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Ancient Animals, New Challenges

Developments in Sponge Research

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Ancient Animals, New Challenges

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SPONGE RESEARCH DEVELOPMENTS

Preface: Sponge research developments

M. Maldonado · X. Turon · M. A. Becerro · M. J. Uriz

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Since 1970, world-wide experts on virtually every aspect of sponge biology have met together once every 4–8 years to present and discuss the latest developments in sponge research. The diverse contributions to each meeting have been published together as monographic proceedings, each book establishing a landmark reference (often more than 500 pages) on Sponge Science, and contributing collectively (7 books) to the establishment of a meaningful tradition. This current book "Ancient animals, new challenges: developments in sponge research," published as a special volume of the international journal *Hydrobiologia*, has attempted to continue this tradition by collecting contributions presented to the VIII World Sponge Conference, held by the Centre d'Estudis Avançats de Blanes (CEAB-CSIC) in Girona, Spain in September 2010.

The Conference hosted a total of 270 attendants from 36 countries, who presented 354 contributions. The Scientific Program included the topics of Evolution and Phylogeny, Organism and Cell Biology, Population Biology, Ecology, Natural Products, Sponges and Society, and Taxonomy. The present volume includes a subset of this research (27 articles), and hopefully will offer a window to the forefront of Sponge Science and its implications in Marine Life Science. The collection of articles reflects hot, ongoing debates in molecular research, such as the monophyletic versus paraphyletic nature of the sponge group, or the new awareness on pros and cons of standard barcodes and other markers in sponge taxonomy and phylogeny. It also features articles showing how the new sequencing technologies reveal the functional and phylogenetic complexity of the "microbial universe" associated to sponge tissues. The ecological interactions of sponges, the effects of nutrients and pollutants, the variability in reproductive patterns, and the processes generating genotypic and phenotypic variability in sponge populations are also covered in several contributions. Zoogeography, population structure and dynamics are also approached with both traditional and molecular tools. The effect of anthropogenic disturbance on the natural environment also finds its place in this volume, with papers dealing with metal accumulation and the potential role of sponges as biomonitors. Biodiversity data from unexplored tropical and deep sea areas are also presented.

Because the number of papers included in the volume is relatively low compared to the total of contributions to the Conference, it can be argued that we, the Guest Editors, missed the tradition of getting the bulk of the Conference published. Massive publication would only have been possible under the

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preceding format of monographic books, which has the potential drawback that all those relevant contributions are not indexed by conventional bibliographic databases, and are thus not readily available to interested readers. Publishing through *Hydrobiologia* guarantees wide diffusion and rapid accessibility. Nevertheless, we acknowledge a bias in the volume content relative to the wider richness and diversity of approaches presented to the VIII World Sponge Conference. The scope of the journal clearly focuses on molecular and experimental, hypothesis-driven studies, and this non-negotiable criterion has left out some solid descriptive work on biology, ecology, and taxonomy. We strived to find a compromise between our wish to see many of the papers presented in the Conference published and the serious limitations of space and scope inherent to an international journal such as *Hydrobiologia*. Honestly, we experienced a bittersweet feeling when seeing the book progressively growing with excellent contributions while we were aware that important work fell outside the scope of this journal and could not be accommodated in this volume. Our apologies if some "regular" contributors to the previous seven Sponge Conference Proceedings may have felt frustrated with the process of manuscript selection. Yet we hope the readers will enjoy the following selection of papers, which we believe represent collectively a significant contribution to our current understanding of sponges.

We would also like to give our explicit thanks to the sponsoring organizations, the people who helped in the organization of the Conference, keynote speakers and all participants, in particular those who submitted manuscripts to be considered in this volume, as well as the constructive reviewers who helped to get the best out of the manuscripts. We also thank Dr. Koen Martens, Editor-in-Chief of *Hydrobiologia*, who made an undeniable extra-effort to seek for excellence in all aspects of the manuscripts.

Finally, we want this volume to be a tribute to the several colleagues who passed away recently, having left behind outstanding pieces of work that have inspired several generations of students and researchers. Our homage to Dr. M[a]. Antonia Bibiloni and Professors Michelle Sara, Max Pavans de Ceccatty Patricia Bergquist, Solange Peixinho, Peter Murphy, and Lidia Scalera-Liaci. Their friendship, either if grown through close, daily work or emerged through sporadic mailing or conference contact, will always remain in our memories.

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 Short Research Note

No longer Demospongiae: Homoscleromorpha formal nomination as a fourth class of Porifera

Eve Gazave · Pascal Lapébie · Alexander V. Ereskovsky · Jean Vacelet · Emmanuelle Renard · Paco Cárdenas · Carole Borchiellini

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Abstract Over the past few years, there has been growing interest among the sponge community in the phylogenetic position of the Homoscleromorpha (i.e. within or outside the class Demospongiae). Recent molecular analyses clearly show that the Homoscleromorpha forms a distinct clade separated from the Demospongiae and is composed of two families, Oscarellidae and Plakinidae. Within the currently more widely accepted hypothesis of a monophyletic Porifera, we formally propose here to raise Homoscleromorpha to the class rank (the fourth one). We, therefore, provide a definition and a formal diagnosis. In the supplementary materials, we also present an alternative classification of the Homoscleromorpha, following the *PhyloCode*.

Keywords Porifera · New class · Homoscleromorpha · Oscarellidae · Plakinidae · *PhyloCode*

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Past and current systematics

For two decades now, phylogenies using genetic and morphological data have provided crucial information toward resolving sponge systematics (Erpenbeck & Wörheide, 2007). While the phylogenetic status of Porifera (monophyly vs. paraphyly) is still debated (Philippe et al., 2009; Sperling et al., 2009), internal phylogenies for the major sponge groups (i.e. the three Recent classes: Demospongiae, Calcarea, and Hexactinellida) are becoming better resolved (Borchiellini et al., 2004; Dohrmann et al., 2006, 2008). However, the affinities and rank of Homoscleromorpha remain unresolved within the Linnaean classification (Muricy, 1999; Muricy & Diaz, 2002).

Traditionally, Homoscleromorpha has been classified as a family or a suborder of the subclass Tetractinellida, within the class Demospongiae, mainly due to the shared presence of siliceous tetractinal-like calthrop spicules (Lévi, 1956). Lévi (1973) later proposed classifying them as a distinct subclass of the Demospongiae. Until 1995, two families were recognized within the Homoscleromorpha, Plakinidae Schulze, 1880 and Oscarellidae Lendenfeld, 1887, distinguished by the presence or absence of the mineral skeleton, respectively. However, in 1990, the discovery of a skeleton-less *Corticium*-like species led Solé Cava et al. (1992) to propose the rejection of the family Oscarellidae. Later, when this species was described as a member of a new genus *Pseudocorticium* (Boury-Esnault et al., 1995), all homoscleromorph genera were merged into a single family, the Plakinidae. *Pseudocorticium* is indeed devoid of a mineral skeleton like the genus *Oscarella*, but is more similar in histological traits (notably the leuconoid aquiferous system and a well-developed ectosome with cortex) to the spiculate genus *Corticium*.

Nowadays, according to the two current synopses of the poriferan classification, *Systema Porifera* (Hooper et al., 2002) and the *World Porifera Database* (http://www.marinespecies.org/porifera/; Van Soest, 2011), Homoscleromorpha is a subclass of the class Demospongiae, containing one order Homosclerophorida Dendy, 1905, one family Plakinidae Schulze, 1880, and seven genera: *Oscarella* Vosmaer, 1884; *Plakina* Schulze, 1880; *Plakortis* Schulze, 1880; *Plakinastrella* Schulze, 1880; *Corticium* Schmidt, 1862; *Pseudocorticium* Boury-Esnault et al., 1995; *Placinolopha* Topsent, 1897.

New insights from molecular phylogenies/phylogenomics studies

Since these two synopses were compiled, molecular phylogenies have challenged these traditional classification schemes. Indeed, in 2004, the first molecular phylogeny of an extensive sampling of Demospongiae *sensu lato* (based on 18S and 28S rDNA) suggested that Homoscleromorpha should form a clade on its own, clearly separated from the rest of the Demospongiae (Borchiellini et al., 2004). Subsequent phylogenic/phylogenomic studies using several nuclear markers corroborated this hypothesis and suggested a sister-group relationship of Homoscleromorpha and calcareous sponges (Calcarea, also known as Calcispongia) (Dohrmann et al., 2008; Philippe et al., 2009; Pick et al., 2010) (Fig. 1). Alternatively, homoscleromorphs were recovered as the sister group of Eumetazoa, albeit with low statistical support (Sperling et al., 2009). Although analysis of complete mitochondrial genomes seemingly supported the traditional placement within the Demospongiae (Lavrov et al., 2008; Wang & Lavrov, 2008), these studies were hampered by a lack of data from Calcarea, preventing a true test of the phylogenetic position of Homoscleromorpha. In any case, the number of homoscleromorph species included in the above-mentioned studies was very low (one or two).

Recently, molecular phylogenetic taxon sampling of Homoscleromorpha was substantially improved to include 18S, 28S rDNA sequences and mitochondrial genomes of six of the seven presently described genera (Gazave et al., 2010a). This study greatly contributed to resolve internal relationships of the group, restored the supra-generic classification of Homoscleromorpha abandoned in 1995 (Boury-Esnault et al., 1995), and reinstated the families Oscarellidae and Plakinidae on the basis of molecular and morphological evidence (Fig. 1). Uncertainties remain concerning the monophyly of *Oscarella* but it clearly appears that *Corticium* and *Plakinastrella* are monophyletic genera. The *Plakina* issue is more challenging and calls for further detailed molecular investigations (Fig. 1).

Linnaean classification of Homoscleromorpha

Taking into account the recent molecular studies, we consider that it is now well-established that Homoscleromorpha is not closely related to other demosponges. We feel that these new insights should be reflected in the Linnaean classification. In this classification system, the rank of both Homoscleromorpha and Calcarea is directly dependent on the phylogenetic status of Porifera. The most complete and robust molecular study of Porifera to date clearly supports the hypothesis of its monophyly, a hypothesis that is also consistent with morphological characters (Philippe et al., 2009; Pick et al., 2010). We thus formally propose, in the present paper, to raise Homoscleromorpha from subclass within Demospongiae to a fourth Recent class of Porifera (there is also a fifth extinct class of Porifera, the Archaeocyatha e.g. Debrenne et al., 2002).

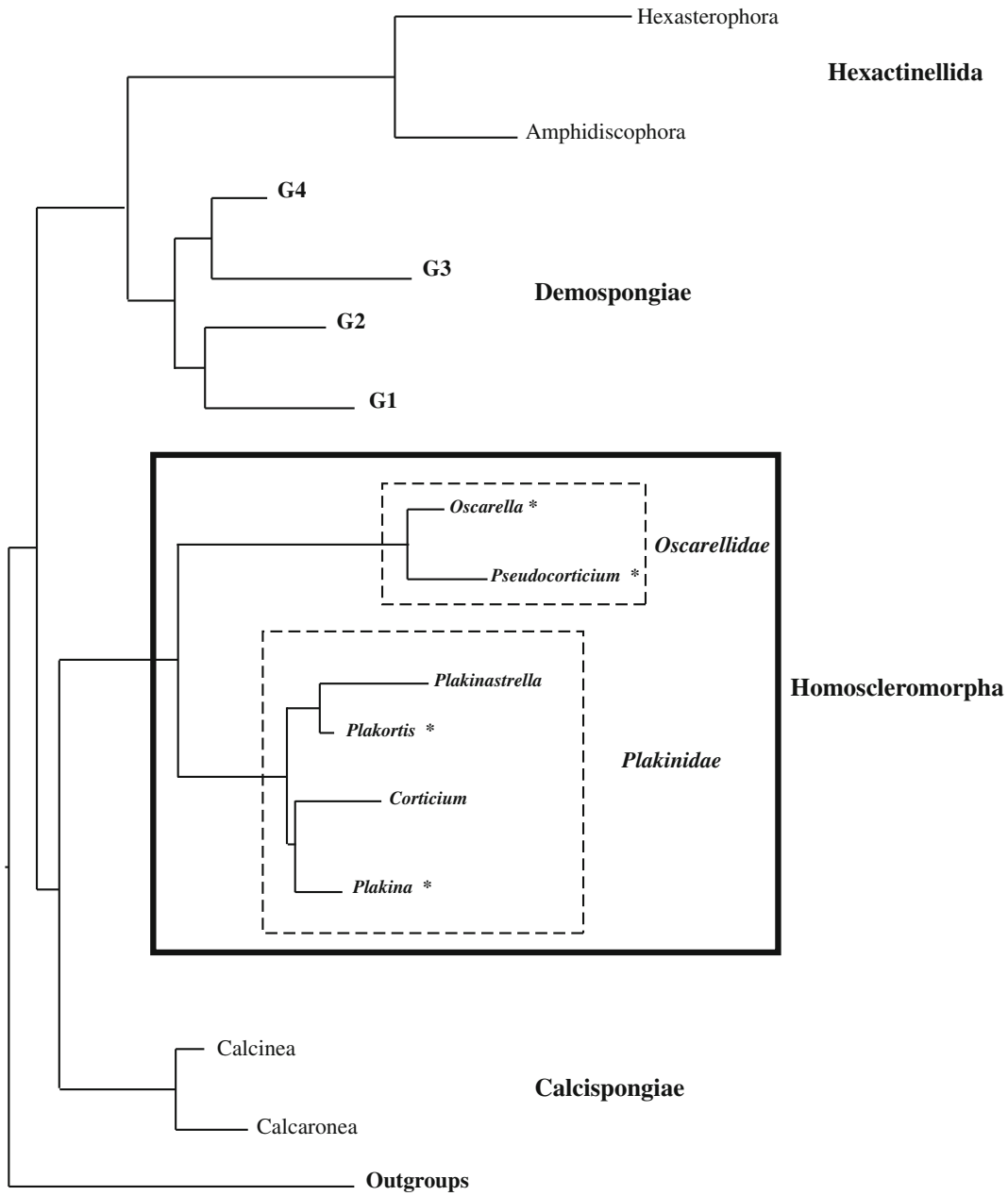

Fig. 1 Porifera simplified tree, following the monophyly hypothesis, mentioning the Linnaean names of the Homoscleromorpha families and genera. Homoscleromorpha genera that are not monophyletic or from which uncertainties subsist are identified with an *asterisk*

Definition (modified from Hooper & Van Soest, 2002)

Class Homoscleromorpha Bergquist, 1978

Other names. Microsclerophora Sollas, 1885. Carnosa Carter, 1875.

Definition

Porifera with cinctoblastula larvae and embryonic incubation; flagellated exo- and endopinacocytes; a basement membrane lining both choanoderm and pinacoderm; skeleton, if present, composed of

tetraxonic siliceous spicules–calthrops- and its derivatives with equal rays (diods, triods, and lophate spicules), arranged around oval to spherical choanocyte chambers reflecting the canal structure (sylleibid-like or leuconoid organization); no differentiation between megascleres and microscleres although size differences do occur between types of spicules; spicules usually small (100 μm or less), not localized in any particular region; choanocyte chambers with large choanocytes.

Remark

Order Homosclerophorida Dendy, 1905 has the same definition as the class Homoscleromorpha (except for the fact that it concerns the Demospongiae instead of the Porifera).

Formal diagnosis (in complement to Muricy & Diaz, 2002)

Diversity and area distribution

Homoscleromorphs are a small group of exclusively marine sponges. Among the 84 presently described species, they are 16 species of *Oscarella*, one of *Pseudocorticium*, 6 of *Corticium*, 6 of *Placinolopha*, 25 of *Plakina*, 11 of *Plakinastrella*, and 19 of *Plakortis*. They are encrusting or lumpy with a smooth surface, usually occurring at shallow depths. Recently, several new species have been described from various areas (from Brazil to Alaska, Africa and Indo-Australian coasts). They mainly pertain to the *Oscarella* and *Plakortis* genera (Moraes & Muricy, 2003; Muricy & Pearse, 2004; Ereskovsky, 2006; Ereskovsky et al., 2009a; Muricy, 2011; Pérez et al., 2011).

Skeleton

If present, it consists of small calthrops (peculiar type of tetractines spicules) and/or their derivatives (lophose calthrops, diodes and triodes). These spicules are evenly distributed in the sponge body and do not form a well-organized skeleton. Spicules are secreted by sclerocytes, pinacocytes of the external epithelium and also, to a lesser degree, by pinacocytes of the internal epithelia (Maldonado & Riesgo, 2007). In contrast to the axial filament of siliceous spicules in Demospongiae and Hexactinellida, the organic core of the spicules of Homoscleromorpha is amorphous, indicating a possible lack of a tertiary structure of the protein contained (Uriz, 2006).

Ultrastructure

The aquiferous system is sylleibid or leuconoid, with large choanocyte chambers (eurypilous, aphodal, or diploid). The homoscleromorphs are the only Porifera that have a true basement membrane with type IV collagen, tenascin and laminin, underlying the choanoderm and the pinacoderm (Boute et al., 1996) and larval ciliated epithelium (Boury-Esnault et al., 2003). Homoscleromorpha possess flagellated exopinacocytes and endopinacocytes, peculiar flagellated apopylar cells, and *zonula adhaerens* cell junctions in adults and larval epithelia (Ereskovsky et al., 2009a).

Development

Homoscleromorpha are sponges with embryos incubation (Ereskovsky, 2010). Spermatogenesis is asynchronous inside one spermatic cyst and a gradient in cell differentiation occurs along the spermatic cysts, with spermatocytes at one side and spermatozoids on the opposite side (Gaino et al., 1986; Ereskovsky, 2005). The spermatozoids have an acrosome (Baccetti et al., 1986; Riesgo et al., 2007). The hollow blastula is formed by means of multipolar egression (centrifugal migration of cells from the center to the periphery of the morula) and presents a central cavity (Ereskovsky & Boury-Esnault, 2002). The cinctoblastula larva possesses a belt of postero-lateral cells with an intranuclear crystalloid and ciliated cells with cross-striated rootlet (Boury-Esnault et al., 2003), which derives from the secondary centriole (Maldonado & Riesgo, 2008). All morphogenesis processes follow the epithelial type (Ereskovsky et al., 2009b).

Scope of the taxon (in complement of Muricy & Diaz, 2002)

Families Oscarellidae and Plakinidae, composed, respectively, of *Oscarella* and *Pseudocorticium* genera and *Plakortis*, *Plakinastrella*, *Corticium*, *Placinolopha,* and *Plakina* genera (Gazave et al., 2010a; Ivanisevic et al., 2010).

Discussion

Although molecular phylogenetic/phylogenomic studies have profoundly increased our understanding of this peculiar group of sponge, one main point is still uncertain: the evolutionary history of the basement membrane (Leys et al., 2009; Philippe et al., 2009). The appearance of a basement membrane as a histological barrier may have important implications concerning cell type specification systems and cell movement mechanisms. This baso-epithelial basement membrane present in both larvae and adult of homoscleromorphs but also in eumetazoans (1) may have been inherited from Urmetazoa (the last common ancestor of animals) and then subsequently lost in the three other sponge classes or (2) may have appeared independently twice in the course of evolution, in Homoscleromorpha sponges and in Eumetazoa. It may be noted that, alternatively, according to the sponge paraphyly hypothesis, this basement membrane may equally represent a synapomorphy of a clade containing Homoscleromorpha and Eumetazoa (named by some authors Epitheliozoa (Sperling et al., 2009)). To date, this issue has not yet been resolved but comparison of basement membrane molecular components between all Porifera classes and Eumetazoa may provide new evidence in the future.

In addition to molecular data, several morphological and developmental differences may be noticed between Homoscleromorpha and Demospongiae that support the molecular topology discussed above. The most remarkable morphological difference is the presence of a true epithelium (basement membrane and apical cell junctions) in Homoscleromorpha and its absence in Demospongiae (as well as in other sponge groups) (Ereskovsky, 2010). Another important cytological character is the flagellated exopinacoderm, which is absent in demosponges, calcareans and hexactinellids. An alveolar choanosomal skeleton, typical of many Plakinidae species, as well as diodes and triodes is also absent in demosponges (Muricy & Diaz, 2002). Concerning the developmental features that are different between those sponges classes, one can note: (1) asynchronous spermatogenesis, whereas it is synchronous in the Demospongiae (Gaino et al., 1986); (2) a multipolar egression during embryonic development (Ereskovsky & Boury-Esnault, 2002); (3) an epithelial invagination during metamorphosis (Ereskovsky et al., 2007); (4) a budding process by morphallactic morphogenesis (Ereskovsky & Tokina, 2007).

Morphological characters supporting the proposed sister-group relationship between Homoscleromorpha and Calcarea are more scarce. A remarkable resemblance is the presence of cross-striated rootlet in larval ciliated cells of both cinctoblastula (Homoscleromorpha) (Boury-Esnault et al., 2003) and amphiblastula (Calcaronea) and calciblastula (Calcinea) (Gallissian & Vacelet, 1992; Ereskovsky & Willenz, 2008). This type of rootlet is absent in other sponge groups and may represent a synapomorphy of a clade (Homoscleromorpha + Calcarea).

Relationships among Homoscleromorpha species also provide a basis for a new hypothesis regarding the evolution of morphological characters. Due to the restoration of the two Homoscleromorpha families (Oscarellidae and Plakinidae), the cortex, aquiferous system organization, and outer morphological similarities encountered between *Corticium* and *Pseudocorticium* (and previously proposed as synapomorphies) would appear to represent homoplasic characters.

Concerning the nonmonophyly of the genus *Plakina*, this may explain the wide variability in morphological characters previously observed in this genus (Muricy et al., 1998). Thus, the genus *Plakina* should be redefined in the future and, potentially subdivided into several genera on the basis of a comprehensive molecular and morphological analysis of extant species.

Conclusion

In this paper, we chose to follow the strongly supported sponge monophyly hypothesis and formally raised the Homoscleromorpha as a fourth poriferan class. Should the alternative hypothesis that Porifera is paraphyletic (Sperling et al., 2009) gain significant support in the future and the sponge monophyly hypothesis be convincingly refuted, Homoscleromorpha and Calcarea would need to change ranks and be reconsidered as potential distinct phyla. This example illustrates the constraints of a rank-based (hierarchical) nomenclatural system whereby molecular data may support a clade but without necessarily the support of morphological synapomorphies. We consider that the information obtained

from molecular (and other) datasets that do not necessarily fit into a hierarchical classification should not be lost (Manuel et al., 2003; Borchiellini et al., 2004; Cárdenas et al., 2010, 2011; Gazave et al., 2010b), and therefore, we provide here an alternative classification of Homoscleromorpha, using the draft recommendations and processes defined by the *PhyloCode* (http://www.ohio.edu/phylocode) (online resources 1 and 2).

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SPONGE RESEARCH DEVELOPMENTS

Molecular phylogeny of glass sponges (Porifera, Hexactinellida): increased taxon sampling and inclusion of the mitochondrial protein-coding gene, cytochrome oxidase subunit I

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Abstract Marine sponges of the class Hexactinellida (glass sponges) are among the most understudied groups of Porifera, and molecular approaches to investigating their evolution have only recently emerged. Although these first results appeared reliable as they largely corroborated morphology-based hypotheses, they were almost exclusively based on ribosomal RNA genes (rDNA) and should, therefore, be further tested with independent types of genetic data, such as protein-coding genes. To this end, we established the mitochondrial-encoded cytochrome oxidase subunit I gene (COI) as an additional marker, and conducted phylogenetic analyses on DNA- and amino-acid level, as well as a supermatrix analysis based on combined COI DNA and rDNA alignments. Furthermore, we increased taxon sampling compared to previous studies by adding seven additional species. The COI-based phylogenies were largely congruent with the rDNA-based phylogeny but suffered from poor bootstrap support for many nodes. However, addition of the COI sequences to the rDNA data set increased resolution of the overall molecular phylogeny. Thus, although obtaining COI sequences from glass sponges turned out to be quite challenging, this gene appears to be a valuable supplement to rDNA data for molecular evolutionary studies of this group. Some implications of our extended phylogeny for the evolution and systematics of Hexactinellida are discussed.

Keywords Porifera · Hexactinellida · Phylogeny · Cytochrome oxidase subunit I · Ribosomal DNA

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Guest editors: M. Maldonado, X. Turon, M. A. Becerro & M. J. Uriz / Ancient animals, new challenges: developments in sponge research

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Introduction

A robust and comprehensive reconstruction of the poriferan Tree of Life is of prime importance for sponge science (and beyond), because all aspects of sponge biology can be best understood in light of the evolutionary context in which the past and current diversity of these animals emerged. Molecular phylogenetics certainly constitutes the most promising approach for attaining this goal, and progress in this field has been rapid over the last two decades or so (Erpenbeck & Wörheide, 2007). However, many gaps in phylogenetic knowledge remain to be filled, and it is a further, much greater challenge to fully reconcile morphology-based taxonomy with molecular phylogenies of Porifera.

With respect to the latter, one of the most understudied groups, the glass sponges (class Hexactinellida; see Leys et al., 2007 for a comprehensive review), may provide the best chances for establishing a systematics that integrates both morphological and molecular information. This is because the glass sponges appear to be an exceptional case, as compared to other sponges (e.g., Dohrmann et al., 2006), where the first published molecular systematic results were largely in line with morphological predictions (Dohrmann et al., 2008, 2009). Nonetheless, some results remain ambiguous, and the monophyly of certain taxa could not be tested due to the lack of sequence data for more than one species. Furthermore, these phylogenies were exclusively based on three ribosomal RNA genes (rDNA)—nuclear 18S, partial nuclear 28S, and partial mitochondrial 16S—and should, therefore, be tested with independent molecular markers.

While a number of protein-coding sequences have been published for a few hexactinellid species in nonphylogenetic studies (e.g., Gundacker et al., 2001; Bebenek et al., 2004; Manuel et al., 2004; Conejo et al., 2008; Rosengarten et al., 2008), molecular phylogenetic studies including this kind of data from glass sponges are scarce (e.g., Borchiellini et al., 1998; Rokas et al., 2003; Haen et al., 2007; Philippe et al., 2009; Sperling et al., 2009), and based on a very limited taxon sampling of Hexactinellida since they did not aim at reconstructing the internal relationships of this group. We, therefore, decided to establish the mitochondrial-encoded cytochrome oxidase subunit I gene (COI) as an additional marker, because (a) this gene is widely regarded as an easily amplifiable "standard" gene for molecular evolutionary studies, and (b) COI sequence data might be useful for other applications besides systematics, such as molecular species identification ("barcoding"; see Bucklin et al., 2011 for a recent review). We also increased taxonomic sampling of hexactinellids by including seven previously unsampled species, and discuss our new results in light of the current taxonomy of the group.

Materials and methods

We added nine additional specimens, seven of which belong to previously unsampled species (Table 1), to the taxon set reported in Dohrmann et al. (2009). 18S, 28S, and 16S rDNA sequences were obtained as previously described (Dohrmann et al., 2008). COI sequences spanning the "Folmer-" and the I3-M11 regions (cf. Erpenbeck et al., 2006; ca. 1.3 kb) were amplified using various combinations of mostly degenerate primers (Supplementary Table S1), Promega's GoTaq (reaction mixes as in Dohrmann et al., 2008), and "touch-down" thermal regimes with final annealing temperatures of 45 or 30°C. Since amplification of this complete region was only rarely successful, 5'- and 3'-halves had to be amplified separately in most cases. To obtain sequences, amplicons were further processed as described (Dohrmann et al., 2008). COI sequences from *Regadrella* sp., *Acanthascus dawsoni*, and *Oopsacas minuta* were taken from ongoing mitochondrial genome sequencing projects (Haen & Lavrov, in prep.); those of *Iphiteon panicea*, *Sympagella nux*, and *Aphrocallistes vastus* were downloaded from GenBank and served as initial templates for primer design (cf. Table S1). Supplementary Table S2 gives an overview of the data set and accession numbers for the newly generated sequences.

Ribosomal DNA sequences were manually aligned to previous alignments (Dohrmann et al., 2009), aided by RNA secondary structure in case of 18S and 28S (cf. Dohrmann et al., 2008); ambiguous regions were excluded from the phylogenetic analysis. COI sequences were pre-aligned in ClustalX 2.0 (Larkin et al., 2007), followed by manual refinement. The COI alignment was largely unambiguous, but contained several instances of single species, 1-bp insertions that were either sequencing errors or

Table 1 Newly sampled specimens

Family	Species	Collection region	Voucher
Rossellidae	***Sympagella nux***	Turks & Caicos Isl.[b]	See Haen et al. (2007)
	Acanthascus dawsoni	British Columbia	Gift of Sally Leys
Euplectellidae	***Regadrella* sp.**	Straits of Florida[b]	HBOI-8-VIII-09-2-001
Leucopsacidae	*Oopsacas minuta*	Mediterranean Sea	Gift of Jean Vacelet
Farreidae	***Aspidoscopulia* n. sp. 1**[c]	Coral Sea, Australia[a]	QM G332077
	***Aspidoscopulia* n. sp. 2**[c]	Coral Sea, Australia[a]	QM G332104
	Lonchiphora antarctica[d]	Antarctica	SMF 10772
	Sarostegia oculata[c]	Florida, W Atlantic[b]	HBOI 25-V-06-2-001
Tretodictyidae	***Psilocalyx wilsoni***[c]	Coral Sea, Australia[a]	QM G331821

Previously unsampled species are highlighted in bold. *HBOI* Harbor Branch Oceanographic Institution, *QM* Queensland Museum, *SMF* Senckenberg Museum Frankfurt

[a] Collected during Deep Down Under Expedition (http://www.deepdownunder.de/)

[b] Collected through HBOI by Johnson-Sea-Link II

[c] Morphological descriptions of these specimens are provided elsewhere (Dohrmann et al., 2011), [d] see Göcke & Janussen (2011)

putative +1 translational frameshifts (Haen et al., 2007; Rosengarten et al., 2008); these sites were removed.

Preliminary analyses recovered essentially the same relationships among nonbilaterian animals as reported in Dohrmann et al. (2008); however, topology and support values for Hexactinellida were not markedly affected when the outgroups were excluded (results not shown). Therefore, we did not include any nonhexactinellid sequences in the final analyses, instead designating the six amphidiscophorans as a multi-species outgroup. This is justified because monophyly of Hexactinellida and its two subclasses, Amphidiscophora and Hexasterophora, is beyond doubt (see Dohrmann et al., 2008), and the deep divergence between the latter two taxa makes them ideal outgroups for each other. Furthermore, investigating relationships between the major nonbilaterian animal lineages is beyond the scope of this article and should better be approached with different, e.g. phylogenomic, data sets (see Philippe et al., 2009; Pick et al., 2010).

Phylogenetic analyses of the COI DNA alignment, the concatenated rDNA alignment, and a supermatrix (cf. de Queiroz & Gatesy, 2007) of all four partitions were conducted in a maximum-likelihood (ML) framework as implemented in RAxML (Stamatakis, 2006) 7.2.6 (http://wwwkramer.in.tum.de/exelixis/software.html), using the Pthreads-parallelized version on a 64-bit Linux cluster at the Molecular Geo- and Palaeobiology Lab, LMU Munich. For the combined rDNA (3328 bp) and supermatrix (4582 bp) analyses, the markers were concatenated in SeaView 4.0 (Gouy et al., 2010) and analyzed under mixed substitution models. Because, in contrast to the previously used Bayesian Markov Chain Monte Carlo (BMCMC) application PHASE (see Dohrmann et al., 2008, 2009), computational limitations are not an issue with RAxML, the least simplifying models could be explored, namely the 16-state paired-sites model (cf. Savill et al., 2001) S16 for 18S + 28S double-stranded regions (stems), and independent GTR models (Lanave et al., 1984) for 18S single-stranded regions (loops), 28S loops, 16S, and COI. However, using the 7- and 6-state paired-sites models S7D and S6B, which do not fully account for mismatch pairs (see Savill et al., 2001), but were found best-fitting in the BMCMC framework among the models tested by Dohrmann et al. (2008, 2009), lead to essentially the same results (not shown). Among-site rate variation was modeled for each partition independently using discrete gamma distributions with four rate categories ($+G_4$; Yang, 1994). We also analyzed the COI data on the amino-acid (aa) level, with DNA sequences translated using the hexactinellid-specific mitochondrial genetic code (Haen et al., 2007), and employing the MtRev+F+G_4 model of aa replacement, as suggested by ProtTest 2.4 (Abascal et al., 2005) under the Akaike Information Criterion (AIC; Akaike, 1974).

In all analyses, clade stability was assessed by rapid bootstrapping (Felsenstein, 1985; Stamatakis et al., 2008) based on 1000 pseudoreplicates.

The final supermatrix and the associated structure-, partition-, and tree files are available at Open Data LMU (http://dx.doi.org/10.5282/ubm/data.40).

Results and discussion

Contrary to our expectations (see Introduction), and for reasons that remain somewhat elusive, obtaining COI sequence data from hexactinellid specimens turned out to be rather challenging. Extremely low annealing temperatures were required to obtain amplicons (see Materials and methods), and in many cases, only very faint bands of target sequences were observed or PCR failed completely for one or both of the fragments (cf. Table S2). Also, different primer combinations worked for different specimens, necessitating that PCRs be optimized individually and no standard protocol could be established after an initial optimization step. Further problems included amplification of nontarget DNA (e.g. prokaryotes; cf. Siddall et al., 2009), multiple

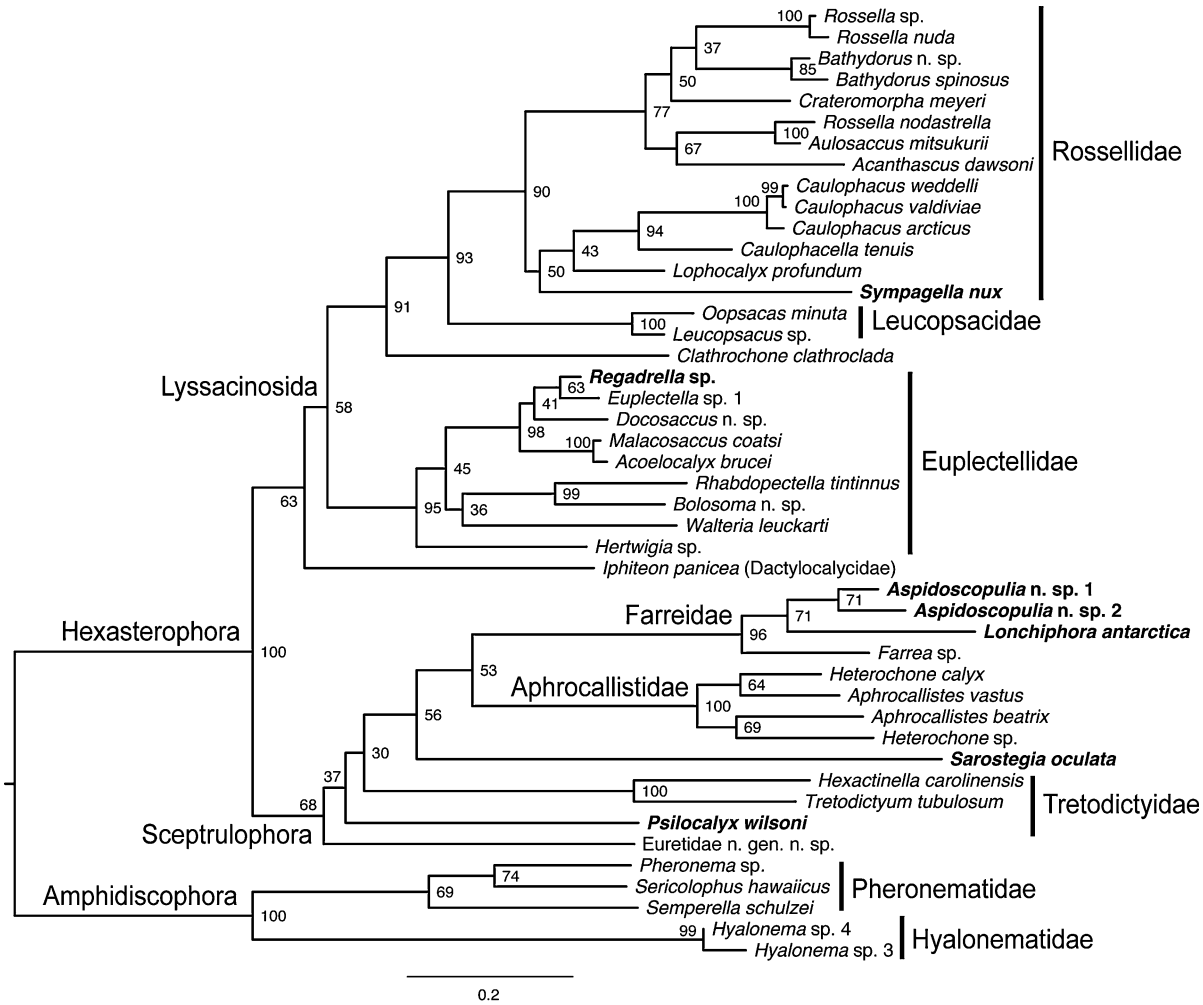

Fig. 1 Maximum likelihood phylogeny of Hexactinellida inferred from COI DNA sequence alignment (RAxML; GTR+G_4 substitution model). Bootstrap support values (1000 replicates) given at nodes. Previously unsampled species are highlighted in *bold*. Scale bar, expected number of substitutions per site. See "Materials and methods" section for further details

bands making gel extraction mandatory, and poor sequence reads leading to nonoverlapping of fragments (cf. Table S2) and requiring increased use of the IUPAC code for ambiguous base calls. Despite these practical difficulties, however, COI proved to be a useful addition to the three established rDNA markers (Dohrmann et al., 2008), as discussed below.

Phylogenies reconstructed from the COI alignments and the combined rDNA alignment, respectively, are largely congruent (Figs. 1, 2, 3), i.e. there are no conflicting clades with high bootstrap support (BS). Despite the overall congruence, many nodes are poorly (BS $< 70\%$) supported in the COI phylogenies, especially in the aa tree (Fig. 2). Strikingly, this is also the case to a lesser extent in the rDNA phylogeny, which appears less robust than the Bayesian trees presented in Dohrmann et al. (2009), even when the different significance levels of bootstrap versus posterior probability values (cf. Hillis & Bull, 1993; Huelsenbeck & Rannala, 2004) are taken into account. For example, support for order Lyssacinosida is very weak and the topology within family Rossellidae is less resolved compared to our previous study. We suspect that these results are due to further methodological and/or implementational differences between RAxML and PHASE, an issue that will be explored elsewhere.

Compared to the results of the separate analyses, robustness and resolution is increased when the two data sets are analyzed together (Fig. 4). For example, support for monophyly of Lyssacinosida is only 58 and 55% in the COI DNA and the rDNA phylogeny,

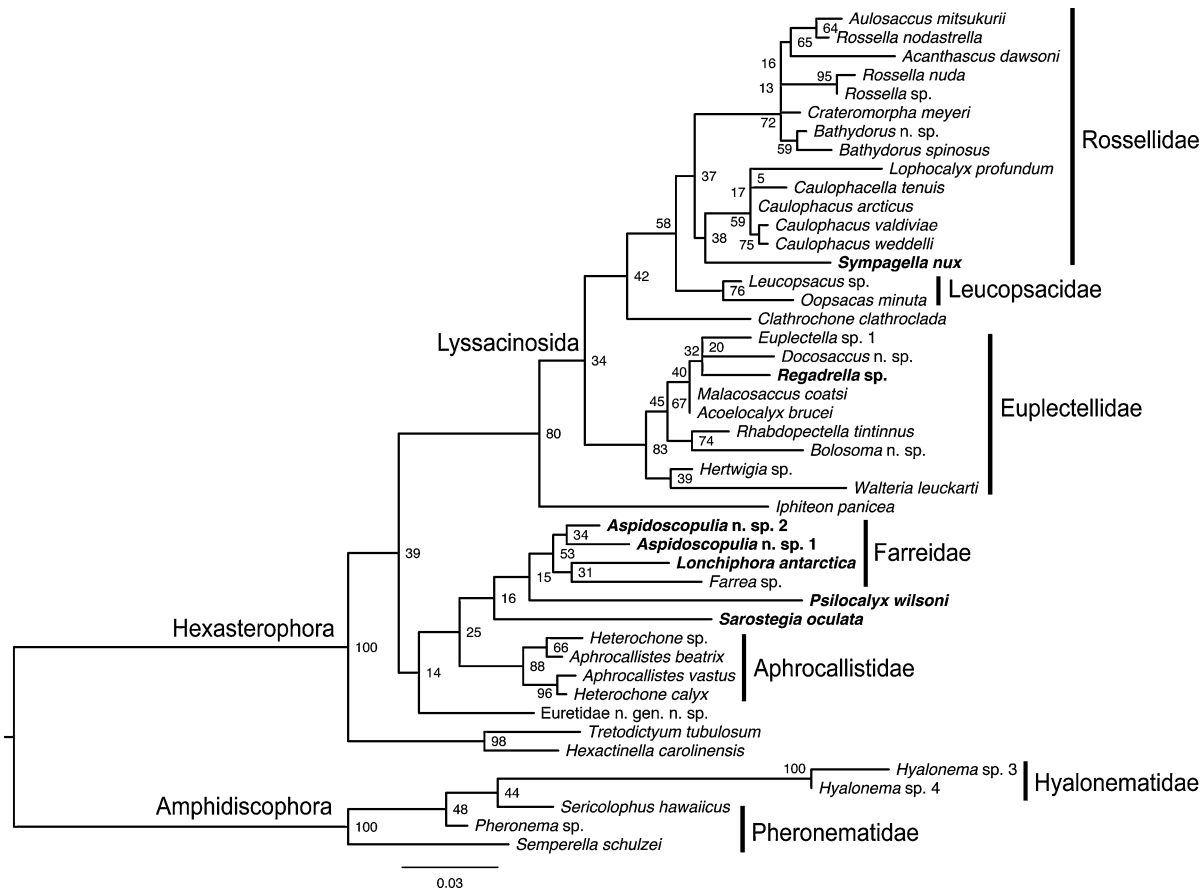

Fig. 2 Maximum likelihood phylogeny of Hexactinellida inferred from COI amino-acid sequence alignment (RAxML; MtRev+F+G_4 substitution model). Bootstrap support values (1000 replicates) given at nodes. Previously unsampled species highlighted in *bold*. Scale bar, expected number of substitutions per site. See "Materials and methods" section for further details

respectively, but rises to 75% in the combined tree, which is significant according to Hillis & Bull (1993). Below, we discuss the placement of the previously unsampled species, as well as some other new results, on the basis of the supermatrix tree (Fig. 4). A more in-depth discussion of the phylogeny of the dictyonal, sceptrule-bearing glass sponges (Sceptrulophora), and implications for spicule evolution is provided elsewhere (Dohrmann et al., 2011).

Within Sceptrulophora, we find that *Sarostegia oculata* does not group with the remaining Farreidae, which form a well-supported clade sister to Aphrocallistidae. Interestingly, *Sarostegia* is the only farreid with a euretoid dictyonal framework and lacks clavules, a spicule type that is typical for, and restricted to, Farreidae. Although this species does not group with the representative of Euretidae (but see Fig. 3), topology-tests indicate that our supermatrix data are consistent with such a placement, and we thus suggest resurrection of *Sarostegia*'s earlier classification in Euretidae (Dohrmann et al., 2011).

Although poorly supported here (BS = 64%), the position of *Psilocalyx wilsoni* as sister to the other two tretodictyids, *Hexactinella* and *Tretodictyum*, receives significant support (BS > 75%) when the taxon set is restricted to dictyonal sponges, allowing for the inclusion of additional rDNA positions (Dohrmann et al., 2011). Thus, monophyly of Tretodictyidae (Dohrmann et al., 2008) is further corroborated. It is particularly noteworthy that this morphologically well-characterized taxon (Mehl, 1992; Reiswig, 2002) was not resolved in the COI and rDNA trees, respectively (Figs. 1, 2, 3). These results indicate that considerable numbers of molecular characters may be required to support certain

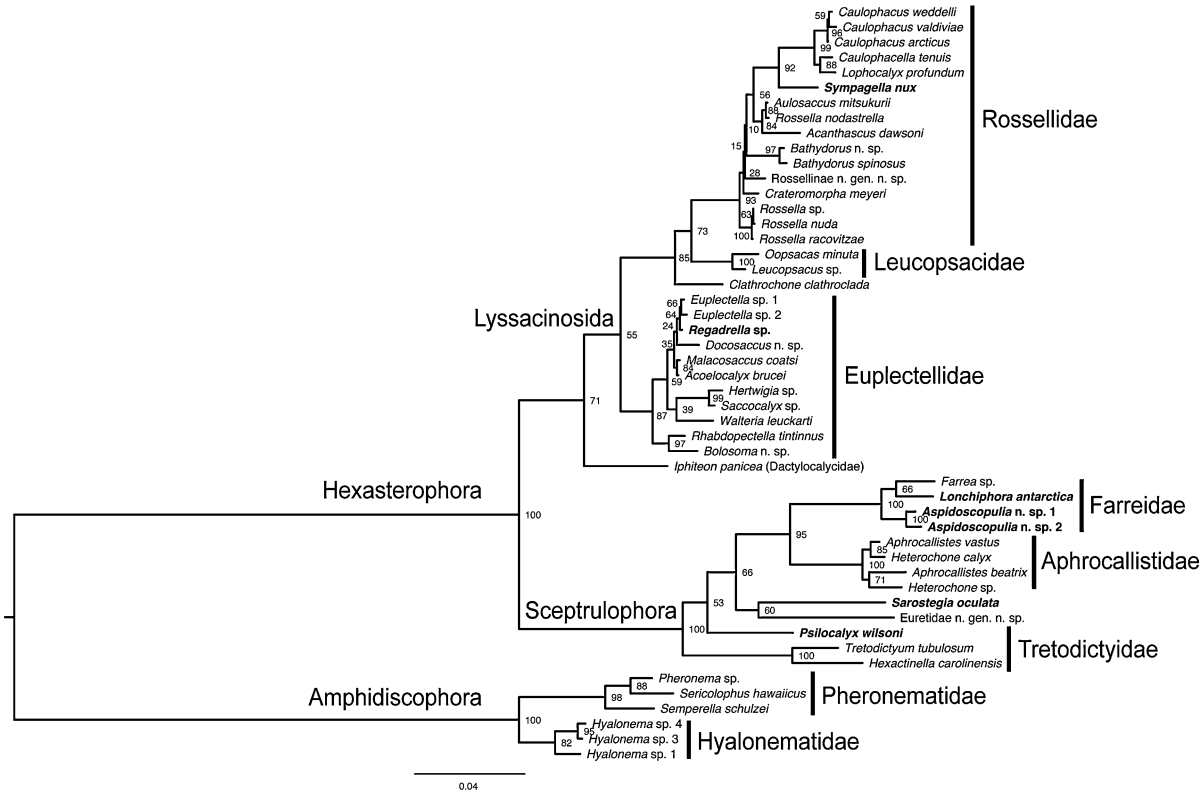

Fig. 3 Maximum likelihood phylogeny of Hexactinellida inferred from concatenated 18S, 28S, and 16S rDNA sequence alignments (RAxML; independent GTR+G_4 substitution models for 18S loops, 28S loops, and 16S; S16+G_4 paired-sites model for 18S+28S stems). Bootstrap support values (1000 replicates) given at nodes. Previously unsampled species highlighted in bold. Scale bar, expected number of substitutions per site. See "Materials and methods" section for further details

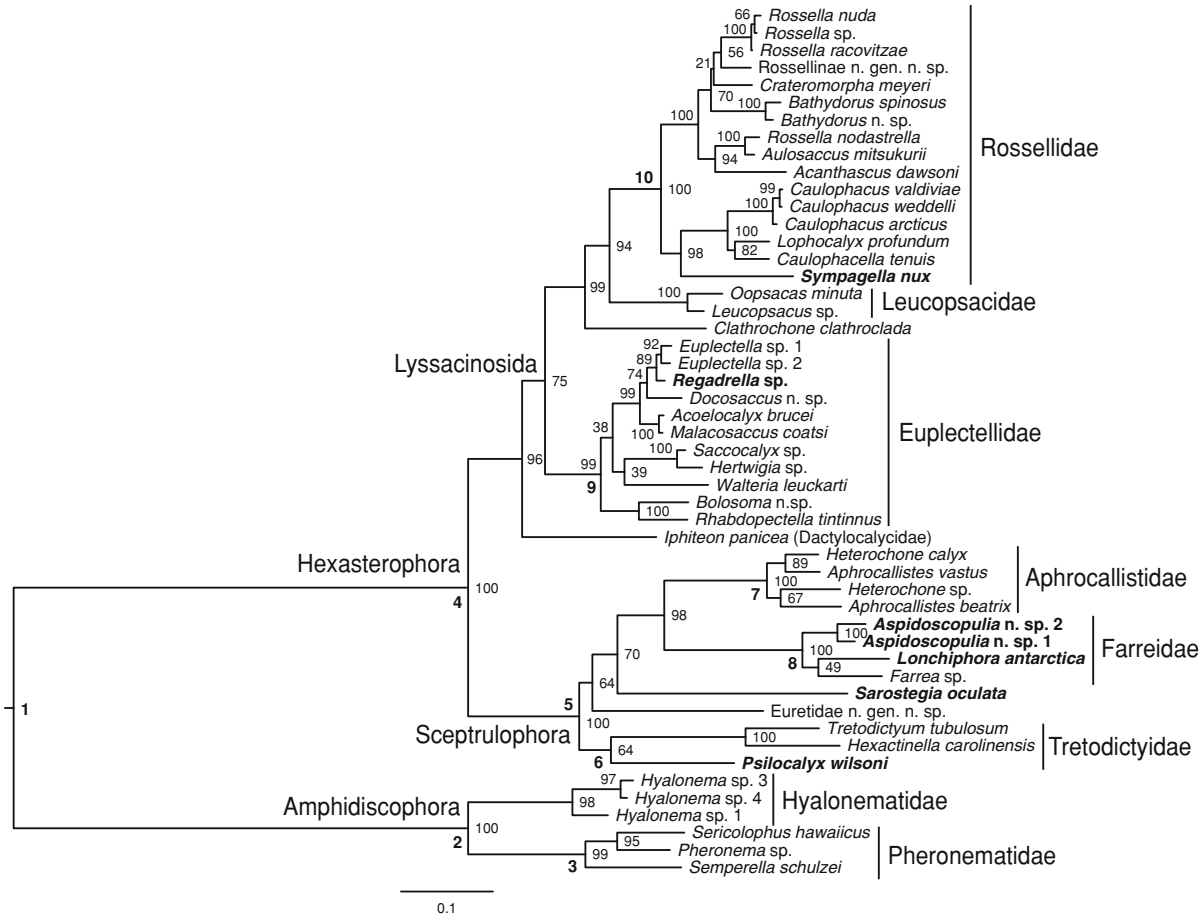

Fig. 4 Maximum likelihood phylogeny of Hexactinellida inferred from concatenated rDNA and COI DNA sequence alignments (RAxML; independent GTR+G_4 substitution models for 18S loops, 28S loops, 16S, and COI; S16+G_4 paired-sites model for 18S+28S stems). Bootstrap support values (1000 replicates) given at nodes. Previously unsampled species are highlighted in *bold*. Scale bar, expected number of substitutions per site. See "Materials and methods" section for further details. *Bold numbers* at nodes refer to the following putatively apomorphic morphological characters (for terminology, see Tabachnick & Reiswig, 2002). *1* triaxonic spicules, syncytial soft tissue, *2* amphidiscs, *3* sceptres, *4* hexasters, capability of spicule fusion, *5* sceptrules, euretoid dictyonal frameworks, *6* schizorhyses, bundled arrangement of dermal uncinates, *7* diarhyses, *8* clavules, farreoid dictyonal frameworks, *9* floricomes, *10* hypodermal pentactins

groups, and demonstrate the beneficial effect of supplementing rDNA evidence with COI sequence data.

Within order Lyssacinosida, we were able to resolve the phylogenetic position of *Clathrochone clathroclada* (see Dohrmann et al., 2009), as sister to Leucopsacidae+Rossellidae [note that this is also recovered in the rDNA tree (Fig. 3) with somewhat weaker support, but is strongly supported in the COI DNA tree (Fig. 1)]. This result rejects our earlier proposal that this species might belong to Leucopsacidae (Dohrmann et al., 2008), and corroborates the hypothesis that it represents an independent evolutionary lineage not belonging to any of the three described families of Lyssacinosida (Tabachnick, 2002a).

Within family Rossellidae, inclusion of *Sympagella nux* allowed us to test monophyly of subfamily Lanuginellinae, which was so far only represented by a single species, *Lophocalyx profundum*. Since Lanuginellinae is morphologically well defined by the presence of strobiloplumicomes (Tabachnick, 2002b), we expected that these two species would group together in the molecular phylogeny. Surprisingly, this hypothesis is not supported because *Sympagella* is resolved as sister to a *Caulophacus/*

Caulophacella/Lophocalyx clade. We consider the convergent evolution of strobiloplumicomes to be unlikely, and speculate that this spicule type was lost in *Caulophacus* and *Caulophacella*. Interestingly, a closer relationship of *Sympagella*, *Caulophacus*, and *Caulophacella* is consistent with earlier classification schemes of Rossellidae (see historical discussion in Tabachnick, 2002b; morphological characters supporting this grouping include the presence of a stalk and pinular hexactins or pentactins, with the latter also found among *Lophocalyx* spp.; however, these features are not unique to these genera). Thus, our results suggest that some abandoned taxonomic hypotheses have to be reconsidered.

Within family Euplectellidae, placement of *Regadrella* sp. (Corbitellinae) in a nested position within Euplectellinae (here: *Euplectella*, *Docosaccus*, *Acoelocalyx*, and *Malacosaccus*) again challenges monophyly of the latter subfamily (see Dohrmann et al., 2008, 2009). Thus, none of the three euplectellid subfamilies are currently supported by molecular data, which suggests that the features used to discriminate these taxa, namely the mode of attachment to the substrate (Tabachnick, 2002c), are highly plastic and of limited phylogenetic value (see also discussion in Dohrmann et al., 2009).

Conclusions

Given the technical difficulties we faced in generating COI sequence data from glass sponges, we consider it unlikely that this gene will play a major role in barcoding hexactinellids, since this approach to species identification relies heavily on easily applicable standard protocols that can be used in a high-throughput context (e.g., Ivanova et al., 2009). However, the additional sequence data proved a valuable supplement to rRNA genes for molecular phylogenetics, so the extra-effort that was required for most specimens certainly paid off. The good congruence between COI and rDNA phylogenies indicates that ribosomal RNA- and protein-coding genes harbor the same phylogenetic signal, thus increasing the reliability of molecular approaches for investigating organismal evolution of glass sponges. Combined analysis of the two data sets led to a more robust and resolved tree, providing a basis for further evolutionary studies such as reconstructing morphological character evolution or estimating divergence times. Finally, the increased taxon sampling of the present study provided some further hints as to where the current Linnean classification needs improvement; we are confident that continued addition of key taxa will ultimately help to resolve remaining taxonomic ambiguities, resulting in a system of Hexactinellida that is as natural as possible.

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SPONGE RESEARCH DEVELOPMENTS

Phylogenetic reconstruction of Polymastiidae (Demospongiae: Hadromerida) based on morphology

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Abstract Phylogeny of the sponge family Polymastiidae was reconstructed based on 25 morphological characters. Twenty-one polymastiid species and three suberitid species, *Suberites domuncula* as outgroup, *Aaptos aaptos* and *A. papillata* as sister groups, were included in the analyses. The reconstructions were done in PAUP* running heuristic search with the parsimony criterion. We analysed three possible evolutionary scenarios based on three alternative interpretations of the body plan of *Quasillina brevis* and *Ridleia oviformis*: first—*Ridleia* possesses aquiferous papillae whereas *Quasillina* lacks them, second—both genera lack papillae and third—the body in both genera is a single hyperdeveloped papilla. All three scenarios excluded the secondary loss of the papillae in the polymastiid evolution. Scenario 2 also excluded the secondary loss of the regular choanosomal skeleton, while scenario 1 assumed its loss in *Ridleia* and scenario 3 admitted its loss in both *Ridleia* and *Quasillina*. We prioritised scenario 2 due to its maximal parsimony and rescaled consistency index and subsequently favoured the clustering of *Ridleia* and *Quasillina* separately from the monophyletic polymastiid clade. In all three scenarios *Pseudotrachya hystrix* clustered separately from other polymastiids in agreement with the molecular evidence, and thus the exclusion of *Pseudotrachya* from Polymastiidae was proposed. The relationships between *A. papillata*, *Tentorium semisuberites*, *Polymastia uberrima*, the clade *Weberella bursa* + *Polymastia boletiformis* and the main polymastiid clade were ambiguous. Meanwhile, all scenarios showed the non-monophyly of *Polymastia* and *Aaptos*. Our hypotheses should be tested by reconstructions based on larger taxon sampling of hadromerid species and larger sets of morphological and molecular characters before any ultimate taxonomic decisions are taken.

Keywords Phylogeny · Morphology · Sponges · Polymastiidae

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Introduction

Polymastiidae Gray, 1867 is a well-known, worldwide distributed demosponge family (Boury-Esnault, 2002). Twenty-four nominal genera with 133 nominal species and additional 10 subspecies and varieties have been allocated to Polymastiidae, but only 118 species plus 4 subspecies belonging to 15 genera are currently recognised as valid (van Soest et al., 2010). Taxonomy based on spicule shape has always been difficult in the case of polymastiids since these sponges possess quite uniform, simple spicules, which are in most cases smooth monactines varying from tylostyles to styles. Thus, the definition of polymastiid genera has been mainly based on the presence of any modified (acanthose, ornamented, etc.) monactines/diactines or more rarely on their peculiar body shapes (Boury-Esnault, 2002). However, in recent decades a number of other morphological characters including the skeleton and aquiferous system architecture of the choanosome, cortex and papillae as well as the number of spicule size categories have been actively used to classify polymastiid taxa (Boury-Esnault, 1987, 2002; Boury-Esnault et al., 1994; Kelly-Borges & Bergquist, 1997; Morrow & Boury-Esnault, 2000; Plotkin, 2004; Plotkin & Boury-Esnault, 2004; Boury-Esnault & Bézac, 2007; Plotkin & Janussen, 2008).

Despite the appearance of this useful morphological approach in the discriminating between the polymastiid taxa, almost 63% of polymastiid species are currently considered to belong to the genus *Polymastia* Bowerbank, 1864a (van Soest et al., 2010), which is in fact distinguished from other polymastiid genera exclusively by its lack of any unique features (Boury-Esnault, 1987, 2002). The evident essential differences between the type species of *Polymastia*, *P. mamillaris* (Müller, 1806), and a number of other *Polymastia* spp. are often ignored, and there is a practice to allocate the species that lack any diagnostic features of other polymastiid genera or possess combinations of the features of different genera to *Polymastia* without any proper argumentation. According to Boury-Esnault (2002) *Polymastia* always has papillae, its principal spicules are arranged in radial tracts, the ectosomal skeleton is composed of at least two layers—the superficial palisade of small tylostyles and the lower layer of intermediary spicules oriented tangential to the surface, the ectosomal spicules are always tylostyles. However, *P. boletiformis* (Lamarck, 1815), *P. zitteli* (von Lendenfeld, 1888) and *P. croceus* Kelly-Borges & Bergquist, 1997 have a reticulated arrangement of principal spicules that is similar to the skeleton of *Weberella* Vosmaer, 1885 (Plotkin & Janussen, 2008). Additionally, *P. boletiformis* has no intermediary spicules since the tylostyles constituting its inner cortical layer are of the same category as the principal choanosomal spicules (Boury-Esnault, 1987), which is again a feature of *Weberella*. *Polymastia invaginata* Kirkpatrick, 1907 lacks the lower ectosomal layer (Plotkin & Janussen, 2008). In *P. grimaldii* (Topsent, 1913) the cortical skeleton from the upper part is different from that of the lower part, which is a characteristic feature of *Radiella* Schmidt, 1870 (Plotkin, 2004). *P. tapetum* Kelly-Borges & Bergquist, 1997 and *P. umbraculum* Kelly-Borges & Bergquist, 1997 possess exotyles with umbrelliform distal extremities in addition to the usual tylostyles in the ectosome that are typical of *Proteleia* Dendy & Ridley, 1886. Furthermore, the choanosomal skeleton of *P. umbraculum* is reticulated (the feature of *Weberella* as stated above) and its body lacks papillae (that resembles suberitid species). There are many other examples of discrepancy, and such a practice has obviously made *Polymastia* a taxonomic dump.

Taxonomic problems exist in other polymastiid genera as well. Spherical distal knobs on the exotyles constitute the diagnostic character of *Sphaerotylus* Topsent, 1898 (Boury-Esnault, 2002). However, *S. borealis* (Swarczewsky, 1906) and *S. antarcticus* Kirkpatrick, 1907 have exotyles with umberilliform extremities, which make them similar to *Proteleia*, although the latter genus is distinguished by the additional cortical palisade (Koltun, 1966; Boury-Esnault, 2002; Plotkin, 2004). Another polymastiid genus with ornamented exotyles, *Tylexocladus* Topsent, 1898, shares the casual presence of centrotylote microxeas with *Atergia* Stephens, 1915 but the latter never possesses any exotyles (Boury-Esnault, 2002).

Finally there is still uncertainty about the relationships between Polymastiidae and the allied family Suberitidae Schmidt, 1870. The suberitid genus *Aaptos* Gray, 1867 possesses in fact the two main diagnostic features of Polymastiidae, the radial choanosomal skeleton and the ectosomal palisade of

small tylostyles. Moreover, *A. papillata* (Keller, 1880) has papillae, which was the reason for this suberitid being misidentified as a new polymastiid species, *Polymastia gleneni* Descatoire, 1966. Radial choanosomal skeletons and small papillae are also recorded in some species of *Suberites* Nardo, 1833, for example in *S. incrustans* Hansen, 1885, *S. caminatus* Ridley & Dendy, 1886 and *S. microstomus* Ridley & Dendy, 1887, despite the fact that, according to the generally accepted diagnosis of this genus, its choanosomal skeleton should be confused or alveolar, and most other *Suberites* spp. lack papillae (van Soest, 2002). It was for this reason that Topsent (1917) allocated *S. caminatus* var. *papillata* Kirkpatrick, 1908 to the polymastiid genus *Tentorium* Vosmaer, 1887. At the same time *Quasillina* Norman, 1869 with its confused choanosomal skeleton and *Ridleia* Dendy, 1888 with its choanosomal skeleton restricted to the tangentially arranged subcortical small tylostyles are both allocated to Polymastiidae by most authors (Topsent, 1898; Boury-Esnault et al., 1994; Boury-Esnault, 2002), although Dendy (1888) suggested that these genera were "the connecting link" between Suberitidae and Polymastiidae.

Evidently, a phylogenetic approach seems to be the only way to solve the taxonomic problems in Polymastiidae; but such methods have been never applied to this family. The aim of the present study was to summarise our knowledge of polymastiid morphology and to reconstruct the phylogeny of Polymastiidae based on these data.

Materials and methods

Selection of taxa

Our reconstruction was predominantly based on the type species of all currently accepted polymastiid genera including *Suberitechinus* de Laubenfels, 1949 but excluding *Trachyteleia* Topsent, 1928. These two monotypic genera were believed to be synonyms, *Trachyteleia* being subsequently considered as the senior name (Boury-Esnault, 2002). However, our re-examination of the type material revealed the differences between *Trachyteleia* and *Suberitechinus* and confirmed their validity. Insufficient data on *Trachyteleia* kept us from considering it in the analyses, whereas the ample data on *Suberitechinus* made it possible to involve the type species in our study (see also "Examined material" section for details).

Due to the considerable heterogeneity and complexity of *Polymastia* we found it reasonable to involve five species of this genus in addition to its type species *P. mamillaris* into our analyses. The species were chosen so that they represented well the morphological diversity of *Polymastia*.

For the similar reasons of heterogeneity and complexity of *Radiella* we considered its two species, *R. hemisphaerica* (Sars, 1872) and *R. sarsi* (Ridley & Dendy, 1886) in our study. The type species *R. sol* (Schmidt, 1870) could not be analysed due to the controversial data on it. On one hand the drawing by Schmidt resembled *R. sarsi*, his description being rather brief. On the other hand the re-description of *R. sol* by Boury-Esnault (2002) based on the assumed holotype considerably differed from Schmidt's drawing and resembled *R. hemisphaerica* (see Plotkin & Janussen, 2008 about the details).

Two suberitid species were involved in the analyses. The type species of *Suberites*, *S. domuncula* (Olivi, 1792) was used as outgroup and two species of *Aaptos*, *A. aaptos* (Schmidt, 1864) and *Aaptos papillata* (Keller, 1880), were enrolled as sister groups.

Thus, altogether 24 species including 21 polymastiid species were involved in our reconstruction (Figs. 1, 2).

Examined material

Among the species treated in the analyses twelve species were studied from both type and comparative material, five species were studied only from the type material and seven species were studied only from the comparative material (Online Resource 1). Data from additional literature sources were taken into account in all cases as well. Two cases require detailed explanation.

The comparison of *Suberitechinus hispidus* (Bowerbank, 1864b) with *Trachyteleia stephensi* Topsent, 1928 based on the type material revealed that all spicules including the exotyles in the former species were uniformly smooth and much longer than the spicules of the respective categories in the latter species with its exotyles being finely spined on their distal parts. These spines were actually considered as the main feature distinguishing *Trachyteleia* from other polymastiid genera (Boury-Esnault, 2002). A