Systematics of 'lithistid' tetractinellid demosponges from the Tropical Western Atlantic – implications for phylodiversity and bathymetric distribution

Astrid Schuster[1,2], Shirley A. Pomponi[3], Andrzej Pisera[4], Paco Cárdenas[5], Michelle Kelly[6], Gert Wörheide[1,7,8], and Dirk Erpenbeck[1,8]

[1]**Department of Earth- & Environmental Sciences, Palaeontology and Geobiology, Ludwig-Maximilians-Universität München, Richard-Wagner Str. 10, 80333 Munich, Germany**
[2]**Current address: Department of Biology, NordCEE, Southern University of Denmark, Campusvej 55, 5300 M Odense, Denmark**
[3]**Harbor Branch Oceanographic Institute, Florida Atlantic University, 5600 U.S. 1 North, Ft Pierce, FL 34946, USA**
[4]**Institute of Paleobiology, Polish Academy of Sciences, ul. Twarda 51/55, 00-818 Warszawa, Poland**
[5]**Pharmacognosy, Department of Medicinal Chemistry, Uppsala University, Husargatan 3, 75123 Uppsala, Sweden**
[6]**National Centre for Coasts and Oceans, National Institute of Water and Atmospheric Research, Private Bag 99940, Newmarket, Auckland, 1149, New Zealand**
[7]**SNSB-Bayerische Staatssammlung für Paläontologie und Geologie, Richard-Wagner Str. 10, 80333 Munich, Germany**
[8]**GeoBio-CenterLMU, Ludwig-Maximilians-Universität München, Richard-Wagner Str. 10, 80333 Munich, Germany**

Corresponding author:

Dirk Erpenbeck[1,8]

Email address: erpenbeck@lmu.de

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.

Methods/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 new tetractinellid suborder, one new genus and several new 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 contribute to a better comparison between present major 'lithistid' faunas and their fossil record in the Mesozoic.

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; 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: 1) 'lithistid' demosponges are dominant components of seamount communities on the Norfolk Ridge and in the South-West Pacific (e.g. Lévi, 1991; Kelly, 2000, 2007; Schlacher-Hoenlinger, Pisera and 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 and Hooper, 2005; Schuster et al., 2015); 2) large 'lithistid' assemblages are reported from continental shelves and caves of the North-East Atlantic (e.g. Carvalho, Pomponi and 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 and Stentoft (1988), Reed and 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 and Cárdenas 2015 (Cárdenas et al., 2011; Morrow and 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, and 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 and Pomponi, 1994; Schuster et al., 2015). With the discovery and description of a new tetractinellid family Stupendidae Kelly and Cárdenas, 2016, a sister group relationship of Stupendidae to a clade consisting of rhizomorine-desma-bearing Scleritodermidae, Siphonidiidae and Azoricidae Sollas, 1888 was recently indicated (Kelly and 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 and 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 and Stentoft, 1988), the Bahamas (Maldonado and Young, 1996; Reed and Pomponi, 1997), Cuba (Pisera, 1999) the deep Florida shelf (Pisera and Pomponi, 2015), and chemotaxonomic studies (Kelly-Borges et al. 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 9 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'.

The present study presents the first molecular systematic attempt to evaluate a large part of the extensive HBOI 'lithistid' collection ($\approx$ 250 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 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 zonations as described in Reed and Pomponi (1997). The phylogenetic affinities of 31 out of the 35 'lithistid' Tetractinellida genera are reconstructed. Furthermore, our results reveal a new clade including all rhizoclone desma bearing lithistids plus Stupendidae and Thrombidae, and document potential new genera/species and occurrences for the TWA. With this systematic groundwork the molecular phylodiversity of eight island regions in the TWA and the relative abundance and bathymetric distribution patterns of 218 samples representing nine desma-bearing families are analysed and discussed suggesting

that the present-day 'lithistid' fauna is as diverse as the fauna from the Mesozoic.

MATERIALS AND METHODS

Specimen collection and identification

Between 1995 and 2011, sponge samples were collected from the Tropical Western Atlantic (TWA) by the *Johnson-Sea-Link* (*JSL*) *I* and *II* submersibles operated by the HBOI, Fort Pierce, Florida and by scuba diving during several expeditions to the Bahamas, Cuba, Florida Keys, Curaçao, Turks and Caicos, Puerto Rico, Honduras, Jamaica, Guadeloupe, Gulf of Mexico and Bonaire (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 (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) 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). Detailed information for all novel samples sequenced is provided in the Supplementary Material.

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 deck pictures, and prepared thick sections as well as spicule and skeleton stubs for scanning electron microscopy (SEM). We used the methodology outlined in Pisera and Pomponi (2015) to illustrate and evaluate morphological characters. Based on this, 249 specimens could be identified to genus and/or species level. Morphological documentation for the 71 representative specimens are provided in the Sponge Barcoding Project (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 under accession numbers XXXX.

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 and 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 and 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 $\approx$ 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 and Renz, 1983), as described in Schuster et al. (2015). For sequencing of the 28S fragment, 6 μl 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 SBP under accession numbers #1794 to #2108.

Phylogenetic reconstructions

Alignments were generated separately for *cox1* and 28S using MAFFT v.7 under the L-INS-I algorithm (Katoh and 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., 2013) 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 and 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 and 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 doi.:XXXXXX. 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 and 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 PDI 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 (RStudio 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 and 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 (Fig. 2) corroborate the monophyly of Tetractinellida, currently including the suborders Astrophorina and Spirophorina (Morrow and Cárdenas, 2015) (Fig. 2C). In addition, the affinity of eight desma-bearing families to the suborder Astrophorina (Cárdenas et al., 2011; Morrow and Cárdenas, 2015; Schuster et al., 2015) is confirmed (Fig. 2A-C). The 28S phylogeny (Fig. 2A) indicates a sister relationship of Astrophorina and Spirophorina. In both gene trees (Fig. 2A, B) 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 and 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) (Fig. 2 and Fig. 3) and needs further investigation, including also 28S for Stupendidae Kelly and Cárdenas 2016, a recently established new family (Kelly and Cárdenas, 2016). In the *cox1* phylogeny (Fig. 2B) Stupendidae is included and 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 and 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 and Cárdenas, 2015; Schuster et al., 2015) (see also Fig. 2A-C). 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 tetraclone desmas and phyllotriaenes to discotriaenes as characteristic megascleres. Typical microscleres are acanthorhabds, spirasters and amphiasters (Pisera and 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 and 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. 4) 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 sp., a potential new genus mainly distinct by the layered network of tetracone desma with smooth rays and strongly tuberculated tips and the less abundant microscleres on the ectosome (SBD2102–2106). The sister relationship of *Manihinea* conferta to *Theonella* sp. 1 is highly supported (PP=0.99) by 28S (Fig. 3), whereas it is not supported by cox1 (Fig. 4). 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 *Discodermia* is sister to a clade consisting of *Manihinea*+*Theonella*+*Siliquariaspongia*, which is sister to *Racodiscula*+Theonellidae sp.

The genus *Racodiscula* is highly supported (PP=1.0) as sister to Theonellidae sp. Although the outer morphology of Theonellidae sp. (SBD2106 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 (SBD2065) building a dense crust on the surface, are rarer or even absent in Theonellidae sp. Instead of microaconthorabds, phyllo- to discotriaenes are the main components of the dense surface crust. In addition, Theonellidae sp. possesses desmas with smooth rays and strongly tuberculated tips (SBD2105 and SBD2106) building a layered network (SBD2102), which clearly differs from *Racodiscula* (Schuster et al. 2018).

Theonella mirabilis (de Laubenfels, 1954) was first named within the homoscleromorphid genus *Placinalopha* (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 *Placinalopha* species were noted by Muricy and Díaz (2002), who suggested that the species *mirabilis* had a more likely affinity with species in family Theonellidae. 28S sequences (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. 4) 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 discotrianes, the latter occasionally recorded in *T. mirabilis*. Our phylogenies clearly place all of the sequenced *Theonella mirabilis* species within the *Theonella*+*Manihinea* clade (Fig. 3), 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 know, only *Macandrewia rigida* Lévi and 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. 3). Morphological differences in desmas (SBD2004) 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* Lendenfeld, 1894) (Fig. 3). 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*. The present comprehensive study does not recover Phymatellidae as monophyletic and is therefore in contrast to Schuster et al. (2015). Instead, a highly supported sister group relationship of *Neoaulaxinia* Pisera and Lévi, 2002 to the neopeltid genus *Daedalopelta* Sollas, 1888 is observed and *Reidispongia coerulea* Lévi and Lévi, 1988 is sister to this clade (Fig. 3). This relationship is in conflict with morphological hypotheses as Phymatellidae is characterized by ectosomal dichotriaenes, while Neopeltidae has ectosomal pseudophyllotriaenes or pseudodiscotriaenes (Pisera, 2002). Until now only one species of *Daedalopelta* is known (*D. nodosa* Sollas, 1888 from Florida, Gulf of Mexico and Southern Caribbean), which clearly differs from our *Daedalopelta* specimens. Therefore, morphological characters of both sequenced *Daedalopelta* species were further investigated and illustrated (SBD1894). Both species are potentially new to science. *Daedalopelta* sp. 1 (SBD1894) possesses the characteristic pseudophyllotriaenes (SBD1894) known from the Neopeltidae, while *Daedalopelta* sp. 2 (SBD1895) possesses dichotriaenes characteristic for the Phymatellidae. These dichotriaenes, however, have a unique shape with indented cladomes (SBD1895). A spicule drawing of *Corallistes tubulatus* Van Soest and Stentoft, 1988 from Barbados, now *Neophrissospongia tubulata*, resembles those unique dichotriaenes implying that *N. tubulata* may need to be reallocated to *Daedalopelta*. Interestingly, dichotriaenes of *Daedalopelta* 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* Moret, 1926 to the family Phymatellidae. *Gignouxia* will include *Daedalopelta* sp. 2 as well as *Daedalopelta 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* de Laubenfels, 1953 (Fig. 3). 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 and 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.

Corallistidae is another major family dominating the HBOI collection and subsequently our phylogenies (Fig. 3 and 4). These taxa were the focus of biomedical investigations (e.g. Haar et al., 1996; Wright, 2010), thus targeted during the HBOI expeditions. Therefore, whether the genera *Discoderma* and *Corallistes* Schmidt, 1870 are over-represented in this study due to their increased sampling, or whether they actually dominate the sponge fauna in the TWA regions

remain obscure and awaits further investigations. 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 and Pomponi, 1994; Chombard, Boury-Esnault and Tillier, 1998; McInerney, Adams and Kelly, 1999). With 52 *Corallistes* specimens sequenced, this study presents the largest data set to data and reveals the monophyly of this genus (28S, PP= 1.0, Fig. 3). *Corallistes typus* Schmidt, 1870 specimens, type species of the genus, were examined (SBD1888, 1889, 1892) and sequenced (Fig. 3 and 4). In addition, a *Corallistes isabela* Desqueyroux-Faúndez and 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 and Van Soest, 1997 and Schuster et al., 2018). In addition, six *Corallistes* (*C.* sp. 2 to *C.* sp. 7, see Fig. 3) differ by 1-3 bp in the 28S fragment, while no differences were found in *cox1*. Morphological differences are observed between *C.* sp. 2 (SBD1872) and *C.* sp. 4 (SBD1879). For example, *C.* sp. 2 has long (700 μm) thin ectosomal oxeas (SBD1872 A), while *C.* sp. 4 has ectosomal styles (SBD1879 D) and subectosomal microxea with spined surfaces (SBD1879 C). Morphological identifications are in progress and necessary to discriminate the remaining *Corallistes* species.

The polyphyletic corallistid genus *Herengeria* Lévi and Lévi, 1983, only known from the Pacific Norfolk Ridge, New Caledonia, and New Zealand (Schlacher-Hoenlinger, Pisera and Hooper, 2005; Kelly et al., 2009), was sequenced (Fig. 3) and morphologically illustrated (SBD1949) 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 and Lévi 1988 and *H. vasiformis* Schlacher-Hoenlinger, Pisera and Hooper, 2005). The morphological observations delimit the new TWA species from *H. auriculata* (Schlacher-Hoenlinger and 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. 3). Our 28S (Fig. 3) and *cox1* (Fig. 4) phylogenies strongly support *Neoschrammeniella* Pisera and Lévi, 2002 as sister to the *Herengeria*/*Isabella* clade. Currently, seven valid species of *Neophrissospongia* Pisera and Lévi, 2002 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. 3). A further Caribbean *Neophrissospongia* species (sp. 2) forms a robust sister clade to *Neophrissospongia* from the Eastern Pacific (Galápagos, Panama) (Fig. 3).

A clade of six as yet unidentified specimens from the HBOI collection (SBD1814) is sister to the monophyletic *Neophrissospongia* (PP=1.0, Fig. 3). 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 (SBD1815), 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 (SBD1814, 1815) differ from those in *Awhiowhio osheai* in sizes and shapes (SBD1814). The *cox1* phylogeny (Fig. 4) 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 (Fig. 3 and 4). *Pleroma menoui* Sollas, 1888 is distant to other *Pleroma* spp. (including the type species *P. turbinatum*) in a close relationship to Corallistidae (Fig. 3 and 4).

Intra-subordinal relationships of spirophorine 'lithistids'

The suborder Spirophorina is characterized by sigmaspire microscleres and its members share triaene spicules with Astrophorina. Currently, three families are known: Samidae Sollas, 1888, Spirasigmidae Hallmann, 1912 and Tetillidae Sollas, 1886, whereas the latter is the largest in terms of genera and species (e.g. Van Soest and Hooper, 2002). The relationships of major clades within our *cox1* and 28S phylogenies (Fig. 3 and 4) were in concordance with the findings of Carella et al. (2016) and Schuster et al. (2017). The latest revised classification of Morrow and Cárdenas (2015) included the desma-bearing families Azoricidae, Scleritodermidae and Siphonidiidae within Spirophorina. Since then, several studies (Schuster et al., 2015, 2017; Kelly and Cárdenas, 2016) using *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 and Cárdenas (2016) provided strong support for families Azoricidae, Scleritodermidae and Siphonidiidae within a new suborder, supported in part by the common possession of rhizomorine desmas.

Regarding **Azoricidae**, 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 and Lévi, 2002b), 11 species are valid to date (WPD access Aug. 2017). In the present study we sequenced the 28S C1-D2 fragment for 52 *Leiodermatium* specimens from several regions in the TWA (Fig. 3) 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. 3). 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 (SBD1966,1967,1959,1965,1956,1955,1975,1977,1976,1994,2000,2001,2003) adumbrate detailed differences of *Leiodermatium* spp., in particular their surfaces (oscules and ostia sizes), diactines and desma morphology. For instance *Leiodermatium* sp. 1 (SBD1955) has large and marginate oscules, while *Leiodermatium* sp. 6 (SBD1994) and *Leiodermatium* sp. 8 (SBD2003) have large but elevated oscules on exterior margins, in contrast to *Leiodermatium* sp. 7, whose oscules are small and closely distributed. Based on molecular and morphological data we propose eight different species of *Leiodermatium* (Fig. 3) 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 and Pomponi, 1994, Kelly and 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 poly- and *Scleritoderma* is paraphyletic, while *Microscleroderma*, *Amphibleptula* Schmidt, 1879 and

Setidium Schmidt, 1879 are monophyletic (Fig. 3). The genus *Amphibleptula* is currently monospecific with *A. madrepora* Schmidt, 1879 from the Caribbean (Pisera and Lévi, 2002c). Morphologically, *A. madrepora* is very similar and easy to confuse with *Microscleroderma spirophora* Lévi, 1960 as discussed in Van Soest and 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 (SBD1802,1803) provide conclusive evidence that our three samples are *Amphibleptula* species, due to their dense tuberculated/blunt spinose rhizoclones, the protruding bundles of oxeas in the oscula area (SBD1802,1803) as well as the presence of sigmaspires (SBD1802). Differences to *A. madrepora* are the diactine spicules present in all three *Amphibleptula* sp.1. In addition, fusiform spined microxeas and acanthorhabds are found in the specimen from Jamaica (HBOI 1-IX-93-1-006, SBD1802). To conclude, we sequenced two potential new species of *Amphibleptula* with clear unique morphological characters, different from *A. madrepora*.

During this study a new suborder Thoosina was suggested by Carballo et al. 2018 including the genera *Thoosa* and *Alectona* so far. However, since no desma-bearing tetractinellids are grouping within this suborder, we decided to not further discuss Thoosina in the scope of this study.

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. 5). The PD_I analyses disclosed a high variation within the TWA locations (Fig. 5). At comparable sampling efforts, the highest PD_I was observed in Jamaica (Fig. 5A) indicating a high biodiversity in this region, closely followed by the Bahamas (Fig. 5B). 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 and Pomponi (1997), and may be explained by the high habitat diversity observed in this region (Reed and Pomponi, 1997) and their close proximity to the species-rich Atlantic (see e.g. Carvalho, Pomponi and Xavier, 2015). Even though Turks and Caicos are close to the Bahamas and the Atlantic, a much lower PD_I 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. 6). 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 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 $\geq$300 m, while those with a less dense desma skeleton like most of the Theonella species (Theonellidae) moved into more shallow water habitats, as less silica is required for their skeleton construction. This trend was also observed in the South West Pacific (Kelly et al., 2007; Hall, Ekins and Hooper, 2014). As Corallistidae and Theonellidae are considered to be polymorphic (Pisera and Lévi, 2002a,d), 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 301–1000 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 and 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 and 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 (Sará and Vacelet, 1973), 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. 6) 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.), and two *Daedalopelta* sp. species 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* 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. 6).

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.

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ADDITIONAL INFORMATION AND DECLARATIONS

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.

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COMPETING INTERESTS

The authors declare there are no competing interests.

AUTHOR CONTRIBUTIONS

Astrid Schuster and Dirk Erpenbeck conceived and designed the study. Dirk Erpenbeck and Gert Wörheide acquired funding, contributed reagents, materials and analysis tools. Astrid Schuster, Andrzej Pisera, Paco Cárdenas, Shirley A. Pomponi and Michelle Kelly identified genera/species. Shirley A. Pomponi (HBOI), Michelle Kelly (NIWA) contributed to the sampling. Astrid Schuster performed the laboratory experiments, did the phylogenetic analyses, bathymetric distribution plot, phylodiversity analysis and SEM. Astrid Schuster wrote the manuscript, prepared figures and tables. Paco Cárdenas, Dirk Erpenbeck, Michelle Kelly, Andrzej Pisera and Gert Wörheide edited versions of the manuscript. All authors reviewed drafts of the paper.

DATA AVAILABILITY

The following information was supplied regarding data availability:

Novel sequences will be archived at the European Nucleotide Archive (ENA) under the accession numbers: XXXX–XXXXX.

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819 ## FIGURES

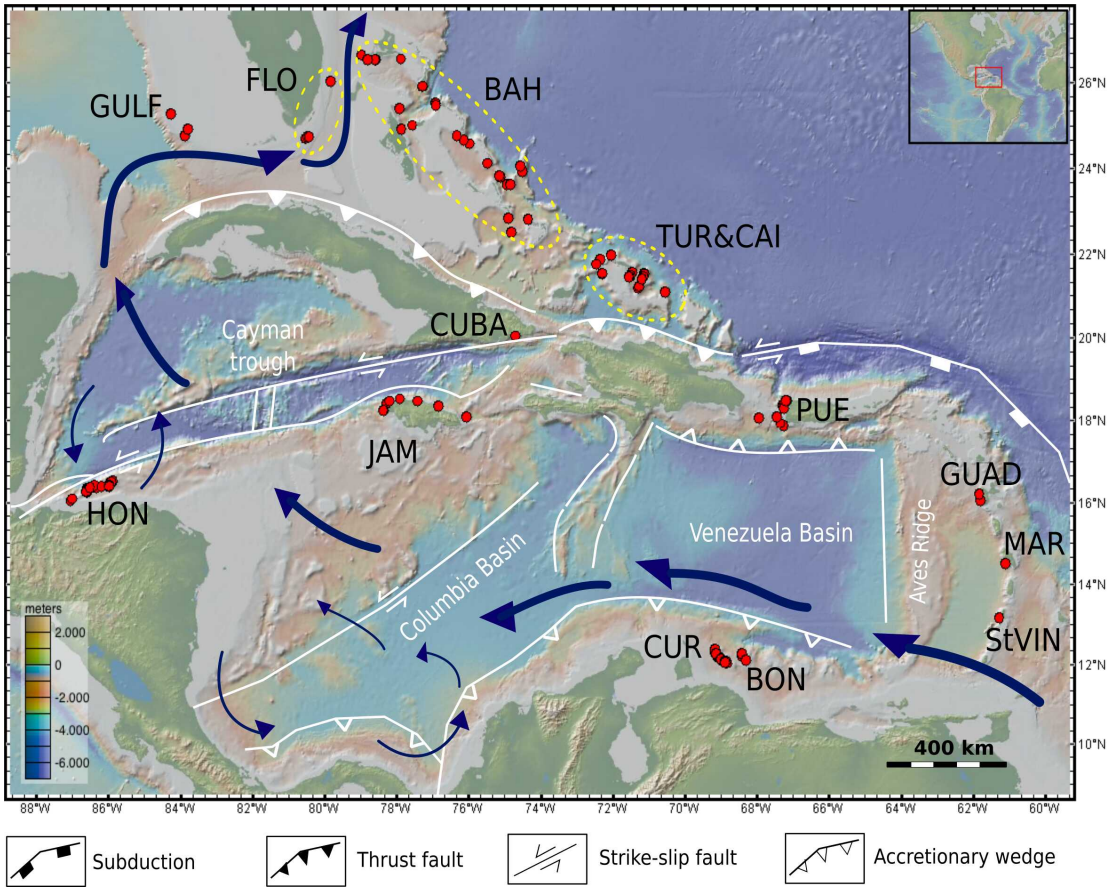


Figure 1. Distribution map of investigated HBOI and other desma-bearing tetractinellids and Vetulinidae from the TWA. Abbreviations correspond to the different locations (CUR=Curaçao, BON=Bonaire, StVIN=St. Vincent, MAR=Martinique, GUAD=Guadaloupe, PUE=Puerto Rico, JAM=Jamaica, HON=Honduras, TUR and CAI=Turks and Caicos, BAH=Bahamas, FLO=Florida). Tectonic settings are schematically indicated (white lines) (Giunta 2011). Main water currents (dark blue arrows) follow Miloslavich et al. (2010). Map generated with GeoMapApp 3.6.3 (http://www.geomapapp.org, Ryan et al., 2009).

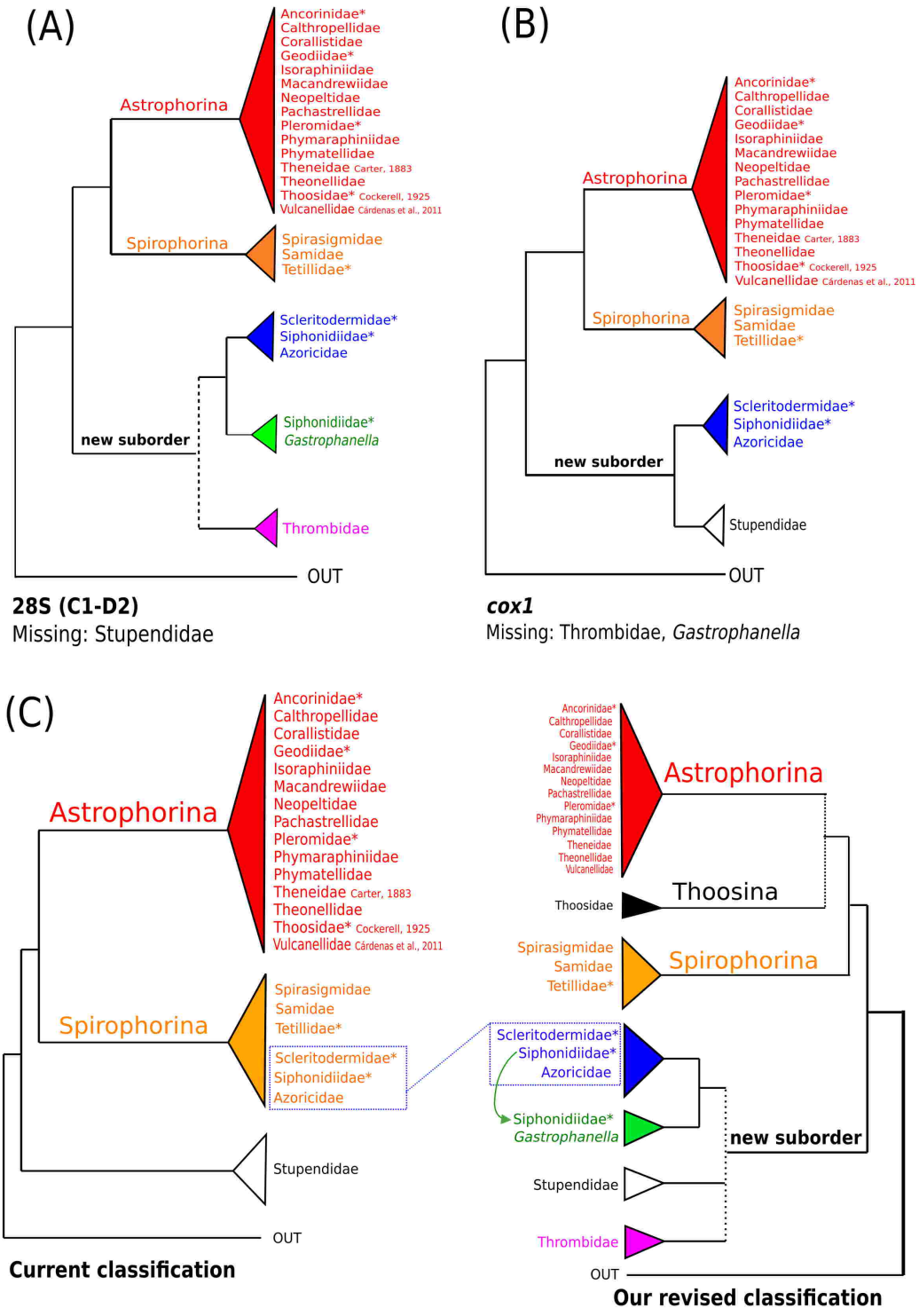
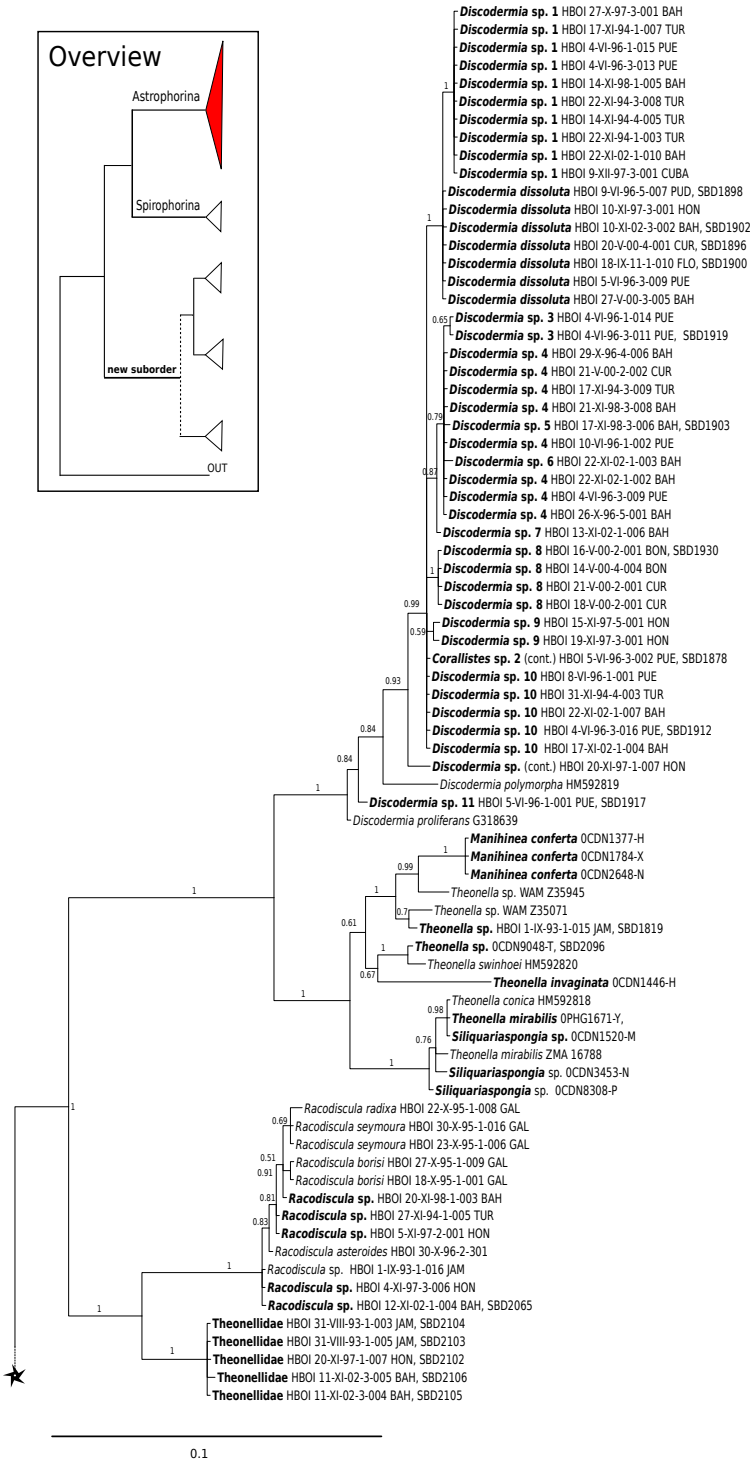


Figure 2. Schematic summary cladograms obtained from the 28S and *cox1* phylogenies indicating the higher-taxa relationships within the order Tetractinellida. (A) 28S (B) *cox1* summary tree with the suborders Astrophorina (red), Spirophorina (orange) and a new suborder (blue, green, pink and light gray) including all rhizoclone desma-bearing families and the family 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 new suborder Thoosina from Carballo et al. (2018).


Overview
Astrophorina
Spirophorina
new suborder
OUT
Discodermia sp. 1 HBOI 27-X-97-3-001 BAH
Discodermia sp. 1 HBOI 17-XI-94-1-007 TUR
Discodermia sp. 1 HBOI 4-VI-96-1-015 PUE
Discodermia sp. 1 HBOI 4-VI-96-3-013 PUE
Discodermia sp. 1 HBOI 14-XI-98-1-005 BAH
Discodermia sp. 1 HBOI 22-XI-94-3-008 TUR
Discodermia sp. 1 HBOI 14-XI-94-4-005 TUR
Discodermia sp. 1 HBOI 22-XI-94-1-003 TUR
Discodermia sp. 1 HBOI 22-XI-02-1-010 BAH
Discodermia sp. 1 HBOI 9-XII-97-3-001 CUBA
Discodermia dissoluta HBOI 9-VI-96-5-007 PUD, SBD1898
Discodermia dissoluta HBOI 10-XI-97-3-001 HON
Discodermia dissoluta HBOI 10-XI-02-3-002 BAH, SBD1902
Discodermia dissoluta HBOI 20-V-00-4-001 CUR, SBD1896
Discodermia dissoluta HBOI 18-IX-11-1-010 FLO, SBD1900
Discodermia dissoluta HBOI 5-VI-96-3-009 PUE
Discodermia dissoluta HBOI 27-V-00-3-005 BAH
Discodermia sp. 3 HBOI 4-VI-96-1-014 PUE
Discodermia sp. 3 HBOI 4-VI-96-3-011 PUE, SBD1919
Discodermia sp. 4 HBOI 29-X-96-4-006 BAH
Discodermia sp. 4 HBOI 21-V-00-2-002 CUR
Discodermia sp. 4 HBOI 17-XI-94-3-009 TUR
Discodermia sp. 4 HBOI 21-XI-98-3-008 BAH
Discodermia sp. 5 HBOI 17-XI-98-3-006 BAH, SBD1903
Discodermia sp. 4 HBOI 10-VI-96-1-002 PUE
Discodermia sp. 6 HBOI 22-XI-02-1-003 BAH
Discodermia sp. 4 HBOI 22-XI-02-1-002 BAH
Discodermia sp. 4 HBOI 4-VI-96-3-009 PUE
Discodermia sp. 4 HBOI 26-X-96-5-001 BAH
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Discodermia sp. 8 HBOI 14-V-00-4-004 BON
Discodermia sp. 8 HBOI 21-V-00-2-001 CUR
Discodermia sp. 8 HBOI 18-V-00-2-001 CUR
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Discodermia sp. 9 HBOI 19-XI-97-3-001 HON
Corallistes sp. 2 (cont.) HBOI 5-VI-96-3-002 PUE, SBD1878
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Discodermia sp. 10 HBOI 31-XI-94-4-003 TUR
Discodermia sp. 10 HBOI 22-XI-02-1-007 BAH
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Discodermia sp. 10 HBOI 17-XI-02-1-004 BAH
Discodermia sp. (cont.) HBOI 20-XI-97-1-007 HON
Discodermia polymorpha HM592819
Discodermia sp. 11 HBOI 5-VI-96-1-001 PUE, SBD1917
Discodermia proliferans G318639
Manihinea conferta 0CDN1377-H
Manihinea conferta 0CDN1784-X
Manihinea conferta 0CDN2648-N
Theonella sp. WAM Z35945
Theonella sp. WAM Z35071
Theonella sp. HBOI 1-IX-93-1-015 JAM, SBD1819
Theonella sp. 0CDN9048-T, SBD2096
Theonella swinhoei HM592820
Theonella invaginata 0CDN1446-H
Theonella conica HM592818
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Siliquariaspongia sp. 0CDN1520-M
Theonella mirabilis ZMA 16788
Siliquariaspongia sp. 0CDN3453-N
Siliquariaspongia sp. 0CDN8308-P
Racodiscula radixa HBOI 22-X-95-1-008 GAL
Racodiscula seymoura HBOI 30-X-95-1-016 GAL
Racodiscula seymoura HBOI 23-X-95-1-006 GAL
Racodiscula borisi HBOI 27-X-95-1-009 GAL
Racodiscula borisi HBOI 18-X-95-1-001 GAL
Racodiscula sp. HBOI 20-XI-98-1-003 BAH
Racodiscula sp. HBOI 27-XI-94-1-005 TUR
Racodiscula sp. HBOI 5-XI-97-2-001 HON
Racodiscula asteroides HBOI 30-X-96-2-301
Racodiscula sp. HBOI 1-IX-93-1-016 JAM
Racodiscula sp. HBOI 4-XI-97-3-006 HON
Racodiscula sp. HBOI 12-XI-02-1-004 BAH, SBD2065
Theonellidae HBOI 31-VIII-93-1-003 JAM, SBD2104
Theonellidae HBOI 31-VIII-93-1-005 JAM, SBD2103
Theonellidae HBOI 20-XI-97-1-007 HON, SBD2102
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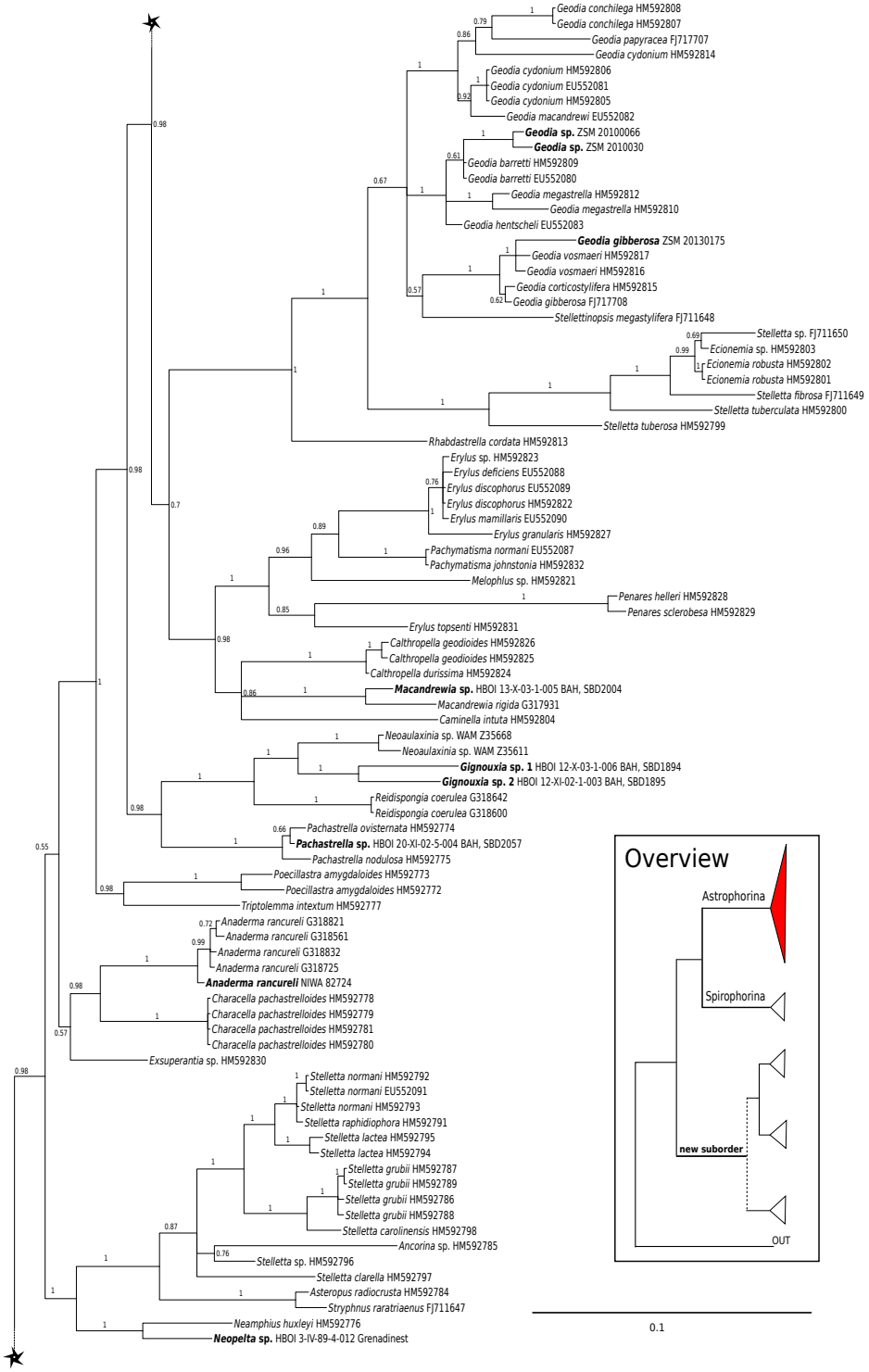
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Geodia hentscheli EU552083
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Geodia vosmaeri HM592817
Geodia vosmaeri HM592816
Geodia corticostylifera HM592815
Geodia gibberosa FJ717708
Stellettinopsis megastylifera FJ711648
Stelletta sp. FJ711650
Ecionemia sp. HM592803
Ecionemia robusta HM592802
Ecionemia robusta HM592801
Stelletta fibrosa FJ711649
Stelletta tuberculata HM592800
Stelletta tuberosa HM592799
Rhabdastrella cordata HM592813
Erylus sp. HM592823
Erylus deficiens EU552088
Erylus discophorus EU552089
Erylus discophorus HM592822
Erylus mamillaris EU552090
Erylus granularis HM592827
Pachymatisma normani EU552087
Pachymatisma johnstonia HM592832
Melophlus sp. HM592821
Penares helleri HM592828
Penares sclerobesa HM592829
Erylus topsenti HM592831
Calthropella geodioides HM592826
Calthropella geodioides HM592825
Calthropella durissima HM592824
Macandrewia sp. HBOI 13-X-03-1-005 BAH, SBD2004
Macandrewia rigida G317931
Caminella intuta HM592804
Neoaulaxinia sp. WAM Z35668
Neoaulaxinia sp. WAM Z35611
Gignouxia sp. 1 HBOI 12-X-03-1-006 BAH, SBD1894
Gignouxia sp. 2 HBOI 12-XI-02-1-003 BAH, SBD1895
Reidispongia coerulea G318642
Reidispongia coerulea G318600
Pachastrella ovisternata HM592774
Pachastrella sp. HBOI 20-XI-02-5-004 BAH, SBD2057
Pachastrella nodulosa HM592775
Poecillastra amygdaloides HM592773
Poecillastra amygdaloides HM592772
Triptolemma intextum HM592777
Anaderma rancureli G318821
Anaderma rancureli G318561
Anaderma rancureli G318832
Anaderma rancureli G318725
Anaderma rancureli NIWA 82724
Characella pachastrelloides HM592778
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Exsuperantia sp. HM592830
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Stelletta normani HM592793
Stelletta raphidiophora HM592791
Stelletta lactea HM592795
Stelletta lactea HM592794
Stelletta grubii HM592787
Stelletta grubii HM592789
Stelletta grubii HM592786
Stelletta grubii HM592788
Stelletta carolinensis HM592798
Ancorina sp. HM592785
Stelletta sp. HM592796
Stelletta clarella HM592797
Asteropus radiocrusta HM592784
Stryphnus raratriaenus FJ711647
Neamphius huxleyi HM592776
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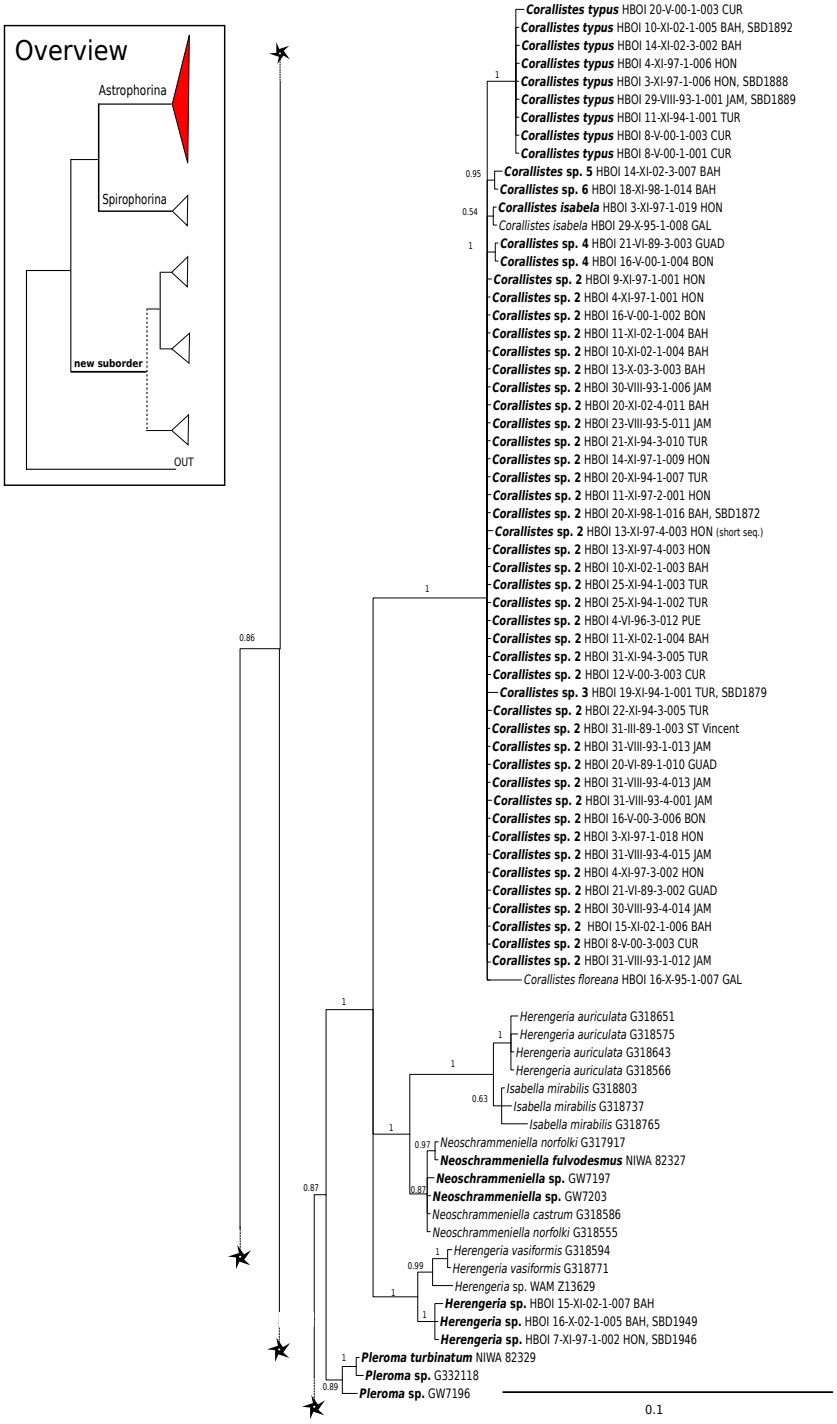
Overview
Astrophorina
Spirophorina
new suborder
OUT
0.1


Overview
Astrophorina
Spirophorina
new suborder
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Corallistes typus HBOI 20-V-00-1-003 CUR
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Corallistes typus HBOI 3-XI-97-1-006 HON, SBD1888
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Corallistes typus HBOI 8-V-00-1-003 CUR
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Corallistes sp. 2 HBOI 25-XI-94-1-003 TUR
Corallistes sp. 2 HBOI 25-XI-94-1-002 TUR
Corallistes sp. 2 HBOI 4-VI-96-3-012 PUE
Corallistes sp. 2 HBOI 11-XI-02-1-004 BAH
Corallistes sp. 2 HBOI 31-XI-94-3-005 TUR
Corallistes sp. 2 HBOI 12-V-00-3-003 CUR
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Corallistes sp. 2 HBOI 20-VI-89-1-010 GUAD
Corallistes sp. 2 HBOI 31-VIII-93-4-013 JAM
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Corallistes sp. 2 HBOI 21-VI-89-3-002 GUAD
Corallistes sp. 2 HBOI 30-VIII-93-4-014 JAM
Corallistes sp. 2 HBOI 15-XI-02-1-006 BAH
Corallistes sp. 2 HBOI 8-V-00-3-003 CUR
Corallistes sp. 2 HBOI 31-VIII-93-1-012 JAM
Corallistes floreana HBOI 16-X-95-1-007 GAL
Herengeria auriculata G318651
Herengeria auriculata G318575
Herengeria auriculata G318643
Herengeria auriculata G318566
Isabella mirabilis G318803
Isabella mirabilis G318737
Isabella mirabilis G318765
Neoschrammeniella norfolki G317917
Neoschrammeniella fulvodesmus NIWA 82327
Neoschrammeniella sp. GW7197
Neoschrammeniella sp. GW7203
Neoschrammeniella castrum G318586
Neoschrammeniella norfolki G318555
Herengeria vasiformis G318594
Herengeria vasiformis G318771
Herengeria sp. WAM Z13629
Herengeria sp. HBOI 15-XI-02-1-007 BAH
Herengeria sp. HBOI 16-X-02-1-005 BAH, SBD1949
Herengeria sp. HBOI 7-XI-97-1-002 HON, SBD1946
Pleroma turbinatum NIWA 82329
Pleroma sp. G332118
Pleroma sp. GW7196
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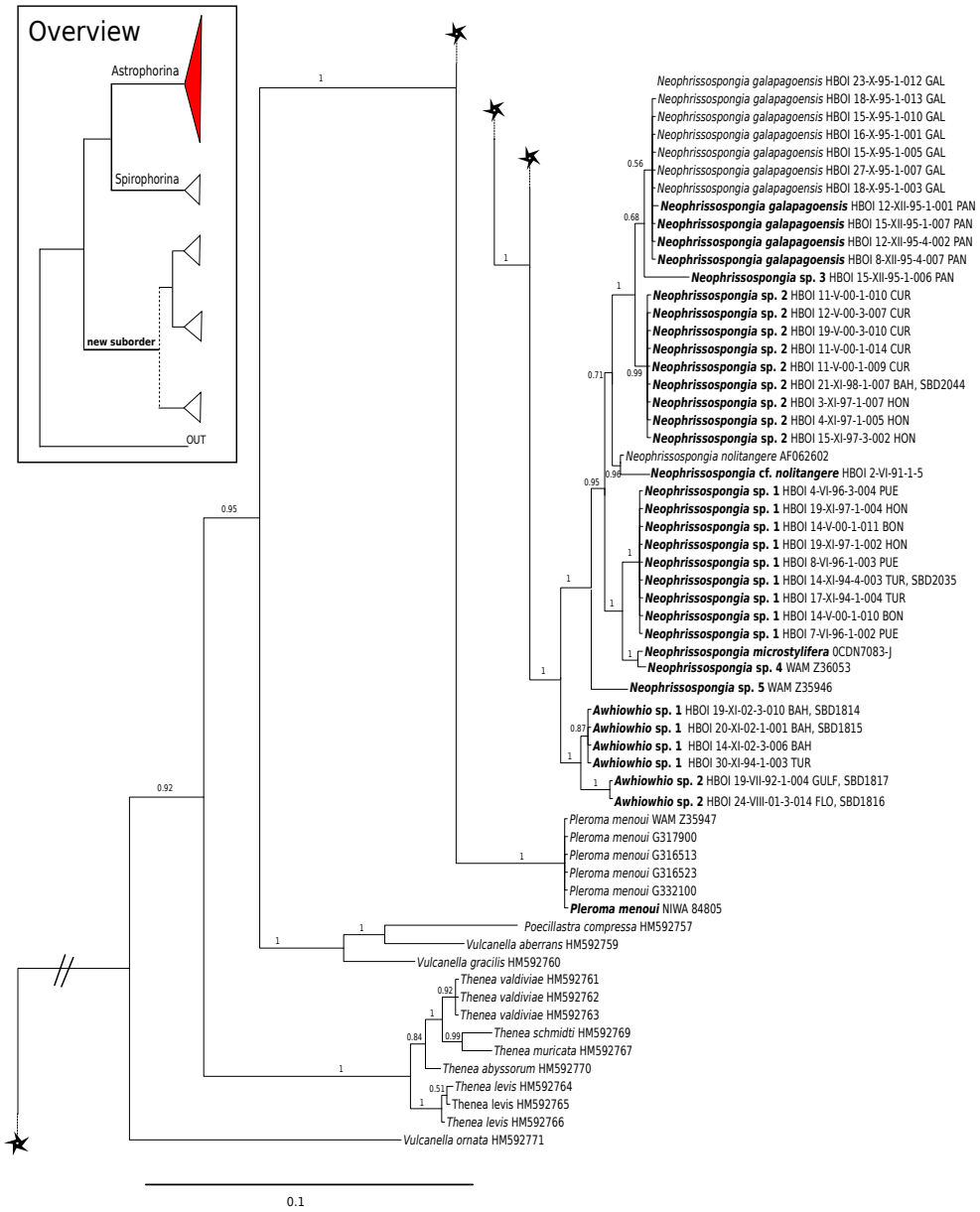
Overview
Astrophorina
Spirophorina
new suborder
OUT
Neophrissospongia galapagoensis HBOI 23-X-95-1-012 GAL
Neophrissospongia galapagoensis HBOI 18-X-95-1-013 GAL
Neophrissospongia galapagoensis HBOI 15-X-95-1-010 GAL
Neophrissospongia galapagoensis HBOI 16-X-95-1-001 GAL
Neophrissospongia galapagoensis HBOI 15-X-95-1-005 GAL
Neophrissospongia galapagoensis HBOI 27-X-95-1-007 GAL
Neophrissospongia galapagoensis HBOI 18-X-95-1-003 GAL
Neophrissospongia galapagoensis HBOI 12-XII-95-1-001 PAN
Neophrissospongia galapagoensis HBOI 15-XII-95-1-007 PAN
Neophrissospongia galapagoensis HBOI 12-XII-95-4-002 PAN
Neophrissospongia galapagoensis HBOI 8-XII-95-4-007 PAN
Neophrissospongia sp. 3 HBOI 15-XII-95-1-006 PAN
Neophrissospongia sp. 2 HBOI 11-V-00-1-010 CUR
Neophrissospongia sp. 2 HBOI 12-V-00-3-007 CUR
Neophrissospongia sp. 2 HBOI 19-V-00-3-010 CUR
Neophrissospongia sp. 2 HBOI 11-V-00-1-014 CUR
Neophrissospongia sp. 2 HBOI 11-V-00-1-009 CUR
Neophrissospongia sp. 2 HBOI 21-XI-98-1-007 BAH, SBD2044
Neophrissospongia sp. 2 HBOI 3-XI-97-1-007 HON
Neophrissospongia sp. 2 HBOI 4-XI-97-1-005 HON
Neophrissospongia sp. 2 HBOI 15-XI-97-3-002 HON
Neophrissospongia nolitangere AF062602
Neophrissospongia cf. nolitangere HBOI 2-VI-91-1-5
Neophrissospongia sp. 1 HBOI 4-VI-96-3-004 PUE
Neophrissospongia sp. 1 HBOI 19-XI-97-1-004 HON
Neophrissospongia sp. 1 HBOI 14-V-00-1-011 BON
Neophrissospongia sp. 1 HBOI 19-XI-97-1-002 HON
Neophrissospongia sp. 1 HBOI 8-VI-96-1-003 PUE
Neophrissospongia sp. 1 HBOI 14-XI-94-4-003 TUR, SBD2035
Neophrissospongia sp. 1 HBOI 17-XI-94-1-004 TUR
Neophrissospongia sp. 1 HBOI 14-V-00-1-010 BON
Neophrissospongia sp. 1 HBOI 7-VI-96-1-002 PUE
Neophrissospongia microstylifera 0CDN7083-J
Neophrissospongia sp. 4 WAM Z36053
Neophrissospongia sp. 5 WAM Z35946
Awhiowhio sp. 1 HBOI 19-XI-02-3-010 BAH, SBD1814
Awhiowhio sp. 1 HBOI 20-XI-02-1-001 BAH, SBD1815
Awhiowhio sp. 1 HBOI 14-XI-02-3-006 BAH
Awhiowhio sp. 1 HBOI 30-XI-94-1-003 TUR
Awhiowhio sp. 2 HBOI 19-VII-92-1-004 GULF, SBD1817
Awhiowhio sp. 2 HBOI 24-VIII-01-3-014 FLO, SBD1816
Pleroma menoui WAM Z35947
Pleroma menoui G317900
Pleroma menoui G316513
Pleroma menoui G316523
Pleroma menoui G332100
Pleroma menoui NIWA 84805
Poecillastra compressa HM592757
Vulcanella aberrans HM592759
Vulcanella gracilis HM592760
Thenea valdiviae HM592761
Thenea valdiviae HM592762
Thenea valdiviae HM592763
Thenea schmidti HM592769
Thenea muricata HM592767
Thenea abyssorum HM592770
Thenea levis HM592764
Thenea levis HM592765
Thenea levis HM592766
Vulcanella ornata HM592771
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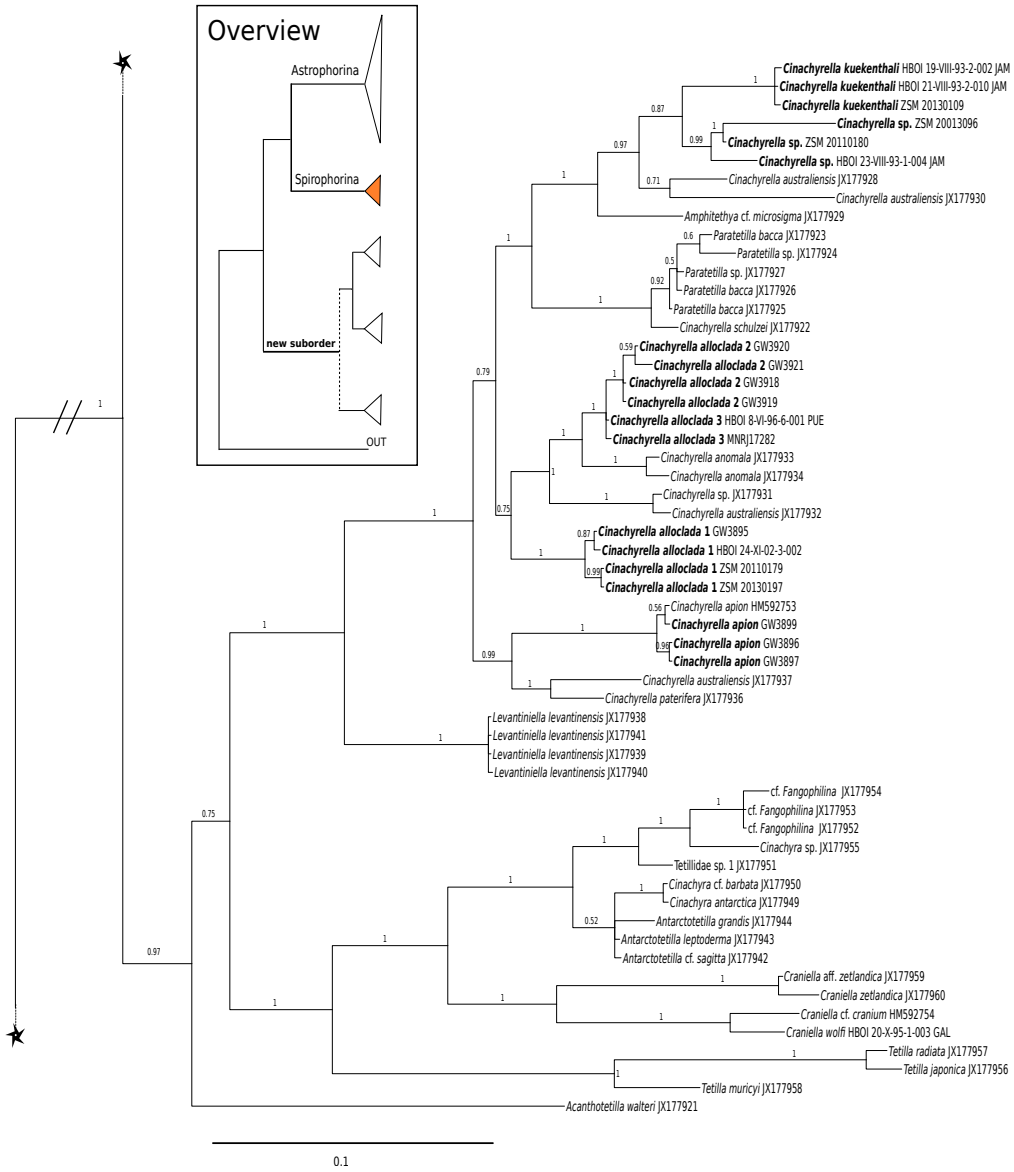
Overview
Astrophorina
Spirophorina
new suborder
OUT
Cinachyrella kuekenthali HBOI 19-VIII-93-2-002 JAM
Cinachyrella kuekenthali HBOI 21-VIII-93-2-010 JAM
Cinachyrella kuekenthali ZSM 20130109
Cinachyrella sp. ZSM 20013096
Cinachyrella sp. ZSM 20110180
Cinachyrella sp. HBOI 23-VIII-93-1-004 JAM
Cinachyrella australiensis JX177928
Cinachyrella australiensis JX177930
Amphitethya cf. microsigma JX177929
Paratetilla bacca JX177923
Paratetilla sp. JX177924
Paratetilla sp. JX177927
Paratetilla bacca JX177926
Paratetilla bacca JX177925
Cinachyrella schulzei JX177922
Cinachyrella alloclada 2 GW3920
Cinachyrella alloclada 2 GW3921
Cinachyrella alloclada 2 GW3918
Cinachyrella alloclada 2 GW3919
Cinachyrella alloclada 3 HBOI 8-VI-96-6-001 PUE
Cinachyrella alloclada 3 MNRJ17282
Cinachyrella anomala JX177933
Cinachyrella anomala JX177934
Cinachyrella sp. JX177931
Cinachyrella australiensis JX177932
Cinachyrella alloclada 1 GW3895
Cinachyrella alloclada 1 HBOI 24-XI-02-3-002
Cinachyrella alloclada 1 ZSM 20110179
Cinachyrella alloclada 1 ZSM 20130197
Cinachyrella apion HM592753
Cinachyrella apion GW3899
Cinachyrella apion GW3896
Cinachyrella apion GW3897
Cinachyrella australiensis JX177937
Cinachyrella paterifera JX177936
Levantiniella levantinensis JX177938
Levantiniella levantinensis JX177941
Levantiniella levantinensis JX177939
Levantiniella levantinensis JX177940
cf. Fangophilina JX177954
cf. Fangophilina JX177953
cf. Fangophilina JX177952
Cinachyra sp. JX177955
Tetillidae sp. 1 JX177951
Cinachyra cf. barbata JX177950
Cinachyra antarctica JX177949
Antarctotetilla grandis JX177944
Antarctotetilla leptoderma JX177943
Antarctotetilla cf. sagitta JX177942
Craniella aff. zetlandica JX177959
Craniella zetlandica JX177960
Craniella cf. cranium HM592754
Craniella wolfi HBOI 20-X-95-1-003 GAL
Tetilla radiata JX177957
Tetilla japonica JX177956
Tetilla muricyi JX177958
Acanthotetilla walteri JX177921
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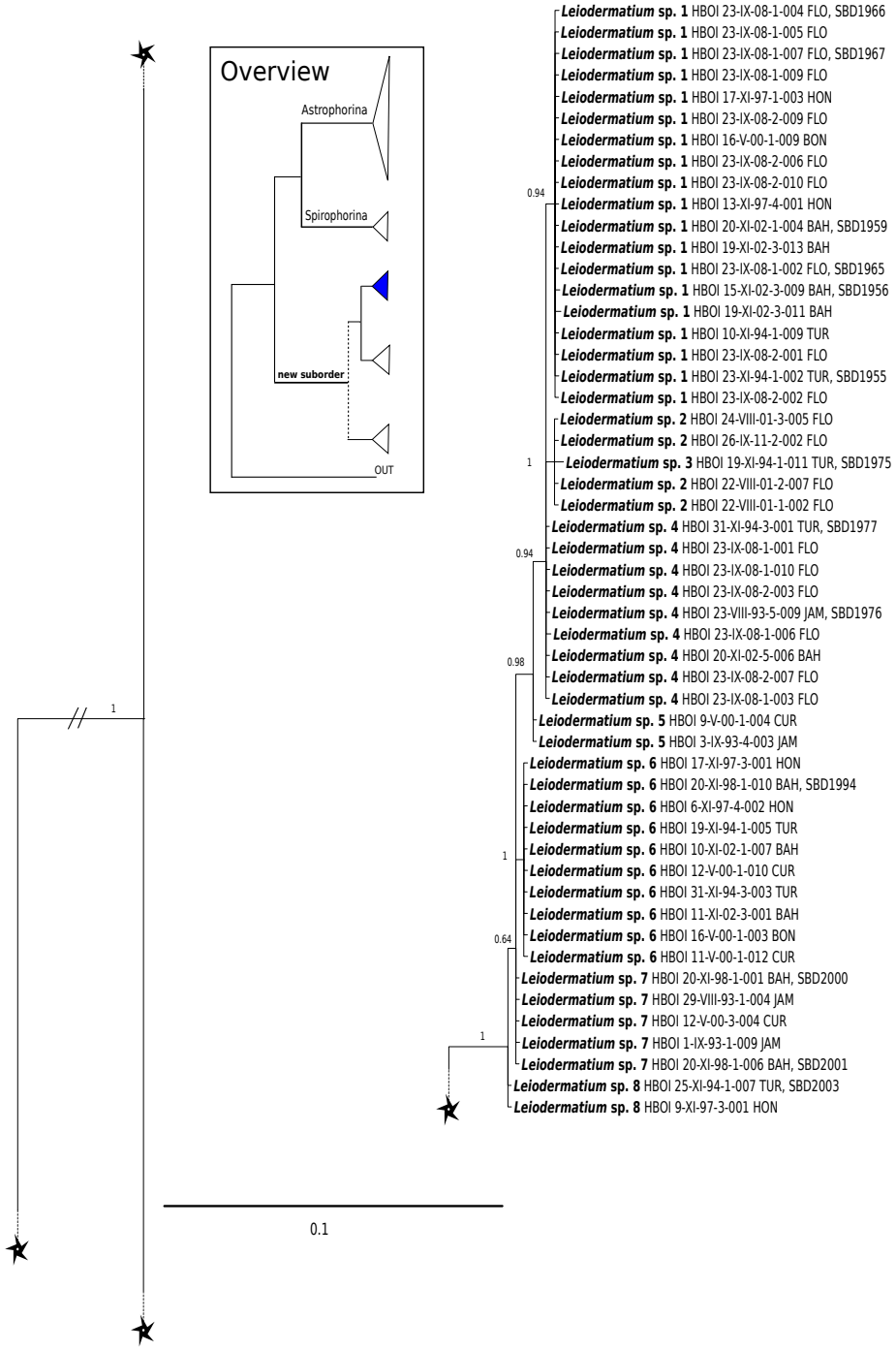

Overview
Astrophorina
Spirophorina
new suborder
OUT
Leiodermatium sp. 1 HBOI 23-IX-08-1-004 FLO, SBD1966
Leiodermatium sp. 1 HBOI 23-IX-08-1-005 FLO
Leiodermatium sp. 1 HBOI 23-IX-08-1-007 FLO, SBD1967
Leiodermatium sp. 1 HBOI 23-IX-08-1-009 FLO
Leiodermatium sp. 1 HBOI 17-XI-97-1-003 HON
Leiodermatium sp. 1 HBOI 23-IX-08-2-009 FLO
Leiodermatium sp. 1 HBOI 16-V-00-1-009 BON
Leiodermatium sp. 1 HBOI 23-IX-08-2-006 FLO
Leiodermatium sp. 1 HBOI 23-IX-08-2-010 FLO
Leiodermatium sp. 1 HBOI 13-XI-97-4-001 HON
Leiodermatium sp. 1 HBOI 20-XI-02-1-004 BAH, SBD1959
Leiodermatium sp. 1 HBOI 19-XI-02-3-013 BAH
Leiodermatium sp. 1 HBOI 23-IX-08-1-002 FLO, SBD1965
Leiodermatium sp. 1 HBOI 15-XI-02-3-009 BAH, SBD1956
Leiodermatium sp. 1 HBOI 19-XI-02-3-011 BAH
Leiodermatium sp. 1 HBOI 10-XI-94-1-009 TUR
Leiodermatium sp. 1 HBOI 23-IX-08-2-001 FLO
Leiodermatium sp. 1 HBOI 23-XI-94-1-002 TUR, SBD1955
Leiodermatium sp. 1 HBOI 23-IX-08-2-002 FLO
Leiodermatium sp. 2 HBOI 24-VIII-01-3-005 FLO
Leiodermatium sp. 2 HBOI 26-IX-11-2-002 FLO
Leiodermatium sp. 3 HBOI 19-XI-94-1-011 TUR, SBD1975
Leiodermatium sp. 2 HBOI 22-VIII-01-2-007 FLO
Leiodermatium sp. 2 HBOI 22-VIII-01-1-002 FLO
Leiodermatium sp. 4 HBOI 31-XI-94-3-001 TUR, SBD1977
Leiodermatium sp. 4 HBOI 23-IX-08-1-001 FLO
Leiodermatium sp. 4 HBOI 23-IX-08-1-010 FLO
Leiodermatium sp. 4 HBOI 23-IX-08-2-003 FLO
Leiodermatium sp. 4 HBOI 23-VIII-93-5-009 JAM, SBD1976
Leiodermatium sp. 4 HBOI 23-IX-08-1-006 FLO
Leiodermatium sp. 4 HBOI 20-XI-02-5-006 BAH
Leiodermatium sp. 4 HBOI 23-IX-08-2-007 FLO
Leiodermatium sp. 4 HBOI 23-IX-08-1-003 FLO
Leiodermatium sp. 5 HBOI 9-V-00-1-004 CUR
Leiodermatium sp. 5 HBOI 3-IX-93-4-003 JAM
Leiodermatium sp. 6 HBOI 17-XI-97-3-001 HON
Leiodermatium sp. 6 HBOI 20-XI-98-1-010 BAH, SBD1994
Leiodermatium sp. 6 HBOI 6-XI-97-4-002 HON
Leiodermatium sp. 6 HBOI 19-XI-94-1-005 TUR
Leiodermatium sp. 6 HBOI 10-XI-02-1-007 BAH
Leiodermatium sp. 6 HBOI 12-V-00-1-010 CUR
Leiodermatium sp. 6 HBOI 31-XI-94-3-003 TUR
Leiodermatium sp. 6 HBOI 11-XI-02-3-001 BAH
Leiodermatium sp. 6 HBOI 16-V-00-1-003 BON
Leiodermatium sp. 6 HBOI 11-V-00-1-012 CUR
Leiodermatium sp. 7 HBOI 20-XI-98-1-001 BAH, SBD2000
Leiodermatium sp. 7 HBOI 29-VIII-93-1-004 JAM
Leiodermatium sp. 7 HBOI 12-V-00-3-004 CUR
Leiodermatium sp. 7 HBOI 1-IX-93-1-009 JAM
Leiodermatium sp. 7 HBOI 20-XI-98-1-006 BAH, SBD2001
Leiodermatium sp. 8 HBOI 25-XI-94-1-007 TUR, SBD2003
Leiodermatium sp. 8 HBOI 9-XI-97-3-001 HON
0.94
1
0.94
0.98
1
0.64
1
1
0.1

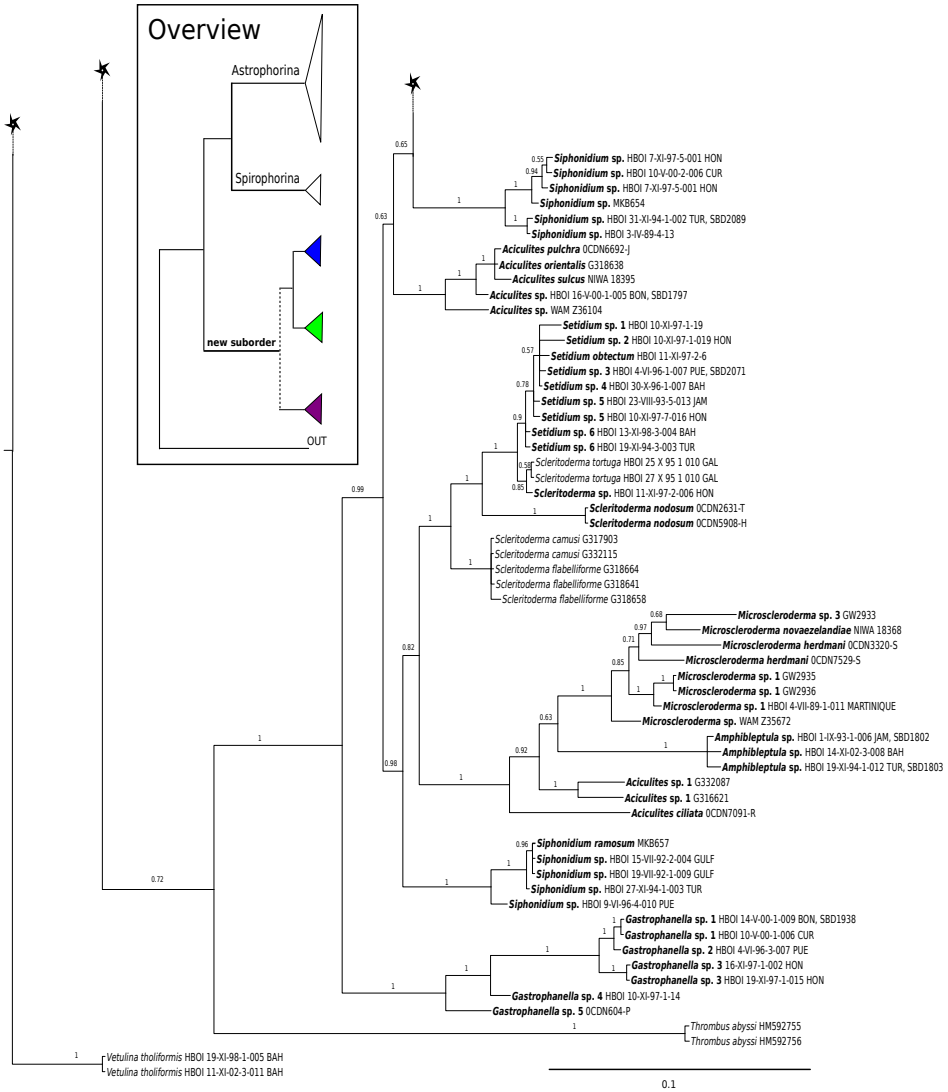
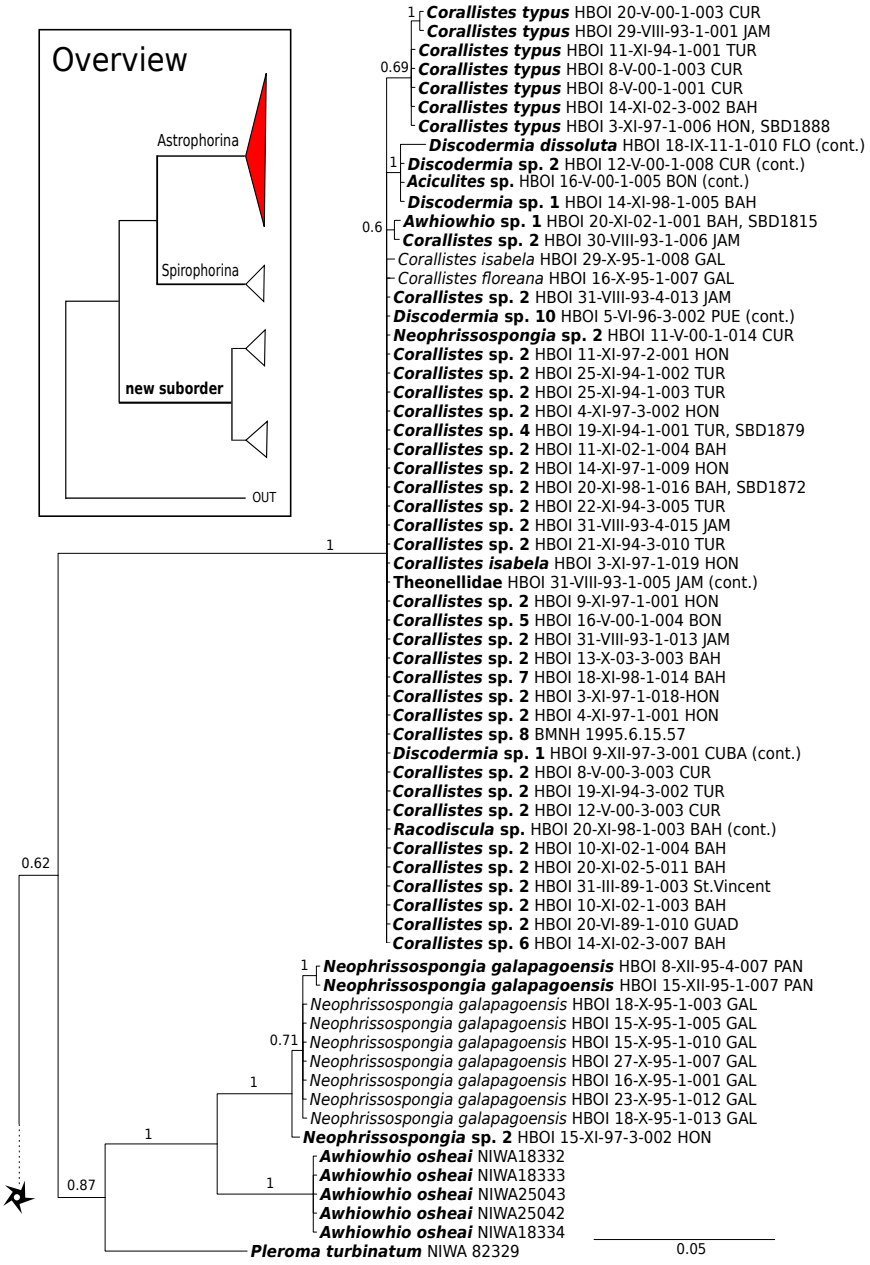
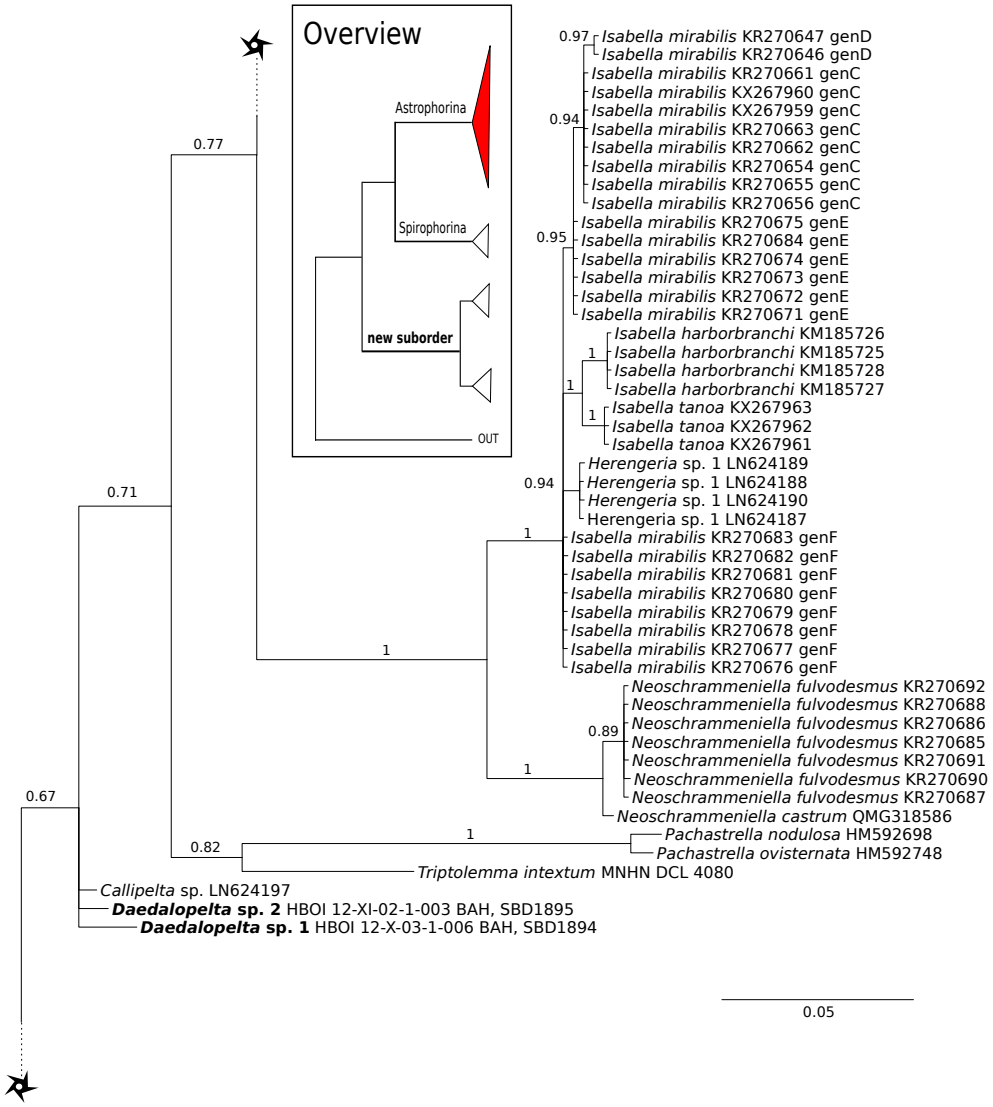
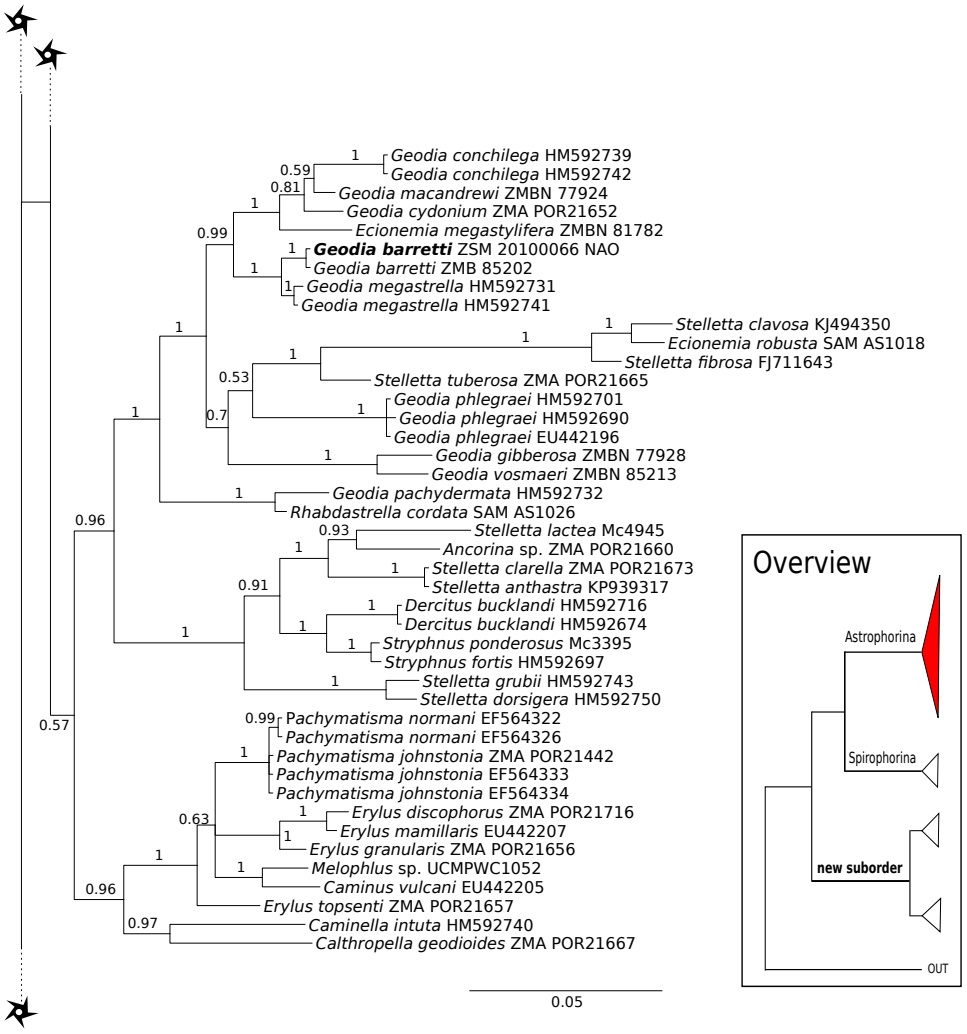


Figure 3. Bayesian Inference phylogeny of Tetractinellida based on 28S (C1-D2). Posterior probability (PP) values are provided above or below branches. Self-generated sequences are in bold. Numbers behind taxon names are either voucher numbers or GenBank/ENA accession numbers. Three letter code behind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


Overview
Astrophorina
Spirophorina
new suborder
OUT
1 Corallistes typus HBOI 20-V-00-1-003 CUR
Corallistes typus HBOI 29-VIII-93-1-001 JAM
Corallistes typus HBOI 11-XI-94-1-001 TUR
0.69 Corallistes typus HBOI 8-V-00-1-003 CUR
Corallistes typus HBOI 8-V-00-1-001 CUR
Corallistes typus HBOI 14-XI-02-3-002 BAH
Corallistes typus HBOI 3-XI-97-1-006 HON, SBD1888
Discodermia dissoluta HBOI 18-IX-11-1-010 FLO (cont.)
1 Discodermia sp. 2 HBOI 12-V-00-1-008 CUR (cont.)
Aciculites sp. HBOI 16-V-00-1-005 BON (cont.)
Discodermia sp. 1 HBOI 14-XI-98-1-005 BAH
0.6 Awhiowhio sp. 1 HBOI 20-XI-02-1-001 BAH, SBD1815
Corallistes sp. 2 HBOI 30-VIII-93-1-006 JAM
Corallistes isabela HBOI 29-X-95-1-008 GAL
Corallistes floreana HBOI 16-X-95-1-007 GAL
Corallistes sp. 2 HBOI 31-VIII-93-4-013 JAM
Discodermia sp. 10 HBOI 5-VI-96-3-002 PUE (cont.)
Neophrissospongia sp. 2 HBOI 11-V-00-1-014 CUR
Corallistes sp. 2 HBOI 11-XI-97-2-001 HON
Corallistes sp. 2 HBOI 25-XI-94-1-002 TUR
Corallistes sp. 2 HBOI 25-XI-94-1-003 TUR
Corallistes sp. 2 HBOI 4-XI-97-3-002 HON
Corallistes sp. 4 HBOI 19-XI-94-1-001 TUR, SBD1879
Corallistes sp. 2 HBOI 11-XI-02-1-004 BAH
Corallistes sp. 2 HBOI 14-XI-97-1-009 HON
Corallistes sp. 2 HBOI 20-XI-98-1-016 BAH, SBD1872
Corallistes sp. 2 HBOI 22-XI-94-3-005 TUR
Corallistes sp. 2 HBOI 31-VIII-93-4-015 JAM
1 Corallistes sp. 2 HBOI 21-XI-94-3-010 TUR
Corallistes isabela HBOI 3-XI-97-1-019 HON
Theonellidae HBOI 31-VIII-93-1-005 JAM (cont.)
Corallistes sp. 2 HBOI 9-XI-97-1-001 HON
Corallistes sp. 5 HBOI 16-V-00-1-004 BON
Corallistes sp. 2 HBOI 31-VIII-93-1-013 JAM
Corallistes sp. 2 HBOI 13-X-03-3-003 BAH
Corallistes sp. 7 HBOI 18-XI-98-1-014 BAH
Corallistes sp. 2 HBOI 3-XI-97-1-018-HON
Corallistes sp. 2 HBOI 4-XI-97-1-001 HON
Corallistes sp. 8 BMNH 1995.6.15.57
Discodermia sp. 1 HBOI 9-XII-97-3-001 CUBA (cont.)
Corallistes sp. 2 HBOI 8-V-00-3-003 CUR
Corallistes sp. 2 HBOI 19-XI-94-3-002 TUR
Corallistes sp. 2 HBOI 12-V-00-3-003 CUR
Racodiscula sp. HBOI 20-XI-98-1-003 BAH (cont.)
Corallistes sp. 2 HBOI 10-XI-02-1-004 BAH
Corallistes sp. 2 HBOI 20-XI-02-5-011 BAH
Corallistes sp. 2 HBOI 31-III-89-1-003 St.Vincent
Corallistes sp. 2 HBOI 10-XI-02-1-003 BAH
Corallistes sp. 2 HBOI 20-VI-89-1-010 GUAD
Corallistes sp. 6 HBOI 14-XI-02-3-007 BAH
1 Neophrissospongia galapagoensis HBOI 8-XII-95-4-007 PAN
Neophrissospongia galapagoensis HBOI 15-XII-95-1-007 PAN
Neophrissospongia galapagoensis HBOI 18-X-95-1-003 GAL
Neophrissospongia galapagoensis HBOI 15-X-95-1-005 GAL
0.71 Neophrissospongia galapagoensis HBOI 15-X-95-1-010 GAL
Neophrissospongia galapagoensis HBOI 27-X-95-1-007 GAL
1 Neophrissospongia galapagoensis HBOI 16-X-95-1-001 GAL
Neophrissospongia galapagoensis HBOI 23-X-95-1-012 GAL
Neophrissospongia galapagoensis HBOI 18-X-95-1-013 GAL
Neophrissospongia sp. 2 HBOI 15-XI-97-3-002 HON
1 Awhiowhio osheai NIWA18332
Awhiowhio osheai NIWA18333
Awhiowhio osheai NIWA25043
Awhiowhio osheai NIWA25042
Awhiowhio osheai NIWA18334
0.87 Pleroma turbinatum NIWA 82329
0.62
0.05


Overview
Astrophorina
Spirophorina
new suborder
OUT
0.97
Isabella mirabilis KR270647 genD
Isabella mirabilis KR270646 genD
Isabella mirabilis KR270661 genC
Isabella mirabilis KX267960 genC
0.94
Isabella mirabilis KX267959 genC
Isabella mirabilis KR270663 genC
Isabella mirabilis KR270662 genC
Isabella mirabilis KR270654 genC
Isabella mirabilis KR270655 genC
Isabella mirabilis KR270656 genC
0.95
Isabella mirabilis KR270675 genE
Isabella mirabilis KR270684 genE
Isabella mirabilis KR270674 genE
Isabella mirabilis KR270673 genE
Isabella mirabilis KR270672 genE
Isabella mirabilis KR270671 genE
Isabella harborbranchi KM185726
1
Isabella harborbranchi KM185725
Isabella harborbranchi KM185728
1
Isabella harborbranchi KM185727
1
Isabella tanoa KX267963
Isabella tanoa KX267962
Isabella tanoa KX267961
0.94
Herengeria sp. 1 LN624189
Herengeria sp. 1 LN624188
Herengeria sp. 1 LN624190
Herengeria sp. 1 LN624187
1
Isabella mirabilis KR270683 genF
Isabella mirabilis KR270682 genF
Isabella mirabilis KR270681 genF
Isabella mirabilis KR270680 genF
Isabella mirabilis KR270679 genF
Isabella mirabilis KR270678 genF
Isabella mirabilis KR270677 genF
Isabella mirabilis KR270676 genF
0.89
Neoschrammeniella fulvodesmus KR270692
Neoschrammeniella fulvodesmus KR270688
Neoschrammeniella fulvodesmus KR270686
Neoschrammeniella fulvodesmus KR270685
Neoschrammeniella fulvodesmus KR270691
Neoschrammeniella fulvodesmus KR270690
Neoschrammeniella fulvodesmus KR270687
Neoschrammeniella castrum QMG318586
1
Pachastrella nodulosa HM592698
Pachastrella ovisternata HM592748
Triptolemma intextum MNHN DCL 4080
0.77
0.71
1
0.67
0.82
Callipelta sp. LN624197
Daedalopelta sp. 2 HBOI 12-XI-02-1-003 BAH, SBD1895
Daedalopelta sp. 1 HBOI 12-X-03-1-006 BAH, SBD1894
0.05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Geodia conchilega HM592742
Geodia macandrewi ZMBN 77924
Geodia cydonium ZMA POR21652
Ecionemia megastylifera ZMBN 81782
Geodia barretti ZSM 20100066 NAO
Geodia barretti ZMB 85202
Geodia megastrella HM592731
Geodia megastrella HM592741
Stelletta clavosa KJ494350
Ecionemia robusta SAM AS1018
Stelletta fibrosa FJ711643
Stelletta tuberosa ZMA POR21665
Geodia phlegraei HM592701
Geodia phlegraei HM592690
Geodia phlegraei EU442196
Geodia gibberosa ZMBN 77928
Geodia vosmaeri ZMBN 85213
Geodia pachydermata HM592732
Rhabdastrella cordata SAM AS1026
Stelletta lactea Mc4945
Ancorina sp. ZMA POR21660
Stelletta clarella ZMA POR21673
Stelletta anthastra KP939317
Dercitus bucklandi HM592716
Dercitus bucklandi HM592674
Stryphnus ponderosus Mc3395
Stryphnus fortis HM592697
Stelletta grubii HM592743
Stelletta dorsigera HM592750
Pachymatisma normani EF564322
Pachymatisma normani EF564326
Pachymatisma johnstonia ZMA POR21442
Pachymatisma johnstonia EF564333
Pachymatisma johnstonia EF564334
Erylus discophorus ZMA POR21716
Erylus mamillaris EU442207
Erylus granularis ZMA POR21656
Melophlus sp. UCMPWC1052
Caminus vulcani EU442205
Erylus topsenti ZMA POR21657
Caminella intuta HM592740
Calthropella geodioides ZMA POR21667
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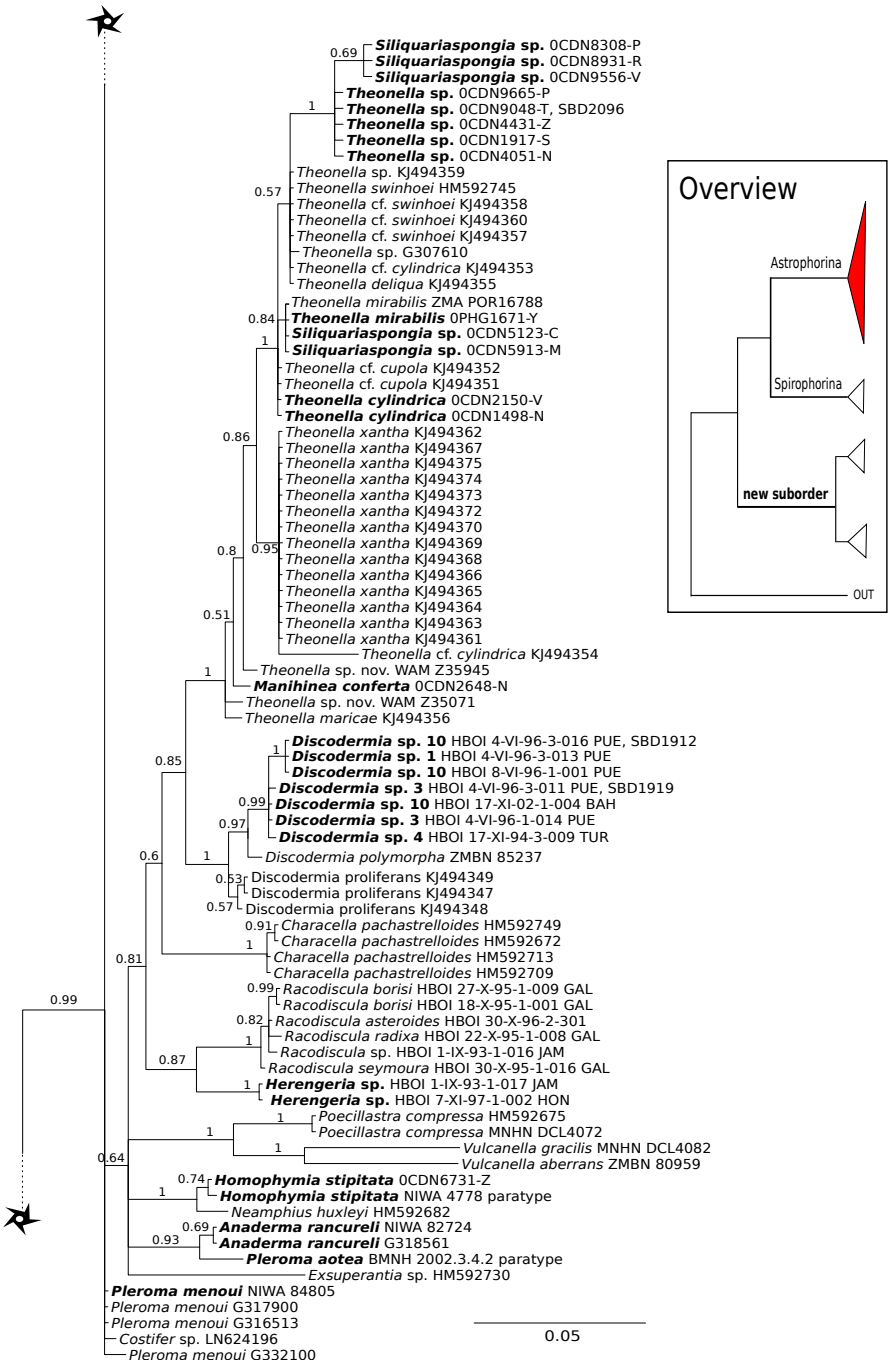
Overview
Astrophorina
Spirophorina
new suborder
OUT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Siliquariaspongia sp. 0CDN8931-R
Siliquariaspongia sp. 0CDN9556-V
Theonella sp. 0CDN9665-P
Theonella sp. 0CDN9048-T, SBD2096
Theonella sp. 0CDN4431-Z
Theonella sp. 0CDN1917-S
Theonella sp. 0CDN4051-N
Theonella sp. KJ494359
Theonella swinhoei HM592745
Theonella cf. swinhoei KJ494358
Theonella cf. swinhoei KJ494360
Theonella cf. swinhoei KJ494357
Theonella sp. G307610
Theonella cf. cylindrica KJ494353
Theonella deliqua KJ494355
Theonella mirabilis ZMA POR16788
Theonella mirabilis 0PHG1671-Y
Siliquariaspongia sp. 0CDN5123-C
Siliquariaspongia sp. 0CDN5913-M
Theonella cf. cupola KJ494352
Theonella cf. cupola KJ494351
Theonella cylindrica 0CDN2150-V
Theonella cylindrica 0CDN1498-N
Theonella xantha KJ494362
Theonella xantha KJ494367
Theonella xantha KJ494375
Theonella xantha KJ494374
Theonella xantha KJ494373
Theonella xantha KJ494372
Theonella xantha KJ494370
Theonella xantha KJ494369
Theonella xantha KJ494368
Theonella xantha KJ494366
Theonella xantha KJ494365
Theonella xantha KJ494364
Theonella xantha KJ494363
Theonella xantha KJ494361
Theonella cf. cylindrica KJ494354
Theonella sp. nov. WAM Z35945
Manihinea conferta 0CDN2648-N
Theonella sp. nov. WAM Z35071
Theonella maricae KJ494356
Discodermia sp. 10 HBOI 4-VI-96-3-016 PUE, SBD1912
Discodermia sp. 1 HBOI 4-VI-96-3-013 PUE
Discodermia sp. 10 HBOI 8-VI-96-1-001 PUE
Discodermia sp. 3 HBOI 4-VI-96-3-011 PUE, SBD1919
Discodermia sp. 10 HBOI 17-XI-02-1-004 BAH
Discodermia sp. 3 HBOI 4-VI-96-1-014 PUE
Discodermia sp. 4 HBOI 17-XI-94-3-009 TUR
Discodermia polymorpha ZMBN 85237
Discodermia proliferans KJ494349
Discodermia proliferans KJ494347
Discodermia proliferans KJ494348
Characella pachastrelloides HM592749
Characella pachastrelloides HM592672
Characella pachastrelloides HM592713
Characella pachastrelloides HM592709
Racodiscula borisi HBOI 27-X-95-1-009 GAL
Racodiscula borisi HBOI 18-X-95-1-001 GAL
Racodiscula asteroides HBOI 30-X-96-2-301
Racodiscula radixa HBOI 22-X-95-1-008 GAL
Racodiscula sp. HBOI 1-IX-93-1-016 JAM
Racodiscula seymoura HBOI 30-X-95-1-016 GAL
Herengeria sp. HBOI 1-IX-93-1-017 JAM
Herengeria sp. HBOI 7-XI-97-1-002 HON
Poecillastra compressa HM592675
Poecillastra compressa MNHN DCL4072
Vulcanella gracilis MNHN DCL4082
Vulcanella aberrans ZMBN 80959
Homophymia stipitata 0CDN6731-Z
Homophymia stipitata NIWA 4778 paratype
Neamphius huxleyi HM592682
Anaderma rancureli NIWA 82724
Anaderma rancureli G318561
Pleroma aotea BMNH 2002.3.4.2 paratype
Exsuperantia sp. HM592730
Pleroma menoui NIWA 84805
Pleroma menoui G317900
Pleroma menoui G316513
Costifer sp. LN624196
Pleroma menoui G332100
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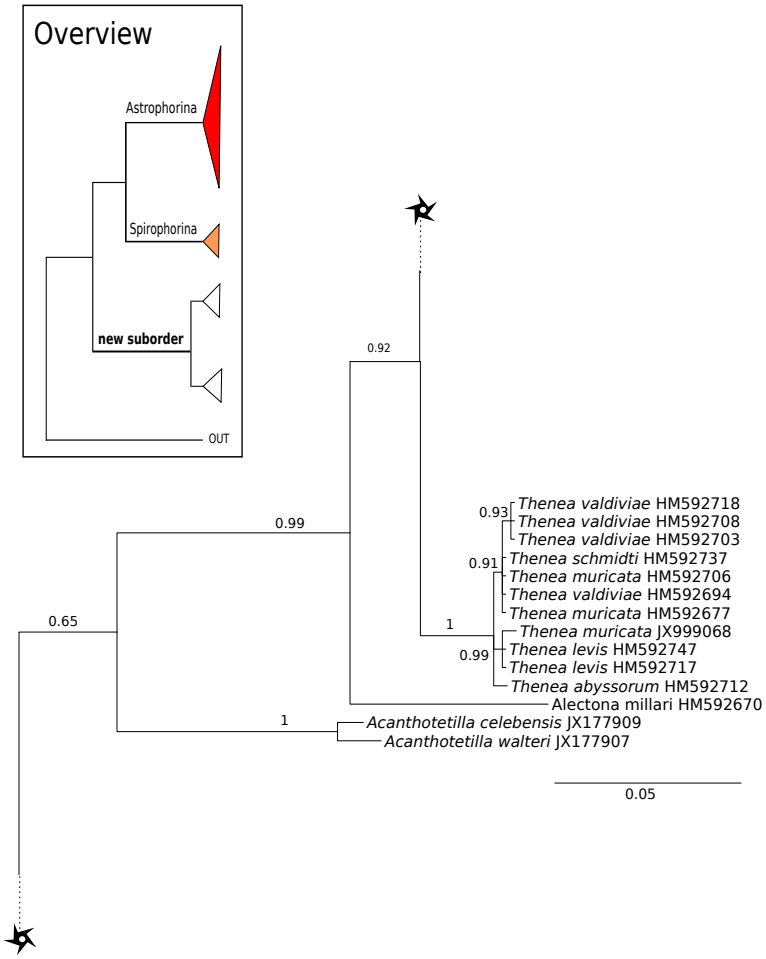
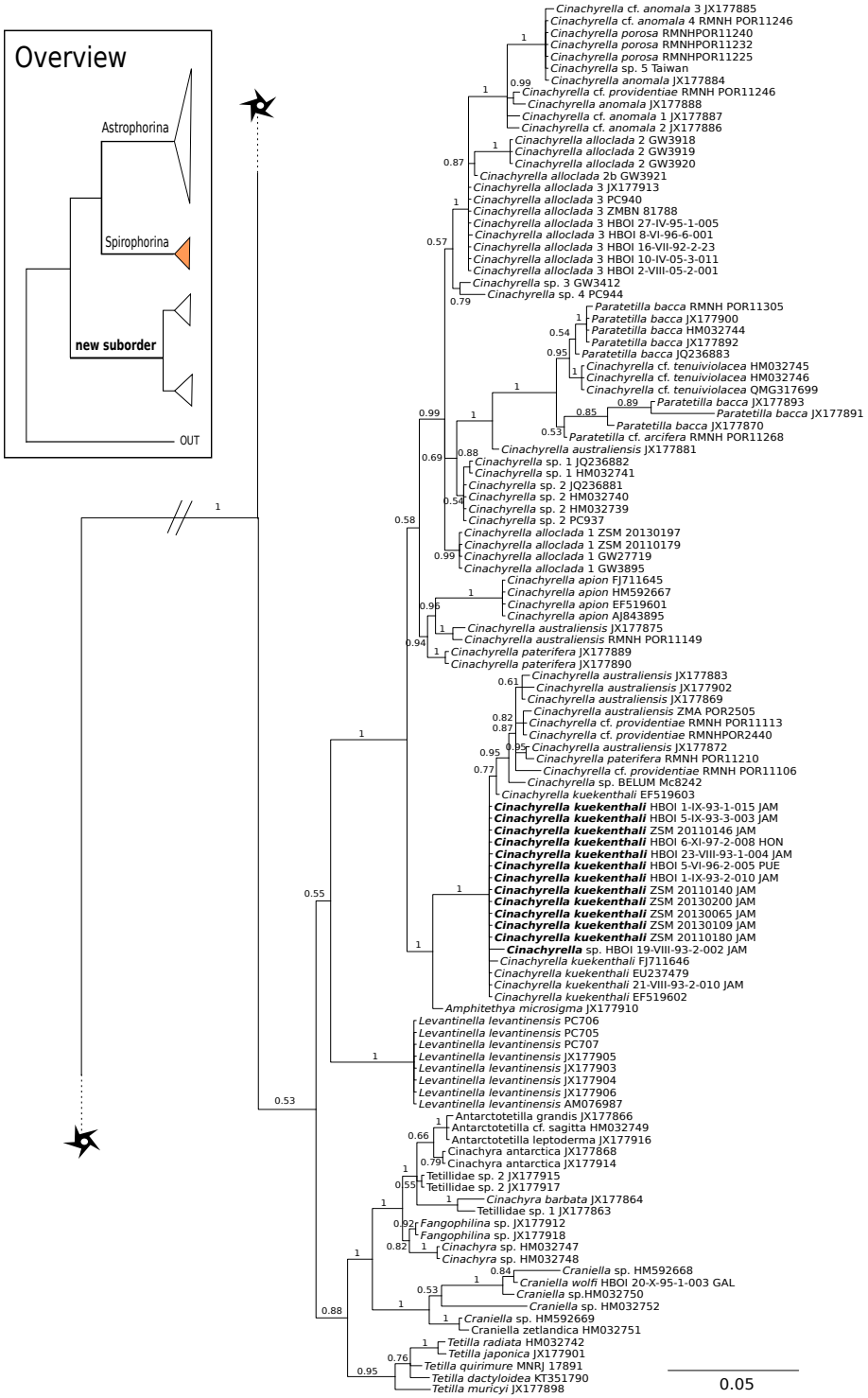
Overview
Astrophorina
Spirophorina
new suborder
OUT




Overview
Astrophorina
Spirophorina
new suborder
OUT
Cinachyrella cf. anomala 3 JX177885
Cinachyrella cf. anomala 4 RMNH POR11246
Cinachyrella porosa RMNHPOR11240
Cinachyrella porosa RMNHPOR11232
Cinachyrella porosa RMNHPOR11225
Cinachyrella sp. 5 Taiwan
Cinachyrella anomala JX177884
Cinachyrella cf. providentiae RMNH POR11246
Cinachyrella anomala JX177888
Cinachyrella cf. anomala 1 JX177887
Cinachyrella cf. anomala 2 JX177886
Cinachyrella alloclada 2 GW3918
Cinachyrella alloclada 2 GW3919
Cinachyrella alloclada 2 GW3920
Cinachyrella alloclada 2b GW3921
Cinachyrella alloclada 3 JX177913
Cinachyrella alloclada 3 PC940
Cinachyrella alloclada 3 ZMBN 81788
Cinachyrella alloclada 3 HBOI 27-IV-95-1-005
Cinachyrella alloclada 3 HBOI 8-VI-96-6-001
Cinachyrella alloclada 3 HBOI 16-VII-92-2-23
Cinachyrella alloclada 3 HBOI 10-IV-05-3-011
Cinachyrella alloclada 3 HBOI 2-VIII-05-2-001
Cinachyrella sp. 3 GW3412
Cinachyrella sp. 4 PC944
Paratetilla bacca RMNH POR11305
Paratetilla bacca JX177900
Paratetilla bacca HM032744
Paratetilla bacca JX177892
Paratetilla bacca JQ236883
Cinachyrella cf. tenuiviolacea HM032745
Cinachyrella cf. tenuiviolacea HM032746
Cinachyrella cf. tenuiviolacea QMG317699
Paratetilla bacca JX177893
Paratetilla bacca JX177891
Paratetilla bacca JX177870
Paratetilla cf. arcifera RMNH POR11268
Cinachyrella australiensis JX177881
Cinachyrella sp. 1 JQ236882
Cinachyrella sp. 1 HM032741
Cinachyrella sp. 2 JQ236881
Cinachyrella sp. 2 HM032740
Cinachyrella sp. 2 HM032739
Cinachyrella sp. 2 PC937
Cinachyrella alloclada 1 ZSM 20130197
Cinachyrella alloclada 1 ZSM 20110179
Cinachyrella alloclada 1 GW27719
Cinachyrella alloclada 1 GW3895
Cinachyrella apion FJ711645
Cinachyrella apion HM592667
Cinachyrella apion EF519601
Cinachyrella apion AJ843895
Cinachyrella australiensis JX177875
Cinachyrella australiensis RMNH POR11149
Cinachyrella paterifera JX177889
Cinachyrella paterifera JX177890
Cinachyrella australiensis JX177883
Cinachyrella australiensis JX177902
Cinachyrella australiensis JX177869
Cinachyrella australiensis ZMA POR2505
Cinachyrella cf. providentiae RMNH POR11113
Cinachyrella cf. providentiae RMNHPOR2440
Cinachyrella australiensis JX177872
Cinachyrella paterifera RMNH POR11210
Cinachyrella cf. providentiae RMNH POR11106
Cinachyrella sp. BELUM Mc8242
Cinachyrella kuekenthali EF519603
Cinachyrella kuekenthali HBOI 1-IX-93-1-015 JAM
Cinachyrella kuekenthali HBOI 5-IX-93-3-003 JAM
Cinachyrella kuekenthali ZSM 20110146 JAM
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Cinachyrella kuekenthali FJ711646
Cinachyrella kuekenthali EU237479
Cinachyrella kuekenthali 21-VIII-93-2-010 JAM
Cinachyrella kuekenthali EF519602
Amphitethya microsigma JX177910
Levantinella levantinensis PC706
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Levantinella levantinensis PC707
Levantinella levantinensis JX177905
Levantinella levantinensis JX177903
Levantinella levantinensis JX177904
Levantinella levantinensis JX177906
Levantinella levantinensis AM076987
Antarctotetilla grandis JX177866
Antarctotetilla cf. sagitta HM032749
Antarctotetilla leptoderma JX177916
Cinachyra antarctica JX177868
Cinachyra antarctica JX177914
Tetillidae sp. 2 JX177915
Tetillidae sp. 2 JX177917
Cinachyra barbata JX177864
Tetillidae sp. 1 JX177863
Fangophilina sp. JX177912
Fangophilina sp. JX177918
Cinachyra sp. HM032747
Cinachyra sp. HM032748
Craniella sp. HM592668
Craniella wolfi HBOI 20-X-95-1-003 GAL
Craniella sp.HM032750
Craniella sp. HM032752
Craniella sp. HM592669
Craniella zetlandica HM032751
Tetilla radiata HM032742
Tetilla japonica JX177901
Tetilla quirimure MNRJ 17891
Tetilla dactyloidea KT351790
Tetilla muricyi JX177898
0.05

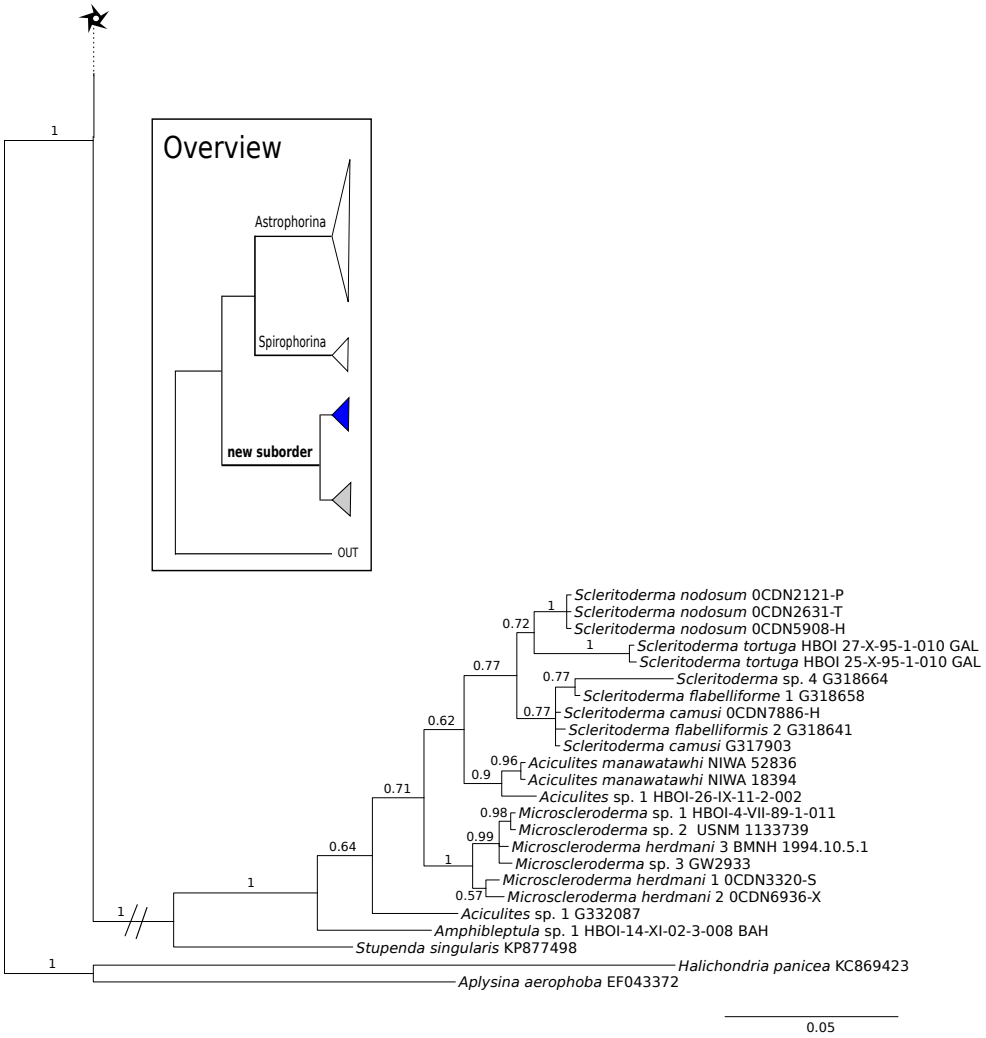

Figure 4. Bayesian Inference phylogeny of Tetractinellida based on *cox1*. Posterior probability (PP) values are provided above or below branches. Self-generated sequences are in bold. Numbers behind taxon names are either voucher numbers or GenBank/ENA accession numbers. Three letter code behind voucher numbers corresponds to the different locations (CUR=Curaçao, BON=Bonaire, StVIN=St. Vincent, MAR=Martinique, GUAD=Guadaloupe, PUE=Puerto Rico, JAM=Jamaica, HON=Honduras, TUR & CAI=Turks & Caicos, BAH=Bahamas, FLO=Florida). Taxa where the morphology was investigated are indicated with their corresponding SBD.

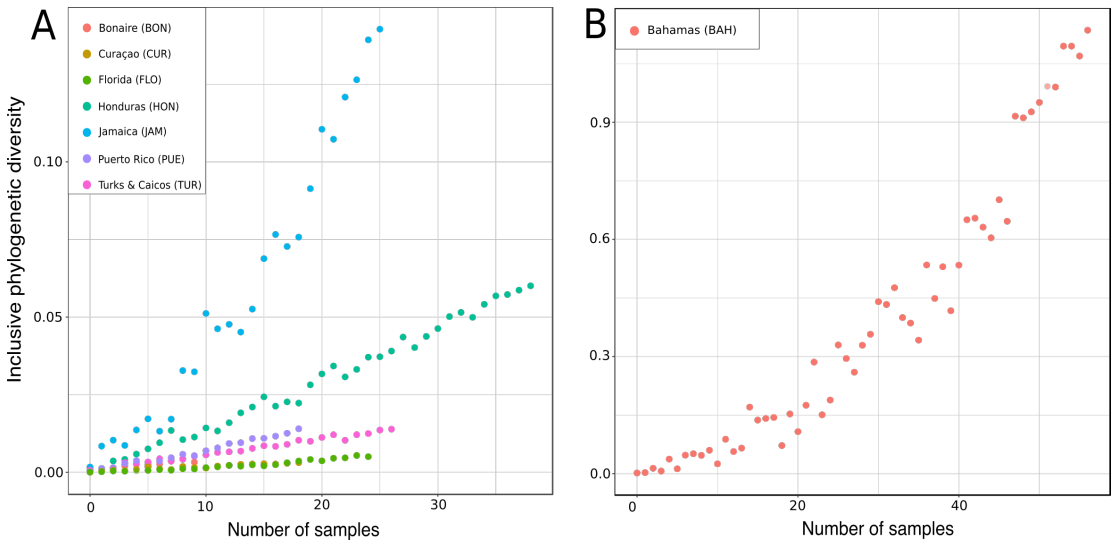

Figure 5. Rarified inclusive phylogenetic diversity (PD_I) curves per marine regions analyzed. For a better visualization the PDI for the Bahamas are illustrated separately (B) due to their larger number of samples.

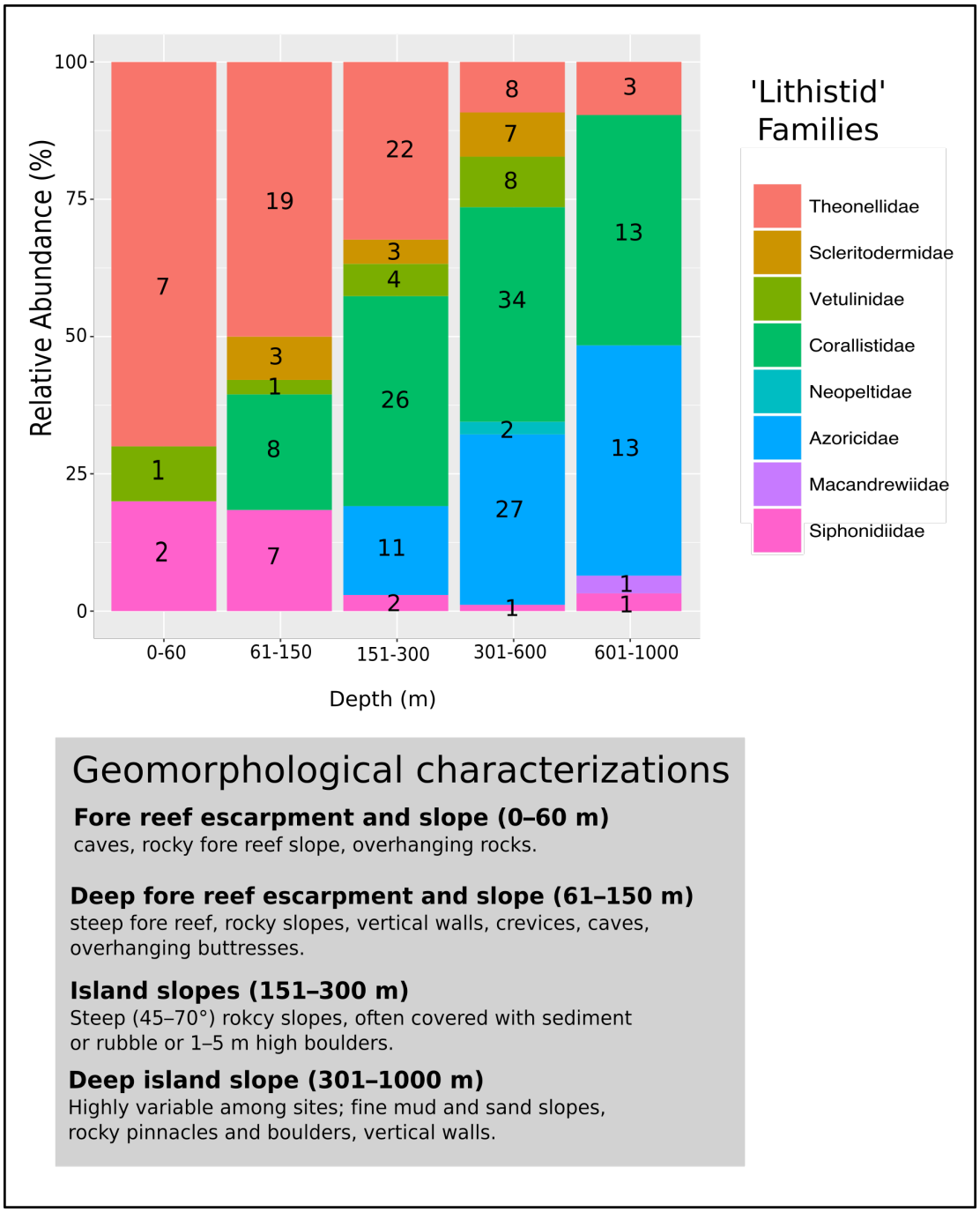

Figure 6. Bathymetric distribution and relative abundance of TWA desma-bearing demosponges based on 234 samples of eight families. Numbers in each bar represent the number of samples investigated. The following genera for each family were included: Leiodermatium (Azoricidae); *Corallistes*, *Herengeria*, *Neophrissospongia* and *Awhiowhio* (Corallistidae); *Macandrewia* (Macandrewiidae); *Daedalopelta* and *Neopelta* (Neopeltidae); *Aciculites*, *Amphibleptula*, *Microscleroderma*, *Scleritoderma* and *Setidium* (Scleritodermidae); *Gastrophanella* and *Siphonidium* (Siphonidiidae); *Discodermia*, *Racodiscula* and *Theonella* (Theonellidae); *Vetulina* (Vetulinidae). Geomorphological characterizations of depth zones are given below the graph and follows Pomponi et al. 2001 and Reed and Pomponi (1997; 2001).