Universidade de Aveiro Departamento de Biologia
Ano 2017

Pedro Emanuel Ferreira dos Reis Vieira

BIODIVERSIDADE E EVOLUÇÃO DA FAUNA DOS PERACARÍDEOS COSTEIROS DA MACARONÉSIA E NORDESTE ATLÂNTICO

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia, realizada sob a orientação científica do Doutor Henrique Queiroga, Professor Associado do Departamento de Biologia da Universidade de Aveiro, Doutor Filipe José Oliveira Costa, Professor Auxiliar da Universidade do Minho e do Doutor Gary Robert Carvalho, Professor do Departamento de Biologia da Universidade de Bangor, País de Gales, Reino Unido.

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Dedico este trabalho à minha mãe e à Sofia.

o júri

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palavras-chave

Peracarida, Crustacea, Nordeste Atlântico, Macaronésia, Biodiversidade Marinha.

resumo

Nas costas complexas e ricas do Nordeste (NE) Atlântico e da Macaronésia, a superordem Peracarida (Crustacea) é um dos taxa de invertebrados marinhos mais abundante e comum, com um papel importante nas comunidades bentónicas. O estudo deste grupo é muitas vezes limitado a listas de inventários ou estudos de comunidade bentónicas e o seu conhecimento genético nesta região é deficiente. O objetivo principal desta tese foi o de melhorar o conhecimento sobre a diversidade e a evolução dos peracarídeos no Atlântico Nordeste e na Macaronésia, com ênfase nos membros presentes nas zonas pouco profundas e nas zonas costeiras rochosas das ordens Amphipoda, Isopoda e Tanaidacea. Esta tese compreende cinco capítulos com pesquisa original, incluindo uma biblioteca de referência de DNA barcodes neste grupo, através da comparação de dados morfológicos e moleculares (capítulo 2), um conjunto de dois estudos dedicados ao género isopode *Dynamene* (capítulos 3 e 4), um capítulo dedicado à família de anfípodes Hyalidae (capítulo 5), e uma abordagem multi-espécies da diversidade e dos padrões filogeográficos dos peracarídios presentes na Macaronésia (capítulo 6).

No primeiro capítulo, relatamos uma biblioteca de referência de DNA barcodes para a superordem Peracarida, que inclui espécimes de costas do Atlântico, principalmente da Península Ibérica, juntamente com membros adicionais do mesmo ou semalhantes taxa de outros locais. Um maior número de *Barcode Index Numbers* (BINs) em comparação com o número de morfo-espécies foi encontrado, com algumas morfo-espécies exibindo até seis BINs. A presença de linhagens profundamente divergentes sugere a existência de uma considerável diversidade taxonómica anteriormente negligenciada, mesmo numa das mais conhecidas faunas de peracarídeos do mundo. Estas descobertas indicam a necessidade de uma revisão ampla, abrangente e integrada da fauna de peracarídeos das costas do Atlântico do Sul da Europa.

No segundo capítulo, o abundante mas controverso género *Dynamene* foi investigado em detalhe, ao examinar vários milhares de individuos amostrados durante mais de cinquenta anos. A distribuição e a taxonomia das seis espécies de *Dynamene* ao longo do eixo Nordeste Atlântico-Mar Negro foram revistas e actualizadas. Novos mapas de distribuição e chaves ilustradas para os machos adultos e fêmeas das espécies deste género, presentes no hemisfério norte, são fornecidas.

Nos três últimos capítulos, as ferramentas de delineamento molecular revelaram uma extensa diversidade críptica no género *Dynamene* (3 morfoespécies *vs* 12 unidades taxonómicas operacionais moleculares - MOTUs), na família de anfípipodes Hyalidae (7 morfoespécies *vs* 32 MOTUs) e em vinte e cinco espécies de peracarídeos (25 morfoespécies *vs* 90 MOTUs). Uma separação entre as populações presentes na Macaronésia e as presentes no continente foi visivel e, na maioria dos casos, as populações presentes na Macaronésia apresentavam maiores níveis de diversidade. Estas descobertas sugerem um papel maior das ilhas oceânicas na diversificação destes invertebrados marinhos do que se anteciparia e contribuiram para expor eventos pouco explorados na filogeografia e evolução da fauna marinha na Macaronésia.

Esta tese mostrou que a biodiversidade marinha, como se observa em peracarídeos presentes no NE Atlântico e na Macaronésia, foi consideravelmente subestimada. O nível de diversidade provavelmente aumentará com a adição de diferentes taxa, diferentes tipos de habitat e de regiões marinhas distintas. Esta tese também sugere que estas ilhas oceânicas podem atuar como impulsionadoras da evolução, da diversificação e do endemismo em organismos marinhos, como acontece nos organismos terrestres.

Keywords Peracarida, Crustacea, Northeast Atlantic, Macaronesia, Marine Biodiversity.

abstract

In the complex and rich Northeast (NE) Atlantic and Macaronesia coasts, the superoder Peracarida (Crustacea) is one of the most abundant and commom marine invertebrate taxa with an important role in benthic communities. The study of this group is often limited to inventory lists or benthic community studies and the genetic knowledge of the group in this region is poor. The main goal of this thesis was to improve knowledge on Peracarida diversity and evolution in the NE Atlantic and Macaronesia, with particular emphasis on shallow water and rocky shore members of the orders Amphipoda, Isopoda and Tanaidacea. The thesis comprises five chapters with original research, entailing a DNA barcode-based screening of the species diversity in this group through the comparison of morphology and molecular-derived data (chapter 2), a set of two studies of the isopod genus *Dynamene* (chapters 3 and 4), one chapter about the amphipod family Hyalidae (chapter 5), and a multi-species analyses of the diversity and broad phylogeographic patterns of Macaronesian peracarideans (chapter 6).

In the first chapter, we reported a DNA barcode reference library for the superorder Peracarida, comprising specimens from marine Atlantic coasts, mainly from Iberian Peninsula, together with additional members of the same or sister taxa from other locations. A higher number of Barcode Index Numbers (BINs) compared with the number of morphospecies was found, with some of them displaying up to six BINs. The presence of deeply divergent intraspecific lineages suggests the existence of considerable overlooked taxonomic diversity, even in one of the most well-known peracaridean faunas in the world. These findings indicate the need for a broad, comprehensive and integrated revision of the peracaridean fauna from the Southern European Atlantic coasts.

In the second chapter, the commom but species-poor and controversial isopod *Dynamene* genus was investigated in detail by examining thousands of specimens records sampled during more than fifty years. The distribution and taxonomy of the six *Dynamene* species along the Northeast Atlantic-Black Sea axis was revised and updated. New distribution maps and illustrated keys to the adult males and females of the northern hemisphere species are provided.

In the last three chapters, molecular delineation tools revealed extensive cryptic diversity in the genus *Dynamene* (3 morphospecies *vs* 12 molecular operational taxonomic units - MOTUs), in the amphipod family Hyalidae (7 morphospecies *vs* 32 MOTUs) and in twenty-five peracaridean species (25 morphospecies *vs* 90 MOTUs). A split between Macaronesian and continental populations was patent, and in most cases the Macaronesian populations displayed high levels of diversity. These findings suggest a much larger role of oceanic islands in the diversification of these marine invertebrates than would have been anticipated, and contributes to expose weakly explored events in the phylogeography and evolution of Macaronesia's marine fauna.

This thesis showed that marine biodiversity, as seen in peracarideans from the NE Atlantic and Macaronesia, has been considerably underestimated. The level of diversity will likely increase with the addition of different taxa, different types of habitat and distinct marine regions. It also suggests that these oceanic islands may act as drivers of evolution, diversification and endemism, just as well for marine organisms as they do for terrestrial ones.

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Chapter 1: General Introduction

1.1 MARINE BIODIVERSITY

Biodiversity, the biological diversity, is the variability among living organisms and those who ever lived from all sources, which includes diversity within species, between species and at ecosystem level (Convention on Biological Diversity 1992). Marine biodiversity has long been underestimated when compared with terrestrial habitats due to the general believe that oceans are homogeneous, with limited habitat diversity, and therefore, limited species diversity and rare speciation events (Briggs 1994, Gray 1997).

So far, "only" 250 000 marine eukaryote species have been described (Bouchet 2006, Jones et al. 2007), but it is expected that around 1 million exist and that most of marine species are still to be discovered (Mora et al. 2011, Appeltans et al. 2012). At higher taxonomic levels, marine diversity is much higher than the terrestrial counterparts (33 of the 36 major phyla of multicellular animals occur in the sea, and 18 of them are marine endemic) (Gray 1997, Roff and Zacharias 2011) and had more 2.7 billion years than terrestrial counterparts for evolutionary diversification (Carvalho et al. 2011). Moreover, oceans cover more than 70% of our planet and with technology improvement and exploration of new habitats, especially in deep sea, the number of new species will increase (Vrijenhoek 2009). Every year around 2000 marine species are discovered (Appeltans et al. 2012, Horton et al. 2017a).

Marine species are sources of food and biotechnological resources, as well as indicators of environmental health and ecosystem functioning. Major threats to marine biodiversity include overharvesting, habitat degradation, pollution, climate change, invasive species and other anthropogenic stressors, most of them impacting coastal areas (Gray 1997). Coastal systems are more susceptible to be affected due to the growing human population concentrating on coastlines (Gray 1997). It has been estimated that almost half of oceans are heavily impacted by humans (Halpern et al. 2008). For instance, overfishing is predicted to cause collapse in fisheries within the next 50 years (Worm et al. 2006), while marine invaders have already increased their ranges and are present worldwide (Molnar et al. 2008). Given these major concerns, it becomes more important than ever to know how many and which species are present in an ecosystem in order to understand and conserve species diversity.

Marine biodiversity has a heterogeneous distribution on the planet, with some areas being more diverse than others (Gaston 2000). For instance, the Arctic and Antarctic regions are less diverse compared with the tropics (Gray 1997). Also, there is an increase of species richness in soft sediments from coastal areas to the deep sea and higher diversity in the benthic realm compared to the pelagic realm (Gray 1997, Tittensor et al. 2010).

Zoobenthic species usually have an adult benthic phase and a larval pelagic phase, with the larvae released into the water column and dispersing over large spatial scales via oceanographic currents (Scheltema 1986, Hohenlohe 2004). Other zoobenthic groups, such as the members of the superoder Peracarida, have direct development with eggs hatching into juveniles or non-dispersive larval forms, thus lacking a pelagic larval phase and therefore being highly restricted in their dispersal (Naylor 1972, Lincoln 1979, Hayward and Ryland 1995). In these taxa, dispersal events are thought to be rarer and to happen locally by swimming or crawling, or passively through rafting on floating objects (usually algae), or mediated by human vectors (e.g., shipping) (Scheltema 1986, Thiel and Gutow 2005). These organisms show stronger genetic structure and are more susceptible to isolation (potentially leading to isolation by distance and allopatric fragmentation) when compared to species with dispersive larval phases. This is supposedly due insufficient gene flow to counterbalance the effects of genetic drift (Varela and Haye 2012).

1.2 SUPERORDER PERACARIDA

Peracarida is a superorder of the subphylum Crustacea, with some disagreement as to which orders should be included. According to World Register of Marine Species (WoRMS), it is composed by 11 orders and more than 20 000 described species, a third of the total number of crustacean species (Horton et al. 2017a). They inhabit marine, freshwater and terrestrial habitats. With a few exceptions (e.g., parasitism, hyperiids or pelagic mysids) (Williams and Boyko 2012), the peracarideans are free living benthic organisms (Naylor 1972). In marine environments, the Peracarida is one of the most diverse and abundant invertebrate groups and inhabit different habitats from shallow water to deep sea all over the world (Cunha et al. 1997, Dauby et al. 2001). They have different ecological roles such as parasitism, predation, detritus feeders and herbivory (Naylor 1972) and have high relevance in trophic interactions as an important source of food for other benthic animals and commercially exploited fish (Beare and Moore 1996, Woods 2009). Additionally, many peracaridean species are also good environmental indicators (Bonsdorff 1984, Ohji et al. 2002, Guerra-García et al. 2006, Lo Brutto et al. 2013).

The superorder Peracarida can be distinguished from other crustacean groups by the following characteristics: a free-living larval stage is absent, except for some parasitic species (Williams and Boyko 2012) meaning that they have direct development with the juveniles being morphological similar with the adults (Naylor 1972, Holdich and Jones 1983); the young are incubated by the female in a ventral structure called marsupium (a brood pouch formed by oostegites on the inner bases of two or more thoracic limbs), where they grow for some days to months depending on the species. Morphologically, usually they are very small (a few mm or cm) and are characterized by the possession of a single pair of maxillipeds, by mandibles with an articulated accessory process between the molar and incisor teeth in the adults, the lacinia mobilis, and the presence of a single thoracic segment fused to the head (Lincoln 1979, Hayward and Ryland 1990). Additionally, sexual dimorphism is common with morphological structures appearing in males before reaching adult phase (Naylor 1972).

The general body structure of peracarideans is divided into a cephalon (head), which normally incorporates one true pereon somite with its associated appendages (maxillipedes), a pereon (thorax) of 7 somites and a pleon (abdomen) of 6 somites (Fig. 1.1). Some or all of the pleon somites may be fused with the terminal telson, forming a pleotelson. The cephalon bears a pair of antennules and a pair of usually larger antennae (often called antenna 1 and antenna 2 respectively), each consisting of a peduncle and multi-articulated flagellum, followed by ventral mandibles, maxillas and maxillipeds (Naylor 1972, Lincoln 1979, Holdich and Jones 1983, Hayward and Ryland 1990). Morphological structures are show in detail in Fig. 1.2.

The orders Amphipoda and Isopoda comprise almost 90% of the total known peracaridean species (Naylor 1972, Lincoln 1979). In rocky shore marine environments in the Northeast (NE) Atlantic (see Annexes 1.1 and 1.2 for details about intertidal rocky shores), additionally to these two orders, the order Tanaidacea is present and common (Pereira et al. 2006, Guerra-García et al. 2011, Vinagre et al. 2016), despite the fact they only constitute less than 5% of known peracaridean diversity worldwide (Holdich and Jones 1983, Blazewicz-Paszkowycz et al. 2012). Mysids and cumaceans can also be present in marine NE Atlantic coasts (Hayward and Ryland 1990, Costello et al. 2001), although they are less commom (Pereira et al. 2006, Izquierdo and Guerra-García 2011, Guerra-García et al. 2011). Below, the three most commom peracaridean orders (Amphipoda, Isopoda, Tanaidacea) present in NE Atlantic coasts are described with greater detail.

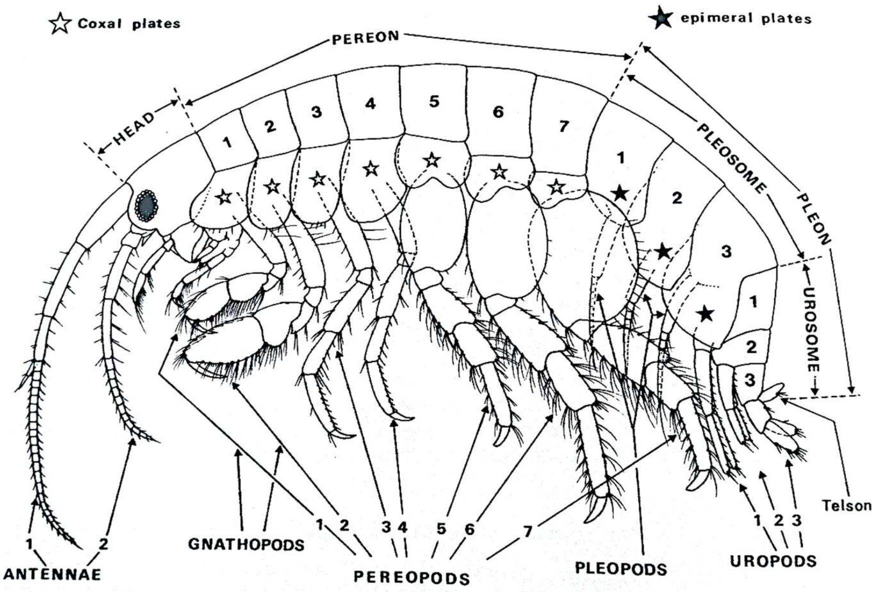


Figure 1.1. Diagram of the anatomy of the gammaridean amphipod *Elasmopus rapax*, here used to represent the general anatomy of a Peracarida.
Adapted from Lincoln 1979.

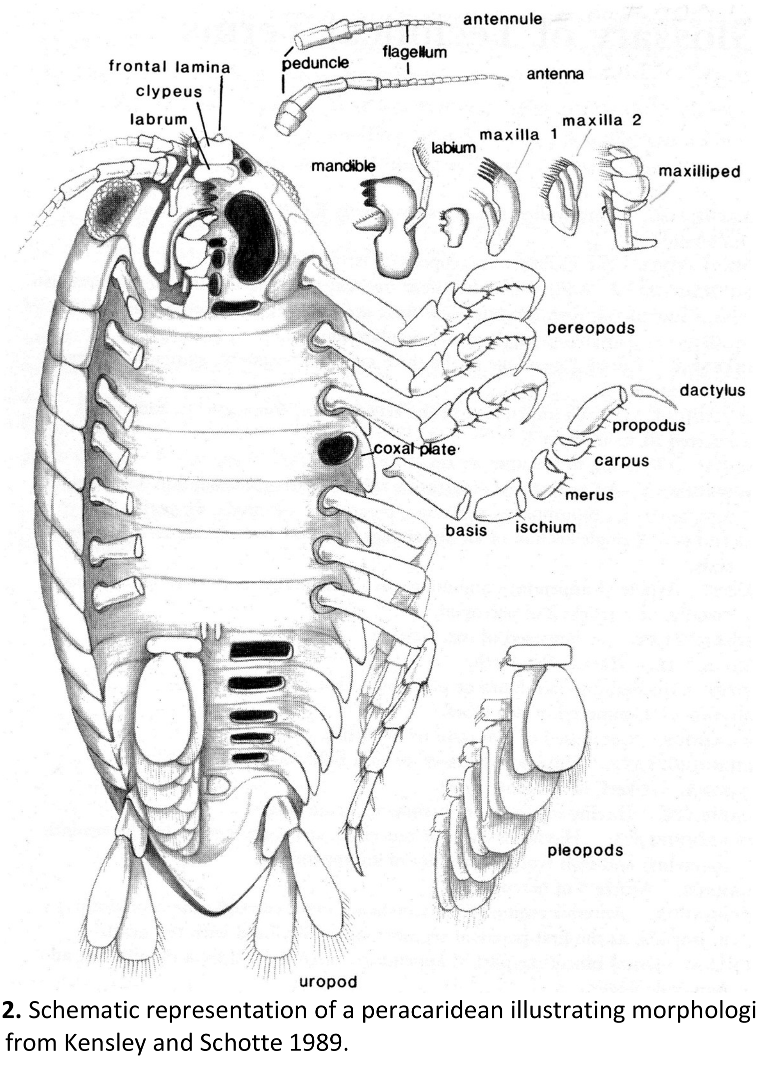


Figure 1.2. Schematic representation of a peracaridean illustrating morphological structures.
Adapted from Kensley and Schotte 1989.

1.2.1 Amphipoda

The order Amphipoda (Fig. 1.3) consists in more than 9 900 known species divided in four sub-orders, recently rearranged (see Lowry and Myers 2013), with around 1/5 of total species living in fresh or inland water (Horton et al. 2017b). Amphipods are thought to have originated in the Lower Carboniferous, however, the fossil record only dates back to the Upper Eocene (Lincoln 1979, Horton et al. 2017b).

Amphipods are characterized by body typically laterally compressed, the absence of carapace, pereon with 7 pairs of uniramous limbs. The first two pairs of pereopods are modified as gnathopods. The name Amphipoda means *different foot*, in reference to the two kinds of pereopods that amphipods possess (in contrast with isopods, see below). Amphipods are unique in the possession of three pairs of pleopods (biramous, multi-articulate used for swimming) and three pairs of uropods (robust, biramous, 1 or 2-articulate appendages). No other malacostracan group possesses more than one pair of uropods. As a group, the amphipods are quite conservative in their overall range of body architecture, in contrast with other peracaridean groups, such as the Isopoda. However, in a small minority of cases, there is reduction in the number and type of appendages, with the pleon rudimentary or absent (Caprellidae) (Lincoln 1979, Hayward and Ryland 1990).

Figure 1.3. An example of an Amphipod: *Ampithoe rubricata*.
Source: www.aphotomarine.com. ©David Fenwick. Accessed on 25-04-2017.

1.2.2 Isopoda

The order Isopoda (Fig. 1.4) comprises more than 10 300 species known to date. Approximately 6 250 of these are marine or estuarine (eight suborders) and the vast majority of species are known from depths of less than 1000 metres (Poore and Bruce 2012). Isopods oldest fossil is from the Carboniferous period, around 300 million years ago (Schram 1970).

The isopod body is usually dorsoventrally flattened, and lacks a carapace. The pereopods are used for locomotion and have similar size, morphology and orientation, giving the order its name "Isopoda", from the Greek *equal foot*. The coxal plates of the pereopods are visible from above. The pleon and telson are wholly or partially fused, forming a pleotelson, with five pairs of biramous pleopods and a pair of uniramous or biramous uropods. In most species, the sexes are separate and sometimes dimorphic, with the main differences in body shape and mouthparts. The male pleopod 2 bears an appendix masculine, or it is sometimes combined with pleopod 1 to form a copulatory structure. In the female, pleopod 1 is missing and pleopod 2 is modified as a flat operculum. The juveniles have a succession of recognisable growth stages (instars) (Naylor 1972, Hayward and Ryland 1990). Marine isopods are among the most morphologically diverse groups of all the Crustacea (Poore and Bruce 2012).

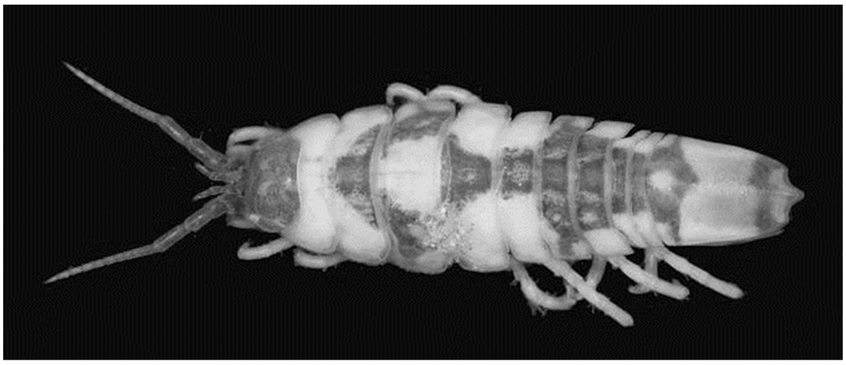


Figure 1.4. An example of an Isopod: *Idotea balthica.*
Source: www.aphotomarine.com. ©David Fenwick. Accessed on 25-04-2017.

1.2.3 Tanaidacea

The order Tanaidacea (Fig. 1.5) is poorly known compared with other peracaridean orders such as Isopoda and Amphipoda (Holdich and Jones 1983) and had an unclear status until the 19th century, commonly being classified within the Isopoda or Amphipoda (Blazewicz-Paszkowycz et al. 2012). They were given separate ordinal status by Hansen (1895) and currently the order includes almost 1400 described species divided in 4 subororders (Anderson 2016). Oldest fossils records date back to the Lower Carboniferous, the Triassic and the Jurassic (Blazewicz-Paszkowycz et al. 2012).

Tanaidaceans are truly demersal organisms which mainly inhabit the surface layer of the sediments, either in burrows, as crevice dwellers or by constructing tubes. They can be found amongst algae in rocky shores, in mud, in crevices between the plats on back of turtles, in the abyssal trenches and some are found in fresh water (Holdich and Jones 1983).

The head and the first two segments of the thorax are fused forming the cephalotorax, covered by the carapace, which is produced into lateral folds enclosing a branchial chamber. They can be dorsoventrally flattened or cylindrical, and tend to the rather elongate. The second fused thoracic segment bears a pair of chelate appendages, the chelipes, and the following ambulatory six pairs of pereops are usually similar. Each pleon segment has a pair of pleopods, used in swimming. The sixth and final segment of the pleon is fused with the telson forming the pleotelson. A single pair of uniramous or biramous uropods is borne by the pleotelson. Some species are hermaphroditic (Holdich and Jones 1983, Hayward and Ryland 1990, Blazewicz-Paszkowycz et al. 2012).

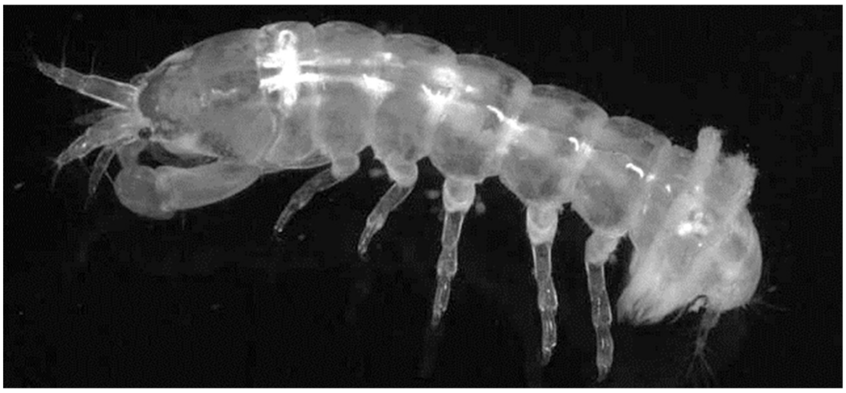


Figure 1.5. An example of a Tanaidacea: *Tanais dulongii*.
Source: www.aphotomarine.com. ©David Fenwick. Accessed on 23-05-2017.

1.3 METHODS IN SPECIES DISCRIMINATION AND DELIMITATION

1.3.1 Morphological approaches

There are significant disparities in taxonomic knowledge across marine species. Larger organisms (e.g., fishes, mammals) are represented by fewer taxa and are usually well-studied groups. Considering how marine vertebrates are relatively well-known compared to most marine invertebrates, the existing gaps in knowledge are particularly disconcerting when attempting to estimate the biodiversity of smaller organisms such as benthic marine invertebrates (Radulovici et al. 2010). For these organisms, apparentely, the extent of taxonomic knowledge depends on the size of the taxonomic community studying it (Bouchet 2006). Due to the many difficulties for biodiversity assessment using current approaches, marine faunal inventories usually fail to identify one third of specimens to the species level when using morphological methods (Schander and Willassen 2005).

The binomial nomenclature system introduced by Carl Linnaeus, based on the anatomical body plan and on morphology, can be easily assessed through the observation of the specimens. It has been the major tool used to describe and classify species diversity. This procedure follows a strict protocol according to the International Code of Nomenclature by which species have unique binomial scientific names (genus and species) and are linked to type specimens (from type localities) preserved in museum collections. However, some limitations are found when using morphological identifications. Morphology-based identifications are very costly and time consuming. Accurate species-level identifications require highly trained specialists with considerable and diverse taxonomic expertise, especially in marine benthic invertebrate fauna due to its great morphological complexity (Radulovici et al. 2010). This leads to a narrow specialization in identifying organisms belonging to a restricted group of taxa (e.g. a carcinologist will likely have difficulties in identifying polychaetes and the other way around; Gordon 2000, Waite et al. 2004). Additionally, the number of experts worldwide is rapidly decreasing due to shortage in funding and to the lack of interest in pursuing a "dead" specialization (Wilson 2003). Because of the resulting "taxonomic impediment" and the current progress in classifying life (Bouchet 2006) the predicted timeframe for an inventory of marine biodiversity alone is more than 1000 years. Considering also the rates of biodiversity loss, it is evident that many species will go extinct before we even know they existed (Mora et al. 2011).

The morphology-based approach can also be problematic for the identification of all life stages (e.g., eggs, larvae), for sexually dimorphic species or those with large phenotypic plasticity

(Ekrem et al. 2007). Moreover, the presence of damaged specimens and the ineffectiveness in diagnosing cryptic species (see below), which have been increasingly reported in marine systems, represent additional limitations (Knowlton 1993). All together, these insufficiencies of the morphology-based approaches strongly limit our ability to monitor biodiversity more extensively, accurately and quickly in benthic ecosystems, and call for alternative or complementary approaches. So, it is no surprise that scientists took the opportunity provided by the development of molecular methods to clarify many ambiguities in traditional taxonomy. Therefore, molecular methods have been increasingly used for species identification and delimitation (Hebert et al. 2004, Jörger et al. 2012) and a universal molecular system has been proposed for identification of eukaryotic life (Hebert et al. 2003).

1.3.2 Molecular approaches

The first type of molecular markers to be used in population genetics and molecular systematic studies were allozymes, alternative forms of enzymes coded by alleles at the same locus (Avise 1975). Subsequently, various methods were developed such as DNA hybridization, random amplified polymorphic DNA, restriction fragment length polymorphism, single strand conformational polymorphic DNA or DNA sequencing (Wong and Hanner 2008). The latter one became the method of choice for species identification and systematics studies, either by using nuclear or/and mitochondrial genes (Bartlett and Davidson 1991, Medeiros-Bergen et al. 1995).

The mitochondrial genes and the nuclear genes encoding ribosomal RNA are easily accessible and very informative and have been particularly important for inferring species phylogenies or to study many systematics' questions (Wakeley 2004). These markers, which have changes that are considered selectively neutral or of little or no functional consequence to the organism (Kimura 1983), have a degree of polymorphisms proportional to the underlying rate of mutation (Drake et al. 1998). Therefore, they have the potential to provide resolution across multiple time scales, with different genes displaying different evolutionary rates (Hillis 1987). However, nuclear markers, on average, have a lower substitution rate, which results in nuclear genes evolving slower than mitochondrial ones. Consequently, they may be better at resolving deeper phylogenetic nodes (Moriyama and Powell 1997).

Studies using 18S (Spears et al. 2005, Meland and Willassen 2007) and 28S rRNA genes (Jarman et al. 2000) exemplify the applications of nuclear loci in Peracarida phylogeny. They have disputed the monophyly of the Peracarida by removing the Mysida, and they have also disproven the monophyly of the Edriophthalma (Isopoda and Amphipoda) and the Mysidacea

(Mysida, Lophogastrida and Pygocephalomorpha) groups. Multi-locus approaches were also applied by Drumm (2010) and Mamos et al. (2016) that used mitochondrial (COI) and nuclear genes (28S rRNA) to resolve the phylogeny within the order Tanaidacea and the *Gammarus balcanicus* complex*,* respectively.

Although several mitochondrial and nuclear loci have been employed as molecular markers for animal species identification and discrimination, DNA barcoding (Hebert et al. 2003), a single-marker approach based on a 658 base pair (bp) fragment at the 5’ end of the mitochondrial gene coding for cytochrome c oxidase subunit I (COI), eventually became the ‘global standard’ (Hebert et al. 2016) and has been widely used, including in crustaceans (e.g., Costa et al. 2007, Raupach and Radulovici 2015, Raupach et al. 2015, Lobo et al. 2016a).

1.3.3 DNA barcoding

The study by Bucklin et al. (1999) was one of the early reports on the use of COl sequences to discriminate sibling species of crustaceans, involving eight species from three genera of planktonic copepods. However, it was Hebert et al. (2003) that suggested a universal DNA-based identification system employing COI DNA sequences as taxon “barcodes”, reportedtly fast, reliable and cost-effective. Several studies (see Hubert and Hanner 2015 for details) indicate that sequence divergences in the COI of most of the animal phyla are larger at the genus than at the species level, enabling the discrimination of closely related species (e.g., Costa et al. 2007). Although DNA barcoding reveals only a tiny segment of the genome, it examines the same core region, so it is possible to compare sequences across species and how they diverge, and therefore assign unidentified specimens to known species (Costa and Carvalho 2010).

DNA barcoding is more than just another method of molecular identification. As its name implies, it involves standardization. ln practice, in any given taxonomic group, there are always markers that can be as good for resolving species as COI. However, by sequencing optimal markers for each group there will be a vast, diverse, but non-comparable array of genetic data. Nevertheless, COl performs sufficiently well across the broadest possible range of taxa to allow standardization (Hebert et al. 2003, Radulovici et al. 2010). ln addition, all the metadata associated with the sequences obtained can be uploaded on-line on BOLD Systems (Ratnasingham and Hebert 2007) and made publicly available.

The choice of mitochondrial DNA (mtDNA) over nuclear DNA is based on the fact that mitochondria are present in large copy numbers in each cell, and are therefore easier to amplify from small amounts of tissue or when DNA is degraded. Additionally, due to maternal inheritance, there is generally no recombination (Galtier et al. 2009). Finally, mtDNA has a higher evolutionary rate and lack of introns (Hebert et al. 2003). Compared with other mtDNA genes, COl has been shown to be a superior marker for species identification and discrimination because it doesn’t have insertions and deletions and it has a higher probability of being amplified in a wide range of species with standard protocols (Hebert et al. 2003, Ratnasingham and Hebert 2013). Using mtDNA sequence information, namely COI barcodes, it is possible to construct phylogenetic trees, to study close to moderately deep interspecific relationships, and disentangle cryptic species (Meyran et al. 1997, Avise and Walker 1999, Hebert et al. 2003, Avise 2004).

1.3.4 Cryptic species

Cryptic species (morphologically similar but genetically distinct) were shown to be a common presence in marine systems (Knowlton 1993, Leray and Knowlton 2016) and in crustaceans in particular (Whiteman et al. 2004, Moura et al. 2008). Cryptic species cannot be identified based on morphological characters, but can be distinguished using molecular methods, such as DNA barcoding (Hebert et al. 2004). Many taxa previously considered cosmopolitan are actually complexes of geographically separated cryptic species (Jaafar et al. 2012, Jörger et al. 2012). Other cryptic complexes follow a sympatric model of speciation, with reproductive barriers resulting from differences in habitat choice or resource use (Miglietta et al. 2011).

Cryptic species are a hidden aspect of marine biodiversity and seem to occur across all marine groups, therefore the extent of marine biodiversity probably is underestimated (Tautz et al. 2003). The identification of cryptic species can be controversial but genetic data can reveal at least the existence of intraspecific genetic groups with separate evolutionary history (Avise et al. 1987, Dawson 2001) and help to understand the process of cryptic speciation in ecological and evolutionary scopes (Whiteman et al. 2004, Moura et al. 2008). However, the task of investigating further the extent of this phenomenon and properly describe a new species needs additional genetic, ecological and behavioral data (Jörger and Schrödl 2013). Unfortunately, since the number of taxonomists is decreasing (Packer et al. 2009) and marine barcodes are rapidly accumulating, the majority of flagged cases stop at the level of cryptic species. Without a larger interest and involvement of highly trained taxonomists in marine barcoding studies, the advancement of the understanding of marine speciation will be slow (Boero 2010).

Nevertheless, uncovering these cryptic species is fundamental for the understanding of evolutionary processes, historical biogeography, ecology and conservation approaches. The lack of morphological characters to distinguish cryptic species should not lead to considerable parts of biological diversity remaining unaddressed (Bickford et al. 2007, Trontelj and Fišer 2009).

1.4 STUDY AREA

1.4.1 Northeast Atlantic

The North Atlantic Ocean comprises the area in the Northern Hemisphere between America continent and the African-European continents (Seton et al. 2012). It was originated during the break-up of Pangaea in the Jurassic period and it has been influenced during its history by climatic oscillations with a rapid cooling in the late Eocene (from subtropical to temperate and cold). These changes led to biological diversification related to emerging environmental conditions (Golikov and Tzvetkova 1972). During the Pliocene, the North Atlantic was invaded by Pacific taxa via the Arctic due to the opening of the Bering Strait (Vermeij 1991).

More recently, the Northeast Atlantic communities were influenced by the Quaternary glaciations, during their glacial and interglacial phases (Wares and Cunningham 2001). During these periods, marine organisms had to move to southern regions such as the Iberian Peninsula or the Macaronesia to escape the ice sheets or survive in glacial refugia. The last glacial maximum (LGM), was around 20 000 years ago, and Europe was covered by massive ice sheets (Fig. 1.6) while the sea level was lower, uncovering the continental shelves (Mix et al. 2001). The present-day Northeast Atlantic marine communities are in great part the result of the above-mentioned historical events.

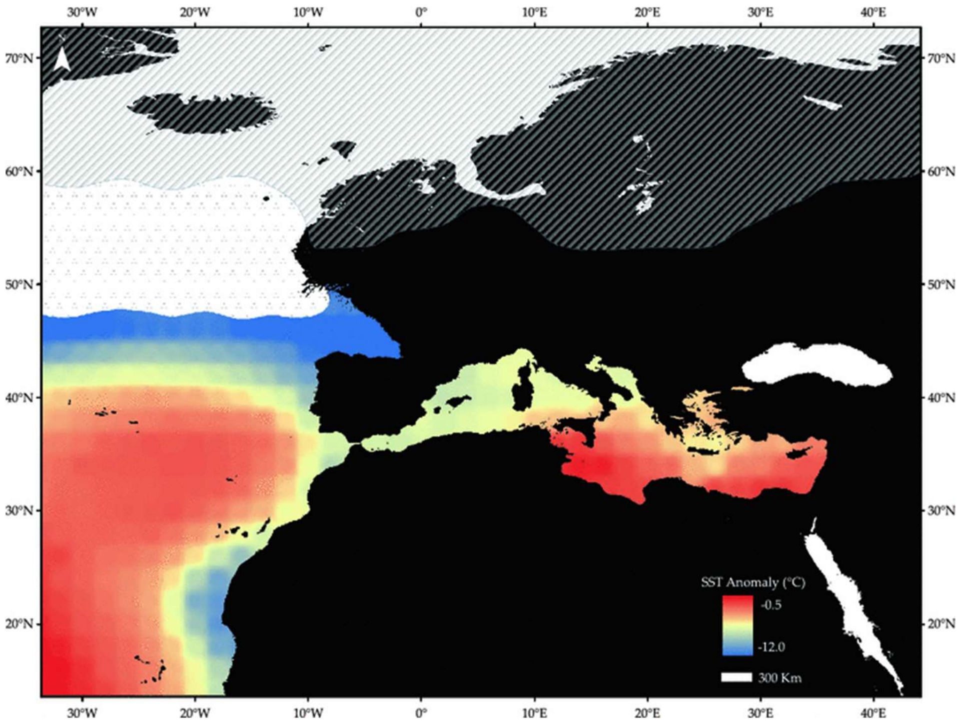


Figure 1.6. Approximate representation of the Northeast Atlantic and Mediterranean at the Last Glacial Maximum. Coastlines extended to a −130 m sea-level. Shaded striped areas represent permanent land and sea ice cover and dotted areas represent seasonal sea ice.
Adapted from Xavier and Van Soest 2012.

1.4.2 Macaronesia

Islands are natural laboratories for evolutionary diversification as well for natural extinction processes (Valente et al. 2014). Volcanic islands arise from the ocean floor and have never been connected to continental landmasses (Thornton 2007). This process begins with initial emergence from an underwater seamount, which is followed by a period of intense island-building, until maximum area and elevation are reached. Islands then enter a slow erosional stage (Price and Clague 2002, Jackson 2013). Several models integrating island ontogeny and/or other factors such as area or distance to near landmass with ecological biogeography have been proposed to explain islands colonization and biodiversity (for details, see Paulay 1994, Stuessy 2006, Whittaker et al. 2008, Fernández-Palacios et al. 2016, Otto et al. 2016). Nevertheless, dispersal from other sources is fundamental to settle life in these new formed habitats (Cowie and Holland 2006).

The Macaronesia is a group of four archipelagos (Azores, Madeira including Selvagens islands, Canary Islands and Cape Verde) (Fig. 1.7) in NE Atlantic Ocean, off the coast of the European and African continents, at distances from the continental shores varying from 96 to 1500 km (Fernández-Palacios et al. 2011). The various archipelagos and their islands all have differing degrees of isolation from the continent, with Flores and Corvo (Azores) in the mid-Atlantic being the most remote (Hawkins et al. 2000).

The oldest "current" island, Selvagem Grande, arose 27 Million years ago (Mya) (Geldmacher et al. 2001) and the newest, Pico island in the Azores, only 0.27 Mya (Carine and Schaefer 2010). The four archipelagos have distinct geneses, although the Selvagens group that belongs to Madeira archipelago is part of Canaries volcanic province (Fernández-Palacios et al. 2011). Cape Verde was not used in this thesis, therefore it will not be explored (for details see Ramalho et al. 2010, Ramalho 2011). Macaronesia's first islands, Gettysburg-Ormonde and Lars, emerged around 60 Mya from the Madeira and Canarian volcanic provinces hotspots respectively, while the most recent ones are Madeira (5 Mya) and El Hierro (1.1 Mya) in the Madeira and Canaries volcanic provinces respectively (Geldmacher et al. 2001, 2005; Fernández-Palacios et al. 2011). In the Azores, the junction between the American, Eurosian and African plates forms a complex zone, the Mid-Atlantic and Terceira-Ridges, where volcanic activity exists (Ferreira 2005). Azores is "younger" when compared with Madeira and Canaries archipelagos, with the oldest island being Santa Maria (8 Mya) and the rest of the islands being younger than 4.1 Mya (Carine and Schaefer 2010).

The sea levels changes during the quaternary glaciations and mainly the period after the LGM deeply affected Macaronesia composition (Lambeck et al. 2002, 2014, Fernández-Palacios et al. 2011). After the LGM, the sea level rose around 130 meters, submerging several islands in the Madeira and Canarian volcanic provinces and changing the shape and coastlines of the remaining emerged islands (Fernández-Palacios and Whittaker 2008). For instance, in the Madeira volcanic province, six of the eight islands existing before the LGM were covered by water and the Madeira island was divided in the Madeira and the Desertas. In the Canarian volcanic province, some islands were also covered by the rise of the sea level, but only three (of ten) islands submerged after the LGM (Fernández-Palacios et al. 2011). These now submerged islands (the Paleo Madeira and Paleo Canaries Seamounts, Fig. 1.7) (Fernández-Palacios et al. 2011, van den Bogaard 2013) could have served as stepping stones for benthic organisms in past dispersal events from and to continental coasts (Hawkins et al. 2000).

During the quaternary glaciations, currents and tides in Northeast Atlantic and Macaronesia changed (Crowley 1981, Keffer et al. 1988, Wilmes and Green 2014), with the Canary and Azores currents playing the major natural roles in large dispersal events in marine organisms (Barton et al. 1998, Arístegui et al. 2009). However, sea surface temperature change was small, namely in Azores and Madeira (between 2-3ºC) and more marked in the eastern islands of the Canaries (Fig. 1.6) (Crowley 1981, Santos et al. 1995). Briggs (1966) proposed a relationship between oceanic islands, endemism, extinction and 'marine paleotemperatures'. He suggested the correlation of the lack of endemism in the shallow marine faunas of several Atlantic oceanic islands to extinctions caused by reduced temperatures associated with the quaternary glaciations. However, Ávila et al. (2008) showed that most of the mollusc species present in the Azores prior to the last glaciation have persisted through to the present day and no signs of 'mass extinctions' were found in the littoral marine molluscs of the Azores. Therefore, it is unlikely that these small changes have affected Macaronesian marine benthic populations, especially considering that the changes in both temperatures and sea level were gradual (Santos et al. 1995, Barton et al. 1998) and peracarideans have the capacity to adapt to changes in temperature and resist small periods of desiccation (Harvey et al. 1973). It is likely that the Macaronesian islands have been glacial refugia for marine benthic organisms (Domingues et al. 2005, Almada et al. 2005). However, changes in islands area due to quaternary glaciations may have affected and shaped species richness, even in marine habitats (Triantis et al. 2012, Hachich et al. 2015).

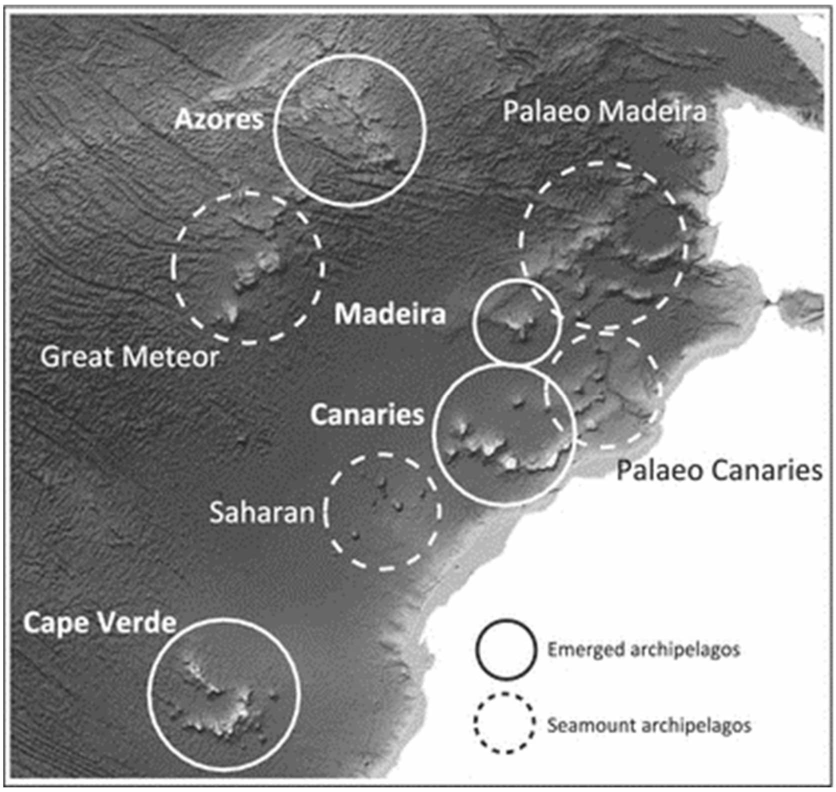


Figure 1.7. Empty ocean bathymetry showing the Northeast Atlantic Ocean region. Besides the actual emerged archipelagos forming the Macaronesia region (Azores, Madeira with Selvagens, Canaries and Cape Verde), other seamount archipelagos, such as Palaeo-Madeira and Palaeo-Canaries (between those island groups and the Iberian Peninsula), the Great Meteor archipelagos (south of the Azores) and the Saharan archipelago (south-west of the Canaries), are also shown.
Adapted from Fernández-Palacios et al. 2011.

1.5 AIMS AND STRUCTURE OF THE THESIS

The main goal of this thesis was to improve the knowledge of Peracarida biodiversity in intertidal rocky shores of the NE Atlantic. The three most dominant peracaridean orders in NE Atlantic: Amphipoda, Isopoda and Tanaidacea, were the focus of the research, which combined morphology-based approaches with molecular methods. The organisms were sampled along the NE Atlantic (see Annexes 1.3 and 1.4 for methodology and Annexes 1.6 and 1.7 for a list of the peracaridean species sampled). More specifically, the objectives of the present thesis were:

- To build, audit and annotate a core reference DNA barcoding library for the peracaridean fauna of the Atlantic coasts of the Iberian Peninsula.
- To update the taxonomy and review the distribution of the isopod genus *Dynamene*.
- To review the diversity of the peracarids in this region, comparing morphology-based species assignments with species boundaries suggested by molecular methods.
- To clarify taxonomic ambiguities and detect potential hidden or cryptic diversity.
- To contribute to the understanding of the role of Macaronesia islands in the diversification and evolution of peracarids.

This thesis is divided in seven chapters, five of which (Chapters 2 to 6) consist on the studies performed in the scope of this thesis and organized in individual sections (Abstract, Keywords, Introduction, Material and Methods, Results, Discussion and Conclusions) and which correspond to one published article in an indexed peer-reviewed international scientific journal, and four articles in preparation to be submitted to indexed peer-reviewed international scientific journals, which are listed listed further below. All five studies were performed under the scope of the FCT research grant DiverseShores (PTDC/BIA-BIC/114526/2009). Financial support for the present thesis was also secured through a PhD grant (SFRH/BD/86536/2012) financed by FCT.

Chapter 1 corresponds to the general introduction. Chapter 2 reports on, and examines, a core DNA barcode library for the superoder Peracarida using novel and publicly available data of the orders Amphipoda, Isopoda and Tanaidacea from the Atlantic Southern European Coasts. In Chapter 3 an extensive update of the taxonomy and distribution of *Dynamene* species in the Northeast Atlantic-Black Sea axis is presented, accompanied by keys and photographs to help in the identification of the males and females of the different species. Chapter 4-6 contrasts morphology-based species assignments with species boundaries suggested by molecular methods to detect hidden or cryptic diversity in different peracaridean species in NE Atlantic, and explores the role of Macaronesia islands in peracaridean diversification and evolution in this oceanographic region. Chapter 4 explores the phylogeny and phylogeography of the isopod genus *Dynamene* present in Northeast Atlantic using a multi-locus approach. In chapter 5, the diversity within the amphipod family Hyalidae in Macaronesia is investigated using DNA barcoding. In chapter 6, a comparative analysis is carried out on the divergence patterns between continental Europe and Macaronesia in populations from twenty-five species from the orders Amhipoda, Isopoda and Tanaidacea. Chapter 7 consists in the global appraisal of the thesis, with the concluding remarks and future perspectives.

Five articles have been produced on the course of this PhD thesis, which have been published or will be submitted for publication in due course:

Chapter 2	Vieira PE, Raupach M, Queiroga H, Costa FO (In preparation) A DNA barcode reference library for the superorder Peracarida (Crustacea) from the Southern European Atlantic coasts.
Chapter 3	Vieira PE, Queiroga H, Costa FO, Holdich DM (2016) Distribution and species identification in the crustacean isopod genus *Dynamene* Leach, 1814 along the Northeast Atlantic-Black Sea axis. ZooKeys. 635: 1-29. doi:10.3897/zookeys.635.10240.
Chapter 4	Vieira PE, Desiderato D, Holdich DM, Creer S, Carvalho G, Costa FO, Queiroga H (In preparation) Macaronesia as an evolutionary hotspot for low dispersal marine invertebrates: genetic evidence from the rocky intertidal isopod genus *Dynamene*.
Chapter 5	Vieira PE, Desiderato D, Abiatti M, Costa FO, Queiroga H (In preparation) Macaronesian islands as drivers of diversification of marine invertebrates in the Northeast Atlantic: the remarkable case of the family Hyalidae (Crustacea: Amphipoda).
Chapter 6	Vieira PE, Azevedo CS, Costa FO, Queiroga H (In preparation) DNA barcoding reveals cryptic diversity in the superorder Peracarida (Crustacea) in Northeast Atlantic: implications for Macaronesia conservation, with new records for several species from Macaronesia.

Chapter 2: A DNA barcode reference library for the superorder Peracarida (Crustacea) from the Southern European Atlantic coast

2.1 ABSTRACT

The superorder Peracarida is a highly diverse crustacean taxon, comprising numerous prominent members in European coastal areas' communities and ecosystems. Here, we report on a DNA barcode reference library for the superorder Peracarida, comprising specimens from marine Atlantic coasts of Iberian Peninsula, together with additional members of the same or sister taxa from other locations. A total of 597 DNA barcodes were compiled in a Barcode of Life Data (BOLD) dataset, with 220 new DNA barcodes. The dataset included specimens of the orders Amphipoda (64.9%), Isopoda (32.1%), and Tanaidacea (3.0%). In total, 140 peracaridean morphospecies were assigned to 160 Barcode Index Numbers (BINs) in BOLD, with 155 (96.9%) represented by single BINs, comprising species collected from geographically distant populations, up to approximately 4000 km in the most extreme cases (e.g., *Apohyale prevostii* from Portugal, Iceland, Scotland, North Sea and Canada). All multiple intraspecific BINs were allopatric, although the geographic distance between members of each BIN lineage ranged from 35 km up to 3000 km. Major splits were detected between upper north and south regions of the Northeast (NE) Atlantic, between Atlantic and the Mediterranean Sea, or sometimes even within countries. The most striking case was revealed for the isopod *Janira maculosa*, which split into six BINs (maximum intraspecific distance 25.16%). The high percentage of morphospecies matching unique BINs (96.9%) shows the good reliability of our DNA barcode library. However, the presence of deeply divergent intraspecific lineages morphospecies suggests the presence of considerable overlooked taxonomic diversity. These findings indicate the need for a comprehensive revision and DNA barcode-based screening of the peracaridean fauna from the Southern European Atlantic coasts.

2.2 KEYWORDS

Peracarida, cytochrome c oxidase subunit I, DNA barcode reference library, Iberian Peninsula, Northeast Atlantic.

2.3 INTRODUCTION

Peracarida is a Superorder of the subphylum Crustacea and one of the most diverse and widely distributed groups of crustaceans. They are also numerically dominant organisms of marine benthic faunas and among the most ecologically important invertebrates (Cunha et al. 1997, Dauby et al. 2001, Lourido et al. 2008, Moreira et al. 2008), with high relevance in trophic interactions (Beare and Moore 1996, Woods 2009). This group currently contains more than 20000 known species listed in World Register of Marine Species (WoRMS) (Horton et al. 2017a), but numerous species still await formal description.

The peracaridean fauna of the Iberian Peninsula coast is rich and diverse, consisting on a mixture of species from adjacent biogeographic regions. Being a biogeographic cross road region where many peracaridean species have their distribution limits (Pereira et al. 2006), this region is particularly relevant for monitoring alterations in distributional ranges driven by different factors, such as climate change, the introduction of alien species or anthropogenic activities (Chainho et al. 2015). Most studies of peracarid crustaceans along the coasts of the Iberian Peninsula have been focusing on the association of the community with algae (e.g., Sánchez-Moyano et al. 2007, Guerra-García et al. 2009, Izquierdo and Guerra-García 2011, Guerra-García et al. 2011, Torrecilla-Roca and Guerra-García 2012) or their ecological distribution (e.g., Reboreda and Urgorri 1995, Castelló and Carballo 2001, Pereira et al. 2006, Vinagre et al. 2016).

Species identifications and delimitation in Peracarida can be rather challenging, due to their small size, sexual dimorphism, morphological variation trough life cycle, and morphological uniformity among closely related species, which further limit the ability to discriminate species based on morphological characters alone (Costa et al. 2004). This leads to the knowledge of peracaridean diversity being constrained by the taxonomic impediment (Coleman 2015).

In recent years, the use of molecular tools like the DNA barcoding for specimen identification and classification has been shown to be successful in several marine groups (Radulovici et al. 2009, Knebelsberger et al. 2014, Raupach et al. 2015). Its usage has become quite widespread, often as a complement to morphological identifications (Hebert et al. 2003, Hajibabaei et al. 2006, Weitschek et al. 2014). A growing number of articles are reporting hidden diversity in peracaridean species (e.g., Witt et al. 2006, Costa et al. 2009, Xavier et al. 2011a, Richards et al. 2012, Raupach et al. 2014), and the availability of reliable, scrutinized and annotated reference libraries of DNA barcodes is a fundamental backbone for making comparisons with morphology-based identifications. Such libraries can be applied to probe and revise the taxonomic diversity of a specific group, provide a quick screening method for highlighting mismatching morphological and molecular data, and detect putative cryptic species, taxonomic complexes, and inaccurate or misleading identifications (Costa and Antunes 2012). Moreover, comprehensive barcode libraries will become essential for biomonitoring applications based on modern high-throughput sequencing technologies (Fonseca et al. 2010, Zhou et al. 2013, Leray and Knowlton 2015).

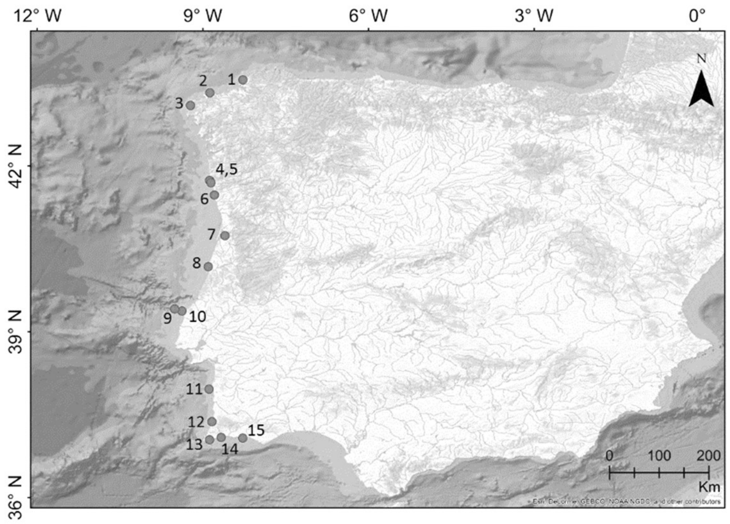
Nevertheless, marine invertebrate species, namely peracarideans, are still poorly represented in the published reference libraries, with only a small fraction of the species occurring in European marine coasts being represented (Raupach et al. 2015 in North Sea and Lobo et al. 2016a in Portugal). In this study, we report and examine a core DNA barcode library for the Peracarida from the Southern European Atlantic Coast (Iberian Peninsula), focusing on representatives of the orders Amphipoda, Isopoda and Tanaidacea.

2.4 MATERIAL AND METHODS

2.4.1 Specimen sampling and taxonomic identification

Specimens were collected between 2008 and 2015 along the Atlantic coasts of mainland Portugal and Spain (Fig. 2.1, Annexes 1.3 and 1.4). Samples were taken from marine rocky shore habitats by scraping of the algal cover or hand picking during low tide (for details see Annex 1.3).

After collection, specimens were preserved in 96% ethanol. Sequence data and specimen metadata were uploaded in the project ‘Peracarida’ (PERAC) within Barcode of Life Data system (BOLD) (Ratnasingham and Hebert 2007). Morphology-based taxonomic identification was supported in specialized literature (Chevreux and Fage 1925, Naylor 1972, Lincoln 1979, Ruffo 1982, Holdich and Jones 1983, Harrison and Ellis 1991, Hayward and Ryland 1995). The identifications were reviewed before and after obtaining the DNA sequences to ensure the correct identification of the specimens. The species’ nomenclature used in this work complies with the accepted nomenclature used in WoRMS and Integrated Taxonomic Informations System (ITIS).



	Collection sites	Latitude	Longitude
1	Pedreira	43.55617	-8.27494
2	Barizo	43.32211	-8.87278
3	Muxía	43.09283	-9.22343
4	Canto Marinho	41.73670	-8.87619
5	Viana Castelo	41.69380	-8.85118
6	Apulia	41.47190	-8.78955
7	Aveiro	40.73604	-8.59895
8	Buarcos	40.17597	-8.90057
9	Berlengas	39.41177	-9.51098
10	Peniche	39.37243	-9.37755
11	Sines	37.96088	-8.88729
12	Vale dos Homens	37.37140	-8.83450
13	Ingrina	37.04525	-8.87804
14	Dona Ana	37.08696	-8.66771
15	Arrifes	37.07605	-8.27678

Figure 2.1. Location and coordinates of the sampling sites where peracaridean specimens were collected in this study.

2.4.2 DNA extraction, amplification and sequencing

DNA extraction was performed using the E.Z.N.A. Mollusc DNA Kit (Omega Bio-tek) according to manufacturer instructions. Depending of the specimen size, only a small amount of tissue or the whole animal was used. Then, a 658 base pair (bp) fragment from the 5′ end of the mitochondrial DNA gene coding for cytochrome oxidase I (COI) was amplified using the primer pairs LCO1490/HCO2198 (Folmer et al. 1994) or LoboF1/LoboR1 (Lobo et al. 2013). When these primers failed to amplify the 658-bp fragment, the primer pair Lobo F1/ ArR5 (Gibson et al. 2014) was used to amplify the first 550 bp of the COI fragment.

All PCR reactions were performed in a 25 µl volume containing 2.5 µl of 10X PCR Buffer, 3 µl of 25 mM MgCl2, 1 µl of 10 mM dNTPs, 0.2 µl of Taq polymerase (ThermoScientific) and 0.55-1.25 µl of each primer (10mM). For the primer pair LCO1490/HCO2198, 0.55 µl of each primer was used and for the pairs LoboF1/LoboR1 and LoboF1/ArR5, 1.25 µl of each primer was used. DNA extraction used varied between 2 µl and 4 µl. Ultrapure water was added until the final volume. Cycling conditions for PCR reactions with the primer pair LCO1490/HCO2198 were: one cycle of 94°C for 1 min, 35 cycles of 94°C for 45 s, 51°C for 90 s and 72°C for 60 s, with a final extension of 72°C for 5 min. Regarding the use of the primer pairs LoboF1/LoboR1 and LoboF1/ArR5, the cycling conditions were: one cycle of 94 °C for 1 min, five cycles of 94°C for 30 s, 45°C for 90 s and 72°C for 60 s, 45 cycles of 94 °C for 30 s, 54 °C for 90 s and 72°C for 60 s, with a final extension of 72°C for 5 min. Amplification success was screened in a 1.5% agarose gel, using 3 µl of PCR product, and successful PCR products were then purified (isopropanol precipitation). Cleaned-up amplicons were sent to external sequencing service suppliers (STAB Vida Ltd, Portugal; Macrogen Europe, Netherlands or GATC Biotech, Germany), for bidirectional sequencing.

2.4.3 Data analysis

All sequences were analysed and edited using MEGA 7.0 (Kumar et al. 2016). Trace files were checked manually, unreadable zones and primers were removed and ambiguous bases corrected. Then, the edited sequences were aligned using Clustal W (Thompson et al. 1994) implemented in MEGA 7.0 (Kumar et al. 2016) and the translation verified for stop codons or indels. GenBank BLASTn search (Altschul et al. 1990) and BOLD Identification System tool (BOLD-IDS) (Ratnasingham and Hebert 2007) were used to search for similarity to confirm the target taxa.

Two dedicated datasets were created in BOLD and used for the analysis in this work. The first, “PERA-IP dataset”, includes the sequences obtained in this study from the Iberian Peninsula plus the available sequences in BOLD for marine and estuarine peracaridean species retrieved from Iberian Peninsula Atlantic coasts. The second dataset, “Global dataset”, comprises the previous dataset plus similar taxa (either from the same morphospecies or the same genus) from other locations outside the Iberian Atlantic coasts, either obtained in this study or retrieved from BOLD. The objective of these datasets was to compare and validate our results.

Intra- and interspecific distances were calculated using Kimura-2-parameter (K2P) model (1000 boostraps) (Kimura 1980) implemented in MEGA 7.0 (Kumar et al. 2016). The Bayesian inference (BI) was conducted in MrBayes 3.2 (Ronquist et al. 2012) to build the Bayesian tree for each order separately using the “Global dataset”. The BI topologies were constructed choosing GTR+G+I as best-fitting model of nucleotide substitution based on its Bayesian Information Criterion, as implemented in MEGA 7.0 (Kumar et al. 2016).

BINs provided by BOLD (Ratnasingham and Hebert 2013) were used as a model for Molecular operational taxonomic units (MOTUs) clustering for all sequences. The ‘BIN Discordance Report’ analysis tool was applied to analyse both datasets used in this study. BINs were identified as taxonomically discordant if species clusters shared a BIN. The concordant BINs mean that one cluster corresponded with one BIN.

A BIN discordance report was generated to enable comparison between morphospecies and MOTUs generated by COI sequence data. The taxonomic reliability of the species records from Iberian Peninsula was ranked using the grades A to E proposed by Costa et al. (2012), and adapted by Borges et al. (2016) and Oliveira et al. (2016). The ranking varies from A to E, where "A" represents highly reliable species barcodes and "E" barcodes with lower reliability: grade A (external concordance: unambiguous BIN match between specimens of the same morphospecies from independent BOLD projects or published sequences), grade B (internal concordance: species' BIN congruent within our data set, with at least 3 specimens of the same species examined but no matching sequences found from independent studies), grade C (suboptimal concordance: at least 3 specimens of the same morphospecies are available within the library, but they are split among more than one nearest neighbouring BIN), grade D (insufficient data: low number of specimens analysed, only 1 or 2 individuals) and grade E (discordant species assignments: sequences for a given species in the data set did not match with the BIN or BINs for the same species in BOLD and the specimen may match with a BIN of a different species or was assigned to a separate non-neighbouring BIN).

2.5 RESULTS

2.5.1 *Morphological identification of species*

A total of 220 novel COI sequences from 58 peracaridean morphospecies (14 specimens identified only to genus level and 5 specimens to family level) belonging to 24 families were generated in this study. Three orders were represented: Isopoda (131 specimens), Amphipoda (75 specimens) and Tanaidacea (14 specimens). The similarity searches in BOLD-IDS returned a significant identity match (98–100%) for 59% of the sequences. Novel barcodes were produced for thirty-one species. For those, the nearest match was found at similarities between 74–89%. Of the 220 barcodes obtained, 160 were retrieved from specimens collected in the Atlantic Continental coast of the Iberian Peninsula (102 from Portugal and 58 from Spain) from a total of 50 morphospecies. All the peracaridean barcodes belonging to the orders Amphipoda, Isopoda or Tanaidacea retrieved from the Atlantic Iberian Peninsula available in BOLD systems (16/01/2017) were mined and added to the alignment (Table 2.1 – "PERA-IP dataset", for sources see Annex 2.1). To avoid adding dubious and/or low quality data, only barcodes with a minimum length of 500 bp, registered as sampled in marine or estuarine coasts of the Atlantic Iberian Peninsula, and with the indication of the respective source were used. In the works of Xavier et al. (2009, 2012), Cabezas et al. (2013a, b, 2014) and Raupach et al. (2014), the authors did extensive studies regarding population structure of a specific species (for further details about *Caprella penantis* in Cabezas et al. 2013a; *Caprella dilatata* in Cabezas et al. 2013b, 2014;

Caprella scaura in Cabezas et al. 2014; *Ligia oceanica* in Raupach et al. 2014; *Stenosoma nadejda* in Xavier et al. 2009 and *Stenosoma lancifer* in Xavier et al. 2009, 2012). Our goal was not to replicate the same conclusions of these authors and also the inclusion of all the sequences obtained by them would result in a massive and repetitive data. In order to simplify the data, only 4-5 barcodes of each species sampled in Iberian Peninsula from these works were added to "PERA-IP dataset". In the end, a total of 217 COI sequences from BOLD were added to our 160 novel COI sequences, resulting in a 377-barcode data set from 106 morphospecies (Table 2.1 – "PERA-IP dataset").

The remaining novel sequences obtained in this study were sampled from Azores (11), Scotland (23), Norway (10) and Iceland (16). To facilitate the analysis of the data and further comparisons, 161 additional COI sequences were mined from BOLD and GenBank from closely related taxa (same species or genus) from non-Atlantic Iberian Peninsula marine coasts and added to the alignment (Table 2.1 – "Global data set", see Annex 2.1 for sources), resulting in a total of 597 barcodes from 140 morphospecies. To avoid adding dubious and/or low quality data, only barcodes with a minimum of 500 bp, the indication where the specimens were sampled and source were added.

Four morphospecies retrieved from BOLD did not have the updated taxonomic nomenclature: *Synisoma lancifer, Synisoma nadejda, Synisoma acuminatum* and *Leptochelia dubia* (for source see Annex 2.1). Using the accepted nomenclature from WoRMS and ITIS, the genus *Synisoma* Collinge, 1917 was updated to *Stenosoma* Leach, 1814, while *Leptochelia dubia* (Kroyer, 1842) was updated to *Chondrochelia savignyi* (Kroyer, 1842). All the analysis in this study took in consideration these changes and the new accepted nomenclature.

Taxonomic classification, number of specimens and their geographical origin are shown in Annex 2.1. COI sequences with 658 bp were obtained for 40.8% of specimens (154) for "PERA-IP dataset" and 47.7 % of specimens (285) for "Global dataset", while the remaining individuals had sequences between 500 and 657 bp. Upon aligning and translating all sequences, no stop codons were found.

The total number of specimens analysed per morphospecies varied from 1 to 35 (34 species were represented by a single specimen). Thirty-seven genera were represented by one species, while twenty-three were represented by 2 to 9 species. Thirteen species were identified only to genus level (12 from Iberian Peninsula), six species were only identified until family level, and one to order.

Table 2.1. Species and sequences number used in this study. SP-Species number in each site/dataset, N – number of sequences.

	SITE	SP	N	SOURCE
IBERIAN PENINSULA	Pedreira	14	25	This study
	Barizo	12	15	
	Muxía	9	18	
	Canto Marinho	9	19	
	Viana Castelo	9	17	
	Apulia	1	2	
	Aveiro	4	9	
	Buarcos	8	13	
	Peniche	4	5	
	Berlengas	2	2	
	Sines	2	3	
	Vale dos Homens	1	1	
	Ingrina	7	8	
	Dona Ana	13	16	
	Arrifes	5	7	
	Novel obtained in this study	**50**	**160**	
	Viana Castelo	33	123	Lobo et al. 2013, 2016a
	Vila do Conde	1	2	Cabezas et al. 2013b
	Aveiro	3	8	Costa et al. 2009, Lobo et al. 2016a
	Ericeira	1	1	Cabezas et al. 2013b
	Foz do Arelho	1	1	Costa et al. 2009
	Setubal	17	30	Costa et al. 2009; Lobo et al. 2013, 2016a
	Sines	1	2	Lobo et al. 2016ª
	Sagres	1	1	Cabezas et al. 2013a
	Ria Alvor	1	1	Costa et al. 2009
	Basc Country	3	3	Aylagas et al. 2014
	Ferol	1	5	Raupach et al. 2014
	Cadiz	1	4	Cabezas et al. unpublished
	Huelva	1	4	Cabezas et al. 2013b
	Portugal unknown locations	2	5	Cabezas et al. 2013a, Larsen et al. unpublished
	North Spain unknown locations	13	13	Aylagas et al. 2014, Sotka et al. 2016
	Iberian Peninsula unknown locations	3	14	Xavier et al. 2009, 2012
	Pera-IP dataset	**106**	**377**	
WORLDWIDE AREAS	Azores	4	11	*This study*
	Belgium	2	3	Costa et al. 2009
	Balearic Islands	1	3	Cabezas et al. unpublished
	Italy	6	14	Maruso et al. unpublished
	France	7	7	Hou et al. 2011, Kilpert et al. 2012, Cowart et al. unpublished, Larsen et al. unpublished
	North Sea	46	107	Raupach et al. 2015
	Hawaii	1	1	Sotka et al. 2016
	Canada	5	9	Radulovici et al. 2009
	Ireland	1	1	Costa et al. 2009
	Netherlands	1	1	Ironside et al. unpublished
	Germany	1	1	Kilpert et al. 2012
	Sweden	1	1	Costa et al. 2009
	Wales	3	3	Costa et al. 2009
	Norway	6	13	*This study*, Costa et al. 2009
	Iceland	9	20	*This study*, Henzler and Ingólfsson 2008, Costa et al. 2009
	Scotland	10	25	*This study*, Costa et al. 2009
	Global dataset	**140**	**597**	

2.5.2 *Intra- and interspecific divergences*

Intra- and interspecific distances concerning all the peracaridean species under analyses, with the exclusion of species with high intra specific distances (ISD) (higher than 3%, see Annex 2.1 for values for each morphospecies) are provided in Table 2.2 for both datasets. This exclusion was to prevent incorrect distance estimations. In the “Global dataset”, the ISD was 0.59% (range 0.00–3.83%), while the average congeneric distance was 20.93% (range 0.00–36.78%) and the average within family distance was slightly higher, 25.53% (range 0.00–47.93%). The “PERA-IP data set” showed similar results: 0.42% for species, 21.56% for genus and 25.61% for families.

2.5.3 *BI phenogram*

Figs 2.2, 2.3 and 2.4 show Bayesian trees using “Global dataset” for Amphipoda (388 sequences), Isopoda (192 sequences) and Tanaidacea (17 sequences) respectively, with clearly defined and well supported clusters. Most species and genera clustered according to their taxonomic hierarchy, although some exceptions were found. Deep intra-specific divergences (more than one BIN) were found for 21 morphospecies, of which 18 were amphipods and 3 were isopods, with eight species with maximum distance higher than 20%: *Ampelisca diadema* (maximum of 25.5%), *Caprella acanthifera* (maximum of 23.2%), *Janira maculosa* (maximum of 25.15%), *Ampithoe rubricauta* (maximum of 24.7%), *Dexamine spinosa* (maximum of 23.6%), *Microdeutopus chelifer* (23.6%), *Jassa pusilla* (maximum of 22.3%) and *Ampelisca spinipes* (maximum of 20.5%).

Some clusters, however, included more than one morphospecies. *Lekanesphaera rugicauda* clustered with *Lekanesphaera hookeri*, while some species that were identified only until genus level clustered with species identified until species level such as *Jassa hermandi* with *Jassa sp.*3 or *Jassa pusilla* with *Jassa sp.1*. In the clusters containing *Caprella acanthifera* and *Caprella danilevski*, *Microdeutopus chelifer* and *Microdeutopus sp.*, *Dexamine spiniventri*s and *Dexamine spinosa*, and *Urothoe poseidonis* and *Urothoe pulchella*, some barcodes were also present in other clusters.

2.5.4 *BINs and ranking system for barcode records*

BIN attribution summary can be found in Table 2.3 for both data sets. The 597 barcode compliant sequences (140 morphospecies) were assigned to 160 BINs. The analysis of the “BIN report” produced by the BOLD system (as on 16 of January 2017) showed 96 concordant BINs, 9 discordant BINs and 55 singletons. The 9 discordant BINs were examined and re-assessed to account for potential artefacts (e.g. misidentifications, specimen mislabelling, contamination, misspelling, synonymies and syntax inaccuracies). After examination, four discordant BINs (AAX8442, AAJ2286, ABU6145, ACH9003) were considered concordant because same specimens did not have updated taxonomy or were not identified to species level. This brings the number of concordant BINs to 100 (62.5%).

Of the 106 putative morphospecies from the Iberian Peninsula used in this study, 20 species were not identified until species level. Therefore, the ranking system was applied to 86 morphospecies (Annex 2.2). After the auditing procedure (for a detailed auditing procedure see Oliveira et al. 2016), the grades A and/or B (high taxonomic reliability) were attributed to 48 species (55.9%); 7 species (8.1%) showed a high intraspecific divergence (grade C): *Janira maculosa, Gammarela fucicola, Talitrus saltator, Microdeutopus chelifer, Corophium multisetosum, Phistica marina and Jassa pusilla*; 22 species (25.6%) were attributed a grade D (insufficient data) and only 9 species (10.4%) were attributed a grade E (incongruent DNA barcodes): *Lekanesphaera hookeri, Dexamine spiniventris, Dexamine spinosa, Ampelisca diadema, Caprella acanthifera, Caprella danilevski, Ampithoe ramondi, Ampithoe rubricata and Urothoe pulchella* (see Annex 2.2 for details).

Table 2.2. Intra and interspecific K2P distances of peracaridean species, genus and families analysed in this study.

	Taxa	Min Dist (%)	Mean Dist (%)	Max dist (%)
Within species				
Pera-IP dataset*	58	0.00	0.42	2.80
Global dataset**	102	0.00	0.59	3.83
Within genus				
Pera-IP dataset*	13	0.59	21.56	31.91
Global dataset**	26	0.00	20.93	36.78
Within family				
Pera-IP dataset*	13	0.00	25.61	47.93
Global dataset**	16	0.00	25.53	47.93

**Dexamine spiniventris, Caprella acanthifera, Microdeutopus chelifer, Corophium multisetosum* were excluded from the intraspecific analysis due to the high divergence values (higher than 3%).

** *Dexamine spiniventris, Caprella acanthifera, Microdeutopus chelifer, Corophium multisetosum, Sphaeroma serratum, Jassa pusilla, Ampelisca brevicornis, Ampelisca tenuicornis, Ampithoe rubricata, Urothoe pulchella, Ampelisca typical, Ampelica spinipes, Ampelisca diadema, Gammarus duebeni, Astacilla intermedia, Janira maculosa, Dexamine spinosa, Ampithoe ramondi, Gammarela fucicola and Talitrus saltator* were excluded from the intraspecific analysis due to the high divergence values (higher than 3%).

Table 2.3. Number of BINs and number of taxonomically concordant, discordant and singleton records for COI sequences used in this study.

	NUMBER OF BINS	
	Pera-IP dataset	Global dataset
CONCORDANCE	70	100
DISCORDANCE	2	5
SINGLETON	41	55
TOTAL	113	160

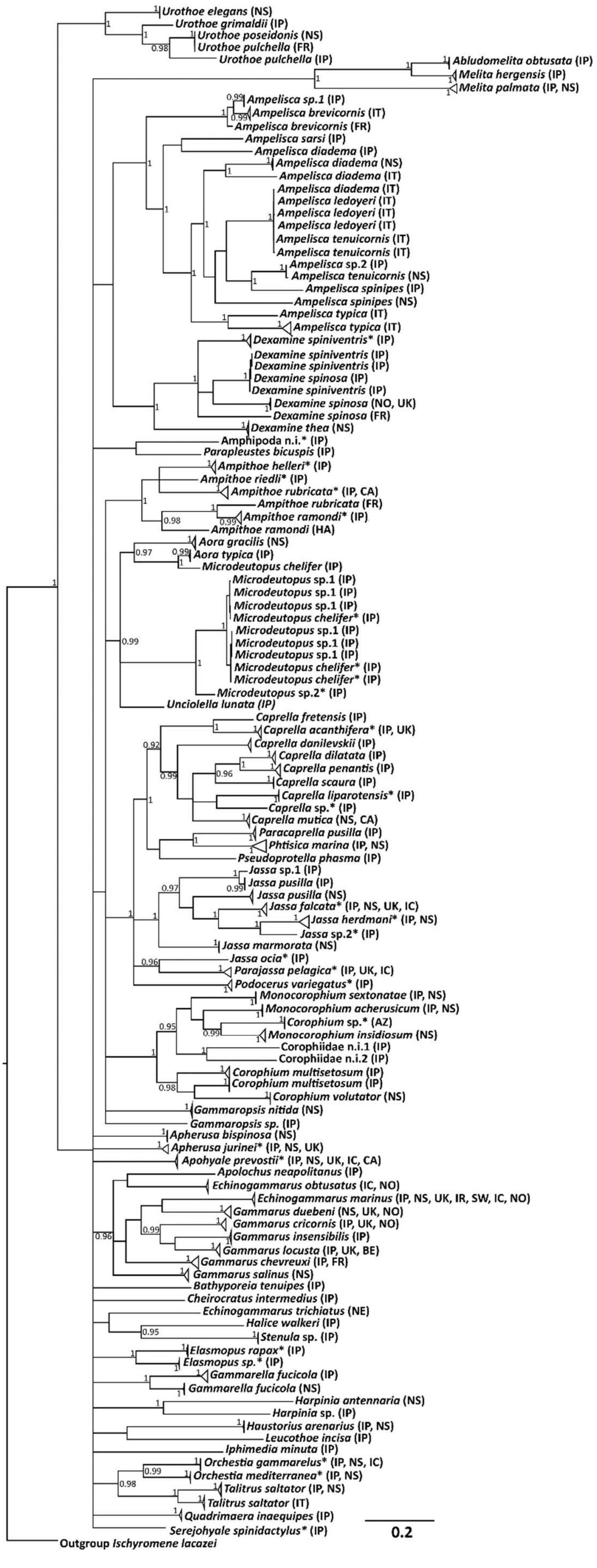


Figure 2.2. The Bayesian tree based on COI sequences from the ninety-seven amphipod species used in this study. Numbers associated with nodes represent posterior probabilities from Bayesian Markov chain Monte Carlo searches conducted in MrBayes (only posterior probabilities higher than 0.90 are shown). Asterisk indicates sequences obtained in this study. The isopod *Ischyromene lacazei* was used as outgroup.

IP- Iberian Peninsula, FR – France, NS - North Sea, IT – Italy, UK – United Kingdom, NO – Norway, CA – Canada, IC – Iceland, AZ – Azores, IR – Ireland, SW – Sweden, BE – Belgium.

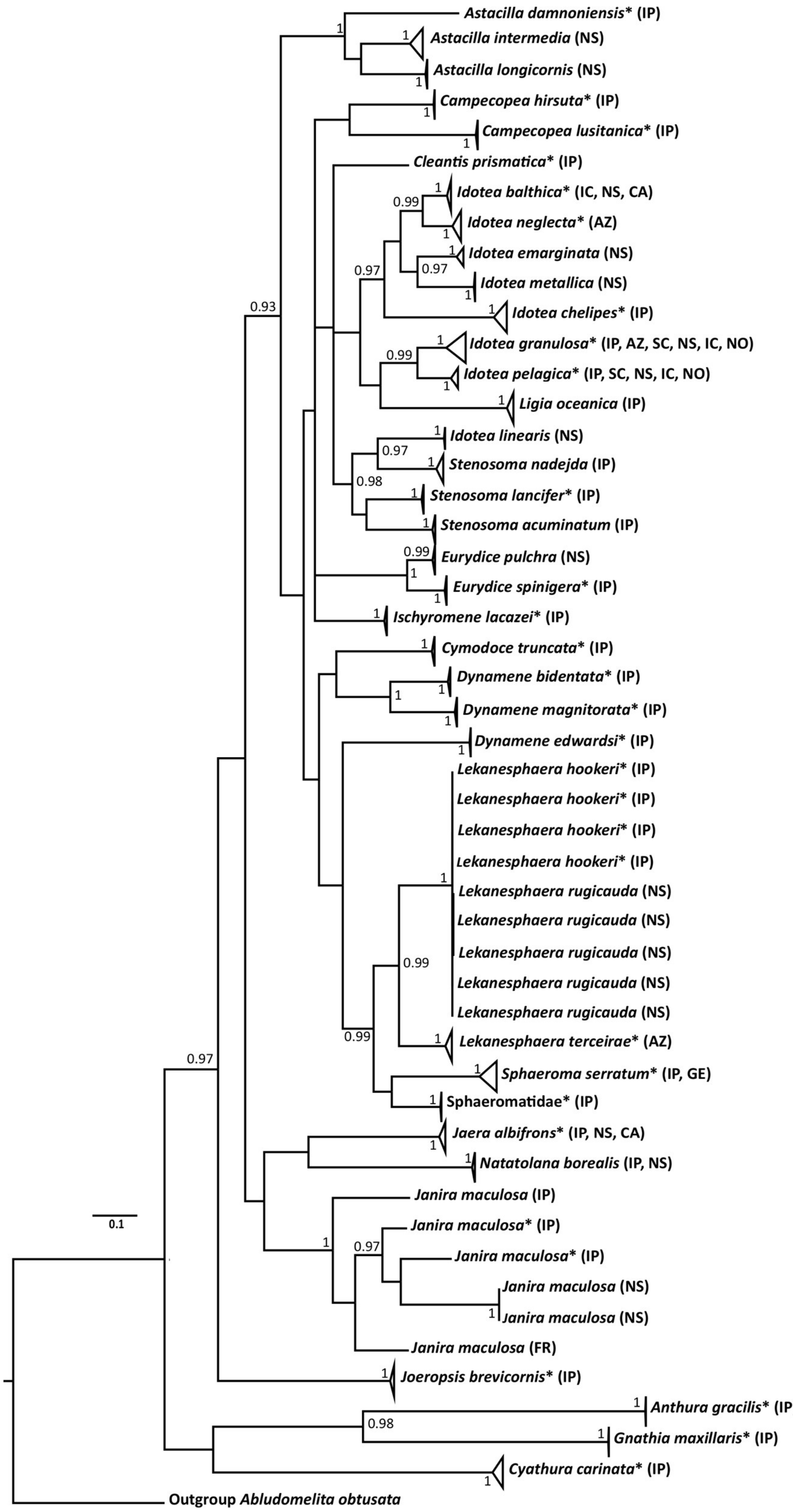


Figure 2.3. The Bayesian tree based on COI sequences from the thirty-seven isopod species used in this study. Numbers associated with nodes represent posterior probabilities from Bayesian Markov chain Monte Carlo searches conducted in MrBayes (only posterior probabilities higher than 0.90 are shown). Asterisk indicates sequences obtained in this study. The amphipod *Abludomelita obtusata* was used as outgroup.

IP- Iberian Peninsula, FR – France, NS - North Sea, IT – Italy, SC – Scotland, NO – Norway, CA – Canada, IC – Iceland, AZ – Azores, GE - Germany.

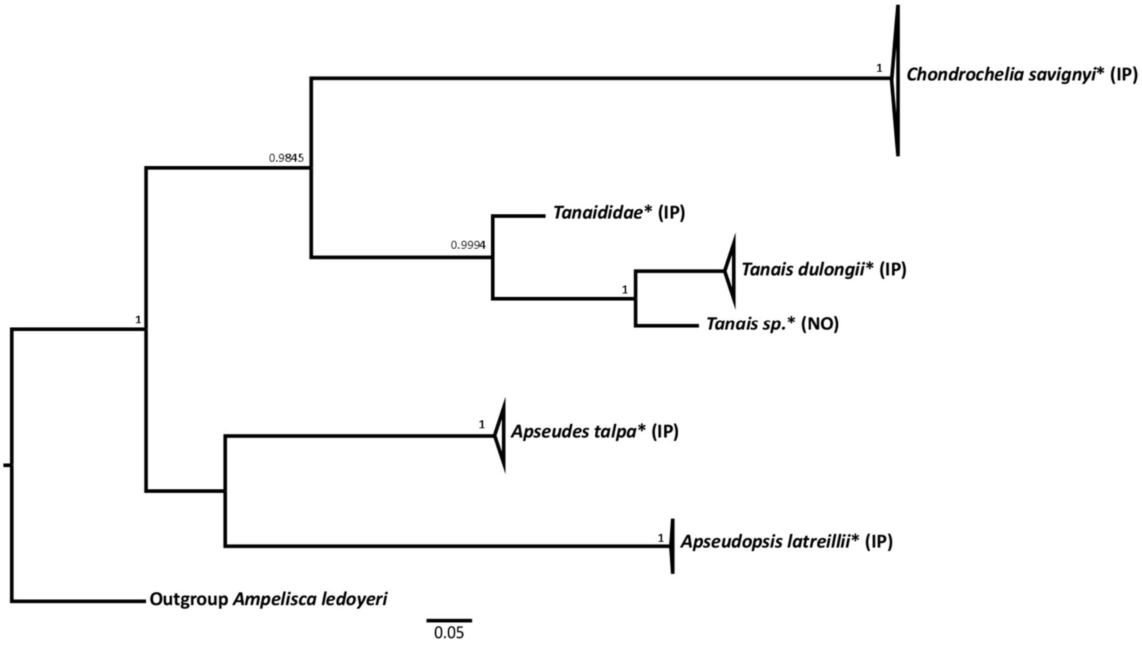


Figure 2.4. The Bayesian tree based on COI sequences from the six tanaid species used in this study. Numbers associated with nodes represent posterior probabilities from Bayesian Markov chain Monte Carlo searches conducted in MrBayes (only posterior probabilities higher than 0.90 are shown). Asterisk indicates sequences obtained in this study. The amphipod *Ampelisca ledoyeri* was used as outgroup.
IP- Iberian Peninsula.

2.6 DISCUSSION

2.6.1 Reference library of DNA barcodes

This study contributes with DNA barcodes for 58 morphospecies from the Atlantic European coasts (50 from Iberian Peninsula), with 33 morphospecies being new additions to the global reference library. The efficiency of DNA barcodes in species discrimination relies on the occurrence of a gap between the maximum intraspecific and minimum congeneric barcode distances (Costa and Carvalho 2010). We have found such distance gap within both datasets (Table 2.2). Average intraspecific (0.42%) and congeneric (21.56%) distances in “PERA-IP dataset” were comparable to those found in other DNA barcoding studies in other marine invertebrate groups such as gastropods, decapods and crustaceans (Matzen da Silva et al. 2011, Raupach et al. 2015, Borges et al. 2016).

We were not able to identify to species level 8 species (neither we retrieved positive matches from BOLD-IDS), with 6 of them from the Iberian Peninsula. This was due to the small size of the specimens and/or lack of distinctive morphological characters. The fact that in most peracaridean species distinctive morphological characters are usually present only in adults or in males, can be an impediment to fully access the taxonomy of the specimens of this group (Costa et al. 2004, Coleman 2015). Raupach et al. (2015) and Ferreira et al. (2016a) also verified this difficulty in the identification of some peracaridean species in their works. A total of 20 morphospecies used in the present study were not identified to species level (18 from the Iberian Peninsula) and 15 did not cluster with a morphospecies identified to species level (13 from the Iberian Peninsula). The fact that it was possible to cluster five previously non-identified species (see Annex 2.3 for details) strenghs the idea that the compilation of data of different sources is important to correct assess species boundaries.

2.6.2 Auditing methods

Considering BINs as MOTUs (Ratnasingham and Hebert 2013), the comparison across both datasets between morphology-based identifications and BINs suggests underestimation of the species diversity. Indeed, the number of BINs here examined (113 for “PERA-IP dataset” and 160 in “Global dataset”, Table 2.3) exceeded by 7 and 20, respectively, the number of morpho-species (Table 2.3). The large number of singleton BINs (41 and 55 respectively, Table 2.3) reinforce the idea that the Peracarida fauna from the Atlantic coasts, including the Iberian Peninsula is still poorly studied, although it comprises 1/3 of the total Crustacea biodiversity worldwide (Horton et al. 2017a). However, the large number of concordant BINs (70 and 100 respectively, Table 2.3) show the reliability of this library, displaying a one-to-one link with morphologically identified species. Some of them include specimens displaying comparatively small K2P distances (<1%), although they originated from populations geographically very distant from each other (e.g*., Apohyale prevostii* and *Idotea granulosa*), contrasting with the idea that these organisms have a low dispersal ability, which was also observed by Xavier (2011a). Concordant BINs comprising a high number of members conveyed higher confidence on the taxonomic identifications of those specimens. COI sequences belonging to the species *Idotea granulosa* and *Jassa falcata* with 20 or more members are two good examples.

In the morphospecies present in Iberian Peninsula, species records with reliability grades A or B amount to 55.9% (for list of species, see Annex 2.2), which is a bit higher than the values found for Polychaeta – 50% (Lobo et al. 2016b), but lower than what has been determined for other reference libraries in Northeast Atlantic: e.g., 84.9% and 78% for fish (Knebelsberger et al. 2014 and Oliveira et al. 2016 respectively); or 70.5% for gastropods (Borges et al. 2016). However, 23 morphospecies (for list of species, see Annex 2.2) lacked matching sequences from other studies for comparison and did not have enough data (grade D – 25.6%) to enable attribution of a higher grade. Interestingly, in two morphospecies, *Ampelisca spinipes* and

Sphaeroma serratum, with two sequences each, two neighbor BINs were retrieved for each species. Each BIN corresponded to different locations and authors. More data is needed to assess if this is a case of possible misidentification or hidden diversity.

Discordant BINs and Grade E may occur for several reasons, such as misidentifications, sample contamination, sample mislabeling or inaccuracies of the BIN delineation algorithm (Hebert et al. 2003, Costa and Antunes 2012, Ratnasingham and Hebert 2013). The discordant BINs (in "Global dataset") resulted from probable misidentification, because in all cases the identification was distinct between different authors. Without access to the specimens or photographies, no definitive conclusion can be made and assumption should be taken carefully. However, looking at associated metadata in BOLD and the BI trees (Figs 2.1-2.3) it seems the specimens of *Dexamine spinosa* and *Caprella acanthifera* obtained by Aylagas et al. (2014) are *Dexamine spiniventris* and *Caprella danilevski* respectively (Annex 2.3). Several cases are harder to evaluate and more sequences and data are needed: *Urothoe pulchella* with *Urothoe poseidonis,* Am*pelisca diadema* with *Ampelisca ledoyeri* and *Ampelisca tenuicornis* and finally, *Lekanasphaera hookeri* with *Lekanasphaera rugicauda*.

2.6.3 High ISD in peracaridean species

Apart from the previous cases, several morphospecies (11) displayed high ISD (Annex 2.1), and therefore more than one BIN, according to the threshold of 3% suggested for Crustacea by Costa et al. (2009). The morphospecies *Gammarela fucicola, Phistica marina, Jassa pusilla, Talitrus saltator* and *Dexamine spinosa* displayed high genetic divergences between distant populations. The first three, between Iberian Peninsula and North Sea, *T. saltator* between Iberian Peninsula/North Sea and Italy (for more details, see Lobo et al. 2016a) and the latter between Scotland/North Sea and France.

Three morphospecies showed sharp genetic discontinuities among proximate populations in Iberian Peninsula: *Dexamine spiniventris*, *Corophium multisetosum* and *Microdeutopus chelifer,* and it does not seem that these lineages are sorted geographically. However, there is a possiblity that the identification of Lobo et al. (2013) of *M. chelifer* from North Portugal is not correct (Annex 2.3), since it is closer with other Aoridae species, such as *Aora gracilis*, supported by high posterior probabilty (>0.90, Fig. 2.2). Members of *Microdeutopus* species are hard to distinguish because an adult male and full develop gnathopds are needed to discriminate correctly between species. We encounter this constrain as we were not able to identify to species level a specimen of *Microdeutopus sp.2* (due to small size).

Janira maculosa displays an interesting case with six different lineages rearranged geographically. Two from North Spain (although with genetic distance of 24.2%), one from North Sea, one from Portugal and one from France (all from different sources, see Annex 2.1). Members of the *Janira* genus can be easily distinguish from other Janiridae genera by the antennae longer than body and uropods longer than pleotelson (Naylor 1972). In our reference library, the different Janiridae genera clusters were well separated (*Janira, Joeropsis* and *Jera*, Fig. 2.3). *Janira maculosa* is present along the North Atlantic Ocean. Only three species of this genus are known and very little is known about the presumably occurrence of the other two *Janira* species (other than *Janira maculosa*) in North Atlantic Ocean and consequently their taxonomy (Horton et al. 2017a). The fact that different authors found and identified only this species within *Janira* genus reinforce the idea that more work related with the taxonomy of this genus is required. Presumably, *Janira maculosa* displays hidden diversity and more work is needed to demonstrate this.

Ampithoe ramondi was reported as a probable widespread cryptic species by Sotka et al. (2016). On our records, Portugal clustered with the record from Spain obtained by Sotka et al. (2016) (Fig. 2.2). One record of *Ampithoe rubricata,* obtained by Cowart et al. unpublished from France appears as neighbour BIN from this group within the *Ampithoe ramondi* “complex” (Fig. 2.2, posterior probability >0.90) and distinct from the rest of the *Ampithoe rubricata* records. We suspect that this record might be in fact part of one lineage of *Ampithoe ramondi* (Annex 2.3). The rest of *Ampithoe rubricata* records obtained by us from Iberian Peninsula and from Radulovici et al. (2009) from Atlantic Canada clustered together. Two groups seem to appear, one from Iberian Peninsula and other from Canada, with maximum genetic distance of just 2.1%. Amphi-Atlantic distributed amphipods with postglacial colonization routes, usually from south to north, was reported before (Costa et al. 2009, Krebes et al. 2011), usually after the last Glacial Maximum around 20 000 years ago and this seems to be the case (Wares and Cunningham 2001, Maggs et al. 2008).

2.7 CONCLUSIONS

Previous studies showed the ability of DNA barcodes to distinguish peracaridean species (e.g., Costa et al. 2009, Raupach et al. 2015, Lobo et al. 2016a), and our data confirmed it. DNA barcoding and specially barcode reference libraries are not restricted to taxonomic or systematic research only. The rise of modern high-throughput sequencing technologies is changing biomonitoring applications and surveys significantly (Fonseca et al. 2010, Leray and Knowlton 2015). As consequence, reference datasets such as ours are essential for the correct identification of specimens sequenced as part of meta barcoding studies. DNA barcodes can help with cases of synonymy or misidentifications, detect distinct genetic populations within a species either separated geographically or within the same region and match non-identified species to well stablish BINs. The high number of BINs compared to morphospecies found in this (and other peracaridean studies such as Raupach et al. 2015 and Lobo et al. 2016a) also suggests a considerable amount of hidden diversity in this group in Iberian Peninsula and Northeast Atlantic Ocean. This and other studies from DNA barcoding can help to understand and improve the knowledge of the biodiversity of Peracarida fauna in Iberian Peninsula and European marine coasts.

2.8 ACKNOWLEDGMENTS

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Chapter 3: Distribution and species identification in the crustacean isopod genus *Dynamene* Leach, 1814 along the Northeast Atlantic-Black Sea axis

3.1 ABSTRACT

Sphaeromatid isopods, such as *Dynamene*, are common and abundant members of the invertebrate fauna of littoral and shallow sublittoral substrates. Six species of *Dynamene* occur in the northern hemisphere. Only two species exist outside this range, in Australia. The distribution of the various species in the Northeast (NE) Atlantic-Black Sea axis has been controversial due to the difficulty in the identification of the different species. This has led to inaccurate records of their distribution, ultimately generating uncertain or faulty assessments on the biodiversity of these habitats. An update and a clarification about the distribution of this genus is therefore in order. In this study, we describe the distribution of *Dynamene* species in the light of new records from the NE Atlantic Ocean and its associated islands, and the Mediterranean, Black and Red Seas, and from re-examination of museum and several authors' personal collections. Based on these observations, we extend the northern and southern limits of *D. bidentata* (Adams); the western and southern limits of *D. magnitorata* Holdich; the northern, eastern and western limits of *D. edwardsi* (Lucas); and the eastern and western limits of *D. bifida* Torelli. The range of *Dynamene tubicauda* Holdich is extended, but is still only known from the eastern Mediterranean. We also clarify the synonymy of *D. torelliae* Holdich with *D. bicolor* (Rathke), and the occurrence of *D. bicolor* in the Black Sea. New distribution maps of the six *Dynamene* species are presented. Illustrated keys to the adult males and females of the northern hemisphere species are provided.

3.2 KEYWORDS

Dynamene, Crustacea, Isopoda, Sphaeromatidae, identification, distribution.

3.3 INTRODUCTION

Isopod crustaceans are common and sometimes abundant members of the invertebrate fauna of the littoral and shallow sublittoral habitats of the world's oceans (Poore and Bruce 2012). Species of the sphaeromatid isopod genus *Dynamene* Leach, 1814 are typical components of these habitats on coasts of the Northeast (NE) Atlantic Ocean and its islands, and the Mediterranean and Black Seas. Six species are endemic to these provinces (Holdich 1968a, 1970): *D. bidentata* (Adams, 1800); *D. bicolor* (Rathke, 1837); *D. edwardsi* (Lucas, 1849); *D. bifida* Torelli, 1930; *D. magnitorata* Holdich, 1968 and *D. tubicauda* Holdich, 1968. *Dynamene torelliae* Holdich, 1968 was considered to be synonymous with *D. bicolor* by Kussakin (1979) and this has been accepted by the current authors. Two additional species occur in, and are endemic to, Australia, but have rarely been recorded: *Dynamene ramuscula* (Baker, 1908) and *Dynamene curalii* Holdich and Harrison, 1980. A number of other *Dynamene* species are incorrectly listed in some databases, e.g., http:/ isopods.nhm.org/, Brusca et al. (1995-2004), Myers et al. (2008). Species attributed to the genus *Dynamene* from the western USA, i.e., *D. angulata* Richardson, 1901; *D. benedicti* (Richardson, 1899); *D. dilatata* Richardson, 1899; *D. glabra* Richardson, 1899 and *D. sheari* Hatch, 1947 do not belong to this genus, as adult males do not possess a bidentate process arising from the sixth pereonite (see below), and are considered incertae sedis (http://www.marinespecies.org/). *Dynamene tuberculosa* Richardson, 1899 from the Aleutian Islands off Alaska is also still listed as such in some databases, but was considered as the female of *Paracerceis cordata* (Richardson, 1899) by Richardson (1905).

The distribution of the various *Dynamene* species associated with the NE Atlantic-Black Sea axis was previously examined by Holdich (1968a, 1970). Since then, many general community studies have been published reporting the presence of *Dynamene* throughout its range (e.g., Pereira et al. 2006 in Portugal; Arrontes and Anadón 1990, Arrontes 1991, Viejo 1997, Castelló and Carballo 2001 in Spain; Castellanos et al. 2003 in northern Africa and Kirkim et al. 2006 in Turkey). In addition, a large number of specimens have become available since Holdich's studies, which make the clarification and updating of distribution maps along the NE Atlantic-Black Sea axis necessary. This is particularly so because many of the records for the Mediterranean and Adriatic relate to *D. torelliae*, which has been synonymized with *D. bicolor*.

In order to be able to identify species of *Dynamene*, and distinguish them from some other sphaeromatid isopods, it is important to understand how the morphology changes during the life history. Adult males (stage 8) of the various *Dynamene* species can be distinguished from those of other sphaeromatid isopods, e.g., *Campecopea* Leach, 1814; *Cymodoce* Leach, 1814;

Ischyromene Racovitza, 1908; *Lekanosphaera* Verhoeff, 1943 and *Sphaeroma* Bosc, 1802, that may be found in the same habitat, by a large two-pronged medial process (the bidentate process) arising from the dorsal posterior margin of the sixth pereonite (Fig. 3.1). This characteristic is unique to the genus (Harrison and Ellis 1991). Some species of *Oxinasphaera* Bruce, 1997 have such a process, but this arises from the pleon (Bruce 1997, Schotte and Kensley 2005), and paired processes arise from the seventh pereonite in *Dynamenella dioxus* Barnard, 1914. Juveniles and females, and even sub-adult males (stages 6 and 7), are more difficult to distinguish between the species, and may also be confused with females of other genera. Vieira et al. (2015) have shown clear differences between *D. bidentata, D. magnitorata* and *D. edwardsi* at the genetic level using cytochrome oxidase I. Details of the changes occurring throughout the life history of the best-studied species, *D. bidentata*, are given below.

Dynamene species are present in a wide-range of habitats, but usually amongst algae and in cryptic habitats, e.g., under rocks, crevices, empty barnacle tests, amongst serpulid and tunicate colonies, mussel beds and encrusting sponges, from midlittoral to shallow sublittoral levels (Holdich 1970, 1976). *Dynamene bidentata*, at least, has a biphasic life cycle with a change of habitat, where the immature stages are present amongst the algal cover (which they eat), whilst the adults occupy cryptic habitats where they reproduce and where females can incubate their broods in relative safety (Holdich 1968b, 1970, 1976). Further details of the habitats occupied by *Dynamene* along the NE Atlantic-Black Sea axis are given for each species below.

Given that fully adult males may not be present in many collections, species identification is often difficult and leads to incorrect assignments, questioning the validity of the information about the actual distribution of the species. The literature is scattered with misidentifications, which have come to light when such authors' material and/ or publications have been examined by us. In the present study, we aim to update and correct the geographical distribution of the six-described species of *Dynamene* from the NE Atlantic-Black Sea axis. To facilitate identification, keys to adult males and females of these six species are provided along with associated photographs. It is hoped that these will enable those involved in littoral and sublittoral surveys in the marine environment to identify species of *Dynamene* more easily.

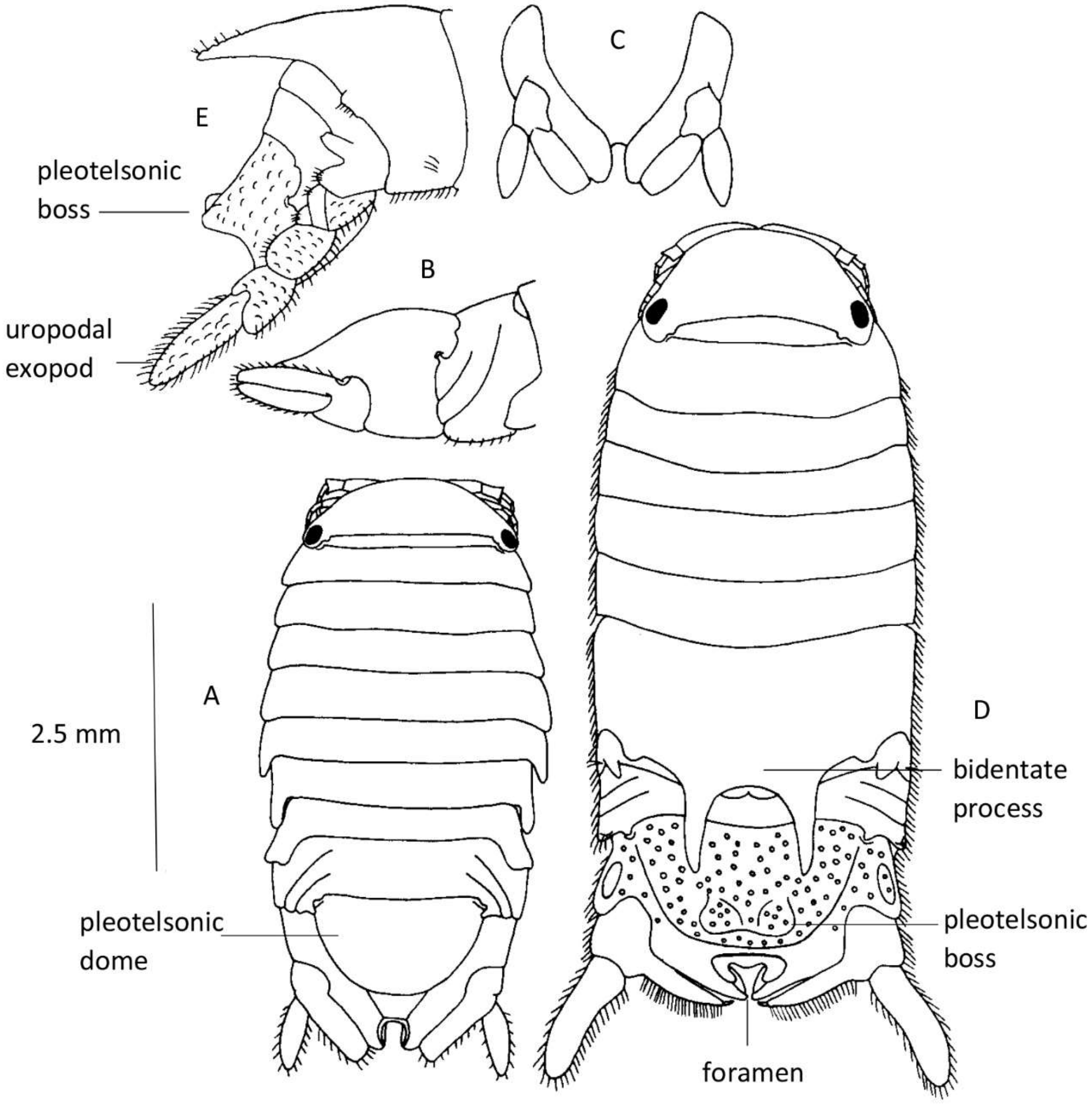

Figure 3.1. Adult male (stage 8) and pre-ovigerous female (stage 7) *Dynamene bidentata*.
A - Dorsal view of stage 7 female. **B** - Lateral view of pleon (with posterior border of pereonite 7), pleotelson and right uropod of stage 7 female. **C** - Ventral view of pleotelson and uropods of stage 7 female. **D** - Dorsal view of stage 8 male. **E** - Lateral view of pereonal segment 6, pleon, and pleotelson and exopod of right uropod of stage 8 male.
Adapted from Holdich 1968b.

3.4 MATERIAL AND METHODS

The records of David Holdich (DMH) used in this study are derived from field work carried out in various localities in the British Isles, Atlantic islands, Atlantic coasts of mainland Europe, and the Mediterranean and Aegean Seas (Holdich 1968a, c, 1970, Holdich and Lincoln 1974, Holdich 1976). In addition, there have been donations from many colleagues between 1970 and 2014 (see Acknowledgments section 3.10). Other samples deposited in several museum collections, particularly those in Leiden, Lisbon, London and Paris (see Acknowledgements section 3.10), and dating back to the 1920s, have been examined. Also, the *Dynamene* specimens (deposited at the Universities of Aveiro and Minho) collected by Pedro Vieira, Henrique Queiroga and Filipe Costa with the help of other colleagues (see Acknowledgments section 3.10) were used to supplement the collections. These samples were collected from the NE Atlantic coasts and the Macaronesian archipelagos of Madeira, Azores and Canary islands, between 2009 and 2015. Samples were taken from rocky shore habitats by scraping of the algal cover and hand picking during low tide.

All specimens of *Dynamene* from DMH's collections have been deposited in the Naturalis Biodiversity Centre, Leiden, The Netherlands under the catalogue numbers: RMNH.CRUS.1. 7517-7616 and 7642-7676. Specimens of *Dynamene* already present in the Leiden collections have the catalogue numbers: RMNH.CRUS.1. 7450-7514.

In most cases the only records considered were of specimens actually seen by the authors, confirmed by molecular tools (unpublished data), or where there were clear diagrams in the literature. Although Holdich (1968c) confirmed many specimens from England and Wales during his surveys, since that time most records of *D. bidentata* have mainly come about as part of the general fauna collected in marine surveys. So, although many records exist in various British databases, particularly those held in the National Biodiversity Network (NBN) Gateway and ERICA (see Acknowledgements section 3.10), the current authors have not tried to track down voucher specimens, but have relied on identifications being correct as only one species of *Dynamene* is indigenous to the British Isles, thus making records more reliable. Details of all the specimens examined in the current study are given in Annex 3.1.

Using information in the databases, maps were constructed of the six *Dynamene* species occurring along the NE Atlantic-Black Sea axis using the software ARCGIS 10.3.

Keys and photographic montages based on the main characters of adult males (stage 8) and females are given in section 3.6 and 3.7 and Figs 3.2, 3.3. To construct the montages, photographs of alcohol preserved specimens were taken with a Dino-Eye Microscope Camera attached to a Wild M5 binocular microscope via a phototube. Images were edited using appropriate software on a computer.

3.5 RESULTS

In this section a generic description of *Dynamene* is given, followed by details of each of the six-species present along the NE Atlantic-Black Sea axis. Keys to and photographs of males and females of each species are given in section 3.6 and 3.7 and Figs 3.2, 3.3. Comparisons are made in the main discussion section 3.8 and overall conclusions are dealt with in the section 3.9. Details of the material examined and geographical coordinates of locations are given in Annexes 3.1 and 3.2.

3.5.1 Dynamene Leach, 1814

Synonymy. *Nesaea* Leach (1814).
Prochonaesea: Hesse (1873).
Sorrentosphaera: Verhoeff (1944).

Diagnosis. Eubranchiate sphaeromatid with body approximately elliptical. Anteriorly, cephalosome separating the bases of the antennules. Eyes set slightly into pereonal tergite 1. Coxal plates of pereonites 1–7 separated from tergites by sutures. The seventh somite is overlapped by the sixth in adult males (stage 8), with the pleura extended postero-laterally into two small processes, which vary in shape according to species. Pleotelson domed or keeled, and terminating in an obvious terminal foramen, which may be enclosed forming a tube. Antennular peduncle articles 1 and 2 dilated and juxtaposed to ventral margins of cephalosome. All pereopods ambulatory. Both rami of pleopods 1-3 bearing margin of plumose setae. Endopods of uropod fused with protopods and juxtaposed to pleotelsonic margin; exopods posteriorly directed. Sexual dimorphism pronounced. Adult male with pereonal tergite 6 longer than those preceding, posterior margin with an elongate, posteriorly directed process either side of the mid-line (the bidentate process). Posterior part of pleotelson with central boss. Penes small, separate. Endopod of pleopod 2 lacking appendix masculina. Female with pereonal tergite 7 similar to those preceding and lacking bidentate process; pleotelson smooth. Ovigerous female with ventral marsupium, formed from four pairs of lamellae, which arise from pereonites 1-4. Mouthparts strongly metamorphosed.

Type species. *Oniscus bidentatus* Adams, 1800

3.5.2 Dynamene bidentata (Adams, 1800)

Restricted synonymy. *Oniscus bidentatus* Adams (1800).

Naesa bidentata: Leach (1815).

Dynamene bidentata: Holdich (1968a, b, c, 1969, 1970, 1971, 1976); Kussakin (1979); Harrison and Ellis (1991).

An extensive synonymy was given by Holdich (1968a, c) for citations prior to 1968.

Material examined. Specimens have been examined from 129 locations in the NE Atlantic, mainly from the British Isles, Channel Islands, France, Spain, Portugal and Morocco – see Annexes 3.1 and 3.2. A number of literature records have been included where the diagrams clearly indicate this species. In addition, there are 76 records from the NBN database.

Key morphological characters. Body convex; in stage 8 males the pleotelsonic boss is large and bilobed, the two halves are separated by a wide v-shaped groove; the arms of the bidentate process taper to a point, and are sparsely rugose dorsally (Fig. 3.2A–B). In stage 7 females the pleotelsonic dome is smoothly rounded in side view and the pleotelsonic foramen is open and flush with the edge of the pleotelson (Fig. 3.3A–B). In populations from Atlantic coasts the smooth outline of the pleotelsonic dome in females and juveniles is key to separating this species from *D. magnitorata* and *D. edwardsi*, where it is keeled in side view. Further details are provided by the scanning electron microscope pictures of the posterior body of a stage 8 male and a stage 7 female in Holdich (1976).

Size. Adult males (stage 8) typically 7.0 × 3.0 mm, although specimens 10 mm in length have been seen; pre-ovigerous females (stage 7) typically 6.0 × 2.9 mm.

Life-history. There are eight life-history stages in both males and females (Holdich 1968b). Sexual dimorphism becomes apparent in stage 6 males with the appearance of a very small bidentate process, this increases in size at the seventh, and is fully developed by the eighth and terminal stage (Figs 3.1D, 3.4–lower row 6–8). This process is absent from juveniles and females (Figs 3.1A-B, 3.3A–M, 3.4–upper row 6–8). Juveniles and females up to and including stage 7 are very similar to each other morphologically. At the moult to stage 8 females become ovigerous and are very similar morphologically between the species. Their mouthparts are strongly metamorphosed, and they die after releasing their broods (Hansen 1905, Holdich 1968b, 1971). Stage 8 males live for two breeding seasons, at least in the British Isles, and remain in their cryptic habitat for the entire period without apparently feeding (Holdich 1971). Those in their second year are recognizable from the growths of algae, and sometimes serpulids, on the pleotelson.

Habitat. All stages can be found on a wide variety of mid- to lower littoral algae, and also in rock pools in the upper littoral zone. Fenwick (pers. comm., July 2016) has found this species commonly amongst lower shore and sublittoral coralline algae in Cornwall, and he has also recorded adults from under large lower shore pebbles. Stage 7 females and stage 8 males move from the algae into cryptic habitats, such as crevices and empty barnacle tests, particularly *Balanus perforatus*, to breed (Holdich, 1970, 1976). Stage 7 females moult into stage 8 females within such a habitat and reach peak numbers in April/May each year (Holdich 1968b).

Colour. Some degree of camouflage in the algal habitat is given by green, yellow and brown 'uniformis' phenotypic varieties, and this is enhanced by the development in some individuals of patterns of white or red, dorsal, non-adaptable chromatophores (Tinturier-Hamelin 1962, 1967, Holdich 1969, Arrontes 2009). In the past some workers have given specific status to the red and green colour varieties, e.g. rubra and viridis (see Holdich 1968c). Adult males are particularly colourful when found amongst red algae on the lower shore, with the margins of the body segments and uropods bordered in orange.

Geographical distribution. The distribution of this species shown in Holdich (1970, 1974) has been extended by the present study. It occurs from the Shetland Islands to Tarfaya in western Morocco and Tenerife and Gran Canaria in the Canary Islands, which are the only two records of the species in Macaronesia (Fig. 3.5A). Within this range *D. bidentata* occurs in the north, northwest (including the outer islands), west and south coasts (as far as the Isle of Wight) of Great Britain, around Northern and Southern Ireland, the Channel Islands, northwest (NW) France, Atlantic Iberian Peninsula and in NW Africa. Arrontes (1991) cites *D. bidentata* as being the most abundant isopod species on shores in northern Spain. It is the only species present in the British Isles (with the exception of a single record of *D. magnitorata* in southern England). It is particularly common in Southwest (SW) England and SW Wales, especially where the large barnacle, *Balanus perforatus* is present. There is one recent record for northeastern England, which may be the result of a stranding, as are records for The Netherlands, where it is not considered indigenous (Holthuis 1956). The closest record to the Mediterranean of *D. bidentata* is Tarifa, in southern Spain (Guerra-García et al. 2011, Izquierdo and Guerra-García 2011, Guerra-García et al. 2012, Torrecilla-Roca and Guerra-García 2012).

Remarks. Maggiore and Fresi (1984) described *D. bidentata* from the Gulf of Naples (publishing descriptions and figures), and several authors (e.g., Castelló and Carballo 2001, Castellanos et al. 2003, Junoy and Castelló 2003) have used Maggiore and Fresi's (1984) observations to justify their findings of *D. bidentata* in the Mediterranean. Yet, examination of the single specimen found by Maggiore and Fresi (1984) showed that it was in fact a *D. magnitorata*. A lot of confusion regarding the identification of D*. bidentata* was caused by Torelli (1930) who figured what she called *D. bidentata* (a stage 8 male and a stage 8 ovigerous female), from the Bay of Naples, Italy. Omer-Cooper and Rawson (1934) used Torelli's figures to illustrate *D. bidentata* from Britain, which was then proliferated in some British identification guides, e.g., Barrett and Yonge (1964), although this has been corrected in more modern guides, e.g., Hayward and Ryland (1995). Pauli (1954) also used Torelli's figures to illustrate *D. bidentata* from the Black Sea. Holdich (1968a) collected material from Naples and decided that Torelli's figures were in fact of a new species, commonly found in the Bay of Naples, which he named *D. torelliae* Holdich, 1968. However, Kussakin (1979) decided that *D. torelliae* was in fact synonymous with *D. bicolor* (Rathke, 1837). This species was in fact unknown to Holdich at the time of his studies. Databases we have consulted indicate that *D. bidentata* commonly occurs around Northern and Southern Ireland. However, we could only find one modern published record, i.e., de Grave and Holmes (1998) from Lough Hyne in County Cork. Unlike most other isopods, stage 8 male *Dynamene bidentata* do not have appendix masculina on the endopods of the second pair of pleopods, this is also the case in the other *Dynamene* species. This phenomenon has also been noted by Messana (2004) in *Sphaeroma terebrans* Bate, 1866. It is very difficult to observe mating in *Dynamene* due to the cryptic habitat of the adults. It is probable that sperm are released directly into the marsupium as the eggs are laid.

3.5.3 *Dynamene bicolor (Rathke, 1837)*

Restricted synonymy. *Campecopea bicolor*: Rathke (1837).
Dynamene bidentata: Torelli (1930); Omer-Cooper and Rawson (1934); Pauli (1954); Holthuis (1956); Barrett and Yonge (1964); [not *D. bidentata* of Adams (1800)].
Dynamene torelliae: Holdich (1968, 1970).
Dynamene bicolor: Kussakin (1979); Maggiore and Fresi (1984).

Material examined. Specimens have been examined from 48 locations in 12 countries in the Mediterranean and Black Seas - see Annexes 3.1 and 3.2. A number of literature records have been included where the diagrams clearly indicate this species.

Key morphological characters. In stage 8 males the pleotelsonic boss is comprised of two right-angled triangular structures separated by a deep groove (however, the boss may be very low lying in some specimens, e.g., those from the Black Sea); the arms of bidentate process taper to a point and are rugose dorsally (Fig. 3.2J–K). In stage 7 females the pleotelsonic dome is keeled in side view and the pleotelsonic foramen is flush with the edge of the pleotelson (Fig. 3.3H, I). The females of this species are very difficult to separate from those of *D. magnitorata*. Maggiore and Fresi (1984) provide a complete description of *D. bicolor*.

Size. Adult males (stage 8) typically 3.5 × 1.5 mm, pre-ovigerous females (stage 7) typically 3.0 × 1.3 mm.

Life-history. Nothing is known of the life-history, other than the fact that sexual dimorphism occurs with males developing the bidentate process characteristic of the genus.

Habitat. Juveniles are usually found in shallow water on a variety of algae down to 3.0 m and adults in empty *Balanus* tests, in mussel beds, in rock crevices, within sponges, and under rocks throughout the Mediterranean. However, occasionally they have been found in deeper water, e.g., off the island of Chios (Greece) specimens were collected from *Cystoseira* at depths from 0.5 – 30 m (see Annex 3.1).

Colour. As with *D. bidentata*, some degree of camouflage in the algal habitat is given by yellow or dull green 'uniformis' phenotypic varieties, and this is enhanced by the development in some individuals of patterns of white or red, dorsal, non-adaptable chromatophores (Holdich 1969).

Geographical distribution. The distribution of this species shown in Holdich (1970) has been extended by the present study. It is the most commonly recorded *Dynamene* species in the Mediterranean, occurring from the Balearic Islands in the west to the coast of Israel in the east, although there are only a few records for the North African coast (Fig. 3.5B). It has been frequently recorded around the Greek islands and mainland coast of both Greece and Turkey. The most northerly record is for Croatia in the Aegean Sea. It has also been recorded for a number of countries around the Black Sea (Bulgaria, Romania, Turkey and Georgia; Fig. 3.5B). Most records in the literature refer to *D. torelliae*, which is now considered synonymous with *D. bicolor*.

Remarks. Many records exist, both published and unpublished, for *Dynamene bicolor* (usually as '*D. torelliae*') in the Mediterranean Sea, particularly from the coasts of Spain, France, Italy and Greece (Holdich 1970, Bakir et al. 2014). However, its presence in Egypt and Israel was unreported until now. Previous observations indicated its presence in the Black Sea (Kussakin

1979), where it was thought to be the only *Dynamene* species present (Gönlügür-Demirci and Katağan 2004). On comparing specimens from the Black and Mediterranean Seas the current authors have accepted the decision of Kussakin (1979) that *D. torelliae* and *D. bicolor* are synonymous. However, it is clear that some of the specimens from the Black Sea have a reduced pleotelsonic boss, and the two may eventually turn out to be separate species when more material is examined. Kirkim et al. (2006) commented on the form of the pleotelsonic boss, stating that this can vary from two small projections to a well-formed boss in specimens of '*D. torelliae*' from the Aegean Sea. Rathke's (1837) drawings of *D. bicolor* show the posterior halves of a female and a stage 7 male. The male has two joined hemispherical pleotelsonic bosses, which are similar to those found in the same stage of '*D. torelliae*' and unlike that of *D. edwardsi* the other species in the region, which is single.

3.5.4 Dynamene bifida Torelli, 1930

Restricted synonymy. *Dynamene bifida*: Torelli (1930).
Dynamene bifida: Holdich (1968, 1970).

Material examined. Specimens were examined from seven locations in Spain, Greece, France, Italy and Turkey in the Mediterranean – see Annexes 3.1 and 3.2. A number of literature records have been included where the diagrams clearly indicate this species.

Key morphological characters. In stage 8 males each arm of the bidentate process is large, tapering and with a well-developed, downwardly-directed accessory process a quarter of the way from the apex; the pleotelsonic boss is very small with raised pointed corners (Fig. 3.2G–H). In stage 7 females the pleotelsonic dome is smoothly rounded in side view and the pleotelsonic foramen is at the end of short tube (Fig. 3.3L–M).

Size. Adult males (stage 8) typically 5.0 × 3.0 mm, although a specimen of 7.0 mm length has been seen; pre-ovigerous females (stage 7) typically 4.0 × 2.0 mm.

Life-history. Nothing is known of the life-history of this species, other than the fact that sexual dimorphism occurs with males developing the bidentate process characteristic of the genus.

Habitat. Adults, including stage 8 females, were found among *Hydroides unicata* colonies and other cryptic habitats in the Bay of Naples (Torelli 1930, Holdich 1970). Ledoyer (1962) recorded it from *Ulva lactuca* at Endoume, southern France, and Holthuis (unpublished records) from rocky shores amongst algae at 0.0–1.0 m at Banyuls-sur-Mer. The latter record included stage 8 females.

Colour. All specimens seen were a pale, sandy yellow. No polychromatism was observed.

Geographical distribution. The distribution of this species shown in Holdich (1970) has been extended by the present study. It has a widespread distribution in the Mediterranean stretching from southern Spain to Turkey (Fig. 3.5C).

Remarks. Originally described by Torelli (1930) from the Bay of Naples, males of this distinctive, and sometimes large species, has been infrequently recorded, and females even less so. The accessory process on each arm of the bidentate process is similar to that found in the Australian species, *D. ramuscula* (Holdich and Harrison 1980). The fact that ovigerous females were found amongst shallow-water algae raises questions about the life-history of this species, although in the Bay of Naples this stage has been recorded with males in more protective habitats.

3.5.5 Dynamene edwardsi (Lucas, 1849)

Restricted synonymy. *Naesa edwardsi*: Lucas (1849).
Dynamene hanseni: Monod (1923).
Dynamene edwardsi: Holdich (1968a, 1970); Harrison (1982).
Dynamene bidentata: Picker and Griffiths (2011).
An extensive synonymy was given by Holdich (1968a, c) for citations prior to 1968.

Material examined. Specimens were examined from 89 locations in NE Atlantic, Mediterranean, Adriatic, Aegean and Red Seas – see Annexes 3.1 and 3.2. A number of literature records, e.g., the Suez Canal, have been included where the diagrams clearly indicate this species.

Key morphological characters. Body convex; in stage 8 males the apices of arms of the bidentate process are swollen, each with a short, downwardly-directed spur; the pleotelsonic boss is plate-like with two forward-facing pegs; the body exhibits various degree of setation (Fig. 3.2E–F), e.g., specimens examined from the Balearic Islands (Spain) and the island of Chios (Greece) are somewhat different from other *D. edwardsi* seen by us in being very hirsute, with a pronounced developing boss and respiratory tube in the stage 7 males. In stage 7 females the pleotelsonic dome is keeled in side view, with a median protuberance; the pleotelsonic foramen is at the end of a short tube (Fig. 3.3E–G). Further details are provided by the scanning electron microscope pictures of the posterior body of a stage 8 male and a stage 7 female in Holdich (1976).

Size. Adult males (Stage 8) typically 5.5 × 2.25 mm; pre-ovigerous females (stage 7) typically 3.0 × 1.1 mm, specimens of 4.4 × 2.3 mm have been seen from the Venice Lagoon, Italy.

Life-history. Nothing is known of the life-history of this species, other than the fact that sexual dimorphism occurs with males developing the bidentate process characteristic of the genus.

Habitat. Juveniles and adults have been found amongst a variety brown, green and red algae in the littoral and sublittoral zones, sometimes in conjunction with *D. bicolor* in the Mediterranean, and with *D. bidentata* and *D. magnitorata* on Atlantic coasts. Adults have also been recorded from amongst mussels and tube worm colonies and barnacle tests in the Bay of Naples (Torelli 1930, Holdich 1970), and elsewhere in the Mediterranean (e.g., Rivosecchi 1961, Bellan-Santini 1962). It has been found associated with encrusting matter on solid surfaces in some harbours and canals. On occasions, it has been found amongst the 'trottoir' on steep-sided cliffs as deep as 10 m. Monod (1932) recorded it from coralline and fucoid algae on the coast of NW Africa. In the Azores, adults have been recorded from empty *Chthamalus stellatus* tests attached to lower shore cobbles, along with *Campecopea lusitanica*. In West Portugal (Buarcos) it is present with *D. bidentata* and *D. magnitorata*. However, while *D. bidentata* adults were present in barnacles, no *D. edwardsi* were found inside barnacles, only among intertidal algae and on a few 'small' algae in shaded crevices at 0-1 m. Also, they were not present among mussels. Unusually, adults, including stage 8 females, were found in upper shore sandstone crevices, along with *Campecopea hirsuta*, in southern Portugal.

Colour. The general body colour is a dull grey-green, individuals sometimes exhibit polychromatism caused by patterns of white, dorsal, non-adaptable chromatophores as seen in some of the other species (Holdich 1969).

Geographical distribution. The distribution of this species shown in Holdich (1970) has been extended by the present study. It is the most meridional of the Atlantic species, occurring from Galicia in NW Spain to Nouadhibou in Mauritania (Fig. 3.5D). This is the currently known southern limit of *Dynamene* species of the NE Atlantic-Black Sea axis. It is widespread in the Macaronesian islands and in the eastern and western Mediterranean (Fig. 3.5D). The most northerly record comes from the Venice Lagoon in the Adriatic Sea. It is also the only *Dynamene* species recorded from the Red Sea, in the Gulf of Aqaba (Fig. 3.5D). Glynn (1972) recorded a species that is clearly *D. edwardsi* from the Suez Canal. Picker and Griffiths (2011) have recorded this species (as *D. bidentata*) from South Africa.

Remarks. *Dynamene edwardsi* occupies a wide vertical range in the littoral zone on NE Atlantic shores, and from the littoral zone down to 10 m in the Mediterranean. In recent field work, it was found to be very abundant in the Canary Islands and Madeira archipelago, whereas *D.*

magnitorata was more common in the Azores and *D. edwardsi* rare. It is the most southerly of the *Dynamene* species extending down the West African coast to Mauritania and the only record for tropical waters. Glynn (1972) suggested that *D. edwardsi* has migrated from the Mediterranean throughout the whole length of the canal. Our study has shown that it has now reached the Gulf of Aqaba in the Red Sea The records for the Suez Canal and Red Sea are interesting as they show movement from the Mediterranean Sea into the Red Sea, whilst many marine species are moving in the opposite direction (Galil et al. 2014). No *Dynamene* species have yet been recorded from the Indian Ocean (Schotte and Kensley 2005). However, a stage 8 male has been recorded from Port Elizabeth harbour in South Africa by Picker and Griffiths (2011). They suggest that it may have been introduced as a fouling organism or in ballast water. It is known that this species can be transported amongst fouling organisms on ships, as evidenced by the finding a stage 8 male on a ship in Tangiers harbour (Morocco) (see Annex 3.1). This species is variable in its morphology and particularly in the degree of hirsuteness. It may be that some of the specimens collected from the Balearic and Greek islands are in fact a new species, but more material is needed to prove this. Ideally, a molecular genetic analysis needs to be carried out on Mediterranean and Adriatic specimens. Such a technique applied to specimens from some NE Atlantic coasts and Macaronesian islands has shown that a number of cryptic species may be present (Vieira et al. 2015, chapter 4 in this thesis).

3.5.6 Dynamene magnitorata Holdich, 1968

Restricted synonymy. *Dynamene magnitorata*: Holdich (1968).
Dynamene bidentata: Monod (1932); Maggiore and Fresi (1984).
Dynamene magnitorata: Holdich (1968a, 1970, 1976).

Material examined. Specimens were examined from 52 locations in the NE Atlantic, and four countries in the Mediterranean - see Annexes 3.1 and 3.2. A number of literature records have been included where the diagrams clearly indicate this species.

Key morphological characters. Body convex; in stage 8 males the pleotelsonic boss is large, bilobed, with the two halves separated by a narrow groove; the arms of the bidentate process are of similar width along their lengths and are dorsally tuberculate (Holdich 1976, fig. 3A, B; Fig. 3.2C–D in this section). In stage 7 females the pleotelsonic dome is keeled in side view and the pleotelsonic foramen is flush with the edge of the pleotelson (Fig. 3.3C–D). Further details are provided by the scanning electron microscope pictures of the posterior body of a stage 8 male and a stage 7 female in Holdich (1976). The females of this species are very difficult to separate from those of *D. bicolor*.

Size. Adult males (stage 8) typically 4.25 × 2.25 mm, pre-ovigerous females (stage 7) typically 4.0 × 2.0 mm.

Life-history. A comparison of the life-histories of *D. bidentata* and *D. magnitorata* from two Atlantic coast locations was made by Holdich (1976). Only a limited number of *D. magnitorata* specimens were available but it showed that this species has a similar sequence of seasonal events (see description for *D. bidentata*). However, whereas *D. bidentata* stage 8 males live for two breeding seasons, those of *D. magnitorata* may only live for one.

Habitat. A mid- to lower littoral and shallow sublittoral species, although sometimes recorded from deeper water. Its range occasionally overlaps that of *D. bidentata*. Juveniles are found associated with a wide range of littoral and shallow water algae, particularly *Corallina sp.*, *Rhodomenia palmata*, *Chondrus cripspus* and *Gigartina stellata*. Adults have been found in empty tests of *Balanus crenatus*, amongst ascidians, and in channels within sponges (including those associated with eel grass beds). In the Roscoff region (northern France) adults were frequently found within the encrusting sponge, *Halichondria sp.* In the Azores (São Miguel island) adults have been found sublittorally in the empty tests of *Megabalanus azoricus*, as well as intertidally among algae on the islands of Terceira, São Miguel and Santa Maria. On Fuerteventura (Canary Islands) adult males were caught using a surface dip net. In the Chafarinas Islands off Mediterranean Morocco they have been recorded from 0.0 m down to 20.0 m on a variety of algae. Like *Dynamene bidentata* (Harvey et al. 1973), *D. magnitorata* adults were found to have a tolerance to high air temperatures, i.e., 38° C (Holdich 1976). However, survival at 5° C was much lower for *D. magnitorata* compared to *D. bidentata* (Holdich 1976) and this may be the reason it has not colonized more northerly regions.

Colour. Individuals exhibit a wide variety of colours, often matching the colour of their background, the predominant colours being coralline-pink and brown, rather than the greens and yellows seen in *D. bidentata*. Individuals sometimes exhibit polychromatism caused by white, dorsal, non-adaptable chromatophores, as seen some other species (Holdich 1969, 1976).

Geographical distribution. The distribution of this species shown in Holdich (1970) has been extended by the present study. It has been recorded from southern England (a single specimen only that may be the result of a stranding), the Channel Islands, around the coasts of Brittany, the Atlantic Iberian Peninsula and NW Africa, the islands of the Azores, Canary Islands and

Madeira in the Macaronesian archipelagos, and in the Mediterranean along the European and African coasts, and also Egypt (Fig. 3.5E).

Remarks. Almost all the *Dynamene* specimens found in the Azores during recent field work belonged to *D. magnitorata*. However, *Dynamene* was less prevalent in the benthic community when comparing with Canaries and Portugal (pers. obs., unpublished data). Maggiore and Fresi (1984) described *D. bidentata* from the Bay of Naples, but in fact examination of the specimen showed it to be a male *D. magnitorata*. If the author's had compared an actual *D. bidentata* with their specimen then they would have realized this, particular as it is so much smaller than any known *D. bidentata* specimen. *Dynamene magnitorata* has only rarely been recorded in the Mediterranean, i.e. twice in Spain, and once in each of Egypt, Italy, Monaco and Tunisia, although it was found to be common on the Chafarinas Islands off Morocco (Castellanos et al. 2003) (see Annex 3.1).

3.5.7 Dynamene tubicauda Holdich, 1968

Restricted synonymy. *Dynamene tubicauda* Holdich (1968).
Dynamene tubicauda: Holdich (1968a, 1970); Lombardo (1984); Borg et al. (2006).

Material examined. Specimens were examined from six Italian locations in the Bay of Naples and off the island of Elba, and one location off Malta - see Annexes 3.1 and 3.2. A number of literature records from Sicily have been included as the diagrams clearly indicate this species (Lombardo 1984).

Key morphological characters. The morphology of this species is unique amongst the known *Dynamene* species - in stage 8 males the pereon length and width are similar; the epimera and front of the head form a shelf; the antennular peduncle is expanded; there are two widely separated, peg-like pleotelsonic bosses; and the pleotelsonic foramen is at the end of a ventrally-closed tube (Fig. 3.2I). In stage 7 females the body is also flattened with the epimera forming a shelf round the body; the pleotelsonic foramen is at the end of a well-developed tube (Fig. 3.3J–K).

Size. Adult males (stage 8) typically 3.0 × 2.0 mm, pre-ovigerous females (stage 7) typically 2.5 × 2.0 mm.

Life-history. Nothing is known of the life-history of this species, other than the fact that sexual dimorphism occurs with males developing the bidentate process characteristic of the genus.

Holdich (1968) only recorded males, but both sexes have been recorded in the present study. Lombardo (1984) was the first to describe the adult female.

Habitat. This species has been found between 2-30 m amongst algae in muddy/ sandy and coralline habitats, rock scrapings, freely swimming at 30 m, and also in sea grass meadows (Lombardo 1984, Borg et al. 2006).

Colour. Pale yellow. No polychromatism was observed.

Geographical distribution. The distribution of this species shown in Holdich (1970) has been extended by the present study. However, it appears to be restricted to the eastern Mediterranean, having only been recorded off the west coast of Italy (Holdich 1968), Sicily (Lombardo 1984) and Malta (Borg et al. 2006). The most northerly record is for the island of Elba and the most southerly is off Malta (Fig. 3.5F).

Remarks. The distribution of this species is the most restricted of all the *Dynamene* species along the NE Atlantic-Black Sea axis. Considering the large number of samples examined during this study this restricted distribution is most likely real. Its unusual flattened shape and the position of the pleotelsonic foramen at the end of a tube, even in adult males, may be an adaptation to inhabiting sediments.

3.5.8 Dynamene sp.

Material examined. Two stage 8 males. See Annexes 3.1 and 3.2.

Key morphological characters. The bilobed pleotelsonic boss has a posteriorlydirected spine not seen in any other stage 8 males. The uropodal exopod is wide and the body markedly hirsute.

Habitat. Known only from the stomach contents of a black scorpionfish *Scorpaena porcus*.

Geographical distribution. Known only known from NW Aegean Sea.

Remarks. Only two specimens have been found, both stage 8 males, and both from the stomach contents of a black scorpionfish, *Scorpaena porcus*. This could well be a new species of *Dynamene*, but more material is needed to confirm this. It may even be related to the hirsute specimens found in the Balearic Islands and the Greek island of Chios. The fish is known to be a bottom feeder in the Black Sea, close to where the specimen came from, which was in the NW Aegean, where it occurs at 20–40 m depth (Başçïnar and Sağlam 2009). Rafrafi-Nouira et al. (2016) examined the diet of *S. porcus* from waters off the coast of Tunisia, but the only isopods they found were listed as unidentified.

3.6 KEY TO THE STAGE 8 MALES OF *DYNAMENE* SPP. ALONG THE NE ATLANTIC-BLACK SEA AXIS

1. With a bidentate process arising from posterior margin of pereonite 6 - sub-adult and adult ♂ *Dynamene* (Figs 1, 2, 3) …………………………………………………………………………………………………… **2**

- Without bidentate arising from posterior margin of pereonite 6 ……………………………………………………**juvenile** and ♀ ***Dynamene*** (see key to females, section 3.7)

2. With large bidentate process arising from posterior margin of pereonite 6: adult ♂ *Dynamene* (Figs 1D, 2A-K) …… **3**

- With small or medium bidentate process arising from posterior margin of pereonite 6 …………………………………………………………………… **sub-adult ♂ *Dynamene*** (Fig. 4–lower row 6-7)

3. Pereon length and width similar; epimera and front of head forming a shelf; antennular peduncle expanded; two widely separated, peg-like pleotelsonic bosses; pleotelsonic foramen at end of a ventrally-closed tube (Fig. 2J) ……………………………………………………………… ***D. tubicauda***

- Pereon length greater than width, pleura and front of head not forming a shelf; antennular peduncle not expanded; pleotelsonic boss single …………………………………………………………………… **4**

4. Bidentate processes large, tapering and with a well-developed, downwardly-directed accessory process a quarter of the way from the apex; pleotelsonic boss very small with raised pointed corners (Fig. 2G-H) ……………………………………………………………………………………… ***D. bifida***

- Bidentate processes without well-developed accessory process; pleotelsonic boss well-developed, without raised pointed corners …………………………………………………………………………… **5**

5. Apices of bidentate processes swollen, each with short, downwardly-directed spur; pleotelsonic boss plate-like with two forward-facing pegs; body exhibiting various degree of setation, sometimes hirsute (Fig. 2E-F) ……………………………………………………………………… ***D. edwardsi***

- Bidentate processes without swollen apices or spurs, pleotelsonic boss not plate-like ………… **6**

6. Pleotelsonic boss comprised of two right-angled triangular structures separated by a deep groove (however, the boss may be very low lying in some specimens, e.g. those from the Black Sea); arms of bidentate process tapering to point, rugose dorsally (Fig. 2I, K) …… ***D. bicolor***

- Pleotelsonic boss comprising two hemispherical structures separated by a wide or a narrow groove, joined at the base ………………………………………………………………………………………… **7**

7. Pleotelsonic boss large, bilobed, two halves separated by a narrow groove; arms of bidentate process of similar width with along length, dorsally tuberculate (Fig. 2C-D) ……… ***D. magnitorata***

- Pleotelsonic boss large, bilobed, two halves separated by a wide v-shaped groove; arms of bidentate process tapering to point, sparsely rugose dorsally (Fig. 2A-B)……………… ***D. bidentata***

3.7 KEY TO STAGE 7 FEMALES AND JUVENILES OF *DYNAMENE* SPP. ALONG THE NE ATLANTIC-BLACK SEA AXIS

1. Sphaeromatid without process arising from the posterior margin of the pereonite 6, and with simple pleotelsonic foramen; with or without dorsal tuberculation …………………………………………………………………………………….**juvenile** and ♀♀ ***Campecopea*, *Dynamene*** and ***Ischyromene***

- Without tuberculation on surface of posterior pereonites, pleonites and/or pleotelsonic dome (Figs 1A, B; 3A-M, 4-upper row 6-8) ………………….. **juvenile** and ♀♀ ***Dynamene*** ……………………….**2**

2. Body flattened, epimera flattened to form a shelf round the body; pleotelsonic foramen at end of a well-developed tube (Fig. 3J-K) …………………………………………………………… ***D. tubicauda***

- Body convex, pleura not flattened to form shelf round body; pleotelsonic foramen either flush with edge of pleotelson or at end of a short tube …………………………………………………………….. **3**

3. Pleotelsonic dome smoothly rounded in side view, pleotelsonic foramen open and flush with edge of pleotelson or at end of short tube …………………………………………………………………………. **4**

- Pleotelsonic dome keeled in side view, with or without a median protuberance ………………….. **5**

4.Pleotelsonic foramen open and flush with edge of pleotelson (Fig. 3A-B) ……. ***D. bidentata***

- Pleotelsonic foramen at end of short tube (Fig. 3L-M) ……………………………………………… ***D. bifida***

5. Pleotelsonic dome keeled in side view, pleotelsonic foramen flush with edge of pleotelson ……………………………………………………………… Fig. 3C-D - ***D. magnitorata*** and Fig. 3H, I - ***D. bicolor***

- Pleotelsonic dome keeled in side view, with median protuberance; pleotelsonic foramen at end of short tube (Fig. 3E, F, G) ……………………………………………………………………………… ***D. edwardsi***

Notes:

When identifying *Dynamene* juveniles and ♀♀ care must be taken not to confuse them with those of *Ischyromene lacazei* Racovitza, 1908 and *Campecopea lusitanica* (Nolting, Reboreda and Wägele, 2008). If in doubt, then consult Schüller and Wägele (2005) and Bruce and Holdich (2002) respectively.

Except for size, juveniles are very similar to stage 7 females. *Dynamene magnitorata* and *D. bicolor* females are very similar and cannot be keyed out, except on size – on average *D. magnitorata* tends to be larger (see section 3.5). Ovigerous females are very similar between species and it is not possible to create a key for them. They are characterized by metamorphosed mouthparts, ventral marsupium, wide body and a pleotelsonic foramen that is more upturned and which gradually becomes closed posteriorly (Fig. 4–upper row 8).

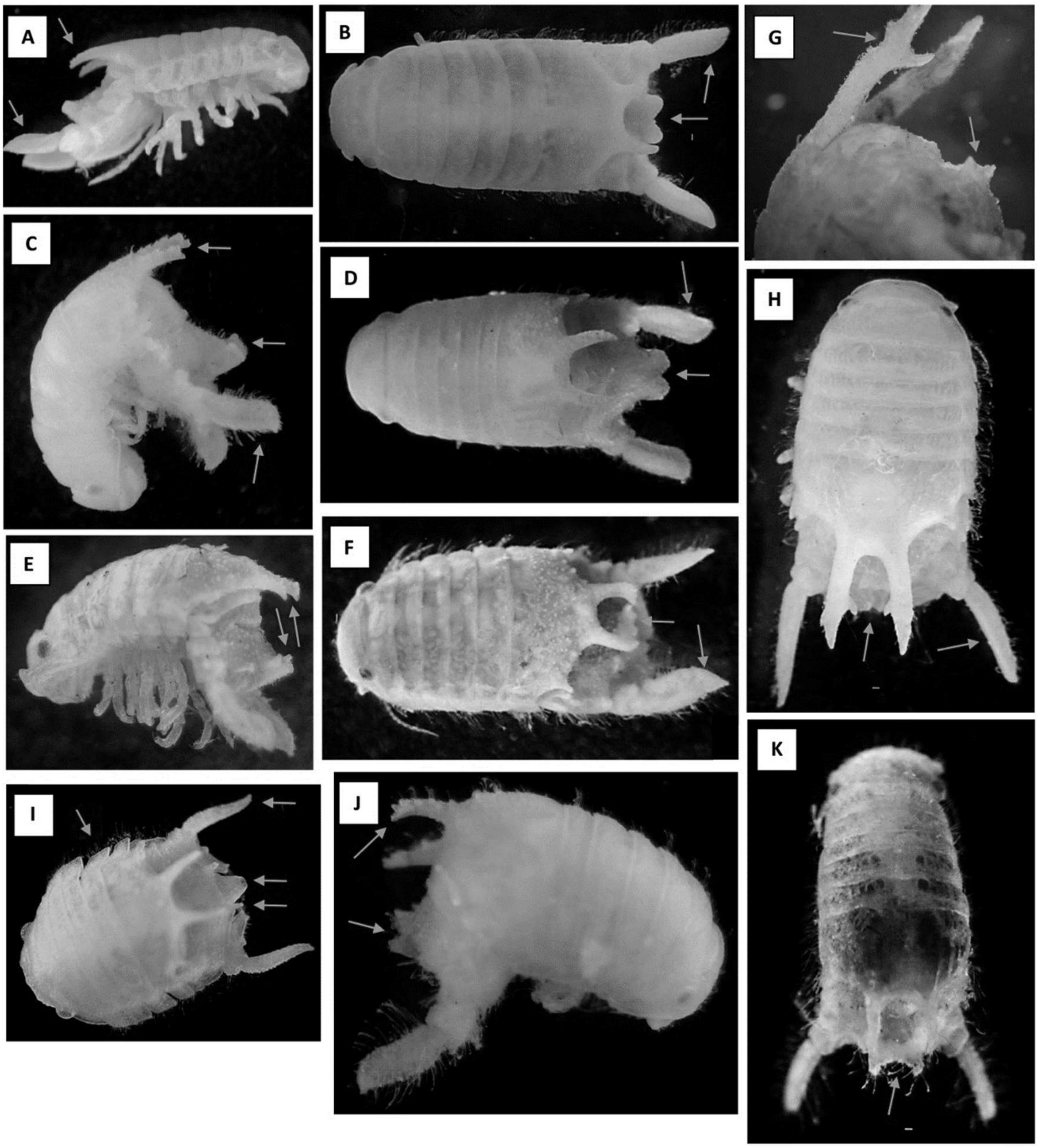

Figure 3.2. Main features of adult males (stage 8) of the NE Atlantic-Black Sea axis *Dynamene spp.* **A**, **B** – *D. bidentata* (South Wales). Arrows indicate shape of the bidentate process (A), uropods (A, B) and pleotelsonic boss (B). **C**, **D** - *D. magnitorata* (Roscoff, France). Arrows indicate shape of the bidentate process (C), the uropods (C, D) and the pleotelsonic boss (C, D). Note the difference in the shape of the boss and the ends of the arms of the bidentate process to those of *D. bidentata*. **E**, **F** – *D. edwardsi* (E – Canaries, F - Azores). Arrows indicate shape of the bidentate process (E, F), uropods (F) and pleotelsonic boss (E, F). Specimen in E shows relatively little dorso-lateral setation, whilst that in F is hirsute. Note the differences in the shape of the boss and the tips of the arms of the bidentate process compared to those of *D. bidentata* and *D. magnitorata*. **G**, **H** - *D. bifida* (France, Mediterranean). Arrows indicate shape of the bidentate process (G, H), uropodal exopod (H) and pleotelsonic boss (G). Note the large accessory process on each arm of the bidentate process, the small sessile pleotelsonic boss and the long narrow uropodal exopods. **I** – *D. tubicauda* (Bay of Naples, Italy). Arrows indicate the unique body shape, tubular respiratory channel, peg-like pleotelsonic bosses, and the curved uropodal exopods. **J**, **K** - *D. bicolor* (Bay of Naples, Italy). Arrows indicate shape of the bidentate process (J), and pleotelsonic boss (J, K). Note in particular, the rugose nature of the dorsal surface of the bidentate arms and the triangular shape of each half of the boss – in specimens from the Black Sea the boss is of a similar shape but much less prominent.

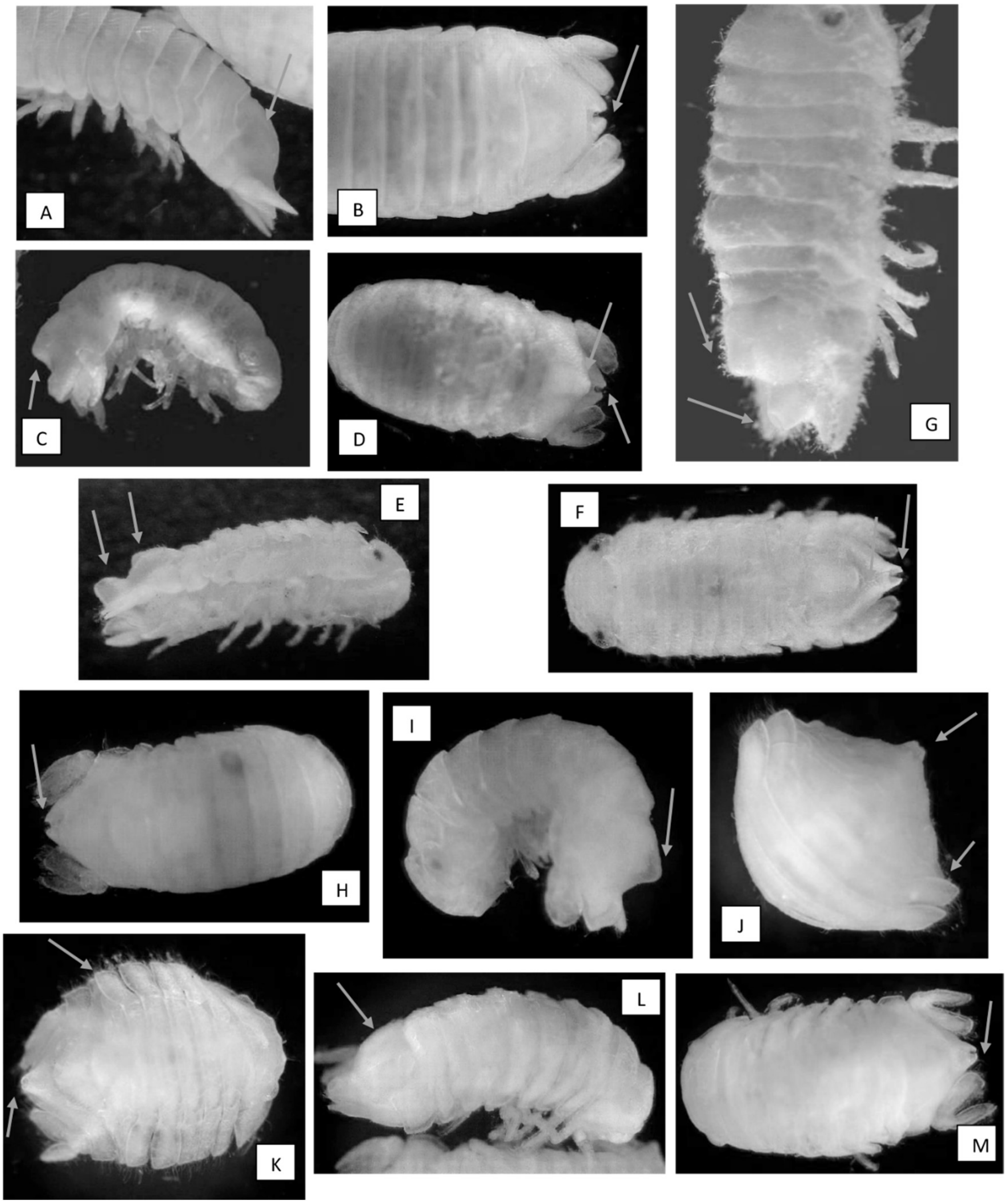

Figure 3.3. Main features of females and juveniles of the NE Atlantic-Black Sea axis *Dynamene spp.*
A, **B** - *Dynamene bidentata* (South Wales). Arrows indicate smooth outline of pleotelsonic dome (A) and non-tubular pleotelsonic foramen (B). **C**, **D** - *Dynamene magnitorata* (Roscoff, France). Arrows indicate angular outline of pleotelsonic dome (C), posterior extension of pleotelsonic keel and non-tubular pleotelsonic foramen (D). **E**, **F**, **G** - *Dynamene edwardsi* (Italy). Arrows indicate angular outline of pleotelsonic dome (E) with central bulge (E, F, G) and tubular pleotelsonic foramen. (E and F from Naples, Italy; G - hirsute female from the Venice Lagoon, Italy). **H**, **I** - *Dynamene bicolor* (Naples, Italy). Arrows indicate angular outline of pleotelsonic dome (I) and non-tubular pleotelsonic foramen (H). **J**, **K** - *Dynamene tubicauda* (Ischia, Italy). Arrows indicate flattened epimera surrounding body that give this species a unique body shape (J, K) and the tubular pleotelsonic foramen (J, K). **L**, **M**. *Dynamene bifida* (Ischia, Italy). Arrows indicate smooth outline to pleotelsonic dome (L) and pleotelsonic foramen at end of short tube (M).

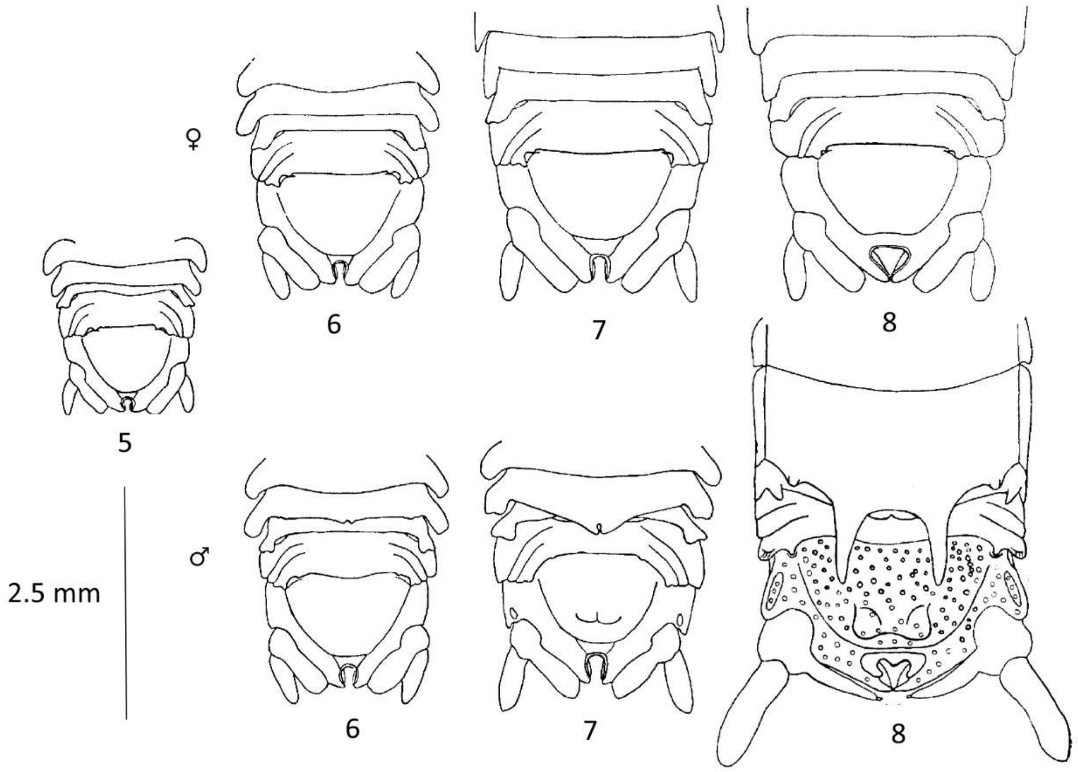

Figure 3.4. Dorsal views of the posterior halves of the bodies of various life history stages (5-8) of *Dynamene bidentata*.
5 – juvenile. **Upper row** – female stages 6, 7 and 8 (ovigerous). **Lower row** – male stages 6, 7 and 8.
Adapted from Holdich 1968b.

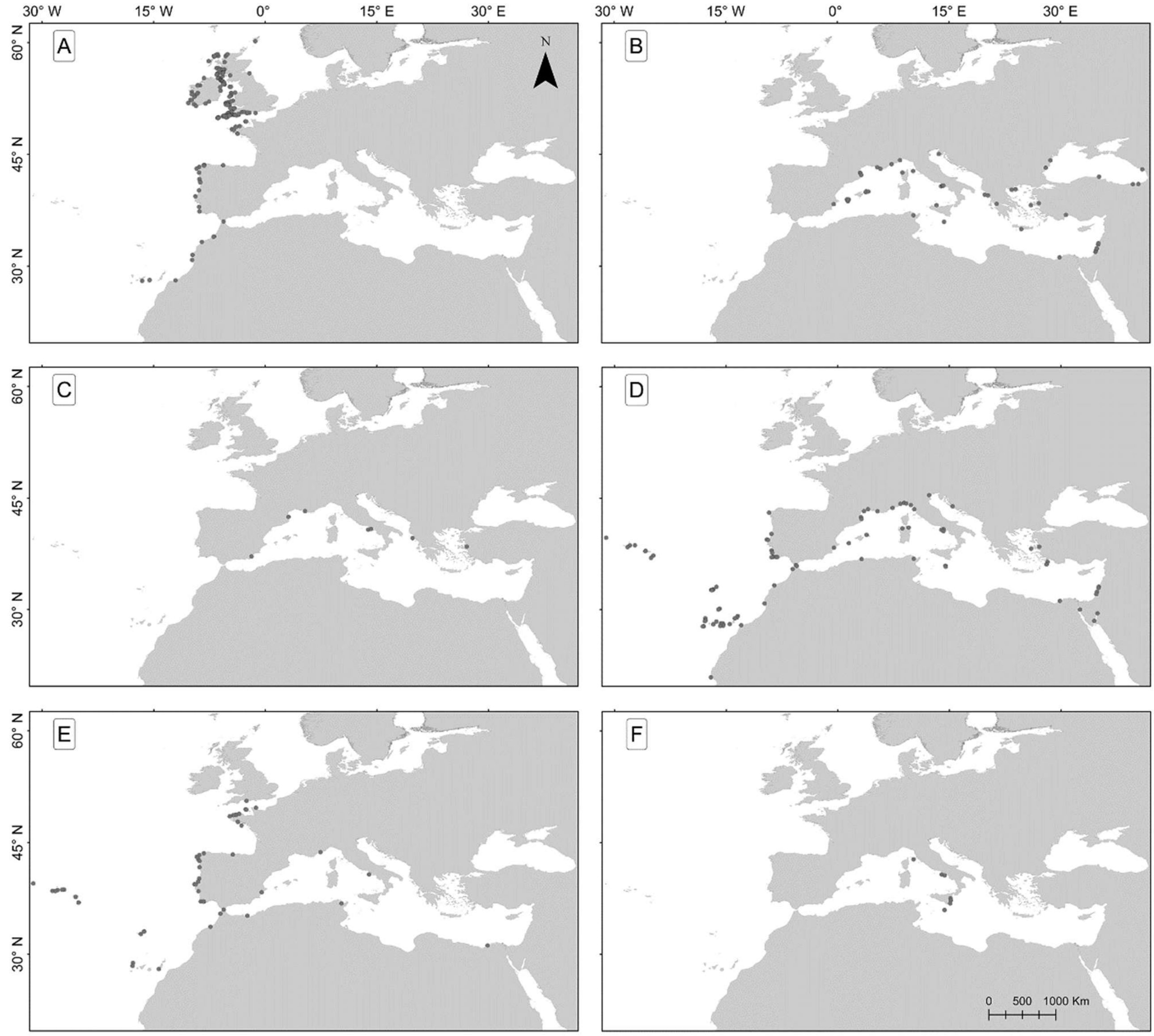

Figure 3.5. Distribution of *Dynamene* species along the NE Atlantic-Black Sea axis based on material validated during the present study.

A - *Dynamene bidentata*. **B** – *Dynamene bicolor*. **C** –*Dynamene bifida*. **D** –*Dynamene edwardsi*. **E** – *Dynamene magnitorata*. **F** - *Dynamene tubicauda*.

3.8 DISCUSSION

Three species of *Dynamene* occur on the shores of the continent and islands of the NE Atlantic Ocean (*D. bidentata, D. magnitorata* and *D. edwardsi*). In recent field work, no *Dynamene* specimens were collected in Scandinavia or Iceland (pers. obs., unpublished data). This is probably due to the fact that members of this genus may not be able to tolerate cold water and weather. For example, studies by Holdich (1968b, c, 1970) were meant to be carried out on the Gower Peninsula in South Wales, but the severe and long-lasting winter of 1962-1963 decimated the populations, as well as those of *Balanus perforatus*, and the study site was relocated to western Pembrokshire in 1964 (SW Wales), where the populations of both were unaffected. Moyse and Nelson-Smith (1964) showed that when sea and air temperatures were below 5°C for a long period, viable broods were not produced by females of *D. bidentata*. Moreover, with lower average air temperatures, populations of *Dynamene* must restrict their growth phases to fewer months of the year (Holdich 1976). The previously known northerly limit of *Dynamene* was Ardrossan in the west of Scotland (Holdich 1970). In this study, we extended the northern range of this genus to Clatholl in the north of Scotland, and recent surveys by British workers have shown that it also occurs in the Shetland Islands north of Scotland. There are a number of records for the Western Isles off Scotland (Fig. 3.5A) that are warmed by the Gulf Stream. However, one record is shown from north-eastern England (Fig. 3.5A), which tends to be colder than the west coast due to a lack of influence from the Gulf Stream, but it is not known if a permanent population exists there. It may represent a stranding from a population elsewhere. Holthuis (1956) recorded *D. bidentata* from the other side of the North Sea in The Netherlands. He was of the opinion that it was not indigenous there, but was occasionally stranded with flotsam and jetsam. There are old records in the literature of *D. bidentata* for eastern Scotland (Scott 1899) and also for south-east England (Butler 1878), but none (other than the record mentioned above) have come to light in the last few decades.

Dynamene bidentata is the only species present in the British Isles (Holdich 1969, 1970; Holdich and Lincoln 1974). Although in our databases there is a record of *Dynamene magnitorata* in southern England, we believe this probably does not represent an actual permanently established population. However, *D. magnitorata* is common on Guernsey (Channel Islands), which is not that far geographically from the south of England. According to Holdich (1970), and confirmed by the current study, *D. bidentata* is distributed along the Atlantic coasts of Europe from the northern British Isles to Portugal. Barrois (1888) recorded *D. bidentata* from the Azores, and it is listed as being present there by Ferraz et al. (2004) and

Borges et al. (2010). Rodrigues (1990) recorded it as being common on the island of Flores. However, none of the specimens we have examined from the Azores have been of this species, and the records may well have been *D. magnitorata* or *D. edwardsi*. Pereira et al. (2006), Guerra-García et al. (2011), Izquierdo and Guerra-García (2011), Guerra-García et al. (2012) and Torrecilla-Roca and Guerra-García (2012) recorded it from southern Portugal and southwest Spain, and indicated that these regions as the most meridional locations where this species was collected. Our observations extend the distribution of *D. bidentata* further south, i.e., Akhfenir in Morocco and Tenerife and Gran Canaria in the Canary Islands. Because *D. bidentata* can survive at temperatures up to 38° C (Harvey et al. 1973), it is possible that this species occurs further south.

During the current study the authors examined many collections from the Mediterranean and we did not find any *D. bidentata*. It has been pointed out above that Torelli's (1930) '*D. bidentata*' from the Bay of Naples is in fact *D. bicolor*, as are a number of other references to *D. bidentata* in the literature. Also, Maggiore and Fresi's (1984) '*D. bidentata*' from the Bay of Naples is a *D. magnitorata*. From the examination of some other collections we also conclude that Castelló's (1986) '*D. bidentata*' is an *Ischyromene sp.*, that Kirkim's (1998) '*D. bidentata*' is *D. bicolor*, and that Castellanos' et al. (2003) '*D. bidentata*' is *D. magnitorata*. It is not impossible that *D. bidentata* occurs in the western Mediterranean as it has been recorded close to the Strait of Gibraltar (Torrecilla-Roca and Guerra-García 2012), but currently there is no evidence for this.

On Atlantic mainland coasts and islands, *D. bidentata, D. edwardsi* and *D. magnitorata* are usually present in the midlittoral to sublittoral zones, although occasionally they are found higher up the shore. Usually the juveniles are present among the fronds of brown, red and sometimes green algae, whilst the adults inhabit cryptic habitats such as crevices, empty barnacle tests, mussel beds and encrusting organisms. Individuals often match the colour of the algae they are feeding on and additional camouflage is afforded by linear and globular patterns of white chromatophores on the dorsal surface (Tinturier-Hamelin 1962, Holdich 1969, 1976). In the Mediterranean and Black Seas, *D. magnitorata, D. bifida, D. bicolor* and *D. edwardsi* usually inhabit shallow water zones, although the last two species can also be present in deeper water off steep-sided islands. Juveniles of these species inhabit algae whilst adults are usually found in more cryptic habitats, but sometimes amongst algae. *Dynamene tubicauda* has been found between 2-30 metres amongst algae in muddy/sandy and coralline habitats, rock scrapings, freely swimming at 30 m, and also in sea grass meadows (Lombardo 1984, Borg et al. 2006, Holdich, pers. obs.). The vertical range of *D. bicolor* is the largest, extending from shallow-water algae and cryptic habitats such as barnacles down to 33 m off steep-sided islands. The vertical ranges of some *Dynamene* species may overlap, e.g., *D. bidentata* and *D. magnitorata* on Atlantic Ocean shores, although the latter usually occurs at a lower level on the shore (Holdich 1970, Arrontes and Anadón 1990a; Castelló and Carballo 2001, Guerra-García et al. 2011, Izquierdo and Guerra-García 2011). *Dynamene bicolor* and *D. edwardsi* frequently inhabit the same shallow-water algae in the Mediterranean.

3.9 CONCLUSIONS

Six species of *Dynamene* are present along the NE Atlantic-Black Sea axis, and one species extends into the Red Sea. It would appear that *D. bidentata* is restricted to coastal habitats of the NE Atlantic, no evidence was found to suggest it inhabits the Mediterranean. *Dynamene magnitorata* has a wider geographical range, occurring on coastal habitats of the NE Atlantic as well as those of the Mediterranean. *Dynamene edwardsi* has the widest geographical range of the six species under consideration, extending from the Macaronesian archipelagos in the NE Atlantic, down the north-western coast of Africa, through the Mediterranean into the Suez Canal and Red Sea. It is not known if a recent record from South Africa represents an introduction or an established population. *Dynamene bicolor, D. bifida* and *D. tubicauda* are restricted to the Mediterranean, although *D. bicolor* also extends into the Black Sea. *Dynamene bicolor* is the most commonly found and most wide-spread *Dyamene* species in the Mediterranean. *Dynamene bifida* has only been recorded at six locations, but its range extends from southern Spain to Turkey. *Dynamene tubicauda* has the smallest geographical range having only been recorded for Italy and Malta. Some species have large vertical ranges, having been found intertidally down to 30 m. It is highly probable that some of the records for the *Dynamene* species are the result of introductions via fouling organisms attached to ocean-going vessels, e.g., *D. magnitorata* and *D. bifida* with their sporadic distribution in the Mediterranean, and D. edwardsi in South Africa.

There are still a number of outstanding issues relating to *Dynamene* that can only be dealt with if more material becomes available. Firstly, the status of the hirsute species from the Balearic Islands and the Greek island of Chios – are these a form of *D. edwardsi* or a new species? Secondly, the status of '*D. torelliae*' – is it really synonymous with *D. bicolor* from the Black Sea? Thirdly, the status of the specimens found in the *Scorpaena porcus* stomach, which appears different from the other species, but cannot be confirmed until more stage 8 males are found.

Fourthly, a genetic analysis of all the species needs to be carried out to ascertain the taxonomic status and species boundaries, and the phylogenetic relationships between species, especially those in the Mediterranean and Black Seas. Currently, only *D. bidentata, D. magnitorata* and *D. edwardsi* from NE Atlantic coasts have been analyzed, and have been found to be distinct.

3.10 ACKNOWLEDGMENTS

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Much of the material used for this study comes from the private collection of DMH, who gives thanks to those below for donating or loaning it to him. All of this material is now deposited in the collection of crustaceans held in the Naturalis Biodiversity Center (Royal Natural History Museum, Leiden, The Netherlands), which already has an extensive collection of *Dynamene*, and which was also used in this study (see Material and Methods section 3.4 for catalogue numbers). Thanks are due to Karen van Dorp for incorporating the new material and looking after the collection. In addition, some material that was examined is held in the crustacean collections of the Natural History Museum, London; the Museum of Natural History, Paris and the Portuguese Museum of Natural History and Science, Lisbon. Thanks are due to following for supplying material for this study: Anadon R (University of Oviedo, Spain); Atta MM (University of Alexandria, Egypt); Băcescu M (Museum of Natural History, Bucharest, Romania); Costa A (University of the Azores, S. Miguel); Castelló J (University of Barcelona, Spain); Ferrario J and Marchini A (University of Pavia, Italy); Fenwick DS Senior (England); Fischelson L (University of Tel Aviv, Israel); Fresi E (Marine Ecological Laboratory, Ischia, Italy); Gönlügür-Demirci G (Ondokuz Mayis University, Turkey); Gözler AM (Rize University, Turkey); Haran T (Tel Aviv University); Jones DA (University of Swansea, Wales); Jones M (University of Plymouth, England); Junoy J (University of Alcalá, Spain); Kirkim F (Ege University, Turkey); Kussakin OG (Far East Science Centre, Vladivostok, Russia); Maggiore F (University of Rome, Italy); McGraff D (University of Galway, Eire); Messana G (University of Florence, Italy), Naturalis Biodiversity

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Chapter 4: Macaronesia as an evolutionary hotspot for low dispersal marine invertebrates: genetic evidence from the rocky intertidal isopod genus *Dynamene*

4.1 ABSTRACT

Diversification and speciation of terrestrial organisms is anticipated in oceanic islands like Macaronesia, a group of Atlantic islands that have arisen from the ocean floor and have never been connected to continental landmasses. Less expected, and investigated, is the diversification of marine organisms in oceanic islands, even in organisms having a putatively lower dispersal capability, such as the case of many peracarid crustaceans that lack larval stages. In this study, we used a multi-locus approach to investigate the role of oceanic islands on the diversity and evolution of the isopod species of the genus *Dynamene* present in Northeast Atlantic. Sequences of two mitochondrial (COI and 16S rRNA) and two nuclear (18S rRNA and 28S rRNA) loci were obtained from specimens of *Dynamene edwardsi* (Lucas, 1849), *Dynamene magnitorata* Holdich, 1968 and *Dynamene bidentata* (Adams, 1800) collected along the Northeast Atlantic Ocean, between Morocco and Scotland, and in Macaronesian archipelagos of Canaries, Madeira and Azores. While for *D. bidentata* and *D. magnitorata* no major phylogeographic structure was detected, within *D. edwardsi*, between 5 to 9 deeply divergent lineages were patent. The 9 cytochrome oxidase I (COI) lineages displayed genetic distances between 4% to 19%, values that compare to those found between established species of peracarids. *D. edwardsi* revealed a long, rich and complex phylogeographic history in Macaronesia, where the geodynamics of islands emergence and submergence, possibly associated with founder effects and subsequent lack of gene flow among populations, frequently appears to supersede geographical distances in justifying diversification. That is the case of the completely sorted lineages of Madeira and Porto Santo, displaying as much as 18% genetic distance despite their vicinity, while haplotypes from the distant Canary island of Tenerife, group in Madeira's clade. These findings suggest a much larger role of oceanic islands in the diversification of marine invertebrates than would have been anticipated, and contributes to expose weakly explored events in the phylogeography of Macaronesia's marine organisms.

4.2 KEYWORDS

Dynamene, Macaronesia, cryptic species, oceanic islands, endemisms, Northeast Atlantic.

4.3 INTRODUCTION

Marine benthic invertebrates that inhabit intertidal coastal areas are unique as they have characteristics that make them different from both terrestrial organisms and other marine taxa (Hachich et al. 2015). This is particularly true on islands, as they are separated from other suitable areas by variable extents of deep water (Hawkins et al. 2000). Many intertidal species have pelagic larvae which may enable them to disperse widely over open water, potentially circumventing the habitat discontinuity. However, some small invertebrates, such as "free-living isopods", are more prone to isolation compared with other marine species with pelagic larvae, because they have direct development, and consequently, putatively lower dispersal capacity. Long-distance dispersion may occur through random events such as rafting on detached macroalgae or floating debris, but this is limited by the capacity of the specimens to survive such events and by their ability to successfully colonize the new habitat (Thiel and Gutow 2005).

Patterns of colonization and gene flow of marine benthic organisms in Macaronesia, which comprises the Atlantic oceanic archipelagos of the Azores, Madeira, Canaries and Cape Verde, have been studied before, but only in organisms with pelagic phase (Chevolot et al. 2006, Sá-Pinto et al. 2008, Xavier et al. 2010). Because these archipelagos have volcanic origin and have never been connected with mainland, their biota is the result of dispersal from distant geographical sources and *in situ* evolution and diversification (Fernández-Palacios et al. 2011). Spread along the Northeast Atlantic Ocean, these islands span a wide range of climatic conditions, holding a highly diverse marine biota which experienced dynamic geological and climatic changes over relatively long periods (e.g., Pleistocene glaciations), thus providing a singular case-study to investigate evolution and phylogeography (Wares and Cunningham 2001, Maggs et al. 2008).

However, understanding the diversity of such organisms is hampered by lack of comprehensive data on species distribution (Witt et al. 2006, Radulovici et al. 2009) as well the difficulty in describing species based solely on morphological characters (Knowlton 1993, Remerie et al. 2006, Beheregaray and Caccone 2007). In isopods, for example, even family-level diagnostic characters might change with development and gender, making difficult their identification (Larsen and Wilson 1998, Larsen 2001). Growing records on the occurrence of cryptic species among marine organisms (Knowlton 2000, Mathews 2006, Witt et al. 2006) further complicate the interpretation of past data records and underline the importance of their recognition in biodiversity monitoring (Knowlton 2000, Cook et al. 2008). Molecular approaches are essential for this purpose, and they have been successfully used among the isopods, to help detection of new species (Xavier et al. 2011b, Khalaji-Pirbalouty and Raupach 2014), discriminate morphologically similar species (Radulovici et al. 2009, Xavier et al. 2012) or unravelling multiple cryptic species complexes (Raupach and Wägele 2006, Markow and Pfeiler 2010, Varela and Haye 2012, Brix et al. 2014, Raupach et al. 2014).

The existence of cryptic species is suspected (but not yet investigated) within most of the large genera in the isopod family Sphaeromatidae such as *Cilicaea, Cymodoce, Dynamenella, Exosphaeroma, Pseudosphaeroma* and *Dynamene* (Poore and Bruce 2012). The members of the genus *Dynamene* Leach, 1814 (Isopoda: Sphaeromatidae) are common and abundant on rocky intertidal and shallow subtidal habitats of the Northeast Atlantic Ocean, and the Mediterranean and Black Seas. Only three species are present in the NE Atlantic (Holdich 1970, Vieira et al. 2016): *D. bidentata* (Adams, 1800), *D. edwardsi* (Lucas, 1849) and *D. magnitorata* Holdich, 1968. In this study, we examine the genetic diversity and phylogeography of *Dynamene* morphospecies from the NE Atlantic using a multi-locus approach. Studies examining the genetic diversity of low dispersal benthic marine organisms in this region have focused only on the mainland shores. Here, the Macaronesian archipelagos of Azores, Madeira and Canary will be also taken into consideration, and this genus will be used to investigate the role of Macaronesian islands in the phylogeography and evolutionary history of marine invertebrates lacking a pelagic dispersal stage.

4.4 MATERIAL AND METHODS

4.4.1 Specimen sampling and taxonomic identification

Dynamene specimens were collected along the distribution range of the genus in the NE Atlantic (Vieira et al. 2016) between 2009 and 2015 in the algae cover of the rocky shore intertidal (Fig. 4.1; see Annexes 1.3, 1.4, 1.5 for details). Three species were sampled: *Dynamene bidentata*, *Dynamene magnitorata* and *D. edwardsi*. *D. bidentata* was collected in Scotland, Iberian Peninsula, Morocco and Gran Canaria (Fig. 4.1A), *D. magnitorata* was found in Iberian Peninsula, Morocco and in the islands of Santa Maria, Terceira, São Miguel and La Palma (Fig. 4.1B) and *D. edwardsi* in Iberian Peninsula, Madeira, Porto Santo, Selvagens, Gran Canaria, La Palma, Tenerife, El Hierro, São Miguel and Morocco (Fig. 4.1C). Two additional individuals sampled in 2014 (see Acknowledgements section 4.8) from algae present at one-meter depth in two harbors from the Mediterranean were also incorporated in the study, one from France and another from Croatia (Fig. 4.1C). After collection, the specimens were immediately preserved in

96% alcohol. Morphology-based taxonomic identification was supported in specialized literature (Holdich 1968a, Vieira et al. 2016). The identifications were reviewed before and after obtaining the DNA sequences to ensure the correct identification of the specimens.

Scanning electron microscope images were produced by David Holdich whilst at the University of Nottingham – see Holdich (1976) for details of preparation and equipment used.

4.4.2 DNA extraction, amplification and sequencing

DNA extraction was performed using the "E.Z.N.A. Mollusc DNA extraction Kit" according to manufacturer instructions. Depending of the specimen size, only a small amount of tissue or the whole animal was used. We used the cytochrome oxidase subunit I DNA barcode region as the prime locus for investigating the genetic diversity of *Dynamene*. A total of 179 sequences were obtained (40 for *D. bidentata*, 101 for *D. edwardsi* and 38 for *D. magnitorata*) (Annex 4.1). Based on the COI phylogeny we selected representative specimens of each region for each species for further analyses of sequence variation using part of the mitochondrial gene 16s rRNA (total of 43 sequences), a partial segment of the nuclear gene coding for 28s rRNA (total of 46 sequences) and the variable regions 2-5 of 18s rRNA (total of 120 sequences). All PCR reactions were performed in a total of 25 µl volume, containing 12.5 µl supreme taq (Nzytech), 0.5-1.25 µl of each primer (10mM) and 1-4 µl DNA extraction. The remaining volume consisted in ultrapure water. For PCR conditions and primers used, see Annex 4.2.

The 658 base pair (bp) barcode region was amplified using the primers LoboF1/LoboR1 (Lobo et al. 2013) or LCO1490/HCO2198 (Folmer et al. 1994) depending on the PCR reaction success. The 16S rRNA fragment was amplified with the primers 16Sar/16Sbr (Palumbi et al. 2002) or D16SAR/D16SBR (Geller et al. 1997) depending on the PCR reaction success. The nuclear genes coding 18S rRNA were amplified with the primers 18sAi/18sBi (Whitting 2002) and 28S with the primers AM-28S-H/AM-28S-T (Tomikawa et al. 2007). Amplification success was verified in a 1.5% agarose gel. DNA templates were purified ("Roche purification kit" according to manufacturer instructions) and sequenced bidirectionally in an external service supplier (STABVida), using an ABI 3730 sequencer and following standard chain-termination sequencing protocols.

All sequences were deposited in Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert 2013) under the project (DYNA - "Dynamene NE Atlantic").

4.4.3 Data analysis

All sequences were analysed and edited using MEGA 7.0 (Kumar et al. 2016). Trace files were checked manually, unreadable zones and primers were removed and ambiguous bases corrected. For the 658 bp COI region, the edited sequences were aligned using Clustal W (Thompson et al. 1994) as implemented in MEGA 7.0 (Kumar et al. 2016) and the translation verified for stop codons or indels.

Sequences of 16S, 18S and 28S were aligned separately using Clustal W (Thompson et al. 1994) tool in MEGA 7.0 (Kumar et al. 2016) as suggested by Talavera and Castresana (2007) and highly variable regions were deleted from the analysis using Gblocks (Castresana 2000), producing a final dataset for 16S, 18S and 28S consisting in, respectively for each species: *D. bidentata* (458 bp, 1120 bp, 781 bp); *D. edwardsi* (451 bp, 1084 bp, 801 bp) and *D. magnitorata* (426 bp, 1125 bp, 780 bp).

4.4.4 Phylogenetic analyses

Phylogenetic analyses for each locus and for the concatenated data (merged together in DNASP 5.10, Librado and Rozas 2009), were performed using maximum likelihood (ML) and Bayesian inference (BI). The software MEGA 7.0 (Kumar et al. 2016) was used to determine the best model of evolution (see Annex 4.3 for list of models). The ML tree was reconstructed using PhyML 3.0 (Guindon et al. 2010) (http://www.atgc-montpellier.fr/phyml/). Branch support was estimated using 1 x 10^3 bootstraps. The Bayesian tree was reconstructed using MrBayes on XSEDE (3.2.6) (Ronquist et al. 2012) (https://www.phylo.org/portal2/createTask!selectTool.action?selectedTool=MRBAYES_XSEDE) through CIPRES Science Gateway (Miller et al. 2010). Two independent runs were conducted with 2 x 10^8 generations each. Parameters were sampled every 1 x 10^3 generations. In the end a Majority rule consensus tree was reconstructed with a burn-in of 10%.

Haplotype genealogy was investigated by building a network of haplotypes using TCS version 1.21 (Clement et al. 2000) with a 90% statistical parsimony connection limit. The networks were edited and drawn in TcsBU (Múrias dos Santos et al. 2015).

4.4.5 Molecular-based species delineation

Two different approaches and five methods of molecular based species deliniation were applied to explore the number of Molecular operational taxonomic units (MOTUs). They were applied to COI, 16s and concatenated data (except for the BIN system that relies only on COI). The first two were based on distance measures. First, COI sequences were automatically subject to the BIN system implemented in BOLD. This approach clusters barcode sequences algorithmically to calculate MOTUs that show high concordance to species (Ratnasingham and Hebert 2013). Then, the Automatic Barcode Gap Discovery (ABGD) species delineation tool on a web interface (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) was applied with default settings using the Kimura-2-parameter (K2P) distance matrix (Puillandre et al. 2012). Finally, three tree-based methods were applied: GMYC single and multi threshold models (Fujisawa and Barraclough 2013) and bPTP (Zhang et al. 2013). Since the GMYC methods requires an ultrametric tree, we first calculated a Bayesian ultrametric phylogenetic tree. The tree was generated in BEAST 2.4.6 (Bouckaert et al. 2014) with the appropriate best model (Annex 4.3), and four independent runs for 7 x 10^7 Markov chain Monte Carlo (MCMC) generations, sampled every 1 x 10^4 generations, were performed. Convergence of the parameters was evaluated using Tracer 1.6 software (Rambaut et al. 2014). The consensus tree was annotated using TreeAnnotator 2.4.6 (Bouckaert et al. 2014). The consensus tree was loaded into the R software package 'SPLITS' (Species Limits by Threshold Statistics; Ezard et al. 2009) in R 3.2.0 (R Core Group, 2015; available at: http://www.r-project.org) and analysed using the single- and multiple-threshold models. In contrast to GMYC, bPTP uses non-ultrametric phylograms. For the input tree, we used ML phylogenies obtained before. Species delimitation analysis was performed using the python code (available at: www.exelixis-lab.org/software.htm, Zhang et al. 2013) with 1 x 10^6 iterations of MCMC and 25% burn-in.

4.4.6 Genetic diversity and structure

Mean and maximum pairwise distances (p-distances) were calculated for each species (intraspecific distances - ISD) using MEGA 7.0 (Kumar et al. 2016), for all loci used in this work (16S, 18S and 28S after the application of Gblocks). Additionally, p-distances for COI and 16S (after the application of Gblocks) within and between MOTUs were also calculated in MEGA 7.0 (Kumar et al. 2016). Indices of genetic diversity, namely haplotype diversity (Hd) and nucleotide diversity (π) were estimated for each locus for each species and MOTU using DNASP 5.10 (Librado and Rozas 2009).

Using the COI data, Fst estimations were made using Arlequin 3.5 (Excoffier and Lischer 2010). Significance of pairwise Fst values was tested by performing 1 x 10^4 permutations between locations, under the null hypothesis of no differentiation. Locations with less than three individuals were excluded from these analyses. Analysis of molecular variance (AMOVA) was performed in order to access the hierarchical population structure at the spatial scales using Arlequin 3.5 (Excoffier and Lischer 2010).

In order to test for a model of isolation by distance we applied a Mantel test to the Fst (as a genetic distance measure) between regions and geographical distance matrices on IBDWS web interface (http://ibdws.sdsu.edu/~ibdws/distances.html, Jensen et al. 2005).

4.4.7 Estimation of approximate time of divergence

To provide a first, rough estimate of the timing of the split between lineages, we applied two methods. Initially, to test the null hypothesis of equal evolutionary rates throughout the tree, and therefore the usefulness of COI divergence rates, the molecular clock test was performed by comparing the ML value for the given topology with and without the molecular clock constraints under the best fitting model (GTR+G+I) in MEGA 7.0 (Kumar et al. 2016). The null hypothesis was not rejected ($P > 0.05$) and therefore a strict clock could be applied. The COI divergence rate has not been previously estimated for *Dynamene* species. However, there are estimates for other isopods: 2.5% per million years (Myr) for *Stenasellus* (Ketmaier et al. 2003) and *Asellus* (Verovnik et al. 2005, Konec et al. 2015) and 1.56 – 1.72% per Myr for *Orthometopon* (Poulakakis and Sfenthourakis 2008). We opted for using 1.5% and 2.5%, that is the lowest and the highest estimated COI divergence rates in isopods, by applying a strict molecular clock and a standard coalescent model in BEAUTI 2.4.6 (Bouckaert et al. 2014). We then analysed the concatenated data in BEAST 2.4.6 (Bouckaert et al. 2014) applying the TN93+G+I model (1×10^9 generations sampled every 1×10^3 trees) and annotated the consensus tree using TreeAnnotator 2.4.6 (Bouckaert et al. 2014). These rates were also applied sucessfully by Xavier et al. (2012) and Panova et al. (2016) in other isopod genus (*Stenosoma* and *Idotea* respectively), and fit within the range of COI rates estimated for other marine invertebrates (Knowlton and Weigt 1998, Wares and Cunningham 2001, Sponer and Lessios 2009, Markow and Pfeiler 2010).

Subsequentely, we applied the Time Tree tool in MEGA 7.0 (Kumar et al. 2016) to access the divergence times for all branching points in a tree. This tool produces a time tree with the same topology as the active tree where all divergence time estimates are based on the branch lengths. The emergence of the most recent islands, El Hierro (1.1 million years ago - Mya) and La Palma (1.7 Mya) (Fernández-Palacios and Wittaker 2008) were used to calibrate the tree. In order to obtain the ultrametric tree, we applied a strict molecular clock and a standard coalescent model in BEAUTI 2.4.6 (Bouckaert et al. 2014). The concatenated data was analysed in BEAST 2.4.6 (Bouckaert et al. 2014) using a TN93+G+I model and four gamma categories for 1×10^9 generations, with sampling every 1×10^3 trees, in order to estimate the time since the most recent common ancestor (tMRCA) with 95% highest posterior density (HPD) intervals. Effective sampling sizes (ESSs, > 200 for all parameters) and convergence of the parameter estimates were assessed in Tracer 1.6 software (Rambaut et al. 2014). A consensus tree was calculated using TreeAnnotator 2.4.6 (Bouckaert et al. 2014).

4.4.8 Ancestral Range Reconstructions and Demographic Inference

Different phylogeographic and different demographic evolution scenarios were tested using the R package BioGeoBars (Matzke 2013a; http://phylo.wikidot.com/biogeobears; for detailed information, methodology and associated packages see Matzke 2013b, 2014). We also used Mesquite 3.2 (Madison and Madison 2017) to access ML estimation of the most probable ancestral using the Markov k-state 1 parameter (Mk1) model (Lewis 2001) assigning the same probability to changes between any two states. For both analysis, the concatenated ML tree obtained in MEGA 7.0 (Kumar et al. 2016) using 1 x 10^3 bootstraps (GTR+G+I) was used with one representative haplotype per MOTU.

4.5 RESULTS

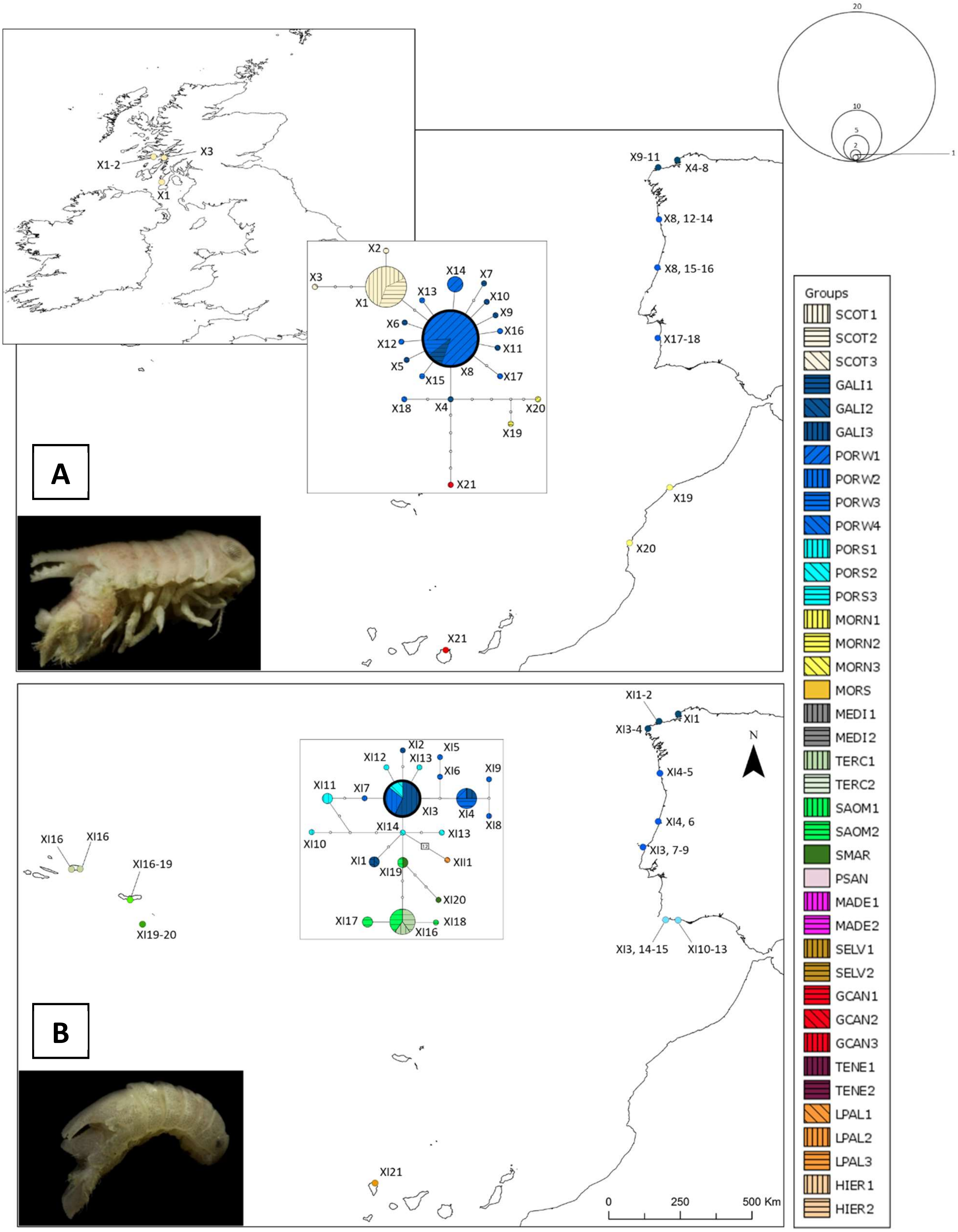
4.5.1 Mophological analysis

Morphologically, *Dynamene edwardsi*, *D. bidentata* and *D. magnitorata* are relatively easy to discriminate among adult males, but harder to separate between sub-adult males, juveniles and females (Holdich 1968a, Vieira et al. 2016). Figure 4.2 shows high magnification power photographs of the three species. The main difference is in the form of the pleotelsonic boss (thinner arrows), which in *D. edwardsi* is an upright plate with peg-like structures at the corners (Fig. 4.2A, B) whilst in *D. bidentata* it is bilobed, the two halves being separated by a wide v-shaped groove (Fig. 4.2C, D). In *D. magnitorata* that structure is also bilobed, but the two halves are more angular and separated by a narrow groove (Fig. 4.2E, F). Also important is the shape of the arms of the bidentate process (thicker arrows), which arises from the posterior margin of sixth pereonite – a feature unique amongst sphaeromatid isopods. In *D. edwardsi* the end of each arm has a downwardly-directed spur (Fig. 4.2A), whilst that of *D. bidentata* tapers to a point (Fig. 4.2C), and that of *D. magnitorata* is more tuberculate and ends bluntly (Fig. 4.2E). No stable diagnostic morphological differences were found among individuals of the same species between different locations.

4.5.2 Molecular analyses and MOTU delimitation

A total of 179 sequences for COI (658 bp), 43 for 16S rRNA, 120 for 18S rRNA and 46 for 28S rRNA were obtained for the three *Dynamene* species (Annex 4.1). All the different locus (individually and concatenated) clearly discriminated the three species (Fig. 4.3A; Annexes 4.7, 4.8, 4.9, 4.10) and BI and ML produced similar topologies. Consequently, we show the BI tree with posterior probabilities from each analysis, complemented with ML bootstrap support (ML concatenated tree displayed in Annex 4.10). *D. edwardsi* showed much higher values of intraspecific variance (Table 4.1) and genetic diversity indices (Annex 4.4) compared with both *D. bidentata* and *D. magnitorata*.

The total number of MOTUs obtained varied between 7 and 20 depending of the locus and method applied (Fig. 4.3B-N). *Dynamene edwardsi* displayed the highest number of MOTUs (between 5 and 11), followed by *D. magnitorata* (between 1 and 5) and *D. bidentata* (between 1 and 4). The majority rule (most common MOTUs across different delimitation methods) delimited 12 MOTUs (Fig. 4.3O), with *D. edwardsi* delimited by 9 MOTUs, *D. magnitorata* by 2 and *D. bidentata* by 1 (Annex 4.1). For the sake of discussion, we assume these numbers of MOTUs as the minimum plausible and most trustworthy given the data, and use them as a reference from here onwards. All MOTUs showed low intra specific genetic variance (< 1%) but high average p-distances between different MOTUs (2.02-23.55% for COI and 0.40-30.02% for 16S) (table 4.2). Within *D. edwardsi* and *D. magnitorata* the individual MOTUs were exclusive to specific regions and sometimes even islands (Annex 4.1). MOTU II displayed the highest value of nucleotide diversity, and MOTU XI was the one with the highest values of haplotype diversity and segregation sites. MOTU III and XII were the only ones displaying single haplotypes (Annex 4.4).


A
B
20
10
5
2
1
X1-2
X3
X1
X2
X14
X7
X13
X10
X9
X6
X16
X12
X11
X5
X15
X8
X17
X18
X4
X20
X19
X21
X9-11
X4-8
X8, 12-14
X8, 15-16
X17-18
XI1-2
XI1
XI3-4
XI4-5
XI4, 6
XI3, 7-9
XI3, 14-15
XI10-13
XI16
XI16-19
XI19-20
XI21
XI2
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XI13
XI10
XII1
XI19
XI20
XI17
XI18
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Groups
SCOT1
SCOT2
SCOT3
GALI1
GALI2
GALI3
PORW1
PORW2
PORW3
PORW4
PORS1
PORS2
PORS3
MORN1
MORN2
MORN3
MORS
MEDI1
MEDI2
TERC1
TERC2
SAOM1
SAOM2
SMAR
PSAN
MADE1
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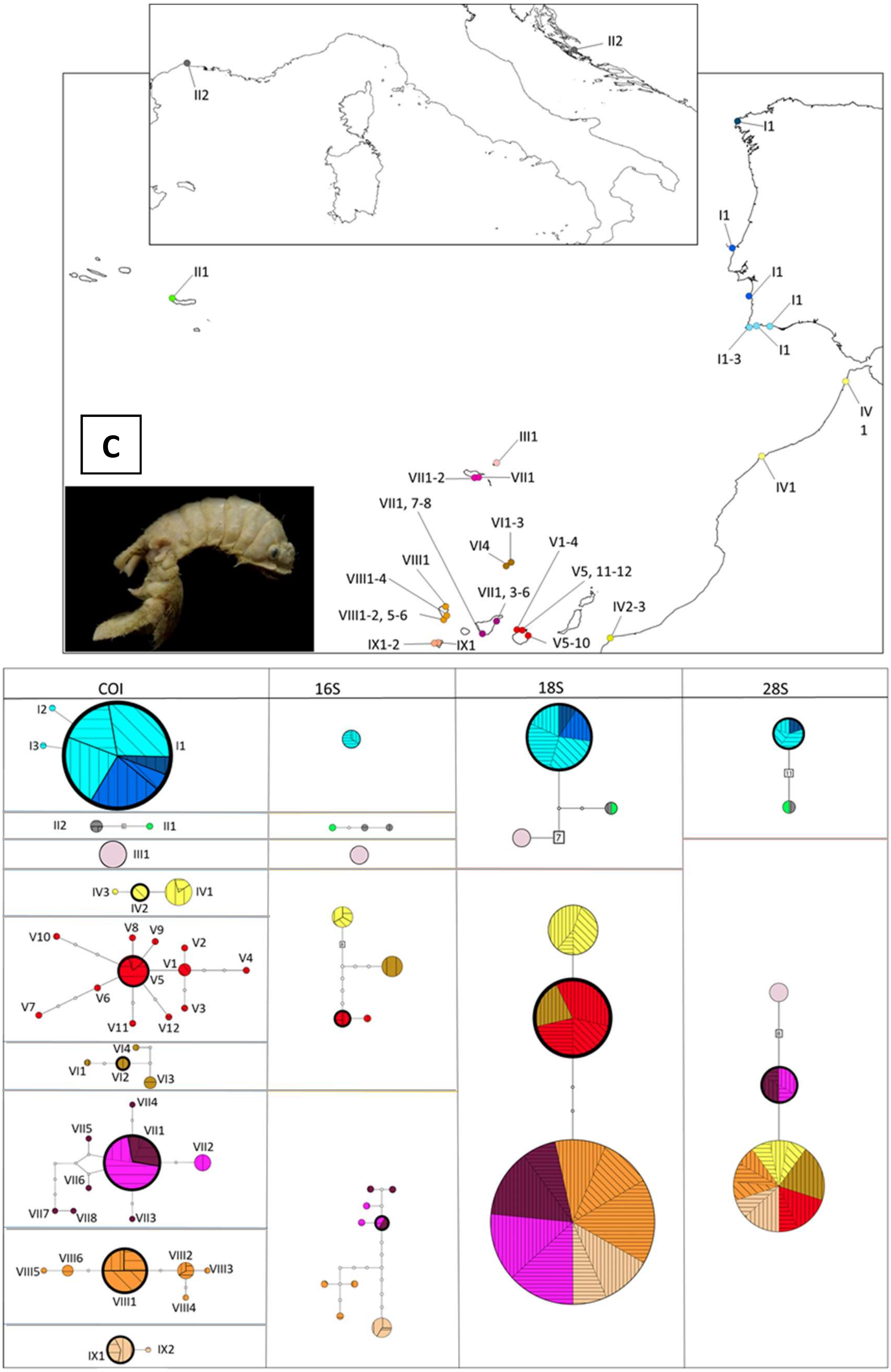

Figure 4.1. Sampling locations and haplotype networks for each *Dynamene* species. **A** – *Dynamene bidentata* (COI haplotype network). **B** – *Dynamene magnitorata* (COI haplotype network). **C** – *Dynamene edwardsi* (COI, 16S, 18S and 28S haplotype networks). COI haplotype numbers according with Annex 4.1 also displayed. Most probable haplotype ancestor according with TCS 1.21 (Clement et al. 2000) highlighted (only displayed in haplotype networks with 3 or more haplotypes).

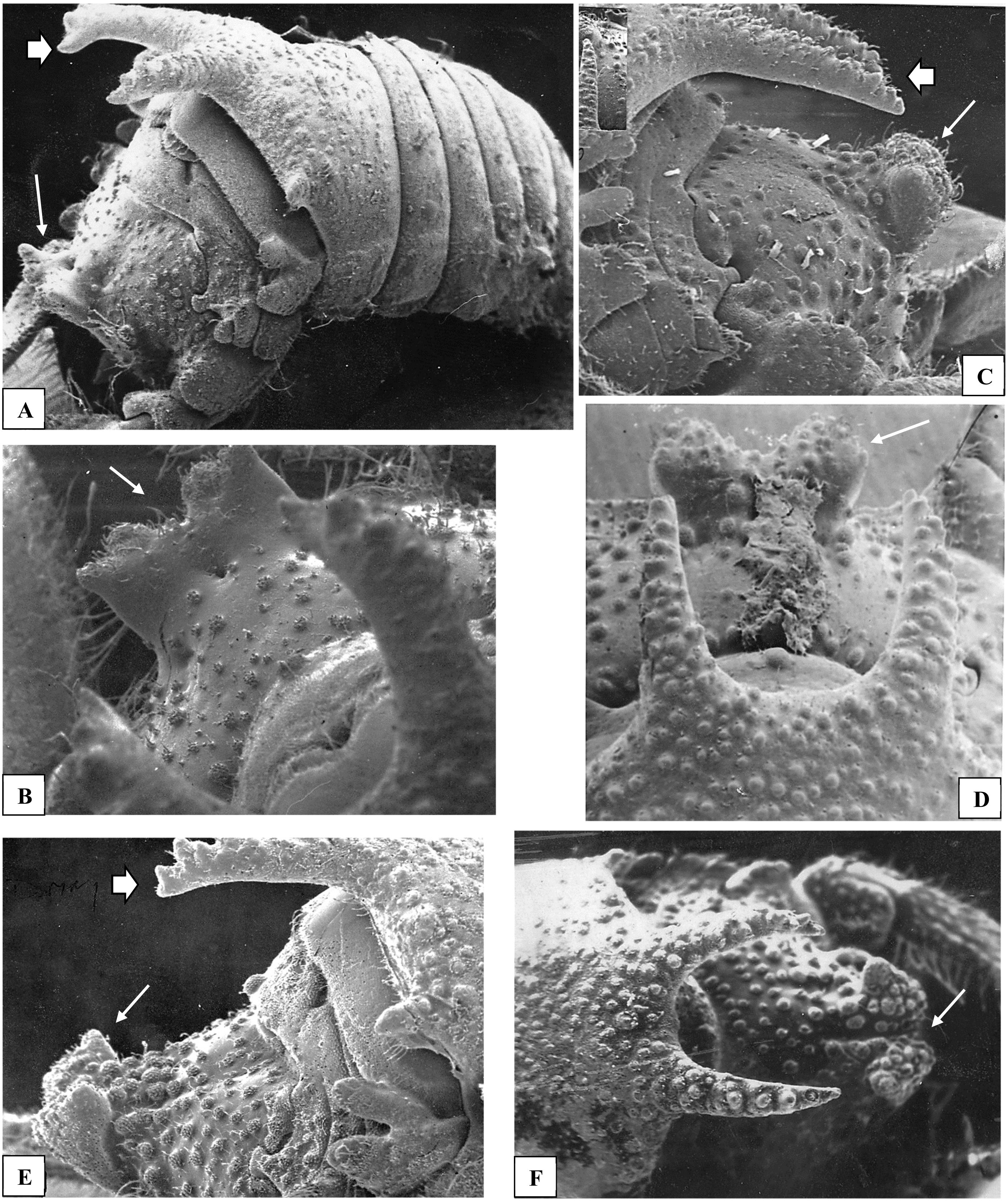

Figure 4.2. Stereoscan electronmicrographs of three species of *Dynamene* showing differences in the posterior pereon and pleotelson. **A** - pereon and pleotelson of stage 8 male *Dynamene edwardsi* (24x). **B** - pleotelsonic boss in dorsal view (30x). **C** - posterior pereon, and pleotelson of stage 8 male *Dynamene bidentata* (45x). **D** - pleotelsonic boss in dorsal view (20x). **E** - posterior pereon and pleotelson of stage 8 male *Dynamene magnitorata* (40x). **F** - pleotelsonic boss in dorsal view (15x).
Adapted by Holdich 1976.

4.5.3 Phylogeographic structure

Results of AMOVA (Annex 4.5) showed that most of the variation in the 3 species was between regions. In *D. edwardsi* 98.14% of the variation occurred between the regions assumed in Annex 4.1 (except Galicia, Portugal West and Portugal South that grouped together). Observed pairwise Fst values between locations from different regions were in general high and close to 1 showing significant differentiation between regions, but lower between locations in the same region (Annex 4.6). In *D. magnitorata,* the variance was best explained by the variation between the group of regions of Iberian Peninsula (Galicia, Portugal West and Portugal South), Azores (Terceira, São Miguel and Santa Maria) and La Palma: 55.63%; while the Fst values were higher than 0.6 between locations from different groups (Iberian Peninsula and Azores), but less than 0.4 between locations within these groups. The results of the AMOVA of *D. bidentata* showed that most of the variation (67.58%) occurred between the groups: Iberian Peninsula (Galicia, Portugal West and Portugal South), Scotland, Morocco North and Gran Canaria. Observed pairwise Fst values between locations from different groups (Iberian Peninsula, Scotland and Morocco) were higher than 0.6 and lower than 0.3 between locations within each group (Annex 4.6).

COI networks of the three species (Fig. 4.1) supported the AMOVA results and clearly discriminated the same regions, with no shared haplotypes between different regions. In *D. edwardsi*, applying 90% of parsimony to COI, resulted in nine networks. The number of networks were different for each locus decreasing gradually from COI to 28S, although the clustering of the clades remained similar. While the network of 16S showed five networks, for both 18S and 28S two networks were displayed. The main difference in the clustering of the networks was in 28S, which exhibited Porto Santo in the same network of Madeira and Canaries archipelagos, contrary with the other three loci (Fig. 4.1C). In both *D. magnitorata* and *D. bidentata* only one network was retrieved in each, and the haplotypes from La Palma and Gran Canaria respectively were the ones more distant from the other haplotypes (Fig. 4.1A, B).

Results of the isolation by distance test showed no significant correlation between genetic distance and geographic distance in any of the studied species ($p>0.05$ for all species).

4.5.4 Ancestral range and time divergence

Both Timetree approach and COI rates used generated similar values (*Dynamene edwardsi* - Fig. 4.4, the other two species not shown). The COI-based divergence time estimates for all the *D. edwardsi* MOTUs (Fig. 4.4) were higher than one million years (except for node 8 with the Timetree approach, see Fig. 4.4 for nodes correpondence), however between Azores and Mediterranean specimens, they were estimated to be between 0.26-0.42 Myr (Fig. 4.4A, B). The most recent estimated divergence was between MOTU VIII and IX: 0.93-1.10 Myr and the first and oldest split within *D. edwardsi* goes back 7.47-9.58 Myr (Fig.4.4). The divergence between *D. magnitorata* and *D. bidentata* was around 6.63-8.51 Myr and the divergence between all the lineages within these 2 species probably occured less than 1 million years (data not shown).

The reconstruction of ancestral range was only possible for *D. edwardsi* (Fig. 4.4), because the two methods use trees with clearly discriminated lineages, which did not occur in *D. magnitorata* and *D. bidentata*. Both analyses used supported similar scenarios, with some differences (Fig. 4.4B). BioGeoBars method suggested that the ancestors of the first big cluster was most probable the MOTU II, with MOTU III with a similar probability, while the Mk1 model suggested MOTU III as the most probable one. In the other *D. edwardsi* cluster, BiogeoBars suggested MOTU VII as the most probable ancestral while Mk1 suggested MOTU VI. The most ancestral MOTU within *D. edwardsi* was suspected to be MOTU II and III according with BioGeoBars method, while Mk1 indicated MOTU III as the most probable one.

The analysis of the dispersal method with BioGeoBars retrieved DIVALIKE+J as the most probable one. From the total events (9.52), 6.64 were founder events with anagenetic dispersal (1.52) and vicariance (1.36) also playing a role. A table with detailed information about the different dispersal methods can be consulted at http://phylo.wdfiles.com/local--files/biogeobears/BioGeoBEARS_supermodel.png (accessed on 01 February 2017).

4.6 DISCUSSION

The diversity and distribution of the genus *Dynamene* in the northern hemisphere has been recently reviewed and updated (Vieira et al. 2016). This review was based on morphology, as well as on new and published occurrence records, which included the Macaronesian archipelagos. Just six species are known for the north hemisphere and, among these, only three are found in the NE Atlantic: *D. bidentata*, *D. magnitorata* and *D. edwardsi.* Our findings, based on both detailed morphological inspection and DNA sequence data from multiple mitochondrial and nuclear loci, challenge those figures. They strongly suggest the existence of at least 7 species, and possibly 4 times more species than currently recognized.

This increase in the diversity of *Dynamene* derives only from molecular data and is due mainly to *D. edwardsi*, which is notably the species with the widest distribution in the Macaronesian islands among the three here investigated. Combined and isolated data from the 4-analysed mitochondrial DNA (mtDNA) and nuclear loci provide compelling evidence for the existence of at least 5 deeply divergent evolutionary units within *D. edwardsi* morphotype, which have been genetically isolated for a long period and therefore could qualify for recognition as separate species. The 5 genetic lineages are completely sorted, consistently recognized as separate MOTUs in all loci, and geographically arranged in such a way that within the same island only one lineage is represented at most, thus excluding any indication of occurrence of sympatric speciation.

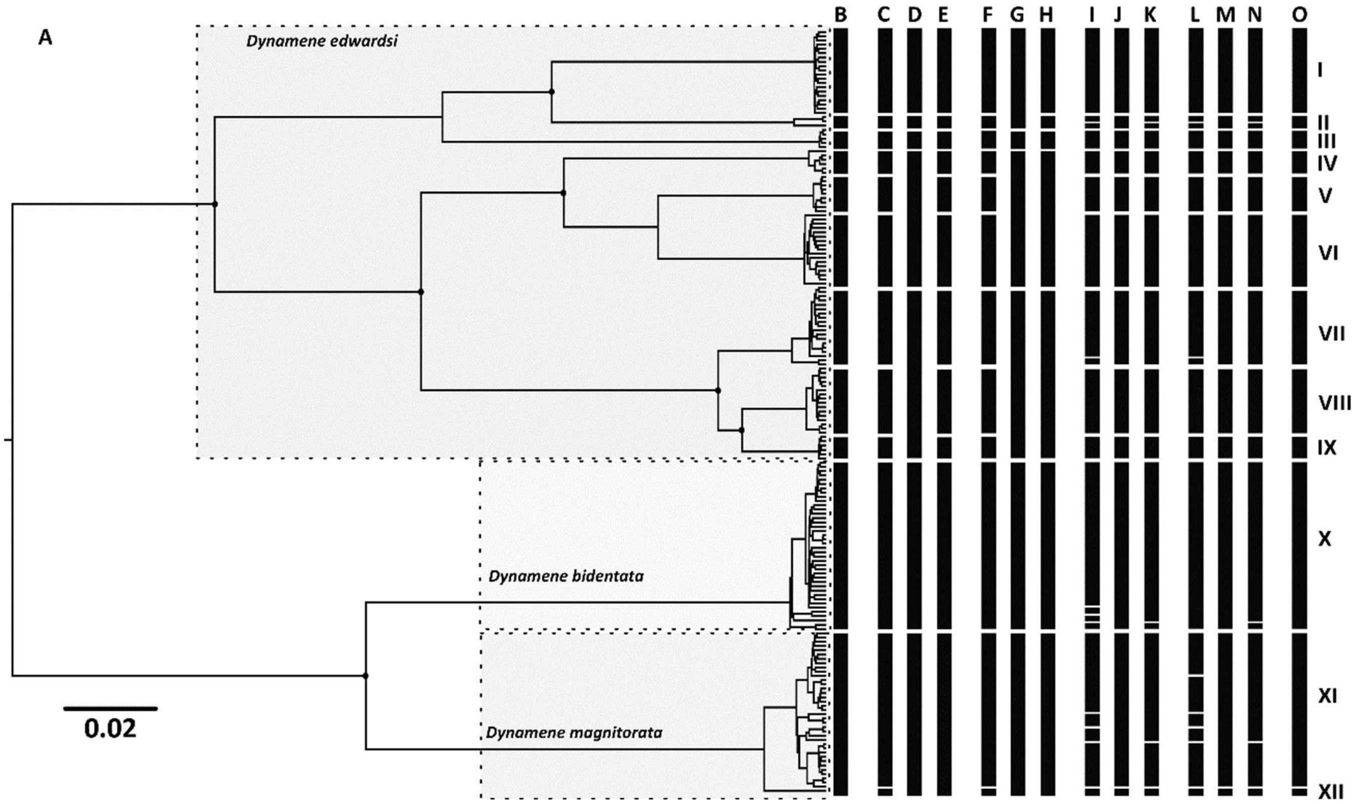

Figure 4.3. A - Bayesian clade credibility tree of *Dynamene* species studied inferred from the 658-bp-long sequence of COI gene. The dots by respective nodes indicate Bayesian posterior probability and maximum likelihood bootstrap values over 0.65 (within MOTUs not shown). Vertical black bars correspond to molecular operational taxonomic units by various methods of species delimitation: **B -** BINs delimitation. **C-E.** bPTP analyses. **C -** COI. **D -** 16S rRNA. **E -** concatenated four-marker dataset. **F-H.** ABGD analyses. **F -** COI. **G -** 16S rRNA. **H -** concatenated four-marker dataset. **I-K.** GMYC single threshold analyses: **I** - COI. **J** - 16S rRNA. **K -** concatenated four-marker dataset. **L-N.** GMYC multiple threshold analyses: **L -** COI. **M -** 16S rRNA. **N -** concatenated four-marker dataset. **O -** Consensus MOTUs.

Table 4.1. Molecular distances (Mean and Max. distances) based on the Kimura 2-parameter model of the analysed specimens for each *Dynamene* species.

Species	COI Mean ISD	COI Max. ISD	16S Mean ISD	16S Max. ISD	18S Mean ISD	18S Max. ISD	28S Mean ISD	28S Max. ISD
Dynamene edwardsi	0.1414	0.2192	0.0829	0.1596	0.0093	0.0236	0.0117	0.0282
Dynamene bidentata	0.0043	0.0154	0.0018	0.0044	0.0002	0.0036	0.0000	0.0000
Dynamene magnitorata	0.0074	0.0265	0.0025	0.0047	0.0052	0.0117	0.0013	0.0064

Table 4.2. Average pairwise distances between MOTUs for COI (lower diagonal) and 16S (upper diagonal) and in diagonal the mean pairwise distances within each MOTU based on COI.

	MOTU I	MOTU II	MOTU III	MOTU IV	MOTU V	MOTU VI	MOTU VII	MOTU VIII	MOTU IX	MOTU X	MOTU XI	MOTU XII
MOTU I	**0.0003**	0.0304	0.0622	0.1314	0.1312	0.1356	0.1272	0.1277	0.1292	0.2884	0.2713	0.2699
MOTU II	0.1222	**0.0091**	0.0631	0.1265	0.1263	0.1307	0.1200	0.1228	0.1198	0.2875	0.2775	0.2786
MOTU III	0.1506	0.1424	**0.0000**	0.1378	0.1354	0.1419	0.1321	0.1319	0.1333	0.3002	0.2706	0.2692
MOTU IV	0.1805	0.1727	0.1746	**0.0012**	0.0267	0.0244	0.0501	0.0453	0.0535	0.2902	0.2709	0.2747
MOTU V	0.1846	0.1842	0.1772	0.0755	**0.0042**	0.0155	0.0533	0.0474	0.0556	0.2920	0.2729	0.2767
MOTU VI	0.1838	0.1930	0.1852	0.0909	0.1178	**0.0037**	0.0555	0.0497	0.0578	0.2918	0.2727	0.2764
MOTU VII	0.1717	0.1713	0.1747	0.1308	0.1404	0.1282	**0.0040**	0.0207	0.0207	0.2910	0.2646	0.2635
MOTU VIII	0.1776	0.1778	0.1732	0.1341	0.1414	0.1281	0.0408	**0.0031**	0.0178	0.2909	0.2693	0.2683
MOTU IX	0.1690	0.1730	0.1781	0.1438	0.1402	0.1302	0.0482	0.0379	**0.0005**	0.2946	0.2661	0.2651
MOTU X	0.2355	0.2343	0.2158	0.2115	0.2083	0.2223	0.2101	0.2166	0.2207	**0.0042**	0.1074	0.1111
MOTU XI	0.2229	0.2258	0.2198	0.2079	0.2027	0.2174	0.2140	0.2048	0.2159	0.1658	**0.0064**	0.0040
MOTU XII	0.2204	0.2264	0.2234	0.2042	0.2061	0.2232	0.2110	0.2023	0.2130	0.1651	0.0227	-

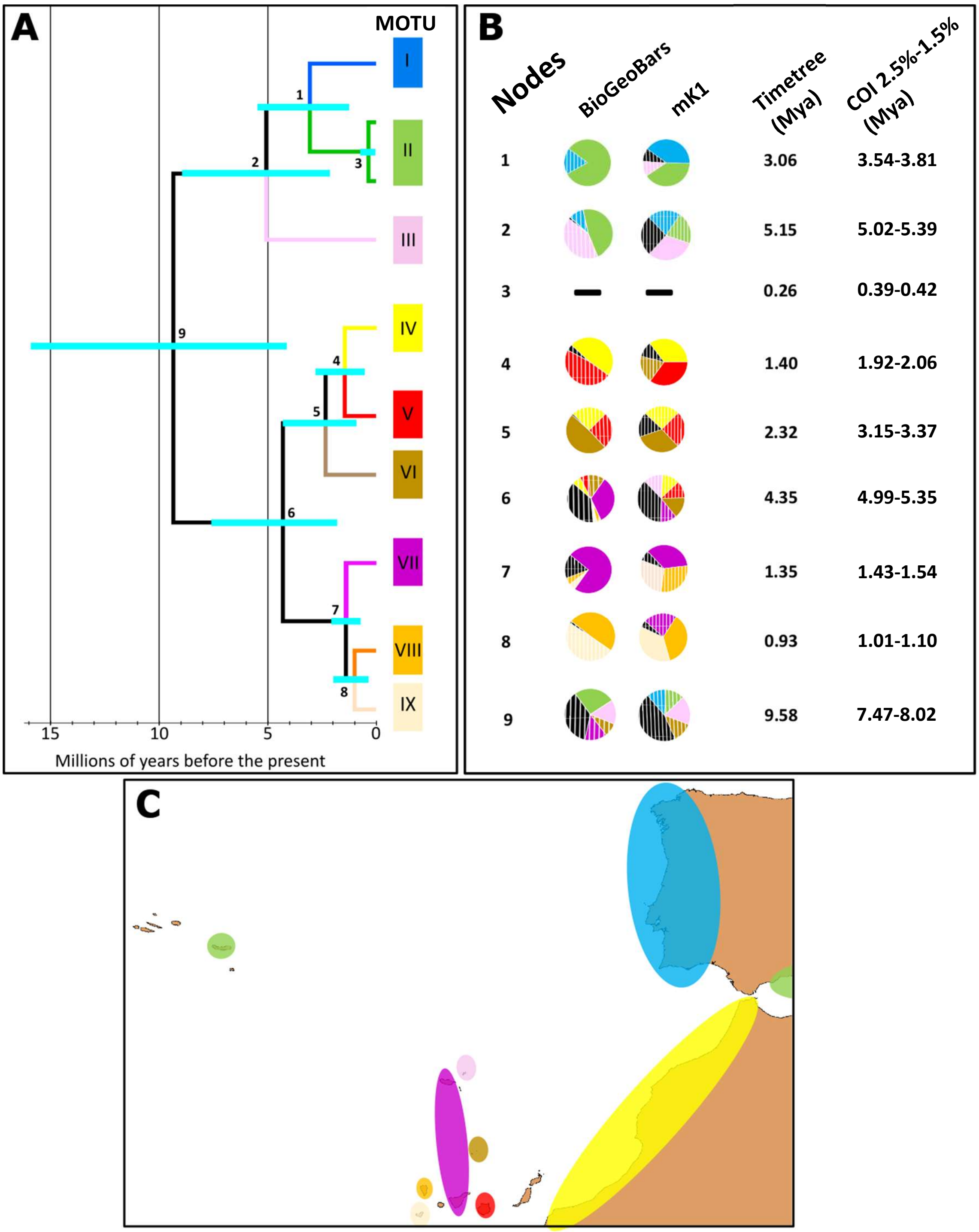

Figure 4.4. Biogeographical scenario for the origin and diversification of *Dynamene edwardsi*. **A** – Timetree concatenated chronogram, with the other two species removed. Blue horizontal bars indicate 95% HPD intervals. **B** – Most probable ancestral in each node with corresponding age. Only probabilities with more than 10% are shown. The most probable(s) MOTU(s) is/are shown in full colour. In black, non-definied MOTUs.
C - The present-day distribution of the different *D. edwardsi* MOTUs. Colours and nodes number match in the three sub-figures.

The number of recognizable sorted lineages and MOTUs within *D. edwardsi* may increase further, depending of the delimitation methods used and if the locus under consideration is a slow nuclear gene (maximum 5 lineages), or a fast mitochondrial one (up to 11 lineages). Lack of divergence or fixed substitutions in the nuclear loci between some of the lineages, which in turn are apparent with mtDNA, does not exclude necessarily the possibility that they still represent separate cryptic species. Since rates of substitution are much slower in the nuclear loci, often highly divergent COI lineages (e.g. >20%) may parallel with very little differentiation in rDNA sequences (e.g. <1%; Borges et al. 2012). In fact, both nuclear loci here used are notoriously known for poor species-level discrimination ability in many groups of animals (e.g., Jörger et al. 2012), despite their robustness for reconstructing deeper phylogenies (e.g., Wetzer et al. 2013). Therefore, a combination of mitochondrial and nuclear multi-locus approach is advised to better access species boundaries and unravel cryptic diversity (Jörger and Schrödl 2013, Grabowski et al. 2017).

Taking COI data as a benchmark for comparison of genetic distances among crustacean species (Costa et al. 2007, Lobo et al. 2016a), even the shortest distances between *D. edwardsi* MOTUs (3.79%) would fall outside the recorded distribution of intraspecific distances in numerous well established morphospecies, including this study's results for *D. bidentata* and *D. magnitorata* (maximum 1.54% and 2.65% respectively; Table 4.1). On the other hand, the highest average distance observed between the 9 MOTUs of *D. edwardsi* (21.92%) surpasses the average distance between *D. bidentata* and *D. magnitorata* (16.55%). The range of COI genetic distances observed within the *D. edwardsi* complex are similar to values reported for a number of cryptic species complexes of isopods, such as *Ligia occidentalis* complex comprising 15 putative cryptic species with a divergence range of 13% to 27% (Markow and Pfeiler 2010), *Excirolana braziliensis* with 3 putative species 14% to 19% (Varela and Haye 2012), *Chelator insignis*, 5 lineages with > 20% divergence (Brix et al. 2014) and *Sphaeroma terebrans* with 4 distinct clades diverging 15 to 18% (Baratti et al. 2005, 2011).

The extensive population structure revealed in *D. edwardsi* was strongly explained by the differences among regions by the AMOVA analysis (98.14% - Annex 4.5) and each MOTU was geographically circumscribed, with the Macaronesia archipelagos of Madeira and Canaries comprising most of the diversity (80% of the total haplotypes). Moreover, the high Fst values obtained (Annex 4.6) also suggest that gene flow is rare or absent between populations from different MOTUs. This idea is also supported by the congruence of the different loci (Fig. 4.3, Annexes 4.7-4.10), and as suggested by Bachtrog et al. (2006), in 'ideal' systems like islands, under strict allopatry (i.e., no gene flow), all regions of the genome will have a single divergence history, and vary only in their coalescence times. This extensive population structure contrasts with the other two well-defined *Dynamene* species that were sampled mainly in Moroccan and European continental coasts. Although in both *D. bidentata* and *D. magnitorata* it is still possible to observe the absence of shared haplotypes (Fig. 4.1) and reduced gene flow between major regions (Annex 4.6), the different loci do not follow the same topology (Fig. 4.3, Annexes 4.7-4.10). The fact that in *D. magnitorata* and *D. bidentata*, Macaronesian populations are distinct from mainland ones (Figs 4.1, 4.3) indicate some level of differentiation and highlight the importance of these islands in the isolation of *Dynamene* species.

The life cycle of the *D. edwardsi* is not known but both *D. bidentata* and *D. magnitorata* are well-studied (Holdich 1970, 1976), although all the *Dynamene* species have sexual dimorphism with males developing the bidentate process characteristic of the genus (Vieira et al. 2016). Both *D. bidentata* and *D. magnitorata* have a biphasic life cycle with a change of habitat, where the immature stages are present amongst the algal cover, whilst the adults occupy cryptic habitats and empty barnacle tests where they stay to reproduce and where females incubate their broods. Dispersal of the juveniles is usually limited to the same “beach” and long dispersal events are rare (Holdich 1968b, 1970, 1976), although these species are present in a wide range of locations (Vieira et al. 2016). In the Atlantic Ocean, the three species have been found amongst a variety of algae in the littoral and sublittoral zones (Vieira et al. 2016). From our personal observations, it seems that their presence is more specific to each location and not to habitat preference, as they can colonize a wide range of habitats (Holdich 1970, Vieira et al. 2016). The same is observed when comparing the different MOTUs, as they are specific to different locations (within each species). Moreover, it seems that these MOTUs do not have the capacity to establish in locations where other MOTUs of the same species are present, or at least their genetic signature is lost by genetic drift.

The fact that the genetic variation is not explained by distance or island emergence (data not shown), together with the fact that the dispersal events occurred mainly by founder events, suggests that complex stochasticity dispersal events were the primary pattern of evolution, which was also observed by Sá-Pinto et al. (2008) in Macaronesia when compared three *Patella* species and none showed the same colonization pattern. The most interesting case is Madeira (within *D. edwardsi*), where this population is closely related with Tenerife, La Palma and El Hierro, a group of islands formed at different times and hundreds of kilometers apart, when comparing with the island of Porto Santo, only 50 km away. Complex evolutionary patterns were also observed in the isopod genus *Ligia* in Hawaii archipelago, with no direct correlation with island genesis or geographical distances (Santamaria et al. 2013).

In other marine invertebrates (sponges and gastropods), a clear ancient split was observed (older than 3 Mya) between Macaronesian and Continental forms (Sá-Pinto et al. 2008, Xavier et al. 2010), which was not so clear in *D. edwardsi*, although in *Dynamene magnitorata* and *Dynamene bidentata*, populations from Macaronesia are clearly distinct from continental coasts, but this diversification occurred more recently (less then 1 Myr). In *D. edwardsi*, the Azores population is genetically closer with the Iberian Peninsula when compared with the other two archipelagos. Phylogeographic studies published so far emphasize the strong affinities of the Azorean populations with those of Madeira, Canaries and western Africa (Santos et al. 1995), which was also observed by Sá-Pinto et al. (2008) in limpets and Domingues et al. (2006) in fish. Although, like we observed in *D. edwardsi*, previous conections between populations of Azores and Iberian Peninsula or Mediterranean were reported before (Xavier et al. 2010).

Macaronesian islands have been proposed as an offshore refugium for several marine organisms (e.g., Chevolot et al. 2006; Domingues et al. 2006; Domingues et al. 2007, 2008, Xavier et al. 2010) during the quaternary glaciations. These groups of islands appeared million years ago (see chapter 1 of this thesis, Fernández-Palacios and Wittaker 2008 and Fernández-Palacios et al. 2011 for details), at different geological times and were shaped by the Pleistocene glaciations (2.58 Myr-present) where the sea level changed several times, and more recently, rised more than 100 m (over the last 20 000 years), and covered several islands that could have served as stepping stones in the past, namely the Paleo Madeira and Paleo Canaries (Fernández-Palacios et al. 2011, 2015). Stepping stone colonizations are commom in many terrestrial invertebrates in Macaronesia (reviewed by Juan et al. 2000) and this scenario can not be discarded for marine invertebrate species. This may explain the genetic proximity in *D. edwardsi* of the Porto Santo Island population with those from the Iberian Peninsula, which it seems to have occurred between 5.02-5.39 Mya, before this glaciation period (Fernández-Palacios et al. 2011). However, the change in sea surface temperature was small (Crowley 1981, Santos et al. 1995) and unlikely to affect Macaronesian marine populations, at least those not close with continental coasts, as these changes in both temperatures and sea level were gradual (Barton et al. 1998). Additionally, members of the genus *Dynamene* have high tolerance to low (5ºC) and high temperatures (>38ºC) (Harvey et al. 1973), which indicates that these species could have survived these multiple geological events.

4.7 CONCLUSIONS

Our study revealed twelve consistent MOTUs within the genus *Dynamene* in NE Atlantic, with nine belonging to *Dynamene edwardsi*, and restricted geographically, in whose genesis Macaronesia's elaborated geomorphological dynamics appear to have played a key role. This contributed to the presence of most of the diversity of this species in the islands, although no clear explanatory evolutionary pattern could be inferred, which may reflect the high stochasticity of long distance dispersal events together with an intricate geomorphological history.

It is noteworthy that *Dynamene* is a genus with only six known species present in the Northern Hemisphere and just three in the Northeast Atlantic (Vieira et al. 2016). If the putative cryptic species here reported are confirmed by further studies, it would represent a staggering increase of 300 % in the known species diversity in the northern hemisphere for this species-poor genus. These findings highlight the relevance of Macaronesia islands in the promotion of isolation and genetic diversity in this genus, and can contribute to the investigation of comparative patterns of evolution and speciation of marine invertebrates in this region. Given the frequent occurrence and dominance of these isopods in the rocky shore communities, this information can be highly pertinent for coastal management and conservation strategies in Macaronesia region. Further studies, namely analysis of other locations in this region and Mediterranean seas are required to fully understand the history of these species and the phylogeographic relationships within this genus.

4.8 ACKNOWLEDGMENTS

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Chapter 5: Macaronesian islands as drivers of diversification of marine invertebrates in the Northeast Atlantic: the remarkable case of the family Hyalidae (Crustacea: Amphipoda)

5.1 ABSTRACT

Pleistocene's glaciations are considered a central element of the phylogeographic history of the Northeast Atlantic, but little is known about the role of the Macaronesian archipelagos in the evolutionary history and diversification of marine invertebrates in this region. Among the amphipod crustaceans, the members of the family Hyalidae are particularly common and abundant in intertidal rockyshores of the Northeast (NE) Atlantic. In this study, we aimed to investigate the genetic variability of Hyalidae species inhabiting the rocky shores of Macaronesia and of the Atlantic European coast. We used the DNA barcoding region to screen the genetic structure and diversity of these species and populations for the first time, with a particular focus on the genetic differentiation between island and continental populations. A total of 159 cytochrome oxidase I (COI) sequences from seven Hyalidae species were amplified from the Macaronesian archipelagos, Morocco, Iberian Peninsula, Iceland, Norway and Scotland. In addition to clearly discriminating the 7 morphospecies studied, DNA barcode sequences also unravelled very high levels of hidden diversity in some of them, making up between 26 and 32 molecular operational taxonomic units (MOTUs) in total, depending of the method used, with as much as 13 MOTU's detected in *Apohyale stebbingi*, and pairwise distances between MOTUs ranging from 1.64 to 16.76 %. In the majority of the cases, the highest number of MOTUs was found in Macaronesian populations, although some morphospecies also displayed a few separate MOTUs in continental populations. *Apohyale prevostii* formed only one MOTU, despite the very large geographical distances among the analysed populations (between Iberian Peninsula and Norway). Most of the MOTUs were also allopatric, with a trend for segregation between islands and continental populations on one side, but also for separation of MOTUs among islands. A notable exception is *A. stebbingi*, which frequently displays several MOTUs within the same island, as in the case of Madeira where up to 4 MOTUs of this species were found. Results suggest distinct evolutionary and diversification patterns among Hyalidae species, but the deep separation between continental and islands lineages appears to be a common feature to all of them. These findings indicate that the complex geologic history of the Macaronesian archipelagos served as an important promoter of remarkable diversification patterns in marine invertebrates of the NE Atlantic, a phenomenon which only now starts to be dully appreciated through the use of molecular data.

5.2 KEYWORDS

Hyalidae, Amphipoda, Macaronesia, DNA barcode, cryptic species.

5.3 INTRODUCTION

The family Hyalidae Bulycheva, 1957 is part of the superfamily Talitroidea, which, according to Serejo (2004), also includes three other families – Chiltoniidae Barnard, 1972, Dogielinotidae Gurjanova, 1953 and Talitridae Rafinesque, 1815. Bousfield and Hendrycks (2002) revised the hyalids, based on the North Pacific fauna and split the large *Hyale* Rathke, 1837 genus into five additional new genera and created 13 new species. After a further revision (Serejo 2004, Horton et al. 2017b) the family Hyalidae was subdivided into two subfamilies (Hyacheliinae Bousfield and Hendrycks, 2002 and Hyalinae Bulycheva, 1957) with 11 genera and more than 110 species worldwide.

The family Hyalidae is predominantly and commonly found among algae of the intertidal and shallow subtidal areas of tropical and subtropical zones (Serejo and Sittrop 2009), although a few species are reported at higher latitudes (McBane and Croker 1984). Like the other peracarideans, hyalids have direct development, lacking the larval phase, which is one of the most common ways of dispersal in the marine environment. The occurrence and abundance of hyalid species are usually related to the complexity of the fronds of the algae, with the juveniles preferring more filamentous algae (*Pterosiphonia*, *Gymnogongrus*), while the adults choosing less ramified and foliaceous algae (*Sargassum*, *Gelidium*, *Ulva*) (Moore 1976, McBane and Croker 1983, Dubiaski-Silva and Masunari 1998). They are mainly detritivores in marine and estuarine habitats and serve as food for many fishes and birds and, like many other amphipods, they play an important role in the food chain (Serejo 2004).

Presently, a complete and corrected checklist of this family, at least for the North Atlantic coasts, is missing. For instance, in World Register of Marine Species (WoRMS) database (Horton et al. 2017a), several species of the genus *Hyale* Rathke, 1837, which now are assigned to different genera, are still included as accepted species with more than one name (e.g., *H. stebbingi* and *Apohyale stebbingi*, *H. schmidti* and *Protohyale* (*Protohyale*) *schmidtii*). At the moment (March 2017), only 13 species are reported in the NE Atlantic Ocean (Ruffo 2006, De Broyer et al. 2007, Horton et al. 2017b).

The NE Atlantic Ocean has a wide range of climatic conditions (from subtropical to subarctic), experienced complex geological and climatological changes during its history (e.g., the Pleistocene glaciation) and has a highly diverse biota. These conditions provide an interesting case study to understand the patterns of genetic diversity and their drivers (Wares and Cunningham 2001, Maggs et al. 2008).

DNA barcodes are recognised, standardised molecular tags for species identification and delimitation (Hebert et al. 2003). The DNA barcode region established for most animal groups is the mitochondrial gene cytochrome c oxidase subunit I. The suitability of the COI gene to deliver species-diagnostic barcode in different vertebrate and invertebrate taxa is well documented (Ward et al. 2005, Costa et al. 2007). Moreover, DNA barcoding may lead to species discovery by flagging cryptic species, which are species with indistinguishable morphology but distinct on a genetic level, although a combination of genetic, ecological and morphological data is needed to describe a new species (Radulovici et al. 2009).

Previous studies have addressed the biology of the Hyalidae family in NE Atlantic, but these have focused mostly on habitat and food preferences (e.g., Guerra-Garcia et al. 2012, Torrecilla-Roca and Guerra-García 2012, Vinagre et al. 2016). In NE Atlantic, *Apohyale prevostii* was the only Hyalidae species studied using molecular tools in DNA barcode reference libraries (e.g., Raupach et al. 2015, Lobo et al. 2016a). Moreover, only a few hyalid species have been studied worldwide (Hiwatari and Kajihara 1984, Dubiaski-Silva and Masunari 1998, Tsoi and Chu 2005). In the present study, we aim to fill this gap by presenting an overview of the genetic variability and phylogeny of Hyalidae species in NE Atlantic Ocean using the COI gene, highlighting the potential of the Macaronesia archipelagos to be hotspots of evolution and speciation and therefore, holding high cryptic diversity in this family.

5.4 MATERIAL AND METHODS

5.4.1 Specimens collection and taxonomic identification

Specimens were collected between 2011 and 2015 during low tide by scrapping the algae cover of the rocky shore intertidal of continental coastal areas (Norway, Scotland, Iceland, Portugal, Spain and Morocco) and archipelagos (Canaries, Madeira and Azores) of the Northeast Atlantic Ocean (Annexes 1.3, 1.4, 1.5, 5.1). After collection, specimens were preserved in 96% ethanol. Morphology-based taxonomic identification was supported in specialized literature (Lincoln 1979, Ruffo 1982, Dallwitz et al. 2000). The identifications were reviewed before and after obtaining the DNA sequences to ensure the correct identification of the specimens. Specimens of *Apohyale media* sampled in 2015 in southwest Atlantic (Rio Janeiro, Brazil; Latitude: -22.9565, Longitude: -43.1642) and identified by Serejo C (see acknowledgments section 5.8) were also included in this study. Sequence data and specimen metadata were uploaded in the project ‘Hyalidae DiverseShores’ (DSHYA) within Barcode of Life Data (BOLD)

(Ratnasingham and Hebert 2013). The species' nomenclature used in this work complies with the accepted nomenclature used in WoRMS and Integrated Taxonomic Informations System (ITIS).

5.4.2 Genetic analysis

From each sample, a piece of isolated trunk muscle tissue or few pereopods or the central part of body were used. DNA extraction was carried out using the E.Z.N.A Mollusc DNA Kit (Omega Biotek), following the manufacturer's instructions. The barcode region of the mitochondrial DNA (mtDNA) gene cytochrome oxidase I was amplified in a MyCycler™ Thermal Cycler (Bio-Rad) thermal cycler using a pre-made PCR master mix and one of the three primer pairs (see Annex 5.2 for details), depending on amplification success. LCO1490/HCO2198 and LoboF1/LoboR1 primers sets were tested first, in this order, for each extraction and when these failed to amplify the 658-base pair (bp) fragment, LoboF1/ArR5 primer pair was used. PCR thermal cycling conditions for each primer pair are also presented in Annex 5.2. Each reaction contained 2.5 µl 10× PCR buffer, 3 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTP mixture, 0.2 µl of 5 U/µl of DNA Taq polymerase (ThermoScientific), 10 µM of each primer (1.25 µl for LoboF1/LoboR1; 0.5 µl for LCO1490/HCO2198; 0.55 µl for ArR5), 2-4 µl of DNA template and completed with sterile milli Q-grade water to make up a total volume of 25 µl.

The PCR products were purified from primers and free nucleotides with the High PCR purification Kit Roche according to manufacturer instructions and then sequenced bidirectionally using the BigDye Terminator 3 kit, and run on an ABI 3730XL DNA analyser (all from Applied Biosystems™) by STAB Vida Lda (Portugal).

5.4.3 Data treatment and analysis

Each trace file was edited individually and manually, unreadable zones and primers were removed and ambiguous bases corrected. The resultant sequences were aligned using Clustal W (Thompson et al. 1994) implemented in MEGA 7.0 (Kumar et al. 2016) and inspected for eventual anomalies, such as stop codons or indels. Sequences of different length were obtained: 658 bp amplified with primers LCO1490/HCO2198 and LoboF1/LoboR1; 550 bp with LoboF1/ArR5. To avoid the problem of increasing artificially the differences, the smallest common fragment of 550 bp was used for diversity and phylogenetic analyses.

5.4.4 Estimate of genetic diversity and MOTU delimitation

Uncorrected pairwise (p) distances for COI within each species and between species were calculated in MEGA 7.0 (Kumar et al. 2016), and were used to estimate genetic divergence between pairs of taxa. Indices of genetic diversity, namely number of haplotypes (H), haplotype diversity (Hd) and nucleotide diversity (π) were estimated for each species using DNASP 5.10 (Librado and Rozas 2009).

For each species, two groups were created based on the location where they were sampled, “Continental” and “Macaronesia”. “Continental” group includes the specimens sampled in Portugal, Spain, Morocco, Scotland, Norway, Iceland and Brazil, while the “Macaronesia” group includes the individuals sampled in Azores, Madeira and Canaries archipelagos. The goal of this comparison was to verify if Macaronesia populations would have general higher values of genetic diversity indices, comparing with the remaining populations. In order to do that, uncorrected p-distances, number of haplotypes, haplotype diversity and nucleotide diversity were calculated for each group in each species.

Four tools were used to determine the minimum threshold between intra- and inter-specific distance and therefore the number of MOTUs. First, through the software R (www.r-project.org) with the libraries APE (Paradis et al. 2004) and SPIDER (function ‘localMinima’; Brown et al. 2012). Additionally, the Automatic Barcode Gap Discovery (ABGD) species delineation tool on a web interface (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) (Puillandre et al. 2012) with the default value of Pmin = 0.001 was used as the minimum allowed intraspecific distance. The maximum allowed intraspecific distance was set to Pmax = 0.03, as this threshold value has been shown to be effective in delimiting crustacean species (Costa et al. 2009). We applied the Kimura-2-parameter (K2P) model sequence correction, which is a standard for barcode analyses (Hebert et al. 2003). Further, the Cluster Sequences tool implemented in BOLD 4 (http://v4.boldsystems.org) (Ratnasingham and Hebert 2013) were used and the generated BINs were used as MOTUs. Finally, opposing to previous methods that consisted on distance methods, we applied the bPTP tree-based method. The bPTP method incorporates the number of substitutions in the model of speciation and assumes that the probability that a substitution gives rise to a speciation event follows a Poisson distribution. The branch lengths of the input tree are supposed to be generated by two independent classes of the Poisson events, one corresponding to speciation and the other to coalescence. Additionally, the bPTP adds Bayesian support (BS) values for the delimited species (Zhang et al. 2013). For the input tree, we used the Maximum-likelihood (ML) tree obtained in section 5.3.5 (see below).

Species delimitation analysis was performed on the bPTP web server (available at: http://species.h-its.org/) with 1 x 10^6 iterations of Markov chain Monte Carlo (MCMC) and 25% burn-in.

5.4.5 Phylogenetic analyses

Phylogenetic analyses of the COI were conducted with the ML and the Bayesian inference (BI) methods. Only one sequence per haplotype was used, which was extracted with the function 'haplotype' of the library PEGAS (Paradis 2010) using the software R (www.r-project.org). The function Best fitting model of MEGA 7.0 (Kumar et al. 2016) was used to search for the most appropriate model of evolution for our dataset. The TN93+I+G model was found to be the best-fit model for the data. The ML tree was reconstructed using the software package PhyML (Guindon et al. 2010) (http://www.atgc-montpellier.fr/phyml/). Branch support was inferred by 1 x 10^3 bootstraps. Bayesian phylogenetic analyses were performed with the software MrBayes on XSEDE (3.2.6) (Ronquist et al. 2012) (https://www.phylo.org/portal2/createTask!selectTool.action?selectedTool=MRBAYES_XSEDE) through CIPRES Science Gateway (Miller et al. 2010). Two independent runs were conducted with 2 x 10^8 generations each. Parameters were sampled every 1 x 10^3 generations. In the end a Majority rule consensus tree was reconstructed with a burn-in of 10%. The sequence of *Gammarus locusta* was retrieved from BOLD (accession number: BNSA073-12) and used as outgroup.

In order to visually compare the two regions ("Continent" and "Macaronesia"), neighbour joining tree (NJT) for each region were constructed using 1 x 10^3 bootstraps of support.

Haplotype genealogy was also investigated by building a network of haplotypes with a 90% statistical parsimony connection limit, with the software TCS 1.21 (Clement et al. 2000). The networks were edited and drawn in TCSbu (Múrias dos Santos et al 2015). Information of the frequency and distribution of haplotypes was also depicted in the network by making circle size proportional to haplotype frequency in the total sample.

5.5 RESULTS

5.5.1 Morphological identification

Seven species belonging to four genera were found and sampled: *Apohyale perieri* (Lucas, 1849), *A. media* (Dana, 1853), *A. prevostii* (Milne Edwards, 1830), *A. stebbingi* (Chevreux, 1888), *Hyale pontica* Rathke, 1847, *Protohyale (Protohyale) schmidtii* (Heller, 1866) (hereafter called *Protohyale schmidtii*), *Serejohyale spinidactylus* (Chevreux, 1926). Representative specimens of each species are shown in Fig. 5.1. No appreciable morphological differences were found between individuals of the same species between different locations.

5.5.2 Estimates of genetic diversity

The COI gene was amplified for a total of 159 individuals (Annex 5.1): 21 for *A. perieri*, 17 for *A. media*, 40 for *A. stebbingi*, 14 for *A. prevostii*, 34 for *P. schmidtii*, 26 for *S. spinidactylus* and 7 for *H. pontica*. Of the 550 bp aligment, 235 variable sites were found, of which 221 were parsimony informative, excluding outgroup species. A total of 103 haplotypes were observed, of which 11 singletons.

The overall Hd was 0.988 and π was 0.1952. Compared to the “Continent” (45 haplotypes), the “Macaronesian” region (60 haplotypes) displayed a slightly higher Hd (0.989 *vs* 0.964) but a considerably higher π (0.2012 *vs* 0.1760) (Table 5.1). Hd ranged from 0.593 in *A. prevostii*, to 0.977 in *P. schmidtii,* although both *A. stebbingi* and *S. spinidactylus* showed similar high values of Hd (0.972). Nucleotide diversity ranged from 0.0015 in *A. prevostii* to 0.1218 in *S. spinidactylus* (Table 5.1). The “Macaronesian” clades showed a higher haplotype and nucleotide diversity than the “Continental” ones, except for *P. schimdtii* and *A. media* which displayed respectively higher nucleotide and haplotype diversity (Table 5.1).

The analysis of pairwise COI nucleotide divergences for all Hyalidae species in our dataset showed a very high divergence among individuals, both between species and within species (Table 5.2). While the overall average distance was 17.8%, the within-species divergence averaged 6.5% (range of 0-12.3%) (Table 5.2) but between-species average divergence was close to 21% (range of 18.2-24.1%) (Table 5.2). The minimum distance among species was detected between *A. prevostii* and *H. pontica* (18.2%) (Table 5.2).

Table 5.1. Number of sequences (N), number of haplotypes (H), haplotype diversity (Hd) and nucleotide diversity (π) for the Hyalidae species included in the present study. *Brazil; **Only present in "Continent".

	Region	***N***	***H***	***Hd***	***π***
All		159	103	0.988	0.1952
	Continent	78	45	0.964	0.1760
	Macaronesia	81	60	0.989	0.2012
Apohyale perieri		21	10	0.776	0.0486
	Continent	12	4	0.455	0.0023
	Macaronesia	9	7	0.944	0.0716
Apohyale media		17	10	0.868	0.0929
	Continent *	5	5	1.000	0.0145
	Macaronesia	12	5	0.727	0.0331
Apohyale stebbingi		40	30	0.972	0.1210
	Continent	19	14	0.959	0.0199
	Macaronesia	21	16	0.971	0.1383
Apohyale prevostii	**	14	5	0.593	0.0015
Protohyale schmidtii		34	28	0.977	0.0680
	Continent	16	10	0.892	0.0264
	Macaronesia	18	18	1.000	0.0226
Serejohyale spinidactylus		26	19	0.972	0.1218
	Continent	5	4	0.900	0.0022
	Macaronesia	21	15	0.962	0.1270
Hyale pontica	**	7	4	0.810	0.0028

Table 5.2. Average pairwise distance between species. In diagonal, pairwise average distance within species.

	1	**2**	**3**	**4**	**5**	**6**	**7**
1) ***A. perieri***	0.049						
2) ***A. media***	0.241	0.093					
3) ***A. prevostii***	0.224	0.206	0.002				
4) ***A. stebbingi***	0.231	0.230	0.185	0.123			
5) ***H. pontica***	0.212	0.209	0.182	0.207	0.003		
6) ***P. schmidtii***	0.227	0.217	0.197	0.231	0.193	0.068	
7) ***S. spinidactylus***	0.225	0.236	0.215	0.233	0.210	0.233	0.120

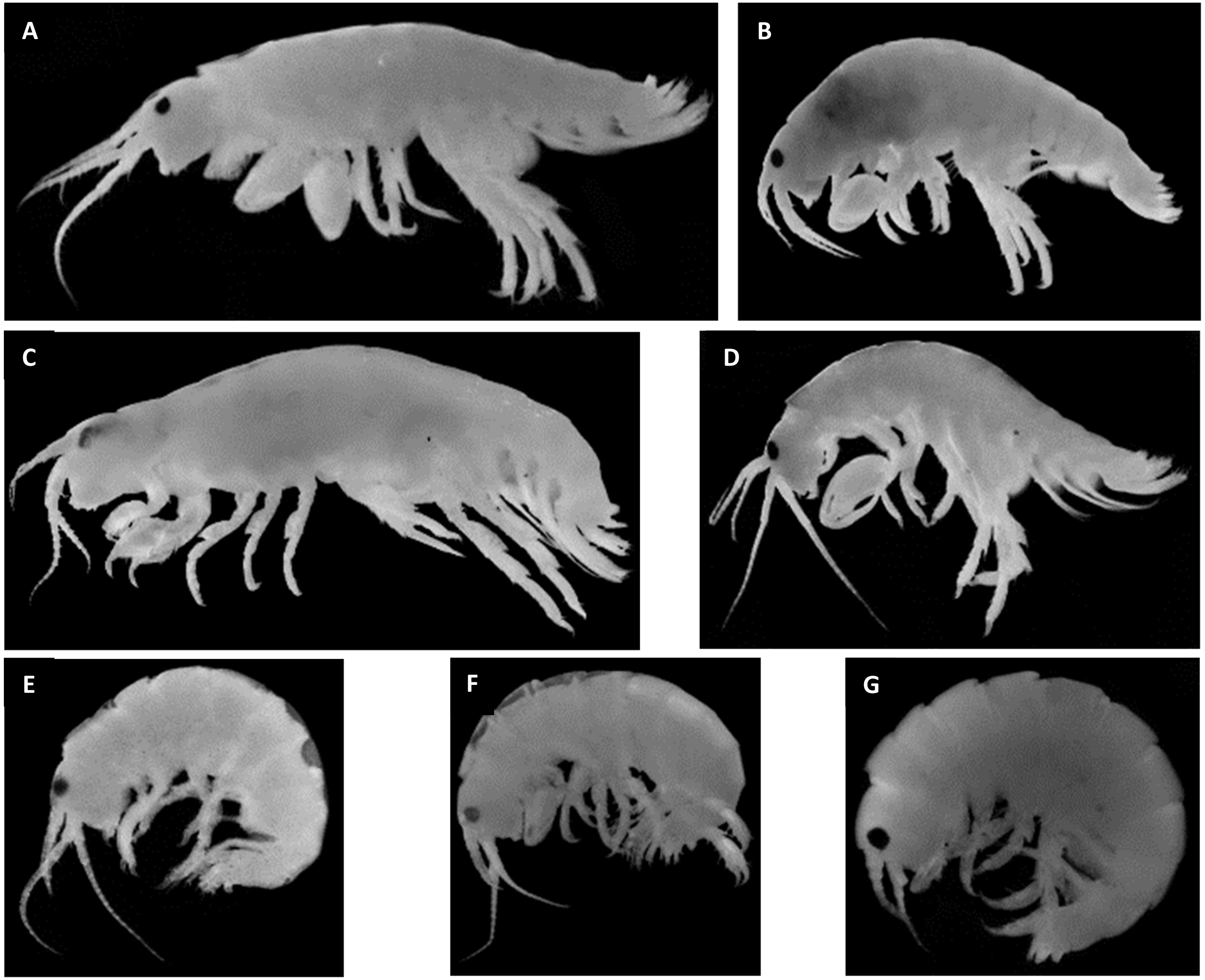

Figure 5.1. Representative specimens of the species sampled and used in this study. **A** - *Apohyale stebbingi*. **B** - *A. prevostii*. **C** - *A. perieri*. **D** - *Serejohyale spinidactylus*. **E** -*Protohyale (Protohyale) schmidtii*. **F** - *A. media*. **G** -*Hyale pontica*. Photos taken by Andrea Desiderato. Specimens not to scale.

5.5.3 MOTUs delimitation

Molecular species delimitation resulted in partitioning our data set into 26–32 MOTUs (Fig. 5.2). The BOLD tool uses a threshold of 2.2% (Annex 5.3), which originated 32 MOTUs. The tool 'local minima' of SPIDER, originated a higher value for this threshold, 5.4% (Annex 5.3), delineating 26 MOTUs. The number of MOTUs generated by ABGD based on K2P was 26 MOTUs and was in concordance with the "local minima". The bPTP retrieved the same MOTUs as the BOLD tool (32). The additional MOTUs identified by BOLD tool and bPTP comparing with ABGD and "local minima" were only in *A. stebbingi* (MOTUs 24, 26 and 29) and *P. schmidtii* (MOTUs 16, 27 and 32) (see Fig. 5.2 and Annexes 5.1, 5.4 for details). Only 2 out of 7 species analysed in this study (*A. prevostii* and *Hyale pontica*) corresponded to just one MOTU, with a within-species distance lower than 1% (Annex 5.4). The other species varied between 3 MOTUs (*A. media*) and 10 to 13 (*A. stebbingi*) depending of the delimitation method used (Fig. 5.2, Annexes 5.1, 5.4). Because there was not a consensus between the methods, we decided to adopt the 32 MOTUs value (Annexes 5.1, 5.4) for two reasons. First, it was the only value obtained by both a distance and tree based method. Second, the BIN system used by BOLD is the standard delimitation method for species when using DNA barcode analyses (Hebert et al. 2003).

5.5.4 Phylogenetic analyses

The topologies of ML and BI were almost identical for the shallow and highly supported nodes of the tree, allowing clear species discrimination by observation of the clustering patterns. All pre-defined MOTUs clustered in generally well supported monophyletic groups, independently of the evolutionary model and tree-building method used. Here we display BI tree (Fig. 5.2). Deeper nodes of the trees showed an overall decrease in node support and more differences among topologies, revealing a polytomy and not significant solved nodes. The most complex clade was the one of *A. stebbingi*. The relations among clades were not clear, but, except for H023 which formed a singleton MOTU (see Annex 5.1 for haplotype number details), all the haplotypes from the "Continent" grouped together in the biggest MOTU (MOTU-8, Annex 5.4), isolating from the Macaronesian clusters. *A. stebbingi* also displayed the highest number of haplotypes (30) and networks (11) (Table 5.1, Fig. 5.4). Six networks were represented just by one haplotype, two by two haplotypes and two by three haplotypes. One network displayed several haplotypes, represented by Scotland, Portugal, Galicia and Morocco, with no shared haplotypes between locations.

Serejohyale spinidactylus displayed five different MOTUs, with high support, each belonging to a different island or coast, except for MOTU-19 that included haplotypes from Galicia and from Azores (H066) and for MOTU-2 which includes haplotypes of Madeira and Selvagem Grande (Fig. 5.2, Annexes 5.1, 5.4). Six networks were retrieved for *S. spinidactylus*, with 21 haplotypes obtained from 26 specimens (Table 5.1, Fig. 5.4), grouped by region (two networks from Gran Canaria, one from La Palma; one shared between Madeira and Selvagens; one from El Hierro and finally one obtained from São Miguel and Galicia). No haplotypes were shared between locations.

Protohyale schmidtii displayed two big and well supported clusters and was the only morphospecies in which the higher number of MOTUs appeared in "Continent" (Fig. 5.2, Annex 5.4). Four different MOTUs, MOTU-31 (from European Coasts), MOTU-16 (from Porto Santo and Madeira) and MOTUs-27, 32 (Morocco) cluster together (Fig. 5.2, Annexes 5.1, 5.4). The other lineage was composed mainly from haplotypes of the Macaronesia, with the exception of a single haplotype from Galicia (Figs 5.2, 5.3). The networks (28 haplotypes from 34 specimens, Table 5.1) also displayed the two big groups (Fig. 5.4) and no shared haplotypes between locations.

Apohyale perieri showed a subdivision into three lineages, one with two singletons from Madeira (H003, MOTU-2) and Gran Canaria (H007, MOTU-3), one with haplotypes from La Palma (MOTU-4) and the last represented by MOTU-1 from different locations (Galicia, Portugal, São Miguel and Madeira) (Fig. 5.2, Annexes 5.1, 5.4). A total of 16 haplotypes from 21 individuals were used and retrieved four networks (Fig. 5.4), although two were represented by just on haplotype each (Madeira and Gran Canaria). A network retrieved from La Palma with three haplotypes was also obtained and finally a star-like network was shared between several distant regions (Galicia, Portugal, São Miguel and Madeira), with one haplotype shared by specimens from Galicia, Portugal and Madeira (Fig. 5.4).

Apohyale media was clustered in two main lineages, however, the "Continental" specimens were not from European coasts but from Brazil (type locality). The high support, exhibited from both analyses, confirmed the taxonomical identification of the new records from Macaronesia of this species. The cluster from Brazil displayed high divergence (Hd: 1.000, π: 0.0145, Table 5.1, Fig. 5.2). Eleven haplotypes from 17 specimens were used and three networks were obtained, one from individuals from Brazil, one from Gran Canaria, La Palma and Madeira and one constituted by two haplotypes from Gran Canaria and Hierro (Fig. 5.4).

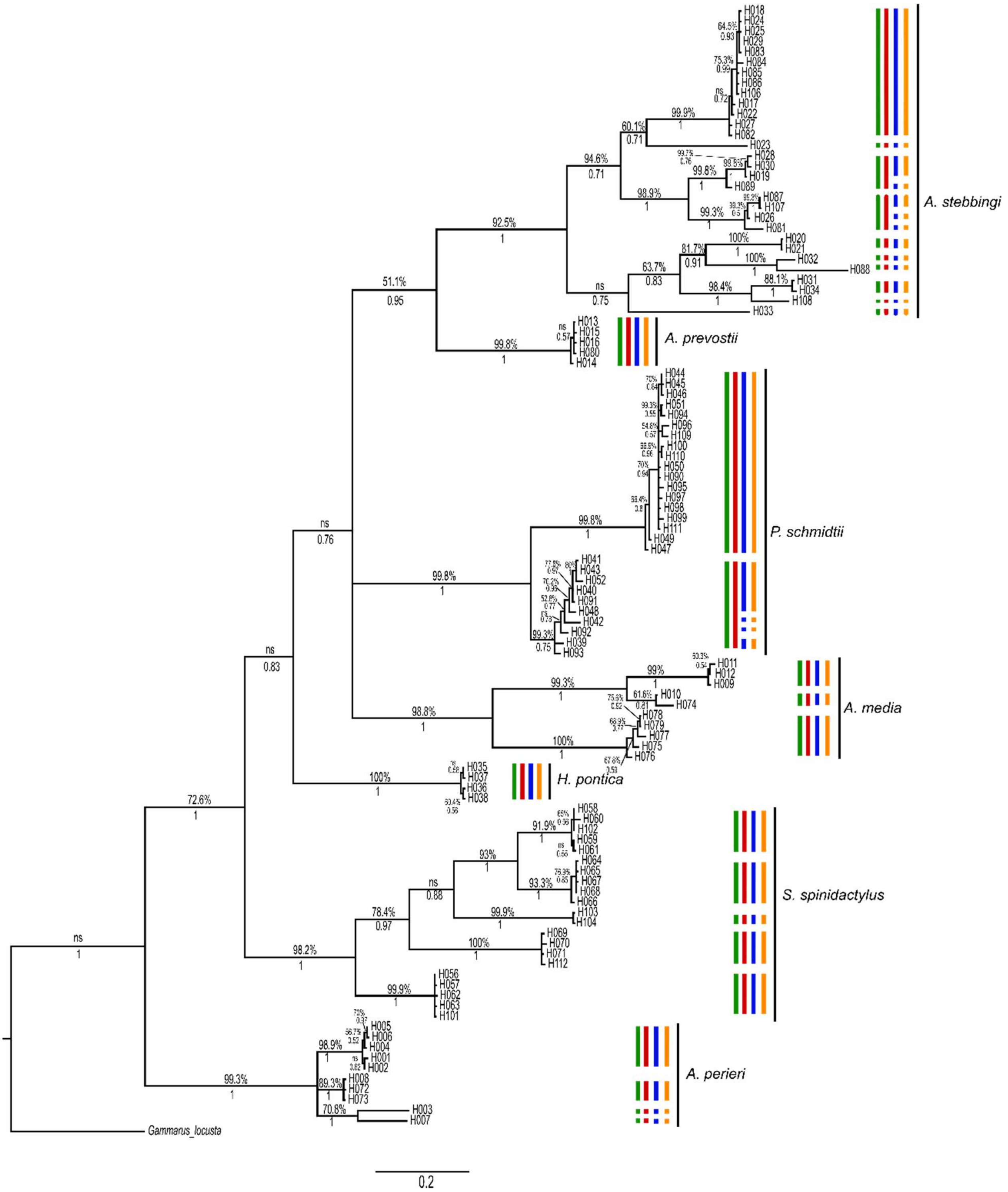

Figure 5.2. Bayesian consensus tree of the cytochrome oxidase I gene of he seven Hyalidae species studied. Values of nodes correspond to maximum likelihood bootstrap (above branches) and to Bayesian posterior probabilities (below branches), respectively. Black lines represent cluster of each morpho-species, coloured lines MOTUs defined with different delimitation methods: green by localminima, red by ABGD, blue by BINs of BOLD and orange by bPTP. n.s. indicates less than 50 % support. See Annex 5.1 for the code of the haplotypes.

Finally, the other two species, *Apohyale prevostii* and *Hyale pontica*, displayed only one lineage (Fig. 5.2) and one network each, with *A. prevostii* displaying a start-like network (Fig. 5.4).

The visual comparison between the NJT of the two regions, showed a greatly higher differentiation along the "Macaronesian" coasts, despite the higher number of morphospecies along the "Continental" coasts (Fig. 5.3). In the "Continental" NJT, only *Apohyale stebbingi* and *P. schmidtii* displayed two highly divergent clades where, in both species, a singleton belonging to the coasts of Galicia departed from the general clade (Fig. 5.3).

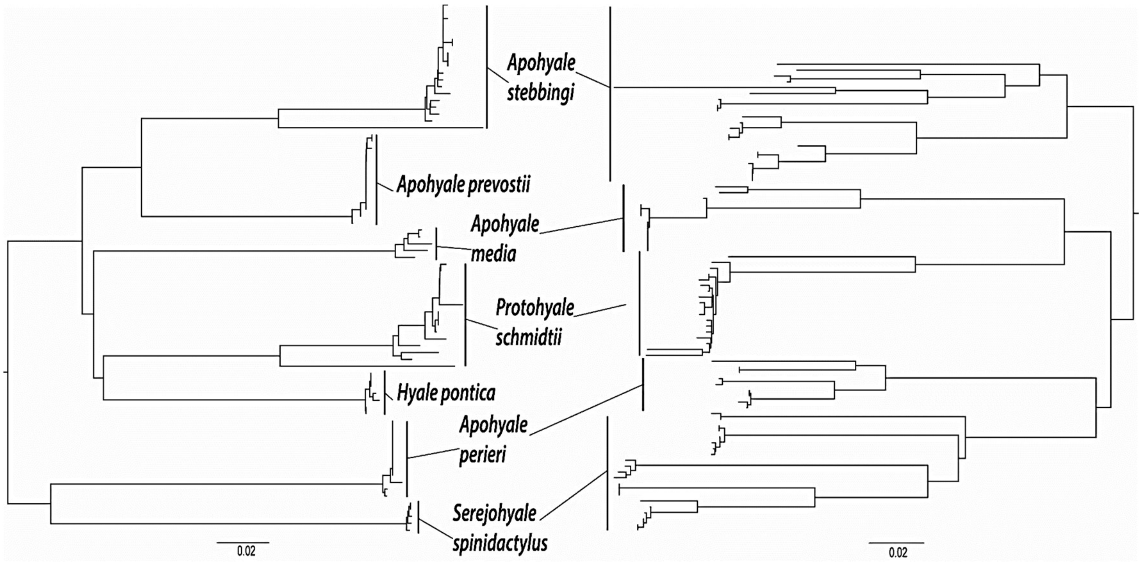

Figure 5.3. Comparison between "Continental" and "Macaronesian" Neighbour Joining Trees, respectively on the left and right.

5.6 DISCUSSION

5.6.1 Hyalidae phylogeny and distribution in Northeast Atlantic

This is the first study that contributes with DNA barcodes for Hyalidae species from Macaronesia and Morocco and for six (all except *Apohyale prevostii*) Hyalidae species in European coasts. Of the thirteen confirmed hyalids recorded in the Northeast Atlantic Ocean (Horton et al. 2017a, b), six were sampled and one (*Apohyale media*) was reported for the first time, increasing the amount to fourteen. *Parhyale eburnea* is recorded on WoRMS also in the North Atlantic Ocean, although it is stated as endemic of the Mediterranean Sea (Ruffo 1982) and the occurrence has not been verified. The absence of *A. prevostii* and *H. pontica* from the Macaronesia was also observed by Krapp-Schickel and Ruffo (1990), although Borges et al.

(2010) reported *A. prevostii* as part of Azorean marine fauna. We observed that the southern limit of these two species was West Portugal, although Pereira et al. (2006) sampled a few *H. pontica* specimens in South Portugal and Guerra-García et al. (2011) in South Spain. These two species are present in Northern European coasts (Costello et al. 2001) and although they are widely distributed (Annex 5.1), they only retrived one MOTU each (Fig. 5.2) and displayed reduced distances between the northern haplotypes from Scotland, Norway and Iceland and the ones from Portugal and Galicia (Fig. 5.4), which was also observed in *A. stebbingi* (Fig. 5.4). This lower diversification in the northern regions could be accounted for the recent recolonization after the last glacial maximum (Maggs et al. 2008).

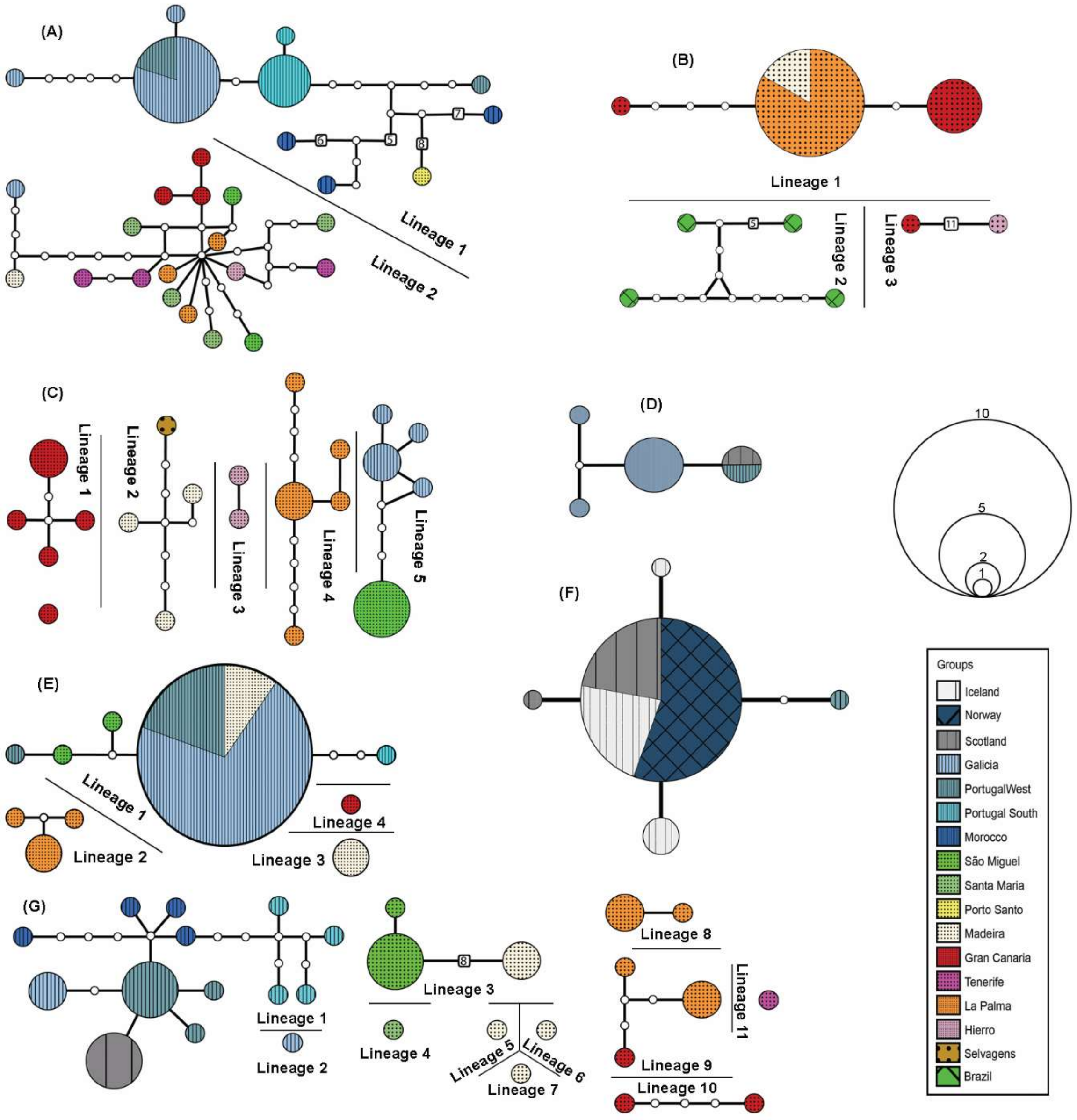

Figure 5.4. Haplotype networks of the seven Hyalidae species studied. **A** - *Protohyale schmidtii*. **B** - *Apohyale media*. **C** - *Serejohyale spinidactylus.* **D** – *Hyale pontica.* **E** - *Apohyale perieri*. **F** – *Apohyale prevostii*. **G** - *Apohyale stebbingi*. Open cirles represent missing haplotypes; open squares with numbers of missing haplotypes are displayed when more than four haplotypes are missing.

It is important to underline that this study is the first record of *Apohyale media* from Madeira and Canaries coasts. This species is cosmopolitan in tropical and subtropical waters (Serejo 1999). It occurs along the coasts of the South Atlantic Ocean, particularly West coasts (Serejo 1999, De Broyer et al. 2007), and in the Gulf of Mexico until Florida (Nelson 1995, LeCroy 2007), while the northernmost record of it, along the East Atlantic coasts, was Cape Verde (De Broyer et al. 2007). It is also documented along the coasts of Pacific and Indian Oceans (Serejo 1999, Martín and Díaz 2003), but many records of this species in the literature, especially Pacific records, may actually refer to other species (LeCroy 2007), as for the specimens from New Zealand, which were recently attributed to the new species *Apohyale papanuiensis* Kilgallen, 2011 (Kilgallen 2011).

Reid (1939) described a new species of hyalid, called *Hyale ramalhoi,* from the coasts of Madeira, which was later added to the *H. spinidactyla* complex (Serejo 2001), and subsequently renamed as the new genus *Serejohyale*, which is represented by four species *S. spinidactylus, S. youngi, S. spinidactyloides* and *S. ramalhoi* (Horton et al. 2017a). *Serejohyale ramalhoi* was never found again after its description and the holotype went missing. Moreover, during this work, close areas to the type one were sampled, but only specimens of *S. spinidactylus* were retrieved, nurturing the hypothesis of a misidentification or a possible extinction. However, it is remarkable how the specimens from Madeira are genetically distant from the others, suggesting a cryptic species in the same area of a previously identified, maybe wrongly, different species.

The average interspecies distance in this family seemed in line with other amphipod works (Raupach et al. 2015, Lobo et al. 2016a), but the average intraspecific distance was considerably high compared with the standard values for Crustacea (Costa 2007). The lowest distance between species was in the not congeneric species *Apohyale prevostii* and *Hyale pontica,* showing signals of a possible paraphyly in the genus *Apohyale*. Moreover, *A. perieri* appeared in a different and distant cluster of the other three congeneric species, although with low node support. The unsolved phylogeny demonstrated a possible saturation of the gene COI, which was not enough to resolve the relationships between the species of this family. More species, and possible additional genes, would be needed to correctly access the phylogeny of the Hyalidae family and perhaps a further taxonomic revision.

5.6.2 Macaronesia role in Hyalidae cryptic diversity

Only the Hyalidae species in this study that inhabit both regions, "Macaronesia" and "Continent" displayed high intraspecific genetic distances (Table 5.2) and several MOTUs (Fig. 5.2). Remarkably, the "Macaronesia" region displayed a higher nucleotide diversity than the "Continent", despite the lower number of morphospecies (Table 5.1). Although amphipods cryptic speciation has been reported before (Witt et al. 2006, Costa et al. 2009, Radulovici et al. 2009), this is the first recorded case for amphipod cryptic taxa existing in the Macaronesia. Here, we propose the roles that Macaronesia could have played in these species (Table 5.3).

Geographic expansion

The presence of two distinct lineages in *Apohyale media* could be related to an ancient migration and speciation, such as the barnacle *Ceratoconcha* with anfiatlantic distribution (Baarli et al. 2017). The higher nucleotide diversity displayed in the lineage of Macaronesia (Table 5.1) and the presence of two different MOTUs in it (Fig. 5.2, Annex 5.1), could be signals of an origin from this region. Nevertheless, one of these MOTUs, was composed by close and abundant haplotypes, with one of them shared between Madeira and La Palma (Annex 5.1), which could mean a recent geographic expansion, maybe also operated by human transport. Because this species is cosmopolitan and present along the coasts of the South Atlantic Ocean (Serejo 1999), more specimens from these regions are needed to understand their roles as possible stepping stones in the expansion of this species.

Glacial refugium

The possible role of Macaronesia region as refugium during Pleistocene glaciations was documented before in marine invertebrates (Sá-Pinto et al. 2008, Xavier et al. 2010). In the species *Serejohyale spinidactylus* and *Apohyale perieri,* Macaronesia may have played a similar role. This hypothesis is strengthened by the reduced haplotype distances in the MOTUs of mainland. Moreover, the presence of haplotypes from Azores, in *S. spinidactylus* and *A. perieri*, within the same MOTUs of the haplotypes from mainland, suggested a colonization from these islands to the "Continent". This scenario is in line with the common opinion that postulates the Azores as glacial refugium (Chevolot et al. 2006, Xavier et al. 2010). In *S. spinidactylus*, the lineage of the "Continent" appears to be one of the shallowest giving support to a recent colonization and speciation of this clade. Further, the haplotype of *A. perieri*, shared between Madeira and European coasts, could be a signal of back colonization, through synanthropic transport. Even though, this common haplotype, could also be a recent colonization from Madeira.

Speciation pool

The incredibly high number of private MOTUs from the Macaronesia region (17-21) is a strong signal of the speciation that has been taking place in it. High mtDNA difference with only one shared haplotype in *A. perieri* indicates a low genetic exchange and suggests the isolation of the different populations (MOTUs). The importance of islands for species evolution is worldwide accepted (Selmi and Boulinier 2001, Villacorta et al. 2008, Losos and Ricklefs 2009, Warren et al. 2015). It is well documented that vicariance events, caused either by the emergence of land barriers or by the isolation within glacial refuges, have prompted allopatric divergence and speciation in many marine organisms (Quesada et al. 1995, Wares and Cunningham 2001, Patarnello et al. 2007, Xavier and Van Soest 2012). Nonetheless, the colonization of oceanic volcanic islands is strictly dependent of the species dispersal capability. In amphipods, which lack a larval phase, dispersal mechanisms are limited to rafting objects and anthropic mediated transport (Thiel and Gutow 2005, Cowie and Holland 2006, Wildish and Pavesi 2012, Cabezas et al. 2013a). Accordingly, the biology of the hyalids as inhabitants of algae with a high rafting dispersal potential, such as species of the genus *Sargassum* (Dubiaski-Silva and Masunari 1998), increase the possibility of these events (Deysher and Norton 1981, Poore 2005). For instance, the situation of *S. spinidactylus* is emblematic, reporting five well-supported MOTUs, with a considerably high average divergence of 12% among them. Furthermore, of the four lineages belonging only to the Macaronesia, three of them are private to single islands (one shared between Madeira and Selvagem Grande), corroborating the segregation scenario. As for the possibility of well-described allopatric speciation in remote islands, in the clade of the morphospecies *A. stebbingi*, there are more MOTUs belonging to same site. For instance, the divergence between the four MOTUs of the site Ponta da Cruz belonging to Madeira, shows the appearance of possible sympatric cryptic species. Sympatric speciation is a phenomenon that is not completely understood. For Mayr (1947), in sympatric speciation, populations first become reproductively isolated and then diverge. This is usually related to a shift in ecological preference of the divergent species, as for the soil predilection of the palms of Lord Howe Island (Savolainen et al. 2006), or the plant host for phytophagous insects (Berlocher and Feder 2002). Nonetheless, the possibility of a shift in the ecological habits of *A. stebbingi*, such as the preference for different algae during their life cycle, is a strong possibility.

However, allopatric speciation in a vicariance scenario is also a possibility, if it would have occurred concurrently with the evolution of the Macaronesia. In fact, it is acknowledged that the present-day Macaronesia is only the residual of a bigger complex that now is submerged for a major part (Fernández-Palacios et al. 2011). The emerged seamounts during the Pleistocene, now eroded and submerged, could have allowed the dispersion between the different archipelagos of the Macaronesia, especially to the Azores from the Paleo Madeira and Paleo Canaries Seamounts (Den Broeck et al. 2008, Fernández-Palacios et al. 2011). Similarly, they could have worked as stepping stones for the colonization of recent islands from Europe and Africa (Carine et al. 2004). The network of *P. schmidtii,* for example, showed the connection between haplotypes from Morocco and Porto Santo, which is the oldest emerged island in the archipelago of Madeira. This could be a remnant of the past connection between the two regions which could have resulted in the speciation of the two different lineages from Macaronesia and Continent.

Table 5.3. Roles and effects of Macaronesia region on the different species of the family Hyalidae.

Role	Effect	Case species
Geographic expansion	Large expansion along Atlantic Ocean with possible ancient split between Macaronesia and South Atlantic populations	*Apohyale media*
Glacial refugium	Due to the climatic stability during the last glaciation, some species sheltered along the coasts of these islands and recolonized the mainland after it.	*Apohyale perieri* and *Serejohyale spinidactylus*
Speciation pool	"De novo" islands of Macaronesia are susceptible to speciation due to the segregation from mainland and reduced gene flow. Possibilities of allopatric and sympatric speciation.	*Serejohyale spinidactylus, Protohyale schmidtii* and *Apohyale stebbingi*

5.7 CONCLUSIONS

The poor-studied family Hyalidae is commom and abundant in Northeast and Macaronesian coasts (Lincoln 1979, Ruffo 1982, Hayward and Ryland 1995). Unexpected high intraspecific variation (4.9-12.3%) and high number of MOTUs (24-30) was found in five Hyalidae morphospecies (*Apohyale media, A. perieri, A. stebbingi, Serejohyale spinidactylus* and *Protohyale schmidtii*). This diversification is manly due to the Macaronesian region which displayed higher genetic diversity values and number of MOTUs when compared with continental coasts. This was the first study to spot cryptic diversity in the order Amphipoda in Macaronesia islands and reinforce two ideas: first, marine invertebrates should be taken in consideration in molecular studies in islands and second, that amphipods can be used as models in molecular delimitation studies. More species should be used to fully understand the phylogeny of Hyalidae and other locus, together with ecological and possible more morphological data should be incorporated to describe these putative new crypic species.

5.8 ACKNOWLEDGEMENTS

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Chapter 6: DNA barcoding reveals cryptic diversity in the superorder Peracarida (Crustacea) in Northeast Atlantic: implications for Macaronesia conservation, with new records for several species from Macaronesia

6.1 ABSTRACT

The Northeast (NE) Atlantic Ocean is a vast and complex marine region. The oceanic islands of Macaronesia, present in NE Atlantic, namely the Madeira and Canaries archipelagos are ideal natural laboratories to study gene flow in benthic marine invertebrates, namely in species with low dispersal capacity, such as the superorder Peracarida, because they are “isolated” from other coasts. In this study, we examined the DNA barcodes in twenty-five peracaridean species (belonging to the orders Amphipoda, Isopoda and Tanaidacea) to investigate allopatric diferentiation between the populations from the archipelagos of Madeira and Canaries and the ones from Iberian Peninsula. This was achieved by detecting a pattern of high genetic distance between populations from these two regions in all species, and by using delimitation molecular methods to find distinct molecular operational taxonomic units (MOTUs) in each species within each region. Globally, between 81 and 98 MOTUs were found in these twenty-five species. Moreover, new records for twenty-one species were found in these regions. The data suggests the existence of a phylogeographic barrier between the archipelagos of Madeira and Canaries and the Iberian Peninsula, which is responsible for a deep genetic differentiation between the populations of peracarideans from these two regions. These results emphasize the genetic heritage hosted by some unprotected areas in Macaronesia, underlining the need to consider organisms with comparatively lower dispersal and the fine-scale endemicity in the design of more effective networks of marine protected areas.

6.2 KEYWORDS

DNA barcoding, Peracarida, Cryptic species, Macaronesia, Northeast Atlantic.

6.3 INTRODUCTION

Establishing species boundaries is crucial for biodiversity assessment (Knowlton 2000, Cook et al. 2008). Traditional approaches for species delimitation only rely on morphological identification. However, relying taxonomy only on morphologic characters might critically underestimate biodiversity, namely in small marine invertebrates with complex morphological traits (Knowlton 1993, Remerie et al. 2006). For instance, in Peracarida, a superorder of the subphylum Crustacea, besides their small size, even family-level diagnostic characters might change with development and gender, making their identification difficult. Moreover, closely related species and genus can have very similar key morphological characters (Harrison and Ellis 1991, Larsen and Wilson 1998, Larsen 2001). Consequently, a combination of multiple approaches (e.g., molecular, morphological and ecological data) is required to accurately access species boundaries (Remerie et al. 2006, Roe and Sperling 2007, Hou et al. 2011). This is particularly true for marine invertebrates species, because some studies have shown the existence of cryptic species, which are species that are genetically distinct, but difficult to distinguish using only morphological characters (Knowlton 2000, Mathews 2006, Witt et al. 2006).

The use of recognized and standardized molecular tools such as the DNA barcoding for specimen identification and delimitation has been shown to be successful in several marine groups (Radulovici et al. 2009, Knebelsberger et al. 2014, Raupach et al. 2015). Its usage has become quite widespread as a tool to species discovery by flagging cryptic species (Radulovici et al. 2009), when complement to morphological identifications (Hebert et al. 2003, Hajibabaei et al. 2006). Despite the growing number of articles reporting hidden diversity in peracaridean species in NE Atlantic (e.g., Costa et al. 2009, Xavier et al. 2011b, Raupach et al. 2014), the Macaronesia region, a group of ocean islands in NE Atlantic, have been neglected in these studies. These islands are the result of volcanic activity and have never been connected with mainland, therefore, their biota is the result of dispersal from distant geographical sources and *in situ* evolution and diversification (Fernández-Palacios et al. 2011). This fact makes these islands an interesting study subject to understand genetic diversity in marine benthic organisms with direct development and theorically low dispersal capacity, and consequently more prone to isolation, such as the Peracarida (Hayward and Ryland 1995).

Recently, we were able to find cryptic diversity with several distinct genetic linages from Macaronesia in the isopod genus *Dynamene* (Chapter 4) and in different morphospecies of the amphipod family Hyalidae (Chapter 5). Using this information as a starting point, we aim in this study, to use DNA barcoding (Cytochrome c Oxidase subunit I - COI) to examine the cryptic diversity of twenty-five peracaridean morphospecies belonging to the orders Amphipoda, Isopoda and Tanaidacea from the NE Atlantic taking in consideration the Macaronesian archipelagos of Madeira, Canaries and Azores, as well the mainland shores of Iberian Peninsula and Morocco. Moreover, we want also, based in chapters 4 and 5, to explore and demonstrate the genetic variation between populations from Iberian Peninsula and the archipelagos of Madeira and Canaries.

6.4 MATERIAL AND METHODS

6.4.1 Specimen sampling and taxonomic identification

Specimens were collected between 2011 and 2015 and sampled during low tide from marine intertidal rocky shores by scraping the algal cover or hand picking during low tide (see Annex 1.3 for sampling details) along the Northeast Atlantic coasts (see Annexes 1.4, 1.5, 6.1 for details).

After collection, specimens were preserved in 96% ethanol. Sequence data and specimen metadata were uploaded in the dataset 'Peracarida Macaronesia vs IberiaPeninsula' (DS-PMACA) within Barcode of Life Data system (BOLD). Morphology-based taxonomic identification was supported in keys for peracarids (Chevreux and Fage 1925, Naylor 1972, Lincoln 1979, Ruffo 1982, Holdich and Jones 1983, Harrison and Ellis 1991, Hayward and Ryland 1995). The species' nomenclature used in this work complies with the accepted nomenclature used in World Register of Marine Species (WoRMS) and Integrated Taxonomic Informations System (ITIS).

For each species, two obligatory groups (Iberian Peninsula *vs* Madeira/Canaries) were created based on the location where they were sampled. The choice of these two regions took in consideration the major genetic differences observed previously in Chapter 4 and 5, in populations sampled in Iberian Peninsula coasts and the archipelagos of Madeira and Canaries (Fig. 6.1). Our aim was to explore if this genetic difference between these two regions would be observable in different peracaridean species. The first group included the specimens sampled in Iberian Peninsula (IP) and the second included the specimens collected in Madeira and Canaries archipelagos (MACA). Additionally, wherever the same species were present in Morocco (MORO) and/or Azores (AZ), these specimens from these regions were added to the analysis (Fig. 6.1).

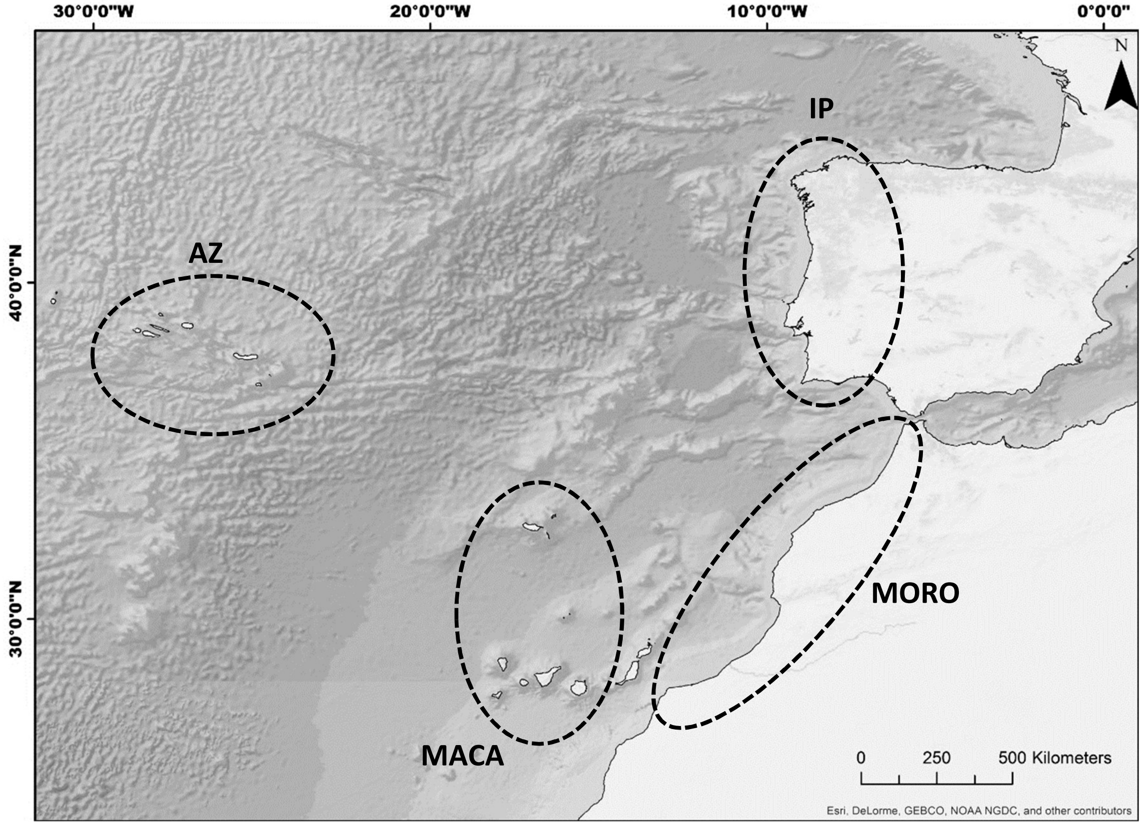

Figure 6.1. Sampling locations according to the groups defined in section 6.4.1.

6.4.2 Genetic analysis and data treatment

DNA extraction, amplification of COI, PCR products purification and sequencing was performed using the methodology previously described in chapter 5 (section 5.3.2). Depending of the specimen size, only a small amount of tissue or the whole animal was used. For details about PCR conditions and primers used, please see section 5.3.2 and Annex 5.2.

Each trace file was edited individually and manually, unreadable zones and primers were removed and ambiguous bases corrected. The resultant sequences were aligned using Clustal W (Thompson et al. 1994) implemented in MEGA 7.0 (Kumar et al. 2016) and inspected for eventual anomalies, such as stop codons or indels in DNASP 5.10 (Librado and Rozas 2009). Sequences of different length were obtained depending of the primer used (see section 5.3.2 and Annex 5.2). Therefore, a common fragment of 520 base pair (bp) obtained from all sequences was used.

GenBank BLASTn search (Altschul et al. 1990) and BOLD Identification System tool (BOLD-IDS) (Ratnasingham and Hebert 2007) were used to search for similarity to confirm the target taxa.

6.4.3 *Genetic diversity and phylogenetic analyses*

Maximum and mean pairwise distances (p-distances) for COI within each species were calculated in MEGA 7.0 (Kumar et al. 2016). Genetic differences between IP and MACA groups were calculated using p-distances in MEGA 7.0 (Kumar et al. 2016).

The Bayesian inference (BI) was conducted in MrBayes 3.2 (Ronquist et al. 2012) to build the Bayesian tree for each order separately. The BI topologies were constructed choosing GTR+G+I as best-fitting model of nucleotide substitution based on its Bayesian Information Criterion as implemented in MEGA 7.0 (Kumar et al. 2016). Runs were conducted with 7×10^6 generations each. Parameters were sampled every 1×10^2 generations. A burn-in of 10% was applied.

6.4.4 *Molecular species delimitation*

Four methods were used to determine the number of MOTUs. Our goal was to understand if each morphospecies corresponded to one or more MOTUs and if these MOTUs matched the groups IP and MACA. Usually, each morphological species matches only a MOTU, however cryptic species match two or more (Hebert et al. 2004). First, we used two distance-based barcode gap approachs. The Automatic Barcode Gap Discovery (ABGD) species delineation tool was performed on a web interface (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) with default settings for the Kimura-2-parameter (K2P) distance matrix (Puillandre et al. 2012). Then, the Cluster Sequences tool implemented in BOLD 4 (http://v4.boldsystems.org) (Ratnasingham and Hebert 2013) was applied.

The web server bPTP (http://species.h-its.org/ptp/), which implements the Poisson tree processes model, was used for the phylogenetic approach (Zhang et al. 2013). Maximum-likelihood (ML) trees for COI were estimated with MEGA 7.0 (Kumar et al. 2016) and used as input. Evolutionary models were selected using also MEGA 7.0 (Kumar et al. 2016) under the corrected Akaike information criterion. Species delimitations were performed using 500 000 Markov chain Monte Carlo iterations with a 20% burn-in.

Finally, the 95% statistical parsimony connection limit was used, by using TCS 1.21 (Clement et al. 2000). This is a common method derived from population genetics to visualize possible intraspecific relationships. Sequences are assigned to networks connected by changes, which are non-homoplastic with a certain probability. Even though this is not equivalent to defining species boundaries, statistical parsimony has also been applied successfully to delimit candidate species before (e.g., Pons et al. 2006, Sauer and Hausdorf 2012).

6.5 RESULTS

6.5.1 Morphological analysis

Twenty-five peracaridean species present in IP and MACA were used in this study. A total of 483 sequences were used, with 137 being novel sequences obtained in this study, 36 obtained in Chapter 2, 168 in Chapter 4 and 116 from Chapter 5 of this thesis. To these, 26 sequences obtained from specimens sampled in Portugal (except for *Stenothoe monoculoides* that was sampled in North Sea and no sequence was available from Iberian Peninsula region) were added, which were retrieved from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) (see Annex 6.1 for number of specimens in each species and source). Of the total number of species, thirteen were amphipod species belonging to ten genera (see Fig. 6.2 for representative of each species): *Apohyale perieri* (Lucas, 1849)*, Apohyale stebbingi* Chevreux, 1888; *Protohyale (Protohyale) schmidtii* (Heller, 1866); *Serejohyale spinidactylus* (Chevreux, 1926); *Caprella acanthifera* Leach, 1814; *Ampithoe ramondi* Audouin, 1826; *Ampithoe helleri* Karaman, 1975; *Ampithoe riedli* Krapp-Schickel, 1968; *Podocerus variegatus* Leach, 1814; *Stenothoe monoculoides* (Montagu, 1815)*; Quadrimaera inaequipes* (A. Costa, 1857)*; Jassa herdmani* (Walker, 1893) and *Elasmopus pectenicrus* (Spence Bate, 1862). Nine were isopod species belonging to seven genera (see Fig. 6.3 for representative of each species)*: Gnathia maxillaris* (Montagu, 1984)*; Anthura gracilis* (Montagu, 1808)*; Joeropsis brevocornis* Koehler, 1885; *Dynamene magnitorata* Holdich, 1968; *Dynamene edwardsi* (Lucas, 1849); *Dynamene bidentata* (Adams, 1800); *Cymodoce truncata* Leach, 1814; *Campecopea lusitanica* (Nolting, Reboreda & Wägele, 1998) and *Janira maculosa* Lach, 1814. Three were tanaidacean species belonging to two genera (see Fig. 6.4 for representative of each species): *Tanais dulongii* (Audouin, 1826); *Tanais grimaldii* (Dollfus, 1897) and *Apseudopsis latreilli* (Milne Edwards, 1828).

Additionally to the general identification keys used before (Chevreux and Fage 1925, Naylor 1972, Lincoln 1979, Ruffo 1982, Holdich and Jones 1983, Harrison and Ellis 1991, Hayward and Ryland 1995), the software package DELTA (DEscription Language for TAxonomy) with the interactive identification keys (INTKEY) for amphipods, isopods and tanaids (Dallwitz et al. 2000, Lowry and Springthorpe 2001, Larsen 2002, Keable et al. 2002, Oliver Coleman et al. 2010) and updated identification keys for the genera *Gnathia* (Hispano et al. 2014), *Dynamene* (Holdich 1968a, Vieira et al. 2016), *Campecopea* (Harrison and Ellis 1991, Nolting et al. 1998, Bruce and Holdich 2002), *Cymodoce* (Harrison and Ellis 1991, Khalaji-Pirbalouty et al. 2013, Khalaji-Pirbalouty and Raupach 2014), *Anthura* (Poore 2001) *Apseudopis* (Esquete et al. 2012, 2016), *Tanais* (Bamber and Robbins 2009, Bamber 2012), *Caprella* (Riera et al. 2003, Lacerda and

Masunari 2011, Guerra-García 2012, Guerra-García et al. 2013), *Jassa* (Conlan 1990, Krapp-Schickel et al. 2008), *Elasmopus* (Lowry and Hughes 2009, Vader and Krapp-Schickel 2012, Gouillieux and Sorbe 2015, Alves et al. 2016), *Stenothoe* (Krapp-Schickel 2006, 2015) and *Ampithoe* (Conlan 1982, Hughes et al. 2008), were also used to accuretly identify the specimens. Checklists for Northeast Atlantic and Macaronesia were used to verify species presence and distribution (Krapp-Schickel and Ruffo 1990, Castelló and Carballo 2001, Costello et al. 2001, Junoy and Castelló 2003, Pereira et al. 2006, Castelló and Junoy 2007, Boyko et al. 2008, Borges et al. 2010, Izquierdo and Guerra-García 2011, Guerra-García et al. 2011, Anderson 2016, Horton et al. 2017a, b).

Twelve of the twenty-five species analysed were present in Azores and nine in Morocco (Table 6.1). *Janira maculosa* and *Apseudopsis latreillii* were the least represented species (only six specimens each), while *Dynamene edwardsi* (99 specimens) and *Apohyale stebbingi* (36 specimens) were the ones with the higher number of individuals.

6.5.2 Molecular analysis

Mean intraspecific distance (ISD) varied between 0.36% (*Dynamene magnitorata*) and 17.16% (*Janira maculosa*), while Maximum (Max) ISD was higher than 3% for all species except *D. magnitorata* (2.46%) *and D. bidentata* (1.23%) (Table 6.1). Mean p-distances between IP and MACA regions were always higher than 3% (except once again for *D. magnitorata and D. bidentata)*, with the highest value observed in the isopod *Anthura gracilis* (28.00%, Table 6.1).

The molecular species delineation methods applied in the twenty-five species retrieved between 81 (by ABGD-K2P) and 98 (TCS 95%) MOTUs depending of the method applied (Table 6.2). Amphipod species originated between 41 and 53 MOTUs (Fig. 6.5, Table 6.2), isopods between 27 and 31 MOTUs (Fig. 6.6, Table 6.2) and tanaidaceans between 11 and 14 MOTUs (Fig. 6.7, Table 6.2). A major rule (most commom number of MOTUs for each species) was applied and in case of a tie, a conservative approach was applied (lowest number of MOTUs). The consensus number of MOTUs was 90 (Table 6.2), with the species *D. bidentata* and *D. magnitorata* with just one MOTU each and the species *D. edwardsi* and *Apohyale stebbingi* with 9 and 11 MOTUs each respectively (Table 6.2).

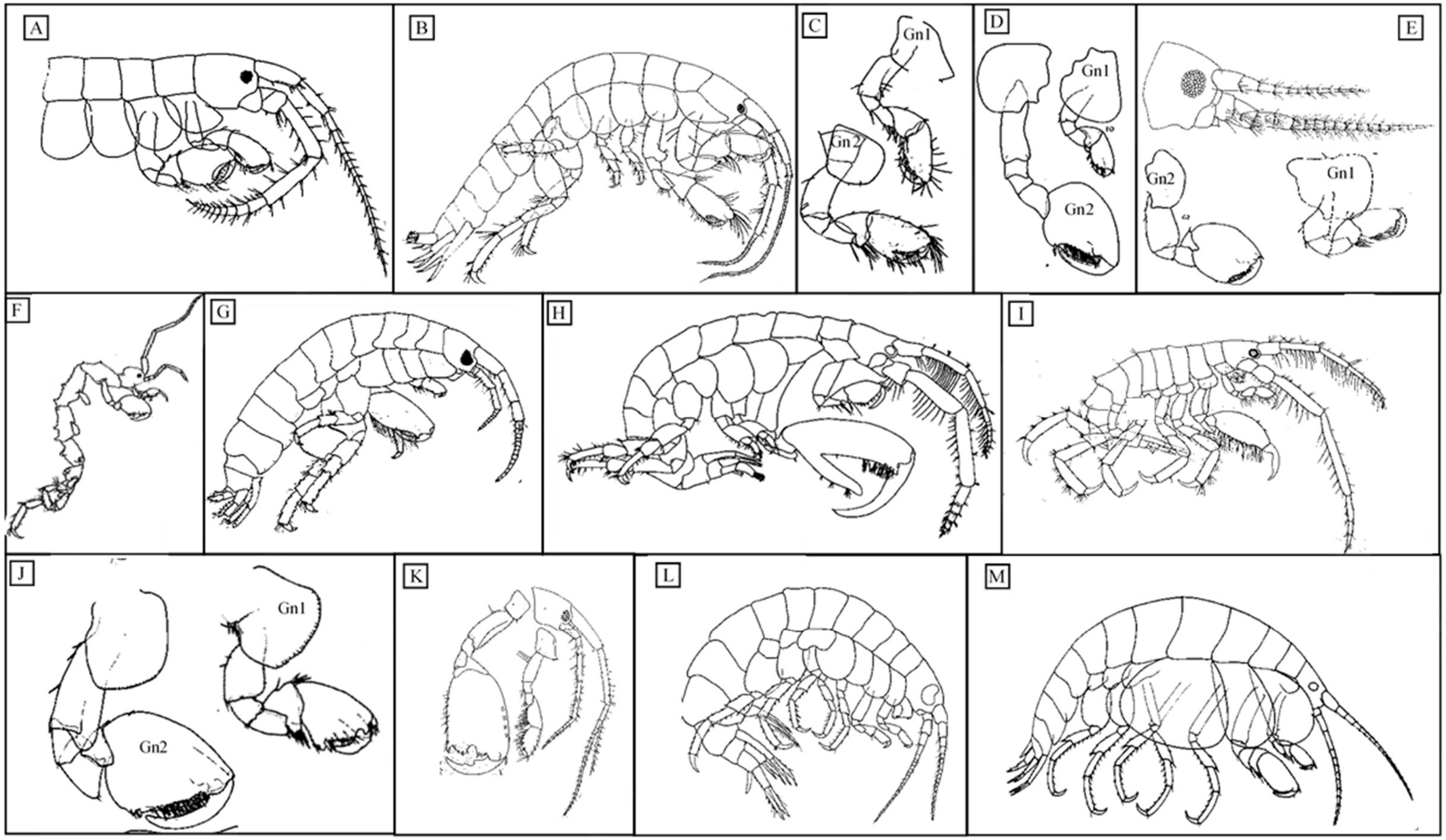

Figure 6.2. Representative figures of the amphipods species used (not to scale). **A**- *Ampithoe helleri* (♂); **B** - *Ampithoe ramondi* (♂); **C** - *Ampithoe riedli* (♂) gnathopods 1 (Gn1) and 2 (Gn2); **D** – *Apohyale perieri* (♂) Gn1 and Gn2; **E** – *Apohyale stebbingi* (♂) head, Gn1 and Gn2; **F** - *Caprella acanthifera* ("sensu lato" ♂); **G** - *Elasmopus pectenicrus* (♂); **H** - *Jassa herdmani* (thumbed ♂ "major form"); **I** – *Podocerus variegatus* (♂); **J** – *Protohyale (Protohyale) schmidtii* ("Hyper form" ♂) Gn1 and Gn2; **K** - *Quadrimaera inaequipes* (♂) head, Gn1 and Gn2; **L** - *Serejohyale spinidactylus* (♂); **M** - *Stenothoe monoculoides* (♀). Adapted from: A (as *Ampithoe neglecta*), E, M - (Lincoln 1979); B, C (as *Hyale perieri*), D (as *Hyale stebbingi*), I, J (as *Hyale schmidtii*), K - (Ruffo 1982); F - (Zeina et al. 2015); G - (Lowry and Hughes 2009); H - (Beerman and Purz 2013); L (as *Hyale spinidactyla*) - (Krapp-Schickel et al. 2011).

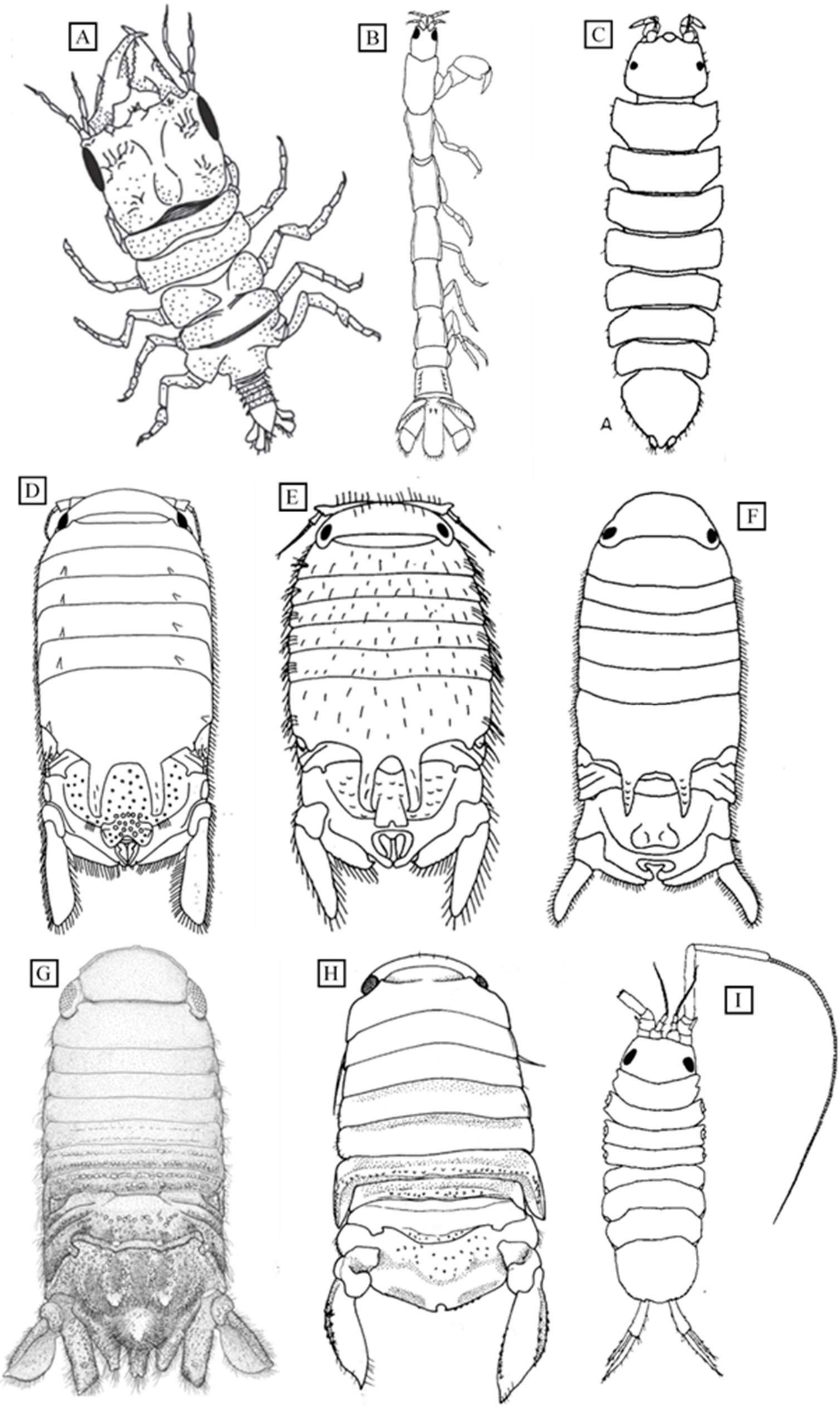

Figure 6.3. Representative figures of the isopod species used (not to scale). **A** - *Gnathia maxillaris* (Adult ♂); **B** -*Anthura gracilis* (♀); **C** - *Joeropsis brevocornis*; **D** - *Dynamene magnitorata* (Adult ♂); **E** - *Dynamene edwardsi* (Adult ♂); **F** - *Dynamene bidentata* (Adult ♂); **G** - *Cymodoce truncata* (Adult ♂); **H** - *Campecopea lusitanica* morpho A (Adult ♂); **I** - *Janira maculosa*. Adapted from: A – (Hispano et al. 2014); B, C, F, I – (Naylor 1972); D, E – (Holdich 1968a); G – (Khalaji-Pirbalouty et al. 2013); H – (Bruce and Holdich 2002).

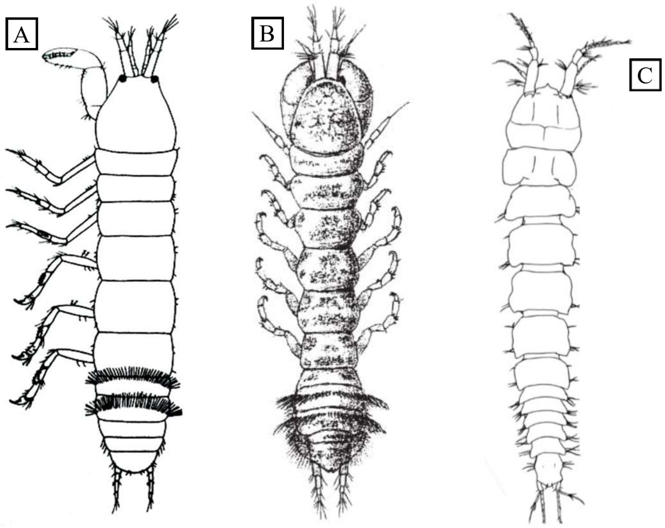

Figure 6.4. Representative figures of the tanaidacean species used (not to scale). **A** – *Tanais dulongii* (Adapted from Holdich and Jones 1983); **B** - *Tanais grimaldii* (Adapted from Bamber and Costa 2009); **C** - *Apseudopsis latreilli* (Adapted from Esquete 2012).

Table 6.1. Presence (●) of the peracaridean species used in this study in each region defined in section 6.4.1. Mean and Max ISD for each species and the Mean p-distance between the Iberian Peninsula (IP) and Madeira and Canaries archipelagos (MACA) for each species are also displayed.
**Stenothoe monoculoides* was retrieved from North Sea instead of IP.

Order	Species	IP	MACA	AZ	MORO	Mean ISD	Max ISD	Mean p-distances between IP and MACA
Amphipoda	*Ampithoe helleri*	●	●			0.0715	0.1327	0.1230
Amphipoda	*Ampithoe ramondi*	●	●	●		0.0181	0.0385	0.0341
Amphipoda	*Ampithoe riedli*	●	●		●	0.0439	0.0827	0.0782
Amphipoda	*Apohyale perieri*	●	●	●		0.0483	0.1135	0.0770
Amphipoda	*Apohyale stebbingi*	●	●	●	●	0.1243	0.2000	0.1574
Amphipoda	*Caprella acanthifera*	●	●	●	●	0.0805	0.1462	0.1374
Amphipoda	*Elasmopus pectenicrus*	●	●		●	0.0381	0.0635	0.0583
Amphipoda	*Jassa herdmani*	●	●	●		0.0751	0.1362	0.1237
Amphipoda	*Podocerus variegatus*	●	●			0.0613	0.1019	0.0974
Amphipoda	*Protohyale schmidtii*	●	●	●	●	0.0693	0.1346	0.1087
Amphipoda	*Quadrimaera inaequipes*	●	●			0.0911	0.1596	0.1357
Amphipoda	*Serejohyale spinidactylus*	●	●	●		0.1152	0.1769	0.1348
Amphipoda	*Stenothoe monoculoides**	●	●			0.1637	0.2765	0.2765
Isopoda	*Anthura gracilis*	●	●	●	●	0.1521	0.2846	0.2800
Isopoda	*Campecopea lusitanica*	●	●			0.1012	0.1981	0.1226
Isopoda	*Cymodoce truncata*	●	●	●		0.1263	0.2019	0.1619
Isopoda	*Dynamene bidentata*	●	●		●	0.0036	0.0154	0.0123
Isopoda	*Dynamene edwardsi*	●	●	●	●	0.1140	0.1865	0.1643
Isopoda	*Dynamene magnitorata*	●	●	●		0.0076	0.0269	0.0246
Isopoda	*Gnathia maxillaris*	●	●			0.1324	0.2038	0.2000
Isopoda	*Janira maculosa*	●	●			0.1715	0.2673	0.2564
Isopoda	*Joeropsis brevicornis*	●	●			0.1252	0.2500	0.2462
Tanaidacea	*Apseudopsis latreilii*	●	●			0.1674	0.2404	0.2372
Tanaidacea	*Tanais dulongii*	●	●		●	0.0840	0.1192	0.1150
Tanaidacea	*Tanais grimaldii*	●	●	●		0.0919	0.1481	0.1065

Table 6.2. Results of the different molecular species delineation methods applied in this study.

Order	Species	ABGD K2P	MOTU BOLD	TCS 95%	bPTP	Consensus number of MOTUs
Amphipoda	*Ampithoe helleri*	3	3	3	3	3
Amphipoda	*Ampithoe ramondi*	1	2	2	2	2
Amphipoda	*Ampithoe riedli*	2	2	3	2	2
Amphipoda	*Apohyale perieri*	4	4	4	4	4
Amphipoda	*Apohyale stebbingi*	9	13	11	11	11
Amphipoda	*Caprella acanthifera*	3	4	4	4	4
Amphipoda	*Elasmopus pectenicrus*	2	3	4	3	3
Amphipoda	*Jassa herdmani*	2	2	3	3	2
Amphipoda	*Podocerus variegatus*	3	3	3	3	3
Amphipoda	*Protohyale (Protohyale) schmidtii*	2	5	6	2	2
Amphipoda	*Quadrimaera inaequipes*	3	3	3	3	3
Amphipoda	*Serejohyale spinidactylus*	5	5	5	5	5
Amphipoda	*Stenothoe monoculoides*	2	2	2	2	2
Isopoda	*Anthura gracilis*	4	5	5	5	5
Isopoda	*Campecopea lusitanica*	2	3	2	3	2
Isopoda	*Cymodoce truncata*	4	4	4	4	4
Isopoda	*Dynamene bidentata*	1	1	1	1	1
Isopoda	*Dynamene edwardsi*	7	9	9	9	9
Isopoda	*Dynamene magnitorata*	1	1	2	1	1
Isopoda	*Gnathia maxillaris*	3	3	3	3	3
Isopoda	*Janira maculosa*	3	3	3	3	3
Isopoda	*Joeropsis brevicornis*	2	2	2	2	2
Tanaidacea	*Apseudopsis latreilli*	3	3	3	3	3
Tanaidacea	*Tanais dulongii*	6	6	6	4	6
Tanaidacea	*Tanais grimaldii*	4	5	5	4	5
	Total	81	96	98	89	90

To better discriminate the different species in the phylogenetic analysis, sister taxa (25 species, 32 sequences) commom in IP and/or MACA (see section 6.5.1 for checklist of references consulted) were added to the analysis (see Annex 6.1 for list of species, number of specimens and source). Of these, 17 species (24 sequences) were obtained in this thesis. In all orders, BI phenogram clearly discriminated the different species (Fig. 6.5 for Amphipoda, Fig. 6.6 for Isopoda and Fig. 6.7 for Tanaidacea). Species of the same genus or family grouped in general together and MOTUs matched the different linages. Two different main clusters were most of the cases patent, corresponding with IP and MACA, with AZ and MORO lineages matching IP or MACA depending of the species (Figs 6.5, 6.6, 6.7).

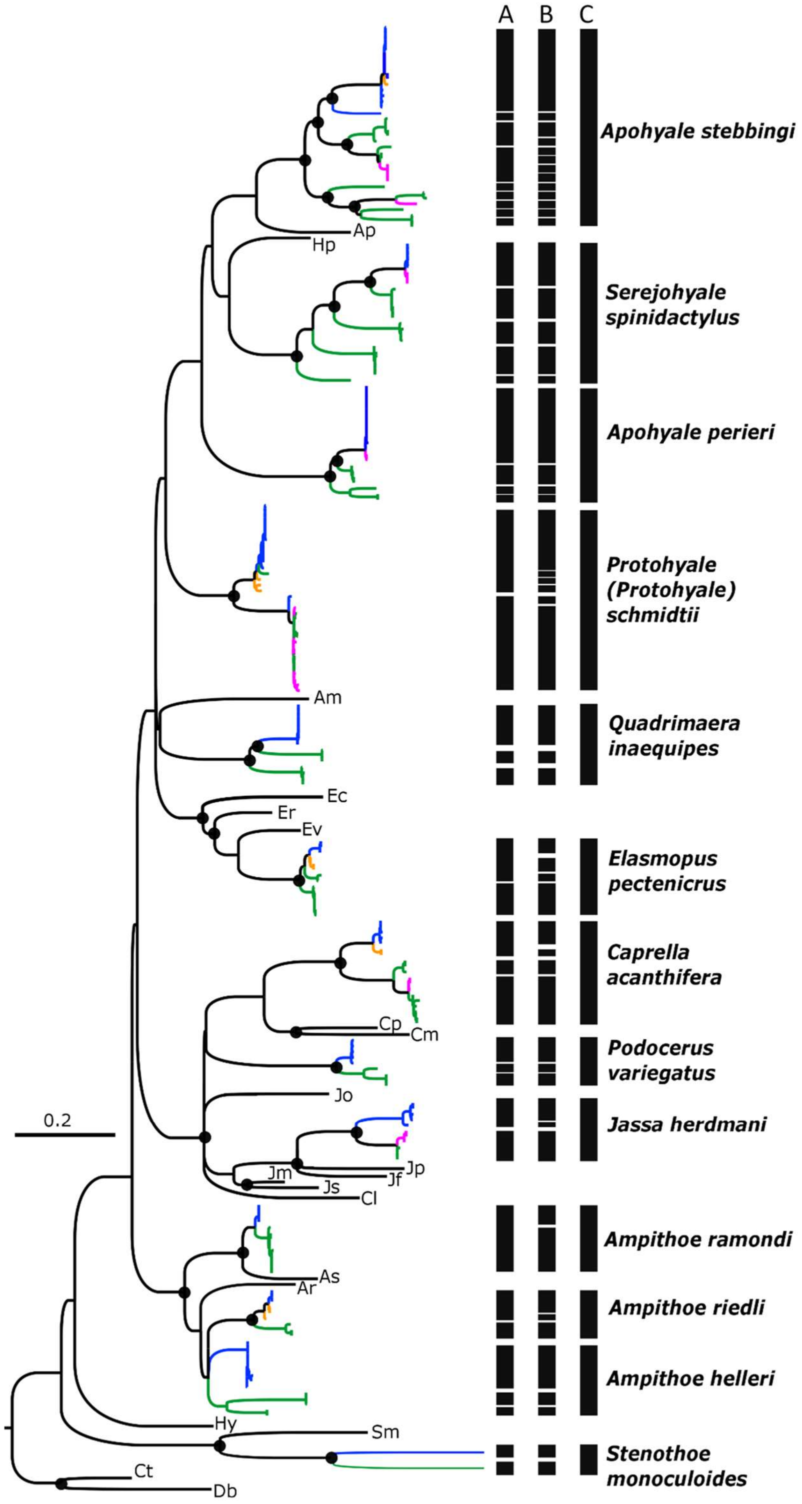

Figure 6.5. Bayesian clade credibility tree based on COI sequences of the amphipod species used in this study. Dotes (●) associated with nodes represent posterior probabilities higher than 0.75. Vertical black bars correspond to MOTUs obtained by the different methods of species delimitation applied (Table 6.2): A – lowest number of MOTUs, B – maximum number of MOTUs, C – morphospecies. *Cymodoce truncata* (Ct) and *Dynamene bidenatata* (Db) were used as outgroup. Lineages colours according with region: blue-IP, green-MACA, purple – AZ, orange – MORO.

Ap - *Apohyale prevostii*; Hp – *Hyale pontica*; Am – *Apohyale media*; Ec – *Elasmopus canarius*; Er – *Elasmopus rapax*; Ev – *Elasmopus vachoni*; Cp – *Caprella penantis*; Cp – *Caprella mutica*; Jo – *Jassa ocia*; Jp – *Jassa pusilla*; Jm – *Jassa marmorata*; Js – *Jassa slatteryi*; Cl – *Capella liparotensis*; As – *Ampithoe sp.*; Ar – *Ampithoe rubricata*; Hy – Hyalinae; Sm – *Stenothoe marina*.

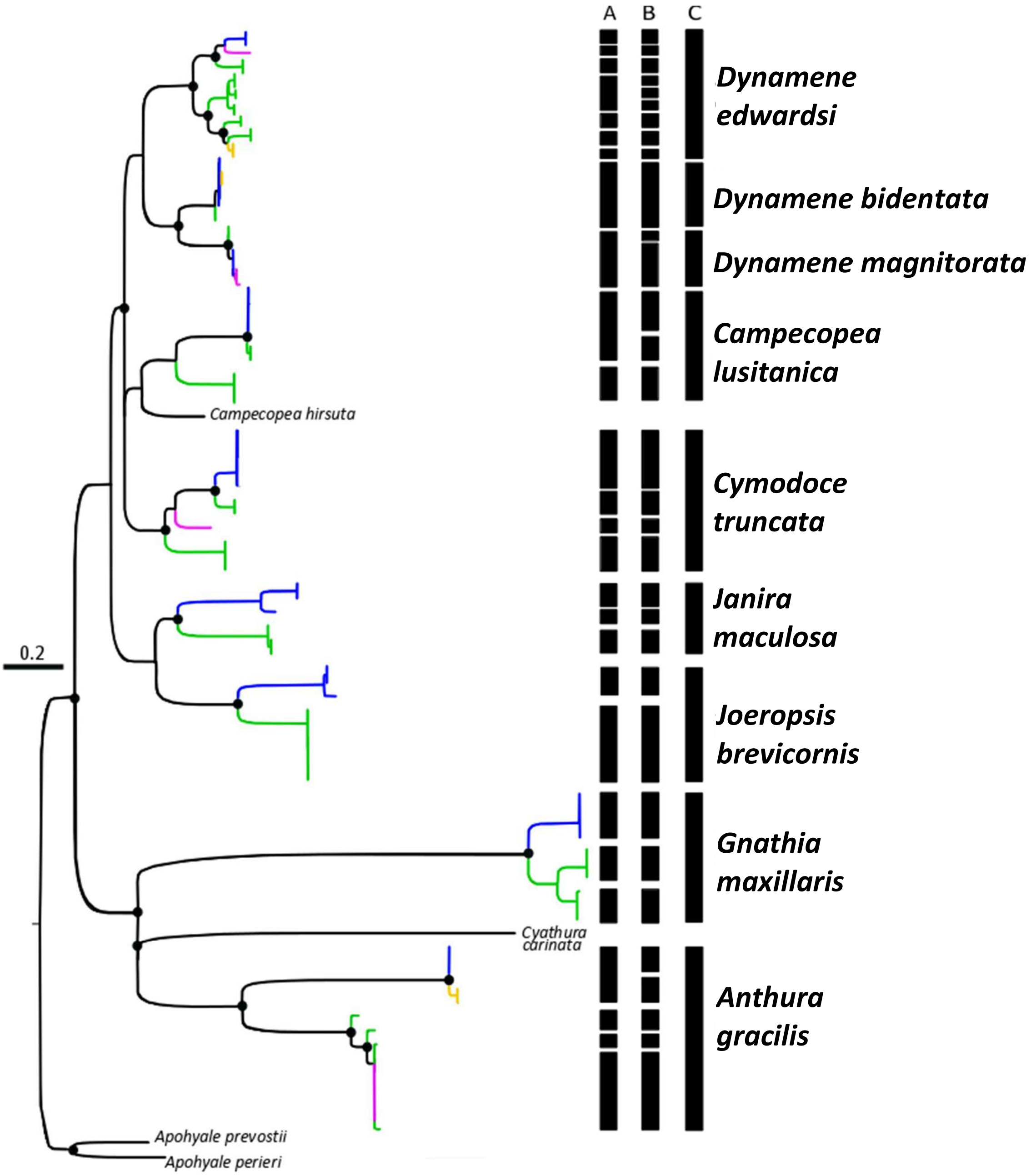

Figure 6.6. Bayesian clade credibility tree based on COI sequences of the isopod species used in this study. Dotes (●) associated with nodes represent posterior probabilities higher than 0.75. Vertical black bars correspond to MOTUs obtained by the different methods of species delimitation applied (Table 6.2): A – lowest number of MOTUs, B – maximum number of MOTUs, C – morphospecies. *Apohyale prevostii* and *Apohyale perieri* were used as outgroup. Lineages colours according with region: blue-IP, green-MACA, purple – AZ, orange – MORO.

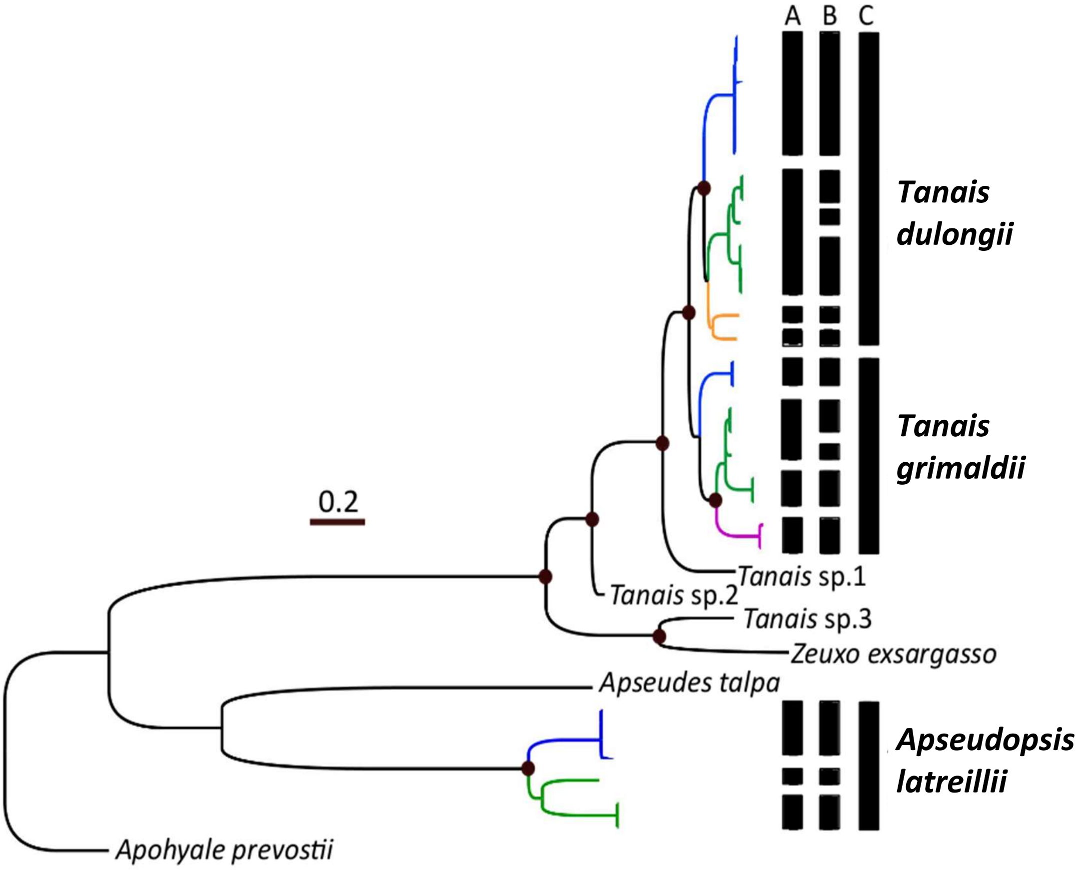

Figure 6.7. Bayesian clade credibility tree based on COI sequences of the tanaid species used in this study. Dotes (●) associated with nodes represent posterior probabilities higher than 0.75. Vertical black bars correspond to MOTUs obtained by the different methods of species delimitation applied (Table 6.2): A – lowest number of MOTUs, B – maximum number of MOTUs, C – morphospecies. *Apohyale prevostii* was used as outgroup. Lineages colours according with region: blue-IP, green-MACA, purple – AZ, orange – MORO.

6.6 DISCUSSION

6.6.1 New records for Macaronesia and Moroccan coasts

By using DNA barcodes of twenty-five representative species of three different peracaridean orders (Amphipoda, Isopoda, Tanaidacea), we were able to unravel hidden cryptic diversity. Up to 81-98 putative cryptic species were discovered, with a clear discrimination between the populations from Southern European Atlantic coasts and the populations from Madeira and Canary Islands. Moreover, to the best of our knowledge, in this study, the following peracaridean species were recorded in Macaronesia Islands and Morocco for the first time: *Anthura gracilis* in Tenerife, Porto Santo, Selvagens, Gran Canaria and La Palma; *Campecopea lusitanica* in Porto Santo, Gran Canaria and La Palma; *Cymodoce truncata* in Porto Santo, Madeira and La Palma; *Gnathia maxillaris* in Gran Canaria, La Palma and Porto Santo; *Janira maculosa* in La Palma; *Joeropsis brevicornis* in Tenerife, La Palma and Madeira; *Apseudopsis* *latreillii* in Porto Santo and Gran Canaria; *Tanais dulongii* in La Palma and Morocco; *Tanais grimaldii* in Selvagens and Porto Santo; *Zeuxo exsargasso* in Porto Santo; *Ampithoe helleri* in La Palma; *Ampithoe ramondi* in Tenerife and Madeira; *Ampithoe riedli* in Morocco, Madeira and La Palma; *Caprella acanthifera* in El Hierro, La Palma, Morocco and Tenerife; *Elasmopus canarius* in La Palma and El Hierro; *Elasmopus pectenicrus* in Tenerife, Porto Santo, Morocco and Madeira; *Elasmopus vachoni* in La Palma; *Jassa herdmani* in Madeira, Porto Santo and São Miguel; *Podocerus variegatus* in La Palma and Porto Santo; *Quadrimaera inaequipes* in Gran Canaria, La Palma and Madeira and *Stenothoe monoculoides* in Tenerife (see Annex 6.1 for details).

6.6.2 Phylogeographic discontinuity between Macaronesia and adjacent continental coasts

Twenty-three species (of the twenty-five used) displayed higher ISD than 3%, which is above the value found in most of the established species of crustaceans where COI barcode variation was examined (Costa et al. 2007). The only species with a lower value were *Dynamene bidentata* (1.54%) and *Dynamene magnitorata* (2.69%). These two species were the only species with just one representative sequence for MACA region (and the only ones with less than three sequences). Interestingly, the use of the same delimitation methods (ABGD-K2P and bPTP) applied to COI used in this study, and in chapter 4, retrieved different number of MOTUs (2 *vs* 1 respectively) for *D. magnitorata*. The reason for this difference was the the length of the COI fragment examined: 520 bp in this study *vs* 658 bp used in chapter 4. This strenghtens the idea that the sequence length, and also multilocus approaches are crucial to correctly delineate species based on molecular data (Delić et al. 2017).

The variation between the populations from IP and MACA regions within each species (excluding *D. bidentata* and *D. magnitorata*) ranged between 3.41% (*Ampithoe ramondi*) and 28.00% (*Anthura gracilis*). As observed in chapters 4 and 5, this information suggests little or no gene flow between populations from these two regions. Phylogeographic discontinuities were reported before in marine environments worldwide e.g., between Patagonia and Falkland islands (Lesse et al. 2008), in the Alboran basin (Xavier et al. 2011a), between east and west Mediterranean (Arnaud-Haond et al. 2007), in the eastern Pacific in North America (Markow and Pfeiler 2010) and in the eastern Pacific in South America (Varela and Haye 2012), but little is known in the NE Atlantic. Sá-Pinto et al. (2008), Xavier et al. (2010) and Domingues et al. (2008) also verified in sponges, gastropods and fish respectively, reduced gene flow and therefore genetic variation between populations from Madeira and Canary islands and those from Iberian Peninsula, suggesting that a possible marine phylogeographic barrier exists for marine fauna.

Peracaridean species have direct development, lacking a pelagic larval phase and therefore being highly restricted in their dispersal (Hayward and Ryland 1995). Long dispersal events are uncommom and dispersal of individuals happens locally by swimming or crawling, or passively through rafting on floating objects or mediated by human vectors (Thiel and Gutow 2005). These species are more susceptible to isolation when compared to species with dispersive larval phases and effective gene flow between distant populations can be rare (Varela and Haye 2012). An exception to direct development (in peracarids) is *Gnathia maxillaris*, which is a fish ectoparasite during some phases of its larval stage, interweaving with periods in benthic habitats, while the adults inhabit shallow waters (Hispano et al. 2014). Nevertheless, and although each peracaridean species has a distinct life cycle, a deep genetic differentiation between the populations from MACA and IP appears to be a common feature to many of them, suggesting that other factors than geographic distance may play a major role in the allopatric fragmentation within these species. Moreover, the fact that this fragmentation has been also observed in species with a larval dispersal phase (e.g., *Gnathia maxillaris*, sponges and gastropods) suggests that even in for organisms with presumably higher dispersal capacity, there is evidence for a sweeping phylogeography discontinuity for marine invertebrates in that region of the NE Atlantic.

While a clear differentiation between MACA and IP populations was patent in all the species studied, the populations from Azores and Morocco displayed affinities to either one, depending on the species. The populations of the Azores archipelago are usually presumed to have higher affinities with the ones from Madeira and Canaries archipelagos (Santos et al. 1995), due to the Canaries current (Arístegui et al. 2009). Previous works showed genetic affinities between these archipelagos in marine invertebrates (Hawkins et al. 2000, Sá-Pinto et al. 2008), while others suggest stronger affinities between Azorean and Iberian populations (Xavier et al. 2010). In our work, the two patterns were observed, with 8 species showing higher proximity between Azorean populations and MACA, and 4 with IP. In most of the peracaridean species here studied (7 out of 9), a higher affinity between Moroccan and IP populations was found, which contradicts other studies that relate the populations from Morocco with those from Macaronesia, due to their proximity, and/or differentiate them from the IP populations (Santos et al. 1995, Cabezas et al. 2013a, Xavier et al. 2011a, 2016).

6.6.3 Macaronesian conservation status

Accurate species delimitation is an essential starting framework for conservation strategies, since species are the basic units for distributional and habitat studies in biodiversity assessment. However, the species concept is probably the most controversial issue in biology (Coyne and Orr 2004). Species boundaries are a conceptual tool used to organize the diversity of life into categories. This started with the classification of organisms into "species" based on their phenotype and inferred reproductive compatibility, but with the emergence of molecular methods, arose also the importance of the genotype for species delineation (Radulovici et al. 2010). Through the use of molecular methods, MOTUs could be considered as the functional units of biodiversity and might act as proxies for estimating diversity (Hey 2006). Concepts such as "Evolutionary Significant Units" (ESU) help surpassing the limitations imposed by rigid species boundaries, enabling the recognition of pertinent infraspecific units for the purpose of biodiversity conservation (Casacci et al. 2014). Hence, regardless of the formal species boundaries of the peracarids here investigated, it appears there is an extraordinary level of endemicity of genetic lineages and ESUs with very small ranges, frequentely no larger than the island that harbours them.

Management strategies greatly benefit from molecular tools in understanding the processes involved in promoting and maintaining biodiversity. For instance, zooplankton species diversity and distribution have been used as an important measure of environmental change in the Arctic Ocean (Bucklin et al. 2007, Hunt et al. 2010). The evolution of natural populations requires genetic variation, and the higher this is, the greater will be the ability to respond satisfactorily to natural stochastic changes or derived from human activities. Therefore, the preservation of genetic diversity should be considered an essential factor in the design of marine conservation strategies by including areas that represent fundamental evolutionary processes (Avise 2000, Allendorf and Luikart 2007).

In the marine environment, priority should be given to the conservation of those species most vulnerable to human activities and those whose populations are in a degree of degradation that endangers the survival of the species or local populations. In this sense, population genetics and empirical studies indicate that island and endemic species are more likely to become extinct than continental or non-endemic species (Frankham 1998). On the other hand, the human activities that mainly affect the marine environment usually take place in coastal areas, whose extension is limited and is where the highest marine productivity is reached (Allendorf and Luikart 2007).

Many Macaronesia islands and sites are included in some kind of marine protected areas (e.g. under Natura 2000, http://ec.europa.eu/environment/nature/natura2000/index_en.htm), and in several cases comprising specific protection regimes for marine mammals or reptiles such as *Caretta caretta* and *Monachus monachus*, or habitats such as reefs. However, marine invertebrates are often neglected, especially in cases of non-engineering species, and little information is known about the status of each species/MOTU/ESU or population. Although some marine invertebrate species have been used to try to understand the genetic affinities between the populations from different islands (Quintero et al. 2012), the peracarideans, which are one of most abundant benthic groups, have received little or nearly none attention (Krapp-Schickel and Ruffo 1990, Castelló and Junoy 2007). This study demonstrated that percacaridean species need to be consider in the design of more effective networks of marine protective areas and genetic information should be consider in order to protect the “endemisms” that each island/group of islands hosts. Moreover, most of the MOTUs detected in this study (and in this thesis) occur in non-protected areas and islands, hence further consideration must be given to the pertinence of their incorporation in marine protected areas.

6.7 CONCLUSIONS

Marine biodiversity is still greatly understimated, namely in what concerns with marine invertebrate fauna (Radulovici et al. 2010). The existence of cryptic diversity is suspected in many small marine invertebrates with complex morphological traits (Knowlton 1993, Remerie et al. 2006), but its occurence in the Peracarida fauna of the NE Atlantic is still poorly documented. Using DNA barcoding, our study revealed a staggering number of MOTUs (between 81 and 98) in only twenty-five peracaridean species belonging to the orders Amphipoda, Isopoda and Tanaidacea from NE Atlantic and Macaronesia. Moreover, a global pattern of deep genetic differentation between the Iberian Peninsula and Macaronesian populations was observed in all species. These remarkable findings indicate that the role of Macaronesia as an hotspot for the evolution and diversification of these marine invertebrates has been greatly unappreciated. Hence, the unique genetic heritage hosted by some non-protected areas in Macaronesia, claims for consideration of the fine-scale endemicity, and for organisms with comparatively lower dispersal, in the design of more effective networks of marine protected areas.

6.8 ACKNOWLEDGEMENTS

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Chapter 7: Concluding remarks and future perspectives

7.1 OVERVIEW: CONTEXT AND ORIGINALITY

Human activities have been generating an increasing impact on the loss of ocean biodiversity (McCauley et al. 2015). However, impact assessments and monitoring initiatives are usually based on large and conspicuous species that represent a minor fraction of marine diversity. Smaller organisms, such as marine invertebrates, which play important ecological roles and that may reflect more comprehensively the impacts of environmental disturbances on the ecosystems, remain overlooked in many biodiversity surveys. This highlights a major limitation in the ability to correctly monitor biological communities (Leray and Knowlton 2016). With most of the marine eukaryotic species still to be described (Mora et al. 2001, Appeltans et al. 2012), it is crucial to improve the knowledge of diagnostic morphological characters, train additional taxonomic experts, increase the number of sampling areas and habitats, and incorporate molecular tools in species descriptions (Wägele et al. 2011, Costello et al. 2012, Leray and Knowlton 2016). Additionally, failure to recognize cryptic diversity, will result in considerable underestimation of the extant biodiversity (Radulovici et al. 2010). Therefore, it is crucial to combine morphological and molecular tools to correctly access biodiversity (Knowlton 1993).

The knowledge of peracaridean fauna of Northeast (NE) Atlantic, namely in southern regions such as Iberian Peninsula, Morocco and Macaronesia, is still poor when compared with other groups or/and regions. Through the comprehensive examination of multiple species in this important order of crustaceans, this thesis contributed not only to considerable progress on the knowledge of the peracaridean biodiversity in NE Atlantic - unravelling unexpectedly high levels of cryptic diversity in a wide range of species - but it also disclosed the prominent role of the Macaronesian archipelagos in promoting the genetic diversification, evolution and speciation of these, and possibly other marine invertebrates, in the NE Atlantic.

7.2 OVERVIEW: MAIN FINDINGS

One of the main contributions of this thesis was the creation of a core reference library of DNA barcodes for marine peracarids of the Iberian Peninsula. Novel and publicly available data was used from more than one hundred species to build this library. Deeply divergent intraspecific variation was found in nineteen morphospecies, suggesting the existence of considerable overlooked taxonomic diversity. With the rise of modern high-throughput sequencing technologies, reference datasets such as ours are essential for the correct identification of specimens sequenced as part of meta barcoding studies (Leese et al. 2016).

Additionally, the correct verification of available data is crucial to detect misidentifications and to detect distinct genetic populations.

A second significant contribution of this thesis was the detailed investigation of the isopod genus *Dynamene* present in the Northern Hemisphere, which had several incorrect assignments, questioning the validity of the information about the correct distribution and taxonomy of the species. This was done by examining new records from the Northeast Atlantic Ocean and its associated islands, the Mediterranean, Black and Red Seas, from re-examination of museum and several authors' personal collections and from literature. Illustrated keys for adult males and females are provided to help other researchers to easily and correctly identify the members of this genus. The distribution of the six *Dynamene* species along the Northeast Atlantic-Black Sea axis was largely extended, namely in Macaronesian islands.

In the fourth chapter, twelve molecular operational taxonomic units (MOTUs) were found among the only three *Dynamene* species present in NE Atlantic, with nine belonging to *Dynamene edwardsi*. Application of multiple mtDNA and nuclear locus essentially confirmed the major and more deeply divergent MOTUs, although, without surprise, nuclear markers could not discriminate all 9 MOTUs observed for COI barcodes (e.g. Delic et al. 2017). All together this constitutes strong evidence for the existence of multiple completed sorted evolutionary lineages within this isopod, and that *D. edwardsii* is in fact a complex of 9 cryptic species. Interestingly, each *D. edwardsi* MOTU was clearly delimited geographically, and some of them were even exclusive for single islands. While it was clear that most of the diversity in this species occured in the islands, no single, all-encompassing evolutionary pattern could be inferred to explain the observed genetic structure. This is probably a result of the high stochasticity of long distance dispersal events and the inability of eventual migrants to genetically displace the resident lineages. Assuming as genuine the putative cryptic species here reported, it would represent a staggering three-fold increase in the known species diversity for this genus in the northern hemisphere, which currently has only six species documented (Vieira et al. 2016).

In the fifth chapter, seven species of the amphipod family Hyalidae were used to contrast DNA barcodes of populations Macaronesia islands with those from continental coasts. Higher genetic diversity and higher number of MOTUs were found in Macaronesian region, which reinforce the role that these islands have in the isolation and diversification of peracaridean species. Moreover, this was the first study to report cryptic diversity in the order Amphipoda in Macaronesia islands.

In the sixth chapter, by means of using a multi-species approach (twenty-five peracaridean species), our study revealed a stunning number of MOTUs (between 81 and 98) and we were able to demonstrate a global pattern of genetic differentation between Iberian Peninsula and Macaronesian populations patent in numerous peracaridean species. These evidences underline the genetic heritage that Macaronesia holds and its role as an hotspot for the evolution and diversification of peracarids and are a strong case underlining the need to consider these marine invertebrates in conservation studies and in the design of more effective networks of marine protected areas.

7.3 FUTURE PERSPECTIVES

This study emphasizes the different kinds of methods and criteria that can be use for species identification/delineation (morphological and phylogenetic). Although, these two main concepts offer limitations (see Hey 2006 for details), the complementary aspects of both aproaches tend to convey more accurate species delineations. This thesis extended considerably the knowledge of biodiversity of NE Atlantic peracaridean fauna and opened innumerous possibilities for further studies, namely in the exploration of the exceptional role of Macaronesian archipelagos for the understanding of the phylogeography and evolution of marine invertebrates in the NE Atlantic. As follow up of this work, we suggest three main topics that should be further investigated and explored to correctly access peracaridean biodiversity in NE Atlantic. In order to proceed with these steps, more individuals along the distribution range of each species should be incorporated and multiple loci examined.

Cryptic species and the revision of species complexes

The concept of species is complex, and even if cryptic species do not have apparent distinct morphological characters, the fact that there is variation at a genetic level reflects different evolutionary histories (Knowlton 1993). With the emergence of ‘molecular species’ where molecular diagnostic characteristics are used to delimit species (e.g., Grabowski et al. 2017) instead of morphological traits, it is fundamental to screen the genetic variation of organisms. The lack of known or visible morphological variation can be a consequence of insufficiency of experts, poor knowledge about individual species or due to the sampling or preservation conditions (Wägele et al. 2011) and the detection of cryptic species using molecular tools can help overcoming this problem. Ocasionally this can lead to the subsequent discovery of unnoticed diagnostic morphological characters, and therefore to the discovery and description of new species (Knowlton 1993, Hawkins et al. 2000). Several peracaridean species are assumed to be complexes of species because their morphology slighty varies, but this variation is not enough to consider them as different species (e.g., Krapp-Schickel and Vader 1998, Bruce and Holdich 2002, Vader and Krapp-Schickel 2012). A detailed examination should be carried out in the species *Campecopea lusitanica*, *Ampithoe ramondi*, *Serejohyale spinidactylus, Caprella acanthifera, Elasmopus pectenirus* and *Tanais dulongii* and a correlation between these different forms with molecular operational taxonomic units should be performed.

Shallow phylogenies of the target peracaridean taxa

One of the biggest gaps still left by this thesis, concerns the shallow phylogenetic relationships of target taxa, namely within the genus *Dynamene* and the family Hyalidae, which could not be totally resolved. The incorporation of the Mediterranean *Dynamene* species could help solving this problem, although some species are uncommon and difficult to find (e.g., *Dynamene tubicauda*, Vieira et al. 2016). As for the family Hyalidae, it seems more complex and difficult to achieve this goal, because more than one hundered species of this family exist worldwide and their taxonomy is complex (Serejo 2004, Horton et al. 2017b). Nonetheless, the early results obtained in chapter 5 indicate that the genera nomenclature in this family is incorrect.

Phylogeographic discontinuities between Macaronesia and the continental coasts

More than fifty species were found in this thesis to be present in both Macaronesia and continental European Atlantic coasts (Annexes 1.6 and 1.7), but in only twenty-five it was possible to obtain data to investigate possible genetic differentiation between Macaronesia and Iberian Peninsula coasts. With the use of more peracaridean species, more robustness could be achieved. Moreover, the addition of other major marine invertebrate groups such as echinoderms, gastropods, polychaetes or other crustacea, could help clarify if the phylogeograhpic discontinuity observed for these peracarids is also present in any other, and which, marine invertebrates with a pelagic phase, as well gaining further insight into the possible role of Macaronesia islands in the diversification of other marine invertebrates.

Annexes

ANNEXES OF CHAPTER 1.

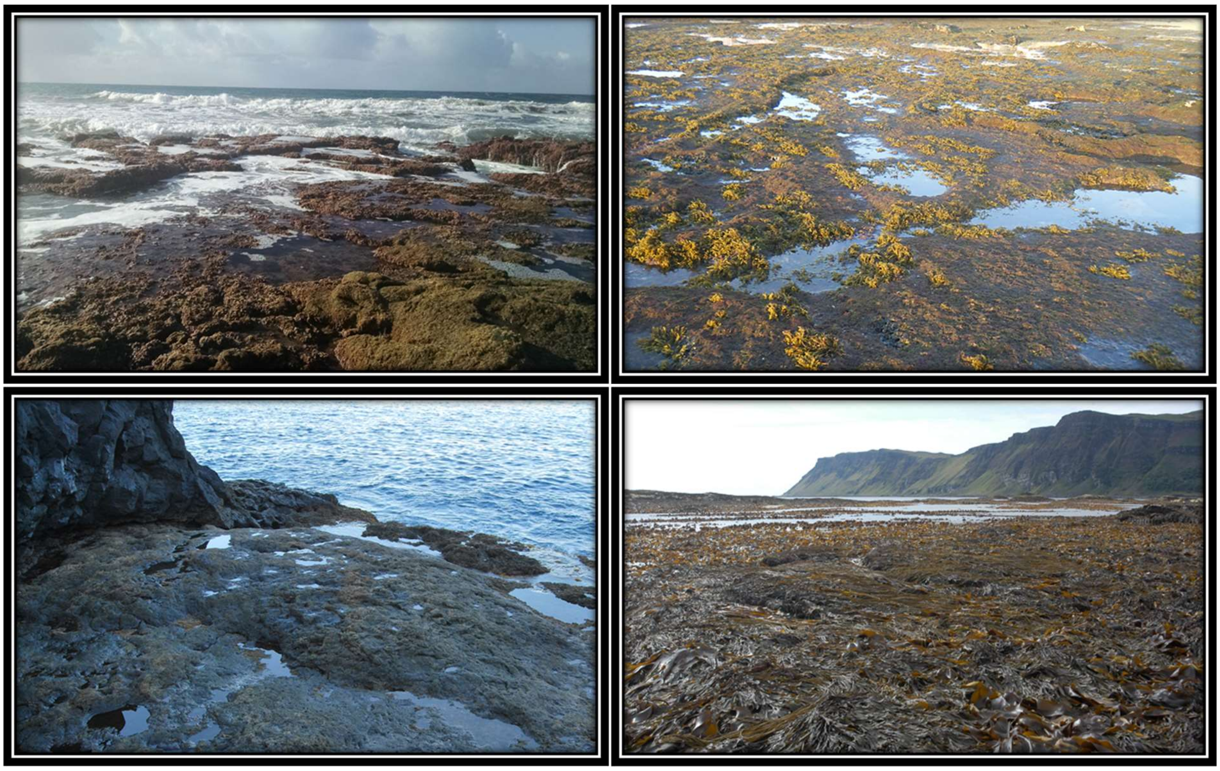
Annex 1.1. Rocky shore intertidal

Rocky shores (Annex 1.2) are intertidal areas of seacoasts that consist of solid rocks. Usually, they present three levels. The upper intertidal level is only flooded during high tides. The mid-littoral level is a turbulent zone that is (un)covered twice a day. This level extends from the upper limit of the barnacles to the upper limit of large brown algae (e.g. Laminariales). The lower intertidal is usually covered with water and it is only uncovered when the tide is extremely low (Lewis 1964, Connell 1972).

Rocky shores are often a biologically rich environment, due to temperate coastal waters that are mixed by waves and convection, maintaining adequate availability of nutrients. Also, the sea brings plankton and broken organic matter in with each tide. The high availability of light (due to low depths) and nutrient levels means that primary productivity of seaweeds can be very high. Therefore, marine benthic invertebrates are abundant and diverse. Despite these favourable factors, these benthic species are limited by salinity, wave exposure, temperature and mainly desiccation, to which they have to adapt. Other threats include predation from birds and other marine organisms, as well as the effects of pollution (Lewis 1964, Connell 1972).

Marine benthic invertebrates that inhabit rocky shores are unique as they have characteristics that make them, in theory, less isolated than terrestrial organisms but more isolated than other marine taxa as the sea is generally viewed as being more homogeneous and lacking clear physical barriers to dispersal (Palumbi 1992, Palumbi 1994). Rocky shore organisms however are isolated from other areas of suitable habitat by deep water. Most hard substrata give way at some depth to sedimentary habitats, and even on continental coastlines rocky areas are usually interspersed with sandy beaches (Hawkins et al. 2000). To circumvent this situation, many littoral organisms have dispersing larval stages (Scheltema 1971). However, rafting is the only dispersal method for animals with direct development such as Peracarida. Long-distance dispersion may occur through random events such as rafting on detached macro algae (Thiel and Gutow 2005), via anthropogenic transfer (Wittmann and Ariani 2009) or even rarer if specimens remain attached to its prey (Sponer and Lessios 2009) or to marine birds (Frisch et al. 2007). Once founded, via transport of one gravid individual or at least two different-sexed individuals, a population can establish and, more importantly, persist in the new habitat (Highsmith 1985).

Annex 1.2. Intertidal marine rocky shores in Northeast Atlantic.



Top left: Praia Formosa, Santa Maria (Photo taken by Pedro Vieira). Top right: Insouane, Morocco (Photo taken by Laura Peteiro). Bottom left: El Faro, La Palma (Photo taken by Mafalda Tavares). Bottom right: Carsaig, Scotland (Photo taken by Henrique Queiroga).

Annex 1.3. Sampling methodology.

Specimens and locations that served as backbone of this thesis were collected in marine rocky shores along the Northeast Atlantic (Annex 1.2), between Norway and Iceland in the north and Morocco and Canary Islands in the south, in 51 locations (Annexes 1.4, 1.5). Two types of sampling protocols were applied. The first one, used for the DiverseShores project (therefore called “DiverseShores”, Annex 1.5), between April and September of 2011, at three regions of NE Atlantic (each comprising three coasts): North (Norway, Iceland and Scotland), Centre (Galicia, West and South Portugal) and South (Gran Canaria, La Palma and Madeira including Porto Santo island). In each coast, samples were collected in three locations, making a total of 27 locations. This nested sampling design attempted to separate regions by 1000s of km, coasts within each region by 100s of km, and sites within each coast by 10s of km. Samples were taken as far as possible from obvious sources of perturbation. As a compromise between effort and efficiency, scrapings of 20x20 cm quadrats of the algal cover were made in each intertidal level (lower, middle and upper), in four microhabitats at each level: immersed-shade, immersed-sunny, emersed-shade and emersed-sunny. Three replicates in each microhabitat were taken, haphazardly distributed within each level.

Twenty-two additional locations in Continental Portugal, Azores, Morocco and Canaries islands were sampled between 2008 and 2015 (henceforth called “Additional sampling”, Annex 1.5) to complement the previous sampling locations. Scrapings of the algal cover were made during low tide along the intertidal level in habitats selected halphazardly. Accompanying fauna was retrieved by washing the algae in freshwater and immediately preserved in alcohol. Data on the algae species sampled were not included in this thesis.

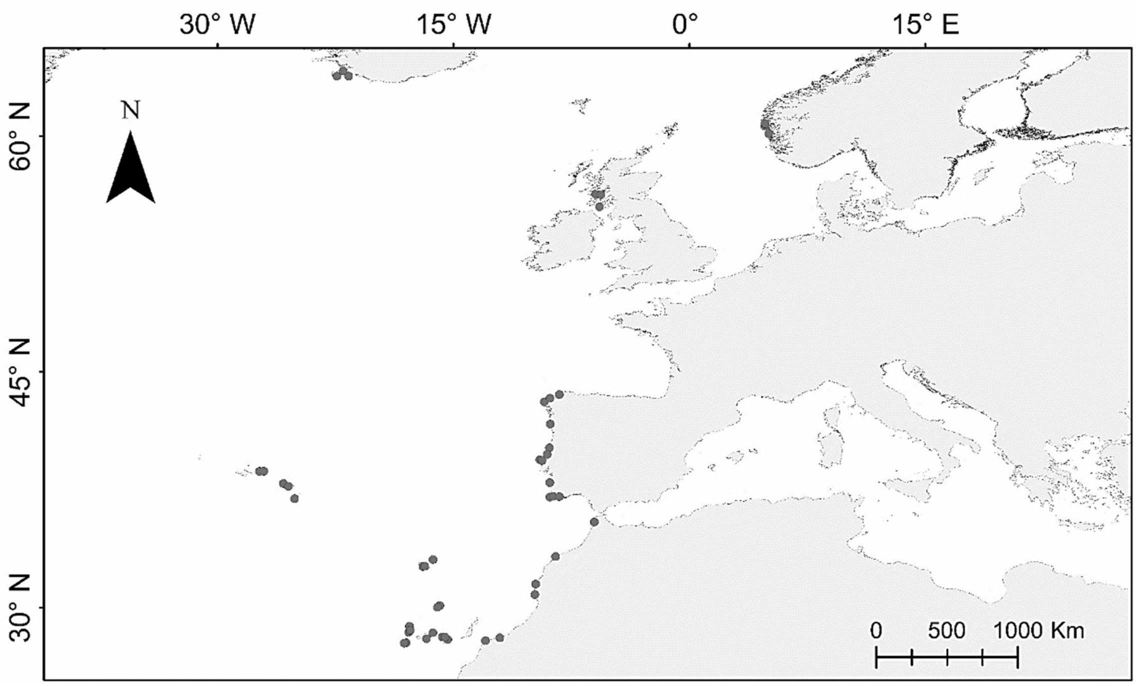
Additional (some) peracaridean specimens from Selvagem Grande and Selvagem Pequena intertidals (therefore called “Selvagens”, Annex 1.5) were also used. These specimens were provided by the Portuguese Museum of Natural History and Science in Lisbon and obtained in 2010 and 2011 during the EMEPC/M@rBis/Selvagens2010 and EMAM/PEPC_M@rBis/2011 campaigns, with catalog numbers M@rBis__001452, M@rBis__000147, M@rBis__001450, M@rBis__000031, M@rBis__001451, M@rBis__000267, M@rBis__000929 and M@rBis__001417.

Taxonomic identification was based on morphologic characteristics using general identification books and papers (e.g., Naylor 1972, Lincoln 1979, Ruffo 1982, Holdich and Jones 1983, Hayward and Ryland 1990, 1995). Specimens collected from “DiverseShores” were identified until genus or species level and the total number of specimens is provided (Annex 1.6).

Only part of the specimens from “Additional sampling” and “Selvagens” were identified until genus or species level. The rest of the specimens were only identified until order or family level and total number of specimens was not possible to access at the time this thesis was written (Annex 1.7).

Although these specimens and locations served as backbone to this thesis, some specimens provided by colleagues from other locations were used to complement specific chapters wherever necessary, namely Canto Marinho, Apulia and Aveiro in chapter 2, Croatia and France in chapter 4 and Brazil in chapter 5 (see each specific material and methods and acknowledgments for more details). All the specimens sampled and used (except for chapter 3) are deposited in the Laboratory of Marine Ecology of the Biology Departament in the University of Aveiro. For the several additional specimens and locations used in chapter 3, see the material and methods and acknowledgments of chapter 3 and Annexes 3.1 and 3.2. The specimens used in chapter 3 are deposited in Naturalis Biodiversity Center (Royal Natural History Museum, Leiden, The Netherlands).

Annex 1.4. Sampling locations map.



Annex 1.5. Sampling locations and respective co-ordinates, organized by sampling protocol.

Project	Country/Region	Locations	Latitude	Longitude
“DIVERSESHORES”	Norway	Baloy	60.805	4.806
		Viksoy	60.175	5.042
		Hellesoy	60.663	4.787
	Iceland	Reykjavik	64.163	-22.009
		Grindavik	63.826	-22.411
		Strandarkirkja	63.823	-21.660
	Scotland	Bellochantuy	55.525	-5.711
		Easdale	56.288	-5.635
		Carsaig	56.319	-5.965
	Galicia	Muxia	43.092	-9.223
		Pedreira	43.556	-8.275
		Barizo	43.322	-8.873
	Portugal West	Buarcos	40.176	-8.901
		Peniche	39.372	-9.378
		São Pedro Moel	39.758	-9.033
	Portugal South	Arrifes	37.076	-8.276
		Ingrina	37.045	-8.878
		Dona Ana	37.087	-8.668
	Madeira (including Porto Santo island)	Ponta Cruz	32.633	-16.943
		Reis Magos	32.646	-16.824
		Porto Frades	33.073	-16.296
	Gran Canaria island	Playa Melenara	27.989	-15.370
		Agaete	28.163	-15.699
		Bañaderos	28.149	-15.540
	La Palma island	La Salemera	28.578	-17.761
		La Fajana	28.842	-17.794
		El Faro	28.457	-17.850
“ADDITIONAL SAMPLING”	Portugal	Viana Castelo	41.689	-8.848
		Agudela	41.241	-8.728
		Berlengas	39.412	-9.511
		Sines	37.961	-8.887
		Vale dos Homens	37.371	-8.834
	São Miguel island	Mosteiros	37.900	-25.817
		Ribeira chã	37.715	-25.487
		Ponta Ferreirinha	37.861	-25.855
	Santa Maria island	São Lourenco	36.988	-25.054
		Praia Formosa	36.949	-25.094
	Terceira island	Porto Martins	38.683	-27.058
		Cinco Ribeiras	38.675	-27.329
	Tenerife island	Mal Paso	28.034	-16.540
		Los Cristianos	28.044	-16.711
	El Hierro island	Los Sargos	27.784	-18.011
		Arenas Blancas	27.767	-18.121
	Morocco	Arzila	35.458	-6.048
		El Jadida	33.264	-8.511
		Essaouire	31.516	-9.771
		Insouane	30.841	-9.821
		Akhfenir	28.097	-12.050
		Tarfaya	27.918	-12.961
“SELVAGENS”	Selvagens	Selvagem Grande	30.140	-15.860
		Selvagem Pequena	30.033	-16.0167

Annex 1.6. List of peracaridean species and number of specimens sampled in "DiverseShores" sampling protocol.

Iceland	**7728**
Reykjavik	871
Ampithoe rubricata	4
Apherusa jurinei	6
Apohyale prevostii	229
Caprella linearis	4
Dexamine spinosa	3
Echinogammarus stoerensis	3
Gammarus finmarchicus	7
Idotea balthica	1
Idotea granulosa	450
Idotea pelagica	10
Jaera (Jaera) praehirsuta	10
Jassa falcata	28
Leptocheirus pilosus	16
Munna kroyeri	15
Parajassa pelagica	85
Grindavik	1418
Ampithoe rubricata	51
Apherusa sp.	24
Apohyale prevostii	12
Caprella linearis	5
Dexamine spinosa	52
Gammarus finmarchicus	14
Idotea granulosa	664
Idotea pelagica	136
Jaera (Jaera) praehirsuta	12
Janira maculosa	5
Jassa falcata	69
Munna kroyeri	7
Parajassa pelagica	359
Stenothoe monoculoides	8
Strandarkirkja	5439
Ampithoe rubricata	2
Apherusa jurinei	16
Apherusa sp.	214
Apohyale prevostii	154
Echinogammarus stoerensis	17
Gammarus finmarchicus	11
Idotea balthica	3311
Idotea granulosa	1654
Idotea pelagica	38
Jaera (Jaera) praehirsuta	22
Norway	**7216**
Baloy	2952
Ampithoe rubricata	7
Apherusa jurinei	27
Apohyale prevostii	1874
Echinogammarus obtusatus	7
Echinogammarus stoerensis	6
Gammarus locusta	3
Idotea granulosa	91
Idotea pelagica	4
Jaera (Jaera) praehirsuta	844
Stenothoe monoculoides	72
Tanais dulongii	17
Hellesoy	3613
Apherusa jurinei	17
Apohyale prevostii	2451
Echinogammarus stoerensis	1
Idotea granulosa	72
Idotea metallica	1
Idotea pelagica	93
Jaera (Jaera) praehirsuta	398
Stenothoe monoculoides	186
Tanais dulongii	394
Viksoy	651
Apherusa jurinei	14
Apohyale prevostii	336
Echinogammarus obtusatus	41
Idotea granulosa	3
Jaera (Jaera) praehirsuta	255
Tanais dulongii	2
Scotland	**8243**
Carsaig	4078
Ampithoe rubricata	63
Apherusa jurinei	85
Apherusa jurinei	32
Apohyale prevostii	71
Apohyale stebbingi	620
Caprella acanthifera	84
Caprella penantis	2
Dexamine spinosa	2
Dynamene bidentata	22
Echinogammarus stoerensis	24
Gammarus locusta	10
Hyale pontica	8
Hyalidae	64
Idotea granulosa	507
Idotea pelagica	368
Jaera (Jaera) praehirsuta	269
Jassa falcata	303
Jassa ocia	1
Leptocheirus pilosus	4
Ligia oceanica	2
Orchestia mediterranea	18
Parajassa pelagica	44
Stenothoe monoculoides	1051
Tanais dulongii	424
Easdale	3195
Ampithoe rubricata	21
Apherusa jurinei	42
Apohyale prevostii	261
Apohyale stebbingi	323
Campecopea hirsuta	4
Caprella acanthifera	20
Dexamine spinosa	1
Dynamene bidentata	14
Gammarus finmarchicus	12
Gammarus locusta	1
Hyale pontica	14
Hyalidae	1
Idotea granulosa	110
Idotea pelagica	370
Jaera (Jaera) praehirsuta	193
Janira maculosa	332
Jassa falcata	292
Jassa ocia	3
Lembos websteri	5
Ligia oceanica	3
Microdeutopus chelifer	1
Munna kroyeri	6
Parajassa pelagica	12
Stenothoe monoculoides	999
Tanais dulongii	155
Bellochantuy	970
Ampithoe rubricata	14
Apherusa jurinei	25
Apohyale perieri	10
Apohyale prevostii	144
Apohyale stebbingi	183
Caprella acanthifera	2
Dexamine spinosa	6
Dynamene bidentata	19
Hyale pontica	5
Idotea granulosa	6
Idotea pelagica	17
Jaera (Jaera) praehirsuta	30
Janira maculosa	13
Jassa falcata	29
Parajassa pelagica	4
Stenothoe monoculoides	84
Sunamphitoe pelagica	37
Tanais dulongii	342
Galicia	**25557**
Pedreira	6556
Ampithoe helleri	32
Ampithoe rubricata	12
Apherusa jurinei	22
Apohyale perieri	25
Apohyale prevostii	16
Apohyale sp.	8
Apohyale stebbingi	532
Astacilla longicornis	1
Campecopea lusitanica	619
Caprella danilevskii	29
Caprella penantis	23
Cyathura carinata	25
Dexamine spinosa	134
Dynamene bidentata	90
Dynamene magnitorata	1486
Elasmopus rapax	89
Gnathia maxillaris	26
Guernea coalita	21
Idotea granulosa	25
Idotea pelagica	259
Ischyrocerus anguipes	5
Ischyromene lacazei	204
Jaera (Jaera) praehirsuta	23
Jassa falcata	333
Jassa ocia	27
Leptochelia savignyi	38
Ligia oceanica	24
Microdeutopus chelifer	439
Munna kroyeri	12
Orchestia mediterranea	16
Parajassa pelagica	299
Podocerus variegatus	13
Protohyale (Protohyale) schmidtii	541
Pseudoprotella phasma	20
Serejohyale spinidactylus	4
Stenosoma lancifer	13
Stenothoe monoculoides	395
Tanais dulongii	680
Barizo	9644
Amphitholina cuniculus	2
Ampithoe gammaroides	12
Anthura gracilis	3
Apherusa jurinei	184
Apohyale perieri	1431
Apohyale prevostii	14
Apohyale stebbingi	300
Campecopea hirsuta	91
Campecopea lusitanica	26
Caprella acanthifera	7

Caprella penantis	280
Cyathura carinata	1
Cymodoce truncata	6
Dynamene bidentata	302
Dynamene magnitorata	88
Hyale pontica	40
Hyalidae	48
Idotea granulosa	154
Idotea pelagica	576
Ischyromene lacazei	979
Jaera (Jaera) praehirsuta	157
Janira maculosa	8
Jassa falcata	140
Joeropsis brevicornis	49
Microdeutopus chelifer	204
Munna kroyeri	36
Parajassa pelagica	1657
Podocerus variegatus	62
Protohyale (Protohyale) schmidtii	1674
Pseudoparatanais batei	7
Serejohyale spinidactylus	363
Stenosoma lancifer	2
Stenothoe monoculoides	643
Tanais dulongii	84
Tanais grimaldii	15
Muxía	9357
Ampithoe gammaroides	1
Apherusa jurinei	127
Apohyale perieri	118
Apohyale prevostii	171
Apohyale stebbingi	113
Astacilla longicornis	1
Campecopea lusitanica	5
Caprella acanthifera	4
Caprella penantis	1223
Caprella scaura	1
Cyathura carinata	23
Cymodoce truncata	6
Dynamene bidentata	69
Dynamene magnitorata	172
Dynamene edwardsi	2
Elasmopus rapax	127
Gnathia maxillaris	8
Hyale pontica	119
Hyalidae	92
Idotea granulosa	465
Idotea pelagica	206
Ischyromene lacazei	1799
Jaera (Jaera) praehirsuta	9
Janira maculosa	7
Jassa falcata	68
Jassa ocia	18
Microdeutopus chelifer	69
Munna kroyeri	2
Parajassa pelagica	2873
Podocerus variegatus	208
Protohyale (Protohyale) schmidtii	307
Pseudoparatanais batei	2
Serejohyale spinidactylus	731
Stenosoma lancifer	21
Stenothoe monoculoides	108
Tanais dulongii	82
Portugal West	**5393**
Buarcos	2016
Ampithoe helleri	6
Apherusa jurinei	4
Apohyale perieri	2
Apohyale prevostii	10
Apohyale stebbingi	2
Apseudopsis latreillii	3
Caprella acanthifera	26
Caprella liparotensis	11
Caprella penantis	296
Caprella scaura	22
Cyathura carinata	9
Cymodoce truncata	18
Dexamine spinosa	11
Dynamene bidentata	100
Dynamene edwardsi	1
Dynamene magnitorata	51
Elasmopus rapax	33
Gammarus locusta	12
Gnathia maxillaris	20
Guernea coalita	11
Hyale pontica	14
Hyalidae	49
Idotea pelagica	237
Ischyrocerus anguipes	6
Ischyromene lacazei	16
Janira maculosa	1
Jassa herdmani	43
Jassa falcata	240
Microdeutopus chelifer	86
Munna kroyeri	128
Parajassa pelagica	6
Podocerus variegatus	23
Protohyale (Protohyale) schmidtii	341
Protohyale (Protohyale) schmidtii	131
Pseudoparatanais batei	12
Stenosoma lancifer	22
Stenothoe monoculoides	37
Tanais dulongii	107
São Pedro Moel	737
Apohyale perieri	56
Apohyale prevostii	40
Apohyale stebbingi	10
Caprella liparotensis	6
Caprella penantis	169
Cymodoce truncata	2
Dexamine spinosa	4
Dynamene magnitorata	4
Hyale pontica	2
Idotea metallica	4
Idotea pelagica	400
Ischyrocerus anguipes	4
Jassa falcata	2
Jassa ocia	2
Podocerus variegatus	6
Stenothoe monoculoides	26
Peniche	2640
Ampithoe helleri	52
Apohyale prevostii	9
Apohyale stebbingi	246
Campecopea lusitanica	284
Caprella acanthifera	1
Caprella penantis	7
Cymodoce truncata	172
Dexamine spinosa	86
Dynamene bidentata	24
Dynamene edwardsi	48
Dynamene magnitorata	448
Eurydice sp.	1
Guernea coalita	8
Hyale pontica	1
Hyalidae	2
Idotea pelagica	1
Jassa herdmani	4
Jassa falcata	68
Jassa ocia	16
Lekanesphaera rugicauda	4
Leptochelia savignyi	30
Melita palmata	8
Microdeutopus chelifer	38
Munna kroyeri	128
Podocerus variegatus	2
Protohyale (Protohyale) schmidtii	38
Pseudoparatanais batei	4
Stenosoma lancifer	1
Stenothoe monoculoides	332
Tanais dulongii	577
Portugal South	**5616**
Dona Ana	1047
Ampelisca sp.	4
Amphilochus neapolitanus	1
Ampithoe ramondi	20
Ampithoe riedli	4
Ampithoe rubricata	11
Apherusa jurinei	35
Apohyale perieri	2
Apohyale stebbingi	21
Apseudes talpa	2
Apseudopsis latreillii	30
Campecopea hirsuta	21
Caprella liparotensis	4
Caprella penantis	16
Corophium sp.	15
Dexamine spinosa	37
Dynamene edwardsi	14
Dynamene magnitorata	52
Elasmopus pectenicrus	7
Elasmopus rapax	184
Ericthonius punctatus	2
Gnathia maxillaris	4
Guernea coalita	22
Hyalidae	9
Janira maculosa	4
Jassa falcata	54
Joeropsis brevicornis	19
Leptochelia savignyi	94
Microdeutopus chelifer	21
Munna kroyeri	32
Podocerus variegatus	1
Protohyale (Protohyale) schmidtii	13
Pseudoparatanais batei	1
Stenothoe monoculoides	44
Stenothoe tergestina	3
Tanais dulongii	244
Arrifes	3302
Ampithoe ramondi	28
Apohyale perieri	32
Apohyale stebbingi	718
Campecopea hirsuta	145
Campecopea lusitanica	1
Caprella acanthifera	4
Caprella penantis	173
Dexamine spinosa	9
Dynamene edwardsi	5
Dynamene magnitorata	140
Elasmopus pectenicrus	52
Elasmopus rapax	172
Gnathia maxillaris	4
Hyale pontica	18
Jassa falcata	72
Joeropsis brevicornis	19
Leptochelia savignyi	141
Microdeutopus chelifer	54
Munna kroyeri	41
Protohyale (Protohyale) schmidtii	231

Serejohyale spinidactylus	4
Stenosoma lancifer	28
Stenothoe monoculoides	32
Tanais dulongii	1171
Ingrina	1267
Ampelisca sp.	5
Ampithoe ramondi	22
Ampithoe riedli	27
Apherusa jurinei	7
Apohyale perieri	4
Apohyale prevostii	2
Apohyale stebbingi	46
Campecopea hirsuta	19
Caprella penantis	5
Cymadusa filosa	4
Cymodoce truncata	1
Dexamine spinosa	5
Dynamene edwardsi	42
Elasmopus rapax	5
Gnathia maxillaris	8
Guernea coalita	10
Hyalidae	14
Jassa falcata	24
Jassa ocia	4
Leptochelia savignyi	17
Microdeutopus chelifer	48
Munna kroyeri	20
Stenothoe monoculoides	40
Tanais dulongii	888
Madeira	**3253**
Porto Frades	628
Amphilochus neapolitanus	5
Ampithoe ferox	60
Ampithoe ramondi	58
Anthura gracilis	5
Apohyale media	2
Apohyale perieri	6
Apohyale sp.	1
Apohyale stebbingi	38
Apseudes talpa	2
Apseudopsis latreillii	10
Campecopea lusitanica	26
Caprella acanthifera	7
Cyathura carinata	2
Cymodoce truncata	3
Dexamine spinosa	13
Dynamene edwardsi	17
Dynamene magnitorata	1
Elasmopus pectenicrus	4
Elasmopus rapax	31
Gnathia maxillaris	4
Janira maculosa	1
Jassa herdmani	5
Joeropsis brevicornis	5
Leptochelia savignyi	14
Quadrimaera inaequipes	4
Microdeutopus chelifer	26
Munna kroyeri	12
Paravireia holdichi	4
Podocerus variegatus	7
Protohyale (Protohyale) schmidtii	6
Serejohyale spinidactylus	23
Stenothoe monoculoides	12
Stenothoe tergestina	1
Sunamphitoe pelagica	2
Tanais dulongii	163
Tanais grimaldii	3
Zeuxo exsargasso	
Zeuxo sp.	47
Reis Magos	925
Ampithoe ferox	99
Ampithoe ramondi	2
Apohyale media	11
Apohyale sp	1
Apohyale stebbingi	203
Campecopea lusitanica	5
Caprella acanthifera	128
Caprella penantis	16
Dynamene edwardsi	194
Elasmopus pectenicrus	27
Janira maculosa	2
Jassa falcata	18
Joeropsis brevicornis	7
Microdeutopus chelifer	1
Munna kroyeri	34
Paravireia holdichi	3
Podocerus variegatus	5
Serejohyale spinidactylus	77
Stenothoe monoculoides	68
Tanais dulongii	19
Zeuxo sp.	5
Ponta Cruz	1700
Ampithoe helleri	12
Ampithoe ramondi	31
Ampithoe riedli	149
Apohyale perieri	70
Apohyale media	6
Apohyale stebbingi	564
Campecopea lusitanica	5
Caprella acanthifera	100
Caprella penantis	67
Cyathura carinata	2
Cymodoce truncata	1
Dynamene edwardsi	228
Elasmopus pectenicrus	12
Hyalidae	2
Janira maculosa	2
Jassa herdmani	33
Jassa falcata	140
Joeropsis brevicornis	27
Quadrimaera inaequipes	4
Munna kroyeri	46
Paravireia holdichi	1
Podocerus variegatus	18
Protohyale (Protohyale) schmidtii	3
Protohyale sp. 1	2
Serejohyale spinidactylus	94
Stenothoe tergestina	8
Tanais dulongii	36
Zeuxo sp.	37
La Palma	**6075**
La Fajana	2103
Ampithoe ferox	20
Ampithoe ramondi	18
Ampithoe riedli	282
Anthura gracilis	2
Apohyale media	8
Apohyale perieri	44
Apohyale stebbingi	435
Campecopea lusitanica	15
Caprella acanthifera	127
Caprella penantis	15
Cyathura carinata	1
Cymadusa filosa	4
Cymodoce truncata	11
Dexamine spinosa	1
Dynamene edwardsi	157
Dynamene magnitorata	1
Elasmopus vachoni	15
Elasmopus rapax	293
Eurydice sp.	2
Gnathia maxillaris	7
Janira maculosa	114
Jassa falcata	147
Joeropsis brevicornis	47
Leptochelia savignyi	5
Leucothoe venetiarum	2
Quadrimaera inaequipes	27
Munna kroyeri	1
Podocerus variegatus	39
Protohyale (Protohyale) schmidtii	142
Serejohyale spinidactylus	54
Stenothoe monoculoides	29
Zeuxo sp.	38
La Salemera	1796
Ampelisca sp.	1
Ampithoe ferox	4
Ampithoe ramondi	42
Ampithoe riedli	111
Apohyale media	262
Apohyale perieri	2
Apohyale stebbingi	416
Apseudes talpa	1
Caprella acanthifera	127
Caprella danilevskii	2
Dexamine spinosa	15
Dynamene edwardsi	39
Elasmopus rapax	98
Gnathia maxillaris	3
Hyalidae	24
Janira maculosa	30
Joeropsis brevicornis	1
Leptochelia savignyi	2
Lysianassa ceratina	4
Quadrimaera inaequipes	38
Protohyale (Protohyale) schmidtii	109
Serejohyale spinidactylus	214
Stenothoe monoculoides	2
Sunamphitoe pelagica	2
Tanais dulongii	245
Zeuxo sp.	2
El Faro	2176
Ampithoe ferox	15
Ampithoe helleri	6
Ampithoe ramondi	129
Ampithoe riedli	102
Apohyale media	22
Apohyale perieri	96
Apohyale prevostii	5
Apohyale stebbingi	590
Campecopea lusitanica	5
Caprella acanthifera	50
Caprella penantis	19
Cymadusa filosa	4
Dynamene edwardsi	108
Dynamene magnitorata	3
Elasmopus canarius	152
Elasmopus rapax	501
Gnathia maxillaris	2
Janira maculosa	5
Joeropsis brevicornis	87
Lekanesphaera levii	4
Quadrimaera inaequipes	6
Podocerus variegatus	5
Protohyale (Protohyale) schmidtii	16
Serejohyale spinidactylus	24
Stenothoe monoculoides	18
Stenothoe tergestina	45

Tanais dulongii	151
Zeuxo sp.	6
Gran Canaria	**5768**
Bañaderos	2018
Amphilochus neapolitanus	8
Ampithoe ferox	60
Ampithoe helleri	8
Ampithoe ramondi	34
Ampithoe riedli	6
Apohyale media	60
Apohyale stebbingi	685
Campecopea lusitanica	28
Caprella acanthifera	9
Caprella penantis	11
Cyathura carinata	4
Cymadusa filosa	17
Cymodoce truncata	1
Dexamine spinosa	2
Dynamene bidentata	1
Dynamene edwardsi	322
Elasmopus canarius	128
Elasmopus rapax	100
Eurydice sp.	1
Gnathia maxillaris	4
Janira maculosa	1
Jassa falcata	29
Joeropsis brevicornis	9
Leucothoe venetiarum	2
Lysianassa ceratina	1
Quadrimaera inaequipes	5
Microdeutopus chelifer	5
Munna kroyeri	55
Protohyale (Protohyale) schmidtii	77
Serejohyale spinidactylus	279
Stenothoe monoculoides	27
Tanais dulongii	30
Zeuxo sp.	9
Agaete	1177
Amphilochus neapolitanus	1
Ampithoe ferox	174
Ampithoe ramondi	6
Anthura gracilis	4
Apohyale media	13
Apohyale stebbingi	280
Apseudopsis latreillii	1
Campecopea lusitanica	1
Caprella acanthifera	47
Caprella penantis	10
Cyathura carinata	2
Cymadusa filosa	26
Dynamene edwardsi	120
Elasmopus rapax	380
Gnathia maxillaris	4
Janira maculosa	3
Jassa falcata	43
Joeropsis brevicornis	2
Munna kroyeri	7
Serejohyale spinidactylus	16
Tanais dulongii	31
Zeuxo sp.	9
Playa Melenara	2573
Ampithoe ferox	93
Ampithoe riedli	29
Apohyale perieri	16
Apohyale stebbingi	571
Apseudes talpa	17
Apseudopsis latreillii	12
Caprella acanthifera	144
Cyathura carinata	37
Cymadusa filosa	49
Dynamene edwardsi	226
Elasmopus rapax	641
Gnathia maxillaris	2
Hyalidae	2
Janira maculosa	12
Joeropsis brevicornis	150
Leptochelia savignyi	47
Microdeutopus chelifer	40
Munna kroyeri	16
Paravireia holdichi	29
Protohyale (Protohyale) schmidtii	2
Serejohyale spinidactylus	156
Stenothoe monoculoides	15
Stenothoe tergestina	6
Tanais dulongii	252
Zeuxo sp.	9
Total "DiverseShores"	**74849**

Annex 1.7. List of peracaridean species and number of specimens sampled in "Additional sampling" and "Selvagens" sampling protocols.

São Miguel	**472**
Mosteiros	38
Dynamene edwardsi	2
Amphipoda n.i.	17
Hyalidae	19
Ponta da Ferraria	14
Dynamene edwardsi	1
Anthuridae	1
Hyalidae	9
Lekanesphaera sp.	3
Ribeira Chã	420
Anthura gracilis	3
Amphipoda n.i.	207
Caprella acanthifera	5
Caprellidae	3
Corophium spp.	47
Cyathura sp.	2
Dynamene edwardsi	1
Dynamene magnitorata	27
Elasmopus vachoni	2
Jassa herdmani	3
Hyalidae	48
Leptochelia sp.	3
Munnidae	62
Tanaidacea n.i.	5
Tanais grimaldii	2
Terceira	**203**
Porto Martins	50
Dynamene magnitorata	42
Amphipoda n.i.	5
Anthuridae	2
Cymodoce truncata	1
Cinco Ribeiras	153
Idotea granulosa	56
Dynamene magnitorata	1
Hyalidae	96
Santa Maria	**249**
São Lourenço	71
Amphipoda n.i.	34
Ampithoe ramondi	2
Dynamene magnitorata	2
Hyalidae	25
Serejohyale spinidactylus	2
Hyalidae	2
Ampithoe sp.	1
Stenothoe sp.	1
Dexamine sp.	1
Elasmopus vachoni	1
Praia Formosa	178
Amphipoda n.i.	132
Ampithoe ramondi	2
Dynamene magnitorata	6
Hyalidae	24
Janiridae	5
Munnidae	1
Tanaidacea n.i.	8
El Hierro	**247**
Los Sargos	79
Amphipoda n.i.	30
Dynamene edwardsi	7
Caprellidae	6
Hyalidae	30
Serejohyale spinidactylus	3
Tanaidacea n.i.	3
Arenas Blancas	168
Amphipoda n.i.	121
Caprellidae	3
Dynamene edwardsi	5
Elasmopus canarius	4
Hyalidae	19
Janiridae	12
Tanaidacea n.i.	4
Tenerife	**143**
Mal Paso	66
Ampithoe ramondi	5
Dynamene edwardsi	8
Elasmopus pectenicrus	4
Hyalidae	21
Amphipoda n.i.	9
Cyathura sp.	1
Tanaidacea n.i.	3
Caprellidae	2
Janiridae	5
Joeropsis brevicornis	2
Stenothoe monoculoides	3
Zeuxo exsargasso	3
Los Cristianos	77
Amphipoda n.i.	8
Anthura gracilis	1
Caprellidae	4
Dynamene edwardsi	7
Hyalidae	50
Isopoda n.i.	3
Tanaidacea n.i.	4
Portugal	**310**
Vale dos Homens	23
Cymodoce sp.	1
Dynamene bidentata	20
Dynamene edwardsi	2
Sines	35
Hyalidae	9
Dynamene spp.	17
Sphaeromatidae	9
Agudela	94
Dynamene bidentata	1
Peracarida n.i.	75
Orchestia gammarelus	5
Hyale pontica	4
Hyalidae	9
Berlengas	54
Campecopea sp.	42
Dynamene edwardsi	4
Amphipoda n.i.	5
Tanaidacea n.i.	3
Viana Castelo	104
Anthura gracilis	4
Cyathura carinata	2
Dynamene spp.	35
Peracarida n.i.	63
Morocco	**1078**
Arzila	633
Anthura gracilis	3
Dynamene edwardsi	30
Ampithoe riedli	4
Amphipoda n.i.	405
Caprellidae	10
Dynamene magnitorata	1
Hyalidae	14
Leptochelia sp.	1
Tanaidacea n.i.	163
Tanais dulongii	2
Akhfehnir	34
Stenothoe sp.	5
Melita sp.	3
Hyalidae	17
Shaeroma serratum	1
Ischyromene sp.	4
Elasmopus pectenicrus	3
Dynamene bidentata	1
El Jadida	279
Amphipoda n.i.	213
Caprella acanthifera	6
Tanaidacea n.i.	17
Isopoda n.i.	21
Dynamene bidentata	19
Caprellidae	1
Dynamene edwardsi	2
Essaouire	76
Dynamene bidentata	27
Amphipoda n.i.	29
Idotea sp.	6
Cymodoce sp.	2
Stenossoma sp.	5
Caprellidae	7
Insouane	14
Dynamene edwardsi	2
Dynamene bidentata	12
Tarfaya	42
Dynamene edwardsi	16
Campecopea lusitanica	1
Hyalidae	25
Total "Additional sampling"	**2702**
Selvagem Grande	**19**
Dynamene edwardsi	8
Hyalidae	7
Anthura gracilis	2
Tanais grimaldii	2
Selvagem Pequena	**17**
Dynamene edwardsi	6
Hyalidae	7
Tanais grimaldii	1
Tanaidacea n.i.	3
Total "Selvagens"	**36**

ANNEXES OF CHAPTER 2.

Annex 2.1. List of species, number of individuals, mean ISD (in bold values higher than 0.03), location and sources used in chapter 2.

Order	Species	Iberian Peninsula	Other sites	Mean ISD	Country/Region	Source
Amphipoda	*Amphipoda n.i.*	1	0	-	Portugal	This study
Amphipoda	*Abludomelita obtusata*	10	0	0.0003	Portugal	Lobo et al. 2016a
Amphipoda	*Ampelisca brevicornis*	0	3	**0.0453**	Italy, Atlantic France	Cowart et al. unpublished; Maruso et al. unpublished
Amphipoda	*Ampelisca diadema*	1	4	**0.1973**	Portugal, North Sea; Italy	Raupach et al. 2015; Lobo et al. 2016a; Maruso et al. unpublished
Amphipoda	*Ampelisca ledoyeri*	0	3	0.0020	Italy	Maruso et al. unpublished
Amphipoda	*Ampelisca sarsi*	1	0	-	Spain	Aylagas et al. 2014
Amphipoda	*Ampelisca sp.1*	2	0	0.0000	Portugal	Lobo et al. 2016a
Amphipoda	*Ampelisca sp.2*	1	0	-	Portugal	Lobo et al. 2016a
Amphipoda	*Ampelisca spinipes*	1	1	**0.2050**	Spain; North Sea	Aylagas et al. 2014; Raupach et al. 2015
Amphipoda	*Ampelisca tenuicornis*	0	3	**0.1172**	North Sea; Italy	Raupach et al. 2015; Maruso et al. unpublished
Amphipoda	*Ampelisca typica*	0	3	**0.1365**	Italy	Maruso et al. unpublished
Amphipoda	*Ampithoe helleri*	13	0	0.0046	Portugal; Spain	This study; Lobo et al. 2016a
Amphipoda	*Ampithoe ramondi*	5	1	**0.0647**	Portugal; Spain; Hawaii	This study; Sotka et al. 2016
Amphipoda	*Ampithoe riedli*	1	0	-	Portugal	This study
Amphipoda	*Ampithoe rubricata*	3	6	**0.0605**	Portugal; Spain; Atlantic France; Atlantic Canada	This study; Radulovici et al. 2009; Cowart et al. unpublished
Amphipoda	*Aora gracilis*	0	3	0.0092	North Sea	Raupach et al. 2015
Amphipoda	*Aora typica*	4	0	0.0018	Portugal	Lobo et al. 2016a
Amphipoda	*Apherusa bispinosa*	0	3	0.0010	North Sea	Raupach et al. 2015
Amphipoda	*Apherusa jurinei*	5	4	0.0085	Portugal; North Sea; Scotland	This study; Raupach et al. 2015; Lobo et al. 2016a
Amphipoda	*Apohyale prevostii*	5	7	0.0019	Portugal; North Sea; Canada, Scotland, Iceland	This study; Radulovici et al. 2009; Raupach et al. 2015; Lobo et al. 2016a
Amphipoda	*Apolochus neapolitanus*	1	0	-	Spain	Aylagas et al. 2014
Amphipoda	*Bathyporeia tenuipes*	1	0	-	Spain	Aylagas et al. 2014
Amphipoda	*Caprella acanthifera*	6	1	**0.0691**	Portugal, Spain, Scotland	This study; Aylagas et al. 2014; Lobo et al. 2016a
Amphipoda	*Caprella danilevskii*	1	0	-	Portugal	Lobo et al. 2016a
Amphipoda	*Caprella dilatata*	4	0	0.0056	Spain	Cabezas et al. 2013b
Amphipoda	*Caprella fretensis*	1	0	-	Spain	Aylagas et al. 2014
Amphipoda	*Caprella liparotensis*	2	0	0.0000	Portugal	This study
Amphipoda	*Caprella mutica*	0	2	0.0109	North Sea, Canada	Radulovici et al. 2009; Raupach et al. 2015
Amphipoda	*Caprella penantis*	6	0	0.0112	Portugal	Cabezas et al. 2013a; Lobo et al. 2016a
Amphipoda	*Caprella scaura*	4	0	0.0000	Portugal	Cabezas et al. 2014
Amphipoda	*Caprella sp.*	1	0	-	Portugal	This study
Amphipoda	*Cheirocratus intermedius*	1	0	-	Spain	Aylagas et al. 2014
Amphipoda	*Corophiidae n.i.1*	1	0	-	Portugal	Lobo et al. 2016a
Amphipoda	*Corophiidae n.i.2*	1	0	-	Portugal	Lobo et al. 2016a
Amphipoda	*Corophiidae n.i.3*	1	0	-	Portugal	Lobo et al. 2016a
Amphipoda	*Corophiidae n.i.4*	1	0	-	Portugal	Lobo et al. 2016a

Order	Species	Iberian Peninsula	Other sites	Mean ISD	Country/Region	Source
Amphipoda	*Corophium multisetosum*	8	0	**0.0977**	Portugal	Lobo et al. 2013, 2016a
Amphipoda	*Corophium sp.*	0	2	0.0039	Azores	This study
Amphipoda	*Corophium volutator*	0	3	0.0000	North Sea	Raupach et al. 2015
Amphipoda	*Dexamine spiniventris*	8	0	**0.0900**	Portugal, Spain	This study; Lobo et al. 2016a
Amphipoda	*Dexamine spinosa*	1	3	**0.1676**	Spain, North Sea, Scotland, France	This study; Aylagas et al. 2014; Raupach et al. 2015; Cowart et al. unpublished
Amphipoda	*Dexamine thea*	0	3	0.0031	North Sea	Raupach et al. 2015
Amphipoda	*Echinogammarus obtusatus*	0	2	0.0094	Iceland, Norway	Costa et al. 2009
Amphipoda	*Echinogammarus marinus*	5	7	0.0068	Portugal, North Sea; Ireland; Scotland; Sweden; Wales; Iceland; Norway	Costa et al. 2009; Raupach et al. 2015; Lobo et al. 2016a
Amphipoda	*Echinogammarus trichiatus*	0	1	-	Netherlands	Ironside et al. unpublished
Amphipoda	*Elasmopus rapax*	10	0	0.0006	Spain; Portugal	This study; Lobo et al. 2013, 2016a
Amphipoda	*Elasmopus sp.*	2	0	0.0019	Portugal	This study
Amphipoda	*Gamarella fucicola*	6	3	**0.0941**	Portugal, North Sea	Raupach et al. 2015; Lobo et al. 2016a
Amphipoda	*Gammaropsis nitida*	0	3	0.0022	North Sea	Raupach et al. 2015
Amphipoda	*Gammaropsis sp.*	1	0	-	Portugal	Lobo et al. 2016a
Amphipoda	*Gammarus chevreuxi*	4	1	0.0131	Portugal, France	Costa et al. 2009; Hou et al. 2011
Amphipoda	*Gammarus crinicornis*	1	4	0.0110	Portugal, Belgium, North Sea	Costa et al. 2009; Raupach et al. 2015
Amphipoda	*Gammarus duebeni*	0	3	0.0265	Wales, North Sea, Norway	Costa et al. 2009; Raupach et al. 2015
Amphipoda	*Gammarus insensibilis*	4	0	0.0031	Portugal	Costa et al. 2009
Amphipoda	*Gammarus locusta*	5	3	0.0159	Portugal, Belgium, Wales, Scotland	Costa et al. 2009
Amphipoda	*Gammarus salinus*	0	3	0.0138	North Sea	Raupach et al. 2015
Amphipoda	*Halice walkeri*	1	0	-	Spain	Aylagas et al. 2014
Amphipoda	*Haripinia sp.*	1	0	-	Portugal	Lobo et al. 2016a
Amphipoda	*Harpinia antennaria*	0	1	-	North Sea	Raupach et al. 2015
Amphipoda	*Haustorius arenarius*	5	1	0.0005	Portugal; North Sea	Raupach et al. 2015; Lobo et al. 2016a
Amphipoda	*Iphimedia minuta*	1	0	-	Spain	Aylagas et al. 2014
Amphipoda	*Jassa falcata*	10	10	0.0071	Portugal, Spain, Scotland, North Sea, Iceland	This study; Raupach et al. 2015; Lobo et al. 2016a
Amphipoda	*Jassa hermandi*	2	3	0.0162	Portugal, North Sea	This study; Raupach et al. 2015
Amphipoda	*Jassa marmorata*	0	3	0.0000	North Sea	Raupach et al. 2015
Amphipoda	*Jassa ocia*	1	0	-	Portugal	This study
Amphipoda	*Jassa pusilla*	2	2	**0.1474**	Portugal, North Sea	Raupach et al. 2015; Lobo et al. 2016a
Amphipoda	*Jassa sp.1*	1	0	-	Portugal	Lobo et al. 2013
Amphipoda	*Jassa sp.2*	1	0	-	Portugal	This study
Amphipoda	*Jassa sp.3*	2	0	0.0265	Portugal	Lobo et al. 2013, 2016a
Amphipoda	*Leucothoe incisa*	1	0	-	Portugal	Lobo et al. 2016a
Amphipoda	*Melita hergensis*	7	0	0.0034	Portugal	Lobo et al. 2016a
Amphipoda	*Melita palmata*	9	3	0.0089	Portugal, North Sea	Raupach et al. 2015; Lobo et al. 2016a
Amphipoda	*Microdeutopus chelifer*	4	0	**0.1224**	Portugal, Spain	This study; Lobo et al. 2013

Order	Species	Iberian Peninsula	Other sites	Mean ISD	Country/Region	Source
Amphipoda	*Microdeutopus sp.1*	6	0	0.0121	Portugal	Lobo et al. 2016a
Amphipoda	*Microdeutopus sp.2*	1	0	-	Spain	This study
Amphipoda	*Monocorophium acherusicum*	0	3	0.0021	North Sea	Raupach et al. 2015
Amphipoda	*Monocorophium insidiosum*	0	3	0.0204	North Sea	Raupach et al. 2015
Amphipoda	*Monocorophium sextonatae*	0	3	0.0000	North Sea	Raupach et al. 2015
Amphipoda	*Orchestia gammarelus*	1	4	0.0000	Portugal, North Sea, Iceland	This study; Hanzler and Ingólfsson 2008; Raupach et al. 2015
Amphipoda	*Orchestia mediterranea*	1	2	0.0000	Spain, North Sea	This study; Raupach et al. 2015
Amphipoda	*Paracaprella pusilla*	4	3	0.0038	Spain, Balearic Islands	Cabezas et al. unpublished
Amphipoda	*Parajassa pelagica*	8	5	0.0132	Portugal, Spain, Scotland, Iceland	This study; Lobo et al. 2016a
Amphipoda	*Parapleustes bicuspis*	1	0	-	Portugal	Lobo et al. 2016a
Amphipoda	*Phtisica marina*	3	2	0.0234	Portugal, North Sea	Raupach et al. 2015; Lobo et al. 2016a
Amphipoda	*Podocerus variegatus*	5	0	0.0077	Spain	This study
Amphipoda	*Pseudoprotella phasma*	1	0	-	Portugal	Lobo et al. 2016a
Amphipoda	*Quadrimaera inaequipes*	10	0	0.0015	Portugal	Lobo et al. 2013, 2016a
Amphipoda	*Serejohyale spinidactylus*	1	0	-	Portugal	This study
Amphipoda	*Stenula sp.*	2	0	0.0031	Portugal	Lobo et al. 2016a
Amphipoda	*Talitrus saltator*	9	4	**0.0426**	Portugal, North Sea, Italy	Raupach et al. 2015; Lobo et al. 2016a; Maruso et al. unpublished
Amphipoda	*Unciolella lunata*	1	0	-	Spain	Aylagas et al. 2014
Amphipoda	*Urothoe elegans*	0	3	0.0000	North Sea	Raupach et al. 2015
Amphipoda	*Urothoe grimaldii*	1	0	-	Portugal	Lobo et al. 2016a
Amphipoda	*Urothoe poseidonis*	0	3	0.0000	North Sea	Raupach et al. 2015
Amphipoda	*Urothoe pulchela*	1	1	**0.1473**	Spain, France	Aylagas et al. 2014; Cowart et al. unpublished
Isopoda	*Anthura gracilis*	3	0	0.0000	Portugal; Spain	This study
Isopoda	*Astacilla damnoniensis*	1	0	-	Portugal	This study
Isopoda	*Astacilla intermedia*	0	3	**0.0317**	North Sea	Raupach et al. 2015
Isopoda	*Astacilla longicornis*	0	3	0.0030	North Sea	Raupach et al. 2015
Isopoda	*Campecopea hirsuta*	5	0	0.0005	Portugal; Spain	This study
Isopoda	*Campecopea lusitanica*	4	0	0.0020	Spain	This study
Isopoda	*Cleantis prismatica*	1	0	-	Portugal	This study
Isopoda	*Cyathura carinata*	8	0	0.0103	Portugal	This study
Isopoda	*Cymodoce truncata*	4	0	0.0027	Portugal, Spain	This study
Isopoda	*Dynamene bidentata*	5	0	0.0031	Portugal	This study
Isopoda	*Dynamene edwardsi*	4	0	0.0008	Portugal	This study
Isopoda	*Dynamene magnitorata*	4	0	0.0040	Portugal	This study
Isopoda	*Eurydice pulchra*	0	3	0.0041	North Sea	Raupach et al. 2015
Isopoda	*Eurydice spinigera*	2	0	0.0034	Portugal	This study
Isopoda	*Gnathia maxillaris*	3	0	0.0000	Portugal; Spain	This study
Isopoda	*Idotea balthica*	0	7	0.0051	Iceland; North Sea, Canada	This study; Radulovici et al. 2009; Raupach et al. 2015
Isopoda	*Idotea chelipes*	3	0	0.0208	Portugal	This study
Isopoda	*Idotea emarginata*	0	3	0.0093	North Sea	Raupach et al. 2015

Order	Species	Iberian Peninsula	Other sites	Mean ISD	Country/Region	Source
Isopoda	*Idotea granulosa*	13	21	0.0057	Portugal; Spain; Azores; Scotland; North Sea; Iceland; Norway	This study; Raupach et al. 2015
Isopoda	*Idotea linearis*	0	3	0.0010	North Sea	Raupach et al. 2015
Isopoda	*Idotea metallica*	0	2	0.0017	North Sea	Raupach et al. 2015
Isopoda	*Idotea neglecta*	0	6	0.0096	Azores	This study
Isopoda	*Idotea pelagica*	9	6	0.0020	Portugal, Spain, North Sea, Scotland, Iceland, Norway	This study; Raupach et al. 2015
Isopoda	*Ischyromene lacazei*	8	0	0.0035	Spain, Portugal	This study
Isopoda	*Jaera albifrons*	5	3	0.0110	Spain, Portugal, North Sea, Canada	This study; Radulovici et al. 2009; Raupach et al. 2015; Lobo et al. 2016a
Isopoda	*Janira maculosa*	3	3	**0.1994**	Spain, Portugal, France, North Sea	This study; Kilpert et al. 2012; Aylagas et al. 2014; Raupach et al. 2015
Isopoda	*Joeropsis brevicornis*	2	0	0.0117	Portugal, Spain	This study
Isopoda	*Lekanesphaera rugicauda*	0	5	0.0009	North Sea	Raupach et al. 2015
Isopoda	*Lekanesphaera hookeri*	4	0	0.0000	Portugal	This study
Isopoda	*Lekanesphaera terceirae*	0	2	0.0243	Azores	This study
Isopoda	*Ligia oceanica*	5	0	0.0103	Spain	Raupach et al. 2014
Isopoda	*Natatolana borealis*	1	3	0.0038	Spain, North Sea	Aylagas et al. 2014; Raupach et al. 2015
Isopoda	*Sphaeroma serratum*	1	1	**0.0578**	Portugal, Germany	This study; Kilpert et al. 2012
Isopoda	*Sphaeromatidae*	3	0	0.0010	Portugal	This study
Isopoda	*Stenosoma acuminatum **	5	0	0.0020	Iberian Peninsula	Xavier et al. 2009, 2012
Isopoda	*Stenosoma lancifer **	8	0	0.0026	Iberian Peninsula	This study; Xavier et al. 2009, 2012
Isopoda	*Stenosoma nadejda **	4	0	0.0100	Iberian Peninsula	Xavier et al. 2009
Tanaidacea	*Apseudes talpa*	3	0	0.0000	Portugal	This study; Larsen et al. unpublished
Tanaidacea	*Apseudopsis latreillii*	2	0	0.0020	Portugal	This study
Tanaidacea	*Condrochelia savignyi ***	3	1	0.0031	Portugal, Spain; France	This study; Aylagas et al. 2014; Larsen et al. unpublished
Tanaidacea	*Tanaididae n.i.*	1	0	-	Portugal	This study
Tanaidacea	*Tanais dulongii*	6	0	0.0074	Portugal, Spain	This study
Tanaidacea	*Tanais sp.*	0	1	-	Norway	This study

* Genus in BOLD with name (*Synisoma*) not updated.

** Process ID GBCM933-13 in BOLD with name not updated: as *Laptochelia dubia*

Annex 2.2. Grade system according with Oliveira et al. 2006 applied to the 87 identified (species level) morphospecies from Iberian Peninsula used in chapter 2.

Species	Grade
Ampithoe helleri	A
Apherusa jurinei	A
Apohyale prevostii	A
Apseudes talpa	A
Caprella penantis	A
Chondrochelia savignyi	A
Echinogammarus marinus	A
Elasmopus rapax	A
Gammarus chevreuxi	A
Gammarus crinocornis	A
Gammarus locusta	A
Haustorius arenarius	A
Idotea granulosa	A
Idotea pelágica	A
Jaera albifrons	A
Jassa falcata	A
Jassa herdmani	A
Melita palmata	A
Orchestia gammarellus	A
Orchestia mediterranea	A
Parajassa pelagica	A
Stenosoma lancifer	A
Abludomelita obtusata	B
Anthura gracilis	B
Aora typica	B
Campecopea hirsuta	B
Campecopea lustitanica	B
Caprella dilatata	B
Caprella scaura	B
Cyathura carinata	B
Cymodoce truncata	B
Dynamene bidentata	B
Dynamene edwardsi	B
Dynamene magnitorata	B
Gammarus insensibilis	B
Gnathia maxillaris	B
Halice walkeri	B
Idotea chelipes	B
Ischyromene lacazei	B
Ligia oceânica	B
Melita hergensis	B
Paracaprella pusilla	B
Podocerus variegatus	B
Quadrimaera inaequipes	B
Stenosoma acuminatum	B
Stenosoma nadejda	B
Tanais dulongii	B
Corophium multisetosum	C
Gammarela fucicola	C
Janira maculosa	C
Jassa pusilla	C
Microdeutopus chelifer	C
Phistica marina	C
Talitrus saltator	C
Ampelisca sarsi	D
Ampelisca spinipes	D
Ampithoe riedli	D
Apolochus neapolitanus	D
Apseudopsis latreilli	D
Astacilla damnoniensis	D
Bathiporeia tenuipes	D
Caprella fretensis	D
Caprella liparotensis	D
Cheirocratus intermedius	D
Cleantis prismatica	D
Eurydice spinigera	D
Iphimedia minuta	D
Jassa ocia	D
Joeropsis brevicornis	D
Leucothoe incisa	D
Natatolana borealis	D
Parapleutes bicuspis	D
Pseudoprotella phasma	D
Serejohyale spinidactylus	D
Sphaeroma serratum	D
Unciolella lunata	D
Urothoe grimaldii	D
Ampelisca diadema	E
Ampithoe ramondi	E
Ampithoe rubricata	E
Caprella acanthifera	E
Caprella danilevski	E
Dexamine spiniventris	E
Dexamine spinosa	E
Lekanesphaera hookeri	E
Urothoe pulchella	E

Annex 2.3. Sugested identifications to previously non-identified species and to possible misidentifications spoted in chapter 2.

Previously identification	Source	Suggested identification
Jassa sp.3	Lobo et al. 2013, 2016a	*Jassa herdmani*
Jassa sp.1	Lobo et al. 2013	*Jassa pusilla*
Microdeutopus sp.1	Lobo et al. 2016a	*Microdeutopus chelifer*
Corophidae n.i.3	Lobo et al. 2016a	*Monocorophium acherusicum*
Corophidae n.i.4	Lobo et al. 2016a	*Monocorophium sextonatae*
Caprella acanthifera	Aylagas et al. 2014	*Caprella danilevski*
Dexamine spinosa	Aylagas et al. 2014	*Dexamine spiniventris*
Microdeutopus chelifer	Lobo et al. 2016a	Aoridae
Ampithoe rubricata	Cowart et al. unpublished	*Ampithoe ramondi*

ANNEXES OF CHAPTER 3.

Annex 3.1. Material examined in chapter 3.

Explanation note: Each entry shows the number of specimens observed for each life history stage, habitat, person who provided the specimen(s), location and country, co-ordinates and other information.

Dynamene bicolor (Rathke, 1837)

Mediterranean

Spain

8 stage 8 ♂♂, 1 stage 7 ♂, 4 ♀♀, 2 juvs; 0-1 m, H. Zibrowius (Endoume Marine Lab.), 1986; Alicante, 38° 20′ N, 0° 29′ E (RMNH.CRUS.1.7573).

2 stage 8 ♂♂ (largest 3 mm x 1.5 mm) 2 stage 7 ♀♀ (largest 3 mm x 1.5 mm); 0.1 m, L. B. Holthuis, 1954; Rosen by Casa Zariguiey (Baai van Cadaques), 42° 14′ N, 3° 12′ E (RMNH.CRUS.1.7493).

3 stage 8 ♂♂, 3 ♀♀; amongst barnacles, shallow water, D. M. Holdich, 1984; Arenal d'en Castell, Menorca, 40° 1′ N, 4° 10′ E (RMNH.CRUS.1.7575).

1 stage 7 ♂, 4 ♀♀; red and brown algae in rock pool amongst the encrusting red algae, D. M. Holdich, 1984; Arenal d'en Castell, Menorca, 40° 1′ N, 4° 10′ E (RMNH.CRUS.1.7576).

1 stage 8 ♀; no habitat details, J. Castelló (University of Barcelona, Spain), 1983; Cap d'Artutx, Spain, 39° 55′ N, 3° 49′ E (RMNH.CRUS.1.7578).

1 ♀: no habitat details, J. Castelló (University of Barcelona), 1983; Cala Morell, Menorca, 40° 3′ N, 3° 53′ E (RMNH.CRUS.1.7579).

2 juvs; no habitat details, J. Castelló (University of Barcelona), 1983; Botafoc, Ibiza, 38° 54′ N, 3° 49′ E (RMNH.CRUS.1.7580).

3 ♀♀; shallow-water encrusting red algae and *Padina*, D. M. Holdich, 1978; Calla Longa, Ibiza, 38° 57′ N, 1° 31′ E (RMNH.CRUS.1.7582).

2 ♀♀; shallow-water algae, D. M. Holdich, 1978; San Antonio, Ibiza, 38° 58′ N, 1° 18′ E (RMNH.CRUS.1.7583).

1 ♀; no habitat details, J. Castelló (University of Barcelona), 1984; Estany d'es Peix, Formentera, 38° 43′ N, 1° 25′ E (RMNH.CRUS.1.7584).

France

13 stage 8 ♂♂, 9 stage 7 ♀♀, 3 stage 8 ♀♀, 6 juvs; 5-6 m, H. Zibrowius (Endoume Marine Lab.), 1980s; Ponteau, nr Marseille, 43° 22′ N, 5° 76′ E, and Marseille, 43° 17′ N, 5° 22′ E (RMNH.CRUS.1.7586).

1 stage 8 ♂, 2 ♀♀; littoral, H. Schmalfuss (Germany), 1962; Villefranche-sur-Mer, 43° 42′ N, 7° 18′ E (RMNH.CRUS.1.7588).

Many ♀♀ and juvs; shallow-water algae, M-L. Roman (University of Marseille), 1978; Lagune du Brusc (near Toulon to the east of Marseille), 43° 04′ N, 5° 48′ E (RMNH.CRUS.1.7589).

1stage 8 ♂, 1 stage 7 ♂, 1 stage 8 ♀; amongst algae at 1m depth; Argelès, 42° 32′ N, 3° 1′ E. No other details. (RMNH.CRUS.1.7495).

15 stage 8 ♂♂, 5 stage7 ♀♀, 2 stage 8 ♀♀; some from 12-15 m, others from rocky face at N. Citadel, H. Zibrowius, pre-1987; NW Corsica (Calvi), 42 ° 34′ N, 8° 45′ E. Pre-1987, but some samples dated from 1950s - these are from previous workers (e.g. Pérès and Picard at Endoume Marine Station). (RMNH.CRUS.1.7585).

1 stage 8 ♂ (4 mm), 1 ♀; there are 4 stage 8 ♀♀, but it is difficult to tell which species they are as both *D. bifida* and *D. torelliae* were found together; rocky shore, between seaweed, 0-1 m, 1957; Banyuls-sur-Mer, 42 ° 29′ N, 3° 7′ E (RMNH.CRUS.1.7498).

Italy

59+ stage 8 ♂♂, 32+ stage 7 ♂♂, 100+ ♀♀, 100+ juvs; shallow water *Cystoseira* and *Sargassum* and crevices, D. M. Holdich, 1967; Mergellina Harbour, Bay of Naples, 40° 49' N, 14° 13' E (RMNH.CRUS.1.7527 and RMNH.CRUS.1.7591).

1 stage 7 ♀; J. A. W. Lucas, 1953; Posillipo, Bay of Naples, 40° 48′ N, 14° 12′ E (RMNH.CRUS.1.7501).

1 stage 7 ♂; amongst algae near Zool. Station, Bay of Naples, L. B. Holthuis, 1950; 40° 49′ N, 14° 13′ E (RMNH.CRUS.1.7502).

4 stage 8 ♂♂, 1 stage 7 ♂; amongst mussels, JAW Lucas. 1953; Via Caracciola (Grand Hotel), Bay of Naples, 40° 50′ N, 14° 13′ E (RMNH.CRUS.1.7503).

1 stage 8 ♂ (damaged); amongst barnacles, J. A. W. Lucas, 1953; Rodonde, Gulf of Naples, 40° 49′ N, 14° 13′ E (RMNH.CRUS.1.7504).

1 stage 7 ♀; amongst barnacles, J. A. W. Lucas, 1953; Rodonde, Bay of Naples, 40° 49′ N, 14° 13′ E (RMNH.CRUS.1.7505).

1 stage 8 ♂; amongst barnacles; J. A. W. Lucas, 1953; Mergellina, Bay of Naples, 40º 49′ N, 14º 13′ E (RMNH.CRUS.1.7506).

3 stage 8 ♂♂, 1 ♀, 1 juv; no habitat details, H. Zibrowius, 1980s; Elba island, 42º 48′ N, 10º 08′ E (RMNH.CRUS.1.7599).

1 stage 8 ♂; 3 stage 7 ♂♂, 1 stage 8 ♀, 2 ♀♀; no habitat details, G. Messana, 1990s; St Andrea, Elba island, 42º 48′ N, 10º 8′ E. Specimens returned to collector.

6 stage 8 ♂♂, 1 stage 8 ♀, 8 ♀♀; no habitat details, H. Zibrowius, 1980s; Isola di Bergegge, Gulf of Genova, 44º 14′ N, 8º 26′ E (RMNH.CRUS.1.7600).

16 males stage 8 ♂♂, 3 stage 7 ♂♂, 6 ♀♀ females and juvs; 3.5 m on algae, U. Schieke and E. Fresi, 1968, 1970; E.La Voru au Wand, Ms Mary, S. Angelo, Ischia island, Bay of Naples, 40º 44′ N, 13º 57′ E (RMNH.CRUS.1.7605).

10 males 8 ♂♂, 8 stage 7 ♂♂, 13 ♀♀, 3 juvs; *Cystoseira* at 4 m (S. Anna), *Hydroides* and algae at 1-3 m, algae and detritus at 35 m (P. S. Pancrazio), algae and sediment at 8-12 m (L'Ameno), U. Schieke and E. Fresi, 1968, 1969; S. Anna, L'Ameno, P.S. Pancrzio, Ischia island, Bay of Naples, 40º 44′ N, 13º 57′ E (RMNH.CRUS.1.7606).

2 stage 8 ♂♂, 2 stage 7 ♂♂, 2 stage 8 ♀♀, 49 ♀♀ and juvs; 3.5 m detritus, *Dictyoptera* by laboratory, 0.5 m (Castelló), U. Schieke and E. Fresi, 1968, 1969; Castello and Ecological Laboratory, Ischia island, Bay of Naples, 40º 44′ N, 13º 57′ E (RMNH.CRUS.1.7607)

16 stage 8 ♂♂, 15stage 6/7 ♂♂, 29 ♀♀ and juvs; 1-4 m on algae below lab., U. Schieke and E. Fresi, 1968; Ecological Laboratory, Ischia island, Bay of Naples, 40º 44′ N, 13º 57′ E (RMNH.CRUS.1.7608).

5 stage 8 ♂♂, 4 stage 7 ♂♂, 30 ♀♀ and juvs; 0.5-4 m on *Cystoseira* and *Dictyoptera*, U. Schieke and E. Fresi, 1968; Castello and below Ecological Laboratory, Ischia island, Bay of Naples, 40º 44′ N, 13º 57′ E (RMNH.CRUS.1.7609).

3 stage 8 ♂♂; trottoir, U. Schieke and E. Fresi, 1968; Palermo, Sicily, 38º 11′ N, 13º 20′ E (RMNH.CRUS.1.7601).

Croatia

1 stage 7 ♂; no other details, 1960; Rovinji, 45º 4′ N, 13º 38′ E, (RMNH.CRUS.1.7507) (labelled as from Yugoslavia).

Greece

3 stage 8 ♂♂,1 stage 7 ♂, 1 stage 8 ♀, 1 ♀; shallow water algae, D. A. Jones, 1967; Emborios Bay, Chios island, 38º 11′ N, 26º 1′ E (RMNH.CRUS.1.7529).

11 stage 8 ♂♂ (averaging 3 mm in length), 1 stage 7 ♂, 2 stage 8 ♀♀, 9 ♀♀ and juvs,; variety of algae, e.g. *Cystoseira*, *Sargassum* at a variety of depths, e.g 0.5 m down to 33 m, D. A. Jones, 1967; Cathedral Rock, Emborios Bay, Cape Maskla, Chios island, 38º 11′ N, 26º 1′ E (RMNH.CRUS.1.7645).

7 stage 8 ♂♂, 2 stage 8 ♀♀; from oscula of sublittoral sponge, D. M. Holdich, 1980; Gerakini Beach, Halkidiki Peninsula, 40º 16′ N, 23º 26′ E (RMNH.CRUS.1.7612).

2 stage 8 ♂♂; from sublitoral coralline algae, D. M. Holdich, 1980; Gerakini Beach, Halkidiki Peninsula, 40º 16′ N, 23º 26′ E (RMNH.CRUS.1.7613).

22 stage 8 ♂♂, 4 stage 8 ♀♀, 1 ♀, (plus 1 male *Campecopea hirsuta*); from surface of empty *Pinna* shell at 2 m, D. M. Holdich, 1980; Gerakini Beach, Halkidiki Peninsula, 40º 16′ N, 23º 26′ E (RMNH.CRUS.1.7614).

4 juvs; from fucoid algae at 0.5 m, D. M. Holdich, 1980; Ouranoupolis, Halkidiki Peninsula, 40º 19′ N, 23º 58′ E (RMNH.CRUS.1.7615).

1 stage 8 ♂ (damaged). 0-15 m, W. J Wolffen and M. Loosies, 1967; Missolonghi (to west of the Gulf of Corinth, now called Mesolongion, 38º 22′ N, 21º 25′ E (RMNH.CRUS.1.7508).

9 stage 7 ♀♀; no habitat details, W. J. Wolff and M. Loosjes, 1964; Sulaora, Epirus (bordering Albania), 39º 30′ N, 20º 15′ E (RMNH.CRUS.1.7509).

1 stage 7 ♂, 1 ♀; weed under stones, <1 m, D. A. Jones, 1967; Corfu island, 39º 36′ N, 19º 49′ E (RMNH.CRUS.1.7643).

1 ♀; encrusting red seaweeds, D. M. Holdich, 1983; Matala, Crete island, 34º 59′ N, 24º 44′ E (RMNH.CRUS.1.7644).

Turkey

2 stage 8 ♂♂ (4.2 x 2 mm), 1 stage 7 ♂; intertidal and shallow water algae and other habitats, F. Kirkim, mid-1990s; Izmir region, 38º 28' N, 27º 6' E (RMNH.CRUS.1.7657).

10 stage 8 ♂♂, 5 stage 7 ♂♂, 17 stage 7 ♀♀, plus 1 *Cymodoce* ♀ sp; 0-50 cm deep, C. Swennen, 1957; Antalya, 36º 53' N, 30º 44' E (RMNH.CRUS.1.7510).

25 stage 8 ♂♂, 2 stage 7 ♂♂, 5 stage 8♀♀, 13 stage 7 ♀♀ and juvs; 5-20 cm deep, C. Swennen, 1959; Antalya harbour, 36º 53' N, 30º 42' E (RMNH.CRUS.1.7511).

1 stage 8 ♀; C. Swennen, 1959; Antalya harbour, 36º 53' N, 30º 42' E (RMNH.CRUS.1.7512).

Israel

2 stage 8 ♂♂, 1 stage 7 ♂, 1 ♀, 1 juv; algae from rocky shore; T. Haran (Tel Aviv University), 1977-78; Mikhmoret (south of Haifa), 32º 24' N, 34º 52' E (RMNH.CRUS.1.7528).

9 stage 8 ♂♂ (largest 4.0 x 2.0 mm), 14 stage 6/7 ♂♂ (3.25 x 1.5 mm), 60 ♀♀ (largest 3.5 x 1.4 mm), 63 juvs; algae on rocky shore (*Ulva*, *Sargassum*, *Jania*, *Acanthophora*, *Cystoseira*), T. Haran (Tel Aviv University), 1977-78; Mikhmoret (south of Haifa), 32º 24' N, 34º 52' E (RMNH.CRUS.1.7649).

1 stage 6 ♂, 9 ♀♀, 4 juvs; littoral algae, L. Fischelson, 1976; Rosh HaNikva, north of Haifa, 33º 5' N, 35º 6' E (for Haifa) (RMNH.CRUS.1.7650).

7 stage 8 ♂♂, 4 stage 7 ♂♂, 6 ♀♀; littoral algae (*Cystoseira*, *Laurencia*, *Jania*), L. Fischelson, 1976; Acre, north of Haifa, 32° 55' N, 35° 4' E (for Haifa) (RMNH.CRUS.1.7652).

3 stage 7 ♂♂, 1 stage 6 ♂, 7 ♀♀, 7 juvs; littoral algae (*Pterocladia*, *Acanthophora*, *Ulva*, *Jania*, *Bryopsis*, *Sargassum*, *Padina*), L. Fischelson, 1976; Bat-Yam, south of Tel Aviv, 32° 5 N', 34° 48' E (for Tel Aviv) (RMNH.CRUS.1.7654).

4 ♀♀, 4 juvs; littoral algae (*Pterocladia*, *Colpomenia*), L. Fischelson, 1977; Michmoreth north of Netania, 32° 20' N, 34° 52' E (for Netania) (RMNH.CRUS.1.7655).

2 ♀♀, 4 juvs; littoral algae (*Jania*, *Centroceras*), L. Fischelson, 1977; Palmhim, north of Ashdod, 31° 55' N, 34° 42' E (RMNH.CRUS.1.7656).

Malta

1 stage 7 ♂, 1 ♀; intertidal rock pool, D. M. Holdich, 1997; Mellieha Bay, 35° 58’ N, 14° 21’ E (RMNH.CRUS.1.7661).

3 stage 8 ♂♂, 3 stage 7 ♀♀; rocky sea bed, 1-2 m, S. Schembri, 2010; Cirkewwa, 35° 59’ N, 14° 19’ E (RMNH.CRUS.1.7662).

Egypt

2 stage 8 ♂♂; M. M. Atta, 1981; Agamy area, Alexandria, 31° 9’ N, 29° 55’ E (RMNH.CRUS.1.7667).

Tunisia

4 stage 8 ♂♂; Th Monod; Tunis (?), 38° 48’ N, 10° 10’ E. Museum National d’Histoire Naturelle, Paris collection. IS 683.

Black Sea

Turkey

1 stage 7 ♂, 1 stage 8 ♀♀, 1 ♀, 1 juv; no habitat details, A. M. Gozler, 2007; Rýze coast, 41° 01' N, 40° 28' E (RMNH.CRUS.1.7530).

1 stage 8 ♂ (3 x 1.3 mm – very small), 5 stage 7 ♂♂ (3.5 x 1.5), 8 ♀♀ (3.5 x 1.5 mm), 1 juv; no habitat details, A. M. Gozler, 2007; Rýze coast, 41° 01' N, 40° 28'E (RMNH.CRUS.1.7670).

1 stage 8 ♂, 1 stage 6 ♂, 2 stage 8 ♀♀, 4 ♀♀, 13 juvs; infralittoral down to 5 m depth, algae (*Cystoseira* spp.), amongst *Mytilus galloproincialis*, G. Gönlügür, 2006; Sinop coast, 42° 00' N, 35° 10' E (RMNH.CRUS.1.7671).

1 stage 7 ♀; 0-5m deep. C. Swennen, 1969; Trabzon harbour (north coast of Turkey near eastern end of Black Sea), 40° 57' N, 39° 43' E (RMNH.CRUS.1.7514).

Romania

1 stage 8 ♂,1 stage 6 ♂, 1 stage 8 ♀, 5 ♀♀, 6 juvs; hard bottoms and silt amongst *Mytilus* and empty shells of *Balanus improvisus* at 5-10 m, M. Băcescu, 1961; eastern Constanta, 44° 12' N, 28° 38' E (RMNH.CRUS.1.7672).

Bulgaria

1 stage 8 ♂ (damaged back end), 2 ♀♀ (one damaged); no habitat details, O. G. Kussakin, 1950s; Varna, 43° 13' N, 28° 00' E (RMNH.CRUS.1.7673).

3 stage 8 ♂♂, 1 stage 6 ♂, 2 stage 8 ♀♀, 3 ♀♀, 2 juvs; no habitat details, O. G. Kussakin; label just says Black Sea, could be Varna, 43° 13' N, 28° 00' E (RMNH.CRUS.1.7674).

Georgia

1 stage 8 ♂; 0.5-1.5 m, O. G. Kussakin, 1900s; Sukhumi Bay - 43° 00' 12'' N, 41° 00' 55'' E (RMNH.CRUS.1.7675).

Dynamene bidentata (Adams, 1800)

Atlantic

Canary Islands (Spain)

1 stage 7 ♂, 2 ♀♀, one juv; algae in upper shore pool, D.M. Holdich, 1970; El Medano, Tenerife, 28° 2’ N, 16° 32’ W (RMNH.CRUS.1.7558).

1 juv; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Bañaderos, Gran Canaria, 28° 8' N, 15° 32' W.

England (including the Isle of Man)

6 stage 8 ♂♂ (second year, with growth of algae on pleotelson and uropods), 4 stage 7 ♀♀, 5 stage 8 ♀♀ (one with epicarid parasite *Ancyroniscus bonnieri*),; empty *Balanus perforatus* tests, D. M. Holdich, 1996; Trevone Bay, Cornwall, 50° 32’ N, 4° 58’ W (RMNH.CRUS.1.7546).

In addition, specimens of *D. bidentata* were examined by DMH from the following locations during the period 1964-1975 (Holdich 1970, 1974, 1976) and subsequently: Bovisand (50°19' 60" N, 04° 06' 60" W); Cape Cornwall (50°07' 01" N, 05° 42' 04" W); Castle Rocks, Falmouth (50° 08' 38" N, 05° 03' 40" W); Derby Haven, Isle of Man (54° 04' 00" N, 04° 37' 00" W); Goodrington Sands (50° 24' 60" N, 03° 33' 60" W); Helford Passage (50° 05' 34" N, 05° 06' 07" W); Ilfracombe (51° 12' 32" N, 04° 07' 46" W); Kennack Sands (50° 00' 00" N, 05° 10' 00" W); Lizard (49° 57' 43" N, 05° 11' 54" W); Marazion (50° 07' 00" N, 05° 27' 00" W); Mullion Cove (49° 59' 49" N, 05° 15' 18" W); Periglio Bay, St Agnes, Scilly Isles (49° 53' 60" N, 06° 24' 00" W); Port Erin, Isle of Man (54° 05' 03" N, 04° 45' 39" W); Portland Harbour (50°

34' 00" N, 02° 26' 00" W); South Milton Sands (50° 15' 00" N, 03° 50' 60" W); St Agnes (50° 19' 01" N, 05° 14' 02" W); St Marys, Isles of Scilly (49° 55' 15" N, 06° 18' 48" W); St Ives (50° 11' 60" N, 05° 28' 00" W); Treyarnon (50° 31' 12" N, 05° 01' 44" W); Wembury, Church Reef (50° 17' 26" N, 04° 46' 46" W); Widermouth (50° 7' 13" N, 04° 33' 37" W); Whitesand Bay (50° 19' 60" N, 04° 15' 60" W).

British Natural History Museum (BMNH) collection:

Port St Mary, Isle of Man (54° 04' 33" N, 04° 44' 21" W); Kimmerridge Bay (50° 36' 39" N, 02° 06' 59" W); Brandy Bay (50° 36' 47" N, 02° 09' 26" W); Hobarrow Bay (50° 36' 33" N, 02° 08' 55" W); St Agnus, Scilly Isles – in sponges and worm tubes (49° 53' 35" N, 06° 20' 25" W); Porth Warne, St Agnus, Scilly Isles - amongst red algae (49° 54' 58" N, 06° 17' 53" W); Porth Hellick, Scilly Isles (49° 55' 07" N, 06° 16' 49" W), Hells Gate Beach, Lundy – intertidal weed (51° 10' 41" N, 04° 20' 02" W).

National Biodiversity Network:

14 records have been used from the NBN database, but details are not given as they can be found on the NBN website: www.searchnbn.net.

ERICA database:

St Martins, Isles of Scilly (49° 58' 48" N, 06° 17' 28" W); Tresco, Isles of Scilly (49° 56' 47"N 06° 20' 18" W); Bryher, Isles of Scilly (49° 57' 08" N, 06° 21' 54" W); Smith Sound, Isles of Scilly (49° 52' 52" N, 06° 21' 59" W); Old Town, Isles of Scilly (49° 54' 09" N, 06° 18' 13" W); Mevagissey (50° 15' 16" N, 04° 46' 01" W); St Mawes (50° 09' 18" N, 05° 01' 20" W); Porthleven (50° 04' 57" N, 05° 19' 21" W); Clovelly (50° 59' 00" N, 04° 23' 00" W), Feock (50° 11' 36" N, 05° 03' 35" W); Gwithian (50° 13' 42" N, 05° 23' 39" W); Mounts Bay (50° 05' 35" N, 05° 22' 27" W); Padstow Bay (50° 34' 49" N, 04° 55' 20" W); Penzance (50° 07' 00" N, 05° 31' 60" W); Portlooe (50° 20' 31" N, 04° 27' 38" W); Portscatho (50° 11' 06" N, 04° 58' 15" W); Rosenithon (50° 02' 48" N, 05° 04' 02" W); Treen (50° 11' 10" N, 05° 36' 05" W); Trevelgue (50° 25' 33" N, 05° 03' 13" W); Weymouth (50° 36' 17" N, 02° 32' 12" W).

Northern Ireland

National Biodiversity Network:

22 records have been used from the NBN database, but details are not given as they can be found on the NBN website: www.searchnbn.net.

Southern Ireland (Eire)

1 stage 7 ♂, 2 stage 8 ♂♂, two ♀♀, 2 juvs; rocky shore, D. McGrath, 1975; Galway, Southern Ireland (Eire), 53° 16’ N, 9° 3’ W (RMNH.CRUS.1.7519).

1 stage 7 ♂, 8 stage 8 ♂♂, 76 ♀♀ and juvs; rocky shore, D. McGrath, 1975; Galway, Southern Ireland (Eire), 53° 16’ N, 9° 3’ W (RMNH.CRUS.1.7550).

British Natural History Museum (BMNH) collection:

Roundstone (53° 23' 36" N, 09° 51' 27" W), Tory Island (55° 15' 55" N, 08° 13' 49" W), Valentia (51° 53' 59" N, 10° 20' 00" W).

National Biodiversity Data Centre (Ireland):

Ballycotton (51° 49' 59" N, 08° 01' 03" W), Carrownedin (54° 13' 55" N, 09° 05' 21" W), Corkagh Beg (54° 16' 11" N, 08° 45' 17" W), Doonbeg (52° 44' 43" N, 09° 31' 26" W), Dungarvan (52° 44' 43" N, 07° 32' 45" W), Fenit (52° 17' 07" N, 09° 52' 34" W), Finvarra (53° 08' 58" N, 09° 08' 22" W), Garrywilliam (52° 18' 34" N, 10° 03' 17" W), Glengariff (51° 44' 52" N, 09° 32' 56" W), Liscannor (52° 56' 10" N, 09° 26' 16" W), Loughshinny (53° 33' 39" N, 05° 58' 55" W), Mullagh (52° 47' 31" N, 09° 29' 06" W), Rathlee (54° 16' 49" N, 09° 03' 31" W), The Seven Hogs (52° 19' 33" N, 10° 01' 13" W).

National Biodiversity Network:

1 record have been used from the NBN database, but details are not given as they can be found on the NBN website: www.searchnbn.net.

Scotland

7 ♀♀ and juvs; on algae in water off rocks at LWM, R. S. Scott, Leicester University Expedition, 1970; Ceann Ear, Monach Islands, NW Scotland, 57° 31’ N, 7° 36’ W (RMNH.CRUS.1.7549).

14 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Carsaig, Scotland, 56° 9' N, 5° 57' W.

1 stage 8 ♂, 14 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Easdale, Scotland, 56° 17' N, 5° 38' W.

3 stage 6-8 ♂♂, 15 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Bellochantuy, Scotland, 55° 31' N, 5° 42' W.

In addition, specimens of *D. bidentata* were examined by DMH from the following locations during the period 1964-1974 (Holdich 1970, 1974): Ardrossan (55° 36' N, 04° 43' W), Clatholl (58° 10' N 00", 05° 19' 00" W).

British Natural History Museum (BMNH) collection:

Oban (56° 24' 54" N, 05° 28' 15" W), Mull (approx. 56° 26' 21" N, 06° 00' 03" W).

National Biodiversity Network:

27 records have been used from the NBN database, but details are not given as they can be found on the NBN website: www.searchnbn.net.

Wales

6 stage 8 ♂♂ (year 1, 5.0-6.0 mm), 1 back end stage 8 ♂, 2 stage 8 ♂♂ (year 2), 1 stage 6 ♂, 8 stage 7 ♀♀ (5.5 mm), 2 stage 8 ♀♀, 2 juvs; adults from empty *Balanus perforatus* tests on mid-shore and juveniles from mid-shore *Fucus serratus*, D.M. Holdich, 1965; St Brides Haven, Pembrokeshire, South Wales, 51° 46’ 00" N, 5° 6’ 00" W (RMNH.CRUS.1.7517).

3 stage 8 ♂♂ (year 2), 6 stage 8 ♂♂ (year 2), 4 stage 6 ♂, 4 stage 7 ♀♀ (5.5 mm), 3 stage 8 ♀♀, 1 juv; adults from empty *Balanus perforatus* tests on mid-shore and juveniles from mid-shore *Fucus serratus*, D.M. Holdich, 1994; St Brides Haven, Pembrokeshire, South Wales, 51° 46’ 00" N, 5° 6’ 00" W (RMNH.CRUS.1.7548).

In addition, specimens of *D. bidentata* were examined by DMH from the following locations during the period 1964-1975 (Holdich 1970, 1976) and subsequently: Aber-Eiddy (51° 56' 30" N, 05° 11' 55" W), Abermawr (51° 56' 17" N, 05° 12' 27" W), Broad Haven (51° 42' 05" N, 05° 09' 11" W), Caer-fai Ba (51° 52' 00" N, 05° 15' 00" W), Dinas Head (52° 01' 15" N, 04° 54' 36" W), Freshwater East (51° 38' 44" N, 04° 51' 34" W), Freshwater West (51° 38' 60" N, 05° 02' 60" W), Goodwick Harbour (51° 59' 60" N, 04° 59 '00" W), Manorbier (51° 37' 60" N, 04° 46' 60" W), Martins Heaven (51° 44' 14" N, 05° 14' 01" W), Monks Haven (51° 42' 60" N, 05° 08' 00" W), Musselwick Sands (51° 42' 60" N, 05° 12' 00" W), Nolton Haven (51° 48' 58" N, 05° 06' 27" W), Porth Colman (52° 52' 00" N, 04° 41' 00" W), Rhoscolyn (53° 15' 00" N, 04° 34' 60" W), Rhosneigre (53° 13' 00" N, 04° 30' 60" W), Sandy Haven (51° 43' 32" N, 05° 06' 41" W), Skomer (51° 43' 60" N, 05° 16' 60" W), Stackpole (51° 37' 41" N, 04° 53' 45" W), West Angle (51° 40' 60" N, 05° 04' 60" W), West Dale Bay (51° 42' 28" N, 05° 11' 19" W), Whitesand Bay (51° 53' 10" N, 05° 18' 18" W. Records for the Gower Peninsula in South Wales prior to 1961 have not been included as the isopod was eliminated from this region in the severe winter of 1961/62, and the authors have seen no new records.

National Biodiversity Network:

12 records have been used from the NBN database, but details are not given as they can be found on the NBN website: www.searchnbn.net.

Channel Islands

14 stage 8 ♂♂, 3 stage 7 ♀♀, 14 stage 8 ♀♀, 2 juvs; upper mid-shore crevice with tubiculous tanaids, mid and lower shore in empty *Balanus perforatus* shells and crevices, D.M.Holdich, 1982; Rocquaine Bay, Guernsey, 49° 26’ N, 2° 39’ W (RMNH.CRUS.1.7551).

4 stage 8 ♂♂, 3 stage ♀♀; mid shore in empty *Balanus perforatus* shells, D.M. Holdich, 1982; Petit Bot Bay, Guernsey, 49° 25’ N, 2° 34’ W (RMNH.CRUS.1.7552).

4 ♀♀; lower shore weed (*Chondrus crispus*), D.M. Holdich, 1982; Rocquaine Bay, Guernsey, 49° 26’ N, 2° 39’ W (RMNH.CRUS.1.7553).

France

2 stage 8 ♂♂ (6.0-6.5 mm), 1 stage 7 ♀ (5.5 mm), 1 stage 8 ♀; adults from empty *Balanus perforatus* test on mid-shore; ♀7 from mid-shore *Ascophyllum nodosum*, D.M. Holdich, 1975, shore by Roscoff Marine Station, Brittany, France, 48° 43’ N, 3° 59’ W (RMNH.CRUS.1.7518).

2 stage 7 ♂♂, 7 stage 8 ♂♂ (one 7 mm), 2 stage 8 ♀♀, 16 ♀♀ and juvs; algae, empty barnacle tests and crevices, mid-lower shore, D.M. Holdich, 1988; Trénez, S. Brittany, 47° 47’ N, 3° 42’ W (RMNH.CRUS.1.7560).

5 ♀♀ and juvs; red algae lower shore, D.M. Holdich, 1988; Trénez, S. Brittany, 47° 47’ N, 3° 42’ W (RMNH.CRUS.1.7561).

4 stage 7 ♂♂, 7 stage 8 ♂♂, 9 females ♀♀ and juvs; rocky mid-shore weed and crevices, D.M Holdich, 1988; Kerfanny, S. Brittany, 47° 51’ N, 3° 38’ W (RMNH.CRUS.1.7562).

1 stage 7 ♂, 2 stage 8 ♂♂, 2 stage 8 ♀♀, 4 ♀♀ and juvs; empty barnacle tests and mid-shore algae, D.M. Holdich, 1975; Roscoff Marine Station Brittany, 48° 43’ N, 3° 59’ W (RMNH.CRUS.1.7563).

2 stage 7 ♀♀; littoral, L. Deckker, 1982; Bestree Pord, Finisterre, 48° 15’ N, 3° 55’ W (RMNH.CRUS.1.7480).

1 juv; H. Nouvel, 1952; Isla Vete de Callo Baie de Morlaiz, not far from Roscoff, 48° 43’ N, 3° 53’ W (RMNH.CRUS.1.7481).

1 stage 8 ♂ (5.5 mm), 1 juv; C. Swennen, 1958; Perros-Guirec, Brittany, 48° 48’ N, 3° 26’ W (RMNH.CRUS.1.7482).

2 stage 8 ♂♂ (8mm and 6 mm), 1 juv; H. Nouvel, 1952; Roscoff, Brittany, 48° 43’ N, 3° 59’ W (RMNH.CRUS.1.7483).

1 stage 7 ♂, 1 stage 7 ♀; H. Nouvel, 1936; Brest, St Ann, 48° 23’ N, 4° 29’ W (RMNH.CRUS.1.7492).

Museum National d’Histoire Naturelles, Paris collection:

1 ♀; 1922; location unknown (IS 1288).

2 ♀♀; 1922; Concarneau, S. Brittany, 48° 48’ N, 3° 26’ W (IS 1292).

1 stage 6 ♂; 1922; Concarneau, 48° 48’ N, 3° 26’ W (IS 1303).

1 female; Concarneau, 48° 48’ N, 3° 26’ (IS 1291).

4 stage 8 ♂♂; 1922; Concarneau, 48° 48’ N, 3° 26’ (IS 1295).

Spain

4 stage 6/7 ♂♂, 1 stage 8 ♂, 6 ♀♀, 31 juvs; *Fucus vesiculosus* zone, R. Anadon, 1982; Bañugues (Asturias), 43º 31’ N, 5º 39’ W (RMNH.CRUS.1.7569).

15 stage 7 ♂♂, 1 stage 8 ♂, 1 stage 8 ♀, 27 ♀♀, 51 juvs; mesolittoral, P. Reboreda, 1984, 1987, 1988; Ria del Ferrol (43º 29’ N, 8º 13’ W), Ria de Arosa (42º 34’ N, 8º 53’ W), Isla Castelo (43º 36’ N, 8º 11’ W) (RMNH.CRUS.1.7570).

1 ♀; littoral amongst *Leathesia*, R.M.N.H., St. 0. 15, 1962; San Vincente, Peninsula del Grove, Ria del Arosa, 42º 34’ N, 8º 53’ W (RMNH.CRUS.17485).

1 stage 7 ♂; R.M.N.H., St. 0. 90,1967; Punta San Vincente del Grove, 42º 34’ N, 8º 53’ W (RMNH.CRUS.17486).

1 stage 8 ♂ (7 mm), 1 stage 7 ♀ (5 mm); littoral in *Saccorhiza*, 1962; San Vincente, 42º 34’ N, 8º 53’ W (RMNH.CRUS.17489).

1 stage 8 ♂, 2 stage 7 ♀♀, 1 juv; 0-1 m, 1963; San Vincente, Peninsula dell Grove, 42º 34’ N, 8º 53’ W (RMNH.CRUS.17491).

1 stage 8 ♂, 77 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Pedreira, Galicia, 43° 33' N, 8° 16' W.

1 stage 8 ♂, 121 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Barizo, Galicia, 43° 19' N, 8° 52' W.

6 stage 6-8 ♂♂, 32 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Muxía, Galicia, 43° 5' N, 9° 13' W.

No specimens available, but a clear photograph by Guerra-Garcia shows a stage 8 ♂♂ from Tarifa island, Spain, 36º 00’ N, 5º 36’ W. For detailed habitat information and number of individuals refer to Izquierdo et al. (2011), Guerra-García et al. (2011), Guerra-García et al. (2012), Torrecilla-Roca and Guerra-García (2012).

Portugal

2 stage 8 ♂♂, 45 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Buarcos, Portugal, 40° 10' N, 8° 54' W.

8 stage 8 ♂♂, 6 stage 8 ♀♀, 80 juvs; intertidal rocky shores among algae, in barnacles and in crevices, during low tide, P. Vieira, 2015; Buarcos, Portugal, 40° 10' N, 8° 54' W.

1 juv; intertidal rocky shores among algae during low tide, P. Vieira, 2015; Agudela, Portugal, 41° 14' N, 8° 43' W.

1 juv; intertidal rocky shores among algae during low tide, P. Vieira, 2014; Praia Vale dos Homens, Portugal, 37° 22' N, 8° 50' W.

3 juvs, intertidal rocky shores among algae during low tide, P. Vieira, 2011; Peniche, Portugal, 39° 22' N, 9° 22' W.

18 juv; among algae, F.O. Costa, 2012; Praia Norte, Portugal, 41° 41' N, 8° 50' W.

4 juv; among algae, F.O. Costa, 2014; Sines, Portugal, 38°28' N, 8°59' W.

2 juv; among algae, F.O. Costa, 2012; Vila do Conde, Portugal, 41°21' N, 8°45' W.

Morocco (NW Africa)

1 ♀ stage 7; Very smooth, no hairs, no keel on pleotelsonic dome, foramen right shape. Label is in Dutch. Station 30 - found 23 km from Rabat (33º 58’ N, 6º 50’ W), NW Morocco, Casablanca. 20 October 1974. RMNH.CRUS.1.7450.

2 stage 7 ♂♂; H. Gantès, 1949; Témara, 33º 55’ N, 6º 54’ W (RMNH.CRUS.1.7451).

2 stage 8 ♂♂, 1 stage 6 ♂, 19 ♀♀ and juvs; among *Laminaria*, P. Vieira, 2015; El Jadida, Morocco, 33° 14' N, 8° 28' W.

1 stage 8 ♀; intertidal rocky shores among algae during low tide, P. Vieira, 2014; Akhfenir, Morocco, 28° 6' N, 12° 3' W.

2 stage 8 ♂♂, 11 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2014; Insouane, Morocco, 30° 50' N, 9° 49' W.

1 stage 8 ♂, 30 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2014; Essaouire, Morocco, 31° 30' N, 9° 46' W.

Dynamene bifida Torelli, 1930

Mediterranean

Spain

1 stage 7 ♂, 1 ♀; under stones 0.5 m, D.M. Holdich, 1985; Pueblo Pier, Mojacar, Spain, 37º 8’ N, 1º 49’ E (RMNH.CRUS.1.7574).

France

1 stage 8 ♂, 1 stage 7 ♂, 1 stage 8 ♀; rocky shore, between seaweed, 0-1 m, L.B. Holthuis, 1955; Banyuls Marine Laboratory, France, 42º 29’ N, 6º 6’ E (RMNH.CRUS.1.7526).

1 stage 8 ♂, 1 stage 7 ♂, 1 stage ♂ 6, 3 stage 7 ♀♀; 0-1m deep, near lab, L.B. Holthuis, 1955; Banyuls-sur-Mer, France 42º 29’ N, 3º 7’ E (RMNH.CRUS.1.7496).

5 stage 8 ♂♂ (4-6 mm), 4 stage 8 ♀♀; rocky shore, between seaweed, 0-1 m, L.B. Holthuis, 1957; Banyuls-sur-Mer, France, 42º 29’ N, 6º 6’ E (RMNH.CRUS.1.7498).

Italy

2 stage 8 ♂♂ (one back end, likely to be >7 mm total length), 2 stage 7 ♂♂, 3 ♀♀; 1.0 m under stones, D.M. Holdich, 1969; Ischia island, Bay of Naples, Italy, 40^{o} 44' N, 13^{o} 56' E (RMNH.CRUS.1.7525).

1 stage 8 ♂ (front end only), 2 stage 8 ♀♀; amongst shallow-water *Hydroides* colony, D.M. Holdich, 1967; Mergellina, Bay of Naples, Italy, 40^{o} 49’ N, 14^{o} 13’ E (RMNH.CRUS.1.7592).

Turkey

1 stage 8 ♂ (4.5 x 2 mm), 1 ♀ (3.2 x 1.75); intertidal and shallow-water algae and other habitats, F. Kirkim, mid-1990s; central location of Aegean coast at Izmir, (RMNH.CRUS.1.7659).

In addition, specimens of *D. bifida* were examined by DMH from Corfu (39^{o} 36’ N, 19^{o} 49’ E) during the period 1964-1970 (Holdich 1970), but no further details are available.

Dynamene edwardsi (Lucas, 1849)

Atlantic

Azores (Portugal)

1 stage 8 ♂ (3 mm), 2 stage 7 ♂♂, 2 stage 8 ♀♀ (3 mm), 6 stage 7 ♀♀ (2.8 mm), 9 juvs; rocky shore with deep tide pools, Tydeman Azores Exp. 1981; CANCAP-V. Stat. 5.K15, south-east coast Corvo, south of Rosario, 39° 40’ N, 31° 07’ W (RMNH.CRUS.1.7452).

3 stage 8 ♂♂ (4 mm), 6 stage 7 ♂♂, 12 stage 7 ♀♀ (3 mm), 1 juv; Tydeman Azores Exp. 1981; CANCAP-V. Stat. 5.K10, north coast São Jorge, Faja da Caldeira, 38° 38’ N, 27° 56’ W (RMNH.CRUS.1.7453).

1 stage 8 ♂, 1 stage 7 ♂, 5 ♀♀, 1 juv, (plus one *Campecopea lusitanica*); rock flat with holes and tide pools, much algae, Tydeman Azores Exp. 1981; CANCAP-V. Stat. 5.KO2, east coast Santa Maria, Baia, S. Lourence, 36° 56’ N, 25° 06’W (RMNH.CRUS.1.7460).

1 stage 8 ♂ (3.5 mm), 1 stage 8 ♀; tide pools and fissures with strong currents, 2 m deep, Tydeman Azores Exp. 1981; CANCAP-V, Stat. 5.KO1, Formigas, 37° 16’ N, 24° 47’ W (RMNH.CRUS.1.7456).

1 stage 8 ♂ (3.5 mm); rocky shore with large protected pool and several smaller pools, all connected with the sea by crevices, considerable growth of algae, Hartog and Lavaleye, 1979; Terceira, south coast, west of Angra do Heroismo, between Baia de Vila Maria and São Mateus da Calheta. Stat. 22, 38° 39’ N, 27° 15’ W (RMNH.CRUS.1.7454).

1 stage 8 ♂ (4 mm); rocky shore collecting and snorkling, Hartog and Lavaleye, 1979; Pico, south coast, Lages harbour, Azores, 38° 24’ N, 28° 15’ W (RMNH.CRUS.1.7457).

1 juv; intertidal rocky shores among algae during low tide, P. Vieira, 2014; Ponta da Ferraria, São Miguel, 37° 51' N, 25° 51' W.

2 juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2014; Mosteiros, São Miguel, 37° 54' N, 25° 49' W.

Madeira (Portugal)

4 stage 8 ♂♂, 1 stage 7 ♀, 1 stage 8 ♀; rocky littoral/sublittoral with boulders, snorkling, ONVERSAAGD-Madeira-Morokko Exp., 1976; south coast of Madeira, Funchal west of harbour, 32° 44’ N, 16° 44’ W (RMNH.CRUS.1.7471).

1 stage 7 ♂, 1 stage 6 ♂, 2 ♀♀, 1 juv; polluted rocky littoral, tide pools, crevices, Tydeman-Madeira-Mauritania Exp., 1978; Stat 3.KO2-CANCAP-III, south coast of Madeira, Funchal, 32° 38’ N, 16° 56’ W (RMNH.CRUS.1.7473).

1 stage 8 ♂, 2 stage 8 ♀♀, (plus one *Cymodoce* sp.); rocky littoral, pools, shallow sublittoral, Tydeman-Madeira-Mauritania Exp. 1978; Stat. 3, KO1-CANCAP-III, south-east coast of Madeira, Caniçal, 32° 44’ N, 16° 44’ W (RMNH.CRUS.1.7474).

1 stage 8 ♂ (4 mm by 2mm); shore-collecting, snorkling and diving, depth 0-22 m, ONVERSAAGD-Madeira-Morocco Exp., 1976; Stat. 14 south-east coast of Madeira near Caniçal, 32° 44’ N, 16° 44’ W (RMNH.CRUS.1.7475).

2 stage 8 ♂♂ (4 mm by 2mm), 1 stage 7 ♂, 1 ♀; polluted rocky littoral pools, Tydeman-Madeira-Mauritania Exp., 1978; Stat 3-KO3-CANCAP III, south coast of Madeira, Funchal, west of harbour pier 32° 38’ N, 16° 58’ W (RMNH.CRUS.1.7476).

7 ♀♀ and juvs; rocky littoral, pools, shallow sublittoral, Tydemena-Madeira-Mauritania Exp., 1978; Stat. 3.KO1-CANCAP-III, south-east coast of Madeira, Caniçal, 32° 44’ N 16° 44’ W (RMNH.CRUS.1.7477).

Many specimens, including stage 8 ♂♂; rocky littoral, pools, shallow sublittoral, Tydeman-Madeira-Mauritania Exp., 1978; Stat. 3.KO1-CANCAP-III, south-east coast of Madeira, Caniçal, 32° 44’ N, 16° 44’ W (RMNH.CRUS.1.7478).

17 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Porto dos Frades, Porto Santo, 33° 4' N, 16° 17' W.

161 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Reis Magos, Madeira, 32° 38' N, 16° 49' W.

2 stage 8 ♂♂, 159 ♀♀; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Ponta da Cruz, Madeira, 32° 37' N, 16° 56' W.

Portuguese Museum of Natural History and Science (MUHNAC) collection:

19 ♀♀ and juvs; intertidal; EMEPC/M@rBis/Selvagens 2010 Mission Report, 2010; Selvagem Grande, 30º 8' N, 15º 52' W (M@rBis__001450; M@rBis__001452; M@rBis__001417; M@rBis__000031).

2 juvs; intertidal; EMEPC/M@rBis/Selvagens 2010 Mission Report, 2010; Selvagem Pequena, 30º 2' N, 16º 1' W (M@rBis__000267; M@rBis__000929).

Canary Islands (Spain)

2 ♀♀; empty barnacle tests amongst tufted coralline algae on mid-shore lava rocks, D. M. Holdich, 2002; Playa Blanca, Lanzarote, 28º 51' N, 13º 49' W (RMNH.CRUS.1.7559).

1 stage 8 ♂; rocks, muddy, polluted littoral, Tydeman-Selvagens-Canary Is. Exp. 1980; Sta. 4.KO2: CANCAP IV, Las Palmas - north coast of Gran Canaria, 28° 9' N, 15° 26' W (RMNH.CRUS.1.7463).

1 stage 8 ♂ (4 mm); rocky littoral, Tydeman-Cancap-II Canary Is. Exp. 1977; Stat. K13, west coast of Fuerteventura near Punta Jandia, 28° 4' N, 14° 30' W (RMNH.CRUS.1.7464).

1 ♀; rocks, tide pools, sandy bay, sea-grass, depth to 5 m, Tydeman - Selvagens-Canary Is. Exp. 1980; Stat. KO6: CANCAP IV, Arinaga, east coast of Gran Canaria, 27° 51' N, 15° 24' W (RMNH.CRUS.1.7466).

2 stage 8 ♂♂ (4.5 mm by 2 mm), one ♀; rocky shore, tide pools, shallow sandy bay, 0-2 m, Tydeman-Selvagens-Canary Is. Exp. 1980; Stat 4. K12: CANCAP IV, Arrecif - south-east coast of Lanzarote, 28° 57' N, 13° 33' W (RMNH.CRUS.1.7467).

1 stage 7 ♂; rocky shore with tide flat and pools, depth 0-4 m, Tydeman-Selvagens-Canary Is. Exp. 1980; Stat. 4.K13 CANCAP IV, Rada de Arrieta - east coast of Lanzarote, 29° 9' N, 13° 25' W (RMNH.CRUS.1.7468).

3 stage 8 ♂♂, 1 stage 8 ♀; littoral sheltered rocky coast, rockpools and skindiving to 6 m, CANCAP-II: Tydeman Canary Is. Exp. 1977; Stat K2, Puerto de Mogan, Gran Canaria, 27° 49' N, 15° 50' W (RMNH.CRUS.1.74693).

93 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; La Fajana, La Palma, 28° 50' N, 17° 47' W.

31 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; La Salemera, La Palma, 28° 34' N, 17° 45' W.

1 stage 8 ♂, 78 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; El Faro, La Palma, 28° 27' N, 17° 51' W.

25 stage 6-8 ♂♂, 282 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Bañaderos, Gran Canaria, 28° 8' N, 15° 32' W.

2 stage 8 ♂♂, 99 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Caleta, Gran Canaria, 28° 9' N, 15° 41' W.

2 stage 6-8 ♂♂, 63 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Playa Melenara, Gran Canaria, 27° 59' N, 15° 22' W.

2 juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2015; Arenas Blancas, El Hierro, 27°46' N, 18° 7' W.

4 juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2015; Los Sargos, El Hierro, 27°47' N, 18° 0' W.

3 juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2015; Los Cristianos, Tenerife, 28° 2' N, 16°42' W.

1 stage 7 ♂, 5 juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2015; Mal Paso, Tenerife, 28°24' N, 16°17' W.

Portugal

14 stage 8 ♂♂, t33 stage 8 ♀♀, 10 ♀♀; upper shore sandstone crevices (with *Campecopea hirsuta*) and mid-shore barnacles, D. M. Holdich, 1981; Dona Ana, Algarve, Portugal, 37º 6' N, 8º 40' W (RMNH.CRUS.1.7567).

1 stage 8 ♂, 1 ♀, 1 juv; amongst mussels on breakwater at mid-tide, D.M. Holdich, 1981; Villamoura, Algarve, Portugal, 37º 5' N, 8º 7' W (RMNH.CRUS.1.7568).

11 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Peniche, Portugal, 39° 22' N, 9° 22' W.

11 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Dona Ana, Algarve, Portugal, 37° 5' N, 8° 40' W.

1 stage 8 ♂, 1 juv; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Arrifes, Algarve, Portugal, 37° 4' N, 8° 16' W.

6 stage 6-8 ♂♂, 25 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Ingrina, Algarve, Portugal, 37° 2' N, 8° 52' W.

2 juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2014; Praia Vale dos Homens, Portugal, 37° 22' N, 8° 50' W.

1 juv; intertidal rocky shores among algae during low tide, P. Vieira, 2014; Buarcos, Portugal, 40° 10' N, 8° 54' W.

1 juv; intertidal rocky shores among algae during low tide, P. Vieira, 2014; Berlengas, Portugal, 39° 24' N, 9° 30' W.

1 juv; among algae, F.O. Costa, 2014; Sines, Portugal, 38° 28' N, 8° 59' W.

Spain

1 ♀; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Muxía, Galicia, Spain, 43° 5' N, 9° 13' W.

No specimens available, but a clear photograph by Guerra-Garcia shows a stage 8 ♂♂ from Tarifa island, Spain, 36° 00′ N, 5° 36′ W. For detailed habitat information and number of individuals refer to Izquierdo et al. (2011), Guerra-García et al. (2011), Guerra-García et al. (2012), Torrecilla-Roca et al. (2012).

Morocco (NW Africa)

1 stage 8 ♂; from fouling organisms on side of ship, H. Zibrowius, 1980s; Tanger (Tangiers) Harbour Tangiers, 35° 53′ N, 5° 30′ W (RMNH.CRUS.1.7571).

1 stage 8 ♂, 29 juvs and ♀♀ and juvs; among algae in pools, P. Vieira, 2015; Arzila, Morocco, 35° 27' N, 6° 2' W.

1 stage 8 ♂, 1 juv; intertidal rocky shores among algae, P. Vieira, 2015; El Jadida, Morocco, 33° 14' N, 8° 28' W.

3 stage 6-8 ♂♂, 16 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2014; Tarfaya, Morocco, 27° 54' , 12° 57' W.

2 juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2014; Insouane, Morocco, 30° 50' N, 9° 49' W.

Mauritania (Western Africa)

Monod's (1923) description of *D. hanseni* from Port Étienne (now Nouadhibou) (20° 56′ N, 17° 2′ W) is clearly *D. edwardsi*. Monod's specimen (Museum National d'Histoire Naturelles, Paris collection) was seen and verified by DMH.

Mediterranean

Spain

1 stage 8 ♂, 1 ♀; no details of habitat, H. Zibrowius, 1980s; Alicante, Spain, 38° 20′ N, 0° 29′ E (RMNH.CRUS.1.7572).

1 stage 7 ♀ (4.5 mm); no habitat details, L.B. Holthuis, 1949; Baai van Cadaqués, Casa Zariguiey, Spain, 42° 17′ N, 3° 15′ E (RMNH.CRUS.1.7494).

1 stage 7 ♀; no habitat details, J. Castelló, 1983; Cala Morell, Menorca, Spain, 40° 3′ N, 3° 53′ E (RMNH.CRUS.1.7577).

1 stage 7 ♂; no habitat details, J. Castelló, 1984; Cala Olivera, Ibiza, Spain, 38° 57′ N, 1° 24′ E (RMNH.CRUS.1.7581).

France

9 stage 8 ♂♂, 2 stage 7 ♂♂, 1 stage 6 ♂, 15 ♀♀, 10 juvs; 5-6 m, H. Zibrowius, 1980s; Ponteau, nr Marseille, 43° 22′ N, 5° 76′ E, and Marseille, France - 43° 17′ N, 5° 22′ E (RMNH.CRUS.1.7586).

1 stage 7 ♂, 1 stage 8 ♀, 4 ♀♀, 2 juvs; from shallow-water *Cystoseira*, I. Gordon, 1952; Banyuls-sur-Mer, France, 42° 29′ N, 3° 7′ E (RMNH.CRUS.1.7587).

4 stage 8 ♂♂, 2 stage 7 ♂♂, 3 ♀♀, 1 stage 8 ♀ (one male 5.5 mm by 3 mm, one female 4 mm by 2.2 mm); 0-1 m depth, 1957; Port Vendres, south of Perpignan, France, 43° 31′ N, 3° 7′ E (RMNH.CRUS.1.7497).

2 stage 8 ♂♂, intertidal on artificial hard surfaces in docks, A. Marchini, 2014; La Grande Motte, France, 43°33' N 4° 5' W.

Monaco

1 stage 7 ♂, one juv.; 1-2 m, 1952; Baiede Garnoles, Monaco, 43° 44′ N, 7° 23′ E (RMNH.CRUS.1.7500).

Italy

2 stage 8 ♂♂ (5.0 mm), 2 stage 7 ♂♂, 3 ♀♀; shallow water crevices and empty barnacle tests, D.M. Holdich, 1967; Mergellina Harbour, Naples, Italy, 40° 49' N, 14° 13' E (RMNH.CRUS.1.7522).

1 stage 7 ♂, 2 ♀♀, 5 juvs; no habitat details, H. Zibrowius, 1980s; Vado Ligure, Gulf of Genova, Italy, 44° 16′ N, 8° 26′ E (RMNH.CRUS.1.7590).

2 stage 8 ♂♂, 5 stage 7 ♂♂, 4 stage 8 ♀♀, 22 ♀♀ and juvs; from shallow-water *Hydroides* colony, *Cystoseira* and *Sargassum*, D. M. Holdich, 1967; V. Galloti, Mergellina, Bay of Naples, Italy, 40° 49′ N, 14° 13′. Two ♀♀ added to this vial; no habitat details, E. Fresi, 1967; Capri island, Bay of Naples, Italy, 40° 33′ N, 14° 13′ E (RMNH.CRUS.1.7593).

12 stage 8 ♂♂, 8 ♀♀; intertidal on artifical hard surfaces in docks, J. Ferrario and A. Marchini, 2014; Genoa Harbour, Italy, 44° 24′ N, 8° 55′ E (RMNH.CRUS.1.7595).

10 stage 8 ♂♂, 5 stage 8 ♀♀, 2 ♀♀; intertidal on artifical hard surfaces in docks, J. Ferrario and A. Marchini, 2014; Santa Margherita Ligure docks, Italy, 44° 19′ N, 9° 12′ E (RMNH.CRUS.1.7596).

2 stage 8 ♂♂, 2 stage 7 ♂♂, 2 ♀♀; walls of canal, R. Sconfietti, 1982-83; Basino di San Marco and Porto di Lido, Venice, Italy, 45° 26′ N, 12° 18′ E (RMNH.CRUS.1.7524).

11 stage 8 ♂♂, 4 stage 7 ♂♂, 3 stage 8 ♀♀; 5 females, 1 juv; walls of canal, R. Sconfietti, 1982-83; Basino di San Marco and Porto di Lido, Venice, Italy, 45° 26′ N, 12° 18′ E (RMNH.CRUS.1.7597).

4 stage 8 ♂♂, 2 stage 7 ♂♂, 1 stage 6 ♂, 5 stage 8 ♀♀, 6 ♀♀, 1 juv; 0.5 m, brown and red algae on rocks, U. Schieke and E. Fresi, 1968, 1969, 1970; Carta Romana, Castello and below Ecological Laboratory, Ischia island, Bay of Naples, Italy, 40° 44′ N, 13° 57′ E (RMNH.CRUS.1.7603).

5 stage 8 ♂♂, 2 stage 6 ♂♂, 8 juvs; intertidal on artificial hard surfaces in docks, J. Ferrario and A. Marchini, 2014; Marina of Porto Retondo, Sardinia, Italy, 41° 1' N 9°32' W.

1 stage 8 ♂; intertidal on artificial hard surfaces in docks, J. Ferrario and A. Marchini, 2014; Marina of Castelsardo, Sardinia, Italy, 40° 54' N 8°42' W.

1 stage 8 ♂, 1 stage 6 ♂, 1 stage 8 ♀, 28 juvs; intertidal on artificial hard surfaces in docks, J. Ferrario and A. Marchini, 2013; Harbour of Leghorn, Tuscany, Italy, 43°33' N 10°17' W.

4 stage 8 ♂♂, 3 stage 6 ♂♂, 2 stage 8 ♀♀, 11 juvs; intertidal on artificial hard surfaces in docks, P. J. Ferrario and A. Marchini, 2013; Harbour of la Spezia, Liguria, Italy, 44° 6' N 9°54' W.

Croatia

2 stage 8 ♂♂, intertidal on artifical hard surfaces in docks, P. M. Maric, 2014; Marina Kornati, Croatia, 43°56' N 15°26' W.

Greece

1 stage 8 ♂; no habitat details; H. Zibrowius, 1980s; Rhodes island, Greece, 36° 25' N, 28° 13' E (RMNH.CRUS.1.7616).

2 stage 8 ♂♂, 3 stage 7 ♂♂, seven ♀♀; under rocks in 20-30 cm water, D.M. Holdich, 2001; Lindos, Rhodes island, Greece, 36° 5' N, 28° 5' E (RMNH.CRUS.1.7642).

3 stage 7 ♂♂, 10 ♀♀ and juvs; variety of algae, e.g. *Cystoseira*, *Sargassum*, from 0.5 m – 33 m, D.A. Jones, 1967; Emborios Bay and Cathedral Rock, Chios island, Greece, 38° 11' N, 26° 1' E (RMNH.CRUS.1.7646).

1 stage 7 ♂, 3 ♀♀; shallow water algae, D.A. Jones, 1967; Emborios Bay, Chios island, Greece, 38° 11' N, 26° 1' E (RMNH.CRUS.1.7532).

Turkey

2 stage 8 ♂♂ (4.5 x 2.3 mm, 5 x 2.5 mm), 1 stage 8 ♀; 1 stage 7 ♀ (4.5 x 2.2 mm); intertidal and shallow-water algae and other habitats, F. Kirkim, 1995; Aegean coast at Izmir, 38° 28' N, 27° 6' E (RMNH.CRUS.1.7658).

Malta

2 stage 8 ♂♂, one ♀; 0.5-1 m, rocky seabed, L. Bonnici, 2010; Birzebbugh, Malta, 35° 47' N, 14° 31' E (RMNH.CRUS.1.7663).

British Natural History Museum (BMNH) collection:

1 stage 7 ♂; fenders; Valetta Harbour, 35° 54' N, 14° 30' E.

Israel

2 stage 8 ♂♂, 1 stage 7 ♂, 2 ♀♀; littoral algae from rocky shore, L. Fischelson, 1976; Acre north of Haifa, Israel, 32° 55' N, 35° 4' E (RMNH.CRUS.1.7523).

1 stage 7 ♂, 4 ♀♀, 1 juv; littoral algae, L. Fischelson, 1976; Rosh HaNikra, Haifa, Israel, 33° 5' N, 35° 6' E (RMNH.CRUS.1.7647).

1 stage 8 ♂, 3 stage 6/7 ♂♂, 3 ♀♀ (one 3.5 x 1.5 mm), 5 juvs; algae on rocky shore (*Ulva*, *Jania*), T. Haran, 1977-78; Mikhmoret, Israel, 32° 24' N, 34° 52' E (RMNH.CRUS.1.7648).

21 stage 8 ♂♂ (4 x 1.75 mm – 3 x 1.5 mm), 17 stage 6/7 ♂♂, 1 stage 8 ♀39 ♀♀, 4 juvs; littoral algae (*Colpomenia*, *Cystoseira*, *Hypnea*, *Laurencia*, *Jania*), from rocky shore, L. Fischelson, 1976; Acre, north of Haifa, Israel, 32° 55' N, 35° 4' E (RMNH.CRUS.1.7651).

1 stage 8 ♂; littoral algae (*Acanthophora*), L. Fischelson, 1976; Bat-Yam, south of Tel Aviv, Israel, 32° 5 N, 34° 48' E (RMNH.CRUS.1.7653).

Egypt

2 stage 7 ♂♂, 5 ♀♀; no habitat details, M.M. Atta, 1981; Alexandria, Egypt, 31° 9' N, 29° 55' E (RMNH.CRUS.1.7666).

Tunisia

No specimen available, but a clear 2009 photograph by R. García, shows a stage 8 ♂ from Tunis, Tunisia, 36° 50' N, 10° 14' E.

Algeria

Naesea edwardsi Lucas, 1849.
No specimens available, but description by Lucas (1849) is clearly of this species. Precise co-ordinates are not known, so approximate ones from Algiers harbour were used: 36° 48' N, 3° 13' E.

Suez Canal

Glynn's (1972) record for the Suez Canal at Tis' A, which is close to Suez and the entrance to the Gulf of Suez, is clearly of this species based his figures. 29° 58' N, 32° 32' E.

Red Sea

Egypt-Israel

1 stage 8 ♂ (4 x 1.75 mm), 1 ♀, one juv.; littoral algae (*Padina*, *Galaxauma*), L. Fischelson, 1976; Eilat Port, Israel, 29° 31' N, 34° 56' E, and Dahab (Egypt) further south on north coast of Gulf of Aqaba, 28° 30' N, 34° 30' E (RMNH.CRUS.1.7665).

Dynamene magnitorata Holdich, 1968

Atlantic

Azores (Portugal)

1 stage 8 ♂ (damaged), 1 stage 7 ♂, 4 ♀♀, 3 juvs; A. Costa (University of the Azores, Ponta Delgada, S. Miguel), 1995 and M. Jones M. (2 juveniles) (University of Plymouth), 1996; São Miguel Island, 37^{o} 46’ N, 25^{o} 29’ W (RMNH.CRUS.1.7555).

3 stage 8 ♀♀; from shallow sub-tidal empty *Megabalanus azoricus* tests (along with *Eurydice affinis* and amphipods), scuba diving, A. Costa, 2010; São Miguel Island, 37^{o} 46’ N, 25^{o} 29’ W (RMNH.CRUS.1.7556).

2 ♀♀; rocky cove with tide pools, cobble beach, subtidal algae, Tydeman. Azores Exp. 1981, CANCAP-V. Stat. 5.KO3, 1981; south coast of Santa Maria, 36° 57’ N, 25° 07’ W (RMNH.CRUS.1.7455).

1 stage 8 ♂ (4 mm), 1 stage 7 ♀ (3 mm), 1 juv; rocky shore collecting, snorkling, Hartog and Lavaleye, 1979. Pico, south coast, Lages harbour, 38° 24’ N, 28° 15’ W (RMNH.CRUS.1.7457).

2 stage 7 ♂♂, 1 stage 6 ♂, 3 ♀♀, 7 juvs, plus 1 *Cymodoce* sp; Depth approx. 10-20 m, sheltered bay, Tydeman Azores Exp, CANCAP-V. Stat. 5. DO1, 1981; south coast of São Miguel, 37° 43’ N, 25° 30’ W (RMNH.CRUS.1.7461).

2 ♀♀, 4 juvs; Tydeman Azores Exp. 1981, CANCAP-V. Stat. 5. DO7, 1981; rocky coast, south of harbour, south-east coast Faial near Horta, 38° 31’ N, 28° 37’ W (RMNH.CRUS.1.7462).

1 stage 8 ♂; Tydeman Azores Exp. 1981, CANCAP-V. Stat. 5.D11, 1981; west entrance to small bay, north coast of Flores, 39° 31’ N 31° 12’ (RMNH.CRUS.1.7459).

1 stage 8 ♂ (4 mm), 1 stage 7 ♀; depth 20 m, cobbles with algae, van Veen grab; Tydeman Azores Exp. 1981, CANCAP-V. Stat. 5.116, 1981; north of Sao Jorge, 38° 38’ N 27° 55’ (RMNH.CRUS.1.7458).

1 stage 8 ♂, 42 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2014; Porto Martins, Terceira, 38° 40' N, 27° 3' W.

2 juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2015; Praia Formosa, Santa Maria, 36° 56' N, 25° 5' W.

2 juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2014; Cinco Ribeiras, Terceira, 38° 40' N, 27° 19' W.

1 stage 6 ♂, 22 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2015; Ribeira Chã, São Miguel, 37° 42' N, 25° 29' W.

British Natural History Museum collection:

♂♂, ♀♀, juvs, lot of samples; habitat and site data apparently available, 1959; Terceira Is, 38° 43’ N, 27° 13’ W – general co-ordinates for island.

Museum National d’Histoire Naturelle, Paris collection:

3 stage 8 ♂♂, 1 stage 8♀, 2 ♀♀; J. Charcot Biacores, 1971; Azores – no other details, IS 770.

1 stage 8 ♂, 2 ♀♀; J. Charcot Biacores, 1971; Azores – no other details, IS 780.

Madeira (Portugal)

1 ♀; Tydeman - Selvagens-Canary Is. Exp. Stat. 4. K26: CANCAP IV, Porto Santo (this is an island north of Madeira), SW coast Baixo, 33° 04’ N, 16° 20’ W (RMNH.CRUS.1.7465).

Numerous ♀♀ and juvs, plus some *Cymodoce* sp. and other crustaceans; rocky littoral pools, shallow sublittoral, Tydeman - Madeira-Maritania Exp. Stat. 3.KO1-CANCAP-III, SE coast of Madeira, Caniçal, 1978; 32° 44’ N, 16° 44’ W (RMNH.CRUS.1.7472).

1 juv; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Porto dos Frades, Porto Santo, 33° 4' N, 16° 17' W.

Canary Islands (Spain)

2 stage 8 ♂♂ (4 mm x 1.75 mm); surface dip net, CANCAP-II: Tydeman Canary Is. Exp. Stat. 17, 1977; south of Fuerteventura, Punta Jandia, 27° 39’ N, 14° 22’ W (RMNH.CRUS.1.7468).

1 juv; intertidal rocky shores among algae during low tide, P. Vieira, 2011; La Fajana, La Palma, 28° 50' N, 17° 47' W.

2 stage 8 ♂♂, 1 juv; intertidal rocky shores among algae during low tide, P. Vieira, 2011; El Faro, La Palma, 28° 27' N, 17° 51' W.

England

1 ♀; red seaweed in rockpool, M. Storey, 2011; Newton’s Cove, Weymouth, Dorset 50^{o} 40’ N, 2^{o} 30’ W (RMNH.CRUS.1.7547).

Channel Islands

4 stage 6/7 ♂♂, 2 stage 8 ♂♂ (one 5 x 2.2 mm), 5 ♀♀; lower shore weed (*Chondrus crispus*) sponge, *Halichondria*, channels (males), D. M. Holdich, 1982; Guernsey, L’Eree, Rocquaine Bay, 49^{o} 26’ N, 2^{o} 39’ W (RMNH.CRUS.1.7553).

2 stage 8 ♂♂ (one 4 mm long), 8 stage 7 ♂♂, 18 ♀♀, 5 juvs; rubbings from red algae on lower shore, D. M. Holdich, 1982 Petit Bot Bay, Guernsey, 49^{o} 25’ N, 2^{o} 34’ W (RMNH.CRUS.1.7554).

France

3 stage 8 ♂♂ (5.0 mm), 1 stage 8 ♀, 2 stage 7 ♀♀; rocky shore crevices, D. M. Holdich, 1975; Roscoff Marine Station, 48° 43’ N, 3° 59’ W (RMNH.CRUS.1.7520).

1 stage 8 ♂, 3 stage 7 ♂♂, 3 ♀♀; red algae on lower shore, D. M. Holdich, 1988; Trénez, S. Brittany, 47° 47’ N, 3° 42’ W (RMNH.CRUS.1.7561).

1 ♀; rocky mid-shore weed and crevices, D. M. Holdich, 1988; Kerfanny, S. Brittany, 47° 47’ N, 3° 43’ W (approx.) (RMNH.CRUS.1.7562).

12 stage 8 ♂♂, 3 stage 7 ♂♂, 4 stage 8 ♀♀, 8 ♀♀ and juvs; empty barnacle test and mid-shore algae, D. M. Holdich, 1975; Roscoff Marine Station shore, Brittany, 48° 43’ N, 3° 59’ W (RMNH.CRUS.1.7564).

1 stage 8 ♂, stage 7 ♂♂, many juveniles, H. Nouvel, 1939; Bisdarz, Roscoff, 48° 43’ N, 3° 59’ (approx.) (RMNH.CRUS.1.7484).

1 stage 8 ♂, 1 stage 7 ♀; H. Nouvel, 1952. Grève Santec, Perarhidy, 48° 41’ N, 3° 58’ W (RMNH.CRUS.1.7487).

1 stage 7 ♀; H. Nouvel, 1952; Roscoff, Térèris, NW France, 48° 43’ N, 3° 59’ W (approx.) (RMNH.CRUS.1.7488).

Museum National d’Histoire Naturelles, Paris collection:

1 ♀; Th Monod; Guitec, NW France, IS 678.

In addition, specimens of *D. magnitorata* were examined by DMH from the following locations during the period 1964-1975 (Holdich 1970, 1976) and subsequently:

Argenton (48° 31' 41" N, 04° 46' 41" W), Barfleur (49° 40' 04" N, 01° 15' 24" W), Brignognan (48° 40' 20" N, 04° 18' 49" W), Isles de Glénans (47° 17' 37" N, 03° 12' 28" W), Trégaster (48° 50' 32" N, 03° 31' 06" W).

Portugal

2 stage 8 ♂♂, 10 stage 7 ♂♂, 7 ♀ and juvs; sub-tidal algae, D. M. Holdich, 1981; Amação de Pêra, Algarve, 37° 6’ N, 8° 21’ W (RMNH.CRUS.1.7565).

1 stage 7 ♂, 6 ♀♀ and juvs; sub-tidal algae, D. M. Holdich, 1981; Dona Ana, Algarve, 37° 6’ N, 8° 40’ W (RMNH.CRUS.1.7566).

2 stage 8 ♂♂, 9 stage 6 ♂♂, 192 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Peniche, Portugal, 39° 22' N, 9° 22' W.

5 ♀♀ and juvs; intertidal rocky shores among algae and in crevices during low tide, P. Vieira, 2014; Peniche, Portugal, 39° 22' N, 9° 22' W.

1 juv; intertidal rocky shores among algae during low tide, P. Vieira, 2011; São Pedro Moel, Portugal, 39° 45' N, 9° 1' W.

1 stage 8 ♂, 39 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Dona Ana, Algarve, Portugal, 37° 5' N, 8° 40' W.

52 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Arrifes, Algarve, Portugal, 37° 4' N, 8° 16' W.

1 juv; among algae, F.O. Costa, 2013; Arrabida, Portugal, 38° 28' N, 9° 59' W.

10 juv; among algae, F.O. Costa, 2012; Praia Norte, Portugal, 41° 41' N, 8° 50' W.

51 juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Buarcos, Portugal, 40° 10' N, 8° 54' W.

2 juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2014; Berlengas, Portugal, 39° 24' N, 9° 30' W.

Spain

1 ♀; mesolittoral, Reboreda, P. (University of Santiago de Compostela, Spain), 1984, 1987, 1988; Puerto Sou, Ria de Noi, 42° 43’ N, 8° 59’ W (RMNH.CRUS.1.7570).

1 stage 8 ♂, 1 stage 7 ♂ 7, 1 stage 7 ♀ (4 mm); 1963; San Vincent, 43° 22’ N, 4° 23’ W (RMNH.CRUS.1.7479).

1 stage 7 ♂, plus 1 stage 6 ♂ and 1 ♀ *D. bidentata*; littoral, 1962; Jidorio Pectregoso, west of Is. de Arosa’ Exc. R.M.N.H. Sta. 0. 75, 42° 33’ N, 8° 51’ W (RMNH.CRUS.17490).

3 stage 8 ♂♂, 607 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Pedreira, Galicia, Spain, 43°33' N, 8°16' W.

1 stage 8 ♂, 48 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Barizo, Galicia, Spain, 43° 19' N, 8° 52' W.

4 stage 6-8 ♂♂, 132 ♀♀ and juvs; intertidal rocky shores among algae during low tide, P. Vieira, 2011; Muxía, Galicia, Spain, 43° 5' N, 9° 13' W.

No specimens available, but a clear photograph by Guerra-Garcia shows a stage 8 ♂♂ from Tarifa island, Spain, 36° 00’ N, 5° 36’ W. For detailed habitat information and number of individuals refer to Izquierdo et al. (2011), Guerra-García et al. (2011), Guerra-García et al. (2012), Torrecilla-Roca et al. (2012).

Morocco (NW Africa)

4 ♀♀ and juvs; among algae in pools, P. Vieira, 2015; Arzila, Morocco, 35° 27' N, 6° 2' W

Museum National d’Histoire Naturelle, Paris collection:

2 stage 8 ♂♂, 5 ♀♀; T. Monod; Fedhala, nr Casablanca, 33° 41’ N, 7° 22’ W, IS 680.

Mediterranean

Spain

3 stage 8 ♂♂; 0-1 m, H. Zibrowius, 1986; Alicante, 38º 20’ N, 0º 29’ E (RMNH.CRUS.1.7573).

2 ♂ stage 8, 1 ♀ stage 8, 1 ♀ stage 7; 0.0 m – 20.0 m on a variety of algae, C. Catellanos et al. (University of Alcalá, Spain), 1991-1993; Chafarinas Islands, off Mediterranean Morocco, Spanish Territory, 35º 11' N, 2º 25' E (RMNH.CRUS.1.7521).

Monaco

1 stage 7 ♂ (damaged); 5-6 m, H. Nouvel, 1971; en face de port Fonteveille, 43º 43’ N, 7º 25’ E (RMNH.CRUS.1.7499).

Italy

1 stage 8 ♂, 1 ♀ (back end); F. Maggiore, 1975; Ischia Porto 40º 44’ N, 13º 57’ E (RMNH.CRUS.1.7610). Labelled as *D. bidentata* (see Maggiore and Fresi 1984).

Egypt

3 stage 8 ♂♂, 2 stage 7 ♂♂, 2 ♀♀, 2 juvs; M. M. Atta, 1981; Alexandria, 31º 9’ N, 29º 55’ E (RMNH.CRUS.1.7668).

Tunisia

Museum National d’Histoire Naturelle, Paris collection:

3 stage 8 ♂♂; T. Monod; Tunis? IS 678.

Dynamene tubicauda Holdich, 1968

Mediterranean

Italy

2 stage 8 ♂♂, 1 stage 8 ♀, 2 ♀♀, 2 juvs; 10 metres, E. Fresi & U. Schieke, 1968; Ischia island, Naples, Italy, 40º 44' N, 13º 56' E (RMNH.CRUS.1.7531).

2 stage 8 ♂♂; 12 m and 20 metres on *Halimeda*, U. Schieke & E. Fresi, E., 1967; Banco S. Croce, Sorrento, Bay of Naples, Italy, 40º 37’ N, 14º 22’ E (RMNH.CRUS.1.7594).

3 stage 8 ♂♂, 2 stage 8 ♀♀, 8 ♀♀, 4 juvs; on *Dictyota*, *Vidalia* and *Halimeda* at 10.5 metres (very muddy) (D.S. Pancrazio); 30 metres on sand and coralline fragments (Secca di ischia), U. Schieke & E. Fresi, 1967, 1968, 1969, 1970; Ischia island, Italy, 40º 44’ N, 13º 57’ E (RMNH.CRUS.1.7611).

3 stage 8 ♂♂, 3 stage 7 ♂♂, 1 ♀, one juvenile; 2-5 metres, H. Zibrowius, 1980; Elba island, Italy, 42 º 47’ N, 10º 08’ E (RMNH.CRUS.1.7598).

From rock scappings and algae at 5-10 metres around Sicily (Italy), Lombardo (1984) recorded 1 stage 8 ♂ from Isola Lachea (37º 33’ N, 15º 9’ E) (Catania), 3 stage 8 ♂♂ from Brucoli (37º 16’ N, 15º 11’ E) (Siracusa), and 1 ♀ from Calaberdardo (36º 52’ N, 15º 85’ E) (Siracusa). The present authors have not seen the specimens for the present study but the published drawings are clearly of this species.

Malta

4 stage 8 ♂♂; *Posidonia oceanica* meadow at 12 m, J. A. Borg, 1998; Mellieha Bay, Malta, 35º 58’ N, 14º 21’ E (RMNH.CRUS.1.7664).

Dynamene sp.

Aegean

1 stage 8 ♂; found in stomach of black scorpionfish *Scorpaena porcus*, M. Băcescu, 1982; N.W. Aegean, 40º N, 25º E (approx.) (RMNH.CRUS.1.7533).

1 stage 8 ♂; found in stomach of black scorpionfish *Scorpaena porcus*, M. Băcescu, 1982; N.W. Aegean, 40º N, 25º E (approx.) (RMNH.CRUS.1.7660).

Annex 3.2. List of locations where Dynamene specimens were recorded in chapter 3.

Species present	**Region**	**Country/Archipelago**	**Location**	**Latitude**	**Longitude**
Dynamene bicolor	Black Sea	Bulgaria	Varna	43°12'52"N	28°00'36"E
Dynamene bicolor	Black Sea	Georgia	Sukhumi Bay	43°00'00"N	41°00'00"E
Dynamene bicolor	Black Sea	Romania	Constanta	44°12'41"N	28°38'40"E
Dynamene bicolor	Black Sea	Turkey	Rýze	41°01'52"N	40°28'23"E
Dynamene bicolor	Black Sea	Turkey	Sinop	42°00'28"N	35°10'57"E
Dynamene bicolor	Black Sea	Turkey	Trabzon harbour	41°00'20"N	39°44'14"E
Dynamene bicolor	Mediterranean Sea	Croatia	Rovinji	45°04'00"N	13°37'60"E
Dynamene bicolor	Mediterranean Sea	Egypt	Alexandria	31°11'07"N	29°52'32"E
Dynamene bicolor	Mediterranean Sea	France	Argelès	42°32'11"N	03°03'23"E
Dynamene bicolor	Mediterranean Sea	France	Banylus-sur-Mer	42°29'14"N	03°07'40"E
Dynamene bicolor	Mediterranean Sea	France	Calvi	42°34'14"N	08°45'00"E
Dynamene bicolor	Mediterranean Sea	France	Toulon	43°04'00"N	05°47'50"E
Dynamene bicolor	Mediterranean Sea	France	Villefranche-Mer	43°42'11"N	07°18'48"E
Dynamene bicolor	Mediterranean Sea	France	Marseilles	43°16'60"N	05°21'60"E
Dynamene bicolor	Mediterranean Sea	Greece	Corfu Island	39°36'00"N	19°48'60"E
Dynamene bicolor	Mediterranean Sea	Greece	Emborious Bay	38°11'01"N	26°00'60"E
Dynamene bicolor	Mediterranean Sea	Greece	Gerakini Beach	40°15'60"N	23°26'00"E
Dynamene bicolor	Mediterranean Sea	Greece	Matala	34°59'00"N	24°44'00"E
Dynamene bicolor	Mediterranean Sea	Greece	Mesolongion	38°22'00"N	21°25'00"E
Dynamene bicolor	Mediterranean Sea	Greece	Ouranoupolis	40°19'55"N	23°57'60"E
Dynamene bicolor	Mediterranean Sea	Greece	Sulaora	39°30'35"N	20°15'30"E
Dynamene bicolor	Mediterranean Sea	Israel	Acre	32°54'60"N	35°04'00"E
Dynamene bicolor	Mediterranean Sea	Israel	Bat-Yam	32°05'42"N	34°46'23"E
Dynamene bicolor	Mediterranean Sea	Israel	Michmoret	32°20'14"N	34°51'00"E
Dynamene bicolor	Mediterranean Sea	Israel	Mikhmoret	32°24'14"N	34°52'00"E
Dynamene bicolor	Mediterranean Sea	Israel	Palmhim	31°55'00"N	34°42'00"E
Dynamene bicolor	Mediterranean Sea	Israel	Rosh Hanikva	33°04'56"N	35°06'16"E
Dynamene bicolor	Mediterranean Sea	Italy	Elba Island	42°47'60"N	10°07'60"E
Dynamene bicolor	Mediterranean Sea	Italy	Grand Hotel	40°50'00"N	14°15'00"E
Dynamene bicolor	Mediterranean Sea	Italy	Ischia Island	40°43'56"N	13°57'59"E
Dynamene bicolor	Mediterranean Sea	Italy	Isola di Bergegge	44°14'00"N	08°26'00"E
Dynamene bicolor	Mediterranean Sea	Italy	Mergellina	40°49'00"N	14°13'00"E
Dynamene bicolor	Mediterranean Sea	Italy	Palermo	38°10'60"N	13°20'00"E
Dynamene bicolor	Mediterranean Sea	Italy	Posillipo	40°47'40"N	14°11'35"E
Dynamene bicolor	Mediterranean Sea	Malta	Cirkewwa	35°58'42"N	14°19'29"E
Dynamene bicolor	Mediterranean Sea	Malta	Mellieha Bay	35°58'04"N	14°21'05"E
Dynamene bicolor	Mediterranean Sea	Spain	Arenal d'en Castell	40°01'30"N	04°10'34"E
Dynamene bicolor	Mediterranean Sea	Spain	Alicante	38°20'08"N	00°29'13"E
Dynamene bicolor	Mediterranean Sea	Spain	Botafoc	38°53'60"N	01°26'00"E
Dynamene bicolor	Mediterranean Sea	Spain	Cadaqués	42°14'25"N	03°12'18"E
Dynamene bicolor	Mediterranean Sea	Spain	Cala Morell	40°02'60"N	03°53'00"E
Dynamene bicolor	Mediterranean Sea	Spain	Calla Longa	38°57'16"N	01°31'36"E
Dynamene bicolor	Mediterranean Sea	Spain	Cap d'Artutx	39°55'44"N	03°49'22"E
Dynamene bicolor	Mediterranean Sea	Spain	Estany d'es Peix	38°43'04"N	01°25'21"E
Dynamene bicolor	Mediterranean Sea	Spain	San Antonio	38°58'05"N	01°17'59"E
Dynamene bicolor	Mediterranean Sea	Tunisia	Tunis Harbour	36°50'00"N	10°13'60"E
Dynamene bicolor	Mediterranean Sea	Turkey	Antalya	36°52'60"N	30°44'00"E
Dynamene bicolor	Mediterranean Sea	Turkey	Izmir	38°27'09"N	27°05'50"E
Dynamene bidentata	Atlantic Ocean	Canary Islands	Bañaderos	28°08'59"N	15°32'24"W
Dynamene bidentata	Atlantic Ocean	Canary Islands	Tenerife Island	28°02'03"N	16°32'27"W
Dynamene bidentata	Atlantic Ocean	Channel Islands	Petit Bot Bay	49°25'03"N	02°33'50"W
Dynamene bidentata	Atlantic Ocean	Channel Islands	Rocquaine Bay	49°25'60"N	02°38'60"W
Dynamene bidentata	Atlantic Ocean	England	Bovisand	50°19'60"N	04°06'60"W
Dynamene bidentata	Atlantic Ocean	England	Cape Cornwall	50°07'01"N	05°42'04"W
Dynamene bidentata	Atlantic Ocean	England	Castle Rocks	50°08'38"N	05°03'40"W
Dynamene bidentata	Atlantic Ocean	England	Clovelly	50°59'00"N	04°23'00"W
Dynamene bidentata	Atlantic Ocean	England	Feock	50°11'36"N	05°03'35"W
Dynamene bidentata	Atlantic Ocean	England	Goodrington Sands	50°24'60"N	03°33'60"W

Species present	**Region**	**Country/Archipelago**	**Location**	**Latitude**	**Longitude**
Dynamene bidentata	Atlantic Ocean	England	Gwithian	50°13'42"N	05°23'39"W
Dynamene bidentata	Atlantic Ocean	England	Helford Passage	50°05'34"N	05°06'07"W
Dynamene bidentata	Atlantic Ocean	England	Kennack Sands	50°00'00"N	05°10'00"W
Dynamene bidentata	Atlantic Ocean	England	Ilfracombe	51°12'32"N	04°07'46"W
Dynamene bidentata	Atlantic Ocean	England	Lizard	49°57'43"N	05°11'54"W
Dynamene bidentata	Atlantic Ocean	England	Marazion	50°07'00"N	05°27'00"W
Dynamene bidentata	Atlantic Ocean	England	Mevagissey	50°15'16"N	04°46'01"W
Dynamene bidentata	Atlantic Ocean	England	Mounts Bay	50°05'35"N	05°22'27"W
Dynamene bidentata	Atlantic Ocean	England	Mullion Cove	49°59'49"N	05°15'18"W
Dynamene bidentata	Atlantic Ocean	England	Padstow Bay	50°34'49"N	04°55'20"W
Dynamene bidentata	Atlantic Ocean	England	Penzance	50°07'00"N	05°31'60"W
Dynamene bidentata	Atlantic Ocean	England	Portland Harbour	50°34'00"N	02°26'00"W
Dynamene bidentata	Atlantic Ocean	England	Kimmerridge Bay	50°36'39"N	02°07'00"W
Dynamene bidentata	Atlantic Ocean	England	Porthleven	50°04'57"N	05°19'21"W
Dynamene bidentata	Atlantic Ocean	England	Portlooe	50°20'31"N	04°27'38"W
Dynamene bidentata	Atlantic Ocean	England	Portscatho	50°11'06"N	04°58'15"W
Dynamene bidentata	Atlantic Ocean	England	Rosenithon	50°02'48"N	05°04'02"W
Dynamene bidentata	Atlantic Ocean	England	South Milton Sands	50°15'00"N	03°50'60"W
Dynamene bidentata	Atlantic Ocean	England	St Agnes	50°19'01"N	05°14'02"W
Dynamene bidentata	Atlantic Ocean	England	St Mawes	50°09'18"N	05°01'20"W
Dynamene bidentata	Atlantic Ocean	England	St Ives	50°11'60"N	05°28'00"W
Dynamene bidentata	Atlantic Ocean	England	Treen	50°11'10"N	05°36'05"W
Dynamene bidentata	Atlantic Ocean	England	Trevelgue	50°25'33"N	05°03'13"W
Dynamene bidentata	Atlantic Ocean	England	Trevone	50°32'41"N	04°58'53"W
Dynamene bidentata	Atlantic Ocean	England	Treyarnon	50°31'12"N	05°01'44"W
Dynamene bidentata	Atlantic Ocean	England	Wembury	50°17'26"N	04°46'46"W
Dynamene bidentata	Atlantic Ocean	England	Weymouth	50°36'17"N	02°32'12"W
Dynamene bidentata	Atlantic Ocean	England	Whitesand Bay	50°19'60"N	04°15'60"W
Dynamene bidentata	Atlantic Ocean	England	Hells Gate Beach	51°10'41"N	04°20'02"W
Dynamene bidentata	Atlantic Ocean	England	Widermouth	50°47'13"N	04°33'37"W
Dynamene bidentata	Atlantic Ocean	France	Bay de Morlaix	48°41'45"N	03°53'11"W
Dynamene bidentata	Atlantic Ocean	France	Brest	48°22'60"N	04°29'00"W
Dynamene bidentata	Atlantic Ocean	France	Finisterre	48°17'55"N	04°12'43"W
Dynamene bidentata	Atlantic Ocean	France	Perron-Quirec	48°47'60"N	03°26'00"W
Dynamene bidentata	Atlantic Ocean	France	Roscoff	48°43'40"N	03°58'09"W
Dynamene bidentata	Atlantic Ocean	France	Kerfanny	47°51'00"N	03°38'00"W
Dynamene bidentata	Atlantic Ocean	France	Trénez	47°46'60"N	03°42'00"W
Dynamene bidentata	Atlantic Ocean	Ireland	Ballycotton	51°49'59"N	08°01'03"W
Dynamene bidentata	Atlantic Ocean	Ireland	Carrownedin	54°13'55"N	09°05'21"W
Dynamene bidentata	Atlantic Ocean	Ireland	Cork	51°30'08"N	09°18'11"W
Dynamene bidentata	Atlantic Ocean	Ireland	Corkagh Beg	54°16'11"N	08°45'17"W
Dynamene bidentata	Atlantic Ocean	Ireland	Doonbeg	52°44'43"N	09°31'26"W
Dynamene bidentata	Atlantic Ocean	Ireland	Dungarvan	52°05'30"N	07°32'45"W
Dynamene bidentata	Atlantic Ocean	Ireland	Fenit	52°17'07"N	09°52'34"W
Dynamene bidentata	Atlantic Ocean	Ireland	Finvarra	53°08'58"N	09°08'22"W
Dynamene bidentata	Atlantic Ocean	Ireland	Galway	53°15'60"N	09°03'00"W
Dynamene bidentata	Atlantic Ocean	Ireland	Garrywilliam	52°18'34"N	10°03'17"W
Dynamene bidentata	Atlantic Ocean	Ireland	Glengariff	51°44'52"N	09°32'56"W
Dynamene bidentata	Atlantic Ocean	Ireland	Liscannor	52°56'10"N	09°26'16"W
Dynamene bidentata	Atlantic Ocean	Ireland	Loughshinny	53°33'39"N	05°58'55"W
Dynamene bidentata	Atlantic Ocean	Ireland	Mullagh	52°47'31"N	09°29'06"W
Dynamene bidentata	Atlantic Ocean	Ireland	Rathlee	54°16'49"N	09°03'31"W
Dynamene bidentata	Atlantic Ocean	Ireland	Roundstone	53°23'36"N	09°51'27"W
Dynamene bidentata	Atlantic Ocean	Ireland	The Seven Hogs	52°19'33"N	10°01'13"W
Dynamene bidentata	Atlantic Ocean	Ireland	Valentia	51°53'60"N	10°20'60"W
Dynamene bidentata	Atlantic Ocean	Isle of Man	Derby Haven	54°04'00"N	04°37'00"W
Dynamene bidentata	Atlantic Ocean	Isle of Man	Port Erin	54°05'03"N	04°45'39"W
Dynamene bidentata	Atlantic Ocean	Isles of Scilly	Bryher	49°57'08"N	06°21'54"W
Dynamene bidentata	Atlantic Ocean	Isles of Scilly	Old Town	49°54'10"N	06°18'13"W
Dynamene bidentata	Atlantic Ocean	Isles of Scilly	Periglio Bay	49°53'60"N	06°24'00"W
Dynamene bidentata	Atlantic Ocean	Isles of Scilly	Porth Hellick	49°54'54"N	06°16'47"W

Species present	Region	Country/Archipelago	Location	Latitude	Longitude
Dynamene bidentata	Atlantic Ocean	Isles of Scilly	Smith Sound	49°52'52"N	06°21'59"W
Dynamene bidentata	Atlantic Ocean	Isles of Scilly	St Agnus	49°53'38"N	06°21'03"W
Dynamene bidentata	Atlantic Ocean	Isles of Scilly	St Martins	49°58'48"N	06°17'28"W
Dynamene bidentata	Atlantic Ocean	Isles of Scilly	St Marys	49°55'15"N	06°18'48"W
Dynamene bidentata	Atlantic Ocean	Isles of Scilly	Tresco	49°56'47"N	06°20'18"W
Dynamene bidentata	Atlantic Ocean	Morocco	Akhfenir	28°05'51"N	12°03'02"W
Dynamene bidentata	Atlantic Ocean	Morocco	El Jadida	33°15'51"N	08°30'38"W
Dynamene bidentata	Atlantic Ocean	Morocco	Essaouire	31°30'58"N	09°46'17"W
Dynamene bidentata	Atlantic Ocean	Morocco	Insouane	30°50'21"N	09°49'23"W
Dynamene bidentata	Atlantic Ocean	Morocco	Rabat	33°59'42"N	06°53'01"W
Dynamene bidentata	Atlantic Ocean	Morocco	Témara	33°56'31"N	06°56'36"W
Dynamene bidentata	Atlantic Ocean	Ireland	Tory Island	55°15'00"N	08°13'00"W
Dynamene bidentata	Atlantic Ocean	Portugal	Agudela	41°14'27"N	08°43'39"W
Dynamene bidentata	Atlantic Ocean	Portugal	Buarcos	40°10'34"N	08°54'02"W
Dynamene bidentata	Atlantic Ocean	Portugal	Peniche	39°22'21"N	09°22'39"W
Dynamene bidentata	Atlantic Ocean	Portugal	Praia Norte	41°41'21"N	08°50'52"W
Dynamene bidentata	Atlantic Ocean	Portugal	Sines	37°57'39"N	08°53'14"W
Dynamene bidentata	Atlantic Ocean	Portugal	Vale dos Homens	37°22'17"N	08°50'04"W
Dynamene bidentata	Atlantic Ocean	Portugal	Vila do Conde	41°21'03"N	08°45'15"W
Dynamene bidentata	Atlantic Ocean	Scotland	Ardrossan	55°36'43"N	04°43'10"W
Dynamene bidentata	Atlantic Ocean	Scotland	Oban	56°24'54"N	05°28'15"W
Dynamene bidentata	Atlantic Ocean	Scotland	Mull	56°26'21"N	06°00'03"W
Dynamene bidentata	Atlantic Ocean	Scotland	Bellochantuy	55°31'32"N	05°42'40"W
Dynamene bidentata	Atlantic Ocean	Scotland	Carsaig	56°19'09"N	05°57'54"W
Dynamene bidentata	Atlantic Ocean	Scotland	Clatholl	58°10'60"N	05°19'60"W
Dynamene bidentata	Atlantic Ocean	Scotland	Easdale	56°17'17"N	05°38'05"W
Dynamene bidentata	Atlantic Ocean	Scotland	Monach Islands	57°30'60"N	07°36'00"W
Dynamene bidentata	Atlantic Ocean	Spain	Bañugues	43°32'36"N	05°38'49"W
Dynamene bidentata	Atlantic Ocean	Spain	Barizo	43°19'20"N	08°52'22"W
Dynamene bidentata	Atlantic Ocean	Spain	Isla Castelo	43°36'17"N	08°11'28"W
Dynamene bidentata	Atlantic Ocean	Spain	Muxía	43°05'34"N	09°13'24"W
Dynamene bidentata	Atlantic Ocean	Spain	Pedreira	43°33'22"N	08°16'30"W
Dynamene bidentata	Atlantic Ocean	Spain	Ria de Arosa	42°34'07"N	08°53'07"W
Dynamene bidentata	Atlantic Ocean	Spain	Ria del Ferrol	43°28'53"N	08°12'50"W
Dynamene bidentata	Atlantic Ocean	Spain	Tarifa Island	36°00'00"N	05°36'38"W
Dynamene bidentata	Atlantic Ocean	Wales	Aber-Eiddy	51°56'30"N	05°11'55"W
Dynamene bidentata	Atlantic Ocean	Wales	Abermawr	51°56'17"N	05°12'27"W
Dynamene bidentata	Atlantic Ocean	Wales	Broad Haven	51°42'05"N	05°09'11"W
Dynamene bidentata	Atlantic Ocean	Wales	Caerfai Bay	51°52'00"N	05°15'00"W
Dynamene bidentata	Atlantic Ocean	Wales	Dinas Head	52°01'15"N	04°54'36"W
Dynamene bidentata	Atlantic Ocean	Wales	Freshwater East	51°38'44"N	04°51'34"W
Dynamene bidentata	Atlantic Ocean	Wales	Freshwater West	51°38'60"N	05°02'60"W
Dynamene bidentata	Atlantic Ocean	Wales	Goodwick Harbour	51°59'60"N	04°59'00"W
Dynamene bidentata	Atlantic Ocean	Wales	Manorbier	51°37'60"N	04°46'60"W
Dynamene bidentata	Atlantic Ocean	Wales	Martins Heaven	51°44'14"N	05°14'01"W
Dynamene bidentata	Atlantic Ocean	Wales	Monks Haven	51°42'60"N	05°08'00"W
Dynamene bidentata	Atlantic Ocean	Wales	Musselwick Sands	51°42'60"N	05°12'00"W
Dynamene bidentata	Atlantic Ocean	Wales	Nolton Haven	51°48'58"N	05°06'27"W
Dynamene bidentata	Atlantic Ocean	Wales	Porth Colman	52°52'00."N	04°41'00"W
Dynamene bidentata	Atlantic Ocean	Wales	Rhoscolyn	53°15'00"N	04°34'60"W
Dynamene bidentata	Atlantic Ocean	Wales	Rhosneigre	53°13'00"N	04°30'60"W
Dynamene bidentata	Atlantic Ocean	Wales	Sandy Haven	51°43'32"N	05°06'27"W
Dynamene bidentata	Atlantic Ocean	Wales	Skomer	51°43'60"N	05°16'60"W
Dynamene bidentata	Atlantic Ocean	Wales	St Brides Haven	51°45'60"N	05°05'60"W
Dynamene bidentata	Atlantic Ocean	Wales	Stackpole	51°37'41"N	04°53'45"W
Dynamene bidentata	Atlantic Ocean	Wales	West Angle	51°40'60"N	05°04'60"W
Dynamene bidentata	Atlantic Ocean	Wales	West Dale Bay	51°42'28"N	05°11'19"W
Dynamene bidentata	Atlantic Ocean	Wales	Whitesands Bay	51°53'10"N	05°18'18"W
Dynamene bifida	Mediterranean Sea	France	Banylus-sur-Mer	42°29'14"N	03°07'40"E
Dynamene bifida	Mediterranean Sea	France	Endoume	43°16'21"N	05°21'33"E
Dynamene bifida	Mediterranean Sea	Greece	Corfu Island	39°36'00"N	19°48'60"E

Species present	Region	Country/Archipelago	Location	Latitude	Longitude
Dynamene bifida	Mediterranean Sea	Italy	Ischia Island	40°43'56"N	13°57'59"E
Dynamene bifida	Mediterranean Sea	Italy	Mergellina	40°49'00"N	14°13'00"E
Dynamene bifida	Mediterranean Sea	Spain	Mojacar	37°07'60"N	01°51'00"W
Dynamene bifida	Mediterranean Sea	Turkey	Izmir	38°27'09"N	27°05'50"E
Dynamene edwardsi	Atlantic Ocean	Azores	Angra do Heroismo	38°38'60"N	27°14'60"W
Dynamene edwardsi	Atlantic Ocean	Azores	Corvo Island	39°39'60"N	31°07'00"W
Dynamene edwardsi	Atlantic Ocean	Azores	Formigas Island	37°15'60"N	24°47'00"W
Dynamene edwardsi	Atlantic Ocean	Azores	Mosteiros	37°54'01"N	25°49'04"W
Dynamene edwardsi	Atlantic Ocean	Azores	Pico Island	38°23'60"N	28°15'00"W
Dynamene edwardsi	Atlantic Ocean	Azores	Ponta da Ferraria	37°51'40"N	25°51'17"W
Dynamene edwardsi	Atlantic Ocean	Azores	Santa Maria Island	36°56'53"N	25°05'34"W
Dynamene edwardsi	Atlantic Ocean	Azores	São Jorge Island	38°37'41"N	27°56'11"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	Arenas Blancas	27°46'02"N	18°07'17"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	Arinaga	27°51'00"N	15°23'60"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	Arrecife	28°57'08"N	13°32'59"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	Bañaderos	28°08'59"N	15°32'24"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	Caleta	28°09'47"N	15°41'57"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	El Faro	28°27'27"N	17°51'01"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	Fuerteventura	28°04'10"N	14°30'36"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	La Fajana	28°50'32"N	17°47'40"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	La Salemera	28°34'41"N	17°45'38"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	Las Palmas	28°08'60"N	15°25'60"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	Los Cristianos	28°02'41"N	16°42'43"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	Los Sargos	27°47'05"N	18°00'42"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	Mal Paso	28°24'58"N	16°17'55"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	Playa Blanca	28°51'00"N	13°49'00"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	Playa Melenara	27°59'20"N	15°22'14"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	Puerto de Mogan	27°49'40"N	15°47'16"W
Dynamene edwardsi	Atlantic Ocean	Canary Islands	Rada de Arrieta	29°09'17"N	13°25'43"W
Dynamene edwardsi	Atlantic Ocean	Madeira	Caniçal	32°44'00"N	16°44'00"W
Dynamene edwardsi	Atlantic Ocean	Madeira	Funchal 1	32°38'44"N	16°58'30"W
Dynamene edwardsi	Atlantic Ocean	Madeira	Funchal 2	32°38'38"N	16°52'35"W
Dynamene edwardsi	Atlantic Ocean	Madeira	Funchal 3	32°38'11"N	16°56'52"W
Dynamene edwardsi	Atlantic Ocean	Madeira	Ponta da Cruz	32°37'59"N	16°56'37"W
Dynamene edwardsi	Atlantic Ocean	Madeira	Porto dos Frades	33°04'21"N	16°17'44"W
Dynamene edwardsi	Atlantic Ocean	Madeira	Reis Magos	32°38'46"N	16°49'27"W
Dynamene edwardsi	Atlantic Ocean	Madeira	Selvagem Grande	30°08'28"N	15°52'12"W
Dynamene edwardsi	Atlantic Ocean	Madeira	Selvagem Pequena	30°01'60"N	16°01'00"W
Dynamene edwardsi	Atlantic Ocean	Mauritania	Nouadhibou	20°56'00"N	17°02'00"W
Dynamene edwardsi	Atlantic Ocean	Morocco	Arzila	35°27'29"N	06°02'53"W
Dynamene edwardsi	Atlantic Ocean	Morocco	El Jadida	33°15'51"N	08°30'39"W
Dynamene edwardsi	Atlantic Ocean	Morocco	Insouane	30°50'21"N	09°49'23"W
Dynamene edwardsi	Atlantic Ocean	Morocco	Tangiers Harbour	35°52'60"N	05°30'00"W
Dynamene edwardsi	Atlantic Ocean	Morocco	Tarfaya	27°55'04"N	12°57'40"W
Dynamene edwardsi	Atlantic Ocean	Portugal	Arrifes	37°04'34"N	08°16'36"W
Dynamene edwardsi	Atlantic Ocean	Portugal	Berlengas	39°24'42"N	09°30'40"W
Dynamene edwardsi	Atlantic Ocean	Portugal	Buarcos	40°10'34"N	08°54'02"W
Dynamene edwardsi	Atlantic Ocean	Portugal	Dona Ana	37°05'13"N	08°40'04"W
Dynamene edwardsi	Atlantic Ocean	Portugal	Ingrina	37°02'43"N	08°52'41"W
Dynamene edwardsi	Atlantic Ocean	Portugal	Dona Ana	37°06'00"N	08°40'00"W
Dynamene edwardsi	Atlantic Ocean	Portugal	Peniche	39°22'21"N	09°22'39"W
Dynamene edwardsi	Atlantic Ocean	Portugal	Sines	37°57'39"N	08°53'14"W
Dynamene edwardsi	Atlantic Ocean	Portugal	Vale dos Homens	37°22'17"N	08°50'04"W
Dynamene edwardsi	Atlantic Ocean	Portugal	Vilamoura	37°04'21"N	08°07'13"W
Dynamene edwardsi	Atlantic Ocean	Spain	Muxía	43°05'34"N	09°13'24"W
Dynamene edwardsi	Atlantic Ocean	Spain	Tarifa Island	36°00'00"N	05°36'38"W
Dynamene edwardsi	Mediterranean Sea	Algeria	Algiers Harbour	36°47'60"N	03°13'00"E
Dynamene edwardsi	Mediterranean Sea	Croatia	Marina Kornati	43°56'32"N	15°26'44"E
Dynamene edwardsi	Mediterranean Sea	Egypt	Alexandria	31°11'07"N	29°52'32"E
Dynamene edwardsi	Mediterranean Sea	France	Banylus-sur-Mer	42°29'14"N	03°07'40"E
Dynamene edwardsi	Mediterranean Sea	France	Endoume	43°16'21"N	05°21'33"E

Species present	Region	Country/Archipelago	Location	Latitude	Longitude
Dynamene edwardsi	Mediterranean Sea	France	La Grande Motte	43°33'17"N	04°05'10"E
Dynamene edwardsi	Mediterranean Sea	France	Port Venders	43°17'47"N	03°32'57"E
Dynamene edwardsi	Mediterranean Sea	Greece	Emborious Bay	38°11'01"N	26°00'60"E
Dynamene edwardsi	Mediterranean Sea	Greece	Lindos	36°05'00"N	28°04'60"E
Dynamene edwardsi	Mediterranean Sea	Greece	Rhodes island	36°25'00"N	28°13'00"E
Dynamene edwardsi	Mediterranean Sea	Israel	Bat-Yam	32°05'42"N	34°46'23"E
Dynamene edwardsi	Mediterranean Sea	Israel	Haifa	32°54'60"N	35°04'00"E
Dynamene edwardsi	Mediterranean Sea	Israel	Michmoret	32°24'14"N	34°51'00"E
Dynamene edwardsi	Mediterranean Sea	Israel	Rosh Hanikva	33°04'56"N	35°06'16"E
Dynamene edwardsi	Mediterranean Sea	Italy	Castelsardo	40°54'46"N	08°42'00"E
Dynamene edwardsi	Mediterranean Sea	Italy	Genoa	44°23'57"N	08°55'47"E
Dynamene edwardsi	Mediterranean Sea	Italy	Harbour of Leghorn	43°33'04"N	10°17'54"E
Dynamene edwardsi	Mediterranean Sea	Italy	Ischia Island	40°43'56"N	13°57'59"E
Dynamene edwardsi	Mediterranean Sea	Italy	La Spezia	44°06'35"N	09°50'27"E
Dynamene edwardsi	Mediterranean Sea	Italy	Mergellina	40°49'00"N	14°13'00"E
Dynamene edwardsi	Mediterranean Sea	Italy	Porto Rotondo	41°01'43"N	09°32'43"E
Dynamene edwardsi	Mediterranean Sea	Italy	Santa Margherita	44°20'04"N	09°12'51"E
Dynamene edwardsi	Mediterranean Sea	Italy	Tiberio	40°32'06"N	14°13'27"E
Dynamene edwardsi	Mediterranean Sea	Italy	Vado Ligure	44°16'00"N	08°26'00"E
Dynamene edwardsi	Mediterranean Sea	Italy	Venice	45°26'00"N	12°18'00"E
Dynamene edwardsi	Mediterranean Sea	Malta	Birzebbugh	35°46'60"N	14°31'00"E
Dynamene edwardsi	Mediterranean Sea	Malta	Valetta Harbour	35°53'60"N	14°30'00"E
Dynamene edwardsi	Mediterranean Sea	Monaco	Monaco	43°44'00"N	07°23'00"E
Dynamene edwardsi	Mediterranean Sea	Spain	Alicante	38°20'00"N	00°29'47"W
Dynamene edwardsi	Mediterranean Sea	Spain	Cadaqués	42°14'25"N	03°12'18"E
Dynamene edwardsi	Mediterranean Sea	Spain	Cala Morell	40°02'60"N	03°53'00"E
Dynamene edwardsi	Mediterranean Sea	Spain	Cala Olivera	38°55'50"N	01°30'04"E
Dynamene edwardsi	Mediterranean Sea	Tunisia	Tunis Harbour	36°50'00"N	10°13'60"E
Dynamene edwardsi	Mediterranean Sea	Turkey	Izmir	38°27'09"N	27°05'50"E
Dynamene edwardsi	Red Sea	Egypt	Dahab	28°30'00"N	34°30'00"E
Dynamene edwardsi	Red Sea	Israel	Port of Eilat	29°30'60"N	34°55'60"E
Dynamene edwardsi	Suez Canal	Egypt	Tis'A	30°01'24"N	32°34'43"E
Dynamene magnitorata	Atlantic Ocean	Azores	Cinco Ribeiras	38°40'31"N	27°19'47"W
Dynamene magnitorata	Atlantic Ocean	Azores	Faial Island	38°31'00"N	28°36'60"W
Dynamene magnitorata	Atlantic Ocean	Azores	Flores Island	39°31'00"N	31°12'00"W
Dynamene magnitorata	Atlantic Ocean	Azores	Pico Island	38°23'60"N	28°15'00"W
Dynamene magnitorata	Atlantic Ocean	Azores	Porto Martins	38°40'60"N	27°03'27"W
Dynamene magnitorata	Atlantic Ocean	Azores	Praia Formosa	36°56'59"N	25°05'42"W
Dynamene magnitorata	Atlantic Ocean	Azores	Ribeira Chã	37°42'00"N	25°29'00"W
Dynamene magnitorata	Atlantic Ocean	Azores	Santa Maria Island	36°56'53"N	25°07'00"W
Dynamene magnitorata	Atlantic Ocean	Azores	São Jorge Island	38°37'41"N	27°56'11"W
Dynamene magnitorata	Atlantic Ocean	Azores	São Miguel island	37°46'00"N	25°29'00"W
Dynamene magnitorata	Atlantic Ocean	Canary Islands	El Faro	28°27'27"N	17°51'01"W
Dynamene magnitorata	Atlantic Ocean	Canary Islands	Fuerteventura	28°04'09"N	14°30'15"W
Dynamene magnitorata	Atlantic Ocean	Canary Islands	La Fajana	28°50'32"N	17°47'40"W
Dynamene magnitorata	Atlantic Ocean	Channel Islands	Petit Bot Bay	49°25'03"N	02°33'50"W
Dynamene magnitorata	Atlantic Ocean	Channel Islands	Rocquaine Bay	49°25'60"N	02°38'60"W
Dynamene magnitorata	Atlantic Ocean	England	Weymouth	50°36'17"N	02°32'12"W
Dynamene magnitorata	Atlantic Ocean	France	Argenton	48°31'41"N	04°46'41"W
Dynamene magnitorata	Atlantic Ocean	France	Barfleur	49°40’04"N	01°15'24"W
Dynamene magnitorata	Atlantic Ocean	France	Brignognan	48°40'20"N	04°18'49"W
Dynamene magnitorata	Atlantic Ocean	France	Grève Santec	48°41'38"N	03°58'09"W
Dynamene magnitorata	Atlantic Ocean	France	Isle de Glénans	47°17'37"N	03°12'28"W
Dynamene magnitorata	Atlantic Ocean	France	Kerfanny	47°47'60"N	03°43'00"W
Dynamene magnitorata	Atlantic Ocean	France	Roscoff	48°43'40"N	03°58'09"W
Dynamene magnitorata	Atlantic Ocean	France	Trégaster	48°50'32"N	03°31'06"W
Dynamene magnitorata	Atlantic Ocean	France	Trénez	47°46'60"N	03°42'00"W
Dynamene magnitorata	Atlantic Ocean	Madeira	Caniçal	32°44'00"N	16°44'00"W
Dynamene magnitorata	Atlantic Ocean	Madeira	Porto dos Frades	33°04'21"N	16°17'44"W
Dynamene magnitorata	Atlantic Ocean	Madeira	Porto Santo	33°04'21"N	16°20'00"W
Dynamene magnitorata	Atlantic Ocean	Morocco	Arzila	35°27'29"N	06°02'53"W

Species present	Region	Country/Archipelago	Location	Latitude	Longitude
Dynamene magnitorata	Atlantic Ocean	Morocco	Fedhala	33°42'21"N	07°22'38"W
Dynamene magnitorata	Atlantic Ocean	Portugal	Armação de Pêra	37°06'00"N	08°20'60"W
Dynamene magnitorata	Atlantic Ocean	Portugal	Arrabida	38°28'10"N	08°59'05"W
Dynamene magnitorata	Atlantic Ocean	Portugal	Arrifes	37°04'34"N	08°16'36"W
Dynamene magnitorata	Atlantic Ocean	Portugal	Berlengas	39°24'42"N	09°30'40"W
Dynamene magnitorata	Atlantic Ocean	Portugal	Buarcos	40°10'34"N	08°54'02"W
Dynamene magnitorata	Atlantic Ocean	Portugal	Dona Ana	37°05'13"N	08°40'04"W
Dynamene magnitorata	Atlantic Ocean	Portugal	Peniche	39°22'21"N	09°22'39"W
Dynamene magnitorata	Atlantic Ocean	Portugal	Praia Norte	41°41'21"N	08°50'52"W
Dynamene magnitorata	Atlantic Ocean	Portugal	São Pedro de Moel	39°45'29"N	09°01'59"W
Dynamene magnitorata	Atlantic Ocean	Spain	Arosa	42°32'48"N	08°51'25"W
Dynamene magnitorata	Atlantic Ocean	Spain	Barizo	43°19'20"N	08°52'22"W
Dynamene magnitorata	Atlantic Ocean	Spain	Muxía	43°05'34"N	09°13'24"W
Dynamene magnitorata	Atlantic Ocean	Spain	Pedreira	43°33'22"N	08°16'30"W
Dynamene magnitorata	Atlantic Ocean	Spain	Puerto Sou	42°43'32"N	08°59'59"W
Dynamene magnitorata	Atlantic Ocean	Spain	San Vincent	43°23'20"N	04°22'43"W
Dynamene magnitorata	Atlantic Ocean	Spain	Tarifa Island	36°00'00"N	05°36'38"W
Dynamene magnitorata	Mediterranean Sea	Chafarinas Islands	Isla del Rey	35°10'60"N	02°24'60"W
Dynamene magnitorata	Mediterranean Sea	Egypt	Alexandria	31°11'07"N	29°52'32"E
Dynamene magnitorata	Mediterranean Sea	Italy	Ischia Island	40°43'55"N	13°57'59"E
Dynamene magnitorata	Mediterranean Sea	Monaco	Fonteville	43°43'00"N	07°25'00"E
Dynamene magnitorata	Mediterranean Sea	Spain	Alicante	38°20'00"N	00°29'47"W
Dynamene magnitorata	Mediterranean Sea	Tunisia	Tunis Harbour	36°50'00"N	10°13'60"E
Dynamene sp.	Mediterranean Sea	NW Aegean	Greece	40°00'00"N	25°00'00"E
Dynamene tubicauda	Mediterranean Sea	Italy	Brucoli	37°15'60"N	15°11'00"E
Dynamene tubicauda	Mediterranean Sea	Italy	Calaberdardo	36°51'18"N	15°07'15"E
Dynamene tubicauda	Mediterranean Sea	Italy	Elba Island	42°47'60"N	10°07'60"E
Dynamene tubicauda	Mediterranean Sea	Italy	Ischia Island	40°43'56"N	13°57'59"E
Dynamene tubicauda	Mediterranean Sea	Italy	Isola Lachea	37°32'60"N	15°09'00"E
Dynamene tubicauda	Mediterranean Sea	Italy	Sorrento	40°37'00"N	14°21'60"E
Dynamene tubicauda	Mediterranean Sea	Malta	Mellieha Bay	35°58'00"N	14°20'60"E

ANNEXES OF CHAPTER 4.

Annex 4.1. List of sampled locations, location codes, co-ordinates, COI haplotype number, number of individuals amplified for each locus in each location, region and MOTU for each Dynamene species used in chapter 4. Code labels correspond with those used in the figures and texts in chapter 4. For corresponding haplotype numbers, see Fig. 4.1.

	Locations	Code	Latitude	Longitude	Hap nr	*COI*	*16S*	*18S*	*28S*	Region	MOTUs
Dynamene edwardsi	Muxia	GALI3	43.092831	-9.223431	I1	1	-	1	1	Galicia	**MOTU I**
	Peniche	PORW3	39.372433	-9.377551	I1	4	-	2	-	Portugal West	
	Sines	PORW4	37.960884	-8.887296	I1	1	-	-	-		
	Arrifes	PORS1	37.076052	-8.27678	I1	4	1	2	1	Portugal South	
	Dona Ana	PORS2	37.086969	-8.667716	I1	5	-	3	1		
	Ingrina	PORS3	37.045257	-8.878047	I1-3	5	2	3	2		
	Mosteiros	SAOM1	37.900153	-25.817875	II1	1	1	1	1	São Miguel	**MOTU II**
	France	MEDI1	43.554809	4.086157	II2	1	1	1	1	Mediterranean	
	Croatia	MEDI2	43.942178	15.445649	II2	1	1	-	-		
	Porto Frades	PSAN	33.072575	-16.295666	III1	5	3	3	3	Porto Santo	**MOTU III**
	Arzila	MORN1	35.458006	-6.047981	IV1	4	1	4	1	Morocco North	**MOTU IV**
	El Jadida	MORN2	33.264036	-8.510717	IV1	1	1	1	1		
	Tarfaya	MORS	27.917817	-12.961147	IV2-3	4	1	4	1	Morocco South	
	Agaete	GCAN1	28.163186	-15.699269	V1-4	6	1	3	1	Gran Canaria	**MOTU V**
	Playa Melenara	GCAN2	27.988891	-15.370485	V5-10	5	-	3	-		
	Bañaderos	GCAN3	28.149658	-15.54018	V5, 11-12	6	2	5	2		
	Selvagem Grande	SELV1	30.141158	-15,870064	VI1-3	4	3	3	3	Selvagens	**MOTU VI**
	Selvagem Pequena	SELV2	30.033233	-16.016675	VI4	2	-	-	-		
	Reis Magos	MADE1	32.646111	-16.824167	VII1	5	1	4	2	Madeira	**MOTU VII**
	Ponta Cruz	MADE2	32.633123	-16.943643	VII1-2	5	2	4	1		
	Mal Paso	TENE1	28.416147	-16.298656	VII1, 3-6	5	2	4	2	Tenerife	
	Los Cristianos	TENE2	28.044714	-16.711856	VII1, 7-8	4	1	2	1		
	La Fajana	LPAL1	28.842276	-17.794324	VIII1	5	1	3	1	La Palma	**MOTU VIII**
	La Salemera	LPAL2	28.577985	-17.760556	VIII1-4	5	1	3	1		
	El Faro	LPAL3	28.457545	-17.85034	VIII1-2, 5-6	6	1	5	1		
	Los Sargos	HIER1	27.784739	-18.011569	IX1	3	2	3	2	El Hierro	**MOTU IX**
	Arenas Blancas	HIER2	27.767189	-18.121308	IX1-2	3	1	2	1		
Dynamene bidentata	Bellochantuy	SCOT1	55.525278	-5.710278	X1	5	1	5	1	Scotland	**MOTU X**
	Carsaig	SCOT2	56.319444	-5.965	X1-2	4	1	3	-		
	Easdale	SCOT3	56.291111	-5.633333	X3	1	-	3	-		
	Pedreira	GALI1	43.55617	-8.274942	X4-8	5	1	4	1	Galicia	
	Barizo	GALI2	43.322113	-8.872784	X9-11	3	-	1	-		
	Viana Castelo	PORW1	41.689194	-8.84787	X8, 12-14	14	-	-	-	Portugal West	
	Buarcos	PORW2	40.175976	-8.900572	X8, 15-16	3	1	5	1		
	Sines	PORW4	37.960884	-8.887296	X17-18	2	-	-	-		
	El Jadida	MORN2	33.264036	-8.510717	X19	1	-	6	1	Morocco North	
	Essaouire	MORN3	31.515982	-9.771497	X20	1	-	1	-		
	Bañaderos	GCAN3	28.149658	-15.54018	X21	1	1	1	1	Gran Canaria	
Dynamene magnitorata	Pedreira	GALI1	43.55617	-8.274942	XI1	1	-	1	-	Galicia	**MOTU XI**
	Barizo	GALI2	43.322113	-8.872784	XI1-2	2	1	1	2		
	Muxía	GALI3	43.092831	-9.223431	XI3-4	5	1	3	1		
	Viana Castelo	PORW1	41.689194	-8.84787	XI4-5	2	-	-	-	Portugal West	
	Buarcos	PORW2	40.175976	-8.900572	XI4, 6	3	1	3	1		
	Peniche	PORW3	39.372433	-9.377551	XI3, 7-9	4	-	3	-		
	Arrifes	PORS1	37.076052	-8.27678	XI10-13	5	-	2	1	Portugal South	
	Dona Ana	PORS2	37.086969	-8.667716	XI3, 14-15	3	-	2	-		
	Cinco Ribeiras	TERC1	38.675414	-27.329717	XI16	1	1	1	1	Terceira	
	Porto Martins	TERC2	38.683328	-27.057522	XI16	2	2	3	2		
	Ribeira Chã	SAOM2	37.715417	-25.486836	XI16-19	6	1	2	1	São Miguel	
	Praia Formosa	SMAR	36.949917	-25.094989	XI19-20	2	-	-	-	Santa Maria	
	La Fajana	LPAL1	28.842276	-17.794324	XI21	1	1	1	1	La Palma	**MOTU XII**

Annex 4.2. Primer pairs and thermal cycling conditions used in chapter 4.

Fragment	Source	Primer	Direction (5’-3’)	PCR termal cycling conditions
COI	(Folmer et al. 1994)	LCO1490	(F) GGTCAACAAATCATAAAGATATTGG	1) 95°C/60s; 2) 35 cycles: 95°C/30s, 51°C/90s, 72°C/60s; 3) 72°C/5 min
		HCO2198	(R) TAAACTTCAGGGTGACCAAAAAATCA	
	(Lobo et al. 2013)	LoboF1	(F) KBTCHACAAAYCAYAARGAYATHGG	1) 95°C/5min; 2) 5 cycles: 95°C/30s, 45°C/90s, 72°C/60s; 3) 45 cycles: 94°C/30s, 54°C/90s, 72°C/60s; 4) 72°C/5 min
		LoboR1	(R) TAAACYTCWGGRTGWCCRAARAAYCA	
16S rDNA	(Palumbi et al. 2002)	16Sar	(F) CGCCTGTTTATCAAAAACAT	1) 95°C/60s; 2) 35 cycles: 95°C/30s, 46°C/90s, 72°C/60s; 3) 72°C/5 min
		16Sbr	(R) CCGGTCTGAACTCAGATCACG	
	(Geller et al. 1997)	D16SAR	(F) CGCCTGTTTAHYAAAAACAT	
		D16SBR	(R) CCGGTCTGAACTCAGMTCAYG	
18S rDNA	(Whitting 2002)	18sAi	(F) CCTGAGAAACGGCTACCACATC	1) 95°C/60s; 2) 35 cycles: 95°C/30s, 45°C/90s, 72°C/60s; 3) 72°C/5 min
		18SBi	(R) GAGTCTCGTTCGTTATCGGA	
28S rDNA	(Tomikawa et al. 2007)	AM-28S-H	(F) GACGCGCATGAATGGATTAACG	1) 95°C/60s; 2) 35 cycles: 95°C/30s, 48°C/90s, 72°C/60s; 3) 72°C/5 min
		AM-28S-T	(R) TGAACAATCCGACGCTTGGCG	

Annex 4.3. Substitution models used for phylogenetic reconstructions used in chapter 4. Number of sequences; total, included and excluded characters and informative sites for each locus are also displayed.

	Gene	Taxa	Total Chars.	Exc. Chars.	Inc. Chars.	Pars. Inf.	AICc	BIC
All species								
	COI	179	658	0	658	247	GTR+G+I	HKY+G+I
	16S rDNA	43	463	2	461	155	GTR+G	TN93+G
	18S rDNA	120	1145	2	1143	129	TN93+G	K2+G
	28S rDNA	46	812	7	805	86	GTR	K2
	Concatenated (*COI+16S+18S+28S*)	42	3078	11	3067	610	GTR+G+I	TN93+G+I

Annex 4.4. Estimates of genetic diversity for each locus for each species used in chapter 4. Also, the estimates of genetic diversity of COI for each MOTU is shown (for MOTU definition see material and methods section 4.4).

	Species/MOTU	N	H	S	Hd	π
COI	*Dynamene edwardsi*	101	41	219	0.945	0.12246
	MOTU I	20	3	2	0.205	0.00032
	MOTU II	3	2	9	0.667	0.00912
	MOTU III	5	1	0	0.000	0.00000
	MOTU IV	9	3	2	0.639	0.00118
	MOTU V	17	12	17	0.919	0.00420
	MOTU VI	6	4	6	0.867	0.00375
	MOTU VII	19	8	15	0.719	0.00403
	MOTU VIII	16	6	8	0.733	0.00314
	MOTU IX	6	2	1	0.333	0.00051
	Dynamene bidentata (MOTU X)	40	21	32	0.890	0.00424
	Dynamene magnitorata	38	21	37	0.942	0.00730
	MOTU XI	37	20	27	0.938	0.00644
	MOTU XII	1	1	-	-	-
16S	*Dynamene edwardsi*	30	16	92	0.954	0.07385
	Dynamene bidentata	5	2	2	0.400	0.00175
	Dynamene magnitorata	8	4	3	0.786	0.00272
18S	*Dynamene edwardsi*	69	5	32	0.682	0.00899
	Dynamene bidentata	32	2	4	0.063	0.00022
	Dynamene magnitorata	22	3	15	0.481	0.00514
28S	*Dynamene edwardsi*	31	5	25	0.712	0.01126
	Dynamene bidentata	5	1	0	0.000	0.00000
	Dynamene magnitorata	10	3	5	0.378	0.00128

N - number of sequences; H - number of haplotypes; S - segregation sites; Hd – haplotype diversity; π – nucleotide diversity.

Annex 4.5. Results of the AMOVA tests comparing variation in COI sequences for each Dynamene species (10 100 permutations).

Species	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fct	Fsc	Fst	P
Dynamene edwardsi									
	Among regions	11	3854.927	44.17783	98.14	0.98139			0.000
	Among populations within regions	15	18.418	0.15057	0.33		0.17971		0.000
	Within populations	72	49.483	0.68727	1.53			0.98473	0.000
	Total	98	3922.828	45.01567	100				
Dynamene bidentata									
	Among regions	3	28.610	1.41875	67.58	0.67582			0.000
	Among populations within regions	6	7.054	0.17030	8.11		0.25024		0.003
	Within populations	29	14.798	0.51026	24.31			0.75694	0.000
	Total	38	50.462	2.09931	100				
Dynamene magnitorata									
	Among regions	2	37.344	1.93645	55.63	0.55635			0.000
	Among populations within regions	10	23.572	0.42821	12.30		0.27730		0.001
	Within populations	25	27.900	1.11600	32.06			0.67937	0.000
	Total	37	88.816	3.48066	100				

Annex 4.6. Pairwise Fst values between sampled locations for each Dynamene species, based on 658 bp mitochondrial cytochrome c oxidase subunit I gene. Only locations with 3 or more sequences were used. In bold, significant values for $P < 0.05$ obtained through 1×10^4 permutations.

Dynamene edwardsi

	PORW3	PORS1	PORS2	PORS3	PSAN	MORN1	MORS	GCAN1	GCAN2	GCAN3	SELV1	MADE1	MADE2	TENE1	TENE2	LPAL1	LPAL2	LPAL3	HIER1	HIER2
PORW3	0.000																			
PORS1	0.000	0.000																		
PORS2	0.000	0.000	0.000																	
PORS3	**0.111**	**0.111**	**0.189**	**0.000**																
PSAN	**1.000**	**1.000**	**1.000**	**0.996**	0.000															
MORN1	**1.000**	**1.000**	**1.000**	**0.996**	**1.000**	0.000														
MORS	**0.998**	**0.998**	**0.998**	**0.993**	**0.998**	**0.800**	0.000													
GCAN1	**0.993**	**0.993**	**0.994**	**0.989**	**0.994**	**0.983**	**0.979**	0.000												
GCAN2	**0.989**	**0.989**	**0.990**	**0.983**	**0.990**	**0.972**	**0.967**	**0.361**	0.000											
GCAN3	**0.984**	**0.984**	**0.985**	**0.978**	**0.985**	**0.961**	**0.956**	0.005	**0.270**	0.000										
SELV1	**0.990**	**0.990**	**0.991**	**0.983**	**0.991**	**0.979**	**0.975**	**0.977**	**0.969**	**0.962**	0.000									
MADE1	**0.994**	**0.994**	**0.995**	**0.989**	**0.995**	**0.992**	**0.990**	**0.986**	**0.981**	**0.975**	**0.979**	0.000								
MADE2	**1.000**	**1.000**	**1.000**	**0.996**	**1.000**	**1.000**	**0.997**	**0.992**	**0.987**	**0.981**	**0.987**	0.500	0.000							
TENE1	**0.984**	**0.984**	**0.986**	**0.977**	**0.986**	**0.979**	**0.976**	**0.977**	**0.970**	**0.965**	**0.966**	0.214	0.000	0.000						
TENE2	**0.980**	**0.980**	**0.983**	**0.972**	**0.983**	**0.974**	**0.971**	**0.972**	**0.965**	**0.960**	**0.958**	0.393	0.369	0.145	0.000					
LPAL1	**1.000**	**1.000**	**1.000**	**0.996**	**1.000**	**1.000**	**0.998**	**0.992**	**0.987**	**0.981**	**0.987**	**0.978**	**1.000**	**0.942**	**0.921**	0.000				
LPAL2	**0.988**	**0.988**	**0.990**	**0.983**	**0.989**	**0.985**	**0.982**	**0.980**	**0.974**	**0.969**	**0.971**	**0.934**	**0.955**	**0.897**	**0.869**	**0.333**	0.000			
LPAL3	**0.986**	**0.986**	**0.988**	**0.981**	**0.987**	**0.982**	**0.979**	**0.979**	**0.973**	**0.969**	**0.970**	**0.929**	**0.947**	**0.896**	**0.868**	0.251	0.116	0.000		
HIER1	**1.000**	**1.000**	**1.000**	0.994	**1.000**	**1.000**	**0.997**	**0.990**	**0.983**	**0.975**	**0.983**	**0.975**	**1.000**	**0.934**	**0.910**	**1.000**	**0.938**	**0.926**	0.000	
HIER2	**0.998**	**0.998**	**0.998**	0.991	**0.998**	**0.997**	**0.994**	**0.988**	**0.981**	**0.973**	**0.980**	**0.968**	**0.993**	**0.928**	**0.902**	**0.991**	**0.930**	**0.919**	0.000	0.000

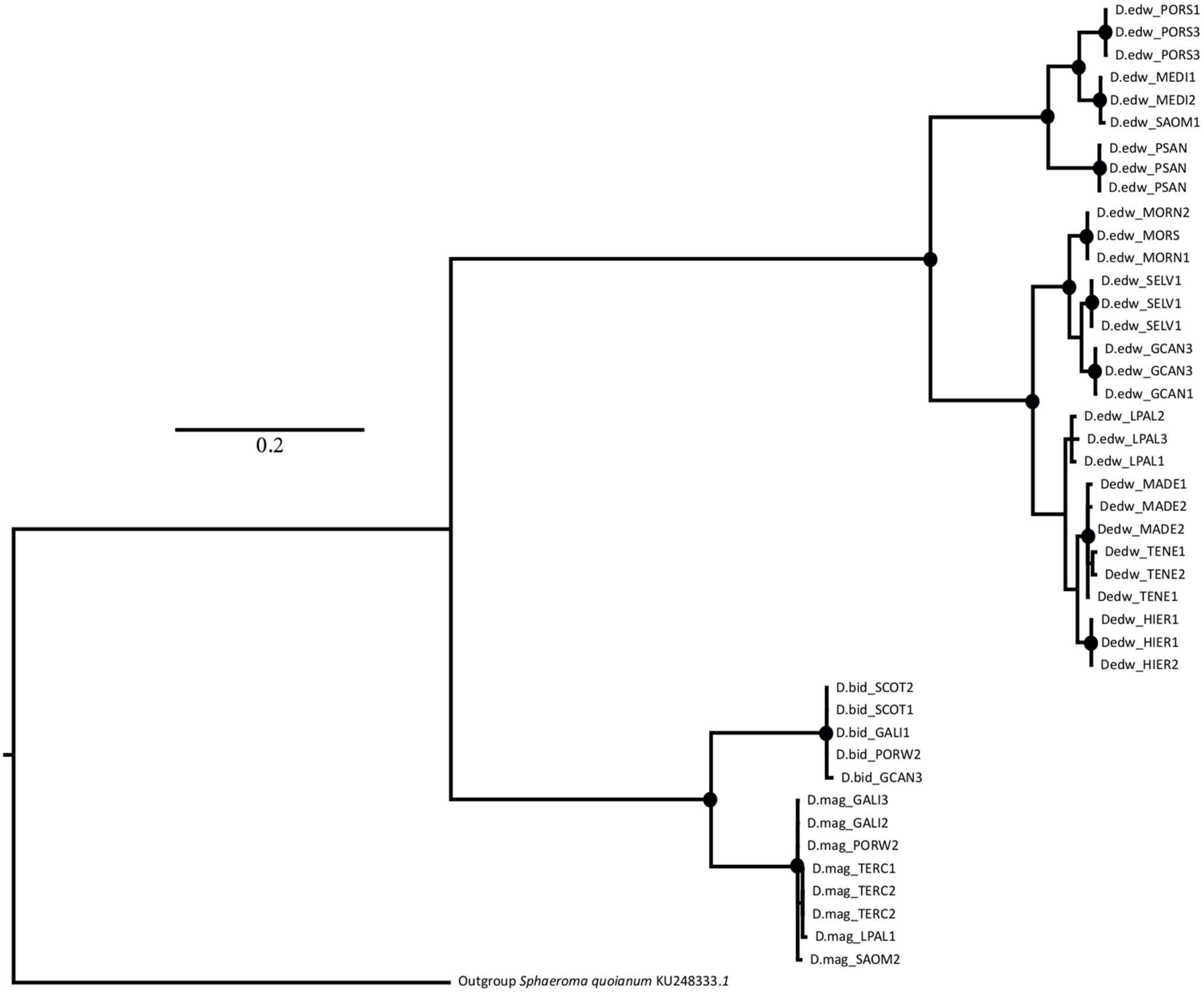
Dynamene bidentata

	SCOT1	SCOT2	GALI1	GALI2	PORW1
SCOT1	0.000				
SCOT2	0.062	0.000			
GALI1	**0.600**	**0.653**	0.000		
GALI2	**0.666**	**0.763**	0.000	0.000	
PORW1	**0.832**	**0.793**	**0.129**	**0.242**	0.000
PORW2	**0.825**	**0.710**	0.000	0.000	0.184

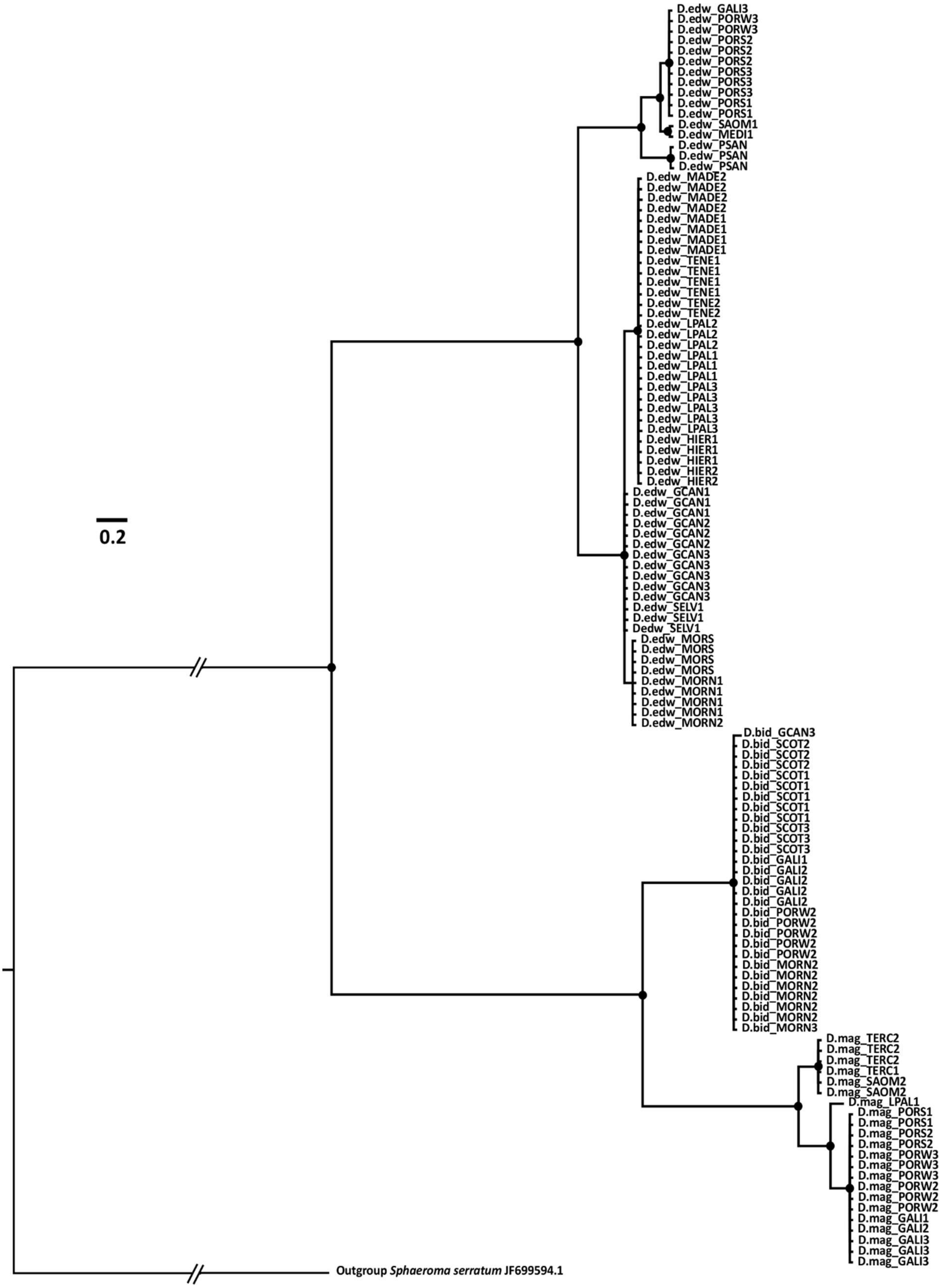
Dynamene magnitorata

	GALI3	PORW2	PORW3	PORS1	PORS2	TERC1	TERC2
GALI3	0.000						
PORW2	0.320	0.000					
PORW3	0.095	0.156	0.000				
PORS1	0.233	**0.382**	0.184	0.000			
PORS2	0.262	0.400	0.247	0.164	0.000		
TERC1	**0.874**	0.845	**0.670**	**0.602**	0.739	0.000	
TERC2	**0.727**	**0.712**	**0.641**	**0.671**	**0.618**	0.000	0.000

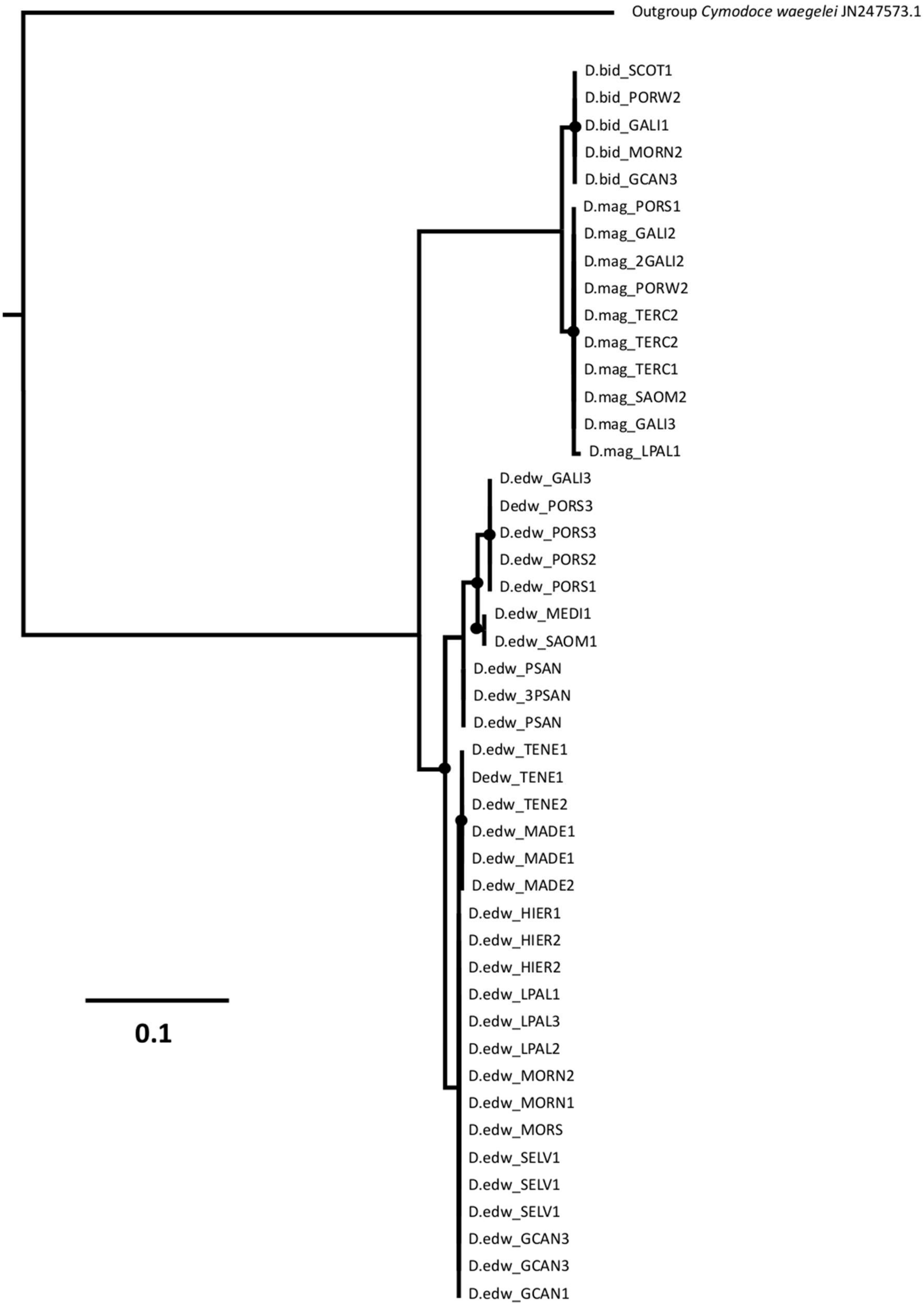
Annex 4.7. BI phylogenetic 16S tree of the three Dynamene species. For codes labels, see Annex 4.1. The dots by respective nodes indicate Bayesian posterior probability and maximum likelihood bootstrap values over 0.65. Sphaeroma quoianum with Genbank accession KU248333.1 was used as outgroup.



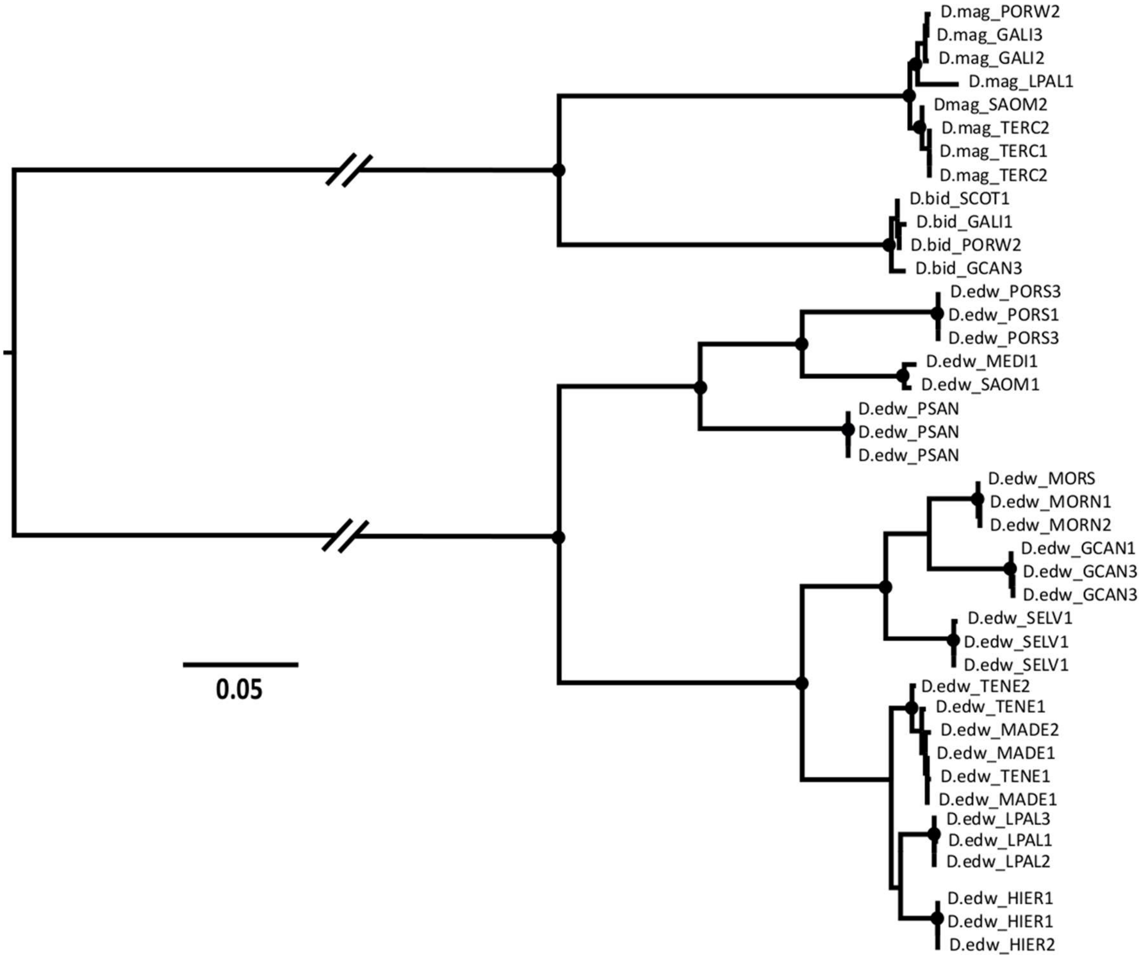
Annex 4.8. BI phylogenetic 18S tree of the three Dynamene species. For codes labels, see Annex 4.1. The dots by respective nodes indicate Bayesian posterior probability and maximum likelihood bootstrap values over 0.65. Sphaeroma serratum with Genbank accession JF699594.1 was used as outgroup.



Annex 4.9. BI phylogenetic 28S tree of the three Dynamene species. For codes labels, see Annex 4.1. The dots by respective nodes indicate Bayesian posterior probability and maximum likelihood bootstrap values over 0.65. Cymodoce waegelei with Genbank accession JN247573.1 was used as outgroup.



Annex 4.10. ML phylogenetic concatenated tree of the three Dynamene species. For codes labels, see Annex 4.1. The dots by respective nodes indicate Bayesian posterior probability and maximum likelihood bootstrap values over 0.65.



ANNEXES OF CHAPTER 5.

Annex 5.1. List of sampled locations, co-ordinates, number of individuals (n) sampled in each location and region for each Hyalidae species used in chapter 5. Accession numbers of BOLD, Haplotypes codes (H) and MOTUs are also shown.

	Locations	Latitude	Longitude	*n*	Accession numbers	H	MOTUs	Country/Island
Apohyale perieri	Muxia	43.092	-9.223	1	DSHYA006-15	H002	MOTU-1	Spain
	Pedreira	43.556	-8.275	3	DSHYA002-15, DSHYA003-15, DSHYA009-15	H002	MOTU-1	
	Barizo	43.322	-8.873	3	DSHYA005-15, DSHYA011-15, DSHYA012-15	H002	MOTU-1	
	Agudela	41.241	-8.728	1	DSHYA018-15	H002	MOTU-1	Portugal
	Buarcos	40.176	-8.901	2	DSHYA017-15, DSHYA013-15	H002, H006	MOTU-1	
	São Pedro Moel	39.758	-9.033	1	DSHYA010-15	H005	MOTU-1	
	Arrifes	37.076	-8.276	1	DSHYA001-15	H001	MOTU-1	
	Agaete	28.163	-15.699	1	DSHYA014-15	H007	MOTU-3	Gran Canaria
	Ponta Cruz	32.633	-16.943	3	DSHYA004-15/ DSHYA007-15, DSHYA117-16	H002, H003	MOTU-1,2	Madeira
	Ponta Ferreirinha	37.861	-25.855	1	DSHYA008-15	H004	MOTU-1	São Miguel
	La Fajana	28.842	-17.794	2	DSHYA016-15,DSHYA116-16	H008, H073	MOTU-4	La Palma
	El Faro	28.457	-17.85	2	DSHYA015-15, DSHYA115-16	H008, H072	MOTU-4	
Apohyale media	Bañaderos	28.149	-15.54	5	DSHYA019-15/ DSHYA020-15/ DSHYA021-15, DSHYA023-15, DSHYA022-15	H009,H011 H010	MOTU-5,6	Gran Canaria
	Los Sargos	27.767	-18.121	1	DSHYA119-16	H074	MOTU-6	El Hierro
	La Salemera	28.577	-17.76	5	DSHYA024-15/ DSHYA025-15/ DSHYA026-15/ DSHYA027-15/ DSHYA118-16	H012	MOTU-5	La Palma
	Ponta Cruz	32.633	-16.943	1	DSHYA028-15	H012	MOTU-5	Madeira
	Rio de Janeiro	-22.957	-43.164	5	DSHYA120-16, DSHYA121-16, DSHYA122-16, DSHYA123-16,DSHYA124-16	H075, H076, H077, H078, H079	MOTU-21	Brazil
Apohyale prevostii	Baloy	60.805	4.806	2	DSHYA029-15/ DSHYA037-15	H013	MOTU-7	Norway
	Viksoy	60.175	5.042	2	DSHYA030-15/ DSHYA041-15	H013	MOTU-7	
	Hellesoy	60.663	4.787	1	DSHYA040-15	H013	MOTU-7	
	Reykjavik	64.163	-22.009	3	DSHYA034-15, DSHYA032-15/ DSHYA033-15	H013, H015	MOTU-7	Iceland
	Grindavik	63.826	-22.411	1	DSHYA035-15	H013	MOTU-7	
	Strandarkirkja	63.823	-21.66	1	DSHYA036-15	H016	MOTU-7	
	Bellochantuy	55.525	-5.711	1	DSHYA038-15	H013	MOTU-7	Scotland
	Easdale	56.288	-5.635	2	DSHYA039-15, DSHYA125-16	H013, H080	MOTU-7	
	São Pedro Moel	39.758	-9.033	1	DSHYA031-15	H014	MOTU-7	Portugal

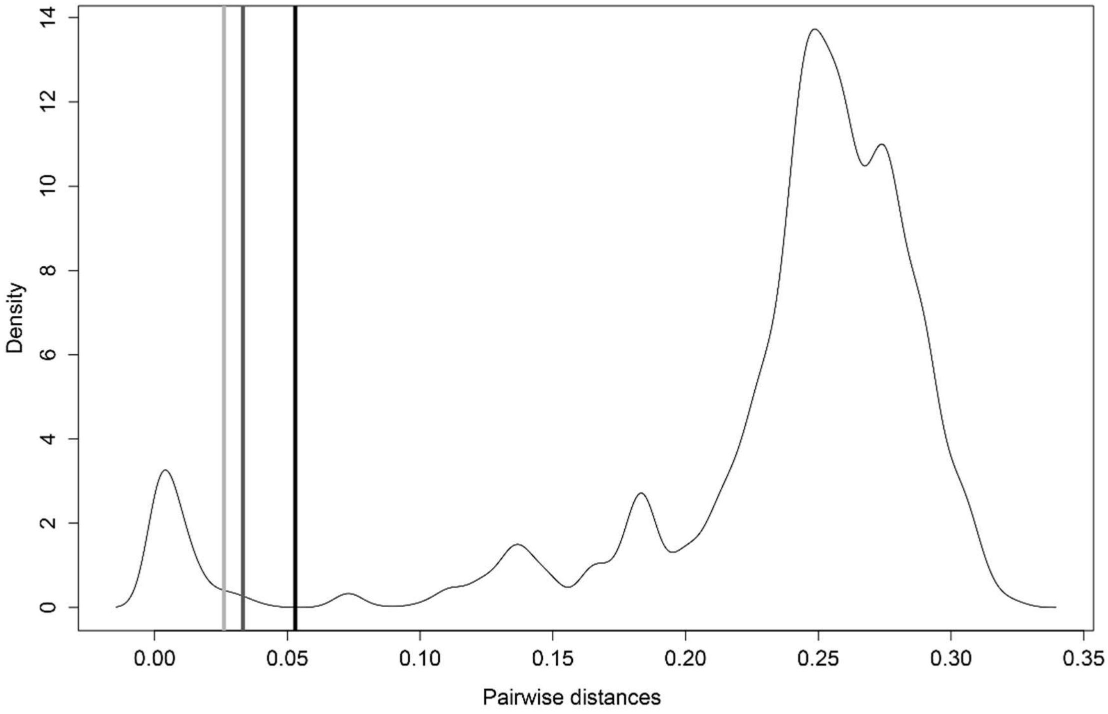
	Locations	Latitude	Longitude	*n*	Accession numbers	H	MOTUs	Country/Island
Apohyale stebbingi	Carsaig	56.319	-5.965	3	DSHYA043-15/ DSHYA044-15/ DSHYA045-15	H018	MOTU-8	Scotland
	Muxia	43.092	-9.223	1	DSHYA050-15	H023	MOTU-11	Spain
	Pedreira	43.556	-8.275	2	DSHYA051-15/ DSHYA052-15	H024	MOTU-8	
	Peniche	39.372	-9.378	2	DSHYA053-15, DSHYA060-15	H025, H029	MOTU-8	
	São Pedro Moel	39.758	-9.033	2	DSHYA058-15/ DSHYA059-15	H029	MOTU-8	Portugal
	Arrifes	37.076	-8.276	2	DSHYA042-15, DSHYA130-16	H017, H082	MOTU-8	
	Ingrina	37.045	-8.878	1	DSHYA055-15	H027	MOTU-8	
	Agudela	41.241	-8.728	1	DSHYA131-16	H083	MOTU-8	
	Dona Ana	37.087	-8.668	1	DSHYA049-15	H022	MOTU-8	
	Arzila	35.458	-6.048	4	DSHYA132-16, DSHYA133-16, DSHYA134-16, DSHYA158-16	H084, H085, H086, H106	MOTU-8	North Morocco
	La Salemera	28.578	-17.761	2	DSHYA056-15, DSHYA057-15	H028	MOTU-9	La Palma
	El Faro	28.457	-17.85	4	DSHYA046-15, DSHYA047-15, DSHYA048-15/ DSHYA127-16	H019, H020, H021	MOTU-9,10	
	Playa Melenara	27.989	-15.37	1	DSHYA065-15	H034	MOTU-12	Gran Canaria
	Agaete	28.163	-15.699	2	DSHYA061-15, DSHYA062-15	H030, H031	MOTU-9,12	
	Los Cristianos	28.044	-16.711	2	DSHYA138-16, DSHYA139-16	H088, H089	MOTU-23,26	Tenerife
	Mosteiros	37.9	-25.817	1	DSHYA159-16	H107	MOTU-30	Sao Miguel
	Ponta Ferreirinha	37.861	-25.855	3	DSHYA135-16/ DSHYA136-16/ DSHYA137-16	H087	MOTU-30	
	Praia Formosa	36.949	-25.094	1	DSHYA160-16	H108	MOTU-28	Santa Maria
	Ponta Cruz	32.633	-16.943	4	DSHYA063-15, DSHYA064-15, DSHYA128-16, DSHYA129-16	H032, H033, H026, H081	MOTU-13,14,29,24	Madeira
	Reis Magos	32.646	-16.824	1	DSHYA054-15	H026	MOTU-29	
Hyale pontica	Easdale	56.288	-5.635	1	DSHYA068-15	H037	MOTU-15	Scotland
	Muxia	43.092	-9.223	3	DSHYA070-15/ DSHYA072-15, DSHYA071-15	H035, H038	MOTU-15	Spain
	Barizo	43.322	-8.873	2	DSHYA066-15, DSHYA067-15, DSHYA085-15	H035, H036, H047	MOTU-15, 18	
	Agudela	41.241	-8.728	1	DSHYA069-15	H037	MOTU-15	Portugal

	Locations	Latitude	Longitude	*n*	Accession numbers	H	MOTUs	Country/Island
Protohyale (Protohyale) schmidtii	Muxia	43.092	-9.223	2	DSHYA091-15, DSHYA092-15	H043, H052	MOTU-31	Spain
	Pedreira	43.556	-8.275	2	DSHYA076-15, DSHYA079-15	H041, H043	MOTU-31	
	Barizo	43.322	-8.873	3	DSHYA078-15/ DSHYA080-15, DSHYA085-15	H043, H047	MOTU-31, 17	
	Buarcos	40.176	-8.901	1	DSHYA086-15	H048	MOTU-31	Portugal
	Peniche	39.372	-9.378	1	DSHYA088-15	H043	MOTU-31	
	Arrifes	37.076	-8.276	4	DSHYA074-15/DSHYA075-15/ DSHYA081-15, DSHYA141-16	H040, H091	MOTU-31	
	Tarfaya	27.918	-12.961	1	DSHYA073-15	H039	MOTU-27	Morocco
	Akhfenir	28.097	-12.050	2	DSHYA142-16, DSHYA143-16	H092, H093	MOTU-32, 27	
	Ponta Cruz	32.633	-16.943	1	DSHYA087-15	H049	MOTU-18	Madeira
	Porto Frades	33.073	-16.296	1	DSHYA077-15	H042	MOTU-16	Porto Santo
	Ribeira Chã	37.715	-25.487	1	DSHYA145-16	H095	MOTU-18	São Miguel
	Mosteiros	37.900	-25.817	1	DSHYA144-16	H094	MOTU-18	
	São Lourenco	36.988	-25.054	2	DSHYA146-16, DSHYA147-16	H096, H097	MOTU-18	Santa Maria
	Praia Formosa	36.949	-25.094	2	DSHYA148-16, DSHYA149-16	H098, H099	MOTU-18	
	Bañaderos	28.149	-15.54	3	DSHYA082-15, DSHYA083-15, DSHYA084-15	H044, H045, H046	MOTU-18	Gran Canaria
	La Salemera	28.578	-17.761	1	DSHYA089-15	H050	MOTU-18	La Palma
	El Faro	28.457	-17.85	1	DSHYA090-15	H051	MOTU-18	
	La Fajana	28.842	-17.794	1	DSHYA140-16	H090	MOTU-18	
	Los Cristianos	28.044	-16.711	1	DSHYA163-16	H110	MOTU-18	Tenerife
	Mal Paso	28.034	-16.54	2	DSHYA150-16, DSHYA162-16	H100, H109	MOTU-18	
	Los Sargos	27.767	-18.121	1	DSHYA164-16	H111	MOTU-18	El Hierro
Serejohyale spinidactylus	Barizo	43.322	-8.872	2	DSHYA105-15, DSHYA106-15	H064, H065	MOTU-19	Galicia
	Muxía	43.092	-9.223	3	DSHYA108-15, DSHYA109-15/ DSHYA110-15	H067, H068	MOTU-19	
	Reis Magos	32.646	-16.824	2	DSHYA113-15, DSHYA114-15	H070, H071	MOTU-22	Madeira
	Ponta Cruz	32.633	-16.943	1	DSHYA111-15	H069	MOTU-22	
	Selvagem Grande	30.14	-15.86	1	DSHYA165-16	H112	MOTU-22	Selvagens
	Mosteiros	37.9	-25.817	1	DSHYA107-15	H066	MOTU-19	São Miguel
	Ponta Ferreirinha	37.861	-25.855	2	DSHYA112-15/ DSHYA154-16	H066	MOTU-19	
	Playa Melenara	27.989	-15.37	2	DSHYA102-15/ DSHYA103-15	H062	MOTU-17	Gran Canaria
	Agaete	28.163	-15.699	1	DSHYA151-16	H101	MOTU-17	
	Bañaderos	28.149	-15.54	3	DSHYA096-15, DSHYA097-15, DSHYA104-15	H056, H057, H063	MOTU-17	
	La Fajana	28.842	-17.794	2	DSHYA100-15, DSHYA101-15	H060, H061	MOTU-20	La Palma
	La Salemera	28.578	-17.761	4	DSHYA098-15/ DSHYA153-16, DSHYA099-15, DSHYA152-16	H058, H059, H102	MOTU-20	
	Los Sargos	27.767	-18.121	1	DSHYA155-16	H103	MOTU-25	El Hierro
	Arenas Blancas	27.767	-18.121	1	DSHYA156-16	H104	MOTU-25	

Annex 5.2. Primers, number of successfully amplified specimens and cycling conditions used in chapter 5.

Reference (number of specimens)	**Primer**	**Primer Direction (5' – 3')**	**PCR thermal cycling conditions**	**bp**
Folmer et al. 1994 (52)	LCO1490 HCO2198	(F) GGTCAACAAATCATAAAGATATTGG (R) TAAACTTCAGGGTGACCAAAAAATCA	1) 94°C (1 min); 2) 5 cycles: 94°C (30 s), 45°C (1 min 30 s), 72°C (1 min); 3) 35 cycles: 94°C (30 s), 51°C (1 min 30 s), 72°C (1 min); 4) 72°C (5 min).	658
Gibson et al. 2014 (44)	LoboF1 ArR5	(F) KBTCHACAAAYCAYAARGAYATHGG (R) GTRATIGCICCIGCIARIACIGG	1) 94°C (2 min); 2) 35 cycles: 94°C (30 s), 46°C (1 min), 72°C (1 min); 3) 72°C (5 min).	550
Lobo et al. 2013 (18)	LoboF1 LoboR1	(F) KBTCHACAAAYCAYAARGAYATHGG (R) TAAACYTCWGGRTGWCCRAARAAYCA	1) 94°C (1 min); 2) 5 cycles: 94°C (30 s), 45°C (1 min 30 s), 72°C (1 min); 3) 45 cycles: 94°C (30 s), 54°C (1 min 30 s), 72°C (1 min); 4) 72°C (5 min).	658

***Annex 5.3. Density plot of the genetic distances. The black line is the minimum transition between intra- and interspecific distances of 5.4%, found with the software R using the function 'localminima' of the library SPIDER (Brown et al. 2012); the lightest line is the maximum distance of 2.2% used for the delimitation of the MOTUs with BINs in BOLD (Ratnasingham and Hebert 2013); the middle line is the threshold of 3% proposed by Costa et al. (2009) for* crustacean species.**



Annex 5.4. Mean and Max distance within MOTUs, number of specimens per MOTU (Count) and distance from the nearest neighbour (NN Dist) used in chapter 5.

	MOTU	Mean	Max	Count	NN Dist
Apohyale perieri	MOTU-1	0.0024	0.0109	14	6.74
	MOTU-2	0.0000	0.0000	2	8.56
	MOTU-3	-	-	1	8.56
	MOTU-4	0.0030	0.0036	4	6.74
Apohyale media	MOTU-5	0.0032	0.0109	10	9.11
	MOTU-6	0.0219	0.0219	2	9.11
	MOTU-21	0.0147	0.0237	5	16.10
Apohyale prevostii	MOTU-7	0.0015	0.0055	14	16.38
Apohyale stebbingi	MOTU-8	0.0068	0.0164	18	12.02
	MOTU-9	0.0052	0.0073	4	2.73
	MOTU-10	0.0012	0.0018	3	13.11
	MOTU-11	-	-	1	12.02
	MOTU-12	0.0073	0.0073	2	8.56
	MOTU-13	-	-	1	8.91
	MOTU-14	-	-	1	16.76
	MOTU-23	-	-	1	8.91
	MOTU-24	-	-	1	2.91
	MOTU-26	-	-	1	2.73
	MOTU-28	-	-	1	8.56
	MOTU-29	0.0000	0.0000	2	1.64
	MOTU-30	0.0009	0.0018	4	1.64
Hyale pontica	MOTU-15	0.0028	0.0055	7	17.85
Protohyale (Protohyale) schmidtii	MOTU-16	-	-	1	2.55
	MOTU-18	0.0106	0.0255	18	11.11
	MOTU-27	0.0182	0.0182	2	2.19
	MOTU-31	0.0059	0.0237	12	2.19
	MOTU-32	-	-	1	2.19
Serejohyale spinidactylus	MOTU-17	0.0038	0.0055	6	15.38
	MOTU-19	0.0051	0.0091	8	9.11
	MOTU-20	0.0039	0.0109	6	9.11
	MOTU-22	0.0082	0.0109	4	13.83
	MOTU-25	0.0020	0.0020	2	14.03

ANNEX OF CHAPTER 6.

Annex 6.1. List of peracaridean species used in chapter 6, with respective source, sampling location and number of individuals used. For respective co-ordinates of the material obtained in this study, see Annex 1.5.

Order	Species	Source/GenBank accession	Country/Island	Location	*n*
Amphipoda	*Ampithoe helleri**	KX223984, KX223981, KX223983, KX223980, KF369110, KF369108, KF369109	Portugal	-	7
Amphipoda	*Ampithoe helleri**	This study	Gran Canaria	Bañaderos	3
Amphipoda	*Ampithoe helleri**	Chapter 2	Spain	Barizo	2
Amphipoda	*Ampithoe helleri**	This study	La Palma[#]	El Faro	2
Amphipoda	*Ampithoe ramondi**	This study	Tenerife[#]	Mal Paso	5
Amphipoda	*Ampithoe ramondi**	Chapter 2	Portugal	Ingrina	1
Amphipoda	*Ampithoe ramondi**	Chapter 2	Portugal	Arrifes	1
Amphipoda	*Ampithoe ramondi**	Chapter 2	Portugal	Dona Ana	2
Amphipoda	*Ampithoe ramondi**	This study	Gran Canaria	Bañaderos	1
Amphipoda	*Ampithoe ramondi**	This study	Madeira[#]	Ponta Cruz	1
Amphipoda	*Ampithoe ramondi**	This study	Santa Maria	Praia Formosa	1
Amphipoda	*Ampithoe ramondi**	This study	Santa Maria	São Lourenço	1
Amphipoda	*Ampithoe riedli**	This study	Portugal	Ingrina	3
Amphipoda	*Ampithoe riedli**	This study	Morocco[#]	Arzila	3
Amphipoda	*Ampithoe riedli**	This study	Madeira[#]	Ponta Cruz	2
Amphipoda	*Ampithoe riedli**	This study	La Palma[#]	La Fajana	1
Amphipoda	*Ampithoe rubricata*	Chapter 2	Portugal	Dona Ana	1
Amphipoda	*Ampithoe sp.*	This study	Gran Canaria	Playa Melenara	1
Amphipoda	*Apohyale media*	Chapter 5	Gran Canaria	Bañaderos	1
Amphipoda	*Apohyale perieri **	Chapter 5	Gran Canaria	Agaete	1
Amphipoda	*Apohyale perieri **	Chapter 5	La Palma	El Faro	3
Amphipoda	*Apohyale perieri **	Chapter 5	La Palma	La Fajana	1
Amphipoda	*Apohyale perieri **	Chapter 5	Madeira	Ponta Cruz	3
Amphipoda	*Apohyale perieri **	Chapter 5	Portugal	Arrifes	1
Amphipoda	*Apohyale perieri **	Chapter 5	Portugal	Buarcos	2
Amphipoda	*Apohyale perieri **	Chapter 5	Portugal	Agudela	1
Amphipoda	*Apohyale perieri **	Chapter 5	Portugal	São Pedro Moel	1
Amphipoda	*Apohyale perieri **	Chapter 5	São Miguel	Ponta Ferreirinha	1
Amphipoda	*Apohyale perieri **	Chapter 5	Spain	Muxía	1
Amphipoda	*Apohyale perieri **	Chapter 5	Spain	Barizo	3
Amphipoda	*Apohyale perieri **	Chapter 5	Spain	Pedreira	3
Amphipoda	*Apohyale prevostii*	KX223997	Portugal	-	1
Amphipoda	*Apohyale stebbingi **	Chapter 5	Gran Canaria	Agaete	2
Amphipoda	*Apohyale stebbingi **	Chapter 5	Gran Canaria	Playa Melenara	1
Amphipoda	*Apohyale stebbingi **	Chapter 5	La Palma	El Faro	4
Amphipoda	*Apohyale stebbingi **	Chapter 5	La Palma	La Salemera	2
Amphipoda	*Apohyale stebbingi **	Chapter 5	Madeira	Ponta Cruz	4
Amphipoda	*Apohyale stebbingi **	Chapter 5	Madeira	Reis Magos	1
Amphipoda	*Apohyale stebbingi **	Chapter 5	Morocco	Arzila	4

Order	Species	Source/GenBank accession	Country/Island	Location	n
Amphipoda	*Apohyale stebbingi* *	Chapter 5	Portugal	Ingrina	1
Amphipoda	*Apohyale stebbingi* *	Chapter 5	Portugal	Arrifes	2
Amphipoda	*Apohyale stebbingi* *	Chapter 5	Portugal	Dona Ana	1
Amphipoda	*Apohyale stebbingi* *	Chapter 5	Portugal	Agudela	1
Amphipoda	*Apohyale stebbingi* *	Chapter 5	Portugal	Peniche	2
Amphipoda	*Apohyale stebbingi* *	Chapter 5	Portugal	São Pedro Moel	2
Amphipoda	*Apohyale stebbingi* *	Chapter 5	Santa Maria	Praia Formosa	1
Amphipoda	*Apohyale stebbingi* *	Chapter 5	São Miguel	Mosteiros	1
Amphipoda	*Apohyale stebbingi* *	Chapter 5	São Miguel	Ponta Ferreirinha	3
Amphipoda	*Apohyale stebbingi* *	Chapter 5	Spain	Muxía	1
Amphipoda	*Apohyale stebbingi* *	Chapter 5	Spain	Pedreira	2
Amphipoda	*Apohyale stebbingi* *	Chapter 5	Tenerife	Los Cristianos	1
Amphipoda	*Caprella acanthifera**	This Study	El Hierro#	Arenas Blancas	1
Amphipoda	*Caprella acanthifera**	This Study	Gran Canaria	Agaete	1
Amphipoda	*Caprella acanthifera**	This Study	La Palma#	El Faro	2
Amphipoda	*Caprella acanthifera**	This Study	La Palma#	La Salermera	1
Amphipoda	*Caprella acanthifera**	This Study	Madeira	Ponta Cruz	2
Amphipoda	*Caprella acanthifera**	This Study	Madeira	Reis Magos	1
Amphipoda	*Caprella acanthifera**	This Study	Morocco#	El Jadida	2
Amphipoda	*Caprella acanthifera**	KX224000, KX223999, KX224001	Portugal	-	3
Amphipoda	*Caprella acanthifera**	Chapter 2	Portugal	Buarcos	2
Amphipoda	*Caprella acanthifera**	This Study	São Miguel	Ribeira Chã	3
Amphipoda	*Caprella acanthifera**	This Study	Tenerife#	Los Cristianos	1
Amphipoda	*Caprella liparotensis*	Chapter 2	Portugal	Dona Ana	1
Amphipoda	*Caprella mutica*	KT208479	North Sea	-	1
Amphipoda	*Caprella penantis*	KF369116	Portugal	-	1
Amphipoda	*Elasmopus canarius*	This study	Gran Canaria	Bañaderos	2
Amphipoda	*Elasmopus canarius*	This study	La Palma#	El Faro	1
Amphipoda	*Elasmopus canarius*	This study	El Hierro#	Arenas Blancas	1
Amphipoda	*Elasmopus pectenicrus* *	This study	Tenerife#	Mal Paso	2
Amphipoda	*Elasmopus pectenicrus* *	This study	Porto Santo#	Porto Frades	2
Amphipoda	*Elasmopus pectenicrus* *	This study	Portugal	Arrifes	1
Amphipoda	*Elasmopus pectenicrus* *	Chapter 2	Portugal	Dona Ana	2
Amphipoda	*Elasmopus pectenicrus* *	This study	Morocco#	Akhfenir	3
Amphipoda	*Elasmopus pectenicrus* *	This study	Madeira#	Ponta Cruz	3
Amphipoda	*Elasmopus pectenicrus* *	This study	Madeira#	Reis Magos	1
Amphipoda	*Elasmopus rapax*	Chapter 2	Spain	Pedreira	1
Amphipoda	*Elasmopus vachoni*	This study	La Palma	La Fajana	1
Amphipoda	*Elasmopus vachoni*	This study	São Miguel	Ribeira Chã	2
Amphipoda	*Elasmopus vachoni*	This study	Santa Maria	São Lourenço	1
Amphipoda	*Hyale pontica*	Chapter 5	Spain	Muxía	1
Amphipoda	*Hyalinae*	This study	Morocco	El Jadida	1
Amphipoda	*Jassa falcata*	Chapter 2	Spain	Pedreira	1
Amphipoda	*Jassa herdmani* *	This study	Madeira#	Ponta Cruz	2
Amphipoda	*Jassa herdmani* *	This study	Porto Santo#	Porto Frades	1
Amphipoda	*Jassa herdmani* *	KX224053, KF369134	Portugal	-	2

Order	Species	Source/GenBank accession	Country/Island	Location	n
Amphipoda	*Jassa herdmani* *	This study	Portugal	Buarcos	3
Amphipoda	*Jassa herdmani* *	This study	São Miguel#	Ribeira Chã	3
Amphipoda	*Jassa marmorata*	KT209366	North Sea	-	1
Amphipoda	*Jassa ocia*	Chapter 2	Portugal	Ingrina	1
Amphipoda	*Jassa pusilla*	KT208423	North Sea	-	1
Amphipoda	*Jassa slatteryi*	EU243815	Pacific	-	1
Amphipoda	*Podocerus variegatus**	This study	La Palma#	El Faro	1
Amphipoda	*Podocerus variegatus**	This study	La Palma#	La Fajana	2
Amphipoda	*Podocerus variegatus**	This study	Porto Santo#	Porto Frades	1
Amphipoda	*Podocerus variegatus**	Chapter 2	Spain	Muxía	2
Amphipoda	*Podocerus variegatus**	Chapter 2	Spain	Barizo	2
Amphipoda	*Podocerus variegatus**	Chapter 2	Spain	Pedreira	1
Amphipoda	*Protohyale schmidtii* *	Chapter 5	Gran Canaria	Bañaderos	3
Amphipoda	*Protohyale schmidtii* *	Chapter 5	La Palma	El Faro	1
Amphipoda	*Protohyale schmidtii* *	Chapter 5	La Palma	La Salemera	1
Amphipoda	*Protohyale schmidtii* *	Chapter 5	La Palma	La Fajana	1
Amphipoda	*Protohyale schmidtii* *	Chapter 5	Madeira	Ponta Cruz	1
Amphipoda	*Protohyale schmidtii* *	Chapter 5	Morocco	Akhfenir	2
Amphipoda	*Protohyale schmidtii* *	Chapter 5	Morocco	Tarfaya	1
Amphipoda	*Protohyale schmidtii* *	Chapter 5	Porto Santo	Porto Frades	1
Amphipoda	*Protohyale schmidtii* *	Chapter 5	Portugal	Arrifes	4
Amphipoda	*Protohyale schmidtii* *	Chapter 5	Portugal	Buarcos	1
Amphipoda	*Protohyale schmidtii* *	Chapter 5	Portugal	Peniche	1
Amphipoda	*Protohyale schmidtii* *	Chapter 5	Santa Maria	Praia Formosa	2
Amphipoda	*Protohyale schmidtii* *	Chapter 5	Santa Maria	São Lourenço	2
Amphipoda	*Protohyale schmidtii* *	Chapter 5	São Miguel	Ribeira Chã	1
Amphipoda	*Protohyale schmidtii* *	Chapter 5	São Miguel	Mosteiros	1
Amphipoda	*Protohyale schmidtii* *	Chapter 5	El Hierro	Los Sargos	1
Amphipoda	*Protohyale schmidtii* *	Chapter 5	Spain	Muxía	2
Amphipoda	*Protohyale schmidtii* *	Chapter 5	Spain	Barizo	3
Amphipoda	*Protohyale schmidtii* *	Chapter 5	Spain	Pedreira	2
Amphipoda	*Protohyale schmidtii* *	Chapter 5	Tenerife	Mal Paso	2
Amphipoda	*Protohyale schmidtii* *	Chapter 5	Tenerife	Los Cristianos	1
Amphipoda	*Quadrimaera inaequipes* *	KX224085, KX224086, KX224087, KX224089, KX224090, KX224091, KF369148, KF369148	Portugal	-	8
Amphipoda	*Quadrimaera inaequipes* *	This study	Gran Canaria#	Bañaderos	1
Amphipoda	*Quadrimaera inaequipes* *	This study	La Palma#	El Faro	1
Amphipoda	*Quadrimaera inaequipes* *	This study	La Palma#	La Salemera	1
Amphipoda	*Quadrimaera inaequipes* *	This study	Madeira#	Ponta Cruz	3
Amphipoda	*Quadrimaera inaequipes* *	This study	La Palma#	La Fajana	1
Amphipoda	*Serejohyale spinidactylus**	Chapter 5	El Hierro	Los Sargos	1
Amphipoda	*Serejohyale spinidactylus**	Chapter 5	Gran Canaria	Bañaderos	3
Amphipoda	*Serejohyale spinidactylus**	Chapter 5	Gran Canaria	Agaete	1
Amphipoda	*Serejohyale spinidactylus**	Chapter 5	Gran Canaria	Playa Melenara	2
Amphipoda	*Serejohyale spinidactylus**	Chapter 5	La Palma	La Salemera	4

Order	Species	Source/GenBank accession	Country/Island	Location	n
Amphipoda	*Serejohyale spinidactylus**	Chapter 5	La Palma	La Fajana	2
Amphipoda	*Serejohyale spinidactylus**	Chapter 5	Selvagens	Selvagem Grande	1
Amphipoda	*Serejohyale spinidactylus**	Chapter 5	Madeira	Ponta Cruz	1
Amphipoda	*Serejohyale spinidactylus**	Chapter 5	Madeira	Reis Magos	2
Amphipoda	*Serejohyale spinidactylus**	Chapter 5	São Miguel	Mosteiros	1
Amphipoda	*Serejohyale spinidactylus**	Chapter 5	São Miguel	Ponta Ferreirinha	2
Amphipoda	*Serejohyale spinidactylus**	Chapter 5	Spain	Muxía	3
Amphipoda	*Serejohyale spinidactylus**	Chapter 5	Spain	Barizo	2
Amphipoda	*Stenothoe marina*	KT209198	North Sea	-	1
Amphipoda	*Stenothoe monoculoides**	KT208458, KT209192, KT208446, KT209271	North Sea	-	4
Amphipoda	*Stenothoe monoculoides**	This study	Tenerife#	Mal Paso	3
Isopoda	*Anthura gracilis **	This study	Tenerife#	Los Cristianos	1
Isopoda	*Anthura gracilis **	This study	Porto Santo#	Porto Frades	1
Isopoda	*Anthura gracilis **	This study	Selvagens#	Selvagem Grande	1
Isopoda	*Anthura gracilis **	This study	Gran Canaria#	Agaete	1
Isopoda	*Anthura gracilis **	Chapter 2	Spain	Barizo	1
Isopoda	*Anthura gracilis **	This study	Morocco#	Arzila	2
Isopoda	*Anthura gracilis **	This study	Terceira	Porto Martins	1
Isopoda	*Anthura gracilis **	Chapter 2	Portugal	Viana Castelo	2
Isopoda	*Anthura gracilis **	This study	São Miguel	Ribeira Chã	3
Isopoda	*Anthura gracilis **	This study	La Palma#	La Fajana	1
Isopoda	*Campecopea hirsuta*	Chapter 2	Portugal	Ingrina	1
Isopoda	*Campecopea lusitanica **	This study	Porto Santo#	Porto frades	2
Isopoda	*Campecopea lusitanica **	This study	Gran Canaria#	Bañaderos	1
Isopoda	*Campecopea lusitanica **	Chapter 2	Portugal	Peniche	1
Isopoda	*Campecopea lusitanica **	This study	La Palma#	El Faro	1
Isopoda	*Campecopea lusitanica **	Chapter 2	Spain	Pedreira	3
Isopoda	*Campecopea lusitanica **	This study	La Palma#	La Fajana	1
Isopoda	*Cyathura carinata*	Chapter 2	Portugal	Viana Castelo	1
Isopoda	*Cymodoce truncata **	This study	Porto Santo#	Porto Frades	2
Isopoda	*Cymodoce truncata **	Chapter 2	Spain	Muxia	1
Isopoda	*Cymodoce truncata **	This study	Portugal	Vale dos Homens	1
Isopoda	*Cymodoce truncata **	Chapter 2	Portugal	Peniche	3
Isopoda	*Cymodoce truncata **	This study	Madeira#	Ponta Cruz	1
Isopoda	*Cymodoce truncata **	This study	Terceira	Porto Martins	1
Isopoda	*Cymodoce truncata **	This study	La Palma#	La Fajana	2
Isopoda	*Dynamene bidentata **	Chapter 4	Gran Canaria	Bañaderos	1
Isopoda	*Dynamene bidentata **	Chapter 4	Morocco	El Jadida	1
Isopoda	*Dynamene bidentata **	Chapter 4	Morocco	Essaouire	1
Isopoda	*Dynamene bidentata **	Chapter 4	Portugal	Buarcos	3
Isopoda	*Dynamene bidentata **	Chapter 4	Portugal	Viana Castelo	14
Isopoda	*Dynamene bidentata **	Chapter 4	Portugal	Sines	2
Isopoda	*Dynamene bidentata **	Chapter 4	Spain	Pedreira	5
Isopoda	*Dynamene bidentata **	Chapter 4	Spain	Barizo	3
Isopoda	*Dynamene edwardsi **	Chapter 4	El Hierro	Arenas Blancas	3

Order	Species	Source/GenBank accession	Country/Island	Location	n
Isopoda	*Dynamene edwardsi* *	Chapter 4	El Hierro	Los Sargos	3
Isopoda	*Dynamene edwardsi* *	Chapter 4	Gran Canaria	Bañaderos	6
Isopoda	*Dynamene edwardsi* *	Chapter 4	Gran Canaria	Agaete	6
Isopoda	*Dynamene edwardsi* *	Chapter 4	Gran Canaria	Playa Melenara	5
Isopoda	*Dynamene edwardsi* *	Chapter 4	La Palma	El Faro	6
Isopoda	*Dynamene edwardsi* *	Chapter 4	La Palma	La Salemera	5
Isopoda	*Dynamene edwardsi* *	Chapter 4	La Palma	La Fajana	5
Isopoda	*Dynamene edwardsi* *	Chapter 4	Madeira	Ponta Cruz	5
Isopoda	*Dynamene edwardsi* *	Chapter 4	Madeira	Reis Magos	5
Isopoda	*Dynamene edwardsi* *	Chapter 4	Morocco	El Jadida	1
Isopoda	*Dynamene edwardsi* *	Chapter 4	Morocco	Arzila	4
Isopoda	*Dynamene edwardsi* *	Chapter 4	Morocco	Tarfaya	4
Isopoda	*Dynamene edwardsi* *	Chapter 4	Porto Santo	Porto Frades	5
Isopoda	*Dynamene edwardsi* *	Chapter 4	Portugal	Ingrina	5
Isopoda	*Dynamene edwardsi* *	Chapter 4	Portugal	Arrifes	4
Isopoda	*Dynamene edwardsi* *	Chapter 4	Portugal	Dona Ana	5
Isopoda	*Dynamene edwardsi* *	Chapter 4	Portugal	Peniche	4
Isopoda	*Dynamene edwardsi* *	Chapter 4	Portugal	Sines	1
Isopoda	*Dynamene edwardsi* *	Chapter 4	São Miguel	Mosteiros	1
Isopoda	*Dynamene edwardsi* *	Chapter 4	Selvagens	Selvagem Pequena	2
Isopoda	*Dynamene edwardsi* *	Chapter 4	Selvagens	Selvagem Grande	4
Isopoda	*Dynamene edwardsi* *	Chapter 4	Spain	Muxia	1
Isopoda	*Dynamene edwardsi* *	Chapter 4	Tenerife	Los Cristianos	4
Isopoda	*Dynamene edwardsi* *	Chapter 4	Tenerife	Mal Paso	5
Isopoda	*Dynamene magnitorata* *	Chapter 4	La Palma	La Fajana	1
Isopoda	*Dynamene magnitorata* *	Chapter 4	Portugal	Arrifes	5
Isopoda	*Dynamene magnitorata* *	Chapter 4	Portugal	Dona Ana	3
Isopoda	*Dynamene magnitorata* *	Chapter 4	Portugal	Buarcos	3
Isopoda	*Dynamene magnitorata* *	Chapter 4	Portugal	Peniche	5
Isopoda	*Dynamene magnitorata* *	Chapter 4	Portugal	Viana Castelo	2
Isopoda	*Dynamene magnitorata* *	Chapter 4	Santa Maria	La Fajana	1
Isopoda	*Dynamene magnitorata* *	Chapter 4	Santa Maria	Praia Formosa	2
Isopoda	*Dynamene magnitorata* *	Chapter 4	São Miguel	Ribeira Chã	6
Isopoda	*Dynamene magnitorata* *	Chapter 4	Spain	Pedreira	1
Isopoda	*Dynamene magnitorata* *	Chapter 4	Spain	Muxía	5
Isopoda	*Dynamene magnitorata* *	Chapter 4	Spain	Barizo	2
Isopoda	*Dynamene magnitorata* *	Chapter 4	Terceira	Cinco Ribeiras	1
Isopoda	*Dynamene magnitorata* *	Chapter 4	Terceira	Porto Martins	2
Isopoda	*Gnathia maxillaris* *	This study	Gran Canaria[#]	Agaete	2
Isopoda	*Gnathia maxillaris* *	This study	La Palma[#]	El Faro	1
Isopoda	*Gnathia maxillaris* *	This study	La Palma[#]	La Fajana	2
Isopoda	*Gnathia maxillaris* *	This study	Porto Santo[#]	Porto Frades	1
Isopoda	*Gnathia maxillaris* *	Chapter 2	Portugal	Ingrina	1
Isopoda	*Gnathia maxillaris* *	This study	Portugal	Buarcos	2
Isopoda	*Gnathia maxillaris* *	Chapter 2	Spain	Pedreira	1

Order	Species	Source/GenBank accession	Country/Island	Location	n
Isopoda	*Janira maculosa* *	Chapter 2	Portugal	Dona Ana	2
Isopoda	*Janira maculosa* *	Chapter 2	Spain	Muxia	1
Isopoda	*Janira maculosa* *	This study	La Palma#	La Salemera	2
Isopoda	*Janira maculosa* *	This study	La Palma#	La Fajana	1
Isopoda	*Joeropsis brevicornis* *	This study	Tenerife#	Los Cristianos	2
Isopoda	*Joeropsis brevicornis* *	This study	Portugal	Dona Ana	2
Isopoda	*Joeropsis brevicornis* *	Chapter 2	Spain	Barizo	1
Isopoda	*Joeropsis brevicornis* *	This study	La Palma#	El Faro	2
Isopoda	*Joeropsis brevicornis* *	This study	Madeira#	Reis Magos	2
Tanaidacea	*Apseudes talpa*	JF927715	Portugal	-	1
Tanaidacea	*Apseudopsis latreillii* *	This study	Porto Santo#	Porto Frades	1
Tanaidacea	*Apseudopsis latreillii* *	This study	Portugal	Dona Ana	3
Tanaidacea	*Apseudopsis latreillii* *	This study	Gran Canaria#	Agaete	2
Tanaidacea	*Tanais dulongii* *	This study	La Palma#	El Faro	1
Tanaidacea	*Tanais dulongii* *	This study	La Palma#	La Salemera	2
Tanaidacea	*Tanais dulongii* *	This study	Madeira	Ponta Cruz	3
Tanaidacea	*Tanais dulongii* *	This study	Morocco#	El Jadida	1
Tanaidacea	*Tanais dulongii* *	This study	Morocco#	Arzila	1
Tanaidacea	*Tanais dulongii* *	HM422239, HM422240	Portugal	-	2
Tanaidacea	*Tanais dulongii* *	Chapter 2	Portugal	Ingrina	1
Tanaidacea	*Tanais dulongii* *	Chapter 2	Portugal	Peniche	1
Tanaidacea	*Tanais dulongii* *	Chapter 2	Portugal	Berlengas	1
Tanaidacea	*Tanais dulongii* *	Chapter 2	Spain	Barizo	1
Tanaidacea	*Tanais grimaldii* *	This study	Selvagens#	Selvagem Pequena	1
Tanaidacea	*Tanais grimaldii* *	This study	Porto Santo#	Porto Frades	2
Tanaidacea	*Tanais grimaldii* *	This study	Selvagens#	Selvagem Grande	2
Tanaidacea	*Tanais grimaldii* *	This study	Spain	Barizo	2
Tanaidacea	*Tanais grimaldii* *	This study	São Miguel	Ribeira chã	2
Tanaidacea	*Tanais sp1*	This study	Santa Maria	Praia Formosa	1
Tanaidacea	*Tanais sp2*	This study	Gran Canaria	Playa Melenara	1
Tanaidacea	*Tanais sp3*	This study	Selvagens	Selvagem Pequena	1
Tanaidacea	*Zeuxo exsargasso*	This study	Tenerife	Mal Paso	1
Tanaidacea	*Zeuxo exsargasso*	This study	Porto Santo#	Porto Frades	1

* Species used in the molecular species delineation.

\# New records obtained in this study.

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