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Two new desma-less species of *Theonella* Gray, 1868 (Demospongiae: Astrophorida: Theonellidae), from the Great Barrier Reef, Australia, and a re-evaluation of one species assigned previously to *Dercitus* Gray, 1867

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Abstract

Extensive surveys of the biodiversity on the seafloor of the inter-reef regions of the Great Barrier Reef, Australia, have resulted in the collection of large numbers of sponges, many of which are likely new to science. Identification of these sponges, however, was made difficult by the absence in some specimens of key diagnostic characters, such as megascleres. We used an integrated approach to the taxonomy of these sponges, incorporating morphological examination by SEM, analysis of DNA sequence data (using the COI barcoding fragment of mtDNA) and preliminary studies of the chemistry of the sponges, to describe the new species, which were found to contain no native spicules other than acanthose microrhabds. Here, we propose two new species of *Theonella* Gray, 1868: *Theonella deliqua* **n. sp.** (found in association with a single unidentified species of siliquariid mollusc) and *Theonella maricae* **n. sp.** from the Great Barrier Reef. Further, we propose the new combination of *Theonella xantha* (Sutcliffe, Hooper and Pitcher 2010) n. comb. for another microrhabd-only-bearing species. On the basis of our gene trees, we recognise *Theonella* (and Theonellidae Lendenfeld, 1903) within Astrophorida Sollas, 1887. We discuss the potential for chemotaxonomic and DNA-based insights into the origins and radiation of species of *Theonella* and explore the evolutionary significance of the reduced morphology of the three additional species recognised here.

Key words: Porifera, Demospongiae, Astrophorida, Theonellidae, *Theonella deliqua* **n. sp.**, *Theonella maricae* **n. sp.**, *Theonella xantha* n. comb., taxonomy, systematics, biodiversity, sponges, Great Barrier Reef, Queensland, Indo-West Pacific

Introduction

Sponges of the Family Theonellidae Lendenfeld, 1903 are distributed worldwide and are found generally in deeper waters. As a lithistid group, theonellids are characterised largely by their megascleres; the spicule complement is dominated by a rigid silica skeleton, formed by an interlocking network of ornate tetraclone desmas. Species of theonellids are known also to contain other tetractinal spicules, notably phyllotriaenes, dichotriaenes and discotriaenes at the surface, in addition to some monactinal megascleres; the microsclere component of theonellids is dominated by the presence of finely spined monactinal microrhabds, although amphiasters and streptasters are known from some members (Pisera and Lévi 2002). Currently, the group is conceived as comprising 51 species classified within five genera: *Theonella* Gray, 1868 (14 spp.), *Discodermia* du Bocage, 1869 (29 spp.), *Manihinea* Pulitzer-Finali, 1993 (2 spp.), *Racodiscula* Xittel, 1878 (5 spp.) and *Siliquariaspongia* Hoshino, 1981 (1 sp.) (see van Soest 2012a, b).

The type-genus for the family, *Theonella* Gray, 1868, is morphologically homogenous, with the group being defined by a surface coat of aligned phyllotriaenes or dichotriaenes, internal tetraclone desma network with tuberculate zygosis, large monactinal spicules, and microscleres, which are only small acanthose microrhabds, often with bends in the middle (Pisera & Lévi 2002; Ilan *et al*. 2004). Ilan *et al.* (2004) studied the reproduction of

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two species of *Theonella* and found both were oviparous, with the possibility of gonochorism. Species of *Theonella* are often marked by a bright yellow-orange pigment (or, less commonly, blue colouration) and are restricted largely to the Indo-West Pacific, although two species are known from western Africa and the Caribbean. Of the Indo-West Pacific species, only *Theonella levior* Lendenfeld, 1907 has been described from Australian waters (being described from the deep sea off Western Australia; Lendenfeld 1907). Few other species have been reported yet from the waters in proximity to Australia, although they are of significant interest to marine natural products chemists (e.g. D'Auria *et al.* 2002). Fromont (1999) recorded an undescribed species of *Theonella* from the Houtman Abrolhos, off the west cost of Australia; and according to van Soest (2012b), only four described species are found in proximity to Australian waters. *Theonella lacerata* Lendenfeld, 1907 is known from Indonesia and *Theonella mirabilis* (de Laubenfels, 1954) is known only from Micronesia and the Marshall Islands. *Theonella swinhoei* Gray, 1868, the type-species for the group, and *Theonella conica* (Kieschnick, 1896) are both distributed widely throughout the Indo-West Pacific, ranging from the Indonesian archipelago to the Red Sea and Madagascar.

To date, very few species from other theonellid genera have been described from the Indo-West Pacific region. Fromont & Pisera (2011) describe *Manihinea lynbeazleyae* Fromont and Pisera, 2011 from deep canyons off the coast of Western Australia. Species of *Manihinea* are unusual among theonellids in bearing only streptasters among their microscleres; species of *Racodiscula* are the only other theonellids known to possess streptasters, although in combination with microrhabds. Fromont & Pisera (2011) suggest that because of this distinctive morphology, these genera may be recognised as a special subgroup within Theonellidae. Further morphological and DNA-based studies are needed to clarify the relationship of these groups to other theonellids.

Theonellids, with their distinctive oily pigmentation, are marked by the presence of unusual chemicals within the sponges themselves. In their 1999 review of the chemosystematics of sponges, van Soest & Braekman stated that lithistids contained an intermediate level of chemical diversity, with 200 chemical structures isolated from them to that time. Species of *Theonella* have been referred to since as noteworthy sources of a potent and diverse array of peptides and polyketide secondary metabolites (Schmidt *et al*. 2000), with *T. swinhoei* containing a high diversity of compounds isolated from bacterial fractions of preparations of these sponges (Faulkner *et al.* 1999). Schmidt *et al*. (2000) isolated the source of some of these metabolites, finding that specific antifungal peptides were produced by an endosymbiotic δ-proteobacterium which they named "*Candidatus* Entotheonella palauensis". A recent review of the natural chemical products of lithistids by Winder *et al*. (2011) suggests that the exceptionally rich diversity of compounds from these sponges is produced predominantly by microbial endosymbionts.

The antifungal metabolites of species of theonellids have been identified from dried pigments isolated from these sponges. Matsunaga *et al*. (1991) characterised cytotoxic chemicals in orange pigments extracted from a sponge identified only as *Theonella* sp.; they called these compounds, which are tetramic acid glycosides, Aurantosides A and B. Subsequently, more aurantosides, with varying degrees of bioactivity, were isolated from other theonellid sponges. Wolf *et al*. (1999) isolated Aurantoside C from *Manihinea conferta* Pulitzer-Finali, 1993 (as *Homophymia conferta*), and Aurantosides D–F were identified from *Siliquariaspongia japonica* Hoshino, 1981 by Sata *et al.* (1999a). Papua New Guinean specimens of *T. swinhoei* were found by Ratnayake *et al*. (2005) to contain novel aurantosides, which they named Aurantosides G through I, and an Indonesian specimen attributed to the same sponge species was found by Angawi *et al*. (2011) to contain a further aurantoside (Aurantoside J), which exhibited bioactivity against fungi. Most recently, an eleventh aurantoside (Aurantoside K), which has demonstrated broad-spectrum antifungal activity, has been isolated from specimens attributed to *Melophlus* Theile, 1899 (Astrophorida: Geodiidae) from Fiji (Kumar *et al.* 2012). Another family of tetramic acid glycosides has been identified from the theonellid species *S. japonica* by Sata *et al*. (1999b); these red pigments, called rubrosides (A through H) are very similar to the aurantosides listed above and have varying degrees of bioactivity. Although an aurantoside has been identified from a species of *Melophlus*, these compounds are not related to melophlins (A through O; Aoki *et al*. 2000, Wang *et al*. 2003), which although sometimes yellow, are clearly distinct structurally from the aurantosides (Kumar *et al*. 2012). With one exception, aurantosides (and related rubrosides) are found exclusively within theonellid sponges and a characteristic yellow, oily pigment, which can be readily extracted into ethanol, marks their presence.

Recent and extensive biodiversity surveys on the shallow inter-reef regions of the Great Barrier Reef, Queensland, Australia (Great Barrier Reef Seabed Biodiversity Project, GBRSBD), have recovered numerous specimens of sponges which contain greasy yellow pigmentation akin ostensibly to that observed in species of

theonellids. Many of these specimens were thought to be species new to science. One such species, containing yellow pigmentation, has been described recently as *Dercitus* (*Stoeba*) *xanthus* Sutcliffe, Hooper and Pitcher, 2010. Evaluation of material attributed to *D.* (*S.*) *xanthus* and additional specimens from the GBRSBD collection stored in the Queensland Museum, using a combination of molecular, morphological and preliminary chemical analyses, suggests that these yellow-pigment containing specimens may be best classified as multiple species of *Theonella*. We used this integrated approach to taxonomy to test the affinities of this material, and based on our results, here, we present two new species and a new combination.

Material and methods

Collection of specimens. All material used in this study was collected as part of the GBRSBD. The study site covered the length and breadth of the Great Barrier Reef, Queensland, Australia, encompassing the seabed of the inter-reef regions. Collection of material from the seafloor was made by epibenthic sled at 1189 sites. Material was also collected by trawl at 457 sites. Sutcliffe *et al*. (2010) provide full details of the specific methodology for the collection and macroscopic sorting of material. Sponge material used in this study was stored frozen in the first instance. Subsamples of this frozen material were made subsequently; these samples were fixed in 70% ethanol for morphological examination and 100% ethanol for DNA studies. Primary type material has been registered and deposited in the Porifera collection at the Queensland Museum (QM), Brisbane, Australia.

Morphological examination. Sponge samples were examined using both light and scanning electron microscopy (SEM). Preparations for light microscopy were made by cutting semi-thin sections of sponge manually; the sections were cleared in phenol-xylene overnight and embedded in Fluka Durcopan™ (Sigma-Aldrich Co., St. Louis, MO, USA). Additional preparations of the spicules were made for light microscopy by digesting small portions of sponge in nitric acid; the sponge-acid mix was heated over a flame and the remaining spicules mounted in Canada balsam. Sponge material was prepared for SEM in two ways to obtain accurate images of the structure; because of the high proportion of non-sponge material incorporated into these specimens, the sponge was very fragile and careful preparation was needed to maintain the structural integrity. Firstly, small pieces of untreated, ethanol-fixed sponge were mounted directly onto stubs covered with carbon and oven-dried. Some of these preparations were subsequently coated in gold. Secondly, we dissolved small portions of sponge in sodium hypochlorite (12.5 % active chlorine). Dissolution of the incorporated carbonate and soft tissue was monitored under a dissecting microscope and halted by the addition of distilled water. The remaining skeleton was washed thoroughly in distilled water twice and rinsed finally in 100 % ethanol prior to mounting on carbon-coated SEM stubs. A low vacuum Hitachi Tabletop Scanning Electron Microscope TM-1000 was used to examine the prepared stubs.

We measured at least 30 spicules for each specimen under SEM. Basic statistics were completed for the sets of measurements for each species, including mean, standard deviation and variance, and sets were compared using boxplots of the distributions of measurements. In addition to the summary statistics, we performed tests for Skewness and Kurtosis to assess the normalness of the distributions, assessed the range of measurements across quartiles, and calculated 95% confidence intervals for the mean, median and standard deviation. These statistical measures were used to look for outliers, to test the normalness of the distributions of the measurements within an hypothesised taxonomic unit (OTU), and to compare measurements and assess the significance of any differences among the hypothesised OTUs. All statistics were generated in the Minitab® 16.1.1 software package (Minitab Inc., State College, PA, USA).

DNA analysis. Extraction and Amplification: DNA was extracted from ~2 mm^3 of sponge (fixed from frozen tissue directly into 100% ethanol) using a NucleoSpin® Tissue DNA extraction kit (Macherey-Nagel, Düren, Germany), following the protocol of the manufacturer. In order to obtain a concentrated extract, the final elution of the genomic DNA was into a volume of 50 µl using pre-warmed elution buffer. We amplified the standard barcoding fragment of the cytochrome oxidase subunit 1 gene (COI mtDNA) using the degenerated Folmer primers (dgLCO1490 and dgHCO2198) of Meyer *et al*. (2005). PCR was performed using HotMaster™ Taq DNA Polymerase (5 Prime GmbH, Hamburg, Germany). Reactions were made to a final volume of 25 µl by this recipe: 2.5 units Taq polymerase, 1× HotMaster™ Taq Buffer with Mg2+ (2.5 mM Mg2+), 0.25 mM dNTPs, 1.0 µM of each primer (forward and reverse), 0.4 µg/µl BSA (Sigma-Aldrich Co.), ~200 ng template DNA and nuclease-free

ddH_2O. Cycling was completed as follows: 94°C /120 sec (1 cycle); 94/20→ 45/10→ 65/45 (10); 95/20→ 48/10→ 65/45(25); 65/600 (1). Completed reactions were held at 10°C. Products were visualised on 1.5% agarose gels (1× TBE buffer) using EZ-Vision™ DNA loading buffer and dye mix (AMRESCO Inc., Solon, OH, USA) and compared against a GeneRuler™ 100 bp DNA Ladder (Fermentas Life Sciences (a part of ThermoFisher Scientific, Waltham, MA, USA)). We used an UltraClean™ PCR Clean-up DNA Purification Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) to purify the amplified DNA fragments; concentrated products were produced by a final elution into a volume of 30 µl. Small volumes of the purified amplicons were again run out on a gel against the GeneRuler ladder to quantify the concentration of the product.

Sequencing: a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems (part of Life Technologies Corporations, Carlsbad, CA, USA) was used to sequence the amplicons in both directions. Sodium acetate and ethanol precipitation was used to purify the completed sequencing reactions; these samples were then sent to the Griffith University DNA Sequencing Facility for visualisation on a 3130xl Genetic Analyser (Applied Biosystems). Chromatograms were viewed and ambiguous base calls resolved using MEGA4 (version 4.0, Tamura *et al*. 2007); contiguous sequences of forward and reverse sequences were completed within the same program. We BLASTed the nucleotide sequences against the NCBI database (=GenBank) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to verify the poriferan origin of the sequences.

Phylogenetic Analysis: our newly generated sequences were aligned against those sequences for the barcoding *COI* fragment available on GenBank (http://ncbi.nlm.nih.gov/genbank) for other Astrophorida Sollas, 1887; sequences of species of "Lithistida" and Spirophorida Bergquist & Hogg, 1969 were included in the alignment for outgroup comparison. Table 1 lists all taxa included in the phylogenetic analysis. We inferred Maximum Likelihood (ML) and Bayesian estimates of the phylogeny for the included taxa. The computer programs RAxML (Stamatakis 2006), implemented via the raxmlGUI (Silvestro & Michalak 2011), and MrBayes (ver 3.1.2; Ronquist & Huelsenbeck 2003) were used to generate these estimates. A General Time Reversible model of evolution, with among site variation modelled using a gamma distribution (with 4 categories), (=GTR + Γ), was applied to the aligned dataset during the analyses. ML trees were bootstrapped through 10,000 replicates (rapid) and a 50% majority rule consensus of these 10,000 trees computed. Bayesian analyses were performed with 2 independent runs, each with 1 cold and 3 heated Metropolis-coupled MCMC chains (4 in total); trees were sampled at every 100 generations, and diagnostics calculated every 1000, for 1.5×10^6 generations. Convergence (when the average deviation of split frequencies was <0.01) was not reached after this time, and the run was terminated when the average deviation was <0.02. The initial proportion of trees with a deviation split frequency >0.1 was then discarded as burn-in and a consensus tree (50% majority rule) calculated.

Taxonomy and systematics

Proposed nomenclatural acts

1. Proposal of two new species of *Theonella* Gray, 1868: *Theonella deliqua* **n. sp.** and *Theonella maricae* **n. sp.**
2. Proposal of one new combination for a species previously recognised within *Dercitus* Gray, 1867 (in *Dercitus* (*Stoeba*) Sollas, 1888) as a species of *Theonella*: *Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010) n. comb.
3. Rediagnoses of Theonellidae Lendenfeld, 1903 and *Theonella* to reflect more accurately the new taxonomic composition of the group. Establishment of a synapomorphy for the group has been hampered by the absence of key diagnostic features in some species; homologies based on DNA motifs and chemotaxonomic characters may require investigation.
4. Proposal to recognise Theonellidae within Astrophorida Sollas, 1887.

Taxonomy

Phylum Porifera Grant, 1836

Class Demospongiae Sollas, 1885

Order Astrophorida Sollas, 1887

Family Theonellidae Lendenfeld, 1903

Diagnosis (after Pisera and Lévi 2002; Fromont and Pisera, 2011): polymorphic, choanosomal spicules as tetraclone desmas; ectosomal spicules as phyllo- to discotriaenes; large choanosomal oxeas sometimes present; megascleres sometimes completely absent (some *Theonella*); microscleres characteristically as small, acanthose microrhabds, sometimes centrangulate or with slight curve, sometimes as streptasters and microrhabds (*Manihinea*) or streptasters only (*Racodiscula*).

Type-genus: *Theonella* Gray, 1868.

Genus *Theonella* Gray, 1868

Rhachella Sollas, 1888

Diagnosis. with characteristics of Theonellidae; ectosomal spicules as phyllo- to dichotriaenes; large choanosomal oxeas sometimes present; megascleres sometimes completely absent; microscleres as small, acanthose microrhabds only, sometimes curved slightly.

Type-species. *Theonella swinhoei* Gray, 1868; by monotypy (Gray 1868).

Theonella deliqua n. sp.

Figs 1–4

Material examined. *Holotype*: QM G329195 (=SBD520375), Australia, Great Barrier Reef, inter-reef sea floor, south of Wreck Island Reef, 23.775°S 15.005°E, 41.3 m (depth), coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Gwendoline May*, 13.Apr.2004, epibenthic sled. *Paratype*: QM G325567 (=SBD518107), Australia, Great Barrier Reef, inter-reef sea floor, south of Wreck Island Reef, 23.375°S 151.975°E, 43.5 m (depth), coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Gwendoline May*, 22.Apr.2004, epibenthic sled.

Description. based on examination of holotype and paratype; both specimens post-fixed in ethanol (70%) after initial frozen storage.

Growth form and gross morphology: sponge consists of very thin sheets, thickness ~50 µm; sheets encrust exclusively over single species of *Tenagodus* Guettard, 1770 (Gastropoda, Caenogastropoda, Siliquariidae); sponge forms mass with snails, cements *Tenagodus* shells, incorporates small amounts of algae, detritus and debris; *Tenagodus* shells in interior of mass appear non-live, shells at perimeter of mass sometimes contain live (at time of fixation) snails; mass incorporates *Tenagodus* of various ages, some tiny (<1 mm diameter), others mature (>5 mm diameter); holotype mass measures ~ 5 × 7.5 × 3 cm (total mass, including shells) (Figs 1A, 2A–D).

Colour: unknown in life; bright orange portions of sponge mixed with green algae and cream snail shells when frozen; colour retained in ethanol; stains ethanol pale golden yellow; yellow pigment greasy.

Oscules: unobserved macroscopically in frozen and fixed material; visible microscopically, few, inconspicuous, shallow, discrete, elliptical, ~100–200 µm (length), distributed sparsely (Fig 3A).

Texture: difficult to determine due to inclusion of large volume of snail shells; sponge very soft, fragile, friable, granular, flaccid, limp, highly compressible, slowly resilient, spongy.

Surface ornamentation: even, smooth.

Ectosomal skeleton: indistinguishable from choanosome.

Choanosomal skeleton: lax, vague; rigid skeleton entirely absent; skeleton consists only of confused arrangement of interstitial microscleres scattered throughout mesohyl; microscleres sparse in patches, distributed singularly, concentrated in other regions, forming dense carpet; collagen homogenous; occasional foreign megascleres (oxeas, regular triacts) incorporated into mesohyl (Figs 2D, 3A–C).

Megascleres: nil.

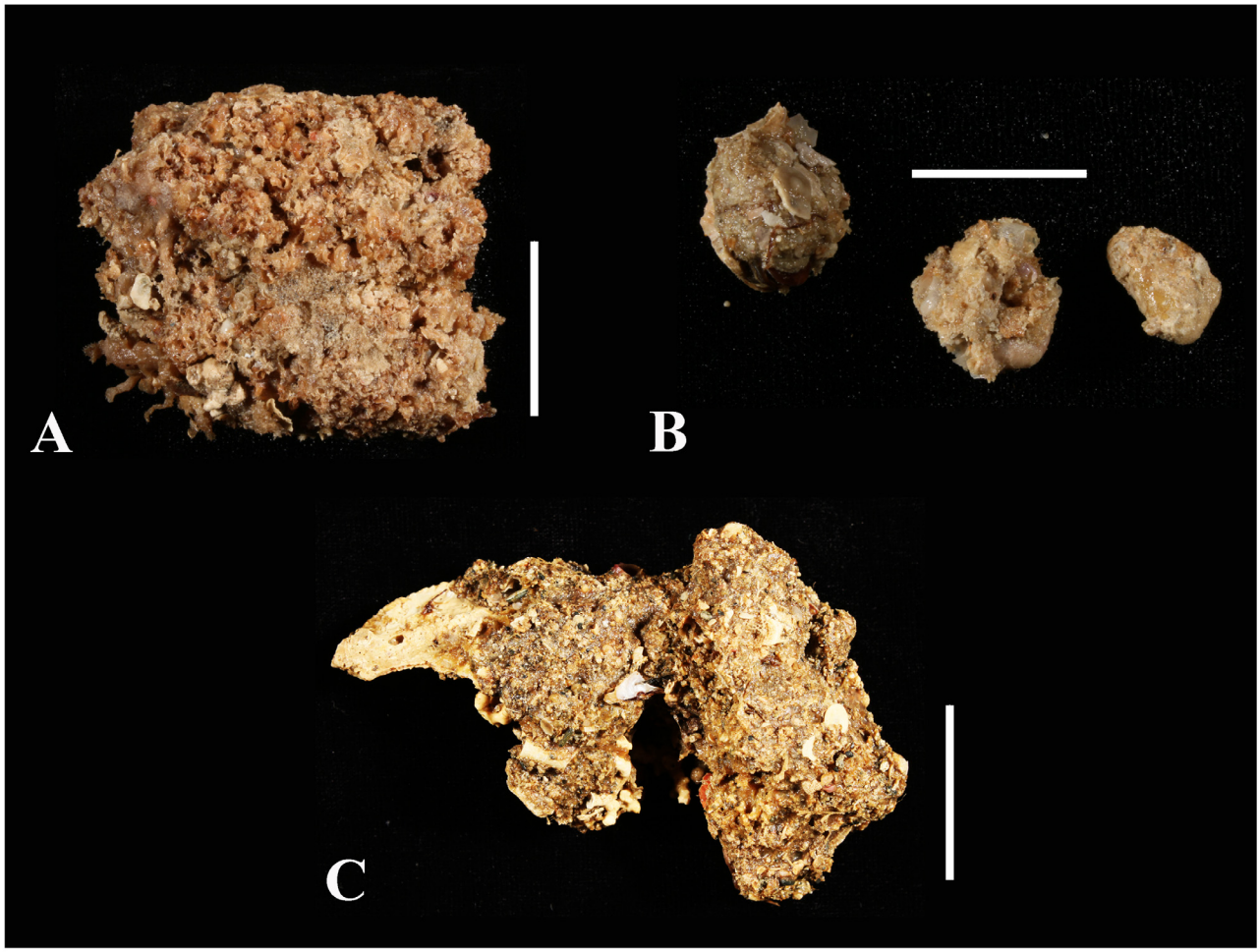

FIGURE 1. Photographs of fixed whole specimens of desma-less species of *Theonella* Gray, 1868. A. *Theonella deliqua* **n. sp.**, habitus, part of holotype (QM G329195), fixed in ethanol; scale bar = 2 cm. B. *Theonella maricae* **n. sp.**, habitus, three parts of holotype (QM G331427), fixed in ethanol; scale bar = 2 cm. C. *Theonella xantha* (Sutcliffe, Hooper and Pitcher 2010) n. comb., habitus, part of holotype (QM G329976), fixed in ethanol; scale bar = 2 cm.

Microscleres: single category of microrhabd; microrhabds as highly spined microxeas, small, isodiametric, slender, fine, slightly curved, curvature irregular, tips sharply hastate, rhabd covered with numerous, fine, narrow, conical spines; spines as long or longer than rhabd width, project prominently from spicule shaft, arise perpendicular to axis; shaft straight, lacks torsion; dimensions 7.2–21.6 (14.6) × 2.5–3.4 (3.0) µm (Fig 3D).

Etymology. The specific epithet *deliqua* derives from the Latin *deliquus* (adjective), meaning lacking or wanting, and refers to the absence of desmas in this species.

DNA sequence data. 1 *COI* barcode sequence was obtained for the holotype (GenBank Accession: KJ494355; see Table 1); this sequence was 709 bp in length (including primers).

Ecology and distribution. Specimens of *T. deliqua* have, to date, been recovered only from the seabed of the inter-reef region of the Great Barrier Reef. Both specimens that we have examined have formed close associations with specimens of a single species of *Tenagodus* (Siliquariidae). Species of *Tenagodus* are known to occur only in obligate relationships with sponges (Bieler 2004), although species-specificity (between sponge and snail) of this obligate relationship has not been established (Pansini *et al*. 1999).

Remarks. During examination of the holotype of *Theonella deliqua* n. sp., a dense mass of regular triactinal spicules (calthrops) was found; many of these calthrops were damaged and had broken rays (Fig 2D). This mass of spicules was found lying in a valley between two *Tenagodus* shells and incorporated broken oxeas and other spicules (from the Family Didemnidae Giard, 1872 (Class Ascidiacea) and some possibly of holothurian origin). Another similar region, containing an accumulation of monactinal spicules, was found in the broken mouth of an empty *Tenagodus* shell (Fig 2C). These regions overlie the thin sheets of *T. deliqua*, but are not incorporated

intimately into the mesohyl of the sponge. The localisation of the spicule masses, in conjunction with their varied composition, indicates clearly that they are of foreign origin, and are not innate components. Further, *T. deliqua* itself encrusts closely over the surface of the *Tenagodus* shells, cementing only the shells together; detritus and debris appears to amass in rafts at low points where two shells are joined by the sponge.

The microrhabds of the holotype and paratype of *T. deliqua* are of similar proportion, averaging 14.6 µm in length, and spanning a range from 7.1 to 21.6 µm. The range of spicule measurements was normally distributed (Fig. 4), although one spicule was detected which lay outside of this normal range, measuring only 6.7 µm. Although the range of microrhabd length is quite large, the majority of spicules ranges between 12 and 17 µm in length, and this size may be interpreted as "typical" for specimens of *T. deliqua*.

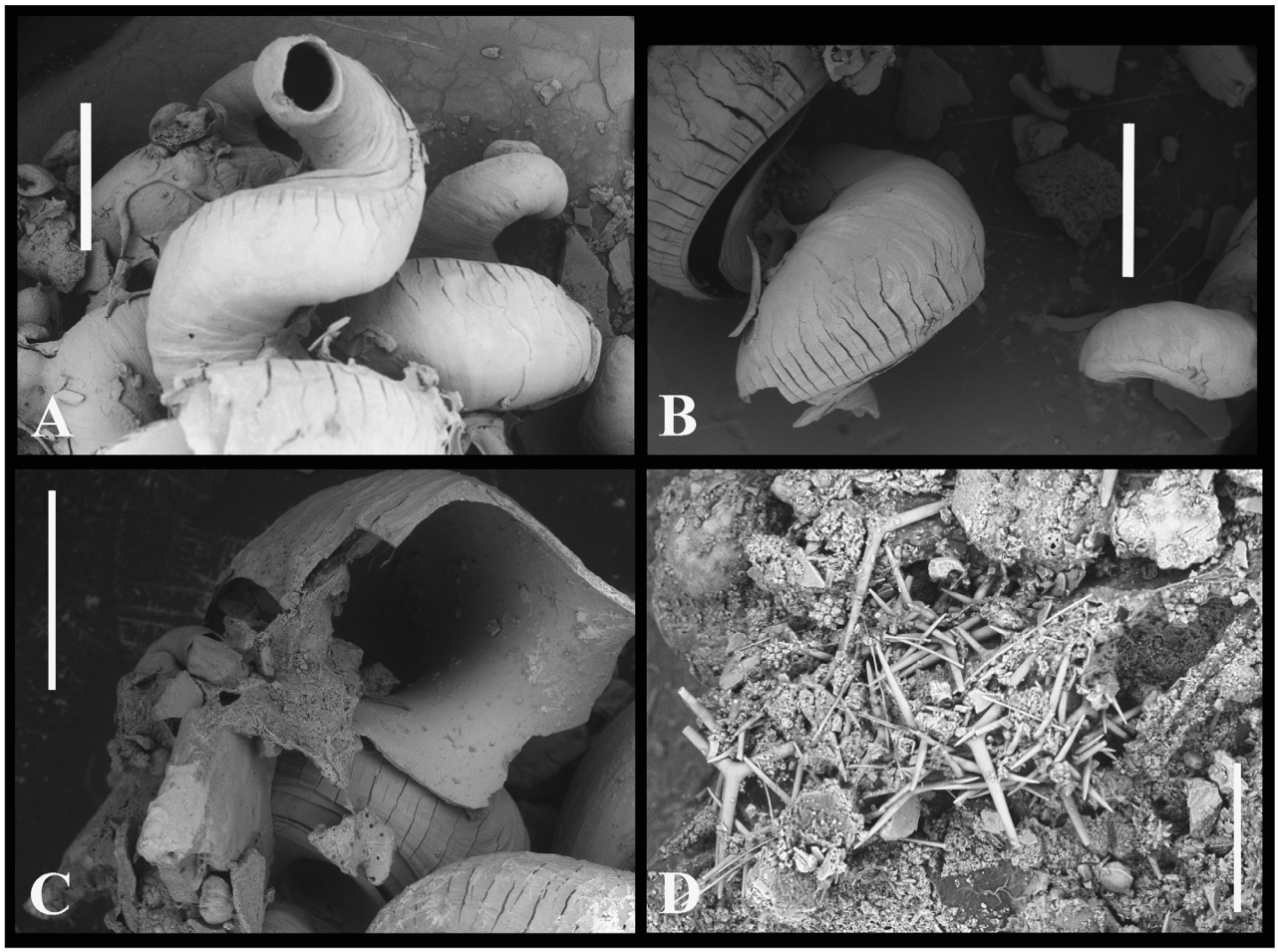

FIGURE 2. *Theonella deliqua* **n. sp.** Micrographs (SEM) of paratype, QM G325567. A. QM G325567, overview of sponge, showing empty shells of a species of *Tenagodus*; scale bar = 1 mm. B. QM G325567, detail of snail shell, showing slit, which is definitive for species of *Tenagodus*; scale bar = 1 mm. C. QM G325567, detail of snail shell, showing *T. deliqua* forming thin encrusting sheets over the shell; scale car = 1 mm. D. QM G325567, detail of region lying between aggregated snail shells; note the accumulation of debris and foreign spicules, including broken calthrops; scale bar = 100 µm.

Comments. Specimens of *T. deliqua* are readily distinguished from the type-species for *Theonella*, *T. swinhoei*, (and all other currently known species), by the absence of tetractinal megascleres. No desmas and no triaenes (phyllotrianes nor dichotriaenes) were observed in either specimen of *T. deliqua* that we examined. The spicule complement of *T. deliqua* comprises only microrhabds; this condition has not been observed to date in any recorded species of *Theonella*. Despite the lack of obvious morphological homologies with *T. swinhoei* and the other members of *Theonella*, membership of this new species to *Theonella* can be asserted confidently. The microrhabds of *T. deliqua* have a similar morphology to those observed in *T. swinhoei* and other species of *Theonella*. Although they are not noted directly in the original description by Gray (1868), we have examined material in the QM Porifera collection which is attributed to *T. swinhoei*, and observed that the microrhabds of *T. swinhoei*, like those in *T. deliqua*, are generally straight; although the rhabd may be bent, the central axis is free

completely of any torsion, with fine, conical spines projecting perpendicularly from the spicule shaft. The lack of torsion is significant and shared between the microscleres of *T. swinhoei* and *T. deliqua.* The straightness of the rods is in contrast to the morphology seen in the streptasters of other astrophorids; this straight morphology justifies our use of the term "microrhabd", rather than sanidaster or streptaster, to describe these microscleres. Further, and perhaps more significantly, the combination of the corroborating molecular analyses (see below) and the presence of shared chemotaxonomic characters (see below) offers strong support to the attribution of this new species to *Theonella.*

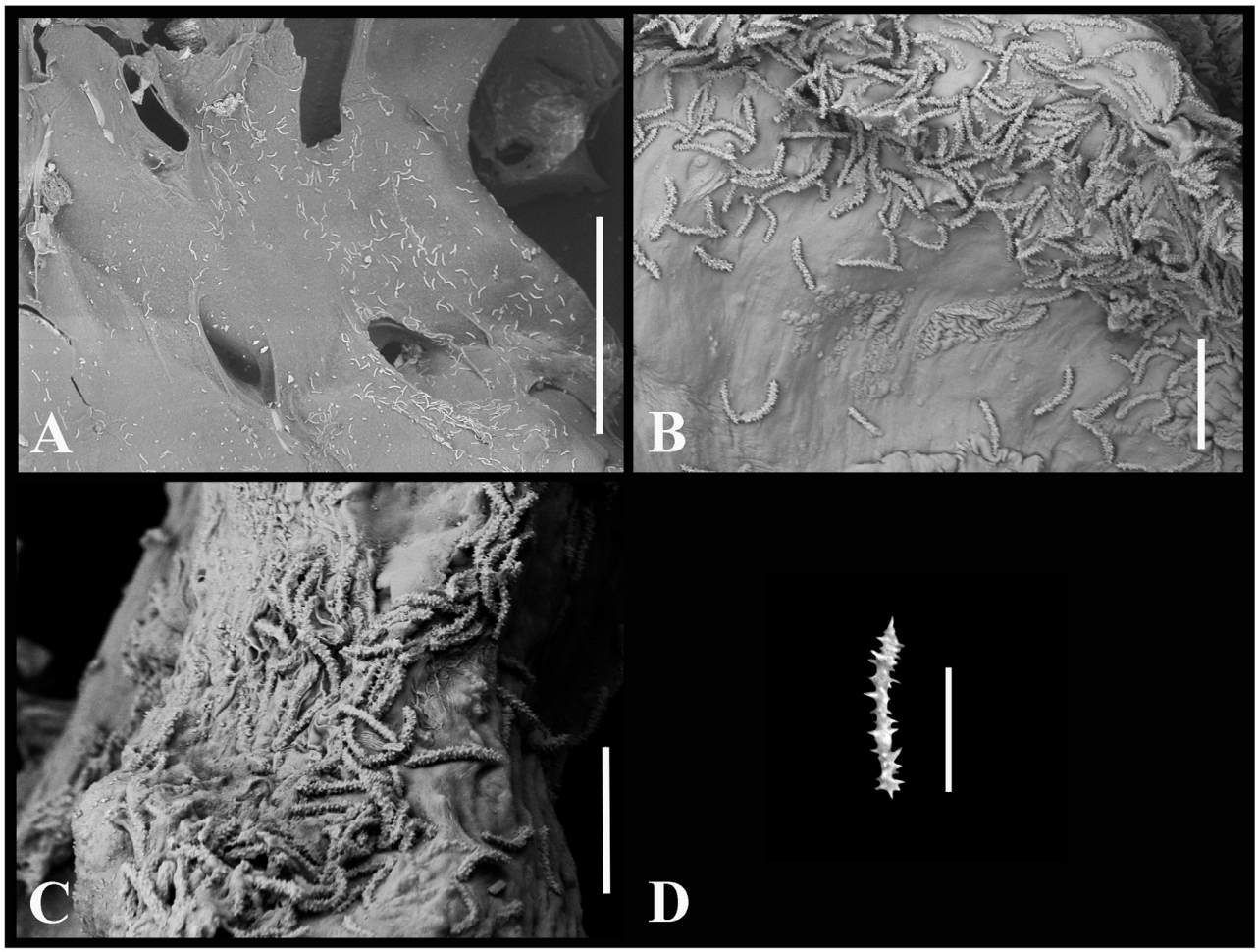

FIGURE 3. *Theonella deliqua* **n. sp.** Micrographs (SEM) of holotype, QM G329195, and paratype, QM G325567. A. QM G329195, thin sheets of *T. deliqua*; showing oscules and density of microrhabds; scale bar = 300 µm. B. QM G325567, detail of sponge, showing random aggregation of microrhabds in a dense carpet; scale bar = 30 µm. C. QM G329195, detail of sponge showing rafts of microrhabds; scale bar = 30 µm. D. QM G325567, single microrhabd; scale bar = 10 µm.

***Theonella maricae* n. sp.**
Figs 1, 4–5

Material examined. *Holotype*: QM G331427 (=SBD513035), Australia, Great Barrier Reef, inter-reef sea floor, south-east of Guthrie Shoal, 23.095°S 151.875°E, 28.0 m (depth), coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Lady Basten*, 22.Sep.2004, epibenthic sled.

Description. Based on examination of holotype; specimen post-fixed in ethanol (70%) after initial frozen storage.

Growth form and gross morphology: *s*ponge consists of very thin sheets, thickness ~50 µm; sheets encrust over assorted non-specific substrates, cements a variety of unidentified broken gastropod shells, diatoms, broken coral debris into single mass; incorporates large amounts of filamentous algae, quartz sand and debris; holotype in three small ovoid masses, largest mass measures ~ 2 × 2 × 1 cm (total mass dimensions) (Figs 1B, 5A–B).

Colour: unknown in life; pale orange to yellow portions of sponge mixed with green algae and dirty cream to brown snail shells, sand and debris when frozen; colour retained in ethanol; stains ethanol pale golden yellow; yellow pigment greasy.

Oscules: unobserved macroscopically in frozen and fixed material; also unobserved microscopically.

Texture: difficult to determine because of large amounts of debris in sponge mass; sponge soft, fragile, friable, granular, flaccid, limp, highly compressible, slowly resilient, spongy.

Surface ornamentation: even, lightly granular.

Ectosomal skeleton: indistinguishable from choanosome.

Choanosomal skeleton: lax, vague; rigid skeleton entirely absent; skeleton consists only of confused arrangement of interstitial microscleres scattered throughout mesohyl; microscleres sparse in patches, distributed singularly, concentrated in other regions, forming moderately dense carpet; collagen homogenous, slightly granular in appearance; occasional foreign spicules (oxeas, rods from ascidians) incorporated into mesohyl (Figs 5B–C).

Megascleres: nil.

Microscleres: single category of microrhabd; microrhabds as highly spined microxeas, robust, large, slightly curved, curvature irregular, tapering at ends, tips pointed, shaft covered with numerous, fine, short, conical spines, tips unspined; spines shorter than rhabd width, raised obviously from spicule shaft, arise perpendicular to axis; shaft straight, lacks torsion; dimensions 18.1–51.6 (37.5) × 2.2–4.4 (3.4) µm (Fig 5D).

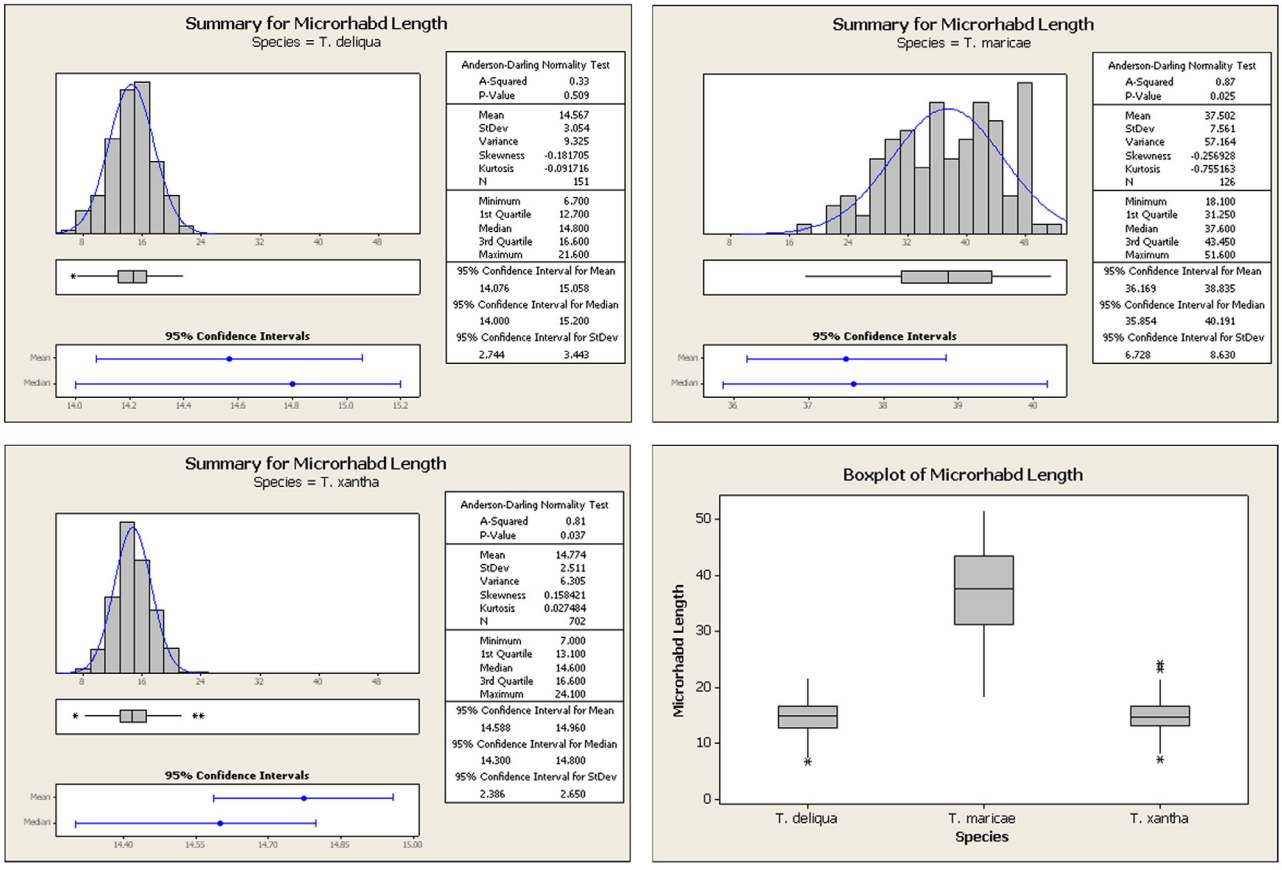

FIGURE 4. Summaries of statistics for sets of measurements of microrhabds from *Theonella deliqua* **n. sp.**, *Theonella maricae* **n. sp.** and *Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010) n. comb. The boxes for each species include the minimum, maximum, mean, standard deviation and variance. A boxplot has been used to demonstrate the distributions of the means of the measurements for each species and to indicate significant differences among them. Outliers are indicated by asterisks (*). The ranges of the measurements for *T. deliqua* and *T. xantha* can be seen to be approximately equivalent, and are normally distributed. Stronger skew and Kurtosis are shown for the measurements for the specimens of *T. maricae*. Most measurements of the microrhabd length for *T. maricae* greatly exceed those of the other two species, as illustrated in the boxplot.

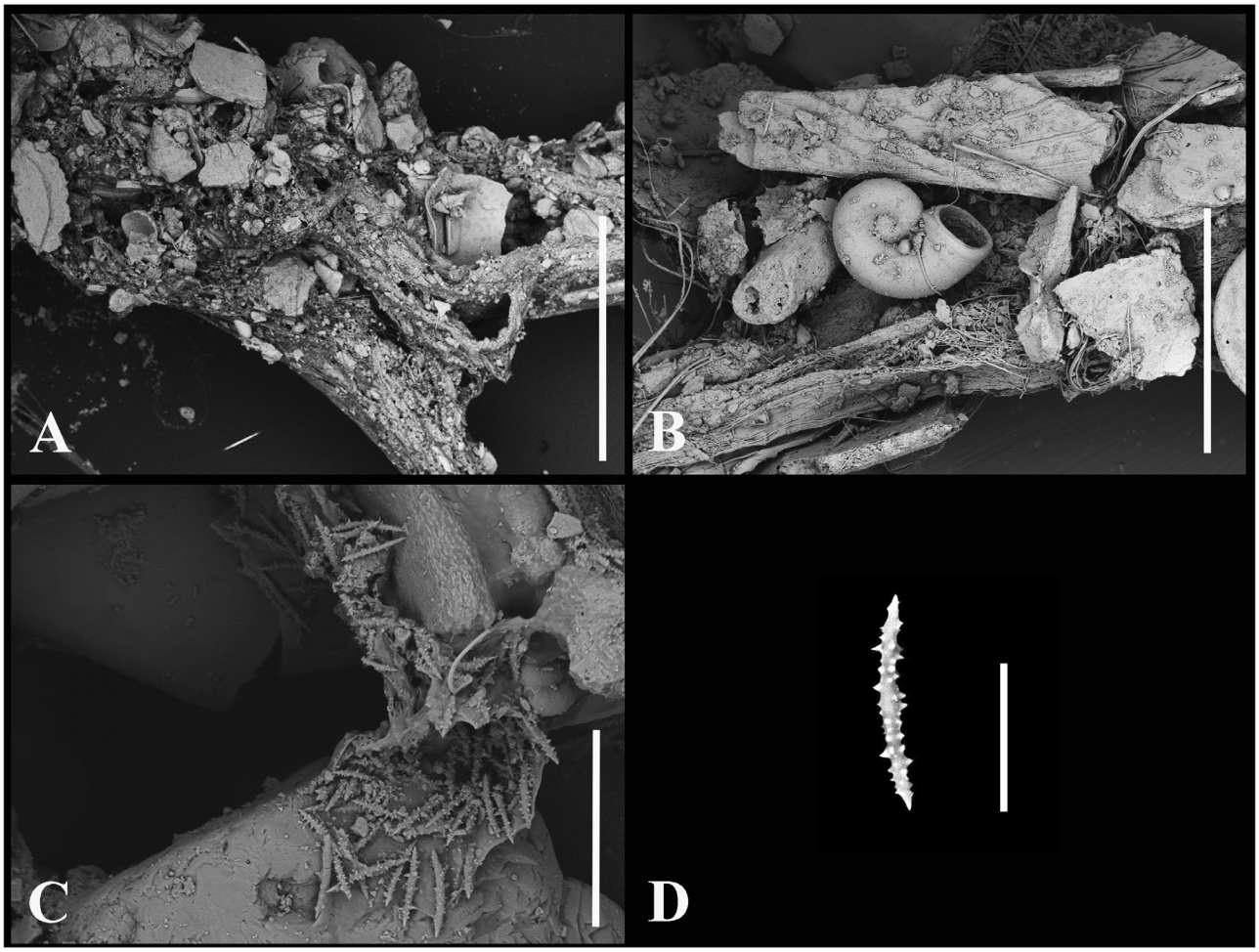

FIGURE 5. *Theonella maricae* **n. sp.** Micrographs (SEM) of holotype, QM G331427. A. Overview of a fragment of sponge, showing the incorporation of large amounts of organic and inorganic debris; scale bar = 1 mm. B. Detail view of sponge, showing thin sheets over debris, including filamentous algae, gastropod shells and other carbonates; scale bar = 500 µm. C. Detail of sheets, showing aggregation of microrhabds in an unstructured, random arrangement; scale bar = 100 µm. D. Single microrhabd; scale bar = 30 µm.

Etymology. This species is named for Mary Kay Harper, College of Pharmacy, University of Utah, who is a close and extensive collaborator on the chemistry of these sponges, and whose painstaking chemical and morphometric observations on theonellids are helping to uncover suites of cryptic species. The chemical complement that Ms Harper has found in specimens of *Theonella* from the western Pacific may be of taxonomic importance and we honour her contribution to sponge chemotaxonomy in naming this species for her.

DNA sequence data. 1 *COI* barcode sequence was obtained for the holotype (GenBank Accession: KJ494356; see Table 1); this sequence was 709 bp in length (including primers).

Ecology and distribution. The single specimen of *T. maricae* was found in the inter-reef region of the Great Barrier Reef, within the Capricorn Bunker group.

Remarks. We have, to date, found only one specimen of *T. maricae* in our collection. This species is difficult to isolate macroscopically, and it is likely that more specimens remain yet to be identified. Our examination of the holotype specimen shows that, like specimens of *T. deliqua*, large amounts of foreign sponge and non-sponge debris are incorporated into the structure of *T. maricae* (Figs 5A–B). Large amounts of filamentous algae, or possibly filamentous bacteria, can be observed within the overall mass of the holotype.

Measurements of the microrhabds of the holotype of *T. maricae* are in one class, with an average length of 37.5 µm and covering a range from 18.1 to 51.6 µm. This range follows a broadly normal distribution (Fig. 4), although it is skewed slightly towards the larger measurements, with the most frequent length approximately reaching 48.0 µm; the 95% confidence interval for the median measurement is 35.9–40.2 µm. Detailed examination of the range

of the spicules indicates that although the smallest recorded microrhabd measured 18.1 μm, the majority of the spicules is much larger, with the microrhabs typically exceeding 36 μm in length.

Comments. The description of *T. maricae* adds a second species which does not contain megascleres to *Theonella*. As with specimens of *T. deliqua*, the specimen of *T. maricae* is characterised largely by the absence of any tetractinal or monactinal structural megascleres; both species possess only microrhabds as the native spicule complement. The holotype of *T. maricae* is distinguished readily, however, from the specimens of *T. deliqua* by the size and shape of the microscleres. The microrhabds of *T. maricae* are typically at least twice as large as those of *T. deliqua* (38 μm *v.* 15 μm). The spines along the rhabd are small and blunt, measuring less than the width of the shaft; this is in contrast to the long and sharply pointed spines along the microrhabds of *T. deliqua.* The overall composition of *T. maricae* incorporates a variety of foreign debris and seafloor rubble, further distinguishing it from *T. deliqua*, which encrusts almost exclusively over the live and dead shells of a single species of *Tenagodus* gastropod.

Theonella xantha **(Sutcliffe, Pitcher & Hooper, 2010) n. comb.**

Figs 1, 4, 6

Dercitus xanthus Sutcliffe, Hooper & Pitcher, 2010, p. 6

Dercitus (*Stoeba*) *xanthus* Sutcliffe, Hooper & Pitcher, 2010; van Soest, Beglinger & de Voogd, 2010, p. 38 (subgenus reassignment); van Soest 2012c (online resource)

Material examined. *Holotype*: QM G329976 (=SBD513022), Australia, Great Barrier Reef, inter-reef sea floor, south-east of Rock Cod Shoal, 23.7249°S 151.665°E, 34.3 m (depth), coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Lady Basten*, 20.Sep.2004, epibenthic sled. *Paratypes*: QM G329977 (=SBD513042), Australia, Great Barrier Reef, inter-reef sea floor, west of Fairfax Island, 23.8849°S 152.105°E, 41.8 m (depth), coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Gwendoline May*, 13.Apr.2004, epibenthic sled; QM G329978 (=SBD505424), Australia, Great Barrier Reef, inter-reef sea floor, west of Old Reef, 19.4049°S 147.935°E, 42.0 m (depth), coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Lady Basten*, 27.Nov.2003, epibenthic sled.

Other material: QM G329095 (=SBD500449), Australia, Great Barrier Reef, inter-reef sea floor, east of Davies Reef, 18.8349°S 147.685°E, 62.9 m (depth), coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Lady Basten*, 22.Sep.2003, trawl; QM G329183 (=SBD517180), Australia, Great Barrier Reef, inter-reef sea floor, north-west of Devlin Reef, 11.805°S 143.825°E, 37.9 m (depth), coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Lady Basten*, 5.Feb.2005, trawl; QM G329186 (=SBD517310), Australia, Great Barrier Reef, inter-reef sea floor, north-west of Devlin Reef, 11.805°S 143.825°E, 34.7 m (depth), coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Lady Basten*, 4.Feb.2005, trawl; QM G329283 (=SBD537784), Australia, Great Barrier Reef, inter-reef sea floor, east of Gladstone, 23.8349°S 151.585°E, 26.9 m (depth), coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Lady Basten*, 14.Nov.2005, trawl; G331398 (=SBD500399), Australia, Great Barrier Reef, inter-reef sea floor, south-west of Little Broadhurst Reef, 19.045°S 147.3949°E, 14.9 m (depth), QM coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Lady Basten*, 21.Sep.2003, epibenthic sled; QM G331401 (=SBD500654), Australia, Great Barrier Reef, inter-reef sea floor, west of Big Broadhurst Reef, 18.925°S 147.525°E, 17.2 m (depth), coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Lady Basten*, 22.Sep.2003, epibenthic sled; QM G331411 (=SBD506498), Australia, Great Barrier Reef, inter-reef sea floor, south-west of Rudder Reef, 16.245°S 145.6149°E, 21.0 m (depth), coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Lady Basten*, 9.Oct.2003, epibenthic sled; QM G331426 (=SBD512852), Australia, Great Barrier Reef, inter-reef sea floor, south-west of Lamont Reef, 23.625°S 151.875°E, 27.3 m (depth), coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Lady Basten*, 21.Sep.2004, epibenthic sled; QM G331429 (=SBD513056),vAustralia, Great Barrier Reef, inter-reef sea floor, north-west of Tryon Island, 23.2249°S 151.7049°E, 28.0 m (depth), coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Lady Basten*, 22.Sep.2004, epibenthic sled; QM G331436 (=SBD513964), Australia, Great Barrier Reef, inter-reef sea floor, north-east of Magnetic Island, 18.995°S 147.095°E, 35.0 m (depth), coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Lady Basten*, 26.Apr.2004, epibenthic sled; G331463 (=SBD525255), Australia, Great Barrier Reef, inter-reef sea floor, east of Gladstone, 23.935°S

151.9333°E, 51.0 m (depth), QM coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Lady Basten*, 19.Sep.2004, epibenthic sled; QM G331662 (=SBD524169), Australia, Great Barrier Reef, inter-reef sea floor, north-east of Mumford Reef, 22.1549°S 150.385°E, 79.2 m (depth), coll. CSIRO Great Barrier Reef Seabed Biodiversity Project on *RV Lady Basten*, 9.May.2004, epibenthic sled; QM G331964, Australia, Great Barrier Reef, inter-reef sea floor, south-west of Polmaise Reef, 23.6383°S 151.5025°E, 26.0 m (depth), coll. Vicki Hall, Northern Fisheries, Cairns (former Department of Employment, Economic Development and Innovation, Queensland Government), 22.Nov.1999, epibenthic sled.

Redescription. Based on examination of holotype, 2 paratypes and 16 vouchers; all specimens post-fixed in ethanol (70%) after initial frozen storage.

Growth form and gross morphology: sponge consists of very thin sheets, thickness ~30 µm; sheets encrust over assorted non-specific substrates, cements a variety of unidentified broken gastropod shells, polychaete tubes, diatoms, broken coral debris into single mass; incorporates large amounts of quartz sand and debris, small amounts of filamentous algae; holotype mass measures ~ 4 × 5 × 3 cm (total mass dimensions) (Figs 1C, 6A–B)

Colour: unknown in life; dark orange to yellow portions of sponge mixed with green algae and dirty cream to brown sand and debris when frozen; colour retained in ethanol; stains ethanol dark golden yellow; yellow pigment greasy.

Oscules: unobserved macroscopically in frozen and fixed material; visible microscopically, few, inconspicuous, shallow, discrete, broadly elliptical, ~100 µm (diameter), distributed sparsely.

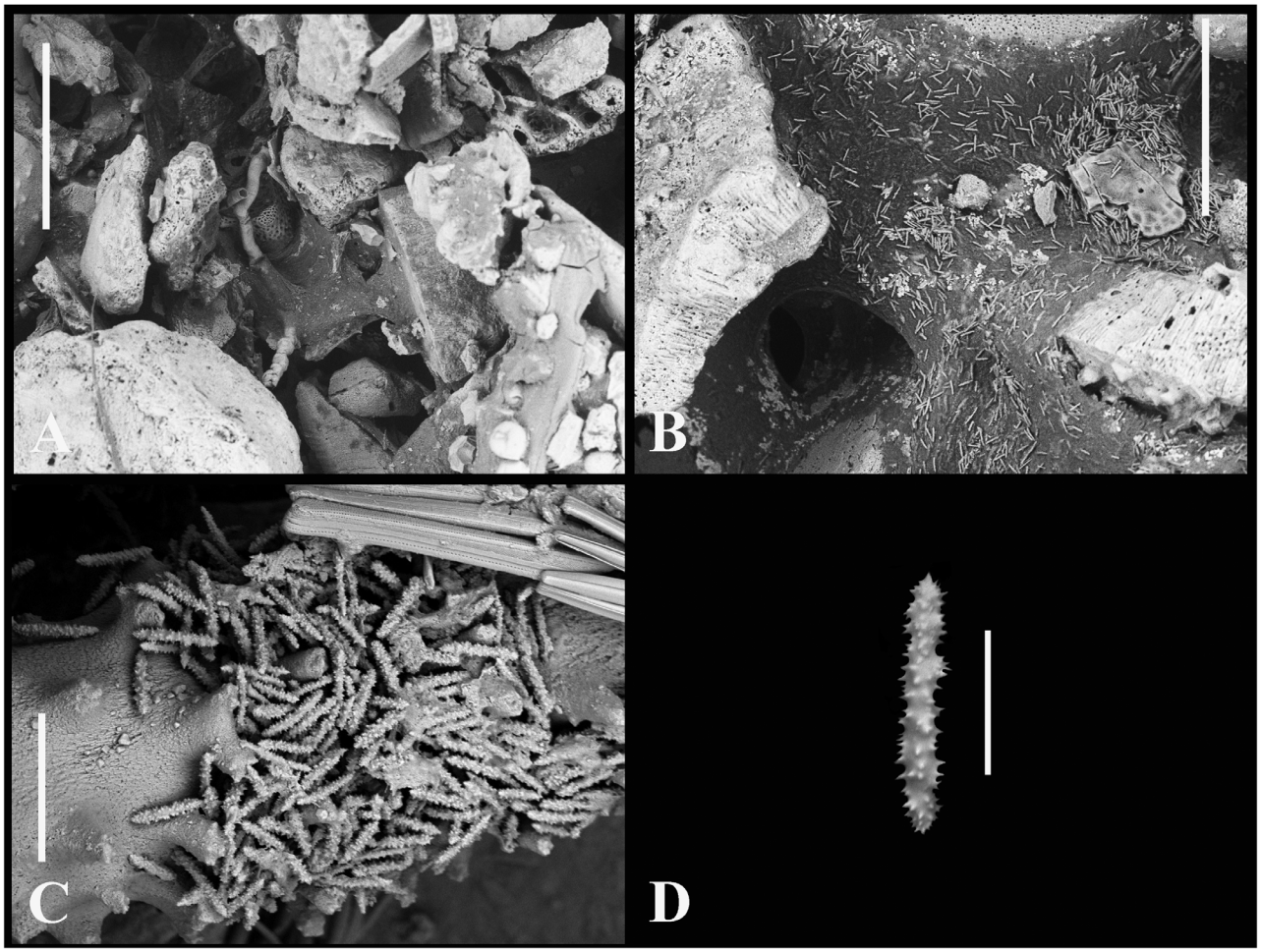

FIGURE 6. *Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010) n. comb. Micrographs (SEM) of paratype, QM G329978, and voucher specimen, QM G329095. A. QM G329095, overview of a fragment of sponge, showing large amounts of organic and inorganic debris, including coral rubble and empty polychaete tubes; scale bar = 500 µm. B. QM G329978, detail of sheets of sponge, showing oscules and carpets of microrhabds; scale bar = 200 µm. C. QM G329978, detail of accumulation of microrhabds *in situ*; scale bar = 30 µm. D. QM G329978, single microrhabd; scale bar = 10 µm.

Texture: difficult to determine because of large amounts of debris in sponge mass; mass friable, fragile; sponge soft, very fragile, friable, granular, flaccid, limp, highly compressible, slowly resilient, spongy.

Surface ornamentation: even, smooth.

Ectosomal skeleton: indistinguishable from choanosome.

Choanosomal skeleton: lax, vague; rigid skeleton entirely absent; skeleton consists only of confused arrangement of interstitial microscleres scattered throughout mesohyl; microscleres sparse in patches, distributed singularly, concentrated in other regions, sometimes forming very dense carpet; collagen homogenous; occasional foreign megascleres (oxeas, regular triacts) incorporated into mesohyl (Figs 6B–C).

Megascleres: nil.

Microscleres: single category of microrhabd; microrhabds as highly spined microxeas, small, isodiametric, robust, generally straight but rarely slightly curved, curvature irregular, tips rounded, rhabd covered with profuse, small, blunt, conical spines; spines shorter than rhabd width, raised obviously from spicule shaft, arise perpendicular to axis; shaft straight, lacks torsion; dimensions 8.1–21.5 (14.7) × 1.3–2.9 (2.2) μm (Fig. 6D).

DNA sequence data. 15 *COI* barcode sequences were obtained for specimens of *T. xantha*, including the holotype and both paratypes (GenBank Accession: KJ494361–KJ494375; see Table 1); each of these sequences was 709 bp in length (including primers), except 4 which were shorter (KJ494367: 597 bp; KJ494365 & KJ494369: 631 bp; KJ494361: 634 bp (including primers)).

Ecology and distribution. Specimens of *T. xantha* have, to date, been found associated with the seabed only in the inter-reef areas of the Great Barrier Reef. Sutcliffe *et al*. (2010) draw attention to enormous biomass that specimens of *T. xantha* represent; they are distributed widely across the entire span of the Great Barrier Reef, extending from regions of low to high latitude, and are found in high densities in the inter-reef area. Sutcliffe *et al.* (2010) did not find any major correlation between the presence or prevalence of *T. xantha* and the composition of the underlying substrate, although specimens were not recovered commonly in areas with a high proportion of mud in the sediment.

Remarks. We re-examined the holotype and both paratypes, in addition to 16 vouchers, of *T. xantha* using SEM and light microscopy. In no specimen were we able to observe any native megascleres; all specimens were found to contain only small, microspined microrhabds. The samples were morphologically homogeneous, with large amounts of debris incorporated into the structure of all specimens, including non-active polychaete tubes and shells, fragments of diatoms, and coralline and siliceous rubble. Small amounts of filamentous algae (or bacteria) were incorporated into the mass also.

The measurements of the microrhabds were consistent among the samples we examined. The average microrhabd length was 14.8 μm (range 8.1 to 21.5 μm); three outlier measurements were detected (7.0 μm, 23.2 μm and 24.1 μm). The lengths fitted a normal distribution, which was not skewed appreciably. The median spicule length was 14.6 μm; there were relatively few spicules which measured less than 13.1 μm. The majority of microrhabds reached lengths of between 13 and 17 μm.

Comments. This species was attributed initially to *Dercitus* Gray, 1867 by Sutcliffe *et al*. (2010) based on their interpretation of the morphology of this species as comprising sanidasters and three-rayed calthrops (calthrops reported in 20% of their samples). Van Soest *et al.* (2010) and van Soest (2012c) classify *D. xanthus* within the subgenus *Dercitus* (*Stoeba*) Sollas, 1888. We have been unable to replicate the sighting of any native calthrops in the holotype or paratypes, nor in any other specimens we examined. We can confirm the common occurrence of broken calthrops distributed sporadically in several of the samples we investigated, however, in no specimen could these be interpreted as native; indeed, in one specimen of *T. deliqua*, dense rafts of non-native broken calthrops were found aggregated in portions of the sponge mass of this species also (as noted above). The geometry of regular calthrops and triods and the thickness of the rays of these megascleres may make these particular spicule morphologies exceptionally robust; the tumbled edges, however, support their foreign origins. The absence of calthrops, and the interpretation of the microscleres as microrhabds, rather than sanidasters, renders the placement of this species within *Dercitus* unjustified. We interpret the morphology of this species as being consistent with other megasclere-lacking species of *Theonella*, and this interpretation is supported by DNA-based studies (see below); based on these data, we designate this species within *Theonella*, as *T. xantha* (Sutcliffe, Hooper and Pitcher, 2010) n. comb.

Morphologically, specimens of *T. xantha* are very similar to those of *T. deliqua* and *T. maricae*, however, they may be distinguished by the shape of the microrhabds and ecological characteristics. Specimens of *T. xantha* are recognisable immediately from those of *T. maricae* by the size of the microrhabds; the spicules of *T. maricae* are

more than twice as long as those of *T. xantha*. Discrimination between *T. xantha* and *T. deliqua* is subtler; boxplots comparing the microrhabd lengths (Fig. 4) show that the range of lengths of the microscleres of both species are broadly equivalent. The microrhabds of *T. xantha*, however, are more robust in appearance than those seen in *T. deliqua*. The spines along the shaft of the microrhabds of *T. xantha* are bluntly conical and generally shorter than the width of the rhabd. Contrastingly, the microrhabds of *T. deliqua* are less robust in appearance, being slender and bearing sharply pointed spines, which are longer than the length of the underlying rhabd. Structurally, *T. xantha*, like *T. maricae*, consolidates the seabed substrates and cements a variety of rubble types, however, these two species can be distinguished from *T. deliqua* by this characteristic, which contrasts the aggregation of only one species of *Tenagodus* shell by specimens of *T. deliqua.*

Key to those species of *Theonella* Gray, 1868, which lack desmas and other megascleres

1a. Encrusting species, forms thin sheets which cement detritus; desmas absent; megascleres absent; microrhabds very small, ~15 µm in length (ranging from 7 to 21 µm) . . . . . 2.

1b. Encrusting species, forms thin sheets which cement detritus; desmas absent; megascleres absent; microrhabds large, conspicuous, ~38 µm in length (ranging from 18 to 52 µm) . . . . . *T. maricae* **n. sp.**

2a. Thin sheets cement exclusively *Tenagodus* shells into discrete clumps; microrhabds slender, usually curved, sharply hastate at ends, spines conspicuous, long, sharp . . . . . *T. deliqua* **n. sp.**

2b. Thin sheets cement a variety of detritus, including quartz sand, foraminifera, algae and coral fragments; microrhabds robust, generally straight, rounded at ends, spines small, blunt, numerous . . . . *T. xantha* (Sutcliffe, Hooper & Pitcher, 2010) n. comb.

Systematics

Phylogenetic analysis

The assembled DNA sequences of the partial fragment of the COI mtDNA genes resulted in an alignment which was 658 bp in total length. Likelihood (ML) and Bayesian inference (BI) analyses were performed on this alignment. Although the Bayesian analysis was run through over 1.5×10^6 generations, the average deviation of split frequencies did not converge (<0.01). At the completion of the analysis, however, the potential scale reduction factors (PSRF) of all parameters approached 1.0 (ranging from 1.008 to 1.178), indicating that convergence had been reached; indeed, for the last 5×10^5 generations of the run, the deviations of the split frequencies hovered around 0.07–0.08. The high average deviations of the split frequencies may be reflected by the lack of resolution of the basal branches on the trees which were inferred. We repeated this analysis, removing sequences of species of another "lithistid" family (2 specimens of *Desmanthus incrustans* (Topsent, 1889), Desmanthidae), however, after another 1.5×10^6 generations, convergence was still not reached. At the termination of this run, the average deviation of split frequencies was 0.016, and the average deviation which had remained in this vicinity (0.017–0.016) for the final 3×10^5 generations. Again, the PSRF of all parameters approached or reached 1.0 (ranging from 1.0 to 1.006), and so we determined that the run had reached convergence, despite the relatively high deviation of split frequencies.

Figure 7 presents a gene tree of the relationships among the taxa included in this study, without the inclusion of the specimens of *Desmanthus*, as hypothesised in the second analysis (ML bootstraps (BS) and BI posterior probabilities (PP) are indicated at the nodes). Neither method of analysis was able to recover high levels of support for the basal branches of the Astrophorida. Resolution of deep nodes has been long acknowledged as difficult using COI mtDNA data alone (*e.g.* Erpenbeck *et al.* 2007), although more recent relationships are recovered with greater confidence. The sequences from all of the new species of *Theonella* described here can be found within a monophyletic assemblage comprising all of the specimens of *Theonella* available (BS 93%; PP 1.0); all of the specimens of *T. xantha* form a monophyletic group (BS 73%), which is a sister to a single specimen of *T. cf. cylindrica* (BS 74%; PP 0.98). The two specimens of *T. cf. cupola* are monophyletic (BS 75%; PP 0.75), and these specimens are the sister (BS 86%; PP 0.99) to a monophyletic assemblage comprising the specimens of *T. deliqua*, one specimen of *T. cf. cylindrica*, *T. cf. swinhoei* and *T. swinhoei* (BS 81%; PP 0.88). The position of *T. maricae* was resolved as basal within *Theonella*, and *T. xantha* is the sister to the other specimens of *Theonella* (except *T. maricae*) (BS 53%; PP 0.93). In none of our analyses is *T. xantha* recovered in a position near to *Dercitus bucklandi* (Bowerbank, 1858) (which is the type-species for *Dercitus*).

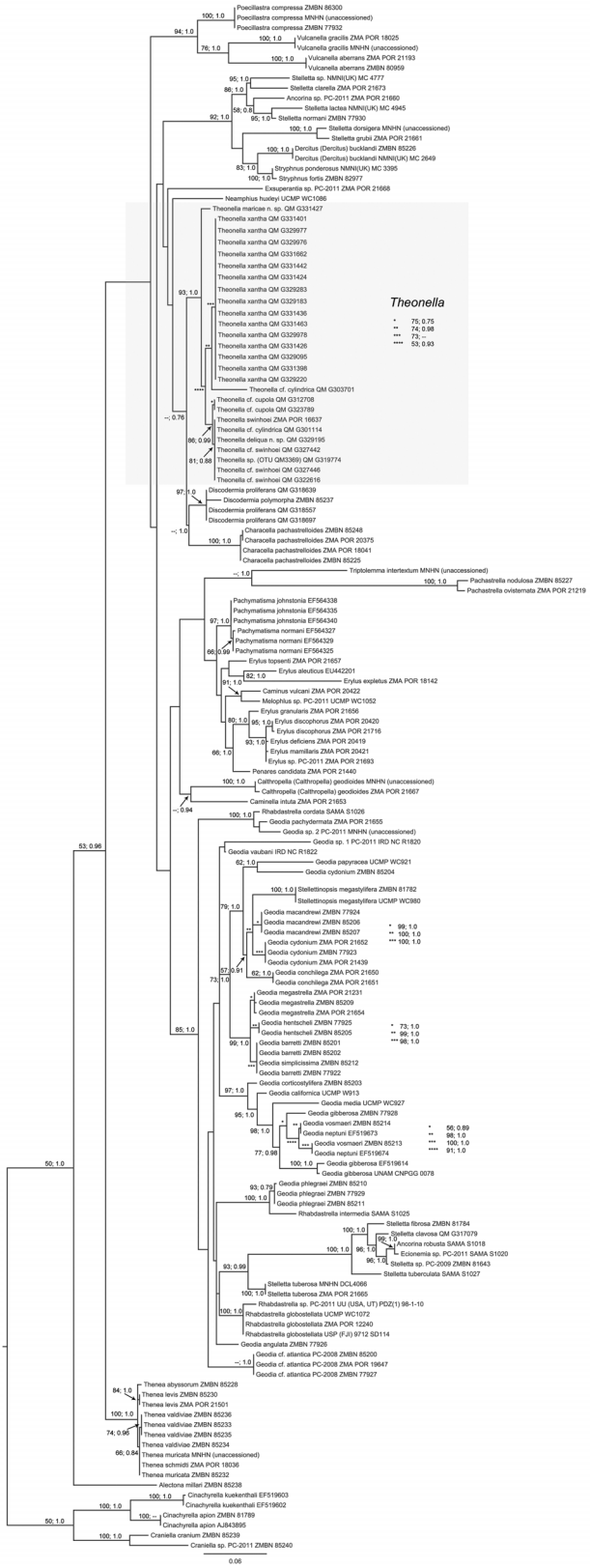

FIGURE 7. Phylogenetic tree of astrophorid relationships. Tree shown is the best tree from the maximum likelihood search. Figures at nodes indicate bootstrap proportion (BS) followed by Bayesian posterior probabilities (PP), shown as "BS;PP"; where a branch was not present in or unsupported by one of the two analyses, the value given is "--". Only ML bootstrap values and Bayesian PPs >50% or 0.5 respectively are shown. Nucleotide substitution rate indicated by scale bar (scale = 0.2 subsitutions/site). Leaves are labelled with the name of the taxon and the corresponding museum specimen registration number (where available); if no voucher specimen is deposited in a registered collection, the GenBank accession number is used to denote the sequence.

Although both analyses support monophyly of the specimens of *Theonella*, monophyly of the Theonellidae is not corroborated by the ML analysis; ML is unable to resolve the position of *Theonella* and *Discodermia* within the Astrophorida. Likelihood and Bayesian analyses do support the monophyly of the specimens of *Discodermia* (BS 97%; PP 1.0). The monophyletic specimens of *Discodermia* are shown in an unresolved, but supported monophyletic group (PP 0.76) comprising *Theonella*, *Discodermia* and *Characella* (Pachastrellidae). The position of *Characella* close to the theonellids is noteworthy, and if "real", may be supported morphologically by the shared presence of dichotriaenes and acanthose microrhabds (see Maldonado 1996; Cárdenas and Rapp 2012); there is very limited support in our analyses for this relationship, however, and it is important not to over-interpret any morphology on the basis of these trees alone.

Despite the absence of strong support at the basal nodes of the trees, our analyses indicate that the three species of megasclere-less theonellids that we present here are supported convincingly within a monophyletic assemblage comprising other desma- and triaene-bearing species of *Theonella*. The relationship of a monophyletic *Theonella* to other known theonellids (such as *Discodermia*) is supported, although equivocally, and a relationship to other triaene-bearing astrophorids (such as *Characella*) is intimated. There is no support for the monophyly of the Pachastrellidae, and no support for the position of the three megasclere-less species of *Theonella* close to *D. bucklandi*. The topology and support for the nodes of our trees underpin the taxonomic decision to include these species of microrhabd-only-bearing species within *Theonella*.

Chemotaxonomy

Aurantosides, a group of tetramic acid glycosides, are found characteristically within specimens of theonellids from the Indo-West Pacific (see Matsunaga *et al*. 1991; Wolf *et al*. 1999; Sata *et al.* 1999a; Ratnayake *et al*. 2005; Angawi *et al*. 2011); a single aurantoside has been identified from a non-theonellid sponge (which was identified only to genus). These compounds were named initially for the golden colour of the pigment from which they were isolated (Matsunaga *et al*. 1991), and are indicated commonly as a yellow oil which is soluble in ethanol. We have observed yellow pigmentation in all three species of *Theonella* considered here, and note that this pigment is soluble in ethanol and appears greasy. Compounds, with mass spectrometric readings matching those of aurantosides, have been identified in this pigment-containing ethanol fraction (Mary Kay Harper, University of Utah, *pers. comm.*). The presence of aurantosides in all of the specimens examined in this study provides further circumstantial corroboration that these specimens are best attributed as species of *Theonella*. Ongoing studies to isolate the compounds and validate their identity as aurantosides are currently in progress and supporting evidence will be presented in a subsequent work (Hall, Harper, Ekins, Ireland & Hooper, in preparation).

Biogeography

Specimens of each of *T. deliqua*, *T. maricae* and *T. xantha* were all recovered from the seafloor of the inter-reef regions of the Great Barrier Reef. Only a few specimens of both *T. deliqua* and *T. maricae* were recovered during this study, and consequently no conclusions about the distributions of these species can be drawn. Sutcliffe *et al*. (2010) discusses in depth the environmental and physical correlates which may influence the distribution of *T. xantha* along the Great Barrier Reef, concluding that this species is very abundant and widespread, although it is not commonly found in geographical regions with substrates containing a high level of muddy silt.

Ecology

The association between siliquariid molluscs and sponges has been known since Aristolean times (Bieler 2004), however, only relatively recently has the nature of this association been characterised. Pansini *et al*. (1999) articulated the interaction between sponges and siliquariids as "facultative mutualism", in as much as the sponge does not require the presence of siliquariids for its survival. The relationship for the siliquariids, however, is obligate; siliquariids are adapted entirely for life inside sponges and most are found only in association with them

(Bieler 2004); specifically, only the slit-shell siliquariids, members of *Tenagodus* and *Petalopoma* Schiaparelli, 2002, are known as obligate infauna of sponges (Schiaparelli 2002). In their major review of siliquariid molluscs associated with sponges, Pansini *et al.* (1999) recorded 35 sponge species as acting as hosts to siliquariids. Their study did not demonstrate strict sponge-specificity on the part of species of *Tenagodus*, although only a restricted group of sponges was found to act as hosts. Pansini *et al*. suggested that the skeletal structure of the sponge host may influence its suitability as a host; siliquariids apparently prefer hosts with dense spiculation or a radial skeleton, which forms a rigid or compact skeleton.

Of the 29 tabled literature records of live interactions in their review, Pansini *et al*. (1999) listed 14 instances where the sponge host was identified to genus at least; of these, 11 of the sponges were astrophorids, and of these, three were members of Theonellidae (*viz.* S*iliquariaspongia japonica*, and two records of *Racodiscula sceptrellifera* (Carter, 1881)). Pansini *et al.* also tabled 20 new observations of sponge-siliquariid interaction: these associations were restricted to astrophorid and halichondriid sponges only, with a single theonellid interaction recorded (with a species attributed to *Discodermia cf. laevidiscus* Carter, 1880). An additional astrophorid interaction not noted by Pansini *et al*., is that described by Hartman & Hubbard (1999), in which *Thrombus sphaeroidocladus* Hartman and Hubbard, 1999 formed enormous mounds, 2 metres in diameter, in association with *Tenagodus modestus* (Dall, 1881) (as *Siliquaria modesta* Dall).

We have found, to date, only two specimens of *T. deliqua*; both of these have been in association with the same unidentified species of *Tenagodus*. Unlike the massive mounds created by the association between *Thr. sphaeroidocladus* and *Te. modestus*, the association between *T. deliqua* and its siliquariid produces much smaller clumps, which are, at most, approximately grapefruit-sized. It should be noted, however, that the collecting of these samples was completed via epibenthic sled, and it is likely that the specimens recovered represent only portions of the original aggregation. It may be possible that, like with other siliquariid-sponge associations, the relationship between *T. deliqua* and the *Tenagodus* sp. may be one of facultative mutualism.

Concluding remarks

Morphological systematics. The three species *T. deliqua*, *T. maricae* and *T. xantha* form a morphologically consistent group, which is distinguished immediately from *T. swinhoei* and the other previously described members of *Theonella* by the absence of any structural megascleres. On the phylogenetic tree which we present, monophyly of the megasclere-less theonellids, however, is not recovered. This suggests that the tetractinal spicules and larger monactinal spicules have been lost on multiple occasions within *Theonella*. The loss of these spicules is remarkable. It seems unusual that these species would lose all structural spicules, however, it could be that this loss is a relatively simple step to achieve in terms of evolution. We speculate that the switching off of genes controlling the production of megascleres may be acquired through a single (or very few) mutation, and may serve an adaptive function, by facilitating the colonisation of new habitats. The specialisation of these three species as seabed rubble-dwellers may be related to the loss of the structural megascleres; in losing the rigid desma skeleton and other large spicules, these species are able to penetrate into the interstitial spaces of the rubble of the seafloor. Further, the aggregation and accretion of seabed material into the structure of these sponges may function analogously to megascleres, with the environmental carbonate (coral fragments, shells, polychaete tubes, foreign non-poriferan spicules), quartz (sand) and silica (foreign sponge spicules, diatoms) elements functioning to provide integrity to the sponge structure.

Although none of the specimens of *T. deliqua*, *T. maricae* or *T. xantha* was found to contain any native megascleres, each of the species is marked by the possession of only profuse, small, straight-shafted microrhabds. Further, these microrhabds completely lack any torsion along the central axis of the microsclere. The straightness of the microsclere is noteworthy because it is in contrast with the morphology of the streptasters and sanidasters described for other astrophorids, especially those of species of *Dercitus*, to which *T. xantha* was attributed initially. The position on our phylogenetic tree of the specimens of *Theonella* in a clade well separated from that of the specimens of *D. bucklandi*, which bears true streptasters, supports the concept of torsion along the central axis of the rhabd as being of taxonomic significance among the Astrophorida.

Early molecular studies of the Astrophorida (Chombard *et al.* 1998), based on 28S rDNA genes, indicated that although streptasters appeared early within the Astrophorida, they were lost subsequently in higher groups on the

tree, such as the Geodiidae and Calthropellidae. Although branch support for deeper relationships is limited, our phylogenetic trees are in contrast with that presented by Chombard *et al*. (1998), indicating that clades bearing streptasters (*e.g.* Pachastrellidae, except *Ch. pachastrelloides*) are derived within Astrophorida; further, Theonellidae, which lacks euasters and streptasters, is basally positioned within Astrophorida. It could be that the evolution of twisted microscleres (such as streptasters and sanidasters) occurred subsequent to the divergence of the theonellid assemblage, and that torsion along the central axis of the monactinal microscleres occurs only in groups sharing the common ancestor which separated from the lineage persisting as Theonellidae. The emergence of twisting along the central axis may have been a profound ontogenetic step in the evolution of astrophorids, an adaptive step which facilitated diversification of the group.

Cárdenas *et al*. (2011) demonstrates inferred high levels of homoplasy among the megascleres and microscleres of astrophorids, concluding that spicule morphology is not a good taxonomic indicator for the group. The phylogeny of Cárdenas *et al*., however, like that which we present here, lacks strong support for the deeper relationships among Astrophorida; and inferring homoplasy in the absence of strong support is difficult. Cárdenas *et al.* presents likelihood estimates of the origins of spicule types, however, character transformations argued from the poorly supported basal relationships should be circumspect; if the basal relationships are not well established, any ontogenetic inferences made on this basis would also lack strong support. The study of Cárdenas *et al.* is important because it explores the evolution of spicule morphology, however, we suggest that without establishment of the basal relationships, the inference of high levels of homoplasy may be a challenge to justify. Further, in any interpretations of homoplasy, it is important to distinguish between "real" homoplasy (such as character loss) and primary homology assessments which are inadequate to account for the evolutionary history of the character. Secondary homology assessments may establish that characters hitherto assumed homologous are, in fact, merely analogous. Kelly *et al*. (1999) drew attention to the importance of the reassessment of character homology in the light of molecularly-based phylogenetic hypotheses (the process of "reciprocal illumination"), and predicted that not only spicule shape, but also patterns of spicule ornamentation, would be instrumental to future studies of lithistid taxonomy. We suggest that the twisting of the monactinal microsclere, as in streptasters and other streptoscleres, is taxonomically important, and difficult to acquire ontogenetically. Following the principle of parsimony, complicated morphologies, such as torsion, are likely therefore to have evolved relatively few times. Further testing of this hypothesis is required, using additional gene regions and increased taxon sampling. A relatively basal position of Theonellidae is recovered in our trees and in those of Cárdenas *et al*. (2011), however, and this, coupled with the absence of twisted microscleres in these animals, suggests that the presence of twisted microscleres may be a derived condition; their absence may be ancestral, rather than homoplasious.

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TABLE 1. List of taxa used in the phylogenetic analysis, museum registration numbers (and author vouchers) where available, GenBank accession numbers and references for the original publication of sequence data; sequences generated newly for this study are indicated in bold type.

Taxon				Museum Registration/Voucher Number	GenBank Accession Number	Reference
Order	Family	Subfamily	Species			
"Lithistida"	Desmanthidae		***Desmanthus incrustans* (Topsent, 1889)**	**QM G303437**	**KJ494345**	**this study**
			***Desmanthus incrustans* (Topsent, 1889)**	**QM G313573**	**KJ494346**	**this study**
	Phymaraphiniidae		*Exsuperantia* sp. PC-2011	ZMA POR 21668	HM592730	Cárdenas *et al.* (2011)
Astrophorida	Ancorinidae		*Ancorina robusta* (Carter, 1883)[α]	SAMA S1018	HM592724	Cárdenas*et al.* (2011)
			Ancorina sp. PC-2011	ZMA POR 21660	HM592744	Cárdenas *et al.* (2011)
			Dercitus (*Dercitus*) *bucklandi* (Bowerbank, 1858)[β]	NMNI (UK) MC 2649	HM592674	Cárdenas *et al.* (2011)
			Dercitus (*Dercitus*) *bucklandi* (Bowerbank, 1858) [β]	ZMBN 85226	HM592716	Cárdenas *et al.* (2011)
			Ecionemia sp. PC-2011	SAMA S1020	HM592725	Cárdenas *et al.* (2011)
			Rhabdastrella cordata Wiedenmayer, 1989	SAMA S1026	HM592727	Cárdenas *et al.* (2011)
			Rhabdastrella globostellata (Carter, 1883)	USP (FJI) 9712 SD114	HM592673	Cárdenas *et al.* (2011)
			Rhabdastrella globostellata (Carter, 1883)	UCMP WC1072	HM592683	Cárdenas *et al.* (2011)
			Rhabdastrella globostellata (Carter, 1883)	ZMA POR 12240	HM592746	Cárdenas *et al.* (2011)
			Rhabdastrella intermedia Wiedenmayer, 1989	SAMA S1025	HM592726	Cárdenas *et al.* (2011)
			Rhabdastrella sp. PC-2011	UU (USA, UT) PDZ(1) 98-1-10	HM592676	Cárdenas *et al.* (2011)
			Stelletta clarella de Laubenfels, 1930	ZMA POR 21673	HM592736	Cárdenas *et al.* (2011)
			***Stelletta clavosa* Ridley, 1884**	**QM G317079**	**KJ494350**	**this study**
			Stelletta dorsigera Schmidt, 1864	MNHN (unaccessioned)	HM592750	Cárdenas *et al.* (2011)
			Stelletta fibrosa (Schmidt, 1870)	ZMBN 81784	FJ711643	Cárdenas *et al.* (2009)
			Stelletta grubii Schmidt, 1862	ZMA POR 21661	HM592743	Cárdenas *et al.* (2011)
			Stelletta lactea Carter, 1871	NMNI(UK) MC 4945	HM592752	Cárdenas *et al.* (2011)
			Stelletta normani Sollas, 1880	ZMBN 77930	EU442193	Cárdenas *et al.* (2010)
			Stelletta sp.	NMNI(UK) MC 4777	HM592751	Cárdenas *et al.* (2011)
			Stelletta sp. PC-2009	ZMBN 81643	FJ711644	Cárdenas *et al.* (2009)
			Stelletta tuberculata (Carter, 1886)			

……*continued on the next page*

TABLE 1. (Continued)

Order	Family	Subfamily	Species	Museum Registration/Voucher Number	GenBank Accession Number	Reference
			Stelletta tuberosa (Topsent, 1892)	MNHN DCL4066	HM592678	Cárdenas *et al.* (2011)
			Stelletta tuberosa (Topsent, 1892)	ZMA POR 21665	HM592735	Cárdenas *et al.* (2011)
			Stellettinopsis megastylifera (Wintermann-Kilian and Kilian, 1984)[γ]	UCMP WC980	AY561980	Nichols (2005)
			Stellettinopsis megastylifera (Wintermann-Kilian and Kilian, 1984)[γ]	ZMBN 81782	FJ711642	Cárdenas *et al.* (2009)
			Stryphnus fortis (Vosmaer, 1885)	ZMBN 82977	HM592697	Cárdenas *et al.* (2011)
			Stryphnus ponderosus (Bowerbank, 1866)	NMNI (UK) MC 3395	HM592685	Cárdenas *et al.* (2011)
	Calthropellidae		*Calthropella* (*Calthropella*) *geodioides* (Carter, 1876)[δ]	MNHN (unaccessioned)	HM592705	Cárdenas *et al.* (2011)
			Calthropella (*Calthropella*) *geodioides* (Carter, 1876)[δ]	ZMA POR 21667	HM592734	Cárdenas *et al.* (2011)
	Geodiidae	Erylinae	*Caminella intuta* (Topsent, 1892)	ZMA POR 21653	HM592740	Cárdenas *et al.* (2011)
			Caminus vulcani Schmidt, 1862	ZMA POR 20422	EU442205	Cárdenas *et al.* (2010)
			Erylus aleuticus Lehnert, Stone and Heimler, 2006		EU442201	Cárdenas *et al.* (2010)
			Erylus deficiens Topsent, 1927	ZMA POR 20419	EU442204	Cárdenas *et al.* (2010)
			Erylus discophorus (Schmidt, 1862)	ZMA POR 20420	EU442206	Cárdenas *et al.* (2010)
			Erylus discophorus (Schmidt, 1862)	ZMA POR 21716	HM592692	Cárdenas *et al.* (2011)
			Erylus expletus Topsent, 1927	ZMA POR 18142	EU442208	Cárdenas *et al.* (2010)
			Erylus granularis Topsent, 1904	ZMA POR 21656	HM592729	Cárdenas *et al.* (2011)
			Erylus mamillaris (Schmidt, 1862)	ZMA POR 20421	EU442207	Cárdenas *et al.* (2010)
			Erylus sp. PC-2011	ZMA POR 21693	HM592687	Cárdenas *et al.* (2011)
			Erylus topsenti Lendenfeld, 1903	ZMA POR 21657	HM592733	Cárdenas *et al.* (2011)
			Melophlus sp. PC-2011	UCMP WC1052	HM592688	Cárdenas *et al.* (2011)
			Pachymatisma johnstonia (Bowerbank in Johnston, 1842)	PC89	EF564335	Cárdenas *et al.* (2007)
			Pachymatisma johnstonia (Bowerbank in Johnston, 1842)	PC170	EF564338	Cárdenas *et al.* (2007)
			Pachymatisma johnstonia (Bowerbank in Johnston, 1842)	PC174	EF564340	Cárdenas *et al.* (2007)
			Pachymatisma normani Sollas, 1888	PC11	EF564325	Cárdenas *et al.* (2007)
			Pachymatisma normani Sollas, 1888	PC105	EF564327	Cárdenas *et al.* (2007)

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TABLE 1. (Continued)

Order	Family	Subfamily	Species	Museum Registration/Voucher Number	GenBank Accession Number	Reference
			Pachymatisma normani Sollas, 1888	PC145	EF564329	Cárdenas *et al.* (2007)
			Penares candidata (Schmidt, 1868)	ZMA POR 21440	HM592719	Cárdenas *et al.* (2011)
		Geodiinae	*Geodia angulata* (Lendenfeld, 1910)	ZMBN 77926	EU442203	Cárdenas *et al.* (2010)
			Geodia barretti Bowerbank, 1858	ZMBN 77922	EU442194	Cárdenas *et al.* (2010)
			Geodia barretti Bowerbank, 1858	ZMBN 85201	HM592684	Cárdenas *et al.* (2011)
			Geodia barretti Bowerbank, 1858	ZMBN 85202	HM592720	Cárdenas *et al.* (2011)
			Geodia californica (Lendenfeld, 1910)	UCMP W913	EU442200	Cárdenas *et al.* (2010)
			Geodia cf. atlantica (Stephens, 1915) PC-2008	ZMBN 77927	EU442195	Cárdenas *et al.* (2010)
			Geodia cf. atlantica (Stephens, 1915) PC-2008	ZMA POR 19647	HM592679	Cárdenas *et al.* (2011)
			Geodia cf. atlantica (Stephens, 1915) PC-2008	ZMBN 85200	HM592695	Cárdenas *et al.* (2011)
			Geodia conchilega Schmidt, 1862	ZMA POR 21650	HM592739	Cárdenas *et al.* (2011)
			Geodia conchilega Schmidt, 1862	ZMA POR 21651	HM592742	Cárdenas *et al.* (2011)
			Geodia corticostylifera Hadju, Muricy, Custodio, Russo and Peixinho, 1992	ZMBN 85203	HM592681	Cárdenas *et al.* (2011)
			Geodia cydonium (Jameson, 1811)	ZMBN 77923	EU442199	Cárdenas *et al.* (2010)
			Geodia cydonium (Jameson, 1811)	ZMA POR 21439	HM592693	Cárdenas *et al.* (2011)
			Geodia cydonium (Jameson, 1811)	ZMBN 85204	HM592715	Cárdenas *et al.* (2011)
			Geodia cydonium (Jameson, 1811)	ZMA POR 21652	HM592738	Cárdenas *et al.* (2011)
			Geodia gibberosa Lamarck, 1815	B27	EF519614	Erpenbeck *et al.* (2008)
			Geodia gibberosa Lamarck, 1815	ZMBN 77928	EU442209	Cárdenas *et al.* (2010)
			Geodia gibberosa Lamarck, 1815	UNAM CNPGG 0078	HM592723	Cárdenas *et al.* (2011)
			Geodia hentscheli Cárdenas, Rapp, Schander and Tendal, 2010	ZMBN 77925	EU442197	Cárdenas *et al.* (2010)
			Geodia hentscheli Cárdenas, Rapp, Schander and Tendal, 2010	ZMBN 85205	HM592671	Cárdenas *et al.* (2011)
			Geodia macandrewi Bowerbank, 1858	ZMBN 77924	EU442198	Cárdenas *et al.* (2010)
			Geodia macandrewi Bowerbank, 1858	ZMBN 85206	HM592689	Cárdenas *et al.* (2011)
			Geodia macandrewi Bowerbank, 1858	ZMBN 85207	HM592696	Cárdenas *et al.* (2011)
			Geodia media Bowerbank, 1973	UCMP WC927	AY561962	Nichols (2005)

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TABLE 1. (Continued)

Order	Family	Subfamily	Species	Museum Registration/Voucher Number	GenBank Accession Number	Reference
			Geodia megastrella Carter, 1876	ZMBN 85209	HM592721	Cárdenas *et al.* (2011)
			Geodia megastrella Carter, 1876	ZMA POR 21654	HM592731	Cárdenas *et al.* (2011)
			Geodia megastrella Carter, 1876	ZMA POR 21231	HM592741	Cárdenas *et al.* (2011)
			Geodia neptuni (Sollas, 1886)[ε]	B74	EF519673	Erpenbeck *et al.* (2008)
			Geodia neptuni (Sollas, 1886)[ε]	K44	EF519674	Erpenbeck *et al.* (2008)
			Geodia pachydermata (Sollas, 1886)	ZMA POR 21655	HM592732	Cárdenas *et al.* (2011)
			Geodia papyracea Hechtel, 1965	UCMP WC921	AY561961	Nichols (2005)
			Geodia phlegraei (Sollas, 1880)	ZMBN 77929	EU442196	Cárdenas *et al.* (2010)
			Geodia phlegraei (Sollas, 1880)	ZMBN 85210	HM592690	Cárdenas *et al.* (2011)
			Geodia phlegraei (Sollas, 1880)	ZMBN 85211	HM592701	Cárdenas *et al.* (2011)
			Geodia simplicissima Burton, 1931	ZMBN 85212	HM592691	Cárdenas *et al.* (2011)
			Geodia sp. 1 PC-2011	IRD NC R1820	HM592680	Cárdenas *et al.* (2011)
			Geodia sp. 2 PC-2011	MNHN (unaccessioned)	HM592707	Cárdenas *et al.* (2011)
			Geodia vaubani Lévi and Lévi, 1983	IRD NC R1822	EU442202	Cárdenas *et al.* (2010)
			Geodia vosmaeri (Sollas, 1886)	ZMBN 85214	HM592711	Cárdenas *et al.* (2011)
			Geodia vosmaeri (Sollas, 1886)	ZMBN 85213	HM592722	Cárdenas *et al.* (2011)
	Pachastrellidae		*Characella pachastrelloides* (Carter, 1876)	ZMBN 85248	HM592672	Cárdenas *et al.* (2011)
			Characella pachastrelloides (Carter, 1876)	ZMBN 85225	HM592709	Cárdenas *et al.* (2011)
			Characella pachastrelloides (Carter, 1876)	ZMA POR 18041	HM592713	Cárdenas *et al.* (2011)
			Characella pachastrelloides (Carter, 1876)	ZMA POR 20375	HM592749	Cárdenas *et al.* (2011)
			Pachastrella nodulosa Cárdenas and Rapp, 2012[ζ]	ZMBN 85227	HM592698	Cárdenas *et al.* (2011)
			Pachastrella ovisternata Lendenfeld, 1894	ZMA POR 21219	HM592748	Cárdenas *et al.* (2011)
			Triptolemma intextum (Carter, 1876)	MNHN (unaccessioned)	HM592710	Cárdenas *et al.* (2011)
	Theneidae		*Thenea abyssorum* Koltun, 1964	ZMBN 85228	HM592712	Cárdenas *et al.* (2011)
			Thenea levis Lendenfeld, 1907	ZMBN 85230	HM592717	Cárdenas *et al.* (2011)
			Thenea levis Lendenfeld, 1907	ZMA POR 21501	HM592747	Cárdenas *et al.* (2011)
			Thenea muricata (Bowerbank, 1858)	ZMBN 85232	HM592677	Cárdenas *et al.* (2011)

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TABLE 1. (Continued)

Order	Family	Subfamily	Species	Museum Registration/Voucher Number	GenBank Accession Number	Reference
			Thenea muricata (Bowerbank, 1858)	MNHN (unaccessioned)	HM592706	Cárdenas *et al.* (2011)
			Thenea schmidti Sollas, 1886	ZMA POR 18036	HM592737	Cárdenas *et al.* (2011)
			Thenea valdiviae Lendenfeld, 1907	ZMBN 85234	HM592694	Cárdenas *et al.* (2011)
			Thenea valdiviae Lendenfeld, 1907	ZMBN 85235	HM592703	Cárdenas *et al.* (2011)
			Thenea valdiviae Lendenfeld, 1907	ZMBN 85233	HM592708	Cárdenas *et al.* (2011)
			Thenea valdiviae Lendenfeld, 1907	ZMBN 85236	HM592718	Cárdenas *et al.* (2011)
	Theonellidae		*Discodermia polymorpha* Pisera and Vacelet, 2011	ZMBN 85237	HM592686	Cárdenas *et al.* (2011)
			***Discodermia proliferans* Lévi and Lévi, 1983**	**QM G318557**	**KJ494347**	**this study**
			***Discodermia proliferans* Lévi and Lévi, 1983**	**QM G318639**	**KJ494348**	**this study**
			***Discodermia proliferans* Lévi and Lévi, 1983**	**QM G318697**	**KJ494349**	**this study**
			***Theonella cf. cupola* Burton, 1928**	**QM G312708**	**KJ494351**	**this study**
			***Theonella cf. cupola* Burton, 1928**	**QM G323789**	**KJ494352**	**this study**
			***Theonella cf. cylindrica* Wilson, 1925**	**QM G301114**	**KJ494353**	**this study**
			***Theonella cf. cylindrica* Wilson, 1925**	**QM G303701**	**KJ494354**	**this study**
			***Theonella cf. swinhoei* Gray, 1868**	**QM G322616**	**KJ494360**	**this study**
			***Theonella cf. swinhoei* Gray, 1868**	**QM G327442**	**KJ494357**	**this study**
			***Theonella cf. swinhoei* Gray, 1868**	**QM G327446**	**KJ494358**	**this study**
			***Theonella deliqua* n. sp. – holotype**	**QM G329195**	**KJ494355**	**this study**
			***Theonella maricae* n. sp. – holotype**	**QM G331427**	**KJ494356**	**this study**
			***Theonella* sp. (OTU QM3369)**	**QM G319774**	**KJ494359**	**this study**
			Theonella swinhoei Gray, 1868	ZMA POR 16637	HM592745	Cárdenas *et al.* (2011)
			***Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010)**	**QM G329095**	**KJ494361**	**this study**
			***Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010)**	**QM G329183**	**KJ494363**	**this study**
			***Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010)**	**QM G329220**	**KJ494365**	**this study**
			***Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010)**	**QM G329283**	**KJ494375**	**this study**
			***Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010) – holotype**	**QM G329976**	**KJ494374**	**this study**

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TABLE 1. (Continued)

Order	Family	Subfamily	Species	Museum Registration/Voucher Number	GenBank Accession Number	Reference
			***Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010)**	**QM G329977**	**KJ494362**	**this study**
			***Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010)**	**QM G329978**	**KJ494370**	**this study**
			***Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010)**	**QM G331398**	**KJ494369**	**this study**
			***Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010)**	**QM G331401**	**KJ494367**	**this study**
			***Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010)**	**QM G331424**	**KJ494366**	**this study**
			***Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010)**	**QM G331426**	**KJ494373**	**this study**
			***Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010)**	**QM G331436**	**KJ494368**	**this study**
			***Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010)**	**QM G331442**	**KJ494364**	**this study**
			***Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010)**	**QM G331463**	**KJ494371**	**this study**
			***Theonella xantha* (Sutcliffe, Hooper and Pitcher, 2010)**	**QM G331662**	**KJ494372**	**this study**
	Thoosidae		*Alectona millari* Carter, 1879	ZMBN 85238	HM592670	Cárdenas *et al.* (2011)
			Neamphius huxleyi (Sollas, 1888)	UCMP WC1086	HM592682	Cárdenas *et al.* (2011)
	Vulcanellidae		*Poecillastra compressa* (Bowerbank, 1866)	ZMBN 77932	EU442192	Cárdenas *et al.* (2010)
			Poecillastra compressa (Bowerbank, 1866)	ZMBN 86300	HM592675	Cárdenas *et al.* (2011)
			Poecillastra compressa (Bowerbank, 1866)	MNHN (unaccessioned)	HM592714	Cárdenas *et al.* (2011)
			Vulcanella aberrans (Maldonado and Uriz, 1996)	ZMBN 80959	HM592699	Cárdenas *et al.* (2011)
			Vulcanella aberrans (Maldonado and Uriz, 1996)	ZMA POR 21193	HM592700	Cárdenas *et al.* (2011)
			Vulcanella gracilis (Sollas, 1888)	ZMA POR 18025	HM592702	Cárdenas *et al.* (2011)
			Vulcanella gracilis (Sollas, 1888)	MNHN (unaccessioned)	HM592704	Cárdenas *et al.* (2011)
Spirophorida	Tetillidae		*Cinachyrella apion* (Uliczka, 1929)		AJ843895	Hess *et al.* (unpublished; GenBank direct submission 2005)
			Cinachyrella apion (Uliczka, 1929)	ZMBN 81789	HM592667	Cárdenas *et al.* (2011)
			Cinachyrella kuekenthali (Uliczka, 1929)	B79	EF519602	Erpenbeck *et al.* (2008)
			Cinachyrella kuekenthali (Uliczka, 1929)	K75	EF519603	Erpenbeck *et al.* (2008)
			Craniella cranium (Müller, 1776)	ZMBN 85239	HM592669	Cárdenas *et al.* (2011)
			Craniella sp. PC-2011	ZMBN 85240	HM592668	Cárdenas *et al.* (2011)

Footnote: (sequences published originally) $^{\alpha}$ as *Ecionemia robusta*; $^{\beta}$ as *Dercitus bucklandi*; $^{\gamma}$ as *Ecionemia megastylifera*; $^{\delta}$ as *Calthropella geodioides*; $^{\varepsilon}$ as *Sidonops neptuni*; $^{\zeta}$ as *Pachastrella* sp. PC-2011 (described as *Pachastrella nodulosa* Cárdenas and Rapp, 2012 in Cárdenas & Rapp (2012)).