Chapter 5

Diversity of the genus *Placospongia* (Porifera: Demospongiae: Hadromerida: Placospongiidae) in the West Pacific

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Zookeys (accepted)

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* is 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 collection of over 100 specimens of *Placospongia* spp. collected from Indonesia (including the Vosmaer & 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, morphology and 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 santodomingoae* 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 santodomingoae* 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 • anchialine pool • ITS • COI

Introduction

Species of the genus *Placospongia* in the tropical Indo-West Pacific occurr in a wide variety of environments 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, de Voogd 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 to Indonesia in 2006 (Sulawesi), 2007 (Papua), 2008 (Berau), 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 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 which is presently housed at the Naturalis Biodiversity Center, 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 are nine evolutionary lineages worldwide within the genus *Placospongia*, of which there are 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.

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 over 100 specimens of *Placospongia* spp. that were collected from Indonesia (including Vosmaer & Vernhout's 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 the aim to determine if growth form and color can be used as diagnostic characteristics to identify different species of *Placospongia* in the field. Finally, an attempt was made to provide species names to the five clades of Indo-Pacific *Placospongia* as published by Nichols & Barnes (2005) by combing their published ITS sequences from GenBank with ITS sequences from identified species of Indo-Pacific *Placospongia.*

Figure 1. *In situ* underwater images of *Placospongia* spp. in Indonesia, displaying natural variation in color and growth form of live specimens. **A** *Placospongia mixta*, **B** *Placospongia carinata,* **C** *Placospongia carinata,* **D** *Placospongia melobesioides.*

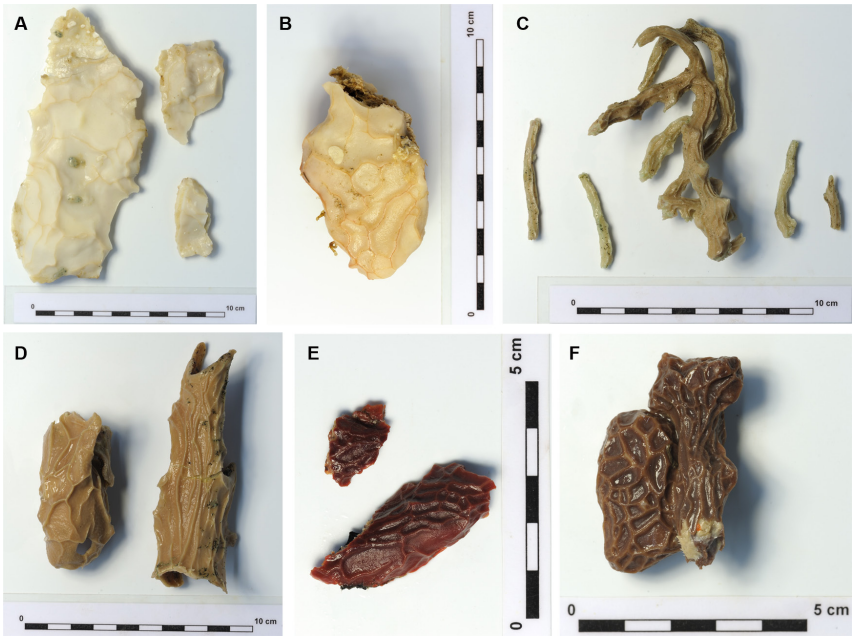


Figure 2. Gradation of external coloration in preserved specimens. A. *Placospongia mixta* RMNH POR. 4492, B. *Placospongia mixta* RMNH POR. 4113, C. *Placospongia carinata* RMNH POR. 4483, D. *Placospongia carinata* RMNH POR. 4483, E. *Placospongia mixta* RMNH POR. 3979, F. *Placospongia melobesioides* RMNH POR. 4114

Material & Methods

Specimens from Indonesia were collected via snorkeling in marine lakes and scuba diving on reefs. Where possible material was preserved in 96% ethanol for DNA analysis, and voucher specimens were preserved in 70% ethanol and deposited in the Porifera collections of the Naturalis Biodiversity Center, Leiden (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 x shaft width X head width; selenasters length x width; spirasters total length x ray length; spherasters diameter; rhabds length x 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.

The following 25 specimens were selected for further molecular analyses:
Placospongia melobesioides: RMNH POR. 2464, 3166, 4497, 3942, 3976, 4495, 4496; ZMA Por. 10496
Placospongia mixta: RMNH POR. 3158, 3936, 4113, 4489, 4490, 4492, 4494, 4493
Placospongia carinata: RMNH POR. 4482, 4483, 4484, 4485, 4486, 4487, 4488; ZMA Por. 10727, ZMA Por. 11367

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 *Placospongia* P-COI-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: GTCCCTGCCCTTTGTACACA 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 & Xie 2001). Species of the families Spirastrellidae and Clionaidae were selected as outgroup for the phylogenetic analyses. For the COI genetree fou specimens of *Spirastrella* aff. *decumbens* (RMNH POR. 4505, 4589, 4614) was 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 as 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 & Ronquist 2001). Each analysis comprised of 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 & 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 (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 (BMNH).

Note on presentation of examined material:
In this study multiple specimens from the same location were examined. As a result, the information of examined material is provided in the following order: country, province, region, island, location, coordinates, habitat type, depth, collector, date: registration number (#fieldnumber in brackets) for all specimens from the specified location. Where certain information is unavailable this is omitted. First the information of the holotype is given, then the material from the Siboga Expedition that was reviewed by Vosmaer & Vernhout (1902), followed by other museum material (first RMNH POR., then ZMA Por.).

Phylum Porifera Grant, 1836
Class Demospongiae Sollas, 1885
Order Hadromerida Topsent, 1894
Family Placospongiidae Gray, 1867
Genus *Placospongia* Gray, 1867

Placospongia Gray, 1867
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 cm^2 to large surfaces of >2m^2 to branching individual with total size of up to 45cm in length and branch diameter between 0.25-1.5cm. 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 this color is usually retained after alcohol preservation. The contact lines between the plates ridge up slightly and are generally a darker shade than 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 & Vernhout (1902).

Spicules: Megascleres are tylostyles two size classes, microscleres are selenasters, and can include choanosomal and ectosomal spirasters, spherasters, spherules, or acanthose microrhabds. Selenasters often remain pigmented after treatment with bleach or nitric acid.

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 of these spicules in specimens.

	region	growthform	color live	Tylostyle blunt end length	max width	head width	Tylostyle sharp end length
P.melobesioides							
BMNH52.4.1.14 (holotype)	Borneo	branching	dark brown	670-**879.6**-1010	10-**13.2**-18	10-**16.3**-20	205-**293.4**-420
RMNH4495	N Kalimantan (Kakaban lake)	encrusting	dark brown	480-**717.6**-1040	5-**9.5**-15	8-**10.3**-15	190-**297.6**-370
RMNH4496	N Kalimantan (Kakaban lake)	branching	dark brown	580-**778.4**-900	8-**11.7**-15	10-**14.1**-18	230-**272.8**-400
RMNH4497	N Kalimantan	branching	dark brown	620-**745.2**-860	10-**12.2**-15	13-**14.8**-18	250-**320.8**-450
RMNH3935	N Kalimantan	encrusting	dark brown	460-**660.9**760	10-**11.6**-15	10-**13.7**-18	210-**325.8**-450
RMNH3166	N Sulawesi	encrusting	dark brown	460-**704.8**-810	8-**11.4**-13	10-**13.2**-15	200-**288**-470
RMNH3976	Moluccas	branching	dark brown	600-**793.6**-910	10-**12**-15	13-**14**-18	190-**321.2**-450
RMNH3977	Moluccas	branching	brown	510-**683.6**-780	10-**11.5**-13	13-**13.9**-15	200-**326**-450
RMNH758	W Papua	branching		630-**853.2**-1020	10-**13.3**-15	13-**15.8**-18	210-**253.2**-310
RMNH757	W Papua	branching		550-**829.2**-960	10-**13.3**-16	13-**15.8**-18	260-**302.1**-370
RMNH2464	Singapore	branching		710-**933.4**-1080	12.5-**15**-17.5	13-**15.7**-20	240-**326.7**-330
ZMA10459	Seychelles	branching	brown	520-**670.8**-820	7.5-**11.4**-12.5	10-**13.4**-17.5	310-**362.5**-430
P. carinata							
R122b-86g-BK1390 (holotype)	"South Sea"			500-**710.4**-800	10-**13.4**-15	10-**15.3**-18	140-**317.4**-450
RMNH4482	N Kalimantan (Haji Buang lake)	branching	orange	660-**726**-800	10-**12.3**-15	10-**14.5**-18	180-**263**-410
RMNH4483	N Kalimantan (Haji Buang lake)	encrusting	crème	610-**703.8**-800	10-**13.1**-15	13-**14.9**-18	190-**286.7**-470
RMNH4484	N Kalimantan (Kakaban lake)	encrusting	crème	560-**709.16**-920	8-**11.7**-18	10-**13.9**-18	175-**267.1**-550
RMNH4485	N Kalimantan (Kakaban lake)	branching	dark	550-**761.2**-930	10-**14**-18	13-**15.5**-18	210-**295.2**-450
RMNH744	Moluccas	encrusting	light purple	450-**748.6**-980	8-**11.1**-13	10-**13.2**-15	195-**256.8**-550
RMNH754	Philippines	branching	white	540-**705.8**-830	10-**12.8**-15	13-**15.2**-18	280-**355.5**-500
RMNH755	W Papua	branching	crème	560-**764.7**-910	8-**12.2**-15	10-**14.7**-18	250-**311.8**-360
ZMA10727	Seychelles	encrusting		620-**738.7**-840	8-**11**-13	13-**15.5**-18	240-**258.3**-270
ZMA9189	India	branching		550-**703.3**-820	10-**12.8**-15	13-**15**-18	210-**318.8**-410
P. mixta							
ZMB3204 (holotype)	Moluccas	encrusting		355-**672.4**-940	8-**12.1**-18	8-**15.6**-20	165-**226.4**-275
RMNH4112	Moluccas	encrusting	red	480-**870**-1040	10-**12.7**-15	13-**15.8**-28	210-**288**-410
RMNH4113	Moluccas	encrusting	crème	550-**817.6**-1030	10-**13.1**-15	13-**15.6**-18	160-**260**-350
RMNH742	Moluccas	branching	red	550-**759.2**-850	10-**11.9**-15	10-**14.9**-20	120-**230**-380
RMNH4489	N Kalimantan (Kakaban lake)	encrusting	crème	630-**886.6**-1010	10-**12.9**-15	13-**15.4**-19	175-**221.5**-320
RMNH4490	N Kalimantan (Kakaban lake)	encrusting	crème	510-**727.6**-970	8-**13.1**20	13-**16.3**-23	150-**240**-310
RMNH4491	N Kalimantan	encrusting	brown	780-**1001.4**-1200	10-**14.8**-18	15-**17.5**-20	240-**284**-350
RMNH4492	N Kalimantan	encrusting	white	610-**995.8**-1250	10-**16**-20	13-**19**-25	260-**274**-290
RMNH3158	N Sulawesi	encrusting	crème	550-**990**-1210	13-**16.9**-20	13-**17.5**-20	130-**267.8**-400
RMNH745	S Sulawesi	encrusting	brown	760-**914.1**-1030	13-**17**-23	10-**18**-25	250-**366.6**-480
RMNH4493	W Papua	encrusting	brown	460-**761.6**-1070	10-**14.6**-23	13-**17.38**-25	220-**323.6**-430
RMNH4494	W Papua	encrusting	brown	540-**758**-900	10-**12.2**-18	10-**13.8**-20	180-**216.9**-350
P. santodomingoae sp.n.							
RMNH4486 (holotype)	N Kalimantan (Hapsi Bulo pool)	branching	brown	430-**605.6**-660	13-**15.5**-20	13-**18.1**-23	240-**261.3**-290
RMNH4487	N Kalimantan (Hapsi Bulo pool)	branching	orange	530-**652.4**-740	13-**16**-20	15-**18.0**-23	220-**274.7**-310
RMNH4488	N Kalimantan (Hapsi Bulo pool)	branching	orange	480-**633.2**-760	15-**17.2**-20	18-**19.6**-23	190-**273.2**-380

		Selenaster		Spheraster	Spiraster		Microrhabd	
max width	head width	length	width	diameter	total length	length ray	length	width
5-**9.9**-13	5-**9.9**-13	58-**63.1**-68	45-**51.7**-68	15-**16.8**-18				
3-**5.8**-8	3-**6.1**-8	45-**56.6**-70	30-**41.6**-50					
5-**7.4**-10	8-**9.1**-10	45-**60**-75	35-**45**-63					
5-**8.8**-10	5-**9.4**-13	63-**70.8**-83	45-**59.6**-65					
3-**7.4**-13	3-**8.3**-13	45-**63.9**-70	38-**51.3**-60	**15-20**				
3-**9.5**-13	5-**10.8**-15	60-**63.6**-70	50-**50.2**-55					
5-**8.5**-13	5-**9.6**-13	48-**66.8**-75	48-**55.2**-65					
5-**7.5**-10	8-**9.5**-13	58-**63.3**-68	40-**46**-53					
5-**9.5**-13	8-**11.8**-15	50-**55.2**-62.5	35-**42.3**-50	**15**				
8-**9.6**-13	10-**11.2**-15	55-**60.4**-65	43-**48.0**-53					
5-**9.2**-13	5-**10.8**-15	67.5-**81**-87.5	60-**72.5**-85					
5-**8.8**-10	5-**10.1**-13	62.5-**68.9**-72.5	50-**55.5**-65					
5-**8.4**-12.5	8-**9.3**-13	80-**90**-98	60-**71.3**-85		23-**33.8**-43	8-**11.6**-15	8-**12.0**-18	2,5
3-**5**-7.5	8-**7.5**-8	65-**71.5**-75	50-**58.5**-65		15-**34**-48	10-**13.0**-15	8-**11.7**-15	2,5
5-**6.4**-10	5-**8.6**-13	60-**80**-85	60-**62.9**-70		20-**33.7**-40	10-**13.2**-15	8-**11.9**-18	2,5
3-**4.4**-10	5-**6.4**-13	50-**61.8**-70	35-**47.4**-55		25-**29.7**-35	8-**11.0**-15	10-**13.3**-18	2,5
3-**5.6**-8	5-**7.6**-10	28-**63**-73	38-**50**-58		20-**27.6**-38	5-**9.0**-13	5-**9.4**-13	<2.5
5-**6.2**-10	5-**6.7**-8	60-**66.3**-70	50-**55.6**-65		25-**29.9**-38	10-**12.9**18	8-**10.8**-13	<2.5
5-**7.0**-10	5-**8.6**-13	55-**67.7**-75	45-**51.8**-55		25-**30.9**-38	8-**9.5**-13	8-**12.3**-18	2,5
5-**7.3**-8	5-**8.2**-10	55-**61.1**-65	38-**47.5**-55		30-**32.9**-38	8-**9.8**-13	8-**10.2**-13	2,5
3-**3.3**-5	3-**4.6**-8	50-**58.8**-78	35-**42.5**-63		25-**27.6**-38	8-**11.1**-15	8-**8.1**-10	<2.5
5-**7.5**-10	5-**9.7**-13	63-**72.2**-78	50-**56.8**-65		30-**35**-48	8-**10.7**-15	8-**9.2**-13	2,5
3-**6.1**-8	3-**7.8**-10	55-**69.8**-75	43-**55.4**-73	20-**25**-30	15-**23.9**-33	3-**7.6**-13	5-**7.1**-10	<2.5
5-**6.2**-10	5-**7.2**-10	50-**66.6**-75	38-**50.7**-58	18-**20.2**-25	18-**23.7**-35	5-**6.4**-10	5-**6.4**-10	<2.5
5-**7.3**-10	5-**8.2**-12.5	62.5-**66**-70	45-**53**-57.5	20-**22.1**-25	20-**24.8**-30	5-**5.7**-8	5-**7.5**-10	2,5
3-**5.9**-10	3-**7.6**-10	50-**65.4**-73	33-**46.5**-56	22-**23.4**-25	15-**22.2**-35	2-**5.7**-8	5-**7.4**-10	<2.5
3-**3.9**-8	2-**7.2**-10	60-**68**-75	43-**50.8**-58	18-**20.6**-25	20-**26.1**-35	8-**10.8**-15	8-**8.5**-10	<2.5
3-**5.3**-8	2-**6.4**-8	55-**70.4**-83	40-**53.3**-65	13-**20.5**-25	15-**21.7**-30	5-**6.4**-13	8-**9.2**-13	<2.5
5-**6.3**-8	5-**8.3**-10	60-**71**-75	48-**57.5**-63	18-**23**-25	20-**27.3**-35	5-**7**-10	5-**6.3**-8	2,5
8-**9**-10	8-**9**-10	58-**71**-78	45-**54.6**-70	15-**20.2**-25	18-**24.8**-33	10-**11.2**-15	5-**8.6**-18	<2.5
5-**8.8**-15	8-**9**-10	65-**71**-75	50-**56.5**-63	23-**23.8**-25	23-**28.4**-35	5-**8.7**-13	5-**6.6**-8	<2.5
3-**8**-13	3-**9**-13	45-**73.6**-80	45-**60**-70	20-**23.9**-25	20-**23.7**-30	3-**6.4-9**	5-**7.5**-10	<2.5
8-**9.1**-13	10-**11.3**-15	73-**80.3**-85	53-**65.3**-73	20-**26.5**-30	18-**23.4**-30	15-**8.1**-10	8-**8.7**-13	<2.5
3-**3.3**-5	4-**4.4**-8	50-**59.1**-68	35-**42.3**-58	15-**20.9**-28	23-**26.9**-30	8-**10.4**-13	8-**8.5**-10	<2.5
5-**7.2**-8	5-**8.8**-10	80-**84.8**-90	60-**67.3**-75				8-**12.3**-18	2.5-**2.7**-3.5
5-**8.2**-13	8-**9.5**-15	*63*-**82.9**-93	60-**66.3**-73				5-**10.5**-20	2.5-**2.6**-3.5
5-**7.9**-10	8-**10.3**-13	80-**87**-93	58-**69**-75				8-**13.5**-18	2.5-**2.9**-3.5

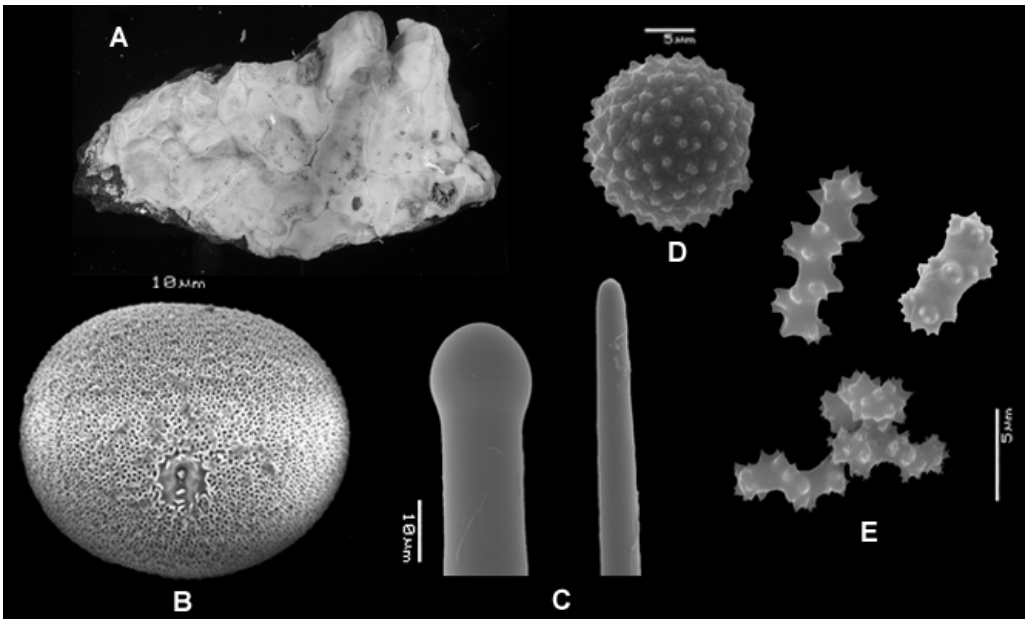


Figure 3. *Placospongia anthosigma* holotype (NSMT-Po R288) A. type specimen (image taken from website of Museum of Nature and Science, Tokyo, Japan), B. selenaster, C large tylostyle (head and blunt end), D. spheraster, E. spirasters referred to as 'anthosigma' by Tanita & Hoshino (1989)

***Placospongia anthosigma* (Tanita & Hoshino, 1989)**
Figure 3

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

Material examined:
Holotype. Japan, Kannonzuka-dashi, Amadaiba, Sagami Bay, depth 62-67m: NSMT-Po R288 (National Museum of Nature and Science, Tokyo, Japan).

Description: Holotype NSMT-Po R288 encrusting specimen in three pieces of 1-2cm^2 and 5mm thick, beige to pink in alcohol (Figure 3A).

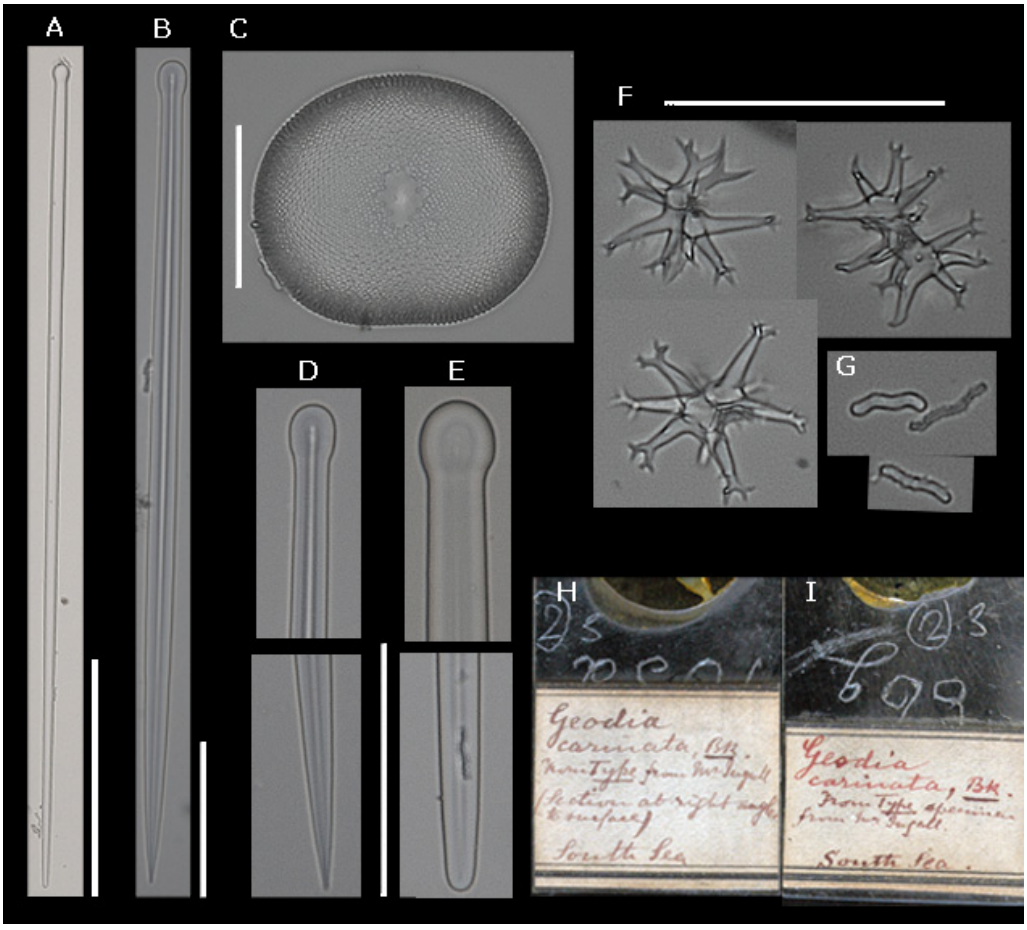
Spicules: Megascleres large tylostyles with blunt point 520-797-930 x 15-18-20 x 18-20-23 μm**,** small tylostyles with blunt point 250-320-410 x 10-12-18 x 13x14-18 μm**;** microscleres selenasters 85-90-98 x 70-73-80 μm, spheraster 15-19-25 μm, stout spirasters with two or three contortions and acanthose spines spirally placed on shaft 8-11-18 x 3-4.5-5 μm (Fig. 3)

Skeleton: as genus description with addition that anthosigma 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 not a 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 spheraster*s*. *Placospongia anthosigma* is distinguished from the other *Placospongia* spp. by the absence of small acanthose microrhabds, presence of stout elongated spirasters referred to by Tanita and Hoshino (1989) as 'anthosigma' and the small class of tylostyles with blunt points. The spirasters of *P. anthosigma* are larger than the acanthose microrhabds of *P. carinata* and *P. mixta* and are furthermore spirally decorated.



F**igure 4.** *Placospongia carinata* slide of holotype (BMNH, R1228, 86g, Bk.1390; R1275, PE01, Bk1390). A. large tylostyle (scale=200 μm), B. small tylostyle (scale=50 μm), C. selenaster (scale=50 μm), D. close up of large tylostyle (scale=50 μm), E. close up of small tylostyle, F. spirasters (scale=50 μm), G. microrhabds, H. original slide of thick section of holotype, I. original slide of spicules of holotype.

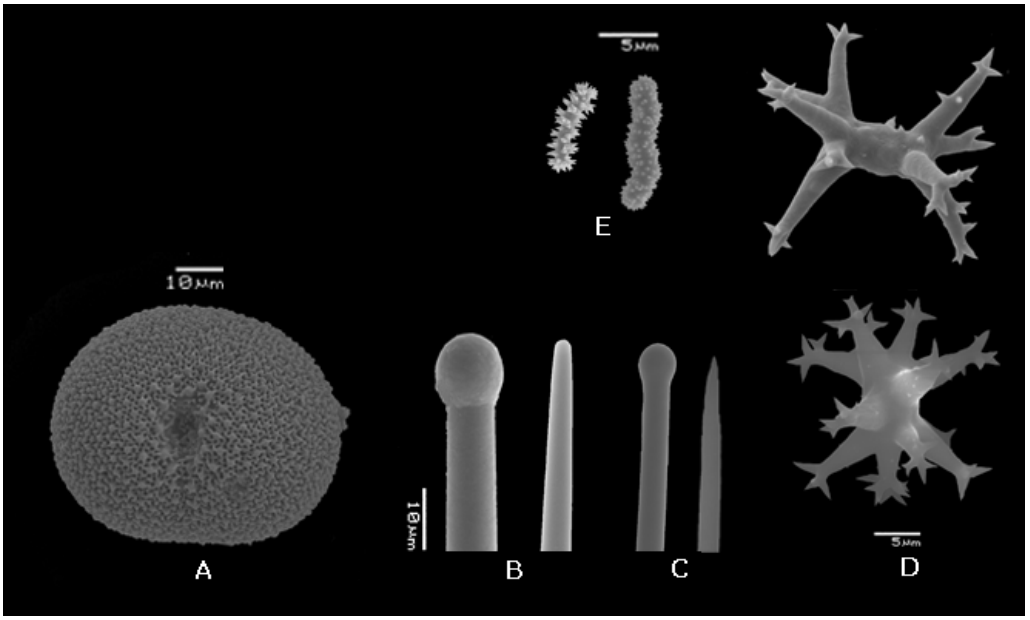


Figure 5. *Placospongia carinata* (RMNH POR. 4483). A. selenaster, B large tylostyle (head and blunt end), C. small tylostyle (head and hastate end), D. spirasters, E. acanthose microrhabds

***Placospongia carinata* (Bowerbank, 1858)**

Figures 4 & 5

Geodia carinata Bowerbank, 1858: plate XXV Fig. 19

Geodia carinata Bowerbank, 1874: plate XLVI Figs 1-5

Material examined:

Lectotype. "South Sea": BMNH R1228 - 86g - Bk.1390 (slide), R1275 - PE01 - Bk1390 (slide).

Vosmaer & Vernhout (1902), Siboga expedition. Indonesia, West Papua province, Raja Ampat region, SE Misool, Siboga stat. 164, S02°28'.5 E131°3'.3 E, 32m. depth: RMNH POR. 755 (#1848); Philippines, Sulu Sea region, Ubian Islands, Siboga stat. 99, 6°7.5'N 120°26'E, 16-23m. depth: RMNH POR. 754 (#1458); Indonesia, Moluccas province, W of Aru, Kur Island, Siboga stat. 250, 20-40m. depth: RMNH POR. 744 (#1500). **Other material.** Indonesia, East Kalimantan province, Berau region, Kakaban island, Kakaban lake, N02° 08' 57.3" E118° 31' 26.4", marine lake, 0-2m. depth, coll. L.E. Becking, ix.2008: RMNH POR. 4484 (#KKB/mol1107), RMNH POR. 3943 (#KKB/mol716), RMNH POR. 3944 (#KKB/mol754), RMNH POR. 4485 (#KKB/mol763), RMNH POR. 3945 (#KKB/mol780), RMNH POR. 3946 (#KKB/mol810), RMNH POR. 3947 (#KKB/mol814), RMNH POR. 3948 (#KKB/mol825), RMNH POR. 3949 (#KKB/mol713), RMNH POR. 3950 (#KKB/mol1068); Indonesia, East Kalimantan province, Berau region, Maratua island, Haji Buang lake, N02° 12'31.2" E118° 35'46.8", marine lake, 0-2m. depth, coll. L.E. Becking, ix.2008: RMNH POR. 3951 (#MA/mol700), RMNH POR. 3952 (#MA/mol975), RMNH POR. 3953 (#MA/mol947), RMNH POR. 3954 (#MA/mol1055), RMNH POR. 3955 (#MA/mol1012), RMNH POR. 4482 (#MA/mol1061), RMNH POR. 3956 (#MA/mol1001), RMNH POR. 3957 (#MA/mol1009), RMNH POR. 4483 (#MA/LE172), RMNH POR. 3958 (#MA/mol1500); Indonesia, Nusa Tenggara province, Komodo, NE cape, Snellius II Expedition, coll. R.W.M. van Soest, 1984: ZMA Por. 8813; Singapore, Pulau Salu: ZMA Por. 09578; Seychelles, Mahé, coll. R.W.M. van Soest, 1992: ZMA Por. 11367, ZMA Por. 16584, ZMA Por. 10727, ZMA Por. 1818, ZMA Por. 10481, ZMA Por. 20735; India, Laccadive Islands, Agatti Island, depth 20-25m, coll. National Institute of Oceanography, 1987: ZMA POR.9189.

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, or cream (Fig. 1 & 2). Color of choanosome is pale beige. After preservation in ethanol colors are similar to live specimens.

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 x 10-**13**-15 X 10-15-18 μm, small straight tylostyles with sharp ends 140-**317**-450 x 5-**8**-25 X 8-**9**-13 μm; microscleres selenasters 80-**90**-98 μm, spirasters with varying number of rays (5-10) with bifurcating endings or tufts 23-**34**-43 x 8-15 μm, acantho microrhabds 8-**12**-18 x 1-2.5 μm, spheraster absent. The range within the examined material (Table 1 & Fig. 5): megascleres large tylostyles 540-990 x 8-18 X 10-18 μm, small tylostyles 175-550 x 3-10 X 3-13 μm; microscleres selenasters 50-85 x 35-70 μm, spirasters 15-48 x 5-18 μm, acantho microrhabds rhabds 5-18 x 1-2.5 μm, spheraster absent.

Skeleton: as genus description 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: 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 (Fig. 9). Distribution may extend further the East.

Ecology: In Indonesia rarely found in reef environment, but in high abundances in marine lakes. Possibly higher prevalence in reef in Eastern Africa, based on the ZMA Por. collection from the reefs in the Seychelles and the publication from Madagascar by Levi (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 spirasters ("arborescent elongo-subsphero-stella"). Subsequently in 1874 Bowerbank published a more extensive description of "*Geodia carinata"* including a drawing of the spirasters (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 are given, 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 spirasters with bifurcating endings and the micro rhabds as indicated in the Bowerbank images and in the images taken from these slides in Fig. 5. The slides clearly do not represent 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*. Furthermore, specimen BMNH 95.6.7.1 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 the author 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 lectotype of *P. carinata*.

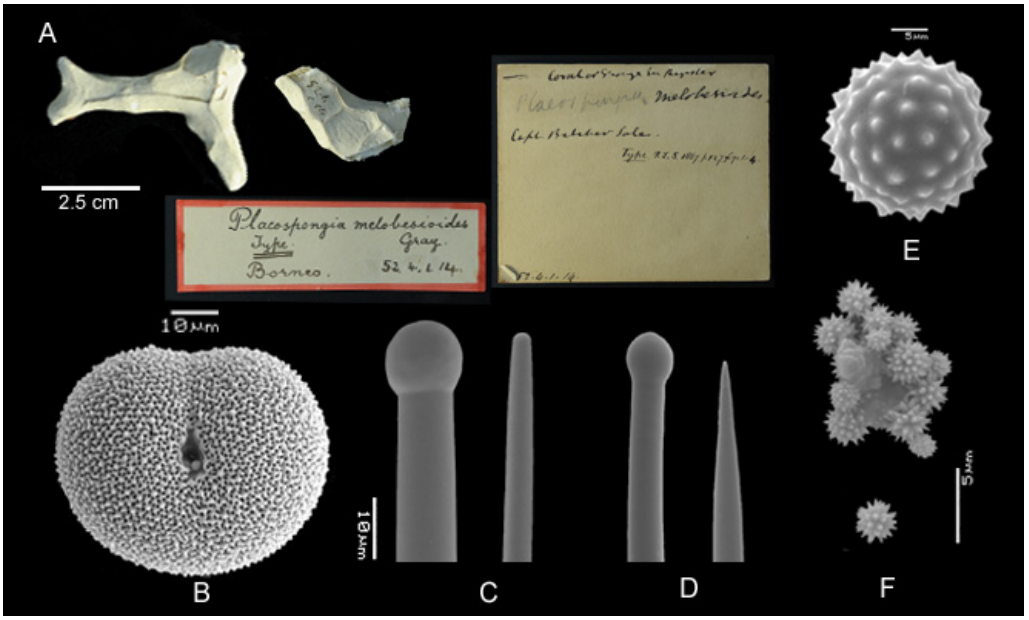


Figure 6. *Placospongia melobesioides* holotype (BMNH 52.4.1.14). A. Holotype with two labels, B. selenaster, C. large tylostyle (head and blunt end), D. small tylostyle (head and hastate end), E. spheraster, F. spherules

***Placospongia melobesioides* Gray, 1867**

Figure 6

Placospongia melobesioides Gray (1867): Figs 1-4

Material examined:

Holotype. Indonesia, Borneo: BMNH 52.4.1.14

Vosmaer & Vernhout 1902. Indonesia, Nusa Tenggara province, N of Sumbawa, S07° 12.6′ S E118° 7.7′, 36m. depth: RMNH POR. 756 (#660); Indonesia, S of Moluccas, E04° 12′ S129° 20.4′, 45m. depth: RMNH POR. 761 (#1033); Indonesia, West Papua province, SE of Misool, Banda Islands, 32m. depth: RMNH POR. 758 (#1847), RMNH POR. 757 (#1849), RMNH POR. 760 (#1851), RMNH POR. 759 (#1853). **Other material.** Indonesia, East Kalimantan province, Berau region, Kakaban island, Kakaban lake, N02° 08′ 57.3″ E118° 31′ 26.4″, marine lake, 0-2m. depth, coll. L.E. Becking, ix.2008: RMNH POR. 3933 (#KKB/mol766), RMNH POR. 3934 (#KKB/mol767), RMNH POR. 4495 (#KKB/mol1075), RMNH POR. 3932 (#KKB/mol866), RMNH POR. 4496 (#KKB/mol776), RMNH POR. 4114 (#KKB/mol795); Indonesia, East Kalimantan province, Berau region, NE Maratua island, N02° 17′ 32.3″ E118° 35′ 26.1″, reef, 5-10m depth, coll. N.J. de Voogd, viii.2008: RMNH POR. 3935 (#BER113/mol689), RMNH POR. 3936 (#BER113/mol687); Indonesia, East Kalimantan province, Berau region, Sangalaki Island, N02° 05′ 36.6″ E118° 24′ 15.2″, reef, 5-10m. depth, coll. L.E. Becking, viii.2008: RMNH POR. 4497 (#BER107/mol603), RMNH POR. 3937 (#BER107/mol604), RMNH POR. 3938 (#BER107/mol608), RMNH POR. 3939 (#BER108/mol601); Indonesia, Moluccas province, Ternate, reef, 5-10m. depth, coll. N.J. de Voogd, xi.2009: RMNH POR. 3976, RMNH POR. 3977, RMNH POR. 3978 (#PM-TER02, #PM-TER08, #PM-TER12); Indonesia, North Sulawesi province, Bunaken, reef, 5-30m. depth, coll. L.E. Becking, ix.2006: RMNH POR. POR3166 (#LEMD13/69), RMNH POR. 3177

(#LEMD22/87), RMNH POR. 3154 (#LEMD05/30); Singapore, Pulau Semakau northwest Side, N01° 13'70", E103° 45'61'", reef, 10-12m. depth, coll. N.J. de Voogd, III.2006: RMNH POR. 2463 (# Sin05/270306/025), RMNH POR. 2464 (# Sin05/270306/026); Micronesia, Yap island, N09° 31' 36.7" E138° 07' 48.7", reefflat in front of mangrove, 1-3m. depth, coll. L.E. Becking, viii.2010: RMNH POR. 3940 (#P-YAP1), RMNH POR. 3941 (#P-YAP2), RMNH POR. 3942 (#P-YAP3); Indonesia, South Sulawesi province, Spermonde archipelago, reef, 5-30m. depth, coll. N.J. de Voogd: ZMA Por. 13097; Seychelles, Mahé, coll. R.W.M. van Soest, xii.1992: ZMA Por. 10459, ZMA Por. 10496.

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 cover larger areas hidden within crevices. Ectosome color in living specimens ranges from purplish brown, 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 x 10-**13**-18 x 10-16-20 μm, small concave to straight tylostyles with sharp ends 205-293-420 x 5-**10**-13 x 5-**10**-13 μm. Microscleres selenasters 58-**63**-68 x 45-**52**-68 μm, spheresters 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 x 5-16 X 8-18 μm, small tylostyles 190-470 x 3-13 X 3-15 μm, selenasters 45-83 x 30-65 μm, spherules 1-3 μm, spheresters only found in singles in some individuals 15-20 μm.

Skeleton: as in genus description with addition of sporadic spheresters lodged amidst selenasters in cortex and high abundance of spherules in choanosome and cortex.

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

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

Remarks: In the original description by Gray (1867) there is no mention of two size classes of tylostyles. Reexamination of the original slide revealed that the holotype contains two size classes of tylostyles; the larger tylostyles with blunt endings and the smaller tylostyles with hastate endings. The Systema Porifera indicates that the holotype has two size classes, the large 720-**963**-1200 x 13-**14.1**-19 μm and the small 350-**438.8**-560 x **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 x 10-**13**-18 μm and 205-**293**-420 x 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 spheresters are only sporadically present, often absent.

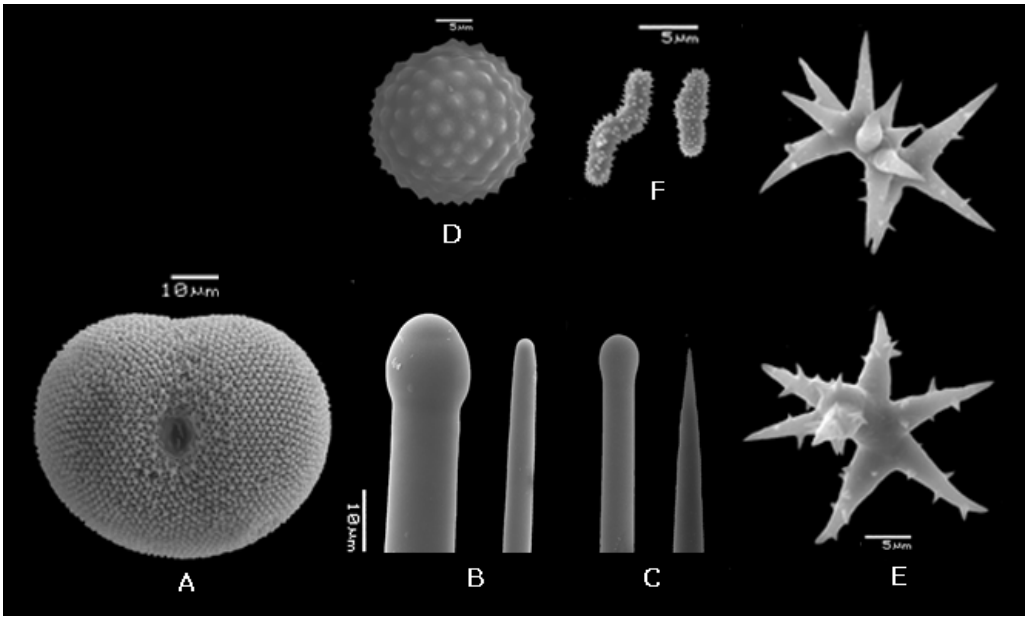


Figure 7. *Placospongia mixta* holotype (ZMB 3204) A. selenaster, B large tylostyle (head and blunt end), C. small tylostyle (head and hastate end), D. spheraster, E. spirasters, F. microrhabds

***Placospongia mixta* Thiele 1900**

Figure 7

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

Material examined:

Holotype. Indonesia, Moluccas, Ternate: ZMB 3204

Vosmaer & Vernhout 1902. Indonesia, Moluccas province, Aru Islands, Siboga stat.273, 13m. depth: RMNH POR. 742 (#163a); Indonesia, South Sulawesi province, N. of Kabia Island, Siboga stat.213, 36m. depth: RMNH POR. 745 (#577); Indonesia, West Papua province, Raja Ampat region, E. of Misool, S01° 42.5' E130° 47.5', 32m. depth: RMNH POR. 753 (#311), RMNH POR. 763 (#1004), RMNH POR. 764 (#1850), RMNH POR. 765 (#1854), RMNH POR. 766 (#1856), RMNH POR. 751 (#1857). **Other material.** Indonesia, East Kalimantan province, Berau region, Kakaban island, Kakaban lake, N02° 08' 57.3" E118° 31' 26.4", marine lake, 0-2m. depth, coll. L.E. Becking, ix.2008: RMNH POR. 4489 (#KKB/mol721), RMNH POR. 4490 (#KKB/mol830), RMNH POR. 3959 (#KKB/mol827), RMNH POR. 3960 (#KKB/mol829), RMNH POR. 3961 (#KKB/mol851), RMNH POR. 3979 (#KKB/mol 779); Indonesia, East Kalimantan province, Berau region, lighthouse near Berau river, N 02° 09' 49.9" E 118° 10' 12.8", reef, 10m. depth, coll. L.E. Becking, viiI.2008: RMNH POR. 4491 (#BER109/mol629); Indonesia, East Kalimantan province, Berau region, Kakaban island, N02° 08' 07.5" E118° 30' 23.3", reef, 10m. depth, coll. N.J. de Voogd, viii.2008: RMNH POR. 4492 (#BER111/mol666), RMNH POR. 3962 (#BER111/mol1203), RMNH POR. 3963 (#BER111/1209), RMNH POR. 3964 (#BER111/1213), RMNH POR. 3965 (#BER111/mol1219); Indonesia, North Sulawesi province, Bunaken, reef, 19m. depth, coll. L.E. Becking, ix.2006: RMNH POR. 3158 (#LEMD08/42), RMNH POR. 3148 (#LEMD40/21), RMNH POR. 3163(#LEMD11/52), RMNH POR. 3155 (#LEMD06/32), RMNH POR. 3157 (#LEMD08/39); Indonesia, Moluccas province, Ternate, reef, coll. N.J. de Voogd, xi.2009: RMNH POR. 4112, RMNH POR. 4113 (#P-TER11, #P-TER22); Indonesia, West Papua province, Raja Ampat region, Gam island, Ctenophore lake, S0°27'17.46" E130°29'33.77", marine lake, 0-2m. depth, coll. L.E. Becking, xi.2007: RMNH POR. 4494 (#RAJ23/mol199), RMNH POR. 3966 (#RAJ23/mol195), RMNH POR. 3967 (#RAJ23/mol187); Indonesia, West Papua province, Raja Ampat region, Waigeo Island, Teluk Mayabilit, S00°18'17.04" E130°54'15.60", reef, 10m. depth, coll. L.E. Becking, xii.2007: RMNH POR. 4493 (#RAJ64/mol428), RMNH POR. 3968 (#RAJ64/mol429), RMNH POR. 3969 (#RAJ64/mol430), RMNH POR. 3970 (#RAJ64/mol431), RMNH POR. 3971 (#RAJ64/mol432), RMNH POR. 3972 (#RAJ64/mol433); Indonesia, West Papua province, Raja Ampat region, Fam Island, S00° 36' 01.5" E130° 45' 08", rocky shore, 0-1m. depth, coll. L.E. Becking, xi.2007: RMNH POR. 3973 (#RAJ39/mol249), RMNH POR. 3974 (#RAJ39/mol250), RMNH POR. 3975 (#RAJ39/mol254); Seychelles, Mahé, southeast coast, near Pointe Cocos, IOP-E stat.738/08, coll. R.W.M. van Soest, 1992: ZMA Por. 10495**;** Indonesia, South Sulawesi province, SW Salayer, reef N of Pulau Bahuluang, Snellius Expedition II stat.079/1, coll. R.W.M. van Soest, 1984: ZMA Por. 0896.

Description: Holotype ZMB3204 encrusting, size 5 x 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 4-10mm, 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.

Spicules: Holotype ZMB 3204 (Fig. 6) Megascleres large straight tylostyles with blunt/rounded point 355-**672**-940 x 7.5-**12**-17.5 x 7.5-**16**-20 μm, small straight tylostyles with sharp point 165-**226**-275 x 2.5-**6**-7.5 x 2.5-**8**-10 μm*;* microscleres selenasters 55-**70**-75 x 42.5-**55**-72.5 μm, spherasters (abundant) 20-**25**-30 μm, spirasters typically with well developed axis and with 4-9 rays with hastate endings, rays can be spined, but do have no bifurcations of the tips 15-**24**-32.5 x 2.5-**8**-12.5 μm; acanthose microrhabs with straight or zig-zag axis 5-7-10 x <2.5 μm. The range within the examined material (Table 1): large tylostyles 460-1250 x 8-23 X 10-25 μm, small tylostyles 120-430 x 3-15 X 2-15 μm, selenasters 50-85 x 22-73 μm, spherasters 13-30 μm, spirasters 15-35 x 2-15 μm, rays 5-18 x 1-2.5 μm .

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. Spherasters amidst selenasters in cortex and scattered in choanosome.

Distribution: East African coast to eastern Indonesia. 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. No *P. mixta* specimens were observed in the Seychelles material deposited at ZMA.

Ecology: Common reef species, also occurs in marine lakes.

Remarks:
In 1900 Thiele described a new species named *P. mixta*, of which the holotype 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 spirasters 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. In the present study the specimens of Vosmaer and Vernhout (1902) were reexamined (see material and Methods). After inspection, the specimens labeled '*P. carinata*' could be clearly and consistently divided into two species: *P. carinata* without spherasters, with spirasters displaying bifurcating endings, and tylostyles up to 980 μm, and *P. mixta* with abundant spherasters, with spirasters displaying hastate endings, and tylostyles up to 1250 μm. In none of the specimens of Vosmaer & Vernhout (1902), nor in the other specimens reviewed for this study was there a mixture of the two types of spirasters. These species also show molecular distinction in both mitochondrial and nuclear markers (Fig. 9, Table 2 & 3).

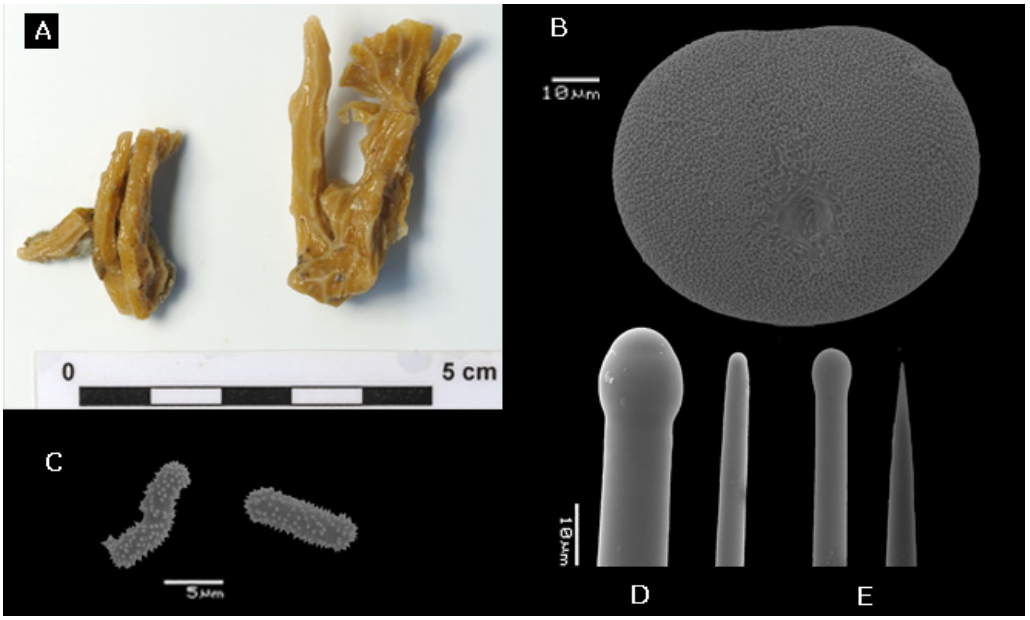


Figure 8. *Placospongia santodomingoae* sp.n. (RMNH POR. 4486) A. ethanol preserved specimen, B. selenaster, C large tylostyle (head and blunt end), D. small tylostyle (head and hastate end), E. microrhabds

***Placospongia santodomingoae* sp.n.**

Figure 8

Holotype. Indonesia, East Kalimantan province, Maratua island, Buli Halo anchialine pool, N02° 11' 16.4" E118° 37' 06.4", anchialine pool, 0-1m. depth, xi.2008, coll. N.K. Santodomingo & Estradivari: RMNH POR. 4486 (#BER128/mol1147). **Paratypes.** Indonesia, East Kalimantan province, Maratua island, Buli Halo anchialine pool, N02° 11' 16.4" E118° 37' 06.4", anchialine pool, 0-1m. depth, xi.2008, coll. N. K. Santodomingo & Estradivari: RMNH POR. 4487 (#BER128/1125), RMNH POR. 4488 (#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 of typical *Placospongia* cortical plates separated by contractible grooves which form a network on the surface. Oscules are present in the grooves. Color of the live holotype was orange-brown, the paratypes were orange, and these colors become slightly lighter after alcohol preservation (Fig. 8A).

Spicules: Holotype (Fig. 8) megascleres large straight tylostyles with blunt point 430-**605**.5-660 x 13-**15**.5-20 x 13-**18.1**-23 μm, small straight tylostyles with sharp point 240-**261.3**-290 x 5-**7.2**-8 x 5-**8.8**-10 μm; microscleres selenasters 80-**84.8**-90 x 60-**67.3**-75 μm, acanthose microrhabds 8-**12.3**-18 x 2.5-**2.7**-3.5 μm. Range of the paratypes (Table 1) large straight tylostyles with blunt point 430-**480**-760 x 13-**20** x 15-23 μm, small straight tylostyles with sharp point 190-380 x 5-13 x 8-15 μm, microscleres selenasters 63-93 x 58-75 μm, acanthose microrhabds with straight axis 5-20 x 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).

Ecology: 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 research on anchialine systems.

Remarks: *Placospongia santodomingoae* sp. n. is similar to *P. carinata,* yet lacks spirasters and has shorter tylostyles. *Placospongia santodomingoae* sp. n. likewise differs from *P. mixta* by the absence of spirasters as well as the absence of spheraster. *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)**
Placospongia labyrinthica Kirkpatrick 1903: Plate V Fig. 1a-b, Plate VI Fig. 1a-f

Reviewed material:

Holotype. South Africa, East London Coast, S33° 06' 30" E028° 11': BMNH 02.11.16.1

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* and has sieve pores, sterrasters with star-like plates, euasters, styles and oxea characteristic of the Geodiidae. In the original description by 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 required as suggested on the World Porifera Database (van Soest et al. 2011).

Identification key for Indo-Pacific species of *Placospongia*

1. Spirasters absent.....2

   Spirasters present.....3

2. Spherules present.....*P. melobesioides*

   Spherules absent, acanthose microrhabds present.....*P. santodomingoae* sp.n.

3. Spirasters with elongated rays of 4-18 μm, acanthose microrhabds present 4

   Spirasters stout with short acanthose spines spirally placed on shaft.....*P. anthosigma*

4. Spirasters have rays with ends bifurcating or with tufts.....*P. carinata*

   Spirasters have rays with hastate ends, spherasters present.....*P. mixta*

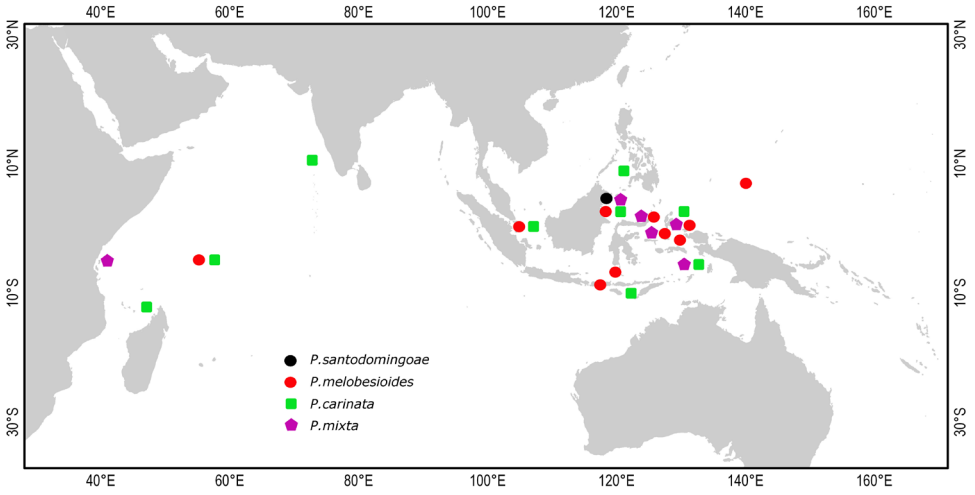


Figure 9. Distribution of *Placospongia* spp. in the Indo-West Pacific. Location of symbols is approximate.

Genetic data analysis

Final alignments (excluding primers) were obtained for the sponge *Placospongia* of 581 bp for COI with three genetic variants (25 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 2). 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 788bp were obtained for ITS with 18 genetic variants from the present study (21 individuals) and 27 genetic variant from GenBank (for GenBank accession numbers see figure 11). 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 3 for uncorrected inter- and intaspecific *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*. Clades 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 sequenced in the present study fell into either C3 or C6 clade. The specimens of *P. carinata* without spirasters represented a separate lineage within the *P. carinata* clade (C4) which was supported by Bayesian inference, but not by maximujm likelihood. The *p*-distance between *P. carinata* specimens and the specimens of *P. santodomingoae* sp.n. was 0.6%.

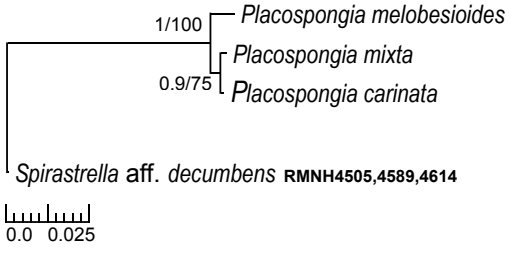


Figure 10 Bayesian/maximum Likelihood phylograms Cytochrome Oxidase I (COI) sequences from Indo-Pacific *Placospongia* spp. *Placospongia melobesioides* was represented by RMNH POR. 2464, 3166, 4497, 3942, 3976, 4495, 4496, ZMA POR.10496; *Placospongia mixta* by RMNH POR. 3158, 3936, 4113, 4489, 4490, 4492, 4494, 4493; *Placospongia carinata* by RMNH POR. 4482, 4483, 4484, 4485, 4486, 4487, 4488, ZMA Por. 10727, ZMA Por. 11367. Only posterior probabilities of >90 and Maximum Likelihood values of >70 indicated. Scale bars indicate substitutions/site.

Table 2 The number of base differences per site from averaging over all COI sequence pairs between *Placospongia* spp. groups are shown (uncorrected *p*-distances). Standard error estimate(s) are shown above the diagonal. The analysis involved 30 nucleotide sequences. There was no within-group difference. *Spirastrella* aff. *decumbens* was used as outgroup in the phylogenetic inference of 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	*

Table 3 The number of base differences per site from averaging over all ITS sequence pairs between *Placospongia* spp. groups are shown (uncorrected *p*-distances). Standard error estimate(s) are shown above the diagonal. The analysis involved 73 nucleotide sequences. All positions with less than 5% site coverage were eliminated. Black cursive along the diagonal indicates within-group uncorrected *p*-distance. C9, C5, C6, C4, C3 refer to five clades with 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***	*0.5*	*0.8*	*0.9*
C6	14.0	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	***0.4***	*0.8*
C3	15.2	7.1	6.1	5.9	14.5	6.9	6.3	5.6	***0.9***

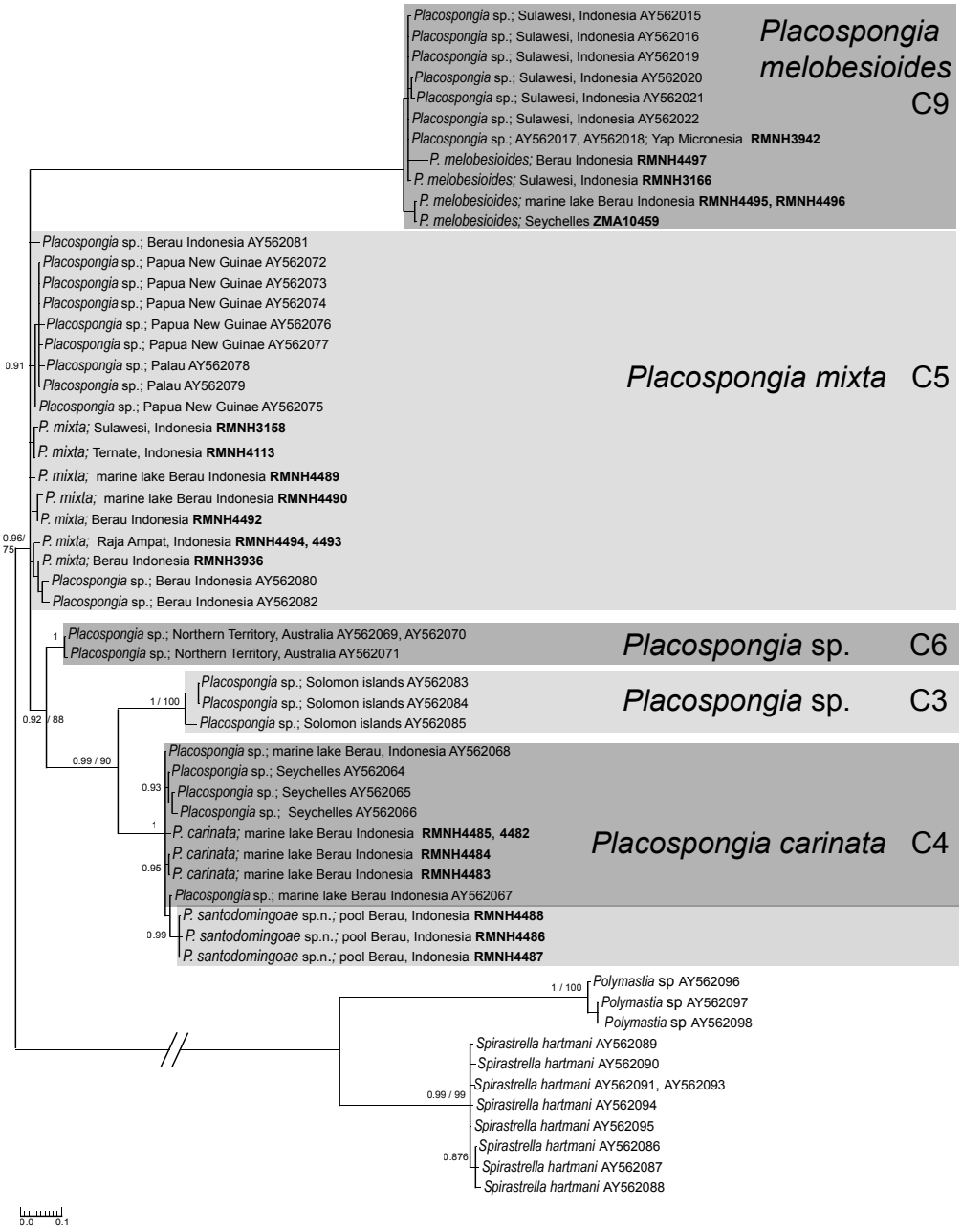


Figure 11 Bayesian/maximum likelihood phylograms of genotypes of the internal transcribed spacer region of nuclear ribosomal operons (ITS) of Indo-Pacific *Placospongia* spp. found in this study and related species from the same genus collected from GenBank. Clades C3,C4,C5,C6 & C9 refer to the clades 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 bars indicate 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. *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 for mitochondrial DNA and particularly COI in sponges is known to be low (Wörheide 2006, Xavier et al. 2010, Pöppe 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 furthermore 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 ZMA Por. 11367 and ZMA Por. 10727 from the Seychelles are identical with specimens RMNH POR. 4483, RMNH POR. 4482, RMNH POR. 4484, RMNH POR. 4485 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 3). *Placospongia santodomingoae* sp.n. should, however, be designated as a new species based on the spicule morphology: the absence of a distinguishing spicule type (spirasters) and consistently shorter and thicker tylostyles (maximum 760 x 20 µm) compared to *P. carinata* (maximum 980 x 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 novel species across a large spectrum of taxa (e.g. Holthuis 1973, Tomascik & Mah 1994, Dawson & Hamner 2005, Becking et al. 2011, CHAPTERS 3, 4, 6 & 7). The divergence of *P. santodomingoae* sp.n. from *P. carinata* is likely too recent to be expressed in the molecular markers I 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 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, Miscronesia and the Seychelles) and clade C5 is of *P. mixta* (specimens from Indonesia, Palau and Papua New Guinae). 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 'Mudmaps' of these specimens in the Queensland Museum portray images of spicules that fit the definition of *P. mixta* (in particular the spirasters with hastate endings). The morphology of these specimens should be further studied in order to 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 spicule composition. 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. All the red specimens appeared to belong 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). Within each species there is also some natural variation in the range of tylostyle length and spicule morphology. The spiraster 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 ornamentation and size of spines can be diverse. For example the spirasters 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 & 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* n.sp. 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 & 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. melobesoides* and *P. carinata.* Rua et al. (2006) and Nichols & Barnes (2005), furthermore, show that there are distinct lineages in the Caribbean 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. The epitome of this sympatry is represented in Kakaban lake where in the 4 km^2 area of the marine lake three common tropical species co-exist. Without review of the spicule morphology the true diversity of species in the study area would perhaps remain concealed.

Acknowledgements

N.J. de Voogd, R.W.M. van Soest, and B.W. Hoeksema 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, and the staff of Conservation International Wayag Station, of Papua Diving, of Nabucco Island Dive Resort, of Derawan Dive Resort, and of WWF/TNC Berau office. The present study is part of a PhD-project funded by NWO, division Earth and Life Sciences (ALW IPJ-07002; # 817.01.008). 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.