



SYMPOSIUM

Molecular Phylogenies Support Homoplasy of Multiple Morphological Characters Used in the Taxonomy of Heteroscleromorpha (Porifera: Demospongiae)

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Synopsis Sponge classification has long been based mainly on morphocladistic analyses but is now being greatly challenged by more than 12 years of accumulated analyses of molecular data analyses. The current study used phylogenetic hypotheses based on sequence data from 18S rRNA, 28S rRNA, and the CO1 barcoding fragment, combined with morphology to justify the resurrection of the order Axinellida Lévi, 1953. Axinellida occupies a key position in different morphologically derived topologies. The abandonment of Axinellida and the establishment of Halichondrida Vosmaer, 1887 *sensu lato* to contain Halichondriidae Gray, 1867, Axinellidae Carter, 1875, Bubaridae Topsent, 1894, Heteroxyidae Dendy, 1905, and a new family Dictyonellidae van Soest et al., 1990 was based on the conclusion that an axially condensed skeleton evolved independently in separate lineages in preference to the less parsimonious assumption that asters (star-shaped spicules), acanthostyles (club-shaped spicules with spines), and sigmata (C-shaped spicules) each evolved more than once. Our new molecular trees are congruent and contrast with the earlier, morphologically based, trees. The results show that axially condensed skeletons, asters, acanthostyles, and sigmata are all homoplasious characters. The unrecognized homoplasious nature of these characters explains much of the incongruence between molecular-based and morphology-based phylogenies. We use the molecular trees presented here as a basis for re-interpreting the morphological characters within Heteroscleromorpha. The implications for the classification of Heteroscleromorpha are discussed and a new order Biemnida ord. nov. is erected.

Introduction

There are approximately 8000 valid species of sponges, but this number is likely to be a gross underestimate given how poorly studied some faunas are, the cryptic nature of many of the habitats, and the occurrence of cryptic species (Cardenas et al. 2012). Of the 8000 described species, approximately 6650 belong to Demospongiae (Morrow et al. 2012). The currently accepted classification of sponges depends almost exclusively on the morphology of spicules and the arrangement of spicules within the sponge tissue. However, some of the most recent

taxonomic studies have taken a more integrative approach using a combination of morphological and molecular characters (Cardenas et al. 2011) and also cytologic and metabolomic fingerprinting (Gazave et al. 2010a). Reconstruction of phylogenetic relationships within sponges is extremely challenging given the relative simplicity and environmental plasticity of the skeletal characters. This task is made more difficult by our lack of knowledge of whether specific skeletal characters indicate a common evolutionary origin (homologous) or whether they are a consequence of convergent evolution, parallel

evolution, or evolutionary reversals (homoplasy). When the number of morphological characters available for analysis is high, the impact of undetected homoplasy may be small (Jenner 2004), but when there is a paucity of morphological characters, which is often the case with sponges, then the consequences of homoplasy can be significant for the classification. Compared with most other groups, the phylogenetic relationships among sponges are still largely unresolved, hindering attempts to achieve a stable classification for the group.

The Lévi-Bergquist-Hartman classification of Demospongiae

Lévi (1953, 1956, 1957, 1973) was the first to provide a modern synthesis of the classification of Demospongiae. He identified two subclasses; Tetractinomorpha for taxa with a radial or axially condensed skeleton and an oviparous mode of reproduction and Ceractinomorpha for taxa with a reticulate skeleton and viviparous reproduction. He erected a new order Axinellida, containing the family Axinellidae, which previously had been classified within Halichondrida (according to the classification of de Laubenfels, 1936). Hallmann (1917) and Lévi (1953, 1956) argued for the removal of Axinellidae from Halichondrida. Lévi (1953) suggested that Axinellida should be given ordinal status. He allocated the new order to the subclass Tetractinomorpha; this was largely based on reproductive strategies. Axinellida was interpreted as containing species that are oviparous and have an axially condensed skeleton whilst Halichondrida *sensu stricto* contained species that are viviparous with a confused or reticulate skeleton. Bergquist (1970), in her study of Axinellida and Halichondrida from New Zealand, concluded that the differences in life-cycle patterns between members of Axinellida and Halichondrida were sufficient to warrant their placement in separate orders. However, Bergquist (1967) pointed out that some axinellids (Raspailiidae Hentschel, 1923 and Sigmamaxinellidae Lévi, 1955) have similar morphological features as some groups of Ceractinomorpha (i.e., Poecilosclerida Topsent, 1928) and are difficult to place between Poecilosclerida and Axinellida. In assigning them to Axinellida she placed emphasis on their reproductive strategies.

Both Bergquist (1970) and Hartman (1982) found support for Lévi's classification, and this became known as the Lévi-Bergquist-Hartman system (L-B-H). Fig. 1A summarizes this classification and shows the families that were assigned to Axinellida.

The Soest-Hooper system

The first studies to utilize morphocladistics in sponge systematics were van Soest (1984a, 1987, 1990, 1991), van Soest et al. (1990), de Weerd (1989), and Hooper (1990a, 1991). These studies were based primarily on skeletal characters. The results led to a new classification which was later adopted by *Systema Porifera* (Hooper and van Soest 2002) and which still underpins the current most widely used reference for sponge nomenclature, the World Porifera Database (van Soest et al. 2013). This classification differs from the L-B-H system primarily by the abandonment of Axinellida and the allocation of Axinellidae, Bubaridae, Heteroxyidae, and Dictyonellidae to Halichondrida; Hemiasterellidae Lendenfeld, 1889 and Trachycladidae Hallmann, 1917 to Hadromerida Topsent, 1894; and Raspailiidae (including Euryporidae Topsent, 1928), Rhabderemiidae Topsent, 1928, and Sigmamaxinellidae to Poecilosclerida. This supports earlier findings that transferred the raspailiids to Poecilosclerida on the basis of shared acanthostyles and similar surface architecture in some species (Hooper 1990a).

Cladistic approaches to systematics were highly critical of the L-B-H system, in particular with regard to the changes Lévi proposed for Halichondrida and Poecilosclerida (van Soest 1987, 1991; van Soest et al. 1990). They argued that reproductive strategies cannot reasonably be interpreted as synapomorphies at the subclass level, and even at lower levels these can be an adaptive response, developed independently. These authors also pointed out that for many taxa reproductive strategies were unknown and were inferred from the skeletal arrangement, thereby making a circular argument. Typical members of Axinellidae, Raspailiidae, Hemiasterellidae, and Sigmamaxinellidae share the possession of an axially condensed skeleton. van Soest et al. (1990) pointed out that each of these families also possessed characters that they interpreted as synapomorphies widely shared by different groups, such as asters in Hemiasterellidae with Hadromerida; acanthostyles in Raspailiidae with some Poecilosclerida; and sigmata in Sigmamaxinellidae with other Poecilosclerida. van Soest et al. (1990) and van Soest (1991) proposed changes to the classification mainly based on the argument that it was more parsimonious to assume that an axially condensed skeleton had arisen independently in different lineages (Hadromerida, Halichondrida, and Poecilosclerida) than to assume that asters, acanthostyles, and sigmata each evolved independently in separate lineages. This classification, which became known as the Soest-Hooper system, is summarized in Fig. 1B.

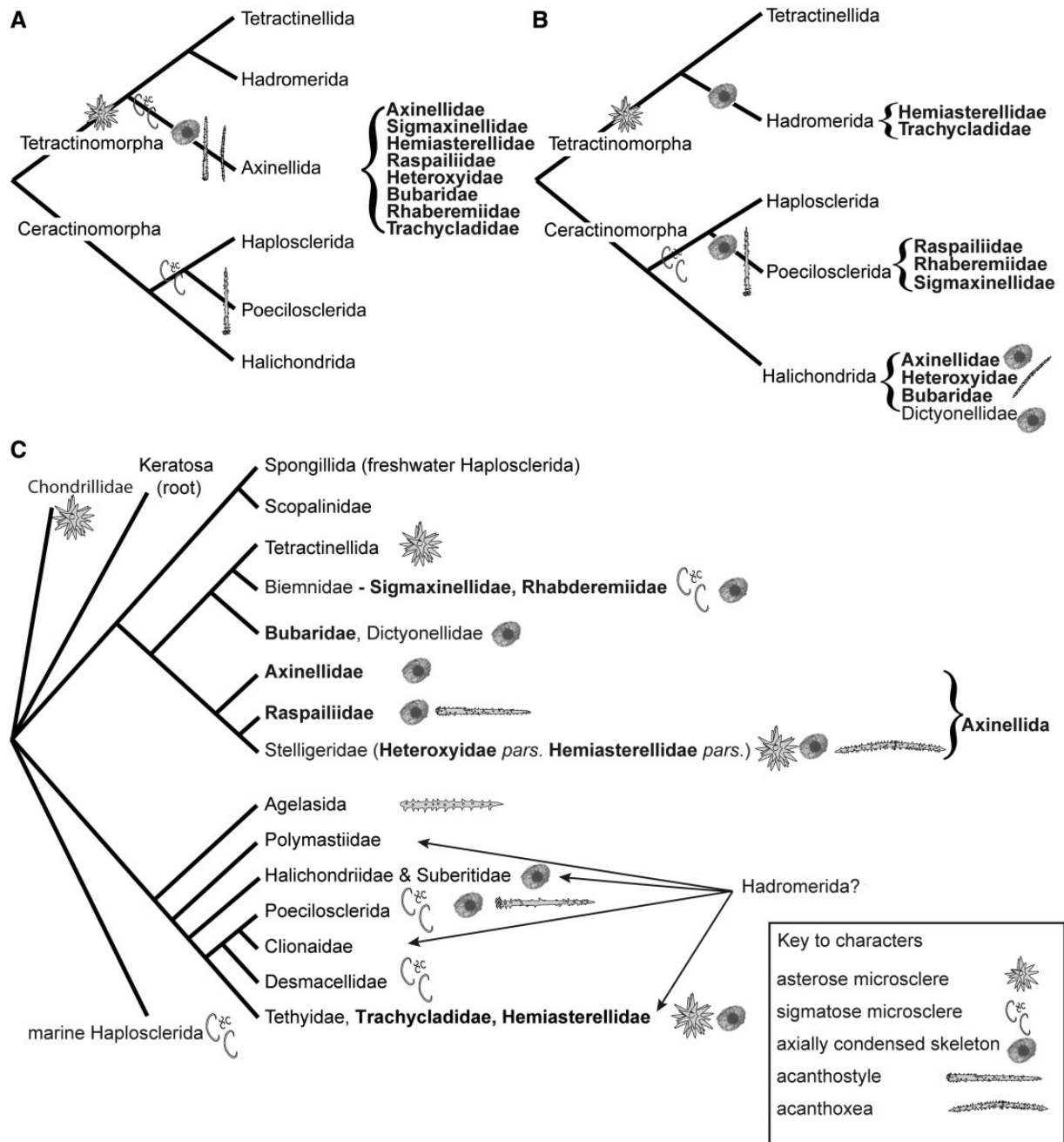


Fig. 1 (A) Summary of the Lévi-Bergquist-Hartman classification based primarily on skeletal architecture and reproductive strategies. (B) Summary of the Soest-Hooper classification based mainly on cladistic analyses of morphological characters. (C) Summary of the molecular results of this study based on full-length 18S rRNA combined with 28S rRNA (D3–D8 region) and CO1 barcoding sequences. Families assigned to Axinellida Lévi, 1953 are shown in bold. The distribution of asterose and sigmatose microscleres; axially condensed skeletons; acanthostyles and acanthoxea are shown on the three cladograms. Families currently assigned to Hadromerida in the World Porifera Database (van Soest et al. 2013) are indicated with an arrow (C).

The molecular classification

Early molecular phylogenetic studies of sponges used full-length sequences of 18S rRNA and the C1-D1 region of 28S rRNA and showed that the class Demospongiae is monophyletic, exclusive of Homoscleromorpha (Borchiellini et al. 2004). These results

showed that Demospongiae consists of four well-supported clades: “G1” and “G2” subsequently named Keratosa and Myxospongiae and marine Haplosclerida (“G3”) and a large clade provisionally called G4. Subsequent molecular studies, e.g., Lavrov et al. (2008) using complete mitochondrial genomes,

and Sperling et al. (2009, 2010) using nuclear house-keeping genes obtained largely congruent results. Sperling et al. (2009) proposed the name *Democlavia* for the G4 clade; however, Cardenas et al. (2012) later formally proposed *Heteroscleromorpha* for this clade. *Heteroscleromorpha* is by far the most important group of demosponges in terms of the number of taxa and contains approximately 5000 described species.

Within *Heteroscleromorpha* there is a large degree of incongruence between phylogenies reconstructed on the basis of molecular sequences and those based on cladistic analysis of morphological characters, as highlighted by Morrow et al. (2012). In the current study we attempted to gain an understanding of the causes of the incongruences by mapping the distribution of asterose and sigmatose microscleres, acanthostyles, and axially condensed skeletons onto updated molecular trees to gain an insight into whether these characters represent homologies or homoplasies (Fig. 1C).

Materials and methods

Samples and specimens

A combination of freshly collected specimens and museum specimens was used together with a number of sequences from Genbank. In total 154 species were included in this study; Table 1 shows the markers obtained and the corresponding catalogue numbers and Genbank accession numbers for each of the species. Most of the fresh material was collected by SCUBA diving, shore collecting, and by the ROV *Holland I* launched from RV *Celtic Explorer*. The sponges were photographed *in situ* prior to collection and samples no bigger than 1 cm³ were collected and fixed in 95% ethanol. When necessary the ethanol was changed after 20 min to fully desiccate the specimen.

DNA extraction

At Queen's University Belfast, DNA was extracted from subsamples following the methods outlined by Morrow et al. (2012). At the University of Alabama at Birmingham, DNA was extracted from subsamples following the procedures outlined by Thacker et al. (2013, this issue). Details of DNA extraction at the National Museum of Natural History are given by Redmond et al. (2013, this issue).

PCR amplification

18S rRNA, 28S rRNA, and CO1 barcoding region were chosen for amplification as these genes have been shown to be useful phylogenetic markers in

sponges (Erpenbeck et al. 2007; Wörheide et al. 2007; Cárdenas 2010; Gazave et al. 2010b). Details of PCR protocols and primers used for amplifying and sequencing are given by Morrow et al. (2012) for 28S rRNA and CO1 sequences, Thacker et al. (2013, this issue) for additional 28S sequences and Redmond et al. (2013, this issue) for 18S sequences.

Phylogenetic analyses

Sequences were managed in Geneious Pro 4.7 software (Drummond et al. 2009). Forward and reverse reads were assembled into contigs using the assembly function of the software and checked for inconsistencies. In cases in which the forward and reverse reads disagreed, Geneious automatically used the better quality of the two reads or introduced an IUPAC ambiguity code into the consensus sequence. The sequences were aligned with MUSCLE v. 3.6 (Edgar 2004a, 2004b) and trimmed in Geneious. Question marks were used for any missing data. JModelTest (Darriba et al. 2012) identified the GTR+G+I model as the best-fit model of molecular evolution for all datasets.

Phylogenetic analyses were conducted using maximum likelihood in RaxML (Stamatakis et al. 2008) and Bayesian inference in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The best tree from RaxML is illustrated showing bootstrap supports >50 and posterior probabilities >0.5 from the Bayesian analysis. Additional partitioned analyses and analyses treating saturation of the third codon in the CO1 barcoding sequences with RY coding gave the same topology.

Whilst previous molecular studies have suggested that Haploscleromorpha (= marine haplosclerids) are the sister group to *Heteroscleromorpha* (Borch-iellini et al. 2004; Lavrov et al. 2008), Erpenbeck et al. (2004) demonstrated that ribosomal sequences in Haploscleromorpha showed increased evolutionary substitution rates, which disqualifies them as a suitable outgroup taxa for rRNA analyses of *Heteroscleromorpha*; therefore *Lamellodysidea herbacea* (Keller, 1889) and *Dysidea arenaria* Bergquist, 1965 (Keratosa: Demospongiae) were chosen for the combined 18S-28S rRNA analysis and the combined 18S-28S-CO1 analysis, respectively. For consistency *Dysidea arenaria* was chosen as the outgroup for our CO1 analysis.

Results

Description of the trees

A genetree based on RaxML analysis of combined full-length 18S and 28S (D3–D8 region) rRNA

Table 1 A list of species used in this study arranged alphabetically with collecting localities

Organism	Voucher	Locality	COX1	28S (D3–5)	28S (D6–8)	18S
<i>Acanthella acuta</i>	Mc7160	Mediterranean	HQ379408	HQ379259	HQ379331	—
<i>Acanthella acuta</i>	—	Mediterranean	—	—	—	GQ466052
<i>Acanthella cavernosa</i>	—	Guam	—	KC869543	—	—
<i>Acanthella cavernosa</i>	0CDN9790-Z	Palau	—	—	—	KC902194
<i>Acanthurypion pilosella</i>	Mc7748	Ireland	—	KC952007	KC883679	KC902379
<i>Acanthostylotella cornuta</i>	0CDN8730-X	Guam	—	KC869600	—	KC902123
<i>Adreus fascicularis</i>	Mc4559	English Channel	HQ379428	HQ379314	HQ379379	KC902329
<i>Adreus sp.</i>	Mc4982	Ireland	—	HQ379311	HQ379377	KC902410
<i>Agelas axifera</i>	G320422	Australia	DQ069299	—	—	—
<i>Agelas conifera</i>	KC869634	Panama	—	KC869634	—	—
<i>Agelas conifera</i>	—	—	—	—	—	AY734443
<i>Agelas dispar</i>	NCI171	USA	—	KC884836	—	—
<i>Agelas dispar</i>	—	—	DQ075710	—	—	AY737640
<i>Amorphinopsis excavans</i>	0CDN9237-Y	Malaysia	—	KC869473	—	KC902330
<i>Amphilectus fucorum</i>	Mc5093	Wales	—	HQ379294	HQ379362	KC902221
<i>Ancorina alata</i>	0CDN6664-C	New Zealand	—	KC884835	—	KC901881
<i>Ancorina alata</i>	0CDN6551-G	New Zealand	—	KC884845	—	KC902129
<i>Anomomycale tibubans</i>	Mc7765	Ireland	—	HQ379297	HQ379365	KC902230
<i>Antho involvens</i>	Mc4262	Scotland	—	HQ379291	HQ379359	KC902050
<i>Astroclera willeyana</i>	0CDN5435-R	Tonga	—	KC869525	—	KC902051
<i>Atergia corticata</i>	Mc7715	Ireland	—	KC883681	KC883680	KC902079
<i>Axechina raspailioides</i>	0M9H2473-G	Australia	—	KC869448	—	KC902059
<i>Axinella infundifoliformis</i>	Mc4438	Scotland	HQ379410	—	—	—
<i>Axinella polypoides</i>	—	Mediterranean	—	DQ299255	—	APU43190
<i>Axinella pyramidata</i>	Mc3385	Ireland	—	HQ379265	HQ379335	KC902269
<i>Axinella vaceleti</i>	Mc4200	Mediterranean	—	HQ379266	HQ379336	KC902004
<i>Axinyssa topsenti</i>	0CDN8822-X	Papua New Guinea	—	KC869558	—	KC902315
<i>Biemna saucia</i>	G303281	Australia	JF773146	—	—	—
<i>Biemna variantia</i>	Mc5405	Wales	HQ379424	HQ379292	HQ379360	KC901961
<i>Ceratopsion axiferum</i>	0M9H2585-A	Australia	—	KC869596	—	KC902000
<i>Cervicornia cuspidifera</i>	0M9G1351-I	USA	—	KC869474	—	KC902382
<i>Cinachyrella kuekenthali</i>	P23	Panama	—	KC869490	—	—
<i>Cinachyrella kuekenthali</i>	—	—	EF519602	—	—	—
<i>Cinachyrella kuekenthali</i>	USNM_1133786	Panama	—	—	—	KC902290
<i>Ciocalyptra penicillus</i>	Mc5051	Roscoff/France	—	HQ379315	HQ379381	KC902049
<i>Clathria armata</i>	Mc4359	Scotland	KC869418	KC869437	KC869445	KC901940
<i>Clathria barleei</i>	Mc4347	Scotland	KC883682	HQ393897	HQ393901	KC902394
<i>Clathria oxeoata</i>	B66	Belize	EF519605	—	—	—
<i>Clathria rugosa</i>	G300696	New Caledonia	HE611604	—	—	—
<i>Clathria schoenus</i>	P10	Panama	—	KC884834	—	—
<i>Clathria schoenus</i>	SI06x33	Panama	—	—	—	KC902370
<i>Cliona celata</i>	Mc5497	Wales	—	HQ379310	HQ379376	KC902383
<i>Cliona celata</i>	—	—	EF519608	—	—	—
<i>Cliona varians</i>	0M9G1439-C	USA	—	KC869519	—	KC902145

(continued)

Table 1 Continued

Organism	Voucher	Locality	COX1	28S (D3–5)	28S (D6–8)	18S
<i>Crella elegans</i>	Mc7174	Mediterranean	KC876698	HQ393898	HQ393902	KC902282
<i>Crella rosea</i>	Mc2418	Ireland	—	HQ379299	HQ379367	KC902058
<i>Cymbaxinella corrugata</i>	USNM_1133767	Panama	—		KC869523	KC902298
<i>Cymbaxinella damicornis</i>	Mc4987	Ireland	—	HQ379261	HQ379333	KC902335
<i>Desmacella cf. annexa</i>	Mc4240a	Scotland	KC876697	HQ379293	HQ379361	KC902284
<i>Desmoxya pelagiae</i>	Mc7764	Ireland	KC876696		—	—
<i>Dictyonella sp.</i>	NCI228	Australia	—		KC884834	—
<i>Dictyonella incisa</i>	Mc2041	Mediterranean	—		—	KC902014
<i>Dragmacidon reticulatum</i>	—	—	AJ843894		—	—
<i>Dysidea arenaria</i>	—	Vanuatu	JQ082809		—	—
<i>Ecionemia acervus</i>	0CDN7076-Z	Palau	—		KC884842	KC902119
<i>Ectyoplasia ferox</i>	USNM_1133718	Panama	EF519612		KC869540	KC901974
<i>Ectyoplasia ferox</i>	—	Caribbean	EF519612		—	—
<i>Ectyoplasia tabula</i>	0M9H2632-C	Australia	—		KC869472	KC901950
<i>Endectyon delaubenfelsi</i>	Mc4527	English Channel	HQ379412		—	—
<i>Ephydatia cooperensis</i>	—	—	DQ087505		—	—
<i>Eurypon clavigerum</i>	Mc4992	Ireland	—	HQ379272	HQ379340	KC901988
<i>Eurypon hispidum</i>	0CDN7586-G	Vanuatu	—		KC869614	KC902068
<i>Forcepia sp.</i>	0CDN7230-S	S. Africa	—		KC869627	KC902407
<i>Geodia vestigifera</i>	0CDN6732-A	New Zealand	—		KC884832	KC901913
<i>Halichondria bowerbanki</i>	Mc4003	Ireland	—	HQ379316	HQ379382	KC902247
<i>Halichondria melanadocia</i>	USNM_1133755	Panama	—		KC869508	KC902080
<i>Halichondria panicea</i>	Mc4070	Ireland	KC869423	HQ379317	HQ379383	KC902238
<i>Halicnemia sp.</i>	Mc5427	Ireland	HQ379422	HQ379287	HQ379355	KC902045
<i>Halicnemia verticillata</i>	Mc5018	Ireland	HQ379414		—	—
<i>Higginsia anfractuosa</i>	0CDN3725-J	Tanzania	—		KC884840	KC902091
<i>Higginsia mixta</i>		Malaysia	—		KC869485	—
<i>Higginsia mixta</i>	0CDN9379-F	Malaysia	—		—	KC902154
<i>Higginsia petrosioides</i>	G300611	Australia	JQ034564		—	—
<i>Homaxinella subdola</i>	Mc5438	Wales	—	HQ379318	HQ379385	KC901944
<i>Hymedesmia pansa</i>	Mc5725	Wales	—	HQ379301	HQ379368	KC902027
<i>Hymeniacion heliophila</i>	0M9G1074-H	USA	—		KC884838	KC901957
<i>Hymeniacion kitchingi</i>	Mc3332	Ireland	—	KC869434	HQ379384	KC902333
<i>Hymenaphia breeni</i>	Mc4693	Ireland	KC869421		—	—
<i>Hymenaphia stellifera</i>	Mc4669	Ireland	—	HQ379275	HQ379343	KC901948
<i>Hymenhabdia typica</i>	Mc4588	Ireland	KC869425	HQ379289	HQ379357	KC902371
<i>Jaspis novaezelandiae</i>	0CDN6804-G	New Zealand	—		KC895549	KC901966
<i>Lamellodysidea herbacea</i>	0PHG1160-T	Malaysia	—		KC869535	KC902214
<i>Latrunculia lunavirdis</i>	0CDN7382-J	S. Africa	—		KC869489	KC902327
<i>Lissodendoryx arenaria</i>	0CDN7285-C	S. Africa	—		KC869561	KC901932
<i>Lissodendoryx colombiensis</i>	USNM_1133712	Panama	—		KC869647	KC902105
<i>Lissodendoryx fibrosa</i>	0CDN9368-R	Malaysia	—		KC869479	KC901973
<i>Lissodendoryx jenjonesae</i>	Mc4281	Scotland	—	HQ379298	HQ379366	KC902088

(continued)

Table 1 Continued

Organism	Voucher	Locality	COX1	28S (D3–5)	28S (D6–8)	18S
<i>Lissodendoryx</i> sp.	0M9I5828-T	Malaysia	—		KC869506	KC902216
<i>Microciona prolifera</i>	—	—	DQ087475		—	—
<i>Microscleroderma herdmanni</i>	0CDN9628-Y	Palau	—		KC884846	KC902255
<i>Monanchora arbuscula</i>	SI06x186	Panama	—		KC869447	KC902187
<i>Mycale macilenta</i>	Mc3618	Ireland	—	KC869436	KC869442	KC901898
<i>Mycale mirabilis</i>	OPHG1422-F	Malaysia	HE611591		KC869613	KC902146
<i>Mycale rotalis</i>	Mc5391	Wales	—	HQ379296	HQ379364	KC902397
<i>Mycale subclavata</i>	Mc3314	Ireland	—	KC869433	KC869441	KC902072
<i>Myrmekioderma granulatum</i>	OPHG1422-F	Malaysia	—		KC869471	KC901877
<i>Myrmekioderma gyroderma</i>	—	—	EF519652		—	—
<i>Myxilla anchorata</i>	Mc3306	Ireland	—	HQ379304	HQ379370	—
<i>Myxilla anchorata</i>	Mc4255	Scotland	—		—	KC902360
<i>Myxilla</i> cf. <i>roseacea</i>	Mc4681	Ireland	—	KC883686	KC883683	KC901935
<i>Neofibularia hartmani</i>	0CDN8100-O	Samoa	JF773145		KC869639	KC901997
<i>Neofibularia nolitangere</i>	—	—	EF519653		—	—
<i>Pachymatisma johnstoni</i>	Mc3504	Scotland	EF564330		—	—
<i>Paratimea</i> cf. <i>duplex</i>	PS70/17-1(1)	Norway	KC869429		—	—
<i>Paratimea</i> sp.	Mc4323	Scotland	HQ379419	HQ379284	HQ379352	HQ379419
<i>Paratimea</i> sp.	Mc5226	Wales	—	HQ379283	HQ379351	KC902401
<i>Penares</i> cf. <i>alata</i>	0CDN7316-M	S. Africa	—		KC869466	KC902193
<i>Phakellia rugosa</i>	Mc7456	Norway	KC869419		—	—
<i>Phakellia ventilabrum</i>	Mc4248	Scotland	HQ379409	HQ379260	HQ379332	KC901915
<i>Phorbas bihamiger</i>	Mc4493	English Channel	—	KC869431	KC869444	KC901921
<i>Phorbas dives</i>	Mc4517	English Channel	—	HQ379303	HQ379369	KC902286
<i>Phorbas punctatus</i>	Mc5343	Wales	—	KC869439	KC869440	KC902093
<i>Pione vastifica</i>	—	Caribbean	EF519665		—	—
<i>Placospongia intermedia</i>	PC-BT-18	Panama	KC869430		—	—
<i>Plocamionida ambigua</i>	Mc4345	Scotland	—	KC869435	KC869443	KC902218
<i>Polymastia boletiformis</i>	Mc5014	Ireland	—	HQ379306	HQ379372	KC902065
<i>Polymastia janeirensis</i>	—	Brazil	EU076813		—	—
<i>Polymastia penicillus</i>	Mc5284	Ireland	—	HQ393899	HQ393903	—
<i>Polymastia penicillus</i>	Mc5065	Ireland	—		—	KC902065
<i>Polymastia</i> sp.	Mc6488	Ireland	KC869420		—	—
<i>Prosuberites longispinus</i>	Mc7173	Mediterranean	—	HQ379320	HQ379387	KC902182
<i>Ptilocaulis spiculifer</i>	0CDN9412-P	Malaysia	—		KC869560	KC902092
<i>Ptilocaulis walpersi</i>	—	Bahamas	EU237488		—	—
<i>Raspaciona aculeata</i>	Mc7159	Mediterranean	HQ379415		—	—
<i>Raspailia hispida</i>	Mc3597	Ireland	HQ379416	HQ379279	HQ379348	KC902385
<i>Raspailia phakellopsis</i>	0M9H2417-T	Australia	—		KC869585	KC902272
<i>Raspailia ramosa</i>	Mc4024	Ireland	HQ379417	HQ379281	HQ379349	KC902299
<i>Raspailia vestigifera</i>	NCI431	Australia	—		KC869583	KC901895
<i>Reniochalina stalagmitis</i>	NCI287	Australia	—		KC869582	—
<i>Reniochalina stalagmitis</i>	—	—	—			EF092272

(continued)

Table 1 Continued

Organism	Voucher	Locality	COX1	28S (D3–5)	28S (D6–8)	18S
<i>Rhabdastrella globostellata</i>	OPHG1710-R	Vietnam	—	KC884843		KC902160
<i>Rhabderemia sorokiniae</i>	G312904	Papua New Guinea	HE611607	—		—
<i>Scopalina hispida</i>	NCI272	USA	—	KC884841		KC902237
<i>Scopalina lophyropoda</i>	Mc4217	Mediterranean	—	HQ379268	HQ379337	KC901894
<i>Scopalina ruetzleri</i>		Panama	—	KC869553		—
<i>Scopalina ruetzleri</i>	—	—	—	—		AJ621546
<i>Spanioplion armaturum</i>	Mc4500	English Channel	EF519602	KC869438	KC869446	KC902324
<i>Sphaerotylus antarcticus</i>	POR21125	Antarctica	KC869424	—		—
<i>Sphaerotylus</i> sp. C	Mc4236	Ireland	—	HQ379307	HQ379373	—
<i>Sphaerotylus</i> sp. C	Mc4697	Ireland	—	—		KC902307
<i>Spongilla lacustris</i>	Mc7351	Ireland	HQ379431	HQ379327	HQ379393	KC902349
<i>Stelletta clavosa</i>	0CDN9840-G	Palau	—	KC884847		KC901967
<i>Stelletta grubii</i>	Mc5043	Ireland	—	HQ379255	HQ379329	KC902213
<i>Stelligera rigida</i>	Mc4357	Scotland	HQ379420	HQ379285	HQ379353	KC902164
<i>Stelligera stuposa</i>	Mc4330	Scotland	HQ379421	HQ379286	HQ379354	KC902232
<i>Stryphnus ponderosus</i>	Mc4240	Scotland	—	HQ379257	HQ379330	—
<i>Suberites aurantiacus</i>	KC869577	Panama	—	KC869577		—
<i>Suberites aurantiacus</i>	SI06x105	Panama	—	—		KC902366
<i>Suberites ficus</i>	Mc4322	Ireland	HQ379429	HQ379322	HQ379389	KC902236
<i>Suberites massa</i>	Mc4528	English Channel	—	HQ379324	HQ379390	KC902066
<i>Suberites pagurorum</i>	Mc4043	Ireland	KC869422	—		—
<i>Svenzea zeai</i>	USNM_1133762	Panama	—	KC869635		KC902075
<i>Tedania strongylostyla</i>	0CDN7611-I	Vanuatu	—	KC869515		KC901911
<i>Terpios aploos</i>	0CDN3602-Y	Tanzania	—	KC869465		KC902316
<i>Terpios gelatinosa</i>	Mc3315	Ireland	—	HQ379325	HQ379391	KC902355
<i>Tethya actinea</i>	SI06x109	Panama	—	KC869527		—
<i>Tethya actinea</i>	—	—	—	—		AY878079
<i>Tethya aurantium</i>	—	Mediterranean	EF584565	—		—
<i>Tethya citrina</i>	Mc5113	Wales	HQ379427	—		—
<i>Tethya norvegica</i>	—	Norway	EF558565	—		—
<i>Tethyopsis mortenseni</i>	0CDN6706-X	New Zealand	—	KC869618		KC902095
<i>Tethyopsis</i> sp.	0CDN6825-C	New Zealand	—	KC869476		KC902234
<i>Tethyspira spinosa</i>	Mc4641	Ireland	HQ379418	HQ379282	HQ379350	KC902120
<i>Theonella cylindrica</i>	0CDN9523-L	Malaysia	—	KC884839		KC902244
<i>Theonella swinhoei</i>	0CDN9465-W	Malaysia	—	KC884844		KC901886
<i>Timea unistellata</i>	Mc7300	Ireland	KC869427	—		—
<i>Topsentia</i> sp.	P126	Panama	—	KC884837		—
<i>Topsentia</i> sp.	0CDN8723-Q	Guam	—	—		KC902261
<i>Trachycladus styliifer</i>	0CDN6656-T	New Zealand	—	KC869453		KC901930
<i>Trachytedania</i> cf. <i>ferrolensis</i>	Mc5348	Wales	—	KC883684	KC883685	KC902219
<i>Tsitsikamma pedunculata</i>	0CDN7414-S	S. Africa	—	KC869512		KC902279
<i>Ulosa stuposa</i>	Mc4523	English Channel	KC869428	HQ379295	HQ379363	KC901912

Catalogue numbers for the voucher specimens are from the Ulster Museum Belfast, Porifera Collection (Mc-); National Cancer Institute (NCI) collection, maintained by the National Museum of Natural History (NMNH) The Queensland Museum, Porifera Collection (G) and a variety of specimens collated by the Porifera Tree of Life project. PC-BT-18 and PS70/70/17-(1) are from Paco Cardenas' private collection. The 18S rRNA, 28S rRNA, and CO1 sequences used in this study are shown with their GenBank accession numbers.

sequences of 121 species was constructed using a wide range of species both from this work and from previous studies (Fig. 2). While it was not always possible to represent the same species, a second tree (Fig. 3), based on mitochondrial CO1 barcoding sequences from 57 taxa, covering the same genera as the 18S-28S tree, was constructed using RaxML. The CO1 tree recovered the same clades as the 18S-28S genetree but had a different branching order and less resolution. A genetree based on RaxML analysis of combined 18S, 28S rRNA and CO1 sequences of 33 taxa was constructed (Fig. 4). In order to have representatives of Axinellidae and Polymastiidae Gray, 1867, the 18S and 28S rRNA sequences of *Axinella vacceleti* Pansini, 1984 were concatenated with the CO1 sequences of *Axinella infundibuliformis* (Linnaeus, 1759) and the 18S and 28S rRNA sequences of *Polymastia penicillus* (Montagu, 1818) were concatenated with *Polymastia* sp. A separate analysis of CO1 sequences (Fig. 3) shows *A. infundibuliformis* grouping within Axinellidae and *Polymastia* sp. within Polymastiidae.

The resulting genetrees (Figs. 2–4) are congruent with the 28S rRNA and CO1 genetrees of Morrow et al. (2012). However, our combined trees (Figs. 2 and 4) have better resolution, particularly of the deeper nodes, and stronger support values. Gazave et al. (2010b) combined full-length 18S rRNA sequences with the C1-D3 region of 28S rRNA; their resulting dataset had 29 species and 2623 positions. Our combined 18S rRNA and 28S rRNA (D3–D8 region) analysis (Fig. 2) is substantially larger and contains 121 species and 3217 positions. This is the first study to do a combined analysis of 18S, 28S, and CO1 sequences for demosponges. Our combined dataset had 33 taxa and the alignment had 3811 positions. Our results conflict with many of the orders, families, and genera of the (morphological) classification of *Systema Porifera* (Hooper and van Soest 2002).

Our results are congruent with previous molecular studies using ribosomal and mitochondrial markers (e.g., Erpenbeck et al. 2007a, 2007b; Nichols 2005) but contrast with the recent results of Hill et al. (2013) which attempted to reconstruct family-level relationships within Demospongiae using seven nuclear housekeeping genes. One of the major differences concerned the relative position of Spongillida (freshwater sponges). In our analyses Spongillida clustered with Scopalinidae and was sister to the main heteroscleromorph clade. However, in Hill et al. (2013) Spongillida did not group with Heteroscleromorpha but was sister to Haploscleromorpha. In that analysis Tetractinellida was the sister group to

the main heteroscleromorph clade but with very low support values. It is difficult to compare our phylogeny with that of Hill et al. which had very low taxon sampling (several of the families we included were not sampled and most of the families were only represented by one taxon) and low support for many of the deeper nodes. Graybeal (1998) and Wiens (1988) demonstrated that increased taxon sampling rather than increased number of characters is more effective in resolving difficult phylogenetic problems.

The 14 clades that are highlighted and named in Figs. 2 and 4 are also those recovered by Morrow et al. (2012). The combined analyses (Figs. 2 and 4) show strong support for a large clade encompassing Axinellidae s.s., Raspailiidae, and Stelligeridae Lendenfeld, 1898. Although Morrow et al. (2012) did not resolve the position of Tetractinellida, Bubaridae (Dictyonellidae), and Biemnidae relative to the rest of the heteroscleromorph clades, our combined analysis in Fig. 4 shows strong support for Biemnidae being the sister group to Tetractinellida with Bubaridae as the sister group to these two clades.

The CO1 genetree (Fig. 3) also supports the clades highlighted in Figs. 2 and 4; however, Scopalinidae was not represented. The CO1 genetree supports a clade with Axinellidae s.s., Raspailiidae, and Stelligeridae; however, the support is much lower than with ribosomal genes (Fig. 2). Erpenbeck et al. (2006, 2007b) pointed out that the CO1 barcoding region did not have sufficient phylogenetic signal to resolve the relationships between clades. Therefore the 18S + 28S tree is our preferred tree for inferring phylogenetic relationships among clades and improving systematics of the group.

Discussion

The division of Demospongiae into two subclasses, Tetractinomorpha (oviparous) and Ceractinomorpha (ovoviviparous), by Lévi (1956) based on reproductive strategies has now been abandoned as several congruent molecular studies have not supported this division (Lafay et al. 1992; Borchiellini et al. 2004; Nichols 2005). Mode of reproduction appears to be a homoplasious character (van Soest et al. 1990; Cardenas et al. 2012). It is possible to reconcile the characters used by traditional taxonomists with our molecular results if we reinterpret the spicule characters used and accept significant levels of homoplasy and character loss. Below we discuss the distribution of asterose and sigmatose microscleres, acanthostyles, and axially condensed skeletons within Heteroscleromorpha. One of the major problems with using cladistics in sponge taxonomy is that

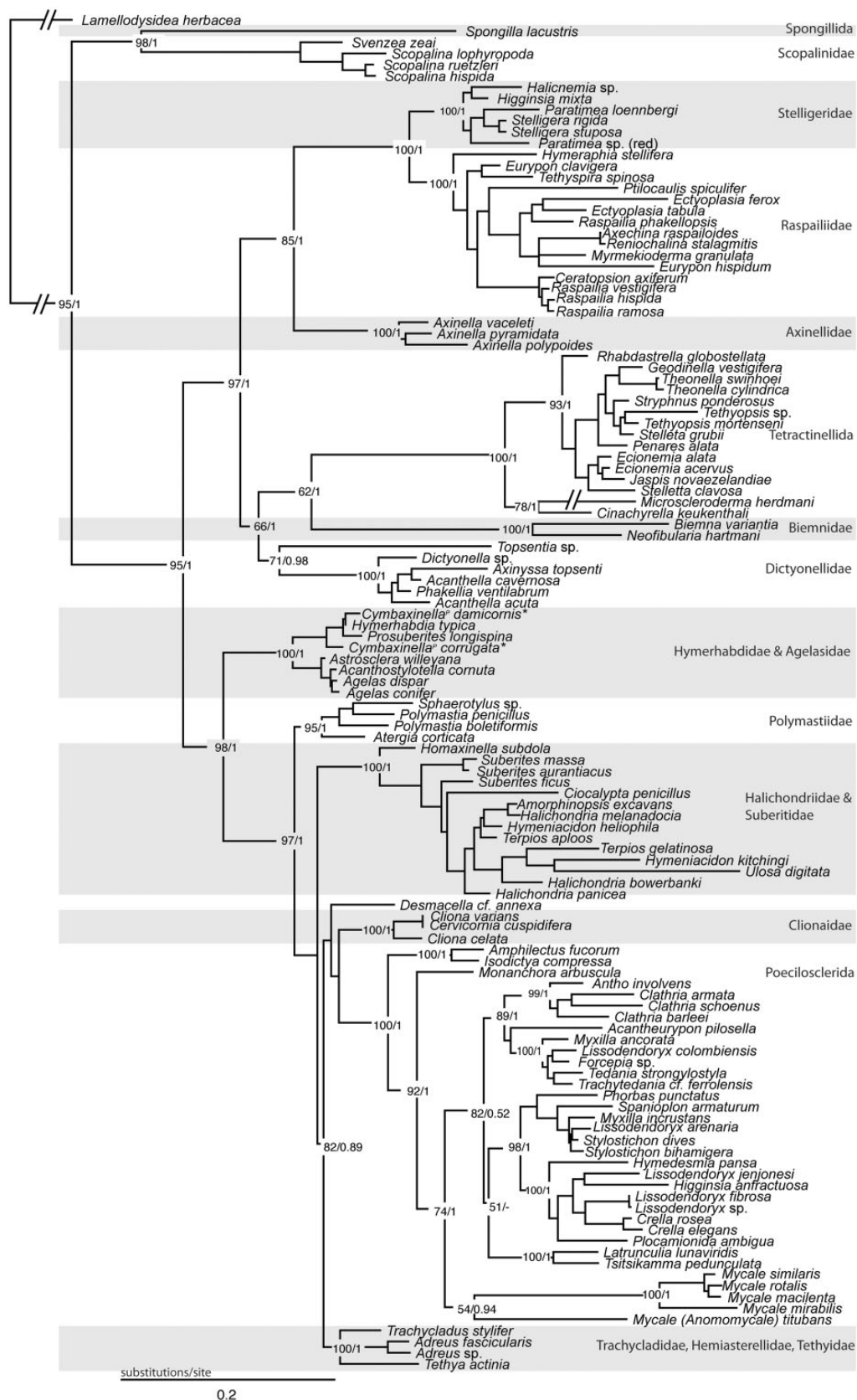


Fig. 2 Best tree output from RaxML analysis of full-length 18S rRNA combined with 28S rRNA (D3–D8 region) sequences from 121 species of demosponges. Figures at nodes correspond to bootstrap support >50 followed by posterior probabilities >0.5 from the Bayesian analysis.

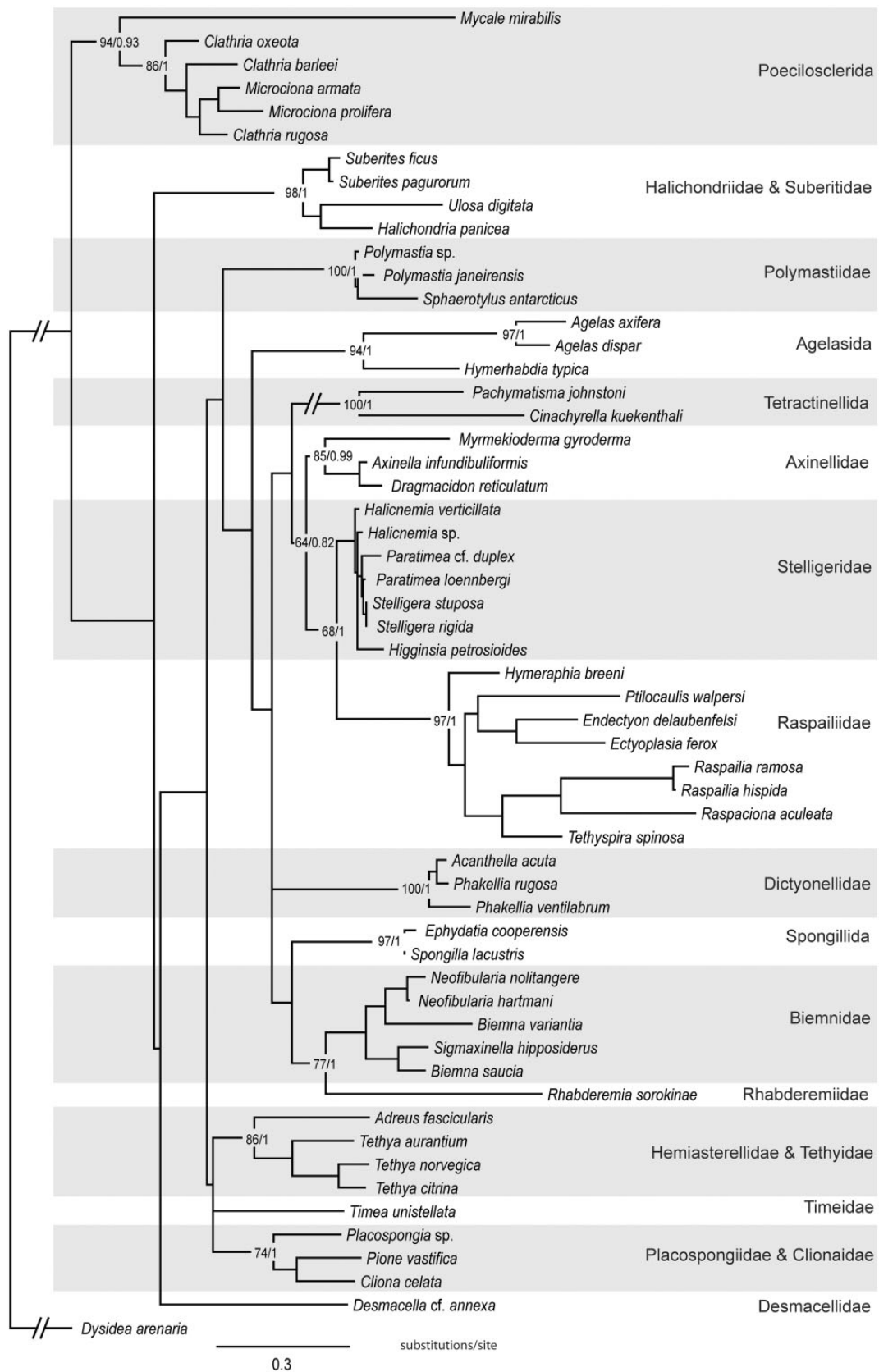


Fig. 3 Best tree output from RaxML analysis of mitochondrial CO1 barcoding fragment from 57 species of demosponges. Figures at nodes correspond to bootstrap support >50 followed by posterior probabilities >0.5 from the Bayesian analysis.

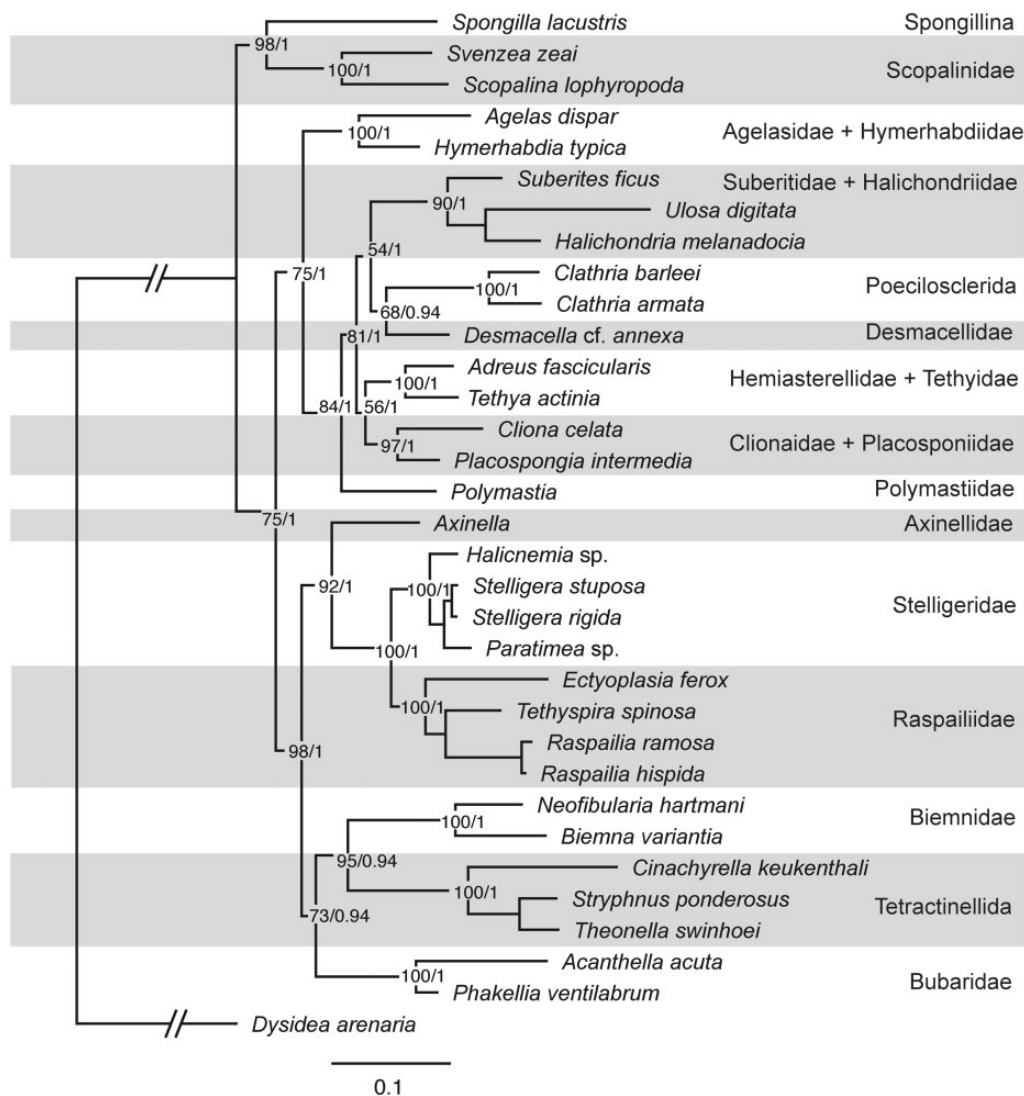


Fig. 4 Best tree output from RaxML combined analysis of full-length 18S rRNA, 28S rRNA (D3–D8 region) and mitochondrial CO1 barcoding fragment from 33 species of demosponges. Figures at nodes correspond to bootstrap support >50 followed by posterior probabilities >0.5 from the Bayesian analysis.

often the name given to a type of spicule is descriptive only and does not imply homology (Boury-Esnault 2006). These new results help to illuminate the evolutionary plasticity of heteroscleromorph skeletal elements.

Sigmata

The term sigma is used for C- or S-shaped microscleres. The Soest-Hooper system placed Haploscleromorpha (= marine haplosclerids) as sister group to Poecilosclerida, primarily on the basis that sigmatose microscleres are found in both (Fig. 1B). Subsequent molecular studies using 18S and 28S rRNA (Borchjellini et al. 2004), 28S rRNA and CO1 (Nichols 2005), 28S rRNA (Holmes and Blanch 2007), complete mitochondrial genomes (Lavrov et al. 2008),

and housekeeping genes (Sperling et al. 2009; Hill et al. 2013) are congruent and show Haploscleromorpha as sister to Heteroscleromorpha. Fromont and Bergquist (1990) studied the different types of sigma found in Haploscleromorpha and Poecilosclerida and concluded that attempts to classify sponges on the basis of general morphological characters such as sigmata was an oversimplification of their diversity and resulted in misleading results. Sigmatose microscleres are found in Biemnidae Hentschel, 1923, Desmacellidae Ridley and Dendy, 1886, Poecilosclerida and Haploscleromorpha; this indicates that the presence of sigmata can be homoplasious (Fig. 1C).

Our CO1 genetree (Fig. 3) shows *Rhabderemia sorokiniae* Hooper, 1990 clustering with *Biemna*

spp., *Neofibularia* spp., and *Sigmaxinella*. On the basis of skeletal characters (mainly the shared possession of sigmata), Hooper (1984) synonymized Sigmaxinellidae (Axinellida) and Biemnidae (Poecilosclerida) into a single family Desmacellidae and assigned Desmacellidae to Axinellida. Lévi (1955) gave a diagnosis of Sigmaxinellidae as “axinellids with sigmoid microscleres;” however, he commented that the status of this family was very uncertain as the spicules might be analogous with those in Biemnidae. van Soest (1984b) transferred Desmacellidae to Poecilosclerida.

Rhabderemiidae

Hooper (1990b) synonymized *Rhabdosigma* Hallmann, 1916 with *Rhabderemia* Topsent, 1890 and transferred Rhabderemiidae from Axinellida to Microcionina Hajdu et al., 1994: Poecilosclerida on the basis that the monactinal megascleres and the structure of the microscleres are homologous with those of poecilosclerids. Rhabderemiidae is a monogeneric family with rhabdostyle megascleres; microscleres (if present) include rugose oxete or toxa-like spicules (thraustoxeas), rugose sigma-like spicules (spirosigmata, thraustosigmata), and rugose microstyles (Hooper 2002). van Soest and Hooper (1993) indicated that there is some doubt over the homology of the sigmoid toxiform microscleres between Rhabderemiidae and other poecilosclerids. *Rhabderemia sorokinae* clusters with *Biemna* spp., *Neofibularia* spp., and *Sigmaxinella hipposiderus* Mitchell et al., 2011 and not with microcionid taxa in Poecilosclerida (Fig. 3).

There is also morphological support for Rhabderemiidae having a close relationship with Biemnidae/Sigmaxinellidae. Cedro et al. (forthcoming) described a new species of *Rhabderemia* that has sigmata with microspined ends, similar to the sigma in some *Biemna* species. e.g., *B. microacanthosigmata* Mothes et al., 2004 and *Sigmaxinella cearense* Salani et al., 2006. *Biemna rhabderemioides* Bergquist, 1961 and *Biemna rhabdostyla* Uriz, 1988 have rhabdose megascleres that resemble those found in *Rhabderemia*. van Soest and Hooper (1993) assumed that the rhabdostyles found in *Rhabderemia* and *Biemna* were homoplasious and did not indicate a close phylogenetic relationship between the two genera. However, in *B. rhabdostyla*, Uriz (1988) highlighted the fact that this species has “normal” *Biemna* spicules, i.e., “normal” styles, sigmata, raphides, and microxea, but in addition it also has rhabdostyles whilst *B. rhabderemioides* has only rhabdose styles. These two species are intermediate between *Biemna* and

Rhabderemia and lend morphological support to the hypothesis that the two families are closely related.

The ribosomal genetree shows Biemnidae as sister group to Tetractinellida Marshall, 1876; this relationship was strongly supported by our Bayesian analysis (p.p.1) but had relatively weak support using RaxML (62 b.s.). The sigmaspires and raphides present in Spirophorina Bergquist and Hogg, 1969 (Tetractinellida) are possibly synapomorphic with the sigmaspires found in *Rhabderemia* and the raphides in *Biemna* and *Neofibularia*. The sigmaspires in *Rhabderemia* are similar to those found in Spirophorina. They are C-shaped or S-shaped, sometimes with a double twist, and the surface is minutely hispid; they also have similar dimensions. The tentative relationship suggested here needs to be tested with other markers, other *Rhabderemia* species, and a more detailed comparison of morphological characters.

Asters

Fig. 1C shows the distribution of asterose microscleres (star-shaped spicules) on our molecular tree. The families Hemiasterellidae and Trachycladidae were included in Axinellida Lévi, 1953. van Soest et al. (1990) assigned them to Hadromerida on the basis of the shared possession of asters. Several molecular studies have now demonstrated that asters are homoplasious (Chombard et al. 1998; Borchellini et al. 2004; Nichols 2005; Morrow et al. 2012). Asterose microscleres have arisen independently on at least four occasions (Fig. 1C): in Myxospongiae Haeckel, 1866 (Chondrillidae Gray, 1872); Tetractinellida (Astrophorina Sollas, 1888); Axinellida (Stelligeridae), and Hadromerida (Hemiasterellidae, Tethyidae Gray, 1848, Trachycladidae, Timeidae Topsent, 1928). Asterose spicules are mainly found in the surface ectosomal layer of sponges. In the phylum Tunicata, calcium carbonate asterose spicules are also found in the surface layer of Didemnidae Giard, 1872 (Kott 2004). The presence of asterose spicules is likely to be a functional response that leads to a strengthening of the surface layer. It is also possible that asters may play an additional role in deterring predators.

Our analyses show that *Trachycladus styliifer* Carter, 1879 clusters with members of Hemiasterellidae (*Adreus* spp.) but our results also show that Hemiasterellidae is polyphyletic (Fig. 2). *Paratimea* Hallmann, 1917 and *Adreus* Gray, 1867 both have euaster microscleres and are currently considered to belong to Hemiasterellidae (van Soest et al. 2013) yet

they do not form a monophyletic assemblage (Fig. 2). Morrow et al. (2012) moved these genera into the family Stelligeridae. Re-examination of the asters in *Paratimea* and *Stelligera* Gray, 1867 shows that they are quite different to those found in *Adreus* and *Tethya* Lamarck, 1817. In *Paratimea* and *Stelligera* they are always smooth-rayed and there is only one size category, whereas in *Adreus*, *Tethya*, and *Hemiasterella* Carter, 1879 the asters often have microspined rays and come in a variety of size classes.

The molecular data presented here and in previous studies show that *Stelligera* and *Paratimea* have a close relationship with *Halicnemia* Bowerbank, 1864 and *Higginsia* Higgin, 1877 (Heteroxyidae), all of which have acanthose oxea (Erpenbeck et al. 2012; Morrow et al. 2012). Topsent (1897) considered the acanthoxea as derived from asters. It is possible that the asters in *Stelligera/Paratimea* are homologous at some level with the acanthoxea in *Halicnemia/Higginsia*, with the latter being an elongate derivative of the former. Fig. 5A shows a normal euaster in *Paratimea* sp.; Fig. 5B an acanthoxea in *Halicnemia* sp.; Fig. 5C an aberrant aster that is transitional between an aster and an acanthoxea; and Fig. 5D an acanthostyle from the raspailiid sponge *Tethyspira spinosa* Topsent, 1890. Similarly, the acanthostyles in Raspailiidae could also have been derived from asters. However, testing these speculations will require detailed examination of the formation and growth of the spicules.

Acanthostyles

Fig. 1C shows the distribution of acanthostyles within Heteroscleromorpha. Acanthostyles are found in Poecilosclerida s.s. (Microcionina; Myxillina Hajdu et al., 1994), Agelasida Hartman, 1980, and Raspailiidae. From their distribution on our tree it seems likely that acanthostyles are homoplasious. Within Agelasida the acanthostyles usually have spines arranged in whorls (verticilles) although in *Acanthostylotella* Burton and Rao, 1932 the spines are not obviously verticillate. van Soest (1991) considered asters to be confined to the group Astrophorida-Hadromerida-Hemiasterellidae (Fig. 1B) and regarded asters as a synapomorphy for a clade composed of these three groups. In his resulting classification, acanthostyles were confined to Raspailiidae-Microcionidae Carter, 1875 -Myxillidae Dendy, 1922 -Agelasidae Verrill, 1907 (Fig. 1B; van Soest 1991). However, uniting this group on the basis of the shared possession of acanthostyles posed some taxonomic problems. van Soest (1991) considered sigmoidose microscleres

synapomorphic for the group Microcioniidae-Myxillidae-Myxillidae-Lundbeck, 1905 -Petrosiidae van Soest, 1980 -Haplosclerida Topsent, 1928, but these are not found in Raspailiidae and Agelasidae. For the raspailiids he attributed this to secondary loss but questioned whether the verticillate acanthostyles found in Agelasidae were homologous. Up to and including Lévi (1973), all authors considered the agelasids to be part of Poecilosclerida. Bergquist (1978), on the basis of reproductive biology and biochemical data, assigned the family to Axinellida. Chombard et al. (1997) found support for this classification using 28S rRNA sequence data. In the same study they also demonstrated a sister relationship between Agelasidae and Astroscleridae. The genus *Axinella* Schmidt, 1862 has been shown to be polyphyletic using ribosomal and also CO1 barcoding sequences (Gazave et al. 2010b; Morrow et al. 2012). Two groups of *Axinella* were recovered, one with the type species *Axinella polypoides* Schmidt, 1862 and another with *A. damicornis* (Esper, 1794). This latter group, also containing *A. corrugata* (George and Wilson, 1919) and *A. verrucosa* (Esper, 1794) is now assigned to *Cymbaxinella*^P (Gazave et al. 2010b) and has been shown to be closely related to agelasids (Morrow et al. 2012).

The acanthostyles in Raspailiidae have a variety of geometries but some are remarkably similar to those found in Microcioniidae. This led Hentschel (1923) to assign Raspailiidae to Poecilosclerida, but other authors (e.g., Ridley and Dendy 1887; Vosmaer 1912) placed *Raspailia* in Axinellidae. Wilson (1921) emphasized an axially condensed skeleton and specialized ectosomal skeleton as the most important taxonomic characters and included Raspailiidae in Axinellidae. Most subsequent authors followed this classification until Hooper (1991), in his revision of Raspailiidae, returned the family to Poecilosclerida. An increasing number of molecular studies has shown that raspailiid taxa are not closely related to Poecilosclerida s.s. (Erpenbeck et al. 2007a, 2007b, 2007c, 2012). Morrow et al. (2012) using 28S rRNA and CO1 barcoding sequences showed that the raspailiids were sister to a redefined Stelligeridae and that the two families clustered with Axinellidae.

We demonstrate strong support for Raspailiidae being sister group to Stelligeridae (Fig. 2), represented in this analysis by *Stelligera* spp., *Paratimea* spp., *Halicnemia* spp., and *Higginsia mixta*. At least some species of the genera *Halicnemia*, *Higginsia*, *Paratimea*, and *Stelligera* share a strikingly similar surface architecture to typical raspailiid species, with large robust styles 2–3 mm long protruding from the surface surrounded by a bouquet of thin

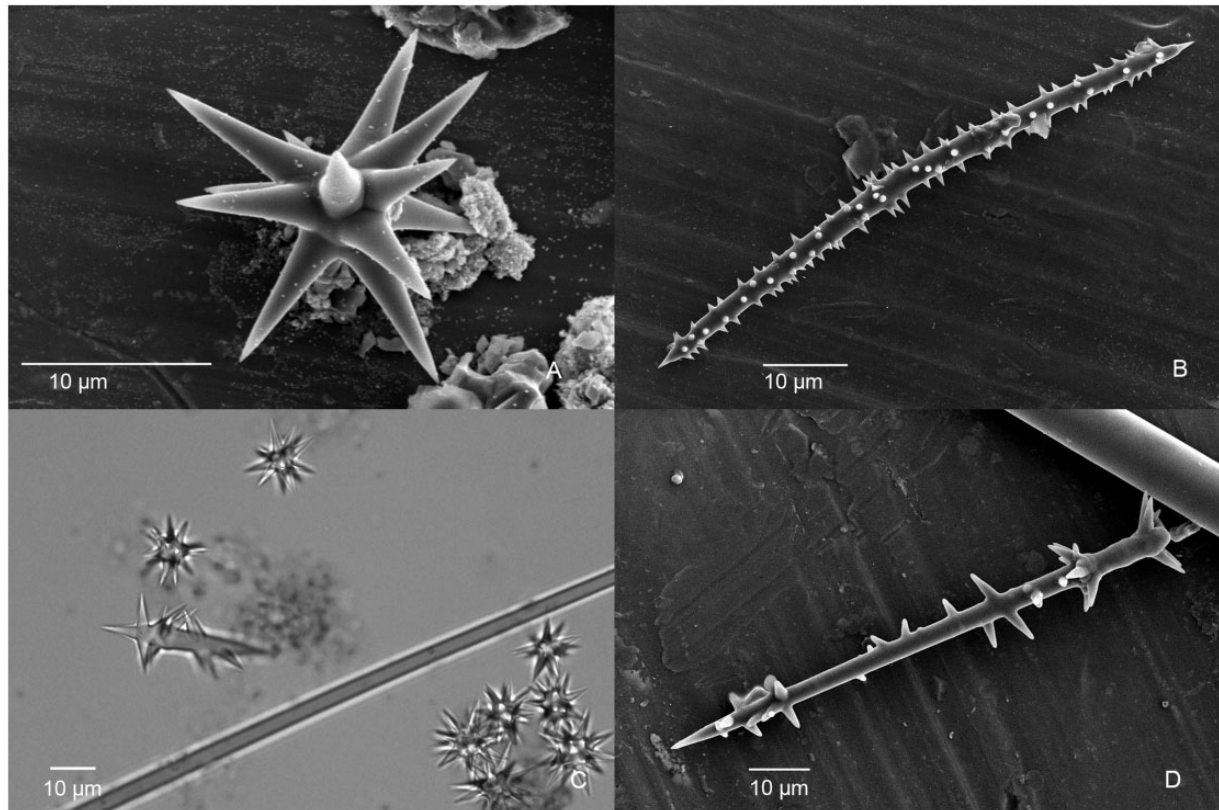


Fig. 5 (A) Scanning electron micrograph (SEM) of euaster from *Paratimea loennbergi* (Mc1590); (B) SEM of acanthoxea from *Halicnemiasp.* (Mc1598); (C) Photomicrograph of an aberrant elongate aster from *Paratimea sp.* (Mc 3163); (D) SEM of acanthostyle from *Tethyspira spinosa* (Mc3163). Catalogue numbers refer to Ulster Museum (BELUM) Porifera collection.

spicules, which in different species are variously described as styles, anisoxea, or oxea (Fig. 6A–D). This specialized ectosomal surface architecture appears to be confined to Raspailiidae and Stelligeridae and gives strong morphological support for a close relationship between these two families; however, it is not ubiquitous for all taxa. This highlights the difficulties in defining higher taxonomic groups on the basis of one or only a few morphological characters. In an undescribed species of *Paratimea*, the centrotylote oxea have fissurate ends; this type of spicule has previously been found only in *Halicnemias verticillata* and some species of *Higginsia* and appears to be apomorphic for Stelligeridae.

Condensed axial skeleton

An axial skeleton consists of a stiff axial region that is clearly distinct from a softer extra-axial region. A cross section through a branch of *Axos cliftoni* Gray, 1867 illustrates the occurrence of axially condensed skeletons (Fig. 1A–C). van Soest (1991) argued that an axially condensed skeleton represents a functional response of erect branching sponges to the problem of obtaining rigidity. It occurs in Biemnidae, Axinellidae,

Raspailiidae, Stelligeridae, Suberitidae Schmidt, 1870, Microcionidae, Trachycladidae, and Hemiasterellidae (Fig. 1C), but within each of these families there are encrusting or cushion-shaped species that do not possess an axially condensed skeleton, thereby lending support to the hypothesis of van Soest (1991).

Proposals for the classification of Heteroscleromorpha

Morrow et al. (2012) proposed the resurrection of Axinellida Lévi, 1953, based mainly on 28S rRNA sequence data. A new definition of the order was formally given to contain Axinellidae s.s., Raspailiidae, and Stelligeridae. The present study finds additional molecular and morphological support for this proposal.

Desmacella cf. *annexa* Schmidt, 1870 does not group with *Biemna* Gray, 1867, *Neofibularia* Hechtel, 1965, or *Sigmaxinella* Dendy, 1897. Molecular data from the type species of *Desmacella* Schmidt, 1870 (Redmond et al. 2013, this issue) indicate that *D. cf. annexa* is representative of the genus and we propose to resurrect Biemnidae (which has seniority over Sigmaxinellidae) for the clade containing *Biemna*

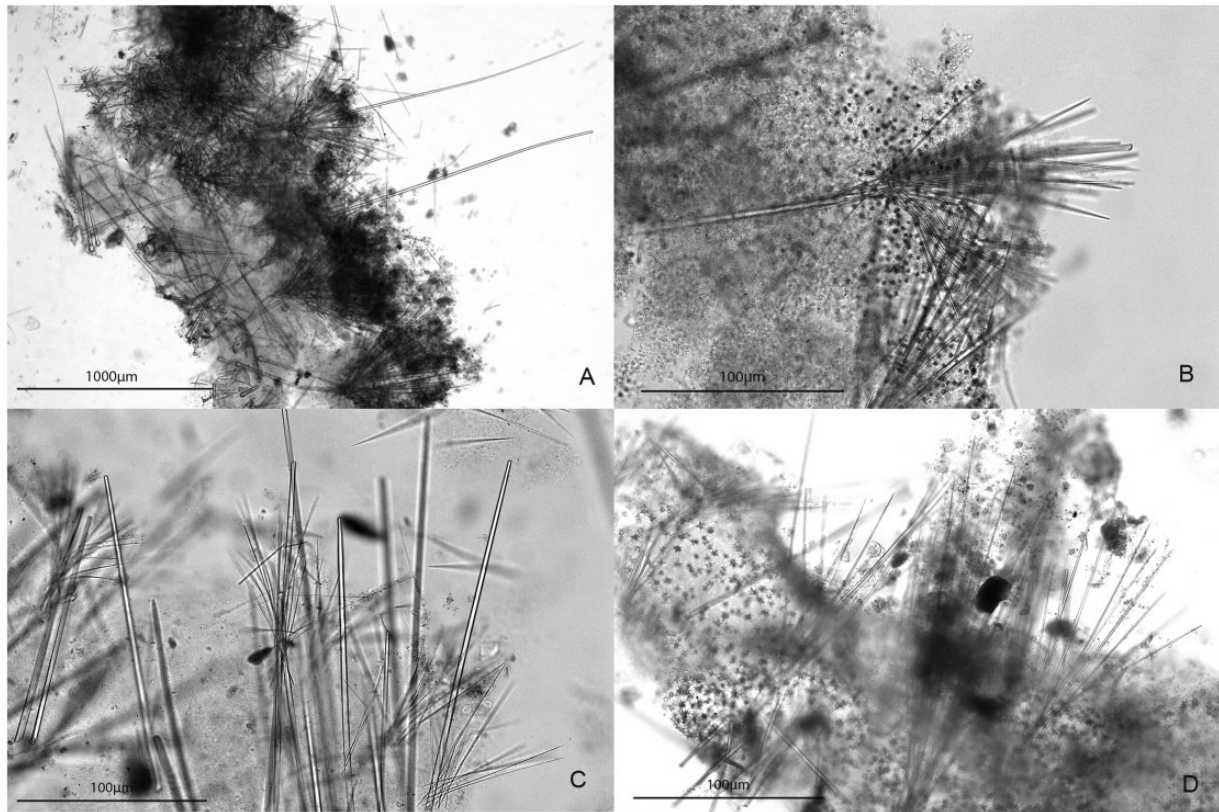


Fig. 6 Photomicrographs showing specialized surface architecture of large robust styles or tylostyles that penetrate the surface surrounded by bouquets of smaller, more slender oxea or styles. (A) *Halcnemia* sp. (Mc5907); (B) *Stelligera stuposa* (Mc4330); (C) *Raspailia hispida* (Mc3597); (D) *Paratimea* sp. (Mc3089). Catalogue numbers refer to Ulster Museum (BELUM) Porifera collection.

spp., *Neofibularia* spp., and *Sigmaxinella hipposiderus*, and use Desmacellidae for species of *Desmacella*. Hajdu and van Soest (2002) pointed out that *Sigmaxinella* is distinguished from *Biemna* mainly by the possession of an axially condensed skeleton. *Sigmaxinella* is only represented in our CO1 genetree (Fig. 3) by a single species. Any decisions regarding the status of this genus will require additional molecular data from a greater number of species.

We recovered a strongly supported clade containing *Biemna* and *Neofibularia* (Fig. 2). Whilst our CO1 tree has a different branching order to our combined 18S-28S rRNA genetree (Fig. 2), it shows strong support for a clade containing Biemnidae and Rhabderemiidae. On the basis of these molecular data and the morphological characters discussed above we propose to formally erect a new order Biemnida.

Biemnida ord. nov. Morrow, 2013

Biemnidae Hentschel, 1923; Rhabderemiidae Topsent, 1928

Encrusting, massive, cup-shaped, fan-shaped, and branching sponges. Megascleres styles, subtylostyles, strongyles, rhabdostyles, or oxea. Spicules typically

enclosed by spongin fibers. Reticulate or plumoreticulate choanosomal skeleton, maybe axially compressed. Extra-axial plumose skeleton usually present. Microscleres sigmata, spirosigmata, toxa, microxeas, raphides, or commata. *Biemna* and *Neofibularia* cause a dermatitis-like reaction when in contact with bare skin.

The problem of Hadromerida

The “hadromerid” families are found in four well-supported clades (Fig. 1C); one contains Polymastiidae Gray, 1867, a second Clionidae d’Orbigny, 1851 + Placospongiidae Gray, 1867 + Spirastrellidae Ridley and Dendy, 1886, a third Suberitidae + Halichondriidae. The fourth equates to Hadromerida: it contains Hemiasterellidae + Trachycladidae + Tethyidae + Timeidae. The order Halichondrida is left with only Halichondriidae and Suberitidae. A decision needs to be made whether to erect orders for each of these clades or suppress the order Poecilosclerida and/or Halichondrida and use Hadromerida for the very large clade containing Polymastiidae, Halichondrida, Suberitidae, Clionidae, Placospongiidae, Spirastrellidae, Poecilosclerida, Trachycladidae,

Hemiasterellidae, Tethyidae, and Timeidae; however, this is beyond the scope of this study.

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References

- Bergquist PR. 1967. Additions to the Sponge Fauna of the Hawaiian Islands. *Micronesica* 3:159–74.
- Bergquist PR. 1970. The Marine Fauna of New Zealand: Porifera Demospongiae, Part 2 (Axinellida and Halichondrida). *NZ Dept Sci Ind Res Bull [NZ Oceanogr Inst Mem 51]* 197:1–85.
- Bergquist PR. 1978. Sponges. London, UK: Hutchinson.
- Borchiellini C, Chombard C, Manuel M, Alivon E, Vacelet J, Boury-Esnault N. 2004. Molecular phylogeny of Demospongiae: implications for classification and scenarios of character evolution. *Mol Phylogenet Evol* 32:823–37.
- Boury-Esnault N. 2006. Systematics and evolution of Demospongiae. *Can J Zool* 84:205–24.
- Cárdenas P. 2010. Phylogeny, Taxonomy and Evolution of the Astrophorida (Porifera, Demospongiae). PhD Thesis, University of Bergen, Norway.
- Cardenas P, Pérez T, Boury-Esnault N. 2012. Sponge systematics facing new challenges. *Adv Mar Biol* 61:79–209.
- Cárdenas P, Xavier JR, Reveillaud J, Schander C, Rapp HT. 2011. Molecular Phylogeny of the Astrophorida (Porifera, Demospongiae) Reveals an Unexpected High Level of Spicule Homoplasy. *PLoS ONE* 6(4):e18318. doi:10.1371/journal.pone.0018318.
- Cedro VR, Hajdu E, Correia MD. Forthcoming. Three new intertidal sponges (Porifera Demospongiae) from Brazil's fringing urban reefs (Maceió Alagoas Brazil), and support for *Rhabderemia's* exclusion from Poecilosclerida. *J Nat Hist*.
- Chombard C, Boury-Esnault N, Tillier S. 1998. Reassessment of homology of morphological characters in tetractinellid sponges based on molecular data. *Syst Biol* 47:351–66.
- Chombard C, Boury-Esnault N, Tiller S, Vacelet J. 1997. Polyphyly of "sclerosponges" (Porifera Demospongiae) supported by 28S ribosomal sequences. *Biol Bull* 193:359–67.
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models new heuristics and parallel computing. *Nat Methods* 9:772.
- De Weerd WH. 1989. Phylogeny and vicariance biogeography of North Atlantic Chalinidae (Haplosclerida Demospongiae). *Beaufortia* 39:55–90.
- Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A. 2009. Geneious Pro v47. (<http://www.geneious.com/>).
- Edgar RC. 2004a. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–7.
- Edgar RC. 2004b. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5:113.
- Erpenbeck D, Hooper JNA, Wörheide G. 2006. CO1 phylogenies in diploblasts and the 'Barcoding of Life'—are we sequencing a suboptimal partition? *Mol Ecol Notes* 6:550–3.
- Erpenbeck D, Cleary DFR, Voigt O, Nichols SA, Degnan BM, Hooper JNA, Wörheide G. 2007a. Analysis of evolutionary biogeographical and taxonomic patterns of nucleotide composition in demosponge rRNA. *J Mar Biol Assoc UK* 87:1607–14.
- Erpenbeck D, Duran S, Rützler K, Paul V, Hooper JNA, Wörheide G. 2007b. Towards a DNA taxonomy of Caribbean demosponges: a gene tree reconstructed from partial mitochondrial CO1 gene sequences supports previous rDNA phylogenies and provides a new perspective on the systematics of Demospongiae. *J Mar Biol Assoc UK* 87:1563–70.
- Erpenbeck D, Hall K, Alvarez B, Büttner G, Sacher K, Schätzle S, Schuster A, Vargas S, Hooper JNA, Wörheide G. 2012. The phylogeny of halichondrid demosponges: past and present re-visited with DNA-barcoding data. *Org Divers Evol* 12:57–70.
- Erpenbeck D, Hooper JNA, List-Armitage SE, Degnan BM, Wörheide G, van Soest RWM. 2007c. Affinities of the

- family SOLLASSELLIDAE (Porifera Demospongiae): II Molecular evidence. *Contrib Zool* 76:95–102.
- Erpenbeck D, McCormack GP, Breeuwer JAJ, van Soest RWM. 2004. Order level differences in the structure of partial LSU across demosponges (Porifera): new insights into an old taxon. *Mol Phylogenet Evol* 32:388–95.
- Fromont JP, Bergquist PR. 1990. Structural characters and their use in sponge taxonomy; when is a sigma not a sigma? In: Rützler K, editor. *New perspectives in sponge biology*. Washington (DC): Smithsonian Institution Press. p. 273–8.
- Gazave E, Lapébie P, Renard E, Vacelet J, Rocher C, Ereskovsky A, Lavrov D, Borchiellini C. 2010a. Molecular Phylogeny restores the supra-generic subdivision of Homoscleromorph sponges (Porifera, Homoscleromorpha). *PLoS One* 5:e14290.
- Gazave E, Carteron S, Chenuil A, Richelle-Maurer E, Boury-Esnault N, Borchiellini C. 2010b. Polyphyly of the genus *Axinella* and of the family Axinellidae (Porifera: Demospongiae^P). *Mol Phylogenet Evol* 57:35–47.
- Graybeal A. 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? *Syst Biol* 47:9–17.
- Hajdu E, van Soest RWM. 2002. Family Desmacellidae Ridley & Dendy 1886. In: Hooper JNA, van Soest RWM, editors. *Systema Porifera. A guide to the classification of sponges*. New York: Kluwer Academic/Plenum Publishers.
- Hallmann EF. 1917. A revision of the genera with microscleres included or provisionally included in the family Axinellidae; with descriptions of some Australian species Part III. *Proc Linn Soc NSW* 41:634–75.
- Hartman WD. 1982. Porifera. In: Parker SP, editor. *Synopsis and classification of living organisms*. Vol. 1. New York: McGraw-Hill. p. 640–66.
- Hentschel E. 1923. Erste Unterabteilung der Metazoa: Parazoa Porifera-Schwämme. In: Kükenthal W, Krumbach T, editors. *Handbuch der Zoologie Eine Naturgeschichte der Stämme des Tierreiches Vol 1 Protozoa Porifera Coelenterata Mesozoa*. Berlin and Leipzig: Walter de Gruyter und Co. p. 307–418. fig 288–377.
- Hill MS, Hill AL, Lopez J, Peterson KJ, Pomponi S, Diaz MC, Thacker RW, Adamska M, Boury-Esnault N, Cárdenas P, et al. 2013. Reconstruction of family-level phylogenetic relationships within Demospongiae (Porifera) using nuclear encoded housekeeping genes. *PLoS One* 8:e50437.
- Holmes B, Blanch H. 2007. Genus-specific associations of marine sponges with group I crenarchaeotes. *Mar Biol* 150:759–72.
- Hooper JNA. 1984. *Sigmaxinella soelae* and *Desmacella ithysetela*, two new desmacellid sponges (Porifera Axinellida Desmacellidae) from the Northwest Shelf of Western Australia with a revision of the family Desmacellidae. *North Territ Mus Arts Sci Mon Ser* 2:1–58.
- Hooper JNA. 1990a. Character stability systematics and affinities between Microcionidae (Poecilosclerida) and Axinellida. In: Rützler K, editor. *New perspectives in sponge biology*. Washington (DC): Smithsonian Institution Press. p. 284–94.
- Hooper JNA. 1990b. A new species of *Rhabderemia* Topsent (Porifera: Demospongiae) from the Great Barrier Reef. The Beagle, Records of the North Territ Mus Arts Sci 7, 1: 65–78.
- Hooper JNA. 2002. Family Rhabderemiidae Topsent 1928. In: Hooper JNA, van Soest RWM, editors. *Systema Porifera. A guide to the classification of sponges*. New York: Kluwer Academic/Plenum Publishers.
- Hooper JNA. 1991. Revision of the family Raspailiidae (Porifera: Demospongiae) with description of Australian species. *Invertebr Taxon* 5:1179–418.
- Hooper JNA, van Soest RWM. 2002. *Systema Porifera. A guide to the classification of sponges*. New York: Kluwer Academic/Plenum Publishers.
- Jenner RA. 2004. When molecules and morphology clash: reconciling conflicting phylogenies of the Metazoa by considering secondary character loss. *Evol Dev* 6:372–8.
- Kott P. 2004. New and little-known species of Didemniidae (Ascidiacea, Tunicata) from Australia (part I). *J Nat Hist* 38:2455–526.
- Lafay B, Boury-Esnault N, Vacelet J, Christen R. 1992. An analysis of partial 28S ribosomal RNA sequences suggests early radiation of sponges. *BioSystems* 28:139–51.
- Lavrov DV, Wang X, Kelly M. 2008. Reconstructing ordinal relationships in the Demospongiae using mitochondrial genomic data. *Mol Phylogenet Evol* 49:111–24.
- Lévi C. 1953. Sur une nouvelle classification des Démosponges. *Compt Rend Hebdom Séan Acad Sci Paris* 236:853–5.
- Lévi C. 1955. Les Clavaxinellides, Démosponges Tétractinomorphes. *Arch Zool exp et gén* 92 (Notes et Revue 2):78–87.
- Lévi C. 1956. Étude des Halisarca de Roscoff. Embryologie et systématique des démousses. *Arch Zool exp et gén* 93:1–181.
- Lévi C. 1957. Ontogeny and systematics in sponges. *Syst Zool* 6:174–83.
- Lévi C. 1973. Systématique de la classe des Demospongiaria (Démousses). In: Grassé P-P, editor. *Traité de Zoologie Vol. 3 Spongiaires*. Paris: Masson et Cie. p. 577–632.
- Morrow CC, Picton BE, Erpenbeck D, Boury-Esnault N, Maggs CA, Allcock AL. 2012. Congruence between nuclear and mitochondrial genes in Demospongiae: a new hypothesis for relationships within the G4 clade (Porifera: Demospongiae). *Mol Phylogenet Evol* 62:174–90.
- Nichols SA. 2005. An evaluation of support for order-level monophyly and interrelationships within the class Demospongiae using partial data from the large subunit rDNA and cytochrome oxidase subunit I. *Mol Phylogenet Evol* 34:81–96.
- Redmond NE, Morrow CC, Thacker RW, Diaz MC, Boury-Esnault N, Cárdenas P, Hajdu E, Lôbo-Hajdu G, Picton BE, Collins AG. 2013. Phylogeny and systematics of Demospongiae in light of new small subunit ribosomal DNA sequences. *Integr Comp Biol* 53:388–415.
- Ridley SO, Dendy A. 1887. Report on the Monaxonida collected by HMS 'Challenger' during the years 1873–1876 Report on the Scientific Results of the Voyage of HMS 'Challenger' 1873–6. *Zoology* 20:i–lxviii. 1–275 pls I-LI, 1 map.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–4.
- Sperling EA, Peterson KJ, Pisani D. 2009. Phylogenetic-signal dissection of nuclear housekeeping genes supports the

- paraphyly of sponges and the monophyly of Eumetazoa. *Mol Biol Evol* 26:2261–74.
- Sperling EA, Robinson JM, Pisani D, Peterson KJ. 2010. Where's the Glass? Biomarkers molecular clocks and microRNAs suggest a 200 million year missing precambrian fossil record of siliceous sponge spicules. *Geobiology* 8:24–36.
- Stamatakis A, Hoover P J, Rougemont A. 2008. Rapid Bootstrap Algorithm for the RAxML Web-Servers. *Syst Biol* 75:758–71.
- Thacker RW, Hill A, Hill M, Redmond N, Collins AG, Morrow CC, Spicer L, Carmack CA, Zappe M, Bangalore P. 2013. Nearly complete 28S rRNA gene sequences confirm new hypotheses of sponge evolution. *Integr Comp Biol*. 53:373–87.
- Topsent E. 1897. Sur le genre *Halicnemia* Bowerbank. *Mém Soc Zool France* 10:235–51.
- Uriz MJ. 1988. Deep-water sponges from the continental shelf and slope off Namibia (Southwest Africa): Classes Hexactinellida and Demospongia. *Monografías de Zoología Marina* 3:9–157.
- van Soest RWM. 1984a. Deficient *Merlia normani* Kirkpatrick 1908 from the Curaçao reefs with a discussion on the phylogenetic interpretation of sclerosponges. *Bijdr Dierkd* 54:211–9.
- van Soest RWM. 1984b. Marine sponges from Curaçao and other Caribbean localities. Part III. Poecilosclerida. In: Hummelinck PW, van der Steen LJ, editors. *Uitgaven van de Natuurwetenschappelijke Studiekring voor Suriname en de Nederlandse Antillen*. No. 112. *Studies on the Fauna of Curaçao and other Caribbean Islands* 62:1–173.
- van Soest RWM. 1987. Phylogenetic exercises with monophyletic groups of sponges. p 227–41. In: Vacelet J, Boury-Esnault N, editors. *Taxonomy of Porifera from the NE Atlantic and Mediterranean Sea NATO ASI Series G13*. Berlin, Heidelberg: Springer Verlag. p. 332.
- van Soest RWM. 1990. Toward a phylogenetic classification of sponges. In: Rützler K, editor. *New perspectives in sponge biology*. Washington (DC): Smithsonian Institution. p. 344–8.
- van Soest RWM. 1991. Demosponge higher taxa classification re-examined. In: Reitner J, Keupp H, editors. *Fossil and recent sponges*. Berlin, Heidelberg: Springer Verlag. p. 54–71.
- van Soest RWM, Boury-Esnault N, Hooper JNA, Rützler K, de Voogd NJ, Alvarez de Glasby B, Hajdu E, Pisera AB, Manconi R, Schoenberg C, et al. 2013. World Porifera database. (<http://www.marinespecies.org/porifera>) on January 20, 2013.
- van Soest RWM, Diaz MC, Pomponi SA. 1990. Phylogenetic classification of the halichondrids (Porifera Demospongiae). *Beaufortia* 40:15–62.
- van Soest RWM, Hooper JNA. 1993. Taxonomy phylogeny and biogeography of the marine sponge genus *Rhabderemia* Topsent, 1890 (Demospongiae Poecilosclerida). In: Uriz M-J, Rützler K, editors. *Recent advances in ecology and systematics of sponges*. *Sci Mar* 57:319–51.
- Vosmaer GCJ. 1912. On the Distinction between the genera *Axinella*, *Phakellia*, *Acanthella* a.o. *Zool Jahrb Jena Suppl* 15:307–22. pls 15–16.
- Wiens JJ. 1998. Does adding characters with missing data increase or decrease phylogenetic accuracy? *Syst Biol* 47:625–40.
- Wilson HV. 1921. The genus *Raspailia* and the independent variability of diagnostic features. *J Elisha Mitchell Sci Soc* 37:54–60.
- Wörheide G, Erpenbeck D, Menke C. 2007. The Sponge Barcoding Project - aiding in the identification and description of poriferan taxa. In: Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy G, editors. *Porifera Research: Biodiversity, Innovation & Sustainability*. Museu Nacional, Série livros 28, Rio de Janeiro. pp. 123–8.