	0	0	0	0	1	1
Spiroplectammina earlandi	0	0	1	2	0	0	0	1	0	1	0	0
Stainforthia fusiformis	1	0	1	1	1	0	0	2	1	0	0	3
***Stainforthia* sp. 1**	10	11	2	0	23	11	12	18	10	21	23	11
Textularia bocki	1	0	0	0	0	0	0	0	0	0	0	1
***Textularia* sp. 1.**	0	0	0	0	1	0	2	0	0	2	0	0
***Textularia* sp.2**	0	0	0	0	0	0	0	0	0	0	1	1
Trifarina fluens	1	0	1	0	0	0	0	0	0	0	0	0
***Trochammina* bradyi**	3	0	0	0	0	0	0	0	0	0	7	3
***Trochammina* sp. 1**	1	0	0	0	1	0	0	1	0	2	1	5

	Sep-08	Oct-08	Nov-08	Dec-08	Jan-09	Feb-09	Mar-09	Apr-08	May-08	Jun-08	Jul-09	Aug-08
Total live counts	424	301	191	244	545	311	537	509	404	563	472	375

Table 6

Linear regressions (Chapter 4)

Table 6 Linear regressions conducted in Chapter 4. The shifted temperatures and salinity values, represent values which have been shifted by a month.

Linear regressions								
	Salinity				Temperature			
	Standing crop		Shifted standing crop		Standing crop		Shifted standing crop	
	r	p	r	p	r	p	R	P
Total standing crop (live foraminiferal per 100ml)	0.119	0.713	0.526	0.79	0.284	0.71	0.48	0.646
Ammonia genotype S5a	0.525	0.8	0.404	0.192	0.525	0.80	0.324	0.34
Ammonia genotype S6	0.455	0.147	0.726	0.108	0.46	0.18	0.32	0.922
Nonionella turgida	0.176	0.584	0.478	0.116	0.42	0.896	0.24	0.942
Eggerelloides scaber	0.586	0.055	0.742	0.06	0.275	0.387	0.057	0.861
Elphidium crispum	0.536	0.072	0.717	0.09	0.07	0.83	0.211	0.511

Figure 1

Personal correspondence with Professor John Haynes (Chapter 6)

Figure 1: Response of Professor Haynes (pers. comms) for detailed description of type site locality of *Elphdium williamsoni* collected in Chapter 6

20/2/13

Dear Angela,

I'm very interested to hear about your work on the Elphium williamsoni group and intended genetic appraisal. The Clettwr Transect is shown on Figure 1 of the enclosed paper (a photocopy of the Haynes and Dobson 1969 paper on the Dovey Estuary) with the foram distribution shown on Figure 14.

The transect runs out from a point about a quarter of a mile east of the railway bridge over the Clettwr. It can be easily approached from the main road (A487) at Treddol by taking the turn to Borth opposite the Wildfowler public house. About a quarter of a mile down this road there is a branch turn to the right which leads past some buildings to a track down by the canalized Clettwr for about a mile to the bridge (where there is a stile and one can cross the bridge and walk up the line to where there used to be a small hut that I used as a guide) fixing my eye on a prominent white cottage on the other side of the estuary, near Trefri.

A problem with the 1969 paper is that it was published before I revised the nomenclature and realized, amongst many other problems dealt with in the 1973 monograph, that *Elphidium 'excavatum'* of authors needed a new name. Another dominant species, *Protelphidium 'depressulum'* has also gone through some name changes, first to *Protelphidium anglicum* in the Monograph, but now *Haynesina germanica.*

After the collection of the first series of samples I went on to collect monthly for a year from the same sites. These samples were eventually worked on by Sherry Penner for her M.Sc. A copy of her thesis and the samples are now in the Natural History Museum in London. It would be very useful for you to read this and see the samples, plus the other Dovey material in the Museum. The title of the thesis is: The ecology and seasonal distribution of foraminifera in the Dovey Estuary.

If there are any problems when you come down to take your samples I would willingly act as a guide to the locality.

Best wishes

Figures 2-6

Classification and regression trees (Chapters 3 and 5)

See enclosed CD for larger images.

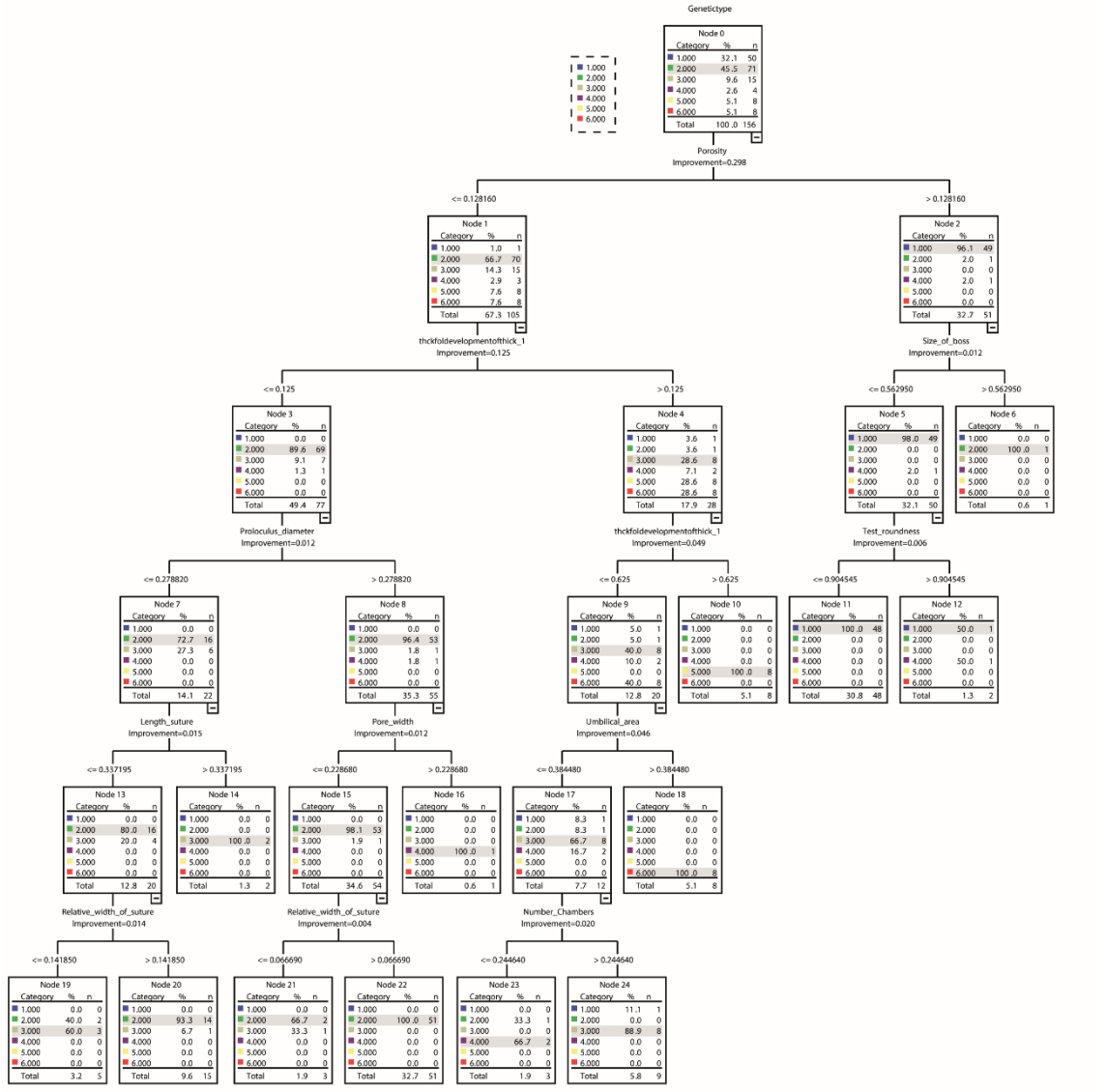

Figure 2 Classification and regression tree conducted on the morphological characteristics of six *Ammonia* genotypes in the NE Atlantic. Results are summarised in Chapter 3, Table 3.6

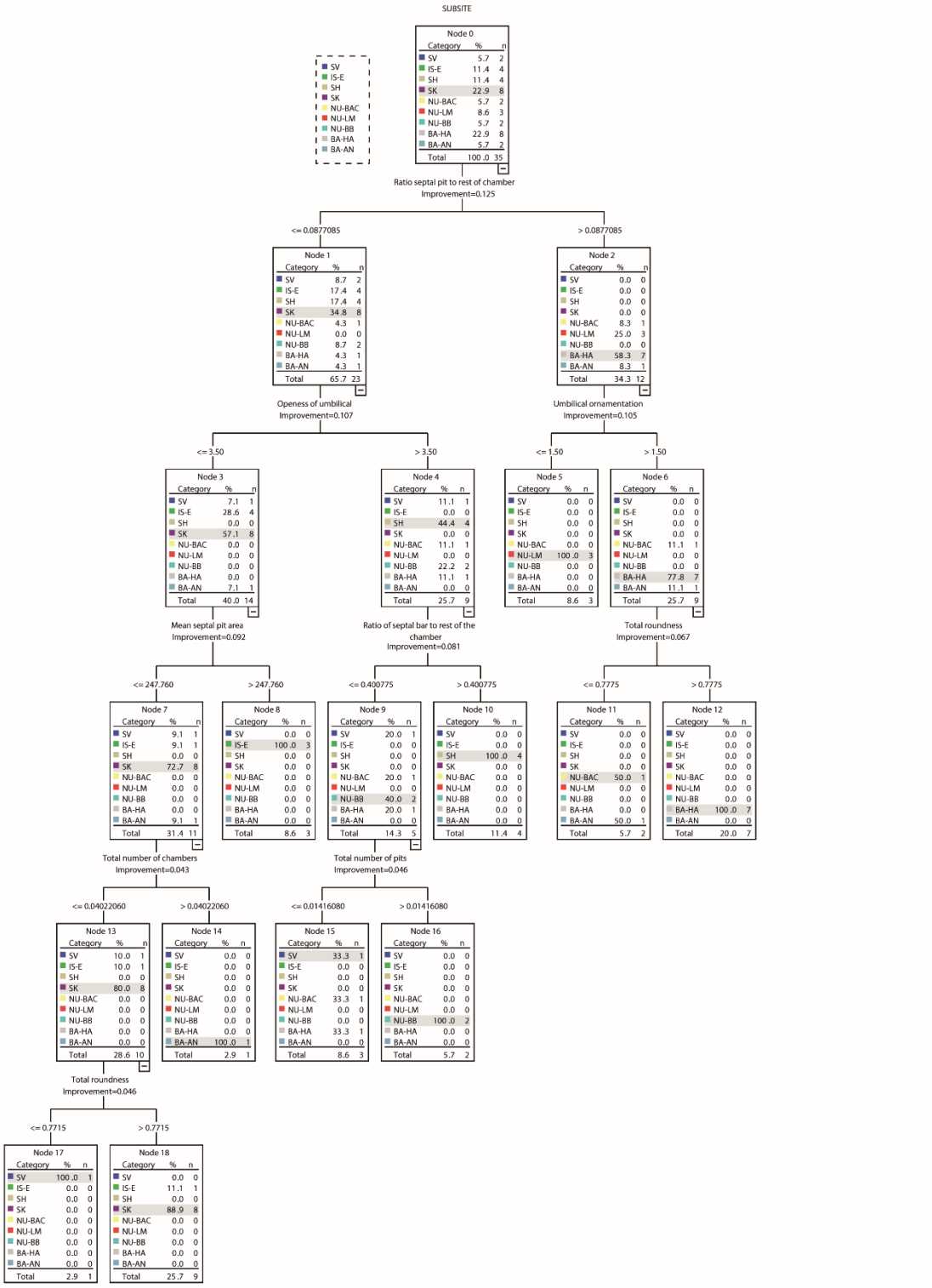

Figure 3 Intraspecific morphological variability of *Ammonia* genotype S1 across four distinctive site localities in the NE Atlantic. Results are summarised in Chapter 3, Table 3.8.

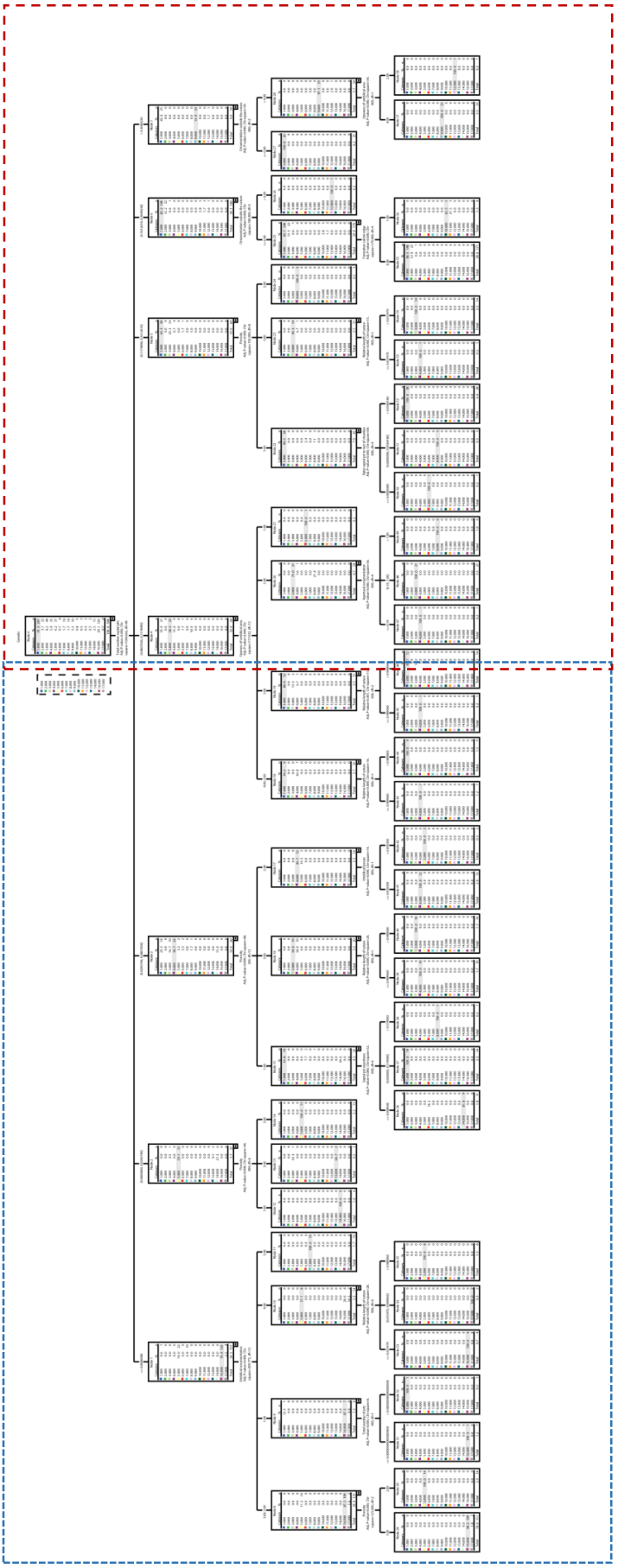

Figure 4 CHAID analysis conducted on 16 Elphidiidae genotypes across the NE Atlantic. This tree is separated into two sections A and B as shown overleaf. The results are summarised in Chapter 5, Table 5.6.

Figure 4:A

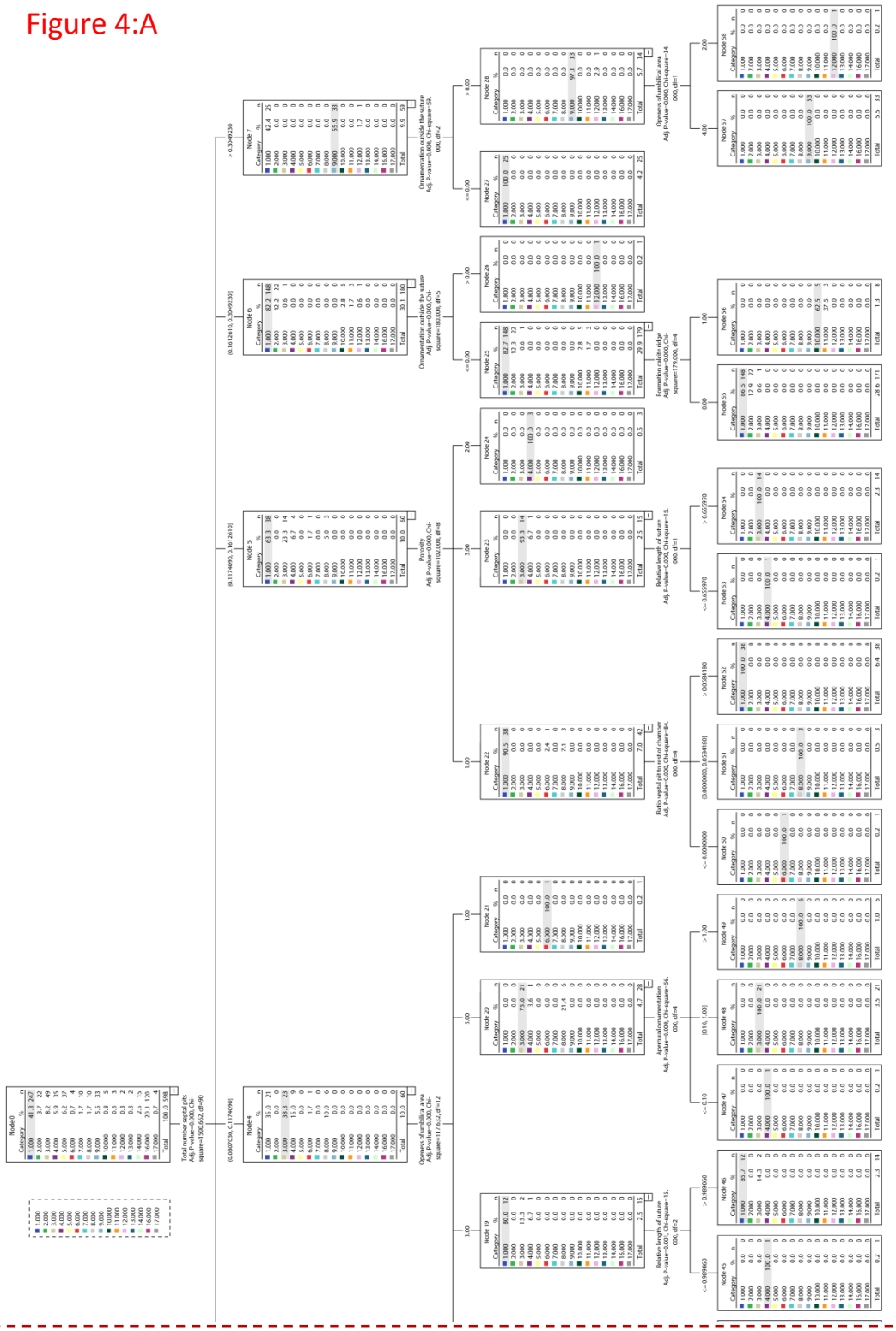

Figure 4A

Figure 4.B

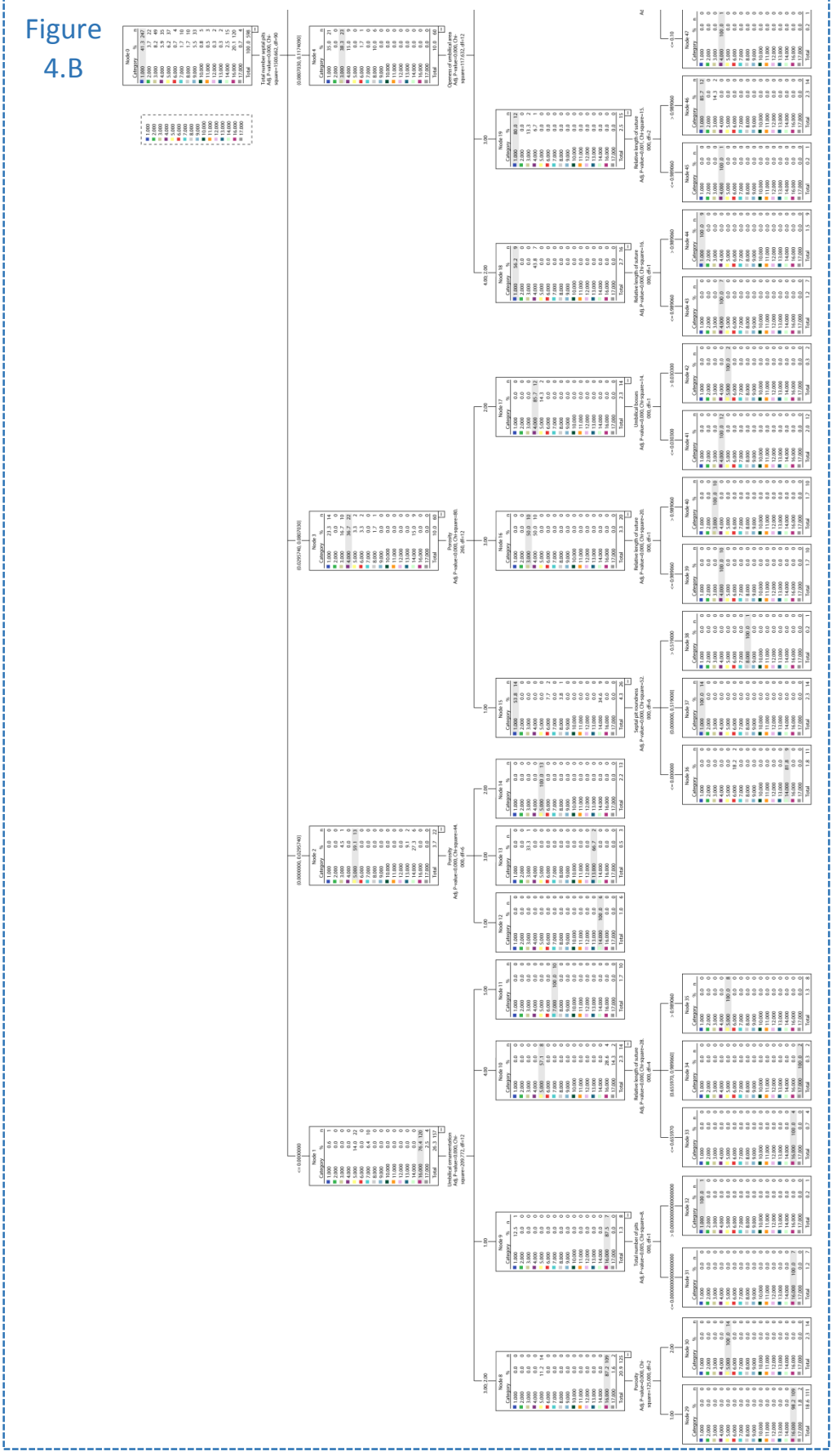

Figure 4B

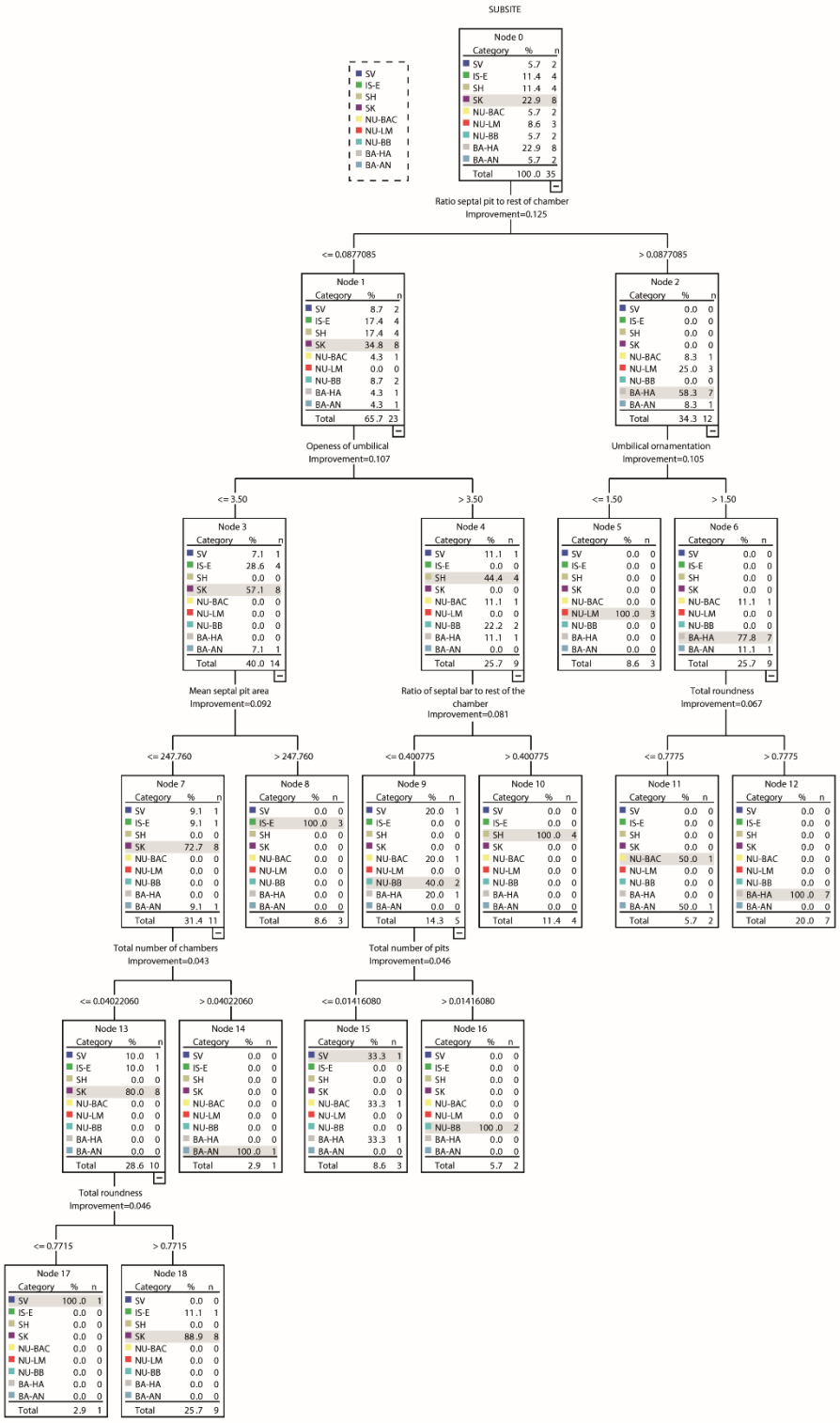

Figure 5 CART tree of the morphological characteristics of *Elphidium* S4 across nine distinct site localities in the NE Atlantic. Results are summarised in Chapter 5, Table 5.14.

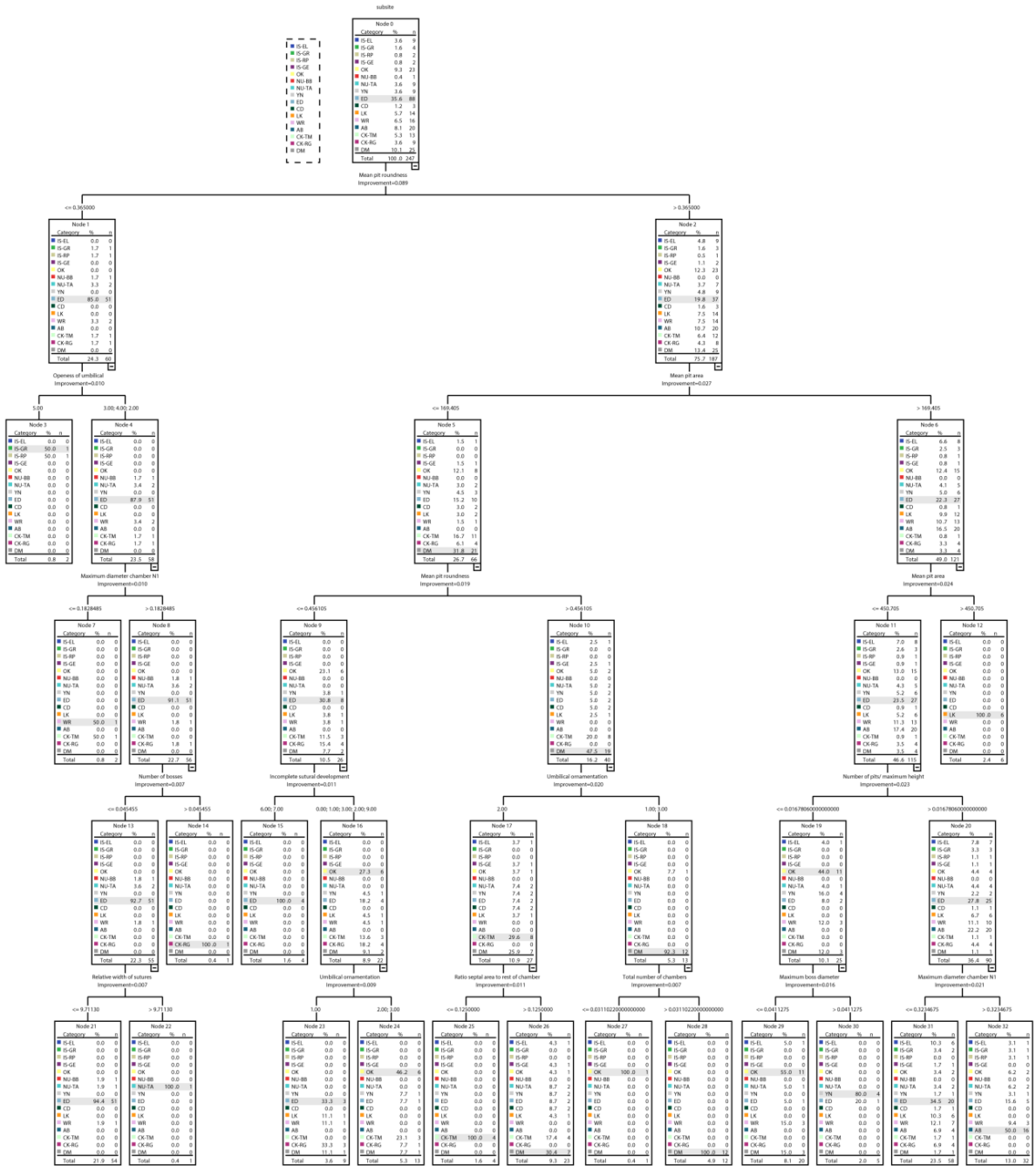

Figure 6 CART tree of morphological traits of *Elphidium* genotype S1 across 16 distinct sampling localities. Results are summarised in Chapter 5, Table 5.11.

Macros 1-2

Macros

Macro 1 for calculating foraminiferal test outline (as illustrated in Chapter 2, Section 2.5.1)

```
dir = getDirectory("image");
name = getTitle;
index = lastIndexOf(name, ".");
if (index!=-1) name = substring(name, 0, index);
name = name + ".xls";
 run("Set Scale...");
waitForUser("set the scale and press OK");
//run("Threshold...");
// Color Thresholder 1.45s
// Autogenerated macro, single images only!
min=newArray(3);
max=newArray(3);
filter=newArray(3);
a=getTitle();
run("HSB Stack");
run("Convert Stack to Images");
selectWindow("Hue");
rename("0");
selectWindow("Saturation");
rename("1");
selectWindow("Brightness");
rename("2");
min[0]=64;
max[0]=89;
filter[0]="pass";
min[1]=128;
max[1]=255;
filter[1]="pass";
min[2]=132;
max[2]=255;
filter[2]="pass";
for (i=0;i<3;i++){
```

```
  selectWindow(""+i);
  setThreshold(min[i], max[i]);
  run("Convert to Mask");
  if (filter[i]=="stop")  run("Invert");
}
imageCalculator("AND create", "0","1");
imageCalculator("AND create", "Result of 0","2");
for (i=0;i<3;i++){
  selectWindow(""+i);
  close();
}
selectWindow("Result of 0");
close();
selectWindow("Result of Result of 0");
rename(a);
// Colour Thresholding----------
run("Analyze Particles...", "size=1-Infinity circularity=0.00-1.00 show=Masks display exclude record");
run("Summarize");
saveAs("Measurements", dir+name);
print(dir+name);
selectWindow("Measurements");
        run("Close" );
```

Macro 2: Macro for calculating septal pit measurements as described in Chapter 2, Section 2.5.1.

```
dir = getDirectory("image");
name = getTitle;
index = lastIndexOf(name, ".");
if (index!=-1) name = substring(name, 0, index);
name = name + ".xls";
run("Set Scale...");
waitForUser("set the scale and press OK");
run("Threshold...");
waitForUser("set the threshold and press OK");
run("Analyze Particles...", "size=1-Infinity circularity=0.00-1.00 show=Masks display exclude record");
run("Summarize");
run("Distribution...", "parameter=Area automatic");
saveAs("Measurements", dir+name);
print(dir+name);
path2 = dir+File.nameWithoutExtension;
selectWindow("Area Distribution");
Plot.getValues(x, y);
for (i=0; i<x.length; i++)
print(x[i], y[i]);
selectWindow("Log");
saveAs("Text",path2+"Area");
print("\\Clear");
run("Distribution...", "parameter=Round automatic");
selectWindow("Round Distribution");
Plot.getValues(x, y);
for (i=0; i<x.length; i++)
print(x[i], y[i]);
selectWindow("Log");
saveAs("Text",path2+"Round");
selectWindow("Round Distribution");
run("Close" );
selectWindow("Results");
      run("Close" );
```