Towards a DNA taxonomy of Caribbean demosponges: a gene tree reconstructed from partial mitochondrial CO1 gene sequences supports previous rDNA phylogenies and provides a new perspective on the systematics of Demospongiae

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We present the most comprehensive cytochrome oxidase subunit 1 gene tree published to date for demosponges based on new sequences. The CO1 barcoding fragment is sequenced for 65 species from the Caribbean Sea, and its gene tree reconstructed. Although its deeper nodes are not particularly well-supported, the gene tree provides a variety of information for new phylogenetic patterns, as well as support for previously published 28S rDNA gene trees. In our analysis Halichondriidae cluster with Suberitidae, supporting previous 28S rDNA data. Chelae-bearing Poecilosclerida are monophyletic but most taxa lacking chelae in this dataset cluster more distantly. Haplosclerida are not resolved monophyletically under this fragment. While some species exhibit distinct barcodes, some genera contain species that share CO1 haplotypes.

INTRODUCTION

The high degree of morphological simplicity and plasticity of characters used for taxonomy in sponges resulted in difficulties in sponge classification at most taxonomic levels. In particular, the distinction of sponges at species level appears frequently problematic, even for experienced taxonomists. These acknowledged limitations of classification based on traditional morphological methods recently stimulated the development of molecular approaches to species identification and the unravelling of species complexes in sponges, especially with mitochondrial markers (e.g. Wörheide et al., 2000), in particular the cytochrome oxidase subunit 1 (CO1) (e.g. Duran et al., 2002; Duran & Rützler, 2006). Recently, a concerted, international initiative the 'Sponge Barcoding Project' (SBP) has been launched to systematically 'tag' sponge species with their CO1 (and other DNA-) signature sequence with the aim to establish a DNA-assisted taxonomy, in congruence with established barcoding initiatives for other organismal taxa (see http://www.spongebarcoding.org and Wörheide & Erpenbeck (this volume) for detailed and related information). Prior to this, a first CO1 DNA-taxonomy campaign had been attempted from the the Smithsonian Marine Station in Fort Pierce, FL, which designed a preliminary sponge barcoding database (Duran, Rützler and Paul: 'DNATaxPor', first presented at the workshop on Barcoding and Molecular Ecology in September 2005 at the Smithsonian Tropical Research Institution, STRI, Panama). It initially comprised sequences of 177 sponge taxa from the Caribbean Sea, but

has recently been merged with the SBP and the data are publicly available in the 'Sponge Barcoding Database'.

The sequences of the former 'DNATaxPor' database are the most comprehensive mitochondrial dataset ever generated for sponges for a defined geographical location to date, as well as the largest mitochondrial gene-dataset generated for sponges in general. In this paper, we analyse 166 sponge sequences from Duran et al.'s data phylogenetically to reconstruct a CO1 gene tree of representative Caribbean Sea demosponges, with the aim to evaluate the phylogenetic resolution capacity of CO1 fragments in Demospongiae and its potential to establish a DNA taxonomic system.

MATERIALS AND METHODS

The list of specimens analysed in this study is provided in Table 1. All the specimens were photographed underwater before being sampled. All sampled specimens were divided into two fragments, one fragment was preserved in 100% ethanol and kept at –20°C until DNA was extracted, the other fragment was fixed in 10% formalin for several hours and then changed to 70–80% ethanol as a voucher for taxonomic studies. All vouchers have been deposited in the National Museum of Natural History, at the Smithsonian Institution, Washington DC, USA. A total of 166 specimens belonging to 65 sponge species from different Caribbean regions and habitats have been sequenced using the universal CO1 primers (Folmer et al., 1994) and procedures described in Duran & Rützler (2006), generating a 584 base pairs fragment of mitochondrial CO1. All sequences are submitted

Table 1. *Species list with their original sample code as provided in GenBank and the Sponge Barcoding Database.*

Order, Suborder, Family	Genus	Species	Code
Agelasida, Agelasidae	*Agelas*	*clathrodes*	B195
Agelasida, Agelasidae	*Agelas*	*clathrodes*	K42
Agelasida, Agelasidae	*Agelas*	*clathrodes*	S29
Agelasida, Agelasidae	*Agelas*	*conifera*	B200
Agelasida, Agelasidae	*Agelas*	*conifera*	B201
Agelasida, Agelasidae	*Agelas*	*conifera*	B59
Agelasida, Agelasidae	*Agelas*	*conifera*	S2
Agelasida, Agelasidae	*Agelas*	*dispar*	B42
Agelasida, Agelasidae	*Agelas*	*dispar*	B75
Agelasida, Agelasidae	*Agelas*	*dispar*	SP1
Agelasida, Agelasidae	*Agelas*	*schmidti*	BH28
Agelasida, Agelasidae	*Agelas*	*schmidti*	K10
Agelasida, Agelasidae	*Agelas*	*schmidti*	K41
Agelasida, Agelasidae	*Agelas*	*clathrodes*	B31
Astrophorida, Geodiidae	*Geodia*	aff. *gibberosa*	B27
Astrophorida, Geodiidae	*Sidonops*	*neptuni*	B74
Astrophorida, Geodiidae	*Sidonops*	*neptuni*	K44
Chondrosida, Chondrillidae	*Chondrilla*	*nucula*	B106
Chondrosida, Chondrillidae	*Chondrilla*	*nucula*	BH13
Chondrosida, Chondrillidae	*Chondrilla*	*nucula*	K18
Chondrosida, Chondrillidae	*Chondrilla*	*nucula*	K24
Chondrosida, Chondrillidae	*Chondrilla*	*nucula*	K35
Chondrosida, Chondrillidae	*Chondrilla*	*nucula*	K36
Chondrosida, Chondrillidae	*Chondrilla*	*nucula*	K80
Chondrosida, Chondrillidae	*Chondrilla*	*nucula*	S35
Chondrosida, Chondrillidae	*Chondrilla*	*nucula*	S36
Chondrosida, Chondrillidae	*Chondrilla*	*nucula*	S47
Chondrosida, Chondrillidae	*Chondrilla*	*nucula*	S9
Chondrosida, Chondrillidae	*Chondrilla*	*nucula*	K1
Chondrosida, Chondrillidae	*Chondrilla*	*nucula*	K29
Chondrosida, Chondrillidae	*Chondrilla*	*nucula*	K3
Chondrosida, Chondrillidae	*Chondrilla*	*nucula*	K30
Chondrosida, Chondrillidae	*Chondrilla*	*nucula*	S37
Chondrosida, Chondrillidae	*Chondrosia*	sp.	P12
Chondrosida, Chondrillidae	*Chondrosia*	sp.	P8
Dendroceratida, Darwinellidae	*Chelonaplysilla*	*erecta*	STAVFP9
Dictyoceratida, Dysideidae	*Pleraplysilla*	sp.	K34
Dictyoceratida, Irciniidae	*Ircinia*	*campana*	K39
Hadromerida, Clionaidae	*Cliona*	aff. *celata*	STAVFP13
Hadromerida, Clionaidae	*Cliona*	*delitrix*	B67
Hadromerida, Clionaidae	*Cliona*	*delitrix*	K53
Hadromerida, Clionaidae	*Cliona*	*delitrix*	S28
Hadromerida, Clionaidae	*Pione*	*vastifica*	STAVFP1
Hadromerida, Clionaidae	*Pione*	*vastifica*	STAVFP7
Hadromerida, Suberitidae	*Suberites*	*aurantica*	STAIFP8
Hadromerida, Suberitidae	*Suberites*	*aurantica*	STAIIFP3
Hadromerida, Suberitidae	*Suberites*	*aurantica*	STAIIFP4
Hadromerida, Suberitidae	*Suberites*	*aurantica*	STAIIFP4B
Hadromerida, Suberitidae	*Suberites*	*aurantica*	STAIFP3
Hadromerida, Suberitidae	*Suberites*	*aurantica*	STAIIFP2
Halichondrida, Axinellidae	*Ptilocaulis*	*marquezi*	K47
Halichondrida, Dictyonellidae	*Svenzea*	*zeai*	B28
Halichondrida, Dictyonellidae	*Svenzea*	*zeai*	S32
Halichondrida, Dictyonellidae	*Scopalina*	*ruetzleri*	BH22
Halichondrida, Dictyonellidae	*Scopalina*	*ruetzleri*	K69
Halichondrida, Dictyonellidae	*Scopalina*	*ruetzleri*	S13
Halichondrida, Dictyonellidae	*Scopalina*	*ruetzleri*	S27
Halichondrida, Halichondriidae	*Ciocalypta*	sp.	STAIVFP2
Halichondrida, Halichondriidae	*Halichondria*	*magniconulosa*	B11
Halichondrida, Halichondriidae	*Halichondria*	*magniconulosa*	S11
Halichondrida, Halichondriidae	*Halichondria*	*melanodocia*	STAIFP4
Halichondrida, Halichondriidae	*Halichondria*	*melanodocia*	K33

Table 1. (*Continued.*)

Halichondrida, Halichondriidae	*Hymeniacidon*	*heliophila*	STAIVFP6
Halichondrida, Halichondriidae	*Hymeniacidon*	*heliophila*	STAVFP12
Halichondrida, Halichondriidae	*Hymeniacidon*	*heliophila*	STAVFP5
Halichondrida, Halichondriidae	*Hymeniacidon*	*heliophila*	STAVFP2
Halichondrida, Heteroxyidae	*Myrmekioderma*	*gyroderma*	B109
Haplosclerida, Callyspongiidae	*Callyspongia*	*armigera*	B193
Haplosclerida, Callyspongiidae	*Callyspongia*	*vaginalis*	BH29
Haplosclerida, Callyspongiidae	*Callyspongia*	*vaginalis*	SP23
Haplosclerida, Callyspongiidae	*Callyspongia*	*vaginalis*	B95
Haplosclerida, Callyspongiidae	*Callyspongia*	*vaginalis*	S42
Haplosclerida, Chalinidae	*Haliclona*	*coerulea*	STAVFP6
Haplosclerida, Chalinidae	*Haliclona*	*implexiformis*	B14
Haplosclerida, Chalinidae	*Haliclona*	*implexiformis*	B190
Haplosclerida, Chalinidae	*Haliclona*	*implexiformis*	STAIFP1
Haplosclerida, Chalinidae	*Haliclona*	*implexiformis*	STAIFP2
Haplosclerida, Chalinidae	*Haliclona*	*implexiformis*	STAIFP6
Haplosclerida, Chalinidae	*Haliclona*	*manglaris*	S15
Haplosclerida, Chalinidae	*Haliclona*	*tubifera*	K32
Haplosclerida, Niphatidae	*Amphimedon*	*compressa*	K37
Haplosclerida, Niphatidae	*Amphimedon*	*compressa*	K43
Haplosclerida, Niphatidae	*Amphimedon*	*compressa*	S34
Haplosclerida, Niphatidae	*Cribrochalina*	*vasculum*	B192
Haplosclerida, Niphatidae	*Niphates*	*alba*	B50
Haplosclerida, Niphatidae	*Niphates*	*digitalis*	BH30
Haplosclerida, Niphatidae	*Niphates*	*digitalis*	K51
Haplosclerida, Niphatidae	*Niphates*	*digitalis*	S3
Haplosclerida, Niphatidae	*Niphates*	*digitalis*	S40
Haplosclerida, Niphatidae	*Niphates*	*erecta*	B179
Haplosclerida, Niphatidae	*Niphates*	*erecta*	BH27
Haplosclerida, Niphatidae	*Niphates*	*erecta*	S41
Haplosclerida, Petrosiidae	*Petrosia*	aff. *dura*	B205
Haplosclerida, Petrosiidae	*Xestospongia*	*muta*	K15
Haplosclerida, Petrosiidae	*Xestospongia*	*muta*	K20
Haplosclerida, Petrosiidae	*Xestospongia*	*muta*	K65
Haplosclerida, Petrosiidae	*Xestospongia*	*muta*	S1
Haplosclerida, Petrosiidae	*Xestospongia*	*muta*	BH8
Haplosclerida, Phleodictyidae	*Aka*	sp.	S24
Homoslerophorida, Plakinidae	*Plakortis*	*angulospiculatus*	B47
Homoslerophorida, Plakinidae	*Plakortis*	*angulospiculatus*	B71
Poecilosclerida, Microcionina, Microcionidae	*Artemisina*	*melana*	B94
Poecilosclerida, Microcionina, Microcionidae	*Artemisina*	*melana*	B97
Poecilosclerida, Microcionina, Microcionidae	*Clathria*	*schoenus*	S18
Poecilosclerida, Microcionina, Microcionidae	*Holopsamma*	*helwigi*	B78
Poecilosclerida, Microcionina, Microcionidae	*Holopsamma*	*helwigi*	S7
Poecilosclerida, Microcionina, Microcionidae	*Pandaros*	*acanthifolium*	B185
Poecilosclerida, Microcionina, Raspailiidae	*Ectyoplasia*	*ferox*	K12
Poecilosclerida, Microcionina, Raspailiidae	*Ectyoplasia*	*ferox*	K2
Poecilosclerida, Mycalina, Desmacellidae	*Neofibularia*	*nolitangere*	B183
Poecilosclerida, Mycalina, Mycalidae	*Mycale*	*laxissima*	B30
Poecilosclerida, Mycalina, Mycalidae	*Mycale*	*laxissima*	K79
Poecilosclerida, Mycalina, Mycalidae	*Mycale*	*laxissima*	S44
Poecilosclerida, Myxillina, Coelosphaeridae	*Lissodendoryx*	*isodictyalis*	K31
Poecilosclerida, Myxillina, Coelosphaeridae	*Lissodendoryx*	*sigmata*	STAIVFP3
Poecilosclerida, Myxillina, Coelosphaeridae	*Lissodendoryx*	sp.	B5
Poecilosclerida, Myxillina, Coelosphaeridae	*Lissodendoryx*	sp.	S12
Poecilosclerida, Myxillina, Coelosphaeridae	*Lissodendoryx*	sp.	STAIIIFP1
Poecilosclerida, Myxillina, Coelosphaeridae	*Lissodendoryx*	sp.	K27
Poecilosclerida, Myxillina, Coelosphaeridae	*Lissodendoryx*	sp.	S19
Poecilosclerida, Myxillina, Crambeidae	*Clathria*	*oxeota*	B66
Poecilosclerida, Myxillina, Crambeidae	*Clathria*	*oxeota*	BH21
Poecilosclerida, Myxillina, Crambeidae	*Monanchora*	*arbuscula*	B110
Poecilosclerida, Myxillina, Crambeidae	*Monanchora*	*arbuscula*	S31
Poecilosclerida, Myxillina, Crambeidae	*Monanchora*	*arbuscula*	B77
Poecilosclerida, Myxillina, Crambeidae	*Monanchora*	*arbuscula*	K45
Poecilosclerida, Myxillina, Iotrochotidae	*Iotrochota*	*birotulata*	K7

Table 1. (*Continued.*)

Poecilosclerida, Myxillina, Iotrochotidae	*Iotrochota*	*birotulata*	K9
Poecilosclerida, Myxillina, Iotrochotidae	*Iotrochota*	*birotulata*	S45
Poecilosclerida, Myxillina, Iotrochotidae	*Iotrochota*	*birotulata*	SP13
Poecilosclerida, Myxillina, Tedaniidae	*Tedania*	*ignis*	B13
Poecilosclerida, Myxillina, Tedaniidae	*Tedania*	*ignis*	BH19
Poecilosclerida, Myxillina, Tedaniidae	*Tedania*	*ignis*	K28
Poecilosclerida, Myxillina, Tedaniidae	*Tedania*	*ignis*	S8
Poecilosclerida, Myxillina, Tedaniidae	*Tedania*	*ignis*	STAIVFP1
Poecilosclerida, Myxillina, Tedaniidae	*Tedania*	*ignis*	STAVFP4
Spirophorida, Tetillidae	*Cinachyrella*	*apion*	B25
Spirophorida, Tetillidae	*Cinachyrella*	*kuekenthali*	B79
Spirophorida, Tetillidae	*Cinachyrella*	*kuekenthali*	K75
Verongida, Aplysinidae	*Aplysina*	*archeri*	B199
Verongida, Aplysinidae	*Aplysina*	*archeri*	K57
Verongida, Aplysinidae	*Aplysina*	*cauliformis*	B194
Verongida, Aplysinidae	*Aplysina*	*cauliformis*	K49
Verongida, Aplysinidae	*Aplysina*	*cauliformis*	K70
Verongida, Aplysinidae	*Aplysina*	*cauliformis*	S39
Verongida, Aplysinidae	*Aplysina*	*cauliformis*	SP7
Verongida, Aplysinidae	*Aplysina*	*fulva*	K48
Verongida, Aplysinidae	*Aplysina*	*insularis*	BH5
Verongida, Aplysinidae	*Aplysina*	*insularis*	K74
Verongida, Aplysinidae	*Aplysina*	*archeri*	K38
Verongida, Aplysinidae	*Aplysina*	*cauliformis*	BH9
Verongida, Aplysinidae	*Aplysina*	*fistularis*	B46
Verongida, Aplysinidae	*Aplysina*	*fistularis*	BH7
Verongida, Aplysinidae	*Aiolochroia*	*crassa*	B187
Verongida, Aplysinidae	*Aiolochroia*	*crassa*	K6
Verongida, Aplysinidae	*Aiolochroia*	*crassa*	K72
Verongida, Aplysinidae	*Aiolochroia*	*crassa*	K76
Verongida, Aplysinidae	*Aiolochroia*	*crassa*	S5
Verongida, Aplysinidae	*Aiolochroia*	*crassa*	SP4
Verongida, Aplysinidae	*Verongula*	*gigantea*	BH18
Verongida, Aplysinidae	*Verongula*	*reiswigi*	SP8
Verongida, Aplysinidae	*Verongula*	*rigida*	B156
Verongida, Aplysinidae	*Verongula*	*rigida*	B43
Verongida, Aplysinidae	*Verongula*	*rigida*	BH17
Verongida, Aplysinidae	*Verongula*	*rigida*	K46
Verongida, Aplysinidae	*Verongula*	*rigida*	S46

to GenBank (www.ncbi.nlm.nih.gov, Accession numbers EF519536-EF519701) and the Sponge Barcoding Database of the SBP (www.spongebarcoding.org, Accession numbers 1–165).

Sequences were managed with MacClade v. 4.06 (Maddison & Maddison, 1992). Alignment was unambiguous due to the protein coding nature of the sequences and was performed manually.

Bayesian analyses on nucleotides were run on the parallel version of MrBayes v. 3.1.2 (Altekar et al., 2004) on a Linux cluster at the Gesellschaft für Wissenschaftliche Datenverarbeitung Göttingen (GWDG), Germany (http://www.gwdg.de), with one processor assigned to each Markov chain. Each Bayesian analysis comprised at least two simultaneous runs of four Metropolis-coupled Markov-chains at the default temperature (0.2). Analyses were terminated either after a maximum of 10,000,000 generations, or after a maximum wall-time of 48 hours, or after the chains converged significantly, as indicated by an average standard deviation of split frequencies

<0.01. We performed the analysis with all three codon positions as well as with the third position excluded. For comparison, Maximum Likelihood bootstrap analyses were conducted using GARLI v. 0.94 (Zwickl, 2006) using a heuristic search with the default option, i.e. under the GTR+G+I of nucleotide substitution under 100 bootstrap replicates model with estimated parameters. Sequences of the Homosclerophorida *Plakortis angulospiculatus* have been chosen as outgroup for all analyses.

RESULTS AND DISCUSSION

The resulting data set comprised 167 taxa and 584 characters. The gene tree representing the results of the different reconstruction methods and approaches is displayed in Figure 1. The different individual topologies are congruent in the higher branches. In contrast, some topological differences are evident between the deeper nodes, where internal splits are frequently weakly supported. The following patterns are consistent among all reconstruction methods and are discussed in further detail:
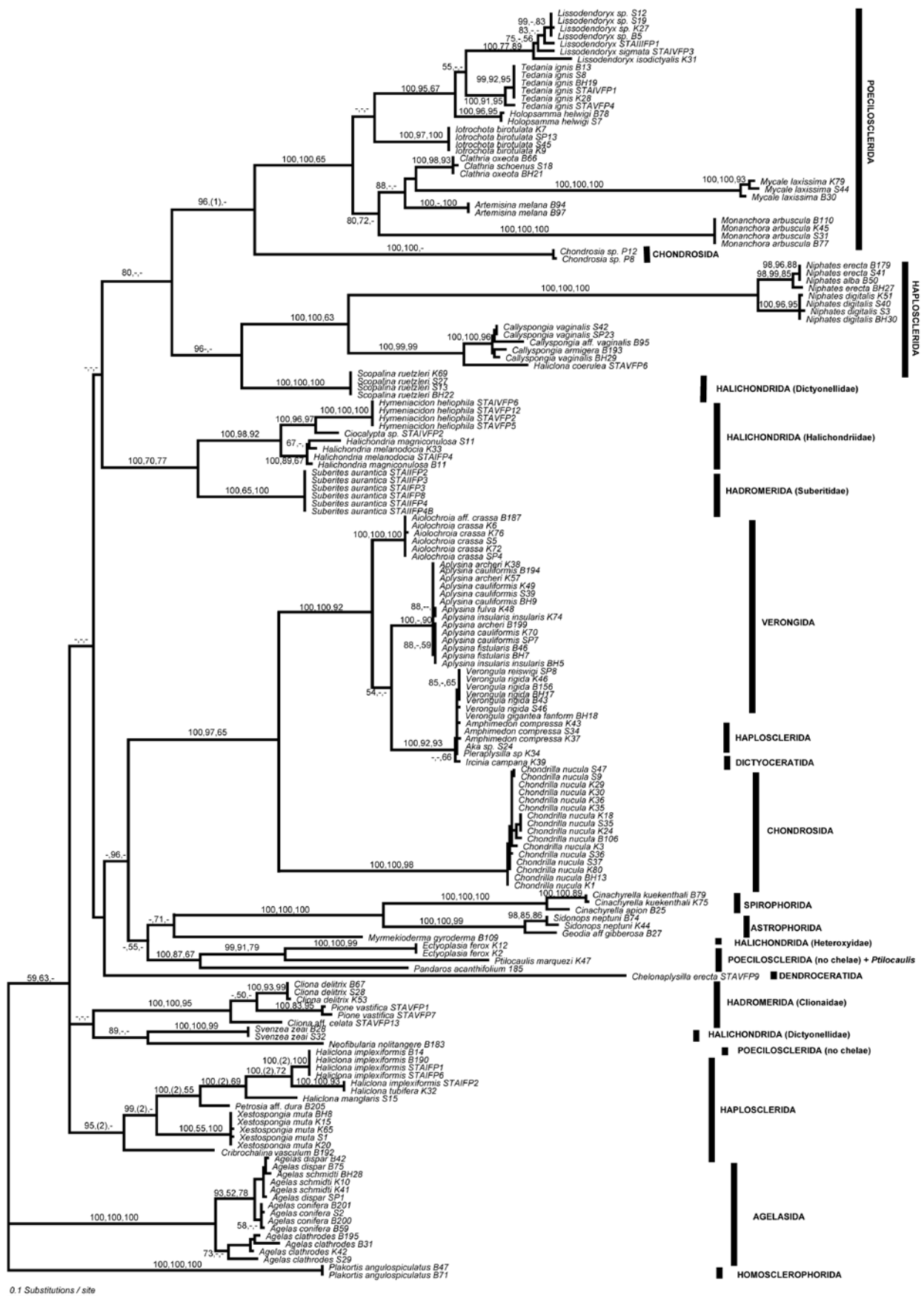
Figure 1. Maximum likelihood phylogram of the present analysis. The taxon names are followed by their sample code as submitted to the Sponge Barcoding Database. Numbers on the branches refer to posterior probabilities and bootstrap values of the following analyses: Bayesian inference with all characters (left); Bayesian inference of the first and second position only (middle); maximum likelihood bootstrap with all characters. The support values of some terminal splits are not displayed to maintain the readability of the figure. Branches with support lower than 50 or incongruent are denoted with a dash. (1) Branch present in this analysis, but including *Scopalina* spp., *Chondrosia* spp. and *Chelonaplysilla erecta*; (2) branch present in this analysis, but including *Neofibularia nolitangere* and/or *Svenzea* spp.

Halichondriidae–Suberitidae clustering

The sequences of *Halichondria* (*Halichondria*) *melanodocia*, *Halichondria* (*Halichondria*) *magniconulosa*, *Hymeniacidon heliophila* and *Ciocalypta* sp. cluster monophyletically as Halichondriidae, but form a well-supported sister-group relationship with the Suberitidae sequences. Closer Halichondriidae–Suberitidae relationships have been hypothesized based on 28S rDNA (Chombard & Boury-Esnault, 1999) but repeatedly discussed as artefacts of the 28S rDNA phylogenies (e.g. Erpenbeck, 2004) due to alternative data sets failing to provide independent corroboration for such a constellation (Erpenbeck et al., 2006). Suberitidae, however, are represented in the current dataset only by specimens of one species (*Suberites aurantica*) and additional taxa would certainly be necessary to further support a Suberitidae and Halichondriidae relationship and previous 28S rDNA results. As molecular (and biochemical) data could not support the clustering of Suberitidae with other Hadromerida to date (Nichols, 2005), a merging of halichondrids with the order Hadromerida, as suggested by Chombard & Boury-Esnault (1999), is not warranted as yet, but a closer relationship between Halichondriidae and Suberitidae should not be completely disregarded any longer. However, studies on sequences of a downstream fragment of CO1 did not support such patterns either (Erpenbeck et al., 2006), and the origins of these differences are under investigation (Erpenbeck et al., unpublished data). Suberitidae differ morphologically from many (but not all) other hadromerid families, e.g. by the absence of microscleres (other than trichodragmata and microrhabds in some) and the absence of a pronounced cortex (van Soest, 2002). Furthermore, in several genera including the type genus *Suberites*, a 'classical' hadromerid radiate skeleton is apparent only at the periphery and becomes rather halichondrid-confused towards the interior.

It should be noted, that the order Halichondrida *sensu* van Soest & Hooper, (2002) does not form a monophylum in gene trees based on this CO1 fragment either. In other, previous analyses 28S rDNA, EF1-alpha and a downstream fragment of CO1 could not support monophyly of the Halichondrida and their families (Erpenbeck, 2004). In the present analyses only the representatives of the halichondrid families Dictyonellidae (*Svenzea zeai* and *Scopalina ruetzleri*), Heteroxyidae (*Myrmekioderma gyroderma*) and Axinellidae (*Ptilocaulis marquesi*) cluster distantly from each other. However, a putative polyphyly of Halichondrida remains to be corroborated with gene fragments evolving at more suitable substitution rates for resolving deeper nodes.

Poecilosclerida phylogeny

Poecilosclerida is by far the largest order of demosponges and comprises some 25 families and 129 genera (Hooper & van Soest, 2002). Despite their obvious important position in the tree of demosponges, the entity of this taxon and its internal phylogenetic relationships have not been analysed with molecular data. Current published phylogenies, which aim to provide a general overview on demosponge systematics, frequently consist of a less representative set of poecilosclerid taxa (Borchiellini et al., 2004; Nichols, 2005). Poecilosclerida is regarded as a monophyletic

taxon particularly due to the common possession of chelae microscleres in most of its genera. These chelae are unique features of sufficient complexity such that they have been regarded as an autapomorphy of the order Poecilosclerida. Some non-chelae bearing taxa such as Raspailiidae or Desmacellidae are assigned to Poecilosclerida because of other similarities in skeletal arrangement and spiculation (see Hooper & van Soest, 2002 for details).

The present CO1 gene tree reconstruction cannot recover a monophyletic Poecilosclerida *sensu* Hooper & van Soest, 2002. There is a well-supported clade comprising *Lissodendoryx* spp., *Tedania ignis*, *Holopsamma helwigi*, *Iotrochota birotulata*, *Clathria* spp., *Mycale laxissima*, *Artemisina melana* and *Monanchora arbuscula*. With the exception of *Tedania ignis* these taxa are all chelae-bearing. Interestingly, the other poeciloscleid sequences, which are not included in this clade, lack this characteristic microsclere: *Ectyoplasia ferox* (Raspailiidae), *Pandaros acanthifolium* (Microcionidae) and *Neofibularia nolitangere* (Desmacellidae)—all from different suborders and/or families. Nevertheless, their apparent polyphyly does not necessarily imply their non-poecilosclerid origin, because the deeper nodes are insufficiently resolvable by the CO1 fragment. The splits between the chelae-bearing and subsequently lacking taxa might be too ancient to be resolved correctly by the CO1 fragment. An independent loss of chelae in *Tedania* could have taken place relatively recently.

However, the monospecific genus *Pandaros* is discussed as a 'borderline taxon that could be legitimately included in either Raspailiidae or Microcionidae' (Hooper, 2002). This estimation is supported in the present analysis by the well-supported clustering of *Pandaros acanthifolium* close to *Ectyoplasia ferox*, the only Raspailiidae in the current taxa set. Van Soest (1984) furthermore observed morphological similarities of *Pandaros* with *Ptilocaulis* (Halichondrida: Axinellidae). Our CO1 gene tree results in a well-supported clade combining *Pandaros acanthifolium* with *Ectyoplasia ferox* and *Ptilocaulis marquezi*. In congruence to this pattern, 28S rDNA gene trees provide independent evidence for a close relationship of *Ptilocaulis* to Raspailiidae (Erpenbeck et al., this volume).

Haplosclerida phylogeny

A monophyly of the order Haplosclerida cannot be shown under the given CO1 fragment either. The Haplosclerida are scattered over several different positions on the tree. However, Haplosclerida clades do not comprise similar families, or their suborders. The largest CO1 haplosclerid clade suggests a monophyletic *Haliclona* spp. clade (*H. implexiformis*, *H. tubifera* and *H. manglaris*, Haplosclerina: Chalinidae) with more basal paraphyletic *Xestospongia muta* and *Petrosia* spp. (both Petrosina: Petrosiidae). The genus *Haliclona* is not retained monophyletically, because *H. coerulea* forms a sister taxon to the *Callyspongia* spp. clade (*C. vaginalis* and *C. armigera*, Haplosclerina: Callyspongiidae) with *Niphates* spp. (*N. alba*, *N. erecta* and *N. digitalis*, Haplosclerina: Niphatidae) branching off earlier. Two further taxa, *Aka* sp. and *Amphimedon compressa* (Petrosina: Phloeodictyidae and Niphatidae respectively) cluster with Verongida and Dictyoceratida distant from all other Haplosclerida.

Alternative gene trees such as 28S rDNA and 18S rDNA (e.g. McCormack et al., 2002; Redmond et al., 2007) repeatedly showed the monophyly of (marine) Haplosclerida, but the internal topologies did not resemble the morphological classification either. A demosponge-wide comparison of 28S rDNA sequences indicated a significantly higher evolutionary rate (Erpenbeck et al., 2004). Similarly, the first published mitochondrial genome of a haplosclerid demosponge revealed a higher mtDNA evolutionary rate (Erpenbeck et al., 2007). It is therefore evident that the present CO1 barcoding fragment would not be suitable to resolve Haplosclerida relationships sufficiently. Instead, the increased evolutionary rate leads to the formation of homoplasies, which cannot be detected and filtered out even with the present phylogeny reconstruction algorithms.

Further patterns

Verongida cluster in a well supported manner, forming an *Aplysina* spp. clade, an *Aiolochroia crassa* clade and a *Verongula* sp. clade. However, the latter clade also contains non-verongid sequences such as the above-mentioned Haplosclerida *Aka* sp. and *Amphimedon compressa*, and furthermore the dictyoceratids *Ircinia campana* and *Pleraplysilla* sp. The *Aplysina* species *A. insularis*, *A. fulva*, *A. archeri*, *A. fistularis* and *A. cauliformis* do not fall into discrete clades but are intermingled. Assuming the *Aplysina* species are 'good' species, this indicates problematic scenarios for DNA barcoding when only this single CO1 fragment is employed.

A well-supported sister group to the Verongida clade is formed by *Chondrilla nucula* (Chondrosida: Chondrillidae). This is in congruence to previously published ribosomal DNA phylogenies (Borchiellini et al., 2004), as is the distant clustering of the two Chondrillidae genera *Chondrilla* and *Chondrosia* (see also Borchiellini et al., 2004 on this topic).

Another well-supported clade combines taxa of the order Astrophorida (*Sidonops neptuni* and *Geodia gibberosa*, both Geodiidae) with *Cinachyrella* spp. of the order Spirophorida (*C. kuekenthali* and *C. apion*). Again, this configuration supports rDNA data (Chombard et al., 1998).

Agelas spp. form a strongly supported monophyletic clade and its internal relationship confirms the phylogenetic results of Parra-Velandia et al. (2006) on this genus.

CONCLUSIONS

The present gene tree based on CO1 fragments sheds new light on poriferan molecular phylogenies. While mitochondrial data were frequently seen as being too uninformative and in contradiction with the more frequently used rDNA data, the present tree not only provides support for existing 28S and 18S rDNA phylogenies (e.g. Suberitidae/Halichondriidae), it also opens up new scenarios for demosponge systematics (e.g. chelae-bearing against chelae-lacking Poecilosclerida taxa). The CO1 fragment is certainly not suitable to resolve deeper demosponge splits, as its power of resolution is too low. Additional markers are required to unravel the deeper nodes and suitable alternative genes should be recruited to double-check the remaining discrepancies between molecular and morphological results, in particular for ancient taxa such as sponges. Nevertheless, CO1 can provide insight into clustering at lower taxonomic

levels, from which further hypotheses could be tested. Certainly the present data set, although currently the largest presently available for demosponges, cannot provide definitive answers and support for all the patterns observed as it is just one gene tree amongst others. However, the addition of further taxa (e.g. Sponge Barcoding Project), with alternative genes and new, sophisticated reconstruction methods will bring the understanding of evolution of early branching Metazoa considerably further.

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