

Soft sponges with tricky tree: On the phylogeny of dictyoceratid sponges

Dirk Erpenbeck^{1,2}  | Adrian Galitz¹ | Merrick Ekins^{3,4}  | Steve de C. Cook⁵ |
Rob W. M. van Soest⁶ | John N. A. Hooper^{3,7} | Gert Wörheide^{1,2,8} 

¹Department of Earth- and Environmental Sciences, Palaeontology & Geobiology, Ludwig-Maximilians-Universität München, Munich, Germany

²GeoBio-Center, Ludwig-Maximilians-Universität München, Munich, Germany

³Biodiversity Program, Queensland Museum, South Brisbane, QLD, Australia

⁴School of Biological Sciences, University of Queensland, St Lucia, QLD, Australia

⁵Formerly Department of Zoology, School of Biological Sciences, University of Auckland, Auckland, New Zealand

⁶Naturalis Biodiversity Center, Leiden, The Netherlands

⁷Griffith Institute for Drug Discovery, Griffith University, Nathan, QLD, Australia

⁸SNSB-Bavarian State Collection of Palaeontology and Geology, Munich, Germany

Correspondence

Dirk Erpenbeck, Department of Earth- and Environmental Sciences, Palaeontology & Geobiology, Ludwig-Maximilians-Universität München, Munich, Germany.
Email: erpenbeck@lmu.de

Funding information

Sixth Framework Programme, Grant/Award Number: MOIF-CT-2004; Deutsche Forschungsgemeinschaft, Grant/Award Number: Er611/5-1; LMUexcellent within the framework of the German Excellence Initiative

Abstract

Keratose (horny) sponges constitute a very difficult group of Porifera in terms of taxonomy due to their paucity of diagnostic morphological features. (Most) keratose sponges possess no mineral skeletal elements, but an arrangement of organic (spongin) fibers, with little taxonomic or phylogenetic information. Molecular phylogenetics have targeted this evolutionary and biochemically important lineage numerous times, but the conservative nature of popular markers combined with ambiguous identification of the sponge material has so far prevented any robust phylogeny. In the following study, we provide a phylogenetic hypothesis of the keratose order Dictyoceratida based on nuclear markers of higher resolution potential (*ITS* and *28S C-region*), and particularly aim for the inclusion of type specimens as reference material. Our results are compared with previously published data of *CO1*, *18S*, and *28S (D3-D5)* data, and indicate the paraphyly of the largest dictyoceratid family, the Thorectidae, due to a sister group relationship of its subfamily Phyllospongiinae with Family Spongiidae. Irciniidae can be recovered as monophyletic. Results on genus level and implications on phylogenetic signals of the most frequently described morphological characters are discussed.

KEYWORDS

Dictyoceratida, Keratosa, Porifera, Spongiidae, Thorectidae

Contributing authors: Adrian Galitz (adrian.galitz@gmx.de); Merrick Ekins (merrick.ekins@qm.qld.gov.au); Steve de C. Cook (steve.cook@scorpius.co.nz); Rob W. M. van Soest (rob.vansoest@naturalis.nl); John N. A. Hooper (john.hooper@QM.QLD.GOV.AU); Gert Wörheide (woerheide@lmu.de)

Galitz is joint first author.

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1 | INTRODUCTION

In the last couple of decades, our knowledge on phylogenetic relationships of sponges, particularly demosponges, experienced major turmoil when molecular data demonstrated serious pitfalls in the classical, morphology-based classification (see, e.g., Boury-Esnault, 2006; Cárdenas, Pérez, & Boury-Esnault, 2012; Erpenbeck & Wörheide, 2007; Redmond et al., 2013; Wörheide et al., 2012). This resulted in a fundamentally revised classification at order level (Morrow & Cárdenas, 2015). However, revisions of most intra-ordinal relationships are still due for revision. A particularly difficult order of sponges is the Dictyoceratida (Subclass Keratosa), which possess a skeleton of organic material (spongin) only and lack mineral skeletal elements (with the exception of *Vaceletia*, which possesses a hypercalcified secondary limestone skeleton instead of spongin fibers, see Wörheide, 2008). Therefore, these sponges were historically assigned to the "horny" sponges. The spongin skeleton renders specimens of some genera useful as bathing sponges, but at the same time limits the suite of diagnostic features for morphological classification and phylogeny. Morphologically, all dictyoceratids share the presence of this anastomosing spongin fiber skeleton that often make up a significant proportion of the body volume. Fibers develop from multiple points and are organized into primary, secondary, and sometimes tertiary fibers (Cook & Bergquist, 2002e). Earlier molecular studies supported monophyly of Dictyoceratida, their sister group relationship to order Dendroceratida as subclass Keratosa, and their distinction from other horny sponge lineages (e.g., Verongiida, subclass Verongimorpha) (Borchiellini et al., 2004; Erpenbeck, Sutcliffe, et al., 2012; Hill et al., 2013; Redmond et al., 2013; Thacker et al., 2013). Internal relationships, however, are still insufficiently understood, although are mandatory for a variety of downstream research (Boufridi et al., 2017; Chianese et al., 2017; see e.g., Erpenbeck, Hooper, et al., 2012).

At the last major (morphology-based) revision of sponge classification, in the *Systema Porifera* (Hooper & Van Soest, 2002), Dictyoceratida were separated into the four taxa at the family level Dysideidae, Irciniidae, Spongiidae, and Thorectidae, with the latter being divided into the subfamilies Thorectinae and Phyllospongiinae (Cook & Bergquist, 2002d, 2002e). A fifth family, Verticillitidae, was added subsequently (Morrow & Cárdenas, 2015; Wörheide, 2008). So far, molecular studies targeting shallow-level relationships of Dictyoceratida provided insufficient resolution or conflicting data: The first comprehensive molecular approach based on the partial mitochondrial *cytochrome c* oxidase subunit 1 gene (CO1) and the D3-D5 partition of the nuclear large ribosomal subunit gene (28S) confirmed monophyly of the families Dysideidae and Irciniidae, and confirmed Dysideidae as sister to all other families as well, but failed to resolve Spongiidae and Thorectidae relationships (Erpenbeck, Sutcliffe, et al., 2012). Likewise, Redmond et al. (2013) and Thacker et al. (2013) confirmed the distinct position of Dysideidae, based on the nuclear small ribosomal subunit gene (18S) and full-length 28S, respectively, but could not robustly resolve the relationship of other dictyoceratid taxa either. Undoubtedly, the molecular markers used

so far bear insufficient resolution potential to answer all dictyoceratid phylogenetic questions.

In the present study, we aim to unravel the phylogenetic relationships of dictyoceratid sponges by employing faster evolving molecular markers. We use the C-region of 28S, which has been successfully used in sponge molecular taxonomic studies (Erpenbeck, Voigt, et al., 2016; e.g., Voigt & Wörheide, 2016), and the internal transcribed spacers 1 and 2 (ITS, including the 5.8S rRNA gene). ITS is a classical marker on species level and below (see, e.g., Borchiellini, Chombard, Lafay, & Boury-Esnault, 2000), but in Dictyoceratida so far recruited for studying metabolite distribution only (Boufridi et al., 2017; Chianese et al., 2017; Erpenbeck, Hooper, et al., 2012).

Conclusive (molecular) phylogenies must be based on well-identified species. Most dictyoceratid phylogenies, however, suffer from incomplete and ambiguous specimen identification (Erpenbeck, Sutcliffe, et al., 2012; Redmond et al., 2013; Thacker et al., 2013) due to the difficult (morphology-based) taxonomy (see also Cook, 2007). Type specimens, particularly holotypes, are the only unambiguous reference points for taxonomic delineation, but not frequently used for sponge molecular phylogenetic studies due to difficult accessibility and bad DNA qualities (see review in Erpenbeck, Ekins, et al., 2016). The present study therefore attempts to use type material where possible, or other well-identified specimens such as *Systema Porifera* reference material. The results of the new dictyoceratid ITS and 28S (C-region) molecular analyses are compared with phylogenies obtained from 18S (Redmond et al., 2013), CO1, and 28S (D3-D5) (Erpenbeck, Sutcliffe, et al., 2012) markers in order to summarize our current knowledge and formulate a phylogenetic hypothesis for dictyoceratids.

2 | MATERIALS AND METHODS

Sponge specimens or fractions thereof, including type material, were borrowed or obtained from the Queensland Museum (Brisbane, Australia), Australian Museum (Sydney, Australia), from the Universalmuseum Joanneum (Graz, Austria; formerly Landesmuseum Joanneum Graz), from the Naturhistorisches Museum Basel (Basel, Switzerland), from the Zoological Museum Amsterdam (now NCB Leiden, the Netherlands), from the Natural History Museum (London, Great Britain), and from the collections of Steve de C. Cook (Auckland, New Zealand) (see Appendix 1 for a complete list of specimens).

PCR amplifications were conducted in 12.5 µl reactions: 5X Green GoTaq® Flexi Reaction Buffer (Promega), 25 mM MgCl₂ (Promega), 10 mM dNTP (Bioline), 5 mM of each primer (Metabion), and 1 unit of Taq polymerase (GoTaq, Promega). Usage of the additive bovine serum albumin (BSA, 10 mg/ml) significantly improved the amplification yields. Polymerase chain reactions (PCRs) for both ITS and 28S were conducted under the following conditions: 3 min at 95°C (denaturation), 35 cycles at 95°C for 30 s (heating), 51°C for 30 s (annealing, for primer combinations, see Table 1), and 72°C for 1 min (extension), followed by 72°C for 5 min (final

TABLE 1 ITS and 28S (C-Region) primers used in this study

Name (reference)	Nucleotide sequence	Target region	Amplicon size
RA2_keratose (fwd) ^a	5' GRA TGG TTT AGT GAG ATC TT 3'	ITS	~660 bp
ITS2.2_keratose (rev) ^a	5' AAA TTC AGC GGG TAG YCT GG 3'		
5.8S_keratose (fwd) ^a	5' TGA CAA CTT CTG ACG GT 3'	ITS-2	~365 bp
28S-C2_keratose (fwd) ^a	5' GAA AAG AAC TTT GRA RAG AGA GTC 3'	28S	~340 bp
28S-D2_keratose (rev) ^a	5' CCG TGT TTC AAG ACG GGT CGR ACG AG 3'		
RA2-fwd ^b	5' GTC CCT GCC CTT TGT ACA CA 3'	ITS	~660 bp
ITS2.2-rev ^b	5' CCT GGT TAG TTT CTT TTC CTC CGC 3'	ITS	~330 bp
5.8S-1-fwd ^c	5' GTC GAT GAA GAA CGC AGC 3'	ITS-2	
28S-C2-fwd ^c	5' GAA AAG AAC TTT GRA RAG AGA GT 3'	28S	~340 bp
28S-D2-rev ^c	5' TCC GTG TTT CAA GAC GGG 3'		

^aGalitz et al. (2018).^bWörheide (1998).^cChombard, Boury-Esnault, and Tillier (1998).

extension). For some samples, touchdown PCRs prove to be more efficient than the standard protocol: 3 min at 95°C (denaturation), 20 cycles at 95°C for 30 s (heating), 55–45°C (annealing; –0.5°C per cycle), and 72°C for 1 min (extension), followed by 20 cycles at 95°C for 30 s (heating), 50°C (annealing), and 72°C for 1 min (extension), concluded by 72°C for 5 min (final extension). PCR products were isolated cleaned up with the freeze-squeeze method (Tautz & Renz, 1983) from 1.5% agarose gels. Cycle sequencing products were generated with BigDye Terminator v3.1 followed by Sanger sequencing on an ABI 3730 in the Genomic Sequencing Unit of the LMU Munich. Forward and reverse reads were assembled and corrected with CodonCode Aligner 3.7.1 (<http://www.codoncode.com>) after checking for contaminants by BLAST against NCBI GenBank. Intragenomic polymorphisms (IGP) were recoded following the IUPAC ambiguity codes for nucleotides. The assembled and checked sequences were aligned with MAFFT (Katoh & Standley, 2013) under default settings as implemented in Geneious Prime® 2019.0.4 (<http://www.geneious.com>; Kearse et al., 2012) and subsequently optimized by eye. The data set was complemented with homologous sequences of the ITS regions and 28S C-region as published in GenBank (see Figures S1–S4). Data for CO1 and 28S (D3–D5) consist predominantly of previously published sequences (see Figures S1–S4), plus 39 yet unpublished sequences (1 of 28S (D3–D5), 38 of CO1) generated in course of the study of Erpenbeck, Sutcliffe, et al. (2012). See boldfaced accession numbers in Appendix 1 and Erpenbeck, Sutcliffe, et al. (2012) for details of sequence generation.

All sequences are submitted to the European Nucleotide Archive (see Appendix 1 for accession numbers [LR#####]). For all four

data sets (ITS, 28S C-region, (28S (D3–D5), and CO1) maximum-likelihood reconstructions were generated with RAxML 8 (Stamatakis, 2014) as implemented in Geneious Prime® 2019.0.4 under the GTR GAMMA I model and 1,000 rapid bootstrap replicates. The alignments used in this study are freely available at <https://github.com/PalMuc/Soft-Sponges-Tricky-Tree>.

3 | RESULTS AND DISCUSSION

For a total of 236 dictyoceratid specimens, new sequences were generated (see Appendix 1). As not all fragments for every specimen were amplifiable and/or available from NCBI GenBank, the data sets for ITS (93 taxa (of which 91 newly sequenced for this study)/ 808 characters), 28S C-Region (148 (121)/ 347), 28S D3–D5 region (76 (1)/ 549), and CO1 (152 (38)/ 495) (see Appendix 1 and Figures S1–S4 for the individual gene trees) differ in their taxon content. The summarizing overview on the phylogenetic results is given in Figure 1.

The dictyoceratid taxa fall into clades differently supported by the individual fragments (see Figure 1). These molecular analyses, as currently the most comprehensive to unravel the phylogenetic relationships of dictyoceratid sponges including type (and other reference) material, demonstrate that family Thorectidae sensu Cook and Bergquist (2002d) cannot be upheld. Thorectidae was erected by Bergquist (1978) who regarded concentric fiber lamination as a distinct and combining feature among dictyoceratid sponges as opposed to the homogeneous fibers in Spongiidae. However, Sanders and van Soest (1996) remarked that several members of Spongiidae

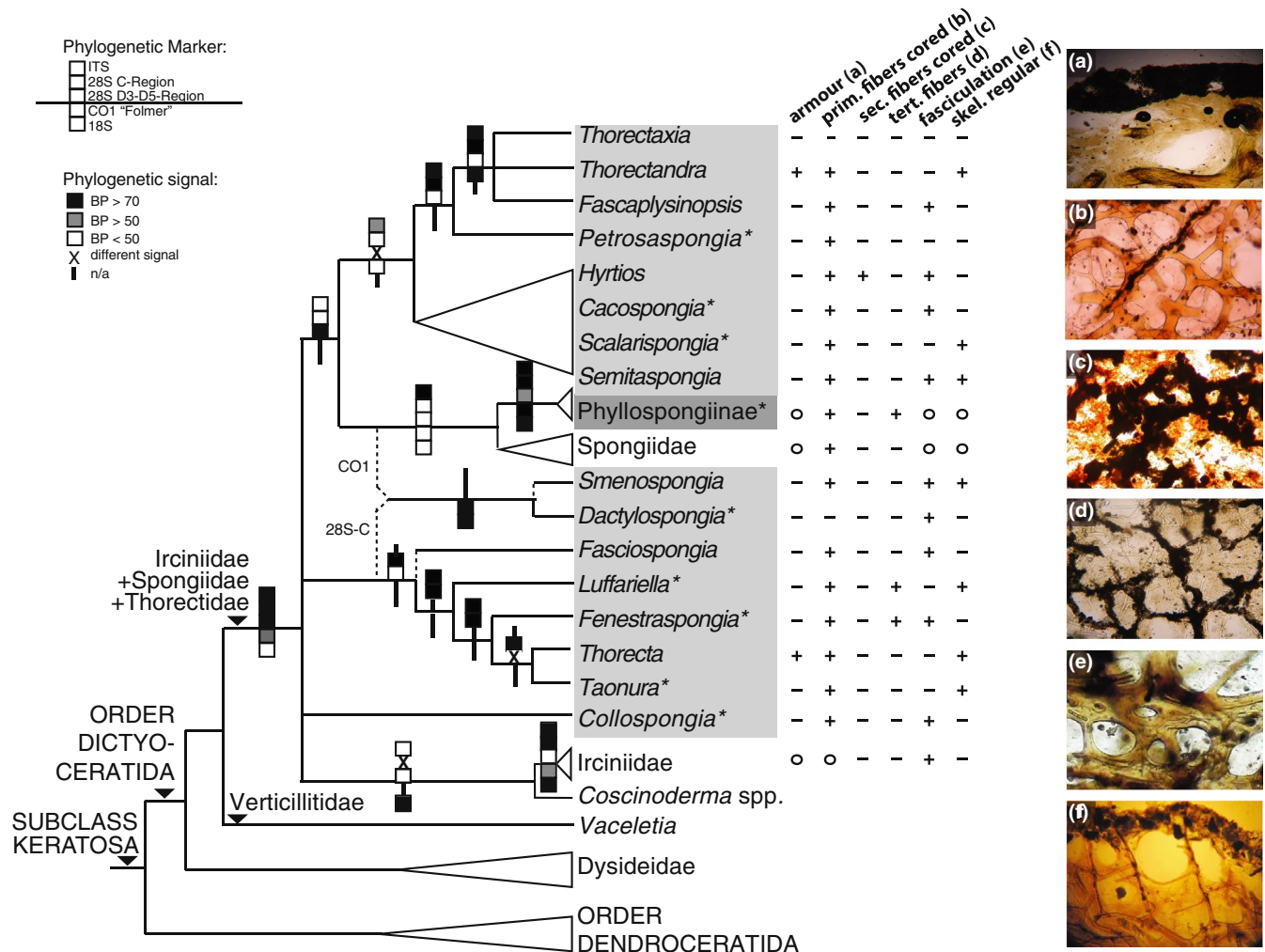


FIGURE 1 Phylogenetic hypothesis combined from the current ITS and 28S (C-region) data yielded in this study, combined with results from 28S (D3-D5), and CO1 reconstructions as calculated in this study and previously published 18S data. The 18S support is selected from Fig. 2 of Redmond et al. (2013), with occasional conflicting data (e.g., due to unverified identifications) disregarded. Thorectid taxa are shaded, of which Thorectinae are highlighted in light gray and Phyllospongiinae in dark gray. Asterisks indicate the presence of type sequences in the taxon (see text for further remarks). Shaded boxes at branches indicate the bootstrap probability (BP) for the different fragments. On the right of the taxon names are presence (+) and absence (-) of morphological features displayed (o indicates both absence and presence occurring between genera within) (cf. Cook & Bergquist, 2002a, 2002b, 2002c, 2002d, 2002e), with examples given by the insert pictures on the right. Inserts are a) armor of *Thorectandra excavatus* (QM G303331); b) cored primary (and uncored secondary) fibers of *Petrosaspongia nigra* (QM G31543); c) cored secondary (and primary) fibers of *Hyrtios erectus* G301248; d) tertiary fibers (connecting uncored secondary fibers) of *Strepsichordaia* sp. (QM G306046); e) fasciculation of a *Fasciospongia* sp. (QM G317952); and f) regular skeletal arrangement of a *Thorecta* sp. (QM G323048)

possess laminated fibers, usually invisible with light microscopy rendering fiber lamination an unsuitable phylogenetic character. Despite these concerns, Bergquist et al. (1999) reclassified foliose Dictyoceratida from Spongiidae into Thorectidae, based on fiber structure, erecting a distinct subfamily Phyllospongiinae (foliose sponges) alongside all other thorectids (which formed Subfamily Thorectinae). Nevertheless, Cook and Bergquist (2002d) regarded Thorectinae as "heterogeneous group of sponges," "difficult to objectively define," and as a "catch-all" for all non-phyllspongiine thorectids. Our studies demonstrate that Thorectidae (particularly Subfamily Thorectinae) constitute a paraphyletic assemblage of dictyoceratid taxa, as indicated in earlier molecular studies (Erpenbeck,

Sutcliffe, et al., 2012; see discussion in Morrow & Cárdenas, 2015; Redmond et al., 2013; Thacker et al., 2013). Fiber laminations (see, e.g., fig. 15 in Cook, 2007), as observed in Thorectidae (see, e.g., Cook & Bergquist, 2002d), are also reported for Dysideidae (Cook & Bergquist, 2002a) that branch first from all other dictyoceratid families. Therefore, such thorectid fiber lamination should be regarded as an ancient dictyoceratid trait, plesiomorph in thorectids, and therefore not suitable to morphologically define any phylogenetic clade within the Dictyoceratida.

Subfamily Phyllospongiinae, however, can be recovered, although with a taxon composition emended back to Keller's (1889) core taxa *Carteriospongia* and *Phyllospongia*, plus *Strepsichordaia*.

This constellation is underlined by several in-depth studies that included types of *Strepsichordaia lendenfeldi* Bergquist, Ayling & Wilkinson (AM Z5026), and *Carteriospongia foliascens* (Pallas) BMNH 1925.11.1.41 (see Abdul Wahab, Fromont, Whalan, Webster, & Andreakis, 2014; Galitz et al., 2018) (sequencing of the holotype of *Phyllospongia papyracea* (Esper) BMNH 1931.4.1.1 was attempted but unsuccessful). Of the remaining phyllospongiine genera (Cook & Bergquist, 2002d), *Candidaspongia* Bergquist, Sorokin & Karuso, 1999 has been identified as Dysideidae (Galitz et al., 2018; Redmond et al., 2013) and *Lendenfeldia* Bergquist, 1980 requires revision—lectotype sequencing of its type species *L. frondosa* (Lendenfeld) (BMNH 1877.5.21.1697) has been attempted, but without success. Further details on the internal relationships of Phyllospongiinae and paraphyly of its genera are given in Abdul Wahab et al. (2014) and Galitz et al. (2018).

However, Phyllospongiinae form a clade with Spongiidae, thereby corroborating the former Spongiidae sensu Gray. Spongiidae were mostly recovered as monophyletic. This clade comprises all the specimens of *Spongia* (including a *Systema Porifera* reference of type species *Spongia officinalis* (Linnaeus) SDCC/RFO01), *Rhopaloeides* (including a *Systema Porifera* reference of type species *Rhopaloeides odorabile* Thompson, Murphy, Bergquist & Evans, SDCC/RFO67), and *Hippospongia* from several different studies as published in NCBI GenBank. Several specimens identified or published as *Spongia* do not form a clade and prompt for a revision of the spongiid taxa (see also Redmond et al., 2013). Unfortunately, success rate of type and reference material of Spongiidae was low, as PCR of the neotype of *S. officinalis* BMNH 1883.12.4.28 failed, likewise sequencing the holotypes of *R. odorabile* (AM Z4965) and *Leiosella levis* (Lendenfeld) (BMNH 1886.8.27.319) furthermore historic comparative material for *Hippospongia communis* (Lamarck) (as *H. equina* (Schmidt) BMNH 1899.5.2.2, see Cook & Bergquist, 2002c) did not result in sequences suitable for phylogenetic analyses. Consequently, we refrain from hypothesizing on the internal phylogenetic relationships of Spongiidae until more molecular data from reference material are obtained. A morphological feature combining Phyllospongiinae and spongiids might be found in the apparently more homogeneous fiber structure in contrast to Thorectinae. Phyllospongiinae were described with "successive fibrous layers," which remain tightly adherent, producing an overall homogeneous structure with visible contiguous laminae" (Cook & Bergquist, 2002e), and Spongiidae are defined by their homogenous fiber structure (Cook & Bergquist, 2002c; objected by Sanders & Van Soest, 1996).

The thorectid genera *Thorectandra*, *Thorectaxia*, *Fascaplysinopsis*, and *Petrosaspongia* form a clade with the latter splitting first. Genus *Petrosaspongia* Bergquist, 1995 currently comprises two species, and the holotype of the type species *Petrosaspongia nigra* Bergquist (QM G304685) was analyzed. *Thorectandra*, *Thorectaxia*, and *Fascaplysinopsis* form a monophyletic group. The holotype for *Thorectandra corticatus* Lendenfeld, type species of *Thorectandra*, is unknown (Hooper & Wiedenmayer, 1994), but its reference material analyzed for the *Systema Porifera*

was sequenced (SDCC/RFO16, see Cook & Bergquist, 2002d). Although histologically regarded as similar (Cook & Bergquist, 2002d), *Thorectandra* is phylogenetically distant to *Thorecta* (see below), prompting a re-evaluation of histological characters for keratose sponge systematics. Instead, *Thorectandra* is recovered close to the monotypic genus *Fascaplysinopsis*. Bergquist (1980) remarks *Fascaplysinopsis* recalling *Thorectandra* species in the "pronounced gelatinous appearance of the matrix, the yellow internal pigmentation and the coarse nature of the fibres" besides similarities in secondary metabolites. Unfortunately, DNA extraction from the holotype of *Fascaplysinopsis reticulata* Bergquist (*Aplysinopsis reticulata* Hentschel SMF904) was yet unsuccessful, but we managed to include the reference sample SDCC/RFO17 from *Systema Porifera* (see Cook & Bergquist, 2002d). However, several additional cf. *Fascaplysinopsis* samples in our data set urge for a revision of this genus. We found a close relationship of *Fascaplysinopsis* and *Thorectandra* to the monotypic genus *Thorectaxia*, of which a sample of *Thorectaxia papuensis* Pulitzer-Finali & Pronzato from the type location (Papua New-Guinea) could be sequenced.

Molecular data reveal phylogenetic signal of a close relationship of (*Thorectandra* + *Thorectaxia* + *Fascaplysinopsis* + *Petrosaspongia*) to *Hyrtios*, *Cacospongia*, *Scalarispongia*, and *Semitaspongia*, whose inter- and intrageneric relationships require revision. Genus *Scalarispongia*, represented by a sequence of the type species' holotype *Scalarispongia scalaris* (Schmidt) LMJG 15406/0, and several *Hyrtios* species, *H. erectus* (Keller), *H. altus* (Poléjaeff), and *H. reticulatus* (Thiele), form a clade, to which *Cacospongia* (including the lectotype LMJG 15405/19 of its type species *C. mollior* Schmidt) is sister. *Cacospongia mycofijiensis* (Kakou, Crews & Bakus), however, is distant, therefore resulting in the paraphyly of *Cacospongia*. Specimens of *Hyrtios proteus* Duchassaing & Michelotti, the nominal type species of *Hyrtios*, fall outside this clade. This confirms earlier findings on non-monophyly of the genus *Hyrtios*, demonstrating the need for a revision of this genus (Erpenbeck et al., 2017; Erpenbeck, Sutcliffe, et al., 2012; Redmond et al., 2013). Cook and Bergquist (2002d), remark that *Cacospongia* species other than *C. mollior* and *C. sarta* (Lendenfeld) require revision. A partial ITS sequence of the *C. sarta* holotype BMNH 1886.8.27.166, so far the only specimen of this species known (Cook & Bergquist, 2000), falls outside this clade, but verification from a longer sequence is required. In the past, *C. mycofijiensis* classification underwent numerous changes in its relatively young taxonomic history, triggered by overlapping morphological characteristics to other genera (see review in Sanders & Van Soest, 1996). An assignment of *C. mycofijiensis* to *Petrosaspongia* (suggested in Bergquist et al., 1999) can be rejected following our data, but assignment to *Cacospongia* (Sanders & Van Soest, 1996) or *Scalarispongia* (objected in Manconi, Cadeddu, Ledda, & Pronzato, 2013) requires thorough revision of the three genera. Both *Scalarispongia* and *Semitaspongia* have been erected by Cook and Bergquist (2000) to accommodate members of the "'*Cacospongia*' group" which is supported by the present data.

A further major clade unites *Luffariella*, *Thorecta*, *Fenestraspongia*, *Taonura*, and *Fasciospongia*. *Thorecta* Lendenfeld is in our data set

represented by *T. reticulata* Cook & Bergquist [reference specimen SDCC/NZ097 in Cook and Bergquist (1996)] and a specimen of *Thorecta freija* Lendenfeld. Sequencing results from the holotype of the type species *T. exemplum* var. *tertia* Lendenfeld (BMNH 1886.8.27.188) were ambiguous. Santos et al. (2010) noted on the shortcomings in the classification of *Thorecta* and regarded eleven species as valid including *T. reticulata*, while *T. freija* was reclassified as *Taonura*. Genus *Taonura* in this analysis is represented by two specimens of the type species *Taonura flabelliformis* Carter (lectotype BMNH 1844.9.13.3 and the *Systema Porifera* reference specimen SDCC/RF024). Although only a partial ITS 2 fragment of the lectotype could be recovered, preventing the resolution of intergeneric relationships, the phylogenetic placement with *Luffariella* + *Thorecta* + *Fenestraspongia* clade is indicated. Our 28S reconstruction recovers *Thorecta* as paraphyletic with a sister group relationship between *T. freija* and *T. flabelliformis*, supporting Santos et al. (2010). Cook and Bergquist (2002d) described *Taonura* as a "hybrid of skeletal morphologies seen in *Cacospongia*, *Semitaspongia*, and *Scalarispongia*," but our molecular results cannot second the phylogenetic signal of *Taonura* skeletal morphology to those genera. Closely related to *Thorecta* is *Fenestraspongia*, represented by the holotype of its type species *F. intertexta* (Carter) BMNH 1886.12.15.238. *Luffariella* Thiele comprises the type species *L. variabilis* (Polejaeff) (holotype BMNH 1885.8.8.52), *L. calculata* Bergquist (holotype QM G304686), and *L. cylindrica* Bergquist (holotype QM G304687) and outside *Thorecta* + *Fenestraspongia*. *Luffariella* and *Fenestraspongia* were regarded as the only Thorectinae with tertiary fibers (Cook & Bergquist, 2002d). A phylogenetic signal of tertiary fibers is not given due to the phylogenetic position of *Thorecta* and the presence of tertiary fibers in Phyllospongiinae and *Petrosaspongia* species (see Uriz & Cebrian, 2006). Genus *Fasciospongia* Burton is in our analyses represented by a *F. costifera* (Lamarck, 1814) from its type locality (Western Australia) and a South African *F. cf. cycni* sequence from GenBank. Type region of *F. cycni* (Lendenfeld) is Western Australia; therefore, the taxonomy of this sample remains to be confirmed.

For *Smenospongia* and *Dactylospongia*, Bergquist relationships to the other dictyoceratid taxa are unresolved as sister to either *Luffariella* + *Thorecta* + *Fenestraspongia* + *Taonura* or Phyllospongiinae + Spongiidae. *Dactylospongia* is here represented by the lectotype (NMB-PORI 44), several samples of the type species *D. elegans* (Thiele), and a reference specimen for the *Systema Porifera* [SDCC/RF047 *D. metachromia* (Laubenfels)]. For *Smenospongia* the type species, *S. aurea* (Hyatt) and other *Smenospongia* samples (Redmond et al., 2013) were considered. *Dactylospongia* was erected to accommodate *Luffariella elegans* Thiele, which appeared morphologically distinct to *Luffariella* (Bergquist, 1965). *Dactylospongia* was subsequently assigned to Thorectidae based on its stratified fiber structure and due to morphological and pigment biochemical similarity to *Smenospongia* (Cook & Bergquist, 2002d). Both, distinction from *Luffariella* and similarity to *Smenospongia*, can be confirmed by our molecular data. A transfer of *D. metachromia* to the genus *Petrosaspongia* as suggested by Kwak, Schmitz, and Kelly (2000)

based on terpenic compounds is in strong conflict with our molecular findings (see Uriz and Cebrian (2006) for a discussion).

Family Irciniidae, currently consisting of the genera *Ircinia*, *Psammocinia*, *Bergquistia*, and *Sarcotragus*, is monophyletic. Irciniidae share the apomorphic fine collagenous filaments in the mesohyl (Cook & Bergquist, 2002b). While molecular studies unequivocally supported irciniid monophyly of its largest genus *Ircinia*, this remains uncertain in respect to *Sarcotragus* (Erpenbeck, Sutcliffe, et al., 2012; see also Pöppe, Sutcliffe, Hooper, Wörheide, & Erpenbeck, 2010). Cook and Bergquist (2002b) regard the status of *Sarcotragus*, which differs from *Ircinia* only by the extent of fiber fasciculation and coring, as uncertain, likewise the distinction of *Bergquistia*, from which so far no molecular marker has been published, to *Sarcotragus* is uncertain (Cook, 2007). Distinction between *Psammocinia* and *Ircinia*, however, has molecularly been shown (Pöppe et al., 2010). Irciniidae frequently resemble species of *Coscinoderma* in shape, texture, and surface (Sim & Kim, 2014). Genus *Coscinoderma* is a disjunct and species-poor genus with rare occurrence (but see Sim & Kim, 2014; Voultsiadou Koukoura, Van Soest, & Koukouras, 1991), currently classified as Spongiidae. Its species possess very fine, meandering ("woolly"), uncured secondary fibers. For example *C. mathewsi* (Lendenfeld), here represented by the reference specimen of the *Systema Porifera* (SDCC/RF077), is repeatedly recovered as sister to (this study) or within (Redmond et al., 2013) Irciniidae. A similar phylogenetic placement is observed from a GenBank specimen published as *C. sporadense* Voultsiadou-Koukoura, van Soest & Koukouras as published (KX866774, see Idan et al., 2018). In contrast, a *C. lanuga* Laubenfels specimen, a species described as poorly known, but valid (Bergquist, 1980; Voultsiadou Koukoura et al., 1991), falls into the Spongiidae resulting in a paraphyletic genus *Coscinoderma*. Clearly, examination of the type species *C. pesleonis* (Lamarck, 1813) is required to resolve the classification of this genus.

For the monospecific genus *Collospongia*, the holotype *C. auris* Bergquist, Cambie & Kernan (AM Z5035) has been analyzed (Galitz et al., 2018). Cook and Bergquist (2002c) remarked on morphological similarities with the Phyllospongiinae, but with different secondary metabolite composition and a unique skeletal structure, which allegedly makes classification into any of the thorectid subclasses difficult. We recover *Collospongia* among the first branching thorectid genera and clearly distant from Phyllospongiinae (see also Galitz et al., 2018).

Genus *Vaceletia* is the only lineage among the dictyoceratids with a mineral (although secondary hypercalcified aragonitic) skeleton. It is regarded as the only extant representative of the fossil family Verticillitidae on the basis of its sphinctozoan bauplan (see Vacelet, 2002). The lack of clear synapomorphies shared with any other extant sponge lineage hampered the (morphological) classification of *Vaceletia* (Vacelet, 2002) until molecular data unequivocally revealed the dictyoceratid origin (Wörheide, 2008), followed by the placement of Verticillitidae as fifth family of Dictyoceratida (Morrow & Cárdenas, 2015). Molecular data recover an early branching of

Vaceletia from the remaining thorectid + spongiid + irciniid taxa, probably as sister group.

3.1 | Implications for dictyoceratid morphological character evolution

Our reconstructed phylogenetic hypothesis has consequences for our current understanding of character evolution in dictyoceratid sponges. The sister group relationship of Dendroceratida to Dictyoceratida with Dysideidae splitting first from all other dictyoceratid families implies an ancestral nature of eurypylous choanocyte chambers for Keratosa in general and Dictyoceratida in particular (Erpenbeck, Sutcliffe, et al., 2012). Verticillitidae (*Vaceletia*) are the only Keratosa with aphodal choanocyte chambers, while the thorectid + spongiid + irciniid sister group can be distinguished by their diploidal choanocyte chambers, which are apomorphic within the Keratosa (Figure 1).

Possession of an armor, that is, a substantial ectosomal layer of foreign material, is frequently used for the discrimination of taxa, but our phylogenetic reconstruction does not indicate any phylogenetic signal in this character. Skeletal features constitute the most important source for phylogenetic and systematic characters in spiculose as well as non-spiculose sponges. Some of these characters have likewise been plotted on the phylogeny in Figure 1. The coring of primary or secondary fibers, that is, the inclusion of foreign mineral material into the fibers, did not harbor any phylogenetic signal. In Dysideidae, coring of both primary and secondary fibers potentially combines *Dysidea*, *Lamellodysidea*, and *Acanthodendrilla*, although the extent of this character as apomorphy in dysideids has yet to be shown (Erpenbeck, Sutcliffe, et al., 2012), particularly as secondaries in *Candidaspongia* are uncored (Cook & Bergquist, 2002a).

The possession of tertiary fibers is a combining character for the Phyllospongiinae, and the tertiary fiber-lacking alleged phyllospongiine *Candidaspongia* was revealed as dysideid (Galitz et al., 2018; Redmond et al., 2013). Tertiary fibers are further present in *Luffariella* and *Fenestraspongia*, two closely related genera. Some *Spongia* possess structures referred to as "pseudo-tertiary fibers" due to structural differences to those found in, for example, *Luffariella* (Cook & Bergquist, 2001), which leaves the possibility of tertiary fiber convergent evolution.

The arrangement of fibers into fascicles or into a regular (e.g., rectangular) skeleton does not constitute a reliable combining character either. While the closely related *Thorecta* and *Taonura* share this feature, histologically similar *Thorectandra* (cf. Cook & Bergquist, 2002d) are clearly distant.

In conclusion, clear-cut and unambiguous morphological apomorphies for the discrimination and classification of dictyoceratid sponges are scarce and too prone to homoplasies. The current morphology-based classification of the inter- and intrafamilial relationships of thorectids, spongiids, Irciniidae, and Verticillitidae is incongruent to phylogenetic hypotheses of independent molecular

markers and prompt for a re-classification and re-evaluation of synapomorphies based on integrative taxonomy.

ACKNOWLEDGEMENTS

We like to thank Dorte Janussen (SMF, Frankfurt), Ulrike Hausl-Hofstätter (Universalmuseum Joanneum, Graz), Carsten Lüter (MfN, Berlin), Emma Sherlock (NHM, London), Urs Wüest (Naturhistorisches Museum Basel), Andreas Dietzel (now JCU), Ratih Aryasari (Universitas Gadjah Mada, Yogyakarta), Gabriele Büttner, Nora Dotzler, and Simone Schätzle (LMU) for various support for this study. DE acknowledges financial support of the European Union under a Marie-Curie outgoing fellowship (MOIF-CT-2004 Contract No 2882) and Deutsche Forschungsgemeinschaft (DFG: Er611/5-1). GW acknowledges funding by LMU Munich's Institutional Strategy LMUexcellent within the framework of the German Excellence Initiative. Renata Manconi, Roberto Pronzato, Editors, and anonymous reviewers are thanked for their constructive comments that improved the manuscript considerably.

ORCID

Dirk Erpenbeck  <https://orcid.org/0000-0003-2716-1085>

Merrick Ekins  <https://orcid.org/0000-0002-4825-462X>

Gert Wörheide  <https://orcid.org/0000-0002-6380-7421>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. ITS reconstruction.

Figure S2. 28S C-region reconstruction.

Figure S3. 28S D3-D5 region reconstruction.

Figure S4. CO1 reconstruction.

How to cite this article: Erpenbeck D, Galitz A, Ekins M, et al. Soft sponges with tricky tree: On the phylogeny of dictyoceratid sponges. *J Zool Syst Evol Res*. 2020;58:27–40. <https://doi.org/10.1111/jzs.12351>

APPENDIX 1

Specimens newly sequenced for this study. "HT", "NT," and "LT" following the voucher number indicate holotype, neotype, and lectotype, respectively. Accession numbers in bold indicate sequences newly obtained in the course of this study. Accession numbers of previously published sequences of the same specimen used in this study are given in regular font.

Species	Voucher number	Accession numbers			
	Type status	CO1	ITS	28S-C	28S-D3D5
Dysideidae					
<i>Candidaspongia flabellata</i>	QM G305536			LR699438	
<i>Candidaspongia flabellata</i>	QM G305606			LR699439	
<i>Candidaspongia flabellata</i>	QM G306588			LR699440	
<i>Candidaspongia flabellata</i>	QM G307326			LR699441	
<i>Candidaspongia flabellata</i>	QM G314439		LR699322		JQ082714
<i>Candidaspongia flabellata</i>	QM G320157		LR699323		JQ082716
<i>Candidaspongia flabellata</i>	QM G322756			LR699442	
<i>Dysidea cf. arenaria</i>	QM G301096			LR699478	
<i>Dysidea cf. arenaria</i>	QM G301107			LR699479	
<i>Dysidea cf. arenaria</i>	QM G304690			LR699480	
<i>Dysidea cf. arenaria</i>	QM G305915			LR699481	
<i>Dysidea cf. arenaria</i>	QM G306542			LR699482	
<i>Dysidea cf. arenaria</i>	QM G306942			LR699483	
<i>Dysidea cf. arenaria</i>	QM G306943			LR699484	
<i>Dysidea cf. arenaria</i>	QM G324696			LR699485	
<i>Dysidea fragilis</i>	QM G301252			LR699486	
<i>Dysidea sp.</i>	QM G333259			LR699487	
<i>Lamellodysidea herbacea</i>	QM G301070			LR699509	
<i>Lamellodysidea herbacea</i>	QM G301191			LR699510	
Irciniidae					
<i>Ircinia sp.</i>	AM Z3989		LR699350		
<i>Ircinia sp.</i>	QM G306067		LR699351		
<i>Ircinia sp.</i>	QM G321282		LR699352		
<i>Ircinia sp.</i>	QM G322564		LR699353		
<i>Psammocinia sp.</i>	QM G303277			LR699528	
<i>Psammocinia sp.</i>	QM G303290			LR699529	
<i>Psammocinia sp.</i>	QM G303916			LR699530	
<i>Psammocinia sp.</i>	QM G304115			LR700203	
<i>Sarcotragus muscarum</i>	ZMA POR19029		LR699420		
<i>Sarcotragus sp.</i>	QM G318919		LR699372		
Spongiidae					
<i>Cf. Coscinoderma nardorus</i>	QM G303003			LR699466	
<i>Cf. Coscinoderma nardorus</i>	QM G304469			LR699467	
<i>Coscinoderma lanuga</i>	ZMA POR17975		LR699329	LR699454	
<i>Coscinoderma mathewsi</i>	QM G301075			LR699455	
<i>Coscinoderma mathewsi</i>	QM G303125			LR699456	
<i>Coscinoderma mathewsi</i>	QM G304249			LR699457	
<i>Coscinoderma mathewsi</i>	QM G304282			LR699458	
<i>Coscinoderma mathewsi</i>	QM G304283			LR699459	
<i>Coscinoderma mathewsi</i>	QM G304295			LR699460	

(Continues)

APPENDIX 1 (Continued)

Species	Voucher number	Accession numbers			
	Type status	CO1	ITS	28S-C	28S-D3D5
<i>Coscinoderma mathewsi</i>	QM G305068			LR699461	
<i>Coscinoderma mathewsi</i>	QM G313086		LR699330		
<i>Coscinoderma mathewsi</i>	QM G322760		LR699331	LR699462	JQ082718
<i>Coscinoderma mathewsi</i>	QM G322762			LR699463	
<i>Coscinoderma mathewsi</i>	QM G322765		LR699332		JQ082719
<i>Coscinoderma mathewsi</i>	QM G324713			LR699464	
<i>Coscinoderma mathewsi</i>	SDCC RF048			LR699465	
<i>Hippospongia ammata</i>	QM G306900		LR699344	LR699493	
<i>Hippospongia communis</i>	ZMA POR14572		LR699345		
<i>Hyattella intestinalis</i>	QM G300839			LR699494	
<i>Hyattella intestinalis</i>	QM G304652			LR699495	
<i>Rhopaloeides odorabile</i>	QM G303923			LR699531	
<i>Rhopaloeides odorabile</i>	QM G304220			LR699532	
<i>Rhopaloeides odorabile</i>	QM G322761	LR699417	LR699369	LR699533	JQ082768
<i>Rhopaloeides odorabile</i>	QM G322813	LR699418	LR699370		JQ082769
<i>Rhopaloeides odorabile</i>	SDCC RF067	LR699419	LR699371	LR699534	
<i>Spongia (Spongia) cf. irregularis</i>	SDCC NZ002		LR699375		JQ082674
<i>Spongia (Spongia) cf. irregularis</i>	SDCC NZ007		LR699376	LR699537	JQ082675
<i>Spongia (Spongia) hispida</i>	QM G303209			LR699538	
<i>Spongia (Spongia) cf. hispida</i>	ZMA POR19756		LR699377		
<i>Spongia (Spongia) officinalis</i>	ZMA POR14396	JQ082842	LR699378		LR699075
<i>Spongia</i> sp.	QM G324326			LR699539	
Spongiidae sp.	QM G304328		LR699379		
Spongiidae sp.	QM G305535		LR699380		
Spongiidae sp.	QM G322786	LR699423	LR699381		
Spongiidae sp.	QM G322830	LR699424	LR699382		
Spongiidae sp.	RMNH 2283	LR699425			
Thorectidae					
<i>Cacospongia cf. mollior</i>	SDCC RF139		LR699316	LR699437	JQ082658
<i>Cacospongia mollior</i>	LMJG 15405, LT		LR699317		
<i>Cacospongia mycofijiensis</i>	QM G301467	LR699396	LR699318	LR699435	
<i>Cacospongia mycofijiensis</i>	QM G312707	LR699398			
<i>Cacospongia mycofijiensis</i>	QM G313245		LR699319		
<i>Cacospongia mycofijiensis</i>	ZMA POR18574	LR699399	LR699320		
<i>Cacospongia mycofijiensis</i>	ZMA POR18575	LR699400	LR699321	LR699436	
<i>Cacospongia</i> sp.	QM G306016	LR699397	LR700205		
<i>Cacospongia</i> sp.	QM G314076		LR700206		
<i>Cacospongia</i> sp.	QM G315096		LR700207		
<i>Carteriospongia contorta</i>	QM G303874			LR699443	
<i>Carteriospongia contorta</i>	SDCC RF018		LR699324	LR699444	JQ082663
<i>Carteriospongia flabellifera</i>	QM G303017			LR699445	
<i>Carteriospongia flabellifera</i>	QM G304084			LR699446	
<i>Carteriospongia flabellifera</i>	QM G304114			LR699447	
<i>Carteriospongia flabellifera</i>	QM G304192			LR699448	

(Continues)

APPENDIX 1 (Continued)

Species	Voucher number	Accession numbers			
	Type status	CO1	ITS	28S-C	28S-D3D5
<i>Carteriospongia flabellifera</i>	QM G306728			LR699449	
<i>Carteriospongia flabellifera</i>	QM G313227	LR699401			JQ082664
<i>Carteriospongia flabellifera</i>	QM G315231		LR699325		JQ082665
<i>Carteriospongia flabellifera</i>	QM G322820				JQ082662
<i>Carteriospongia flabellifera</i>	QM G315298			LR699450	JQ082666
<i>Carteriospongia foliascens</i>	BMNH 1925.11.1.411, NT		LR699326	LR699451	
<i>Carteriospongia foliascens</i>	QM G304326			LR699452	
<i>Carteriospongia foliascens</i>	QM G317494	LR699402			
<i>Carteriospongia foliascens</i>	QM G322818		LR699327		JQ082667
<i>Collosporgia auris</i>	AM Z5035 HT			LR699453	
<i>Dactylospongia elegans</i>	NMB-PORI 44, LT		LR699333		
<i>Dactylospongia elegans</i>	QM G304125			LR699468	
<i>Dactylospongia elegans</i>	QM G304225			LR699469	
<i>Dactylospongia elegans</i>	QM G304296			LR699470	
<i>Dactylospongia elegans</i>	QM G305092			LR699471	
<i>Dactylospongia elegans</i>	QM G305998			LR699472	
<i>Dactylospongia elegans</i>	QM G306931			LR699473	
<i>Dactylospongia elegans</i>	QM G307754			LR699474	
<i>Dactylospongia elegans</i>	QM G313054	JQ082802	LR699334		
<i>Dactylospongia elegans</i>	QM G313637		LR699335		JQ082683
<i>Dactylospongia elegans</i>	QM G325555			LR699475	
<i>Dactylospongia metachromia</i>	SDCC RF047		LR699336	LR699476	JQ082684
<i>Dactylospongia</i> sp.	QM G311348	LR699408	LR699337		JQ082682
<i>Cf. Fascaplysinopsis reticulata</i>	QM G322803	JQ082812	LR699338		JQ082812
<i>Cf. Fascaplysinopsis reticulata</i>	SDCC RF017		LR699339	LR699489	JQ082706
<i>Cf. Fascaplysinopsis</i> sp.	CASIZ300177			LR699488	
<i>Cf. Fascaplysinopsis</i> sp.	QM G307325	LR699405			
<i>Cf. Fascaplysinopsis</i> sp.	QM G313004	LR699406	LR699340		
<i>Cf. Fascaplysinopsis</i> sp.	QM G314831		LR700208		
<i>Cf. Fascaplysinopsis</i> sp.	QM G320018	LR699407	LR699341	LR699490	
<i>Cf. Fascaplysinopsis</i> sp.	QM G331054		LR699342	LR699491	
<i>Cf. Fascaplysinopsis</i> sp.	QM G333241		LR700209		
<i>Cf. Fascaplysinopsis</i> sp.	QM G333299		LR700210	LR700202	
<i>Fenestraspongia intertexta</i>	BMNH 1886.12.15.238, HT		LR699343	LR699492	
<i>Hyrtios altus</i>	QM G311014	LR699410			
<i>Hyrtios erectus</i>	QM G301134			LR699496	
<i>Hyrtios erectus</i>	QM G301248			LR699497	
<i>Hyrtios erectus</i>	QM G303305			LR699498	
<i>Hyrtios erectus</i>	QM G303883			LR699500	
<i>Hyrtios erectus</i>	QM G303906			LR699501	
<i>Hyrtios erectus</i>	QM G303445			LR699499	
<i>Hyrtios erectus</i>	QM G303917			LR699502	
<i>Hyrtios erectus</i>	QM G304193			LR699503	
<i>Hyrtios erectus</i>	QM G304223			LR699504	

(Continues)

APPENDIX 1 (Continued)

Species	Voucher number	Accession numbers			
	Type status	CO1	ITS	28S-C	28S-D3D5
<i>Hyrtios erectus</i>	QM G304346			LR699505	
<i>Hyrtios erectus</i>	QM G304354			LR699506	
<i>Hyrtios erectus</i>	QM G304362			LR699507	
<i>Hyrtios erectus</i>	QM G305776			LR699508	
<i>Hyrtios erectus</i>	SDCC RF049		LR699346		
<i>Hyrtios erectus</i>	SNSB-BSPG.GW6170		LR699347		
<i>Hyrtios proteus</i>	ZMA POR14381	JQ082820	LR699348		
<i>Hyrtios reticulatus</i>	SDCC RF031		LR699349		
<i>Lendenfeldia chondrodes</i>	SNSB-BSPG.GW27611			LR699513	
<i>Lendenfeldia chondrodes</i>	SNSB-BSPG.GW27619			LR699514	
<i>Lendenfeldia chondrodes</i>	SNSB-BSPG.GW27699			LR699515	
<i>Lendenfeldia chondrodes</i>	SNSB-BSPG.GW8481		LR699354	LR699516	
<i>Lendenfeldia plicata</i>	QM G303343			LR699517	
<i>Lendenfeldia plicata</i>	QM G304093			LR699518	
<i>Lendenfeldia plicata</i>	QM G319507		LR699356		
<i>Lendenfeldia plicata</i>	QM G322766	LR699412	LR699394		
<i>Lendenfeldia plicata</i>	QM G312964	LR699411	LR699392		
<i>Lendenfeldia cf. plicata</i>	QM G304324			LR699512	
<i>Luffariella caliculata</i>	QM G304686, HT		LR699357	LR699519	
<i>Luffariella cylindrica</i>	QM G304687, HT		LR699358	LR699520	
<i>Luffariella variabilis</i>	BMNH 1885.8.8.52, HT		LR699359		
<i>Petrosaspongia nigra</i>	QM G304685, HT		LR699360	LR699521	
<i>Petrosaspongia nigra</i>	QM G313020	LR699413	LR699361		JQ082747
<i>Petrosaspongia nigra</i>	QM G315543	LR699414	LR699362		JQ082748
<i>Phyllospongia lamellosa</i>	QM G304169			LR699522	
<i>Phyllospongia lamellosa</i>	QM G304677			LR699523	
<i>Phyllospongia lamellosa</i>	QM G322790		LR699363		JQ082749
<i>Phyllospongia lamellosa</i>	QM G322848	LR699415	LR699364		
<i>Phyllospongia papyracea</i>	QM G300316			LR699524	
<i>Phyllospongia papyracea</i>	QM G304332			LR699525	
<i>Phyllospongia papyracea</i>	QM G307267	LR699416	LR699365	LR699526	
<i>Phyllospongia papyracea</i>	QM G307268			LR699527	
<i>Phyllospongia papyracea</i>	QM G318009		LR699366		JQ082750
<i>Phyllospongia papyracea</i>	QM G322855		LR699367		JQ082751
<i>Phyllospongia papyracea</i>	QM G322863		LR699368		JQ082752
<i>Phyllospongiinae</i> sp.	SNSB-BSPG.GW26545		LR735997		
<i>Scalarispongia scalaris</i>	LMJG 15406		LR699373		
<i>Semitaspongia</i> sp.	SDCC NZ066			LR699535	
<i>Semitaspongia</i> sp.	SDCC NZ121	LR699421	LR699374	LR699536	
<i>Smenospongia aurea</i>	ZMA POR13807	LR699422			
<i>Strepsichordaia aliena</i>	RMNH 2284	LR699426	LR699383		
<i>Strepsichordaia caliciformis</i>	QM G311299	JQ082843	LR699384		
<i>Cf. Strepsichordaia lendenfeldi</i>	QM G322810		LR700211		JQ082775

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Species	Voucher number	Accession numbers			
	Type status	CO1	ITS	28S-C	28S-D3D5
<i>Strepsichordaia lendenfeldi</i>	AM Z5026 HT	LR699427	LR699385	LR699540	JQ082776
<i>Strepsichordaia lendenfeldi</i>	QM G303854			LR699541	
<i>Strepsichordaia</i> sp.	QM G306046	LR699403	LR699328		JQ082669
<i>Strepsichordaia</i> sp.	QM G306072	LR699404			
<i>Taonura flabelliformis</i>	BMNH 1844.9.13.3, HT		LR699386		
<i>Taonura flabelliformis</i>	SDCC RF024			LR699542	JQ082777
<i>Thorecta freija</i>	QM G303743		LR699387	LR699543	JQ082778
<i>Thorecta reticulata</i>	SDCC NZ097			LR699544	JQ082779
<i>Thorecta</i> sp.	QM G303206				JQ082780
<i>Thorectandra excavatus</i>	QM G303331	LR699428	LR699389	LR699545	JQ082781
<i>Thorectandra excavatus</i>	QM G303563			LR699546	
<i>Thorectandra excavatus</i>	QM G303575			LR699547	
<i>Thorectandra excavatus</i>	ZMA POR14042	JQ082845	LR699390		JQ082782
<i>Thorectandra</i> sp.	SDCC RF016		LR700212	LR700204	
<i>Thorectaxia papuensis</i>	ZMA POR19767			LR699548	
Thorectidae sp.	SNSB-BSPG.GW26569		LR700215		
Thorectidae sp.	CASIZ302695			LR699549	
Thorectidae sp.	QM G306003		LR700213		JQ082707
Thorectinae sp.	CASIZ302698			LR699550	
Thorectinae sp.	QM G301060			LR699551	
Thorectinae sp.	QM G307378	LR699431	LR699391		JQ082710
Thorectinae sp.	QM G313051	LR699432	LR699393		
Thorectinae sp.	SDCC RF053			LR699552	JQ082743
Thorectinae sp.	SNSB-BSPG.GW26644	LR699430			
Thorectinae sp.	ZMA POR11466	LR699433			
Thorectinae sp.	ZMA POR15722	JQ082831			JQ082744
Thorectinae sp.	ZMA POR16798	JQ082813	LR700214		
Thorectinae sp.	ZMA POR17995	LR699434	LR699395		
Uncategorized					
Dictyoceratida sp.	SDCC NZ147	LR700201			
Dictyoceratida sp.	SNSB-BSPG.GW27609			LR699477	