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Horny sponges and their affairs: On the phylogenetic relationships of keratose sponges

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ABSTRACT

The demosponge orders Dictyoceratida and Dendroceratida are historically assigned to the keratose (or "horny") sponges, which are mostly devoid of primary skeletal elements, but possess an elaborate skeleton of organic fibres instead. This paucity of complex mineral skeletal elements makes their unambiguous classification and phylogenetic reconstruction based on morphological features difficult. Here we present the most comprehensive molecular phylogeny to date for the Dendroceratida, Dictyoceratida, and also other sponge orders that largely lack a mineral skeleton or skeletal elements at all (i.e. Verongida, Halisarcida, Chondrosida), based on independent mitochondrial and nuclear markers. We used molecular data to validate the coherence of all recognised orders, families and subfamilies that are currently defined using morphological characteristics. We discussed the significance of morphological and chemotaxonomic characters for keratose sponges, and suggested adapted definitions for the classification of dendroceratid, dictyoceratid, and verongid higher taxa. Also, we found that chondrosid sponges are non-monophyletic with respect to Halisarcida. Verongida and Dendroceratida were monophyletic, however most of their classically recognised families were not recovered. This indicated that the current distinction between dendritic and mesh-like fibre skeletons is not significant at this level of classification. Dysideidae were found to be the sister-group to the remaining Dictyoceratida. Irciniidae formed a distinct clade, however Thorectidae and Spongiidae could not be separated with the molecular markers used. Finally, we are establishing the name Verongimorpha for the clade combining verongid, chondrosid and halisarcid taxa and readjust the content of its sister-clade Keratosa.

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1. Introduction

Sponges (Phylum Porifera) are a group of sessile benthic organisms, important filter feeders in almost all aquatic ecosystems (see e.g., Vacelet, 1979) and were the dominant reef builders in the Cambrian (see e.g., Wood, 1999). Approximately 8300 recent species have been described, of which Demospongiae comprise about 90% and are therefore by far the largest and most successful group of extant sponge clades (see Van Soest et al., 2011). (Demo)sponges are also among the most challenging Metazoa in regards to understanding evolutionary relationships (see e.g., Boury-Esnault, 2006). The skeleton provides the most important morphological characters to identify and classify sponge groups. In most demosponges the skeleton consists of mineral elements such as primary siliceous spicules, a secondary calcified basal skeleton, or a combination thereof. However, skeletal complexity and phylogenetic information content are limited and plagued by secondary losses or homoplasies (e.g., Erpenbeck et al., 2006).

Particularly difficult sponge taxa to characterise based on morphological characters are the "horny" or "keratose" demosponges, which mostly lack a mineral skeleton but possess skeletal structures of organic collagenous material, or spongin. The commonly known bath sponge *Spongia officinalis* Linnaeus belongs to this important group and is the nominal archetype of all Porifera. In addition to this traditional economic function, several keratose sponges have received particular attention on account of their

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bioactive secondary compounds (see e.g., Faulkner, 1998 and subsequent publications of this series). Nevertheless, the lack of mineral components increases the difficulties for classification and phylogenetic reconstruction for these sponges.

Historically, horny sponges formed the "Keratosa" (Grant, 1861). After changes in their classification (Minchin, 1900; von Lendenfeld, 1889) this group was elevated to the ordinal level (Burton, 1934; De Laubenfels, 1936, 1948; Vacelet, 1959). Analyses on amino acids divided the Keratosa into two different orders Dictyoceratida and Dendroceratida (Bergquist and Hartman, 1969), from which two additional orders Verongida (Bergquist, 1978) and Halisarcida (Bergquist, 1996) were separated. The latter order, devoid of any skeletal elements, received ordinal status due to its uncertain relationship to any other sponge taxon. Recent data based on ribosomal genes (Borchiellini et al., 2004) and complete mitochondrial genomes (Lavrov et al., 2008) provided the first molecular evidence that Dictyoceratida, Dendroceratida, Verongida and Halisarcida are closely related in a sister group to all other, predominantly mineral skeleton bearing, demosponges. Dictyoceratida + Dendroceratida form a "Keratosa" clade (initially termed "G1", Borchiellini et al., 2004), which is sister group to Verongida + Halisarcida and also Order Chondrosida. Chondrosida is a taxon of predominantly, but not exclusively spicule-lacking sponges. Some chondrosid taxa possess asterose spicules which led to a presumed affinity with hadromerid or tetractinellid families (see Boury-Esnault (2002) for details). This Verongida + Halisarcida + Chondrosida clade was subsequently termed "Myxospongiae" (Borchiellini et al., 2004), and is morphologically supported by their cellular ultrastructure (see Maldonado, 2009). Additionally, a striking similarity in the fertilisation process and embryo development was discovered between members of Verongida (i.e., *Aplysina*) and Chondrosida (i.e., *Chondrosia*), which led Maldonado (2009) to formally erect the Verongida + Halisarcida + Chondrosida to the subclass "Myxospongia". Authorship for the higher taxa as presently interpreted, Keratosa Bowerbank, 1864, and "Myxospongia" (sensu Borchiellini et al., 2004; Maldonado, 2009) as Verongida + Chondrosida + Halisarcida, for which taxonomic concepts have been supported by independent molecular datasets (Borchiellini et al., 2004; Lavrov et al., 2008), need to be firmly established.

The monophyly of the keratose sponge orders as defined in the Systema Porifera (Hooper and Van Soest, 2002) has never been verified in phylogenetic analyses. Features overlap among several orders, e.g., in the reticulation of dictyoceratid and the dendroceratid skeletons (Dictyodendrillidae) (see also on the position of Dysideidae, Vacelet et al., 1989). Combinations and alternative characters were used to re-define taxa, including biochemical compounds and choanocyte chamber form (Bergquist, 1980).

However, the robustness of these biochemical and cytological characters has never been confirmed and character state overlaps are present in the current classification: For example eurypylous *and* diplodal choanocyte chambers are present in genera of Verongida and Dictyoceratida. Furthermore, families may overlap superficially in their chemistry e.g., the diterpenoids of Dysideidae (Dictyoceratida) and Darwinellidae (Dendroceratida). Additionally biochemical characters suffer from sample bias because only a limited number of species are checked for metabolites and the homologisation of their biosynthetic pathways is often ambiguous (see also Erpenbeck and Van Soest, 2007; Van Soest and Braekman, 1999).

Consequently, a comprehensive phylogenetic analysis of these economically important keratose sponges, is needed to provide better taxonomic certainty in classification and clearer understanding of demosponge skeletal character evolution. Here we reconstruct mitochondrial and nuclear ribosomal gene trees for the largest set of keratose and myxospongid species to date. We incorporate a wide range of taxa in order to reconstruct the phylogenetic relationships and to gain insight into the evolution and taxonomic relevance of sponge morphological characters.

2. Material and methods

DNA was extracted from sponge tissue of the Porifera collection of the Queensland Museum, (Brisbane, Australia), from the Porifera collection of the Zoological Museum Amsterdam and Naturalis Leiden, the Bavarian State collection for Palaeontology and Geology Munich and from the collection of Steve de C. Cook of which many samples were used as reference specimens for the keratose sponge chapters of the Systema Porifera (Hooper and Van Soest, 2002).

Fragments of the mitochondrial cytochrome oxidase subunit I (CO1) were amplified using a twofold-degenerated version of the universal barcoding primers: dgLCO1490 (GGT CAA CAA ATC ATA AAG AYA TYG G) and dgHCO2198 (TAA ACT TCAG GGT GAC CAA ARA AYC A) (Meyer et al., 2005). PCR primers employed for the 28S rDNA fragment were (RD3A: GACCCGTCTTGAAACACGA and RD5B2: ACACACTCCTTAGCGGA (McCormack et al., 2002). Both fragments were amplified under the following temperature regime: 94 °C 2 min, 35 cycles at 94 °C 30 s; 45 °C 20 s; 65 °C 60 s, followed by 72 °C 10 min. PCR reactions contained 11.25 μl ddH_2O, 4.15 μl dNTP (10 mM), 3.25 μl $MgCl_2$ (25 mM), 2.5 μl 10× HotMaster PCR Buffer, 2.5 μl BSA (100 mM, Sigma), 0.5 μl primer (10 mM) and 2u HotMaster polymerase (Eppendorf).

Fragments were sequenced on an ABI 3730 capillary sequencer of the Genomic Sequencing Units of the Griffith University (Nathan, Australia) and the LMU Munich. Forward and reverse sequences were assembled with CodonCodeAligner (www.codoncode.com) and checked for potential contamination against Genbank (www.ncbi.nlm.nih.gov). For the phylogenetic analyses, the taxon sets were extended by additional sequences from Genbank. Due to its protein coding nature, alignment of CO1 sequences has been unambiguous. The alignment of the 28S ribosomal data set were analysed under secondary structure specific models (as suggested in the recent literature (Hudelot et al., 2003) and subsequently successfully applied for sponges (Erpenbeck et al., 2007)). 28S rDNA sequences were aligned in SEAVIEW (Galtier et al., 1996) following published secondary structure models (e.g., Schnare et al., 1996) and the alignments of the Sponge Genetree Server (Erpenbeck et al., 2008). Non-alignable regions were omitted from the analyses.

Bayesian analyses on nucleotide sequences were run with parallel version of MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) on the 64-node Linux cluster of the Molecular Geo- and Palaeobiological Labs, LMU Munich with one processor assigned to each Markov chain. Each Bayesian analysis comprised at least two simultaneous runs of eight Metropolis-coupled Markov-chains at the default temperature (0.2) under the most generalising model (GTR + G + I) because it has been reported that overparametrisation does not negatively affect Bayesian analyses (Huelsenbeck and Ranala, 2004). Analyses were terminated after the chains converged significantly, as indicated by the average standard deviation of split frequencies <0.01. Maximum-likelihood reconstructions were inferred with RAxML 7.2.5 (Stamatakis, 2006) under the GTRGAMMA model of nucleotide substitution for unpaired sites respectively, the S16 model for paired sites under 100 fast bootstrap replicates.

3. Results and discussion

3.1. Phylogenetic signal of the selected markers

The mitochondrial cytochrome oxidase subunit 1 (COI) and the large nuclear ribosomal subunit (28S) data set comprised 86 and 141 taxa and resulted in data sets of 575 and 698 characters respectively. CO1 and 28S are markers of different genomes and

therefore evolve independently. Consequently, congruence in 28S and CO1 topologies indicate strong support for a given clade. Fig. 1 displays the summary cladogram of the gene trees reconstructed from the diverse reconstruction methods, and all underlying gene trees are provided in the Supplementary figures.

In the past, the congruent patterns of mitochondrial and ribosomal data (Borchiellini et al., 2004; Lavrov et al., 2008) led to increased confidence in demosponge molecular phylogenies. In this study, relationships from subclass to genus level are investigated using CO1 and 28S. Consequently, although a broad congruence between 28S and CO1 is observed, we notice that in some cases both genes do not provide the same level of support for individual clades. In almost every case this is due to the lack of resolution for a clade resulting in contradictory supported topologies. This lack of resolution affected the assessment of the phylogenetic position of Spongiidae and Thorectidae (see below and branch length in the Supplementary data).

Every marker also has evolutionary peculiarities (see e.g. Lavrov et al., 2008 for CO1). The 28S rDNA marker applied in this study consists of a very variable 5′ part and a more conserved 3′ end. For the verongid *Hexadella* only the conservative 3′ part could be used, which apparently led to reconstruction artefacts during assessment of its subsequent phylogenetic position. Likewise, the complete 28S fragment displayed too long branches in other ianthellid taxa (see Supplementary data), which lead to artifactual branching patterns (see e.g. Bergsten, 2005).

Nevertheless, when 28S was not available, support for CO1 could be retrieved in previous analyses on 18SrDNA, or on the intergenic transcribed spacers (ITSs), which are part of the nuclear ribosomal cistron as is 28S. Therefore our conclusions are drawn in light of the individual shortcomings of the markers. Consequently, Fig. 1 and our discussion should (as every phylogenetic tree) be regarded as phylogenetic hypothesis only, particularly where only a single gene contributes to the topology.

3.2. Phylogenetic implications

Previous analyses of rDNA and complete mtDNA indicated that Dictyoceratida, Dendroceratida, Halisarcida, Verongida and Chondrosida form a monophyletic group (Borchiellini et al., 2004; Lavrov et al., 2008). While other demosponge orders may also contain spicule-deficient taxa, probably as a result of secondary loss (e.g., Haplosclerida: *Dactylia*), the absence of mineral skeletal material can be regarded as a dominant feature in these five orders. The G1 clade "Keratosa" (sensu Borchiellini et al., 2004, *i.e.* Dendroceratida and Dictyoceratida) is a sister-group to the G2 clade "Myxospongia" (Verongida, Chondrosida and Halisarcida), as demonstrated by mitochondrial and nuclear data (Borchiellini et al., 2004; Lavrov et al., 2008).

3.2.1. Verongimorpha subclass. nov.

The name "Myxospongiae" is wrongly attributed to Zittel (Zittel, 1878, refers to Haeckel), despite being introduced by Haeckel (Haeckel, 1866). More importantly, it remains unclear why the name "Myxospongiae" has been revived for such an assemblage of orders, different to the original group of Haeckel, 1866. Myxospongiae literally means 'slime sponges', and was originally intended for sponges like *Halisarca*. Later, other genera such as *Oscarella* (the latter now Class Homoscleromorpha) were added. Consequently, Myxospongiae describes a polyphyletic assemblage (see also Maldonado, 2009). Furthermore, the name "Myxospongiae" is now justified only for one genus (*Halisarca*) and clearly not representative for Verongida + Chondrosida. Although higher taxa names are 'free' from ruling of the ICZN rules, we believe that common sense dictates that an assemblage with a radically different content, as present here, should get a new and informative name, because neither Verongida nor Chondrosida were part of any Myxospongiae or resemble 'slime sponges'. We therefore propose the name Verongimorpha for the clade combining Verongida + Chondrosida + Halisarcida. The name Verongimorpha is derived from the taxon with the largest set of families and genera (Verongida) while its suffix (*-morpha*) classically indicates a taxon equivalent to subclass level in sponges and simultaneously avoids confusion with polyphyletic taxon concepts.

The Verongimorpha comprise sponge taxa of heterogeneous morphologies as they may possess spicules, spongin skeleton only, or no skeleton at all. Synapomorphies are found in the orientation of the accessory centriole, the nuclear apex, and the Golgi apparatus as observed in the ultrastructure of epithelial and larval cells, furthermore in embryo development similarities (Maldonado, 2009). In complete mitochondrial and 18SrDNA gene trees of Verongimorpha, Verongida are sister to a clade comprising Halisarcida and chondrosid taxa (Lavrov et al., 2008).

3.2.1.1. Halisarcida and chondrosids. Halisarcida (represented by its only genus *Halisarca*) lack a mineral or fibrous skeleton but possess a highly organised ectosomal and subectosomal collagen and have tubular and branched choanocyte chambers (Bergquist and Cook, 2002h). Two of the four chondrosid genera, do not possess a skeleton (*Chondrosia* and *Thymosiopsis*). In contrast, for the two remaining genera, *Thymosia* possesses a spongin skeleton, and *Chondrilla* has siliceous skeleton elements. All chondrosid genera are defined by their marked cortex of fibrillar collagen and inhalant apertures in pore-sieves or cribriporal chones (Boury-Esnault, 2002). The complex collagen structure could provide a synapomorphic feature combining *Halisarca* with chondrosid genera. Nevertheless, the monophyly of the order Chondrosida could not be corroborated by molecular data (Erpenbeck and Wörheide, 2007). Gene trees of both ribosomal (18S and 28S) and mitochondrial markers (CO1) resolved *Chondrosia*, the nominal genus of Chondrosida, distant from *Chondrilla, Thymosiopsis* and *Thymosia*, which formed a clade with *Halisarca*. In 18S rDNA phylogenies Chondrosida and Halisarcida formed a clade, with *Chondrosia* as sister to the other chondrosids and *Halisarca* (see also www.spongegenetrees.org, Erpenbeck et al., 2008). Our CO1 data suggested an even earlier split in congruence with the B9–B21 region of 28S (see www.spongegenetrees.org Erpenbeck et al., 2008). However, as the resolution power of CO1 for such deep splits yet has to be shown (Lavrov et al., 2008) and 18S and 28S result in different topologies, we refrain from suggestions for new classifications (see also Ereskovsky et al., 2011).

3.2.1.2. Verongida. Verongida formed a distinct taxon in the Verongimorpha. Verongid sponges generally possess spongin fibres with a well-laminated bark, and a dark, cellular pith (except for the skeleton lacking genus *Hexadella*). Their fibres can be dendritic as well as polygonal-anastomosing but lack a hierarchic organisation (Bergquist and Cook, 2002i). Besides the morphological characters, the production of bromotyrosine derivatives is discussed as a distinctive feature of verongid taxa (see also Erpenbeck and Van Soest, 2007).

Morphologically, Verongida were classified into four families primarily based on choanocyte chamber shape (eurypylous vs. diplodal) and branching pattern of the spongin skeleton (anastomose vs. dendritic): Ianthellidae Hyatt, 1875: Eurypylous + anastomose; Aplysinidae Carter, 1875: Diplodal + anastomose; Aplysinellidae Bergquist, 1980: Diplodal + dendritic with dominant bark; Pseudoceratinidae Carter, 1885 Diplodal + dendritic with dominant pith (Bergquist and Cook, 2002a,b,e,f).

In our reconstructions, only the CO1 tree recovered monophyletic Verongida, while the 28S gene tree placed Ianthellidae in an equivocal position at the base of the tree. Here, the 28S data might

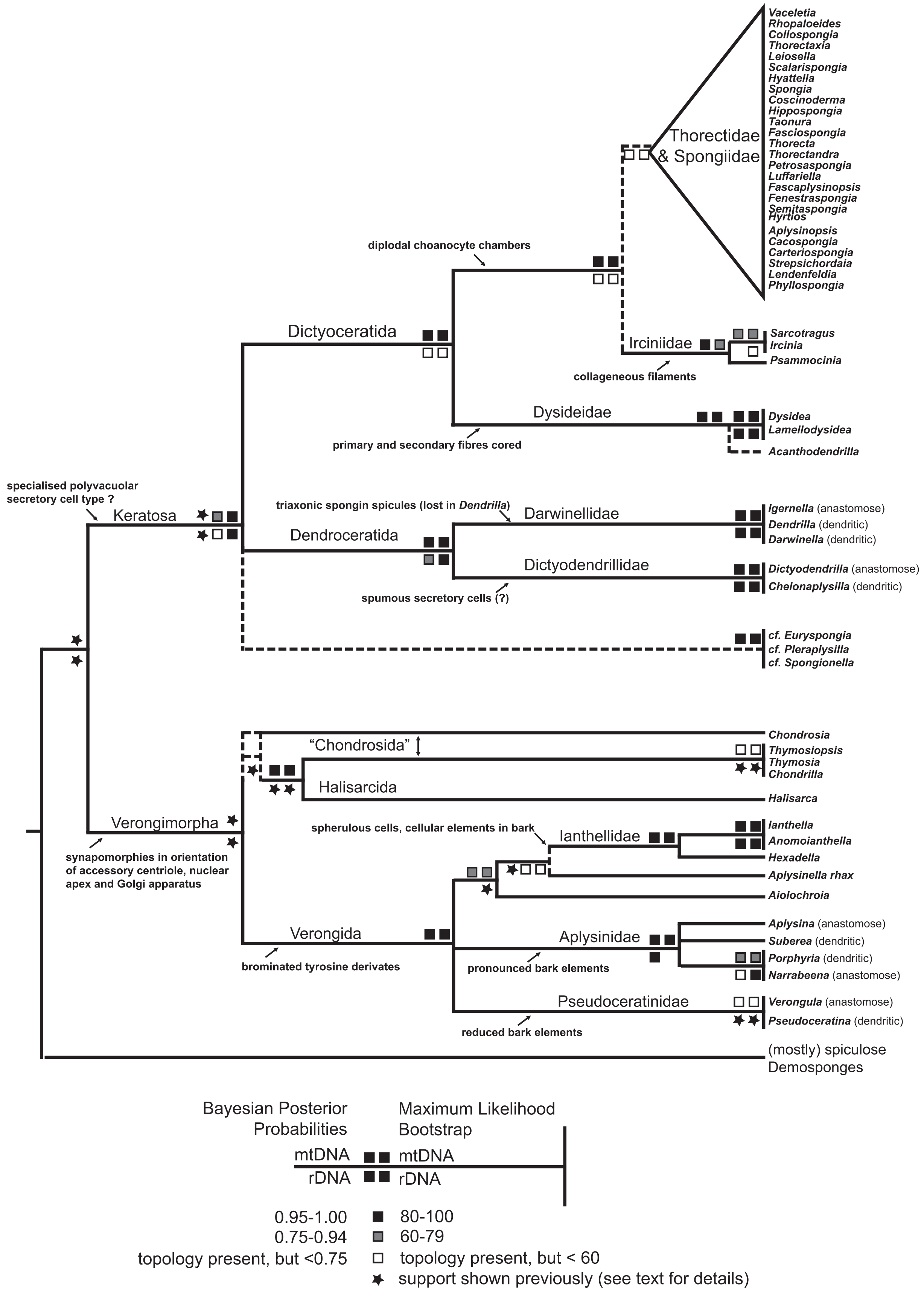

Fig. 1. Schematic figure of the phylogenetic relationships for Keratosa and Verongimorpha as evident from the 28S and CO1 fragment, and different phylogenetic reconstruction methods (see Supplementary figures). The relative filling of the boxes indicates the level of support; a star indicates support demonstrated in earlier publications (see text for details).

be biased by as *Ianthella* and *Anomoianthella* display long branches in 28S (see Supplementary data) and *Hexadella* is only represented by shorter sequences derived from Genbank (Reveillaud et al., 2010).

The four families were not recovered in molecular phylogenies. CO1 and 28S data resulted in a clade comprising Ianthellidae, and *Aiolochroia* corroborating earlier hypotheses based on a different nuclear marker (ITS, Erwin and Thacker, 2007). In Erwin and Thacker's reconstructions, this clade forms a sister-group to all other verongids. In our CO1 data set this branching pattern only resulted under non-probabilistic reconstruction methods (e.g. distance methods or parsimony, not shown). The remaining families, all with diplodal choanocyte chambers, were not recovered as monophyletic in nuclear and CO1 gene trees (with exception of the monogeneric Pseudoceratinidae). Instead we retrieved two distinct clades (*Aplysina* + *Suberea* + *Porphyria* + *Narrabeena* and *Pseudoceratina* + *Verongula*) in which the original distinction of the families based on dendritic vs. anastomosing spongin skeletons was not upheld for either clade. Although the branching order of the three clades was not recovered, nuclear and mitochondrial data recovered the following clades:

3.2.1.2.1. Ianthellidae + Aiolochroia. Ianthellidae *s.s.* (i.e. sensu Bergquist and Cook, 2002e) can be recovered by CO1 only due to long branches and only partial sequences in 28S (as mentioned above). *Ianthella*, represented by several species (including its type species *I. flabelliformis*) and *Anomoianthella* (represented among others by its type species *A. popae*) formed a clade with *Hexadella* (represented by the type species *H. racovitzai*) as a sister group. Ianthellidae were distinguished among the Verongida by the possession of eurypylous choanocyte chambers, typical spherulous secretory cells and, if present, a strongly anastomosing fibre skeleton in which bark incorporates cellular elements in contrast to all other verongids (Bergquist and Cook, 2002e).

Previous analyses based on ITS2-28S found *Aplysinella rhax* closely related to ianthellids (Erwin and Thacker, 2007). In our 28S tree *Aplysinella rhax* grouped closely with *Hexadella*, however, and CO1 data is lacking to draw further conclusions.

Aiolochroia is a monotypic genus, classified as *Aplysina incertae sedis* after removal from the Pseudoceratinidae (Bergquist and Cook, 2002b). Our CO1 data now supports previous nuclear data (Erwin and Thacker, 2007) in that *Aiolochroia* appears in fact closely related to Ianthellidae.

3.2.1.2.2. Aplysina + Suberea + Porphyria + Narrabeena. Aplysinidae, described as a cohesive group and sharply distinct from other fibrous sponges (Bergquist and Cook, 2002b) is paraphyletic in all molecular analyses, which again corroborates previous findings (Erwin and Thacker, 2007) using an independent marker. While the 28S data is occasionally hampered by the partial *Hexadella* sequence (discussed above), CO1 analyses resulted in a large clade that included Aplysinidae (represented by its type species *Aplysina aerophoba* plus several additional species, published in Genbank) and several species and genera of Aplysinellidae such as *Porphyria* (represented by its type species *P. flintae*) and *Suberea clavata*. The molecular differences between *Aplysina*, *Porphyria* and *Suberea* are very small in comparison to other verongid taxa, so that internal relationships between these groups are not resolvable.

Suberea was erected by Bergquist, 1995 for Aplysinellidae with dominant pith elements but bark still present. Unfortunately, it was not possible to PCR-amplify COI and 28S from the type species of *Suberea*, *S. creba*. Morphologically, *S. creba* differs from *Suberea clavata* by its spreading habit, smooth surface and fibre structure (Bergquist, 1995).

Additionally, our CO1 and 28S reconstructions resolved *Narrabeena* (monotypic, *N. lamellata*), which is currently classified to the dictyoceratid family Thorectidae, with the verongids. *Narrabeena* had been erected for *Smenospongia lamellata*, which possesses fibres with a high amount of pith, whereas *S. aurea*, the type species, only possesses traces of pith. *Smenospongia* has been regarded as the "point of closest similarity between Verongida and Dictyoceratida" (Bergquist, 1980), but despite its verongid morphology, *Narrabeena* was previously placed into Dictyoceratida due to chemotaxonomic considerations, as dictyoceratids do not produce brominated tyrosine derivatives, typical of verongids. Our results recovered *Narrabeena* as the only verongid species without brominated tyrosine derivates, suggesting a secondary loss of the ability to produce this metabolite.

The differences in skeletal architecture between the taxa of this clade indicate that skeletal architecture cannot be used as synapomorphies: *Aplysina* and *Narrabeena* are anastomose, while *Suberea* and *Porphyria* are dendritic. A possible shared derived character could be the pronounced nature of the bark elements, unlike Pseudoceratinidae and *Verongula* (see below), but this will require more thorough morphological analyses of a greater range of taxa than examined here.

3.2.1.2.3. Pseudoceratinidae + Verongula. The position of Pseudoceratinidae in our molecular analyses also supports the paraphyly of Aplysinidae. Its nominal genus *Pseudoceratina* (represented by its type species *P. durissima* and several additional species) forms a clade with *Verongula* (Aplysinidae, represented by *V. rigida* and *V. gigantea*) in CO1, and is congruent with previous findings on independent nuclear markers (Erwin and Thacker, 2007) (No 28S data could be obtained for *Verongula*). Both genera have substantial differences in their skeletal arrangement: *Verongula* is anastomose, while *Pseudoceratina* is dendritic. This again indicates that skeletal architecture cannot be used as synapomorphic characters (see also the *Aplysina* + *Suberea* + *Porphyria* + *Narrabeena* paragraph above). Additionally, the skeleton of *Verongula* is described as very similar to *Aplysina* (i.e. a reticulum with polygonal meshes), from which it can be distinguished by a honeycomb-like surface structure, but a close relationship between both genera is contradicted by the molecular analyses. A possible synapomorphy could be the reduction of skeletal fibre bark elements, unlike in most other Verongida with diplodal choanocyte chambers (see above), but this will require more thorough morphological studies.

3.2.2. Keratosa

The Keratosa formed a monophyletic group – corresponding to subclass level. Our reconstructions (COI and 28S) resolved Dictyoceratida and Dendroceratida as clades plus *Spongionella pulchella*, *Pleraplysilla spinifera* and a *Euryspongia* sp. with yet uncertain affinities. A potential distinguishing character to Verongimorpha might be the presence of a specialised polyvacuolar secretory cell type, described in the dendroceratid *Aplysilla* (see e.g., Schneider, 1902) and subsequently detected in the dictyoceratid *Dysidea*, but not in the verongimorphs *Chondrosia*, *Chondrilla*, *Halisarca* or *Aplysina* or in other sponges (Michael Nickel, pers. comment).

3.2.2.1. Dendroceratida. In our analyses, dendroceratid sponges formed a well-supported monophyletic group with the exception of *Spongionella* (represented by its type species *S. pulchella*) and *Acanthodendrilla* (represented by the holotype of its type species *A. australis*). All Dendroceratida have eurypylous choanocyte chambers and a more reduced fibre skeleton, arising from a basal plate into a dendritic, or likewise anastomosing skeleton (Bergquist and Cook, 2002g). Such dendritic skeletal arrangement defined the family Darwinellidae Merejkowsky, 1879, while Dictyodendrillidae Bergquist, 1980, the second family of this order, is characterised by a reticulated skeleton (Bergquist and Cook, 2002c,d). Dictyodendrillidae was erected to accommodate species with "clear dendroceratid affinities but reticulate skeletons with fibres of darwinellid morphology" (Bergquist, 1980; Bergquist and Cook, 2002d). Nevertheless, this classic generic composition of both

families Darwinellidae and Dictyodendrillidae was not supported by our molecular data. Consequently the distinction of dendroceratid families based on dendritic vs. anastomosing spongin skeletons was not upheld in Dendroceratida either, which corroborates earlier morphological conclusions (Maldonado and Uriz, 1999).

3.2.2.1.1. Darwinellidae. Our reconstructions (28S and CO1) find the two darwinellid genera *Darwinella* (represented by *D. oxeata* and *D. gardineri*) and *Dendrilla* (represented by its type species *D. rosea*) in a clade with the dictyodendrillid taxon *Igernella* (represented by its type species *I. notabilis*), strongly supported by both mitochondrial and nuclear data. *Darwinella* and *Dendrilla* possess a strictly dendritic skeleton, while *Igernella* is anastomosing and therefore rejects the suitability of spongin skeletal architectures as a synapomorphy of this group. Spongin spicules are shared derived characters, as they are present in *Darwinella* and *Igernella,* assuming a secondary loss in *Dendrilla*. Spongin spicules are suggested to be distinct based on the type of collagen they are composed of (Garrone, 1978), consequently of different developmental pathways (Bergquist and Cook, 2002d). Our data now suggests that some yet undiscovered biogenetic features makes spongin spicules more suitable as a uniting character than previously anticipated. The spongin spicules in *Darwinella* and *Igernella* are triaxonic and therefore different from their strongylote counterparts in *Aplysinella* (Verongida).

3.2.2.1.2. Dictyodendrillidae. *Dictyodendrilla*, nominal genus of the Dictyodendrillidae (represented by its type species *D. cavernosa* and several other species) and *Chelonaplysilla* (Darwinellidae, represented by *C. aurea*, *C. erecta* and *C. delicata*) form a strongly supported clade in both CO1 and 28S reconstructions. *Dictyodendrilla* possesses an anastomosing skeleton, while *Chelonaplysilla* is strictly dendritic (see also Bergquist and Cook (2002c) on seemingly anastomosing chelonaplysillids). A synapomorphy for this new dictyodendrillid composition might be the presence of spumous cells in both genera. Spumous cells have a yet unknown secretory function and to our knowledge there is no record of their presence in *Darwinella*, *Dendrilla* or *Igernella.*

3.2.2.2. Dictyoceratida. Dictyoceratida have been defined as sponges with an anastomosing spongin fibre skeleton which develops from multiple points, is hierarchically organised into primary, secondary and sometimes tertiary fibres, and makes up a significant proportion of the body volume (Cook and Bergquist, 2002e). With the exception of *Narrabeena* and putative *Euryspongia* sequences, the Dictyoceratida were supported as monophyletic in our reconstructions.

3.2.2.2.1. Dysideidae. A distinguishing feature between Dysideidae and the other three dictyoceratid taxa is their possession of eurypylous choanocyte chambers as opposed to diplodal choanocyte chambers in the other families. Dysideidae also possess laminated fibres, which are found in Thorectidae and Irciniidae but not Spongiidae (Cook and Bergquist, 2002a).

In our CO1 phylogenies, Dysideidae s.s. were the well-supported sister group to the remaining Dictyoceratida, which is in congruence of most 28S gene trees. This clade was comprised of *Dysidea* (several species) and *Lamellodysidea* (represented by its type species *L. herbacea*), which was separated from *Dysidea* based on the presence of an encrusting basal plate, and the lack of skeletal orientation, with respect to the surface (Cook and Bergquist, 2002a).

Citronia (represented by its holotype of the type species *C. vasiformis*), *Euryspongia* (not represented by a designated species but only by several unidentified species in our collections) and *Pleraplysilla* (represented by its type species *P. spinifera*) do not form a clade with Dysideidae s.s. The familial assignment of *Pleraplysilla* was doubted by Cook and Bergquist because pith elements more so resemble dendroceratid taxa, although frequently masked by coring material. *Pleraplysilla* was eventually placed into the Dysideidae based on shared chemotaxonomic traits (sesquiterpenes opposed to diterpenes in Dendroceratida, Cook and Bergquist, 2002a). Ribosomal data indicated the close relationship to Dysideidae (e.g. Borchiellini et al., 2004). The phylogenetic positions of these genera is currently the subject to a more detailed study and will be published elsewhere.

Acanthodendrilla (represented by the holotype of its type species *A. australis*) is currently assigned to the Dictyodendrillidae and here forms a clade with the Dysideidae in CO1 reconstructions. *Acanthodendrilla* is the only Dictyodendrillidae with cored primary and secondary fibres in combination with eurypylous choanocyte chambers and shares these features with *Lamellodysidea* and *Dysidea*. Nevertheless, this combination of characters might not be diagnostic for this clade, because two putative species of *Lamellodysidea* and *Dysidea* have been discovered in the Great Barrier Reef, in which secondary fibres are not cored (J. Hooper, unpublished).

3.2.2.2.2. Irciniidae + Spongiidae + Thorectidae. In contrast to the other keratose sponge families, the remaining three dictyoceratid families possess diplodal choanocyte chambers. Irciniidae was erected (Bergquist, 1995) for thorectid sponges with collagenous filaments in addition to their fibre skeleton (Cook and Bergquist, 2002b, therefore strictly speaking leaving Thorectidae paraphyletic). Spongiidae and Thorectidae, the largest groups among keratose sponges, are distinguished by the structure of their skeletal fibres. Spongiidae Gray, 1867 have homogeneous skeletal fibres, without "distinct laminations" (Cook and Bergquist, 2002c), while Thorectidae Bergquist, 1978 possess characteristic lamination (Cook and Bergquist, 2002d). Irciniidae + Spongiidae + Thorectidae formed a monophyletic clade, strongly supported by the CO1 gene tree – however, genetic distances between the clades were low and it could not be unambiguously assessed whether Irciniidae were the sister group to thorectids and spongiids.

Irciniidae: Irciniidae was recovered in CO1 analyses as a distinct clade, although 28S did not provide support for this outcome. The characteristic collagenous filaments of irciniids can be regarded as good morphological synapomorphies. *Psammocinia* (represented by its type species *P. halmiformis* and other species) is distinguished from *Ircinia*, the nominal genus (represented by several species) by the presence of a sandy armour – this distinction has been shown earlier with CO1 data (Pöppe et al., 2010). The third irciniid genus, *Sarcotragus* (represented by its type species *S. spinosulus* and also *S. muscarum*) displays a close relationship to *Ircinia* (both share a lack of cortical armour). However, our molecular data also indicate that *Ircinia* may be paraphyletic with respect to *Sarcotragus* and therefore corroborates the most recent revision of Irciniidae, in which its status is "viewed as uncertain" (Cook and Bergquist, 2002b).

Spongiidae + Thorectidae: Spongiidae and Thorectidae currently could not be distinguished, as branch length for both CO1 and 28S gene trees were too short, and an unambiguous phylogenetic signal could not be extracted.

4. Conclusion

Our study highlights the utility of molecular data to progress the evaluation of phylogenetic signals of morphological characters, in order to assess their evolution and consequently deduct the phylogenetic relationships of sponges. It is apparent that the simple morphological bauplan of sponges possesses individual lineage-specific peculiarities that force us to carefully reassess the phylogenetic importance of characters for each taxon separately (see e.g., Erpenbeck et al., 2006). This applies to skeletal characters in particular: Bergquist (1980) remarked that the dictyoceratid skeleton

characterises all Dictyoceratida, occurs to a great part in Verongida and to a minor part in Dendroceratida, which indicates that gross morphology of skeletal features is poorly discretionary at the large scale. However, skeletal apomorphic features may be indicative at shallower taxonomic levels (e.g., dendritic vs. anastomosing fibre skeletons may not distinguish Dictyodendrillidae from Darwinellidae but is still useful to differentiate *Chelonaplysilla* from *Dictyodendrilla*).

We found several cases in which skeletal fibre arrangement could not provide us with robust phylogenetic characters, particularly for the deeper splits. Other characters (such as the choanocyte chamber shape) still appear distinctive at higher taxonomic levels. In the future, phylogenetic hypotheses will have to rely on robust molecular phylogenies (preferably consisting of independent datasets of several genes), from which diagnostic, morphological characters can be tested and supported or refuted, as attempted here, and from which character evolution in these earliest-branching extant metazoan phylum can be deduced unambiguously.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2012.02.024.

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