Description of a new species of sponge encrusting on a sessile gastropod in the Singapore Strait

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Abstract. *Theonella laena* n. sp. (Porifera: Tetractinellida: Theonellidae) is a bright orange sponge thinly encrusting on colonies of living and dead *Tenagodus cf. sundaensis* Dharma, 2011 (Mollusca: Gastropoda: Siliquariidae) found at 40m depth in the Singapore Strait. The sponge has an intricate aquiferous system built around openings found along the length of the tubular shells of the sessile gastropod, where water expelled by the latter flows into the sponge incumbent canal system. Incurrent water enters the two closely associated organisms through both the gastropod shell aperture and ostia of the sponge located externally but the excurrent water exits only through the oscula of the sponge. The new sponge species is distinguished from its congeners in having acanthose microrhabds (13.6–27 × 2.5–4.2 μm) with bifid spine terminuses, which together form a thin layer on the sponge surface. The entwined tubes of *Tenagodus* were almost entirely encrusted with the sponge except at the aperture openings of the gastropod. This is also the first report of a *Theonella* species associated with slit-wormsnails.

Keywords. new species, Porifera, Tetractinellida, *Theonella*, Siliquariidae, *Tenagodus*, Singapore

INTRODUCTION

Members of the sponge genus *Theonella* were previously placed in a polyphyletic and informal group, characterised by choanosomal articulating desmas forming a rigid skeleton, collectively known as ‘lithistid’ demosponges in ‘Systema Porifera’ (Hooper & Van Soest, 2002). This group consisted of 13 extant families with 36 genera and about 200 species (see Pisera & Lévi, 2002a), exhibiting a wide array of desmas geometries, ectypeosomal spicules and microscleres. The term ‘lithistid’ was derived from the taxon ‘Lithistida’ proposed by Schmidt (1870), who defined it as ‘tetractinellida comprised of a consistent skeleton by the zygosis of modified spicules or desmas’. The polyplypetic nature of Lithistida was widely recognised since the early 20th century (see Pisera & Lévi, 2002a) but there was little agreement as to where these families could be assigned within the Demospongiae. Recent work on lithistid demosponges (Cárdenas et al., 2012; Schuster et al., 2015) and revision of higher taxa of the Demospongiae by Morrow & Cárdenas (2015) have now divided the ‘lithistids’ among three disparate demosponge orders Tetractinellida, Sphaerocladina and Bubarida. As a result, the genus *Theonella* (family Theonellidae) is now placed in the order Tetractinellida resurrected by Morrow & Cárdenas (2015) under the subclass Heteroscleromorpha erected by Cárdenas et al. (2012). Within the order Tetractinellida, the suborder Astrophytonora is now recognised (derived from the order Astrophorida sensu Lévi, 1973) in the revision of Morrow & Cárdenas (2015). This suborder retains the same taxonomic composition as in previous classifications, but with desma-bearing families added to the group (Van Soest et al., 2015), including the family Theonellidae based on molecular data.

Species comprising of the genus *Theonella* Gray, 1868 are morphologically homogenous. All of them have a surface coat of aligned phyllotriaenes to discotriaenes over an internal tetractine desma network, tuberculated zygmoid terminal, large monactinal spicules, and microscleres, which are only small acanthose microrhabds, often bent in the middle (Pisera & Lévi, 2002b). However, three species comprising only acanthose microrhabds and without desmas, phyllotriaenes and megasmere, were discovered recently by Hall et al. (2014). There are currently 19 species worldwide that are placed in the genus *Theonella* (see Van Soest et al., 2015) and they are well represented in tropical Pacific and Southeast Asian waters (see Lim et al., 2016). Only four species are recorded from the Atlantic Ocean and the Caribbean (see Pisera & Pomponi, 2015), whilst as many as five species are known just from the South China Sea (Gray, 1868; Wilson, 1925; Hooper et al., 2000; Longakit et al., 2005; Lim et al., 2016).

Ten sponge genera in four disparate families (Geodidae: *Erylus* and *Penares*; Ancorinidae: *Holoxea* and *Jaspis*; Halichondriidae: *Epipolasis*, *Spongiosorites*, and *Topsenia*; Theonellidae: *Discoderma*, *Racodiscula*, and *Siliquariaspongia*) are known to be associated with the molluscan slit wormsnails - the Siliquariidae. Members of this sessile, filter-feeding caenogastropod family (not to be confused with the Vermetidiae) have tubular shells that become uncoiled during growth. Individuals are usually entwined and
attached to each other to form aggregates which are invariably associated with sponges (see Pensini et al., 1999 for a review of sponge-siliquariid associations; also Hartman & Hubbard, 1999; Bieler, 2004). The family Siliquariidae comprises five genera Caporbis, Hummelinckiella, Petalopoma, Stephopoma and Tenagodus with some 30 species. Siliquariids are obligate sponge infauna (Schiaparelli, 2002) but the sponge-siliquariid association is not species-specific (Pansini et al., 1999). In addition, sponges associated with siliquariids may occur in the absence of the gastropod (see Pensini et al., 1999). Morton (1955) was probably the first to comment on the association between the siliquariid slit and the sponge aquiferous system. Subsequently, the nature of movement of water across sponge and gastropod was discussed by various authors, including Savazzi (1996), Pensini et al. (1999), Schiaparelli (2002), and Bieler (2004). Additionally, Bieler (2004) made direct observations using living material of Tenagodus squamatus, associated with either Spongosorites rueizi or S. siliquaria, and showed that the gastropod expelled water through the shell slits into the sponge canal system. Sponge workers including Annandale (1911), Hoshino (1981), and Van Soest & Stentoft (1988) described species associated with siliquariids but did not comment on water movement between the siliquariid and the sponge.

In this study, a new species of Theonella associated with living siliquariid molluscs was collected during the ‘Comprehensive Marine Biodiversity Survey’ conducted in Singapore and is herein described. The five-year survey was carried out between November 2010 and October 2015 as a national initiative to take stock of the state of marine biodiversity in Singapore waters. The main objective of this survey was to obtain samples from as many marine ecosystems as possible, with emphasis on poorly known habitats. The subtidal benthos below the busy port waters where few previous surveys have been conducted. Dredging and trawling the seabed off Singapore Island have resulted in many new records and new species of invertebrates (see Tan et al., 2015 and also this volume).

MATERIAL AND METHODS

Sponge material was collected off Raffles Lighthouse from a depth of about 40 m in the Singapore Strait using a rectangular dredge during the Singapore Strait International Workshop in May 2013 and again from the same locality in March 2016. A portion was preserved in 95% denatured ethanol within six hours of collection, while remaining colonies of the sponge and molluscs were kept alive in flow-through aquaria at St John’s Island to observe the animals over several days after collection. Fluorescein and milk were used as tracers to determine gross current flow through the two animals. Type material was deposited at the Zoological Reference Collection (ZRC) of the Lee Kong Chian Natural History Museum (LKCNHM, formerly Raffles Museum of Biodiversity Research), National University of Singapore. To examine skeletal architecture, paraffin-embedded sponge tissue was sectioned either by hand or by using a microtome. The sections were then cleared in phenol-xylene solution and mounted in Dpex® on glass slides. Spicule preparations were made on a glass slide by dissolving a small piece of the specimen in a few drops of concentrated nitric acid over an alcohol flame. These were mounted either in Dpex® on glass slides for light microscopy or transferred onto brass stubs for SEM (scanning electron microscopy). SEM mounts were platinum-coated and viewed using JEOL JSM-6510 scanning electron microscope. Spicule measurements (25 for each type, unless stated otherwise) were made by light microscopy and from SEM images. Spicule size range was estimated and presented as lowest value-mean-highest value of both length and width. Phyllotriaenes and tetracline desmas were measured based on their maximum diameters. The classification of Theonella used here follows Morrow & Cádenceas (2015) as accepted by the World Porifera Database (Van Soest et al., 2015).

TAXONOMY

Class Demospongiae Sollas, 1885

Subclass Heteroscleromorpha Cádenceas, Perez & Boury-Esnault, 2012

Order Tetractinellida Marshall, 1876

Suborder Astrophorina Sollas, 1887

Family Theonellidae Lendenfeld, 1903

Genus Theonella Gray, 1868

Theonella laena n. sp.

(Figs. 1–9)

Material examined. Holotype, ZRC.POR.0278, Singapore Strait off Raffles Lighthouse, dredged from 40 m depth; encrusting on an aggregation of living and dead shells of the sessile gastropod Tenagodus cf. sunaensis Dharma, 2011,
coll. Lim S.C., 5 June 2013. Paratype, ZRC.POR.0279, Singapore Strait off Raffles Lighthouse, dredged from 40 m depth; encrusting on an aggregation of living and dead shells of the sessile gastropod *Tenagodus* cf. *sundaensis*, coll. Lim S.C., 30 March 2016. Three other aggregations of sponge and gastropod shells were also examined from the same locality obtained in 2016.

**Description.** The sponge was thinly encrusting, typically less than 1 mm in thickness, but may be up to 3 mm thick (Fig. 1). The sponge covered almost the entire external surface of the densely intertwined shells of *Tenagodus* cf. *sundaensis* Dharma, 2011 (Mollusca: Gastropoda: Siliquariidae) measuring 7 × 5 × 5 cm in size with the gastropods inside. Sand grains and pieces of broken shells also formed part of the sponge. It was bright orange when alive, beige when preserved in ethanol, with a smooth surface. Consistency was firm but brittle. Oscula and ostia were not visible to the naked eye when sponge was out of water. However, oscula can be observed in living material in water under a stereomicroscope with the aid of soluble tracer (fluorescein and milk). Oscula were rare (approximately one per 10 cm²) and were each about 2 mm in diameter. The considerably smaller ostia ranged between 10–60 µm in diameter (Fig. 2).
Ectosomal skeleton. The surface skeleton was approximately 100 µm thick, forming a surface crust comprising acanthose microrhabds on top of a single layer of phyllotriaenes (Fig. 2) and surrounding the phyllotriaenes (Fig. 3). The periphery of the encrusting sponge consisted mainly of the surface crust with little or no evidence of a choanosomal skeleton (Fig. 3). The thin surface crust, consisting of phyllotriaenes and acanthose microrhabds at the periphery, detached easily from the thin layer of choanosomal skeleton on the shell when excised. Acanthose microrhabds were strongly spined (Fig. 4A) and spines were often bifid at the terminus (Fig. 4B). The acanthose microrhabds were cylindrical in shape, often curved or bent in the mid-region, and varied widely in length and diameter, i.e., 13.6–20.9–27 × 2.5–3.4–4.2 µm (Fig. 4C). Phyllotriaenes were smooth, with long narrow cladi that were sinuous, sometimes branched, whose tips were slightly rounded, approximately 350–800 µm in diameter; rhabds were short and approximately 55–87 µm in length (Fig. 5).

Choanosomal skeleton. The choanosome comprised mainly of tetraclone desmas that were smooth in the middle and tuberculate towards the zygomes (Fig. 6). The thickness of choanosomal skeleton on the gastropod shell was typically less than 1 mm in thickness (Fig. 7). The tetraclone desmas measured approximately 300–660 µm in diameter. The other type of choanosomal megascleres comprised the strongyles. They were slender, slightly curved, sometimes sinuous and approximately 600–630 × 5 µm in size. Strongyles (Fig. 8) were rare, oriented radially or irregularly and sparsely strewed singly or in small bundles perpendicular to the surface between the tetraclines in the choanosomal skeleton. Acanthose microrhabds were sparsely and irregularly distributed in the choanosome. At the base of the choanosomal skeleton where the sponge came into contact with the longitudinal slit of the Tenagodus cf. sundaensis, canal openings of about 500 µm in diameter (Fig. 9A) corresponded to the contiguous fenestrae along the shell slit (Fig. 9B). The tetraclone desmas of choanosomal skeleton extended into the shell slit of T. cf. sundaensis (Fig. 9B). The canals of the sponge were narrower and branched towards the interior of the choanosomal skeleton.

Ecology. The sponge encrusting the siliquariid colonies obtained by dredging were presumably unattached and lying...
freely on the seafloor at about 40 m depth in the Singapore Strait on coral rubble, sand and mud. They have not been recorded at depths shallower than 30 m despite extensive surveys carried out in the immediate vicinity and elsewhere in Singapore. The sponge is closely associated to Tenagodus cf. sundaensis, a siliquariid mollusc described recently from the Natuna Islands, Indonesia (Dharma, 2011) in the South China Sea. The gastropod shell has a longitudinal slit, and an elaborated sponge aquiferous system is built around the slit. Both the sponge and the siliquariid are filter feeders. Water enters the siliquariid through their aperture openings and ostia on the external surface of the sponge, but the excurrent flow is restricted through the oscula of the sponge found amongst the ostia (pers. obs. using soluble tracer).

Distribution. The sponge is hitherto known only from a small area of seabed at 40 m depth off the Raffles Lighthouse in the Singapore Strait, although T. sundaensis is known from Natuna Islands, Anambas Islands, and West Kalimantan, Borneo (Dharma, 2011).

Etymology. Laena refers to the thin, cloak-like habit of the sponge that wraps around the siliquariid colony. The name is used as a noun in apposition.

Remarks. Perhaps the most distinguishing feature that sets the new species apart from its 19 other valid congeners (van Soest et al., 2015) is the thinly encrusting habit on the shell surfaces of the siliquariid Tenagodus cf. sundaensis, as compared to the typically cylindrical, digitate and massive habit of most other Theonella species. It has the thinnest encrusting habit among all congeners that bear desmas. Only T. complicata (Carter, 1880) and T. pulchrifolia Dendy, 1922 from the Indian Ocean are known to be thinly encrusting. However, T. complicata has calthrophilic desmas and T. pulchrifolia has discotriaenes, and these characters clearly separate the two species from Theonella laena n. sp. which has typical theonellid tetracleone desmas, and lacking discotriaenes. Three recently described species, namely Theonella deliqua Hall et al., 2014, T. maricae Hall et al., 2014 and T. xantha (Sutcliffe et al., 2010), all from the Great Barrier Reef, are thinly-encrusting as well but these three species only possess acanthose microrhabds and lack megascles such as phyllotriaenes and tetracleones that are typical of members in the genus Theonella. Other than the new species, these five encrusting theonellids were not associated with siliquariid molluscs.

In addition to its distinct encrusting habit, T. laena n. sp. has acanthose microrhabds with bifid spine terminus that are reported for the first time in the genus Theonella, and these form a thin layer on the sponge surface. Theonella laena n. sp. also has an unique size combination of slender strongyles (600–630 × 5 µm) and acanthose microrhabds (13.7–27–2.5–4.7 µm). Theonella cylindrica Wilson, 1925 has similar strongyles but possess much smaller acanthose microrhabds (8 × 3 µm). Theonella cupola Burton, 1928 has acanthose microrhabds size similar to T. laena n. sp., but T. cupola is clearly distinct in having oxeas and tylotes in addition of strongyles and the unique character of tubercles on phyllotriaenes. Theonella lacerata Lendenfeld, 1907 also has similar acanthose microrhabds and slightly thicker strongyles (400–630 × 6–10 µm) but it is clearly distinct in having discotriaenes. Theonella levior Lendenfeld, 1907 also has similar acanthose microrhabds (15–30 × 2–4 µm) and strongyles that are thicker (500–770 × 5–11 µm) but T. levior is clearly distinct in having tylotes in addition to strongyles.

The surface crust of a typical Theonella consists of phyllotriaenes with acanthose microrhabds that fill up the gaps. The surface crust of Theonella laena n. sp. consists of a layer of microrhabds on the surface and over the phyllotriaenes (Figs. 2, 3). Acanthose microrhabds on the surface and phyllotriaenes have only been reported in T. invaginata Wilson, 1925 and T. atlantica Van Soest & Stentoft, 1988 but the extent of the coverage of acanthose microrhabds were unclear. However, the surface crust of microrhabds over phyllotriaenes can be patchy and sometimes absent in the same species of Theonella (A. Pisera, pers. comm.). All five specimens of T. laena n. sp. examined exhibit extensive acanthose microrhabds on the surface especially around the oscules. These differences clearly indicate that Theonella laena n. sp. is distinct from all other Theonella species described so far.
DISCUSSION

*Theonella laena* is the second species of *Theonella* to be recorded from Singapore after *T. cylindrica*, which was documented by Hooper et al. (2000). Two other *Theonella* species, *T. invaginata* from the Philippines and *T. swinhoei* from Taiwan, have been recorded from the South China Sea (Hooper et al., 2000; Lim et al., 2016). The latter three *Theonella* species have a massive cylindrical habit that is quite distinct from the encrusting *Theonella laena* n. sp. and they have not been reported to be associated with siliquariid gastropods. *Theonella invaginata* has not been reported since it was described by Wilson (1925) but *T. swinhoei*, the type species for the genus, has a wide geographical distribution. It has been reported from China, Indonesia, the Indian Ocean, Red Sea, and Gulf of Aqaba (see Gray, 1868; Sollas, 1888; Wilson, 1925; Burton & Rao, 1932; Lévi & Lévi, 1989; Hooper & Van Soest, 2002; Li, 2008; Van Soest et al. 2015). However there is a significant distribution gap in the South China Sea; it has not been recorded in Singapore, Peninsular Malaysia, Gulf of Thailand and Vietnam despite extensive surveys (see Lindgren, 1897; Dawydoff, 1952; Lévi, 1961; Putchakarn, 2006, 2007; Azzini et al., 2007; Nyguen, 1977; Li, 2008; Lim et al., 2012; Thai, 2013; Lim et al., 2016).

Desmas are distinctive spicules with zygomes that form the irregularly articulated, interlocking, rigid chaoosomal skeleton in sponges. *Theonella laena* n. sp. is only the fourth demosponge observed in Singapore waters that possess desmas. Apart from its congener *T. cylindrica*, *Aciculites* sp. of De Voogd & Cleary (2009) and *Desmanthus* sp. of Lim et al. (2012) are two species known from the shallow waters of Singapore that can be easily distinguished from *Theonella laena* n. sp. The species of *Aciculites* belongs to the family Scleritodermidae with rhizoclone desmas, has an ectosomal layer of acanthose strongyles or styles, but lacks microscleres, whilst the species of *Desmanthus* (Bubaridia) has monocrepidial desmas and lacks microscleres as well. The difference in the shape of the desmas and the absence of microscleres in *Theonella laena* n. sp. separate this species easily from the other two demosponges that contain desmas.

The sponge-siliquariid association have been reported to be non-species specific (Pansini et al., 1999) but all five *T. laena* n. sp. specimens examined in our study are associated with the same *Tenagodus* species, i.e., *T. cf*. *sundaensis*. Although its protoconch was not observed, the small (< 4 mm) diameter of the tubes and the numerous lamellae (up to 15) of the conical operculum with short bristles are congruent with the description provided by Dharma (2011). The sponge has not been recorded existing alone or alone with other organisms but it could most probably exist alone without the siliquariid. The siliquariid probably obtains the following benefits from their association with sponges: 1) protection from predators, mechanically and possibly chemically; 2) a steady and elevated platform which may confer trophic advantages to both filter-feeders; and 3) the sponge pumping activity provide a continuous water flow into the siliquariid through both gastropod and sponge aperture openings, bringing food to both gastropod and sponge in the process (see Pansini et al., 1999; Bieler, 2004). Perhaps possible gains by the sponge include the constantly growing surface area provided by the gastropod shell surface for the sponge to grow on, and the energy saved through water movement brought about by the ciliary action of the siliquariid ctenidia into the incumbent canal system of the sponge. Thus, the association between the sponge and siliquariid is either facultative mutualism or commensal symbiosis where the sponge does not depend entirely on the siliquariid for its survival. On the other hand, siliquariids are thought to be obligate sponge commensals (Pansini et al., 1999; Bieler, 2004) and depend upon the substratum provided by the sponges for their survival.

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