

Seven new deep-water Tetractinellida (Porifera: Demospongiae) from the Galápagos Islands – morphological descriptions and DNA barcodes

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The Galápagos Islands, positioned in the confluence of warm and coldwater currents in the Eastern Pacific, is well known for the high degree of endemism of its marine invertebrate fauna. This fauna has been studied extensively in recent years: the echinoderms, corals and other benthic cnidarians, but little is known about the deep- and shallow-water sponge faunas. To date, only 70 sponge species have been described from the Galápagos Islands, 37 of which are endemic. Of these 70 species, only one shallow-water species of desma-bearing Tetractinellida (Demospongiae), *Corallistes isabela*, has been reported. In 1995, Harbor Branch Oceanographic Institution, Florida, led an expedition around the Galápagos archipelago, focussed on the collection of deep-water Porifera. Here, we describe seven new species and provide DNA barcodes for the tetractinellids from these collections. Phylogenetic relationships of these new species are discussed and compared with other material from the Caribbean, the Central and West Pacific Oceans. The new species represent five genera (*Craniella*, and desma-bearing Tetractinellida *Neophrissospongia*, *Corallistes*, *Racodiscula* and *Scleritoderma*). Phylogenetic reconstructions combining independent markers (mtDNA and rDNA) support the generic affiliation of these new species and confirm the separation of Eastern Pacific species from Caribbean and Central to West Pacific species.

ADDITIONAL KEYWORDS: Astrophorina – *COI* – Corallistidae – DNA barcode – endemism – Galápagos Islands – lithistids – Scleritodermidae – Spirophorina – Theonellidae.

INTRODUCTION

The Galápagos Islands are located on the Nazca Plate, in the Eastern equatorial Pacific Ocean, about 926

km west of Ecuador (Fig. 1). Plate tectonic analyses demonstrate proximity to the so-called ‘Galápagos Triple Junction’, where the Pacific, Cocos and Nazca plates concur (Holden & Dietz, 1972). The position of Galápagos archipelago at the confluence of three major cold oceanic currents (Peru Coastal, Peru Oceanic and Cromwell) and one warm (Panamá) oceanic current (Chavez & Brusca, 1991) is further influenced by

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anomalous events such as the El Niño and La Niña, which seasonally change the ocean surface temperatures and are known to impact shallow coral reef communities (Feingold, 2011). Due to its extremely isolated geographic position, diverse underwater geomorphological settings as a result of ongoing tectonic and volcanic activity (Geist *et al.*, 2008), in combination with complex mixing of water masses (Chavez & Brusca, 1991), the Galápagos archipelago constitutes a unique marine laboratory to investigate the ecology, evolution and biogeography of marine animals in an isolated region of the Eastern Pacific. In 1978, the Galápagos Islands were protected by UNESCO and described as a 'unique living museum and showcase of evolution'. In 2001, the status of World Heritage Site was granted to the Galápagos Marine Reserve (133 000 km²), which comprises the second largest Marine Reserve in the world (UNESCO, 1978).

Despite the young age of the Galápagos platform (10–15 Myr) and its biogeographic isolation (e.g. Chavez & Brusca, 1991), the marine invertebrate fauna is relatively diverse and reveals large numbers of described endemic species, including echinoderms, molluscs, corals and other benthic cnidarians (James, 1991). However, little is known about the deep- and shallow-water sponge fauna; to date, only 70 demosponge species have been described from the Galápagos Islands, of which 37 (~50%) are endemic. Only seven deep-water species (>100 m) have been described (Wilson, 1904; de Laubenfels, 1939; Desqueyroux-Faúndez & Van Soest, 1997). Among these 70 species, 11 were reported as belonging to the order Tetractinellida Marshall, 1876 (Desqueyroux-Faúndez & Van Soest, 1997), of which ten belong to Astrophorina Sollas, 1887 (families Ancorinidae Schmidt, 1870; Pachastrellidae Carter, 1875; Geodiidae Gray, 1867; Corallistidae Sollas,

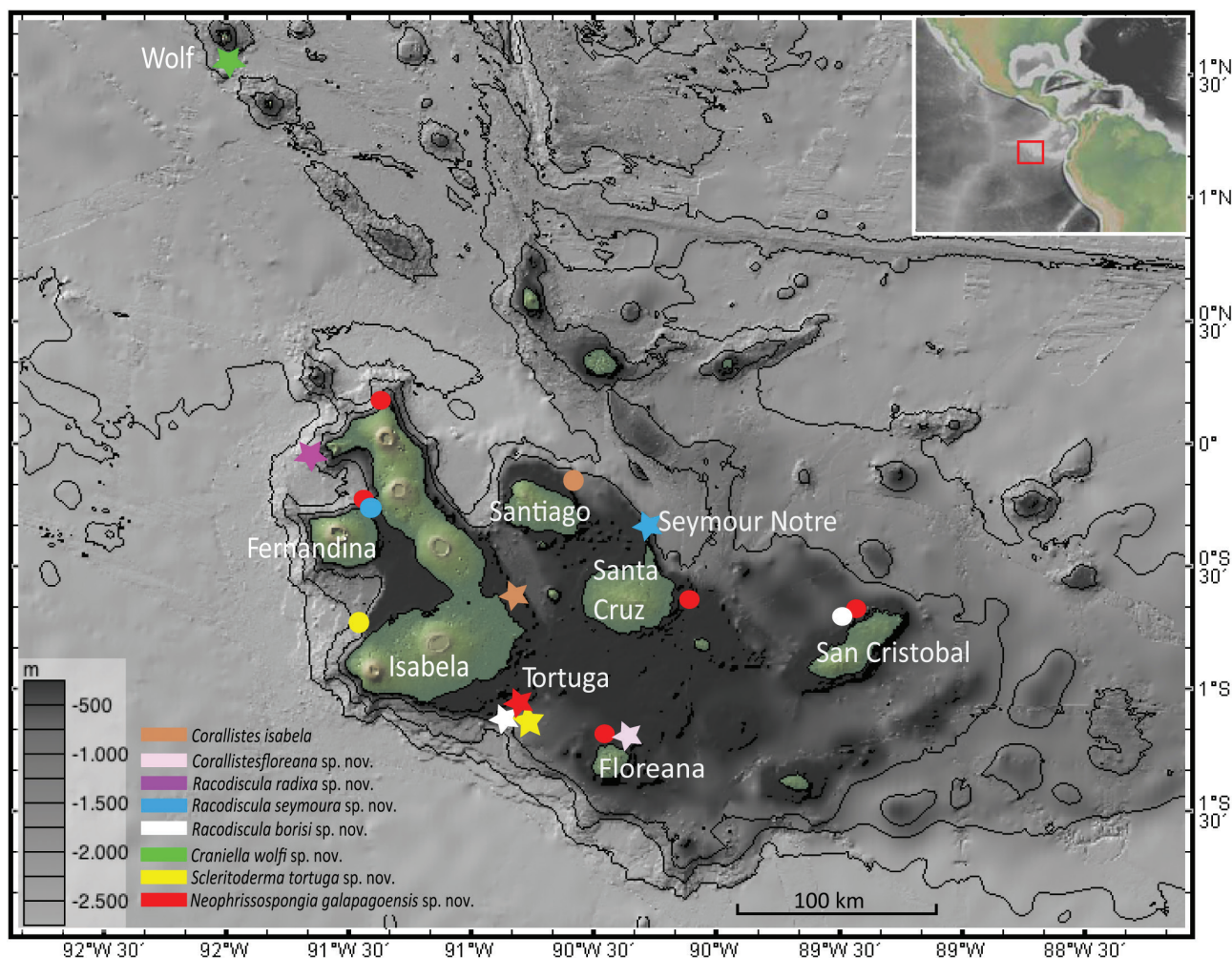


Figure 1. Map of the Galápagos archipelago with sampling locations and species collected. Colour code denotes species and records (stars = holotype location; circles = paratypes). Map made with GeoMapApp, <http://www.geomapapp.org>, using the implemented Global Multi-Resolution Topography (GMRT Grid Version 3.2) (Ryan *et al.*, 2009).

1888) and one (*Cinachyrella globulosa* Desqueyroux-Faúndez & Van Soest, 1997) to the Spirophorina Bergquist & Hogg, 1969 (Tetillidae Sollas, 1886). The Galápagos archipelago constitutes one of the largest island groups in the Pacific, but has the lowest demosponge diversity compared to the Central Indo-Pacific and Tropical Eastern Pacific (Van Soest *et al.*, 2012).

Besides the discovery of one shallow-water species of the family Corallistidae (*Corallistes isabela* Desqueyroux-Faúndez & Van Soest, 1997), no further desma-bearing tetractinellids have been described from this area. This study is focused primarily on the morphological description of seven new deep-water tetractinellids from the Galápagos archipelago, the majority of which are desma-bearing, and provides additional supportive molecular phylogenies for the generic affiliation of these new species using three markers (mtDNA *COI* and 28S, 18S rDNA).

MATERIAL AND METHODS

SAMPLING

Sponges were collected using the *Johnson-Sea-Link I* research submersible, operated by Harbor Branch Oceanographic Institution (HBOI), during an expedition to the Galápagos Islands, conducted 14–31 October 1995. The objective of the expedition was to conduct a biodiversity inventory and carry out biodiscovery research on sponges, octocorals and algae. Sponges analyzed in the present study were collected from ten stations around the Galápagos archipelago between 21 and 396 m depth (Fig. 1). A detailed list of these specimens, with their corresponding vouchers, location, latitude, longitude and National Center for Biotechnology Information (NCBI) accession number is given in Supporting Information, Table S1. Frozen (–80 °C) as well as formalin and 70% ethanol-preserved specimens, stubs, spicule slides and skeletal sections of the type materials are archived at the Harbor Branch Oceanographic Museum (HBOM), Fort Pierce, Florida, USA.

MOLECULAR INVESTIGATIONS

Two independent molecular markers were used to amplify 18 samples from the Galápagos Islands and 16 comparative samples, following the protocol described in Schuster *et al.* (2015): the ‘Folmer’ fragment of mitochondrial *COI* (c. 650 bp) and the C1-D2 region of the nuclear 28S rDNA (c. 800 bp), using, respectively, the primers dgLCO1490 and dgHCO2198 (Meyer, Geller & Paulay, 2005) and C1’ASTR (Cárdenas *et al.*, 2009) and D2 (Lê, Lecointre & Perasso, 1993).

Additionally, we amplified and sequenced near-complete 18S rDNA sequences (~1800 bp) for all Galápagos Islands samples. Amplification of ~1800 bp of 18S was

performed using primers SP18aF and SP18gR (Lavrov, Wang & Kelly, 2008) with PCR settings of 95 °C, 3 min (95 °C, 30 s; 50 °C, 40 s; 72 °C, 2.5 min) × 35 cycles; 72 °C, 7 min. Primers SP18aF, SP18gR (Lavrov *et al.*, 2008), and 400F18S, 1200F18S, 600R18S and 1350R18S (Lavrov *et al.*, 2008) were used to sequence ~1800 bp of overlapping fragments. In order to confirm the sponge origin of newly generated sequences, we assessed their homology by BLAST searches against NCBI GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Sequences were assembled into contigs using Geneious v.8.1.8 (<http://www.geneious.com>, Kearse *et al.*, 2012). 28S (C1-D2) and *COI* were independently aligned against alignments from Schuster *et al.* (2015). In addition to these sequences, we included *Stupenda singularis* Kelly & Cárdenas, 2016, in the alignment, taxonomic updates on the Antarctic Tetillidae made by Carella *et al.* (2016), *Theonella* species from Hall, Ekins & Hooper (2014), and corrected *Isabella tanoa* Ekins, Erpenbeck, Wörheide & Hooper, 2016, which was re-identified by Ekins *et al.* (2016) and previously named as *Isabella mirabilis* (Schuster *et al.*, 2015). *Isabella harborbranchi* Carvalho, Pomponi & Xavier, 2015, was added from Carvalho *et al.* (2015) (see also Supporting Information, Table S1). A representative concatenated matrix was compiled from the independent *COI* and 28S alignments in Geneious, assuring that for most taxa, sequences of both markers were present. The concatenated alignment (28S rDNA and *COI* mtDNA) was 1440 bp long; 658 bp were constant, 105 bp parsimony uninformative and 677 bp parsimony informative. Sequences of 18S rDNA were only obtained from the Galápagos Islands material, thus we did not incorporate these sequences into the final concatenated alignment. Bayesian inference (BI) and maximum likelihood (ML) analyses were conducted as described in Schuster *et al.* (2015). Partitionfinder v.1.1.1 (Lanfear *et al.*, 2012) was used to select the best-fit partitioning scheme and model, which was the generalizing GTR+G+I model for both markers. New sequences from this study are stored at GenBank (NCBI). GenBank accession numbers are: *COI* KY652805–KY652825; 28S C1-D2 KY652771–KY652804; 18S KY652826–KY652840. Additionally, *COI* sequences of all type material are deposited at the Sponge Barcoding Project (SBP) (<http://www.spongebarcoding.org>) (record numbers 1702–1733). The concatenated alignment from this study is freely available at OpenDataLMU (<https://doi.org/10.5282/ubm/data.113>).

MORPHOLOGICAL INVESTIGATIONS

In order to carry out scanning electron microscopy (SEM) of the sponge skeleton and spicules, a section of formalin-preserved sponge tissue, perpendicular to the sponge surface, was cut under a binocular microscope, dried and digested in 66%

nitric acid (HNO₃) for 15–20 min. The digested product was boiled until all organic material was dissolved. The remaining skeletons and spicules were rinsed at least five times with ultrapure water and twice with 99% ethanol, as described in [Pisera & Pomponi \(2015\)](#). The zygoed skeletons were mounted on SEM stubs using Carbon-Leit-C (PLANO, Germany) with the ectosomal surface uppermost. Loose spicules were prepared on glass coverslips attached to SEM stubs. All stubs were sputter-coated with gold and examined with a Leo 1430VP SEM at 5–25 kV at the Zoological State Collection (Munich). For thick histological sections, the same tissue orientation as described above was used. Tissues were resuspended overnight in 70% ethanol, dehydrated in graded ethanol series (1 h 90%, 12 h 99%) at room temperature and embedded into L.R. White (PLANO) after two to three changes. For polymerization, the blocks were placed at 46 °C for 1 h and 60 °C for 12 h. Thick sections (50–200 µm) were cut using a Leica SP1600 saw microtome (Germany) and examined under a Leica M165FC microscope.

Measurements of spicules are given as minimum–**mean**–maximum length × minimum–**mean**–maximum width (µm) with total number of measured spicules (*N*) in parentheses. Measurements of the triaene cladome refer to the total diameter of the cladome, across the endpoints of the clades; measurements of the triaene rhabdome refer to the distance from just below the cladome to the tip. Microscleres in the desma-bearing Tetractinellida are predominantly streptasters, a general term for microscleres with rays that emanate from an elongate centrum (amphiasters, metasters, spirasters). Metasters have a few quite long rays emanating from a short, elongate centrum; spirasters have sparse or numerous rays emanating from a single bent or multiple spiraled elongate centrum; amphiasters have numerous rays emanating from either end of a straight shaft. Strict distinction between these microscleres is often not clear-cut, so we chose to cite combinations of forms used in the present study, for example, streptaster/spiraster or streptaster/amphiaster when distinction was difficult. Measurements of microscleres are given as the complete length of the microsclere and the maximum width of the actin.

RESULTS

SYSTEMATICS

The classification used here follows [Morrow & Cárdenas \(2015\)](#). The taxonomic authority for all new species described is restricted to the first author.

CLASS DEMOSPONGIAE SOLLAS, 1885
 SUBCLASS HETEROSCLEROMORPHA CÁRDENAS, PÉREZ
 & BOURY-ESNAULT, 2012
 ORDER TETRAC TINELLIDA MARSHALL, 1876
 SUBORDER ASTROPHORINA SOLLAS, 1887
 FAMILY CORALLISTIDAE SOLLAS, 1888
 GENUS *NEOPHRISSOSPONGIA* PISERA & LÉVI, 2002
NEOPHRISSOSPONGIA GALAPAGOENSIS SP. NOV.
 (FIGS 2–4; TABLE 1)

Diagnosis: Massive ear-shaped, flabellate, *Neophrissospongia* with rhabdome of dichotriaenes showing a large variation from 172 to 617 µm length with spined cladome and blunt to acute tips.

Type material: Holotype: HBOM 003:02010, Coll. Johnson Sea-Link I (JSL-I) dive 3923 [27 October 1995, Galápagos, 4 nautical miles (NM) SE of Tortuga Island, 01°04'59"S, 90°51'56"W, 241 m]. Paratypes: HBOM 003:01097 (227 m), HBOM 003:01098 (189 m), Galápagos, NE of Rocas Gordin, Santa Cruz Island, 0°32'53"S, 90°7'47"W, Coll. JSL-I dive 3900. HBOM 003:02002 (204 m), HBOM 003:02003 (120 m), Galápagos, 5 NM NE of Kicker Rock at San Cristobal Island, 01°43'8"S, 89°27'14"W, Coll. JSL-I dive 3905. HBOM 003:01099 (177 m), Galápagos, N-Side 1.25 NM NW of Cormorant PT at the Santa Maria Island (Floreana), 01°12'6"S, 90°26'10"W, Coll. JSL-I dive 3901. HBOM 003:02008 (235 m), Galápagos, West Coast 2 NM NW of PTA Tortuga at Isabela Island, 0°14'31"S, 91°24'56"W, Coll. JSL-I dive 3915. HBOM 003:02005 (328 m), Galápagos, NW Coast, 66 NM offshore, Isabela Island, 0°8'35"S, 91°23'42"W, Coll. JSL-I dive 3912.

Comparative material: *Neophrissospongia nolitangere* Schmidt, 1870, HBOI 2-VI-91-1-005 (358 m), Portugal, Madeira, Savage Islands, Selvagem Grande, South Coast, 30°07'21"N, 15°52'7.8"W, Coll. JSL-I dive 3006, June 1991, identified by Michelle Kelly. *Neophrissospongia microstylifera* Lévi & Lévi, 1983 OCDN7083-J (111 m), Palau, Koror Reef, W-Side of Uchelbeluu Reef, wall, rock, 7°16'25"N, 134°31'25"E, 2001, identified by Michelle Kelly; OCDN5043-Q (109 m), Palau, Koror Reef, W-Side of Uchelbeluu Reef, overhang, rock, 7°16'25"N, 134°31'26"E, 1997, identified by Michelle Kelly.

Type locality: Tortuga Island, Galápagos Islands (241 m) (Fig. 1).

Distribution: Tortuga Island, Isabela Island, Fernandina, Santa Cruz Island, San Cristobal, Floreana Island (Fig. 1).

Habitat: Attached to hard substratum, depth range 120–328 m.

Description: Morphology, in the shape of an ear, flared cup or vase, or foliose with folded margins when mature; walls 1–1.5 cm thick, margins flattened with rounded edges (Fig. 2); dimension of holotype, 12 cm diameter, 16 cm high (Fig. 2A); paratypes range 20–30 cm diameter, 30–45 cm high or larger (Fig. 2C, D). **Surface,** with horizontal growth lines; concave inhalant surface with numerous ostia, 25–35 μm diameter; convex (exhalant surface) has numerous, barely visible, oscules. **Texture,** stony, especially towards the base of the sponge. **Colour,** beige in life, brown in ethanol preservative. **Ectosomal skeleton,** 1.2–1.5 mm thick, outermost surface a densely packed, 100- μm -thick layer of microscleres, within which lies the cladomes of the dichotriaenes, the rhabdomes of which penetrate deeply into the choanosome (Fig. 4A, B). **Choanosomal skeleton** composed of zygosed dicranoclone desmas and acanthose microtylostyles,

often located around the pore openings. **Megascleres,** desmas and triaenes; dicranoclone desmas, 280–450 μm (N15) maximum diameter, with a massive, arched, central core, from which four to six arms splay away from the underside of the arch, both the central core and the arms are covered irregularly with tubercles (Fig. 3B, C, F), the tubercles on the desma core are larger, fungiform and multituberculate; dichotriaenes, rhabdome 172–422–617 μm (N30) long, cladome, 175–227–372 μm (N30) wide, upper surface of clads irregularly spined (Fig. 4G, H). **Microscleres,** irregular amphiasters with short, thick rays, 15.2–11.2–18 μm long \times 4.9–7.2–10.7 μm (N25) wide; acanthose microtylostyles, 44–91–133 μm (N25) long.

Etymology: Named after the locality and distribution of the species, the Galápagos Islands.

DNA barcodes: We sequenced partial COI, 28S (C1-D2) and complete 18S of the holotype and the paratypes from different localities of the Galápagos Islands

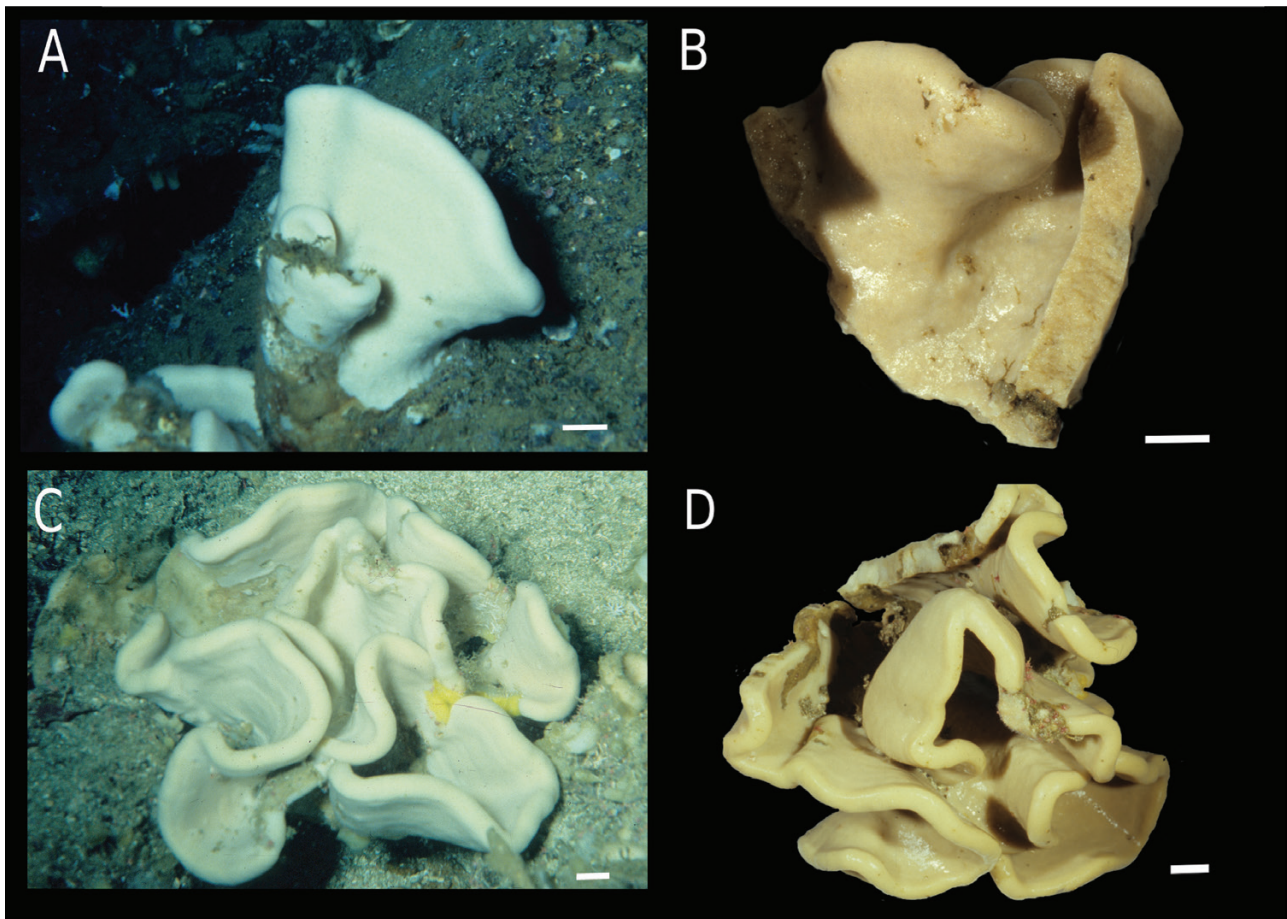


Figure 2. *In situ* and deck pictures of *Neophrissospongia galapagoensis* sp. nov. A, B, cup-shaped holotype Harbor Branch Oceanographic Museum (HBOM) 003:02010. C, D, massive flabellate paratype HBOM 003:01099. Scale bar is 1 cm.

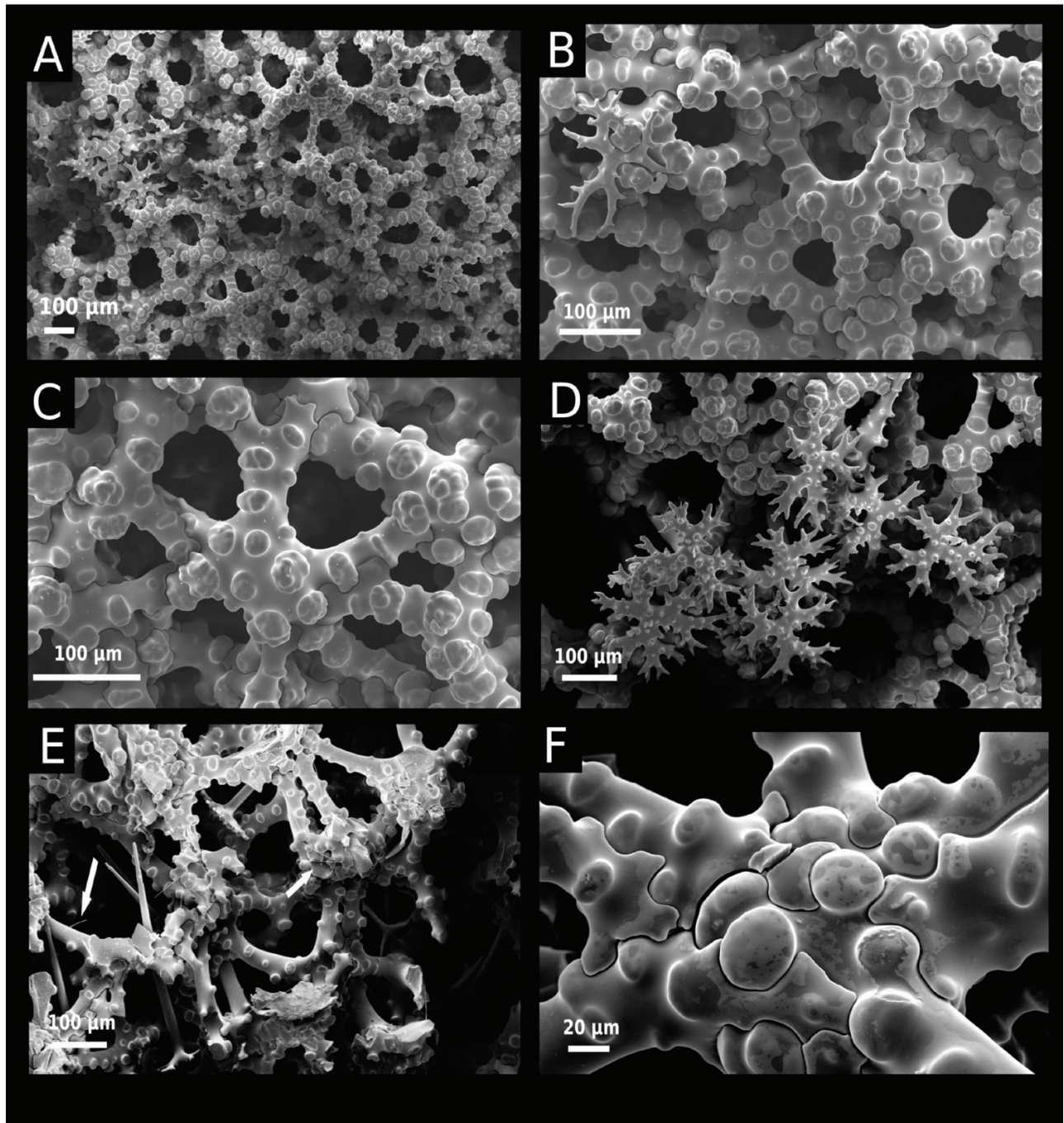


Figure 3. Various views of the choanosome skeleton of *Neophrissospongia galapagoensis* sp. nov. holotype Harbor Branch Oceanographic Museum (HBOM) 003:02010. A, overview of dicranoclone desmas. B, dicranoclone desmas with a spinose dichotriaene *in situ*. C, details of the dicranoclones. D, *in situ* picture of a bundle of spinose dichotriaenes between the ectosome and choanosome. E, choanosome cut with disordered partly broken dicranoclones, arrows pointing to numerous microtylostyles. F, detailed view of dicranoclone articulation (zygome).

(Fig. 1). *COI* sequences of all type material sequenced were identical with the exception of HBOM 003:02005 (no *COI* sequenced). 28S and 18S sequences of all

type material were identical with the exception of HBOM 003:02005 (no 18S sequenced). GenBank accession numbers: *COI* KY652805–KY652811; 28S

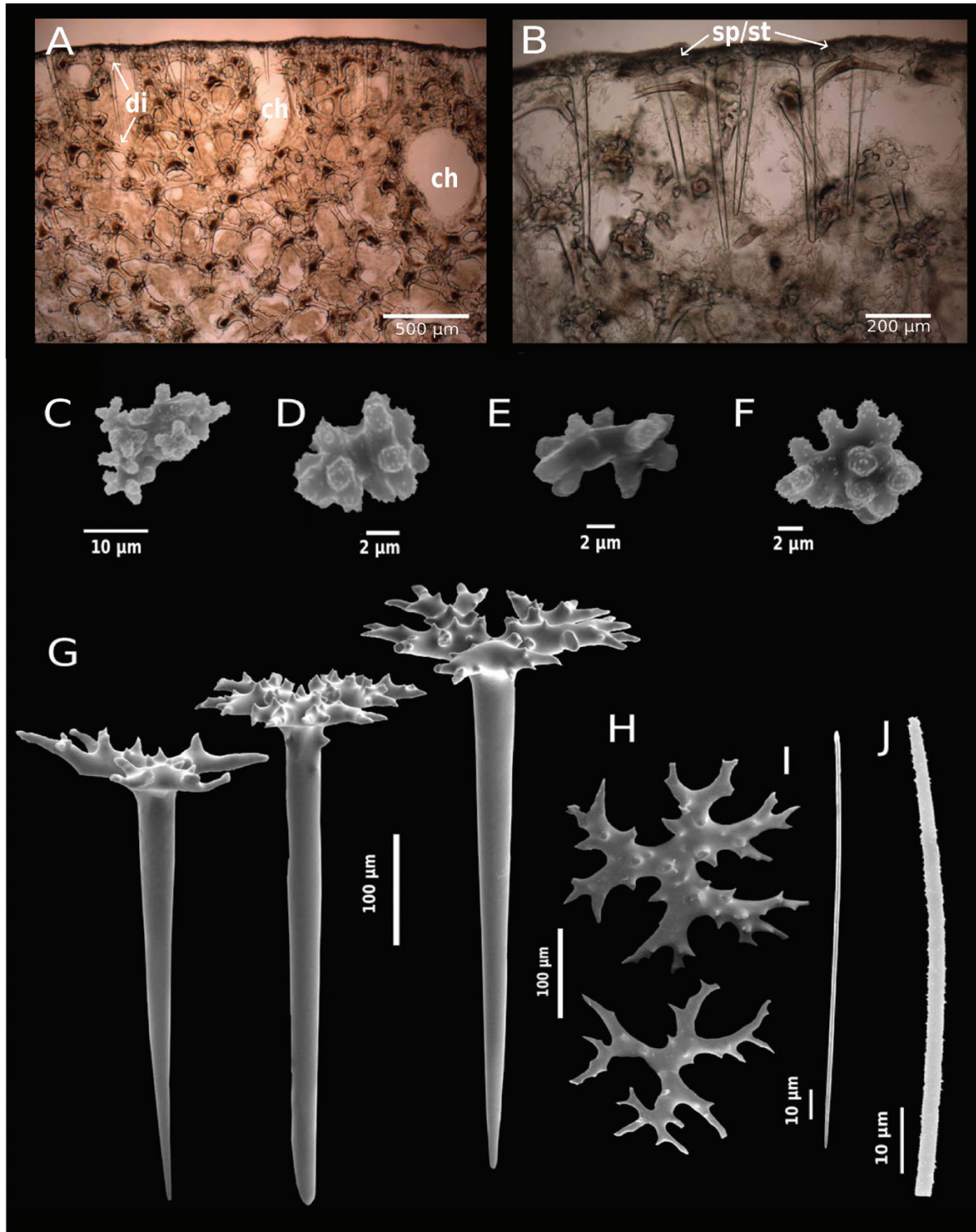


Figure 4. Section and mega- and microscleres of *Neophrissospongia galapagoensis* sp. nov., holotype Harbor Branch Oceanographic Museum (HBOM) 003:02010. A, thin section of the ectosome and choanosome showing channel (ch) and spinose dichotriaenes (di) penetrating deeply into the choanosome. B, detailed thin section illustrating a thick layer of spiraster/streptaster (sp/st) on top of the ectosomal region. C–F, thick streptasters from the ectosomal regions. G, various ectosomal dichotriaenes. H, top view of tubercled cladomes of dichotriaenes. I, J, microspinose choanosomal microtylostyles, general view (I), detailed view (J).

KY652771–KY652778; 18S KY652826–KY652832; SBP No. 1702–1709.

Remarks: According to the World Porifera Database (WPD) (Van Soest *et al.*, 2017, <http://www.marinespecies.org/porifera/>), only six different species of *Neophrissospongia* have been described: *N. nolitangere* (Table 1; Pisera & Vacelet, 2011) and *N. tubulata* Van Soest & Stentoft, 1988 from the tropical Western Atlantic; *N. endoumensis* Pisera & Vacelet, 2011, *N. nana* Manconi & Serusi, 2008 and *N. radjae* Pisera & Vacelet, 2011, from the Mediterranean Sea, and *N. microstylifera* Lévi & Lévi, 1983 from New Caledonia in the Pacific Ocean.

Neophrissospongia galapagoensis sp. nov., which is cup-shaped to foliose, clearly differs from the encrusting *N. nana*, discovered in a Mediterranean marine cave. The club-shaped *N. radjae* (Table 1; Pisera & Vacelet, 2011) from a cave in the Mediterranean with an apical oscule is also distinguished from our species by gross morphology. Minor differences between the dichotriaenes of *N. galapagoensis* sp. nov. and *N. radjae* (Table 1) also distinguish both species: *N. radjae* has a more distinctly tuberculated cladome than in our new species.

Neophrissospongia nolitangere, from the Azores, Cape Verde Islands, off Portugal (e.g. Pisera & Lévi, 2002a), Madeira (Carvalho *et al.*, 2015), submarine caves in the Mediterranean (Table 1; Pisera & Vacelet, 2011) and the Bahamas (Pomponi *et al.*, 2001), is distinguished from *N. galapagoensis* sp. nov. in the

possession of smaller streptasters with longer rays; 28S sequences of *N. nolitangere* by Chombard, Boury-Esnault & Tillier (1998) and here (HBOI 2-VI-91-1-005) are both 100% identical and differ by 1% from our new species. Molecular analyses (Fig. 5) further separate *N. galapagoensis* sp. nov. from *N. nolitangere*. Sequence comparison with material from the West Pacific (WAM Z35946, 36053; probably both new species, A. Pisera, personal observation) disclose a 1.6% difference.

Neophrissospongia tubulata from Barbados is characterized by small, single or clustered tubes, in which the dichotriaenes have short rhabds (30–50 µm) and a non-tuberculate cladome. Rather, the clads have dentate margins, clearly distinguishing them from the dichotriaenes of *N. galapagoensis* sp. nov. (Table 1) and all other members of the genus. This lateral dentation of the dichotriaene clads of *N. tubulata* points to the need for a revision of this species.

The dichotriaenes of *N. endoumensis*, from the Mediterranean (cladome width: 248–283 µm; rhabdome length: 531–615 µm) are larger and thicker and are less variable in size (Table 1; Pisera & Vacelet, 2011) compared to those of *N. galapagoensis* sp. nov.

In the original description of tubular *N. microstylifera* from New Caledonia, Lévi & Lévi (1983) documented microstyles of 110–170 µm length, which are absent in our new species, and the dichotriaenes are more robust than those of *N. galapagoensis* sp. nov.

KEY TO SPECIES OF *NEOPHRISSOSPONGIA* (AFTER MANCONI & SERUSI, 2008 AND THE PRESENT STUDY)

- (1) Growth form ear- to cup-shaped or large flabellate massive foliose with small inhalant and large exhalant apertures on opposite sides of the lamella, ectosomal skeleton of tuberculated dichotriaenes, styles/subtylostyles, smaller streptaster microscleres with long arms *N. nolitangere*
- Spinose microtylostyles and streptaster with massive arms as microscleres..... ***N. galapagoensis* sp. nov.**
- Cup-shaped growth with spinose and massive cladome of dichotriaenes and regular massive streptaster *N. endoumensis*
- Growth form clavate to plate-like with inhalant and exhalant areas in different regions of the same sponge surface..... 2
- (2) Growth form clavate, ectosomal skeleton of dichotriaenes and strongyle/ subtylostyles *N. microstylifera*
- Spined dichotriaenes and spinose microtylostyles..... *N. radjae*
- Plate-like grow form, ectosomal skeleton of dichotriaenes, styles/subtylostyles and aster-like microscleres..... *N. nana*

Table 1. Outer morphology and spicule measurements of the known seven *Neophrissospongia* species including the new species *N. galapagoensis* sp. nov.

Species Distribution Depth	Grow form/shape	Dicranoclone desma	Dichotriane Cladome tuberculated/ spinose	Dichotriane smooth	Monaxial spicule	Streptaster/ amphiaster
<i>N. nolitangere</i> Schmidt, 1870* Portugal	Flabellate massive	350–500	Tuberculate clad 230–310* clad	Not recorded	Oxea not measured, not recorded*	10–13 × 9–10
Depth unknown			450–1200* rhabd			
<i>N. nolitangere</i> Schmidt, 1870 Azores, Fayal 365 m	Flabellate massive	35 thick	Tuberculated clad 45–50 prot 90–100 deut 450–1200 rhabd	Not recorded	Oxea 70–100	10–13
<i>N. nolitangere</i> Schmidt, 1870 Azores, Fayal	Flabellate massive	Not measured	Tuberculate clad 151–280 clad (N36)	Not measured	Style/subtylostyle, slightly bent/sinuuous	Not measured
MNH DT 781, re-examined in: Manconi & Serusi (2008) 440 m			35–50 × 18–20 prot 50–100 × 18–20 deut 366–1030 × 28 rhabd (N15)		51–87 × < 3 (N50)	
<i>N. nolitangere</i> Azores, AZR 71	Flabellate massive	Not measured	Tuberculated clad 47–58 prot (N20)	47–120 prot 47–151 deut	Style-like 52–96 × < 3 (N30)	9–14 (N15)
Pulitzer-Finali collection re-examined in Manconi & Serusi (2008)			478–1200 × 23–35 rhabd (N16)			
<i>N. nolitangere</i> von Lendenfeld, 1903 Sao Tiago, Cape Verde Island	Flabellate massive	35 thick	Tuberculated clad 550 rhabd	Not recorded	Not measured	Not recorded
<i>N. nolitangere</i> (Carvalho et al., 2015) Selvagens (358 m) Madeira (317 m)	Flabellate massive	Not recorded	Not recorded	Not recorded	Not recorded	Not recorded
Canaries (295–450 m)						
<i>N. microstylifer</i> Lévi & Lévi, 1983 MNH-DCL 2773 New Caledonia 355–360 m	Clavate	250–300	250–350 clad 30–40 prot 100 deut 350–600 × 30–45 rhabd	Not recorded	Strongyle 25–45 × 3 Subtylostyle 110–170 × 2	Not recorded
<i>N. nana</i> Manconi & Serusi, 2008 MSNG 54599 W-Sardinia Terrazze Cave 6 m	Encrusting plate-like	280	145–270 clad (N30) 20–50 × 10–20 prot 50–100 × 10–20 deut 290–540 rhabd (N30)	93–172 clad	59–86 × < 3 (N30) Subtylostyle	5–15 (N30)
<i>N. endoumensis</i> Pisera & Vacelet, 2011 MNH-DJV-120 Endoume Cave (France)	Cup-shaped	318–490	248–283 massive and densely spined clad 320–615 × 32–45 rhabd	Not recorded	Spinose microtylostyle 68.8–123 × 1.6–1.7	12.7–20.1 × 11.4–16.6
Depth not reported						

Table 1. Continued

Species Distribution Depth	Grow form/shape	Dicranoclone desma	Dichotriane Cladome tuberculated/spinose	Dichotriane smooth	Monaxial spicule	Streptaster/amphiaster
<i>N. radjajae</i> Pisera & Vacelet, 2011 MNHN-DJV-1119 Dalmatian Cave (Croatia) Depth not reported	Clavate 45 mm high and 30 mm in diameter	280–500	160–340 spined clad 220–639 × 20–30 rhabd	Not recorded	Spinose microtylostyles 80–140 × 0.8–2.1	9.55–15.5 × 8.35–12
<i>N. galapagoensis</i> sp. nov. HBOM 003:02010 Tortuga Island (Galápagos Islands) 241 m	Cup- to ear-shaped, flabellate massive, 12 cm in diameter and 16 cm in height	280–450 (N15)	175–227–372 spined clad (N30) 172–422–617 rhabd (N30)	Not recorded	Spinose microtylostyles 44–91–133 (N25)	15.2–11.2–80 × 4.9–7.2–10.7 (N25)

If not otherwise stated measurements are given in micrometres. Measurements are given for the holotype (in bold = new species) and indicated for *N. nolitangere* with ⁶⁶. Measurements and outer morphology are taken from Van Soest & Stentoft (1988), Pisera & Stentoft (2011), Pisera & Vacelet (2008), Pisera & Serusi (2008), Manconi & Serusi (2011) and Carvalho et al. (2015). clad = cladome in diameter; dent = deuteroclade; prot = protoclade; rhabd = rhabdome.

The key to species of *Neophrissospongia* by Manconi & Serusi (2008) is updated from the work of Pisera & Vacelet (2011) and the present study.

GENUS *CORALLISTES* SCHMIDT, 1870

***CORALLISTES FLOREANA* SP. NOV.**

(FIGS 6, 7; TABLE 2)

Diagnosis: *Corallistes* with smooth dichotriaenes in a broad range of sizes (93–558 µm rhabd; 72–272 µm cladome) and amphioxees of 327–887 µm length; choanosomal megascleres are tuberculate dicranoclone desmas, about 211–345 µm diameter.

Type material: Holotype: HBOM 003:02000, Coll. JSL-I dive 3901 [16 October 1995, Galápagos, N-Side 1.25 NM NW of Cormorant Point, Floreana Island, Santa Maria, 01°12'5.88"S, 90°26'9.96"W, 121 m].

Comparative material: *Corallistes isabela* HBOM 003:02013 (203 m), Galápagos, Santiago Island, 0°11'29"S, 90°34'58"W, Coll. JSL-I dive 3927, identified by A. Schuster.

Type locality: Floreana Island, Galápagos Islands (121 m) (Fig. 1).

Distribution: Only known from type locality.

Habitat: Attached to hard substratum (121 m).

Description: **Morphology**, a shallow bowl with a slightly folded or indented margin, or ear-shaped, walls about 5–6 mm thick with rounded margins (Fig. 6A, B), holotype, about 8 cm diameter, about 6 cm high (Fig. 6A, B). **Surface**, uneven, partly covered with algae, coral and shell fragments, bundles of projecting spicules trap sediment on the upper surface, otherwise the surface is smooth, oscules are not visible (Fig. 6B). **Texture**, stony. **Colour**, beige in life (Fig. 6A), light brown to cream in ethanol preservative (Fig. 6B). **Ectosomal skeleton**, irregular, composed of dichotriane cladomes, aligned tangentially to surface with rhabdome pointing in towards choanosome (Figs 6C, D, 7J, K), with scattered microscleres (Fig. 7B, E). **Choanosomal skeleton** composed of zygosd dicranoclone desmas (Fig. 6E, F) between which are large subdermal spaces and scattered microscleres (Fig. 7C–G). **Megascleres**, desmas, triaenes, microxeas; dicranoclone desmas, 211–354 × 176–261 µm (N15). Dichotriaenes in a large size range with long and short rhabdomes: smallest dichotriaenes, cladome, 72–82–106 µm, rhabdome 93–110–130 µm (N25), largest dichotriaenes, cladome, 130–186–278 µm, rhabdome, 208–314–558 µm (N25) (Figs 6D, 7J, K); microxeas, 327–630–887 × 4.8–6.6–10.8 µm

(N6) (Fig. 7H, I). **Microscleres**, metasters with long, pointed, acanthose rays, 16.8–29.9–42 × 14.3–23.6–30.7 µm (N21), amphisters and spirasters with short, pointed, microspined rays, 8.2–11.9–15.1 × 6.4–10.1–13.5 µm (N21).

Etymology: Named after the type locality, Floreana Island.

DNA barcodes: We sequenced *COI*, 28S (C1-D2) and 18S (1303 bp) from the holotype; GenBank accession numbers: *COI* KY652816, 28S KY652784, 18S KY652833. SBD record no. 1715.

Remarks: *Corallistes floreana* sp. nov. resembles *C. isabela*, previously described from the Galápagos Islands, but there are notable differences in the size and ornamentation of the megascleres. The dicranoclone desmas in *C. floreana* sp. nov. are smaller and tuberculate rather than knobbed as in *C. isabela* (Table 2). Furthermore, the alternating sizes of the *C. floreana* sp. nov. cladomes differ by their ratio from *C. isabela* (Table 2). Amphisters / spirasters are smaller in size as well (Table 2). Pairwise sequence differences of 1.2% (28S) and 0.8% (*COI*) between *C. floreana* sp. nov. and *C. isabela* (HBOM 003:02013) further confirm integrity of these two species.

FAMILY THEONELLIDAE VON LENDENFELD, 1903

GENUS *RACODISCUA* ZITTEL, 1878

RACODISCUA RADIXA SP. NOV.

(FIGS 8, 9; TABLE 3)

Diagnosis: *Racodiscula* with a deep, apical, cavity/ osculum, into which subdermal canals converge from the margins; desmas are irregular, loosely zygoed tetraclone desmas with root-like protrusions.

Type material: Holotype: HBOM 003:02006, Coll. JSL-I dive 3913. [22 October 1995, Galápagos, N-Side 1.25 NM W of point Vincente Roca NW Coast, Isabela Island, 0°02'55"S, 91°35'16"W, 378 m].

Comparative material: *Racodiscula seymoura* sp. nov. HBOM 003:02014 (396 m) (see below), Galápagos, Seymour Island, 0°22'51"S, 90°16'06"W, Coll. JSL-I dive 3529; HBOM 003:02007 (319 m), Galápagos, Isabela Island, 0°14'31"S, 91°24'56"W, Coll. JSL-I dive 3015. *Racodiscula borisi* sp. nov. HBOM 003:02011 (242 m) (see below), Galápagos Island, Tortuga Island, 1°4'59"S, 90° 51'56"W, Coll. JSL-I dive 3923; HBOM 003:02001 (215 m), Galápagos Island, San Cristobal Island, 0°43'08"S, 89°27'14"W, Coll. JSL-I dive 3905.

Type locality: Isabela Island, Galápagos Islands (378 m) (Fig. 1).

Distribution: Only known from type locality.

Habitat: Attached to hard substratum at a depth of 378 m.

Description: **Morphology**, massive subspherical folded with a deep, apical, cavity/osculum, about 1 cm longest diameter, situated on the elevated apex (Fig. 8A, B); dimension of the holotype, about 7 cm diameter, about 5 cm high (Fig. 8A, B). **Texture**, medium hard. **Surface**, smooth, with branched, meandering, subdermal canals, converging from the margins (Fig. 8B) towards the apical cavity; lateral inhalant surface with pores, 450–500 µm diameter. **Colour**, white in cream (Fig. 8A), dark cream in ethanol preservative (Fig. 8B). **Ectosomal skeleton**, detachable in places due to large subdermal canals, composed of phyllostriaene cladomes, stacked and overlapping, (Figs 8C–F, 9G), interspersed with microrhabds and other microscleres (Fig. 9B, E) forming a thick outer layer; tylostyles are located at the subectosomal region (Fig. 8D–F). **Choanosomal skeleton** contains loosely zygoed tetraclone desmas (Fig. 8D), loose bundles of oxeads and scattered microscleres (Fig. 9C, D). **Megascleres**, tetraclone desmas, phyllostriaenes and oxeads: tetraclone desmas, relatively smooth, from which emerge thin, root-like branches, 405–532 × 254–276 µm (N5) (Fig. 8C–F); phyllostriaenes with a leafy cladome, 305–389–494 µm (N20), short, irregular rhabdome, 93–116–160 × 32–42–55 µm (N20) (Fig. 9A, B, G); tylostyles, 450–315–605 µm (N15) (Fig. 9A). **Microscleres**, acanthose microrhabds with irregularly distributed spined tubercles, 21–25–30 × 4 µm (N30); metasters with long rays, 15.6–18.5–20.2 × 11.3–14.7–19.2 µm (N20).

Etymology: Named for the root-like ornamentation of the tetraclone desmas in this species (*radix*, root; Latin).

DNA barcodes: we sequenced *COI*, 28S (C1-D2) and 18S (1,623 bp) of the holotype. GenBank accession number: *COI* KY652819, 28S KY652788, 18 KY652836. SBD record no. 1720.

Remarks: *Racodiscula radix* sp. nov. is differentiated from the two other new *Racodiscula* species from the Galápagos Islands, *R. seymoura* sp. nov. and *R. borisi* sp. nov. (see species description below), in having the cavity/oscula at an elevated centre with vein-like furrows to the margins, while *R. seymoura* sp. nov. have the oscula in a centred depression and *R. borisi* sp. nov. has a perfectly circular and centred oscula on the surface without an elevated/compressed centre. The oscula of all three new *Racodiscula* species

Code for biogeographical distributions
 CAR=Caribbean
 PAC=Pacific
 ATL=Atlantic
 MED=Mediterranean
 RED=Red Sea
 GLS=Greenland Sea
 ANT=Antarctica
 INO=Indian Ocean
 NOT=not available

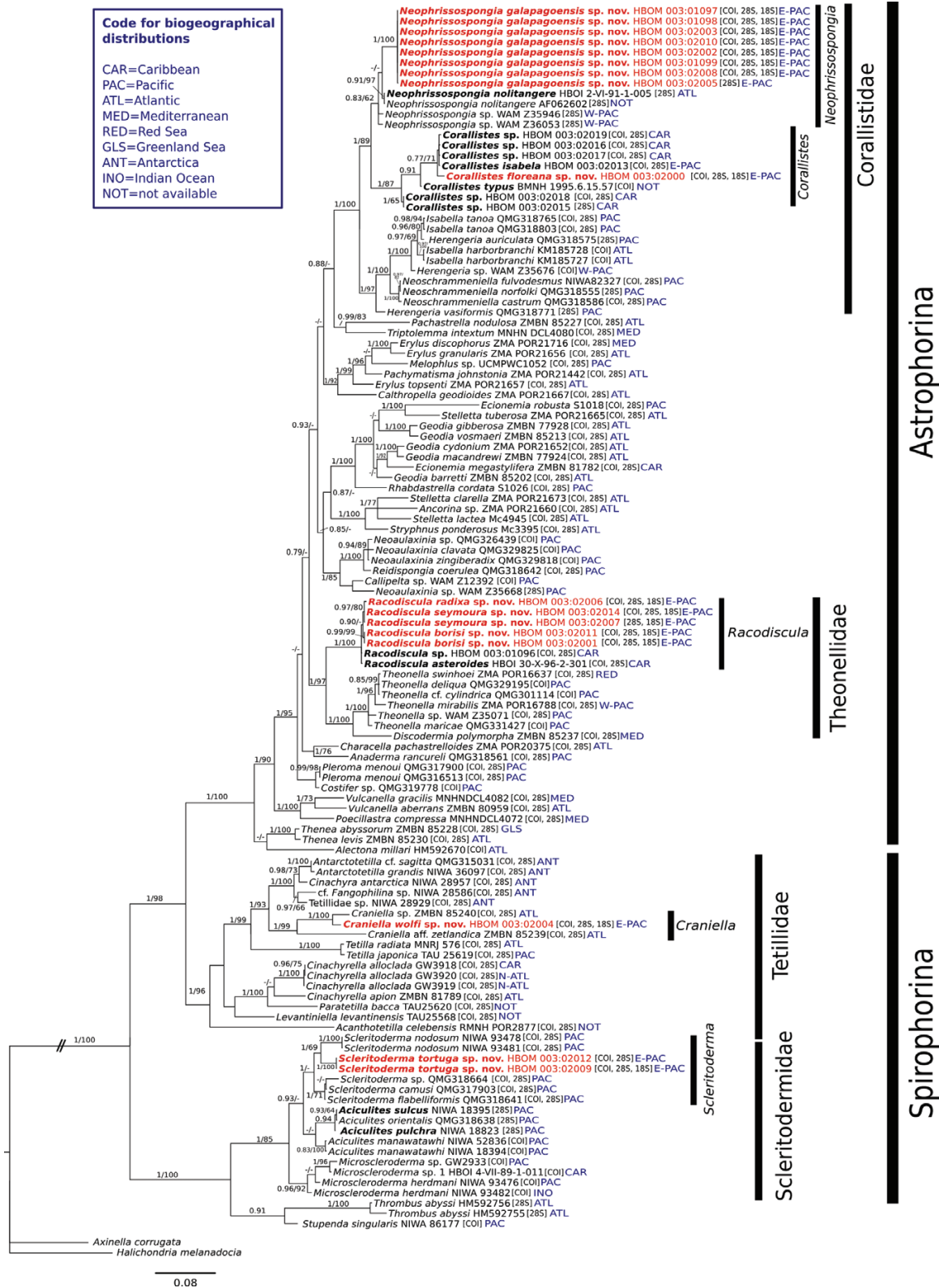


Figure 5. Bayesian inference (BI) reconstruction of the concatenated dataset (COI and 28S) showing the relationship of the seven new species from the Galápagos to other tetractinellids from the Caribbean, Pacific and Atlantic. Maximum likelihood (ML) tree is mostly congruent, otherwise no support values are given. Newly generated sequences are in bold (red: new species,

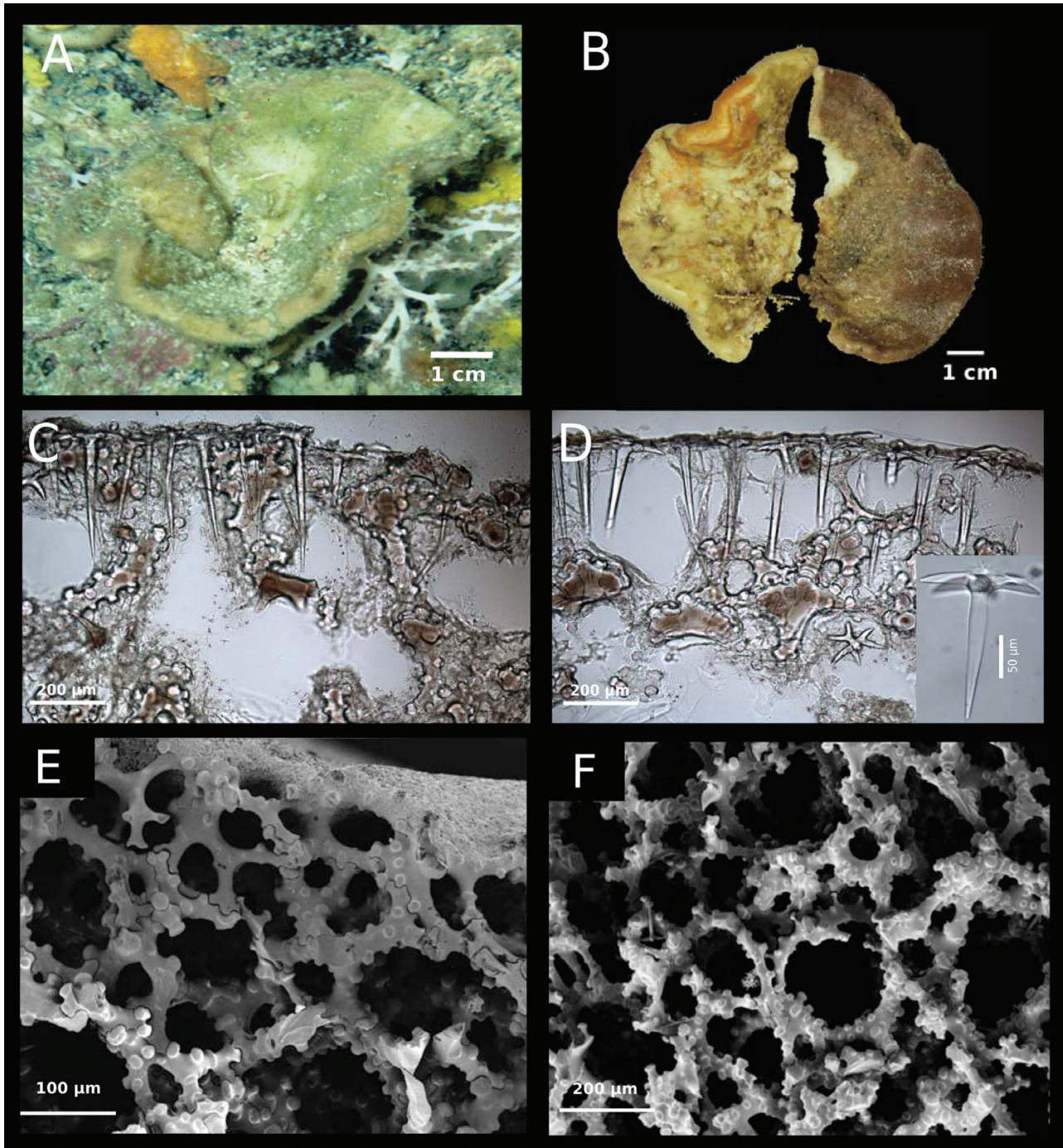


Figure 6. *Corallistes floreana* sp. nov. Harbor Branch Oceanographic Museum (HBOM) 003:02000. A, *in situ* picture (121 m). B, deck picture with inner surface: left part and outer surface: right part. C, D, thick sections of the ectosomal part showing diverse dichotriaenes penetrating to the choanosome. E, F, scanning electron microscopy pictures of cross-section showing articulated dicranoclone desmas.

black: comparative samples). Bayesian posterior probabilities and ML bootstrap support values are indicated for clades $>0.75/60$, otherwise marked with a dash. Numbers following the taxon names are collection numbers or NCBI Genbank accession numbers. Markers sequenced are indicated in square brackets. Three letter code in dark blue indicates the biogeographical location.

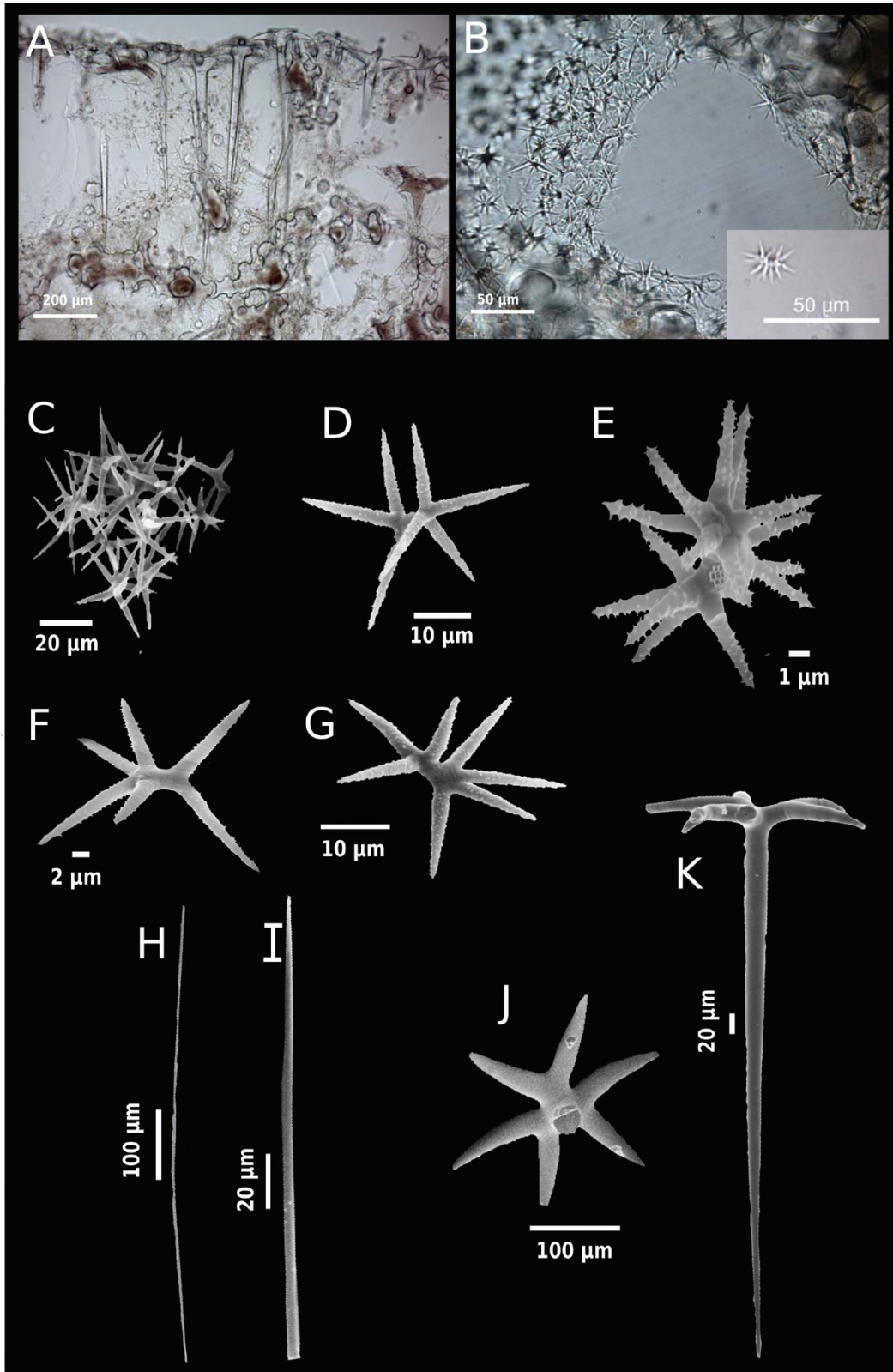


Figure 7. *Corallistes floreana* sp. nov. Harbor Branch Oceanographic Museum (HBOM) 003:02000. A, B, thick sections showing the accumulation of streptaster at the subectosomal region (A) and around channel openings (B). C, bundle of metasters/amphiasters. D–G, diverse streptaster with long pointed rays. H, I, amphioxea. J, K, ectosomal dichotriaene.

described are different to *Racodiscula asteroides* Zittel, 1878 in having only one opening, while *R. asteroides* has multiple oscules in a shallow depression (Pisera & Lévi, 2002b). However, intraspecific variations of this character cannot be excluded. Thick strongyles as found in *R. seymoura* sp. nov. and *R. borisi* sp. nov. (see below) could not be detected in any of the spicule preparations for *R. radixa* sp. nov., which may indicate that this second type of microsclere was secondarily lost (Table 3). The cladome of phyllo- to discotriaenes is smaller than in *R. seymoura* sp. nov. and *R. borisi* sp. nov. The rhabdome is also slightly shorter than in *R. seymoura* sp. nov. but larger than in *R. borisi* sp. nov. (Table 3). *R. radixa* sp. nov. has irregularly rather loose root-like desmas with shorter branches than *R. seymoura* sp. nov. In contrast, *R. borisi* sp. nov. shows strong tuberculated tetraclone desmas similar to *R. asteroides*. Differences were also found in our molecular analyses (Fig. 5). *R. radixa* sp. nov. differ to *R. seymoura* sp. nov. with 3.3% (COI), 0.3% (28S) and 1.8% (18S) and to *R. borisi* sp. nov. with 4.8% (COI), 0.5% (28S), 2.3% (18S). The sister-group relationship of *R. radixa* sp. nov. and *R. seymoura* sp. nov. is supported [posterior probability (PP) = 0.97 and bootstrap support (BS) = 80]. The clade with the three new Galápagos *Racodiscula* species is well supported by PP = 0.90, but not by BS. The sister-group relationship of this

clade with *R. asteroides* and *Racodiscula* sp. from the Caribbean is well supported (Fig. 5).

***RACODISCUA SEYMOURA* SP. NOV.**

(FIGS 10, 11; TABLE 3)

Diagnosis: *Racodiscula* with one oval to circular cavity/osculum (0.7 cm) opening in a shallow centred depression; choanosomal spicules are zygosied root-like tetraclone desmas with long and thin branches; choanosomal spicules are thick strongyles.

Type material: Holotype: HBOM 003:02014, Coll. JSL-I dive 3529, [30 October 1995, Galápagos, 66 NM NE of Seymour Island, 0°22'51"S, 90°16'06"W, 396 m]. Paratype: HBOM 003:02007 (319 m), Galápagos, N-Side 1.25 NM W of PTA Vincente Roca NW Coast, Isabela Island, 0°14'31"S, 91°24'56"W, Coll. JSL-I dive 3915.

Comparative material: *Racodiscula radixa* sp. nov. HBOM 003:02006. *Racodiscula borisi* sp. nov. HBOM 003:02011 (242 m), Galápagos Island, Tortuga Island, 1°4'59"S, 90°51'56"W, Coll. JSL-I dive 3923; HBOM 003:02001 (215 m), Galápagos Island, San Cristobal Island, 0°43'08"S, 89°27'14"W, Coll. JSL-I dive 3905.

Type locality: Seymour Island, Galápagos Islands (396 m) (Fig. 1).

Table 2. Spicule sizes in micrometres of *Corallistes floreana* sp. nov. compared to different *Corallistes typus* material and *Corallistes isabela*

Species	Locality	Desmas	Dichotriaenes, alternating in sizes of: I: large and II: small	Oxeas	Amphiasters/spirasters/metaster (in diameter)
		Dicranoclone			
<i>Corallistes typus</i> (Schmidt, 1870)	Florida	Not recorded	Not recorded	Not recorded	Not recorded
<i>Corallistes typus</i> (Sollas, 1888)	Pernambuco	Tuberculated	Rhabd: 238–320 × 32	701 × 4	Spirasters: 20–24
<i>Corallistes typus</i> (Van Soest & Stentoft, 1988)	Barbados	300–360 × 15–24	Rhabd: 130–380 × 15–24 Clad: 90–300	700–1260 × 4–8	Spirasters: 14–26
<i>Corallistes isabela</i> (Desqueyroux-Faúndez & Van Soest, 1997)	Galápagos (Isabela Island)	Knobbe-like 526–959 × 129–327	I: Rhabd: 14–40 Clad: 329–540 II: Rhabd: 120 Clad: 229–269 × 23	>1000 × 1–5	Amphiasters: 42 Spirasters: 23
ZMA POR 11237 MHNG 20599 HBOM 003:02013					
<i>Corallistes floreana</i> sp. nov.	Galápagos (Floreana Island)	Tuberculated 211–354 × 176–261	I: Rhabd: 208–558 Clad: 130–278 II: Rhabd: 93–130 Clad: 72–106	327–887 × 4–10	Amphiasters: 29 Metaster: 10
HBOM 003:02000					

Information for *Corallistes isabela* taken from table 9 of Desqueyroux-Faúndez & Van Soest (1997). clad = cladome; rhabd = rhabdome.

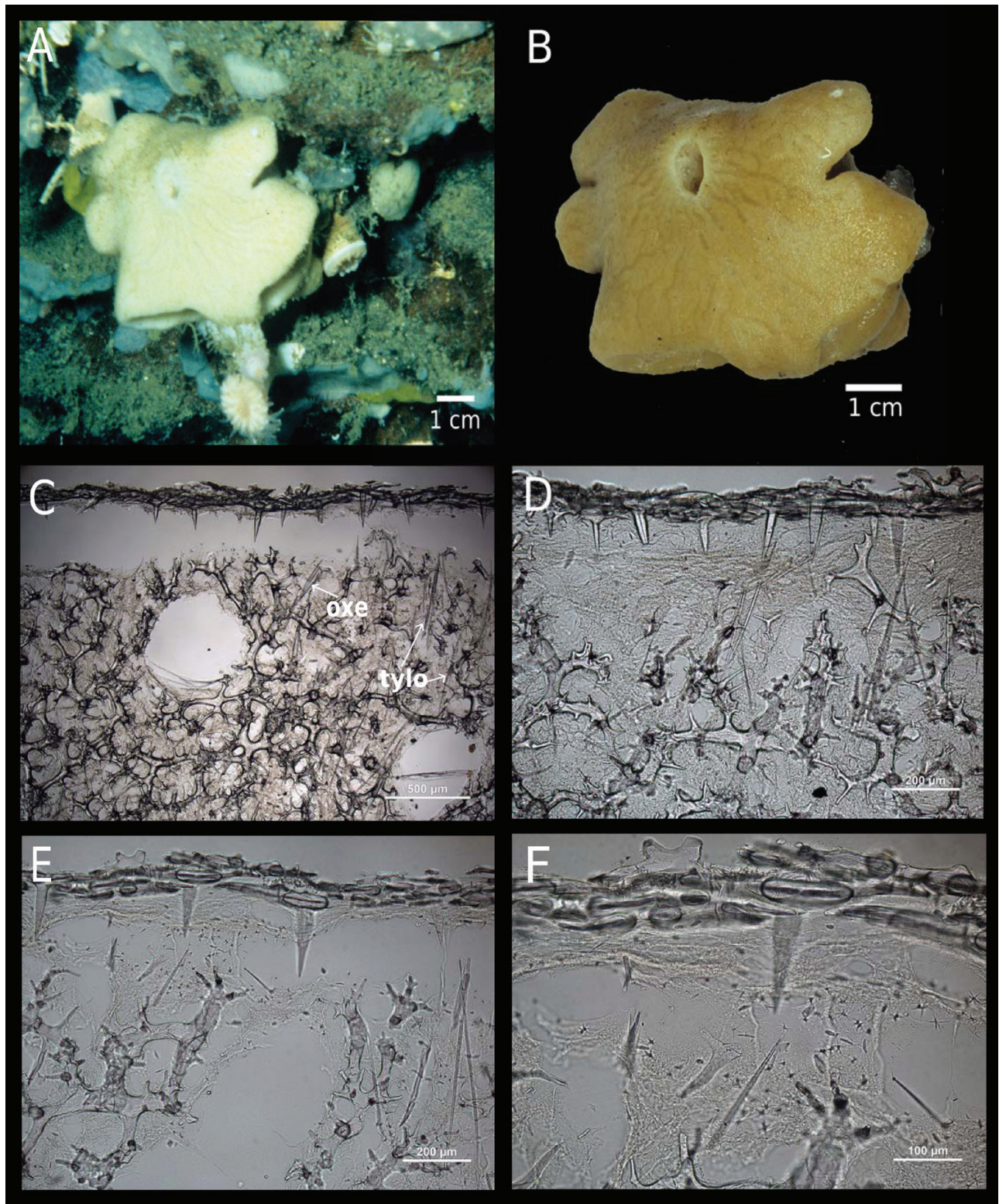


Figure 8. *Racodiscula radixa* sp. nov. Harbor Branch Oceanographic Museum (HBOM) 003:02006. A, *in situ* picture (378 m). B, deck picture. C, D, thick section of the ecto- and choanosome, representing disorganized tetracene, phyllotri-aenes with rhabdomes protruding perpendicular from the surface and bounds of tylostyles (tylo) and oxeas (oxe). Gap between ecto- and choanosome (C) is an artefact from sectioning. E, F, thick section showing the poor articulation of tetrac- lone desmas and diverse streptasters.

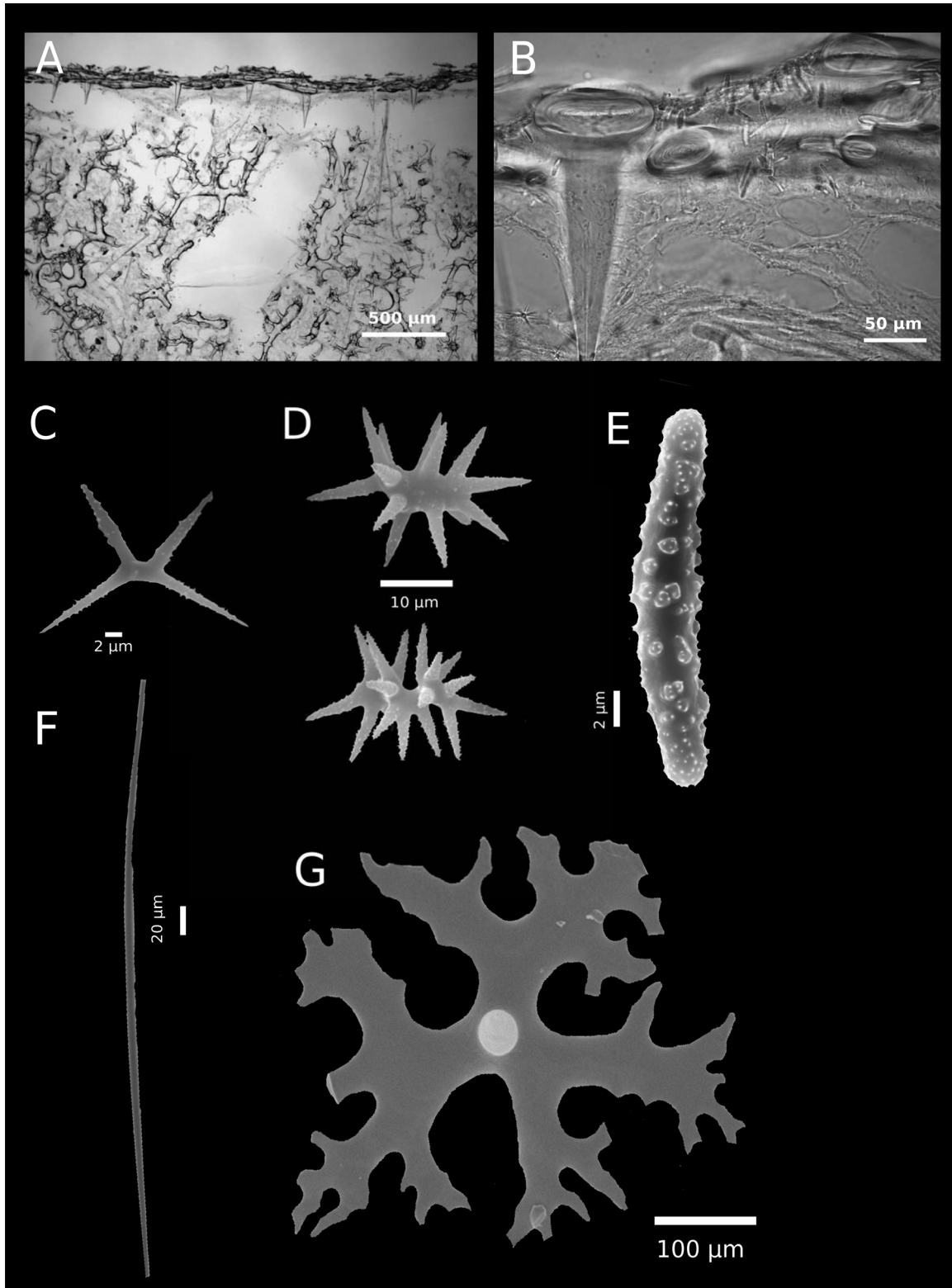


Figure 9. *Racodiscula radixa* sp. nov. Harbor Branch Oceanographic Museum (HBOM) 003:02006. A, B, thick sections showing phyllotriasenes with acanthorhabds on top (A) and in between (B), forming a thick crust on the surface. C, metaster. D, spiraster. E, acanthorhabds. F, tylostyles. G, ectosomal phyllotriaene.

Table 3. Morphology and spicule sizes of the three new *Racodiscula* species from the Galápagos Islands compared to *Racodiscula asteroides* Zittel, 1878 (neotype)

Species	Locality	Cavity/oscula	Tetraclone desma	Phyllo- to discotriaenes	Tylostyles	I: oxeas	Microscleres
<i>Racodiscula radixa</i> sp. nov.	Isabela Island	One oval oscula at elevated centre, vein-like furrows from the oscula to the margins; diameter: 1.0 cm	Irregular, loosely zygosd tetraclone desmas with root-like protrusions, not tuberculated, 405–532 by 254–276	I: 305–389–494 II: 93–116–160 × 32–42–55	450–315–605	I: present, but difficult to measure as only seen in the sections (Fig. 9C–F) between desmas; II: not present	I: 15.6–18.5–20.2 × 11.3–14.7–19.2 II: 21–25–30 × 4
HBOM 003:02006							
<i>Racodiscula seymoura</i> sp. nov.	Seymour Island, Isabela Island	One oval to circular oscula in a shallow centred depression; diameter: 0.7 cm	Zygosd root-like with long thin branches, not tuberculated, 632–736 by 395–406	I: 447–536–636 II: 121–150–185 × 22–37–40	434–317–719	I: 120–125–134 × 4–5 II: 95–100–112 × 7–10	I: 17.3–20.5–22.2 × 12.5–15.6–20.0 II: 5–17–20 × 3–4
HBOM 003:02014, HBOM 003:02007							
<i>Racodiscula borisi</i> sp. nov.	San Cristobal Island, Tortuga Island	One perfectly circular oscula located at the centre; diameter: 0.8 cm	Zygosd with short thick root-like branches, strongly tuberculated, 540–650 by 385–400	I: 395–504–605 II: 20–66–93 × 18–21–25	440–410–904	I: 140–176–204 × 4–5 II: 95–194–112 × 15–28	I: 12.0–14.5–17.2 × 11.5–15.4–20.0 II: 12–19–27 × 3–4
HBOM 003:02001, HBOM 003:02011							
<i>Racodiscula asteroides</i> Zittel, 1878	Barbados	Multiple oscules located at shallow depression, iris-like diaphragm	Sparsely tuberculated rays and strongly tuberculated tips	I: 250–420	Present, but no measurement given	Not present	I: 14.7–20.7 × 11.2–19.4 II: 6.7–10.6 × 4.8–5.3 Pseudospherasters: 6.53–7.41
Neotype ZMA POR5264 (=ZPAL Pf.15/1)							

Measurements are given in micrometres, if not otherwise stated. Measurements for new species are given for the holotype (bold) and for *R. asteroides* (neotype) taken from Pisera & Lévi (2002b).

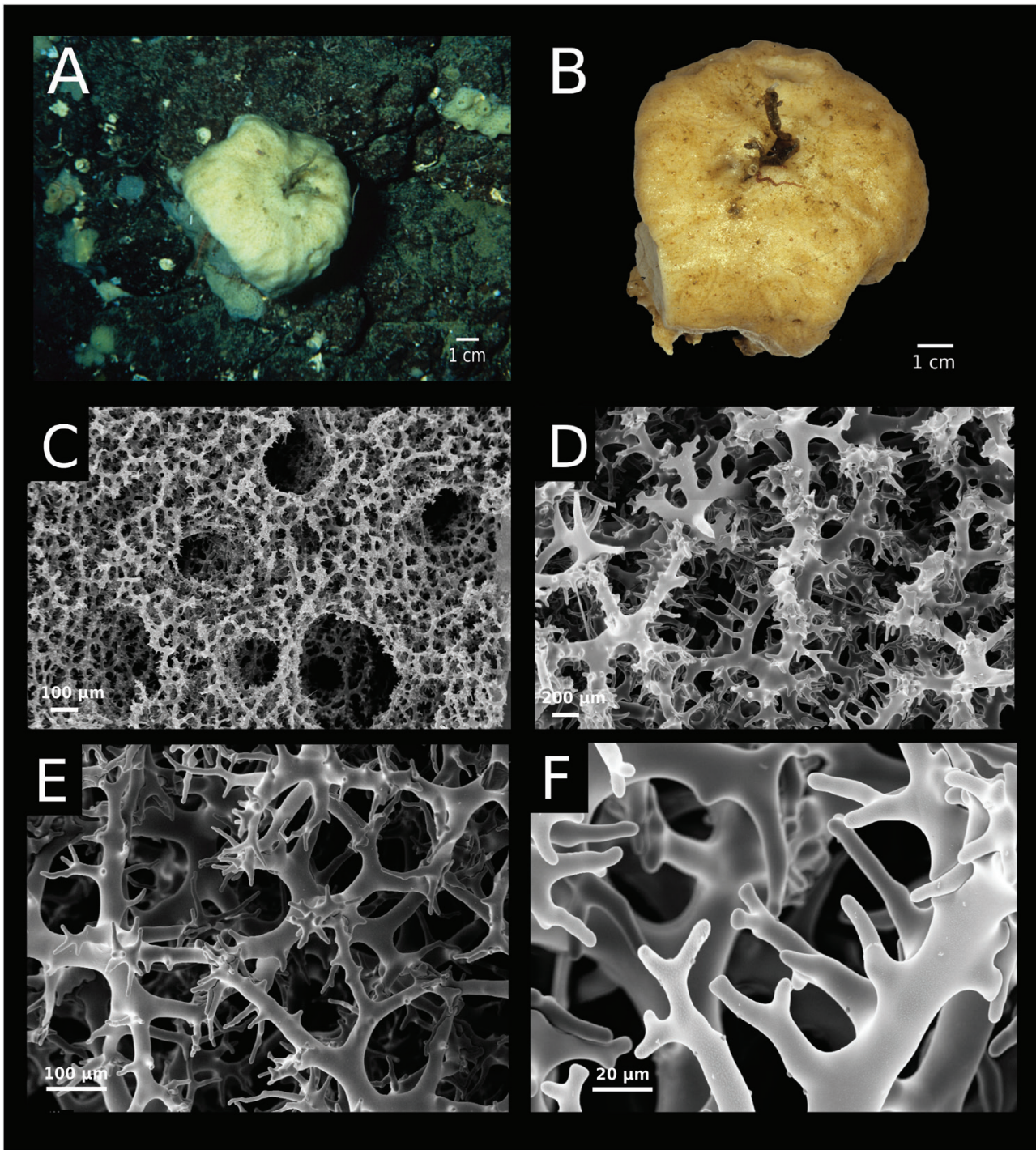


Figure 10. *Racodiscula seymoura* sp. nov. Harbor Branch Oceanographic Museum (HBOM) 003:02014. A, *in situ* picture of the paratype (378 m). B, deck picture of the paratype. C, outer surface of the choanosomal skeleton from the holotype. D, tetraclone desmas with phyllostriaene *in situ* of the holotype. E, F, detailed view of the articulated tetraclone desmas from the holotype.

Distribution: Known from type locality, Seymour Island, and Isabela Island, Galápagos Islands.

Habitat: Attached to hard substratum, depth range, 319–396 m.

Description: Morphology, massive subspherical with an apical cavity/osculum opening on top of the surface in a shallow centred depression (Fig. 10A, B). Dimension of the holotype is about 7 cm in diameter and

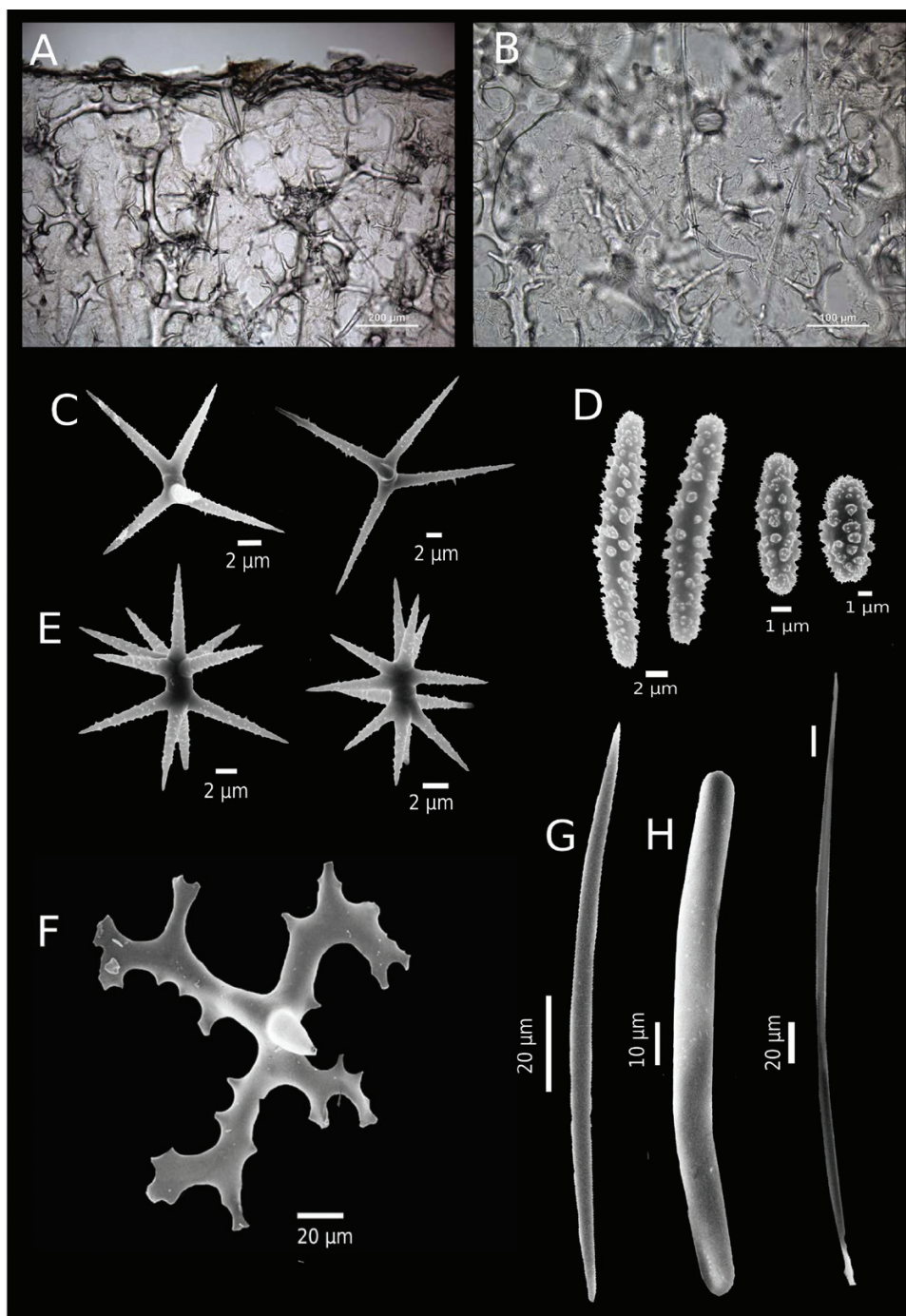


Figure 11. *Racodiscula seymoura* sp. nov. Harbor Branch Oceanographic Museum (HBOM) 003:02014. A, B, thick sections showing phyllotrias with acanthorhabds on top (A) and in subectosomal regions (B). C, metaster. E, amphiaster. D, acanthorhabds in various sizes. F, ectosomal phyllotriaene. G, ecto- to subectosomal oxea with pointed tips. H, thick strongyle. I, choanosomal style.

about 5 cm in height (Fig. 10A, B). **Texture**, medium hard. **Surface**, smooth and uneven (Fig. 10B). **Colour**, white *in vivo* (Fig. 10A) and light brownish in ethanol (Fig. 10B). **Ectosomal skeleton** contains phyllo- to discotriasenes (Figs 10B, 11A, F) with acanthorhabds

in between and on top of this layer (Fig. 11A, B, D). Tylostyles are located at the subectosomal region (Fig. 11A, B, I). **Choanosomal skeleton** contains articulated tetraclone desmas, which form a dense root-like skeleton (Fig. 10C–F). Spinose metasters

(Fig. 11C) and amphiasters (Fig. 11E). Oxeas (Fig. 11A, G) with pointed tips and thick strongyles (Fig. 11H). **Megascleres** are tetraclone desmas with root-like structures, measuring $632\text{--}736 \times 395\text{--}406 \mu\text{m}$ (N15) (Fig. 10C–F). Phyllo- to discotriaenes with cladomes $447\text{--}536\text{--}636 \mu\text{m}$ (N20) and rhabdomes of $121\text{--}150\text{--}185 \times 22\text{--}37\text{--}40 \mu\text{m}$ (N20) (Figs 10D, 11F). Tylostyles are $434\text{--}317\text{--}719 \mu\text{m}$ long (N15) (Fig. 11I). Oxea with pointed tips $120\text{--}125\text{--}134 \times 4\text{--}5 \mu\text{m}$ (N7). Thick strongyles with rounded ends $95\text{--}100\text{--}112 \times 7\text{--}10 \mu\text{m}$ (N4); **Microscleres** are massive acanthorhabds with irregularly distributed spined tubercles $5\text{--}17\text{--}20 \times 3\text{--}4 \mu\text{m}$ (N30). Spinose metastar/amphiasters with long rays $17.3\text{--}20.5\text{--}22.2 \times 12.5\text{--}15.6\text{--}20.0 \mu\text{m}$.

Etymology: Named after the type locality: Seymour Island (Galápagos Islands).

DNA barcodes: We sequenced all three markers of the holotype and 28S (C1-D2) and 18S of the paratype. 28S sequences of type material were identical as well as were the 18S. GenBank accession numbers: COI KY652820, 28S KY652789–KY652790, 18S KY652837–KY652838. SBD record no. 1721–1722.

Remarks: Apart from the differences observed in the oscula opening (see remarks for *R. radixa* sp. nov. and Table 3) *Racodiscula seymoura* sp. nov. show clear spiculation differences compared to the two other *Racodiscula* species described in this study. The presence of oxeas and strongyles and the root-like tetraclone desmas with long thin branches (Fig. 10F) along with slightly differences observed in the sizes of phyllo- to discotriaenes (Table 3) separates this species from *R. radixa* sp. nov. Tetraclone desmas, which are not tuberculated in *R. seymoura* sp. nov. but strongly tuberculated in *R. borisi* sp. nov., and the longer and thicker rhabdome size clearly separate this new species from *R. borisi* sp. nov. Molecular markers suggest that *R. seymoura* sp. nov. is more closely related to *R. radixa* sp. nov. than to *R. borisi* sp. nov. (Fig. 5), and positioned within the clade of *Racodiscula* from the Galápagos. This is in accordance with the observed morphological differences.

RACODISCUA BORISI SP. NOV.

(Figs 12, 13; TABLE 3)

Diagnosis: *Racodiscula* with one perfectly circular apical cavity/osculum (0.8 cm) located at the centre; choanosomal spicules are articulated tetraclone desmas with short thick and strongly tuberculated branches, choanosomal spicules are thick strongyles with rounded tips.

Type material: Holotype: HBOM 003:02001, Coll. JSL-I dive 3905 [18 October 1995, Galápagos, 5NM NE of Kicker Rock, San Cristobal Island, $0^{\circ}43'08''\text{S}$, $89^{\circ}27'14''\text{W}$, 215 m]. Paratype: 003:02011 (242 m), Galápagos, 4 NM SE of Tortuga Island, $1^{\circ}04'59''\text{S}$, $90^{\circ}51'56''\text{W}$, Coll. JSL-I dive 3923.

Comparative material: *Racodiscula radixa* sp. nov. HBOM 003:02006. *Racodiscula seymoura* sp. nov. HBOM 003:02014; HBOM 003:02007.

Type locality: San Cristobal Island, Galápagos Islands (215 m) (Fig. 1).

Distribution: Known from the type locality and Tortuga Island, Galápagos.

Habitat: Attached to hard substratum, depth range 215–242 m.

Description: **Morphology**, massive subspherical with a centralized circular apical cavity/osculum opening on top of the surface (Fig. 12A, B). Dimension of the holotype is 5.5 cm in diameter and about 4–5 cm high (Fig. 12A, B). **Texture**, medium hard. **Surface**, smooth (Fig. 12B). **Colour**, white *in vivo* (Fig. 12A) and beige in ethanol (Fig. 12B). **Ectosomal skeleton** contains phyllo- to discotriaenes (Fig. 13B, F) with acanthorhabds in between and on top of this layer (Fig. 13A, B, E). Tylostyles are located at the subectosomal region (Fig. 12A, B, I). **Choanosomal skeleton** contains articulated tetraclone desmas, which form a dense skeleton (Fig. 12C–F). Spinose metastar/amphiasters (Fig. 13C, D). Oxeas (Fig. 13G) with pointed tips and thick strongyles (Fig. 13H). **Megascleres**, tetraclone desmas with short branches measuring $540\text{--}650 \times 385\text{--}400 \mu\text{m}$ (N15) (Fig. 12E). Phyllo- to discotriaenes with cladomes $395\text{--}504\text{--}605 \mu\text{m}$ (N25) and rhabdomes of $20\text{--}66\text{--}93 \times 18\text{--}21\text{--}25 \mu\text{m}$ (N10) (Fig. 13B, F). Tylostyles are $440\text{--}410\text{--}904 \mu\text{m}$ (N15) (Fig. 13I). Oxeas with pointed tips $140\text{--}176\text{--}204 \times 4\text{--}5 \mu\text{m}$ (N7). Thick strongyles with round ends $95\text{--}194\text{--}112 \times 15\text{--}28 \mu\text{m}$ (N4); **Microscleres** are massive acanthorhabds with irregularly distributed spined tubercles $12\text{--}19\text{--}27 \times 3\text{--}4 \mu\text{m}$ (N30). Spinose streptaster/amphiasters with long rays $12.0\text{--}14.5\text{--}17.2 \times 11.5\text{--}15.4\text{--}20.0 \mu\text{m}$.

Etymology: Named in memory of the first author's friend Dipl. Biol. Boris Knerr (†20 July 2012), for encouraging her scientific curiosity in sponge taxonomy.

DNA barcodes: We sequenced COI, 28S (C1-D2) and 18S of the holotype and paratype. All three markers show identical sequences. GenBank accession

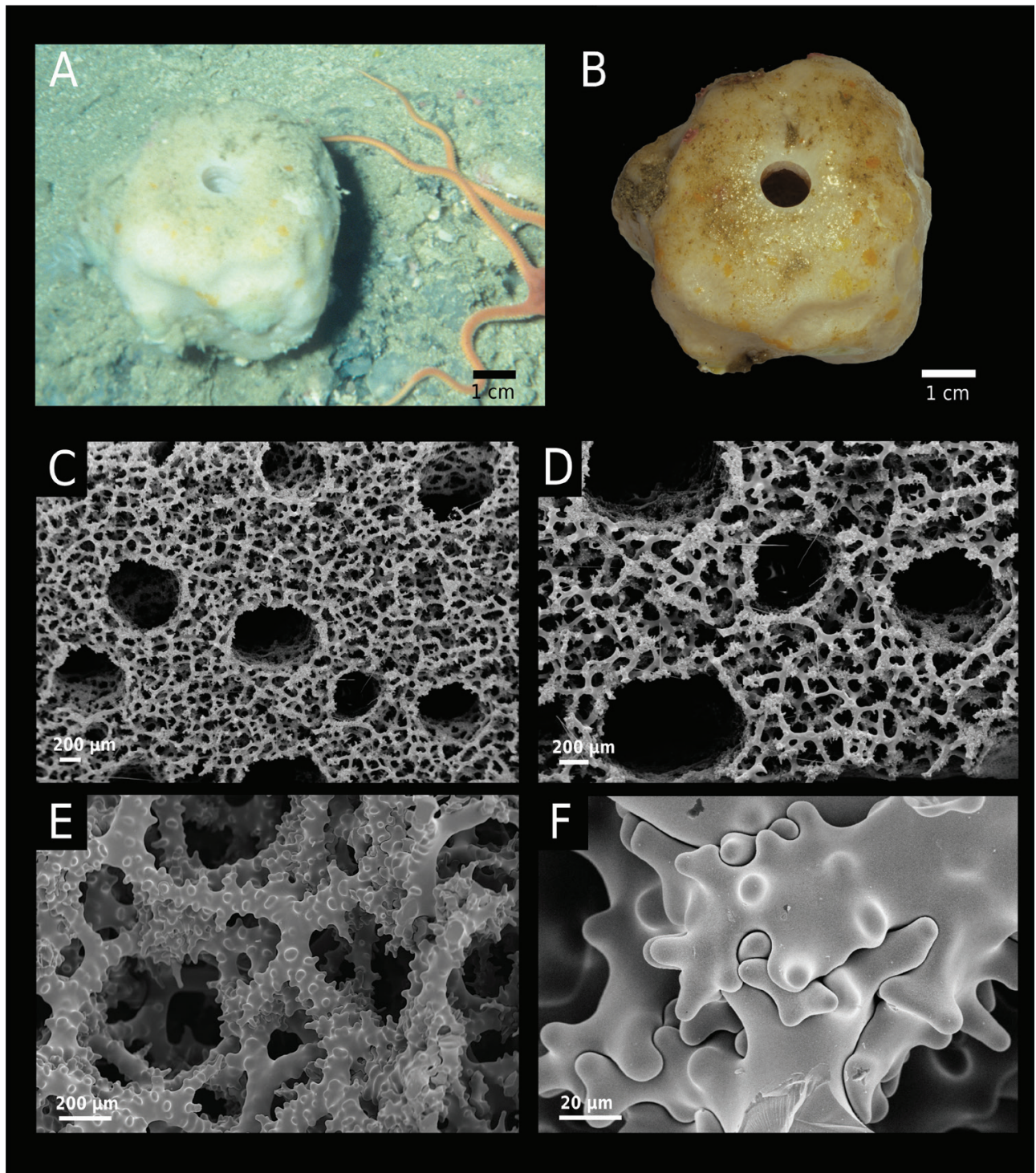


Figure 12. *Racodiscula borisi* sp. nov. Harbor Branch Oceanographic Museum (HBOM) 003:02001. A, *in situ* picture of the holotype (215 m). B, deck picture of the holotype. C, outer surface of the choanosomal skeleton with pore openings. D, tetraclone desmas with tylostyles lying on top. E, F, detailed views of the articulated tetraclone desmas from the holotype.

numbers: COI KY652821–KY652822; 28S KY652791–KY652792; 18S KY652834–KY652835. SBD record no. 1723, 1724.

Remarks: The most obvious key diagnostic character which differentiates this new species from the other two new *Racodiscula* species described here,

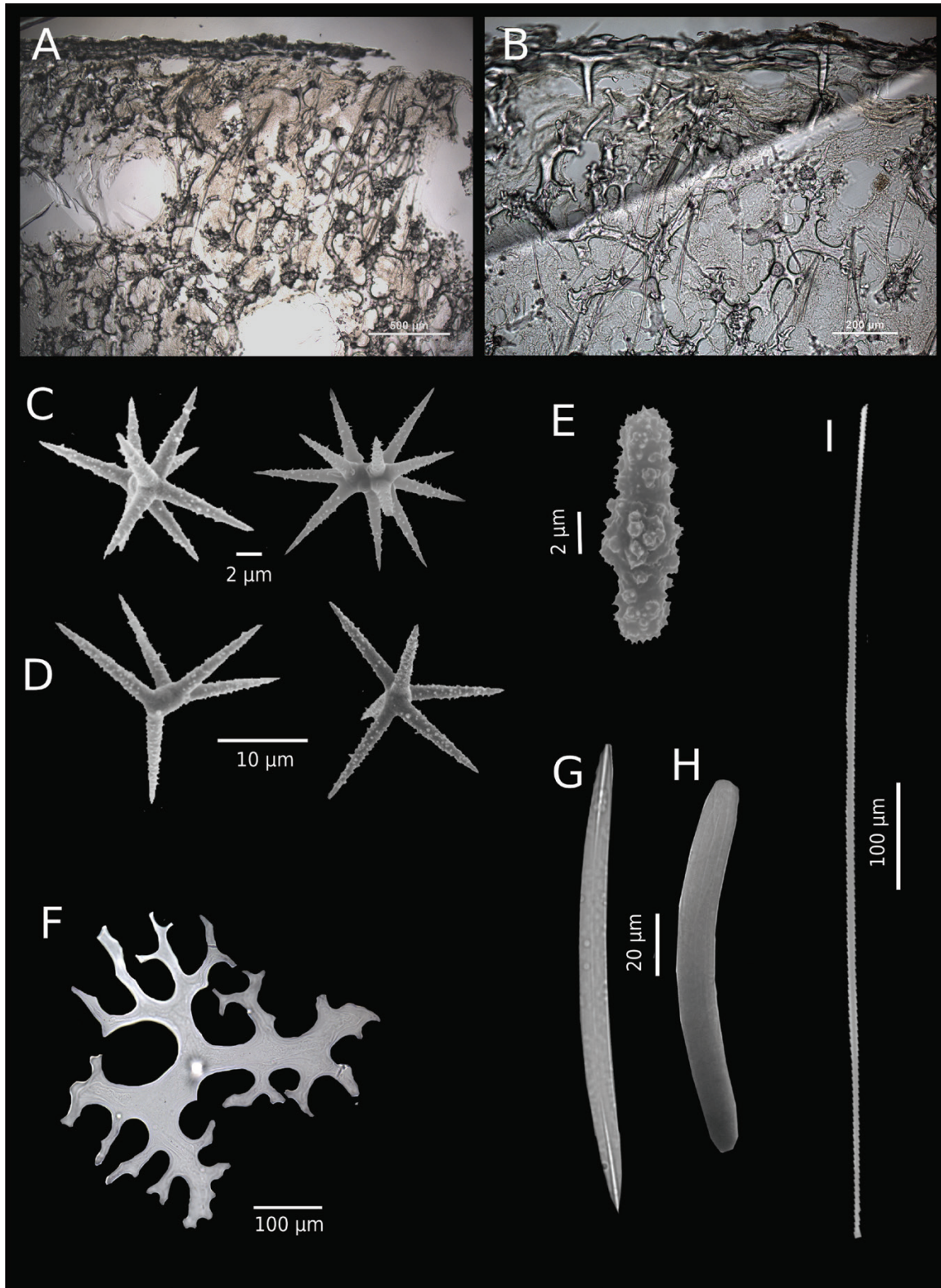


Figure 13. *Racodiscula borisi* sp. nov. Harbor Branch Oceanographic Museum (HBOM) 003:02001. A, B, thick sections of the ectosomal to choanosomal region showing phyllotriaenes, with acanthorhabds and bundles of tylostyles. C, D, scanning electron microscopy (SEM) of diverse amphiaster/metaster. E, SEM of acanthorhabd. F, light microscopy picture of ectosomal phyllotriaene. G, light microscopy picture of ecto- to subectosomal oxea with pointed tips. H, thick strongyle with rounded ends. I, SEM of choanosomal style.

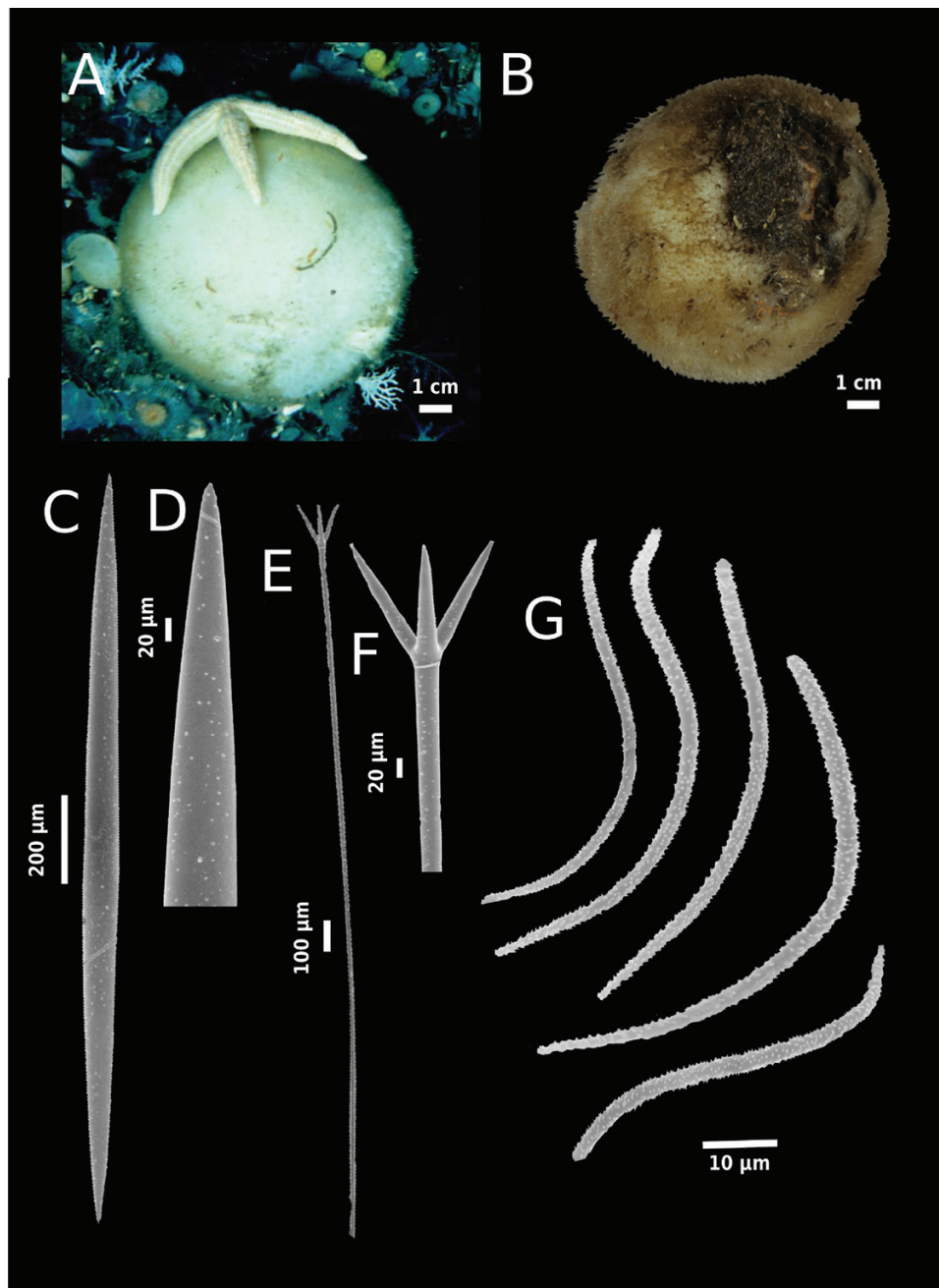


Figure 14. *Craniella wolfi* sp. nov. holotype Harbor Branch Oceanographic Museum (HBOM) 003:02004. A, *in situ* picture (355 m). B, deck picture. C, oxea. D, detailed view of oxea. E, protriaene with equal clads. F, details of protriaene. G, long sigmaspires.

is the presence of tetraclone desmas, which are strongly articulated with short root-like branches showing a strong tuberculation (Fig. 12C–F). This strong tuberculation of the tetraclone desmas is not present in the two other new species described. *Racodiscula asteroides* also shows sparsely tuberculated rays and strongly tuberculated tips

of tetraclone desmas; however, it lacks oxeas and strongyles (Pisera & Lévi, 2002b). The rhabdome of phyllo- to discotrianes from *R. borisi* sp. nov. is much shorter and a bit thinner in size compared to the others newly described species (Table 3). Oxeas and strongyles appear larger in size compared to *R. seymoura* sp. nov. *R. borisi* sp. nov. groups within

the *Racodiscula* clade from the Galápagos and forms a sister-group relationship to the other two species (Fig. 5).

SUBORDER SPIROPHORINA BERGQUIST & HOGG, 1969

FAMILY TETILLIDAE SOLLAS, 1886

GENUS *CRANIELLA* SCHMIDT, 1870

***CRANIELLA WOLFI* SP. NOV.**

(FIG. 14)

Diagnosis: *Craniella* with worm-like long (62–91 µm) sigmaspires.

Type material: Holotype: HBOM 003:02004, Coll. JSL-I dive 3909 [20 October 1995, Galápagos, S. Coast, approx. 5 NM NE of Wolf Island, 1°21'28"N, 91°48'41"W, 355 m].

Type locality: Wolf Island, Galápagos Islands (355 m) (Fig. 1).

Distribution: Only known from type locality.

Habitat: Attached to hard substratum, 355 m.

Description: **Morphology**, globular small hispid sponge with small apical oscule and 1-mm-thick cortex (Fig. 14A, B). Dimension of the holotype is 3 cm in diameter and about 4–5 cm high (Fig. 14A, B). **Texture**, soft. **Surface**, conulose and hispid. **Colour**, white *in vivo* (Fig. 14A) and light brown in ethanol (Fig. 14B). **Ectosomal skeleton** contains bundles of oxeas with few protriaenes sticking out of these bundles. No anatriaenes detected. **Choanosomal skeleton** contains oxeas (Fig. 14C, D) and abandoned uncoiled sigmaspires. **Megascleres** are thick oxeas measuring 134–356–627 × 8–15–25 µm (N30) (Fig. 14C, D). Protriaenes, equal clads measuring 121–150–177 µm (N11) and rhabdome 3380–4864–5640 × 19–27–30 µm (N11). Anatriaenes are not present in our spicule preparations. **Microscleres** are thin and uncoiled long sigmaspires 62–79–91 × 1–3 µm (N30) (Fig. 14G).

Etymology: Named after point of origin: Wolf Island (Latin, *wolfi*).

DNA barcodes: We sequenced *COI*, 28S (C1-D2) and 18S (1,670 bp) of the holotype. GenBank accession number: *COI* KY652825, 28S KY652795, 18S KY652840. SBD record no. 1727.

Remarks: The absence of porocalices and the presence of a conspicuous double-layered cortex clearly places this species into the genus *Craniella*. There are currently 34 valid *Craniella* species (P. Cárdenas, unpublished results), ten of which have lost their sigmaspires. The remaining 24 species have classical C- or S-shaped sigmaspires 8–30 µm long; none have these unusually worm-like long (62–91 µm) sigmaspires. Molecular investigations confirm that *C. wolfi* sp. nov. is closely related to *Craniella* aff. *zetlandica* (ZMBN 85239) from Western Norway (Korsfjord), a topology which is highly supported (PP = 1, BS = 100) (Fig. 5).

FAMILY SCLERITODERMIDAE SOLLAS, 1888

GENUS *SCLERITODERMA* SOLLAS, 1888

Emended diagnosis: Scleritodermidae with ectosomal acanthostrongyles or acanthorhabds, occasionally with ectosomal tylostyles, and sigmaspires as microscleres (modified after Pisera & Lévi, 2002c).

Remarks: Our new species has all the characters of typical *Scleritoderma*, for example, rhizoclone desmas, sigmaspire microscleres occurring in the choanosome; however, this is the first time tylostyles have been observed. Careful analysis demonstrated, that these are clearly not a contamination (see remarks below). The occurrence of tylostyles resulted in the emended diagnosis.

***SCLERITODERMA TORTUGA* SP. NOV.**

(FIGS 15, 16)

Diagnosis: *Scleritoderma* with tylostyles vertically protruding from the skeletons surface.

Synonymy: *Scleritodermida* sp. 1 and *Scleritodermida* sp. 2 (Schuster *et al.*, 2017, additional file 1, Fig. 4).

Type material: Holotype: HBOM 003:02012, Coll. JSL-I dive 3923 [27 October 1995, Galápagos, SW Coast, Caleta Webb, 0.5 NM offshore of Tortuga Island, 1°04'59"S, 90°51'56"W, 242 m]. Paratype: HBOM 003:02009 (239 m), Galápagos, Isabela Island, 0°47'04"S, 91°26'32"W, Coll. JSL-I dive 3919.

Comparative material: *Scleritoderma camusi* Lévi & Lévi, 1983, Holotype MNHN DCL 2787, paratypes: MNHN DCL 2788, 355–30 m, Norfolk Ridge. *S. camusi* (described in Schlacher-Hoenlinger, Pisera & Hooper, 2005), Norfolk Ridge 278–410 m, QMG318549, QMG318706. *S. nodosum* Thiele, 1900,

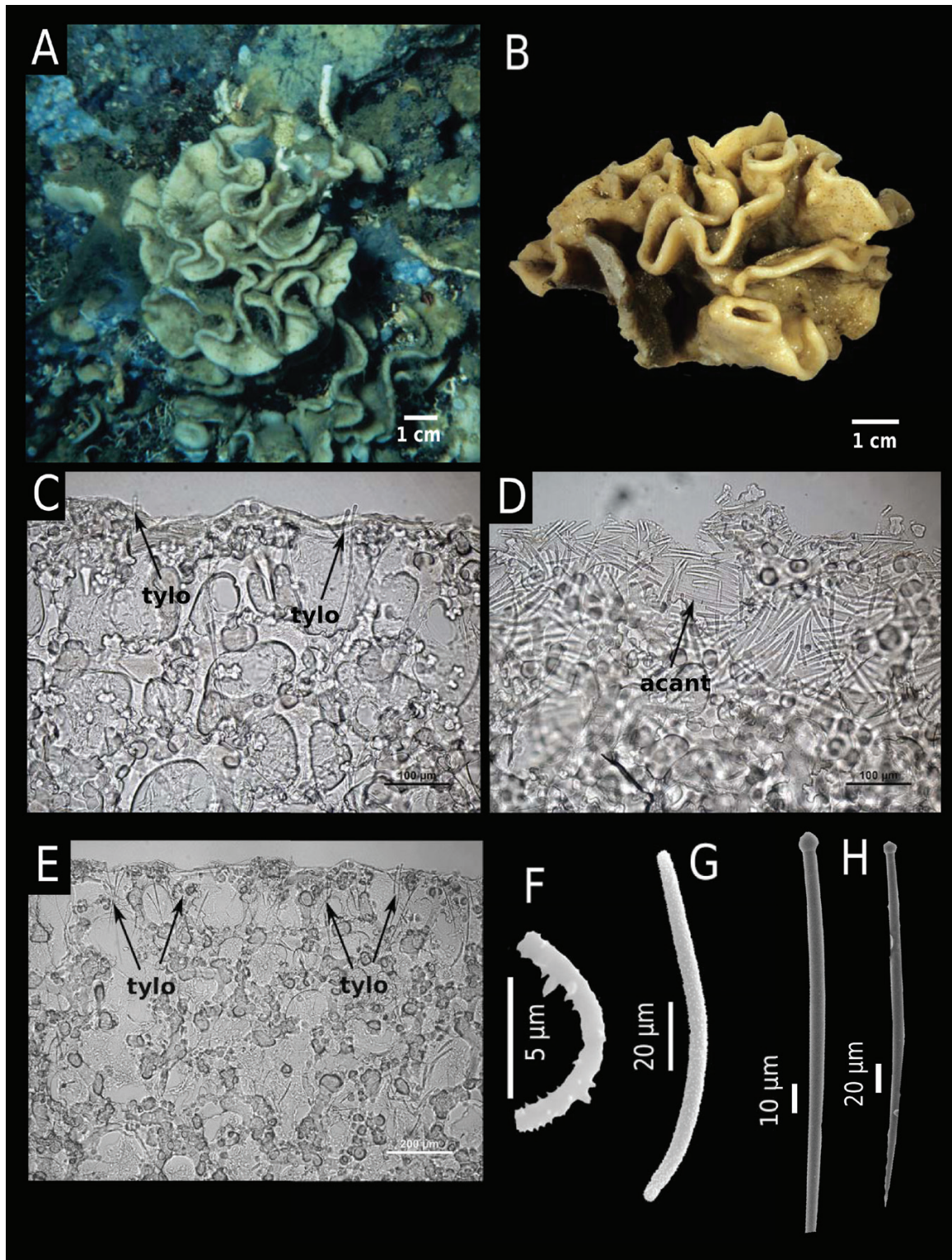


Figure 15. *Scleritoderma tortuga* sp. nov. holotype Harbor Branch Oceanographic Museum (HBOM) 003:02012. A, *in situ* picture 242 m. B, deck picture. C, thick section of the ectosome and part of the choanosome. Arrows pointing to tylostyles (tylo). D, detailed view the ectosome with a layer of acanthorhabds (acant). E, ectosomal view with tylostyles (tylo). F, thick sigma-spire. G, spinose acanthorhabd. H, detailed views of tylostyles.

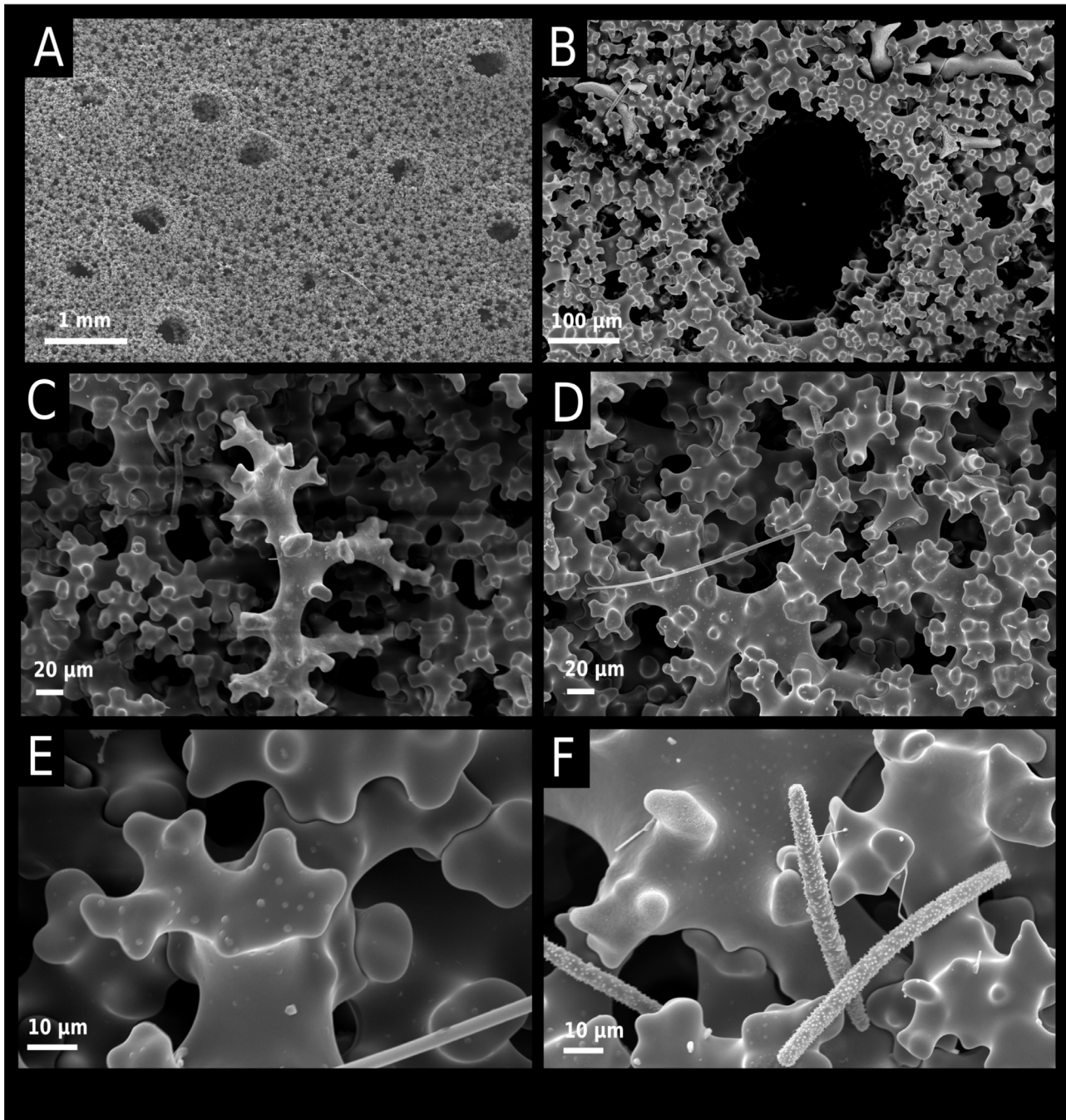


Figure 16. Diverse desmas of *Scleritoderma tortuga* sp. nov. holotype Harbor Branch Oceanographic Museum (HBOM) 003:02012. A, overview of the choanosomal skeleton with several oscules. B, detailed view of one oscula opening. C, a loose rhizoclone desma. D, rhizoclone desmas with a tylostyle. E, F, detailed view of spines of rhizoclones with acanthorhabds.

MNH DCL 3233, 90–110 m, Philippines (described in Lévi & Lévi, 1989). *S. flabelliforme* Sollas, 1888 (described in Schlacher-Hoenlinger *et al.*, 2005), Norfolk Ridge, 458–68 m, QMG318641, QMG318658, QMG318664.

Type locality: Tortuga Island, Galápagos Islands (242 m) (Fig. 1).

Distribution: Known from type locality and Isabela Island.

Habitat: Attached to hard substratum, depth range 239–242 m.

Description: **Morphology**, massive and flabelliform (Fig. 15A, B). Dimension of the holotype is 8 cm in diameter and about 3–5 cm in height (Fig. 15A, B). **Texture**, stony and hard. **Surface**, covered with small oscule openings 0.2–0.3 mm in diameter. **Colour**, beige *in vivo* (Fig. 15A) and light brown in ethanol (Fig. 15B). **Ectosomal skeleton** contains a thick layer of acanthorhabds (Fig. 15D) on the surface. The heads of tylostyles project beyond the surface layer (Fig. 15C, E). Very rare sigmaspires. **Choanosomal skeleton** composed of dense articulated slightly thorny rhizoclones (Fig. 16). Large oscula openings on the surface of the choanosomal skeleton 300–400 µm in diameter (Fig. 16A). **Megascleres** are rhizoclone desmas 225–350 µm in diameter and tylostyles measuring 153–256–316 µm (N15) (Fig. 15E, H). **Microscleres** are C-shaped thick spinose sigmaspires 9–13 × 1–2 µm (N3) (Fig. 15F) and slightly curved acanthostrongyles 61–84–101 × 5–6 µm (N30) (Fig. 15D, G).

Etymology: Named after the type locality: Tortuga Island.

DNA barcodes: *COI* sequences as in Schuster *et al.* (2017), 28S (C1-D2) of the holotype and paratype as well as 18S (1770 bp) of the paratype (GenBank accession numbers: 28S KY652801, KY652802; 18S KY652839, SBD record no. 1730, 1731). The 28S sequences were identical. The obtained *COI* sequence of HBOM 003:02012 was shorter (393 bp) compared to HBOM 003:02009 (630 bp). The 393 bp were identical (100%).

Remarks: Our new species *Scleritoderma tortuga* sp. nov. differs from the four other *Scleritoderma* species, *S. camusi* Lévi & Lévi, 1983, *S. cyaneum* Van Soest & Stentoft, 1988, *S. flabelliforme* Sollas, 1888 and *S. nodosum* Thiele, 1900 (Van Soest *et al.*, 2017), by the presence of tylostyles. Since tylostyles have never been observed in *Scleritoderma* species before, we first questioned if these could be due to a contamination from another sponge. However, we are convinced here that these are proper to the specimen, as we observed at least 15 tylostyles in both the holotype and paratype sections, all orientated in a vertical position protruding from the skeleton's surface (Fig. 15C, E). Additionally, in all of our preparations, no other spicule types were found, which may have indicated a possible contamination from another sponge. Furthermore, our concatenated molecular analyses including sequences of all known *Scleritoderma* species (Fig. 5) indicate that *Scleritoderma tortuga* sp. nov. forms a sister-group

relationship to *S. nodosum* from the Indo-Pacific (17–27 m). Additional supportive evidence of the new species is given by the presence of a 1109 bp long 'intron 723' located within the *COI* region (Schuster *et al.*, 2017) in both specimens. Our concatenated phylogenetic analysis supports the monophyly of the genus *Scleritoderma* which was first reported by Schuster *et al.* (2017) on single mitochondrial data. Based on the presence of tylostyles in *S. tortuga* sp. nov. the definition of *Scleritoderma* was amended. Tylostyles are actually not uncommon within Scleritodermidae, for example, they are known to occur in *Aciculites* and may have been overlooked in other species. We therefore recommend that future revisions of this family reassess the presence of tylostyles within this group.

DISCUSSION AND CONCLUSION

Previous knowledge on tetractinellids from the Galápagos archipelago was scarce (about 11 species described) and mainly limited to shallow-water habitats (<100 m) (Wilson, 1904; de Laubenfels, 1939; Desqueyroux-Faúndez & Van Soest, 1997). In fact, only one single desma-bearing astrophorid species (*Corallistes isabela*) and one species from the suborder Spirophorina (*Cinachyrella globulosa*), both endemic to the Galápagos, were described by Desqueyroux-Faúndez & Van Soest (1997). Applying the morphological as well as the phylogenetic species concept we enlarge the current knowledge with the discovery of five new deep-water desma-bearing Astrophorina (*Neophrissospongia galapagoensis* sp. nov., *Corallistes floreana* sp. nov., *Racodiscula radixa* sp. nov., *Racodiscula seymoura* sp. nov. and *Racodiscula borisi* sp. nov.) and report for the second time the occurrence of *Corallistes isabela*. Additionally, *Craniella wolffi* sp. nov. of the family Tetillidae and *Scleritoderma tortuga* sp. nov. of the family Scleritodermidae (Spirophorina) are described. The definition of the genus *Scleritoderma* required subsequent amendment. All seven new species are most likely endemic to the Galápagos islands. Statistical methods such as Principle Component Analysis (PCA) or haplotype networks which may help discriminate the closely related species *C. floreana* sp. nov. from *C. isabela* and *R. radixa* sp. nov. from *R. seymoura* sp. nov. were not feasible in this study, due to the low sample size (only one or two samples per species). A further statistical probabilistic based test is the coalescent theory which is based on the evolutionary history of alleles. The application of this test is widespread in integrative taxonomic studies to analyze species structures (Fujita *et al.*, 2012). However, these methods recently have been shown to overestimate structure and consequently falsely suggest speciation events (Sukumaran & Knowles, 2017). This

test again requires a higher sample size than given by our data. Therefore, we refrained from using multispecies coalescent analysis in this study as it may provide unambiguous results. Nevertheless, this study provides the first comprehensive dataset to investigate the deep-water 'lithistid' fauna of the Galápagos by providing descriptions of unique specimens collected in one of the most remote marine areas. Our phylogenetic analyses (Fig. 5) including other material from the Caribbean, Central and West Pacific provide strong evidence of biogeographical differences and support an apparent high level of demosponge endemism in this region (Desqueyroux-Faúndez & Van Soest, 1997), but should be corroborated by further sampling from other areas of the Eastern Pacific. The long paleontological history of most families like Corallistidae ~155 Myr and Theonellidae ~167 Myr, as well as rhizoclone desmas present since the Early Paleozoic (e.g. Pisera, 2002), may indicate that the current tetractinellid biodiversity in the Eastern Pacific is much higher than previously assumed.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Table S1. Specimen list.