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Integrative taxonomy of the genus *Thenea* (Porifera, Demospongiae, Tetractinellida) of the Portuguese shelf and slope: new records and new species for science

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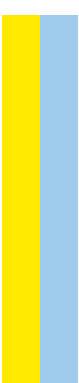
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2021

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Acknowledgements

Abstract

Sponge grounds are highly structured habitats that play key roles in benthic communities and are currently recognized as a vulnerable marine ecosystem (VME). The genus *Thenea*, known to form dense aggregations, encompasses 38 species that inhabit soft bottom habitats in deep waters. While the genus is relatively well documented at high latitudes in the Northeast Atlantic, and in the Mediterranean Sea, knowledge from the Portuguese continental slope and in the Azores is relatively scarce. This study aims to investigate the diversity of the genus *Thenea* from the southern Portuguese continental shelf and the Azores archipelago, through the implementation of an integrative taxonomic approach. Taxonomic identifications were performed based on external (shape, type of oscule and pores) and internal morphological characters (size and organization of skeletal elements). Molecular-based identifications were performed through the generation of DNA barcodes (partial mitochondrial COI and ribosomal 28S rRNA markers) followed by phylogenetic reconstructions under Bayesian and Maximum Likelihood approaches. We report the presence of six distinct species, including *Thenea muricata* (Bowerbank, 1858), *Thenea schmidtii* Sollas, 1886, from the Portuguese continental slope and four morphologically and/or genetically distinct species new to science, *Thenea* sp. nov. 1, *Thenea* sp. nov. 2 and *Thenea* sp. nov. 3 from the Azores region and *Thenea* sp. nov. 4 from the Gulf of Cadiz, which occurs sympatrically with *T. muricata*. The very subtle morphological differences between *Thenea* sp. nov. 4, *T. schmidtii* and *T. muricata* suggests the occurrence of a morphologically cryptic complex of species on the Gulf of Cadiz. This means that previous records and widespread *T. muricata* identified with morphological characters need to be revisited. Spatial and bathymetric patterns of distribution of the genus are discussed in the context of the biogeography of the Northeast Atlantic bathyal fauna, and implications of the findings herein reported for the conservation of the habitats they form presented.

Keywords: Sponge grounds; integrative taxonomy; Portuguese continental shelf; Azores; deep-sea

Resumo

As agregações de esponjas são habitats de estrutura complexa que desempenham papéis importantes nas comunidades bentónicas e são atualmente reconhecidos como ecossistemas marinhos vulneráveis (VME). O género *Thenea*, conhecido por formar este tipo de agregações densas, é composto por 38 espécies que habitam sedimentos moles no mar profundo. Este género encontra-se relativamente bem documentado a altas latitudes no Noroeste Atlântico, mas também no mar Mediterrâneo, no entanto, o conhecimento das *Thenea* na costa continental portuguesa e nos Açores é relativamente escasso. Este projeto tem como objetivo investigar a diversidade das esponjas do género *Thenea* da plataforma continental portuguesa e dos Açores com a implementação de uma abordagem de taxonomia integrativa. A identificação foi realizada com base em caracteres morfológicos externos (forma, tipo de ósculo, e poros) e internos (tamanho e organização de elementos do esqueleto). A identificação com base molecular foi realizada através da geração de códigos de barras de ADN (marcadores parciais do gene mitocondrial COI e ribossomal 28S rRNA) seguido de reconstruções filogenéticas usando o método Bayesiano e Máxima verossimilhança. É reportada a presença de seis espécies distintas, incluindo *Thenea muricata* (Bowerbank, 1858), *Thenea schmidtii* Sollas, 1886, da plataforma continental portuguesa e quatro espécies morfologicamente e/ou geneticamente distintas novas para a ciência *Thenea* sp. nov. 1, *Thenea* sp. nov. 2 and *Thenea* sp. nov. 3 da região dos Açores e *Thenea* sp. nov. 4, do golfo de Cádis, que ocorre simpatricamente com *T. muricata*. As variações morfológicas subtis entre *Thenea* sp. nov. 4, *T. schmidtii* e *T. muricata*, sugere a ocorrência de um complexo de espécies morfologicamente crípticas no golfo de Cádiz. Isto significa que registos prévios e amplamente distribuídos da espécie *T. muricata*, identificados com base em caracteres morfológicos necessitam de ser revisitados. Os padrões espaciais e batimétricos da distribuição do género são discutidos no contexto da biogeografia da fauna batial do Nordeste Atlântico, e as implicações dos resultados aqui relatados para a conservação dos habitats que eles formam são apresentadas.

Palavras-chave: Agregações de esponjas; taxonomia integrativa; plataforma continental portuguesa; Açores; mar profundo

Declaração de Honra

Declaro que a presente dissertação é da minha autoria e não foi utilizada previamente noutro curso ou unidade curricular, desta ou de outra instituição. As referências a outros autores (afirmações, ideias, pensamentos) respeitam escrupulosamente as regras de atribuição, e encontram-se devidamente indicadas no texto e nas referências bibliográficas, de acordo com as normas de referenciação. Tenho consciência de que a prática de plágio e auto-plágio constitui um ilícito académico.

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Introduction

Sponges (phylum Porifera) are benthic organisms capable of forming dense aggregations on all oceans and at a wide depth range occurring at different regions like continental shelves, slopes, seamounts, and oceanic ridges. These aggregations provide intricate habitats, substantially increasing biodiversity, often affecting the hydrodynamics, circulation, and recycling of marine nutrients, and facilitating energy transfer in the food web (Maldonado *et al.*, 2016). Sponge aggregations in the deep-sea are typically composed of multiples species from the classes Hexactinellida and Demospongiae and their high biodiversity makes them one of the most important habitats in this wast realm (Hogg *et al.*, 2010; OSPAR, 2010). In some sponge aggregations, up to 90% of the benthic invertebrate biomass is made of sponges (Murillo, Kenchington, Lawson, Li, & Piper, 2016). Sponges provide mankind with various goods, ranging from being used as bath sponges to the development of drugs (Hooper & Van Soest, 2002). In the pharmaceutical field, sponges are the main source of marine natural products, with more than 5000 compounds isolated, comprising about 30% of all marine compounds, which can have antibacterial, anticancer, antifungal, anti-HIV, anti-inflammatory, and antimalarial properties (Han *et al.*, 2019).

Deep sea sponge grounds are considered a vulnerable marine ecosystem (VME) and are threatened by various disturbances (FAO, 2009) such as bottom fishing, deep sea mining, oil exploitation and climate change (Clark *et al.*, 2016; Martín *et al.*, 2014). Sponge grounds that have been damaged by human activities such as bottom trawling have low densities of associated biota and very slow recovery rates, possibly taking decades for complete recovery (Morrison *et al.*, 2020). Other threats include deep sea mining, oil exploration and climate change, due to sponge dislodgement and physical damage, release of pollutants and changes in ocean geochemistry (Hogg *et al.*, 2010). Deep sea littering is another potential threat that sponge grounds face. Litter in the deep-sea is commonly seen, and although still unknown, smothering, damage and chemical leaching are potential effects these habitats may face (Ramirez-Llodra *et al.*, 2010). In order to implement conservation policies for the protection of deep-sea habitats such as sponge grounds, knowledge of their biology, ecology, diversity and distribution is needed. It is important for a species to be properly described and named for it and its habitat to have conservation policies implemented (Pante, Schoelinck, & Puillandre, 2015). Initially, sponge taxonomy was mostly conducted by the analysis of spicules and other morphological characters (Hooper & Van Soest, 2002). However, many of these characters are influenced by environmental factors (Bell, Barnes & Turner, 2002). In some cases, spicules

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have been shown to be plesiomorphic and homoplastic characters (Cárdenas *et al.*, 2011). Sponges are a group that possess many cryptic species, with no morphological differences, only revealed with aid of genetic analysis (Pöppe *et al.*, 2010; Reveillaud *et al.*, 2010, Xavier *et al.*, 2010). The lack of reliable morphological features, resulting in the difficulty of sponge identification, occurs throughout the phylum (Boury-Esnault *et al.*, 2013) and, in order to establish a solid framework of sponge DNA sequences to aid taxonomists, the sponge barcoding project (https://www.spongebarcoding.org/approach.php) was created (Wörheide, Erpenbeck, & Menke, 2007). The combination of two or more methodologies, such as morphology and genetics, for taxonomy is referred to as an integrative taxonomy approach (Dayrat, 2005; Pante, Schoelinck, & Puillandre, 2015).

Thenea Gray, 1867, is a genus of demosponges documented on all oceans ranging from 100-4000 m in depth, comprising a total of 38 valid species according to the World Porifera Database (de Voogd *et al.*, 2021). Although many sponge grounds are typically documented on hard substrates, *Thenea* assemblages are mostly found on soft sediments (Santín *et al.*, 2018). Deep-sea sponge aggregations are considered vulnerable marine ecosystems (VMEs) of high conservation concern (FAO, 2009). The identification of VMEs can be done either through direct observation of the seafloor with imaging systems (cameras, autonomous or remotely operated vehicles), or through collection and identification of species that are considered indicators (VMEIs) of the presence of VMEs. The list of taxa classified as VMEIs is regularly reassessed and updated during workshops of the ICES Working Group on Deep-Water Ecology (WGDEC). In 2020 *Thenea* species were included, supported by direct evidence of their functional significance, fragility and structural complexity that fit the designation of a VME indicator (FAO, 2009, ICES, 2020). Since *Thenea* species mostly inhabit soft sediments, the type of substrate most exploited by trawl fishing, they are vulnerable to this activity (Pansini & Musso, 1991). These sponges vary from flat, spherical, oval to elongated in shape, ranging from 1-15 cm in size and often possess root-like structures (Cárdenas & Rapp, 2012; Steenstrup & Tendal, 1982). The family encompassing *Thenea*, Theneidae Gray, 1872, has gone through multiple changes, originally described as Theneadae Gray, 1872, encompassing *Thenea muricata* and a number of species from other genera, later synonymized with *Thenea* (Gray, 1872). The family Theneanina was erected by (Carter 1883) encompassing the same genera and species as Theneadae, and are both interpreted as the same family, Theneidae. Theneidae was then merged with Pachastrellidae Carter, 1875, as at the time, morphological characters did not allow for a reliable distinction between the two families (Maldonaldo, 2002).

However, genetic analysis showed that this arrangement resulted in a polyphyletic family and that Theneidae was a valid monophyletic family, therefore resurrected, now including the genus *Annulastrella* Maldonado, 2002, and, based on morphological characters, the monotypic genus *Cladothenea* Koltun, 1964 as well (Cárdenas *et al.*, 2011). The family is diagnosed by the presence of long shafted triaenes, and diverse streptaster spicules (Cárdenas *et al.*, 2011). The genus *Thenea* is diagnosed by the presence of tetraxonic megascleres and without cladotyles Cárdenas & Rapp, 2012). The genus is reported worldwide, but better studied in the Northeast Atlantic (de Voogd *et al.*, 2021). The species found in this area are *T. muricata* (Bowerbank, 1858), *T. abyssorum* Koltun, 1964, *T. valdiviae* Lendenfeld, 1907, *T. levis* Lendenfeld, 1907 and *T. schmidtii* Sollas, 1886. Other less known species in the Atlantic include *T. bojeadori* Lendenfeld, 1907, *T. echinata* (Verrill, 1874), *T. fenestrata* (Schmidt, 1880), *T. megastrella* Lendenfeld, 1907, and *T. microclada* Lendenfeld, 1907.

Thenea species form dense aggregations in the Northeast Atlantic (Maldonado *et al.*, 2016). In the Arctic, in the Norwegian and Greenland Seas, *Thenea abyssorum* was very abundant and was the preferred substrate of the calcareous sponges: *Sycon abyssale* Borojevic & Graat-Kleeton, 1965*, Clathrina blanca* (Miklucho-Maclay, 1868), and *Clathrina* sp. Gray, 1867 (Barthel & Tendal, 1993). It was shown in the Faroe Islands that the sponges that hosted the higher number of associated taxa were *T. levis* and *T. valdiviae* (Klitgaard, 1995). In previous studies, high numbers of *T. muricata* were sampled from the Ibero-Moroccan Gulf which may suggest the presence of these aggregations in the area (Boury-Esnault *et al.*, 1994; Sitjá *et al.*, 2019). The species *T. abyssorum* is gonochoric and oviparous, with successive hermaphroditism not excluded, and also capable of asexual reproduction through budding (Witte, 1996). *T. muricata* is a species capable of hermaphroditism, as individuals have been observed with both oocytes and spermatocytes (Babiç 1915). Water masses were shown to structure habitat distribution in the deep-sea sponges of the genus *Geodia* (Roberts et al., 2021). A similar phenomenon may occur in *Thenea* species. Although most research involving this genus has been related to taxonomy, there are still limitations on the reliable morphological distinction of some species, such as the case of the widespread *Thenea muricata* and its sister species, *T. schmidtii*, reported in more temperate regions (Cárdenas & Rapp, 2015). Some hybridization may also occur between species, as evidenced by genetic analysis (Cárdenas & Rapp, 2012). There are also some doubtful records within the species *T. muricata*, where some morphological variability is found, notably in the size of spicules (plesiasters) of sponges from different regions, which could indicate that sponges are being misidentified. A great deal of

morphological and spicular variation of *T. muricata* collected from the Ibero-Moroccan Gulf and from the Alboran Sea were reported (Boury-Esnault *et al.*, 1994).

In comparison with the Northeast Atlantic and the Mediterranean, knowledge on sponge grounds from the Portuguese continental slope is scarce. This study aims to investigate the diversity and distribution of *Thenea* species sampled from the Portuguese deep-sea, using an integrative taxonomy approach based on morphological features combined with the generation of voucher-linked DNA barcodes of two genetic markers and the assembly of a distribution and bathymetric dataset.

2. Material and Methods

2.1. Study areas and sampling

Samples of *Thenea* collected in two study areas, the south and southwestern continental margin of the Portuguese mainland and the Azores archipelago, were selected for this work.

The Azores is an archipelago situated in the North Atlantic formed by volcanic and tectonic activity, situated near the Mid-Atlantic Ridge between 28º 00′ N – 49º 00′ N, and 17º 00′ W – 41º 00′ W with an extension of approximately 8,051,544 km^2 that includes the Exclusive Economic Zone (EEZ) along with the Portugal's claimed extended continental shelf area around the archipelago (George *et al.*, 2018; Perán *et al.*, 2016). Its seafloor is characterized by the presence of various geologic formations, such as seamounts, and a vast area covered by mud, (Perán *et al.*, 2016; Morato *et. al.*, 2008). It is a region affected by numerous water masses (Bashmachnikov *et al.*, 2015). Specimens from the Azores region were collected with an Agassiz trawl by Francisca Carvalho during the M150 BIODIAZ cruise in 2018 on the RV Meteor (George *et al.*, 2018).

Southern Portugal is partly covered by the Gulf of Cadiz, which starts east Cabo de Santa Maria, is situated south of the Iberian Peninsula and the Gibraltar Strait connects the Atlantic Ocean with the Mediterranean. Southwest Portugal, Southern Portugal and the Gulf of Cadiz contain complex geological formations, seamounts and mud volcanoes which harbor extensive fauna (Xavier & Van Soest 2007; Sitjá *et al.*, 2019; Vázquez *et al.*, 2015). The two main water masses, the North Atlantic Surface Water and the Mediterranean outflow of water influence the dispersal of both shallow and deep-sea fauna (Sitjá *et al.*, 2020; Xavier *et al.*,

2012). The Mediterranean outflow of water is known to run along the Iberian coast and flows through the Portuguese continental rise (Zenk & Armi, 1990). Some specimens from Southern Portugal were collected with a beam trawl during the ISUNEPCA 0619 cruise in 2019 onboard the RV Ángeles Alvariño by Jorge Lobo Arteaga. The specimens from Southwest Portugal and some from Southern Portugal were collected with a bottom trawl during the PNAB in 2021 onboard the RV Mário Ruivo by Celso Domingos.

Collected samples were sorted onboard and preserved in 96% ethanol for subsequent analysis. Vouchers of all analyzed specimens are deposited in the reference collection of the Deep-Sea Biodiversity and Conservation Lab at the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR). Type-material and supplementary samples of the new species herein described will be deposited at the Museu de História Natural e da Ciência da Universidade do Porto (MHNC-UP) and Museu Nacional de História Natural e da Ciência (MUHNAC). Locations and details of the sampling stations presented in Figure 1. and Table 1.
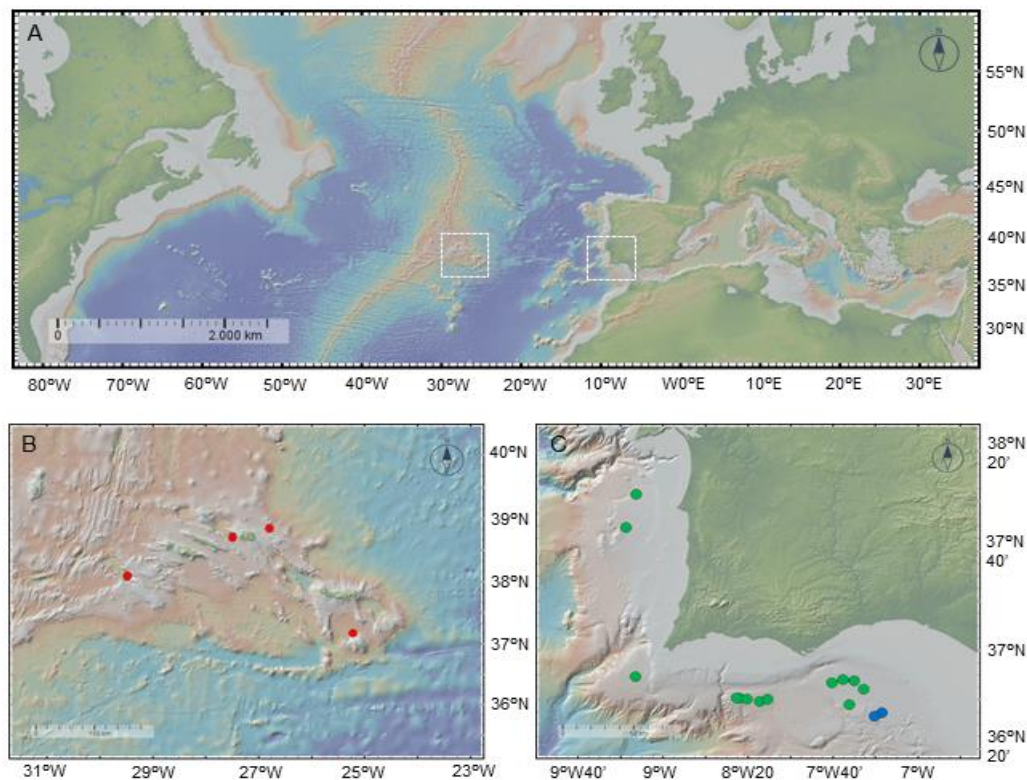
Figure 1. Study areas and sampling sites. A - Context map of our study area's location in the North Atlantic. B - Azores, four stations from BIODIAZ (red). C - Southern and Southwestern

Portugal, two stations from ISUNEPCA (blue) and 13 stations from PNAB Crustáceos 2021 campaign (green)

Table 1. Station list of collected samples used in this study.

Campaign	Station	Date	Latitude	Longitude	Depth (m)	no. of specimens
BIODIAZ M150	178	08/09/2018	38.091	-29.475	2547	7
BIODIAZ M150	322	14/09/2018	38.850	-26.790	1535	1
BIODIAZ M150	475	21/09/2018	37.173	-25.216	2065	2
BIODIAZ M150	534	24/09/2018	38.708	-27.489	1819	4
ISUNEPCA	BT12_E4	13/06/2019	36.580	-7.285	543	114
ISUNEPCA	BT10_E5	13/06/2019	36.556	-7.342	532.5	23
PNAB	1	15/06/2021	38.047	-9.212	588	14
PNAB	10	19/06/2021	36.678	-8.402	698	3
PNAB	11	19/06/2021	36.680	-8.424	704	8
PNAB	12	20/06/2021	36.672	-8.342	701.5	54
PNAB	13	20/06/2021	36.656	-8.242	766	8

Table 1. Continued

Campaign	Station	Date	Latitude	Longitude	Depth (m)	no. of specimens
PNAB	20	21/06/2021	36.671	-8.180	763	1
PNAB	38	25/06/2021	36.635	-7.539	551	7
PNAB	41	26/06/2021	36.739	-7.427	550	12
PNAB	42	26/06/2021	36.796	-7.502	538	93
PNAB	49	28/06/2021	36.804	-7.590	545	13
PNAB	50	28/06/2021	36.782	-7.676	568	28
PNAB	61	01/07/2021	36.824	-9.216	661	7
PNAB	66	04/07/2021	37.826	-9.292	528.5	10

2.2. Morphological characterization and identification

All specimens were photographed after preservation. For spicule analysis, fragments of the sponges measuring roughly 0.5 cm x 0.2 cm of each sample were used and digested in 65% nitric acid, then washed twice in water and 96% ethanol. The spicules were placed on a microscopic slide with Eukitt® Quick-hardening mounting medium and the slides were then left to dry in a fume hood between 4 to 6 days. The slides were observed under a Leica DM500 light microscope mounted with a ICC50W camera and the spicules were photographed and

measured using the Leica Application Suite (LAS 4.12) programme (Leica Microsystems). For each analyzed specimen, and unless otherwise stated, 30 spicules of each type were measured as follows: megascleres (triaene: rhabdome length and width; clad length; and oxea length and width) and microscleres (plesiaster: actine length and width; and spiraster/metaster length) (Supplementary Material 1 for morphological terminology). Often, megascleres were broken which limited the number of measurements obtained. However, the sizes of these spicules have been shown of limited taxonomic relevance in previous studies due to their similarity between species (Cárdenas & Rapp, 2015). For a subset of samples, spicules were also prepared for scanning electron microscope (SEM) and were placed onto a glass cover slip glass, glued on top of a stub, and covered with a fine film of Au/Pd using a SPI Module Sputter Coater. Spicules were then observed and photographed with a FEI Quanta 400FEG ESEM / EDAX Genesis X4M at the IMICROS lab of the Materials Centre of the University of Porto (CEMUP).

2.3. Distribution and bathymetric data assembly

Data on *Thenea* species' occurrence across the Northeast Atlantic was compiled from specialised literature (Cárdenas & Rapp 2015; Steenstrup & Tendal, 1982; Boury-Esnault, Pansini & Uriz, 1994), the World Porifera Database (WPD, http://www.marinespecies.org/porifera/) and the Global Biodiversity Information Facility (GBIF, https://www.gbif.org), and complemented with our own records (Supplementary Material 6). All maps depicting the distribution of the various species were produced using GeoMapApp 3.06.10. Ridge plots depicting the bathymetric distribution of the various species were produced using R statistical software R version 4.1.1 (R Core Team 2020).

2.4. DNA extraction and sequencing

The genomic DNA of a small portion (about 3 mm^3) of the sponges was extracted with the DNeasy® Blood & Tissue Kit (QIAGEN) following the manufacturer's protocol. The quality and quantity of DNA extracted were checked with a DS-11 Series Spectrophotometer (DeNovix). The extracted DNA was amplified through polymerase chain reaction (PCR) targeting the mitochondrial cytochrome *c* oxidase subunit I (COI-5P) and the 28S ribosomal gene partial fragment (C1–D2 domains) (Lê *et al.*, 1993; Cárdenas *et al.*, 2010; Folmer *et al.*, 1994). For the COI region, the primers LCO1490 (5' GGT CAA CAA ATC ATA AAG ATA TTG G 3') and HCO2198 (5' TAA ACT TCA GGG TGA CCA AAA AAT CA 3') were used with the

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following cycle details (1 cycle 5min/94ºC; 37 cycles 1 min/94ºC, 1min/47ºC, 1min30s/72ºC; 1 cycle 72ºC) (Folmer *et al.*, 1994). For the 28S rRNA region the primers C1'ASTR (5' ACC CGC TGA ACT TAA GCA T 3') and the D2 (5' TCC GTG TTT CAA GAC GGG 3') were used with the following cycle details (1 cycle 3min/95ºC; 35 cycles 30s/95ºC, 45s/56ºC, 1min/72ºC; 1 cycle 6 min/72ºC) (Cárdenas *et al.*, 2010; Lê *et al.*, 1993). The quality of the amplicons was checked in 1 % agarose gel electrophoresis using the Fisher BioReagents; exACTGene; 100 bp PCR DNA ladder. The amplified products were purified using ExoSAP-IT™ Product Cleanup Reagent and the purification protocol followed by the manufacturer (Thermo Fisher Scientific). The purified samples were sequenced through sanger sequencing serviced by STABVIDA using the BigDye Terminator v 3.1 kit on an ABI 3730XL DNA analyser (Applied BiosystemsTM, Foster City, CA, USA).

2.5. Phylogenetic reconstructions

Sequences were aligned manually using MEGA 7 software (Kumar, Stecher, and Tamura, 2015) and were checked using Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov). Additional sequences of *Thenea* spp., and other species in the families Theneidae and Vulcanellidae were obtained from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Our sequences were distributed in three alignment datasets: COI, 28S and COI+28S concatenated. To find the most appropriate model for the phylogenetic analysis of each dataset for maximum likelihood analysis, MrModeltest2 (Nylander, 2004) and PAUP4 (Swofford, 1998) were used. Maximum likelihood analysis was conducted in IQ-TREE (Minh *et al.*, 2020) using the following models: GTR+I for the COI dataset and GTR+I+G for the 28S and Concatenated datasets with 2000 bootstrap replicates. Bayesian Inference was conducted using Mrbayes version 3.2.7 (Ronquist *et al.*, 2012), running Markov Chain Monte Carlo runs of four chains, with 5 million generations, sampling every 1000th tree and discarding the first 25% as burn-in. The phylogenetic trees were visualized in FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

To assess intra- vs interspecific genetic variation, uncorrected p-distances were calculated using pairwise comparisons in MEGA7 under default parameters. A record list with all specimens used in this study containing their respective photographs, sample location, depth, associated DNA sequences and trace files was created on The Barcode of Life Data

System (BOLD) (https://www.boldsystems.org/) under the code name "THENE" (see example page in Supplementary Material 2).

3. Results

From a total of 409 samples of the genus *Thenea* from the three collections, 35 samples were sub-selected for this work. Seventeen from Southern Portugal, four from Southwest Portugal and 13 from the Azores region, and taxonomically identified as representing six species of which four are new to science (*Thenea* sp. nov. 1, *Thenea* sp. nov. 2, *Thenea* sp. nov. 3 and *Thenea* sp. nov. 4). Morphological descriptions of the examined material and phylogenetic reconstructions are provided in the following sections and Supplementary Material 3,4.

3.1. Taxonomic descriptions

<div align="center">

Phylum Porifera Grant, 1836

Class Demospongiae Sollas, 1885

Subclass Heteroscleromorpha Cárdenas et al., 2012

Order Tetractinellida Marshall, 1876

Suborder Astrophorina Sollas, 1887

Family Theneidae Gray, 1872

Genus *Thenea* Gray, 1867

Thenea muricata (Bowerbank, 1858)

</div>

Examined material: Gulf of Cadiz: IS2 and IS3: 36.556, -7.342, 532.5 m depth, Centro Oceanográfico de Cádiz (IEO), station BT10_E5. IS4, IS5, IS9, IS10, IS12 and IS14: 36.556, -7.342, 543 m depth, Centro Oceanográfico de Cádiz (IEO), station BT12_E4; PN199 and PN202: 36.796, -7.502, 538 m depth, Instituto Português do Mar e da Atmosfera (IPMA), station 42; Southwest Portugal: PN4, PN8 and PN12: 38.047, -9.212, 588 m depth, Instituto Português do Mar e da Atmosfera (IPMA), station 1; PN285, 37.826, 9.292, 528.5 m depth, Instituto Português do Mar e da Atmosfera (IPMA), station 66.

Outer morphology: Small sponges (< 1cm-2.5 cm diameter), shape is subspherical and slightly compressible (except IS14, not compressible). Color whitish to light brown in ethanol. Surface minutely hispid (except IS10, IS14, hispid). Some specimens are fragments (PN4, PN8 and PN12). A single uncovered oscule is located at the top center of the sponge (except in PN199,

which has no oscule). The pore area is covered by a sieve but can be difficult to find/non-existent (IS9, IS12). Roots are in general, long, and thin (Figure 2A-C).
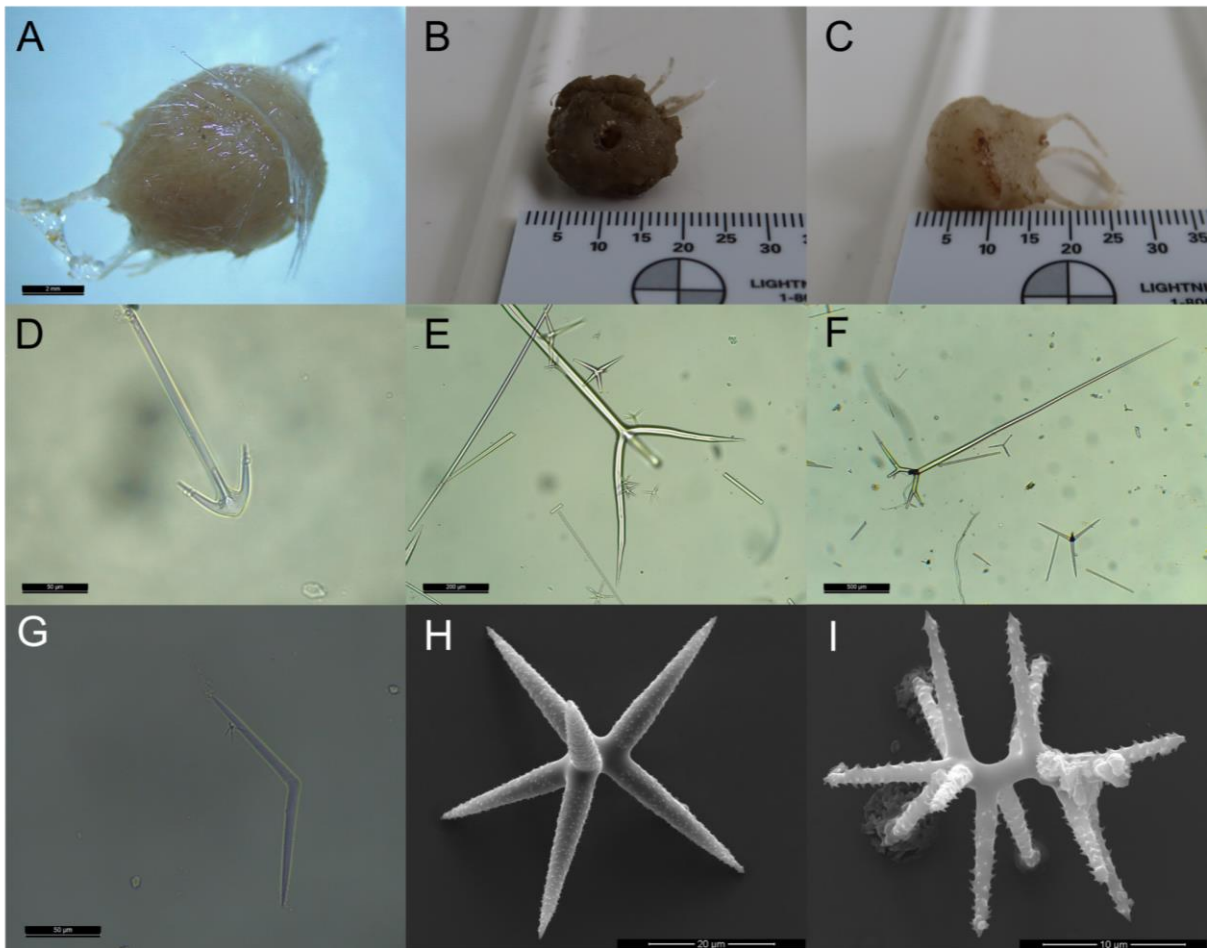
Figure 2. External morphology and spicule composition of *Thenea muricata* (Bowerbank, 1858). A-C, specimens preserved in ethanol; A, IS10; B, PN285; C, PN199; D-G, spicules in optical microscopy; D, anatriaene [PN8]; E, protriaene [PN199]; F, dichotriaene [PN8]; G, plesiaster [IS4]; H-I, spicules in SEM; H, metaster [IS14]; I, spiraster [IS4].

Spicules: Megascleres are oxeas, anatriaenes (IS12 and IS14), protriaenes (IS4, PN199 and PN202) and dichotriaenes (except in IS2, IS3, IS14, PN4 and PN285). Oxeas 873.4-1156.0-1438.7 µm long and 7.2-10.0-12.8 µm thick (PN12). Anatriaenes have 51.3-93.3-141.0 µm long clads, 1737.7 µm long and 6.1 µm thick rhabdomes (PN12) (Figure 2D). Protriaenes have 474.4 µm long clads (PN199) (Figure 2E). Dichotriaenes have 123.4-134.6-148.3 µm long protoclads, 348 µm long deuteroclads, 2219.7 µm long and 40.9 µm thick rhabdomes (PN8) (Figure 2F). Microsclers are plesiasters, metaster and spirasters. Plesiasters are minutely spined, almost

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smooth in larger ones, mostly 4 actines, but can have 2-5, sometimes even 7 actines (Figure 2G). Actine length: 35.0-79.1-175.7 µm, width: 3.1-9.7-22.3 µm (IS4) but the length can be up to 389.01 µm (IS9). Spirasters and metasters (Figure 2H,I) spined, length 13.6-23.1-29.6 µm (IS4) (Table 3).

DNA sequences: The COI and 28S fragments were sequenced from the 13 specimens identified as *T. muricata* in this work. No genetic divergences were found between our specimens and COI sequences were identical to *T. muricata* (GenBank accession ns. HM592677.1 and HM592706.1) and *T. schmidtii* (GenBank accession n. HM592737.1). The 28S fragments of these specimens are identical to *T. muricata* (GenBank accession ns. HM592768.1 and HM592767.1). The COI p-distance between the closest species, *Thenea* sp. nov. 3 and *Thenea valdiviae* was 0.2% for each and the 28S p-distance between the closest species, *Thenea* sp. nov. 4 was 0.8%.

Taxonomic remarks: Morphologically, our specimens match with the redescription of *T. muricata* (Steenstrup & Tendal, 1982) except for the absence of oscules in PN199 and pore areas in IS9 and IS12. We report the presence of plesiasters reduced to two actines (IS2, IS4, IS5, IS14) (Figure 2G), a feature not observed in this species before.

Thenea schmidtii Sollas, 1886

Examined material: South Portugal: PN74, PN75 and PN83: 36.672, -8.342, 701.5 m depth, Instituto Português do Mar e da Atmosfera (IPMA) station 12; PN92: 36.656, -8.242, 766 m depth, Instituto Português do Mar e da Atmosfera (IPMA) station 13.

Outer morphology: Subspherical sponges (Figure 3A-C). Size may vary from 1-4 cm diameter. Most samples are small sponges (PN75, PN75 and PN83), with size ranging from 1-2 cm diameter, only one sample (PN92) is massive (4 cm diameter). Sponges are slightly compressible, color whitish to light brown in ethanol and with the surface minutely hispid. A single uncovered oscule is located at the top center of the sponge surrounded by a whitish sphincter (Figure 3A). The pore area is partly covered by a sieve (Figure 3B). Roots are short and thick in PN74, PN75, long and thin in PN83 and PN92 (Figure 3B).
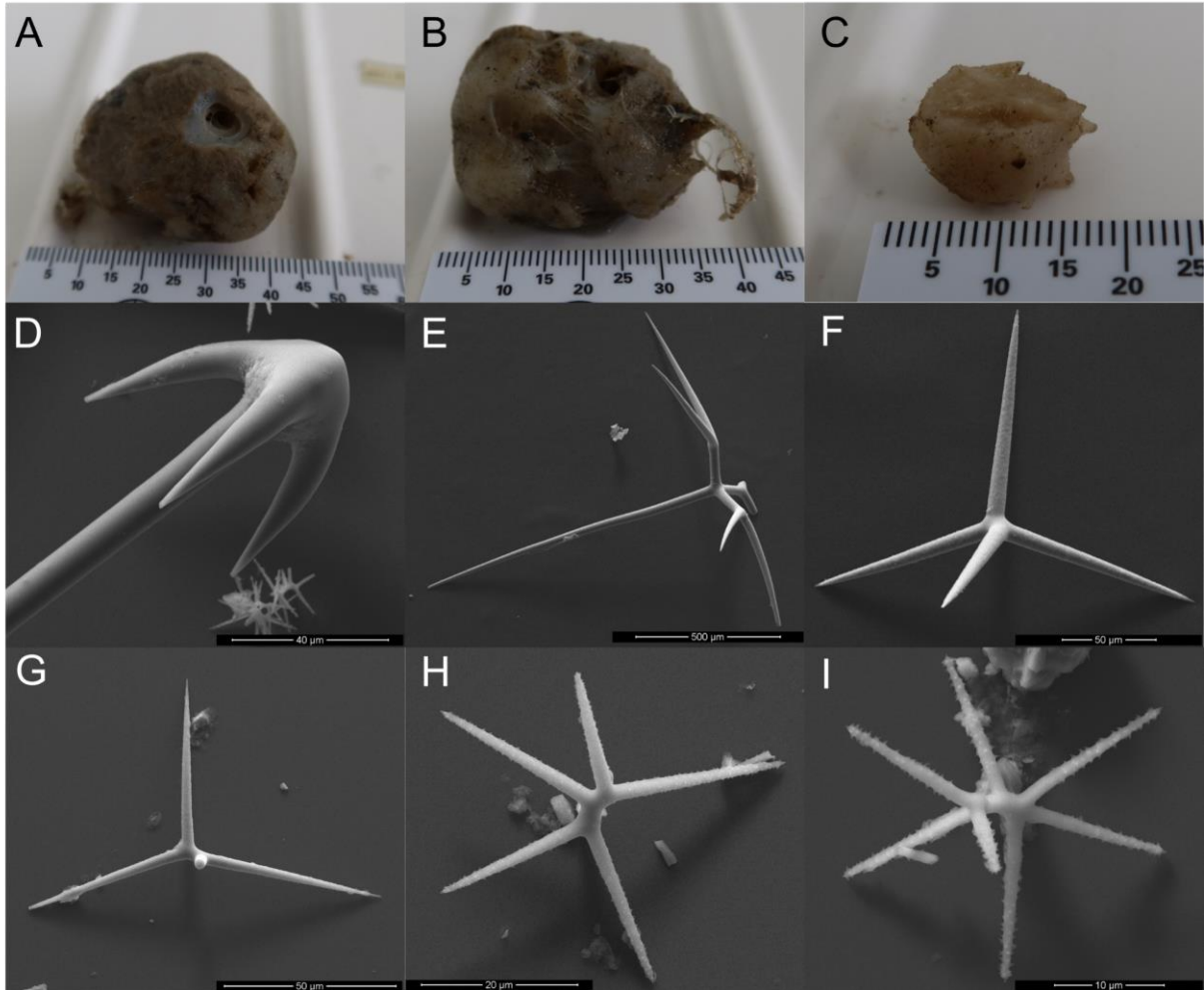
Figure 3. External morphology and spicule composition of *Thenea schmidtii* Sollas, 1886. A-C, specimens preserved in ethanol; A, PN92; B, PN92 pore area; C, PN75; D-I, spicules in SEM; D, anatriaene [PN74]; E, dichotriaene [PN83]; F, plesiaster [PN83]; G, plesiaster [PN74]; H, metaster [PN74]; I, spiraster [PN83].

Spicules: Megascleres are oxeas, anatriaenes (except PN75) and dichotriaenes (PN83). Oxeas are 2328.0 µm long and 10.9 µm thick (PN92). Anatrianes have 85.3 µm long clads (PN92) (Figure 3D). (Figure 3E). Microscleres are plesiasters, metasters and spirasters. Plesiasters are minutely spined with normally 4 actines, however sometimes they can have two to five actines. Actine length: 50.3-94.1-176.3 µm; Width: 3.7-7.2-16.5 µm (PN83) (Figure 3F). Metasters (Figure 3I) and spirasters (Figure 3H) are spined. Total length: 15.5-25.8-41.4 µm (Table 3).

DNA sequences: Genetic sequences obtained for the COI-5P fragment of all four specimens did not show divergences with the available sequences of *Thenea muricata* (GenBank accession ns. HM592677.1 and HM592706.1) and *T. schmidtii* (GenBank accession n. HM592737.1) as reported previously by (Cárdenas and Rapp, 2012), which can be explained by the low evolution rate of this region among these species (Cárdenas & Rapp, 2012). The 28S rRNA sequence of these specimens, out of all *Thenea* species, match the most with the single sequence available on genbank of *T. schmidtii* (GenBank accession n. HM592769.1) from Gulf of Cadiz, despite the rather high number of ambiguous b.p. (11) of this sequence. Our specimens may help to differentiate *T. muricata* from *T. schmidtii* as our sequences have up to sixteen b.p. difference in the 28S rRNA from *T. muricata* (GenBank accession ns. HM592768.1 and HM592767.1), due to the lower number of ambiguous b.p. of our sequences. Although the 28S rRNA sequences of our specimens and the *Thenea schmidtii* (GenBank accession n. HM592769.1) have slight variations due to the ambiguous b.p., the intraspecific variation of all *T. schmidtii* sequences was 0%.

Taxonomic remarks: Past studies have mentioned that there are no reliable morphological features to differentiate *Thenea muricata* and *Thenea schmidtii* (Cárdenas & Rapp, 2015). Cárdenas & Rapp, (2012) reported that the white broad sphincter surrounding the oscule could be a more reliable character for the distinction of the two species. We report this feature in our largest specimen, PN92, but not in our smaller ones, which shows that this feature should not be used as a reliable diagnostic feature for this species but will be discussed further. Our specimens are also difficult to distinguish from *T. muricata* and a reliable identification would not have been possible without genetic analysis of the 28S rRNA.

Thenea sp. nov. 1

Examined material: Azores, Off Faial: PR7, PR8, PR9, PR10, PR11 and PR12: 38.091, -29.475, 2547 m depth, German Centre for Marine Biodiversity Research (DZMB) station 178-1.

Outer morphology: Small to medium flattened-sub globular sponges with 1-3 cm in diameter. The color is light brown in ethanol (Figure 4A-C). The surface is fairly hispid, and the oscules are uncovered and can be very small, off-centered (PR11) and surrounded by a fringe (PR11, PR12) (Figure 4C). All have long and thin, filamentous roots (Figure 4 A,B). PR8 is the only

with a pore area covered by a sieve, the rest have a very thin pore area protected by a thick overhang (Figure 4A).

Spicules: Megascleres are oxeas, protriaenes and dichotriaenes. Oxeas are 1422.8 μm long and 11.2 μm thick. Dichotriaenes have 165.0-117.9-87.4 μm long protoclads and 783.9-563.1-417.0 μm long deuteroclads. Microscleres are plesiasters, amphiasters and rare metasters/spirasters. Plesiasters are small, minutely spined with 4-9 thin actines. Actine length: 48.9-62.5-92.7 μm; Width: 3.8-4.9-6.5 μm (PR7). Amphiasters (Figure 4H) and the rare metasters/spirasters (Figure 4I) are spined, length: 18.6-30.6-37.7 μm (PR7) (Table 4).
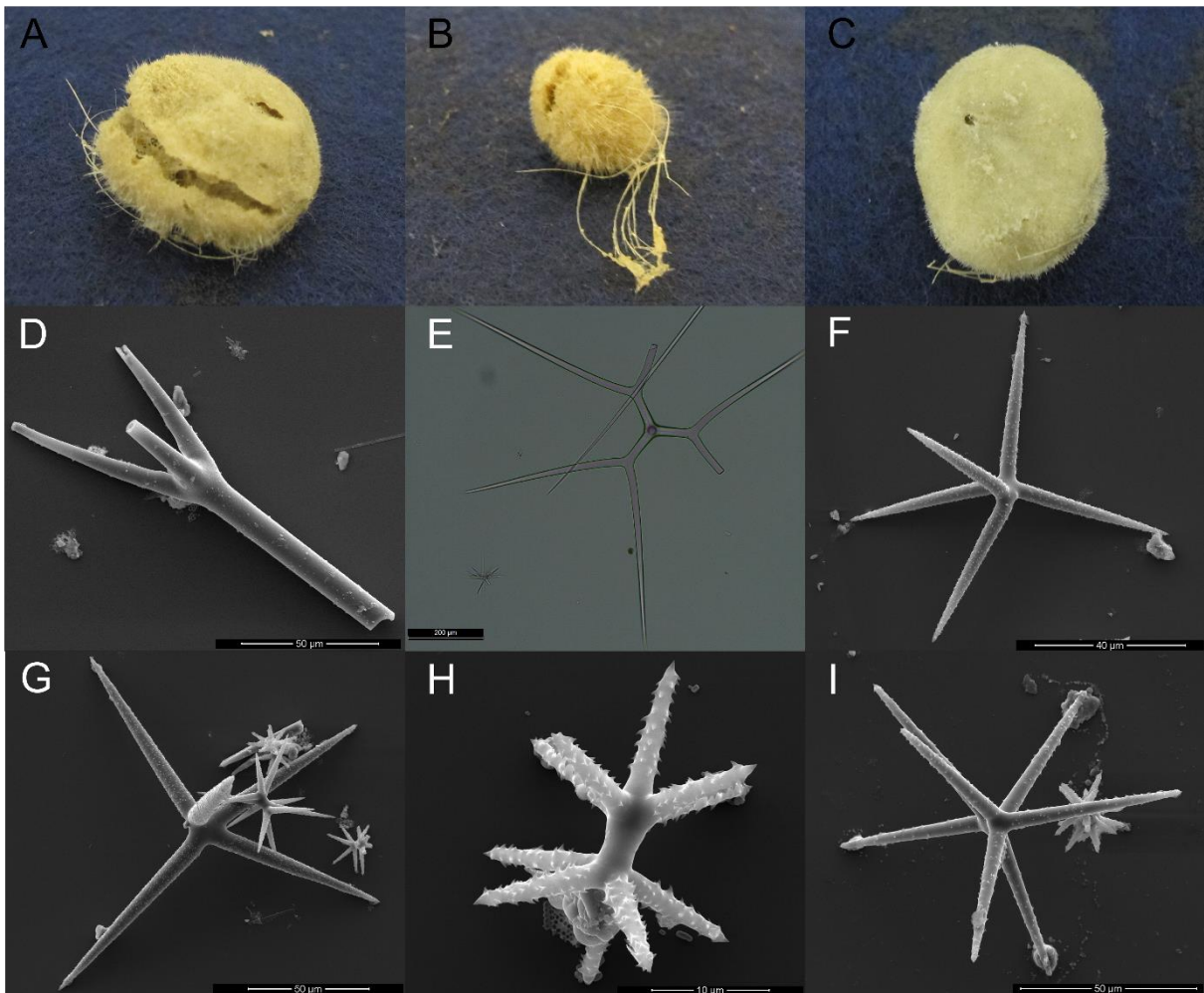
Figure 4. External morphology and spicule composition of *Thenea* sp. nov. 1. A-C, specimens preserved in ethanol; A, PR8; B, PR9; C, PR11, notice the small off-centered oscule; D, F-I, spicules in SEM; E, spicule in optical microscopy; D, protriaene [PR11]; E, dichotriaene [PR7]; F, plesiaster [PR11]; G, plesiaster [PR11]; H, amphiaster [PR11]; I, spiraster [PR11].

DNA sequences: Genetic analysis of this group of six specimens for the COI fragment revealed a notable separation from other *Thenea* species forming a strongly supported clade (Figure 8). The sequences of these specimens are mostly identical, PR9 and PR10 with one and two different b.p. respectively but have 0% intraspecific p-distance. The p-distance with its closest species, *Thenea levis* (GenBank accession n. HM592717.1) and *Thenea* sp. nov. 2 is 3.7%, with 25 different b.p. with *Thenea levis*. This difference is remarkable, considering that the COI fragment is a rather slow evolving gene in comparison with the 28S rRNA (Cárdenas & Rapp, 2012). Out of the six specimens only one (PR7) had a good quality 28S sequence generated. Nonetheless, this sequence was also shown to be significantly different from other *Thenea* sequences, with the 8.7% p-distance between *Thenea* sp. nov. 2, *Thenea* sp. nov. 4 and *T. abyssorum* (GenBank accession n. HM592770.1). Comparing *Thenea* sp. nov. 1 with all the remaining *Thenea* species, the p-distance is 3.8% for the COI fragment and 8.5% for the 28S rRNA.

Taxonomic remarks: *Thenea* sp. nov. 1 specimens matches with the description of *Thenea* cf. *valdiviae* reported by (Cardenas & Rapp, 2015). These specimens were collected between 2961 m and 3046 m depth from the Azores region, close to where our specimens were collected and also in the bathyal zone. The external shape of the new species, with the exception of the sieved oscules, are identical to the described before, being slightly flattened, sub globular and fairly hispid. The authors highlight that the MAR-Eco specimens described for the Azores region differ from the boreo-artic region in the absence of anatriaenes, common presence of plesiasteres with thinner actines that are higher in actin number (4-9) in the azorean specimens (Cardenas & Rapp, 2015), characters also found in our specimens. Although there is no genetic data on the individuals examined by Cardenas & Rapp (2015), we think that those specimens are from the same species as the ones examined here.

<center>

***Thenea* sp. nov. 2**

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Examined material: Azores, West Ilha Terceira: PR4: 38.708, -27.489, 1819 m depth, German Centre for Marine Biodiversity Research (DZMB) M150_534-1.

Outer morphology: Circular-flattened sponge with hair like spicules covering the surface (Figure 5A,B). The only sample collected of this species is a fragment measuring 3.5 cm in diameter. The color in ethanol is light-brown. The pore area is concealed due to the high density of hair-

like spicules surrounding it (Figure 5B). The pores are not covered by a mesh or sieve. Its consistency is slightly compressible.

Spicules: Megascleres are oxeas, anatriaenes, protriaenes and dichotriaenes. Anatriaenes are common with 49.2-82.9-108.6 µm long clads, and the rhabdomes are 994.9-1211.0-1442.2 µm long and 9.7-11.9-14.7 µm (Figure 5C). Microscleres are plesiasters, metasters and spirasters. Plesiasters are small, minutely spined with typically 4 actines, although some have 2,3,5,7 actines (Figure 5F). Actine length: 22.3-52.5-79.3 µm and width: 2.4-8.3-12.0 µm. Metasters (Figure 5H,I) and spirasters spined: Length 17.9- 29.3-40.4 µm (Table 4).
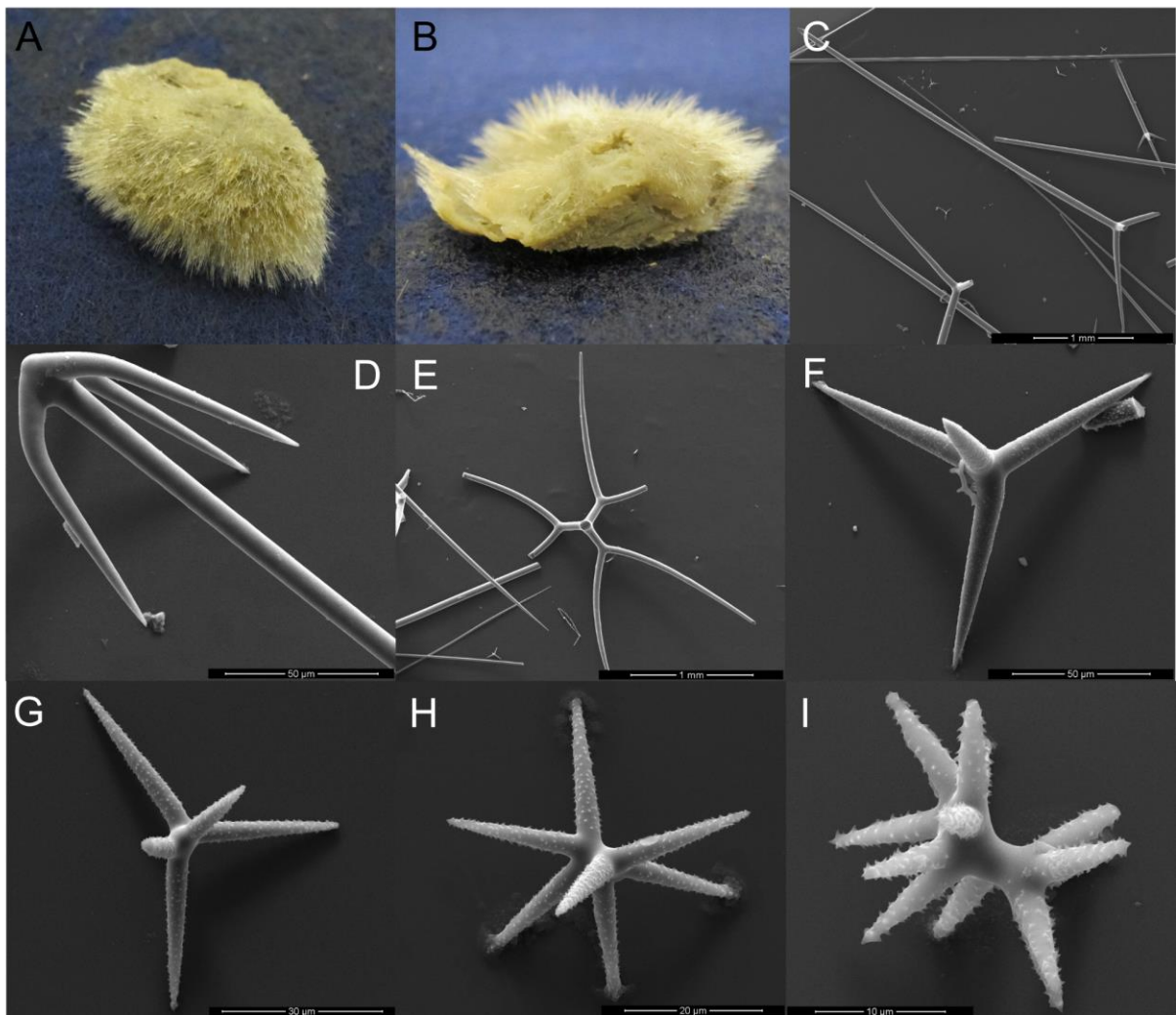
Figure 5. External morphology and spicule composition of *Thenea* sp. nov. 2 [PR4]. A-B, specimen preserved in ethanol [PR4]; A, upperside; B, underside; C-I, spicules in SEM; C, protriaene; D, anatriaene; E, dichotriaene; F, plesiater; G, plesiaster; H, metaster; I, spiraster.

DNA sequences: Genetic analysis placed this specimen close to *Thenea levis*, a species with distinct sequences for COI and 28S from other *Thenea* species (Supplementary Material 5), for both genetic markers with strong support (Figure 8, 9). This specimen is also shown to be different from *T. levis*, with a p-distance of 0.2% on COI and 8.7% on 28S. This specimen has two different b.p. with the COI sequence of *Thenea levis* (HM592747.1 and HM592717.1) and 12 different b.p. with the 28S (HM592765.1) sequence.

Taxonomic remarks: The external morphology of our specimen is different from the usually described *T. levis*. with the elongated shape, it is flattened and circular (Lendenfeld, 1907, Cárdenas & Rapp, 2015). The surface is very hispid with hair-like spicules covering the surface, a feature observed in a specimen from Sotbakken, Northern Norway at 286 m depth (Cárdenas & Rapp, 2012). We report the presence of plesiasters reduced to two actines, a feature reported in specimens from the Flemish Cap and West Greenland (Cárdenas & Rapp, 2015). "Fat" or bullet-shaped plesiasters were not seen and we lack sufficient anatriaene measurements, so spicule characters for discrimination between *T. levis* and other *Thenea* are not confirmed/ cannot be checked (Cárdenas & Rapp, 2015). The plesiasters in this specimen are smaller and the spirasters are longer than *Thenea levis* and its external morphology looks closer to the boreal form of *T. valdiviae* (Lendenfeld, 1907). The size of spicules in our specimen are closer to that of *T. valdiviae* as well, except for the anatriaenes (49-108/90-260 μm) and plesiaster (22-79/60-200 μm), which are smaller in our species. In addition, in *T. valdiviae* anatriaenes are rare to non-existent (Cárdenas & Rapp, 2012, Lendenfeld, 1907) and in *Thenea* sp. nov. 2 they are common. The top oscule of *T. valdiviae* is covered by a sieve, but in our specimen, the top oscule was not seen.

Thenea sp. nov. 3

Examined material: Azores: East Terceira Island: PR2, 38.85, -26.79, 1535 m depth, German Centre for Marine Biodiversity Research (DZMB) station M150_322-1; North Santa Maria Island: PR3 and PR14: 37.173, -25.216, 2065 m depth, German Centre for Marine Biodiversity Research (DZMB) station M150_475-1; West Ilha Terceira Island: PR5, PR13 and PR15, 38.708, -27.489, 1819 m depth, German Centre for Marine Biodiversity Research (DZMB) station M150_534-1; Southwest Faial Island: PR6, 38.091, -29.475, 2547 m depth, German Centre for Marine Biodiversity Research (DZMB) station M150_178-1.

<u>Outer morphology:</u> Massive, slightly compressible, and sub-spherical sponges (up to 5 cm in diameter). The color of the sponges is light brown in ethanol and the surface is minutely hispid (Figure 6A-D). A single uncovered oscule can be found at the top (Figure 6B) with a whitish sphincter (PR2 has a fringe surrounding the oscule, Figure 6B) and a pore area that runs along the equatorial side of the sponge that is covered by a sieve and is protected by an overhang (Figure 6A). PR5 is smaller (2.5 cm diameter), more hispid and flattened (Figure 6C). It also has a fringe surrounding the oscule surrounded by a whitish sphincter and is slightly compressible. PR3 is also a fragment but the uncovered oscule surrounded by a whitish sphincter can be seen (Figure 6D). PR2 and 13 had roots, but not PR6, PR13 and the fragmented specimens, although damage/removal may have occurred during sampling. A pore area runs along the equatorial side of the sponges covered by a sieve protected by an overhang.

<u>Spicules:</u> Megascleres are oxeas, anatriaenes and dichotriaenes. Oxeas are 1780.5 µm long and 11.6 µm thick (PR2). Anatriaenes have 26.1-<u>38.5</u>-50.7 µm long clads and the rhabdomes are 618.6 µm long and 4.5 µm thick (PR2) (Figure 6E). Microscleres are plesiasters, metasters and spirasters. Plesiasters are minutely spined with 4 actines, but sometimes 2-7 (Figure 6G). Actine length: 18.1-<u>60.1</u>-118.1 µm (PR2). Actine width: 1.6-<u>6.3</u>-14.2 µm (PR2). Metasters (Figure 6H) and spirasters (Figure 6I) spined. Length: 21.9-<u>32.9</u>-54.6 µm (PR2) (Table 4).

<u>DNA sequences:</u> The genetic sequence for the COI fragment of our specimens match the most with *T. muricata* (HM592677.1), differing in one (PR2, PR5, PR13, PR14) or two (PR6, PR13) b.p.s, and with a p-distance of 0.2 % (Supplementary Material 5). Phylogenetic analysis placed these specimens in a strongly supported clade (PP=0.91, BS=89). For the 28S gene, our specimens match the most with *T. valdiviae* with nine (PR14) or ten (the remaining four) different b.p.s (Figure 9), and with a p-distance of 1.7 %. Phylogenetic analysis placed these specimens in a strongly supported clade (PP=0.99, BS=89).

<u>Taxonomic remarks:</u> Our specimens are very similar to the ones designated as *Thenea* cf. *schmidtii* described by Cárdenas & Rapp (2015), which were sampled North of the Azores region at a similar depth range (1650-2078 m depth). The outer morphology, spicule morphology and size correspond to those previously mentioned, although we lack reliable thick sections of our specimens. We also report the presence of plesiasters reduced to two actines in some of our specimens. Although there are no genetic sequences from the *Thenea* cf. *schmidtii* examined in Cárdenas & Rapp (2015), we think those individuals are from the

same species as the ones examined here. The plesiasteres of this species are slightly smaller than *Thenea muricata* and *Thenea* sp. nov. 4 and have a wide range, much wider than *Thenea valdiviae*, the genetically closest species. In addition, the metasters in this species can be quite large, and their distinction with the smaller plesiasters can be difficult, which is not the case for *Thenea muricata*/*Thenea* sp. nov. 4/*Thenea schmidtii*.
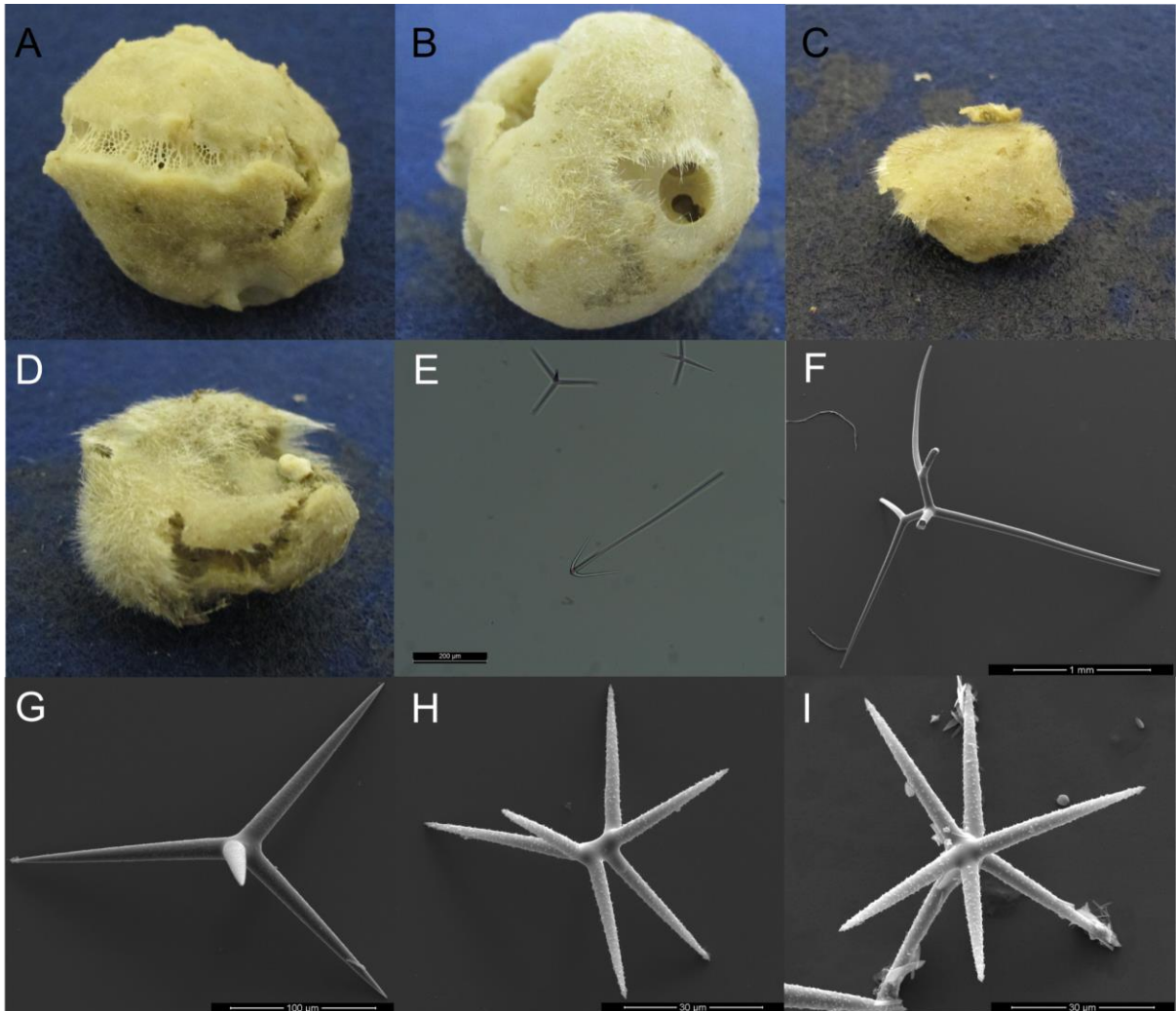
Figure 6. External morphology and spicule composition of *Thenea* sp. nov. 3. A-D, specimens preserved in ethanol; A, PR6; B, PR2; C, PR13; D, PR5; E, spicule in optical microscopy; E, anatriaene [PR13]; F-I, spicules in SEM; F, dichotriaene [PR3]; G, plesiaster [PR6]; H, metaster [PR3]; I, spiraster [PR6].

Thenea sp. nov. 4

<u>Examined material:</u> Gulf of Cadiz: IS7: 36.58, -7.285, 532.5 m depth Centro Oceanográfico de Cádiz (IEO) station BT10_E5; IS8 and IS11: 36.556, -7.342, 543 m depth, Centro Oceanográfico de Cádiz (IEO) station BT12_E4.

<u>Outer morphology</u>: Small and pear-shaped sponges (1-2 cm diameter) (Figure 7A-C). The consistency is slightly compressible and the color varies from brown (IS7) to white (IS8, IS11). The surface is smooth, with a single uncovered oscule situated at the center of the top area of the sponge (Figure 7C). Long and thin roots are present (Figure 7A,B) and the pore area is absent in these specimens.

<u>Spicules</u>: Megascleres are oxeas, anatriaenes, protriaenes and dichotriaenes. Oxeas are 409.8-<u>1742.1</u>-2764.6 µm long and 5.0-<u>16.7</u>-25.0 µm thick (IS11). Anatriaenes have 102.7-245.1-426.9 µm long clads and the rhabdomes are 1129.1-<u>1812.8</u>-2932.0 µm long and 16.4-<u>23.3</u>-31.9 µm thick (IS7). Dichotriaenes have 69.3-<u>93.2</u>-130.0 µm long protoclads, 105.8-<u>393.1</u>-470.0 µm long deuteroclads and the rhabdomes are 1140.6 µm long and 27.6 µm thick (IS7). Microscleres are plesiasters, metasters and spirasters. Plesiasters and spirasters are similar in all specimens. Plesiasters are minutely spined with 4 or sometimes 5 actines (Figure 7F, G). Actine length: 34.8-<u>80.8</u>-250.2 µm, width: 11.0-<u>27.0</u>-44.0 µm (IS7). Spirasters (Figure 7I) and metasters (Figure 7H) are minutely spined, length: 13.7-<u>22.0</u>-32.9 µm (IS7) (Table 3).

<u>DNA sequences</u>: These specimens share the same sequence for the COI fragment with *T. muricata* (HM592677.1 and HM592706.1) and *T. schmidtii* (HM592737.1) and were therefore placed close to these species. For the 28S fragment, these specimens match the closest with *T. muricata* (HM592768.1 and HM592767.1) although with some differences. Our sequences are different from *T. muricata* in seven b.p. and with a p-distance of 0.8 % (Supplementary Material 5). Four of those different b.p. are matching with *T. schmidtii* (HM592769.1) and the remaining three do not match with either species. There are eight sites for the 28S fragment where *T. muricata* (HM592768.1 and HM592767.1) and *T. schmidtii* (HM592769.1) are different, these specimens have four sites where the b.p. are different from *T. muricata*, but equal to *T. schmidtii* and vice versa. However, using our sequences of *T. schmidtii*, which have fewer ambiguous b.p., it shows that these specimens are closer to *T. muricata* than *T. schmidtii*. The three specimens were placed in a nearly maximum supported clade (PP=0.99, BS=98) for the 28S rRNA tree and (PP=0.99, BS=91) for the concatenate tree.
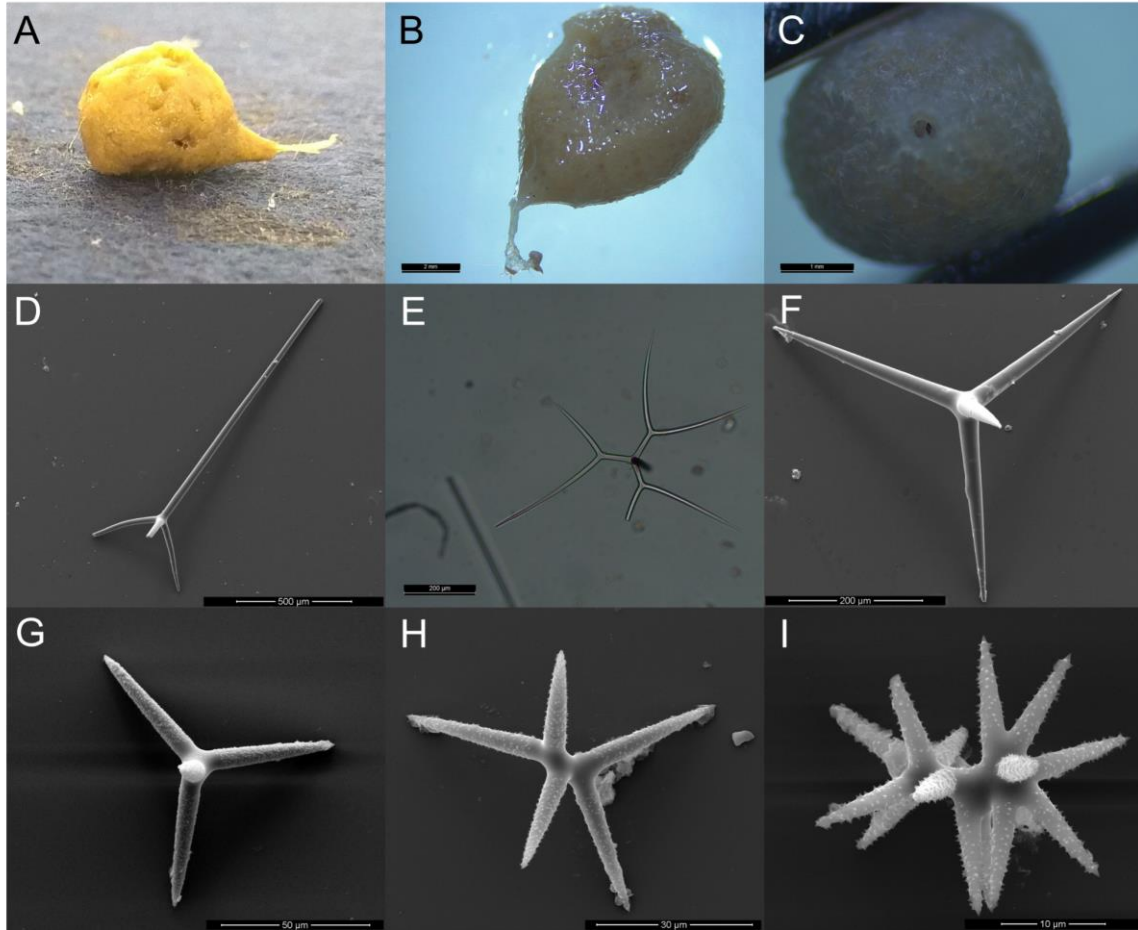
Figure 7. External morphology and spicule composition of *Thenea* sp. nov. 4. A-C, specimens preserved in ethanol; A, IS7; B, IS11; C, IS8; D,F-I, spicules in SEM; E, spicules in optical microscopy; D, protriaene [IS7]; E, dichotriaene [IS11]; F, plesiaster [IS7]; G, plesiaster [IS7]; H, metaster [IS7]; I, spiraster [IS7].

Taxonomic remarks: Our specimens match the morphological description of *T. muricata*, except for the lack of a pore area (Bowerbank, 1858; Steenstrup & Tendal, 1982), a feature seen in some of our smaller specimens of *T. muricata* (Figure 2B). Plesiasters reduced to two actines were not found in our specimens. The morphological distinction of this species from *T. muricata* is challenging at the moment and the future analysis of more specimens will make possible differences more apparent. These specimens were collected from the same stations as some of *T. muricata* analyzed in this study (Figure 1), meaning that these two species are sympatric. As *T. muricata* and *Thenea* sp. nov. 4 have no reliable morphological distinctive features, it could mean that *T. muricata* is a morphologically cryptic complex of species, and its records, identified solely on morphological characters should be revisited.

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Table 2. Comparison of morphological characteristics for *Thenea* species of the Arctic and Northern Atlantic. Spicule measurements (min-<u>mean</u>-max) provided in μm. L – length, W – width.

Species (source of description)	External morphology	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (Actin L x W)	Metaster spirasters (L)	Locality, depth
Thenea abyssorum Koltun, 1964 (Koltun, V. M. 1964)	Shape round, Mushroom-like, 2 cm in height with roots; Equatorial pore area with long spicules. Hispid. Color light grey to brown. Apical oscula with fringe. Pores at the bottom of pore area.	Prot L:100 x 180 μm

Deut L: 200-1400 μm

Rhab LxW: 700-2500 μm x 30-70 μm | Clad L: 70-110 μm

Rhab LxW: 900-1200 μm x 8-10 μm | Clad L:100-900 μm

Rhab LxW: 1000-3700 μm x 20-50 μm | LxW: 900-5500 μm x 8-60 μm | Thorny – 100-280 μm (diameter), Smooth 70-150 μm (diameter) | 20-35 μm | Arctic Russia, Spitsbergen, and East Greenland, 1130-2750 m |
| *Thenea levis* Lendenfeld, 1907 (Steenstrup & Tendal 1982) | Flattened, elongated/crescent shaped, 1-15 cm long, 0.8-4.5 cm high, 0.5-3cm wide. Non compressible. Pore and oscule sieved opposing. | Cladome total length: 1000 μm

Rhabd L: 750-4000 μm | Clad L: 100-200 mm | Protriaene prodiaene Clad L: 100-800 μm Mesoprotriaenes prolongation twice the length of clad. | L: 4500-11000 μm | 30-275 μm diameter | 12 μm | Denmark Strait, Faroe Islands, 250-1480 m |

...Continued on next page

Table 2. Continued

Species (source of description)	External morphology	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (Actin L x W)	Metaster spirasters (L)	Locality, depth
Thenea valdiviae Lendenfeld, 1907 (Steenstrup & Tendal 1982)	Flat/spherical/lumpy 2-11 cm diameter. Greyish brown, not compressible. Sieved oscule and pore area situated in the equatorial side.	Prot L: 1000 µm Deut L: 3000-8000 µm Rhab L: 9000-12000 µm	Clad L: 90-260 µm Rhab L: 11000-14000 µm	Clad L: 1000-2000 µm Rhab: 8000-13000 µm	L: <13000 µm	60-200 µm diameter	20-40 µm	Arctic, Kara Sea Norwegian Sea, East Greenland, Barent sea Laptev Sea, East Siberian Sea, Polar Basin, 110 -1900 m
Thenea echinata (Verrill, 1874)	Broad, convex, hemispherical, 5-10 cm diameter, 5-7.5 cm in height, supported on a broad stout, but short root. Hispid Large fringed apical oscules covered by sieve.	Not provided	Not provided	Not provided	Not provided	Not provided	Not provided	Cashe's Ledge, 75 m

Table 3. Comparison of morphological characteristics for *Thenea* species of the temperate Northeast Atlantic. Spicule measurements (min-mean-max) provided in μm. L – length, W – width

Species (source of description)	External morphology	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (Actin L x W)	Metaster spirasters (L)	Locality, depth
Thenea muricata (Bowerbank, 1858) (Boury-Esnault, et. al 1994)	Symmetrical, globular, mushroom like shape. Diameter 0.2-2cm Apical oscule, surrounded by an area of collagen.	Prot L:150-350 μm Deut L:300-560-1000 μm Rhab LxW: 4000-4900-5200 μm x 50-90-110 μm	Clad L: 50-105-180 μm Rhab LxW: 2000-2850-3200 μm x 21-23-14 μm	Clad L: 250-286-360 μm Rhab LxW: 5000 μm x 24-25-26 μm	LxW: 5000-5400-20000 μm 10-15-25 μm	Actin L: 15-69-110 μm	13-22.6-32 μm	North occidental Atlantic to the Atlantic, 109-4020 m
Thenea muricata (Bowerbank, 1858) PN12 This study	Small sponges (<1cm-2.5cm diameter), subspherical, slightly compressible light brown and minutely hispid Apical oscula with no sieve. Pore area in equatorial side covered by sieve.	Prot L: 123.4-134.6-148.3 μm Deut L: 348 μm Rhab LxW: 2219.7 μm x 40.9 μm (PN8)	Clad L: 51.26-93.3-141.0 μm Rhab LxW: 1737.7 μm x 6.1 μm	Clad L: 474.4 μm (PN199)	LxW: 873.4-1156.0-1438.7 μm x 7.24-10.0-12.8 μm	33.4-104.5-285.4 μm x 2.9-13.3-31.9 μm	16.1-21.9-28.9	Southwest Portugal, 588 m

Table 3. Continued

Species (source of description)	External morphology	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (Actin L x W)	Metaster spirasters (L)	Locality, depth
Thenea schmidtii Sollas, 1886 (Sollas, W.J. 1888)	Spherical, hispid, almost white, faintly grey Pore area either restricted to one side, opposing oscule, or surrounding the entire equatorial area	Prot L: 175 µm Deut L: 1500 µm Rhab LxW: 9300 x 87 µm	Clad L: 143-196 µm Rhab LxW: 17900-21500 x 20-25 µm	Clad L: 1070 µm x Rhab LxW: 5400 µm x 71 µm	LxW: 14300 µm x 79 µm	Actin LxW: 175-205 µm x 17-27.6 µm	32 µm	Gibraltar, 1097 m; Culebra Island, 713 m; Azores, 1829 m;
Thenea schmidtii Sollas, 1886 PN92 This study	Massive subspherical, 4 cm in diameter, minutely hispid, whitish in color. Long thin roots. Apical oscula not sieved, surrounded by broad white sphincter. Equatorial pore area fringed.	Not measured	Clad L: 85.3 µm	Not measured	LxW: 2328.0 x 10.9 µm	Actin LxW: 48.4-91.7-150.9 µm x 6.8-11.1-20.5 µm	16.9-23.7-35.6	Gulf of Cádiz, 766 m
Thenea sp. nov. 4 IS7 This study	Pear shaped, 1-2 cm diameter, slightly compressible light brown, smooth surface, long and thin roots Single uncovered apical oscule. Pore area absent.	Prot L:69.3-93.2-130.0 µm Deut L: 105.8-393.1-470.0 µm Rhab LxW: 1140.6 µm x 27.6 µm	Clad L: 102.7-245.1-426.9 µm Rhab LxW: 1129.1-1812.8-2932.0 µm x 16.4-23.3-31.9 µm	Not measured	LxW: 409.8-1742.1-2764.6 x 5.0-16.7-25 µm (IS11)	Actin LxW: 34.8-80.8-250.2 µm x 11.0-27.0-44.0 µm	13.7-22.0-32.9 µm	Gulf of Cádiz, 532.5 m

Table 4. Comparison of morphological characteristics for *Thenea* species of the Azores region. Spicule measurements (min-<u>mean</u>-max) provided in µm. L – length, W – width.

Species (source of description)	External morphology	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (actin L x W)	Amphiasters metasters spirasters (L)	Locality, depth
Thenea cf. *schmidtii* Sollas, 1886 (Cardenas & Rapp, 2015)	Subspherical, hispid sponges, 1.5–3 cm in diameter. Apical oscule without sieve. Equatorial cribiporal oscules.	Not provided	Not provided	Not provided	Not provided	28–<u>89</u>–188 µm x 4–16 µm	24 –<u>29.9</u>–36 µm	Azores, 1650-2078 m
Thenea sp. nov. 3 PR2 This study	Massive sponge 5 cm in diameter, sub-spherical, minutely hispid, slightly compressible, brown in color. Apical oscule surrounded by fringe, with a whitish sphincter. Equatorial pore area covered by sieve and protected by overhang.	Not measured	26.1-<u>38.5</u>-50.7 µm x 618.6 µm x 4.5 µm	Not measured	1780.5 µm x 11.6 µm	18.1-<u>60.1</u>-118.1 µm x 1.6-<u>6.3</u>-14.2 µm	21.9-<u>32.9</u>-54.6 µm	Azores, 1535- 2547 m

...Continued on next page

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Table 4. Continued

Species (source of description)	External morphology	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (actin L x W)	Amphiasters metasters spirasters (L)	Locality, depth
Thenea cf. *valdiviae* Lendenfeld, 1907 (Cardenas & Rapp, 2015)	Sub-globular, slightly flattened, triangular. Fairly hispid surface. dirty colour. One to several apical oscules with large meshed sieves. Equatorial poral area also sieved.	Not provided	Not provided	Not provided	Not provided	44–<u>55.3</u>–64 µm x 3–5 µm	20 –26.6–32 µm	Azores, 2961-3046 m
Thenea sp. nov. 1 PR7 This study	Small flattened, 3 cm diameter, hispid with filamentous roots. Color brown. Apical oscule small, uncovered.	Prot L: 165.0-<u>117.9</u>-87.4 µm Deut L: 783.9-563.1-417.0 µm	293.8 µm (PR11)	Not measured	1422.8 µm x 11.2 µm	48.9-62.5-92.7 µm x 3.8-4.9-6.5 µm	18.6-<u>30.6</u>-37.7 µm	Azores, 2547 m
Thenea sp. nov. 2 PR4 This study	Flattened, circular sponge, 3.5 cm diameter, with hair like spicules covering the surface. Slightly compressible Color brown. Equatorial pore area hidden under the high density of hair like spicules surrounding it. Pores are not covered by sieve.	Not measured	49.2-<u>82.9</u>-108.6 µm x 994.9-<u>1211.0</u>-1442.2 µm x 9.7-<u>11.9</u>-14.7 µm	Not measured	Not measured	22.3-52.5-79.3 µm x 2.4- 8.3-12.0 µm	17.9- 29.3-40.4 µm	Azores, 1819 m

Table 5. Comparison of morphological characteristics for *Thenea* species of the Tropical Atlantic. Spicule measurements (min-<u>mean</u>-max) provided in μm. L – length, W – width.

Species (source of description)	External morphology	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (actin L x W)	Metasters spirasters (L)	Locality, depth
Thenea bojeadori Lendenfeld, 1907 ZMH (holotype) (Lendenfeld, R. von. 1907)	Pear-shaped; 7 mm long and 4 mm wide, root 6 mm long, hispid. Color light brown. Apical oscules, 400-700 μm wide.	Prot L: 110 μm Deut L: 620 μm Rhab LxW: 1500 μm x 37-40 μm.	Rhab W: 20-37 μm thick	Clad L: 200-300 μm long Rhab W: 20-28 μm	LxW: 3000 μm x 30 μm	Actin LxW: 90-120 μm x 7-8 μm	15-70 μm	Cap Bojeador, Morocco, 146 m
Thenea fenestrata (Schmidt, 1880) (Sollas, W.J. 1888)	Cushion-shaped with an oval margin; surface rounded, Hispid. Roots are small. Apical oscula surrounded by fringe. An equatorial pore area with six to seven pores protected by fringe	Prot L: 163 μm Deut L: 678 μm Rhab LxW: 314 μm x 30-50 μm	Clad L: 400 μm Rhab LxW: 4641-5560 μm x 12.5 μm	Clad L: 785 μm Rhab LxW: 3900 μm x 52 μm	LxW: 5000-9300 μm x 60-50 μm	Actin LxW: 60-90 μm x 3.9 μm	39.5-47.4 μm Metaster absent	South Atlantic Lower Bathyal Province, 3137 m

Table 5. Continued

Species (source of description)	External morphology	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (actin L x W)	Metasters spirasters (L)	Locality, depth
Thenea megastrella Lendenfeld, 1907 ZMB (holotype) (Lendenfeld, R. von. 1907)	Massive, spherical, 13 mm in diameter. Several roots. Light brown. Apical oscule Completely closed equatorial pore area surrounds the body.	Prot L: 150-175 µm Deut L: 450-500 µm Rhab LxW: 1800 -2500 µm x 65-100 µm	Clad L: 50 µm Rhab L: 9000 µ	Rhab LxW: 350-370 µm x 40-50 µm	LxW:3300-5900 µm x 40 -70 µm	Actin LxW: 230-400 µm x 22-40 µm	17-18 µm	Cape Verde, 217 m
Thenea microclada Lendenfeld, 1907 ZMH (holotype) (Lendenfeld, R. von. 1907)	Pear-shaped body 4-10 mm long, 4-7 mm wide ending in a 4-24 mm long root. Brownish white. Not provided	Prot L: 100-140 µm Deut L: 300-500 µm Rhab LxW: 760-2100 µm x 30-40 µm	Clad L: 22-65 µm Rhab W: 6 - 24 µm	Clad L: 200-350 µm Rhab W: 17-25 µm	LxW: 3000-8000 µm x 20-44 µm.	Actin LxW: 50-205 µm x 15-20 µm	17-70 µm	Saharan Upwelling 146 m

3.2. Phylogenetic affinities

In total, we obtained 34 and 29 sequences of the mtDNA COI and C1-D2 rRNA 28S gene fragments, respectively (Table 6). Phylogenetic reconstructions performed separately for the two markers, as well as for both markers concatenated were largely congruent in topological arrangement, but with greater sub-structuring and nodal support in the 28S tree (Figures 8-10). All analysis retrieve a Theneidae clade with very high to maximum support (PP=1 and BS>0.98), encompassing all *Thenea* spp. and *Annulastrella ornata* Sollas, 1888.

In the COI tree, two major clades are retrieved, one encompassing six specimens of *Thenea* sp. nov. 1 (PR7–PR12) collected in the Azores at 2547 m depth with maximum support (PP=1; BS=98) sister to a larger clade comprising all remaining sequences of the various Northeast Atlantic and Arctic *Thenea* species, i.e. *T. levis*, *T. abyssorum*, *T. schmidtii*, *T. muricata* and *T. valdiviae* (PP=0.96, BS=89). The former clade is represented in the 28S and, subsequently, the concatenated tree by a single sequence, but it is clearly separated from all other sequences (8.5% p-distance) for 28S. Within the larger COI clade, some sub-structuring is observed with *Thenea levis* sequences from GenBank (accession ns. HM592747 and HM592717) grouping with one, likely misidentified, *Thenea muricata* sequence (accession n. JX999068) and one specimen (PR4), here identified as *Thenea* sp. nov. 2 collected in the Azores at 1819 m depth with strong support (PP=0.99, BS=84). The same clade is found with high support for 28S (PP=0.90, BS=77) and (PP=0.99, BS=92) for the concatenated dataset, but the Azorean specimen branches off from the remaining three *T. levis* GenBank sequences which are found together with maximum support for 28S (PP=1, BS=95) and (PP=1, BS=96) for the concatenated dataset (PP=1, BS=96). A polytomy is formed with all sequences, from GenBank and newly generated for specimens assigned to *T. schmidtii, T. muricata* and *Thenea* sp. nov. 4 and a single *T. valdiviae* sequence, discussed in Cárdenas & Rapp 2012 as a potential hybrid, in the COI tree (Figure 8). However, in the 28S and in the concatenated phylogeny, all sequences are grouped in a highly supported clade for 28S (PP=0.99, BS=71) and for the concatenated dataset (PP=0.98, BS=86) which is further split into three specific sub-clades: one formed by all *T. schmidtii* from the Southern margin of Portugal (PN74, PN75, PN83, PN92), alongside a GenBank sequence from a specimen collected in the Gulf of Cadiz (HM592769) for 28S (PP=1, BS= 99) and for the concatenated dataset (PP=1, BS= 97); another one formed by three *Thenea* sp. nov. 4 specimens (IS7, IS8, IS11) collected between 532–543 m depth for 28S (PP=1, BS=98) and for the concatenated dataset (PP=1, BS=91), sister to another maximally supported clade for 28S (PP=1, BS= 98) and for the concatenated

dataset (PP=1, BS=98), comprising several *T. muricata* specimens collected both on the South and Southwest margin of mainland Portugal and GenBank sequences collected both in Western Norway (HM592768) and the Mediterranean Sea (HM592767) at upper bathyal depths (Figure 9,10).

Seven specimens collected in the Azores between 1535 and 2065 m, assigned to *Thenea* sp. nov. 3, form a well-supported clade in the COI (PP=0.91, BS=86), 28S (PP=0.99, BS=89) and concatenated (PP=1, BS=89) trees. Finally, all *T. valdiviae* COI sequences from GenBank form a clade with relatively high support (PP=0.86, BS=87) with exception of a single sequence (HM592694) collected from the Greenland Sea at 578 m depth, which is found among the remaining *Thenea muricata/ schmidtii*/sp. nov. 4 sequences. Interestingly, in the 28S and concatenated trees, the corresponding sequence is found in a strongly supported *T. valdiviae* clade (PP=0.92, BS=81) for 28S and (PP=0.99, BS=86) for the concatenated dataset.

Pairwise COI p-distances varied between 0-0.24% and 0.2-3.9%, within and between species, respectively. Within species, *Thenea levis* had 0.24% p-distance while the remaining species had 0%. Species *Thenea* sp. nov. 1 and *Thenea muricata/schmidtii*/sp. nov. 4 were the most genetically divergent, with 3.86% p-distance, while *Thenea muricata/schmidtii*/sp. nov. 4 and *Thenea valdiviae* were the least genetically divergent, with 0.16% p-distance. The most divergent species was *Thenea* sp. nov. 1, with a mean distance of 3.75% with the remaining *Thenea* species. Pairwise 28S p-distances varied between 0-0.09% and 0.80-9.34% within and between species, respectively. Within species, *Thenea* sp. nov. 3 had 0.08% p-distance while the remaining species had 0%. The species *Thenea* sp. nov. 1 and *Thenea muricata* were the most genetically divergent, with 9.34% p-distance, while *Thenea muricata* and *Thenea* sp. nov. 4 were the least genetically divergent, with 0.80% p-distance. The most divergent species was *Thenea* sp. nov. 1. with a mean distance of 8.46% with the remaining *Thenea* species.

Table 6. List of sequences, mtDNA COI and rRNA 28S, used in the phylogenetic analysis with the respective voucher and GenBank accession nos. Sequences generated in the present study are highlighted in bold and will be deposited in GenBank.

Species	Voucher	Locality, depth (m)	mtDNA COI	rRNA 28S	Source
Thenea abyssorum	ZMBN:85228	Greenland Sea, 2425–2463 m	HM592712.1	HM592770.1	Cárdenas *et al.* 2010
Thenea levis	ZMBN 85229	Northern Norway, 286m	-	HM592764.1	Cárdenas *et al.* 2010
Thenea levis	ZMBN 85230	Northern Norway, 300m	HM592717.1	HM592765.1	Cárdenas *et al.* 2010
Thenea levis	ZMAPOR 21501	SW Rockfall Bank, Ireland 844-857 m	HM592747.1	HM592766.1	Cárdenas *et al.* 2010
Thenea muricata	ZMBN 85231	Western Norway, 300m	-	HM592768.1	Cárdenas *et al.* 2010
Thenea muricata	ZMBN 85232	Western Norway, 100m	HM592677.1	-	Cárdenas *et al.* 2010
Thenea muricata	MNHN DCL4083	Southern Italy 585m	HM592706.1	HM592767.1	Cárdenas *et al.* 2010
Thenea muricata (?)	MCZ:DNA105738	No data	JX999068.1	-	Riesgo *et al.* 2014
Thenea muricata	IS4	Gulf of Cadiz 543m	**XXXXXX**	**XXXXXX**	**This study**
Thenea muricata	PN8	Southwest Portugal 588m	**XXXXXX**	**XXXXXX**	**This study**

Table 6. Continued

Species	Voucher	Locality, depth (m)	mtDNA COI	rRNA 28S	Source
Thenea schmidtii	ZMAPOR 18036	Gulf of Cadiz 443m	HM592737.1	HM592769.1	Cárdenas *et al.* 2010
Thenea schmidtii	PN92	Gulf of Cadiz 766m	**XXXXXX**	**XXXXXX**	**This study**
Thenea valdiviae	ZMBN 85233	Barents Sea 297m	HM592708.1	-	Cárdenas *et al.* 2010
Thenea valdiviae	ZMBN 85234	Greenland Sea 578m	HM592694.1	HM592761.1	Cárdenas *et al.* 2010
Thenea valdiviae	ZMBN 85235	Northern Norway 330 m	HM592703.1	HM592762.1	Cárdenas *et al.* 2010
Thenea valdiviae	ZMBN 85236	Western Norway 300 m	HM592718.1	HM592763.1	Cárdenas *et al.* 2010
Thenea sp. nov. 1	PR7	Azores 2547 m	**XXXXXX**	**XXXXXX**	**This study**
Thenea sp. nov. 1	PR8	Azores 2547 m	**XXXXXX**	**XXXXXX**	**This study**
Thenea sp. nov. 1	PR9	Azores 2547 m	**XXXXXX**	**XXXXXX**	**This study**
Thenea sp. nov. 1	PR10	Azores 2547 m	**XXXXXX**	**XXXXXX**	**This study**
Thenea sp. nov. 1	PR11	Azores 2547 m	**XXXXXX**	**XXXXXX**	**This study**
Thenea sp. nov. 1	PR12	Azores 2547 m	**XXXXXX**	**XXXXXX**	**This study**

Table 6. Continued

Species	Voucher	Locality, depth (m)	mtDNA COI	rRNA 28S	Source
Thenea sp. nov. 2	PR4	Azores 1819 m	**XXXXXX**	**XXXXXX**	**This study**
Thenea sp. nov. 3	PR2	Azores 1535 m	**XXXXXX**	**XXXXXX**	**This study**
Thenea sp. nov. 3	PR3	Azores 2065 m	**XXXXXX**	**XXXXXX**	**This study**
Thenea sp. nov. 3	PR6	Azores 2547 m	**XXXXXX**	**XXXXXX**	**This study**
Thenea sp. nov. 3	PR5	Azores 1819 m	**XXXXXX**	**XXXXXX**	**This study**
Thenea sp. nov. 3	PR13	Azores 1819 m	**XXXXXX**	**XXXXXX**	**This study**
Thenea sp. nov. 3	PR14	Azores 2065 m	**XXXXXX**	**XXXXXX**	**This study**
Thenea sp. nov. 3	PR15	Azores 1819 m	**XXXXXX**	**XXXXXX**	**This study**
Thenea sp. nov. 4	IS7	Gulf of Cadiz 543 m	**XXXXXX**	**XXXXXX**	**This study**
Thenea sp. nov. 4	IS8	Gulf of Cadiz 532.5 m	**XXXXXX**	**XXXXXX**	**This study**
Thenea sp. nov. 4	IS11	Gulf of Cadiz 543 m	**XXXXXX**	**XXXXXX**	**This study**

(?) doubtful identity.
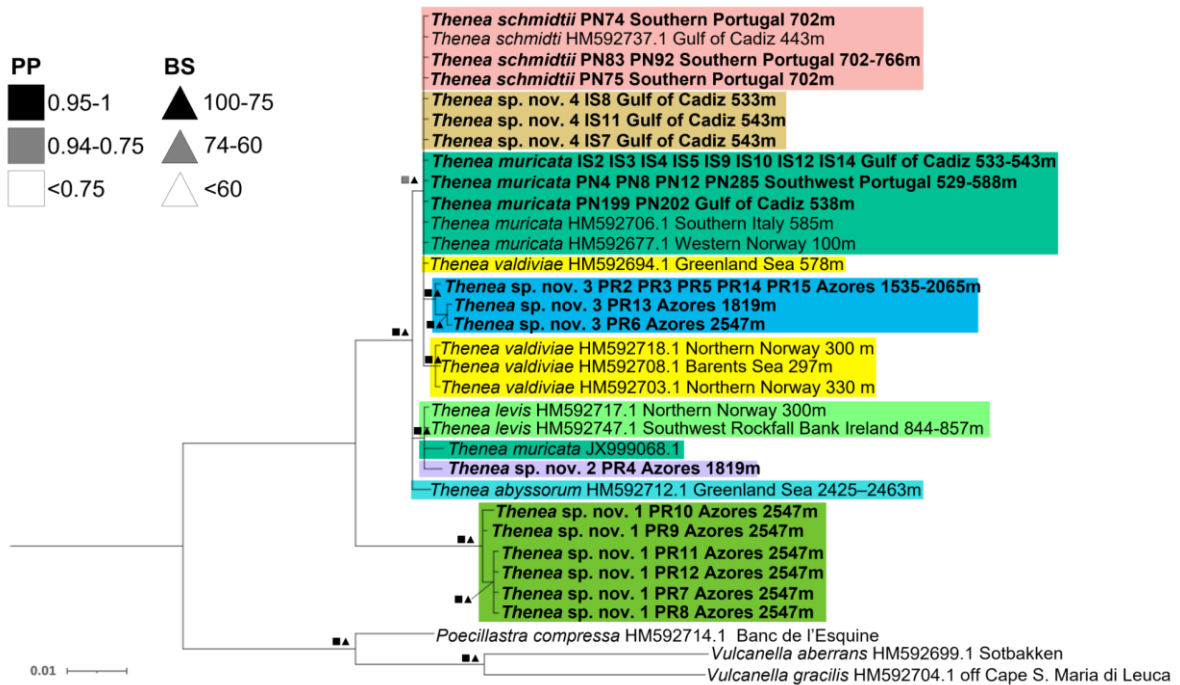
Figure 8. Phylogenetic relationships of the Northeast Atlantic and Arctic *Thenea* species based on the mtDNA COI gene. Bayesian posterior probabilities (PP, left squares) and maximum likelihood bootstrap support (BS, right triangles) are given on the branches. Sequences generated in this study are highlighted in bold.
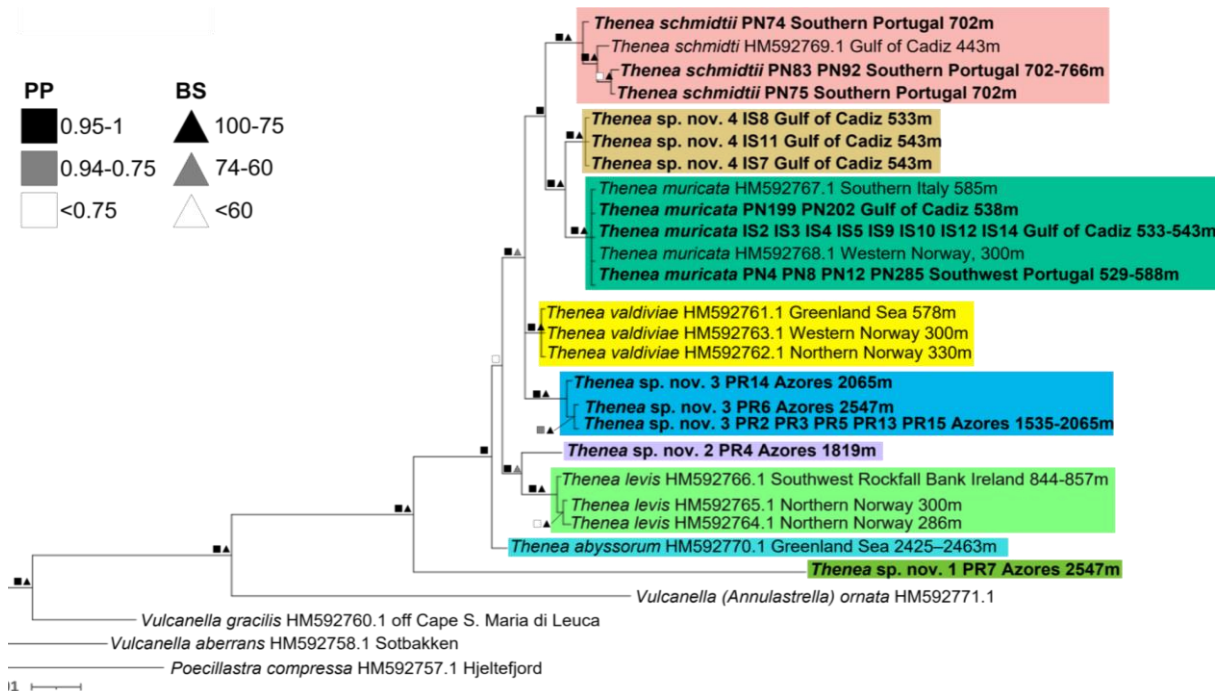
Figure 9. Phylogenetic relationships of the Northeast Atlantic and Arctic *Thenea* species based on the rRNA 28S (C1-D2) gene. Bayesian posterior probabilities (PP, left squares) and

maximum likelihood bootstrap support (BS, right triangles) are given on the branches. Sequences generated in this study are highlighted in bold.
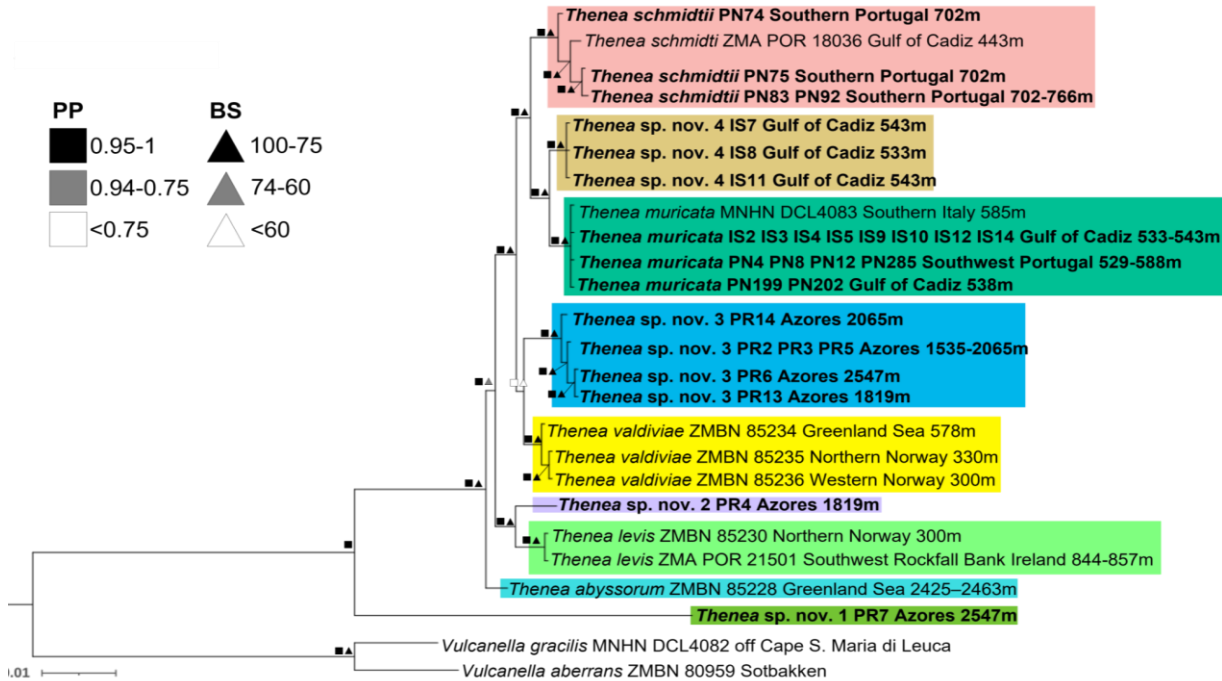
Figure 10. Phylogenetic relationships of the Northeast Atlantic and Arctic *Thenea* species based on the concatenated dataset comprising the mtDNA COI and rRNA 28S (C1-D2) regions. Bayesian posterior probabilities (PP, left squares) and maximum likelihood bootstrap support (BS, right triangles) are given on the branches. Sequences generated in this study are highlighted in bold.

3.3. Spatial and bathymetric distribution

A total of 362 records were obtained from the literature (Supplementary Material 6) and complemented with the records of 409 specimens obtained from the collection campaigns (Table 1). Some spatial and bathymetric patterns were detected. The species *Thenea abyssorum, Thenea valdiviae* and *Thenea levis* are mostly distributed throughout the boreo-Arctic and Northeast Atlantic (Figure 11) with *T. valdiviae* and *T. levis* having similar depth distribution, up to 2000 m with rare reports from deeper regions, and *T. abyssorum* is scattered throughout the bathyal zone (Figure 16). *Thenea echinata* has a single record from the Western Atlantic at 75 m, a shallow depth for *Thenea* species (Figure 11, 16). The species *Thenea muricata* is the most widely distributed species, found throughout the boreo-Arctic and along the Northeast Atlantic coast, as well as the Mediterranean (Figure 12). It also has a wide depth range, but, similarly to *T. valdiviae* and *T. levis*, most of its records are from down to 2000 m

(Figure 16). In the Azores, the species *Thenea* sp. nov. 1, *Thenea* sp. nov. 2 and *Thenea* sp. nov. 3, restricted to deeper waters, as well few records of *Thenea muricata* and *Thenea schmidtii* are reported (Figure 11, 13, 16). *Thenea schmidtii*, a species with a somewhat similar bathymetric distribution to *T. valdiviae* and *T. levis*, although more evenly distributed, also has some records from the Gulf of Cadiz, along with *Thenea* sp. nov. 4, a species with few records at similar depths (Figure 14, 16). The temperate and tropical Atlantic are inhabited by the shallower inhabiting species *Thenea bojeadori, Thenea microclada* and *Thenea megastrella,* as well as *Thenea fenestrata* a species with a seemingly divided bathymetric distribution, both in shallow, and deep waters (Figure 15, 16)*.*
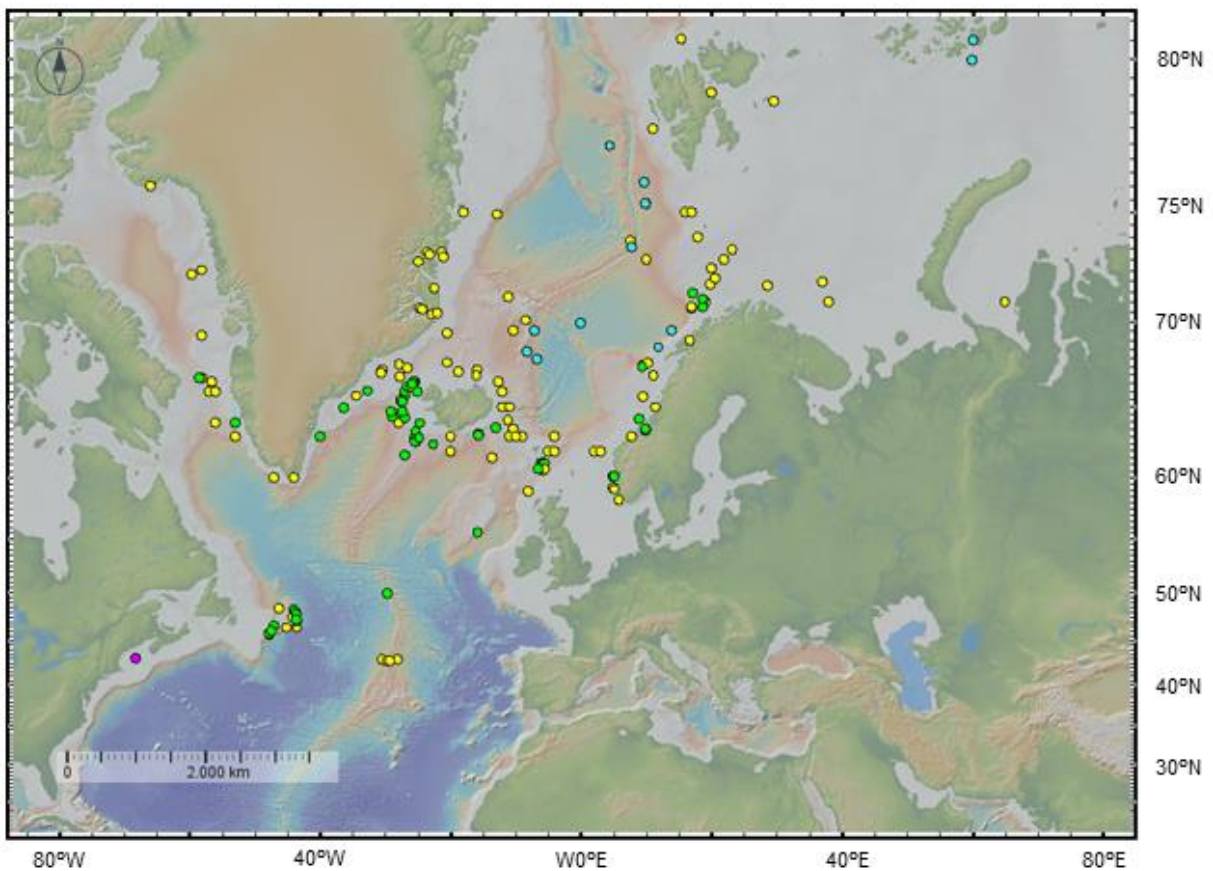
Figure 11. Geographical distribution of *Thenea valdiviae* Lendenfeld, 1907 (yellow)*, Thenea levis* Lendenfeld, 1907 (green), *Thenea abyssorum* Koltun, 1964 (blue)*,* and *Thenea echinata* (Verrill, 1874) (purple) across the Arctic and the temperate Northern Atlantic realms.
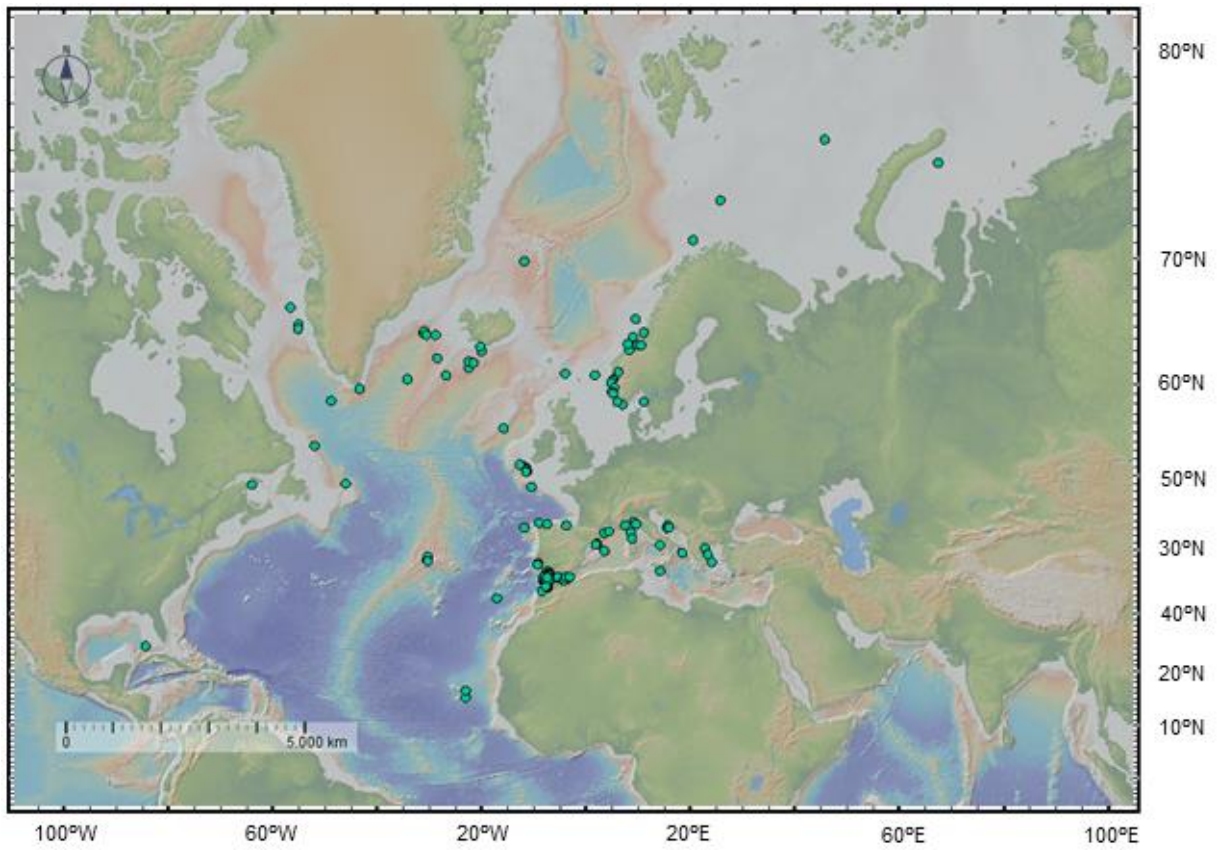
Figure 12. Geographical distribution of *Thenea muricata* (Bowerbank, 1858) across the Arctic and the temperate Northern Atlantic realms.
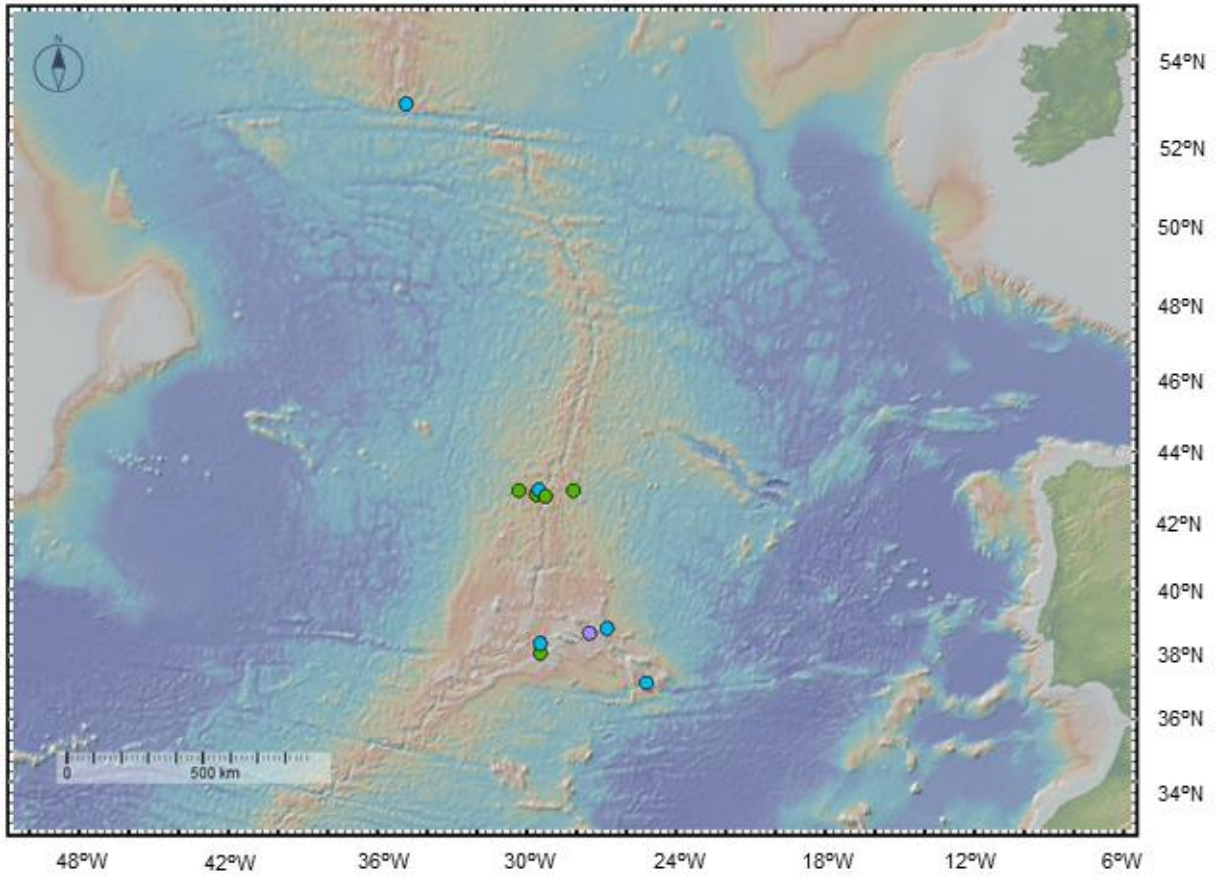
Figure 13. Geographical distribution of *Thenea* sp. nov. 1 (green), *Thenea* sp. nov. 2 (purple) and *Thenea* sp. nov. 3 (blue) on the Azores archipelago and northern Mid-Atlantic Ridge, temperate Northern Atlantic realm.
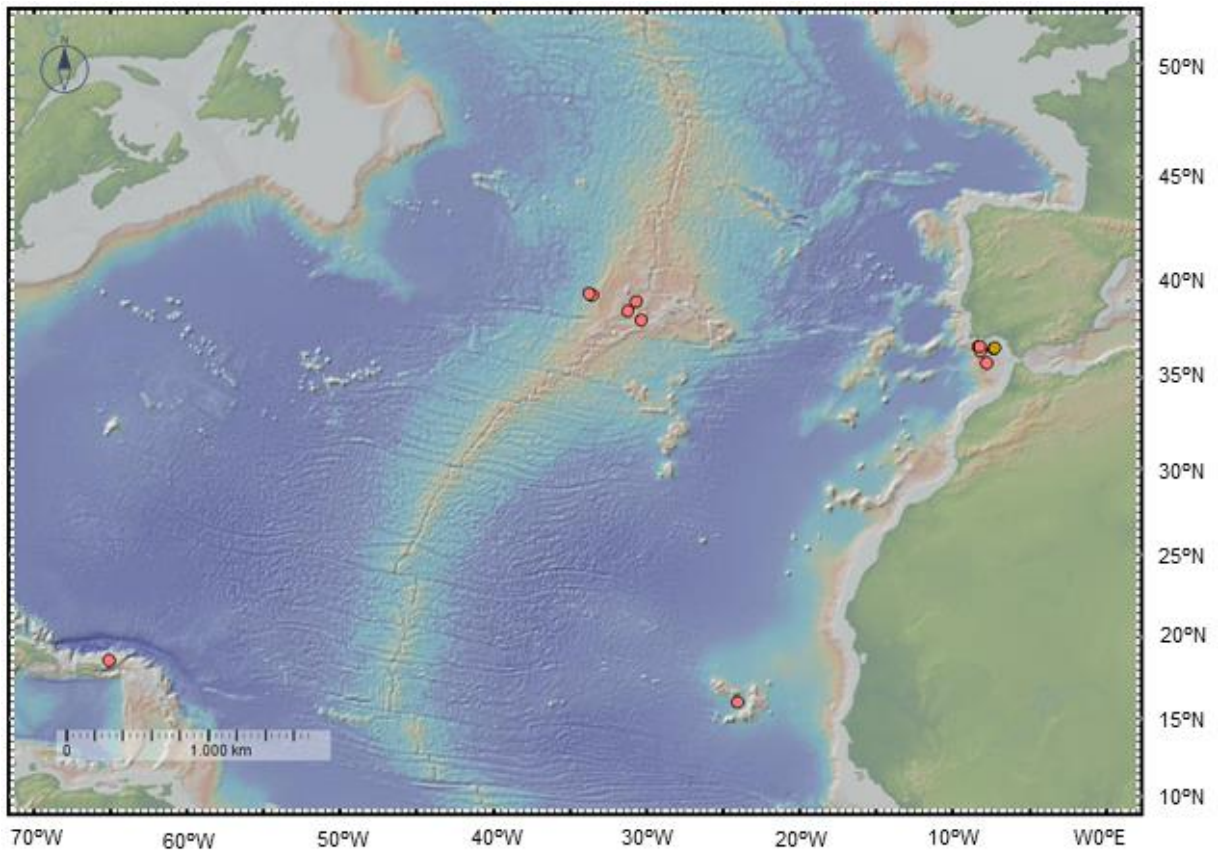
Figure 14. Geographical distribution of *Thenea schmidtii* Sollas, 1886 (red) and *Thenea* sp. nov. 4 (brown) across the temperate Northern and tropical Atlantic realms.
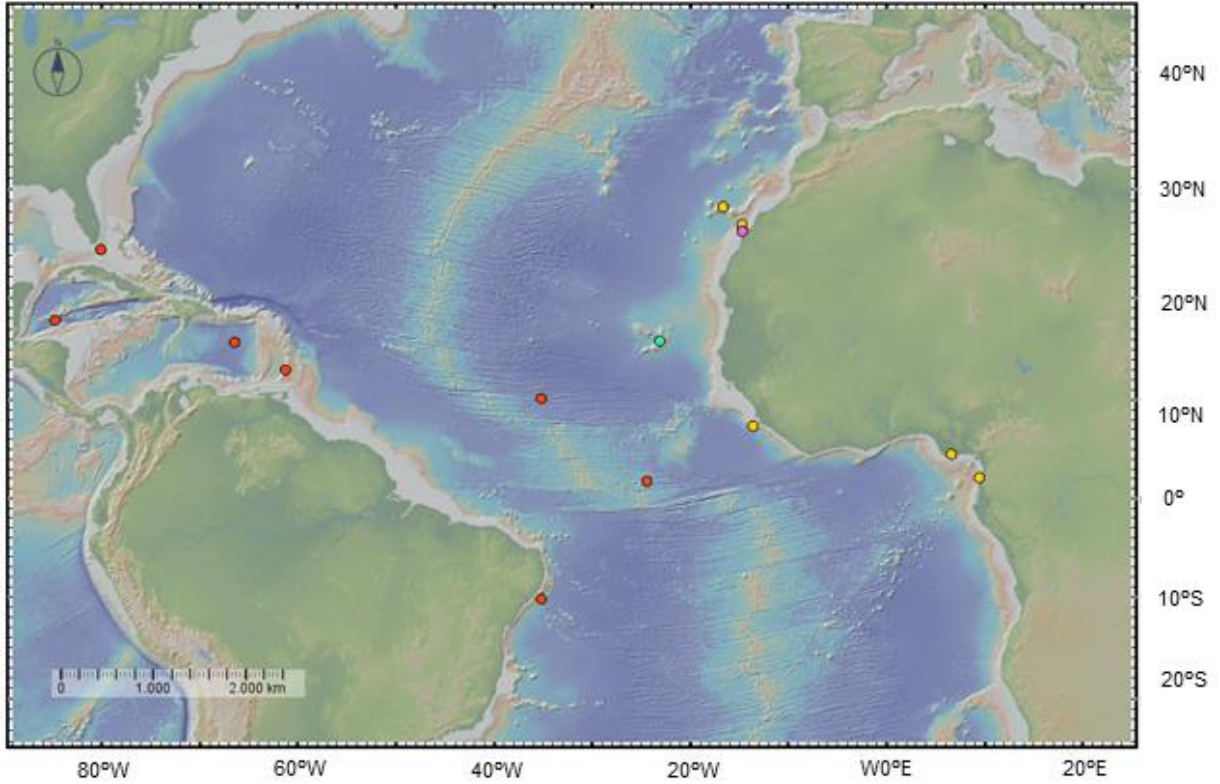
Figure 15. Geographical distribution of *Thenea megastrella* Lendenfeld, 1907 (green)*, Thenea microclada* Lendenfeld, 1907 (pink)*,* and *Thenea bojeadori* Lendenfeld, 1907 (yellow), and *Thenea fenestrata* (Schmidt, 1880) (red) across the temperate Northern and tropical Atlantic realms.
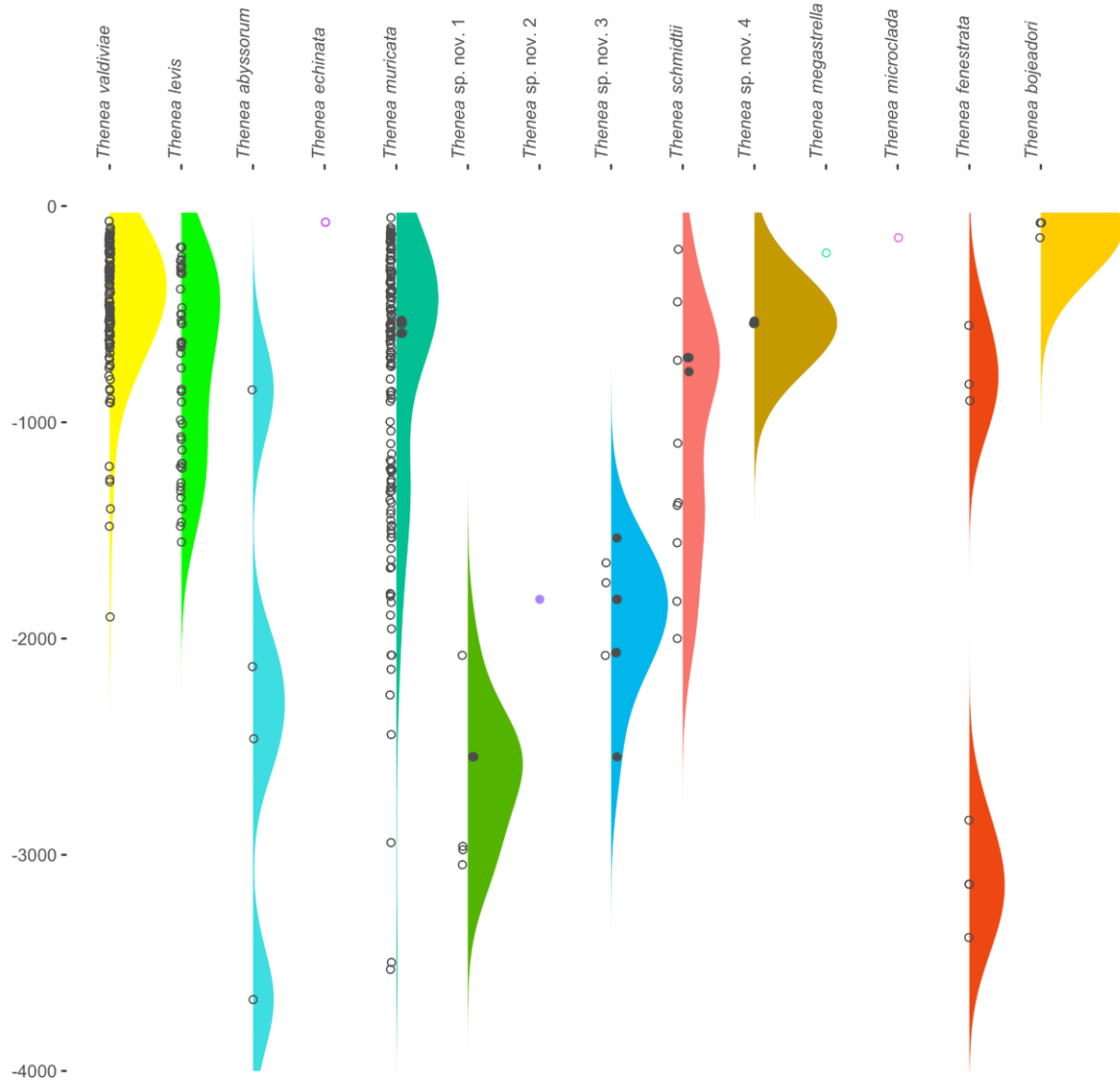
Figure 16. Ridge plots of bathymetric distribution records of *Thenea* species across the Arctic, temperate Northern and tropical Atlantic realms. Open circles represent records from the literature, whereas closed circles indicate records from this study. Colors correspond to the phylogenetic trees and maps.

4. Discussion

4.1. Hidden diversity of the *Thenea* genus

Thenea are sponges that, although relatively easy to identify at genus level, can have subtle morphological variation, making them a challenging taxa to identify to the species level (Cárdenas & Rapp, 2012, 2015). In this study, we reveal the presence of a new species in the Gulf of Cadiz, *Thenea* sp. nov. 4, which has little morphological differentiation from its sister species, *Thenea muricata*, making it difficult to be accurately identified on a morphological basis alone. It is a species that cohabits with *T. muricata*, being sampled together from the same stations. This species does not have an equatorial pore area, and the plesiasters are more uniform (Figure 7F,G), with little variation on the number of actines (4-5), whereas in *T. muricata* the plesiasters are more variable in actine number (Boury-Esnault *et al.*, 1994; Steenstrup & Tendal, 1982; Figure 2G) (2-7). However, we also find *T. muricata* with the aforementioned characteristics. The absence of a pore area was seen in our small, similarly sized specimens and not every *T. muricata* has plesiasters with great actine number variation, making these variations unreliable for the distinction of both species. There could be morphological variations that were not analyzed, such as the abundance of plesiasters on the different parts of the sponge (eg. ectosome, choanosome) and this will be further investigated. Only three specimens of *Thenea.* sp. nov. 4 were studied, the analysis of more specimens could make morphological variations more apparent. Based on molecular analysis of the 28S rRNA gene, there is clear separation between the two species, that form two reciprocally monophyletic and maximally supported clades without any intraspecific genetic variability. However, the COI gene of the two species is identical. The COI gene is a slower evolving gene than the 28S gene (Cárdenas & Rapp, 2012), which might indicate that the two species might have diverged relatively recently, therefore showing little morphological variations. *Thenea muricata* might as of now be a morphologically cryptic species complex, with specimens identified solely based on morphological features possibly misidentified. Cryptic speciation is not an uncommon phenomenon for sponges, with various species with no apparent morphological differences but clearly distinct when their DNA is analyzed (e.g., Pöppe *et al.*, 2010; Reveillaud *et al.,* 2010, Xavier *et al.*, 2010).

Thenea schmidtii is a species whose status has gone through some changes. It was originally described based on three specimens from various locations: Eastern Caribbean, Azores and Ibero-Moroccan Gulf (Sollas, 1888). Although the distinction of this species with

Thenea muricata was not fully established, there were a number of characters in which the two species appeared to differ, such as the size of plesiasters, reported to be much larger and abundant in *T. schmidtii*, the choanossome tissue is underdeveloped in *T. schmidtii*, the choanocyte chambers being on average, twice as large in *T. schmidtii*, and a large sphincter surrounding the oscule (Sollas, 1888). However, some authors were not convinced about the status of this species (Uriz, 1981; Steenstrup & Tendal, 1982) and some considered it to be a variety of *Thenea muricata* by (Topsent, 1894). *T. schmidtii* was considered to be a subspecies of *T. muricata*, named *Thenea muricata schmidtii* by (Babiç, 1917). The large size and high number of plesiasters in various *T. muricata* from the Mediterranean showed that this character is not reliable for the distinction between the two species, and thus, *T. schmidtii* was synonymized with *T. muricata* (Uriz, 1981), also agreed by (Steenstrup & Tendal, 1982). However, genetic analysis revealed that *T. schmidtii* has the same COI sequence as *T. muricata*, but with clear differences in the 28S rRNA gene (C1-D2) and therefore it is strongly suggested that it could be considered a valid species (Cárdenas & Rapp, 2012). However, prior to this study, a single 28S rRNA sequence of *T. schmidtii* was available, from a specimen (ZMAPOR 18036), from the Gulf of Cadiz which had 11 ambiguous b.p. rendering its phyletic status uncertain. Our molecular results generated four additional sequences of *T. schmidtii*, which had the same COI sequence as *T. muricata*/*schmidtii*/sp. nov. 4, and for the 28S fragment, our sequences had fewer ambiguous b.p., and formed a maximum supported clade, further evidencing that *Thenea schmidtii* is a valid species (see "DNA sequences" of *T. muricata*, *T. schmidtii* and *T.* sp. nov. 4 in the Results section). The size of the plesiasters of our *T. schmidtii* specimens are not considerably larger than the ones from our *T. muricata*, actually being smaller than some of our *T. muricata* specimens. We did, however, notice that the spirasters/metasters are slightly larger in *T. schmidtii* (Supplementary Material 3) but this will have to be investigated further. The final differentiating character originally described the broad white sphincter that surrounds the oscule was seen only in our largest specimen (PN92), with the remaining smaller ones resembling *T. muricata* in outer morphology. None of our *T. muricata* specimens have an oscule with a large whitish sphincter. This could mean that the absence of this feature does not rule out a specimen from being a *T. schmidtii*, but the presence could indicate that the specimen is a *T. schmidtii*. In our 2021 PNAB collection we notice that: all exceptionally large sized specimens have a large whitish sphincter and come from the same collection sites; in some stations, nearly all medium sized specimens also have this feature and that none of the smaller sized specimens have this feature. Could the large whitish sphincter

be a feature that develops with age/size? Are all *Thenea* with a large whitish sphincter *Thenea schmidtii*? These are questions that can only be answered with certainty with an in-depth analysis of a larger number of specimens across a wider size range. First it would have to be confirmed that all *Thenea* with a large whitish sphincter are in fact *Thenea schmidtii*, which can only be, as of now, confirmed with molecular analysis of the 28S rRNA gene (C1-D2).

In this study we identify three new species from the Azores region, *Thenea* sp. nov. 1, *Thenea* sp. nov. 2 and *Thenea* sp. nov. 3. *Thenea* sp. nov. 1 specimens, collected from deep waters (2547 m) can vary on their outer morphology, but all specimens have long and thin filamentous roots, are fairly hispid and are light brown in ethanol (Figure 4A-C). Their plesiasters have a narrow size range, are considerably smaller and thinner, and have a higher number of actines than the other species in this study (Figure 4F,G). They also have numerous amphiasters, rather than the usual spirasters (Figure 4H), a type of spicule unusual for *Thenea*, and metasters were not seen. Sponges with these characters were reported by Cárdenas & Rapp (2015) sampled from northern Azores ranging from 2961-3046 m in depth, and were named *Thenea* cf. *valdiviae*, due to the similarity of their outer morphology, spicule size with the boreal form of *Thenea valdiviae*, but with noticeable differences. Our specimens of *Thenea* sp. nov. 1 match with every character except for the oscules, which are sieved in *Thenea* cf. *valdiviae*, and a sieved pore area was only seen in one of our specimens. Nonetheless, we consider that the specimens examined by Cárdenas & Rapp (2015) are the same species as *Thenea* sp. nov. 1. Molecular analysis revealed that this species is genetically very divergent from other *Thenea* species, placing them in a basal clade within *Thenea* (Figure 8, 9, 10). Unlike *Thenea schmidtii* and *Thenea* sp. nov. 4, species that share the COI fragment with *Thenea muricata*, the COI fragment of *Thenea* sp. nov. 1 was also very divergent from any other *Thenea*, with all six specimens forming a clade with strong support (Figure 8). Only one 28S rRNA sequence was generated for this species, therefore, a clade with support was not formed (Figure 9). Nonetheless, this sequence was clearly separated from other *Thenea* species. No sequences of the Cárdenas & Rapp (2015) specimens were generated. Another species found in the Azores is *Thenea* sp. nov. 3. collected from deep waters (1535-2547 m). In general, the specimens are massive subspherical sponges with a single uncovered oscule centered at the top, and a sieved equatorial pore area (Figure 6A-D). The plesiasters have a wide size range and are slightly smaller than *Thenea muricata*/*schmidtii*/sp. nov. 4, the metasters in this species can be quite large and similar to the smallest plesiasters (Supplementary Material 3). Due to the morphological similarities, both external and spicules,

the relatively close collection site and similar depth, we think that *Thenea* sp. nov. 3 is the same species as *Thenea* cf. *schmidtii* reported by Cárdenas & Rapp (2015). Molecular analysis of these specimens revealed that they are divergent from other *Thenea* species, forming a strongly supported clade for both markers (Figures 8-10). There is 0.09% p-distance of the 28S rRNA within this species due to 1 b.p. difference among the sequences (Supplementary Material 5). The other species found in the Azores at 1819 m depth, is *Thenea* sp. nov. 2, represented by a single specimen, is circular, flattened and very hispid (Figure 5A,B). Its megascleres are abundant, specially anatriaenes, and the plesiasters small, while the metasters can be quite large, with the smaller plesiasters overlapping in size with the larger metasters (Supplementary Material 3). There is no single species that quite matches with all of the mentioned features. *Thenea levis* can have abundant anatriaenes, but the sponge is in most cases elongated in shape, with larger plesiasters and smaller metasters/spirasters (Lendenfeld, 1907; Steenstrup & Tendal, 1982; Table 2). Molecular analysis placed this species close to *Thenea levis* for both markers (Figure 8, 9, 10). In the case of the COI fragment, a strongly supported clade was formed by *Thenea levis, Thenea* sp. nov. 2 and a single *Thenea muricata* (Figure 8) (likely misidentification). For the 28S rRNA, a strongly supported clade was formed by *Thenea* sp. nov. 2 with a subclade formed by *Thenea levis*. *Thenea* sp. nov. 2 is genetically the closest to *T. levis* (Figure 9), but still fairly divergent, with 0.2% COI intraclade genetic p-distance and 1.4% 28S interclade genetic p-distance (Supplementary Material 3). There is sufficient evidence, supported by both morphological and molecular characters to determine that this is a new species.

4.2. Biogeographic patterns

There seems to be a geographic pattern affecting the distribution of Arctic and Atlantic *Thenea* species. The northernmost species are *T. abyssorum* and *T. valdiviae* followed by *T. levis* and *T. echinata*. *T. valdiviae* and *T. levis* have a similar depth range, not found deeper than 2000 m. Both species can commonly be found together hosting a wide variety of associated biota (Klitgaard, 1995). The species *T. abyssorum* is found the deepest, at 3670 m, but has a wide depth range, being also reported at 850 m. The single *Thenea echinata* record is at 75 m depth, considered a shallow depth for the genus and given that it has not been found since its description, it should be investigated further. The mid-Atlantic ridge is inhabited by the new species *Thenea* sp. nov. 1, *Thenea* sp. nov. 2, and *Thenea* sp. nov. 3 in the Azores, all

found in deeper waters, ranging from 1535 m to 3046 m. There are some records for this area of *Thenea schmidtii* (Sollas, 1888) and *Thenea muricata* (Topsent, 1892) with no taxonomic descriptions, which hamper the confirmation of the species. In a study of lithistid sponges, it was found that assemblages from the Azores share some similarities with nearby seamounts, followed by the Canaries and Madeira archipelagos, but had no species shared with the Iberian slope (Xavier *et al.*, 2021). A similar pattern is observed here, although the old records of *Thenea muricata* and *T. schmidtii* reported from the mid-Atlantic ridge, may need to be revisited. This separation of species could mean that the Azores archipelago and the Iberian slope are too far apart for species of each location to colonize the other, and without sufficient geomorphological structures, such as seamounts, to serve as "stepping-stones", colonization may be limited. The temperate northeast Atlantic and Mediterranean is mainly inhabited by *Thenea muricata, T. schmidtii* and *Thenea* sp. nov. 4. *Thenea schmidtii* can be found down to 2000 m. *Thenea* sp. nov. 4 has a narrow depth distribution, as all specimens collected up to date originated from two collection sites (533 and 543 m) *Thenea muricata* is widely distributed, ranging from the boreo-Arctic throughout the Northeast Atlantic and in the Mediterranean. It also has a wide depth range, down to 3530 m, but most records are no deeper than 2000 m. The species has been shown to characterise the soft sediment sponge assemblage on the shelf break (110-180 m depth) in the Menorca Channel in the Western Mediterranean Sea (Santín *et al.*, 2018). This species is also reported in soft sediments throughout the boreo-Arctic continental plateaus or seamounts in deep waters (Cárdenas & Rapp, 2012). Sitjá *et al.* (2019) refer to *T. muricata* as the most abundant species in the mud volcanoes of the Gulf of Cadiz (366 collected individuals), forming aggregations in the extensive fields of at least three of the mud volcanoes studied – Anastasya, Pipoca and Aveiro – which are notably one of the shallowest (489–546 m) and the deepest (1136–1146 m). It is also reported that the Mediterranean Outflow Water has an effect of exporting marine fauna towards the Atlantic along the Iberian coast. It is therefore possible that *Thenea* sp. nov. 4 is a species originating from the Mediterranean. Whether the specimens reported by Sitjá *et al.* (2019) are all representative of *T. muricata* or possibly also *T. schmidtii* and/or *Thenea* sp. nov. 4 will require further investigation. It is likely that in some of these areas, two (or more) *Thenea* species occur in sympatry. The temperate North Atlantic and the tropical Atlantic are inhabited by the shallow species *T. megastrella, T. microclada* and *T. bojeadori*, (max depth 217 m), as well as *T. fenestrata* which has a wide depth separation, with a group forming between 552-900 m and another much deeper, between 2840-3383 m which might need revisiting. These geographic

patterns could be influenced by water masses, which have shown to affect the distribution of the deep-sea sponges of the genus *Geodia* (Roberts *et al.*, 2021). It is thought that water masses have an influence in larval and juvenile dispersal, which may be limited by environmental factors, such as temperature, salinity, and food availability, which could be unfavorable in adjacent water masses (Roberts *et al.*, 2021). In the future it would be interesting to investigate, with a dedicated analysis, if similar patterns are observed in the Thenea genus.

4.3. Conservation considerations

Species of the genus *Thenea* have been considered to meet the "Functional significance", "Fragility" and "Structural complexity" VME criteria defined in scope of the International Guidelines for the Management of Deep-Sea Fisheries (FAO, 2009) and therefore classified as indicators of VMEs (ICES, 2020). Soft sediments are the type of seafloor most intensely exploited by trawling activity, and aggregations of *Thenea*, being mostly associated with soft bottom habitats, are vulnerable to bottom contact fishing gear (Pansini, & Musso, 1991). *Thenea* assemblages are known to harbor a high number of associated fauna (Klitgaard, 1995). Thus, understanding their patterns of distribution is critical to ensure that appropriate management and conservation measures are implemented, to prevent significant adverse impacts over the habitats formed by these species. At present such measures encompass the establishment of marine protected areas (MPAs), fisheries closure areas that protect VMEs and a "move-on" rule that for the NEAFC area establishes that upon incidental collection of 400 kg of live sponge of VME indicators (in a trawl tow), the fishing vessel must cease its activity and move out of an area defined as a 2 nautical mile wide band on both sides of the trawl track (NEAFC, 2014). However, this is only applicable outside of countries' EEZs, and given the small size (and weight) of *Thenea* species, reaching such a biomass threshold would require the capturing of tens of thousands of specimens in a single tow. As such, a revision of these thresholds should be reassessed for these and other species to ensure effectiveness of measures aimed at conserving the habitats they form and the ecological services they provide.

5. Funding

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7. Supplementary Material

Supplementary Material 1. Glossary of the most relevant morphological terminology used in the taxonomy of *Thenea* (from Boury-Esnault & Rutzler, 1997; Hajdu, Peixinho and Fernandez, 2011)

Hispid: Surface with long and scattered spicular projections.
Oscule: Opening through which the water leaves the sponge.
Pore: Any opening in the sponge through which the water enters.
Ectosome: The superficial region of a sponge.
Choanosome: The internal region of a sponge.
Spicule: A component of the mineral skeleton, typically composed of silica or calcium carbonate.
Megasclere: Relatively large spine, main component of the sponge skeleton, often with structural function.
Triaene: General term for a tetractinal megasclere having one unequal ray (termed rhab) that is commonly much longer than the other three (termed clads).
Clad: Any ray or axial branch containing an axis or axial canal confluent with that of the protoclad or protorhab in any type of spicule; term chiefly used in triaene spicules.
Rhabdome: the rhab of a triaene and or a triaene derived spicule (rhab a collective term for a monaxic megasclere, later applied to the one ray of a triaene that is distinct from the other three, mostly in length).
Anatriaene: A triaene in which the clads are sharply curved backward (see below).
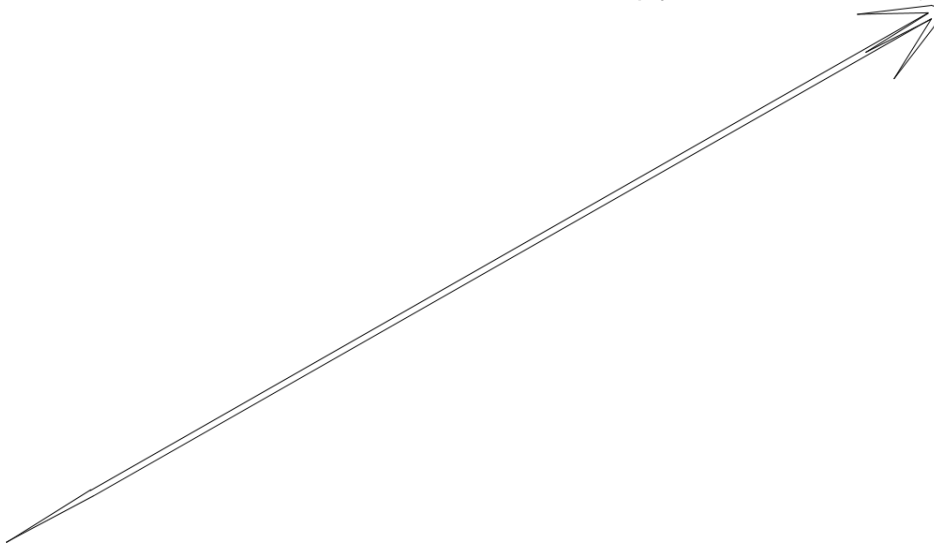
Dichotriaene: A triaene in which the clads are bifurcate (see below).
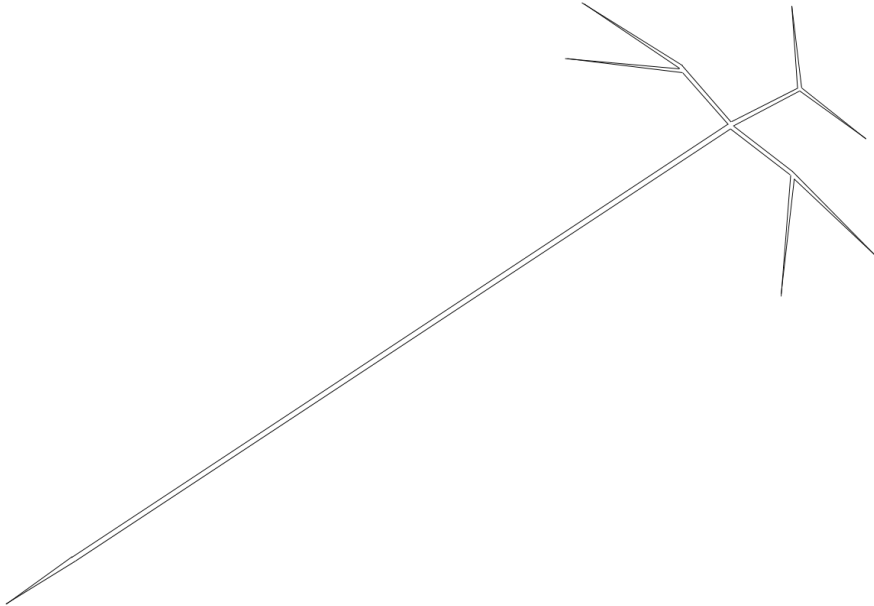
Protoclad: The proximal, unbranched portion of a branched ray (see above).
Deuteroclad: An actinal branch or a distal, branched portion of a ray (see above).
Protriaene: A triaene in which the clads are directed or sharply curved forward, away from the rhab (see below).
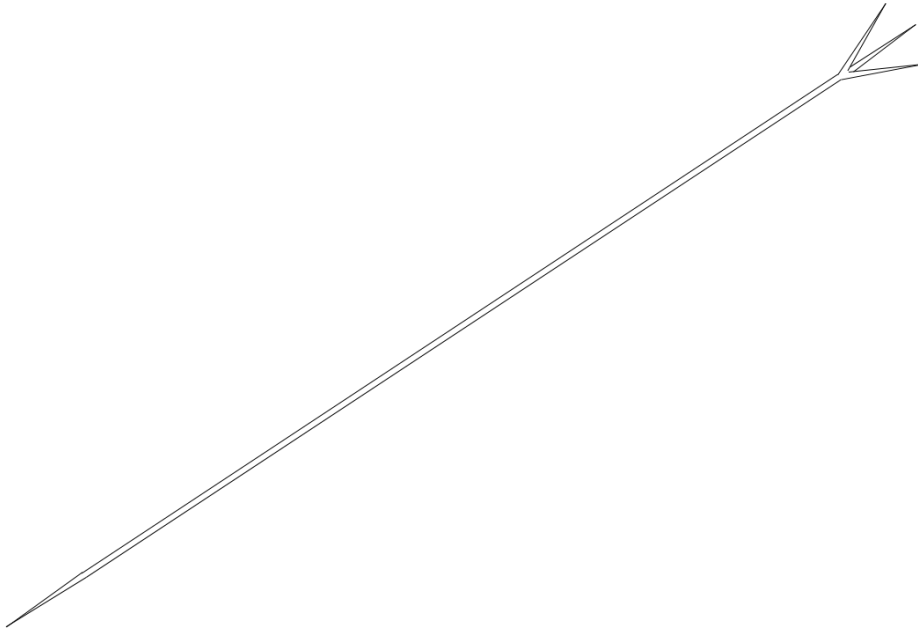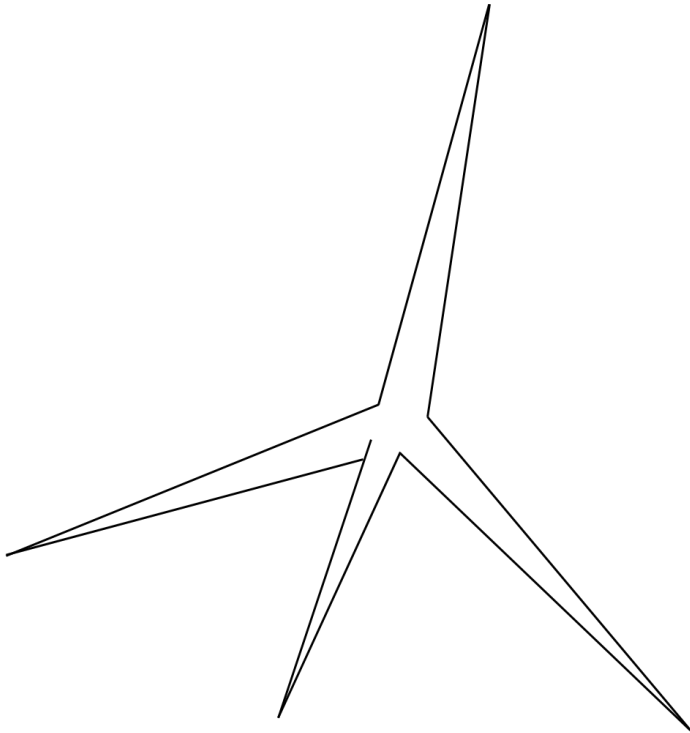
Oxea: monaxon (diactinal) spicule pointed at both ends (see below).
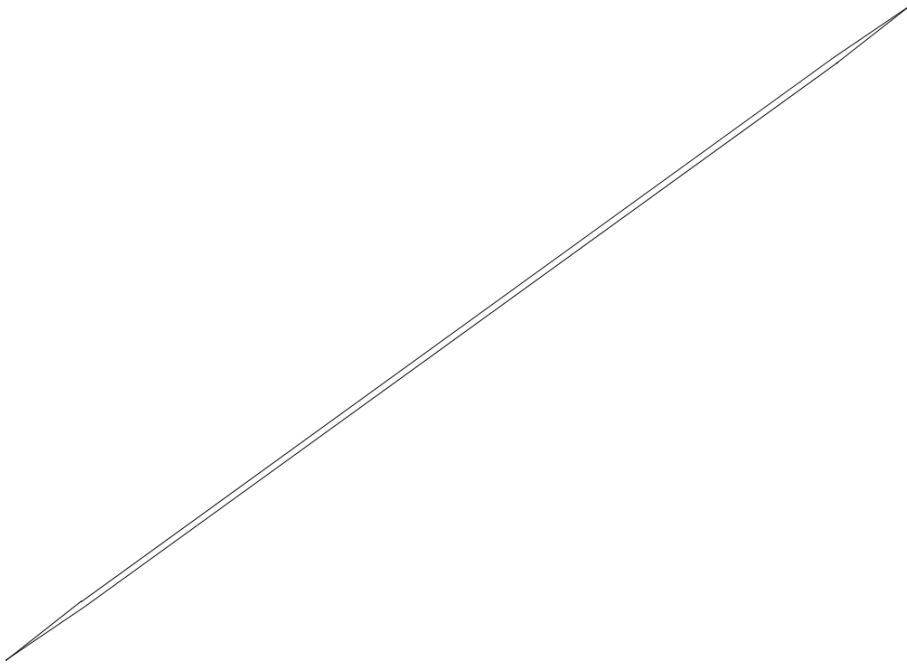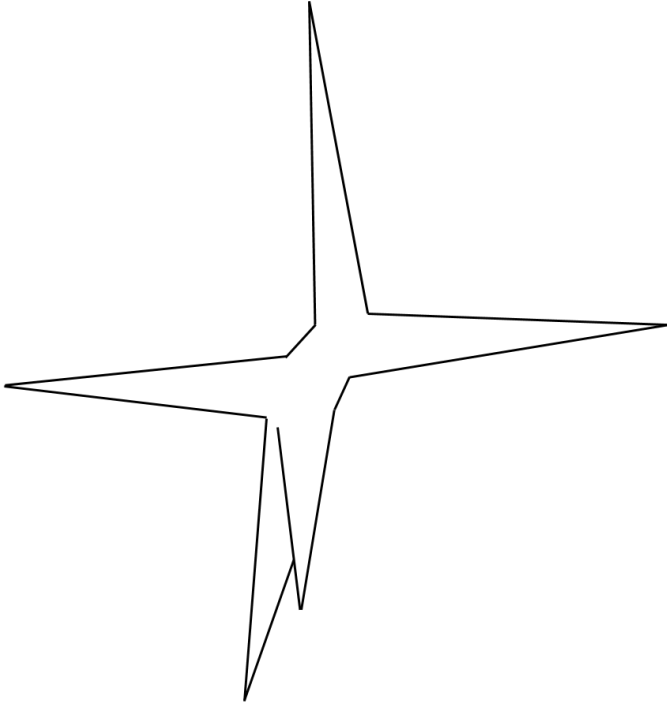
Microsclere: Small spicule, often ornate in shape.
Plesiaster: A microsclere with a very short shaft, always shorter than the spines (see below).
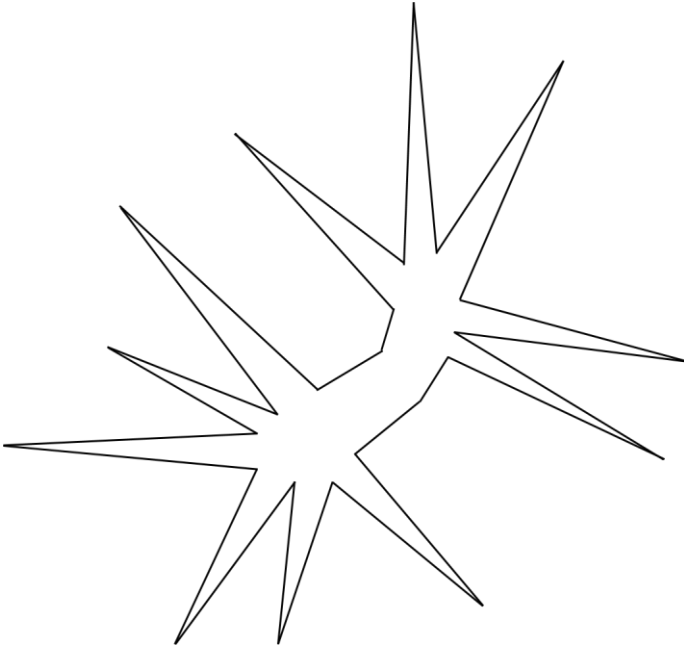
Actine: A centered ray containing an axis or axial canal (See above).
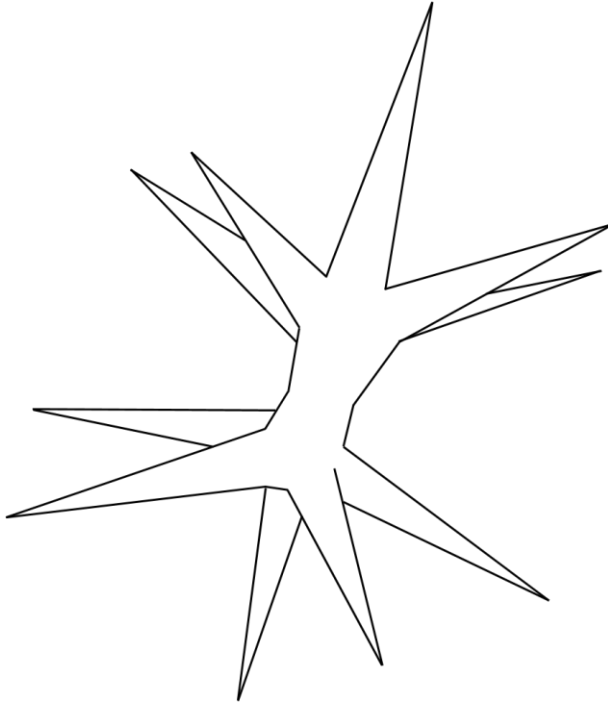
<u>Metaster</u>: A microsclere with a twisted shaft no longer than the spines; intermediate between plesiaster and spiraster (see below).

<u>Spiraster</u>: A spiral, rod shaped microsclere with spines peripherally arranged (see below).

Amphiaster: A microsclere with microspined rays radiating from both ends of a shaft; the rays are shorter than the shaft (see below).

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Supplementary Material 2. Example of BOLD page generated for *Thenea muricata*, bin BOLD:ACB4291

BT_IS2

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Interdisciplinary Centre for Marine and
Environmental Research

Lateral   Anterior

Process ID: THENE001-21
Identification: Thenea
Identified by   Daniel J Despujols
♀ Collected in   North Atlantic Ocean
by   Jorge Lobo Arteaga
Institution Storing: Interdisciplinary Centre for Marine and
                     Environmental Research
Field ID: BT10_E5

🐛 **Specimen Details**   ⌄ ✕

⛃ **Taxonomy**   ⌄ ✕

▥ **Barcode Index Numbers**   ⌃ ✕

BIN: BOLD:ACB4291	**Phylum:** Porifera [27]
Type: Member	**Class:** Demospongiae [27]
Max Divergence in BIN: 0.83% (p-dist)	**Order:** Tetractinellida [27]
Distance to NN: 4.01% (p-dist)	**Family:** Theneidae [27]
	Subfamily:
	Genus: Thenea [27]
	Species: Thenea valdiviae [4]
	Thenea muricata [3]
	Thenea levis [2]
	Thenea abyssorum [1]
	Thenea schmidti [1]

🌐 **Collection Data**   ⌃ ✕

Country: North Atlantic Ocean	**Collector:** Jorge Lobo Arteaga
Province/State:	**Date Collected:** 13-Jun-2019
Region/County: Algarve, Gulf of Cadiz	**Date Accuracy:**
Sector:	**Time Collected:**
Exact Site:	**Site Code:**
Lat/Lon: 36.556, -7.342	**Habitat:**
Elevation:	**Sampling Protocol:**
Elevation Accuracy:	**Coord. Source:**
Depth: 532.5 m	**Coord. Accuracy**
Depth Accuracy:	
Collection Event ID:	

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Supplementary Material 3. Measurement tables of each species analyzed in this study.

Spicule measurements of *Thenea muricata* specimens of this study. Spicule measurements (min-mean-max) provided in µm. L – length, W – width, from 30 measurements, unless stated otherwise, in brackets.

Thenea muricata specimens	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (Actin L x W)	Metasters/ spirasters (L)
IS2	Prot L: 11.7 µm (1) Deut L: 27.1-1400 µm (1)	Not measured	Not measured	LxW: 620.7-<u>1534.9</u>-2449.0 µm x 8.0-<u>8.2</u>-8.4 µm (2)	LxW: 26.1-84.6-<u>219.7</u> µm x 3.1-<u>11.0</u>-29.8 µm	16.2-<u>23.7</u>-31.9 µm
IS3	Prot L: 10.4 µm (1) Deut L: 67.6 µm (1)	Not measured	Not measured	LxW: 1116.7-<u>1734.6</u>-2712.0 µm x µm (3)	LxW: 34.7-<u>54.7</u>-98.4 µm x 3.0-<u>5.7</u>-9.8 µm	12.1-<u>21.6</u>-30.6 µm
IS4	Prot L: 92.8-<u>131.2</u>-149.5 µm Deut L: 372.4-<u>391.0</u>-417.8 µm (4)	Not measured	Not measured	LxW: 1340.2 µm x 10.2 µm (1)	LxW: 35-<u>79.1</u>-175.7 µm x 3.1-<u>9.7</u>-22.3 µm	13.63-23.1 µm

Spicule measurements of *Thenea muricata* specimens of this study continued.

Thenea muricata specimens	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (Actin L x W)	Metasters/ spirasters (L)
IS5	Prot L: 150.0-<u>164.0</u>-177.9 µm (2) Deut L: 386.7-<u>296.4</u>-406-2 µm (2)	Not measured	Not measured	LxW: 2865.1 µm x 10.8 µm (1)	LxW: 35.7-<u>86.1</u>-165.4 µm x 4.3-<u>8.4</u>-16.3 µm	13.9-21.4-38.0 µm
IS9	Prot L: 62.8-<u>99.3</u>-122.8 µm (9) Deut L: 152.9-<u>339.5</u>-421.2 µm (9) Rhab LxW: 1250.4-<u>1559.7</u> µm x 22.6-<u>25.9</u>-29.2 µm (2)	Not measured	Not measured	LxW: 413.6-<u>1279.0</u>-2238.0 µm x 2.9-<u>14.5</u>-24.3 µm (8)	LxW: 35.1-<u>161.0</u>-389.0 µm x 3.1-<u>14.8</u>-40.5 µm	20.7-<u>25.7</u>-33.9 µm
IS10	Prot L: 129.4-<u>135.4</u>-141.4 µm (2) Deut L: 122.2-<u>320.7</u>-446.6 µm (3)	Not measured	Not measured	LxW: 1039.9-<u>1184.5</u>-1329.0 µm x 5.8-<u>10.7</u>-15.6 µm (2)	LxW: 32.8-<u>85.2</u>-160.9 µm x 3.7-<u>6.9</u>-16.2 µm	17.8-<u>23.2</u>-29.8 µm

Spicule measurements of *Thenea muricata* specimens of this study continued.

Thenea muricata specimens	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (Actin L x W)	Metasters/ spirasters (L)
IS12	Prot L: 83.7-<u>113.2</u>-142.7 µm (9) Deut L: 319.3-<u>418.8</u>-493.8 µm (5)	Clad L: 28.2-<u>34.5</u>-43.4 µm (4)	Not measured	LxW: 1848.5 µm x 9.7 µm (1)	LxW: 29.3-<u>98.5</u>-233.1 µm x 3.6-<u>11.5</u>-25.7 µm	14.3-<u>22.3</u>-31.6 µm
IS14	Prot L: 142.4 µm (1) Deut L: 352.1 µm (1)	Clad L: 84.3 µm (1)	Not measured	LxW: 168.7-<u>454.6</u>-1384.1 µm x 5.5-<u>9.4</u>-13.9 µm (22)	LxW: 25.3-<u>65.0</u>-138.5 µm x 3.3-<u>7.1</u>-14.0 µm	15.7-<u>22.0</u>-32.2 µm
PN4	Not measured	Clad L: 189.7 µm (1)	Not measured	LxW: 1386.4-<u>3166.3</u>-5489.1 µm x 8.7-<u>27.1</u>-46.9 µm (3)	LxW: 49.4-<u>148.6</u>-268.7 µm x 5.8-<u>20.6</u>-40.2 µm	13.5-<u>21.8</u>-26.6 µm

Spicule measurements of *Thenea muricata* specimens of this study continued.

Thenea muricata specimens	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (Actin L x W)	Metasters/ spirasters (L)
PN8	Prot L: 123.8-<u>134.6</u>-148.3 μm (3) Deut L: 348.1 μm (1) Rhab LxW: 2219.7 μm x 40.8 μm (1)	Clad L: 54.7-<u>54.9</u>-55.1 μm (2)	Not measured	Not measured	LxW: 36.6-<u>74.9</u>-260.4 μm x 2.7-<u>8.3</u>-28.3 μm	13.5-<u>19.8</u>-25.2 μm
PN12	Prot L: 114.0-<u>139.3</u>-163.4 μm (6) Deut L: 640.4 μm (1)	Clad L: 51.3-<u>93.3</u>-141.0 μm (8) Rhab LxW: 1737.7 μm x 6.1 μm (1)	Not measured	LxW: 873.4-<u>1156.0</u>-1438.7 μm x 7.2-<u>10.0</u>-12.8 μm (2)	LxW: 33.4-<u>104.5</u>-285.4 μm x 2.9-<u>13.3</u>-31.9 μm	16.1-<u>21.9</u>-28.9 μm
PN199	Prot L: 127.0 μm (1) Deut L: 438.7 μm (1)	Not measured	Clad L: 474.4 μm (1)	Not measured	LxW: 24.9-<u>74.2</u>-154.3 μm x 4.3-<u>10.5</u>-20.2 μm	13.5-<u>19.9</u>-29.3 μm

Spicule measurements of *Thenea muricata* specimens of this study continued.

***Thenea muricata* specimens**	**Dichotriaenes** (prot L, deut L, rhab L x W)	**Anatriaenes** (clad L x rhab LxW)	**Protriaenes** (clad L x rhab LxW)	**Oxeas** (L x W)	**Plesiasters** (Actin L x W)	**Metasters/ spirasters** (L)
PN202	Prot L: 127.0-<u>142.8</u>-164.0 µm (3) Deut L: 261.0 µm (1)	Not measured	Not measured	Not measured	LxW: 30.9-<u>82.6</u>-219.6 µm x 5.6-<u>12.3</u>-33.2 µm	9.9-<u>20.9</u>-27.0 µm
PN285	Not measured	Not measured	Not measured	Not measured	LxW: 34.7-<u>92.6</u>-228.5 µm x 3.5-<u>11.1</u>-30.8 µm	16.8-<u>22.2</u>-28.7 µm

Spicule measurements of *Thenea schmidtii* specimens of this study. Spicule measurements (min-mean-max) provided in µm. L – length, W – width, from 30 measurements, unless stated otherwise, in brackets.

Thenea schmidtii specimens	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (Actin L x W)	Metasters/ spirasters (L)
PN74	Not measured	Clad L: 40.7 µm (1)	Not measured	LxW: 1749.5-<u>2124.3</u>-2698.2 µm x 8.2-<u>9.7</u>-12.4 µm (3)	LxW: 32.3-<u>121.8</u>-183.7 µm x 3.7-<u>11.8</u>-19.6 µm	17.2-<u>26.5</u>-37.5 µm
PN75	Not measured	Not measured	Not measured	LxW: 299.0 µm x 18.7 µm (1)	LxW: 57.7-<u>132.2</u>-188.5 µm x 4.8-<u>13.2</u>-21.0 µm	13.4-<u>26.9</u>-43.2 µm
PN83	Not measured	Clad L: 98.5 µm (1)	Not measured	Not measured	LxW: 50.3-<u>94.1</u>-176.3 µm x 3.7-<u>7.2</u>-16.5 µm	15.5-<u>25.8</u>-41.4 µm
PN92	Not measured	Clad L: 85.3 µm (1)	Not measured	LxW: 2328.0 µm x 10.9 µm (1)	LxW: 48.4-<u>91.7</u>-150.9 µm x 6.8-<u>11.1</u>-20.5 µm	16.9-<u>23.7</u>-35.6 µm

Spicule measurements of *Thenea* sp. nov. 1 specimens of this study. Spicule measurements (min-mean-max) provided in µm. L – length, W – width, from 30 measurements, unless stated otherwise, in brackets.

Thenea sp. nov. 1 specimens	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (Actin L x W)	Amphiasters/metasters/ spirasters (L)
PR7	Prot L: 87.4-<u>117.9</u>-165.0 µm (4) Deut L: 417.0-<u>563.1</u>-783.9 µm (3)	Not measured	Not measured	LxW:964.9 -<u>1422.8</u>-2281.0 µm x 5.8-<u>11.2</u>-15.1 µm (11)	LxW: 48.9-<u>62.5</u>-92.7 µm x 3.8-<u>4.9</u>-6.5 µm (29)	18.5-<u>30.6</u>-37.7 µm
PR8	Not measured	Not measured	Not measured	LxW: 247.1-<u>1008.1</u>-1965.6 µm x 5.0-<u>9.4</u>-12.7 µm (19)	LxW: 39.5-<u>50.7</u>-70.2 µm x 3.4-<u>4.6</u>-6.1 µm	20.0-<u>27.5</u>-34.4 µm
PR9	Not measured	Not measured	Clad L: 293.8 µm (1)	LxW: 224.6-<u>923.5</u>-1407.3 µm x 6.1-<u>10.2</u>-16.1 µm (11)	LxW: 41.2-<u>51.5</u>-61.5 µm x 3.5-<u>5.3</u>-6.2 µm	22.6-<u>27.9</u>-37.2 µm

Spicule measurements of *Thenea* sp. nov. 1 specimens of this study continued.

Thenea sp. nov. 1 specimens	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (Actin L x W)	Amphiasters/metasters/ spirasters (L)
PR10	Not measured	Not measured	Not measured	LxW: 352.2-1117.7-2142.4 µm x 6.4-11.6-20.1 µm (25)	LxW: 41.7-52.5-73.8 µm x 3.2-4.8-6.1 µm	17.6-25.9-34.5 µm
PR11	Not measured	Not measured	Not measured	LxW: 1796.4 µm x 7.2 µm (1)	LxW: 24.0-45.6-55.7 µm x 2.2-4.7-6.1 µm	21.9-29.0-36.3 µm
PR12	Not measured	Not measured	Not measured	LxW: 300.8-358.3-393.2 µm x 7.4-11.1-15.3 µm (3)	LxW: 43.3-54.4-68.9 µm x 3.2-4.7-6.9 µm	18.7-26.8-36.8 µm

Spicule measurements for *Thenea* sp. nov. 2 specimen of this study. Spicule measurements (min-mean-max) provided in µm. L – length, W – width, from 30 measurements, unless stated otherwise, in brackets.

Thenea sp. nov. 2 specimens	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (Actin L x W)	Metasters spirasters (L)
PR4	Not measured	Clad L: 49.2-<u>82.9</u>-108.6 µm (8) Rhab LxW: 994.9-<u>1211.0</u>-1442.2 µm x 9.7-<u>11.9</u>-14.7 µm (4)	Not measured	Not measured	LxW: 22.3-<u>52.5</u>-79.3 µm x 2.4-<u>8.3</u>-12.0 µm	17.9-<u>29.3</u>-40.4 µm

Spicule measurements for Thenea sp. nov. 3 specimens of this study. Spicule measurements (min-mean-max) provided in µm. L – length, W – width, from 30 measurements, unless stated otherwise, in brackets.

| *Thenea* sp. nov. 3 specimens	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas(L x W)	Plesiasters (Actin L x W)	Metasters spirasters (L)
PR2	Not measured	Clad L: 26.1-_38.5_-50.8 µm (2) Rhab LxW: 618.6 µm x 4.5 µm (1)	Not measured	LxW: 1780.5 µm x 11.6 µm (1)	LxW: 18.1-_60.1_-118.1 µm x 1.6-_6.3_-14.2 µm	21.9-_32.9_-54.6 µm
PR3	Not measured	Not measured	Not measured	LxW: 426.6 µm x 13.5 µm (1)	LxW: 36.2-_90.2_-145.0 µm x 3.6-_8.0_-11.7 µm	19.4-_27.7_-35.6 µm
PR5	Not measured	Not measured	Not measured	Not measured	LxW: 43.5-_66.8_-113.1 µm x 4.9-_8.0_-12.4 µm	20.6-_27.9_-40.7 µm
PR6	Not measured	Not measured	Not measured	Not measured	LxW: 27.7-_94.2_-151.9 µm x 3.1-_11.0_-17.4 µm	16.3-_35.1_-45.7 µm

Spicule measurements for Thenea sp. nov. 3 specimens of this study continued.

| *Thenea* sp. nov. 3 specimens	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas(L x W)	Plesiasters (Actin L x W)	Metasters spirasters (L)
PR13	Not measured	Clad L: 108.8-<u>144.0</u>-163.1 µm (3)	Not measured	LxW: 1015.5 µm x 23.6 µm (1)	LxW: 36.1-<u>103.3</u>-132.4 µm x 3.2-<u>9.4</u>-12.5 µm	23.5-<u>30.1</u>-45.0 µm
PR14	Not measured	Not measured	Not measured	LxW: 165.3 µm x 4.4 µm (1)	LxW: 14.8-<u>68.3</u>-123.5 µm x 1.7-<u>8.0</u>-13.7 µm	19.5-<u>28.0</u>-39.2µm
PR15	Not measured	Clad L: 135.3-<u>138.1</u>-141.0 µm (2)	Not measured	LxW: 3092.1 µm x 19.4 µm (1)	LxW: 25.5-<u>74.6</u>-151.4 µm x 2.3-<u>7.4</u>-16.1 µm	17.4-<u>30.5</u>-43.3 µm

Spicule measurements for Thenea sp. nov. 4 specimens of this study. Spicule measurements (min-mean-max) provided in μm. L – length, W – width, from 30 measurements, unless stated otherwise, in brackets.

Thenea sp. nov. 4 specimens	Dichotriaenes (prot L, deut L, rhab L x W)	Anatriaenes (clad L x rhab LxW)	Protriaenes (clad L x rhab LxW)	Oxeas (L x W)	Plesiasters (Actin L x W)	Metasters spirasters (L)
IS7	Prot L: 69.3-93.2-113.0 μm (8) Deut L: 105.8-393.1-469.8 μm (9) Rhab LxW: 1140.6 μm x 27.6 μm (1)	Clad L: 102.7-245.1-426.9 μm (3) Rhab LxW: 1129.1-1812.8-2932.0 μm x 16.4-23.3-31.9 μm (5)	Not measured	Not measured	LxW: 34.8-80.8-250.2 μm x 3.8-10.0-28.2 μm	13.7-21.8-30.7 μm (26)
IS8	Prot L: 26.2-90.2-123.0 μm (8) Deut L: 74.9-304.1-514.5 μm (8)	Not measured	Not measured	Not measured	LxW: 25.3-81.5-191.6 μm x 4.1-8.6-16.2 μm	16.6-24.0-35.8 μm
IS11	Prot L: 27.8-114.2-160.8 μm (29) Deut L: 76.8-363.9-549.2 μm (27) Rhab LxW: 1230.0-1656.2-2709.5 μm x 19.9-25.3-33.8 μm (6)	Not measured	Not measured	LxW: 409.8-1742.2-2764.6 μm x 5.0-16.7-26.0 μm (14)	LxW: 40.8-125.1-234.4 μm x 5.3-12.4-27.2 μm	13.1-22.4-30.3 μm

Supplementary Material 4. Individual phylogenetic trees obtained by Bayesian Inference and Maximum Likelihood for the mtDNA COI, rRNA 28S, and concatenated datasets.
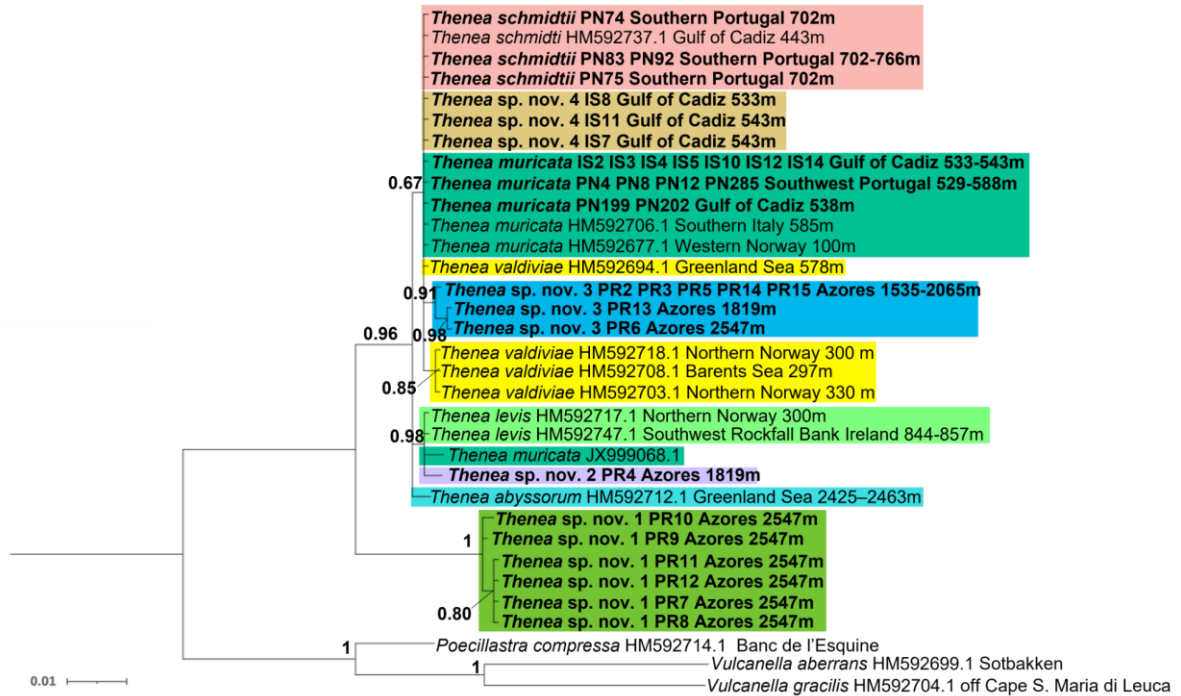
Phylogenetic tree obtained by bayesian inference for the mtDNA COI gene, bayesian posterior probabilities displayed. Sequences generated in this study are highlighted in bold.
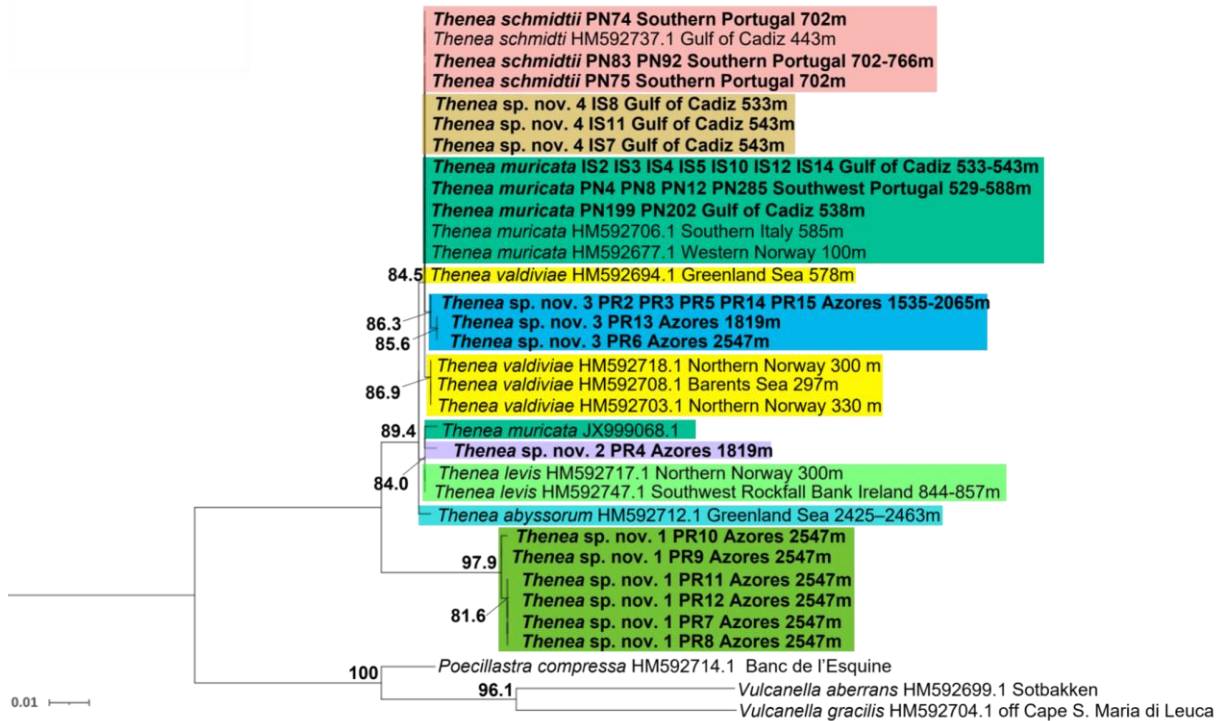
Phylogenetic tree obtained by Maximum Likelihood for the mtDNA COI, maximum likelihood bootstrap support displayed. Sequences generated in this study are highlighted in bold.
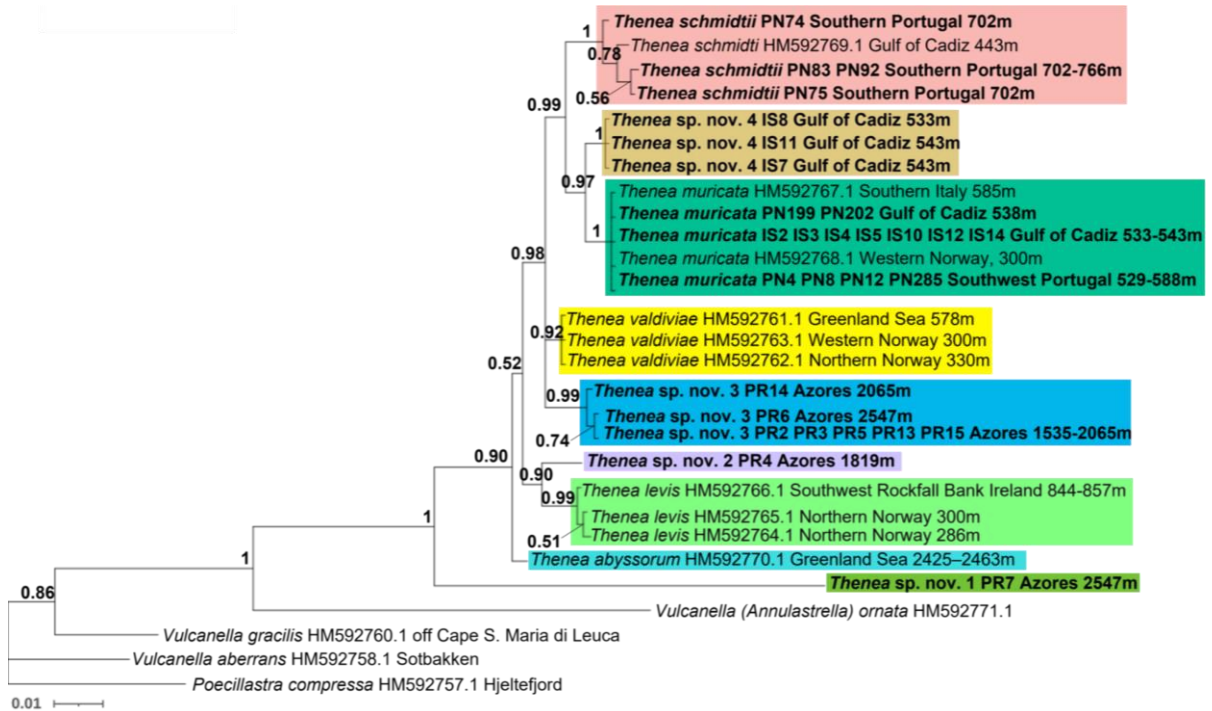
Phylogenetic tree obtained by bayesian inference for the rRNA 28S gene (C1-D2), bayesian posterior probabilities displayed. Sequences generated in this study are highlighted in bold.
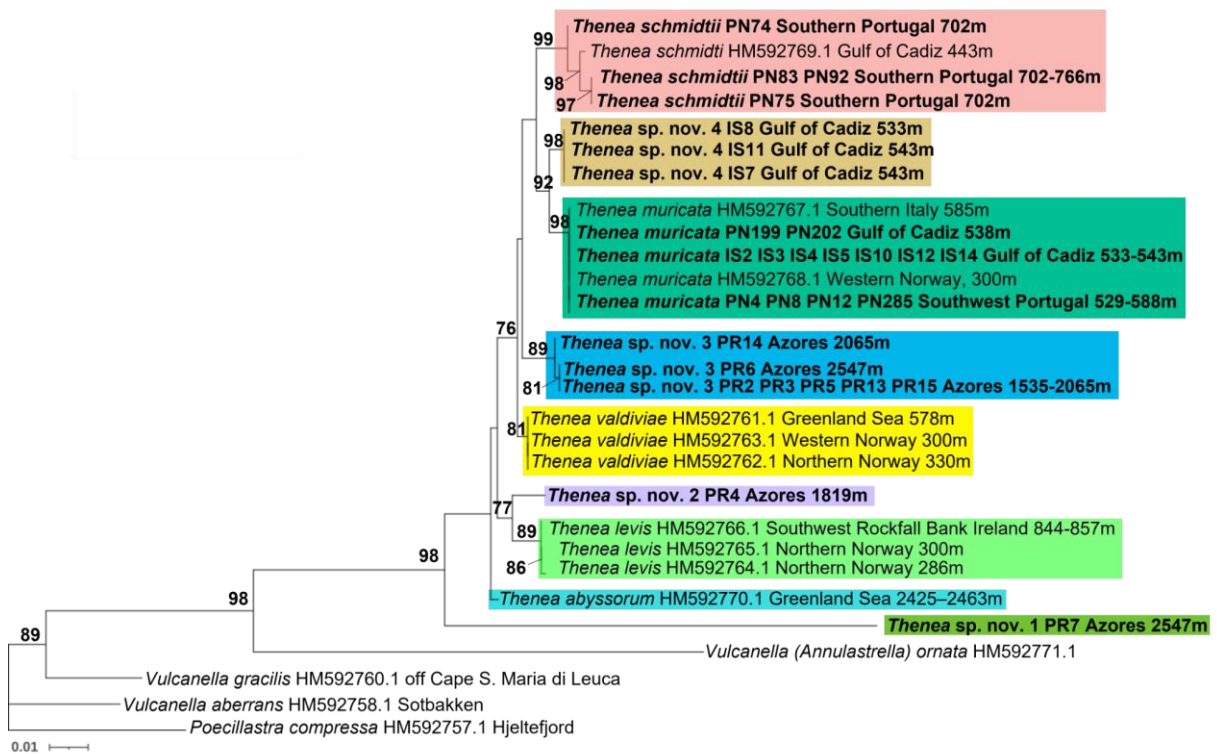
Phylogenetic tree obtained by Maximum Likelihood for the rRNA 28S gene (C1-D2), maximum likelihood bootstrap support displayed. Sequences generated in this study are

highlighted in bold.
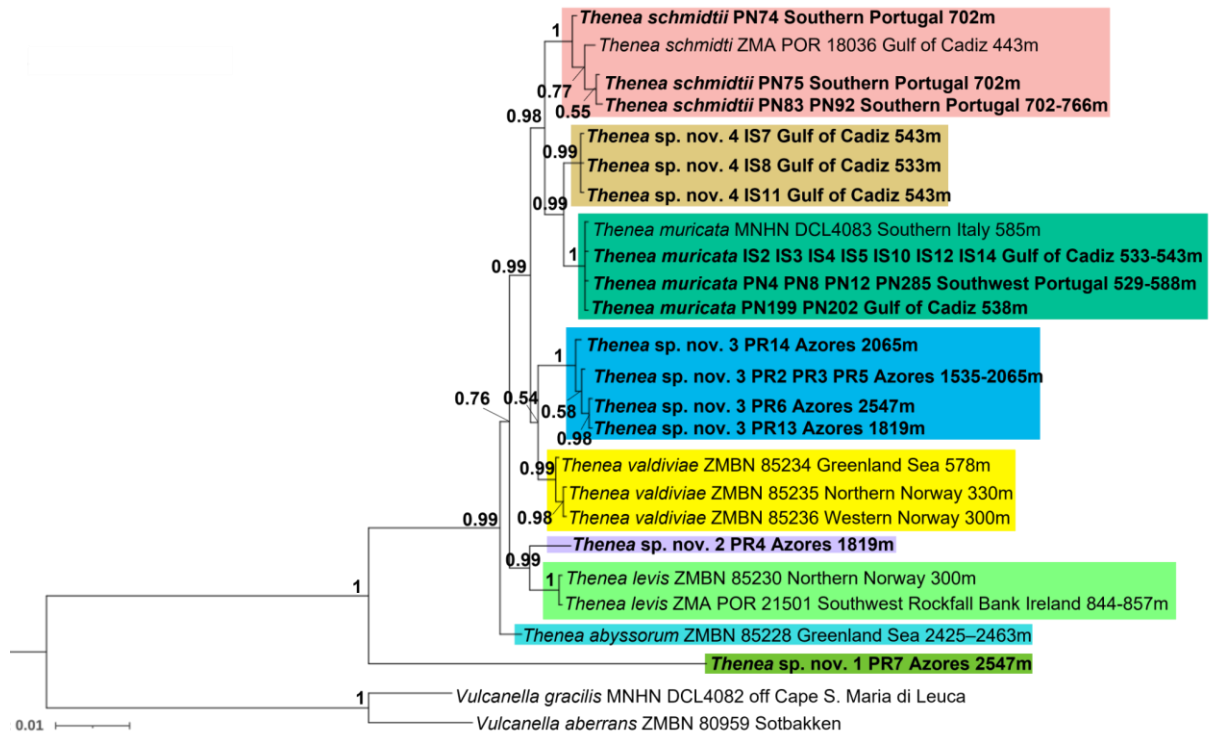
Phylogenetic tree obtained by Maximum Likelihood for the concatenated dataset comprising the mtDNA COI gene and rRNA 28S gene (C1-D2), maximum likelihood bootstrap support displayed. Sequences generated in this study are highlighted in bold.
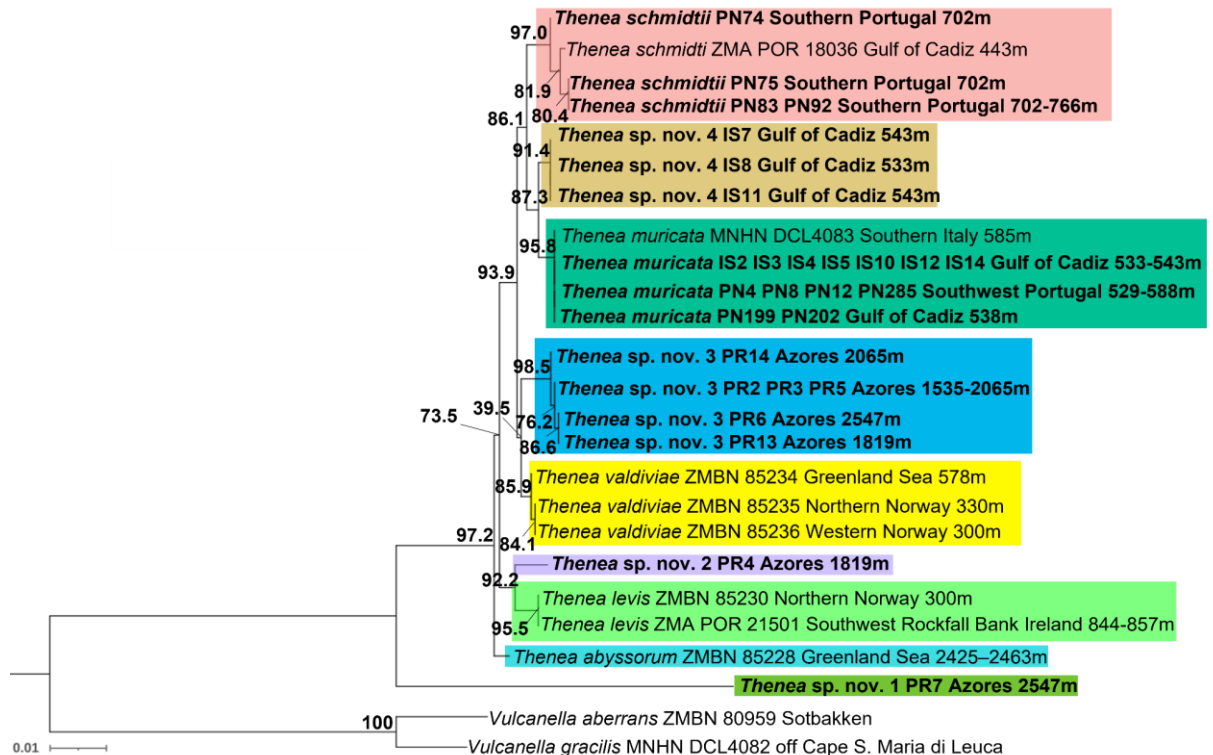
Phylogenetic tree obtained by bayesian inference for the concatenated dataset comprising the mtDNA COI gene and rRNA 28S gene (C1-D2), bayesian posterior probabilities displayed. Sequences generated in this study are highlighted in bold.

Supplementary Material 5. Intraclade (diagonal line) and interclade (below diagonal) mean genetic p-distances (%) in the mtDNA COI (upper table) and rRNA 28S regions (lower table).

COI	*Thenea schmidtii/muricata/* sp. nov. 4	*Thenea* sp. nov. 1	*Thenea* sp. nov. 3	*Thenea levis/* sp. nov. 2	*Thenea valdiviae*	*Thenea abyssorum*
Thenea schmidtii/muricata/ sp. nov. 4	0					
Thenea sp. nov. 1	3.7	0				
Thenea sp. nov. 3	0.2	3.9	0			
Thenea levis sp. nov. 2	0.4	3.7	0.6	0.2		
Thenea valdiviae	0.2	3.9	0.3	0.6	0	
Thenea abyssorum	0.5	3.9	0.6	0.6	0.6	n/c

28S	*Thenea* sp. nov. 1	*Thenea levis*	*Thenea schmidtii*	*Thenea* sp. nov. 2	*Thenea* sp. nov. 4	*Thenea muricata*	*Thenea abyssorum*	*Thenea* sp. nov. 3	*Thenea valdiviae*
Thenea sp. nov. 1	n/c								
Thenea levis	8.9	0							
Thenea schmidtii	9.0	1.9	0						
Thenea sp. nov. 2	8.7	1.4	1.9	n/c					
Thenea sp. nov. 4	8.7	1.9	0.8	1.9	0				
Thenea muricata	9.3	2.0	1.1	1.9	0.8	0			
Thenea abyssorum	8.7	1.4	1.4	1.4	1.6	1.6	n/c		
Thenea sp. nov. 3	9.3	2.0	1.0	2.1	1.4	1.7	1.5	0.089	
Thenea valdiviae	9.2	1.5	1.2	2.0	0.9	1.2	1.2	1.0	0

Supplementary Material 6. Number of records in the literature/this work and depth range of the species of the genus *Thenea* in the Atlantic.

Species	Number of records	Depth m (min-max)	References
Thenea abyssorum	4	675-3670	1, 2, 3
Thenea bojeadori	5	74-146	4, 5, 6, 7
Thenea echinata	1	-	8
Thenea fenestrata	8	552-3383	9, 10, 11, 12, 13
Thenea levis	52	100-1481	3, 14, 15, 16, 17
Thenea megastrella	1	212	6
Thenea microclada	1	146	6
Thenea muricata	168	51-3498	2, 3, 12, 13, 14, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, this work
Thenea schmidtii	16	200-2000	2, 13, 20, 33, 42, 43, this work
Thenea valdiviae	125	60-1900	2, 3, 6, 14, 15, 16, 35, 44, 45, 46
Thenea sp. nov. 1	6	2547	14, this work
Thenea sp. nov. 2	1	1819	this work
Thenea sp. nov. 3	6	1535-2547	19, this work
Thenea sp. nov. 4	3	532-543	this work

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