Revision of the genus *Placospongia* (Porifera, Demospongiae, Hadromerida, Placospongiidae) in the Indo-West Pacific

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Abstract

Species of the genus *Placospongia* are common within the tropical Indo-West Pacific, demonstrating a wide variety of colors and either branching or encrusting growth forms. A revision of Indo-West Pacific *Placospongia* was undertaken based on a redescription of the holotypes of species of *Placospongia* from the Indian Ocean and Western Pacific and an examination of an additional 103 specimens of *Placospongia* ssp. collected from Indonesia (including Vosmaer and Vernhout 1902 material), Seychelles, India, Singapore and Micronesia. One mitochondrial (COI) and one nuclear (ITS) marker were subsequently used to differentiate species. All *Placospongia* species are characterized by selenasters and tylostyles in two size classes. The combination of microsclere diversity and morphology as well as megasclere size were shown to be informative morphometric characters, supported by molecular evidence. Live coloration and growth form is shown to be unreliable for diagnoses. The study of holotypes found that *P. mixta* is a valid species and that two genus transfers are necessary: *Geodinella anthosigma* is a *Placospongia* and *P. labyrinthica* is a *Geodia*. A new species is also described from an anchialine pool in Indonesia, *Placospongia santodomingae* sp. n.; bringing the total fauna of *Placospongia* species in the Indo-West Pacific to five: *Placospongia anthosigma, Placospongia carinata, Placospongia mixta, Placospongia melobesioides, and Placospongia santodomingae* sp. n. An identification key is given. Two additional species, possibly morphologically cryptic, have been identified by molecular markers.
Keywords
Sponge, Indonesia, marine lake, coral reef, mangrove, anchialine pool, ITS, COI

Introduction

Species of the genus *Placospongia* in the tropical Indo-West Pacific occur in a wide variety of habitats such as marine lakes, coral reefs and mangroves. They may display a variety of colors and growth forms, from encrusting to branching (Figs 1, 2). Generally only two species have been recorded in species checklists within the Indo-West Pacific (e.g. Burton 1959, Hooper and Wiedenmeyer 1994, Hooper et al. 2000, Becking et al. 2006, de Voogd et al. 2008, de Voogd et al. 2009): *Placospongia melobesioides* Gray 1867, and *Placospongia carinata* (Bowerbank 1858). A recent collection of over 100 *Placospongia* specimens during fieldtrips by the author to Indonesia in 2006 (Sulawesi), 2007 (Papua), 2008, 2009 (Berau), and to Micronesia in 2010 (Yap) revealed, however, that there were more than two species present in these faunas.

The taxonomic literature records six valid species of *Placospongia* worldwide, of which there are three from the Indian Ocean and Western Pacific: *P. carinata* (type locality “South Sea”, presumably in the Pacific), *Placospongia labyrinthica* Kirkpatrick

![Figure 1](image-url). *In situ* underwater images of *Placospongia* spp. in Indonesia, displaying natural variation in color and growth form of live specimens. **A** *Placospongia mixta* (by L.E. Becking) **B** *Placospongia carinata* (by L.E. Becking) **C** *Placospongia carinata* (by L.E. Becking) **D** *Placospongia melobesioides* (by N.J. de Voogd).
1903 (type locality East London, South Africa, Indian Ocean), *P. melobesioides* (type locality Borneo, Pacific). In 1900 Thiele described the species *Placospongia mixta* from Ternate (Indonesia), which was later synonymized with *P. carinata* by Vosmaer and Vernhout in 1902. Vosmaer and Vernhout (1902) based their conclusions on a review of 26 specimens collected during the Siboga expedition to Indonesia, and this collection is presently housed at the Naturalis Biodiversity Center (Leiden, The Netherlands). Subsequently, according to the World Porifera Database (van Soest et al. 2011) *Geodinella anthosigma* Tanita and Hoshino 1989 (type locality Sagami Bay, Japan) should be transferred to the genus *Placospongia*, and *P. labyrinthica* should in fact be transferred to the genus *Geodia*. These suggested genus transfers have, however, not yet been published in the peer-reviewed literature. A molecular phylogeny constructed using the internal transcribed spacer region (ITS) indicated that there were nine evolutionary lineages worldwide within the genus *Placospongia* of which there were five distinct clades in the Indo-Pacific (clades C3, C4, C5, C6 & C9) that may represent five species (Nichols & Barnes 2005). The authors did not investigate the spicule morphology of the specimens in their study, therefore it is unclear which species name can be assigned to the different clades.

**Figure 2.** Gradation of external coloration in preserved specimens. A *Placospongia mixta* RMNH POR. 4492 B *Placospongia mixta* RMNH POR. 4113 C *Placospongia carinata* RMNH POR. 4482 D *Placospongia carinata* RMNH POR. 4483 E *Placospongia mixta* RMNH POR. 3979 F *Placospongia melobesioides* RMNH POR. 4114.
The objectives of the present study were to revise the genus *Placospongia* in the Indo-West Pacific by examining the holotypes of *P. melobesioides*, *P. carinata*, *P. mixta*, as well as 103 specimens of *Placospongia* spp. that were collected from Indonesia (including the Vosmaer & Vernhout material), Singapore, Seychelles, Madagascar, and Micronesia. In order to obtain a full view of the species from the Western Pacific and Indian Ocean the holotypes of the temperate species *G. anthosigma*, and *P. labyrinthica* were also examined. Subsequently it was determined if growth form and color can be used as diagnostic characteristics to identify different species of *Placospongia* in the field. Finally, the aim was to provide species names to the five clades of Indo-Pacific *Placospongia* as published by Nichols and Barnes (2005) by combining their published ITS sequences from GenBank with ITS sequences from identified species of Indo-Pacific *Placospongia*.

**Material and methods**

Specimens from Indonesia were collected via snorkeling in marine lakes and mangroves, and scuba diving in reefs. For a detailed description of marine lakes in Indonesia see Becking et al. (2011). Where possible material was preserved in 96% ethanol for DNA analysis, and voucher specimens were preserved in 70% ethanol and deposited in the collections of the Naturalis Biodiversity Center, Leiden, The Netherlands (RMNH POR.). Records were made on the external morphology, skeletal architecture and spicules of all material. Spicule dimensions were measured of a subset of specimens indicated in Table 1, based on 25 measurements (unless noted otherwise) and given in the text as minimum-average-maximum. The following dimensions were measured: tylostyles length × shaft width × head width; selenasters length × width; streptasters total length × ray length; spherasters diameter; rhabds length × width. Only fully developed spicules were measured. To study the skeletal architecture hand-cut perpendicular sections of the choanosome were made. The sections were air-dried, mounted in Durcupan® ACM on a microscope slide, and studied under a Leica 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.

DNA extractions were made with Qiagen DNEasy animal blood and tissue extraction kit following the manufacturer’s protocol. The polymerase chain reaction (PCR) reaction volume was 25 μl and contained 5 μl Phire® Hot Start reaction buffer, 1 unit Hotstart Phire® Hot Start DNA polymerase (Finnzymes), 2 μl 1 mM dNTPs (Gibco), 1 μl DNA template (5-20 ng) and 0.625 μl of 10mM each primer. The standard DNA-barcoding fragment of the mitochondrial cytochrome oxidase subunit I (COI) fragment was amplified by using a specific forward primer designed by the author for
Table 1. Measurements of spicules of *Placospongia carinata*, *Placospongia melobesioides*, *Placospongia mixta*, and *Placospongia santodomingoae* sp. n. Sample location, growth form, color and spicule measurements provided per specimen. Spicule dimensions are based on 25 measurements and given in the text as minimum-average-maximum. Spheraster measurements in *P. melobesioides* based on less than ten measurements, due to low of abundance in specimens.

|                | tylostyle blunt end | tylostyle sharp end |
|----------------|---------------------|---------------------|
|                | growthform | color live | length | max width | head width | length | max width | head width |
| *P. carinata*  |            |            |        |           |            |        |           |            |
| RMNH POR. 4482 | branching | orange     | 660-726-800 | 10-12.3-15 | 10-14.5-18 | 180-263-410 | 3-5-7.5 | 8-7.5-8   |
| RMNH POR. 4483 | encrusting  | light brown | 610-703.3-800 | 10-13.1-15 | 13-14.9-18 | 190-286.7-470 | 5-6.4-10 | 5-8.6-13  |
| RMNH POR. 4484 | encrusting  | cream      | 560-709.16-920 | 8-11.7-18 | 10-13.9-18 | 175-267.1-550 | 3-4.4-10 | 5-6.4-13  |
| RMNH POR. 4485 | branching   | dark brown | 550-761.2-930 | 10-14-18 | 13-15.5-18 | 210-295.2-450 | 3-5.6-8 | 5-7.6-10  |
| RMNH POR. 744  | encrusting  | purple     | 450-748.6-980 | 8-11.1-13 | 10-13.2-15 | 195-256.8-550 | 5-6.2-10 | 5-6.7-8   |
| RMNH POR. 754  | encrusting  | white      | 540-705.8-830 | 10-12.8-15 | 13-15.2-18 | 280-355.5-500 | 5-7-10   | 5-8.6-13  |
| RMNH POR. 755  | encrusting  | cream      | 560-764.7-910 | 8-12.2-15 | 10-14.7-18 | 250-311.8-360 | 5-7.3-8 | 5-8.2-10  |
| ZMA Por. 10727 | encrusting  | -          | 620-738.7-840 | 8-11.3-13 | 13-15.5-18 | 240-258.3-270 | 3-3.5-6  | 3-4.6-8   |
| ZMA Por. 9189  | branching   | -          | 550-703.3-820 | 10-12.8-15 | 13-15.1-18 | 210-318.8-410 | 5-7.5-10 | 5-9.7-13  |
| *P. melobesioides* |            |            |        |           |            |        |           |            |
| BMNH52.4.1.14  | branching   | dark brown | 670-879.6-1010 | 10-13.2-18 | 10-16.3-20 | 205-293.4-420 | 5-9.9-13 | 5-9.9-13  |
| RMNH POR. 4495 | encrusting  | dark brown | 480-717.6-1040 | 5-9.5-15 | 8-10.3-15 | 190-297.6-370 | 3-5.8-8 | 3-6.1-8   |
| RMNH POR. 4496 | branching   | dark brown | 580-778.4-900 | 8-11.7-15 | 10-14.1-18 | 230-272.8-400 | 5-7.4-10 | 8-9.1-10  |
| RMNH POR. 4497 | branching   | dark brown | 620-745.2-860 | 10-12.2-15 | 13-14.8-18 | 250-320.8-450 | 5-8.8-10 | 5-9.4-13  |
| RMNH POR. 3935 | encrusting  | dark brown | 460-660.9-760 | 10-11.6-15 | 10-13.7-18 | 210-325.8-450 | 3-7.4-13 | 3-8.3-13  |
| RMNH POR. 3166 | encrusting  | dark brown | 460-704.8-810 | 8-11.4-13 | 10-13.2-15 | 200-288.4-470 | 3-9.5-13 | 5-10.8-15 |
| RMNH POR. 3976 | branching   | dark brown | 600-793.6-910 | 10-12-15  | 13-14.8-18 | 190-321.2-450 | 5-8.5-13 | 5-9.6-13  |
| RMNH POR. 3977 | branching   | brown      | 510-683.6-780 | 10-11.5-13 | 13-13.9-15 | 200-326.4-500 | 5-7.5-10 | 8-9.5-13  |
| RMNH POR. 758  | branching   | purple     | 630-853.2-1020 | 10-13.3-15 | 13-15.8-18 | 210-253.2-310 | 5-9.5-13 | 8-11.8-15 |
| RMNH POR. 757  | branching   | white      | 550-829.2-960 | 10-13.3-16 | 13-15.8-18 | 260-302.1-370 | 8-9.6-13 | 10-11.2-15|
| RMNH POR. 2464 | branching   | -          | 710-933.4-1080 | 12.5-15-17.5 | 13-15.7-20 | 240-326.7-330 | 5-9.2-13 | 5-10.8-15 |
| ZMA Por. 10459 | branching   | brown      | 520-670.8-820 | 7.5-11.4-12.5 | 10-13.4-17.5 | 310-362.5-430 | 5-8.8-10 | 5-10.1-13 |
| *P. mixta*     |            |            |        |           |            |        |           |            |
| ZMB3204 (holotype) | encrusting | -          | 355-672.4-940 | 8-12.1-18 | 8-15.6-20 | 165-226.4-275 | 3-6.1-8 | 3-7.8-10  |
| growthform | color live | length | max width | head width | length | max width | head width |
|------------|------------|--------|-----------|------------|--------|-----------|------------|
| encrusting | red        | 480-870 | 10-12.7-15 | 13-15.8-28 | 210-288 | 5-6.2-10 | 5-7.2-10   |
| encrusting | cream      | 550-817 | 10-13.1-15 | 13-15.6-18 | 160-260 | 5-7.3-10 | 5-8.2-12.5 |
| branching  | red        | 550-759 | 10-11.9-15 | 10-14.9-20 | 120-230 | 3-5.9-10 | 3-7.6-10   |
| encrusting | cream      | 630-886 | 10-12.9-15 | 13-15.4-19 | 175-221 | 3-3.9-8  | 2-7.2-10   |
| branching  | cream      | 510-727 | 10-13.6-23 | 150-340 | 3-5.3-8 | 2-6.4-8   |
| encrusting | brown      | 780-1001| 10-14.8-18 | 15-17.5-20 | 240-284 | 5-6.3-8 | 5-8.3-10   |
| encrusting | white      | 610-995 | 10-16-20   | 13-19-25 | 260-274 | 8-9-10  | 8-9-10   |
| encrusting | cream      | 550-990 | 13-16.9-20 | 13-17.5-20 | 130-267 | 5-8.8-15 | 5-9-10    |
| encrusting | red        | 760-914 | 13-17-23   | 10-18-25 | 250-366 | 3-8-13  | 3-8-13    |
| branching  | brown      | 460-761 | 10-14.6-23 | 13-17.38-25 | 220-323 | 6-11.3-15 | 6-11.3-15 |
| encrusting | brown      | 540-758 | 10-12.2-18 | 10-13.8-20 | 180-216 | 3-3.3-5 | 4-4-8     |

Table 1. Continued.

| selenaster | width | spheraster | diameter | streptaster | microrhabd |
|------------|-------|------------|----------|-------------|------------|
| length     |       | total length |            | length ray  | length     | width      |

P. carinata

R122b-BGK1390 (holotype) | 80-90-98 | 60-71.3-85 | 23-33.8-43 | 8-11.6-15 | 8-12.0-18 | 2.5    |
| RMNH POR. 4482 | 65-71.5-75 | 50-58.5-65 | 15-34-48 | 10-13-0.15 | 8-11.7-15 | 2.5    |
| RMNH POR. 4483 | 60-80-85 | 60-62.9-70 | 20-35.7-40 | 10-13.2-15 | 8-11.9-18 | 2.5    |
| RMNH POR. 4484 | 50-61.8-70 | 35-47.4-55 | 25-29.7-35 | 8-11.0-15 | 10-13-1.8 | 2.5    |
| RMNH POR. 4485 | 28-63-73 | 38-50-58 | 20-27.6-38 | 5-9.0-13 | 5-9.4-13 | <2.5  |
| RMNH POR. 744 | 60-66.3-70 | 50-55.6-65 | 25-29.9-38 | 10-12-91.8 | 8-10.8-13 | <2.5  |
| RMNH POR. 754 | 55-67.7-75 | 45-51.8-55 | 25-30.9-38 | 8-9.5-13 | 8-12.3-18 | 2.5    |
| RMNH POR. 755 | 55-61.1-65 | 38-47.5-55 | 30-32.9-38 | 8-9.8-13 | 8-10.2-13 | 2.5    |
| ZMA Por. 10727 | 50-58.8-78 | 35-42.5-63 | 25-27.6-38 | 8-11.1-15 | 8-8.1-10 | <2.5  |
Revision of the genus Placospongia (Porifera, Demospongiae, Hadromerida, Placospongiidae)...

| Specimen | Length | Width | Diameter | Total Length | Length Ray | Width Ray | Micronubert | Length | Width |
|----------|--------|-------|----------|-------------|------------|-----------|-------------|--------|-------|
| ZMA Por. 9189 | 63.72 - 72.78 | 50 - 56.65 | | | | | | 8.92 - 13 | 8.40 - 27.15 |
| P. orocentroides | 58 - 62.6 | 45 - 51.7 - 68 | 15 - 36.8 | | | | | | |
| RANH POR. 4935 | 45 - 50.7 - 75 | 35 - 45.63 | 35 | | | | | | |
| RANH POR. 49164 | 45 - 60.7 - 93 | 35 - 50.65 | 15 - 20 | | | | | | |
| RANH POR. 3156 | 43 - 50.68 | 40 - 52.65 | 15 | | | | | | |
| RANH POR. 3157 | 45 - 60.58 | 40 - 48.53 | | | | | | | |
| RANH POR. 10490 | 45 - 60.58 | 40 - 48.53 | | | | | | | |
| RANH POR. 10491 | 45 - 60.58 | 40 - 48.53 | | | | | | | |
| ZMA Por. 10493 | 45 - 60.75 | 35 - 50.65 | 15 | | | | | | |
| P. mixta | 50 - 55.75 | 35 - 42.63 | 15 | | | | | | |
| ZMB3204 (holotype) | 55 - 69.8 | 43 - 50.65 | 20 - 30 | 15 - 23.93 | 5 - 6.13 | | | 8.85 - 10 | 8.79 - 10.5 |
| ZMA Por. 10490 | 45 - 60.58 | 40 - 52.65 | 15 | | | | | | |
| P. santodomingoae | 63 - 78.75 | 50 - 56.65 | 15 | | | | | | |
| RMNH POR. 44860 (holotype) | 80 - 84.8 | 60 - 72.75 | 20 - 28.12 | 15 - 22.73 | 5 - 6.41 | | | 8.85 - 10 | 8.79 - 10.5 |
| RMNH POR. 44861 | 80 - 84.8 | 60 - 72.75 | 20 - 28.12 | 15 - 22.73 | 5 - 6.41 | | | 8.85 - 10 | 8.79 - 10.5 |


Placospongia P-FO-I-F: GCA GG ATG ATA GGA ACA GGW TTT AG and the degenerated reverse primer from Folmer et al. (1994) designed by Meyer et al. (2005): dgHCO2198: TAA ACT TCA GGG TGA CCA AAR AAY CA. Temperature regime: 94°C for 30s; followed by 35 cycles of 94°C for 5s; 50°C for 5s; 72°C for 12 s; followed by 71°C for 1 min). ITS was amplified with primers from Wörheide (1998) RA2: GTC CCT GCC CTT TGT ACA CA and ITS2.2: CCT GGT TAG TTT CTT TTC CTC CGC). PCR products were purified and sequenced by Macrogen Inc (Korea and The Netherlands). The poriferan origin of the obtained sequences was verified through BLAST searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were handled in SEQUENCHER 4.10.1 (Gene Codes Corporation) and aligned with CLUSTALW and MUSCLE implemented in DAMBE (Xia and Xie 2001). Species of the family Spirastrellidae were selected as outgroup for the phylogenetic analyses. For the COI genetree four specimens of Spirastrella aff. decumbens (RMNH POR. 4505, 4589, 4614) were taken. For the ITS genetree sequences of species from Spirastrellidae were taken from GenBank, as well as ITS sequences of Indo-Pacific Placospongia spp. from the study by Nichols & Barnes (2005), for GenBank accession numbers see Figure 11. The best-fit DNA substitution model was selected by the Akaike Information Criterion deployed in jMODELTEST v. 0.1.1 (Posada 2008) and this model (HKY for COI and GTR+G+I for ITS) was used for subsequent Bayesian and maximum likelihood phylogeny inferences. Phylogenetic reconstructions were performed under Bayesian inference criteria implemented in MrBayes v. 3.1.2. (Huelsenbeck and Ronquist 2001). Each analysis comprised two independent runs of four Metropolis-coupled Markov-chains, sampled at every 1000th generation at the default temperature (0.2). Analyses were terminated after the chains converged significantly as indicated by an average standard deviation of split frequencies <0.001. Convergence was also checked in Tracer v. 1.5.0 (Rambaut and Drummond 2007). For comparison, maximum likelihood bootstrap analyses were conducted using MEGA v. 5.01 (Tamura et al. 2011) using a heuristic search with 1000 bootstrap replicates. Within-group and between-group uncorrected p-distances were calculated in MEGA.

Abbreviations used in this manuscript: Naturalis Biodiversity Center, Leiden, The Netherlands (RMNH POR.), the Zoological Museum of the University of Amsterdam (ZMA Por.), Zoologisches Museum für Naturkunde an der Universität Humboldt zu Berlin, Berlin, Germany (ZMB), The Natural History Museum, London, United Kingdom (BMNH).

**Taxonomy**

Phylum Porifera Grant, 1836  
Class Demospongiae Sollas, 1885  
Order Hadromerida Topsent, 1894  
Family Placospongiidae Gray, 1867  
Genus Placospongia Gray, 1867
Placospongia Gray, 1867
http://species-id.net/wiki/Placospongia

**Type species:** Placospongia melobesioides Gray, 1867 by monotypy

*Description, amended from Systema Porifera (Hooper and van Soest 2002).*

Encrusting to branching growth forms. Small encrustations of 3 cm² to large surfaces of >2m² to branching individual with total size of up to 45 cm in length and branch diameter between 0.25-1.5 cm. Total size of specimens is hard to establish as parts of the body may be encrusting within cracks. Dried material is hard, alcohol preserved and live specimens remain compressible as the choanosome is of more pliant material than the cortex. The surface is made up of smooth cortical plates separated by contractible grooves which form a kind of network on the surface while these are firmly closed in preserved specimens. See Vosmaer & Vernhout (1902) and Rützler (2002) for an extensive description of the genus. In live specimens grooves are open and oscules are visible inside contractile ridges, running between plates. Live color white, cream, orange, reddish brown to dark black-brown (Fig. 1, 2) and come color is usually retained after alcohol preservation. The contact lines between the plates ridge up slightly and are generally a different shade of the color of the plates.

**Skeleton.** The cortical plates consist of densely packed selenasters and can also contain auxiliary microscleres. Developmental stages of selenasters occur throughout the choanosome. Tylostyle tracts support the margins of the cortical plates. In branching specimens radial tylostyle tracts run from the centre core (consisting of densely packed selenaster) to the cortical plates, in encrusting specimens tracts run in direction from substrate to cortex. The sharp ends of the smaller tylostyles are projected beyond the cortex surface. Microscleres occur in the cortex and scattered in choanosomal skeleton. For a detailed description of external morphology and anatomy see Vosmaer and Vernhout (1902).

**Spicules.** Megascleres are tylostyles in two size classes, microscleres are selenasters, and can include choanosomal and ectosomal spirasters (slender-spined streptasters and acanthose microrhabds), spherasters, and/or spherules. Selenasters often remain pigmented after treatment with bleach or nitric acid.

Placospongia anthosigma (Tanita & Hoshino, 1989)
http://species-id.net/wiki/Placospongia_anthosigma

Figure 3

Geodinella anthosigma Tanita & Hoshino, 1989: Fig. 16, Plate III Fig. 1

**Material examined.** Holotype. NSMT-Po R288 (National Museum of Nature and Science, Tokyo, Japan), Japan, Kannonzuka-dashi, Amadaiba, Sagami Bay, 62–67 m depth.
Description. Holotype NSMT-Po R288 encrusting specimen in three pieces of 1–2cm² and 5mm thick, beige to pink in alcohol (Figure 3A).

Spicules. Megascleres large tylostyles with blunt point 520-797-930 × 15-18-20 × 18-20-23 μm, small tylostyles with blunt point 250-320-410 × 10-12-18 × 13-14-18 μm; microscleres selenasters 85-90-98 × 70-73-80 μm, spherasters 15-19-25 μm, stout spirasters with two or three contortions and acanthose spines spirally placed on shaft 8-11-18 × 3-4.5-5 μm (Fig. 3).

Skeleton. As description of genus with addition that spirasters form a layer over and amidst the selenaster cortex and are also prevalent in choanosomal tissue. Spherasters amidst selenaster cortex and dispersed in choanosome.

Distribution. Type locality Sagami Bay, Eastern Japan, presently not recorded from any other locality.

Ecology. On rock substrate in deep temperate waters.

Remarks. Originally described by Tanita and Hoshino (1989) as *Geodinella anthosigma*. *Geodinella* is no longer a valid genus. *Geodinella anthosigma* should be transferred to the genus *Placospongia* based on the external morphology with the characteristic cortical plates and the presence of selenasters, tylostyles and spherasters. *Placospongia anthosigma* is distinguished from the other Indo-Pacific *Placospongia* spp. by the presence of contorted, spirally ornamented spirasters referred to by Tanita and Hoshino (1989) as ‘anthosigma’ and the small class of tylostyles with blunt points.
Revision of the genus Placospongia (Porifera, Demospongiae, Hadromerida, Placospongiidae)

Placospongia carinata (Bowerbank, 1858)
http://species-id.net/wiki/Placospongia_carinata
Figures 4, 5

Geodia carinata Bowerbank, 1858: plate XXV Fig. 19.
Geodia carinata Bowerbank, 1874: plate XLVI Figs 1–5.

Material examined. Holotype. “South Sea”: BMNH R1228 - 86g - Bk.1390 (slide), R1275 - PE01 - Bk1390 (slide).

Vosmaer and Vernhout (1902), Siboga expedition: RMNH POR. 755; RMNH POR. 754; RMNH POR. 744. Other material: RMNH POR. 4484, RMNH POR. 3943, RMNH POR. 3944, RMNH POR. 4485, RMNH POR. 3945, RMNH POR. 3946, RMNH POR. 3947, RMNH POR. 3948, RMNH POR. 3949, RMNH POR. 3950; RMNH POR. 3951, RMNH POR. 3952, RMNH POR. 3953, RMNH POR. 3954, RMNH POR. 3955, RMNH POR. 4482, RMNH POR. 3956, RMNH POR. 3957, RMNH POR. 4483, RMNH POR. 3958; ZMA Por. 8813ZMA Por. 09578; ZMA Por. 11367, ZMA Por. 16584, ZMA Por. 10727, ZMA Por. 1818, ZMA Por. 10481, ZMA Por. 20735; ZMA POR. 9189. (See Table 2 for full details per specimen)

Description. Reviewed material is encrusting and/or branching. External morphology follows the description of the genus. Color of live specimens can be purple brown, chocolate brown, milk coffee brown, orange brown, orange, cream, or white (Fig. 1, 2). Color of choanosome is pale beige. After preservation in ethanol specimens retain some color of the live coloration.

Spicules. Holotype slide with spicules R1228-86g-Bk.1390 (BMNH) and slide with thick section R1275-PE01-Bk1390 (BMNH) (Fig. 4): megascleres large straight tylostyles with blunt ends 500-710-820 × 10-13-15 × 10-15-18 µm, small straight tylostyles with sharp ends 140-317-450 × 5-8-25 × 8-9-13 µm; microscleres selenasters 80-90-98 µm, streptasters with varying number of (spined) rays (5-10) with bifurcating endings or tufts 23-34-43 × 8-15 µm, acantho microrhabds 5-18 × 1-2.5 µm, spherasters absent. The range within the examined material (Table 1 & Fig. 5): megascleres large tylostyles 540-990 × 8-18 × 10-18 µm, small tylostyles 175-550 × 3-10 × 3-13 µm; microscleres selenasters 50-85 × 35-70 µm, streptasters 15-48 × 5-18 µm, acanthose microrhabds 5-18 × 1-2.5 µm, spherasters absent.

Skeleton. As description of genus with addition that microrhabds form a layer over and amidst the selenaster cortex and are also prevalent in choanosomal tissue. Spirasters scattered in choanosome.

Distribution. East African coast to eastern Indonesia (Fig. 9 , Table 2). Originally described from the ‘South Sea’, presumably the South Pacific Ocean. This has been interpreted by some (Rützler 2002, van Soest et al. 2011) to be Palau or Vanuatu, but this remains speculative. Based on the reviewed material and literature the minimal distribution is from Madagascar (Lévi 1956), to the Seychelles, and across Indonesia to the Aru Islands (Table 2). Distribution may extend further East.
Ecology. Depth 0–45m. In Indonesia rarely found in reef environment, but high abundance in marine lakes. Possibly higher prevalence in reefs in Eastern Africa, based on the ZMA Por. collection from the Seychelles and the publication from Madagascar (Lévi 1956).

Remarks. The Bowerbank description from 1858 should be considered as the original description of ‘Geodia carinata’, now accepted as P. carinata, with plates XXV Fig. 19 and XXVI Fig. 10 representing the streptasters (“arborescent elongo-subsphero-stella”). Subsequently in 1874 Bowerbank published a more extensive description of “Geodia carinata” including a drawing of the streptasters (Fig. 3, p.299) and spined microrhabds (“minute multiangulated cylindrical retentive spicula”, fig.2, p.299) that he described as characteristic of the species. In neither
publication registration numbers were provided, however. The habitus drawing in Fig. 5, p299 of Bowerbank publication in 1874 is identical to the specimen BMNH95.6.7.1 that I received from the BMNH after requesting the holotype for *P. carinata*. In addition, I received the slides of spicules (codes: R1228, 86g, Bk.1390) and of the thick cut (codes: R1275, PE01, Bk1390) that were labeled to belong to the holotype (Fig. 5). Upon inspection I discovered that the specimen BMNH 95.6.7.1 is in fact a *P. melobesioides*, while the two slides do indeed represent *P. carinata* containing the characteristic streptasters with bifurcating endings and the microrhabds as indicated in the Bowerbank images and in the images taken from these slides in Fig. 5. The slides clearly do not come from the specimen BMNH 95.6.7.1. In the 16 years between Bowerbank’s 1858 and 1874 publications, I fear that there has been some exchange or misinterpretation of the labels of the specimens resulting in the incorrect assignment of specimen BMNH 95.6.7.1 to the slides and as the holotype of *P. carinata*. This specimen BMNH 95.6.7.1, furthermore, has two labels attached to it: one with “*Geodia carinata*”, and one with “*Placospongia melobesioides*”. According to Bowerbank (1874) three specimens had been reviewed for his manuscript: one received from his friend Mr. Thos. Ingall in 1854, one placed by Dr. Baird from the coral to the sponge collection in the BMNH, and one specimen purchased by Bowerbank in 1864. The first mentioned specimen is presumably the holotype, but as this specimen has not been located, I propose to designate the slides R1228- 86g-Bk.1390 and R1275-PE01-Bk1390 as representing the holotype of *P. carinata*. 

**Figure 5. Placospongia carinata** (RMNH POR. 4483). A selenaster B large tylostyle (head and blunt end) C small tylostyle (head and hastate end) D streptasters, E. acanthose microrhabds.
| registration number | field code | country | province | island | region | locality | habitat | latitude | longitude | depth (m.) | date | collector |
|---------------------|------------|---------|----------|--------|--------|----------|---------|----------|-----------|-----------|------|-----------|
| RMNH POR. 744 #1500 | #1500      | Indonesia | Moluccas | W of Aru | Sula | Sea of Aru | benthic hard | 06°7.5'N | 120°26'E | 0-2 | 6.xii.1899 | Siboga expedition |
| RMNH POR. 754 #1458 | #1458      | Philippines | Mindanao | Mindanao | Mindanao | Mindanao | benthic soft | 02°28.5'S | 123°53'E | 0-2 | 28.viii.1899 | Siboga expedition |
| RMNH POR. 755 #1484 | #1484      | Indonesia | Java | Java | Java | Java | lithothamnion | 131°3'3'E | 120°9'19'E | 0-2 | 20.viii.1899 | L.E. Becking |
| RMNH POR. 3943 #KKB/mol716 | #KKB/mol716 | Indonesia | East Kalimantan | East Kalimantan | East Kalimantan | East Kalimantan | marine lake | 118°31'26.4'E | 0-2 | 0-2 | L.E. Becking |
| registration number | fieldcode | country | province | region | island | locality | collector | depth (m.) | date | collector |
|---------------------|-----------|---------|----------|--------|--------|----------|-----------|------------|------|-----------|
| RMNH POR. 3954      | #MA/mol1055 | Indonesia | East Kalimantan | Berau | Maratua | Haji Buang lake | marine lake | 02°12'31.2"N 118°35'46.8"E | 0-2 | ix.2008 | L.E. Becking |
| RMNH POR. 3955      | #MA/mol1012 | Indonesia | East Kalimantan | Berau | Maratua | Haji Buang lake | marine lake | 02°12'31.2"N 118°35'46.8"E | 0-2 | ix.2008 | L.E. Becking |
| RMNH POR. 3956      | #MA/mol1001 | Indonesia | East Kalimantan | Berau | Maratua | Haji Buang lake | marine lake | 02°12'31.2"N 118°35'46.8"E | 0-2 | ix.2008 | L.E. Becking |
| RMNH POR. 3957      | #MA/mol1097 | Indonesia | East Kalimantan | Berau | Maratua | Haji Buang lake | marine lake | 02°12'31.2"N 118°35'46.8"E | 0-2 | ix.2008 | L.E. Becking |
| RMNH POR. 3958      | #MA/mol1500 | Indonesia | East Kalimantan | Berau | Maratua | Haji Buang lake | marine lake | 02°12'31.2"N 118°35'46.8"E | 0-2 | ix.2008 | L.E. Becking |
| RMNH POR. 4482      | #MA/mol1061 | Indonesia | East Kalimantan | Berau | Maratua | Kakaban lake | marine lake | 02°08'57.3"N 118°31'26.4"E | 0-2 | ix.2008 | L.E. Becking |
| RMNH POR. 4483      | #MA/mol110 | Indonesia | East Kalimantan | Berau | Maratua | Kakaban lake | marine lake | 02°08'57.3"N 118°31'26.4"E | 0-2 | ix.2008 | L.E. Becking |
| RMNH POR. 4484      | #KKB/mol1110 | Indonesia | East Kalimantan | Berau | Maratua | Kakaban lake | marine lake | 02°08'57.3"N 118°31'26.4"E | 0-2 | ix.2008 | L.E. Becking |
| RMNH POR. 4485      | #KKB/mol763 | Indonesia | East Kalimantan | Berau | Maratua | Kakaban lake | marine lake | 02°08'57.3"N 118°31'26.4"E | 0-2 | ix.2008 | L.E. Becking |
| ZMA Por. 1818       | #MA/mol1018 | Indonesia | Maluku | Palau | Banda Islands | reef | reef | 04°32'23.3"S 129°54'28.8"E | 9-45 | 22.xi.1899 | Siboga expedition |
| ZMA Por. 9578       | #KKB/mol978 | Singapore | | | | reef | reef | 01°12'59.0"N 105°42.25.2"E | 2 | 22.xii.1977 | H. Moll |
| ZMA Por. 8813       | #KKB/mol763 | Indonesia | Nusa Tenggara | Komodo | | reef | reef | 08°26'40.0"S 119°34'48.8"E | 30 | 19.x.1984 | National Institute of Oceanography |
| ZMA Por. 9189       | #MA/mol1061 | India | | | | reef | reef | | | | 20-25 | 1987 |
| registration number | fieldcode | country | province | region | island   | locality                        | habitat | latitude | longitude | depth (m.) | date       | collector   |
|---------------------|-----------|---------|----------|--------|----------|---------------------------------|---------|----------|-----------|------------|------------|-------------|
| ZMA Por. 10481      | Seychelles| Mahé    | Mahé     | SE     | SE coast, near Pointe Cocos      | reef    |          |           |            | 35-45      | 24.xii.1992 | R.W.M. van Soest |
| ZMA Por. 10727      | Seychelles| Mahé    | Mahé     | NE     | Pointe Cocos                      | reef    | 04°34'59.9"S | 055°28'0.1"E | 1          | 14.xii.1992 | R.W.M. van Soest |
| ZMA Por. 11367      | Seychelles| Mahé    | Mahé     | N      | Aride                             | reef    | 04°10'59.9"S | 055°40'0.1"E | 40         | 19.xii.1992 | R.W.M. van Soest |
| ZMA Por. 16584      | Seychelles| Mahé    | Mahé     | SW     | coast, Baie Lazare, Anse Gaulettes | reef    | 04°10'59.9"S | 055°40'0.1"E | 1-4        | 6.xii.1992  | R.W.M. van Soest |
| ZMA Por. 20735      | Seychelles| Mahé    | Mahé     |        |                                   | reef    |          |           |            | 1992       |             | R.W.M. van Soest |
Revision of the genus *Placospongia* (*Porifera, Demospongiae, Hadromerida, Placospongiidae*)...

*Placospongia melobesioides* Gray, 1867

http://species-id.net/wiki/Placospongia_melobesioides

Figure 6

*Placospongia melobesioides* Gray (1867): Figs 1–4.

**Material examined.** **Holotype.** BMNH 52.4.1.14, Indonesia, Borneo island.

*Vosmaer and Vernhout (1902), *Siboga expedition*: RMNH POR. 756, RMNH POR. 761, RMNH POR. 758, RMNH POR. 757, RMNH POR. 760, RMNH POR. 759. **Other material**: RMNH POR. 4497, RMNH POR. 4496, RMNH POR. 4495, RMNH POR. 4114, RMNH POR. 3978, RMNH POR. 3977, RMNH POR. 3976, RMNH POR. 3942, RMNH POR. 3941, RMNH POR. 3940, RMNH POR. 3939, RMNH POR. 3938, RMNH POR. 3937, RMNH POR. 3935, RMNH POR. 3934, RMNH POR. 3933, RMNH POR. 3932, RMNH POR. 3177, RMNH POR. 3166, RMNH POR. 3154, RMNH POR. 2464, RMNH POR. 2463, ZMA Por. 13097, ZMA Por. 10459 (See Table 3 for full details per specimen)

**Description.** Holotype BMNH 52.4.1.14 dry, chalky white angular branches, hard. Other examined material encrusting to branching, hard, thicker specimens slightly compressible. External morphology follows the description of the genus. Size ranging between 5-50 cm, though encrusting specimens may be larger growing within crevices. Ectosome color in life ranging from purple, dark black brown, chocolate brown, orange brown to light beige (Fig. 1, 2). Choanosome pale beige. After preservation color of ectosome is similar to live color.

**Spicules.** Holotype BMNH 52.4.1.14 (Fig. 6): Megascleres large straight tylostyles with blunt ends 670-880-1010 × 10-13-18 × 10-16-20 μm, small concave to straight tylostyles with sharp ends 205-293-420 × 5-10-13 × 5-10-13 μm. Microscleres selenasters 58-63-68 × 45-52-68 μm, spherasters 15-17-18 μm (five measurements, not abundant), spherules 1-2-3 μm. The range within the examined material (Table 1): large tylostyles 460-1040 × 5-16 × 8-18 μm, small tylostyles 190-470 × 3-13 × 3-15 μm, selenasters 45-83 × 30-65 μm, spherules 1-3 μm, spherasters only found in singles in some individuals 15-20 μm. Streptasters and microrhabds absent.

**Skeleton.** As description of genus with addition of sporadic spherasters lodged amidst selenasters in cortex and high abundance of spherules in choanosome and cortex.

**Ecology.** Depth: 0-45m. Reefs, rocky shores, reefflats, mangroves, and marine lakes.

**Distribution.** Type locality: Borneo. Distribution from Seychelles to Micronesia (Fig. 9, Table 3). Possibly further east to Central Pacific.

**Remarks.** In the original description by Gray (1867) there is no mention of two size classes of tylostyles. I reexamined the original slide and conclude that the holotype does contain two size classes of tylostyles. The Systema Porifera indicates that the holotype has two size classes, the large 720-963-1200 × 13-14.1-19 μm and the small 350-438.8-560 × 8-9.1-10.5 μm, based on 10 measurements per spicule type (Rützler 2002). These measurements deviate from the holotype measurements in the present
study that were based on 25 measurements per spicule type (670-880-1010 × 10-13-18 μm and 205-293-420 × 5-10-13 μm respectively), and also deviate from the range of sizes within the examined material of this study (Table 1). There is great variation in tylostyle length and spherasters are only sporadically present, often absent.

*Placospongia mixta* Thiele 1900
http://species-id.net/wiki/Placospongia_mixta
Figure 7

*Placospongia mixta* Thiele, 1900: Plate III, Fig. 25.

**Material examined.** Holotype. ZMB 3204, Indonesia, Moluccas, Ternate.  
Vosmaerand Vernhout (1902), Siboga expedition: RMNH POR. 753, RMNH POR. 751, RMNH POR. 745, RMNH POR. 742. Other material: RMNH POR. 4494, RMNH POR. 4493, RMNH, POR. 4492, RMNH POR. 4491, RMNH POR. 4490, RMNH POR. 4489, RMNH POR. 4113, RMNH POR. 4112, RMNH, POR. 3979, RMNH POR. 3975, RMNH POR. 3974, RMNH POR. 3973, RMNH POR. 3972, RMNH POR. 3971, RMNH POR. 3970, RMNH POR. 3969, RMNH POR. 3968, RMNH POR. 3967, RMNH POR. 3966, RMNH POR. 3965, RMNH
Revision of the genus Placospongia (Porifera, Demospongiae, Hadromerida, Placospongiidae)

POR. 3964, RMNH POR. 3963, RMNH POR. 3962, RMNH POR. 3961, RMNH POR. 3960, RMNH POR. 3959, RMNH, POR. 3163, RMNH POR. 3158, RMNH POR. 3157, RMNH POR. 3155, RMNH POR. 3148, ZMA Por. 10495, ZMA Por. 896 (See Table 4 for full details per specimen)

**Description.** Holotype ZMB 3204 encrusting, size 5 × 2.5 cm and thickness 1–5 mm (as described by Thiele, now very small fragment), white after preservation in alcohol. The majority of the reviewed material is encrusting with a thickness of 4–10 mm, but branching specimens also occur. External morphology follows the description of the genus. Color of the ectosome can be red, orange, brown orange, dark brown, chocolate brown, milk coffee brown, cream, or white (Fig. 1, 2). Color of choanosome is pale beige. After preservation in ethanol color is similar to live specimens, but lighter shade.

**Spicules.** Holotype ZMB 3204 (Fig. 6) Megascleres large straight tylostyles with blunt/rounded point 355-672-940 × 7.5-12-17.5 × 7.5-16-20 μm, small straight tylostyles with sharp point 165-226-275 × 2.5-6-7.5 × 2.5-8-10 μm; microscleres selenasters 55-70-75 × 42.5-55-72.5 μm, spherasters (abundant) 20-25-30 μm, streptasters typically with well developed axis and with 4-9 rays with hastate tips, rays are smooth or can be spined, but do not have bifurcations of the tips 15-24-32.5 × 2.5-8-12.5 μm; acanthose microrhabs with straight or zig-zag axis 5-7-10 × <2.5 μm. The range within the examined material (Table 1): large tylostyles 460-1250 × 8-23 × 10-25 μm, small tylostyles 120-430 × 3-15 × 2-15 μm, selenasters 50-85 × 22-73 μm, spherasters 13-30 μm, streptasters 15-35 × 2-15 μm, rays 5-18 × 1-2.5 μm.

**Figure 7.** *Placospongia mixta* holotype (ZMB 3204). **A** selenaster **B** large tylostyle (head and blunt end) **C** small tylostyle (head and hastate end) **D** spheraster **E** streptasters **F** microacanthose microrhabds.
### Table 3. Location details of reviewed specimens of *Placospongia melobesioides.*

| registration number | fieldcode | country     | province | region      | island            | locality | habitat       | latitude     | longitude     | depth (m.) | date   | collector            |
|--------------------|-----------|-------------|----------|-------------|-------------------|----------|--------------|--------------|--------------|------------|--------|---------------------|
| RMNH POR. 761      | #1033     | Indonesia   | S of Moluccas | S of Moluccas | sand & rock       | 04°12'S 129°20.4'E | 45           | 1899         | Siboga expedition |
| RMNH POR. 756      | #660      | Indonesia   | Nusa Tenggara | N of Sumbawa | sand & rock       | 07°12.6'S 118°7.7'E | 36           | 14.ii.1900 | Siboga expedition |
| RMNH POR. 757      | #1849     | Indonesia   | Moluccas    | SE of Misool | sand & rock       | 32          | 1899         | Siboga expedition |
| RMNH POR. 758      | #1847     | Indonesia   | Moluccas    | SE of Misool | sand & rock       | 32          | 1899         | Siboga expedition |
| RMNH POR. 759      | #1853     | Indonesia   | Moluccas    | SE of Misool | sand & rock       | 32          | 1899         | Siboga expedition |
| RMNH POR. 760      | #1851     | Indonesia   | Moluccas    | SE of Misool | sand & rock       | 32          | 1899         | Siboga expedition |
| RMNH POR. 2463     | #Sin05/270306/025 | Singapore | Semaku     | Pulau Semakau NW side | reef | 01°13'70"N 103°45'61"E | 10-12 | iii.2006 | N.J. de Voogd |
| RMNH POR. 2464     | #Sin05/270306/026 | Singapore | Semaku     | Pulau Semakau NW side | reef | 01°13'70"N 103°45'61"E | 10-12 | iii.2006 | N.J. de Voogd |
| RMNH POR. 3154     | #LEMD05/30 | Indonesia   | North Sulawesi | Bunsaken     | Pangalisang       | 01°37'26"N 124°46'55"E | 9            | 24.ix.2006 | L.E.Beaiking |
| RMNH POR. 3166     | #LEMD13/69 | Indonesia   | North Sulawesi | Bunsaken     | Pangalisang       | 01°37'26"N 124°46'55"E | 19           | 28.xx.2006 | L.E.Beaiking |
| RMNH POR. 3177     | #LEMD22/87 | Indonesia   | North Sulawesi | Bunsaken     | Likuan2           | 01°35'78"N 124°46'06"E | 21           | 13.x.2006 | L.E.Beaiking |
| RMNH POR. 3932     | #KKB/mol866 | Indonesia   | East Kalimantan | Berau        | Kakaban lake      | 0°20'57.3"N 118°31'26.4"E | 0-2          | ix.2008  | L.E.Beaiking |
| RMNH POR. 3933     | #KKB/mol766 | Indonesia   | East Kalimantan | Berau        | Kakaban lake      | 0°20'57.3"N 118°31'26.4"E | 0-2          | ix.2008  | L.E.Beaiking |
| Registration number | Collector | Collecting date | Habitat | Latitude | Longitude | Depth (m.) | Island | Province | Region | Country | Fieldcode |
|---------------------|-----------|-----------------|---------|----------|-----------|------------|--------|----------|--------|---------|-----------|
| RMNH POR. 3934 | L.E. Becking | ix.2008 | lake | 02°08'57.3"N | 118°31'26.4"E | 0-2 | East Kali-mantan | Indonesia | East Kali-mantan | Kakaban |
| RMNH POR. 3935 | L.E. Becking | 15.viii.2008 | reef | 02°17'32.3"N | 118°35'26.1"E | 5-10 | NE Maratua | Indonesia | East Kali-mantan | Berau |
| RMNH POR. 3937 | L.E. Becking | 15.viii.2008 | reef | 02°05'36.6"N | 118°24'15.2"E | 5-10 | E Sangalaki | Indonesia | East Kali-mantan | Berau |
| RMNH POR. 3938 | L.E. Becking | 15.viii.2008 | reef | 02°05'36.6"N | 118°24'15.2"E | 5-10 | W Sangalaki | Indonesia | East Kali-mantan | Berau |
| RMNH POR. 3939 | L.E. Becking | 28.viii.2010 | reef in front of mangrove | 09°31'36.7"N | 138°07'48.7"E | 1-3 | Yap | Micronesia | Yap | \ |
| RMNH POR. 3940 | L.E. Becking | 28.viii.2010 | reef in front of mangrove | 09°31'36.7"N | 138°07'48.7"E | 1-3 | Yap | Micronesia | Yap | \ |
| RMNH POR. 3941 | L.E. Becking | 28.viii.2010 | reef in front of mangrove | 09°31'36.7"N | 138°07'48.7"E | 1-3 | Yap | Micronesia | Yap | \ |
| RMNH POR. 3942 | L.E. Becking | 28.viii.2010 | reef in front of mangrove | 09°31'36.7"N | 138°07'48.7"E | 1-3 | Yap | Micronesia | Yap | \ |
| RMNH POR. 3943 | L.E. Becking | 28.viii.2010 | reef in front of mangrove | 09°31'36.7"N | 138°07'48.7"E | 1-3 | Yap | Micronesia | Yap | \ |
| RMNH POR. 3944 | L.E. Becking | 28.viii.2010 | reef in front of mangrove | 09°31'36.7"N | 138°07'48.7"E | 1-3 | Yap | Micronesia | Yap | \ |
| RMNH POR. 3945 | L.E. Becking | 28.viii.2010 | reef in front of mangrove | 09°31'36.7"N | 138°07'48.7"E | 1-3 | Yap | Micronesia | Yap | \ |
| RMNH POR. 3946 | L.E. Becking | 28.viii.2010 | reef in front of mangrove | 09°31'36.7"N | 138°07'48.7"E | 1-3 | Yap | Micronesia | Yap | \ |
| registration number | fieldcode | country       | province       | region         | island       | locality     | habitat       | latitude       | longitude       | depth (m.) | date       | collector         |
|---------------------|-----------|---------------|----------------|----------------|--------------|--------------|---------------|----------------|----------------|------------|------------|------------------|
| RMNH POR. 4496      | #KKB/mol776 | Indonesia     | East Kaltimantan | Berau          | Kakaban      | lake         | marine lake   | 02°08'57.3"N  | 118°31'26.4"E | 0-2        | ix.2008    | L.E. Becking     |
| RMNH POR. 4497      | #BER107/mol603 | Indonesia     | East Kaltimantan | Berau          | Sangalaki    | E Sangalaki  | reef          | 02°05'36.6"N  | 118°24'15.2"E | 5-10       | 15.viii.2008 | L.E. Becking     |
| ZMA Por. 10459      |           | Seychelles    | Mahé           | Mahé           | NE coast,    | North East Point | reef         | 04°34'59.9"S  | 055°28'0.1"E   | 5          | 8.xii.1992  | R.W.M. van Soest  |
| ZMA Por. 10496      |           | Seychelles    | Mahé           | Mahé           | North East Point | reef       | 04°34'59.9"S  | 055°28'0.1"E   | 14.xii.1992 | R.W.M. van Soest    |
| ZMA Por. 13097      |           | Indonesia     | South Sulawesi | Spermonde archipelago | Samalona | reef         |               | 5-30       | 27.iv.1997   | N.J. de Voogd |
Skeleton. As description of genus with addition that microrhabds form a layer over and amidst the selenaster cortex and are also prevalent in choanosomal tissue. Streptasters scattered in choanosome. Spherasters amidst selenasters in cortex and scattered in choanosome.

Distribution. East African coast to eastern Indonesia (Fig. 9, Table 4). Possibly further east to Central Pacific. Pulitzer-Finali (1993) identified a ‘P. carinata’ from East Africa (Mombasa) that fits the description of *P. mixta* based on the length of the tylostyles (up to 1200 μm) and the presence of spherasters, but no *P. mixta* specimens were observed in the Seychelles material deposited at ZMA.

Ecology. Depth 0–45m. Common in reefs, also occurs in marine lakes.

Remarks. In 1900 Thiele described a new species named *P. mixta*, which was originally identified as *P. melobesioides* by Kieschnick (1896). The specific epithet *mixta* was given because the specimen contained a mixture of spicules: both spirasters like *P. carinata* as well as large spherasters like *P. intermedia* and *P. melobesioides*, which are absent in *P. carinata*. In 1902 Vosmaer & Vernhout decided that *P. mixta* was a junior synonym of *P. carinata*, because they saw no distinction between the different shapes of streptasters and stated that spherasters are never very abundant – in some ‘exceedingly rare and in some we failed to find them at all’ – and could therefore not be seen as a distinguishing character. The specimens that were studied by Vosmaer and Vernhout (1902) were collected in Indonesia during the Siboga Expedition (1899-1900) and are housed in the collection of the Naturalis Biodiversity Center (Leiden, The Netherlands). In the present study these specimens were reexamined. After inspection, the specimens labeled ‘*P. carinata*’ could be clearly and consistently divided into two species: *P. carinata* without spherasters, with streptasters displaying bifurcating tips, and tylostyles up to 980 μm, and *P. mixta* with abundant spherasters, with streptasters displaying hastate tips, and tylostyles up to 1250 μm. In none of the specimens of Vosmaer & Vernhout (1902), nor of the other specimens reviewed for this study was there a mixture of the two types of streptasters. These two species also show molecular distinction in both mitochondrial and nuclear markers (Fig. 10, 11, Table 6, 7).

**Placospongia santodomingoae** sp. n.

urn:lsid:zoobank.org:act:3C4F2599-15C0-4075-BD3B-8C6439C8F821
http://species-id.net/wiki/Placospongia_santodomingoae

Figure 8

**Holotype.** RMNH POR. 4486, Indonesia, East Kalimantan province, Maratua island, Buli Halo anchialine pool, 02°11’16.4”N, 118°37’06.4”E, 0–1m. depth, xi.2008, coll. N.K. Santodomingo & Estradivari, #BER128/mol1147. **Paratypes.** RMNH POR. 4487, Indonesia, East Kalimantan province, Maratua island, Buli Halo anchialine pool, 02°11’16.4”N, 118°37’06.4”E, 0–1m. depth, xi.2008, coll. N. K. Santodomingo & Estradivari; RMNH POR. 4488, Indonesia, East Kalimantan province, Marat-
Table 4. Location details of reviewed specimens of *Placospongia mixta*.

| Registration number | Fieldcode | Country | Province | Region | Island | Locality | Habitat | Latitude | Longitude | Depth | Date | Collector |
|---------------------|-----------|---------|----------|--------|--------|----------|---------|----------|-----------|-------|-------|-----------|
| RMNH POR. 753 | #311 | Indonesia | West Papua | E. of West | Misool | E. of Misool | sand & rock | 01°42.5'S | 130°47.5'E | 32 | 20.viii.1899 | Siboga expedition |
| RMNH POR. 751 | #1857 | Indonesia | West Papua | E. of West | Misool | E. of Misool | sand & rock | 01°42.5'S | 130°47.5'E | 32 | 20.viii.1899 | Siboga expedition |
| RMNH POR. 754 | #577 | Indonesia | South Sulawesi | | | N. of Kabia | Selayar anchorage | 01°42.5'S | 130°47.5'E | 36 | 23.viii.1899 | Siboga expedition |
| RMNH POR. 742 | #63a | Indonesia | Moluccas | | | Aru | Pearl Banks anchorage | 01°42.5'S | 130°47.5'E | 13 | 23.viii.1899 | L.E. Becking |
| RMNH POR. 3148 | #LEMD04/21 | Indonesia | North Sulawesi | | | Lembah Lemuken | reef | 01°27.57'S | 124°46.06'E | 15 | 24.vi.2006 | L.E. Becking |
| RMNH POR. 3155 | #LEMD08/32 | Indonesia | North Sulawesi | | | Nudi Reef | reef | 01°27.57'S | 124°46.06'E | 21 | 25.vii.2006 | L.E. Becking |
| RMNH POR. 3157 | #LEMD08/39 | Indonesia | North Sulawesi | | | Nudi Fals | reef | 01°27.57'S | 124°46.06'E | 6 | 25.vii.2006 | L.E. Becking |
| RMNH POR. 3158 | #LEMD11/52 | Indonesia | South Sulawesi | | | 0-5 km W of Lembah Lemuken | reef | 01°27.57'S | 124°46.06'E | 8 | 25.vii.2006 | L.E. Becking |
| RMNH POR. 3163 | #LEMD09/42 | Indonesia | East Kalimantan | | | Beru | reef | 01°27.57'S | 124°46.06'E | 10 | 25.vii.2006 | L.E. Becking |
| RMNH POR. 3959 | #KKB/mol827 | Indonesia | East Kalimantan | | | Beru | reef | 02°08.57'N | 118°31.26'E | 21 | 27.vii.2008 | L.E. Becking |
| RMNH POR. 3960 | #KKB/mol829 | Indonesia | East Kalimantan | | | Beru | reef | 02°08.57'N | 118°31.26'E | 8 | 27.vii.2008 | L.E. Becking |
| RMNH POR. 3961 | #KKB/mol851 | Indonesia | East Kalimantan | | | Beru | reef | 02°08.57'N | 118°31.26'E | 21 | 27.vii.2008 | L.E. Becking |
| RMNH POR. 3962 | #BER111/mol203 | Indonesia | East Kalimantan | Berau | | reef | 02°08.07'N | 118°30.23'E | 10 | 27.vii.2008 | N.J. de Voogd |
Revision of the genus Placospongia (Porifera, Demospongiae, Hadromerida, Placospongiidae)...

| Registration number | Fieldcode | Country | Province | Island | Region | Collector | Latitude | Longitude | Depth | Date | Habitat        |
|---------------------|-----------|---------|----------|--------|--------|-----------|----------|-----------|-------|------|----------------|
| RMNH POR. 3963      | #BER11/1  | Indonesia | East Kali- | Kakaban | SW Kakaban | N.J. de Voogd | 02°08'07.7"N | 118°30'23.5"E | 10   | 17.vii.2008 | reef             |
| RMNH POR. 3964      | #BER11/2 | Indonesia | East Kali- | Kakaban | SW Kakaban | N.J. de Voogd | 02°08'07.7"N | 118°30'23.5"E | 10   | 17.vii.2008 | reef             |
| RMNH POR. 3965      | #BER11/3 | Indonesia | East Kali- | Kakaban | SW Kakaban | N.J. de Voogd | 02°08'07.7"N | 118°30'23.5"E | 10   | 17.vii.2008 | reef             |
| RMNH POR. 3966      | #BER11/4 | Indonesia | East Kali- | Kakaban | SW Kakaban | N.J. de Voogd | 02°08'07.7"N | 118°30'23.5"E | 10   | 17.vii.2008 | reef             |
| RMNH POR. 3967      | #BER11/5 | Indonesia | East Kali- | Kakaban | SW Kakaban | N.J. de Voogd | 02°08'07.7"N | 118°30'23.5"E | 10   | 17.vii.2008 | reef             |

| Registration number | Fieldcode | Country | Province | Island | Region | Collector | Latitude | Longitude | Depth | Date | Habitat        |
|---------------------|-----------|---------|----------|--------|--------|-----------|----------|-----------|-------|------|----------------|
| RMNH POR. 3968      | #RAJ129/  | Indonesia | West Papua | Waigeo | Waigeo | L.E. Becking | 00°18'17.5"S | 130°54'15.6"E | 10   | xi.2007 | reef             |
| RMNH POR. 3969      | #RAJ130/  | Indonesia | West Papua | Waigeo | Waigeo | L.E. Becking | 00°18'17.5"S | 130°54'15.6"E | 10   | xi.2007 | reef             |
| RMNH POR. 3970      | #RAJ131/  | Indonesia | West Papua | Waigeo | Waigeo | L.E. Becking | 00°18'17.5"S | 130°54'15.6"E | 10   | xi.2007 | reef             |
| RMNH POR. 3971      | #RAJ132/  | Indonesia | West Papua | Waigeo | Waigeo | L.E. Becking | 00°18'17.5"S | 130°54'15.6"E | 10   | xi.2007 | reef             |
| RMNH POR. 3972      | #RAJ133/  | Indonesia | West Papua | Waigeo | Waigeo | L.E. Becking | 00°18'17.5"S | 130°54'15.6"E | 10   | xi.2007 | reef             |
| RMNH POR. 3973      | #RAJ134/  | Indonesia | West Papua | Waigeo | Waigeo | L.E. Becking | 00°18'17.5"S | 130°54'15.6"E | 10   | xi.2007 | reef             |
| RMNH POR. 3974      | #RAJ135/  | Indonesia | West Papua | Waigeo | Waigeo | L.E. Becking | 00°18'17.5"S | 130°54'15.6"E | 10   | xi.2007 | reef             |
| RMNH POR. 3975      | #RAJ136/  | Indonesia | West Papua | Waigeo | Waigeo | L.E. Becking | 00°18'17.5"S | 130°54'15.6"E | 10   | xi.2007 | reef             |
| RMNH POR. 3976      | #RAJ137/  | Indonesia | West Papua | Waigeo | Waigeo | L.E. Becking | 00°18'17.5"S | 130°54'15.6"E | 10   | xi.2007 | reef             |
| RMNH POR. 3977      | #RAJ138/  | Indonesia | West Papua | Waigeo | Waigeo | L.E. Becking | 00°18'17.5"S | 130°54'15.6"E | 10   | xi.2007 | reef             |

| Registration number | Fieldcode | Country | Province | Island | Region | Collector | Latitude | Longitude | Depth | Date | Habitat        |
|---------------------|-----------|---------|----------|--------|--------|-----------|----------|-----------|-------|------|----------------|
| RMNH POR. 3978      | #RAJ139/  | Indonesia | East Kali- | Berau | Berau | L.E. Becking | 00°36'01.5"S | 130°45'05.8"E | 0-1  | xi.2007 | rocky shore   |
| RMNH POR. 3979      | #RAJ140/  | Indonesia | East Kali- | Berau | Berau | L.E. Becking | 00°36'01.5"S | 130°45'05.8"E | 0-1  | xi.2007 | rocky shore   |
| RMNH POR. 3980      | #RAJ141/  | Indonesia | East Kali- | Berau | Berau | L.E. Becking | 00°36'01.5"S | 130°45'05.8"E | 0-1  | xi.2007 | rocky shore   |
| RMNH POR. 3981      | #RAJ142/  | Indonesia | East Kali- | Berau | Berau | L.E. Becking | 00°36'01.5"S | 130°45'05.8"E | 0-1  | xi.2007 | rocky shore   |
| RMNH POR. 3982      | #RAJ143/  | Indonesia | East Kali- | Berau | Berau | L.E. Becking | 00°36'01.5"S | 130°45'05.8"E | 0-1  | xi.2007 | rocky shore   |
| RMNH POR. 3983      | #RAJ144/  | Indonesia | East Kali- | Berau | Berau | L.E. Becking | 00°36'01.5"S | 130°45'05.8"E | 0-1  | xi.2007 | rocky shore   |

| Registration number | Fieldcode | Country | Province | Island | Region | Collector | Latitude | Longitude | Depth | Date | Habitat        |
|---------------------|-----------|---------|----------|--------|--------|-----------|----------|-----------|-------|------|----------------|
| RMNH POR. 3984      | #KKB/mol  | Indonesia | East Kali- | Berau | Kakaban lake | N.J. de Voogd | 02°08'37.1"N | 118°31'26.4"E | 10   | xi.2008 | reef             |
| RMNH POR. 4112      | #P-TER11  | Indonesia | Moluccas | Ternate | Ternate | N.J. de Voogd | 02°08'37.1"N | 118°31'26.4"E | 10   | xi.2009 | reef             |
| registration number | fieldcode | country | province | region | island | locality | habitat | latitude | longitude | depth | date   | collector |
|---------------------|-----------|---------|----------|--------|--------|----------|---------|----------|-----------|-------|--------|-----------|
| RMNH POR. 4113      | #P-TER22  | Indonesia | Moluccas | Ternate | Ternate | reef     |         | 02°08'57.3"N | 118°31'26.4"E | 0-2   | xi.2009| N.J. de Voogd |
| RMNH POR. 4489      | #KKB/mol721 | Indonesia | East Kalimantan | Berau | Kakaban | Kakaban lake | marine lake | 02°08'57.3"N | 118°31'26.4"E | 0-2   | ix.2008| L.E. Becking |
| RMNH POR. 4490      | #KKB/mol830 | Indonesia | East Kalimantan | Berau | Kakaban | Kakaban lake | marine lake | 02°08'57.3"N | 118°31'26.4"E | 0-2   | ix.2008| L.E. Becking |
| RMNH POR. 4491      | #BER109/mol629 | Indonesia | East Kalimantan | Berau | | lighthouse near Berau river | reef | 02°09'49.9"N | 118°10'12.8"E | 10    | 16.viii.2008| L.E. Becking |
| RMNH POR. 4492      | #BER111/mol666 | Indonesia | East Kalimantan | Berau | | SW Kakaban | reef | 02°08'07.5"N | 118°30'23.3"E | 10    | 17.viii.2008| N.J. de Voogd |
| RMNH POR. 4493      | #RAJ64/mol428 | Indonesia | West Papua | Raja Ampat | Waigeo | Teluk Mayabilit | reef | 00°18'17.0"S | 130°54'15.6"E | 10    | xii.2007| L.E. Becking |
| RMNH POR. 4494      | #RAJ23/mol199 | Indonesia | West Papua | Raja Ampat | Gam | Ctenophore lake | marine lake | 00°27'17.5"S | 130°29'33.8"E | 0-2   | xii.2007| L.E. Becking |
| ZMA Por. 896        |          | Indonesia | South Sulawesi | SW Salayer | | reef N of Pulau Bahuluang | reef | 06°27'00"S | 120°25'48"E | 10-45 | 30.ix.1984| R.W.M. van Soest (Snellius Expedition II) |
| ZMA Por. 10495      |          | Seychelles | Mahé | Mahé | SE coast near Pointe Cocos | reef | 04°45'00"S | 055°32'60"E | 35-45 | 24.xii.1992| R.W.M. van Soest |
Revision of the genus Placospongia (Porifera, Demospongiae, Hadromerida, Placospongiidae)...

ua island, Buli Halo anchialine pool, 02°11’16.4”N, 118°37’06.4”E, 0–1m. depth, xi.2008, coll. N. K. Santodomingo & Estradivari, #BER128/1156.

Description. Holotype and paratypes are branching and encrusting, size 8cm in length. Total size of specimens in situ is hard to establish as parts of the body may be encrusting within cracks. Alcohol preserved and live specimens are hard but slightly compressible. The surface is made up with typical Placospongia cortical plates separated by contractible grooves which form a network on the surface. Oscules are present in the grooves. Live color of holotype was dark brown, the paratypes were orange, and these colors were mostly retained after alcohol preservation (Fig. 8A).

Spicules. Holotype (Fig. 8) megascleres large straight tylostyles with blunt point 430-605.5-660 × 13-15.5-20 × 13-18.1-23 μm, small straight tylostyles with sharp point 240-261.3-290 × 5-7.2-8 × 5-8.8-10 μm; microscleres selenasters 80-84.8-90 × 60-67.3-75 μm, acanthose microrhabds 8-12.3-18 × 2.5-2.7-3.5 μm. Range of the paratypes (Table 1) large straight tylostyles with blunt point 430-760 × 13-20 × 15-23 μm, small straight tylostyles with sharp point 190-380 × 5-13 × 8-15 μm, microscleres selenasters 63-93 × 58-75 μm, acanthose microrhabds 5-20 × 2.5-3.5 μm.

Skeleton. The cortical plates consist of densely packed selenasters, microrhabds form a layer over and amidst this selenaster cortex and are also prevalent in choanosomal tissue. Developmental stages of selenasters occur throughout the choanosome. Tylostyle tracts support the margins of the cortical plates in radial tracts from the centre core (consisting of densely packed selenaster) to the cortical plates. The sharp ends of the smaller tylostyles can be projected beyond the cortex surface.
Distribution. Presently only recorded from Buli Halo anchialine pool on Maratua island, Berau, East Kalimantan, Indonesia (Fig. 9). For a full description of the pool, see Becking et al. (2011)

Ecology. Depth 0–2m. occurs in anchialine pool, can be exposed to air during low tide and can tolerate great fluctuations in salinity (from 24 to 33 ‰).

Etymology. Named in honor of Nadiezhda K. Santodomingo, the collector of the types, for her years of tireless work in marine science including anchialine research.

Remarks. Placospongia santodomingoae sp. n. is similar to P. carinata, yet lacks streptasters and has shorter tylostyles. Placospongia santodomingoae sp. n. likewise differs from P. mixta by the absence of streptasters as well as the absence of spherasters. Placospongia santodomingoae sp. n. differs from P. anthosigma by the absence of anthosigma, and by having hastate endings of the smaller tylostyles.

Geodia labyrinthica (Kirkpatrick, 1903)
http://species-id.net/wiki/Geodia_labyrinthica

Placospongia labyrinthica Kirkpatrick 1903: Plate V Fig. 1a–b, Plate VI Fig. 1a–f.

Reviewed material. Holotype. BMNH 02.11.16.1, South Africa, East London Coast, 33°06’30”S, 028°11’E.

Spicules. Megascleres styles, oxea; microscleres sterrasters, chiasters

Remarks. This species was originally described as ‘Placospongia labyrinthica’, but does not have the characteristic cortical plates of Placospongia. The specimen furthermore has sieve pores, sterrasters with star-like plates, euasters, styles and oxea characteristic of the Geodiidae. In the original description, Kirkpatrick (1903) stated “the presence of chiasters is so exceptional that I thought at first that I had to deal with a geodine sponges, but there were no triaenes to be found” and as a result placed this species in the Placospongia rather than Geodia. Genus transfer to Geodia is, however, required as suggested on the World Porifera Database (van Soest et al. 2011).

Identification key for Indo-Pacific species of Placospongia

1 Streptasters absent.................................................................2
– Streptasters present ..........................................................3
2 Spherules present.........................................................P. melobesioides
– Spherules absent............................................................4
3 Streptasters have rays with birfurcating ends.....................P. carinata
– Streptasters have rays with hastate ends, spherasters present........P. mixta
4 Spherasters present, microrhabds with short spines spirally places on shaft....
.................................P. anthosigma
– Spherasters absent, acanthose microrhabds present....P. santodomingoae sp.n.
Revision of the genus Placospongia (Porifera, Demospongiae, Hadromerida, Placospongiidae)...

Genetic data analysis

All sequences were submitted to GenBank with accession numbers KC848421 - 41 (Table 5). Final alignments (excluding primers) were obtained for the sponge Placospongia of 581 bp for COI with three genetic variants (28 individuals) and 13 polymorphic sites. The three genetic variants correspond to the three species P. melobesioides, P. mixta, and P. carinata that represent monophyletic groups which are strongly supported by both Bayesian and maximum likelihood inference methods (Fig. 10). There was no intra-specific variation within each species, regardless of geographic locality. The inter-specific p-distances ranged between 0.5-2.1% (Table 6). There were 11 substitutions between P. melobesioides and P. carinata, 12 substitutions between P. melobesioides and P. mixta, and three substitutions between P. mixta and P. carinata. The specimens of P. carinata and of P. santodomingoae sp. n. had identical genotypes for COI. No molecular work could be done on the dried holotype of Placospongia anthosigma and fresh material was not available.

Final alignments (excluding primers) of 720 bp were obtained for ITS with 18 genetic variants from the present study (22 individuals). An additional 27 genetic variants from GenBank (for GenBank accession numbers see Fig. 11) were included in the phylogenetic analysis. The ITS sequences represented five clades that were strongly supported by both Bayesian and maximum likelihood inference methods (Fig. 11). These five divergent clades (see Table 7 for uncorrected p-distances) correspond to the clades C3, C4, C5, C6, and C9 as presented by the study of Nichols & Barnes (2005). Clade C9 represents specimens of the species P. melobesioides, clade C5 P. mixta, and clade C4 P. carinata. Clade C6 is represented by one specimen from the Solomon Islands (QM317896) and clade C3 by one specimen from Bynoe Harbour, Northern Territory, Australia (QM303439); none of the samples that were sequenced in the

Figure 9. Distribution of Placospongia spp. in the Indo-West Pacific. Location of symbols is approximate.
Table 5. Specimens of Placospongia studied for DNA analysis. Genbank accession numbers provided for sequences of Cytochrome Oxidase I (COI) and internal transcribed spacer region (ITS).

| Registration number | Species            | COI        | ITS        |
|---------------------|--------------------|------------|------------|
| RMNH POR. 4482      | P. carinata        | KC848441   | KC848429   |
| RMNH POR. 4483      | P. carinata        | KC848441   | KC848427   |
| RMNH POR. 4484      | P. carinata        | KC848441   | KC848428   |
| RMNH POR. 4485      | P. carinata        | KC848441   | KC848429   |
| ZMA Por. 10727      | P. carinata        | KC848441   | -          |
| ZMA Por. 11367      | P. carinata        | KC848441   | -          |
| RMNH POR. 2464      | P. melobesioides   | KC848439   | -          |
| RMNH POR. 3942      | P. melobesioides   | KC848439   | KC848422   |
| RMNH POR. 3976      | P. melobesioides   | KC848439   | -          |
| RMNH POR. 4114      | P. melobesioides   | KC848439   | KC848426   |
| RMNH POR. 4495      | P. melobesioides   | KC848439   | KC848436   |
| RMNH POR. 4496      | P. melobesioides   | KC848439   | KC848436   |
| RMNH POR. 4497      | P. melobesioides   | KC848439   | KC848437   |
| RMNH POR. 3166      | P. melobesioides   | KC848439   | KC848422   |
| ZMA Por. 10459      | P. melobesioides   | KC848439   | KC848438   |
| RMNH POR. 3158      | P. mixta           | KC848440   | KC848421   |
| RMNH POR. 3960      | P. mixta           | KC848440   | KC848423   |
| RMNH POR. 3979      | P. mixta           | KC848440   | -          |
| RMNH POR. 4113      | P. mixta           | KC848440   | KC848425   |
| RMNH POR. 4489      | P. mixta           | KC848440   | -          |
| RMNH POR. 4490      | P. mixta           | KC848440   | KC848433   |
| RMNH POR. 4491      | P. mixta           | KC848440   | KC848433   |
| RMNH POR. 4492      | P. mixta           | KC848440   | KC848434   |
| RMNH POR. 4493      | P. mixta           | KC848440   | KC848435   |
| RMNH POR. 4494      | P. mixta           | KC848440   | KC848435   |
| RMNH POR. 4486      | P. santodomingoae sp. n. | KC848441 | KC848430   |
| RMNH POR. 4487      | P. santodomingoae sp. n. | KC848441 | KC848431   |
| RMNH POR. 4488      | P. santodomingoae sp. n. | KC848441 | KC848432   |

Table 6. The number of base differences per site from averaging over all Cytochrome Oxidase I (COI) sequence pairs between Placospongia spp. groups are shown (uncorrected p-distances). Standard error estimate(s) are shown above the diagonal in italic. The analysis involved 30 nucleotide sequences. There was no within-group difference. Spirastrella aff. decumbens was used as outgroup in the phylogenetic inference (see Fig. 10).

| % p-distance COI | P. melobesioides | P. mixta | P. carinata | Spirastrella aff. decumbens |
|------------------|------------------|---------|-------------|-----------------------------|
| P. melobesioides | *                | 0.6     | 0.6         | 1.3                         |
| P. mixta         | 2.1              | *       | 0.3         | 1.2                         |
| P. carinata      | 1.9              | 0.5     | *           | 1.3                         |
| Spirastrella aff. decumbens | 12.2              | 11.5     | 11.7       | *                           |

The present study fell into either C3 or C6 clade. The specimens of P. santodomingoae sp. n. represented a separate lineage within the P. carinata clade (C4) which was supported by Bayesian inference, but not by maximum likelihood analysis. The p-distance between P. carinata specimens and the specimens of P. santodomingoae sp. n. was 0.6%.
Table 7. The number of base differences per site from averaging over all internal transcribed spacer (ITS) sequence pairs between *Placospongia* spp. groups are shown (uncorrected p-distances). Standard error estimate(s) are shown above the diagonal. All positions with less than 5% site coverage were eliminated. Black cursive along the diagonal indicates within-group uncorrected p-distance. The analysis involved 73 nucleotide sequences. C9, C5, C6, C4, C3 refer to five clades in the Indo-West Pacific *Placospongia* as presented in Fig. 11.

| % p-distance ITS | *P. melobesioides* | *P. mixta* | *P. carinata* | *P. santodomingoae* sp. n. | C9   | C5   | C6   | C4   | C3   |
|------------------|-------------------|------------|---------------|----------------------------|------|------|------|------|------|
| *P. melobesioides* | 0.1               | 1.3        | 1.4           | 1.4                        | 0.3  | 1.3  | 1.3  | 1.3  | 1.3  |
| *P. mixta*       | 13.8              | 0.7        | 0.9           | 0.9                        | 1.2  | 0.2  | 0.5  | 0.9  | 0.9  |
| *P. carinata*    | 14.7              | 6.3        | 0.4           | 0.2                        | 1.3  | 0.9  | 0.9  | 0.2  | 0.9  |
| *P. santodomingoae* sp. n. | 13.2 | 5.8 | 0.6 | **1.6** | 1.3 | 0.9 | 0.9 | 0.3 | 0.9 |
| C9               | 0.9               | 13.5       | 14.6          | 13.6                       | 0.1  | 1.2  | 1.2  | 1.2  | 1.2  |
| C5               | 13.5              | 0.9        | 6.6           | 6.1                        | 12.9 | 0.7  | 0.8  | 0.9  | 0.9  |
| C6               | 14                | 2.2        | 6.4           | 6.1                        | 13.2 | 2.2  | 0.1  | 0.8  | 0.8  |
| C4               | 14.8              | 6.3        | 0.5           | 0.9                        | 14.3 | 6.3  | 6    | **0.4** | 0.8 |
| C3               | 15.2              | 7.1        | 6.1           | 5.9                        | 14.5 | 6.9  | 6.3  | 5.6  | **0.9** |

Figure 10. Bayesian/maximum likelihood phylograms of Cytochrome Oxidase I (COI) sequences from Indo-Pacific *Placospongia* spp. See Table 5 for GenBank accession numbers. Only posterior probabilities of >90 and maximum likelihood values of >70 indicated. Scale bar indicates substitutions/site.

Discussion

Different species

In the Indo-West Pacific at least five species of the genus *Placospongia* can be identified based on spicule morphology: *P. anthosigma*, *P. carinata*, *P. mixta*, *P. melobesioides*, and *P. santodomingoae* sp. n.. *Placospongia melobesioides*, *P. carinata*, and *P. mixta* can be distinguished with the DNA barcode marker (COI) and a nuclear marker (ITS). The species *P. santodomingoae* sp. n. and *P. carinata* have the same sequence of COI. The sequence variation of COI in sponges can be low (e.g. Wörheide 2006, Xavier et al. 2010, Pöppel et al. 2011) and this is also the case in species of *Placospongia*, e.g. only 0.5% nucleotide distance between the species *P. mixta* and *P. carinata*. There is further-
Figure 11. Bayesian/maximum likelihood phylograms of genotypes of the internal transcribed spacer region of nuclear ribosomal operons (ITS) of Indo-Pacific *Placospongia* spp. found in this study and related species from the same genus collected from GenBank. Clades C3, C4, C5, C6 & C9 refer to the clades presented in the study by Nichols & Barnes (2005). Taxon labels are organized as follows: Specimen - Locality - Genbank code or RMNH POR. Number. Only posterior probabilities of >90 and maximum likelihood values of >70 indicated. Scale bar indicate substitutions/site.
more no intraspecific variation in COI within each of the *Placospongia* species, not even between populations at 1000s of km distance from each other (e.g. specimens from the Seychelles are identical with specimens from Indonesia). The phylogenetic inference based on the ITS sequences does show a supported clade of *P. santodomingoae* sp. n. within the clade of *P. carinata* (Fig. 11), though the degree of divergence between the two species is low (0.6%) (Table 7). *Placospongia santodomingoae* sp. n. should, however, be designated as a new species based on the spicule morphology: the absence of a distinguishing spicule type (streptasters) and consistently shorter and thicker tylostyles (maximum 760 × 20 μm) compared to *P. carinata* (maximum 980 × 17.5 μm) are valid arguments to distinguish a separate species within this genus. The specimens of *P. santodomingoae* sp. n. were collected from an anchialine pool. This kind of isolated environment has previously been shown to contain small, rapidly evolving populations, and many rare species across a large spectrum of taxa (e.g. Holthuis 1973, Tomascik & Mah 1994, Dawson & Hamner 2005, Becking et al. 2011, Becking et al. in press). The divergence of *P. santodomingoae* sp. n. from *P. carinata* is likely too recent to be expressed in the molecular markers that were used. Other, faster evolving, molecular markers might show a more distinct separation between species, but for the present significant morphometric differences in spicules are reliable characters in separating these sister species.

A molecular phylogeny using the internal transcribed spacer region (ITS) showed that there were five distinct clades within the genus *Placospongia* in the Indo-West Pacific (clades C3, C4, C5, C6 & C9) (Nichols & Barnes, 2005). Nichols & Barnes (2005) indicated that their results presented a conundrum that “specimens collected from Indonesian marine lakes that have been isolated from the surrounding marine environment since the Pleistocene are undifferentiated from individuals collected from the Seychelles indicating that populations from these geographically disparate regions are, or have recently been, connected by gene flow despite the lack of evidence of connectivity between these lakes and nearby reefs.” It is important to note here that the authors did not investigate the spicule morphology of the specimens in their study, while it is in fact the spicules that can largely explain the presented conundrum. In the present study over 30 specimens from the marine lakes Kakaban and Maratua and the adjacent reefs have been reviewed as well as the specimens from the ZMA Por. collection that were used in the Nichols & Barnes (2005) study. Clade C4 represents the material from the Seychelles (ZMA Por.11367) together with the marine lakes and can all be morphologically identified as *P. carinata sensu stricto*. The samples from the lakes and the Seychelles are thus conspecific, but the populations of the two locations are necessarily connected by gene flow. Subsequently clade C9 is *P. melobesioides* (specimens from Indonesia, Micronesia and the Seychelles) and clade C5 is of *P. mixta* (specimens from Indonesia, Palau and Papua New Guinea). This explains three of the five clades from the Indo-West Pacific and leaves two undetermined: clade C3 represented by one specimen from Bynoe Harbour, Northern Territory, Australia (QM303439), and clade C6 represented by one specimen from the Solomon Islands (QM317896). The morphology of these specimens should be further studied in order to correctly identify the species and determine if they may represent morphologically cryptic species.
Natural variation

Each of the five species of the genus *Placospongia* in the Indo-West Pacific can be distinguished based on the composition and morphology of spicules. The external morphology, however, does not allow species distinction. The most common species from the tropical Indo-West Pacific (*P. melobesioides, P. mixta, and P. carinata*) can have both encrusting and branching growth forms displaying a variety of colors from white to dark brown. The only observed consistent pattern was that all the red specimens belonged to *P. mixta*, while all the dark black-brown specimens belonged to *P. melobesioides*. These two colors may be useful for field identifications, yet both species can also display the range of other colors (white, cream, beige, light brown) as well. The density of canals/ridges (or size of cortical plates) appears to be related to environment as this is higher in specimens from high sediment locations such as the marine lakes than in specimens from the reefs (Fig. 1, 2). Within each species there is also some natural variation in the range of tylostyle length and spicule morphology. The streptaster morphology varies within species and even within individuals. Within one individual the number of rays can vary from 4-10 (Figs 3, 4) and between individuals the decoration and size of spines can be diverse. For example the streptasters of *P. carinata* specimens from Haji Buang marine lake are micro-acanthose while the specimens from other locations are not. Spherasters are always present and abundant in *P. mixta* and *P. anthosigma*, but are in low abundances or absent in *P. melobesioides*, as has been indicated previously by Vosmaer & Vernhout (1902). In *P. carinata* and *P. santodomingoae* sp. n. spherasters are always absent.

Ecology and distribution

*P. melobesioides* and *P. mixta* are common in the reef environment. Most of the collected material from the reefs in Indonesia were one of these two species. *P. carinata* appears to be rare in the reefs, in Indonesia at least, while it is highly abundant in the marine lakes Haji Buang and Kakaban in East Kalimantan, Indonesia. *Placospongia santodomingoae* sp. n. is restricted to an anchialine pool. *Placospongia anthosigma* was not found in any of the examined collections from the tropical Western Pacific, this species is restricted to more temperate and deeper waters. *Placospongia melobesioides* is indicated in the Systema Porifera to have a distribution from the Indo-West Pacific to the Tropical Atlantic (Rützler 2002). Both *P. melobesioides* and *P. carinata* have been recorded from the Atlantic (e.g. de Laubenfels 1936, Hechtel 1976, Coelho and Mello-Leitão 1978, Pulitzer-Finali 1986, González-Farías 1989), which would imply that these are pantropical species. Recent molecular and more detailed morphological studies have, however, shown that many cosmopolitan sponge species are in fact species complexes either delineated by morphology or molecules (e.g. Reveilleud et al. 2010, Xavier et al. 2010). Van Soest (2009) has indicated that there are at least five species of *Placospongia* in the Caribbean that are morphologically different from the holotypes of *P. melobesioides* and *P. carinata*. Rua et al. (2006) and Nichols and Barnes (2005), furthermore, show that there are distinct lineages in the Carib-
bean and Western Pacific, that are not shared between the two regions and that most likely represent undescribed species in the Caribbean. Considering these results as well as the large geographic distance between the Caribbean and the type localities of *P. melobesioides* and *P. carinata* (both Indo-West Pacific), it is highly unlikely that these species occur in the Tropical Atlantic. Further revision of the Atlantic and Eastern Pacific material will shed more light on this issue.

Future biodiversity surveys and species checklists both in the Atlantic as well as in the Pacific are advised to check the spicule morphology of *Placospongia* specimens in order to identify species, as the external morphology and color will not give an indication to the number of species. The different *Placospongia* spp. can occupy the same type of habitats in the tropics. An example of such sympatry is represented in Kakaban lake where in the 4 km² area of the marine lake the three common tropical species of *Placospongia* co-exist side by side. Neglecting to review the spicule morphology would mean possibly missing the true diversity of species that are present in the location of study.

**Acknowledgements**

N.J. de Voogd, R.W.M. van Soest, and three reviewers provided valuable comments on the original manuscript. For the loan of the type-specimens I thank: E. Sherlock (British Museum of Natural History), H. Namikawa (National Museum of Nature and Science, Tokyo, Japan), and C. Eckert (Zoologisches Museum für Naturkunde). I am also grateful to the following people for their help in various ways in field- or labwork logistics: B.W Hoeksema, Suharsono, Y. Tuti, Y. Ise, S. Hoshino, Y. Nakao, N. K. Santodomingo, Estradivari, Bahruddin, M. Erdmann, M. Ammer, D. Erpenbeck, S. Menken, H. Breeuwer, J. van Ooyen, E. Beglinger, E. Kruidenier, and the staff of Papua Diving, of Nabucco Island Dive Resort, of Derawan Dive Resort, and of WWF/TNC Berau office. The present study was funded by the Netherlands Organisation for Scientific Research (NWO), division Earth and Life Sciences (ALW IPJ-07002, # 817.01.008; ALW-Rubicon # 825.12.007). Fieldwork in Indonesia was made possible through additional financial support of World Wildlife Foundation Netherlands-INNO Fund, the Schure-Beijerinck-Popping Fund of the Royal Dutch Academy of Science (KNAW), the Treub-Maatschappij Fund, the Lerner-Gray Fund for Marine Research (American Natural History Museum), the Leiden University Fund (LUF)/Slingelands, Singapore Airlines, the A.M. Buitendijk Fund and the J.J. ter Pelkwijk Fund. I thank the Indonesian Institute of Sciences (LIPI) and the Indonesian State Ministry of Research and Technology (RISTEK) for providing research permits in Indonesia.

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