



## 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

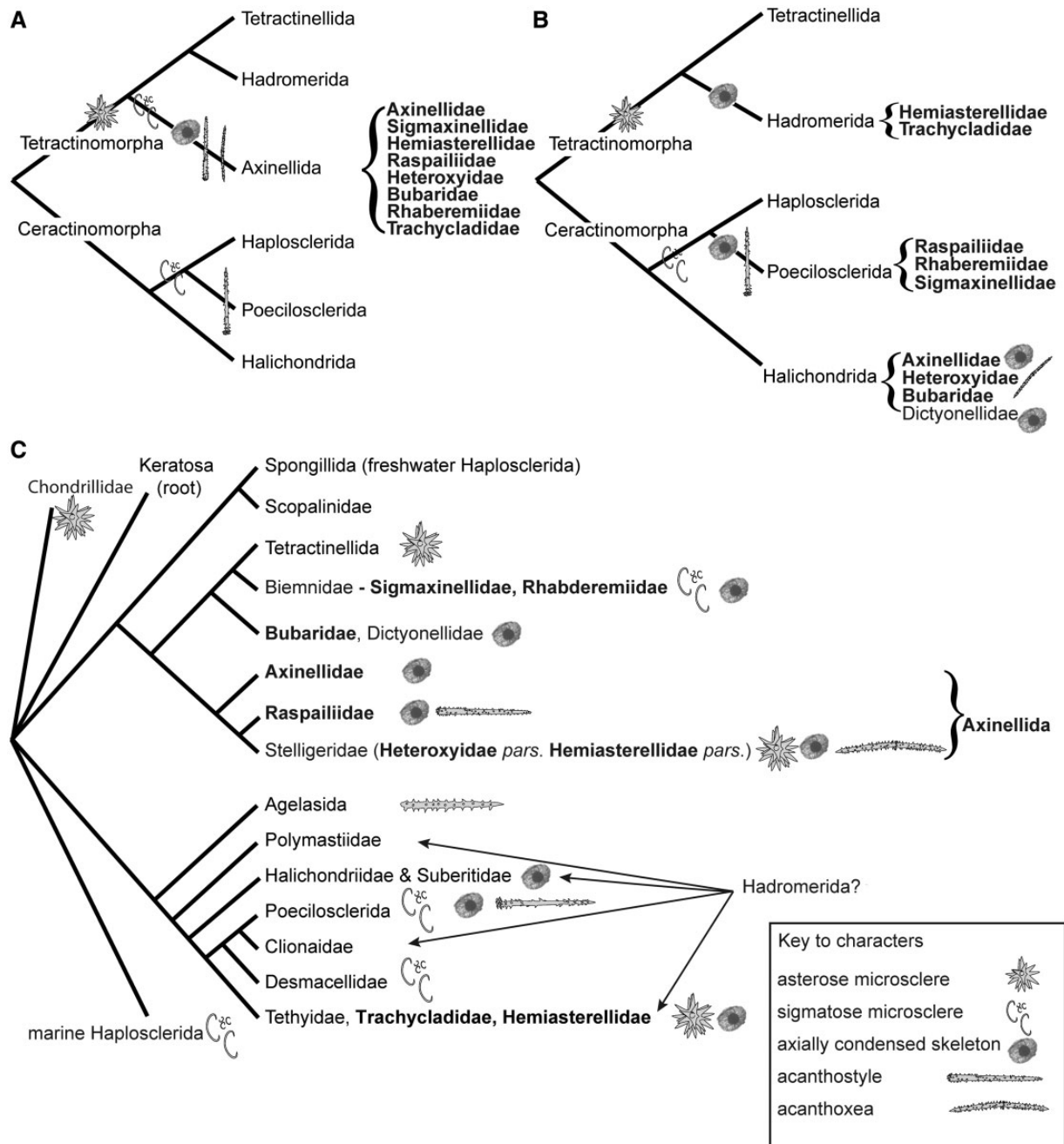
Lévi (1953, 1956, 1957, 1973) was the first to provide a modern synthesis of the classification of Demospongiae. He identified two subclasses; Tetractinomorpha for taxa with a radial or axially condensed skeleton and an oviparous mode of reproduction and Ceractinomorpha for taxa with a reticulate skeleton 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 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 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 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, thereby 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 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 cm<sup>3</sup> 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 oxecta</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

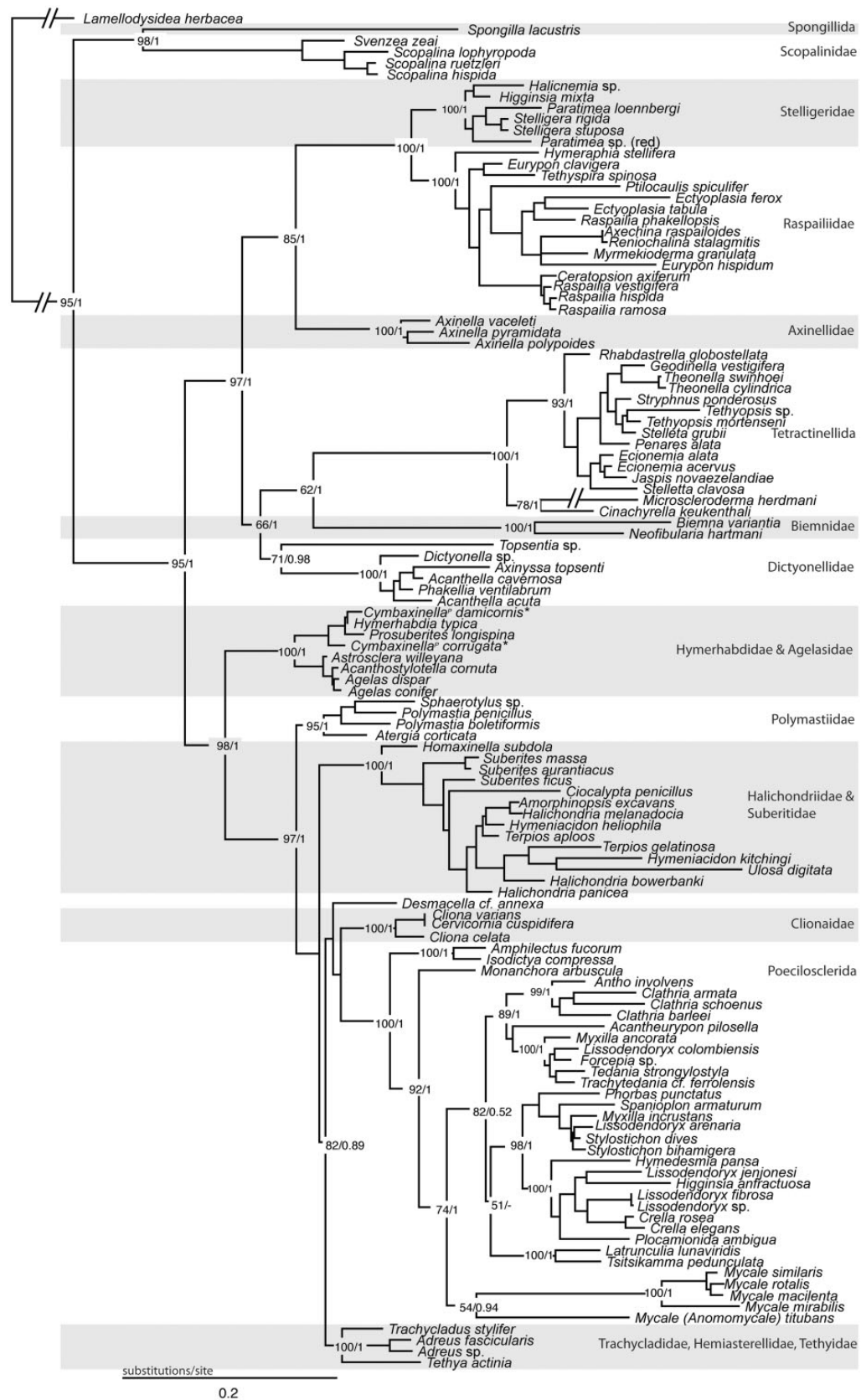
the main heteroscleromorph clade but with very low support values. It is difficult to compare our phylogeny with that of Hill et al. which had very low taxon sampling (several of the families we included were not sampled and most of the families were only represented by one taxon) and low support for 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



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**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.

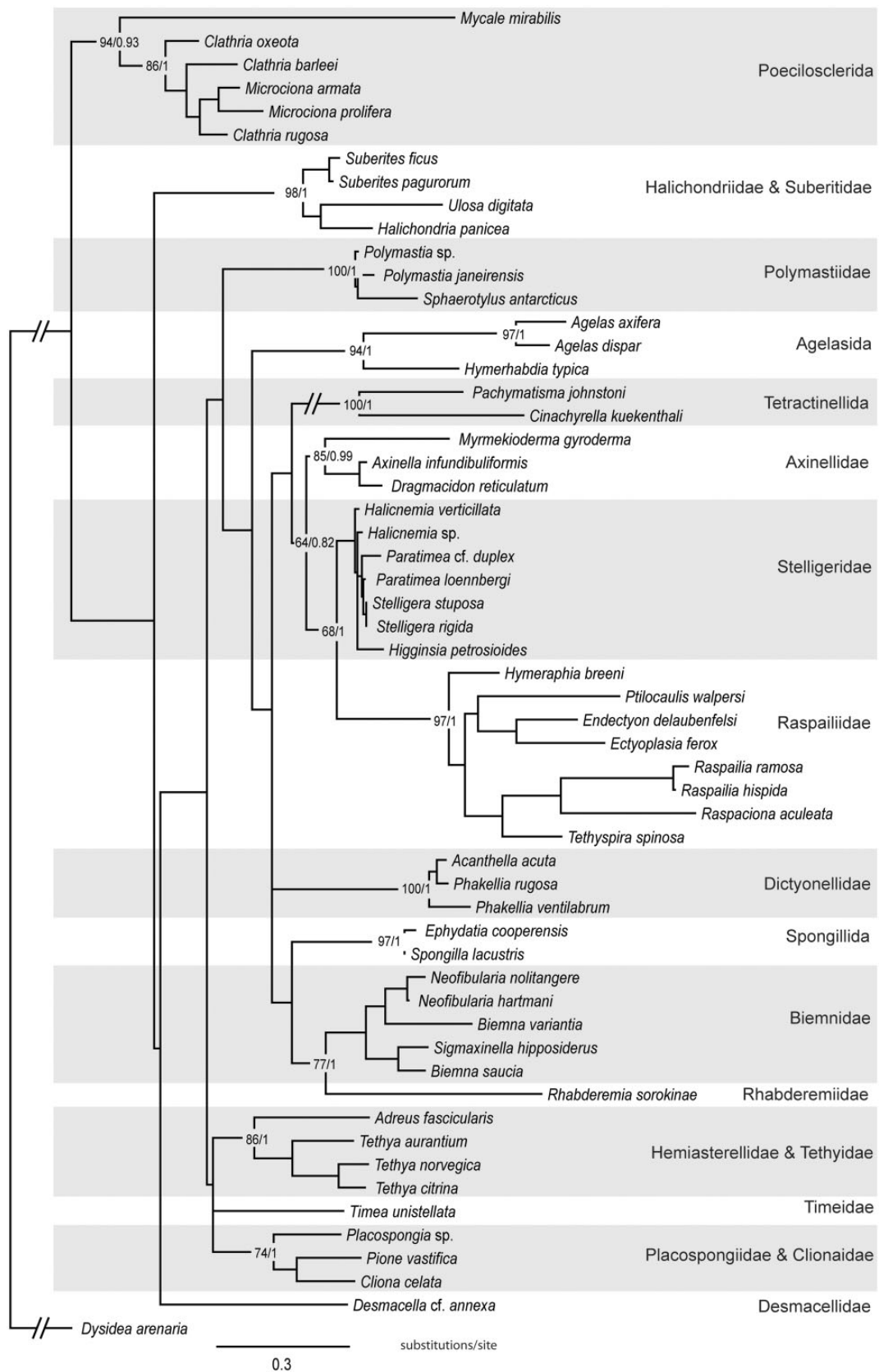
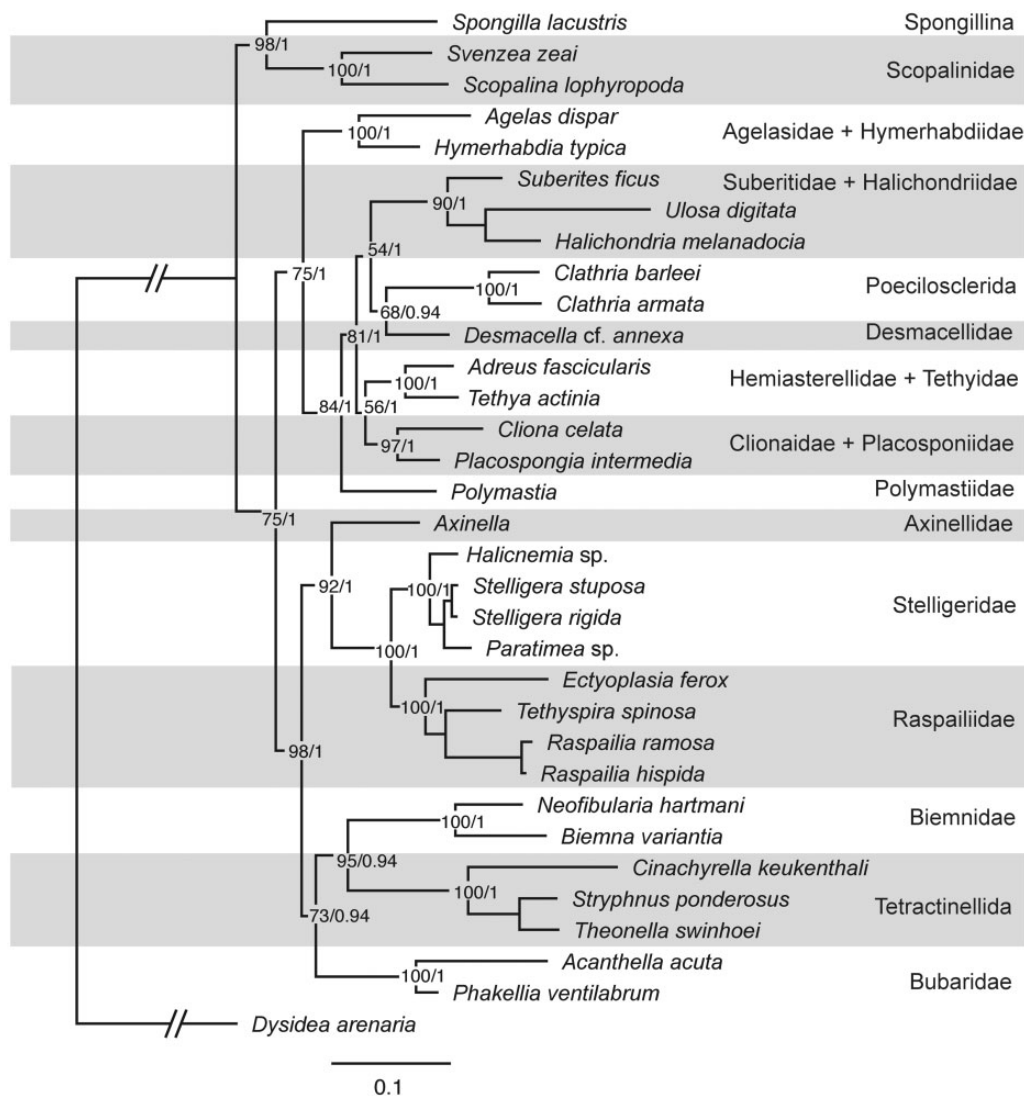


Fig. 3 Best tree output from RaxML analysis of mitochondrial CO1 barcoding fragment from 57 species of demosponges. Figures at nodes correspond to bootstrap support >50 followed by posterior probabilities >0.5 from the Bayesian analysis.



**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.

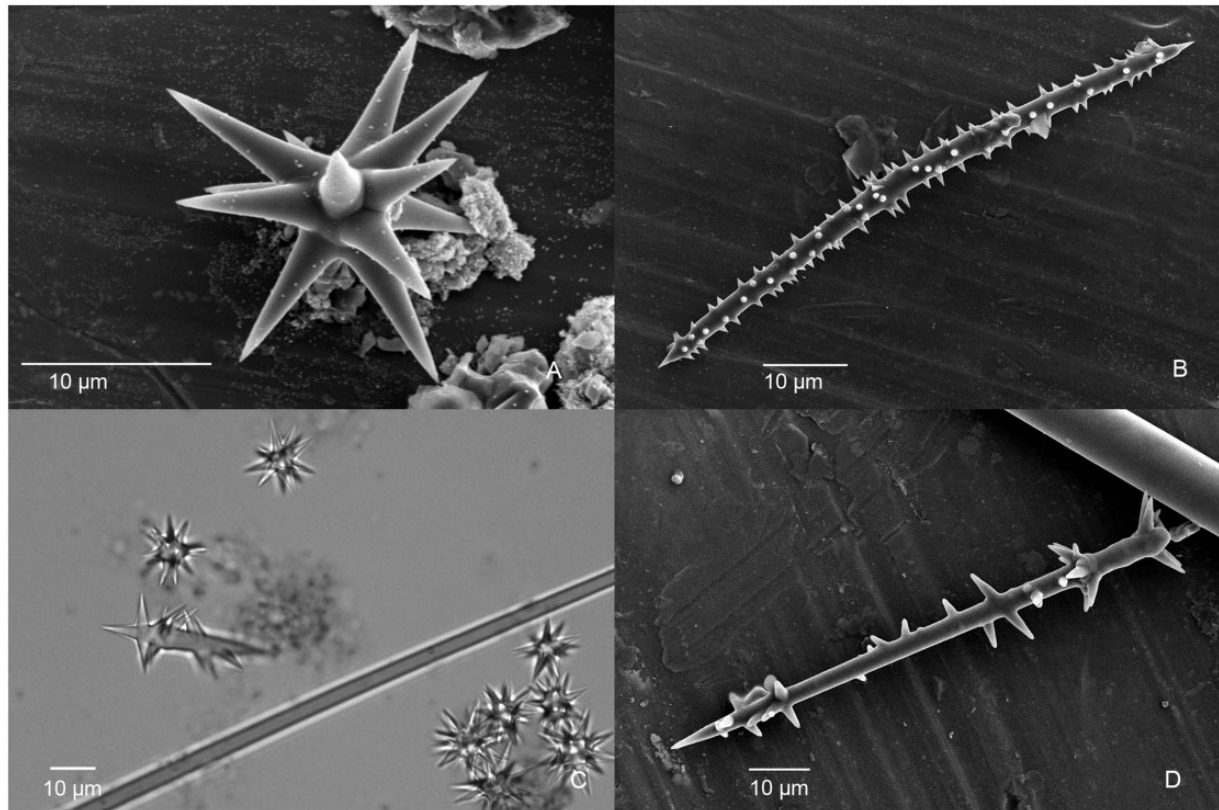
### Acanthostyles

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*<sup>P</sup> (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

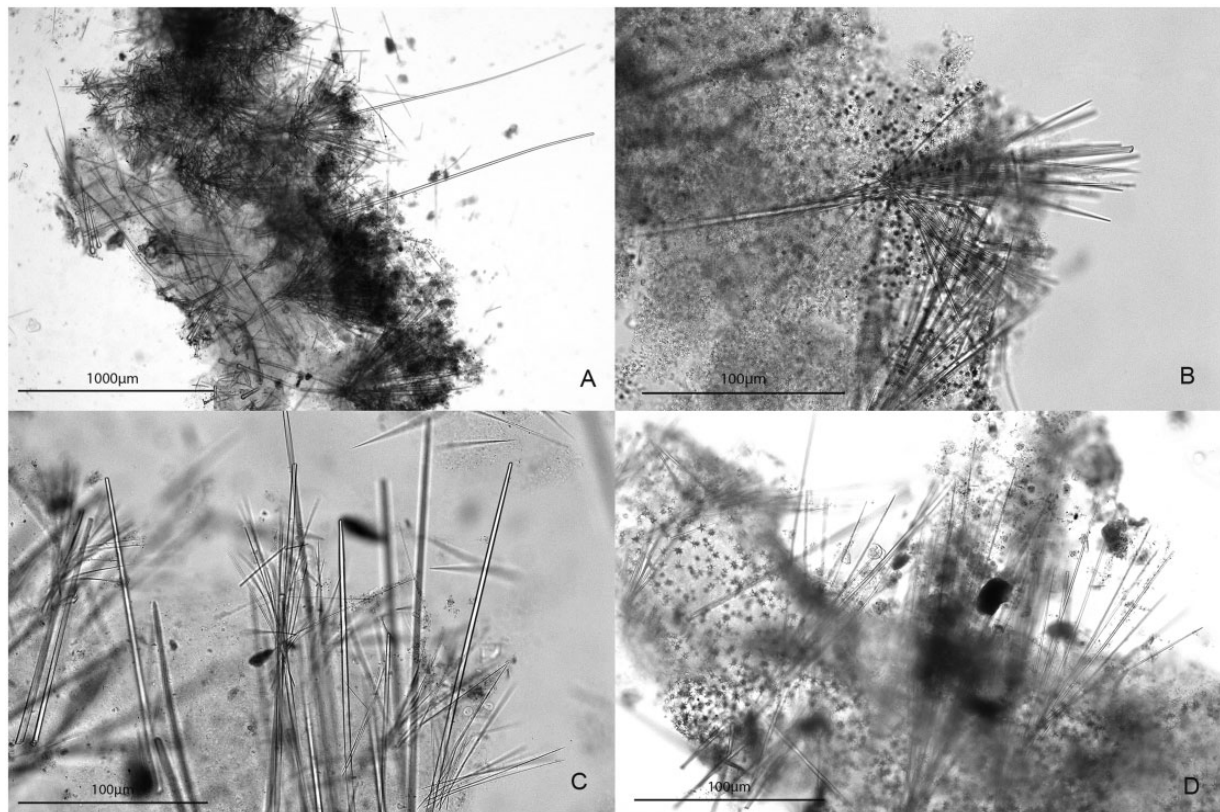
An axial skeleton consists of a stiff axial region that is clearly distinct from a softer extra-axial region. A cross section through a branch of *Axos cliftoni* Gray, 1867 illustrates the occurrence of axially condensed skeletons (Fig. 1A–C). van Soest (1991) argued that an axially condensed skeleton 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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