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SYMPOSIUM

# Mycalina: Another Crack in the Poecilosclerida Framework

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**Synopsis** This is the first phylogenetic analysis integrating both morphological and molecular data of the sponge suborder Mycalina (Poecilosclerida), which was erected in 1994. A cladistic analysis of morphology supported the monophyly of Cladorhizidae (including *Eucheliplumа*), Guitarridae (excluding *Eucheliplumа*), Isodictyidae, Latrunculiidae, and Podospongiidae but rejected monophyly for Desmacellidae, Esperiopsidae, Hamacanthidae, and Mycalidae. Analyses of partial 16S and partial 28S rRNA datasets combined, as well as that of a complete 18S rDNA dataset, suggest that Mycalina is not monophyletic; Biemnidae is only distantly related to other poecilosclerids; *Merlia* and *Desmacella* branch near the base of a diverse Poecilosclerida clade; Mycalidae is monophyletic (excluding *Mycale [Anomomycale] titubans* in 18S); and Esperiopsidae and Isodictyidae form a clade. Analyses of the two molecular datasets differed on the monophyly of Podospongiidae and about the relationship of Podospongiidae to Isodictyidae + Esperiopsidae.

## Introduction

The conceptual framework for understanding the phylogenetic relationships within Poecilosclerida (Porifera) dates back to the pioneering studies by Nardo (1833), Gray (1867), Schmidt (1870), Carter (1875), Ridley and Dendy (1887), and Topsent (1894). These authors proposed classifications based on characters, such as spicules or skeletal architecture, shared among subsets of the many taxa already known in the 19th century. By the beginning of the 21st century, the *Systema Porifera* compiled the most comprehensive classification of the phylum and 25 families were recognized within Poecilosclerida (Hooper and van Soest 2002). Notwithstanding seven of these dating from the 19th century, and another 16 from the early 20th century, the foundations for an unambiguous grouping of those taxa into suprafamilial groups were only established much later. The phylogenetic rationales laid down by van Soest (1984) and Bergquist and Fromont (1988) resulted in Hajdu et al. (1994) proposing the suborders Microcionina, Myxillina, and Mycalina, to which Latrunculina was subsequently added on the basis of revisions undertaken by Kelly-Borges and Vacelet (1995) and Kelly and Samaai (2002b). This article focuses on the phylogenetic status of Mycalina.

Mycalina consisted of five families in 1994: Cladorhizidae, Desmacellidae, Guitarridae, Hamacanthidae, and Mycalidae. Two underlying putative synapomorphies supported the proposal of this suborder: the widespread occurrence of mycalostyles and derivatives of sigmancistras pertaining to a postulated transformation series (sigmancistras—diancistras or cyrtancistras—clavidiscs). These characters, albeit not universally present at the generic and specific levels, occurred in all five of the above families, with the notable exception of derivatives of sigmancistras in Mycalidae. In addition, none of these families possessed true acanthostyles or

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echinating megascleres of any kind. Soon afterward, the *Systema Porifera* placed another four families in the suborder (van Soest and Hajdu 2002a)—Esperiopsidae (van Soest and Hajdu 2002b), Isodictyidae (Hajdu and Lôbo-Hajdu 2002), Merliidae (van Soest and Hajdu 2002c), and Podospongiidae (as *incertae sedis*; Kelly and Samaai 2002a). Esperiopsidae and Merliidae were raised from within the Mycalidae and Desmacellidae, respectively, while the other two families were brought into the suborder from outside Poecilosclerida. These taxa possess only a meager match, if any at all, to the original phylogenetic rationale for Mycalina.

Relationships among these families were hypothesized based on overviews of the distribution of characters, beginning in the late 19th century. Ridley and Dendy (1887) postulated a close relationship between *Hamacantha* (as *Vomerula*) and *Mycale* (as *Esperella*), on account of the neatly reticulated ectosomal skeleton observed in both and of the peculiar disposition of diancistra microscleres in *Hamacantha esperioides*, thought to match the pattern seen in the large sigmas of *Mycale simonis*. Then, Kirkpatrick (1911) remarked upon the obvious similarity between diancistras and the clavidiscs of *Merlia normani* (Merliidae), and van Soest (1984) noted the shared occurrence of commas between the latter and *Biemna* (Desmacellidae). Thus, it appeared that a close relationship existed among Desmacellidae, Hamacanthidae, and Mycalidae, and Hajdu et al. (1994) noted the shared occurrence of sigmodragmas as well as toxodragmas as further corroboration, in contrast to the likely loss of toxas and trichodragmas observed in Cladorhizidae and Guitarridae. Since publication of *Systema Porifera*, no further attempt to elucidate the phylogenetic history of the expanded Mycalina has been made, either on the basis of morphology or of DNA. The objective of the present study is to contribute toward filling that gap.

## Materials and methods

The assessment of phylogenetic relationships undertaken here consists of an evaluation of morphological diversity at the generic level—contrasted to datasets generated for combined partial 16S and 28S rRNA gene sequences, as well as near complete 18S rRNA sequences obtained from various species representing Mycalina. 16S and 28S sequences were originally generated for a study of subgeneric relationships within *Mycale* (de Paula 2013), while 18S sequences are part of a much larger effort to retrieve a solid phylogenetic framework for Demospongiae, undertaken as part of the Porifera Tree of Life (PorToL) Project (see Redmond et al. 2013, this issue).

### Specimens

The majority of samples used for molecular work were obtained by SCUBA or snorkeling and preserved in 93–96% ethanol upon collection. Vouchers are deposited in the Porifera collections of Museu Nacional (MNRJ, Rio de Janeiro), the National Museum of Natural History (several field codes, Smithsonian Institution, Washington D.C.), the Naturalis Biodiversity Center (ZMA, Leiden), the Bernice Pauahi Bishop Museum (BPBM, Honolulu), the Museo Civico di Storia Naturale 'Giacomo Doria' (MSNG, Genova), the Harbor Branch Oceanographic Institute (JR, Florida Atlantic University, Fort Pierce), and the Ulster Museum (CCM, Belfast). Supplementary Table S1 provides an annotated list of samples and sequences used in this study.

### Identifications

Brazilian samples were identified by E.H. and additional Brazilian specialists and those from other regions by colleagues working in the above listed institutions. The latter were double-checked by E.H. Samples from Bocas del Toro were usually identified through the collective efforts of several taxonomists and sponge biologists, E.H. among them for collections undertaken in 2012.

### Morphology

The morphological study included 44 Operational Taxonomic Units (OTU): 39 genera of Poecilosclerida (Mycalina 28, Myxillina 5, Microcionina 4, and Latrunculina 2), 2 from Hadromerida, 2 from Haplosclerida, and 1 from Halichondrida as the outgroup, and 28 characters (Supplementary Material 2). The possibility of non-independence of characters was evaluated, with no deletions undertaken when the likelihood of evolutionary congruence could not be easily discarded a priori. The data matrix was compiled using NDE 0.5.0 (Page 2001) and analyzed by the Traditional Search heuristic algorithm for Maximum Parsimony (MP) as implemented in TNT 1.1 (Goloboff et al. 2008), with 1000 addition sequences (up to 10 trees saved per replicate), Tree Bisection and Reconnection (TBR), under implied weights (Goloboff 1993).

### 18S rDNA

PCR amplification, DNA sequencing, editing, alignment, and phylogenetic analyses were conducted as described by Redmond et al. (2013, this issue). Of

particular interest here, the choice of taxa comprised 40 Mycalina, 33 Microcionina, 69 Myxillina, and 5 Latrunculina. The results illustrated here (155 OTUs, see below) made use of *Xenospongia*, *Timea*, and *Tectitethya* as outgroups. Nevertheless, five distinct analyses were undertaken with different combinations of outgroups to establish whether outgroup selection affected ingroup topology. In the most comprehensive of these, 163 OTUs were used, with outgroups including *Cliona*, *Halichondria*, *Hymeniacidon*, *Placospongia*, and *Suberites*, apart from those already mentioned.

### 16S and 28S rRNA

DNA extraction and PCR amplification were conducted as described by de Paula et al. (2012). PCR products were sent to Macrogen (Macrogen Inc, Seoul, Korea) for purification and sequencing with both forward and reverse primers. Sequences generated in this work have been deposited in GenBank under accession numbers KC952717–KC952736 and KC961641–KC961731. The fragment of the 16S rRNA gene (ca. 630 bp) was amplified with primers diplo-rnl-f1 and diplo-rnl-r1 (Lavrov et al. 2008) and the D3–D5 region of the 28S rRNA gene (ca. 390 bp) with primers 28sCallyF and 28sCallyR (López-Legentil et al. 2010). All sequences were edited using the SeqMan Pro program (Lasergene DNASTAR©), and each marker aligned independently with MAFFT (Katoh et al. 2005) using two different algorithms for local alignment searches (L-INS-i and E-INS-i) with default parameters. These two alignments were contrasted using SOAP (Löytynoja and Milinkovitch 2001), and only positions congruent in both, and positions without gaps or missing data were included in the phylogenetic analyses. Phylogenetic reconstructions were performed through maximum likelihood (ML) and conducted with RAxML 7.0.4 (Stamatakis 2006), where both markers were combined into a single matrix. The best-scoring ML tree was inferred using GTRGAMMA, the best-fitting model estimated for each partition individually, after rapid Bootstrap analysis with 1000 replicates.

## Results

### Morphology

The MP analysis of generic-level morphological data (Supplementary Material 2) is summarized in the majority-rule consensus shown in Fig. 1. MP recovered 821 trees (Tree Length (TL) = 227, Consistency Index (CI) = 0.74, Homoplasy Index (HI) = 0.81, Rescaled Consistency Index (RC) = 0.41). This analysis permitted recognition of the following monophyletic clades: Cladorhizidae (including *Eucheliplumа*), Guitarridae (excluding *Eucheliplumа*), Isodictyidae, and Podospongiidae. Several mycaline families were not supported by these results: Desmacellidae, Esperiopsidae, Hamacanthidae, and Mycalidae. In neither analysis is monophyly of the suborder supported, as all the clades mentioned above, as well as some spurious ones, form a basal polytomy with several non-mycaline and non-poecilosclerid taxa (the hadromerids *Suberites* and *Tethya*).

### 18S rDNA

The 155 OTUs considered in the analysis of large and/or complete 18S sequences yielded several highly supported clades (Fig. 2). A few unidentified taxa clustered inside Poecilosclerida, as did two supposedly non-poecilosclerid taxa, namely, *Desmoxya pelagiae* van Soest and Hooper, 2005 and *Higginsia anfractuosa* Hooper and Lévi, 1993. In summary, Mycalina, apart from Mycalidae and *Guitarra*, is inferred to be paraphyletic, with a series of lineages sprouting at the base of Poecilosclerida.

Monophyly of the order, exclusive of Biemnidae, which branches elsewhere within Demospongiae (Morrow et al. 2012, 2013; Redmond et al. 2013, this issue), had 100% bootstrap support with a restricted choice of outgroups. *Merlia* (type species, two OTUs) is revealed as the earliest diverging lineage of the order, and *Desmacella* (two spp. including the type, two OTUs) is supported as the sister group of all poecilosclerids other than *Merlia* (Fig. 2A). Support for these specific relationships and monophyly of the order dwindles with increased taxon sampling. Redmond et al. (2013, this issue, Figures 6 and S9), with a larger representation of Heteroscleromorpha (=G4) found the same arrangement, with 57% bootstrap support and 0.76 posterior probability for Poecilosclerida with Merlia diverging first, and 75% bootstrap support and 0.96 posterior probability for the position of Desmacella. In all analyses, the remaining core group of poecilosclerids received high support (see Redmond et al. 2013, this issue).

This core clade has a basal dichotomy (Fig. 2), only one branch of which finds high support (90%): Podospongiidae (98% support; *Diacarnus*, *Negombata*, and *Neopodospongia*), *Amphilectus fucorum* (Esper, 1794) (Esperiopsidae) and *Isodictya* spp. (Isodictyidae). The branch with low support is composed of two well-supported and again highly unbalanced branches. On one side, there is 99%

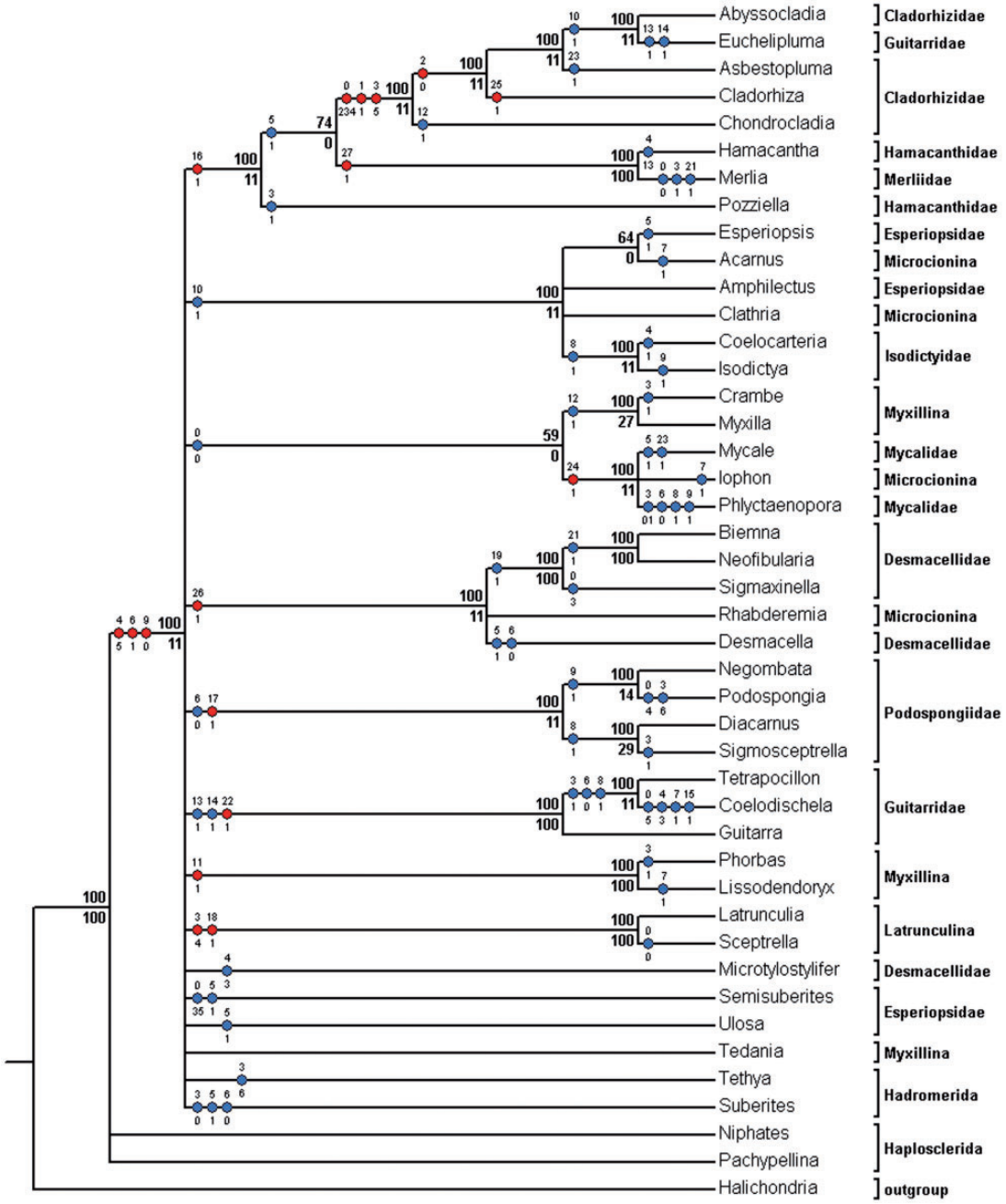


**Fig. 1** Majority-rule consensus of 821 (227 steps, RC = 0.41) most parsimonious phylogenetic trees obtained for the morphological data compiled in Supplementary Material 3. Majority-rule fractions are shown on top of branches, Bremer support underneath. Filled (red online) circles indicate synapomorphies. Unfilled (blue online) circles indicate homoplasies. Color figure is available online.

support for the myxillines (Crambeidae) *Crambe* + *Monanchora*; on the other, 80% support for all the rest. This vast majority of the Poecilosclerida houses a complex dendrogram of unsupported basal branches, which requires jumping higher in the tree for highly supported clades. A threshold support of 90% was achieved by *Mycale* (12 spp.), Latrunculina (three genera, five spp.), and Microcionina (five genera of two families, 27 OTUs). The latter excluded the raspailiid genera *Eurypon* and *Cyamon*, and the acarnid *Cornulella*, thus pointing to polyphyly of Acarnidae, as *Acarnus* remained within the large microcionine clade. On the other hand, 80% threshold support was retrieved for two large, separate myxilline clades, thereby underscoring the polyphyly of this suborder. Species of *Lissodendoryx* (Coelosphaeridae) and *Myxilla* (Myxillidae) dispersed over both clades, while those of *Phorbas* (Hymedesmiidae) and *Tedania* (Tedaniidae) stood separate. Similar support was also attained by subsets

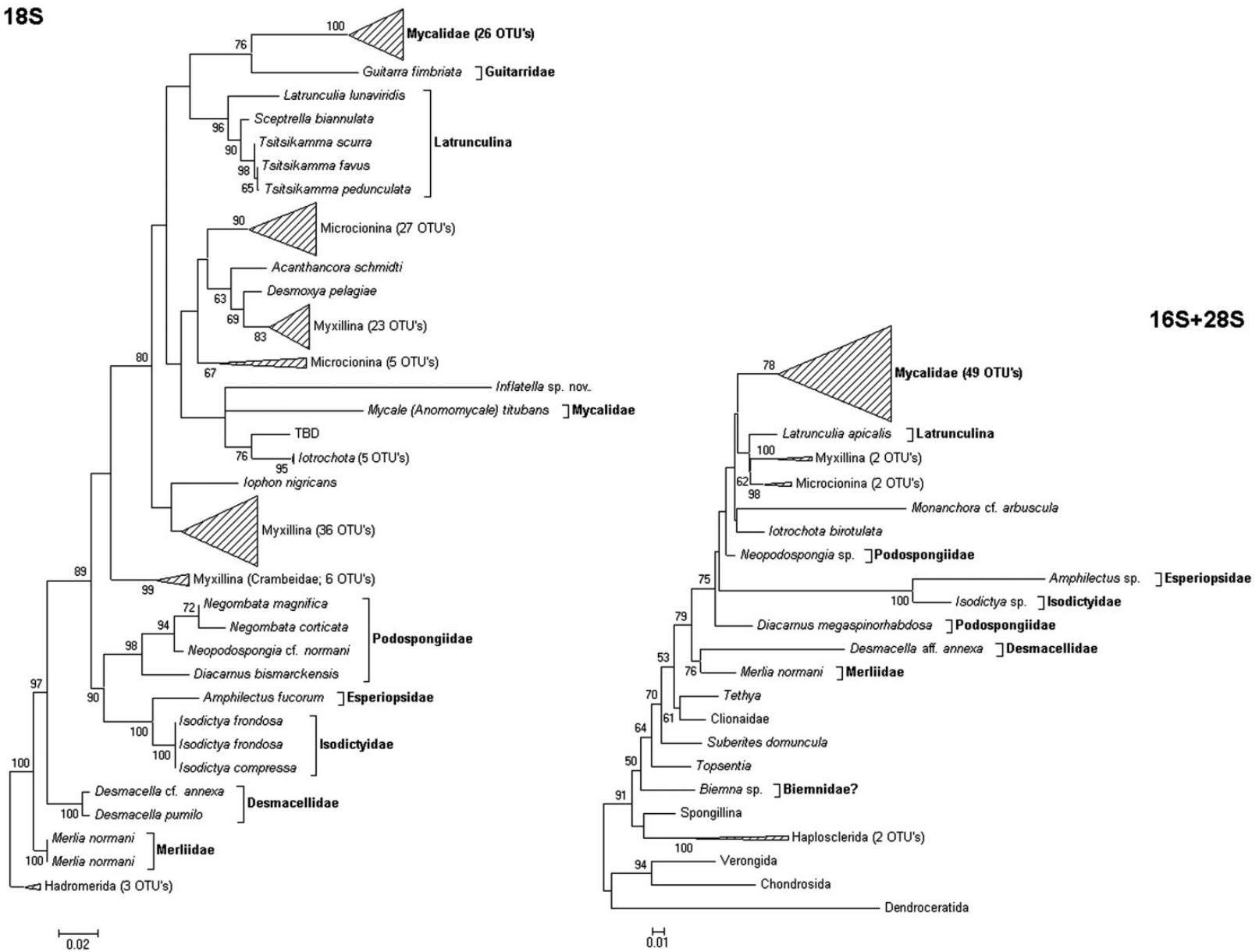


**Fig. 2** ML phylogenetic trees obtained from analysis of complete 18S rDNA and combined partial 16S and 28S rRNA sequences. Bootstrap support values are indicated. Further information on OTUs appears in Supplementary Table S1.

of the species of *Clathria* (Microcionidae). Lowering the threshold to 70% adds a clade containing all the *Mycale* spp. included in the analysis, and their sister taxon, namely, *Guitarra* (Guitarridae). Apart from this, only minor clades are supported, comprising either one or two genera, for example, *Clathria/Echinoclathria* (Microcionidae), *Hymedesmia* (Hymedesmiidae)/*Lissodendoryx*, *Iotrochota* (Iotrochotidae), *Tedania* (Tedaniidae), *Clathria* spp.

### 16S and 28S rRNA combined dataset

The combined analysis of partitioned 16S and 28S rRNA data retrieved a well-supported Mycalidae (49 OTUs, bootstrap 78%, Fig. 1B). Nevertheless, *Phlyctaenopora*, the sole additional genus in the family, could not be included for lack of successful amplification with the chosen markers. Similarly high bootstrap support (79%) was obtained for a large clade including the above clade and several additional poecilosclerid sponges, with the notable exception of *Biemna* (Biemnidae), a result also obtained by Morrow et al. (2012, 2013), and in the 18S analysis (Redmond et al. 2013, this issue). This large clade comprises elements of the Latrunculina (one OTU), Microcionina (two OTUs), Myxillina (four OTUs), monophyletic *Desmacella* + *Merlia* (76% support), *Amphilectus* + *Isodictya* (Isodictyidae) (100% support), and a polyphyletic Podospongiidae (two genera, two OTUs). Another highly supported clade (100%) is (Latrunculina [Myxillina without *Iotrochota* and *Monanchora*, Microcionina]). Again, Mycalina sponges, in this case, apart from Mycalidae only, comprise a series of early paraphyletic branches in poecilosclerid evolution.

## Discussion

Previously, the phylogenetic relationships of Mycalina were only meagerly understood. As emphasized above, no formal phylogenetic analysis had ever been made of the readily available morphological data. Neither had molecular systematists managed to establish a comprehensive phylogenetic framework. Recently, however, this situation has been changing.

In most of the 2000s, a single representative of Mycalina was used in molecular studies, namely, *Mycale fibrexilis* Wilson, 1894. 18S (GenBank AF100946) was originally derived by Collins (1998) and the same extract was used to derive near complete 28S (AY026376) by Medina et al. (2001). Using these sequences, Borchiellini et al. (2001, 2004) found *M. fibrexilis* grouping with the microcionine *Clathria* (*C.*) *prolifera* (Ellis & Solander, 1786), in contrast to the findings of Nichols (2005) and Kober and Nichols (2007), who retrieved it side by side with the myxilline *Tedania ignis* (Duchassaing & Michelotti, 1864). At this point, it was not yet possible to realize how unexpected these results were in view of the extremely limited taxon sampling undertaken.

Albeit including only two species, *Biemna fistulosa* (Topsent, 1897) and *Negombata magnifica* (Keller, 1889), Rot et al. (2006) produced the first evidence of Mycalina being non-monophyletic, as their mycaline taxa clustered with *Axinella corrugata* (George & Wilson, 1919). Gazave et al. (2010) found the latter species to be closer to *Agelas* (Agelasidae) and proposed the Phylocode name *Cymbaxinella*$^{P}$ to accommodate this species as well as *Axinella* spp. that do not belong in the same clade as *Axinella polypoides* Schmidt, 1862 (*Axinella*'s type species). Holmes and Blanch (2007) provided additional evidence of Mycalina polyphyly, by inclusion of *Coelocarteria* (Isodictyidae) and *Diacarnus* in the conundrum, intermingled with microcionines and myxillines, as well as some tetractinellids.

In the current decade this scenario changed by inclusion of additional putative mycaline families and additional species of *Mycale*. Morrow et al. (2012) confirmed the polyphyletic nature of Mycalina, as Desmacellidae (*Desmacella* cf. *annexa* Schmidt, 1870), Esperiopsidae (*Amphilectus fucorum* [Esper, 1794], and *Ulosa stuposa* [Esper, 1794] as *Ulosa digitata* [Schmidt]), and Mycalidae (*Mycale rotalis* [Bowerbank, 1874]) did not cluster together. They found *M. rotalis* branching basally in a clade with several myxillines; *Amphilectus* and *Desmacella*, basal to a larger clade, including the above, several microcionines, and further myxillines; and *Ulosa* nesting among several hadromerids and halichondrids. New results were brought up by Vargas et al. (2013), who sampled Cladorhizidae (four genera, five OTUs), Isodictyidae (*Coelocarteria*, two OTUs), Mycalidae (*Mycale,* three OTUs), and Podospongiidae (two genera, two OTUs). In contrast to our results, their three *Mycale* spp. do not form a monophylum, which strongly suggests a problem with the sequence of *M. fibrexilis* (the name by which the original source material is locally known is thought to be erroneous by one of us: A.G.C.), as this is the only *Mycale* among tens of species tested so far that nests elsewhere in the Poecilosclerida tree. Therefore, we strongly recommend that Genbank sequences labeled *M. fibrexilis* from northeastern USA (e.g., AF100946, AY026376, and AJ843890) be avoided or used cautiously in further phylogenetic studies, pending the collection of new sequence

data from carefully identified *M. fibrexilis* from the Woods Hole, Massachusetts (USA) area.

In addition, Vargas et al. (2013) found support for a monophyletic Cladorhizidae and for the sister-group relationship between this family and *Mycale* (non-*M. fibrexilis*), but found Podospongiidae to be possibly polyphyletic and confirmed the finding by Morrow et al. (2012) that Mycalina is also polyphyletic. Moreover, *Coelocarteria* ended up in a microcionine + myxilline clade, representing further support for the hypothesis of mycaline polyphyly. Given the series of families assigned to Mycalina by the *Systema Porifera* (Hooper and van Soest 2002), and currently represented in the *World Porifera Database* (van Soest et al. 2013), and the insufficient sampling of the families hitherto studied, much remained to be verified, using expanded taxon sampling and an integrated approach to systematics.

Data presented here intended to reduce this gap substantially. Accordingly, the morphologic analysis took into account all nine mycaline families and nearly every genus included in these. Genera excluded from our analyses were the monotypic *Cercicladia* Rios, Kelly & Vacelet, 2011 (Cladorhizidae), *Dragmatella* Hallmann, 1917 (Desmacellidae), *Lollipocladia* Vacelet, 2008 (Cladorhizidae), *Neocladia* Koltun, 1970 (Cladorhizidae), *Sceptrintus* Topsent, 1898 (Podospongiidae); and *Diplopodospongia* Sim-Smith & Kelly, 2011 (Podospongiidae), with three species described. Families Hamacanthidae and Merliidae were included for the first time in formal phylogenetic studies, the latter involving both morphologic and molecular approaches. The genus *Merlia* shifts around the base of the Poecilosclerida tree, and as stated above (results of 18S), the more comprehensive the taxon sampling, the weaker the support for its inclusion in this order. Analysis of morphology reached an entirely different conclusion, no doubt a consequence of treating sigmas with fimbriae (sigmancistras, diancistras, cyrtancistras, clavidiscs) and sigmas with notches (diancistras, clavidiscs), as two independent characters. In this case, *Merlia* was retrieved as the sister taxon of *Hamacantha*, both being sister to Cladorhizidae (including *Eucheliplum*a). The affinities of Desmacellidae (excluding *Microtylostylifer*) + *Rhabderemia*, Guitarridae (excluding *Eucheliplum*a), and Podospongiidae remained unresolved from the analysis of morphological characters.

Cladorhizidae was shown to be likely monophyletic by Vargas et al. (2013) on the basis of partial CO1 and partial 28S sequences, which is in relative agreement with our morphological data. On the other hand, morphology brought *Eucheliplum*a (Guitarridae) within the family, despite the shared occurrence of placochelae (characters alae with flat discs and alae with denticulated inner surfaces) with Guitarridae. Hajdu (1994) argued for a closer relation between Cladorhizidae and Guitarridae because both lack toxas and trichodragmas, but this hypothetical close relationship has not been recovered here, nor in any previous formal phylogenetic analysis.

The possible monophyly of Cladorhizidae determines the likely parallel development of several morphotypes of chelae microscleres, as already recognized by Hajdu et al. (1994) and highlighted by Vargas et al. (2013). Their results with the CO1 marker are highly supported and imply the Cladorhizidae has reproduced the same transformation series proposed by Hajdu et al. (1994) for the entire Poecilosclerida (no Latrunculina at that time), with later amendments by Lopes et al. (2011). According to this hypothesis, palmate chelae are the basalmost morphotype, from which arcuate chelae developed. The latter gave rise to cleistochelae and abyssochelae on one side and anchorate chelae on the other. This is implicit in the *Asbestopluma*, *Abyssocladia*, *Chondrocladia*, and *Cladorhiza* branching sequence. On the other hand, our results imply a distinct transformation series, where the anchorate morphotype is the basalmost one (*Chondrocladia*, *Cladorhiza*), giving rise to palmate (*Asbestopluma*), and only then to arcuate, cleistochelate and placochelate (*Abyssocladia*, *Eucheliplum*a). An important persistent question about the evolutionary history of Poecilosclerida is ''How frequently did parallel development of the main chelae morphotypes occur?'' Answering this question is pivotal for understanding how much phylogenetic signal exists among the various complex microsclere morphologies observed in Demospongiae.

Desmacellidae was found to be polyphyletic (Morrow et al. 2012) and this is now confirmed by data from additional molecular markers as well as from morphological evidence. The 18S analysis retrieved *Desmacella* branching separately from *Biemna*, *Neofibularia*, and *Sigmaxinella*. The latter three genera are quite close morphologically, only distantly related to Poecilosclerida, and may be the sister group to Tetractinellida (Redmond et al. 2013; Morrow et al. 2013, this issue), which is why they are excluded from our 18S analysis (Fig. 2A). Tetractinellida is absent in the combined 16S and 28S analysis presented here, but this analysis found *Biemna* branching earlier than Halichondrida and Hadromerida, in regard to Poecilosclerida. Our morphological evidence retrieved *Microtylostylifer* in a

large basal polytomy comprising eight poecilosclerid clades and another five genera (including the two hadromerid ones). Furthermore, *Rhabderemia* (formerly Rhabderemiidae, Microcionina; but see Cedro et al. forthcoming; Morrow et al. 2013, this issue) was found to belong in the same clade as the remaining Desmacellidae, recognized on the basis of the shared occurrence of terminally microspined sigmas, and thus suggesting that a monophyletic Desmacellidae may depend solely on the selection of the appropriate taxa to include in it.

Evidence for the polyphyly of Esperiopsidae was also apparent from the results of Morrow et al. (2012), as *Amphilectus* and *Ulosa* did not cluster together. 18S data confirm this result (Redmond et al. 2013, this issue). On the other hand, while a basal position in regard to Poecilosclerida is advocated for *Amphilectus* through published 28S data, both our 18S as well as combined 16S and 28S data point to its sister-taxon relationship with *Isodictya* (Fig. 2) and a close relation with Podospongiidae. *Amphilectus* and *Isodictya* form a highly supported monophyletic clade with the latter family in 18S (Fig. 2). Our morphological results distinguished chelae-bearing Esperiopsidae (*Amphilectus*, *Esperiopsis*) from those devoid of chelae. The former clustered with Isodictyidae and microcionine taxa with palmate isochelae (*Acarnus*, *Clathria*). On the other hand, *Semisuberites* and *Ulosa*, Esperiopsidae without chelae, remain unresolved, in a large basal polytomy.

Guitarridae, as defined in *Systema Porifera* (Hajdu and Lerner 2002), did not prove to be monophyletic, which is surprising given the complexity of its placochelae and other apparently related microscleres (the placocheloid forms). The presence of a denticulated inner surface in the alae of these microscleres is absolutely unique and remarkable, and it is surprising that *Eucheliplum*a nested in a derived position among the chadorhizids. Nevertheless, *Eucheliplum*a shares with Cladorhizidae the possession of sigmancistras and a pinnate habit as well as likely being carnivorous. Our 18S data provide additional evidence for the unexpected polyphyly of the family, as *Guitarra* appears related to *Mycale*, whereas *Tetrapocillon* is revealed to be a close relative of *Diacarnus spinipoculum* (Podospongiidae) and *Raspailia* spp. (Raspailiidae) (Redmond et al. 2013, this issue).

No molecular data have been published for Hamacanthidae, but morphology suggests that the family is polyphyletic. If 18S data are correct in suggesting that *Merlia* branches at, or near, the very base of Poecilosclerida, and morphology is also correct in bringing *Hamacantha* close to *Merlia*, it is then conceivable that the former genus will eventually also prove to be a lineage of Poecilosclerida that diverged early. There is no surprise in the retrieved affinity of both genera given the striking similarity exhibited by diancistras and clavidiscs, already pointed out by Kirkpatrick (1911). Nonetheless, it is quite unexpected that *Pozziella* did not cluster together with both genera. Our cladistic analysis proposed *Pozziella* as the earliest diverging taxon in a clade from which successively branch the sister pair *Hamacantha* and *Merlia*, and then all the cladorhizids, and *Eucheliplum*a, as stated above.

It is still not possible to fully assess the monophyly of Isodictyidae from molecular data, as its two genera have been the subject of separate analyses. There is, nevertheless, an indication of possible polyphyly, as Vargas et al. (2013) retrieved *Coelocarteria* sister to a large myxilline clade, while we obtained a close relationship for *Isodictya* and the mycalines *Amphilectus* and Podospongiidae. Our assessment of relationships on the basis of morphological data retrieved only a poorly supported monophyletic Isodictyidae, brought together by the possession of strongyles, amidst the clade containing every genus considered which has palmate isochelae (*Acarnus*, *Amphilectus*, *Clathria*, *Esperiopsis*). The occurrence of strongyles is highly homoplastic, found in *Phlyctaenopora*, and supporting the sister-taxon relationship of *Coelodischela* and *Tetrapocillon* (Guitarridae), as well as of *Diacarnus* and *Sigmosceptrella* (Podospongiidae).

Mycalidae is currently accepted as a very unbalanced family. Its alleged two evolutionary lines contrast the extremely species-poor *Phlyctaenopora* with one of Porifera's richest genera, *Mycale*. Such a remarkable contrast, unsurprisingly, mirrors the polyphyly of the family based on morphological data. Our finding that *Iophon* (Acarnidae, Microcionina) may be close to Mycalidae, based on the shared possession of basally spurred palmate anisochelae is, nevertheless, probably an artifact amenable to correction by further sampling of characters from non-mycaline poecilosclerids. Remarkably, the possession by *Iophon* of acanthostyles (character 6.2) and tylotes (character 7.1) did not change these results, in spite of their absence from both Mycalidae genera considered. Acanthostyles occur in another eight genera, tylotes in another five, none of which had enough impact to attract *Iophon*. The phylogenetic position of *Mycale* has been sought in several molecular studies, including ours. Leaving aside *M. fibrexilis* (see rationale for this above), there are still indications of

evolutionary proximity to Myxillina (Morrow et al. 2012, one *Mycale* OTU), Cladorhizidae (Vargas et al. 2013, seven *Mycale* OTUs), to Guitarridae (our 18S data, 26 *Mycale* OTUs), and a composite clade including microcionines, myxillines, and Podospongiidae (our combined 16S + 28S data, 49 *Mycale* OTUs). It appears to us that an integrated analysis of these apparently conflicting datasets has to be sought before a better understanding of the affinities of *Mycale* can be achieved.

Finally, monophyly of Podospongiidae is supported by morphological data (Fig. 1), but not quite with molecular data (Fig. 2, and Redmond et al. 2013, this issue). Morphology retrieved the ((*Diacarnus, Sigmosceptrella*) (*Negombata, Podospongia*)) relationship, with no indication as regards the affinities of the family. Our 18S tree shows (*Diacarnus bismarckensis* (*Negombata, Neopodospongia*)), but allocated *Diacarnus spinipoculum*, the genus' type species, outside the core poecilosclerids (Redmond et al. 2013, this issue). The combined 16S and 28S data failed to recover a monophyletic relationship between the podospongiids *Diacarnus megaspinorhabdosa* and *Neopodospongia* sp.

Our expanding discussion on Mycalina and the phylogenetic affinities of its various taxa highlights several areas in need of greater study. In some instances, the applied datasets do not yield consensus among competing phylogenetic hypotheses, some of which are quite unexpected. Not every limitation, however, is due to lack of appropriate markers. Some questions may not have been answered because of lack of biological materials. Even when genera were represented, one wonders whether the hypothesis of its phylogenetic position will be stable when additional species are considered. *Mycale* is the sole genus that can be said to be well represented in the phylogenetic analyses conducted up to the present time, with its tens of species sequenced and analyzed, and yet its affinities remain obscure. The next best sampled taxon is *Tsitsikamma*, with three species included. Obviously much still needs to be done in terms of "good, old science." We need to do a lot of collecting.

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## Supplementary data

Supplementary Data are available at *ICB* online.

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