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A FIRST RECORD OF FRESHWATER SPONGE FROM SINGAPORE AND REDESCRIPTION OF *EUNAPIUS CONIFER* (ANNANDALE, 1916) (HAPLOSCLERIDA: SPONGILLINA: SPONGILLIDAE)

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ABSTRACT. — *Eunapius conifer* is reported for the first time from Singapore, extending its distribution significantly south to the equator from China. The identity of *E. conifer* has been confusing and uncertain since Annandale described the species in 1916 from Tai Hu near Shanghai, China. Smooth gemmuloscleres (oxeas), 65–115 µm in length, and gemmules, 250–350 µm in diameter, are characteristic of type material which do not agree with Annandale's original description, where it was stated that gemmules were not more than 140 µm in diameter and short, spiny gemmuloscleres were 30 µm in length. We conclude that Annandale's original description of *E. conifer* is in error and we provide a redescription based on type material as well as living specimens from Singapore. The latter specimens also constitute the first record of freshwater sponge from Singapore.

KEY WORDS. — Porifera, freshwater sponge, *Eunapius conifer*, redescription, Singapore, biodiversity

INTRODUCTION

Freshwater sponges are a fairly successful group of animals with about 250 species (see Manconi & Pronzato, 2008; Van Soest, 2013) distributed around the world, with the exception of Antarctica, in lakes, ponds, rivers and streams. Some species are common and widespread, such as *Spongilla lacustris* (Linnaeus, 1759) and *Eunapius carteri* (Bowerbank, 1863), while about half of them appear to be considerably restricted in their distribution (Manconi & Pronzato, 2008). They are generally able to live in wide variety of habitats with fluctuating environmental conditions by having tough and resilient gemmules that can survive extreme temperatures without water and can be transported over long distances by insects, birds, mammals (including humans) and wind (Smith, 2001).

Freshwater sponges remain poorly known in most countries of Southeast Asia including Cambodia, Myanmar (Burma), Thailand and Indonesia. There appears to be no records of freshwater sponges from Laos, Vietnam, Malaysia (both peninsular and East Malaysia), Brunei and Singapore. Indonesia has some 15 species of freshwater sponges (see Weber, 1890; Weltner, 1901; Koningsberger, 1915; Vorstman, 1927, 1928; Gee, 1930, 1932c; Annandale, 1918; Penney & Racek, 1968), Myanmar has some nine species (see Kirkpatrick, 1908; Annandale, 1911, 1918; Gee, 1930, 1932c; Penney & Racek, 1968), and Thailand has eight species (see Evans, 1901; Annandale, 1918; Gee, 1932c; Penney & Racek, 1968; Manconi et al., 2012; Ruengsawang et al., 2012), with two new species described recently. Three species were recorded from Cambodia in a recent study by Masuda (2004). The total number of freshwater species recorded from these countries in Southeast Asia is around 25 species. This number is comparable to India (31 species) and China (26 species) where freshwater sponges have been well studied (see Annandale, 1911, 1918; Soota, 1991; Gee, 1927a, 1927b, 1931; Chen et al., 1991, respectively).

The freshwater sponge fauna is uncharacteristically depauperate in peninsular Malaysia (see Addis, 2004) and Singapore (Annandale, 1918), despite the ubiquitous presence of freshwater bodies fed and maintained by abundant rainfall and high temperature throughout the year. Singapore is a small island approximately 700 km^2 in area at the southern tip of Malay peninsula. The terrain is relatively flat and natural bodies of water are absent, although streams and rivers occur throughout the land. More recently, artificial freshwater

reservoirs were formed by damming rivers and streams at various locations along their paths, which were also canalised (see Koninck et al., 2008). These now support both native and alien biodiversity (Yeo & Lim, 2011) but freshwater sponges have not been recorded. Annandale (1918) came to Singapore and failed to find freshwater sponges, in spite of his conviction that these locations were very favourable for the growth of sponges. He also concluded "There can be no doubt, therefore, that in most parts of Malaya, as in Ceylon, some unknown obstacle to the growth of sponges is wide-spread in fresh water".

This study reports the first record of a freshwater sponge, *Eunapius conifer*, from Singapore. The identity of *E. conifer* has been confusing and uncertain since Annandale (1916) described the species from Tai Hu near Shanghai, China. We examined the differing accounts of *E. conifer* and verified its identity through examination of holotype and paratype material from the Zoological Survey, India and Smithsonian Institution, USA. A redescription is provided with additional observations on the species from examination of living material.

MATERIAL AND METHODS

Freshwater sponge survey was carried by visual census from a boat, as well as by walking and wading along the edge of freshwater bodies. Sponges were photographed in situ before preserving in 70% ethyl alcohol. Observations were made using both light microscopy (LM) and scanning electron microscopy (SEM). To examine skeletal architecture, paraffin-embedded sponge tissue was sectioned either by hand or by using a microtome. The sections were then cleared in either Histoclear™ or a phenol-xylene mixture 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, following the methods described in Hooper (2000). Gemmules were dried, sputter coated with platinum and observed under SEM (Jeol LV6510). Gemmule size range was estimated by measuring 25 gemmules from each specimen. Spicule size range was estimated by measuring 25 spicules from each specimen, unless stated otherwise, and presented as lowest value of range–mean–highest value of range of length, by lowest value of range–mean–highest value of range of width. Gemmules and spicules from a total of seven specimens were examined from China and Singapore. The classification used here follows Manconi & Pronzato (2002).

Acronyms: National Museum of Natural History, Smithsonian Institution, Washington D.C., USA (USNM), Zoological Survey of India, Kolkata, India (ZSI), Zoological Reference Collection, Raffles Museum of Biodiversity Research, National University of Singapore (ZRC).

TAXONOMY

Class Demospongiae Sollas, 1888
Order Haplosclerida Topsent, 1928
Sub Order Spongillina Manconi & Pronzato, 2002
Family Spongillidae Gray, 1867
Genus *Eunapius* Gray, 1867

Eunapius conifer (Annandale, 1916)

(Figs. 1–6)

Spongilla (*Eunapius*) *conifera* – Annandale, 1916: 51 (no illustration provided)
Spongilla conifera – Annandale, 1918: 203, pl. IX, figs. 3–5; Gee, 1926: 110; 1927a: 3; 1927b: 184; Gee & Wu, 1927b: 8, fig. 9; Gee, 1931: 36; 1932b: 37; 1932c: 54; Sasaki, 1969: 163
Spongilla (*Eunapius*) *conifera* – Gee & Wu, 1927a: 258, figs. a–d
Eunapius coniferus – Penney, 1960: 15; Penney & Racek, 1968: 33; Masuda & Satoh, 1989: 80
Eunapius conifer – Van Soest, 2013, World Porifera Database webpage

Materials examined. — Holotype (in ethanol) ZEV 7105 – 6/7, *Spongilla* (*Eunapius*) *conifera* Annandale, mouth of Moo-Too Creek, Tai Hu, Kiang Su Province, China, 'stn 12', 5 Dec.1915.

Paratype USNM 21524 (dry material, labeled as Co-Type), *Spongilla conifera* Annandale, mouth of Moo-Too Creek, Tai Hu, Kiang Su Prov., China, EX.ZEV 7106/7, Dec.1915.

Paratype USNM 21524 (slide, labeled as Type and Schizoholotype), *Spongilla conifera* Annandale, mouth of Moo-Too Creek, Tai Hu, Kiang Su Prov., China, Gee no. 54388.

ZRC.POR.0274. Singapore, 2 Feb.2007, on concrete wall of canal, Yishun, Singapore

ZRC.POR.0275. Singapore, 7 Jan.2011, on concrete wall of canal, Yishun, Singapore

USNM P0039458 (dry material), *Spongilla conifera* Annandale, China; Shandong; Qingdao (as Tsingtao), Gist Gee Freshwater Sponge Collection, collection date unknown.

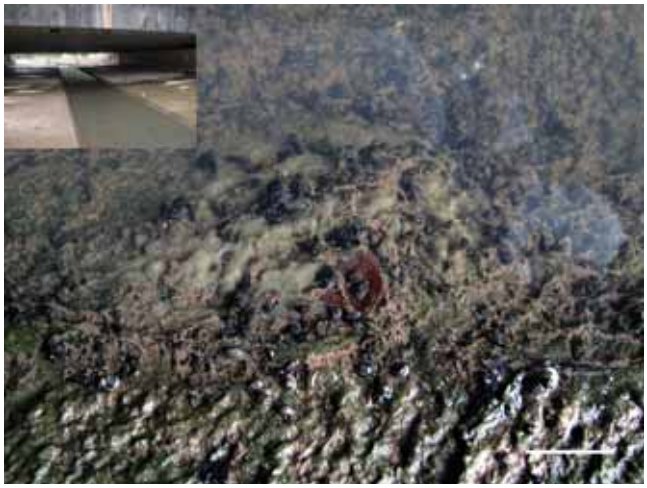

Fig. 1. *Eunapius conifer* individual encrusting on sloping concrete surface of a shallow drain channel in a storm canal in Singapore. Scale bar = 2 cm. Inset shows the drain channel (water depth about 20 cm) inside storm canal (about 10 m wide).

USNM P0040644 (dry material), *Spongilla conifera* Annandale, China; Jiangsu, Nanjing, Gist Gee Freshwater Sponge Collection, collection date unknown.

Description. — Material from Singapore and Qingdao, China (Gee Freshwater Sponge Collection), are encrusting, typically 5–8 cm wide and 2–3 cm in height (Fig. 1). The type material (ZSI and USNM, Fig. 2) from Tai Hu (Tai Lake) near Shanghai are very thin, approximately 1 mm in thickness, growing on the leaf blade of an eel grass, *Vallisneria spiralis* (Hydrocharitaceae). It is interesting to note that gemmules occupied a significant volume of the thin sponge. Colour ranged from almost colourless white to green or brown. Consistency of the living sponge was moderately firm and compact but fragile and friable. Surface smooth, hispid under the light microscope. Oscules fairly numerous, mostly 1–2 mm in diameter. Ostia numerous, slightly less than 1 mm in diameter. Subectosomal cavities not common. The dark gemmules were numerous and can be easily seen through the skeleton of the sponge in the field. Ectosomal skeleton undifferentiated; choanosomal skeleton consists of irregular anisotropic paucispicular tracts: primary tracts typically 2–6 spicules thick; secondary tracts 1–3 spicules thick (Fig. 3). Spongin sparse. Oxeas, 210–232.7–255 µm × 7.5–8.8–11 µm, smooth, straight or slightly bent (Fig. 4A). Spicules from type material were larger, ranging 180–350 µm × 8–17 µm. Microscleres absent. Gemmules (Figs. 5, 6) were conical in shape with a flattened subspherical base (250–315–350 µm) in all type material examined. Pneumatic layer present, thickest at the foramen, becoming thinner towards the base and was thinnest at the base of the gemmule. Gemmuloscleres

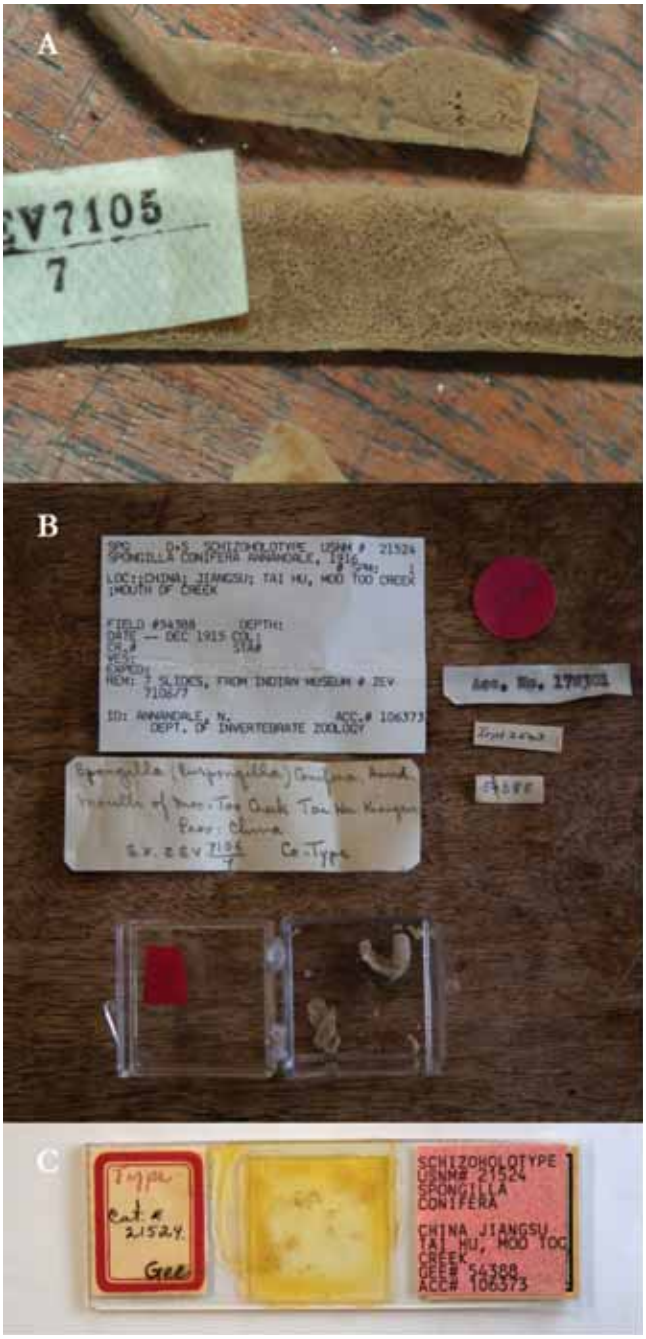

Fig. 2. *Eunapius conifer*. A, Holotype ZEV 7105 – 6/7 from ZSI showing encrusting sponge on surface of leaf blade of *Vallisneria spiralis*. B, Paratype USNM 21524 (dry material). C, Paratype USNM 21524 (slide).

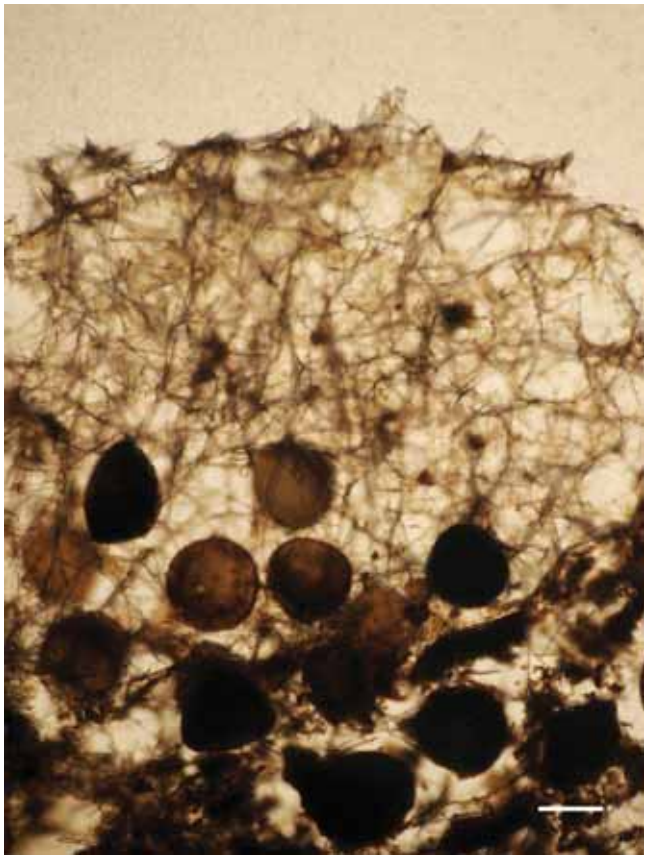

Fig. 3. *Eunapius conifer* (ZRC.POR.0274). Skeletal cross-section, with gemmules scattered at the base. Scale bar = 200 µm.

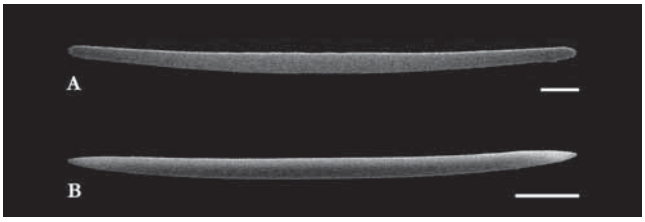

Fig. 4. *Eunapius conifer* (ZRC.POR.0274). A, gemmulosclere. Scale bar = 5 µm. B, skeletal oxea. Scale bar = 20 µm.

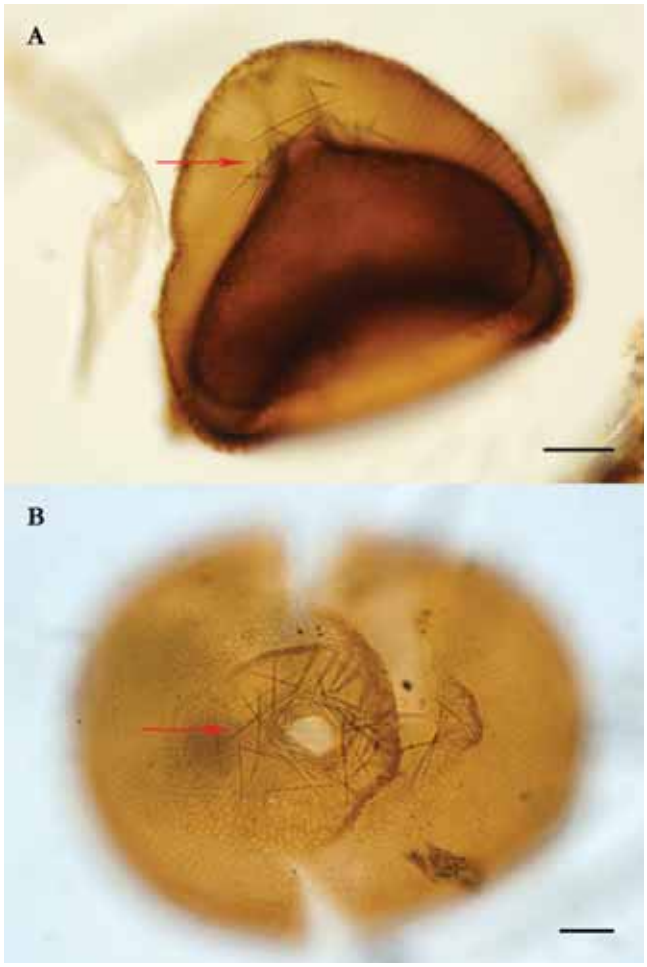

Fig. 5. *Eunapius conifer*. A, ZRC.POR.0274, Gemmule viewed from the side to show gemmuloscleres localised around the foramen (indicated by arrow). Scale bar = 60 µm. B, Paratype USNM 21524, smooth gemmuloscleres around foramen (indicated by arrow). Scale bar = 50 µm.

were embedded in the pneumatic layer only around the foramen (Figs. 5, 6) and were absent on gemmular surface as well as in other parts of the pneumatic layer. Foramen single with a simple foraminal tubule, simple without collar. Gemmular theca tri-layered. Outer layer consists of outlines of pneumatic chambers evident at the gemmular surface. Pneumatic layer 10–100 µm in thickness with regular lines of polygonal chambers. Foramen opening about 25 µm in diameter, foramen tube straight and simple, without collar. Gemmules are singly scattered throughout the body and are most numerous at the base of (Fig. 3). Gemmuloscleres are oxeas measuring 65–81.5–115 µm × 2–2.6–3 µm. The oxeas are smooth, straight, sometimes slightly bent, with blunt tips (Fig. 4B).

Habitat. *Eunapius conifer* was fairly common on a concrete wall lining a storm canal at Yishun, Singapore. It could only be found at the upper, non-tidal reaches of the Yishun–Khatib Bongsu storm canal (Fig. 1). Six specimens of this species were observed on the concrete wall of the canal over a three-meter stretch, just below the surface of running freshwater in Jan.2011 about 50 m downstream of the Yishun Pond, where road runoff accumulated. However, repeated observations made along the edge of the pond itself did not detect the presence of freshwater sponges inside Yishun pond. Similarly, no sponges were found further downstream toward the mouth of the canal leading into the Khatib Bongsu mangroves. Despite visiting 17 localities across reservoirs and streams in Singapore, no other specimens of this species was observed.

DISCUSSION

This study reports *Eunapius conifer* for the first time from Singapore, which is also the first discovery of a freshwater sponge from this country. The occurrence of this species

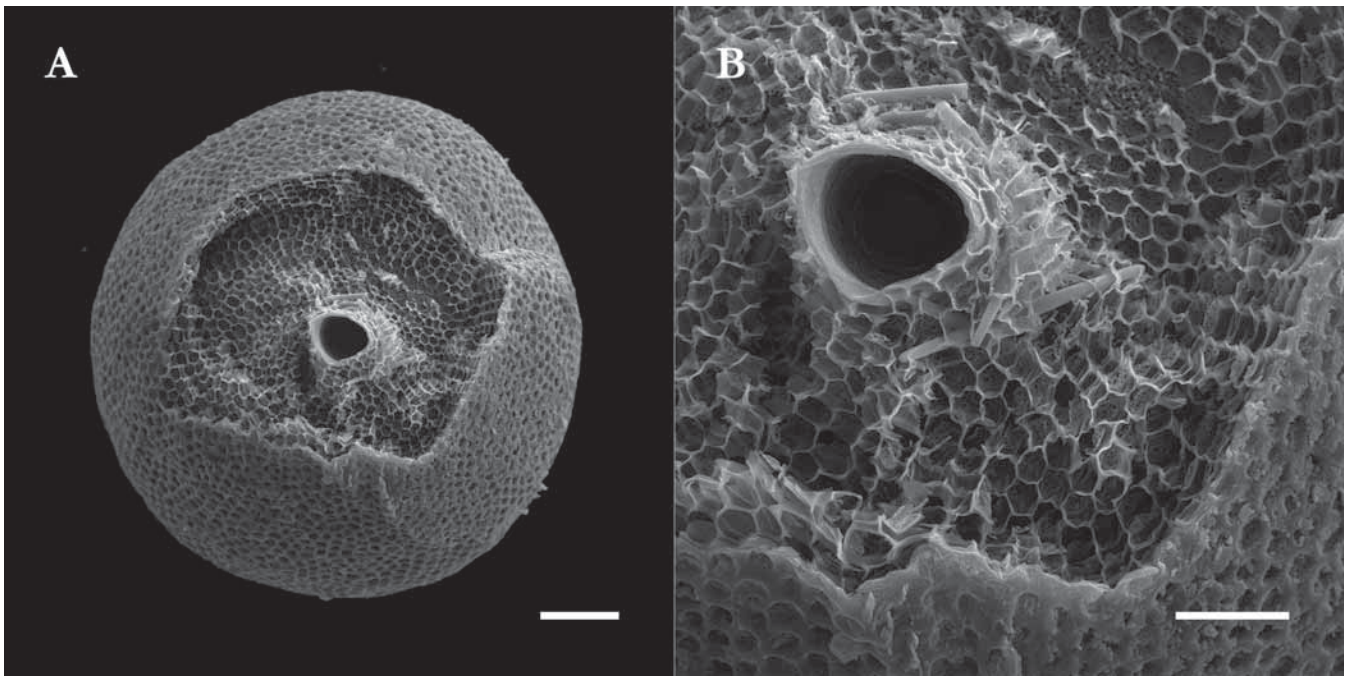

Fig. 6. *Eunapius conifer* (ZRC.POR.0274). A, Tangentially cut section of the foramen tip to reveal gemmuloscleres embedded around it. Scale bar = 50 µm. B, Magnified view of gemmuloscleres embedded in pneumatic layer around foramen. Scale bar = 30 µm.

in Singapore extends its previously known geographical distribution from China and Japan to the equator. A disjunct distribution of some 30° in latitude appears to occur between China and Singapore but this is probably due to lack of studies in this region.

***Identity of* Eunapius conifer.** — There are a number of differing historical accounts on the morphological characteristics of *Eunapius conifer* that have caused considerable confusion regarding its identity. After the original description of the species in 1916, Annandale provided additional notes on *E. conifer* [sic] two years later (Annandale, 1918: 203): "The most remarkable features of this sponge are the small size of all its parts and the peculiar structure of the gemmules; this is clearly shown in the figures on pl. IX. Round the base of the gemmule there is often a circle of minute spinelets formed owing to an imperfect development of the pneumatic cells in this region. I have discovered a few free-microscleres in specimens since the original description was published. These microscleres are cylindrical, straight, blunt at the extremities and covered with short spines. Minute smooth amphioxi occur occasionally in the parenchyma, but are probably young macroscleres, also spiny amphioxi and amphistrongli which are apparently adventitious. The macroscleres are occasionally amphistrongylous and vary greatly in size, proportions and outline; they are always smooth." This account contains new observations of the gemmule base and adventitious spicules, but there was no amendment to his original description.

Following Annandale's accounts of *E. conifer* in 1916 and 1918, Gee & Wu (1927a) redescribed *E. conifer* based on paratype material (labeled as Co-Type). The descriptions by Annandale (1916) and Gee & Wu (1927a) have significant differences: 1) Gemmuloscleres are smooth, 80–110 µm × 4–6 µm in Gee & Wu (1927a) but were described as spiny and 30 µm in length in Annandale (1916); 2) Gemmule diameter is 255–290 µm in Gee & Wu (1927a) but not more than 140 µm in Annandale (1916). The gemmules and gemmuloscleres are important characters in freshwater sponge taxonomy and these considerable differences in the descriptions would likely constitute two different species.

Gee & Wu (1927a: 259) mentioned: "The following description of the gemmules is a modification of the one given by Annandale". However, they did not state the reasons for the redescription explicitly. Their account seemed to suggest that Annandale was mistaken in his observations. It is important to note that Gee and Annandale had a close working relationship. Gee hosted Annandale in China during the collection of *E. conifer* (see Gee & Wu, 1927a). Annandale brought the sponge back to India whilst Gee kept a small piece of paratype material. However, Gee probably had better knowledge of *E. conifer* since he was based in that region and had access to living populations of the sponge at the type locality and surrounding areas.

There remains a possibility that Annandale and Gee & Wu were looking at different species, but Annandale passed away in 1924 (see Clover, 1924; Ramakrishna et al., 2010) before Gee & Wu's (1927a) article was published. Much later, Penney & Racek (1968) in their seminal work provided a description of *Eunapius conifer* (as *Eunapius coniferus*) similar to Annandale (1916). They had access to "fraction of paratype, and several slides of paratype obtained by Gee; material and slides from China (N. Gist Gee)" in their "Material" section but gave a similar description of *E. conifer* as Annandale (1916). Penney & Racek (1968) cited four of Gee's works; of these, *E. conifer* only appears in species and distribution lists, descriptions being absent (Gee, 1931, 1932b, 1932c). The fourth, "Gee, N. G., and Wu, C. F. 1927. Descriptions of some freshwater sponges from China. The China Journal of Science & Arts, Shanghai 4, pp. 136, 235-237, 258-260", was cited erroneously. The redescription of *E. conifer* is in volume 6 instead of volume 4. In any case, Penney & Racek (1968) did not mention or discuss the discrepancies of *E. conifer* described by Annandale (1916: 51) and Gee & Wu (1927a: 258).

Interestingly, Sasaki (1969) reported *Eunapius conifer* (as *Spongilla conifera*) that conformed to the description provided by Gee & Wu (1927a) from Japan in the following year. He cited Annandale (1916) and Gee & Wu (1927b) but Penney & Racek's (1968) work was not mentioned. However, Sasaki did not cite Gee & Wu (1927a) which contains the redescription of *E. conifer* but cited Gee & Wu (1927b) which only provides a key to species (*Spongilla conifera*, *S. gee*, *S. carteri*, *S. fragilis*) and a drawing of *E. conifer* spicules, gemmule and, most importantly, the smooth gemmuloscleres. Sasaki (1969) did not provide a detailed description and dimensions of *E. conifer* and probably identified the sponge in Japan based only on Gee & Wu (1927b). There was no mention of the discrepancies in the descriptions of *E. conifer* provided by Annandale (1916) and Gee & Wu (1927a) as well. Masuda & Satoh (1989) produced detailed SEM images of *E. conifer* (as *E. coniferus*) to complement Sasaki's (1969) description of Japanese *E. conifer* and referred to the accounts of Annandale (1916, 1918), Penney & Racek (1968) and the differing Sasaki (1969), but dismissed the smooth gemmuloscleres in Japanese material as immature gemmuloscleres and did not discuss them further. Apparently, the little-known redescription by Gee & Wu (1927a) was not known to them as well.

Prior to this study, the original description of *Eunapius conifer* was widely accepted as it is supported by Penney & Racek (1968) and the "World Porifera Database" (Van Soest, 2013). Hence, the identity and characteristics of *E. conifer* had been confusing and uncertain. In order to find what *E. conifer* really is, i.e., whether it possesses the small spiny gemmuloscleres and small gemmules described by Annandale (1916) or the larger smooth gemmuloscleres and larger gemmules described by Gee & Wu (1927a), holotype material, ZEV 7105/7 (Fig. 2A) at ZSI, India and paratype material, USNM 21524 (Fig. 2B & C), slides and dry fragment at Smithsonian Institute, USA were examined. The larger smooth gemmuloscleres and gemmules described in Gee & Wu (1927a) in both holotype paratype material in collections of the Zoological Survey and USNM, were observed. The small spiny gemmuloscleres, and the small gemmules described in Annandale (1916)

were absent. Additional material from Qingdao and Nanjing, China (USNM P0039458 and USNM P0040644) in the "Gee Freshwater Sponge Collection" of the Smithsonian Institution labelled "*Spongilla conifera*" were also examined and observed to be similar to the description provided by Gee & Wu (1927a).

Similar species. — We examined other *Eunapius* species to ensure that *Eunapius conifer* redescribed by Gee & Wu (1927a) is valid. There are 17 valid *Eunapius* species worldwide (Manconi et al., 2008; Manconi & Pronzato, 2007, 2009; Van Soest, 2013). Most *Eunapius* species have only spined gemmuloscleres. To date, only two species, *Eunapius conifer* and *E. carteri*, possess only smooth gemmuloscleres. Both *E. conifer* and *carteri* are variable in morphology and can look similar. The main distinguishing character separating the gemmules of *E. conifer* from those of *E. carteri* is the localisation of gemmuloscleres in the pneumatic layer exclusively around the foramen in *E. conifer*. Unlike *E. carteri*, which typically has abundant gemmuloscleres lying tangentially on the surface of the gemmular layer, the localisation of gemmuloscleres solely in the pneumatic layer was observed in *E. conifer*. This is also the case for the members of the genus *Spongilla*. However, the absence of both microscleres and spiny gemmuloscleres indicates *E. conifer* should be placed in the genus *Eunapius*, and not *Spongilla*. The second key difference is the small size of gemmuloscleres (65–81.5–115 µm × 2–2.6–3 µm) belonging to *E. conifer*. The gemmuloscleres of *E. carteri* are more than twice as large in both length and width, having an average of 166–180–207 µm × 6.1–6.7–9.3 µm compared to those of *E. conifer*. The oxeas and gemmules of *E. conifer* are also significantly smaller than those of *E. carteri*, falling outside the size range recorded for *E. carteri* by Carter (1849), Bowerbank (1863), Annandale (1911), Arndt (1923), Gee (1930, 1932a), Penney & Racek (1968), Soota (1991), Gugel (1995), Manconi & Pronzato (2002), Masuda (2004), Manconi et al. (2008). We also examined BMNH material of *E. carteri* and a fresh *E. carteri* collected from the type locality of var. *mollis* first described by Annandale (1911), and these confirmed the differences in spicule dimension between *E. carteri* and *E. conifer*.

We conclude that *Eunapius conifer* consists of smooth gemmuloscleres 65–115 µm in length and gemmules 250–350 µm in diameter and does not possess spiny gemmuloscleres (30 µm in length) nor minute gemmules (diameter, not more than 140 µm) as erroneously stated in the original description (Annandale, 1916).

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