Affinities of the family Sollasellidae (Porifera, Demospongiae). II. Molecular evidence

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Abstract

This is the second part of a revision and re-classification of the demosponge family Sollasellidae, and an example of a successful use of combined morphological and molecular data. *Sollasella* had been a poorly known, long forgotten taxon, placed *incertae sedis* in the order Hadromerida in the last major revision of the demosponges. It has recently been suggested to belong to Raspailiidae in the order Poecilosclerida due to striking morphological similarities. The present analysis verified this re-classification using molecular markers. Comparing 28S rDNA fragments of *Sollasella cervicornis*, a newly described species *S. moretonensis* and a representative set of raspailiid and hadromerid samples. In our analyses *Sollasella* clearly clusters inside the Raspailiidae clade, and distantly from hadromerid taxa. Supporting morphological hypothesis of Van Soest *et al.* (2006), that *Sollasella* is a raspailiid sponge.

Contents

Introduction

A morphological re-examination of material of the demosponge family Sollasellidae has shed a new light on its classification (Van Soest *et al.,* 2006). This monospecific, previously poorly known family, with the type locality in East Australia (*Sollasella digitata* Lendenfeld, 1888), had been nearly completely forgotten since Hall-

mann (1914), and consequently assigned more recently as *incertae sedis* in the order Hadromerida based on its cortex and radiating skeleton (Hooper and Van Soest, 2002). However, the recent collection and examination of a morphologically similar sponge from Oman identified as *Raspailopsis cervicornis* Burton, 1959 (= *Raspailia (Parasyringella) cervicornis* sensu Hooper, 2002b), raised evidence for a placement of *Sollasella* in the family Raspailiidae (order Poecilosclerida, Van Soest *et al.,* 2006). After further morphological analyses, *R. cervicornis* was merged with *Sollasella* forming the twentieth valid genus in the Raspailiidae (Van Soest *et al.,* 2006). In the same publication Van Soest and colleagues describe a similar species from subtropical Australia, *Sollasella moretonensis*, which shares the characteristic polygonal perforation-like surface pattern of *S. cervicornis* and *S. digitata*, in addition to other features. These polygonal surface structures remain a distinguishing feature for the genus.

The aim of our analysis is to test the hypothesis that *Sollasella* is more closely related to poecilosclerid taxa as concluded recently based on morphologic data in the family Raspaillidae than to hadromerid sponges (Van Soest *et al.,* 2006). Among candidate hadromerid taxa are Polymastiidae, with which *Sollasella* shares the presence of a cortex (particularly the genus *Pseudotrachya*, with which *Sollasella* shares the combination of choanosomal styles and ectosomal oxeas), and Suberitidae, with which *Sollasella* shares the stalked habit and axially condensed skeleton (genera *Homaxinella, Plicatellopsis, Rhizaxinella*, see Van Soest, 2002a). There is also an apparent slight affinity with Stylocordylidae (Van Soest, 2002b).

Reliability of morphological systematics in sponges is hampered by the paucity of complex characters, many

of which are also prone to homoplasies. The family Raspailiidae, as in many other demosponge taxa, does not possess unique autapomorphies, and is defined by a combination of characters that may each be found also in some other demosponge orders and families (see Hooper, 2002b). DNA sequence data should provide independent evidence to test morphological hypotheses. However, sponge molecular systematics has pitfalls of its own such that phylogenies arising from these analyses need to be cautiously interpreted pending better insight into demosponge molecular evolution. Molecular data occasionally generate hypotheses that conflict dramatically with phylogenies based on morphological characters (*e.g.,* McCormack *et al.,* 2002; Erpenbeck *et al.,* 2006). Improved algorithms may aid to filter the correct phylogenetic signal from random noise and provide explanations for the occasionally 'odd' phylogenies. Nevertheless, sponge molecular systematics has repeatedly been shown to outcompete morphological approaches (*e.g.* Erpenbeck *et al.,* 2006).

In our present approach we generate and analyse 28S rDNA data from specimens of *Sollasella cervicornis* (sensu Van Soest *et al.,* 2006) from Oman, *Sollasella moretonensis* from northeastern Australia, and several species of other Raspailiidae. The sequences are compared with data sets from Erpenbeck *et al.* (2005) and partially from Nichols (2005), which is currently the most comprehensive 28S rDNA data set with a strong representation of hadromerid sequences.

Material and methods

The list of specimens used in this analysis is given in Table 1. We chose the 28S D3-D5 fragment for which a comprehensive taxon set has been sequenced. However, no amplifiable DNA could be obtained from material of the type specimen of the family Sollasellidae, *Sollasella digitata,* from the Australian Museum in Sydney (AM G9107). Sequences of *Sollasella cervicornis*, *S. moretonensis* and a representative set of Raspailiidae have been recently generated and are deposited in GenBank (see table 1). Their DNA was extracted with Qiagen DNeasy kits. PCR primers employed were taken from McCormack *et al.* (2002) (RD3A: GACCCGTCTTGAAACACGA and RD5B2: ACACACTCCTTAGCGGA, temperature regime: 94°C 2 min, 35 cycles at 94°C 30 sec; 47°C 20 sec; 65°C 30 sec, followed by 65°C 10 min). PCR amplifications contained 11.25 µl ddH$_2$O, 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 4 µl HotMaster polymerase (Eppendorf). The BSA was used only on the *Sollasella moretonensis* DNA extracts and replaced with ddH$_2$O for all other samples. Cycle sequencing of both strands was performed with BigDye terminator v1.1 (ABI) and a capillary sequencer (ABI). MacClade 4.06 (Maddison and Maddison, 1992) was used for sequence management and manual alignment. The sequences were incorporated into two modified alignments based on secondary structure information. They consisted of the full length D3-D5 data set as published in Erpenbeck *et al.* (2005) and a second, shorter data set with additional overlapping sequences of Nichols (2005). As this overlap data set is comparatively short - it resulted in an alignment with 441 characters - we restricted ourselves to incorporate only those sequences of Nichols (2005) with a complete 5' region (see Table 1) and used it for comparative purposes only.

Phylogenetic relationships were reconstructed with Bayesian inference methods (BI) using MrBayes 3.1 (Ronquist and Huelsenbeck, 2003). Potential overparameterization does not influence the correctness of BI reconstructions in MrBayes (Huelsenbeck *et al.,* 2001). Therefore, the GTR+G+I model was chosen for all BI analyses of the D3-D5 data set and non-pairing sites in secondary structure specific analyses with the Nichols' (2005) data set (SH for paring sites, see Erpenbeck *et al.* 2007 for details). Results were compared with Minimum-evolution (ME) reconstructions under Maximum-likelihood (ML) distances. Modeltest 3.06 (Posada and Crandall, 1998) estimated the relatively best-fitting ML-model. Bayesian inference analyses consisted of two runs of four Markov chains each for maximal 10,000,000 generations. Runs were stopped automatically when the average standard deviation of split frequencies dropped below 0.01. All other phylogenetic analyses were performed with PAUP*b10 (Swofford, 2002). The ME bootstrap values were calculated on 1000 replicates on maximum likelihood distances. Different phylogenetic hypotheses were tested using the COUNSEL 0.942 package (Shimodaira and Hasegawa, 2001) under default settings.

Results

The different phylogenetic reconstruction methods of both data sets displayed identical results on the position of the *Sollasella cervicornis* sequence. In the longer

data set this sequence clusters inside a clade comprising all the other Raspailiidae sequences including *Raspailia (Raspailia)*, *R. (Raspaxilla)* and *Eurypon* (Fig. 1). This pattern is supported by high posterior and bootstrap probabilities (BI: 100, ME: 94). Similarly, sequences of the newly described species *Sollasella moretonensis* cluster in the same clade as the Raspailiidae, but the genus *Sollasella* is not recovered as

Table 1. Sample list with the accession numbers for the particular data sets. (New sequences are highlighted in bold.)

Order	Family	Taxon	Acc.Nr.
Agelasida	Agelasidae	*Agelas oroides*	AY319311
Agelasida	Agelasidae	*Agelas* sp.	AY561929
Agelasida	Astroscleridae	*Astrosclera willeyana*	AY561928
Astrophorida	Ancorinidae	*Ecionemia* sp.	AY561938
Astrophorida	Ancorinidae	*Holoxea* sp.	AY561942
Astrophorida	Ancorinidae	*Melophlus* sp.	AY561940
Astrophorida	Ancorinidae	*Rhabdastrella* sp.	AY561939
Astrophorida	Geodiidae	*Geodia media*	AY561937
Astrophorida	*Incertae Sedis*	*Lamellomorpha* sp.	AY561941
Calcaronea	Leucosoleniidae	*Leucosolenia* sp.	AY026372
Chondrosida	Chondrillidae	*Chondrilla australiensis*	AY561867
Chondrosida	Chondrillidae	*Chondrilla* sp.	AY561868
Chondrosida	Chondrillidae	*Chondrosia* sp.	AY561866
Chondrosida	Chondrillidae	*Chondrosia* sp.	AJ005916
Dictyoceratida	Dysideidae	*Lamellodysidea chlorea*	AY561957
Dictyoceratida	**Irciniidae**	***Ircinia ramosa***	**EF507818**
Dictyoceratida	Spongiidae	*Spongia* sp.	AY561951
Hadromerida	Clionaidae	*Cliona* sp.	AY561886
Hadromerida	Clionaidae	*Spheciospongia vagabunda*	AY319310
Hadromerida	Hemiasterellidae	*Hemiasterella* sp. 1	AY561901
Hadromerida	Hemiasterellidae	*Hemiasterella* sp. 2	AY561946
Hadromerida	Hemiasterellidae	*Hemiasterella* sp. 3	AY561947
Hadromerida	Placospongiidae	*Placospongia* sp.	AY561896
Hadromerida	Polymastiidae	*Polymastia invaginata*	AY561922
Hadromerida	Polymastiidae	*Pseudotrachya* sp.	AY561965
Hadromerida	Spirastrellidae	*Diplastrella megastellata*	AY561893
Hadromerida	Suberitidae	*Aaptos suberitoides*	AY319308
Hadromerida	Suberitidae	*Pseudosuberites* sp.	AY561917
Hadromerida	Suberitidae	*Rhizaxinella* sp.	AY561910
Hadromerida	Suberitidae	*Suberites ficus*	AY026381
Hadromerida	Suberitidae	*Suberites suberia*	AY319309
Hadromerida	Tethyidae	*Stellitethya ingens*	AY561899
Hadromerida	Timeidae	*Timea lowchoyi*	AY561871
Hadromerida	Timeidae	*Timea* sp.	AY561907
Halichondrida	Axinellidae	*Axinella damicornis*	AY319314
Halichondrida	Axinellidae	*Axinella polypoides*	AY618728
Halichondrida	Axinellidae	*Axinella verrucosa*	AY319312
Halichondrida	Heteroxyidae	*Didiscus oxeata*	AY319320
Halichondrida	Heteroxyidae	*Didiscus* sp.	AY561948
Halichondrida	Heteroxyidae	*Myrmekioderma granulata*	AY319319
Halichondrida	Dictyonellidae	*Acanthella acuta*	AY319322
Halichondrida	Dictyonellidae	*Acanthella* sp.	AY561936
Halichondrida	Dictyonellidae	*Dictyonella* sp.	AY319325
Halichondrida	Dictyonellidae	*Liosina paradoxa*	AY319318
Halichondrida	Dictyonellidae	*Scopalina lophyropoda*	AY319323
Halichondrida	Dictyonellidae	*Scopalina ruetzleri*	AY561872
Halichondrida	Dictyonellidae	*Stylissa flabelliformis*	AY319316
Halichondrida	Dictyonellidae	*Svenzea zeai*	AF441349
Halichondrida	Halichondriidae	*Amorphinopsis excavans*	AY319313

Halichondrida	Halichondriidae	*Axinyssa aplysinoides*	AY319324
Halichondrida	Halichondriidae	*Halichondria bowerbanki*	AY646836
Halichondrida	Halichondriidae	*Halichondria panicea*	AY319315
Halichondrida	Halichondriidae	*Hymeniacidon perlevis*	AY618715
Haplosclerida	Callyspongiidae	*Callyspongia (Callyspongia) multiformis*	AF441344
Haplosclerida	Callyspongiidae	*Callyspongia (Cladochalina) plicifera*	AF441343
Haplosclerida	Chalinidae	*Haliclona (Gellius) toxius*	AF441342
Haplosclerida	Chalinidae	*Haliclona (Halichoclona) vansoesti*	AF441346
Haplosclerida	Chalinidae	*Haliclona (Haliclona) oculata 2*	AF441330
Haplosclerida	Chalinidae	*Haliclona (Haliclona) oculata 1*	identical
Haplosclerida	Chalinidae	*Haliclona (Soestella) xena*	AY319327
Haplosclerida	Chalinidae	*Haliclona cinerea*	AF441339
Haplosclerida	Niphatidae	*Amphimedon compressa*	AF441351
Haplosclerida	Niphatidae	*Amphimedon paraviridis*	AF441350
Haplosclerida	Niphatidae	*Niphates olemda*	AF441353
Haplosclerida	Niphatidae	*Pachychalina* sp.	AF441352
Haplosclerida	Petrosiidae	*Acanthostrongylophora ashmorica*	AF441354
Haplosclerida	Petrosiidae	*Acanthostrongylophora ingens*	AY319326
Haplosclerida	Petrosiidae	*Neopetrosia subtriangularis*	AF441341
Haplosclerida	Petrosiidae	*Petrosia ficiformis*	AF441347
Haplosclerida	Petrosiidae	*Petrosia* sp.	AY561859
Haplosclerida	Petrosiidae	*Xestospongia caminata*	AF441348
Haplosclerida	Phloeodictyidae	*Aka coralliphaga*	AF441345
Haplosclerida	Phloeodictyidae	*Oceanapia* sp.	AY561857
Homosclerophorida	Plakinidae	*Plakinastrella* sp. 1	AY561869
Homosclerophorida	Plakinidae	*Plakinastrella* sp. 2	AY561870
"Lithistida"	Corallistidae	*Corallistes* sp.	AJ005913
"Lithistida"	Scleritodermidae	*Aciculites* sp.	AY561945
"Lithistida"	Theonellidae	*Discodermia dissoluta*	AJ005914
"Lithistida"	Theonellidae	*Theonella* sp.	AJ005917
"Lithistida"	Vetulinidae	*Vetulina* sp.	AJ005915
Poecilosclerida	Coelosphaeridae	*Lissodendoryx topsenti*	AY561876
Poecilosclerida	Isodictyidae	*Coelocarteria singaporensis*	AY561874
Poecilosclerida	Isodictyidae	*Coelocarteria* sp.	AY561875
Poecilosclerida	Mycalidae	*Mycale fibrexilis*	AY026376
Poecilosclerida	**Raspailiidae**	***Eurypon* sp. G305782**	**EF507817**
Poecilosclerida	**Raspailiidae**	***Raspailia (Raspailia)* sp. G312932**	**EF507819**
Poecilosclerida	**Raspailiidae**	***Raspailia (Raspailia)* n. sp. G315208**	**EF507820**
Poecilosclerida	**Raspailiidae**	***Raspailia (Raspaxilla) topsenti* G312182**	**EF507821**
Poecilosclerida	**Raspailiidae**	***Raspailia (Raspaxilla)* sp. G304865**	**EF507823**
Poecilosclerida	**Raspailiidae**	***Raspailia (Raspaxilla)* sp. G314949**	**EF507822**
Poecilosclerida	**Raspailiidae**	***Raspailia (Raspaxilla)* sp. G315648**	**EF507824**
Poecilosclerida	**Raspailiidae**	***Aulosponges* n. sp. G320085**	**EF507825**
Poecilosclerida	**Raspailiidae**	***Sollasella cervicornis* POR 17450**	**EF507826**
Poecilosclerida	**Raspailiidae**	***Sollasella moretonensis* G303059**	**EF507827**
Poecilosclerida	**Raspailiidae**	***Sollasella moretonensis* G303205**	**EF507828**
Poecilosclerida	**Raspailiidae**	***Sollasella moretonensis* G303227**	**EF507829**
Poecilosclerida	**Raspailiidae**	***Sollasella moretonensis* G303996**	**EF507830**
Poecilosclerida	**Raspailiidae**	***Sollasella moretonensis* G306153**	**EF507831**
Poecilosclerida	**Raspailiidae**	***Sollasella moretonensis* G315719**	**EF507832**
Poecilosclerida	**Raspailiidae**	***Sollasella moretonensis* G315759**	**EF507833**
Poecilosclerida	**Raspailiidae**	***Sollasella moretonensis* G321402**	**EF507834**
Spirophorida	Tetillidae	*Cinachyrella* sp.	AY561943
Spirophorida	Tetillidae	*Tetilla arb*	AY561944
Verongida	Aplysinellidae	*Aplysinella* sp.	AY561865
Verongida	Aplysinidae	*Aplysina fistularis*	AY561864
Verongida	Pseudoceratinidae	*Pseudoceratina* sp.	AY561956
Cnidaria (outgroup)	Zoantharia	*Antipathes galapagensis*	AY026365

99 ┌ *Sollasella moretonensis* G303205
├ *Sollasella moretonensis* G303996
└ *Sollasella moretonensis* G303059
├ *Sollasella moretonensis* G303227
├ *Sollasella moretonensis* G303204
75,87 ├ *Sollasella moretonensis* G321402
├ *Sollasella moretonensis* G315759
├ *Sollasella moretonensis* G303204
75 ├ *Sollasella moretonensis* G315719
├ *Sollasella cervicornis* POR 17450
├ *Eurypon* sp. G315208
└ *Aulospongus* n. sp. G320085
├ *Raspailia (Raspaxilla)* G315648
├ *Raspailia (Raspaxilla)* G314949
├ *Raspailia (Raspaxilla)* G304865
├ *Raspailia (Raspaxilla) topsenti* G312182
100,94 78 ├ *Raspailia (Raspailia)* n. sp. G315208
99 ├ *Raspailia (Raspailia)* G312932
81 ├ *Myrmekioderma granulata*
└ *Didiscus oxeata*

90 ┌ *Callyspongia multiformis*
98 ├ *Haliclona toxius*
├ *Haliclona cinerea*
85 100 ├ *Haliclona oculata* 1
96 ├ *Haliclona oculata* 2
100,97 ├ *Haliclona xena*
├ *Callyspongia plicifera*
92,76 └ *Neopetrosia subtriangularis*
100,96 100,89 ├ *Haliclona vansoesti*
└ *Xestospongia caminata*
├ *Aka coralliphaga*
100,99 100 ┌ *Amphimedon compressa*
98 ├ *Amphimedon paraviridis*
├ *Niphates olemda*
100,87 100,100 90 ├ *Pachychalina* sp.
├ *Acanthostrongylophora ashmorica*
└ *Acanthostrongylophora ingens*
└ *Petrosia ficiformis*

100,97 ┌ *Suberites ficus*
78 ├ *Suberites suberia*
├ *Aaptos suberitoides*
96,96 ├ *Amorphinopsis excavans*
99,80 ├ *Hymeniacidon perlevis*
├ *Halichondria bowerbanki*
├ *Halichondria panicea*
94,87 ┌ *Axinella verrucosa*
96 ├ *Stylissa flabelliformis*
100 ├ *Agelas oroides*
├ *Axinella damicornis*
├ *Axinella polypoides*
├ *Liosina paradoxa*
└ *Spheciospongia vagabunda*
100,100 ┌ *Theonella* sp. 1
100,98 ├ *Theonella* sp. 2
100,100 ├ *Discodermia dissoluta*
└ *Corallistes* sp.
100,98 ┌ *Scopalina lophyropoda*
95 ├ *Svenzea zeai*
└ *Vetulina* sp.
99 ┌ *Axinyssa aplysinoides*
100 ├ *Dictyonella* sp.
└ *Acanthella acuta*
├ *Mycale flagellifera*
└ *Chondrosia* sp.
└ *Antipathes galapagensis*
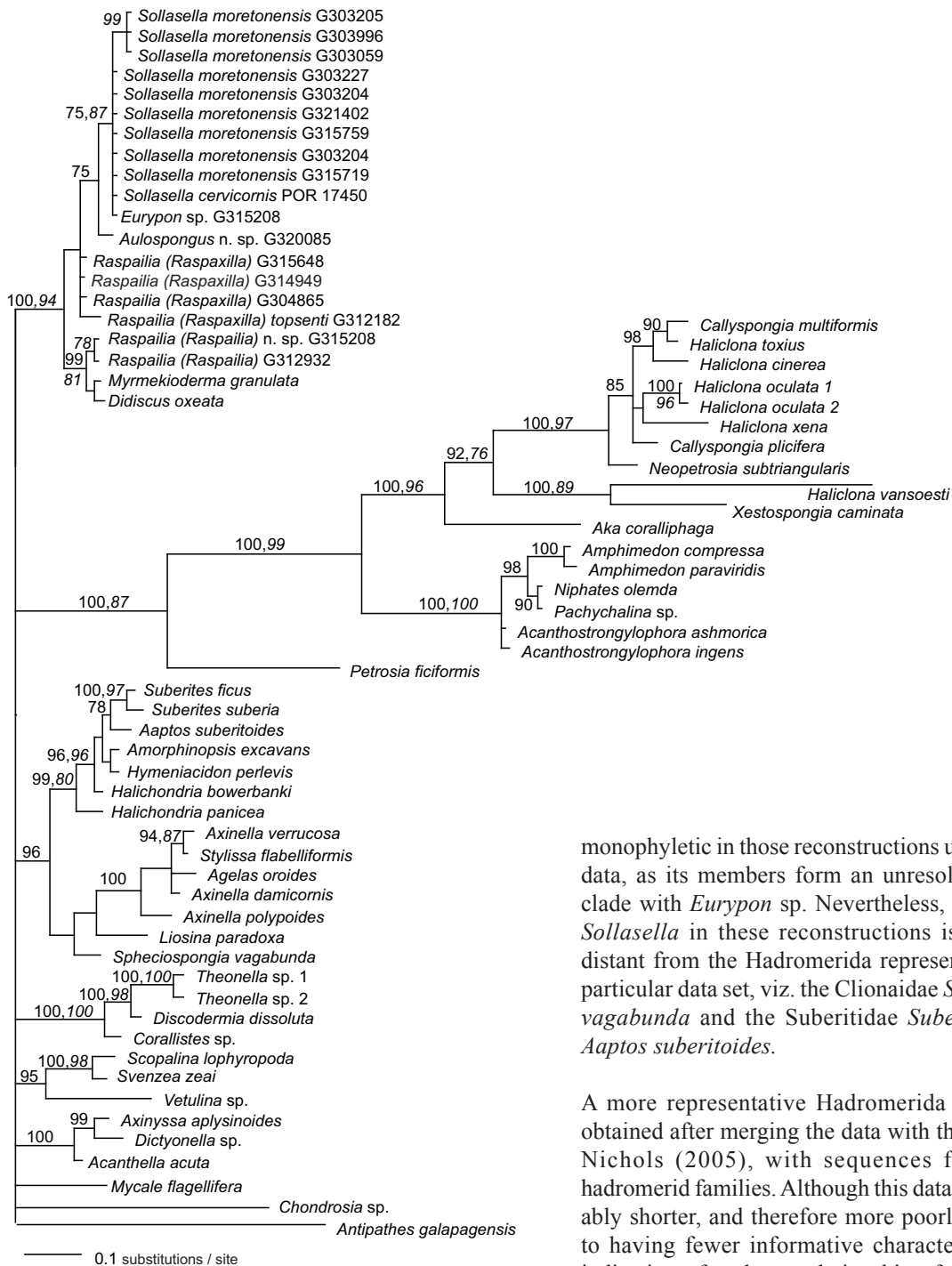
— 0.1 substitutions / site

Fig. 1. Bayesian inference consensus tree of the D3-D5 data set.
The non-italic numbers refer to Bayesian posterior probabilities.
Numbers in italics are Minimum-evolution bootstrap support
values of corresponding clades. Values lower than 75 are omitted
from both methods. Numbers behind taxon names are QM
voucher specimen numbers.

monophyletic in those reconstructions using molecular
data, as its members form an unresolved polytomic
clade with *Eurypon* sp. Nevertheless, the position of
Sollasella in these reconstructions is clearly more
distant from the Hadromerida representatives of this
particular data set, viz. the Clionaidae *Spheciospongia
vagabunda* and the Suberitidae *Suberites* spp. and
Aaptos suberitoides.

A more representative Hadromerida taxon set was
obtained after merging the data with the sequences of
Nichols (2005), with sequences from multiple
hadromerid families. Although this data set is consider-
ably shorter, and therefore more poorly resolved due
to having fewer informative characters, there is no
indication of a closer relationship of *Sollasella* with
any Hadromerida (Fig. 2). Regarding the position of
Sollasella the resulting phylogenetic topology is con-
gruent with the previous results using the smaller
taxon set. The *Sollasella cervicornis* and *S. moreton-
ensis* cluster is well-supported within the Raspailiidae,
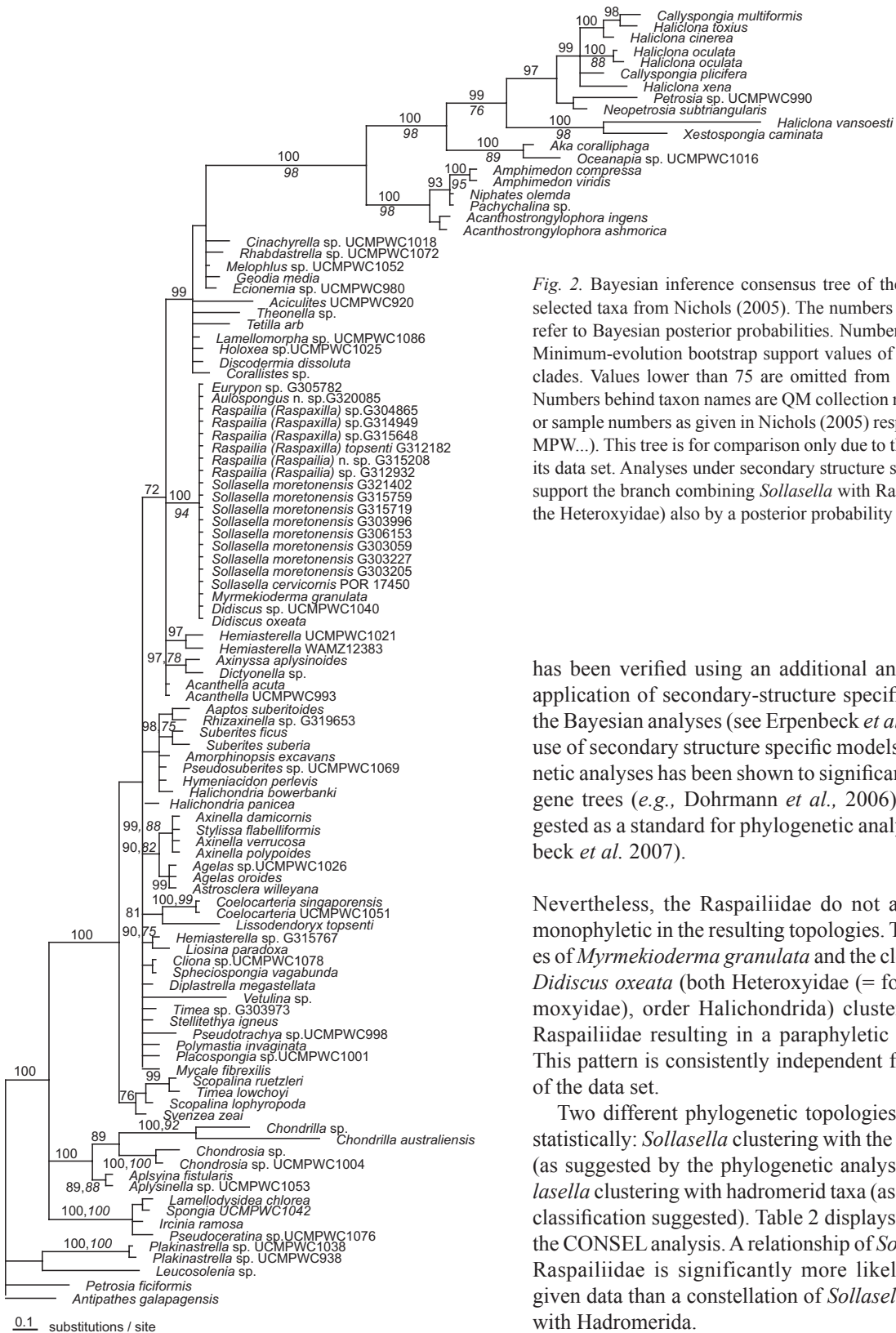which forms a strongly supported clade. This pattern

Fig. 2. Bayesian inference consensus tree of the overlap with selected taxa from Nichols (2005). The numbers in regular font refer to Bayesian posterior probabilities. Numbers in italics are Minimum-evolution bootstrap support values of corresponding clades. Values lower than 75 are omitted from both methods. Numbers behind taxon names are QM collection numbers (G....) or sample numbers as given in Nichols (2005) respectively (UC-MPW...). This tree is for comparison only due to the shortness of its data set. Analyses under secondary structure specific models support the branch combining *Sollasella* with Raspailiidae (and the Heteroxyidae) also by a posterior probability of 100.

has been verified using an additional analysis under application of secondary-structure specific models in the Bayesian analyses (see Erpenbeck *et al.* 2007). The use of secondary structure specific models in phylogenetic analyses has been shown to significantly improve gene trees (*e.g.,* Dohrmann *et al.,* 2006) and is suggested as a standard for phylogenetic analyses (Erpenbeck *et al.* 2007).

Nevertheless, the Raspailiidae do not appear to be monophyletic in the resulting topologies. The sequences of *Myrmekioderma granulata* and the closely related *Didiscus oxeata* (both Heteroxyidae (= formerly Desmoxyidae), order Halichondrida) cluster within the Raspailiidae resulting in a paraphyletic assemblage. This pattern is consistently independent from the size of the data set.

Two different phylogenetic topologies were tested statistically: *Sollasella* clustering with the Raspailiidae (as suggested by the phylogenetic analysis); and *Sollasella* clustering with hadromerid taxa (as the previous classification suggested). Table 2 displays the result of the CONSEL analysis. A relationship of *Sollasella* with Raspailiidae is significantly more likely under the given data than a constellation of *Sollasella* clustering with Hadromerida.

Table 2. Output of COUNSEL 0.942b on the support of two different hypotheses (*Sollasella* + Raspailiidae (Sol+Ras) against *Sollasella* + Hadromerida (Sol+Had). See Shimodaira and Hasegawa (2001) for further details and references.

rank	Hypoth.	obs	au [a]	np [b]	bp [c]	pp [d]	kh [e]	sh [f]	wkh [g]	wsh [h]
1	Sol+Ras	-445.1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
2	Sol+Had	445.1	5e-042	3e-015	0	5e-194	9e-005	9e-005	9e-005	9e-005

[a] *P*-value of the approximately unbiased test.

[b] Bootstrap probability of the selection.

[c] Bootstrap probability calculated directly from the replicates.

[d] Bayesian posterior probability (PP) calculated by the BIC approximation.

[e] *P*-value of the Kishino-Hasegawa (KH) test.

[f] *P*-value of the Shimodaira-Hasegawa (SH) test.

[g] *P*-value of the weighted Kishino-Hasegawa (WKH) test.

[h] *P*-value of the weighted Shimodaira-Hasegawa (WSH) test.

Discussion

The molecular analysis of the 28SrDNA provides a clear picture of the phylogenetic position of *Sollasella* in the classification of demosponges. These 28S sequence data fully support the morphological hypothesis of Van Soest *et al.* (2006) that *Sollasella* should be classified within the Raspailiidae of the order Poecilosclerida and not as a hadromerid sponge. The family Sollasellidae Lendenfeld, 1887, which was previously assigned as *incertae sedis* in the Hadromerida (Van Soest, 2002), has therefore been abandoned.

Our data provides an example of the successful application of molecular tools to sponge phylogeny without contradicting current morphological hypotheses and posing additional questions (*e.g.,* McCormack *et al.,* 2002; McCormack and Kelly 2002; Nichols, 2005; Erpenbeck *et al.,* 2005). Conversely, the resulting phylogenies provide us with another pattern that needs further explanation: the clustering of the Heteroxyidae *Myrmekioderma granulata* and *Didiscus oxeata* with the Raspailiidae. Although the coherence of the family Heteroxyidae is unverified and molecular data could not unambiguously assign them to the Halichondrida (Erpenbeck *et al.,* 2005), a relationship with Raspailiidae appears unlikely based on morphometric characteristics. The largely confused arrangement of oxeote megascleres with ectosomal microxeas in Heteroxyidae differs fundamentally from the structured raspaliid skeleton. Although the polygonal grooves found in *Sollasella* are remarkably similar to those seen in *Didiscus* and *Myrmekioderma, t*hese have probably have been acquired independently. In *Didiscus* the surface is "strongly grooved with angular striations forming po-lygonal plates; plates contractile with oscula in between". In *Myrmekioderma* the surface is "convoluted with large conules or rounded or polygonal plates, each separated by shallow but distinct grooves, excavated channels containing large oscula" (Hooper, 2002a). This is morphologically and functionally different from *Sollasella*, whose surface is "provided with a characteristic polygonal pattern of lines of round shallow depressions presumed to be inhalant openings" (Van Soest, 2002, from Hallmann, 1914). Such similarity of 28S rDNA sequences is comparable with other instances in which 28S rDNA resulted in "odd" phylogenies (*e.g.* McCormack *et al.* 2002). More intensive studies on the molecular evolution of 28S rRNA genes in demosponges are required to explain such phenomena. Nevertheless, the present analysis has demonstrated that the clustering of *Sollasella* with the Raspailiidae is clearly no such '28S rDNA' artefact because an alternative data set (morphology) provides independent evidence for this scenario.

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