# A new *Suberites* (Demospongiae: Hadromerida: Suberitidae) from the tropical Indo-West Pacific

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Key words: Porifera; Hadromerida; *Suberites*; new species; Indonesia; Singapore; anchialine lakes; brackish water.
In this paper we describe *Suberites diversicolor* spec. nov. (Porifera: Demospongiae: Hadromerida: Suberitidae) from four enclosed anchialine lakes located in Indonesia and from a confined system in Singapore. Initially this species was thought to be specific to anchialine lakes, but further comparison to coastal areas indicated that it is more widespread in inshore systems. We have used morphological characters to distinguish this species and a molecular marker to confirm that all types are the same species. *Suberites diversicolor* spec. nov. is encrusting or massive with small protrusions or larger globular branches. The external colour can be olive-green, blue, purple, red-orange, or orange-yellow. *Suberites diversicolor* spec. nov. differs from known shallow water species of the genus *Suberites* in the tropical Indo-Pacific due to its diverse display of colour-morphs and the presence of larger tylostyles with a wide size range.

## Introduction

Anchialine lakes, *sensu* Holthuis (1973), are small bodies of seawater that are entirely surrounded by land (Fig. 2A). These lakes are variably connected to the open sea through porous rock or through small subterranean channels. The land-locked pools of water are subjected to a tidal regime which is typically delayed (ranging from 20 minutes to 4 hours) and damped (ranging from 20 cm to 1.5 m) compared to the adjacent sea. The flora and fauna of anchialine lakes are sparsely documented, with a notable exception of a study by Azzini et al. (2007) on eight lakes in Vietnam. Recently, three campaigns were held in Indonesia in 2003 (NWO-KNAW East Kalimantan Program), 2007 (E-WIN Naturalis Raja Ampat Expedition) and 2008 (fieldtrip by first author to anchialine lakes in East Kalimantan) at which time 15 lakes were located and their sponge fauna was documented. Sponges are one of the most dominant taxa in anchialine lakes in terms of species diversity and biomass (de Voogd et al., 2006; Becking & de Voogd, 2008). During the surveys of the lakes one species of the genus *Suberites* was frequently observed and collected. Not all anchialine lakes that were visited contained this species, but when present it was typically rather abundant (often > 1 individual per $m^2$). Individuals were generally large (> 8 cm), growing in the mud, on mangrove roots or on limestone rock. As this *Suberites* species had not been recorded in the coral reefs, it was initially suspected to be a species unique to isolated anchialine lake systems (de Voogd et al. 2006; Azzini et al. 2007). This thought was further enforced by records of previous authors who have described endemic subspecies and new species of other

taxa from these isolated systems, such as the ascidean *Styela complexa* Kott 1995 and the decapod crab *Orcovita saltatrix* Ng & Tomascik, 1994. However, in the same period of the lake surveys a very similar species of *Suberites* was observed in Singapore (by the second author). Though the external colour and growth forms can vary greatly between specimens depending on locality and even within localities, we have concluded that these are indeed the same species based on a closer inspection of the morphology and a molecular marker. We have subsequently encountered this species in coastal mangrove systems in Indonesia and received material from similar lake systems in Vietnam and a man-made marine pool in Darwin, northern Australia. This species is therefore not necessarily restricted to anchialine lakes, yet all localities seem to have in common that the salinity is on average low (29 promille or less) and in all these localities the sponges have the potential to be exposed to air and the strong sun for up to four hours a day. This species appears to be able to tolerate and thrive in such an extreme, intertidal, estuarine environment. We conclude that this is an inshore species with a wide distribution in the Indo-West Pacific in areas with lower salinity than fully marine. Comparison with available literature on *Suberites* species and examination of type material show that this species is new to science.

## Material and methods

Specimens from Indonesia, Vietnam and Australia were collected while snorkelling and the specimens from Singapore were collected from intertidal reef flats during low tides. Where possible the material was preserved in 96% ethanol for DNA analysis and the voucher specimens were preserved in 70% ethanol and deposited in the collections of the National Museum of Natural History, Leiden (RMNH Porifera) and the Zoological Reference Collection, Raffles Museum of Biodiversity Research, National University of Singapore (ZRC). The external morphology, skeletal architecture and shape and size of spicules were examined for all material. Spicule dimensions are based on 25 measurements and given as minimum-average-maximum length × minimum-average-maximum width in the text. To examine the skeletal architecture, hand-cut tangential sections of the ectosome and perpendicular sections of the choanosome were made. The sections were air-dried, mounted in Durcupan® ACM on a microscope slide, and studied under a Leitz high power microscope. Spicule preparations were made by dissolving the organic tissue of a small fragment of the specimen in commercial bleach, after which the spicules were washed 10 times with distilled water and once with 96% ethanol. The spicules were air-dried on microscope slides and mounted with Durcupan® ACM. The spicules were also mounted on aluminium stubs, coated with gold-palladium and studied with a Jeol Scanning Electron Microscope.

For RMNH.POR 4670, 4672, 4673, 4675, 2433, 2434, 2263, 1716 we amplified and sequenced part of the mitochondrial cytochrome oxidase subunit I (COI) using the universal primers and protocol as described by Folmer et al. (1994). We compared the obtained sequences to those of other species of *Suberites* available on GenBank and computed a basic similarity matrix in BioEdit version 5.0.9 (Hall, 1999).

Further abbreviations used in this paper: Zoologisches Museum für Naturkunde an der Universität Humboldt zu Berlin, Berlin, Germany (ZMB), British Museum of Natural History (BMNH).

## Systematics

Phylum Porifera Grant, 1835
Class Demospongiae Sollas, 1885
Order Hadromerida Topsent, 1894
Family Suberitidae Schmidt, 1870
Genus *Suberites* Nardo, 1833

*Suberites diversicolor* spec. nov.
(figs 1A-B, 2B-D, 3A-C; table 1)

*Suberites flabellatus*; *sensu* Dendy, 1916: 135 (not Carter, 1886)

Material.— **Holotype**: RMNH Por. 4672, Indonesia, W Papua, Raja Ampat, Mansuar Island, anchialine lake; 0°35'19.6"S 130°35'48.8"E; 1 m. depth, 20.xi.2007, coll. L.E. Becking, #RAJ04/MOL037. **Paratypes**: RMNH Por. 4673 , Indonesia, W Papua, Raja Ampat, Mansuar island, anchialine lake; 0°35'19.6"S 130°35'48.8"E; 1 m. depth, 20.xi.2007, coll. L.E. Becking, #RAJ04/MOL035; RMNH Por. 2263, Indonesia, NE Kalimantan, Berau, Maratua Island, Danau Haji Buang, anchialine lake; 02°12'31.2"N 118°35'46.8"; 0.5-2 m. depth, 17.x.2003, coll. N.J. de Voogd, #BER18/171003/NV187; RMNH Por. 4670, Indonesia, NE Kalimantan, Berau, Maratua Island, Danau Tanah Bamban, anchialine lake; 02°13'48.8"N 118°34'48.0"E, 0.5-2 m. depth, 26.x.2003, coll. R. Moolenbeek, #BER44/RM15; RMNH.Por. 4675 Singapore; Johor Strait, 0 m. depth, 01°26'02.34"N, 104°02'54.31"E; 20.viii.2008, coll. S.C. Lim; ZRC. Por. 0005 Singapore; Johor Strait, 0 m. depth, 01°26'02.34"N 104°02'54.31E"; 20.viii.2008, coll. S.C. Lim.
Additional material examined: BMNH 1925.11.1.350, labelled '*Suberites glabellatus*' (spelling mistake of *Suberites flabellatus*), Okhamandal, H.I.S.II.1, Dendy Coll..
RMNH Por. 4674, Vietnam, Ha Long Bay, Dau Be Island, small anchialine lake; 20°45'01"N 107°08'53"E, 1-2 m. depth, 27.iv.2004, coll. M. Pansini, F. Azzini & B. Calcinai, #HL182; RMNH Por. 4677, Australia, Northern Territory, Darwin, Lake Alexander, man-made marine lake; 12°25'S, 130°50'E, 0.5-1 m. depth, 15.ii.2008, coll. B. Alvarez, # BAG080215-07. RMNH Por. 4680, Indonesia, W Papua, Raja Ampat, Mansuar island, anchialine lake; 0°35'19.6"S 130°35'48.8"E; 1 m. depth, 20.xi.2007, coll. L.E. Becking, #RAJ04/MOL010; RMNH Por. 4681, Indonesia, W Papua, Raja Ampat, Mansuar island, anchialine lake; 0°35'19.6"S 130°35'48.8"E; 1 m. depth, 20.xi.2007, coll. L.E. Becking, #RAJ04/MOL043; RMNH Por. 4682, Indonesia, W Papua, Raja Ampat, Mansuar island, anchialine lake; 0°35'19.6"S 130°35'48.8"E; 1 m. depth, 20.xi.2007, coll. L.E. Becking, #RAJ04/MOL471; RMNH Por.1716, Indonesia, NE Kalimantan, Berau, Maratua Island, Danau Haji Buang, anchialine lake; 02°12'31.2"N 118°35'46.8"E; 0.5-2 m. depth, 17.x.2003, coll. N.J. de Voogd, #BER18/171003/NV189; RMNH Por. 2433, Indonesia, NE Kalimantan, Berau, Maratua Island, Danau Tanah Bamban, anchialine lake; 02°13'48.8"N 118°34'48.0"E, 0.5-2 m. depth, 26.x.2003, coll. R. Moolenbeek, # BER44/RM16; RMNH Por. 2434, Indonesia, NE Kalimantan, Berau, Maratua Island, Danau Tanah Bamban, anchialine lake; 02°13'48.8"N 118°34'48.0"E, 0.5-2 m. depth, 26.x.2003, coll. R. Moolenbeek, # BER44/RM07; ZRC. Por. 0012 Singapore; Johor Strait, 0 m. depth, 01°26'02.34"N 104°02'54.31"E; 25.vii.2003, coll. S.C. Lim; ZRC. Por. 0014 Singapore; Johor Strait, 0 m. depth, 01°26'02.34"N 104°02'54.31"E; 11.x.2006, coll. S.C. Lim; ZRC. Por. 0015 Singapore; Johor Strait, 0 m. depth, 01°26'02.34"N 104°02'54.31"E; 25.vi.2006, coll. S.C. Lim.

Shape and size. — The holotype is irregularly massive with short, rounded, finger-shaped processes of up to 4 cm in length (fig. 1A&B), approximately 10 × 6 × 9 cm (length × width × height). Larger specimens can occur up to 40 cm in height. Shape is to some degree dependant on the type of habitat. In exposed areas such as coastal tidal mangrove systems and buoys in the sea, this species is encrusting up to 2 cm thick. In areas with little exposure the sponge is massive, irregularly shaped with at times erect,

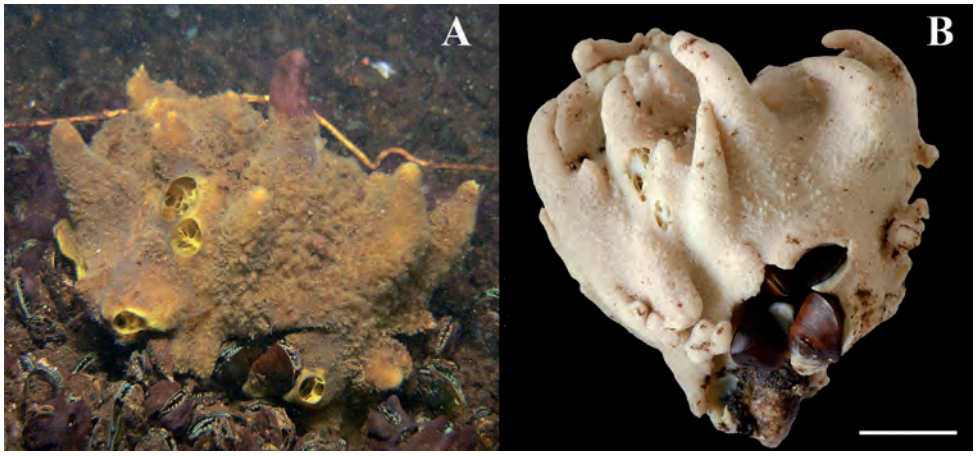


Fig. 1 *Suberites diversicolor* spec. nov. holotype (RMNH Por. 4672), A. habit *in situ*, B. after preservation in ethanol (scale bar = 2 cm).

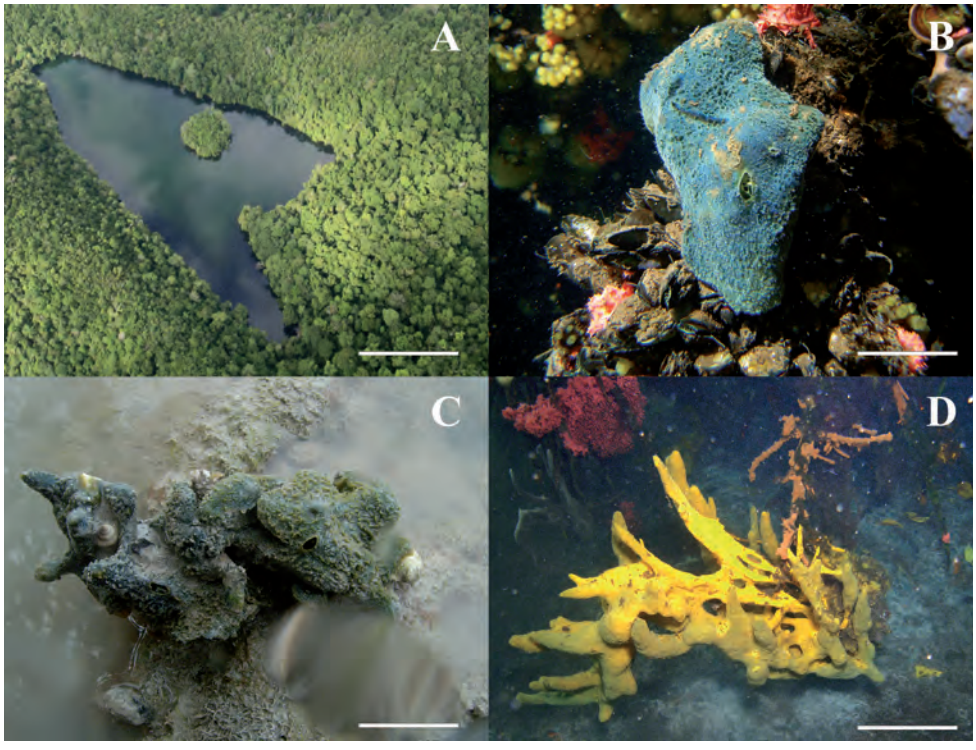


Fig. 2. A, aerial photograph of anchialine lake on Mansuar Island in Raja Ampat, West Papua, Indonesia (scale bar = 100 m.); B, blue morphotype (scale bar = 5 cm.); C, green morphotype exposed to air (scale bar = 5 cm.); D, green-yellow morphotype with globular branches (scale bar = 10 cm.).

globular and slightly flattened branches (fig. 2B-D). Paratype RMNH Por.4670 has globular branches projecting from an ill-defined stalk. The terminal ends of the processes are rounded and without oscules. Compound oscules with a wide diameter range (0.2-2.0 cm) occur sparingly on the upper part of the sponge. When encrusting, oscules are not visible to the naked eye and when in protected areas with high sedimentation, the oscules are greatly enlarged.

Colour.— The holotype is purple-brown externally. Living individuals have a wide range of external colours: olive-green, blue-green, blue-purple, purple, or red-orange (fig. 2B-D); most of the Singapore encrusting material exhibits the same external and internal colour, yet all other material is bright to dark yellow internally. The variable external colouration may be due to the presence and type of photosynthesizing symbionts. All specimens turned light beige after preservation in ethanol.

Surface.— Regularly microhispid to velvety, but can be papillate with irregular rounded protrusions of approximately 30 mm length and 10 mm diameter.

Consistency.— Firm, slightly compressible and elastic with a meat-like consistency.

Skeleton.— Ectosomal skeleton consists of smaller tylostyles (up to 500 μm) at the periphery directed outwards in palisade, carried by tracts of larger tylostyles. Tangential spicules absent. There is no recognizable cortex. The interior skeleton comprises of densely packed tylostyles in vague tracts and/or in confusion. Peripheral choanosomal skeleton consists of closely packed diverging tracts of 60-100 μm in diameter (fig. 3A). The tylostyles have a wide size range and the typical two size categories tylostyles found in *Suberites* species (see definition in Van Soest, 2002) are overlapping in this species.

Spicules.— The megascleres are tylostyles (fig. 3B, C). These are straight, smooth, and sharply pointed at the end; for holotype tylostyle dimensions are 165-499-810 μm length × 2.5-8.9-17.5 μm width (please refer to Table 1 for details of tylostyle dimensions

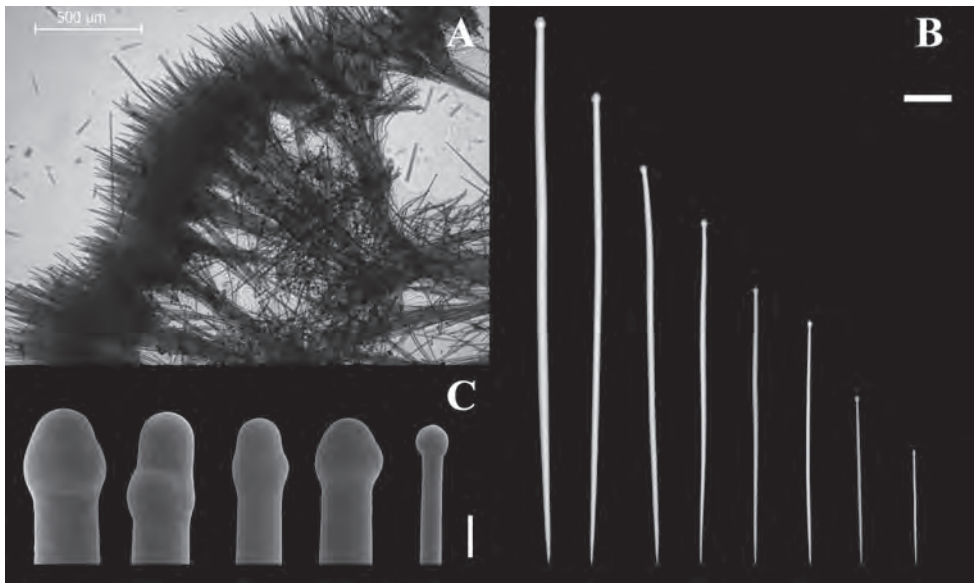


Fig 3. *Suberites diversicolor* spec. nov. A, Cross section of choanosomal skeleton of holotype; B, Sequence of size ranges of tylostyles from paratype ZRC. Por. 0005 (scale bar = 100 μm); C, Tylostyle head variations of paratype RMNH Por. 2263 (scale bar = 10 μm).

Table 1. Region of collection, colour, habit, and tylostyle dimensions (in μm) of types of *Suberites diversicolor* spec. nov. and additional material.

| | | | | Tylostyle length | | | Tylostyle width | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Region** | **Colour** | **Habit** | **Min.** | **Average** | **Max.** | **Min.** | **Average** | **Max.** |
| **RMNH POR.4672** | Papua | purple | massive with processes | 165 | 499 | 810 | 2.5 | 8.85 | 17.5 |
| **RMNH POR.4673** | Papua | olive green | massive with processes | 183 | 470 | 830 | 2.5 | 8.4 | 15 |
| **RMNH POR.4680** | Papua | blue | massive | 127 | 486 | 850 | 5 | 10 | 20 |
| **RMNH POR.4681** | Papua | olive green | massive | 185 | 570 | 860 | 2.5 | 9.7 | 20 |
| **RMNH POR.4682** | Papua | blue | massive | 210 | 572 | 830 | 2.5 | 10.1 | 17.5 |
| **RMNH POR.2263** | Kalimantan | orange | massive with globular branches | 160 | 472 | 790 | 2.5 | 8.4 | 15 |
| **RMNH POR.1716** | Kalimantan | olive green | massive with globular branches | 165 | 476 | 760 | 2.5 | 8.1 | 12.5 |
| **RMNH POR.4670** | Kalimantan | orange | massive with globular branches | 138 | 537 | 930 | 2.5 | 7.2 | 15 |
| **RMNH POR.2433** | Kalimantan | orange | massive with globular branches | 155 | 481 | 770 | 2.5 | 6.5 | 10 |
| **RMNH POR.2434** | Kalimantan | orange | massive with globular branches | 133 | 491 | 890 | 2.5 | 6.7 | 12.5 |
| **RMNH POR.4675** | Singapore | olive green | encrusting | 143 | 486 | 840 | 2.5 | 8.1 | 15 |
| **ZRC.POR.0005** | Singapore | olive green | encrusting | 160 | 522 | 830 | 2.5 | 8.8 | 17.5 |
| **ZRC.POR.0012** | Singapore | olive green | encrusting | 180 | 556 | 930 | 2.5 | 8.7 | 17.5 |
| **ZRC.POR.0013** | Singapore | olive green | encrusting | 110 | 453 | 790 | 2.5 | 7.7 | 17.5 |
| **ZRC.POR.0014** | Singapore | olive green | encrusting | 140 | 466 | 780 | 2.5 | 8.1 | 17.5 |
| **ZRC.POR.0015** | Singapore | olive green | encrusting | 150 | 521 | 960 | 2.5 | 8.2 | 17.5 |
| **RMNH POR.4674** | Vietnam | olive green | massive with globular branches | 220 | 541 | 960 | 2.5 | 6.4 | 15 |
| **RMNH POR.4677** | Australia | olive green | thick encrusting | 117 | 493 | 890 | 2.5 | 7.9 | 15 |

per specimen). Modifications of shape and position of the tylostyles were observed; some tylostyles were slightly curved and variation in form and position of the tylostyle-heads (fig. 3C) was observed.

Ecology.— Found at 0-7 m. depth in anchialine lakes, mangrove systems, and tidal reef flats; growing in mud, on rock, on mangrove roots, on submerged treetrunks/branches, on buoys, and among beds of mussels. Salinity range from 26 to 29 promille. Both solitary and associated with other invertebrates.

DNA Barcode.— The holotype, four paratypes and three specimens of the additional material from three regions described in this paper have one identical genotype for COI and this representative nucleotide sequence of 534 bp in length has been deposited in GenBank with Accession Number: FJ968448. Comparison of this nucleotide sequence to the three available of the same fragment of other *Suberites* species in GenBank showed that *S. diversicolor* spec. nov. differs by 1% to *Suberites aurantiacus* from the Caribbean (Accession number EF519681), 12% to *Suberites ficus* from the northern Atlantic (AJ843891), and 3% from an unidentified *Suberites* sp. from Australia (AY561966).

Distribution.— India, Singapore, Vietnam, Indonesia, and Northern Australia.

Etymology.— Name refers to wide variety of colours that this species can display.

## Discussion

The genus *Suberites* is highly speciose, consisting of more than 70 species (Van Soest et al., 2008). However, most of them are described from temperate seas and only six species are described from the tropical Indo-Pacific of which one is from Indonesia (Van Soest et al., 2008). We will discuss and compare by region these six species as well as three additional species collected from the tropical Indo-Pacific to *Suberites diversicolor* spec. nov., concluding with the additional material examined for this paper and some reports of unidentified *Suberites* sp. from brackish water systems.

The singular description from Ternate, Indonesia, of *Suberites radiatus* Kieschnick (1896) is extremely brief and vague. No mention is made of the dimensions of spicules. Thiele (1900) re-examined and described a part of Kieschnick's material from Ternate, but this species was not included. We could not locate Kieschnick's material at the ZMB (Zoologisches Museum für Naturkunde an der Universität Humboldt zu Berlin, Berlin, Germany). According to Thiele (1900), a part of his collection was lost and it is likely that *S. radiatus* might be among it.

Annandale (1914, 1915) reported two *Suberites* species from the brackish water Chilka Lake in Orissa, India. One is *Suberites aquaedulcioris* (Annandale, 1914) which can occur in a variety of colours (deep orange yellow to bright green). The largest tylostyles in *S. aquaedulcioris* are 330 μm in length (Annandale, 1914). In a later description Annandale (1915) designated this specimen as *Laxosuberites aquaedulcioris,* which in the present accepted classification would be *Protosuberites aquadulcioris*. *Suberites sericeus* Thiele 1898, originally described from Enoshima in Japan, was also reported by Annandale (1915) from Chilka Lake as both encrusting and irregularly massive with spicule dimensions of maximum 440 × 11.7 μm. We have made measurements in specimens of *S. diversicolor* spec. nov. with a variety of size classes (from 5-40 cm in maximum length) and a variety of growth forms (thinly encrusting to massive with globular branches) from three different environmental situations (brackish water protected anchialine lakes, exposed reef flats, more saline inshore systems). All of these *S. diversicolor* specimens examined have a maximum tylostyle length of between 760-960 μm, which is at least two times as long as the maximum lengths reported for the Chilka Lake specimens. In fact, the maximum lengths reported for the Chilka lake specimens are lower than the average lengths of all the specimens of *S. diversicolor* spec. nov (see Table 1). We consider the spicule length of twice the size in *S. diversicolor* spec. nov. as a strong distinguishing character. It is unfortunate that we could not examine Annandale's type materials, but we are confident that he was a trustworthy and reliable taxonomist, as there are no records of significant erroneous spicule measurements in his species descriptions. He described close to one hundred sponges species and the majority of them remain valid.

Two deep sea species *Suberites bengalensis* Lévi, 1964 and *Suberites pisiformis* Lévi, 1993 have been described from India (1190 m. depth) and New Caledonia (400-700 m. depth) respectively. *Suberites bengalensis* differs from the shallow water *S. diversicolor* spec. nov. not only in its distinct deep sea habitat, but also in containing substantially larger tylostyles in two size categories measuring 280-1000 μm × 7-20 μm and 1200-1600 μm × 30-32 μm. *Suberites pisiformis* Lévi, 1993 also differs from the *S. diversicolor* spec. nov. in having either an egg-shaped or spherical growth form and containing tylostyles in three size classes: principal tylostyles 500-700 μm × 8-10 μm, base tylostyles 300-450 μm × 5-8 μm, and peripheral tylostyles, 150-275 μm × 6-10 μm.

Examination of the type material of *Suberites clavatus* Keller, 1891 from the Eritrean Red Sea (ZMB 2696), revealed tylostyles measuring 300-449-530 μm × 5-9.8-15 μm. These are shorter than those of *S. diversicolor* spec. nov. Similar spicule dimensions for *S. clavatus* were reported by Lévi (1965) from the island of Abulat, close to Jeddah in the Red Sea. Another Red Sea species *Suberites tylobtusa* Lévi, 1958 contains tylostrongyles which are not present in *S. diversicolor* spec. nov. Both tylostyles and tylostrongyles in *S. tylobtusa* furthermore have much thicker spicule widths of up to 25 μm.

Based on examination of the type material (slide) of *Suberites laxosuberites* Sollas,

1902 (BMNH 1938.8.17.4) from peninsular Malaysia, we conclude that this species belongs to a different Suberitid genus, namely *Aaptos*. This species contains abundant strongyloxeas in the skeleton instead of tylostyles.

Dendy (1916) reported *Suberites flabellatus* (Carter, 1886), BMNH 1925.11.1.350, from Okhamandal, Western India. The specimen described by Dendy has similar spiculation, skeletal structure and habit to *S. diversicolor* spec. nov., but unfortunately the live colour was not recorded. The tylostyles have a similar wide size range, 110-475.5-755 μm × 4-8.4-15 μm, and there are no apparent size categories, comparable with *S. diversicolor* spec. nov. However, *S. flabellatus sensu* Dendy is probably not conspecific with *S. flabellatus* (Carter, 1886). The latter was originally described from South Australia as flabellate and stipitate with a thick stem. In contrast, Dendy's material is encrusting, massive, irregularly shaped, with short digits. The average length of tylostyles of Carter's *S. flabellatus* is much shorter, about 241.3 μm in length, thus only about half the length of Dendy's (size range was provided by Carter). There are no subsequent reports of *S. flabellatus* which extend its distribution from South Australia to other areas besides India. It is also notably absent in recent studies conducted in the Dampier Archipelago (Fromont, 2004) and Great Barrier Reef (Hooper et al., 1999), suggesting that *S. flabellatus* may be confined to South Australia where it was first described.

Finally, we examined two specimens from an anchialine lake in Vietnam (RMNH Por.4674) and from a man-made marine to brackish water lake in Australia (RMNH Por.4677). This material has the same habit as *S. diversicolor* spec. nov. and has a similarly wide tylostyle size range (see Table 1) without discrete size categories. As we have not been able to obtain the COI sequences, we have chosen not to include this material as paratypes in the present description. Though we strongly suspect that they are conspecific with *S. diversicolor* spec. nov., we prefer to prevent possible confusion in the future in the event that cryptic speciation is demonstrated.

Interestingly, some undetermined *Suberites* species were reported from Lake Motitoi on the island of Satonda in Indonesia which is a brackish water lake in an old volcano crater. Reitner et al. (1999) recorded two morphotypes belonging to a *Suberites* sp. and "*Laxosuberites* sp. (= *Protosuberites*)". They recorded green, brown, yellow-brown and yellow specimens with encrusting or somewhat erect growth forms and ectosomal plumose bundles of short tylostyles (150-200 μm) and larger tylostyles in the choanosome (300-500 μm). There have been additional reports of an olive-green coloured *Suberites* sp. from from Ongeim'l Tketau (Jellyfish Lake) in Palau by L.J. Bell (Coral Reef Research Foundation). These reports may represent *S. diversicolor* spec. nov. or possibly a closely related species.

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