

**DIVERSITY, DISTRIBUTION AND
PHYLOGENETIC RELATIONSHIPS
OF ROCK SPONGES (PORIFERA,
'LITHISTID' DEMOSPONGIAE) OF
THE MACARONESIAN ISLANDS
AND NORTHEAST ATLANTIC
SEAMOUNTS**

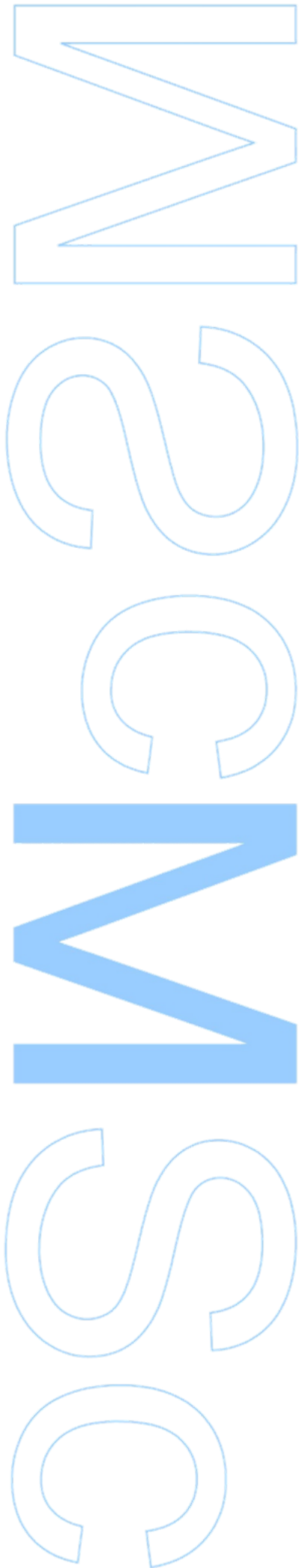
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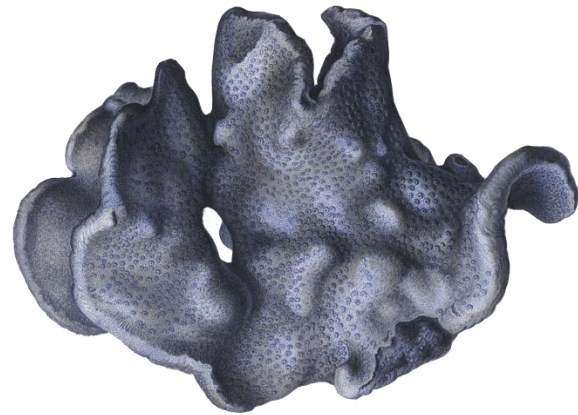
Dissertação de Mestrado apresentada à

Faculdade de Ciências da Universidade do Porto em

Biodiversidade, Genética e Evolução

2013





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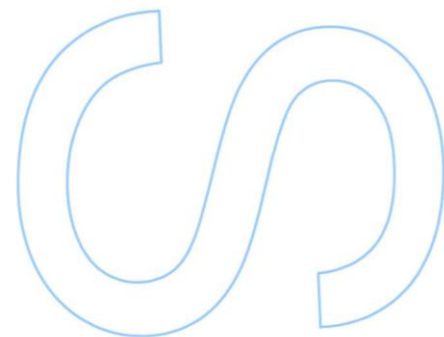
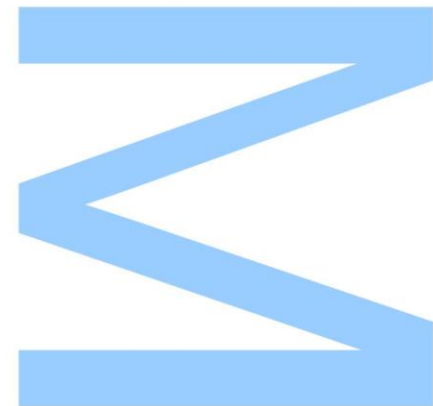
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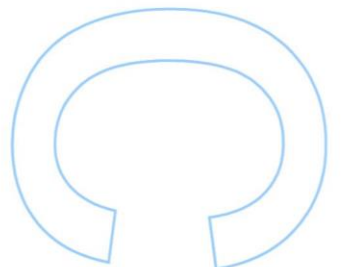
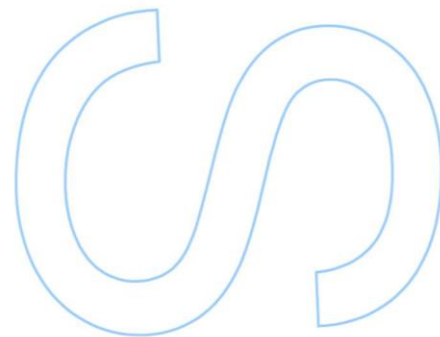
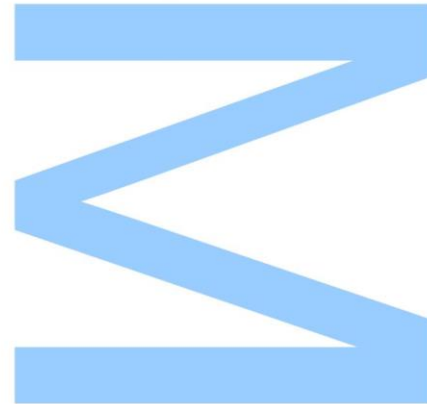
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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.
O Presidente do Júri,
Porto, ____/____/____



ACKNOWLEDGEMENTS

My work was only possible with the help of many people that during this period of time supported me in many ways.

I am deeply thankful to Joana Xavier, my supervisor. For making me believe. For all the hours spent sharing your knowledge, strength, support and friendship. For all the enthusiastic hours spent on the microscope. For all the forgiveness when I was late. For supporting me since the first day and make my confidence come back even when it seemed to be lost. It was an amazing experience to work and learn with you. Thank you so much!!

I also wish to thank James Harris for accepting to co-supervise me and dedicate so much of his time and patience to a very important part of this work. It wouldn't be possible without your support. Thank you for all the kindness and availability when I needed the most.

To the Spongelab team, a very special thanks. It was really an enriching experience to work with all of you. To Francisco Pires for being a friend, and a teacher at the lab. And for converting a simple boring lab room into a nice and cool place to work. To Raquel Pereira for the endless patience helping me doing the maps. And for the help whenever I needed. I am going to miss all these times.

I am also very thankful to Raquel Xavier for receiving me so well and helping me in the most difficult hours during the molecular work. Thank you for teaching me and for always clarifying my doubts. All the people of CIBIO's lab for being there, always available when I needed.

This work also wouldn't be possible without the help of Jorge Medeiros with the SEM microscopy. Thank you for all the hours spent in search of microscleres and for your great work. To Isadora Moniz for helping me to finish the huge amount of preparations that seemed to be endless!

To Joana Xavier once again, for providing me the chance to meet exceptional researchers and to participate in the "International Workshop on Taxonomy of Atlanto-Mediterranean Deep-Sea Sponge Fauna". It was an incredible and fascinating experience where I could learn more and more about sponge taxonomy. To Andrzej Pisera for sharing his expert knowledge with me. To Shirley Pomponi for providing so many interesting samples to complete my work and for all her kindness. It was very nice to meet and get to

work with you. To Paco Cárdenas for his help every time I needed during my thesis and to work with me.

To Dr. Filipe Porteiro for making the Azorean samples available for this study. To all the people, technicians and researchers who have done an incredible work on collecting samples and take a good care of them in the collections.

To Joana Vilela, Íris Sampaio and Susana Freitas. A huge thank you. For being more than just colleagues and partners. For the friendship, all the good conversations in beer's company. For being so kind, helpful and for preventing my "panic attacks" to be more serious. Thank you!

To my Mom and Dad. Always present in the most difficult moments. Always supporting - and sponsoring - my decisions and making my act of dreaming possible. For helping me whenever I needed and giving me wings since ever.

To my brothers and sisters for being themselves. Simply for being themselves. My life wouldn't be the same and I wouldn't be the same without you. My dreams have grown with you. They wouldn't be possible without your love, support and care. And the courage you all give me. Obrigada Susana, Bábá, Zé e Gui. :)

To my three grandmothers. Vó Zecas, Vó Glória e Zairinha. I can't tell how much your love fulfills me and makes me a better person each day. Your embrace and your smile every time I see you warms and deeply encourages me.

To my uncle Maria Rui, for passing me the enthusiasm for Biology and for always supporting my decisions.

To you, for making part of my life.

To all my friends and family. For making me so happy. It feels so good to have people like you on my side.

This thesis is dedicated to all of you.

Obrigada!!!

ABSTRACT

'Lithistid' Demospongiae *sensu* Pisera & Lévi, 2002 is a much understudied polyphyletic group of sponges usually found at bathyal or bathyal-like environments, such as caves, in tropical and warm temperate regions.

In this study we investigated the diversity, distribution and phylogenetic relationships of the lithistid sponge fauna of the northeast Atlantic with a focus on the Macaronesian Islands (Azores, Madeira, Selvagens and Canaries) and neighbouring seamounts (of the Great Meteor group). Specimens were collected in the course of several research campaigns conducted by the Azores University in the Azores EEZ, by the MNHN of Paris in the seamounts southern of the Azores (the Seamount-2 campaign) and by the Harbor Branch Oceanographic Institute (HBOI) in Madeira, Selvagens and Canary islands.

A total of 162 specimens were analyzed and assigned to 11 taxa representing five families and nine genera. Eight species were recorded for the first time on the seamounts southern of the Azores, of which seven are shared with this archipelago. Three species constitute new records for Madeira Island (*Neophrissospongia nolitangere*, *Discodermia verrucosa* and *Racodiscula clava*), two for Selvagens (*N. nolitangere* and *Macandrewia azorica*), and two for the Canaries, of which one is a new species for science of the genus *Isabella*. Of the 14 species found to occur in the Macaronesian islands, only three viz. *N. nolitangere*, *Leiodermatium pfeifferae* and *R. clava* are shared among all archipelagos, the remainder being restricted to one or two island groups. Similarly, only four species seem to be shared between the Mediterranean Sea and these Atlantic archipelagos: *N. nolitangere* (with the Azores, Madeira, Selvagens and the Canaries) *Neoschrammeniella bowerbankii* (with Madeira); *Siphonidium ramosum* (with the Azores) and *L. lynceus* (with the Azores and the Canaries). Furthermore, several changes of taxonomic significance were proposed based on the new findings such as re-definition of the genera *Neophrissospongia* and *Isabella*, and the species *N. bowerbankii*. A discussion concerning the validity of Topsent's *Racodiscula clava* is also presented.

Phylogenetic reconstructions, by means of Bayesian analyses, of the mtDNA COI and the rDNA 28S genes confirmed the polyphyly of this group. The astrophorid affinity of the families Corallistidae, Macandrewiidae and Theonellidae was shown. Species and genera were always retrieved as well-supported monophyletic clades. The COI analysis has revealed for the first time the monophyly of the family Corallistidae and a sister-taxa

relationship was found between the genera *Neoschrammeniela/Neophrissospongia* and *Herengeria/Isabella* within this family. The family Theonellidae (*Discodermia*, *Theonella* and *Racodiscula*) was revealed non-monophyletic in both analysis. Lastly the family Azoricidae, here represented by *Leiodermatium*, exhibits a basal position in relation to the astrophorid/lithistid clade, but its phylogenetic affinity remains unclear.

This study greatly expands the knowledge on the diversity, distribution and phylogenetic relationships of the lithistid sponges associated with the bathyal environment of the Macaronesian Islands and the northeast Atlantic seamounts.

Keywords: Lithistids, Macaronesian Islands, seamounts, diversity, distribution, phylogenetic analyses.

RESUMO

'Lithistid' Demospongiae *sensu* Pisera & Lévi, 2002 é um grupo polifilético de esponjas muito pouco estudado que normalmente se encontra na zona batial ou em ambientes semelhantes, como grutas, em regiões tropicais e temperadas quentes.

Neste estudo investigou-se a diversidade, distribuição e relações filogenéticas da fauna de esponjas lithistidas do nordeste Atlântico com foco nas ilhas da Macaronésia (Açores, Madeira, Selvagens e Canárias) e nas montanhas submarinas adjacentes (do grupo do Great Meteor). Os exemplares foram recolhidos no decurso de várias expedições científicas realizadas pela Universidade dos Açores na ZEE dos Açores, pelo MNHN de Paris nas montanhas submarinas localizadas a sul dos Açores (a campanha Seamount-2) e pelo Harbor Branch Oceanographic Institute (HBOI) nas ilhas da Madeira, Selvagens e Canárias.

Um total de 162 espécimes foram analisados, tendo sido identificados 11 taxa representantes de cinco famílias e nove géneros. Pela primeira vez, foram registadas oito espécies de lithistidas nas montanhas submarinas a sul dos Açores, das quais sete são partilhadas com este arquipélago. Na Madeira, foram encontrados três novos registos (*Neophrissospongia nolitangere*, *Discodermia verrucosa* e *Racodiscula clava*), dois nas Selvagens (*N. nolitangere* and *Macandrewia azorica*) e dois nas Canárias, dos quais um representa uma espécie nova para a ciência do género *Isabella*. Das 14 espécies registadas para as ilhas da Macaronésia, apenas três são partilhadas entre todos os arquipélagos, *N. nolitangere*, *Leiodermatium pfeifferae* e *R. clava*, sendo as restantes espécies restritas a um ou dois grupos de ilhas. Similarmente, apenas quatro espécies parecem ser partilhadas entre o mar Mediterrâneo e estes arquipélagos do Atlântico: *N. nolitangere* (com os Açores, Madeira, Selvagens e Canárias), *Neoschrammeniella bowerbankii* (com a Madeira); *Siphonidium ramosum* (com os Açores) e *L. lynceus* (com os Açores e as Canárias). Para além disso, várias alterações de significância taxonómica foram aqui propostas, com base nos resultados obtidos, tais como a re-definição dos géneros *Neophrissospongia* e *Isabella*, e da espécie *Neoschrammeniella bowerbankii*. A validade da espécie *Racodiscula clava* descrita por Topsent, também é aqui discutida.

Reconstruções filogenéticas, por métodos de inferência Bayesiana, dos genes mtDNA COI e do rDNA 28S confirmam a polifilia deste grupo. A afinidade das famílias Corallistidae, Macandrewiidae e Theonellidae com as astrophoridas foi verificada. Espécies

e géneros foram sempre obtidos em clados monofiléticos bem suportados. Na análise do COI verificou-se, pela primeira vez, a monofilia da família Corallistidae e foi encontrada uma relação de grupo-irmão entre os géneros *Neoschrammeniella/Neophrissospongia* e *Herengeria/Isabella*. A família Theonellidae (*Discodermia*, *Theonella* e *Racodiscula*) revelou-se não monofilética em ambas as análises. Por último, a família Azorecidae, representada nas análises pelo género *Leiodermatium*, demonstra uma posição basal em relação ao clade astrophoridas/lithistidas, mas as suas afinidades filogenéticas continuam por esclarecer.

Este estudo expande significativamente o nosso conhecimento da diversidade, distribuição e relações filogenéticas das esponjas lithistidas associadas ao ambiente batial das ilhas da Macaronésia e das montanhas submarinas do nordeste Atlântico.

Palavras chave: Lithistidas, Ilhas da Macaronésia, montanhas submarinas, diversidade, distribuição, análise filogenética.

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LIST OF ACRONYMS AND ABBREVIATIONS

| | |
|-------------------------|--|
| AIC | Akaike Information Criterion |
| CaCO₃ | Calcium carbonate |
| COI | Mitochondrial cytochrome c oxidase subunit I |
| Coleta | Sponges' collection of the Department of Oceanography and Fisheries of Azores University, Portugal |
| DB/Uaç | Biology Department of Azores University, Ponta Delgada, Portugal |
| DBUA.Por | Sponges' collection of the Biology Department of Azores University, Portugal |
| DNA | Deoxyribonucleic acid |
| DOP/Uaç | Department of Oceanography and Fisheries of Azores University, Horta, Portugal |
| HBOI | Harbour Branch Oceanographic Institute, Florida, USA |
| HNO₃ | Nitric acid |
| MNHN | Muséum National d'Histoire Naturelle, Paris, France |
| OM | Optical Microscopy |
| PCR | Polymerase Chain Reaction |
| PorTOL | Porifera Tree of Life Project |
| ROV | Remotely Operated Vehicle |
| SEM | Scanning electron microscopy |
| SGS | Sponge GeneTree Server |
| SiO₂ | Silica |
| SPD | Sponge Barcoding Project |
| WoRMS | World Register of Marine Species |
| WPD | World Porifera Database |
| 28S rDNA | 28S ribosomal DNA nuclear gene |

CHAPTER 1. INTRODUCTION

1.1. PHYLUM PORIFERA

The phylum Porifera (from Latin *porus*= pore and *ferō* = bearer) comprises a diverse and ecologically important group of filter-feeding invertebrates (van Soest *et al.*, 2012). Thought to exist since the late Neoproterozoic (635 Myr ago), they are regarded amongst the most primitive extant animal groups (Love *et al.*, 2009). Sponges inhabit a variety of environments from the poles to the equator and from shallow to deep-waters. They are mostly marine although a group of species also occurs in freshwater. They also present a variety of shapes and sizes, from thinly incrusting to large cup-shapes as a consequence of their high adaptation capacity (van Soest *et al.*, 2012).

Sponges are sessile metazoans and efficient filter-feeders that exhibit a simple body organization lacking true tissues or organs. The body organization is divided into three distinct layers, pinacoderm, mesohyl and choanoderm. The first one is the "skin" of the sponge and is coated by flattened cells, the pinacocytes, that also covers the surface of the internal canal system; the mesohyl possesses a matrix of collagen/spongin fibres, and inorganic skeleton (spicules) composed by silica (SiO₂) or calcium carbonate (CaCO₃) that confer firmness to the sponge; other specialized cells such as amebocytes and archaeocytes are also present in this layer; lastly, the choanoderm is composed by a layer of flagellated cells, the choanocytes that are responsible for the uni-directional water flow in the interior of the sponge allowing its filtration (e.g. Hooper *et al.*, 2002a; Hajdu *et al.*, 2011; van Soest *et al.*, 2012). Sponges reproduce asexually by fragmentation and budding for example (Fell, 1993). Regarding sexual condition, sponges can be either gonochoristic or hermaphroditic. With respect to reproductive condition, sponges can be either oviparous, releasing the gametes into the water column; or viviparous, by brooding the embryos until their release as larvae (Maldonado & Riesgo, 2008; Riesgo *et al.*, 2013). There are nine types of sponge larvae (reviewed in Maldonado & Bergquist, 2002), being the parenchymella the most common one within the class Demospongiae. This type of larva is lecithotrophic and its dispersal may take from a few hours to several days before attachment, depending on the species. Upon attachment the larva undergoes a metamorphosis and becomes a juvenile sponge (Maldonado *et al.*, 2003).

Sponges play key-roles in the ecosystems. As filter-feeders, they are important in the benthic-pelagic coupling by either directly removing nutrients from the environment (e.g. Yahel *et al.*, 2003; Goeij *et al.*, 2008) or through the symbiotic microorganisms (Radax *et*

al., 2011; Schläppy, *et al.*, 2010); they are also responsible for the elimination of some viruses (Hadas *et al.*, 2006). Furthermore, sponges are major players in substrate stabilisation, consolidation and regeneration, bioerosion, reef formation, oxygen depletion, and provide shelter for other animals (reviewed in Wulff, 2001; Bell, 2008).

Sponges have been known since Greek antiquity to bring benefits for men such as in cleansing and bathing (still used today), medicine, pharmacology and also as a household object (Voultsiadou, 2007). Nowadays, sponges have a leading role in Blue Biotechnology due to their high potential in the production of marine natural products with antiviral, antibiotic, anticancer, antitumor and fungicidal properties (e.g. Pomponi, 2006; Grasela *et al.*, 2012). These bioactive compounds are thought to be produced by sponges as a chemical defense against predators, as antifouling or for spatial competition purposes (e.g. Becerro *et al.*, 1997, 2003).

Sponges were recognized as a Phylum for the first time by Grant, in 1836 but questions regarding the evolutionary history and phylogenetic relationships of the group perdure to this very day. The taxonomy and systematics of sponges is primarily based on external (shape, colour, texture) and internal morphological characters (mainly on type and nature of skeletal structures) (Hooper *et al.*, 2002a). However high levels of spicule homoplasy have been found (e.g. Manuel *et al.*, 2003; Cárdenas *et al.*, 2011), rendering sponge classification based on morphological characters alone problematic and, to some extent, unreliable. In 2002 a group of authors got together to establish and summarize the classification of sponges based on classic taxonomy – the *Systema Porifera* (Hooper & van Soest, 2002). An online database, the World Porifera Database (WPD, <http://www.marinespecies.org/porifera>), was also created with the aim of listing all sponge species and their distribution, as well as reference taxonomic literature. Information concerning the taxonomic status (e.g. valid or not valid) of sponge *taxa* is also given.

For the past two decades, the development of molecular tools was shown to be an important resource in the clarification of some questions concerning the classification and evolution of sponges, including: the mono- vs. paraphyletic status of the phylum (Philippe, 2009); the resurrection of the class Homoscleromorpha (Gazave *et al.*, 2012); phylogenetic relationships within the group at various taxonomic levels (e.g. Cárdenas *et al.*, 2011; Morrow *et al.*, 2012; Redmond *et al.*, 2013); and the detection of several cases of cryptic speciation (e.g. Blanquer & Uriz, 2007; Xavier *et al.*, 2010). International initiatives such as

the Sponge Barcoding Project (SPD, <http://www.spongebarcoding.org>), the Sponge GeneTree Server (SGS, <http://www.spongegenetrees.org>) and the Porifera Tree of Life Project (PorTOL, <https://www.portol.org>) were created with the goals of making the identifications of sponges easier (through molecular barcodes), to improve the estimate of phylogenetic relationships at various taxonomic levels, and to improve knowledge regarding the evolutionary history of the phylum.

The phylum Porifera comprises four extant classes (Homoscleromorpha Bergquist, 1978, Demospongiae Sollas, 1885, Calcarea Bowerbank, 1862 and Hexactinellida Schmidt, 1870) and one extinct (Archaeocyatha Bornemann, 1884), 25 orders, 128 families, 680 genera and a total of 8553 currently accepted species (WPD, van Soest *et al.*, 2012) but this number is estimated to represent just half of the true diversity of the phylum (van Soest *et al.*, 2012).

1.2. LITHISTID SPONGES

Within Porifera, the class Demospongiae is by far the largest and most diverse, accounting for approximately 85% (*i.e.* 7000+) of all described species. This class is characterized by the possession of siliceous spicules and/or a fibrous skeleton (Hooper & van Soest, 2002b). Within this class are the "Lithistid" Demospongiae *sensu* Pisera & Lévi, 2002a, a group of species characterized by the shared possession of a hypersilified skeleton composed of articulated spicules (desmas). These interlocking spicules often give a very firm or rock-hard consistency to the sponges, the reason for which the lithistids are also known as rock sponges (Kelly-Borges & Pomponi, 1994; Pisera & Lévi, 2002a).

Lithistid sponges were formerly considered a group (Order Lithistida Schmidt, 1870), which included all desma-bearing sponges. In 1888, Sollas established the first clear classification of lithistids based on ectosomal (surface) spicules and microscleres (small spicules) instead of shape of desmas. Two suborders were created: Hoplophora, which contained ectosomal spicules and microscleres, and Anoplia, characterized by the absence of spicules. For Sollas (1888), desmas represented a single evolutionary event, and therefore Lithistida was a monophyletic group. However a substantial body of mostly morphological and some molecular evidence has revealed the polyphyletic nature of lithistids and the astrophorid, halichondrid and spirophorid affinities of several of its families

(e.g. Kelly-Borges & Pomponi, 1994; McInerney *et al.*, 1999; Cárdenas *et al.*, 2011; Morrow *et al.*, 2012; Redmond *et al.*, 2013). However, for practical purposes, lithistids were kept together. Globally, 14 families, 51 genera and 198 extant species are currently accepted (van Soest *et al.*, 2012; Tab. I), many of which are thought to be relicts of a much more diverse and abundant fauna occurring in the Mesozoic (Reid, 1967; Lévi, 1991). Given the high fossilization potential of the desmas, lithistids are, of all sponges, the best represented ones in the fossil record (Finks, 1970; Rigby, 1991; Pisera, 2002) and are thus considered a key group for the understanding of the evolutionary history of the phylum (Kelly-Borges & Pomponi, 1994).

Tab. I - Current classification and diversity account of 'Lithistid' Demospongiae (Source: Pisera & Lévi, 2002 and WPD, van Soest *et al.*, 2012)

| Family | Genus | Nº of species |
|----------------------------------|---|---------------|
| AZORICIDAE Sollas, 1888 | <i>Desmascula</i> de Laubenfels, 1950 | 1 |
| | <i>Jereicopsis</i> Lévi & Lévi, 1983 | 1 |
| | <i>Leiodermatium</i> Schmidt, 1870 | 11 |
| CORALLISTIDAE Sollas, 1888 | <i>Awhiowhio</i> Kelly, 2007 | 3 |
| | <i>Corallistes</i> Schmidt, 1870 | 14 |
| | <i>Herengeria</i> Lévi & Lévi, 1988 | 2 |
| | <i>Isabella</i> Schlacher-Hoenlinger, Pisera & Hooper, 2005 | 1 |
| | <i>Neophrissospongia</i> Pisera & Lévi, 2002 | 6 |
| | <i>Neoschrammeniella</i> Pisera & Lévi, 2002 | 6 |
| DESMANTHIDAE Topsent, 1894 | <i>Desmanthus</i> Topsent, 1894 | 5 |
| | <i>Paradesmanthus</i> Pisera & Lévi, 2002 | 1 |
| | <i>Petromica</i> Topsent, 1898 | 8 |
| | <i>Sulcastrella</i> Schmidt, 1879 | 3 |
| ISORAPHINIIDAE Schrammen, 1924 | <i>Costifer</i> Wilson, 1925 | 2 |
| MACANDREWIIDAE Schrammen, 1924 | <i>Macandrewia</i> Gray, 1859 | 7 |
| NEOPELTIDAE Sollas, 1888 | <i>Callipelta</i> Sollas, 1888 | 6 |
| | <i>Daedalopelta</i> Sollas, 1888 | 1 |
| | <i>Homophymia</i> Vaclet & Vasseur, 1971 | 3 |
| | <i>Neopelta</i> Schmidt, 1880 | 5 |
| PHYMARAPHINIIDAE Schrammen, 1924 | <i>Exsuperantia</i> Özdikmen, 2009 | 1 |
| | <i>Kaliapsis</i> Bowerbank, 1869 | 3 |
| | <i>Lepidothenea</i> de Laubenfels, 1936 | 1 |
| PHYMATELLIDAE Schrammen, 1910 | <i>Neoaulaxinia</i> Pisera & Lévi, 2002 | 3 |
| | <i>Neosiphonia</i> Sollas, 1888 | 4 |
| | <i>Reidispongia</i> Lévi & Lévi, 1988 | 2 |
| PLEROMIDAE Sollas, 1888 | <i>Anaderma</i> Lévi & Lévi, 1983 | 1 |

| | | |
|---------------------------------------|--|-------------------------------------|
| | <i>Pleroma</i> Sollas, 1888 | 4 |
| SCLERITODERMIDAE Sollas, 1888 | <i>Aciculites</i> Schmidt, 1879 | 12 |
| | <i>Amphibleptula</i> Schmidt, 1879 | 1 |
| | <i>Microscleroderma</i> Kirkpatrick, 1903a | 7 |
| | <i>Pomelia</i> Zittel, 1878 | 1 |
| | <i>Scleritoderma</i> Schmidt, 1879 | 4 |
| | <i>Setidium</i> Schmidt, 1879 | 1 |
| | SIPHONIDIIDAE Lendenfeld, 1903 | <i>Gastrophanella</i> Schmidt, 1879 |
| <i>Lithobactrum</i> Kirkpatrick, 1903 | | 1 |
| <i>Siphonidium</i> Schmidt, 1879 | | 5 |
| THEONELLIDAE Lendenfeld, 1903 | <i>Discodermia</i> Du Bocage, 1869 | 29 |
| | <i>Manihinea</i> Pulitzer-Finali, 1993 | 2 |
| | <i>Racodiscula</i> Zittel, 1878 | 5 |
| | <i>Siliquariaspongia</i> Hoshino, 1981 | 1 |
| | <i>Theonella</i> Gray, 1868 | 14 |
| VETULINIDAE Lendenfeld, 1903 | <i>Vetulina</i> Schmidt, 1879 | 1 |
| LITHISTIDA <i>incertae sedis</i> | <i>Arabescula</i> Carter, 1873 | 1 |
| | <i>Collectella</i> Schmidt, 1870 | 1 |
| | <i>Plakidium</i> Lendenfeld, 1907 | 1 |

Lithistids are known to produce a diverse array of structurally complex natural products of great interest for biotechnology companies. In fact, to date more than 300 different compounds with antimicrobial (Matsunaga *et al.*, 2001), antitumor (Sun & Sakemi, 1991), antifungal (Gunaskera *et al.*, 1991), immunosuppressive (Longley *et al.*, 1991) and anticancer (Gulavita *et al.*, 1992; Haar *et al.*, 1996) properties have been isolated from lithistids (reviewed in Winder *et al.*, 2011).

Most lithistid species are found at bathyal or bathyal-like environments, such as caves, in tropical and warm temperate regions (e.g. Pomponi *et al.*, 2001; Pisera & Vacelet, 2011). Diversity and distribution patterns of the lithistid sponge fauna are largely understudied, with the exception of some regions such as the continental shelf and slope of the tropical western Atlantic (e.g. Schmidt, 1870, 1880; van Soest & Stentoft 1988; Pomponi *et al.*, 2001), the southwest Pacific archipelagos of New Caledonia, New Zealand and the seamounts of the Norfolk Ridge (e.g. Lévi & Lévi, 1983, 1988; Lévi, 1991; Schlacher-Hoenlinger *et al.*, 2005; Kelly *et al.*, 2007), and the Mediterranean Sea (e.g. Manconi & Serusi, 2008; Pisera & Vacelet, 2011).

In the Northeast Atlantic, knowledge of the lithistid sponge fauna is mostly restricted to the Azores archipelago for which 10 species belonging to seven genera and seven families were reported in the course of several expeditions (e.g. Topsent 1892a, 1904b, 1928). In fact, previous to the present study, only five lithistid species, viz. *Neophrissospongia nolitangere* (Schmidt, 1870), *Discodermia verrucosa* Topsent, 1928, *Leiodermatium lynceus* (Schmidt, 1870), *Macandrewia azorica* Gray, 1859 and *Racodiscula clava sensu* Topsent, 1892a were known to occur in the Canary Islands; and only three species, *Corallistes masoni* (Bowerbank, 1869), *Neoschrammeniella bowerbankii* (Johnson, 1863) and *Leiodermatium pfeifferae* (Carter, 1876) were reported to Madeira.

1.3. GOALS

The main aims of this thesis were: 1) to characterize the diversity and distribution of the lithistid sponge fauna of the Macaronesian islands (Azores, Madeira, Selvagens and Canaries) and the seamounts located southern of the Azores (Great Meteor group); and 2) to investigate the phylogenetic relationships of the identified species and the affinities of their families.

CHAPTER 2. MATERIAL AND METHODS

2.1. SAMPLES AND STUDY AREA

The lithistid sponges used in this work were collected in the course of several research expeditions conducted by various research institutes along the Macaronesian archipelagos of the Azores, Madeira, Selvagens and Canaries as well as on the seamounts located southern of the Azores (Fig. 1). These expeditions were conducted by: the Biology and Oceanography and Fisheries Departments of the Azores University (DB and DOP) between 2006 and 2011 to the Azores archipelago; the Harbor Branch Oceanographic Institute to Madeira, Selvagens and Canaries in 1991 aboard the RV Seward Johnson and employing the Johnson.Sea-Link I submersible; and the MNHN of Paris to the seamounts southern of the Azores (the Seamount 2 expedition) in 1993 aboard the NO Cryos. Specimens were collected by dredge, trawl, and submersible or as bycatch of longline fishing gear. Most specimens were fixed and preserved in ethanol (70%, 90%, 96%), however the specimens from the Seamount-2 expedition were fixed in formalin. Sampling information regarding the examined material (location, coordinates, sampling method and depth) is presented in Appendix I.

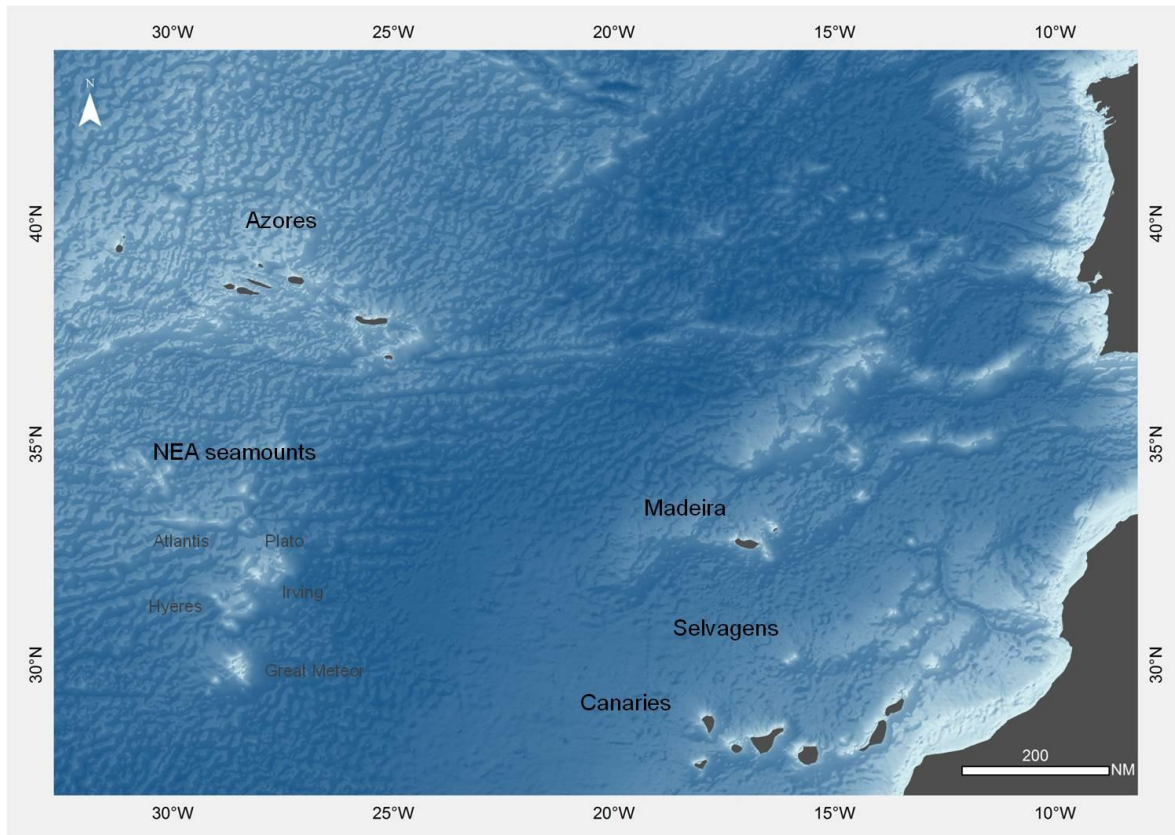


Fig. 1 - Study area: the Macaronesian archipelagos of the Azores, Madeira, Selvagens and Canaries, and the northeast Atlantic seamounts located southern of the Azores.

2.2. TAXONOMY

Taxonomic and distribution data on lithistid sponges of the entire northeast Atlantic (including the Macaronesian islands) and the Mediterranean Sea were extracted from the literature.

Identifications were made from the analysis of external and internal morphological characteristics following the *Systema Porifera* classification system (Hooper & van Soest, 2002) and some later references (e.g. Schlacher-Hoenlinger, *et al.* 2005; Pisera & Vacelet, 2011). Original descriptions of all lithistid species reported for the study area were also consulted. Permanent slides of the skeleton and spicules were made for all specimens for observation under optical microscopy (OM). Skeleton slides were prepared by making thick cross and tangential tissue sections that were mounted in Canada balsam. For spicules preparations, small tissue portions were digested in bleach during 24 hours (or 48h for some

specimens), and then the bleach was removed by washing several times with distilled water and ethanol. Some drops of the spicules' suspensions were placed on microscopy slides and then dried and fixed with Canada balsam.

Eight specimens were also prepared for observation under a scanning electron microscopy (SEM). Small portions of sponge tissue were digested in hot nitric acid (HNO₃). When all organic matter was digested, nitric acid was removed by a washing series with distilled water and ethanol. The suspension of spicules was set on the stub surface, dried and covered with gold-palladium for observations under a JEOL 5410 scanning electron microscope. A total of 162 specimens were analysed and identified to the lowest taxonomic rank possible. Small fragments of all analysed specimens, as well as spicules and skeleton preparations, are deposited in the sponge collection of the Biology Department of the University of the Azores (DBUA.Por).

2.3. PHYLOGENETIC RELATIONSHIPS

2.3.1. DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

In order to investigate the phylogenetic relationships of the identified species two gene fragments were sequenced: the "Folmer fragment" of the mitochondrial cytochrome *c* oxidase subunit I gene (COI) (Folmer *et al.*, 1994) and the D3-D5 domains of the nuclear ribosomal 28S gene (Morrow *et al.*, 2012). Both genes are being used in the Porifera Tree of Life project (www.portol.org) to resolve species-level questions and in combination with other markers also allow to address higher level relationships. For this purpose only specimens belonging to the COLETA, DBUA.Por and HBOI collections were used. Specimens from the MNHN Paris Seamount-2 collection were fixed in formalin and were therefore unsuitable for molecular work. A total of 70 specimens, representing all of the identified species were used.

A small fragment of choanosomal tissue (internal region of a sponge; see Glossary) was used for DNA extraction using the QIAGEN DNeasy blood and tissue kit according to the manufacturer's instructions. Since spicules are not digested by proteinase K (initial

digestion) one centrifugation step was added to remove all spicules, as suggested by Cárdenas and co-workers (2011). For the amplification of COI (612 bp) the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994) were used; the polymerase chain reaction (PCR) conditions were the following: 4 min/94°C; 5 cycles [30 s/93°C, 1 min/45°C, 30 s/72°C]; 30 cycles [30 s/93°C, 45 s/50°C, 30 s/72°C]; 7 min/72°C. For the amplification of the 28S rRNA gene (653 bp) the primers Por28S-830F (5'-CAT CCG ACC CGT CTT GAA-3') and Por28S-1520R (5'-GCT AGT TGA TTC GGC AGG TG-3') (Morrow *et al.*, 2011) were used and the PCR conditions were: 3 min/94°C; 36 cycles [3 min/94°C, 30 s/94°C, 45 s/58°C, 40 s/72°C]; 7 min/72°C. In some cases other temperatures (60-62°C) were adopted on the annealing step to get better results on PCR products.

The PCR products were purified and sequenced (in the majority of samples one direction -forward- was sufficient; in some cases both directions were used) by Macrogen, Europe.

2.3.2. DATA ANALYSIS

The obtained sequences were checked through BLAST searches (<http://blast.ncbi.nlm.nih.gov/>), aligned and edited in BioEdit v 7.0.5 (Hall, 2005). Additional lithistid, Astrophorida, Spirophorida, Hadromerida, Halichondrida and Poecilosclerida sequences were extracted from GenBank and the Sponge Barcoding Project website to be used in the analyses. The analysed COI and 28S datasets included 102 and 52 sequences in total of which 36 and 29 were new, respectively (Appendix II, Tab. I and Tab. II).

Bayesian analyses were implemented using Mr. Bayes v.3.1 (Huelsenbeck & Ronquist, 2001) with parameters estimated as part of the analysis. The best-fitting models for COI and 28S datasets were derived from and jModeltest 2.1.4 (Posada, 2008) under the Akaike Information Criterion (AIC). For COI the model used was GTR+I+G and for 28S was TrN+I+G. In both cases the analyses were run for 1×10^6 generations, saving one tree each 1000 generations. The log-likelihood values of the sample point were plotted against the generation time and all the trees prior to reaching stationary were discarded, ensuring that burn-in samples were not retained. Remaining trees were combined in a 50% majority consensus tree, in which frequency of any particular clade represents the posterior probability (Huelsenbeck & Ronquist, 2001). Members of the order Spirophorida were

designated as outgroup for the COI analysis. For the 28S analysis members of the orders Hadromerida and Halichondrida were chosen as outgroups. These outgroups were chosen on the basis of sequences being available on GenBank and the Sponge Barcoding Project, of species that have demonstrated to be phylogenetically close to the ingroup.

CHAPTER 3. RESULTS

3.1. DIVERSITY AND DISTRIBUTION OF LITHISTIDS ON THE NORTHEAST ATLANTIC OCEAN

Records of lithistid sponges were collected from the literature for the entire northeast Atlantic Ocean and Mediterranean Sea (e.g. Topsent 1892a, 1904b for the Macaronesian islands; Schmidt, 1870 and Du Bocage, 1869 for the Portuguese mainland; Manconi & Serusi, 2006 and Pisera & Vacelet, 2011 for the Mediterranean Sea). In total, 22 lithistid species representing 14 genera and eight families have been reported for these areas (Fig. 2; Tab. II).

In this study, 162 lithistid specimens were analysed and assigned to 11 taxa representing five families and nine genera (Fig. 2; Tab. II). Of these, the Corallistidae Sollas, 1888 is the best represented of all families with a total of four genera and four species. The remaining families are represented by one or two genera maximum.

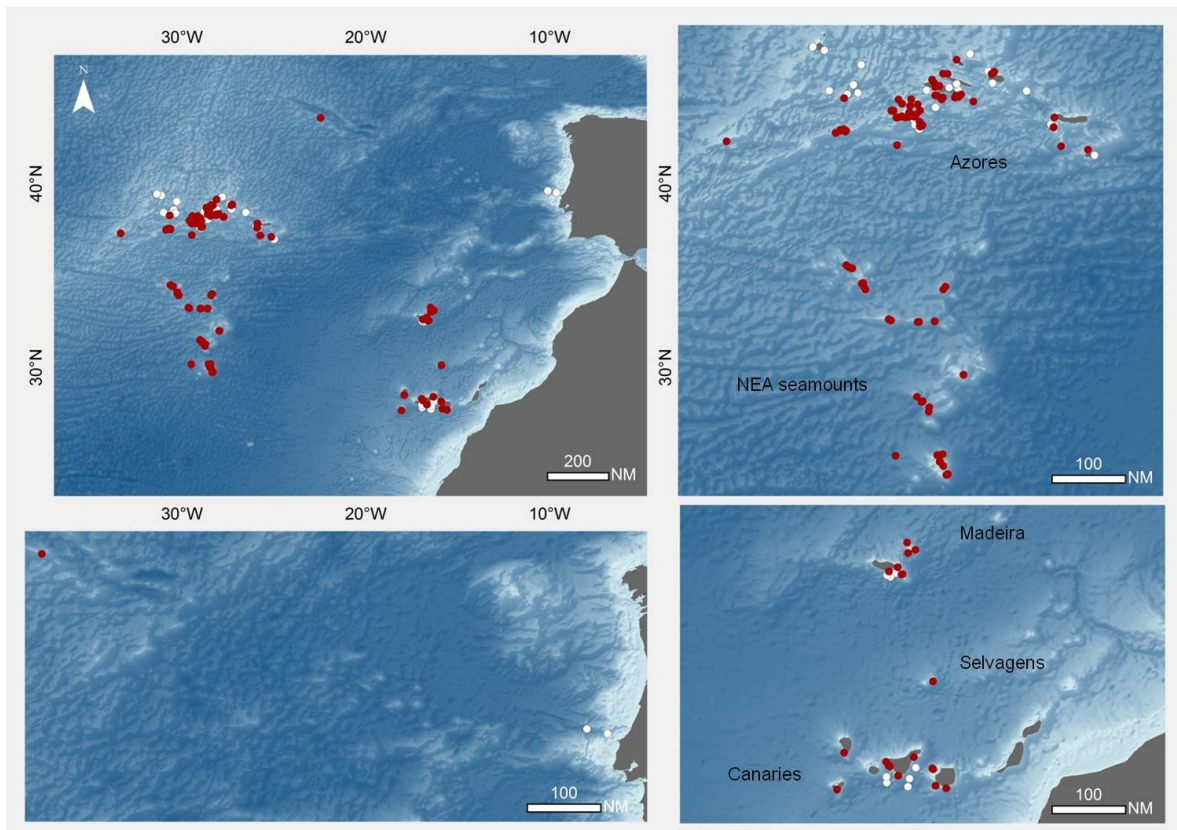


Fig. 2 - Distribution of lithistids in Macaronesian islands and neighboring seamounts: white-filled circles represent records from the literature and red-filled circles represent the specimens analyzed in this study.

Of the 10 species previously recorded from the Azores archipelago, only seven, representing five families and six genera were found in this study. Eight species were recorded for the first time on the seamounts south of the Azores, of which seven are shared with this archipelago. Three species constitute new records for Madeira Island (*Neophrissospongia nolitangere* (Schmidt, 1870), *Discodermia verrucosa* Topsent, 1928 and *Racodiscula clava sensu* Topsent, 1892), two for Selvagens (*N. nolitangere* and *Macandrewia azorica* Gray, 1859), and two for the Canaries, of which one is a new species for science of the genus *Isabella*. Of the 14 species found to occur in the Macaronesian islands, only three viz. *N. nolitangere*, *Leiodermatium pfeifferae* (Carter, 1876) and *R. clava* are shared among all archipelagos, with the remainder being restricted to one or two islands' group. Similarly, only four species seem to be shared between the Mediterranean Sea and these Atlantic archipelagos: *N. nolitangere* (with the Azores, Madeira, Selvagens and the Canaries), *Neoschrammeniella bowerbankii* (Johnson, 1863) (with Madeira), *Siphonidium ramosum* (Schmidt, 1870) (with the Azores) and *Leiodermatium lynceus* Schmidt, 1870 (with the Azores and the Canaries).

Most species were found in the upper bathyal (200-800 m) although some were found at lower depths (e.g. *N. nolitangere* at 91 m depth and *R. clava* at 168 m in the Azores; *Isabella harborbranchi* at 133 m in Canaries). The deepest records were those of *Macandrewia robusta* and *R. clava* found at 1000 m on the northeast Atlantic seamounts.

Tab. II - Records of lithistid sponges from the literature and found in this study with an indication of their bathymetric range; (-) no record; (*) occurring inside sublittoral caves.

| | Azores | | Madeira | | Selvagens | | Canaries | | NEA Seamounts | Portugal (mainland) | Mediterranean |
|---|------------|------------|------------|------------|------------|------------|-------------|------------|---------------|---------------------|---------------------|
| | Literature | This study | Literature | This study | Literature | This study | Literature | This study | This study | Literature | Literature |
| AZORICIDAE Sollas, 1888 | | | | | | | | | | | |
| <i>Leiodermatium</i> sp. | - | 182-706 m | - | 402-741 m | - | - | - | 281-291 m | 330-660 m | - | - |
| <i>Leiodermatium lynceus</i> Schmidt, 1870 | 370-1730 m | - | - | - | - | - | 1340-1530 m | - | - | - | 430-760 m |
| <i>Leiodermatium pfeifferae</i> (Carter, 1876) | 454-1250 m | - | unknown | - | - | - | - | - | - | - | - |
| CORALLISTIDAE Sollas, 1888 | | | | | | | | | | | |
| <i>Corallistes elegantior</i> Schmidt, 1870 | - | - | - | - | - | - | - | - | - | unknown | - |
| <i>Corallistes masoni</i> (Bowerbank, 1869) | - | - | unknown | 480-610 m | - | - | - | 415-417 m | - | - | - |
| <i>Isabella harborbranchi</i> n. sp. | - | - | - | - | - | - | - | 133-366 m | - | - | - |
| <i>Neophrissospongia endoumensis</i> Pisera & Vacelet, 2011 | - | - | - | - | - | - | - | - | - | - | 5 m* |
| <i>Neophrissospongia nana</i> Manconi & Serusi, 2008 | - | - | - | - | - | - | - | - | - | - | 6 m* |
| <i>Neophrissospongia nolitangere</i> (Schmidt, 1870) | 440 m | 91-435 m | - | 317 m | - | 358 m | 10-20 m* | 295-450 m | 461-501 m | - | 20-22 m* |
| <i>Neophrissospongia radjae</i> Pisera & Vacelet, 2011 | - | - | - | - | - | - | - | - | - | - | unknown* |
| <i>Neoschrammeniella bowerbankii</i> (Johnson, 1863) | - | - | 113-131 m | 519-669 m | - | - | - | - | 480-695 m | - | 12-30 m*; 400-640 m |
| DESMANTHIDAE Topsent, 1894 | | | | | | | | | | | |
| <i>Desmanthus incrustans</i> (Topsent, 1889) | - | - | - | - | - | - | - | - | - | - | 2-3 m*; 10-40 m |
| <i>Sulcastrella tenens</i> (Vacelet, 1969) | - | - | - | - | - | - | - | - | - | - | 210-240 m |
| <i>Petromica (Petromica) grimaldii</i> Topsent, 1898 | 200-650 m | - | - | - | - | - | - | - | - | - | - |
| MACANDREWIIDAE Schrammen, 1924 | | | | | | | | | | | |
| <i>Macandrewia azorica</i> Gray, 1859 | 454-1495 m | 256-770 m | - | - | - | 361 m | 1340-1530 m | - | 480-900 m | - | - |
| <i>Macandrewia ramifera</i> Topsent, 1904 | 1360 m | - | - | - | - | - | - | - | - | - | - |
| <i>Macandrewia robusta</i> Topsent, 1904 | 1165 m | 635 m | - | - | - | - | - | - | 705-1000 m | - | - |
| SCLERITODERMIDAE Sollas, 1888 | | | | | | | | | | | |
| <i>Aciculites mediterranea</i> Manconi, Serusi & Pisera, 2006 | - | - | - | - | - | - | - | - | - | - | 7.5 m* |
| <i>Microscleroderma lamina</i> Perez, Vacelet, Bitar & Zibrowius, 2004 | - | - | - | - | - | - | - | - | - | - | 2 m* |
| SIPHONIDIIDAE Lendenfeld, 1903 | | | | | | | | | | | |
| <i>Gastrophanelia phoeniciensis</i> Perez, Vacelet, Bitar & Zibrowius, 2004 | - | - | - | - | - | - | - | - | - | - | 2 m* |
| <i>Siphonidium ramosum</i> (Schmidt, 1870) | 349-793 m | 530 m | - | - | - | - | - | - | 470-545 m | - | 20-1000 m |
| THEONELLIDAE Lendenfeld, 1903 | | | | | | | | | | | |
| <i>Discodermia polydiscus</i> (Bowerbank, 1869) | - | - | - | - | - | - | - | - | - | unknown | 12-30 m*; 60-360 m |
| <i>Discodermia polymorpha</i> Pisera & Vacelet, 2011 | - | - | - | - | - | - | - | - | - | - | 3-20 m*; 210-360 m |
| <i>Discodermia ramifera</i> Topsent, 1892 | 98-318 m | 237-475 m | - | - | - | - | - | - | 300-580 m | - | - |
| <i>Discodermia verrucosa</i> Topsent, 1928 | - | - | - | 476-497 m | - | - | 400 m | 303-322 m | - | - | - |
| <i>Racodiscula clava sensu</i> Topsent, 1892 | 200-1229 m | 168-770 m | - | 563 m | - | - | 1340-1530 m | 408 m | 280-1000 m | - | - |

3.2. SYSTEMATICS

Phylum PORIFERA Grant, 1836

Class DEMOSPONGIAE Sollas, 1885

'Lithistid' Demospongiae *sensu* Pisera & Lévi, 2002

DEFINITION: Polyphyletic group of demosponges related by the presence of choanosomal articulated spicules (desmas) that form a rigid skeleton in the majority of the species. Lithistids are highly polymorphic and may be encrusting, massive irregular, cup- or ear-shaped, flabellate cylindrical, branched, globular, with or without pedicel, and with or without atrial cavity. Ectosomal megascleres are discotriaenes, pseudodiscotriaenes, phyllotriaenes, pseudophyllotriaenes, dichotriaenes, rhabds, oxeas, and in some species they are absent. Several types of choanosomal megascleres such as tetraxial (tetraclones), monaxial (rhizoclonas, megaclones, dicranoclonas, heloclonas, or various complex branched forms), polyaxial or anaxial (sphaeroclonas). Microscleres may be different types of rhabds, oxeas, spirasters, amphiasters, sigmaspires and raphides, or several combinations of these spicules (Pisera & Lévi, 2002a).

Family CORALLISTIDAE Sollas, 1888

DEFINITION: Sponges similar to a cup, vase, massive ear or with a lamellar shape; Ectosomal megascleres are dichotriaenes and in some cases, simple triaenes; choanosomal desmas are dicranoclonas; microscleres can be two types of spirastres, streptasters/amphiasters, microxeas, microstyles and microstrongyles (Pisera & Lévi, 2002c).

Genus *Corallistes* Schmidt, 1870

TYPE SPECIES: *Corallistes typus* Schmidt, 1870

DEFINITION: Cup- to vase-shape sponges, with simple or folded plates; ectosomal megascleres are smooth dichotriaenes and large oxeas can be present; choanosomal desmas are dicranoclonas very tuberculated; microscleres are usually two types of spirasters (Pisera & Lévi, 2002c).

Corallistes masoni (Bowerbank, 1869)

(Figs. 3A-H)

EXAMINED MATERIAL: HBOI 27-V-91-3-006, 29-V-91-3-017, 31-V-91-1-015, 10-VI-91-1-003, 11-VI-91-1-003.

EXTERNAL MORPHOLOGY: Polymorphic sponges with cup-to vase-shape or simple to folded plates. Colour beige to light brown. Inner surface with oscules in small elevations, that may be bright blue; outer surface is smooth (Figs. 3A-B).

SKELETON: Ectosomal skeleton (Fig. 3C) contains a compact layer of smooth dichotriaenes transverse to the surface, long oxeas as megascleres, and two types of abundant spirasters with pointed arms as microscleres. Choanosomal skeleton (Fig. 3D) composed of tuberculated dicranoclone desmas that form a compact and rigid mesh (Fig. 3E); spirasters are spread throughout the choanosome but less numerous than in the ectosome.

SPICULES: Ectosomal dichotriaenes are smooth with some of the tips of the cladome directed towards the rhabdome and/or bifurcated; the cladome is very variable in length within the same specimen 80 – 202- 459 μm diameter; the rhabdome is long with pointed extremity, 124 – 425 – 950 μm length and 6 – 19 – 37 μm width (Fig. 3F). Oxeas are long, thin, and smooth and have blunt tips, 184 – 555 – 995 x 2.3 – 4.8 – 7.5 μm (Fig. 3G). Dicranoclone desmas are compact with large and rough tubercles, with brownish tone (Fig. 3E). Microscleres are two types of spirasters: type I with thin, short and numerous rays, 11 – 18 – 26 μm ; type II with thin, long and few rays, 17 – 30 – 74 μm (Fig. 3H).

DISTRIBUTION: Individuals of *C. masoni* were sampled in Madeira and Canary islands between 415 – 610 m depth. Until the present study this species was only known from its type locality in the Madeira archipelago (unknown depth).

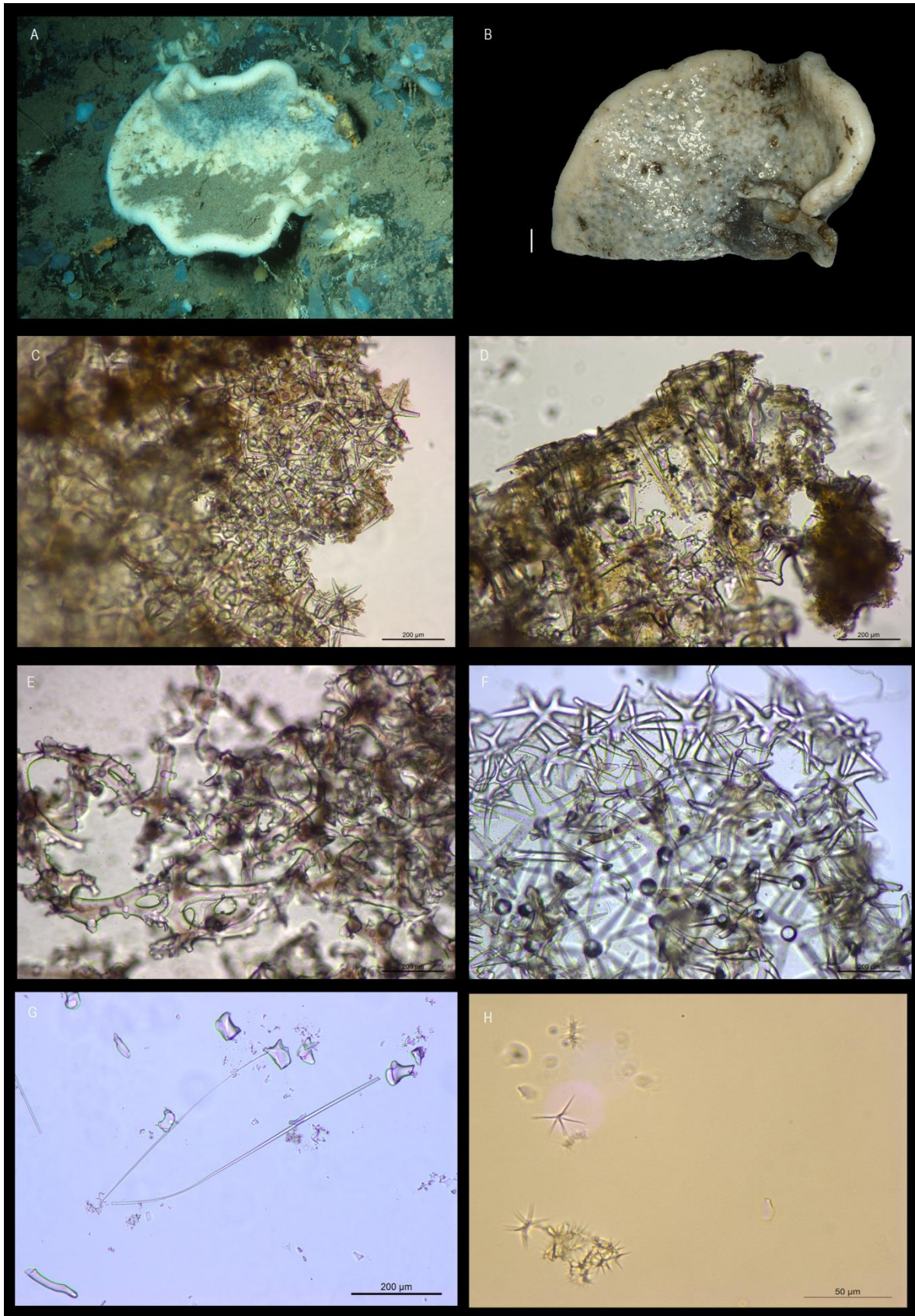


Fig. 3 - *Corallistes masoni* (Bowerbank, 1869). A, specimen from Madeira *in situ*; B, specimen collected in Madeira, photo ex

situ (scale 1 cm); **C**, ectosomal skeleton showing a layer of dichotriaenes (scale 200 µm); **D**, choanosomal and ectosomal skeleton (scale 200 µm); **E**, dicranoclone desmas (scale 200 µm); **F**, dichotriaenes (scale 200µm); **G**, oxeas (scale 200 µm); **H**, two types of spirasters: type I with thin, short and numerous rays, and type II with thin, long and few rays (scale 50 µm).

REMARKS: *C. masoni* was originally described by Bowerbank (1869) based on a specimen collected at an unknown depth in the Madeira archipelago. Since then the species was never reported again for the NEA but it has been often reported (misidentified) in the Mediterranean Sea as *Neoschrammeniella bowerbankii* (Pisera & Vacelet, 2011). With this study the known geographic range, as well as the bathymetric distribution of this species was expanded.

Genus *Neophrissospongia* Pisera & Lévi, 2002c

TYPE SPECIES: *Corallistes nolitangere* Schmidt, 1870.

DEFINITION: Sponges with irregular cup or ear shape, contorted flabellate masses or clavate growth forms; ectosomal megascleres are dichotriaenes with strong spines and/or tubercles in the upper surface of the cladome; choanosomal desmas are dicranoclones; Microscleres are streptasters/amphiasters and spinose microtylostyles (Pisera & Vacelet, 2011).

Neophrissospongia nolitangere (Schmidt, 1870) (Figs. 4A-D; 5A-H)

EXAMINED MATERIAL: HBOI- 28-V-91-2-001, 2-VI-91-1-005, 9-VI-91-4-003, 9-VI-91-4-006, 11-VI-91-1-001, 11-VI-91-1-008, 13-VI-91-4-003, 16-VI-91-2-004; DOP- 1134, 1135, 1241, 1609, 2048.1, 2048.2, 3147, 3774, 4037, 4166, 4602, 4905, 5323, 5850, 5853, 5871, 5879, 6192, 6370, 6565, 6614, 6623, 6686; DBUA.Por- SAB2011.DR12.B1.

EXTERNAL MORPHOLOGY: Large flabellate masses in old individuals (Fig. 4A) and ear-shaped forms in young specimens (Fig. 4B); large individuals are attached to the substrate by the entire lower base whereas the youngest individuals are attached by a pedicel. Colour of the specimens varies from beige to light brown, in life and alcohol. Walls are approximately 1.3 cm thick. There are significant differences between the inner and the outer surface of the sponge: the inner surface has several oscular openings in small elevations

and is smooth on the borders; some specimens have a bright blue colour on the oscular openings; the outer surface is smooth with ostia distributed over the entire surface.

SKELETON: The ectosome (Fig. 4C) is composed of a compacted layer of dichotriaenes transverse to the surface, covered by an extremely dense and compact crust of spirasters-streptasters; abundant microtylostyles can also be observed in the ectosome. Choanosomal skeleton (Fig. 4D) is constituted by strongly tuberculated dicranoclone desmas, in a compacted net with irregular cavities (Figs. 5A-B); both microtylostyles and spirasters-streptasters are also present.

SPICULES: Ectosome megascleres are dichotriaenes with cladome $87 - \underline{225} - 367 \mu\text{m}$ in diameter that have small pointed tubercles $3 - \underline{12} - 22 \mu\text{m}$ in size, on their upper surface; blunt rhabdome with $276 - \underline{610} - 1117 \times 5 - \underline{18} - 35 \mu\text{m}$, straight or sometimes slightly curved (Figs. 5C-D); in small specimens smooth dichotriaenes can be found (Fig. 5E). Choanosomal desmas are massive dicranoclones extremely articulated, covered with mushroom-shaped tubercles (Figs. 5A-B); dicranoclones are brownish. Microscleres are microtylostyles and spirasters/streptasters; microtylostyles are pin-shaped with a spinous head and some few sparse spines all over the spicule, with $30 - \underline{77} - 188 \mu\text{m}$ long and $0.8 - \underline{1.8} - 4.5 \mu\text{m}$ large (Fig. 5F). The spirasters/streptasters are $6 - \underline{11} - 18 \mu\text{m}$ long, with thick and spiny arms or with long and smooth arms (Figs. 5G-H).

DISTRIBUTION: Specimens of *N. nolitangere* were collected in all archipelagos from the Macaronesian region (Azores, Madeira, Selvagens and Canaries), between 91 and 502 m depth. This species was previously known from its type-locality in Portugal (uncertain locality), and from the archipelagos of the Azores and Cape Verde. The records for Madeira and Selvagens here reported for the first time, make therefore for a much more continuous geographical range of the species.

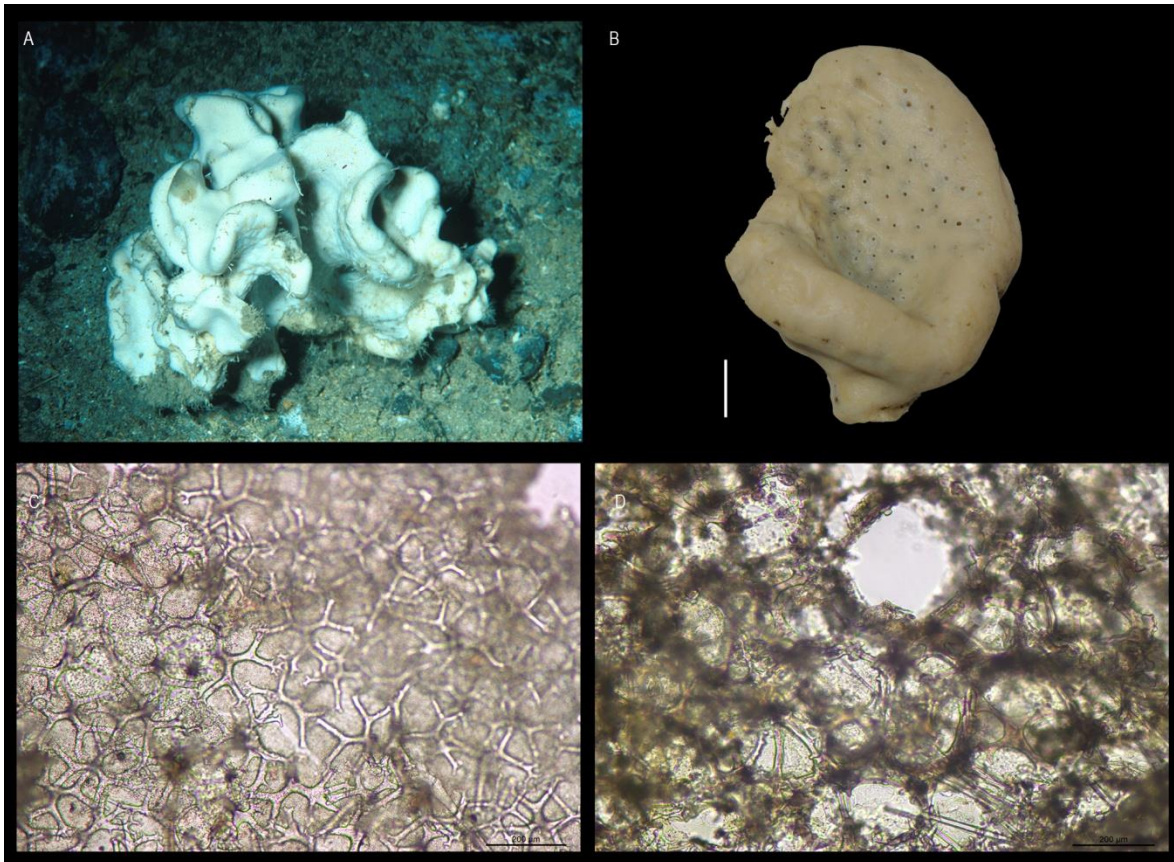


Fig. 4 - *Neophrissospongia nolitangere* (Schmidt, 1870). **A**, adult individual from the Canaries, *in situ*; **B**, young specimen collected in the Azores, *ex situ* (scale 1 cm); **C**, ectosomal skeleton with a compact layer of dichotriaenes and microscleres (scale 200 μ m); **D**, choanosomal skeleton (scale 200 μ m).

REMARKS: Manconi & Serusi (2008) suggested the revision of the genus *Neophrissospongia* erected by Pisera & Lévi (2002c) due to the rare presence of smooth dichotriaenes in young individuals and microstyles in some specimens, that were not described before. In their work concerning the lithistid sponges of the Mediterranean Sea Pisera & Vacelet (2011) redescribed this genus by adding the rare presence of spinose microtylostyles but they did not mention the presence of smooth dichotriaenes. In some specimens analysed in the present work smooth dichotriaenes in young ear- to cup-shaped individuals were also found. Furthermore, in the specimens here analysed, spinose microtylostyles were not at all rare, they were actually quite abundant. Accordingly, the inclusion of smooth dichotriaenes and the presence of microtylostyles in the genus definition is hereby proposed.

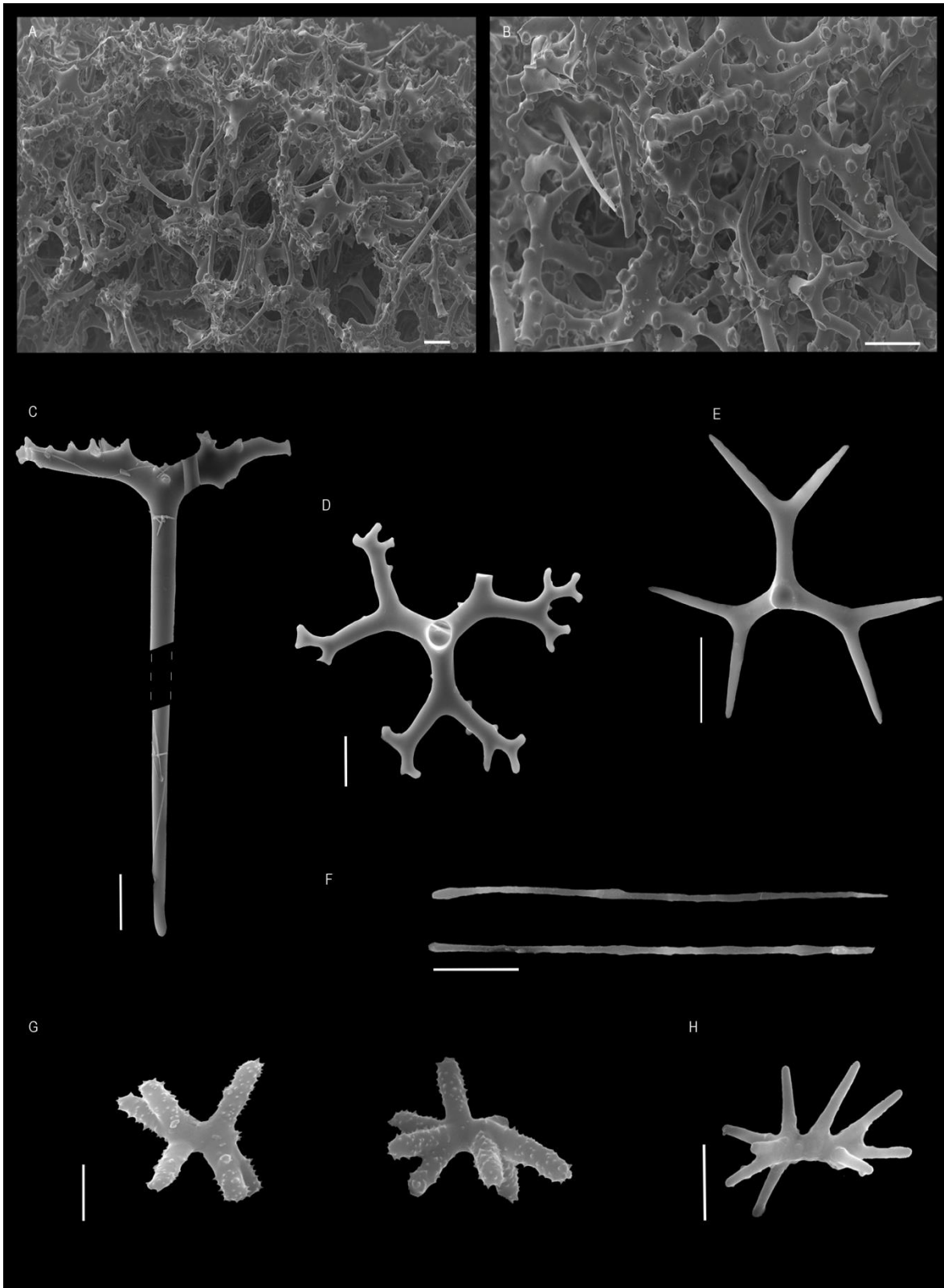


Fig. 5 - *Neophrissospongia nolitangere* (Schmidt, 1870). **A**, dicranoclone desmas (scale 100 μ m); **B**, detail of dicranoclones where one can observe the circular-shape elevated tubercles (scale 100 μ m); **C**, ectosomal dichotriaene (scale 50 μ m); **D**, cladome of dichotriaene of an adult individual, view from below (scale 50 μ m); **E**, smooth cladome of dichotriaene of a young

individual, view from below (scale 50 μm); **F**, microstyles (scale 10 μm); **G**, spirasters/streptasters with thick and spiny arms (scale 5 μm); **H**, spirasters/streptasters with long and smooth arms (scale 5 μm).

Genus *Neoschrammeniella* Pisera & Lévi, 2002c

TYPE SPECIES: *Iouea moreti* Lévi & Lévi, 1988.

DEFINITION: Polymorphic sponges, cylindrical to cup- vase- shaped; ectosomal megascleres are smooth dichotriaenes; choanosomal megascleres are anisoxeas, dicronoclone desmas, and some species may have big fringing diactines; Microscleres are two or three types of acanthose spirasters, with short blunt rays, long or short pointed rays (Pisera & Vacelet, 2011).

Neoschrammeniella bowerbankii (Johnson, 1863) (Figs. 6A-D; 7A-G)

EXAMINED MATERIAL: HBOI- 29-V-91-3-008, 30-V-91-4-007; MNHN- IP-2008-233, IP-2008-234.

EXTERNAL MORPHOLOGY: Polymorphic sponges, cup-shaped to undulate lamellate masses (Figs. 6A-B); colour beige to brown in life and alcohol; the walls are very thick, 1-2 cm, and both surfaces are smooth. The sponges are attached to the substrate by a small pedicel or by the entire lower base.

SKELETON: The ectosome (Fig. 6C) has a layer of smooth dichotriaenes and microscleres (two types of spirasters). In some specimens it is possible to observe some thin and long oxeas protruding the surface out from the choanosome. The choanosome (Fig. 6D) is formed by solid and brownish dicranoclone desmas (Figs. 7A-B), and dispersed spirasters of two types.

SPICULES: Ectosomal megascleres are smooth dichotriaenes and two types of spinous spirasters; dichotriaenes are smooth with 144 – 255 – 429 μm in diameter (cladome), and in some cases, with the point of the cladome directed towards the rhabdome or bifurcated; rhabdome is long and sharp 201- 470 – 755 μm long and 8 - 15 – 24 μm width (Figs. 7C-D). Two distinct types of spinous spirasters are found: type I are short, thick and have blunt

arms, 17 – 21 – 28 μm long (Fig. 7E) and type II are long, thin and have pointed arms, 13 – 25 – 39 μm long (Fig. 7F). Choanosome megascleres are dicranoclone desmas and thin oxeas; dicranoclones are dense with a lot of irregular tubercles which may be divided into small callosities (Figs. 7A-B); oxeas with blunt points measure 124 – 260 – 713 μm in length and 1.9 – 2.8 – 4.1 μm in width; most of the oxeas were broken, so this was the maximum length measured.

DISTRIBUTION: *N. bowerbankii* was previously known from its type-locality, Madeira Island (unknown depth), and from the Mediterranean Sea (20-22 m, occurring inside sublittoral caves). In the present study this species was found in Madeira (519-669 m) and on the Plato and Hyères seamounts (480-695 m), being the first records of this species to these seamounts.

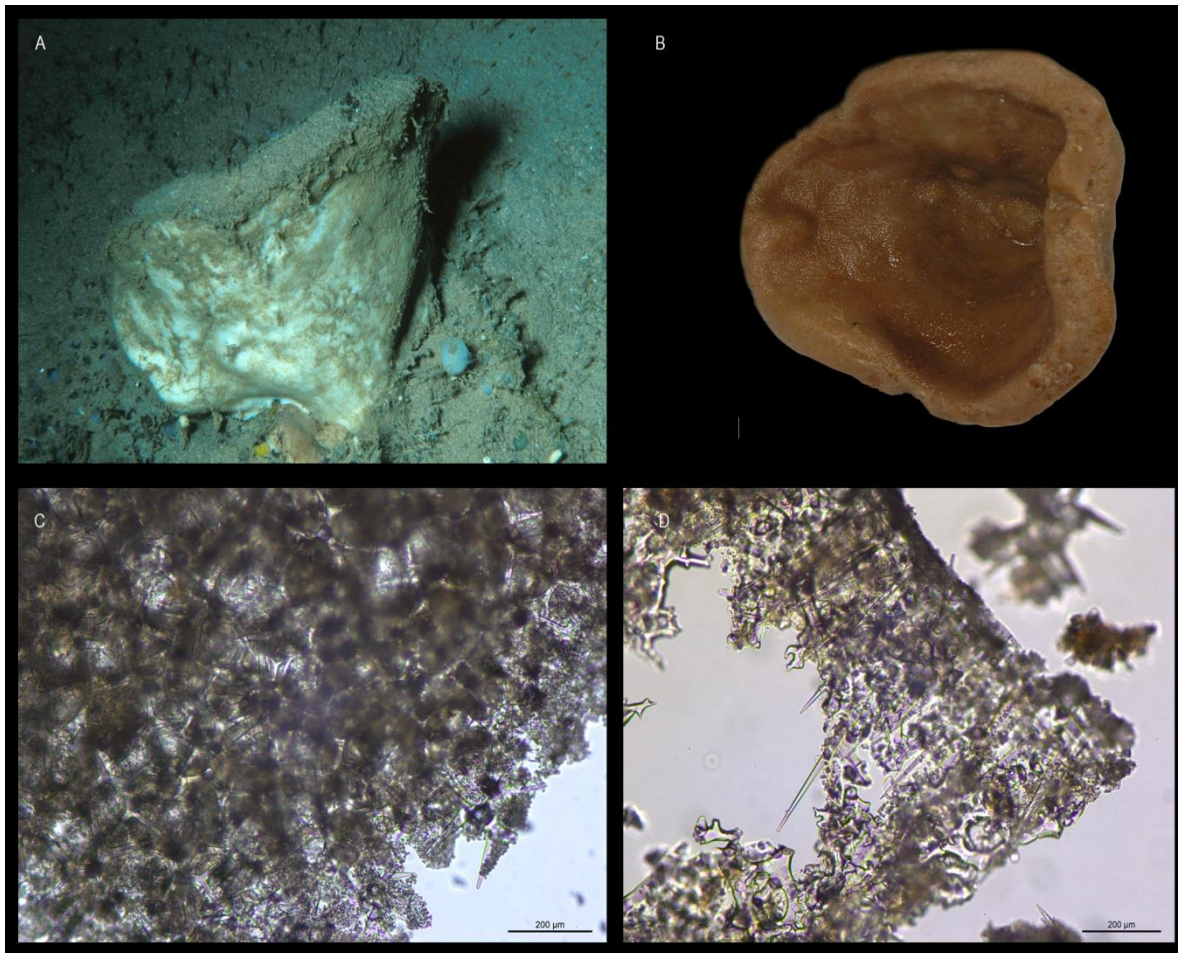


Fig. 6 - *Neoschrammeniella bowerbankii* (Johnson, 1863). **A**, specimen collected in Madeira Island, *in situ*; **B**, specimen collected in Hyeres seamount, *ex situ* (scale 1 cm); **C**, top view of ectosomal skeleton where one can observe a layer of dichotriaenes and abundant microscleres (scale 200 μm); **D**, transversal view of choanosomal skeleton (scale 200 μm).

REMARKS: The genus *Neoschrammeniella* was formally diagnosed by Pisera & Lévi (2002c) and the knowledge of the distribution and morphological characters of *N. bowerbankii* consequently increase. In their work, images of SEM were presented allowing a better observation of microscleres, namely the two types of spirasters. In the present work SEM analysis allowed the observation of a third type of spirasters (with short, thick and pointed arms; Fig. 6G) occurring in much lower quantity. This type III seems an intermediate form of the two types previously described by Pisera & Lévi (2002c). SEM examination of further specimens will be necessary to ascertain the taxonomic significance of this finding.

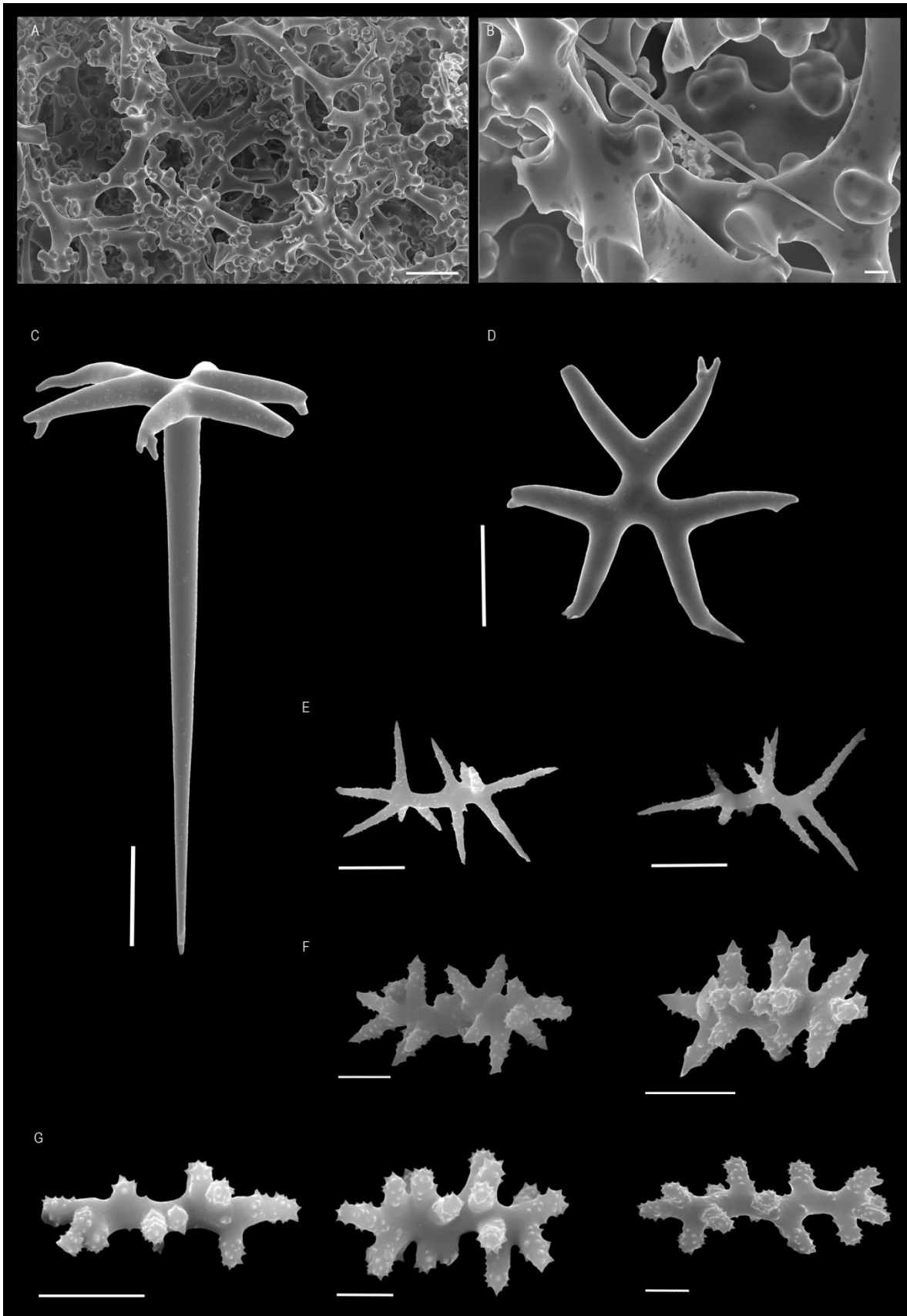


Fig. 7 - *Neoschrammeniella bowerbankii* (Johnson, 1863). A, overview of dicranoclone desmas (scale 100 μ m); B, detail of

dicranoclone desmas, note the tubercles divided into small tubercles and the oxea with blunt tip (scale 10 μm); **C**, dichotriaene (scale 100 μm); **D**, smooth cladome of a dichotriaene (scale 100 μm); **E**, spirasters of type I, with short, thick and blunt arms (scale 5 μm); **F**, spirasters of type II, with long, thin and pointed arms (scale 10 μm); **G**, spirasters of type III found in this present work, with short, thick and pointed arms (scale 5 μm).

Genus *Isabella* Schlacher-Hoenlinger, Pisera & Hooper 2005

TYPE SPECIES: *Isabella mirabilis* Schlacher-Hoenlinger, Pisera & Hooper 2005.

DEFINITION: Massive and globular lithistid; ectosomal dichotriaenes, short and long-shafted triaenes, all smooth; choanosomal dicranoclones desmas are slender, smooth and with small tubercles in some parts; two types of oxeas: type I long and thick with blunt tips, type II long, thin and curved with acerate tips; four types of microscleres: spinous fusiform microxeas, spinous centrotylotes, spirasters with small, thick and thorny arms and streptasters with long, thin and also thorny arms (Schlacher-Hoenlinger *et al.*, 2005).

Isabella harborbranchi Carvalho, Pomponi & Xavier (in prep.)

(Figs. 8A-E; 9A-N)

EXAMINED MATERIAL: HBOI- 8-VI-91-4-005, 9-VI-91-4-013, 11-VI-91-1-002, 14-VI-91-1-003.

MORPHOLOGY: Massive and globular Corallistidae. Surface is irregular and slightly compressible, while the inner part of the sponge is rigid. Oscules/pores cannot be seen with naked eye. Colour dark brown in life and in ethanol (Fig. 8A).

SKELETON: Ectosome is rather difficult to distinguish from the choanosome due the huge quantity of microscleres spread throughout the sponge and the dark colour of its tissue. Ectosomal skeleton contains a small number of dichotriaenes and both long- and short-shafted triaenes spread throughout the sponge surface along with a large quantity of microacanthoxeas (Fig. 8B). Choanosomal skeleton (Fig. 8C) consists on dicranoclone desmas (Figs. 8D-E), two types of long oxeas and microscleres (microacanthoxeas, spirasters/streptasters).

SPICULES: Ectosomal dichotriaenes and triaenes (long and short-shafted) are irregular. The cladome of the dichotriaenes is 150 - 334 - 623 μm in diameter, 3.6 - 9.1 - 12.9 μm in width and have blunt tips; the rhabdome is 107 - 283 - 578 x 6.5 - 9.3 - 14.8 μm (Fig. 9A); some of these megascleres exhibit small arms, with blunt tips and, in some cases

downwards, in the rhabdome with $62 - \underline{78} - 95 \times 7.7 - \underline{8.9} - 10.2 \mu\text{m}$. Short-shafted triaenes measure $162 - \underline{306} - 397 \times 8.1 - \underline{11.8} - 14.3 \mu\text{m}$ (cladome) and $19.9 - \underline{36.2} - 49.3 \times 7.8 - \underline{10.7} - 15.9 \mu\text{m}$ (rhabdome)(Fig. 9B). Long-shafted triaenes have also blunt tips in the cladome and rhabdome; cladome is $174 - \underline{242} - 436 \mu\text{m}$ in diameter and $5.6 - \underline{9} - 12.4 \mu\text{m}$ in width, and rhabdome measures $210 - \underline{395} - 587 \times 6.6 - \underline{9.6} - 12.3 \mu\text{m}$ (Fig. 9C); as in dichotriaenes, small arms were also observed. Microscleres are microacanthoxeas, slightly curved with acerate tips, spirasters and streptasters. The larger and most abundant microacanthoxeas are fusiform and have acerate tips, $85 - \underline{101} - 118 \times 4.9 - \underline{5.4} - 7.1 \mu\text{m}$ (Figs. 9D and 9F); whereas the smaller and rarer ones are centrotylote, $57 - \underline{69} - 88 \times 4.5 - \underline{5.4} - 6.2 \mu\text{m}$ (Figs. 9E and 9G); spirasters with long, thin and thorny arms, measure $10.9 - \underline{11.3} - 11.6 \mu\text{m}$ in length, and are very variable in shape and number of actines (Figs. 9H-J); streptasters have short, thick and thorny arms, and measure $13.7 - \underline{14.7} - 15.7 \mu\text{m}$ in length (Figs. 9K-N). Choanosomal dicranoclone desmas are slender, smooth, dark brown, and with few small smooth tubercles. The desmas do not form a compact net as in most lithistid species. They are irregular and form big spaces between them; in these spaces, large quantities of microacanthoxeas and some spirasters/streptasters can be found. Oxeas, of two different types, can form bundles in the choanosome. Type I are long and thick oxeas with blunt tips (in some oxeas, the tip has a small thorn), with $358 - \underline{777} - 1395 \times 5.5 - \underline{9.1} - 18.2 \mu\text{m}$; type II are long, thin and curved oxeas with acerate tips, with $378 - \underline{810} - 1286 \times 3.04 - \underline{3.9} - 4.8 \mu\text{m}$. Most oxeas were broken so these are the maximum values measured.

DISTRIBUTION: Known only from the circalittoral and upper bathyal of the Canary Islands (W and S coasts of Tenerife and SW coast of La Palma), between 133 and 366 m depth.

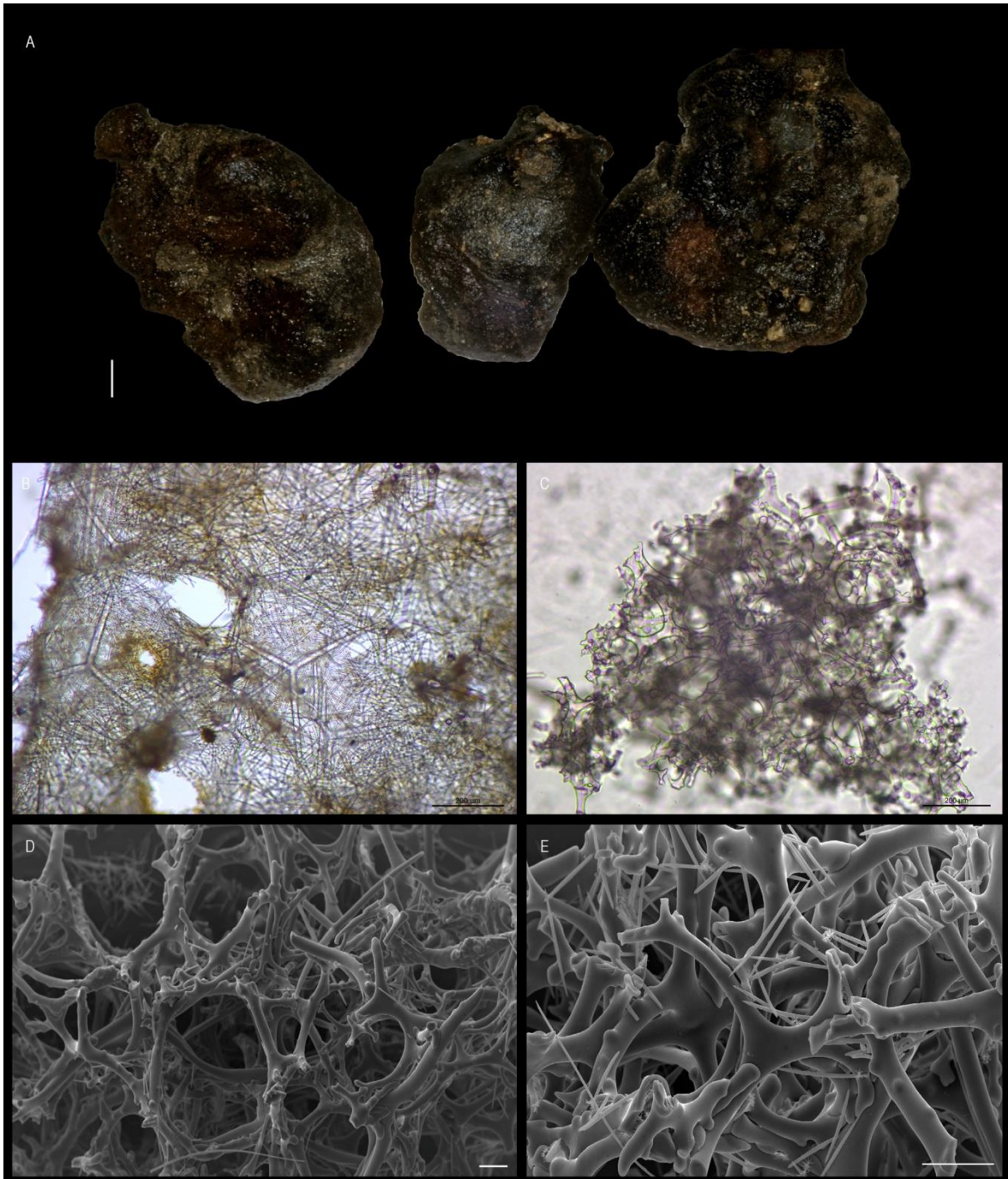


Fig. 8 - *Isabella harborbranchi* Carvalho, Pomponi & Xavier (in prep.). **A**, specimens collected in the Canaries, *ex situ* (scale 1 cm); **B**, top view of the ectosome where one can observe the huge quantity of microscleres and some dichotriaenes and triaenes (scale 200 µm); **C**, choanosomal skeleton and dicranoclone desmas (scale 200 µm); **D**, overview of dicranoclone desmas (scale 100 µm); **E**, detail of dicranoclone desmas, note the abundant microacanthoxeas and part of an oxea (in the lower-left corner) (scale 100 µm).

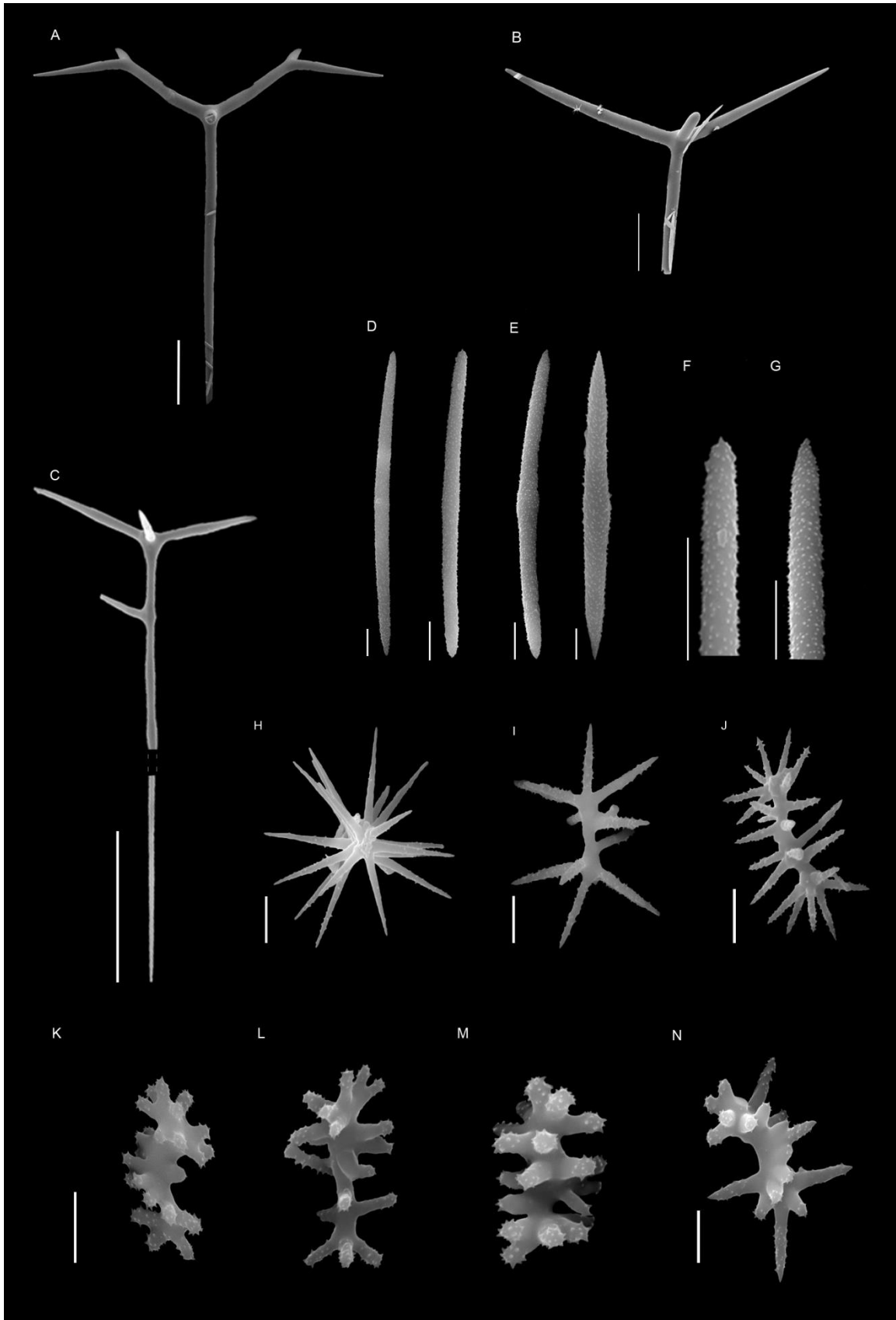


Fig. 9 - *Isabella harborbranchi* Carvalho, Pomponi & Xavier (in prep.).. **A**, dichotriaene (scale 100 μ m); **B**, short-shafted triaene

(scale 100 μm); **C**, long-shafted triaene with a small arm in the rhabdome (scale 100 μm); **D**, microacanthoxeas (scale 10 μm); **E**, centrotylotes (scale 10 μm); **F**, detail of the extremity of a microacanthoxea (scale 10 μm); **G**, detail of the extremity of a centrotylote (scale 10 μm); **H-J**, spirasters with long, thin and thorny arms (scale 5 μm); **K-N**, streptasters with short, thick and thorny arms (scale 5 μm).

REMARKS: *I. harborbranchi* conforms to the diagnostic characters of the recently erected genus *Isabella*, until now represented by a single species *I. mirabilis* (Schlacher-Hoenlinger *et al.*, 2005). The morphology, colour, and texture are similar in both species. The main differences between the two species lie in the arrangement and shape of mega- and microscleres. It is in the ectosome that these differences are notable: *I. harborbranchi* possesses long- and short-shafted triaenes that are absent in *I. mirabilis*. In addition, its dichotriaenes, are irregular, less abundant and have longer cladome and shorter rhabdome.

While in *I. mirabilis* the microacanthoxeas are only present in the ectosome, in *I. harborbranchi* they are copiously present throughout the ectosome and the choanosome and they are also slightly longer. Both species share the presence of abundant spirasters and streptasters in the ectosome and choanosome. Spirasters are very similar in these two species, but bigger in *I. mirabilis*. Streptasters are different in size and shape; in *I. mirabilis* they are asymmetrical and bigger. We therefore propose a redefinition of the genus as follows: massive cup to cushion shaped lithistids, dark brown in colour and discolouring the ethanol purplish-brown; with smooth ectosomal dichotriaenes that can be accompanied by short and/or long-shafted triaenes and choanosomal root/vine-like dicranoclone desmas; microscleres are microacanthoxeas (some may be centrotylote) and two types of spirasters/streptasters variable in shape but one euaster-like.

Family THEONELLIDAE Lendenfeld, 1903

DIAGNOSIS: Polymorphic sponges; ectosomal megascleres are phyllo- to discotriaenes; choanosomal megascleres are tetracclone desmas and large oxeas can be present in some species; several types of microscleres such as spinose microrhabds, and/or microxeas, streptasters and/or pseudospherasters (Pisera & Lévi, 2002).

Genus *Discodermia* du Bocage, 1869

TYPE SPECIES: *Dactylocalyx polydiscus* Bowerbank, 1869.

DEFINITION: Polymorphic sponges, from massive irregular to cup-shaped, branched or cylindrical; ectosomal megascleres are smooth discotriaenes; choanosome megascleres are tetracclone desmas (regular or irregular) that can be smooth or tuberculated, and oxeotes or stylotes; microscleres are acanthoxeas and acanthorhabds (Pisera & Vacelet, 2011).

Discodermia ramifera Topsent, 1892

(Figs. 10A-C; 11A-I)

EXAMINED MATERIAL: DOP – 1640, 5862, 6595, 6476; MNHN – IP-2008-204; IP-2008-205, IP-2008-206, IP-2008-207, IP-2008-208, IP-2008-210, IP-2008-211, IP-2008-212, IP-2008-213, IP-2008-214 (all specimens from the MNHN collection were identified as *Discodermia cf. ramifera*).

EXTERNAL MORPHOLOGY: Polymorphic lithistid sponges, shape varies from elongated to spherical masses with big or small protuberances. Small sponges, 19 to 47 mm high. Surface is smooth. Colour varies from beige to light brown (Fig. 10A).

SKELETON: Ectosome (Fig. 10B) is composed of a layer of overlapping discotriaenes and abundant microxeas and acanthorhabds. Choanosomal skeleton (Fig. 10C) has tetracclone desmas (Figs. 11A-B) and smooth oxeas as megascleres; microxeas and acanthorhabds are also present but in smaller quantity.

SPICULES: Ectosomal discotriaenes are very variable in shape from round/oval to irregular and indented forms; cladome is plane or slightly concave, 142 – 279 – 532 μm diameter; rhabdome is short and conical, 25 – 57 – 113 μm in length and 7 – 22 – 47 μm in width (Figs. 11C-E). Microscleres are microxeas, rarely centrotylotes, and acanthorhabds; microxeas are thorny, slightly curved with pointed ends, measuring 29 - 45 – 67 μm in length and 1.7 – 3.4 - 5.5 μm in width (Figs. 11G-H); the acanthorhabds are similar to the microxeas with the exception that they are smaller and have blunt tips, 8.8 – 18 – 38 x 1.4 – 2.5 – 4.2 μm (Fig. 11I). Tetracclone desmas have smooth rays and are extremely tuberculated in their extremities/zygoses; tubercles are smooth and in some cases one tubercle may be divided into various smaller tubercles; tetracclones form a regular and compact net (Figs. 11A-B). Oxeas are long, smooth with rounded extremities; the vast majority of oxeas were broken,

so this was the maximum value measured 884 μm in length with 2.9 – 5.7 – 9.2 μm width (Fig. 11F).

DISTRIBUTION: Specimens of *D. ramifera* were sampled in the Azores archipelago and on the neighbouring seamounts (Meteor, Atlantis and Plato) between 237- 580 m depth. Until now, *D. ramifera* was only recorded from the Azores (type-locality), and therefore these constitute the first records of this species for the northeast Atlantic seamounts.

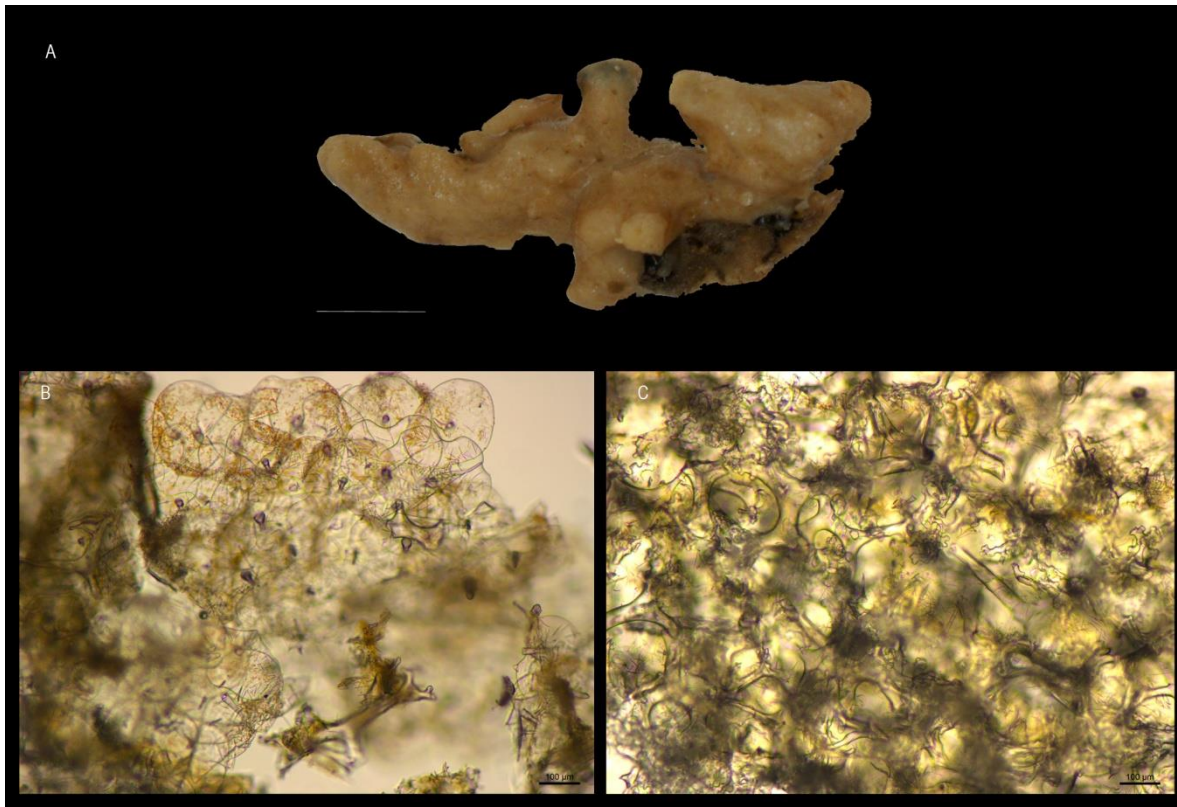


Fig. 10 - *Discodermia ramifera* Topsent, 1892. **A**, specimen collected in the Azores, *ex situ* (scale 1 cm); **B**, ectosome, where one can observe the compact layer of discotriaenes (scale 100 μm); **C**, choanosomal skeleton (scale 100 μm).

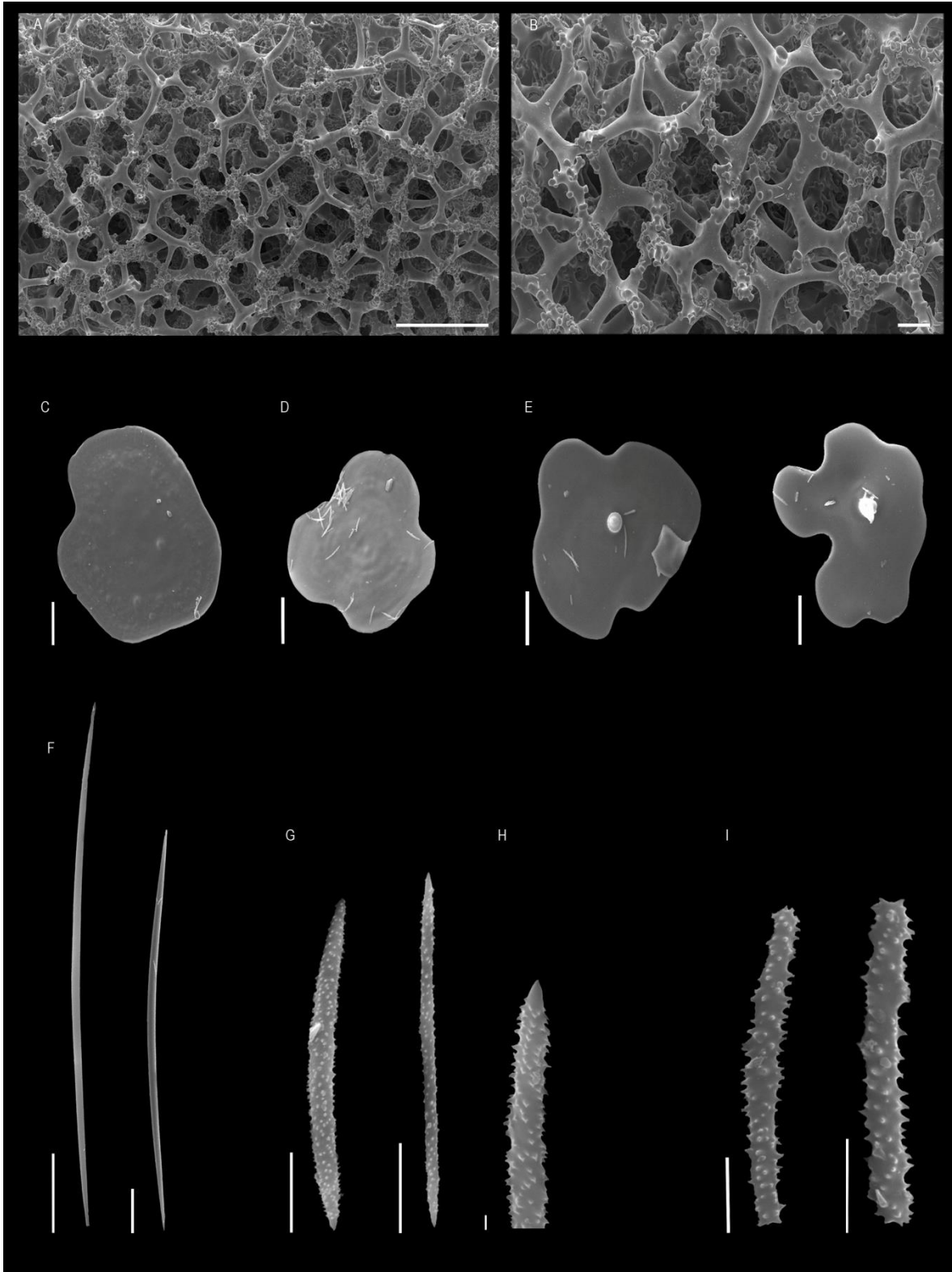


Fig. 11 - *Discodermia ramifera* Topsent, 1892. **A**, overview of tetraclone desmas (scale 500 μm); **B**, detail of tetraclones; **C**, top view of discotriaenes (scales 50 and 100 μm , respectively); **D**, bottom view of discotriaenes, note the variability of shapes (scales 100 μm); **E**, long oxeas (scales 50 and 100 μm , respectively); **F**, microxeas (scales 10 μm); **G**, detail of the extremity of microxeas (scale 1 μm); **H**, acanthorhabds (scales 5 μm).

Discodermia verrucosa Topsent, 1928

(Figs. 12A-G)

EXAMINED MATERIAL: HBOI - 31-V-91-1-006, 31-V-91-3-003, 2-VI-91-1-012, 8-VI-91-1-002.

EXTERNAL MORPHOLOGY: Small, irregular to spherical lithistids, 18 mm high and 14 mm wide, with lumpy surface. Oscules or pores cannot be seen with naked eye. Colour varies from whitish to light brown (Fig. 12A).

SKELETON: Ectosome (Fig. 12B) is composed of a compact layer of discotriaenes and abundant microscleres (microxeas and acanthorhabds); oxeas are also present and can form bundles. Choanosomal skeleton (Fig. 12C) has tetracclone desmas (Fig. 12D) that form an irregular net where it is possible to observe dispersed microxeas and acanthorhabds.

SPICULES: Ectosome megascleres are discotriaenes and oxeas; discotriaenes have an irregular shape that varies from round to oval (sometimes indented); the cladome is smooth, slightly concave with 147 – 394 – 607 µm in diameter and the rhabdome has a conical shape with 48 – 85 – 153 x 10 – 24 – 50 µm (Fig. 12E). Choanosomal tetracclone desmas are very tuberculated in the extremities and with some tubercles in the rays (Fig. 12D). Oxeas are long, smooth with rounded ends, similar to a crochet nail (Fig. 12F); most oxeas were broken due to their large size, thus the maximum value obtained was 1066 µm in length and 3.8 – 7.6 – 14.7 µm in width. Microxeas are spinous, slightly curved with pointed ends, 32 – 45 – 62 x 1.7 – 3 – 4.4 µm (Fig. 12G). Acanthorhabds are also spinous with blunt tips, 8 – 14 – 21 µm in length and 1.3 – 2.6 – 4.1 µm in width (Fig. 12G).

DISTRIBUTION: This species was found in Madeira, Selvagens and Canaries in a depth range of 303-497 m. *D. verrucosa* was previously known only from Madeira, the species' type-locality, and therefore with the present study both the geographic and bathymetric ranges of this species are considerably expanded.

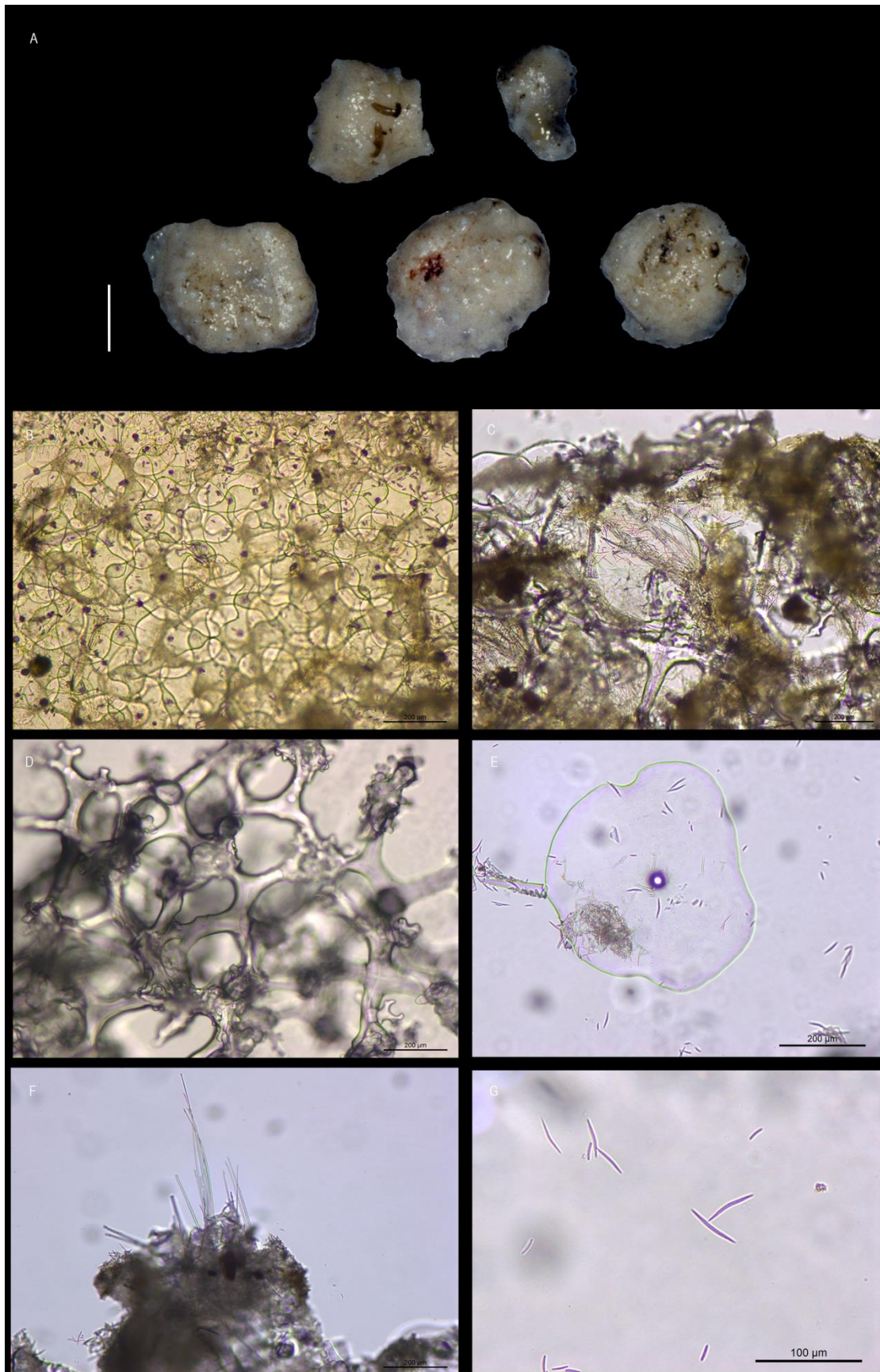


Fig. 12- *Discodermia verrucosa* Topsent, 1928. A, specimens collected in Madeira Island, ex situ (scale 1 cm); B, ectosome

with a compact layer of discotriaenes (scale 200 µm); **C**, transversal image of choanosomal skeleton (scale 200 µm); **D**, tetraclore desmas (scale 200 µm); **E**, discotriaene (scale 200 µm); **F**, long oxeas (scale 200 µm); **E**, microxeas (bigger) and acanthorhabds (smaller)(scale 100 µm).

REMARKS: The analysis of the genus *Discodermia* is very difficult, given the few and very variable morphological characters available for the distinction between the different species (Pisera & Vacelet, 2011). This is particularly challenging in the case of the three species described for the northeast Atlantic, *D. polydiscus* Bowerbank, 1869, *D. ramifera* Topsent, 1892 and *D. verrucosa* Topsent, 1928 since: there are only the original descriptions, some size classes of spicules are identical or they fall within the same range in different species, discotriaenes and microscleres (acanthorhabds and acanthoxeas) are very variable in shape and size within the same species and even specimen. This high variability of characters is also found in the Mediterranean *D. polymorpha* Pisera & Vacelet, 2011, for which the authors attributed a series of highly variable specimens to one same morphospecies. They further propose the use of molecular markers to help to identify *Discodermia* species.

Genus Racodiscula Zittel, 1878

TYPE SPECIES: *Racodiscula asteroides* Zittel, 1878

DEFINITION: Massive pillow to subspherical lithistids; megasclere spicules are phyllo- to discotriaenes; microscleres are streptasters/amphiasters with long and slender rays and two types of acanthorhabds which may vary to pseudospherasters (Pisera & Lévi, 2002e).

Racodiscula clava sensu Topsent, 1892

(Figs. 13A-C; 14A-G)

EXAMINED MATERIAL: HBOI - 29-V-91-3-003, 9-VI-91-4-008; MNHN - IP-2008-191, IP-2008-192, IP-2008-193, IP-2008-195, IP-2008-196, IP-2008-197, IP-2008-198, IP-2008-199, IP-2008-200, IP-2008-201, IP-2008-202, IP-2008-240, IP-2008-243; DOP – 1976, 1977, 2973, 2944, 5048, 5814, 5883, 6212, 6238, 6248, 6255, 6582.

EXTERNAL MORPHOLOGY: Columnar to ficiform lithistids, that may exhibit lateral protuberances or branches. Surface is smooth with marked channels. Oscules are not visible with the naked eye. Small sponges with approximately 21 mm length and 11 mm width, attached to the substrate by the entire lower base. Colour varies from whitish to light brown (Fig. 13A).

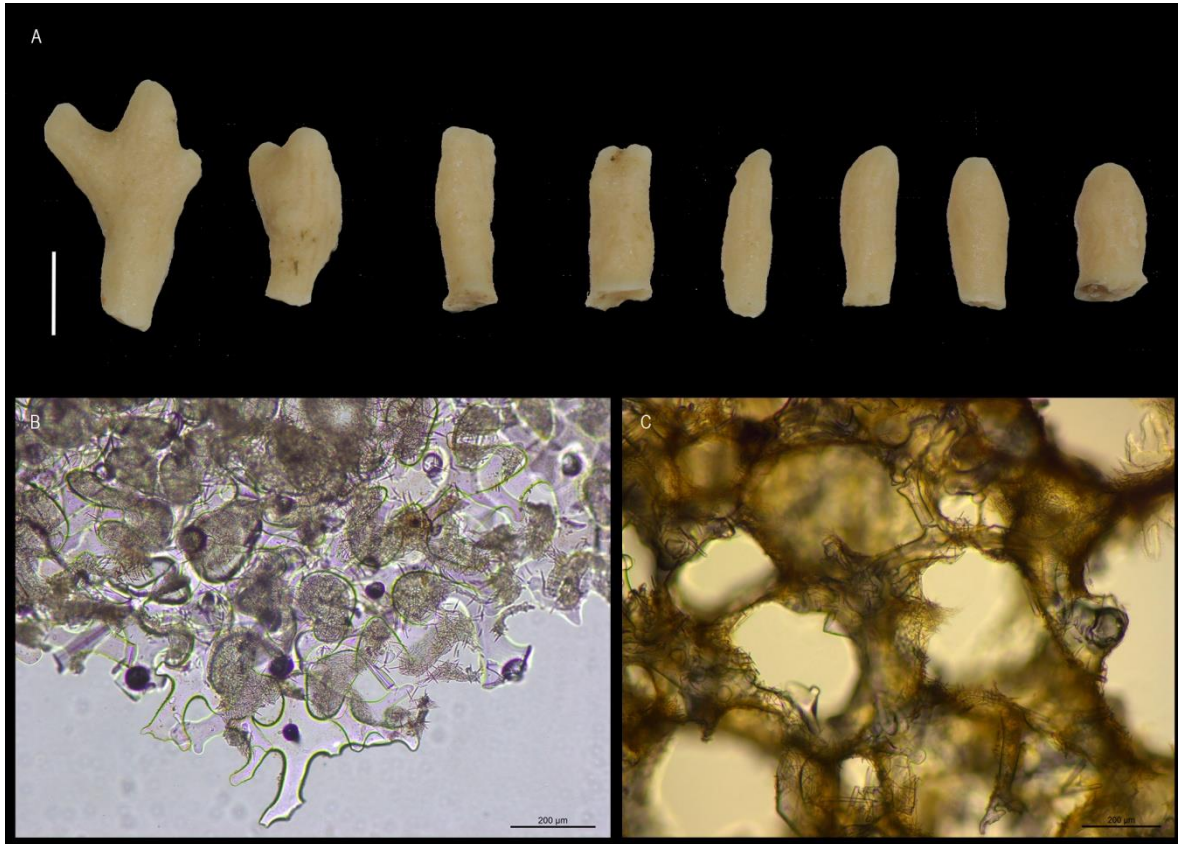


Fig. 13- *Racodiscula clava sensu* Topsent, 1892. **A**, specimens collected in the Hyeres seamount, *ex situ* (scale, 1cm); **B**, ectosome, where one observes a layer of phyllostriaenes and abundant microscleres (scale 200 µm); **C**, choanosomal skeleton composed of tetractones (scale 200 µm).

SKELETON: Ectosomal surface (Fig. 13B) constituted by a layer of phyllostriaenes, abundant acanthorhabds and acanthoxeas, and some spirasters. Pores are surrounded by these microscleres. Oxeas (or bundles of oxeas in some specimens) transverse to the surface cross both parts of the skeleton. Choanosomal skeleton (Fig. 13C) composed of tuberculated tetractones in an irregular net (Figs. 14A-B) as megascleres; acanthorhabds and acanthoxeas are also abundant and spread through the entire skeleton; spirasters are few and dispersed.

SPICULES: Ectosomal megascleres are phyllostriaenes and oxeas; the cladome of phyllostriaenes is large, strongly incised and very variable in shape, 231 – 534 – 742 µm in

diameter, and the rhabdome has a conical shape and pointed end, $60 - \underline{137} - 325 \times 12 - \underline{38} - 66 \mu\text{m}$ (Fig. 14C); oxeas are smooth, and have rounded and slightly curved extremities, $160 - \underline{459} - 1386 \times 4.4 - \underline{11} - 25 \mu\text{m}$ (only some oxeas were unbroken) (Fig. 14D). Tetraclone desmas are robust with smooth and large tubercles, mainly in its extremities/zygoses, forming big meshes (Figs. 14A-B). Acanthoxeas are slightly curved with pointed ends, $20 - \underline{38} - 68 \mu\text{m}$ length and $1.4 - \underline{3.4} - 5 \mu\text{m}$ width (Fig. 14E); acanthorhabds are $7.5 - \underline{17.7} - 35.4 \mu\text{m}$ in length and $1.2 - \underline{2.6} - 11.2 \mu\text{m}$ in width (Fig. 14F); spirasters have thin and spinous arms, $6.2 - \underline{11.4} - 27.6 \mu\text{m}$ length (Fig. 14G).

DISTRIBUTION: The specimens of *R. clava* analysed were sampled in the Azores, Madeira, and Canary islands and on several seamounts (Tyro, Atlantis, Hyères, and Plato) in a depth range of 280-1000 m. All records, except those for the Azores and the Canaries, are new.

REMARKS: In 1879, Schmidt described the species *Rimella clava* (family Phymaraphinidae) from specimens collected in the Caribbean Sea in the vicinity of Havana at 534 m depth. In his monographs, Topsent (1892a, 1904b) assigned several specimens collected in the Azores and Canary Islands to *Racodiscula clava* Schmidt and synonymized *Rimella* with *Racodiscula*. Later on, Pisera & Lévi (2002n) re-identified Topsent's material as belonging to a similar yet undescribed species of *Racodiscula* (family Theonellidae). Schmidt's *Rimella* was in the meantime found to be a pre-occupied genus and thus changed to *Exsuperantia* (Ozdkimen, 2009). Since the original specimens of Schmidt (also analysed by A. Pisera and C. Lévi) were deciduous, it is unclear what the spicular complement of *Exsuperantia clava* is. The specimens analysed in the present study match the traits described by Topsent (1892a, 1904b) for *Racodiscula clava* and certainly belong to the family Theonellidae, *i.e.* they have tetraclone desmas and not triders as in Phymaraphiniidae. Consequently the nominal species *Racodiscula clava* Topsent, 1892 should be kept.



Fig. 14 – *Racodiscula clava* sensu Topsent, 1892. A, general view of tetraclone desmas (scale 100 μ m); B, detail of the

tetracles' shapes, note the smooth and large tubercles (scale 50 µm); **C**, bottom view of phyllotriaenes, where is clear the variability of shapes (scale 100 µm); **D**, oxea (scale 100 µm); **E**, acanthoxeas (scale 5 µm); **F**, acanthorhabds (scale 5 µm); **G**, spirasters (scale 5 µm).

Family SIPHONIDIIDAE Lendenfeld, 1903

DIAGNOSIS: Polymorphic lithistids, massive to globular with fistules or vase-shaped; no distinct ectosomal spicules; choanosomal spicules are rhizoclone desmas and exotylostyles with ornamented spinose head or styles; no microscleres (Pisera & Lévi, 2002f).

Genus *Siphonidium* Schmidt, 1879

TYPE SPECIES: *Leiodermatium ramosum* Schmidt, 1870.

DEFINITION: Polymorphic Siphonidiidae, encrusting, massive irregular, hemispherical or irregularly cylindrical to club-shape with fistules; without special ectosomal spicules; rhizoclone desmas and exotylostyles as choanosmal spicules (Pisera & Lévi, 2002f).

Siphonidium ramosum (Schmidt, 1870) (Figs. 15A-E)

EXAMINED MATERIAL: MNHN – IP-2008-232, IP-2008-236, IP-2008-245, IP-2008-256b; DOP – 5048, 5846, 6608.

EXTERNAL MORPHOLOGY: Polymorphic Siphonidiidae, cylindrical, arborescent, attached to the surface by the base. They are usually quite small, 27 – 44 mm high and 2 – 10 mm wide, and extremely hard (difficult to cut). Colour varies from beige to light brown. Surface is smooth and often exhibits several fistules (1.4 – 5 x 1.1 – 1.7 mm) (Fig. 15A).

SKELETON: The ectosome (Fig. 15B) is difficult to distinguish from the choanosome, since there are no special ectosomal spicules in this species. Choanosomal skeleton is formed by rhizoclone desmas and exotylostyles (Fig. 15C). Rhizoclones form a compacted and extremely branched network. Exotylostyles are pin-shaped and are spread throughout the choanosome.

SPICULES: Rhizoclone desmas are extremely compact and dense (Fig. 15D); this species' skeleton is basically composed of these rhizoclones what makes them extremely hard; the rhizoclones are extremely branched, especially in the surface, becoming less branched and slightly more compact in the interior. Exotylostyles have spiny heads and pointed ends, with 80 – 262 – 487 μm in length and 2.2 – 4.8 – 9.5 μm in width (Fig. 15E).

DISTRIBUTION: The specimens of *S. ramosum* were collected in the Azores archipelago and on the Atlantis and Hyères seamounts at 457-950 m depth. This is the first record of this species, whose type-locality is in Florida, for the northeast Atlantic seamounts.

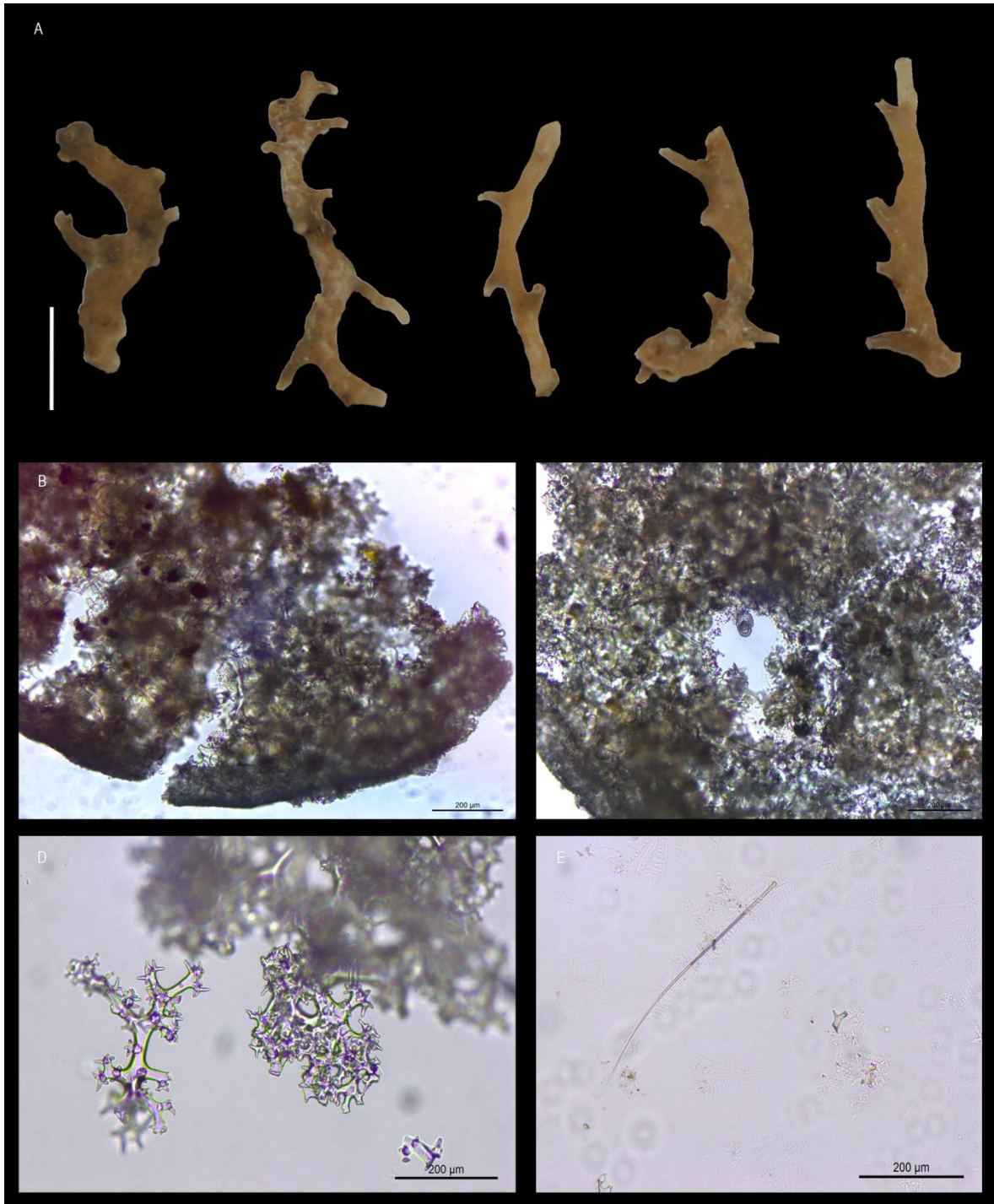


Fig. 15- *Siphonidium ramosum* (Schmidt, 1870). **A**, specimens collected in the Canaries, *ex situ* (scale 1 cm); **B**, image of the ectosomal (lower part) and choanosomal skeleton (scale 200 µm); **C**, choanosomal skeleton formed by extremely compacted rhizoclonal desmas (scale 200 µm); **D**, rhizoclonal desmas (scale 200 µm); **E**, exotylostyle (scale 200 µm).

Family AZORICIDAE Sollas, 1888

DEFINITION: Polymorphic lithistids, foliose, vase-shaped or massive conical sponges; ectosomal megascleres are diactines, that may emerge beyond the sponge surface; choanosomal megascleres are extremely spined rhizoclones; microscleres, if present, are raphides (Kelly, 2007, after Pisera & Lévi, 2002h).

Genus *Leiodermatium* Schmidt, 1870

TYPE SPECIES: *Leiodermatium lynceus* Schmidt, 1870.

DEFINITION: Lamellate, plate-like, foliose, vase- or ear-shaped Azoricidae; oscules are visible; ectosomal spicules and microscleres are absent; Choanosomal desmas are spiny rhizoclones; megascleres are diactines (Kelly, 2007; modified from Pisera & Lévi, 2002h).

Leiodermatium sp.

(Figs. 16A-D; 17A-E)

EXAMINED MATERIAL: HBOI – 26-V-91-1-006, 27-V-91-3-013, 29-V-91-1-002, 14-V-91-1-004, 14-V-91-3-020; MNHN – IP-2008-237, IP-2008-239, IP-2008-249, IP-2008-251, IP-2008-253, IP-2008-255; DOP – 810, 1062, 1190, 1946, 1996, 2028, 2063, 2766, 2975, 4412, 4466, 4862, 5424, 6446, 6480, 6482, 6483, 6484, 6670, 6671, 6674.

EXTERNAL MORPHOLOGY: Lamellate, vase- to ear-shaped lithistids, attached to the substrate by the entire lower base (Figs. 16A-B). Inner and outer surface have marked differences: inner surface with large and elevated oscules and smooth edges (Fig. 16B) while the outer surface is apparently smooth but with small pores distributed over the entire surface. Large specimens may be confused with *N. nolitangere*, due to their lamellate form; the most distinguishing feature between the two species is the thickness of the walls that is much thinner in *Leiodermatium* species, 4.5 to 5.5 mm. The colour is beige to light brown (Fig. 16A) and blue (Fig. 16B) in life and alcohol; in some specimens, the blue colour disappeared over time in the alcohol.

SKELETON: The ectosome is difficult to distinguish from the choanosome, as both comprise rhizoclonal desmas; in the ectosome (Fig. 16C) it is possible to observe oxeas as megascleres (may formed bundles) and/or raphides as microscleres; both oxeas and raphides occasionally extend to the choanosome. Choanosomal skeleton (Fig. 16D) is formed by spiny rhizoclonal desmas (Figs. 17A-B).

SPICULES: Ectosomal oxeas are smooth, very long with pointed ends, 42 - 487 - 1919 μm length and 2.3 - 5.3 - 11.6 μm width (Figs. 17 C and D). Raphides are short, very thin, 37 - 192 - 508 \times 1 - 1.7 - 3 μm in size (Fig. 17E). Rhizoclone desmas form a compact and dense mesh extremely spiny; rhizoclonal desmas have multifurcating spines (Fig. 17B).

DISTRIBUTION: Specimens of *Leiodermatium* sp. were sampled in Madeira, Canary Islands and the Azores archipelagos, as well as on Atlantis, Great Meteor and Hyères seamounts, between 200-741 m depth. The genus *Leiodermatium* in this study area is represented by the species *Leiodermatium lynceus* Schmidt, 1870 (Portugal and St. Iago; depth unknown) and *Leiodermatium pfeifferae* (Carter, 1873) (Madeira, Portuguese mainland off Cape St. Vincent; 684 m depth). Although the identification down to species level was not possible, with this study more information concerning the distribution and bathymetric range of *Leiodermatium* was acquired; these are the first records of this genus to the northeast Atlantic seamounts and to the Canary Islands.

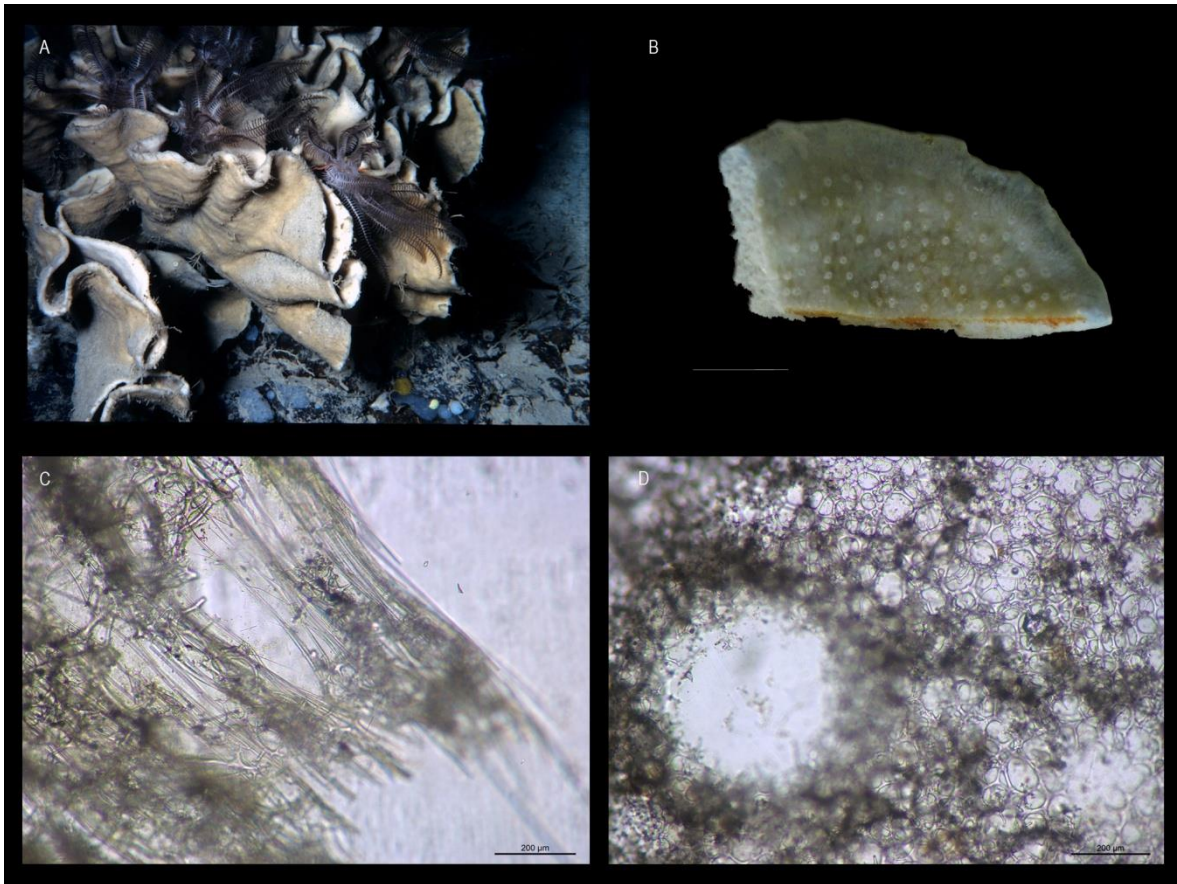


Fig. 16 - *Leiodermatium* sp.. **A**, specimen collected in Madeira, *in situ*; **B**, specimen collected in the Azores, *ex situ* (scale 1 cm); **C**, ectosome, composed by desmas, bundles of oxeas and some raphides (scale 200 μm); **D**, choanosome, showing rhizoclone desmas surrounding a pore (scale 200 μm).

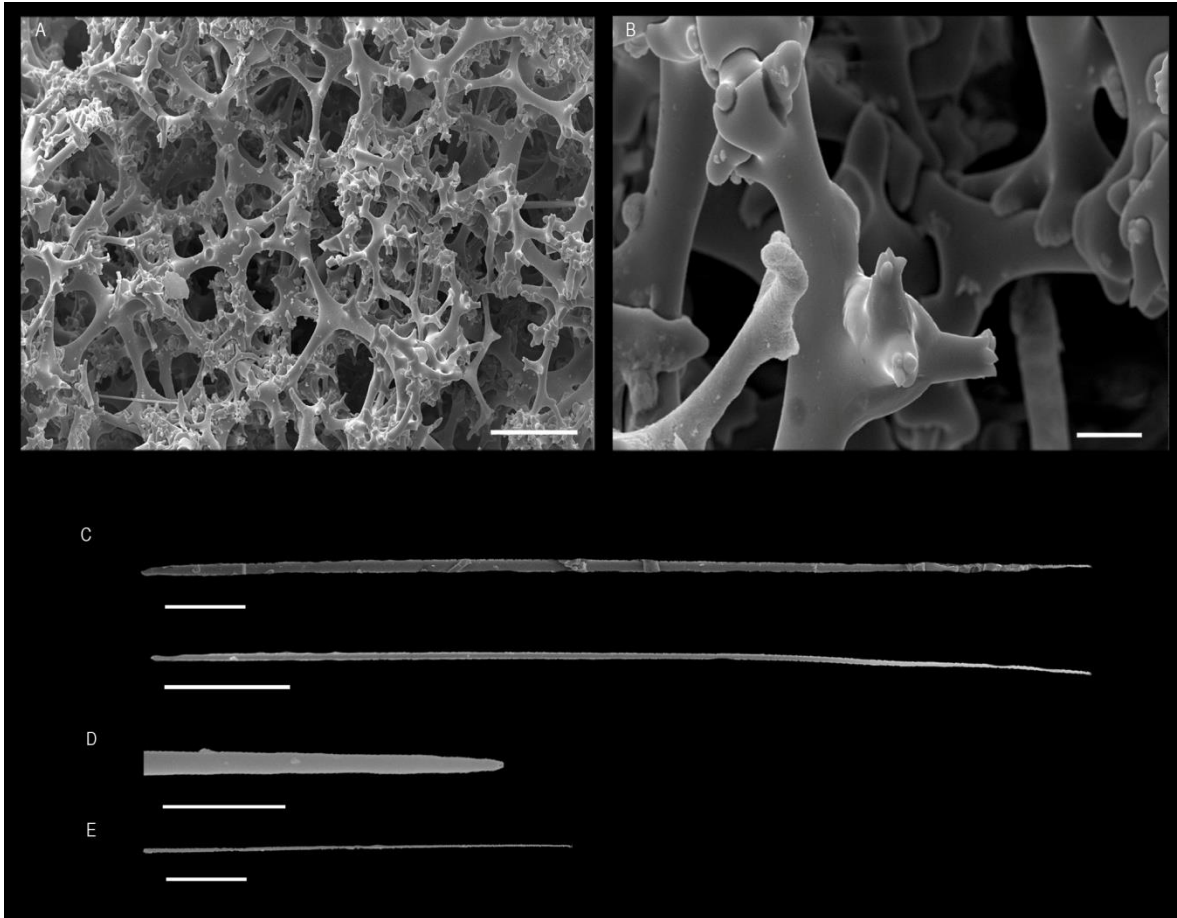


Fig. 17 - *Leiodermatium* sp.. **A**, overview of rhizoclone desmas (scale 100 µm); **B**, detail of the sculpture and terminations of rhizoclones, note the multifurcating spines of the terminations (scale 10 µm); **C**, oxeas (scale 100 µm); **D**, detail of an oxea's extremity (scale 10 µm); **E**, raphide (scale 100 µm).

REMARKS: The identification of species belonging to the genus *Leiodermatium* is very difficult due to the few diagnostic characters present in this genus, *i.e.* a simple skeleton composed uniquely of rhizoclones desmas and diactines (Pisera & Lévi, 2002h; Kelly, 2007). The identification of species within the genus is made based on the shape, disposition of oscules/ostia in both surfaces and on the ornamentation and termination of desmas. For these reasons, the use of SEM is pivotal for the differentiation between species of this genus (Kelly, 2007). In fact many authors refer that this genus is in great need of a systematic revision (Pisera & Lévi, 2002h; Kelly, 2007)

In the northeast Atlantic *Leiodermatium* is represented by two species only, *L. lynceus* Schmidt, 1870 and *L. pfeifferae* Carter, 1873. Despite being very similar, they were considered by Carter as two different species: 1) *L. lynceus* has oscules on the inner side of the lamellae, whereas *L. pfeifferae* has oscules on the outer side, and 2) the number of diactines around the margin is different in both species, *L. lynceus* with few and *L. pfeifferae*

with several diactines. In *Systema Porifera*, Pisera & Lévi (2002h) described *L. lynceus* the type species of the genus *Leiodermatium* based on slides of the holotype (MZUS PO145); in their description they do not appear to have found oxeas/diactines or any kind of mega- or microscleres in the holotype, but refer that van Soest & Stentoft (1988) found oxeas in their specimens from Barbados identified as *L. lynceus*. Topsent (1928) also mentioned the presence of two classes of oxeas in their specimens of *L. lynceus* (from Tenerife), but he only reported the size of one of the classes (larger than 1.8 mm and 7-9 μm width; he only refers the width of the other class, about 2 μm).

The material examined in this work, did not allow a differentiation into the two species reported for the northeast Atlantic Ocean due to: 1) the large quantities of oxeas/diactines/raphides observed on both surfaces in all specimens (never mentioned in the literature); and 2) the impossibility to distinguish the inner from the outer surface of the examined material since only small fragments were available for analysis, and the photos of the original material (when available) did not have enough resolution that would allow to detect the differences between the surfaces.

Family MACANDREWIIDAE Schrammen, 1924

DEFINITION: Polymorphic sponges; ectosomal megascleres are phyllo/discotriaenes very carved and dentate; choanosomal desmas with triaenose (rarely monaxial) crepsis; only one type of microscleres: microxeas in the ectosome (Pisera & Lévi, 2002m).

Genus *Macandrewia* Gray, 1859

TYPE SPECIES: *Macandrewia azorica* Gray, 1859.

DEFINITION: Polymorphic Macandrewiidae; ectosomal spicules are dentate phyllotriaenes and/or discotriaenes; desmas are smooth with a predominant terminal zygotis with a triaenose crepsis (rarely monaxial); microscleres are microxeas (Pisera & Lévi, 2002m).

Macandrewia azorica Gray, 1859

(Figs. 18A-D; 19A-G)

EXAMINED MATERIAL: HBOI – 2-VI-91-1-009; MNHN – IP-2008-217, IP-2008-219, IP-2008-220, IP-2008-222, IP-2008-226, IP-2008-228, IP-2008-229, IP-2008-230, IP-2008-249; DOP – 1012, 1037, 2398, 2409, 2843, 3796, 4079, 4468, 4845, 5334, 5602, 5774, 5882, 6247, 6249, 6601.

EXTERNAL MORPHOLOGY: Polymorphic Macandrewiidae, ficiform in young individuals and cyathiform to flabellate in developed ones; in small specimens the stem is inconspicuous, and is similar to *Macandrewia robusta* (see description below); in large ones, the stem is short, 10 – 27 mm, and they have undulated margins; the walls are thick, 4 - 10 mm. Inner and outer surfaces are very different; the inner surface is covered by several oscules, visible to the naked eye, while the outer surface is smooth, with many small pores. The colour of these sponges varies from beige to light brown (Figs. 18A-B).

SKELETON: The ectosome (Fig. 18C) is constituted by a compact layer of phyllostriaenes as megascleres and fusiform microxeas as microscleres; phyllostriaenes are transverse to the surface while microxeas are arranged radially around the pores/oscules. Choanosomal skeleton has two types of megascleres, rhizoclone desmas which form a regular network (Figs. 19A-B) and smooth fusiform oxeas with blunt tips; microscleres are dispersed fusiform microxeas, also smooth and with blunt tips.

SPICULES: Phyllostriaenes have the cladome extremely incised with 209 – 300 – 480 µm in diameter, and a short conical-shape rhabdome, 51 – 118 – 184 x 11.5 – 17.9 – 30.4 µm in size (Figs. 19C-E). Rhizoclone desmas form a compact and regular mesh with large spaces between them; rhizoclones are smooth and quite branched, with blunt ends (Figs. 19A-B). Oxeas are smooth, slightly curved with pointed ends, with 124 – 322 – 699 µm length and 3.3 – 7.6 – 13.3 µm width (Fig. 19F). Microscleres are smooth fusiform microxeas, measuring measure 28.3 – 56.9 – 95.4 µm in length and 2.3 – 6.9 – 7.7 µm in width (Fig. 19G).

DISTRIBUTION: Specimens of *M. azorica* were found in the Azores (type-locality), Selvagens and Canary islands, as well as on several of the northeast Atlantic seamounts

(Atlantis, Tyro, Great Meteor, Antialtair, Plato, Hyères, Irving) at 256-990 m depth. These are the first records of this species to Selvagens and the seamounts of the northeast Atlantic.

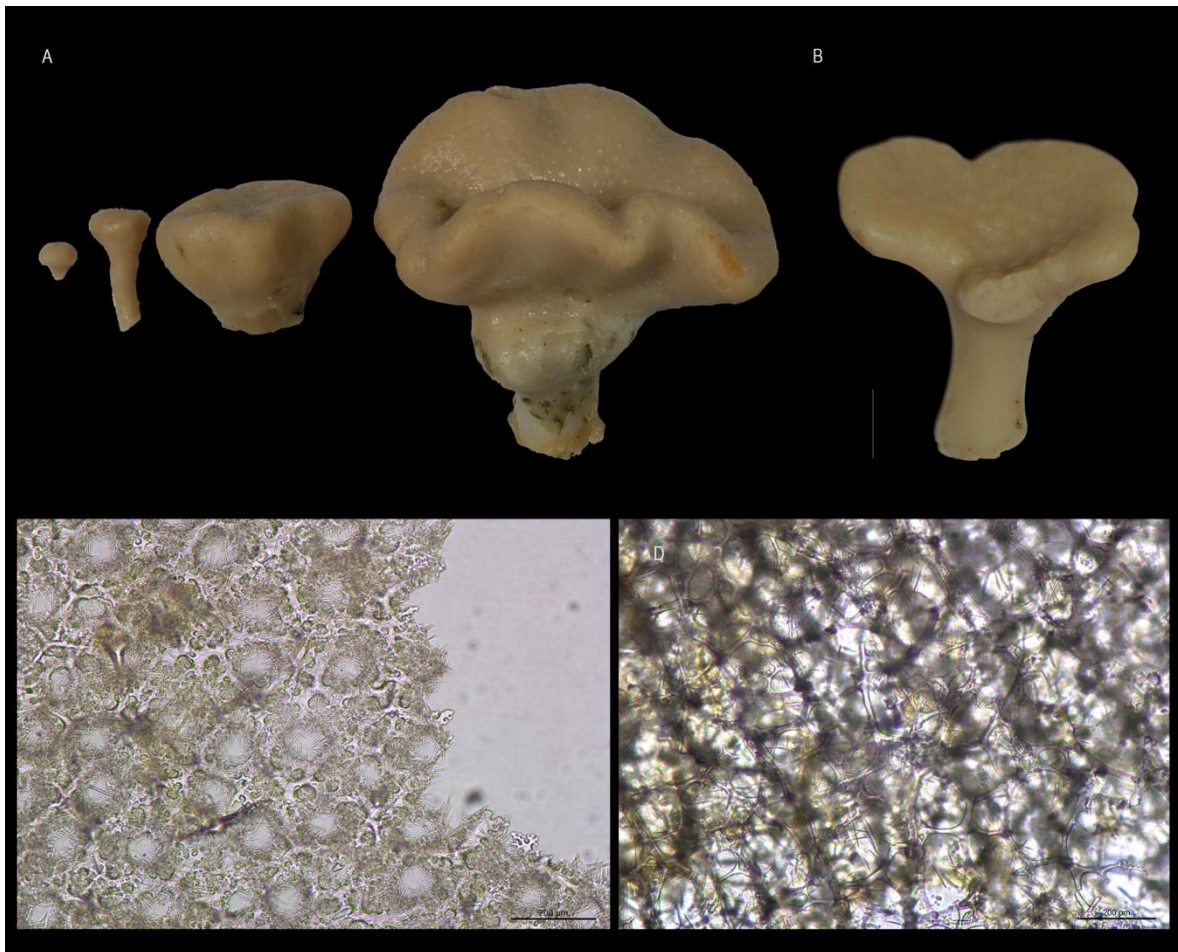


Fig.18 - *Macandrewia azorica* Gray, 1859. **A**, specimens collected on the Tyro seamount, *ex situ*, note the diversity of shapes in different stages (no scale); **B**, specimen collected in Azores, *ex situ* (scale 1 cm); **C**, ectosome showing a layer of abundant phyllostriaenes and microscleres around the pores (scale 200 μ m); **D**, choanosomal skeleton formed by rhizoclones desmas (scale 200 μ m).

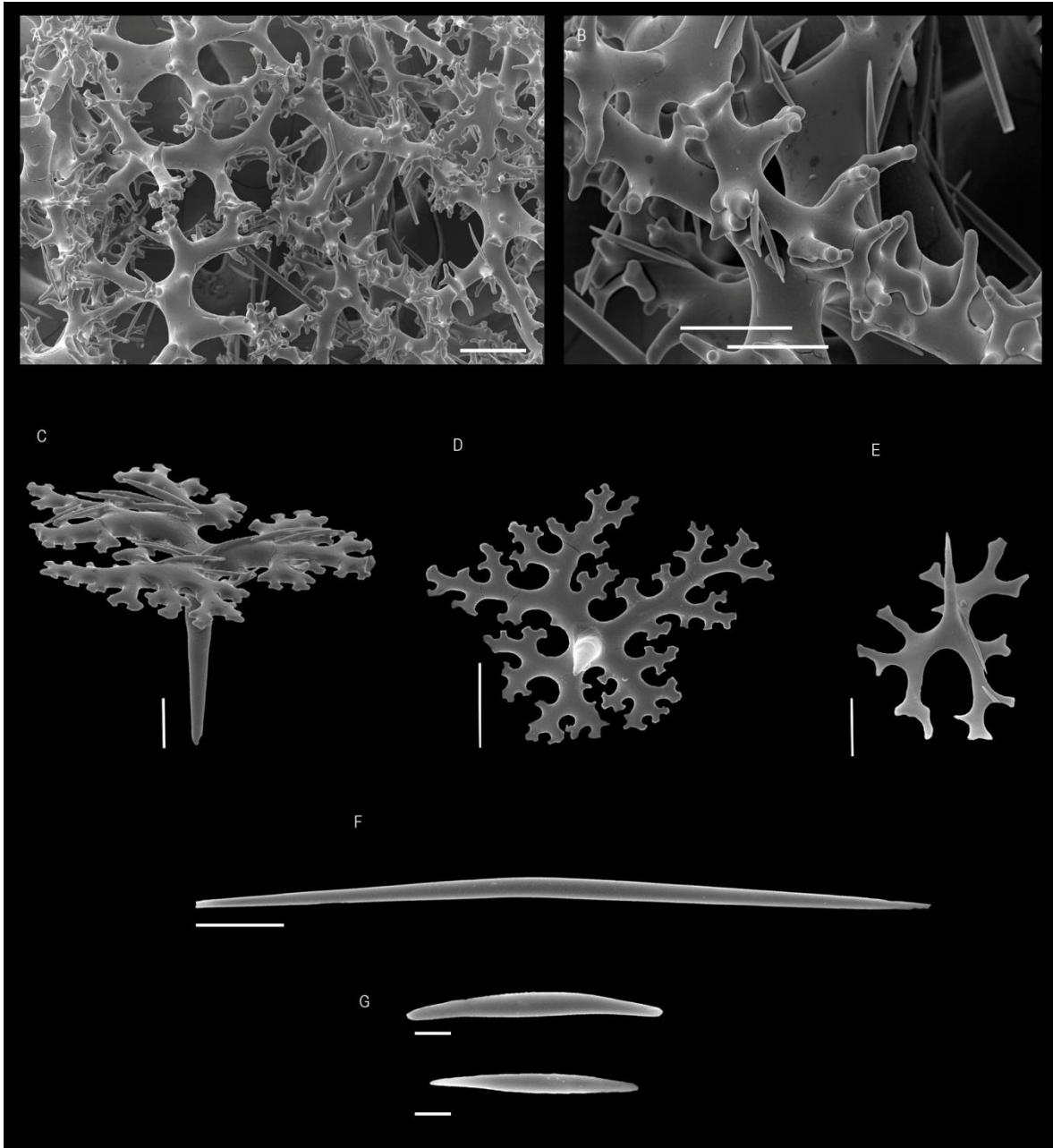


Fig. 19 - *Macandrewia azorica* Gray, 1859. **A**, general view of rhizoclone desmas (scale 100 μ m); **B**, detail of articulation and shape of the rhizoclones (scale 50 μ m); **C**, phyllotriaene (scale 50 μ m); **D**, bottom view of phyllotriaene showing the extremely indented cladome (scale 100 μ m); **E**, small phyllotriaene in development (scale 50 μ m); **F**, oxea (scale 50 μ m); **G**, fusiform microxeas (scales 10 μ m).

Macandrewia robusta Topsent, 1904

(Figs. 20A-C; 21A-E)

EXAMINED MATERIAL: MNHN – IP-2008-216, IP-2008-224, IP-2008-225; DOP – 2866.

EXTERNAL MORPHOLOGY: Sponges with ficiform to globular shape, with or without a short and thick pedicel. When the pedicel is present, 5-7 mm length and 7-14 mm width, the sponges are attached to the substrate by this pedicel; and when it is absent they are attached by the entire lower base. Inner and outer surfaces are quite different; the upper surface is irregular with small and well evident oscules, while the outer surface is smooth with small pores spread throughout the entire surface. In some individuals the upper surface has a slight depression. The colour varies from beige to light brown (Fig. 20A).

SKELETON: Ectosome (Fig. 20B) is composed of a layer of phyllostriaenes and abundant fusiform microxeas; the pores are surrounded by radially disposed microxeas. Choanosomal skeleton (Fig. 20C) constituted by highly branched rhizoclone desmas with blunt extremities (Figs. 21A-B) and smooth oxeas; microscleres are fusiform microxeas dispersed throughout the choanosome.

SPICULES: Ectosome formed by a compact layer of phyllostriaenes which have an extremely indented cladome with 89 - 266 - 429 μm in diameter, and a conical rhabdome 35 - 85 - 183 x 2.6 - 18.7 - 25.7 μm in size (Fig. 21C). Rhizoclones are smooth, very branched with blunt endings, forming a regular mesh (Figs. 21A-B). Oxeas are smooth with rounded ends, 112 - 206 - 388 μm in length and 2.7 - 7.8 - 15.2 μm in width (Fig. 21D). Microxeas are smooth, slightly curved with pointed ends, 35 - 68 - 101 x 2.4 - 5.1 - 8.3 μm in size (Fig. 21E).

DISTRIBUTION: Specimens of *M. robusta* were sampled in the Azores archipelago and on the Hyères and Tyro seamounts, at 635-1000 m depth. Previous to this study, this species was only known to occur in the Azores at 1165 m depth. Thus, knowledge into the geographical and bathymetric range of the species is hereby considerably expanded.

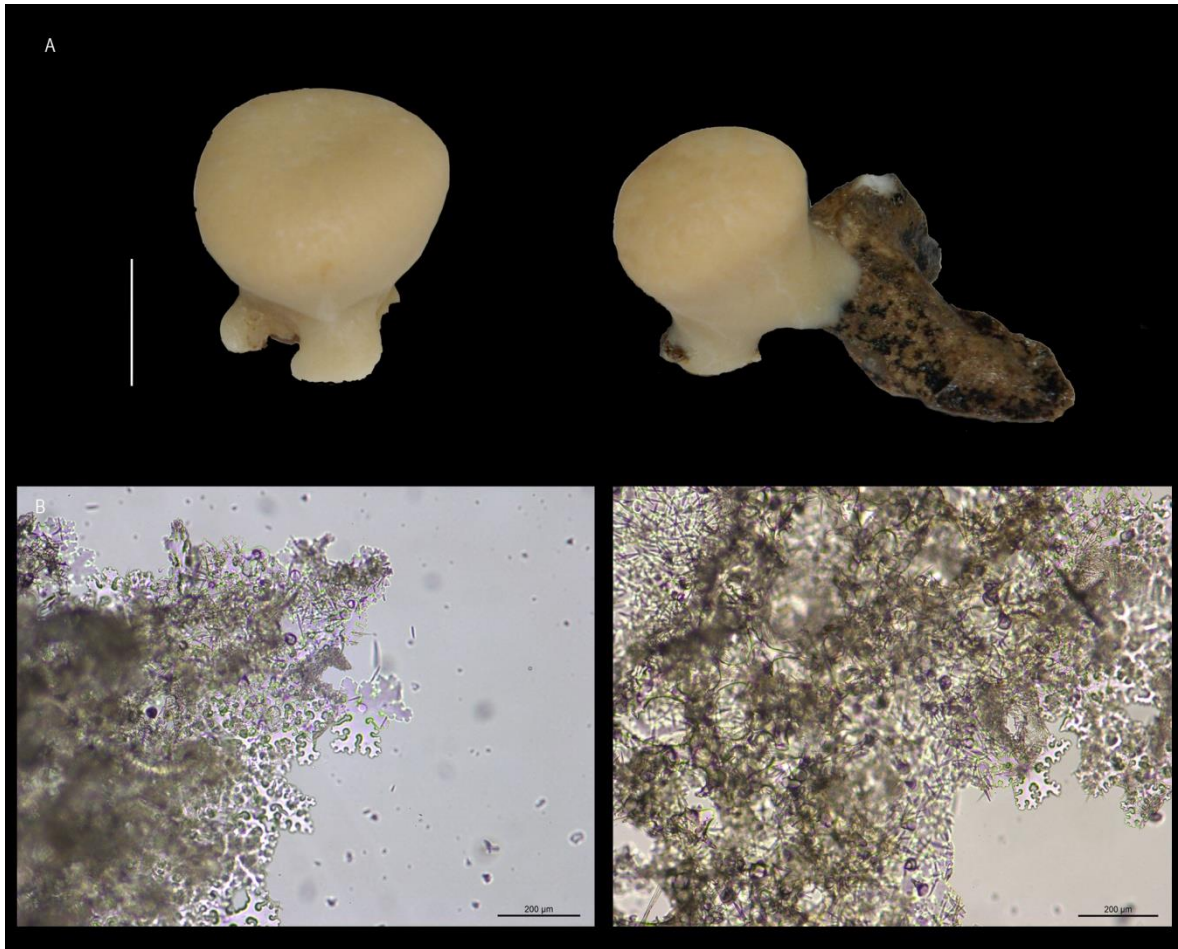


Fig. 20- *Macandrewia robusta* Topsent, 1904. **A**, Specimens collected on the Hyères seamount, *ex situ* (scale 1 cm); **B**, ectosome composed of abundant phyllotrias and microscleres (scale 200 µm); **C**, transversal view of choanosomal skeleton showing rhizoclones and part of the ectosome (200 µm).

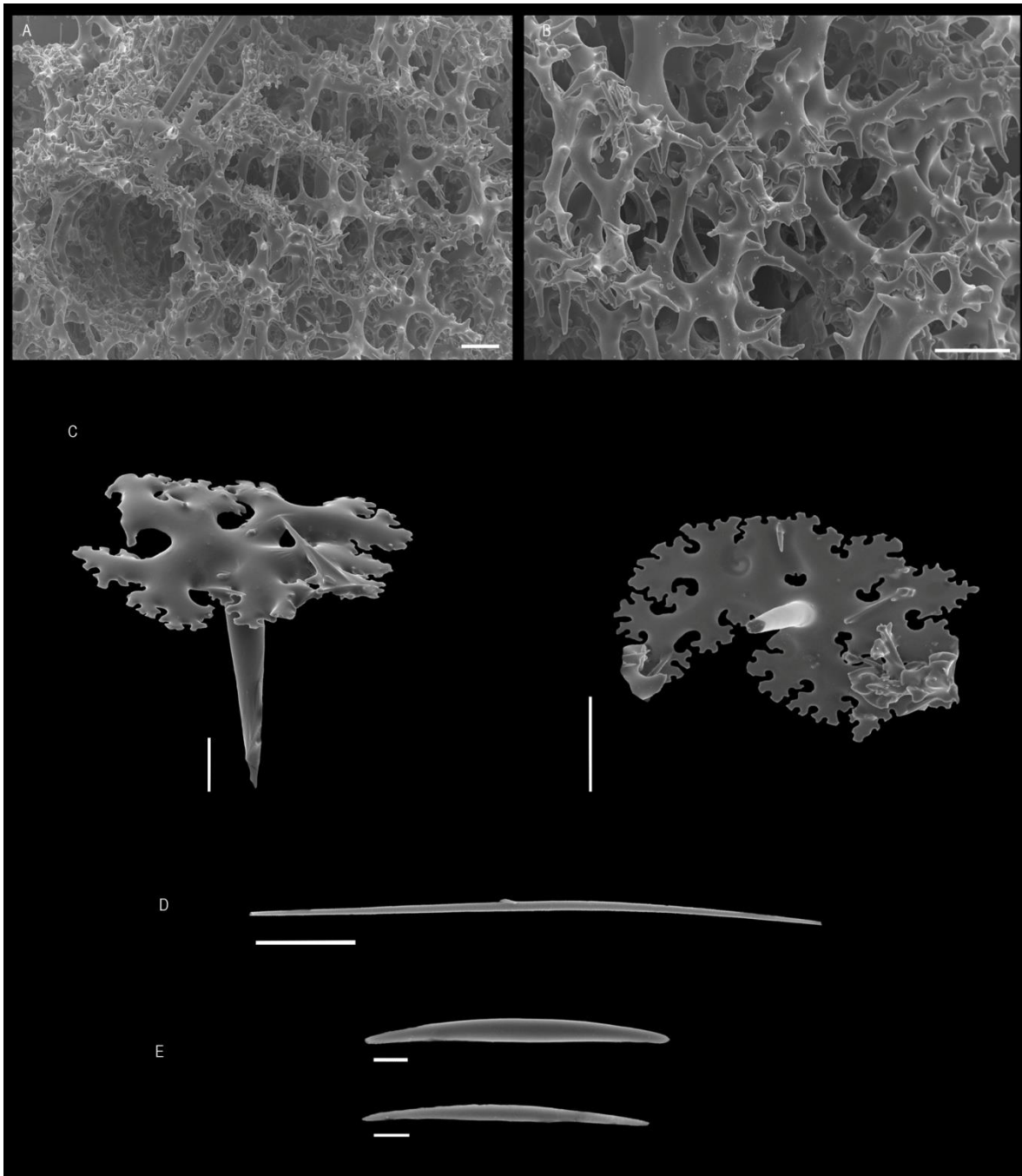


Fig. 21 - *Macandrewia robusta* Topsent, 1904. **A**, overview of rhizoclone desmas (scale 100 μ m); **B**, detail of rhizoclones (scale 100 μ m); **C**, phyllotriaenes (scales 50 and 100 μ m, from left to right); **D**, oxea (scale 100 μ m); **E**, fusiform microxeas (scale 10 μ m).

3.3. PHYLOGENETIC RELATIONSHIPS

A total of 65 sequences (36 from the mtDNA COI gene and 29 from the rDNA 28S gene) were generated in the course of this study. Sequences from the mtDNA COI gene represent seven genera and eight species of which those of *C. masoni*, *I. harborbranchi*, *N. bowerbankii*, *M. azorica*, *D. ramifera* and *D. verrucosa* were generated for the first time. The rDNA 28S sequences represent six genera and six species, viz. *C. masoni*, *N. nolitangere*, *N. bowerbankii*, *D. ramifera*, *R. clava* and *Leiodermatium* sp. (all generated for the first time). Unfortunately, not all lithistid species analyzed in this study could be sequenced due to either preservation mode (e.g. the entire Seamount-2 collection was originally preserved in formaldehyde) or unsuccessful results in extraction/amplification/sequencing. Examples are the species *M. robusta*, *S. ramosum* and *Leiodermatium* sp.; and *I. harborbranchi* n. sp., *M. azorica*, *M. robusta*, *S. ramosum* and *D. verrucosa* for which no sequences of COI and 28S were obtained, respectively.

In the phylogenetic analysis of the mt DNA COI gene lithistids were represented by three families (Corallistidae, Theonellidae and Macandrewiidae) and by seven genera, in a total of nine species: *C. masoni*, *N. nolitangere*, *N. bowerbankii*, *I. harborbranchi*, *D. verrucosa*, *D. ramifera*, *D. polymorpha*, *Theonella swinhoei* and *M. azorica*; and additional sequences of the orders Spirophorida and Astrophorida were used (Appendix II, Tab. I). The best tree resulting from the phylogenetic reconstruction, by means of Bayesian inference of the mtDNA COI gene is shown in Fig. 22 and confirms the astrophorid affinity of the studied species. The family Corallistidae was revealed monophyletic (posterior probability of 0.97) and a sister-taxa relationship was found between the genera *Neoschrammeniella/Neophrissospongia* (posterior probability of 1) and *Herengeria/Isabella* (posterior probability 1). This family forms a poorly supported clade with Pachastrellidae (posterior probability 0.74) here represented by *Pachastrella nodulosa*, *P. ovisternata* and *Triptolemma intextum*. The family Theonellidae was non-monophyletic, where the genus *Discodermia* forms a poorly supported clade with *Theonella* and *Characella pachastrelloides* (posterior probability of 0.65), while *Racodiscula clava* is closest to the family Vulcanellidae (*Poecilastra compressa*, *Vulcanella aberrans* and *V. gracilis*) (posterior probability of 0.78). It is important to mention that the sequence deposited in GenBank as *Exsuperantia* sp. is in fact from a specimen of *Racodiscula clava sensu* Topsent, 1892, thus belonging to the family Theonellidae and not Phymaraphiniidae. It is for that reason that this sequence groups with the other *Racodiscula clava* sequences. The placement of the family Macandrewiidae within

Astrophorida remains unclear. The status at species and genus level is clear and well supported for all clades, with exception of *Isabella mirabilis* (from the Pacific Ocean) that was retrieved paraphyletic in regards to *I. harborbranchi* (posterior probability of 1).

In the phylogenetic analysis of the rDNA 28S lithistids were represented by four families (Azoricidae, Corallistidae, Desmanthidae and Theonellidae), in a total of eight genera and 11 species: *Leiodermatium* sp., *Corallistes* sp., *C. masoni*, *N. nolitangere*, *N. bowerbankii*, *Desmanthus incrustans*, *Discodermia dissoluta*, *D. ramifera*, *R. clava*, *Theonella swinhoei* and *Theonella* sp.; and sequences of the orders Astrophorida, Hadromerida, Halichondrida and Poecilosclerida were added (Appendix II, Tab. II). The best tree resulting from the phylogenetic reconstruction, by means of Bayesian inference of the rDNA 28S gene is shown in Fig. 23. The overall topology is similar to that of the COI tree and the status at genus and species level are well resolved. The family Corallistidae forms a monophyletic clade (posterior probability of 0.99) including the genera *Corallistes*, *Neophrissospongia* and *Neoschrammeniella* within which the last two also form a highly supported clade (posterior probability of 0.89). The family Theonellidae is non-monophyletic, and includes several members of the family Ancorinidae Schmidt, 1870 (*Stelletta* and *Stryphnus*) but this clade is, however, poorly supported (posterior probability of 0.67). Nonetheless, the species/genera of the members within Theonellidae form well supported clades (posterior probabilities of 1 in *Theonella* and *Racodiscula*; posterior probability of 0.94 in *Discodermia*). The families Corallistidae and Theonellidae, seem to exhibit an astrophorid affinity, but the support for this is not very high (posterior probability of 0.79). The family Azoricidae is only represented by the genus *Leiodermatium* in this analysis. Nevertheless, this clade is well supported (posterior probability of 1) and exhibits a basal position in relation to the astrophorid/lithistid clade. Curiously, the species *Desmanthus incrustans*, considered a lithistid in Sytema Porifera, falls within a strongly supported clade with members of the order Halichondrida (posterior probability of 1).

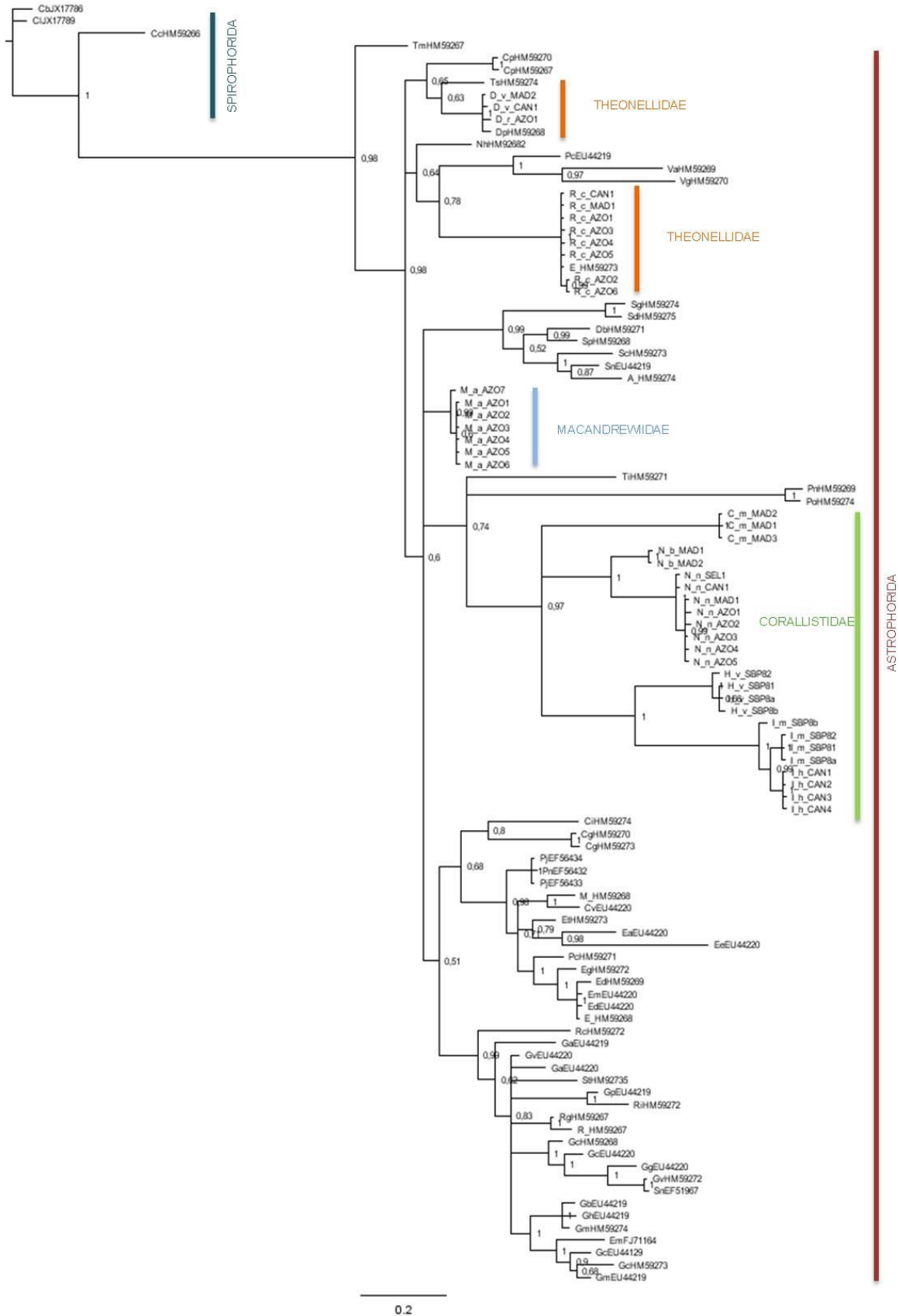


Fig. 22 – Bayesian phylogeny of lithistid demospongiae based on mtDNA COI gene sequences. Lithistids are represented by: *Discodermia verrucosa* (D_v); *Discodermia ramifera* (D_r); *Discodermia polydiscus* (D_p); *Racodiscula clava* (R_c); *Macandrewia azorica* (M_a); *Corallistes masoni* (C_m); *Neoschrammeniella bowerbankii* (N_b); *Neophrissospongia nolitangere* (N_n); *Herengeria vasiformis* (H_v); *Isabella mirabilis* (I_m); and *Isabella harborbranchi* (I_h). For further information on sequences codes and accession nos. see Table I in Appendix II.



Fig. 23 - Bayesian phylogeny of lithistid Demospongiae based on rDNA 28S (D3-D5) gene fragment. Lithistids are represented by: *Discodermia ramifera* (Dr); *Discodermia dissolute* (Ds); *Racodiscula clava* (Rc); Tsp as *Theonella* sp. Cm as *Corallistes masoni*; Nb as *Neoschrammeniella bowerbankii*; Nn as *Neophrissospongia nolitangere*; Lsp as *Leiodermatium* sp.; Di as *Desmanthus incrustans*.

CHAPTER 4. DISCUSSION

4.1 LITHISTID SPONGES OF THE NORTH ATLANTIC OCEAN AND MEDITERRANEAN SEA

Knowledge concerning the distribution and diversity of the lithistid fauna in Macaronesian region was mainly restricted to the Azores archipelago, mostly as the result of the late XIX and, early XX centuries' scientific campaigns of Prince Albert I of Monaco, and the remarkable work on sponge systematics made by Émile Topsent, a renowned French taxonomist (Topsent, 1892, 1904, 1928). Of a total of 13 species recorded for these islands (e.g. Bowerbank, 1869; Carter, 1873; Topsent, 1892, 1904, 1928), 10 were found in this study. Only the species *Petromica (Petromica) grimaldii* and *Macandrewia ramifera* Topsent, 1904 (recorded from Azores) were not found in the examined collections, and additionally the identification of the *Leiodermatium* specimens, down to species level was not possible. The family Corallistidae Sollas, 1888 is the best represented in the area with a total of four genera: *Corallistes* Schmidt, 1870, *Neoschrammeniella* Pisera & Lévi, 2002, *Neophrissospongia* Pisera & Lévi, 2002 and *Isabella* Schlacher-Hoenlinger, Pisera & Hooper 2005 and four species. The family Theonellidae Lendenfeld, 1903 is represented by two genera, *Discodermia* du Bocage, 1869 and *Racodiscula* Zittel, 1878, and three species, with the remaining families Siphonidiidae Lendenfeld, 1903, Azoricidae Sollas, 1888 and Macandrewiidae Schrammen, 1924 only represented by one genus each.

Previous to this work, the difference in the diversity of lithistids between the Azores and the other Macaronesian Islands was substantially larger (10 species in the Azores, three in Madeira, none in Selvagens, and five in the Canaries; Tab. I) as a result of an uneven sampling effort in this area. With the new records here reported these differences are considerably reduced. Furthermore, here are presented the first records of lithistids in the seamounts located south of the Azores, with a total of 8 species. It is important to note, that these results were product of the identification of 162 specimens of which 81 were sampled in the Azores, 32 in Madeira, Selvagens and the Canaries, and 49 on the seamounts, which may still account for some diversity bias towards the most sampled locations.

The bathymetric distribution of lithistids in the northeast Atlantic was also poorly known for the majority of the species. The depth at which *Corallistes masoni* occurred was unknown and the bathymetric distribution of the remaining species was in general, only known from the Azores archipelago. In this study, most species were found in the upper bathyal (200-800m) although some were found at shallower circalittoral depths (above

200m) or at the upper lower bathyal (800-2000m). The known bathymetric ranges of the species *N. nolitangere*, *N. bowerbankii*, *D. ramifera* and *D. verrucosa* were considerably expanded with the newly examined specimens. *N. nolitangere* was only recorded from 400m depth or in sublittoral caves (10-20m); now its bathymetric distribution ranges from 91 to 501m depth. *N. bowerbankii* was only recorded from 113-131m, in contrast with the range of 480-695m depth found in this study. *D. ramifera* was recorded from the Azores between 98-318m and now the bathymetric range was expanded until 475m depth; similarly, *D. verrucosa* (known from 400m) has now a larger bathymetric range, 300-497m depth. The species *R. clava* and *S. ramosum* were found at previously reported depths, while *Leiodermatium* sp., *M. azorica* and *M. robusta* were collected at much shallower depths than previously known.

Besides the several new records for all areas, in the course of this study a new species of *Isabella*, a genus only known from the southwest Pacific, was discovered and fully described. This genus, until now monotypic, was described to accommodate the species *Isabella mirabilis* discovered on two seamounts of the Norfolk Ridge, a submarine ridge running between New Caledonia and New Zealand (Schlacher-Hoenlinger *et al.*, 2005). This is therefore the first record of this genus to the Atlantic Ocean, where the new species *I. harborbranchi* was found exclusively on the Canary Islands at 133-366m depth (Carvalho *et al.*, in prep). The identification based on morphological characters was further supported by the obtained molecular data, as the phylogenetic reconstruction based on the mtDNA COI gene shows that *I. harborbranchi* specimens form a strongly supported monophyletic clade sister to *I. mirabilis*.

It is important to note, that the detailed analysis of all specimens used in this work contributed to important taxonomic questions regarding the re-definition of some genera and species. This work proposed the re-definition of the genus *Neophrissospongia*, adding the presence of smooth dichotriaenes and microtylostyles (not rare) in its diagnosis (for more details see remarks in page 51). The genus *Isabella* was also re-defined here due to the finding of a second species belonging to this genus (see page 58). Based on the discovery of a third type of spiraster (microsclere) – with short, thick and pointed arms – in the species *Neoschrammeniella bowerbankii*, a future thorough revision of the species is required in order to determine the taxonomic significance of this finding (see page 55). A careful revision of the species *Leiodermatium pfeifferae* and *L. lynceus*, both recorded for the Atlantic Ocean was also suggested. Species belonging to this genus are very difficult to identify due to the

presence of few diagnostic characters (Pisera & Lévi, 2002h; Kelly, 2007). Furthermore, these two species are very similar and the finding of several diactine/oxeas in all analysed specimens, raised the question whether in fact it is only one species instead of two (as previously recorded). Unfortunately the material examined in the present study couldn't be confidently assigned to any of the two species recorded for the Atlantic Ocean. The morphological and molecular examination of additional specimens and the holotypes will be needed to ascertain these species' validity (see page 77). Finally, the validity of the species *Racodiscula clava* Topsent, 1892 as a member of the family Theonellidae was clarified here.

In the Mediterranean Sea lithistids are normally associated with sublittoral caves, and records in deeper areas are scarce. This region harbours a total of 14 species, representing 10 genera and six families (Perez *et al.*, 2004; Manconi *et al.*, 2006; Manconi & Serusi, 2008; Pisera & Vacelet, 2011). The family Corallistidae Sollas, 1888 is also the predominant family with a total of five species, while the other families are represented by one or two species. Comparing the lithistid faunas of the northeast Atlantic with the Mediterranean Sea, it is possible to note some contrasts. Although the family Corallistidae is the best represented in both regions (with five species each), in the Mediterranean Sea the number of genera is half than reported from the northeast Atlantic. Interestingly, the genus *Neophrissospongia* represented by a single species in the Macaronesian region is particularly rich in Mediterranean caves (4 spp). In general, the families known from the northeast Atlantic harbour more genera, with the exception of the families Desmanthidae Topsent, 1894 and Siphonidiidae Lendenfeld, 1903. The families Macandrewiidae Schrammen, 1924 and Scleritodermidae Sollas, 1888 are restricted to the northeast Atlantic and Mediterranean Sea, respectively. Of the total of 25 species reported for the Northeast Atlantic and Mediterranean only five species are shared between these areas, viz. *Neophrissospongia nolitangere* (Schmidt, 1870), *Neoschrammeniella bowerbankii* (Johnson, 1863), *Discodermia polydiscus* (Bowerbank, 1869), *Siphonidium ramosum* (Schmidt, 1870) and *Leiodermatium lynceus* Schmidt, 1870. The high level of "endemism" and few shared species (NEA vs MED) found for the lithistid fauna are in accordance with the diversity and biogeographical patterns found for the shallow-water sponge fauna of the Atlanto-Mediterranean region (Xavier & Van Soest, 2012). However, it seems that the lithistids are more widely distributed within each area, which may indicate that larger geographical ranges are to be expected for deep-sea sponges.

Pisera and Vacelet (2011) suggested that the Mediterranean lithistids represent migrants from the Atlantic, pre-Messinian relicts or recently evolved endemics. It would be interesting to test these hypotheses with a deeper phylogenetic analysis of the genera *Neophrissospongia* and *Discodermia*, both of which have species restricted to each area.

The lithistid fauna of both sides of the North Atlantic is very similar, both in terms of diversity and in terms of higher-level taxonomic composition (provided the Mediterranean is included in the NEA). In the northwest Atlantic 26 species representing 18 genera and nine families are recorded (Schmidt, 1870, 1879, 1880; van Soest & Zea, 1986; van Soest & Stentoft, 1988; Lehnert & van Soest, 1996; Maldonado & Young, 1996; Pisera, 1999; Pomponi *et al.*, 2001). The predominant families in terms of numbers of species are the Theonellidae (6 spp), Scleritodermidae (5 spp), and Corallistidae (5 spp). The remaining families, Siphonidiidae, Azoricidae, Desmanthidae, Macandrewiidae, Neopeltidae Sollas, 1888 and Vetulinidae Lendenfeld, 1903 are represented by one to three species each. The main differences between both sides of the north Atlantic is the absence of the families Vetulinidae and Neopeltidae and the genera *Amphibleptula* Schmidt, 1879, *Scleritoderma* Sollas, 1888, *Setidium* Schmidt, 1879, and *Vetulina* Schmidt, 1879, in the northeast region (Appendix III; Tab. I). Despite these similarities, only nine species are shared between the two areas viz., *N. nolitangere*, *D. polydiscus*, *Discodermia verrucosa* Topsent, 1928, *Racodiscula clava sensu* Topsent, 1892, *S. ramosum*, *L. lynceus*, *L. pfeifferae*, *Desmanthus incrustans* (Topsent, 1898) and *Sulcastrella tenens* (Vacelet, 1969). A similar pattern of low overlap between western and eastern Atlantic was found in another group of deep-sea sponges, the so-called carnivorous sponges (family Cladorhizidae) (Hestetun *et al.*, 2013).

Recent studies in the southwestern Pacific and western Australia (Pisera & Fromont, 2010; Pisera, unpublished) suggest that for these areas the diversity of lithistids is similar, in terms of number of species, to the lithistid fauna of the Mesozoic (Pisera, 2012), contradicting the previous view of a far more diverse fauna in that era as evidenced in the fossil record of this group (Finks, 1970; Rigby, 1991). This simply highlights the need for further research in this as yet much understudied, albeit pivotal, deep-sea group.

4.2 PHYLOGENETIC RELATIONSHIPS OF LITHISTIDS

In the past few decades, several studies based on morphological (e.g. Topsent, 1928; Reid, 1970; Lévi, 1991) and molecular data (Kelly-Borges & Pomponi, 1994; Chombard *et al.*, 1998; Cárdenas *et al.*, 2011; Morrow *et al.*, 2012; Redmond *et al.*, 2013) have revealed the polyphyletic nature of lithistid demosponges and the affinities of several of its families with various orders such as the Astrophorida Sollas, 1888, Spirophorida Bergquist & Hogg, 1969 and Halichondrida Gray, 1867 (Kelly-Borges & Pomponi, 1994; Morrow *et al.*, 2012; Redmond *et al.*, 2013). These results show that the emergence of desmas in sponges occurred independently several times throughout the evolutionary history of the phylum, and not in a single event as proposed by Sollas (1888).

Despite the relevance of lithistid sponges to the comprehension of the evolutionary history of the phylum Porifera, to date only two studies were made with the specific aim of addressing the phylogenetic relationships among this group (Kelly-Borges & Pomponi, 1994; McInerney *et al.*, 1999) and these were limited in their taxonomic coverage.

This is the first study where two independent markers (the mt DNA COI and the rDNA 28S) have been included to try to clarify the phylogenetic relationships of this artificial group of sponges. Furthermore, it is also one of the most comprehensive both in terms of number of the species (14 spp, 17 taxa) and the number of sequences used (90 sequences of which 64 were newly generated).

In the work here presented the trees resulting from the analysis of the mt DNA COI gene and the rDNA 28S gene were for the most part congruent and confirmed the astrophorid affinity of the families Corallistidae and Theonellidae. This affinity was supported in both trees with posterior probabilities of 0.98 in COI and 0.79 in 28S (Figs. 22 and 23). The astrophorid affinity of Corallistidae and Theonellidae was originally proposed based on a phylogenetic analysis of the rDNA 18S gene (Kelly-Borges & Pomponi, 1994) and further confirmed in more recent phylogenetic works using mt DNA COI, rDNA 28S, and rDNA 18S (Cárdenas *et al.*, 2011; Redmond *et al.*, 2013). However, in neither of the analysis performed in the present study, have these families exhibited sister-clade relationships as found in a previous work (Kelly-Borges & Pomponi, 1994). Recently, the family Neopeltidae Sollas, 1888 was also shown to belong to the Astrophorida (Redmond *et al.*, 2013).

The monogeneric family Macandrewiidae was here included in a phylogenetic analysis for the first time. This family had been proposed to belong to the Astrophorida based on morphological evidence only (Cárdenas *et al.*, 2012). Despite being represented by a single species in this study, the astrophorid affinity of this family is well supported in the COI analysis (posterior probability 0.99).

The allocation of several lithistid families in orders other than Astrophorida has also been confirmed in molecular works (Kelly-Borges & Pomponi, 1994; Morrow *et al.*, 2012; Redmond *et al.*, 2013). The family Azoricidae, here represented by *Leiodermatium* had been proposed as having a spirophorid affinity (Kelly-Borges & Pomponi, 1994). In the 28S analysis this genus appears on a basal position with regard to the lithistid/astrophorid clade. However, the failure to obtain COI sequences for the *Leiodermatium* specimens and the limited number of 28S sequences available for comparison did not allow further clarification regarding its evolutionary relationships. Scleritodermidae and Siphonidiidae were also proposed belong to Spirophorida based on molecular (Kelly-Borges & Pomponi, 1994; Redmond *et al.*, 2013) and morphological data (Cárdenas *et al.*, 2012). The family Desmanthidae Topsent, 1894 was shown to be polyphyletic and revealed an affinity with the order Halichondrida (Morrow *et al.*, 2012; Redmond *et al.*, 2013). Vetulinidae Lendenfeld, 1903, is a very peculiar monotypic family among lithistids in that its skeleton is exclusively composed of desmas. Recently, this family was found to form a strongly supported monophyletic clade with freshwater sponges, family Spongillidae Gray, 1867 but no reasonable explanation has been found for this affinity (Redmond *et al.*, 2013).

The monophyly of the family Corallistidae, represented in this study by five of the six corallistid genera (all except *Awhiowhio* Kelly, 2007), was shown for the first time and this was highly supported in both analyses (0.97 in COI and 0.99 in 28S). However, these results contrast with the findings by Redmond and co-workers (2013) that based on an analysis of the rDNA 18S gene found a paraphyletic Corallistidae (see Fig. 10 in Redmond *et al.*, 2013). Furthermore, the discovery of the genus *Isabella* in the Atlantic Ocean is also well supported by the COI analysis, where the species *I. mirabilis* and *I. harborbranchi* formed a strongly supported monophyletic clade.

Despite weak support, the non-monophyly of the family Theonellidae was congruent in the analyses of both gene fragments. These results contrast those found in two previous studies where this family appeared monophyletic (Kelly-Borges & Pomponi, 1994; Cárdenas *et al.*, 2011). In the study made by Kelly-Borges & Pomponi (1994) based on rDNA 18S

sequences three of the five genera of this family were analyzed (*Discodermia*, *Theonella* and *Racodiscula*) and revealed a monophyletic Theonellidae, but this was mainly due to limited availability of astrophorid sequences for comparison. In Cárdenas *et al.* (2011) the species *Exsuperantia* sp. was allocated to the family Phymaraphiniidae Schrammen, 1924, this specimen actually appears to be a sample of *Racodiscula clava sensu* Topsent, 1892, a member of the family Theonellidae; taking this into account, the family Theonellidae was non-monophyletic in their analyses. This family was found paraphyletic in the study by Redmond and co-workers (see Fig. 10 in Redmond *et al.*, 2013).

In conclusion, the present study greatly expands the knowledge on the diversity, distribution and phylogenetic relationships of the lithistid sponges associated with the bathyal environment of the Macaronesian Islands and northeast Atlantic seamounts. Ongoing efforts aimed at increasing the taxonomic and geographical coverage of this study, and including additional genetic markers, will help to further clarify phylogenetic relationships, and contribute to a new classification of lithistid sponges.

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GLOSSARY

GLOSSARY OF TERMS USED IN TAXONOMY OF 'LITHISTD' DEMOSPONGIAE ¹

Actine, a ray of a spicule.

Amphiaster, a microsclere with microspined rays radiating from both ends of a shaft; the rays are shorter than the shaft.

Acantho-, prefix meaning spined.

Acerate, pointed like a needle; adjective referring to the extremities of megascleres.

Aniso-, prefix meaning unequal; generally referring to the ends of a spicule.

Archaeocyte, amoeboid cell with large nucleolus and capable of phagocytosis. May differentiate into other types of cells.

Centrotylote, spicule with a median tyle (globular swelling).

Choanoderm, surface lined by choanocytes.

Choanocyte, cell having a flagellum that is surrounded by a collar of cytoplasmic microvilli linked by bridges of glycocalyx. Several are typically arranged in spherical, ovoid, or elongated chambers. Fundamental cell of sponges.

Choanosome, the internal region of a sponge, including the choanocyte chambers.

Choanosomal skeleton, skeleton of the main body, supporting the canal system and responsible for the form of the sponge.

Clad, any ray or axial branch containing an axis or axial canal confluent with that of the protoclad or protorhabd in any type of spicule; term chiefly used in triaene spicules.

Cladome, the clads of a triaene or triaene-derivative spicule.

Clones, ray like arms of the desmas which are partly (i.e., crepis extends only in the part of the desma close to the its center) or entirely anaxial (crepis absent); number of clones is governed by the crepis geometry.

Crepis, the inceptional body of a desma (axial filament) visible as canal (monocrepid desma) or canals (tetracrepid desma).

Ectosome, the superficial region of a sponge that has no choanocyte chambers.

Ectosomal skeleton, skeleton found in the superficial region of a sponge, distinct from that of the choanosome.

Euaster, a collective term for astrose microscleres in which the rays radiate from a central point.

Exotyle, styles or tylostyles with enlarged or ornated distal end, projecting from the sponge surface.

Desmas, articulating choanosomal megascleres of various geometry and usually complex morphology, often secondarily modified and very irregular, in lithistids.

Diactine, a spicule composed of two actines.

Dichotriaene, ectosomal spicule of triaenose symmetry with regularly developed cladome (may be smooth or tuberculated or spinose) which is tangential to the sponge surface; rhabdome, which is perpendicular to the cladome usually long, axial canals extend along the whole length of the rhabdome and clads.

Dicranoclone, monaxial arched desma bearing well developed tubercles, it may be bi, tri or sometimes tetrapodial. Zygomes terminal (articulating with upper tuberculated portion of other desma).

Discotriaene, ectosomal spicule with tangential part rounded to oval in shape (flat or slightly concave) and entire or incised margin, with short rhabd, crepis tetraxial, very short in the disc.

Heloclone, elongated monocrepidial desmas with sinuous shaft, no branches, and zygomes as lateral notches; axial canal usually present along the whole desma length.

Micro-, prefix used for naming microscleres that are similar in shape to megascleres (e.g., microxea, microstyle).

Megaclone, arch-shaped, usually branched, smooth or rarely mounded desma (with an uneven surface), with short monaxial (when visible at all) crepis; zygomes terminal (articulating with lateral and or upper part of other desma).

Megascleres, structural spicule.

Mesohyl, part of sponge enclosed by pinacoderm and choanoderm.

Microscleres, small spicule, often ornate in shape.

Osculum (pl. oscula), opening through which the water leaves a sponge.

Ostium (pi. ostia) (= pore), any opening in the exopinacoderm through which water enters the sponge.

Oxea, monaxon (diactinal) spicule pointed at both ends. Different types are distinguished by shape and tip morphology.

Phyllostriaene, ectosomal spicule with one ray called rhabdome, usually perpendicular to the sponge surface and penetrating it, and three others are more or less flat (tangential to the sponge surface) and irregular, branching, called cladome; crepis tetraxial, very short in the cladome.

Pinacoderm, surface lined by pinacocytes.

Pinacocyte, cell delimiting the sponge from the external milieu and always only in a layer one-cell deep.

Pseudodiscotriaene, ectosomal spicule resembling discotriaene and analogous to it but monocrepid (monaxial).

Pseudophyllostriaene, ectosomal spicule closely resembling phyllostriaene and analogous to it, but monocrepid (crepis may be located in the rhabd or in the cladome).

Raphide, a very thin, hair-like microsclere, often in bundles called trichodragmas.

Rhabd, a collective term for a monaxonic megasclere, later applied to the one ray of a triaene that is distinct from the other three, mostly in length (= rhabdome).

Rhabdome, the rhabd of a triaene and of a triaene-derived spicule.

Rhizoclone, monaxial (monocrepidial) desma, usually with numerous spines and or/pointed tubercles which serve as zygomes (mostly lateral).

Sigmaspire, a contorted-sigmoid microspined microsclere.

Sphaeroclone, anaxial desma in which several ray-like arms extend from a globular center, which may be spinose.

Spicule, a component of the mineral skeleton, typically composed of silica or calcium carbonate.

Spiraster, spiral, rod-shaped microsclere with spines peripherally arranged.

Streptaster, aster in which the rays proceed from an axis that is usually spiral.

Strongyle, an isodiametric, diactinal megasclere with rounded ends.

Style, monaxon spicule with one end pointed, the other (head or base) blunt.

Tetraclone, tetraaxial (tetracrepidial) desma, may be smooth or tuberculated; very regular, but often secondarily modified (some rays shorter, angles between clones different from 120 degrees, clones branching, sometimes resembling rhizoclone etc.), its real nature may be recognized by crepis observation which is usually very short; zygomes mostly terminal (tips of desma articulating with other tips).

Triaene, general term for a tetractinal megasclere having one unequal ray (termed rhabd) that is commonly much longer than the other three (termed clads, forming the cladome).

Tylostyle, a style with a tyle (globular swelling) at the base.

Zygone, articulating part of the desma.

¹The terms used in this glossary were adapted from Boury-Esnault & Rützler (1997) and Pisera & Lévi (2002a).

APPENDIX I

Tab. 1 - List of the examined material from the Harbor Branch Oceanographic Institute (HBOI) collected in the course of the 1991 research expedition aboard the RV Seward Johnson.

| Specimen code | Locality | Sample method | Latitude | Longitude | Depth (m) |
|----------------------|--------------------|----------------------|-----------------|------------------|------------------|
| 26-V-91-1-006 | Madeira | Submersible | 32° 36' 83"N | 16° 52' 34"W | 741 |
| 27-V-91-3-006 | Madeira | Submersible | 32° 42' 41"N | 16 40' 25"W | 482 |
| 27-V-91-3-013 | Madeira | Submersible | 32° 42' 41"N | 16 40' 25"W | 402 |
| 28-V-91-2-001 | Madeira | Submersible | 33° 06' 28"N | 16 16' 11"W | 317 |
| 29-V-91-1-001 | Madeira | Submersible | 33° 01' 75"N | 16 26' 00"W | 659 |
| 29-V-91-1-002 | Madeira | Submersible | 33° 01' 75"N | 16 26' 00"W | 657 |
| 29-V-91-3-003 | Madeira | Submersible | 33° 01' 90"N | 16 26' 27"W | 563 |
| 29-V-91-3-008 | Madeira | Submersible | 33° 01' 90"N | 16 26' 27"W | 669 |
| 29-V-91-3-017 | Madeira | Submersible | 33° 01' 90"N | 16 26' 27"W | 610 |
| 30-V-91-4-007 | Madeira | Submersible | 32° 26' 50"N | 16 27' 49"W | 519 |
| 31-V-91-1-006 | Madeira | Submersible | 32° 32' 51"N | 16 35' 08"W | 476 |
| 31-V-91-1-015 | Madeira | Submersible | 32° 32' 51"N | 16 35' 08"W | 480 |
| 31-V-91-3-003 | Madeira | Submersible | 32° 30' 00"N | 16 33' 50"W | 497 |
| 2-VI-91-1-005 | Selvagens | Submersible | 30° 07' 35"N | 15 52' 13"W | 358 |
| 2-VI-91-1-009 | Selvagens | Submersible | 30° 07' 35"N | 15 52' 13"W | 361 |
| 2-VI-91-1-012 | Selvagens | Submersible | 30° 07' 35"N | 15 52' 13"W | 322 |
| 8-VI-91-1-002 | Canaries | Submersible | 27° 45' 50"N | 15 48' 75"W | 303 |
| 8-VI-91-4-005 | Canaries, Tenerife | Submersible | 28° 12' 69"N | 16 50' 72"W | 292 |
| 9-VI-91-4-003 | Canaries | Submersible | 28° 18' 00"N | 16 56' 10"W | 439 |
| 9-VI-91-4-006 | Canaries | Submersible | 28° 18' 00"N | 16 56' 10"W | 422 |
| 9-VI-91-4-008 | Canaries | Submersible | 28° 18' 00"N | 16 56' 10"W | 408 |
| 9-VI-91-4-013 | Canaries, Tenerife | Submersible | 28° 18' 00"N | 16 56' 10"W | 366 |
| 10-VI-91-1-003 | Canaries | Submersible | 27° 40' 20"N | 18 02' 81"W | 417 |
| 11-VI-91-1-001 | Canaries | Submersible | 28° 30' 25"N | 17 53' 25"W | 450 |
| 11-VI-91-1-003 | Canaries | Submersible | 28° 30' 25"N | 17 53' 25"W | 415 |
| 11-VI-91-1-008 | Canaries | Submersible | 28° 30' 25"N | 17 53' 25"W | 302 |
| 11-VI-91-1-012 | Canaries, La Palma | Submersible | 28° 30' 25"N | 17 53' 25"W | 133 |
| 13-VI-91-4-003 | Canaries | Submersible | 28° 11' 65"N | 16 51' 21"W | 295 |
| 14-VI-91-1-003 | Canaries, Tenerife | Submersible | 27° 59' 00"N | 16 39' 60"W | 282 |
| 14-VI-91-1-004 | Canaries | Submersible | 27° 59' 00"N | 16 39' 60"W | 281 |
| 14-VI-91-3-020 | Canaries | Submersible | 28° 24' 23"N | 16 18' 33"W | 291 |
| 16-VI-91-2-004 | Canaries | Submersible | 27° 41' 85"N | 15 34' 20"W | 363 |

Tab. II - List of the examined material from the collection of the Muséum National d'Histoire Naturelle (MNHN Paris) collected in the course of the 1993 Seamount-2 research expedition aboard the NO Cryos.

| Specimen code | Locality | Sample method | Latitude | Longitude | Depth (m) |
|----------------------|-------------------|----------------------|-----------------|------------------|------------------|
| IP-2008-191 | Tyro Bank | no data | 34° 00'N | 28° 21'W | 1000 |
| IP-2008-192 | Atlantis Bank | no data | 34° 29'N | 30° 36'W | 545 |
| IP-2008-193 | Hyères Bank | no data | 31° 10'N | 28° 44'W | 845 |
| IP-2008-195 | Atlantis Bank | no data | 34° 04'N | 30° 15'W | 338 |
| IP-2008-196 | Hyères Bank | no data | 31° 30'N | 29° 00'W | 310 |
| IP-2008-197 | Atlantis Bank | no data | 34° 05'N | 30° 14'W | 280 |
| IP-2008-198 | Gran Canaria | no data | 28° 09'N | 15° 53'W | 660 |
| IP-2008-199 | Atlantis Bank | no data | 34° 00'N | 30° 12'W | 420 |
| IP-2008-200 | Plato Bank | no data | 33° 12'N | 28° 57'W | 710 |
| IP-2008-201 | Hyères Bank | no data | 31° 23'N | 28° 54'W | 480 |
| IP-2008-202 | Atlantis Bank | no data | 34° 05'N | 30° 13'W | 280 |
| IP-2008-204 | Great Meteor Bank | no data | 30° 02'N | 28° 29'W | 300 |
| IP-2008-205 | Atlantis Bank | no data | 34° 04'N | 30° 15'W | 338 |
| IP-2008-206 | Plato Bank | no data | 33° 14'N | 29° 36'W | 520 |
| IP-2008-207 | Great Meteor Bank | no data | 30° 01'N | 28° 28'W | 308 |
| IP-2008-208 | Plato Bank | no data | 33° 14'N | 29° 35'W | 580 |
| IP-2008-210 | Atlantis Bank | no data | 34° 00'N | 30° 12'W | 420 |
| IP-2008-211 | Great Meteor Bank | no data | 29° 44'N | 28° 20'W | 330 |
| IP-2008-212 | Great Meteor Bank | no data | 30° 01'N | 28° 28'W | 304 |
| IP-2008-213 | Great Meteor Bank | no data | 29° 56'N | 28° 24'W | 320 |
| IP-2008-214 | Great Meteor Bank | no data | 30° 10'N | 28° 29'W | 335 |
| IP-2008-216 | Hyères Bank | no data | 31° 24'N | 28° 52'W | 705 |
| IP-2008-217 | Atlantis Bank | no data | 34° 26'N | 30° 32'W | 610 |
| IP-2008-219 | Tyro Bank | no data | 33° 56'N | 28° 24'W | 805 |
| IP-2008-220 | Atlantis Bank | no data | 34° 00'N | 30° 12'W | 420 |
| IP-2008-222 | Great Meteor Bank | no data | 30° 12'N | 28° 25'W | 615 |
| IP-2008-224 | Hyères Bank | no data | 31° 24'N | 28° 52'W | 705 |
| IP-2008-225 | Tyro Bank | no data | 34° 00'N | 28° 21'W | 1000 |
| IP-2008-226 | no data | no data | no data | no data | 500 |
| IP-2008-228 | Antialtair Bank | no data | 43° 35'N | 22° 26'W | 900 |
| IP-2008-229 | Gran Canaria | no data | 28° 08'N | 15° 52'W | 480 |
| IP-2008-230 | Plato Bank | no data | 33° 14'N | 29° 36'W | 520 |
| IP-2008-232 | Gran Canaria | no data | 28° 08'N | 15° 52'W | 470 |
| IP-2008-233 | Hyères Bank | no data | 31° 23'N | 28° 54'W | 480 |
| IP-2008-234 | Plato Bank | no data | 33° 12'N | 28° 59'W | 695 |
| IP-2008-236 | Atlantis Bank | no data | 34° 29'N | 30° 36'W | 545 |
| IP-2008-237 | Atlantis Bank | no data | 34° 29'N | 30° 36'W | 545 |
| IP-2008-239 | Hyères Bank | no data | 31° 23'N | 28° 54'W | 480 |
| IP-2008-240 | Plato Bank | no data | 33° 14'N | 29° 36'W | 520 |

| | | | | | |
|--------------|-------------------|---------|----------|----------|---------|
| IP-2008-243 | Atlantis Bank | no data | 34° 00'N | 30° 12'W | 420 |
| IP-2008-245 | Hyères Bank | no data | 31° 23'N | 28° 54'W | 480 |
| IP-2008-248 | Great Meteor Bank | no data | 29° 45'N | 28° 18'W | 950 |
| IP-2008-249 | Hyères Bank | no data | 31° 16'N | 28° 43'W | 640 |
| IP-2008-251 | Great Meteor Bank | no data | 29° 44'N | 28° 20'W | 330 |
| IP-2008-253 | Great Meteor Bank | no data | 29° 44'N | 28° 20'W | 330 |
| IP-2008-254 | Great Meteor Bank | no data | 30° 11'N | 28° 27'W | 340 |
| IP-2008-255 | Great Meteor Bank | no data | 28° 09'N | 15° 53'W | 660 |
| IP-2008-256a | no data | no data | no data | no data | no data |
| IP-2008-256b | no data | no data | no data | no data | no data |

Tab. III - List of examined material from the Biology and Oceanography and Fisheries departments of the University of the Azores collected in the course of several research expeditions.

| Specimen code | Locality | Sample method | Latitude | Longitude | Depth (m) |
|---------------|---------------------|-----------------|--------------|--------------|-----------|
| 810 | no data | Bottom longline | 38° 31' 26"N | 28° 35' 06"W | 457 |
| 1012 | Baixo de São Mateus | no data | 38° 19' 30"N | 28° 32' 16"W | 362 |
| 1037 | Baixo de São Mateus | no data | 38° 19' 30"N | 28° 32' 16"W | 362 |
| 1062 | Baixo de São Mateus | no data | 38° 32' 56"N | 28° 29' 13"W | 384 |
| 1134 | São Jorge | Bottom longline | no data | no data | 366 |
| 1135 | São Jorge | Bottom longline | no data | no data | 366 |
| 1190 | Princess Alice Bank | Bottom longline | 37° 56' 41"N | 29° 10' 33"W | 201 |
| 1241 | Faial | Handline | 38° 36' 54"N | 28° 36' 21"W | 219 |
| 1609 | Princess Alice Bank | Bottom longline | 37° 54' 05"N | 29° 01' 01"W | 238 – 329 |
| 1640 | Princess Alice Bank | Bottom longline | 37° 54' 05"N | 29° 01' 01"W | 238 |
| 1946 | Graciosa | Bottom longline | 39° 08' 31"N | 28° 06' 03"W | 326 |
| 1976 | Açores Bank | Bottom longline | 38° 05' 59"N | 29° 08' 59"W | 168 - 594 |
| 1977 | Açores Bank | Bottom longline | 38° 05' 59"N | 29° 08' 59"W | 168 – 594 |
| 1996 | Princess Alice Bank | Bottom longline | 37° 54' 05"N | 29° 01' 40"W | 183 – 366 |
| 2028 | Pico | Handline | no data | no data | 200 |
| 2028 | Pico | Handline | no data | no data | 200 |
| 2048 | Pico | Handline | no data | no data | 200 |
| 2048 | Pico | Handline | no data | no data | 200 |
| 2063 | Princess Alice Bank | Bottom longline | 37° 56' 41"N | 29° 10' 33"W | 201 |
| 2409 | Irving | Bottom longline | 32° 00' 00"N | 27° 56' 24"W | 503 - 550 |
| 2422 | Irving | Bottom longline | 32° 00' 00"N | 27° 56' 24"W | 452 - 500 |
| 2762 | no data | Bottom longline | no data | no data | no data |
| 2766 | Mar da Prata | Bottom longline | 37° 36' 01"N | 25° 53' 59"W | 279 |
| 2866 | Mar da Prata | Bottom longline | 37° 10' 48"N | 25° 43' 47"W | 635 |
| 2973 | no data | Bottom longline | no data | no data | no data |

| | | | | | |
|------|---------------------|-----------------|--------------|--------------|-----------|
| 2944 | Voador Bank | Bottom longline | 37° 32' 59"N | 30° 44' 23"W | 442 |
| 3147 | Faial | Bottom longline | no data | no data | 91 |
| 3774 | Princess Alice Bank | Bottom longline | 37° 38' 16"N | 28° 54' 18"W | 384 - 402 |
| 3796 | Princess Alice Bank | Bottom longline | 37° 51' 28"N | 29° 20' 20"W | 413 - 430 |
| 4037 | Azores Bank | Bottom longline | 37° 54' 50"N | 29° 10' 15"W | 238 |
| 4079 | Voador Bank | Bottom longline | 37° 30' 46"N | 30° 36' 53"W | 457 |
| 4166 | Pico-Faial Canal | Bottom longline | 37° 30' 46"N | 30° 36' 53"W | 434 |
| 4412 | Princess Alice Bank | Bottom longline | 37° 30' 46"N | 30° 36' 53"W | 404 - 439 |
| 4466 | Princess Alice Bank | Bottom longline | 37° 30' 46"N | 28° 56' 34"W | 280 - 479 |
| 4468 | Princess Alice Bank | Bottom longline | 37° 30' 46"N | 28° 56' 20"W | 280 - 479 |
| 4602 | Princess Alice Bank | Bottom longline | 37° 56' 23"N | 29° 09' 35"W | 192 - 366 |
| 4845 | Azores Bank | Bottom longline | 38° 19' 40"N | 29° 25' 01"W | 554 |
| 4862 | Azores Bank | Bottom longline | 38° 08' 23"N | 29° 20' 05"W | 622 - 706 |
| 4863 | Azores Bank | Bottom longline | 38° 08' 23"N | 29° 20' 05"W | 622 - 706 |
| 4905 | Azores archipelago | Bottom longline | no data | no data | no data |
| 5043 | Pico | Bottom longline | 38° 11' 38"N | 27° 43' 08"W | 457 - 530 |
| 5048 | Pico | Bottom longline | 38° 11' 38"N | 27° 43' 08"W | 457 - 530 |
| 5323 | no data | Bottom longline | 38° 49' 01"N | 27° 18' 14"W | 102 |
| 5334 | no data | Bottom longline | 38° 52' 01"N | 27° 14' 52"W | 303 |
| 5424 | no data | Bottom longline | no data | no data | no data |
| 5602 | Princess Alice Bank | Bottom longline | no data | no data | 256 - 348 |
| 5749 | no data | no data | no data | no data | no data |
| 5774 | Sarda Bank | no data | 38° 52' 01"N | 27° 14' 52"W | 448 |
| 5814 | Princess Alice Bank | no data | 37° 52' 01"N | 27° 14' 52"W | 432 |
| 5846 | Plato | Bottom longline | 33° 15' 25"N | 29° 38' 13"W | 485 |
| 5850 | Plato | Bottom longline | 33° 15' 32"N | 29° 38' 13"W | 501 |
| 5853 | Plato | Bottom longline | 33° 15' 14"N | 29° 38' 06"W | 461 |
| 5862 | Plato | Bottom longline | 33° 15' 14"N | 29° 38' 06"W | 461 |
| 5871 | Princess Alice Bank | Bottom longline | 37° 51' 54"N | 29° 07' 51"W | 435 |
| 5879 | Chino Bank | Bottom longline | 37° 59' 13"N | 29° 31' 48"W | 371 |
| 5882 | Chino Bank | Bottom longline | 37° 59' 13"N | 29° 31' 48"W | 371 |
| 5883 | Chino Bank | Bottom longline | 37° 59' 13"N | 29° 31' 48"W | 371 |
| 6192 | Voador Bank | Bottom longline | 37° 28' 58"N | 30° 50' 34"W | 418 |
| 6212 | Voador Bank | Bottom longline | 37° 29' 13"N | 30° 50' 45"W | 353 |
| 6238 | Voador Bank | Bottom longline | 37° 31' 22°N | 30° 36' 28"W | 470 |
| 6247 | Voador Bank | Bottom longline | 37° 31' 51°N | 30° 36' 25"W | 770 |
| 6248 | Voador Bank | Bottom longline | 37° 31' 22°N | 30° 36' 28"W | 770 |
| 6249 | Voador Bank | Bottom longline | 37° 31' 44°N | 30° 36' 28"W | 711 |
| 6255 | Voador Bank | Bottom longline | 37° 31' 30°N | 30° 36' 28"W | 578 |
| 6370 | Voador Bank | Bottom longline | 37° 31' 22°N | 30° 44' 45"W | 264 |
| 6565 | Azores Bank | Bottom longline | 38° 07' 48"N | 28° 58' 58"W | 395 |
| 6582 | Princess Alice Bank | Bottom longline | 37° 50' 16"N | 29° 23' 29"W | 429 |
| 6595 | Princess Alice Bank | Bottom longline | 37° 12' 14"N | 29° 26' 49"W | 476 |

| | | | | | |
|-----------------|---------------------|-----------------|--------------|--------------|-----|
| 6601 | Princess Alice Bank | Bottom longline | 37° 59' 06"N | 29° 34' 44"W | 398 |
| 6608 | Princess Alice Bank | Bottom longline | 37° 59' 24"N | 29° 34' 19"W | 398 |
| 6614 | Princess Alice Bank | Bottom longline | 37° 59' 24"N | 29° 33' 54"W | 393 |
| 6623 | Princess Alice Bank | Bottom longline | 37° 58' 29"N | 29° 31' 51"W | 367 |
| 6670 | Azores Bank | Bottom longline | 37° 58' 20"N | 28° 55' 48"W | 327 |
| 6671 | Azores Bank | Bottom longline | 37° 37' 30"N | 28° 55' 37"W | 400 |
| 6674 | Azores Bank | Bottom longline | 37° 37' 44"N | 28° 55' 58"W | 373 |
| 6686 | Princess Alice Bank | Bottom longline | 37° 59' 34"N | 28° 34' 47"W | 402 |
| 6476-6477 | Voador Bank | Bottom longline | 37° 32' 34"N | 30° 42' 03"W | 271 |
| SAB2011.DR12.B1 | S. Miguel | Dredging | 37° 42' 31"N | 25° 20' 14"W | 330 |

APPENDIX II

Tab. I - Sequences of the mtDNA COI gene used in the phylogenetic analysis. Analysis code corresponds to the code of each species in the phylogenetic tree. GB - GenBank; SPB - Sponge Barcoding Project.

| Systematics | Analysis code | Source | Author |
|---|----------------------|---------------|---------------------------------|
| Order SPIROPHORIDA Bergquist & Hogg, 1969 | | | |
| TETILLIDAE Sollas, 1886 | | | |
| <i>Craniella cranium</i> (Müller, 1776) | CcHM59266 | GB HM592669 | Cárdenas <i>et al.</i> , 2011 |
| <i>Craniella cf. leptoderma</i> (Sollas, 1886) (now <i>Tetilla leptoderma</i>) | CIJX17789 | GB JX177895 | Szitenberg <i>et al.</i> , 2013 |
| <i>Cinachyra barbata</i> Sollas, 1886 | CbJX17786 | GB JX177864 | Szitenberg <i>et al.</i> , 2013 |
| Order ASTROPHORIDA Sollas, 1888 | | | |
| ANCORINIDAE Schmidt, 1870 | | | |
| <i>Ancorina sp.</i> Schmidt, 1862 | AHM59274 | GB HM592744.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Dercitus bucklandi</i> (Bowerbank, 1858) | DbHM59271 | GB HM592716.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Ecionemia megastylifera</i> Wintermann-Kilian & Kilian, 1984 (now <i>Stellettinopsis megastylifera</i>) | EmFJ71164 | GB FJ711642.1 | Cárdenas <i>et al.</i> , 2009 |
| <i>Rhabdastrella cordata</i> Wiedenmayer, 1989 | RcHM59272 | GB HM592727.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Rhabdastrella globostellata</i> (Carter, 1883) | RgHM59267 | GB HM592673.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Rhabdastrella intermedia</i> Wiedenmayer, 1989 | RiHM59272 | GB HM592726.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Rhabdastrella sp.</i> Thiele, 1903 | RHM59267 | GB HM592676.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Stelletta clarella</i> de Laubenfels, 1930 | ScHM59273 | GB HM592736.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Stelletta dorsigera</i> Schmidt, 1864 | SdHM59275 | GB HM592750.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Stelletta grubii</i> Schmidt, 1862 | SgHM59274 | GB HM592743.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Stelletta normani</i> Sollas, 1880 | SnEU44219 | GB EU442193.1 | Cárdenas <i>et al.</i> , 2010 |
| <i>Stelletta tuberosa</i> (Topsent, 1892) | StHM92735 | GB HM592735.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Stryphnus ponderosus</i> (Bowerbank, 1866) | SpHM59268 | GB HM592685.1 | Cárdenas <i>et al.</i> , 2011 |
| CALTHROPELLIDAE Lendenfeld, 1907 | | | |
| <i>Calthropella geodioides</i> (Carter, 1876) (now <i>Calthropella (Calthropella) geodioides</i>) | CgHM59270 | GB HM592705.1 | Cárdenas <i>et al.</i> , 2011 |
| GEODIIDAE Gray, 1867 | | | |
| Subfamily ERYLINAЕ Sollas, 1888 | | | |
| <i>Caminella intuta</i> (Topsent, 1892) | CiHM59274 | GB HM592740.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Caminus vulcani</i> Schmidt, 1862 | CvEU44220 | GB EU442205.1 | Cárdenas <i>et al.</i> , 2010 |
| <i>Erylus aleuticus</i> Lehnert, Stone & Heimler, 2006 | EaEU4420 | GB EU442201.1 | Cárdenas <i>et al.</i> , 2010 |
| <i>Erylus deficiens</i> Topsent, 1927 | EdEU44220 | GB EU442204.1 | Cárdenas <i>et al.</i> , 2010 |
| <i>Erylus discophorus</i> (Schmidt, 1862) | EdHM59269 | GB HM592692.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Erylus expletus</i> Topsent, 1927 | EeEU44220 | GB EU442208.1 | Cárdenas <i>et al.</i> , 2010 |

| | | | |
|--|-----------|---------------|-------------------------------|
| <i>Erylus granularis</i> Topsent, 1904 | EgHM59272 | GB HM592729.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Erylus mamillaris</i> (Schmidt, 1862) | EmEU44220 | GB EU442207.1 | Cárdenas <i>et al.</i> , 2010 |
| <i>Erylus</i> sp. Gray, 1867 | EHM59268 | GB HM592687.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Erylus topsenti</i> Lendenfeld, 1903 | EtHM59273 | GB HM592733.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Melophlus</i> sp. | MHM59268 | GB HM592688.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Pachymatisma johnstonia</i> (Bowerbank in Johnston, 1842) | PjEF56433 | GB EF564337.1 | Cárdenas <i>et al.</i> , 2007 |
| <i>Pachymatisma normani</i> Sollas, 1888 | PnEF56432 | GB EF564329.1 | Cárdenas <i>et al.</i> , 2007 |
| <i>Penares candidata</i> (Schmidt, 1868) | PcHM59271 | GB HM592719.1 | Cárdenas <i>et al.</i> , 2011 |
| Subfamily GEODIINAE Sollas, 1888 | | | |
| <i>Geodia angulata</i> (Lendenfeld, 1910) | GaEU44220 | GB EU442203.1 | Cárdenas <i>et al.</i> , 2010 |
| <i>Geodia barretti</i> Bowerbank, 1858 | GbEU44219 | GB EU442194.1 | Cárdenas <i>et al.</i> , 2010 |
| <i>Geodia californica</i> (Lendenfeld, 1910) | GcEU44220 | GB EU442200.1 | Cárdenas <i>et al.</i> , 2010 |
| <i>Geodia cf. atlantica</i> (Stephens, 1915) | GaEU44219 | GB EU442195.1 | Cárdenas <i>et al.</i> , 2010 |
| <i>Geodia conchilega</i> Schmidt, 1862 | GcHM59273 | GB HM592739.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Geodia corticostylifera</i> Hajdu, Muricy, Custodio, Russo & Peixinho, 1992 | GcHM59268 | GB HM592681.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Geodia cydonium</i> (Jameson, 1811) | GcEU44219 | GB EU442199.1 | Cárdenas <i>et al.</i> , 2010 |
| <i>Geodia gibberosa</i> Lamarck, 1815 | GgEU4422 | GB EU442209.1 | Cárdenas <i>et al.</i> , 2010 |
| <i>Geodia hentscheli</i> Cárdenas, Rapp, Schander & Tendal, 2010 | GhEU44219 | GB EU442197.1 | Cárdenas <i>et al.</i> , 2010 |
| <i>Geodia macandrewii</i> Bowerbank, 1858 | GmEU44219 | GB EU442198.1 | Cárdenas <i>et al.</i> , 2010 |
| <i>Geodia megastrella</i> Carter, 1876 | GmHM59274 | GB HM592741.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Sidonops neptuni</i> (Sollas, 1886) (now <i>Geodia neptuni</i>) | SnEF51967 | GB EF519674.1 | Cárdenas <i>et al.</i> , 2007 |
| <i>Geodia phlegraei</i> (Sollas, 1880) | GpEU44219 | GB EU442196.1 | Cárdenas <i>et al.</i> , 2010 |
| <i>Geodia vaubani</i> Lévi & Lévi, 1983 | GvEU44220 | GB EU442202.1 | Cárdenas <i>et al.</i> , 2010 |
| <i>Geodia vosmaeri</i> (Sollas, 1886) | GVHM2722 | GB HM592722.1 | Cárdenas <i>et al.</i> , 2011 |
| PACHASTRELLIDAE Carter, 1875 | | | |
| <i>Characella pachastrelloides</i> (Carter, 1876) | CpHM59270 | GB HM592709.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Pachastrella nodulosa</i> Cárdenas & Rapp, 2012 | PnHM59269 | GB HM592698.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Pachastrella ovisternata</i> Lendenfeld, 1894 | PoHM59274 | GB HM592748.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Triptolemma intextum</i> (Carter, 1876) | TiHM59271 | GB HM592710.1 | Cárdenas <i>et al.</i> , 2011 |
| THENEIDAE Carter, 1883 | | | |
| <i>Thenea muricata</i> (Bowerbank, 1858) | TmHM59267 | GB HM592706.1 | Cárdenas <i>et al.</i> , 2011 |
| THOOSIDAE Cockerell, 1925 | | | |

| | | | |
|--|------------|---------------|--|
| <i>Neamphius huxleyi</i> Sollas, 1888 VULCANELLIDAE Cárdenas, Xavier, Reveillaud, Schander & Rapp, 2011 | NhHM592682 | GB HM592682.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Poecillastra compressa</i> (Bowerbank, 1866) | PcEu44219 | GB EU442192.1 | Cárdenas <i>et al.</i> , 2010 |
| <i>Vulcanella aberrans</i> (Maldonado & Uriz, 1996) | VaHM59269 | GB HM592699.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Vulcanella gracilis</i> (Sollas, 1888) | VgHM59270 | GB HM592702.1 | Cárdenas <i>et al.</i> , 2011 |
| LITHISTID' Demospongiae sensu Pisera & Lévi, 2002 | | | |
| CORALLISTIDAE Sollas, 1888 | | | |
| <i>Corallistes masoni</i> (Bowerbank, 1869) | Cm MAD1 | | This study |
| | Cm MAD2 | | This study |
| | Cm MAD3 | | This study |
| <i>Herengeria vasiformis</i> Schlacher-Hoelinger, Pisera & Hooper, 2005 | HvSBP82 | SBP SBP#822 | Schlacher 2001 |
| | HvSBP81 | SBP SBP#818 | Schlacher-Hoelinger <i>et al.</i> , 2005 |
| | HvSBP82 | SBP SBP#820 | Schlacher-Hoelinger <i>et al.</i> , 2005 |
| | HvSBP82 | SBP SBP#826 | Schlacher-Hoelinger <i>et al.</i> , 2005 |
| <i>Isabella harborbranchi</i> n. sp. | IhCAN3 | | This study |
| | IhCAN4 | | This study |
| | IhCAN1 | | This study |
| | IhCAN2 | | This study |
| <i>Isabella mirabilis</i> Schlacher-Hoelinger, Pisera & Hooper, 2005 | ImSBP81 | SBP SBP#814 | Schlacher-Hoelinger <i>et al.</i> , 2005 |
| | ImSBP82 | SBP SBP#823 | Schlacher-Hoelinger <i>et al.</i> , 2005 |
| | ImSBP82 | SBP SBP#824 | Schlacher-Hoelinger <i>et al.</i> , 2005 |
| | ImSBP82 | SBP SBP#828 | Schlacher-Hoelinger <i>et al.</i> , 2005 |
| <i>Neophrissospongia nolitangere</i> (Schmidt, 1870) | NnMAD1 | | This study |
| | NnSEL1 | | This study |
| | NnCAN1 | | This study |
| | NnAZO1 | | This study |
| | NnAZO2 | | This study |
| | NnAZO3 | | This study |
| | NnAZO4 | | This study |
| | NnAZO5 | | This study |
| | NnAZO7 | | This study |
| <i>Neoschrammeniella bowerbankii</i> (Johnson, 1863) | NbMAD1 | | This study |
| | NbMAD2 | | This study |

| | | | |
|--|-----------|---------------|--------------------------------|
| MACANDREWIIDAE Schrammen, 1924 | | | |
| <i>Macandrewia azorica</i> Gray, 1859 | MaAZO1 | | This study |
| | MaAZO2 | | This study |
| | MaAZO3 | | This study |
| | MaAZO4 | | This study |
| | MaAZO5 | | This study |
| | MaAZO6 | | This study |
| | MaAZO7 | | This study |
| THEONELLIDAE Lendenfeld, 1903 | | | |
| <i>Discodermia polymorpha</i> Pisera & Vacelet, 2011 | DpHM59268 | GB HM592686.1 | Cárdenas <i>et al.</i> , 2011 |
| <i>Discodermia ramifera</i> Topsent, 1892 | DrAZO1 | | This study |
| <i>Discodermia verrucosa</i> Topsent, 1928 | DvMAD2 | | This study |
| | DVCAN1 | | This study |
| <i>Racodiscula clava sensu</i> Topsent, 1892 | RcCAN1 | | This study |
| | RcMAD1 | | This study |
| | RcAZO1 | | This study |
| | RcAZO2 | | This study |
| | RcAZO3 | | This study |
| | RcAZO4 | | This study |
| | RcAZO5 | | This study |
| | RcAZO6 | | This study |
| * as <i>Exsuperantia</i> sp. | EHM59273 | GB HM592730.1 | Cárdenas <i>et al.</i> , 2011* |
| <i>Theonella swinhoei</i> Gray, 1868 | TsHM59274 | GB HM592745.1 | Cárdenas <i>et al.</i> , 2011 |

Tab. II - Sequences of the ribosomal 28S (D3-D5) gene used in the phylogenetic analysis. Analysis code corresponds to the code of each species in the phylogenetic tree. GB: GenBank; SPB Sponge Barcoding Project.

| Systematics | Analysis code | Source | Author |
|---|---------------|---------------|-----------------------------|
| Order ASTROPHORIDA Sollas, 1888 | | | |
| ANCORINIDAE Schmidt, 1870 | | | |
| <i>Holoxea</i> sp. | HspJF506040 | GB JF506040.1 | Liu & Li, 2011 |
| <i>Stelletta grubii</i> Schmidt, 1862 | SgHQ379255 | GB HQ379255.1 | Morrow <i>et al.</i> , 2012 |
| <i>Stelletta lactea</i> Carter, 1871 | SIHQ379256 | GB HQ379256.1 | Morrow <i>et al.</i> , 2012 |
| <i>Stryphnus ponderosus</i> (Bowerbank, 1866) | SpHQ379257 | GB HQ379257.1 | Morrow <i>et al.</i> , 2012 |
| GEODIIDAE Gray, 1867 | | | |
| Subfamily ERYLINAЕ Sollas, 1888 | | | |
| <i>Pachymatisma johnstonia</i> (Bowerbank in Johnston, 1842) | PjHQ379258 | GB HQ379258.1 | Morrow <i>et al.</i> , 2012 |
| Order HADROMERIDA Topsent, 1928 | | | |
| HEMIASTERELLIDAE Lendenfeld, 1889 | | | |
| <i>Adreus fascicularis</i> (Bowerbank, 1866) | AfHQ379314 | GB HQ379314.1 | Morrow <i>et al.</i> , 2012 |
| 'LITHISTID' Demospongiae sensu Pisera & Lévi, 2002 | | | |
| AZORICIDAE Sollas, 1888 | | | |

| | | | |
|--|--------------|---------------|--------------------------------|
| <i>Leiodermatium</i> sp. | LspMAD1* | | This study |
| | LspAZO1* | | This study |
| | LspAZO2* | | This study |
| | LspAZO3* | | This study |
| | LspAZO4* | | This study |
| | LspAZO5* | | This study |
| | LspAZO6* | | This study |
| CORALLISTIDAE Sollas, 1888 | | | |
| <i>Corallistes</i> sp. | Csp AJ005913 | GB AJ005913.1 | McInerney <i>et al.</i> , 1999 |
| <i>Corallistes masoni</i> (Bowerbank, 1869) | Cm MAD2* | | This study |
| <i>Neophrissospongia nolitangere</i> (Schmidt, 1870) | NnMAD1* | | This study |
| | NnSEL1* | | This study |
| | NnCAN2* | | This study |
| | NnCAN1* | | This study |
| | NnCAN3* | | This study |
| | NnAZO1* | | This study |
| | NnAZO3* | | This study |
| | NnAZO6* | | This study |
| | NnAZO4* | | This study |
| | NnAZO5* | | This study |
| | NnAZO7* | | This study |
| <i>Neoschrammeniella bowerbankii</i> (Johnson, 1863) | NbMAD1* | | This study |
| DESMANTHIDAE Topsent, 1894 | | | |
| <i>Desmanthus incrustans</i> (Topsent, 1889) | DiHQ379254 | GB HQ379254.1 | Morrow <i>et al.</i> , 2012 |
| THEONELLIDAE Lendenfeld, 1903 | | | |
| <i>Discodermia dissoluta</i> Schmidt, 1880 | DsAJ005914 | GB AJ005914.1 | McInerney <i>et al.</i> , 1999 |
| <i>Discodermia ramifera</i> Topsent, 1892 | DrAZO1* | | This study |
| | DrAZO2* | | This study |
| <i>Racodiscula clava sensu</i> Topsent, 1892 | RcAZO7* | | This study |
| | RcAZO8* | | This study |
| | RcAZO2* | | This study |
| | RcAZO3* | | This study |
| | RcAZO4* | | This study |
| | RcAZO5* | | This study |
| | RcAZO6* | | This study |
| <i>Theonella</i> sp. | TspAJ005917 | GB AJ005917.1 | McInerney <i>et al.</i> , 1999 |
| <i>Theonella</i> sp. | TspAJ0059181 | GB AJ005918.1 | McInerney <i>et al.</i> , 1999 |
| Order POECILOSCLERIDA Topsent, 1928 | | | |
| RASPAILIIDAE Nardo, 1833 | | | |
| <i>Eurypon</i> sp. | EspHQ379274 | GB HQ379274.1 | Morrow <i>et al.</i> , 2012 |
| DESMACELLIDAE Ridley & Dendy, 1886 | | | |
| <i>Biemna variantia</i> (Bowerbank, 1858) | BmHQ379292 | GB HQ379292.1 | Morrow <i>et al.</i> , 2012 |

| | | | |
|---|-------------|---------------|------------------------------------|
| MYCALIDAE Lundbeck, 1905 | | | |
| <i>Mycale rotalis</i> (Bowerbank, 1874) (now <i>Mycale (Aegogroplia) rotalis</i>) | MrHQ379296 | GB HQ379296.1 | Morrow <i>et al.</i> , 2012 |
| Order HALICHONDRIDA Gray, 1867 | | | |
| AXINELLIDAE CARTER, 1875 | | | |
| <i>Axinella pyramidata</i> Stephens, 1916 | ApHQ379265 | GB HQ379265.1 | Morrow <i>et al.</i> , 2012 |
| <i>Cymbastela stipitata</i> (Bergquist & Tizard, 1967) | Cs EF646836 | GB EF646836.1 | Gerasimova <i>et al.</i> , 2007 |
| <i>Phakellia ventilabrum</i> (Linnaeus, 1767) | PvHQ379260 | GB HQ379260.1 | Morrow <i>et al.</i> , 2012 |
| BUBARIDAE Topsent, 1894 | | | |
| <i>Bubaris cf. carcasic</i> Vacelet, 1969 | BcHQ379267 | GB HQ379267.1 | Morrow <i>et al.</i> , 2012 |
| DICTYONELLIDAE Van Soest, Diaz & Pomponi, 1990 | | | |
| <i>Acanthella acuta</i> Schmidt, 1862 | AaHQ379259 | GB HQ379259.1 | Morrow <i>et al.</i> , 2012 |
| <i>Acanthella cavernosa</i> Dendy, 1922 | AcAY618703 | GB AY618703.1 | Erpenbeck <i>et al.</i> , 2005 |
| HALICHONDRIIDAE Gray, 1867 | | | |
| <i>Amorphinopsis excavans</i> Carter, 1887 | AeAY319313 | GB AY319313.1 | Erpenbeck <i>et al.</i> , 2005 |
| <i>Axinyssa ambrosia</i> (de Laubenfels, 1936) | Aa AY618707 | GB AY618707.1 | Erpenbeck <i>et al.</i> , 2005 |
| <i>Axinyssa aplysinoides</i> (Dendy, 1922) | Aa AY319324 | GB AY319324.1 | Erpenbeck <i>et al.</i> , 2005 |

APPENDIX III

Tab. I - List of lithistid species recorded from the northwest Atlantic (NWA), northeast Atlantic (NEA) and the Mediterranean Sea (MED).

| | NWA | NEA | MED |
|--|-----|-----|-----|
| AZORICIDAE Sollas, 1888 | | | |
| <i>Leiodermatium lynceus</i> Schmidt, 1870 | ✓ | ✓ | ✓ |
| <i>Leiodermatium pfeifferae</i> (Carter, 1876) | ✓ | ✓ | |
| CORALLISTIDAE Sollas, 1888 | | | |
| <i>Corallistes elegantior</i> Schmidt, 1870 | | ✓ | |
| <i>Corallistes masoni</i> (Bowerbank, 1869) | | ✓ | |
| <i>Corallistes typus</i> Schmidt, 1870 | ✓ | | |
| <i>Corallistes paratypus</i> van soest & Stentoft, 1988 | ✓ | | |
| <i>Corallistes cf. undulatus</i> Lévi & Lévi, 1983 | ✓ | | |
| <i>Isabella harborbranchi</i> n. sp. | | ✓ | |
| <i>Neophrissospongia endoumensis</i> Pisera & Vacelet, 2011 | | | ✓ |
| <i>Neophrissospongia nana</i> Manconi & Serusi, 2008 | | | ✓ |
| <i>Neophrissospongia nolitangere</i> (Schmidt, 1870) | ✓ | ✓ | ✓ |
| <i>Neophrissospongia radjae</i> Pisera & Vacelet, 2011 | | | ✓ |
| <i>Neophrissospongia tubulata</i> (van Soest & Stentoft, 1988) | ✓ | | |
| <i>Neoschrammeniella bowerbankii</i> (Johnson, 1863) | | ✓ | ✓ |
| DESMANTHIDAE Topsent, 1894 | | | |
| <i>Desmanthus incrustans</i> (Topsent, 1889) | ✓ | | ✓ |
| <i>Sulcastrella tenens</i> (Vacelet, 1969) | ✓ | | ✓ |
| <i>Petromica (Chaladesma) ciocalyptoides</i> (van Soest & Zea, 1986) | ✓ | | |
| <i>Petromica (Petromica) grimaldii</i> Topsent, 1898 | | ✓ | |
| MACANDREWIIDAE Schrammen, 1924 | | | |
| <i>Macandrewia azorica</i> Gray, 1859 | | ✓ | |
| <i>Macandrewia clavatella</i> (Schmidt, 1870) | ✓ | | |
| <i>Macandrewia ramifera</i> Topsent, 1904 | | ✓ | |
| <i>Macandrewia robusta</i> Topsent, 1904 | | ✓ | |
| NEOPELTIDAE Sollas, 1888 | | | |
| <i>Daedalopelta nodosa</i> (Schmidt, 1879) | ✓ | | |
| <i>Neopelta perfecta</i> Schmidt, 1880 | ✓ | | |
| SCLERITODERMIDAE Sollas, 1888 | | | |
| <i>Aciculites cribrophora</i> (Schmidt, 1880) | ✓ | | |
| <i>Aciculites higginsii</i> Schmidt, 1879 | ✓ | | |
| <i>Aciculites mediterranea</i> Manconi, Serusi & Pisera, 2006 | | | ✓ |
| <i>Amphibleptula cf. madrepora</i> Schmdit, 1879 | ✓ | | |
| <i>Microscleroderma lamina</i> Perez, Vacelet, Bitar & Zibrowius, 2004 | | | ✓ |
| <i>Scleritoderma cyaneum</i> van Soest & Stentoft, 1988 | ✓ | | |

| | | | |
|---|---|---|---|
| <i>Setidium obtectum</i> Schmidt, 1879 | ✓ | | |
| SIPHONIDIIDAE Lendenfeld, 1903 | | | |
| <i>Siphonidium ramosum</i> (Schmidt, 1870) | ✓ | ✓ | ✓ |
| <i>Gastrophanella implexa</i> Schmidt, 1879 | ✓ | | |
| <i>Gastrophanella phoeniciensis</i> Perez, Vacelet, Bitar & Zibrowius, 2004 | | | ✓ |
| THEONELLIDAE Lendenfeld, 1903 | | | |
| <i>Discodermia dissoluta</i> Schmidt, 1880 | ✓ | | |
| <i>Discodermia polydiscus</i> (Bowerbank, 1869) | ✓ | ✓ | ✓ |
| <i>Discodermia polymorpha</i> Pisera & Vacelet, 2011 | | | ✓ |
| <i>Discodermia ramifera</i> Topsent, 1892 | | ✓ | |
| <i>Discodermia verrucosa</i> Topsent, 1928 | ✓ | ✓ | |
| <i>Racodiscula asteroides</i> Zittel, 1878 | ✓ | | |
| <i>Racodiscula clava sensu</i> Topsent, 1892 | ✓ | ✓ | |
| <i>Theonella atlantica</i> van Soest & Stentoft, 1988 | ✓ | | |
| VETULINIDAE Lendenfeld, 1903 | | | |
| <i>Vetulina stalactites</i> Schmidt, 1879 | ✓ | | |

Source literature: Bowerbank, 1869; Carter, 1876; Du Bocage, 1869; Gray, 1859; Johnson, 1863; Lenhart & van Soest, 1996; Longo *et al.*, 2005; Magnino, 1999; Maldonado & Young, 1996; Manconi *et al.*, 2006; Manconi & Serusi, 2008; Perez *et al.*, 2004; Pisera, 1999; Pisera & Lévi, 2002c, 2002e, 2002f, 2002h, 2002m; Pisera & Vacelet, 2011; Pomponi *et al.*, 2001; Schmidt, 1870, 1879, 1880; Topsent, 1892, 1889, 1904, 1928; Vacelet, 1969; van Soest & Stentoft, 1988; van Soest & Zea, 1986.

