Universidade de Aveiro Departamento de Biologia
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Sistemática e evolução de Hydrozoa de águas costeiras e profundas do Atlântico NE

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia, realizada sob a orientação científica da Sra. Prof. Doutora Marina Ribeiro Cunha, Professora Auxiliar do Departamento de Biologia da Universidade de Aveiro, e do Sr. Prof. Doutor Alex David Rogers, Tutorial Fellow in Conservation Biology, Somerville College, Universidade de Oxford, Reino Unido.

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… e ao meu afilhado Henrique por toda a sua força de viver.
Eles terão sempre um lugar especial no meu coração.

I dedicate this work to my Mother for all the encouragement…
… and to my godchild Henrique for all his strength of living.
They will ever have a special place in my heart.

o júri / the jury

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Professora Auxiliar, Universidade de Aveiro, Portugal

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O Prof. Fran Ramil (Univ. de Vigo – Espanha) recebeu-me na sua instituição (por uma semana no primeiro ano do doutoramento) para discutir a taxonomia de hidrários Plumulariidae e Lafoeidae. Fran é um reconhecido taxonomista de hidrários e foi um grande prazer conhecê-lo e aprender com ele.

O Dr Peter Schuchert (Museu de História Natural de Genebra – Suiça) é talvez o melhor taxonomista de hidrozoários da actualidade. Sinto-me sortudo por ele me ter treinado na sistemática de hidrozoários, durante um total de duas

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Deverei também agradecer as organizações dos (dois últimos) workshops da Hydrozoan Society. Esses workshops deram-me ótimas oportunidades de conhecer e acompanhar o trabalho de grandes referências da sistemática e ecologia de hidrários, tais como os professores Wim Vervoort, Ferdinando Boero, Alvaro Peña Cantero, Stefano Piraino, Allen Collins, etc. Foi também interessante conhecer a nova geração de colegas com trabalho algo similar, destacando o Dr Lucas Leclère.
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O staff do Museu de História Natural de Paris – França (nomeadamente Aude Andouche, Pierre Lozouet e Pascale Joannot) permitiu-me o estudo de espécimes de *Nemertesia* depositados nesse museu. As coleções biológicas e bibliográficas do MHN de Paris sustêm grande parte da história da biologia marinha e senti-me privilegiado em investigar parte dessas coleções.

Um grande objetivo desta tese foi amostrar hidrários bentónicos por mergulho com escafandro autónomo em diversas localizações de águas costeiras Portuguesas, incluindo os Açores e Madeira. Pessoalmente também ambicionei conhecer as costas Portuguesas debaixo de água, e muitas pessoas e instituições ajudaram-me a alcançar esses propósitos.
Iniciei a amostragem de hidrários por mergulho no verão de 2007, na costa Algarvia e Vicentina, em colaboração com o colega André Moura (Univ. de Durham – UK) que estava estudando golfinhos no seu veleiro. André e os colegas Claudia Ferraz, Catarina Silva e José Póvoa foram os companheiros de mergulho e providenciaram uma ótima companhia de proximidade debaixo de água. Os centros de mergulho ‘Aveirosub’ de Ílhavo e ‘Pinguim sub’ de Portimão, providenciaram generosamente apoio logístico para os mergulhos.
Em 2008 tive oportunidade de participar na ‘LusoExpedição Olympus 2008’, para amostrar hidrários dos picos do Banco Gorringe. Experienciei o melhor mergulho da minha vida nesta expedição, mas também os maiores níveis de adrenalina por mergulhar em local tão remoto. Felizmente a expedição foi um sucesso e tenho portanto de agradecer aos Professores Pinto Abreu e Gonçalo Calado (ambos da Universidade Lusófona – Portugal) o convite de participação. Estou também grato aos mergulhadores/colegas José Tourais, Pedro Neves, José Pedro Borges, Joana Xavier, João Tiago Tavares e Mónica Albuquerque por me facilitarem a amostragem de hidrários. No geral a tripulação dessa expedição foi fantástica.
Em Julho de 2008 viajei para os Açores para amostrar hidrários por mergulho. A gente açoriana recebeu-me muito bem, e tenho de enviar um especial obrigado a: Dinis Geraldes e Paulo Luís (Nerus diving center – São Miguel); Maria Ana Dionisio, João Brum, Andreia Cunha e a Prof. Ana Costa (Univ.

agradecimentos (cont.)

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Prof. Fran Ramil (Univ. of Vigo – Spain) received me in his institution (for one week in the first year of Ph.D.) to discuss the taxonomy of Plumulariidae and Lafoeidae hydroids. Fran is a renewed taxonomist of hydroids and it was a great pleasure to met him and learn from him.

Dr Peter Schuchert (Natural History Museum of Geneva – Switzerland) is perhaps the best hydrozoan taxonomist presently. I feel lucky that he trained me on hydrozoan systematics, during a total of two weeks that I spent in Geneva (one in 2005 during the M.Sc., the other by the middle of Ph.D.). Peter

also taught me patiently the protocols for molecular analyses applied to hydroids. We kept regular scientific contact by email since we met; being Peter Schuchert my most complete professor of hydrozoan systematics.
I must also acknowledge the organizations of the (last two) workshops of the Hydrozoan Society. These workshops gave me great opportunities to meet and follow the work of great references of hydrozoan systematics and ecology, such as the professors Wim Vervoort, Ferdinando Boero, Alvaro Peña Cantero, Stefano Piraino, Allen Collins, etc. It was also interesting to meet the new generation of colleagues with work somewhat similar, evidencing Dr Lucas Leclère.
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A great aim of this thesis was to collect benthic hydroids by scuba-diving at several locations of Portuguese shallow waters, including the Azores and Madeira. Personally I also intended to perceive Portuguese coasts underwater, and many people and institutions helped me to achieve these endeavors.
I started the hydroid sampling by scuba-diving during the summer of 2007, in the coasts of Algarve and Alentejo, in collaboration with the colleague André Moura (Univ. of Durham – UK) that was studying dolphins in his sailing boat. André and the colleagues Claudia Ferraz, Catarina Silva and José Póvoa were the buddy-divers and provided a great company of proximity underwater. The diving centers ‘Aveirosub’ from Ílhavo and ‘Pinguim sub’ from Portimão, provided kind logistical support for the dives.
In 2008 I had opportunity to participate in the ‘LusoExpedição Olympus 2008‘ to collect hydroids from the peaks of the Gorringe Bank. I experienced the best dive of my life in this expedition, but also the higher levels of adrenaline for diving at such a remote place. Fortunately the expedition was a success and therefore I must thank Professors Pinto Abreu and Gonçalo Calado (both from the University Lusófona – Portugal) the invitation of participation. I am also indebt to the divers/collegues José Tourais, Pedro Neves, José Pedro Borges, Joana Xavier, João Tiago Tavares and Mónica Albuquerque for facilitating me the hydroid sampling. In general the crew of that expedition was fantastic.
In July of 2008 I travelled to the Azores in order to collect hydroids by scuba-diving. The Azorean people received me very good, and I must send a special thanks to: Dinis Geraldes and Paulo Luís (Nerus diving center – São Miguel Island); Maria Ana Dionisio, João Brum, Andreia Cunha and Prof. Ana Costa (University of Azores – São Miguel island); and the diving centers teams of ‘Dive Azores’ (Faial Island) and ‘Mareocidental’ (Flores Island).
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In August of 2009 I collected hydroids in the beautiful archipelago of Berlengas. The Natural Reserve of Berlengas kindly provided accommodation and transport by boat between diving spots, on the behalf of Lurdes Morais, António Casanova and the other staff of the reserve that I met. My buddy-divers were Teresa Amaro and the experienced 'local' diver Pedro Andrade e Silva. The legendary António Pata had the pacience to send us the diving-tanks refilled with air by boat to the island.
Prof. Pedro Gomes (Univ. Minho – Portugal) accompanied me underwater along two extraordinary dives (organized by him) close to Viana do Castelo – northern Portugal.

Work at the Zoological Society of London was extraordinary. I worked amidst scientists of great quality, increased my knowledge on animal conservation issues and I learned several techniques of molecular phylogenetic analyses. I highlight the fantastic colleagues that helped me most at ZSL: Bill Jordan, Dada Gotelli, Chris Yesson, Robert Pickles, Michelle Taylor, Kirsty Kemp and Ruth Brown.
The colleagues from the LEME (Laboratório de Ecologia Marinha e Estuarina) at the University of Aveiro broke the monotony during the many hours I spent in front of the microscope staring at the animals. Susana Oliveira, Fábio Matos and Isabel Oliveira were great colleagues and friends. The colleagues Clara Rodrigues, Ana Hilário and Luciana Génio collected many of the deep-sea hydroids herein mentioned from the Gulf of Cadiz and/or Alboran Sea.

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palavras-chave

Hydrozoa; Leptothecata; Taxonomia; Filogenética; Filogeografia; Evolução; espécies cripticas; Plumulariidae; Sertulariidae; Lafoeidae; Aglaopheniidae; costa Portuguesa; Golfo de Cádis; Açores; Madeira; Mar de Alboran; Banco Gorringe; Atlântico; Mediterrâneo.

resumo

O estudo da fauna Portuguesa de Hydrozoa estava abandonado há mais de meio século, exceto para o arquipélago dos Açores. Um dos principais objetivos do projecto de doutoramento foi contribuir com novos registos de hidrozoários para uma perceção mais precisa da diversidade real de hidrozoários de águas Portuguesas, incluindo os arquipélagos dos Açores e Madeira, e áreas geográficas próximas, para habitats compreendidos entre o mar profundo e o nível intertidal. Hidrários de águas superficiais de diversas regiões marinhas Portuguesas (incluindo o Banco Gorringe) foram amostradas por mergulho com escafandro autónomo. Hidrários do mar profundo, dos Açores, Madeira, Golfo de Cádis e Mar de Alboran, foram colhidos por investigadores de diferentes instituições durante variadas campanhas oceanográficas. Foi efetuada amostragem esporádica de hidrários por mergulho com escafandro autónomo no Reino Unido, Malta e Espanha.

Foram identificadas mais de 300 espécies de hidrários e foram geradas cerca de 600 sequências do ‘DNA barcode’ de hidrozoários 16S mRNA. As families Sertulariidae, Plumulariidae, Lafoeidae, Hebellidae, Aglaopheniidae, Campanulinidae, Halopterididae, Kirchenpaueriidae, Haleciidae e Eudendriidae, foram estudadas em detalhe. Foram geradas cerca de 350 sequências do marcador genético 16S para esses taxa, com a finalidade de providenciar inferências filogenéticas, filogeográficas e evolucionárias, e também identificações taxonómicas mais precisas.

As análises filogenéticas moleculares foram integradas com carateres morfológicos. Os resultados subsequentes revelaram: níveis particularmente elevados de biodiversidade críptica, polifilia em muitos grupos taxonómicos, pares de espécies que eram sinónimas, a identidade de diversas variedades como espécies válidas, e evidenciaram associações filogeográficas de hidrários em águas superficiais e profundas do Atlântico nordeste e Mediterrâneo oeste. Provou-se que muitas (mas não todas) espécies de hidrários marinhos com distribuição geográfica supostamente ampla vertical e/ou horizontalmente, de fato correspondem a complexos de taxa cripticos. Este estudo também revelou, que no Atlântico nordeste, os ambientes de águas costeiras sustêm maior diversidade e abundância de hidrozoários, mas a importância dos ambientes batiais como fonte de diversidade filogenética foi também revelada. As montanhas submarinas dos Açores revelaram ser importantes na segregação de populaçoes de hidrários com potencial dispersivo reduzido.

resumo (cont.)

Os habitats batiais do Golfo de Cádiz provaram suster um número considerávelmente grande de espécimes cripticas, o que poderá ser consequência da heterogeneidade de habitats e convergência de variadas massas de água no Golfo.

As principais causas propostas para a especiação e divergência populacional dos hidrários foram: as dimensões das populações de espécies, os mecanismos de dispersão e plasticidade que permita os hidrários habitarem diferentes condições ambientais, mas também a influência das correntes oceânicas (e suas propriedades), heterogeneidade dos habitats, alterações climáticas e deriva continental. A alta resolução filogenética obtida para a família Plumulariidae revelou particularmente que ciclos glaciais poderão ter facilitado divergência de populações, ultimamente especiação, e também transições evolucionárias faunísticas de águas profundas para superficiais.

keywords

Hydrozoa; Leptothecata; Taxonomy; Phylogenetics; Phylogeography; Evolution; Cryptic species ; Plumulariidae; Sertulariidae; Lafoeidae; Aglaopheniidae; Portuguese coast; Gulf of Cadiz; Azores; Madeira; Alboran Sea; Gorringe Bank; Atlantic; Mediterranean.

abstract

The study of the Portuguese Hydrozoa fauna has been abandoned for more than half a century, except for the Azores archipelago. One of the main aims of this Ph.D. project was to contribute new hydrozoan records leading to a more accurate perception of the actual hydrozoan diversity found in Portuguese waters, including the archipelagos of Azores and Madeira, and neighbouring geographical areas, for habitats ranging from the deep sea to the intertidal. Shallow water hydroids from several Portuguese marine regions (including the Gorringe Bank) were sampled by scuba-diving. Deep-water hydroids, from the Azores, Madeira, Gulf of Cadiz and Alboran Sea, were collected by researchers of different institutions during several oceanographic campaigns. Occasional hydroid sampling by scuba-diving was performed in the UK, Malta and Spain.

Over 300 hydroid species were identified and about 600 sequences of the hydrozoan ‘DNA barcode’ 16S mRNA were generated. The families Sertulariidae, Plumulariidae, Lafoeidae, Hebellidae, Aglaopheniidae, Campanulinidae, Halopterididae, Kirchenpaueriidae, Haleciidae and Eudendriidae, were studied in greater detail. About 350 16S sequences were generated for these taxa, allowing phylogenetic, phylogeographic and evolutionary inferences, and also more accurate taxonomic identifications.

Phylogenetic analyses integrated molecular and morphological characters. Subsequent results revealed: particularly high levels of cryptic biodiversity, polyphyly in many taxonomic groups, pairs of species that were synonymous, the identity of several varieties as valid species, and highlighted phylogeographic associations of hydroids in deep and shallow-water areas of the NE Atlantic and W Mediterranean. It was proved that many (but not all) marine hydroid species with supposedly widespread vertical and/or horizontal geographical distributions, correspond in fact to complexes of cryptic taxa. This study further revealed that, in the NE Atlantic, shallow environments sustain higher hydrozoan diversity and abundance, but the importance of bathyal habitats as a source of phylogenetic diversity was also revealed. The Azorean seamounts were shown to be particularly important in the segregation of populations of hydroids with reduced dispersive potential. The bathyal habitats of the Gulf of Cadiz proved to harbour a considerably high number of cryptic species, which may mainly be a consequence of habitat heterogeneity and convergence of various water masses in the Gulf.

abstract (cont.)

The main causes proposed for speciation and population divergence of hydroids were: species population size, dispersal mechanisms and plasticity to inhabit different environmental conditions, but also the influence of oceanic currents (and its properties), habitat heterogeneity, climate change and continental drift. Higher phylogenetic resolution obtained for the family Plumulariidae revealed particularly that glacial cycles likely facilitated population divergence, ultimately speciation, and also faunal evolutionary transitions from deep to shallow waters.

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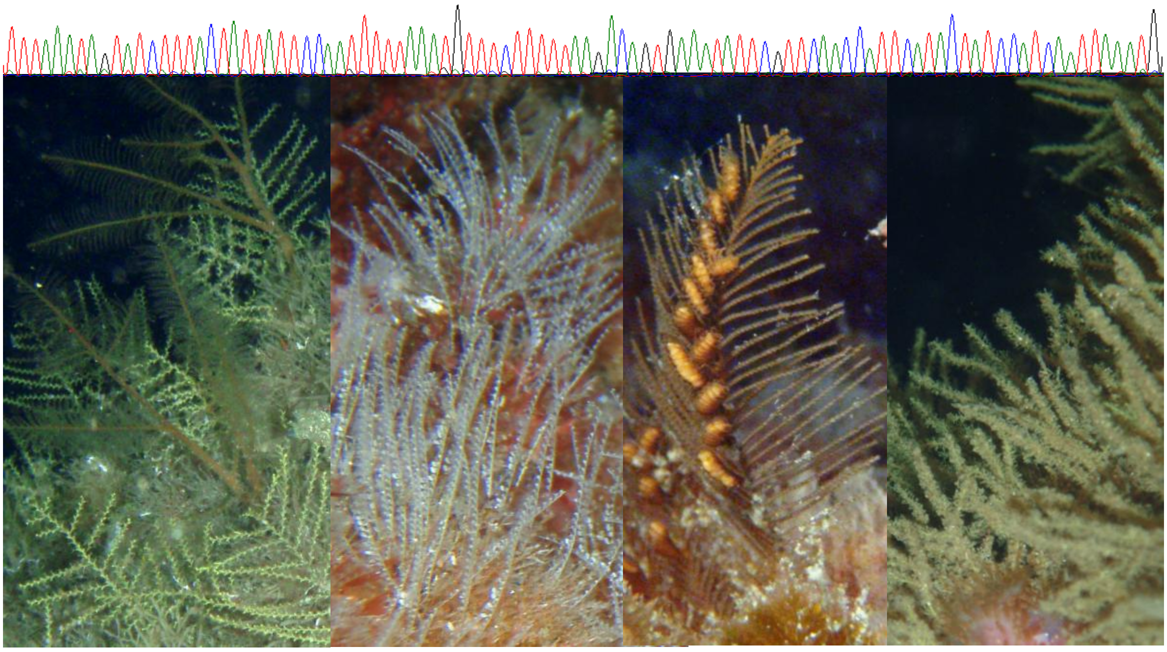
Part I

Introduction



Chapter 1

1.1 GENERAL INTRODUCTION

The oceans are the cradle of life on Earth and they harbour a higher diversity at upper taxonomic levels (phylum, class, orders), than terrestrial or freshwater ecosystems. Out of *c.* 46 phyla of animals: 35 are found in the oceans, 14 of which are exclusively marine and several others are mainly marine (e.g. Cnidaria; Sala & Knowlton 2006). However, the opposite appears true at the species level with *ca.* 1.4 million species having been described from terrestrial environments but only about 194.5 thousand from marine ecosystems (Mora *et al*. 2011). This can be explained essentially by the present lack of knowledge of biodiversity of marine environments, and by the higher investment that has been made in biological terrestrial studies. These results can be also partially explained by the high diversity of terrestrial arthropods but may also reflect the large, open-nature of marine ecosystems, resulting in large geographical distributions of populations and species and lower rates of allopatric speciation over geological time scales. This is thought to have resulted in near cosmopolitan distributions of many species, with perhaps the most striking examples being among the hydroids (Stepanyants 1980; Cornelius 1995a, b).

Hydroids or Hydrozoa were classified under the 'Vermes' (invertebrates) as 'Zoophyta' (plant-animals) by Carl Linnaeus in the 10th Edition of *Systema Naturae* in 1758. Hydroids consist of polyps which primitively have a central mouth surrounded by tentacles armed with nematocysts. These polyps can occur as solitary individuals in some species but in most usually occur as colonies where each is joined by a tube of tissue and the whole is protected by a rigid exoskeleton, the perisarc (Cornelius 1995a,b). Almost all colonies are fixed to the seabed (sessile) and have a variety of forms from root-like stolons to erect tree-like structures that can be conspicuous amongst the emergent epifauna of coastal and deep-sea ecosystems. Many common hydroids from the coastal waters of Europe were described originally by Linnaeus and, as many species in the region, have subsequently been recorded as having a near cosmopolitan distribution (Cornelius 1995a,b, e.g. *Obelia geniculata* (Linnaeus, 1758); *Plumularia setacea* (Linnaeus, 1758); *Amphisbetia operculata* (Linnaeus, 1758)). In some cases, the widespread distribution of hydroids may result from their dispersal capabilities. Some forms (e.g. *O. geniculata*) have a reproductive pelagic medusoid stage which releases gametes that undergo external fertilization producing a pelagic planula stage. In other cases (e.g. most of the Plumulariidae) the medusoid stage is reduced, effectively being retained by the hydroid colony and fertilization is external with most species being dioecious (colonies that have separate sexes). The resultant planula larvae swim or crawl away and are generally short-lived, although those of some species may take up to several days to settle and metamorphose. Many hydroids have the ability to raft on natural substrata, such as kelp holdfasts (hydrochory, e.g. Cornelius 1992, 1995a,b), and many can grow on artificial surfaces, often being a significant element of marine fouling communities (e.g. Lewbel *et al*. 1987; Relini *et al*. 1998; Yan *et al*. 2006) so passive dispersal on natural and anthropogenic materials is likely for many species. The hydroids are anatomically simple animals with few morphological characters, great phenotypic plasticity and many shared characters that represent homoplasies. There are about 3700 described species (Bouillon *et al*. 2006) but the taxonomy of the group is problematic and the potential for morphologically cryptic taxa is high (e.g. Govindarajan *et al*. 2005a; Schuchert 2005a; Moura *et al.* 2008, 2011a, c, d). Often identification of species can only be accomplished at a particular life-stage or when reproductive structures are present. Furthermore, taxonomic expertise in the group is diminishing and existing taxonomists tend to focus their studies only on particular life-stages and/or taxonomic groups. This ‘taxonomic impediment’ makes resolution of species difficult for many hydroids and the perceived widespread cosmopolitanism of the group may be erroneous.

Marine hydroids have a great variety of forms, are important components of coastal and deep-sea benthic habitats, provide substratum for the settlement of other taxa and are some of the most important sessile predators and competitors for bottom space (e.g. Bouillon *et al.* 2006). However, despite their importance in marine ecosystems, the scientific study of these animals has been highly neglected and their systematic relations are contentious and poorly resolved largely because of their morphological simplicity and plasticity. Given both the ecological significance and taxonomic difficulties of the hydrozoans, the clarification of their true diversity and a better understanding of their evolution is important. An incorrect discrimination of taxa may negatively influence ecological, biogeographic, evolutionary and applied-biotechnological studies, as well as management and conservation planning (e.g. Knowlton 2000; Bickford *et al.* 2007).

Barcoding life with standardized molecular biomarkers is increasingly being used successfully as an aid for the identification of taxa that may represent distinct and/or new species (after works of Floyd *et al*. 2002; Hebert *et al*. 2003a, b). At a time when biodiversity studies are being seen as important because of human-induced environmental change but are inhibited by the taxonomic impediment (e.g. Boero 2010), DNA barcoding has revitalized taxonomy by revealing a hitherto overlooked but significant cryptic diversity (e.g. Hebert *et al.* 2004a, b; Janzen *et al.* 2005; Smith *et al.* 2006). Such approaches also allow: the connection of life stages and genders, where species are sexually dimorphic (Beskansky *et al.* 2003; Janzen *et al.* 2005); the clarification of problems of synonymy (Alroy 2002), and the attraction of more funds and public interest in taxonomic research (Hebert & Gregory 2005). Moreover, DNA barcoding approaches have revealed the potential to test taxonomic hypotheses, including phylogenetic relationships, and can also provide new insights into evolution, especially if it is integrated with other types of data, including molecular, morphological, distributional, ecological and behavioral (e.g. Dayrat 2005; Hebert & Gregory 2005; Savolainen *et al.* 2005; Smith *et al.* 2005; Will *et al.* 2005; Padial *et al.* 2010; Moura *et al.* 2011a, b, c).

Most 'DNA barcoding' studies have focused on *ca.* 650 base pairs of the cytochrome *c* oxidase subunit 1 (COI) gene as the main barcoding gene for metazoans. This is because COI is easily amplified from diverse taxa using broad-range primers under variable laboratory conditions and protocols (Folmer *et al*. 1994; Hebert *et al*. 2003a,b; Ivanova *et al*. 2007; Kerr *et al*. 2007). It is also readily aligned for sequence comparisons, and is sufficiently divergent to discriminate species effectively across a variety of phyla, and therefore a large set of data from this gene has already been accumulated in publicly accessible data bases (Hebert *et al*. 2004a, b). Presently, over 1 300 000 DNA 'barcodes' corresponding to more than 110 800 species (www.barcodinglife.org) are already gathered. However, other genes have also been used as a basis for barcoding or for species discovery (e.g. Markmann & Tautz 2005; Mathews 2006), especially where COI does not amplify reliably for the taxon under study (Cook *et al*. 2005; Bhadury *et al*. 2006), has failed to resolve species as a result of slow evolution of the mitochondrial genome (e.g. Cnidaria; Porifera; France *et al*. 1996; van Oppen *et al*. 1999; Shearer *et al*. 2002; citing R. Watkins, pers. comm.; Duran *et al*. 2004; Hellberg 2006; Wörheide 2006), or where divergent COI sequences have been found within species (Vences *et al*. 2005). DNA barcoding, especially when used for species discovery, remains contentious, especially amongst taxonomists (Moritz & Cicero 2004; DeSalle *et al*. 2005; Ebach & Holdrege 2005; Brower 2006). Drawbacks identified with DNA barcoding approaches, have centred around the resolution of intra- and interspecific variation in DNA sequences (Hewitt 1996; Avise & Johns 1999; Hebert *et al*. 2003b; Moritz & Cicero 2004; Meyer & Paulay 2005) including problems associated with inherited symbionts (Hurst & Jiggins 2005), with introgression/ hybridization (e.g. Masta *et al*. 2002; Babik *et al*. 2005) or with very recent speciation (e.g. Johnson & Cicero 2004); the reliance on single-gene trees (DeSalle *et al*. 2005); the lack of comprehensive sampling of species of the taxa under study (Moritz & Cicero 2004; DeSalle *et al*. 2005); and failure to sample voucher specimens or to take account of existing taxonomic knowledge. Whilst these criticisms are valid, the linking of DNA sequence data with other information, such as geographical, ecological, biological or morphological data can provide a powerful way to break out of the tautological or circular reasoning that lies behind an oversimplified application of barcoding, especially when identifying new taxa (DeSalle *et al*. 2005). Examples of this include the identification of covariation between morphology and barcode sequence clusters in South American Lepidoptera (Hajibabaei *et al*. 2006) and the use of mating trials to confirm that deep genetic lineages within the bryozoan *Celleporella hyalina* are reproductively isolated (Gómez *et al*. 2007). Thus, DNA barcoding or DNA taxonomy can be a modern complement to the classification of the most basic unit of biodiversity, the species, testing and making systematics more practical, rigorous and complete.

In Hydrozoa the mitochondrial 16S rRNA gene has been more widely accepted for barcoding purposes than COI (Moura *et al.* 2008, 2011a). COI and other mitochondrial genes (including 16S) exhibit slow evolutionary rates in most Anthozoa (McFadden *et al*. 2000, 2011; Shearer *et al.* 2002; Hellberg 2006; Shearer & Coffroth 2008; Huang *et al*. 2008) and therefore COI has been assumed to be useless for DNA barcoding in this taxon. However, Sinniger *et al*. (2008) recommended that both COI and 16S markers can be useful as DNA barcodes of species of the order Zoantharia, as each gene has advantages and disadvantages. COI has been the preferred mitochondrial gene to investigate species boundaries and phylogenetics of Scyphozoa (e.g. Dawson & Jacobs 2001; Dawson & Martin 2001; Holland *et al*. 2004; Dawson 2005a, b). The 16S marker has been less used to investigate the phylogenetic relationships of the Scyphozoa (e.g. 597 COI sequences are deposited in GenBank, in contrast to only 48 sequences of 16S), but has also been used successfully in the demarcation of scyphozoan species (e.g. Schroth et *al*. 2002; Licandro *et al*. 2010). Recently Ortman *et al*. (2010) and Bucklin *et al*. (2011) extrapolated that COI is ‘broadly useful’ for DNA barcoding of species across the Medusozoa (sister clade to Anthozoa, containing Scyphozoa, Cubozoa and Hydrozoa), but is not phylogenetically informative for higher taxonomic ranks. However, the medusozoans barcoded with COI by Ortman *et al*. (2010) and Bucklin *et al*. (2011) included a considerable number of Siphonophora (subclass of Hydrozoa), but only few sequences of Cubozoa, Scyphozoa or other subclasses of the Hydrozoa. Shearer *et al.* (2002) demonstrated that the mutational rate of COI in one hydrozoan species was low, as found in the Anthozoa, but Govindarajan *et al*. (2005a) found COI to be a faster evolving gene and a more phylogenetically informative one for hydrozoan species. Despite such incongruence or lack of comprehensive studies that test the efficacy of COI as a DNA barcode across Meduzosoa, from a practical point of view, the 16S gene has been found to be much easier to amplify than COI, particularly in the Anthoathecata and Leptothecata groups (Miglietta *et al.* 2009; C. J. Moura, pers. observation). As a result of the resolution and effectiveness of 16S rDNA for barcoding, DNA taxonomy and phylogenetic analyses of Hydrozoa, 1204 16S rDNA sequences are deposited in GenBank at the present time, compared to only 476 COI sequences (an increase of about 200% of 16S sequences ‘banked’ since the last count published by Moura *et al.* 2008). The 16S gene has allowed recognition and discrimination of nominal and cryptic hydrozoan species (including invasive taxa), but also revealed reasonable potential for phylogenetic, phylogeographic and evolutionary analyses (Bridge *et al.* 1995; Romano & Palumbi 1997; Watkins & Beckenbach 1999; Schierwater & Ender 2000; Ender & Schierwater 2003; Collins *et al.* 2005; Dunn *et al.* 2005; Govindarajan *et al.* 2005a, b; Schuchert 2005a, b, 2006; Fraser *et al*. 2006; Govindarajan *et al*. 2006; Schuchert & Reiswig 2006; Galea & Leclère 2007; Hemmrich *et al*. 2007; Leclère *et al*. 2007, 2009; Miglietta *et al*. 2007, 2009; Moura *et al*. 2007, 2008, 2011a, b, c, d; Cartwright *et al*. 2008; Altuna 2009; Cartwright & Nawrocki 2010; Nawrocki *et al*. 2010;

Peña Cantero *et al*. 2010; Miranda *et al*. 2010). For hydrozoans, 16S genetic information has been found to be consistent with nuclear genetic markers (e.g. Elongation Factor 1α, Miglietta *et al*. 2009; and ITS1 and ITS2, Miranda *et al*. 2010) and also morphology (e.g. Schuchert 2005a; Miglietta *et al*. 2009; Moura *et al*. 2011a, b, c, d), further evidence of its potential as a DNA barcoding gene.

The advent of molecular techniques has made available new additional characters for objective discrimination of taxa. As a result, cryptic or pseudocryptic species have been discovered across nearly all metazoan phyla (e.g. reviews of Knowlton 2000; Bickford *et al.* 2007; Trontelj & Fišer 2009). In the Cnidaria, the reported cases of cryptic diversity are comparatively low compared to other major metazoan clades, such as the Porifera (Trontelj & Fišer 2009**)**. The limited discovery of cryptic species in cnidarians may result from a slow rate of genetic evolution, and/or it may be a consequence of regular hybridization (reviewed by Knowlton 2000). However, past studies have primarily focused on Anthozoa with the Hydrozoa having attracted relatively little attention. Recent studies have revealed cryptic or pseudocryptic species within the Hydrozoa (Govindarajan *et al*. 2005a; Schuchert 2005a; Miglietta *et al.* 2007, 2009; Moura *et al*. 2008, 2011a, c, d; Folino-Rorem *et al*. 2009), suggesting that hydrozoan diversity might be significantly underestimated. Furthermore, vast geographic areas remain unexplored and poorly sampled for Hydrozoa, including most of the deep sea. Despite a long history of research on hydrozoan systematics, dating back to the pre-Linnaean times, new taxa, even at the level of families are still being discovered (e.g. Moura *et al*. 2007).

The study of the Portuguese Hydrozoa fauna was abandoned for more than half a century (except for the Azores archipelago and more recently for the Madeira island) until the herein presented taxonomic and phylogenetic investigations of Moura (2011) and Moura *et al*. (2008, 2011a, b, c, d). Many ‘classical’ taxonomic studies of hydroids exist elsewhere in Europe, but molecular phylogenetic or evolutionary studies with these animals were still scarce in the old continent, uniquely with works of Schuchert (2005a) and Leclère *et al*. 2007, 2009). This lack of knowledge on systematics of hydroids of Portugal but also from the rest of European waters deserves scientific interest, especially when taking into consideration the usual ubiquity of hydroids in marine biotopes and their great biological significance (e.g. Bouillon *et al*. 2006). Furthermore, the Portuguese marine waters are in a key geographic position for biodiversity and biogeographic studies, because such territory represents an important biogeographic transition zone of the Boreal, Mediterranean and Mauritanian faunas. The small amount of research on hydroids of Portuguese continental waters was carried out by Nobre (1931, 1937) and Da Cunha (1940, 1941, 1944, 1950), who provided almost all our knowledge on hydroids of the Portuguese continental coast. Besides the fact that the taxonomic framework used by these authors is now obsolete, many localities of the coast were still unexplored. The Azores have been investigated in more detail by Rees & White (1966) and Cornelius (1992). Despite their addition of new records to the faunal list and the important ideas on how these hydroids may have reached these islands, these authors did not sample the archipelago uniformly and many areas remained to investigate. The archipelago of Madeira is the region of Portugal that has received the least attention, with only very few records of some deep-sea hydroid species (Medel & Vervoort 2000). More recently a list of hydroid species found in shallow-waters of Madeira was provided by Wirtz (2007).

The Plumulariidae, Aglaopheniidae, Lafoeidae and Sertulariidae, were the taxa studied in greater detail in this thesis, and are amongst the most specious hydrozoan families (Bouillon *et al*. 2006), being usually common in benthic communities of coastal to deep marine habitats worldwide. In these hydrozoan families, the medusoid phase is apparently suppressed from the life cycle of most of these hydroids (e.g. Bouillon *et al*. 2006). Instead colonies release planulae that are carried in the water column, before they settle on a substratum and metamorphose into a erect sessile colony. Because their dispersive pelagic stage is potentially short, gene-flow may be highly constrained by population size, the local current regime and availability and type of substrata. Such life-history traits suggest that dispersal in these hydroids likely fits a stepping-stone model. Therefore, because the organisms selected represent the earliest mobile metazoans, are extremely diverse, play important ecological roles, and a great part of these taxa have supposedly widespread horizontal and vertical distributions but limited dispersal mechanisms; they are ideal to test species boundaries, effective distributions of species, gene-flow between localities, and to reveal processes underlying marine phylogeography and speciation, including faunistic responses to environmental changes.

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Chapter 2

THE DISSERTATION

Contents

2.1 AIMS AND RATIONALE

The primary purpose of this work is to provide a first comprehensive insight of Hydrozoa diversity in Portuguese waters, including the Azores and Madeira and encompassing shallow- to deep-water habitats (e.g. Gulf of Cadiz). The numerous phylogenetic problems associated with hydrozoan systematics were approached by integrating diagnostic morphological characters with molecular techniques, namely phylogenetic analyses with 16S mRNA sequence data. The high 16S haplotype sampling obtained further allowed some phylogeographic and evolutionary inferences for the hydrozoan fauna of both shallow and deep waters of the NE Atlantic and western Mediterranean.

To achieve the above mentioned general aims, this project included the following specific tasks/aims:

- To collect shallow-water hydroids from several locations representative of the Portuguese territory;

- To obtain (for taxonomic identification and phylogenetic analyses) hydrozoan samples previously collected by other researchers/staff of diverse scientific institutions, especially biological material from deep waters of Portugal and adjacent areas;

- To further obtain hydroids from occasional scuba diving sampling from different locations around Europe;

- To identify the hydrozoans collected personally or obtained through collaborative research, down to the taxonomic level of species/genus, whenever possible;

- To 'DNA barcode' the 16S mRNA marker, according to the diversity and ubiquity of taxa, and dedicate higher sampling effort to the hydrozoan families better represented in both shallow and deep waters of NE Atlantic and western Mediterranean;

- To apply molecular phylogenetic analyses with the 'sequence' data obtained and published in Genbank;

- To evaluate the potential of the mitochondrial 16S rRNA gene as a tool for DNA barcoding of hydroid species;

- To evaluate the systematic resolution of the mt 16S rRNA above the species level for addressing phylogenetic questions within the Class;

- To test the monophyletic status of the hydrozoan families, and to better represent its genera and species in phylogenetic analyses;

- To solve cases of complicated synonymies and erroneous species assignments to genera;

- To investigate whether hydroid species are widespread in geographical distribution or if they are in fact complexes of cryptic taxa segregated by depth, geographical barriers (e.g. continents) or distance;

- To explain the causes underlying cryptic or pseudocryptic hydrozoan diversity detected;

- To investigate potential patterns of morphological divergence accompanying speciation;

- To investigate the extent and direction of gene flow between non-contiguous and contiguous populations;

- To investigate the evolution of Hydrozoa morphs from deep to shallow waters (or the reverse), and whether or not this has occurred multiple times;

- To contribute for the understanding of how, from where and when hydroids reached the seamounts/islands of Azores;

- To investigate the correlation between the patterns and causes of speciation found, with major events of previous geological ages, such as alterations in oceanic currents and land barriers, glaciations and other large-scale climatic changes.

2.2 THESIS ORGANIZATION

This dissertation is organized into three main parts: (I) *Introduction*; (II) *Research Work*; and (III) *Final Remarks*. Each of these is in turn composed of different chapters, in a total of ten and organized as follows.

Part I – *Introduction.* This section consists of a brief introduction for framing the reader in the basis for the development of this project. It starts by summarising a *General Introduction* (Chapter 1) regarding the need of the execution of this thesis and ends presenting *The Dissertation* (Chapter 2) aims, rationale and organization.

Part II – *Research Work*. This section is presented in seven chapters (Chapter 3 to 9). Chapters 4 to 9 were structured as scientific manuscripts, and therefore each one contains its own abstract, theoretical introduction, specific aims, applied procedures, obtained results, respective discussion and conclusions. The sequence of inclusion of chapters (and of the manuscripts) in this dissertation, is intended to reveal the logical progression of this work and its increasing complexity, particularly in chapter 9.

- Chapter 3 describes the data associated with the sampling stations studied specifically for this dissertation, and the respective taxa identified for each station. Some hydrozoan families (e.g. Haleciidae, Eudendriidae) mentioned particulary in Chapter 5 were not mentioned in Chapter 3 due to deadline constraints and the complex taxonomic problems of these groups. The list of stations and species of the hydroids collected from the Gorringe Bank are mentioned in Chapter 4.

- Chapter 4 presents the hydrozoan fauna (including all the Leptothecata and Anthoathecata species) collected from the peaks of the Gorringe Bank during the 'LusoExpedição Olympus 2008'. Some ecological and biogeographic considerations are presented.

- Chapter 5 investigates the potential of the 16S gene to 'DNA barcode' hydroids, and highlights the potential of cryptic diversity in hydroids, as well as other taxonomic inconsistencies. This chapter was the basis for the research work developed in chapters 6 to 9.

- Chapter 6 presents a manuscript concerning the phylogenetic relations and taxonomic problems associated with the family Sertulariidae. This research work revealed many probable cases of cryptic and pseudo-cryptic biodiversity, as well of potential synonymies. Some phylogeographic inferences are also presented.

- Chapter 7 addresses phylogenetic relations of the putative Superfamily Lafoeoidea and of hydroids with campanulated hydrothecae (e.g. family Campanulinidae).This manuscript/chapter highlights profound problems in the taxonomy of these taxa. For example, the Superfamily Lafoeoidea was found polyphyletic, as well as its supposed families Lafoeidae and Hebellidae. High levels of potential cryptic diversity were also found. Some phylogeographic relations are also resolved.

- Chapter 8 describes phylogenetic relationships of Aglaopheniidae from the north-east Atlantic and west Mediterranean. The highlights of this manuscript include the finding of several polyphyletic groups, probable cases of synonymies, and the identification of two undescribed species of *Aglaophenia*. Phylogeographic associations were again revealed.

- Chapter 9 resolves phylogenetic relations of plumulariids by an integrative molecular approach supported by morphologic characters and by a ‘molecular clock’ approach. High levels of cryptic diversity are revealed, as well as important inferences on the mechanisms of hydrozoans population divergence and speciation. Phylogeographic assumptions supported by the dating of phylogenetic nodes were also performed.

Part III – *Final Remarks*. Because specific discussions and conclusions from Chapter 4 to 9 are offered, this final part is a brief compilation of the main findings of all the research work presented in this dissertation.

Part II

Research Work

Chapter 3

TAXONOMIC LIST AND THE SAMPLING STATIONS OF LEPTOTHECATA HYDROIDS (CAMPANULINIDAE, TIARANNIDAE, HEBELLIDAE, LAFOEIDAE, SERTULARIIDAE, KIRCHENPAUERIIDAE, HALOPTERIDIDAE, PLUMULARIIDAE, AGLAOPHENIIDAE) FROM THE NE ATLANTIC AND WEST MEDITERRANEAN

Contents

3.1 INTRODUCTION

This chapter presents the data associated with the sampling stations studied for this dissertation, with the respective taxa of 9 hydrozoan families (Campanulinidae, Tiarannidae, Hebellidae, Lafoeidae, Sertulariidae, Kirchenpaueriidae, Halopterididae, Plumulariidae and Aglaopheniidae) identified at each of these stations. These were the hydrozoan families best studied phylogenetically in the course of this Ph.D. thesis. The taxa with specimens ‘DNA barcoded’ for the 16S mRNA gene are identified in bold in the following faunal list presented in this chapter.

A high sampling effort of hydroids at selected localities along the Portuguese coasts was achieved by scuba diving up to depths of around 40 m. The mainland of Portugal was extensively sampled from north to south by scuba diving, as well as the Madeira Island, and three islands (São Miguel, Faial, Flores) of the Archipelago of the Azores. The hydrozoan fauna from the peaks of the Gorringe Bank (also located in waters of Portuguese jurisdiction) was also extensively studied taxonomically, but it is only presented in the next chapter of this dissertation. In order to complement faunal lists and phylogenetic analyses, occasional sampling by scuba diving was additionally performed in Terceira Island (Azores), Malta Island, Benidorm (Spain) and in the SW of the United Kingdom.

Some shallow water hydroids were also sampled from ship hulls, pontoons, and artificial substrata in marinas and naval ports in selected points of the Portuguese coast, especially in Algarve, Sines, Madeira Island and in the marinas of Horta and Ponta Delgada (Azores). This sampling strategy was meant to provide ecological information on native and invasive species, on the role of ships in the introduction of the latter and to posteriorly presume their environmental impact in the native assemblages. The recognition of the taxa with potential of being transported by boats was considered in the molecular phylogenetic inferences presented in chapters 5 to 9 of this Ph.D. dissertation.

An extensive collection of hydroids from bathyal depths of the Gulf of Cadiz, collected during 14 oceanographic campaigns, was studied in great detail. One of these oceanographic campaigns (TTR17) also collected hydroids from bathyal depths of the Alboran Sea, which were also included in taxonomic and molecular studies. A wide range of sampling devices was used to collect hydroids in these oceanographic campaigns; these included: Tv-grabs, remoted operated vehicles (ROVs), box-cores and dredges.

Representative collections of hydroid specimens essentially from deep waters of the Azores and Madeira, principally sampled as part of by-catch studies, were also investigated. That biological material was borrowed for the completion of this thesis aims, respectively, from the Department of Fisheries and Oceanography – University of the Azores, and the Municipal Museum of Funchal – Biological Station of Funchal, Madeira. The fishing devices used to collect these hydroids included ‘long lines with multiple hooks’ (in Portuguese: ‘palangre de fundo’, ‘gorazeira’, ‘trole’ and ‘espinhel’) and ‘fish traps' (in Portuguese: 'covos'). The 'CoralFish' project also provided specimens sampled by submersible from the Pico-Faial channel (Azores).

3.2 LIST OF THE STATIONS AND SPECIES STUDIED

3.2.1 PORTUGAL (main land)

3.2.1.1 Coastal waters

Southern coast

Albufeira: marina, 37°05.019'N 8º15.903'W, 0-0,5 m, dock; 28 March 2007.

Sertulariidae undet..

Alvor beach: 37º07.000'N 8º35.000'W, *ca.* 10 m, scuba diving, rocky seafloor; 14 July 2007.

Lafoeina tenuis Sars, 1874, - *Cuspidella* sp., - *Dynamena disticha* (Bosc, 1802), - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Antennella secundaria* (Gmelin, 1791), - ***Aglaophenia pluma* (Linnaeus, 1758)**.

Albufeira: Olhos d'Água beach, 37°05.319'N 8°11.452'W, 2-3 m, scuba diving, rocky seafloor; 23 July 2007.

Modeeria rotunda (Quoy & Gaimard, 1827), - *Lafoeina tenuis* Sars, 1874, - *Scandia gigas* (Pieper, 1828), - *Sertularella mediterranea* Hartlaub, 1901, - ***Salacia desmoides* (Torrey, 1902)**, - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Kirchenpaueria similis* (Hincks, 1861), - ***Aglaophenia pluma* (Linnaeus, 1758)**.

Carvoeiro: Hotel Tivoli beach, Lagoa, 1-8 m, snorkling, rocky wall of northern side of islet; March 2007.

Sertularella lagenoides Stechow, 1919, - *Aglaophenia pluma* (Linnaeus, 1758).

Carvoeiro: Lagoa, 37º05.419'N 8º27.269'W, 1-8 m, scuba diving, rocky wall; 21 July 2007.

Lafoeina tenuis Sars, 1874, - ***Scandia* cf. *gigas* (Pieper, 1828)**, - *Salacia desmoides* (Torrey, 1902), - *Kirchenpaueria similis* (Hincks, 1861), - *Halopteris diaphana* (Heller, 1868), - ***Plumularia setacea* (Linnaeus, 1758)**, - ***Aglaophenia pluma* (Linnaeus, 1758)**.

Lagos: Ponta da Piedade, 37°04.753'N 8°40.088'W, 1-8 m, scuba diving, rocky wall; 24 July 2007.

Phialella quadrata (Forbes, 1848), - cf. *Hebella scandens* (Bale, 1888), - *Lafoea* cf. *dumosa* (Fleming, 1820), - *Sertularella mediterranea* Hartlaub, 1901, - ***Salacia desmoides* (Torrey, 1902)**, - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Antennella secundaria* (Gmelin, 1791), - *Halopteris diaphana* (Heller, 1868), - *Monotheca pulchella* (Bale, 1882), - *Plumularia setacea* (Linnaeus, 1758), - ***Aglaophenia pluma* (Linnaeus, 1758)**.

Olhão: naval club, 37°01.399'N 7°50.189'W, 0-0,5 m, 3 docks, cables and bouys; 23 July 2007.

Lafoeina tenuis Sars, 1874, - *Kirchenpaueria bonnevieae* (Billard, 1906).

Sagres: Ilhas do Martinhal, 37º00.897'N 8º55.080'W, 5-15 m, scuba diving, rocky seafloor and islet walls; 1 August 2007.

Campanulina pumila (Clarke, 1875), - *Sertularella mediterranea* Hartlaub, 1901, - *Sertularia distans* Lamouroux, 1816, - *Kirchenpaueria similis* (Hincks, 1861), - *Antennella secundaria* (Gmelin, 1791), - *Halopteris diaphana* (Heller, 1868), -

***Plumularia setacea* (Linnaeus, 1758)**, - ***Aglaophenia pluma* (Linnaeus, 1758)**, - *Aglaophenia kirchenpaueri* (Heller, 1868).

Sagres: outside harbour, 37°00.514'N 8°55.500'W, 4-20 m, scuba diving, rocky wall; 2 August 2007.

cf. *Hebella scandens* (Bale, 1888), - *Synthecium evansi* (Ellis & Solander, 1786), - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Antennella secundaria* (Gmelin, 1791), - *Halopteris catharina* (Johnston, 1833), - *Plumularia setacea* (Linnaeus, 1758), - *Aglaophenia pluma* (Linnaeus, 1758), - *Gymnangium montagui* (Billard, 1912).

Sagres: Ponta de Sagres, 36º59.700’N 8º56.715’W, 4-22 m, scuba diving, rocky bottom and wall; 11 August 2007.

Filellum cf. *serratum* (Clarke, 1879), - *Sertularella mediterranea* Hartlaub, 1901, - *Sertularia distans* Lamouroux, 1816, - ***Amphisbetia operculata* (Linnaeus, 1758)**, - *Diphasia margareta* (Hassall, 1841), - *Antennella secundaria* (Gmelin, 1791), - ***Nemertesia antennina* (Linnaeus, 1758)**, - ***Plumularia setacea* (Linnaeus, 1758)**, - *Aglaophenia acacia* Allman, 1883, - *Aglaophenia pluma* (Linnaeus, 1758), - *Aglaophenia kirchenpaueri* (Heller, 1868), - ***Gymnangium montagui* (Billard, 1912)**.

Sagres: Ponta dos Caminhos, 37º01.21ºN 8º54.33’W, 12-18 m, scuba diving, rocky bottom and wall; 12 August 2007.

cf. *Lafoeina tenuis* Sars, 1874, - *Sertularella ellisii* (Deshayes & Milne Edwards, 1836), - *Salacia desmoides* (Torrey, 1902), - *Sertularia distans* Lamouroux, 1816, - *Amphisbetia operculata* (Linnaeus, 1758), - *Kirchenpaueria similis* (Hincks, 1861), - *Antennella secundaria* (Gmelin, 1791), - *Halopteris diaphana* (Heller, 1868), - *Monotheca pulchella* (Bale, 1882), - ***Nemertesia antennina* (Linnaeus, 1758)**, - *Plumularia setacea* (Linnaeus, 1758), - *Aglaophenia pluma* (Linnaeus, 1758), - *Aglaophenia kirchenpaueri* (Heller, 1868), - *Gymnangium montagui* (Billard, 1912).

Sagres: Ponta da Atalaia, 37º00.033'N 8º55.882'W, 16-22 m, scuba diving, rocky bottom; 8 June 2008.

***Nemertesia antennina* (Linnaeus, 1758)**

Vilamoura: marina, 37°04.729'N 8º07.221'W, 0-0,5 m, mussels over dock; 28 March 2007.

Lafoeina tenuis Sars, 1874.

South-western coast

Aljezur: island close to restaurant 'Sitio do Forno', 37°10.158'N 8°54.226'W, 2-11 m, scuba diving, rocky wall; 4 August 2007.

Modeeria rotunda (Quoy & Gaimard, 1827), - *Lafoeina tenuis* Sars, 1874, - *Lafoea dumosa* (Fleming, 1820), - ***Sertularella mediterranea* Hartlaub, 1901**, - ***Salacia desmoides* (Torrey, 1902)**, - *Sertularia distans* Lamouroux, 1816, - *Amphisbetia operculata* (Linnaeus, 1758), - *Kirchenpaueria similis* (Hincks, 1861), - *Antennella secundaria* (Gmelin, 1791), - *Halopteris diaphana* (Heller, 1868), - *Monotheca pulchella* (Bale, 1882), - *Plumularia setacea* (Linnaeus, 1758), - ***Aglaophenia parvula* Bale, 1882**.

Odemira: Vila Nova de Milfontes, near local fishing port, 37°44.242'N 8°48.100'W, 3-5 m, scuba diving, rocky sea-floor; 23 August 2007.

Scandia gigas (Pieper, 1828), - *Salacia desmoides* (Torrey, 1902), - *Sertularia distans* Lamouroux, 1816, - *Kirchenpaueria similis* (Hincks, 1861), - *Monotheca pulchella* (Bale, 1882).

Porto Covo: Aljezur, 37°51.192'N 8º48.211'W, 15-20 m, scuba diving, rocky sea-floor and wall; 2 September 2007.

Lafoea cf. *dumosa* (Fleming, 1820), - cf. *Hebella scandens* (Bale, 1888), - *Sertularella mediterranea* Hartlaub, 1901, - *Sertularia distans* Lamouroux, 1816, -

Amphisbetia operculata (Linnaeus, 1758), - *Synthecium evansi* (Ellis & Solander, 1786), - *Antennella secundaria* (Gmelin, 1791), - ***Nemertesia antennina* (Linnaeus, 1758)**, - ***Plumularia setacea* (Linnaeus, 1758)**, - *Aglaophenia pluma* (Linnaeus, 1758), - ***Gymnangium montagui* (Billard, 1912)**.

Sines (marina): ship-hull of 'Indiana', 37°04.729'N 8º07.221'W, 1 m, boat from the UK; 18 August 2007.

Sertularia distans Lamouroux, 1816, - *Campomma* cf. *hincksii* (Hartlaub, 1897).

Sines (marina): ship-hull of 'Chiquitita', 37°04.729'N 8º07.221'W, 1 m, ship-hull not cleaned for 2 years, boat from Sweden; 18 August 2007.

Filellum cf. *serratum* (Clarke, 1879), - *Plumularia setacea* (Linnaeus, 1758), - *Campomma* cf. *hincksii* (Hartlaub, 1897).

Sines (marina): ship-hull of 'Shatatu': 37°04.729'N 8º07.221'W, 1-3 m, ship-hull moored at the marina for more than 1 year, boat from the Mediterranean; 18 August 2007.

Lafoeina tenuis Sars, 1874, - *Kirchenpaueria halecioides* (Alder, 1859), - *Campomma* cf. *hincksii* (Hartlaub, 1897).

Sines: outside harbour, 37º56.323'N 8º53.330'W, 10-15 m, scuba diving, rocky sea-floor and wall; 27 August 2007.

Sertularella mediterranea Hartlaub, 1901, - *Sertularia distans* Lamouroux, 1816, - *Kirchenpaueria similis* (Hincks, 1861), - *Antennella secundaria* (Gmelin, 1791), - *Halopteris diaphana* (Heller, 1868), - ***Monotheca obliqua* (Johnston, 1847)**, - ***Plumularia setacea* (Linnaeus, 1758)**, - *Aglaophenia pluma* (Linnaeus, 1758).

Sines (marina): 37°04.729'N 8º07.221'W, 0-0.5 m, mussels over dock; 27 August 2007.

Lafoeina tenuis Sars, 1874.

Sines: São Torpes, 37º54.770'N 8º48.467'W, 9-13 m, scuba diving, rocky tunnels; 02 September 2007.

***Lafoea* cf. *dumosa* / sp.1 (Fleming, 1820)**, - cf. *Hebella scandens* (Bale, 1888), - *Dynamena disticha* (Bosc, 1802), - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Antennella secundaria* (Gmelin, 1791), - ***Nemertesia antennina* (Linnaeus, 1758), - *Plumularia setacea* (Linnaeus, 1758)**, - ***Aglaophenia pluma* (Linnaeus, 1758).**

Western coast

Berlengas: Berlenga Island (southern side), 39°24.540'N 9°30.708'W, 5-14 m, scuba diving, rocky walls and tunnels; 11 August 2009.

Lafoeina tenuis Sars, 1874, - *Campomma hincksii* (Hartlaub, 1897), - *Lafoea* cf. *dumosa* (Fleming, 1820), - *Filellum* cf. *serratum* (Clarke, 1879), - ***Filellum* cf. *scandens* (Hassall, 1848)**, - *Sertularella mediterranea* Hartlaub, 1901, - ***Salacia desmoides* (Torrey, 1902)**, - ***Sertularia distans* Lamouroux, 1816**, - ***Diphasia margareta* (Hassal, 1848)**, - *Antennella secundaria* (Gmelin, 1791), - *Halopteris diaphana* (Heller, 1868), - *Aglaophenia pluma* (Linnaeus, 1758), - ***Aglaophenia kirchenpaueri* (Heller, 1868)**.

Berlengas: Farilhões, 39º28.494'N 9º32.640'W, 17-22 m, scuba diving, rocky wall and seafloor; 12 August 2009.

***Sertularelloides cylindritheca* (Allman, 1888), - *Nemertesia ramosa* (Lamarck, 1816)**.

Berlengas: Farilhões (cave), 39º28.351'N 9º32.821'W, 28-32 m, scuba diving, rocky wall and seafloor; 12 August 2009.

Lafoea* cf. *dumosa* (Fleming, 1820) / sp1**, - *Filellum* cf. *serratum* (Clarke, 1879), - ***Sertularella gayi* (Lamouroux, 1821)**, - *Sertularella mediterranea* Hartlaub, 1901, - ***Sertularella polyzonias* (Linnaeus, 1758)**, - ***Sertularelloides cylindritheca

(Allman, 1888), - *Sertularia distans* Lamouroux, 1816, - ***Diphasia delagei*** **(Billard 1912)**, - *Antennella secundaria* (Gmelin, 1791), - ***Nemertesia ramosa*** **(Lamarck, 1816)**, - ***Plumularia setacea*** **(Linnaeus, 1758)**, - *Aglaophenia parvula* Bale, 1882.

Berlengas: Rinchão, 39º25.437'N 9º29.941'W, 25-35 m, scuba diving, rocky wall; 13 August 2009.

Campomma hincksii (Hartlaub, 1897), - *Filellum* cf. *serratum* (Clarke, 1879), - ***Hebella scandens*** **(Bale, 1888) / sp1**, - ***Lafoea*** **cf.** ***dumosa*** **(Fleming, 1820) / sp1**, - *Sertularella gayi* (Lamouroux, 1821), - ***Sertularella ornata*** **Broch, 1933**, - *Sertularelloides cylindritheca* (Allman, 1888), - *Sertularella mediterranea* Hartlaub, 1901, - *Dynamena disticha* (Bosc, 1802), - *Sertularia distans* Lamouroux, 1816, - ***Diphasia margareta*** **(Hassal, 1848)**, - ***Diphasia rosacea*** **(Linnaeus, 1758)**, - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Antennella secundaria* (Gmelin, 1791), - ***Plumularia setacea*** **(Linnaeus, 1758)**, - *Aglaophenia acacia* Allman, 1883, - *Aglaophenia parvula* Bale, 1882, - *Aglaophenia kirchenpaueri* (Heller, 1868), - ***Aglaophenia tubulifera*** **(Hincks, 1861)**, - ***Gymnangium montagui*** **(Billard, 1912)**.

Berlengas: Estelas, 39º25.225'N 9º32.084'W, 15-26 m, scuba diving, rocky wall and bottom; 13 August 2009.

Lafoeina tenuis Sars, 1874, - *Campomma hincksii* (Hartlaub, 1897), - *Lafoea* cf. *dumosa* (Fleming, 1820), - ***Sertularella ellisii*** **(Deshayes & Milne Edwards, 1836)**, - *Sertularia distans* Lamouroux, 1816, - ***Diphasia margareta*** **(Hassal, 1848)**, - ***Diphasia rosacea*** **(Linnaeus, 1758)**, - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Antennella secundaria* (Gmelin, 1791), - *Monotheca pulchella* (Bale, 1882), - ***Nemertesia antennina*** **(Linnaeus, 1758)**, - ***Plumularia setacea*** **(Linnaeus, 1758)**, - ***Aglaophenia pluma*** **(Linnaeus, 1758)**, - *Aglaophenia kirchenpaueri* (Heller, 1868), - ***Aglaophenia tubulifera*** **(Hincks, 1861)**, - ***Gymnangium montagui*** **(Billard, 1912)**.

Berlengas: Estelas, Baixa Alagada, 39º25.477'N 9º32.120'W, 30-38 m, scuba diving, rocky wall and seafloor; 14 August 2009.

Lafoeina tenuis Sars, 1874, - *Campomma hincksii* (Hartlaub, 1897), - **cf. *Hebella scandens* / sp.2 (Bale, 1888)**, - ***Lafoea* cf. *dumosa* (Fleming, 1820) / sp1**, - ***Filellum* cf. *serratum* (Clarke, 1879) / sp1**, - cf. *Staurodiscus* sp., - *Sertularella gayi* (Lamouroux, 1821), - ***Sertularella ornata* Broch, 1933**, - *Sertularella* cf. *mediterranea* Hartlaub, 1901, - *Sertularella* cf. *lagenoides* Stechow, 1919, - ***Sertularelloides cylindritheca* (Allman, 1888)**, - ***Dynamena disticha* (Bosc, 1802)**, - *Sertularia distans* Lamouroux, 1816, - *Diphasia margareta* (Hassal, 1848), - ***Diphasia rosacea* (Linnaeus, 1758)**, - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Antennella secundaria* (Gmelin, 1791), - *Aglaophenia pluma* (Linnaeus, 1758), - *Aglaophenia kirchenpaueri* (Heller, 1868), - ***Aglaophenia tubulifera* (Hincks, 1861)**, - *Gymnangium montagui* (Billard, 1912).

Berlengas: Berlenga Island (northern side), Ilhéu do Soldado, 39º24.981'N 9º30.784'W, 5-16 m, scuba diving, rocky walls; 14 August 2009.

Campomma hincksii (Hartlaub, 1897), - *Filellum* cf. *serpens* (Hassall, 1848), - *Sertularella mediterranea* Hartlaub, 1901, - *Salacia desmoides* (Torrey, 1902), - *Sertularia distans* Lamouroux, 1816, - *Halopteris diaphana* (Heller, 1868), - ***Monotheca obliqua* (Johnston, 1847)**, - ***Plumularia setacea* (Linnaeus, 1758)**, - ***Aglaophenia parvula* Bale, 1882**, - ***Aglaophenia kirchenpaueri* (Heller, 1868)**.

Cascais (marina): Cascais, 38º41.454'N 9º25.165'W, 0-0,5 m, dock; 24 May 2007.

***Plumularia setacea* (Linnaeus, 1758)**.

Cascais: Boca do Inferno, 38°41.442'N 9°25.866'W, 5-12 m, scuba diving, rocky wall; 3 September 2007.

Campanulinidae undet., - *Sertularella ellisii* (Deshayes & Milne Edwards, 1836), - *Sertularia distans* Lamouroux, 1816, - *Kirchenpaueria pinnata* (Linnaeus, 1758), - ***Aglaophenia parvula* Bale, 1882**, - *Aglaophenia pluma* (Linnaeus, 1758).

Ericeira: Coxos, 39º00.151’N 9º25.672’W, Ericeira, 4-8 m, scuba diving, rocky seafloor; 4 September 2007.

Sertularella mediterranea Hartlaub, 1901, - *Salacia desmoides* (Torrey, 1902), - *Sertularia distans* Lamouroux, 1816, - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Kirchenpaueria similis* (Hincks, 1861), - ***Monotheca obliqua*** **(Johnston, 1847)**, - ***Plumularia setacea*** **(Linnaeus, 1758)**, - ***Aglaophenia parvula*** **Bale, 1882**.

Northern coast

Ilhavo: Ria de Aveiro, ‘triângulo’, 40°38.690'N 8°44.032'W, *ca.* 3 m; mussels on rock; July 2005.

Plumularia setacea (Linnaeus, 1758).

Ilhavo: Praia da Barra, beach, 40° 37.917'N 8°45.050'W, 0 m, floating algae; 13 September 2009.

Dynamena pumila (Linnaeus, 1758).

Viana do Castelo: close to shore, 41º41.680’N 8º52.711’W, 29-33 m, scuba diving, rocky canyons; 19 August 2009.

Sertularella polyzonias (Linnaeus, 1758), - *Sertularia distans* Lamouroux, 1816, - ***Thuiaria articulata*** **(Pallas, 1766)**, - ***Nemertesia antennina*** **(Linnaeus, 1758)**, - ***Plumularia setacea*** **(Linnaeus, 1758)**.

Off Viana do Castelo: 41º37.379’N 8º53.805’W, 25-33 m, scuba diving, rocky wall and bottom, 19 August 2009.

Lafoeina tenuis Sars, 1874, - *Tiaropsis multicirrata* (Sars, 1835), - *Lafoea* cf. *dumosa* (Fleming, 1820), - *Filellum* cf. *serratum* (Clarke, 1879), - ***Sertularella* cf. *mediterranea*** **Hartlaub, 1901**, - *Salacia desmoides* (Torrey, 1902), - ***Sertularia***

***distans* Lamouroux, 1816**, - *Amphisbetia operculata* (Linnaeus, 1758), - *Abietinaria abietina* (Linnaeus, 1758), - ***Thuiaria articulata* (Pallas, 1766)**, - *Halopteris diaphana* (Heller, 1868), - ***Nemertesia antennina* (Linnaeus, 1758)**, - *Aglaophenia pluma* (Linnaeus, 1758), - ***Aglaophenia tubulifera* (Hincks, 1861)**, - ***Gymnangium montagui* (Billard, 1912)**.

3.2.1.2 Deep waters

Underwater mound close to Lisbon

Stn AT-688-Gr: TTR17 - leg 2, 39°04.327'N 10°33.992'W, 1294 m, TV-assisted grab, big pieces of volcanic and magmatic rocks, coral debris; 03 July 2008.

***Stegopoma bathyale* Vervoort, 1966**, - ***Zygophylax sagamiensis* Hirohito, 1983**, - ***Zygophylax sibogae* Billard, 1918**.

Stn AT-690-D: TTR17 – leg 2, 39°04.775'N 10°34.114'W, 1419 m, dredge, big pieces of volcanic and magmatic rocks, coral debris; 03 July 2008.

***Zygophylax leloupi* Ramil & Vervoort, 1992a**.

Stn AT-691-D: TTR17 – leg 2, 39°05.220'N 10°34.060'W, 1384 m, dredge, big pieces of volcanic and magmatic rocks, coral debris; 03 July 2008.

***Cryptolaria pectinata* (Allman, 1888)**.

3.2.2 AZORES

3.2.2.1 Coastal waters

São Miguel Island

Caloura: 37º42.815'N 25º29.705'W, 5-10 m, scuba diving, rock; 5 July 2008.

Sertularella cf. *fusiformis* (Hincks, 1861), - ***Dynamena disticha* (Bosc, 1802)**, - *Kirchenpaueria similis* (Hincks, 1861), - *Aglaophenia pluma* (Linnaeus, 1758).

Doris wreck: near Ponta Delgada, 37º44.591'N 25º37.647'W, 16-21 m, scuba diving; 10 July 2008.

Filellum cf. *serratum* (Clarke, 1879), - *Hebellopsis scandens* (Bale, 1888), - ***Sertularella* sp.**, - *Salacia desmoides* (Torrey, 1902), - *Dynamena disticha* (Bosc, 1802), - *Kirchenpaueria similis* (Hincks, 1861), - *Antennella secundaria* (Gmelin, 1791), - *Halopteris diaphana* (Heller, 1868), - *Aglaophenia pluma* (Linnaeus, 1758).

Feteiras do Sul: 37º42.836’N 25º29.756’W, 5-13 m, scuba diving, rocky wall of islet; 6 July 2008.

Lafoeina tenuis G.O. Sars, 1874, - *Sertularella* cf. *fusiformis* (Hincks, 1861), - *Salacia desmoides* (Torrey, 1902), - ***Dynamena disticha* (Bosc, 1802)**, - *Antennella secundaria* (Gmelin, 1791), - ***Plumularia setacea* (Linnaeus, 1758)**, - ***Aglaophenia pluma* (Linnaeus, 1758)**.

Mosteiros: 37º53.372’N 25º50.132’W, 18-23 m, scuba diving, rocky bottom and walls; 07 July 2008.

Filellum cf. *serratum* (Clarke, 1879), - ***Sertularella mediterranea* Hartlaub, 1901**, - ***Salacia desmoides* (Torrey, 1902)**, - *Dynamena disticha* (Bosc, 1802), - ***Sertularia marginata* (Kirchenpauer, 1864)**, - ***Amphisbetia operculata* (Linnaeus, 1758)**, - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Antennella secundaria* (Gmelin, 1791), - ***Monotheca obliqua* (Johnston, 1847)**, - ***Aglaophenia pluma* (Linnaeus, 1758)**, - ***Aglaophenia picardi* Svoboda, 1979**.

Recife da Cadeia: 37º44.415'N 25º39.234'W, 15-19 m, scuba diving, rocky bottom; 10 July 2008.

Filellum cf. *serratum* (Clarke, 1879), - *Sertularella* cf. *fusiformis* (Hincks, 1861), - *Dynamena disticha* (Bosc, 1802), - *Kirchenpaueria similis* (Hincks, 1861), - *Antennella secundaria* (Gmelin, 1791), - *Halopteris diaphana* (Heller, 1868), - *Aglaophenia pluma* (Linnaeus, 1758).

Santa Iria: N side of island, port, 37º50.150'N 25º29.081'W, 5-18m, scuba diving, rock; 9 July 2008.

Lafoeina tenuis G.O. Sars, 1874, - *Sertularella* cf. *fusiformis* (Hincks, 1861), - *Dynamena disticha* (Bosc, 1802), - *Kirchenpaueria similis* (Hincks, 1861), - *Antennella secundaria* (Gmelin, 1791), - *Aglaophenia pluma* (Linnaeus, 1758).

Ponta Delgada naval club: 37°44.446'N 25°39.476'W, 0-0.5 m, buoys and dock; 10 July 2008.

Opercularella lacerata (Johnson, 1847), - *Kirchenpaueria halecioides* (Alder, 1859), - ***Plumularia setacea* (Linnaeus, 1758)**.

Vila Franca do Campo: 37º42.332'N 25º26.755'W, 8-18 m, scuba diving, rocky wall; 8 July 2008.

Filellum cf. *serratum* (Clarke, 1879), - *Sertularella* cf. *fusiformis* (Hincks, 1861), - ***Salacia desmoides* (Torrey, 1902)**, - *Dynamena disticha* (Bosc, 1802), - *Antennella secundaria* (Gmelin, 1791), - ***Aglaophenia picardi* Svoboda, 1979**.

Terceira Island

Hotel Caracol: 37º44.590'N 25º37.647ºW, 4-10 m, scuba diving, rocky bottom and underwater pipe; 11 July 2008.

***Sertularella mediterranea* Hartlaub, 1901**, - *Salacia desmoides* (Torrey, 1902), - *Dynamena disticha* (Bosc, 1802), - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Antennella secundaria* (Gmelin, 1791).

Faial Island

Boca das Caldeirinhas: 38º30.938'N 28º37.625'W, 15-40 m, scuba diving, rocky wall; 15 July 2008.

Lafoeina tenuis G.O. Sars, 1874, - ***Sertularella* cf. *fusiformis* (Hincks, 1861) / sp2**, - *Salacia desmoides* (Torrey, 1902), - *Dynamena disticha* (Bosc, 1802), - *Antennella secundaria* (Gmelin, 1791), - *Halopteris diaphana* (Heller, 1868), - *Plumularia setacea* (Linnaeus, 1758), - ***Aglaophenia pluma* (Linnaeus, 1758)**.

Entre Montes: near Horta, 38º31.800’N 28º37.662’W, 3-18 m, scuba diving, rocky seafloor; 17 July 2008.

Sertularella cf. *fusiformis* (Hincks, 1861), - ***Dynamena disticha* (Bosc, 1802)**, - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Antennella secundaria* (Gmelin, 1791), - *Monostaechas quadridens* (Mccrady, 1859), - *Halopteris diaphana* (Heller, 1868), - ***Monotheca margaretta* Nutting, 1900**, - *Aglaophenia pluma* (Linnaeus, 1758).

Gruta da Pedra: 38º30.950'N 28º37.618'W, 5-16 m, scuba diving, rocky cave and islet nearby; 15 July 2008.

***Sertularella* cf. *fusiformis* (Hincks, 1861) / (sp2)**, - ***Salacia desmoides* (Torrey, 1902)**, - *Dynamena disticha* (Bosc, 1802), - ***Amphisbetia operculata* (Linnaeus, 1758)**, - ***Plumularia setacea* (Linnaeus, 1758)**, - *Aglaophenia pluma* (Linnaeus, 1758).

Horta port: 38°32.015'N 28°37.304'W, 5-18 m, scuba diving, bottom rocks; 17 July 2008.

cf. *Anthohebella parasitica* (Ciamician, 1880), - ***Sertularella* cf. *fusiformis* (Hincks, 1861) / (sp2)**, - *Kirchenpaueria halecioides* (Alder, 1859), - ***Aglaophenia picardi* Svoboda, 1979**.

Horta (marina): ship-hull of ‘Sun Magic 44’, 38°31.685'N 28° 37.552'W, 0.5-2 m, scuba diving, last clean of metallic hull in France 2 years ago, from Guadelupe but had been previously in the Mediterranean; July 2008.

Cuspidella sp..

Horta (marina): ship-hull of ‘ SEA Gypoy’, 38°31.685'N 28° 37.552'W, 0.5-2 m, scuba diving, hull of wood, last clean in USA in April 2007, from Rhoad Island directly to Azores; July 2008.

Sertularella cf. *fusiformis* (Hincks, 1861), - *Plumularia setacea* (Linnaeus, 1758).

Horta (marina): ship-hull of ‘ Wondering Albatroz’, 38°31.685'N 28° 37.552'W, 0.5-2 m, scuba diving, last clean of metallic hull in May 2008, from Ascencion Island to Faial; July 2008.

Filellum cf. *serpens* (Hassall, 1848), - cf. *Zygophylax* sp., - *Plumularia setacea* (Linnaeus, 1758).

Horta (marina): pontoon A, 38°31.685'N 28° 37.552'W, 0-0,5 m; July 2008.

Lafoeina tenuis G.O. Sars, 1874, - *Plumularia setacea* (Linnaeus, 1758).

Ilhéu Negro: near Horta, 38º31.205ºN 28º37.929ºW, 5-16 m, scuba diving, rocky walls; 13 July 2008.

Sertularella sp., - ***Dynamena disticha* (Bosc, 1802)**, - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Antennella secundaria* (Gmelin, 1791), - *Aglaophenia pluma* (Linnaeus, 1758).

Pico-Faial channel: 46 m, R/V Águas Vivas, voucher DOP C3021; 16 June 2008.

***Aglaophenia pluma* (Linnaeus, 1758)**.

Flores Island

Fajã Grande: 39°27.575'N 31°15.721'W, 3-10 m, scuba diving, rocky wall, 19 July 2008.

Lafoeina tenuis G.O. Sars, 1874, - *Sertularella* cf. *fusiformis* (Hincks, 1861), - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Monotheca pulchella* (Bale, 1882), - *Aglaophenia pluma* (Linnaeus, 1758).

Ponta Delgada: 39º31.714'N 31º12.148'W, 20-27 m, scuba diving, rocky bottom; 20 July 2008.

Lafoeina tenuis G.O. Sars, 1874, - ***Sertularella* cf. *fusiformis* (Hincks, 1861) / (sp2)**, - *Dynamena disticha* (Bosc, 1802), - ***Plumularia setacea* (Linnaeus, 1758)**, - ***Aglaophenia pluma* (Linnaeus, 1758)**.

Ponta Delgada: 39º31.092'N 31º12.437'W, 5-24 m, scuba diving, rocky bottom and wall; 20 July 2008.

Sertularella cf. *fusiformis* (Hincks, 1861), - *Salacia desmoides* (Torrey, 1902), - ***Dynamena disticha* (Bosc, 1802)**, - ***Amphisbetia operculata* (Linnaeus, 1758)**, - *Plumularia setacea* (Linnaeus, 1758), - *Aglaophenia pluma* (Linnaeus, 1758).

NE Flores: 39º28.531'N 31º08.479'W, 10-16 m, rocky canyons; 21 July 2008.

Lafoeina tenuis G.O. Sars, 1874, - *Filellum* cf. *serratum* (Clarke, 1879), - ***Anthohebella parasitica* (Ciamician, 1880)**, - *Sertularella* sp- ***Salacia desmoides* (Torrey, 1902)**, - ***Dynamena disticha* (Bosc, 1802)**, - ***Dynamena quadridentata* (Ellis & Solander, 1786)**, - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Halopteris diaphana* (Heller, 1868), - *Monotheca pulchella* (Bale, 1882), - ***Plumularia setacea* (Linnaeus, 1758)**, - ***Aglaophenia picardi* Svoboda, 1979**.

NE Flores: Islet, 39º29.044'N 31º08.739'W, 5-30 m, scuba diving, rocky wall, 21 July 2008.

Lafoeina tenuis G.O. Sars, 1874, - *Filellum* cf. *serratum* (Clarke, 1879), - *Sertularella* cf. *fusiformis* (Hincks, 1861), - *Salacia desmoides* (Torrey, 1902), - *Dynamena disticha* (Bosc, 1802), - *Halopteris diaphana* (Heller, 1868), - ***Aglaophenia picardi*** **Svoboda, 1979**.

3.2.2.2 Deep waters

Açor Bank: ArqDAÇO, Estrato 7 I.S., voucher DOP C2315; 14 June 2007.

Diphasia pinastrum (Cuvier, 1830), - *Polyplumaria flabellata* G.O. Sars, 1874.

Atlantis Seamount : TTR12, 34°00.355’N 30°00.460'W, R/V Prof. Logachev, Stn AT-421-D, 555-614 m, dredge; 24 August 2002.

Acryptolaria cf. *conferta* (Allman, 1877), - *Lafoea* cf. *dumosa* (Fleming, 1820), - *Sertularella gayi* (Lamouroux, 1821), - *Sertularelloides cylindritheca* (Allman, 1888), - *Polyplumaria flabellata* G.O. Sars, 1874, - *Antennella secundaria* (Gmelin, 1791), - ***Nemertesia norvegica*** **(Sars, 1873)**.

Atlantis Seamount: TTR12, 34°05.831'N 30°11.719'W, R/V Prof. Logachev, Stn AT-422-Gr, 375 m, TV-guided grab; 24 August 2002.

Filellum cf. *serratum* (Clarke, 1879).

Azores Bank: 38°09.420'N 29°01.560'W, 307 m, F/V Neuza Mar, espinhel, hydroids associated with *Leiopathes* cf. *glaberrina*, vouchers: DOP C1349, C1364; 23 March 2007.

Filellum cf. *serratum* (Clarke, 1879), - *Acryptolaria* sp1., - *Diphasia margareta* (Hassall, 1841), - *Antennella secundaria* (Gmelin, 1791).

Azores Bank: 38°13.440'N 28°59.700'W, 402 m, F/V Conde de Porto Pim, hand-line, voucher DOP C1407; 28 March 2007.

***Aglaophenopsis cartieri* (Bedot, 1921)**.

Azores Bank: 38°06.480'N 29°00.720’W, 307 m, F/V Neuza Mar, palangre de fundo, vouchers: DOP C1431, C1433; 29 March 2007.

***Aglaophenopsis cartieri* (Bedot, 1921)**, **- *Nemertesia* cf. *belini* (Quelch, 1885)**.

Azores Bank: 38º10.020’N 29º00.000’W, 219-329 m, F/V Manuel Arriaga, espinhel, vouchers: DOP C1709, C1711; 6 May 2007.

***Nemertesia norvegica* (Sars, 1873)**.

Azores Bank: 38º14.280’N 28º54.120’W, 452-492 m, R/V Arquipélago, ARQDAÇO-27-P07, Palangre de fundo, voucher DOP C2363; 06 June 2007.

***Nemertesia* cf. *antennina* (Linnaeus, 1758) */* sp6**.

Azores Bank: 38°13.800' 28°59.400'W, 310 - 350 m, R/V Arquipélago ARQDAÇO-27-P07, palangre de fundo, voucher DOP C2316; 10 June 2007.

***Diphasia pinastrum* (Cuvier, 1830)**.

Azores Bank: 38°13.200'N 29°02.400'W, 258 m, R/V Arquipelago, ARQDAÇO-27-P07, palangre de fundo, voucher DOP C2377; 27 June 2007.

***Lytocarpia myriophyllum* (Linnaeus, 1758)**.

Azores Bank: 38°06.000'N 29°09.000'W, 169 – 594 m, F/V Manuel Arriaga, espinhel, voucher DOP C1974; 3 July 2007.

Lytocarpia myriophyllum (Linnaeus, 1758).

Azores Bank: 38°12.300'N 28°58.140'W, 183 m, F/V Manuel Arriaga, espinhel, vouchers: DOP C1981, C1982, C1985; 10 July 2007.

cf. *Lafoea dumosa* (Fleming, 1820), - *Antennella secundaria* (Gmelin, 1791), - *Aglaophenia* sp1., - *Aglaophenia tubulifera* (Hincks, 1861).

Azores Bank: 38°13.200'N 28°55.200'W, 434 m, R/V Arquipelago, ARQDAÇO-27-P07, palangre de fundo, voucher DOP C2647.

***Lytocarpia myriophyllum* (Linnaeus, 1758)**.

Azores Bank: 38°18.000'N 28°53.340'W, 468 m, F/V Manuel Arriaga, espinhel, voucher DOP C1500; 2 November 2007.

***Sertularella* cf. *unituba* Calder, 1991**.

Azores Bank: 219-329 m, Horta fishing fleet, voucher DOP C2120; 13 April 2008.

Polyplumaria flabellata (Sars, 1873).

Azores Bank: 38°12.900'N 28°59.400'W, F/V Baia das Lajes, palangre, voucher DOP C398;26 May 2008.

Antennella secundaria (Gmelin, 1791), - *Polyplumaria flabellata* (Sars, 1873).

Azores Bank: 38°12.900'N 28°59.100'W, 236 m, F/V Baia das Lajes, palangre, voucher DOP 2542; 26 May 2008.

Zygophylax sp., - *Diphasia margareta* (Hassall, 1841), - *Diphasia pinastrum* (Cuvier, 1830), - *Aglaophenia tubulifera* (Hincks, 1861).

Azores Bank: 38°12.900'N 28°59.400'W, 236 m, F/V Baia das Lajes, palangre, voucher DOP 2545; 26 May 2008.

Diphasia margareta (Hassall, 1841), - *Aglaophenia tubulifera* (Hincks, 1861).

Azores Bank (32 miles): 38°16.800'N 28°51.660'W, 368 m, F/V Manuel Arriaga, espinhel, voucher DOP C1496; 11 April 2007.

***Nemertesia norvegica* (Sars, 1873)**, - ***Aglaophenia tubulifera* (Hincks, 1861)**.

Azores Bank (35 miles): 38°36.000'N 28°53.340'W, 468 m, F/V Manuel Arriaga, espinhel, voucher DOP C1517; 11 April 2007.

***Aglaophenia* sp1**.

Azores Bank (35 miles): 37°58.140'N 20°03.600'W, 329 -365 m, F/V Tatiana, espinhel, vouchers: DOP C1817, C1823, C1824; 15 May 2007.

cf. *Lafoea dumosa* (Fleming, 1820), - *Diphasia margareta* (Hassall, 1841), - *Polyplumaria flabellata* (Sars, 1873), - *Aglaophenia* sp1.

Baixa de São Mateus: 38°19.500'N 28°32.280'W, 362 m, F/V Ana Miguel, voucher DOP 1026; 8 November 2006.

Acryptolaria sp1.

Cabeço do Luís: east of Pico, 200 m, sampling with jigs, voucher DOP C2039; 2 November 2007.

Filellum cf. *serratum* (Clarke, 1879), - *Acryptolaria* sp1.

Condor de Terra Bank: 38°32.880'N 29°02.760'W, 210 – 217 m, R/V Esperanza, Greenpeace ‘Defending our Oceans’, hydroids attached to camera suspended, voucher DOP C2485; 1 May 2006.

Diphasia margareta (Hassall, 1841), - *Antennella secundaria* (Gmelin, 1791), - *Aglaophenia* sp1.

Condor de Terra: 38°30.840'N 29°00.120'W, 256-274 m, F/V Mistério Azul, gorazeira, voucher DOP C889; 27 October 2006.

***Acryptolaria* cf. *crassicaulis* (Allman, 1888)**.

Condor Bank: 38°30.840'N 28°59.280'W, 238 m, F/V Baia das Lajes, voucher DOP C1223; 8 March 2007.

***Aglaophenia* sp1**.

Condor Bank: 38°32.460'N 29°02.940'W, 229 m, F/V Baia das Lajes, gorazeira, voucher DOP C1399; 27 March 2007.

***Cryptolaria pectinata* (Allman, 1888)**.

Condor de Terra Bank: 38°30.000'N 28°55.980'W, 256 - 384 m, F/V Manuel Arriaga, voucher DOP C1549; 15 April 2007.

Polyplumaria flabellata (Sars, 1873).

Condor de Terra Bank: 38°31.020'N 28°55.980'W, 220 – 366 m, F/V Baia das Lajes, espinhel, voucher DOP C1764; 7 May 2007.

Cryptolaria pectinata (Allman, 1888), - *Zygophylax* sp..

Condor Bank: 38°31.320'N 29°01.020'W, 238 – 243 m, F/V Baia das Lajes, espinhel, voucher DOP C1917; 26 May 2007.

Aglaophenia sp1.

Condor Bank: 38°31.320'N 29°01.620'W, 240 m, F/V Baia das Lajes, espinhel, voucher DOP C1932; 27 May 2007.

Filellum cf. *serratum* (Clarke, 1879), - *Antennella secundaria* (Gmelin, 1791), - ***Aglaophenia* sp1**.

Condor de Terra Bank: 38°32.640'N 29°00.600'W, 439 m, F/V Manuel Arriaga, palangre de fundo, over coral, vouchers: DOP C2024, C2025, C2395; 29 October 2007.

Filellum cf. *serratum* (Clarke, 1879), - *Acryptolaria* sp1, - *Diphasia margareta* (Hassall, 1841), - ***Diphasia pinastrum* (Cuvier, 1830)**, - *Sertularella* cf. *unituba* Calder, 1991, - *Aglaophenia tubulifera* (Hincks, 1861).

Corvo Island: 39°38.400'N 31°07.800'W, 727-735, R/V Arquipélago ARQDAÇO-27-P07, palangre de fundo, voucher DOP C2352; 22 June 2007.

Antennella secundaria (Gmelin, 1791).

Flores Island: 39°30.000'N 31°07.200'W, 109 – 147 m, R/V Arquipélago ARQDAÇO-27-P07, palangre de fundo, vouchers: DOP C2324, C2334, C2340; 19 June 2007.

Filellum cf. *serratum* (Clarke, 1879), - ***Acryptolaria* cf. *crassicaulis* (Allman, 1888)**, - ***Sertularella gayi* (Lamouroux, 1821)**.

Formigas: trole (fishing device); August 2005.

Acryptolaria sp1, - *Antennella secundaria* (Gmelin, 1791).

Giant Bank: between Flores and Faial, 38º59.040’N 29º52.980’W, 248 m, F/V Manuel Arriaga, espinhel, voucher DOP C1919; 26 May 2007.

***Nemertesia norvegica* (Sars, 1873)**.

Graciosa/Terceira channel: 38°53.960'N 27°33.120'W, 421 m, F/V Mestre Bobicha, palangre de fundo, voucher DOP C2057; 20 November 2007.

Filellum cf. *serratum* (Clarke, 1879), - ***Diphasia margareta* (Hassall, 1841)**, - *Antennella secundaria* (Gmelin, 1791).

Graciosa: 39°01.800'N 28°06.600'W, 267-300, R/V Arquipélago, ARQDAÇO-27-P07, palangre de fundo, voucher DOP 2215; 8 May 2007.

Polyplumaria flabellata (Sars, 1873).

Great Meteor: south of Azores, 30°09.600'N 28°32.400'W, 355 – 399 m, R/V Arquipélago, DEECON-28-V07, palangre de fundo, vouchers DOP: C2396, C2395; 6 July 2007.

***Diphasia margareta* (Hassall, 1841)**, - ***Diphasia pinastrum* (Cuvier, 1830)**, - *Aglaophenia* sp1.

Great Meteor: 30°10.800'N 28°32.400'W, 552-596 m, R/V Arquipélago DEECON-28-V07, palangre de fundo, voucher DOP C3062; 6 July 2007.

***Acryptolaria* sp1**.

Grupo Central: F/V Baia das Lajes, espinhel, voucher DOP C2052; 11 November 2007.

***Nemertesia norvegica* (Sars, 1873)**.

Irving: 32°00.000'N 27°57.000'W, 452 - 500 m, R/V Arquipélago, DEECON-28-V07, palangre de fundo, voucher DOP C2416; 8 July 2007.

***Cryptolaria pectinata* (Allman, 1888)**.

Lucky Strike segment: TTR12, 34°45.376'N 32°13.792'W, Mid-Atlantic Ridge, R/V Prof. Logachev, Stn AT-429-D, 1846-1916 m, dredge; 29 August 2002.

Nemertesia norvegica (Sars, 1873).

Lucky Strike segment: TTR12, 37°14.444'N 32°16.680'W, Mid-Atlantic Ridge, R/V Prof. Logachev, Stn AT-434-D, 1945-2030 m, dredge; 29 August 2002.

Stegopoma bathyale Vervoort, 1966.

Mar da Prata: 37°34.800'N 25°54.000'W, 174-192 m, R/V Arquipélago, ARQDAÇO-27-P07, palangre de fundo, voucher DOP C2152; 16 April 2007.

Polyplumaria flabellata (Sars, 1873).

Mar da Prata (near São Miguel): 37°36.600'N -25°54.000'W, 279 m, R/V Arquipélago ARQDAÇO-29-P08, palangre de fundo, over sponge, voucher DOP C2932; 8 June 2008.

***Cryptolaria pectinata* (Allman, 1888)**.

Mar Falso: 33°22.200'N 29°01.200'W, 435 m, F/V Manuel Arriaga, Espinhel, vouchers: DOP C1887, C1891; 18 May 2007.

***Acryptolaria* cf. *crassicaulis* (Allman, 1888)**.

Menez Gwen: 2 June 2003.

Cryptolaria pectinata (Allman, 1888).

Pico Island: 38°22.200'N 28°13.320'W, 311-348 m, F/V Renato Alexandre, palangre de fundo, voucher DOP C2087; 26 October 2007.

***Aglaophenia tubulifera* (Hincks, 1861)**.

Pico-Faial Channel: Fishing fleet of Horta, vouchers: DOP C2110, C2111, C2112; 9 November 2005.

***Sertularella polyzonias* (Linnaeus, 1758)**, **- *Aglaophenia tubulifera* (Hincks, 1861)**, **- *Nemertesia ramosa* Lamarck, 1816**.

Pico-Faial Channel: ‘fora do calhau’, 130 m, F/V Pantera azul, squid fishing, voucher DOP C972; 31 October 2006.

Acryptolaria cf. *crassicaulis* (Allman, 1888).

Pico-Faial Channel: 38°30.541’N 28°36.544’W, 150 m, submersible Lula, LULA dive #133, Hydrozoa from pannel II - 2 years; 19 September 2008.

Filellum cf. *serratum* (Clarke, 1879), - *Zygophylax* sp., - ***Sertularella polyzonias* (Linnaeus, 1758)**, - *Antennella secundaria* (Gmelin, 1791), - ***Nemertesia ramosa* (Lamarck, 1816)**.

S Pico-Faial Channel: 38º29.379’N 28º37.149’W to 38º29.410’N 28º37.326’W, 340 – 400 m, submersible Lula, Lula dive #143 hydroids over *Isidella* sp.; 12 June 2009.

***Lafoeina tenuis* G.O. Sars, 1874**, - ***Filellum* cf. *serratum* (Clarke, 1879) / sp3.**

Princess Alice Bank: 37º39.480’N 28º53.520’W, 567 m, F/V Açor Ocidental, palangre de fundo,vouchers DOP: C848, C849; 13 October 2006.

***Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp7**.

Princess Alice Bank: 37°57.360'N 29°12.840'W, 201 m, F/V Manuel Arriaga, gorazeira, voucher DOP C1374; 26 March 2007.

***Aglaophenia tubulifera* (Hincks, 1861)**.

Princess Alice Bank, 37°54.480'N 29°10.920'W, 269 m, F/V Manuel Arriaga, espinhel, voucher DOP C1463; 30 March 2007.

***Acryptolaria* sp1**.

Princess Alice Bank: 37°54.480'N 29°10.920'W, 201 m, F/V Manuel Arriaga, gorazeira, voucher DOP C2482; 30 March 2007.

***Acryptolaria* sp1**.

Princess Alice Bank: 37°54.000'N 29°01.200'W, 229 – 238 m, F/V Mistério Azul, gorazeira, voucher DOP C2122; 4 May 2007.

Filellum cf. *serratum* (Clarke, 1879).

Princess Alice Bank: 37°54.000'N 29°01.020'W, 238 – 329 m, F/V Mistério Azul, espinhel, voucher DOP C1676; 4 May 2007.

Acryptolaria sp1.

Princess Alice Bank: 37°46.560'N 29°09.120'W, 274 m, F/V Manuel Arriaga, espinhel, voucher DOP C1844; 16 May 2007.

***Cryptolaria pectinata* (Allman, 1888)**.

Princess Alice Bank: 37°54.000'N 29°01.800'W, 366 m, F/V Neuza Mar, espinhel, voucher DOP C2005; 11 July 2007.

Polyplumaria flabellata G.O. Sars, 1874.

Princess Alice Bank: 38°00.000'N 29°16.020'W, R/V Arquipelago, ARQDAÇO-21-P04/1PAL(4)P04, Estrato 5, voucher DOP 405 HZ.

Lytocarpia myriophyllum (Linnaeus, 1758).

Ponta das Contendas: near Terceira Island, 38º37.020’N 27º04.020’ W, 51-100 m, R/V Arquipélago ARQDAÇO-21-P04, palangre de fundo, voucher DOP C408.

***Nemertesia* cf. *perrieri* (Billard, 1901) / sp5**.

Rainbow: hydrothermal field, SW Azores, R/V Pourquois Pas, MOMARDREAM, submersible, voucher DOP C2506; 19 July 2007.

***Stegopoma bathyale* Vervoort, 1966**.

Salão: 38°37.140'N 28°37.200'W, 250 m, R/V Arquipélago, palangre, voucher DOP C1967; 29 June 2007.

Filellum cf. *serratum* (Clarke, 1879), - *Diphasia margareta* (Hassall, 1841), - *Diphasia pinastrum* (Cuvier, 1830).

São Jorge: Queimada, 38º39.960’N 28º15.960’W, 1062-1100 m, R/V Arquipélago, ARQDAÇO-27-P07, Stn 2SJO(1)P07 (Est. 22), palangre de fundo, vouchers: DOP C2232, C2235, C2293; 10 June 2007.

Filellum cf. *serratum* (Clarke, 1879), - ***Nemertesia belini* (Quelch, 1885)**, - *Antennella secundaria* (Gmelin, 1791).

São Jorge: 38°40.200'N 28°30.000'W, 1102-1137 m, R/V Arquipélago, ARQDAÇO-27-P07, palangre de fundo, voucher DOP 2252; 10 June 2007.

Antennella secundaria (Gmelin, 1791).

3.2.3 MADEIRA

3.2.3.1 Coastal waters

Madeira Island

Calheta: port, 32°43.054'N 17°10.341'W, 0-0.5 m, dock; 22 September 2008.

Kirchenpaueria halecioides (Alder, 1859).

Caniçal: old fishing port, 32º44.094'N 16º44.43'W, 4-18 m; rocky wall; 13 September 2008.

Lafoeina tenuis G.O. Sars, 1874, - *Filellum* cf. *serratum* (Clarke, 1879), - *Sertularella* cf. *fusiformis* (Hincks, 1861), - *Salacia desmoides* (Torry, 1902), - *Dynamena disticha* (Bosc, 1802), - *Kirchenpaueria* sp., - *Antennella secundaria* (Gmelin, 1791), - *Halopteris diaphana* (Heller, 1868), - Plumulariidae undet., - ***Aglaophenia* sp2**.

Caniçal: estern side, outside new fishing port, 32°44.275'N 16° 43.718'W, 5-23 m, scuba diving, sandy bottom with rocky patches and algae; 14 September 2008.

Lafoeina tenuis G.O. Sars, 1874, - *Sertularella* cf. *fusiformis* (Hincks, 1861), - *Dynamena disticha* (Bosc, 1802), - *Kirchenpaueria* sp., - *Antennella secundaria* (Gmelin, 1791), - *Monotheca pulchella* (Bale, 1882), - *Aglaophenia* sp2, - *Macrorhynchia philippina* Kirchenpauer, 1872.

Caniço de Baixo: Hotel RocaMar, 38º32.057'N 28º37.500´W, 7-12 m, scuba diving, fishing line and rocky walls and bottoms; 20 September 2008.

Lafoeina tenuis G.O. Sars, 1874, - *Filellum* cf. *serratum* (Clarke, 1879), - *Aglaophenia* sp2.

Garajau: Baixa das Mamas, natural reserve of Garajau, 32º38.302'N 16º51.651'W, 15-29 m, scuba diving, rocky walls; 24 September 2008.

Lafoeina tenuis G.O. Sars, 1874, - *Filellum* cf. *serratum* (Clarke, 1879), - *Halopteris diaphana* (Heller, 1868), - *Aglaophenia picardi* Svoboda, 1979.

Garajau: Baixa do Garajau, natural reserve of Garajau, 32º38.217'N 16º51.242'W, 15-23 m, scuba diving, rock; 24 September 2008.

Filellum cf. *serratum* (Clarke, 1879), - *Halopteris diaphana* (Heller, 1868), - *Aglaophenia* sp2.

Lido: Funchal naval club, 32º38.046’N 16º56.351’W, 3-13 m, scuba diving, rocks at bottom and cables; 16 September 2008.

cf. *Tiaropsidium mediterraneum* (Metschnikoff, 1886), - ***Sertularella* cf. *fusiformis* (Hincks, 1861) (sp1)**, - ***Dynamena disticha* (Bosc, 1802)**, - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Antennella secundaria* (Gmelin, 1791), - *Monostaechas quadridens* (Mccrady, 1859), - *Halopteris diaphana* (Heller, 1868), - ***Monotheca margaretta* Nutting, 1900**, - ***Aglaophenia* sp2, -** *Macrorhynchia philippina* Kirchenpauer, 1872.

Lido: Baixa do Carneiro, 32°38.127'N 16°55.909'W, 10-15 m, scuba diving, rocky bottom and walls; 19 September 2008.

***Dynamena disticha* (Bosc, 1802)**, - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Monostaechas quadridens* (Mccrady, 1859), - *Halopteris alternata* (Nutting, 1900), - *Monotheca pulchella* (Bale, 1882), - ***Aglaophenia* sp2,** - ***Macrorhynchia philippina* Kirchenpauer, 1872**.

Machico: 32º41.333'N 16º46.248'W, 6-24 m, scuba diving, rock; 23 September 2008.

Lafoeina tenuis G.O. Sars, 1874, - *Filellum* cf. *serratum* (Clarke, 1879), - *Antennella secundaria* (Gmelin, 1791), - *Halopteris diaphana* (Heller, 1868), - ***Aglaophenia* sp2**, - *Aglaophenia kirchenpaueri* (Heller, 1868), - *Aglaophenia picardi* Svoboda, 1979.

Madalena do Mar: 32°42.166'N 17°08.385'W, 5-9 m, scuba diving, rocks; 22 September 2008.

Lafoeina tenuis G.O. Sars, 1874, - *Sertularella* cf. *fusiformis* (Hincks, 1861), - ***Dynamena disticha* (Bosc, 1802)**, - ***Sertularia marginata* (Kirchenpauer, 1864)**, - *Kirchenpaueria halecioides* (Alder, 1859), - *Monostaechas quadridens* (Mccrady, 1859), - *Halopteris diaphana* (Heller, 1868), - *Monotheca pulchella* (Bale, 1882), - ***Aglaophenia pluma* (Linnaeus, 1758)**, - *Macrorhynchia philippina* Kirchenpauer, 1872.

Paul do Mar: fishing port, 32º45.019'N 17º13.551'W, 4-8 m, algae; 22 September 2008.

Campanulinidae undet., - *Antennella secundaria* (Gmelin, 1791).

Porto da Cruz: port, 32°46.516'N 16°49.585'W, 4-12 m, scuba diving, rocky bottoms and walls; 21 September 2008.

***Sertularella* cf. *fusiformis* (Hincks, 1861) / (sp1)**, - *Dynamena disticha* (Bosc, 1802), - ***Sertularia marginata* (Kirchenpauer, 1864)**, - *Antennella secundaria*

(Gmelin, 1791), - *Monotheca pulchella* (Bale, 1882), - *Aglaophenia pluma* (Linnaeus, 1758), - ***Macrorhynchia philippina*** **Kirchenpauer, 1872**.

Ribeira brava: fishing port, 32°40.075'N 17°03.787'W, 3-8 m, scuba diving, rocky wall and algae on sand; 15 September 2008.

Sertularella cf. *fusiformis* (Hincks, 1861), - *Dynamena disticha* (Bosc, 1802), - *Kirchenpaueria halecioides* (Alder, 1859), - *Antennella secundaria* (Gmelin, 1791), - *Halopteris diaphana* (Heller, 1868), - ***Monotheca margaretta*** **Nutting, 1900**, - ***Aglaophenia picardi*** **Svoboda, 1979**.

Santa Cruz: Baixa de Sta Cruz; 32º45.016´N 17º13.553'W, 33-38 m, scuba diving, rocky walls; 23 September 2008.

Lafoeina tenuis G.O. Sars, 1874, - *Cuspidella* sp., - *Aglaophenia* sp2.

Desertas Island

Desertas: voucher MMF 25124, 32º23.00’N 16º27.23’W, 30 – 91 m, R/V Johnson Sea Link, submersible; 30 May 1991.

Sertularia distans **(Lamouroux, 1816)**, - ***Nemertesia ramosa*** **(Lamarck, 1816)**.

3.2.3.2 Deep waters

Madeira Island

Calheta: voucher MMF 39075, Stn 23, 1300 m, covo, coral; 08 May 2008.

Sertularella gayi **(Lamouroux, 1821)**, - *Antennella secundaria* (Gmelin, 1791).

Off Caniçal: voucher MMF39095, Stn 34, 600 m, covo; 02 June 2008.

***Zygophylax biarmata* Billard, 1905**, - *Acryptolaria* sp., - ***Lytocarpia myriophyllum* (Linnaeus, 1758)**.

Off Garajau: voucher MMF 36727, 32º37.49'N 16º50.58'W, 700 m, F/V Baía de Câmara de Lobos; 21 October 2006.

***Zygophylax levinseni* (Saemundsson, 1911)**, - ***Nemertesia belini* (Quelch, 1885)**.

Paul do Mar: voucher MMF, 1000 m, Project PescProf – 3 Chacmad -3, station 7, covo; 29 April 2008.

***Stegopoma bathyale* Vervoort, 1966**.

Ponta do Pargo: MMF 39060, Stn 17, 1000 m, Apparatus abandoned, over deep-sea anemones; 29 April 2008.

Zygophylax levinseni (Saemundsson, 1911).

Seixal: voucher MMF 39027, 500m, Stn 10 Covo 10; 21 April 2008.

Lytocarpia myriophyllum (Linnaeus, 1758).

?: voucher MMF 39419, SAL 19/150798, associated with dead *Lophelia pertusa.*

Acryptolaria cf. *conferta* (Allman, 1877), - *Cryptolaria pectinata* (Allman, 1888).

Desertas Island

Desertas: voucher MMF 25124, 32º23.00'N 16º27.23'W, 30-91 m, R/V Johnson Sea Link, submersible; 06 June 2008.

Dynamena disticha (Bosc, 1802), - *Diphasia margareta* (Hassal, 1848), - *Nemertesia ramosa* (Lamarck, 1816), - *Plumularia setacea* (Linnaeus, 1758).

3.2.4 GULF OF CADIZ

3.2.4.1 Deep waters

Al Idrisi – Active mud volcano. It is located at the very edge of the Atlantic Moroccan shelf, where it forms a rounded promontory. This is the largest mud volcano of the El Arraiche mud volcano field, it is conical-shaped, 225 m high, approximately 5.3 km in diameter at the base and 1.4 Km at the top. Mud flows are very large especially at the western side. Slow activity in terms of sediment eruption: of just about 1.33 % to 5 % of the time during the last 3 Ma. Crater rims are not as well developed as in the neighbouring mud volcanoes.

Stn AT-412-D: TTR12, 35º14.193’N 6º36.609’W, 230-235 m, dredge, rusty-yellowish sandstones and siltstones with a characteristic honeycomb pattern as the result of chemical weathering; 17 July 2002.

Acryptolaria cf. *conferta* (Allman, 1877), - *Nemertesia* cf. *antennina* (Linnaeus, 1758), - *Aglaophenia lophocarpa* Allman, 1877, - *Cladocarpus sinuosus* Vervoort, 1966.

Cadiz channel – Submarine channel on the southern Iberian margin with areas of carbonate chimneys.

Stn AT-599-D: TTR15, 36º06.538'N 07º53.942'W, 1275-1418 m, dredge, carbonate chimneys; 03 August 2005.

Modeeria rotunda (Quoy & Gaimard, 1827), - *Zygophylax bathyphila* Leloup, 1940, - ***Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp4**.

Captain Arutyunov – Active mud volcano. Located in the central part of the Gulf of Cadiz at 35º39.5’N – 7º20.0’W, at a water depth of around 1200 m. It is conical-shaped, 100 high, with 3 km diameter at base and 800 m at top. This mud volcano was recently active and is characterized by gas venting and presence of gas hydrates.

Stn AT-399-Gr: TTR12, 35º39.805'N 07º19.997'W, 1339 m, TV-assisted grab, grey mud breccias with randomly scattered rock fragments (gravel size) recovered from the crater outermost part; 13 July 2002.

Cladocarpus cf. *corneliusi* Ramil & Vervoort, 1992a.

Stn 344-FLUFO5-Recovery: MS MERIAN 01-03, 35°39.697'N 07°20.038'W, 1320 m, FLUFO Recovery; 16 May 2006.

Sertularella robusta Allman, 1873.

Coral Patch Seamount

Stn GeoB12767-1: 64PE284, 34°56.480'N 11°57.730'W, 700 m, ROV, basaltic rock; 07 March 2008.

Cryptolaria pectinata (Allman, 1888), - *Zygophylax biarmata* Billard, 1905.

Fíuza – Active mud volcano (one of the most active). It has a conical shape, length of 125 m, diameter of around 2.24 Km at the base and 0.5 Km at the top. The water depth at the top of the mud volcano is approximately 400 m. It is characterised by series of concentric rims with high-backscatter mud flows extending down the flanks, and up to 1.3km from the crater.

Stn AT-566-Gr: TTR14, 35º15.510'N 06º41.702'W, 414 m, TV-assisted grab, mud breccia covered by maerl; 09 August 2004.

Campanulinidae complex spec., - *Acryptolaria* cf. *conferta* (Allman, 1877), - *Filellum* cf. *serratum* (Clarke, 1879), - *Zygophylax biarmata* Billard, 1905, - *Sertularella robusta* Allman, 1873, - *Aglaophenia lophocarpa* Allman, 1877.

Formosa ridge - elongated diapiric ridge covered with carbonate crusts and chimneys located by the middle of the Gulf of Cadiz.

Stn AT-388-Gr: TTR12, 36º10.263'N 07º43.819'W, 1079 m, TV-assisted grab, oxidized carbonate chimneys with aragonite as cement, up to 2 cm of diameter and 25 cm of length, with a large proportion of these joined together as doublets or even triplets; many cemented bivalve and *Madrepora oculata* fragments in the chimneys; 08 July 2002.

Acryptolaria cf. *conferta* (Allman, 1877), - *Kirchenpaueria pinnata* (Linnaeus, 1758), - *Nemertesia* cf. *antennina* (Linnaeus, 1758), - *Polyplumaria flabellata* G.O. Sars, 1874.

Stn AT-389-D: TTR12, 36º10.123'N 07º44.121'W, 1068-1080 m, dredge, oxidized carbonate chimneys with aragonite as cement, up to 2 cm in diameter and 25 cm in length, with a large proportion of these joined together as doublets or even triplets; many cemented bivalve and *Madrepora oculata* fragments attached; also large samples of authigenic carbonate rocks; 08 July 2002.

Polyplumaria flabellata G.O. Sars, 1874.

Channel West of Gibraltar – Submarine channel on the Atlantic Moroccan shelf relatively close to the Strait of Gibraltar. Fields of inactive carbonate chimneys.

Stn AT-550-D: TTR14, 35º42.257’N 6º30.000’W, 368-392 m, dredge, carbonate chimneys; 07 August 2004.

Lafoeina tenuis G.O. Sars, 1874, - *Modeeria rotunda* (Quoy & Gaimard, 1827), - *Acryptolaria* cf. *conferta* (Allman, 1877), - *Cryptolaria pectinata* (Allman, 1888), - *Lafoea* cf. *dumosa* (Fleming, 1820), - *Zygophylax biarmata* Billard, 1905, - *Lytocarpia myriophyllum* (Linnaeus, 1758), - *Cladocarpus sinuosus* Vervoort, 1966, - *Kirchenpaueria bonnevieae* (Billard, 1906), - *Polyplumaria flabellata* G.O. Sars, 1874, - *Diphasia margareta* (Hassal, 1848), - *Sertularella robusta* Allman, 1873.

Stn AT-551-D: TTR14, 35º42.769’N 6º30.305’W, 393-445 m, dredge, carbonate chimneys; 07 August 2004.

Acryptolaria cf. *conferta* (Allman, 1877), - *Cladocarpus sinuosus* Vervoort, 1966, - *Polyplumaria flabellata* G. O. Sars, - *Diphasia margareta* (Hassal, 1848).

Stn AT-552-Gr: TTR14, 35º42.816’N 6º30.234’W, 428 m, carbonate chimneys; 07 August 2004.

Lafoeina tenuis G.O. Sars, 1874, - *Acryptolaria* cf. *conferta* (Allman, 1877), - *Cryptolaria pectinata* (Allman, 1888), - *Aglaophenia lophocarpa* Allman, 1877, - *Cladocarpus sinuosus* Vervoort, 1966, - *Nemertesia* cf. *antennina* (Linnaeus, 1758), - *Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp.2, - *Polyplumaria flabellata* G. O. Sars, 1874.

Gil Eanes Channel – Contourite channel located at the deep Moroccan shelf of the Gulf of Cadiz.

Stn AT-342-G: TTR11, 35º46.983'N 07º29.355'W, 1291 m, grab, contourite channel with coarsening upwards units at the upper reaches of a system of small submarine channels beyond the Gil Eanes contourite channel mouth; 27 August 2001.

Aglaophenia tubulifera (Hincks, 1861), - *Salacia desmoides* (Torry, 1902).

Guadalquivir Ridge – Carbonate chimneys form large fields especially on the tops and slopes of the outcropping diapiric structures. Their presence might be evidence of intensive fluid escape from the sediments involved in the diapiric process.

Stn AT-339-D: TTR11, 36º07.591'N 7º46.587'W, 1021 – 1086 m, dredge, ridge of carbonate chimneys on the northwest side of the Ibérico mud volcano; 26 August 2001.

Modeeria rotunda (Quoy & Gaimard, 1827), - *Diphasia pinastrum* (Cuvier, 1830), - *Antennella secundaria* (Gmelin, 1791), - *Polyplumaria flabellata* G.O. Sars, 1874, - *Aglaophenia lophocarpa* Allman, 1877, - ***Aglaophenia tubulifera*** **(Hincks, 1861)**.

Kidd – Active mud volcano with relatively high abundance of Pogonophora worms and methane in the sediment that mostly consists of mud breccia usually covered by hemipelagic sediments or maerl. It is positioned at the eastern Moroccan field in the Gulf of Cadiz.

Stn AT-528-Gr: TTR14, 35º25.281'N 06º43.972W', 489 m, TV-assisted grab, mud breccia with strong smell of H_2S covered by hemipelagic sediments; 03 August 2004.

Lafoeina tenuis G.O. Sars, 1874, - *Lovenella producta* (G.O. Sars, 1874), - *Campanulina panicula* G.O. Sars, 1874, - *Acryptolaria longitheca* (Allman, 1877), - *Zygophylax biarmata* Billard, 1905, - *Zygophylax levinseni* (Saemundsson, 1911), - *Sertularella robusta* Allman, 1873, - ***Aglaophenia lophocarpa*** **Allman, 1877**.

Stn AT-559-B: TTR14, 35º24.777'N 06º43.782'W, 552 m, box corer, Kidd outskirts, hemipelagic sediments; 08 August 2004.

Acryptolaria cf. *conferta* (Allman, 1877).

Meknès – Active mud volcano on the southern part of the Moroccan shelf of the Gulf of Cadiz.

Stn AT-542-G: TTR14, 34º59.175'N 07º04.364'W, 703 m, grab, mud breccia with strong smell of H_2S; 06 August 2004.

Zygophylax biarmata Billard, 1905.

Stn AT-581-Gr: TTR15, 34º59.178'N 07º04.353'W, 700 m, TV-assisted grab; 28 July 2005.

***Aglaophenia lophocarpa* Allman, 1877**.

Stn 321-GKG-22: MS MERIAN 01-03, 34°58.796'N 07°04.394'W, 731 m, GKG; 14 May 2006.

Acryptolaria cf. *conferta* (Allman, 1877).

Stn 335-GKG-23: MS MERIAN 01-03, 34°59.035'N 07°04.552'W, 703 m, GKG; 15 May 2006.

Stegopoma bathyale Vervoort, 1966, - *Acryptolaria* cf. *conferta* (Allman, 1877).

Meknès Carbonate Mound Provinces

Stn GeoB12728-1: 64PE284, 35°10.830'N 06°56.530'W, 753 m, ROV, coral framework with live polyps. Central Carbonate Mound Province, northern area; 26 February 2008.

***Acryptolaria* cf. *conferta* (Allman, 1877)**, - ***Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp.2**.

Stn GeoB12729-1: 64PE284, 35°10.830'N 06°56.530'W, 754 m, box corer, brown silty clay to olive grey clay. Abundant coral fragments throughout the core. Central Carbonate Mound Province, northern area; 26 February 2008.

Acryptolaria cf. *conferta* (Allman, 1877), - *Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp.1.

Stn GeoB12738-1: 64PE284, 34°59.980'N 07°04.510'W, 738 m, ROV, live *Lophelia* framework. Small mounds in the northern area; 28 February 2008.

Acryptolaria cf. *conferta* (Allman, 1877), *Zygophylax biarmata* Billard, 1905, - ***Sertularella robusta* Allman, 1873**, - *Kirchenpaueria pinnata* (Linnaeus, 1758).

Stn GeoB12739-1: 64PE284, 35°00.010'N 07°04.470'W, 736 m, box corer, slightly over the top. Brownish silty clay to grayish clay; abundant coral fragments throughout the core. Small mounds in the northern area; 28 February 2008.

Acryptolaria cf. *conferta* (Allman, 1877), - ***Sertularella robusta* Allman, 1873**, - *Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp.1, - ***Nemertesia ramosa* (Lamarck, 1816)**.

Mercator – Active mud volcano. It is conical, about 125 m high, around 1.84 Km wide at the base and 0.5 Km at the top. The water depth at the top of the mud volcano is 350 m. It has a series of semi-concentric rims (rose petal pattern) with high-backscatter mud flows extending down the flanks.

Stn AT-408-G: TTR12, 35°17.756'N 368 m, grab, grey mud breccia covered with light brown pelagic maerl recovered from the volcano crater. The absence of gas smell suggests that the volcano is not active in the sampled area at the present time; 16 July 2002.

Campanulina panicula G.O. Sars, 1874.

Stn AT-409-D: TTR12, 35º17.929’N 6º38.903’W, 375-397 m, dredge, a large quantity of sandstones, siltstones, claystones and calcite vein fragments recovered; 16 July 2002.

Campanulina panicula G.O. Sars, 1874, - *Cladocarpus sinuosus* Vervoort, 1966.

Stn AT-410-D: TTR12, 35º18.014'N 06º38.986'W, 366-392 m, dredge, a large quantity of sandstones, siltstones, claystones and calcite vein fragments recovered; 16 July 2002.

Lafoeina tenuis G.O. Sars, 1874 - *Acryptolaria* cf. *conferta* (Allman, 1877), - *Sertularella gayi* (Lamouroux, 1821), - *Lytocarpia myriophyllum* (Linnaeus, 1758).

Stn AT-569-Gr: TTR15, 35º17.917'N 06º38.717'W, 358 m, TV-assisted grab, top of the volcano; 25 July 2005.

Campanulina panicula G.O. Sars, 1874 - *Acryptolaria* cf. *conferta* (Allman, 1877), - *Sertularella polyzonias* (Linnaeus, 1758), - *Nemertesia* cf. *antennina* (Linnaeus, 1758).

Stn AT-575-B: TTR15, 35º17.903'N 06º38.715'W, 355 m, box corer, top of the volcano; 26 July 2005.

Acryptolaria cf. *conferta* (Allman, 1877), - *Zygophylax biarmata* Billard, 1905, - *Sertularella robusta* Allman, 1873, - *Nemertesia* cf. *antennina* (Linnaeus, 1758), - ***Nemertesia ventriculiformis* (Marktanner-Turneretscher, 1890)**, - *Aglaophenia lophocarpa* Allman, 1877.

Stn AT-576-B: TTR15, 35º17.657'N 06º39.129'W, 428 m, box corer, mud flow; 26 July 2005.

Acryptolaria cf. *conferta* (Allman, 1877), - ***Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp1**, - *Lytocarpia myriophyllum* (Linnaeus, 1758).

Stn 237#2 MUC 12 A & 12 B: MS MERIAN 01-03, 35°17.914'N 06°38.687'W, 352 m, MUC; 06 May 2006.

Modeeria rotunda (Quoy & Gaimard, 1827), - ***Sertularella gayi*** **(Lamouroux, 1821)**, - *Sertularella robusta* Allman, 1873.

Stn 241-GKG-12: MS MERIAN 01-03, 35°17.918'N 06°38.717'W, 353 m, GKG; 06 May 2006.

Sertularella gayi (Lamouroux, 1821), - *Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp1, - *Aglaophenia lophocarpa* Allman, 1877, - ***Streptocaulus multiseptatus*** **(Bale, 1915)**.

Stn 242-GKG-13: MS MERIAN 01-03, 35°17.870'N 06°38.810'W, 350 m, GKG; 06 May 2006.

Zygophylax biarmata Billard, 1905, - ***Nemertesia*** **cf.** ***antennina*** **(Linnaeus, 1758) / sp1**, - ***Nemertesia ventriculiformis*** **(Marktanner-Turneretscher, 1890)**, - ***Aglaophenia lophocarpa*** **Allman, 1877**.

Stn 267-MUC-13A: MS MERIAN 01-03, 35°17.875'N 06°38.789'W, 350 m, MUC; 09 May 2006.

Stegopoma bathyale Vervoort, 1966, - *Filellum* cf. *serratum* (Clarke, 1879), *Acryptolaria* cf. *conferta* (Allman, 1877), - *Zygophylax biarmata* Billard, 1905.

Stn 287-MUC-14B: MS MERIAN 01-03, 35°17.890'N 06°39.059'W, 380 m, MUC; 11 May 2006.

Nemertesia **cf.** ***antennina*** **(Linnaeus, 1758) / sp1**, - *Aglaophenia lophocarpa* Allman, 1877.

Stn 01A Belgica 09/14b - Out: 35º17.916’N 06º38.709’W, 631 m, hydroids over ropes of sampling device deployed on the seabed, ROV; 19 May 2007 until 19 May 2009.

Campanulina panicula G.O. Sars, 1874, - *Filellum* cf. *serratum* (Clarke, 1879), - *Acryptolaria* cf. *conferta* (Allman, 1877), - *Cryptolaria pectinata* (Allman, 1888), - *Zygophylax biarmata* Billard, 1905.

Stn 01W Belgica 09/14b - Out: 35º17.916’N 06º38.709’W, 631 m, hydroids over ropes of sampling device deployed on the seabed, ROV; 19 May 2007 until 19 May 2009.

***Modeeria rotunda* (Quoy & Gaimard, 1827)**, - ***Campanulina panicula* G.O. Sars, 1874, - *Filellum* cf. *serratum* (Clarke, 1879) / sp2**, - ***Acryptolaria* cf. *conferta* (Allman, 1877)**, - ***Cryptolaria pectinata* (Allman, 1888)**, - *Zygophylax biarmata* Billard, 1905.

Stn 01C Belgica 09/14b - Out: 35º17.916’N 06º38.709’W, 631 m, hydroids over ropes of sampling device deployed on the seabed, ROV; 19 May 2007 until 19 May 2009.

Filellum cf. *serratum* (Clarke, 1879), - *Acryptolaria* cf. *conferta* (Allman, 1877), - *Cryptolaria pectinata* (Allman, 1888), - *Zygophylax biarmata* Billard, 1905.

Stn 01A Belgica 09/14b - alfalfa: 35º17.916’N 06º38.709’W, 631 m, hydroids over ‘alfalfa’ in sampling device deployed on the seabed, ROV; 19 May 2007 until 19 May 2009.

Campanulina panicula G.O. Sars, 1874, - *Filellum* cf. *serratum* (Clarke, 1879), - *Cryptolaria pectinata* (Allman, 1888), - *Zygophylax biarmata* Billard, 1905.

Stn 01W Belgica 09/14b - wood: 35º17.916’N 06º38.709’W, 631 m, hydroids over wood in sampling device deployed on the seabed, ROV; 19 May 2007 until 19 May 2009.

Modeeria rotunda (Quoy & Gaimard, 1827), - *Campanulina panicula* G.O. Sars, 1874, - *Filellum* cf. *serratum* (Clarke, 1879), - *Cryptolaria pectinata* (Allman, 1888), Stn 01C Belgica 09/14b - Carb In (net 60 um): 35º17.916’N 06º38.709’W, 631 m, hydroids over carbonates in sampling device deployed on the seabed, ROV; 19 May 2007 until 19 May 2009.

Campanulina panicula G.O. Sars, 1874, - *Zygophylax biarmata* Billard, 1905.

Stn 02C Belgica 09/14b - Carb In: 35º17.916’N 06º38.709’W, 631 m, hydroids over carbonates in sampling device deployed on the seabed, ROV; 19 May 2007 until 19 May 2009.

Zygophylax biarmata Billard, 1905.

Stn 03W Belgica 09/14b - Wood: 35º17.916’N 06º38.709’W, 631 m, hydroids over wood in sampling device deployed on the seabed, ROV; 19 May 2007 until 19 May 2009.

Campanulina panicula G.O. Sars, 1874.

NE Mercator mud volcano

Stn Dive 27 Rock 1: JC10, 35°18.755'N 06°37.422'W, 428 m, rock sample; 18 May 2007.

Campanulina panicula G.O. Sars, 1874, - *Acryptolaria* cf. *conferta* (Allman, 1877), - *Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp3, - ***Nemertesia ventriculiformis* (Marktanner-Turneretscher, 1890)**, - *Aglaophenia lophocarpa* Allman, 1877.

Stn Dive 27 Rock 2: JC10, 35°18.823'N 06°37.377'W, 432 m, rock sample; 18 May 2007.

Cuspidella sp., - Campanulinidae incertae sedis, - *Acryptolaria* cf. *conferta* (Allman, 1877), - *Kirchenpaueria pinnata* (Linnaeus, 1758), - ***Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp3**, - *Nemertesia ventriculiformis* (Marktanner-Turneretscher, 1890), - *Aglaophenia lophocarpa* Allman, 1877, - ***Streptocaulus dollfusi* (Billard, 1924)**.

Stn Dive 27 Rock 3: JC10, 35°18.839'N 06°37.354'W, 426 m, rock sample; 18 May 2007.

***Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp3**, - *Nemertesia ventriculiformis* (Marktanner-Turneretscher, 1890), - *Aglaophenia lophocarpa* Allman, 1877.

Stn Dive 27 Rock 5: JC10, 35°18.825'N 06°37.154'W, 373 m, rock sample; 18 May 2007.

Modeeria rotunda (Quoy & Gaimard, 1827), - ***Acryptolaria* cf. *conferta* (Allman, 1877)**, - ***Zygophylax biarmata* Billard, 1905**, - *Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp3, - *Nemertesia ventriculiformis* (Marktanner-Turneretscher, 1890).

Stn Dive 27 Rock 6: JC10, 35°18.827'N 06°37.058'W, 376 m, rock sample, ROV; 18 May 2007.

Lafoea cf. *dumosa* (Fleming, 1820), - ***Acryptolaria* cf. *conferta* (Allman, 1877)**, - *Acryptolaria* cf. *longitheca,* - *Zygophylax biarmata* Billard, 1905, - ***Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp4**, - ***Nemertesia ventriculiformis* (Marktanner-Turneretscher, 1890)**, - *Polyplumaria flabellata* G.O. Sars, 1874, - *Aglaophenia lophocarpa* Allman, 1877, - *Cladocarpus* cf. *multiseptatus* (Bale, 1915).

Stn Dive 27 Rock 7: JC10, 35°18.840'N 06°37.039'W, 381 m, rock sample, ROV; 18 May 2007.

Lafoea cf. *dumosa* (Fleming, 1820), - *Acryptolaria* cf. *conferta* (Allman, 1877).

Chechouan mud volcano

Stn AT-610-Gr: TTR16, 35°28.468'W 07°15.477'W, 1177 m, MAKAT135, mud breccia (clasts, carbonate crusts); 30 May 2006.

Lytocarpia myriophyllum (Linnaeus, 1758).

Pen Duick Escarpment - Fault scarp of about 4.5 Km long, west of Gemini mud volcano. The escarpment changes from the NW-SE direction to almost N-S, it is around 100 m high, the water depth at the top is 525 m. At the top of the escarpment, a platform with irregular backscatter pattern occurs. The eastern part platform is characterized by a hummocky topography. To the west the pattern changes to parallel elongated ridges. Gullies occur at the base of the escarpment. The coral banks consist of dead coral framework with terrigeneous mud matrix and few living corals at the top. It is indicative of a more favourable coral habitat in the past, perhaps related to strong currents in this area. The carbonate slabs consists of carbonate cement with shells, corals and other debris, which might indicate present or past expulsion of hydrocarbon gases, probably along a fault zone. Fault zone with carbonate slabs.

Recent studies on the Pen Duick escarpment revealed that cold water corals can colonise fields of mud breccia and form large build-ups associated with mud volcanoes.

Stn AT-406-Gr: TTR12, 35º18.148'N 06º47.666'W, 550 m, TV-assisted grab, dead coral bank escarpment; 15 July 2002.

Lafoeina tenuis G.O. Sars, 1874, - *Modeeria rotunda* (Quoy & Gaimard, 1827), - *Acryptolaria* cf. *conferta* (Allman, 1877), - *Filellum* cf. *serratum* (Clarke, 1879), - ***Zygophylax biarmata* Billard, 1905**, - *Antennella secundaria* (Gmelin, 1791), - *Nemertesia* spec. 1, - *Plumulariinae* spec..

Stn AT-407-Gr: TTR12, 35º17.695'N 06º47.082'W, 560 m, TV-assisted grab, dead coral bank escarpment; 15 July 2002.

Campanulina panicula G.O. Sars, 1874, - *Filellum* cf. *serratum* (Clarke, 1879).

Stn AT-534-G: TTR14, 35º18.410'N 06º47.812'W, 550 m, grab, coral debris in clay covered by maerl; 04 August 2004.

Aglaophenia lophocarpa Allman, 1877.

Stn AT-565-Gr: TTR14, 35º18.180'N 06º47.656'W, 544 m, TV-assisted grab, coral debris, clay; 09 August 2004.

Acryptolaria cf. *conferta* (Allman, 1877), - *Zygophylax biarmata* Billard, 1905.

Stn 10 B: M2005, 35º18.988'N 6º47.667'W, 538 m, box core; 24 May 2005.

Campanulina panicula? spec. 1.

Stn 14 AB: M2005, 35º18.330'N 6º47.724'W, 546 m, box core; 25 May 2005.

Filellum cf. *serratum* (Clarke, 1879).

Stn 15 B: M2005, 35º18.326'N 6º47.927'W, 570 m, box core; 25 May 2005.

Lafoeina tenuis G.O. Sars, 1874, - *Acryptolaria* cf. *conferta* (Allman, 1877), - *Sertularella robusta* Allman, 1873, - *Antennella secundaria* (Gmelin, 1791), - *Nemertesia* cf. *antennina* (Linnaeus, 1758).

Stn 16 AB: M2005, 35º18.310'N 6º48.205'W, 660 m, box core; 26 May 2005.

Modeeria rotunda (Quoy & Gaimard, 1827), - ***Sertularella robusta* Allman, 1873**, - ***Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp2**.

Stn 20 B: M2005, 35º18.173'N 6º46.653'W, 516 m, box core; 30 May 2005.

Campanulina panicula? spec. 2.

Stn 21 B: M2005, 35º19.084'N 6º46.397'W, 498 m, box core; 30 May 2005.

Lafoeina tenuis G.O. Sars, 1874, - *Modeeria rotunda* (Quoy & Gaimard, 1827), - ***Acryptolaria* cf. *conferta* (Allman, 1877)**, - *Zygophylax biarmata* Billard, 1905, - Plumulariidae spec. 2, - *Cladocarpus sinuosus* Vervoort, 1966.

Stn 22 B: M2005, 35°19.105'N 06°46.256'W, 518, m, box core; 30 May 2005.

Plumulariidae spec. 2.

Stn 28 B/AB: M2005, 35º18.200'N 6º48.527'W, 622 m, box core; 31 May 2005.

Lafoeina tenuis G.O. Sars, 1874, - *Lovenella producta* (G.O. Sars, 1874), - *Acryptolaria* cf. *conferta* (Allman, 1877), - *Lafoea* cf. *dumosa* (Fleming, 1820), - *Zygophylax biarmata* Billard, 1905.

Stn 29 B: M2005, 35º18.431'N 6º48.539'W, 628 m, box core; 01 June 2005.

Acryptolaria cf. *conferta* (Allman, 1877).

Stn 30 B: M2005, 35º18.755'N 6º47.895'W, 550 m, box core; 01 June 2005.

Acryptolaria cf. *conferta* (Allman, 1877).

Stn AT-600-Gr: TTR16, 35°18.779'N 06°48.453'W, 610 m, MAKAT127, mud breccia (?) covered by coral debris and carbonate crusts; 28 May 2006.

Lafoeina tenuis G.O. Sars, 1874, - *Lafoea* cf. *dumosa* (Fleming, 1820), - ***Acryptolaria* cf. *conferta* (Allman, 1877)**, - *Sertularella robusta* Allman, 1873, - *Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp2.

Stn AT-602-Gr: TTR16, 35°17.693'N 06°47.089'W, 556 m, MAKAT126, hemipelagic sediments, shell debris cemented by carbonate crusts; 28 May 2006.

Acryptolaria cf. *conferta* (Allman, 1877).

Stn 2: M2005, 35°17.701'N 06°47.279'W, 526 m, box core; 24 September 2006.

Modeeria rotunda (Quoy & Gaimard, 1827), - *Filellum* cf. *serratum* (Clarke, 1879), - ***Cryptolaria pectinata* (Allman, 1888)**, - ***Zygophylax biarmata* Billard, 1905**, - ***Zygophylax levinseni* (Saemundsson, 1911)**, - *Sertularella robusta* Allman, 1873.

Stn 6: M2006, 35°16.880'N 06°45.350'W, 418 m, box core, western top of Gemini mud volcano; 06 October 2006.

Zygophylax levinseni (Saemundsson, 1911), - *Aglaophenia lophocarpa* Allman, 1877.

Stn 8: M2006, 35°16.750'N 06°45.720'W, 444 m, box core; 07 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877), - *Zygophylax levinseni* (Saemundsson, 1911), - *Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp2, - ***Aglaophenia lophocarpa* Allman, 1877**, - *Lytocarpia myriophyllum* (Linnaeus, 1758).

Stn 9: M2006, 35°16.760'N 06°45.760'W, 451 m, box core; 07 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877), - *Zygophylax levinseni* (Saemundsson, 1911), - *Polyplumaria flabellata* G.O. Sars, 1874, - *Aglaophenia lophocarpa* Allman, 1877.

Stn 10: M2006, 35°16.830'N 06°45.540'W, 432 m, box core; 07 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877), - *Zygophylax* sp., - Lafoeidae sp., - *Aglaophenia lophocarpa* Allman, 1877.

Stn 11: M2006, 35°16.790'N 06°27.350'W, 438 m, box core; 07 October 2006.

Filellum cf. *serratum* (Clarke, 1879), - *Zygophylax levinseni* (Saemundsson, 1911), - *Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp2.

Stn 13: M2006, 35°16.650'N 6°46.110'W, 516 m, box core; 08 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877), - *Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp2, - *Lytocarpia myriophyllum* (Linnaeus, 1758).

Stn 21: M2006, 35°19.000'N 06°48.050'W, 560 m, box core; 10 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877).

Stn 22: M2006, 35°19.000'N 6°48.160'W, 557 m, box core; 10 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877).

Stn 23: M2006, 35°19.020'N 06°48.220'W, 557 m, box core, hydroids over dead *Lophelia*; 10 October 2006.

Lafoeina tenuis G.O. Sars, 1874, - ***Acryptolaria* cf. *conferta* (Allman, 1877)**, - *Zygophylax biarmata* Billard, 1905, - *Antennella secundaria* (Gmelin, 1791), - *Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp2.

Stn 24: M2006, 35°19.020'N 06°48.340'W, 571 m, box core; 10 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877).

Stn 28: M2006, 35°19.010'N 06°49.000'W, 642 m, box core; 10 October 2006.

Zygophylax biarmata Billard, 1905.

Stn 33: M2006, 35°18.930'N 06°47.590'W, 542 m, box core; 11 October 2006.

Filellum sp., - *Acryptolaria* cf. *conferta* (Allman, 1877).

Stn 34: M2006, 35°18.920'N 06°47.410'W, 543 m, box core; 11 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877), - *Zygophylax* sp.

Stn 38A: M2006, 35°19.090'N 06°46.400'W, 494 m, box core; 12 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877), - *Zygophylax biarmata* Billard, 1905, - *Lytocarpia myriophyllum* (Linnaeus, 1758).

Stn 38B: M2006, 35°19.090'N 06°46.400'W, 497 m, box core; 12 October 2006.

Aglaophenia lophocarpa Allman, 1877.

Stn 38C: M2006, 35°19.090'N 6°46.400'W, 497 m, box core; 12 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877).

Stn 38D: M2006, 35°19.090'N 06°46.400'W, 497 m, box core; 12 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877).

Stn 39: M2006, 35°18.900'N 06°47.900'W, 560 m, box core; 13 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877).

Stn 40: M2006, 35°18.900'N 06°47.020'W, 542 m, box core; 13 October 2006.

Lafoeina tenuis G.O. Sars, 1874, - *Filellum* cf. *serratum* (Clarke, 1879), - *Acryptolaria* cf. *conferta* (Allman, 1877), - *Zygophylax biarmata* Billard, 1905.

Stn 40A: M2006, 35°18.910'N 06°47.030'W, 560 m, box core; 13 October 2006.

Lafoeina tenuis G.O. Sars, 1874, - *Filellum* cf. *serratum* (Clarke, 1879), - *Acryptolaria* cf. *conferta* (Allman, 1877), - *Zygophylax biarmata* Billard, 1905, - *Zygophylax levinseni* (Saemundsson, 1911), - ***Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp2**.

Stn 41: M2006, 35°18.910'N 06°48.180'W, 568 m, box core; 13 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877).

Stn 42: M2006, 35°18.920'N 06°48.390'W, 637 m, box core; 13 October 2006.

Lafoeina tenuis G.O. Sars, 1874, - ***Lafoea* cf. *dumosa* (Fleming, 1820) / sp3**, - *Acryptolaria* cf. *conferta* (Allman, 1877).

Stn 43: M2006, 35°18.900'N 6°49.240'W, 654 m, hopper camera; 13 October 2006.

Nemertesia cf. *antennina* (Linnaeus, 1758) / sp2.

Stn 44A: M2006, 35°18.900'N 06°48.900'W, 640 m, box core; 13 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877).

Stn 49: M2006, 35°17.900'N 06°38.640'W, 360 m, box core; 14 October 2006.

Nemertesia cf. *antennina* (Linnaeus, 1758) / sp2.

Stn 51: M2006, 35°18.570'N 6°48.280'W, 624 m, box core; 15 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877).

Stn 52: M2006, 35°18.440'N 6°48.240'W, 622 m, box core; 15 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877).

Stn 54: M2006, 35°18.090'N 06°48.120'W, 634 m, box core; 15 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877).

Stn 57: M2006, 35°17.920'N 06°47.940'W, 598 m, box core; 16 October 2006.

Campanulina panicula G.O. Sars, 1874, - *Acryptolaria* cf. *conferta* (Allman, 1877), - *Zygophylax biarmata* Billard, 1905.

Stn 58: M2006, 35°17.86000'N 06°47.880'W, 606 m, box core; 16 October 2006.

Nemertesia cf. *antennina* (Linnaeus, 1758) / sp2.

Stn 59: M2006, 35°17.790'N 06°47.770'W, 637 m, box core; 16 October 2006.

Acryptolaria cf. *conferta* (Allman, 1877), - *Zygophylax biarmata* Billard, 1905.

Stn unknown: M2007, net trapped on sampling device; May 2007.

Filellum cf. *serratum* (Clarke, 1879), - *Acryptolaria* cf. *flabellum* (Allman, 1888), - *Cryptolaria pectinata* (Allman, 1888), - *Zygophylax biarmata* Billard, 1905, - ***Zygophylax brownei* Billard, 1924**, - *Sertularella robusta* Allman, 1873, - ***Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp2**, - ***Nemertesia ventriculiformis* (Marktanner-Turneretscher, 1890)**.

Stn 15: M2007, 35°17.905'N 06°44.286'W, 444 m, box core; 06 May 2007.

***Acryptolaria* cf. *conferta* (Allman, 1877)**.

Stn 17: M2007, 35°17.061'N 06°42.617'W, 416 m, box core; 09 May 2007.

Acryptolaria cf. *conferta* (Allman, 1877), - ***Sertularella robusta* Allman, 1873**, - ***Nemertesia ventriculiformis* (Marktanner-Turneretscher, 1890)**, - *Streptocaulus dollfusi* Billard, 1924.

Stn 23: M2007, 35°17.772'N 06°43.964'W, 498 m, box core; 10 May 2007.

***Lytocarpia myriophyllum* (Linnaeus, 1758)**.

Stn 39: M2007, 35°00.230' 06°44.894'W, 441 m, box core; 11 May 2007.

Acryptolaria cf. *conferta* (Allman, 1877), - ***Zygophylax biarmata* Billard, 1905**, - ***Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp2**, - *Streptocaulus dollfusi* (Billard, 1924).

Stn 40: M2007, 35°00.137'N 06°44.806'W, 473 m, box core; 12 May 2007.

***Lafoea* cf. *dumosa* (Fleming, 1820) / sp3**, - *Acryptolaria* cf. *flabellum* (Allman, 1888), - ***Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp2**, - ***Aglaophenia lophocarpa* Allman, 1877**, - *Lytocarpia myriophyllum* (Linnaeus, 1758).

Stn 41: M2007, 35°00.113'N 06°44.815'W, 461 m, box core; 12 May 2007.

Modeeria rotunda (Quoy & Gaimard, 1827), - *Filellum* cf. *serratum* (Clarke, 1879), - *Lafoea* cf. *dumosa* (Fleming, 1820), - ***Acryptolaria* cf. *flabellum* (Allman, 1888)**, - *Zygophylax biarmata* Billard, 1905, - ***Sertularella robusta* Allman, 1873**, - *Antennella secundaria* (Gmelin, 1791), - ***Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp2**, - ***Nemertesia ventriculiformis* (Marktanner-Turneretscher, 1890)**, -

***Aglaophenia lophocarpa* Allman, 1877**, - ***Streptocaulus dollfusi* (Billard, 1924)**, - ***Campanulina panicula* G.O. Sars, 1874**.

Pipoca – mud volcano.

Stn GeoB12706-1: 64PE284, 36°26.810'N 07°12.700'W, 702 m, box core, sediment disturbed top: light yellowish brown sand with few shells, coral rubble, live cnidarians; southern flank mud volcano; 21 February 2008.

Aglaophenia lophocarpa Allman, 1877.

Student - Active mud volcano with carbonates located by the deep Atlantic Moroccan shelf.

Stn AT-232-G: TTR10, 35º30.849'N 7º08.744'W, 940 m, grab, seepage area with carbonates: grey mud enriched with foraminiferans, bivalve shells and many fragments of dead *Lophelia pertusa* and *Madrepora oculata*, also found a large (5 cm in diameter) carbonate crust in the middle of the recovered sequence; 22 July 2000.

Acryptolaria cf. *conferta* (Allman, 1877).

Stn AT-239-Gr: TTR10, 35º30.853'N 7º08.816'W, 955 m, TV-assisted grab, seepage area mainly characterised by a relatively flat relief with discontinuously distributed carbonate mounds and slabs; the video profile confirmed the presence of active seepage through the area and showed the presence of gas-related carbonate build-ups, live corals and coral debris; a large amount of dead bivalves and coral fragments trapped during the crust's cementation process were found in the sample; 22 July 2000.

Cuspidella spec., - *Acryptolaria* cf. *conferta* (Allman, 1877)

TTR – Active mud volcano located in the eastern Moroccan Field.

Stn AT-416-Gr: TTR12, 35º21.87'N 06º52.00'W, 695 m, TV-assisted grab, coral debris, light brownish very water-saturated maerl and grey dense carbonate clay; 17 July 2002.

Lafoeina tenuis G.O. Sars, 1874, - *Acryptolaria* cf. *conferta* (Allman, 1877), - *Filellum* cf. *serratum* (Clarke, 1879), - *Lafoea* cf. *dumosa* (Fleming, 1820), - *Zygophylax biarmata* Billard, 1905, - *Sertularella robusta* Allman, 1873.

Vernadsky ridge – NW-SE oriented tectonic ridge border a side of the El Arraiche mud volcano field located on the upper slope of the Moroccan shelf.

Stn AT-574-D: TTR15, 35º25.982’N 6º46.661’W, 508-512 m, dredge, one ton of sediment recovered rich with anthozoans (diverse octocorals and one antipatharian); 26 July 2005.

Acryptolaria cf. *conferta* (Allman, 1877), - *Cryptolaria pectinata* (Allman, 1888), - *Filellum* cf. *serratum* (Clarke, 1879), - *Zygophylax biarmata* Billard, 1905, - *Cladocarpus sinuosus* Vervoort, 1966, - *Sertularella robusta* Allman, 1873.

Stn GeoB12759-1: 64PE284, 35°26.570'N 06°46.780'W, 524 m, box core, brownish to grayish silty clay, abundant coral fragments, distinct pteropod layer; 04 March 2008.

***Zygophylax biarmata* Billard, 1905**.

Yuma – Active mud volcano with considerable densities of pogonophorans living amongst mud breccia. It is positioned by the middle Atlantic Moroccan Field.

Stn AT-523-G: TTR14, 35º24.960'N 07º05.475'W, 962 m, grab, mud breccia covered by hemipelagic sediments; 02 August 2004.

Acryptolaria longitheca (Allman, 1877).

Stn AT-524-Gr: TTR14, 35º24.958'N 07º05.510'W, 968 m, TV-guided grab, mud breccia covered by hemipelagic sediments; 02 August 2004.

Acryptolaria longitheca (Allman, 1877).

Stn AT-605-Gr: TTR16, 35°25.046'N 07°05.450'W, 975 m, TV-guided grab, mud breccia covered by thin layer of maerl; south-eastern crater; 29 May 2006.

***Lafoea* cf. *dumosa* (Fleming, 1820) / sp3**, - *Acryptolaria* cf. *conferta* (Allman, 1877).

Carbonate Mound Province SE of Yuma MV

Stn GeoB12722-1: 64PE284, 35°18.630'N 07°00.990'W, 907 m, 0-28cm: brown silty/sandy clay. Southern deep mounds; 25 February 2008.

***Nemertesia ramosa* (Lamarck, 1816)**.

3.2.5 ALBORAN SEA

3.2.5.1 Deep waters

Carmen – Mud volcano.

Stn MS-387-Gr: TTR17 – Leg 1, 35°43.292'N 04°44.022'W, 809 m, TV-assisted grab, 13 June 2008.

Lovenella producta (G.O. Sars, 1874).

Dhakan – Mud volcano.

Stn MS-412-G: TTR17 – Leg 1, 35°25.423'N 4°31.829'W, 360 m, grab, mud breccia; 19 June 2008.

***Aglaophenia lophocarpa* Allman, 1877**.

MS-413-Gr: TTR17 – Leg 1, 35°25.401'N 04°32.021'W, 377 m, TV-assisted grab; 19 June 2008.

Campanulina panicula G.O. Sars, 1874, - *Antennella secundaria* (Gmelin, 1791), - *Aglaophenia lophocarpa* Allman, 1877.

Granada - Mud volcano.

Stn MS-420-Gr: TTR17 – Leg 1, 35°33.854'N 04°37.435'W, 600 m, TV-assisted grab; 20 June 2008.

Antennella secundaria (Gmelin, 1791), - ***Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp1**, - ***Nemertesia ventriculiformis* (Marktanner-Turneretscher, 1890)**, - ***Streptocaulus dollfusi* Billard, 1924**.

Melilla Carbonate Mound

Stn MS-393-B: TTR17 – Leg 1, 35°19.799'N 02°33.067'W, 245 m, box corer, coral; 16 June 2008.

***Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp1**, - ***Lytocarpia myriophyllum* (Linnaeus, 1758)**.

Stn MS-394-B: TTR17 – Leg 1, 35°26.064'N 02°30.585'W, 332 m, TV-assisted grab, coral, 15 June 2008.

Antennella secundaria (Gmelin, 1791), - ***Nemertesia ventriculiformis* (Marktanner-Turneretscher, 1890)**.

Stn MS-395-Gr: TTR17 – Leg 1, 35°26.415'N 02°31.075'W, 300 m, TV-assisted grab, coral, 15 June 2008.

***Campanulina panicula* G.O. Sars, 1874**, - ***Acryptolaria* cf. *flabellum* (Allman, 1888)**, - *Antennella secundaria* (Gmelin, 1791), - ***Nemertesia* cf. *belini* Bedot, 1916**, - ***Aglaophenia lophocarpa* Allman, 1877**, - ***Streptocaulus dollfusi* Billard, 1924**.

Mulhacen – Mud volcano.

Stn MS-416-Gr: TTR17 – Leg 1, 35°24.431'N 04°34.119'W, 365 m, TV-assisted grab, mud breccia and coral, 20 June 2008.

Acryptolaria cf. *conferta* (Allman, 1877), - ***Nemertesia* cf. *antennina* (Linnaeus, 1758) / sp1**, - *Aglaophenia lophocarpa* Allman, 1877.

3.2.6 SPAIN (southern coast)

3.2.6.1 Shallow waters

Benidorm Island: 38º29.914'N 0º07.746'W, 8-26 m, scuba diving, rocky seafloor; 13 November 2008.

Lafoea cf. *dumosa* (Fleming, 1820), - ***Filellum* cf. *serpens* (Hassall, 1848)**, - ***Scandia gigas* (Pieper, 1884)**, - *Sertularella ellisii* (Deshayes & Milne Edwards, 1836), - *Halopteris diaphana* (Heller, 1868), - *Aglaophenia pluma* (Linnaeus, 1758).

3.2.6 UNITED KINGDOM

3.2.6.1 Shallow waters

Lundy Island: 51º09.24’N 4º39.18’W, 5-40 m, scuba diving, collector: J. Hall-Spencer; 2006.

***Diphasia rosacea* (Linnaeus, 1758)**, - ***Hydrallmania falcata* (Linnaeus, 1758)**, - ***Gymnangium montagui* (Billard, 1912)**, - ***Sertularia cupressina* Linnaeus, 1758**,- ***Halopteris catharina* (Johnston, 1833)**, - ***Nemertesia antennina* (Linnaeus, 1758)**, - ***Nemertesia ramosa* Lamouroux, 1816**, - ***Aglaophenia kirchenpaueri* (Heller, 1868)**, - ***Aglaophenia pluma* (Linnaeus, 1758)**, - ***Aglaophenia tubulifera* (Hincks, 1861)**, - ***Gymnangium montagui* (Billard, 1912)**.

Plymouth: 5-40 m, scuba diving, collector: J. Hall-Spencer; 2006.

***Diphasia rosacea* (Linnaeus, 1758)**, - ***Hydrallmania falcata* (Linnaeus, 1758)**, - ***Nemertesia antennina* (Linnaeus, 1758)**, - ***Aglaophenia kirchenpaueri* (Heller, 1868)**.

Plymouth: Mewstone, 50º17.60’N 4º05.60’W, 18-25 m, scuba diving, rocky bottom; June 2007.

***Sertularella polyzonias* (Linnaeus, 1758)**, - ***Nemertesia ramosa* Lamouroux, 1816**, - ***Aglaophenia pluma* (Linnaeus, 1758)**.

3.2.8 MALTA ISLAND

3.2.8.1 Shallow waters

Cirkewwa: NW Malta, 35°59.3480'N 14°19.680'E, 16 - 26 m, scuba diving, rocky bottom; 15 April 2010.

Lafoeina tenuis G.O. Sars, 1874, - *Anthohebella parasitica* (Ciamician, 1880), - ***Scandia gigas* (Pieper, 1884)**, - *Sertularia distans* (Lamouroux, 1816), - ***Sertularia perpusilla* Stechow, 1911**, - *Antennella secundaria* (Gmelin, 1791), - *Monotheca obliqua* (Johnston, 1847), - ***Aglaophenia harpago* Schenck, 1965**.

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Pfannkuche, O. & cruise participants. CRUISE REPORT, MARIA S. MERIAN 1/3, 12 April – 19 May 2006; IFM-GEOMAR, Leibniz-Institut für Meereswissenschaften, Kiel, Germany. 115 pp.

Chapter 4

THE HYDROZOAN FAUNA (CNIDARIA: HYDROZOA) FROM THE PEAKS OF THE GORRINGE BANK, NE ATLANTIC

Moura, C. J. (2011). [submitted].

Contents

ABSTRACT

28 hydroid species were collected from the summits (35-42 m) of the Gorringe Bank (NE Atlantic) during the oceanographic campaign 'LusoExpedição Olympus 2008'. This taxonomic survey presents 20 new records of hydroid species for the Gorringe Bank which is known to contain a total of 38 hydroid species. *Eudendrium armatum* and *Eudendrium* cf. *merulum* were supposedly restricted to the Mediterranean and were now found in the Gorringe. The Gorringe seems highly connected zoogeographically with the Mediterranean and neighbouring continental coasts, in terms of hydrozoan fauna. The large densities of algae at the peaks of the seamounts sustain a considerable hydrozoan diversity (23 species). Conversely, algae may also inhibit the direct attachment of hydroid species to the rocky substrata. All the hydrozoan species known from the peaks of the Gorringe were collected during summer only, and therefore sampling in other seasons, despite being

somewhat adventurous by scuba diving, may reveal further hydrozoan diversity due to seasonal patterns of growth of algae and hydroids. Nevertheless, the reasonably high levels of hydrozoan biodiversity demonstrated only from the summits of the Gorringe, is further indication of this seamount as a 'biodiversity hotspot'.

4.1 INTRODUCTION

Seamounts are prominent undersea mountains usually associated with volcanic activity (e.g. Epp & Smoot 1989; Rogers 1994). Seamounts are known as 'underwater oases' because compared to surrounding environments, they host richer communities of seabed organisms (e.g. corals, sponges), as well as aggregations of fishes (e.g. Rogers 1994; Koslow 1997; Koslow 2000; Stocks 2004). The high establishment of biodiversity in seamounts is mainly due to the availability of hard-substrata and interaction of seamounts with oceanic currents that tend to increase water-flow velocities around these geologic structures, giving rise to hydrographic phenomena like upwellings, turbulence, Taylor cones and eddies (e.g. Eriksen 1991; Kunze & Stanford 1993; Rogers 1994; Koslow *et al*. 2001; Coelho & Santos 2003).

There is an estimation of up to 100,000 seamounts in the world's oceans rising from the seabed more than 1 Km (Wessel 2001). Although only a very small fraction of these structures have been properly sampled and studied in terms of biodiversity (Stocks 2009), despite their well-recognized importance in terms of marine life (e.g. Rogers 1994).

The Gorringe Bank is located at about 125-150 miles WSW off the Cape St. Vincent in SW Portugal. It covers an area of over 180 Km long, that rises from depths of over 5000 m up to depths of about 35 m in its two major peaks: the Gettysburg (36º31'N, 11º34'W) and Ormonde (38º42'N, 11º09'W). The seamounts Gettysburg and Ormonde are set apart by about 30 miles. (Oceana 2005; Tourais 2008; Fig. 4.1)

The Gorringe Bank is included in a ridge with other seamounts oriented in a NE-SW direction, covering an area of about 9500 Km^2 (Oceana 2005). The Gorringe Bank is assumed to be one of the oldest Atlantic seamounts (Conti *et al*. 2004). It is located in an area of conjunction of the African and Euro-Asiatic tectonic plates, and is sometimes associated with geological seismicity (e.g. Purdy 1975; Moreira 1985). The Gorringe is influenced by several important major currents that converge in or nearby the seamounts (e.g. the Gulf, Antarctic, African and Mediterranean currents) being therefore a site potentially enriched with nutrients and consequently of fauna and algae (e.g. Oceana 2005).

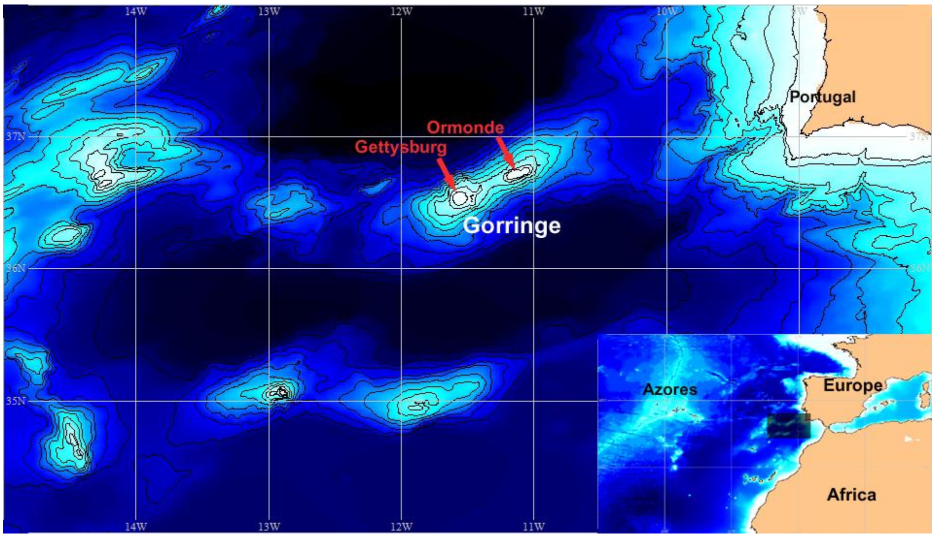

Fig. 4.1. Map with the location of the Gorringe Bank.

Despite the topographic prominence of the Gorringe Bank, its relative proximity to shore and recognizable importance as biodiversity 'hotspot' with need for protection as marine protected area (WWF 2001; Oceana 2005; Oceana 2011), the Gorringe has only been subject to few biological studies in recent years (Ramil *et al*. 1998; Ávila & Malaquias 2003; Dijkstra & Gofas 2004; Gonçalves *et al*. 2004; Oceana 2005; Xavier & van Soest 2007; Abecasis *et al*. 2009).

The hydrozoan fauna of the Gorringe Bank is little studied, only with records of some deep-sea hydroids (Ramil *et al.* 1998) and very few shallow-water taxa (Oceana 2005). The latter study probably did not include a taxonomic revision by a hydrozoan specialist.

The aim of the present study was to identify the shallow-water hydrozoan fauna of the peaks of Gorringe Bank (Gettysburg and Ormonde) collected by SCUBA diving during the 'LusoExpedição Olympus 2008' between the 8th and 13th July 2008 onboard of the NT 'Creoula'. This study may also contribute to further arguments for the implementation of a marine reserve at the Gorringe Bank area (WWF 2001; Oceana 2005; Oceana 2011).

8.2 LIST OF STATIONS

Stn 1: Ormonde peak, 10 June 2008, 16:00h, collector: C. J. Moura, 36º42.857'N 11.09.968'W, 35-37 m.

Eudendrium armatum Tichomiroff, 1887, - *Eudendrium* sp., - *Lafoeina tenuis* G.O. Sars, 1874, - *Filellum* cf. *serratum* (Clarke, 1879), - *Sertularella ellisii* (Deshayes & Milne Edwards, 1836), - *Antennella secundaria* (Gmelin, 1791), - *Aglaophenia kirchenpaueri* (Heller, 1868), - *Orthopyxis integra* (MacGillivray, 1842), - *Clytia hemisphaerica* (Linnaeus, 1767).

Stn 2: Ormonde peak, 13 June 2008, 12:00h, collector: J. Xavier, 36º42.839'N 11º09.944'W, *ca.* 40 m.

Lafoeina tenuis G.O. Sars, 1874, - *Sertularella ellisii* (Deshayes & Milne Edwards, 1836), - *Plumularia setacea* (Linnaeus, 1758), - *Aglaophenia pluma* (Linnaeus, 1758), - *Clytia hemisphaerica* (Linnaeus, 1767).

Stn 3: Ormonde peak, 13 June 2008, 16:00h, collector: J. Xavier, 36º42.945'N 11º09.313'W, unknown depth.

Eudendrium cf. *merulum* Watson, 1985, - *Lafoeina tenuis* G.O. Sars, 1874, - *Halecium* sp., - *Sertularella ellisii* (Deshayes & Milne Edwards, 1836), - *Obelia dichotoma* (Linnaeus, 1758), - *Clytia hemisphaerica* (Linnaeus, 1767).

Stn 4: Ormonde peak, 13 June 2008, 16:30h, collector: C.J. Moura, 36º42.833'N 11º09.956'W, 37-42 m.

Coryne eximia Allman, 1859, - *Hydractinia* sp., - *Eudendrium armatum* Tichomiroff, 1887, - *Eudendrium* cf. *merulum* Watson, 1985, - *Eudendrium ramosum* (Linnaeus, 1758), - *Lafoeina tenuis* G.O. Sars, 1874, - *Filellum* cf. *serratum* (Clarke, 1879), - *Halecium pusillum* (M. Sars, 1857), - *Halecium* sp., - *Sertularella ellisii* (Deshayes & Milne Edwards, 1836), - *Obelia dichotoma* (Linnaeus, 1758), - *Obelia geniculata* (Linnaeus, 1758), - *Orthopyxis integra*

(MacGillivray, 1842), - *Clytia gracilis* (M. Sars, 1850), - *Clytia hemisphaerica* (Linnaeus, 1767).

Stn 5: Gettysburg peak, 11 June 2008, 10:30h, collector: J. Xavier, 36º31.092'N 11º34.284'W, 35-37 m.

Coryne cf. *pusilla* Gaertner, 1774, - *Eudendrium armatum* Tichomiroff, 1887, - *Eudendrium* cf. *merulum* Watson, 1985, - *Eudendrium* sp., - *Lafoeina tenuis* G.O. Sars, 1874, - *Filellum* cf. *serpens* (Hassal, 1848), - *Sertularella ellisii* (Deshayes & Milne Edwards, 1836), - *Sertularella ornata* Broch, 1933, - *Plumularia setacea* (Linnaeus, 1758), - *Aglaophenia pluma* (Linnaeus, 1758), - *Aglaophenia kirchenpaueri* (Heller, 1868), - *Clytia gracilis* (M. Sars, 1850), - *Clytia hemisphaerica* (Linnaeus, 1767).

Stn 6: Gettysburg peak, 11 June 2008, 11:00h, collector: P. Neves, 36º31.091'N 11º34.284'W, *ca.* 38 m.

Filifera undet., - *Eudendrium* sp., - *Lafoeina tenuis* G.O. Sars, 1874, - *Cuspidella* sp., - *Sertularella ellisii* (Deshayes & Milne Edwards, 1836), - *Plumularia setacea* (Linnaeus, 1758), - *Aglaophenia pluma* (Linnaeus, 1758), - *Aglaophenia kirchenpaueri* (Heller, 1868), - *Clytia gracilis* (M. Sars, 1850), - *Clytia hemisphaerica* (Linnaeus, 1767).

Stn 7: Gettysburg peak, 11 June 2008, 14:30h, collector: C.J. Moura, 36º31.076'N 11º34.277'W, 33-37 m.

Bougainvillidae undet., - *Eudendrium armatum* Tichomiroff, 1887, - *Eudendrium ramosum* (Linnaeus, 1758), - *Eudendrium* sp., - *Lafoeina tenuis* G.O. Sars, 1874, - *Cuspidella* sp., - *Halecium* sp., - *Filellum* cf. *serpens* (Hassall, 1848), - *Sertularella ellisii* (Deshayes & Milne Edwards, 1836), - *Antennella secundaria* (Gmelin, 1791), - *Aglaophenia pluma* (Linnaeus, 1758), - *Aglaophenia kirchenpaueri* (Heller, 1868), - *Clytia gracilis* (M. Sars, 1850), - *Clytia hemisphaerica* (Linnaeus, 1767).

Stn 8: Gettysburg peak, 12 June 2008, 10:30h, collector: J. Pedro, 36º31.071'N 11º34.275'W, *ca.* 33 m.

Eudendrium armatum Tichomiroff, 1887, - *Eudendrium* sp., - *Lafoeina tenuis* G.O. Sars, 1874, - *Cuspidella* sp., - *Halecium tenellum* Hincks, 1861, - *Sertularella ellisii* (Deshayes & Milne Edwards, 1836), - *Antennella secundaria* (Gmelin, 1791), - *Plumularia setacea* (Linnaeus, 1758), - *Aglaophenia pluma* (Linnaeus, 1758), - *Aglaophenia kirchenpaueri* (Heller, 1868), - *Clytia gracilis* (M. Sars, 1850), - *Clytia hemisphaerica* (Linnaeus, 1767).

Stn 9: Gettysburg peak, 12 June 2008, 14:30h, collector: C.J. Moura, 36º31.071'N 11º34.275'W, 37-41 m.

Filifera undet., - *Eudendrium armatum* Tichomiroff, 1887, - *Eudendrium* cf. *merulum* Watson, 1985, *Filellum* cf. *serratum* (Clarke, 1879), - *Lafoeina tenuis* G.O. Sars, 1874, - *Cuspidella* sp., - *Halecium* sp., - *Filellum serratum* (Clarke, 1879), - *Sertularella ellisii* (Deshayes & Milne Edwards, 1836), - *Antennella secundaria* (Gmelin, 1791), - *Aglaophenia pluma* (Linnaeus, 1758), - *Clytia gracilis* (M. Sars, 1850), - *Clytia hemisphaerica* (Linnaeus, 1767).

Stn 10: Gettysburg peak, 12 June 2008, 16:30h, collector: J. Pedro, 36º31.071'N 11º34.275'W, 37-39 m.

Eudendrium armatum Tichomiroff, 1887, - *Eudendrium* sp., - *Lafoeina tenuis* G.O. Sars, 1874, - *Halecium tenellum* Hincks, 1861, - *Halecium pusillum* (M. Sars, 1857), - *Sertularella gayi* (Lamouroux, 1821), - *Sertularella ellisii* (Deshayes & Milne Edwards, 1836), - *Plumularia setacea* (Linnaeus, 1758), - *Aglaophenia pluma* (Linnaeus, 1758), - *Aglaophenia kirchenpaueri* (Heller, 1868), - *Clytia gracilis* (M. Sars, 1850), - *Clytia hemisphaerica* (Linnaeus, 1767).

4.3 TAXONOMIC REPORT

ANTHOATHECATA

Filifera undet.
(Fig. 4.2A)

Material inspected. **Gettysburg, stn 6**: over cauli of algae *Zonaria tourneforti*, orange color after preservation. **Gettysburg, stn 9**: over cauli of algae *Zonaria tourneforti*, orange color after preservation.

Remarks. Phenotype resembling Hydractiniidae species.

Family Corynidae Johnston, 1836

***Coryne eximia* Allman, 1859**
(Fig. 4.2B)

Coryne eximia Allman, 1859: 141. - Schuchert 2001: 773-776, Figs 13A-D & 18B-C.

Material inspected. – **Ormonde, stn 4**: very abundant over diving cable lost two years before; fertile colonies; epibionts: *Lafoeina tenuis*, *Clytia gracilis*, small algae and calcified Bryozoa.

Remarks. This species was only found over anthropogenic materials and was genetically undifferentiated from a 16S sequence from the Mediterranean (GenBank accession: AJ878715.1). It may correspond to an unintentional human-introduced species in the Gorringe.

Distribution. Circumglobal (Schuchert 2005). This is the first record of *C. eximia* in the Gorringe.

***Coryne* cf. *pusilla* Gaertner, 1774**
(Fig. 4.2C)

Coryne pusilla Gaertner, 1774: 40, pl. 4 Fig. 8. - Schuchert 2001: 776-780, Fig. 14.

Material inspected. **Gettysburg, stn 5**: scarce material, several hydrocauli over caulus of *Zonaria tourneforti*; ramification up to 1st order; perisarc annulated throughout; stenotele length *ca.* 21 µm.

Remarks. The absence of fertile material makes impossible the accurate identification of this material. Nevertheless this is the first record of this species in the Gorringe.

Distribution. *Coryne pusilla* is presumably present in coasts of Europe, South Africa, Kerguelen, Seychelles, Korea, Japan, New Zealand, eastern Canada and Mediterranean Sea (Schuchert 2001).

Family Bougainvilliidae Lütken, 1850

Bougainvillidae undet.

(Fig. 4.2D)

Material inspected. **Gettysburg, stn 7**: abundant, creeping over algae and *Eudendrium armatum,* colonies maximum size is 1200 µm; nematocysts: 7x3 µm; perisarc tube usually annulated basally, occasionally distally, usually straight; pseudohydrotheca present, one row of filiform tentacles, hypostome salient.

Remarks. The phenotype is similar to that of *Rhizorhagium roseum* Sars, 1874. The absence of fertile specimens made impossible an accurate identification. This is the first record of this species in the Gorringe.

Family Hydractiniidae L. Agassiz, 1862

Hydractiniidae undet.

(Fig. 4.2E, F)

Material inspected. **Ormonde, stn 4**: sparse polyps over cauli of *Zonaria tourneforti*.

Fig. 4.2. (A) Filifera undet. – polyp; Stn 9. (B) *Coryne eximia* Allman, 1859 – large colonies overgrowing a cable; Stn 4. (C) *Coryne* cf. *pusilla* Gaertner, 1774 – part of a colony; Stn 5. (D) Bougainvillidae undet. – fragment of a colony; Stn 10. (E, F) Hydractiniidae undet. **-** Individual polyps detached from the colony; Stn 4. (G) *Eudendrium armatum* Tichomiroff, 1887 – live bushy colony little exposed to sunlight; Stn 7. (H, I) *Eudendrium* cf. *merulum* Watson, 1985 - part of colony; Stn 4. (H) colony overgrowing one crustacean; Stn 9. (I, J) *Eudendrium ramosum* (Linnaeus, 1758) – dense tufts of well-developed monosiphonic colonies; Stn 4. (K) *Eudendrium* sp. – hydranth and pedicel; Stn 5. Scale bars: 0.2 mm (A, E, F); 1 mm (C, D, H, K); 5 mm (I); 1 cm (J). Photo credits. C.J. Moura.

Remarks. The sample material was not fertile preventing an accurate identification of this species. This is the first record of this species in the Gorringe.

Family Eudendriidae Agassiz,1862

Eudendrium armatum Tichomiroff, 1887

(Fig. 4.2G)

Eudendrium armatum Tichomiroff, 1887: 31, Fig. 25, pl. 1 Figs 3-4, pl. 2, Figs 3-4. – Schuchert 2008: 744-746, Fig. 35.

Material inspected. **Ormonde, stn 1**: few individual colonies, over rock, epibionts: small algae, calcified Bryozoa (infesting a dead colony). **Ormonde, stn 4**: one colony, over rock, epibionts: *Clytia hemisphaerica*, calcified Bryozoa and small algae. **Gettysburg, stn 5**: some colonies over rocks (found amidst sponges), epibionts: algae and calcified Bryozoa. **Gettysburg, stn 7**: many specimens (including large colonies), overgrowing rocks, algae, sponges and Bryozoa; fertile colonies; epibionts: Bougainvillidae undet., *Halecium sibogae*, *Clytia gracilis*, foraminifera, calcified Bryozoa, polychaetes, algae. **Gettysburg, stn 8**: few small colonies, epibionts: small algae. **Gettysburg, stn 9**: large colonies, fertile, over rock and dead octocoral, epibionts: small algae, calcified Bryozoa and foraminifera. **Gettysburg, stn 10**: two colonies (one quite large), over rock, epibionts: small algae, two species of calcified Bryozoa.

Remarks. Genetics confirmed the identity of this species (unpublished data). This is the first record of *E. armatum* in the Gorringe and outside the Mediterranean basin.

Distribution. *E. armatum* was assumed to be endemic to the Mediterranean (e.g. Schuchert 2008).

Eudendrium cf. *merulum* Watson, 1985

(Fig. 4.2H,I)

Eudendrium merulum Watson, 1985: 200, Figs 53-58. - Schuchert 2008: 715-719, Figs 19-20.

Material inspected. **Ormonde, stn 4**: all over the brown algae *Zonaria tourneforti* Linnaeus, overgrowing other algae with *Orthopyxis integra*. **Gettysburg, stn 9**: abundant, over small shrimp and cauli of algae *Zonaria tourneforti*.

Remarks. This is the first record of *E. merulum* in the Gorringe. The inconclusive taxonomic status is due to the probable cryptic biodiversity associated with this species (Schuchert 2008). Identification verified genetically (unpublished data).

Distribution. *Eudendrium merulum* was originally described from Australia, but has been recorded in the Mediterranean possibly as misidentified (see Schuchert 2008).

Eudendrium ramosum (Linnaeus, 1758)

(Fig. 4.2J)

Tubularia ramosa Linnaeus, 1758: 804.
Eudendrium ramosum – Schuchert 2008: 702-708, Figs 12-14.

Material inspected. **Ormonde, stn 4**: dense aggregations of monosiphonic, branched, tall and robust colonies overgrowing sponges; epibionts: small algae. **Gettysburg, stn 7**: four robust monosiphonic colonies, over sponges, epibionts: *Halecium* spand small algae.

Remarks. This is the first record of *E. ramosum* in the Gorringe.

Distribution. *E. ramosum* seems accurately identified from the Mediterranean and European coasts from the Artic to Africa (see Schuchert 2008).

Eudendrium sp.

(Fig. 4.2K)

Material inspected. **Ormonde, stn 1**: fertile specimens (sporosac developing on hydranth), frequent over cauli of the algae *Zonaria tourneforti*. **Ormonde, stn 3**: few exemplars, delicate, unbranched and monosiphonic colonies, overgrowing cauli of *Zonaria tourneforti*; hydrocauli with few annulations. **Gettysburg, stn 5**: fertile specimens; small, delicate and unbranched colonies; pedicel of hydrothecae annulated; overgrowing *Zonaria tourneforti* and *Aglaophenia pluma*. **Gettysburg, stn 6**: abundant on *Zonaria tourneforti*, fertile and monosiphonic colonies. **Gettysburg, stn 7**: frequent over cauli of *Zonaria tourneforti*, hydrocauli somewhat annulated. **Gettysburg, stn 8**: fertile, predominantly over cauli of *Zonaria tourneforti*, less common over hydrorhizae of *Sertularella ellisii*. **Gettysburg, stn 10**: few exemplars over cauli of *Zonaria tourneforti*, pedicels strongly annulated.

Remarks. This species did not reveal any particular phylogenetic relation with a variety of *Eudendrium* species (Schuchert, unpublished; Moura, unpublished). This represents the first record of this inconspicuous species in the Gorringe.

LEPTOTHECATA

Family Campanulinidae Hincks, 1868

Lafoeina tenuis G.O. Sars, 1874

(Fig. 4.3A)

Lafoeina tenuis G.O. Sars, 1874: 95, 119-121, pl. 5 Figs 1-5; Bouillon *et al*. 2004: 131, Figs 69E-G.

Material inspected. **Ormonde, stn 1**: few polyps, over cauli of *Zonaria tourneforti*. **Ormonde, stn 2**: few polyps, all over some *Zonaria tourneforti*. **Ormonde, stn 3**: very abundant, over cauli of *Zonaria tourneforti* and calcified bryozoa. **Ormonde, stn 4**: frequent, all over some *Zonaria tourneforti*. – Gettysburg, stn 5: few polyps, over *Zonaria tourneforti*. **Gettysburg, stn 6**: few polyps, over two species of algae. **Gettysburg, stn 7**: frequent over algae. **Gettysburg, stn 8**: few polyps, over *Zonaria tourneforti*. **Gettysburg, stn 9**: very frequent over *Zonaria tourneforti*, also overgrowing *Sertularella ellisii*. **Gettysburg, stn 10**: very abundant; over algae and some colonies of *Sertularella gayi* and *S. ellisii*.

Remarks. This is the first record of this species in the Gorringe.

Distribution. North Atlantic, Indian Ocean and Mediterranean (Bouillon *et al*. 2004).

***Cuspidella* sp.**

(Fig. 4.3B)

Material inspected. **Gettysburg, stn 6**: relatively frequent, over cauli of *Zonaria tourneforti*, basal line in hydrothecal cusps present. **Gettysburg, stn 7**: many polyps detected over cauli of one specimen of *Zonaria tourneforti*. **Gettysburg, stn 8**: over *Zonaria tourneforti*, basal line in hydrothecal cusps present. **Gettysburg, stn 9**: relatively frequent, over cauli of *Zonaria tourneforti* and dead bits of octocorals.

Remarks. This is the first record of this species in the Gorringe.

Family Haleciidae Hincks, 1868

***Halecium pusillum* (M. Sars, 1857)**

(Fig. 4.3C)

Eudendrium pusillum M. Sars, 1857: 154, pl. 1 Figs 14-16.
Halecium pusillum - Peña Cantero & Carrascosa 2002: 71-74, Fig. 14d-e. - Bouillon *et al*. 2004: 142, Figs 76D-G.

Material inspected. **Ormonde, stn 4**: few individual colonies, over algae. **Gettysburg, stn 10**: few specimens, over *Aglaophenia kirchenpaueri* , *Sertularella gayi* and algae.

Remarks. The identity of species was verified genetically with exemplars from station 10. This is the first record of this species in the Gorringe.

Distribution. Eastern and western Atlantic, Mediterranean, Indian Ocean and possibly in the Pacific (Bouillon *et al*. 2004).

***Halecium tenellum* Hincks, 1861**

(Fig. 4.3D)

Halecium tenellum Hincks, 1861: 252, pl. 6 Figs 1-4. - Ramil *et al*. 1998: 8; Medel & Vervoort 2000: 23-25; Peña Cantero & Carrascosa 2002: 75-77, Fig. 12c-e. - Bouillon *et al*. 2004: 143, Figs 77A-E.

Material inspected. **Gettysburg, stn 8**: over *Zonaria tourneforti* and *Sertularella ellisii*. **Gettysburg, stn 10**: common over *Sertularella ellisii* and *Sertularella gayi*.

Remarks. This nominal species was previously recorded in the Gorringe at a depth of 54-62 m (Ramil *et al*. 1998).

Distribution. Atlantic, Mediterranean, Indo-Pacific, Arctic, Antarctic (Bouillon *et al*. 2004).

Halecium sp.

(Fig. 4.3E)

Material inspected. **Ormonde, stn 3**: few colonies overgrowing *Zonaria tourneforti*. **Ormonde, stn 4**: considerable (but not abundant) representation of colonies, over cauli of *Zonaria tourneforti*. **Gettysburg, stn 7**: very abundant, some well-developed colonies, over: *Eudendrium armatum* and *E. ramosum*, sponges, different sorts of algae. **Gettysburg, stn 9**: few colonies, over *Zonaria tourneforti*.

Remarks. This species seems phylogenetically close to *Halecium muricatum* as indicated by 16S haplotype data (results not shown). It is distinct from any other *Halecium* species ever recorded in the Gorringe. This represents the first record of this species in the Gorringe Bank.

Family Lafoeidae Hincks, 1868

Filellum cf. *serpens* (Hassall, 1848)

(Fig. 4.3F)

Campanularia serpens Hassal, 1848: 2223.

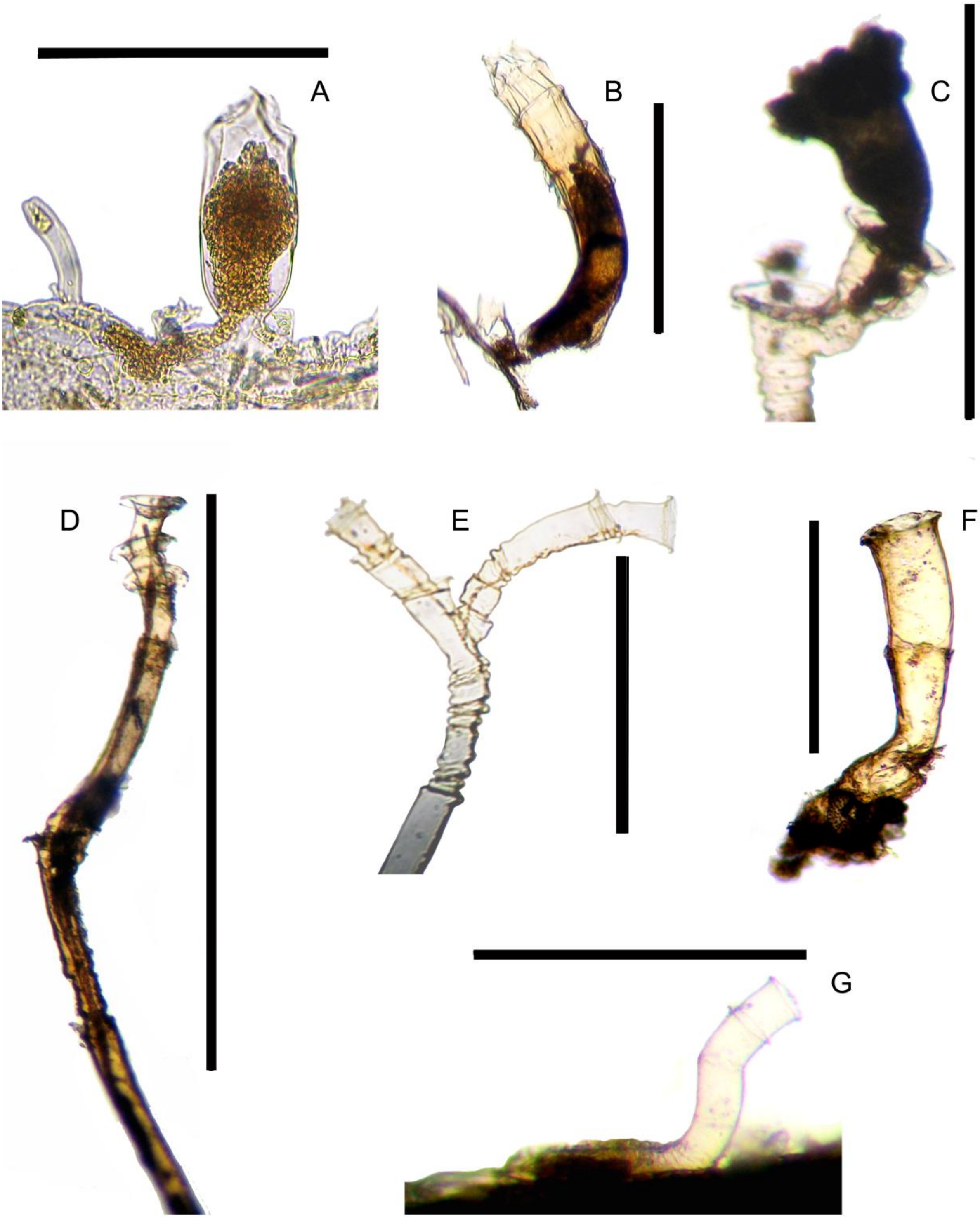

Fig. 4.3. (A) *Lafoeina tenuis* G.O. Sars, 1874 – hydrotheca with hydranth (right) and nematotheca (left); Stn 10. (B) *Cuspidella* sp. – Hydrotheca with hydranth; Stn 9. (C) *Halecium pusillum* (M. Sars, 1857) **-** part of a colony, hydrothecae and hydranths; Stn 10. (D) *Halecium tenellum* Hincks, 1861- part of a colony; Stn 10. E: *Halecium* sp. – portion of a colony; Stn 4. (F) *Filellum* cf. *serpens* (Hassall, 1848) – hydrotheca; Stn 7. (G) *Filellum* cf. *serratum* (Clarke, 1879) – hydrotheca; Stn 4. Scale bars: 0.2 mm (A); 0.5 mm (B, C, E-G); 1 mm (D). Photo credits. C.J. Moura.

Coppinia mirabilis Hassall, 1848: 2223.
Filellum serpens - Bouillon *et al.* 2004: 156, Figs 84 G-H.

Material inspected. **Gettysburg, stn 5**: one colony with few hydrothecae over frond of *Zonaria tourneforti*. **Gettysburg, stn 7**: abundant over *Eudendrium armatum*, less common over *Zonaria tourneforti*.

Remarks. The absence of coppinia prevented an accurate classification. This is the first record of this species in the Gorringe.

Distribution. *Filellum serpens* is regarded as cosmopolitan (Bouillon *et al.* 2004).

***Filellum* cf. *serratum* (Clarke, 1879)**

(Fig. 4.3G)

Lafoëa serrata Clarke, 1879: 242, pl. 4 Fig. 25.
Filellum serratum - Bouillon *et al*. 2004: 156, Figs 84I-J.

Material inspected. **Ormonde, stn 1**: few polyps found over the hydrocaulus of *Aglaophenia kirchenpaueri*. **Ormonde, stn 4**: few polyps over cauli of *Zonaria tourneforti*, – **Gettysburg, stn 9**: some polyps encrusting *Zonaria tourneforti*.

Remarks. Transversal perisarc ridges visible on the basal upper-parts of hydrothecae. The absence of coppinia impeded an accurate classification of this material. This is the first record of this species in the Gorringe.

Distribution. *Filellum serratum* is regarded as cosmopolitan species (Bouillon *et al*. 2004) but previous records of this nominal species may correspond to a complex of species (Peña Cantero *et al*. 1998; Moura *et al*. 2011d).

Family Sertulariidae Lamouroux, 1812

***Sertularella ellisii* (Deshayes & Milne Edwards, 1836)**

(Fig. 4.4A)

Sertularia Ellisii Deshayes & Milne Edwards, 1836: 142-143.

Sertularella ellisi ellisi - Peña Cantero & Carrascosa 2002: 124-125, Fig. 24A-C.
Sertularella ellisi - Bouillon *et al*. 2004: 181, Figs 99A-E.

Material inspected. **Ormonde, stn 1**: abundant, over *Zonaria tourneforti*, fertile colonies, epibionts: small algae and calcified Bryozoa. **Ormonde, stn. 2**: abundant, over *Zonaria tourneforti*, epibionts: algae and calcified Bryozoa. **Ormonde, stn 3**: abundant, fertile colonies, over *Zonaria tourneforti*; epibionts: small algae and calcified bryozoa. **Ormonde, stn 4**: very abundant, fertile colonies, over *Zonaria tourneforti*, epibionts: calcified bryozoa, small algae and *Clytia hemisphaerica*. **Gettysburg, stn 5**: few colonies, over *Zonaria tourneforti*, overgrown by small algae. **Gettysburg, stn 6**: abundant, fertile colonies, over *Zonaria tourneforti* and hydrocauli of *Aglaophenia pluma*, epibionts: algae. **Gettysburg, stn 7**: over *Zonaria tourneforti*, epibionts: Bryozoa. **Gettysburg, stn 8**: many colonies, fertile specimens, over *Zonaria tourneforti*, epibionts: *Halecium tenellum, Eudendrium* sp(on hydrorizae), *Clytia gracilis*. **Gettysburg, stn 9**: abundant, fertile colonies, over *Zonaria tourneforti*, epibionts: *Lafoeina tenuis*, small algae, Bryozoa. **Gettysburg, stn 10**: some specimens, over *Zonaria tourneforti*, epibionts: *Lafoeina tenuis* and *Halecium tenellum*.

Remarks. This is the first record of *S. ellisi* in the Gorringe.

Distribution. Mediterranean and eastern Atlantic; less recorded in the western Atlantic and Pacific (Bouillon *et al*. 2004).

Sertularella gayi (Lamouroux, 1821)

(Fig. 4.4B)

Sertularia Gayi Lamouroux, 1821:12, pl. 66 Figs 8,9.
Sertularella gayi - Bouillon *et al*. 2004: 156, Figs 99F-I, 100A.

Material inspected. **Gettysburg, stn 10**: nine well-developed colonies, over rock; epibionts: *Halecium tenellum, Halecium pussilum, Clytia gracilis, Clytia hemisphaerica, Lafoeina tenuis,* Bryozoa and small algae.

Remarks. This species was previously recorded by Oceana (2005) in the Gorringe Bank but its identification was not given accurately. The present study corroborates its presence in shallow waters of the Gorringe Bank.

Distribution. Eastern Atlantic and Mediterranean (Bouillon *et al*. 2004).

Sertularella ornata Broch, 1933

(Fig. 4.4C)

Sertularella fusiformis f. *ornata* Broch, 1933: 74-76, Fig. 29.

Sertularella ornata - Medel Soteras *et al*. 1991: 526, Fig. 10; Peña Cantero & Carrascosa 2002: 126-128, Fig. 24D.

Material inspected. **Gettysburg, stn 5**: a number of colonies, over *Zonaria tourneforti* and sponge, epibionts: *Aglaophenia kirchenpaueri*, small algae and calcified Bryozoa.

Remarks. This is the first record of this species in the Gorringe.

Distribution. Mediterranean, Cape Verde and western Portugal (see Peña Cantero & Carrascosa 2002; Moura *et al*. 2011a).

Family Halopterididae Millard, 1962

Antennella secundaria (Gmelin, 1791)

(Fig. 4.4D)

Sertularia secundaria Gmelin, 1791: 3856.

Antennella secundaria - Peña Cantero & Carrascosa 2002: 100-102, Fig. 18E-H. - Bouillon et al. 2004: 143, Figs 77P, 78A-D.

Material inspected. **Ormonde, stn 1**: scarce material, over *Zonaria tourneforti* and *Aglaophenia kirchenpaueri*. **Gettysburg, stn 7**: abundant, over *Zonaria tourneforti*. **Gettysburg, stn 9**: few colonies over *Zonaria tourneforti*.

Remarks. This species was previously recorded in the Gorringe but in deeper waters: 250-320 m (Ramil *et al*. 1998).

Distribution. Cosmopolitan with preference for warmer waters (Bouillon *et al*. 2004).

Family Plumulariidae Hincks, 1868

Plumularia setacea (Linnaeus, 1758)

(Fig. 4.4E)

Sertularia setacea Linnaeus, 1758: 813.
Plumularia setacea - Peña Cantero & Carrascosa 2002: 117-119, Fig. 21c-d. - Bouillon *et al*. 2004: 175-176, Figs 95A-E.

Material inspected. **Ormonde, stn 2**: fertile, ramification of hydrocladia of 2nd order, over hydrorizae and hydrocauli of *Aglaophenia pluma*. **Gettysburg, stn 5**: juvenile and fertile colonies, over *Aglaophenia pluma*; with hydrocladia ramified. **Gettysburg, stn 6**: fertile, over *Aglaophenia pluma*. **Gettysburg, stn 8**: some small colonies over colonies of *Aglaophenia kirchenpaueri*. **Gettysburg, stn 10**: some small cormoids over hydrocauli of *Aglaophenia kirchenpaueri*.

Remarks. This species was previously recorded at 250 m depth in the Gorringe (Ramil *et al*. 1998).

Distribution. Cosmopolitan (Bouillon *et al*. 2004).

Family Aglaopheniidae Marktanner-Turneretscher, 1890

Aglaophenia kirchenpaueri (Heller, 1868)

(Fig. 4.4F)

Plumularia kirchenpaueri Heller, 1868: 40, 82, pl. 2 Fig. 4.
Aglaophenia kirchenpaueri - Peña Cantero & Carrascosa 2002: 87-88, Fig. 17C. - Bouillon *et al*. 2004: 121, Figs 63A-D.

Material inspected. **Ormonde, stn 1**: one colony over *Zonaria tourneforti*, epibionts over hydrocauli: *Filellum* cf. *serratum* and *Antenella secundaria*. **Gettysburg, stn 5**: eight cormoids, over *Zonaria tourneforti* and *Sertularella ornata*, with colonies of *Clytia gracilis* attached. **Gettysburg, stn 6**: seven cormoids, over calcified Bryozoa possibly encrusted on rock, epibionts: calcified Bryozoa. **Gettysburg, stn 7**: three cormoids, over cauli of *Zonaria tourneforti*. **Gettysburg, stn 8**: one cormoid with gonotheca, over *Zonaria tourneforti*, epibionts: *Plumularia setacea*, small algae. **Gettysburg, stn 10**: four cormoids, one colony fertile, over carbonated substrate, epibionts: *Halecium pusillum*, *Plumularia setacea* and *Clytia hemisphaerica*.

Remarks. This is the first record of *A. kirchenpaueri* in the Gorringe.

Distribution. NE Atlantic and Mediterranean (Bouillon *et al*. 2004).

Aglaophenia pluma (Linnaeus, 1758)

(Fig. 4.4G)

Sertularia pluma Linnaeus, 1758: 811.
Aglaophenia tubiformis Marktanner-Turneretscher 1890: 269-270, pl. 7 Figs 4,5, 6,17.
Aglaophenia pluma - Peña Cantero & Carrascosa 2002: 92-95, Fig. 17G, H. - Bouillon *et al*. 2004: 122-123, Figs 64I-L, 65A-L.

Material inspected. **Ormonde, stn 2**: very abundant, over *Zonaria tourneforti*, epibionts: *Plumularia setacea*, *Clytia hemisphaerica* and algae. **Gettysburg, stn 5**: very abundant, fertile colonies, over *Zonaria tourneforti*, epibionts: algae, foraminifera, *Clytia hemisphaerica*, *Plumularia setacea* and *Eudendrium* sp.. **Gettysburg, stn 6**: very abundant, fertile colonies, some colonies ramified, over *Zonaria tourneforti*, epibionts: algae, calcified Bryozoa, *Plumularia setacea* and *Sertularella ellisii*. **Gettysburg, stn 7**: one small cormoid over *Zonaria tourneforti*. **Gettysburg, stn 8**: very abundant, fertile colonies, over *Zonaria tourneforti*. **Gettysburg, stn 9**: some developed and juvenile colonies over *Zonaria tourneforti*. **Gettysburg, stn 10**: some cormoids over *Zonaria tourneforti* (some are ramified).

Remarks. *Aglaophenia tubiformis* was reported in the Gorringe Bank by Ramil *et al*. (1998) at 54-62 m. An undetermined species of *Aglaophenia* was also detected in shallow waters of the Gorringe Bank by Oceana (2005). Because *A. tubiformis* seems to be conspecific with *A. pluma* (Moura *et al*. 2008; Moura *et al*. 2011b) and *A. pluma* was herein detected in high abundances at the Gorringe peaks, I synonymise to *A. pluma* the previously reported identifications of *Aglaophenia* species in the Gorringe.

Distribution. North-eastern Atlantic and Mediterranean, but possibly cosmopolitan (Bouillon *et al*. 2004).

Family Campanulariidae Hincks, 1868

Obelia dichotoma (Linnaeus, 1758)

(Fig. 4.4H)

Sertularia dichotoma Linnaeus, 1758: 812.

Obelia dichotoma - Peña Cantero & Carrascosa 2002: 161-162, Figs 31B-C. - Bouillon *et al*. 2004: 200, Figs 114C-G.

Material inspected. **Ormonde, stn 3**: few scattered colonies, over *Zonaria tourneforti*. **Ormonde, stn 4**: abundant over tuff of algae, well-developed colonies, epibionts: small algae.

Remarks. This is the first record of this species in the Gorringe.

Distribution. Cosmopolitan (Bouillon *et al*. 2004).

Obelia geniculata (Linnaeus, 1758)

(Fig. 4.4I)

Sertularia geniculata Linnaeus, 1758: 812.

Obelia geniculata - Peña Cantero & Carrascosa 2002: 162-163, Fig. 31D. - Bouillon *et al*. 2004: 200, Figs 114H-I, 115A-E.

Material inspected. **Ormonde, stn 4**: fertile colonies, very abundant over the brown algae *Laminaria ochroleuca* Bachelot de la Pylaie.

Remarks. This species was previously identified with uncertain taxonomic identity by Oceana (2005). Therefore its presence at the Gorringe peaks is herein confirmed.

Distribution. Cosmopolitan (Bouillon *et al*. 2004).

Fig. 4.4. (A) *Sertularella ellisii* (Deshayes & Milne Edwards, 1836) – Hydrotheca and internode; Stn 5. (B) *Sertularella gayi* (Lamouroux, 1821) – hydrothecae in piece of hydrocladium; Stn 10. (C) *Sertularella ornata* Broch, 1933 – hydrotheca; Stn 5. (D) *Antennella secundaria* (Gmelin, 1791) – part of a colony; Stn 7. (E) *Plumularia setacea* (Linnaeus, 1758) – part of a colony, hydrocaulus and hydrocladia; Stn 10. (F) *Aglaophenia kirchenpaueri* (Heller, 1868) – hydrothecae; Stn 5. (G) *Aglaophenia pluma* (Linnaeus, 1758) – hydrothecae; Stn 10. (H) *Obelia dichotoma* (Linnaeus, 1758) – fragment of stem with hydrothecae; Stn 4. (I) *Obelia geniculata* (Linnaeus, 1758) – stem internodes and hydrotheca. (J) *Orthopyxis integra* (MacGillivray, 1842) – hydrotheca and pedicel; Stn 4. (K) *Clytia gracilis* (M. Sars, 1850) – hydrotheca and distal part of pedicel; Stn 8. (L) *Clytia hemisphaerica* (Linnaeus, 1767) - hydrotheca and distal part of pedicel; Stn 10. Scale bars: 0.5 mm (G, K, L); 1 mm (A-E, F, H-J). Photo credits. C.J. Moura.

***Orthopyxis integra* (MacGillivray, 1842)**

(Fig. 4.4J)

Campanularia integra Macgillivray, 1842: 465.

Orthopyxis integra - Peña Cantero & Carrascosa 2002: 166-167, Fig. 27G-I. - Bouillon *et al*. 2004: 201-202, Figs 116I-S.

Material inspected. **Ormonde, stn 1**: abundant, all over algae (not *Zonaria tourneforti*). **Ormonde, stn 4**: very abundant, over different species of algae (not *Zonaria tourneforti*).

Remarks. This is the first record of this species in the Gorringe.

Distribution. Atlantic, Mediterranean and Indo-Pacific (Bouillon *et al*. 2004).

***Clytia gracilis* (M. Sars, 1850)**

(Fig. 4.4K)

Laomedea gracilis p.p. M. Sars, 1850: 138.

Clytia gracilis - Peña Cantero & Carrascosa 2002: 145-146, Fig. 28a-b. - Bouillon *et al*. 2004: 193-194, Figs 108B-G.

Material inspected. **Ormonde, stn 4**: considerable number of hydrothecae over *Coryne eximia*. **Gettysburg, stn 5**: few polyps, over *Aglaophenia kirchenpaueri* and *Zonaria tourneforti*. **Gettysburg, stn 6**: few hydrothecae, over *Zonaria tourneforti* (especially over cauli). **Gettysburg, stn 7**: few colonies, over algae and *Eudendrium armatum*. **Gettysburg, stn 8**: few hydrothecae over *Sertularella ellisii* and *Zonaria tourneforti*. **Gettysburg, stn 9**: several hydrothecae, over *Zonaria tourneforti* and calcified Bryozoa. - **Gettysburg, stn 10**: considerable number of hydrothecae over some colonies of *Sertularella gayi*.

Remarks. This is the first record of this species in the Gorringe.

Distribution. Atlantic, Indo-Pacific and Mediterranean (Bouillon *et al*. 2004).

Clytia hemisphaerica (Linnaeus, 1767)

(Fig. 4.4L)

Medusa hemisphaerica Linnaeus, 1767: 1098.
Clytia hemisphaerica - Peña Cantero & Carrascosa 2002: 147-148, Fig. 28C-D. - Bouillon *et al*. 2004: 194, Figs 108H-K.

Material inspected. **Ormonde, stn 1**: very abundant, fertile colonies, over *Zonaria tourneforti*. **Ormonde, stn 2**: few specimens, fertile colonies, over *Zonaria tourneforti* and *Aglaophenia pluma*. **Ormonde, stn 3**: very abundant, fertile colonies, over *Zonaria tourneforti*. **Ormonde, stn 4**: very abundant, fertile specimens, over *Zonaria tourneforti* (in higher abundance), *Eudendrium armatum*, *Sertularella ellisii* and other species of algae; usually on different algae of those overgrown by *Orthopyxis integra*. **Gettysburg, stn 5**: abundant, fertile colonies, over *Zonaria tourneforti* and *Aglaophenia pluma*. **Gettysburg, stn 6**: very abundant, fertile, over *Zonaria tourneforti*. **Gettysburg, stn 7**: very abundant, fertile colonies, over *Zonaria tourneforti*. **Gettysburg, stn 8**: some specimens, fertile colonies, over *Zonaria tourneforti*. **Gettysburg, stn 9**: very abundant, fertile colonies, over *Zonaria tourneforti* and calcified Bryozoa. **Gettysburg, stn 10**: very abundant, overgrowing *Aglaophenia kirchenpaueri*, *Sertularella gayi* and *Zonaria tourneforti*.

Remarks. This was the most abundant and frequent hydroid species collected. Pedicels of hydrothecae in some colonies extremely annulated. This is the first record of this species in the Gorringe.

Distribution. Atlantic, Indo-Pacific, Mediterranean (Bouillon *et al*. 2004).

Table 4.1. List of hydroid species recorded from the Gorringe Bank (in red: first records for the Gorringe; in green: species not recorded; in blue: species previously recorded and found in this study).

Species	Dist	LC	Stn 1	Stn 2	Stn 3	Stn 4	Stn 5	Stn 6	Stn 7	Stn 8	Stn 9	Stn 10	Oceana (2005)	Ramil *et al.* (1998)
Filifera sp	?	?						+			+			
Coryne eximia	C	m				+								
Coryne cf. *pusilla*	?	?					+							
Bougainvillidae sp.	?	?							+					
Hydractinia sp	?	?				+								
Eudendrium armatum	M	g	+			+	+		+	+	+	+		
Eudendrium cf. *merulum*	?	g				+					+			
Eudendrium ramosum	C	g				+			+					
Eudendrium sp.	?	g	+		+		+	+	+	+		+		
Velella velella	CT	m											+	
Lafoeina tenuis	IP	g	+	+	+	+	+	+	+	+	+	+		
Cuspidella sp.	?	g						+	+	+	+			
* *Halecium beanii*	C	g												+
Halecium pusillum	TA	g				+						+		
* *Halecium sessile*	C	g												+
Halecium tenellum	C	g								+		+		+
Halecium sp .	?	g			+	+			+		+			
Filellum cf. *serpens*	?	g					+		+					
Filellum cf. *serratum*	?	g	+			+					+			
Sertularella ellisii	TA	g	+	+	+	+	+	+	+	+	+	+		
Sertularella gayi	C	g										+	+	
Sertularella ornata	AM	g					+							
* *Kirchenpaueria bonnevieae*	IP	g												+
* *Kirchenpaueria pinnata*	C	g												+
Antennella secundaria	C	g	+						+		+			+
Plumularia setacea	C	g		+			+	+		+		+		+
* *Pseudoplumularia sabinae*	X	g												+
* *Nemertesia antennina*	?	g												+
Aglaophenia kirchenpaueri	AM	g	+				+	+	+	+		+		
Aglaophenia pluma	AM	g		+			+	+	+	+	+	+	+	+
* *Aglaophenia tubulifera*	AM	g												+
* *Cladocarpus elongatus*	NEA	g												+
* *Streptocaulus corneliusi*	EA	g												+
* *Lytocarpia myriophyllum*	C	g												+
Obelia dichotoma	C	m			+	+								
Obelia geniculata	C	m				+							+	
Orthopyxis integra	C	mg	+			+								
Clytia gracilis	C	m				+	+	+	+	+	+	+		
Clytia hemisphaerica	C	m	+	+	+	+	+	+	+	+	+	+		

Symbols: ‘+’ – presence; ‘*’ – species found in the Gorringe only in deep waters.
Abbreviations: ‘Stn’ – sampling station; ‘Dist’ – geographical distribution of species; ‘M’ – Mediterranean-endemic; ‘AM’ – Atlantic and Mediterranean; ‘TA’ – tropical-Atlantic; ‘NEA’ – North-east Atlantic; ‘EA’ – eastern Atlantic; ‘IP’ – Indo-Pacific and Mediterranean; ‘CT’ – circumtropical; ‘C’ – cosmopolitan; ‘Rep’ – life cycle pattern of species; ‘g’ – fixed gonophores; ‘mg’ – liberate medusoids; ‘m’ – medusa; ‘?’ – doubtful.

4.4 CONCLUSIONS

This study is the first that provides a comprehensive checklist of the hydroids from the summits of the Gorringe Bank. The hydroid samples were collected by scuba diving from the Ormonde and Gettysburg seamount peaks at depths between 35 and 42 m. Only few records of hydroids from the Gorringe existed (Ramil *et al*. 1998; Oceana 2005).

The 28 hydroid species herein revealed were identified amongst the biological material collected during the ‘LusoExpedição Olympus 2008’. 22 of these species represent new records for the Gorringe Bank (Table 4.1). The total of hydroid species now known at the Gorringe seamounts ascends to 38 (Table 4.1). The present work consequently contributes to an increase of over 130% of new records of hydroid species for the Gorringe; and only the summits of the seamounts were sampled.

The relationship between the shallow waters of the Mediterranean and the Gorringe was evident by the presence of *Eudendrium armatum* and *E.* cf. *merulum* in the Gorringe, outside the Mediterranean. Additionally, apart from the taxa with uncertain identity, all the other species herein recorded are also present in the Mediterranean.

Eudendrium ramosum and the two and three species of *Aglaophenia* and *Sertularella* respectively, have geographical ranges limited to the eastern Atlantic and the Mediterranean (Table 4.1). The other 11 hydrozoan taxa identified nominally to the species level present (supposedly) much larger distributions (see Table 4.1).

The majority of the hydroid specimens collected specifically for this study were found as epibiont of algae and/or of other hydroids. Only two hydroid species (*Eudendrium armatum* and *Sertularella gayi*) were found directly attached to rock. The extremely high density of algae populating the summits of the Gorringe Bank (Oceana 2005; Moura, pers. observation) visibly inhibits the establishment of hydroids directly over the rocky substrate. *E. armatum* and *S. gayi* were found in relatively high abundances on solid substrata, but only on vertical walls with limited exposure to sunlight and consequently fewer algae. On the other hand, the high abundance and diversity of algae on the Gorringe peaks facilitated the direct establishment of 23 hydroid species.

The abundance of algae on the peaks of the Gorringe may decrease in winter especially due to more limited sunlight exposure, and other hydroid species (not reported yet for the seamount) could be also present on the summits of the Gorringe outside summer

- e.g. see the evidence of high levels of seasonality of benthic hydroids (and algae) in a Mediterranean location by Boero & Fresi (1986) and Bavestrello *et al*. (2006).

Coryne eximia was found just once (but extremely abundant) over a cable that had been lost in the Gorringe two years before the hydroid sampling. *C. eximia* can be found at the low water mark and overgrowing floating objects like buoys and rafts (Schuchert 2001), and therefore this species might have been transported unintentionally by humans to the Gorringe.

The majority of the hydroids collected during the 'LusoExpedição Olympus 2008' (75% of the species with life cycle pattern known), have the medusoid stage suppressed from the life-cycle and therefore their mode of dispersal is practically limited to short-lived planulae and rafting. As so, the most probable explanation for the presence of these hydroids in the Gorringe is precisely by rafting on algae; taking into account the distance of the Gorringe to the coast, the absence of these hydrozoan species in deeper waters (exception for *S. gayi*), and their direct or indirect occurrence over algae. The five Campanulariidae hydroid species recorded liberate medusae or medusoids, are cosmopolitan and abundant, and therefore might have also arrived in the Gorringe directly through oceanic currents. *Sertularella gayi* does not liberate medusae or medusoids and was not detected overgrowing algae; but the effective presence of this nominal species both in shallow and deep waters (Moura *et al*. 2011a), does not exclude the possibility that this species may have reached the peaks of the seamount from deeper waters.

In conclusion, this study: (1) revealed relatively high specific richness of hydroids only from the summits of the Gorringe Bank sampled; (2) confirmed that the Gorringe is a 'biodiversity hotspot' still with many unreported species; (3) highlighted that most of the hydrozoan biodiversity found are phylogeographically associated with the Mediterranean and close continental shores of the eastern Atlantic; (4) discussed the probable vagility of the hydroids found in the Gorringe; (5) provided further arguments to protect the Gorringe as a marine protected area.

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Chapter 5

DNA BARCODING REVEALS CRYPTIC DIVERSITY IN MARINE HYDROIDS (CNIDARIA, HYDROZOA) FROM COASTAL AND DEEP-SEA ENVIRONMENTS

Moura, C. J., Harris, D. J., Cunha, M. R. & Rogers, A. D. (2008). *Zoologica Scripta, 37*, 93–108.

Contents

ABSTRACT

Fifty-six sequences of the mitochondrial 16S RNA gene were generated for hydroids, belonging to six nominal families — Eudendriidae, Lafoeidae, Haleciidae, Sertulariidae, Plumulariidae and Aglaopheniidae — collected from bathyal environments of the Gulf of Cadiz (22 haplotypes), Greenland (1 haplotype), Azores (1 haplotype), the shallow waters of the UK (17 haplotypes) and Portugal (2 haplotypes). When combined and analysed with 68 additional sequences published in GenBank, corresponding to 63 nominal species of these families (nine species in common between the GenBank sequences and those presented by the authors), cryptic species were detected (e.g. two species of *Nemertesia* and another of *Lafoea*), as well as apparent cases of conspecificity (e.g. *Nemertesia* *antennina* and *N. perrieri* and *Aglaophenia octodonta*, *A. pluma* and *A. tubiformis*). Other taxonomic inconsistencies were found in the data including cases where species from different genera clustered together (e.g. *Sertularia cupressina*, *Thuiaria thuja*, *Abietinaria abietina* and *Ab. filicula*). The mitochondrial 16S rRNA proved to be a useful DNA 'barcode' gene for hydroids, not only allowing discrimination of species, but also in some cases of populations, genera and families, and their intra- or interphylogenetic associations. Although still under-represented in public data bases, the 16S rRNA gene is starting to be used frequently in the study of hydroids. These data provide powerful complementary evidence for advancing our understanding of hydrozoan systematics.

5.1 INTRODUCTION

The oceans are the cradle of life on Earth and they harbour a higher diversity at upper taxonomic levels (phylum, class, orders), than terrestrial or freshwater ecosystems. Out of *ca.* 46 phyla of animals: 35 are found in the oceans, 14 of which are exclusively marine and several others are mainly marine (e.g. Cnidaria; Sala & Knowlton 2006). However, the opposite appears true at the species level with *ca.* 1.5 million species having been described from terrestrial environments but only about 300 000 from marine ecosystems (Reaka-Kudla 1997). This partially results from the high diversity of terrestrial arthropods but may also reflect the large, open-nature of marine ecosystems, resulting in large geographical distributions of populations and species and lower rates of allopatric speciation over geological time scales. This is thought to have resulted in near cosmopolitan distributions of many species, with perhaps the most striking examples being among the hydroids (Stepanyants 1980; Cornelius 1995a,b).

Hydroids or Hydrozoa were classified under the *Vermes* (invertebrates) as *Zoophyta* (plant-animals) by Carl Linnaeus in the 10th Edition of *Systema Naturae* in 1758. Hydroids consist of polyps which primitively have a central mouth surrounded by tentacles armed with nematocysts. These polyps can occur as solitary individuals in some species but in most usually occur as colonies where each is joined by a tube of tissue and the whole is protected by a rigid exoskeleton, the perisarc (Cornelius 1995a,b). Almost all colonies are fixed to the seabed (sessile) and have a variety of forms from root-like stolons to erect tree-like structures that can be conspicuous amongst the emergent epifauna of coastal and deep-sea ecosystems. Many common hydroids from the coastal waters of Europe were described originally by Linnaeus and, as with many species in the region, have subsequently been recorded as having a near cosmopolitan distribution (Cornelius 1995a,b, e.g. *Obelia geniculata* (Linnaeus, 1758); *Plumularia setacea* (Linnaeus, 1758); *Amphisbetia operculata* (Linnaeus, 1758)). In some cases, the widespread distribution of hydroids may result from a dispersive life-history. Some forms (e.g. *O. geniculata*) have a reproductive pelagic medusoid stage which releases gametes that undergo external fertilization producing a pelagic planula stage. In other cases (e.g. most of the Plumulariidae) the medusoid stage is reduced, effectively being retained by the hydroid colony and fertilization is external with most species being dioecious (colonies that have separate sexes). The resultant planula larvae swim or crawl away and are generally short-lived, although those of some species may take up to several days to settle and metamorphose. Many hydroids have the ability to raft on natural substrata, such as kelp holdfasts (hydrochory, e.g. Cornelius 1992, 1995a,b), and many can grow on artificial surfaces, often being a significant element of marine fouling communities (e.g. Lewbel *et al*. 1987; Relini *et al*. 1998; Yan *et al*. 2006) so passive dispersal on natural and anthropogenic materials is likely for many species.

The hydroids are anatomically simple animals with few morphological characters, great phenotypic plasticity and many shared characters that represent homoplasies. There are about 3700 described species (Bouillon *et al*. 2006) but the taxonomy of the group is problematic and the potential for morphologically cryptic taxa is high (e.g. Govindarajan *et al*. 2005a; Schuchert 2005a). Often identification of species can only be accomplished at a particular life-stage or when reproductive structures are present. Furthermore, taxonomic expertise in the group is diminishing and existing taxonomists tend to focus their studies only on particular life-stages and/or taxonomic groups. This ‘taxonomic impediment’ makes resolution of species difficult for many hydroids and the perceived widespread cosmopolitanism of the group may be erroneous. A similar situation exists for many groups of marine invertebrates and coupled with the very poor sampling of marine habitats (e.g. in the deep sea) may have led to a significant underestimation of the global diversity of metazoans in the world’s oceans.

Nucleotide sequence data have been applied successfully to the study of phylogenetic relationships between taxa because of the work of Woese & Fox (1977) on bacteria. However, the idea of large-scale screening of standardized short genetic markers (DNA ‘barcodes’), to rapidly assign unknown individuals, life-history stages and forensic or subfossil material to known species or even populations or to aid the discovery of cryptic biodiversity, was first proposed by Floyd *et al*. (2002) and Hebert *et al*. (2003a,b). To date, DNA barcoding projects have been broadly tested on known taxa, and have tended to successfully identify > 95% of target species (Hebert *et al*. 2004a,b; Hebert & Gregory 2005; Ward *et al*. 2005; Clare *et al*. 2006; Cywinska *et al*. 2006; Hajibabaei *et al*. 2006). Studies have also indicated the potential of barcoding genes to identify putative cryptic taxa indicating the power of this approach in species discovery (Monaghan *et al*. 2005; Barber & Boyce 2006; Clare *et al*. 2006; Hajibabaei *et al*. 2006; Witt *et al*. 2006; Gómez *et al*. 2007; Kerr *et al*. 2007). These studies have all focused on *ca*. 650 base pairs of the cytochrome *c* oxidase subunit 1 (COI) gene as the main barcoding gene for metazoans. This is because COI is easily amplified from diverse taxa using broad-range primers under variable laboratory conditions and protocols (Folmer *et al*. 1994; Hebert *et al*. 2003a,b; Ivanova *et al*. 2007; Kerr *et al*. 2007). It is also readily aligned for sequence comparisons, and is sufficiently divergent to discriminate species effectively across a variety of phyla, with the result that a lot of data from this gene has already been accumulated in publicly accessible data bases (Hebert *et al*. 2004a,b). Presently, over 200 000 DNA ‘barcodes’ corresponding to more than 26 000 species (www.barcodinglife.org) are already gathered. However, other genes have also been used as a basis for barcoding or for species discovery (e.g. Markmann & Tautz 2005; Mathews 2006), especially where COI does not amplify reliably for the taxon under study (Cook *et al*. 2005; Bhadury *et al*. 2006), has failed to resolve species as a result of slow evolution of the mitochondrial genome (e.g. Cnidaria; Porifera; France *et al*. 1996; van Oppen *et al*. 1999; Shearer *et al*. 2002; citing R. Watkins, pers. comm.; Duran *et al*. 2004; Hellberg 2006; Wörheide 2006), or where divergent COI sequences have been found within species (Vences *et al*. 2005).

DNA barcoding, especially when used for species discovery, remains contentious, especially amongst taxonomists (Moritz & Cicero 2004; DeSalle *et al*. 2005; Ebach & Holdrege 2005; Brower 2006). Drawbacks identified with DNA barcoding approaches, have centred around the resolution of intra- and interspecific variation in DNA sequences

(Hewitt 1996; Avise & Johns 1999; Hebert *et al*. 2003b; Moritz & Cicero 2004; Meyer & Paulay 2005) including problems associated with inherited symbionts (Hurst & Jiggins 2005), with introgression/ hybridization (e.g. Masta *et al*. 2002; Babik *et al*. 2005) or with very recent speciation (e.g. Johnson & Cicero 2004); the reliance on single-gene trees (DeSalle *et al*. 2005); the lack of comprehensive sampling of species of the taxa under study (Moritz & Cicero 2004; DeSalle *et al*. 2005); and failure to sample voucher specimens or to take account of existing taxonomic knowledge. Whilst these criticisms are valid, the linking of DNA sequence data with other information, such as geographical, ecological, biological or morphological data can provide a powerful way to break out of the tautological or circular reasoning that lies behind an oversimplified application of barcoding, especially when identifying new taxa (DeSalle *et al*. 2005). Examples of this include the identification of covariation between morphology and barcode sequence clusters in South American Lepidoptera (Hajibabaei *et al*. 2006) and the use of mating trials to confirm that deep genetic lineages within the bryozoan *Celleporella hyalina* are reproductively isolated (Gómez *et al*. 2007). Thus, DNA barcoding or DNA taxonomy can be a modern complement to the classification of the most important unit of biodiversity, the species, testing and making systematics more practical, rigorous and complete.

The orthologous barcoding gene, COI, has rarely been used and studied in the hydroids (Shearer *et al*. 2002; Govindarajan *et al*. 2005a, 2006). This is because studies have suggested that it evolves at a slow rate (Shearer *et al*. 2002), although in some cases the gene has been revealed as phylogenetically informative (see Govindarajan *et al*. 2005a for *O. geniculata*). Instead the mitochondrial 16S rRNA gene has been preferentially used to resolve phylogenetic problems from the subfamily to population levels (e.g. Bridge *et al*. 1995; Schierwater & Ender 2000; Collins *et al*. 2005; Govindarajan *et al*. 2005a,b, 2006; Schuchert 2005a,b; Fraser *et al*. 2006; Schuchert & Reiswig 2006; Galea & Lecrère 2007; Hemmrich *et al*. 2007; Leclère *et al*. 2007; Moura *et al*. 2007); whereas the nuclear 18S rRNA and 28S rRNA genes have been used for evolutionary studies at higher taxonomical levels (Bridge *et al*. 1995; Collins 2000; Collins *et al*. 2006; Govindarajan *et al*. 2006).

The present study evaluates the potential of the mitochondrial 16S rRNA gene as a tool for DNA barcoding of hydroid species. It also evaluates the systematic resolution of the mt 16S rRNA above the species level for addressing phylogenetic questions within the Class (e.g. in the Plumularioidea). The data obtained are also used to test whether hydroid species are widespread in geographical distribution or whether they are in fact complexes of cryptic taxa segregated by depth, geographical barriers (e.g. continents) or distance.

5.2 MATERIALS AND METHODS

Hydroids collected from bathyal environments of the Gulf of Cadiz, Azores and Greenland, and shallow waters of the southwest of England and south of Portugal (Table 5.1) were identified and the total DNA was extracted (DNeasy Kit, Qiagen Ltd, Crawley, West Sussex, UK). Whenever possible, the skeletons of the colonies were mounted in permanent slides and deposited, in conjunction with their original sample, in the Department of Biology, University of Aveiro, Portugal. The primers SHA (ACGGAATGAACTCAAATCATGT) and SHB (TCGACTGTTTACCAAAAACATA) (Cunningham & Buss 1993) were used to amplify *ca.* 600 base pairs of the mitochondrial 16S rRNA gene. A 50 µL of PCR reaction contained 30.5–35.5 µL of ultrapure water, 5 µL of 10x PCR buffer, 4 µL of $MgCl_2$ (25 mM), 4 µL of dNTPs (10 mM), 2 µL of each primer (10 pM/µL) and 1–5 µL of DNA template (all reagents Qiagen apart from water which was from the Sigma Chemical Company). The thermal cycle parameters of the PCR reaction consisted of one cycle of 5 min 94 ºC; 35 cycles of 1 min 94 ºC, 1 min 48–50 ºC and 1.5 min 72 ºC and a final cycle of 5 min 72 ºC. After purification of the PCR product (Qiaquick, Qiagen), the same primers were used to sequence both strands using the ABI BigDye Terminator v. 3.1 kit. Sequencing reactions were purified using a Dye-Ex v. 2 Spin Kit (Qiagen) according to the manufacturer’s instructions. Purified sequencing reactions were dried using a vacuum centrifuge and then resuspended in 10 µL of formamide and heated for 3 min at 96 ºC and cooled for 3 min on ice prior to sequencing on an Applied Biosystems 3100 Prism DNA Analyser.

Both strands of corresponding sequences were aligned in BIOEDIT using CLUSTALW with default alignment parameters, and then corrected by eye to produce a consensus sequence. Sixty-eight additional 16S sequences of hydroids were obtained from GenBank to complement the analysis for the six families of Hydrozoa treated in the present study. These sequences were analysed together in a single data set with *Tubiclavoides* *striatum* as the outgroup, and in separate data sets for each family using one or two haplotypes of a different (but closely related) family as outgroups. For each data set sequences were aligned in MEGA 3.1 (Kumar *et al*. 2004) with CLUSTALW using the default alignment settings, and trimmed to the shortest sequence. Positions with gaps were removed from the alignment of the data set containing all sequences of all the represented families. For the other data sets, the full-length of gene was used. The program MODELTEST 3.7 (Posada & Crandall 1998) was used to determine the optimal probabilistic model of sequence evolution by using the Akaike Information Criterion for each alignment. 'Maximum-likelihood' phylogenetic analyses were performed, for each data set, through the PHYML website (http://atgc.lirmm.fr/phyml) (Guindon *et al*. 2005) using default parameters except for the model of nucleotide substitution, which was specified as GTR. This was the closest model available to those selected by MODELTEST (GTR + I + G with the exception of the data sets of Lafoeidae and Eudendriidae, where GTR + G was selected). A 'bioneighbor-joining' starting tree was also estimated using the PHYML website. PAUP 4.1 (Swofford 1998) was used to determine pairwise uncorrected sequence differences ('*P*' distances) within the data sets of each family, for simple comparisons of percentage sequence divergence within species, within genera and between genera.

5.3 RESULTS AND DISCUSSION

A total of 56 sequences of the 16S RNA gene, of 580 nucleotides in length, corresponding to 43 distinct genotypes of 35 nominal and 36 phylogenetically distinct species of hydroids were determined for the present analysis. Of these, 24 distinct haplotypes represent hydroids from bathyal environments of the Gulf of Cadiz (22), Greenland (1), and the Azores (1), and 19 from shallow waters of the United Kingdom (17) and Portugal (2). The new sequences herein presented were complemented with 68 more taken from the GenBank (http://www.ncbi.nlm.nih.gov), corresponding to an additional 63 nominal species, nine of which were also identified among the authors' samples.

Amplification of 16S sequences was impeded by DNA deterioration in many colonies, as a result of preservation in 70% ethanol and/or the excessive exposure of the biological material to air and/or sun prior to fixation. It is notable that material that was rapidly preserved in 96% ethanol following the collection from the bathyal environment of the Gulf of Cadiz, gave a markedly greater rate of successful amplification and sequencing.

The alignment of all the 16S sequences (Table 5.1) revealed a high number of insertion/deletions (indels) making alignment of the sequences difficult and potentially ambiguous. Where taxonomic sampling is broad, fewer characters of ribosomal genes are likely to be homologous, justifying the choice to consider a smaller number of base pairs. Comparison of nodal supports of the trees with indels included and those with indels excluded indicated that bootstrap indices, especially of clades representing families and genera, tended to be higher in the latter. It was therefore necessary to exclude all positions in the alignment containing gaps, in order to provide a more reliable alignment and phylogenetic analysis. However, the removal of these characters from sequences potentially reduced the resolution of diversity at the population and species level. For example, indel regions proved to be useful in detecting specific populations of species (e.g. *Aglaophenia*). Therefore, analyses for each family were carried out separately on full-length sequences containing the whole gene.

Tubiclavoides striatum (family Tubiclavoididae) was used as outgroup for the analysis containing all the 124 16S sequences herein analysed. This was because the closest related taxon to this is the Eudendriidae, as both families belong to the Order Anthoathecata and Suborder Filifera. This follows the suggestion that outgroups should be monophyletic with the ingroup in a wider phylogenetic context (e.g. Donoghue & Cantino 1984; Smith 1994). The 16S sequence of *T. striatum* also does not have many insertions compared to the other taxa analysed, simplifying the alignment. However, the support for the hypothesized relationship between *T. striatum* and the Eudendriidae was weak in the current analysis (Fig. 5.1). All the other five families herein examined (Haleciidae, Lafoeidae, Sertulariidae, Aglaopheniidae and Plumulariidae) belong to the putatively monophyletic order Leptothecata, which is a sister group to the Anthoathecata. The use of the Siphonophorae or even Trachylina as outgroups is unlikely to be useful as these taxa are highly divergent to those in the present analysis giving a high probability that changes in nucleotide sequences are effectively random as a result of multiple substitutions at homologous sites (Wheeler 1990; Smith 1994). For the phylogenetic analyses of the families separately, outgroups were selected from branches close to the family identified in the overall phylogenetic tree.

High-level phylogenetic analysis

The inferred tree of the data set containing all the 124 distinct 16S sequences, with 368 bp (indels excluded), is essentially concordant with the other trees recovered for each of the seven families characterized, especially where nodes are well-supported (Fig. 5.1). It comprises a distinctive well characterized congruent monophyletic clade representing the Plumularioidea; and a less-resolved/supported group of clades containing the haplotypes of the other families. It is notable that regions of DNA used for barcoding often give poor information at higher taxonomic levels because the rate of evolution that renders them suitable for differentiating species is too high for resolving higher phylogeny (Moritz & Cicero 2004). However, the topology of even the poorly resolved part of the tree supports the monophyly of the Eudendriidae, and suggests that the Lafoeidae comprises two distinct clades, the Haleciidae is subdivided into weakly associated clades, and all but one species (*Symplectoscyphus tricuspidatus*) of Sertulariidae are monophyletic (Fig. 5.1). Regarding the monophyletic superfamily Plumularioidea, high bootstrap values in the analysis for this particular group corroborate the subdivision of this superfamily into the monophyletic families Aglaopheniidae and Plumulariidae, with the remainder subdivided into the subfamilies Halopteriinae, Kirchenpaueriinae and Plumulariinae, as proposed by Cornelius (1995a,b). The prominence of the Plumularioidea clade in the tree when compared to the other thecate and athecate families analysed suggest a faster rate of evolution of the 16S gene in this superfamily (also noted by Leclère *et al*. 2007). This is further supported by the high number of indels in the alignment of sequences.

Family Plumulariidae

Probably the most remarkable aspect of the phylogenetic analysis of this family, are the high genetic divergences that occur within the subfamilies Halopteriinae, Kirchenpaueriinae and the genera *Monotheca*, *Dentitheca* and *Plumularia* of the

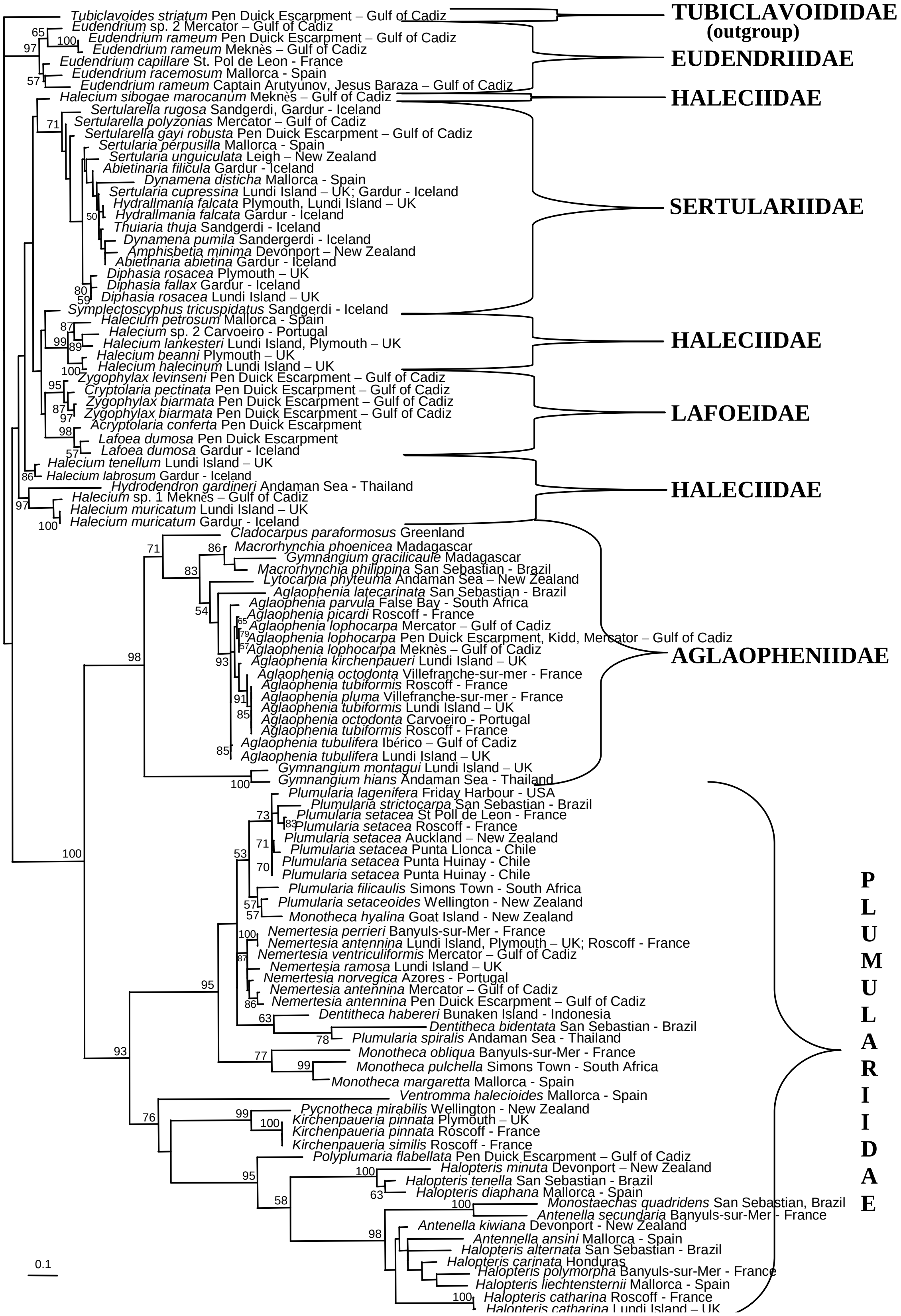

Fig. 5.1 High-level 'maximum-likelihood' phylogenetic analysis (500 pseudoreplicates) of the data set containing all the 16S sequences used in this study with indels removed. Numbers near the nodes indicate the percent bootstrap values. Nodes without number mean that the bootstrap values were < 50%. The branch length indicator represents 0.1 substitutions per site.

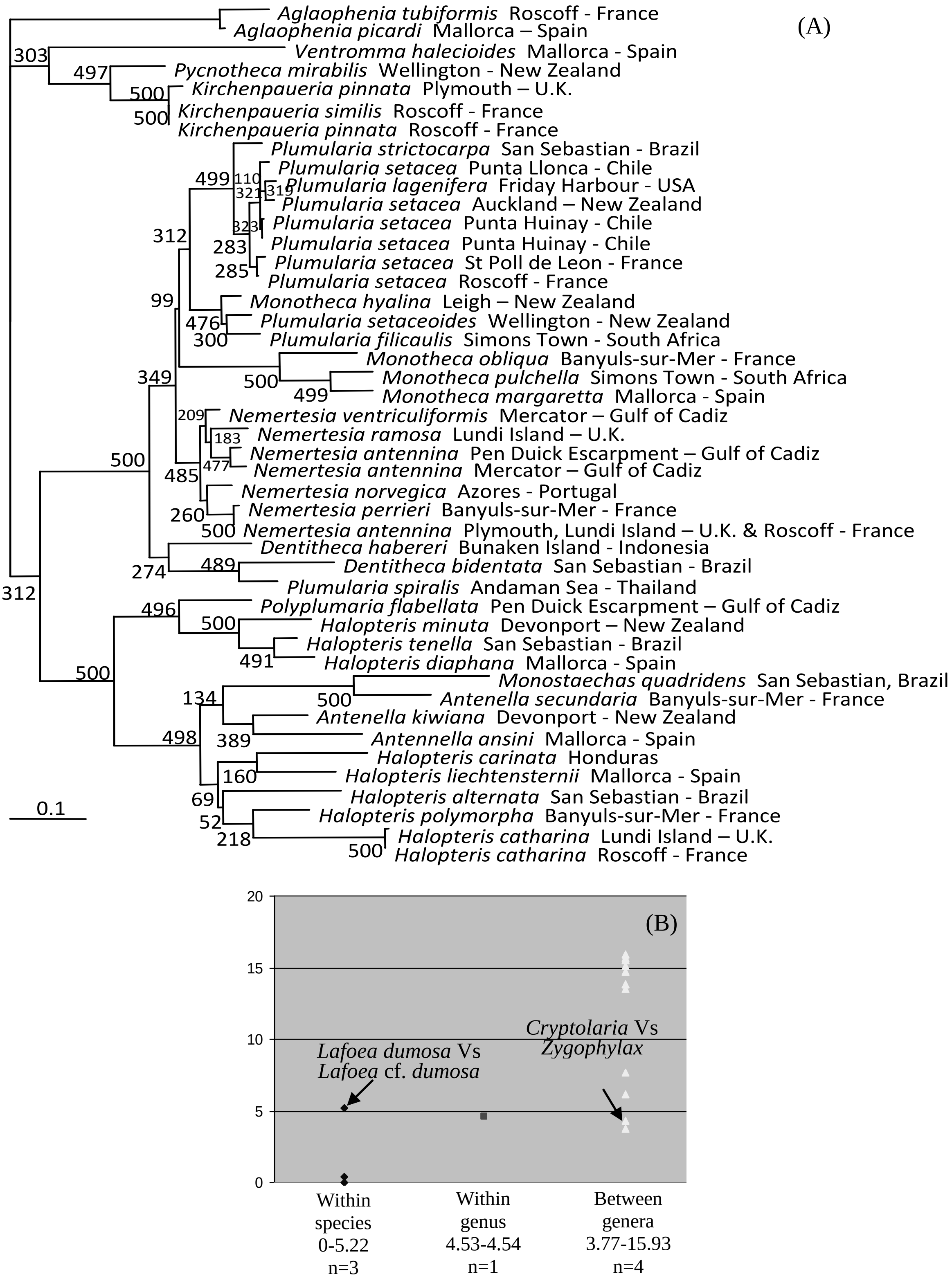

Fig. 5.2 A, B. Phylogenetic analysis of the data set containing the 16S sequences of the family Plumulariidae. (A) 'Maximum-likelihood' tree for 500 pseudoreplicates. *Aglaophenia tubiformis* and *A. picardi* are the outgroup taxa. Numbers near the nodes indicate the percent bootstrap values. The branch length indicator represents 0.1 substitutions per site. (B) Graphic of the 16 S sequence divergences as uncorrected *P*-values (%): within species, within genera and between genera. Abbreviation: n, indicates the number of taxa with two or more haplotypes at those taxonomic levels.

Plumulariinae (Fig. 5.2A). The sequence divergence between species of genera from these taxonomic ranks reaches 26%, 25.4% and 19.4%, respectively. This suggests a rapid evolution of the 16S gene within these groups. The Plumulariidae is resolved as a well-supported monophyletic group with two main sister clades: the well-supported monophyletic subfamily Plumulariinae, and another clade with less bootstrap support containing the subfamilies Halopteriinae and Kirchenpaueriinae (Fig. 5.2A). In general the obtained topology of trees and results are in accordance to those obtained by Leclère *et al*. (2007).

The subfamily Halopteriinae is monophyletic with strong bootstrap support, comprising species with a high interspecific 16S sequence divergence (Fig. 5.2B). It is notable that *Polyplumaria flabellata* clusters within the Halopteriinae instead of inside the Plumulariinae, as has been proposed by some authors (Fig. 5.2A). In evolutionary terms *P. flabellata* is the closest species to the common ancestor of this subfamily. It groups with *Halopteris minuta*, *H. tenella*, *H. diaphana* in a different clade to that containing other species of *Halopteris* and the genera *Antenella* and *Monostaechas* (Fig. 5.2A). This last clade comprises very divergent species, the relationships between which are not resolved by means of 16S data. It is therefore not possible to confirm the validity or monophyly of the genera *Antenella* and *Monostaechas* and the haplotype data remains insufficient to clarify the phylogenetic relations within this subfamily.

The subfamily Kirchenpaueriinae is represented by only four nominal species of three genera, and appears monophyletic, though without high bootstrap support (Fig. 5.2A). The extremely high sequence divergence in this subfamily between *Ventromma halecioides* and the other *Kirchenpauerinae* species represented (*ca*. 25%) is notable. Also there is almost no variation in 16S sequences between the haplotypes of *Kirchenpaueria similis* and *K. pinnata* (Fig. 5.2B). If this is not a result of contamination or misidentification, it is likely that these two latter species are conspecific. Again, more haplotype data from representatives of this subfamily are required for a better understanding of the phylogenetic relationships of its member taxa.

The subfamily Plumulariinae is recovered as a well supported monophyletic group (Fig. 5.2A). The genera *Nemertesia* and *Dentitheca* appear monophyletic; *Plumularia* and *Monotheca* are polyphyletic. *Plumularia lagenifera*, *Pl. strictocarpa* and *Pl. setacea* group closely, and the haplotypes of the former species represented in the analyses reflect genetic population subdivision, with higher genetic divergences observed between the Pacific and Atlantic individuals.

The genus *Nemertesia* is recovered as monophyletic but the systematic relationships between its species are not fully resolved on the basis of 16S haplotype data (Fig. 5.2A). Nevertheless, more than five distinct *Nemertesia* species are recovered: *Nemertesia norvegica*, *N. antennina* (from shallow waters), *N. ventriculiformis*, *N. ramosa* and a species complex within *N. antennina* (from deep waters). *Nemertesia antennina* is a conspicuous species considered to be distributed in boreal, temperate and subtropical Atlantic waters, from the low water mark to about 2300 m depth. It has also been reported from localities in the Indian Ocean, on coast of South Africa and from Japan (Ansín Agís *et al*. 2001). One haplotypic group of *N. antennina* from Roscoff, France, and Plymouth and Lundy Island, UK, corresponds to colonies presenting the diagnostic morphological features of the species (i.e. with one nematotheca per athecate internode). *Nemertesia perrieri* that differs from *N. antennina* by the constant presence of two nematothecae per athecate internode (or two consecutive athecate internodes with one nematothecae) (Ansín Agís *et al*. 2001), appears to be conspecific to *N. antennina* from shallow waters based on 16S gene data (Fig. 5.2B). It thus confirms that the morphology of *N. antennina* from shallow waters can vary. However, the specimens of '*N. antennina*' collected from deep waters of the Gulf of Cadiz, which is a geographical area between the Mediterranean, English and French Atlantic coasts, form a very distant clade to that of *N. antennina* and *N. perrieri* from shallow-waters (Fig. 5.2A,B). From this evidence it seems that the nominal species *N. antennina* corresponds in fact to a complex of species segregated by depth. Additionally, the deep-water group of *'N. antennina*' in turn seems to be segregated into two distinct haplotypes representing species (or populations). These new species are morphologically distinct from *N. antennina* and *N. perrieri* and will be described and discussed in a forthcoming publication. Thus the socalled taxonomic circle will be closed following the revelation from 16S of at least two cryptic species of *Nemertesia* in the NE Atlantic. The occurrence of cryptic species living at different depths on the continental shelf/slope or in the water column has been observed for many taxa including echinoderms, crustaceans and molluscs (e.g. France & Kocher 1996; Howell *et al*. 2004; Zardus *et al*. 2006) and reinforces the perception of bathyal species having narrow depth ranges (e.g. Howell *et al*. 2002). This suggests that depth or physical factors correlated with depth have an important influence on speciation in some major groups of marine organisms.

Family Aglaopheniidae

This family is resolved as monophyletic with strong bootstrap support. The genus *Gymnangium*, represented by a well supported clade containing *G. montagui* and *G. hians*, is the sister group of a clade containing the genera *Cladocarpus*, *Lytocarpia*, *Aglaophenia* and *Macrorhynchia* (Fig. 5.3A). *Cladocarpus* is sister to the other three genera and *Lytocarpia* and *Macrorhynchia* are sister groups to a monophyletic clade containing nine nominal species of *Aglaophenia*. *Gymnangium gracilicaule* clusters with strong support (Fig. 1) within the genus *Macrorhynchia*, suggesting that that species should be moved to *Macrorhynchia* (see also Fig. 5.3B). The genus *Aglaophenia* is recovered as monophyletic, but the phylogenetic relationships between its species are not well-resolved. Three different haplotypes of *A. lophocarpa* occur on the Moroccan margin of the Gulf of Cadiz, despite the species practically having no morphological variation. The intraspecific genetic differences between these specimens of *A. lophocarpa* sampled in proximate localities, although within the normal range, can be twice as great as those of between specimens of *A. tubulifera* from shallow waters of England and bathyal depths of the Gulf of Cadiz. Although based on few haplotype data that are insufficient to draw firm conclusions on the intraspecific variation of these taxa, it may suggest genetically effective dispersal between these shallow and deep localities for *A. tubulifera*, possibly via the influence of the Mediterranean Outflow Water (MOW). The MOW flows through the Gulf of Cadiz and part of it flows north along the continental slope off Portugal, France and the Celtic Sea (e.g. McCartney & Mauritzen 2001; Hernández-Molina *et al*. 2006; White 2007). *Aglaophenia tubiformis*, *A. octodonta* and *A. pluma* show extremely low levels of 16S sequence divergence and genetic distances are more consistent with intraspecific comparisons in the group than interspecific (Fig. 5.3B). Comparison of the morphologies of *A. tubiformis*, *A. octodonta* and *A. pluma*, show that these ‘species’ are very similar. Phenotypic characters including shape or inclination of hydrothecal cusps, colour of structures and presence of zooxanthellae are the only diagnostic features separating these three species (Bouillon *et al*. 2004). Molecular data thus strongly suggests that these ‘diagnostic’ features probably represent environmentally driven plasticity or different populations/varieties within a single species, with the name *A. pluma*, having priority.

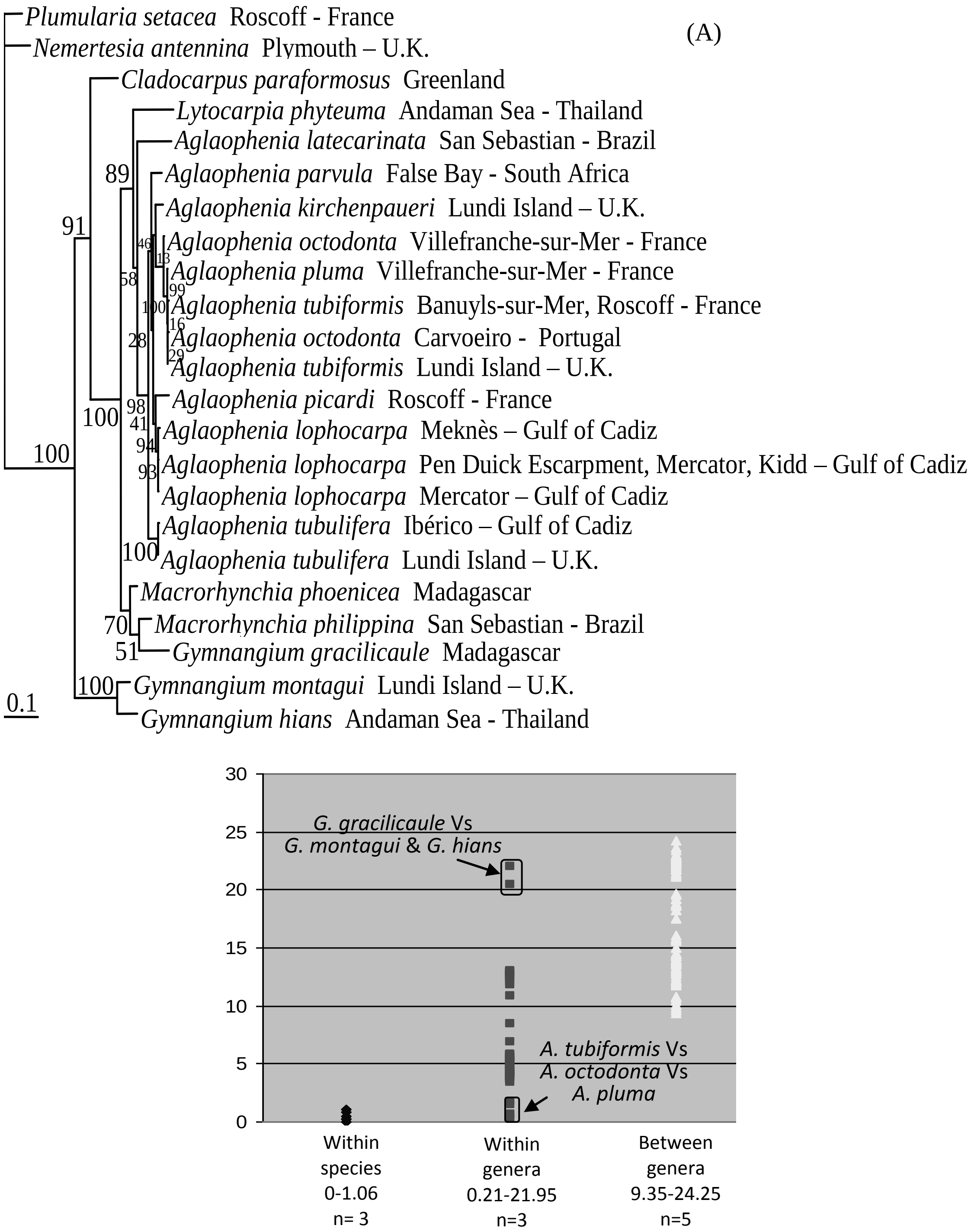

Fig. 5.3 A, B. Phylogenetic analysis of the data set containing the 16S sequences of the family Aglaopheniidae. (A) ‘Maximum-likelihood’ tree for 500 pseudoreplicates. *Plumularia setacea* and *Nemertesia antennina* are the outgroup taxa. Numbers near the nodes indicate the percent bootstrap values. The branch length indicator represents 0.1 substitutions per site. (B) Graphic of the 16S sequence divergences as uncorrected *P*-values (%): within species, within genera and between genera.

Family Sertulariidae

The relationship between the Sertulariidae and the superfamily Plumularioidea, both from the Suborder Plumulariida, was not determined by 16S sequence data. The Sertulariidae was recovered as polyphyletic in the analysis because the sequence of *S. tricuspidatus* tends to cluster more closely to a group of *Halecium* species (note on Fig. 5.4A the *S. tricuspidatus* is closer to the outgroup of *Halecium* species) rather than to the eight genera of Sertulariidae represented in the analysis, although with weak support. Furthermore, the high genetic distance between the *Symplectoscyphus* haplotype and the genus *Sertularella* is strong evidence that these genera are not congeneric as thought previously (see notes in Cornelius 1995b: 94). Unless the *S. tricuspidatus* sequence is a result of contamination or misidentification, the inclusion of a species with a 'typical' Sertulariidae appearance in the Haleciidae clade is surprising. *Symplectoscyphus tricuspidatus* may represent a transitory stage between Sertulariidae and Haleciidae although without evidence from further taxa within the group or other gene(s) this is speculation. All the other Sertulariidae species represented form a well-supported monophyletic clade (Fig. 5.4A). The genus *Sertularella*, represented by *Se. rugosa*, *Se. gayi robusta* and *Se. polysonias* (the latter two species looking distinct — see Fig. 5.4B), form a monophyletic group that is a sister taxon to a clade containing the genera *Amphisbetia*, *Dynamena*, *Diphasia*, *Sertularia*, *Thuiaria*, *Abietinaria* and *Hydrallmania*. Within this clade, the genera *Amphisbetia*, *Hydrallmania* and *Diphasia* form three distinctive isolated stems corroborating their generic status. *Dynamena* and *Sertularia* haplotypes group together only weakly, highlighting the possibility that these two genera are polyphyletic. *Sertularia cupressina*, *Thuiaria thuja*, *Abietinaria abietina* and *Ab. filicula* surprisingly all fall within a well supported clade suggesting that they may be congeneric species, but the relationships amongst these taxa are not fully resolved (Fig. 5.4A,B). The intraspecific genetic distances between the represented specimens of *Hydrallmania falcata* and *Diphasia rosacea* are within a 'normal' range, although the divergence is high between the two genotypes of the latter species sampled from relatively close geographical localities along the south-western coast of England.

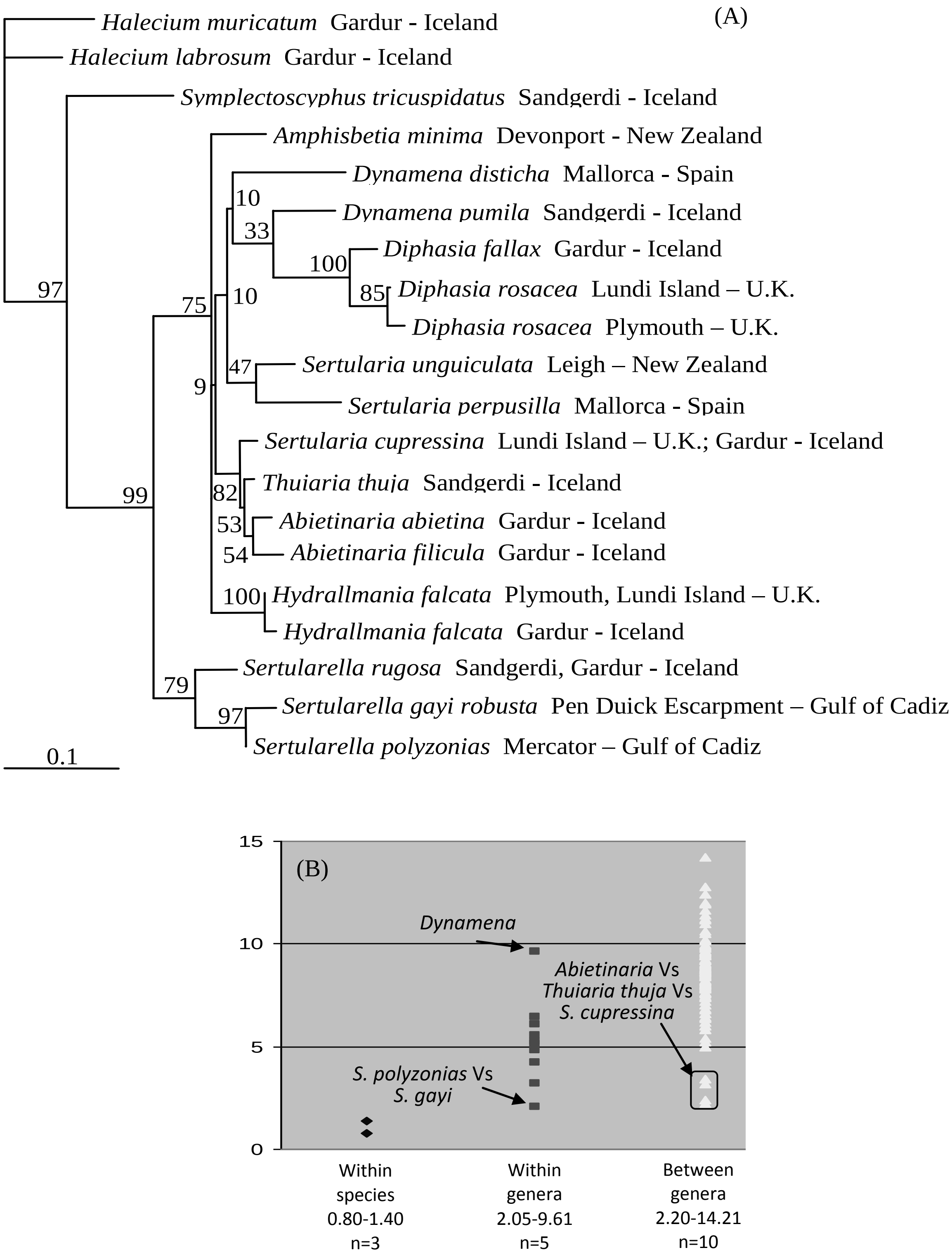

Fig. 5.4 A, B. Phylogenetic analysis of the data set containing the 16S sequences of the family Sertulariidae. (A) ‘Maximum-likelihood’ tree for 500 pseudoreplicates. *Halecium muricatum* and *Ha. labrosum* are the outgroup taxa. Numbers near the nodes indicate the percent bootstrap values. The branch length indicator represents 0.1 substitutions per site. Note that *Symplectoscyphus tricuspidatus* is genetically closer to the outgroup. (B) Graphic of the 16 S sequence divergences as uncorrected *P*-values (%): within species, within genera and between genera.

Family Haleciidae

In this family the sequence of *Hydrodendron* diverges markedly from the 10 *Halecium* species represented, supporting its inclusion in a different genus, but also giving low support for the monophyly of Haleciidae (Figs 5.1 and 5.5A). The genus *Halecium* also cannot be confirmed as monophyletic on the basis of 16S haplotype data (Figs 5.1 and 5.5A). Two divergent main clusters are consistently recovered within *Halecium*. One clade comprises the *Ha. sibogae marrocanum* haplotype which is considerably divergent to the sister group that is subdivided into a clade containing *Ha. tenellum* and *Ha. labrosum*, and another with *Ha. muricatum* and an unidentified deep-water species from an active mud volcano from the Gulf of Cadiz (Fig. 5.5A). This unnamed species has morphological similarities to *Ha. muricatum*. In the other main branch of *Halecium*, *Ha. halecinum* and *Ha. beanni* can be found tightly grouped (Fig. 5.5B) and sister to *Ha. petrosum*, *Ha. lankesteri* and *Halecium* sp2, the last two species being more closely related (Fig. 5.5B).

Family Lafoeidae

The monophyly of Lafoeidae was not unequivocally supported by the present 16S data (Figs 5.1 and 5.6A). This family is represented by two well-supported main clades, divergent and of an uncertain relationship (Figs 5.1 and 5.6A). One clade corresponds to the nominal subfamily Zygophylacinae containing the genera *Zygophylax* and *Cryptolaria*. The other represents the subfamily Lafoeinae including the genera *Lafoea* and *Acryptolaria*. The high 16S sequence dissimilarity between these subfamilies, supports the elevation of the Zygophylacinae and Lafoeinae to the categories of family, as suggested by Stepanyants (1979). Within the ‘Zygophylacinae’ clade there is a good demarcation of species, and *Cryptolaria pectinata* appears to be derived from the genus *Zygophylax*, to which it is closely related (Fig. 5.6B). There are two distinct haplotypes of *Zygophylax biarmata* collected from the Pen Duick Escarpment in Gulf of Cadiz in different years (3 years interval). Among the ‘Lafoeinae’ clade, *A. conferta* seems to present a unique haplotype along the Pen Duick Escarpment, and appears to be a sister taxon to a clade containing two very divergent genotypes of *Lafoea dumosa*: one from the bathyal depths of the Gulf of Cadiz, the other from Iceland (Fig. 5.6B). This suggests that the well-known and widespread *L. dumosa* could also be a species complex and clearly warrants further investigation in the waters of the NE Atlantic.

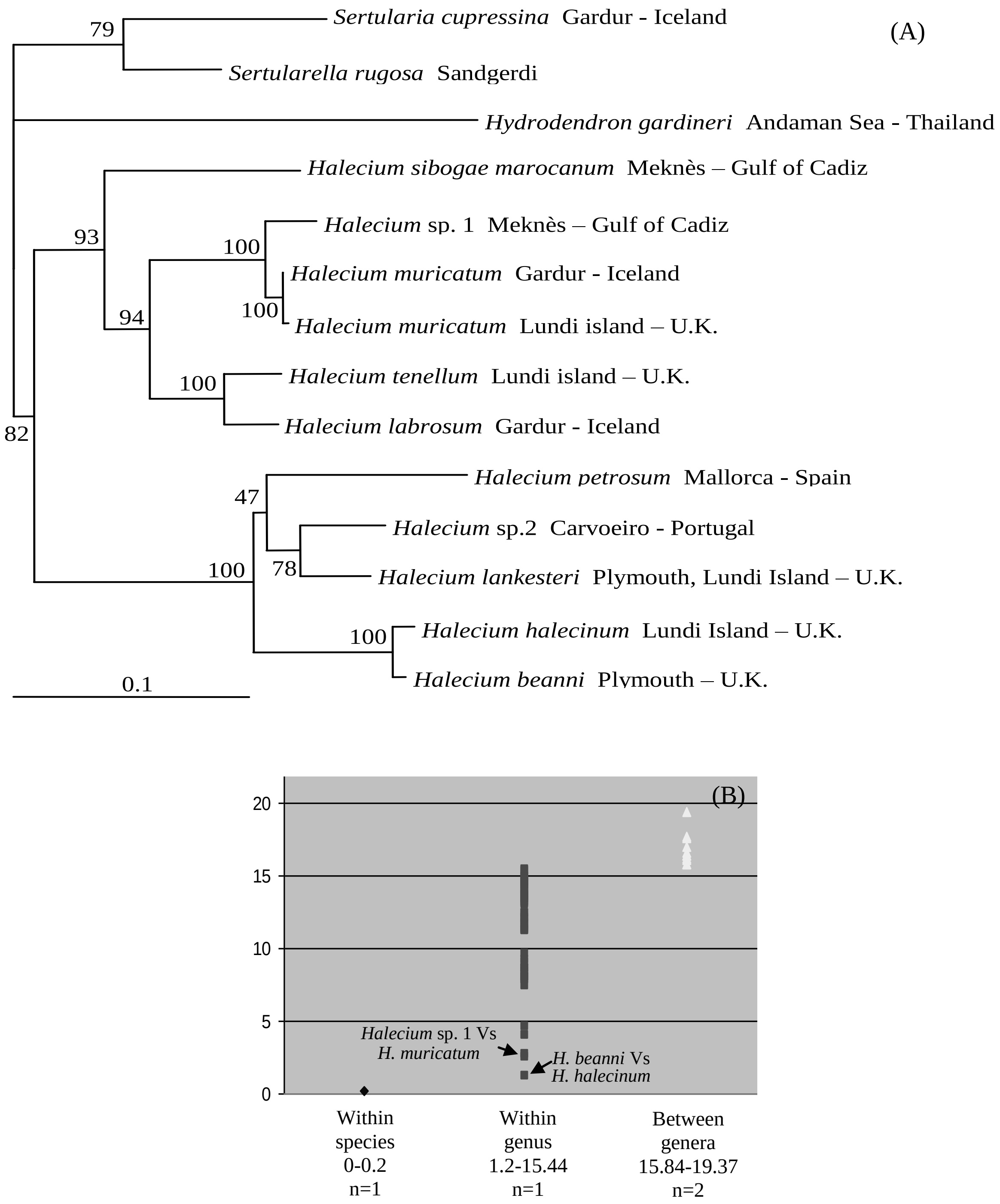

Fig. 5.5 A, B. Phylogenetic analysis of the data set containing the 16S sequences of the family Haleciidae. (A) 'Maximum-likelihood' tree for 500 pseudoreplicates. *Sertularia cupressina* and *Sertularella rugosa* are the outgroup taxa. Numbers near the nodes indicate the percent bootstrap values. The branch length indicator represents 0.1 substitutions per site. (B) Graphic of the 16 S sequence divergences as uncorrected *P*-values (%): within species, within genera and between genera.

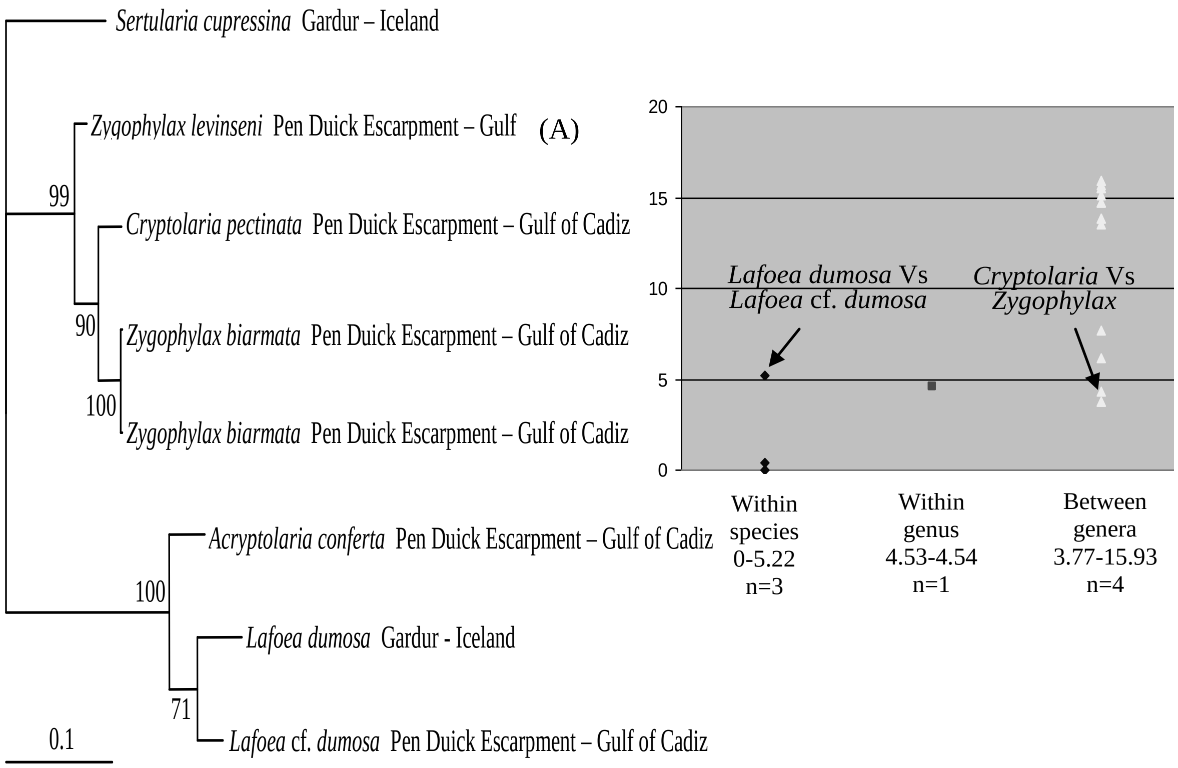

Fig. 5.6 A, B. Phylogenetic analysis of the data set containing the 16S sequences of the family Lafoeidae. (A) ‘Maximum-likelihood’ tree for 500 pseudoreplicates. *Sertularia cupressina* is the outgroup taxon. Numbers near the nodes indicate the percent bootstrap values. The branch length indicator represents 0.1 substitutions per site. (B) Graphic of the 16 S sequence divergences as uncorrected *P*-values (%): within species, within genera and between genera.

Family Eudendriidae

As noted above, the monophyly of the genus *Eudendrium*, as represented in the analysis, is well-supported (Fig. 5.1). Overall *E. racemosum* represents the earliest diverging lineage included in the analysis. The remaining *Eudendrium* haplotypes together occur as a clade although this is not very well supported (Fig. 5.7A,B). This clade may split into two, one containing *E. capillare* and a divergent species from two active mud volcanoes located in the middle of the Gulf of Cadiz (below 1000 m). The other clade contains three haplotypes from the Moroccan margin of the Gulf of Cadiz. One of these haplotypes from the Mercator mud volcano (*ca*. 350 m) represents an unidentifiable monosiphonic colony, that is, a clearly divergent evolutionary unit to haplotypes representing polysiphonic colonies morphologically similar to *E. rameum* (Fig. 5.7A). Polysiphonic colonies of *E. rameum* comprise genetically distinct populations from the Meknès mud volcano (700 m) and the Pen Duick Escarpment (*ca*. 500 m; Fig. 5.7A). The diversity of *Eudendrium* haplotypes in the bathyal environments from the Gulf of Cadiz, resolved by the 16S data, is therefore higher than previously recognized. Morphologically these specimens look very similar to each other. For example, the polysiphonic colonies of *Eudendrium* sp1 have gonophores and cnidocysts of the same type and size range, to specimens of *E. rameum* from the Moroccan margin. Without the additional information provided by 16S sequence analysis it is likely that these colonies would have been regarded as conspecific. However, much of the material collected from the Gulf of Cadiz is infertile and in a poor condition and is therefore unsuitable for taxonomic and/or molecular studies. More specimens will have to be collected, identified and the 16S gene sequenced, in order to understand the correct identity and distribution of species and populations across the active mud volcanoes and the surrounding environments of the Gulf of Cadiz.

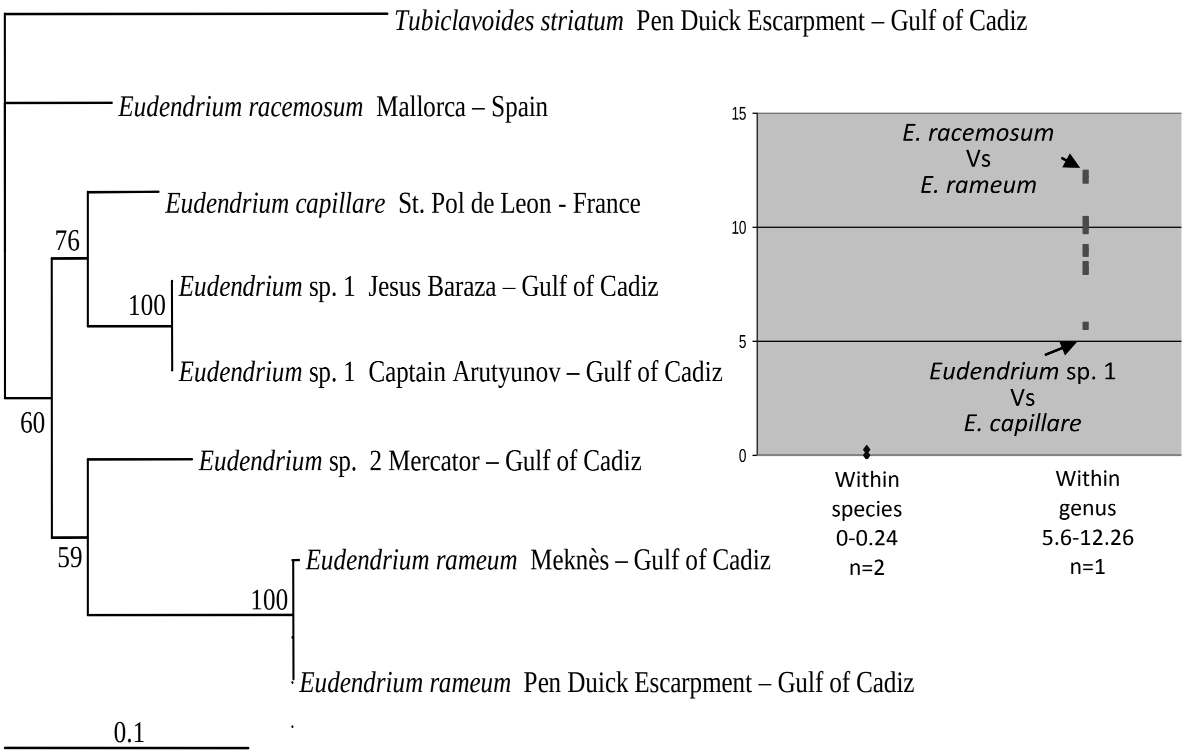

Fig. 5.7 A, B. Phylogenetic analysis of the data set containing the 16S sequences of the family Eudendriidae. (A) ‘Maximum-likelihood’ tree for 500 pseudoreplicates. *Tubiclavoides striatum* is the outgroup taxon. Numbers near the nodes indicate the percent bootstrap values. The branch length indicator represents 0.1 substitutions per site. (B) Graphic of the 16S sequence divergences as uncorrected *P*-values (%): within species, within genera and between genera.

5.4 CONCLUSIONS

This study has demonstrated that in the Hydrozoa the mitochondrial 16S rRNA provides a level of resolution that differentiates between species and is therefore useful as a barcoding gene. However, it also provides sufficiently broad resolution to distinguish genera, families and even a superfamily (Plumularoidea), and to infer relationships amongst and between many of these taxonomic groups at different hierarchical levels. In some cases, the 16S rRNA may provide information at the population level although sample sizes were not sufficiently large to investigate this in the present study.

As a barcoding gene the most useful aspect of the 16S sequence data is its ability to resolve cryptic species (e.g. within *N. antennina* and *L. dumosa*) and by contrast cases of apparent conspecificity (e.g. *N. antennina* and *N. perrieri*, and *A. octodonta*, *A. pluma* and *A. tubiformis*), congenerity (e.g. case of *Se. cupressina*, *Th. thuja*, *Ab. abietina* and *Ab. filicula*) or other systematic problems. In the present study the 16S sequence data demonstrated that species previously considered as common and widely distributed are in fact species complexes, a result that does not support the view of Hydrozoa as having cosmopolitan or widespread distributions.

The identification of many of the hydrozoan species using morphology is especially difficult as diagnostic features (e.g. reproductive structures) may not always be visible for ontogenetic reasons, because of environmental influences on morphology or because of damage during sampling and preservation. Characters are often subject to a high degree of phenotypic variation driven by the environment and they may be often homoplasious through evolutionary convergence. The systematics of the medusoid phase of hydroids is also long overdue for revision and there are many cases where the pelagic and benthic stages of hydroids have double taxonomic status.

Presently, about 450 hydrozoan 16S sequences have been published in public genetic data bases (mainly GenBank). This still under-represents the roughly 3700 described species (Bouillon *et al*. 2006), but the number of phylogenetic studies including 16S sequences of Hydrozoa have accelerated dramatically in recent years (Bridge *et al*. 1995; Romano & Palumbi 1997; Watkins & Beckenbach 1999; Schierwater & Ender 2000; Ender & Schierwater 2003; Collins *et al*. 2005; Dunn *et al*. 2005; Govindarajan *et al*.

2005a,b; Schuchert 2005a,b, 2006; Fraser *et al*. 2006; Govindarajan *et al*. 2006; Schuchert & Reiswig 2006; Galea & Lecrère 2007; Hemmrich *et al*. 2007; Leclère *et al*. 2007; Miglietta *et al*. 2007; Moura *et al*. 2007) and is set to grow in the near future. A well-populated sequence data base will greatly enhance the systematics of the hydrozoans and will promote their study in ecology and other aspects of biology.

Molecular taxonomy (barcoding) has been sometimes seen as a threat to Linnean systematics, but the present study clearly demonstrates that additional evidence from morphological analysis and other sources of data (i.e. geographical origin of specimens) are essential when interpreting genetic information. Thus, 16S nucleotide sequence data has the potential to successfully complement classical Linnean taxonomy in order to resolve distinct evolutionary units and better understand their associations, especially when exploring new, under-sampled habitats, such as the deep sea, that may contain many new taxa.

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Table 5.1 16S rRNA sequence data. Sequences marked with asterisk were determined in the present study.

Species	Family	Acession number	Geographical locality of samples
Tubiclavoides striatum	Tubiclavoididae	AM403520	Pen Duick Escarpment, Stn 16A, M2005, 660 m - Gulf of Cadiz
Eudendrium rameum *	Eudendriidae	AM888307	Meknès, Stn AT-581-Gr, TTR 15, 700 m - Gulf of Cadiz
Eudendrium rameum *	Eudendriidae	AM888309	Pen Duick Escarpment, Stn 21B, M2005, 498 m - Gulf of Cadiz
Eudendrium rameum *	Eudendriidae	AM888308	Pen Duick Escarpment, Stn 2, M2005, 526 m - Gulf of Cadiz
Eudendrium sp1 *	Eudendriidae	AM888310	Captain Arutyunov, Stn AT-546-Gr, TTR14, 1339 m - Gulf of Cadiz
Eudendrium sp1 *	Eudendriidae	AM888311	Jesus Baraza, Stn AT-391-Gr, TTR 12, 1105 m - Gulf of Cadiz
Eudendrium sp2 *	Eudendriidae	AM888312	Mercator, Stn 241, MS Merian 01-03, 353 m – Gulf of Cadiz
Eudendrium capillare	Eudendriidae	AY787884	St. Pol de Leon – France
Eudendrium racemosum	Eudendriidae	AY787896	Cala Murada, Mallorca – Spain
Acryptolaria conferta *	Lafoeidae	AM887980	Pen Duick Escarpment, Stn 21Bx, M2005, 498 m - Gulf of Cadiz
Acryptolaria conferta *	Lafoeidae	AM887981	Pen Duick Escarpment, Stn AT-600-Gr, TTR16, 660 m - Gulf of Cadiz
Acryptolaria conferta *	Lafoeidae	AM888345	Pen Duick Escarpment, Stn 23, M2006, 557 m - Gulf of Cadiz
Cryptolaria pectinata *	Lafoeidae	AM887994	Pen Duick Escarpment, Stn 2, M2005, 526 m - Gulf of Cadiz
Lafoea dumosa	Lafoeidae	AY787917	Gardur – Iceland
Lafoea cf. *dumosa* *	Lafoeidae	AM888327	Pen Duick Escarpment, Stn 42, M2006, 637 m - Gulf of Cadiz
Zygophylax biarmata *	Lafoeidae	AM888343	Pen Duick Escarpment, Stn AT-406-Gr, TTR12, 550 m - Gulf of Cadiz
Zygophylax biarmata *	Lafoeidae	AM888342	Pen Duick Escarpment, Stn 2, M2005, 526 m - Gulf of Cadiz
Zygophylax levinseni *	Lafoeidae	AM888344	Pen Duick Escarpment, Stn 2, M2005 , 526 m - Gulf of Cadiz
Halecium beanni *	Haleciidae	AM888314	Plymouth - UK
Halecium halecinum *	Haleciidae	AM888315	Lundy Island – UK
Halecium labrosum	Haleciidae	AY787916	Gardur – Iceland
Halecium lankesteri *	Haleciidae	AM888317	Plymouth - UK
Halecium lankesteri *	Haleciidae	AM888316	Lundy Island – UK
Halecium muricatum *	Haleciidae	AM888318	Lundy Island – UK
Halecium muricatum	Haleciidae	AY787915	Gardur – Iceland
Halecium petrosum	Haleciidae	AY787893	Mallorca – Spain
Halecium sibogae marrocanum *	Haleciidae	AM888319	Meknès, Stn AT-586-Gr, TTR15, 701 m – Gulf of Cadiz
Halecium tenellum *	Haleciidae	AM888322	Lundy Island – UK
Halecium sp1 *	Haleciidae	AM888321	Meknès, Stn 321 GKG22, MS Merian 01/03, 731 m - Gulf of Cadiz
Halecium sp2 *	Haleciidae	AM888320	Carvoeiro, 2-6 m, Algarve – Portugal

Hydrodendron gardineri	Haleciidae	AY787923	Andaman Sea, Koh Pee Pee Don, Ao Nui - Thailand
Abietinaria abietina	Sertulariidae	AY787898	Gardur – Iceland
Abietinaria filicula	Sertulariidae	AY787899	Gardur – Iceland
Amphisbetia minima	Sertulariidae	AY787903	Cheltenham Beach, Devonport - New Zealand
Diphasia fallax	Sertulariidae	AY787901	Gardur – Iceland
Diphasia rosacea *	Sertulariidae	AM888306	Plymouth - UK
Diphasia rosacea *	Sertulariidae	AM888305	Lundy Island – UK
Dynamena disticha	Sertulariidae	AY787909	Mallorca – Spain
Dynamena pumila	Sertulariidae	AY787902	Sandgerdi – Iceland
Hydrallmania falcata	Sertulariidae	AY787900	Gardur – Iceland
Hydrallmania falcata *	Sertulariidae	AM888325	Plymouth - UK
Hydrallmania falcata *	Sertulariidae	AM888324	Lundy Island – UK
Sertularella rugosa	Sertulariidae	AY787906	Sandgerdi & Gardur – Iceland
Sertularella gayi robusta *	Sertulariidae	AM888339	Pen Duick Escarpment, Stn 16AB, M2005, 660 m - Gulf of Cadiz
Sertularella polyzonias *	Sertulariidae	AM888340	Mercator, St237, MS Merian 01/03, 353 m - Gulf of Cadiz
Sertularia cupressina	Sertulariidae	AY787905	Gardur – Iceland
Sertularia cupressina *	Sertulariidae	AM888341	Lundy Island – UK
Sertularia perpusilla	Sertulariidae	AY787894	Mallorca – Spain
Sertularia unguiculata	Sertulariidae	AY787904	Leigh - New Zealand
Symplectoscyphus tricuspidatus	Sertulariidae	AY787907	Sandgerdi – Iceland
Thuiaria thuja	Sertulariidae	AY787908	Sandgerdi – Iceland
Aglaophenia kirchenpaueri *	Aglaopheniidae	AM887982	Lundy Island – UK
Aglaophenia kirchenpaueri *	Aglaopheniidae	AM887983	Plymouth - UK
Aglaophenia latecarinata	Aglaopheniidae	DQ855936	San Sebastian – Brazil
Aglaophenia lophocarpa *	Aglaopheniidae	AM887985	Meknès, Stn AT-581-Gr, TTR15, 700 m - Gulf of Cadiz
Aglaophenia lophocarpa *	Aglaopheniidae	AM887988	Mercator, Stn AT-575-B, TTR15, 355 m – Gulf of Cadiz
Aglaophenia lophocarpa *	Aglaopheniidae	AM887987	Mercator, Stn 242 GKG13, MS Merian 01/03, 350 m - Gulf of Cadiz
Aglaophenia lophocarpa *	Aglaopheniidae	AM887984	Kidd, Stn AT-528-Gr, TTR14, 489 m - Gulf of Cadiz
Aglaophenia lophocarpa *	Aglaopheniidae	AM887986	Pen Duick Escarpment, Stn 8, M2006, 444 m - Gulf of Cadiz
Aglaophenia picardi	Aglaopheniidae	AY787891	Cala Murada, Mallorca – Spain
Aglaophenia parvula	Aglaopheniidae	DQ855914	False Bay - South Africa
Aglaophenia pluma	Aglaopheniidae	DQ855916	Villefranche-sur-Mer – France
Aglaophenia octodonta *	Aglaopheniidae	AM887989	Carvoeiro, 2-6 m, Algarve – Portugal
Aglaophenia octodonta	Aglaopheniidae	DQ855915	Villefranche-sur-Mer – France
Aglaophenia tubiformis	Aglaopheniidae	AY787914	Roscoff – France
Aglaophenia tubiformis	Aglaopheniidae	DQ855917	Banuyls-sur-Mer – France
Aglaophenia tubiformis *	Aglaopheniidae	AM887990	Lundy Island – UK
Aglaophenia tubulifera *	Aglaopheniidae	AM887992	Lundy Island – UK
Aglaophenia tubulifera *	Aglaopheniidae	AM887991	Guadalquivir Ridge, Stn AT-339-D, TTR11,

			1021 m - Gulf of Cadiz
Cladocarpus paraformosus *	Aglaopheniidae	AM887993	Canyon area, Stn AT-486-D, TTR13, 1940-2080 m – Greenland
Gymnangium hians	Sertulariidae	AY787922	Andaman Sea, Pee Pee Island, Hin Dot – Thailand
Gymnangium montagui *	Aglaopheniidae	AM888313	Lundy Island – UK
Gymnangium gracilicaule	Aglaopheniidae	DQ855934	Madagascar
Lytocarpia phyteuma	Aglaopheniidae	AY787921	Andaman Sea, Ko Pee Pee, Aow Maa Yaa - Thailand
Macrorhynchia philippina	Aglaopheniidae	DQ855937	San Sebastian – Brazil
Macrorhynchia phoenicea	Aglaopheniidae	DQ855935	Madagascar
Antennella ansini	Plumulariidae	AY787890	Cala Murada, Mallorca – Spain
Antennella kiwiana	Plumulariidae	DQ855918	Devonport – New Zealand
Antennella secundaria	Plumulariidae	DQ883445	Banyuls-sur-Mer – France
Dentitheca habereri	Plumulariidae	DQ855927	Bunaken Island – Indonesia
Dentitheca bidentata	Plumulariidae	DQ855942	San Sebastian – Brazil
Halopteris alternate	Plumulariidae	DQ855939	San Sebastian – Brazil
Halopteris carinata	Plumulariidae	DQ855919	Honduras
Halopteris catharina *	Plumulariidae	AM888323	Lundy Island – UK
Halopteris catharina	Plumulariidae	DQ855920	Roscoff – France
Halopteris diaphana	Plumulariidae	DQ855921	Mallorca – Spain
Halopteris liechtensternii	Plumulariidae	AY787888	Cala Murada, Mallorca – Spain
Halopteris minuta	Plumulariidae	AY787912	Cheltenham Beach, Devonport - New Zealand
Halopteris polymorpha	Plumulariidae	DQ855922	Banyuls-sur-Mer – France
Halopteris tenella	Plumulariidae	DQ855938	San Sebastian – Brazil
Kirchenpaueria pinnata *	Plumulariidae	AM888326	Plymouth - UK
Kirchenpaueria pinnata	Plumulariidae	AY787911	Roscoff – France
Kirchenpaueria similis	Plumulariidae	DQ855923	Roscoff – France
Monostaechas quadridens	Plumulariidae	DQ855941	San Sebastian, Brazil
Monotheca hyalina	Plumulariidae	AY787913	Goat Island, Leigh - New Zealand
Monotheca margaretta	Plumulariidae	AY787892	Mallorca – Spain
Monotheca obliqua	Plumulariidae	DQ855929	Banyuls-sur-Mer – France
Monotheca pulchella	Plumulariidae	DQ855930	Simons Town - South Africa
Nemertesia cf. *antennina* *	Plumulariidae	AM888334	Mercator, Stn AT-576-B, TTR15, 428 m – Gulf of Cadiz
Nemertesia cf. *antennina* *	Plumulariidae	AM888332	Mercator, Stn 242 GKG13, MS Merian 01/03, 350 m - Gulf of Cadiz
Nemertesia cf. *antennina* *	Plumulariidae	AM888333	Mercator, Stn 287, MS Merian 01/03, 352 m - Gulf of Cadiz
Nemertesia cf. *antennina* *	Plumulariidae	AM888335	Pen Duick Escarpment, Stn 40A, M2006, 560 m - Gulf of Cadiz
Nemertesia antennina *	Plumulariidae	AM888328	Lundy Island – UK
Nemertesia antennina *	Plumulariidae	AM888329	Plymouth - UK
Nemertesia antennina	Plumulariidae	AY787910	Roscoff – France
Nemertesia perrieri	Plumulariidae	DQ855925	Banyuls-sur-Mer – France
Nemertesia norvegica *	Plumulariidae	AM888330	Atlantis Seamount, Stn AT-421-D, TTR12, 555-

			614 m – Azores
Nemertesia ramosa *	Plumulariidae	AM888331	Lundy Island – UK
Nemertesia ventriculiformis *	Plumulariidae	AM888337	Mercator, Stn AT-575-B, TTR15, 355 m – Gulf of Cadiz
Nemertesia ventriculiformis *	Plumulariidae	AM888336	Mercator, Stn 242 GKG13, MS Merian 01/03, 350 m - Gulf of Cadiz
Plumularia filicaulis	Plumulariidae	DQ855926	Simons Town - South Africa
Plumularia lagenifera	Plumulariidae	DQ855928	Friday Harbour – USA
Plumularia setacea	Plumulariidae	AY787885	Roscoff – France
Plumularia setacea	Plumulariidae	AY787886	St. Pol de Leon – France
Plumularia setacea	Plumulariidae	AY787887	Army Bay, Whangaparoa, Auckland - New Zealand
Plumularia setacea	Plumulariidae	EF472682	Comao, Punta Huinay – Chile
Plumularia setacea	Plumulariidae	EF472683	Comao, Punta Huinay – Chile
Plumularia setacea	Plumulariidae	EF472684	Huinay, Punta Llonca – Chile
Plumularia setaceoides	Plumulariidae	DQ855931	Wellington - New Zealand
Plumularia spiralis	Plumulariidae	AY787920	Andaman Sea, Koh Bida Nok – Thailand
Plumularia strictocarpa	Plumulariidae	DQ855940	San Sebastian – Brazil
Pycnotheca mirabilis	Plumulariidae	DQ855924	Wellington - New Zealand
Polyplumaria flabellata *	Plumulariidae	AM888338	Pen Duick Escarpment, Stn 9, M2006, 451 m - Gulf of Cadiz
Ventromma halecioides	Plumulariidae	AY787895	Mallorca – Spain

Abbreviations: Stn, station; M, oceanographic campaign ‘Microsystem’; TTR, oceanographic campaign ‘Training Trough Research’; UK, United Kingdom.

Chapter 6

THE USE OF THE DNA BARCODE GENE 16S MRNA FOR THE CLARIFICATION OF TAXONOMIC PROBLEMS WITHIN THE FAMILY SERTULARIIDAE (CNIDARIA, HYDROZOA)

Moura, C. J., Cunha, M. R., Porteiro, F. M. & Rogers, A. D. (2011a). *Zoologica Scripta, 40*, 520-537.

Contents

ABSTRACT

The Sertulariidae are the most speciose family of the Hydrozoa and their systematics are problematic. Here, 77 new 16S mtDNA sequence 'barcodes' of Atlantic and Mediterranean sertulariids were subject to phylogenetic analyses along with another 29 already deposited in GenBank. Among the 12 nominal genera and around 40 putative species analysed, several taxonomic inconsistencies and corroborations were highlighted by the genetic data. Patterns of morphological characters between the specimens analysed genetically were congruent with molecular estimates. The genera *Sertularia, Dynamena* and *Symplectoscyphus* were found to be polyphyletic, and the genus *Sertularella* paraphyletic. The validity of *Sertularella robusta* at a species level was confirmed, along with the exclusion of *Sertularelloides cylindritheca* from the genus *Sertularella*. Potential

cryptic species were detected within *Sertularella fusiformis*, *Sertularella mediterranea, Sertularella ellisii*, *Sertularia distans*, *Dynamena disticha*, *Thuiaria articulata* and *Diphasia margareta*. Conversely, probable cases of synonymy were revealed for the species pairs: *Sertularia cupressina* and *Sertularia argentea*, *Amphisbetia minima* and *Amphisbetia operculata*, and *Thuiaria thuja* and *T. articulata*. 16S haplotype data also revealed probable phylogeographic structure across localities of the NE Atlantic and Mediterranean. As an alternative 'DNA barcode', the 16S gene has the great advantage of being easily amplified and sequenced across hydrozoan taxa. Furthermore, the marked increase in deposited 16S sequences in public databases facilitates the identification of hydrozoan samples to the level of family, genus, and especially species or even populations, in a manner that is coherent with phenotypic and geographic/environmental differences.

6.1 INTRODUCTION

Barcoding life with standardized molecular biomarkers is increasingly being used successfully as an aid for the identification of taxa that may represent distinct and/or new species (after works of Floyd *et al*. 2002; Hebert *et al*. 2003a, b). At a time when biodiversity studies are being seen as important because of human-induced environmental change but are inhibited by the taxonomic impediment (e.g. Boero 2010), DNA barcoding has revitalized taxonomy by revealing a hitherto overlooked but significant cryptic diversity (e.g. Hebert *et al.* 2004a, b; Janzen *et al.* 2005; Smith *et al.* 2006) . Such approaches also allow: the connection of life stages and genders, where species are sexually dimorphic (Beskansky *et al.* 2003; Janzen *et al.* 2005); the clarification of problems of synonymy (Alroy 2002), and the attraction of more funds and public interest in taxonomic research (Hebert & Gregory 2005). Moreover, DNA barcoding approaches have revealed the potential to test taxonomic hypotheses, including phylogenetic relationships, and can also provide new insights into evolution, especially if it is integrated with other types of data, including molecular, morphological, distributional, ecological and behavioral (e.g. Dayrat 2005; Hebert & Gregory 2005; Savolainen *et al.* 2005; Smith *et al.* 2005; Will *et al.* 2005; Padial *et al.* 2010; Moura *et al.* 2011c).

The mitochondrial cytochrome c oxidase I (COI) gene was proposed and adopted as the preferred gene to barcode animal life (Hebert *et al.* 2003a, b). However, in Hydrozoa the mitochondrial 16S rRNA gene has been more widely accepted for barcoding purposes than COI (Moura *et al.* 2008). COI and other mitochondrial genes (including 16S) exhibit slow evolutionary rates in most Anthozoa (McFadden *et al*. 2000, 2011; Shearer *et al.* 2002; Hellberg 2006; Shearer & Coffroth 2008; Huang *et al*. 2008) and therefore COI has been assumed to be useless for DNA barcoding in this taxon. However, Sinniger *et al*. (2008) recommended that both COI and 16S markers can be useful as DNA barcodes of species of the order Zoantharia, as each gene has advantages and disadvantages. COI has been the preferred mitochondrial gene to investigate species boundaries and phylogenetics of Scyphozoa (e.g. Dawson & Jacobs 2001; Dawson & Martin 2001; Holland *et al*. 2004; Dawson 2005a, b). The 16S marker has been less used to investigate the phylogenetic relationships of the Scyphozoa (e.g. 597 COI sequences are deposited in GenBank, in contrast to only 48 sequences of 16S), but has also been used successfully in the demarcation of scyphozoan species (e.g. Schroth et *al*. 2002; Licandro *et al*. 2010). Recently Ortman *et al*. (2010) and Bucklin *et al*. (2011) extrapolated that COI is ‘broadly useful’ for DNA barcoding of species across the Medusozoa (sister clade to Anthozoa, containing Scyphozoa, Cubozoa and Hydrozoa), but is not phylogenetically informative for higher taxonomic ranks. However, the medusozoans barcoded with COI by Ortman *et al*. (2010) and Bucklin *et al*. (2011) included a considerable number of Siphonophora (subclass of Hydrozoa), but only few sequences of Cubozoa, Scyphozoa or other subclasses of the Hydrozoa. Shearer *et al.* (2002) demonstrated that the mutational rate of COI in one hydrozoan species was low, as found in the Anthozoa, but Govindarajan *et al*. (2005a) found COI to be a faster evolving gene and a more phylogenetically informative one for hydrozoan species. Despite such incongruence or lack of comprehensive studies that test the efficacy of COI as a DNA barcode across Meduzosoa, from a practical point of view, the 16S gene has been found to be much easier to amplify than COI, particularly in the Anthoathecata and Leptothecata groups (Miglietta *et al.* 2009; C. J. Moura, pers. observation). As a result of the resolution and effectiveness of 16S rDNA for barcoding, DNA taxonomy and phylogenetic analyses of Hydrozoa, 1204 16S rDNA sequences are deposited in GenBank at the present time, compared to only 476 COI sequences (an increase of about 200% of 16S sequences 'banked' since the last count published by Moura *et al.* 2008). The 16S gene has allowed recognition and discrimination of nominal and cryptic hydrozoan species (including invasive taxa), but also revealed reasonable potential for phylogenetic, phylogeographic and evolutionary analyses (Bridge *et al.* 1995; Romano & Palumbi 1997; Watkins & Beckenbach 1999; Schierwater & Ender 2000; Ender & Schierwater 2003; Collins *et al.* 2005; Dunn *et al.* 2005; Govindarajan *et al.* 2005a, b; Schuchert 2005a, b, 2006; Fraser *et al*. 2006; Govindarajan *et al*. 2006; Schuchert & Reiswig 2006; Galea & Leclère 2007; Hemmrich *et al*. 2007; Leclère *et al*. 2007, 2009; Miglietta *et al*. 2007, 2009; Moura *et al*. 2007, 2008, 2011c; Cartwright *et al*. 2008; Altuna 2009; Cartwright & Nawrocki 2010; Nawrocki *et al*. 2010; Peña Cantero *et al*. 2010; Miranda *et al*. 2010). For hydrozoans, 16S genetic information has been found to be consistent with nuclear genetic markers (e.g. Elongation Factor 1α, Miglietta *et al*. 2009; and ITS1 and ITS2, Miranda *et al*. 2010) and also morphology (e.g. Schuchert 2005; Miglietta *et al*. 2009; Moura *et al*. 2011c), further evidence of its potential as a DNA barcoding gene.

The family Sertulariidae is the most speciose within the Hydrozoa, containing over 500 species (Bouillon *et al*. 2006). Sertulariids are essentially benthic polypoid erect colonies, representing a regular faunistic component of seabed communities from coastal to abyssal waters (see examples of Fig. 6.1). Species of Sertulariidae are generally easily recognized at family level, although the differentiation of genera (and often species) is quite difficult, as most of the diagnostic morphological characters (mainly skeletal) are highly variable, even within an individual colony (Bouillon *et al*. 2006). As a consequence, it is common to find many taxa of Sertulariidae that have been misclassified, complicating the interpretation of many lists of species. The systematics of the family Sertulariidae is recognizably still far from resolved, and the introduction of other sources of diagnostic data, such as DNA sequence information, are critical to improve the taxonomy of the group. The phylogenetics of Sertulariidae was studied by means of genetic markers by Moura *et al*. (2008), Leclère *et al*. (2009) and Peña Cantero *et al*. (2010), but many taxonomic problems still persist in the family that require further investigation.

In the present study, we ‘barcoded’ several sertulariid species, including specimens from the shallow and deep waters of the NE Atlantic and western Mediterranean, by means of 16S rDNA sequence data. These DNA sequences along with others of Sertulariidae, already deposited in GenBank, were analysed phylogenetically with the objective of solving taxonomical problems within the family, namely cases of complicated synonymies, cryptic biodiversity and erroneous species assignments to genera. This barcoding approach was also applied to the identification of specimens unclassifiable by conventional morphological methods (e.g. broken and/or unfertile colonies), and for phylogeographic analyses.

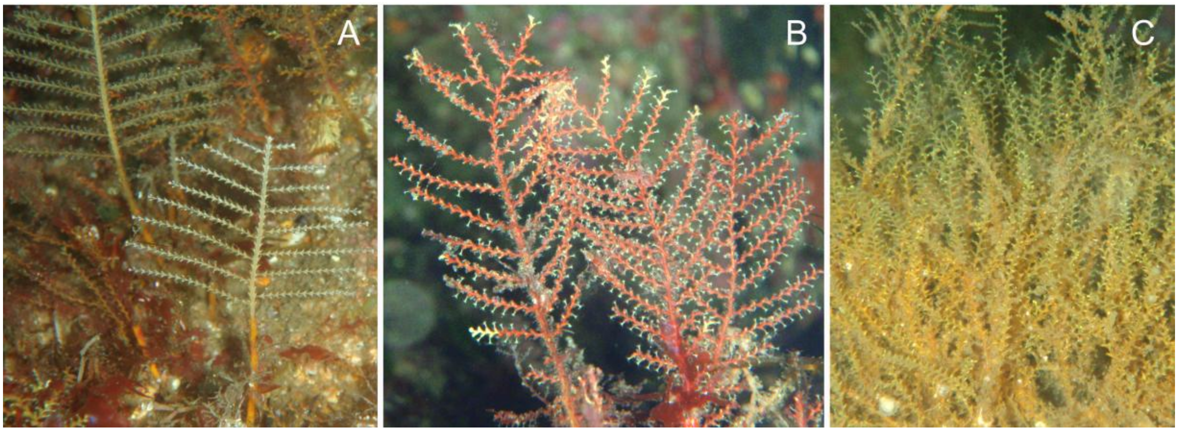

Fig. 6.1: Examples of sertulariids in shallow waters of the Berlengas archipelago – W Portugal: (A) *Diphasia margareta*; (B) *Sertularelloides cylindritheca*; (C) *Sertularella mediterranea*. Credits: C.J. Moura.

6.2 MATERIALS AND METHODS

Collection and identification of samples

Specimens of Sertulariidae and Thyroscyphidae were collected from 47 distinct sampling sites of shallow and deep-water localities of the northern Atlantic and western Mediterranean (Fig. 6.2, Table 6.1). Specimens were fixed and preserved mostly in 96% ethanol, although sometimes in 70% ethanol. The material collected in the Azores and Madeira included specimens obtained as part of a study on by-catch from deep-sea fisheries and some were frozen prior to fixation in ethanol. Taxa were classified taxonomically in the laboratory by C. J. Moura mainly based on publications of Picard (1956), Cornelius (1979, 1995), Ramil & Vervoort (1992), Medel (1996), Medel & Vervoort (1998), and Peña Cantero & García Carrascosa (2002).

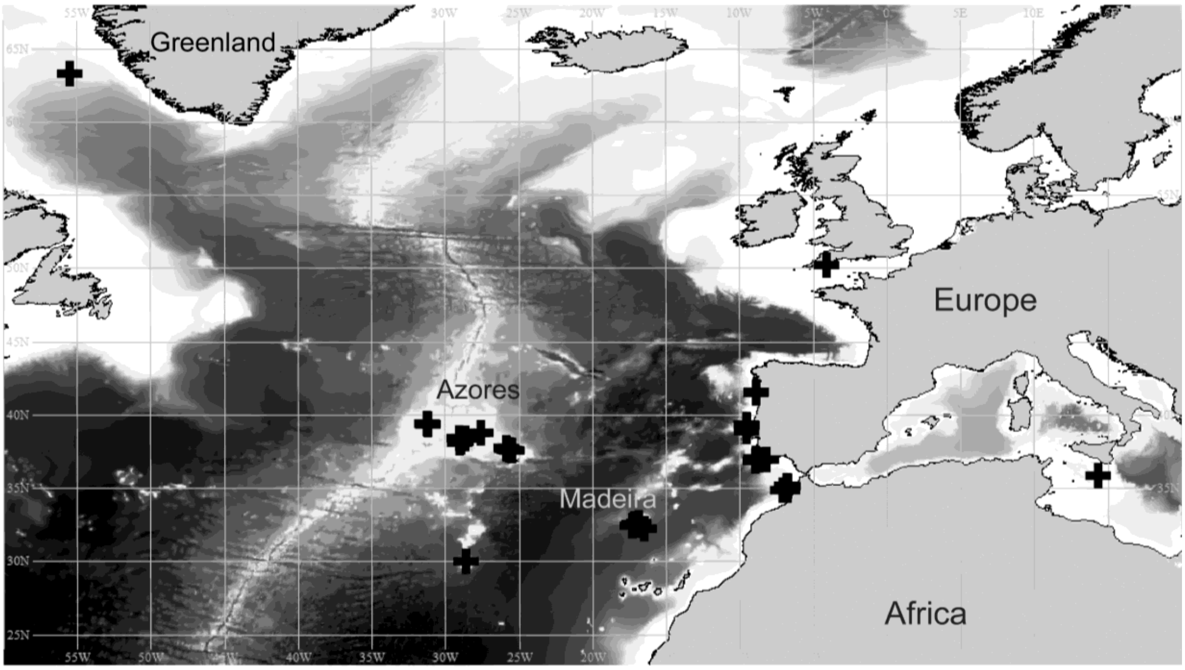

Fig. 6.2: Map representing the sampling sites (marked with crosses) from where hydrozoan DNA sequences were determined for the present study.

DNA extraction, amplification and sequencing

Protocols for DNA extraction, amplification and sequencing of the mitochondrial large ribosomal subunit (16S) rDNA gene followed the methodology described in Moura *et al.* (2008), except that the volumes of reagents used for PCR reactions were reduced to half, and that some of the purified PCR products were sent to 'STAB VIDA, Investigação e Serviços em Ciências Biológicas, Lda', Oeiras, Portugal for sequencing. The primers used were SHA (ACGGAATGAACTCAAATCATGT) and SHB (TCGACTGTTTACCAAAAACATA) (Cunningham & Buss 1993). The skeletons of voucher specimens were mounted in permanent slides and temporarily deposited in the Department of Biology, University of Aveiro (DBUA), Portugal. The material from the Portuguese coast and Gulf of Cadiz is temporarily deposited in the Biological Research Collection of the Department of Biology, University of Aveiro (DBUA), Portugal. Specimens from Madeira remained in the Municipal Museum collection at the Marine Biology Station of Funchal, Portugal. Azorean specimens gathered by the Department of

Oceanography and Fisheries (DOP), University of the Azores, Portugal, were returned to that institution. Sampling details, GenBank, Morphbank and Museums accession numbers are described in Table 6.1.

Phylogenetic analyses

The sequences assembled for this study were clustered with sequences of Sertulariidae published on GenBank. They were then aligned in MEGA version 4 (Tamura *et al*. 2007) with CLUSTALW using the default alignment settings, and trimmed to the shortest sequence. MrModeltest version 2.3 (Nylander 2004) was used to determine the optimal probabilistic model of sequence evolution. The 'general time reversible plus gamma' was the model selected by the Akaike Information Criterion. 'Maximum-likelihood' phylogenetic analyses were executed through the PHYML website (http://www.atgc-montpellier.fr/phyml/) (Guindon & Gascuel 2003) using the GTR model. Bayesian analyses were also performed with MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001), using the same model. The search was run for 10^7 generations, with trees sampled every 1000 generations (the first million generations were discarded as 'burnin'). Clade stability was assessed using non-parametric bootstrapping of ML analyses (1000 replicates) and Bayesian posterior probability values. MEGA version 4 (Tamura *et al*. 2007) was used to calculate pairwise distances for simple comparisons of percentage sequence divergence between taxa.

6.3 RESULTS AND DISCUSSION

In the present paper phylogenetic analyses were undertaken for 484 base-pairs of partial 16S rDNA sequences (Fig. 6.3) from 106 sertulariids (Sertulariidae and Thyroscyphidae) corresponding to 77 haplotypes of 12 nominal genera and about 40

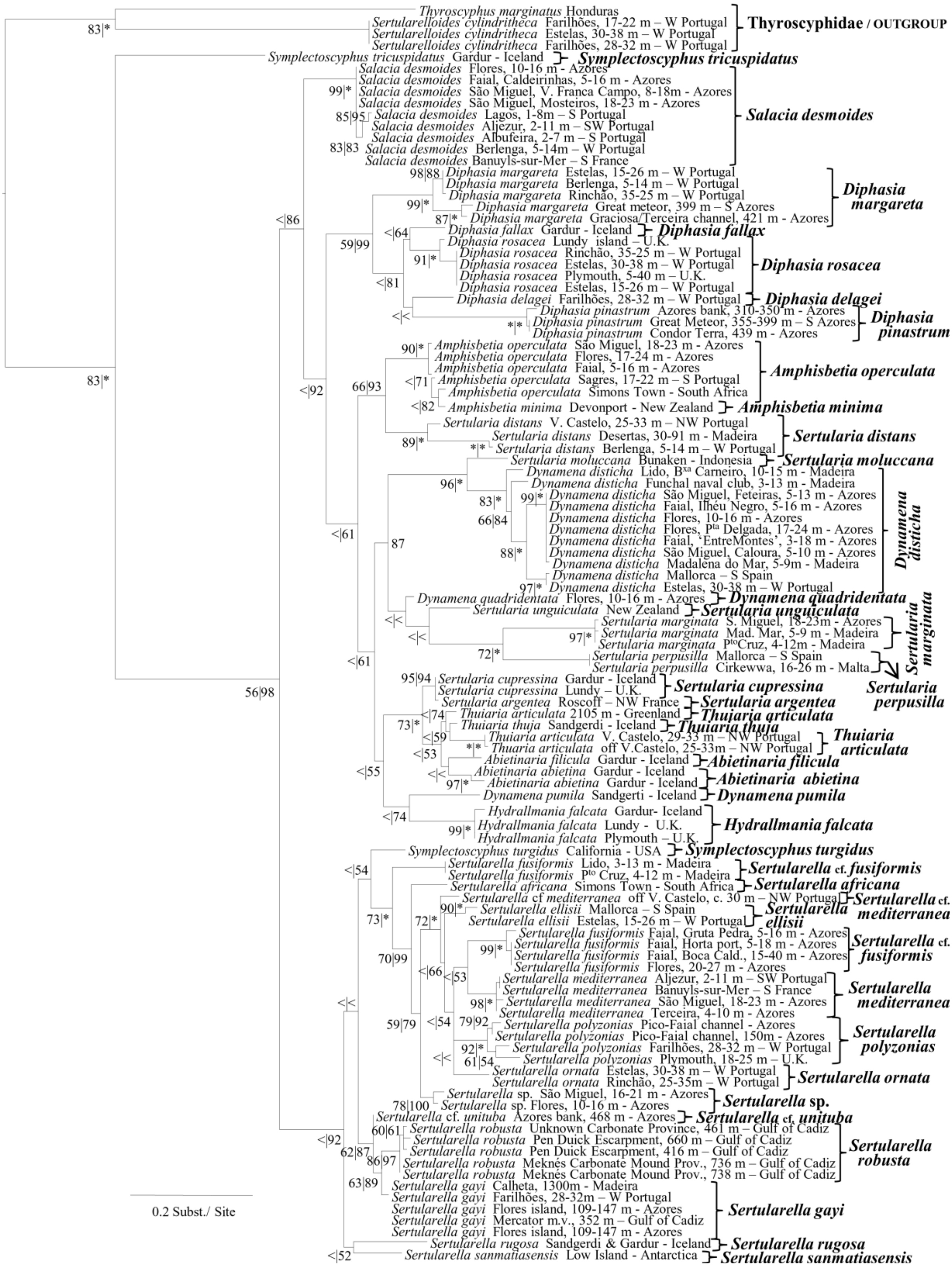

Fig. 6.3: 'Maximum likelihood' phylogenetic hypothesis derived from mitochondrial 16S rRNA gene sequences of sertulariids. Numbers near the nodes indicate values of bootstrap (left) and posterior probabilities (right) in percentage. The symbols '*' and '<' indicate bootstrap or posterior probability values equal to 100%, or less than 50%, respectively. The branch length indicator represents 0.2 substitutions per site.

putative species. Seventy-six sequences were determined for this study, 11 by C. W. Cunningham (direct EMBL submission), eight by Moura *et al*. (2008), 10 by Leclère *et al*. (2009) and one by Peña Cantero *et al*. (2010). Two species of Thyroscyphidae (*Sertularelloides cylindritheca* and *Thyroscyphus marginatus*), from a hydrozoan family easily confounded morphologically with Sertulariidae were used as an outgroup. These Thyroscyphidae sequences, despite being considerably divergent (9% *P* distance), cluster together with high nodal support and are genetically very distant from all the other Sertulariidae represented that are recovered as monophyletic (Fig. 6.3). However, if setting for instance the Haleciidae as the outgroup, the Thyroscyphidae is recovered as a divergent long branch nested amongst Sertulariidae, as sister clade to the genus *Sertularella* (more specifically to *Sertularella sanmatiensis*). Concomitantly the Sertulariidae is recovered paraphyletic (similarly to some results of Leclère *et al*. 2009). In fact, some authors (e.g. Millard 1975) have synonymised these two families, but in our analyses, the placement of Thyroscyphidae as a long branch away from the other Sertulariidae supports both families as being valid. Furthermore *Ser. cylindritheca* is very divergent genetically from the other *Sertularella* species (Fig. 6.3), thus supporting the argument that this species should be kept in *Sertularelloides* and not in *Sertularella*. The re-inclusion of *Ser. cylindritheca* (and *Thyroscyphus marginatus*) in Thyroscyphidae is a conservative, although provisional interpretation of these data. Further genetic studies (including other genetic markers) and haplotype sampling is needed for families phylogenetically close to Sertulariidae to test this hypothesis further.

The great disadvantage of DNA barcoding with 16S instead of COI includes the frequent presence of indels (insertions/deletions) in 16S sequences, which may complicate the alignment of sequences and consequently may alter the value of sequence divergence according to the alignment adopted. However, the presence of indels can also be advantageous as they may be taxonomically informative of infra- to supraspecific taxonomic levels (e.g. Sinniger *et al*. 2008; the present study). A particular region (197-231 bp) of the alignment herein analysed corresponded to a major indel region that was difficult to align. Therefore to search for any bias related to the alignment, we also ran several phylogenetic analyses with alignments generated through MAFFT, version 6 (http://mafft.cbrc.jp), and alignments with and without gap positions or sequences that represented long branches (namely *Symplectoscyphus tricuspidatus* and Thyroscyphidae taxa). The topologies of the phylogenetic trees obtained in these analyses were consistently identical, especially when referring to the clades with higher nodal support of Fig. 6.3.

Genus Symplectoscyphus

The genus *Symplectoscyphus* is herein represented by two species – *Sy. tricuspidatus* and *Symplectoscyphus turgidus* that appear at divergent phylogenetic positions (Fig. 6.3). The 16S rDNA gene is inconclusive about the real phylogenetic position of these two species within the family Sertulariidae, but their genetic distances (8%) seem good evidence to assign them to different genera. Whilst *Sy. tricuspidatus* is recovered as monophyletic with *Symplectoscyphus* species present in Antarctica (not shown, see Peña Cantero *et al*. 2010), *Sy. turgidus* seems to cluster more closely to *Sertularella* species (Leclère *et al*. 2009; the present study), and likely should be moved again to the genus *Sertularella* (as firstly proposed by Clark 1876) or even to another (perhaps new) genus. Further studies are therefore required to resolve the phylogenetic position of *Sy. turgidus*.

Genus Sertularella

The genus *Sertularella* - represented by 12 nominal species, two (or three) cryptic species and one undetermined species - appears monophyletic (or paraphyletic if we do not consider '*Symplectoscyphus' turgidus* as *Sertularella*), with low bootstrap support (Fig. 6.3). Leclère *et al*. (2009), using more genetic markers, also recovered the genus *Sertularella* as monophyletic (or paraphyletic with '*Symplectoscyphus' turgidus*), although without the divergent clades of *Sertularella rugosa* and *Sertularella sanmatiasensis* presently represented.

Sertularella gayi is recovered as monophyletic with genetically undifferentiated samples from deep waters of Madeira (Calheta - 1300 m), Azores (Flores Island - 109 -147 m), Gulf of Cadiz (Mercator mud volcano - 352 m) and shallow depths of western Portugal (Berlengas - *ca*. 30 m) (Fig. 6.3). The species is indeed genetically close to the morphologically similar taxon *Sertularella robusta* , which was previously considered as a variety of *S. gayi*, but the consistent monophyly of the '*gayi*' phenotype (Fig. 6.3) supports the recent reseparation of these species (e.g. Bouillon *et al*. 2006).

Sertularella robusta is recovered as monophyletic with four lineages co-occurring at the deep waters of the Gulf of Cadiz (Fig. 6.3). Genetic distances between the *S. robusta* haplotypes reach 0.5%.

The represented haplotype of *Sertularella* cf. *unituba* Calder, 1991 corresponds to one infertile colony from 468 m in the Azores Bank that is morphologically similar to *S. robusta* (by the sizes of hydrothecae and mode of ramification) but seems rather more phylogenetically close to *S. gayi* (Fig. 6.3). That Azorean lineage is recovered as a clear distinct species, with a similar phenotype to that of *S. unituba* namely by the form of hydrothecae, but these structures are distinctly larger in our sample than that of *S. unituba* measured by Medel & Vervoort (1998) (including type material). *Sertularella unituba* was also previously considered as a variety of *S. gayi*, and the present 16S data recognizes the phylogenetic association with these species but also confirms that they are distinct (Fig. 6.3).

Sertularella rugosa and *S. sanmatiasensis*, which are only represented by one sample each, were collected from the polar regions and are found genetically divergent from all the other *Sertularella* species herein represented from the NE Atlantic and the Mediterranean (Fig. 6.3). The phylogenetic position of *S. rugosa* and *S. sanmatiasensis* is uncertain (Fig. 6.3), possibly because of the limitation of the 16S gene (by itself) to establish some intergeneric relationships amongst Sertulariidae.

All other *Sertularella* species represented in the analyses cluster in a well-supported clade comprising the nominal species *Sertularella fusiformis*, *Sertularella mediterranea*, *Sertularella ellisii*, *Sertularella ornata* and *Sertularella polyzonias* (Fig. 6.3). These taxa are indeed morphologically similar so that taxonomic debates on their validity or synonymy have been extensive over years (e.g. Cornelius 1979; Ramil & Vervoort 1992; Medel & Vervoort 1998). The 16S genetic data (Fig. 6.3) proved again to be in agreement with phenotype-based taxonomical classifications, by the recognition of these nominal species and their phylogenetic association. Furthermore the 16S gene provided evidence of cryptic diversity associated with *S. mediterranea* and *S. fusiformis*, and resolved an unidentified species from the shallow waters of the Azores ('*Sertularella* sp.') (Fig. 6.3).

The morphology of the latter species resembles somewhat *S. ellisii* and the hydrothecae of *S. polyzonias* but phylogenetic analyses (Fig. 6.3) confirm it is distinct.

This species has already been identified by Medel & Vervoort (1998) as a variety (‘form 3’) of *S. ellisii,* also collected in relatively shallow waters of the Azores. We have therefore the evidence that this corresponds to a new species that can potentially be misidentified as *S. ellisii* and that this taxon needs to be described.

Sertularella mediterranea has its type locality in Yugoslavia in the Mediterranean Sea. We have represented haplotypes of the species from the Mediterranean (southern France), SW Portugal and Azores (S. Miguel and Terceira Islands) with little genetic divergence, possibly revealing a recent influence of the Mediterranean Sea in the latter two geographical areas (or vice-versa). A quite divergent haplotype (up to 2.5% of genetic distance), originally from northern Portugal (Viana do Castelo), also classified morphologically as *S. mediterranea,* seems to correspond to a new cryptic species (Fig. 6.3).

Sertularella fusiformis was originally described from the coasts of Devon (UK). We have no representatives of it from its type locality of temperate cold waters; instead we have samples of that nominal species in distinct clades from the subtropical Madeira and the Azores Islands (each clade corresponding to one archipelago) (Fig. 6.3). These clades likely correspond to different species, according to genetic distances (up to 2.5% between major clades) and some morphological differences (see Table 6.2), and therefore two (or at least one) cryptic species within *S. fusiformis* were discovered in the present study.

S. polyzonias is considered a cosmopolitan species (e.g. Ramil & Vervoort 1992), present in both shallow and deep waters (e.g. see Peña Cantero & García Carrascosa 2002). This species is herein represented by samples recovered from the Portuguese and English coasts and from relatively deep waters of the Azores. Apart from the UK, *S. polyzonias* does not occur frequently either at the Azores or at the Portuguese coasts (C. J. Moura, pers. observ.), and despite its well-supported monophyletic recovery, the four haplotypes found are somewhat divergent (reach a maximum of 1.9% of sequence divergence). The phylogenetic relationship of its haplotypes appears to be coherent with geographical and bathymetrical distances (perhaps also because of low population sizes in the studied areas), and further studies are therefore required to understand whether *S. polyzonias* has such a widespread distribution.

Genus Salacia

This genus is represented in the analyses solely by specimens of *Salacia desmoides* which cluster monophyletically (Fig. 6.3). For that species, four genetically close haplotypes (genetic distant up to 1%) were found in nine samples, respectively, from the Azores (Flores, S. Miguel and Faial Islands), western Portugal and the Mediterranean, and south and southwest Portugal (Algarve and Alentejo) (Fig. 6.3). As with the pattern found for *S. mediterranea*, there is evidence of recent gene flow between the Mediterranean and western/southern Portugal, and the Azores.

Genus Diphasia

The genus *Diphasia* is recovered as monophyletic (although with relatively low bootstrap support) with the nominal species *Diphasia margareta*, *Diphasia fallax*, *Diphasia rosacea*, *Diphasia delagei* and *Diphasia pinastrum* (Fig. 6.3). Species-relationships within the genus are not, however, clearly resolved with 16S sequence data.

Diphasia margareta is recovered as monophyletic containing a clade with samples from shallow waters of Berlengas and a slightly divergent deep-water lineage from the Azores with two close haplotypes (Fig. 6.3). The genetic distance (up to 2%) between these two main clades may emphasize that gene flow for this species does not presently occur between shallow waters of central Portugal and the bathyal depths of the Azores, contrary to what was found for *S. gayi*. Furthermore, *D. margareta,* especially found in deep waters, was known as shallow as 24 m depth (Medel & Vervoort 1998), and we recovered the species at about 10 m in western Portugal, altering its shallowest bathymetrical record.

Diphasia rosacea was found monophyletic with two close haplotypes co-occurring in the UK (genetically distant 1%) (Fig. 6.3). One of the haplotypes is also present in western Portugal (Berlengas), likely revealing the influence of northern Atlantic waters up to central Portugal (Moura *et al.* 2011c).

Diphasia delagei is a quite uncommon species known from depths between *ca.* 41 (Medel 1996) and 1250 m (Ramil & Vervoort 1992). Our exclusive sample of the species represents its first record in Portugal, and the shallowest record for the species to date (*ca.* 30 m).

Diphasia pinastrum was found in deep Azorean waters without much genetic divergence (0-0.7%): two close haplotypes in three samples.

Genus Amphisbetia

The genus *Amphisbetia* is recovered as monophyletic with two nominal species represented: *Amphisbetia operculata* and *Amphisbetia minima* (Fig. 6.3). In five samples of *A. operculata*, we found two haplotypes in the Azores, one in southern Portugal and the other in South Africa. These are genetically close (up to 1.2% of sequence divergence) and in the 'normal' range of intraspecific sequence variation of sertulariids. *A. minima* collected from New Zealand diverge very little (1% of genetic distance) from the haplotypes of *A. operculata*, raising the strong possibility that these two nominal species, that are morphologically similar, are synonymous.

Genus Sertularia

The genus *Sertularia* is found polyphyletic when represented by seven nominal species: *Sertularia distans*, *Sertularia moluccana*, *Sertularia unguiculata*, *Sertularia marginata*, *Sertularia perpusilla*, *Sertularia cupressina* and *Sertularia argentea* (Fig. 6.3).

Sertularia distans is represented by two monophyletic but highly divergent haplotypes (Fig. 6.3). One haplotype was found in northern Portugal, the other in Madeira (Desertas Island) and western Portugal (Berlenga Island). Sequence divergence between these haplotypes is quite high (3.2%), suggesting that they correspond to different species. The corresponding morphotypes share similar characters, although more haplotype sampling of *Se. distans* (and further genetic and morphologic analyses) is required to determine whether the species is a complex. Furthermore, the phylogenetic position of *Se. distans* (sensu lato), dislocated from other *Sertularia* taxa (Fig. 6.3), suggests it should be included in another genus, perhaps the presently invalid genus *Tridentata*.

Sertularia marginata was recovered as monophyletic with genetically close haplotypes occurring in Madeira and Azores (distant genetically up to 0.5 %) (Fig. 6.3). This species appears as sister to the morphological similar taxon *Se. perpusilla* (Fig. 6.3), which is endemic to the Mediterranean Sea. For the latter species we did not find genetic differentiation between Mallorca and the island of Malta.

Sertularia cupressina is found with high nodal support in a clade with the phenotypically similar *Se. argentea* (Fig. 6.3). Sequence divergence between the two species is too low (0.2%) and is in the range of intraspecific variation of other Sertulariidae species. Genetic data therefore suggests the synonymization of *Se. cupressina* and *Se. argentea* (following Pallas (1766)), but more haplotype sampling of both nominal species is required for confirmation. Furthermore, as in Moura *et al*. (2008) and Leclère *et al*. (2009), the clade of *Se. cupressina* clusters tightly with *Thuiaria* and *Abietinaria* species, suggesting that these taxa may correspond to the same genus. Indeed, these taxa share a synapomorphic morphological character (see Schuchert 2001) of having an ectodermal pad in the inner part of the adcauline wall of hydrothecae that further supports such a phylogenetic relationship.

Genus Thuiaria

The genus *Thuiaria* represented by *Thuiaria articulara* and *Thuiaria thuja*, is recovered as monophyletic, although with low nodal support (Fig. 6.3). These two species are morphological similar, but differ on the general structure of colonies and projection of hydrothecal walls out of the axis (see Cornelius 1995). Our analyses (Fig. 6.3) show that two divergent clades of *T. articulata* (one from coastal waters of northern Portugal, the other from bathyal depths of Greenland, genetic distance up to 1.7%) cluster with an intermediate lineage containing *T. thuja* from deep waters of Iceland. The type localities of *T. thuja* and *T. articulata* are from coastal waters of Scotland and England (respectively), and therefore the phylogeny suggests that *T. 'articulata'* found in deep waters of Greenland likely corresponds to a cryptic species that can also exhibit the typical phenotype described for *T. thuja*, which consequently may be synonymous to *T. articulata*. We therefore hypothesize that there is a distinct *Thuiaria* species in the deep waters of Greenland and Iceland, presenting morphological characters close to *T. thuja* and *T. articulata*.

Genus Abietinaria

The genus *Abietinaria* is recovered as monophyletic with low nodal support (Fig. 6.3). It comprises two reciprocally monophyletic clades comprising *Abietinaria filicula* and

Abietinaria abietina, respectively, without much interspecific sequence divergence (up to 4%).

Genus Dynamena

The genus *Dynamena* herein represented by *Dynamena quadridentata*, *Dynamena pumila* and *Dynamena disticha,* is recovered as polyphyletic, with these species falling at relatively distant positions of the cladogram (Fig. 6.3).

Dynamena disticha is the only species of the genus that was sampled from a wide geographic area. No sequence differentiation was found in samples from three Azorean islands (S. Miguel, Faial and Flores). Three more slightly divergent haplotypes are found in Madeira, and a different haplotype is shared between the western Mediterranean (Mallorca) and western Portugal (Berlengas). One lineage from Madeira is very close genetically to the Azorean haplotypes (genetic distance of 0.2%), but two other evolutionary branches also present in Madeira diverge with relatively high distances (up to 2.7%) from the other *Dy. disticha* clades (Fig. 6.3). Further studies are therefore required to determine whether cryptic diversity may be associated with this nominal species.

Genus Hydrallmania

The genus *Hydrallmania* is recovered as monophyletic, represented by two close haplotypes of *Hydrallmania falcata* (diverging 0.5%), from the UK and Iceland, respectively. The phylogenetic position of the genus within Sertulariidae is not sufficiently resolved solely by 16S haplotype data (Fig. 6.3).

6. 4 CONCLUSIONS

The present work confirmed the utility of the mitochondrial 16S rDNA sequence data as ‘DNA-barcodes’, providing sufficiently high species-level resolution to identify hydrozoan species (Govindarajan *et al.* 2005a, b; Schuchert 2005a, b; Govindarajan *et al.* 2006; Miglietta *et al.* 2007, 2009; Moura *et al.* 2008, 2011c; Miglietta & Lessious 2009; Nawrocki *et al.* 2009; Miranda *et al.* 2010; Peña Cantero *et al.* 2010). At taxonomic ranks superior to species, the 16S gene (by itself) could not resolve many of the phylogenetic associations within sertulariids possibly because of a relatively slow evolutionary rate of 16S for these taxa (Peña Cantero *et al.* 2010). Therefore, most of the relationships between (and sometimes within) genera of sertulariids presented in the phylogeny were not conclusive. Regardless of this, the genetic data presented resolved many areas of phylogenetic topology, refuted assignments of species and genera, and gave probable insights into phylogeographic associations within species. DNA barcoding with 16S genetic data also highlighted potential cryptic and synonymous species and provided correspondence of morphologically unidentifiable specimens (e.g. unfertile or degraded) with accurately classified biological material.

All the characterized genera were recovered monophyletic, with exception of *Sertularia, Dynamena, Symplectoscyphus* and *Sertularella*. The former genus is recovered paraphyletic (as in Leclère *et al*. 2009) only if *Sy. turgidus* is not re-included in *Sertularella*, the other genera were found polyphyletic. *Symplectoscyphus* is polyphyletic for the reason previously stated, whilst the genera *Sertularia* and *Dynamena* comprise several species which are very divergent, sometimes more related to other genera. Our data therefore suggested that *Sertularia* and *Dynamena* are truly polyphyletic genera that require significant taxonomic revisions.

At a supra-specific phylogenetic level the 16S data also confirmed that *Ser. cylindritheca* does not belong to the genus *Sertularella* and possibly neither to the family Sertulariidae. We suggested its re-inclusion in the genus *Sertularelloides* and (at least provisionally) in the family Thyroscyphidae.

At a taxonomic species rank, evidence was presented for the validation of *S. robusta* as a distinct species from *S. gayi*. However, it was also demonstrated that two pairs of nominal species might be synonymous (*Se. cupressina* and *Se. argentea*, and *A. minima* and *A. operculata*). Furthermore the 16S gene also resolved potential cryptic diversity within *S. fusiformis, S. mediterranea, S. ellisii, Se. distans*, *Dy. Disticha, T. articulata* and *D. margareta*. Furthermore, because the cryptic species of *T.* '*articulata*' found in Greenland seemed conspecific to *T. thuja* found in Iceland, we also question the validity of this latter species.

At an infraspecific taxonomical level some probable phylogeographic affinities were revealed. Identical intraspecific 16S sequences, possibly revealing ongoing or recent genetic migration, were detected in shallow-water samples of different Azorean islands (for *S. desmoides*, *A. operculata*, *Dy. disticha*, *S.* cf. *fusiformis*); from W and SW Portugal and the Mediterranean (for *S. desmoides and S. mediterranea*); from W Portugal and the UK (for *D. rosacea*); and from shallow waters of W Portugal and deep-water localities of the Azores, Madeira and Gulf of Cadiz (for *S. gayi*). Low levels of sequence divergence were also detected between samples of: shallow waters of W Portugal and Azores (for *A. operculata*); W Portugal and Madeira (for *S. distans*); Azores and Madeira (for *Dy. disticha* and *S. marginata*); W Portugal and Mediterranean (for *Dy. disticha*); and between Azores, SW Portugal and the Mediterranean (for *S. mediterranea*). These null or slight nucleotide differences of 16S sequences between hydrozoan samples probably reveal present or relatively recent gene flow between the different geographical areas clustered, especially when the same haplotypes are shared between localities. Such potential phylogeographical affinities detected are likely influenced by water currents and properties (e.g. temperature), dispersal modes of species (including rafting on biologic and/or artificial substrata for some taxa), population sizes, geographical distance and potential environmentally driven physiological constrains.

Finally, the 16S genetic data supported the morphological similarity and discontinuities of taxa. For example, *Se. marginata* revealed sister to the phenotypically similar *Se. perpusilla; A. minima* is genetically close to *A. operculata*; and *S. ellisii*, *S. ornata*, *S. fusiformis*, *S. polyzonias* and *S. mediterranea* that have been synonymized and set apart over years are indeed distinct but genetically closely-related species. Furthermore, consistent morphological differences can also be detected in probable cryptic species identified genetically, after a subsequent closer inspection of voucher specimens (e.g. in the cryptic *S. mediterranea*).

The present work based on 16S sequence data proved to resolve many taxonomic problems of the complicated serturaliid nomenclature and sets another starting point for further investigations on these taxa. More haplotype sampling coupled with the sequencing of other genetic markers, and the posterior search of diagnostic morphological characters will be important for the scrutiny of the various systematic troubles highlighted (and others) in such a highly diverse marine invertebrate family. Whilst higher genetic representation is still required for the establishment of genetic distance thresholds to differentiate hydrozoan species solely with 16S sequence data, we point (conservatively) to a maximum of about 2% divergence for 16S intraspecific sequence divergence in Sertulariidae (depending on the alignment and totality of bp analysed). Failing the recognition of a strict 16S 'barcoding gap' for Sertulariidae (or other Hydrozoa), specific genetic distance limits between species for particular taxonomic groups may be required possibly because of heterogeneity of mutational rates across hydrozoan taxa, for example as verified between Plumulariidae and Sertulariidae (Leclère *et al.* 2007; Moura *et al*. 2008). Nevertheless the dramatic increase in 16S sequences deposits in GenBank is already facilitating accurate correspondences or phylogenetic affinities of new hydrozoan barcodes to previously established species, genera or families.

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Table 6.1: 16S rRNA sequence data. Sequences of specimens marked with asterisk were determined in the present study.

Species	GenBank accession Number	Morphbank Specimen Id	Voucher specimen	Cruise/ collector	Collection method	Sample location
Thyroscyphus marginatus	FJ550495		MHNG INVE35477			Honduras
Sertularelloides cylindritheca *	JF897999		DBUA 1167.01	C. J. Moura	Hand	Berlengas, Farilhões (cave), 28-32 m 39º28.351'N 9º32.821'W W Portugal
Ser. cylindritheca *	JF897998		DBUA 1167.02	C. J. Moura	Hand	Berlengas, Farilhões, 17-22 m 39º28.494'N 9º32.640'W W Portugal
Ser. cylindritheca *	JF897997		DBUA 1167.03	C. J. Moura	Hand	Berlengas, Estelas, Baixa Alagada, 30-38 m 39º25.477'N 9º32.120'W W Portugal
Abietinaria filicula	AY787899		MHNG INVE29947			Gardur Iceland
Abietinaria abietina	AY787898		MHNG INVE29446			Gardur Iceland
Amphisbetia operculata *	JF898021	643639	DBUA 1168.01	C.J. Moura	Hand	São Miguel Island, Mosteiros, 18-23 m 37º53.372'N 25º50.132'W Azores
A. operculata *	JF898020		DBUA 1168.02	C.J. Moura	Hand	Flores Island,Ponta Delgada, 17-24 m 39º31.714'N 31º12.148'W Azores
A. operculata *	JF898018		DBUA 1168.03	C.J. Moura	Hand	Faial Island, 'Gruta da Pedra', 5-16 m 38º30.950'N 28º37.618'W Azores
A. operculata *	JF898019		DBUA 1169.01	C.J. Moura	Hand	Sagres, Ponta de Sagres, 17-22 m 36º59.700'N 8º56.715'W S Portugal
A. operculata	FJ550489		MHNG INVE34014			Simons Town South Africa
Amphisbetia minima	AY787903		MHNG INVE25071			Devonport, Cheltenham Beach New Zealand
Diphasia delagei *	JF898056	643691	DBUA 1170.01	C. J. Moura	Hand	Berlengas, Farilhões (cave), 28-32 m 39º28.351'N 9º32.821'W W Portugal
Diphasia fallax	AY787901		MHNG INVE29950			Gardur Iceland
Diphasia margareta *	JF898059		DBUA 1171.01	C.J. Moura	Hand	Berlengas, Estelas, 15-26 m 39º25.225'N 9º32.084'W W Portugal
D. margareta *	JF898057		DBUA 1171.02	C.J. Moura	Hand	Berlengas, Berlenga Island, 5-14 m 39°24.540'N 9°30.708'W W Portugal
D. margareta *	JF898060		DBUA 1171.03	C.J. Moura	Hand	Berlengas, Rinchão, 25-35m 39º25.437'N 9º29.941'W W Portugal
D. margareta *	JF898055	643677	DOP C2396	R/V Arquipélago DEECON-28-V07	Palangre de fundo	Great Meteor, 399 m 30°09.600'N 28°32.400'W S Azores
D. margareta *	JF898058	643674	DOP C2057	F/V Mestre Bobicha	Palangre de fundo	Graciosa/Terceira channel, 421m 38°53.960'N 27°33.120'W Azores

Diphasia pinastrum *	JF898061		DOP C2316	R/V Arquipélago ARQDAÇO-27-P07	Palangre de fundo	Azores Bank, 310 - 350 m 38°13.800' 28°59.400'W Azores
D. pinastrum *	JF898053		DOP C2395	R/V Arquipélago DEECON-28-V07		Great Meteor, 355 – 399 m 30°09.600'N 28°32.400'W S Azores
D. pinastrum *	JF898054	643680	DOP C2024	F/V Manuel Arriaga	Palangre de fundo	Condor de Terra Bank, 439 m 38°32.640'N 29°00.600'W Azores
Diphasia rosacea	AM888305		DBUA 1172.01	J. Hall-Spencer	Hand	Lundy Island, 5-40 m 51º09.400'N 4º39.300'W United Kingdom
D. rosacea	AM888306		DBUA 1172.02	J. Hall-Spencer	Hand	Plymouth, 5-40 m United Kingdom
D. rosacea *	JF898064	643688	DBUA 1173.01	C.J. Moura	Hand	Berlengas, Rinchão, 25-35m 39º25.437'N 9º29.941'W W Portugal
D. rosacea *	JF898063		DBUA 1173.02	C. J. Moura	Hand	Berlengas, Estelas, Baixa Alagada, 30-38 m 39º25.477'N 9º32.120'W W Portugal
D. rosacea *	JF898062		DBUA 1173.03	C.J. Moura	Hand	Berlengas, Estelas, 15-26 m 39º25.225'N 9º32.084'W W Portugal
Dynamena disticha *	JF898069	643664	DBUA 1174.01	C.J. Moura	Hand	Madeira Island, Lido, B^{xa} do Carneiro, 10-15 m 32°38.127'N 16°55.909'W Madeira
Dy. disticha *	JF898003	643670	DBUA 1174.02	C.J. Moura	Hand	Lido, Funchal naval club, 3-13 m 32º38.046'N 16º56.351'W Madeira
Dy. disticha *	JF898011		DBUA 1175.01	C.J. Moura	Hand	São Miguel Island, Feteiras, 5-13 m 37°48.145'N 25°48.116'W Azores
Dy. disticha *	JF898072		DBUA 1175.02	C.J. Moura	Hand	Faial Island, Ilhéu Negro, 5-16 m 38º31.205ºN 28º37.929ºW Azores
Dy. disticha *	JF898068		DBUA 1175.03	C.J. Moura	Hand	Flores Island, 10-16 m 39º28.531'N 31º08.479'W Azores
Dy. disticha *	JF898071		DBUA 1175.04	C.J. Moura	Hand	Flores Island,Ponta Delgada, 17-24 m 39º31.714'N 31º12.148'W Azores
Dy.disticha *	JF898065	643667	DBUA 1175.05	C.J. Moura	Hand	Faial Island, near Horta, Entre Montes, 3-18 m 38°31.370'N 28°37.394'W Azores
Dy. disticha *	JF898013		DBUA 1175.06	C.J. Moura	Hand	São Miguel Island, Caloura, 5-10 m 37º42.815'N 25º29.705'W Azores
Dy. disticha *	JF898070		DBUA 1174.03	C.J. Moura	Hand	Madeira Island, Madalena do Mar, 5-9 m 32°42.166'N 17°08.385'W Madeira
Dy. disticha *	JF898066	643646	DBUA 1176.01	C.J. Moura	Hand	Berlengas, Estelas, Baixa Alagada, 30-38 m 39º25.477'N 9º32.120'W W Portugal
Dy. Disticha	AY787909		MHNG INVE29754			Mallorca, Cala Murada Spain
Dynamena pumila	AY787902		MHNG INVE29026			Sandgerdi Iceland

Dynamena quadridentata *	JF898067	643684	DBUA 1177.01	C.J. Moura	Hand	Flores Island, 10-16 m 39º28.531’N 31º08.479’W Azores
Hydrallmania falcata	FJ550487		MHNG INVE29948			Gardur Iceland
H. falcata	AM888325		DBUA 1178.01	J. Hall-Spencer	Hand	Plymouth, 5-40 m United Kingdom
H. falcata	AM888324		DBUA 1178.02	J. Hall-Spencer	Hand	Lundy Island, 5-40 m 51º09.400’N 4º39.300’W United Kingdom
Salacia desmoides *	JF898001		DBUA 1179.01	C.J. Moura	Hand	Flores Island, 10-16 m 39º28.531’N 31º08.479’W Azores
S. desmoides *	JF898000		DBUA 1179.02	C.J. Moura	Hand	Faial Island, ‘Gruta da Pedra’, 5-16 m 38º30.950'N 28º37.618'W Azores
S. desmoides *	JF898005		DBUA 1179.03	C.J. Moura	Hand	S. Miguel Island, V. Franca do Campo, 8-18 m 37º42.332'N 25º26.755'W Azores
S. desmoides *	JF898002		DBUA 1179.04	C.J. Moura	Hand	São Miguel Island, Mosteiros, 18-23 m 37º53.372’N 25º50.132’W Azores
S. desmoides *	JF898008	643707	DBUA 1180.01	C.J. Moura	Hand	Lagos, Ponta da Piedade, 1-8 m 37°04.753'N 8°40.088'W S Portugal
S. desmoides *	JF898006		DBUA 1180.02	C.J. Moura	Hand	Aljezur, island, 2-11 m 37°10.158'N 8°54.226'W SW Portugal
S. desmoides *	JF898007		DBUA 1180.03	C.J. Moura	Hand	Albufeira, Olhos d’Água beach, 2-3 m 37°05.319'N 8°11.452'W S Portugal
S. desmoides *	JF898004		DBUA 1181.01	C.J. Moura	Hand	Berlengas, Berlenga Island, 5-14 m 39°24.540'N 9°30.708'W W Portugal
S. desmoides	FJ550464					Banyuls-sur-Mer France
Sertularella africana	FJ550490		MHNG INVE34017			Simons Town South Africa
Sertularella ellisii	FJ550478		MHNG INVE32156			Mallorca S Spain
S. ellisii *	JF898049	643725	DBUA 1182.01	C.J. Moura	Hand	Berlengas, Estelas, 15-26 m 39º25.225’N 9º32.084’W W Portugal
Sertularella cf. *fusiformis* (sp.1) *	JF898041	643727	DBUA 1183.01	C.J. Moura	Hand	Lido, Funchal naval club, 3-13 m 32º38.046’N 16º56.351’W Madeira
S. cf. *fusiformis* (sp.1) *	JF898042	643736	DBUA 1183.02	C.J. Moura	Hand	Madeira Island, Porto da Cruz, 4-12 m 32°46.516'N 16°49.585'W Madeira
S. cf. *fusiformis* (sp2) *	JF898047		DBUA 1184.01	C.J. Moura	Hand	Faial Island, ‘Gruta da Pedra’, 5-16 m 38º30.950'N 28º37.618'W Azores
S. cf. *fusiformis* (sp2) *	JF898050	643740	DBUA 1184.02	C.J. Moura	Hand	Faial Island, Horta port, 5-18 m 38°32.015'N 28°37.304'W Azores
S. cf. *fusiformis* (sp2) *	JF898030	643743	DBUA 1184.03	C.J. Moura	Hand	Faial Island, Boca das Caldeirinhas, 15-40 m 38º30.938'N 28º37.625'W Azores

S. cf. *fusiformis* (sp2) *	JF898038		DBUA 1184.04	C.J. Moura	Hand	Flores Island,Ponta Delgada, 20-27 m 39º31.092'N 31º12.437'W Azores
Sertularella gayi *	JF898029		DBUA 1185.01	C. J. Moura	Hand	Berlengas, Farilhões (cave), 28-32 m 39º28.351’N 9º32.821’W W Portugal
S. gayi *	JF898046		MMF 39075		Covo	Madeira Island, Calheta, Stn 23, 1300 m Madeira
S. gayi *	JF898033		DOP C2340	R/V Arquipélago ARQDAÇO-27-P07	Palangre de fundo	Flores Island, 109-147 m 39°30.000'N 31°07.800'W Azores
S. gayi	AM888340	643713	DBUA 1186.01	Maria S. MERIAN No. 1 Leg 3	MUC	Mercator MV, Stn 237, 352 m 35°17.914'N 6°38.687'W Gulf of Cadiz
S. gayi *	JF898032		DOP C2334	R/V Arquipélago ARQDAÇO-27-P07	Palangre de fundo	Flores Island, 109-147 m 39°30.000’N 31°07.800’W Azores
Sertularella mediterranea *	JF898037	643750	DBUA 1187.01	C.J. Moura	Hand	Aljezur, island, 2-11 m 37°10.158'N 8°54.226'W SW Portugal
S. mediterranea *	JF898039	643747	DBUA 1188.01	C.J. Moura	Hand	São Miguel Island, Mosteiros, 18-23 m 37º53.372’N 25º50.132’W Azores
S. mediterranea *	JF898040		DBUA 1188.02	C.J. Moura	Hand	Terceira Island, Hotel Caracol, 4-10 m 37º44.590'N 25º37.647ºW Azores
S. mediterranea	FJ550479		MHNG INVE32948			Banyuls-sur-Mer France
S. cf. *mediterranea* *	JF898028	643745	DBUA 1189.01	C.J. Moura	Hand	Off Viana do Castelo, 25-33 m 41º37.379’N 8º53.805’W NW Portugal
Sertularella ornata *	JF898051		DBUA 1190.01	C. J. Moura	Hand	Berlengas, Estelas, Baixa Alagada, 30-38 m 39º25.477'N 9º32.120'W W Portugal
S. ornata *	JF898052	643710	DBUA 1190.02	C.J. Moura	Hand	Berlengas, Rinchão, 25-35m 39º25.437’N 9º29.941’W W Portugal
Sertularella polyzonias *	JF898044		DBUA 1191.01	Submersible Lula	Submersible	Pico-Faial Channel, LULA dive #133, 150 m 38°30.541’N 28°36.544’W Azores
S. polyzonias *	JF898031	643701	DOP C2110	Fishing fleet of Horta		Pico-Faial Channel Azores
S. polyzonias *	JF898026	643733	DBUA 1192.01	C. J. Moura	Hand	Berlengas, Farilhões (cave), 28-32 m 39º28.351’N 9º32.821’W W Portugal
S. polyzonias *	JF898025		DBUA 1193.01	C. J. Moura	Hand	Plymouth, Mewstone, 18-25 m 50º17.600’N 4º05.600’W United Kingdom
Sertularella robusta *	JF898034		DBUA 1194.01	R/V Pelagia M2007	Box-corer	Unknown Carbonate Province, Stn 41, 461 m 35°00.113'N 6°44.815'W Gulf of Cadiz
S. robusta	AM888339		DBUA 1195.01	R/V Pelagia M2005	Box-corer	Pen Duick Escarpment, Stn 16, 660 m 35º18.310’N 6º48.205’W Gulf of Cadiz

S. robusta *	JF898035	643716	DBUA 1194.02	R/V Pelagia M2007	Box-corer	Pen Duick Escarpment, Stn 17, 416 m 35°17.061'N 6°42.617'W Gulf of Cadiz
S. robusta *	JF898036		DBUA 1196.01	R/V Pelagia 64PE284	ROV	Meknés Carbonate Prov., Stn 12738-1 , 738 m 34°59.980'N 7°04.510'W Gulf of Cadiz
S. robusta *	JF898043		DBUA 1196.02	R/V Pelagia 64PE284	ROV	Meknés Carbonate Prov., Stn 12739-1, 736 m 35°00.010'N 7°04.470'W Gulf of Cadiz
S. rugosa	AY787906		MHNG INVE29032			Sandgerdi & Gardur Iceland
Sertularella sanmatiasensis	FN424141					Low Island Antarctica
Sertularella sp*	JF898027	643732	DBUA 1197.01	C. J. Moura	Hand	São Miguel Island, Doris wreck, 16-21 m 37º44.591'N 25º37.647'W Azores
Sertularella sp*	JF898048	643729	DBUA 1197.02	C.J. Moura	Hand	Flores Island, 10-16 m 39º28.531’N 31º08.479’W Azores
Sertularella cf. *unituba* *	JF898045	643704	DOP C1500	F/V Manuel Arriaga	Espinhel	Azores Bank, 468 m 38°18.000'N 28°53.340'W Azores
Sertularia argentea	FJ550461					Roscoff NW France
Sertularia cupressina	FJ550475		MHNG INVE29949			Gardur Iceland
Se. cupressina	AM888341		DBUA 1198.01	J. Hall-Spencer	Hand	Lundy Island, 5-40 m 51º09.400’N 4º39.300’W United Kingdom
Sertularia distans *	JF898012	643697	DBUA 1199.01	C.J. Moura	Hand	Off Viana do Castelo, 25-33 m 41º37.379’N 8º53.805’W NW Portugal
Se. distans *	JF898009	643694	MMF 25124	R/V Johnson Sea Link	Submersible	Desertas, 30 – 91 m 32º23.00’N 16º27.23’W Madeira
Se. distans *	JF898014		DBUA 1200.01	C.J. Moura	Hand	Berlengas, Berlenga Island, 5-14 m 39°24.540'N 9°30.708'W W Portugal
Sertularia marginata *	JF898010		DBUA 1201.01	C.J. Moura	Hand	São Miguel Island, Mosteiros, 18-23 m 37º53.372’N 25º50.132’W Azores
Se. marginata *	JF898016		DBUA 1202.01	C.J. Moura	Hand	Madeira Island, Madalena do Mar, 5-9 m 32°42.166'N 17°08.385'W Madeira
Se. marginata *	JF898017		DBUA 1202.02	C.J. Moura	Hand	Madeira Island, Porto da Cruz, 4-12 m 32°46.516'N 16°49.585'W Madeira
Sertularia moluccana	FJ550494					Bunaken Island Indonesia
Sertularia perpusilla	AY787894		MHNG INVE29765			Mallorca, Cala Murada S Spain
Se. perpusilla *	JF898015		DBUA 1203.01	C.J. Moura	Hand	Cirkewwa, 16-26 m 35°59.348'N 14°19.680'E Malta
Sertularia unguiculata	AY787904		MHNG INVE29969			Leigh New Zealand
Symplectoscyphus tricuspidatus	AY787907		MNHN INVE29953			Iceland, Sandgerdi
Symplectoscyphus turgidus	FJ550462		MHNG INVE29467			California USA

Thuiaria articulata *	JF898024	643719	DBUA 1204.01	TTR13	Dredge	Stn 498, 2105 m 63°18.659'N 55°27.031'W Greenland
T. articulata *	JF898023	643721	DBUA 1205.01	C.J. Moura	Hand	Off Viana do Castelo, 25-33 m 41º37.379'N 8º53.805'W NW Portugal
T. articulata *	JF898022		DBUA 1205.02	C.J. Moura	Hand	Viana do Castelo, 29-33 m 41º41.680'N 8º52.711'W NW Portugal
Thuiaria thuja	AY787908		MHNG INVE29951			Sandgerdi Iceland

Abbreviations: DBUA, 'Departamento de Biologia da Universidade de Aveiro'; DOP, 'Departamento de Oceanografia e Pescas'; F/V, Fishing Vessel; M, oceanographic campaign 'Moundforce'; MV, mud volcano; MMF, 'Museu Municipal do Funchal'; MHNG, Muséum d'Histoire Naturelle de Genève, Switzerland; PE, Pelagia; ROV, Remote Operated Vehicle; R/V, Research Vessel; Stn, station; TTR, oceanographic campaign 'Training Trough Research'.

Table 6.2: Morphological measurements (in µm) of *Sertularella* species.

Species	*S.* cf. *fusiformis*	*S.* cf. *fusiformis*	*S.* cf. *fusiformis*	*S.* cf. *fusiformis*	*S.* cf. *fusiformis*	*S. ellisii*	*'Sertularella* sp.'	*'Sertularella* sp.'	*S.* cf. *mediterranea*	*S. mediterranea*	*S. mediterranea*
Locality	Lido	P^{to} Cruz	Horta port	Boca Cald.	Flores	Estelas	São Miguel	Flores	off V. Castelo	Aljezur	São Miguel
	Madeira	Madeira	Azores	Azores	Azores	W Portugal	Azores	Azores	NW Portugal	SW Portugal	Azores
Internodes, length	400-570	330-600	500-700	650-760	430-460	450-690	330-700	280-380	340-470	300-600	530-630
diameter at node	120-130	110-170	160-170	210-230	120-130	180-200	270-290	100-130	200-250	140-180	210-220
Hydrotheca											
length abcauline wall	400-500	430-500	500-550	560-570	440-580	550-610	490-550	330-410	600-700	580-610	560-610
length free part adcauline wall	290-350	270-360	320-370	320-380	310-430	320-400	230-350	200-250	400-550	370-400	300-440
length adnate part adcauline wall	230-250	230-270	240-300	300-330	190-250	290-310	310-350	210-265	300-340	280-330	310-350
diameter at margin	150-180	150-160	190-220	210-230	170-200	250-260	200-270	120-170	220-250	210-280	180-230
maximum diameter	220-260	210-250	250-290	280-290	240-280	280-320	250-280	190-210	310-340	270-280	300-330
Gonotheca											
length		1280-1500									
maximum diameter		570-700									
diameter at aperture		320									
number of annulations		8-11									
cusps		3-4									

Chapter 7

POLYPHYLY AND CRYPTIC DIVERSITY IN THE HYDROZOAN FAMILIES LAFOEIDAE AND HEBELLIDAE (CNIDARIA: HYDROZOA)

Moura, C. J., Cunha, M. R., Porteiro, F. M. & Rogers, A. D. (2011d). [in review]

Contents

ABSTRACT

The taxonomy of the putative superfamily Lafoeoidea (Hydrozoa) which includes the families Hebellidae and Lafoeidae, has been widely disputed at all systematic levels, mainly because these are morphologically simple and plastic animals. We used the molecular marker 16S mRNA to address phylogenetic relations of 'Lafoeoidea' hydroids mainly from shallow and deep waters of the north-east Atlantic and west Mediterranean. This study proves that the morphological simplicity of the 'Lafoeoidea' has led to several erroneous taxonomic assignments. We demonstrate that the superfamily 'Lafoeoidea' is not monophyletic and thus not valid; the families Hebellidae and Lafoeidae are polyphyletic. The 'Lafoeidae' subfamilies 'Lafoeinae' and 'Zygophylacinae' were recovered monophyletic but quite distantly related and should be erected to the level of distinct families. The 16S haplotype data analyzed in conjunction with morphological

characters and distribution data was useful in differentiating morphologically undistinguishable, nominal and cryptic or pseudo-cryptic species, including undescribed taxa. Particularly within the 'Lafoeinae' group, unexpectedly high genetic biodiversity (including cryptic species) was encountered in the possibly not monophyletic genera *Lafoea*, *Acryptolaria* and *Fillelum.* Cryptic diversity is also likely associated with the 'Zygophylacinae' nominal species *Cryptolaria pectinata.* The indications of genetic segregation by geographical distance of the 'Lafoeoidea' hydroids, particularly verified in deep waters, is probably a consequence of their reduced potential for large-scale dispersal which likely interacts with the influence of the seabed topography, oceanographic circulation and adaptability to tolerate different abiotic conditions.

7.1 INTRODUCTION

According to the phylogenetic hypotheses formulated by Cartwright *et al.* (2008) and Leclère *et al.* (2009), the hydrozoan family Lafoeidae A. Agassiz, 1865 may represent the most primitive forms of Leptothecate hydroids. The gross morphology of Lafoeidae species ranges from minute stolonal, to bushy erect colonies, always with very simply structured hydrothecae, usually with basic tubular to campanulate forms, sometimes supported by a pedicel (cf. diagnoses in Bouillon *et al.* 2006). 'Lafoeids' are especially abundant and therefore ecologically important components of the deep-sea benthic faunas, but sometimes also thrive at coastal depths (e.g. Fig. 7.1).

Fig. 7.1: Hydrozoan colonies of *Lafoea dumosa* (Lafoeidae) overgrowing rocky bottoms in coastal waters of central Portugal (Berlenga Island, *ca.* 10 meters). Photo credits: C. J. Moura.

The systematics of Lafoeidae has been largely disputed at various taxonomic ranks. The family Hebellidae Fraser, 1912 has been included in the superfamily Lafoeoidea A. Agassiz, 1865 alongside with Lafoeidae, or within the Lafoeidae (see Marques *et al.* 2006). However Cartwright *et al.* (2008) and Leclère *et al.* (2009) found 'hebelliids' phylogenetically closer to 'Statocysta' rather than to the 'lafoeiid' *Lafoea dumosa* (Fleming, 1820). This suggests that Hebellidae does not even belong to the Lafoeoidea, which confirmed the hypotheses of Schuchert (2001) that there is no synapomorphy uniting the Lafoeidae and Hebellidae and that the similarity of their hydrothecae likely corresponds to a plesiomorphy. Within the Lafoeidae, Moura *et al.* (2008) found that its sub-families Lafoeinae A. Agassiz, 1865 and Zygophylacinae Quelch, 1885 seem unrelated, and therefore suggested that these groups should be re-elevated to the level of family (as previously suggested by Stepanjants 1979). The systematics of Lafoeidae *sensu lato* (*s. l.*) at the levels of genera and species within these sub-families (or independent families) is far from well-resolved, mostly because of their simple and highly plastic structure, despite some recent efforts to resolve morphological characters (e.g. Marques *et al.* 2004, 2005a, b, 2006; Peña Cantero *et al.* 1998, 2007).

Cryptic diversity within Lafoeidae *s. l.* was previously detected by using molecular approaches solely in a single nominal species – *Lafoea dumosa* (Moura *et al.* 2008). The simple structure of these animals means that more cryptic diversity is likely to be discovered within the group. The medusoid phase is apparently suppressed in the life cycle of 'lafoeiids' (excluding some of the Hebellidae) (e.g. Bouillon *et al*. 2006). Instead colonies release planulae that are carried in the water column, before they settle on a substratum and metamorphose into a stolonal or erect sessile colony (e.g. Hughes 1977; Sommer 1992). Because the dispersive pelagic stage is potentially short (e.g. Hughes 1977; Sommer 1992), gene-flow may be highly constrained by population size, the local current regime and availability and type of substrata (including substrata that may raft). In the northeast Atlantic and west Mediterranean, 'lafoeiids' are often locally abundant and have the ability to overgrow a multitude of surfaces including artificial substrata and other hydrozoan colonies (C.J. Moura, pers. observ.). They may be pioneer faunal colonizers, and can also raft on anthropogenic or natural materials (Moura, pers. observ.). Many species of Lafoeidae *s. l.* (e.g. *Acryptolaria conferta* (Allman, 1877), *Lafoea dumosa* and

Filellum serratum (Clarke, 1879)) are assumed to have widespread vertical and horizontal distributions, but these assumptions have never been tested by means of molecular methods.

Nucleotidic sequence data of the mitochondrial 16S rDNA gene have been notably useful, coherent and popularly used to extrapolate phylogenetic relations of hydrozoans from the taxonomic levels of families to populations (cf. review of Moura *et al*. 2011a). For these reasons and because the 16S is easily amplified through PCR, it has also been more used for 'DNA barcoding' purposes of hydrozoans in detriment of the COI (cf. review of Moura *et al*. 2011a).

In the present paper, we 'barcoded' with the 16S marker (*sensu* Moura *et al*. 2011a) a relatively high representation and diversity of taxa within the Hebellidae and Lafoeidae from the North-east Atlantic and West Mediterranean. We aimed to resolve the phylogenetic relationships between taxa and resolve significant taxonomic problems within these groups, taking into account the phylogenetic hypotheses of Leclère *et al*. (2009) obtained with more conserved genetic markers but with considerably less Lafoeoidea *s. l.* sampled. For this reason, specimens of Campanulinidae were also included, in order to test the phylogenetic placement of the 'Lafoeinae' clade (see Leclère *et al.* 2009). We also intended to investigate cryptic diversity and phylogeographic associations in genetically well-represented taxa.

7.2 MATERIALS AND METHODS

Collection and identification of samples

Specimens of Lafoeidae, Hebellidae and Campanulinidae were collected from 41 sampling sites of various shallow and deep-water localities of the northern Atlantic and western Mediterranean (Fig. 7.2, Appendix A). Specimens were fixed and preserved mostly in 96% ethanol, although sometimes in 70% ethanol. The material collected in Azores and Madeira included specimens obtained as part of a study on by-catch from deep-sea fisheries and some were frozen prior to fixation in ethanol. Taxa were sorted and classified taxonomically in the laboratory by C. J. Moura mainly based on publications of

Cornelius (1975), Millard (1975), Ramil and Vervoort (1992), Medel (1996), Boero *et al.* (1997), Peña Cantero and García Carrascosa (2002), Bouillon *et al.* (2006), and Peña Cantero *et al.* (2007). Morphometric measurements were taken using a graticule on a compound stereo microscope in order to assess the diagnostic value of several characters in representatives of nominal species or genus within genetically distinct clades.

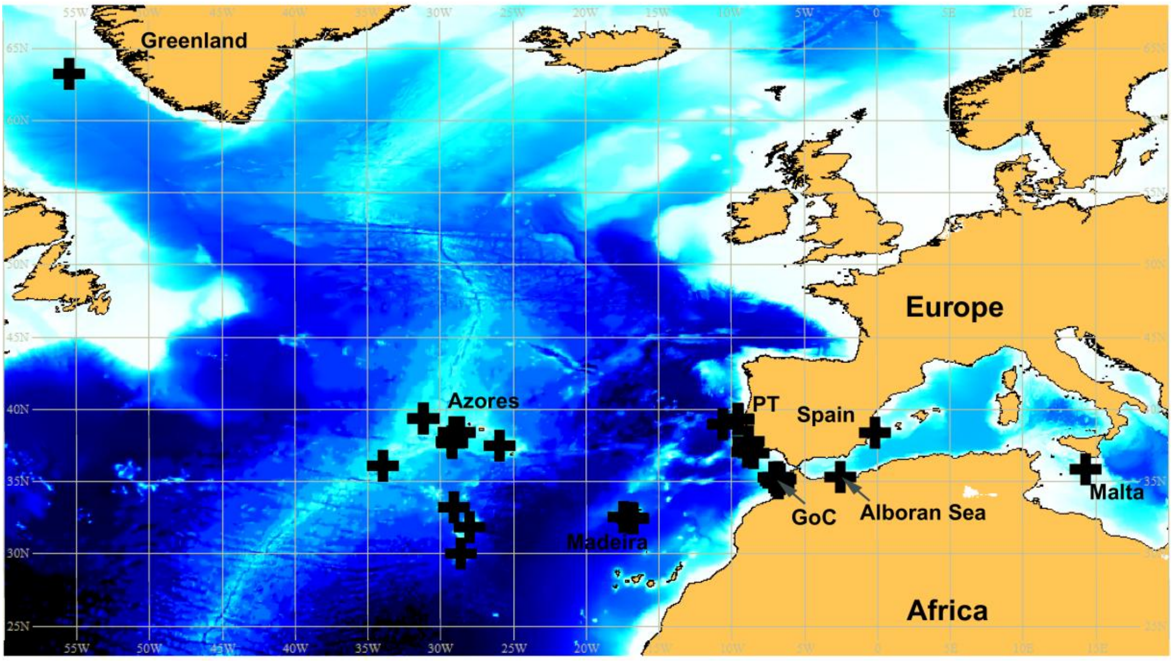

Fig. 7.2: Map representing the sampling sites (marked with crosses) from where hydrozoan DNA sequences were determined for the present study. Abbreviations: GoC, Gulf of Cadiz; PT, Portugal.

DNA extraction, amplification and sequencing

Protocols for DNA extraction, amplification and sequencing of the mitochondrial large ribosomal subunit (16S) rDNA gene followed the methodology described in Moura *et al.* (2011a). The treatment and deposition of voucher specimens in museums is analogous also to that of Moura *et al.* (2011a). Sampling details, GenBank, Morphbank and Museums accession numbers are presented in Table 7.5. The sequence datasets used for the phylogenetic inferences herein presented were deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S12036).

Phylogenetic analyses

The 16S sequences of the putative superfamily Lafoeoidea (including Hebellidae and Lafoeidae *s. l.*) and family Campanulinidae assembled for this study were clustered with phylogenetically close sequences previously published on GenBank. The choice of sequences for inclusion in the analyses, including the use of *Bougainvillia* sequences as outgroup (see all metadata of sequences in Table 7.5), was especially influenced by the phylogenetic hypothesis presented by Marques *et al.* (2006) and Leclère *et al.* (2009). Based on the phylogenetic hypotheses of Leclère *et al.* (2009) that placed *Lafoea dumosa* (Lafoeidae, subfamily Lafoeinae) at a basal phylogenetic position to other Leptothecata hydroids, we included for the analyses presented in Fig. 7.3 a sequence of 'athecate' hydrozoan (*Stylaster cf. multiplex*) as the outgroup. A 'blast' search in Genbank for a 16S sequence of *Lafoea dumosa* retrieved the sequence of *Stylaster cf. multiplex* as the more identical, further supporting its choice for the outgroup of the phylogenetic reconstructions of Fig. 7.3. The outgroups selected (not shown) for the phylogenetic analyses of the subsets of sequences of Zygophylacinae (Fig. 7.4) and Lafoeinae (Fig. 7.5) were *Phialella quadrata* and *Tiaropsis multicerrata,* and *Melicertum octocostatum,* respectively for each subfamily, taking into account the phylogenetic structure presented in Fig. 7.3. The datasets were aligned in *MEGA* version 5 (Tamura *et al.* 2011) with MUSCLE using the default alignment settings, and trimmed to the shortest sequence. *Gblocks* version 0.91b (Castresana 2000) was used for the phylogenetic reconstructions of Fig. 7.3, with the default settings, to remove ambiguous aligned regions and to select conserved blocks of the alignment. MrModeltest version 2.3 (Nylander 2004) was used to determine the optimal probabilistic model of sequence evolution. The 'general time reversible plus gamma' was the model selected (in all datasets of sequences) by the Akaike Information Criterion. 'Maximum-likelihood' phylogenetic analyses were executed through the PHYML website (http://www.atgc-montpellier.fr/phyml) (Guindon & Gascuel 2003) using the GTR model. Bayesian analyses were performed with MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001), using again the same model. The searches were run for 10^7 generations, with trees sampled every 1000 generations (the first 1 million generations were discarded as 'burnin'). Clade stability was assessed using non-parametric bootstrapping of ML analyses (1000 replicates) and Bayesian posterior probability values. MEGA version 5 (Tamura *et al.* 2011) was used to calculate pairwise uncorrected sequence differences (*P* distances) in the alignments of the datasets used for the analyses of Figs 7.4 and 7.5, for simple comparisons of percentage sequence divergence between taxa.

7.3 RESULTS AND DISCUSSION

Fifty-seven new 16S sequences of Lafoeidae *s. l.*, Hebellidae, and hydroids with operculated hydrothecae (42, eight and seven sequences, respectively for each group) were determined for the present study. These sequences were analysed phylogenetically in conjunction with twenty-six sequences previously published on GenBank of phylogenetically close taxa – nine by Leclère *et al.* (2009), eight by Moura *et al.* (2008), five by Peña Cantero *et al.* (2010), three by C. W. Cunningham (direct EMBL submission), and one by Cartwright *et al.* (2008) (see Table 7.5). The topologies of the phylogenetic trees obtained through Bayesian and ML analyses, and with different combinations of sequences and outgroups (not all results shown), were consistently identical, especially when referring to the clades with higher nodal support of Figs 7.3, 7.4 and 7.5. Our phylogenetic reconstructions (Fig. 7.3) were also coherent with those of Leclère *et al*. (2009), enabling inferences at supra-specific taxonomic levels.

Phylogenetic analysis between the major clades of the 'Lafoeoidea'

The monophyly of Lafoeidae *s. l.* was not supported by the 16S haplotype data (cf. Moura *et al.* 2008; Fig. 7.3). Two major clades were recovered within the nominal family Lafoeidae, corresponding respectively to the monophyletic subfamilies Zygophylacinae and Lafoeinae (Fig. 7.3). These clades are quite distantly related and highly divergent (Fig. 7.3), supporting their elevation to the family rank (Stepanjants 1979; Moura *et al.* 2008). Furthermore the 'Lafoeinae' clade appears phylogenetically more closely related to *Stegella lobata* (Vanhöffen, 1910) and *Melicertum octocostatum* (Sars, 1835) (families Campanulinidae Hincks, 1868 and Melicertidae Agassiz, 1862, respectively), and the 'Zygophylacinae' group may be more closely related to clades of Hebellidae *s. l.*, Phialellidae Russell, 1953, Tiaropsidae Boero, Bouillon & Danovaro, 1987 and two other distinct clades of Campanulinidae *s. l.* (see Fig. 7.3 and compare with phylogenetic hypothesis of Leclère *et al.* 2009).

We have also tested the relation of Hebellidae with Lafoeidae *s. l.*, and the relation of these families with hydroids of the ''*Cuspidella*'' type (with operculated hydrotheca), based on results of Cartwright *et al.* (2008) and Leclère *et al.* (2009). *Billardia subrufa*

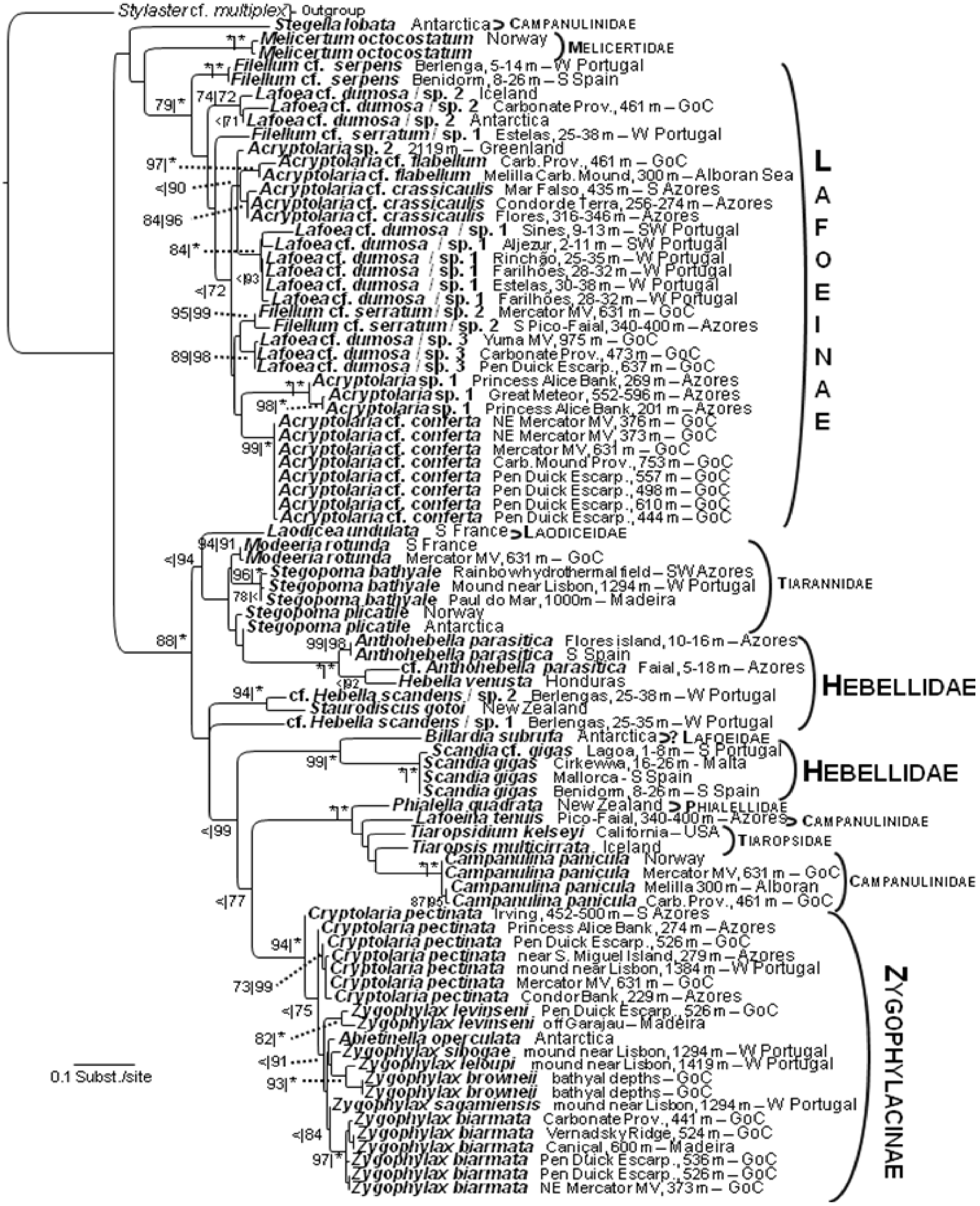

Fig. 7.3: ‘Maximum-likelihood’ phylogenetic hypothesis derived from mitochondrial 16S rRNA gene sequences of the hydrozoan nominal families: Lafoeidae *s. l.*, Hebellidae s. l., Campanulinidae *s. l.*, Melicertidae, Laodiceidae, Tiarannidae, Phialellidae, and Tiaropsidae. Numbers near the nodes indicate values of bootstrap (left) and posterior probabilities (right) in percentage. If these values were less than 70%, they were replaced by the sign ‘<‘. Values of bootstrap and posterior probabilities are omitted when both were less than 70% for the same node. The symbol ‘*’ indicates bootstrap or posterior probability values equal to 100%. The branch length indicator represents 0.1 substitutions per site.

Abbreviations: Carb., carbonate; Escarp., escarpment; GoC, Gulf of Cadiz; MV, mud volcano; Prov., province.

(Jäderholm, 1904) (with uncertain systematic position in Lafoeidae *s. l.*) and *Scandia gigas* (Hebellidae *s. l.*) cluster in a well-supported clade, which in turn group with the 'Zygophylacinae' and another evolutionary lineage containing members of the Campanulinidae (*Lafoeina tenuis* G.O. Sars, 1874 and *Campanulina panicula* G.O. Sars, 1874), Tiaropsidae (*Tiaropsidium kelseyi* Torrey, 1909 and *Tiaropsis multicirrata* (Sars, 1835)) and Phialellidae (*Phialella quadrata* (Forbes, 1848)). Assuming these phylogenetic results (Fig. 7.3) are accurate: the 'Zygophylacinae' may outgroup closely (or even ingroup) the clade of 'Statocysta' as pointed out by Leclère *et al.* (2009) despite the unshared synapomorphy of medusae production by the 'Zygophylacinae'. Nevertheless the results indicate that the 'Zygophylacinae' and 'Lafoeinae' do not even belong to the same superfamily (Fig. 3), revealing that their common trait of presenting aggregated gonothecae (in coppinia or scapus) (e.g. see Bouillon *et al*. 2006) represents in fact a homoplasy or symplesiomorphy. Furthermore, it is demonstrated (Fig. 7.3) that Hebellidae is likely a polyphyletic group with numerous highly divergent and distantly related clades, and that the genera of Hebellidae: *Hebella* Allman, 1888, *Anthohebella* Boero, Bouillon & Kubota, 1997 and *Staurodiscus* Haeckel 1879, seem more closely related to the families Tiarannidae Russell, 1940 [genera *Modeeria* Forbes, 1848 and *Stegopoma* Levinsen, 1893) and Laodiceidae Agassiz, 1862 (*Laodicea undulata* (Forbes & Goodsir, 1851)], rather than to *Scandia gigas* (Pieper, 1884) or *Billardia subrufa* (Jäderholm, 1904). The latter two taxa are also currently classified into Hebellidae but are more related to a clade containing 'Zygophylacinae' amongst other phenotypically-simple hydroid families (see above) (Fig. 7.3). In fact, Andrade and Migotto (1997) and Migotto and Andrade (2000) had already demonstrated the morphological resemblance of medusae of *Hebella scandens* (Bale, 1888) and *Hebella furax* Millard, 1957 with the Laodiceidae, but the verified polyphyletic status of Hebellidae (Fig. 7.3) had not been questioned.

Some of the above mentioned results were also partially verified by Leclère *et al.* (2009) and Peña Cantero *et al.* (2010), but with much less taxonomic coverage. The present study therefore provides further support to treat Lafoeinae, Zygophylacinae and Hebeliidae *s. l.* as independent and quite distantly families.

Phylogenetic analyses within the 'Zygophylacinae' clade

The 'Zygophylacinae' clade is represented as a distinctive monophyletic group that includes, with high support, the genera *Zygophylax* Quelch, 1885, *Abietinella* Levinsen, 1913 and *Cryptolaria* Busk, 1857 (Fig. 7.3). However, the phylogenetic relationships between these three nominal groups are not clear (Figs 7.3 and 7.4). Reciprocal clusters of nominal species of 'Zygophylacinae' are easily discriminated in the trees (Figs 7.3 and 7.4) and are also concordant with the morphological diagnostic characters of species.

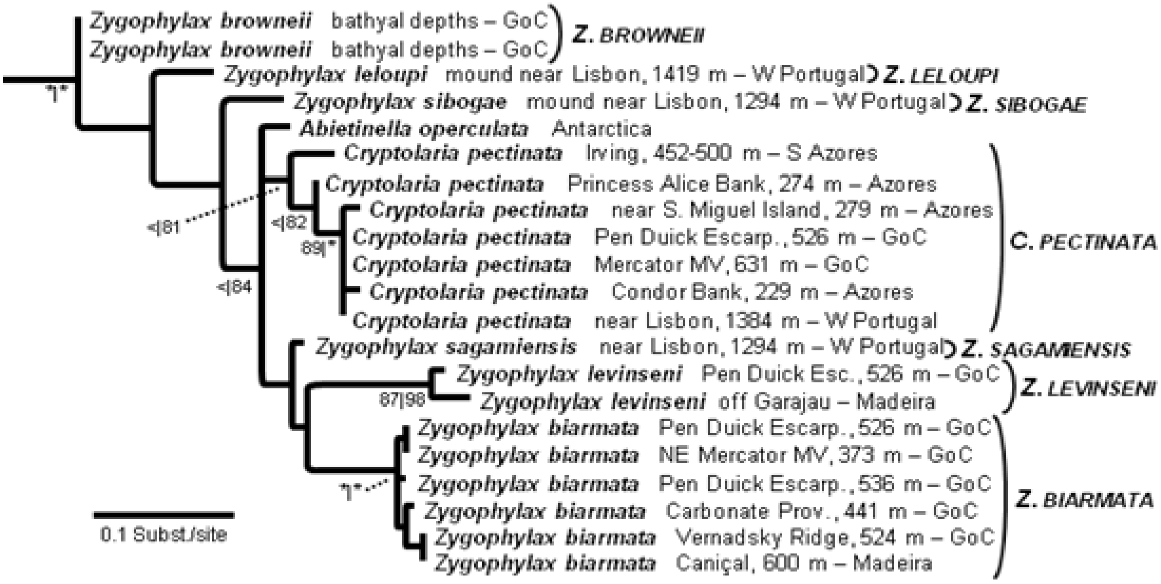

Fig. 7.4: 'Maximum-likelihood' phylogenetic analysis of the dataset containing 16S rRNA gene sequences of the 'Zygophylacinae' clade. The outgroup (now shown) included sequences of *Tiaropsis multicirrata* and *Phialella quadrata*. Numbers near the nodes indicate values of bootstrap (left) and posterior probabilities (right) in percentage. If these values were less than 70%, they were replaced by the sign '<'. Values of bootstrap and posterior probabilities are omitted when both were less than 70% for the same node. The symbol '*' indicates bootstrap or posterior probability values equal to 100%. The branch length indicator represents 0.1 substitutions per site. Abbreviations: Carb., carbonate; CM, carbonate mound; Escarp., escarpment; GoC, Gulf of Cadiz; MV, mud volcano; Prov., province.

Cryptolaria pectinata (Allman, 1888)

Within the 'Zygophylacinae' clade intraspecific sequence divergence was found to be 'abnormally' higher in the nominal species *Cryptolaria pectinata* (Allman, 1888) (up to 2.2%), and therefore a reexamination of vouchers specimens was needed in order to certify the taxonomic identifications. We maintained the taxonomical decisions with respect to the assignment of the specimens to that nominal species, and no consistent morphological differences (Table 7.1) were found between the lineages discovered (Figs 7.3 and 7.4).

Therefore we highlight here the possible existence of cryptic diversity within *C. pectinata* based solely on molecular phylogenetic analyses (Figs 7.3 and 7.4), requiring further investigations on this nominal taxon.

Table 7.1: Comparative morphometric data (in μm) of the haplotypes of *Cryptolaria pectinata*.

Station	**Hydrothecae**				**Nematothecae**		**Nematocysts (large group)**	
	Adnate adcauline wall	**Free adcauline wall ***	**Free adcauline wall ****	**Diameter at rim**	**Length**	**Diameter**	**Size ranges**	**Mean Sizes ± SD (n=10)**
Irving, 452-500 m – S Azores	230-340	150-250	190-300	80-100	60-120	15-20	12-14 x 4.5-5	12.75 ± 2.33 x 4.95 ± 0.16
Princess Alice Bank, 274 m – Azores	310-350	125-200	125-200	100-120	50-80	10-20	12-14 x 4.5-6	12.9 ± 2.42 x 5.45 ± 0.55
near S. Miguel Island, 279 m – Azores	230-370	100-160	190-260	90-110	40-100	18-20	12-14 x 4.2-5.5	12.95 ± 2.40 x 4.97 ± 0.46
Pen Duick Escarp., 526 m – GoC	260-300	150-210	190-210	100-130	80-110	20-30		
Mercator MV, 631 m – GoC	220-300	120-205	140-205	90-105	60-80	20-25	12.5-13.2 x 4.5-5.5	12.87 ± 2.28 x 5.0 ± 0.33
near Lisbon, 1384 m – W Portugal	200-280	75-160	130-240	60-80	40-110	15-20		
Condor Bank, 229 m – Azores	350-360	120-210	190-250	100-120	45-70	20-27	12-14 x 5-6.5	13.1 ± 2.21 x 5.6 ± 0.39

* = **no renovations**
**** = with renovations**

Genera Zygophylax and Abietinella

Our analyses indicated that the genus *Zygophylax* may be paraphyletic (Fig. 7.3) as some *Zygophylax* congeners show more divergence between them (e.g. *Zygophylax biarmata* Billard, 1905, or *Zygophylax levinseni* (Saemundsson, 1911)) than *Abietinella operculata* (Jäderholm, 1903), from *Zygophylax* taxa (Figs 7.3 and 7.4). Indeed *A. operculata* was originally described as *Zygophylax* (Jäderholm, 1903) but was thereafter disputed taxonomically between taxa of Sertulariidae and Lafoeidae (see Peña & García Carrascosa 1993). This study suggests its re-inclusion in the genus *Zygophylax* (Fig. 7.3), but using only 16s rDNA sequences there is insufficient nodal support and congruency between the tree-topologies obtained (Figs 7.3 and 7.4) to support the monophyletic status of *Zygophylax* and consequently we refrain to reclassify this species at the present time. The only well-supported phylogenetic relation between nominal species of 'Zygophylacinae' in our analyses (only in Fig. 7.3) is that between *Zygophylax leloupi*

Ramil & Vervoort, 1992 and *Zygophylax brownei* Billard, 1924, which are morphologically well distinctive (e.g. Ramil & Vervoort 1992).

Phylogeographic inferences

Some phylogeographic affinities have been detected within the species *Z. biarmata* and *C. pectinata* (Figs 7.3 and 7.4). A relatively good representation of haplotypes was obtained for these taxa because they are commonly found in bathyal environments of the NE Atlantic (C.J. Moura, pers. observ.).

For *Z. biarmata*, four distinct but genetically-close haplotypes (up to 0.7% of *P* distance) co-occurred at different localities of the Moroccan margin of the Gulf of Cadiz, one of which was also encountered in deep waters of Madeira (Figs 7.3 and 7.5). The multitude of different habitats found in the Gulf of Cadiz (e.g. mud volcanoes, areas of carbonate crusts and/or cold-water coral stands), coupled with the influence of different water-masses and currents in the area (e.g. Perez *et al.* 1998; Hernández-Molina *et al.* 2006), may be correlated to the diversity of *Z. biarmata* haplotypes discovered in this region (Fig. 7.3), but this supposition requires further testing.

The marked phylogeographic structure obtained for *Cryptolaria pectinata* (Figs 7.3 and 7.5) suggests that this nominal species may not be so widely distribution as supposed (e.g. Gili *et al*. 1989) and in fact may correspond to a complex of cryptic species with morphological similarities. Two haplotypes of *C. pectinata* were detected in the bathyal habitats of the Gulf of Cadiz, one of these occurring in the Mercator mud volcano (354 meters) is also present in central-western Portugal at a much greater depth (1384 meters). In Azorean waters four distinct haplotypes (0. 8 – 1.9 % of sequence divergence) from three different seamounts were encountered, and a less-related haplotype from the remote Irving seamount (452 - 500 m), located south of the Azores, diverges considerably from all the other 16S sequences of *C. pectinata* represented in the present study (1.3 –2.2 % of sequence divergence). This genetic diversity discovered in a few samples from geographically proximate locations in the deep sea around the Azores (as similarly verified for *Acryptolaria* sp1, and *A.* cf. *craussicaulis* – see bellow) possibly reveals the impact of retention mechanisms generated by water currents at seamounts or effective barriers of deep-water habitat in the segregation of populations with reduced dispersive potential (see Moura *et al.* 2011c).

Phylogenetic analyses within the 'Lafoeinae' clade

'Lafoeinae' includes the nominal genera *Lafoea* Lamouroux, 1821, *Acryptolaria* Norman, 1875 and *Filellum* Hincks, 1868 (Fig. 7.3). The 'Lafoeinae' clade herein represented includes at least 10 putative species (Figs 7.3 and 7.5), most of these difficult to identify solely based on morphological characters. The taxonomic history of the 'Lafoeinae' is complicated. Species-identification depends highly on the presence of reproductive structures, but these are often absent or uninformative. Therefore, 16S haplotype data was crucial to complement phenotypic classifications of 'Lafoeinae' species. As in the case of 'Zygophylacinae', the phylogenetic relations between most of the identified species of 'Lafoeinae' are not completely resolved with 16S haplotype data, but species can be fairly easily discriminated when analyzing a phylogram (Figs 7.3 and 7.5).

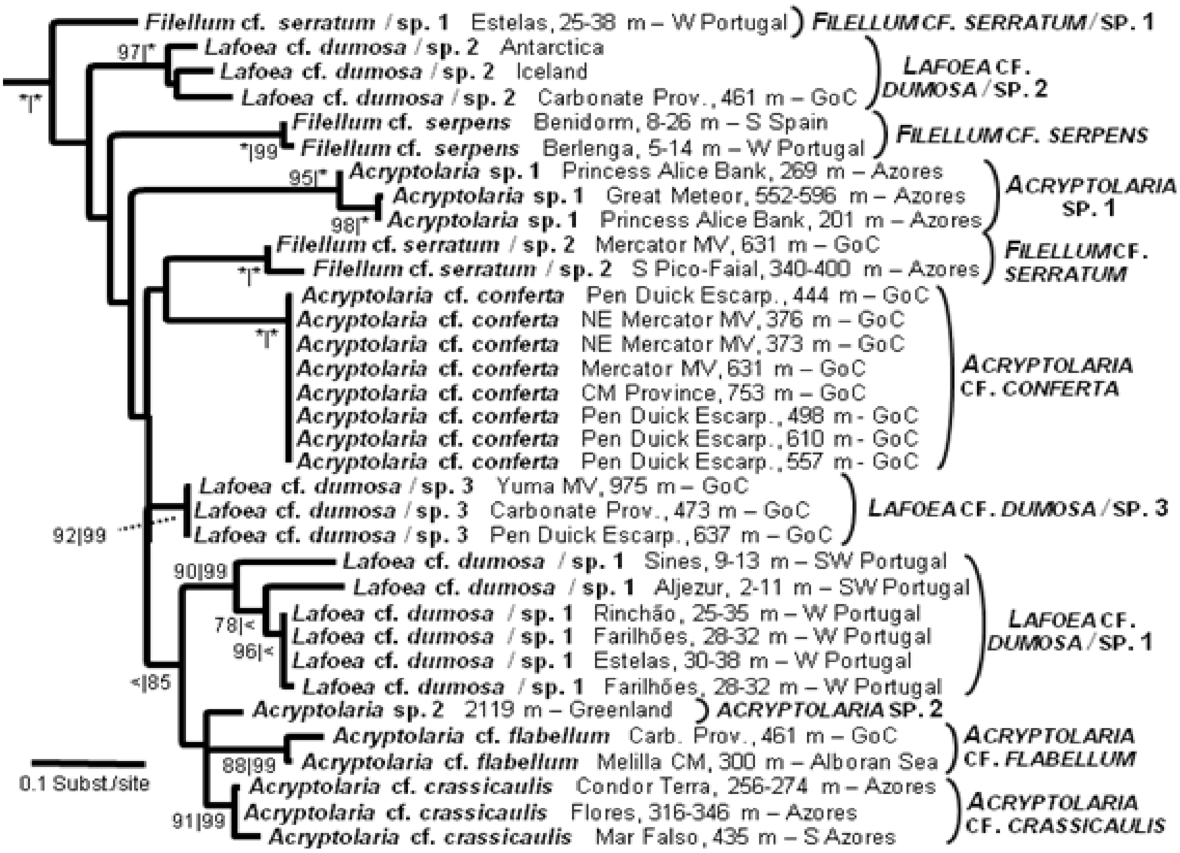

Fig. 7.5: 'Maximum-likelihood' phylogenetic analysis of the dataset containing 16S rRNA gene sequences of the 'Lafoeinae' clade. The outgroup (now shown) included sequences of *Melicertum octocostatum*. Numbers near the nodes indicate values of bootstrap (left) and posterior probabilities (right) in percentage. If these values were less than 70%, they were replaced by the sign '<'. Values of bootstrap and posterior probabilities are omitted when both were less than 70% for the same node. The symbol '*' indicates bootstrap or posterior probability values equal to 100%. The branch length indicator represents 0.1 substitutions per site. Abbreviations: Carb., carbonate; Escarp., escarpment; GoC, Gulf of Cadiz; MV, mud volcano; Prov., province.

Regarding the few well-supported phylogenetic relations between the putative species of ‘Lafoeinae’ outlined (Figs 7.3 and 7.5) we note that one clade of *Lafoea* (‘*Lafoea* cf. *dumosa* / sp1*’*) consistently grouped with some lineages of *Acryptolaria* in our analyses (e.g. Figs 7.3 and 7.5). This may suggest that the nominal genera *Lafoea* and *Acryptolaria* are polyphyletic. Furthermore, and despite the absence of satisfactory nodal support, the genus *Filellum* does not seem to be a monophyletic group either, taking into account the genetic divergence (up to 8.2%) and the segregated phylogenetic placement between the three main lineages of *Filellum* (Figs 7.3 and 7.5).

Genus Acryptolaria

Five putative species of *Acryptolaria* were distinguished by morphological characters (e.g. colonies-growth mode, general appearance of hydrothecae, presence/absence of ‘diaphragm’, morphometric characters - Table 7.2) and 16S genetic data (Figs 7.3 and 7.5), but their correspondence to described species was questionable in all cases. Three of these species were matched to nominal described taxa (namely *Acryptolaria flabellum* (Allman, 1888)*,* *Acryptolaria crassicaulis* (Allman, 1888) and *A. conferta*), but their type localities are geographically relatively far from the locations of the biological material studied herein. Because tree topologies (Figs 7.3 and 7.5) indicate segregation by geographical distance of the different species outlined, it is suggested that dispersal of *Acryptolaria* taxa may be highly constrained spatially. Thus, because the potential to find cryptic diversity is high (also due to morphological simplicity), the systematic classifications of the *Acryptolaria* species herein discriminated remain to be confirmed by further haplotype sampling of nominal species of *Acryptolaria* closer to their respective type localities.

The lack of morphological characters to characterize *Acryptolaria* species is well recognized (e.g. Peña Cantero *et al*. 2007) and taxonomists have relied highly on morphometric characters to differentiate species (c.f. Peña Cantero *et al*. 2007). Comparing our phylogenetic reconstructions (Figs 7.3 and 7.5) and morphometric measurements on *Acryptolaria* specimens (Table 7.2), there is evident concordance between both analyses: - *Acryptolaria* sp1 has the smaller hydrothecae; - *Acryptolaria* cf. *conferta* and *Acryptolaria* cf. *flabellum* present hydrothecae of intermediate size but the latter taxon has colonies ramified in one plane; - *Acryptolaria* sp2 and *Acryptolaria* cf. *crassicaulis* are not so divergent molecularly (2-3.1%) and present the largest hydrothecae. Within the latter 'group' with larger hydrothecae, *Acryptolaria* sp2 and *Acryptolaria* cf. *crassicaulis* from Azores are difficult to distinguish morphologically, but the lineage of *Acryptolaria* cf. *crassicaulis* from the south of the Azores is somewhat divergent molecularly (1% *P* distance) from their 'conspecifics' and is the one with the largest hydrothecae (Table 7.2). We highlight however that the sizes of the nematocysts varied too much within lineages and therefore such character is likely useless to diagnose *Acryptolaria* species (at least the ones presented herein), contrarily to the observations of Peña Cantero *et al*. (2007).

Table 7.2: Comparative morphometric data (in μm) of the putative species of *Acryptolaria*.

Species & Station	**Hydrothecae**				**Nematocysts (large group)**	
	Adnate adcauline wall	**Free adcauline wall ***	**Free adcauline wall ****	**Diameter at rim**	**Size ranges**	**Mean Sizes ± SD (n=13)**
Acryptolaria cf. *conferta* Pen Duick Escarp., 498 m - GoC	500 – 550	230 - 400	300 - 560	150 - 190		
Acryptolaria cf. *conferta* Pen Duick Escarp., 557 m - GoC	300-520	320-420	400-720	135-190		
Acryptolaria cf. *conferta* NE Mercator MV, 373 m – GoC	490-650	260-390	270-460	145-190	22-25 x 8-9	23.1 ± 0.74 x 8.4 ± 0.45
Acryptolaria cf. *conferta* Mercator MV, 631 m – GoC	380-480	310-400	360-510	150-180	23-25 x 8-9	23.8 ± 0.7 x 8.2 ± 0.3
Acryptolaria cf. *conferta* NE Mercator MV, 376 m – GoC	410-510	300-450	300-680	150-180	19-21 x 8	19.7 ± 0.67 x 8
Acryptolaria cf. *conferta* Pen Duick Escarp., 610 m - GoC	670-760	230-430	230-510	180-230	14-15 x 4.5-5	14.5 ± 0.41 x 4.9 ± 0.19
Acryptolaria cf. *conferta* Pen Duick Escarp., 444 m – GoC	540-650	200-460	320-515	175-200	19-22 x 8-8.2	20.6 ± 0.72 x 8 ± 0.06
Acryptolaria sp1 Princess Al. Bank, 269 m – Azores	440-570	130-200	160-230	170-205	16.8 – 18 x 6-7	17.2 ± 0.33 x 6.3 ± 0.38
Acryptolaria sp1 Princess Al. Bank, 201 m – Azores	440-510	80-190	80-300	95-130	14.5-16 x 5-5.5	15.27 ± 0.44 x 5.05 ± 0.15
Acryptolaria sp1 Great Meteor, 552-596 m – Azores	510-580	110-190	160-210	120-150	16-17 x 5-6	16.69 ± 0.43 x 5.6 ± 0.5
Acryptolaria sp2 2119 m – Greenland	610-1000	370-590	430-890	190-280	12.5-14 x 4-5	13.19 ± 0.43 x 4.17 ± 0.37
Acryptolaria cf. *flabellum* Melilla CM, 300 m – Alboran Sea	650-770	330-450	370-760	160-190	14.5-15.5 x 4.5-5	15 ± 0.32 x 4.9 ± 0.22
Acryptolaria cf. *flabellum* Carb. Prov., 461 m – GoC	580-730	380-470	500-660	170-180	20-21.3 x 7.2-8	20.4 ± 0.5 x 7.65 ± 0.31
Acryptolaria cf. *craussicaulis* Flores, 316-346 m – Azores	710-830	300-510	310-760	190-240	14-16 x 4-4.7	14.58 ± 0.54 x 4.36 ± 0.2
Acryptolaria cf. *craussicaulis* Condor Terra, 256-274 m – Azores	700-960	490-710	530-830	230-280	12-13.5 x 4	13.04 ± 0.43 x 4± 0
Acryptolaria cf. *craussicaulis* Mar Falso, 435 m – S Azores	1050-1280	790-1000	1000-1330	270-310	14.5-16 x 4-4.5	15.23 ± 0.49 x 4.25 ± 0.22

* = **no renovations**
**** = with renovations**

Genus Filellum

Provisional identifications were also provided for the tiny and morphologically simple *Filellum* species, which cannot be correctly identified morphologically without the examination of reproductive structures – the coppinia (e.g. Peña Cantero *et al.* 1998). At least three species of *Filellum* were identified genetically in our analyses (Figs 7.3 and 7.5), two of each, possibly segregated by depth (one lineage from deep, another from shallow waters), share phenotypic similarities with *Filellum serratum*– striations on the base of hydrothecae. *Filellum* cf. *serratum* from Azores and from the Gulf of Cadiz differ by seven base-pairs (1.4% of divergence) in the generated 16S sequences, and morphologically they also differ substantially by the sizes of the largest nematocysts observed) which appear to be larger in the Azorean lineage (Table 7.3). We refrain in this study to further split these two genetically close haplotypes as different species, and further study of their classification is required in the future. The morphological characters of *Fillelum* species are few; their measurements (with the probable exception of nematocysts) can be highly variable (e.g. Peña Cantero *et al.* 1998; Table 7.3) and do not distinguish between genetically different species (cf. Figs 7.3 and 7.5, and Table 7.3), and therefore are potentially useless to set species apart.

Table 7.3: Comparative morphometric data (in μm) of the putative species of *Filellum.*

Species & Station	**Hydrothecae**				**Nematocysts (large group)**	
	Adnate adcauline wall	**Free adcauline wall ***	**Free adcauline wall ****	**Diameter at rim**	**Size ranges**	**Mean Sizes ± SD**
Filellum cf. *serratum* / sp.1 Estelas, 25-38 m – W Portugal	150-330 (n=4) 252.5 ± 75.9	230-270 (n=4) 250 ± 18.3	260-440 (n=4) 332.5 ± 84.6	105-170 (n=6) 131.7 ± 24.6	18-20 x 4.5-6	19.55 ± 0.69 x 5.15 ± 0.47 (n=10)
Filellum cf. *serratum* / sp.2 Mercator MV, 631 m – GoC	110-250 (n=10) 175 ± 51.3	180-520 (n=10) 336 ± 98.1	350-890 (n=10) 609 ± 154.6	130-165 (n=10) 143 ± 13.6	7-8 x 2-3	7.5 ± 0.5 x 2.43 ± 0.35 (n=7)
Filellum cf. *serratum* / sp.3 S Pico-Faial, *ca.* 370 m – Azores	150-250 (n=10) 203 ± 31.6	170-550 (n=10) 287 ± 123.4	260-820 (n=10) 400 ± 160.9	100-160 (n=10) 130 ± 22.1	14-15 x 3-4	14.2 ± 0.76 x 3.67 ± 0.58 (n=3)
Filellum cf. *serpens* Berlenga, 5-14 m – W Portugal	120-270 (n=10) 191 ± 47.2	130-340 (n=10) 268 ± 66.3	140-430 (n=10) 325 ± 91.4	120-150 (n=10) 130 ± 11.5	21-25 x 4.5-6	23.4 ± 1.15 x 5.2 ± 0.48 (n=10)

* = **no renovations**
** = **with renovations**

Genus Lafoea

Cryptic biodiversity was already encountered within *Lafoea dumosa* by Moura *et al.* (2008). These authors found two genetically divergent clades of hydroids classified as *Lafoea dumosa.* In the present study we substantially increased the haplotype representation for this nominal species, and now at least three different species classified under *L. dumosa* can be distinguished genetically (Figs 7.3 and 7.5). Taxonomy of *L. dumosa* has been widely debated until Cornelius (1975), who established the synonymy of all described species morphologically similar to *Lafoea dumosa*, namely *Lafoea cornuta*

(Lamouroux, 1821), *Lafoea pocillum* Hincks, 1868, *Lafoea gracillima* (Alder, 1856) and *Lafoea fruticosa* (Sars, 1850). Before Cornelius (1975), hydrozoan researchers (e.g. Vervoort, 1972; Millard, 1975) generally accepted as valid species in that complex only *L. fruticosa* and *L. dumosa*, differing by the presence or absence of pedicels in the hydrothecae, respectively. Shuchert (2001) found substantial differences in the sizes of nematocysts related to the 'varieties' with pedicellate or stalkless hydrothecae, and pointed out that Fleming's original description of *L. dumosa* likely corresponded to a form with stalked hydrothecae, contrary to what had been widely assumed (e.g. Cornelius 1975). Despite suspecting that two separate species were included within *L. dumosa*, Shuchert (2001) refrained from splitting species without other sources of data, such as mitochondrial DNA sequences. In the present study, we have indeed verified that specimens with apedicellate hydrothecae collected in shallow waters of Portugal, correspond to a species distinct from the specimens with pedicellate hydrothecae, herein represented by deep-water organisms (Figs 7.3 and 7.5). The larger nematocysts of the species with stalkless hydrothecae are also considerably smaller than those of the morphs with pedicellate hydrothecae (in accordance with Schuchert 2001; see Table 7.4). Regarding the '*L. dumosa*' with pedicels in hydrothecae, we found two distinct lineages (Moura *et al.* 2008; Peña Cantero *et al.* 2010) distantly related by 16S data (up to 5 % of sequence divergence between clades), which we believe corresponds to at least two genetically distinct species (Figs 7.3 and 7.5). Both putative 'species' were found to co-occur in close proximity at bathyal depths of the Gulf of Cadiz. One of these 'species' was recorded only in three localities of the Gulf of Cadiz (two haplotypes diverging only by one base-pair) including one mud volcano; the other was found at geographically distant localities including the Gulf of Cadiz, Iceland and Antarctica. Not surprisingly, these showed relatively high sequence divergence between samples (genetic distances of 1.8, 2.7 and 3.1 %) (Figs 7.3 and 7.5). Therefore, we strongly suspect that further cryptic diversity might be associated with this main lineage of '*L. dumosa'*, and that gene flow in these animals is likely limited in deep waters. It is also noteworthy that no substantial morphological differences were detected between the two main lineages of pedicellate '*L. dumosa'*, including in the sizes of the largest nematocyst types (see Table 7.4). While it seems imperative to re-split taxa within the nominal *L. dumosa* based on the hypotheses and arguments discussed here, further haplotype sampling is required, especially in the immediate vicinity of the type localities of the described species and varieties presently in the synonymy of *L. dumosa*, before conclusions can be reached.

Table 7.4: Comparative morphometric data of cnidocysts (in µm) of the putative species of *Lafoea.*

Species & Station	Nematocysts (large group)	
	Size ranges	Mean Sizes ± SD
Lafoea cf. *dumosa* / sp.1 São Torpes, 9-13 m	11.5-13 x 4.7-5	12.45 ± 0.6 x 4.97 ± 0.09 (n=10)
Lafoea cf. *dumosa* / sp2 Carb. Prov., 461 m – GoC	22-25 x 9-10.5	23.5 ± 0.95 x 9.7 ± 0.44 (n=21)
Lafoea cf. *dumosa* / sp3 Carb. Prov., 473 m – GoC	25-27 x 10-11	26 ± 0.74 x 10.29 ± 0.33 (n=10)
Lafoea cf. *dumosa* / sp3 Yuma MV, 975 m – GoC	26-32 x 9.5-11	29.25 ± 2 x 10.3 ± 0.48 (n=10)
Lafoea cf. *dumosa* / sp3 Pen Duick Escarp., 637 m – GoC	26-29 x 9-10	27.35 ± 0.94 x 9.65 ± 0.34 (n=10)

Phylogeographic inferences

In terms of phylogeographic associations indicative of fairly recent and/or actual gene-flow of ‘Lafoeinae’ taxa between localities, the same 16S haplotype of *Acryptolaria* cf. *conferta* is shared between seven localities of the Gulf of Cadiz (including mud volcanoes and areas of carbonates or dead cold-water coral stands) at depths between 373 and 753 meters (Figs 7.3 and 7.5). Another genetically close haplotype of that putative species, far less common than the other haplotype of the Gulf of Cadiz, was found only once at one sampling station (444 meters) also in this region (Figs 7.3 and 7.5). *Acryptolaria* cf. *conferta* is a highly abundant and widespread species in the deep waters in the Gulf of Cadiz. The low genotypic diversity encountered for this species may reflect the effect of its large population sizes, which should be also interconnected with the large dispersal capability of the species, at least regionally. The other ‘Lafoeinae’ species represented are not so frequently found at neighboring sampling localities, probably because of relatively smaller population sizes and reduced dispersal capacity for those taxa. These life history traits coupled with the effects of oceanic currents, seafloor features (like seamounts, mud volcanoes and extensive abyssal plains) and in some cases glacial cycles (see Moura *et al.* 2011c), may explain the much higher sequence divergence amongst conspecific specimens and potential for cryptic diversity.

7.4 CONCLUSIONS

As a final conclusion, the 16S sequence data when analyzed conjunctively with morphologic and sampling data, proved to be extremely useful to rectify or refine many taxonomic classifications and phylogenetic/phylogeographic assumptions of difficult to resolve hydrozoan taxa like the Lafoeidae *s. l.* or Hebellidae *s. l.*. The present study provided several new and important insights into the systematics of those groups, which deserve further investigation and comprehensive taxonomic work to resolve nominal species, genera and even families.

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Table 7.5: 16S rRNA sequence data. Sequences of specimens marked with asterisk were determined in the present study.

Species	GenBank accession Number	MorphBank specimen ID	Voucher specimen	Cruise / collector	Collection method	Sample location
Abietinella operculata	FN424136 (Peña Cantero *et al*. 2010)					Ross Sea Antarctica
Acryptolaria cf. *conferta* *	JN714632	781202	DBUA 127201	R/V Pelagia M2007	Box-corer	Pen Duick Escarpment, Stn. 15, 444 m 35°17.905'N 6°44.286'W Gulf of Cadiz
Acryptolaria cf. *conferta* *	JN714633		DBUA 1273.01	R/V Pelagia 64PE284	ROV	Central Carbonate Mound Province (N area), Stn. 12728-1, 753 m 35°11.020’N 6°56.620’W Gulf of Cadiz
Acryptolaria cf. *conferta* *	JN714634		DBUA 1274.01	James Cook 10	ROV	NE Mercator MV, Stn. Dive 27 Rock 5, 373 m 35°18.825'N 6°37.154'W Gulf of Cadiz
Acryptolaria cf. *conferta* *	JN714635		DBUA 1128.01	R/V Belgica	ROV	Mercator MV, Stn. B09-01W out, 631 m 35º17.916’N 06º38.709’W Gulf of Cadiz
Acryptolaria cf. *conferta* *	JN714636		DBUA 1274.02	James Cook 10	ROV	NE Mercator MV, Stn. Dive 27 Rock 6, 376 m 35°18.827’N 6°37.058’W Gulf of Cadiz
Acryptolaria cf. *conferta*	AM888345 (Moura *et al.* 2008)	781208	DBUA 1275.01	R/V Pelagia M2006	Box-corer	Pen Duick Escarpment, Stn. 23, 557 m 35°19.020'N 6°48.220'W Gulf of Cadiz
Acryptolaria cf. *conferta*	AM887981 (Moura *et al.* 2008)	781183	DBUA 1276.01	R/V Prof. Logachev TTR 16	Grab	Pen Duick Escarpment, Stn. 600, 610 m 35°18.779'N 6°48.453'W Gulf of Cadiz
Acryptolaria cf. *conferta*	AM887980 (Moura et al. 2008)		DBUA 925.06	R/V Pelagia M2005	Box-corer	Pen Duick Escarpment, Stn. 21, 498 m 35°19.084'N 6°46.397'W Gulf of Cadiz
Acryptolaria cf. *crassicaulis* *	JN714637		DOP C889	F/V Mistério Azul	LLMH	Condor de Terra, 256-274 m 38°30.840'N 29°00.120'W Azores
Acryptolaria cf. *crassicaulis* *	JN714638	781205	DOP C2324	R/V Arquipélago ARQDAÇO-27-P07	LLMH	Flores Island, 316 – 346 m 39°30.000'N 31°06.000'W Azores
Acryptolaria cf. *crassicaulis* *	JN714639		DOP C1887	F/V Manuel Arriaga	LLMH	Mar Falso, 435 m 33°22.200'N 29°01.200'W S Azores
Acryptolaria cf. *flabellum* *	JN714640	781211	DBUA 1277.01	R/V Pelagia M2007	Box-corer	Carbonate Province, Stn. 41, 461 m 35°00.113'N 6°44.815'W Gulf of Cadiz
Acryptolaria cf. *flabellum* *	JN714641	781215	DBUA 1278.01	R/V Prof. Logachev TTR17-leg1	Grab	Melilla Carb. Mound, Stn. 395, 300 m 35º26.415’N 2º31.075’W Alboran Sea
Acryptolaria sp1 *	JN714642		DOP C1463	F/V Manuel Arriaga	LLMH	Princess Alice Bank, 269 m 37°54.480'N 29°10.920'W Azores
Acryptolaria sp1 *	JN714643	781199	DOP C3062	R/V Arquipélago DEECON-28-V07	LLMH	Great Meteor, 552-596 m 30°10.800'N 28°32.400'W S Azores
Acryptolaria sp1 *	JN714644		DOP C2482	F/V Manuel Arriaga	LLMH	Princess Alice Bank, 201 m 37°54.480'N 29°10.920'W Azores
Acryptolaria sp2 *	JN714645	781196	DBUA 1279.01	R/V Prof. Logachev TTR 13	Dredge	Stn. 456, 2119-2186 m 63°14.135'N 55°26.239'W Greenland
Anthohebella parasitica	AY787918 (C.W.Cunningham EMBL subm.)		MHNG INVE29762			Cala Murada, Mallorca S Spain
Anthohebella parasitica *	JN714646		DBUA 1304.01	C.J. Moura	Hand	Flores Island, 10-16 m 39º28.531’N 31º08.479’W Azores
cf. *Anthohebella parasitica* *	JN714647	781238	DBUA 1305.01	C.J. Moura	Hand	Faial Island, Horta port, 5-18 m 38°32.015'N 28°37.304'W Azores
Billardia subrufa	FN424117 (Peña Cantero *et al*. 2010)					Antarctic Peninsula Antarctica

Campanulina panicula	FJ550511 (Leclère *et al.* 2009)					Korsfjord Norway
Campanulina panicula *	JN714648		DBUA 1308.01	R/V Belgica	ROV	Mercator MV, Stn. B09-01W out, 631 m 35º17.916’N 06º38.709’W Gulf of Cadiz
Campanulina panicula *	JN714649		DBUA 1310.01	R/V Prof. Logachev TTR17-leg1	Grab	Melilla Carb. Mound, Stn. 395, 300 m 35º26.415’N 2º31.075’W Alboran Sea
Campanulina panicula *	JN714650		DBUA 1311.01	R/V Pelagia M2007	Box-corer	Carbonate Province, Stn. 41, 461 m 35°00.113'N 6°44.815'W Gulf of Cadiz
Cryptolaria pectinata *	JN714651	781191	DOP C2416	R/V Arquipélago DEECON-28-V07	LLMH	Irving, 452 - 500 m 32°00.000'N 27°57.000'W S Azores
Cryptolaria pectinata *	JN714652		DOP C1844	F/V Manuel Arriaga	LLMH	Princess Alice Bank, 274 m 37°46.560'N 29°09.120'W Azores
Cryptolaria pectinata *	JN714653		DOP C2932	R/V Arquipélago ARQDAÇO-29-P08	LLMH	Mar da Prata (near São Miguel), 279 m 37°36.600'N 25°54.000'W Azores
Cryptolaria pectinata	AM887994 (Moura *et al.* 2008)	781221	DBUA 1280.01	R/V Pelagia M2005	Box-corer	Pen Duick Escarpment, Stn. 2, 526 m 35°17.701'N 6°47.279'W Gulf of Cadiz
Cryptolaria pectinata *	JN714654		DBUA 1281.01	R/V Belgica	ROV	Mercator MV, Stn. B09-01W out, 631 m 35º17.916’N 06º38.709’W Gulf of Cadiz
Cryptolaria pectinata *	JN714655	781218	DBUA 1282.01	R/V Prof. Logachev TTR17 – leg 2	Dredge	Mound near Lisbon, Stn. 691, 1384 m 39°05.220'N 10°34.060'W W Portugal
Cryptolaria pectinata *	JN714656		DOP C1399	F/V Baia das Lajes	LLMH	Condor Bank, 229 m 38°32.460'N 29°02.940'W Azores
Filellum cf. *serpens* *	JN714657	781242	DBUA 1283.01	C.J. Moura	Hand	Benidorm Island, 8-26 m 38º29.914'N 0º07.746'W S Spain
Filellum cf. *serpens* *	JN714658	781248	DBUA 1284.01	C.J. Moura	Hand	Berlengas, Berlenga Island, 5-14 m 39°24.540'N 9°30.708'W W Portugal
Filellum cf. *serratum* / sp1*	JN714659	781250	DBUA 1285.01	C. J. Moura	Hand	Berlengas, Estelas, B^{xa} Alagada, 25-38 m 39º25.477'N 9º32.120'W W Portugal
Filellum cf. *serratum* / sp.2 *	JN714660		DBUA 1286.01	R/V Belgica	ROV	Mercator MV, Stn. B09-01W out, 631 m 35º17.916’N 06º38.709’W Gulf of Cadiz
Filellum cf. *serratum* / sp.3*	JN714661	781245	DBUA 1287.01	Submersible Lula	Submersible	S Pico-Faial channel, dive 143, 340 – 400 m 38º29.379’N 28º37.149’W to 38º29.410’N 28º37.326’W Azores
cf. *Hebella scandens* / sp.1 *	JN714662	781188	DBUA 1288.01	C.J. Moura	Hand	Berlengas, Rinchão, 25-35m 39º25.437’N 9º29.941’W W Portugal
cf. *Hebella scandens* / sp.2*	JN714663	781185	DBUA 1289.01	C. J. Moura	Hand	Berlengas, Estelas, B^{xa} Alagada, 25-38 m 39º25.477'N 9º32.120'W W Portugal
Hebella venusta	FJ550496 (Leclère *et al.* 2009)		MHNG INVE35476			Honduras
Lafoea cf. *dumosa* / sp1*	JN714664	781261	DBUA 1290.01	C.J. Moura	Hand	Sines, São Torpes, 9-13 m 37º54.770’N 8º48.467’W SW Portugal
Lafoea cf. *dumosa* / sp1*	JN714665		DBUA 1290.02	C.J. Moura	Hand	Aljezur, islander, 2-11 m 37°10.158'N 8°54.226'W SW Portugal
Lafoea cf. *dumosa* / sp1*	JN714666		DBUA 1291.01	C.J. Moura	Hand	Berlengas, Rinchão, 25-35m 39º25.437’N 9º29.941’W W Portugal
Lafoea cf. *dumosa* / sp1*	JN714667		DBUA 1292.02	C. J. Moura	Hand	Berlengas, Estelas, B^{xa} Alagada, 30-38 m 39º25.477'N 9º32.120'W W Portugal
Lafoea cf. *dumosa* / sp1*	JN714668 JN714669		DBUA 1291.03	C. J. Moura	Hand	Berlengas, Farilhões (cave), 28-32 m 39º28.351’N 9º32.821’W W Portugal
Lafoea cf. *dumosa* / sp2	FN424137 (Peña Cantero *et al.* 2010)					Antarctic Peninsula Antarctica

Lafoea cf. *dumosa* / sp2	AY787917 (C.W. Cunningham, EMBL subm.)		MHNG INVE29952			Gardur Iceland
Lafoea cf. *dumosa* / sp2*	JN714670	781258	DBUA 1292.01	R/V Pelagia M2007	Box-corer	Carbonate Province, Stn. 41, 461 m 35°00.113'N 6°44.815'W Gulf of Cadiz
Lafoea cf. *dumosa* / sp3*	JN714671	781253	DBUA 1293.01	R/V Prof. Logachev TTR 16	Grab	Yuma mud volcano, Stn. 605, 975 m 35°25.046'N 7°05.450'W Gulf of Cadiz
Lafoea cf. *dumosa* / sp3	AM888327 (Moura *et al.* 2008)		DBUA 1294.01	R/V Pelagia M2006	Box-corer	Pen Duick Escarpment, Stn. 42, 637 m 35°18.920'N 6°48.390'W Gulf of Cadiz
Lafoea cf. *dumosa* / sp3*	JN714672	781256	DBUA 1295.01	R/V Pelagia M2007	Box-corer	Carbonate Province, Stn. 40, 473 m 35°00.137'N 6°44.806'W Gulf of Cadiz
Lafoeina tenuis *	JN714673		DBUA 1312.01	Submersible Lula	Submersible	S Pico-Faial channel, dive 143, 340 – 400 m 38º29.379’N 28º37.149’W to 38º29.410’N 28º37.326’W Azores
Laodicea undulata	FJ550471 (Leclère *et al*. 2009)		MHNG INVE31753			Villefranche-sur-Mer S France
Melicertum octocostatum	EU305479 (Cartwright *et al*. 2008)		USNM 1073342173342			
Melicertum octocostatum	FJ550510 (Leclère *et al*. 2009)		MHNG INVE48744			Raunefjord Norway
Modeeria rotunda	FJ550476 (Leclère *et al*. 2009)		MHNG INVE32967			Banyuls-sur-Mer S France
Modeeria rotunda *	JN714674		DBUA 1314.01	R/V Belgica	ROV	Mercator MV, Stn. B09-01W out, 631 m 35º17.916’N 06º38.709’W Gulf of Cadiz
Phialella quadrata	FJ550474 (Leclère et al. 2009)		MHNG INVE33466			Whangaparoa New Zealand
Scandia cf. *gigas* *	JN714675		DBUA 1306.01	C.J. Moura	Hand	Lagoa, Carvoeiro, 1-8 m 37º05.419’N 8º27.269’W S Portugal
Scandia gigas *	JN714676		DBUA 1307.01	C.J. Moura	Hand	Benidorm Island, 8-26 m 38º29.914'N 0º07.746'W S Spain
Scandia gigas *	JN714677		DBUA 1308.01	C.J. Moura	Hand	Cirkewwa, 16-26 m 35°59.348'N 14°19.680'E Malta
Scandia gigas	AY787919 (CW Cunningham EMBL subm.)		MHNG INVE29764			Cala Murada, Mallorca S Spain
Staurodiscus gotoi	FJ550472 (Leclère *et al*. 2009)		MHNG INVE33467			Devonport New Zealand
Stegella lobata	FN424119 (Peña Cantero *et al*. 2010)					Peter I Island, Antarctica
Stegopoma bathyale *	JN714678		DOP C2506	R/V Pourquois Pas MOMARDREAM	Submersible	Rainbow (hydrothermal field) SW Azores
Stegopoma bathyale *	JN714679			PescProf – 3 Chacmad -3	FT	Paul do Mar, Stn. 7, 1000 m Madeira
Stegopoma bathyale *	JN71468		DBUA 1313.01	R/V Prof. Logachev TTR17 – leg 2	Dredge	Mound near Lisbon, Stn. 688, 1294 m 39°04.327'N 10°33.992'W W Portugal
Stegopoma plicatile	FN424152 (Peña Cantero *et al*. 2010)					Bellingshausen Sea Antarctica
Stegopoma plicatile	FJ550513 (Leclère et al. 2009)					Raunefjord Norway
Tiaropsidium kelseyi	EU305485 (Cartwright *et al*. 2008)					Central California USA
Tiaropsis multicirrata	FJ550468 (Leclère *et al*. 2009)					Sandgerdi Iceland
Zygophylax leloupi *	JN714681	781230	DBUA 1296.01	R/V Prof. Logachev TTR17 – leg 2	Dredge	Mound near Lisbon, Stn. 690, 1419 m 39°04.775'N 10°34.114'W W Portugal
Zygophylax brownei *	JN714682	781225	DBUA 1297.01	R/V Pelagia M2007	Box-corer	Unknown Station, bathyal environment Gulf of Cadiz

Zygophylax biarmata *	JN714683		DBUA 1298.01	R/V Pelagia M2007	Box-corer	Carbonate Province, Stn. 39, 441 m 35°00.230'N 6°44.894'W Gulf of Cadiz
Zygophylax biarmata *	JN714684		MMF 39095		FT	Off Caniçal, Stn. 34, 600 m Madeira
Zygophylax biarmata *	JN714685		DBUA 1299.01	R/V Pelagia 64PE284	Box-corer	Vernadsky Ridge, Stn. 12759, 524 m 35°26.570'N 6°46.780'W Gulf of Cadiz
Zygophylax biarmata	AM888343 (Moura *et al.* 2008)		DBUA 919.02	R/V Prof. Logachev TTR-12	Grab	Pen Duick Escarpment, Stn. 406, 536 m 35°18.174'N 6°45.564'W Gulf of Cadiz
Zygophylax biarmata	AM888342 (Moura *et al.* 2008)		DBUA 921.03	R/V Pelagia M2005	Box-corer	Pen Duick Escarpment, Stn. 2, 526 m 35°17.701'N 6°47.279'W Gulf of Cadiz
Zygophylax biarmata *	JN714686		DBUA 1300.01	James Cook 10	ROV	NE Mercator MV, Stn. Dive 27 Rock 5, 373 m 35°18.825'N 6°37.154'W Gulf of Cadiz
Zygophylax levinseni	AM888344 (Moura *et al.* 2008)	781232	DBUA 1301.01	R/V Pelagia M2005	Box-corer	Pen Duick Escarpment, Stn. 2, 526 m 35°17.701'N 6°47.279'W Gulf of Cadiz
Zygophylax levinseni *	JN714687	781387	MMF 36727	F/V Baía de Câmara de Lobos		Off Garajau, 700 m 32º37.49'N 16º50.58'W Madeira
Zygophylax sagamiensis *	JN714688	781236	DBUA 1302.01	R/V Prof. Logachev TTR17 – leg 2	Dredge	Mound near Lisbon, Stn. 688, 1294 m 39°04.327'N 10°33.992'W W Portugal
Zygophylax sibogae *	JN714689		DBUA 1303.01	R/V Prof. Logachev TTR17 – leg 2	Dredge	Mound near Lisbon, Stn. 688, 1294 m 39°04.327'N 10°33.992'W W Portugal
Bougainvillia fulva	EU305470 (Cartwright *et al.* 2008)					
Bougainvillia muscus	AY787880 (C.W. Cunningham, EMBL subm.)		MHNG INVE29410			St. Pol de Leon S France

Abbreviations: DBUA, 'Departamento de Biologia da Universidade de Aveiro'; DOP, 'Departamento de Oceanografia e Pescas'; F/V, fishing vessel'; FT, fish traps; LLMH, long lines with multiple hooks; M, oceanographic campaign 'Moundforce'; MV, mud volcano; MMF, 'Museu Municipal do Funchal'; MHNG, 'Muséum d'Histoire Naturelle de Genève, Switzerland '; PE, Pelagia; ROV, Remote Operated Vehicle; R/V, Research Vessel; Stn, station; TTR, oceanographic campaign 'Training Trough Research'.

Chapter 8

A MOLECULAR PHYLOGENETIC APPRAISAL OF THE SYSTEMATICS OF THE AGLAOPHENIIDAE (CNIDARIA: HYDROZOA, LEPTOTHECATA) FROM THE NORTH-EAST ATLANTIC AND WEST MEDITERRANEAN

Moura, C. J., Cunha, M. R., Porteiro, F. M. & Rogers, A. D. (2011b). *Zoological Journal of the Linnean Society*. [in press]

Contents

ABSTRACT

The hydrozoan family Aglaopheniidae (Cnidaria) is widespread worldwide and contains some of the most easily recognizable hydroids because of their large colony size and characteristic microscopic structure. The systematics of the group has, however, been controversial and dedicated molecular analyses are lacking. We therefore analysed existing and new 16S rRNA sequences of Aglaopheniidae, in a total of 98 16S sequences corresponding to 25 putative species (25 nominal and three undescribed) from seven genera. The monophyly of the subfamilies Gymnangiinae and Aglaopheniinae, and tribes

Aglaopheniini and Cladocarpini were not verified with 16S sequence data. The genera *Gymnangium* and *Aglaophenia* can only be considered valid if both *Gymnangium gracicaule* and *Aglaophenia latecarinata* are removed from their respective genera. The phenotypically similar *Cladocarpus* and *Streptocaulus* are probably monophyletic and clearly distinct genetically. The genus *Lytocarpia* may be polyphyletic. The nominal species *Aglaophenia pluma*, *Aglaophenia tubiformis* and *Aglaophenia octodonta* are likely conspecific, as are also the species *Aglaophenia acacia* and *Aglaophenia elongata*. The 16S data revealed the existence of two potentially unnamed species of *Aglaophenia* respectively from the Azores and Madeira. The phylogeographic structure of the taxa with the greatest representation of haplotypes from the NE Atlantic and Mediterranean, revealed the influence of Mediterranean waters in Madeira and the Azores, and gene flow between deep waters of the Mediterranean and Atlantic. The last glaciations in Europe may have caused genetic bottlenecks but also high intraspecific haplotype diversity. Finally, *Macrorhynchia philippina* was detected in samples from Madeira and possibly represents an invasive species.

8.1 INTRODUCTION

Marine hydroids (Cnidaria, Hydrozoa) are important components of coastal and deep-sea benthic habitats, being some of the most important sessile predators and competitors for bottom space (e.g. Bouillon *et al.* 2006). However, despite their importance in marine ecosystems, the scientific study of these animals has been highly neglected and their systematic relations are contentious and poorly resolved largely because of their morphological simplicity and plasticity.

Some of the more frequent and prominent hydroids found worldwide in shallow and deep waters belong to the cosmopolitan family Aglaopheniidae Agassiz, 1862. The Aglaopheniidae comprise about 250 valid species included within eight genera (Bouillon *et al.* 2006). Colonies of Aglaopheniidae are passive suspension feeders with colonies having a typical appearance of feathers (see examples of Fig. 8.1).

Molecular phylogenetic analyses of Leclère *et al*. (2007), Moura *et al.* (2008), Leclère *et al*. (2009) and Peña Cantero *et al*. (2010) have all recovered the family Aglaopheniidae as monophyletic with a maximum number of 16 nominal species and five genera of aglaopheniids represented. These authors also confirmed that the Aglaopheniidae belongs to the superfamily Plumularoidea. Peña Cantero *et al.* (2010) found that the Schizotrichidae are likely to be the most closely related group to the Aglaopheniidae. At an intra-family level Leclère *et al.* (2007) and Moura *et al*. (2008) found the genus *Gymnangium* Hincks, 1874 to be polyphyletic and the genus *Aglaophenia* Lamouroux, 1812 monophyletic. All other genera were insufficiently represented in their molecular phylogenetic analyses to investigate their relationships. Moura *et al*. (2008) further highlighted the possibility that the nominal species *Aglaophenia pluma* (Linnaeus, 1767), *Aglaophenia octodonta* Nutting, 1900 and *Aglaophenia tubiformis* Marktanner-Turneretscher, 1890 are conspecific, the name *Aglaophenia pluma* having priority.

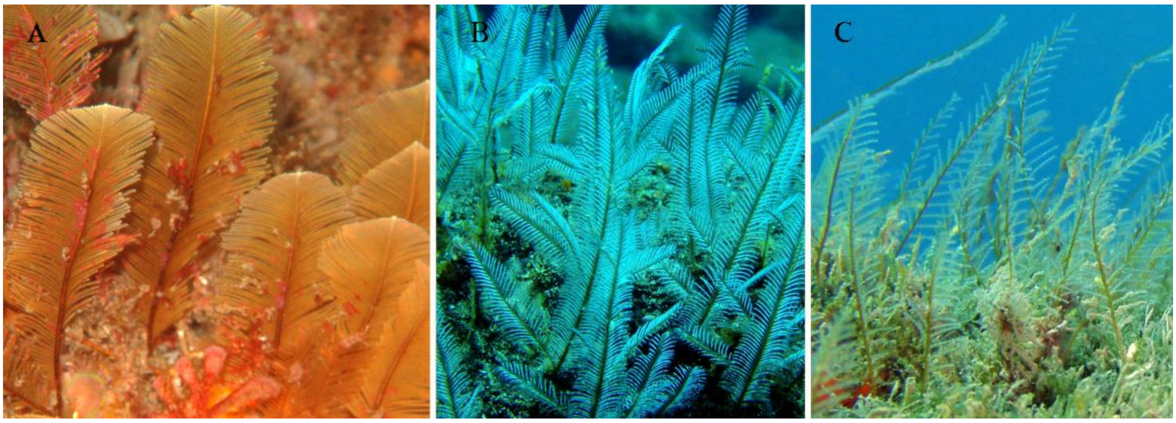

Fig. 8.1. Examples of Aglaopheniidae colonies: (A) *Gymnangium montagui* off Berlengas – west Portugal; (B) *Macrorhynchia philippina* off Madeira Island; (C) Undetermined *Aglaophenia* species off Madeira Island. Credits: C.J. Moura.

Based on morphological characters, Calder (1997) ordered the genera of Aglaopheniidae into two subfamilies: Gymnangiinae Calder, 1997, in which the gonothecae (reproductive structures) are unprotected, and Aglaopheniinae Marktanner-Turneretscher, 1890, in which the gonothecae are protected by ‘phylactocarps’ or ‘corbulae’. Calder (1997), based on the structure of the reproductive organs, further subdivided the subfamily Aglaopheniinae into two tribes: the Aglaopheniini Marktanner-Turneretscher, 1890, and the Cladocarpini Calder, 1997. However, these subdivisions of the family Aglaopheniidae were never tested genetically because of insufficient haplotype representation of these groups in molecular phylogenetic analyses.

In the present study, the number of taxa of Aglaopheniidae represented by 16S mRNA haplotypes was increased considerably with the main aim to test phylogenetic relations within the family, including the monophyletic status, of its subfamilies, tribes, genera, and species. 16S rDNA data may also help to understand phylogeographical relations of aglaopheniids in the north-east Atlantic and west Mediterranean as this gene resolves some level of intraspecific variation in other hydroid taxa (e.g. Moura *et al*. 2008, 2011a, c, d). The utility of the 16S sequence data for 'DNA barcoding' purposes in the hydroids of Aglaopheniidae is also assessed.

8.2 MATERIALS AND METHODS

Collection and identification of samples

Specimens of Aglaopheniidae were collected from 57 distinct sampling sites of various shallow and deep-water localities of the northern Atlantic and western Mediterranean (Fig. 8.2, Table 8.1). Specimens were fixed and preserved mostly in 96% ethanol, although sometimes in 70% ethanol. The material collected in deep waters of the Azores and Madeira included specimens obtained as part of a study on by-catch from deep-sea fisheries and some were frozen prior to fixation in ethanol. Taxa were classified taxonomically in the laboratory by C. J. Moura mainly based on the publications of Cornelius (1995), Svoboda & Cornelius (1991), Ramil & Vervoort (1992, 2004), Medel & Vervoort (1995), Ramil *et al.* (1998) and Ansín Agís *et al*. (2001).

DNA extraction, amplification and sequencing

Protocols for DNA extraction, amplification, and sequencing of the mitochondrial large ribosomal subunit (16S) rDNA gene followed the methodology described in Moura *et al*. (2008), except that the volume of reagents used in PCR reactions was reduced by half, and some of the purified PCR products were sent to 'STAB VIDA, Investigação e Serviços

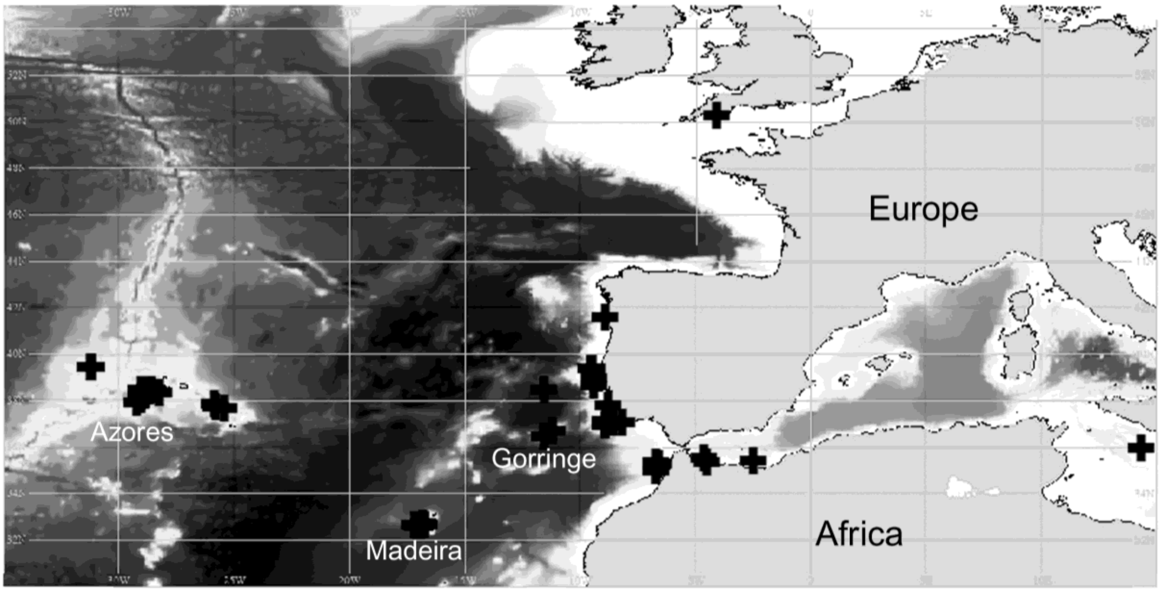

Fig. 8.2. Map of the north-east Atlantic and west Mediterranean with representation of the sites with Aglaopheniidae haplotypes sampled specifically for this study.

em Ciências Biológicas, Lda’, Oeiras, Portugal for sequencing. The primers used were SHA (ACGGAATGAACTCAAATCATGT) and SHB (TCGACTGTTTACCAAAAACATA) (Cunningham & Buss 1993). The skeletons of voucher specimens were mounted in permanent slides and deposited in the Museu Nacional de História Natural, Lisbon, Portugal. The material from the Portuguese coast and Gulf of Cadiz is temporarily deposited in the Biological Research Collection of the Department of Biology, University of Aveiro (DBUA), Portugal. Specimens from Madeira remained in the Municipal Museum collection at the Marine Biology Station of Funchal, Portugal. Azorean specimens gathered by the Department of Oceanography and Fisheries (DOP), University of the Azores, Portugal, were returned to that institution. Sampling details, GenBank and Museum accession numbers are described in Table 8.1.

Phylogenetic analyses

The sequences assembled for this study were clustered with sequences of Aglaopheniidae published on GenBank. They were then aligned in MEGA version 4 (Tamura *et al.* 2007) with CLUSTALW using the default alignment settings, and trimmed to the shortest sequence. MrModeltest version 2.3 (Nylander 2004) was used to determine the optimal probabilistic model of sequence evolution. The ‘general time reversible plus gamma’ was the model selected by the Akaike Information Criterion. Maximum likelihood

(ML) phylogenetic analyses were performed through the PHYML website (http://www.atgc-montpellier.fr/phyml) (Guindon & Gascuel 2003) using the GTR model. Bayesian analyses were also performed with MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001), using again the same model. The search was run for 10^7 generations, with trees sampled every 1000 generations (the first 1 million generations were discarded as 'burnin'). Clade stability was assessed using non-parametric bootstrapping of ML analyses (1000 replicates) and Bayesian posterior probability values. MEGA version 4 (Tamura *et al*. 2007) was used to calculate pairwise distances for simple comparisons of percentage sequence divergence between taxa.

8.3 RESULTS AND DISCUSSION

A total of 98 16S mRNA sequences (comprising 60 haplotypes) of Aglaopheniidae, with 566 base pairs (298 parsimony informative sites, ts/tv ratio =1.48), were analysed phylogenetically; 66 sequences out of the 98 were determined specifically for the present study. Peña Cantero *et al*. (2010) found that the most likely sister group of the Aglaopheniidae is the family Schizotrichidae Peña Cantero, Sentandreu & Latorre, 2010. For this reason, sequences of *Schizotricha* Allman, 1883 were used as an outgroup in the present phylogenetic reconstructions (Fig. 8.3). As in the phylogenetic hypotheses of Leclère *et al*. (2007, 2009), Moura *et al*. (2008) and Peña Cantero *et al*. (2010), the family Aglaopheniidae is monophyletic with high nodal support (Fig. 8.3). Within Aglaopheniidae four main divergent clades were recovered: (1) species of *Cladocarpus* Allman, 1874 and *Aglaophenopsis* Fewkes, 1881; (2) *Streptocaulus* Allman, 1883, species; (3) *Gymnangium hians* (Busk, 1852) and *Gymnangium montagui* (Billard, 1912); and (4) *Gymnangium gracilicaule* (Jäderholm, 1903) with species of *Aglaophenia*, *Macrorhynchia* Kirchenpauer, 1872 and *Lytocarpia* Kirchenpauer, 1872 (Fig. 8.3). The only statistically supported phylogenetic relationship between these main evolutionary branches is that between species of *Gymnangium* (i.e. *G. hians* and *G. montagui*) and *Streptocaulus* (Fig. 8.3). The present study therefore does not support the subdivision of the Aglaopheniidae into the subfamilies Gymnangiinae and Aglaopheniinae, and the latter nominal group into

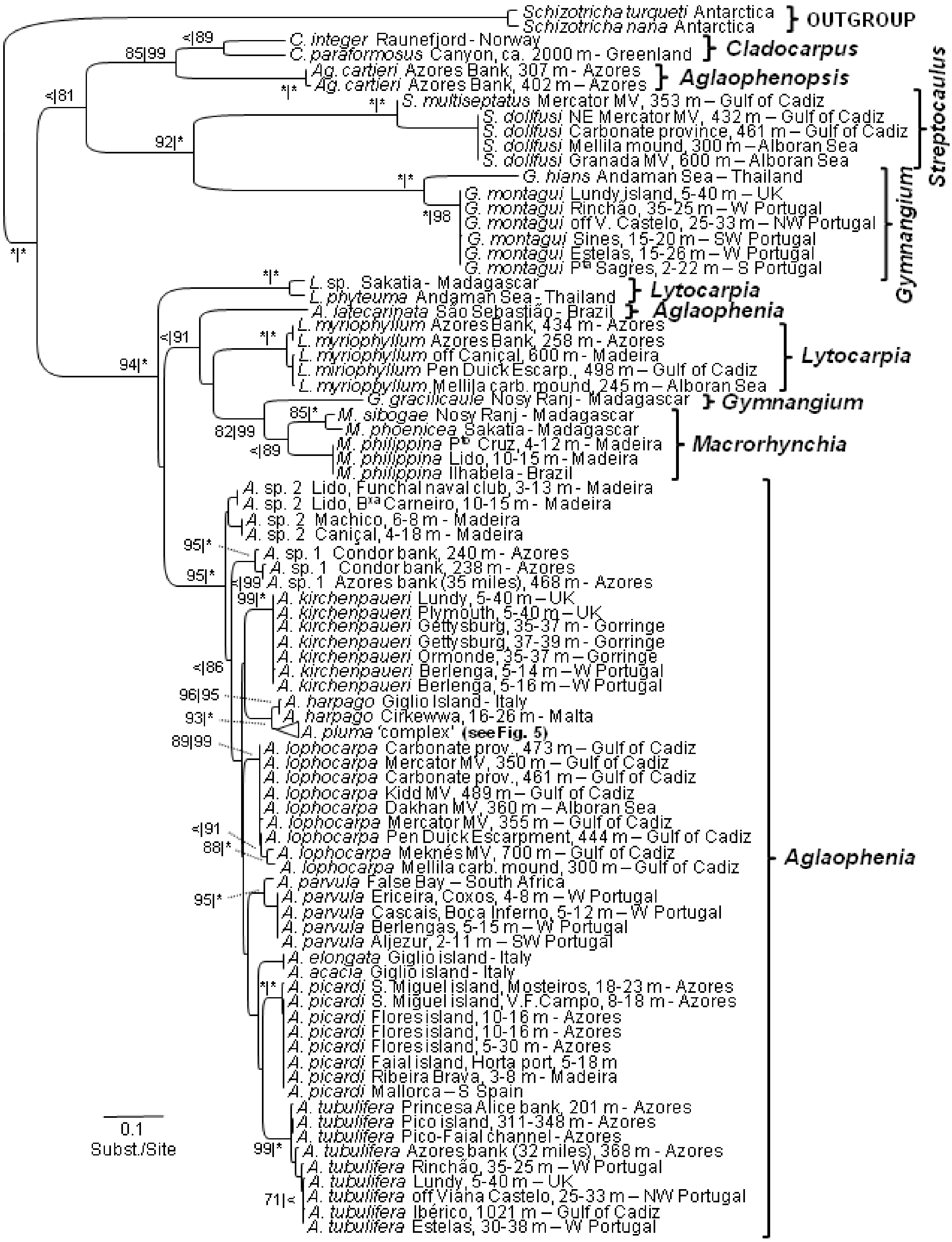

Fig. 8.3. 'Maximum-likelihood' phylogenetic analysis of the dataset containing all the 16S Aglaopheniidae sequences used in this study. Note that the part of the phylogenetic tree with all the branches of the *Aglaophenia pluma* complex is represented in a separate figure - Fig. 8.5. Numbers near the nodes indicate the values of bootstrap (left) and posterior probabilities (right) in percentages. Values were less than 70% are replaced by <; if equal to 100%, an asterisk is used instead. Values of bootstrap and posterior probabilities are omitted when both were less than 70% for the same node. The branch length indicator represents 0.1 substitutions per site.

the tribes Aglaopheniini and Cladocarpini, as proposed by Calder (1997) based on the morphology of reproductive structures. The genetic distinctiveness of the clade containing *G. hians* and *G. montagui* (Fig. 8.3) may support the demarcation of the subfamily Gymnangiinae, but the placement of *G. gracilicaule* closer to *Macrorhynchia* species indicates that the diagnostic character established for Gymnangiinae (gonothecae unprotected) is not valid and that unprotected gonothecae arose independently in the evolutionary history of Aglaopheniidae. The subfamily Gymnangiinae may therefore be considered a valid group that excludes the taxon *'Gymnangium' gracilicaule*. The monophyletic status of the subfamily Aglaopheniinae, and of its tribes Aglaopheniini and Cladocarpini was also not verified.

Within Aglaopheniidae two of the seven genera represented (*Gymnangium* and *Aglaophenia*) were recovered as polyphyletic (Fig. 8.3). The genera *Cladocarpus* and *Streptocaulus*, represented by two species each (none of which were the type of the respective genus), are recovered as monophyletic. The monophyletic nature of the genera *Macrorhynchia* and *Lytocarpia* cannot be refuted at the present time (Fig. 8.3) because nodal support is low for the clades encompassing representatives of each genus.

The eastern Atlantic species *G. montagui* clusters tightly on a distinct evolutionary branch with *G. hians* collected from Thailand, but the 16S sequence of *Gymnangium gracilicaule* from Madagascar is found in another clade more closely related to *Macrorhynchia* species. Therefore the genus *Gymnangium* can be considered polyphyletic (Leclère *et al*. 2007; Moura *et al*. 2008; Fig. 8.3). The representatives of *Macrorhynchia* may form a monophyletic group that together with '*Gymnangium' gracilicaule* comprise a sister clade to species of *Aglaophenia* and *Lytocarpia* (Fig. 8.3).

The diagnostic characters of the genera *Cladocarpus* and *Streptocaulus* have been much debated, as some species possess phenotypic characteristics of both nominal genera and others possess none of them (e.g. Calder 1997; Schuchert 2001; Bouillon *et al*. 2006). Our phylogenetic analyses (Fig. 8.3), although still limited by under-representation of taxa of these genera, recover both genera monophyletic and genetically quite divergent (genetic distance of 23-26% between taxa of both genera). However, neither the type species of the genus *Streptocaulus* (= *Streptocaulus pulcherrimus* Allman, 1883) nor the type species of

Cladocarpus (= *Cladocarpus formosus* Allman, 1874a) were included in our molecular analyses in order to extrapolate the monophyletic status of both genera. However, species of *Streptocaulus* and *Cladocarpus* cluster into two quite separated clades, suggesting that both genera could coincide with two distinct clades. This result also provide support to the hypothesis of Ramil & Vervoort (2008) that *Streptocaulus multiseptatus* (Bale, 1915) belongs to the genus *Streptocaulus* (instead of *Cladocarpus*), according to the morphology of the phylactocarps (modified hydrocladia that protect the gonothecae). The genus *Streptocaulus* [represented by *Streptocaulus dollfusi* (Billard, 1924) and *S. multiseptatus*] form a separate divergent clade that is sister to the genus *Gymnangium* (Fig. 8.3). The genus *Cladocarpus* [represented by *Cladocarpus paraformosus* Schuchert, 2000 and *Cladocarpus integer* (G.O. Sars, 1874)] forms a clade sister to *Aglaophenopsis cartieri* (Bedot, 1921) (Fig. 8.3), previously classified in the genus *Cladocarpus* until Ramil & Vervoort (2004) transferred it to *Aglaophenopsis* (genetic distance of 12-13% between both clades). Our analyses did not clarify if the clade containing *Aglaophenopsis cartieri* and *Cladocarpus* is sister to the clade with *Gymnangium* and *Streptocaulus*, or instead to the evolutionary branch containing ‘*Gymnangium’ gracilicaule* and species of *Aglaophenia* and *Lytocarpia* (Fig. 8.3).

The genus *Lytocarpia* herein represented by three species comprises two main clades with uncertain phylogenetic relationships: one clade contains the nominal species *Lytocarpia myriophyllum* (Linnaeus, 1758), the other (genetically divergent from the latter by 10-11 %) includes *Lytocarpia phyteuma* (Kirchenpauer, 1876) and an undetermined species of *Lytocarpia* from Madagascar. Such a result may indicate a polyphyletic status for the genus *Lytocarpia*.

The genus *Aglaophenia* is recovered as polyphyletic because the sequence of *Aglaophenia latecarinata* Allman, 1877, lies outside of all other *Aglaophenia* taxa, which themselves cluster into a well-supported group (Fig. 8.3). It is unlikely that the sequence of *Aglaophenia latecarinata* Allman, 1877 may correspond to a misidentification or contamination (A. Lindner & E. Migotto, pers. communication), and therefore, the genus *Aglaophenia* is monophyletic only with the exclusion of *A. latecarinata*. The genus *Aglaophenia* is very common in the shallow and deep waters of the NE Atlantic (Moura, pers. observ.) and all the eleven nominal species of *Aglaophenia* reported for European and Mediterranean waters are represented in our phylogeny (Fig. 8.3; sensu Svoboda &

Cornelius 1991). Additionally to these species, our phylogenetic hypotheses (Fig. 8.3) also confirmed the presence of two (possibly new undescribed) species: one that was found widespread in coastal waters of southern Madeira, the other present in the bathyal depths of the Azores. None of these two new species revealed any particular phylogenetic relation with other *Aglaophenia* species (Fig. 8.3), further suggesting their distinctiveness. Morphologically, the deep-water species from the Azores can be distinguished by the pronounced intrathecal septum and the more protruding mesial nematotheca (Fig. 8.4A), whereas the taxon from Madeira is peculiar by the prominent mesial tooth of the hydrotheca and the fusion of the mesial nematotheca up to the middle of the abcauline wall of hydrothecae (Fig. 8.4B). These morphological characteristics lend further support to the specific distinctness of these two clades (Fig. 8.4).

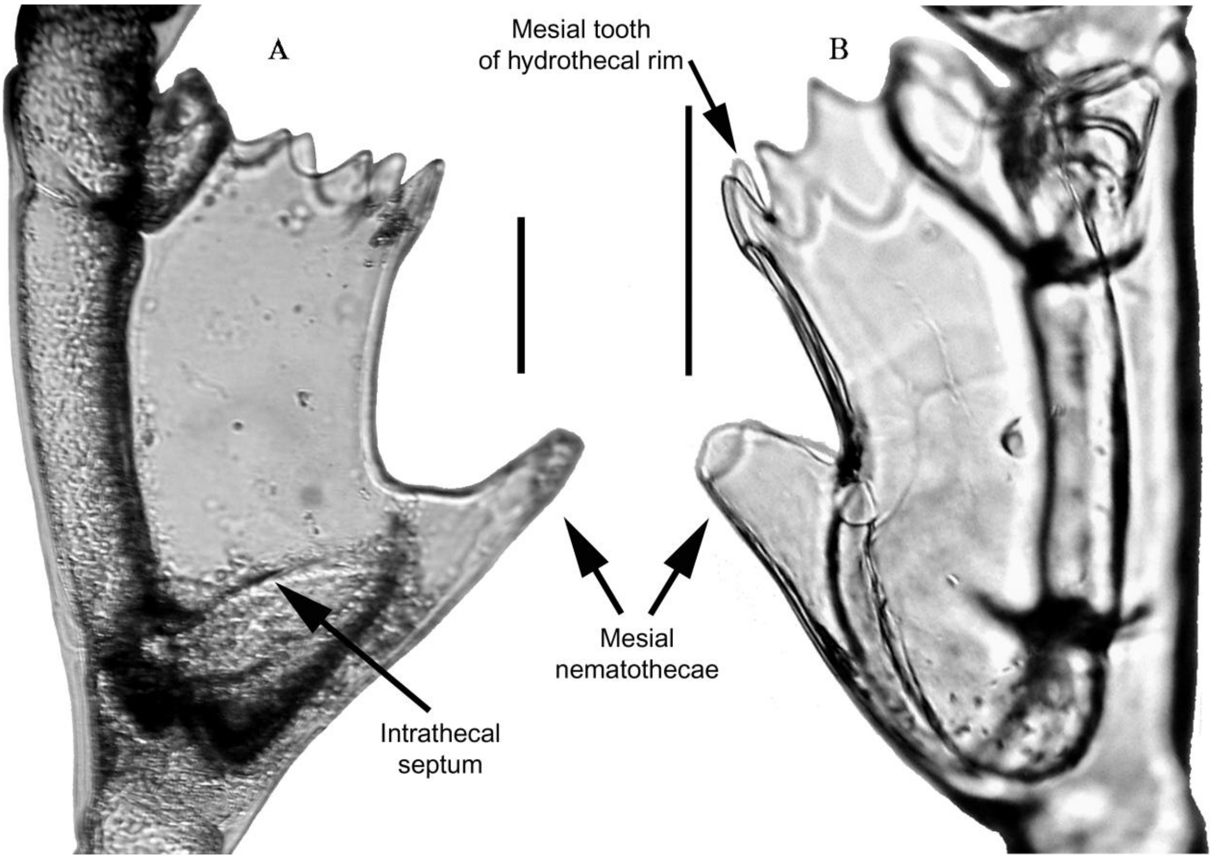

Fig. 8.4. Hydrothecae of *Aglaophenia* species revealed as distinct by genetic data: (A) *Aglaophenia* sp1 from deep waters of the Azores; (B) *Aglaophenia* sp2 from coastal waters of Madeira Island. Scale bars: 0.1 mm. Credits: C.J. Moura.

Few phylogenetic relationships amongst *Aglaophenia* species are well supported by 16S haplotype data (Fig. 8.3). However, it is worth mention that *Aglaophenia elongata* and *Aglaophenia acacia* share the same 16S haplotype (Fig. 8.3), raising the possibility of synonymy of these morphologically similar nominal species. Furthermore, the predictions of Moura *et al*. (2008) that *Aglaophenia tubiformis*, *Aglaophenia octodonta* and *Aglaophenia pluma* are synonymous nominal species are confirmed (Fig. 8.5). This is because specimens presenting the supposedly morphologic diagnostic characters of these three ‘species’ (according to the taxonomic key by Svoboda & Cornelius 1991) do not form reciprocal phylogenetic clusters (Fig. 8.5). All the different lineages identified within that complex of three nominal species (with maximal genetic distance of 2 % between its haplotypes) seem to rather be segregated by geographical region (see Fig. 8.5). We therefore conclude that if any species need to be split within the *Aglaophenia pluma* complex, new diagnostic criteria will have to be found. Also note that *Aglaophenia harpago* Von Schenck, 1965, which is an endemic species from the Mediterranean, and inhabits exclusively the leaves of the seagrasses (*Posidonia* and *Cymodocea*), outgroup and cluster tightly with the complex of *Aglaophenia pluma* (with interspecific genetic distance of 1.1-2.5 %) (Fig. 8.5). However, despite the morphological resemblance between the two taxa, *Aglaophenia harpago* is recovered as monophyletic (Fig. 8.5) and therefore we do not question its validity on the basis of the evidence presented here.

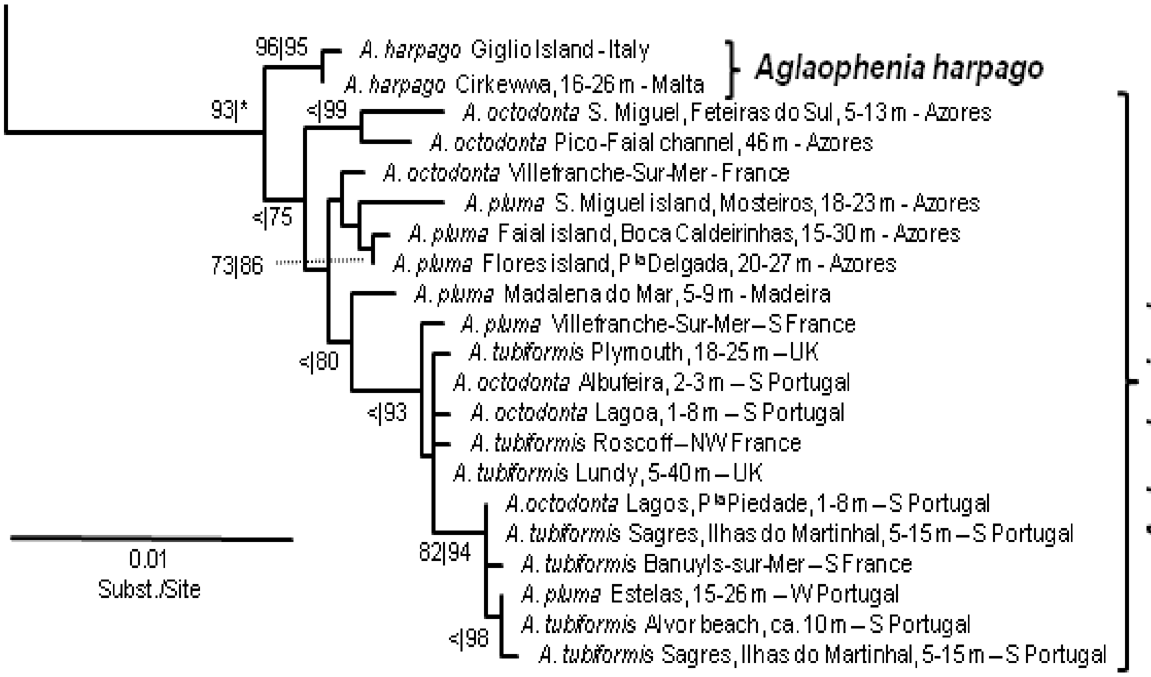

Fig. 8.5. Part of the phylogenetic tree of Fig. 8.3 with all the branches of the *Aglaophenia pluma* complex represented. Numbers near the nodes indicate the values of bootstrap (left) and posterior probabilities (right) in percentages. Values less than 70% are replaced by <; if equal to 100%, an asterisk is used instead. Values of bootstrap and posterior probabilities are omitted if both were less than 70% for the same node. The branch length indicator represents 0.1 substitutions per site.

The intraspecific sequence variation of nominal species of Aglaopheniidae, is usually relatively low (maximum of 2 % for *A. parvula*) and the species *Gymnangium montagui*, *Aglaophenia kirchenpaueri*, *Macrorhynchia philippina* (Kirchenpauer, 1872) and *Streptocaulus dollfusi* present no intraspecific divergence amongst specimens from relatively far geographical locations (Fig. 8.3).

For *G. montagui* the same 16S haplotype is shared between coastal waters of Portugal (from south to north) and southern UK (Fig. 8.3). A relatively similar pattern is detected for *Aglaophenia kirchenpaueri* that presents the same haplotype in specimens from central Portugal, southern U.K and from the peaks of the Gorringe Bank (Fig. 8.3). Geographical distributions of both *G. montagui* and *A. kirchenpaueri* are restricted to shallow waters of the eastern Atlantic (and also west Mediterranean for *Aglaophenia kirchenpaueri*) (e.g. Ansín Agís *et al.* 2001) and therefore these species may have a fairly recent origin and/or suffered recent genetic bottlenecks, reasons that may explain the presence of identical genetic sequences amongst such distant geographical locations. Nevertheless we cannot discount the possibility of recent effective gene flow amongst the localities for which we obtained haplotype data for these two species. Large population sizes and/or oceanic currents or even possibly a slow rate of genetic evolution for these two species may be responsible for such genetic homogeneity.

Macrorhynchia philippina is considered a circumglobal species present in tropical and subtropical oceanic waters (e.g. see Ansín Agís *et al*. 2001). The presence of the same 16S haplotype for this species in Brazil and Madeira (Fig. 8.3) is remarkable. *M. philippina* develops prominent colonies that have potential to be very abundant locally (Moura, pers. observ.). Although the hypotheses mentioned above to explain the sharing of the same 16S haplotypes in *G. montagui* and *Aglaophenia kirchenpaueri* may be valid for *M. philippina*, we hypothesize that this species may be more likely to have been introduced via transport on ship-hulls or in ballast waters and became invasive in Madeira. This hypothesis is supported by the apparent absence of *M. philippina* in the archipelagos of Selvagens and Azores (Moura, pers. observ.). Furthermore, Zenetos *et al*. (2011) also considered *M. philippina* to be a well-established alien species in the eastern Mediterranean.

For *S. dollfusi* we detected the same 16S haplotype in deep waters of the Alboran Sea (west Mediterranean) and Gulf of Cadiz (Fig. 8.3), which probably represents recent (or fairly recent) gene flow between both deep-water areas, analogous to what was found in other bathyal hydroids by Moura *et al.* (2011c, d).

Other phylogeographic associations can be also detected in other taxa, for which a higher representation of haplotypes were obtained, namely for *Aglaophenia parvula*, *Aglaophenia picardi*, *Aglaophenia lophocarpa*, *Aglaophenia tubulifera,* and for the *Aglaophenia pluma* complex (Fig. 8.3).

For *Aglaophenia parvula* two genetically relatively distant haplotypes (with 2 % sequence divergence) were encountered: one from South Africa, the other distributed from the western to the south-western Portuguese coast (Fig. 8.3). It thus seems likely that gene flow may have ceased between Portugal and South Africa for *Aglaophenia parvula,* and that presently gene flow likely occurs between the Portuguese locations from where the species was collected.

Two close haplotypes of *Aglaophenia picardi* were detected (Fig. 8.3), one of which was shared amongst three Azorean islands (São Miguel, Faial and Flores), Madeira and the western Mediterranean. This may result from the influence of Mediterranean waters on these Atlantic islands (e.g. similarly to patterns revealed in molluscs by Ávila 2000).

For *Aglaophenia lophocarpa* five haplotypes were found in a relatively small geographical area. One of these, although apparently more common in the Gulf of Cadiz, was also detected in a mud volcano of the Alboran Sea (similar to the pattern described for *S. dollfusi*) (Fig. 8.3). One haplotype of *Aglaophenia lophocarpa* was exclusively found in a carbonate mound of the Alboran Sea whereas the other three haplotypes of that species were discovered at different bathyal locations of the Gulf of Cadiz (Fig. 8.3). The wide habitat heterogeneity at bathyal depths of the Gulf of Cadiz and Alboran Sea, coupled with past variation in oceanographic conditions, may have promoted the observed high levels of genetic diversity for *Aglaophenia lophocarpa* (see Moura *et al*. 2011c for *Nemertesia* species in the Gulf of Cadiz).

Seven 16S haplotypes of *Aglaophenia tubulifera* were detected in shallow and deep-water specimens from very distant geographical areas, including the coastal waters of Portugal, the UK, and deep waters of the Azores and Gulf of Cadiz. In the Azores, *Aglaophenia tubulifera* was detected only in deep waters (Moura, pers. observ.) and the four haplotypes represented from that geographical area seem to be more related to each other in comparison to the haplotypes from other locations. This provides sound evidence that *Aglaophenia tubulifera* arrived in the Azorean archipelago via deep waters, and that the genetic divergence observed in specimens from that area (Fig. 8.3) might be a consequence of the isolation by distance from other suitable habitats and/or current-topography interactions of the seamounts leading to larval retention. The other deep-water haplotype of *Aglaophenia tubulifera* represented is from the Gulf of Cadiz and is apparently more related to shallow-water specimens of European waters rather than to the deep-water samples from the Azores (Fig. 8.3). The two shallow-water haplotypes of *Aglaophenia tubulifera*: one present in central and northern Portugal, and another from southern UK, diverge little from the deep-water conspecifics represented (Fig. 8.3). Therefore *Aglaophenia tubulifera* constitutes a good example of a species effectively present in both shallow and deep waters.

Within the complex of *Aglaophenia pluma* (also comprising the nominal species *Aglaophenia octodonta* and *Aglaophenia tubiformis*) 17 closely related haplotypes were detected (Fig. 8.5). As previously stated, the segregation of haplotypes within this complex seems to be correlated to their geographical origin rather than to the classification into morphotypes of the nominal species *Aglaophenia pluma*, *Aglaophenia octodonta*, and *Aglaophenia tubiformis* (Fig. 8.5). Two related haplotypes, slightly more divergent from others of the established complex, are respectively from the Central and Eastern group of the Azorean islands (sampling sites are: Pico-Faial channel and São Miguel) (Fig. 8.5). Another clade, with four closely related haplotypes, corresponds to specimens from the three Azorean groups of islands (Central, Eastern and Western) and one from the Mediterranean (Fig. 8.5); again revealing the influence of Mediterranean waters in the Azorean archipelago (as suggested above for *Aglaophenia picardi*). A haplotype from Madeira does not reveal any particular association with other specimens of the complex represented (Fig. 8.5). Another clade of the *Aglaophenia pluma* complex is represented by nine haplotypes: two from the Mediterranean, two from the southern UK (one of these is also present in southern Portugal), one from north-west France, four from southern Portugal, and another from central Portugal (Fig. 8.5). Such high levels of intraspecific variability within the *Aglaophenia pluma* complex seem to be relatively recent and may possibly reflect the effects of the past glaciations that impacted Europe (similarly to the results also found for *Nemertesia* and *Plumularia* species by Moura *et al.* 2011c).

Finally, some specimens collected specifically for the present study increase the known geographical and bathymetrical range of some species herein reported. For the first time *Aglaophenia kirchenpaueri* has been recorded from the Gorringe Bank, *S. multiseptatus* is found in the Gulf of Cadiz, *L. myriophyllum* has been recorded in the Alboran Sea, and *C. paraformosus*, previously known only from one locality (905 m depth) off the northern coast of Iceland (Schuchert, 2000), has now been recorded in the waters of Greenland at about 2000 m. The deepest bathymetric record of *S. dollfusi* has also been extended from 380 m (Billard 1934) to 600 m; *Aglaophenopsis cartieri* had its shallowest bathymetrical record at 318 m (Bedot 1921) and has been herein recorded at 307 m, and *G. montagui* that was only known from a maximum depth of 20 m (Medel & Vervoort 1995) was herein found at depths of around 30 m.

8.4 CONCLUSIONS

The present study analysed 98 Aglaopheniidae sequences (68 of which were new) of the mitochondrial gene 16S rRNA, corresponding to 25 putative species (25 nominal and three undescribed) of seven nominal genera. The family Aglaopheniidae was recovered as monophyletic with four divergent and well-supported main clades that rejected the monophyly of the subfamilies Gymnangiinae and Aglaopheniinae, and tribes Aglaopheniini and Cladocarpini as recently proposed by Calder (1997) based on the morphology of reproductive structures. The four main evolutionary branches found within Aglaopheniidae are: (1) *Streptocaulus* species; (2) *G. hians* and *G. montagui*, that may demarcate the subfamily Gymnangiinae; (3) *Cladocarpus* and *Aglaophenopsis* species; and (4) *G. gracilicaule* with species of *Aglaophenia*, *Macrorhynchia* and *Lytocarpia.* Despite being genetically distinct, the first two aforementioned clades cluster together; the other two clades have uncertain phylogenetic relationships within Aglaopheniidae. Note however that the 16S is a fast-evolving marker and may not be the most appropriate to resolve phylogenetic relationships above the taxonomic level of genus. Therefore the above-mentioned phylogenetic relations of Aglaopheniidae at high taxonomic ranks are provisional (but in agreement with phylogenetic hypotheses of Leclère *et al*. 2009), and further genetic sampling is required.

The Aglaopheniidae genera *Gymnangium* and *Aglaophenia* were found to be monophyletic only with the exclusion and taxonomic revision of the biological material represented by the sequences of *G. gracicaule* and *Aglaophenia latecarinata*, respectively for each genus. The genera *Cladocarpus* and *Streptocaulus*, although not represented in this study by the type species of both genera, were recovered as monophyletic, and clearly distinct genetically from each other, despite their morphological resemblances. The monophyletic nature of the genera *Macrorhynchia* and *Lytocarpia* was not refuted because of highly divergent clades among these genera and because of the consequent low nodal support involved with their corresponding lineages. The genus *Aglaophenopsis* represented only by *Aglaophenopsis cartieri* was genetically distinct from the sister genus *Cladocarpus*.

The genus *Aglaophenia* was the best-characterized in our analyses with representatives of all the 11 species known from European and Mediterranean waters (sensu Svoboda & Cornelius 1991), *Aglaophenia latecarinata*, plus two undescribed species (possibly new to science): one from shallow waters of Madeira, the other from deep waters of the Azores. Apart from *Aglaophenia latecarinata*, all other species of *Aglaophenia* cluster together and are easily distinguished in a phylogram and by the monophyly of species with concordant morphological diagnostic characters. However, the nominal species *Aglaophenia pluma*, *Aglaophenia tubiformis* and *Aglaophenia octodonta* were not recognized as monophyletic and share very small genetic distances, suggesting that these may be conspecific. In case of established synonymy, the name *A. pluma* has priority (Moura *et al*. 2008). Specimens referred in Leclère *et al*. (2009) to *A. acacia* and *A. elongata*, using material identified by A. Svoboda, share the same 16S haplotypes and therefore are likely to be conspecific. Within the genus *Aglaophenia*, the only other interspecific relationship investigated was that between *Aglaophenia harpago* and the complex of *Aglaophenia pluma*, that are very close genetically.

The relatively good haplotype representation obtained for certain taxa also highlighted phylogeographical associations in coastal and deep waters of the north-east Atlantic and the west Mediterranean. Probable evidence of recent gene flow, supported by shared 16S sequences, were revealed between the following localities: (1) the southern UK and southern and central Portugal (for *G. montagui* and *Aglaophenia kirchenpaueri*); (2) southern and western Portugal (for *Aglaophenia parvula*); (3) western and northern Portugal (for *Aglaophenia tubulifera*); (4) Azorean islands (São Miguel, Faial, and Flores), Madeira, and the western Mediterranean (for *Aglaophenia picardi*); (5) deep waters of the Alboran Sea and Gulf of Cadiz (for *S. dollfusi* and *Aglaophenia lophocarpa*). The presence of the same 16S genotype of *M. philippina* in Brazil and Madeira was interpreted as the result of an unintentional introduction of that species in Madeira possibly by shipping, whereas the phylogeographical patterns verified for *G. montagui* and *Aglaophenia kirchenpaueri* may be associated to fairly recent origins and/or recent genetic bottlenecks perhaps enhanced by the last glacial cycle that affected Europe. The phylogeographical patterns verified for *Aglaophenia picardi*, and to some extent for the *Aglaophenia pluma* complex, probably reveal the influence of oceanic currents in the relatively recent gene flow amongst shallow waters of the Mediterranean, Madeira, and the Azores.

Relatively high haplotype diversity was encountered in the Gulf of Cadiz (e.g. for *Aglaophenia lophocarpa*) and coastal European waters (e.g. for *Aglaophenia pluma*). The latter may be explained by the effects of previous glaciations in the Northern hemisphere causing fragmentation of populations followed by secondary contact. The former may be explained by the consequence of the habitat heterogeneity and complex oceanographic settings in the Gulf of Cadiz.

Finally the 16S sequence data were consistent with the morphologically defined species and indicated the existence of two unnamed species and the possible conspecificity of two species groups. The partial 16S gene sequence is therefore suitable as a ‘DNA barcode’ marker for aglaopheniids. Further genetic representation of the other species of this diverse family should resolve problems of synonymy and other doubts in taxonomic assignment, and should also clarify the true diagnostic morphological characters for species and genera. Greater sampling of haplotypes from other localities and the calibration of the ‘molecular clock’ of the 16S gene for the Aglaopheniidae will also probably contribute to a better understanding of the actual diversity in oceans and of past events such as alterations in the circulation of water currents and the effects of climatic change.

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Table 8.1. 16S rRNA sequence data. Sequences of specimens marked with asterisk were determined in the present study.

Species	**GenBank accession Number**	**Voucher specimen**	**Cruise/collector**	**Collection method**	**Sample location**
Gymnangium hians	AY787922 (C.W. Cunningham, EMBL submission)	MHNG INVE32586			Pee Pee Island, Andaman Sea Thailand
Gymnangium montagui *	JN560075	DBUA 1235.01	C.J. Moura	Hand	Berlengas, Rinchão, 25-35 m 39º25.437'N 9º29.941'W W Portugal
G. montagui	AM888313 (Moura *et al*. 2008)	DBUA 945.01	J. Hall-Spencer	Hand	Lundy Island, 5-40 m 51º09.24'N 4º39.18'W United Kingdom
G. montagui *	JN560076	DBUA 1236.01	C.J. Moura	Hand	Sines, Porto Covo, 15-20 m 37°51.192'N 8º48.211'W SW Portugal
G. montagui *	JN560077	DBUA 1235.02	C.J. Moura	Hand	Berlengas, Estelas, 15-26 m 39º25.225'N 9º32.084'W W Portugal
G. montagui *	JN560078	DBUA 1236.02	C.J. Moura	Hand	Sagres, P^{ta} de Sagres, 17-22 m 36º59.700'N 8º56.715'W S Portugal
G. montagui *	JN560079	DBUA 1237.01	C.J. Moura	Hand	Off Viana do Castelo, 25-33 m 41º37.379'N 8º53.805'W NW Portugal
G.gracilicaule	DQ855934 (Leclère *et al*. 2007)	MHNG INVE36839			Nosy Ranj Madagascar
Streptocaulus multiseptatus *	JN560080	DBUA 1238.01	Maria S. MERIAN No. 1 Leg 3	GKG	Mercator MV, Stn 241, 353 m 35°17.918'N 6°38.717'W Gulf of Cadiz
Streptocaulus dollfusi *	JN560081	DBUA 1239.01	R/V Pelagia M2007	Box-corer	Unknown Carbonate Province, Stn 41, 461 m 35°00.113'N 6°44.815'W Gulf of Cadiz
S. dollfusi *	JN560082	DBUA 1240.01	R/V Prof. Logachev TTR17-leg1	Grab	Granada MV, Stn 420, 600 m 35º33.854'N 4º37.435'W Alboran Sea
S. dollfusi *	JN560083	DBUA 1241.01	James Cook 10	ROV	NE Mercator MV, Stn Dive 27 Rock 2, 432 m 35°18.823'N 6°37.377'W Gulf of Cadiz
S. dollfusi *	JN560084	DBUA 1240.02	R/V Prof. Logachev TTR17-leg1	Grab	Melilla Carbonate Mound, Stn 395, 300 m 35º26.415'N 2º31.075'W Alboran Sea
Aglaophenopsis cartieri *	JN560085	DOP C1407	F/V Conde de Porto Pim	Hand-line	Azores Bank, 402 m 38°13.440'N 28°59.700'W Azores
Ag. cartieri *	JN560086	DOP C1431	F/V Neuza Mar	Palangre de fundo	Azores Bank,307 m 38°06.480'N 29°00.720'W Azores
Cladocarpus paraformosus	AM887993 (Moura *et al*. 2008)	DBUA 1242.01	R/V Prof. Logachev TTR13	Dredge	Canyon area, Stn 487, 1940–2080 m 63°18.5400'N 55°27.195'W Greenland
Cladocarpus integer	FJ550512 (Leclère *et al*. 2009)	MHNG INVE48754			Raunefjord Norway
Macrorhynchia phoenicea	DQ855935 (Leclère *et al*. 2007)	MHNG INVE36813			Sakatia Madagascar
Macrorhynchia sibogae	FJ550500 (Leclère *et al*. 2009)	MHNG INVE36832			Nosy Ranj Madagascar
Macrorhynchia philippina	DQ855937 (Leclère *et al*. 2007)				Ilhabela Brazil
M. philippina *	JN560087	DBUA 1243.01	C.J. Moura	Hand	Madeira Island, Lido, B^{xa} do Carneiro, 10-15 m 32°38.127'N 16°55.909'W Madeira
M. philippina *	JN560088	DBUA 1243.02	C.J. Moura	Hand	Madeira Island, Porto da Cruz, 4-12 m 32°46.516'N 16°49.585'W Madeira

Aglaophenia latecarinata	DQ855936 (Leclère *et al*. 2007)				São Sebastião, Ponta do Baleeiro Brazil
Lytocarpia phyteuma	AY787921 (C.W. Cunningham, EMBL submission)	MHNG INVE32597			Andaman Sea, Ko Pee Pee Thailand
Lytocarpia sp.	FJ550505 (Leclère *et al*. 2009)	MHNG INVE36828			Sakatia Madagascar
Lytocarpia myriophyllum *	JN560089	DBUA 1244.01	R/V Prof. Logachev TTR17-leg1	Gravity-corer	Melilla Carbonate Mound, Stn 393B, 245 m 35º19.799’N 2º33.067’W Alboran Sea
L. myriophyllum *	JN560090	DBUA 1245.01	R/V Pelagia M2007	Box-corer	Pen Duick escarpment, Stn 23, 498 m 35°17.772'N 6°43.964'W Gulf of Cadiz
L. myriophyllum *	JN560091	MMF 39095		Covo	Off Caniçal, Stn. 34, 600 m Madeira
L. myriophyllum *	JN560092	C2377	R/V Arquipelago ARQDAÇO-27-P07	Palangre de fundo	Azores Bank, 258 m 38°13.200'N 29°02.400'W Azores
L. myriophyllum *	JN560093	C2647	R/V Arquipelago ARQDAÇO-27-P07	Palangre de fundo	Azores Bank, 434 m 38°13.200'N 28°55.200'W Azores
Aglaophenia sp1 *	JN560094	C1517	F/V Manuel Arriaga	Espinhel	Azores Bank (35 miles), 468 m 38°36.000'N 28°53.340'W Azores
A. sp1 *	JN560095	C1223	F/V Baia das Lajes		Condor Bank, 238 m 38°30.840'N 28°59.280'W Azores
A. sp1 *	JN560096	C1932	F/V Baia das Lajes	Espinhel	Condor Bank, 240 m 38°31.320'N 29°01.620'W Azores
Aglaophenia parvula	DQ855914 (Leclère *et al*. 2007)	MHNG INVE34013			False Bay, Simons Town South Africa
A. parvula *	JN560097	DBUA 1246.01	C.J. Moura	Hand	Aljezur, island, 2-11 m 37°10.158'N 8°54.226'W SW Portugal
A. parvula *	JN560098	DBUA 1246.02	C.J. Moura	Hand	Cascais, Boca do Inferno, 5-12 m 38°41.442'N 9°25.866'W W Portugal
A. parvula *	JN560099	DBUA 1247.01	C.J. Moura	Hand	Berlenga Island, Ilhéu do Soldado, 5-16 m 39º24.981’N 9º30.784’W W Portugal
A. parvula *	JN560100	DBUA 1246.03	C.J. Moura	Hand	Ericeira, Coxos, 4-8 m 39º00.151’N 9º25.672’W W Portugal
Aglaophenia sp2 *	JN560101	DBUA 1248.01	C.J. Moura	Hand	Madeira Island, Caniçal, 4-18 m 32º44.094'N 16º44.43'W Madeira
A. sp2 *	JN560102	DBUA 1248.02	C.J. Moura	Hand	Madeira Island, Machico,6-8 m 32º41.333'N 16º46.248'W Madeira
A. sp2 *	JN560103	DBUA 1248.03	C.J. Moura	Hand	Madeira Island, Lido, Funchal naval club, 3-13 m 32º38.046’N 16º56.351’W Madeira
A. sp2 *	JN560104	DBUA 1248.04	C.J. Moura	Hand	Madeira Island, Lido, B^{xa} do Carneiro, 10-15 m 32°38.127'N 16°55.909'W Madeira
Aglaophenia picardi	AY787891 (C.W. Cunningham, EMBL submission)	MHNG INVE29758			Mallorca, Cala Murada S Spain
A. picardi *	JN560105	DBUA 1249.01	C.J. Moura	Hand	São Miguel Island, Mosteiros, 18-23 m 37º53.372’N 25º50.132’W Azores

A. picardi *	JN560106	DBUA 1249.02	C.J. Moura	Hand	Faial Island, Horta port, 5-18 m 38°32.015'N 28°37.304'W Azores
A. picardi *	JN560107	DBUA 1250.01	C.J. Moura	Hand	Madeira Island, Ribeira Brava, fishing port, 3-8 m 32º40.086’N 17º03.781’W Madeira
A. picardi *	JN560108 JN560109	DBUA 1249.03	C.J. Moura	Hand	Flores Island, 10-16 m 39º28.531’N 31º08.479’W Azores
A. picardi *	JN560110	DBUA 1249.04	C.J. Moura	Hand	S. Miguel Island, V. Franca do Campo, 8-18 m 37º42.332'N 25º26.755'W Azores
A. picardi *	JN560111	DBUA 1249.05	C.J. Moura	Hand	Flores Island, islet, 5-30 m 39º29.044’N 31º08.739’W Azores
Aglaophenia lophocarpa *	JN560112	DBUA 1251.01	R/V Prof. Logachev TTR17-leg1	Grab	Melilla Carbonate Mound, Stn 395, 300 m 35º26.415’N 2º31.075’W Alboran Sea
A. lophocarpa	AM887985 (Moura *et al*. 2008)	DBUA 900.02	R/V Prof. Logachev TTR15	Grab	Meknès, Stn 581, 700 m 34°59.182'N 7°04.344'W Gulf of Cadiz
A. lophocarpa *	JN560113	DBUA 1252.01	R/V Pelagia M2007	Box-corer	Carbonate Province, Stn 40Bx, 473 m 35°00.137'N 6°44.806'W Gulf of Cadiz
A. lophocarpa *	JN560114	DBUA 1252.02	R/V Pelagia M2007	Box-corer	Unknown Carbonate Province, Stn 41, 461 m 35°00.113'N 6°44.815'W Gulf of Cadiz
A. lophocarpa *	JN560115	DBUA 1253.01	Maria S. MERIAN No. 1 Leg 3	GKG	Mercator MV, Stn 242, 350 m 35º17.870'N 6º38.810'W Gulf of Cadiz
A. lophocarpa	AM887988 (Moura *et al*. 2008)	DBUA 900.01	R/V Prof. Logachev TTR15	Gravity-corer	Mercator MV, Stn 575, 355 m 35º17.903'N 6º38.715'W Gulf of Cadiz
A. lophocarpa	AM887987 (Moura *et al*. 2008)	DBUA 1253.02	Maria S. MERIAN No. 1 Leg 3	GKG	Mercator MV, Stn 242, 350 m 35º17.870'N 6º38.810'W Gulf of Cadiz
A. lophocarpa *	JN560116	DBUA 1251.02	R/V Prof. Logachev TTR17-leg1	Grab	Dakhan MV, Stn 412, 360 m 35°25.423'N 4°31.829'W Alboran Sea
A. lophocarpa	AM887986 (Moura *et al*. 2008)	DBUA 1254.01	R/V Pelagia M2006	Piston core	Pen Duick Escarpment, Stn 8, 444 m 35°16.750'N 6°45.720'W Gulf of Cadiz
A. lophocarpa	AM887984 (Moura *et al*. 2008)	DBUA 899.01	R/V Prof. Logachev TTR14	Grab	Kidd MV, Stn 528, 489 m 35°24.973'N 6°43.972'W Gulf of Cadiz
Aglaophenia acacia	FJ550507 (Leclère *et al*. 2009)				Giglio Island Italy
Aglaophenia elongata	FJ550508 (Leclère *et al.* 2009)	MHNG INVE37539			Giglio Island Italy
Aglaophenia tubulifera	AM887992 (Moura *et al*. 2008)	DBUA 938.01	J. Hall-Spencer	Hand	Lundy Island, 5-40 m 51º09.400’N 4º39.300’W United Kingdom
A. tubulifera	AM887991 (Moura *et al*. 2008)	DBUA 906.01	R/V Prof. Logachev TTR11	Dredge	Guadalquivir Ridge, Stn 339, 1021 m 36°07.591'N 7°46.587'W Gulf of Cadiz
A. tubulifera *	JN560117	DBUA 1255.01	C. J. Moura	Hand	Berlengas, Estelas, Baixa Alagada, 30-38 m 39º25.477'N 9º32.120'W W Portugal
A. tubulifera *	JN560118	DBUA 1256.01	C.J. Moura	Hand	Off Viana do Castelo, 25-33 m 41º37.379’N 8º53.805’W NW Portugal
A. tubulifera *	JN560119	DBUA 1255.02	C.J. Moura	Hand	Berlengas, Rinchão, 25-35 m 39º25.437’N 9º29.941’W W Portugal

A. tubulifera *	JN560120	C1496	F/V Manuel Arriaga	Espinhel	Azores Bank (32 miles), 368 m 38°16.800'N 28°51.660'W Azores
A. tubulifera *	JN560121	C1374	F/V Manuel Arriaga	Gorazeiras	Princess Alice Bank, 201 m 37°57.360'N 29°12.840'W Azores
A. tubulifera *	JN560122	C2087	F/V Renato Alexandre	Palangre de fundo	Pico Island, 311-348 m 38°22.200'N 28°13.320'W Azores
A. tubulifera *	JN560123	C2111	Fishing fleet of Horta		Pico-Faial channel Azores
Aglaophenia kirchenpaueri	AM887982 (Moura *et al*. 2008)	DBUA 937.01	J. Hall-Spencer	Hand	Lundy Island, 5-40 m 51º09.400’N 4º39.300’W United Kingdom
A. kirchenpaueri	AM887983 (Moura *et al*. 2008)	DBUA 1257.01	J. Hall-Spencer	Hand	Plymouth, 5-40 m United Kingdom
A. kirchenpaueri *	JN560124	DBUA 1258.01	LusoExpedição Olympus 2007 / Joana Xavier	Hand	Gettysburg, 35-37 m 36º31.092'N 11º34.284'W Gorringe Bank
A. kirchenpaueri *	JN560125	DBUA 1259.01	C.J. Moura	Hand	Berlenga Island, Ilhéu do Soldado, 5-16 m 39º24.981’N 9º30.784’W W Portugal
A. kirchenpaueri *	JN560126	DBUA 1258.02	LusoExpedição Olympus 2008 / C.J. Moura	Hand	Ormonde, 35-37 m 36º42.857'N 11º09.968'W Gorringe Bank
A. kirchenpaueri *	JN560127	DBUA 1258.03	LusoExpedição Olympus 2008 / José Pedro Borges	Hand	Gettysburg, 37-39 m 38º31.071'N 11º34.275'W Gorringe Bank
A. kirchenpaueri *	JN560128	DBUA 1259.02	C.J. Moura	Hand	Berlengas, Berlenga Island, 5-14 m 39°24.540'N 9°30.708'W W Portugal
Aglaophenia harpago	FJ550506 (Leclère *et al*. 2009)	MHNG INVE37531			Giglio Island Italy
A. harpago *	JN560129	DBUA 1260.01	C.J. Moura	Hand	Cirkewwa, 16-26 m 35°59.348'N 14°19.680'E Malta
Aglaophenia octodonta / pluma *	JN560130	C3021	R/V Águas Vivas / Valentina Matos		Pico-Faial channel, 46 m Azores
A. octodonta / pluma *	JN560131	DBUA 1261.01	C.J. Moura	Hand	São Miguel Island, Feteiras do Sul, 5-13 m 37º42.836’N 25º29.756’W Azores
A. pluma *	JN560132	DBUA 1261.02	C.J. Moura	Hand	São Miguel Island, Mosteiros, 18-23 m 37º53.372’N 25º50.132’W Azores
A. pluma *	JN560133	DBUA 1261.03	C.J. Moura	Hand	Flores Island,Ponta Delgada, 20-27 m 39º31.092’N 31º12.437’W Azores
A. pluma *	JN560134	DBUA 1261.04	C.J. Moura	Hand	Faial Island, Boca das Caldeirinhas, 15-30 m 38º30.938'N 28º37.625'W Azores
A. pluma *	JN560135	DBUA 1262.01	C.J. Moura	Hand	Madeira Island, Madalena do Mar, 5-9 m 32°42.166'N 17°08.385'W Madeira
A. pluma *	JN560136	DBUA 1263.01	C.J. Moura	Hand	Berlengas, Estelas, 15-26 m 39º25.225’N 9º32.084’W W Portugal
A. tubiformis / pluma *	JN560137 JN560138	DBUA 1264.01	C.J. Moura	Hand	Sagres, Ilhas do Martinhal, 5-15 m 37º00.897’N 8º55.080’W S Portugal
A. tubiformis / pluma *	JN560139	DBUA 1264.02	C.J. Moura	Hand	Alvor beach, *ca*. 10 m 37º07.000’N 8º35.000’W S Portugal

A. octodonta / pluma *	JN560141	DBUA 1264.03	C.J. Moura	Hand	Lagos, Ponta da Piedade, 1-8 m 37°04.753'N 8°40.088'W S Portugal
A. tubiformis / pluma *	JN560140	DBUA 1265.01	C. J. Moura	Hand	Plymouth, Mewstone, 18-25 m 50º17.60’N 4º05.60’W United Kingdom
A. octodonta / pluma *	JN560142	DBUA 1264.04	C.J. Moura	Hand	Albufeira, Olhos d’Água beach, 2-3 m 37°05.319'N 8°11.452'W S Portugal
A. octodonta / pluma	DQ855915 (Leclère *et al*. 2007)	MHNG INVE32875			Villefranche-sur-Mer S France
A. octodonta / pluma	AM887989 (Moura *et al*. 2008)	DBUA 1264.05	C.J. Moura	Hand	Lagoa, Carvoeiro, 1-8 m 37º05.419’N 8º27.269’W S Portugal
A. pluma	DQ855916 (Leclère *et al*. 2007)	MHNG INVE38220			Villefranche-sur-Mer France
A. tubiformis / pluma	DQ855917 (Leclère *et al*. 2007)	MHNG INVE32960			Banyuls-sur-Mer France
A. tubiformis / pluma	AY787914 (C.W. Cunningham, EMBL submission)	MHNG INVE29967			Roscoff France
A. tubiformis / pluma	AM887990 (Moura *et al.* 2008)	DBUA 1266.01	J. Hall-Spencer	Hand	Lundy Island, 5-40 m 51º09.400’N 4º39.300’W United Kingdom

Abbreviations: DBUA, ‘Departamento de Biologia da Universidade de Aveiro’; DOP, ‘Departamento de Oceanografia e Pescas’; F/V, Fishing Vessel; M, oceanographic campaign ‘Moundforce’; MV, mud volcano; MMF, ‘Museu Municipal do Funchal’; MHNG, Muséum d’Histoire Naturelle de Genève, Switzerland; PE, Pelagia; ROV, Remote Operated Vehicle; R/V, Research Vessel; Stn, station; TTR, oceanographic campaign ‘Training Trough Research’

Chapter 9

EVOLUTION OF *NEMERTESIA* HYDROIDS (CNIDARIA: HYDROZOA, PLUMULARIIDAE) FROM THE SHALLOW AND DEEP WATERS OF THE NE ATLANTIC AND WESTERN MEDITERRANEAN

Moura, C. J., Cunha, M. R., Porteiro, F. M., Yesson, C. & Rogers, A. D. (2011c). *Zoologica Scripta* [in press].

Contents

ABSTRACT

Hydroid species from the genus *Nemertesia* develop some of the largest and most complex hydrozoan colonies. These colonies are abundant and ecologically important in both shallow and deep waters worldwide. Here we analyse the systematics of most *Nemertesia* species from the NE Atlantic and Mediterranean using morphology and phylogenetic inferences of 16S rRNA haplotype data. Phylogeographical analysis revealed multiple movements of taxa to and from the Mediterranean after the Messinian salinity crisis through shallow and deep waters. The nominal species *Nemertesia belini* and *Nemertesia antennina* revealed multiple genetic lineages representing cryptic species diversity. Molecular phylogenetic evidence was supported by consistent phenotypic differences between lineages and three and seven putative species were resolved within the *N. belini* and *N. antennina* complexes respectively. Three putative species of the *N. antennina* complex found at different seamounts of the Azores grouped in a clade clustered amongst the other four cryptic species present at neighbouring bathyal localities of the Gulf of Cadiz. These cryptic species, mostly from the deep sea, form a clade distantly related to the typical *N. antennina* from European coastal waters. Depth or environmental correlates of depth seem to influence the reproductive strategies of *Nemertesia* colonies and ultimately speciation. In particular, speciation of these hydroids must have been influenced by hydrography, habitat heterogeneity, isolation by distance and larval dispersal capacity. The deep sea is shown as an important environment in the generation and accumulation of lineages that may occasionally invade coastal waters in the NE Atlantic. Glacial cycles of cooling, along with changes in sea level, and eradication of some coastal faunas likely facilitated speciation and evolutionary transitions from deep to shallow waters.

9.1 INTRODUCTION

The Hydrozoa live in a wide range of aquatic systems (mainly marine) and are the most diverse medusozoan Class with around 3700 described species (Bouillon *et al.* 2006). They have a great variety of forms, provide substratum for the settlement of other taxa and are planktonic and benthic predators that play an important role in food webs (Bouillon *et al.* 2006). Given both the ecological significance and taxonomic difficulties of the hydrozoan group, the clarification of their true diversity and a better understanding of their evolution are important. An incorrect discrimination of taxa may negatively influence ecological, biogeographic, evolutionary and applied biotechnological studies, as well as management and conservation planning (e.g. Knowlton 2000; Bickford *et al.* 2007). However, like in many marine invertebrate groups, the taxonomy of hydroids is poorly resolved and taxonomic expertise is diminishing. A paucity of diagnostic morphological characters is often found to categorise taxa, a consequence of their structural simplicity. Furthermore the high levels of morphological plasticity that hydrozoans display often make it difficult to differentiate intraspecific from interspecific variation.

The advent of molecular techniques has made available additional characters for objective discrimination of taxa. As a result, cryptic or pseudocryptic species have been discovered across nearly all metazoan phyla (e.g. reviews of Knowlton 2000; Bickford *et al.* 2007; Trontelj & Fišer 2009). In the Cnidaria, reported cases of cryptic diversity are comparatively low compared with other major metazoan clades, such as the Porifera (Trontelj & Fišer 2009**)**. The limited discovery of cryptic species in cnidarians may result from a slow rate of genetic evolution, and/or it may be a consequence of regular hybridisation (reviewed by Knowlton 2000). However, past studies have primarily focused on Anthozoa but the Hydrozoa have attracted relatively little attention. Recent studies have revealed cryptic or pseudocryptic species within the Hydrozoa (Govindarajan *et al*. 2005; Schuchert 2005; Miglietta *et al.* 2007, 2009; Moura *et al*. 2008; Folino-Rorem *et al*. 2009), suggesting that hydrozoan diversity might be significantly underestimated. Furthermore, vast geographic areas remain unexplored and poorly sampled for Hydrozoa, including most of the deep sea. Despite a long history of research on hydrozoan systematics, dating back to the pre-Linnaean times, new taxa, even at the level of families are still being discovered (e.g. Moura *et al*. 2007).

Moura *et al*. (2008) revealed the potential of the mitochondrial 16S mRNA gene to distinguish hydrozoan species and reported cryptic diversity within the genus *Nemertesia* Lamouroux, 1812. Species of *Nemertesia* usually develop prominent, complex, arborescent sessile colonies and are frequently present, sometimes with high abundance, in oceans globally, from abyssal depths to shallow coastal waters. *Nemertesia* species are mainly found on soft bottoms, but can also overgrow hard substrata including rocks, shells or coral debris. An individual colony of *Nemertesia* usually consists of a basal network of reticulate tubules (hydrorhiza) that serve as holdfast of a hydrocaulus (stem) with hydrocladia (lateral branches) carrying polyps for feeding (hydrothecae) and defensive and predatory functions (nematothecae) (see Fig. 9.1). The hydrocauli, generally also covered with nematothecae, are divided into internodes that usually bear apophyses that support the hydrocladia arranged alternately or typically on whorls (see Fig. 9.1A,B, or check glossary of morphological features in Bouillon *et al*. 2006). The hydrocladia are also subdivided into internodes: thecates with hydrothecae and nematothecae, and sometimes also athecates without hydrothecae but usually with nematothecae (see Fig. 9.1B,C). Gonothecae (the reproductive structures) are sessile, usually elongated and ovoid, and borne singly or in pairs in apophyses (see Fig. 9.1B). Some *Nemertesia* species may display sexual dimorphism, revealed by the contours of gonothecae (e.g. Ansín Agís *et al*. 2001; Ramil & Vervoort 2006). The morphological characterisation of *Nemertesia* species includes the presence or absence of stem branching, the segmentation mode of hydrocladia, the number and arrangement of nematothecae on hydrocladial internodes, the form of gonothecae and hydrothecae, and the dimensions of structures such as the hydrothecae (Ramil & Vervoort 2006). The phenotypic plasticity of the diagnostic characters is high, and taxonomy within *Nemertesia* has been controversial for a long time. Presently, nearly sixty species of *Nemertesia* are accepted (Ramil & Vervoort 2006), but consensus about the validity of many of these or other currently synonymised species is still far from being achieved (e.g. Bouillon *et al*. 2006 recognized only forty *Nemertesia* species). In this sense, the genus exemplifies many of the systematic problems associated with the Class as a whole.

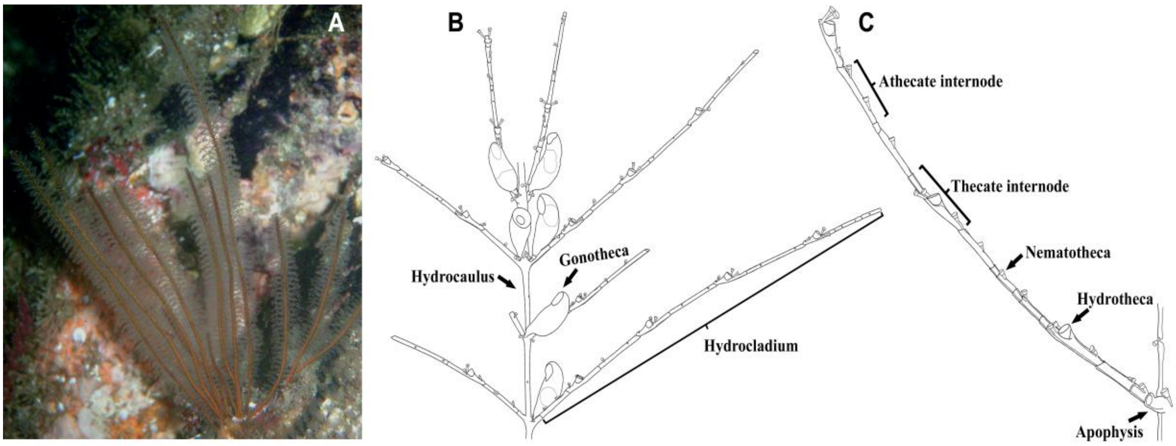

Fig. 9.1. Characteristics of *Nemertesia*: (A) aggregation of conspecific colonies of *N. antennina* at Berlengas, 15-25 m; (B) Part of a fertile colony of *N*. ‘sp2’; (C) basal hydrocladium of a *N*. ‘sp6’ specimen. Credits: (A) C.J. Moura, (B, C) C.J. Moura and J. Oliveira.

Little is known about the life-histories of *Nemertesia* species, although some details of *N. ramosa* (Lamarck, 1816) and *N. antennina* (Linnaeus, 1758) are known (Hughes 1975, 1977; Williams 1976). Like most hydroids, *Nemertesia* species do not develop free-swimming medusae, instead colonies release planulae (e.g. Millard 1975; Williams 1976; Hughes 1977). The short-lived planula larvae are not active swimmers but represent the only mobile stage of these hydroids that are locally dispersed by currents (Hughes 1977). Close proximity is required to achieve routine cross-fertilisation, and once planulae are released, long-distance dispersal away from the natal conspecific colonies is therefore probably highly limited. Such life-history traits suggest that dispersal in *Nemertesia* likely fits a stepping-stone model.

In contrast to many other hydroids, and except for *N. antennina* that has been recorded from both sides of the Atlantic, the Mediterranean, the Pacific and Indian oceans (see Ansín Agís *et al.* 2001), *Nemertesia* species typically do not show large distributional ranges (cf. Ramil & Vervoort 2006). The NE Atlantic and the Mediterranean *Nemertesia* species are particularly abundant in cooler waters, at high latitudes and/or in deep waters, but can also be found, usually at lower abundances in shallow depths of temperate/subtropical waters (C.J. Moura, pers. observ.). Given such a widespread vertical and horizontal distribution, the genus *Nemertesia* may also be a good candidate to study the evolutionary relationships between shallow- and deep-water forms.

Aims

This study uses the mitochondrial 16S mRNA gene along with morphological data to investigate cryptic diversity in *Nemertesia*. The focus is on processes causing speciation, phylogeographic affinities and evolution of the genus *Nemertesia* in the Atlantic and Mediterranean with respect to past geological and environmental events and in the context of the global diversity of these hydroids.

9.2 MATERIALS AND METHODS

Collection and identification of samples

Nemertesia colonies were collected from 60 sampling sites of the north-east Atlantic and west Mediterranean (Fig. 9.2, Table 9.1). Specimens were fixed and preserved mostly in 96% ethanol, although sometimes in 70% ethanol. The material collected in the Azores and Madeira included specimens obtained as part of a study on by-catch from deep-sea fisheries and, in some cases, colonies were frozen prior to fixation in ethanol. Taxa were identified by C.J. Moura following Ramil & Vervoort (1992, 2006) and Ansín Agís *et al*. (2001). Measurements and other observations of putative diagnostic characters were undertaken on representatives identified under the nominal species *N. antennina* (Tables 9.2, 9.3, 9.5; Fig. 9.5) and *N. belini* Bedot, 1916 (Tables 9.4, 9.5), using calipers and a graticule on a compound stereo microscope. Morphological data were analysed by means of a Principal Component analyses (PCA) executed in *R* environment (version 2.8.1, The *R* Foundation for Statistical Computing; http://www.r-project.org).

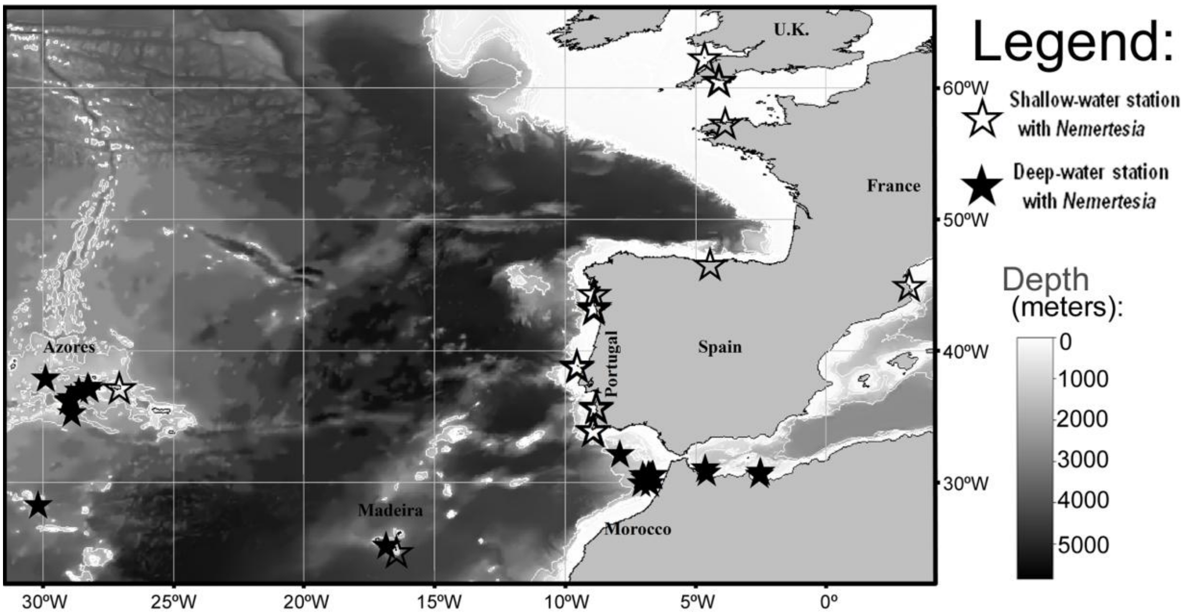

Fig. 9.2. Map of the NE Atlantic and western Mediterranean with representation of the sites with *Nemertesia* haplotypes herein studied.

Plumularia and *Monotheca* hydroid colonies, used for the molecular dating analyses, were collected from shallow waters of the Azores, Madeira, Gorringe Bank, and several localities of mainland Portugal, by scuba diving (see Table 9.1). Specimens were sampled, fixed in 96% ethanol and identified in the laboratory by C. J. Moura.

DNA extraction, amplification and sequencing

Protocols for DNA extraction, amplification and sequencing of the mitochondrial large ribosomal subunit (16S) rDNA gene followed the methodology described by Moura *et al*. (2008), except that the volume of reagents used in PCRs was reduced by half, and some of the purified PCR products were sent to 'STAB VIDA, Investigação e Serviços em Ciências Biológicas, Lda', Oeiras, Portugal, for sequencing. The skeletons of voucher specimens were mounted in permanent slides and temporarily deposited in the Biological Research Collection of the Department of Biology, University of Aveiro (DBUA), Portugal. The material from the Portuguese coast and Gulf of Cadiz is temporarily deposited in the DBUA, Portugal. Specimens from Madeira remained in the Municipal

Museum collection at the Marine Biology Station of Funchal, Portugal. Azorean specimens gathered by the Department of Oceanography and Fisheries (DOP), University of the Azores, Portugal, were returned to that institution. Sampling details, GenBank and Museum accession numbers are described in Table 9.1.

Phylogenetic analyses

The final dataset comprised 63 sequences of *Nemertesia* along with two species of *Plumularia* as outgroup (see Table 9.1). Previous phylogenetic analysis had established the *Plumularia* as the sister taxon to *Nemertesia* (Moura *et al*. 2008; Leclère *et al*. 2009). Sequences were aligned in MEGA version 4 (Tamura *et al*. 2007) using CLUSTALW with the default alignment settings and trimmed to the shortest sequence. MrModeltest version 2.3 (Nylander 2004) was used to determine the optimal probabilistic model of sequence evolution. The general time reversible model (GTR+I+G) was the selected using the Akaike Information Criterion. 'Maximum-likelihood' phylogenetic analyses were performed through the PHYML website (http://www.atgc-montpellier.fr/phyml) (Guindon & Gascuel 2003) using the GTR+I+G model. Bayesian analyses were also performed with MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001), again using the GTR+I+G model. The search was run for 10^7 generations, with trees sampled every 1000 generations (the first million generations were discarded as 'burnin'). Clade stability was assessed using non-parametric bootstrapping of ML analyses (1000 replicates) and Bayesian posterior probability values. PAUP 4.1 (Swofford 1998) was used to determine pairwise uncorrected sequence differences (*P* distances) for simple comparisons of percentage sequence divergence between taxa. A test of the monophyly of *N. antennina* was conducted with an SH test (Shimodaira & Hasegawa 1999) using PAUP* version 4.0b10 (Swofford 1998). The most likely tree from the unconstrained analysis was compared with the optimal ML tree resulting from a search with the monophyly of *N. antennina* constrained. Depth characters (i.e. shallow or deep water) were mapped onto the selected phylogeny of Figs 9.3 and 9.7, using the maximum parsimony character reconstruction optimisation in Mesquite v2.6 (Maddison & Maddison 2008).

A preliminary molecular dating analysis was performed with the analysed 63 *Nemertesia* sequences, in conjunction with 34 *Plumularia* sequences from diverse localities of the Atlantic and Pacific, 11 Atlantic *Monotheca* sequences, and two

Halopteriidae sequences as outgroup. Because *Plumularia* and *Monotheca* species are preferentially found in shallow waters, we included in the analyses a considerable haplotype sampling for these taxa to search for a preferred 16S nucleotide substitution rate (or valid substitution rate ranges) for the Plumulariidae based on well-known geological events (rise of the isthmus of Panama, and of Azorean islands) and on the correspondent geographical location of taxa. Given that, we could set potential approximate age ranges for the nodes of the *Nemertesia* phylogeny of Fig. 9.3. Molecular clock analyses were performed using BEAST v 1.5.2 that conducts a Bayesian MCMC analysis with the option of incorporating a strict or relaxed molecular clock model (Drummond & Rambaut 2007). A strict molecular clock model was enforced as the data could not reject the molecular clock (p=0.97) according to the likelihood ratio test (Felsenstein 1988). The analysis was run for 30 million replicates, sampling every thousandth record. The initial first million replicates were discarded as burnin. The output chronograms were rescaled to fit mean substitution rates observed for related taxa (Govindarajan *et al*. 2005; Lindner *et al*. 2008). As the basis for the choice of a preferred substitution rate, a prior age constraint of 3.5 million years ago (Ma) (e.g. Cronin & Dowsett 1996; Collins *et al*. 1996; Wares & Cunningham 2001) was used for the divergence between Pacific and Atlantic *Plumularia setacea* lineages. However, a potential source of error might be associated with this choice because the process of the closure of the Central American Isthmus started approximately 15 Ma and was not fully complete until about 2.8Ma (Lessios 2008). Therefore we have an additional constraint by including the apparently endemic Azorean *P. setacea* lineage from Flores which could only have diverged from sister populations after the rise of the first Azorean island ~8 Ma (e.g. Hughes & Malmqvist 2005). (For further details of the presented molecular dating analyses see Appendix A).

9.3 RESULTS AND DISCUSSION

16S rDNA nucleotide sequence data were successfully obtained from 51 distinct *Nemertesia* colonies collected from various depths (between approx. 10 and 1400 m) in areas of the eastern Atlantic (Gulf of Cadiz, Azores, Madeira, United Kingdom, Spain and

Portugal continental) and western Mediterranean (Alboran Sea). Most of the diverse habitats sampled in this study are still poorly known, e.g. deep-sea mud volcanoes, areas of carbonate chimneys and crusts, seamounts, cold-water coral bioherms, and even many shallow-water habitats of the Portuguese coast.

The sequence alignment analysed contained a total of 65 sequences of 565 base-pairs length (nucleotide frequencies are A = 37.51%, T = 29.44%, C = 15.18%, and G = 17.88%; proportion of invariant sites = 0.25; gamma shape parameter = 0.1319). The monophyly of the genus *Nemertesia* was evident with strong bootstrap support (Figs 9.3 and 9.7), as in Leclère *et al*. (2007) and Moura *et al*. (2008). Within the *Nemertesia* clade, *N. ventriculiformis* (Marktanner-Turneretscher, 1890), *N. ramosa*, *N. norvegica* (G.O. Sars, 1874) and *N. antennina* (from shallow waters) are represented as well defined monophyletic species, with little intraspecific variation between haplotypes from relatively distant geographical areas. In contrast, the sequence variation is much higher in *N. belini* and especially within *N. antennina* sensu lato (s.l.) with the latter appearing to be polyphyletic and with sympatric specimens falling into separate clades (Fig. 9.3).

N. ramosa appears as sister to a clade containing all the other *Nemertesia* species. Within the latter clade the phylogenetic relationships between the recognized genealogical species is not fully resolved. *N. antennina* from European coastal waters forms a sister clade to *N. belini*, which in turn are sister to a clade containing the several cryptic lineages of deep-sea *'N. antennina*'. However, nodal support for this topology is low (bs=61%, pp=84%), and constrained analyses do not reject the monophyly of the nominal species *N. antennina*. The monophyly of *N. antennina* plus *N. belini* relative to *N. ventriculiformis*, *N. norvegica* and *N. ramosa* receives low support (bs=34%, pp=67%), but is consistent with morphological data. The latter three species typically display supracalycine nematothecae and usually do not contain athecate internodes, in contrast to the former species.

Nemertesia ramosa

N. ramosa usually develops prominent branched colonies and has been observed frequently in the eastern Atlantic and Mediterranean, from very shallow waters (3 m), where it can be abundant, to bathyal depths (1425 m). It has also been reported from the Indian Ocean, on the coasts of South Africa and Mozambique (see detailed distributional ranges in Ansín Agís *et al*. 2001). This species is represented by five haplotypes with high

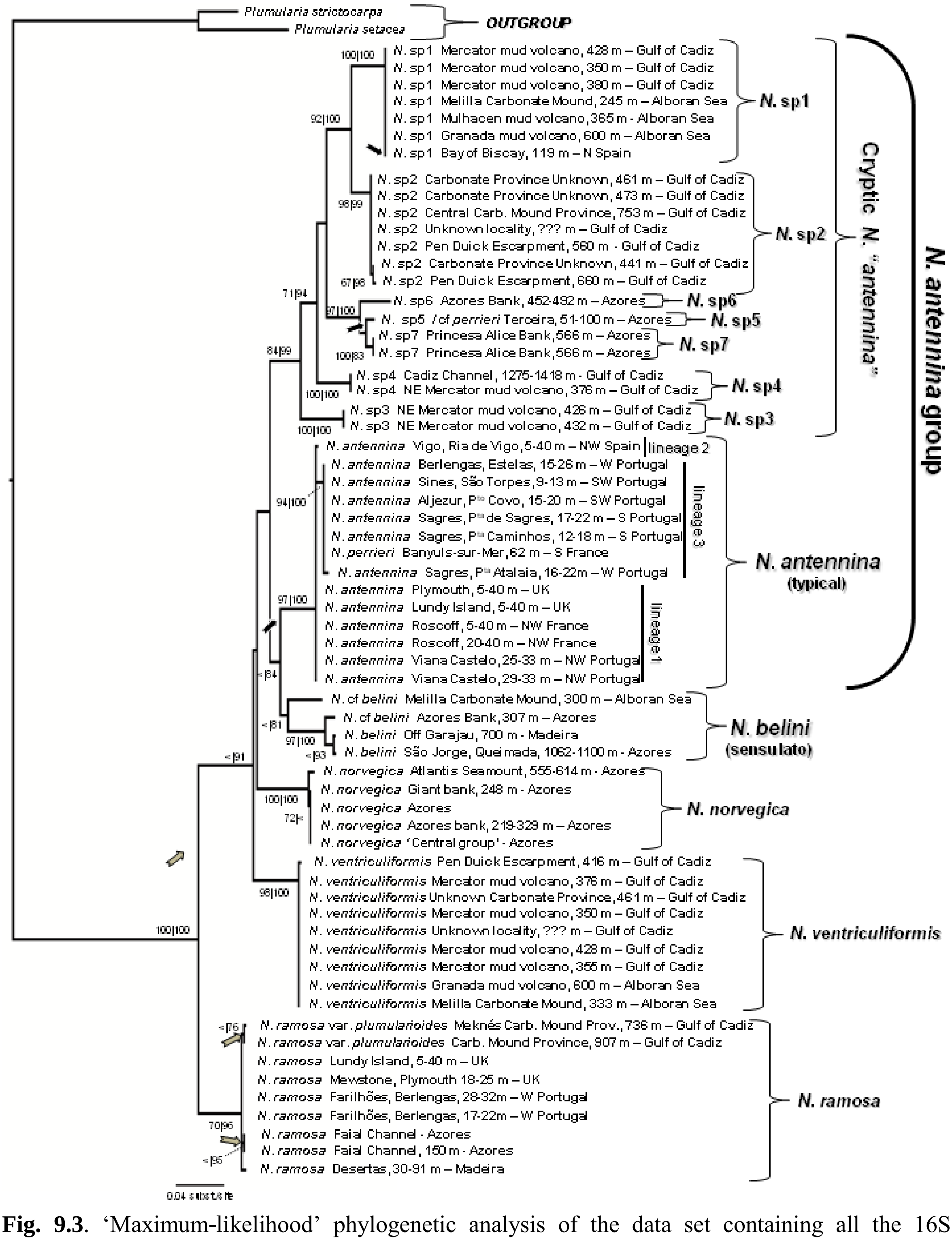

Fig. 9.3. 'Maximum-likelihood' phylogenetic analysis of the data set containing all the 16S Nemertesia sequences used in this study. Numbers near the nodes indicate the values of bootstrap (left) and posterior probabilities (right) in percentages. If these values were <70%, they were replaced by the sign '<'. Values of bootstrap and posterior probabilities are omitted when both were <70% for the same node. The branch length indicator represents 0.04 substitutions per site. Black arrows represent evolutionary transitions from deep to shallow waters. Grey arrows represent the reverse. A bathymetric limit of 130 metres was chosen to differentiate shallow from deep water (e.g. Davis 1977) samples. Abbreviations: N., Nemertesia; Stn, station.

genetic similarity (0 – 0.7 % uncorrected *P* distance), although the samples originate from relatively distant geographical areas and varied depths including: Madeira (30-91 m), Azores (*ca.* 150 m), Gulf of Cadiz (736 and 907 m), SW England and central mainland Portugal (5-40 m). These low levels of intraspecific haplotypic diversity could be due to wide geographic connectivity of populations, recent bottleneck events, or poor resolution of the gene at the intraspecific level.

Nemertesia ramosa taxonomy

The low genetic distances among the represented *N. ramosa* haplotypes support the rejection of the variety *Nemertesia ramosa* var. *plumularioides* Billard (1906) (e.g. Ansín Agís *et al*. 2001). This variety was identified from colonies sampled in the deep waters of the Gulf of Cadiz which presented hydrocladia alternating in one plane along the axis of the hydrocaulus, instead of arranged in typical decussate verticils. Hughes (1977) found such a deviant mode of colony development in *N. antennina* when cultivated in laboratory conditions (but not in the field). This abnormal growth mode was also observed but only distally to a point of regeneration of the hydrocaulus in a few *N. antennina* colonies from shallow Portuguese waters (C.J. Moura, pers. observ.). Many of the cryptic '*N. antennina*' colonies found on the bathyal depths of the Gulf of Cadiz, and *N. ramosa* from the same geographical area, also appear to reduce effort in the clonal propagation of colonies possibly as a result of poor food supply and/or unsuitable abiotic conditions in deep-sea environments. Such a mode of growth has sometimes been attributed to the juvenile lifestage of colonies (e.g. Bedot 1917; Millard 1975; Ansín Agís *et al*. 2001), but the above-mentioned specimens were collected in different periods of the year from deep waters of the Gulf of Cadiz and were consistently delicate, predominantly with alternating hydrocladia in one plane, whilst the other *Nemertesia* colonies inspected from the Azores, Madeira and coastal European waters were, by the given order, much more robust with the apophyses distributed in verticils. As further discussed in the *N. antennina* sub-section 'morphology and speciation', these morphological adaptations may have influenced the evolution of *Nemertesia* species.

Onshore to offshore evolutionary pattern

A survey of the literature on *N. ramosa* (check list of synonymies by Ansín Agís *et al*. 2001) shows a much higher number of reports of this species in shallow rather than deep waters. While such reports could represent a sampling bias, they may also suggest that *N. ramosa* has shallow-water preference and perhaps origin, as proposed by our ancestral character state reconstruction analysis (not conclusive because of limited haplotype sampling). If this is the case, the occurrence of *N. ramosa* in deep waters of the Gulf of Cadiz may constitute evidence of a submergence of the species into the deep sea. Such an evolutionary pattern resembles that found for *Oculina* Lamarck, 1816 coral populations (Eytan *et al*. 2009), and it is in agreement with the general perception that deep-sea communities derived from shallow waters (Jablonski *et al*. 1983). It is also notable that the two deep-sea clades in the phylogeny are distinctly segregated in an otherwise unresolved *N. ramosa* clade (Fig. 9.3).

Nemertesia norvegica

N. norvegica is a relatively poorly studied species known to occur in the NE Atlantic, from Norway to Senegal, at depths between 65 and 1250 m (Ansín Agís *et al*. 2001). Our analysis supports the monophyly of this species and shows little genetic differentiation between five colonies of this species collected from the Azores, at depths ranging from about 200 to 600 m. The haplotype of *N. norvegica* represented by a colony from the remote Atlantis Seamount (*ca*. 600 m) differs by only three base-pairs (0.5% *P* distance) from all the other colonies collected from shallower waters.

Nemertesia ventriculiformis

N. ventriculiformis has been sporadically found in the eastern Atlantic, from France to the Cape Verde islands, and in the western Mediterranean (including the Adriatic), especially from deep waters (up to 900 m), but has also been recorded in coastal waters (below 10 m depth) (Ansín Agís *et al*. 2001; Vervoort 2006). The *N. ventriculiformis* clade, represented by nine colonies from the bathyal zone of the Gulf of Cadiz and Alboran Sea (333-600 m), included only two haplotypes differing by a single nucleotide. It is worth noting that the same haplotype was observed between individuals from the bathyal environments of both the Alboran Sea and the Atlantic Moroccan margin.

Nemertesia belini

N. belini is a quite uncommon species mainly found in deep waters (91-1229 m) from the Azores and Cape Verde islands (Ansín Agís *et al*. 2001). It shows such a high level of morphological variation that diagnostic characters have not been fixed for this species (Bedot 1916, 1921; Ansín Agís *et al*. 2001). Our sampling, from the the Azores, Madeira and Alboran Sea, spans a large part of the known distributional range of the species. Data indicate that *N. belini* is monophyletic with three considerably divergent lineages (in four specimens). A colony from the Azores is found genetically very close to another from Madeira (0.4% *P* distance). Another somewhat divergent lineage of *N. belini* is also found in the Azores (1.4% *P* distance between clades). The two lineages from the Azores are divergent from the other '*N. belini*' colony from the Alboran Sea (4.5 – 4.8% *P* distance), suggesting that these two clades have been genetically isolated for a long time (likely since the Pliocene period – Fig. 9.7).

Nemertesia belini species-complex taxonomy

The syntype of this species was originally sampled from Pico (Azores) at a depth of 1160 m, and it is likely that any of the colonies sampled in the Azores may represent the same taxon. Our sample from São Jorge, Queimada, comes from a similar depth (1062-1100 m) and a locality nearby Pico, and along with the closely related sample from Madeira, it is the most morphologically similar to the type material (see Table 9.4). The sample from the Azores Bank differs from all the others by the frequent absence of athecate internodes (whenever present they are of small size), the occasional occurrence of two hydrothecae on thecate internodes, and the comparatively smaller hydrothecae. Median nematothecae of this sample and the one from the Alboran Sea were found to be smaller than the others. The representative of the Alboran Sea lineage differs from all the other material identified as *N. belini* by the strict absence of supracalycine nematothecae and notably smaller size of gonothecae. This sample also has much smaller nematocysts of the largest type, compared with the specimens from São Jorge. The morphological and genetic data indicate that the samples from the Alboran Sea and the Azores Bank may correspond to two cryptic species but this requires study of further samples.

Nemertesia antennina

Nemertesia antennina was amongst the first hydroids ever described, and it is the type species of the genus. *N. antennina* or 'the sea beard' is one of the most abundant and well-recognized hydroids because of its extremely large colonies that can reach approximatelly 30 cm, its habit of growing in clumps (see Fig. 9.1A), and its wide distribution in various habitats. It can be extremely abundant in northern temperate waters of the Atlantic but is less common in warm temperate and Mediterranean waters (C.J. Moura, pers. observ.). Further sporadic observations have been reported from the eastern coast of South Africa, and the Pacific Ocean from Indochina and Japan (Ansín Agís *et al*. 2001). *N. antennina* can be found from shallow sublittoral (usually bellow 10 m; C.J. Moura, pers. observ.) to deeper offshore waters (2400 – 2450 m; Ansín Agís *et al*. 2001).

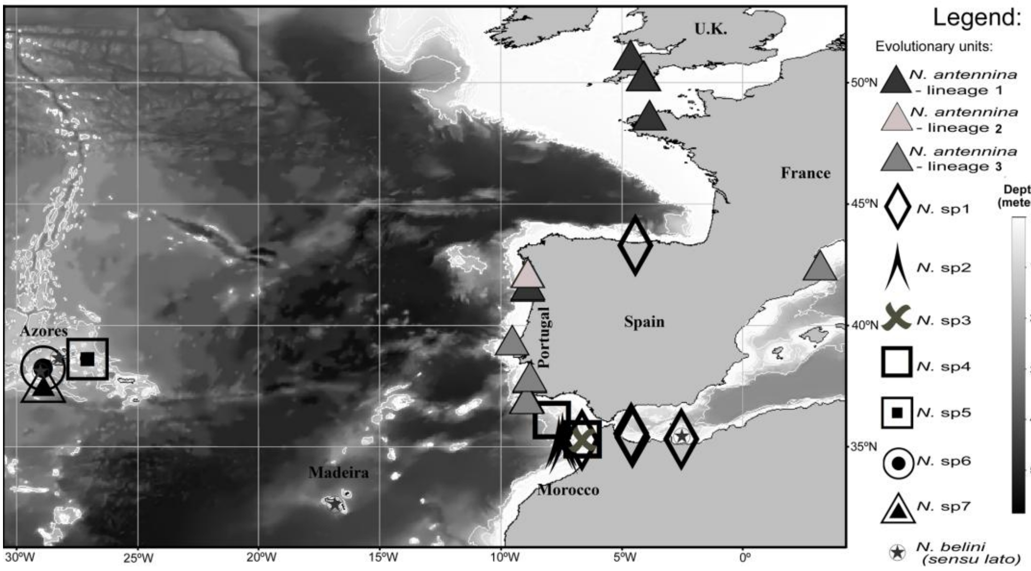

Fig. 9.4. Geographical location of the lineages discovered within the *Nemertesia antennina* and *N. belini* species complexes.

Molecular phylogenetic studies testing the relationships of the *N. antennina* complex were performed by Moura *et al*. (2008). The authors found two cryptic lineages from the bathyal environments of the Gulf of Cadiz and suggested that *N. perrieri* is synonymous to the typical *N. antennina* from European coastal waters. In the present study, a surprisingly large number of distinct evolutionary units have now been discovered by sampling mostly the eastern Atlantic deep waters in a relatively small part of the distributional range of '*N. antennina*'. Two main clades are recovered for the paraphyletic '*N. antennina* group': one from shallow coastal waters of Europe (*ca.* 10-62 m depth), and the other representing deep-sea colonies from the Gulf of Cadiz, Alboran Sea, Bay of Biscay and Azores (*ca.* 70-1400 m depth). The clade containing typical *N. antennina* from shallow waters of Europe seems more closely related to *N. belini* than to the other '*N. antennina*'. However, support for this relationship is poor and we cannot reject the monophyly of *N. antennina s.l.* (P = 0.244). If we assume support of this topology, it appears there has been a long divergence time between the two main clades of the nominal *N. antennina*, probably since the Late Miocene (see Fig. 9.7).

Typical Nemertesia antennina clade

A highly supported compact clade of *N. antennina* is represented by colonies from shallow European coastal waters. The type locality of *N. antennina* is in the UK, and therefore, this clade is inferred as representing the *N. antennina* described by Linnaeus. The position of the typical *N. antennina* clade nested amongst deep-sea clades suggests an evolutionary transition from deep to shallow waters, in accordance with the pattern found for stylasterid corals (Lindner *et al*. 2008) and to some extent also for scleractinian corals (Kitahara *et al*. 2010), although our study has a narrower taxonomic scope.

Despite the limited haplotype sampling for this clade, our data suggest a segregation of populations between northern and southern European coastal waters (genetic distances between 0.5 and 1 %). The formation of these lineages is likely to have been associated with the glaciations of the early–mid Pleistocene which probably fragmented *N. antennina* into a number of refugial populations, including on the Atlantic coast of Europe and in the Mediterranean (see Fig. 9.7 and Table 9.6). These populations accumulated genetic differences in allopatry, and with ice-retreat and subsequent expansion of populations, they have come into secondary contact somewhere along the coast of northern Portugal or Spain. A similar pattern has been found amongst the populations of several marine species in European waters and points to a shared history of the impacts of past glacial cycles on the European marine fauna (e.g. Roman & Palumbi 2004; Schuchert 2005; Gómez *et al*. 2007; Hoarau *et al*. 2007; Campo *et al*. 2010).

Cryptic Nemertesia 'antennina' clade

This clade is represented by samples from the Gulf of Cadiz, Azores, Bay of Biscay and Alboran Sea, at depths between 51-100 m and 1418 m. It comprises a highly supported group, structured into numerous divergent cryptic lineages (Figs 9.3 and 9.4), most of which receive high nodal support. Many of these lineages are from deep waters although there is no clear pattern in bathymetric distribution and separation of putative taxa. Apart from the Azorean haplotypes that cluster separately, all the other divergent cryptic evolutionary units encountered (four well defined genealogical species) occur in very close spatial proximity in the Gulf of Cadiz. *Nemertesia* sp1 is the only species we identified as present in the Mediterranean and on a single mud volcano (Mercator) of the Gulf of Cadiz. In contrast, the closely related *N*. sp2 is unknown in the Mediterranean or at the Mercator mud volcano but occurs in many other localities along the Moroccan margin of the Gulf of Cadiz. Our preliminary molecular dating analyses (see Fig. 9.7 and Table 9.6) suggest that we cannot exclude the hypothesis that the common ancestors of *N*. sp1 and *N*. sp2 might have diverged as a result of one or more glaciation events that retained the ancestral *N*. sp1 in the Mediterranean basin, isolated from its Atlantic sister taxon because of regression(s) in sea level that prevented gene flow through deep waters of the Strait of Gibraltar (similar to Patarnello *et al*. 2007). The presence of a haplotype of *N*. sp1 in the deep waters of the western Mediterranean, Gulf of Cadiz and relatively shallow waters at Bay of Biscay (119 m) could be explained by the influence of Mediterranean outflow waters (e.g. see current regimes given by Iorga & Lozier 1999). Furthermore, the Spanish specimen probably represents a recent emergence of *N*. sp1 from deep to shallow waters. *N*emertesia sp1 is the only species of this clade detected living on active cold seep habitats, revealing its adaptation to or tolerance of these environments. *Nemertesia* sp4 and the clade of Azorean colonies (composed of *N*. sp5, *N*. sp6 and *N*. sp7) group with the clade containing *N*. sp1 and *N*. sp2, but the phylogenetic relations between these three evolutionary branches are not clear.

The three haplotypes of colonies from the Azores cluster with high bootstrap support. However, both morphological and nucleotide differences suggest that they might be considered distinct but related species. The haplotype of *N*. sp5 from Terceira, Azores, sampled from a relatively shallow depth (between 51 and 100 m) clusters amongst lineages represented by colonies of bathyal origin, again representing another evolutionary shift from deep to shallow waters. The divergence between *N*. sp5 and *N*. sp7 likely occurred in the Pleistocene during the 100 KY orbitally forced glaciations (e.g. see Shackleton & Hall 1984; Raymo *et al*. 1989; Cronin & Raymo 1997; Raymo *et al.* 1998; Mc Intyre *et al*.

2001) (Fig. 9.7). Environmental changes during these cycles, along with changes in sea level, may have driven coastal faunal extinctions opening the way for recolonization of shallow waters from the deep sea. These results provide further evidence that the 'sea beard' reported for the Azorean archipelago arrived there via deep waters and that the shallow-water fauna of ocean archipelagos may derive not just through larval or adult dispersal of shallow-water species (similar to the findings of Lindner *et al*. 2008 for *Distichopora robusta* Lindner, Cairns and Guzman, 2004). This is different from a classic 'island' view of population isolation by distance and allopatric or peripatric speciation and suggests that oceanic islands and shallow seamounts may have played a role in speciation of hydroids in this region. Such divergence, occurring in a continuously distributed species along a strong environmental gradient associated with depth, is compatible with a parapatric model of speciation.

Nemertesia sp4 is poorly represented in the analyses but corresponds to a distinct cryptic species of the *N. antennina* complex found at both the Moroccan and Iberian margins of the Gulf of Cadiz, at a wide depth range (376 and 1418 m, respectively). Morphologically, it resembles *N.* sp3 and although they may occur very close geographically, the genetic distance is almost 5%. *Nemertesia* sp3 is represented by a highly divergent haplotype sister to a clade containing all other represented species of cryptic '*N. antennina'*. So far, *N.* sp3 has only been detected in two proximate localities of the Moroccan margin of the Gulf of Cadiz (next to Mercator mud volcano) at about 400 m depth.

N. antennina species-complex taxonomy

The nominal species *N. antennina* is morphologically variable, including in some of its diagnostic characters: unbranched hydrocaulus with hydrocladia comprising a basal ahydrothecate internode followed by thecate internodes with three nematothecae, alternated with ahydrothecate internodes with one or two nematothecae (e.g. Billard 1906; Ansín Agís *et al*. 2001). These features separate it from other *Nemertesia* species including the morphologically similar *Nemertesia perrieri* (Billard, 1901), which differs by having ahydrothecate internodes regularly with two nematothecae, or occasionally two successive ahydrothecate internodes with one nematotheca each between thecate internodes (Ansín Agís *et al*. 2001). Ansín Agís *et al*. (2001) compared the holotypes of both species and added differences such as the morphology of the hydrotheca and the presence/absence of nematothecae on the hydrocaulus (the latter difference erroneously assigned – C.J. Moura, pers. observ.). However, it is difficult to reconcile the use of such variable characters as diagnostic for *N. antennina* (e.g. Billard 1906). Many species and varieties have been described as separate from the '*N. antennina* group' (complete synonymies given by Ansín Agís *et al*. 2001). Some of these taxa were inadequately described and their status is controversial. Recently (e.g. Ramil & Vervoort 1992; Ansín Agís *et al*. 2001; Bouillon *et al*. 2006), the only species recognised as separate from *N. antennina* have been, without general consensus: *N. fraseri, N. pinnata*, *N. perrieri* and *N. irregularis,* originally described from the northern Pacific coast (18-37 m), Atlantic American coast (106-682 m), Canaries (100 m) and Cape Verde Islands (800 m), respectively. All other species or varieties proposed were regarded as synonymous to *N. antennina* or to *N. perrieri*. *Nemertesia irregularis,* a species similar to *N. perrieri,* but differing from the latter by having athecate internodes sporadically with only one nemathotheca, was recently re-included in the synonymy of *N. antennina* by Ansín Agís *et al.* (2001).

Morphological differences between shallow- and deep-water lineages

Morphologically, the main difference detected between the two major clades of the '*N. antennina*' complex (shallow- vs. deep-water clades) relates to the dimensions of gonothecae. These reproductive structures are larger in specimens from coastal European waters with a greater maximum length and diameter (see Fig. 9.5A, B and Table 9.2). However, gonothecae were not found in some of the resolved cryptic lineages, and their dimensions can vary intraspecifically by location as reported for *N*. sp1 (see Table 9.3). The length of both thecate and athecate internodes were found to be lower in the clade of typical *N. antennina*, though sometimes these ranges overlap those for some cryptic lineages (see Fig. 9.5C,D and Table 9.2). The clade containing typical *N. antennina* has a relatively larger diameter of the rim of hydrothecae than the other cryptic lineages, excluding *N*. sp5 and *N*. sp7 (Fig. 9.5E and Table 9.2). While further haplotype sampling is needed to test whether these morphological differences vary intraspecifically with depth or habitat, our data suggests that the hydroid species from coastal waters generally have more robust colonies, wider hydrothecae (that sustain the feeding polyps), larger gonothecae (reproductive structures) and smaller internodes, than in deep waters. This is consistent with the evidence that in coastal waters nutrient availability and competition for space are higher.

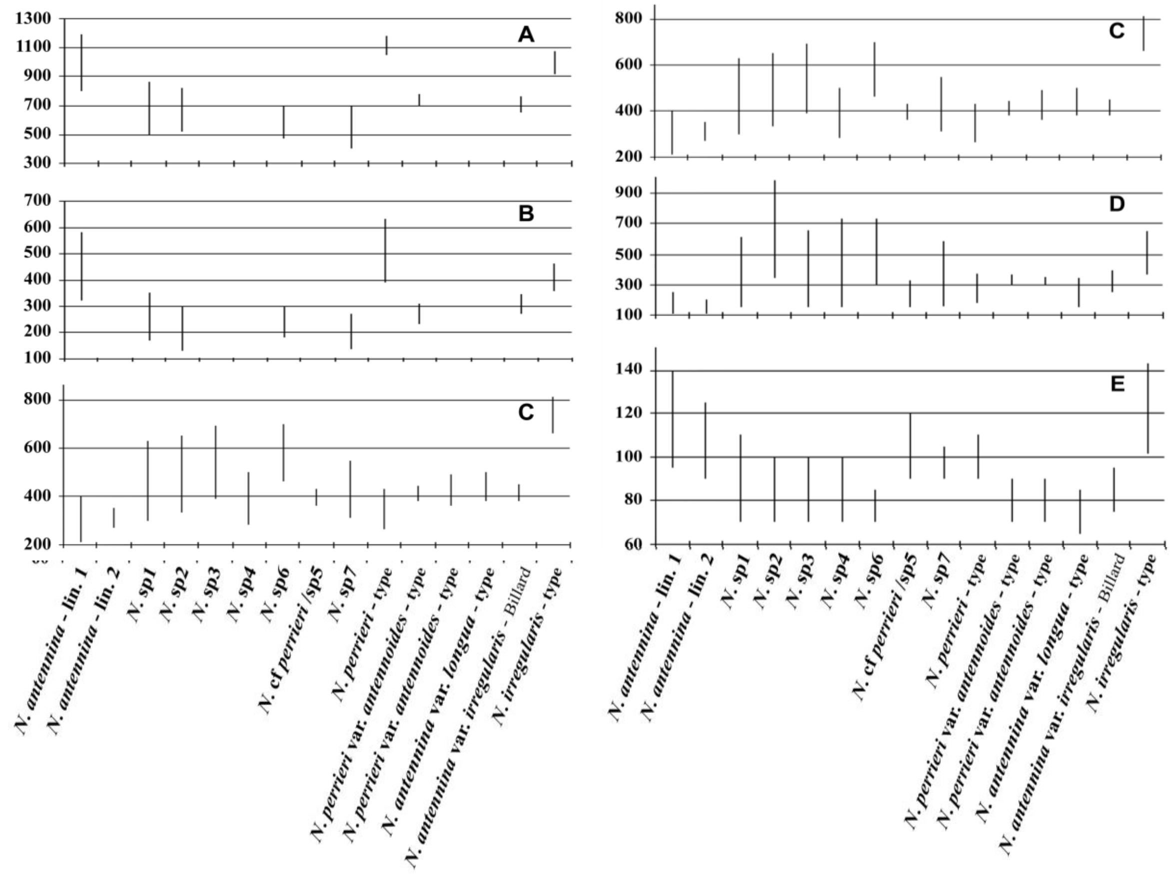

Fig. 9.5. Spatial representation of the main morphological differences found within the '*N. antennina*' group: (A) maximum length of gonothecae, (B) maximum diameter of gonothecae, (C) length thecate internodes, (D) length athecate internodes, (E) diameter of the rim of hydrothecae.

Morphological differences between cryptic species

Morphologically the differences between the recognized cryptic species of the *N. antennina* group can be practically imperceptible and have been overlooked or regarded as intraspecific variation (see Fig. 9.5, Tables 9.2, 9.3). However, by combining the observed and measured morphological characters, phenotypic differences can be sufficiently obvious and consistent (see Fig. 9.6) to clearly identify them without the usage of molecular markers.

The results of a principal component analysis (Fig. 9.6) show that typical *N. antennina* can be differentiated from all other lineages by the size of hydrothecae and hydrocladial internodes. *N.* sp7 specimens demarcates from the others mainly by the great length of nematocysts, in comparison with the other putative species of the complex.

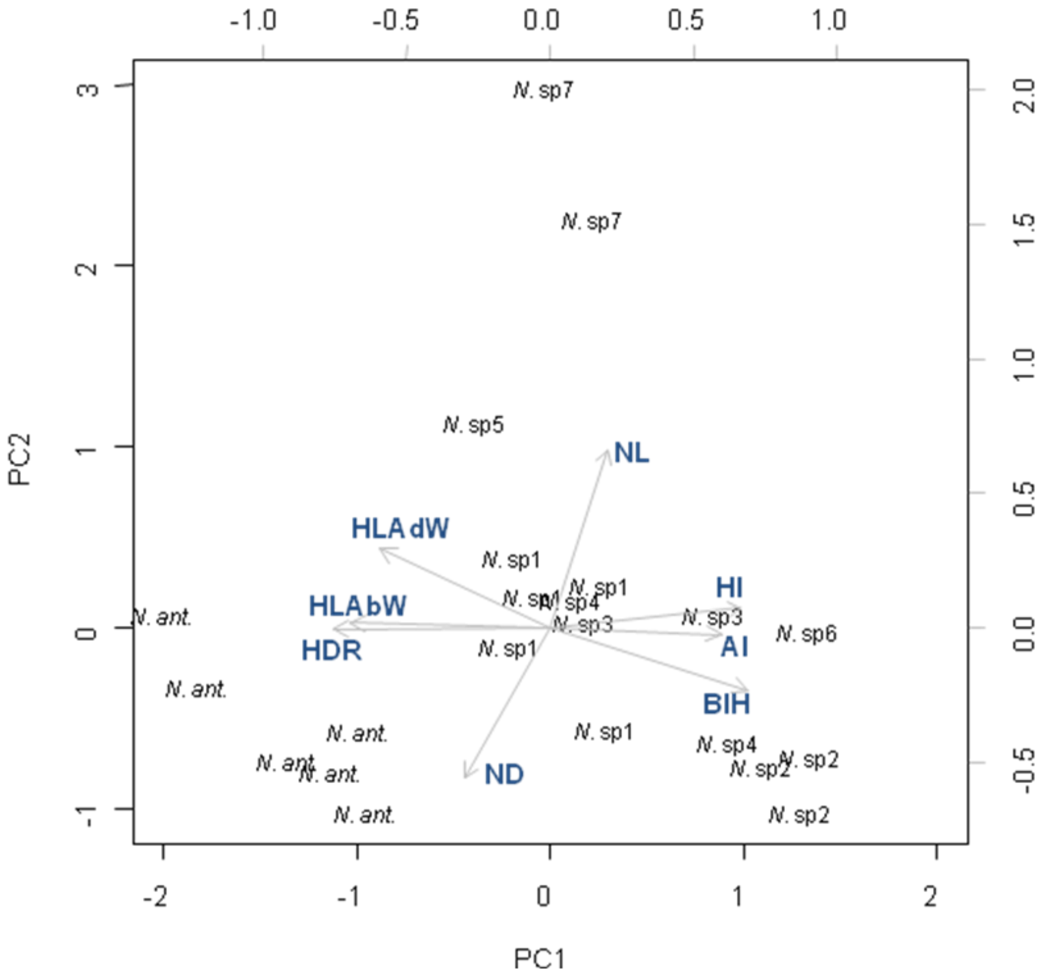

Fig. 9.6. Principal Component Analyses (PCA) of morphologic measurements of specimens of the *Nemertesia antennina* species complex.
Abbreviations: BIH = Basal internode of hydrocladium length, HI = Hydrothecate internode length, AI = Ahydrothecate internode length , HLAbW = Hydrotheca length of abcauline wall:, HLAdW = Hydrothecae length of adcauline wall:, HDR = Hydrothecae diameter at rim:, NL = Nematocysts length, ND = Nematocysts diameter.

The other two putative species herein reported for the Azores (*N.* sp5 and *N.* sp6), despite represented uniquely by one specimen each, are well differentiated by our PCA analyses (Fig. 9.6). *Nemertesia* sp2 specimens stand out grouped, but morphologically close to one specimen of *N.* sp4 (Fig. 9.6). These two putative species can be in fact easily confounded, but *N.* sp4 tends to have smaller ahydrothecate internodes (Table 9.5). This PCA analyses (Fig. 9.6) do not distinguish morphologically the putative species *N.* sp3, *N.* sp1 and *N.* sp4, which are curiously relatively well divergent by 16S haplotype data (Fig. 9.3).

A direct reading of the present diagnostic characters of *N. antennina* suggests that *N.* sp1 is the more morphologically similar to the typical *N. antennina* from shallow waters of the European coasts, having usually one nematotheca per ahydrothecate internode. All the other cryptic lineages are represented mainly by colonies with two nematothecae on the ahydrothecate internodes (exception for *N.* sp7 that has 1-2 nematothecae). Additionally *N.* sp2 is easily differentiated across all lineages by the significantly longer ahydrothecate internodes (Fig. 9.6, Table 9.5). *Nemertesia* sp3 and *N.* sp4 are morphologically extremely similar species found in close spatial proximity but they differ by the size of the hydrothecate internodes that tend to be smaller in *N.* sp4. *Nemertesia* sp6 is genetically and morphologically the most divergent from the other cryptic Azorean taxa (Fig. 9.6). It differs by the longer thecate internodes, smaller hydrothecae and nematocysts and usually two nematothecae on the basal internodes of hydrocladia. Sequence divergence (0.9 % *P* distance) is relatively slight between *N.* sp5 and *N.* sp7, but they differ by the size of athecate internodes and of the adcauline wall of hydrothecae, which are usually higher for the latter species. *Nemertesia* sp7 also have nematocysts with larger size than other Azorean lineages. *Nemertesia* sp5 has similar characteristics to the type material of *N. perrieri*, original from Canary Islands (*ca.* 100 m), and may prove to be conspecific with that species. Despite genetic and morphological differences that induce the separation of the detected Azorean lineages as different species, we still do not have enough samples to show reciprocally monophyletic clades for these putative cryptic taxa, and therefore, the taxonomic status of *N.* sp5, *N.* sp6 and *N.* sp7 is not yet certain.

The major phenotypic differences detected between the cryptic evolutionary units of the *N. antennina* group relate to the size of gonothecae, hydrocladial internodes, hydrothecae and nematocysts. However, these characteristics can be variable, homoplastic and somewhat intergraded between sympatric species (Figs 9.5 and 9.6). It is understandable that many species and varieties were previously distinguished from *N. antennina* as a result of extensive differences between morphotypes, but these were later synonymised during taxonomic revisions (e.g. Bedot 1917; Ramil & Vervoort 1992; Ansín Agís *et al*. 2001). Even with the given phylogenetic analyses, the taxonomic status of these previously described species (apart from the typical *N. antennina*) and varieties remains doubtful as their specified diagnostic characters are insufficient or even incorrect, taxonomic descriptions are inadequate and many have insufficient sampling information or representation. Furthermore, the revision of old type material is highly constrained by its deterioration or loss and the infeasibility of extraction of pure DNA. Ultimately, several of the taxa detected in the present analysis may remain truly cryptic species if they cannot be reliably identified on the basis of morphological features comparable to previously described species and/or varieties.

Despite demonstrating a demarcation of the established putative species with 16S sequence information integrated with morphologic characters, we suggest that further geographical representation of the haplotypes and phylogenetic information with other molecular markers (e.g. nuclear) are needed to ascertain the real number of biological species within the *N. antennina* complex. Regardless of the number of cryptic species proposed in this study, it was evidently demonstrated that the '*N. antennina'* reported both both in the Azores and in the continental margins of Europe and Africa, does not in fact correspond to the true species *N. antennina* that is found in European coasts.

Morphology and speciation

Phylogenetic reconstruction suggests that the ancestor of the cryptic clade of '*N. antennina'* would have a deep-sea origin. Consequently, the great majority of lineages of that cryptic clade correspond to specimens with two or three nematothecae per ahydrothecate internode. Such a morphological condition is consistent with a hypothetical adaptation to food-limited habitats in the deep sea, where the production of more nematothecae by colonies would be advantageous for the efficient capture of prey at low densities. The phylogenetic position of *N.* sp1 and *N.* sp7 demonstrates that the reduction from several nemathotecae per ahydrothecate internodes to one evolved multiple times. However, the phylogenetic information provided by this morphological condition should be interpreted with caution given the conspecificity between shallow-water '*N. perrieri'* from the Mediterranean, consistently with two nematothecae per ahydrothecate internode, and the characteristic *N. antennina* from southern Portugal. The number of nematothecae per athecate internode seems most likely to be driven environmentally. Perhaps more important in explaining the observed speciation patterns are the morphological adaptations of colonies to the heterogeneity of deep-sea habitats (e.g. active and inactive mud-volcanoes, carbonate mounds, seamounts and coralline and/or poriferan bioherms) such as the maximum height of colonies and the number of apophyses per internodes of hydrocaulus. For example, the colonies of *N.* sp1 collected from the Alboran Sea were much taller and consequently with more apophyses per internode, more feeding polyps and with higher production of gonothecae, than colonies of the same species (or of other species of the 'cryptic '*N. antennina*' clade') that were found on the Moroccan margin of the Gulf of Cadiz. The maximum size of colonies registered from the sample containing *N*. sp5 collected at shallow waters of the Azores was about four times greater than the size of *N*. sp6 and *N*. sp7 colonies collected from deeper waters of the same archipelago (Table 9.2). As previously reported for *N. ramosa*, the cryptic *Nemertesia* found at bathyal depths in the Gulf of Cadiz also develop smaller delicate colonies with hydrocladia mainly arranged alternately along the hydrocaulus. Again, this may be an adaptation to low food availability and/or unfavourable environmental conditions where intensive clonal propagation of colonies would not be advantageous for the survival of individuals. Such adaptation has the cost of developing less apophyses and gonothecae per colony, and therefore, the process of sexual reproduction - the only known mode of spatial dispersal in *Nemertesia* - is constrained by a more reduced chance of fertilisation and release of fewer planulae per colony. Furthermore, if gonothecae in shallow waters are in fact commonly larger (see Fig. 9.5, Tables 9.2, 9.3), less gametes may be produced per gonotheca in the deep sea, also limiting reproductive output per individual offshore. These factors result in the production of less reproductive units per population and should limit dispersal at large spatial scales. Such impediments for dispersal might be further enhanced by habitat availability. For example, all the represented cryptic *Nemertesia* colonies found in the Gulf of Cadiz, with exception of *N*. sp1, were associated with areas of carbonate crusts or chimney build-ups, usually within scattered patches of (fossil) scleractinian frameworks and rubble. These seafloor features provide suitable substrata for settlement of planulae and enhance local productivity and/or biodiversity by harbouring other taxa usually rare in the surrounding deep sea.

Evolution over geological ages

Our molecular dating analyses (Fig. 9.7) reveal the existence of a small number of Plumulariidae clades that have persisted since the middle Miocene - an epoch of global (oceanic) warm temperatures (e.g. Raymo 1994; Zachos *et al*. 2001; Retallack 2002) and extinctions (e.g. Sepkoski 1992). In contrast, high levels of cladogenesis are seen in the late Miocene, including the evolutionary transition from shallow to deep waters of the ancestor of all *Nemertesia* species represented, excluding *N. ramosa* (Fig. 9.7). These

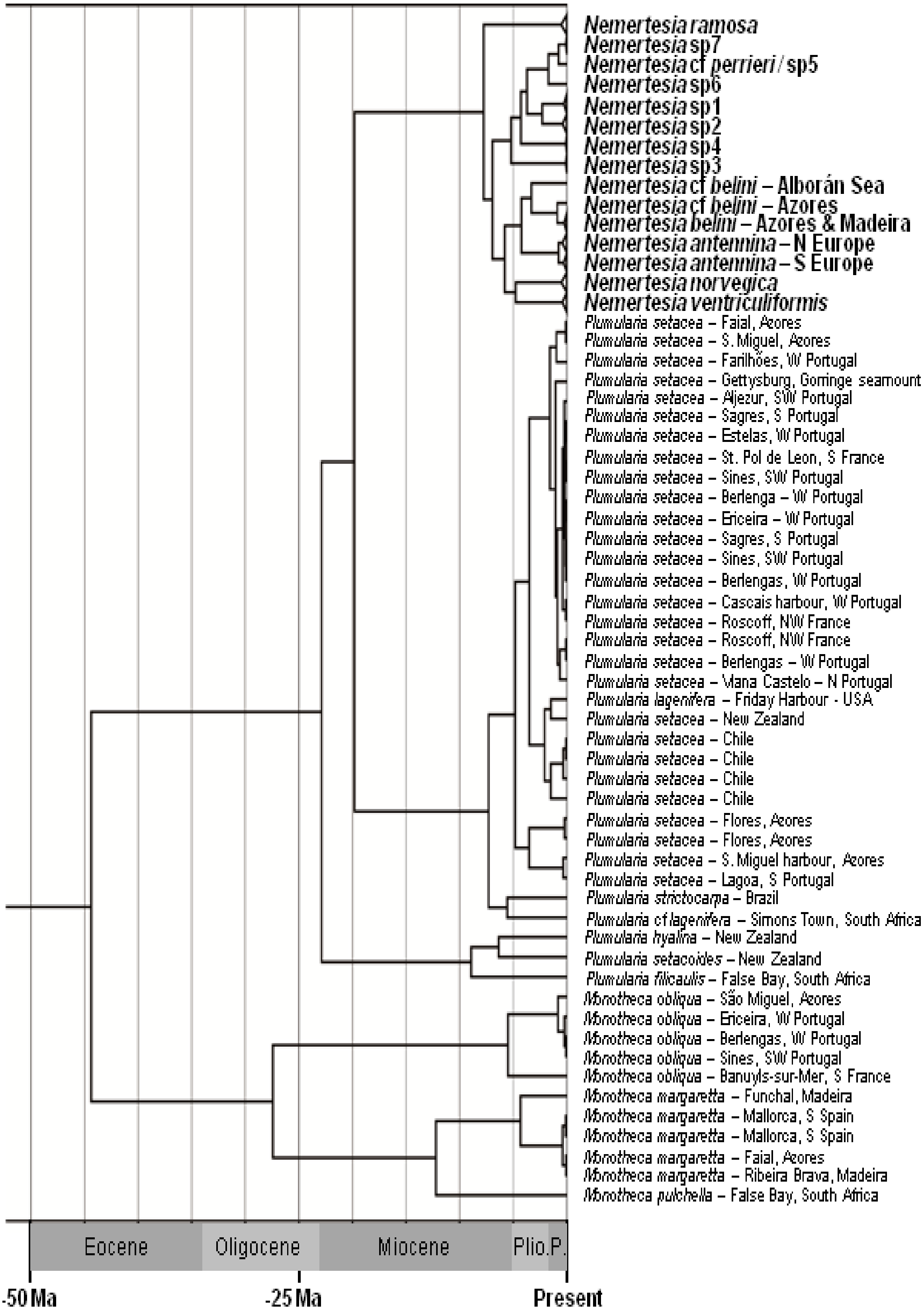

Fig. 9.7. Preliminary molecular clock study on Plumulariids. Bayesian MCMC analysis calibrated with a chosen rate of 9.69×10^{-09} substitutions per site per year. Note that some terminal branches of the *Nemertesia* clade end in triangles that represent well-established lineages.

phenomena can be associated with a period of global cooling (e.g. Shackleton & Kennett 1975; Ciesielski *et al*. 1982) interspersed by warmer and arid episodes (e.g. Van Dam & Weltje 1999) and intensification of the thermohaline circulation with the formation of the North Atlantic Deep Water (e.g. Wright *et al*. 1991; Billups 2002) in the late Miocene. The early-mid-Pliocene was an epoch of climatic warmth (e.g. Cronin 1991; Lawrence *et al*. 2009) that included the Mediterranean salinity crisis (e.g. Shackleton & Kennett 1975; McKenzie & Oberhaensli 1985; Hodell *et al*. 1986, 2001) and coincides with a period of cladogenesis of some of the deep-sea clades of *Nemertesia*. Interestingly, all lineages represented from the Mediterranean seem to have been generated after the Messinian salinity crisis, which likely eradicated all 'plumulariids' that were established in the basin. Finally, several glacial episodes that occurred through the late Pliocene and early Pleistocene (e.g. Shackleton & Hall 1984; Raymo *et al*. 1989; Cronin & Raymo 1997; Raymo *et al*. 1998; McIntyre *et al*. 2001) may explain: (1) the differentiation of *N*. sp1 as a species distinct to *N*. sp2 (possibly because of the isolation of the ancestral of *N*. sp1 in the Mediterranean, as previously referred in section 'Cryptic *'Nemertesia antennina'* clade'); (2) the split of *N*. cf. *perrieri* / sp5 from *N*. sp7 and the evolutionary transition of the former lineage to shallow waters (see section 'Cryptic *Nemertesia 'antennina'* clade'); (3) the genesis of genetically distinct populations of *N. antennina* (see section 'Typical *Nemertesia antennina* clade') and *Plumularia* and *Monotheca* species (Fig. 9.7).

9.4 CONCLUSIONS

The phylogenetic analyses based on mitochondrial 16S mRNA haplotype data of *Nemertesia* species collected from shallow and deep waters of the eastern Atlantic and western Mediterranean proved extremely useful in delineating species and clarifying some of their intra- and inter-specific phylogenetic associations. Haplotype data for *Nemertesia* species revealed genetic similarities between intraspecific populations in the deep waters of the western Mediterranean and the Gulf of Cadiz (for *N*. sp1 and *N. ventriculiformis*), deep waters of Madeira and Azores (for *N. belini* and *N. ramosa*), shallow waters of central Portugal and UK (for *N. ramosa*), shallow waters of central and southern Portugal and the Mediterranean (for *N. antennina*) and between the shallow waters of North Portugal, UK and NW France (for *N. antennina*). The zero genetic distance encountered between samples of *N.* sp1 from deep waters of the western Mediterranean, Gulf of Cadiz and relatively shallow waters of southern Bay of Biscay potentially represents a recent emergence to shallow waters from the deep sea and may implicate a role of Mediterranean outflow waters in dispersal and population connectivity. Indeed, several recent shifts to and from the eastern Atlantic to the Mediterranean were proved to have occurred since the last enclosure of the Strait of Gibraltar during the Messinian salinity crisis (see Fig. 9.7, Table 9.6). *Nemertesia ramosa* is widely dispersed in the eastern Atlantic and western Mediterranean and revealed practically no sequence divergence between haplotypes from Madeira, Azores, Gulf of Cadiz and UK, from deep and shallow waters.

In contrast, surprisingly high cryptic diversity was revealed within the nominal species *N. belini* and *N. antennina.* For the deep-sea species *N. belini*, a monophyletic group was found comprising a highly divergent lineage from the western Mediterranean and a clade with two fairly distinct haplotypes both occurring in the Azores. *Nemertesia antennina* is possibly polyphyletic with two very divergent main clades with unsolved phylogenetic relationships within the genus *Nemertesia*. One clade from coastal waters of Europe, probably originated in the deep sea, likely represents the type species described by Linnaeus (1758). The other, essentially present in deep waters (51-100 m and 1418 m) of the eastern Atlantic and western Mediterranean, is represented by several new taxa. Within this deep-sea clade, seven putative cryptic species were discovered: three from the Azores and two geographically proximate seamounts clustered in a distinct lineage, and the remaining four found close to each other in the Gulf of Cadiz.

An ancient founder event from the continental margins of the eastern Atlantic could explain the divergence of the lineage represented by the Azorean cryptic '*N. antennina'* colonies. However, the existence of different taxa on nearby seamounts raises the possibility that dispersal is restricted between seamounts and the islands in this region, and speciation or incipient speciation has occurred as a result. This would be contrary to recent evidence of gene flow between seamount populations (e.g. O'Hara 2007; McClain *et al*. 2009; Thoma *et al*. 2009), but would fit with some observations of genetic differentiation between seamounts for populations of species with limited dispersal (Samadi *et al*. 2006; Castelin *et al*. 2010, Clark *et al*. 2010). It suggests that geographic dispersal distance in *Nemertesia* may be limited. Therefore, dependence on the intensity and characteristics of water currents for dispersal and speciation of these animals seems highly probable. The consequence of different morphological adaptations to the food-limited but heterogeneous deep-sea habitats may also have contributed to speciation in this genus. Additionally, the effects of past glacial cycles seem to have promoted population divergences (and ultimately speciation) and favoured evolutionary shifts from deep to shallow waters. Lindner *et al*. (2008) similarly found that stylasterid corals originated and diversified in the deep sea and invaded temperate and tropical shallow waters multiple times. In the present paper, we also demonstrate that the genus *Nemertesia* has diversified more extensively in deep waters and emerged to shallow waters more than once, but in a setting of temperate and subtropical latitudes of the NE Atlantic. In contrast, *N. ramosa* may present a recent case of submergence into deep waters, in agreement with the general notion that deep-sea forms derived from shallow waters (Jablonski *et al.* 1983; Eytan *et al*. 2009).

Morphological and molecular dating analyses further supported the considerable long divergence times between the recognized distinct cryptic (or pseudo-cryptic) species within the *N. antennina* and *N. belini* complexes. The events that triggered diversification in the deep sea still remain difficult to identify. Nevertheless, the importance of deep-sea habitats as a source of phylogenetic diversity is evident from this and other studies (e.g. Lindner *et al*. 2008). Further hydrozoan studies will continue to bring important insights into the possible mechanisms of speciation and anatomical evolution in sessile marine invertebrates in both shallow- and deep-sea environments.

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APPENDIX

Details of the molecular dating analysis

A phylogenetic analysis (see Fig. 9.7) containing several monophyletic Plumulariidae sequences revealed that *Nemertesia* species are monophyletic and evolutionary derived from *Plumularia* species. The genus *Monotheca* was recovered as monophyletic with two widely diverged main clades, which are sister to a branch containing *Plumularia* and *Nemertesia* species. The latter genus is found sister to a clade containing morphologically similar species: *Plumularia lagenifera,* Allman, 1885, *Plumularia strictocarpa,* Pictet, 1893 and *Plumularia setacea* (Linnaeus, 1758). *P. setacea* is regarded as a common widespread near-cosmopolitan species (apparently absent from the Antarctic), especially abundant in shallow waters but present at least down to 604 m (see distributional details and synonymy in Ansín Agís *et al.* 2001). This study includes coastal *Plumularia setacea* haplotypes from the Azores, Madeira, the Gorringe Bank, and mainland Portugal, along with conspecific sequences published in GenBank from Chile, New Zealand, western USA, and northern and southern France (see Table 9.1). These *P. setacea* sequences represent several distinct haplotypes clustered monophyletically. Pacific samples group together separate from the other Atlantic *P. setacea* samples. The marked

Pacific/Atlantic segregation would have been triggered by the rise of the Isthmus of Panama. However, it is likely that the split of the represented Atlantic and Pacific *P. setacea* lineages would have been prior to this event. *P. setacea* collected from one of the most pristine and remote islands of the Azores - Flores, were found genetically quite diverse and with (so far) exclusive haplotypes for that archipelago when compared with both Pacific and other Atlantic samples (Fig. 9.7, and P. Schuchert, pers. comm.). Other haplotypes representative of Azorean *P. setacea*, namely from São Miguel/Faial and São Miguel harbour, are shared with populations from Madeira and the Mediterranean, respectively (Schuchert, unpublished). Therefore, these shallow-water and apparently endemic Azorean lineages from Flores would have emerged after the rise of the first Azorean island (*ca.* 8 Ma). *Plumularia setacea* from cooler waters of northern Europe (namely from NW France, northern and central mainland Portugal) form distinct but closely related clades to conspecific specimens from central Portugal down to the Mediterranean, resembling the pattern seen for shallow-water *N. antennina*. However, intraspecific haplotype diversity for *P. setacea* seems higher, possibly as a result of rafting, larger populations sizes and more undifferentiated choice of substrata for settlement of planulae. It also likely represents the influence of past Pleistocene glaciations on the fragmentation of *P. setacea* populations along the European coastline, as with typical *N. antennina*. These data do not support a cosmopolitan distribution for *P. setacea*.

The above-mentioned geological events were, by the given order, used to choose a preferred substitution rate for the 16S gene for plumulariids (see Table 9.6). Additionally, we were expecting a relatively rapid substitution rate for this gene in the Plumulariidae family, as previously suggested by Leclère *et al.* (2007) and Moura *et al*. (2008). The preferred rate appearing to fit most consistently with the geological events was $9.69\text{x}10^{-09}$ substitutions per site per year, which allowed setting (provisional) mean ages for the tree nodes of the phylogeny of Fig. 9.3 (Fig. 9.7, Table 9.6). Nevertheless, rates between approximately $4.49\text{x}10^{-09}$ and $1.21\text{x}10^{-08}$ substitutions per site per year were considered also as possible (see Table 9.6). Additionally, the genetic data comfortably fit a molecular clock with a very high P value ($P = 0.97$), suggesting constrained rates within all taxa represented in the analyses.

Table 9.1. 16S rRNA sequence data. Sequences of specimens marked with asterisk were determined for the present study.

Species	GenBank accession Number	Voucher specimen	Cruise/collector	Collection method	Sample location
Nemertesia ventriculiformis *	JN714555	DBUA 1096.01	R/V Prof. Logachev TTR17-leg1	Grab	Granada MV, Stn 420, 600 m 35º33.854’N 4º37.435’W Alboran Sea
Nemertesia ventriculiformis *	JN714556	DBUA 1096.02	R/V Prof. Logachev TTR17-leg1	Grab	Melilla Carbonate Mound, Stn 394, 333 m 35º26.064’N 2º30.585’W Alboran Sea
Nemertesia ventriculiformis *	JN714557	DBUA 1098.01	R/V Pelagia M2007	Box-corer	Unknown Carbonate Province, Stn 41, 461 m 35°00.113'N 6°44.815'W Gulf of Cadiz
Nemertesia ventriculiformis	AM888337 (Moura *et al*. 2008)	DBUA 1099.01	R/V Prof. Logachev TTR15	Gravity-corer	Mercator MV, Stn 575, 355 m 35º17.903'N 6º38.715'W Gulf of Cadiz
Nemertesia ventriculiformis	AM888336 (Moura *et al*. 2008)	DBUA 1100.01	Maria S. MERIAN No. 1 Leg 3	GKG	Mercator MV, Stn 242, 350 m 35º17.870'N 6º38.810'W Gulf of Cadiz
Nemertesia ventriculiformis *	JN714558	DBUA 1101.01	James Cook 10	ROV	Mercator MV, Stn Dive 27 Rock 1, 428 m 35°18.755’N 6°37.422’W Gulf of Cadiz
Nemertesia ventriculiformis *	JN714559	DBUA 1101.02	James Cook 10	ROV	Mercator MV, Stn Dive 27 Rock 6, 376 m 35°18.827’N 6°37.058’W Gulf of Cadiz
Nemertesia ventriculiformis *	JN714560	DBUA 1098.02	R/V Pelagia M2007	Box-corer	Pen Duick Escarpment, Stn 17, 416 m 35°17.061'N 6°42.617'W Gulf of Cadiz
Nemertesia ventriculiformis *	JN714561	DBUA 1098.03	R/V Pelagia M2007	Box-corer	Unknown station, net trapped on sampling device, bathyal environment Gulf of Cadiz
*Nemertesia ramosa**	JN714562	DOP C2112	Fishing fleet of Horta		Pico-Faial Channel Azores
*Nemertesia ramosa**	JN714563	DBUA 1119.01	Submersible Lula	Submersible	Pico-Faial Channel, LULA dive #133, 150 m 38°30.541’N 28°36.544’W Azores
Nemertesia ramosa var. *plumularioides* *	JN714564	DBUA 1097.01	R/V Pelagia 64PE284	Box-corer	Carbonate Mound Province SE Yuma MV, Stn Geob12722-1, 907 m 35°18.630’N 7°00.990’W Gulf of Cadiz
Nemertesia ramosa var. *plumularioides* *	JN714565	DBUA 1097.02	R/V Pelagia 64PE284	Box-corer	Meknés Carbonate Mound Provinces, Stn Geob12739-1, 736 m 35°00.010’N 7°04.470’W Gulf of Cadiz
Nemertesia ramosa	AM888331 (Moura *et al*. 2008)	DBUA 943.01	J. Hall-Spencer	Hand	Lundy Island, 5-40 m 51º09.400’N 4º39.300’W United Kingdom
Nemertesia ramosa *	JN714566	MMF 25124	R/V Johnson Sea Link	Submersible	Desertas, 30 – 91 m 32º23.00’N 16º27.23’W Madeira
Nemertesia ramosa *	JN714567	DBUA 1120.01	C. J. Moura	Hand	Berlengas, Farilhões, 17-22 m 39º28.494’N 9º32.640’W W Portugal
Nemertesia ramosa *	JN714568	DBUA 1120.02	C. J. Moura	Hand	Berlengas, Farilhões, 28-32 m 39º28.351’N 9º32.821’W W Portugal
Nemertesia ramosa *	JN714569	DBUA 1106.01	C. J. Moura	Hand	Plymouth, Mewstone, 18-25 m 50º17.60’N 4º05.60’W United Kingdom
Nemertesia norvegica *	JN714570	DOP C1919	F/V Manuel Arriaga	Espinhel	Giant bank, between Flores and Faial, 248 m 38º59.040’N 29º52.980’W Azores
Nemertesia norvegica	AM888330 (Moura *et al*. 2008)	DBUA 1107.01	R/V Prof. Logachev TTR12	Dredge	Atlantis Seamount, Stn 421, 555-614m 34º10.350’N 30º10.360’W Azores
Nemertesia norvegica *	JN714571	DOP C2052	F/V Baia das Lajes	Espinhel	‘Central Group’ Azores

Nemertesia norvegica *	JN714572	DOP C1496			Azores bank, 368 m 38°16.800'N 28°51.660'W Azores
Nemertesia norvegica *	JN714573	DOP C1709	F/V Manuel Arriaga	Espinhel	Azores bank, 219-329 m 38º10.020’N 29º00.000’W Azores
Nemertesia belini *	JN714574	DOP C2232	R/V Arquipélago ARQDAÇO-27-P07	Palangre de fundo	São Jorge, Queimada, Stn 2SJO(1)P07 (Est. 22), 1062-1100 m 38º39.960’N 28º15.960’W Azores
Nemertesia belini *	JN714575	MMF 36727	F/V Baía de Câmara de Lobos		Off Garajau, 700 m 32º37.49’N 16º50.58’W Madeira
Nemertesia cf *belini* *	JN714576	DOP C1433	F/V Neuza Mar	Palangre de fundo	Azores Bank, 307 m 38º06.480’N 29º00.720’W Azores
Nemertesia cf *belini* *	JN714577	DBUA 1108.01	R/V Prof. Logachev TTR17-leg1	Grab	Melilla Carbonate Mound, Stn 395, 300 m 35º26.415’N 2º31.075’W Alboran Sea
Nemertesia antennina *	JN714578	DBUA 1117.01	C.J. Moura	Hand	Berlengas, Estelas, 15-26 m 39º25.225’N 9º32.084’W W Portugal
Nemertesia antennina *	JN714579	DBUA 1116.01	C.J. Moura	Hand	Aljezur, Porto Covo, 15-20 m 37°51.192'N 8º48.211’W SW Portugal
Nemertesia antennina *	JN714580	DBUA 1116.02	C.J. Moura	Hand	Sagres, Ponta dos Caminhos, 12-18 m 37º01.21ºN 8º54.33’W S Portugal
Nemertesia antennina *	JN714581	DBUA 1116.03	C.J. Moura	Hand	Sagres, Ponta de Sagres, 17-22 m 36º59.700’N 8º56.715’W S Portugal
Nemertesia antennina *	JN714582	DBUA 1129.01	C.J. Moura LusoExpedição Olympus 2008	Hand	Sagres, Ponta da Atalaia, 16-22 m 37º00.033’N 8º55.882’W S Portugal
Nemertesia antennina *	JN714583	DBUA 1116.04	C.J. Moura	Hand	Sines, São Torpes, 9-13 m 37º54.770’N 8º48.467’W SW Portugal
Nemertesia perrieri / antennina	DQ855925 (Leclère *et al.* 2007)	MHNG INVE32971		Dredge	off Banyuls-sur-Mer, 62 m 42°30.360N 3°10.620’E S France
Nemertesia antennina *	JN714584	DBUA 1118.01	C.J. Moura	Hand	Off Viana do Castelo, 25-33 m 41º37.379’N 8º53.805’W NW Portugal
Nemertesia antennina *	JN714585	DBUA 1118.02	C.J. Moura	Hand	Viana do Castelo, 29-33 m 41º41.680’N 8º52.711’W NW Portugal
Nemertesia antennina *	JN714586	DBUA 1109.01	F. Ramil	Hand	Vigo, Ria de Vigo, 10-40 m NW Spain
Nemertesia antennina	AM888329 (Moura *et al*. 2008)	DBUA 948.01	J. Hall-Spencer	Hand	Plymouth, 5-40 m United Kingdom
Nemertesia antennina	AM888328 (Moura *et al*. 2008)	DBUA 942.01	J. Hall-Spencer	Hand	Lundy Island, 5-40 m 51º09.24’N 4º39.18’W United Kingdom
Nemertesia antennina	AY787910 (Cunningham, EMBL submission)	MHNG INVE29954		Hand	Roscoff, near Carantec, 20-40 m 48º40.620’N 3º51.960’W NW France
Nemertesia antennina	FJ550458 (Leclère *et al*. 2009)				Roscoff NW France
Nemertesia cf *antennina* / sp1 *	JN714587	DBUA 1110.01	R/V Prof. Logachev TTR17-leg1	Gravity-corer	Melilla Carbonate Mound, Stn 393B, 245 m 35º19.799’N 2º33.067’W Alboran Sea
Nemertesia cf *antennina* / sp1	AM888333 (Moura *et al*. 2008)	DBUA 1111.01	Maria S. MERIAN No. 1 Leg 3	MUC	Mercator MV, Stn 287, 379 m 35º17.890'N 6º39.059'W Gulf of Cadiz
Nemertesia cf *antennina* / sp1	AM888332 (Moura *et al*. 2008)	DBUA 1111.02	Maria S. MERIAN No. 1 Leg 3	GKG	Mercator MV , Stn 242, 350 m 35º17.870'N 6º38.810'W Gulf of Cadiz
Nemertesia cf *antennina* / sp1 *	JN714588	DBUA 1110.02	R/V Prof. Logachev	Grab	Granada MV, Stn 420, 600 m 35º33.854’N 4º37.435’W

			TTR17-leg1		Alboran Sea
Nemertesia cf *antennina* / sp1 *	JN714589	DBUA 1110.03	R/V Prof. Logachev TTR17-leg1	Grab	Mulhacen MV, Stn 416, 365 m 35º24.431'N 4º34.119'W Alboran Sea
Nemertesia cf *antennina* / sp1	AM888334 (Moura *et al*. 2008)	DBUA 1112.01	R/V Prof. Logachev TTR15	Gravity-corer	Mercator MV, Stn 576, 428 m 35º17.657'N 6º39.129'W Gulf of Cadiz
Nemertesia cf *antennina* / sp1	JN714590	DBUA 1121.01	Trebol 2005 survey		Bay of Biscay, 119 m 43°18.792'N 04°26.826'E Spain
Nemertesia cf *antennina* / sp2 *	JN714591	DBUA 1102.01	R/V Pelagia M2007	Box-corer	Unknown Carbonate Province, Stn 39, 441 m 35°00.230'N 6°44.894'W Gulf of Cadiz
Nemertesia cf *antennina* / sp2 *	JN714592	DBUA 1103.01	R/V Pelagia M2005	Box-corer	Pen Duick Escarpment, Stn 16, 660 m 35º18.310'N 6º48.205'W Gulf of Cadiz
Nemertesia cf *antennina* / sp2 *	JN714593	DBUA 1104.01	R/V Pelagia 64PE284	ROV	Central Carbonate Mound Province (N area), Stn 12728-1, 753 m 35°11.020'N 6°56.620'W Gulf of Cadiz
Nemertesia cf *antennina* / sp2	AM888335 (Moura *et al*. 2008)	DBUA 1105.01	R/V Pelagia M2006	Piston core	Pen Duick Escarpment, Stn 40A, 560 m 35°18.910'N 6°47.030'W Gulf of Cadiz
Nemertesia cf *antennina* / sp2 *	JN714594	DBUA 1102.02	R/V Pelagia M2007	Box-corer	Unknown Carbonate Province, Stn 40Bx, 473 m 35°00.137'N 6°44.806'W Gulf of Cadiz
Nemertesia cf *antennina* / sp2 *	JN714595	DBUA 1102.03	R/V Pelagia M2007	Box-corer	Unknown Carbonate Province, Stn 41, 461 m 35°00.113'N 6°44.815'W Gulf of Cadiz
Nemertesia cf *antennina* / sp2 *	JN714596	DBUA 1102.04	R/V Pelagia M2007	Box-corer	Unknown station, net trapped on sampling device, bathyal environment Gulf of Cadiz
Nemertesia cf *antennina* / sp3 *	JN714597	DBUA 1113.01	James Cook 10	ROV	NE Mercator MV, Stn Dive 27 Rock 3, 426 m 35°18.839'N 6°37.354'W Gulf of Cadiz
Nemertesia cf *antennina* / sp3 *	JN714598	DBUA 1113.02	James Cook 10	ROV	NE Mercator MV, Stn Dive 27 Rock 2, 432 m 35°18.823'N 6°37.377'W Gulf of Cadiz
Nemertesia cf *antennina* / sp4 *	JN714599	DBUA 1114.01	R/V Prof. Logachev TTR15	Dredge	Cadiz Channel, carbonate chimneys, Stn 599, 1275-1418 m 36º06.538'N 7º53.942'W Gulf of Cadiz
Nemertesia cf *antennina* / sp4 *	JN714600	DBUA 1115.01	James Cook 10	ROV	NE Mercator MV, Stn Dive 27 Rock 6, 376 m 35°18.827'N 6°37.058'W Gulf of Cadiz
Nemertesia cf *perrieri* / sp5 *	JN714601	DOP C408	R/V Arquipélago ARQDAÇO-21-P04	Palangre de fundo	Terceira, Ponta das Contendas, 51-100 m 38º37.020'N 27º04.020' W Azores
Nemertesia cf *antennina* / sp6 *	JN714602	DOP C2363	R/V Arquipélago ARQDAÇO-27-P07	Palangre de fundo	Azores Bank, 452-492 m 38º14.280'N 28º54.120'W Azores
Nemertesia cf *antennina* / sp7 *	JN714603	DOP C848	F/V Açor Ocidental	Palangre de fundo	Princesa Alice Bank, 567 m 37º39.480'N 28º53.520'W Azores
Nemertesia cf *antennina* / sp7 *	JN714604	DOP C849	F/V Açor Ocidental	Palangre de fundo	Princesa Alice Bank, 567 m 37º39.480'N 28º53.520'W Azores
Plumularia setacea *	JN714605	DBUA 1122.06	C.J. Moura	Hand	Faial Island, Gruta da Pedra, 5-16 m 38º30.950'N 28º37.618'W Azores
Plumularia setacea *	JN714606	DBUA 1122.02	C.J. Moura	Hand	São Miguel Island, Feteiras do Sul, 5-13 m 37º42.836'N 25º29.756'W

					Azores
Plumularia setacea *	JN714607	DBUA 1123.01	C.J. Moura	Hand	Berlengas, Farilhões (cave), 28-32 m 39º28.351’N 9º32.821’W W Portugal
Plumularia setacea *	JN714608	DBUA 1124.01	J. Pedro Luso-Expedição Olympus 2007	Hand	Gorringe seamount, Gettysburg, 37-39 m 36º31.071'N 11º34.275'W E Atlantic
Plumularia setacea *	JN714609	DBUA 1125.01	C.J. Moura	Hand	Aljezur, Porto Covo, 15-20 m 37°51.192'N 8º48.211’W SW Portugal
Plumularia setacea *	JN714610	DBUA 1125.02	C.J. Moura	Hand	Sagres, Ponta de Sagres, 17-22 m 36º59.700’N 8º56.715’W S Portugal
Plumularia setacea *	JN714611	DBUA 1123.02	C.J. Moura	Hand	Berlengas, Estelas, 15-26 m 39º25.225’N 9º32.084’W W Portugal
Plumularia setacea	AY787886 (Cunningham, EMBL subm.)	MHNG INVE29393			St. Pol de Leon S France
Plumularia setacea *	JN714612	DBUA 1125.03	C.J. Moura	Hand	Sines, São Torpes, 9-13 m 37º54.770’N 8º48.467’W SW Portugal
Plumularia setacea *	JN714613	DBUA 1123.03	C.J. Moura	Hand	Berlenga Island, Ilhéu do Soldado, 5-16 m 39º24.981’N 9º30.784’W W Portugal
Plumularia setacea *	JN714614	DBUA 1125.04	C.J. Moura	Hand	Ericeira, Coxos, 4-8 m 39º00.151’N 9º25.672’W W Portugal
Plumularia setacea *	JN714615	DBUA 1125.05	C.J. Moura	Hand	Sagres, Ilhas do Martinhal, 5-15 m 37º00.897’N 8º55.080’W S Portugal
Plumularia setacea *	JN714616	DBUA 1125.06	C.J. Moura	Hand	Sines, outside harbour, 10-15 m 37º56.323’N 8º53.330’W SW Portugal
Plumularia setacea *	JN714617	DBUA 1123.01	C.J. Moura	Hand	Berlengas, Farilhões (cave), 28-32 m 39º28.351’N 9º32.821’W W Portugal
Plumularia setacea *	JN714618	DBUA 1126.01	C.J. Moura	Hand	Cascais, marina, dock, 0-0.5 m 38º41.454’N 9º25.165’W W Portugal
Plumularia setacea	FJ550459 (Lecrére *et al*. 2009)				Roscoff NW France
Plumularia setacea	AY787885 (Cunningham, EMBL subm.)	MHNG INVE29392			Roscoff NW France
Plumularia setacea *	JN714619	DBUA 1123.04	C.J. Moura	Hand	Berlengas, Rinchão, 25-35m 39º25.437’N 9º29.941’W NW Portugal
Plumularia setacea *	JN714620	DBUA 1127.01	C.J. Moura	Hand	Viana do Castelo, 29-33 m 41º41.680’N 8º52.711’W NW Portugal
Plumularia lagenifera	DQ855928 (Leclère *et al*. 2007)				Friday Harbour USA
Plumularia setacea	AY787887 (Cunningham, EMBL subm.)	MHNG INVE29966			Auckland, Whangaparoa, Arm Bay New Zealand
Plumularia setacea	EF472685 (Galea & Leclère 2007)	MNHG INVE38773			Chile
Plumularia setacea	EF472683 (Galea & Leclère 2007)	MNHG INVE35933			Comao, Punta Huinay Chile
Plumularia setacea	EF472682 (Galea & Leclère 2007)	MNHG INVE35935			Comao, Punta Huinay Chile
Plumularia setacea	EF472684 (Galea & Leclère 2007)	MNHG INVE38774			Huinay, Punta Llonca Chile
Plumularia setacea *	JN714621	DBUA 1122.03	C.J. Moura	Hand	Flores Island,Ponta Delgada, 17-24 m 39º31.714’N 31º12.148’W Azores

Plumularia setacea *	JN714622	DBUA 1122.04	C.J. Moura	Hand	Flores Island, 10-16 m 39º28.531'N 31º08.479'W Azores
Plumularia setacea *	JN714623	DBUA 1122.05	C.J. Moura	Hand	São Miguel Island, Ponta Delgada naval club, 0-0.5 m 37º44.460'N 25º39.476'W Azores
Plumularia setacea *	JN714624	DBUA 1125.07	C.J. Moura	Hand	Lagoa, Carvoeiro, 1-8 m 37º05.419'N 8º27.269'W S Portugal
Plumularia strictocarpa	DQ855940 (Leclère *et al*. 2007)				São Sebastião, Praia do Segredo Brazil
Plumularia cf *lagenifera*	FJ550491 (Leclère *et al*. 2009)	MHNG INVE34019			Simons Town South Africa
Plumularia hyalina	AY787913 (Cunningham, EMBL subm.)	MHNG INVE25333			Leigh New Zealand
Plumularia setaceoides	DQ855931 (Leclère *et al*. 2007)	MHNG INVE33460			Wellington, Te Raikaihau New Zealand
Plumularia filicaulis	DQ855926 (Leclère *et al*. 2007)	MHNG INVE34020			False Bay, Simons Town, Boulder Beach South Africa
Monotheca obliqua *	JN714625	DBUA 1267.01	C.J. Moura	Hand	São Miguel Island, Mosteiros, 18-23 m 37º53.372'N 25º50.132'W Azores
Monotheca obliqua *	JN714626	DBUA 1268.01	C.J. Moura	Hand	Ericeira, Coxos, 4-8 m 39º00.151'N 9º25.672'W W Portugal
Monotheca obliqua *	JN714627	DBUA 1269.01	C.J. Moura	Hand	Berlenga Island, Ilhéu do Soldado, 5-16 m 39º24.981'N 9º30.784'W W Portugal
Monotheca obliqua *	JN714628	DBUA 1268.02	C.J. Moura	Hand	Sines, outside harbour, 10-15 m 37º56.323'N 8º53.330'W SW Portugal
Monotheca obliqua	DQ855929 (Leclère *et al*. 2007)				Banyuls-sur-Mer France
Monotheca margaretta *	JN714629	DBUA 1270.01	C.J. Moura	Hand	Lido, Funchal naval club, 3-13 m 32º38.046'N 16º56.351'W Madeira
Monotheca margaretta	AY787892 (Cunningham, EMBL subm.)	MHNG INVE29760			Mallorca, Cala Murada S Spain
Monotheca margaretta	FJ550483 (Leclère *et al*. 2009)	MHNG INVE29760			Mallorca S Spain
Monotheca margaretta *	JN714630	DBUA 1271.01	C.J. Moura	Hand	Faial Island, near Horta, Entre Montes, 3-18 m 38º31.800'N 28º37.662'W Azores
Monotheca margaretta *	JN714631	DBUA 1270.02	C.J. Moura	Hand	Ribeira Brava, fishing port, 3-8 m 32º40.086'N 17º03.781'W Madeira
Monotheca pulchella	DQ855930 (Leclère *et al*.2007)	MHNG INVE34016			False Bay, Simons Town, Boulder Beach South Africa
Antenella secundaria	FJ550467 (Leclère *et al*. 2009)				Roscoff NW France
Halopteris diaphana	DQ855921 (Leclère *et al*.2007)	MHNG INVE30116			Mallorca, Cala Murada S Spain

Abbreviations: DBUA, 'Departamento de Biologia da Universidade de Aveiro'; DOP, 'Departamento de Oceanografia e Pescas'; F/V, Fishing Vessel; M, oceanographic campaign 'Moundforce'; MV, mud volcano; MMF, 'Museu Municipal do Funchal'; MHNG, Muséum d'Histoire Naturelle de Geneve, Switzerland; PE, Pelagia; ROV, Remote Operated Vehicle; R/V, Research Vessel; Stn, station; TTR, oceanographic campaign 'Training Trough Research'.

Table 9.2. Morphological features and measurements (in µm) of the *N. antennina* species complex observed in each lineage.

Species	*N. antennina* - lineage 1	*N. antennina* - lineage 2	*N.* sp1	*N.* sp2	*N.* sp3	*N.* sp4	*N.* sp5 / cf *perrieri*	*N.* sp6	*N.* sp7	*N. perrieri* (type material)	*N. irregularis* (type material)
Maximum height of hidrocaulus (in cm):	14.7	10.9	13.1	3.6	1.5	4.5	ca. 16	4.3	3.4	25	ca 9.6
Hydrocaulus diameter:	470-670	330-780	110-540	70-250	65-250	70-230	290-710	160-250	65-340	590-980	255-408
Maximum number of cauline nematothecae per apophysis:	5	4	6	4	4	5	4	4	4	5	4
Number of ahydrothecate internodes proximal to the first hydrothecal internode:	0-2	0-2	0-3	1-2	0-3	0-2	1-3 (mostly 1)	1-3 (mostly 1)	1-2 (usually 1)	0-2 (usually 1)	1-4
Number of ahydrothecate internodes between hydrothecal internodes:	1-2 (occasionally 2)	1-2 (rarely 2)	1-2 (occasionally 2)	1-2 (occasionally 2)	1-2 (usually 1)	1-3 (usually 1)	1-2 (rarely 2)	1-3 (mostly 1)	1-2 (occasionally 2)	1-2 (occasionally 2)	1-2 (occasionally 2)
Basal internode of hydrocladium,											
Length:	70-150	110-160	160-300	210-600	170-460	145-490	110-160	160-420	120-260	170	214-326
Diameter at node:	70-120	60-85	50-100	40-80	45-80	40-65	60-100	65-90	60-80	110-115	112-143
Nematothecae:	1-2 (rarely 2)	1	1	1-2 (usually 1)	1	1-2 (variable)	1	1-2 (mostly 2)	1	0-1	1
Hydrothecate internode,											
Length:	210-400	270-350	300-630	330-650	390-690	280-500	360-430	460-700	310-550	260-430	663-816
Ahydrothecate internode,											
Length:	110-250	110-200	150-610	340-980	150-655	150-730	150-330	300-730	160-580	180-370	363-643
Diameter at node:	50-90	40-75	30-80	30-65	30-65	30-60	50-60	30-55	35-80	45-60	82-112
Hydrotheca,											
Length abcauline wall:	55-110	60-90	50-85	40-70	55-80	50-70	60-80	50-70	50-80	60-80	92-122
Length adcauline wall:	70-120	70-100	70-100	50-85	65-100	60-100	70-85	60-75	70-115	70-90	102-133
Diameter at rim:	95-140	90-125	70-110	70-100	70-100	70-100	90-120	70-85	90-105	90-110	102-143
Lateral nematothecae,											
Length:	85-130	80-125	100-200	80-140	65-100	70-120	90-110	90-120	80-110	95-120	163-224
Diameter at rim:	40-55	50-70	40-60	30-50	25-45	30-50	40-45	35-50	40-55	50-70	61-82
Median nematothecae,											
Length:	80-110	65-105	70-120	65-95	50-80	50-100	60-100	60-90	60-90	65-100	112-143
Diameter at rim:	30-50	35-55	30-45	25-40	25-40	30-50	30-50	30-40	35-45	45-60	41-51
Number per ahydrothecate internode:	1-2 (occasionally 2)	1-2 (variable)	0-2 (usually 1)	2-3 (usually 2)	1-2 (mostly 2)	1-3 (usually 2, 3 frequently)	1-2 (mostly 2)	2-3 (rarely 3)	1-2 (variable)	1-2 (mostly 2)	1-2 (mostly 2)
Axial nematothecae,											
Length:	90-150	80-120	60-120	70-120	75-120	70-115	90-120	70-90	80-100	90-100	112-204
Diameter at rim:	40-55	40-60	25-40	25-50	30-40	20-50	40-50	30-40	30-50	40-50	51-82
Gonothecae,		Not found			Not found	Not found	Not found				
Length:	800-1190	-	490-860	520-820	-	-	-	470-700	400-700	1050-1180	918-1071
Maximum diameter:	320-580	-	170-350	130-300	-	-	-	180-300	135-270	390-630	357-459

Table 9.3. Detailed morphological features and measurements (in µm) of the *N. antennina* species complex.

Species	*N. antennina* - lineage 1	*N. antennina* - lineage 1	*N. antennina* - lineage 1	*N. antennina* - lineage 3	*N. antennina* - lineage 3	*N. antennina* - lineage 3	*N.* sp 1	*N.* sp 1	*N.* sp 1	*N.* sp 1	*N.* sp 1
Locality	Lundy island, U.K.	Plymouth, U.K.	Viana Castelo, Portugal	Estelas, Portugal	Ponta Sagres, Portugal	Porto Covo, Portugal	Stn AT569Gr, Mercator MV - GoC	Stn AT575B, Mercator MV - GoC	Stn MS416GR, Mulhacen MV - Alboran Sea	Stn MS420GR, Granada MV - Alboran Sea	Bay of Biscay, N Spain
Maximum height of hidrocaulus (in cm):	11.8	14.7	8.8	10.9	8.7	8.4	3.5	2.4	8.3	13.1	
Axis, diameter:	470-600	500-670	610-630	530-780	330-480	575-580	110-350	205-280	180-270	260-540	380-430
Maximum number of cauline nematothecae per apophysis:	4	5	4	4	4	4	4	4	6	3	4
Number of ahydrothecate internodes proximal to the first hydrothecal internode:	0-1	0-1	0-2	0-1	1	1-2 (usually 1)	1	1	0-2	1-3	1
Number of ahydrothecate internodes between hydrothecal internodes:	1	1-2 (occasionally 2)	1	1	1	1-2 (mostly 1)	1-2 (occasionally 2)	1-2 (occasionally 2)	1-2	1-2 (occasionally 2)	1
Basal internode of hydrocladium,											
Length:	120-150	100-150	70-120	110-160	120-150	130-140	180-240	180-220	170-210	230-300	160-210
Diameter at node:	100	70-75	80-120	60-70	60-85	70-80	50-90	50-75	60-75	80-90	80-100
Nematothecae:	1	1-2 (rarely 2)	1	1	1	1	1	1	1	1	1
Hydrothecate internode,											
Length:	210-400	250-375	230-360	280-330	270-340	310-350	300-550	350-500	430-570	460-630	490-550
Ahydrothecate internode,											
Length:	150-230	110-240	130-250	110-150	120-200	125-175	150-480	150-410	150-500	280-610	200-330
Diameter at node:	50-90	50-60	50-90	40-60	50-75	50-70	40-80	40-60	30-50	40-50	50-75
Hydrotheca,											
Length abcauline wall:	70-100	55-85	65-110	60-80	70-90	60-80	50-80	55-70	60-70	55-80	60-85
Length adcauline wall:	100-120	70-95	80-100	75-90	70-100	75-80	70-80	80-100	75-100	70-80	75-90
Diameter at rim:	100-140	95-130	115-125	90-120	95-125	90-125	70-100	90-100	80-110	90-110	80-110
Lateral nematothecae,											
Length:	100-130	85-105	90-120	90-125	90-100	80-100	100-180	115-155	120-200	150-190	140-160
Diameter at rim:	45-55	45-55	40-50	50-70	45-55	40-50	40-50	40-55	50-60	45-50	45-55
Median nematothecae,											
Length:	80-90	80-105	80-110	65-100	70-105	70-80	70-115	75-105	90-110	100-120	80-100
Diameter at rim:	30-40	40-50	40-50	40	35-40	40-55	30-45	35-40	35-40	35-45	35-40
Number per ahydrothecate internode:	1	1	1-2 (usually 1)	1	1	1-2 (mostly 1)	0-1 (usually 1)	1 (usually 1)	1-2 (usually 1)	1-2 (usually 1)	1
Axial nematothecae,											
Length:	90-150	100-120	95-130	100-120	100-110	80-110	60-120	70-100	70-90	85-100	70-110
Diameter at rim:	40-50	40-55	50	55-60	45-50	40-60	30-40	30-35	25-40	30-40	30-40
Gonothecae,											
Length:	800-1150	910-1190	860-1040	-	-	-	490-700	-	700-850	710-860	-
Maximum diameter:	490-580	320-540	480-530	-	-	-	170-220	-	300-350	250-290	-

Table 9.3. (Continued)

N. sp 3	*N.* sp 3	*N.* sp 4	*N.* sp 4	*N.* sp 5 / cf *perrieri*	*N.* sp 6	*N.* sp 7	*N.* sp 7	*N. perrieri* (type material)	*N. perrieri* var *antennoides* (type material)	*N. perrieri* var *antennoides* (type material)	*N. antennina* var *longua* (type material)	*N. antennina* var *irregularis*	*N. irregularis* (type material)
Stn Dive 27 - Rock 2, NE Mercator, GoC	Stn Dive 27 - Rock 3, NE Mercator, GoC	Stn AT-599-D, GoC	Stn Dive 27 Rock 6, GoC	DOP C408, Azores	DOP C2363, Azores	DOP C849, Azores	DOP C848, Azores	Blanc cape, Canary Islands	Canary Islands	Cap Blanc, Canary Islands	Spartel cape, coast of Morocco	'Vanneau' Stat CX	Off a cable, Cape Verde Islands
1.5	1.2	1.2	4.5	*ca.* 16	4.3	3.4	3.4	25					*ca.* 9.6
80-250	65-160	70-220	75-330	290-710	160-250	65-340	120-210	590-980	530-560	280-360	450-570	260-340	255-408
3	3	5	3	4	4	4	3	5	4	4	5	4	4
0-3 (mostly 1)	1-2	0-1	1-2 (occasionally 2)	1-3 (mostly 1)	1-3 (mostly 1)	1	1-2 (usually 1)	0-2 (usually 1)	1-2 (rarely 2)	0-1	1-2 (mostly 1)	0-1	1-4
1-2 (mostly 1)	1-2 (mostly 1)	1-3 (occasionally 2, rarely 3)	1 -3 (mostly 1, occasionally 2, rarely 3)	1-2 (rarely 2)	1-3 (mostly 1)	1-2 (occasionally 2)	1-2 (occasionally 2)	1-2 (occasionally 2)	1-2 (occasionally 2)	1	1	1	1-2 (occasionally 2)
170-350	225-460	170-490	145-410	110-160	160-420	120-260	120-170	170	150-220	80-180	140-230	140	214-326
50-80	45-60	40-60	50-65	60-100	65-90	70-80	60-75	110-115	70-75	70-80	60-70	70	112-143
1	1	1-2 (usually 2)	1-2 (occasionally 2)	1	1-2 (mostly 2)	1	1	0-1	1	0-1	1	1	1
390-600	415-690	350-500	280-440	360-430	460-700	370-550	310-540	260-430	380-440	360-490	380-500	380-450	663-816
190-655	150-640	170-730	150-570	150-330	300-730	250-550	160-580	180-370	290-360	290-350	150-340	250-390	363-643
30-60	40-65	30-60	40-50	50-60	30-55	35-80	40-75	45-60	50-60	45-55	50-60	45-60	82-112
60-80	55-80	50-60	50-70	60-80	50-70	55-80	50-75	60-80	45-70	50-60	50-60	45-60	92-122
70-100	65-90	60-80	70-100	70-85	60-75	80-115	70-85	70-90	60-80	60-80	50-60	50-80	102-133
80-100	70-90	70-90	85-100	90-120	70-85	90-105	90-100	90-110	70-90	70-90	65-85	75-95	102-143
65-100	70-80	70-120	110-120	90-110	90-120	80-110	100	95-120	130-140	110-140	120	120-135	163-224
30-45	25-30	30-45	45-50	40-45	35-50	40-55	40-45	50-70	40-60	50	50	45-50	61-82
50-80	60-70	50-90	60-100	60-100	60-90	60-90	60-80	65-100	70-105	80-100	80-105	75-100	112-143
25-40	25-30	30-40	30-50	30-50	30-40	35-40	40-45	45-60	40-45	40-45	40-45	35-45	41-51
2	1-2 (usually2)	1-3 (usually 2, frequently 3)	1-3 (usually 2)	1-2 (mostly 2)	2-3 (rarely 3)	1-2 (usually 1)	1-2 (usually 2)	1-2 (mostly 2)	1-2 (rarely 1)	1-2 (rarely 1)	1-2 (variable)	1-2 (variable)	1-2 (mostly 2)
75-120	75-90	70-100	80-115	90-120	70-90	80-100	90	90-100	100-140	80-100	80-100	90-100	112-204
30-40	30-35	20-35	35-50	40-50	30-40	30-50	40	40-50	40	40	40	40	51-82
-	-	-	-	-	470-700	530-700	400-590	1050-1180	700-780	-	-	650-760	918-1071
-	-	-	-	-	180-300	135-270	150-180	390-630	230-310	-	-	270-345	357-459

Table 9.4. Morphological features and measurements (in µm) of the *N. belini* species complex observed in each lineage.

Locality	Pico, Azores (type material)	São Jorge, Queimada, Azores	Off Garajau, Madeira	Azores Bank, Azores	Mellila carb. mound, Alboran Sea
Maximum height of hidrocaulus (in cm):	25	9,9	23	2,1	3,3
Axis, diameter:	350-950	280-430	190-700	180-280	110-190
Maximum number of cauline nematothecae per apophysis:	9	10	8	5	8
Number of ahydrothecate internodes proximal to the first hydrothecal internode:	0-3 (usually none)	1-3	0	0	0-1
Number of ahydrothecate internodes between hydrothecal internodes:	0-3	0-2	0-2	0-1 (mostly none)	0-2
Basal internode of hydrocladium,					
Length:	190-340	190-250	-	-	170-320
Diameter at node:	100-110	100-110	80-90	60-80	50-70
Nematothecae:	1-3	1-4			1
Hydrothecate internode,				(occasionally with 2 hydrothecae)	
Length:	590-1000	490-1020	600-1230	420-1400	360-970
Ahydrothecate internode,					
Length:	160-520	190-430	170-580	75-200	160-650
Diameter at node:	50-90	50-70	50-60	45-60	30-45
Hydrotheca,					
Length abcauline wall:	90-120	100-110	75-100	65-80	80-100
Length adcauline wall:	100-140	110-150	100-130	80-105	110-120
Diameter at rim:	110-150	110-160	100-135	70-90	100-110
Lateral nematothecae,	(occasionally 3)				
Length:	80-115	110-120	80-105	70-90	80-90
Diameter at rim:	45-60	55-60	30-50	30-40	35-45
Median nematothecae,					
Length:	90-125	100-110	80-100	65-80	60-75
Diameter at rim:	40-50	40-50	30-40	30-40	30-35
Number per ahydrothecate internode:	1-3	1-3	1-2	1-2	1-3
Number of supracalicine on thecate internodes	1-3	0-3	1-3	2-3	0
Number bellow hydrothecae on thecate internodes	1-5	2-4	1-3	1-3	1-4
Axial nematothecae,					
Length:	110-190	120-150	110-150	65-75	65-90
Diameter at rim:	40-55	40-60	35-45	25-30	35-40
Gonothecae,				Not found	
Length:	540-650	660-720	610-700	-	380-450
Maximum diameter:	270-380	300-320	280-310	-	180-200

Table 9.5. Measurements (in µm) of the largest groups of nematocysts (length, diameter, respective ratio) of species of the *N. antennina* and *N. belini* complexes.

Species	**Range (Dimentions/Ratio)**	**Mean ± SD (n=10)**
N. antennina	9.2-10 x 4-4.5	9.7 ± 0.4 x 4.1 ± 0.2
- lineage 1 Stn Lundy	2.2-2.5	2.4±0.1
N. antennina	9-10 x 4-4.8	9.6 ± 0.4 x 4.2 ± 0.3
- lineage 1 Stn Lundy	2.1-2.5	2.3±0.1
N. antennina	8.8-10 x 3.5-4.4	9.5 ± 0.3 x 4 ± 0.3
- lineage 1 Stn V. Castelo	2-2.8	2.4±0.2
N. antennina	8.5-10 x 3.5-4.1	9.3 ± 0.5 x 3.9 ± 0.3
- lineage 3 Stn Estelas	2.1-2.7	2.4 ± 0.2
N. antennina	9-10 x 4-4.7	9.6 ± 0.4 x 4.3 ± 0.3
- lineage 3 Stn Pto Covo	2.1-2.5	2.2 ± 0.1
N. antennina	8.1-10 x 3.1-5	9.3 ± 0.6 x 4.1 ± 0.5
- lineage 3 Stn Pta Sagres	1.9-3	2.3 ± 0.3
N. sp1	10.5-11 x 3.7-4.5	10.8 ± 0.2 x 4.1 ± 0.2
- Stn Bay Biscay	2.4-3	2.6 ± 0.2
N. sp1	10-11.8 x 3.5-4.7	10.6 ± 0.5 x 4.1 ± 0.4
- Stn MS420Gr	2.3-3	2.6 ± 0.3
N. sp1	10.2-11.5 x 3.5-4.5	11 ± 0.3 x 4 ± 0.2
- Stn MS416Gr	2.5-2.9	2.8 ± 0.1
N. sp1	10-11.2 x 3.2-4	10.7 ± 0.4 x 3.5 ± 0.2
- Stn AT569B	2.8-3.2	3 ± 0.1
N. sp1	10-11.6 x 3.5-4.1	10.7 ± 0.4 x 3.7 ± 0.2
- Stn AT575B	2.6-3	2.9 ± 0.1
N. sp2	9.4-10.6 x 3.5-4.1	10.1 ± 0.3 x 3.9 ± 0.2
- Stn M2007-39	2.3-2.8	2.6 ± 0.2
N. sp2	10-11.2 x 3.8-4	10.8 ± 0.4 x 3.9 ± 0.1
- Stn M2007-40	2.6-2.9	2.7 ± 0.1
N. sp2	10.8-11.5 x 4	11 ± 0.2 x 4
- Stn GEOB12728-1	2.7-2.9	2.8 ± 0
N. sp3	9.6-11 x 3.5-4	10.7 ± 0.5 x 3.8 ± 0.2
- Stn Dive27Rock2	2.5-3	2.8 ± 0.2
N. sp3	10-11.8 x 3.2-4.1	10.6 ± 0.5 x 3.5 ± 0.2
- Stn Dive27Rock3	2.8-3.2	3 ± 0.1
N. sp4	8.8-11.3 x 3.1-3.8	10.1 ± 0.7 x 3.4 ± 0.3
- Stn Dive27Rock6	2.3-3.4	3 ± 0.3
N. sp4	10.2-11.2 x 3.2-4	10.7 ± 0.3 x 3.9 ± 0.3
- Stn 599D	2.5-3.4	2.8 ± 0.3
N. sp5	10-11.2 x 2.6-3.5	10.7 ± 0.3 x 2.9 ± 0.2
- Stn C408	3-4	3.7 ± 0.3
N. sp6	8.2-9.4 x 2.6-2.9	8.8 ± 0.3 x 2.8 ± 0.1
- Stn C2363	2.8-3.4	3.1 ± 0.2
N. sp7	13.5-15.2 x 3-3.4	14.2 ± 0.5 x 3.2 ± 0.2
- Stn C848	4.1-5.1	4.5 ± 0.3
N. sp7	14-15.2 x 3-3.2	14.6 ± 0.5 x 3.1 ± 0.1
- Stn C849	4.3-5	4.7 ± 0.2
N. belini	10.1-11.3 x 3.7-4.4	10.9 ± 0.4 x 4 ± 0.2
-Stn C2232	2.4-2.9	2.7 ± 0.2
N. cf *belini*	8.8-9.9 x 3.4-4	9.1 ± 0.4 x 3.7 ± 0.2
-Stn MS395Gr	2.3-2.8	2.5 ± 0.2

Table 9.6. Age profiles for selected nodes on the phylogeny, calibrated by previously set substitutions per site per year (sources: 1 - Govindarajan *et al*. 2005; 2 - Lindner *et al*. 2008). Calibration 3 and 4 assume different dates for the rise

Node	**Age (Ma)**	1,25E-09[1]	2,44E-09[1]	4,49E-09[2]	6,54E-09[1]	7,71E-09[2]	**9,69E-09**[3]	1,21E-08[4]	1,95E-08[1]
Root	Mean	1152,3	590,3	320,8	220,2	186,8	**148,6**	118,9	73,9
	5th %ile	821,7	420,9	228,8	157,1	133,2	**106,0**	84,8	52,7
	95th %ile	1555,0	796,6	432,9	297,2	252,1	**200,5**	160,4	99,7
Ingroup (*Nemertesia* + *Plumularia* + *Monotheca*)	Mean	344,0	176,2	95,8	65,7	55,8	**44,4**	35,5	22,0
	5th %ile	274,7	140,7	76,5	52,5	44,5	**35,4**	28,3	17,6
	95th %ile	424,4	217,4	118,1	81,1	68,8	**54,7**	43,8	27,2
Monotheca	Mean	212,7	108,9	59,2	40,6	34,5	**27,4**	21,9	13,6
	5th %ile	166,5	85,3	46,3	31,8	27,0	**21,5**	17,2	10,7
	95th %ile	267,3	136,9	74,4	51,1	43,3	**34,5**	27,6	17,1
Nemertesia + Plumularia	Mean	177,3	90,8	49,4	33,9	28,7	**22,9**	18,3	11,4
	5th %ile	141,7	72,6	39,4	27,1	23,0	**18,3**	14,6	9,1
	95th %ile	219,5	112,5	61,1	42,0	35,6	**28,3**	22,6	14,1
Plumularia	Mean	56,5	28,9	15,7	10,8	9,2	**7,3**	5,8	3,6
	5th %ile	43,8	22,4	12,2	8,4	7,1	**5,6**	4,5	2,8
	95th %ile	71,5	36,6	19,9	13,7	11,6	**9,2**	7,4	4,6
P. setacea US/Chile (Stem age)	Mean	27,3	14,0	7,6	5,2	**4,4**	**3,5**	2,8	1,7
	5th %ile	20,4	10,5	5,7	3,9	3,3	**2,6**	2,1	1,3
	95th %ile	35,8	18,4	10,0	6,8	5,8	**4,6**	3,7	2,3
P. setacea (N & Central Portugal) Vs (S Portugal) *(15 taxa)*	Mean	7,4	3,8	2,1	1,4	1,2	**1,0**	**0,8**	**0,5**
	5th %ile	4,6	2,3	1,3	0,9	0,7	**0,6**	0,5	0,3
	95th %ile	11,1	5,7	3,1	2,1	1,8	**1,4**	1,1	0,7
P. setacea Azores (S. Miguel & Faial) & W Portugal (Farilhões) Vs 'all other P. setacea sequences' (19 taxa)	Mean	12,1	6,2	3,4	2,3	2,0	**1,6**	1,2	0,8
	5th %ile	8,2	4,2	2,3	1,6	1,3	**1,1**	0,8	0,5
	95th %ile	16,9	8,7	4,7	3,2	2,7	**2,2**	1,7	1,1
P. setacea Azores (S. Miguel & Faial) Vs *P. setacea* W Portugal *(3 taxa)*	Mean	11,9	6,1	3,3	2,3	1,9	**1,5**	1,2	0,8
	5th %ile	7,6	3,9	2,1	1,4	1,2	**1,0**	0,8	0,5
	95th %ile	16,9	8,7	4,7	3,2	2,7	**2,2**	1,7	1,1
M. pulchella Azores Vs *M. pulchella* W Portugal *(4 taxa)*	Mean	1,5	0,8	0,4	0,3	0,2	**0,2**	0,2	0,1
	5th %ile	0,2	0,1	0,0	0,0	0,0	**0,0**	0,0	0,0
	95th %ile	3,4	1,7	0,9	0,6	0,5	**0,4**	0,3	0,2
M. margaretta Mallorca Vs *M. margaretta* Azores & Madeira (3 taxa)	Mean	3,0	1,5	0,8	0,6	0,5	**0,4**	0,3	0,2
	5th %ile	1,0	0,5	0,3	0,2	0,2	**0,1**	0,1	0,1

Table 9.6. (Continued)

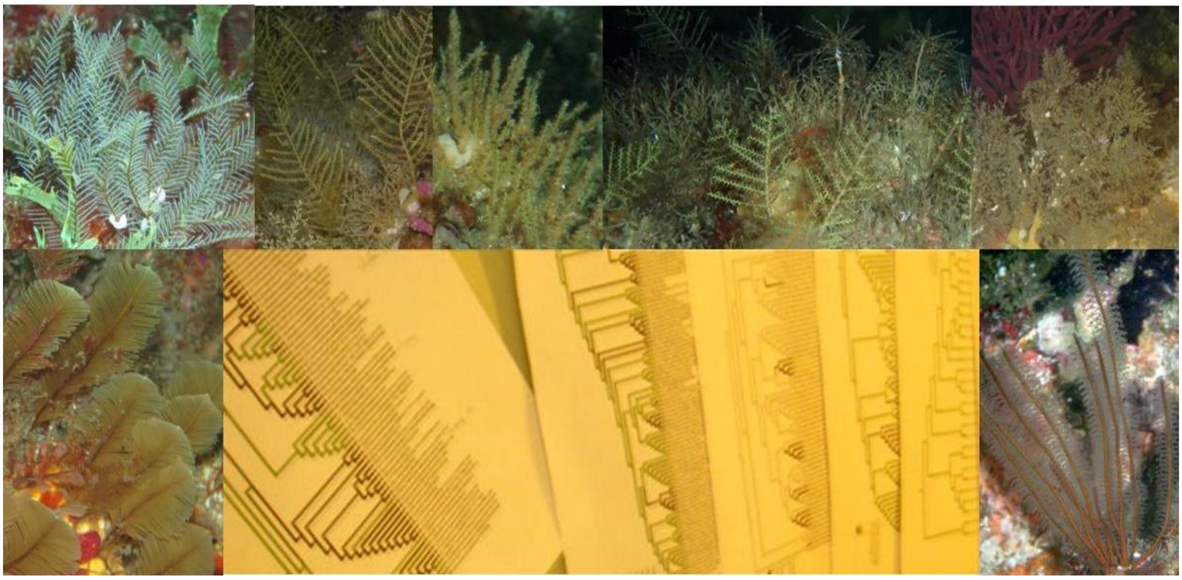
	95th %ile	5,9	3,0	1,6	1,1	1,0	**0,8**	0,6	0,4
Plumularia spfrom Flores (Stem age)	Mean	37,5	19,2	10,4	**7,2**	6,1	**4,8**	3,9	2,4
	5th %ile	28,9	14,8	8,0	5,5	4,7	**3,7**	3,0	1,9
	95th %ile	47,5	24,3	13,2	9,1	7,7	**6,1**	4,9	3,0
Plumularia from Flores (2 taxa)	Mean	1,7	0,9	0,5	0,3	0,3	**0,2**	0,2	0,1
	5th %ile	0,3	0,1	0,1	0,1	0,0	**0,0**	0,0	0,0
	95th %ile	4,0	2,0	1,1	0,8	0,6	**0,5**	0,4	0,3
Nemertesia (63 taxa)	Mean	60,1	30,8	16,7	11,5	9,7	**7,8**	6,2	3,9
	5th %ile	48,2	24,7	13,4	9,2	7,8	**6,2**	5,0	3,1
	95th %ile	75,0	38,4	20,9	14,3	12,2	**9,7**	7,7	4,8
N. ramosa (9 taxa)	Mean	4,0	2,0	1,1	0,8	0,6	**0,5**	0,4	0,3
	5th %ile	2,1	1,1	0,6	0,4	0,3	**0,3**	0,2	0,1
	95th %ile	6,6	3,4	1,8	1,3	1,1	**0,8**	0,7	0,4
Nemertesia excl. *N. ramosa* (54 taxa)	Mean	56,1	28,7	15,6	10,7	9,1	**7,2**	5,8	3,6
	5th %ile	45,3	23,2	12,6	8,7	7,3	**5,8**	4,7	2,9
	95th %ile	68,3	35,0	19,0	13,1	11,1	**8,8**	7,1	4,4
N. norvegica + *N. ventriculiformis* (14 taxa)	Mean	43,3	22,2	12,1	8,3	7,0	**5,6**	4,5	2,8
	5th %ile	28,0	14,3	7,8	5,3	4,5	**3,6**	2,9	1,8
	95th %ile	61,9	31,7	17,2	11,8	10,0	**8,0**	6,4	4,0
N. norvegica (5 taxa)	Mean	3,8	2,0	1,1	0,7	0,6	**0,5**	0,4	0,2
	5th %ile	1,6	0,8	0,4	0,3	0,3	**0,2**	0,2	0,1
	95th %ile	7,0	3,6	2,0	1,3	1,1	**0,9**	0,7	0,5
N. ventriculiformis (9 taxa)	Mean	3,1	1,6	0,9	0,6	0,5	**0,4**	0,3	0,2
	5th %ile	1,4	0,7	0,4	0,3	0,2	**0,2**	0,1	0,1
	95th %ile	5,6	2,9	1,6	1,1	0,9	**0,7**	0,6	0,4
N. belini + *N. antennina* (18 taxa)	Mean	33,1	16,9	9,2	6,3	5,4	**4,3**	3,4	2,1
	5th %ile	24,9	12,8	6,9	4,8	4,0	**3,2**	2,6	1,6
	95th %ile	42,5	21,8	11,8	8,1	6,9	**5,5**	4,4	2,7
N. belini (4 taxa)	Mean	26,4	13,5	7,4	5,1	4,3	**3,4**	2,7	1,7
	5th %ile	18,7	9,6	5,2	3,6	3,0	**2,4**	1,9	1,2
	95th %ile	35,8	18,3	10,0	6,8	5,8	**4,6**	3,7	2,3
N. antennina (North vs South) (Shallow water clade) (14 taxa)	Mean	5,8	3,0	1,6	1,1	0,9	**0,8**	**0,6**	**0,4**
	5th %ile	3,2	1,7	0,9	0,6	0,5	**0,4**	0,3	0,2
	95th %ile	9,2	4,7	2,6	1,8	1,5	**1,2**	1,0	0,6
N. sp 1-7 (22 taxa)	Mean	33,8	17,3	9,4	6,5	5,5	**4,4**	3,5	2,2
	5th %ile	26,1	13,4	7,3	5,0	4,2	**3,4**	2,7	1,7
	95th %ile	42,5	21,8	11,8	8,1	6,9	**5,5**	4,4	2,7

Table 9.6. (Continued)

N. sp 5,6,7 (Azores) (4 taxa)	Mean	13,1	6,7	3,7	2,5	2,1	**1,7**	1,4	0,8
	5th %ile	8,4	4,3	2,3	1,6	1,4	**1,1**	0,9	0,5
	95th %ile	18,8	9,6	5,2	3,6	3,1	**2,4**	1,9	1,2
N. belini (Azores) (3 taxa)	Mean	6,9	3,5	1,9	1,3	1,1	**0,9**	0,7	0,4
	5th %ile	3,7	1,9	1,0	0,7	0,6	**0,5**	0,4	0,2
	95th %ile	10,9	5,6	3,0	2,1	1,8	**1,4**	1,1	0,7
N. sp5 stem lineage (Shallow water clade) (2 taxa)	Mean	1,4	0,7	0,4	0,3	0,2	**0,2**	0,1	0,1
	5th %ile	0,2	0,1	0,0	0,0	0,0	**0,0**	0,0	0,0
	95th %ile	3,2	1,6	0,9	0,6	0,5	**0,4**	0,3	0,2
N. sp1 (7 taxa)	Mean	2,1	1,1	0,6	0,4	0,3	**0,3**	0,2	0,1
	5th %ile	0,9	0,4	0,2	0,2	0,1	**0,1**	0,1	0,1
	95th %ile	3,9	2,0	1,1	0,8	0,6	**0,5**	0,4	0,3
N. sp1 + *N*. sp2 (14 taxa)	Mean	17,8	9,1	5,0	3,4	2,9	**2,3**	1,8	1,1
	5th %ile	12,3	6,3	3,4	2,4	2,0	**1,6**	1,3	0,8
	95th %ile	24,5	12,6	6,8	4,7	4,0	**3,2**	2,5	1,6

Part III

Final Remarks



Chapter 10

10.1 FINAL REMARKS

A great sampling effort along the Portuguese coasts, including in Madeira and Azores, coupled with the study of hydroids sampled by several research campaigns in deep waters (of the Gulf of Cadiz, Alboran Sea, Madeira and Azores), contributed to a better perception of the actual hydrozoan biodiversity of the north-east Atlantic and western Mediterranean. A large number of hydroid species were identified morphologically and genetically from these geographical regions, increasing dramatically the numbers of hydrozoan records, especially in Portugal where hydrozoan research has been dormant for over half-century. The integration of genetic and morphological data allowed accurate identifications and a more realistic insight of the biogeographic, phylogenetic and evolutionary relations of taxa.

In the course of this Ph.D. thesis, over 300 hydroid species were identified in Portuguese waters only, and about 600 sequences of the hydrozoan ‘DNA barcode’ 16S mRNA gene were obtained. For phylogenetic, phylogeographic and evolutionary purposes, higher haplotype sampling was generated for the hydrozoan families: Sertulariidae, Plumulariidae, Aglaopheniidae, Lafoeidae, Hebeliidae, Campanulinidae, Halopterididae, Kirchenpaueriidae, Haleciidae, Tiarannidae and Eudendriidae. However, the latter six families were not studied in as great detail in this dissertation, as the others. The systematics of Hydrozoa were revealed to be complex and the time was limited to describe all the findings of this Ph.D.; therefore the unresolved morphologicas and phylogenetic analyses on the other hydrozoan families identified and 16S sequences determined, will be only released in future publications.

Around 350 16S sequences (of the ten hydrozoan families mentioned on the last paragraph) determined specifically for this Ph.D. thesis are mentioned in this dissertation, and analysed phylogenetically with other 16S sequences of phylogenetically close taxa previously published on GenBank.

The molecular phylogenetic analyses (integrated with morphological data) enabled the correction of taxonomic identifications based on morphological characters only, but also provided important insights to the systematic positions of taxa, and on their evolution and phylogeographic affinities along the shallow and deep waters of the NE Atlantic and western Mediterranean. Whether some hydroids species have in fact wide (vertical and horizontal) geographical distributions, or if they are in fact complexes of cryptic or pseudo-cryptic taxa was investigated. Molecular data were also used to investigate the evolution of Hydrozoa morphotypes from deep to shallow waters (or the reverse), and whether or not this has occurred multiple times.

The value of mt 16S rDNA sequence data was tested and confirmed by the integration and comparison with morphological data of various phenotypes. The 16S mRNA gene was revealed to be useful and consistent as a ‘barcode’ for hydrozoans, providing sufficiently high resolution to discriminate hydrozoans at the species-level. The few cases when 16S sequences of different nominal species were identical indeed corresponded to very morphologically similar taxa that may be synonymous. The phylogenetic information provided by 16S data was also extremelly informative and the well-supported clusters of species or specimens were practically in complete agreement with morphological characters.

Genetic data highlighted many polyphyletic groups of Hydrozoa, such as: (1) the genera *Gymnangium* and *Aglaophenia* (Aglaopheniidae); (2) the genera *Sertularia*, *Dynamena* and *Sertularella* (Sertulariidae); (3) the superfamily Lafoeiodea and its two ‘families’: Hebellidae and Lafoeidae; (4) the genera *Filellum*, *Lafoea* and *Acryptolaria* (Lafoeidae).

The potential cryptic diversity observed in some taxa by the abnormal phylogenetic position and high genetic divergence within some nominal species, was congruent with morphological divergence of specimens. High levels of cryptic biodiversity were revealed both in shallow and deep waters, within the nominal species: (1) *Sertularella fusiformis, S. mediterranea, Sertularia distans, Dynamena disticha, Thuiaria articulata* and *Diphasia margareta* (Sertulariidae); (2) *Lafoea dumosa*, *Zygophylax levinseni* and *Cryptolaria pectinata* (Lafoeidae); (3) *Nemertesia antennina, N. belini, Plumularia setacea, Monotheca obliqua*, *M. margaretta* (Plumulariidae). Two undescribed species were also revealed within Aglaopheniidae: one in deep waters of the Azores and the other from coastal waters of Madeira.

On the other hand, cases of probable synonymies, indicated by identical 16S sequences and/or low genetic distances coupled with paraphyly, were revealed between: (1) *Aglaophenia elongata* and *A. acacia*; (2) *Aglaophenia pluma*, *A. tubiformis* and *A. octodonta* (Aglaopheniidae); (3) *Amphisbetia operculata* and *A. minima* (Sertulariidae), and d) *Sertularia cupressina* and *S. argentea* (Sertulariidae).

The phylogeographic patterns highlighted by genetic analyses were concordant with geographic and/or bathymetric distance between samples. Morphological differences or similarities between haplotypes were also supported by phylogeographic inferences. Many of the phylogeographic patterns observed were also extremely similar across the hydrozoan families herein studied in greater detail. Other hydrozoan researchers have also consistently highlighted phylogeographic structure of hydroids based on 16S haplotype data (e.g. Govindarajan *et al.* 2005a, b, Schuchert 2005, Miglietta et al. 2009, Miranda *et al*. 2010). The phylogeographic relations of hydroids had not been studied in detail in the north-east Atlantic and western Mediterranean; therefore the majority of the phylogeographic inferences of the shallow and deep-water hydroids within the geographical setting covered by this thesis, are new to science.

Identical intraspecific partial 16S sequences, possibly revealing ongoing or recent genetic migration, were detected between: (1) shallow waters of Azorean islands (for *Salacia desmoides*, *Amphisbetia operculata*, *Dynamena disticha*, *Sertularella* cf. *fusiformis* and *Aglaophenia picardi*); (2) shallow waters of western Portugal and the Mediterranean (for *Salacia desmoides, Sertularella mediterranea*, *Aglaophenia parvula*, *N. antennina*); (3) shallow waters of western Portugal and the United Kingdom (for *Diphasia rosacea*, *Gymnangium montagui*, *Aglaophenia kirchenpaueri*, *Aglaophenia tubulifera*, *Nemertesia ramosa* and *N. antennina*); (4) deep waters of Madeira and Azores (for *Nemertesia belini* and *N. ramosa*); (5) deep waters of the Alboran Sea and Gulf of Cadiz (for *Streptocaulus dollfusi, Aglaophenia lophocarpa, Campanulina panicula, Nemertesia* sp1 and *N. ventriculiformis*); (6) contrasting bathyal depths of the Gulf of Cadiz (for *Acryptolaria* cf. *conferta & Nemertesia* sp4); (7) deep waters of the Gulf of Cadiz and: Madeira (for *Z. biarmata*) and Norway (for *Campanulina panicula*); (8) deep-water localities of the Azores, Madeira, Gulf of Cadiz and coastal waters of western Portugal (for *Sertularella* *gayi*). The existence of the same 16S haplotype of *Macrorhynchia philippina* in shallow waters of Brazil and Madeira, was also associated to an unintentional human-introduction of that species in Madeira by boat(s). The presence of *Coryne eximia* in the Gorringe Bank was also likely to a human-induced introduction.

Low levels of sequence divergence, possibly revealing relatively recent gene flow, was further detected between samples of: (1) shallow waters of western Portugal and Azores (for *Amphisbetia operculata; Aglaophenia pluma* and *Sertularella mediterranea*); (2) shallow waters of western Portugal and Madeira (for *Sertularia distans* and *Aglaophenia pluma*); (3) Azores and Madeira (for *Dynamena disticha* and *Sertularia marginata* in shallow waters, and for *Nemertesia belini* and *N. ramosa* by deep waters); (4) shallow waters of western Portugal and the Mediterranean (for *Dynamena disticha* and *S. mediterranea*); (5) between distant localities and contrasting bathyal depths of the Gulf of Cadiz (for *Zygophylax biarmata* and *Acryptolaria* cf. *conferta*); (6) deep waters of the Gulf of Cadiz and central-western Portugal (for *Cryptolaria pectinata*).

The considerable genetic diversity discovered in hydrozoan samples from relatively close deep-sea locations of the Azores (e.g. for *Aglaophenia tubulifera*, *Acryptolaria* sp1, *A.* cf. *craussicaulis* , *Cryptolaria pectinata, Nemertesia belini*, *Nemertesia ‘antennina’*), likely reveal the impact of water-currents generated by seamounts in the segregation of populations with reduced dispersive potential.

The high genetic diversity found in the bathyal environments of the Gulf of Cadiz (e.g. for *Aglaophenia lophocarpa* and *Nemertesia ‘antennina’*) may be a consequence of the great heterogeneity of habitats and diversity of water masses that converge in the depths of the Gulf of Cadiz.

The high haplotypic variability and/or geographical segregation found in European coastal waters (e.g. for *Aglaophenia pluma, Lafoea dumosa*, *Plumularia setacea, Nemertesia antennina*) may be explained by the effects on the isolation of populations during the last glaciations in the northern hemisphere. The effects of past glacial episodes probably promoted not only the divergence of populations, but also speciation (e.g. between *Nemertesia* sp.1 and *N.* sp2) and favoured evolutionary shifts from deep to shallow waters. On the other hand, warmer geological periods may have caused extinctions of marine (hydroid) species.

The high phylogenetic resolution obtained for the Plumulariidae, raised further suspicion that the Messinian salinity crises eradicated hydroids from the Mediterranean basin, but after that event several episodes of gene flow may have occurred between the Atlantic and Mediterranean, in both directions in shallow and deep waters.

This study showed that, in the NE Atlantic, coastal marine environments sustain higher hydrozoan diversity and abundance, but the importance of deep sea habitats as a source of phylogenetic diversity (both in shallow and deep waters) was also evident. The present account revealed that hydrozoan diversity has been substantially underestimated at various taxonomic levels; it further provided evidence that many sessile marine invertebrates previously recorded with wide geographic distributions probably comprise complexes of morphologically similar but distinct species. It was also demonstrated that some nominal species of hydroids (e.g. *Sertularella gayi* and *Aglaophenia tubulifera*) are actually present in both shallow and deep waters, whilst other hydroid species with supposedly wide vertical distributions (e.g. *Nemertesia antennina* and *Lafoea dumosa)* represent in fact complexes of cryptic taxa.

The main explanatory causes proposed for speciation and population divergence of benthic hydroids were: species population size, dispersal mechanisms and ecological plasticity to inhabit different abiotic conditions, but also the influence of oceanic currents (and its properties), habitat heterogeneity, climate change and continental drift.

Finnally this Ph.D. project should contribute to a better perception of the biodiversity and speciation mechanisms of sessile animals in the marine realm. In particular, this Ph.D. thesis will contribute to a new, more comprehensive insight of hydrozoan diversity in the NE Atlantic and western Mediterranean. The taxa more intensively studied during this Ph.D., have profound ineherent taxonomic problems, but are important and frequent components of coastal and deep-sea habitats. The knowledge of their true diversity is important for more accurate studies of ecology, biogeography, evolution and applied-biotechnology, as well as management and conservation planning (e.g. Knowlton). The comprehension of processes underlying speciation throughout past geological ages is particularly relevant, especially in the current context of a fast-changing world threatened by large-scale losses of biodiversity (much of these remaining undescribed). The understanding of phenomena implicated in past and present speciation events will be important to predict extinctions and genesis of species.

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