II

Paper II

Cárdenas, P., Menegola, C., Rapp, H.T. & Díaz, M.C. (2009) Morphological description and DNA barcodes of shallow-water *Tetractinellida* (Porifera: Demospongiae) from Bocas del Toro, Panama, with description of a new species. *Zootaxa*, 2276, 1-39.

Article

Morphological description and DNA barcodes of shallow-water *Tetractinellida* (Porifera: Demospongiae) from Bocas del Toro, Panama, with description of a new species

PACO CÁRDENAS[1], CARLA MENEGOLA[2], HANS TORE RAPP[1, 3] & MARIA CRISTINA DÍAZ[4]

[1] *Department of Biology, University of Bergen, High-Technology Center, PO Box 7800, N-5020 Bergen, Norway. E-mail: paco.cardenas@bio.uib.no, hans.rapp@bio.uib.no*

[2] *Instituto de Biologia, Departamento de Zoologia, Universidade Federal da Bahia, Campus de Ondina, Rua Barão de Geremoabo, s/n – CEP 40170–180, Salvador, BA, Brasil. E-mail: carlamms@ufba.br*

[3] *Centre for Geobiology, University of Bergen, Allégaten 41, 5007 Bergen, Norway.*

[4] *Museo Marino de Margarita, Boca del Río, Macanao, Edo. Nueva Esparta, Venezuela. E-mail: crisdiaz@ix.netcom.com*

Table of contents

Abstract

Tetractinellida Marshall 1876 [Borchiellini *et al.* 2004] comprises the Orders Spirophorida and Astrophorida. A survey of their diversity in the Bocas del Toro archipelago (Panama) was conducted. A total of ten species of *Tetractinellida* were encountered: *Cinachyrella alloclada*, *Cinachyrella apion*, *Cinachyrella kuekenthali*, *Ecionemia megastylifera*, *Stelletta fibrosa*, *Stelletta* sp., *Stryphnus raratriaenus* **sp. nov.**, *Erylus formosus*, *Geodia gibberosa* and *Geodia papyracea*. Five of these species are new to the Atlantic sponge fauna of Panama, one of which is new to science. *Stryphnus raratriaenus* sp. nov. is very similar to Caribbean *Asteropus* species but it possesses triaenes. It is the first species of this genus in the Caribbean. The description of *Ecionemia megastylifera* Wintermann–Kilian & Kilian, 1984 is here revised and the species fully redescribed. A cytochrome *c* oxidase subunit I (COI) gene partial fragment and/or a 28S ribosomal gene partial fragment (C1–D2 domains) were sequenced for some of the species collected.

Key words: Taxonomy, barcoding, COI, 28S, Spirophorida, Astrophorida, *Geodia*, *Erylus*, *Ecionemia*, *Stryphnus*, *Stelletta*, *Cinachyrella*.

Abstract [Spanish]

Tetractinellida Marshall 1876 [Borchiellini *et al.* 2004] incluye los Ordenes Spirophorida y Astrophorida. Se realizó un inventario de su diversidad en el archipiélago de Bocas del Toro (Panamá), encontrándose diez especies de *Tetractinellida*: *Cinachyrella alloclada*, *Cinachyrella apion*, *Cinachyrella kuekenthali*, *Ecionemia megastylifera*, *Stelletta fibrosa*, *Stelletta* sp., *Stryphnus raratriaenus* **sp. nov.**, *Erylus formosus*, *Geodia gibberosa* y *Geodia papyracea*. Cinco de estas especies son nuevos reportes para la fauna atlántica de esponjas de Panamá y una de ellas es nueva para la ciencia. *Stryphnus raratriaenus* sp. nov. es muy parecida a las especies de *Asteropus* del Caribe, pero posee triaenes. Es la primera especie de este género en el Caribe. La descripción de *Ecionemia megastylifera* Wintermann–Kilian & Kilian, 1984 ha sido revisada y la especie ha sido redescrita en este artículo. Estudios moleculares que incluyeron una secuencia parcial del gen citocromo *c* oxidase subunidad I (COI) y/o una secuencia parcial del gen ribosómico 28S (dominios C1–D2) fueron realizados en algunas de las especies encontradas.

Introduction

On the western Caribbean shore of Panama, the Bocas del Toro region includes two large water bodies, Almirante Bay and Chiriquí Lagoon; it is a shallow coastal zone (maximum depths of 20–50 m). More than 68 islands and mangrove keys form the Bocas del Toro archipelago (Fig. 1). This archipelago offers a wide range of marine habitats such as reefs, mangroves and seagrass beds. As in other Caribbean sites the sponge fauna represents one of the most diverse benthic animal group with 123 shallow-water sponges identified (Collin *et al.* 2005; Díaz 2005; Díaz *et al.* 2007; Valderrama *et al.* 2009).

Tetractinellida Marshall 1876 [Borchiellini *et al.* 2004] is a worldwide monophyletic group comprising the sister-orders Spirophorida Bergquist & Hogg, 1969 and Astrophorida Sollas, 1888 (Borchiellini *et al.* 2004; Nichols 2005). *Tetractinellida* share four-rayed megascleres with usually one unequal ray longer than the other three. These 'triaenes' are, up to now, the single morphological synapomorphy of the group (Chombard *et al.* 1998) although they can sometimes be secondarily reduced or lost (Uriz 2002a;b).

The first sponge survey in the Bocas del Toro region, focusing on reefs, encountered two tetractinellids (Guzman & Guevara 1999; Guzman & Guevara 2001): *Cinachyrella alloclada* (Uliczka, 1929) and *Cinachyrella* sp.. Later, a more comprehensive study listed four tetractinellid species (Díaz 2005): *Geodia papyracea* Hechtel, 1965, *Erylus formosus* Sollas, 1886, *Cinachyrella alloclada* and *Cinachyrella apion* (Uliczka, 1929). This is a relatively low number of species compared to neighboring regions with similar habitats and where sponge surveys are more comprehensive. For example, along the Caribbean Colombian coast there has been a total of 11 tetractinellid species identified (Wintermann-Kilian & Kilian 1984; Díaz 2007; van Soest 2009) while Cuba has 26 (Alcolado 2002). If we consider only shallow-water tetractinellids, Belize has about 10 species (Rützler *et al.* 2000; Erpenbeck *et al.* 2007), Curacao has 11 (van Soest 1981)

while Jamaica has 12 (Lehnert & van Soest 1998). We thus suspected that the number of tetractinnelids in the Bocas del Toro region was higher and took advantage of the 3rd 'Taxonomy and Ecology of Caribbean Sponges' Workshop at the Bocas del Toro Smithsonian Tropical Research Station (STRI) to assess the *Tetractinellida* diversity of this region.

DNA barcoding is an identification method that compares short specific DNA sequences from unidentified specimens to sequences of previously identified voucher specimens (Hebert *et al.* 2003). Tagging identified specimens with their DNA markers, as it is done in this paper, is the first important step towards building the barcode database. Tagging requires i) species identifications by specialists, ii) sequencing of the DNA markers and iii) storage of the voucher specimens in museum collections. To make tagging even more reliable, morphological data of the voucher should also be recorded and made easily accessible to the public in case of future developments in the taxonomy. This is now possible with the 'Sponge Barcoding Database' (SBD) (www.spongebarcoding.org) that links a GenBank tag sequence (http://www.ncbi.nlm.nih.gov) to its voucher's description, and the 'World Porifera Database' (www.marinespecies.org/porifera) (Wörheide *et al.* 2007; van Soest *et al.* 2009). But species identifications may require more space than is available on the SBD: especially because of additional figures, tables, references to comparative material, discussion, etc. In this regard, a scientific publication offers more space and therefore a more nuanced and detailed identification. It also gives the opportunity to describe specimens only identified to the genus level (at present, not accessible in the SBD). Furthermore, simultaneous publication of morphological and molecular data (such as in the present paper) unambiguously tie the DNA sequences to its vouchers and provide future molecular taxonomic and phylogenetic studies with significant and accurate data. This integrative taxonomic approach (also advocated by the SBD) is a straightforward way to reduce identification errors and contributes to the reliability of the sponge DNA barcoding tool. Our sequences will also promote the DNA barcoding of Caribbean demosponges initiative (Erpenbeck *et al.* 2007) thus helping the sponge biodiversity survey of this area.

Material and methods

Sponges were collected by M. C. Díaz, R. Thacker and P. Cárdenas in August 2007, February 2008 and July 2009 by snorkeling and scuba-diving. S. Nichols provided additional samples collected in March 2000. Sites surveyed included mangroves, reefs and seagrass habitats of the Bocas del Toro archipelago. We surveyed some sites already visited by Díaz (2005; Table 1) but also new localities: Point Hospital (East of Solarte Island), South Solarte 4 (09°17'37.8''N, 82°11'32.2''W) and South Solarte 5 (09°17'51.8''N, 82°11'47.5''W), Sache and Salt Creek (both off Bastimento Island), Buoy 19 in the Almirante Bay and Zapatilla Cayes (Fig. 1). Material from different institutions were also examined for comparison. Digital pictures of the species were taken *in situ* or upon return at the STRI station. All samples were fixed in ethanol 95% soon after collection and stored at room temperature. Specimens are deposited in the Bergen Museum. Species and collecting localities are given in Table 1.

Spicule mounts were made according to the standard procedure described in Rapp (2006). 30 spicules per spicule type were measured, unless otherwise stated (N: number of spicules measured). Long and fragile megascleres such as triaenes and oxeas often ended up broken in spicule preparations so they were preferably measured in the sections. Measurements of spicules are given in μm throughout the article as follows: minimum-*mean*-maximum. Measurements of all spicules were made with a light microscope, except for the measurements of sigmaspires and rosettes (tips of fused actins in sterrasters and aspidasters), made with the scanning electron microscope (SEM). Rhabdome widths of triaenes were measured at the base of cladomes. Some of these spicules were placed directly on top of a cover slip glass taped to a stub and coated with a gold/ palladium mix. They were then observed with a ZEISS Supra 55V SEM at the University of Bergen. We followed Boury-Esnault *et al.* (2002) for making thick sections (100–800 μm). Instead of epoxy resin, we used an Agar Low Viscosity Resin kit (© Agar Scientific) in accordance with the manufacturer's mixing

instructions to make a hard embedding medium. The sections were not polished or stained but directly embedded in the resin between the cover slip and the slide. Digital pictures of these sections were taken with a Nikon camera fixed to a stereomicroscope (Leica M216 A).
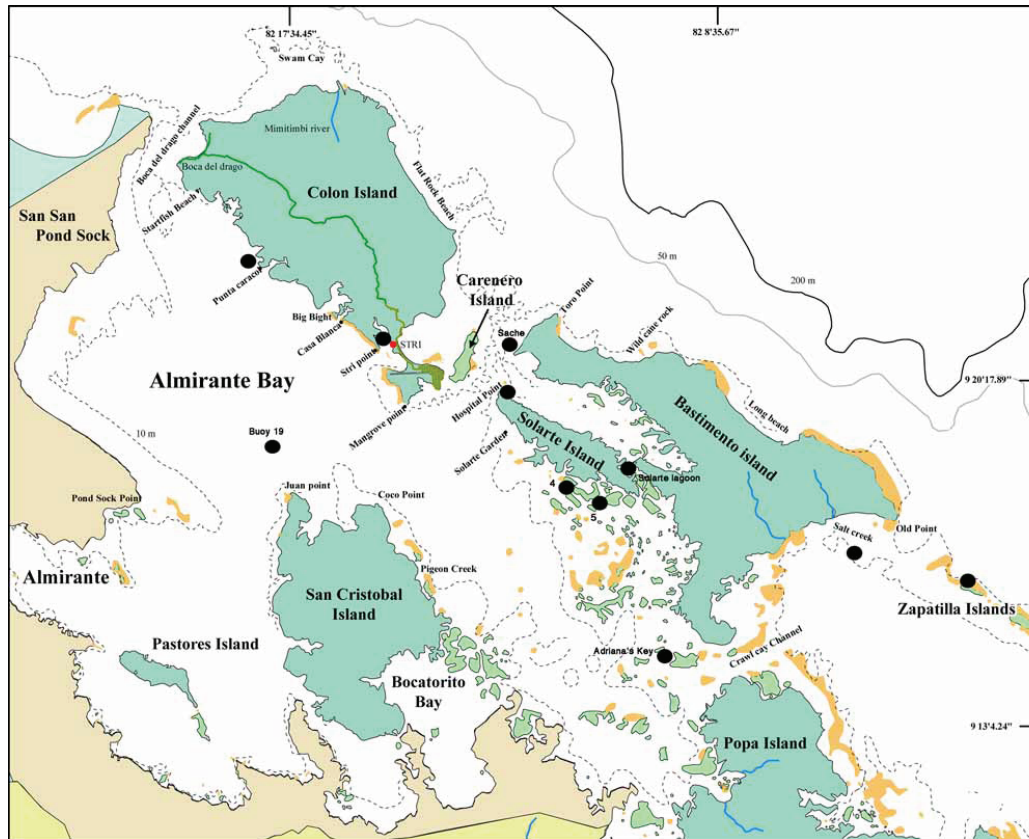

FIGURE 1. Map of the Bocas del Toro archipelago. (●) Sites surveyed in August 2007, and February 2008.

DNA extraction from choanosome samples was performed using the Tissue Genomic DNA extraction kit (Viogene, Sunnyvale, U.S.A.) in accordance with the manufacturer's instructions. We sequenced two widely used molecular markers in sponge barcoding: the mitochondrial cytochrome *c* oxidase subunit I (COI) gene and the 5' end terminal part of the 28S rRNA gene (C1–D2 domains). The Folmer primers (LCO1490 and HCO2198) were used to amplify a 659 bp. region of COI (Folmer *et al.* 1994) (5 min/94°C; 5 cycles [30 s/ 94°C, 1 min30s/45°C, 1 min/72°C]; 30-35 cycles [30 s/94°C, 1 min30s/50°C, 1 min/72°C]; 7 min/72°C). The primers C'1ASTR (5'–ACC CGC TGA ACT TAA GCA T–3') and D2 (5'–TCC GTG TTT CAA GAC GGG–3') were used to amplify a 797–801 bp. 5' end of 28S (Lê *et al.* 1993; Chombard *et al.* 1998; Cárdenas *et al.* 2009) (1 cycle [4 min/95°C, 2 min/59°C, 2 min/72°C]; 35 cycles [1 min/94°C, 45 s/59°C, 1 min/72°C]; 7 min/72°C). COI sequences of some of these specimens had been obtained previously (Nichols 2005). The new and existing COI/28S sequences, along with the specimen descriptions, were all submitted to the SBD. Voucher museum numbers and GenBank accession numbers are given in Table 1.

Abbreviations used in the text are as follows: BMNH (British Museum of Natural History, London); INVPOR (Universidad Nacional de Colombia, INVEMAR, Santa Marta); MC (National Museums Northern Ireland); MCZ (Museum of Comparative Zoology, Harvard University); MNHN (Muséum National d'Histoire

Naturelle, Paris); MSNG (Museo Civico di Storia Naturale "G. Doria", Genoa); UFBA (Universidade Federal da Bahia, Salvador); UMPCW (University of California Museum of Paleontology, Berkeley); USNM (National Museum of Natural History, Washington DC); YPM (Yale Peabody Museum, New Haven); ZMA (Zoölogisch Museum, Universiteit van Amsterdam); ZMB (Zoologisches Museum für Naturkunde an der Universität Humboldt zu Berlin); ZMBN (Bergen Museum, Universitetet i Bergen).

TABLE 1. *Tetractinellida* species collected in the Bocas del Toro region, museum accession numbers of the specimens sequenced, GenBank accession numbers of partial COI and 28S sequences (in bold, new sequences from this article). (–) means that no sequence was obtained. UMPCW (University of California Museum of Paleontology, Berkeley); ZMBN (Bergen Museum).

Species	Localities	Museum number	COI	28S (C1-D2)
Spirophorida				
Tetillidae				
Cinachyrella alloclada (Uliczka, 1929)	Swan Key*, TINC*, Cristobal Island reef*, Punta Caracol reef[†], Isla Popa (Cayo Deer)[‡], Cayos Tigre medio[‡], Península Valiente[‡]	ZMBN 81788	–	–
Cinachyrella apion (Uliczka, 1929)	Solarte 1*, Solarte 2*, STRI Point*[†], Big Bight*, Adriana's key*[†]	ZMBN 81785	**FJ711645**	–
Cinachyrella kuekenthali (Uliczka, 1929)	Adriana's Key[†], Punta Caracol reef[†], Cayos Zapatilla[†], Buoy 19[†]	ZMBN 81787	**FJ711646**	–
Astrophorida				
Ancorinidae				
Ecionemia megastylifera Wintermann–Kilian & Kilian, 1984	STRI Point[†], Point Hospital[†], Sache[†], Solarte 4[†]	ZMBN 81782 UCMPWC980	**FJ711642** AY561938	**FJ711648** –
Stelletta fibrosa (Schmidt, 1870)	Punta Caracol reef[†]	ZMBN 81784	**FJ711643**	**FJ711649**
Stelletta sp.	Solarte 1[†]	ZMBN 81643	**FJ711644**	**FJ711650**
Stryphnus raratriaenus sp. nov.	STRI Point[†]	ZMBN 81642	–	**FJ711647**
Geodiidae				
Erylus formosus Sollas, 1886	Adriana's Key*[†], Pastores Island*	ZMBN 81644–81645	–	–
Geodia gibberosa Lamarck, 1815	Solarte 1[†], Solarte 2[†]	ZMBN 77928	EU442209	**FJ717708**
Geodia papyracea (Hechtel, 1965)	Solarte 1*, Solarte 2*	UMPCW921	AY561961	**FJ717707**

[‡] Guzman and Guevara (1999; 2001)

* Díaz, 2005

[†] present study

Results and discussion

Systematics

The classification used here follows the *Systema Porifera* (Hooper & van Soest 2002).
Class: Demospongiae Sollas, 1885

Order: Spirophorida Bergquist and Hogg, 1969

Family: Tetillidae Sollas, 1886

Genus: *Cinachyrella* Wilson, 1925

***Cinachyrella alloclada* (Uliczka, 1929)**
(Figure 2)

Synonyms (Rützler & Smith 1992).
Cinachyra alloclada Uliczka, 1929: 41, figs. 11–15, pl. I, figs. 2–3.
Trachygellius cinachyra de Laubenfels, 1936b: 158, pl. 18, fig. 1.
Cinachyrella alloclada (Uliczka, 1929): Rützler 1987: 200, figs. 1d, f; 2a–d; 3a; 5a–b.

Holotype. ZMB 4921, Dry Tortugas, Florida (not seen).
    Material. ZMBN 81788, fragment collected at Caracol Reef, 15 m depth.
    Additional material examined. Cinachyrella alloclada, UFBA-POR 2565, Salvador, Bahia, Brazil; ZMBN 82980, Sweetings Cay, Grand Bahama Island, Bahamas, 15 m depth; ZMBN 82981, Key Largo, Florida, U.S.A.
    Outer morphology (Fig. 2A). Massive, subglobular orange (ca 10 cm of diameter), hispid. One obvious oscule at the top of the sponge. Covered with algae and sediments.
    Skeleton (Fig. 2B). Radial bundles of oxeas cross the choanosome until and beyond the surface. A whitish ectosome layer is visible with the naked eye (ca 0.9 mm), it has less sigmas than in the choanosome. Anatriaenes are tangled with the oxea bundles. The cladomes of anatriaenes are placed in or just under the ectosome layer, or protruding beyond the surface. Anatriaenes outnumber protriaenes. Many brownish crystalline round structures (diameter of 68–90 µm) are present in the choanosome, only visible in thick sections. Sediments, foreign spicules, foraminifera and diatoms are fairly abundant in the sponge.
    Spicules (Fig. 2C–F). *Megascleres*: (a) oxeas I, large, length: 2054–*2989.1*–3666 µm; width: 30–*40.2*–50 µm. (b) oxeas II (Fig. 2C–D), smooth, slightly bent, less common than oxeas I, length: 95–*147.5*–170 µm; width: 2–*5.3*–10.6 µm. (c) protriaenes, width of rhabdome tends to increase from cladome base, rhabdome length: 3110–*3486*–4200 µm (N=3); rhabdome width: 3.5–*8.2*–13.5 µm (N=17); clad length: 54–*122.5*–182 µm (N=17). (d) anatriaenes (Fig. 2E), very common, rhabdome length: 1330–*2208.7*–2640 µm (N=8); rhabdome width: 3.5–*5.3*–7 µm; clad length: 48–*73.2*–95 µm. *Microscleres*: (e) sigmaspires (Fig. 2F), spiny, length: 8.2–*9.8*–12.4 µm; width: 0.7–*1*–1.6 µm.
    Habitat in the Bocas del Toro region. Reef, 5–20 m depth.
    Distribution. North and South Carolina, U.S.A. (Wells *et al.* 1960; Rützler & Smith 1992); Georgia, U.S.A. (Freeman *et al.* 2007); Florida, U.S.A. (Uliczka 1929; de Laubenfels 1936b; Little 1963; Rützler & Smith 1992); Bermuda (de Laubenfels 1950); Bahamas (Wiedenmayer 1977; Rützler & Smith 1992); Cuba (Alcolado 2002); Jamaica (Lehnert & van Soest 1998); Curaçao (van Soest 1981); Costa-Rica (Cortés 1996); Panama (Collin *et al.* 2005; Díaz 2005); Colombia (Díaz 2007); Venezuela (Alvarez *et al.* 1991)*;* Brazil (Mothes de Moraes 1980; Lazoski *et al.* 1999; Cedro *et al.* 2007); Azores and Senegal: doubtful identifications because of geographical distance (Barnathan *et al.* 1992; Moss 1992).
    Remarks and discussion. Our specimen of *C. alloclada* fitted with the description of the species. We had two size categories of oxeas instead of three, but it has been shown that one size class could be absent (Rützler & Smith 1992). The size of the smallest oxea category is very variable, averaging 147.5 µm in our specimen, 159 µm in South-East Brazil (Mothes de Moraes 1980), 355 µm in South-Eastern U.S.A. (Rützler & Smith 1992) and 837 µm in Bahia, Brazil (Lazoski *et al.* 1999). The Florida specimen had sigmaspires with a much wider size range (11–21 µm). Our specimen had anatriaenes with an oval umbrella shape (Fig. 2E), just as the ones illustrated by Rützler and Smith (1992). On the other hand, anatriaenes of UFBA 2565 POR (Bahia, Brazil) had a depressed apex and clads that start growing horizontally before bending down.

C. alloclada has been the most common *Cinachyrella* reported in the Bocas del Toro region (Guzman & Guevara 1999; Guzman & Guevara 2001; Díaz 2005; Erwin & Thacker 2007).

For an unknown reason, amplification of the molecular markers did not succeed. PCRs were also difficult with the other *Cinachyrella* specimens of this study.
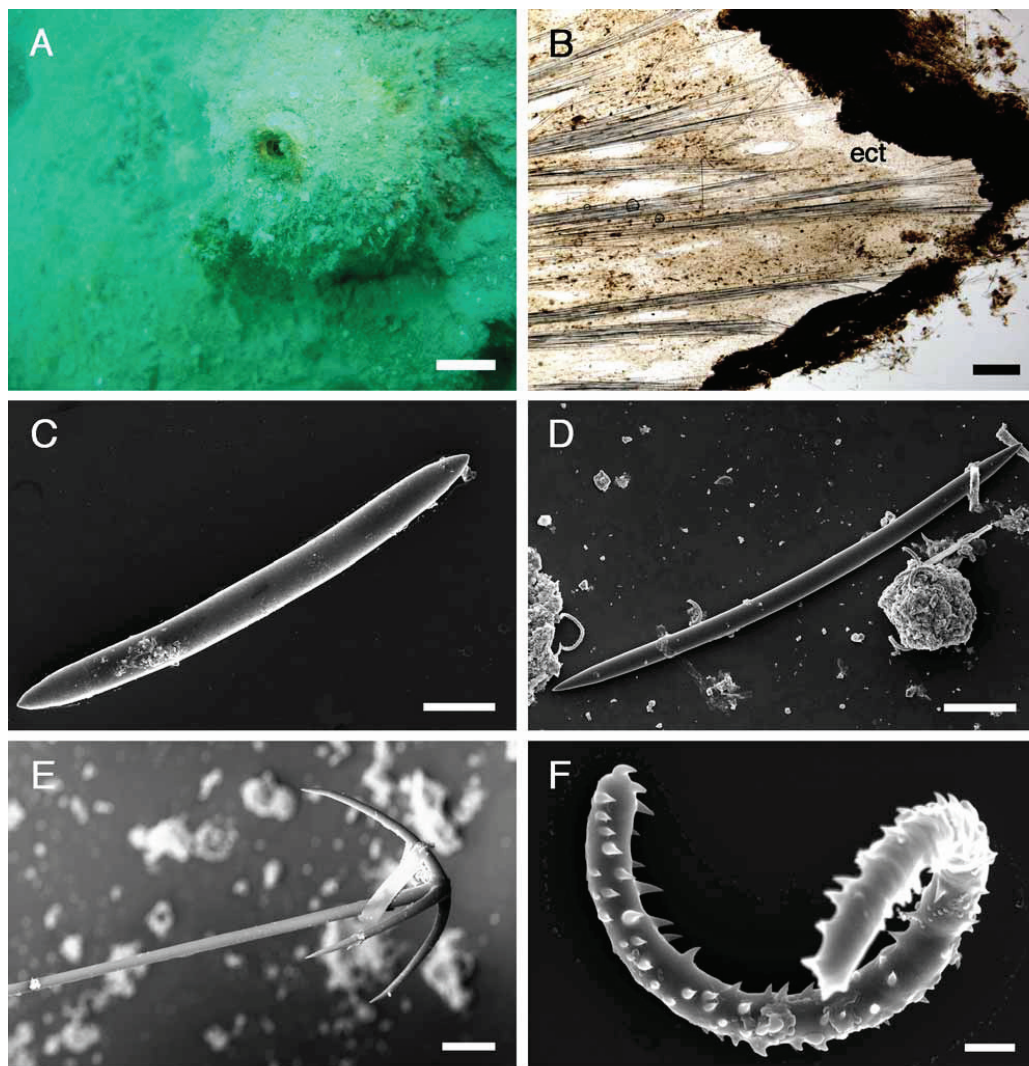

FIGURE 2. *Cinachyrella alloclada* (Uliczka, 1929) [ZMBN 81788]: A. Specimen *in situ*. Scale: 1 cm; B. Cross–section showing the skeletal architecture. Scale: 1 mm; C. Oxea II. Scale: 20 μm; D. Oxea II. Scale: 20 μm; E. Anatriaenes. Scale: 20 μm; F. Sigmaspire. Scale: 1 μm.

Cinachyrella apion (Uliczka, 1929)
(Figure 3)

Synonyms (Rützler & Smith 1992).
Cinachyra apion Uliczka, 1929: 43, figs. 16–21, pl. I, fig. 4.
Cinachyra rizophyta Uliczka, 1929: 38, figs. 1–10, pl. I, fig. 1.

Cinachyra cavernosa Lamarck, 1815 *sensu* de Laubenfels, 1950: 128, fig. 56, pl. II, fig. 7.
Cinachyra subterranea van Soest & Sass, 1981: 337, fig. 4, pl. II, fig. 2.
Cinachyrella apion (Uliczka, 1929): Rützler 1987: 200, figs. 2g; 3b–g; 4a, c; 5d, e.

Holotype. ZMB 4911, St. Thomas, Virgin Islands (not seen).

Material. ZMBN 80958, Adriana's reef, 1 m depth; ZMBN 81785, STRI Point, 1 m depth; 4 other specimens collected.

Additional material examined. Cinachyrella apion, ZMBN 81789, Key Largo, Florida; UFBA-POR 2232, Barra/Ondina, Salvador, Bahia, Brazil, 13°00'42" S / 38°31'12" W, C. Menegola col., intertidal.

Outer morphology (Fig. 3A). Massive, small spherical sponge that can reach 7 cm in diameter. External color alive is orange, as well as the choanosome. In ethanol, color becomes yellowish. The surface is very hispid, which hides the true color of the sponge, and gives it a rather sandy color. Generally one oscule (ca 3 mm of diameter), which can be found in any part of the sponge. Porocalices (2 mm of diameter) are numerous and evenly distributed, especially on the sides. No macro-epibionts were found growing on this species, except filamentous red algae. No budding was observed.

Skeleton (Fig. 3B). The skeleton organization is similar to that of *C. alloclada* except for the presence of trichodragmata. Radial bundles of oxeas cross the choanosome until and beyond the surface. A whitish ectosome layer is visible with the naked eye (ca 0.9 mm), it has less sigmas than in the choanosome. Anatriaenes are tangled with the oxea bundles. The cladomes of anatriaenes are placed in or just under the ectosome layer, or protruding beyond the surface. Protriaenes I and protriaenes II are present only around the oscules and porocalices (Fig. 3C). Protriaene cladomes were not found in the sponge, but only at its surface. Trichodragmata were common in the choanosome (never in the ectosome), with no particular orientation. Smaller stout oxeas, Haplosclerida-like, dispersed in the choanosome with no particular orientation. A few crystalline round structures (diameter of 91–127 µm) are present in the choanosome, only visible in thick sections. In the choanosome there are also some foreign spicules, foraminifera and diatoms.

Spicules (measurements of ZMBN 80958) (Fig. 3D–F). *Megascleres*: (a) oxeas I, large, length: 2088–*3526.2*–4800 µm (N=14); width: 11–*31.3*–37 µm (N=14). (b) oxeas II (Fig. 3D), foreign?, short, stout, length: 141–*169.9*–219 µm; width: 6–*9.4*–13.6 µm. (c) protriaenes I, rhabdome length: > 2040 µm (rhabdomes broken); rhabdome width: 4–*5.2*–7 µm; clad length: 33–*74*–113 µm. (d) protriaenes II, rhabdome length: > 1440 (rhabdomes broken); rhabdome width: 2–*2.9*–4 µm; clad length: 8–*20.7*–29 µm. (e) anatriaenes (Fig. 3E), very common, rhabdome with whip–like end, rhabdome length: 2232–*3708*–4800 µm (N=4); rhabdome width: 2–*4.1*–7 µm; clad length: 11–*44.7*–68 µm. *Microscleres*: (f) trichodragmata, length: 135–*203.3*–248 µm; width: 7–*14.1*–30 µm. (g) sigmaspires (Fig. 3F), spiny, length: 8.7–*10.4*–12.5 µm (N=13); width: 0.9–*1.2*–1.4 µm (N=13).

Habitat in the Bocas del Toro region. Common on very shallow reefs and sand (0.4–1.5 m depth), often below mangrove trees. Other reports at Bocas del Toro found it growing on mangrove roots (Collin *et al.* 2005; Díaz 2005), as it is often found in Belize (Rützler & Smith 1992).

Distribution. North and South Carolina (Rützler & Smith 1992); Bermuda (de Laubenfels 1950; Rützler & Smith 1992); Florida (Rützler & Smith 1992); Bahamas (van Soest & Sass 1981; Rützler & Smith 1992); Virgin Islands (Uliczka 1929); Belize (Rützler & Smith 1992; Rützler *et al.* 2000); Panama (Wulff 2000; Díaz 2005); Colombia (Wintermann-Kilian & Kilian 1984); Brazil (Lazoski *et al.* 1999; Cedro *et al.* 2007).

Remarks and discussion. C. apion was considered to be rare in Bocas del Toro (Díaz 2005), but we found it to be fairly common, especially in Adriana's reef. Despite the presence of oxeas II (Fig. 3D), the morphology of our specimens completely agreed with the description of *C. apion*. It is interesting to note that the short oxeas II were never previously reported in *C. apion*, and that they were not observed in our specimens from Florida. Therefore, we cannot rule out the possibility that these oxeas II were foreign.

The crystalline structures in the choanosome were previously reported in this species and *C. alloclada* (Rützler & Smith 1992; Lazoski *et al.* 1999). These structures have also been observed in other species of Tetillidae: *Cinachyrella levantinensis* Vacelet *et al.* (Vacelet *et al.* 2007), *Craniella cranium* (Müller) and *Craniella zetlandica* (Carter) (P. Cárdenas, personal observation). Their function is still unknown, as well as their diagnostic relevance. We nonetheless emphasize the fact that these were never found in *C. kuekenthali* (in previous records or our specimens).

Our COI sequence (FJ711645) was strictly identical to those of a *C. apion* from Twin Cays Mangroves, Belize (EF519601) and from Flatts Inlet, Bermuda (AJ843895).
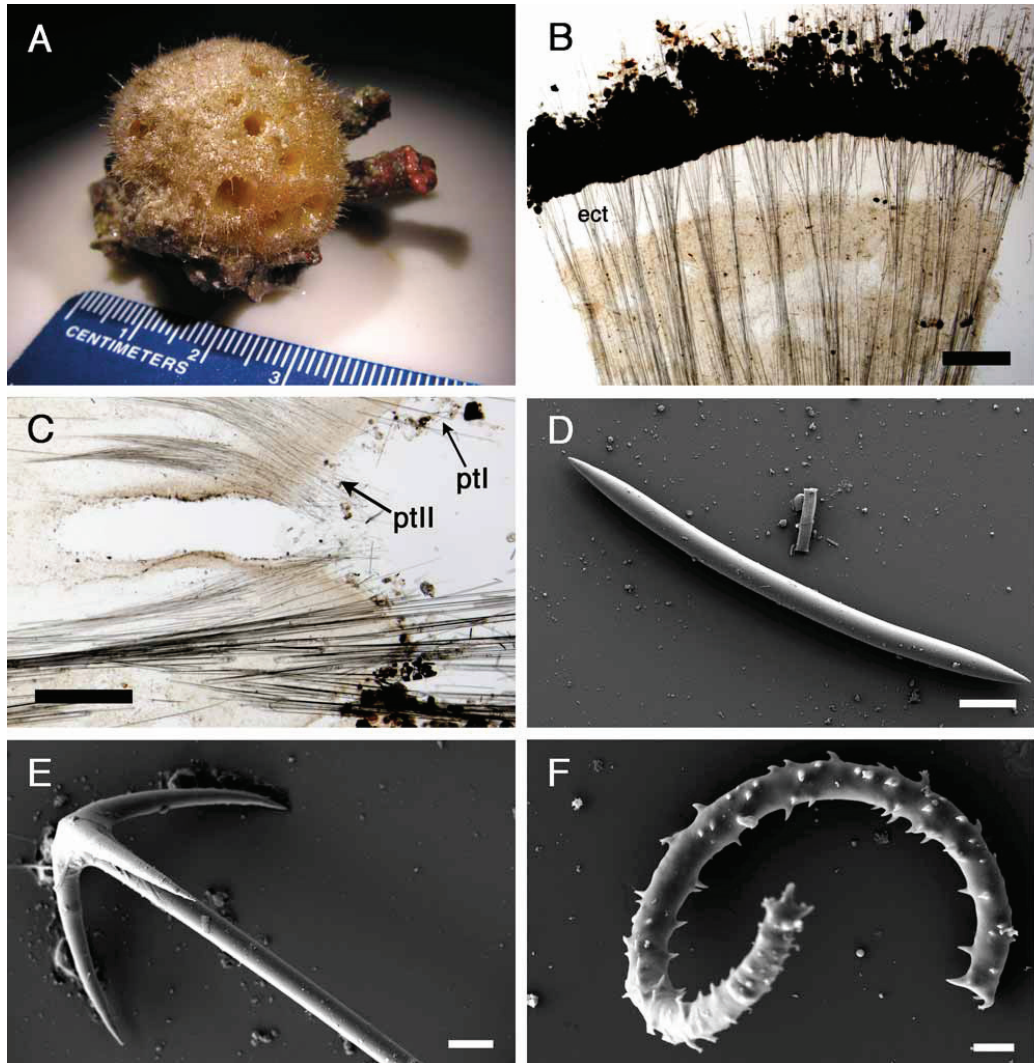

FIGURE 3. *Cinachyrella apion* (Uliczka, 1929) [ZMBN 80958]: A. Gross morphology; B. Cross–section showing the skeletal architecture. ect: ectosome. Scale: 1mm; C. Cross–section of oscule. ptI: protriaenes I, ptII: protriaenes II. Scale: 0.5 mm; D. Oxea II. Scale: 20 μm; E. Anatriaene. Scale: 10 μm; F. Sigmaspire. Scale: 1 μm.

***Cinachyrella kuekenthali* (Uliczka, 1929)**
(Figure 4)

Synonyms (Rützler & Smith 1992)*.*
Cinachyra kuekenthali Uliczka, 1929: 43, figs. 16–21, pl. I, fig. 4.
Cinachyra schistospiculosa Uliczka, 1929: 45, figs. 27–30, pl. I, fig. 6.
Cinachyrella kuekenthali (Uliczka, 1929): Rützler 1987: 200, figs. 2f, 5c.

Neotype. USNM 31491, off St. John, Virgin Islands, 16 m depth (not seen).

Material. 3 specimens collected at Caracol Reef and Adriana's reef, 3–15 m depth. Two specimens deposited: ZMBN 81786–81787, both from Adriana's reef, 3 m depth.

Outer morphology (Fig. 4A). Massive, subglobular, it can reach 15 cm of diameter. Strongly hispid. Often covered with sand, green and red algae or more rarely macro-epibionts (ascidians, sponges). It can therefore appear grayish or reddish in the field, while its true surface color is orange. Choanosome is orange when alive, yellowish in ethanol. Few oscules (ca 1 cm), sometimes only one. Porocalices (0.3– 0.5 cm) are numerous, unevenly distributed but usually not on the top. Oscules and porocalices can contract.

Skeleton (Fig. 4B). The skeleton organization is fairly similar to that of *C. apion*. Radial bundles of oxeas cross the choanosome until and beyond the surface. The ectosome layer is somewhat larger (1.2–1.5 mm). The cladomes of anatriaenes are still placed in this layer or protruding beyond the surface. Protriaenes are less abundant than anatriaenes and mainly placed at the surface. Numerous spiny microxeas are randomly positioned in the choanosome, and in lower densities in the ectosome. Sigmaspires are present throughout the choanosome. Foreign spicules and diatoms are rare in the sponge. No crystalline structures were observed.

Spicules (measurements of ZMBN 81786) (Fig. 4C–D). *Megascleres*: (a) oxeas, large, length: 2088–*2853.4*-3840 μm (N=15); width: 15–*39.6*–58 μm. (b) microxeas (Fig. 4C), spiny, straight or slightly bent, length: 141–*184.3*–215.5 μm; width: 2.7–*5.1*–5.3 μm. (c) protriaenes, with rare prodiaenes, width of rhabdome tends to increase from cladome base, rhabdome length: 2200–3500 μm (N=2); rhabdome width: 4–*8.5*–11 μm; clad length: 73–*122.4*–177 μm. (d) anatriaenes, very common, rhabdome length: 1600–*2735*–3600 μm (N=6); rhabdome width: 3–*5.7*–11.4 μm; clad length: 30–*67.9*–107 μm. *Microscleres*: (e) sigmaspires (Fig. 4D), spiny, with occasional central or terminal bulge, length: 11.1–*13.8*–16.5 μm (N=22); width: 1–*1.2*–1.6 μm (N=22).

Habitat in the Bocas del Toro region. Common on reef and coral rubble, 3–18 m depth.

Distribution. North Carolina and Florida, U.S.A. (Rützler & Smith 1992); Bahamas (Wiedenmayer 1977; van Soest & Sass 1981); Cuba (Alcolado 2002); Virgin Islands (Uliczka 1929; Rützler & Smith 1992); Jamaica (Rützler & Smith 1992; Lehnert & van Soest 1998); Barbados (Uliczka 1929; van Soest & Stentoft 1988); Curaçao (van Soest 1981); Belize (Rützler & Smith 1992); Panama (this study); Colombia (Rützler & Smith 1992; Valderrama 2001; Díaz 2007); Brazil (Rützler & Smith 1992).

Remarks and discussion. This is the first record of *C. kuekenthali* in Panama although it had already been studied at Bocas del Toro, as *Cinachyrella* sp. (Erwin & Thacker 2007). Most descriptions state that *C. kuekenthali* is characterized by a depression on top (Wiedenmayer 1977; Rützler & Smith 1992; Díaz 2007). We did not observe this, as in some Colombian specimens (Valderrama 2001). This character difference is not considered of great importance since variability of shape with respect to different environment conditions is well documented in the Tetillidae (McDonald *et al.* 2002; Meroz-Fine *et al.* 2005). We found it very difficult to distinguish the two categories of oxeas previously observed in *C. kuekenthali* (van Soest & Stentoft 1988; Rützler & Smith 1992) and therefore considered only one size in our measurements. The microxeas were always very numerous, so their absence in a record from Venezuela (Amaro & Liñero-Arana 2002) makes this identification doubtful.

There was a 42 bp. difference between the COI Folmer fragment of *C. apion* and *C. kuekenthali* from Bocas. Contrary to *C. apion*, there was high intra-specific genetic polymorphism of COI for *C. kuekenthali* in the Caribbean. Four COI sequences are now known from Florida, Belize and Panama; they group in 3 haplotypes (Table 2). *C. kuekenthali* from Bocas del Toro had the most diverging sequence with four to five base pair differences with specimens from Florida and Belize. Therefore, the Folmer partition of COI would possibly be a suitable mitochondrial marker for future population studies of *C. kuekenthali*.
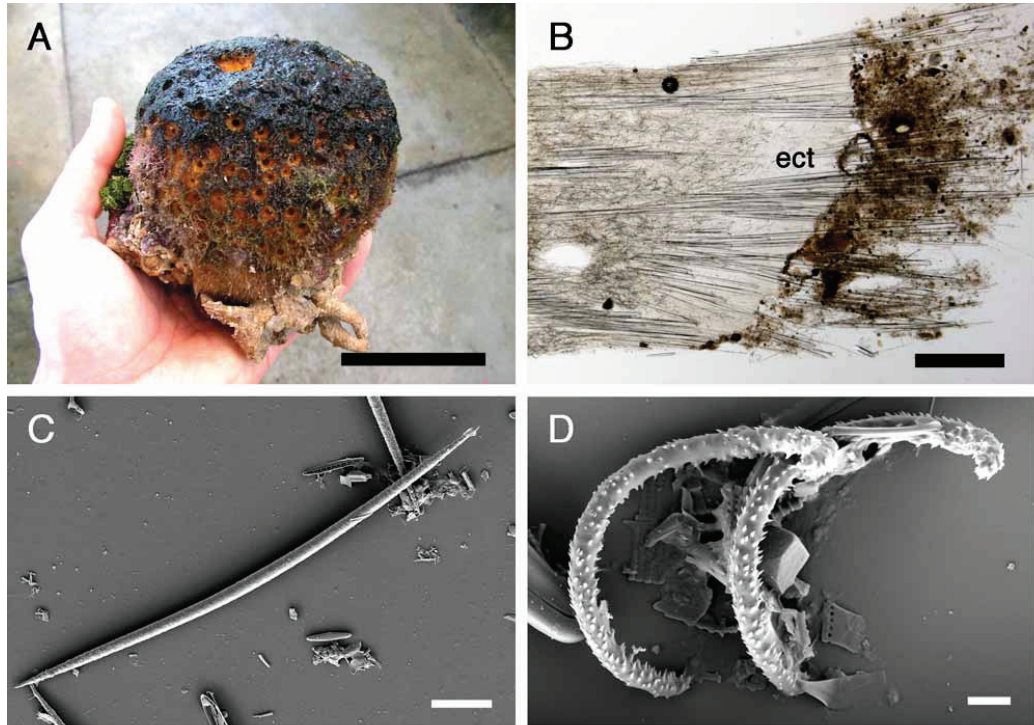
FIGURE 4. *Cinachyrella kuekenthali* (Uliczka, 1929) [ZMBN 81787]: A. Gross morphology. Scale: 5 cm; *C. kuekenthali* [ZMBN 81786]: B. Cross–section showing the skeletal architecture. Scale: 1 mm; C. Microxeas. Scale: 20 μm; D. Sigmaspire. Scale: 2 μm.

TABLE 2. Distribution of polymorphic sites in the COI sequences (Folmer fragment) for 4 specimens of *Cinachyrella kuekenthali*. Positions of mutations refer to the complete mitochondrial genome of *C. kuekenthali* (EU237479).

		Positions of mutations				
Genbank accession number	Locality	51	144	237	264	573
EU237479	Tennessee Reef, FL, USA	C	G	T	T	C
EF519603*	Delta Reef, Keys, FL, USA	?	G	G	T	C
EF519602*	Patch Reef, Belize	?	G	T	T	C
FJ711646*	Bocas del Toro, Panama	T	A	T	C	Y

* in Sponge Barcoding Database: www.spongebarcoding.org
? = missing data; Y = T/C.

C. *alloclada*, *C. apion* and *C. kuekenthali* are sympatric species in Bocas del Toro. Often covered by sediments and algae, they can be challenging to differentiate in the field without careful spicule observation. *C. apion* is usually small (ca 4 cm in diameter) and found near mangrove areas, in very shallow waters (0–1 m). *C. kuekenthali* is fairly large (ca 10–20 cm in diameter) and found deeper in the reefs (2–18 m). *C. alloclada* is ca 8–10 cm in diameter and also found in reefs (> 5 m).

SEM observations showed that all three species of *Cinachyrella* have similar spiny sigmaspire morphologies. The main difference concerned the size of these miscroscleres: the sigmaspires of *C. kuekenthali* were, on average, longer than in *C. apion* and *C. alloclada*.

Order: Astrophorida Sollas, 1888

Family: Ancorinidae Schmidt, 1870

Genus: *Ecionemia* Bowerbank, 1862

***Ecionemia megastylifera* Wintermann-Kilian & Kilian, 1984**
(Figures 5–6–7)

Synonyms.
Ecionemia megastylifera Wintermann-Kilian & Kilian, 1984: 122, figs. 1–2.
Ancorina megastylifera (Wintermann–Kilian & Kilian, 1984): Alcolado 2002: p. 56.
Stellettinopsis dominicana Pulitzer-Finali, 1986: 67, figs. 1–2 (new synonym).
Ecionemia dominicana (Pulitzer-Finali, 1986): Rützler et al. 2000: Table 1 (new synonym).

Holotype. INVPOR 1148, Bahia Nenguange, Colombia, 3 m depth.

   Material. 5 specimens, from STRI Point, Solarte Island and Bastimento Island, 0.4–20 m depth. Two specimens deposited: ZMBN 81782–81783.

   Additional material examined. Ecionemia megastylifera, INVPOR 1148, holotype, Bahia Nenguange, Colombia, 3 m; ZMAPOR 7772, Bahamas; ZMAPOR 14265, off Slangebaai, Curacao, 3.8 m. *Ecionemia dominicana* (Pulitzer-Finali, 1986), MSNG 47679, holotype, Boca Chica, Dominican Republic, 15–25 m. *Ecionemia demera* (de Laubenfels, 1934), MNHN DNBE-17, slide of holotype, Puerto Rico, 60–73 m; ZMAPOR 03512, Puerto Rico.

   Outer morphology (Fig. 5A–D). Thickly encrusting to massive lobate sponge. One very large specimen found on coral rubble in Solarte south was sub-circular, 8 cm high and with a diameter of 15 cm (Fig. 5B); it had cemented and integrated coral rubble pieces. Other specimens had an elongated shape and were 20 cm long (Fig. 5C). Surface is rugose. Slightly compressible. Choanosome has a hairy aspect due to the high density of large oxeas. External color on living specimen and in ethanol is light brownish to black. Internal color on living specimen is whitish to cream, whitish in ethanol. Oscules are 3–10 mm of diameter and distributed on the top surface of the sponge (Fig. 5A–C). There is a thin contractile membrane around the oscule. Oscules lead into one or more cloacae chambers where uniporal exhalant openings are present. Pores are cribriporal (Fig. 5D) and evenly distributed over large surfaces of the sponge, there is no separation between pore plates. A single pore plate has a diameter of around 0.5 mm. Macro-epibionts (e.g. sponges, algae) can be found growing on large specimens. Gammarid amphipods were found living in an oscule.

   Skeleton (Fig. 5E). Small acanthostrongylasters lay on a paratangential layer of acanthomicrorhabds, thus forming a very thin cortex (ca 140–170 µm thick) supported by bundles of long–shafted robust dichotriaenes. Thin microxeas, sometimes in bundles, cross the cortex. In certain parts where the sponge is hispid, large oxeas, dichotriaenes, anatriaenes and protriaenes also cross the cortex. Sub-cortical canals cross transversely this layer of triaenes. Under the triaenes, large oxeas paratangentially positioned form a dense layer (ca 1.2 mm thick). Deeper, this arrangement becomes looser and more irregular; oxeas II, some dichotriaenes and rare anatriaenes can be found there also. Acanthoxyasters are found dispersed in the choanosome, and are abundant around canals. Large granular cells (diameter: 16–27 µm) are especially abundant in the cortex but are also present, albeit in lower density, in the choanosome. The cortex of cloacae chambers is somewhat different. It has fewer acanthomicrorhabds and is not supported by triaenes; it is mainly tangential large oxeas that stretch and support the acanthostrongylaster layer. It is only crossed by a few microxeas.
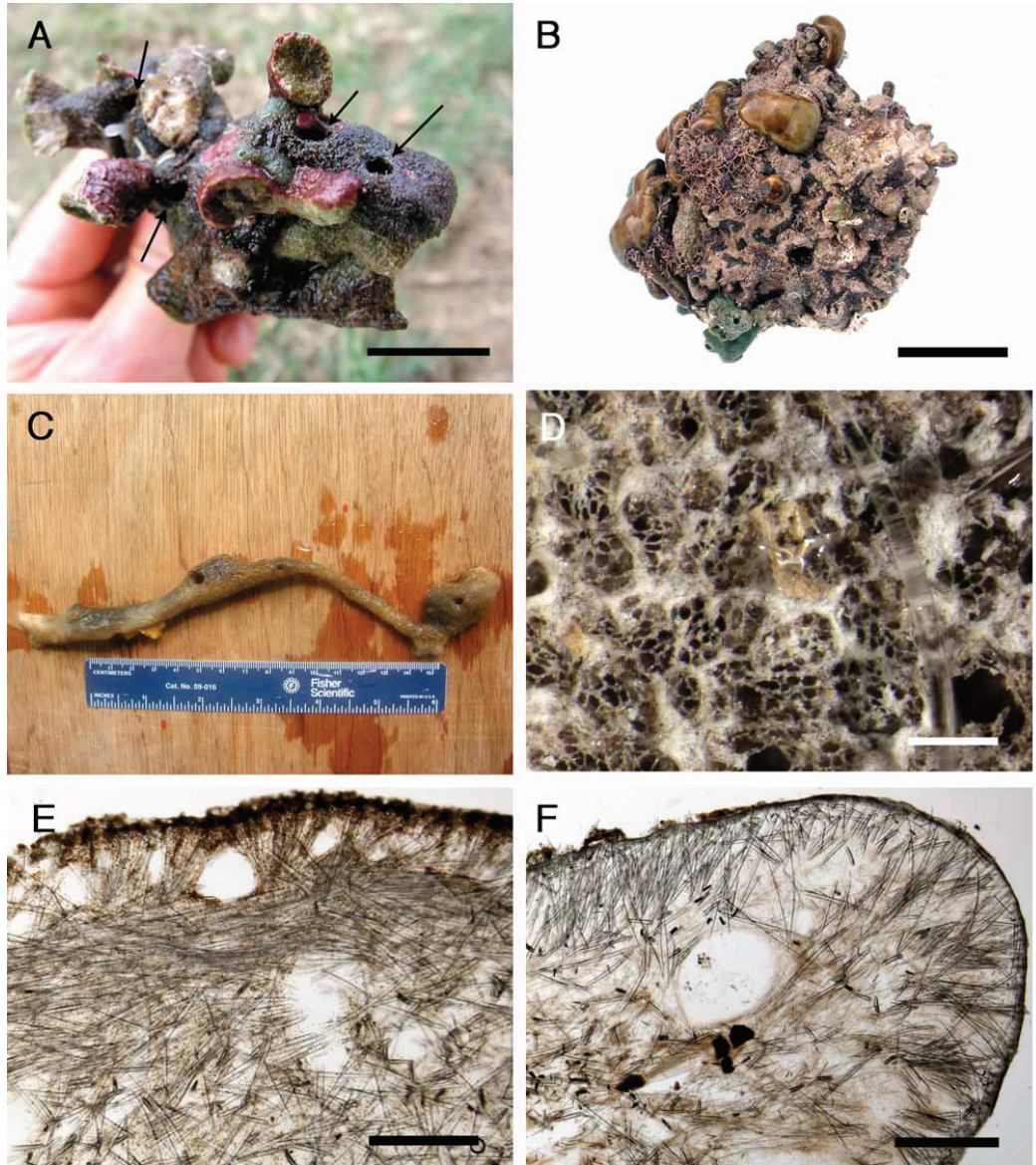
FIGURE 5. *Ecionemia megastylifera* Wintermann-Kilian & Kilian, 1984: A. Dark specimen found growing under coral rubble (1 m depth). Arrows point to its oscules. Scale: 4 cm; B. Large dark specimen found on Solarte South 4 (0.4 m depth) growing on coral rubble. It is covered with sediments and overgrown by sponges (*Chondrilla* aff. *nucula* and *Amphimedon erina*) and algae. Scale: 5 cm; C. Elongated coffee–colored specimen collected in a small cavity on Sache (15 m depth); D. Close up of the surface (dried) revealing the cribriporal pores. Scale: 0.5 mm; E. Cross–section showing the skeletal architecture: cortex and choanosome [ZMBN 81782]. Scale: 1 mm; F. Cross–section of cortex and choanosome [INVPOR 1148, holotype]. Scale: 1 mm.
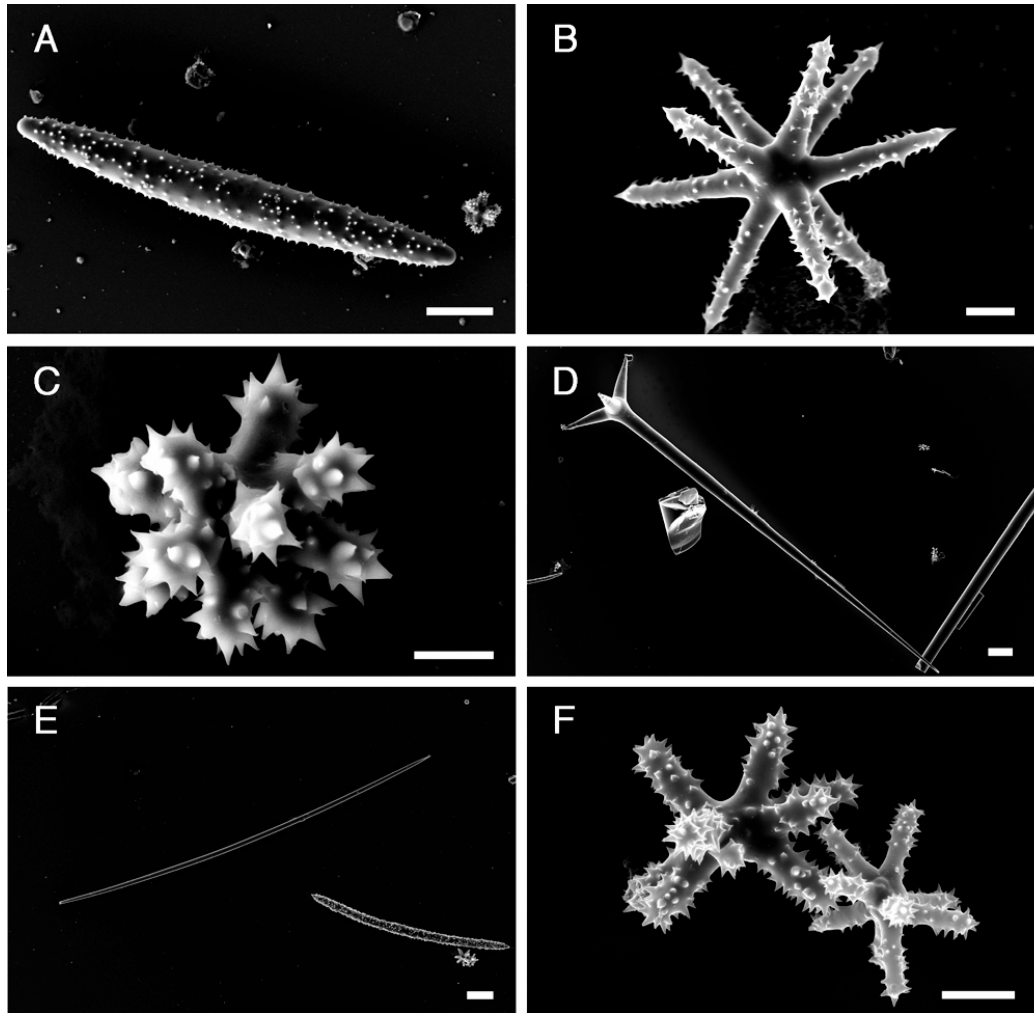
FIGURE 6. *Ecionemia megastylifera* Wintermann-Kilian & Kilian, 1984 [ZMBN 81782]: A. Microrhabd and acanthostrongylaster. Scale: 10 µm; B. Acanthoxyaster. Scale: 2 µm; C. Acanthostrongylaster. Scale: 2 µm; *E. megastylifera* [INVPOR 1148, holotype]: D. Young triaene. Scale: 20 µm; E. Microxea, microrhabd and acanthostrongylaster. Scale: 10 µm; F. Acanthostrongylasters. Scale: 2 µm.

Spicules (measurements of ZMBN 81782, except for protriaenes, only found in ZMBN 81783) (Figs. 6–7). *Megascleres*: (a) oxeas I, very large, slightly bent, sometimes modified to styles, length: 1332–*1524*–1764 µm; width: 56–*64.9*–70 µm. (b) oxeas II, straight or slightly bent, length: 520–*815.2*–1260 µm; width: 14–*22.9*–42 µm. (c) microxeas, usually straight but can be slightly bent, length: 182–*215.4*–317 µm; width: 2.5–*4*–5 µm. (d) dichotriaenes to orthotriaenes, stout, rare in some specimens, the rhabdome can be slightly bent, and in some rare cases extra spines are present, thus becoming acanthotriaenes, rhabdomes can have an oxeote or strongylote end, rhabdome length: 790–*997.6*–1222 µm; width: 50–*64.7*–80 µm; clad length: 120–*151.7*–180 µm (some young triaenes observed, rhabdome length: 400–*530*–651 µm; width: 18–*27*–41 µm; clad length: 53–*76.2*–104 µm). (e) anatriaenes, rare, rhabdome slightly inflated, rhabdome length: 228–*278.3*–334 µm (N=3); rhabdome width: 1–*1.3*–2 µm (N=6); clad length: 3–*4*–5 µm (N=6). (f) protriaenes, rare,

sometimes modified to mesoprotriaenes, rhabdome length: 417 µm (N=1); rhabdome width: 2.5–*3.75*–5 µm (N=2); clad length: 27–*32.5*–38 µm (N=2). *Microscleres*: (g) acanthomicrorhabds (Fig. 6A), with oxeote or strongylote ends, sometimes centrotylote, length: 59–*98.6*–138.3 µm; width: 4.5–*8.2*–10.6 µm. (h) acanthoxyasters (Fig. 6B), 6–9 actines, a characteristic spike at the end of each actine, diameter: 9–*13.1*–19 µm. (i) acanthostrongylasters (Fig. 6C), 4–11 actines, diameter: 4–*6.7*–10 µm.
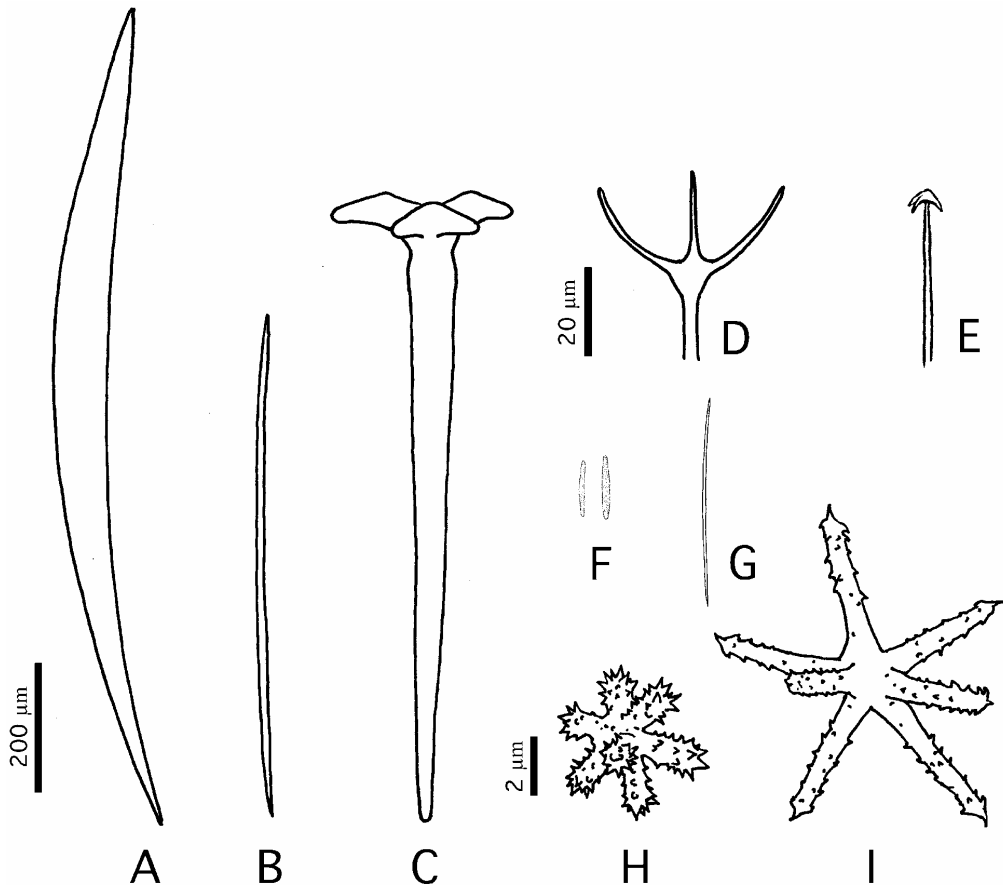

FIGURE 7. Spicule repertoire of *Ecionemia megastylifera* Wintermann-Kilian & Kilian, 1984. A. Oxea I; B. Oxea II; C. Dichotriaene; D. Cladome of protriaene; E. Cladome of anatriaene; F. Acanthomicrorhabds; G. Microxeas; H. Acanthotylaster; I. Acanthoxyaster. Scale 200 µm for A–B–C and F–G. Scale 20 µm for D–E. Scale 2 µm for H–I.

Habitat in the Bocas del Toro region. Common in coral rubble or in sheltered areas of reefs (such as small cavities). Usually sciophilous 1–25 m depth.

Distribution. Bahamas (this study); Cuba (Alcolado 2002); Dominican Republic (Pulitzer-Finali 1986); Belize (Rützler *et al.* 2000); Costa Rica (Cortés 1996); Panama (this study); Colombia (Wintermann-Kilian & Kilian 1984; Valderrama 2001; Díaz 2007); Curaçao (this study).

Remarks and discussion. This species possesses true spiny microrhabds (Fig. 6A) (not sanidasters) so it does not belong to the *Ancorina* genus. The original description by Wintermann–Kilian and Kilian (1984) mentions only one size class of euasters, but after reexamination of the holotype it is quite obvious that there are two kinds of euasters: the small strongylasters placed in the ectocortex and the larger oxyasters in the choanosome. Wintermann–Kilian and Kilian (1984) note the presence of microstrongyles, but these are

simply variations of microrhabds. They also overlooked the presence of microxeas present in the holotype (Fig. 6E). Furthermore, we observed in the Bocas material and the holotype two size classes of oxeas that had not been identified before. There were some differences between the Bocas del Toro specimens and the holotype. The holotype had smaller oxeas, smaller triaenes (Fig. 6D), thinner and smaller microrhabds (Fig. 6E) (length: 48–*69.2*–92, width: 2–*3.1*–5), smaller microxeas, asters with thinner actines (Fig. 6F), and more oxeas II (most of them found under the oxea I layer) (Fig. 5F). Anatriaenes and protriaenes observed in the Bocas del Toro specimens were not found in the holotype. The holotype of *E. dominicana* had also two size classes of euasters and the microxeas, both of which had been overlooked by Pulitzer-Finali (1986). The microrhabds' measurements in the *E. dominicana* holotype were very similar to those found in the holotype of *E. megastylifera*. As a result of these observations, *E. dominicana* becomes a junior synonym of *E. megastylifera*. Lehnert (1993) also described a *Stelletinopsis* sp. in Cozumel (Mexico) which resembles *E. megastylifera*. However, the vase shape, the blue and yellow colors and the absence of triaenes do not match with our species. The other Caribbean species of *Ecionemia*, *E. demera* from Puerto Rico differs from *E. megastylifera* in having plagiotriaenes, much larger anatriaenes, smaller acanthomicrorhabds (ca 7 μm) and only one kind of euasters. The dark color of *E. megastylifera* is due to large pigmented cells, more or less present with respect to light conditions. These pigments may serve for light protection since their abundance is greater in light environments; this has been suggested in other sponges (Renouf 1934; Bandaranayake *et al.* 1996; Cavalcanti *et al.* 2007; Valderrama *et al.* 2009). This is the species collected in Bocas del Toro by Nichols (2005) and identified as *Ecionemia* sp. We sequenced three of our specimens and obtained strictly identical COI sequences as Nichols (2005).

Genus: *Stelletta* Schmidt, 1862

***Stelletta fibrosa* (Schmidt, 1870)**
(Figures 8–9)

Synonyms.
Ancorina fibrosa Schmidt, 1870: 67.
Pilochrota fibrosa (Schmidt, 1870): Sollas 1888: 180.
Myriastra fibrosa (Schmidt, 1870): de Laubenfels 1936: 169.
?*Pilochrota fibrosa* var. *globuliformis* Wilson, 1902: 385.
?*Stelletta fibrosa* var. *globuliformis* (Wilson, 1902): Díaz 2007: 21.
?*Stelletta incrustata* Uliczka, 1929: 47, figs. 31–35, pl. I, fig. 7.
?*Stelletta variastra* Pulitzer-Finali, 1986: 70, figs. 4–5.

Lectotype. MCZ 8020, Tortugas, Florida, dry, Schmidt material (here designated).
    Material. ZMBN 81784, Caracol Reef, 15 m depth.
    Additional material examined. Stelletta fibrosa, MCZ 8020, holotype, Tortugas, Florida, dry; ZMAPOR 04585a, Blauwbaai, Curaçao, 10 m; ZMAPOR 12608, Santa Marta, Colombia, 5 m. *Stelletta* cf. *anancora* Sollas, 1886, INVPOR 1061, La Guajira, Colombia, 50 m.
    Outer morphology (Fig. 8A–C). Massive, sub–globular sponge, 9 cm of diameter. Color alive is whitish with dark–purple tiny spots mainly concentrated around the oscules. Bottom of the sponge is pure white (Fig. 8C). Choanosome color alive is cream. In ethanol, cortex and choanosome are whitish. Slightly compressible. Rugose surface. The cortex is thick (3 mm) and lighter than the choanosome. One large oscule (diameter: 1.5 cm) is placed at the top with a brown contractile membrane with whitish spots on it; the oscule leads into a cloaca 5 cm deep where uniporal excurrent canal openings meet (Fig. 8A–B). Smaller oscules (1–5 mm) are generally surrounded by an elevated thick margin, they are unevenly distributed on the sides; the underlying cloacae are around 2 cm deep (Fig. 8A–B). Pores are covered with cribriporal plates (ca 1 mm) and are evenly distributed. A few short root–like structures (ca 1 cm) are present at the base of the sponge (Fig. 8C). The

green sponge *Amphimedon erina* (de Laubenfels) is growing on it. Ophiuroids were found living in the cloacae of the small oscules.

Skeleton (Fig. 8D). The cortex is composed of radially and densely arranged plagiotriaenes. Subcortical cavities are conspicuous and visible to the naked eye. Plagiotriaenes and oxeas under the cortex are more or less radially arranged; they are not as densely packed as in the cortex. Acanthotylasters are common and present throughout the cortex and choanosome.

Spicules (Fig. 9A–D). *Megascleres*: (a) oxeas (Fig. 9A), large, very slightly bent or straight, length: 660–*1151.4*–1536 µm; width: 7–*30.1*–57 µm. (b) plagiotriaenes (Fig. 9A), stout, horn–shaped clads, rhabdome length: 511–*1032.9*–1280 µm; rhabdome width: 7–*33.3*–50 µm; clad length: 14–*61.7*–98 µm. (c) anatriaenes (Fig. 9B), rare, with depressed apex, rhabdome length: ? (rhabdomes broken); rhabdome width: 31–*32.5*–34 µm (N=2); clad length: 45–*49.5*–54 µm (N=2). *Microscleres*: (d) acanthotylasters (Fig. 9C–D), 4–8 actines, diameter: 7–*11*–16 µm.

TABLE 3. Shape, depth of collection, size, cortex thickness, and spicules measurements (in µm) of *Stelletta fibrosa* specimens from Bocas del Toro and previous records. Measurements of *Stelletta incrustata*, a possible junior synonym, are also included. In bold are the museum specimens measured for this study. Means are in bold italics; other values are ranges; N=30 unless stated otherwise between parentheses. – = not refered; n.f. = not found; n.o. = not observed. ? = missing data.

Specimen	Shape	Depth (m)	Size (cm)	Cortex (mm)	Plagiotriaene (rhabdome length x width / clad length)	Anatriaene (rhabdome length x width / clad length)	Oxea (Length / width)	Tylasters (diameter)
MCZ 8020 Florida, lectotype	Thick incrustation	217	0.5–1 x 1.9 (fragment)	1.3, with sediments	846–*1065*–1224 x 9–*22.6*–28 / 11–*33.4*–54	n.f.	465–*937.2*–1248 / 4.5–*15.5*–30	7–*9.9*–13
ZMAPOR 12608 Colombia	n.o.	5	n.o.	2, with sediments	648–*1114.4*–1368 x 7–*22.6*–30 / 7–*41.8*–66	818–*988.3*–1248 (N=11) x 10–*17.5*–23 / 11–*26.8*–43	641–*1108.2*–1368 / 4–*21.6*–39	9–*11.1*–13
ZMAPOR 04585a Curaçao	n.o.	10	n.o.	1.7, few sediments	558–*901.8*–1104 x 4.5–*13.2*–23 / 4.5–*17.6*–38	? x 25 / 32 (N=1)	465–*765.9*–1032 / 3–*12.7*–27	9–*10.5*–16
Puerto-Rico (Wilson, 1902)	Spheroidal, massive	40	3.5	–, shell fragments	1000 x 24 / 40	1450 x 16 / 25 (rare)	1420 / 27	12
Puerto-Rico (Pulitzer–Finali, 1986)	Irregularly massive	0.5–1	6 x 4 x 2.5	–	1100–1300 x 13–29 / 43–58	1170–1290 x 12–23 / 19 (rare)	1170–1500 / 23–28	10–15
ZMBN 81784 Panama	Spheroidal, massive	15	9	3	511–*1032.9*–1280 x 7–*33.3*–50 / 14–*61.7*–98.	? x 31–*32.5*–34 / 45–*49.5*–54 (N=2)	660–*1151.4*–1536 / 7–*30.1*–57	7–*11*–16
S. incrustata St. Thomas, holotype (Uliczka, 1929)	Spheroidal, massive	–	7–8	–, with shells, corals	970–1300 x 20–27 / 25–50	n.f.	990–1250 / 17.5–28	5–10

Habitat in the Bocas del Toro region. Reefs, rare, 15 m depth.

Distribution. As *S. fibrosa*: Florida, U.S.A. (Schmidt 1870); Curaçao (van Soest 1981); Panama (this study); Colombia (this study). As *S. fibrosa* var. *globuliformis*: Puerto–Rico (Wilson 1902).

Remarks and discussion. A thorough revision of the Caribbean *Stelletta* species is greatly needed but beyond the scope of this study. In the Caribbean, four species of *Stelletta* share tylasters: *S. fibrosa* (Schmidt,

1870), *S. variabilis* (Wilson, 1902), *S. kallitetilla* (de Laubenfels, 1936b) and *S. pudica* (Wiedenmayer, 1977). These four species are poorly known.
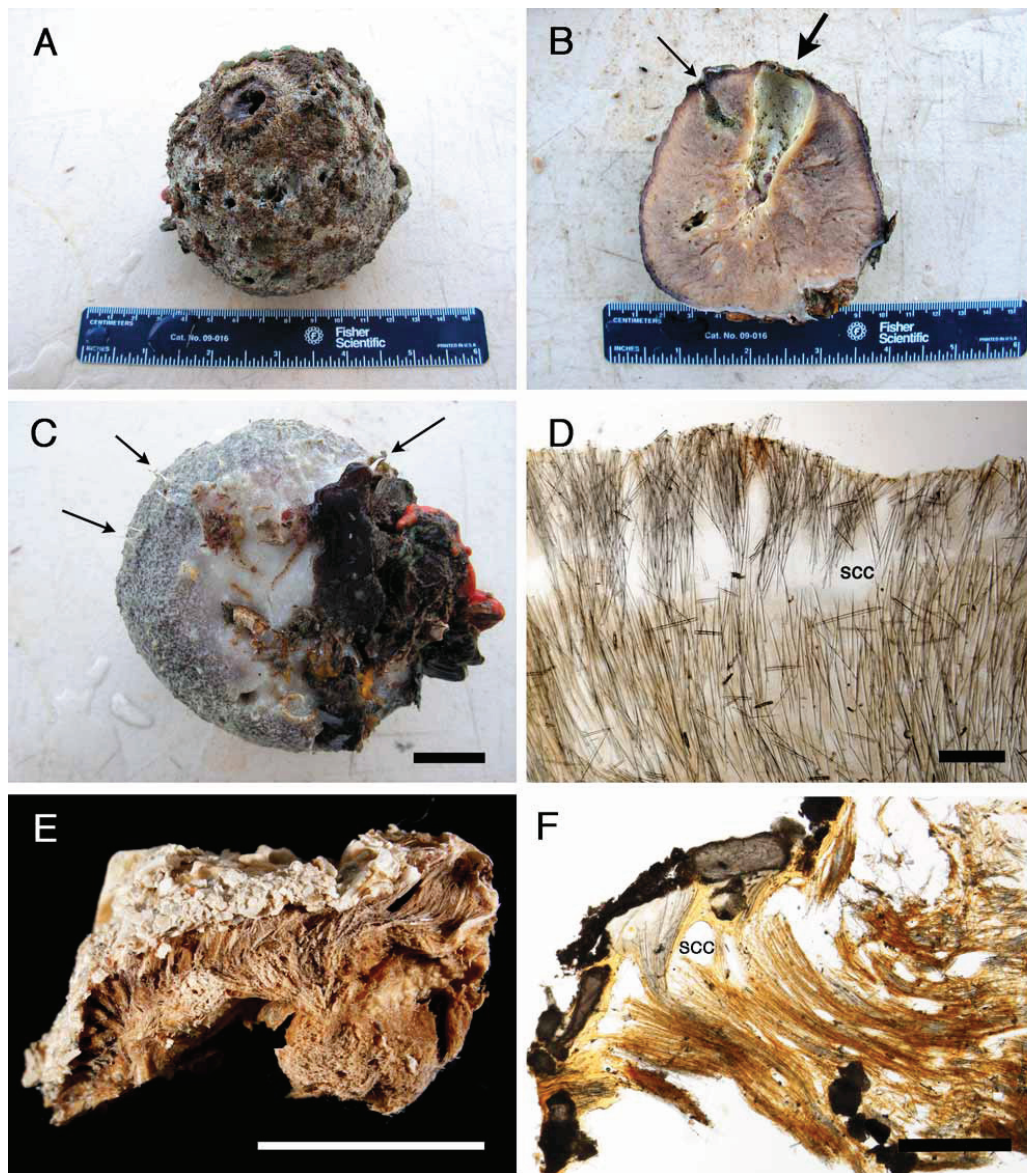

FIGURE 8. *Stelletta fibrosa* (Schmidt, 1870) [ZMBN 81784]: A. Specimen with large oscule on top and smaller oscules on the sides (picture taken shortly after collection); B. Cross–section of specimen showing large and small oscules and their cloacae (large and small arrow). Cortex is clearly visible; C. Base of specimen: color is lighter and small roots are present (arrows). Scale: 2 cm; D. Cross–section showing the skeletal architecture: cortex and choanosome. scc: sub–cortical canal. Scale: 1 mm; *S. fibrosa* [MCZ 8020, lectotype]: E. Lectotype specimen. Scale: 1 cm; F. Cross–section of cortex and the choanosome. Scale: 1 mm.
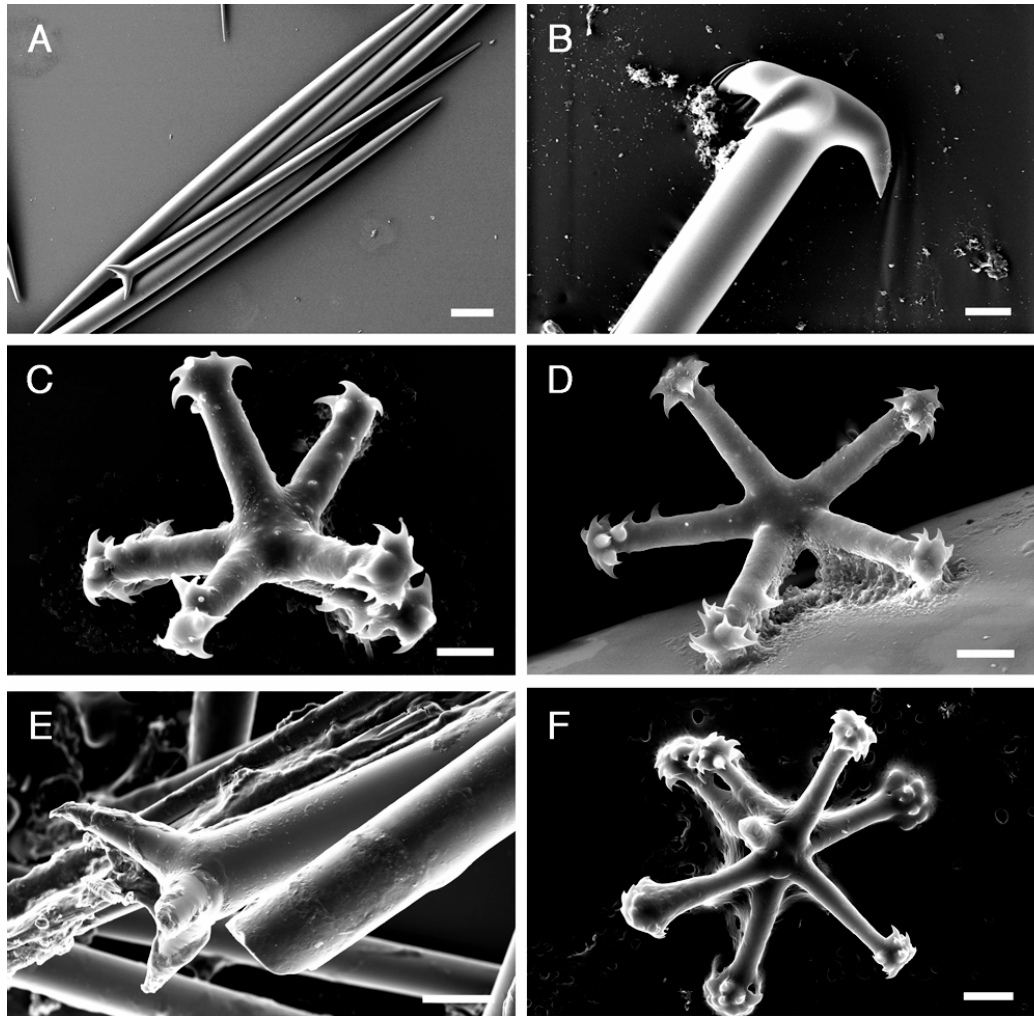
FIGURE 9. *Stelletta fibrosa* (Schmidt, 1870) [ZMBN 81784]: A. Plagiotriaene and oxeas. Scale: 100 µm; B. Anatriaene. Scale: 20 µm; C–D. Tylaster. Scale: 2 µm; *S. fibrosa* [MCZ 8020, lectotype]: E. Plagiotriaene. Scale: 20 µm; F. Tylaster. Scale: 2 µm.

We have had difficulty to assign a species name to this specimen, never observed before in Panama. The color alive of our specimen (whitish with purple spots) corresponded to that of *S. fibrosa* from Puerto–Rico (Pulitzer-Finali 1986). This species is unfortunately very poorly described by Schmidt (1870) and the type is not formally identified. However, the original label "*Ancorina fibrosa* n. n." of a dry specimen (MCZ 8020) from the type locality (Tortugas, Florida) strongly suggests that it is type material from the Schmidt collection. This small fragment 1.9 cm long (Fig. 8E) is here designated as the lectotype of *Stelletta fibrosa*. The shape of the spicules and the skeleton organization of the Bocas del Toro specimen fitted with the lectotype (Figs. 8F, 9E-F) and comparative material. A few differences between the lectotype and the Bocas specimen were nonetheless observed: the Bocas specimen had (i) several anatriaenes, (ii) no wavy to strongly bent oxeas and rhabdomes (such as those found in the lectotype), (iii) larger width of triaenes and oxeas and (iv) longer clad lengths. The triaenes and oxeas of the Bocas specimen were also bigger than in our comparative material and

any previous descriptions of *S. fibrosa* (Table 3). Although anatriaenes were absent in the lectotype, they have been found in low numbers in previous records (Wilson 1902; Pulitzer-Finali 1986) and also in our comparative material. Anatriaenes are therefore fairly common in this species. As for the strongly bent oxeas found in the lectotype, we believe they are not diagnostic since these were absent in the comparative material. Tylasters of our specimen had actines with few spines (Fig. 9C–D), whereas these were completely smooth in the lectotype (Fig. 9F). To conclude, the size of the megascleres remained the main difference between our material and previous records; it might be explained by the size of our specimen, the largest of this species described so far. The lectotype and ZMAPOR 12608 both had abundant sand grains incorporated in the ectocortex (Fig. 8E), while ZMAPOR 04585a had few and our specimen none (Fig. 8D).

Examination of INVPOR 1061 from Colombia, identified as *S. fibrosa* var. *globuliformis* (Díaz, 2007), showed that it had different sizes of triaenes, the largest size having significantly longer and thinner clads than our *S. fibrosa* specimens. With respect to its triaenes and its external morphology (massive spherical, single osculum on top, surface with numerous foreign material), it appeared to us similar to *S. anancora*. But a revison of this species is required to confirm this. For the moment, we agree with Pulitzer-Finali (1986) that *S. fibrosa* and *S. fibrosa* var. *globuliformis* are the same species: their spicule measurements match. This species could therefore have an encrusting or a massive spherical shape. *S. variabilis* (Wilson, 1902) appears to be a valid species, different from *S. fibrosa*: orthotriaenes have long clads, there are smaller oxeas II in cortex, and common anatriaenes. Following this, *S. fibrosa* identified by Wells (1960) is rather *S. variabilis* (as noted before us by Pulitzer-Finali (1986)). As for *S. pudica*, it has no or only few regressed plagiotriaenes (Wiedenmayer 1977; Pulitzer-Finali 1986).

Stelletta sp.
(Figure 10)

Material. ZMBN 81643, Solarte lagoon (9° 18.35 N, 82° 10.39 W), on mangrove root, 1 m depth.

Additional material examined. Stelletta soteropolitana Cosme & Peixinho, 2007, UFBA-POR 500, holotype, Salvador, Bahia State, Brazil, <10 m.

Outer morphology (Fig. 10A–B). Massive, sub–globular sponge, 10 cm high, with a diameter of 9 cm. Surface color alive is dark–purple to whitish. Choanosome color is cream In ethanol, cortex and choanosome are grayish. One large oscule (diameter: 2 cm) with a contractile membrane is on the top surface. Under the oscule is a large cloaca (6 cm deep) with cribriporal exhalant apertures. Unconspicuous cribriporal pores are evenly distributed, each pore is 100–1000 μm large. Slightly–compressible, rugose surface with irregular ridges, grooves and knobs (2–7 mm high). Mussels and oysters are embedded in it. A second specimen was observed (but not collected): it was about twice the size of ZMBN 81643, with two oscules.

Skeleton (Fig. 10C–D). Dense radial bundles of plagiotriaenes and anatriaenes, especially near the surface. Their cladomes are often found at or beyond the surface of the sponge. There is a slightly differentiated cortex, not visible with the naked eye, except for the pigmentation. Bundles of megascleres are looser under the cortex and triaenes less abundant. Acanthotylasters, oxeas I and II are abundant throughout the sponge.

Spicules (Fig. 10E–G). *Megascleres*: (a) oxeas I, large, slightly bent or straight, length: 1080–*1236.3*–1440 μm; width: 18–*24.4*–32 μm. (b) oxeas II (Fig. 10E), smaller, with very thinly pointed ends, almost whip–like, generally with a bend or a double bend, length: 303–*724.7*–1000 μm; width: 3–*9.5*–14 μm. (b) plagiotriaenes (Fig. 10F), with rare dichotriaenes, rhabdome can have a straight pointed or whip–like end, rhabdome length: 511–*850.9*–1056; rhabdome width: 13–*31.5*–39 μm; clad length: 50–*121.3*–152 μm. (c) anatriaenes (Fig. 10G), common but less frequent than plagiotriaenes, and rare dichoanatriaenes, with depressed apex, rhabdome length: 716–*1363.2*–2280 μm; rhabdome width: 8–*21.7*–29 μm; clad length: 38–*65.1*–91 μm. *Microscleres*: (d) acanthotylasters (Fig. 10H), 5–8 actines with scarce spines, actine is only slightly tylote, one single spine at the tip of each actine, diameter: 9–*13.5*–20 μm.
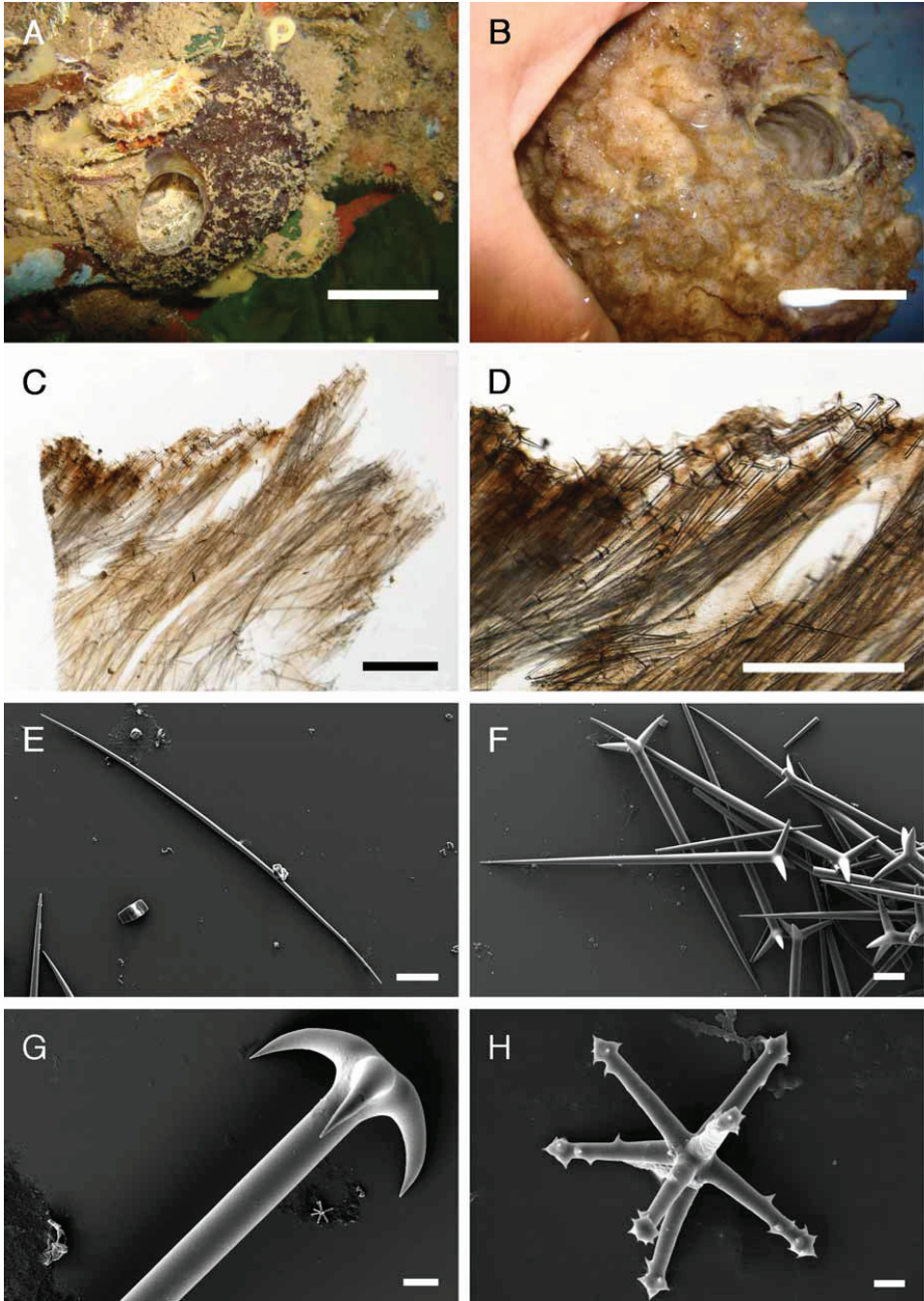
FIGURE 10. *Stelletta* sp. [ZMBN 81643]: A. Specimen *in situ* on mangrove root, August 2007. Notice large oscule leading to a cloaca. Scale: 2 cm; B. Bleached specimen maintained alive in a tank, July 2009. Scale: 2 cm; C. Cross–section showing the skeletal architecture. Scale: 1 mm; D. Close-up of cross-section showing bundles of triaenes at the surface of the sponge. Scale: 1 mm; E. Oxea II. Scale: 100 μm; F. Plagiotriaenes. Scale: 100 μm; G. Anatriaene and tylaster. Scale: 20 μm; H. Tylaster. Scale: 4 μm.

Habitat in the Bocas del Toro region. Mangrove roots, rare, 1 m depth.

Distribution. Panama (this study).

Remarks and discussion. This species appears to be rare in Bocas del Toro. We only sighted two specimens during our survey in 2007, both a few meters apart in Solarte lagoon. At this time, we only took a small piece from ZMBN 81643. In July 2009, both specimens were still there, but were slightly bigger and bleached (Fig. 10B). The bleaching could be due to shading from the algae growing around it, because when we finally collected ZMBN 81643 and kept it alive alone in an open tank, it started to gain back some purple colouration.

At present there are four known species of *Stelletta* with tylasters in the Caribbean (*S. fibrosa*, *S. variabilis*, *S. kallitetilla* and *S. pudica*) and three in Brazil (*S. anancora*, *S. beae* Hajdu & Carvalho, 2003 and *S. soteropolitana*). Of these, only the three Brazilian species share long clads (average of clad length > 100 μm) with our Bocas del Toro specimen. However, when compared with *Stelletta* sp., *S. anancora* has (i) only one category of oxeas, (ii) 2–3 triaene sizes, (iii) no anatriaenes and (iv) no dense accumulation of megascleres in its cortex; *S. beae* has (i) shorter plagiotriaenes (rhabdome length ca 490 μm) with longer clads (ca 200 μm), (ii) shorter anatriaenes with reduced clads (ca 23 μm), (iii) one size of oxea, and (iv) smaller acanthotylasters (ca 11 μm). When it comes to *S. soteropolitana* it has i) plagiotriaenes twice the size of those in *Stelletta* sp., ii) protriaenes and iii) no anatriaenes. As we have already shown for *S. fibrosa*, anatriaenes can be present or absent in the same species, and the missing anatriaenes in *S. soteropolitana*, at the present time, cannot be used to distinguish the species from our *S.* sp. The protriaenes found in *S. soteropolitana* could simply be plagiotriaenes that are more forward oriented. Also, it is important to stress that *S. soteropolitana* is described from a single specimen and therefore, we have no data on its intra-specific morphological variation, especially concerning spicule sizes. To conclude, we cannot exclude *S. soteropolitana* as being conspecific with our specimen but in our opinion more data is required to settle this matter.

Genus: *Stryphnus* Sollas, 1886

***Stryphnus raratriaenus* sp. nov.**
(Figures 11–12)

Material. ZMBN 81642, holotype, STRI Point (9°21'05''N, 82°15'34''W), Isla Colón, under coral rubble, 1 m depth. Fixed in ethanol 96%. Collected by M. C. Díaz, 18th August 2007, specimen has been cut in pieces for transport.

Additional material examined. Stryphnus ponderosus (Bowerbank, 1866), MC 3395, Rathlin Island, Northern Ireland. *Stryphnus fortis* (Vosmaer, 1885), ZMBN 82977, Korsfjord, Bergen region, Norway, 200–400 m. *Stryphnus mucronatus* (Schmidt, 1868), MNHN Coll. Chombard, Mediterranean Sea, La Ciotat, France. *Asteropus niger* Hajdu & van Soest, 1992, ZMAPOR 14182, Curaçao, 39.5 m.

Outer morphology (Fig. 11A). Thickly encrusting sponge, 4 x 3 cm. Color alive is dark–brown with a whitish choanosome. In alcohol, these colors are preserved. No visible openings. Consistency is firm, choanosome is dense. Surface is rugose and flat.

Skeleton (Fig. 11B–C). Sanidasters and oxyasters are abundant in the thin cortex. Underneath, abundant paratangantial large oxeas fill the choanosome. Sanidasters and oxyasters are also abundant in the choanosome. A total of three dichotriaenes were found, two were observed in the thick sections, both perpendicular to the external layer, one with its cladome at the surface of the sponge, the other in the choanosome (Fig. 11C). Large brown granular cells (diameter: 14–23 μm) are observed mainly in the cortex but are also present in lower density in the choanosome.

Spicules (Figs. 11D–F, 12). *Megascleres*: (a) oxeas I (Fig. 11B), stout, straight or slightly bent, length: 1742–*1971*–2210 μm; width: 45–*62*–80 μm. (b) oxeas II, straight or slightly bent, length: 310–*448*–560 μm; width: 15–*22.2*–30 μm. (c) styles, length: 590–*603*–620 μm; width: 17.3–*18*–18.6 μm. (c) dichotriaenes, very

rare, rhabdome with a strongyle end, deuteroclads can be further subdivided, rhabdome length: 447–620 µm (N=2); rhabdome width: 33–44 µm (N=2); protoclad length: 67–113 µm (N=2); deuteroclad length: 77–87 µm (N=2). *Microscleres*: (d) sanidasters (Fig. 11D), spiny, length: 16–*20.2*–23.9 µm; width (axis and actines included): 2.1–*2.9*–4 µm. (h) oxyasters (Fig. 11E–F), 5–8 actines, faintly spiny, diameter: 29.3–*33.8*–37.2 µm.

Habitat in the Bocas del Toro region. Under coral ruble, 1 m depth.

Distribution. Panama (this study).
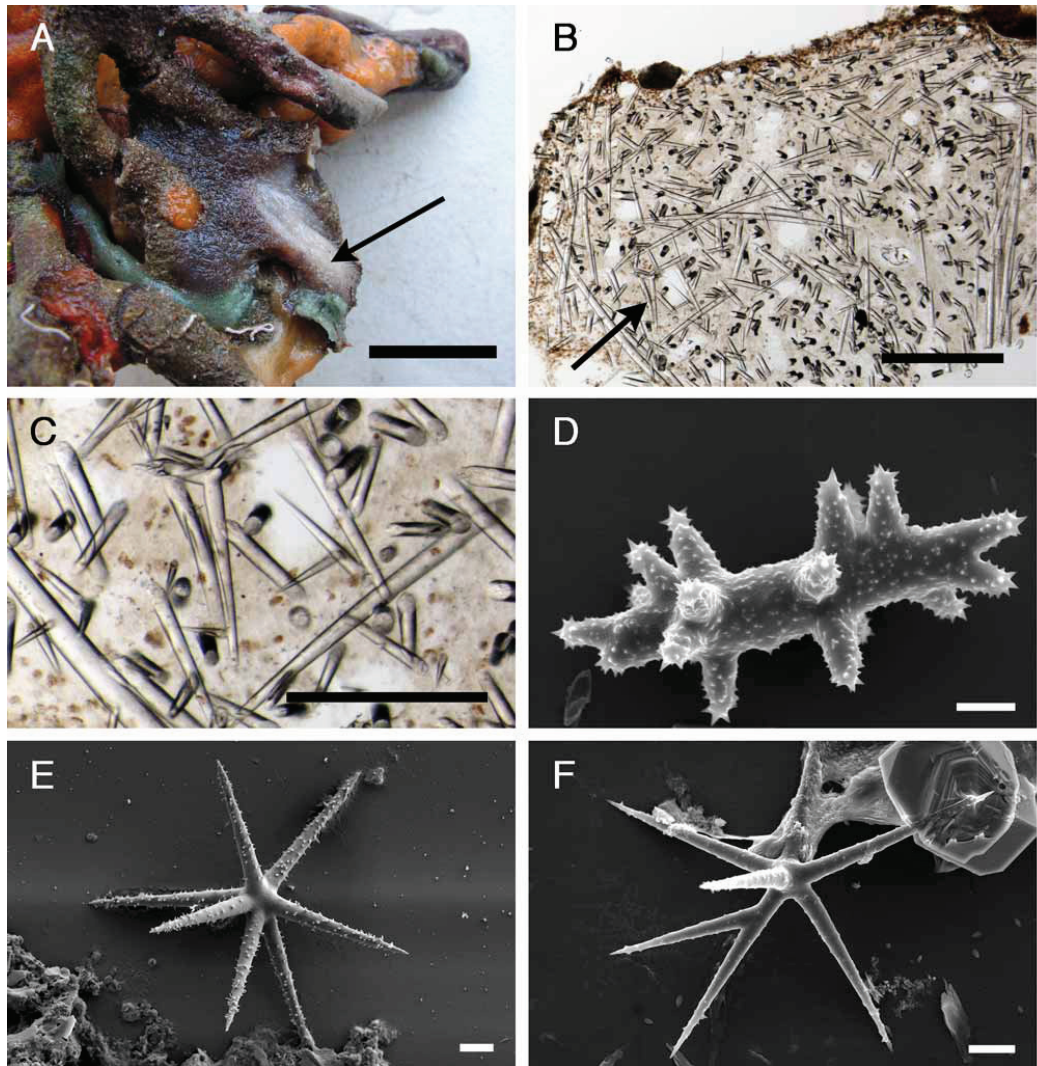
FIGURE 11. *Stryphnus raratriaenus* sp. nov. [ZMBN 81642]: A. Holotype, alive, growing on coral rubble. A tangential cut in the specimen shows the whitish choanosome (arrow). Scale: 2 cm; B. Cross–section showing the skeletal architecture: cortex and choanosome. Arrow points to a dichotriaene. Scale: 1 mm; C. Detail of cross section (B) showing the dichotriaene. Note the large granular brown cells. Scale: 500 µm; D. Sanidaster. Scale: 2 µm; E. Oxyaster. Scale: 4 µm; F. Oxyaster with a dichotomous actine. Scale: 4 µm.
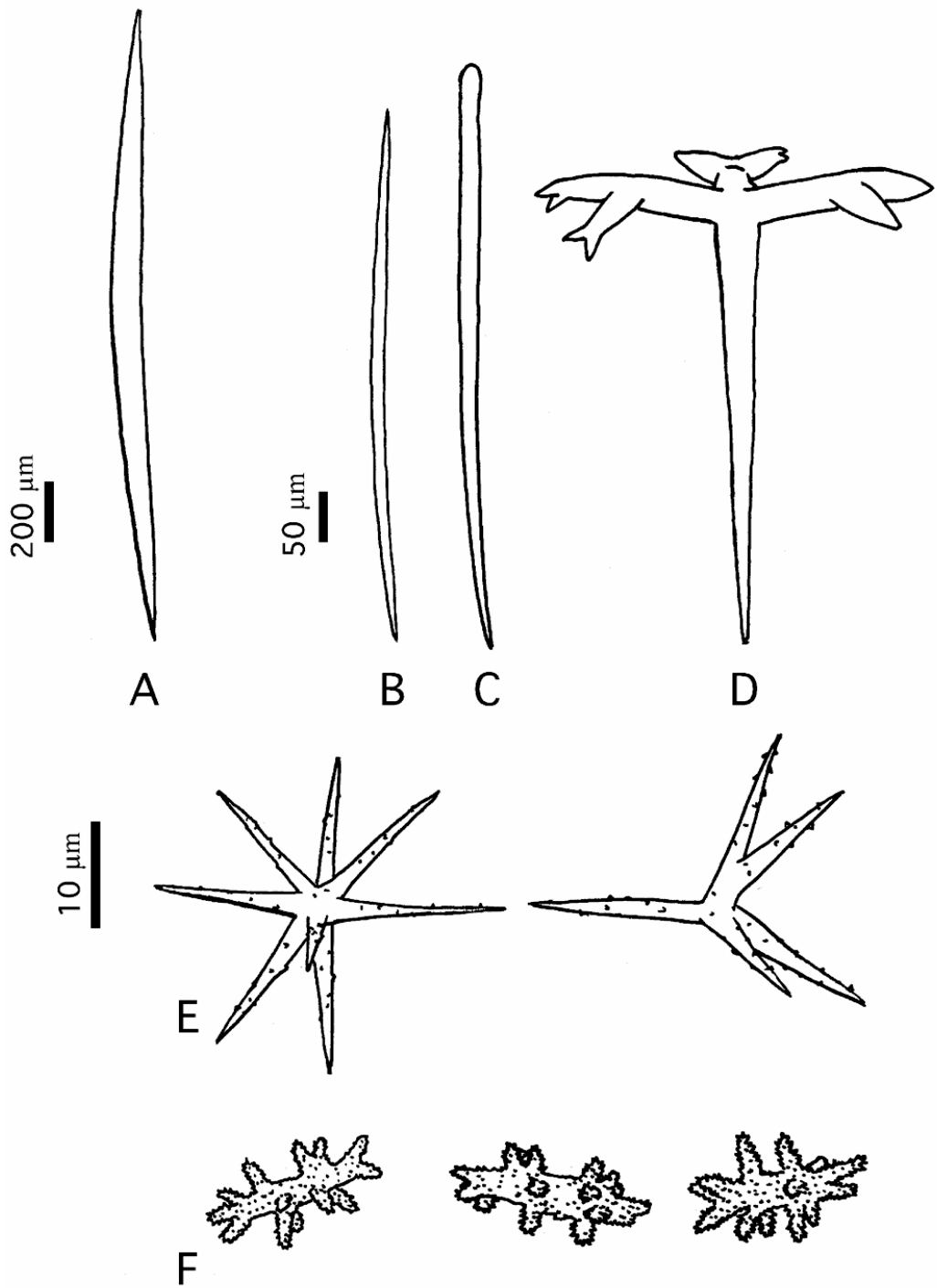
FIGURE 12. Spicule repertoire of *Stryphnus raratriaenus* sp. nov [ZMBN 81642]: A. Oxea I; B. Oxea II; C. Style; D. Dichotriaene; E. Oxyasters; F. Sanidasters. Scale 200 μm for A. Scale 50 μm for B–C–D. Scale 10 μm for E–F.

Remarks and discussion. This species appears to live in cryptic habitats and is therefore difficult to encounter. Its color is due to large granular cells. This species illustrates once again the close relationship of the genera *Asteropus* Sollas, 1888 and *Stryphnus* (Sollas 1888; van Soest & Stentoft 1988; Hajdu & van Soest 1992; Uriz 2002a; Carvalho 2008). Indeed, if it were not for the presence of triaenes (Fig. 11C), this species would have been assigned to *Asteropus*. In the Caribbean, *S. raratriaenus* sp. nov. could certainly be mistaken with the shallow–water *Asteropus brasiliensis* Hajdu & van Soest, 1992, but the later has one size class of oxea, rare oxyasters (13–42 μm) and no triaenes. Our species is even closer to *Asteropus ketostea* de Laubenfels, 1950 from Bermuda, but the latter has thinner oxeas, smaller oxyasters (17–27 μm) and no triaenes. As for *Asteropus niger*, it has a dark choanosome, smaller oxeas II, larger oxyasters (42–98 μm), trichodragmata and no triaenes. The differences between *Ancorina* and *Stryphnus* are also ambiguous. Sollas (1888, p. 171) and Uriz (2002a) state that *Stryphnus* species can have amphiasters (e.g. *Stryphnus fortis*) but in our opinion these are clearly just sanidasters with few actines on the main shaft. Sollas (1888, p. 171) and Uriz (2002a) also state that *Stryphnus* species have large oxeas (2–3 mm long, ca. 40–60 μm) but *Ancorina* species present the same range of oxea sizes, as well as *Asteropus* species. Both *Stryphnus* and *Ancorina* genera have similar spicule repertoires (large oxeas, triaenes, oxyasters and sanidasters) except for the presence of anatriaenes in *Ancorina*. We also noticed that *Stryphnus* generally possesses dichotriaenes, whereas these are rare in *Ancorina*. Furthermore, according to Sollas (1888, p. cxxxvii), *Stryphnus* has a more irregular spicule arrangement and no fibrous cortex (the cortex can be densely filled with large oxeas and triaenes) whereas *Ancorina* has a radially arranged skeleton and a more conspicuous cortex (often partly fibrous). We acknowledge that the definitions of these genera are still not satisfying at the moment, but until their comprehensive revision is initiated, our new species is assigned to *Stryphnus* based on the absence of anatriaenes, presence of dichotriaenes, its irregular spicule arrangement and lack of thick cortex. It is furthermore quite different from the only described Caribbean *Ancorina* species, namely *Ancorina fenimorea* de Laubenfels, 1934. *A. fenimorea* is known from rather deep water and has much larger sanidasters (60 μm) than our *S. raratriaenus* (average of 20.2 μm). As for *Stryphnus* species, they are all quite different from *S. raratriaenus*. *Stryphnus fortis* (Vosmaer, 1885), *Stryphnus ponderosus* (Bowerbank, 1866), *Stryphnus mucronatus* (Schmidt, 1868), *Stryphnus unguiculus* Sollas, 1886 and *Stryphnus niger* Sollas, 1886 all have smooth oxyasters and abundant dichotriaenes. *Stryphnus progressus* (Lendenfeld, 1907) also has abundant dichotriaenes. *S. raratriaenus* sp. nov. is the seventh species of this genus described in the world and the first in the Caribbean. It should be noted that *S. raratriaenus* had numerous large dark granular cells, similar to those observed in our *S. mucronatus* and *S. ponderosus* specimens.

Despite substantial efforts, we were not able to obtain a COI sequence for this specimen. We strongly suspect that DNA contaminants block the PCR reaction. After extra cleaning of the DNA (precipitation, drying, extra washing with 70% ethanol), we nonetheless managed to obtain a partial 28S sequence.

Etymology. From the latin word 'rara' meaning 'rare' and the spicule name 'triaene'.

Family: Geodiidae Gray, 1867

Genus: *Erylus* Gray, 1867

***Erylus formosus* Sollas, 1886**
(Figure 13)

Synonyms.
Erylus formosus Sollas, 1886: 195; Sollas 1888: 209, pl. XXVIII.
Erylus bahamensis Pulitzer-Finali, 1986: 78, figs. 12–13 (new synonym).
?*Erylus clavatus* Pulitzer-Finali, 1986: 80, figs. 14–15.

Holotype. BMNH 1889.1.1.77, off Bahia, Brazil, 12–36 m (not seen).

Material. ZMBN 81644–81645, Adriana's Reef, Bocas del Toro, 3 m depth.

Additional material examined. Erylus bahamensis, ZMBN 82978, Little San Salvador Island, Bahamas, det. P. Cárdenas; ZMBN 82979, Sweetings Cay, Grand Bahama Island, Bahamas, det. P. Cárdenas. *Erylus formosus*, MNHN DNBE–997, off Brazil, 7°29'S, 34°30' W, 45 m, Calypso expedition; ZMBN 81781, Cat Cay, Bimini Island, Bahamas, det. P. Cárdenas.
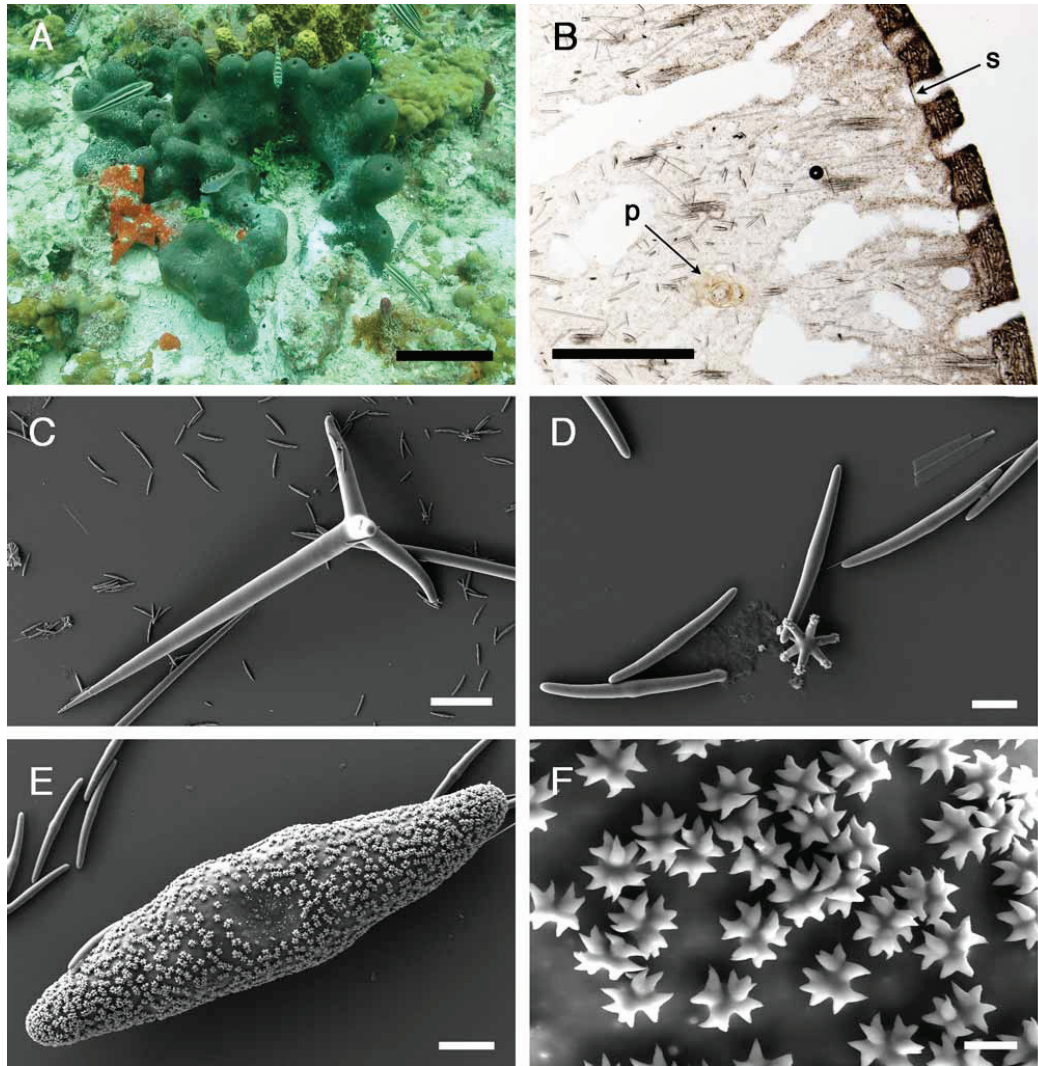

FIGURE 13. *Erylus formosus* Sollas, 1886: A. *In situ,* Adriana's reef (Picture: Deborah Gochfeld). Note the uniporal oscules. Scale: 6 cm; [ZMBN 81644]: B. Cross–section of the uniporal pores showing the skeletal architecture: cortex and choanosome. s: sphincter; p: polychaete living in the canals. Scale: 1 mm; C. Orthotriaene. Scale: 100 µm; D. Microrhabds and acanthotylaster. Scale: 10 µm; E. Aspidaster. Scale: 20 µm; F. Smooth rosettes with bifid or trifid rays on the surface of an aspidaster. Scale: 2 µm.

Outer morphology (Fig. 13A). Massive, lobate, 15 to 30 cm long, dark–brown sponge. Choanosome is whitish and dense. Cortex becomes blackish in alcohol while the choanosome remains whitish. Surface is

very smooth, no epibionts were observed. Uniporal oscules (ca 3 mm) are placed at the top of vertical lobes. Each oscule leads into a cloaca in which exhalant canals flow in; no sphincters could be seen in these openings. Uniporal pores (ca 0.15 mm) are evenly distributed on the whole surface of the sponge; each pore has a sphincter (Fig. 13B).

Skeleton (Fig. 13B). The cortex (168–336 µm thick) is composed of a very poorly developed ectocortex of microrhabds and a thick endocortex of aspidasters, tangentially arranged. Orthotriaenes and oxeas under the cortex are more or less radially arranged, in small bundles; the cladomes of the orthotriaenes are juxtaposed to the endocortex. Under this peripheral arrangement (1 mm thick), bundles of oxeas tend to be paratangential. Acanthotylasters are present in the choanosome, and in the walls of canals. Microrhabds are present in the choanosome too. Dark granular cells (11–14 µm) are present from the cortex to the choanosome although their density decreases under the peripheral layer.

Spicules (measurements of ZMBN 81645) (Fig. 13C–F). *Megascleres*: (a) oxeas, stout, straight or slightly bent, sometimes modified to styles, length: 780–*943.8*–1118 µm; width: 20–*24.2*–30 µm. (b) orthotriaenes to plagiotriaenes (Fig. 13C), rhabdome with an oxeote end, rhabdome length: 286–*577.2*–728 µm; rhabdome width: 20–*31*–40 µm; clad length: 150–*197.3*–260 µm. *Microscleres*: (c) aspidasters (Fig. 13E–F), irregular rod–shape, with enlarged center, smooth rosettes at their surface (diameter: ca. 2–3 µm), each one with 3–4 bifid or trifid rays, length: 180–*206.2*–226.1 µm; width: 37–*47.1*–55.9 µm; length/width: 4.1–5.9. (d) microrhabds (Fig. 13D), smooth, usually centrotylote, straight or slightly bent, with strongylote ends, length: 34.6–*45.8*–55.9 µm; width: 2.7–*3.5*–5.3 µm. (e) oxyasters, 2–5 actines, irregular with no centrum, with slightly tylote end or not, diameter: 34.6–*47.3*–63.8 µm. (f) acanthotylasters (Fig. 13D), 4–9 actines, diameter: 11–*17.4*–21.6 µm.

Habitat in the Bocas del Toro region. Patchy reef, 3–6 m depth.

Distribution. Florida Keys, USA (Chanas & Pawlik 1995; Kubanek *et al.* 2000); Bahamas (Wiedenmayer 1977; Pulitzer-Finali 1986; Stead *et al.* 2000); Cuba (Alcolado 2002); Puerto-Rico (Carballeira & Negrón 1991); Jamaica (Lehnert & van Soest 1998); Virgin Islands (USNM 31489, 31579, 41227); Dominican Republic (Pulitzer-Finali 1986); Curaçao (van Soest 1981); Mexico (Lehnert 1993; Antonov *et al.* 2007); Belize (USNM 32377); Panama (Collin *et al.* 2005; Díaz 2005); Venezuela (Alvarez *et al.* 1991); Brazil (Sollas 1888; Boury-Esnault 1973; Volkmer-Ribeiro & de Moraes 1975; Solé Cava *et al.* 1981; Mothes *et al.* 1999)

Remarks and discussion. ZMBN 81644 fitted the description of *E. bahamensis* Pulitzer-Finali, 1986 while ZMBN 81645 fitted the description of *E. formosus*. Indeed, the single morphological difference between *E. bahamensis* and *E. formosus* is the absence of the largest category of asters in the former. Apart from this unique spicule difference, both specimens were identical, from the same locality and depth. Wiedenmeyer (1977) had noticed these single aster *E. formosus* but thought it was not a strong enough character to create a different species. However, a few years later, Pulitzer-Finali (1986) decided it was enough to create a different species. We think the largest asters can be more or less widespread in the choanosome, and may even be totally absent. Depending where the section or spicule preparation are made, they can be overlooked, hence records of single aster *E. formosus* (Wiedenmayer 1977; Lehnert & van Soest 1998) and *E. bahamensis* (Pulitzer-Finali 1986; Alcolado 2002). Mothes *et al.* (1999) suggested that *E. bahamensis* further differed from *E. formosus* by its aspidaster proportions (length/width). We reviewed this proportion in the literature and found a proportion of 4.5–6.5 for most *E. formosus*, including the holotype (Sollas 1888; Volkmer-Ribeiro & de Moraes 1975; Mothes *et al.* 1999), 4.1–5.9 for the Bocas del Toro specimens and 2.3–7.1 for the holotype of *E. bahamensis* (Pulitzer-Finali, 1986, Figure 13). To conclude, having shown that (i) large asters can be missing or overlooked and that (ii) there is no clear difference in aspidaster proportion between the two species, we consider *E. bahamensis* to be a junior synonym of *E. formosus*. Interestingly, we noticed that a few specimens from Brazil are out of the length/width range found above, and possess unusually narrow aspidasters (width: 11–20 µm) and thus present an aspidaster proportion of respectively 12.5 and 11.5 (Boury-Esnault 1973; Mothes *et al.* 1999). However, after reexamination of one of these specimens (MNHN DNBE–997), we could find no other character to distinguish it from *E. formosus*. Another Caribbean *Erylus*, *E.*

clavatus Pulitzer-Finali, 1986 from Jamaica, has two types of asters but an aspidaster proportion of 2.2–3.5, a bit lower than most *E. formosus*. We think that its club-shape and its slightly lower aspidaster proportions are not enough to consider *E. clavatus* a valid species. It is most likely a junior synonym of *E. formosus*, as earlier suggested (Lehnert & van Soest, 1998).

Few morphological differences were observed between our Bahamas and Bocas del Toro samples. The Bahamas samples had a thinner cortex (144–160 µm) with flatter aspidasters; also, their asters had thinner actines.

We noticed that the rosettes on the aspidasters of the Bocas del Toro specimens (Fig. 13F) were more complex than the ones of a Brazilian *E. formosus* specimen (MCNPOR 3379) (Mothes *et al.* 1999, Fig. 5F). More data is required to ascertain if the rosettes' morphology is a relevant discriminating microstructure in *Erylus* species.

E. formosus was found on two sites in Bocas del Toro (Table 1) and is relatively common in Adriana's reef. Studies show that it harbors no photosynthetic symbionts (Erwin & Thacker 2007). On the other hand, we found small 4–5 mm long Syllidae polychaetes (F. Pleijel, personal communication) living in the canals of our specimen (Fig. 13B), as well as a copepod. The Bahamas specimens examined also harbored numerous polychaetes.

Unfortunately and despite substantial efforts (DNA washing notably), we were not able to obtain a molecular sequence for this species, even from our Bahamas specimens. It has been previously shown that this species is particularly rich in proteins, carbohydrates and lipids when compared to other reef sponge species (Chanas & Pawlik 1995). Triterpene glycosides, for example, are used by this species for chemical defense against fish predation, fouling and microbial attachment (Kubanek *et al.* 2000; Kubanek *et al.* 2002). Some of these compounds may act as DNA contaminants and block the PCR reactions.

Genus: *Geodia* Lamarck, 1815

Geodia gibberosa Lamarck, 1815
(Figures 14–15)

Synonyms (modified from da Silva, 2002).
Geodia gibberosa Lamarck, 1815: 334.
Pyxitis gibberosa Lamarck, 1815: Schmidt 1870: 70.
Geodia (*Geodia*) *gibberosa* Lamarck, 1815: Hechtel 1965: 68, pl. VIII, fig. 2.
Geodia cariboea Duchassaing de Fonbressin and Michelotti, 1864 (in part): 105, pl. XXV, fig. 8.
Geodia tumulosa Bowerbank, 1872: 628, pl. XLVII.
Geodia media Bowerbank, 1873 (*non G. media* Lendenfeld, 1910): 13, pl. II.
Geodia dysoni Bowerbank, 1873: 14, pl. III.
Geodia reticulata Bowerbank, 1874: 300, pl. XLVI, figs. 14–20.
Sidonops stromatodes Uliczka, 1929: 54, figs. 51–56, pl. I, fig. 10.
Geodia media var. *leptoraphes* Uliczka, 1929: 56, figs. 57–67, pl. I, fig. 11.
Geodia flexisclera Pulitzer-Finali, 1986: 76, figs. 10–11.

Holotype. MNHN DT–608, dry, French Guiana.

Material. 5 specimens, all collected in Solarte lagoon, on mangrove roots, 0.5–1 m depth. Two specimens (fragments) are deposited: ZMBN 77928 and 81780.

Additional material examined. Geodia gibberosa, MNHN DT–608, holotype, French Guiana; YPM 5302, 5304, 5311, mangrove boat channel, Port Royal, Jamaica (from Hechtel, 1965); USNM 4997, off Florida, USA, 14 m; ZMAPOR 03772b, Plaja Kalkie, Westpunt, Curaçao; UFBA-POR 207, Barra do Pote, Veracruz, Bahia, Brazil, 12°59'00" S/ 38°36'00" W, V. Almeida coll., intertidal.
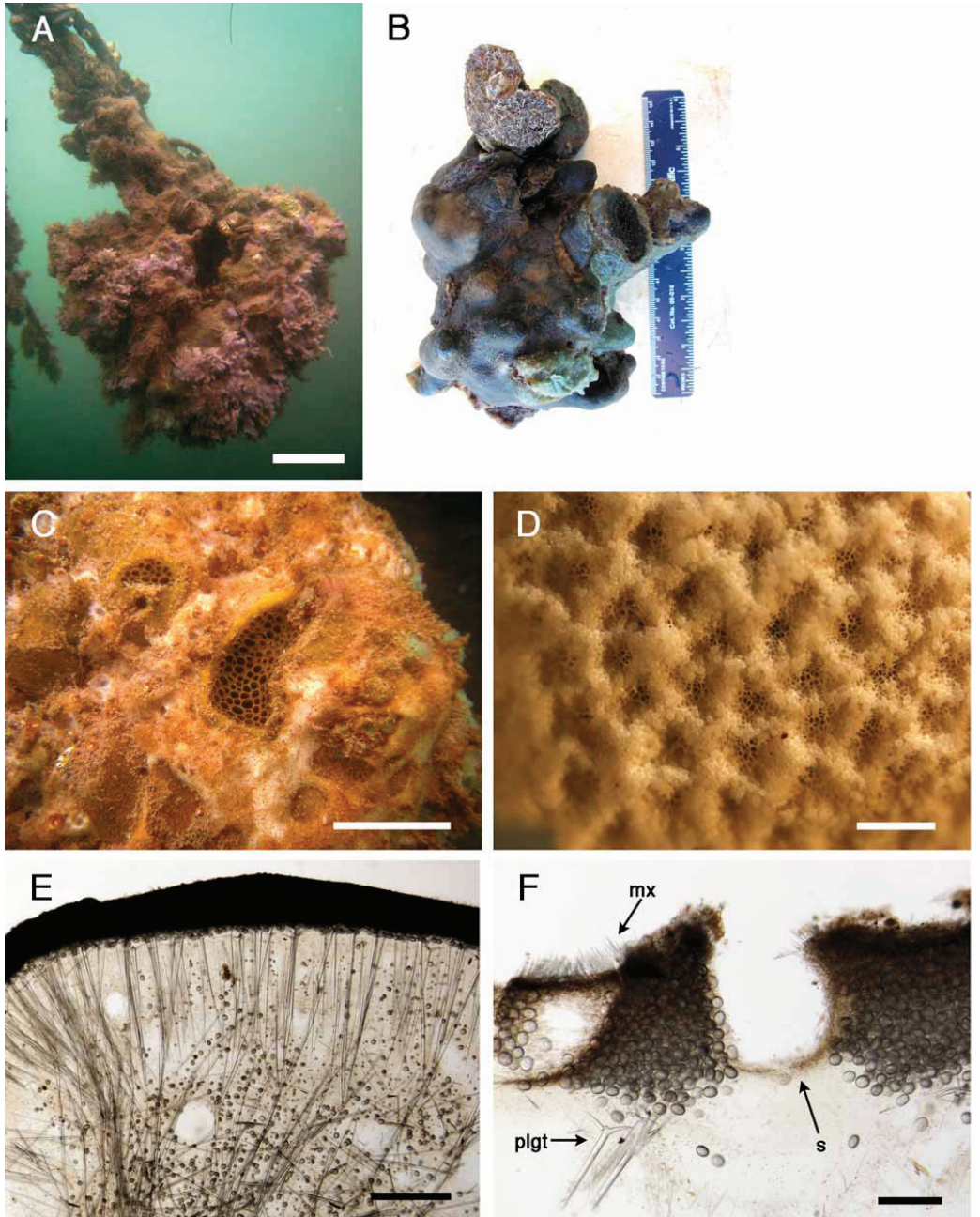
FIGURE 14. *Geodia gibberosa* Lamarck, 1815: A. Large specimen hanging at the tip of a mangrove root. It is overgrown by the purple sponge *Chalinula molibta*, algae and oysters. Scale: 6 cm; B. Specimen with characteristic tumulose shape; C. Close up of oscular plates of an overgrown specimen *in situ*. Each oscule is uniporal. Scale: 4 cm; *G. gibberosa*: [ZMBN 81780]: D. Close up of the cribriporal pores. Scale: 1 mm; E. Cross–section showing the skeletal architecture: cortex and choanosome. Note the radial arrangement of the triaenes right under the cortex. Scale: 1 mm; F. Cross–section of a uniporal oscule. mx: cortical microxeas. s: sphincter. plgt: plagiotriaene. Scale: 0.5 mm.
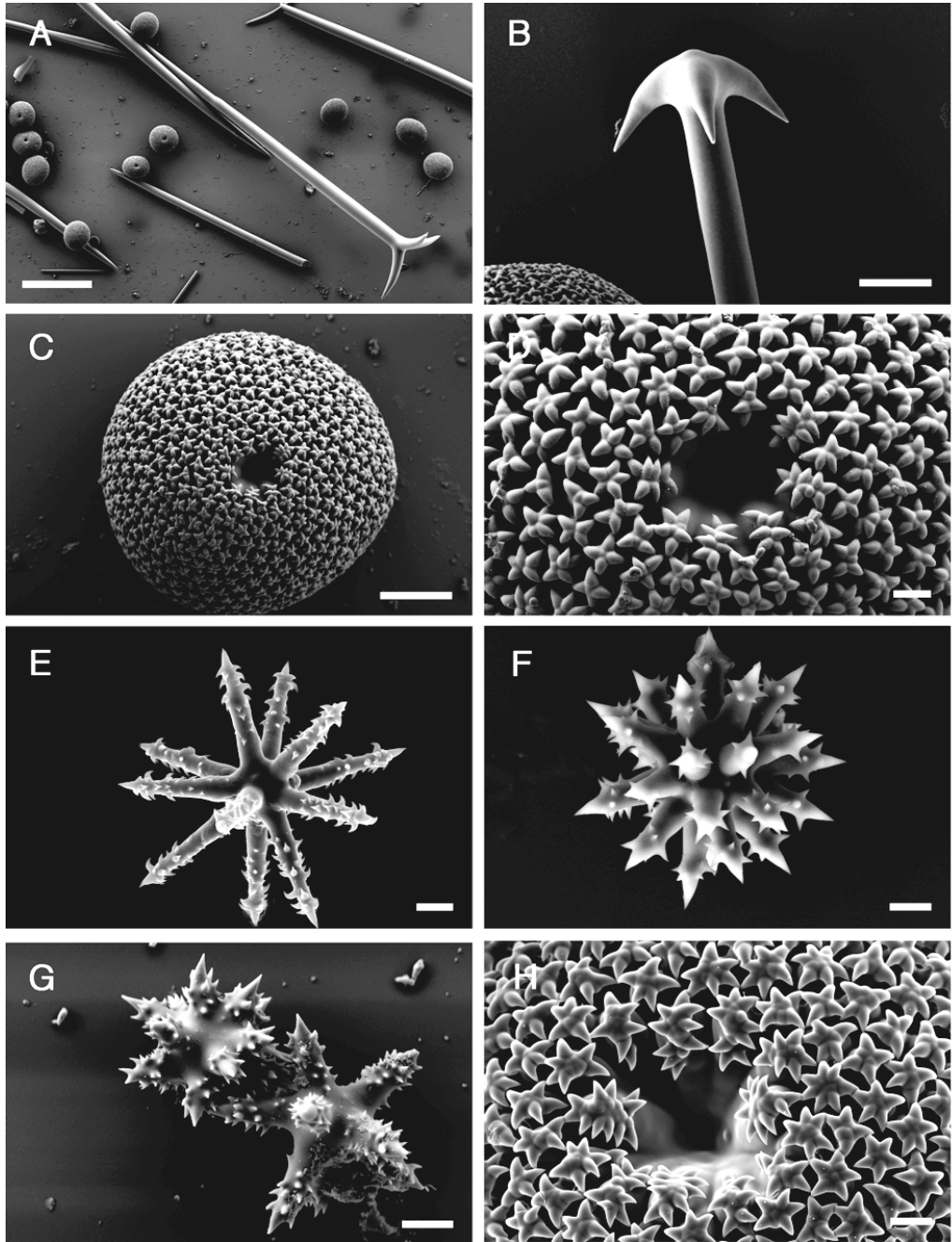
FIGURE 15. *Geodia gibberosa* Lamarck, 1815 [ZMBN 81780]: A. Plagiotriaene and sterrasters. Scale: 200 µm; B. Anatriaene cladome. Scale: 20 µm; C. Sterraster. Scale: 20 µm; D. Star–shaped smooth rosettes and hilum from a sterraster surface. Scale: 4 µm; E. Acanthoxyaster I. Scale: 4 µm; F. Acanthoxyaster II. Scale: 2 µm; G. Acanthoxyasters III. Scale: 2 µm; *G. gibberosa* [MNHN DT–608, holotype]: H. Smooth rosettes and hilum from a sterraster surface. Scale: 4 µm.

Outer morphology (Fig. 14A–D). Massive, irregularly lobate large sponge ('gibberosa' means 'hunchbacked') (Fig. 14A–B). Size can be up to 30 cm in diameter. Color alive is dark–green to light–brown when exposed to light, otherwise whitish. Color in ethanol is whitish, except the oscule area that stays dark brown. Choanosome color, alive and in ethanol, is whitish. However, in live green specimens, the choanosome just below the cortex can also be greenish. It is a slightly compressible sponge with a dense choanosome, and a tough cortex, difficult to break. Surface is usually smooth, but can be hispid in some areas. Often overgrown by other sponges (e.g. *Tethya actinia* de Laubenfels, *Chalinula molitba* (de Laubenfels), *Haliclona* spp.), ascidians, polychaetes, clams and algae. Oscules are uniporal (0.5–1.5 mm in diameter), each with a sphincter (Fig. 14F), grouped in circular slightly depressed areas (2–4 cm diameter) (Fig. 14C). These oscular plates can be situated at the end of lobate projections or not; they are never covered by ectosymbionts. These plates usually are of darker color, brownish. Pores (Fig. 14D) are cribriporal (diameter of a pore plate: 0.5–1 mm), numerous and evenly distributed over the whole surface.

Skeleton (Fig. 14E–F). The cortex is 0.4–0.9 mm thick and is subdivided between a very thin ectocortex of acanthoxyasters III (ca 20 μm) and a thicker endocortex of sterrasters (ca 500 μm). Oxeas I and plagiotriaenes are radially positioned under the cortex with cladomes supporting the endocortex. Under this layer of plagiotriaenes the radial arrangement is less obvious. Most acanthoxyasters II are present right under the cortex while acanthoxyasters I and III are quite abundant throughout the choanosome, as well as developing sterrasters. Oxeas II can be found in the choanosome but are especially placed on the cortex around the oscules (Fig. 14F).

Spicules (measurements from ZMBN 77928, except for the rosette diameter measured in ZMBN 81780) (Fig. 15). *Megascleres*: (a) oxeas I, stout, straight or very slightly bent, length: 1044–*1342.1*–1824 μm; width: 16–*31.2*–42 μm. (b) oxeas II, usually straight, length: 157–*201.5*–238 μm; width: 3.6–*4.3*–7.2 μm. (c) plagiotriaenes (Fig. 15A), rhabdome length: 792–*1266*–1620 μm; rhabdome width: 35–*48.1*–70 μm; clad length: 98–*211.4*–308 μm. (d) anatriaenes (Fig. 15B), rare, rhabdome length: 1433 μm (N=1); rhabdome width: 6–*7.9*–11 μm (N=3); clad length: 23–*26.5*–33 μm (N=3). *Microscleres*: (e) sterrasters (Fig. 15C–D), oval, with smooth 3–5 branched rosettes at their surface (diameter: ca. 4 μm), length: 75–*84.1*–93.1 μm; width: 54–*79.5*–93.1 μm. (f) acanthoxyasters I (Fig. 15E), 6–12 thin actines, diameter: 16–*21.3*–34 μm. (g) acanthoxyasters II (Fig. 15F), large centrum, with thicker and shorter actines than acanthoxyasters I, 14–20 actines, diameter: 9.7–*13.4*–16.2 μm. (h) acanthoxyasters III (Fig. 15G), 9–17 actines, diameter: 4.3–*6.5*–7.6 μm.

Habitat in the Bocas del Toro region. Common on mangrove roots, 1–2 m depth.

Distribution. Georgia, Florida, Texas, U.S.A. (de Laubenfels 1936b;1953; Little 1963; Freeman *et al.* 2007); Bermudas (de Laubenfels 1950); Bahamas (de Laubenfels 1949; Wiedenmayer 1977); Cuba (Alcolado 2002); Jamaica (Bowerbank 1872; Hechtel 1965; Lehnert & van Soest 1998); Dominican Republic (Bowerbank 1873); Puerto Rico (Pulitzer-Finali, 1986); St. Thomas (Duchassaing de Fonbressin & Michelotti 1864); St. John (Uliczka 1929); Barbados (Uliczka 1929; van Soest & Stentoft 1988); Mexico (Lehnert 1993); Honduras (Bowerbank 1872); Belize (Rützler *et al.* 2000); Costa–Rica (Loaiza Coronado 1991; Cortés 1996); Panama (de Laubenfels 1936a; Wulff 2000); Colombia (Díaz 2007); Curaçao (van Soest 1981); Venezuela (Carter 1882; Sutherland 1980); French Guiana (Lamarck 1815); Brazil (da Silva 2002).

Remarks and discussion. After the Galeta and Panama Canal locality records, this is the third record of *G. gibberosa* in Panama. In fact, *G. gibberosa* is one of the sponge species that have been able to cross to the Pacific side using the Panama Canal (de Laubenfels 1936a). The spicule sizes and morphologies of our specimens fitted previous descriptions (da Silva 2002) and the comparative material, except for the Florida specimen (USNM 4997) which had a more regular gross morphology and smaller sterrasters with a different rosette pattern (data not shown). We suggest that the status of Florida populations should be tested in the future. This is the first observation of anatriaenes in *G. gibberosa* but due to their rarity, they could have easily been overlooked in previous observations. It is also the first time that oxeas II are observed in high density around the oscules (Fig. 14F). The lobate morphology of our specimens (Fig. 14B) was very similar to that of the holotype of *Geodia tumulosa* (Bowerbank 1872, pl. XLVII), later synonymized with *G. gibberosa* (Carter,

1882; da Silva, 2002). *G. gibberosa* is a common wide-spread Tropical western Atlantic species. In the literature it appears that *G. gibberosa* is very polymorphic when it comes to its gross morphology (lobate to flat, massive to encrusting) and its color (white, brown, green and black). A pattern emerges when one considers its two habitats (reef and mangrove). Reef specimens tend to be smaller in size with common encrusting forms, and are usually white to brown. On the other hand, mangrove specimens like ours tend to be large, massive, lobate, and darker colored. Ecology studies have shown that *G. gibberosa* is very palatable for reef fishes and has no chemical defenses (Pawlik *et al.* 1995). Therefore, it uses secondary metabolites to promote overgrowth of other species better equipped to defend themselves from fish predation (Engel & Pawlik 2005). All the specimens we observed in Bocas del Toro were indeed covered with numerous sponges, ascidians, algae, etc. Fish predation being lower in mangroves (Dunlap & Pawlik 1996), *G. gibberosa* can reach bigger sizes and grow in more open habitats thereby receiving more sunlight and having darker colors. Conversely, predation pressure in reefs being higher, *G. gibberosa* is usually smaller and prefers cryptic habitats (under rocks or other sponges, crevices). When not exposed to light it is of lighter color, often white. Seemingly, two other mangrove sponges (*Tedania (Tedania) ignis* (Duchassaing de Fonbressin & Michelotti) and *Chondrosia* sp.) were found in cryptic habitats when collected on reefs (Dunlap & Pawlik 1996). An alternative to the polymorphism hypothesis is that we simply have two (or more) cryptic species. Both hypotheses should be tested combining morphology and molecular data.

Geodia papyracea (Hechtel, 1965)
(Figure 16)

Synonyms.
Geodia (Cydonium) papyracea Hechtel, 1965: 71, text-fig. 13, pl. VIII, figs.1–2.
Geodia papyracea Hechtel, 1965: Alcolado 1981, p. 38.

Holotype. YPM 5045, mangrove boat channel, Port Royal, Jamaica.

    Material. UMPCW921, Solarte Island, on a mangrove root, 0.5–1 m depth.

    Additional material examined. G. papyracea, YPM 5045, holotype, mangrove boat channel, Port Royal, Jamaica; YPM 5309, 5311, paratypes, mangrove boat channel, Port Royal, Jamaica; USNM 42662, mangrove, Twin Cays, Belize.

    Outer morphology (Fig. 16A). Whitish massive sponge with green tinges when alive. It is ca 6x12 cm. The sub-sample we have is whitish in alcohol; the cortex being lighter than the choanosome. Oscules are uniporal, each with a sphincter and grouped in a sub–circular, slightly depressed plate. The oscule plate can be dark green. Pores are cribriporal and widely distributed over the entire surface. Each cribriporal plate has a diameter of 1 mm.

    Skeleton (Fig. 16B). The cortex is 480–840 µm thick and composed of a thin ectocortex of strongylasters (0–72 µm) and a thicker endocortex made of sterrasters (480–840 µm). The cortex is easily torn and detachable from the choanosome. Oxeas I and plagiotriaenes are positioned under the cortex, more or less radially. The plagiotrianes usually cross the cortex so that the cladomes end up in the ectocortex or at the surface of the cortex. The radial arrangement of megascleres is less obvious 1 mm under the cortex. Oxyasters I, oxyasters II and strongylasters can be found in the whole choanosome, as well as developing sterrasters. Oxeas II, smaller than oxeas I, are only found in the choanosome.

    Spicules (Fig. 16C–G). *Megascleres*: (a) oxeas I, stout, straight or slightly bent, length: 651–*1039*–1248 µm; width: 7–*24.3*–29 µm. (b) oxeas II, rare, sometimes with strongyle ends, usually straight, length: 95–*124.3*–244 µm; width: 1–*2*–3 µm (N=14). (c) plagiotriaenes, rhabdome length: 641–*962.7*–1080; rhabdome width: 13–*24.6*–34 µm; clad length: 57–*97.1*–132 µm. (d) anatriaenes, rare, rhabdome length: 354–*633.2*–885 µm (N=5); rhabdome width: 2.5–*3.2*–4 µm (N=8); clad length: 5–*13.8*–27 µm (N=8). (e) mesoprotriaenes, very rare, rhabdome length: 632 µm (N=1); rhabdome width: 3 µm (N=1); clad length: 9–16 µm (N=2).
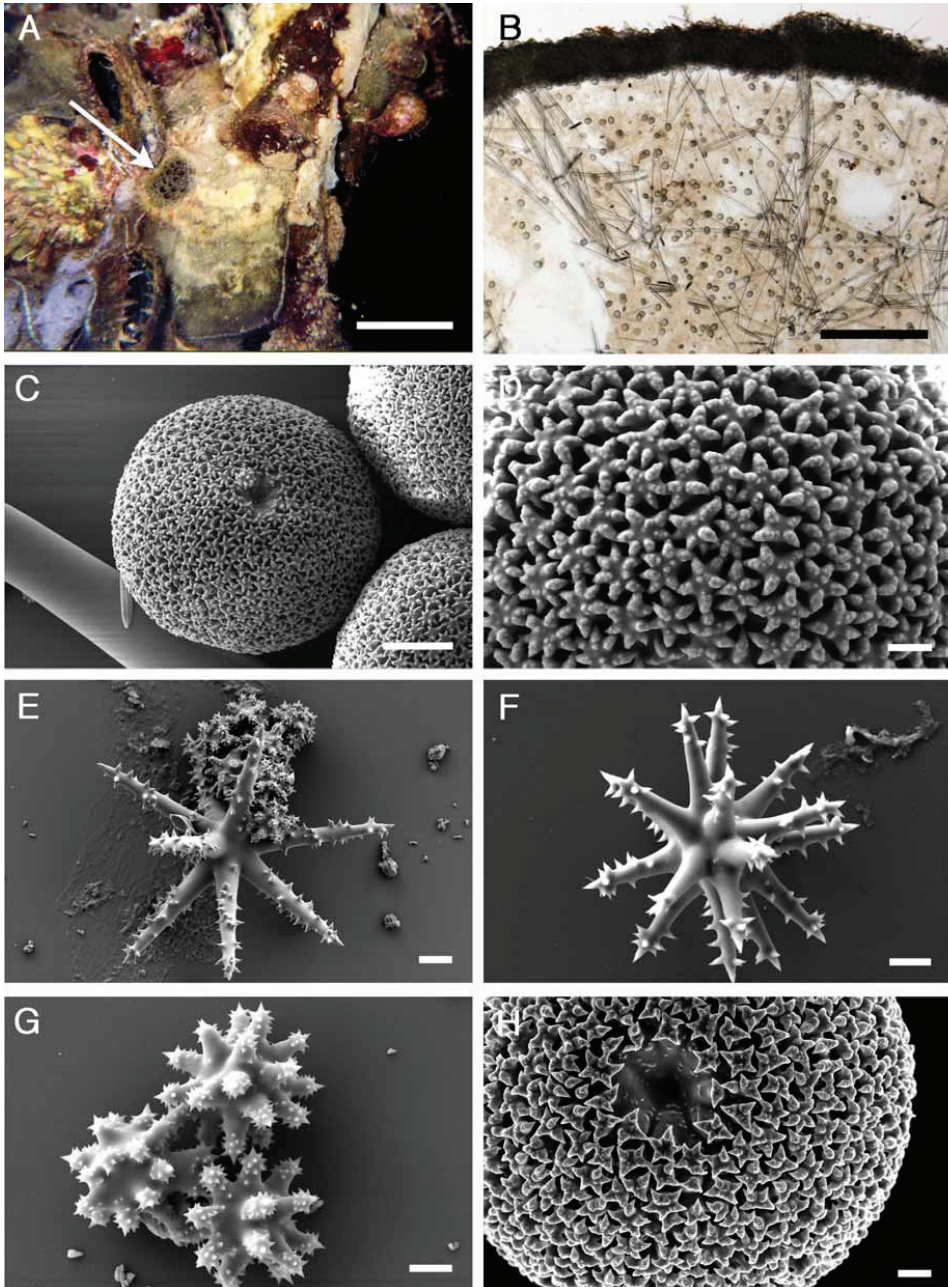
FIGURE 16. *Geodia papyracea* (Hechtel, 1965) [UMPCW921]: A. Specimen *in situ*, growing on a mangrove root, arrow points to the oscule plate with uniporal oscules (Picture: Scott Nichols). Scale: 3 cm; B. Cross–section showing the skeletal architecture: cortex and choanosome. Note the confused arrangement of the megascleres in the choanosome. Scale: 1 mm; C. Sterraster, with an acanthostrongylaster by the warty hilum. Scale: 20 µm; D. Star–shaped warty rosettes from a sterraster surface. Scale: 4 µm; E. Acanthoxyaster I and acanthostrongylasters. Scale: 4 µm; F. Acanthoxyaster II. Scale: 4 µm; G. Acanthostrongylasters. Scale: 2 µm; *G. papyracea* [YPM 5045, holotype]: H. Warty rosettes and hilum from a sterraster surface. Scale: 4 µm.

Microscleres: (f) sterrasters (Fig. 16C–D), subglobular, with warty 4–7 branched rosettes at their surface (diameter: ca 4 µm), length: 65–*71.6*–77 µm; width: 63–*70.3*–75 µm; thickness: 50–*55.9*–61 µm. (g) acanthoxyasters I (Fig. 16E), 9–12 thin actines, center more or less developed, diameter: 22–*27.5*–36 µm. (h) acanthoxyasters II (Fig. 16F), with large centrum and thicker actines than acanthoxyasters I, 9–17 actines, less common than acanthoxyasters I. diameter: 14–*23*–29 µm. (i) acanthostrongylasters (Fig. 16G), 14–20 actines, diameter: 3–*4.9*–8.8 µm.

Habitat in the Bocas del Toro region. On mangrove roots, 0.5–1.5 m depth.

Distribution. Cuba (Alcolado 2002); Belize (Rützler 1988; Rützler *et al.* 2000); Panama (Díaz 2005); Jamaica (Hechtel 1965); Colombia (Wintermann-Kilian & Kilian 1984); Curaçao (van Soest 1981); Brazil (Burton 1940; da Silva *et al.* 2004; Cedro *et al.* 2007).

Remarks and discussion. The fragile and easily peeled cortex of *G. papyracea* might be due to the peculiar disposition of the triaenes, with the cladomes not supporting the cortex as in most *Geodia* species. The ectocortex of the Bocas del Toro specimen was much thinner (0–72 µm) than in the Belize sample (120–325 µm) and the holotype/paratypes (93–232 µm thick). Conversely, the sterrastreal layer in the Bocas del Toro specimens was thicker (480–840 µm) than in the Belize sample (93–279 µm) and the holotype/paratypes (186–418 µm). This is partly a consequence of the larger sterrasters in comparison with the type material and previous *G. papyracea* records (da Silva *et al.* 2004). We also noted that the surface of the Bocas del Toro specimen was less folded, the parchment–like appearance characteristic of this species was therefore not as obvious as in the samples from Jamaica and Belize. The rest of our measurements were in accordance with the species description. This species was very common in Solarte lagoon in 2005 and 2006 (M. C. Díaz, personal observation). We were not able to find it anymore in 2007 and 2008 as it appeared to have been replaced by the sympatric species *Geodia gibberosa*.

In the field, *G. papyracea* can be mistaken with the previously described *G. gibberosa* since they are both found living on mangrove roots and can even be found growing on one another (Hechtel 1965). Again, gross morphology is here important: *G. papyracea* has very poor relief and a fragile cortex while *G. gibberosa* has characteristic lobes and a tough and thicker cortex. *G. gibberosa* seems to be more overgrown than *G. papyracea*. In thick sections, the skeleton was more radially organized in *G. gibberosa* (Fig. 14E)*,* with plagiotriaenes supporting the cortex and not crossing it like in *G. papyracea* (Fig. 16B). This probably accounts for the firm cortex in the former. Then, *G. gibberosa* has larger oxeas and plagiotriaenes; the sterrasters are also a bit larger and more oval shaped. SEM pictures of asters revealed additional microstructural differences. When comparing the surface of sterrasters, the *G. gibberosa* rosettes (Fig. 15D) had less rays than those of *G. papyracea* (Fig. 16D). In *G. gibberosa*, rosettes also had a smaller center and no warts on the rays. Seemingly, the hilum of the sterrasters were different (the hilum is a circular depression, a remnant of where the nucleus of the sclerocyte used to be): smooth in *G. gibberosa*, warty in *G. papyracea*. These differences were confirmed by SEM observation of the holotypes' sterrasters (Figs. 15H, 16H). We also noticed that the actines of the acanthoxyasters III in *G. gibberosa* (Fig. 15G) have a more prominent spine at their tip when compared with the acanthostrongylasters of *G. papyracea* (Fig. 16G). Finally, these two species had significantly different Folmer COI sequences (60 bp. difference) and 28S partial sequence (63 bp. difference). A molecular phylogenetic study shows that they belong to two clearly seperated *Geodia* clades (Cárdenas *et al.* 2009).

Although the holotype (YPM 5045) has been fixed and preserved in alcohol, the DNA we extracted from it was poorly preserved. Consequently, we were unable to amplify the Folmer COI fragment or a 28S sequence from the type material.

Conclusion

We found all four *Tetractinellida* species reported by Díaz (2005). Six additional *Tetractinellida* species were encountered, five of them new for Panama: *Cinachyrella kuekenthali, Ecionemia megastylifera, Stelletta*

fibrosa, *Stelletta* sp. and *Stryphnus raratriaenus* sp. nov.. Therefore, a total of ten species of *Tetractinellida* were collected: 7 Astrophorida and 3 Spirophorida (Table 1). This is still a lower number of *Tetractinellida* than some neighboring regions (for references, cf. Introduction), so we suspect that in the future more species will be found in the Bocas del Toro region (e.g. *Geodia*, *Caminus*, *Erylus*, *Stelletta* and *Jaspis*). Our results bring the number of identified sponge species living in the Bocas del Toro shallow-waters to 129. DNA tagging of these species should further help their identification (*Geodia* spp. and *Cinachyrella* spp.) or even their revision (*Stelletta* spp.).

Acknowledgments

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