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SYMPOSIUM

Phylogeny and Systematics of Demospongiae in Light of New Small-Subunit Ribosomal DNA (18S) Sequences

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Synopsis The most diverse and species-rich class of the phylum Porifera is Demospongiae. In recent years, the systematics of this clade, which contains more than 7000 species, has developed rapidly in light of new studies combining molecular and morphological observations. We add more than 500 new, nearly complete 18S sequences (an increase of more than 200%) in an attempt to further enhance understanding of the phylogeny of Demospongiae. Our study specifically targets representation of type species and genera that have never been sampled for any molecular data in an effort to accelerate progress in classifying this diverse lineage. Our analyses recover four highly supported subclasses of Demospongiae: Keratosa, Myxospongiae, Haploscleromorpha, and Heteroscleromorpha. Within Keratosa, neither Dendroceratida, nor its two families, Darwinellidae and Dictyodendrillidae, are monophyletic and Dictyoceratida is divided into two lineages, one predominantly composed of Dysideidae and the second containing the remaining families (Irciniidae, Spongiidae, Thorectidae, and Verticillitidae). Within Myxospongiae, we find Chondrosida to be paraphyletic with respect to the Verongida. We amend the latter to include species of the genus *Chondrosia* and erect a new order Chondrillida to contain remaining taxa from Chondrosida, which we now discard. Even with increased taxon sampling of Haploscleromorpha, our analyses are consistent with previous studies; however, *Haliclona* species are interspersed in even more clades. Haploscleromorpha contains five highly supported clades, each more diverse than previously recognized, and current families are mostly polyphyletic. In addition, we reassign *Janulum spinispiculum* to Haploscleromorpha and resurrect *Reniera filholi* as *Janulum filholi* comb. nov. Within the large clade Heteroscleromorpha, we confirmed 12 recently identified clades based on alternative data, as well as a sister-group relationship between the freshwater Spongillida and the family Vetulinidae. We transfer *Stylissa flabelliformis* to the genus *Scopalina* within the family Scopalinidae, which is of uncertain position. Our analyses uncover a large, strongly supported clade containing all heteroscleromorphs other than Spongillida, Vetulinidae, and Scopalinidae. Within this clade, there is a major division separating Axinellidae, Biemnida, Tetractinellida, Bubaridae, Stelligeridae, Raspailiidae, and some species of *Petromica*, *Topsentia,* and *Axinyssa* from Agelasida, Polymastiidae, Placospongiidae, Clionaidae, Spirastrellidae, Tethyidae, Poecilosclerida, Halichondriidae, Suberitidae, and *Trachycladus*. Among numerous results: (1) Spirophorina and its

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family Tetillidae are paraphyletic with respect to a strongly supported Astrophorina within Tetractinellida; (2) Agelasida is the earliest diverging lineage within the second clade listed above; and (3) *Merlia* and *Desmacella* appear to be the earliest diverging lineages of Poecilosclerida.

Introduction

Sponges (Porifera) constitute one of the most diverse metazoan phyla, with more than 8500 known species (van Soest et al. 2012a) and as many as 29,000 species yet to be described (Appeltans et al. 2012). Traditional taxonomy, classification, and identification of sponges are based on anatomical, cytological, and reproductive characteristics, particularly spicules and skeletal architecture (see Hooper and van Soest 2002). Unfortunately, there are a relatively small number of such characters, and many exhibit high levels of plasticity and homoplasy, which have made the taxonomy and classification of sponges (particularly Calcarea and Demospongiae) at all hierarchical levels extremely challenging (e.g., Hooper and van Soest 2002; Boury-Esnault 2006). Fortunately, an ever-growing number of sponge molecular phylogenetic studies have been conducted (e.g., Alvarez et al. 2000; Borchiellini et al. 2000, 2004; McCormack et al. 2002; Erpenbeck et al. 2002, 2004, 2005a, 2005b, 2006, 2007a, 2007d, 2011, 2012a, 2012b; Lavrov et al. 2005, 2008; Nichols 2005; Dohrmann et al. 2006, 2008, 2009, 2011, 2012; Erwin and Thacker 2007; Kober and Nichols 2007; Raleigh et al. 2007; Redmond et al. 2007, 2011; Cárdenas et al. 2010, 2011; Gazave et al. 2010a, 2010b; Morrow et al. 2012; Voigt et al. 2012), most of which increasingly involve the integration of molecular and morphological observations. These studies are rapidly contributing to a better understanding of phylogenetic relationships and evolution within various groups of sponges. Moreover, names of taxa and their meanings, as befits their nature as hypotheses, are in a state of flux as an improved integrative systematics of Porifera emerges. Nevertheless, numerous questions remain about the evolutionary history of Porifera and how best to classify and communicate about its various lineages.

As it currently stands, Phylum Porifera Grant, 1836 comprises four classes: Calcarea Bowerbank, 1864, Demospongiae Sollas, 1885, Hexactinellida Schmidt, 1870, and Homoscleromorpha Bergquist, 1978. Although some early molecular phylogenetic work cast doubt about whether the group is monophyletic (e.g., Collins 1998; Kruse et al. 1998; Borchiellini et al. 2001; Medina et al. 2001; Peterson and Eernisse 2001; Peterson and Butterfield 2005; Sperling et al. 2007) and the debate is not entirely closed (Sperling et al. 2009, 2010), recent phylogenomic analyses have found significant support for a monophyletic Porifera (Philippe et al. 2009; Pick et al. 2010; Nosenko et al. 2013).

Of the four Poriferan classes, Demospongiae is by far the most diverse. Whereas, Calcarea, Homoscleromorpha, and Hexactinellida have approximately 700, 90, and 600 known species, respectively, Demospongiae has roughly 7000 (Cárdenas et al. 2012; van Soest et al. 2012a). As described in numerous recent papers (Cárdenas et al. 2012; Morrow et al. 2012, 2013; Wörheide et al. 2012; Diaz et al. 2013; Hajdu et al. 2013; Hill et al. 2013; Thacker et al. 2013), the higher classification of Demospongiae is in flux as new evidence is brought to bear on relationships within the group. However, progress in understanding the phylogeny of a group proceeds faster than the creation of a phylogenetically sound classification, primarily stemming from issues related to incomplete taxon sampling. First, relevant features that distinguish any particular clade (especially if not corresponding to a traditional taxon) are not easily discerned in the absence of thorough sampling. Second, type species are intimately tied to nomenclatural issues and their absence in a study may prevent changes to the definition of a group even when it becomes known that the group is not monophyletic.

The Porifera Tree of Life project (PorToL) set out to unravel poriferan phylogeny at multiple levels (Thacker et al. 2013). This study more than triples the number of nearly complete small subunit rRNA (18S) gene sequences available, from 220 to 726, in an attempt to further resolve relationships and advance the systematics of Demospongiae. Prior to this study, 94 of 504 demosponge genera, as recognized in the World Porifera Database (van Soest et al. 2013), had 18S sequences in Genbank (January 2013). Our study includes 144 additional genera sampled for 18S, including 44 genera that have never been sampled for any molecular data. Of the 227 genera represented in our dataset, 114 type species are represented, including 78 generated by PorToL.

Materials and methods

Specimens and DNA aliquots

Specimens, tissue subsamples, and DNA aliquots were derived from a number of different sources, including: (1) PorToL-supported expeditions to the

Smithsonian Tropical Research Institute at Bocas del Toro, Panama, in 2009, 2010, 2011, and 2012, where specimens were photographed *in situ*, when possible, collected by SCUBA diving or snorkeling, and placed in 95% ethanol with multiple changes to ensure desiccation; (2) subsamples of specimens from the collections at the Harbor Branch Oceanographic Institute-Florida Atlantic University (HBOI-FAU); (3) subsamples from the National Cancer Institute (NCI) sponge collection held at the National Museum of Natural History, Smithsonian Institution (NMNH); (4) the DNA and tissue collections of one of us (C.C.M.; Morrow et al. 2012, 2013); and (5) DNA aliquots from the Moorea Biocode project, Moorea, French Polynesia. Details of each sample used in this study, including voucher numbers, are presented in Supplementary Table S1.

Extraction, amplification and sequencing of DNA

Extraction of DNA was carried out using either AutoGenPrep 965 high-throughput DNA extraction robotic system (AutoGen) in accordance with the manufacturer's instructions for Whole Blood extraction, or the Biosprint 96 workstation (Qiagen) in conjunction with the QIAGEN Biosprint 96 DNA Blood Kit (cat. no. 940057). Extracted genomic DNA was visualized using gel electrophoresis.

Due to the considerable number and high taxonomic diversity of the samples, there was a large variety in PCR protocols and thermocycling conditions employed. For the majority of samples, the complete 18S rRNA gene was amplified using primers SP18aF and SP18gR (Supplementary Table S2). Degraded DNA samples were amplified in three overlapping fragments using primers SP18aF and 600R18S; 400F18S and 1350R18S; 1200F18S and SP18gR, respectively (Redmond et al. 2007, this study). Polymerase chain reaction (PCR) was carried out in 10 μl aliquots and comprised final concentrations of the following: 0.5 units *Taq* (Biolase DNA polymerase [Bioline USA Inc., Taunton, MA] or GoTaq Flexi DNA polymerase [Promega, Madison, WI]), 0.3 mM of each primer, 0.5 mM dNTPs (Bioline), 1.5 mM magnesium chloride, 2.5× Bovine serum albumin (BSA) (New England BioLabs Inc., Ipswich, MA), and 1× Buffer, DNAase-free Water to bring the volume to 10 μl.

The standard thermocycling protocol was an initial denaturation step of 94°C for 5 min, 30 cycles of 94°C for 1 min, 40–65°C for 30 s, 72°C for 2.5 min, followed by a final extension step of 72°C for 5–10 min. Other modifications included increasing reaction volume to 20 μl, excluding BSA, increasing thermocycling cycles to 40, varying concentration of template DNA and changes to magnesium chloride concentration. For some of the NCI samples, a nested PCR method was used. After initial amplification of the complete gene as described above, 1 μl of this amplicon was used as the template in subsequent PCRs for each of the three overlapping fragments. All PCR products were visualized on 1.5% agarose gel.

To purify the PCR products, 3 μl of a 1 in 5 dilution of ExoSAP-IT for PCR Product Clean-Up (Affymetrix, USB Products) was added to each PCR reaction. These reactions were run through a thermocycling program of 37°C for 30 min followed by 80°C for 20 min. 1 μl of the purified PCR product was subsequently used in the cycle sequencing reaction, which was performed using a dye-labelled dideoxy terminator (Big Dye Terminator v. 3.1). Sephadex G-50 Fine (GE Healthcare Life Sciences, Pittsburgh, PA) was used with MultiScreenHTS-HV Plates (Millipore, Billerica, MA) for clean up of the cycle sequencing reaction products which were then analyzed on either Applied Biosystems 3130xl Genetic Analyzer or Applied Biosystems 3730xl DNA Analyzer.

Phylogenetic analyses

All forward and reverse sequence reads were processed in Geneious (Drummond et al. 2011), which was used for assessing quality, trimming read ends, assembling contigs, and checking for possible non-sponge contaminants using BLAST (blast.ncbi.nlm.nih.gov). A large demosponge dataset was assembled containing 18S rDNA sequences from 742 demosponge taxa (538 generated in this project, 204 from Genbank). The minimum length for inclusion of sequences was put at 1000 bp, which allowed the inclusion of the largest set of taxa (748 unique sequences including outgroups) without hindering phylogenetic signal (data not shown). Smaller datasets of subclasses and various highly supported clades were created and analyzed separately (details in Table 1).

All datasets were analyzed as follows. The online version of the multiple sequence alignment program MAFFT (version 7) (Katoh and Standley 2013) was used for alignment. For larger datasets (>240 taxa), the FFT-NS-i alignment strategy was employed and for the smaller datasets (<240 taxa), the Q-INS-i alignment strategy was used. The latter strategy seeks to incorporate information on secondary structure of RNA when creating the alignment. Gblocks (Castresana 2000; Talavera and Castresana 2007) was utilized to select conserved blocks of the alignments for use in subsequent phylogenetic analyses. Default parameters were used with the exception of "allow

Table 1 Details of all datasets analysed in this study including the MAFFT alignment strategy employed

Dataset	Figure No.	Total No. taxa excluding outgroups	No. newly generated sequences	No. GenBank sequences	MAFFT alignment strategy
Demospongiae	1	742	538	204	FFT-NS-i
Keratosa	2	110	92	18	Q-INS-i
Myxospongiae	3	61	42	19	Q-INS-i
Haploscleromorpha	4	76	50	26	Q-INS-i
Heteroscleromorpha	6	495	354	141	FFT-NS-i
Scopalinidae	7	9	7	2	Q-INS-i
Axinellidae	8	21	11	10	Q-INS-i
Stelligeridae + Raspailiidae	9	43	35	9	Q-INS-i
Tetractinellida	10	115	67	48	Q-INS-i
Bubaridae	11	20	15	5	Q-INS-i
Agelasidae + Hymerhabdiidae	12	32	17	15	Q-INS-i
Large Heteroscleromorpha subclade	13	235	195	40	Q-INS-i
Halichondriidae + Suberitidae	14	48	32	16	Q-INS-i
Core Poecilosclerida	15	148	132	16	Q-INS-i

gap positions,'' which was changed to *All*. For phylogenetic analysis, RAxML version 7.2.6—multithread (Stamatakis 2006) for maximum likelihood (ML) and MrBayes version 3.2.1 for Bayesian (MB) were employed on the computing cluster of the NMNH's Laboratories of Analytical Biology. Under ML, the GTRGAMMA model was used for all analyses. One thousand bootstrap replicates were carried out on each dataset. Under MB, both GTR and mixed models were used. Analyses consisted of two runs of four chains each (three cold and one heated) for 10,000,000 generations and sampled every 1000th tree after a 25% burn-in. Phylogenetic trees were visualized and edited in FigTree v. 1.4 (tree.bio.ed.ac.uk/software/figtree/).

Results

A total of 538 demosponge 18S rRNA gene sequences were generated as part of this study. These 18S sequences represent 225 genera, 130 of which had no 18S sequence data in GenBank (January 2013). Forty-four had no molecular data available and, to our knowledge, have never been included in molecular phylogenetic analyses. Of the 107 type species included in the dataset, 76 were generated by this study. A restricted search in GenBank for demosponge 18S rRNA gene sequences >1000 nt in length resulted in 220 records (2 January 2013), of which 181 were included in this study. In addition, 24 unreleased (as of 19 February 2013) Tetractinellida sequences were kindly provided by Amir Szitenberg (subsequently published in Szitenberg et al. 2013) and included in this study. The largest dataset contained 18S rRNA gene sequences from 742 demosponges plus six hexactinellids employed as outgroups. Table 1 gives details of each; most likely trees based on them are presented in Figs. 1–4 and 6–15, and Bayesian topologies under GTR and mixed models can be found in Supplementary Figs. S1–S28. For purposes of discussion, we define ''high,'' ''moderate,'' ''weak,'' and ''no support'' in terms of support values. High support = 90–100, Moderate = 70–89, Weak = 50–69, No = <49, but present in ML topologies. Similarly, under MB posterior probabilities were defined as: High support = 0.99–1, Moderate = 0.96–0.98, Weak = 0.90–0.95, No = <0.89. These words will be used consistently in the text from this point forward.

Both MB and ML runs yielded similar trees, with minor differences in topologies (see below; Supplementary Figs. S1–S28). In MB analyses, we used the mixed model to explore the GTR model space of substitution rates. For most datasets, a derivate of the HKY model displayed the highest probability, yet none were significant (values < 0.60; Supplementary Table S3).

Discussion

Relationships of the four subclasses

Our broad analysis confirms the existence of the four demosponge subclasses—Keratosa Grant, 1861, Myxospongiae Haeckel, 1866 (also known as

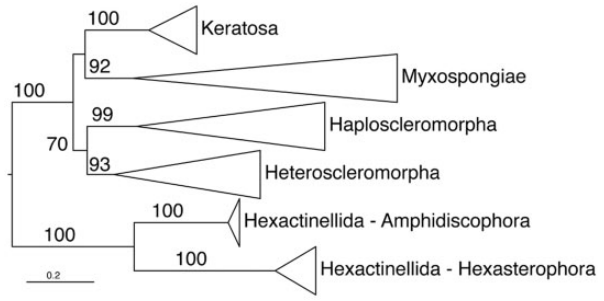

Fig. 1 ML topology of the complete Demospongiae dataset with bootstrap support indices. Topology is rooted on six Hexactinellida. The branches to the four demosponge subclasses have been collapsed. Bootstrap values below 50% have been removed from the tree.

Verongimorpha; Erpenbeck et al. 2012a), Haploscleromorpha Cárdenas, Pérez and Boury-Esnault, 2012 (includes the marine Haplosclerida), and Heteroscleromorpha Cárdenas, Pérez and Boury-Esnault, 2012 (equivalent to the G4 clade of Borchiellini et al. [2004] and Democlavia of Sperling et al. [2009])—each with high support (Fig. 1). Our analyses do not provide strong evidence for the relationships among these groups. However, we consistently find, albeit with no support, Keratosa, a clade consisting of species that do not produce mineral spicules and which are classified in two orders, Dendroceratida and Dictyoceratida, to form the sister group of Myxospongiae, which contains the aspiculose orders Verongida and Chondrosida (exception *Chondrilla* Schmidt, 1862). Some molecular studies have Keratosa and Myxospongiae as sister taxa (Borchiellini et al. 2004; Lavrov et al. 2008; Wang and Lavrov 2008; ML analyses of Hill et al. 2013), whereas others have suggested that Myxospongiae may be the sister group to all other demosponges (Sperling et al. 2009; Bayesian analyses of Hill et al. 2013; Thacker et al. 2013). All analyses at this broad level, however, agree that the subclasses Haploscleromorpha and Heteroscleromorpha, containing species that produce mineral skeletons, share a common ancestor. In our 18S analyses, the support for this relationship is moderate to high (Fig. 1 and Supplementary Figs. S1 and S2). The weight of evidence from so many independent analyses, employing different methods and datasets, suggests that this relationship is very likely true.

Keratosa Grant, 1861

Keratosa constitutes a highly monophyletic group, here (Fig. 1) and elsewhere (Borchiellini et al. 2004; Erpenbeck et al. 2012a), and contains all demosponges that do not have a mineral skeleton, but instead have either a hypercalcified skeleton (*Vaceletia* Pickett, 1982) or a skeleton composed entirely of spongin, which equates to members of the orders Dendroceratida and Dictyoceratida. Erpenbeck et al. (2012a) proposed the presence of a specialized polyvacuolar cell type as a possible distinguishing character for the group. However, this type of cell has been described only within two keratose genera (*Dysidea* Johnston, 1842 and *Aplysilla* Schulze, 1878).

Dendroceratida has two families, Darwinellidae and Dictyodendrillidae, each containing four genera. According to Bergquist and Cook (2002a), the separation of these two families is based on differences in the fiber skeleton. Darwinellids have a completely dendritic fiber skeleton, whereas dictyodendrillids have a skeleton that is reticulate, with the meshes ranging from perfectly regular to slightly irregular (Bergquist and Cook 2002b, 2002c). The 18S data from seven taxa of Dendroceratida, representing both Darwinellidae and Dictyodendrillidae, suggest that the order and both families are not monophyletic (Fig. 2 and Supplementary Figs. S3 and S4). Erpenbeck et al. (2012a), employing both mitochondrial and nuclear data, found the order monophyletic but only with the exclusion of *Spongionella* Bowerbank, 1862 and *Acanthodendrilla* Bergquist, 1995; the distinction of dendroceratid families based on dendritic versus anastomosing spongin skeletons was not upheld. Earlier evidence for the polyphyly of the order appeared in work by Borchiellini et al. (2004) and Schmitt et al. (2005).

Within our analyses, two *Chelonaplysilla* group together, and these form the earliest diverging clade within Keratosa. However, this pairing and their position in relation to the other keratose sponges are not resolved with high support (Fig. 2 and Supplementary Figs. S3 and S4). Three *Igernella* Topsent, 1905 (including the type *I. notabilis* (Duchassaing and Michelotti, 1864)) and *Spongionella* cf. *foliascens* (both Dictyodendrillidae) group with *Aplysilla sulfurea* Schulze, 1878 (Dictyodendrillidae) in a highly supported monophyletic group that forms a sister group to Dictyoceratida. Prior authors have noted problems in the assignment of *Igernella* and *Spongionella* at the familial level (Bergquist and Cook 2002c). Although the first sequence data from *Spongionella* were recently included in a molecular phylogeny, the affinities of the type species *Spongionella pulchella* (Sowerby, 1804) was uncertain in those analyses (Erpenbeck et al. 2012a). In addition, the genus *Aplysilla* Schulze, 1878 is notoriously difficult to describe as the main distinctive feature is an encrusting growth form and all other dendroceratids pass through this growth state prior

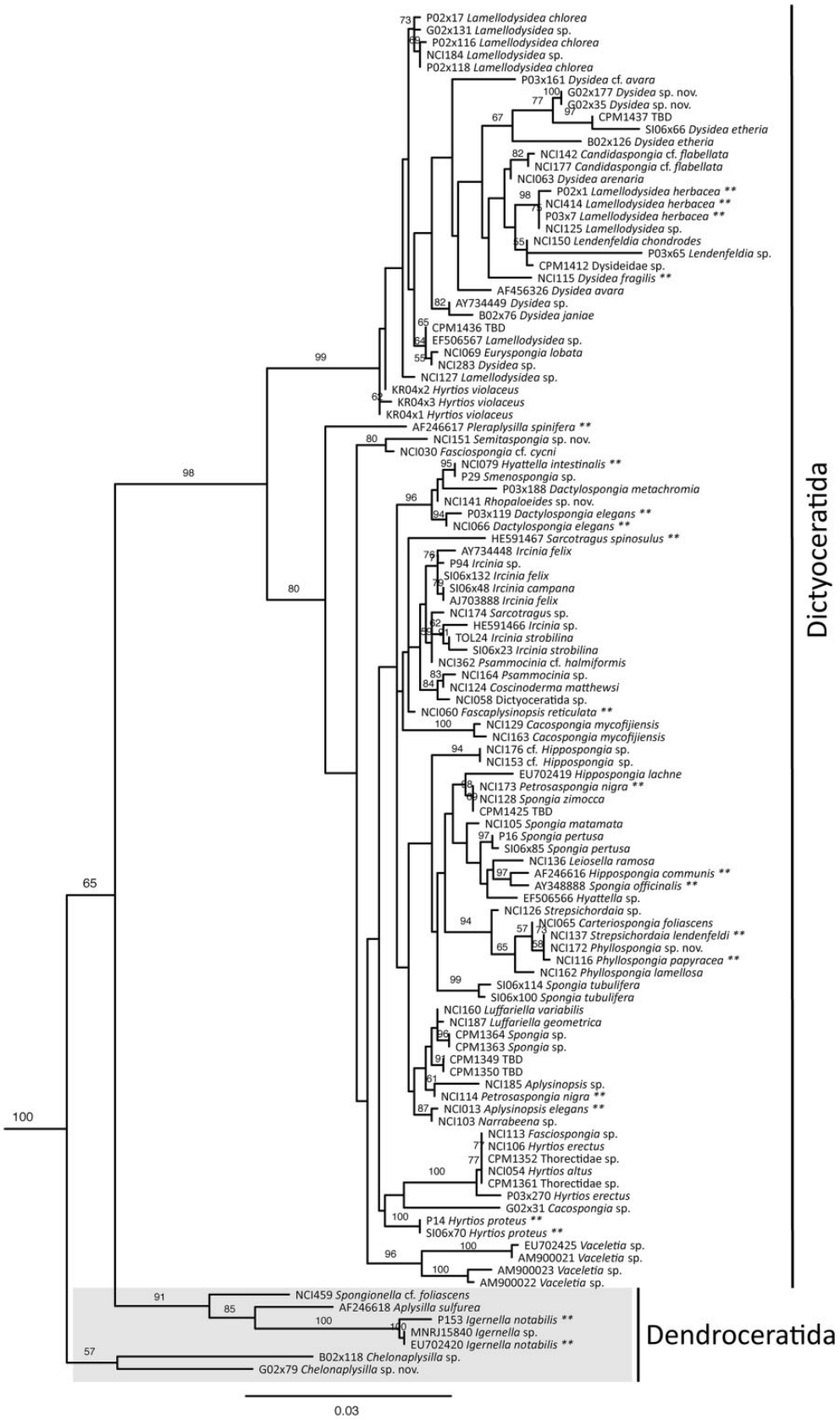

Fig. 2 ML topology of the Keratosa dataset with bootstrap support indices. Topology is rooted on six representatives of the three other subclasses—JR172 *Vetulina* sp., P24 *Stelletta fibrosa*, P07 *Amphimedon compressa*, DQ927307 *Haliclona oculata*, P32 *Halisarca* sp. nov. and L03x26 *Aplysina fistularis* (not shown in the figure). Bootstrap support values below 50% have been removed from the tree. All type species are indicated with **.

to reaching their final form (Bergquist and Cook 2002b). We cannot draw any firm conclusions about the status of this order or its two families until data from more taxa are included, especially from more type species and under-represented and non-represented genera.

The order Dictyoceratida, as currently classified, contains five families: Dysideidae, Irciniidae, Spongiidae, Thorectidae, and Verticillitidae. Verticillitidae had been placed in its own order Verticillitida (Vacelet 2002) until Wörheide (2008) utilized rDNA sequences to conclusively show its affinities within Dictyoceratida. This result was subsequently supported by mitochondrial and additional ribosomal data (Lavrov et al. 2008; Erpenbeck et al. 2012a). Although dictyoceratids can be difficult to deal with taxonomically, 18S supports the grouping of 103 taxa in a monophyletic Dictyoceratida and its division into two major clades (Fig. 2 and Supplementary Figs. S3 and S4), one consisting predominantly of dysideids and the other containing the rest of the families (Irciniidae, Spongiidae, Thorectidae, and Verticillitidae). This result is consistent with other gene-tree topologies (Borchiellini et al. 2004; Erpenbeck et al. 2012a; Thacker et al. 2013).

The first dictyoceratid clade, predominated by Dysideidae, has high support, but many of its internal relationships are not well supported. It includes representatives of a small number of species of Thorectidae—*Candidaspongia* Bergquist, Sorokin and Karuso, 1999, *Lendenfeldia* Bergquist, 1980 (represented by *Lendenfeldia chondrodes* and *Lendenfeldia* sp.), as well as three representatives of *Hyrtios violaceus* (Duchassaing and Michelotti, 1864). These results indicate that several thorectid taxa should be transferred to Dysideidae and highlight the difficulty of assigning taxonomic affiliations for species whose morphological features display a high degree of plasticity and homoplasy. For example, consider the sponge currently known as *H. violaceus*, which was originally described as *Acamas violacea* (Poecilosclerida: Mycalidae) by Duchassaing and Michelotti (1864). de Laubenfels (1936) provided a new description of this species as *Oligoceras hemorrhages* (Dictyoceratida: Thorectidae); subsequently, Bergquist (1980) transferred it to the genus *Hyrtios* Duchassaing and Michelotti, 1864 (Dictyoceratida: Thorectidae). Thacker et al. (2007) reported that the cyanobacterial symbionts (*Oscillatoria spongeliae*) of this species are closely related to those of *Lamellodysidea* Cook and Bergquist, 2000 (Dictyoceratida: Dysideidae); furthermore, the choanocyte chambers of this species are generally obscured by the presence of a large amount of sand. Our analyses support the conclusion that this species is part of Dysideidae. However, *Lamellodysidea* does not form a monophyletic group within Dysideidae; additional morphological data and more quickly evolving genetic markers are needed to determine whether *H. violaceus* should be assigned to *Dysidea*, *Lamellodysidea*, or another genus within Dysideidae. *Lendenfeldia* and *Candidaspongia* also contain filamentous cyanobacterial symbionts (*O. spongeliae*) similar to those present in *Lamellodysidea* (Bergquist et al. 1999; Ridley et al. 2005). Notably, this dataset includes the only molecular data yet collected for *Candidaspongia* and suggests that the subfamily Phyllospongiinae must be reexamined to determine which genera and species should be assigned to Dysideidae and which to Thorectidae.

The second dictyoceratid clade is moderately to weakly supported and contains most of the species representing the families Irciniidae, Spongiidae, Thorectidae, and Verticillitidae (Fig. 2 and Supplementary Figs. S3 and S4). There was very limited resolution within this clade and only a small number of internal relationships have high support. Many of the results are consistent with the mtCOI and 28S gene-sequence data of Erpenbeck et al. (2012a). One exception was the position of *Narrabeena* Cook and Bergquist, 2002. Erpenbeck et al. (2012a) recovered *Narrabeena lamellata* Bergquist, 1980 with the verongids; however, the *Narrabeena* specimen in our analyses groups within this second dictyoceratid clade. Until further specimens of this genus are included in other analyses, no firm conclusion can be drawn.

A review of feeding specificity in the sponge-feeding Chromodorididae (Nudibranchia: Mollusca) by Rudman and Bergquist (2007) found that different genera of the nudibranch family Chromodorididae were specialized feeders on either Dysideidae or Thorectidae, which implies taxonomic acumen in these nudibranchs. This specialization by the nudibranch predators probably indicates significant chemical anti-feedant compounds manufactured by the separate clades of sponges and further corroborates the separation of Dysideidae from Thorectidae as seen in our results.

Myxospongiae Haeckel, 1866

Myxospongiae was elevated to the rank of sub-class by Maldonado (2009) for the clade containing Chondrosida (Chondrillidae and Halisarcidae) and Verongida (Aplysinellidae, Aplysinidae, Ianthellidae, Pseudoceratinidae). Erpenbeck et al. (2012a) proposed the alternative name Veronginomorpha for this same clade based on a lack of correspondence

between the original and present composition of Myxospongiae, but this is true for many taxa throughout biology and the present understanding of Myxospongiae is well established (Borchiellini et al. 2004; Maldonado 2009; Cárdenas et al. 2012; Wörheide et al. 2012; Hill et al. 2013; Thacker et al. 2013). Nearly, all sponges in this clade lack an authigenic mineral skeleton, with the exception of *Chondrilla,* and megascleres are never present. As Erpenbeck et al. (2012a) pointed out, the group is quite heterogeneous, also including taxa that lack skeletons completely, and united only by cellular and developmental characters. Our dataset is composed of 12 Chondrosida, 47 Verongida, and 1 GenBank sequence labeled *Smenospongia aurea* (Hyatt, 1875) (Dictyoceratida: Keratosa). The topology of the trees obtained show two sister-clades: one with *Chondrilla* spp. and *Halisarca* spp. and the other with *Chondrosia* spp. and Verongida (Fig. 3 and Supplementary Figs. S5 and S6).

The order Chondrosida was erected for the unique family Chondrillidae (Boury-Esnault and Lopès 1985). Currently, this order contains two families: Chondrillidae (with four accepted genera—*Chondrilla*, *Chondrosia* Nardo, 1847, *Thymosia* Topsent, 1895, and *Thymosiopsis* Vacelet and Perez, 1998) and the recently added, monogeneric Halisarcidae (Ereskovsky et al. 2011). A more in-depth discussion of the history of this group can be found in Cárdenas et al. (2012).

All members of *Halisarca* Johnston, 1842 lack a fibrous or mineral skeleton (Bergquist and Cook 2002d). Three specimens of a new species of *Halisarca* are newly included in our analyses and cluster in a highly supported clade with *Halisarca dujardinii* Johnston, 1842 (Fig. 3 and Supplementary Figs. S5 and S6). A highly supported sister-group relationship is found between *Halisarca* and representatives of *Chondrilla* (Fig. 3 and Supplementary Figs. S5 and S6). However, species of *Chondrosia*, the nominal genus of Chondrosida, do not group with *Chondrilla* and *Halisarca*, but instead are highly supported as the sister to all verongid representatives (Fig. 3 and Supplementary Figs. S5 and S6), rendering the order Chondrosida paraphyletic. Previous molecular phylogenetic studies have demonstrated the non-monophyletic nature of Chondrillidae, also indicating that *Chondrosia* is not most closely related to the other genera of Chondrillidae (Borchiellini et al. 2004; Erpenbeck et al. 2007a, 2012a; Thacker et al. 2013). The bundles of interstitial collagen fibrils in the cortex of *Chondrosia* could be homologous to those present within the pith of the fibers of *Aplysina* (Garrone 1978). Chondrosiidae Schultze, 1877, is considered a junior synonym of Chondrillidae (Boury-Esnault 2002) but in light of available evidence we recommend: (1) the family Chondrosiidae be resurrected for *Chondrosia* and included within an amended Verongida and (2) a new order Chondrillida be created to contain Chondrillidae and Halisarcidae (see definitions below).

Verongida currently has four families and 10 valid genera. In the 18S tree reconstruction, a total of 48 taxa grouped within the monophyletic clade, 35 of which were new sequences. The number of genera without a skeleton and placed within Verongida is increasing with new molecular phylogenies based on a higher number of taxa. Diaz et al. (2013) described a number of new askeletal taxa from this group and deals with their relationships within this clade (using analyses both of 18S and of mitochondrial COI).

In our 18S analyses (Fig. 3 and Supplementary Figs. S5 and S6), four representatives of the askeletal *Hexadella* Topsent, 1896 (Ianthellidae) were the first to diverge. There was high support for *Anomoianthella* Bergquist, 1980 and *Ianthella* Gray, 1869 clustering with a new genus of askeletal Ianthellidae (see Diaz et al. 2013), but this family is not supported as monophyletic (Fig. 3 and Supplementary Figs. S5 and S6). Taking into account, the sister relationship of the askeletal *Chondrosia* to the verongids and the basal position of the askeletal *Hexadella* within this group, the ancestral verongid likely lacked a skeleton.

The remaining verongids, representing three families characterized by the possession of diplodal choanocyte chambers (Bergquist and Cook 2002e)—Aplysinellidae (represented by *Suberea* Bergquist, 1995), Aplysinidae (represented by *Aiolochroia* Wiedenmayer, 1977, *Aplysina* Nardo, 1834, *Verongula* Verrill, 1907), and Pseudoceratinidae (represented by *Pseudoceratina* Carter, 1885)—are highly supported as a clade (Fig. 3 and Supplementary Figs. S5 and S6). Within this clade, 18S rRNA has low variability and most relationships are uncertain. Nevertheless, this clade is split into two lineages, one highly supported containing most sampled representatives of *Aplysina*, the other unsupported containing *Aiolochroia crassa* (Hyatt, 1875), *Aplysina lacunosa* (Lamarck, 1814), *Pseudoceratina arabica* Keller, 1889, *Pseudoceratina purpurea* (Carter, 1885), two distinct *Pseudoceratina* spp., *Suberea creba* Bergquist, 1995, *Verongula gigantea* (Hyatt, 1875), representatives of two new Pseudoceratinidae, and *Smenospongia aurea*.

Smenospongia Wiedenmayer, 1977 is classified with Thorectidae (Dictyoceratida) due to the

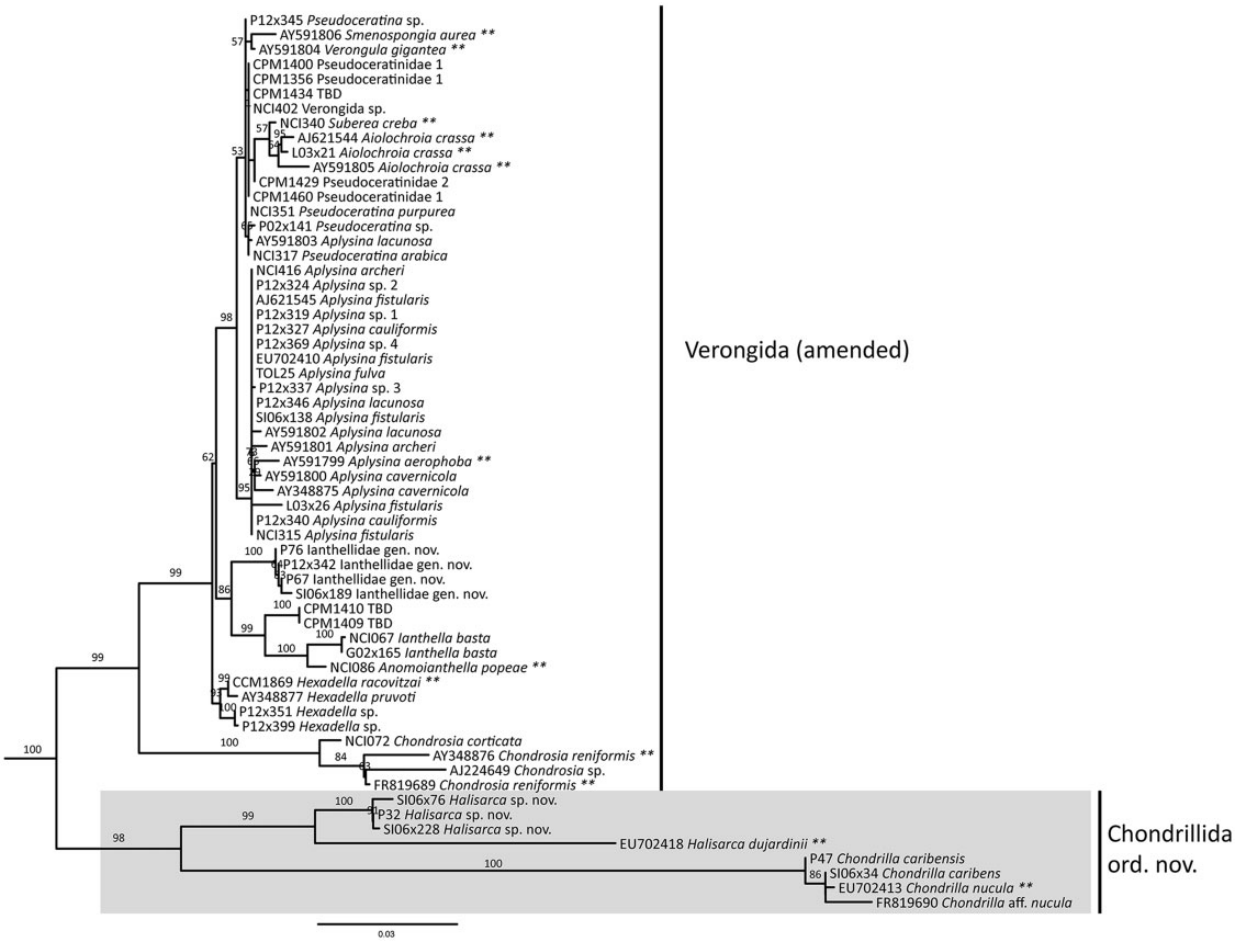

Fig. 3 ML topology of the Myxospongiae dataset with bootstrap support indices. Topology is rooted on six representatives of the three other subclasses—JR172 *Vetulina* sp., P24 *Stelletta fibrosa*, P07 *Amphimedon compressa*, DQ927307 *Haliclona oculata*, G02x131 *Lamellodysidea* sp., G02x79 *Chelonaplysilla* sp. nov. (not shown in the figure). Bootstrap support values below 50% have been removed from the tree. All type species are indicated with **.

presence of tryptophane-related secondary metabolites—compounds that are not found in verongids (Bergquist 1980). In our analyses, one representative of *Smenospongia* (*S.* sp.) groups with dictyoceratids as expected (Fig. 2 and Supplementary Figs. S3 and S4, Thacker et al. 2013); however, *S. aurea,* the type species of the genus, falls within the verongids. The latter sequence is from GenBank and identification of the specimen could not be confirmed, preventing firm conclusion on the status of this genus.

At the familial level, Aplysinidae is paraphyletic due to the inclusion of Pseudoceratinidae and Aplysinellidae, a result seen previously (Erwin and Thacker 2007; Erpenbeck et al. 2012a). *Aiolochroia* is within Aplysinidae, although its familial affinity is uncertain due to its fiber skeleton possessing features of both Aplysinidae and of Aplysinellidae (Bergquist and Cook 2002f; Erwin and Thacker 2007). The high support for the placement of *A. crassa* within a large clade of Aplysinidae, and not with Ianthellidae, contradicts the results of Erwin and Thacker (2007) and Erpenbeck et al. (2012a) and might reflect the much higher level of taxon sampling used in this study.

New Taxonomic Definitions within Myxospongiae

Verongida Bergquist, 1978

Five families: Aplysinidae Carter, 1875; Aplysinellidae Bergquist, 1981; Pseudoceratinidae Carter, 1885; Ianthellidae Hyatt, 1875 and Chondrosiidae Schulze, 1877.

Definition: Verongida includes Myxospongiae in which the fibrous skeleton, when present, is either anastomosing or dendritic in construction. Reproduction is always oviparous (Bergquist and Cook 2002d).

Chondrosiidae Schulze, 1877 new definition

Monogeneric family: Genus *Chondrosia.*

Definition: Verongida without skeleton, but with a well-developed cortical skeleton made of interlacing

bundles of several hundred elementary collagen fibrils (each fibrils 15–22 nm in diameter). Reproduction is oviparous and the larva is a blastula.

Chondrillida new order
Two families: Halisarcidae Schmidt, 1862 and Chondrillidae Gray, 1872.
Definition: Myxospongiae in which the skeleton can be absent, but when present is composed of nodular spongin fibers or aster microscleres.

Halisarcidae Schmidt, 1862

Monogeneric family: Genus *Halisarca*.
Definition: Chondrillida without skeleton, in which the choanocyte chambers are tubular, branched, and wide-mouthed. Reproduction viviparous; the larvae are incubated dispherulae. Skeleton is fibrillar collagen only, highly organized in the ectosome (modified from Bergquist and Cook 2002c).

Chondrillidae Gray, 1862

Three genera: *Chondrilla* with aster microscleres, *Thymosia* with nodular spongin fibers, *Thymosiopsis* without skeleton.
Definition: Chondrillida in which the skeleton can be absent, but when present is composed of nodular spongin fibers or aster microscleres (Boury-Esnault 2002).

Haploscleromorpha Cárdenas, Pérez and Boury-Esnault 2012

Haploscleromorpha refers to marine sponges of the order Haplosclerida Topsent, 1928 (see Cárdenas et al. 2012). This group comprises an enormous diversity of sponges in terms of habitat and species and is characterized by isodictyal skeletons composed of diactinal megascleres (van Soest and Hooper 2002). Classification of these marine sponges is hindered by high species richness, combined with low diversity of morphological characters (de Weerdt 1985; van Soest and Hooper 2002). Although various molecular phylogenetic results have recovered a highly supported monophyletic Haploscleromorpha, many of the internal relationships contradict traditional classification (the two suborders, five of the six families, and many genera are not monophyletic), highlighting the severity of the problem of classifying this group (McCormack et al. 2002; Erpenbeck et al. 2004; Raleigh et al. 2007; Redmond et al. 2007, 2011; Thacker et al. 2013).

The 18S data provide strong support for the monophyly of Haploscleromorpha (Fig. 1 and Supplementary Figs. S1 and S2), as well as five distinct clades within it (Fig. 4 and Supplementary Figs. S7 and S8). Each of these five distinct clades contains members of multiple families, indicating that almost every family within Haploscleromorpha is polyphyletic (Calcifibrospongiidae was not represented). Three of these clades (A, B, and C) have been described previously by Redmond et al. (2011) but in our analyses they are larger and more species-rich due to the addition of 51 new haplosclerid sequences (Fig. 4). Similarly, diversity within the new clades D and E, which were previously represented by *Dasychalina fragilis* Ridley and Dendy, 1886 and *Siphonodictyon mucosum* Bergquist, 1965, respectively (Redmond et al. 2007, 2011), are now known to be more diverse (Fig. 4).

Chalinidae has a worldwide distribution and contains five genera with approximately 450 species (van Soest et al. 2013). The taxonomic history of this family is long and complicated. Twenty-seven genera have been described but de Weerdt (2002) only included four valid genera, with six subgenera of *Haliclona* Grant, 1836. As a result, *Haliclona* is the most species-rich (>400 species) genus of the phylum Porifera. *Haliclona* is distributed across Haploscleromorpha (Fig. 4 and Supplementary Figs. S7 and S8). For the first time, members of this genus are positioned within Clade C with Niphatidae van Soest, 1980 and Petrosiidae van Soest, 1980. *Haliclona curacaoensis* van Soest, 1980 and multiple *Haliclona walentinae* Díaz, Thacker, Rützler and Piantoni, 2007 cluster strongly with two *Niphates* Duchassaing and Michelotti, 1864. *Haliclona* is also positioned within Clade B and there is high support for the sister relationship of (*Haliclona vansoesti* de Weerdt, de Kluijver and Gomez, 1999 + *H. tubifera* George and Wilson, 1919) and (*Xestospongia bocatorensis* Díaz, Thacker, Rützler and Piantoni, 2007 + *Neopetrosia carbonaria* (Lamarck, 1814)). Clade A is predominantly Chalinidae and Callyspongiidae with multiple species of *Haliclona* interspersed with species of *Callyspongia* Duchassaing and Michelotti, 1864. In comparison to previous studies (Redmond et al. 2007, 2011), this clade contains three additional *Haliclona* species: *H. manglaris* Alcolado, 1984, *H. fascigera* (Hentschel, 1912), and *H. tubifera* (George and Wilson, 1919). Since, the skeletal architecture of *Haliclona* is quite simple, repeated secondary losses of characters (specifically masses of spicules at the base of the sponge and multispicular fiber tracts) in multiple lineages might explain the polyphyletic pattern we observe.

The position of *D. fragilis* as a lone taxon within Haploscleromorpha was previously inconclusive (Redmond et al. 2007). With the addition of further taxa, 18S data suggest that it is part of a highly supported clade (Clade D) with *Gelliodes callista* de

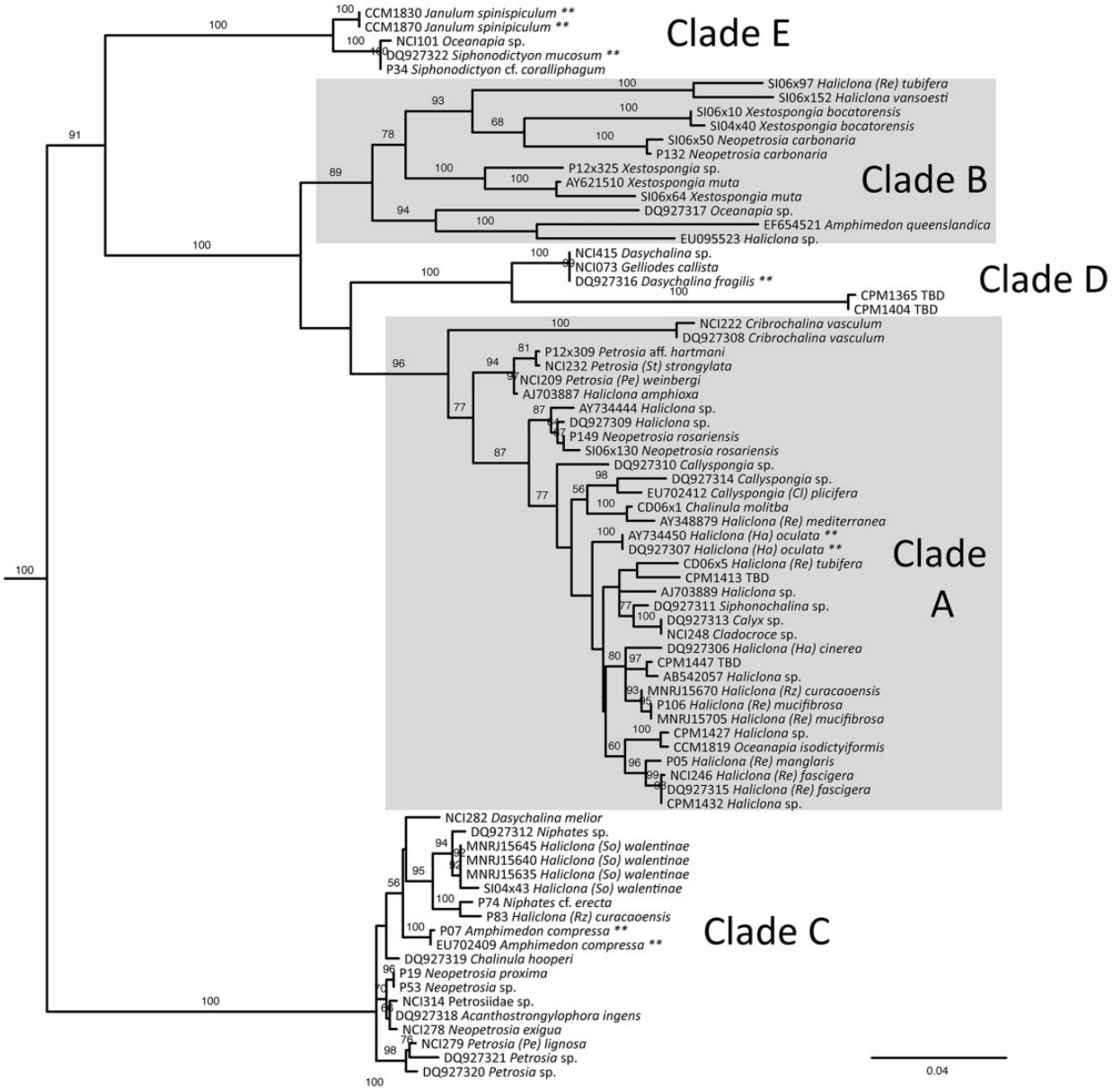

Fig. 4 ML topology of the Haplosclerida dataset with bootstrap support indices. Topology is rooted on six representatives of the three other subclasses—JR172 *Vetulina* sp., P24 *Stelletta fibrosa*, P32 *Halisarca sp. nov.*, and L03x26 *Aplysina fistularis*, G02x131 *Lamellodysidea* sp., G02x79 *Chelonaplysilla* sp. nov. (not shown in the figure). Bootstrap support values below 50% have been removed from the tree. All type species are indicated with **. Subgenera are indicated as follows: *Cl* = *Callyspongia*, *Ha* = *Haliclona*, *Rz* = *Rhizoneria*, *Re* = *Reneira*, *So* = *Soestella*.

Laubenfels, 1954 and two sequences from unidentified sponges collected as part of the Moorea Biocode project. The branch to these latter two taxa is long and could possibly represent a new genus, but the two specimens collected were extremely small and an exact identification has not been possible.

What initially seemed to be an unexpected relationship in our tree was the grouping of two representatives of *Janulum spinispiculum* (Carter, 1876) (collected on the West Coast of Ireland) with *Siphonodictyon mucosum* and *Oceanapia* sp. *Janulum* is considered a monospecific genus most recently assigned, with question, to *Lithoplocamia* Dendy, 1922 (Raspailiidae Nardo, 1833; Poecilosclerida) Hooper (2002; see below for discussions on Raspailiidae and Poecilosclerida). *Janulum spinispiculum* was originally described as *Isodictya spinispiculum* Carter, 1876 not long after Bowerbank (1864) described *Isodictya,* for which he had a haplosclerid-like concept, with emphasis laid solely on the reticulated skeleton with ascending primary fibres and transverse secondary ones. This indicates that

Carter (1876), when describing *Isodictya spinispiculum*, had a more haplosclerid idea for this species, a concept with which Vacelet (1969) agreed. *Janulum spinispiculum* has a skeleton composed of acanthostrongyles arranged in an isodictyal reticulation. In the acanthostrongyles, the spination is restricted to the middle region and the ends are smooth and rounded (Fig. 5). Hooper (2002), in assigning *J. spinispiculum* to *Lithoplocamia,* questioned whether the acanthostrongyles were indeed the only class of spicule present or whether some types of spicules had been overlooked. Careful examination of our material confirms that acanthostrongyles are the only spicule type found in this species. Both morphological and molecular evidence strongly suggest that *Janulum spinispiculum* should be reassigned to Haplosclerомorpha, as already suggested by Vacelet (1969). In the course of our study, one of us (C.C.M.) examined type material from *Reniera filholi* Topsent 1890, an allied species that has been synonymized with *Janulum spinispiculum* (van Soest et al. 2013). While the spicules of the two species are similar, there are also clear differences (Fig. 5). Externally *J. spinispiculum* is a thin white crust while specimens of *R. filholi* are subspherical in shape and yellowish/gray in color. We do not have molecular data from *Reniera filholi*, but we take this opportunity to resurrect it as a distinct species and assign it to *Janulum*, as *Janulum filholi* comb. nov.

Heteroscleromorpha Cárdenas, Pérez and Boury-Esnault 2012

Heteroscleromorpha is by far the largest subclass of Demospongiae, containing approximately 5000 species (Cárdenas et al. 2012). Morrow et al. (2012) recently carried out a large molecular (nuclear 28S and mitochondrial COI) and morphological assessment of this subclass. This work proposed and described a number of new clades and relationships in this group and also suggested several amendments to taxa. The 18S analyses presented here continues this work as many specimens overlap between the two studies. The 14 named clades from Morrow et al. (2012) are retrieved (Fig. 6; branches collapsed, but see subsequent figures and Supplementary Figs. S9 and S10 for all taxa).

The first group to diverge within our ML analysis of Heteroscleromorpha comprises Spongillida plus the family Vetulinidae (Fig. 6). Only a small representation of freshwater taxa was included in our

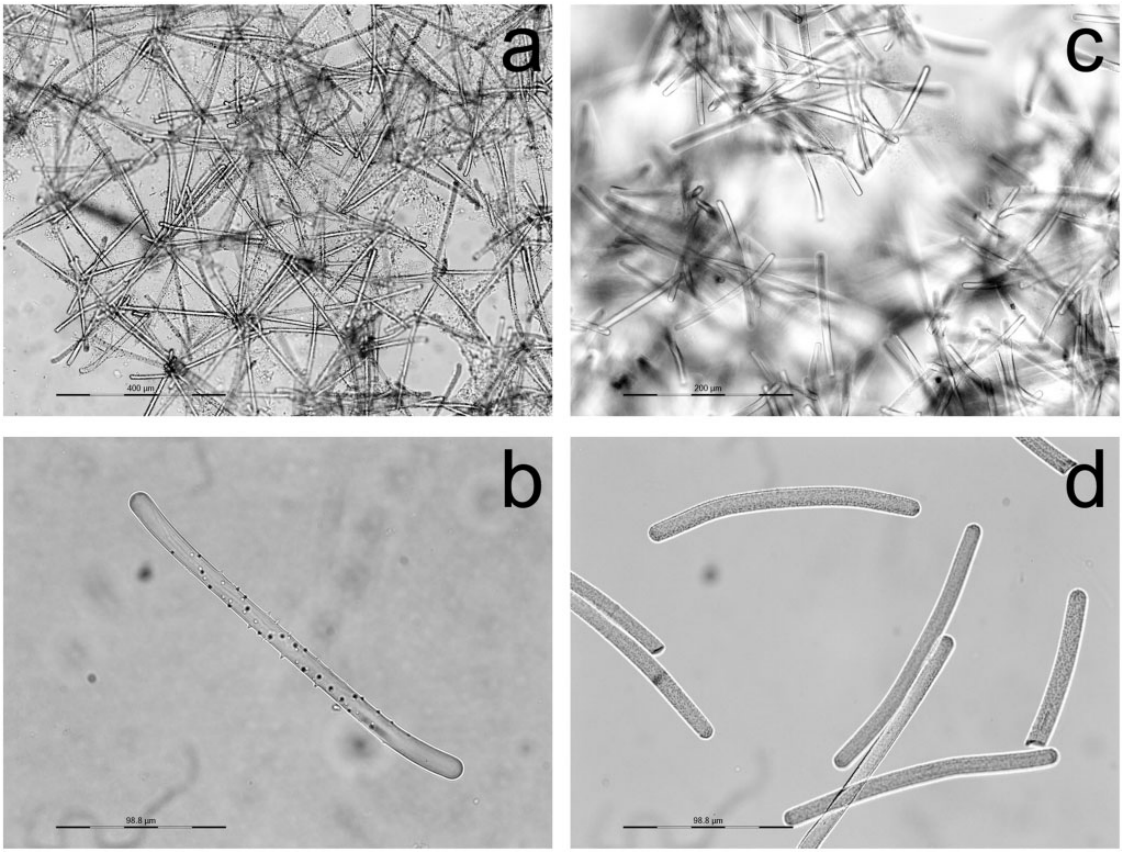

Fig. 5 *Janulum spinipiculum* (**A**, **B**) and *Janulum filholi* comb. nov. (**C**, **D**) skeleton and spicules.

analyses and they formed a monophyletic group with 100% support, but we did not intend to address their internal relationships. Recent work on Spongillida can be found elsewhere (e.g., Addis and Peterson 2005; Meixner at al. 2007; Itskovich et al. 2008; Erpenbeck et al. 2011; Cárdenas et al. 2012; Wörheide et al. 2012). Of considerable interest is the reaffirmation that the genus *Vetulina* Schmidt, 1879 is strongly supported as the sister group of the freshwater sponges (Fig. 6 and Supplementary Figs. S9 and S10). *Vetulina* is a monospecific genus in a monogeneric family Vetulinidae known only from the Caribbean. The species has neither ectosomal spicules nor microscleres and choanosomal spicules are articulated (the so-called lithistid condition) acrepid polyaxial desmas (Pisera and Lévi 2002). Addis and Peterson (2005) included *Vetulina stalactites* Schmidt, 1879 in their phylogenetic analyses of freshwater sponges; however, the 18S rRNA gene sequence used was only 1251 bp in length and shorter than the other taxa in that study. Here a 1741-bp long 18S sequence from *Vetulina* sp. was generated and the two *Vetulina* group together and are consistently and highly supported as sister to Spongillida. It is unclear how to relate the morphology of *Vetulina* to that of freshwater sponges.

Scopalinidae was recovered as a highly supported monophyletic group (Fig. 6 and Supplementary Figs. S9 and S10) containing a total of nine taxa. Previous studies have shown *Scopalina ruetzleri* (Wiedenmayer, 1977) as sister to the freshwater sponges (Nichols 2005; Redmond et al. 2007); however, in our various 18S analyses, this relationship is taxon-dependent. There was high support in the larger demosponge trees for Scopalinidae + Spongillida + *Vetulina* (ML not shown; Supplementary Figs. S1 and S2) but this relationship was not supported when just Heteroscleromorpha was analyzed (Fig. 6 and Supplementary Figs. S9 and S10). Scopalinidae currently has *Scopalina* Schmidt, 1862, *Svenzea* Alvarez, van Soest and Rützler, 2002, and *Stylissa flabelliformis* (Hentschel, 1912) assigned to it (Morrow et al. 2012), the latter assignment based on molecular data presented by Alvarez et al. (2000).

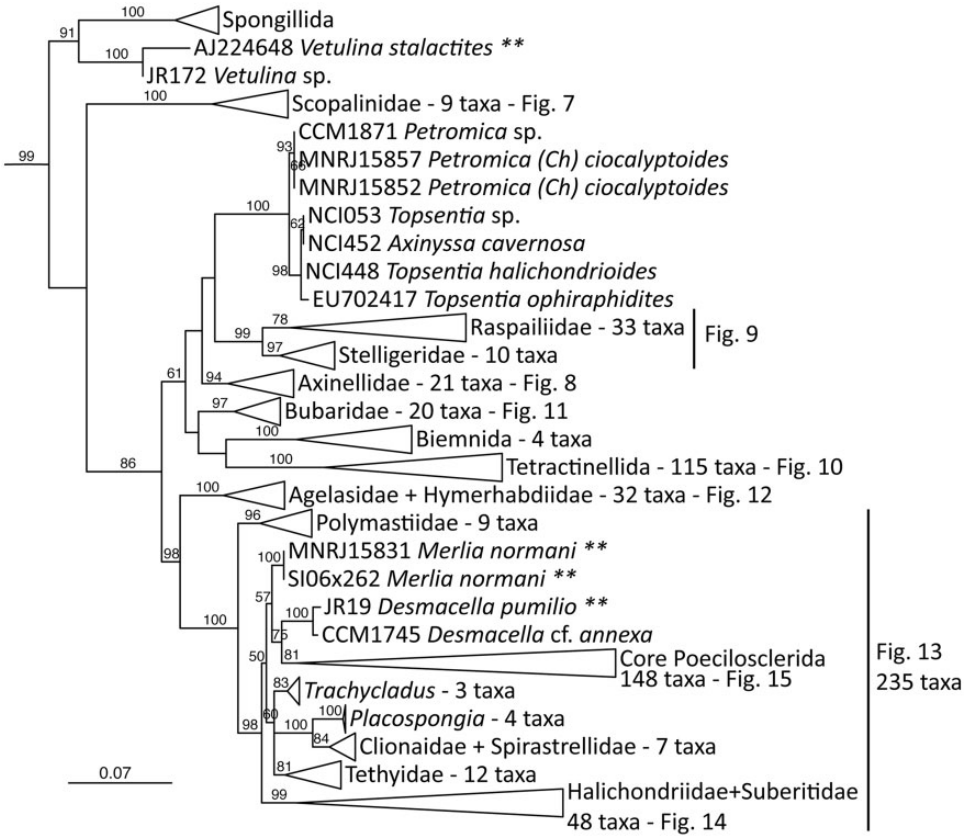

Fig. 6 ML topology of the large Heteroscleromorpha dataset with bootstrap support indices. Topology is rooted on six representatives of the three other subclasses—P32 *Halisarca sp. nov.* and L03x26 *Aplysina fistularis*, G02x131 *Lamellodysidea* sp., G02x79 *Chelonaplysilla* sp. nov., P07 *Amphimedon compressa* and DQ927307 *Haliclona oculata* (not shown in the figure). The branches to a number of highly supported groups have been collapsed. Bootstrap support values below 50% have been removed from the tree. All type species are indicated with **.

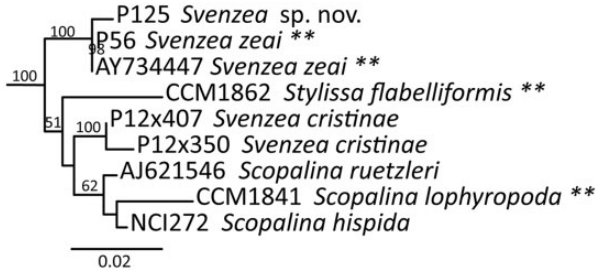

Fig. 7 ML topology of Scopalinidae dataset using with bootstrap support indices. Topology is rooted on JR172 *Vetulina* sp., DQ176775 *Baikalospongia bacillifera*, DQ167166 *Spongilla vastus*, P12x362 *Myrmekioderma rea*, AY769087 *Agelas clathrodes*, and P111 *Agelas* sp. nov. (not shown in the figure). Bootstrap support values below 50% have been removed from the tree. All type species are indicated with **.

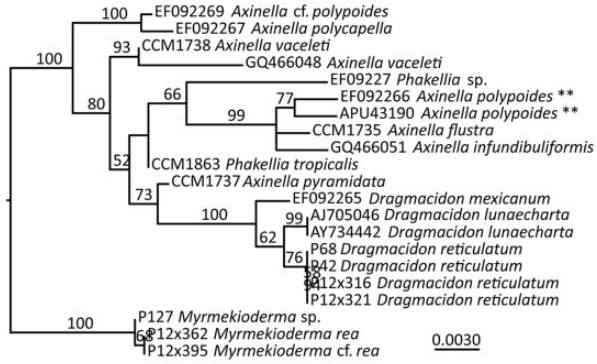

Fig. 8 ML topology of Axinellidae dataset with bootstrap support indices. Topology is rooted according to ML analysis of the large Heteroscleromorpha dataset (seen in Fig. 6). Bootstrap support values below 50% have been removed from the tree. All type species are indicated with **.

Our analyses confirm a group of *Svenzea, Scopalina,* and *S. flabelliformis* (Fig. 7 and Supplementary Figs. S11 and S12). Still other species of *Stylissa* (specifically *S. massa* (Carter, 1887) and *S. carteri* (Dendy, 1889)) have been shown to fall within Agelasida (Erpenbeck et al. 2006; Alvarez and Hooper 2010; Morrow et al. 2012). *Stylissa flabelliformis* is the type species of *Stylissa,* a genus currently assigned to Dictyonellidae (Halichondrida). It has surface morphological characters similar to those of *Scopalina* and skeletal characters similar to those of *Svenzea* and *Scopalina* (Alvarez and Hooper 2010). Since *S. flabelliformis* is the type species of *Stylissa*, and *Scopalina* has precedence over *Stylissa*, *S. flabelliformis* should be transferred to *Scopalina*, while other *Stylissa* spp. falling within Agelasida will need to be placed within an existing or new genus. A revision of the species currently assigned to "*Stylissa*" is currently in progress and will reflect the new taxonomic status or taxonomic combinations of this group (B. Alvarez, unpublished data). By the phylogenetic definition provided by Gazave et al. (2010a), these *Stylissa* species appear to belong in the clade *Cymbaxinella*^{P}.

The remaining heteroscleromorphs exhibit a major dichotomy. The first is weakly to highly supported and contains Axinellidae, Biemnida (*sensu* Morrow et al. 2013), Tetractinellida, Bubaridae (=Dictyonellidae of Morrow et al. 2012, 2013), Stelligeridae, Raspailiidae, and some species of *Petromica* Topsent, 1898, *Topsentia* Berg, 1899, and *Axinyssa* Lendenfeld, 1897 (Fig. 6 and Supplementary Figs. S9 and S10). The second is highly supported and contains Agelasida, Polymastiidae, Placospongiidae, Clionaidae, Spirastrellidae, Tethyidae, Poecilosclerida (including *Merlia* Kirkpatrick, 1908 and *Desmacella* Schmidt, 1870), Halichondriidae, Suberitidae, and *Trachycladus* Carter, 1879 (Fig. 6 and Supplementary Figs. S9 and S10). Both of these clades, albeit with fewer representatives, are also revealed as robust clades by combined 18S and 28S data (Morrow et al. 2013) and suggest a previously unrecognized, fundamental division within Heteroscleromorpha.

Axinellidae is a well-supported clade of 21 taxa in our analyses (Supplementary Figs. S6, S8, S9, S10, S13, and S14), with two species of *Myrmekioderma* Ehlers, 1870 forming the sister group to a larger clade containing several species of *Axinella* Schmidt, 1862, two of *Phakellia* Bowerbank, 1862, and a clade of three species of *Dragmacidon* Hallmann, 1917. *Myrmekioderma* is currently assigned to Heteroxyidae (Halichondrida) and it is found in two separate clades in Heteroscleromorpha. *Myrmekioderma rea* (de Laubenfels, 1934) clusters in Axinellidae (Fig. 8 and Supplementary Figs. S13 and S14) while the position of *Myrmekioderma granulatum* (Esper, 1794) (type species of *Myrmekioderma*) and *Myrmekioderma* sp. is highly supported with *Didiscus* Dendy, 1922 in Raspailiidae (Fig. 9 and Supplementary Figs. S15 and S16). The identifications of both *Myrmekioderma rea* and *Myrmekioderma* sp. from Gazave et al. (2010a) have been confirmed (Diaz and Boury-Esnault, respectively). *Axinella* has just fewer than 100 species assigned to it (van Soest et al. 2013), but its polyphyletic nature is well established (e.g., Gazave et al. 2010a; Morrow et al. 2012). We confirm this result by finding other *Axinella* species (=*Cymbaxinella*^{P}) within Agelasida (see below). Morrow et al. (2013) give a more in-depth discussion about the homoplasious nature of multiple morphological characters in Heteroscleromorpha, and call for the resurrection of Axinellida Lévi, 1953, including the families Axinellidae Carter,

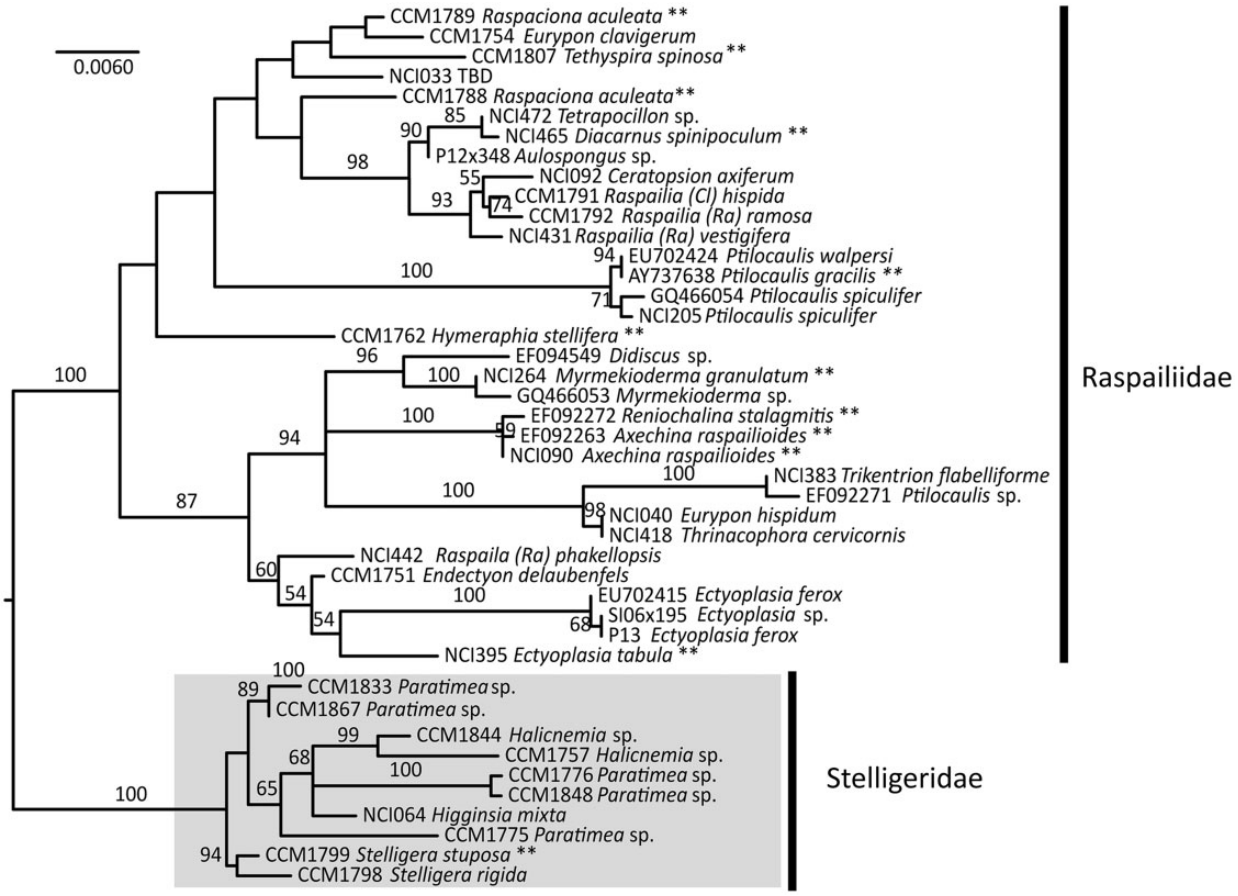

Fig. 9 ML topology of Raspailiidae + Stelligeridae dataset with bootstrap support indices. Topology is rooted according to ML analysis of the large Heteroscleromorpha dataset (seen in Fig. 6). Bootstrap support values below 50% have been removed from the tree. All type species are indicated with **. Subgenera are indicated as follows: *Cl* = *Clathriodendron*, *Ra* = *Raspailia*.

1875, Raspailiidae Nardo, 1833, and Stelligeridae Lendenfeld, 1878. Our 18S analyses provide no compelling evidence for or against this hypothesis (Fig. 6 and Supplementary Figs. S9 and S10).

We do, however, recover a strongly supported relationship between two clades representing Raspailiidae and Stelligeridae (Fig. 6 and Supplementary Figs. S9 and S10). Many of the internal branches within Raspailiidae, containing 33 taxa, are highly supported (Fig. 9 and Supplementary Figs. S15 and S16). Within this family, *Axechina raspailioides* Hentschel, 1912 groups with *Reniochalina* Lendenfeld, 1888; the latter is classified within Axinellidae (van Soest et al. 2013). Our 18S data provide independent evidence supporting the assertion of Morrow et al. (2012) that *Axechina* Hentschel, 1912 and *Reniochalina* are raspailids that have lost their acanthostyles. Similarly, *Ptilocaulis* Carter, 1833 is classified within Axinellidae (van Soest et al. 2013), but two species (*Ptilocaulis walpersi* (Duchassaing and Michelotti, 1864) and *Ptilocaulis spiculifer* (Lamarck, 1814)) in our analyses fall within Raspailliidae, supporting an earlier finding (Erpenbeck et al. 2007b). Alvarez et al. (2000) utilizing 28S data also found strong support for the grouping of *Ptiloaulis* and *Reniochalina stalagmitis* Lendenfeld, 1888. Another surprising member of this clade is *Diacarnus spinipoculum* (Carter, 1879), the type species of its genus. *Diacarnus* Burton, 1934 is currently classified in Poecilosclerida, and its congener *D. bismarkensis* Kelly-Borges and Vacelet, 1995 is highly supported as grouping with *Neopodospongia* Sim-Smith and Kelly, 2011 and *Negombata* de Laubenfels, 1936 (all in, Podospongiidae) as expected (Fig. 15 and Supplementary Figs. S23 and S24). Nomenclatural changes will be necessitated when the respective families are revised. Within Stelligeridae, we find *Higginsia mixta* Hentschel, 1912 clustered with *Stelligera* Gray, 1867, *Paratimea* Hallmann, 1917, and *Halicnemia* Bowerbank, 1864 (Fig. 9 and Supplementary Figs. S15 and S16), similar to the mtCOI results of Erpenbeck et al. (2012b) although different species of *Higginsia* were included.

Mirroring results derived from mtCOI and 28S data (Mitchell et al. 2011; Morrow et al. 2012), 18S

data suggest a close common ancestry for *Sigmaxinella* sp. nov., *Neofibularia hartmani* Hooper and Lévi, 1993, *Biemna variantia* (Bowerbank, 1858) (type species of *Biemna* Gray, 1867) and *Biemna fistulosa* (Topsent, 1897) (Supplementary Figs. S9 and S10). This clade was provisionally called Desmacellidae by Morrow et al. (2012) in the absence of evidence from the type species of *Desmacella*. Here, we have been able to include the type *Desmacella pumillo* Schmidt, 1870, and find it to represent an early diverging lineage of the order Poecilosclerida (see below). Here, we follow Morrow et al. (2013) and recognize the clade consisting of *Sigmaxinella* Dendy, 1897, *Neofibularia* Hechtel, 1965, and *Biemna* as the new order Biemnida (Fig. 6).

In agreement with other studies, our 18S analysis robustly supports the existence of a diverse clade, Tetractinellida (Supplementary Figs. S6, S10, S9, S10, S17, and S18), containing the suborders Astrophorina (Ancorinidae, Calthropellidae, Geodiidae, Pachastrellidae, Theneidae, Thoosidae, Thrombidae, Vulcanellidae, and the lithistid families Corallistidae, Isoraphiniidae, Macandrewiidae, Neopeltidae, Phymaraphiniidae, Phymatellidae, Pleromidae, and Theonellidae) and Spirophorina (Samidae, Spirasigmidae, Tetillidae, and the lithistid families Scleritodermidae and Azorecidae) (cf. Cárdenas et al. 2012, p. 172, concerning the reallocation of lithistid families). The sister group of Tetractinellida was not resolved in our analyses, but among the possible candidates (Axinellidae, *Topsentia* + *Axinyssa* + *Petromica*, Bubaridae, Biemnida, or Stelligeridae + Raspailiidae), Biemnida is slightly favored (Fig. 6 and Supplementary S9 and S10). This hypothesis receives even greater support from analysis of combined 18S and 28S data (Morrow et al. 2013).

Spirophorina is paraphyletic with respect to Astrophorina (Fig. 10 and Supplementary Figs. S17 and S18), with lineages of Tetillidae being more closely related to Astrophorina than to the other members of Spirophorina (lithistids: *Aciculites* Schmidt, 1879, *Microscleroderma* Kirkpatrick, 1903). This topology suggests that the ancestor of Tetractinellida may have had sigmaspires. It is possible that these spicules, along with raphides, are shared with members of Biemnida (see Morrow et al. 2013). Also, since members of Scleritodermidae do not have triaenes, these may have appeared later, in the ancestor of Tetillidae plus Astrophorina, or were possibly lost in the ancestor of Scleritodermidae. Unexpectedly, Tetillidae is paraphyletic with two main groups (Fig. 10 and Supplementary Figs. S17 and S18): (1) an unresolved earlier diverging group including *Cinachyrella* Wilson, 1925, *Paratetilla* Dendy, 1905 and one *Acanthotetilla* Burton, 1959 (=I + II clade from Szitenberg et al. 2013) and (2) a moderately to highly supported clade with *Tetilla* Schmidt, 1868, *Craniella* Schmidt, 1870, *Fangophalina* Schmidt, 1880, and *Cinachyra* Sollas, 1886 (=III + IV clade from Szitenberg et al. 2013). This latter clade of tetillids appears to be the sister clade to Astrophorina, conflicting with previous molecular studies, including the most complete to date (Szitenberg et al. 2013), in which combined datasets of COI, 18S, and 28S supported a sister group relationship between Astrophorina and a monophyletic Tetillidae. It should be emphasized that the main difference between our sampling and that of Szitenberg et al. (2013; Supplementary Fig. S1) is our inclusion of Scleritodermidae. Paraphyly of Tetillidae would suggest that Astrophorina is derived from a tetillid-like sponge with sigmaspires. The presence of an *Acanthotetilla* species with *Cinachyrella* and *Paratetilla* contradicts previous COI molecular results showing that *Acanthotetilla* species diverge prior to all other Tetillidae (Szitenberg et al. 2013).

Within the highly supported Astrophorina (Fig. 10 and Supplementary Figs. S17 and S18), we find several lithistids belonging to Corallistidae and Theonellidae, confirming previous results suggested by COI and 28S data (Cárdenas et al. 2011). For the first time, two species of Neopeltidae are included in a molecular phylogeny. Although the 18S results confirm that this lithistid family belongs in Astrophorina, the two species *Callipelta cavernicola* (Vacelet and Vasseur, 1965) and *Homophymia stipitata* Kelly, 2000 do not group together; the first has an uncertain position, and the second forms a strongly supported clade with *Neamphius huxleyi* (Sollas, 1888) and *Penares nux* (de Laubenfels, 1954). The phylogenetic relationship of *N. huxleyi* with Astrophorina lithistids confirms previous molecular studies (Cárdenas et al. 2011) while the recent reallocation of *Pachastrissa nux* to the genus *Penares* (van Soest et al. 2010) probably needs to be reconsidered in light of our results. Branching at the base of the Astrophorina we find *Lamellomorpha strongylata* Bergquist, 1968, sequenced here for the first time. This Astrophorina *incertae sedis* completely lacks triaenes but possesses microstrongyles and amphiasters, which suggests that it may be close to *Characella* (Sollas, 1886) or *Pachastrella* Schmidt, 1868 (Cárdenas et al. 2011), which is not in contradiction with our present results. The basal position of *Poecillastra laminaris* Sollas, 1886 is also in accordance with previous studies, which sequenced other *Poecillastra* Sollas, 1888 species (Cárdenas et al.

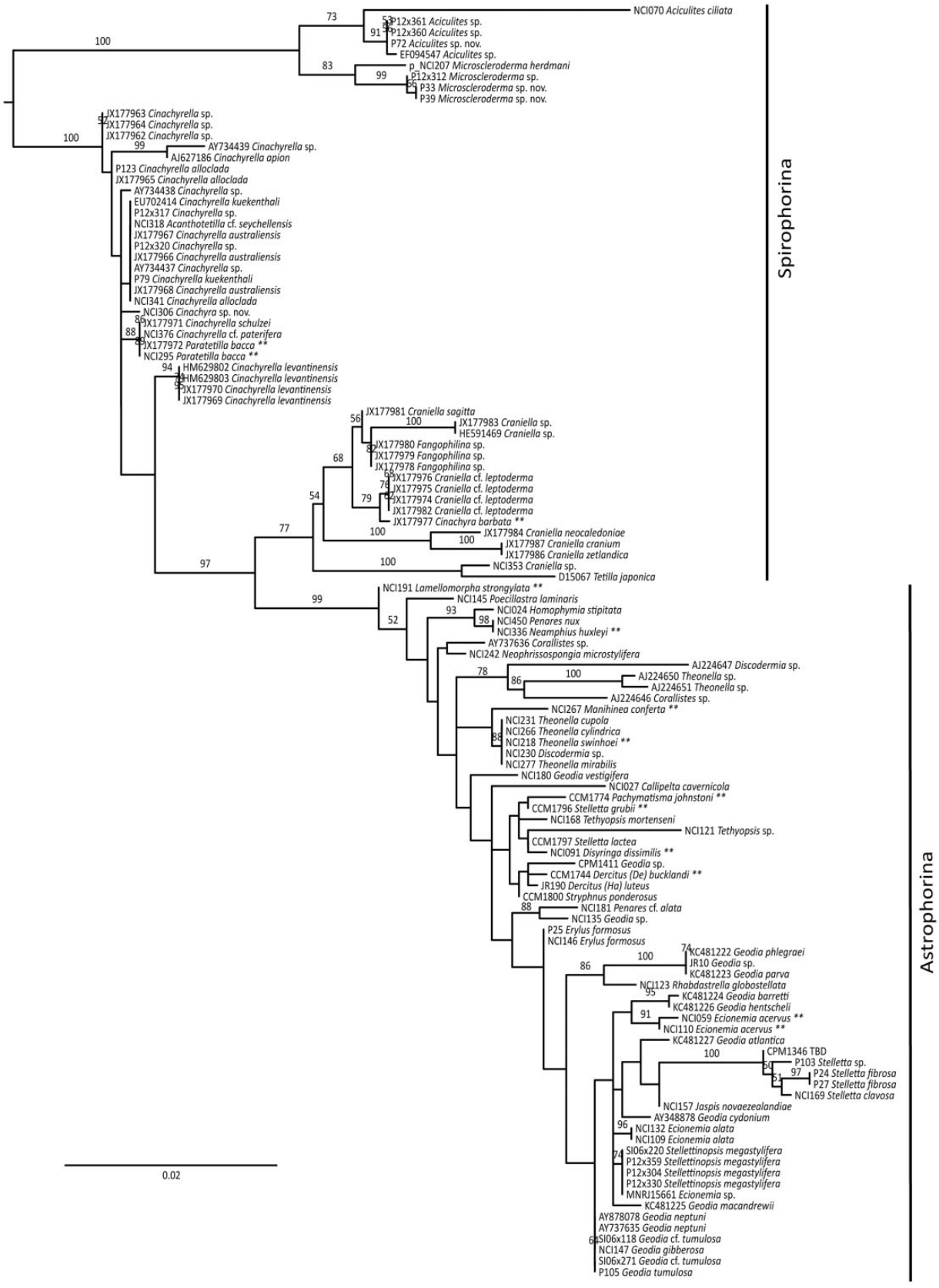

Fig. 10 ML topology of Tetractinellida dataset with bootstrap support indices. Topology is rooted according to ML analysis of the large Heteroscleromorpha dataset (seen in Fig. 6). Bootstrap support values below 50% have been removed from the tree. All type species are indicated with **.

2011). The rest of the Astrophorina species form low-supported groups mixing species of Geodiidae and Ancorinidae. The Ancorinidae *sensu stricto* (*Disyringa* Sollas, 1888, *Stryphnus* Sollas, 1886, *Dercitus* Gray, 1867, *Tethyopsis* Stewart, 1870, some *Stelletta* Schmidt, 1862) do not form a clade, although they tend to group together, which would confirm previous studies including the same *Dercitus*, *Stryphnus,* and North Atlantic species of *Stelletta*, but with different markers (Cárdenas et al. 2011). The first sequences for *Disyringa* and *Tethyopsis* confirm their belonging to the Ancorinidae. However, it is interesting to note that *Tethyopsis* does not seem to be monophyletic and could be a junior synonym of *Stelletta*. The only difference between the two genera is the presence of conspicuous tubes in *Tethyopsis,* a character that could have evolved independently in different *Stelletta*. Species of Erylinae (*Pachymatisma johnstonia* (Bowerbank in Johnston, 1842), *Erylus formosus* Sollas, 1888, *Penares* spp.) do not group together; however, *E. formosus* and *Penares* spp. (*Penares nux, Penares* cf. *alata*) have never been sequenced before. *Penares nux* was discussed previously; *P. alata* (Lendenfeld, 1907) from South Africa is also a special *Penares* since it has calthrops and no euasters so that it used to belong to the Pachastrellidae. The identifications of some of the basal Geodiidae specimens (e.g., *Geodia vestigifera* (Dendy, 1924), *Penares* cf. *alata and Geodia* sp.) may need to be revisited in light of these results. Although there is low resolution within Astrophorina, some of the 18S results confirm previous results with mtCOI and 28S of Cárdenas et al. (2011). There is a large clade (albeit not supported) that includes mainly *Geodia* species as well as *Rhabdastrella globostellata* (Carter, 1883); *Stellettinopsis megastylifera* (Wintermann-Kilian and Kilian, 1984); *Ecionemia acervus* Bowerbank, 1864; *Ecionemia alata* (Dendy, 1924); *Jaspis novaezelandiae* Dendy, 1924; and some *Stelletta*. The presence of *R. globostellata*, *S. megastylifera* and some of these *Stelletta* in the Geodiinae was also found by Cárdenas et al. (2011). The presence of *E. acervus*, type species of the genus *Ecionemia* Bowerbank, 1864, suggests that this genus should be synonymized with *Geodia* Lamarck, 1815, and raises the question of the origin of large and small acanthomicrorhabds (found in *S. megastylifera*, *E. alata*, and *E. acervus*) in the Geodiinae. The highly supported *Stelletta* clade corresponds to the *PhyloCode*-defined clade *Geostelletta*[P] (Cárdenas et al. 2011). The presence of *J. novaezelandiae* at the base of the *Geostelletta*[P] confirms that some *Jaspis* species have lost their sterrasters and need to be moved from Ancorinidae to Geodiidae. The *Phylocode* clades *Geodia*[P] Lamarck, 1815 (Cárdenas et al. 2010) ("*gibberosa*/*neptuni*/*tumulosa*" complex) and *Depressiogeodia*[P] (Cárdenas et al. 2010) (*G. barretti* Bowerbank, 1858 and *G. hentscheli* Cárdenas, Rapp, Schander and Tendal, 2010) are also retrieved.

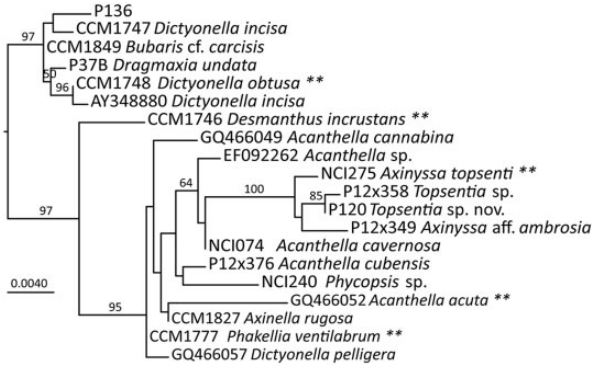

Fig. 11 ML topology of Bubaridae dataset with bootstrap support indices. Topology is rooted according to ML analysis of the large Heteroscleromorpha dataset (seen in Fig. 6). Bootstrap support values below 50% have been removed from the tree. All type species are indicated with **.

Dictyonellidae (herein = Bubaridae Topsent, 1894) as defined by Morrow et al. (2012) was recovered with high support and contained 20 taxa (Figs. 6 and 11 and Supplementary Figs. S9, S10, S19, and S20). Due to the inclusion of *Bubaris* cf. *carcisis*, we propose that the name of this clade be changed from Dictyonellidae to Bubaridae because of the seniority of the family name. The clade is divided into two highly supported lineages. In one, *Desmanthus incrustans* (Topsent, 1889) is highly supported as the sister to a number of *Acanthella* species, *Phakellia rugosa* (Bowerbank, 1866), *Acanthella cannabina* (Esper, 1794), *Dictyonella pelligera* (Schmidt, 1864), *Phakellia ventilabrum* (Linnaeus, 1767) (type species of the genus), *Phycopsis* sp., two *Topsentia* and, two *Axinyssa* (including the type species of this genus). The second main clade of Bubaridae contains two discrete *Dictyonella incisa* (Schmidt, 1880); *Dictyonella obtusa* (Schmidt, 1862); *Dragmaxia undata* Alvarez, van Soest and Rützler, 1998 (type species of the genus); *Bubaris* cf. *carcisis;* and an unnamed specimen P136. Identification of this latter specimen has proved to be extremely difficult; its classification cannot be verified at this time. The lithistid Desmanthidae (including *Desmanthus* Topsent, 1894; *Paradesmanthus* Pisera and Lévi, 2002; *Sulcastrella* Schmidt, 1879, and *Petromica*) is clearly polyphyletic with *Desmanthus* and *Petromica* in different clades (Fig. 6 and Supplementary Figs. S9 and S10). Vacelet (1969) also showed how *Lithobubaris* (=*Sulcastrella*) is similar in many ways to bubarid species. Therefore, the genera *Desmanthus, Sulcastrella,* along with the monotypic *Paradesmanthus*, are formally reallocated to the Bubaridae. *Petromica* remains

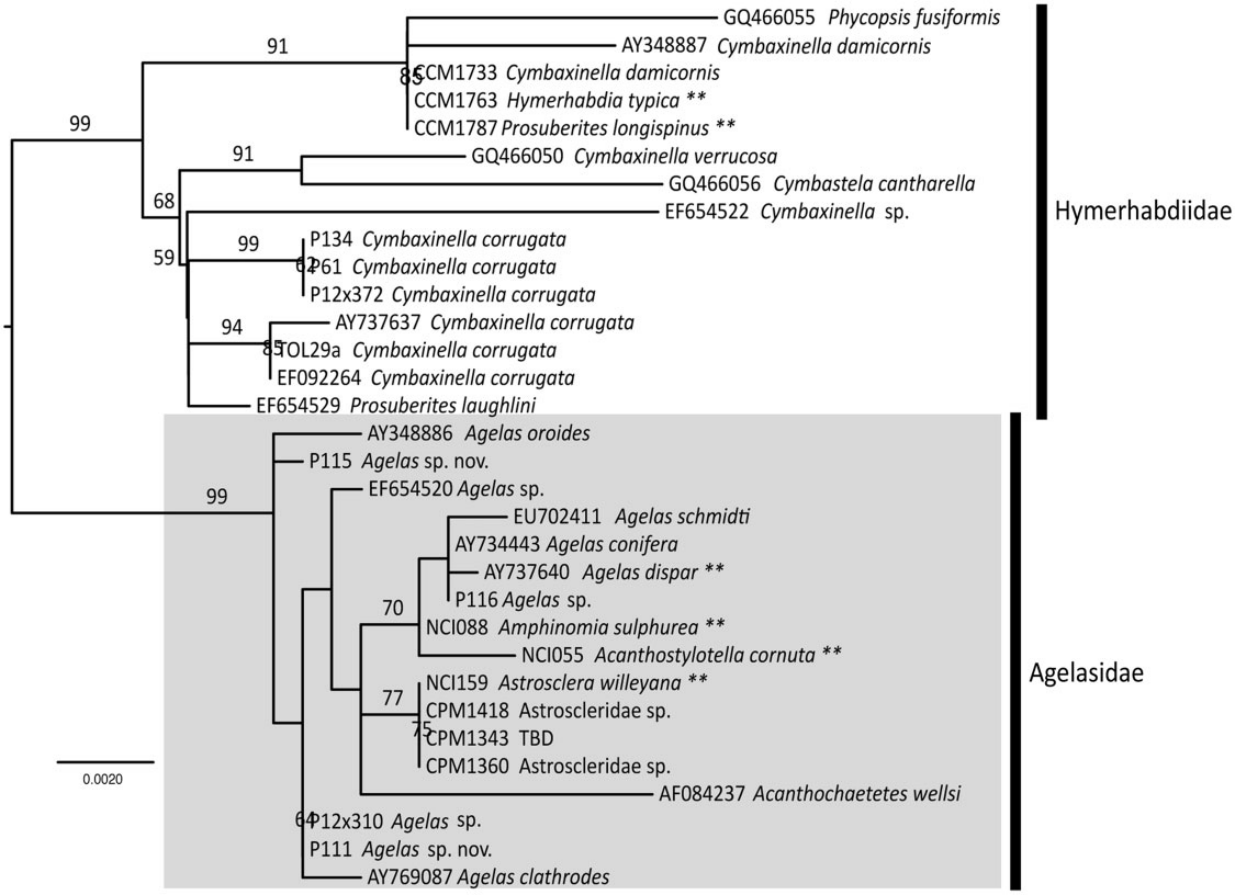

Fig. 12 ML topology of Agelasida dataset with bootstrap support indices. Topology is rooted according to ML analysis of the large Heteroscleromorpha dataset (seen in Fig. 6). Bootstrap support values below 50% have been removed from the tree. All type species are indicated with **.

a Heterosclermorpha *incertae sedis* along with some *Topsentia* and *Axinyssa*.

Agelasida is the earliest diverging lineage of the major clade containing Agelasida, Polymastiidae, Placospongiidae, Clionaidae, Spirastrellidae, Tethyidae, Poecilosclerida (including the genera *Merlia* and *Desmacella*), Halichondriidae, Suberitidae, and representatives of *Trachycladus* (Fig. 6 and Supplementary Figs. S9 and S10), matching results of Morrow et al. (2013) using combined 18S and 28S data. We sampled 32 taxa in Agelasida that fall into two highly supported clades corresponding to Hymerhabdiidae and Agelasidae (Fig. 12 and Supplementary Figs. S21 and S22). The first contains predominantly *Prosuberites* Topsent, 1893; *Axinella* with *Cymbastela cantharella* Lévi, 1983; *Hymerhabdia typica* Topsent, 1892; and *Phycopsis fusiformis* (Lévi, 1967), and conforms to the phylogenetic definition of *Cymbaxinella*^{P} (Gazave et al. 2010a), which corresponds to the family Hymerhabdiidae (following Morrow et al. 2012). Agelasidae contains species of *Agelas* Duchassaing and Michelotti, 1864, and Asteroscleridae, as well as the monospecific genera *Acanthostylotella* Burton and Rao, 1932 and *Amphinomia* Hooper, 1991. These taxa are currently assigned to Raspailiidae, Poecilosclerida (van Soest et al. 2013), but de Voogd et al. (2010) presented chemical, molecular, and morphological data suggesting that *Amphinomia* was synonymous with *Acanthostylotella* and that the former should be reassigned to Agelasidae. Our results corroborate the work of de Voogd et al. (2010).

A GenBank sequence from *Acanthochaetetes wellsi* Hartman and Goreau, 1975 (Acanthochaetetidae: Hadromerida) also grouped within Agelasidae. However, we suspect that this is an identification error as *Astrosclera* Lister, 1900 and *Acanthochaetetes* Fischer, 1970 can be relatively easily confused in the field (J. Vacelet, personal communication) and the identification of this specimen was not re-examined for our study. Previous molecular studies using 28S rRNA (Chombard et al. 1997) suggested a close relationship between *Acanthochaetetes* and Clionaidae and Spirastrellidae. Morphologically, the tylostyle megascleres and streptaster microscleres in

Acanthochaetetes are very similar to those in Spirastrellidae (Rützler and Vacelet 2002). The spicules of *Agelas*, *Astrosclera,* and *Acanthostylotella*, acanthostyles with verticillate spines, are strikingly different from those of *Acanthochaetetes.*

The sister group to Agelasida is a large and very strongly supported clade containing families currently classified within the order Hadromerida (Clionaidae, Placospongiidae, Polymastiidae, Spirastrellidae, and Suberitidae), the family Halichondriidae, and the order Poecilosclerida (Fig. 6 and Supplementary Figs. S9 and S10). Within this large assemblage, our 18S-based hypothesis highly supports Polymastiidae as the monophyletic sister group (Fig. 6 and 13 and Supplementary Figs. S9, S10, S23, and S24) to all the remaining taxa. Included in the latter family were *Atergia corticata* Stephens, 1915; *Polymastia pachymastia* de Laubenfels, 1932*; Polymastia boletiformis* (Lamarck, 1815); *P. penicillus* (Montagu, 1818)*; Proteleia sollasi* Dendy and Ridley, 1886; *Quasilina brevis* (Bowerbank, 1861)*;* two *Sphaerotylus* spp., and *Tentorium semisuberites* (Schmidt, 1870). This well-supported separation of Polymastiidae from the ''classic'' hadromerids with microscleres suggests that Hadromerida is paraphyletic or polyphyletic, but a lack of resolution for the remaining assemblage of hadromerids and poecilosclerids prevents a more definitive conclusion.

As shown in several other studies, the two families Halichondriidae and Suberitidae are closely related (Chombard 1998; Chombard and Boury-Esnault 1999; Erpenbeck et al. 2007a, Morrow et al. 2012; Hill et al. 2013). Our analysis included 50 taxa (Fig. 14 and Supplementary Figs. S25 and S26) and many of the internal relationships are similar to those seen in Morrow et al. (2012). *Spongosorites genitrix* (Schmidt, 1870) and *Homaxinella subdola* (Bowerbank, 1866) were first to diverge and *Suberites* Nardo, 1833 was split over two smaller clades. *Suberites aurantiacus* (Duchassaing and Michelotti, 1864); *S. massa* Nardo, 1847; *and* two *Suberites* sp. group with *Rhizaxinella* sp., whereas the second clade consists of *S. ficus* (Johnston, 1842)*; S. domuncula* (Olivi, 1792); and *Aaptos suberitoides* (Brøndsted, 1934) (Fig. 14). *Hymeniacidon* Bowerbank, 1858 was not monophyletic as a large clade of *H. perlev*is (Montagu, 1818); *H. sinapium* de Laubenfels, 1930; and *H.* sp. had high support whereas *H. caerulea* Pulitzer-Finali, 1986; *H. heliophila* (Parker, 1910); and *H. kitchingi* (Burton, 1935) were spread across the Halichondriidae clade. *Terpios* Duchassaing and Michelotti, 1864 was also polyphyletic. There was moderate to high support for the split of *Terpios* spp. into three distinct clades. *Terpios manglaris* Rützler and Smith, 1993

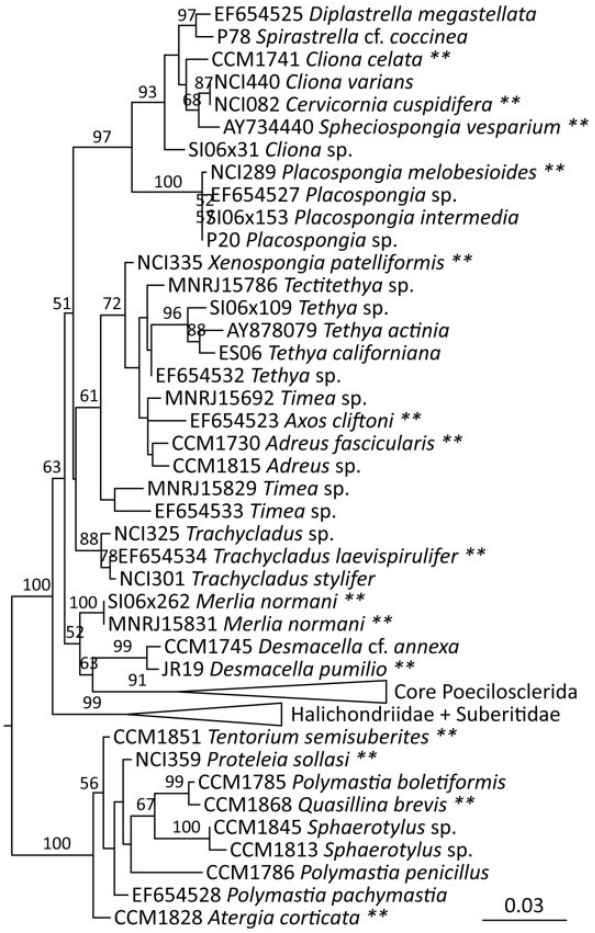

Fig. 13 ML topology of large subclade of Heteroscleromorpha dataset with bootstrap support indices. Topology is rooted on Polymastiidae (as seen in Fig. 6). Bootstrap support values below 50% have been removed from the tree. All type species are indicated with **.

and *T.* sp. branched at the base of a large clade comprising *Amorphinopsis* Carter, 1887; *Halichondria* Fleming, 1828; *Hymeniacidon; Pseudosuberites; Ulosa* de Laubenfels, 1936; and additional species of *Terpios.* Our analysis confirms the results of Morrow et al. (2012) who transferred *Ulosa* from Esperiopsidae (Poecilosclerida) to Halichondriidae (and see Hajdu et al. 2013).

Our 18S analyses recover the Hemiasterellidae + Tethyidae + Timeidae clade described by Morrow et al. (2012), but weak to high support depending on method of analysis (Fig. 13 and Supplementary Figs. S23 and S24). Due to lack of resolution, our analyses neither support nor conflict with the hypothesis that Trachycladidae is the sister group of this assemblage. We find highly supported relationships among three hadromerid taxa, *Placospongia* Gray, 1867 as the sister group to a clade containing Clionaidae and Spirastrellidae (Fig. 13 and Supplementary Figs. S23 and S24). As also found

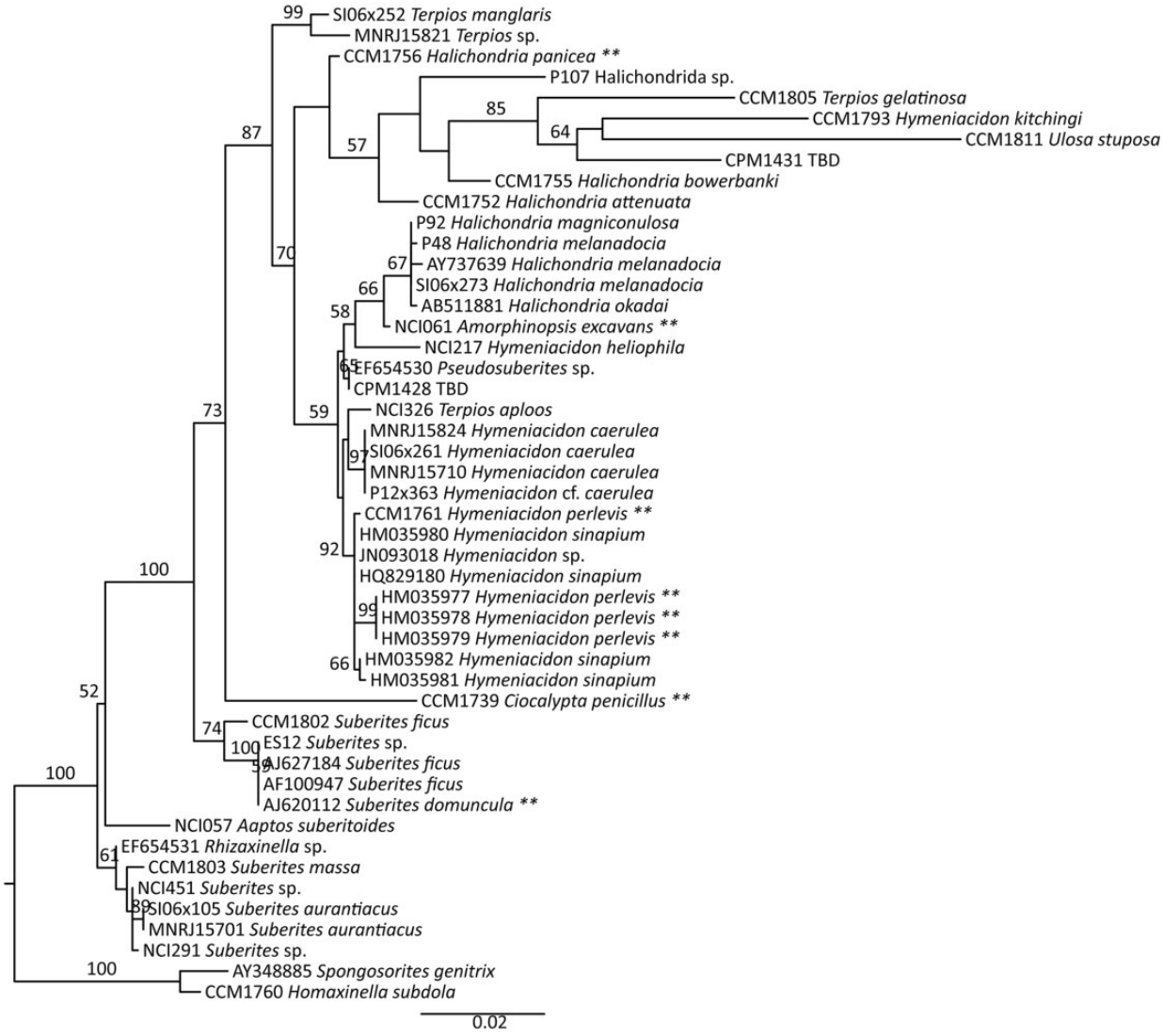

Fig. 14 ML topology of Halichondriidae + Suberitidae dataset with bootstrap support indices. Topology is rooted according to ML analysis of the large Heteroscleromorpha dataset (seen in Fig. 6). Bootstrap support values below 50% have been removed from the tree. All type species are indicated with **.

by Morrow et al. (2012), Clionaidae appears to be paraphyletic with respect to Spirastrellidae, which will necessitate future nomenclatural changes for these taxa and their component genera and species.

Poecilosclerida is a highly diverse group of sponges composed of four suborders, 25 families and 142 genera (van Soest et al. 2013). This study has greatly expanded the dataset of 18S gene sequences. Hajdu et al. (2013) provide a comprehensive discussion of the suborder Mycalina and their observations will not be repeated here. The majority of Poecilosclerida, which we consider to be the core poecilosclerids (exclusive of Raspailiidae, Biemnida, *Ulosa*, and *Janulum* discussed above, and *Merlia* and *Desmacella* discussed here) are highly supported as a clade in our broad analyses of Heteroscleromorpha (Fig. 6 and Supplementary Figs. S9 and S10), as well as in our narrower analyses of the clade containing poecilosclerids, hadromerids, and Halichondriidae (Fig. 13 and Supplementary Figs. S23 and S24). 18S data provide moderate support for *Desmacella* being the sister group of the core poecilosclerids and weak support for *Merlia* being the earliest diverging lineage of Poecilosclerida. The combined partial 28S and mitochondrial 16S analysis of Hajdu et al. (2013) also suggests that *Desmacella* and *Merlia* branch near the base of the core poecilosclerids, suggesting that these findings are robust. These are the first studies to include *Merlia*, a poecilosclerid bearing clavidiscs and possessing a basal skeleton of layered calcareous chambers, that is, a chaetedid organization (see Hajdu et al. 2013).

Fig. 15 ML topology of core Poecilosclerida dataset with bootstrap support indices. Topology is rooted on NCI289 *Placospongia melobesioides*, SI06x153 *Placospongia intermedia*, NCI335 *Xenospongia patelliformis*, MNRJ15786 *Tectitethya* sp., MNRJ15692 *Timea* sp. Bootstrap support values below 50% have been removed from the tree. All type species are indicated with **. Subgenera are indicated as follows: *Ac* = *Acarnia*, *Ad* = *Acanthodoryx*, *Ae* = *Aegogropila*, *An* = *Antho*, *Ca* = *Carnia*, *Cl* = *Clathria*, *Cr* = *Crella*, *Ec* = *Ectyodoryx*, *Hy* = *Hymedesmia*, *Li* = *Lissodendoryx*, *Mi* = *Microciona*, *My* = *Mycale*, *Pa* = *Paresperella*, *Py* = *Pytheas*, *Te* = *Tedania*, *Th* = *Thalysias*, *Tr* = *Trachytedania*, *Yv* = *Yvesia*, *Zy* = *Zygomycale*.

The genus *Acantheurypon* Topsent, 1927 was synonymized with *Eurypon* Gray, 1867 by Hooper (1991). However, Morrow et al. (2012) demonstrated *Eurypon (Acantheurypon) pilosella* (Topsent, 1904) clustering within Poecilosclerida, thereby supporting the view of Picton et al. (2010) that *Acantheurypon* is a poecilosclerid that had lost chelae. In our analyses, *Eurypon (Acantheurypon) pilosella* groups with *Cyamon spinispinosum* (Topsent, 1904) with high support deep within Poecilosclerida (Fig. 15 and Supplementary Figs. S27 and S28). *Cyamon* Gray, 1867 is currently assigned to the subfamily Cyamoninae in Raspailiidae (van Soest et al. 2013) and this genus is closely related to *Trikentrion* Ehlers, 1870 (van Soest et al. 2012b). However, *Trikentrion flabelliforme* Hentschel, 1912 grouped in a highly supported clade with *Eurypon hispidum* Bergquist, 1970; *Thrinacophora cervicornis* Ridley and Dendy, 1886; and a suspect *Ptilocaulis* sp. in the distantly related Raspailiidae (Figs. 6 and 9) as would be expected. *Cyamon spinispinosum* displays a number of characteristics that distinguish it from the other species in this genus. The styles of *Cyamon* are usually smooth; however, *C. spinispinosum* lacks long thin styles and instead it possesses unusual acanthostyles (van Soest et al. 2012b). Ultrastructural analyses of the spicules of *Cyamon spinispinosum* (Fig. 16A–C) and a microcionid style with microspined end from *Microciona fallax* Bowerbank, 1866 (Fig. 16D; van Soest et al. 2012b) raise questions as to whether *Cyamon spinispinosum* is really a *Cyamon*. *Cyamon spinispinosum* was assigned to *Acantheurypon* (Topsent 1928), and given that it clustered with *A. pilosella* (type species of *Acantheurypon*), we propose the resurrection of *Acantheurypon* Topsent, 1927 and the reassignment of *C. spinispinosum* to this genus. *Acantheurypon,* currently assigned to Raspailiidae in WPD, is a valid genus of Poecilosclerida while the remaining *Eurypon* species are raspailiids.

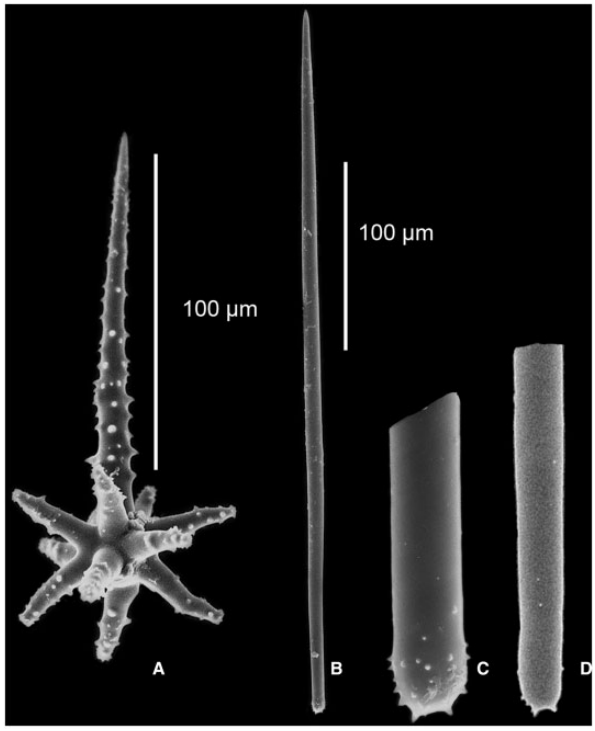

Fig. 16 SEM photos of spicules of *Acantheurypon spinispinosum* and *Microciona fallax*. (**A**) and (**B**) are from *A. spinispinosum*. (**C**) A close up of the end of (**B**); (**D**) The microspined ends on spicules of *M. fallax*.

Conclusion

This study has more than tripled the number of 18S sequences available from demosponges (from 220 to 726), more than doubled the number of represented genera (from 91 to 227), including 44 that had never before been sampled for any molecular data, and more than tripled the representation of type species (from 36 to 114). This marker, the gene coding for the small subunit of the ribosome or 18S, was instrumental in early molecular phylogenetic studies of Porifera (e.g., Borchiellini et al. 2004; Redmond et al. 2007, etc.). In subsequent years, it was used less frequently as researchers started conducting more taxonomically narrow analyses using more rapidly evolving markers (e.g., Morrow et al. 2012; Redmond and McCormack 2009; Erpenbeck et al. 2005a, 2006, 2007a, 2007b, 2007c, 2012a, 2012b). Our analyses, using a vastly expanded 18S dataset confirm numerous recent findings based on alternative datasets. For example, this dataset recovers the four major clades corresponding to the demosponge subclasses, most of the keratosan clades of Erpenbeck et al. (2012a), the non-monophyly of several verongid families as revealed by Erwin and Thacker (2007), the presence of five distinct clades within Haplosclerromorpha, following Redmond et al. (2007, 2011), a sister group relationship between Spongillida and Vetulinidae, and all of the heteroscleromorphan clades of Morrow et al. (2012). In addition, these data have uncovered new alliances of taxa, for instance the position of poecilosclerid *Janulum spinispiculum* within Haplosclerromorpha, *S. flabelliformis* within Scopalinidae, and two large clades within Heteroscleromorpha, exclusive of Spongillida, Vetulinidae, and Scopalinidae: clade 1: Axinellidae, Biemnida, Tetractinellida, Bubaridae, Stelligeridae, Raspailiidae, and some species of *Petromica*, *Topsentia*, and *Axinyssa*, and clade 2: Agelasida, Polymastiidae, Placospongiidae, Clionaidae, Spirastrellidae, Tethyidae, Poecilosclerida,

Halichondriidae, Suberitidae, and *Trachycladus*, etc. Our findings allow us to make numerous improvements to demosponge systematics, including amending the definition of Verongida to incorporate the inclusion of *Chondrosia*, creating the order Chondrillida for the chondrosid taxa other than *Chondrosia*, confirming the new order Biemnida, and resurrection of the old order Axinellida within Heteroscleromorpha. The Porifera Tree of Life project and numerous efforts from laboratories around the globe are contributing to the rapid development of a new systematics for Porifera, one that is more firmly based on a morphological appreciation of natural taxa, as inferred from studies of molecular sequence data. This new systematics will enhance communication about all aspects of the biology of sponges.

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Supplementary data

Supplementary Data available at *ICB* online.

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