Zootaxa 2901: 19–34 (2011)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

A new species of *Sigmaxinella* Dendy, 1897 (Demospongiae, Poecilosclerida, Desmacellidae) from the Tasman Sea

KIRSTY D. MITCHELL[1], KATHRYN A. HALL & JOHN N. A. HOOPER
Biodiversity Program, Queensland Museum, South Brisbane, Qld, 4101, Australia
[1]Corresponding author. E-mail: kirsty.mitchell@qm.qld.gov.au

Abstract

Sigmaxinella hipposiderus **sp. nov.** is described from morphological and molecular datasets, based on a single known specimen collected from the upper margin of a submarine canyon on the edge of the continental shelf, south-east of coastal Victoria (Tasman Sea), Australia. Morphologically, the species is clearly assigned to the genus *Sigmaxinella*, and preliminary molecular data (COI mt DNA) support the close relationship of this new species to other specimens attributed to Desmacellidae. This is the thirteenth species of *Sigmaxinella* and the seventh described for the Australian EEZ. Remarkably, 12 of the 13 known species are recorded predominantly from temperate or subantarctic Australian, New Zealand or South African waters, with only a single species described so far from the temperate Atlantic Ocean.

Key words: Porifera, Poecilosclerida, Mycalina, Desmacellidae, *Sigmaxinella*, taxonomy, Australia, Tasman Sea

Introduction

Desmacellidae Ridley & Dendy, 1886, has received moderate taxonomic attention over the past decade (*e.g.* Hajdu & Van Soest 2002; Salani *et al* 2006; Jeon & Sim 2008). The number of new species is continuing to rise worldwide, with currently 107 species recognised as valid. There are 13 nominal genera assigned to the family, of which six are presently recognised as valid (Van Soest, 2011). Nevertheless, there is still some uncertainty about generic boundaries between some of these, in particular the relationship between the morphologically similar genera *Sigmaxinella* Dendy, 1897 and *Biemna* Gray, 1867. This uncertainty concerns the phylogenetic value of an axially compressed skeleton in desmacellid sponges, as present in *Sigmaxinella*, that are otherwise morphologically very similar to *Biemna*. Ultimately, this uncertainty can only be resolved using datasets other than traditional morphometric features (Hooper & Van Soest 2002).

Within the genera of Desmacellidae the following number of valid species are currently recognised (following Van Soest, 2011): *Biemna* Gray, 1867 (56), *Desmacella* Schmidt, 1870 (30), *Dragmatella* Hallmann, 1917 (1), *Microtylostylifera* Dendy, 1924 (3), *Neofibularia* Hechtel, 1965 (5) and *Sigmaxinella* (12). Within the last genus, 21 nominal species or subspecies have been assigned at one time or another, but nearly half of these are considered synonyms. The most recent publication on *Sigmaxinella* (Salani *et al.* 2006) also included *S. megastyla* Burton, 1959, but this species has been since transferred to *Biemna* (Van Soest, 2011). Species of the genus are predominantly temperate to subantarctic in distribution, with 12 living in the Indo-Pacific and only a single species recorded from the Atlantic Ocean.

In this paper we describe a new species of *Sigmaxinella* from the upper margins of a submarine canyon on the edge of the continental shelf, south-east Australia, Tasman Sea. The species is compared to all others in this genus based on morphology and some preliminary molecular data.

Accepted by D. Erpenbeck: 4 Apr. 2011; published: 1 Jun. 2011

Material and methods

Collection of material. The holotype was collected from the edge of the continental shelf of Australia, Tasman Sea (Fig. 1), using a Sherman Sled during a CSIRO Marine & Atmospheric Research survey of southern Australian benthic marine resources. The specimen was initially fixed by freezing and subsequently preserved in 70% ethanol, and registered in the QM collection.

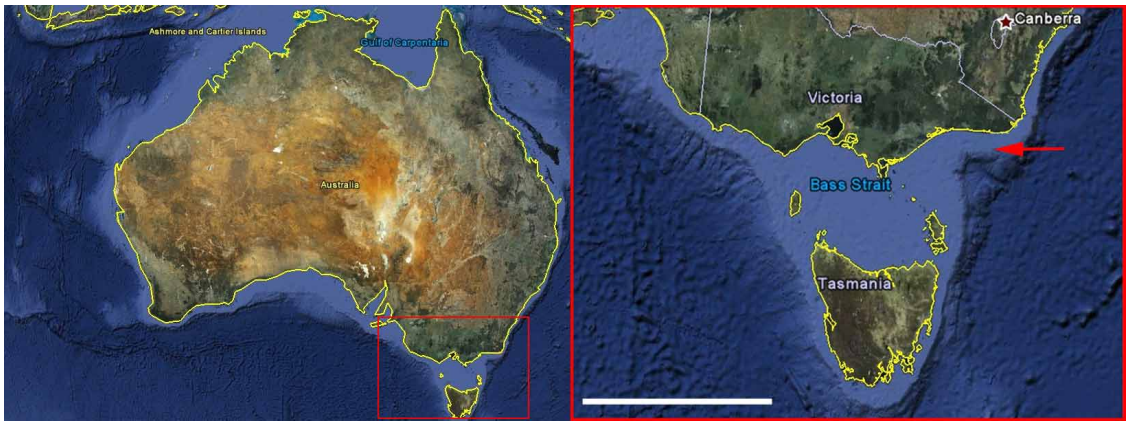

FIGURE 1. Type locality of *Sigmaxinella hipposiderus* **sp. nov.** in the Pacific ocean (Tasman Sea), off the southeastern coast of Australia. The specific location is at the Big Horseshoe Canyon, a side canyon to the Bass Canyon system off the eastern Victorian boarder, lying on the edge of the continental shelf, and at a depth of 160 metres. Image modified from Google Earth (©2009 Google™); scale bar = 500 km.

Morphology (Table 1). Two types of preparations were made for light microscopy. The first technique involved thin cross-sections through a branch of the holotype, including components of the ectosome and choanosome. This section was placed in a saturated solution of xylene-phenol and left for 12 hours to clear the mesohyl, embedded in Fluka Durcopan™ (Sigma-Aldrich Co., St. Louis, MO, USA), then oven dried at 40°C for 12 hours. The second preparation was for spicules. Preparations were made from small (~3 mm^3) pieces of the sponge, including both ectosome and choanosome, and digested using nitric acid gently heated over a low flame; the spicules were then mounted in Canada balsam.

SEM preparations were made by dissolving small pieces (~2 mm^3) of sponge in 12.5% sodium hypochlorite to remove soft tissue, monitoring the digestion using a dissecting microscope to ensure skeletal structure was not destroyed. Rate of digestion was controlled by the addition of demineralised water, and the reaction terminated using 70% ethanol. The resulting fragment was mounted on a blackened SEM stub using a soft clear adhesive gum and then left to air dry. Some stubs were sputter coated in gold and others left uncoated. Stubs were examined using a low vacuum Hitachi Tabletop Scanning Electron Microscope TM-1000. Images were recorded and plates assembled for publication using Adobe Photoshop CS5 (version 12.0.1x32) (Adobe Systems Inc., San Jose, CA, USA).

DNA analysis (Table 2). Extraction: DNA was extracted from specimens stored in 70% ethanol. Small pieces of specimen (~3 mm^3) were frozen in liquid nitrogen and then crushed in a mortar and pestle over additional liquid nitrogen. DNA was extracted from the macerated sponge using a NucleoSpin® Tissue DNA extraction kit (Macherey-Nagel, Düren, Germany). We followed the instructions provided by the manufacturer with the following exceptions: final DNA was eluted after an on-bench incubation period of 5 minutes and then using 2 × 50 µl volumes of pre-warmed elution buffer. These modifications produced a high yield and high concentration genomic DNA extract.

Amplification: we amplified the standard barcoding fragment (Folmer fragment) of mitochondrial DNA (partial cytochrome oxidase subunit 1 (COI mtDNA)) using degenerate "Folmer" primers (dgLCO1490 and dgHCO2198) designed by Meyer *et al*. (2005). HotMaster™ *Taq* DNA Polymerase (5 Prime GmbH, Hamburg, Germany) was used in PCR reactions. PCR reactions were made to a final volume of 25 µl using the following recipe: 2.5 units *Taq* polymerase, 1× HotMaster™ *Taq* Buffer with Mg^{2+} (2.5 mM Mg^{2+}), 0.25 mM dNTPs, 1.0 µM of each primer, 0.4 µg/µl BSA (Sigma-Aldrich Co.), ~200 ng template DNA and nuclease-free ddH_2O. PCR products were obtained through the following temperature regime: 94°C /120 sec (1 cycle); 94/20→ 45/10→ 65/45 (10);

TABLE 1. Comparison between species of *Sigmaxinella* Dendy, 1897. All measurements are in micrometres, cited as length × width, except length of sigmas measured as chord length. Data from original sources, re-examination of some type material, redescriptions (*e.g.*: Dendy 1897; Hallmann 1916), and subsequent revisions or summaries of taxa (modified from Hooper 1984; Salani *et al.* 2006).

Species	Growth form	Skeleton	Megascleres				Microscleres		Distribution	Depth
			Styles	Strongyles	Oxeas	Sigmas	Microxeas	Raphides		
arborea Kirkpatrick, 1903	Erect, stipitate, ramose, cylindrical-compressed dichotomous branching in more than 1 plane, surface hispid from protruding choanosomal tracts.	Dense axis of reticulating multispicular tracts bound by spongin, tufts, simple or branched, radiating out horizontally from the axis.	800–1150 × 25-37	700–870 × 25-30	825 × 12.5 (rare)	15 × 1	70 (single or in bundles)	absent	South Africa, Natal S. African EEZ	110–200 m
australiana Dendy, 1897	Erect, ramose, stipitate with short stalk, slender subcylindrical or compressed dichotomously branching, some anastomosing branching in 2 planes, surface granular or non hispid tough, compressible, resilient.	Thick, dense axial skeleton, extra-axial skeleton with slender fibres curved outwards towards the surface, non-plumose, abundant spongin, ectosomal skeleton with sparse, slightly projecting tufts of spicules; spongin fibres strongly developed.	120–450 × 2–17	(some styles transformed into strongyles)		I) 9–16 × 25 II) 25–50 × 1 (often in bundles)	absent	20–45 (hairlike, mostly in trichodragmata, very abundant)	Southeast Australia, Port Phillip Heads, Victoria, and Port Jackson, NSW, Australian EEZ	?
cearense Salani *et al*., 2006	Stipitate, short bush on narrow peduncle, bushy part composed of fusiform branches parallel to the main axis of the sponge, branches composed secondary branchlets producing bush appearance, surface conulose with spatuliferous projections.	Axially compressed, extra-axially plumo-reticulate, multispicular tracts coated with abundant spongin, with diverging choanosomal tracts protruding through the surface.	320–764 × 2.6–15			15–25	absent	absent	Northeastern Brazil, Ceara State, Brazilian EEZ	21 m
dendroides Whitelegge, 1907	Erect, stipitate, ramose, slender cylindrical branches dichotomously branching in 1 plane, tapering at ends, surface even.	Compressed axial reticulation, abundant spongin, extra-axial radiating skeleton poor in spongin, only few anastomosing fibres, plumose spicule bundles at surface forming a tuft.	300–640 × 10–26		(rare anisoxeas)	I) 12–20 × 2 II) 25–40 × 2 (all contort, s-shaped)	25–35 × 1.5 (rare, single)	absent	Southeast Australia, Port Hacking, Wattamolla, NSW, Australian EEZ	5–133 m
flabellata Carter, 1885 *sensu* Dendy, 1897	Stipitate, short stalked, flabelliform, with compressed lobate lamallate branches arising from the short stalk, irregular margins, firm consistency, even, granulated or slightly hispid surface.	Dense skeleton of loose, plumose tracts bound by sparse spongin, plumose towards the surface, few connecting paucispicular fibres, surface with ascending fibres projecting slightly.	290–350 × 16–20	200–580 × 1.5–7 (rare)		15–20 × 1 (contort)	37–60 (in bundles or singly)	15–28 (only in dragmata) ("trichites in sheaf-like bundles")	Southeast Australia, Bass Strait, Victoria, and New Zealand EEZ, Australian EEZ & NZ EEZ	33 m
florida Brøndsted, 1924	Distinctive branching coralline shape, with conical radiating branches from a central axis.	Compressed fibres branching at acute angles, compacted and radiating towards surface in dense bundles.	416–858 × 20 (some subtylote)			50–70 (mostly contorted)	35–50	I) 200–270 II) 70	Southern New Zealand, NZ EEZ	"rather shallow water"

continued next page

TABLE 1. (continued)

Species	Growth form	Skeleton	Megascleres			Microscleres			Distribution	Depth
			Styles	Strongyles	Oxeas	Sigmas	Microxeas	Raphides		
incrustans Kirkpatrick, 1903	Thinly encrusting, 6mm thick, 'woolly-looking' surface.	Basal compression, branched plumose columns of megascleres rising vertically from base to surface, ectosomal with layer of microscleres, collagenous spongin moderate.	1085 × 33			27.5 × 2.7	absent	60 (single or in bundles)	South Africa, Natal, S. Africa EEZ	156 m
papillata Brøndsted, 1924	Lump shaped base, digitate branches even thickness, hispid surface with choansomal tracts forming small papillae, firm consistency.	Anastomosing axial skeleton of stout spicule tracts bound by spongin, becoming plumose at the periphery, with smaller styles on the surface.	286–650 × 9–17		145–416 × 7–11	30 × 2	absent	50 × 1 (rare)	Southern New Zealand, Carnley Harbour, NZ EEZ	"rather shallow water"
ramosa (Carter, 1883)	Erect, stipitate, ramose, thick cluster of tapering compressed polychotomous branches, arising from peduncle, branches with expanded ends or spatuliferous surface projections, surface hispid, even, consistency firm, resilient, becoming hard, compact, and rigid towards the base.	Skeleton uniformely fibro-reticulate, solid, without mention of axial and extra-axial differentiation.	681 × 27.2			12.3	20.5 (single or in bundles, "variable in size")	absent	Southeast Australia, Bass Strait, Australian EEZ	?
soelae Hooper, 1984	Erect, stipitate, arborescent, ramose, cylindrical compressed branches, dichotomous branching in 1 plane, firm, barely compressible, smooth, even, hispid surface.	Distinct axial, extra-axial and peripheral plumos components of the skeleton, light spongin fibres cored by styles.	I) 311–519 × 17–28 II) 210–389 × 5–12	(rare anisostron-gyles)		8–15 × 1-1.5	I) 59–86 (single or in bundles) II) 12–26 × 1	absent	Northwest Australia, Port Hedland. Australian EEZ	83 m
stylotata Brøndsted, 1924	Lump shaped basis giving rise to many papillae or columns tapering to sharp points.	Axially compressed, radiating extra-axial skeleton, dense tracts.	I) 455–676 × 20–33 II)190–402 × 8–17			40 (most contorted)	50	absent	Southern New Zealand, Carnley Harbour, NZ EEZ	"rather shallow water"
viminalis Hallmann, 1916	Erect, stipitate, ramose, very elongate thin cylindrical branches irregularly disposed, tapering ends, surface minutely hispid, even.	Central axis with loosely defined tracts, extra-axial skeleton without transverse fibres but with numerous, short, paucispicular tracts running to the surface, nearly perpendicular.	I) 700–1525 × 18 II) 320–700 (rare)			I)12–18 × 1 (c-shaped) II)27–50 × 1.5 (s-shaped)	absent	22–48 × 0.5–0.75 (single or in trichodragmata)	South Australia, Great Australian Bight, Australian EEZ	?
***hipposiderus* sp. nov.**	Arborescent, stipitate, erect, flattened into 1 plane, irreg. dichotomously branched, cylindrical compressed branches, surface conulose with spatuliferous projections.	Axial skeleton strongly compressed reticulation of multispicular tracts enclosed in abundant collagenous spongin, extra-axial region plumose, with ends of terminal spicules forming brushes at the surface.	500–1300 × 15–25			10–41 × 1–1.5	27–55 × 1.2 (single or in strong bundles, clearly not trichodragmata, however)	absent	Southeast Australia, Tasman Sea, Australian EEZ	159.6 m

95/20→ 48/10→ 65/45(25); 65/600 (1). Samples were held at 10°C and amplified products were visualised on a 1.5% agarose gel (1× TBE buffer) using EZ-Vision™ DNA Dye (AMRESCO Inc., Solon, OH, USA) as a loading buffer. Fragment sizes were verified against a GeneRuler™ 100 bp DNA Ladder (Fermentas Life Sciences (a part of ThermoFisher Scientific, Waltham, MA, USA)). Products were purified using an UltraClean™ PCR Clean-up DNA Purification Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) using the manufacturer's protocol, with the exception of a final elution in a volume of 30 μl. Product concentration was quantified on a gel by comparison to GeneRuler 100 bp DNA Ladder.

Sequencing: PCR fragments were sequenced using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems (part of Life Technologies Corporations, Carlsbad, CA, USA)). Fragments were sequenced in both directions in quarter reactions (10 μl) using the protocols of the manufacturer. Samples were held at 10°C until purification and precipitation. Completed sequencing reactions were purified using a sodium acetate and ethanol precipitation and visualised at the Griffith University DNA Sequencing Facility using a 3130xl Genetic Analyser (Applied Biosystems). Sequences were verified against chromatograms and ambiguous base calls resolved within MEGA v. 4.0 (Tamura *et al*. 2007). Contiguous sequences of forward and reverse reads were assembled in MEGA4 and compared to the NCBI (GenBank) database using BLAST searching (Altschul *et al*. 1990) to assess their general identity. New sequences have been deposited in GenBank (http://www.ncbi.nlm.nih.gov/) and the Sponge Barcoding Database (SBD) (http://www.spongebarcoding.org/) (accession details in Table 2). Sequences were aligned with sequences from specimens of other Poecilosclerida Topsent, 1928, in addition to Halichondrida Gray, 1867; sequences of Verongida Bergquist, 1978 and Spirophorida Bergquist & Hogg, 1969 were used for outgroup comparison (see Table 2 for list of all specimens used in the phylogenetic analysis).

Phylogenetic analysis. Aligned sequences were analysed using maximum likelihood methods implemented in RAxML (Stamatakis 2006) for a GTR + Γ model of sequence evolution; 1000 fast bootstrap replicates were performed. Bayesian methods were also used to estimate a likelihood tree. We implemented a GTR + Γ + I model of sequence evolution in MrBayes (ver. 3.1) (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) using default priors. We ran the Bayesian analysis through 10^6 generations of 2 runs with 4 chains (1 cold and 3 hot); 15% of the samples (sampling frequency = 10^2) were discarded as burn-in (established as when average standard deviation of split frequencies ≤ 0.02).

Systematic description

Order Poecilosclerida Topsent, 1928

Suborder Mycalina Hajdu, Van Soest & Hooper, 1994

Family Desmacellidae Ridley & Dendy, 1886

Genus *Sigmaxinella* Dendy, 1897

Diagnosis. Desmacellidae with axially compressed reticulate skeleton and extra-axially plumose skeleton; spicules styles, sigmas and microxeas in most cases (Hajdu & Van Soest 2002).

Type species: *Sigmaxinella australiana* Dendy, 1897 (by subsequent designation; Hallmann 1916: 535).

Sigmaxinella hipposiderus sp. nov.

Figs 2–5

Holotype. QM G323175, Big Horseshoe Canyon (West Bank), Bass Canyon system, Tasman Sea, Australia, -38.1148 S, 149.3565 E, depth 159.6 m, coll. CSIRO 'Southern Surveyor' cruise SS0404, Sherman sled, 26.iv.2004.

Diagnosis. *Sigmaxinella* with a single category of styles as megascleres (mean length 791 μm, mean width 19.2 μm); microscleres include a single category of sigmas (mean length 19.6 μm) and microxeas singly or in bundles (mean length 42 μm).

TABLE 2. List of specimens used in phylogenetic analysis.

Taxon	#	QM[α] Registration	GenBank[β] Accession	SBD[γ] Accession	Reference
Poecilosclerida					
Mycalina					
Desmacellidae					
Biemna fistulosa (Topsent, 1897)			AM076982		Rot *et al*. (2006)
Biemna saucia Hooper, Capon & Hodder, 1991		G303281	JF7731346	1054	[δ]
Neofibularia hartmani Hooper & Lévi, 1993	A	G306606		642/592	
	B	G206628		644/594	
	C	G324632	JF773145	1055	[δ]
Neofibularia irata Wilkinson, 1978		G307266		650/600	
Neofibularia nolitangere (Duchassaing & Michelotti, 1864)			EF519653	156/156	Erpenbeck *et al*. (2007)
Sigmaxinella hipposiderus **sp. nov.**		G323175	JF773147	1053	[δ]
Esperiopsidae					
Esperiopsis challengeri (Ridley, 1885)		G306063		732/762	
Isodictyidae					
Coelocarteria singaporensis (Carter, 1883)		G319331		669/619	
Mycalidae					
Mycale fibrexilis (Wilson, 1894)			AJ843890		Hess *et al*. (direct submission)
Mycale (*Arenochalina*) *laxissima* (Duchassaing & Michelotti, 1864)[1]			EF519649		Erpenbeck *et al*. (2007)
Mycale (*Arenochalina*) *mirabilis* (Lendenfeld, 1887)		G300561		572/522	
Mycale (*Mycale*) *sulcata* Hentschel, 1911		G304666		693/719	
Podospongiidae					
Diacarnus spinipoculum (Carter, 1879)			AY561975		Nichols (2005)
Microcionina					
Microcionidae					
Artemisina melana Van Soest, 1984			EF519575		Erpenbeck *et al*. (2007)
Clathria (*Clathria*) *prolifera* (Ellis & Solander, 1786)[2]			AJ843888		Hess *et al*. (direct submission)
Clathria (*Thalysias*) *oxeota* (Van Soest, 1984)[3]			EF519606		Erpenbeck *et al*. (2007)
Clathria (*Thalysias*) *schoenus* (de Laubenfels, 1936)[4]			EF519607		Erpenbeck *et al*. (2007)
Pandaros acanthifolium Duchassaing & Michelotti, 1864			EF519662		Erpenbeck *et al*. (2007)

continued next page

TABLE 2. (continued0

Taxon	#	**QM[α] Registration**	**GenBank[β] Accession**	**SBD[γ] Accession**	**Reference**
Raspailiidae					
Ectyoplasia ferox (Duchassaing & Michelotti, 1864)			EF519613		Erpenbeck *et al*. (2007)
Myxillina					
Chondropsidae					
Strongylacidon bermuda (de Laubenfels, 1950)[5]			AJ843889		Hess *et al*. (direct submission)
Coelosphaeridae					
Lissodendoryx (*Lissodendoryx*) *isodictyalis* (Carter, 1882)[6]			EF519638		Erpenbeck *et al*. (2007)
Lissodendoryx (*Lissodendoryx*) *sigmata* (de Laubenfels, 1949)[7]			EF519643		Erpenbeck *et al*. (2007)
Crambeidae					
Crambe crambe (Schmidt, 1862)			AF526297		Duran *et al*. (2004)
Monanchora arbuscula (Duchassaing & Michelotti, 1864)			EF519648		Erpenbeck *et al*. (2007)
Desmacididae					
Desmapsamma anchorata (Carter, 1882)[8]			EF519627		Erpenbeck *et al*. (2007)
Iotrochotidae					
Iotrochota birotulata (Higgin, 1877)			EF519636		Erpenbeck *et al*. (2007)
Tedaniidae					
Tedania (*Tedania*) *ignis* (Duchassaing & Michelotti, 1864)[9]			DQ133896		Wulff (2006)
Tedania (*Tedania*) *klausi* Wulff, 2006[10]			DQ133899		Wulff (2006)
Halichondrida					
Axinellidae					
Axinella corrugata (George & Wilson, 1919)			AY791693		Lavrov & Lang (2005)
Axinella sp. (OTU 0955)[11]		G300253		674/624	
Dragmacidon reticulatum (Ridley & Dendy, 1886)[12]			AJ843894		Hess *et al*. (direct submission)
Dragmacidon sp. (OTU 0817)[11]		G300605		568/518	
Ptilocaulis marquezi (Duchassaing & Michelotti, 1864)			EF519668		Erpenbeck *et al*. (2007)
Dictyonellidae					
Dictyonella sp.			AM498649		Blanquer & Uriz (2007)
Scopalina blanensis Blanquer & Uriz, 2008			AM498643		Blanquer & Uriz (2007)
Scopalina canariensis Blanquer & Uriz, 2008			AM498644		Blanquer & Uriz (2007)
Scopalina ceutensis Blanquer & Uriz, 2008			AM698646		
Scopalina lophyropoda Schmidt, 1862			AM498640		Blanquer & Uriz (2007)

continued next page

TABLE 2. (continued)

Taxon	#	QM[α] Registration	GenBank[β] Accession	SBD[γ] Accession	Reference
Scopalina ruetzleri (Wiedenmayer, 1977)			EF519669		Erpenbeck *et al*. (2007)
Svenzea zeai (Alvarez, Van Soest & Rützler, 1998)			EF519682		Erpenbeck *et al*. (2007)
Halichondriidae					
Halichondria (*Halichondria*) *magniconulosa* Hechtel, 1965[13]			EF519615		Erpenbeck *et al*. (2007)
Halichondria (*Halichondria*) *melanadocia* de Laubenfels, 1936[14]			EF519618		Erpenbeck *et al*. (2007)
Halichondria (*Halichondria*) *panicea* (Pallas, 1766)[15]			EF095183		Itskovich *et al*. (2007)
Hymeniacidon heliophila (Parker, 1910)			EF519632		Erpenbeck *et al*. (2007)
Heteroxyidae					
Didiscus sp.			AY561972		Nichols (2005)
Myrmekioderma gyroderma (Alcolado, 1984)			EF519652		Erpenbeck *et al*. (2007)
Verongida					
Aplysinidae					
Aplysina fulva (Pallas, 1766)			NC_010203		Lavrov *et al*. (2008); Wang & Lavrov (2008).
Spirophorida					
Tetillidae					
Cinachyrella apion (Uliczka, 1929)			AJ843895		Hess *et al*. (direct submission)
Cinachyrella kuekenthali (Uliczka, 1929)			EF519603		Erpenbeck *et al*. (2007)

[α] Queensland Museum; not all specimens have vouchers in the Queensland Museum collection.
[β] NCBI database, available at: http://www.ncbi.nlm.nih.gov/
[γ] Sponge Barcoding Database, available at: http://www.spongebarcoding.org/; number provided as record number then sequence number (xxx/xxx).
[δ] sequence generated newly in this study

[1] as *Mycale laxissma*
[2] as *Microciona prolifera*
[3] as *Clathria oxeota*
[4] as *Clathria schoenus*
[5] as *Strongylacidon bermudae*
[6] as *Lissodendoryx isodictyalis*
[7] as *Lissodendoryx sigmata*
[8] as *Holopsamma helwigi*
[9] as *Tedania ignis*
[10] as *Tedanis klausi*
[11] this species is undescribed but has been identified as an operational taxonomic unit by the Queensland Museum, as designated by an OTU number.
[12] as *Pseudaxinella reticulata*
[13] as *Halichondria magniconulosa*
[14] as *Halichondria melanodocia*
[15] as *Halichondria panicea*

Description. Growth form arborescent, erect, flattened into one plane, branching dichotomously, but irregularly, basal attachment small and on well formed stalk (now detached in fixed specimen); branches cylindrical, laterally compressed, ellipsoidal in cross-section, matting of spicules gives appearance of external segmentation in fixed specimen (Fig. 2B). Dimensions: overall height 103.0 mm; maximum breadth 69.0 mm; stalk length is approximately 15 mm; width of main axis 1.5 mm at base, widening to 3.8 mm at first bifurcation and 5.7 mm at apex; longest branch 62.2 mm in length from point of bifurcation to branch apex, maximum width 7.5 mm; shortest branch 35.7 mm in length, maximum width 2.6 mm. Colour light beige to pale grey in ethanol. Oscules inconspicuous, shallow, less than 1mm diameter, few, roughly circular; fine oscules observed on proximal portion of main axis in fixed specimen.

Surface uneven, velvety, hispid, with hair-like conulose projections forming a pile which becomes matted distally on branches, axial region villose. Texture barely compressible, tough, difficult to tear although easily cut; branches flexible, pliable; main axis flexible, but more resistant to bending than branches.

Skeleton with markedly differentiated axial and extra-axial construction; axis strongly compressed, extra-axial region plumose (Fig. 3). Compressed axial core occupies approximately half the diameter of the sponge in the region of the stalk, and one third the diameter in the branches. Choanosomal skeleton an axially condensed reticulation of multispicular tracts enclosed in abundant collagenous spongin; extra-axial skeleton plumose, with ends of terminal spicules forming brushes at the surface; microscleres densely packed along and between megascleres in plumose tracts; packing of microscleres more dense near axis than in periphery of skeleton, and density of microscleres decreases towards ectosome. Ectosome indistinct from choanosome, without any conspicuous specialisation aside from plumose terminal spicule bundles protruding from the choanosomal extra-axial skeleton.

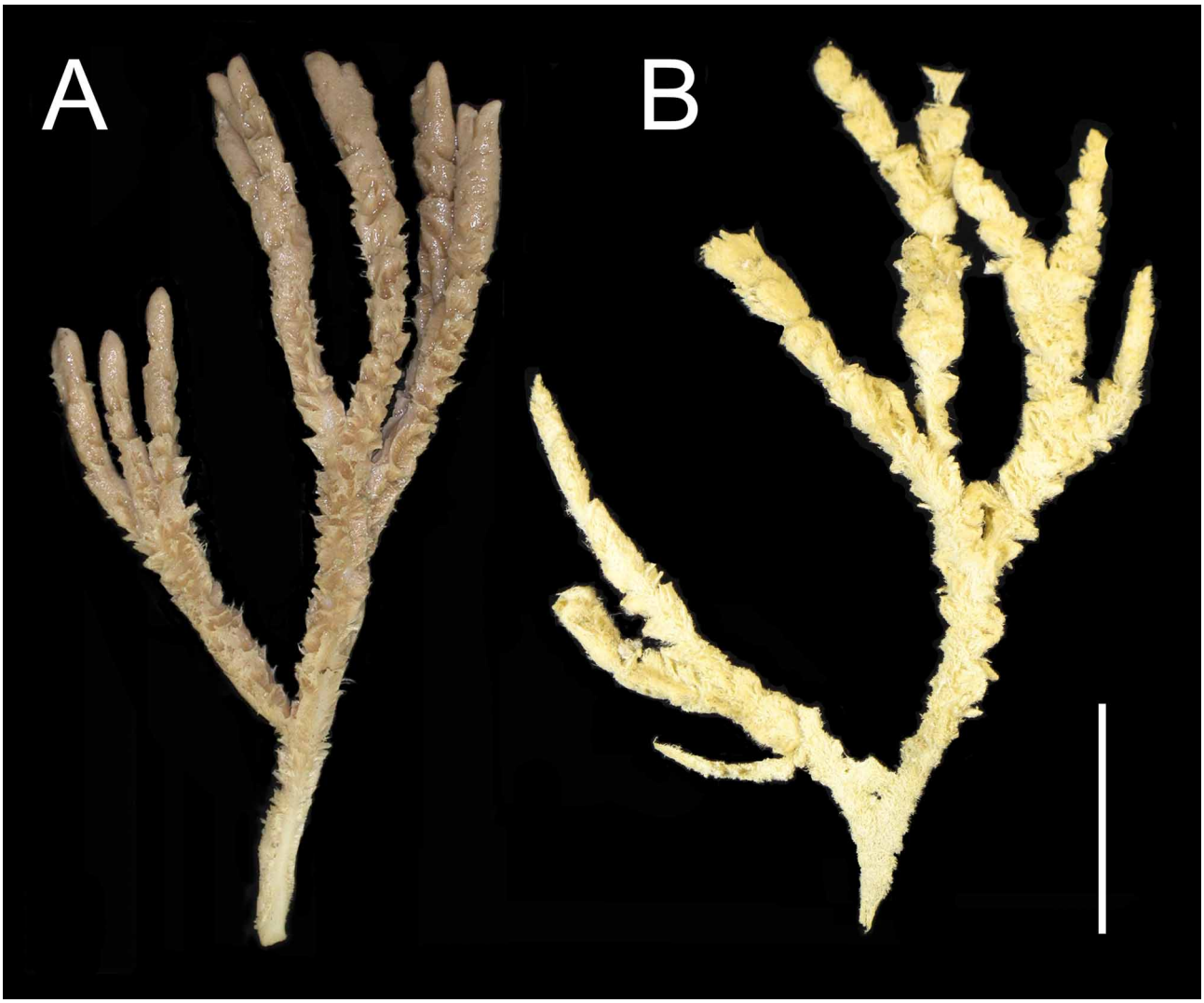

FIGURE 2. *Sigmaxinella hipposiderus* **sp. nov.** Holotype (QMG 323175), *habitus*, scale bar = 25 mm. A, specimen on deck; B, specimen after preservation in ethanol (70%).

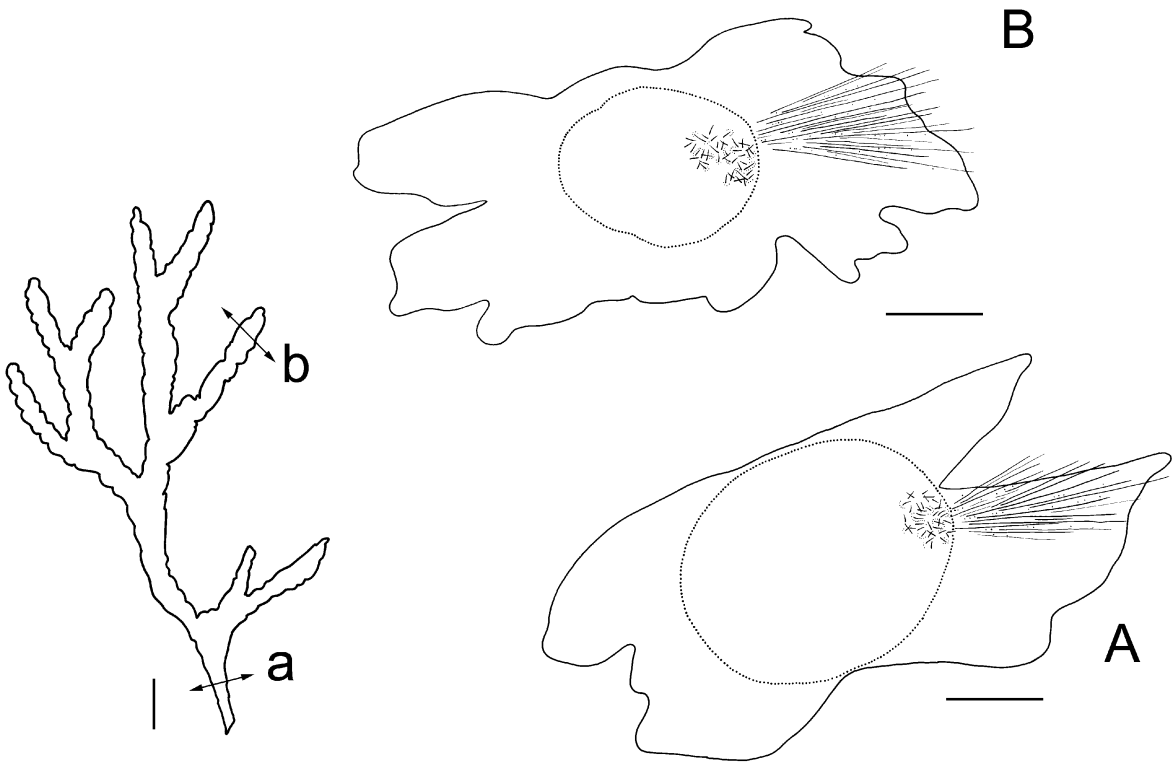

FIGURE 3. Schematic representation of skeletal arrangement of *Sigmaxinella hipposiderus* **sp. nov.**, scale bars = 1000 μm. A, schematic of cross-section taken through upper branch of specimen (as indicated by the double-arrow 'a' on whole specimen image); B, schematic of cross-section taken through lower stalk of specimen (as indicated by the double-arrow 'b').

Spicules (n = 25) (Table 1, Fig. 5):

Megascleres: single category of styles as megascleres, occasionally bent, 500–1300 (791.0) × 15–25 (19.2) μm.

Microscleres: single categories of both sigmas and microxeas; sigmas c-shaped, elongate, entirely smooth, 10–41 (19.6) × 1–2 (1.4) μm (chord length and widest axis), microxeas single or in compact bundles, isodiametric, completely smooth, tips hastate, 27–55 (42.1) × 1–1.5 (1.2) μm (length and maximum width).

Ecology and distribution. Known only from the holotype collected from the shallower margins of the Big Horseshoe Canyon, a side canyon to the Bass Canyon system off the eastern Victorian boarder, lying on the edge of the southeastern continental shelf. The specimen was collected from 160 m in a habitat dominated by rock and silt.

Etymology. The epithet is made as a masculine noun in apposition from the ancient Greek *hipposideros* (= horseshoe) in reference to the type locality, Big Horseshoe Canyon, Tasman Sea.

DNA sequence data. 1 sequence: COI mt DNA (partial, Folmer fragment, 658 bp, 1 replicate).

Remarks. This species is clearly assigned to *Sigmaxinella* on the basis of its compressed axial and plumose extra-axial skeletons, which is otherwise similar to *Biemna*. By having a single category of unmodified style megascleres, and single categories of both sigmas and microxeas, and in specific sizes of its spicules, this species differs from the 12 valid species of *Sigmaxinella* as follows (refer to Table 1 for the comparison among species of *Sigmaxinella*).

Sigmaxinella hipposiderus **sp. nov.** clearly differs from the type species, *S. australiana* Dendy, 1897, in having a single category of styles, sigmas and microxeas. The latter has much smaller styles, some of which are modified into strongyles and oxeas, two categories of sigmas and one category of much thinner raphides (*i.e*. those in the present species are thicker and more obviously microxeas than raphides, and, even though they form bundles, these bundles are not trichodragmata) (Table 1, Fig 5B). Nevertheless, both species have a similar external morphology (see Fig. 2, 6A) and a differentiated axial and extra-axial skeletal architecture, features which can only be described as 'typical' of *Sigmaxinella*.

In growth form, *S. hipposiderus* also vaguely resembles *S. dendroides* Whitelegge, 1907 from southern New South Wales (Fig. 6C), but the latter has distinctly smaller megascleres, two categories of sigmas, all s-shaped, and

rare microxeas not forming bundles (Table 1). The present species should also be compared to the Atlantic *S. cearense* Salani *et al*., 2006; both species show simple, reduced spiculation, but the present species also has microxeas and sigmas. The sigmas of *S. hipposiderus* lack the *Paresperella*-like spines on the outer edge as is seen in the Atlantic species.

There has been some confusion by previous authors in the descriptions of some species of Desmacellidae (including *Sigmaxinella* and *Biemna*) about the possession of raphides versus microxeas. Usually these spicule categories have been combined into a single category (*e.g.*: Hooper 1984; Salani *et al*. 2006), yet they are differentiated clearly under electron microscopy (*e.g.*: Hooper & Lévi 1993). This is the case for *S. flabellata* Carter, 1885 *sensu* Dendy, 1897 redescribed by Hallmann (1916). This species was described as having two categories of raphides, yet further examination indicates that the larger category, occurring both in bundles and singly, are microxeas, not raphides.

A further note on *S. flabellata* is appropriate, as re-examination of one of the syntypes of *Axinella flabellata* Carter, 1885 (BMNH 1886.12.15.471 from Port Phillip Heads, Victoria), the type species of the genus *Sigmaxia* Hallmann, 1916, shows this to actually be a species of *Raspailia* (*Raspailia*) Nardo, 1833. Unfortunately, the other syntype (BMNH 1886.12.15.143 wet) has not yet been located in the collections of the BMNH. Therefore, for the time being, we must use the concept of *Sigmaxinella flabellata* in the sense of Dendy (1897: 241). Hallmann (1916: 535) subsequently redescribed *S. flabellata* much more comprehensively based on new material, which he also stated he compared to "one of Dendy's specimens". Carter's (1885) original concept of *A. flabellata* remains uncertain.

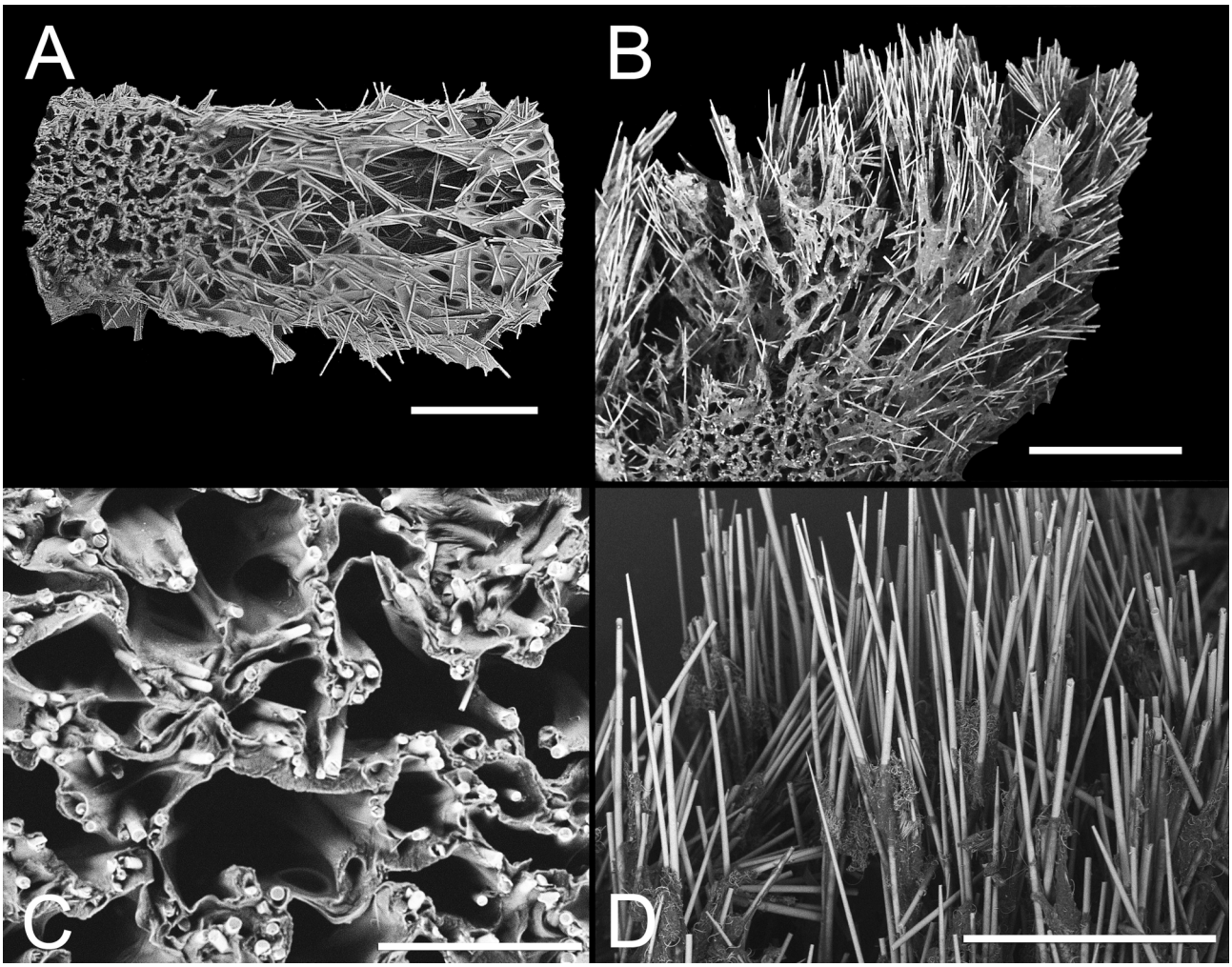

FIGURE 4. Scanning electron micrographs of skeleton structure from *Sigmaxinella hipposiderus* **sp. nov.** A, overview of skeleton, showing axial compression and plumose extra-axial region, scale bar = 1000 μm; B, extra-axial region, showing plumose tracts of styles radiating from central axis, scale bar = 1000 μm; C, axial region, showing strong compression with styles arranged in parallel into tightly packed bundles surrounded by dense spongin, scale bar = 200 μm; D, styles arranged in plumose brushes in extra-axial region and penetrating ectosome, scale bar = 500 μm.

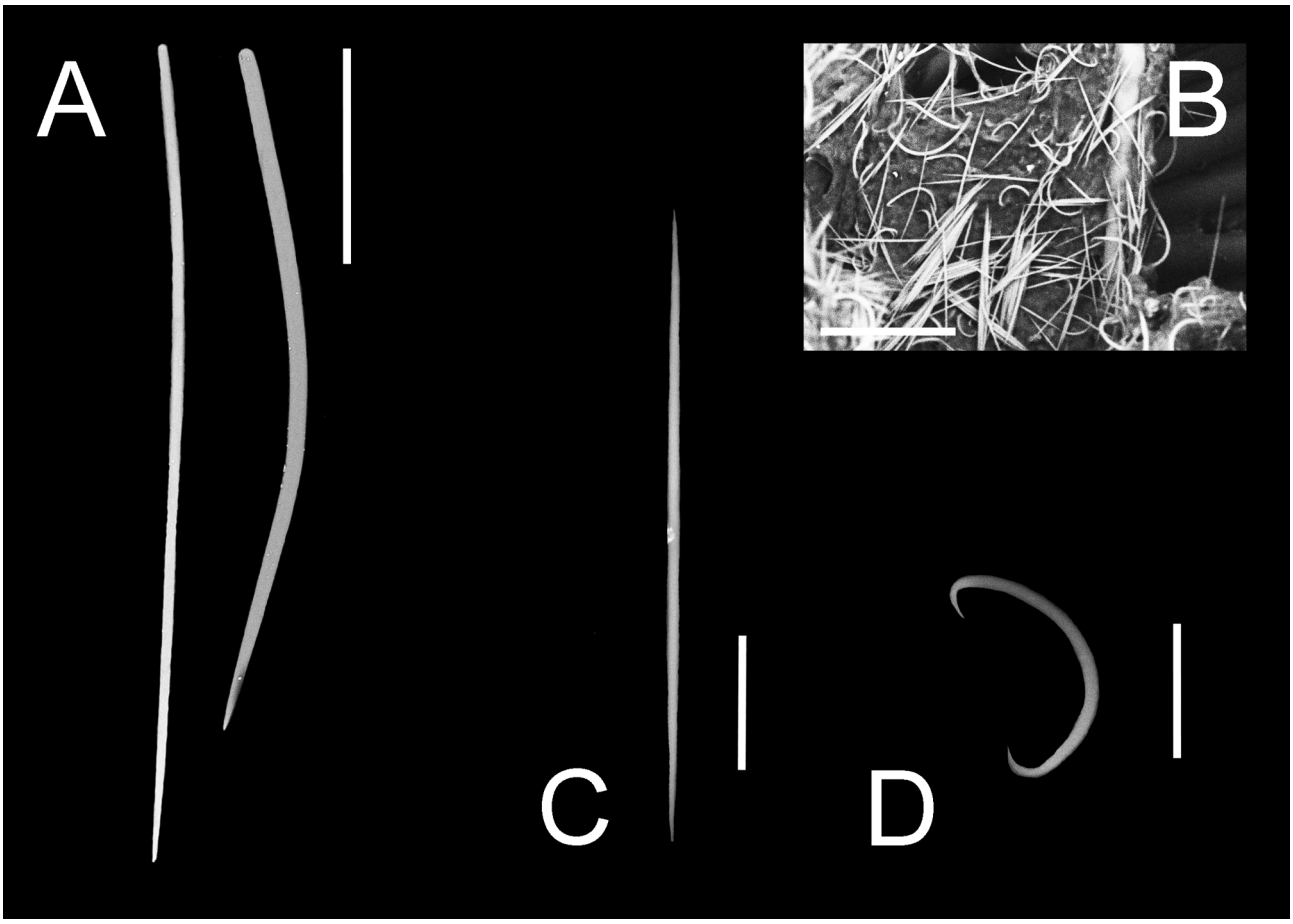

FIGURE 5. Scanning electron micrographs of spicule complement from *Sigmaxinella hipposiderus* **sp. nov.** A, megascleres are represented by two types of styles, with the long straight styles dominant, scale bar = 200 μm; B, image of microscleres in the mesohyl, showing microxeas forming bundles, but not organised into true trichodragmata, scale bar = 50 μm; C, microxea, smooth and isodiametric, scale bar = 10 μm; D, sigma, c-shaped and without any spines on hooks, scale bar = 10 μm.

Phylogenetic analysis

The alignment of the COI mtDNA gene region comprised 51 taxa, of which eight were nominally designated as belonging to Desmacellidae. This study has used all desmacellid material available on public databases, such as GenBank and SBD, supplemented by new sequences presented here. An additional 21 poecilosclerid taxa and an additional 19 halichondrid taxa were included in the alignment; one verongid and two spirophorids were included as outgroups. The final alignment comprised 584 base pairs. Maximum likelihood and Bayesian estimates of phylogeny were inferred for this dataset (Fig. 7).

In our phylogenies, monophyly of the desmacellid taxa was recovered with good support (98% maximum likelihood bootstrap (ML); 1.0 Bayesian posterior probability (PP)), and our new species, *S. hipposiderus* lies within this desmacellid clade. Although we cannot assert with confidence that the Desmacellidae is monophyletic without the inclusion of specimens of *Desmacella pumilio* Schmidt, 1870 and additional types for the remaining unrepresented genera (which were unavailable for this study), we are confident that the taxa assigned here to Desmacellidae form a well-supported, monophyletic assemblage. In our trees, *S. hipposiderus* is shown as a sister to *Biemna saucia* Hooper, Capon & Hodder, 1991, but with only moderate support (70% ML; 0.92 PP). Sequences of specimens of *Neofibularia* formed a well-supported clade (100% ML; 1.0 PP) with one specimen attributed to *Biemna* (*B. fistulosa* (Topsent, 1897)) by Rot *et al*. (2006), although monophyly of the specimens of *Neofibularia* themselves was not well supported. According to our phylogenies, *Biemna* (and possibly *Neofibularia*) as currently conceived, is paraphyletic, although it should be noted here that only two sequences of specimens of *Biemna* were currently available.

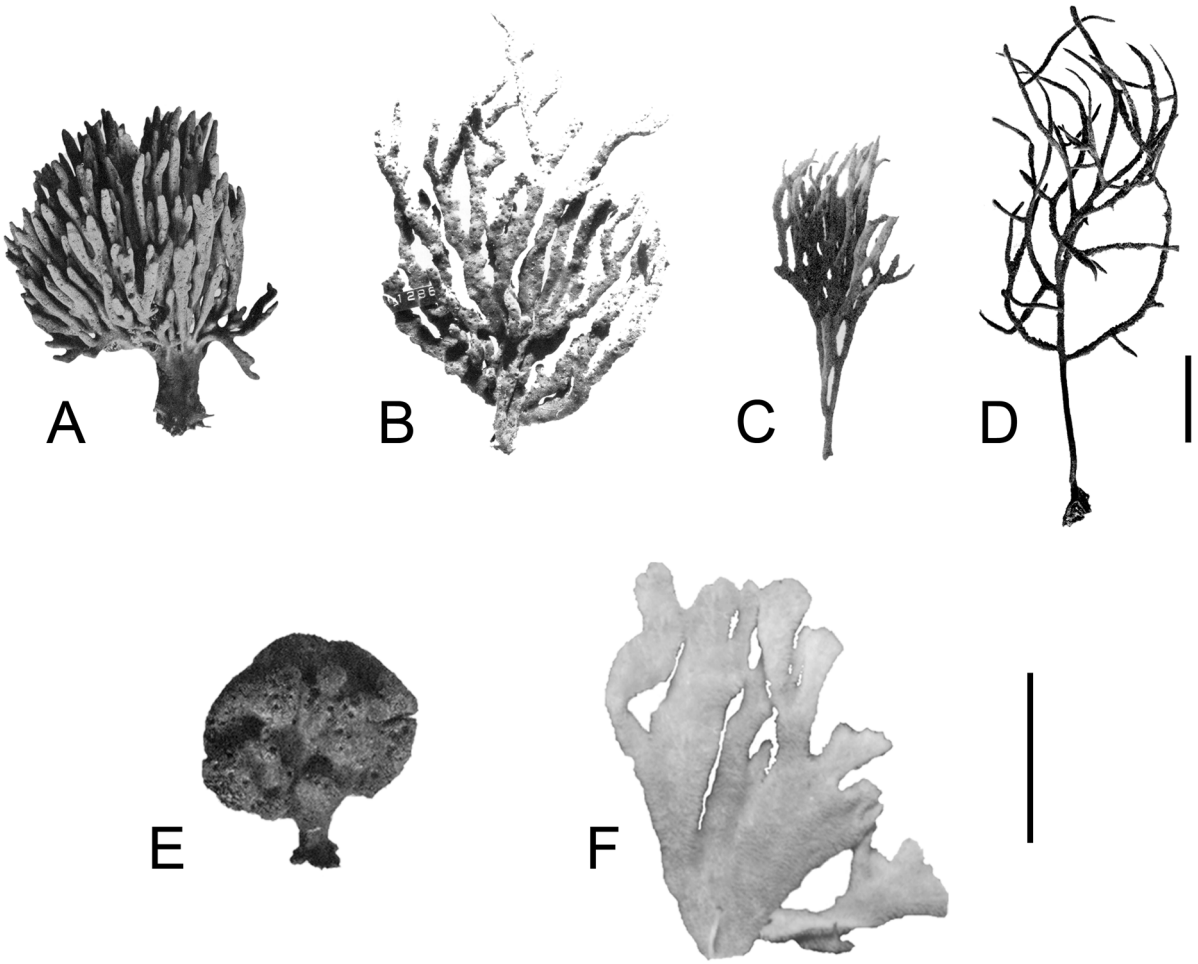

FIGURE 6. Comparison of gross morphology of species of *Sigmaxinella* Dendy, 1897 found in Australian waters. A, *S. australiana* Dendy, 1897; B, *S. soelae* Hooper, 1984; C, *S. dendroides* Whitelegge, 1907; D, *S. viminalis* Hallmann, 1916; E, *S. flabellata* (Carter 1885) *sensu* Dendy, 1897; F, *S. ramosa* (Carter, 1883); image A from Hallmann (1916, pl. XXXIII, Fig. 1); image B from Hooper (1984, p. 8, Fig. 1); image C from Whitelegge (1907, pl. XLVI, Fig. 42); image D from Hallman (1916, pl. XXXIII, Fig. 4); image E from Hallmann (1916, pl. XXXIII, Fig. 5); image F original, syntype of *Phakellia ramosa* Carter, 1883, specimen BMNH 1884.4.14.2, collected from Sydney region; images A–D and E–F to same proportion, both scale bars = 50 mm.

The position of the Desmacellidae in our phylogeny is unresolved. There is an estimated association with some axinellid and dictyonellid taxa, however, this relationship is not supported with any confidence. Despite the lack of deep resolution on our tree, it is clear that our desmacellid taxa do not have any close relationship to chelae-bearing poecilosclerids. Desmacellidae is classified currently within Mycalina, and not one of our trees was able to recover a relationship between desmacellids and other groups from Mycalina (*e.g.*: Mycalidae, Esperiopsidae, Isodictyidae, Podospongiidae). Monophyly of the chelae-bearing poecilosclerids is well-supported on our trees (97% ML; 1.0 PP), although there is little internal resolution of this group. (NB: although Tedaniidae Ridley & Dendy, 1886 lack true chelae, this interpreted as a secondary absence (*e.g.* Erpenbeck *et al*. 2007)). Polyphyly of the Poecilosclerida (including non-monophyly of Mycalina and Myxillina) was established by Erpenbeck *et al*. (2007); our results are in broad agreement with their phylogeny. Further, our results also agree with Erpenbeck *et al*. (2007) that there is support for monophyly of the chelae-bearing poecilosclerids. Our trees indicate that Desmacellidae (which is non-chelae-bearing) is not best placed within Mycalina and the higher systematics of the family should be addressed. Additional molecular data (including the 28S rDNA gene) should be analysed to establish the higher relationships of Desmacellidae.

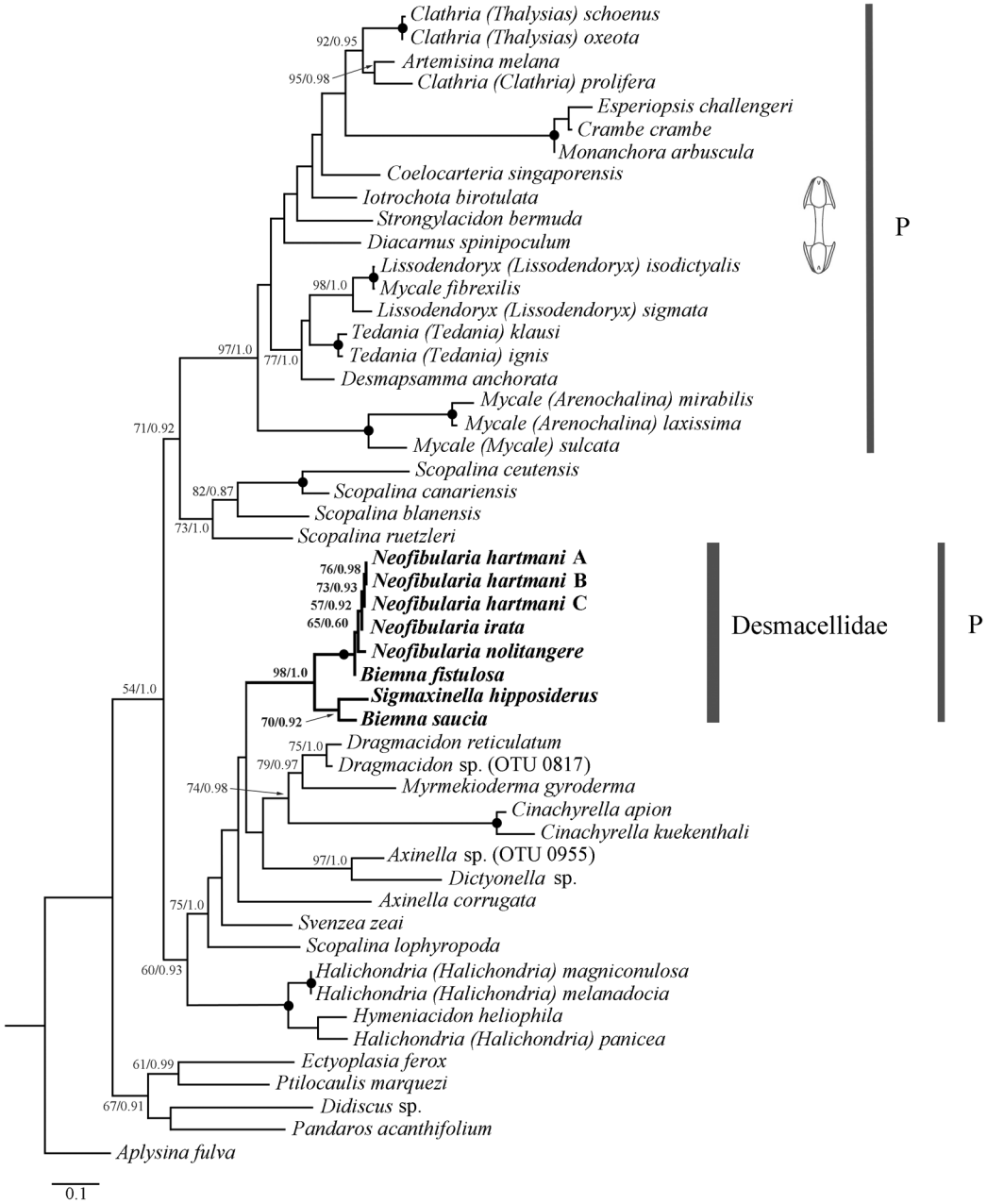

FIGURE 7. Phylogram of relationships among nominal desmacellid taxa. Tree shown is a maximum likelihood phylogeny (-lnL = 8811.690278) based on a GTR + Γ model generated in RAxML. Maximal bootstrap values and posterior probabilities (100% and 1.0) are indicated by closed circles at the nodes. ML bootstrap support values < 50% are not shown; bootstrap values ≥ 50% but < 100% are indicated at nodes. Posterior probabilities are mapped onto nodes (where node is supported by bootstrapping), following bootstrap values (BS/0.PP). Clades of poecilosclerid taxa are denoted "P" and primary chelae- (and their derivatives-) bearing poecilosclerids are indicated by the image of a chela; it should be noted that although species of *Tedania* Gray, 1867 lack true chelae, this has been interpreted by Erpenbeck *et al.* (2007) as a secondary absence.

Acknowledgements

This study was funded by the Australian Biological Resources Study National Taxonomy Research Grant Program (grant 208-63). We are grateful to Dr Monika Schlacher-Hoenlinger (QM) for bringing this species to our attention through her initial identification of the collections from the CSIRO Southeast Canyons surveys. KDM also thanks Dr Merrick Ekins and Dr Robert Raven (QM) for their patience in scanning electron microscopy instruction. We also thank Ms Nicole Hogg and the team at Griffith University DNA Sequencing Facility for their work in visualising our sequences.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology*, 215, 3, 403–410.

Blanquer, A. & Uriz, M.J. (2007) Cryptic speciation in marine sponges evidenced by mitochondrial and nuclear genes: a phylogenetic approach. *Molecular Phylogenetics and Evolution*, 45 (1), 392–397.

Brøndsted, H.V. (1924) Papers from Dr. Th. Mortensen's Pacific Expedition 1914–16. XV. Sponges from the Auckland and Campbell Islands. *Videnskabelige Meddelelser fra Dansk naturhistorisk Forening i Kjøbenhavn*, 75, 117–167.

Carter, H.J.(1883) Contributions to our knowledge of the Spongida. *Annals and Magazine of Natural History*, 12(71), 308–329, pls. XI–XIV.

Carter, H.J. (1885) Descriptions of sponges from the neighbourhood of Port Phillip Heads, South Australia. *Annals and Magazine of Natural History*, 16(94), 277–294, 347–368.

Dendy, A. (1897) Catalogue of non-calcareous sponges collected by J. Bracebridge Wilson, Esq., M.A., in the neighbourhood of Port Phillip Heads. Part III. *Proceedings of the Royal Society of Victoria*, new series, 9, 230–259.

Duran, S., Pascual, M. & Turon, X. (2004) Low levels of genetic variation in mtDNA sequences over the biogeographic range of the sponge *Crambe crambe* (Poecilosclerida). *Marine Biology*, 144, 31–35.

Erpenbeck, D., Duran, S., Rützler, K., Paul, V., Hooper, J.N.A. & Wörheide, G. (2007) Towards a DNA taxonomy of Caribbean demosponges: a gene tree reconstructed from partial mitochondrial CO1 gene sequences supports previous rDNA phylogenies and provides a new perspective on the systematics of Demospongiae. *Journal of the Marine Biological Association* (*UK*), 87 (6), 1563–1570.

Hajdu, E. & Van Soest, R.W.M. (2002) Family Desmacellidae Ridley & Dendy, 1886. *In*: Hooper, J.N.A. & Van Soest, R.W.M. (Eds), *Systema Porifera: A Guide to the Classification of Sponges*. Kluwer Academic/Plenum Publishers, New York, USA, pp. 642–650.

Hallmann, E.F. (1916) A revision of the genera with microscleres included, or provisionally included, in the family Axinellidae; with descriptions of some Australian species. Part I and Part II, issued 1916; Part III issued 1917. *Proceedings of the Linnean Society of New South Wales*, 41 (3–4), 453–491, 495–552, 634–675.

Hooper, J.N.A. (1984) *Sigmaxinella soelae* and *Desmacella ithystela*, two new desmacellid sponges (Porifera, Axinellida, Desmacellidae) from the northwest shelf of western Australia, with a revision of the family Desmacellidae. *Northern Territory Museum of Arts and Sciences Monograph Series*, 2, 58 pp.

Hooper, J.N.A. & Lévi, C. (1993) Poecilosclerida from the New Caledonia lagoon (Porifera: Demospongiae). *Invertebrate Taxonomy*, 7(5), 1221–1302.

Hooper, J.N.A. & Van Soest, R.W.M. (2002) *Systema Porifera: A Guide to the Classification of Sponges.* Kluwer Academic/Plenum Publishers, New York, USA.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, 17, 754–755.

Itskovich, V., Belikov, S., Efremova, S., Masuda, Y., Perez, T., Alivon, E., Borchiellini, C. & Boury-Esnault, N. (2007) Phylogenetic relationships between freshwater and marine Haplosclerida (Porifera, Demospongiae) based on the full length 18S rRNA and partial COXI gene sequences. *In*: Custódio, M.R., Lobo-Hajdu, G., Hajdu, E. & Muricy, G. (Eds), *Porifera Research: Biodiversity, Innovation & Sustainability.* Museu Nacional, Rio de Janeiro, Brazil, pp. 383–391.

Jeon, Y.J. & Sim, C.J. (2008) A new species of the genus *Biemna* (Demospongiae: Poecilosclerida: Desmacellidae) from Korea. *Animal Cells and Systems*, 12, 241–243.

Kirkpatrick, R. (1903) Descriptions of South Africa sponges. Part III. *Marine Investigations South Africa*, 2, 233–264, pls V–VI.

Lavrov, D.V. & Lang, B.F. (2005) Transfer RNA gene recruitment in mitochondrial DNA. *Trends in Genetics*, 21 (3), 129–133.

Lavrov, D.V., Wang, X. & Kelly, M. (2008) Reconstructing ordinal relationships in the Demospongiae using mitochondrial genomic data. *Molecular Phylogenetics and Evolution*, 49 (1), 111–124.

Meyer, C.P., Geller, J.B. & Paulay, G. (2005) Fine scale endemism on coral reefs: archipelagic differentiation in turbinid gastropods. *Evolution*, 59, 113–125.

Nichols, S.A. (2005) An evaluation of support for order-level monophyly and interrelationships within the class Demospongiae using partial data from the large subunit rDNA and cytochrome oxidase subunit I. *Molecular Phylogenetics and Evolution*, 34 (1), 81–96.

Ronquist, F. & Huelsenbeck, J.P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.

Rot, C., Goldfarb, I., Ilan, M. & Huchon, D. (2006) Putative cross-kingdom horizontal gene transfer in sponge (Porifera) mitochondria. *BMC Evolutionary Biology*, 6, 71.

Salani, S., Monteiro da Cruz Lotufo, T. & Hajdu, E. (2006) *Sigmaxinella cearense* sp. nov. from sandstone reefs of Fortaleze (Céara State, Brazil) (Desmacellidae, Mycalina, Poecilosclerida, Demospongiae). *Zootaxa*, 1369, 43–53.

Stamatakis, A. (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22, 2688–2690.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24, 1596–1599. [Download available at http://www.megasoftware.net/mega4/mega.html, last accessed 4 March, 2011.]

Van Soest, R.W.M. (2011) Desmacellidae. *In*: Van Soest, R.W.M, Boury-Esnault, N., Hooper, J.N.A., Rützler, K, de Voogd, N.J., Alvarez de Glasby, B., Hajdu, E., Pisera, A.B., Manconi, R., Schoenberg, C., Janussen, D., Tabachnick, K.R., Klautau, M., Picton, B., Kelly, M. (Eds), *World Porifera Database*. [Available at http://www.marinespecies.org/porifera/porifera.php?p=taxdetails&id=131645, last accessed 23 March, 2011.]

Wang, X. & Lavrov, D.V. (2008) Seventeen new complete mtDNA sequences reveal extensive mitochondrial genome evolution within the Demospongiae. *PLoS ONE*, 3(7), E2723.

Whitelegge, T. (1907) Sponges. Part 1. — Addenda. Part II. Monaxonida continued. *Memoirs of the Australian Museum*, 4(10), 487–515, pls. XLV–XLVI.

Wulff, J.L. (2006) Sponge systematics by starfish: predators distinguish cryptic sympatric species of Caribbean fire sponges, *Tedania ignis* and *Tedania klausi* n. sp. (Demospongiae, Poecilosclerida). *Biological Bulletin*, 211 (1), 83–94.