



Systematics of ‘lithistid’ tetractinellid demosponges from the Tropical Western Atlantic—implications for phylodiversity and bathymetric distribution

Astrid Schuster^{1,2}, Shirley A. Pomponi³, Andrzej Pisera⁴, Paco Cárdenas⁵, Michelle Kelly⁶, Gert Wörheide^{1,7,8} and Dirk Erpenbeck^{1,7}

¹ Department of Earth and Environmental Sciences, Ludwig-Maximilians-Universität München, Munich, Germany

² Current affiliation: Department of Biology, Nordsee, Southern University of Denmark, Odense, Denmark

³ Harbor Branch Oceanographic Institute, Florida Atlantic University, Ft Pierce, FL, USA

⁴ Institute of Paleobiology, Polish Academy of Sciences, Warszawa, Poland

⁵ Pharmacognosy, Department of Medicinal Chemistry, Uppsala University, Uppsala, Sweden

⁶ National Centre for Coasts and Oceans, National Institute of Water and Atmospheric Research, Newmarket, Auckland, New Zealand

⁷ GeoBio-Center, Ludwig-Maximilians-Universität München, Munich, Germany

⁸ SNSB-Bayerische Staatssammlung für Paläontologie und Geologie, Munich, Germany

ABSTRACT

Background. Among all present demosponges, lithistids represent a polyphyletic group with exceptionally well-preserved fossils dating back to the Cambrian. Knowledge of their recent diversity, particularly in the Tropical Western Atlantic Ocean (TWA) where they are common in deep waters, is scarce making any comparison between present and past major ‘lithistid’ faunas difficult. In addition, the lack of sufficient molecular and morphological data hamper any predictions on phylogenetic relationships or phylodiversity from this region. The Harbor Branch Oceanographic Institute (HBOI, Fort Pierce, Florida) holds the largest collection of TWA lithistid sponges worldwide, however, the majority remain to be taxonomically identified and revised.

Principal Findings. In this study we provide sequences of 249 lithistid demosponges using two independent molecular markers (28S rDNA (C1-D2) and *cox1* mtDNA). In addition, a morphological documentation of 70 lithistid specimens is provided in the database of the Sponge Barcoding Project (SBP). This integrated dataset represents the largest and most comprehensive of the TWA lithistids to date. The phylogenetic diversity of ‘lithistid’ demosponges in the Bahamas and Jamaica are high in comparison to other TWA regions; Theonellidae and Corallistidae dominate the fauna, while Neopeltidae and Macandrewiidae are rare. A proposed tetractinellid suborder, one undescribed genus and several undescribed species are recognized and the Pacific ‘lithistid’ genera, *Herengeria* and *Awhiowhio*, are reported from the TWA for the first time. The higher-taxa relationships of desma-bearing tetractinellids are discussed and topics for revision suggested.

Conclusion. This first integrative approach of TWA ‘lithistid’ demosponges contributes to a better understanding of their phylogenetic affinities, diversity and bathymetric distribution patterns within the TWA. As in the Pacific, the TWA ‘lithistid’ demosponges dominate deep-water habitats. Deeper taxonomic investigations will undoubtedly

Submitted 3 October 2019
Accepted 22 December 2020
Published 2 April 2021

Corresponding author
Dirk Erpenbeck, erpenbeck@lmu.de

Academic editor
James Reimer

Additional Information and
Declarations can be found on
page 31

DOI 10.7717/peerj.10775

© Copyright
2021 Schuster et al.

Distributed under
Creative Commons CC-BY 4.0

OPEN ACCESS

contribute to a better comparison between present major 'lithistid' faunas and their fossil record in the Mesozoic.

Subjects Biodiversity, Evolutionary Studies, Marine Biology, Taxonomy

Keywords Tetractinellida, Integrative taxonomy, Tropical Western Atlantic, Lithistid demosponges

INTRODUCTION

Among all present demosponges, lithistids represent a palaeontologically important polyphyletic group, with exceptionally well-preserved fossils dating back to the Cambrian (e.g., [Pisera, 2002](#); [Pisera, 2006](#)), and several relict genera represented in living faunas today (e.g., [Lévi, 1991](#); [Pisera, 2002](#); [Kelly, 2007](#); [Kelly et al., 2003](#)). Several key 'lithistid' demosponge faunas are relatively well known: 'lithistid' demosponges are dominant components of seamount communities on the Norfolk Ridge and in the South-West Pacific (e.g., [Lévi, 1991](#); [Kelly, 2000](#); [Kelly, 2007](#); [Schlacher-Hoenlinger, Pisera & Hooper, 2005](#); [Kelly et al., 2007](#)), and their inventory, morphological identification and molecular systematics has been the focus of several studies (e.g., [Schlacher-Hoenlinger, Pisera & Hooper, 2005](#); [Schuster et al., 2015](#)). Furthermore, large 'lithistid' assemblages are reported from continental shelves and caves of the North-East Atlantic (e.g., [Carvalho, Pomponi & Xavier, 2015](#)), and from seamounts in the Mediterranean (e.g., [Maldonado et al., 2015](#)).

However, the present-day lithistid species and their phylogenetic diversity in several marine bioregions including the Western Indian Ocean, Subantarctic regions including South Africa, Northern Pacific and Tropical Western Atlantic (TWA) are incompletely understood. While 'lithistid' demosponges in the TWA are reported from continental shelves, caves and slopes by [Van Soest & Stentoft \(1988\)](#), [Reed & Pomponi \(1997\)](#), and [Pomponi et al. \(2001\)](#), and many earlier reports of individual species (e.g., [Sollas, 1888](#)), the fauna is still poorly known with few descriptions and no molecular data. This greatly limits the understanding of their phylogenetic relationships, diversity and evolution.

Desma-bearing demosponges, historically referred to as 'lithistid' demosponges, form a polyphyletic group. Molecular systematics now group the majority of 'lithistid' demosponges (11 out of 13 families) to the order Tetractinellida [Marshall, 1876](#). Eight of these families are assigned to the suborder Astrophorina [Sollas, 1887](#) and three to the suborder Spirophorina *sensu* [Morrow & Cárdenas, 2015](#) ([Cárdenas et al., 2011](#); [Morrow & Cárdenas, 2015](#); [Schuster et al., 2015](#)). [Schuster et al. \(2015\)](#) showed several 'lithistid' families such as Pleromidae [Sollas, 1888](#), Desmanthidae [Topsent, 1894](#) and Scleritodermidae ([Sollas, 1888](#)) to be polyphyletic, and Corallistidae ([Sollas, 1888](#)), Theonellidae [Von Lendenfeld, 1903](#) and Phymatellidae [Schrammen, 1910](#) to be monophyletic. However, the systematic affinities for families such as e.g., Siphonidiidae [Von Lendenfeld, 1903](#), Azoricidae [Sollas, 1888](#) and Neopeltidae [Sollas, 1888](#), remain obscure due to few molecular data available. Hence, only 21 out of 40 'lithistid' genera were evaluated in [Schuster et al. \(2015\)](#). The same study indicated that several spicule types convergently evolved within this sponge group. The families Scleritodermidae and Siphonidiidae were suggested to form a separate clade

within Tetractinellida, but outside the two suborders Astrophorina and Spirophorina (Kelly Borges & Pomponi, 1994; Schuster et al., 2015). With the discovery and description of a new tetractinellid family Stupendidae (Kelly & Cárdenas, 2016), a sister group relationship of Stupendidae to a clade consisting of rhizomarine-desma-bearing Scleritodermidae, Siphonidiidae and Azoricidae Sollas, 1888 was recently indicated (Kelly & Cárdenas, 2016). However, understanding the higher taxonomic relationships within Tetractinellida including its lithistid lineages is still hindered by incomplete taxon sampling and sequencing of key taxa such as Thrombidae Sollas, 1888 or *Gastrophanella* Schmidt, 1879 (e.g., Kelly Borges & Pomponi, 1994; Cárdenas et al., 2011).

Aside from the report of ‘lithistids’ in some specific island regions of the TWA, such as Barbados (Van Soest & Stentoft, 1988), the Bahamas (Maldonado & Young, 1996; Reed & Pomponi, 1997), Cuba (Pisera, 1999), Dutch Antilles (Van Soest, Meesters & Becking, 2014), the deep Florida shelf (Pisera & Pomponi, 2015), and chemotaxonomic studies (Kelly Borges & Pomponi, 1994) the most comprehensive taxonomy based survey comprising nearly all island groups in the TWA was conducted by Pomponi et al. (2001). The main focus of a study of Pomponi et al. (2001) was the documentation of biodiversity and bathymetric distributions of ‘lithistids’, thus no morphological species descriptions, sequences or phylogenetic affinities of these specimens were included. Although Pomponi et al. (2001) concluded that ‘lithistids’ are an important and dominant group of deep, hard-bottom habitats in the TWA, no comprehensive integrative taxonomic approach using molecular and morphological data has yet been made to evaluate this large and unique collection of TWA ‘lithistid’ demosponges, which is to a large extent unidentified and awaits taxonomic revision. Their study was based on 36 expeditions and 450 submersible transects led by the Harbor Branch Oceanographic Institute (HBOI) from 1984 to 2000, and aimed to provide an inventory of the biodiversity and bathymetric distribution of TWA ‘lithistids’. As a result, 28 ‘lithistid’ species representing 18 genera and nine families were reported from the TWA. However, knowledge of the TWA ‘lithistid’ fauna still remains comparatively poorly known, but crucial for a better knowledge of their global diversity and their comparison to the Mesozoic ‘lithistids’.

Therefore, the aim of this study is to (1) provide a general molecular phylogenetic overview of the subordinal classification within Tetractinellida; (2) focus on desma-bearing taxa from the TWA to provide a robust phylogeny for further analyses. In order to achieve this aim we investigated a large part of the extensive HBOI ‘lithistid’ collection (249 specimens) by means of generating independent molecular markers (*cox1* and 28S, C1-D2 region) from material collected between 1985 and 2011. Complementary to this we included *in situ* and SEM pictures of 71 taxa into the Sponge Barcoding Project (SBP). This study includes samples from almost all island groups in the TWA (Fig. 1) from depths ranging between 2 and 950 m, covering different geomorphological zonation as described in Reed & Pomponi (1997). The phylogenetic affinities of 31 out of the 35 ‘lithistid’ Tetractinellida genera are reconstructed.

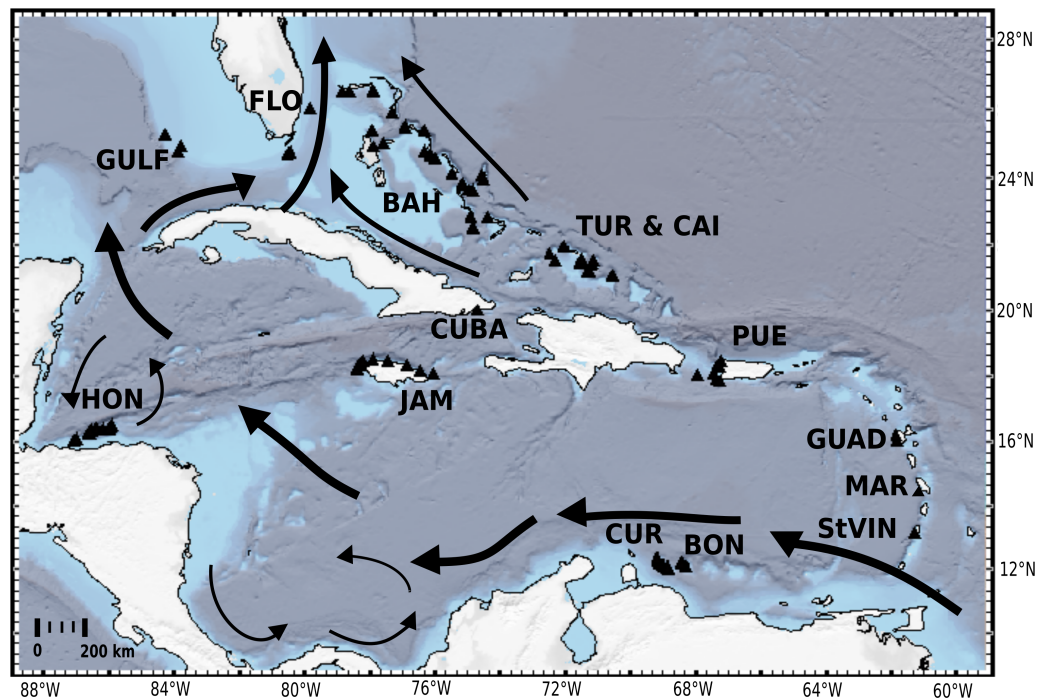


Figure 1 Distribution map of investigated HBOI and other desma-bearing tetractinellids and Vetulinidae from the TWA. Abbreviations correspond to the different locations (GULF, Gulf of Mexico; CUR, Curaçao; BON, Bonaire; StVIN, St. Vincent; MAR, Martinique; GUAD, Guadeloupe; PUE, Puerto Rico; JAM, Jamaica; HON, Honduras; TUR and CAI, Turks and Caicos; BAH, Bahamas; FLO, Florida). Arrows depict main surface currents. Map generated with GeoMapApp 3.6.3 (<http://www.geomapp.org>, Ryan *et al.*, 2009).

Full-size  DOI: 10.7717/peerj.10775/fig-1

MATERIALS AND METHODS

Specimen collection and identification

Sponge samples were collected from the Tropical Western Atlantic (TWA) using the Johnson-Sea-Link manned submersibles with permission granted to Harbor Branch Oceanographic Institute by: the United States National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Dry Tortugas National Park (letters of acknowledgement F/SER25:KM, F/SER23:PE, SER02-130) and the Florida Keys National Marine Sanctuary (permit numbers 2001-049, 2001-043); the Government of the Bahamas, Department of Fisheries; the Government of Honduras, Department of Fisheries (DIGEPESCA-638/97); the Government of Portugal, Parque Natural de Madeira; the Government of Jamaica, Jamaican Ministry of Foreign Affairs (diplomatic note no. 7/703/315); the Government of Bonaire, Bonaire Marine Park, and Netherlands Ministry of Foreign Affairs (note no. VADV-172/00); the Government of Panama (American Embassy Panama telegram no. 5570); the Puerto Rico Department of Natural And Environmental Resources; the Government of St. Vincent; the Government of France; and the Government of Turks and Caicos (American Embassy London telegram no. 12514). Sampling locations are indicated on the map (Fig. 1). These expeditions aimed to conduct a biodiversity

inventory and collect samples for biomedical research focused particularly on sponges, octocorals and algae. Various habitats from the fore reef slopes and escarpments to the deep shelf slopes were sampled using either a claw, suction tube or scoop in depths from 0–1000 m. Sponge samples from this collection, were pre-identified by S.P. and M.K., and frozen and/or stored in 70% ethanol. For comparison, additional material from the Southwest Pacific (New Caledonia and New Zealand), and Indo-Pacific region, in the National Institute of Water and Atmospheric Research (NIWA) collection in Auckland and its invertebrate collection (NIWA Invertebrate Collection, NIC) in Wellington, New Zealand, were subsampled for molecular investigations. This material included subsamples of tetractinellids, which were collected by SCUBA diving during several expeditions across the Indo-Pacific and New Zealand, led by the Coral Reef Research Foundation (CRRF) under contract to the U.S. National Cancer Institute in Republic of Palau, identified by M.K. Six specimens (three *Geodia* spp. and three *Cinachyrella* spp.) from Jamaica and Norway were added from the Bavarian State Collection of Zoology (ZSM) in Munich, Germany (identification by Helmut Lehnert). Material from Marquesas were provided by Cécile Debitus (Institute de Recherche pour le Développement, IRD) sampled during the cruise DEBITUS Cécile (2009) BSMPF-1, RV Alis: [10.17600/9100030](https://doi.org/10.17600/9100030). More detailed information for all novel samples sequenced is provided in the Supplementary Material as well as in PANGAEA (<http://www.pangaea.de>) under the digital object identifier (DOI): [10.1594/PANGAEA.924148](https://doi.org/10.1594/PANGAEA.924148).

Undetermined samples from the TWA (all HBOI subsamples) were identified to the genus level according to their phylogenetic position relative to known species. Based on this, we selected 71 samples with distinct genotypes for a deeper morphological investigation. For those taxa we examined collection pictures, prepared thick sections and spicule and skeleton stubs for Scanning Electron Microscopy (SEM). We used the methodology outlined in *Pisera & Pomponi (2015)* to illustrate and evaluate morphological characters. Based on this, 249 specimens were identified to genus and/or species level. Morphological documentation for the 71 representative specimens are provided in the SBP (<http://www.spongebarcoding.org/>). SEM stubs and spicule slides including thick sections are deposited at the Bavarian State Collection for Paleontology and Geology (BSPG) Munich, Germany.

Molecular investigations

Genomic DNA was isolated from small pieces of sponge tissue preserved in 70% ethanol using a modified protocol of the DNeasy (Qiagen) Blood and Tissue Kit, which included an additional centrifugation step just before transferring the lysate to the spin column. A Nano-Drop 1000 Spectrophotometer (Thermo Scientific) was used to quantify the isolated genomic DNA. Amplification of a fragment of the mitochondrial cytochrome c oxidase subunit 1 (*cox1*, partial \approx 659 bp) was performed using the primers dgLCO1490 and dgHCO2198 (*Meyer, Geller & Paulay, 2005*). Additionally, a fragment of an unlinked nuclear ribosomal gene (28S; partition C1-D2, 768-832 bp) was amplified using the forward C1' ASTR (*Cárdenas et al., 2010*) and the reverse universal D2 (*Lê, Lecointre & Perasso, 1993*) primers. Both amplifications follow the PCR protocol and settings outlined

in *Schuster et al. (2015)*. Amplification success was checked on a 1.5% agarose gel. For the majority of the 28S fragments we observed an additional non-specific shorter band at ≈ 650 bp, which was subsequently identified as originating from a bacterial template. Therefore, separation of double bands and PCR clean-up was performed using a modified freeze-squeeze method (*Tautz & Renz, 1983*), as described in *Schuster et al. (2015)*. For sequencing of the 28S fragment, 6 μ l of the remaining supernatant from the clean-up was used with the PCR primers and BigDye Terminator v3.1 (Applied Biosystems, Forster City, CA, USA) chemicals. For sequencing of *cox1* we used a 1:10 dilution of the PCR products together with the PCR primers and BigDye Terminator v3.1 chemicals. Sequencing was carried out on an ABI 3730 Genetic Analyzer at the Sequencing Service of the Department of Biology (LMU München). Sponge origin of novel sequences were tested by BLAST searches against NCBI GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Raw trace files were post-processed by base-calling using CodonCode Aligner v.3.7.1.1 (CodonCode Corporation). Geneious v.8.1.8 (<http://www.geneious.com>, *Kearse et al., 2012*) was used for the assembly of forward and reverse reads. Sequences will be deposited at the European Nucleotide Archive (ENA) and the Sponge Barcoding Database (SBD) of the SBP under accession numbers SBD#1794 to SBD#2108.

Phylogenetic reconstructions

Alignments were generated separately for *cox1* and 28S using MAFFT v.7 under the L-INS-I algorithm (*Katoh & Standley, 2013*) because of heterogeneous taxon sampling and moderate sequencing success of *cox1*. Saturation of both markers was evaluated using Xia's test (*Xia et al., 2003*) as implemented in DAMBE v5.1.5 (*Xia, 2013*) which compares an estimated substitution saturation index (Iss) to a critical substitution saturation index (Iss.c). For the *cox1* dataset, sequences of *Halichondria panicea* (*Pallas, 1766*) (subclass: Heteroscleromorpha *Cárdenas, Pérez & Boury-Esnault, 2012*, order Suberitida *Schmidt, 1870*) and *Aplysina aerophoba* (*Nardo, 1833*) (subclass: Verongimorpha *Erpenbeck et al., 2012*, order Verongiida *Bergquist, 1978*) were chosen as outgroups. For the 28S dataset sequences of the order Sphaerocladina were chosen as outgroup. All outgroups have been used in earlier phylogenetic studies on tetractinellids (see e.g., *Schuster et al., 2015*; *Kelly & Cárdenas, 2016*). The final *cox1* alignment comprised 307 sequences of which 122 are newly generated sequences for this study. The alignment was 635 bp long, of which 295 bp were constant, 40 bp were parsimony uninformative and 300 bp were parsimony informative. The final 28S alignment comprised 474 sequences of which 305 are newly generated sequences for this study. In total this alignment was 905 bp long, of which 325 bp were constant, 66 bp parsimony uninformative and 514 bp parsimony informative. Both alignments from this study are freely available at OpenDataLMU [10.5282/ubm/data.221](https://doi.org/10.5282/ubm/data.221). Phylogenetic tree reconstructions for both datasets were performed on a parallel version of MrBayes v3.2.4 (*Ronquist et al., 2012*) on a Linux cluster. The most generalized GTR+G+I evolutionary model, indicated as the most suitable by jModelTest v.2.1.7 (*Darriba et al., 2012*), was used. Analyses were run in two concurrent runs of four Metropolis-coupled Markov-chains (MCMCMC) for 100,000,000 generations and stopped when the average

standard deviation of split frequencies dropped below 0.01. The first 20% of the sampled trees were removed as Burn-in from further analyses.

Inclusive molecular phylodiversity and abundance analyses

The Inclusive Phylogenetic Diversity (PD_I) is the sum of all branch lengths of a gene tree connecting a set of taxa from the root of the tree to the tips of all phylogenetic branches spanned by this set of taxa (see e.g., *Lewis & Lewis, 2005*). To evaluate the PD_I, a Maximum Likelihood (ML) tree was first calculated from the most comprehensive dataset (28S, C1-D2 partition) using RAxML 7.2.8 (*Stamatakis, 2014*). The GTRGAMMA nucleotide evolutionary model selected by jModelTest v.2.1.7 (*Darriba et al., 2012*) was taken with 1000 fast pseudo-replicated bootstraps. The resulting tree topology was used to calculate the PD_I for several areas in the TWA using a modified python script from *Vargas et al. (2015)*. All non-TWA genera and all TWA genera less than five were excluded from this analysis. In total, the PD_I of Bonaire, Curaçao, Florida, Honduras, Jamaica, Puerto Rico and Turks and Caicos was calculated. In order to compensate for different sampling efforts across the seven regions, rarefaction curves (*Sanders, 1968*) were used for each location. The rarefaction curves were generated in RStudio (*R Studio Team, 2014*). Both scripts are available at <https://bitbucket.org/molpalmuc/>.

The relative abundance of eight 'lithistid' families from five depth zones (0–60 m; 61–150 m; 151–300 m; 301–600 m; 601–914 m) from the TWA was plotted and illustrated using ggplot2 (*Wickham, 2009*) as implemented in RStudio. These depth zonations follow *Reed & Pomponi (1997)* and *Pomponi et al. (2001)*, which are based on the geomorphological observations of the sites sampled.

RESULTS AND DISCUSSION

Integrative morphological and molecular systematics of 'lithistid' demosponges with focus on TWA species

Higher-taxa relationships of desma-bearing tetractinellids

The 296 lithistid sequences of at least 88 species from 27 genera (35 known) constitute the largest and most comprehensive taxon set on desma-bearing tetractinellids to date. Our phylogenies (overview in *Fig. 2*, see *Figs. 3–8* (28S) and *Figs. 9–16* (*cox1*) for details) corroborate the monophyly of Tetractinellida, currently including the suborders Astrophorina and Spirophorina (*Morrow & Cárdenas, 2015*) (*Fig. 2C*). In addition, the affinity of eight desma-bearing families to the suborder Astrophorina (*Cárdenas et al., 2011; Morrow & Cárdenas, 2015; Schuster et al., 2015*) is confirmed (*Figs. 2A–2C*). The 28S phylogeny (*Fig. 2A*) indicates a sister relationship of Astrophorina and Spirophorina. In both gene trees (*Figs. 2A–2B*) desma-bearing tetractinellids do not group with the Spirophorina (only represented by the Tetillidae in our sampling). In both gene trees the rhizomorine-bearing families Scleritodermidae, Siphonidiidae and Azoricidae form a clade (*Fig. 2*). However, *Gastrophanella* (Siphonidiidae) is distinct and sister (1.0 Posterior Probability (PP)) to Scleritodermidae/Siphonidiidae/Azoricidae in the 28S phylogeny (*Fig. 2A*). This sister-group relationship could not be corroborated by *cox1* analysis as no sequence of *Gastrophanella* could be generated. We suspected an intron

insertion within *cox1* due to the discovery of these in closely related rhizomorine-bearing genera (*Setidium* Schmidt, 1879, *Microscleroderma* Kirkpatrick, 1903, *Aciculites* Schmidt, 1879, *Scleritoderma* Sollas, 1888) (Schuster et al., 2017). Based on this, various primer sets suggested by Schuster et al. (2017) were tested, however, without success. We suspect that *Gastrophanella* has one or several intron insertions in the *cox1* gene in a yet unknown position. By including several additional rhizomorine-bearing genera such as *Gastrophanella*, *Leiodermatium*, *Siphonidium* and *Amphibleptula* in our datasets, the family Thrombidae could not be recovered within Astrophorina as hypothesized by the Systema Porifera (Hooper & Van Soest, 2002). The 28S gene tree recovers Thrombidae as sister to all rhizomorine-bearing tetractinellids, but this relationship is not supported (0.72 PP) (Figs. 2 and 8) and needs further investigation, including also 28S for Stupendidae Kelly & Cárdenas, 2016, a recently established new family (Kelly & Cárdenas, 2016). In the *cox1* phylogeny (Fig. 2B) Stupendidae is a highly supported sister taxon to Scleritodermidae/Siphonidiidae/Azoricidae. It should be noted that Thrombidae and *Gastrophanella* are missing in the *cox1* phylogeny (Fig. 2B).

Intra-subordinal relationships of astrophorine ‘lithistids’

The majority (15 out of 23) of the currently known tetractinellid families are located within the Astrophorina (Morrow & Cárdenas, 2015). This includes eight desma-bearing families (Corallistidae, Isoraphiniidae Schrammen, 1924, Macandrewiidae Schrammen, 1924, Neopeltidae, Pleromidae, Phymaraphiniidae Schrammen, 1924, Phymatellidae and Theonellidae) and seven non-desma bearing families (Cárdenas et al., 2011; Morrow & Cárdenas, 2015; Schuster et al., 2015) (see also Figs. 2A–2C). Thus, the present study supports earlier findings, which were based on lower taxon sampling and additionally provides deeper insights into the intraspecific relationship of desma-bearing astrophorids.

The family **Theonellidae** consists of the genera *Discodermia* Du Bocage, 1869, *Manihinea* Pulitzer-Finali, 1993, *Racodiscula* Zittel, 1878, *Siliquariaspongia* Hoshino, 1981 and *Theonella* Gray, 1868. Theonellidae possesses tetracelone desmas and phyllostriaenes to discotriaenes as characteristic megascleres. Typical microscleres are acanthorhabds, spirasters and amphiasters (Pisera & Lévi, 2002a). Until now, only *Theonella* and *Discodermia* species as well as one *Manihinea* sp. were sequenced in different phylogenetic studies using 18S, 28S and *cox1* (see e.g., Redmond et al., 2013; Hall, Ekins & Hooper, 2014; Schuster et al., 2015). By providing sequences for all known genera, our 28S phylogeny (Fig. 3) recovers Theonellidae as monophyletic (PP = 1.0), thus conclusively support earlier findings of Schuster et al. (2015), while the *cox1* phylogeny (Fig. 13) lacks support in this respect. The 28S phylogeny indicates the monophyly of the genera *Discodermia*, *Manihinea*, *Racodiscula* and a potential new taxon, here denoted as Theonellidae gen. sp., a potential new genus mainly distinct by the layered network of tetracelone desmas with smooth rays and strongly tuberculated tips and the less abundant microscleres on the ectosome (SBD#2102–2106). The sister relationship of *Manihinea conferta* to *Theonella* sp. is highly supported (PP = 0.99) by 28S (Fig. 3), whereas it is not supported by *cox1* (Fig. 13). A close relationship of *Theonella* and *Manihinea* was observed in an earlier study by Redmond et al. (2013) using a nearly complete 18S gene fragment, but unsupported. The genus

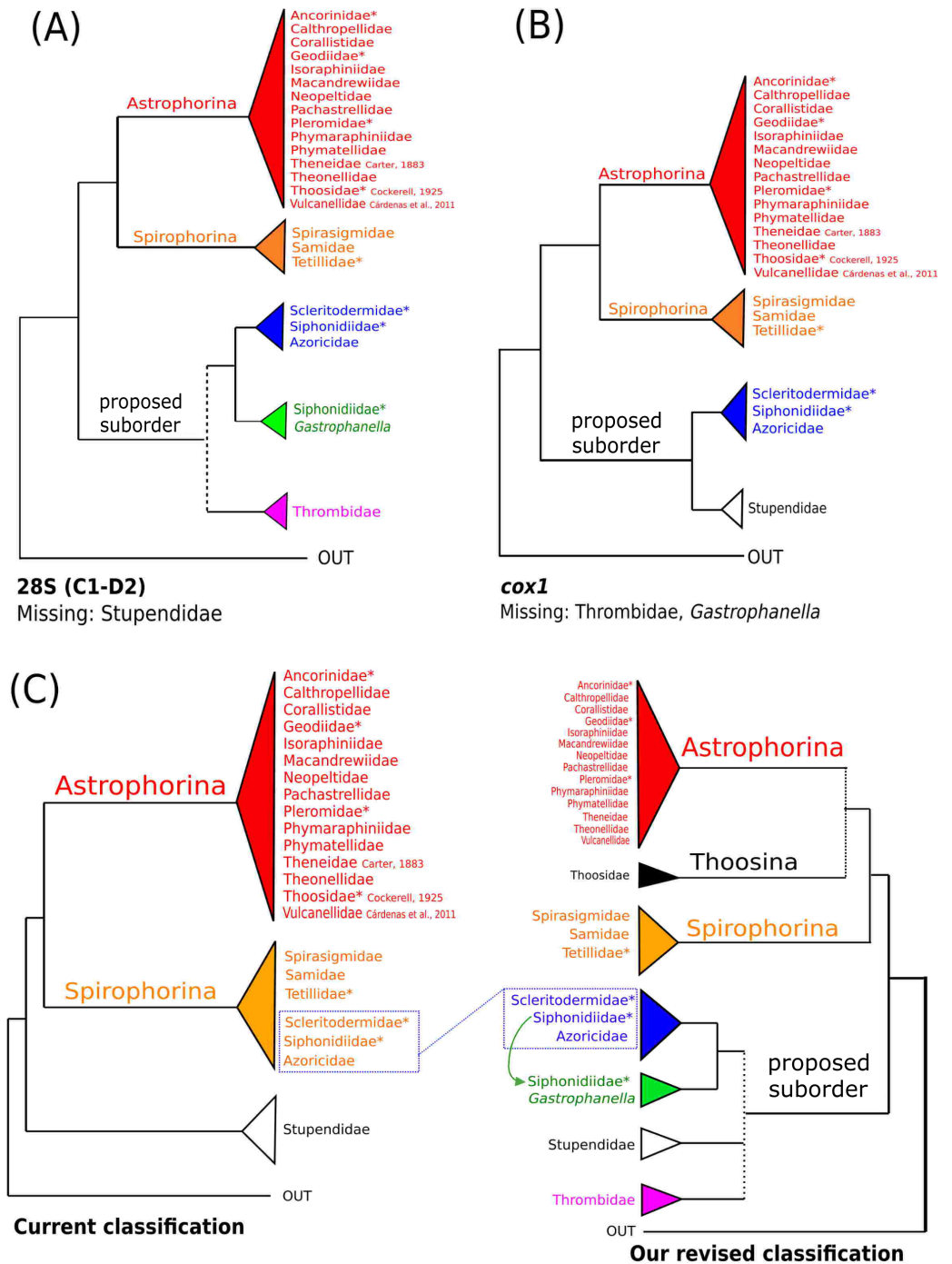


Figure 2 Schematic summary cladograms obtained from the 28S and *cox1* phylogenies indicating the higher-taxa relationships within the order Tetractinellida. (A) 28S (B) *cox1* summary tree with the suborders Astrophorina (red), Spirophorina (orange) and a proposed suborder (blue, green, pink and light gray) including all rhizoclone desma-bearing families and the families Thrombidae and Stupendidae. Stars behind family names indicate their proposed polyphyly. Dashed lines indicate the uncertainties of not supported topologies. (C) Comparison of current and revised classification including the proposed suborder Thoosina from Carballo et al. (2018).

Full-size DOI: 10.7717/peerj.10775/fig-2

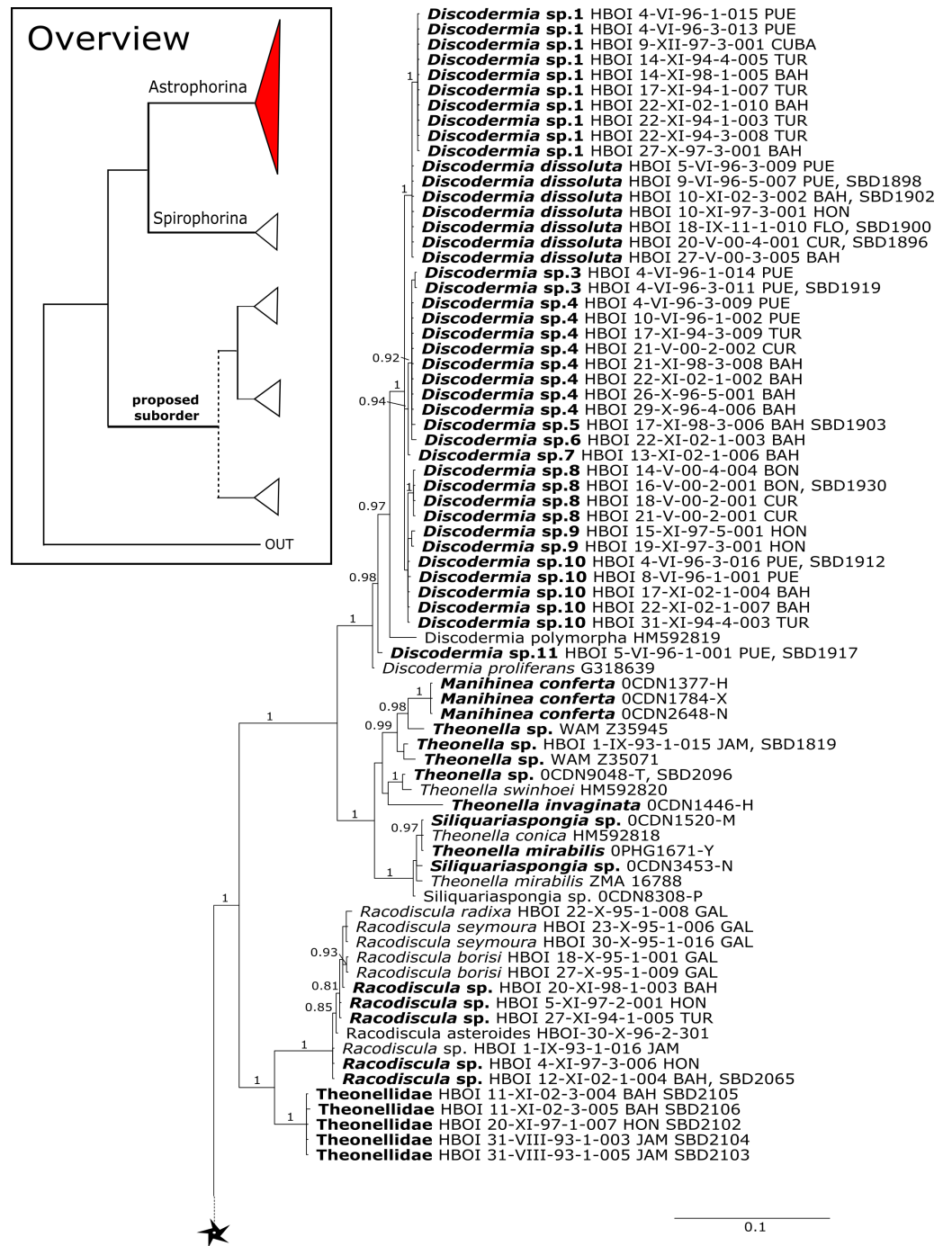


Figure 3 Bayesian Inference phylogeny of Tetractinellida based on 28S (C1-D2). Posterior probability (PP) values are provided above or below branches. Self-generated sequences are in bold. Numbers behind taxon names are either voucher numbers or GenBank/ENA accession numbers. Three letter code behind voucher numbers corresponds to the different locations (GULF, Gulf of Mexico; CUR, Curaçao; BON, Bonaire; GUAD, Guadaloupe; PUE, Puerto Rico; JAM, Jamaica; HON, Honduras; TUR, Turks & Caicos; BAH, Bahamas; FLO, Florida; GAL, Galápagos). Taxa where the morphology was investigated are indicated with their corresponding SBD#.

Full-size DOI: [10.7717/peerj.10775/fig-3](https://doi.org/10.7717/peerj.10775/fig-3)

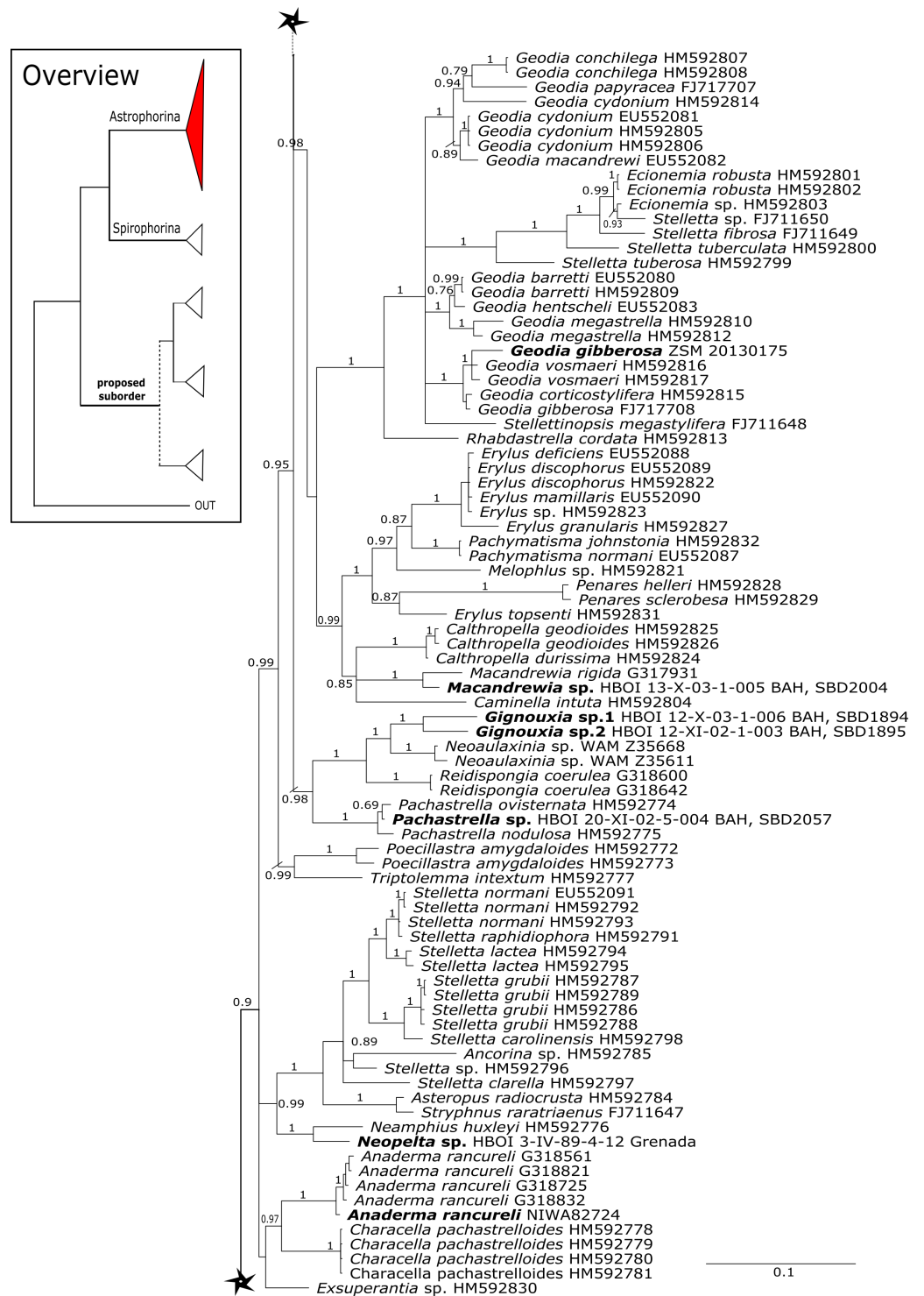


Figure 4 28S phylogeny continued. See caption in Fig. 3.

Full-size DOI: 10.7717/peerj.10775/fig-4

Discodermia is sister to a clade consisting of *Manihinea* + *Theonella* + *Siliquariaspongia*, which is sister to *Racodiscula* + Theonellidae gen. sp.

The genus *Racodiscula* is highly supported (PP=1.0) as sister to Theonellidae gen. sp. Although the outer morphology of Theonellidae gen. sp. (SBD#2106 A–D) is very similar to that of *Racodiscula*, it differs in spicule composition, desma and skeleton structure: the usually abundant spinose microacanthorhabds, covering the surface of *Racodiscula* species (SBD#2065) building a dense crust on the surface, are rarer or even absent in Theonellidae gen. sp. Instead of microacanthorhabds, phyllo- to discotriaenes are the main components of the dense surface crust. In addition, Theonellidae gen. sp. possesses desmas with smooth rays and strongly tuberculated tips (SBD#2105 and SBD#2106) building a layered network (SBD#2102), which clearly differs from *Racodiscula* ([Schuster et al., 2018](#)).

Theonella mirabilis ([De Laubenfels, 1954](#)) was first named within the homosclerophorid genus *Placinolopha* (Class Homoscleromorpha, Order Homosclerophorida, Family Plakinidae) on the possession of what de Laubenfels described as 'lophotetractines'. The key size and shape differences between the 'tetralophs' of *T. mirabilis* and other *Placinolopha* species were noted by [Muricy & Diaz \(2002\)](#), who suggested that the species *mirabilis* had a more likely affinity with species in family Theonellidae. Sequences of 28S ([Fig. 3](#)) unite specimens identified as *Theonella mirabilis* in a single clade with a specimen identified as *T. conica* ([Kieschnick, 1896](#)) which also has tetraloph-like desmas, suggesting that species with non-articulated 'tetraloph' desmas may be monophyletic and separate from other *Theonella* spp. However, *cox1* sequences ([Fig. 13](#)) separate *T. mirabilis* into two groups, nesting them within diverse species of *Theonella*. *Theonella mirabilis* is very similar in spicule complement to the type species of the genus *Siliquariaspongia*, *S. japonica* [Hoshino, 1981](#) (Family Theonellidae), although the latter lacks the strongyles and possesses frilly discotriaenes, the latter occasionally recorded in *T. mirabilis*. Our phylogenies clearly place all of the sequenced *Theonella mirabilis* species within the *Theonella* + *Manihinea* clade ([Figs. 3](#) and [13](#)), confirming that this species belongs to the family Theonellidae. This result is supported by the discovery of potent new depsipeptides mirabamides A–D, that inhibit HIV-1 infection, adding to a small class first exemplified by the papuamides from various *Theonella* spp. ([Plaza et al., 2007](#)).

The family **Macandrewiidae** is monogeneric with currently seven valid species ([Van Soest et al., 2018a](#)). Until now, only *Macandrewia rigida* [Lévi & Lévi, 1989](#) from the Solomon Islands has been sequenced (28S C1–D2 region, LN624160, G317931) ([Schuster et al., 2015](#)). The present study includes a further sequence of an undescribed *Macandrewia* sp. from the Bahamas (909 m depth), which clearly differs from *M. rigida* ([Fig. 4](#)). Morphological differences in desmas (SBD#2004) corroborate the genetic difference to *M. rigida* and provide further evidence of a possible new species, which would be the first record in the TWA. Nevertheless, further morphological observations and comparison with the type material of *M. rigida* as well as its sequences are needed to conclusively describe and distinguish this potential new species. Both *Macandrewia* species group within the Geodiidae, close to the Erylinae [Sollas, 1888](#), within a clade of non-desma bearing astrophorins (*Calthropella* [Sollas, 1888](#), *Caminella* [Von Lendenfeld, 1894](#)) ([Fig. 4](#)). This relationship is currently not supported by morphology ([Cárdenas et al., 2018](#)) and in any

case suggests a distinct evolutionary history of *Macandrewia* to other 'lithistid' families Corallistidae and Neopeltidae (Schuster et al., 2015), where *Macandrewia* was previously allocated (Kelly, 2000; Pomponi et al., 2001).

The family **Phymatellidae** currently includes three genera: *Neoaulaxinia*, *Neosiphonia* and *Reidispongia*. A highly supported sister group relationship of *Neoaulaxinia* *Pisera & Lévi, 2002e* to the genus *Gignouxia* *Moret, 1926* is observed and *Reidispongia coerulea* *Lévi & Lévi, 1988* is sister to this clade (Fig. 4). Morphological characters of both sequenced *Gignouxia* species were further investigated and illustrated (SBD#1894). Both species are potentially new to science. *Gignouxia* sp. 1 (SBD#1894) possesses the characteristic pseudophyllotriaenes (SBD#1894) known from the Neopeltidae, while *Gignouxia* sp. 2 (SBD#1895) possesses dichotriaenes characteristic for the Phymatellidae. These dichotriaenes, however, have a unique shape with indented cladomes (SBD#1895). A spicule drawing of *Corallistes tubulatus* *Van Soest & Stentoft, 1988* from Barbados, now *Neophrissospongia tubulata*, resembles those unique dichotriaenes implying that *N. tubulata* may need to be reallocated to *Gignouxia*. Interestingly, dichotriaenes of *Gignouxia* sp. 2 resemble those of the fossil *Gignouxia niciensis* *Moret, 1926* (Corallistidae) from the Late Cretaceous (Pl. XVIII, Fig. 2, 2' fig-txt 37). We suggest to allocate *Gignouxia* to the family Phymatellidae. *Gignouxia* will include *Gignouxia* sp. 2 as well as *N. tubulata* comb. nov.

Neopeltidae polyphyly is given by the highly supported (PP = 1.0) sister relationship of the newly sequenced species *Neopelta* sp. to the non-desma bearing astrophorid *Neamphius huxley* *Sollas, 1888* (Fig. 4). Morphologically, these two species only share choanosomal amphiasters with spiny rays and microxeas. Thus, monocrepid desmas and pseudodiscotriaenes characterizing *Neopelta* were lost in *Neamphius*. Spicule losses and gains are not uncommon within tetractinellids and have frequently been shown (Chombard, Boury-Esnault & Tillier, 1998; Cárdenas et al., 2011; Schuster et al., 2015). Nevertheless, these two genera form a robust sister clade to a non-desma bearing Ancorinidae clade consisting of *Stelletta* *Schmidt, 1862*, *Ancorina* *Schmidt, 1862*, *Asteropus* *Sollas, 1888* and *Stryphnus* *Sollas, 1886* (see Fig. 4).

Corallistidae is another major family dominating the HBOI collection and subsequently our phylogenies (Figs. 5 and 9). These taxa were the focus of biomedical investigations (e.g., Haar et al., 1996; Wright, 2010), thus targeted during the HBOI expeditions. Even though *Corallistes* were frequently sampled in the past and 15 species are described to date (Van Soest et al., 2018b), only five sequences are published (Kelly Borges & Pomponi, 1994; Chombard, Boury-Esnault & Tillier, 1998; McInerney, Adams & Kelly, 1999). With 52 *Corallistes* specimens sequenced, this study presents the largest data set to date and reveals the monophyly of this genus (28S, PP = 1.0, Fig. 5). *Corallistes typus* (Schmidt, 1870) specimens, type species of the genus, were examined (SBD#1888, 1889, 1892) and sequenced (Figs. 5 and 9). In addition, a *Corallistes isabela* *Desqueyroux-Faúndez & Van Soest, 1997* sample from Honduras was sequenced. Until this study *Corallistes isabela* was only known from the Eastern Pacific (Galápagos) and discussed as endemic to the Galápagos (Desqueyroux-Faúndez & Van Soest, 1997 and Schuster et al., 2018). In addition, six *Corallistes* (C. sp. 2 to C. sp. 7, see Fig. 5) differ by 1–3 bp in the 28S fragment, while no differences were found in *cox1* (Fig. 9). Morphological differences are observed between

C. sp. 2 (SBD#1872) and *C. sp. 4* (SBD#1879). For example, *C. sp. 2* has long (700 μm) thin ectosomal oxeas (SBD#1872 A), while *C. sp. 4* has ectosomal styles (SBD#1879 D) and subectosomal microxea with spined surfaces (SBD#1879 C). Morphological identifications are in progress and necessary to discriminate the remaining *Corallistes* species.

The polyphyletic corallistid genus *Herengeria* Lévi & Lévi, 1988, only known from the Pacific Norfolk Ridge, New Caledonia, and New Zealand (Schlacher-Hoeningler, Pisera & Hooper, 2005; Kelly et al., 2009), was sequenced (Fig. 5) and morphologically illustrated (SBD#1949) in the present study for the first time from the Bahamas and Honduras, representing a new genus for the Atlantic. Only two species of *Herengeria* are described (*H. auriculata* Lévi & Lévi, 1988) and *H. vasiformis* Schlacher-Hoeningler, Pisera and Hooper, 2005). The morphological observations delimit the new TWA species from *H. auriculata* (Schlacher-Hoeningler, Pisera & Hooper, 2005) due to a lack of subectosomal rhabd-like spirasters. With respect to molecular markers, *Herengeria* spp. from the TWA are distinct from *H. vasiformis*/*Herengeria* sp. from the Pacific (Fig. 5). Our 28S (Fig. 5) and *cox1* (Fig. 10) phylogenies strongly support *Neoschrammeniella* Pisera & Lévi, 2002d as sister to the *Herengeria*/*Isabella* clade. Currently, seven valid species of *Neophrissospongia* (Pisera & Lévi, 2002d) are described (Van Soest et al., 2018c). Only a few *Neophrissospongia* sequences (two 28S and one 18S) from the Pacific Ocean are currently published. However, in order to gain a better understanding of their geographical distribution and genetic differences, additional material from the Caribbean were sequenced in this study: Their resulting 28S phylogeny clearly separates *Neophrissospongia* from the Pacific and the Caribbean Islands. *Neophrissospongia* sp. 1 from different Caribbean Islands is sister (PP=1.0) to *N. microstylifera* and *Neophrissospongia* sp. 3, from the Pacific (Fig. 6). A further Caribbean *Neophrissospongia* species (sp. 2) forms a robust sister clade to *Neophrissospongia* from the Eastern Pacific (Galápagos, Panama) (Fig. 6).

A clade of six as yet unidentified specimens from the HBOI collection (SBD#1814) is sister to the monophyletic *Neophrissospongia* (PP = 1.0, Fig. 6). We assume that this clade consists of species from the as yet unsequenced genus *Awhiowhio* Kelly, 2007 from the Pacific based on morphological evidence. These show similar mega- and microsclere types to *Awhiowhio* such as dicranoclone desmas and smooth dichotriaenes in *Awhiowhio* sp. 1 from the Bahamas (SBD#1815), most similar to the *Awhiowhio osheai* from New Zealand (Kelly, 2007), but slightly different in terms of desma ornamentation. Streptaster microscleres and acanthose microrhabds in *Awhiowhio* sp. 1 (SBD#1814, 1815) differ from those in *Awhiowhio osheai* in sizes and shapes (SBD#1814). The *cox1* phylogeny (Fig. 9) indicates the sister group relationship of *Awhiowhio osheai* Kelly, 2007 to *Neophrissospongia*. A close relationship of *Awhiowhio* to *Herengeria* as suggested by Kelly (2007) based on morphological features is not supported by any of our phylogenies. Instead, both markers independently suggest a close relationship (strongly supported by PP = 1.0) to *Neophrissospongia*. The genus *Pleroma* Sollas, 1888 (family Pleromidae) is recovered as paraphyletic in both phylogenies (Figs. 5, 6, 9 and 13). *Pleroma menoui* (Sollas, 1888) is distant to other *Pleroma* spp. (including the type species *P. turbinatum*) in a close relationship to Corallistidae (Figs. 5 and 9).



Figure 5 28S phylogeny continued. See caption in Fig. 3.

Full-size DOI: 10.7717/peerj.10775/fig-5



Figure 7 28S phylogeny continued. See caption in Fig. 3.

Full-size DOI: 10.7717/peerj.10775/fig-7

latest revised classification of *Morrow & Cárdenas (2015)* included the desma-bearing families Azoricidae, Scleritodermidae and Siphonidiidae within Spirophorina. Since then, several studies (*Schuster et al., 2015; Schuster et al., 2017; Kelly & Cárdenas, 2016*) using

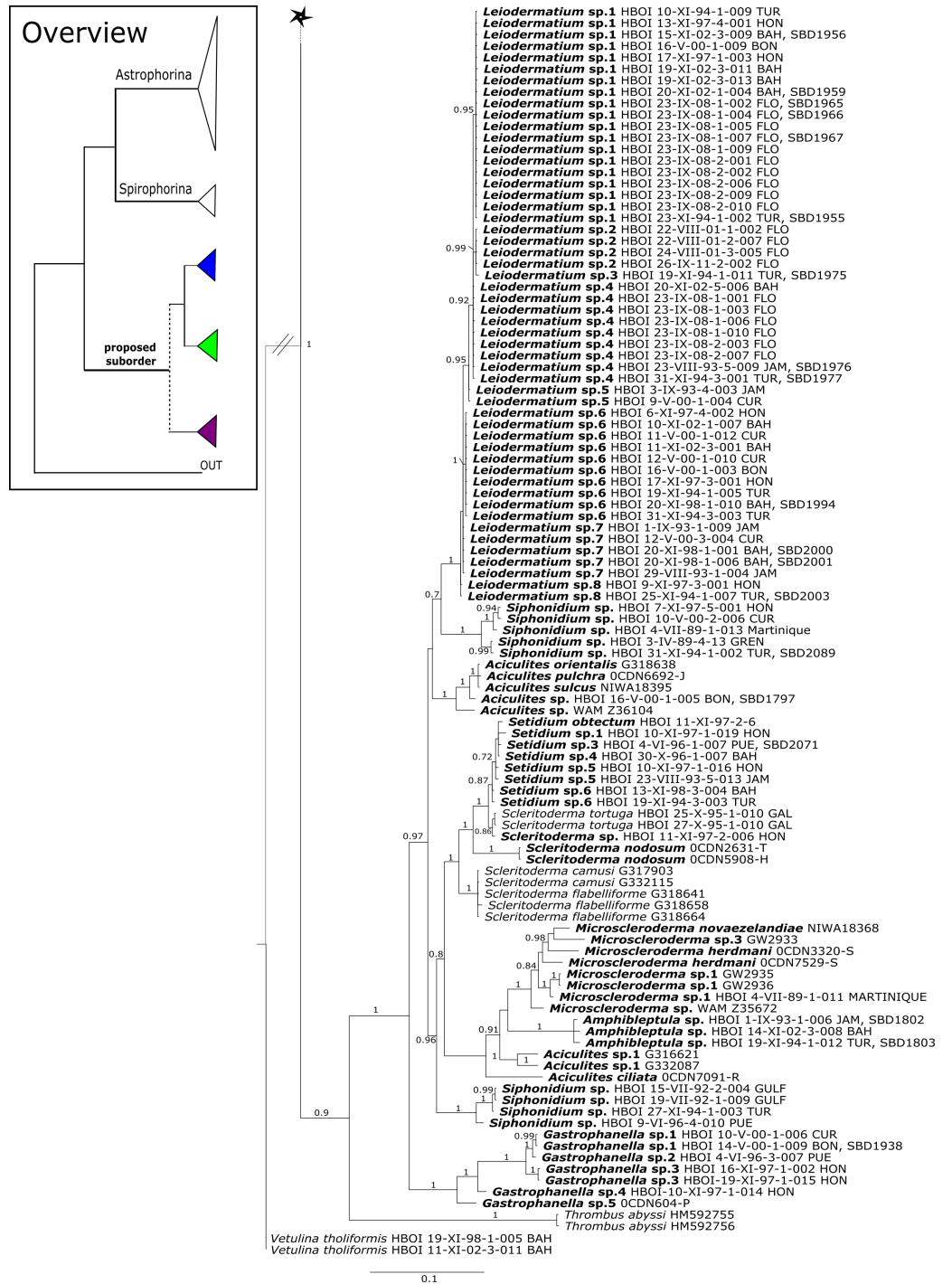


Figure 8 28S phylogeny continued. See caption in Fig. 3.

Full-size DOI: 10.7717/peerj.10775/fig-8

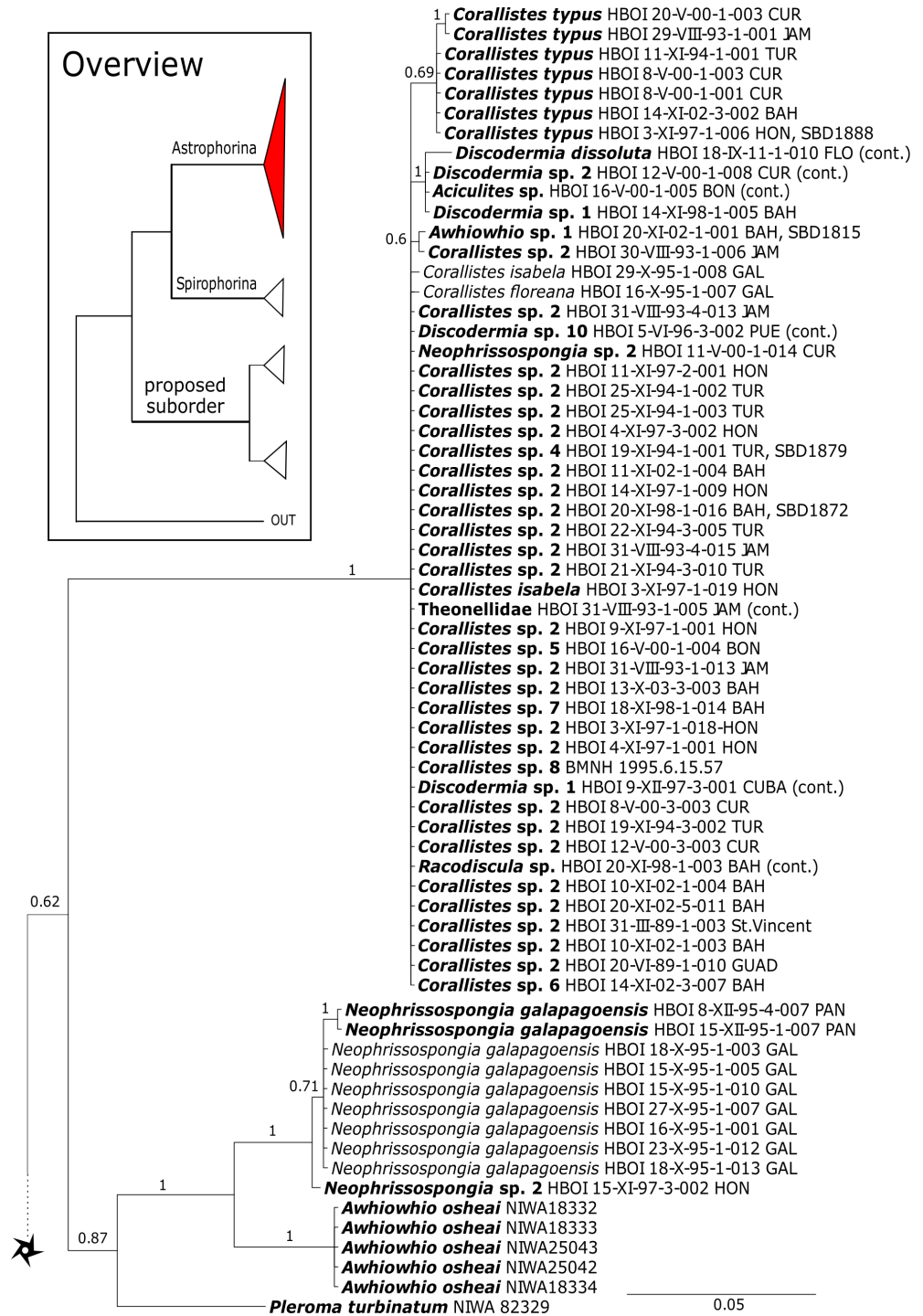


Figure 9 Bayesian Inference phylogeny of Tetractinellida based on *cox1*. Posterior probability (PP) values are provided above or below branches. Self-generated sequences are in bold. Numbers behind taxon names are either voucher numbers or GenBank/ENA accession numbers. Three letter code behind voucher numbers corresponds to the different locations (GULF, Gulf of Mexico; CUR, Curaçao; BON, Bonaire; GUAD, Guadeloupe; PUE, Puerto Rico; JAM, Jamaica; HON, Honduras; TUR, Turks & Caicos; BAH, Bahamas; FLO, Florida; GAL, Galápagos). Taxa where the morphology was investigated are indicated with their corresponding SBD#.

Full-size DOI: 10.7717/peerj.10775/fig-9

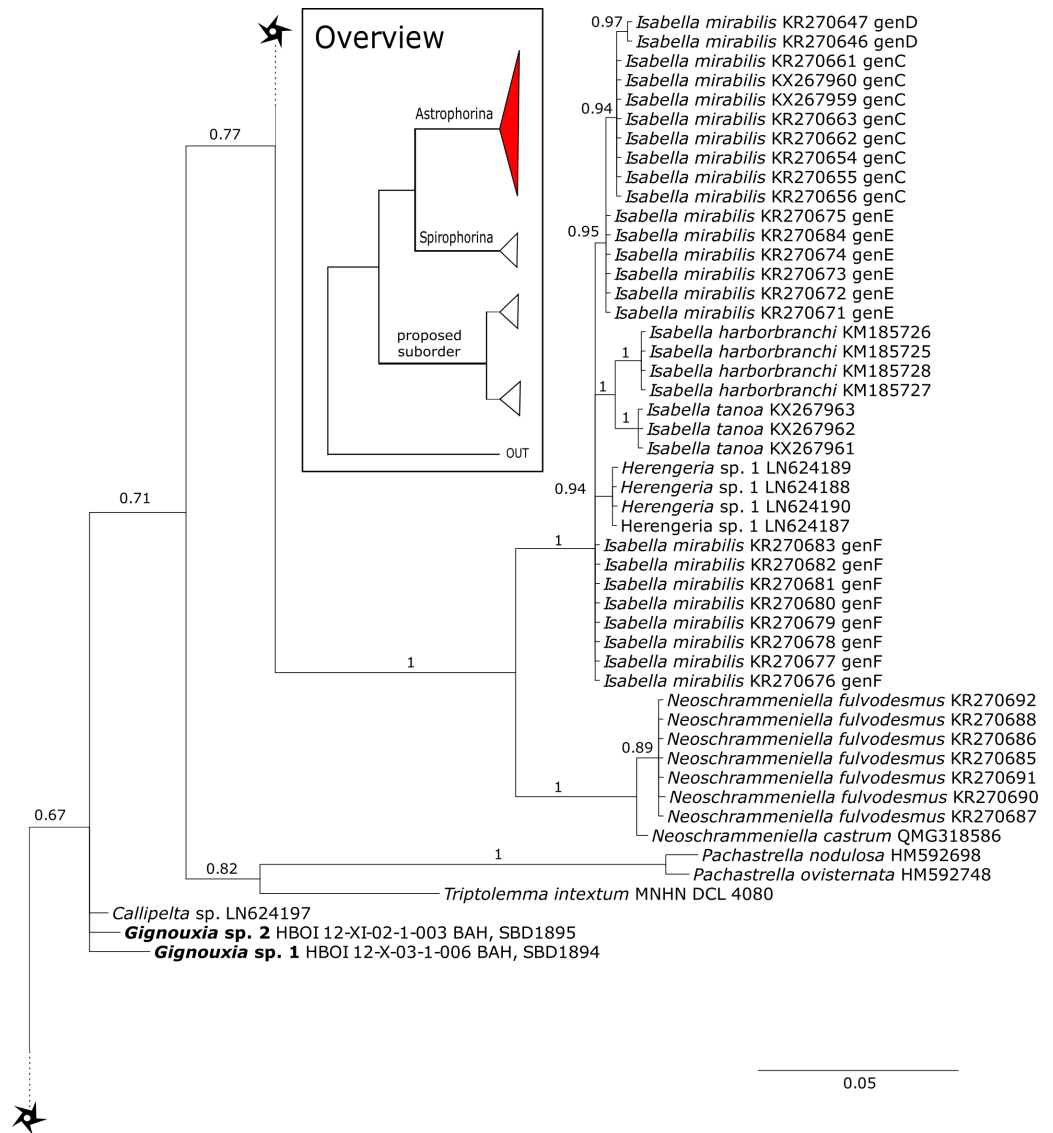


Figure 10 *Cox1* phylogeny continued. See caption in Fig. 9.

Full-size DOI: 10.7717/peerj.10775/fig-10

cox1, 18S and 28S markers showed the separation of all rhizomorine-bearing sponges from Spirophorina (=Tetillidae). The present enlarged dataset corroborates again the absence of desma-bearing sponges in Spirophorina and their grouping in a well supported clade along with the Stupendidae and the Thrombidae (Fig. 2). In order to establish this clade as a new taxa, we await further molecular data from the latter two families (work in progress, MK and PC).

Subordinal structure of Tetractinellida

Kelly & Cárdenas (2016) provided strong support for families Azoricidae, Scleritodermidae and Siphonidiidae within a proposed suborder, supported in part by the common possession of rhizomorine desmas.

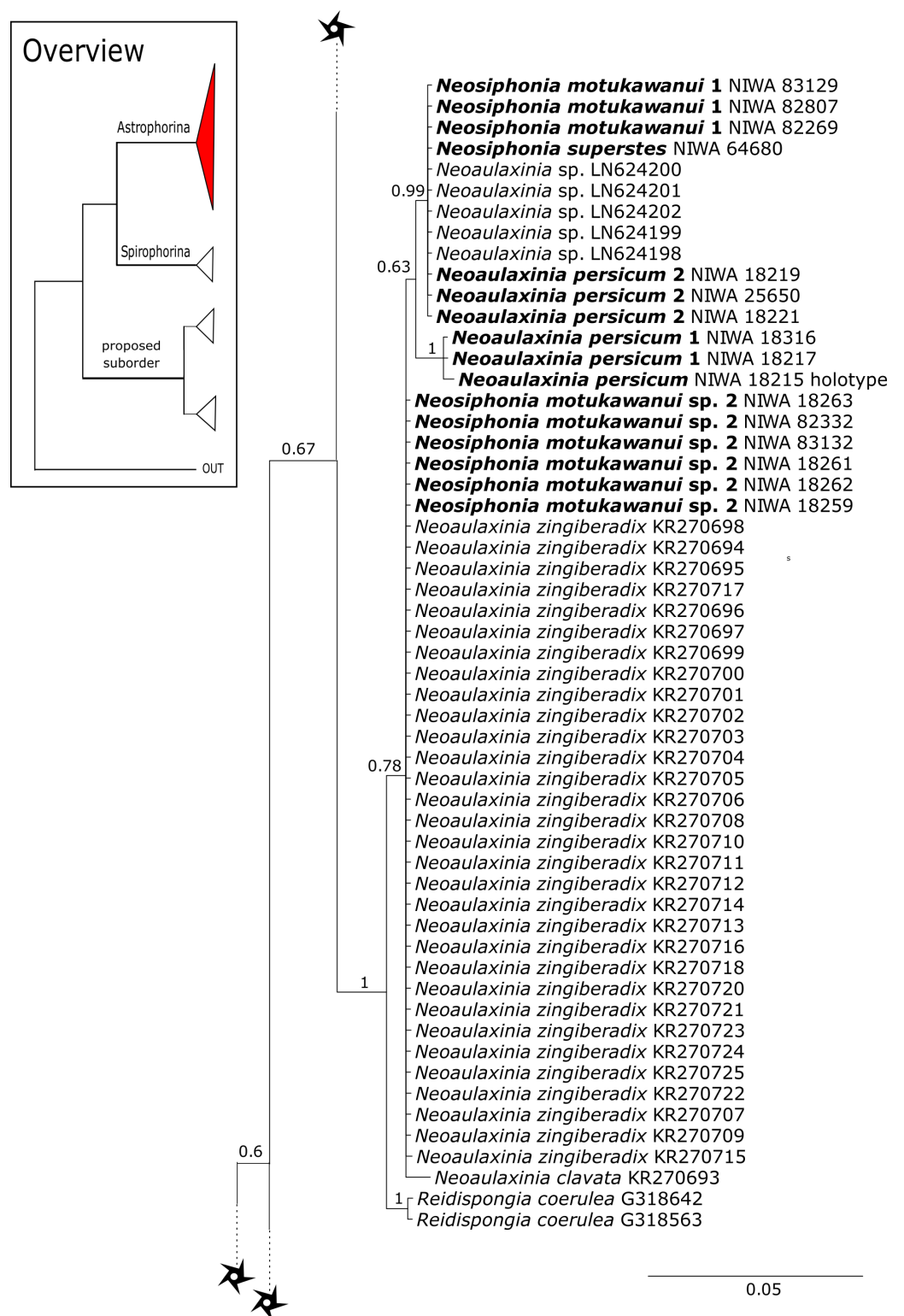


Figure 11 *Cox1* phylogeny continued. See caption in Fig. 9.

Full-size DOI: 10.7717/peerj.10775/fig-11

Regarding **Azoriciidae**, *Maldonado et al. (2015)* discovered a dense and large aggregation of *Leiodermatium pfeifferae* Carter, 1873 on seamounts in the Mediterranean building complex reef-forming structures. Even though *Leiodermatium Schmidt, 1870* has very few diagnostic characters (no microscleres) to discriminate between species (*Pisera, 2002; Pisera & Lévi, 2002b*), 11 species are valid to date (WPD access Jan. 2020). In the present study we sequenced the 28S C1-D2 fragment for 52 *Leiodermatium* specimens from several regions in the TWA (*Fig. 8*) representing at least 8 species and the largest sequenced dataset for this genus to date. The monophyly of *Leiodermatium* is highly supported by our 28S phylogeny (PP = 1.0; *Fig. 8*). The amplification of *cox1* unfortunately failed, most likely due to the presence of introns similar to other rhizomorine-bearing genera like e.g., *Microscleroderma* and *Scleritoderma* (*Schuster et al., 2017*). Preliminary morphological investigations (SBD# 1966, 1967, 1959, 1965, 1956, 1955, 1975, 1977, 1976, 1994, 2000, 2001, 2003) adumbrate detailed differences of *Leiodermatium* spp., in particular their surfaces (oscles and ostia sizes), diactines and desma morphology. For instance *Leiodermatium* sp. 1 (SBD#1955) has large and marginate oscles, while *Leiodermatium* sp. 6 (SBD#1994) and *Leiodermatium* sp. 8 (SBD#2003) have large but elevated oscles on exterior margins, in contrast to *Leiodermatium* sp. 7, whose oscles are small and closely distributed. Based on molecular and morphological data we propose eight different species of *Leiodermatium* (*Fig. 8*) in the TWA, however further morphological investigations are needed to corroborate this assumption. *Leiodermatium* is unsupported (PP = 0.65) sister to a clade of *Siphonidium* spp. (Siphonidiidae); the same relationship was revealed with small fragment of the 18S gene (482 bp) for *Leiodermatium* sp. (*Kelly Borges & Pomponi, 1994; Kelly & Cárdenas, 2016*). Further investigation and a review of all extant and fossil *Leiodermatium* species is suggested to better understand the geographical distribution and recent diversification of this paleontological important group.

Within the polyphyletic rhizomorine family **Scleritodermidae**, its genera *Aciculites* is polyphyletic and *Scleritoderma* is paraphyletic, while *Microscleroderma*, *Amphibleptula Schmidt, 1879* and *Setidium Schmidt, 1879* are monophyletic (*Fig. 8*). The genus *Amphibleptula* is currently monospecific with *A. madrepora Schmidt, 1879* from the Caribbean (*Pisera & Lévi, 2002c*). Morphologically, *A. madrepora* is very similar and easy to confuse with *Microscleroderma spirophora Lévi, 1960* as discussed in *Van Soest & Stentoft (1988)*. *Amphibleptula* is here sequenced for the first time and our 28S phylogeny shows *Microscleroderma* and *Amphibleptula* sp. 1 as sister groups, although unsupported in the 28S phylogeny (PP = 0.63). Morphological observations (SBD#1802,1803) provide conclusive evidence that our three samples are *Amphibleptula* species, due to their dense tuberculated/blunt spinose rhizoclones, the protruding bundles of oxeads in the oscula area (SBD#1802,1803) as well as the presence of sigmaspires (SBD#1802). Differences to *A. madrepora* are the diactine spicules present in all three *Amphibleptula* sp. 1. In addition, fusiform spined microxeads and acanthorhabds are found in the specimen from Jamaica (HBOI 1-IX-93-1-006, SBD#1802). To conclude, we sequenced two potential new species of *Amphibleptula* with clear unique morphological characters, different from *A. madrepora*.

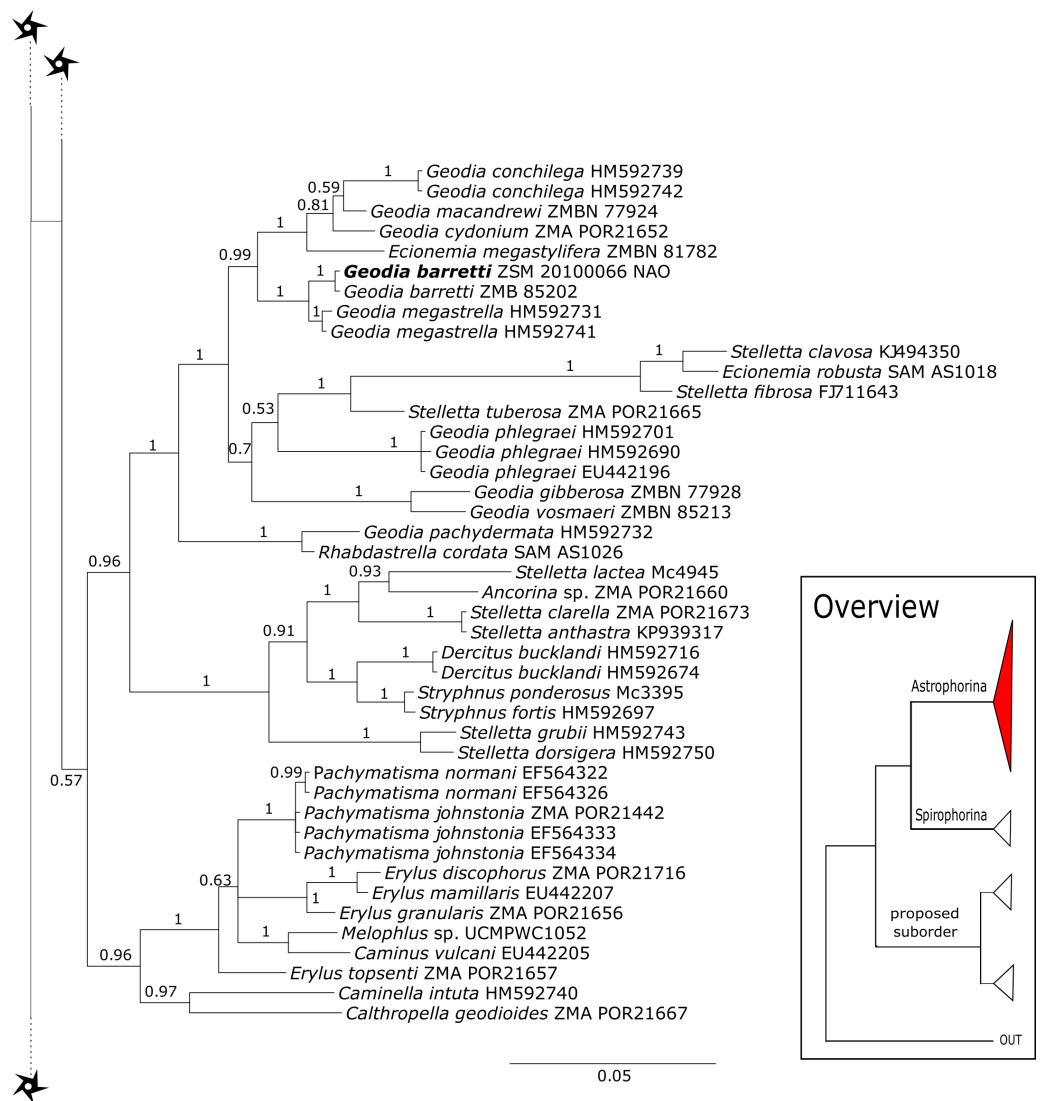


Figure 12 *Cox1* phylogeny continued. See caption in Fig. 9.

Full-size DOI: 10.7717/peerj.10775/fig-12

Molecular phylodiversity of TWA desma-bearing demosponges

In the present study the inclusive phylodiversity was calculated for Bonaire, Curaçao, Florida, Honduras, Jamaica, Puerto Rico, Turks and Caicos and the Bahamas (Fig. 17). The PD_I analyses disclosed a high variation within the TWA locations (Fig. 17). At comparable sampling efforts, the highest PD_I was observed in Jamaica (Fig. 17A) indicating a high biodiversity in this region, closely followed by the Bahamas (Fig. 17B). At sample size of 20, Curaçao and Florida showed the lowest PD_I , followed by Turks and Caicos and Honduras. The high PD_I of 'lithistid' demosponges calculated for the Bahamas is in agreement with the findings of Reed & Pomponi (1997), and may be explained by the high habitat diversity observed in this region (Reed & Pomponi, 1997) and their close proximity to the species-rich Atlantic (see e.g., Carvalho, Pomponi & Xavier, 2015). Even though Turks and Caicos are

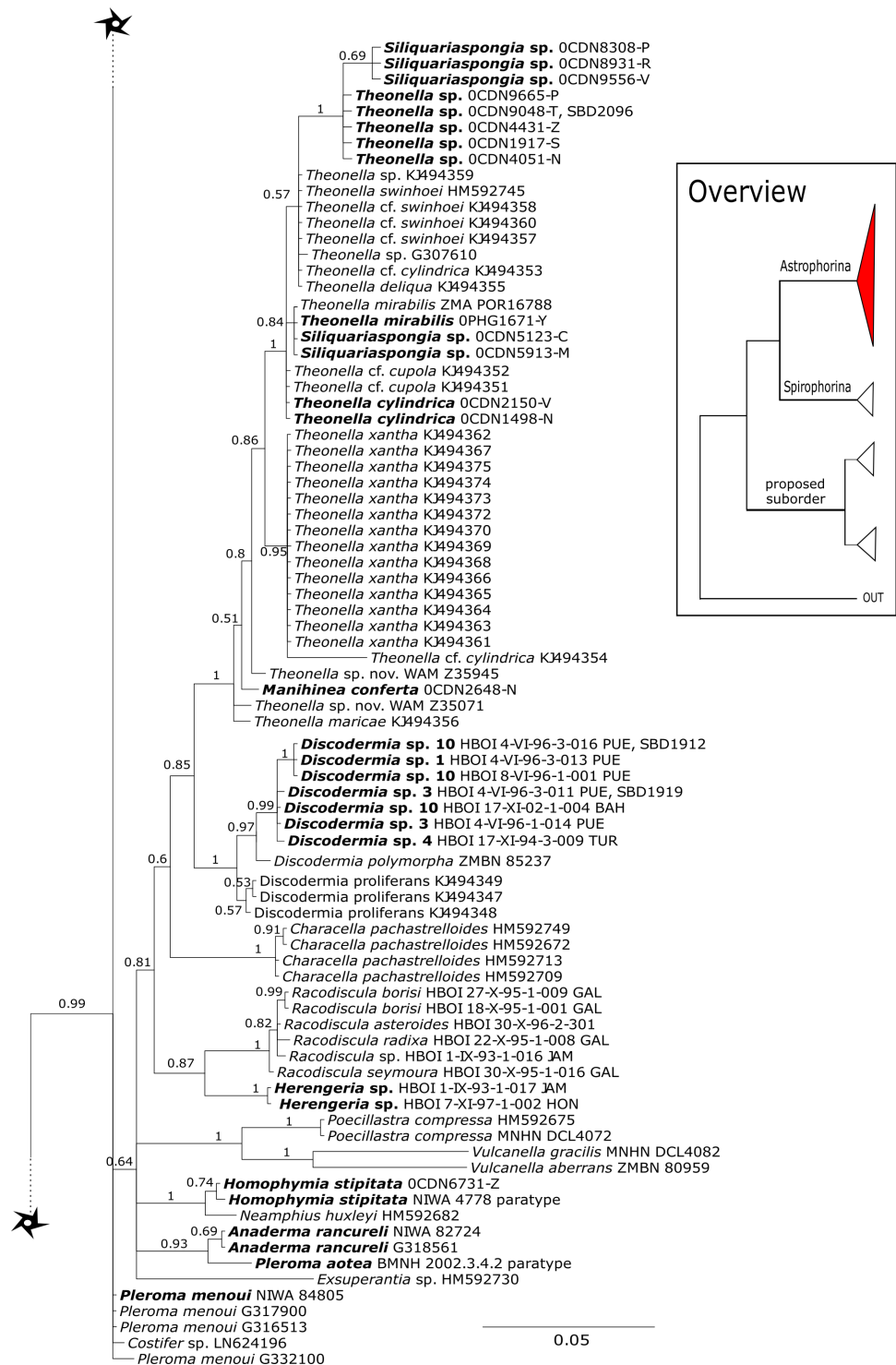


Figure 13 *Cox1* phylogeny continued. See caption in Fig. 9.

Full-size DOI: 10.7717/peerj.10775/fig-13

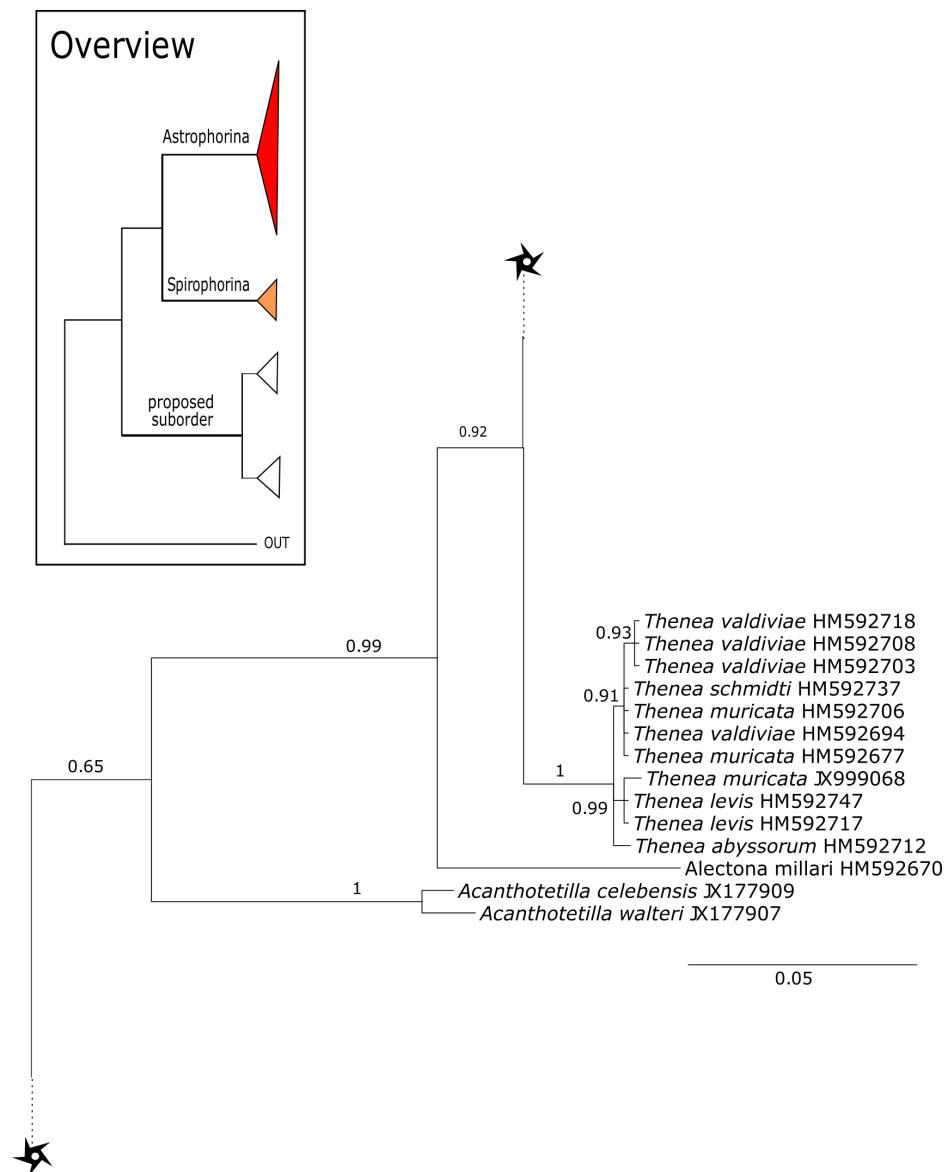


Figure 14 *Cox1* phylogeny continued. See caption in Fig. 9.

Full-size  DOI: [10.7717/peerj.10775/fig-14](https://doi.org/10.7717/peerj.10775/fig-14)

close to the Bahamas and the Atlantic, a much lower PD_1 was calculated, maybe due to less habitat diversity.

Bathymetric distribution and relative abundance of TWA desma-bearing families

The evaluation of the relative abundance of eight 'lithistid' families within each depth zone is based upon 234 specimens collected from eight localities in the TWA (Fig. 18). Theonellidae and Corallistidae are the two dominant families in the present dataset, and assumed to be the dominant families in the TWA (Pomponi et al., 2001). While Theonellidae dominate depth zones of 0–151 m, Corallistidae are more abundant in depth zones of 151–600 m. This

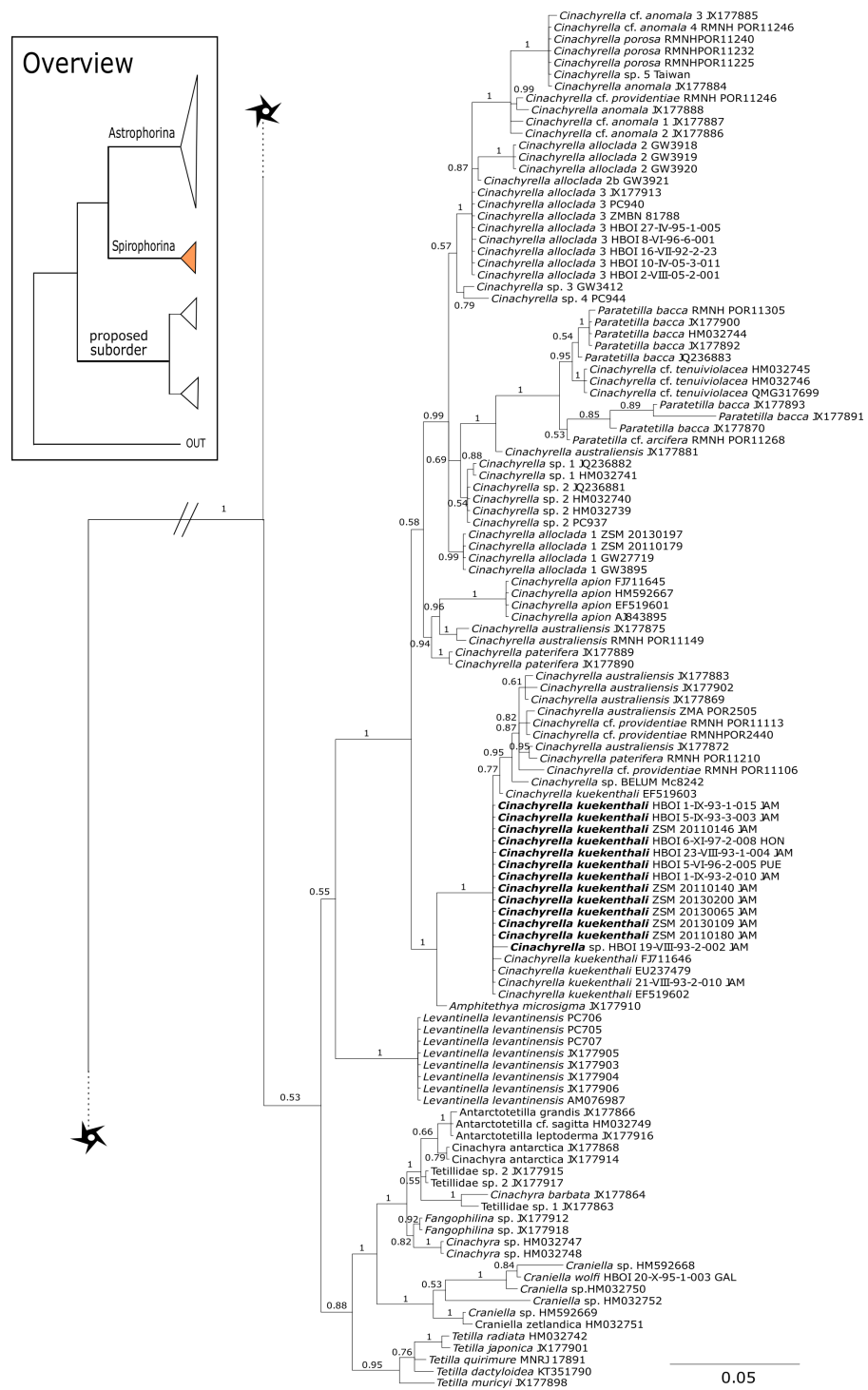


Figure 15 *Cox1* phylogeny continued. See caption in Fig. 9.

Full-size DOI: 10.7717/peerj.10775/fig-15

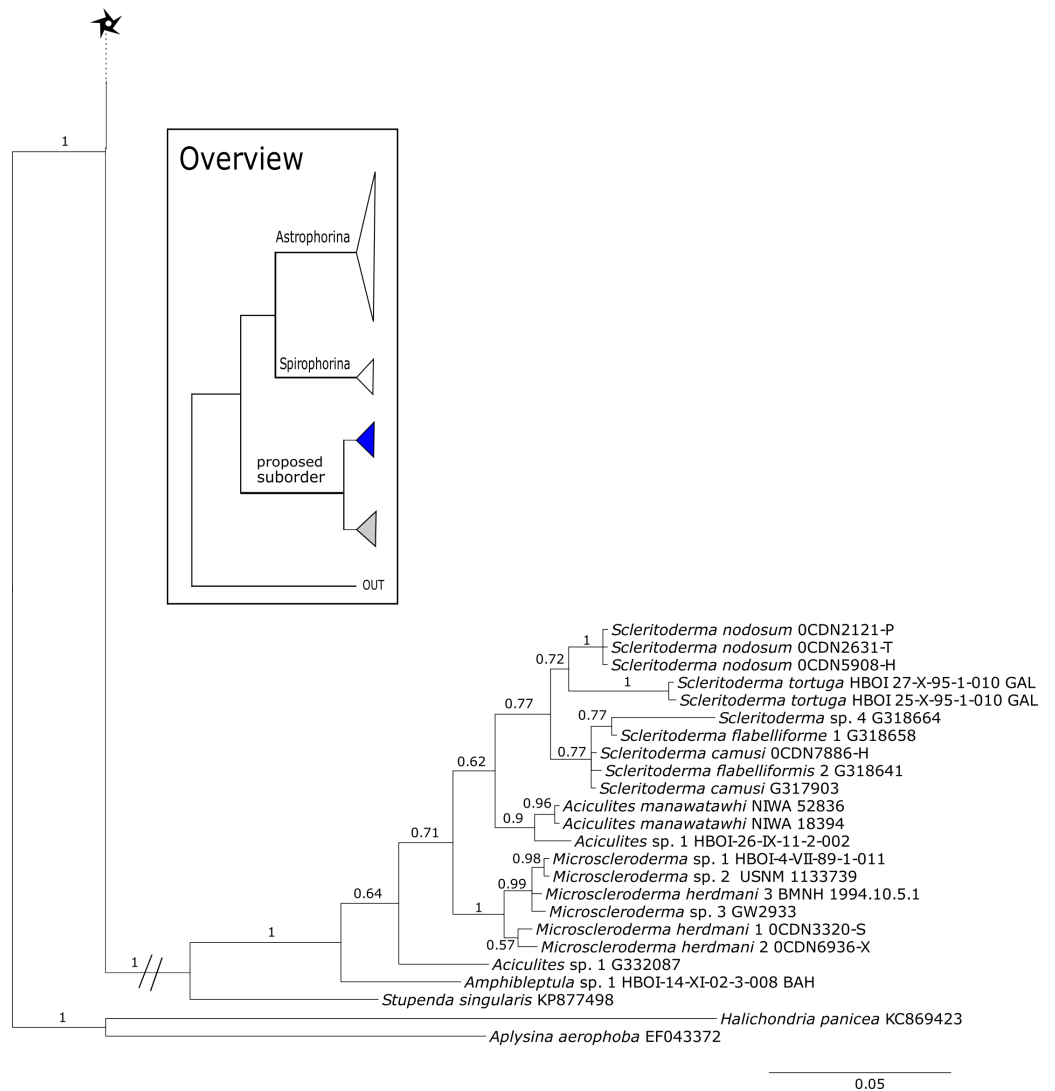


Figure 16 *CoxI* phylogeny continued. See caption in Fig. 9.

Full-size DOI: [10.7717/peerj.10775/fig-16](https://doi.org/10.7717/peerj.10775/fig-16)

corroborates the result of *Pomponi et al. (2001)* showing that *Discodermia* (Theonellidae) is the dominant genus from 0–151 m, while *Corallistes* (Corallistidae) dominates the zone of 151–914 m. An explanation for this might be that Corallistidae have a dense rigid skeleton of dicranoclone desmas, while Theonellidae possess a less articulated skeleton of tetraclone desmas. Thus, it can be hypothesized, that ‘lithistids’ with a hyper-silicified dense desma skeleton like Corallistidae occur in deeper zones ≥ 300 m, while those with a less dense desma skeleton like most of the *Theonella* species (Theonellidae) moved into more shallow water habitats, where less silica is available to build their skeleton (e.g., *Tréguer et al., 1995*). This trend was also observed in the South West Pacific (*Kelly et al., 2007; Hall, Ekins & Hooper, 2014*). As Corallistidae and Theonellidae are considered to be polymorphic (*Pisera*

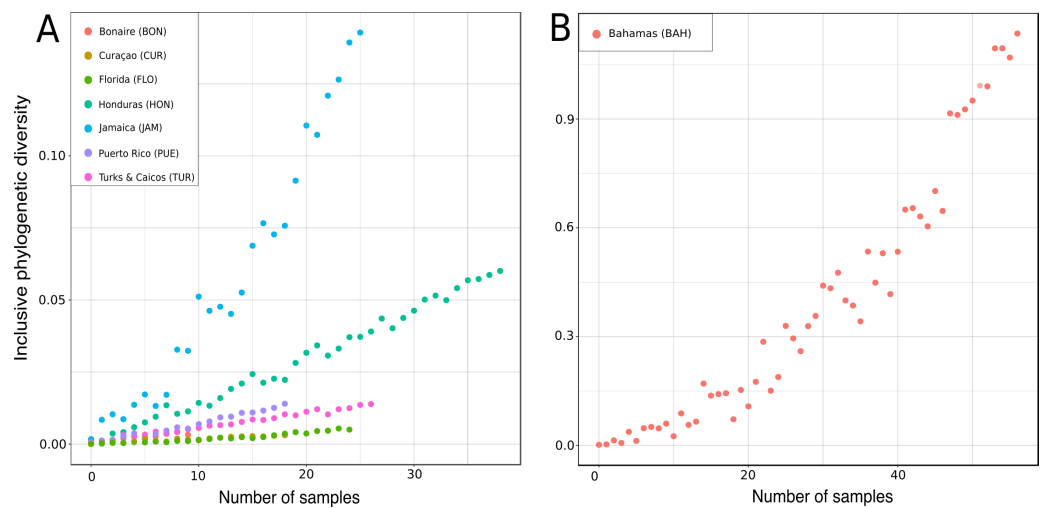


Figure 17 Rarefied inclusive phylogenetic diversity (PD_i) curves per marine regions analyzed. For a better visualization the PD_i for the Bahamas are illustrated separately (B) due to their larger number of samples.

Full-size DOI: [10.7717/peerj.10775/fig-17](https://doi.org/10.7717/peerj.10775/fig-17)

& Lévi, 2002a, Pisera & Lévi, 2002d), it is difficult, to draw any conclusion of different depth zones or habitats influencing growth form patterns in these two families.

However, further ‘lithistid’ families with a similar bathymetric trend are observed and growth forms are suggested to play a role in the bathymetric distributions of ‘lithistids’. For instance *Leiodermatium* spp. (Azoricidae) are abundant (27 specimens) in depth zones 301–1,000 m. Similar to Corallistidae *Leiodermatium* possess a dense heavily articulated skeleton, but of strongly spinose rhizoclone desmas. The growth form of *Leiodermatium* species are described as being foliated or vase to ear-shaped (Pisera & Lévi, 2002b). Such growth forms are suggested to improve the water circulation in sponges, in particular of those in the deep-sea habitats, and to be more resistant to higher water viscosity and scarcity of particles (Levinton, 1982; Gage & Tyler, 1991). Many vase to cup or ear-shaped sponges have their inhalant pores facing the outer side and exhalant openings on the upper side separating incoming and processed water (Sarà & Vacelet, 1973; Pronzato, Bavestrello & Cerrano, 1998), which may reduce any negative effect on filtering due to a sedimentation. This is in contrast to Siphonidiidae, a family represented in this analysis by the genera *Gastrophanella* and *Siphonidium*, which are rather encrusting or irregular cylindrical, thus more abundant in the depth zone of 61–150 m.

Scleritodermidae occurred more often on vertical walls in depth 301–600 m, but was also not observed to be a major component of the ‘lithistid’ fauna in the TWA. The greatest number of desma-bearing demosponges were found in depth zone 301–600 m (87 specimens), with Corallistidae as the dominant family (34 specimens) followed by Azoricidae with 27 specimens. Diverse habitats from fine mud and sand slopes to rock pinnacles, boulders and vertical walls in this depth zone (Fig. 18) could be a possible explanation. The families Neopeltidae and Macandrewiidae are rare in our study with only one species discovered at 909 m depth on a vertical wall in the Bahamas (*Macandrewia* sp.),

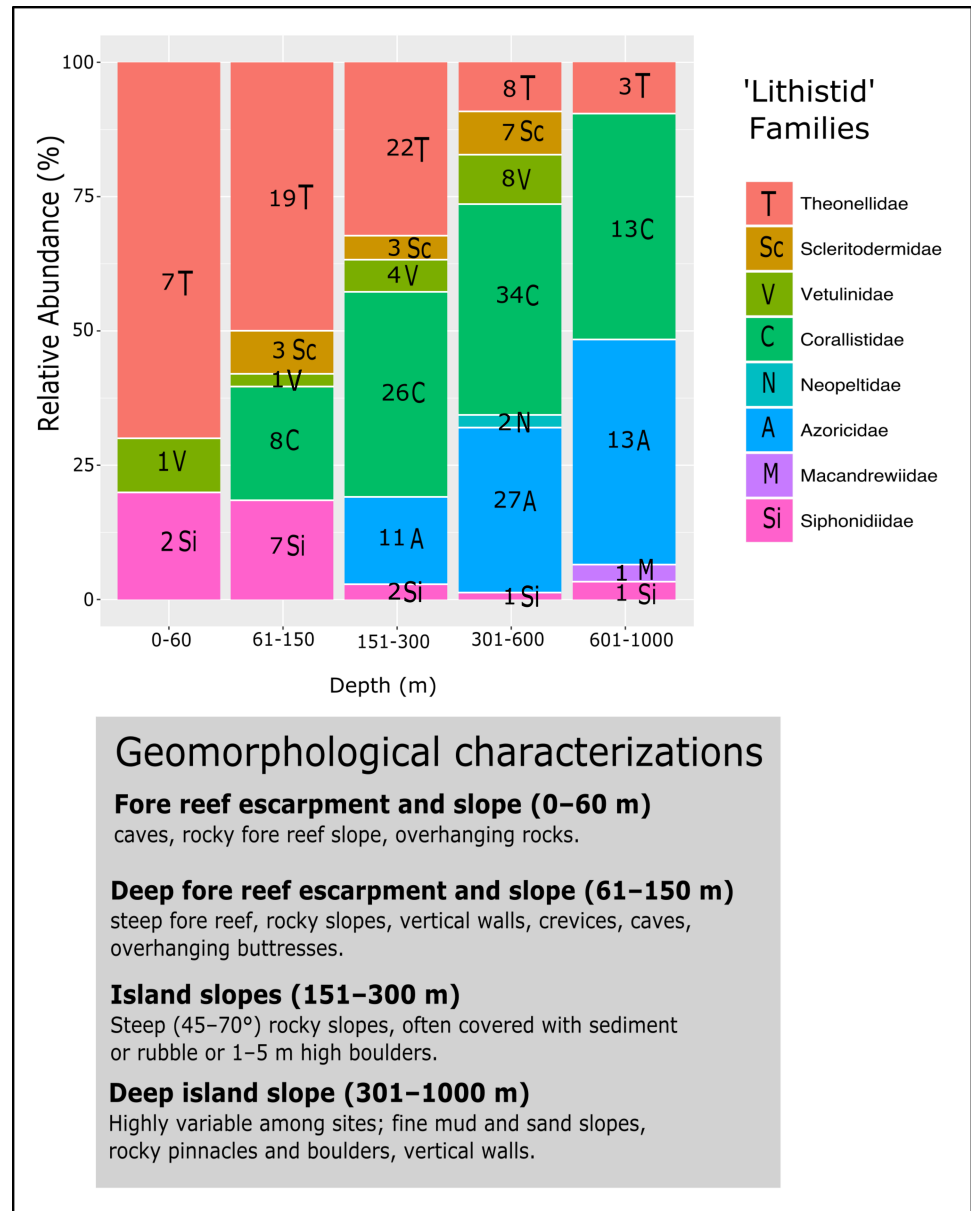


Figure 18 Bathymetric distribution and relative abundance (%) of TWA desma-bearing demosponges based on 234 samples of eight families. Numbers in each bar represent the number of samples investigated. The following genera for each family were included: *Leiodermatium* (Azoricidae); *Corallistes*, *Herenigeria*, *Neophrissospongia* and *Awhiowhio* (Corallistidae); *Macandrewia* (Macandrewiidae); *Daedalopelta* and *Neopelta* (Neopeltidae); *Aciculites*, *Amphibleptula*, *Microscleroderma*, *Scleroderma* and *Setidium* (Sclerodermidae); *Gastrophanella* and *Siphonidium* (Siphonidiidae); *Discoderma*, *Racodiscula* and *Theonella* (Theonellidae); *Vetulina* (Vetulinidae). Geomorphological characterizations of depth zones are given below the graph and follows [Pomponi et al. \(2001\)](#) and [Reed & Pomponi \(1997\)](#).

Full-size DOI: [10.7717/peerj.10775/fig-18](https://doi.org/10.7717/peerj.10775/fig-18)

and two *Daedalopelta* spp. collected from the Bahamas at 301–600 m. This corroborates the findings of *Pomponi et al. (2001)*, because they found one species of *Daedalopelta nodosa* *Schmidt, 1879* at 452 m in the Bahamas, one *Neopelta perfecta* in 116 m depth from Grenada and one *Macandrewia clavatella* in the southwest coast of Florida. These families and species are also found to be rare in the Southwest Pacific (*Lévi, 1991; Kelly, 2000*). Besides the tetractinellid ‘lithistid’ sponges, we noted that other desma-bearing sponge lineages, such as family Vetulinidae (Order Sphaerocladina) constitute only a minor component in any depth-zone in the TWA.

Further testing is required to assess whether geomorphological conditions resulting of a variety of complex tectonic interactions (e.g., strike-slip faults, thrust fault, subduction and seafloor spreading in Cayman Trough, see *Fig. 1*), directly affect diversity and bathymetric distribution of ‘lithistids’ in the TWA (*Fig. 18*).

CONCLUSION

In summary, this is the first integrative approach using molecular and morphological data on TWA ‘lithistid’ demosponges, thus contributing to a better understanding of their phylogenetic affinities, diversity and bathymetric distribution patterns. The present study points to specimens/groups in need of deeper taxonomic investigations and revision, however, additional morphological as well as other independent markers are needed. With recent evidence (*Pomponi et al., 2001*) that ‘lithistids’ are dominant components among all investigated TWA regions, we suggest a comparable diversity to the Pacific ‘lithistids’ as well as to the Mesozoic fauna. Furthermore, there is a clear shift of lithistids with a rigid and heavily articulated desma towards deeper habitats (Corallistidae and Azoricidae), whereas ‘lithistids’ with a less articulated skeleton tend to occur in more shallow habitats (Theonellidae and Siphoniidae). A major effect causing this shift is the availability of silica in the ocean throughout time. Our robust phylogeny enables relaxed molecular clock analyses in conjunction with the rich fossil record of lithistids to better correlate such shifts to geological/geochemical events in the past.

ACKNOWLEDGEMENTS

Thirty-two new specimens and associated data were supplied by the NIWA Invertebrate Collection (NIC) for sequencing; we are particularly grateful to Sadie Mills for her diligent assistance with loans. A further 31 specimens and associated images and data were supplied by Lori J. Bell and the Coral Reef Research Foundation (CRRF) for sequencing; these were collected under contract to the US National Cancer Institute. We thank colleagues Amy Wright, John Reed and Megan Conkling (HBOI-FAU) for their assistance with the HBOI collection of samples and associated data, likewise John Hooper for the Queensland Museum samples (QMG), Cécile Debitus and IRD for Marquesas and Tahiti samples, Jane Fromont and Oliver Gomez for the samples from the Western Australian Museum (WA), and Bernhard Ruthensteiner for access to the collection of the Zoologische Staatssammlung München (ZSM). Patrick Colin, Lori J. Bell, Renata Manconi and one anonymous reviewer are thanked for comments that improved the MS considerably. AS thanks Sergio Vargas for

his help with the modification of the phylodiversity script. Nicole Enghuber is thanked for her help in spicule preparations and Simone Schätzle is thanked for sequencing assistance (all at the Dept. of Earth and Environmental Sciences, LMU München, Munich, Germany).

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

Financial support for this study was provided by the German Science Foundation to Dirk Erpenbeck and Gert Wörheide (DFG ER 611/3-1, DFG Wo869/15-1, respectively). The LMU Mentoring and the HELGE AX:Son JOHNSON STIFTELSE provided funding for Astrid Schuster to visit HBOI (Florida, USA) and NIWA (National Institute of Water and Atmospheric Research, Auckland and Wellington in New Zealand). AS was further funded by Villum Fonden grant no. 16518. Financial support for the R/V Seward Johnson/Johnson Sea Link I+II expedition was provided by HBOI. Michelle Kelly's participation was funded by NIWA under Coasts and Oceans Research Programme 2 Marine Biological Resources: Discovery and definition of the marine biota of New Zealand (2016/2017 and 2017/2018 SCI). Paco Cárdenas and Shirley A. Pomponi received support from the European Union's Horizon 2020 research and innovation program through the SponGES project (Grant agreement No. 679849). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:

The German Science Foundation: DFG ER 611/3-1, DFG Wo869/15-1.

Astrid Schuster to visit HBOI (Florida, USA).

NIWA (National Institute of Water and Atmospheric Research, Auckland and Wellington in New Zealand).

HBOI.

NIWA under Coasts and Oceans Research Programme 2 Marine Biological Resources.

The marine biota of New Zealand: 2016/2017, 2017/2018 SCI.

The European Union's Horizon 2020 research and innovation program through the SponGES project: 679849.

Competing Interests

The authors declare there are no competing interests. This document reflects only the authors' view and the Executive Agency for Small and Medium-sized Enterprises (EASME) is not responsible for any use that may be made of the information it contains.

Author Contributions

- Astrid Schuster conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, identified genera/species, and approved the final draft.

- Shirley A. Pomponi and Michelle Kelly analyzed the data, authored or reviewed drafts of the paper, identified genera/species and contributed to the sampling, and approved the final draft.
- Andrzej Pisera and Paco Cárdenas analyzed the data, authored or reviewed drafts of the paper, identified genera/species, and approved the final draft.
- Gert Wörheide analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Dirk Erpenbeck conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

Permission was granted to Harbor Branch Oceanographic Institute by: the United States National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Dry Tortugas National Park (letters of acknowledgement F/SER25:KM, F/SER23:PE, SER02-130) and the Florida Keys National Marine Sanctuary (permit numbers 2001-049, 2001-043); the Government of the Bahamas, Department of Fisheries; the Government of Honduras, Department of Fisheries (DIGEPESCA-638/97); the Government of Portugal, Parque Natural de Madeira; the Government of Jamaica, Jamaican Ministry of Foreign Affairs (diplomatic note no. 7/703/315); the Government of Bonaire, Bonaire Marine Park, and Netherlands Ministry of Foreign Affairs (note no. VADV-172/00); the Government of Panama (American Embassy Panama telegram no. 5570); the Puerto Rico Department of Natural And Environmental Resources; the Government of St. Vincent; the Government of France; and the Government of Turks and Caicos (American Embassy London telegram no. 12514).

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

Novel sequences are archived at the European Nucleotide Archive: [LR655885–LR656163](#) (28S) and [LR698845–LR698952](#) (CO1).

Data Availability

The following information was supplied regarding data availability:

Data is available at the Sponge Barcoding Database: [SBD#1794–SBD#2108](#).

Data is available at GenBank: [LR655885–LR656163](#) and [LR698845–LR698952](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.10775#supplemental-information>.

REFERENCES

- Bergquist PR. 1978.** *Sponges*. Hutchinson University Library. Berkeley and Los Angeles: University of California Press 1–268.

- Carballo JL, Bautista-Guerrero E, Cárdenas P, Cruz-Barraza JA, Aguilar-Camacho JM. 2018.** Molecular and morphological data from Thoosidae in favour of the creation of a new suborder of Tetractinellida. *Systematics and Biodiversity* **2018**:1–10.
- Cárdenas P, Pérez T, Boury-Esnault N. 2012.** Sponge systematics facing new challenges. In: Becerro MA, Uriz MJ, Maldonado M, Turon X, eds. *Advances in Sponge Science: Phylogeny, Systematics, Ecology, Advances in Marine Biology*. 61. 79–209 DOI [10.1016/B978-0-12-387787-1.00010-6](https://doi.org/10.1016/B978-0-12-387787-1.00010-6).
- Cárdenas P, Rapp HT, Schander C, Tendal OS. 2010.** Molecular taxonomy and phylogeny of the Geodiidae (Porifera, Demospongiae, Astrophorida) –combining phylogenetic and Linnaean classification. *Zoologica Scripta* **39**:89–106.
- Cárdenas P, Vacelet J, Chevaldonné P, Pérez T, Xavier JR. 2018.** From marine caves to the deep sea, a new look at *Caminella* (Demospongiae, Geodiidae) in the Atlanto-Mediterranean region. *Zootaxa* **4466**:174–196 DOI [10.11646/zootaxa.4466.1.14](https://doi.org/10.11646/zootaxa.4466.1.14).
- Cárdenas P, Xavier JR, Reveillaud J, Schander C, Rapp HT. 2011.** Molecular phylogeny of the Astrophorida (Porifera, Demospongiae) reveals an unexpected high level of spicule homoplasy. *PLOS ONE* **6**:e18318 DOI [10.1371/journal.pone.0018318](https://doi.org/10.1371/journal.pone.0018318).
- Carella M, Agell G, Cárdenas P, Uriz M. 2016.** Phylogenetic reassessment of Antarctic Tetillidae (Demospongiae, Tetractinellida) reveals new genera and genetic similarity among morphologically distinct species. *PLOS ONE* **11**:e0160718 DOI [10.1371/journal.pone.0160718](https://doi.org/10.1371/journal.pone.0160718).
- Carvalho FC, Pomponi SA, Xavier JR. 2015.** Lithistid sponges of the upper bathyal of Madeira, Selvagens and Canary Islands, with description of a new species of *Isabella*. *Journal of the Marine Biological Association of the United Kingdom* **95**:1287–1296 DOI [10.1017/S0025315414001179](https://doi.org/10.1017/S0025315414001179).
- Chombard C, Boury-Esnault N, Tillier S. 1998.** Reassessment of homology of morphological characters in tetractinellid sponges based on molecular data. *Systematic Biology* **47**:351–366 DOI [10.1080/106351598260761](https://doi.org/10.1080/106351598260761).
- Darriba D, Taboada GL, Doallo R, Posada D. 2012.** jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**:772–772.
- de Laubenfels MW. 1954.** The sponges of the West-Central Pacific. Oregon State Monographs. *Studies in Zoology* **7**:248–249.
- Desqueyroux-Faúndez R, Van Soest RWM. 1997.** Shallow waters Demosponges of the Galápagos Islands. *Revue Suisse de Zoologie* **104**:379–467 DOI [10.5962/bhl.part.80003](https://doi.org/10.5962/bhl.part.80003).
- Du Bocage JVB. 1869.** Eponges siliceuses nouvelles du Portugal et de l'île Saint-Iago (Archipel de Cap-Vert). *Jornal de Ciencias Mathematicas, Physicas e Naturaes* **2**:159–162.
- Erpenbeck D, Sutcliffe P, Cook SDC, Dietzel A, Maldonado M, Van Soest RWM, Hooper JNA, Wörheide G. 2012.** Horny sponges and their affairs: On the phylogenetic relationships of Keratose sponges. *Molecular Phylogenetic and Evolution* **63**:809–816 DOI [10.1016/j.ympev.2012.02.024](https://doi.org/10.1016/j.ympev.2012.02.024).
- Gage JD, Tyler PA. 1991.** *Deep-sea biology: a natural history of organisms at the deep-sea floor*. Cambridge: Cambridge University Press.

- Gray JE. 1868.** Note on *Theonella* a new genus of coralloid sponges from Formosa. *Proceedings of the Zoological Society of London* **37**:565–566.
- Haar ET, Kowalski RJ, Hamel E, Lin CM, Longley RE, Gunasekera SP, Rosenkranz HS, Day BW. 1996.** Discodermolide, a cytotoxic marine agent that stabilizes microtubules more potently than taxol. *Biochemistry* **35**:243–250 DOI [10.1021/bi9515127](https://doi.org/10.1021/bi9515127).
- Hall KA, Ekins MG, Hooper J. 2014.** Two new desma-less species of *Theonella* Gray, 1868 (Demospongiae: Astrophorida: Theonellidae), from the Great Barrier Reef, Australia, and a re-evaluation of one species assigned previously to *Dercitus* Gray, 1867. *Zootaxa* **3814**:451–477 DOI [10.11646/zootaxa.3814.4.1](https://doi.org/10.11646/zootaxa.3814.4.1).
- Hallmann EF. 1912.** Report on the Sponges obtained by the F.I.S. ‘Endeavour’ on the Coasts of New South Wales, Victoria, South Australia, Queensland, and Tasmania, 1909–10. Part I. *Zoological Results of the Fishing Experiments carried out by F.I.S. ‘Endeavour’* 11909–14.
- Hooper JNA, Van Soest RWM. 2002.** *Systema porifera. A guide to the classification of sponges*. New York: Kluwer Academic/Plenum Publishers.
- Hoshino P. 1981.** Shallow-water demosponges of Western Japan 2. *Journal of Science of the Hiroshima University (B)* **29**:207–289.
- Katoh K, Standley DM. 2013.** MAFFT Multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**:772–780 DOI [10.1093/molbev/mst010](https://doi.org/10.1093/molbev/mst010).
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012.** Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**:1647–1649 DOI [10.1093/bioinformatics/bts199](https://doi.org/10.1093/bioinformatics/bts199).
- Kelly M. 2000.** Description of a new lithistid sponge from northeastern New Zealand, and consideration of the phylogenetic affinities of families Corallistidae and Neopeltidae. *Zoosystema* **22**:265–284.
- Kelly M. 2007.** The Marine Fauna of New Zealand: Porifera: Lithistid Demospongiae (rock Sponges). *NIWA Biodiversity Memoir* **121**:1–100.
- Kelly M, Cárdenas P. 2016.** An unprecedented new genus and family of Tetractinellida (Porifera, Demospongiae) from New Zealand’s Colville Ridge, with a new type of mitochondrial group I intron. *Zoological Journal of the Linnean Society* **177**:335–352 DOI [10.1111/zoj.12365](https://doi.org/10.1111/zoj.12365).
- Kelly M, Edwards AR, Wilkinson MR, Alvarez B, de Cooks C, Bergquist PR, Buckeridge S, Campbell HJ, Reiswig HM, Valentine C, Vacelet J. 2009.** Phylum Porifera: sponges. In: Gordon DP ed. *New Zealand inventory of biodiversity: 1 Kingdom Animalia: Radiata, Lophotrochozoa, Deuterostomia. New Zealand Inventory of Biodiversity* **1**:23–46.
- Kelly M, Ellwood M, Tubbs L, Buckeridge J. 2007.** The lithistid Demospongiae in New Zealand waters: species composition and distribution. In: Custódio MR, Lôbo-Hajdu G, Hajdu E, Murcy G, eds. *Porifera research: biodiversity, innovation and sustainability. Série Livros* **28**. Rio de Janeiro: Museu Nacional, 393–404.

- Kelly M, Lee D, Kelly S, Buckeridge JS. 2003.** A recent sponge, *Pleroma aotea* Kelly (Order Lithistida: Family Pleromidae), in the late Eocene Ototara Limestone of Otago, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 37:129–148 DOI [10.1080/00288330.2003.9517152](https://doi.org/10.1080/00288330.2003.9517152).
- Kelly Borges M, Pomponi SA. 1994.** Phylogeny and classification of lithistid sponges (Porifera: Demospongiae): a preliminary assessment using ribosomal DNA sequence comparisons. *Molecular Marine Biology and Biotechnology* 3:87–103.
- Kieschnick O. 1896.** Silicispongiae von Ternate nach den Sammlungen von Herrn Prof. Dr. W. Kükenthal. *Zoologischer Anzeiger* 19:526–534.
- Kirkpatrick R. 1903.** Descriptions of South African Sponges. *Marine Investigations in South Africa* 2:171–180.
- Lê HLV, Lecointre G, Perasso R. 1993.** A 28S rRNA-based phylogeny of the gnathostomes: first steps in the analysis of conflict and congruence with morphologically based cladograms. *Molecular Phylogenetics and Evolution* 2:31–51 DOI [10.1006/mpev.1993.1005](https://doi.org/10.1006/mpev.1993.1005).
- Lévi C. 1960.** Spongiaires des côtes occidentales africaines. *Bulletin de l'Institut français d'Afrique noire (A. Sciences naturelles)* 22:743–769.
- Lévi C. 1991.** Lithistid sponges from the Norfolk Rise, recent and Mesozoic genera. In: Reitner J, Keupp H, eds. *Fossil and recent sponges*. Berlin, Heidelberg: Springer-Verlag, 72–82.
- Lévi C, Lévi P. 1988.** Nouveaux Spongiaires Lithistidés bathyaux à affinités crétacées de la Nouvelle-Calédonie. *Bulletin du Muséum national d'Histoire naturelle* 10:241–263.
- Lévi C, Lévi P. 1989.** Spongiaires (MUSORSTOM 1 and 2). *Mémoires du Muséum National d'Histoire naturelle* 4:25–103.
- Levinton JS. 1982.** *Marine Ecology*. New Jersey: Prentice-Hall.
- Lewis LA, Lewis PO. 2005.** Unearthing the molecular phylodiversity of desert soil green algae (Chlorophyta). *Systematic Biology* 54:936–947 DOI [10.1080/10635150500354852](https://doi.org/10.1080/10635150500354852).
- Maldonado M, Aguilar R, Blanco J, García S, Serrano A, Punzón A. 2015.** Aggregated clumps of lithistid sponges: A singular, reef-like bathyal habitat with relevant paleontological connections. *PLOS ONE* 10:e0125378 DOI [10.1371/journal.pone.0125378](https://doi.org/10.1371/journal.pone.0125378).
- Maldonado M, Young CM. 1996.** Bathymetric patterns of sponge distribution on the Bahamian slope. *Deep Sea Research Part I: Oceanographic Research Papers* 43:897–915 DOI [10.1016/0967-0637\(96\)00042-8](https://doi.org/10.1016/0967-0637(96)00042-8).
- Marshall W. 1876.** Ideen über die Verwandtschaftsverhältnisse der Hexactinelliden. *Zeitschrift für wissenschaftliche Zoologie* 21:113–136.
- McInerney JO, Adams CI, Kelly M. 1999.** Phylogenetic resolution potential of 18S and 28S rRNA genes within the lithistid Astrophorida. *Memoirs of the Queensland Museum* 44:343–351.
- Meyer CP, Geller JB, Paulay G. 2005.** Fine scale endemism on coral reefs: Archipelagic differentiation in turbinid gastropods. *Evolution* 59:113–125 DOI [10.1111/j.0014-3820.2005.tb00899.x](https://doi.org/10.1111/j.0014-3820.2005.tb00899.x).
- Moret L. 1926.** Contribution à l'étude des spongiaires siliceux du Crétacé supérieur français. *Mémoires de la Société Géologique de France* 5:1–327.

- Morrow C, Cárdenas P. 2015.** Proposal for a revised classification of the Demospongiae (Porifera). *Frontiers in Zoology* **12**:1–27 DOI [10.1186/s12983-014-0093-6](https://doi.org/10.1186/s12983-014-0093-6).
- Muricy G, Diaz MC. 2002.** Order Homosclerophorida Dendy, 1905. Family Plakinidae Schulze, 1880. In: Hooper JNA, Van Soest RWM, eds. *Systema porifera. A guide to the classification of sponges*. New York: Kluwer Academic/Plenum Publishers, 71–82.
- Nardo GD. 1833.** Auszug aus einem neuen System der Spongiarien, wonach bereits die Aufstellung in der Universitäts-Sammlung zu Padua gemacht ist De Spongiis, *Isis, oder*. In: *Encyclopädische Zeitung Coll. Oken, Jena*. 519–523.
- Pallas PS. 1766.** *Elenchus zoophytorum sistens generum adumbrationes generaliores et specierum cognitarum succintas descriptiones, cum slectis auctorum synonymis*. Hagae: Fransiscum Varrentrapp, 451.
- Pisera A. 1999.** Lithistid sponge *Setidium obtectum* Schmidt, 1879, rediscovered. In: Hooper JNA, ed. *Memoirs of the Queensland Museum, Proceedings of the 5th International sponge symposium, Brisbane, June-1998*. 473–478.
- Pisera A. 2002.** Fossil “Lithistids”: An Overview. In: Hooper JNA, Van Soest RWM, eds. *Systema Porifera. A guide to the classification of sponges*. New York: Kluwer Academic/Plenum Publishers, 388–402.
- Pisera A. 2006.** Palaeontology of sponges —a review. *Canadian Journal of Zoology* **84**:242–261 DOI [10.1139/z05-169](https://doi.org/10.1139/z05-169).
- Pisera A, Lévi C. 2002a.** Family Theonellidae Von Lendenfeld, 1903. In: Hooper JNA, Van Soest RWM, eds. *Systema Porifera. A guide to the classification of sponges*. New York: Kluwer Academic/ Plenum Publishers, 327–337.
- Pisera A, Lévi C. 2002b.** Family Azoricidae (*Sollas, 1888*). In: Hooper JNA, Van Soest RWM, eds. *Systema Porifera. A guide to the classification of sponges*. New York: Kluwer Academic/ Plenum Publishers, 352–355.
- Pisera A, Lévi C. 2002c.** Family Scleritodermidae (*Sollas, 1888*). In: Hooper JNA, Van Soest RWM, eds. *Systema Porifera. A guide to the classification of sponges*. Kluwer Academic/ Plenum Publishers, New York, Boston, Dordrecht, London, Moscow 302–311.
- Pisera A, Lévi C. 2002d.** Family Corallistidae (*Sollas, 1888*). In: Hooper JNA, Van Soest RWM, eds. *Systema Porifera. A guide to the classification of sponges*. New York, Boston, Dordrecht, London, Moscow: Kluwer Academic/ Plenum Publishers, 312–320.
- Pisera A, Lévi C. 2002e.** Family Phymatellidae (*Schrammen, 1910*). In: Hooper JNA, Van Soest RWM, eds. *Systema Porifera. A guide to the classification of sponges*. New York, Boston, Dordrecht, London, Moscow: Kluwer Academic/ Plenum Publishers, 312–320.
- Pisera A, Pomponi SA. 2015.** New data on lithistid sponges from the deep Florida shelf with description of a new species of *Theonella*. *Journal of the Marine Biological Association of the United Kingdom* **95**:1297–1309 DOI [10.1017/S0025315414001477](https://doi.org/10.1017/S0025315414001477).
- Plaza A, Gustchina E, Baker HL, Kelly M, Bewley CA. 2007.** Mirabamides A–D, Depsipeptides from the sponge *Siliquariaspongia mirabilis* that inhibit HIV-1 fusion. *Journal of Natural Products* **70**:1753–1760 DOI [10.1021/np070306k](https://doi.org/10.1021/np070306k).

- Pomponi SA, Kelly M, Reed JA, Wright EA. 2001.** Diversity and bathymetric distribution of lithistid sponges in the tropical western Atlantic region. *Bulletin of the Biological Society of Washington* **10**:344–353.
- Pronzato R, Bavestrello G, Cerrano C. 1998.** Morpho-functional adaptations of species of *Spongia* (Porifera, Demospongiae) from a Mediterranean vertical cliff. *Bulletin of Marine Science* **63**:317–328.
- Pulitzer-Finali G. 1993.** A collection of marine sponges from East Africa. *Annali del Museo Civico di Storia Naturale Giacomo Doria* **89**:247–350.
- Redmond NE, Morrow CC, Thacker RW, Diaz MC, Boury-Esnault N, Cárdenas P, Hajdu E, Lobo-Hajdu G, Picton BE, Pomponi SA, Kayal E, Collins AG. 2013.** Phylogeny and systematics of Demospongiae in light of new small-subunit ribosomal DNA (18S) Sequences. *Integrative and Comparative Biology* **53**:388–415 DOI [10.1093/icb/ict078](https://doi.org/10.1093/icb/ict078).
- Reed JK, Pomponi SA. 1997.** Biodiversity and distribution of deep and shallow water sponges in the Bahamas. *Proceedings of the 8th International Coral Reef Symposium* **2**:1387–1392.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012.** MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space. *Systematic Biology* **61**:539–542 DOI [10.1093/sysbio/sys029](https://doi.org/10.1093/sysbio/sys029).
- R Studio Team. 2014.** RStudio: Integrated Development for R, Inc. Boston, MA. Available at <http://www.rstudio.com/>.
- Ryan W, Carbotte SM, Coplan JO, O’Hara S, Melkonian A, Arko R, Weissel RA, Ferrini V, Goodwillie A, Nitsche F, Bonczkowski J, Zemsky R. 2009.** Global multi-resolution topography synthesis. *Geochemistry, Geophysics, Geosystems* **10**:1–9.
- Sanders HL. 1968.** Marine benthic diversity: a comparative study. *The American Naturalist* **102**:243–282 DOI [10.1086/282541](https://doi.org/10.1086/282541).
- Sará M, Vacelet J. 1973.** Écologie des démosponges. In: Grassé P-P, ed. *Traité de zoologie. Anatomie, systématique, biologie*. Paris: Masson et Cie, 472–576.
- Schlacher-Hoenlinger MA, Pisera A, Hooper JNA. 2005.** Deep-sea “lithistid” assemblages from the Norfolk Ridge (New Caledonia), with description of seven new species and a new genus (Porifera, Demospongiae). *Zoosystema* **27**:649–698.
- Schmidt O. 1862.** *Die Spongien des adriatischen Meeres*. i-viii. Leipzig: Wilhelm Engelmann, 1–88.
- Schmidt O. 1870.** *Grundzüge einer Spongien-Fauna des atlantischen Gebietes*. Leipzig: Verlag von Wilhelm Engelmann,.
- Schmidt O. 1879.** *Die Spongien des Meerbusen von Mexico. I Abt. Lithistiden*. Verlag G. Fischer.
- Schrammen A. 1910.** Neue Kieselschwämme aus der oberen Kreide von Nordwestdeutschland. I. Teil. Tetraxonia, Monaxonia und Silicea incertae sedis. *Palaeontographica* **5**:1–175.
- Schrammen A. 1924.** Die Kieselspongien der oberen Kreide von Nordwestdeutschland. III. und letzter Teil. *Monographien zur Geologie und Paläontologie* **1**:1–159.

- Schuster A, Cárdenas P, Pisera A, Pomponi SA, Kelly M, Wörheide G, Erpenbeck D. 2018. Seven new deep-water Tetractinellida (Porifera: Demospongiae) from the Galápagos Islands –Morphological descriptions and DNA barcodes. *Zoological Journal of the Linnean Society* **184**:1–31 DOI [10.1093/zoolinnean/zlx097](https://doi.org/10.1093/zoolinnean/zlx097).
- Schuster A, Erpenbeck D, Pisera A, Hooper J, Bryce M, Fromont J, Wörheide G. 2015. Deceptive Desmas: Molecular phylogenetics suggests a new classification and uncovers convergent evolution of lithistid demosponges. *PLOS ONE* **10**:e116038 DOI [10.1371/journal.pone.0116038](https://doi.org/10.1371/journal.pone.0116038).
- Schuster A, Lopez JV, Becking LE, Kelly M, Pomponi SA, Wörheide G, Erpenbeck D, Cárdenas P. 2017. Evolution of group I introns in Porifera: new evidence for intron mobility and implications for DNA barcoding. *BMC Evolutionary Biology* **17**:1–21 DOI [10.1186/s12862-016-0855-1](https://doi.org/10.1186/s12862-016-0855-1).
- Sollas WJ. 1886. Preliminary account of the tetractinellid sponges dredged by H.M.S. ‘Challenger’ 1872–76. Part I. The Choristida. *Scientific Proceedings of the Royal Dublin Society (new series)* **5**:177–199.
- Sollas WJ. 1887. Sponges. In: Black AC, ed. *Encyclopaedia Britannica*. 9th edition. 22 (Edinburgh), 412–429.
- Sollas WJ. 1888. Report on the Tetractinellida collected by H.M.S. Challenger, during the years 1873–1876. *Zoology* **25**:1–458.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**:1312–1313 DOI [10.1093/bioinformatics/btu033](https://doi.org/10.1093/bioinformatics/btu033).
- Tautz D, Renz M. 1983. An optimized freeze-squeeze method for the recovery of DNA fragments from agarose gels. *Analytical Biochemistry* **132**:14–19 DOI [10.1016/0003-2697\(83\)90419-0](https://doi.org/10.1016/0003-2697(83)90419-0).
- Topsent E. 1894. Etude monographique des spongiaires de France. I. Tetractinellida. *Archives de Zoologie Expérimentale et Générale* **3**:259–400.
- Tréguer P, Nelson DM, Bennekoum AJVan, DeMaster DJ, Leynaert A, Quéguiner B. 1995. The silica balance in the world ocean: a reestimate. *Science* **268**:375–379 DOI [10.1126/science.268.5209.375](https://doi.org/10.1126/science.268.5209.375).
- Van Soest RWM, Boury-Esnault N, Hooper JNA, Rützler K, De Voogd NJ, Alvarez B, Hajdu E, Pisera AB, Manconi R, Schönberg C, Klautau M, Picton B, Kelly M, Vacelet J, Dohrmann M, Díaz MC, Cárdenas P, Carballo JL, Ríos P, Downey R. 2018c. World Porifera database. *Neophrissospongia* Pisera and Lévi, 2002. Available at <http://www.marinespecies.org/porifera/porifera.php?p=taxdetails&id=171085> (accessed on 20 September 2018).
- Van Soest RWM, Boury-Esnault N, Hooper JNA, Rützler K, De Voogd NJ, Alvarez B, Hajdu E, Pisera AB, Manconi R, Schönberg C, Klautau M, Picton B, Kelly M, Vacelet J, Dohrmann M, Díaz MC, Cárdenas P, Carballo JL, Ríos P, Downey R. 2018a. World Porifera database. *Macandrewia* Gray, 1859. Available at <http://www.marinespecies.org/porifera/porifera.php?p=taxdetails&id=132087> (accessed on 20 September 2018).
- Van Soest RWM, Boury-Esnault N, Hooper JNA, Rützler K, De Voogd NJ, Alvarez B, Hajdu E, Pisera AB, Manconi R, Schönberg C, Klautau M, Picton B, Kelly M,

- Vacelet J, Dohrmann M, Díaz MC, Cárdenas P, Carballo JL, Ríos P, Downey R. 2018b. World Porifera database. *Corallistes* Schmidt, 1870. Available at <http://www.marinespecies.org/porifera/porifera.php?p=taxdetails&id=132080> (accessed on 20 September 2018).
- Van Soest RWM, Hooper JNA. 2002. Order Spirophorida Bergquist and Hogg, 1969. In: Hooper JNA, Van Soest RWM, eds. *Systema Porifera. A guide to the classification of sponges*. New York: Kluwer Academic/ Plenum Publishers, 83–84.
- Van Soest RWM, Meesters E, Becking L. 2014. Deep-water sponges (Porifera) from Bonaire and Klein Curaçao, Southern Caribbean. *Zootaxa* **3878**:401–443 DOI [10.11646/zootaxa.3878.5.1](https://doi.org/10.11646/zootaxa.3878.5.1).
- Van Soest RWM, Stentoft N. 1988. Barbados deep-water sponges. *Studies on the Fauna of Curaçao and other Caribbean Islands* **70**:1–175.
- Vargas S, Kelly M, Schnabel K, Mills S, Bowden D, Wörheide G. 2015. Diversity in a cold hot-spot: DNA-barcoding reveals patterns of evolution among antarctic demosponges (Class Demospongiae, Phylum Porifera). *PLOS ONE* **10**:e0127573 DOI [10.1371/journal.pone.0127573](https://doi.org/10.1371/journal.pone.0127573).
- Von Lendenfeld R. 1894. Die Tetractinelliden der Adria (Mit einem Anhang über die Lithistiden). *Denkschriften der Kaiserlichen Akademie der Wissenschaften Wien. Mathematisch-Naturwissenschaften Klasse* **61**:91–204.
- Von Lendenfeld R. 1903. Tetraxonia. In: Schulze FE, ed. *Das Tierreich*. Friedländer: Berlin Publishers, 1–168.
- Wickham H. 2009. ggplot2: elegant graphics for data analysis. New York: Springer-Verlag.
- Wright AE. 2010. The Lithistida: important sources of compounds useful in biomedical research. *Current Opinion in Biotechnology* **21**:801–807 DOI [10.1016/j.copbio.2010.09.012](https://doi.org/10.1016/j.copbio.2010.09.012).
- Xia X. 2013. DAMBE5: a comprehensive software package for data analysis in molecular biology and evolution. *Molecular Biology and Evolution* **30**:1720–1728 DOI [10.1093/molbev/mst064](https://doi.org/10.1093/molbev/mst064).
- Xia X, Xie Z, Salemi M, Chen L, Wang Y. 2003. An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution* **26**:1–7 DOI [10.1016/S1055-7903\(02\)00326-3](https://doi.org/10.1016/S1055-7903(02)00326-3).
- Zittel KA. 1878. Studien über fossile Spongien. II. Lithistidae. *Abhandlungen der Mathematisch-Physikalischen Classe der Königlich-Bayerischen Akademie der Wissenschaften* **13**:65–154.