

The phylogeny of halichondrid demosponges: past and present re-visited with DNA-barcoding data

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Abstract Halichondrid sponges play a pivotal role in the classification of demosponges as changes in their classification has had direct consequences for the classification of Demospongiae. Historically, the systematics of halichondrids has been unstable. During the 1950s, the order was divided into two subclasses, which were based on empirical and assumed reproductive data. Subsequent morphological and biochemical analyses postulated the re-merging of halichondrid families, but recent molecular data indicate their polyphyly. Here we review the classification history of halichondrid taxa, compare it with the current and predominantly ribosomal molecular data, and support the new phylogenetic hypotheses with mitochondrial data from DNA barcoding.

Keywords Sponges · Porifera · Demospongiae · Halichondrida · Phylogeny · Barcoding · CO1

Introduction

Sponges, particularly demosponges, which represent approximately 85% of all sponge species, are among the most difficult Metazoa in terms of classification. Indeed, Lévi (1957) dubbed the Porifera as the last major group of Metazoa in which the orders were still not clearly defined—a situation that still permeates Porifera today. The difficulties are due largely to the primitive bauplan of sponges from which few characters can be assessed rigorously; in the absence of robust character assessments, robust phylogenetic reconstruction is impossible. This situation is evident for all sponges, but is apparent particularly in halichondrid demosponges. This group of demosponges inhabits all world oceans, in almost all latitudes, from the surface down to great depths. Halichondrid sponges are named after the type genus, *Halichondria* Fleming, 1828, which translates from ancient Greek as “cartilage of the sea”, in reference to the amorphous shape and cartilaginous consistency of many taxa (Hansson 1994). The “breadcrumb-sponge”, *Halichondria panicea* Pallas, 1766, is perhaps the best studied of all sponges—although also possibly still the most ambiguous in terms of decisive morphometric features.

Halichondrids, as for many other sponge groups, lack unambiguous synapomorphic characters to indicate their monophyly such as e.g. chelae in Poecilosclerida s.s., verticillate acanthostyles in Agelasida, or incubated cincto-blastula larvae for Homosclerophorida. In the latest comprehensive classification of sponges (Hooper and Van Soest 2002), Order Halichondrida Gray, 1867 comprised 45 valid genera, arranged in five families: Halichondriidae Gray 1867, Axinellidae Carter 1875; Bubaridae Topsent 1894a;

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Dictyonellidae Van Soest, Diaz & Pomponi 1990 and Heteroxyidae Dendy, 1905. Their definitions of the families are generally based upon combinations of absent characters or character sets that did not include autapomorphies, and consequently the diagnoses of the families overlapped. For instance, according to Hooper and Van Soest (2002), Axinellidae, Bubaridae and Dictyonellidae all lack a specialized ectosomal skeleton. Discord and overlap in the familial diagnoses of halichondrid groups are clear. Bubaridae are defined as being entirely encrusting, and bearing a skeleton that is differentiated into a basal layer of interlacing spicules and a perpendicular layer of monactines with bases embedded in the basal skeleton (Alvarez and Van Soest 2002). Axinellidae bear ascending spiculo-fibers connected irregularly by loose spicules, or are plumoreticulated with connected ascending plumose tracts (Alvarez and Hooper 2002). Dictyonellidae have a dense organic ectosomal layer, giving the sponge a fleshy appearance and the choanosomal skeleton is built predominantly of styles, though occasionally oxeas or strongyles are known to occur (Van Soest et al. 2002). Heteroxyids (Hooper 2002a, better known under the junior synonym of Desmoxiidae) and halichondriids both bear an ectosomal skeleton (except in *Axinyssa* Lendenfeld, 1897 and *Laminospongia* Pulitzer-Finali, 1987). In the heteroxyids, the ectosomal skeleton consists of microxeote microscleres or modified forms, while in halichondriids the ectosomal skeleton consists of a densely confused tangentially arranged crust of styles and/or oxeote megascleres. The choanosomal skeleton in both families is a confused arrangement of spicules. In heteroxyids, a widely spaced reticulation of bundles occasionally occurs, whereas in halichondriids, only vague tracts of spicules may lead to the surface (Erpenbeck and Van Soest 2002).

In the following, firstly we summarise, review and document the pivotal position of halichondrid demosponges within the greater demosponge classification and phylogeny. Numerous sponge classifications have been erected in the past, and only a few selected schemas will be highlighted. Revisions of halichondrid classifications up to the end of the nineteenth century have been deduced mostly from the historical systematic of sponges outlined in Vosmaer (1887), which provides a very detailed recapitulation of the history of sponge science up to that time. Subsequently, we compare the past and current morphologically based classifications with analyses from the reconstruction of a mitochondrial gene tree from current DNA barcoding data and compare this tree to previously published nuclear ribosomal gene trees.

Material and methods

Molecular data were obtained during the barcoding of the Queensland Museum sponge collection in the course of the Sponge Barcoding Project (see <http://www.spongebarcoding.org>).

org, Wörheide and Erpenbeck 2007 for details). The DNA extraction was conducted following a plate based extraction method, developed for the barcoding project (Vargas et al. 2010). Fragments of the mitochondrial cytochrome oxidase subunit 1 (standard barcoding fragment) were amplified using the degenerated version of universal barcoding primers: dgLCO1490 (GGT CAA CAA ATC ATA AAG AYA TYG G) and dgHCO2198 (TAA ACT TCA GGG TGA CCA AAR AAY CA) (Meyer et al. 2005) with an annealing temperature of 43°C. The PCR products were purified with ExoSAP-IT (Affymetrix; <http://www.affymetrix.com>) or standard Ammonium acetate-Ethanol precipitation before cycle sequencing using the BigDye-Terminator Mix v3.1 (Applied Biosystems, Foster City, CA) following the manufacturer's protocol. Both strands of the template were sequenced on an ABI 3730 automated sequencer. The poriferan origin of the sequences was checked by a BLAST search against the NCBI Genbank database (<http://www.ncbi.nlm.nih.gov/>). Sequences were basecalled, trimmed and assembled in CodonCode Aligner v 3.7.1.1 and subsequently aligned in Sea-View 4 (Galtier et al. 1996) using Muscle 3.6 (Edgar 2004). Sequences are deposited in GenBank under accession numbers JQ034526–JQ034587, and in the Sponge Barcoding Database (<http://www.spongebarcoding.org>). For phylogenetic analyses, additional samples representing all major taxa of the demosponge "G4" clade (the major subgroup of all primarily spiculate demosponges (excluding marine Haplosclerida), Borchini et al. 2004) were included in the alignment.

Bayesian analyses of nucleotide sequences were run with the parallel version of MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) on a Linux cluster with one processor assigned to each Markov chain under the most generalising model (GTR+G+I) because overparametrisation has been shown to not negatively affect Bayesian analyses (Huelsenbeck and Ranala 2004). Each Bayesian analysis comprised at least two simultaneous runs of four Metropolis-coupled Markov-chains at the default temperature (0.2). Analyses were terminated after the chains converged significantly, as indicated by the average standard deviation of split frequencies < 0.01. Maximum likelihood reconstructions were performed using RAxML 7.2.5 (Stamatakis 2006) under the GTR model of nucleotide substitution with CAT approximation of rate heterogeneity and fast bootstrap (1,000 bootstrap replicates).

Results and discussion

Historic (pre-molecular) overview of halichondriids within the Demospongiae

Aristotle was probably the first to describe sponges scientifically [as *Spongia* and "*Aplysina*" (= *Sarcotragus* cf. Schmidt 1862)] and among the first to assume that they

are animals (cf. Johnston 1842). Since Aristotle, the systematic placement of sponges has undergone somewhat of an odyssey through the classification of organisms. Belon (1553), for example, placed sponges as a new kingdom between plants and animals. Linnaeus (1759) put them in the Algae (Cryptogamae) and later returned them to Animalia Linnaeus (1767). Donati (1750) discovered the presence of spicules as skeletal elements in sponges and used them in his first classification. Then followed the pioneering work of de Monet JBPA (1813), who placed the marine sponges into his “Polypiers polymorphs” (see Vosmaer 1887). Nardo separated sponges into three orders: (1) a group comprising horny sponges, (2) sponges with siliceous spicules, and (3) calcareous sponges. He expanded this system in 1833, independently (cf. Vosmaer 1887) from Hogg (1842) to include two more orders comprising siliceous-horny and calcareous-horny sponges. Grant (1836) published, also independently from Nardo (cf. Vosmaer 1882; 1886), a similar three-order system in which sponges were called “Porifera” for the first time.

Grant's system was refined by Bowerbank (1864, 1866, 1874), who named the three orders “Silicea” (for the siliceous sponges), “Calcareae” (for the calcareous sponges) and “Keratosae” (for the “horny” sponges). Halichondrid genera were distributed widely throughout Bowerbank's system. *Halichondria*, *Hymeniacidon* Bowerbank, 1858, and *Ciocalypa* Bowerbank, 1862 have been placed variously in three (out of seven) separate groups of the order “Silicea”, which they shared with members of several other currently valid orders. *Phakellia* Bowerbank, 1862 and *Halicnemis* Bowerbank 1864 were placed with *Ciocalypa*. Already halichondrids were off to an early, shaky start.

Schmidt (1866) criticized Bowerbank's system sharply for not being genealogical and therefore unnatural. In an earlier (1862) monograph on Adriatic sponges, Schmidt had divided all sponges into six groups, which can be defined roughly as:

- (1) Calcispongiae=calcareous sponges;
- (2) Ceraospongiae=horny-fibrous sponges
- (3) Gumminae=globular siliceous sponges with cortex
- (4) Halisarcinae=sponges without skeleton
- (5) Corticatae=true siliceous sponges
- (6) Halichondriae=Corticatae “with negative characters”

Schmidt admitted (a century before Hennigian philosophy) that his Halichondriae were based on negative characters and therefore indefensible. He placed his (Adriatic) samples of *Axinella* Schmidt 1862, *Acanthella* Schmidt 1862, *Halichondria* (as *Reniera* Schmidt 1862) and *Scopalina* Schmidt 1862 in the Halichondriae together with different species currently in Poecilosclerida, Hadromerida, and Haplosclerida. In Schmidt 1869, he published one of the first phylogenetic trees of sponges based on a 12-order system (see also Schmidt 1870), which was later reduced to 6 in order to equalise their ranks (Schmidt 1880, emended by Zittel). The taxon Halichondriae

disappeared in his system, which consisted of: (1) Hexactinellidae, (2) Calcispongiae, (3) Ceraospongiae, (4) Halisarcinae & Gumminaceae, (5) Tetractinellidae and (6) Monactinellidae. The last order (“monactin”=spicules with only one ray and one axis) comprised the current halichondrid taxa among others:

- (1) Renierinae (with reticulate skeleton, with oxeas and strongyles), including *Halichondria* (*Eumastia*), *Auletta* Schmidt 1870 and a mixture of Haplosclerida and Poecilosclerida)
- (2) Chalinopsidinae (with styles, but no chelae and sigma): *Axinella*, *Phakellia*, *Acanthella* and Dictyonellidae, in addition to *Raspailia* Nardo 1833 and *Clathria* Schmidt 1862 (now Poecilosclerida) and others
- (3) Desmacidinae (with chelae, sigma and toxa): mostly Poecilosclerida and *Scopalina*
- (4) Chalinae (with anisotropic skeleton, with oxeas and strongyles)
- (5) Suberitinae (with tylote spicules).

Gray (1867) published a system of “Poriphora calcarea” and “Poriphora silicea” independently and obviously ignored the work of Schmidt (cf. Vosmaer 1887). He divided the siliceous sponges by assumed reproductive features (“ovisacs”), and therefore was one of the first to use this type of character in sponge science. Gray founded the family “Halichondriadae” as a mixture of Halichondriidae (*Halichondria*, *Ciocalypa*), and many Haplosclerida, Hadromerida and Poecilosclerida. His families “Chalinidae” (including *Axinella* and *Acanthella*) and the newly erected “Phakelliadae” (monotypic for *Phakellia*) were placed closer to keratose sponges than to the Halichondriadae. Gray's system was criticised strongly by Bowerbank (1874), Schmidt (1868, who did not use the taxon Halichondriadae in his work), Haeckel (1872) and Vosmaer (1882–1886). Subsequently, Gray (1872) published a modified system, which united “Halichondriidae”, “Chalinidae”, and “Phakellidae” with six other families in the taxon “Keratospongia” (Vosmaer 1887).

The system of Carter (e.g. 1875) comprised eight orders. He erected the taxon “Axinellida” as a family inside his order “Echinonemata” (with mostly styles and tylostyles in fibers), as sister group to “Ectyonida”, which comprise actual raspailid taxa (among others). The Halichondrina were distant in his Family Renierida of the order Holoraphidota (with various spicules, minimum spongin) besides other hadromerid families such as Suberitida.

The distinction between siliceous and calcareous sponges was more important for Vosmaer (1886). He based his system on a dichotomous separation of sponges in “Porifera calcarea” and the “Porifera non-calcarea”, which were organised as follows:

- (1) Hyalospongiae (= Hexactinellida),

- (2) Spiculispongiae (no spongin to cement the spicules, spicules loose). Suborders:
 - (A) Lithistina
 - (B) Tetractina (Plakinidae etc.);
 - (C) Oligosilicina (cf. Chondrosida, Halisarcidae, etc.)
 - (D) Pseudotetraxonina (cf. Hadromerida).
- (3) Cornacuspongiae (with spongin to cement the spicules and the suborders:
 - (A) Halichondrina (basically every Cornacuspongiae except keratose sponges)
 - (a) Halichondridae (with *Halichondria*, *Axinella*, *Acanthella*, *Dictyonella* Schmidt 1868, *Phakellia*, *Auletta*, *Eumastia* Schmidt 1870 and several Haplosclerida)
 - (b) Ectyonidae (with *Phycopsis* Carter, 1883, *Ptilocaulis* Carter, 1883, *Leucophloeus* Carter, 1883, *Ciocalypa*, *Hymenaphia* Bowerbank 1864, *Higginsia* Higgin, 1877)
 - (c) Desmacidonidae
 - (d) Spongillidae
 - (B) Ceratina (keratose sponges).

With Vosmaer's "Spiculispongiae" and "Cornacuspongiae" began a long tradition of dividing non-hexactinellid siliceous sponges (= demosponges) among two clades. These two clades were based on different definitions by Lendenfeld, Sollas, Hentschel, Dendy and Lévi until the recent past (see below), but their contents were more-or-less comparable.

Sollas (1885) introduced the name "Demospongiae" into sponge systematics. He gave calcareous sponges class-level status and opposed them to non-Calcareous ("Plethospongiae"). Those "Plethospongiae" were divided into the orders:

- (1) Hexactinellida (glass sponges)
- (2) Myxospongiae (all skeleton-lacking sponges)
- (3) Demospongiae
 - (A) Monaxonidae (spicules with one axis)
 - (B) Tetractinellidae (spicules with four axes)
 - (C) Cerospongiae (keratose sponges, assumed to be polyphyletic by Sollas).

Ridley and Dendy (1886) divided the Monaxonidae in "Clavulina" (with radiate skeleton and/or cortex) and "Halichondrina" (with reticulated skeleton and without cortex). Halichondrina were divided into:

- (1) Homorrhaphidae (with *Halichondria*) cylindrical spicules
- (2) Heterorrhaphidae
- (3) Desmacidonidae (with *Agelas* Duchassaing & Michelotti, 1864)
- (4) Axinellidae (with *Hymeniacidon*, *Axinella*, *Phakellia*).

Von Lendenfeld (1887) adopted Sollas' system of opposing Calcareous and non-calcareous ("Silicea"), but, in contrast to Vosmaer, he regarded microscle shape as a more valuable character. His classification (congruent with Ridley and Dendy 1886, 1887) divides the "Subclassis Silicea" into the following orders:

- (1) Hexactinellida
- (2) Chondrospongiae (no spongin to cement the spicules; asteroid microscleres)
- (3) Cornacuspongiae (spongin cements the spicules; meniscoid microscleres) with the suborder Halichondrina including:
 - (A) Spongillidae (freshwater sponges)
 - (B) Homorrhaphidae: including Family Renieridae Schmidt 1870 (with *Halichondria*, and mostly haplosclerids)
 - (C) Heterorrhaphidae
 - (D) Desmacionidae (cf. Ridley and Dendy 1887) (with *Agelas*)
 - (E) Axinellidae: (with *Dendropsis* (= *Higginsia*) Ridley and Dendy, 1886, *Hymeniacidon*, *Phakellia*, *Ciocalypa*, *Acanthella*, *Axinella*, *Drumacidon* Hallmann, 1917 and *Raspailia* (now Poecilosclerida)).

With Vosmaer, Sollas and Lendenfeld a "certain period of stability" (Lévi 1957) was reached in sponge systematics. Calcareous were distinguished from non-Calcareous, and Hexactinellida obtained a specific position among siliceous sponges. Further classificational work was done around that time by Schulze, Ridley, Dendy, Carter, Thiele, Lundbeck, Kirkpatrick, Wilson, Minchin and Hallmann. Their studies lead to multiple changes of taxon names but were without major impact on the system. Lévi (1957) remarked that the only "original contribution" at that time was Dendy's (1922) classification of the siliceous demosponges, by giving microscleres a higher priority in the classification than megascleres. Dendy's system of siliceous sponges is as follows:

- (1) Triaxonida (Hexactinellidae)
- (2) Myxospongida (no skeleton)
- (3) Euceratosa (horny sponges)
- (4) Tetraaxonida, which were divided into the following groups based on Hentschel's (1909, 1911) suggestions:
 - (A) Astrotetaxonida (with asterose microscleres, if not secondarily lost)
 - (B) Sigmatotetaxonida (with sigmatose microscleres, if not secondarily lost) including families:
 - (a) Lithistidae
 - (b) Tetillidae
 - (c) Haploscleridae with *Halichondria*, and most actual Haplosclerida

- (d) Desmacioidae with *Bubaris* Gray, 1867, *Axinella*, *Phakellia*, *Auletta*, *Hymeniacidon*, *Leucophloeus*, *Higginsia*, *Spongosorites*, *Halicnemis* and most actual Poecilosclerida s.s.
- (e) Clavulidae with *Didiscus* Dendy, 1922 and many actual Hadromerida

Hallmann (1917) erected the taxon “Desmoxyinae” including its actual taxa *Halicnemis*, *Higginsia* (*Higginsia*) and *Higginsia* (*Desmoxya*) (Hallmann 1917) (as *Desmoxya*).

Comprehensive classifications were proposed by Hentschel (1923), Topsent (1928) and de Laubenfels (1936). Hentschel (1923) maintained Vosmaer's and Von Lendenfeld's groups, but placed Dendroceratida as separate order equivalent to Calcarea, Hexactinellidae, and the demosponge orders. Additionally, he modified the taxon “Bubaridae”, which was founded by Topsent, (1894b, containing the currently valid genera *Bubaris*, *Cerbaris* Topsent, 1898 and *Hymerhabdia* Topsent, 1892), by adding (poecilosclerid) taxa. Hentschel's system was structured as follows:

- (1) Calcarea
- (2) Hexactinellidae
- (3) Tetraxonida with the suborders:
 - (A) Homosclerophora
 - (B) Sigmatorhiza
 - (C) Astrophora
 - (D) Desmophora
 - (E) Astromonaxonellina (most hadromerids)
- (4) Cornacuspongida with the suborders:
 - (A) Protorhabdina (microscleres and monactine megascleres)
 - (B) Poikilorhabdina (various megascleres, often microscleres) including:
 - (a) Coelosphaeridae with *Topsentia* Berg, 1899,
 - (b) Crellidae with *Didiscus*
 - (c) Bubaridae with *Bubaris*, *Hymerhabdia*, *Cerbaris*, *Monocrepidium* Topsent, 1898, but also actual Poecilosclerida.
 - (C) Phthirorhabdina: (simple megascleres, barely microscleres)
 - (a) Axinellidae (no ectosomal skeleton), with *Auletta*, *Phakellia*, *Acanthella*
 - (b) Ciocalyptidae (with ectosomal skeleton) with *Halichondria*, *Eumastia*, *Axinyssa*, *Hymeniacidon*, *Ciocalypta*, *Liosina* Thiele, 1899, *Spongosorites* Topsent, 1896 and actual Haplosclerida,
 - (D) Aporhabdina (cf. Dictyoceratida)
- (5) Dendroceratida (with spongin spicules).

Topsent (1928) divided the demosponges into three subclasses based on megasclere morphology. He united most of

the halichondrid taxa and re-defined Hentschel's Bubaridae, including the four valid species:

- (1) Tetractinellida (four-rayed spicules: orders Lithistida and Choristida with Homosclerophora)
- (2) Monaxonellida (one-rayed spicules):
 - (A) Hadromerina: family Spongosoritidae with *Spongosorites*
 - (B) Halichondrina, with the families:
 - (a) Axinellidae with most modern Halichondriidae: *Halichondria*, *Hymeniacidon*, *Ciocalypta*, *Axinella*, *Ptilocaulis*, *Phakellia*, *Dragmacidon*, *Microxistyla* Topsent, 1928, *Topsentia*, *Higginsia*, *Ptilocaulis*, *Dragmacidon*, *Auletta*)
 - (b) Astraxinellidae (*Halicnemis*, and some Hadromerida)
 - (c) Heteroxyidae with *Heteroxya* Topsent, 1898
 - (d) Bubaridae (*Bubaris*, *Cerbaris*, *Monocrepidium*, *Hymerhabdia*)
 - (C) Poecilosclerina with *Didiscus*
 - (D) Haplosclerina
- (3) Ceratellida (horny sponges, no spicules, orders: Dictyoceratina, Dendroceratina).

De Laubenfels (1936) modified Topsent's system and published a monograph that aimed to unite all known sponge genera known at that time. He placed Topsent's Astraxinellidae over various taxa and put *Halichondria* and *Hymeniacidon* in different families. The resulting family Hymeniacidonidae was based on its fleshy appearance. De Laubenfels' system was structured as follows:

- (1) Keratosa
- (2) Haplosclerina (with Desmacionidae: *Liosina*)
- (3) Poecilosclerina: with Agelasidae and Phorbasinae (*Didiscus*, *Myrmekioderma* Ehlers, 1870, *Heteroxya*)
- (4) Halichondrina (ectosomal skeleton, no specially localized megascleres) with the families
 - (A) Axinellidae (with hispid ectosome and axial core), which were separated in:
 - (a) Axinellinae: (no microscleres) *Ptilocaulis*, *Auletta*, *Axinella*, *Dragmacidon*, *Acanthella*, *Pararhaphoxya* Burton, 1934, *Phakellia*, *Phakettia* de Laubenfels, 1936, *Phycopsis*, *Spongosorites*, *Stylissa* Hallmann, 1914, *Bubaris*
 - (b) Higginsinae: (with spined microscleres) *Desmoxya*, *Higginsia*, *Negombo*
 - (B) Halichondriidae (simple spiculation, smooth spicules, ectosomal skeleton) *Ciocalypta*, *Ciocalapata* de Laubenfels, 1936, *Leucophloeus*, *Halichondria*
 - (C) Semisuberitidae as a Poecilosclerida/hadromerid assemblage

- (D) Hymeniacionidae: (simple spiculation, smooth spicules, fleshy appearance) *Hymeniacion*, *Acanthella*, *Dictyonella*
- (E) Monanthidae
- (5) Hadromerina
- (6) Epipolasida (like Hadromerina, but with oxeas and styles instead: *Negombata* de Laubenfels, 1936, *Parahigginsia* Dendy, 1924, *Topsentia*, *Epipolasis* de Laubenfels, 1936, *Axinyssa*)
- (7) Choristida
- (8) Carnosa

Lévi (1951) compared Topsent's findings with his own ontogenetic observations. The orders Halisarca, Poecilosclerida and Haplosclerida were found to be viviparous, and Hadromerida, Homosclerophoridae (Lévi 1953a) to be oviparous. The order Halichondrida was "artificiel et hétérogène" (Lévi 1951) and the viviparous groups (such as *Halichondria* and *Hymeniacion*) were recommended to be separated from the oviparous (such as *Axinella*).

Lévi (1957) stated that three classes Hexactinellida, Calcarea and Demospongiae in their entities were well defined, but their reciprocal relationships were completely unknown. He remarked on the congruence of most previous authors in dividing the demosponges into two groups, but criticized the poor internal division, especially of the Cornacuspongiae/Sigmaxinellidae group (cf. Halichondrida, Poecilosclerida, Haplosclerida and keratose sponges). He found sponges without skeletons impossible to classify, the position of the Plakinidae (now separated as Homosclerophorida) ambiguous, and the Halichondrida (*sensu* Topsent), especially the family Axinellidae, a pitfall in the sponge systematics. Lévi regarded Axinellidae and the skeleton-lacking Myxospongida [consisting of *Halisarca* Johnston, 1842 (now order Halisarcida) and *Oscarella* Vosmaer, 1884 (now class Homoscleromorpha)] as intermediate morphological forms connecting the two demosponge groups (Dendroceratida—Dictyoceratida—Haplosclerida—Poecilosclerida) and (Homosclerophorida—Tetractinellida—Hadromerida—Epipolasida).

Based on his ontogenetic data, which was subsequently extrapolated for the entire class, Lévi divided demosponges into two groups (Lévi 1953b). These groups were defined primarily by reproductive features, based on his own observations. Halichondrida and Myxospongida were split. The first group comprised the viviparous "Ceractinomorpha" with their solid, incubated parenchymella larvae, consisting of the Dendroceratida, Dictyoceratida, Haplosclerida, Poecilosclerida, and Halichondrida *s.s.* (without Axinellidae), with *Halisarca* (now Halisarcida) as the most primitive taxon. Opposed to the Ceractinomorpha were the oviparous Tetractinomorpha with *Oscarella* (viviparous, now Homosclerophorida) as the most primitive taxon, and Hadromerida, Tetractinellidae, and Axinellidae separated from Halichondrida. Lévi resurrected the

order Axinellida, with nine (assumed oviparous) families, and combined Axinellida, Hadromerida, and Epipolasida in the superorder Clavaxinellida - presuming their close relationship (Lévi 1956; 1955; 1957). The Homosclerophorida subsequently received subclass status, equal to Ceractinomorpha and Tetractinomorpha (Lévi 1973), together with the rediscovered (Hartman 1969) Sclerospongiae.

Lévi's system of the demosponges (1973) was structured as follows:

- (1) Homosclerophorida (viviparous)
- (2) Tetractinomorpha (oviparous)
 - (A) Astrophorida
 - (B) Spirophorida
 - (C) Desmophorida (and other lithistid sponges)
 - (D) Hadromerida
 - (E) Axinellida (choanosomal skeleton axial condensed and indiscriminate presence of styles and oxeas)
 - (a) Axinellidae: *Axinella*, *Phakellia*, *Auleta*, *Ptilocaulis*, *Acanthella*, *Drumacidon*
 - (b) Bubaridae: *Bubaris*, *Monocrepidium*
 - (c) Desmoxyidae: *Higginsia*, *Parahigginsia*, *Halicnema*
 - (d) Trachycladidae
 - (e) Rhabderemiidae
 - (f) Sigmaxinellidae
 - (g) Raspailiidae
 - (h) Euryponidae
 - (i) Hemiasterellidae
- (3) Ceractinomorpha (viviparous)
 - (A) Dictyoceratida
 - (B) Dendroceratida
 - (C) Haplosclerida
 - (D) Poecilosclerida with families Agelasidae (*Agelas*) and Latrunculidae (*Didiscus*)
 - (E) Halichondrida (defined by the ectosomal tangential skeleton and confused choanosomal skeleton)
 - (a) Halichondriidae: (primarily oxeas): *Halichondria*, *Trachyopsis* Dendy, 1905 (= *Topsentia*), *Ciocalypa*, *Amorphinopsis* Carter, 1887
 - (b) Hymeniacionidae: (primarily styles): *Hymeniacion*, *Leucophloeus*
- (4) Sclerosponges [raised to class status (Hartman and Goreau 1970)]

Lévi's system was extended by Bergquist (1978) and Hartman (1982), who explored additional supporting (mostly biochemical, reproductive and ultra-histological) characters. Bergquist, however, expressed doubts about the monophyly of Tetractinomorpha (1978). Nevertheless, she placed Bubaridae within Axinellidae.

Hartman (1982) elevated Agelasida in his demosponge classification inside the Tetractinomorpha to ordinal level:

- 1) Homoscleromorpha: Order Homosclerophorida
- 2) Tetractinomorpha: Orders Astrophorida (Choristida), Spirophorida, Lithistida, Hadromerida, Axinellida, and Agelasida
- 3) Ceractinomorpha: Orders Dendroceratida, Dictyoceratida, Verongida, Haplosclerida, Petrosida, Poecilosclerida, Halichondrida

Several morphological (Vacelet 1985; Van Soest 1984; Reitner 1992), molecular (Chombard et al. 1997) and chemotaxonomic data-sets (see review in Wörheide 1998) have demonstrated unequivocally the polyphyly of Sclerospongia and that its sole family, Astroscleridae, is in fact closely related to Agelasidae.

Van Soest (1984) and Hooper (1984) pointed out inconsistencies in the current classifications in their independent analyses on Poecilosclerida and Axinellida, respectively. They found the Levi-Bergquist-Hartman classification with its distinction between Ceractinomorpha and Tetractinomorpha unparsimonious and subsequently suggested the re-merging of Axinellida with Halichondrida. Van Soest, after introducing cladistic character analyses in sponge systematics (1985), found the taxon Tetractinomorpha to be based only on the absence of primitive reproductive features (1987). Furthermore, the placement of Axinellida in Tetractinomorpha was based solely on the possession of hemiasterellid-type asters and plesiomorphic oviparity, and therefore problematic. Such a classification, if natural, would mean that the plumoreticulate architecture must have evolved twice. The placement of halichondrids in Ceractinomorpha was also problematic, as the larval morphology of these sponges differs from the remaining Ceractinomorpha, and a diversity of types of larvae had been observed in halichondrids (Wapstra and Van Soest 1987). As an additional complication to reproduction-based classification, several ceractinomorphic taxa were found to be oviparous (verongids, agelasids, petrosiids, desmacellids, (e.g. Bergquist 1978; Bergquist 1980b; Hoppe and Reichert 1987). Consequently, it seemed unlikely that viviparity evolved only once in sponges as reported for several other invertebrate groups such as molluscs and ascidians. Independently, Hooper observed closer relationships of the tetractinomorphic Family Raspailiidae to the ceractinomorphic Family Microcionidae, than to other axinellid groups, supporting the concept of de Laubenfels (1936) (Hooper 1990, 1991). Consequently, (Clav-) Axinellida *sensu* Lévi was abandoned and its families were partially (Heteroxyidae (as Desmoxyidae) and Axinellidae) redistributed within Halichondrida (Van Soest et al. 1990). In this schema, Family Dictyonellidae was erected to cope with genera of the polyphyletic order Axinellida. Dictyonellidae was predicted to be close to Halichondriidae, based on the loss of the reticulate skeleton. Both families

formed a putative sister-group relationship to Heteroxyidae (as Desmoxyidae) based on the possession of a fleshy ectosome (Van Soest et al. 1990) (see also Carballo et al. 1996 for subsequently suggested emendations for Dictyonellidae). The combination of non-localized interchangeable monaxons (styles and oxeas), sinuous strongyles, plumoreticulate architecture, and collagenous mesohyl united the three families with Axinellidae into the order Halichondrida. Previously, a series of halichondrid genera with intermediate forms between an axinellid plumo-reticulated skeleton and a halichondrid confused skeleton has been shown (Diaz 1997). The segregation of families based on the predominance of either styles or oxeas (e.g. Halichondriidae against Hymeniacidonidae) was no longer upheld. Additionally, the placement of the heteroxyid genera *Myrmekioderma* and *Didiscus* back in the halichondrids was proposed. This classification comprised the following halichondrid genera:

Halichondriidae: (high density of spicules in confusion and in ill-defined tracts) *Halichondria*, *Hymeniacidon*, *Amorphinopsis*, *Myrmekioderma*, *Didiscus*, *Epipolasis*, *Spongisorites*, *Axinyssa* Lendenfeld, 1897;

Axinellidae: (choanosomal skeleton axially condensed, extra-axially plumoreticulated): *Axinella*, *Phakellia*, *Auleta*, *Bubaris*, *Monocrepidium*, *Hymenhabdia*, *Cerbaris*;
Dictyonellidae: (spongin reinforced tracts, fleshy-conulose surface) *Dictyonella*, *Scopalina*, *Acanthella*, *Dactylella* Thiele, 1898, *Tethyspira* Topsent, 1890, *Liosina*;

Desmoxyidae: (=Heteroxyidae; reticulate-fasciculate choanosomal skeleton of spongin-enveloped tracts with a fleshy surface): *Higginsia*, *Halcnemia*, *Ptilocaulis*.

Van Soest (1991) supported his classification with a cladistic analysis. The resulting consensus combined Poecilosclerida and Haplosclerida, Halichondrida (with Axinellida) and the keratose sponges in a clade separated from Tetractinellida Hadromerida, Homoscleromorpha and Calcarea. The axial compression of the skeleton, a synapomorphy for the Order Axinellida *sensu* Lévi, was suggested to be homoplasious in demosponges.

Although this new classification gained broader acceptance in the ensuing years (e.g. Lévi 1997), morphological and biochemical data indicated that Axinellidae and Heteroxyidae might not be homogeneous groups and the genera might not be easily differentiated (Bergquist and Hartman 1969; Sole-Cava et al. 1991; Hooper and Bergquist 1992; Hooper et al. 1992). In subsequent revisions, the genera nominated for Axinellidae were reduced to 11 (Alvarez et al. 1998; Alvarez de Glasby 1996; Hooper and Lévi 1993) and morphological as well as molecular phylogenies of this family were inferred (Alvarez and Crisp 1994; Alvarez et al. 2000). *Myrmekioderma* and *Didiscus* were returned to Desmoxyidae (subsequently Heteroxyidae, see Van Soest and Hooper 2005),

together with the genera *Higginsia*, *Halicnemis*, *Heteroxya*, *Myrmekioderma*, *Didiscus*, and *Julavis* de Laubenfels 1936 (Van Soest and Lehnert 1997). Finally, in *Systema Porifera* (Hooper and Van Soest 2002), Halichondrida were classified as an order containing five families: Halichondriidae (Erpenbeck and Van Soest 2002), Axinellidae (Alvarez and Hooper 2002), Dictyonellidae (Van Soest et al. 2002), Heteroxyidae (Hooper 2002a), and the Bubaridae (Alvarez and Van Soest 2002), which re-attained family status (Van Soest and Hooper 2002). However, with the advent of molecular techniques in sponge systematics (Kelly Borges et al. 1991), early indications were that the strictly morphological classification of the halichondrids was in many aspects likely to be incongruent with molecular hypotheses (Lafay et al. 1992). The molecular hypotheses will be in the highlighted in the following section, which is based on phylogenetic reconstructions incorporating our new halichondrid barcoding data.

The molecular view on halichondrid sponges

Halichondrida (and several of its constituent families) has never been recovered as monophyletic; there is no analysis, be it based on morphological, biochemical (e.g., Erpenbeck and Van Soest 2005) or molecular datasets. This is in accord with the phylogenetic trees we reconstructed from the CO1 mtDNA barcoding fragment. Our tree comprises 584 characters from a total of 163 halichondrid and selected other demosponges to obtain the most representative possible data set. Of these, 62 sequences were newly generated (Identifier: Gxxxxxx in Fig. 1). CO1 cannot support the deeper splits; however, it is evident that halichondrid CO1 sequences fall in distant clades (Fig. 1, grey boxes) and no halichondrid family is recovered as monophyletic (with the exception of Bubaridae, which are not sampled here).

Most Halichondriidae, the nominal family of the halichondrids, form a clade with suberitid taxa. A close halichondrid+suberitid relationship was previously inferred using ribosomal data (e.g. Chombard and Boury-Esnault 1999; McCormack and Kelly 2002; Erpenbeck et al. 2004; 2005b), and also using CO1 data Erpenbeck et al. (2007a), as well as recent combined analyses (Morrow et al. 2011). Morphologically, Suberitidae and Halichondriidae share the presence of a (mostly) confused choanosomal skeleton, although in suberitids it can tend to form radial arrangements. Consequently the (approximate) distinction into taxa with oxaeas (halichondriids) and tylostyles (suberitids) is invalid. This is particularly obvious in the genus *Vosmaeria* Fristedt, 1885, which combines both spicule types (see Gerasimova et al. 2008 for a molecular analysis).

Halichondriids and suberitids both possess an ectosomal skeleton with overlapping traits, but a distinction based on its (mostly) tangential shape in Halichondriidae and its brush- or palisade-type in Suberitidae is not warranted. For example,

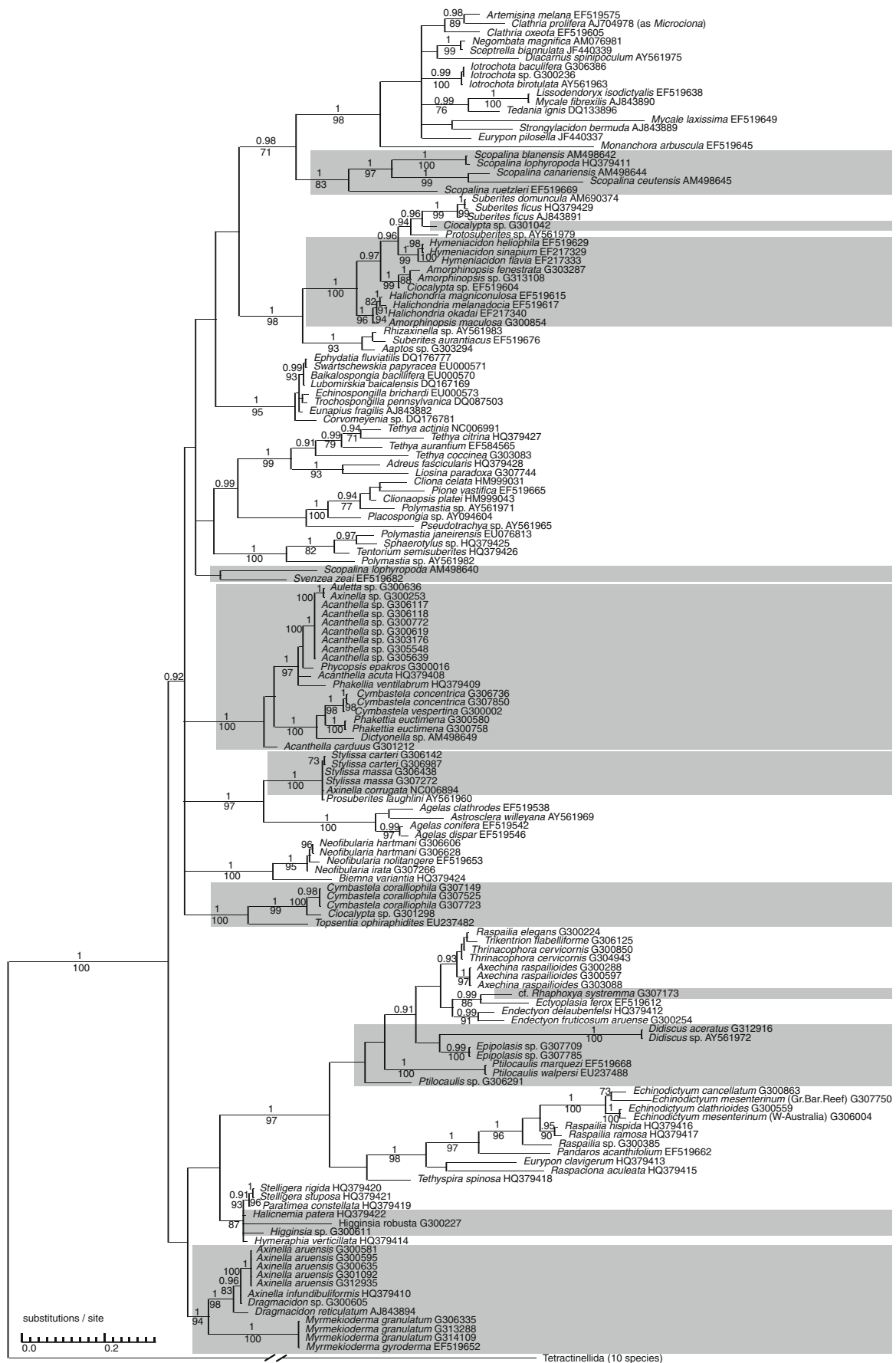
Fig. 1 Bayesian inference phylogram on CO1 barcoding samples. Values at branches are support values. Below: Maximum-likelihood bootstrap values (>70), above: Bayesian posterior probabilities (> 0.9). Support values for some branches of lesser importance of this analysis (inside outgroups, Spongillina, Poecilosclerida s.s.) may not be provided to maintain legibility. Grey boxes indicate halichondrid taxa sensu Hooper and Van Soest (2002). The taxon names are followed by Genbank accession numbers. In case the sequences have not been published earlier, collection numbers of the Queensland Museum Brisbane (Gxxxxxx) are provided

paratangential forms may be present in *Hymeniacion* and other Halichondriidae, and species of the suberitid genus *Caulospongia* Kent, 1871 possess a tangential ectosomal skeleton. *Caulospongia* and other taxa with overlapping morphological traits (e.g., *Pseudosuberites*) represent pivotal taxa to investigate to fully understand the evolution of this clade.

In our reconstruction, the non-monophyly of Halichondrida and halichondrid families is demonstrated in other aspects. *Stylissa carteri* (Dendy, 1889) and *S. massa* (Carter, 1887) are distant to other Dictyonellidae and join the sister group to Order Agelasida with several axinellid taxa (termed as Hymerhabdiidae, Morrow et al. 2011). This corroborates one of the first molecular phylogenies on sponges (Lafay et al. 1992) and biochemical analyses (Van Soest and Braekman 1999) and subsequent independent data sets (see Erpenbeck et al. 2005a, 2006; Nichols 2005; Morrow et al. 2011).

Dictyonellidae were classically defined based mostly on negative characters, and were therefore likely candidates to form an assemblage of unrelated sponges (e.g. Van Soest et al. 2002). Dictyonellidae possess predominantly styles and display a fleshy appearance due to a comparatively low spicule content. Sponges from several demosponge lineages can resemble dictyonellids after a (presumable) reduction of their skeletal elements. Consequently, we find dictyonellid taxa scattered all over the demosponge phylogeny (Fig. 1). For example, CO1 recovers *Liosina paradoxa* Thiele, 1899, classified "tentative[ly] in the absence of any definite synapomorphies with either Halichondrida and Poecilosclerida" (Van Soest et al. 2002), in a clade with tethyid and other hemiasterellid sequences. This result agrees with earlier findings on *Liosina* based on nuclear ribosomal DNA sequence data (Kober and Nichols 2007), and supports the placement of *Liosina* in a redefined family Tethyidae (Morrow et al. 2011). Likewise, analyses of molecular data (CO1 and ribosomal) infer the positions of *Scopalina lophyropoda* Schmidt 1862 (and *Svenzea* Alvarez, Van Soest & Rützler, 2002) as unrelated to most other halichondrids (Nichols 2005; Erpenbeck et al. 2007a) and with uncertain relationship to other taxa; this result triggered the proposal of a separate family (see Morrow et al. 2011). We also observe long interspecific branches in *Scopalina* CO1 preventing the formation of single *Scopalina* clade, unlike in nuclear ribosomal data (see also Morrow et al. 2011).

Several species allocated to former axinellid/dictyonellid genera display "fuzzy boundaries" due to overlapping



characters and phenotypic plasticity (Alvarez and Hooper 2009). In the clade containing *Acanthella acuta* (type species of *Acanthella*) and *Dictyonella* we recover representatives from the erstwhile axinellids *Auletta*, *Phakellia*, and *Phycopsis*. *Phakellia* is represented by its type species *P. ventilabrum* and its affinity to a Dictyonellidae clade has been shown earlier (Morrow et al. 2011). Several species of *Acanthella* have been mistaken consistently for *Phakellia*. For example, *Acanthella carduus* has been a suggested new combination for *Phakellia carduus* (Alvarez and Hooper 2010), nevertheless, its molecular affinities are equivocal in this data set. *Acanthella*, *Phakellia* plus species from other genera might form a species complex, the resolution of which requires additional data.

Phakettia euctimena (Hentschel, 1912) was initially assigned to *Acanthella* before being reclassified on the grounds of the general skeletal architecture and the spicule composition (Alvarez and Hooper 2010). Our molecular tree lacks the comparison to the type species *P. cactoides* (Burton, 1928) but corroborates a genealogical distance to *Acanthella*.

The skeletal organisation of *Auletta* is comparable to *Phakellia* (see Alvarez and Hooper 2002) and discussed as homologous (Morrow et al. 2011). *Auletta*, *Phycopsis* and *Phakellia* all share the flexuous strongyles with *Acanthella* and our analyses raise further evidence that the possession of strongyles is of greater taxonomic relevance than presently recognised (see also Morrow et al. 2011). For example, close relationships between the genera *Ptilocaulis* and *Reniochalina* are well established (e.g. Hooper and Lévi 1993, 1994; Alvarez et al. 2000), but molecular and morphological data rejected a monophyletic *Ptilocaulis*. Therefore, the strongyle-bearing species *Ptilocaulis fusiformis* Lévi, 1967 and *P. epakros* Hooper and Lévi, 1993 were assigned to *Phycopsis* opposed to the strongyle-lacking type species, *Ptilocaulis walpersi* (Duchassaing & Michelotti, 1864) and *Ptilocaulis marquezii* (Duchassaing & Michelotti, 1864) (Alvarez et al. 1998; Alvarez and Hooper 2002). Our data support this placement within *Phycopsis*, as the type specimen of *P. epakros* falls in a clade with *Acanthella* and *Phakellia*, distant to *P. walpersi* and *P. marquezii*.

Nevertheless, the affinities of other strongyle bearing-taxa *Rhaphoxya systemma* Hooper and Lévi 1993 (represented here only by a putative specimen) or certain raspilid taxa, e.g. *Ceratopsion rugosum* (Schmidt 1870, not represented here) await confirmation. The possession of strongyles is not an obligatory feature for this clade, because *Axinyssa*, previously classified as the Halichondriidae (Erpenbeck and Van Soest 2002) is likely to fall into this clade, as was indicated by rDNA analyses (Erpenbeck et al. 2005b; Morrow et al. 2011). *Axinyssa* also lacks the halichondriid ectosomal skeleton, which makes its different phylogenetic position plausible.

As with *Axinyssa*, species of the axinellid genus *Cymbastela* Hooper and Bergquist, 1992 only possess oxeas.

Our CO1 reconstructions, including the holotype of *Cymbastela vespertina* Hooper and Bergquist, 1992, corroborates independent nuclear ribosomal data that *Cymbastela* shares a close relationship to *Acanthella* and *Dictyonella* (e.g. Alvarez et al. 2000; Erpenbeck et al. 2005b; Gazave et al. 2010; Morrow et al. 2011) although skeleton and spiculation of both groups is relatively different (Alvarez and Hooper 2010). Our CO1 data also supports, for the first time with a mitochondrial marker, that *Cymbastela coralliophila* Hooper and Bergquist, 1992 is in fact unrelated to other *Cymbastela* species (see the ribosomal data of Alvarez et al. 2000). *Cymbastela coralliophila* differs from *C. vespertina* by the possession of a paratangential ectosomal skeleton (absent in *C. vespertina*) and two size categories of oxeas in the primary skeleton (only one in *C. vespertina*).

Cymbastela coralliophila forms a clade with an as yet unidentified oxea-bearing species of *Ciocalypta* (resulting paraphyly of *Ciocalypta*), and a GenBank specimen submitted as *Topsentia ophiraphidites* (Halichondriidae). *Cymbastela coralliophila* and *Topsentia* share the possession of oxeas in several different size classes, and the possession of a paratangential skeleton, but differ in choanosomal skeletal architecture (confused vs plume-reticulate). A closer examination of this *Topsentia* specimen is required. It displays the same position distant to Halichondriidae in 18S analyses (see <http://www.spongegenetrees.org>, Erpenbeck et al. 2008), but is different to another specimen of *T. ophiraphidites*, which is related to Halichondriidae in 28S and CO1 analyses (Erpenbeck et al. 2005b; Raleigh et al. 2007).

The non-monophyly of *Axinella*—the nominal genus for the Axinellidae—has been shown earlier (Alvarez et al. 2000; Gazave et al. 2010). Our reconstruction recovers a clade of *Dragmacidon*, *A. aruensis* (Hentschel, 1912) and *A. infundibuliformis* (Linnaeus, 1759). *D. reticulatum* (Ridley and Dendy, 1886) and *A. aruensis* have nearly identical choanosomal skeletons, spicule compositions and sizes. Nevertheless, *Axinella* and *Dragmacidon* are distinguished largely by the relative density of the spiculation and the degree of axial compression in the skeleton, with the skeleton of *Axinella* being less dense and more compressed. *Dragmacidon* is further distinguished from *Axinella* by its growth form and surface characteristics. These characteristics tend to grade into one another and can be difficult to separate.

Our CO1 data corroborates the closer relationship of *Ptilocaulis* (s.s.) to Raspailiidae (previously classified to the order Poecilosclerida, Hooper 2002b), as has been indicated previously by several ribosomal and mitochondrial analyses (Erpenbeck et al. 2007a; Erpenbeck et al. 2007b; Erpenbeck, et al. 2007c; Holmes and Blanch 2007). Species of Raspailiidae (mostly) possess a typical ectosomal skeleton, consisting of small thin spicules forming bouquets around long styles, or oxeas that penetrate the surface, in combination

with echinating acanthostyles in the choanosomal skeleton (Hooper 2002b). However, these features are found neither in *Ptilocaulis* nor in *Epipolasis*, for which specimens are recovered among raspailiids. *Epipolasis* was assigned to the Halichondriidae based on its confused choanosomal skeleton and an ectosomal crust of intercrossing spicules (Erpenbeck and Van Soest 2002). The molecular results lack a satisfactory morphological explanation, yet they support earlier hypotheses on the homoplasious nature of extra-axially plumo-reticulate and axially compressed skeletons, as found in axinellids, raspailiids and other (e.g. hadromerid) taxa (Hooper 1991).

Our reconstruction corroborates the polyphyly of heteroxyid sponges (Erpenbeck et al. 2005a, b; Morrow et al. 2011). *Didiscus* is related more closely to Raspailiidae than to other heteroxyid taxa (Erpenbeck et al. 2007b). This tree also indicates, for the first time, the phylogenetic position of *Higginsia* (close to *Halicnemis* and former hemiasterellid taxa, recently combined as Stelligeridae, Morrow et al. 2011).

In conclusion, COI underlines the polyphyletic nature of the halichondrids, one of the most pivotal and controversial groups in demosponge classification. This result supports previous, predominantly ribosomal DNA-based, analyses. Despite the fact that the lower resolution power of COI for deeper splits has been described in detail (Lavrov et al. 2008), COI gene trees clearly contain sufficient signal to analyse the extend of halichondrid polyphyly. Indeed, this is particularly emphasised when our COI gene tree is congruent to trees based on independent genes such as nuclear ribosomal sequences (18S, 28S, ITS). Independent loci likely reflect different evolutionary histories and substitution patterns (see e.g. Moore 1995), therefore congruent topologies provide strong evidence for “correctness” and should be regarded as more important than high branch support, which solely measures the power of the underlying characters (e.g. Felsenstein 1985).

The results from our study highlight the need for stringent typification in phylogenetic studies. Molecular phylogenetic studies that aim to contribute to the classification of sponges require clear definition of the type species. This should not necessarily be based solely on the type material, which is often unavailable because of historical factors (specimens lost, destroyed, inadequately or inappropriately fixed) or cultural reasons (museum regulations), but also on genetic and morphometric variation of the type species in their type localities. It is certainly insufficient to raise new taxa based on single specimens, without proper comparisons of the intra-population variability and the likelihoods of misidentifications.

Importantly, through this study, we see that the evolution of demosponge, and particularly halichondrid taxa, underwent scrutiny from several different viewpoints. Early views focused on megascleres, with more importance on microscleres (e.g. von Lendenfeld 1887), with later views stressing other

features (e.g. Lévi 1951). These different viewpoints were likely to be triggered by unsatisfying earlier classifications that originated from the paucity of phylogenetically relevant sponge characters, and reflect the struggles of early workers to find these characters to establish a robust sponge classification. Nevertheless, it is difficult to extract particular historic schemes of the authors, which would fit the current molecular hypotheses in more than just a few higher taxa.

The phylogeny inferred by Morrow et al. (2011) unites most of the currently proposed molecular hypotheses as far as it concerns the halichondrids and summarizes several hypotheses, which have never before been discussed. Their study agrees in large part with the findings presented here. Together, these molecular studies provide strong evidence that molecular methods, when they are associated with good and correct taxonomic identifications (strengthened by the process of typification and morphological vouchering), can provide alternative ways of interpreting morphological data. Importantly, these studies also (as frequently underestimated) demonstrate that thorough and careful morphological classifications can be “right” and natural.

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