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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

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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 Sigmaxinellidae 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 Weerdt (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 Euryponidae Topsent, 1928), Rhabderemiidae Topsent, 1928, and Sigmaxinellidae 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, therebv making a circular argument. Typical members of Axinellidae, Raspailiidae, Hemiasterellidae, and Sigmaxinellidae 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 Sigmaxinellidae 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 housekeeping 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 $1\,cm^3$ 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 (Borchiellini 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
Acanthella acuta	Mc7160	Mediterranean	HQ379408	HQ379259	HQ379331	—
Acanthella acuta	—	Mediterranean	—	—		GQ466052
Acanthella cavernosa		Guam	—	KC869543		—
Acanthella cavernosa	0CDN9790-Z	Palau	—	—		KC902194
Acantheurypon pilosella	Mc7748	Ireland	—	KC952007	KC883679	KC902379
Acanthostylotella cornuta	0CDN8730-X	Guam	—	KC869600		KC902123
Adreus fascicularis	Mc4559	English Channel	HQ379428	HQ379314	HQ379379	KC902329
Adreus sp.	Mc4982	Ireland	—	HQ379311	HQ379377	KC902410
Agelas axifera	G320422	Australia	DQ069299	—		—
Agelas conifera	KC869634	Panama	—	KC869634		—
Agelas conifera	—	—	—	—		AY734443
Agelas dispar	NCI171	USA	—	KC884836		—
Agelas dispar	—	—	DQ075710	—		AY737640
Amorphinopsis excavans	0CDN9237-Y	Malaysia	—	KC869473		KC902330
Amphilectus fucorum	Mc5093	Wales	—	HQ379294	HQ379362	KC902221
Ancorina alata	0CDN6664-C	New Zealand	—	KC884835		KC901881
Ancorina alata	0CDN6551-G	New Zealand	—	KC884845		KC902129
Anomomycale titubans	Mc7765	Ireland	—	HQ379297	HQ379365	KC902230
Antho involvens	Mc4262	Scotland	—	HQ379291	HQ379359	KC902050
Astrosclera willeyana	0CDN5435-R	Tonga	—	KC869525		KC902051
Atergia corticata	Mc7715	Ireland	—	KC883681	KC883680	KC902079
Axechina raspailioides	0M9H2473-G	Australia	—	KC869448		KC902059
Axinella infundifuliformis	Mc4438	Scotland	HQ379410	—		—
Axinella polypoides	—	Mediterranean	—	DQ299255		APU43190
Axinella pyramidata	Mc3385	Ireland	—	HQ379265	HQ379335	KC902269
Axinella vaceleti	Mc4200	Mediterranean	—	HQ379266	HQ379336	KC902004
Axinyssa topsenti	0CDN8822-X	Papua New Guinea	—	KC869558		KC902315
Biemna saucia	G303281	Australia	JF773146	—		—
Biemna variantia	Mc5405	Wales	HQ379424	HQ379292	HQ379360	KC901961
Ceratopsion axiferum	0M9H2585-A	Australia	—	KC869596		KC902000
Cervicornia cuspidifera	0M9G1351-I	USA	—	KC869474		KC902382
Cinachyrella kuekenthali	P23	Panama		KC869490		—
Cinachyrella kuekenthali	—	—	EF519602	—		—
Cinachyrella kuekenthali	USNM_1133786	Panama	—	—		KC902290
Ciocalypta penicillus	Mc5051	Roscoff/France	—	HQ379315	HQ379381	KC902049
Clathria armata	Mc4359	Scotland	KC869418	KC869437	KC869445	KC901940
Clathria barleei	Mc4347	Scotland	KC883682	HQ393897	HQ393901	KC902394
Clathria oxeota	B66	Belize	EF519605	—		—
Clathria rugosa	G300696	New Caledonia	HE611604	—		—
Clathria schoenus	P10	Panama	—	KC884834		—
Clathria schoenus	SI06x33	Panama	—	—		KC902370
Cliona celata	Mc5497	Wales		HQ379310	HQ379376	KC902383
Cliona celata			EF519608			
Cliona varians	0M9G1439-C	USA	—	KC869519		KC902145

(continued)

Table 1 Continued

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

(continued)

Table 1 Continued

Organism	Voucher	Locality	COX1	28S (D3–5)	28S (D6–8)	18S
***Lissodendoryx* sp.**	0M9I5828-T	Malaysia	—	KC869506		KC902216
Microciona prolifera	—	—	DQ087475	—		—
Microscleroderma herdmanni	0CDN9628-Y	Palau	—	KC884846		KC902255
Monanchora arbuscula	SI06x186	Panama	—	KC869447		KC902187
Mycale macilenta	Mc3618	Ireland	—	KC869436	KC869442	KC901898
Mycale mirabilis	0PHG1422-F	Malaysia	HE611591	KC869613		KC902146
Mycale rotalis	Mc5391	Wales	—	HQ379296	HQ379364	KC902397
Mycale subclavata	Mc3314	Ireland	—	KC869433	KC869441	KC902072
Myrmekioderma granulatum	0PHG1422-F	Malaysia	—	KC869471		KC901877
Myrmekioderma gyroderma	—	—	EF519652	—		—
Myxilla anchorata	Mc3306	Ireland	—	HQ379304	HQ379370	—
Myxilla anchorata	Mc4255	Scotland	—	—		KC902360
Myxilla* cf. *rosacea	Mc4681	Ireland	—	KC883686	KC883683	KC901935
Neofibularia hartmani	0CDN8100-O	Samoa	JF773145	KC869639		KC901997
Neofibularia nolitangere	—	—	EF519653	—		—
Pachymatisma johnstoni	Mc3504	Scotland	EF564330	—		—
Paratimea* cf. *duplex	PS70/17-1(1)	Norway	KC869429	—		—
***Paratimea* sp.**	Mc4323	Scotland	HQ379419	HQ379284	HQ379352	HQ379419
***Paratimea* sp.**	Mc5226	Wales	—	HQ379283	HQ379351	KC902401
Penares* cf. *alata	0CDN7316-M	S. Africa	—	KC869466		KC902193
Phakellia rugosa	Mc7456	Norway	KC869419	—		—
Phakellia ventilabrum	Mc4248	Scotland	HQ379409	HQ379260	HQ379332	KC901915
Phorbas bihamiger	Mc4493	English Channel	—	KC869431	KC869444	KC901921
Phorbas dives	Mc4517	English Channel	—	HQ379303	HQ379369	KC902286
Phorbas punctatus	Mc5343	Wales	—	KC869439	KC869440	KC902093
Pione vastifica	—	Caribbean	EF519665	—		—
Placospongia intermedia	PC-BT-18	Panama	KC869430	—		—
Plocamionida ambigua	Mc4345	Scotland	—	KC869435	KC869443	KC902218
Polymastia boletiformis	Mc5014	Ireland	—	HQ379306	HQ379372	KC902065
Polymastia janeirensis	—	Brazil	EU076813	—		—
Polymastia penicillus	Mc5284	Ireland	—	HQ393899	HQ393903	—
Polymastia penicillus	Mc5065	Ireland	—	—		KC902065
***Polymastia* sp.**	Mc6488	Ireland	KC869420	—		—
Prosuberites longispinus	Mc7173	Mediterranean	—	HQ379320	HQ379387	KC902182
Ptilocaulis spiculifer	0CDN9412-P	Malaysia	—	KC869560		KC902092
Ptilocaulis walpersi	—	Bahamas	EU237488	—		—
Raspaciona aculeata	Mc7159	Mediterranean	HQ379415	—		—
Raspailia hispida	Mc3597	Ireland	HQ379416	HQ379279	HQ379348	KC902385
Raspailia phakellopsis	0M9H2417-T	Australia	—	KC869585		KC902272
Raspailia ramosa	Mc4024	Ireland	HQ379417	HQ379281	HQ379349	KC902299
Raspailia vestigifera	NCI431	Australia	—	KC869583		KC901895
Reniochalina stalagmitis	NCI287	Australia	—	KC869582		—
Reniochalina stalagmitis	—	—	—			EF092272

(continued)

Table 1 Continued

Organism	Voucher	Locality	COX1	28S (D3–5)	28S (D6–8)	18S
Rhabdastrella globostellata	0PHG1710-R	Vietnam	—	KC884843		KC902160
Rhabderemia sorokinae	G312904	Papua New Guinea	HE611607	—		—
Scopalina hispida	NCI272	USA	—	KC884841		KC902237
Scopalina lophyropoda	Mc4217	Mediterranean	—	HQ379268	HQ379337	KC901894
Scopalina ruetzleri		Panama	—	KC869553		—
Scopalina ruetzleri	—	—	—	—		AJ621546
Spanioplon armaturum	Mc4500	English Channel	EF519602	KC869438	KC869446	KC902324
Sphaerotylus antarcticus	POR21125	Antarctica	KC869424	—		—
***Sphaerotylus* sp. C**	Mc4236	Ireland	—	HQ379307	HQ379373	—
***Sphaerotylus* sp. C**	Mc4697	Ireland	—	—		KC902307
Spongilla lacustris	Mc7351	Ireland	HQ379431	HQ379327	HQ379393	KC902349
Stelletta clavosa	0CDN9840-G	Palau	—	KC884847		KC901967
Stelletta grubii	Mc5043	Ireland	—	HQ379255	HQ379329	KC902213
Stelligera rigida	Mc4357	Scotland	HQ379420	HQ379285	HQ379353	KC902164
Stelligera stuposa	Mc4330	Scotland	HQ379421	HQ379286	HQ379354	KC902232
Stryphnus ponderosus	Mc4240	Scotland	—	HQ379257	HQ379330	—
Suberites aurantiacus	KC869577	Panama	—	KC869577		—
Suberites aurantiacus	SI06x105	Panama	—	—		KC902366
Suberites ficus	Mc4322	Ireland	HQ379429	HQ379322	HQ379389	KC902236
Suberites massa	Mc4528	English Channel	—	HQ379324	HQ379390	KC902066
Suberites pagurorum	Mc4043	Ireland	KC869422	—		—
Svenzea zeai	USNM_1133762	Panama	—	KC869635		KC902075
Tedania strongylostyla	0CDN7611-I	Vanuatu	—	KC869515		KC901911
Terpios aploos	0CDN3602-Y	Tanzania	—	KC869465		KC902316
Terpios gelatinosa	Mc3315	Ireland	—	HQ379325	HQ379391	KC902355
Tethya actinea	SI06x109	Panama	—	KC869527		—
Tethya actinea	—	—	—	—		AY878079
Tethya aurantium	—	Mediterranean	EF584565	—		—
Tethya citrina	Mc5113	Wales	HQ379427	—		—
Tethya norvegica	—	Norway	EF558565	—		—
Tethyopsis mortenseni	0CDN6706-X	New Zealand	—	KC869618		KC902095
***Tethyopsis* sp.**	0CDN6825-C	New Zealand	—	KC869476		KC902234
Tethyspira spinosa	Mc4641	Ireland	HQ379418	HQ379282	HQ379350	KC902120
Theonella cylindrica	0CDN9523-L	Malaysia	—	KC884839		KC902244
Theonella swinhoei	0CDN9465-W	Malaysia	—	KC884844		KC901886
Timea unistellata	Mc7300	Ireland	KC869427	—		—
***Topsentia* sp.**	P126	Panama	—	KC884837		—
***Topsentia* sp.**	0CDN8723-Q	Guam	—	—		KC902261
Trachycladus stylifer	0CDN6656-T	New Zealand	—	KC869453		KC901930
Trachytedania* cf. *ferrolensis	Mc5348	Wales	—	KC883684	KC883685	KC902219
Tsitsikamma pedunculata	0CDN7414-S	S. Africa	—	KC869512		KC902279
Ulosa stuposa	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 vaceleti* 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

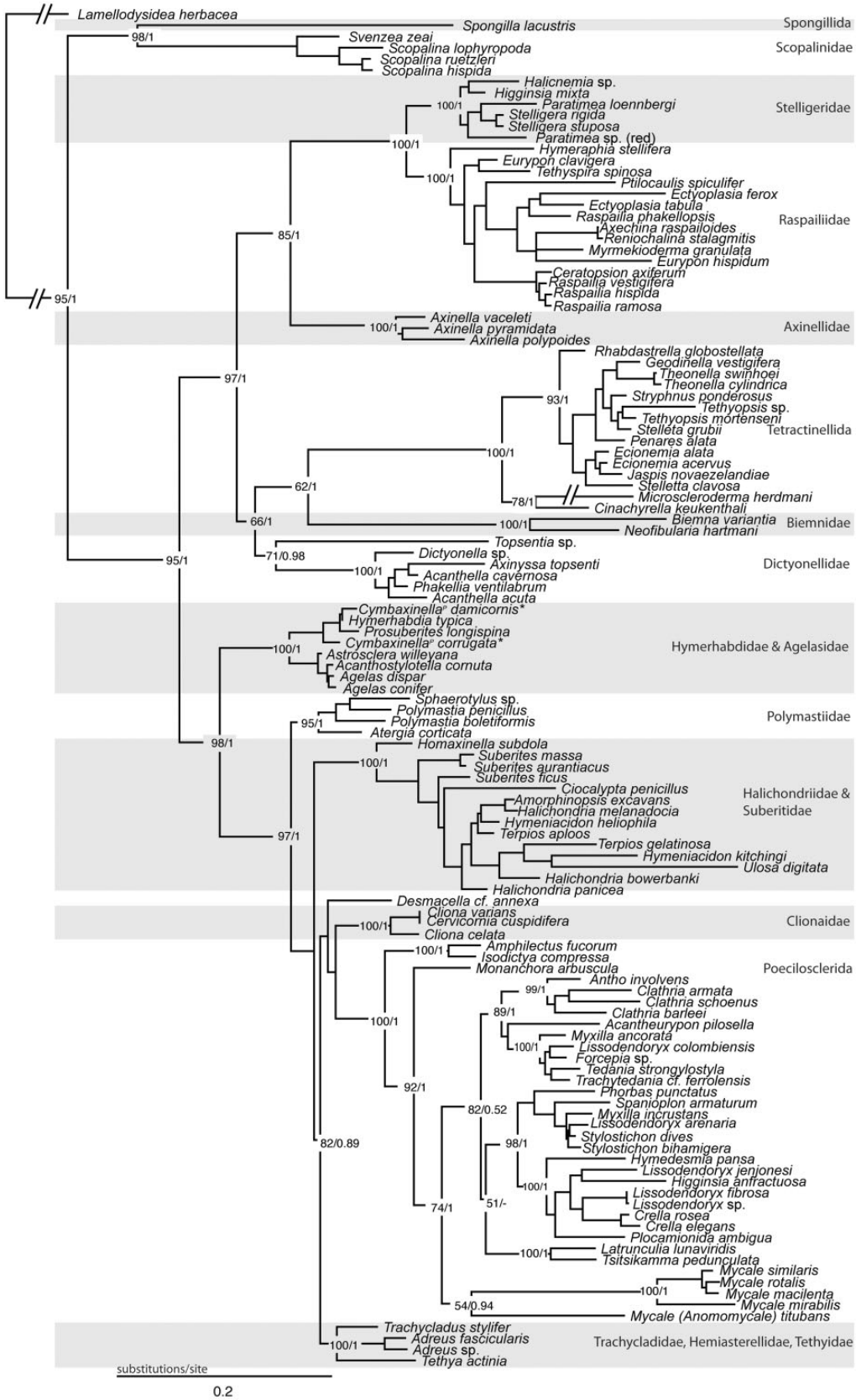

Fig. 2 Best tree output from RaxML analysis of full-length 18S rRNA combined with 28S rRNA (D3–D8 region) sequences from 121 species of demosponges. Figures at nodes correspond to bootstrap support >50 followed by posterior probabilities >0.5 from the Bayesian analysis.

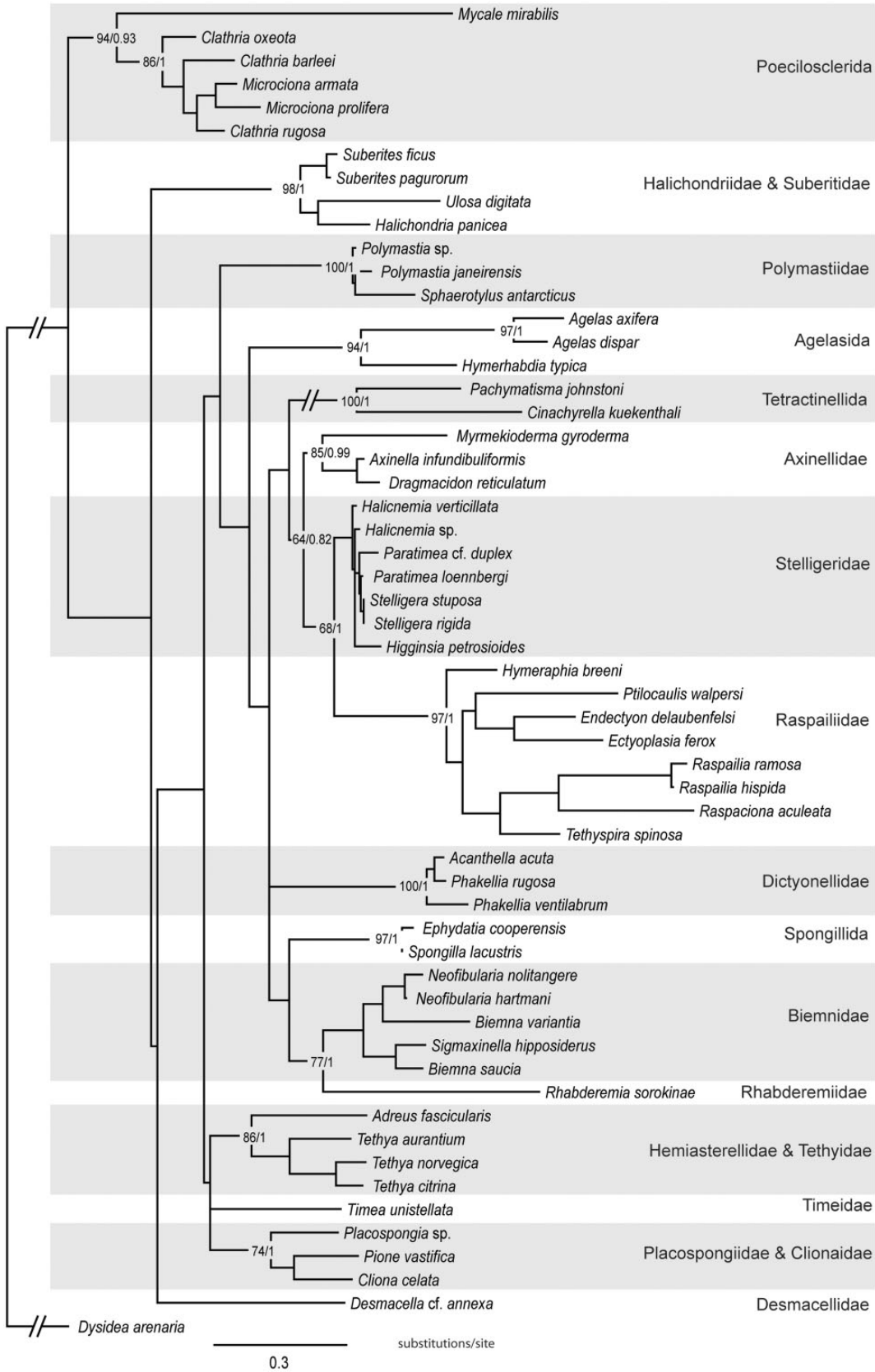

Fig. 3 Best tree output from RaxML analysis of mitochondrial CO1 barcoding fragment from 57 species of demosponges. Figures at nodes correspond to bootstrap support >50 followed by posterior probabilities >0.5 from the Bayesian analysis.

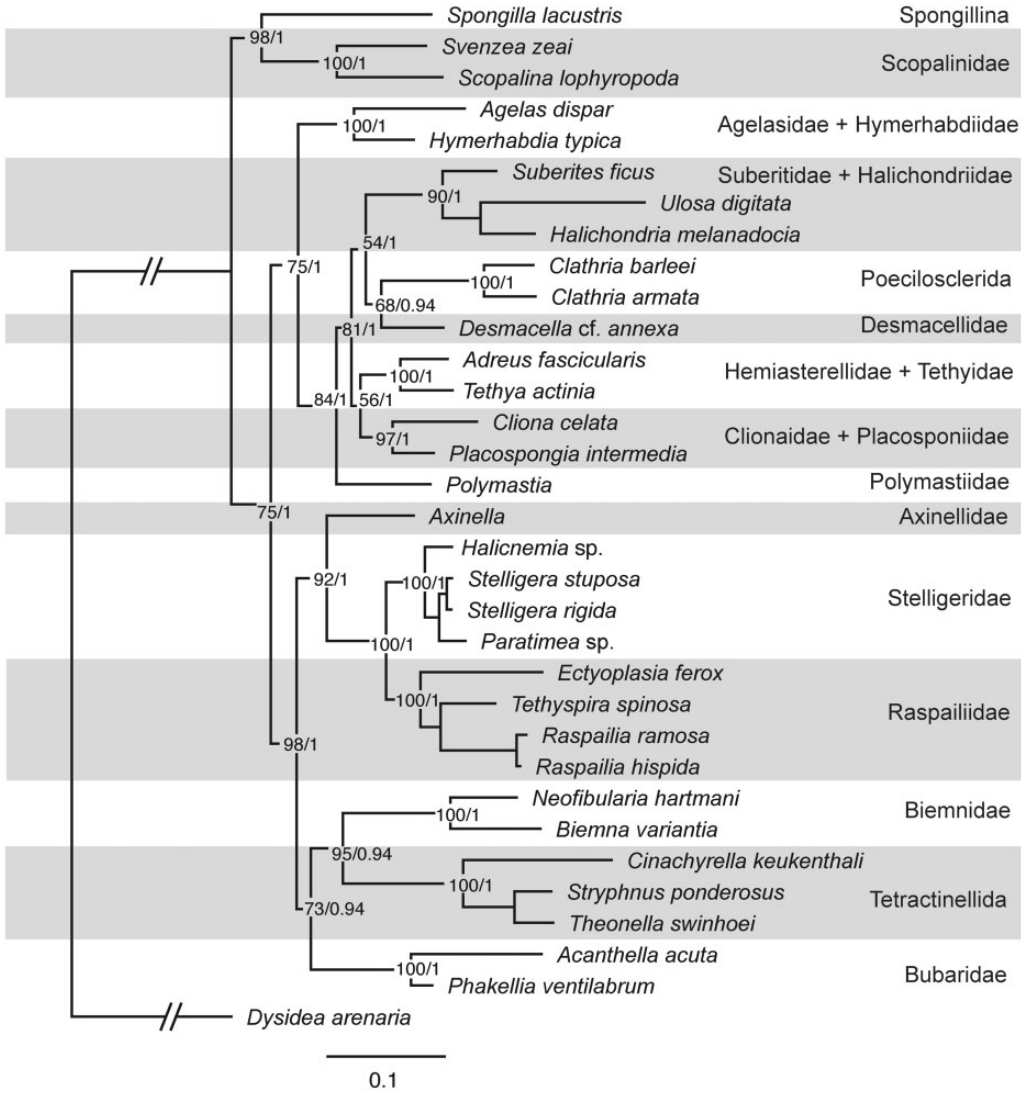

Fig. 4 Best tree output from RaxML combined analysis of full-length 18S rRNA, 28S rRNA (D3–D8 region) and mitochondrial CO1 barcoding fragment from 33 species of demosponges. Figures at nodes correspond to bootstrap support >50 followed by posterior probabilities >0.5 from the Bayesian analysis.

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 (Borchiellini 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 sorokinae* 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 oxeote 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; Borchiellini 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 stylifer* 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 sigmatose microscleres synapomorphic for the group Microcioniidae-Myxillidae-Mycalidae 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

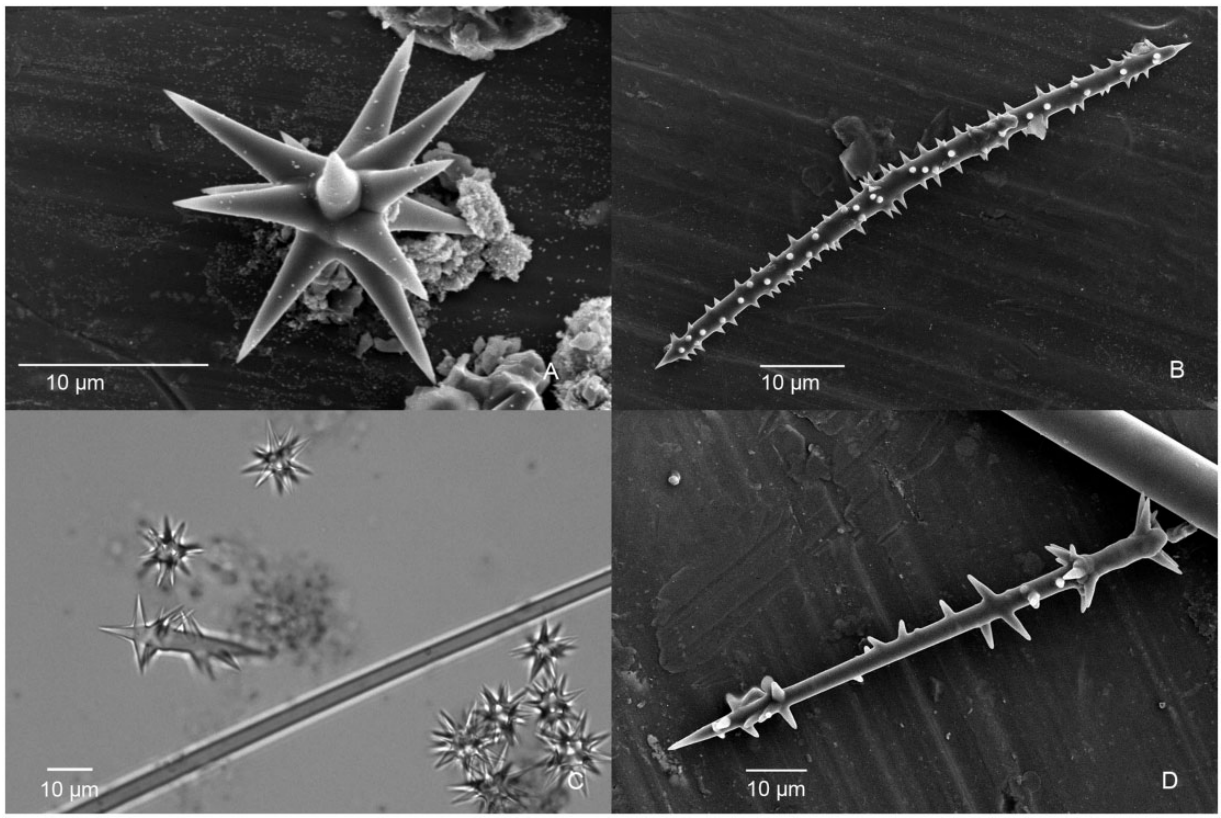

Fig. 5 (**A**) Scanning electron micrograph (SEM) of euaster from *Paratimea loennbergi* (Mc1590); (**B**) SEM of acanthoxea from *Halicnemia* sp. (Mc1598); (**C**) Photomicrograph of an aberrant elongate aster from *Paratimea* sp. (Mc 3163); (**D**) SEM of acanthostyle from *Tethyspira spinosa* (Mc3163). 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 *Halicnemia 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*

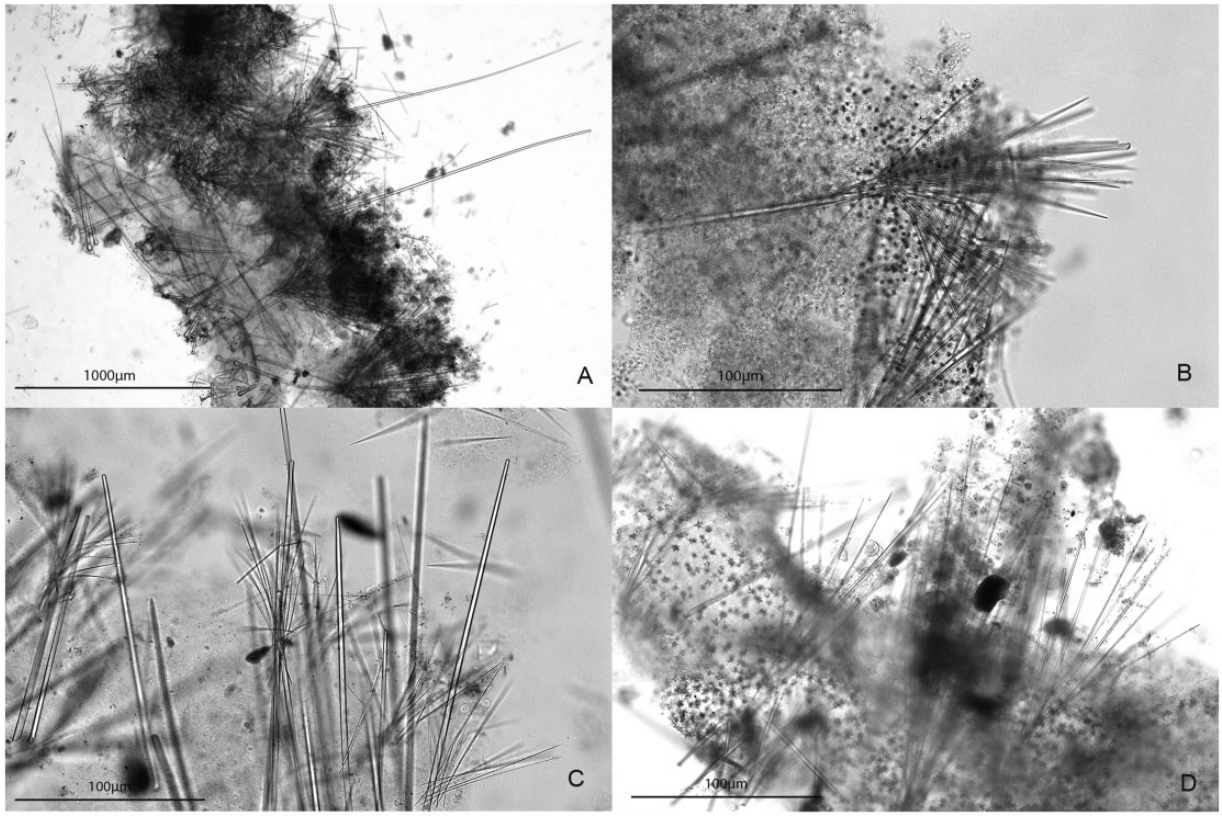

Fig. 6 Photomicrographs showing specialized surface architecture of large robust styles or tylostyles that penetrate the surface surrounded by bouquets of smaller, more slender oxea or styles. (**A**) *Halicnemia* sp. (Mc5907); (**B**) *Stelligera stuposa* (Mc4330); (**C**) *Raspailia hispida* (Mc3597); (**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 Clionaidae 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, Clionaidae, 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, Demospongiaep) 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 Weerdt 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://wwwgeneiouscom/).

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 Sollasellidae (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 Naturgeschichteder 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, BouryEsnault 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 ithystela*, 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 Didemnidae (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 systématiquedes démosponges. 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 (Démosponges). 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): Classses 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.marinespeciesorg/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.