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Systematics and spicule evolution in dictyonal sponges (Hexactinellida: Sceptrulophora) with description of two new species

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In this paper we report on recently collected specimens of glass sponges belonging to Farreidae Gray, 1872, and Tretodictyidae Schulze, 1886 (Porifera: Hexactinellida: Hexactinosida). All specimens represent new geographical records for their genera: Coral Sea for *Aspidoscopulia* Reiswig, 2002 (Farreidae) and *Psilocalyx* Ijima, 1927 (Tretodictyidae); north-west Atlantic for *Sarostegia* Topsent, 1904 (Farreidae). Two new species, ***Aspidoscopulia australia*** Dohrmann, Göcke & Janussen **sp. nov.** and ***Aspidoscopulia ospreya*** Dohrmann, Göcke & Janussen **sp. nov.**, are described. To investigate further the evolution of hexactinosidan sponges, we sequenced two nuclear (18S and 28S rDNA) and two mitochondrial [16S ribosomal rDNA, cytochrome oxidase subunit I (COI)] genes from these specimens, as well as from a recently described new species of *Lonchiphora* Ijima, 1927 (Farreidae). Besides corroborating the monophyly of Tretodictyidae, our molecular phylogenetic analyses support a clade of clavule-bearing sponges with a farreoid dictyonal framework (i.e. Farreidae *sensu stricto*). In contrast, *Sarostegia*, which lacks these features, appears unrelated to this clade – instead our data are consistent with an earlier placement of this genus in Euretidae Zittel, 1877. We introduce formally the taxon Sceptrulophora Mehl 1992, and emend the classification of Hexactinosida to reflect this move and our new findings regarding the position of *Sarostegia*. Finally, we discuss implications of the molecular phylogeny for the evolution of sceptrules, the defining autapomorphy of Sceptrulophora.

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ADDITIONAL KEYWORDS: *Aspidoscopulia* – Deep Down Under Expedition – Farreidae – *Lonchiphora* – molecular systematics – Porifera – *Psilocalyx* – *Sarostegia* – sceptrules.

INTRODUCTION

Glass sponges (Porifera, Hexactinellida) are a diverse group of mainly deep-sea dwelling siliceous sponges that are globally important in benthic ecosystems (Leys, Mackie & Reiswig, 2007). Approximately 600 extant species are currently described, but given their remote habitats and the low number of taxonomic experts for the group, hexactinellid diversity is probably much higher (Reiswig, 2002a). The enormous

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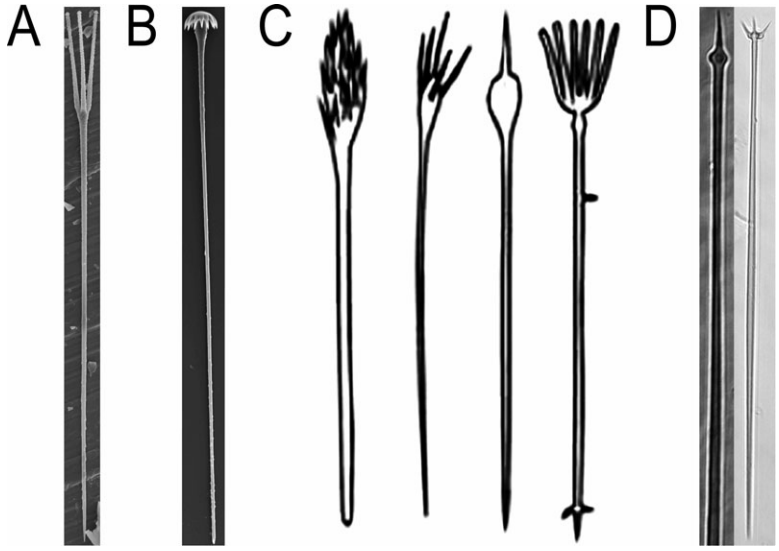

Figure 1. Sceptrules. A, regular scopule (from *Heterochone* sp.). B, regular (pileate) clavule from *Farrea* sp. (courtesy H. M. Reiswig). C–D, unusual sceptrule types. C, from left to right: sarule (left: *Sarostegia*, right: *Claviscopulia*), lonchiole (*Lonchiphora*; interpreted from text-description), aspidoscopule (*Aspidoscopulia*). Redrawn from Reiswig (2002b); D, two types of lonchioles from *Lonchiphora antarctica* (Göcke & Janussen, 2011).

variety of spicule forms and the complex skeletal anatomy of Hexactinellida provide rich grounds for delineating natural taxa, which is rather exceptional for sponges and probably responsible for a generally good agreement between morphology-based systems and molecular phylogenies of this group (Erpenbeck & Wörheide, 2007; Dohrmann *et al*., 2008; Dohrmann, Collins & Wörheide, 2009). Nonetheless, some taxonomic hypotheses, such as the designation of certain genera to their respective families remain ambiguous, and molecular phylogenetic investigation of many important groups is still pending, especially amongst the so-called dictyonal taxa, i.e. those possessing rigid skeletons of fused hexactine megascleres (cf. Leys *et al*., 2007: ch. 6).

Dictyonal skeletons (frameworks) appear in the Late Devonian (Rigby *et al*., 2001), and are especially prominent in the Mesozoic, often in reef settings (e.g. Mehl, 1992; Pisera, 1999); modern analogues of dictyonal sponge reefs can be found on the west Canadian continental shelf (Conway *et al*., 1991; Krautter *et al*., 2001). Whereas morphological and molecular evidence suggest that dictyonal sponges do not represent a natural group (Mehl, 1992; Dohrmann *et al*., 2008), the presence of sceptrules (Fig. 1) in most of the extant species supports the monophyly of the taxon Sceptrulophora (Mehl, 1992), which was recently corroborated by molecular data (Dohrmann *et al*., 2008, 2009). However, a division of Sceptrulophora into Scopularia and Clavularia (Schulze, 1886; Mehl, 1992), according to the mutually exclusive occurrence of scopules and clavules (Fig. 1), is probably artificial as indicated by strong molecular evidence for the paraphyly of Scopularia (Dohrmann *et al*., 2008, 2009). Although clavules are likely to be synapomorphic, to date it has not been possible to test the monophyly of Clavularia – i.e. Farreidae Gray, 1872 – with molecular phylogenetic methods because of the lack of DNA sequence data from more than one species (see Dohrmann *et al*., 2009).

Farreidae currently contains six genera: *Farrea* Bowerbank, 1862 (26 spp.), and the monospecific genera *Asceptrulum* Duplessis & Reiswig, 2004, *Aspidoscopulia* Reiswig, 2002, *Claviscopulia* Schulze, 1899, *Lonchiphora* Ijima, 1927, and *Sarostegia* Topsent, 1904.

Whereas sceptrules in *Farrea* species exclusively occur in the form of clavules (Reiswig, 2002b) and *Asceptrulum* lacks sceptrules altogether (Duplessis & Reiswig, 2004), three types of unusual sceptrule forms are found amongst the remaining genera, namely aspidoscopules, lonchioles, and sarules (Fig. 1). These latter types of spicules are of particular interest to understanding sceptrule evolution because they appear somewhat 'intermediate' between scopules and clavules. For example, sarules have been hypothesized to represent the ancestral sceptrule-type ('protosceptrule') from which both scopules and clavules evolved (Schulze, 1899; Reid, 1958; Mehl, 1992). However, without a better understanding of sceptrulophoran phylogeny, one can only speculate about scenarios of spicule evolution.

Here, we report on recently collected material of sceptrulophoran sponges, comprising four species of

Farreidae, as well as the rarely found *Psilocalyx wilsoni* Ijima, 1927 (Tretodictyidae Schulze, 1886). We provide morphological descriptions of the specimens, including two new species of *Aspidoscopulia*, and incorporate them into a molecular phylogenetic analysis based on four genes. We found that *Sarostegia* does not group with the remaining farreids, consistent with previous suggestions based on framework construction (see discussion in Reiswig, 2002b). In contrast, *Farrea*, *Aspidoscopulia*, and *Lonchiphora* appear closely related. Monophyly of Tretodictyidae (Mehl, 1992; Dohrmann *et al*., 2008) is further corroborated. Based on our findings, we transfer *Sarostegia* back to Euretidae Zittel, 1877, and accordingly emend family diagnoses of Euretidae and Farreidae. We also formally introduce Sceptrulophora Mehl, 1992 with diagnosis and scope, provisionally suggesting subordinal rank within Hexactinosida Schrammen, 1912. Finally, we discuss the evolution of sceptrules in light of the molecular phylogeny.

MATERIAL AND METHODS

Specimen collection and morphology

Two specimens belonging to new species of *Aspidoscopulia* and one specimen of *P. wilsoni* were collected during the December 2009 Deep Down Under expedition (DDU, http://www.deepdownunder.de/) at Osprey Reef, Queensland Plateau, Coral Sea, Australia, using a remotely operated vehicle (ROV) 'Cherokee' (MARUM, Bremen, Germany) (see supporting movies available at Open Data LMU http://dx.doi.org/10.5282/ubm/data.36), and preserved in 96% ethanol. One specimen of *Sarostegia oculata* was collected in 2006 by Harbor Branch Oceanographic Institution (HBOI) off Miami Terrace, Florida, USA, south-west of Bimini, using the manned submersible 'Johnson-Sea-Link II', cryopreserved, kindly provided to M. D. by Karri Haen (Iowa State University, Ames), and transferred to 96% ethanol. These specimens were initially identified at LMU Munich, based on light-microscopic (LM) investigation of skeletal fragments and provisional spicule preparations. Subsamples were then processed at Senckenberg Museum Frankfurt (SMF). Skeletal preparations were produced and studied by LM and scanning electron microscopy (SEM) as previously described (e.g. Janussen & Reiswig, 2009). In addition, a subsample of the holotype of the recently described farreid *Lonchiphora antarctica* (Göcke & Janussen, 2011) was preserved in RNAlater (Ambion) upon collection, stored at –80 °C at the University of Göttingen, and later transferred to LMU Munich for molecular investigation. For comparison, a specimen of *Claviscopulia facunda* Schmidt, 1870 (from Harvard Museum of Comparative Zoology, MCZ) and *P. wilsoni*, and an unpublished specimen of *S. oculata*, were studied at the Zoological Museum of Amsterdam (ZMA). The original material published herein is deposited at the Queensland Museum (QM), the Harbor Branch Oceanographic Museum (HBOM), and the Bavarian State Collections for Zoology. Schizotypes and voucher samples are deposited at SMF.

Molecular methods

Nuclear 18S and 28S, and mitochondrial (mt) 16S ribosomal DNA (rDNA) fragments were amplified and sequenced as described previously (Dohrmann *et al*., 2008). In addition, an ~1.3 kb fragment of the mt-encoded cytochrome oxidase subunit I (COI) gene – spanning the 'Folmer-' and the internal loop 3 – transmembrane helix 11 (I3–M11) partitions (cf. Erpenbeck, Hooper & Wörheide, 2006) – was amplified from the above specimens, as well as from further dictyonal sponges used in previous studies (Dohrmann *et al*., 2008, 2009), using various combinations of mostly degenerate primers (Table 1), Promega's GoTaq (reaction mixes as in Dohrmann *et al*., 2008),

Table 1. PCR primers used to amplify cytochrome oxidase subunit I

#	Position	Sequence (5′–3′)	Remarks
1	F	GCTGACTATNTTCNACNAACCACAAAG	This study
2	F	TCTACCAACCACAAAGATATCGG	K. Haen (pers. comm.)
3	R int	TAGCATTGTTATTCCTCCGGCTA	K. Haen (pers. comm.)
4	R int	TARCATNGTNATTCCNCCNGCTA	Modified from no. 3
5	R int	TARCATTGTTATTCCBCCDGCTA	Modified from no. 3
6–8	F int		Reverse-complements of nos 3–5
9	R	TCCTAGAAANTGTTGNGGGAAGAAAG	This study
10	R	TCCTARAAANTGTTGNGGGAARAANG	Modified from no. 9
11	R	TCCTARAAARTGTTGDGGGAARAASG	Modified from no. 9

F, forward primer; F int, forward primer for 3′-half; R, reverse primer; R int, reverse primer for 5′ half.

Table 2. Overview of molecular data set, with accession numbers for newly generated sequences and voucher numbers for previously unsampled specimens

Family	Species	18S	28S	16S	COI
Farreidae	***Aspidoscopulia ospreya* sp. nov.** QM G332104	FR846206*	FR846207	FR846208	FR848904
	***Aspidoscopulia australia* sp. nov.** QM G332077	FR846203*	FR846204	FR846205	FR848903
	Sarostegia oculata HBOI 25-V-06-2-001	FR846212	FR846213	FR846214	FR848907†
	Lonchiphora antarctica SMF 10772	FR846209*	FR846210	FR846211	FR848905‡
	Farrea sp.	2	2	2	FR848906†
Euretidae	**gen.** et **sp. nov.**	2	2	2	FR848908
Tretodictyidae	*Psilocalyx wilsoni* QM G331821	FR846215	FR846216	FR846217	FR848909*
	Tretodictyum tubulosum	1	1	1	FR848910
	Hexactinella carolinensis	1*	1	1	FR848911
Aphrocallistidae	*Aphrocallistes beatrix*	2	2	2	FR848902
	Aphrocallistes vastus	1	1	1	3
	Heterochone calyx	1	1	1	FR848901
	Heterochone sp.	1	1	1	FR848900
Dactylocalycidae	*Iphiteon panicea*	1	1	1	4

1, from Dohrmann *et al*. (2008); 2, from Dohrmann *et al*. (2009); 3, from Rosengarten *et al*. (2008); 4, from Haen *et al*. (2007); *only 5′-half; †fragments did not overlap, leaving an internal gap; ‡only 3′-half.
HBOI, Harbor Branch Oceanographic Institution; QM, Queensland Museum; SMF, Senckenberg Museum Frankfurt.

and 'touch-down' thermal regimes with final annealing temperatures of 45 or 30 °C. Complete amplification of this region was only rarely successful; for most specimens 5′- and 3′-halves were thus amplified separately. Amplicons were further processed as described in Dohrmann *et al*. (2008). Table 2 gives an overview of data completeness and accession numbers for the newly generated sequences.

Phylogenetic analysis

Ribosomal DNA sequences were manually aligned to previous alignments (Dohrmann *et al*., 2009), aided by RNA secondary structure in the case of 18S and 28S (cf. Dohrmann *et al*., 2008); ambiguous regions were removed. COI sequences were pre-aligned in ClustalX 2.0 (Larkin *et al*., 2007), followed by manual refinement. The COI alignment was largely unambiguous, containing only a few instances of single-species, 1-bp insertions (probably a result of sequencing errors); these sites were removed. Preliminary analyses showed that topology and support values for Sceptrulophora were not markedly influenced by a larger taxon sampling including all available hexactinellid orthologues plus outgroups (results not shown). Therefore, only those taxa listed in Table 2 were kept for final analysis as this allowed inclusion of additional sites whose alignment was ambiguous within the complete taxon set. The non-sceptrulophoran dictyonal species *Iphiteon panicea* Bowerbank, 1869 (Dactylocalycidae Gray, 1867) served as an outgroup.

The four markers were concatenated (4686 bp) in SeaView v. 4.0 (Gouy, Guindon & Gascuel, 2010) and analysed in a partitioned maximum likelihood (ML) framework as implemented in RAxML (Stamatakis, 2006) v. 7.2.6 (http://wwwkramer.in.tum.de/exelixis/software.html) using independent substitution models for 18S + 28S double-stranded regions (stems), 18S single-stranded regions (loops), 28S loops, 16S, and COI. The final alignment and the corresponding structure, partition, and tree files are available at Open Data LMU (http://dx.doi.org/10.5282/ubm/data.36). As computational limitations were not an issue with this small data set, the most general (i.e. least simplifying) models were employed, namely the 16-state paired-sites model (cf. Savill, Hoyle & Higgs, 2001) S16 for 18S + 28S stems, and general time reversible (GTR; Lanave *et al*., 1984) for the other partitions. However, additional analyses under six- and seven-state paired-sites models (S6A, S7A), which do not fully account for mismatch pairs (Savill

et al., 2001), led to essentially the same results (not shown). Among-site rate variation was modelled for each partition independently using discrete gamma distributions with four rate categories (+G_4; Yang, 1994). Clade stability was assessed by rapid bootstrapping (Felsenstein, 1985; Stamatakis, Hoover & Rougemont, 2008), based on 1000 pseudoreplicates.

Additional tests of phylogenetic hypotheses (see Results) were performed using the approximately unbiased (AU) test (Shimodaira, 2002) as implemented in TREEFINDER (Jobb, von Haeseler & Strimmer, 2004; October 2008 version). As paired-sites models are not supported by TREEFINDER, only GTR + G_4 models, partitioned by gene, were employed in these analyses.

RESULTS

Molecular phylogeny

The phylogeny of Sceptrulophora inferred from our four-gene data set (Fig. 2) is largely congruent with previous results (Dohrmann *et al*., 2009), except that there is now strong support for paraphyly of *Aphrocallistes* and *Heterochone* (Aphrocallistidae Gray, 1867). Monophyly of Tretodictyidae is further corroborated, although the position of *Psilocalyx* as sister to

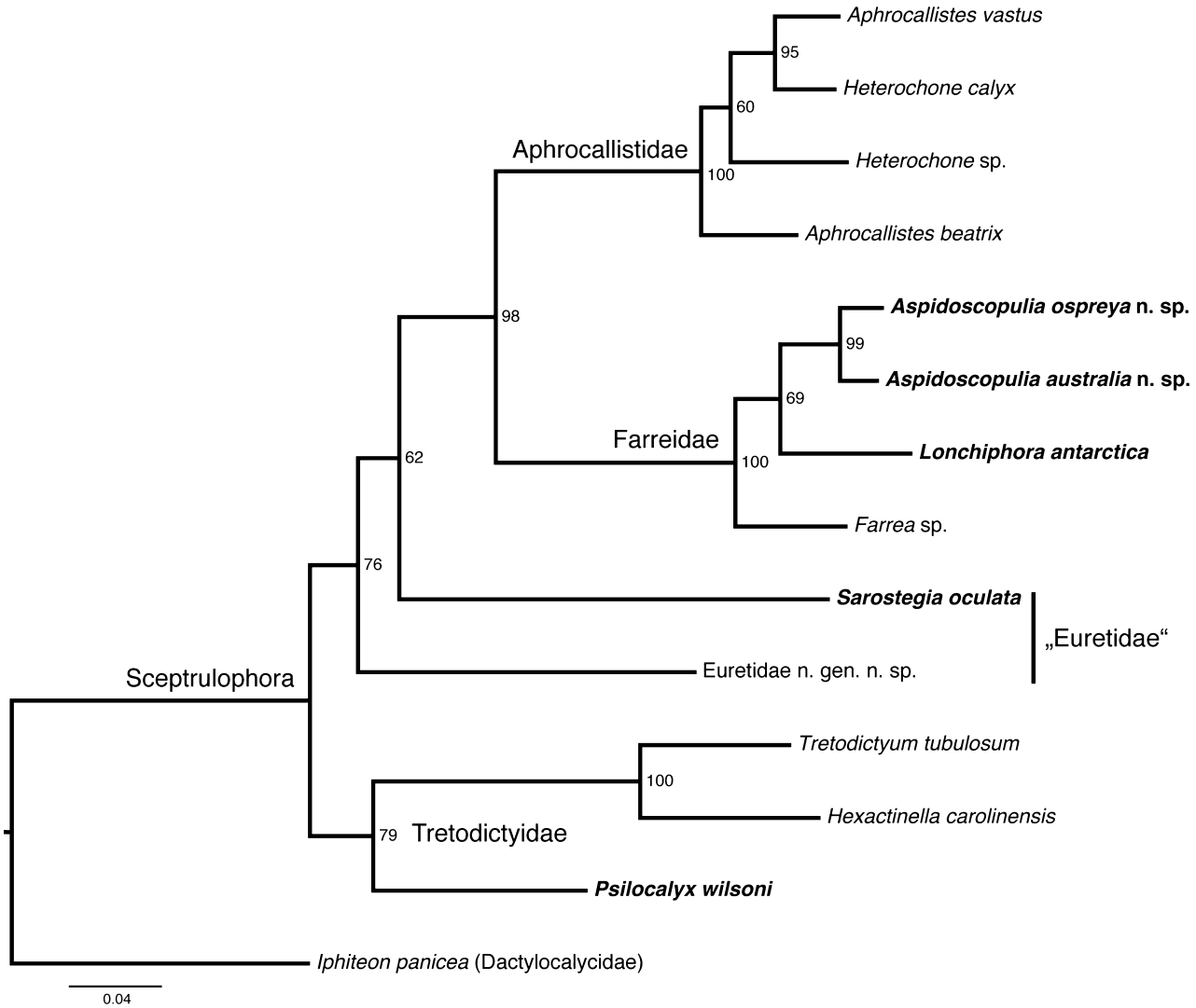

Figure 2. Maximum likelihood phylogeny of Sceptrulophora inferred from combined 18S, 28S, 16S, and COI sequences. Previously unsampled species are highlighted in bold. Bootstrap percentages (based on 1000 pseudoreplicates) are given at nodes. Scale bar indicates number of expected substitutions per site. See Material and methods for further details.

Tretodictyum + *Hexactinella* is only moderately supported. *Aspidoscopulia* and *Lonchiphora* form sister groups [with weak bootstrap support (BS)] and firmly group together with *Farrea*, whereas *Sarostegia* falls outside this clade, as sister group to Aphrocallistidae + Farreidae *s.s.* Although this exact position remains ambiguous because of low BS, a close relationship of *Sarostegia* to the remaining farreids was rejected by the AU test ($P = 0.0000$). A topology displaying *Sarostegia* as sister to the remaining sceptrulophorans was likewise rejected ($P = 0.0000$). In contrast, topologies with *Sarostegia* as sister to Euretidae gen. nov. and sister to Tretodictyidae, respectively, could not be rejected (Euretidae: $P = 0.4494$, Tretodictyidae: $P = 0.1060$). Thus, although monophyly of Farreidae *s.s.* is supported by our analyses, the molecular data clearly reject the taxonomic hypothesis that *Sarostegia* belongs in this family. As an affinity to Euretidae had been previously proposed (Topsent, 1928; Reid, 1958), and *Sarostegia* does not display any diagnostic/apomorphic characters of Tretodictyidae (see Discussion), we hereafter treat *Sarostegia* as belonging to Euretidae (as further discussed below).

MORPHOLOGICAL DESCRIPTIONS AND SYSTEMATIC ACCOUNT

Porifera Grant, 1836

Hexactinellida Schmidt, 1870

Hexasterophora Schulze, 1886

Hexactinosida Schrammen, 1912

Sceptrulophora Mehl, 1992

Diagnosis: Dictyonal Hexactinellida with sceptrules; if sceptrules lacking (which is rare), dictyonal skeleton usually euretoid or farreoid. Uncinates and oxy- and/or discohexasters usually present.

Remarks: Mehl (1992) did not give a formal diagnosis for Sceptrulophora, and this taxon is not recognized in *Systema Porifera* (Hooper & van Soest, 2002). However, monophyly of the group is strongly supported by morphology (Mehl, 1992; Dohrmann *et al*., 2008) and by molecular data (Dohrmann *et al*., 2008, 2009; this study). Sceptrulophora is here formally introduced for the sake of a more natural (i.e. phylogeny-based) classification; for the time being it is best given subordinal rank within Hexactinosida, although the latter taxon is likely to be a paraphyletic assemblage in need of revision (Mehl, 1992; Dohrmann *et al*., 2008, 2009). Although this might require erection of a second suborder for the remaining hexactinosidans – essentially Dactylocalycidae, which might itself be an artificial group – we refrain from such a move here. Instead, we follow Ebach & Williams (2010) and designate Hexactinosida and Dactylocalycidae as 'aphyletic' taxa.

Scope: The following families are included in Sceptrulophora: Aphrocallistidae Gray, 1867, Auloplacidae Schrammen, 1912 (=Auloplax Schulze, 1904), Craticulariidae Rauff, 1893 (=*Laocoetis* Pomel, 1872), Cribrospongiidae Roemer, 1864 (=*Stereochlamis* Schrammen, 1912), Euretidae Zittel, 1877, Farreidae Gray, 1872, Fieldingiidae Tabachnick & Janussen, 2004 (=*Fieldingia* Kent, 1870), and Tretodictyidae Schulze 1886. The order Fieldingida, which was solely erected for *Fieldingia* (Tabachnick & Janussen, 2004) is consequently abolished. Inclusion of *Stereochlamis* is somewhat provisional because loose spiculation has not been reported yet for this poorly known taxon (Reiswig, 2002c); however, it has a euretoid skeletal organization and therefore fits the diagnosis given above. *Tretopleura* Ijima, 1927 will also have to be moved to Sceptrulophora because it includes one species with scopules [*Tretopleura styloformis* (Tabachnick, 1988); loose spicules from the second (type) species are unknown]. Although currently classified in Aulocalycoida: Uncinateridae (Reiswig, 2002d), an affinity of *Tretopleura* to Euretidae has been suggested previously (see Tabachnick & Reiswig, 2000); this problematic genus (cf. Reiswig, 2002d) is currently under revision (K. R. Tabachnick, pers. comm.).

Euretidae Zittel, 1877

Revised diagnosis (emended from Reiswig & Wheeler, 2002, for inclusion of *Sarostegia*; addition highlighted in bold): Body form either of branching and/or anastomosing tubes, or cup-funnel formed of a ring of tubes, or of a single tube, or of a single-wall funnel with or without lateral oscula extended on marginal tubes, or blade form; dictyonal meshes mainly rectangular or triangular or irregular; meshes usually equal-sided but elongate prismatic mesh series with transverse lamellae developed in some species; dictyonal strands, if developed, orientated longitudinally; with or without dictyonal cortices composed of primary or secondary dictyonalia; dermalia and atrialia are commonly pentactins or pinular hexactins with rays of approximately equal length, or both forms lacking; scopules, **which might also be represented by sarules**, and uncinates are usually present but are lacking in two genera; microscleres occur as oxyhexasters and/or discohexasters.

Remarks: Euretidae, being the most species-rich family of Hexactinosida (Reiswig & Wheeler, 2002; Leys *et al*., 2007), is morphologically highly diverse and taxonomically poorly defined (see above); in lack

of potential autapomorphies, its validity remains to be tested with molecular data.

Sarostegia Topsent, 1904

Sarostegia oculata Topsent, 1904

Material examined: One specimen (HBOI 25-V-06-2-001, SMF 11034) collected 25.v.2006 by Johnson-Sea-Link II east of Miami Terrace, south-west of Bimini, 25°38′N, 79°24′W, on a 10–20° slope, depth 745 m.

Additional material examined: One specimen (ZMA Por.3868) collected 8.vi.1982 at Sao Tiago, Cap Verde, 14°49′N, 24°45′W, depth 600–675 m. One specimen of *Claviscopulia facunda* (MCZ 6711n) collected 21.ii.1879 during the Agassiz expedition 1878–79, Blake Caribbean Island Exploration (C.I.E.), St. Vincent, st. 232, 13°07′N, 61°07′W, 159 m depth.

Description: Body shape tubular, arborescent, more or less branching, attached by a basal plate, branches cylindrical to subcylindrical (Fig. 3). Specimen examined is 53 mm high and diameter of the tubes 3–7 mm, gradually increasing from base to top. Atrial cavity narrow, 2–3 mm diameter, extending throughout most of the body. Surface of the specimen occupied by (?symbiotic) zoanthids as previously reported for this species (see Reiswig, 2002b). One of the tubes contained a large polychaete, which filled out the entire inner cavity (Fig. S1). Parietal oscula 0.6–2 mm distributed at the sides of branches at intervals of 6–8 mm. Colour in ethanol preserved white to pale brown.

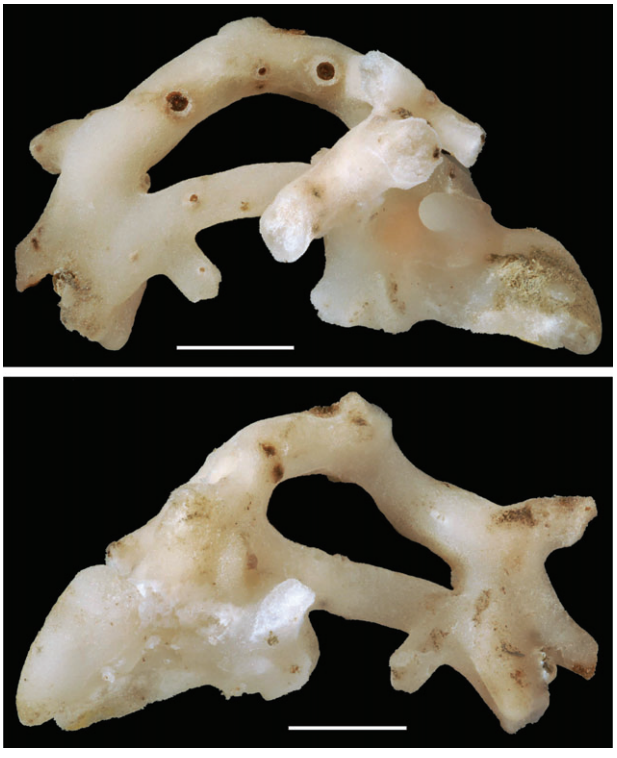

Figure 3. *Sarostegia oculata*. Piece of the original specimen described herein seen from both sides. Scale bars = 10 mm.

Skeleton (Figs 4–5, Table 3): Irregular dictyonal framework of hexactins with triangular and quadrangular meshes. Beams are microtuberculated, mesh sides 119–333 μm, beam thickness 14–48 μm. Dermalia are microspined hexactins with short distal ray, tangential rays 250–320 μm and proximal ray 230–340 μm length, probably choanosomal oxyhexactins spiny with unpaired rays measuring 78–150 μm and tangential rays 58–100 μm. Microhexactins of similar dimensions are commonly found attached by one ray to the dictyonal framework. Sceptrules are sarules, 308–400 mm long, with 15–30 secondary rays, some of which are fused almost all the way to the top. The axial cross of the sarules lies in the basal part of the head. Uncinates are present in two size ranges:

Table 3. Spicule measurements of *Sarostegia oculata* (HBOI 25-V-06-2-001, SMF 11034)

Parameter	Mean	SD	Range	*N*
Free spicules				
Dermal hexactin				
Tangential ray (L)	154	32.3	250–320	28
Proximal ray (L)	144	67.4	230–340	18
Choanosomal hexactin				
Proximal ray (L)	104	18.2	78–150	31
Tangential ray (L)	81	13.1	58–100	31
Large uncinate (L)	842	83.1	720–1030	21
Small uncinate (L)	552	57.7	400–650	33
Sarule				
(L)	361	22.4	308–400	31
(D)	10	0.6	9–12	31
Oxyhexaster				
(D)	74	6.5	60–90	31
Primary ray (L)	37	3.6	30–43	31
Secondary ray (L)	26	2.4	20–30	31
Number of secondary rays	3	0.6	3–5	31
Discohexaster				
(D)	51	7.0	35–60	28
Primary ray (L)	23	3.7	16–30	28
Secondary ray (L)	16	2.5	12–20	28
Number of secondary rays	5	0.0	5–7	28
Framework				
Length between nodes	232	52.7	119–333	30
Central beam width	26	9.9	14–48	30

All measurements in μm.
D, diameter; L, length.

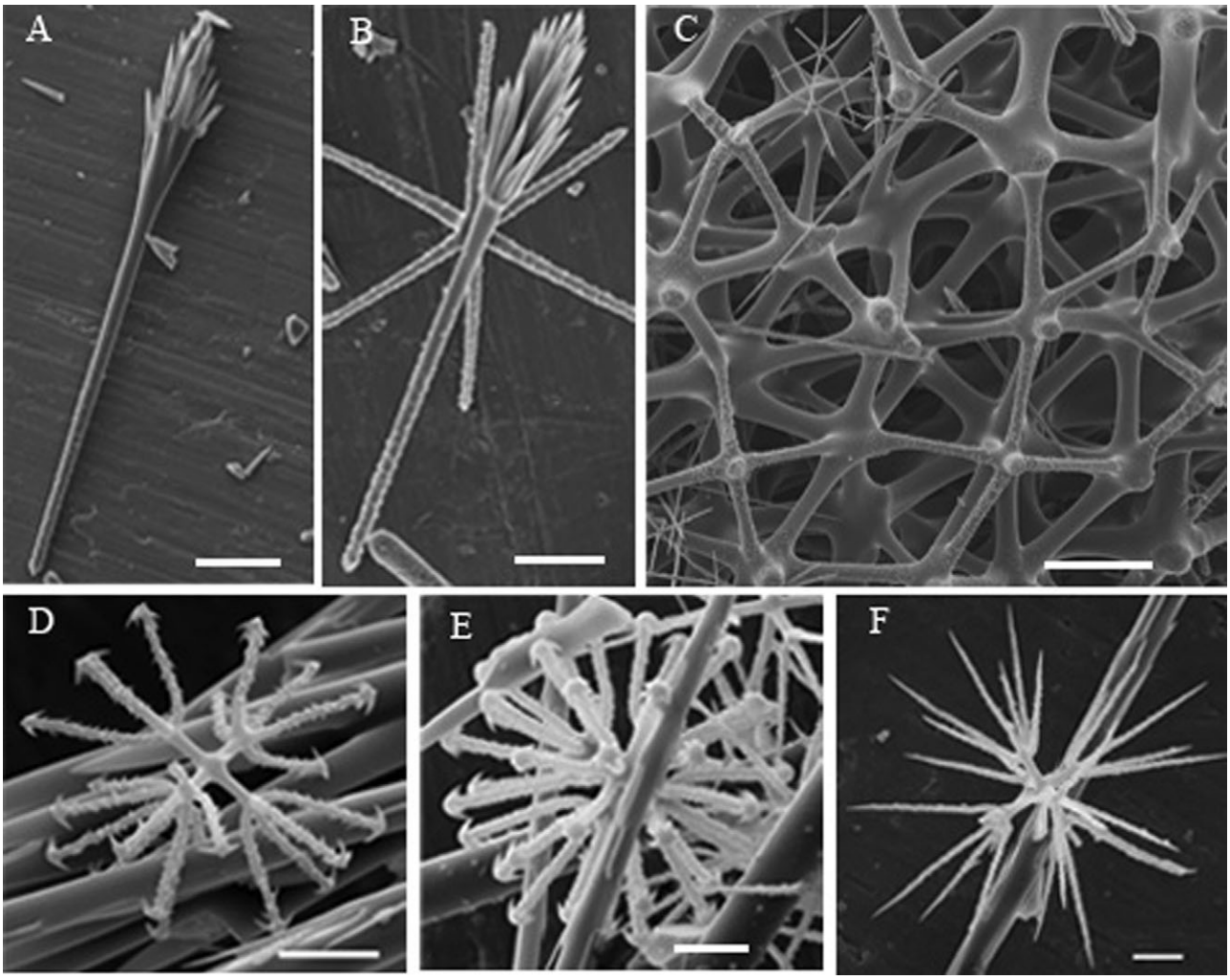

Figure 4. *Sarostegia oculata*, skeleton. A–B, sarules (A, scale bar = 30 μm; B, scale bar = 50 μm). C, dictyonal framework (scale bar = 150 μm). D–E, discohexasters (scale bars = 10 μm). F, oxyhexaster (scale bar = 10 μm).

large ones, length 720–1030 μm, and smaller ones, length 400–650 μm. Microscleres are oxyhexasters, 60–90 μm diameter, primary rays 30–43 μm long, usually occurring as holoxyhexasters (Fig. 4F), but also occasionally as hemioxyhexasters (Fig. 5E), and discohexasters with hooked spines, variable in size and number of secondary rays, diameter 35–60 μm, length of primary rays 16–30 μm, number of secondary rays five to seven.

Remarks: The placement of *Sarostegia* in Farreidae (Reiswig, 2002b) was based on the hypothesis that its sarules are homologous to those of *Claviscopulia*. However, this interpretation is debatable, as the sarules of the two genera show clear differences. In *Sarostegia* the sarules tend to have short, scale-like secondary rays often fused almost to the top, leading to a pine-cone like appearance of the sceptrule head (Figs 1, 4, 5). The sarules of *Claviscopulia facunda* (Schmidt, 1870) tend to have fewer secondary rays (ten to 15), which are clearly differentiated from the sceptrule head giving it a brush-like shape (Fig. 1; see also Mehl, 1992: pl. 6, figs 1, 2). Considering the euretoid nature of the dictyonal framework in *Sarostegia*, the absence of clavules, and the strong molecular evidence against its placement in Farreidae (see above), we transfer *Sarostegia* back to Euretidae, in line with Topsent (1928) and Reid (1958; see also Reid, 1963).

Sarostegia oculata was previously only known from the Indian Ocean, Cap Verde Is., and south-east Brazil (Reiswig, 2002b; Tabachnick *et al*., 2009); this is the first report from the north-west Atlantic. The collection depth of the new specimen (745 m) is well within the known range for this species (256–1829 m; Reiswig, 2002b).

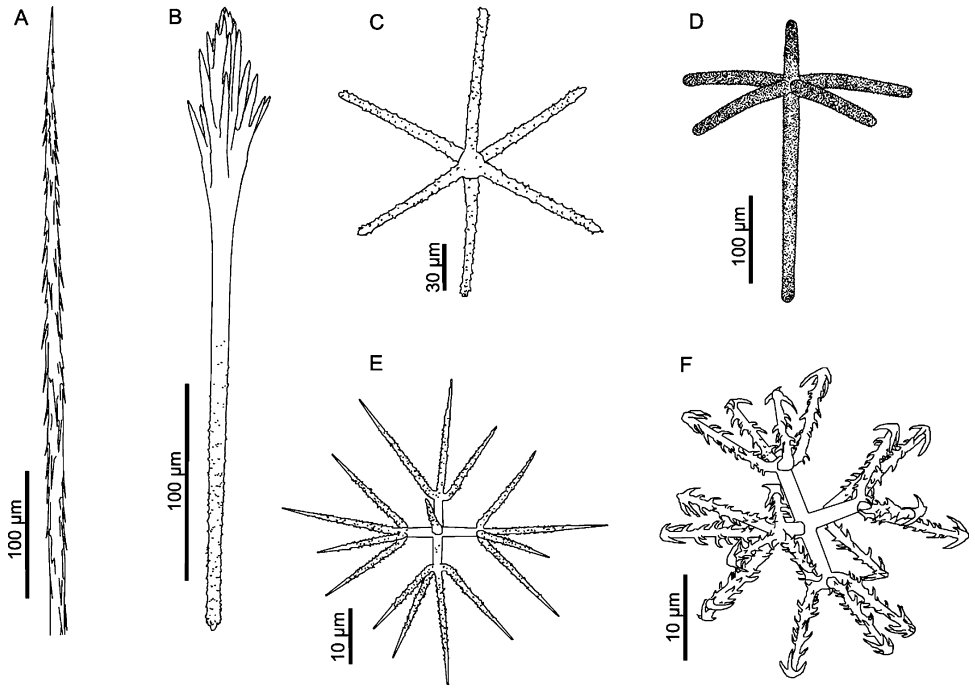

Figure 5. *Sarostegia oculata*, spicules: A, uncinate. B, sarule. C, choanosomal hexactin. D, dermal hexactin. E, hemioxyhexaster. F, discohexaster.

FARREIDAE GRAY, 1872

Revised diagnosis (emended from Duplessis & Reiswig, 2004, for exclusion of *Sarostegia*): Sceptrulophora with a farreoid framework construction, typically with sceptrules in the form of clavules. Aspidoscopules, lonchioles, or sarules may also be present.

ASPIDOSCOPULIA REISWIG, 2002

Revised diagnosis (emended from definition in Reiswig, 2002b, for inclusion of the new species described below): Tubular Farreidae with sceptrules as distinctive scopules (aspidoscopules) and/or anchorate clavules. Aspidoscopules have a shield-like or discoid, flattened head; scopule tines emanate from the head in a single marginal whorl. Anchorate clavules are very spiny with a flattened head and few large marginal spines and a prominent whorl of rounded hooks just below the head. Pileate clavules and uncinates may be present.

Remarks: The genus *Aspidoscopulia* was previously only known from the type material of *Aspidoscopulia furcillata* (Lévi, 1990) collected west of Celebes, Indonesia (Reiswig, 2002b). The collection depths of the new specimens are somewhat shallower (656 and 747 m for QM G332077 and QM G332104, respectively) than that of the type species (798 m; Reiswig, 2002b). Anchorate clavules with a whorl of hooks below the head also occur in several *Farrea* species (see Lopes, Hajdu & Reiswig, 2011).

ASPIDOSCOPULIA AUSTRALIA DOHRMANN, GÖCKE & JANUSSEN **SP. NOV.**

Material examined: One specimen, the holotype (QM G332077, SMF 11031), collected 10.xii.2009 during the DDU expedition at Osprey Reef, ROV Dive #4, 13°50.74S, 146°32.88E, on a coral reef wall, depth 656 m.

Description: Body branching with an anastomosing system of tubes, attached to a basal plate, holotype about 0.50 m tall (Fig. 6, supporting movie M1, available at Open Data LMU http://dx.doi.org/10.5282/ubm/data.36). The live sponge was covered by numerous orange zoanthids and by several actinians. Walls thin, outer edges of tubes *c*. 1 mm; diameter of the oscular tubes about 15 to 25 mm, increasing distally, skeletal channels absent. Colour white, both the living sponge and in ethanol preservation.

Skeleton (Figs 7, 8, Table 4): Dictyonal framework of smooth hexactins forming rectangular, occasionally triangular meshes, mesh width 281–787 µm, beam thickness 34–113 µm, the thinnest outer framework parts consist of only one layer of hexactins. Distal rays of dermal outer layer are thickened and more or

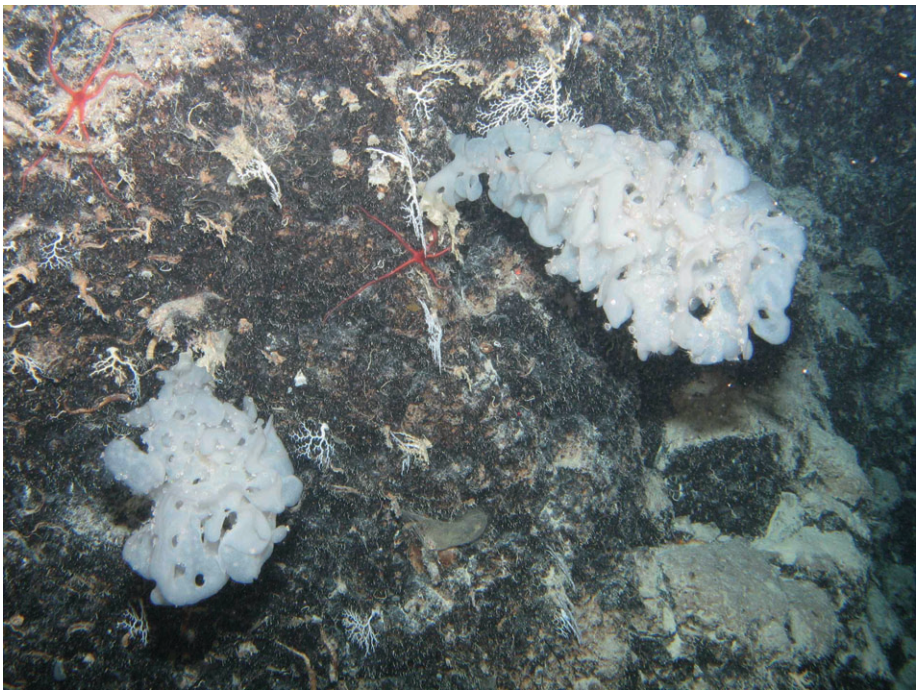

Figure 6. ***Aspidoscopulia australia*** **sp. nov.**, live photograph taken by the remotely operated vehicle 'Cherokee' (http://www.marum.de). Only the specimen on the right was collected (see supporting movie M1). Approximate size of specimen: 0.50 m.

less tuberculate, no microhexactins were found attached to the dictyonal framework. Loose spicules include dermal and atrial pentactins, microspined with proximal ray length of 88–263 µm and tangential ray lengths 113–300 µm. Sceptrules are anchorate clavules, 313–433 µm in length, microspiny, with a flattened head showing only little expansion and with six to 11 (mean eight) large, separated marginal spines, length 25–35 µm. The shaft shows a conspicuous whorl of large rounded hooks, 10–25 µm long, just below the head, which contains a swelling of the central canal, representing the axial cross. Other sceptrules are pileate clavules, 263–313 µm in length, with an umbrella-like head, 22–30 µm diameter, framed by *c*. 25 fused marginal spines, and aspidoscopules, 213–225 µm length, with a flattened, plate-like head and six to seven tines, 25–35 µm in length. Shaft of aspidoscopules with or without long projecting spines below the head. Rare uncinates observed, 100–125 µm in length. Microscleres are oxyhexasters, 50–82 µm diameter, with primary rays, 30–40 µm length, four to six secondary rays, and discohexasters, microspined, 57–77 µm diameter, with long primary rays, 25–37 µm length, and four to six secondary rays.

Remarks: Aspidoscopulia australia sp. nov. differs from the type species of the genus, *Asp. furcillata*, mainly by the presence of anchorate clavules. Additionally, a small cross at the end of the aspidoscopule shaft, occasionally occurring downward-bending aspidoscopule tines, and microhexactins fused by one ray to the dictyonal framework, as described from *Asp. furcillata* (Lévi, 1990; Reiswig, 2002b), were not observed in *Asp. australia*. Furthermore, the hexasters of the latter are more variable in size than those of the type species.

Etymology: The species name refers to the first record of the genus *Aspidoscopulia* from Australia.

ASPIDOSCOPULIA OSPREYA DOHRMANN, GÖCKE & JANUSSEN SP. NOV.

Material examined: One specimen, the holotype (QM G332104, SMF 11032), collected 14.xii.2009 during the DDU expedition at Osprey Reef, ROV Dive #8, 13°49′S, 146°32′W, depth 747 m.

Description: Body branching with an anastomosing system of tubes attached to a basal plate, holotype about 0.70 m tall, walls thin, outer edges of tubes *c*. 1 mm; diameter of the oscular tubes about 20 to 30 mm, increasing distally, skeletal channels absent (Fig. 9, supporting movie M2, available at Open Data LMU http://dx.doi.org/10.5282/ubm/data.36). Colour white, both the living sponge and in ethanol preservation.

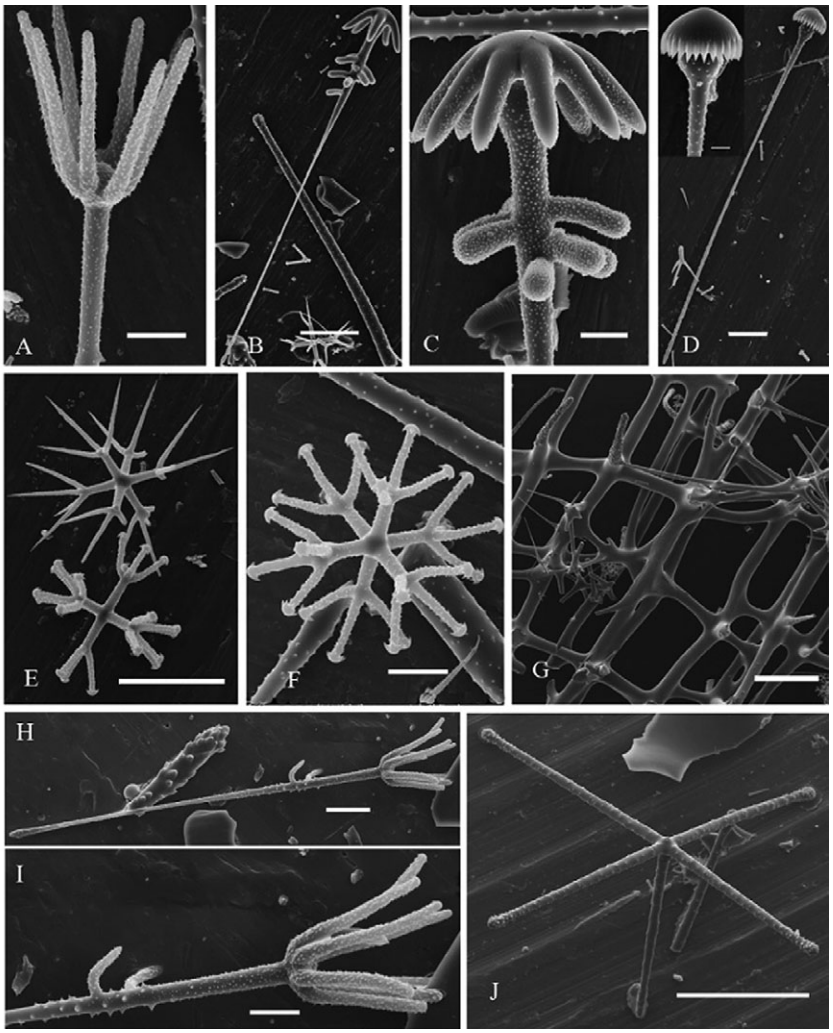

Figure 7. ***Aspidoscopulia australia*** **sp. nov.**, skeleton. A, head of aspidoscopule (scale bar = 10 μm). B–C, anchorate clavules (B, scale bar = 50 μm; C, scale bar = 10 μm). D, pileate clavule (scale bar = 30 μm) and clavule head (inset; scale bar = 5 μm). E, disco- and oxyhexaster (scale bar = 30 μm). F, discohexaster (scale bar = 10 μm). G, dictyonal framework (scale bar = 300 μm). H–I, aspidoscopule with lateral spines (H, scale bar = 30 μm; I, scale bar = 10 μm). J, surface pentactin (scale bar = 100 μm).

Skeleton (Figs 10–11, Table 5): Dictyonal framework of smooth hexactins forming rectangular, occasionally triangular meshes, mesh width 217–652 μm, beam thickness 54–109 μm (mean values), the thinnest outer framework parts probably (not observed) consist of only one layer of hexactins. Distal rays of dermal outer layer are thickened and more or less tuberculate, no microhexactins were observed attached to the dictyonal skeleton. Dermalia and atrialia are microspined pentactins with proximal ray length 230–340 μm, paratangential ray lengths 250–340 μm. Only sceptrules observed are microspined anchorate clavules similar to those in *Asp. australia*, length 275–338 μm, with four to eight (mean six) large, separated marginal spines, length 15–27 μm. The shaft shows a whorl of large rounded hooks, 7–20 μm long, just below the head. Head contains the axial cross represented by a swelling of the axial canal. Rare uncinates observed, 250–950 μm in length. Microscleres are oxyhexasters, 95–145 μm in diameter, with primary rays 43–70 μm length, two to three secondary rays, and discohexasters, microspined, 66–105 μm in diameter with primary rays, 30–50 μm length, three to four secondary rays.

Remarks: Aspidoscopulia ospreya differs from the type species, *Asp. furcillata*, by the presence of anchorate clavules and the absence of aspidoscopules. Furthermore, microhexactins fused by one ray to the

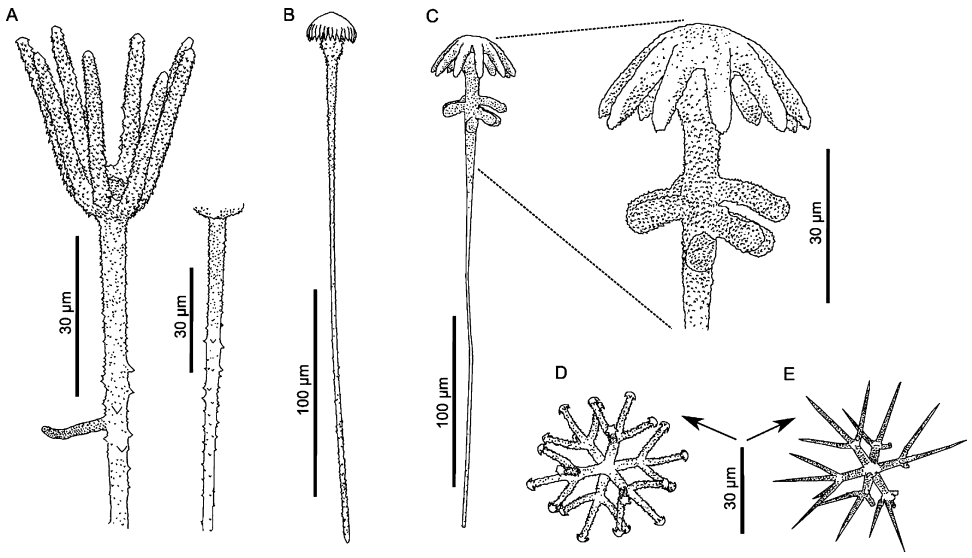

Figure 8. ***Aspidoscopulia australia*** **sp. nov.**, spicules. A, aspidoscopule. Left, head and neck of specimen with lateral spine; right, detail of specimen without spines. B, pileate clavule. C, anchorate clavule. D, discohexaster. E, oxyhexaster.

dictyonal framework, if present at all, are not as abundant as in the type species. A close relationship of *Asp. ospreya* to *Asp. australia* is clearly indicated by the very similar appearance of the anchorate clavules in these two species. However, *Asp. ospreya* differs from *Asp. australia* by the absence of other sceptrules – pileate clavules or aspidoscopules were not observed in this species, whereas they are common in *Asp. australia*. The anchorate clavules are generally smaller and show a lower number of marginal spines in *Asp. ospreya* (mean six) compared to *Asp. australia* (mean eight); also, both oxy- and discohexasters are generally larger, but with fewer secondary rays, in *Asp. ospreya* than in *Asp. australia*.

Assignment of QM G332104 and QM G332077 to two distinct species is further justified by significant differences at the molecular level, with uncorrected p-distances of 0.0009, 0.0034, 0.0498, and 0.0687 for 18S, 28S, 16S, and COI sequences, respectively.

Etymology: Species name refers to the type locality, Osprey Reef.

TRETODICTYIDAE SCHULZE, 1886

PSILOCALYX IJIMA, 1927

Diagnosis: As given in Reiswig (2002e: 1347 under 'Definition').

PSILOCALYX WILSONI IJIMA, 1927

Material examined: One specimen (QM G331821, SMF 11033) collected 12.xii.2009 during DDU from Osprey Reef, ROV Dive #6, 13°48′S, 146°32′E, depth 508 m.

Additional material examined: Psilocalyx wilsoni Ijima 1927, syntype, Zoological Museum of Amsterdam (ZMA Por. 3402).

Description: Small thick-walled cup or short tube attached by broad base to hard substrate, superior round osculum (Fig. 12, supporting movie M3, available at Open Data LMU http://dx.doi.org/10.5282/ubm/data.36). Specimens observed by video were colonized by small actinians. Colour of the living sponge and in ethanol fixation is white. Specimen examined measures about 100 mm high and 50 mm in diameter, wall 10–14 mm thick and permeated by 5–10 mm wide ovoid to irregular apertures, which open into a labyrinthic schizorhysial channel system.

Skeleton (Figs 13–14, Table 6): Dictyonal skeleton with regular rectangular and triangular meshes, mesh width of the inner skeleton 170–343 µm, dictyonal beams are spiny and 21–86 µm in diameter. The external dictyonal surface layer is thickened into a hard crust, hypersilicified surface is tuberculate with beam length 166–500 µm and ray thickness 111–200 µm. Loose dermal spicules are strongyloscopules, 560–860 µm total length, with four to six rough tines, 80–120 µm long, and uncinates, 450–670 µm length, with very small barbs. Dermal and atrial hexactins or pentactins are absent. Microscleres are lophodiscohexasters, 50–66 µm total diameter, with primary rays, 10–15 µm long, and five to ten microspined secondary rays, 15–18 µm long.

Table 4. Spicule measurements of ***Aspidoscopulia australia* sp. nov.** (holotype, QM G332077, SMF 11031)

Parameter	Mean	SD	Range	*N*
Free spicules				
Pentactin				
Tangential ray (L)	216	44.2	113–300	33
Proximal ray (L)	200	52.3	88–263	12
Discohexaster				
(D)	68	6.2	57–77	30
Primary ray (L)	32	3.3	25–37	30
Secondary ray (L)	17	2.8	12–22	30
Number of secondary rays	5	0.7	4–6	26
Oxyhexaster				
(D)	68	7.7	50–82	31
Primary ray (L)	34	2.9	30–40	31
Secondary ray (L)	20	3.2	15–27	31
Number of secondary rays	5	0.9	4–6	28
Anchorate clavules				
(L)	374	38	313–433	23
Shaft (D)	10	1	7–12	38
Head (D)	60	7.1	42–70	38
Number of marginal spines	8	0.7	6–11	38
Pileate clavules				
(L)	287	15.3	263–313	15
Shaft (D)	5	0.4	4–6	16
Head (D)	26	2.4	22–30	16
Aspidoscopules				
(L)	220	6.1	213–225	4
Shaft (D)	4.8	0.5	4–5	4
Tines (L)	30	4.8	25–35	4
Framework				
Length between nodes	536	117.9	281–787	30
Central beam width	63	15.7	34–113	30

All measurements in μm.
D, diameter; L, length.

Remarks: Together with a few specimens recently collected off New Zealand (Reiswig & Kelly, 2011), this is one of the first findings of this species since the description of the type material (Ijima, 1927), collected from Arafura and Banda Seas, Indonesia, during the Dutch Siboga expedition (1899–1900). The collection depth (508 m) is well within the range of 424–1045 m reported by Reiswig & Kelly (2011) for their specimens of this species. The here-described material confirms the true absence of dermalia and atriala in *Psilocalyx* (see Reiswig, 2002e). The Australian specimen differs from the holotype by having generally smaller scopules and hexasters as described by Reiswig (2002e). Furthermore, the hexasters of the holotype show longer primary rays relative to the secondary rays and they have generally more secondary rays than the hexasters of our specimen. However, according to spicule composition, skeletal architecture, and body shape, we consider the attribution to *P. wilsoni* as justified.

The thickening of the dermal dictyonal layers of *Psilocalyx* into a hard surface crust is very similar to the extreme hypersilicification of the dictyonal outer layers found in many Mesozoic hexactinosidan and lychniscosidan genera (e.g. Schrammen, 1912).

DISCUSSION

Phylogeny of Sceptrulophora

The evolutionary history of hexactinellid sponges has only very sparsely been investigated using cladistic methods (Mehl, 1992; Dohrmann *et al*., 2008) and has only recently begun to be investigated with molecular

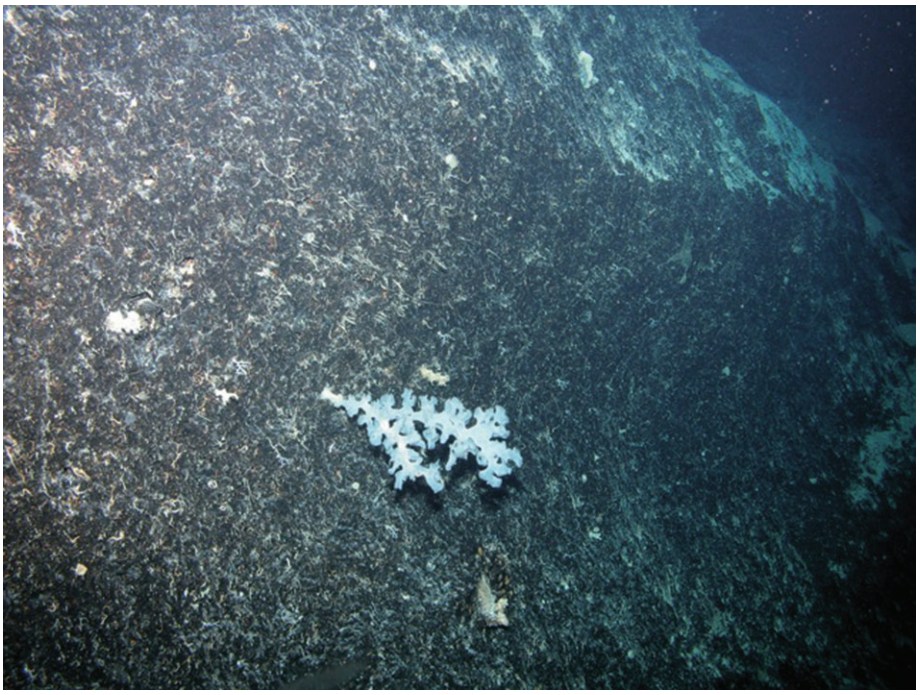

Figure 9. ***Aspidoscopulia ospreya*** **sp. nov.**, live photograph taken by the remotely operated vehicle 'Cherokee' (http://www.marum.de). Approximate size of specimen: 0.70 m.

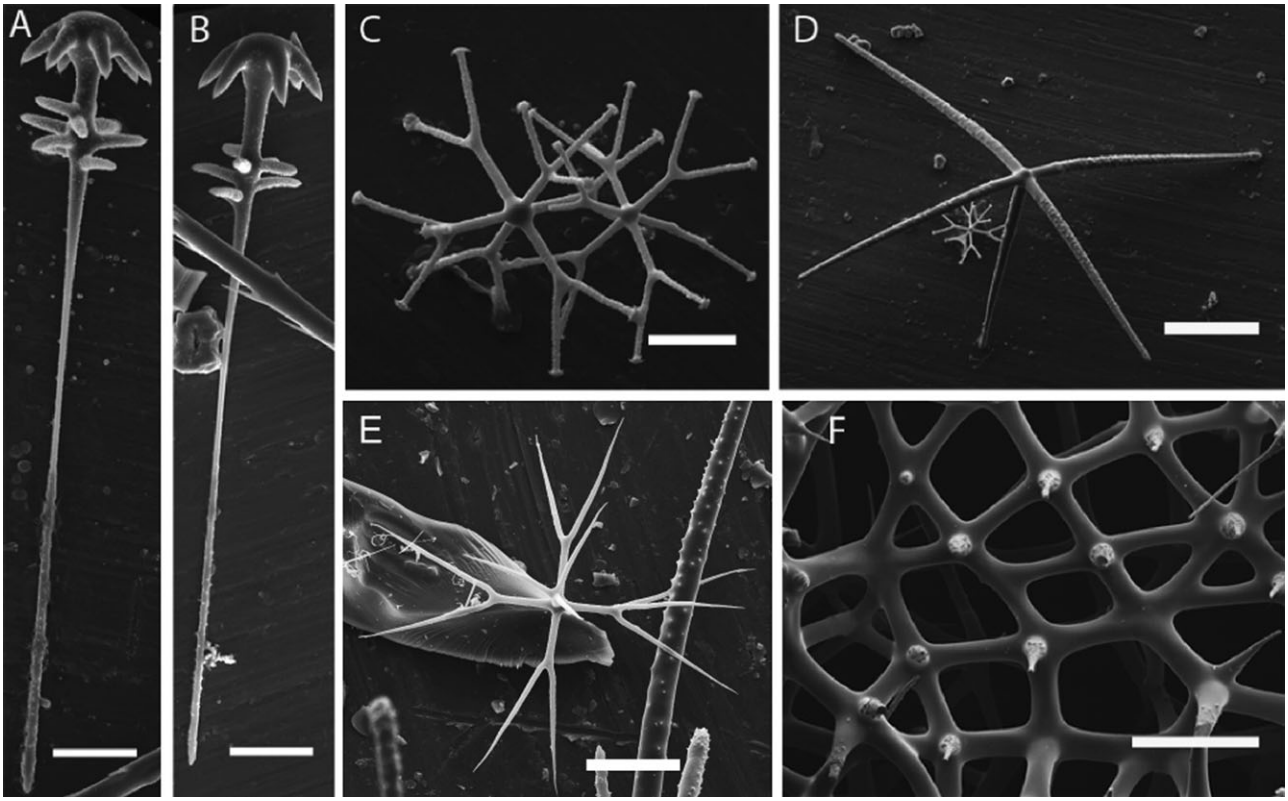

Figure 10. ***Aspidoscopulia ospreya*** **sp. nov.**, skeleton. A–B, anchorate clavules (scale bars = 25 μm). C, discohexasters (scale bar = 30 μm). D, surface pentactin (scale bar = 100 μm). E, oxyhexaster (scale bar = 30 μm). F, dictyonal framework (scale bar = 300 μm).

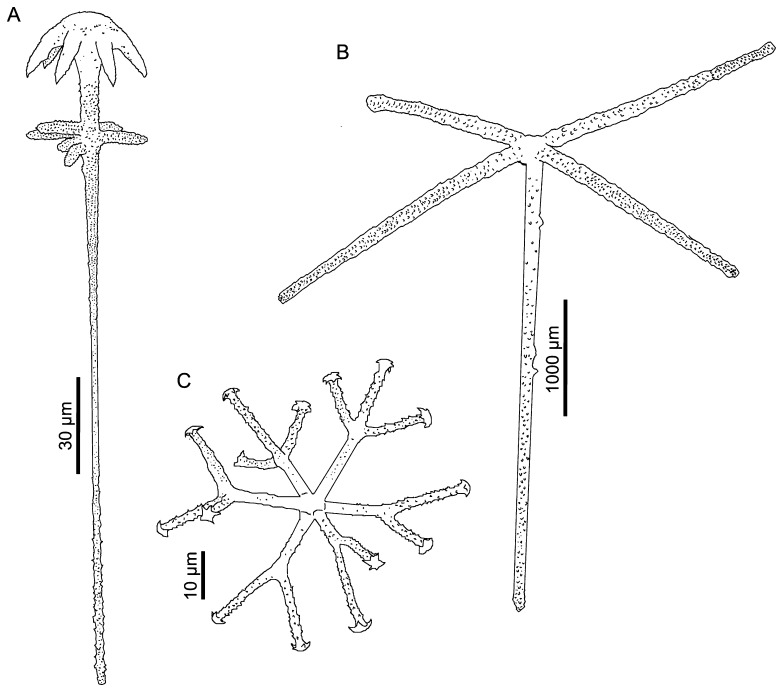

Figure 11. ***Aspidoscopulia ospreya*** **sp. nov.**, spicules. A, anchorate clavule. B, surface pentactin. C, discohexaster.

phylogenetic approaches (Dohrmann *et al*., 2008, 2009). Although taxonomic coverage in these analyses was already comparably good, phylogenetic placement of a number of important taxa was (and still is) pending, preventing insights into the evolution of key characters such as certain spicule types. Here, recently collected material of the sceptrulophoran families Farreidae and Tretodictyidae allowed us to increase the taxon sampling of these underrepresented groups.

Monophyly of Tretodictyidae is supported by a unique channelization of the dictyonal framework (schizorhyses), and the bundled arrangement (at least in some genera) of dermal uncinates and scopules (Mehl, 1992; Reiswig, 2002e). Although previous molecular results were consistent with monophyly of the family (Dohrmann *et al*., 2008), only two species belonging to morphologically very close genera, *Hexactinella* Carter, 1885, and *Tretodictyum* Schulze, 1886, were included. Here, we recovered *Psilocalyx* as the sister group to those two genera, further corroborating monophyly of Tretodictyidae. However, although significant following Hillis & Bull (1993), statistical support for this clade is not strong, and *Psilocalyx* is separated by a long branch from *Hexactinella* + *Tretodictyum*, indicating deep genetic divergence within this group. With 30 species in eight genera (Leys *et al*., 2007; Reiswig *et al*., 2008; Reiswig, 2010; Reiswig & Kelly, 2011), tretodictyids are the second-most diverse dictyonal family;

Table 5. Spicule measurements of ***Aspidoscopulia ospreya*** **sp. nov.** (holotype, QM G332104, SMF 11032)

Parameter	Mean	SD	Range	*N*
Free spicules				
Pentactin				
Tangential ray (L)	279	20.7	250–320	30
Proximal ray (L)	293	31.9	230–340	16
Oxyhexaster				
(D)	126	14.0	95–145	11
Primary ray (L)	59	7.7	43–70	11
Secondary ray (L)	40	7.0	30–53	11
Number of secondary rays	2.4	0.5	2–3	11
Discohexaster				
(D)	82	9.3	66–105	30
Primary ray (L)	38	5.0	30–50	30
Secondary ray (L)	20	4.3	12–27	30
Number of secondary rays	3	0.2	3–4	30
Anchorate clavules				
(L)	307	18.4	275–338	30
Shaft (D)	10	1.5	5–12	30
Head (D)	54	5.2	40–65	29
Number of marginal spines	6	1.4	4–8	30
Framework				
Length between nodes	375	93.5	217–652	30
Central beam width	72	16.8	54–109	30

All measurements in µm.
D, diameter; L, length.

Figure 12. *Psilocalyx wilsoni*, live photograph taken by the remotely operated vehicle 'Cherokee' (http://www.marum.de). Approximate size of specimen in the middle: 100 mm.

although we consider nonmonophyly of this taxon unlikely, this remains to be tested by sampling more genera for molecular systematics.

Farreidae was so far only represented by a single species in molecular phylogenetic data sets (Dohrmann *et al*., 2008, 2009). The specimens described herein and in Göcke & Janussen (2011) made it possible to test the family's monophyly for the first time with molecular data. We found a well-supported clade consisting of *Farrea*, *Lonchiphora*, and *Aspidoscopulia*, which is morphologically supported by a farreoid dictyonal framework (see Tabachnick & Reiswig, 2002 for definition) and the presence of clavules [although clavules are lacking in *Lonchiphora antarctica* (Göcke & Janussen, 2011), they may be present in the type species, *L. inversa* Ijima, 1927 (Reiswig, 2002b)]. These features are also characteristic of *Claviscopulia* (Reiswig, 2002b) and *Asceptrulum*, which both have a farreoid skeleton, although the latter is lacking sceptrules (Duplessis & Reiswig, 2004). Thus, we predict that these two genera also fall within this clade. *Sarostegia*, by contrast, has a euretoid dictyonal framework (see Tabachnick & Reiswig, 2002 for definition), and lacks clavules (Reiswig, 2002b). Reiswig (2002b) assumed homology of *Sarostegia*'s sarules with those of *Claviscopulia*, and therefore retained *Sarostegia* in Farreidae. However, this interpretation is debatable: although the distinction between the sarules of these two genera is sometimes blurred because of variable morphology, in our view there is no strong argument for homology of sarules between them (see above). Finally, the molecular evidence presented here clearly rejects a farreid affinity of *Sarostegia*, implying convergent evolution of sarules (see next section). Although *Sarostegia* did not group with the single included representative of Euretidae, such a clade could not be rejected by the AU test; furthermore, many more species will need to be sampled in order to test monophyly of this morphologically poorly supported family (see above). For the time being, *Sarostegia* is therefore best classified in Euretidae, where it was previously placed (Topsent, 1928; Reid, 1958).

Branching order within Aphrocallistidae (= *Aphrocallistes* Gray, 1858 and *Heterochone* Ijima, 1927) had previously been found to be unstable in rDNA trees, but with ambiguous support for alternative topologies (Dohrmann *et al*., 2009). In contrast, we here found strong support for nonmonophyly of *Aphrocallistes* and *Heterochone*. Although the two genera are morphologically similar (Reiswig, 2002f), morphological support for their paraphyly remains elusive. Interestingly, the 28S partition appears to harbour significant signal for the monophyly of *Aphrocallistes*, whereas in the other single-gene analyses paraphyly was always recovered, albeit significantly only in the case of the

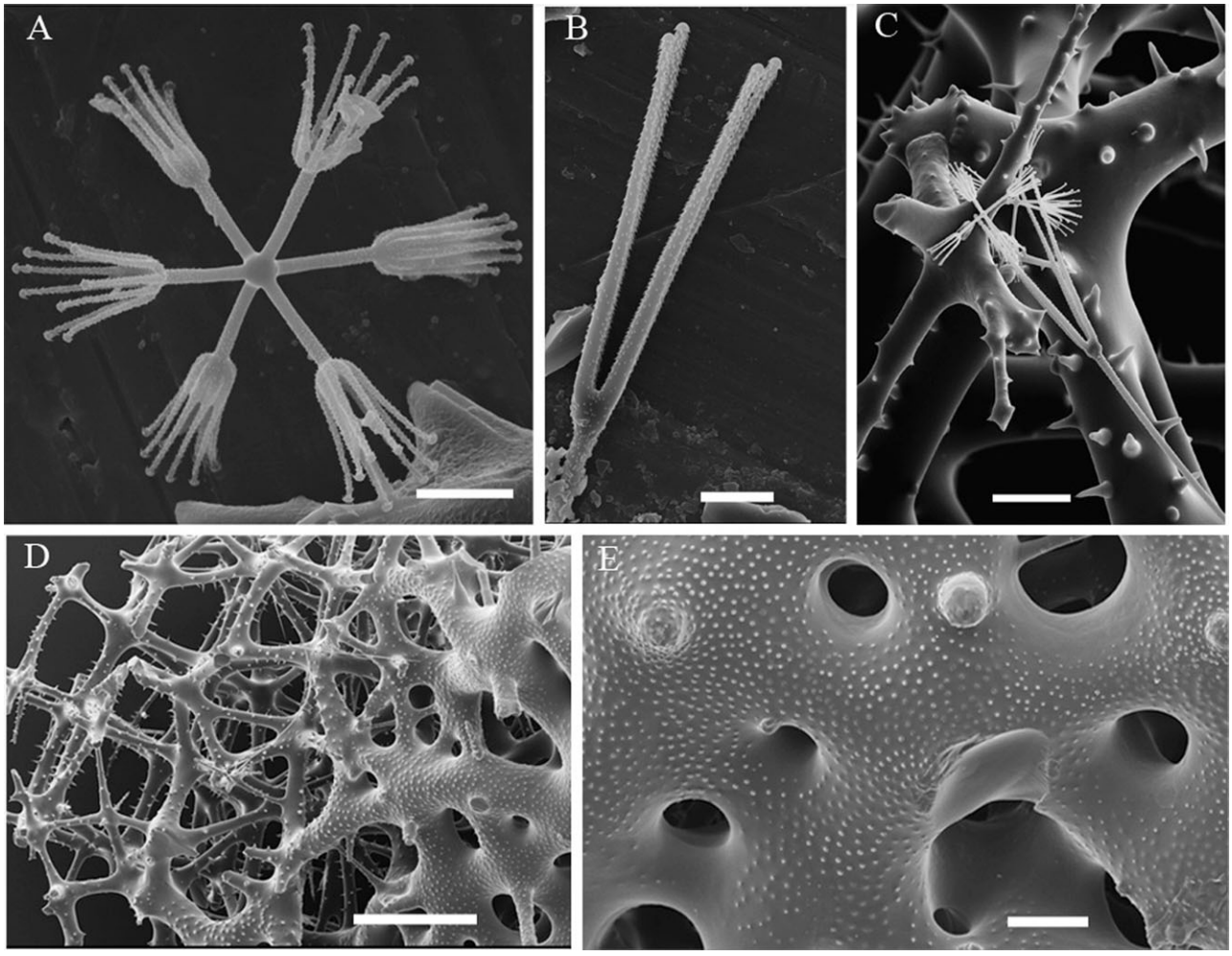

Figure 13. *Psilocalyx wilsoni*, skeleton. A, lophodiscohexaster (scale bar = 10 μm). B, scopule head (scale bar = 15 μm). C, hexasters and scopule within the dictyonal skeleton. D, dictyonal framework, transition to the thickened dermal layer (scale bar = 300 μm). E, dermal dictyonal (hypersilicified) cortex layer (scale bar = 100 μm).

mitochondrial (16S and COI) partitions (results not shown). This suggests that the mt genome of Aphrocallistidae might evolve differently from the nuclear genome, and our results do not reflect the true species tree (cf. Edwards, 2009). The family therefore deserves further attention in future studies, ideally with complete taxon sampling (see discussion in Dohrmann *et al*., 2009) and coalescent-based approaches to phylogenetic inference (reviewed in Degnan & Rosenberg, 2009; Liu *et al*., 2009).

In line with previous studies based on a lower taxon sampling (Dohrmann *et al*., 2008, 2009), our analyses strongly support a sister-group relationship between Aphrocallistidae and Farreidae. Based on the skeletal morphology a similar hypothesis was suggested by Mehl (1992), who compared the one to two-layered, dictyonal walls between the diarhysis channels of *Aphrocallistes* with the thin-walled tubes of *Farrea* and stated that the diarhysis might be interpreted as a system of small adjacent farreoid tubes. The ancestor of Aphrocallistidae would accordingly be a species with a skeleton of thin-walled, anastomosing tubes similar to *Farrea* (Mehl, 1992: 65). Further synapomorphies may be the similar shape of oxyhexasters, with transitions to tylo- or discohexasters in *Aphrocallistes* (e.g. *Aphrocallistes vastus* Schulze, 1886) and in some Farreidae (e.g. *Aspidoscopulia* spp.).

Evolution of sceptrules

Whether the sceptrule is an evolutionary novelty (see, e.g. Müller & Newman, 2005; Moczek, 2008 for discussion of this problematic concept) or evolved from a pre-existing spicule type such as the pinular

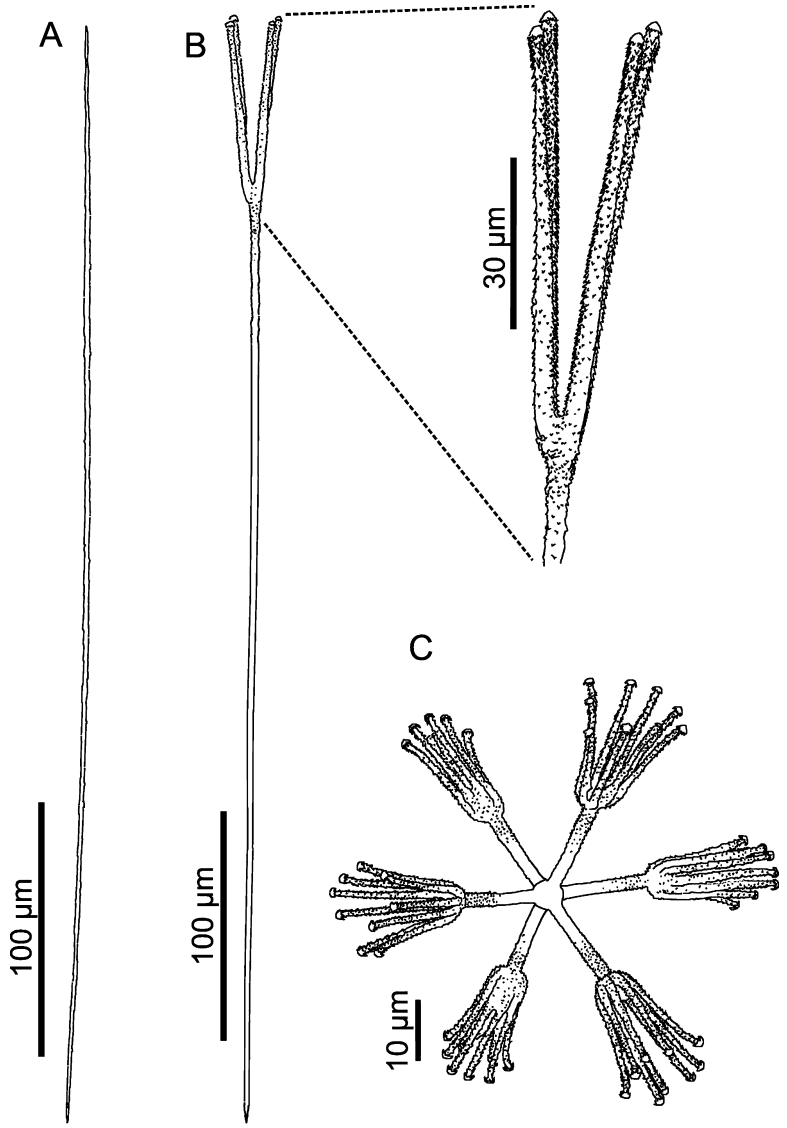

Figure 14. *Psilocalyx wilsoni*, spicules. A, uncinate. B, scopule. C, lophodiscohexaster.

pentactin (Mehl, 1992), remains an open question. In light of the phylogeny (Fig. 2) it is clear, however, that the interpretation of sarules as ancestral-like sceptrules (Schulze, 1899; Reid, 1958; Mehl, 1992) is highly unparsimonious, given the nested position of *Sarostegia*. Instead, the last common ancestor of extant Sceptrulophora probably already possessed regular scopules, which subsequently repeatedly evolved into more unusual sceptrule types – sarules (twice convergently), lonchioles, and aspidoscopules (Fig. 15). Given that scopules can safely be assumed as the sole sceptrule type in the sceptrulophoran ground pattern (Fig. 15), clavules are probably derived from them, too, and clavules and scopules initially co-occurred in the stem lineage of Farreidae – this arrangement is seen in *Asp. furcillata* and *Asp. australia* sp. nov. amongst the recent fauna. Whether aspidoscopules are an apomorphy of the latter genus (Fig. 15, left) or were inherited from the farreid ground pattern (Fig. 15, right) cannot be answered with certainty. However, scopules with tines arising from a shield-like head (as in aspidoscopules) have been reported from Early Jurassic and Late Triassic strata (Mostler, 1990; Donofrio, 1991); if these forms are homologous to recent ones, the latter hypothesis would have to be favoured because it is unlikely that *Aspidoscopulia* dates back to the early Mesozoic.

According to our scenario (Fig. 15), scopules (or aspidoscopules) were secondarily lost in *Farrea* (although more frequent losses would have to be assumed should this taxon turn out to be nonmono-

Table 6. Spicule measurements of *Psilocalyx wilsoni* (QM G331821, SMF 11033)

Parameter	Mean	SD	Range	*N*
Free spicules				
Discohexaster				
(D)	59	6.1	50–66	10
Primary ray (L)	11	1.9	10–15	10
Secondary ray (L)	16	1.3	15–18	10
Reduced discohexaster				
(D)	75	0.7	74–75	2
Primary ray (L)	13	2.1	11–14	2
Secondary ray (L)	24	2.1	22–25	2
Scopule				
(L)	696	120.4	560–860	7
Shaft (D)	4	1.1	3–6	7
Primary ray (L)	597	108.6	450–740	7
Secondary ray (L)	99	18.6	80–120	7
Uncinate				
(L)	579	61.5	450–670	17
Shaft (D)	3	0.6	2–4	17
Framework				
Interior skeleton				
Length between nodes	249	53.9	171–343	30
Central beam width	34	12.6	21–86	30
Surface skeleton				
Length between nodes	300	87.6	166–500	30
Central beam width	144	27.9	111–200	30

All measurements in μm.
D, diameter; L, length.

phyletic). Likewise, clavules were lost in *Lonchiphora antarctica* (and probably *Asceptrulum*, although the ancestral sceptrule type of this genus is unknown). Curiously, anchorate clavules might have evolved convergently from aspidoscopules within *Aspidoscopulia*. This hypothesis gains some support from the occurrence of lateral spines on the shaft of both the anchorate clavules of *Asp. australia* sp. nov. and *A. ospreya* sp. nov. and the aspidoscopules of *Asp. australia* and *Asp. furcillata*. More strikingly, aspidoscopules of *Asp. furcillata* can have some tines bending downwards (see Reiswig, 2002b: fig. 2), resembling an intermediary state between scopule-like and clavule-like spicules. However, it is unclear if scopule tines and clavule marginal spines are homologous structures. Therefore, the anchorate clavules of the newly described *Aspidoscopulia* spp. might alternatively be homologous to similar spicules found in several *Farrea* spp., which also possess a whorl of hooks below the head (see Lopes *et al*., 2011). Clearly, a better knowledge of the genetic bases of spicule formation, the functional roles of different spicule types, and the extent of phenotypic plasticity of these structures (see Maldonado *et al*., 1999) is required for a better understanding of their homology-relationships.

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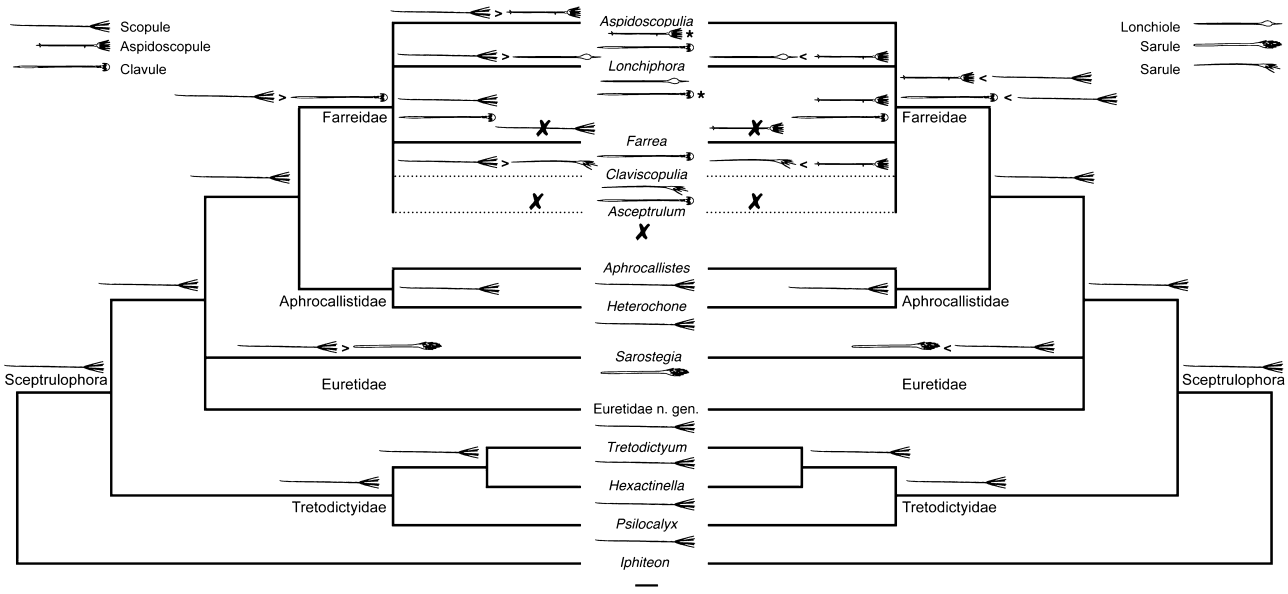

Figure 15. Evolution of sceptrules, with two alternative scenarios for the origin of aspidoscopules. The trees are based on the phylogeny shown in Figure 2, reduced to genus level, and nodes with < 70% bootstrap support collapsed. The two farreid genera not sampled here, *Claviscopulia* and *Asceptrulum*, are shown with dotted lines in their predicted position (polytomies indicate uncertainty of exact placement within Farreidae). Sceptrule types of terminal taxa, inferred sceptrule types at internal nodes, and inferred character state transitions along branches are shown. Left scenario: the stem species of Farreidae possessed regular scopules, which evolved into aspidoscopules in the stem species of *Aspidoscopulia* (i.e. aspidoscopules are an autapomorphy of *Aspidoscopulia*). Right scenario: alternatively, aspidoscopules might already have evolved from regular scopules in the stem lineage of Farreidae and were subsequently lost or transformed in all farreid genera except *Aspidoscopulia* (i.e. aspidoscopules are a plesiomorphy of *Aspidoscopulia*). Crosses indicate spicule loss (all sceptrules in the case of *Asceptrulum*); asterisks indicate spicule loss within genera (aspidoscopules in ***Aspidoscopulia ospreya* sp. nov.** and clavules in *Lonchiphora antarctica*). See text for further discussion.

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NOTE ADDED IN PROOF

While this paper went to press, Tabachnick *et al*. (2011) published a revision of the genus *Aspidoscopulia*, in which they report on extensive material collected in the W. Pacific and describe two new species. The latter are similar to those described herein in possessing anchorate clavules with lateral hooks. However, they show clear differences: *Aspidoscopulia tetrasymmetrica* Tabachnick *et al*., 2011 differs from *A. australia* sp. nov. mainly in lacking discohexasters and by the morphologies of the anchorate clavules and aspidoscopules, which include a range of aberrant forms (see below); it differs from *A. ospreya* sp. nov. mainly in lacking discohexasters, by the morphologies of the anchorate clavules, and the presence of pileate clavules and aspidoscopules. *Aspidoscopulia bisymmetrica* Tabachnick *et al*., 2011, differs from *A. australia* sp. nov. mainly by the presence of onychohexasters and by the morphologies of the anchorate clavules and aspidoscopules (which include aberrant forms similar to those in *A. tetrasymmetrica*); it differs from *A. ospreya* sp. nov. mainly by the presence of onychohexasters, pileate clavules, and aspidoscopules, and by the morphologies of the anchorate clavules. Some of the aberrant sceptrules from *A. tetrasymmetrica* and *A. bisymmetrica* appear strikingly intermediate between aspidoscopules and anchorate clavules, which strengthens our hypothesis that these spicule types are derived from each other. However, as an alternative to our scenario, Tabachnick *et al*. (2011) suggest that aspidoscopules have evolved from anchorate clavules, not the other way around.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Fragment of *Sarostegia oculata* HBOI 25-V-06-2-001 with polychaete worm (undetermined species) in gastral cavity of the left, partially open tube (scale 10 mm).

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