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Five new species of Homoscleromorpha (Porifera) from the Caribbean Sea and re-description of *Plakina jamaicensis*

ALEXANDER V. ERESKOVSKY[1,2], DENNIS V. LAVROV[3] AND PHILIPPE WILLENZ[4]

[1]Mediterranean Institute of Biodiversity and Ecology (IMBE), UMR 7263, Aix-Marseille Université, CNRS, IRD, Station Marine d'Endoume, Marseille, France, [2]Biological and Soil Science Faculty, Saint-Petersburg State University, Russia, [3]Department of Ecology, Evolution and Organismal Biology, Iowa State University, 253 Bessey Hall, Ames, IA, USA, [4]Department of Invertebrates, Royal Belgian Institute of Natural Sciences, 29 rue Vautier, 1000 Brussels, Belgium

Five new species of Homoscleromorpha (Porifera) of four genera, Oscarella, Plakortis, Plakina *and* Corticium, *are described from vertical walls of reef caves at depths ranging from 23 to 28 m in the Caribbean Sea.* Oscarella nathaliae *sp. nov. has a leaf-like thinly encrusting, flat body, loosely attached to the substrate and a perforated, not lobate surface.* Oscarella nathaliae *sp. nov. contains two bacterial morphotypes and is characterized by two mesohylar cell types with inclusions.* Plakortis myrae *sp. nov. has diods of two categories: abundant large ones (83–119 μm long) and rare small ones (67–71 μm long) with sinuous, S-bent centres; triods Y- or T-shaped (18–5 μm long), and abundant microrhabds (5–12 μm long).* Plakortis edwardsi *sp. nov. has diods of one category with thick, sinuous, S-bent centres (110 to 128 μm long); triods T-shaped (actines 28–59 μm long). It is the only species of this genus showing small diods (22–31 μm long).* Plakortis dariae *sp. nov. has diods of two categories: large ones (67–112 μm long) and small, rare, irregular ones, slightly curved, often deformed with one end blunt (30–59 μm long); triods rare and regular (actines 20–44 μm long).* Corticium diamantense *sp. nov. has oscula situated near its border, regular non-lophose calthrops of one size-class, very rare tetralophose calthrops and candelabra with the fourth actine ramified basally in 4–5 microspined rays. In addition, a re-description of* Plakina jamaicensis *is based on newly collected material and the type specimen.* Plakortis jamaicensis *has a convoluted brain-like surface; well developed sub-ectosomal cavities; irregular sinuous diods, triods, calthrops, rare monolophose calthrops, rare dilophose calthrops, rare trilophose calthrops and common tetralophose calthrops. Molecular 'barcoding' sequences for mitochondrial cob are given for* Plakortis edwardsi *sp. nov.,* P. dariae *sp. nov.,* Plakina jamaicensis *and* Corticium diamantense *sp. nov. An identification key for all western Atlantic Homoscleromorpha is provided.*

Keywords: taxonomy, Porifera, Homoscleromorpha, biodiversity, *Oscarella*, *Plakortis*, *Plakina*, *Corticium*, Jamaica, Martinique

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INTRODUCTION

Homoscleromorpha represents a small group with two families (<100 described species) of exclusively marine sponges, generally located in shallow waters ranging from 8 to 60 m, but a few were also recorded from abyssal depths, up to 2460 m (Muricy & Diaz, 2002). All species are dwellers of hard substrate communities often in semi-dark or dark conditions. Some species may exclusively grow on coralligenous substrate. In some places, Homoscleromorpha can be predominant and seem to be strong competitors for space, overgrowing massive sponges, sea fans and erect bryozoans (Diaz & van Soest, 1994; Muricy & Diaz, 2002; Ereskovsky *et al.*, 2009a; Pérez *et al.*, 2011).

Homoscleromorpha have a great variability of shapes but their general organization and the shared features of their cytology and embryology argue for the monophyly of this group. This sponge clade is characterized by an aquiferous system of either sylleibid-like or leuconoid organization with eurypylous, diplodal or aphodal choanocyte chambers. Skeletal structures, when present, harbour a peculiar type of tetractinal spicules (calthrops), distinguishable from calthrops of Demospongiae and their derivatives by their small size, ramification of one to all four actines (lophose calthrops) or reduction (diods and triods) (Muricy & Diaz, 2002). These spicules do not form a well-organized skeleton. Homoscleromorpha possess flagellated exopinacocytes and endopinacocytes, a cinctoblastula larva, cross-striated ciliar rootlets in larval cells, a basement membrane underlying both choanoderm and pinacoderm, and zonula adherens cell junctions in adults and larval epithelia (for review see Muricy & Diaz, 2002; Ereskovsky *et al.*, 2009a; Gazave *et al.*, 2010).

Until recently, Homoscleromorpha was classified as a subclass of the class Demospongiae, containing one order (Homosclerophorida Dendy, 1905), one family (Plakinidae Schulze, 1880) and seven genera (Boury-Esnault *et al.*, 1995; Muricy & Diaz, 2002). Molecular phylogenies challenged this traditional classification schema: several phylogenetic/phylogenomic studies using several nuclear markers have corroborated the hypothesis suggesting that Homoscleromorpha

Corresponding author:
A.V. Ereskovsky
Email: alexander.ereskovsky@imbe.fr

forms a clade on its own, clearly separated from Demospongiae (Borchiellini *et al.*, 2004). Recently, molecular phylogenetic studies of Homoscleromorpha, including six of the seven genera presently known, brought a new interpretation of the relationships within this group (Gazave *et al.*, 2010, 2012; Ivanišević *et al.*, 2010). These studies restored the Oscarellidae Lendenfeld, 1887 and Plakinidae (Schulze, 1880) families, a suprageneric classification of Homoscleromorpha abandoned in 1995 (Boury-Esnault *et al.*, 1995). Therefore, Homoscleromorpha are considered to represent a separate class of sponges, with one order (Homosclerophorida Dendy, 1905), two families (Plakinidae Schulze, 1880 and Oscarellidae Lendenfeld, 1887) and seven genera (van Soest *et al.*, 2013). Five genera belong to Plakinidae (*Corticium* Schmidt, 1862; *Plakina* Schulze, 1880; *Plakinastrella* Schulze, 1880; *Placinolopha* Topsent, 1897; and *Plakortis* Schulze, 1880) and two belong to Oscarellidae (*Oscarella* Vosmaer, 1887; and *Pseudocorticium* Boury-Esnault *et al.*, 1995) (van Soest *et al.*, 2013). The two latter genera are characterized by the lack of spicules and by an aquiferous system consisting of spherical, eurypylous choanocyte chambers regularly organized around large and even exhalant canals. To countervail this lack of morphological characters, attempts were made to recognize secondary metabolites, but only *Oscarella lobularis* from Mediterranean brought to light cytotoxic epoxy-sterols (Aiello *et al.*, 1990).

Homoscleromorpha, with the exception of the genus *Pseudocorticium*, have a more or less worldwide distribution. Nevertheless, until now no Homoscleromorpha from the genera *Placinolopha, Oscarella* and *Pseudocorticium* have been registered in the Caribbean region.

The genus *Oscarella* has a worldwide distribution and consists of 16 valid species (van Soest *et al.*, 2013), but with rare incursions into cooler temperate waters (Muricy & Pearse, 2004; Ereskovsky, 2006; Ereskovsky *et al.*, 2009b; Pérez *et al.*, 2011). Until now the genus was reported but indeterminate in the Caribbean, from the Virgin Islands (Díaz & van Soest, 1994), Belize (Rützler *et al.*, 2000; Díaz *et al.*, 2004; Díaz & Rützler, 2009), Panama (Díaz, 2005; Díaz & Rützler, 2009) and the Bahamas (Zea *et al.*, 2009). Only one *Oscarella* sp. is registered from the south-east Brazilian coasts (Muricy & Hajdu, 2006) and two other ones from north-east (NE) Brazil (Muricy & Moraes, 1998), all three yet to be described.

The genus *Plakortis* is cosmopolitan with 19 species known from different oceans around the world (Muricy, 2011; van Soest *et al.*, 2013) and with six species described from the Caribbean Sea, north-eastern Brazil and north-western Atlantic: *Plakortis simplex* Schulze, 1880, *P. angulospiculatus* (Carter, 1879), *P. halichondrioides* Wilson, 1902, *P. zyggompha* de Laubenfels, 1934, *P. insularis* Moraes & Muricy, 2003 and *P. microrhabdifera* Moraes & Muricy, 2003 (Carter, 1879; de Laubenfels, 1934; Hechtel, 1965; Boury-Esnault, 1973; Wiedenmayer, 1977; Pulitzer-Finali, 1986; Zea, 1987; Mothes & Bastian, 1993; Diaz & van Soest, 1994; Lehnert & van Soest, 1998; Moraes & Muricy, 2003).

Plakortis species are mainly characterized by a skeleton formed by small diods with triods of varying abundance, even though some species may present diactine-derived microrhabds and deformed calthrops (Diaz & van Soest, 1994; Muricy & Diaz, 2002; Muricy, 2011). Due to similarity of most species and simplicity of their spiculation (composed mainly of irregular diods of a single size-class and of rare triods), the species diagnosis is quite difficult without a detailed observation of external and anatomical features (e.g. architecture of the aquiferous system) and skeletal characters (Diaz & van Soest, 1994; Muricy & Diaz, 2002; Moraes & Muricy, 2003; Muricy, 2011).

Species from the genus *Plakortis* were proven to be a prolific source of secondary metabolites with a large structural diversity. They produce biologically active oxygenated polyketides, cyclic peroxides, peroxylactones, and other secondary metabolites (Faulkner, 2002), many of which are biologically active (Gochfeld & Hamann, 2001; Muricy & Diaz, 2002; Rudi *et al.*, 2003; Berrué *et al.*, 2005; Holzwarth *et al.*, 2005).

The genus *Plakina* includes 25 species (van Soest *et al.*, 2013) with worldwide distribution. In the Atlantic, six species were described: *Plakina brachylopha* Topsent, 1927 from the Azores, *P. versatilis* (Schmidt, 1880) from the Gulf of Mexico, *P. elisa* (de Laubenfels, 1936a), *P. jamaicensis* Lehnert & van Soest, 1998 and *P. tetralopha* (Hechtel, 1965) from the Caribbean Sea and *P. trilopha* sensu Boury-Esnault (1973), from NE Brazil.

Plakina species have a distinctive spiculation consisting of diods, triods, and calthrops of a single size-class, and homolophose calthrops with one to four lophate rays. Antimicrobial steroidal alkaloids were reported in *Plakina* sp. (Rosser & Faulkner, 1984).

Finally, the genus *Corticium* with only six species has also a wide-ranging distribution, from NW Mediterranean to Indian Ocean, Western Pacific. A single species was recorded in the Caribbean, *Corticium quadripartitum* (Topsent, 1923). *Corticium* species are defined as thin to thick plakinids, with a skeleton dominated by non-lophose calthrops and heterolophose calthrops (candelabra). Certain species also possess homolophose calthrops. Some news steroidal alcaloids have been described from different *Corticium* species (De Marino *et al.*, 1998, 1999; Lee *et al.*, 2001; Borbone *et al.*, 2002; Ridley & Faulkner, 2003; Zampella *et al.*, 2005).

In this paper we describe a new *Oscarella* occurring in Martinique and Jamaica, three new *Plakortis* from Jamaica and a new *Corticium* from Martinique. In addition a revision of *Plakina jamaicensis* Lehnert & van Soest 1998 is based on newly collected material from Jamaica and the type specimen. A key to Caribbean species of Homoscleromorpha is provided.

MATERIALS AND METHODS

In Martinique, specimens were collected by SCUBA in early June 2003 from the vertical walls and roof of a coralligenous reef cave ('Fer à Cheval' Cave) off Le Diamant at depths of 22–26 m. In Jamaica, specimens were collected by SCUBA in March and April 2005 from vertical walls of coralligenous reef caves at Pear Tree Bottom, 5 km east of Discovery Bay at depths of 28 m and at Chalet Caribe, west of Montego Bay (Figure 1). Geographical coordinates are given in taxonomic descriptions. All underwater photographs were taken with a Nikon Coolpix 950 digital camera. Vouchers for microscopy were fixed in 4% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.4, supplemented with 0.35 M sucrose and 0.1 M NaC1 to obtain a final osmotic pressure of 1700 mOsM for 24 h at 4°C. Specimens were washed six times for 10 min in 0.2 M sodium cacodylate buffer, pH 7.4. Samples were postfixed for 3 h in 1% osmium tetroxide in

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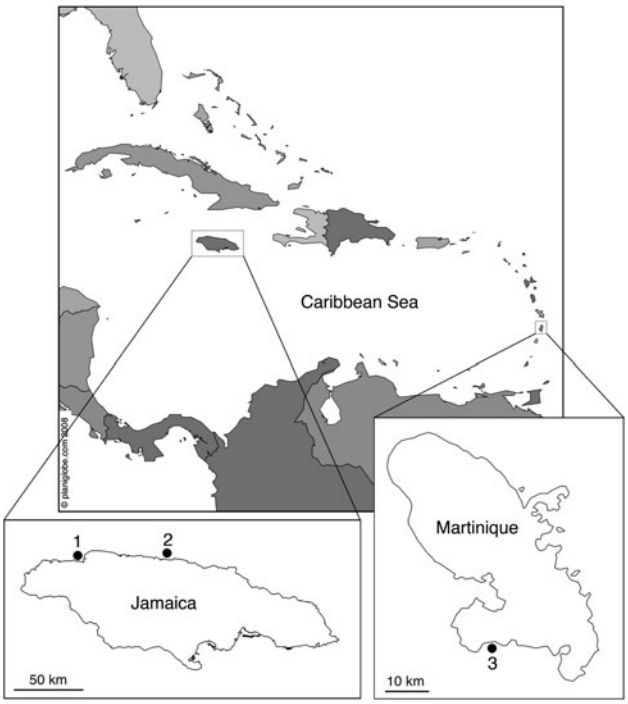

Fig. 1. Collection sites of Homoscleromorpha from Jamaica and Martinique. 1, Chalet Caribe; 2, Pear Tree Bottom; 3, Le Diamant.

0.2 M sodium cacodylate and 0.3 M NaC1, dehydrated through a graded ethanol series, and embedded in ERL 4206 according to Spurr (1969). Sections were obtained with a diamond knife on a Leica Ultracut UCT ultramicrotome. Prior to sectioning, the siliceous spicules at the section plane were dissolved with 15 to 20% hydrofluoric acid in distilled water for 5 min. Semithin sections were stained with toluidine blue, observed in light microscopy (LM) and photographed with a Nikon Coolpix 950 digital camera. For transmission electron microscopy (TEM), thin sections double-stained with uranyl acetate and lead citrate (Reynolds, 1963) were examined with a Zeiss EM 900, a FEI Tecnai 10 and a LEO 906 E transmission electron microscopes at 60 and 80 kV. For scanning electron microscopy (SEM), specimens were fractured in liquid nitrogen, critical-point-dried, sputter-coated with gold-palladium, and observed under a XL30 ESEM Philips SEM. Whole samples destined to reference collection were fixed and preserved in ethanol 70°. For skeletal architecture observation, sections were cut from ERL embedded fragments with a low speed diamond saw (Bennet Labcut 1010) using a diamond wafering blade, mechanically wet ground on a series of diamond grinding disks (Buehler ultra-prepTM) using a semiautomatic grinder (Buehler Minimet 1000) to a thickness of 5–10 µm and observed under a Nikon Optiphot-2 microscope.

Total DNA was prepared from a small piece of each specimen preserved in ethanol by proteinase K digestion in the CTAB (hexadecyltrimethylammonium bromide) buffer, followed by phenol-chloroform extraction and ethanol precipitation (Saghai-Maroof *et al.*, 1984). A region of *cob* was PCR amplified with Promega PCR kit using the diplo-cob primers (Lavrov *et al.*, 2008) and the sequences were determined at the Eurogene company. Partial mitochondrial *cob* sequences from species described in this study and complete *cob* sequences from species used in our previous study were aligned with the MAFFT program (Katoh & Toh, 2008) and the ML phylogeny was estimated with the PhyML 3 program (Guindon *et al.*, 2010). Abbreviations used: DBML (Discovery Bay Marine Laboratory), MNHN (Muséum National d'Histoire Naturelle, Paris, France), RBINS (Royal Belgian Institute of Natural Sciences), SME (Station Marine d'Endoume, Marseille, France), RBINS (Royal Belgian Institute of Natural Sciences), WPD (World Porifera Database), ZIN RAS (Zoological Institute of the Russian Academy of Science). All type material described here is deposited at RBINS under the general iventory number IG 32243.

SYSTEMATICS

Phylum PORIFERA Grant, 1836
Class HOMOSCLEROMORPHA Bergquist, 1978
Order HOMOSCLEROPHORIDA Dendy, 1905
Family OSCARELLIDAE Lendenfeld, 1887
Genus *Oscarella* Vosmaer, 1887

SYNONYMY

[*Oscaria*] Vosmaer, 1881: 163 (preocc. by *Oscaria* Gray, 1873 – Reptilia). *Oscarella* Vosmaer, 1884: pl. 8 (explanation). *Oscarella* Vosmaer, 1887: 326; pl. 2 fig. 3, pl. 8 (nom. nov. for *Oscaria* Vosmaer). *Octavella* Tuzet and Paris, 1963: 88 (no type specimens designated). Taxonomic decision after Vosmaer (1887: 326); Boury-Esnault *et al.*, (1984: 13, 1992b: 282); Diaz & van Soest (1994: 102).

TYPE SPECIES

Halisarca lobularis Schmidt, 1862 (by monotypy).

DIAGNOSIS (MODIFIED FROM MURICY & DIAZ, 2002)

Homoscleromorpha without skeleton, with thinly encrusting to lobate shape. Thin ectosome (<100 µm), often limited to pinacoderm; true cortex absent. Mesohyl poorly developed, with a proportion of mesohyl to chambers varying from 0.5:1 to 1.2:1. The aquiferous system has a sylleibid organization, with spherical, eurypylous choanocyte chambers uniformly arranged around large, regular exhalant canals, and a large basal cavity.

Oscarella nathaliae sp. nov.
(Figures 2–6)

TYPE MATERIAL

Holotype: RBINS POR 90, Martinique, Le Diamant, 'Fer à Cheval' Cave, 14°28′04.72″N 61°00′59.37″W, 22 m depth, Coll. Ph. Willenz and B. Rosenheim, 7 June 2003.

Paratypes: RBINS POR 91; RBINS POR 92, same locality, fixed for EM, coll. Ph. Willenz and B. Rosenheim, 9 June 2003; RBINS POR 67; RBINS POR 78, Jamaica, Pear Tree Bottom Cave, 5 km east of Discovery Bay, 18°27′57.31″N 77°21′18.49″W, 28 m depth, coll. A. Ereskovsky and Ph. Willenz, 1 April 2005. RBINS POR 94 Guadeloupe, Cathedral Cave, Pointe de la Fontaine (Anse-Bertand) 16°27′40.48″N 61°31′50.67″W; depth 15 m, coll. A. Ereskovsky, 18 May 2012.

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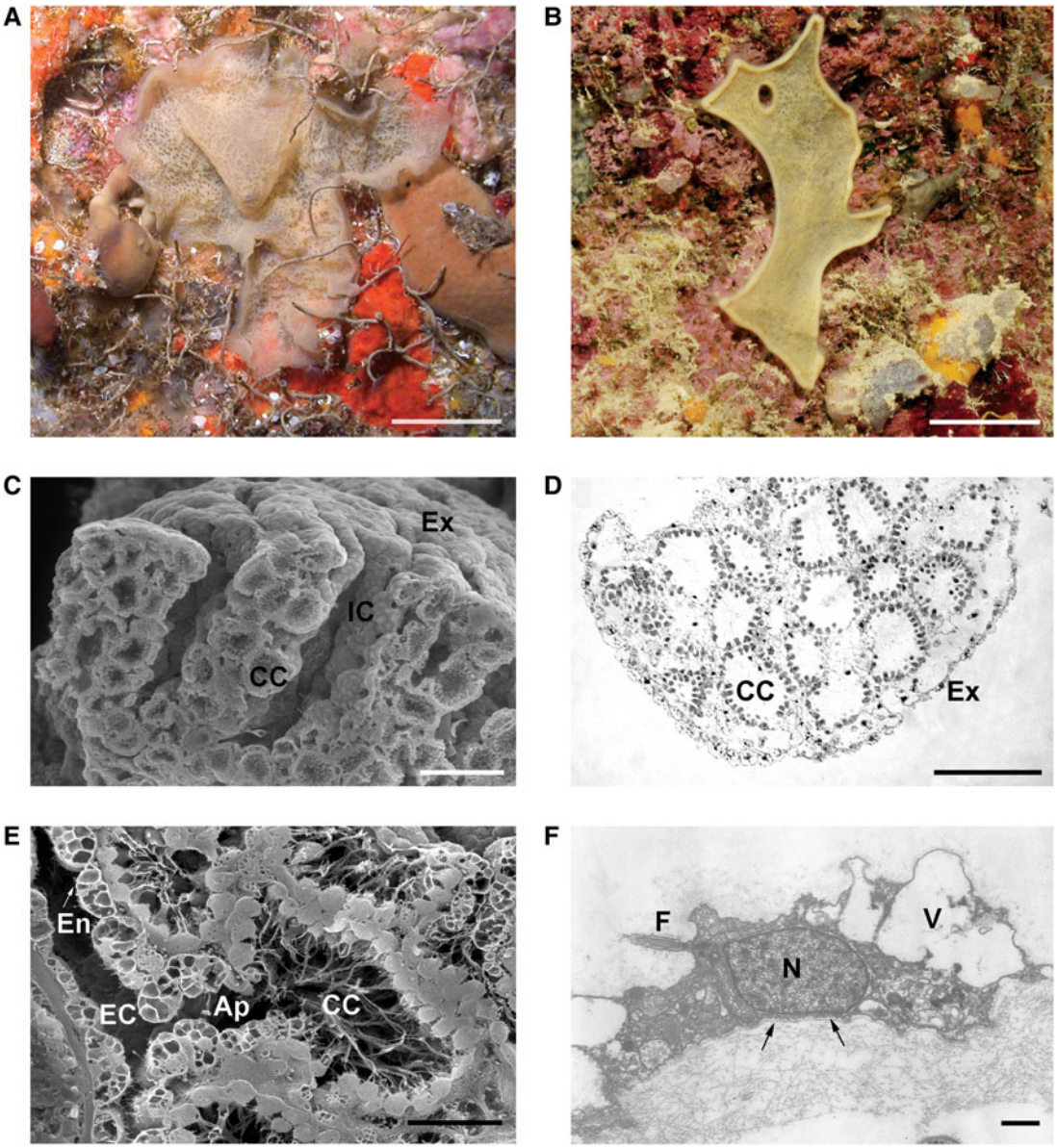

Fig. 2. *Oscarella nathaliae* sp. nov.: (A) from Martinique; (B) from Jamaica (*in situ*); (C) transverse cryofracture through ectosome and choanosome (SEM); (D) transverse section through ectosome and choanosome (LM); (E) cryofracture of a choanocyte chamber and its apopyle (SEM); (F) apopylar cell (TEM). Ap, apopyle; CC, choanocyte chamber; EC, exhalant canal; En, endopinacocyte; Ex, exopinacocytes; F, flagellum; IC, inhalant canals; N, nucleus; V, vacuole. Arrows, basement membrane. Scale bars: A, 3 cm; B, 2 cm; C, 200 μm; D, 50 μm; E, 20 μm; F, 1 μm.

COMPARATIVE MATERIAL EXAMINED

Oscarella malakhovi Ereskovsky, 2006 (ZIN RAS 10697, ZIN RAS 10698: Japan Sea). *Oscarella kamchatkiensis* Ereskovsky, Sanamyan & Vishnyakov, 2009 (ZIN RAS 11058, ZIN RAS 11059 and ZIN RAS 11060: North Pacific, Avacha Gulf). *Oscarella lobularis* (Schmidt, 1862) (SME AE 008) and *Oscarella tuberculata* (Schmidt, 1868) (SME AE 003). NE Mediterranean Sea (Marseille region), underwater cave of Maire Island. *Oscarella microlobata* Muricy, Boury-Esnault, Bézac, Vacelet, 1996 (SME AE 010) and *Oscarella viridis* Muricy, Boury-Esnault, Bézac, Vacelet, 1996 (SME AE 009). NE Mediterranean Sea (Marseille region), underwater cave of Jarre Island. *Oscarella balibaloi,* Pérez, Ivanišević, Dubois, Pedel, Thomas, Tokina, Ereskovsky, 2011 (MNHN-DJV 129). NE Mediterranean Sea (Marseille region), underwater cave of Maire Island.

DIAGNOSIS

Cave dwelling thin and translucent *Oscarella*, yellowish-pale green or milky-beige in colour, forming hanging lobes with perforated surface curled up at the periphery, soft, slimy consistency and two mesohylar types of cells with inclusions: large, abundant vacuolar cells and small rare granular ones.

DESCRIPTION (FIGURE 2)

Sponge leaf-like, thinly encrusting on vertical surfaces, flat, irregular with fragile lobes hanging from the surface. Size up to 3 × 8 cm wide by 1–4 mm thick. Colour *in vivo* yellowish-pale green or milky-beige. Preserved fragments are light brown to ivory white. Sponge loosely attached to the substrate at intervals only. Surface is finely lobate and is perforated by abundant pores 9–12 μm in diameter. Bulge of tissue without perforation along periphery of individuals

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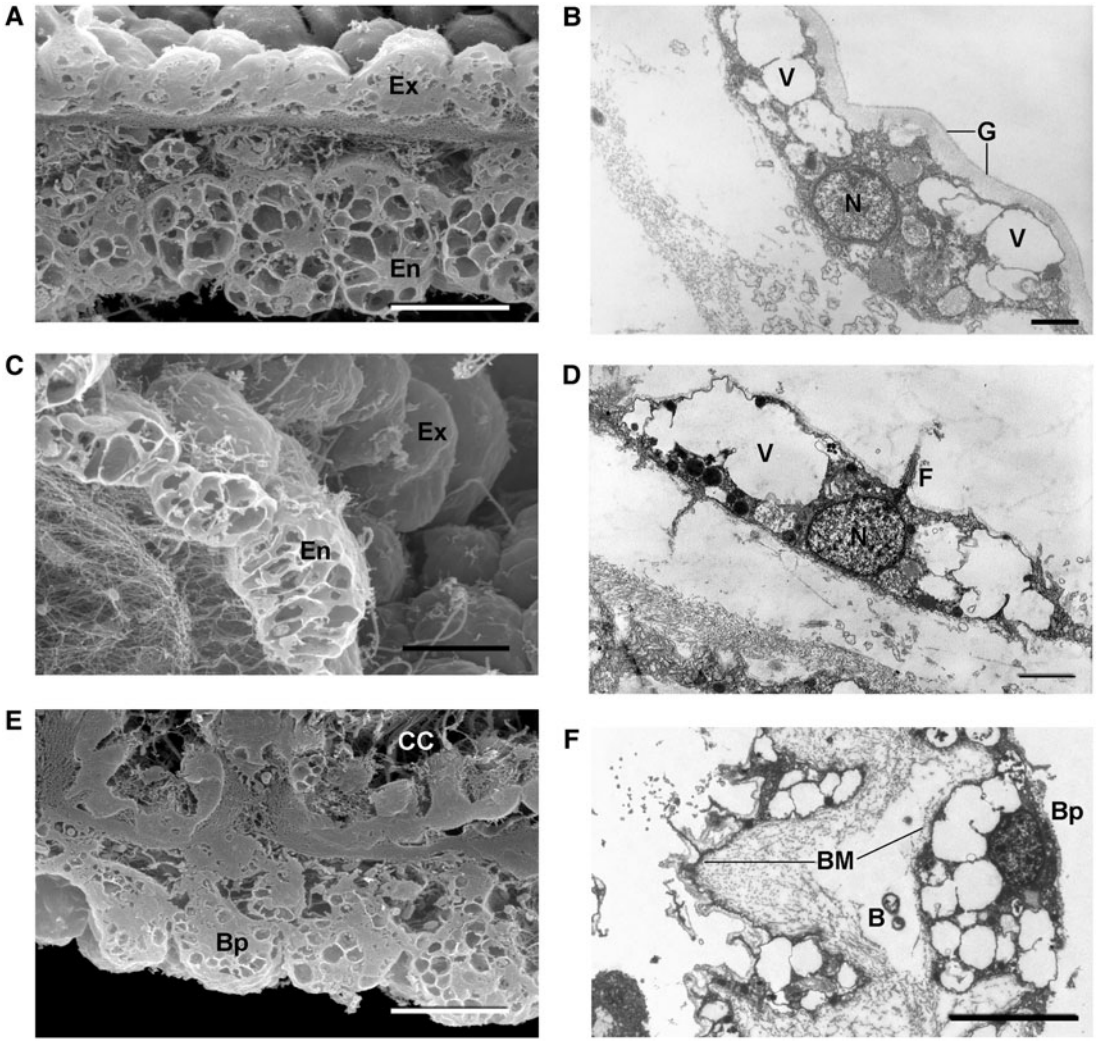

Fig. 3. *Oscarella nathaliae* sp. nov.: Pinacocytes (SEM and TEM). (A, B) Exopinacocytes; (C, D) endopinacocytes; (E, F) basopinacocytes. B, endobiotic bacteria; BM, basement membrane; Bp, basopinacocytes; CC, choanocyte chamber; En, endopinacocytes; Ex, exopinacocytes; F, flagellum; G, glycocalyx; N, nucleus; V, vacuole. Scale bars: A, 10 μm; B, 1 μm; C, E, 10 μm; D, 2 μm; F, 5 μm.

corresponding to exhalant canals. Oscula rare and located at the edge of the sponge, oscular tubes about 1–2 mm high. Consistency soft, slimy, very fragile and easy to tear.

SOFT TISSUE ORGANIZATION (FIGURE 2C, D)

Spicule and (or) fibre skeleton absent. Ectosome from 6 to 15 μm thick. Inhalant canals (15–40 μm in diameter) run perpendicular to the surface (Figure 2C). Choanocyte chambers ovoid to spherical, eurypilous, 27.1–*49.6*–61.2 μm in diameter (Figure 2C, D, E). Exhalant canals run towards a well developed system of basal cavities with diameter 7.2–*37.6*–78.4 μm, leading to the oscula situated on the external border of specimens. Ostia 8–23 μm in diameter.

CYTOLOGY (FIGURES 2F; 3A–F; 4A–C; 5A–C)

Exopinacocytes flagellated with oval to flat (in the ostia regions) shape, 7.6 μm wide by 5.5 μm high (N = 15; Figure 3A, B). Flagellae very poorly developed. Cytoplasm filled with electron translucent vacuoles 4.5–7.5 μm in diameter. Nucleus about 2.2 μm in diameter with basal or central position in flat cells. External surface of exopinacocytes covered by dense layer of glycocalyx 0.14–0.30 μm thick (Figure 3B).

Endopinacocytes flagellated, about 12.1 μm wide by 4.8 μm high (N = 15; Figure 3C, D). Cell free surface smooth or wavy (Figure 3C). Nucleus ovoid (2.2 μm in diameter), without nucleolus. Cytoplasm filled with electron translucent vacuoles 0.8–3.8 μm in diameter and rare phagosomes 0.8–1.1 μm in diameter.

Basopinacocytes oval, rare, flat, 10.5 μm wide by 6.5 μm high and similar to exopinacocytes (N = 8; Figure 3E, F). Flagellae absent. Cytoplasm filled with electron translucent vacuoles 0.6–3.2 μm in diameter. Nucleus centrally located about 2.5 μm in diameter.

Choanocytes ovoid to pyramidal, irregular, basal part about 4.2 μm wide by 5.4 μm high (N = 20; Figure 4A, B). Nucleus apical or centrally positioned, about 2.2 μm in diameter with rare small nucleolus. Cytoplasm includes from one to eight phagosomes 0.7–2.2 μm in diameter. Collar 4 μm in width, with about 38 microvilli. Choanocytes contact each other at their middle or basal parts (Figure 4A, B). Basal parts often show large outgrowth, anchoring the cell to the underlining collagen layer.

Apopylar cells roughly triangular in section, 6.9 μm wide by 4.3 μm high (N = 8; Figures 2F, 4C). Nucleus spherical,

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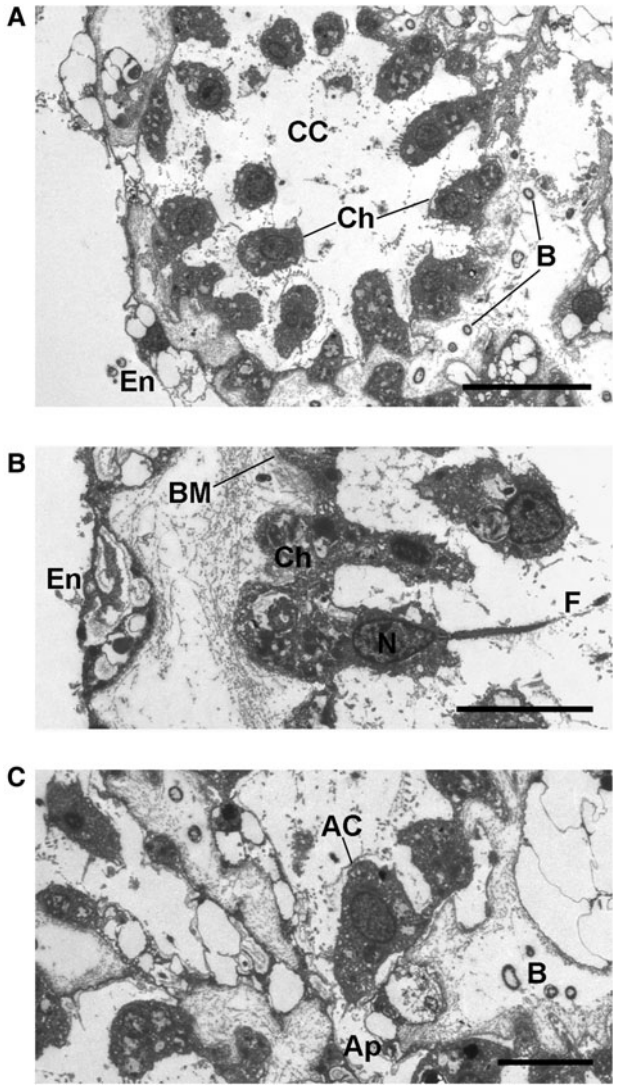

Fig. 4. *Oscarella nathaliae* sp. nov.: Choanocytes and apopylar cells (TEM). (A) Choanocyte chamber; (B) detail of a choanocyte; (C) apopylar cell. AC, apopylar cell; Ap, apopyle; B, endobiotic bacteria; BM, basement membrane; CC, choanocyte chamber; Ch, choanocytes; En, endopinacocytes; F, flagellum; N, nucleus. Scale bars: A, 10 μm; B, C, 5 μm.

up to 2.5 μm in diameter. Cytoplasm contains mitochondria, digestive vacuoles, and small osmiophilic inclusions.

Surface of endopinacocytes, choanocytes and apopylar cells covered by thin irregular layer of glycocalyx. Choanoderm and pinacoderm underlined by continuous basement membrane-like dense collagen layer 0.9–1.7 μm thick (Figures 3B, D, F, 4B).

Two types of cells with inclusions occur within the mesohyl.

Vacuolar cells ovoid to triangular, 18.3 μm × 11.3 μm (N = 10; Figure 5A). Nucleus ovoid, about 2.7 μm in diameter, with nucleolus. Cytoplasm thick with large electron translucent vacuoles 1.6–14.5 μm in diameter, often connected to each other and with very rare small electron-dense homogeneous granules 0.5 μm in diameter. Abundant cells often forming compact aggregates.

Granular cells ovoid to elongate, approximately 6.5 × 10.4 μm, sometimes amoeboid-like or irregular (N = 8; Figure 5B, C). Cytoplasm filled with small (approximately 0.2 μm in diameter) and larger (0.9–2.4 μm in diameter) electron translucent vacuoles, with vacuoles 1.6–3.4 μm in diameter, containing heterogeneous material, and irregular (0.5–1.2 μm) vacuoles with opaque homogeneous contents. Nucleus irregular, about 1.9 μm in diameter.

Archaeocytes absent in all samples examined.

ENDOBIOTIC BACTERIA (FIGURE 5D, E)

Two morphological types of extracellular endobiotic bacteria occur in the mesohyl: B1, B2 (Figure 5E). Type B1 are the most abundant, rod-like, slightly curved shape 0.9–*1.56*–2.0 μm in length and 0.7–*0.73*–0.87 μm in diameter (N = 22; Figure 5E). Cell wall consists of two membranes, and a tight periplasmic space. Cytoplasm forming a dark irregular layer under the cell wall. Small cavities often formed between cell wall and dark cytoplasm layer. Nucleoid filamentous network is irregular, with thick elements in the centre and thin filaments closer to the periphery. Surface covered with thin filamentous outgrowths, but in some bacteria, this layer forms a well developed capsule. Type B1 appears sometimes in the vacuoles of granular cells. Type B2 are very rare and small, spherical to oval about 0.3 μm in diameter (N = 3; Figure 5E). Cell wall consists of two membranes with a developed intermediate space. Cytoplasm forms a dark irregular layer under the cell wall. Nucleoid region with dense filamentous network.

REPRODUCTION (FIGURE 6A, B)

In sponges, collected in Martinique spermatogenesis observed in early June. Spermatic cysts surrounded by a thick layer of collagen located in the choanosome (Figure 6A). Spermatocysts resulting from choanocyte chambers differentiation ovoid, about 65 μm in diameter. Spermatogenesis asynchronous. Cysts generally with several generations of male germ cells forming clusters (Figure 6A, B). Mature spermatozoa with long terminal flagella and slightly elongated head containing acrosome and a large mitochondria (Figure 6B). Oogenesis asynchronous; oocytes occur simultaneously at different stages in the same individual, as well as embryos and larvae (Figure 6C). Embryos at different stages of development and mature larvae in the lower part of the choanosome of specimens collected at the end of March and early April in Jamaica and in May in Guadeloupe. Cinctoblastula larvae typical for Homoscleromorpha with oval shape about 153 × 255 μm. Posterior pole pink, surrounded by a belt of cells with intranucleolar paracrystalline inclusion. Basal side of ciliated larval cells lined by basement membrane (Figures 6D). Larval cavity filled with extracellular matrix (Figure 6C) including endobiotic bacteria of both morphotypes (Figure 6D).

HABITAT

Exclusively sciaphilous, occurring on vertical walls or ceilings of reef caves between 15 and 28 m.

DISTRIBUTION

Caribbean: north of Jamaica, Martinique, Guadeloupe (present study).

ETYMOLOGY

The specific name is given in honour of Nathalie Deneumoustier for her support while describing this sponge.

TAXONOMIC REMARKS

The external morphology and colour of *Oscarella nathaliae* sp. nov. are unique. The surface is unusually perforated by abundant pores. *Oscarella nathaliae* sp. nov. has small lobes hanging from

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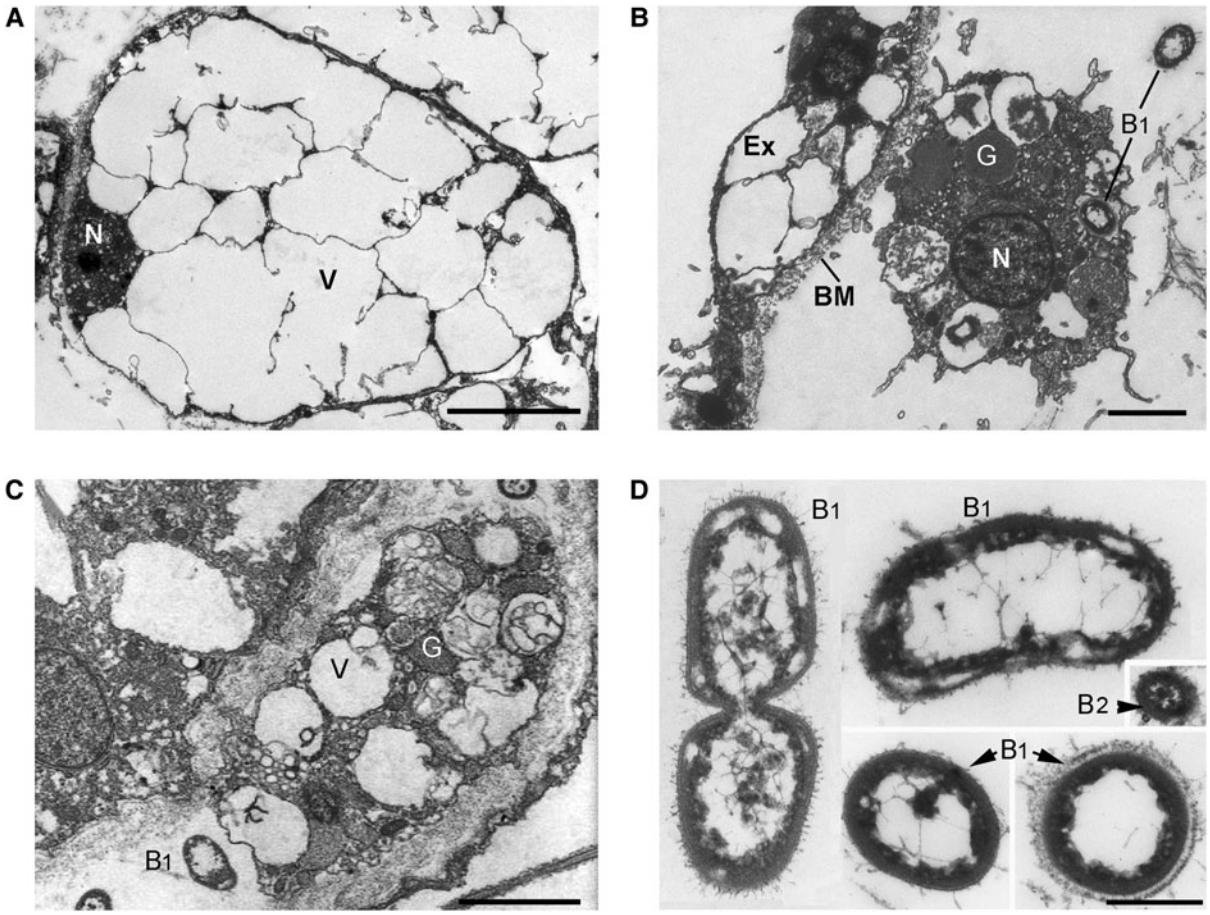

Fig. 5. *Oscarella nathaliae* sp. nov.: Mesohylar cells (TEM). (A) Vacuolar cell; (B, C) granular cells; (D, E) endobiotic bacteria. B, endobiotic bacteria; B1, endobiotic bacteria of morphotype 1; B2, endobiotic bacteria of morphotype 2; BM, basement membrane; Ex, exopinacocyte; G, granules; N, nucleus; V, vacuole. Scale bars: A, 5 μm; B, C, 3 μm; D, 0.5 μm.

the surface and a milky-grey or yellowish-pale green colour *in vivo*. These colours are different from any known *Oscarella* species. Unlike other members of this genus, the new species shows a leaf-like flat body with a bulgy edging lacking perforation. The loose, irregular and punctual attachment to the substrate is another external exclusive character of this new species. Cytological characters, such as cell types with different inclusions or pinacocytes features are important clues to identify *Oscarella* at the species level in modern taxonomy. Spherulous cells, vacuolar cells and granular cells are abundant in *Oscarella* and possess different varieties of structures in each species (Boury-Esnault *et al.*, 1992; Muricy *et al.*, 1996; Muricy & Pearse, 2004; Ereskovsky, 2006; Ereskovsky *et al.*, 2009a, b; Pérez *et al.*, 2011). The cell contents of *O. nathaliae* sp. nov. like in all other *Oscarella* species are simple, with only two kinds of cells with inclusions: vacuolar and granular cells. Vacuolar cells are the most characteristic cells of *O. nathaliae* sp. nov. As in *O. tuberculata,* they are abundant and large, forming compact masses. However, they are much larger in *O. nathaliae* sp. nov. (up to 18.3 × 11.3 μm) than in *O. tuberculata* (10.0 × 7.0 μm) (Boury-Esnault *et al.*, 1992). As for many other *Oscarella* species, the mesohyl of *O. nathaliae* sp. nov. lacks archaeocytes.

In contrast with the other *Oscarella* species, the pinacoderm cells (exo- and endopinacocytes) of *O. nathaliae* sp. nov. have large electron translucent vacuoles. An additional character useful to differentiate Homoscleromorpha is the presence of different types of endosymbiotic bacteria. Their ultrastructure and the number of types are specific to each species studied so far (Boury-Esnault *et al.*, 1992; Muricy *et al.*, 1996, 1999; Ereskovsky *et al.*, 2009b; Vishnyakov & Ereskovsky, 2009; Gloeckner *et al.*, 2012). *Oscarella nathaliae* sp. nov. has two types of endosymbiotic extracellular bacteria, like *O. tuberculata, O. viridis, O. carmela, O. malakhovi* and *O. balibaloi*. While *O. lobularis, O. imperialis* and *O. kamchatkensis* have three bacterial types, and *O. microlobata* has six distinct morphotypes (Vishnyakov & Ereskovsky, 2009).

Reproduction features of the new species are typical to all *Oscarella* (Ereskovsky, 2010).

Family PLAKINIDAE Schulze, 1880
Genus *Plakortis* Schulze, 1880

SYNONYMY
Plakortis Schulze, 1880: 449; *Placortis* Topsent, 1895:557; *Roosa* de Laubenfels, 1934: 2 (after Topsent, 1937:7).

TYPE SPECIES
Plakortis simplex Schulze, 1880.

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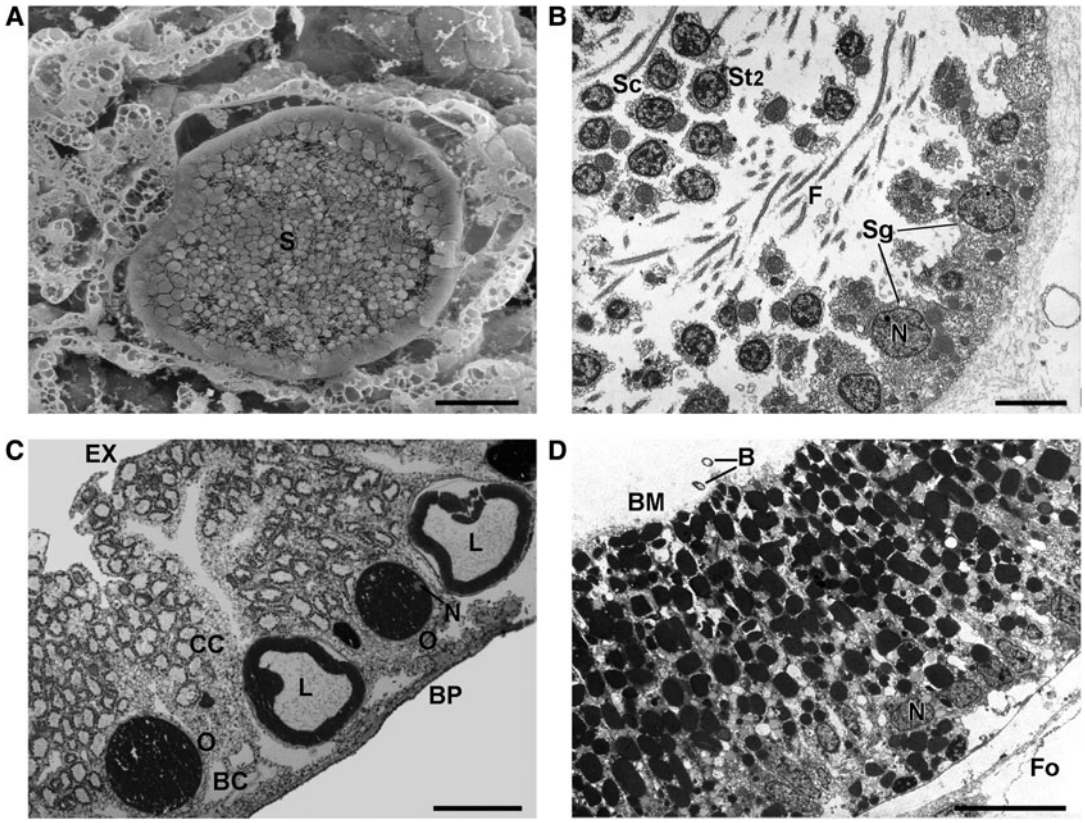

Fig. 6. *Oscarella nathaliae* sp. nov.: Reproduction (A) Spermatocyste in the choanosome (SEM); (B) detail of a spertmatocyste (TEM); (C) oocytes and cinctoblastulae larvae in the choanoderm (LM); (D) cell wall of a larva (TEM). B, endobiotic bacteria inside a larva; BC, basal cavity; BP, basopinacoderm; BM, basement membrane; CC, choanocyte chamber; EX, exopinacoderm; F, flagella; Fo, follicle; L, larva; N, nucleus; O, oocyte; S, spermatic cyste; Sg, spermatogonia; St, spermatide; St2 spermatocyte 2. Scale bars: A, 20 μm; B, 5 μm; C, 100 μm; D, 50 μm.

DIAGNOSIS (FROM MURICY & DIAZ, 2002)

Thinly to massively encrusting plakinids with a skeleton mostly formed by small (50–200 μm) diods with triods in varying abundance. Deformed calthrops can be found in some specimens. Some species have microrhabds (5–20 μm) distributed regularly in the sponge body. Aquiferous system intermediate between sylleibid-like and leuconoid, with eurypylous choanocyte chambers regularly distributed around exhalant canals. Both ectosomal inhalant cavities and basal exhalant cavities are usually present. Skeleton confused, dense, without ectosomal specialization or differential location of spicules.

Plakortis myrae sp. nov.
(Figure 7)

TYPE MATERIAL

Holotype: RBINS POR 68, Jamaica, Pear Tree Bottom Cave, 5 km east of Discovery Bay, 18°27′57.31″N 77°21′18.49″W, 28 m depth, coll. A. Ereskovsky and Ph. Willenz, 1 April 2005.

Paratype: none.

COMPARATIVE MATERIAL EXAMINED

Plakortis dariae (RBINS POR 65 and RBINS POR 76): Jamaica. *Plakortis edwardsi* sp. nov. (RBINS POR 69): Jamaica. *Plakortis simplex* Schulze, 1880 (SME AE 005): NE Mediterranean Sea (Marseille region), underwater cave 3PP.

DIAGNOSIS

Plakortis light brown. Diods of one size-class, 66.6–119.0 μm, with lightly to strongly marked centre, some sinuous-bent. Triods Y- or T-shaped with actines 17.8–53.0 μm long. Microrhabds abundant 5.0–12.4 μm long.

DESCRIPTION (FIGURE 7A)

Sponge thickly encrusting to cushion shaped, irregular. Size up to 4 × 10 cm wide by 1–2 cm thick. Colour *in vivo* is homogeneous light brown. Preserved specimens are dark brown. Sponge firmly attached to the substrate. Surface even and smooth. Oscules slightly elevated with translucent inner rim around the oscula 2–5 mm in diameter, contracted in alcohol. Consistency soft, compressible.

SKELETON (FIGURE 7B, C)

Ectosome is distinct in transversal sections, 0.5–0.6 mm thick with multispicular tracts 20–40 μm in diameter, oriented perpendicular to the surface; forming irregularly elliptical meshes, spicules rarely cross the surface. Ectosome with a tangential reticulation of spicule tracts forming elliptical meshes (Figure 7B, C). Choanosome formed by a dense and relatively confused alveolar arrangement of diods and microrhabds with rounded meshes 60–80 μm in diameter (Figure 7D).

SPICULES (FIGURE 7E–G)

Diods abundant, thin, irregular, slightly curved, lightly to sometimes strongly marked protuberant, sometimes sinuous

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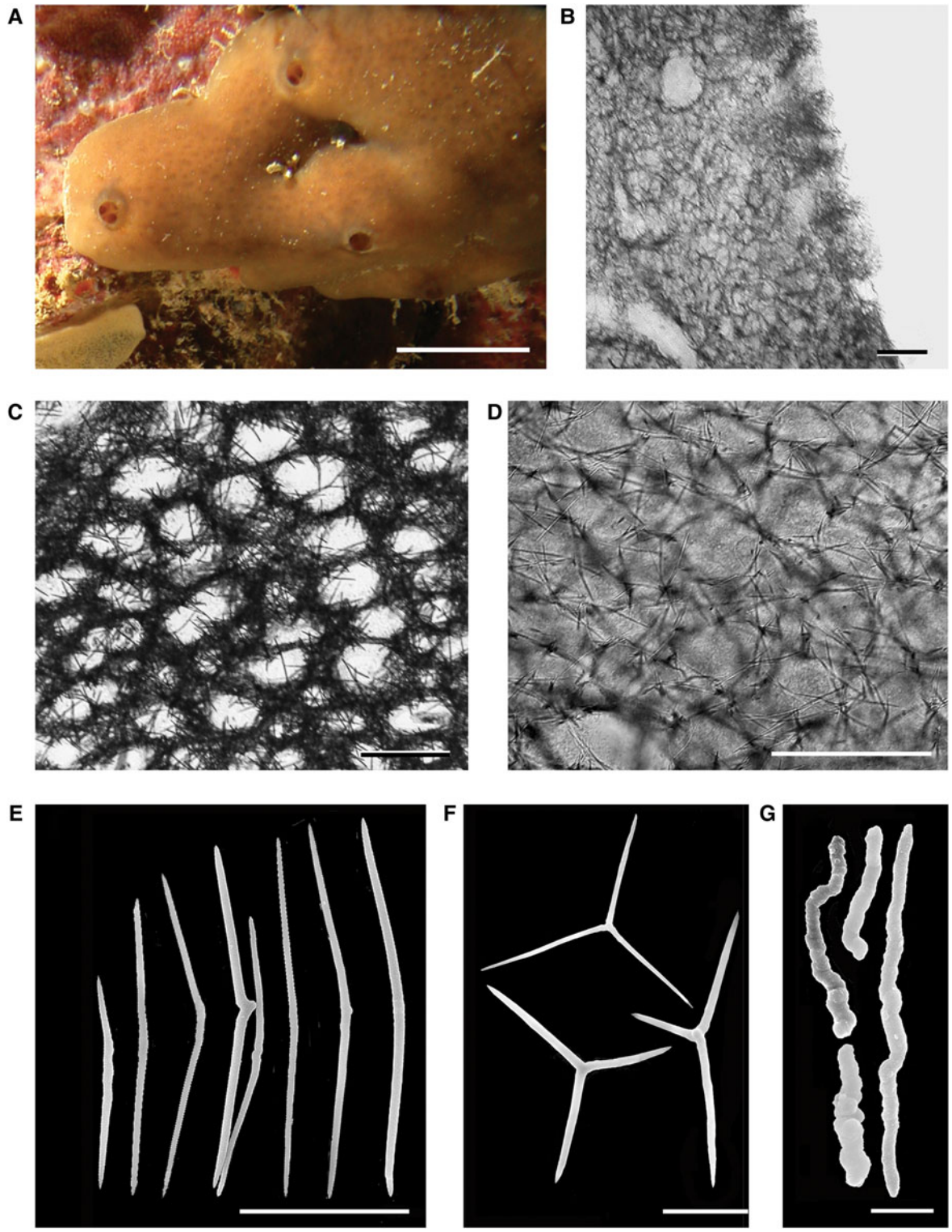

Fig. 7. *Plakortis myrae* sp. nov. (A) *In situ* close-up; (B) transverse section through the ectosomal skeleton (LM); (C) tangential section through the ectosome (LM); (D) transverse section through the choanosomal skeleton (LM); (E) diods; (F) triods; (G) microrhabds. Scale bars: A, 2 cm; B–D, 200 μm; E, 50 μm; F, 20 μm; G, 2 μm.

S-bent centre and sharp endings with high length variations: 66.6–*100.2*–119.0/2–4 μm (N = 20; Figure 7E). Triods abundant, regular to irregular Y- or T-shaped, with sharp endings; actines 17.8–*37.1*–53.5/1.8–3.3 μm (N = 8; Figure 7F). Microrhabds abundant, irregularly twisted, sinuous: 5.0–*7.5*–12.4/0.44–0.97 μm (N = 5; Figure 7G). Microdiods or small diods absent.

HABITAT

Exclusively sciaphilous, occurring on vertical walls of a coralligenous reef cave between 26 and 28 m depth.

DISTRIBUTION

Caribbean: north of Jamaica (present study).

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ETYMOLOGY
The specific name is given in honour of Myriam Doriaux to celebrate 40 years of patient assistance to the last author.

TAXONOMIC REMARKS
Nineteen species of the genus *Plakortis* are known throughout the world, four of which occur in the Caribbean Sea and more precisely in the Jamaican region: *P. angulospiculatus, P. zyggompha, P. halichondrioides* and *P. simplex* (Carter, 1879; Schulze, 1880; de Laubenfels, 1934; Hechtel, 1965; Boury-Esnault, 1973; Wiedenmayer, 1977; Pulitzer-Finali, 1986; Zea, 1987; Mothes & Bastian, 1993; Diaz & van Soest, 1994; Lehnert & van Soest, 1998; Moraes & Muricy, 2003). Two other species are known from the Western Atlantic Ocean: *Plakortis insularis* and *P. microrhabdifera* (Moraes & Muricy, 2003).

Plakortis myrae sp. nov. is part of the *P. lita* species group which is characterized by the presence of microrhabds. The group includes three species (*P. hooperi* Muricy, 2011 from Papua New Guinea, *P. lita* de Laubenfels, 1954 from Indonesia and Papua New Guinea, and *P. microrhabdifera* Moraes & Muricy, 2003 from NE Brazil). *Plakortis myrae* sp. nov. differs from *P. microrhabdifera* in that it presents triods which are absent in *P. microrhabdifera*; it differs from *P. hooperi* in its reticulated ectosome, whereas the latest is characterized by a confused ectosomal skeleton; and it differs from *P. lita* in its light brown colour (in *P. lita* the colour is reddish-brown externally and lighter internally). *Plakortis myrae* sp. nov. differs from all other *Plakortis* species in that it has irregular microrhabds.

Plakortis edwardsi sp. nov.
(Figure 8)

TYPE MATERIAL
Holotype: RBINS POR 69 Jamaica, Pear Tree Bottom Cave, 5 km east of Discovery Bay, 18°27′57.31″N 77°21′18.49″W, 25 m depth, coll. A. Ereskovsky and Ph. Willenz, 28 March 2005.

Paratype: none.

COMPARATIVE MATERIAL EXAMINED
Plakortis dariae sp. nov. (RBINS POR 65 and RBINS POR 76): Jamaica. *Plakortis myrae* sp. nov. (RBINS POR 68): Jamaica. *Plakortis simplex* Schulze, 1880 (SME AE 005): NE Mediterranean Sea (Marseille region), underwater cave 3PP.

DIAGNOSIS
Plakortis light to dark brown with different colour patches on the same specimens. Consistency soft, compressible, ectosome without alveolar arrangement of skeleton. Diods abundant of one size-class (110–128 μm) with thick, sinuous, S-bent centres; small diods rare, slightly twisted, sinuous (22–31 μm), triods not abundant Y- or T-shaped, some sinuous close to the centre, actines 28–59 μm. Microrhabds absent.

DESCRIPTION (FIGURE 8A)
Sponge thickly encrusting to massive, lobate, irregular. Size up to 2 × 8 cm by 1.0 – 2.5 cm thick. Colour *in vivo* light to dark brown with different colour patches on the same specimens. Choanosome is green-brown. Preserved specimens are dark brown. Sponge firmly attached to the substrate. Surface smooth, regular. Oscules flush with the surface, 2–4 mm in diameter, contracted in alcohol. Consistency soft, compressible, fragile.

SKELETON (FIGURE 8B–D)
Ectosome in transversal sections 60–80 μm thick, with a loose confused arrangement of diods in low density, without signs of alveolar arrangement (Figure 8B). Spicules never cross the surface. Ectosome with a tangential reticulation of spicule tracts forming rounded meshes (Figure 8C). Choanosome formed by a confused, dense mass of diods without a clear alveolar arrangement (Figure 8D).

SPICULES (FIGURE 8E–G)
Diods of two categories: large and small. Large diods irregular to almost straight, slightly curved, centre thick, sinuous, S-bent centre, sometimes with small protuberance; endings acerate: 110.0–*118.0*–128.0/2.6–3.0 μm (N = 20; Figure 8E). Small diods rare, slightly irregularly twisted, sinuous with rugged surface and stub edges: 22.4–*27.9*–31.1/0.56–*0.77*–1.09 μm (N = 5; Figure 8F). Triods not abundant, regular to irregular, Y- or T-shaped, generally sinuous close to the centre, with sharp endings; actines sometimes slightly curved 28.1–*42.9*–59.4/2–2.6 μm (N = 7; Figure 8G). Microrhabds absent.

HABITAT
Occurs on vertical shaded sides of massive reef boulders among coralline algae between 23 and 26 m depth.

DISTRIBUTION
Caribbean: north of Jamaica (present study).

ETYMOLOGY
The specific name is given to honour Tracey Edwards, who first collected this sponge.

TAXONOMIC REMARKS
Plakortis edwardsi sp. nov. is the only species of this genus with very small diods (< 30 μm). See the same section after *Plakortis dariae* sp. nov. description.

Plakortis dariae sp. nov.
(Figure 9)

TYPE MATERIAL
Holotype: RBINS POR 65, Jamaica, Pear Tree Bottom Cave, 5 km east of Discovery Bay, 18°27′57.31″N 77°21′18.49″W, 28 m depth, coll. A. Ereskovsky and Ph. Willenz, 27 March 2005.

Paratype: RBINS POR 76, the same site, coll. A. Ereskovsky and Ph. Willenz, 28 March 2005.

COMPARATIVE MATERIAL EXAMINED
Plakortis myrae sp. nov. (RBINS POR 68): Jamaica. *Plakortis edwardsi* sp. nov. (RBINS POR 69): Jamaica. *Plakortis simplex* Schulze, 1880 (SME AE 005): NE Mediterranean Sea (Marseille region), underwater cave 3PP.

DIAGNOSIS
Plakortis light green with thin slightly brownish patches. Diods abundant of two size-classes; large: 67.3–112.2 μm, with lightly to strongly marked centre, some sinuous-bent;

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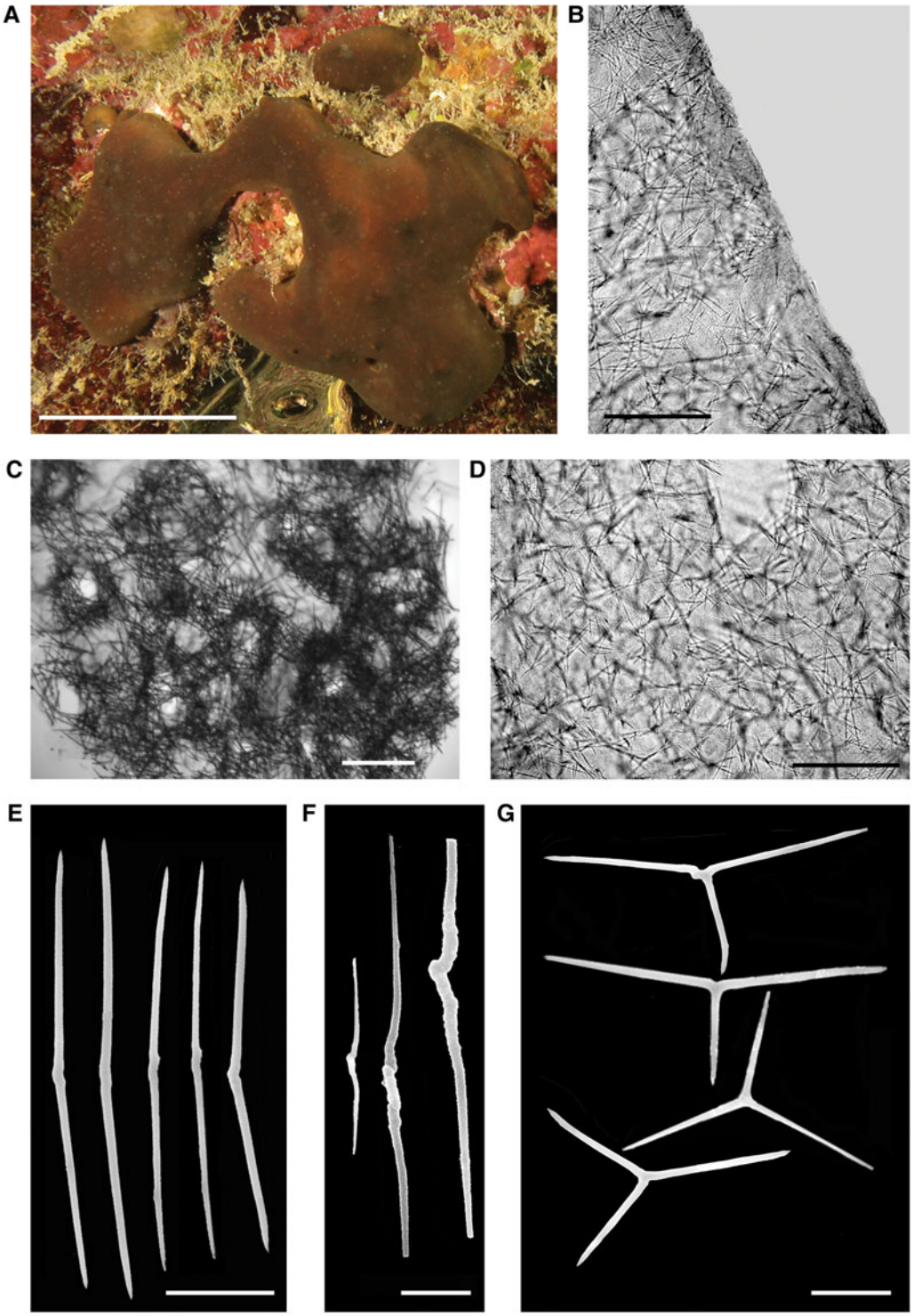

Fig. 8. *Plakortis edwardsi* sp. nov. (A) *In situ* close-up; (B) transverse section through the ectosome (LM); (C) tangential section through the ectosome (LM); (D) transverse section through the choanosome (LM); (E) diods; (F) small diods; (G) triods. Scale bars: A, 2 cm; B–D 200 μm; E, 25 μm; F, 5 μm; G, 20 μm.

small diods rare, irregular, slightly curved, with protuberant centre, often deformed with one end blunt: 30–59.5 μm. Triods rare, regular; actines 20–43.5 μm long. Microrhabds and microdiods absent.

DESCRIPTION (FIGURE 9A)

Sponge thickly encrusting to cushion shaped, irregular. Size up to 2 × 8 cm wide by 1–3 cm thick. Colour *in vivo* is light green with thin slightly brownish patches. Sponge firmly attached to the substrate. Surface even and smooth. Oscules flush with the surface, 3–8 mm in diameter, contracted in alcohol. Consistency soft, compressible.

SKELETON (FIGURE 9B–D)

Ectosomal skeleton is distinctly reticulate, with multispicular tracts oriented perpendicular to the surface, forming circular or irregular meshes in tangential sections (Figure 9C). Spicules cross the surface (Figure 9B). The ectosome

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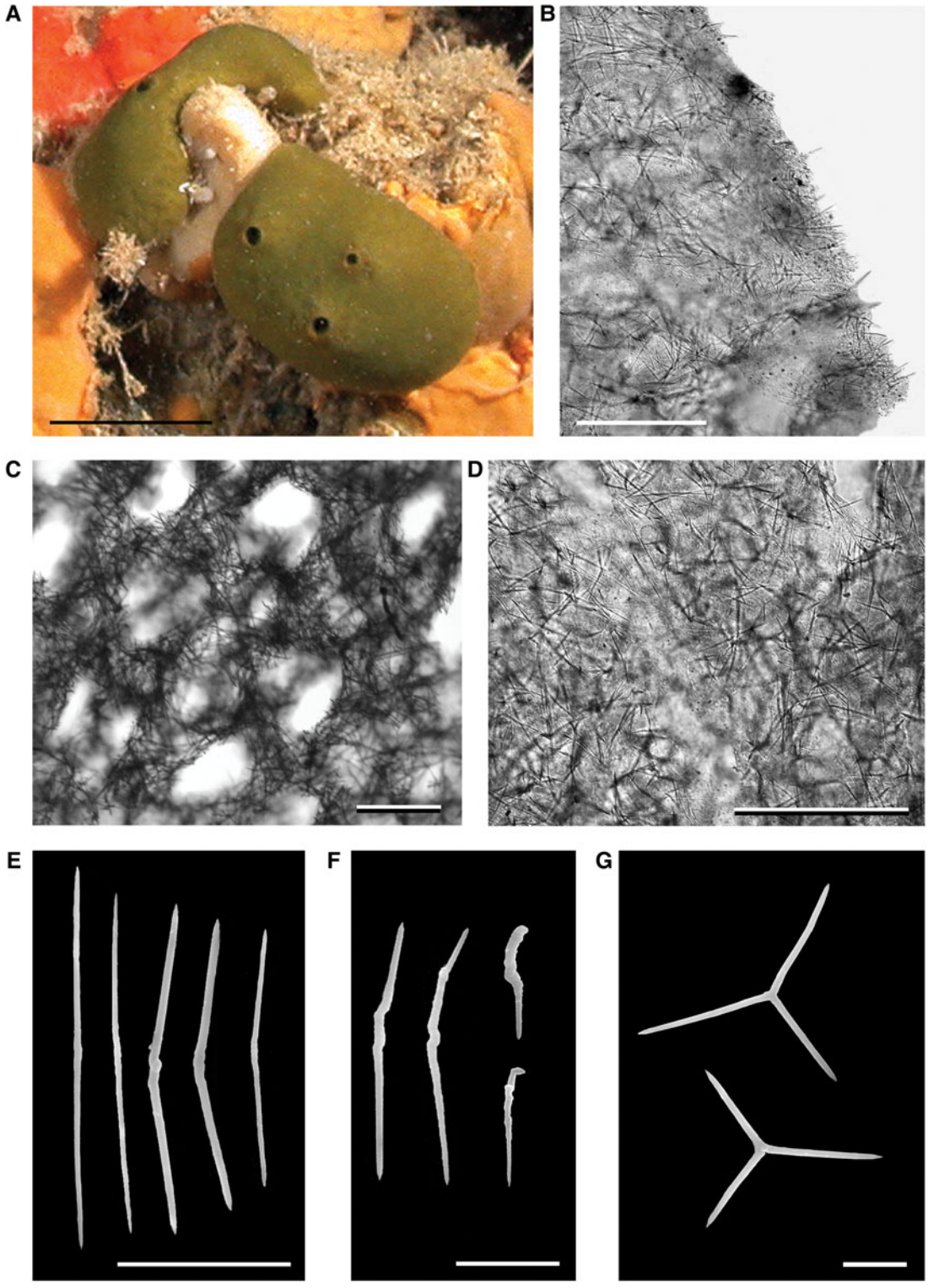

Fig. 9. *Plakortis dariae* sp. nov. (A) *In situ* close-up; (B) transverse section through the ectosome (LM); (C) tangential section through the ectosome (LM); (D) transverse section through the choanosome (LM); (E) diods; (F) small diods; (G) triods. Scale bars: A, 2 cm; B–D, 200 μm; E, 50 μm; F, G, 20 μm.

(120–170 μm thick) is poorly differentiated, slightly denser than the choanosome, without subectosomal lacunae. Choanosomal skeleton confused to vaguely reticulate (Figure 9D).

SPICULES (FIGURE 9E–G)

Diods of two categories: large and small. The large diods are abundant, thin, irregular, slightly curved, lightly to sometimes strongly marked protuberant centre, sometimes sinuous S-bent centre and sharp endings: 67.3–*89.7*–112.2/1.6–2.8 μm (N = 20; Figure 9E). The small diods are rare, irregular, slightly curved, marked protuberant, often deformed with one end blunt: 30–*46.1*–59.5/0.8–2.1 μm (N = 9; Figure 9F). Triods rare, regular to irregular form, with sharp endings; actines 20–*35.8*–43.5/2.2–2.7 μm

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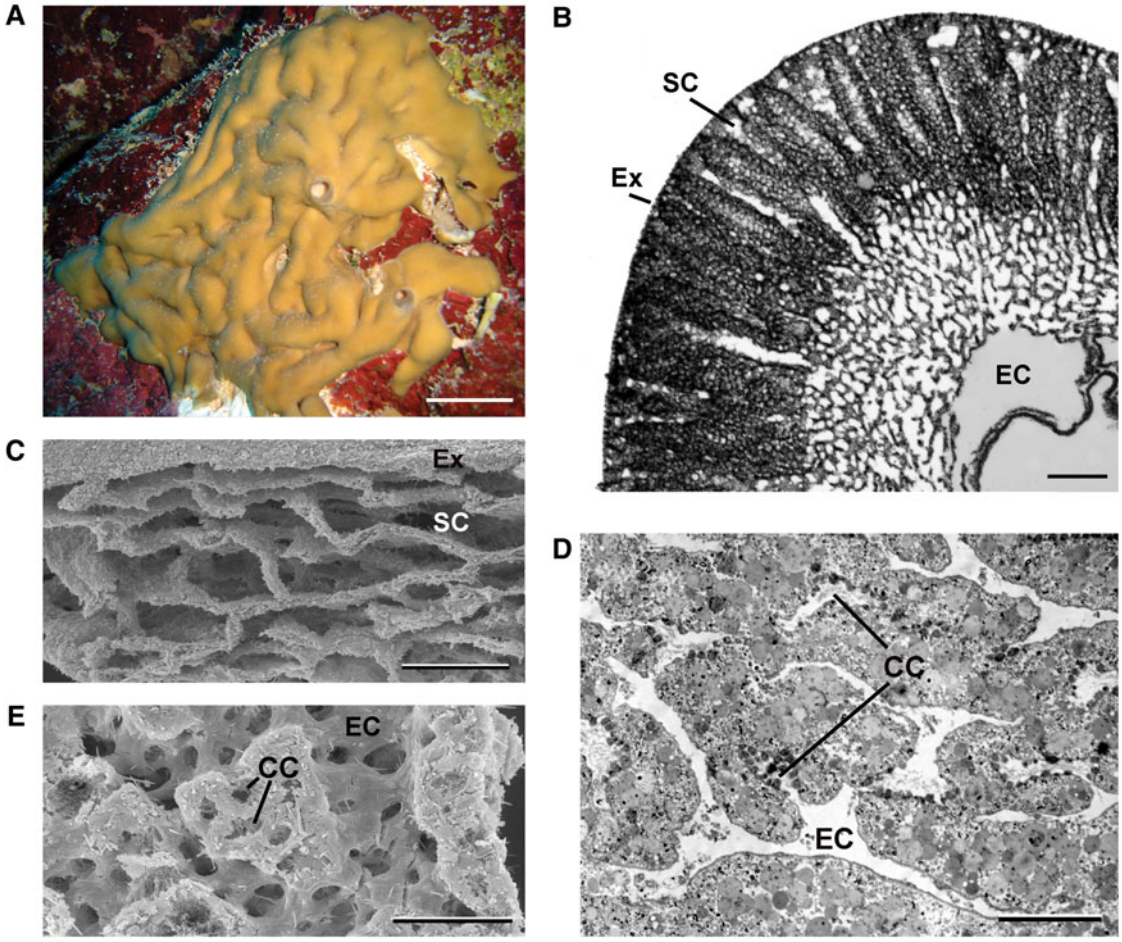

Fig. 10. *Plakina jamaicensis* RBINS POR 70. (A) *In situ* close-up; (B) transverse section through the tissue, showing the general organization of the skeleton and aquiferous system (LM); (C) transverse section through the ectosome (SEM); (D) transverse section through the choanosome (LM); (E) transverse section through the choanosome (SEM). CC, choanocyte chambers; EC, exhalant canals; Ex, exopinacoderme; SC, subdermal inhalant cavities. Scale bars: A, 2 cm; B, 500 μm; C, 200 μm; D, 50 μm ; E, 100 μm.

(N = 6; Figure 9G). Microrhabds and microdiods are absent.

HABITAT

Exclusively sciaphilous, occurring on vertical walls of coralligenous reef caves between 26 and 28 m depth.

DISTRIBUTION

Caribbean: north of Jamaica (present study).

ETYMOLOGY

The specific name is given in honour of Daria Tokina, without whom this research would have been impossible.

TAXONOMIC REMARKS

Based on the spicule size and composition, *Plakortis edwardsi* sp. nov. and *P. dariae* sp. nov. should be placed in the *P. simplex* species group, which is characterized by the absence of any distinctive characters such as microrhabds, quasiamphiasters, or large spicules (Muricy, 2011). This group includes 12 species (*P. albicans* Cruz-Barraza & Carballo, 2005, *P. communis, P. copiosa* Pulitzer-Finali, 1993, *P. edwardsi* sp. nov., *P. erythraena* Lévi, 1958, *P. galapagensis, P. insularis, P. japonica* (Hoshino, 1977), *P. nigra* Lévi, 1953, *P. dariae* sp. nov., *P. simplex* and *P. zyggompha*). However, such placement is not supported by molecular data for which *P. edwardsi* sp. nov. is grouped with *P. halichondroides/P. angulospiculatus* while *P. dariae* sp. nov. is grouped with *P. simplex* (Figure 13).

Plakortis edwardsi sp. nov. and *P. dariae* sp. nov. both differ from other members of this group and from other *Plakortis* species by the presence of diods of two size-classes separated by a size gap. Although both species display diods of similar sizes (*P. edwardsi* sp. nov.: smaller 22–31 μm and larger 110–128 μm; *P. dariae* sp. nov.: smaller 30–59.5 μm and larger: 67.3–112 μm) their skeletons are notably different. The ectosomal skeleton of *P. edwardsi* sp. nov. has a loose confused arrangement of diods in low density, without signs of alveolar arrangement and the spicules do not cross the surface. The ectosomal skeleton of *P. dariae* sp. nov. is distinctly reticulate, with multispicular tracts oriented perpendicular to the surface, forming circular or irregular meshes. Spicules cross the surface. Two other *Plakortis* species with diods of two size-categories were previously reported: *P. bergquistae* Muricy, 2011 and *P. galapagensis* Desqueyroux-Faúndez and van Soest, 1997.

Plakortis bergquistae differs from the new species described here in diods sizes and shape: smaller 91–163/2–6 μm, larger 202–356/5–11 μm; both are straight or slightly curved, smooth, regular, with slightly thickened centre. On the

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contrary, diods of *P. edwardsi* sp. nov. are almost straight, slightly curved, but irregular, with a thick sinuous S-bent centre, sometimes with small protuberance. Diods of *P. dariae* sp. nov. are slightly curved, irregular, with a lightly to sometimes strongly marked protuberant, sometimes sinuous S-bent centre. *Plakortis galapagensis* differs also from both new species in that it has larger diods: smaller 27–92/1.5–4 μm and larger 126–165/4–8 μm.

Genus *Plakina* Schulze, 1880

SYNONYMY

[*Achinoe*] Gray, 1867a: 546 (unavailable name). *Plakina* Schulze, 1880: 448. *Placina* Topsent, 1890d: 231. *Plakoosa* de Laubenfels, 1936b: 462 (after Topsent, 1937: 7).

TYPE SPECIES

Plakina monolopha Schulze, 1880.

DIAGNOSIS (MURICY & DIAZ, 2002)

Thinly to massively encrusting Plakinidae with a spiculation of diods, triods, and calthrops, and with homogeneously ramified lophocalthrops with one, two, three, or four lophate rays. Candelabra (heterolophose calthrops) absent. Lophocalthrops usually concentrated at the sponge surface and along bordering canals. Development of the ectosome is variable, and subectosomal cavities may be present. A large basal cavity is present in most species. Proportion of mesohyl to chambers varies from 0.7 to 1.8:1. Choanocyte chambers are eurypylous or aphodal, usually with a radial arrangement around incurrent and excurrent canals (called sylleibid-like arrangement).

Plakina jamaicensis Lehnert and van Soest, 1998 (Figures 10, 11)

MATERIAL EXAMINED

Holotype: ZMA POR. 12736, Jamaica, Discovery Bay, # J319, 17.7.1993, fore reef, underneath *Montastrea annularis*, 35 m depth.

Paratype: RBINS POR 70 and RBINS POR 79, Jamaica, Chalet Caribe Cave, west of Montego Bay, 18°27′14.78″ N 77°58′17.29 W, 23 m depth, coll. A. Ereskovsky and Ph. Willenz, 3 April 2005.

COMPARATIVE MATERIAL EXAMINED

Plakina trilopha Schulze, 1880 (SME AE 006), *Plakina jani* Muricy, Boury-Esnault, Bézac, Vacelet, 1998 (SME AE 007), NE Mediterranean Sea (Marseille region, La Ciotat), underwater cave 3PP.

DIAGNOSIS

Plakina slightly tough, compressible, creamy orange to yellowish or fawn, encrusting, up to 1 cm thick, surface brain-like convoluted. Spicule types irregular and variable in shape. Diods common with central swelling smooth or with one to two spines. Triods abundant, never lophose. Monolophose calthrops rare (1m, ts or only 1m). Dilophose calthrops rare (1m, 2d, ts). Trilophose calthrops very rare (1m, 2d, ts). Tetralophose calthrops common (1m, 2d, ts).

DESCRIPTION (FIGURE 10A)

Sponge firm but compressible, firmly attached to the substrate, thinly encrusting with convoluted brain-like aspect due to swelled sub-ectosomal exhalant canals converging to rare and short oscules. Size from 9 × 9 to 30 × 40 cm wide by about 1 cm thick. Colour *in vivo* is yellowish or fawn. Preserved fragments are light brown. Surface smooth, irregular borders. Oscules, about 5 mm in diameter, surrounded by a thin transparent oscular rim. Found on vertical shaded areas of coral reefs.

GENERAL ORGANIZATION (FIGURE 10B–E)

Ectosome is 17–43 μm thick, separated from the choanosome by well developed system of irregular inhalant/exhalant canals 30–140 μm wide (Figure 10B, C). Aquiferous system (Figure 10C–E) is leuconoid, choanocyte chambers spherical or ovoid, diplodal, 35–50 μm in diameter. Spicules of all kinds are haphazardly dispersed throughout the mesohyl. The spiculation is denser in the ectosome, The choanosome is somewhat less densely packed with spicules.

SPICULES (FIGURE 11A–H)

All spicule types are irregular and variable in shape.

Diods abundant, irregular, sinuous, with actines gradually pointing to sharp endings. Central swelling typically S-bent, with one or two spines or almost smooth: 69.0–*78.2*–91.1/2.3–2.9 μm (N = 20; Figure 11A). Triods abundant, central swelling, actine size, and angle between actines variable. Never lophose or with bifurcated rays. Actines: 22.1–*27.5*–32.1/1.3–2.0 μm (N = 9; Figure 11B). Calthrops common, actines unequal, never lophose. Actines: 5.5–*9.6*–11.6/0.8–2.9 μm (N = 9; Figure 11C). Monolophose calthrops rare, irregular, with some bifurcated or trifurcated actines (ramification pattern 1m, ts or only lm). Total length: 22.4–*27.1*–31.7/1.1–1.6 μm (N = 4; Figure 11D). Dilophose calthrops rare, irregular, some lophose bifurcated actines with one or two distal spines (ramification pattern 1m, 2d, ts). Total length: 24.9–*28.3*–35.7/1.3–1.9 μm (N = 6; Figure 11E). Trilophose calthrops rare, actines bifurcated or trifurcated, lophose actines with two distal spines (ramification pattern 1m, 2d, ts). Total length: 25.7–*28.4*–29.2/1.4–1.54 μm (N = 3; Figure 11F). Tetralophose calthrops common, actines bifurcated, rarely trifurcated or quadrifurcated, with one or two distal spines (ramification pattern 1m, 2d, ts). Total length: 17.6–*19.8*–23.3/1.1–1.8 μm (N = 12; Figure 11G). Distally tetralophose calthrops very rare with two to four distal spines (ramification pattern 1d, ts). Total length 26–*30*–31.8 μm (N = 2; Figure 11H).

HABITAT

On vertical shaded areas of coral reef, 20–25 m depth.

DISTRIBUTION

Caribbean: Bahamas, Panama (Diaz and Rützler, 2009), North Jamaica (Lehnert & van Soest, 1998; present study).

TAXONOMIC REMARKS

Our redescription of *Plakina jamaicensis* Lehnert and van Soest, 1998, based on the revision of the holotype as well as on a newly collected specimen, reveals essential differences with the original description of spicule composition and size. In addition to diods, triods, rare calthrops and tetralophose calthrops originally reported, our investigations reveal abundant simple calthrops and additional monolophose, dilophose and trilophose calthrops.

Plakina jamaicensis differs markedly from the two other known Caribbean *Plakina* by its convoluted brain-like

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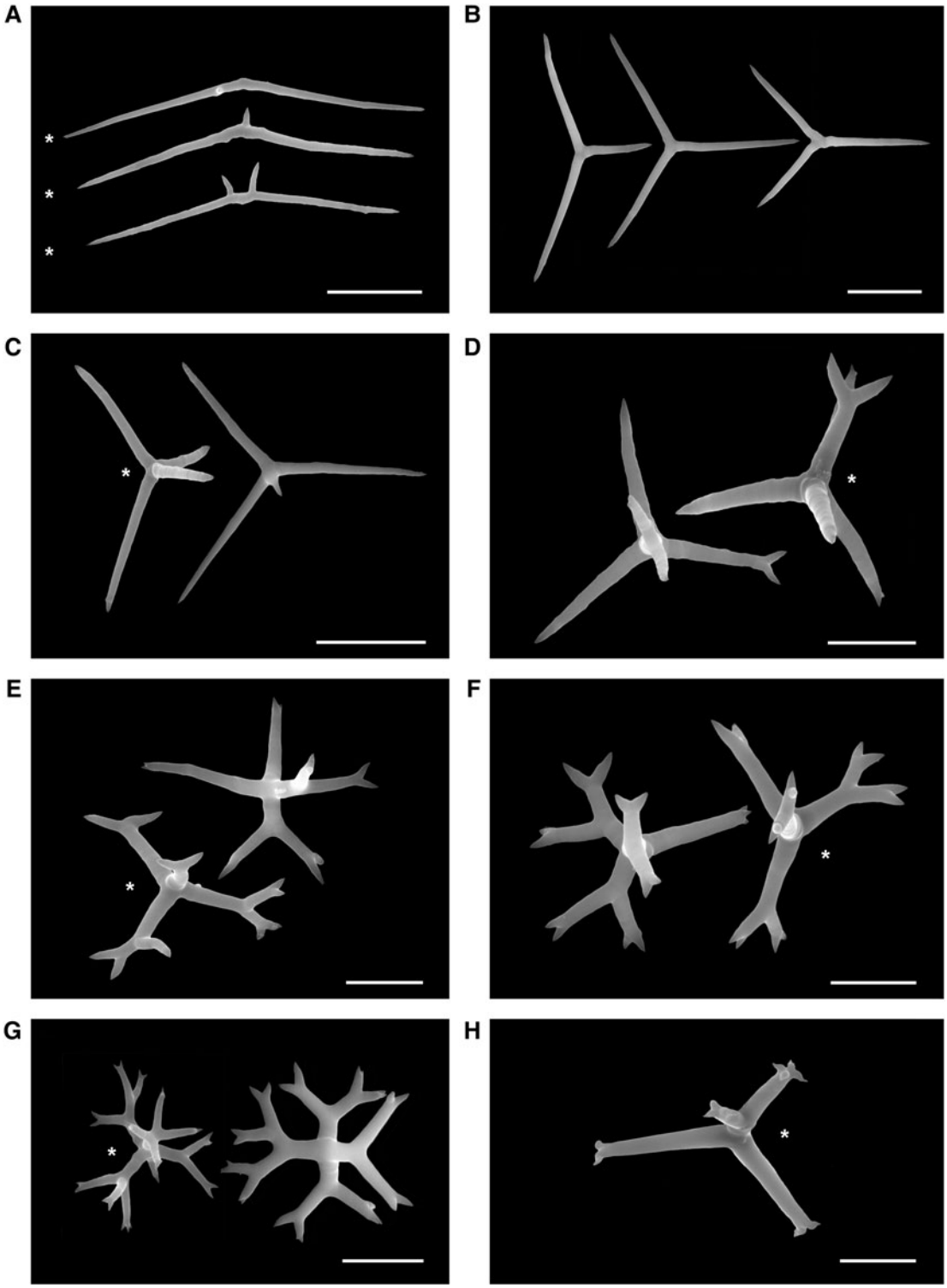

Fig. 11. Spicules of *Plakina jamaicensis*. Holotype ZMA POR 12736; paratype RBINS POR 70 (*) (SEM). (A) Diods; (B) triods; (C) calthrops; (D) monolophous calthrops; (E) dilophous calthrops; (F) trilophous calthrops; (G) tetralophous calthrops; (H) distally tetralophose calthrop. Scale bars: A–C, 20 μm; D–H, 10 μm.

surface, spicule assortment and dimensions, as well as from the six Atlantic species. However, among Mediterranean *Plakina* species, *P. jani* and *P. trilopha* display a similar external shape. *Plakina brachylopha* has only calthrops and monolophose calthrops, both larger than in *P. jamaicensis* (Topsent, 1927). *Plakina elisa* possesses diods, triods and monolophose calthrops which are smaller than in *P. jamaicensis* and in addition it has a typical blue colour *in vivo* (de Laubenfels, 1936a). Spicules of *P. tetralopha* include triods, calthrops, trilophose and tetralophose calthrops that all have different dimensions than in *P. jamaicensis* (Hechtel, 1965). *Plakina versatilis* (Schmidt, 1880) differs from *P. jamaicensis* in the presence of monolophose and trilophose calthrops only, according to the original description. The type specimen,

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Corticium versatile TYPE = MCZ8140 (Orig n° 342) Coll A. Agassiz, was accidentally mixed up with a *Halichondria* and lost in the Harvard University Museum collection as previously mentioned by B. Austin (personal communication to A. Johnson, Museum of Comparative Zoology, Harvard University, 1986). Our analysis of the spicules of this specimen confirmed the only presence of oxea.

Interestingly, part of the Mediterranean *Plakina* species shows the same spicules composition as *P. jamaicensis* (*P. trilopha*, *P. jani*, *P. endoumensis* Muricy, Boury-Esnault, Bézac, Vacelet 1998, and *P. weinbergi* Muricy, Boury-Esnault, Bézac, Vacelet, 1998), whereas the other Mediterranean species have a different spicule combination (*P. monolopha* Schulze, 1880, *P. dilopha* Schulze, 1880 and *P. crypta* Muricy, Boury-Esnault, Bézac, Vacelet, 1998). Here we provide the comparative analysis of *P. jamaicensis* with *P. trilopha*, *P. jani*, *P. endoumensis*, and *P. weinbergi* (Table 1).

Diods of *P. jamaicensis* are different from other species by their size; triods are abundant with size similar only with *P. jani* and *P. weinbergi* but in the later these spicules are rare. Calthrops of *P. jamaicensis* are smaller than all compared species. Rare monolophose calthrops have similar sizes in *P. jamaicensis, P. endoumensis, P. trilopha*, *P. jani*, as well as dilophose calthrops, in addition, in *P. endoumensis* these spicules are abundant. Rare trilophose calthrops of *P. jamaicensis* are close in size with the same but abundant spicule type of *P. trilopha* and *P. jani*. The dimensions of tetralophose calthrops of *P. jamaicensis* are different from all other *Plakina* species. Finally the ramification patterns of the lophose calthrops actines of *P. endoumensis* and *P. weinbergi* are very different compared to *P. jamaicensis, P. jani* and *P. trilopha* (Table 1). The ramification pattern of *P. jamaicensis* and *P. jani* is the same (1m, 2d, ts) except that monolophose calthrops of *P. jamaicensis* have 1m, ts or only lm.

Genus *Corticium* Schmidt, 1862

SYNONYMY

Corticium Schmidt, 1862, p. 42.

TYPE SPECIES

Corticium candelabrum Schmidt, 1862.

DIAGNOSIS (AMENDED AFTER MURICY & DIAZ 2002)

Thinly encrusting to cushion-shaped Plakinidae with a spiculation consisting almost exclusively of non-lophose calthrops in one size-class and heterolophose calthrops ('candelabra'). Homolophose calthrops may also be present, and nonlophose calthrops are absent in some species. Aquiferous system leuconoid, with aphodal choanocyte chambers. The species of this genus present variable but usually thick cortex (100–300 μm), and a proportion of mesohyl to chambers of about 1:1.

Corticium diamantense sp. nov.
(Figure 12)

MATERIAL EXAMINED

Holotype: RBINS POR 85, Martinique, Le Diamant, Grotte du Fer à Cheval, 14°28′04.72″N 61°00′59.37″W, 22 m depth, coll. Ph. Willenz and Brad Rosenheim, 7 June 2003.

Paratypes: RBINS POR 86 and RBINS POR 87, same locality, same collectors, 9 June 2003.

COMPARATIVE MATERIAL EXAMINED

Corticium candelabrum Schmidt, 1862 (SME AE 011), NE Mediterranean Sea (Marseille region), underwater cave of Maire Island.

DIAGNOSIS

Corticium leaf-like, thin, partly encrusting. Colour *in vivo* light brown, preserved specimens are dark brown. Loosely attached to the substrate, dense ectosome with abundant regular calthrops, rare tetralophose calthrops and candelabra with equally ramified (2–4 rays) conical actines (the fourth one basally ramified in 4–5 microspined rays).

DESCRIPTION

Sponge thin, ear- or leaf-shaped, lobate. Size up to 9 × 13 cm wide by 1.5 cm thick, Colour *in vivo* light brown. Preserved samples are darker brown. Sponge adhering to the substrate punctually, easily removed. Surface uneven, thin exhalant canals organized in an irregular mesh, slightly rough to touch. Oscula short and discrete, at the convergence of swelled exhalant canals, 1–3 mm in diameter, located on the margin of the sponge and forming short oscular chimneys up to 1–5 mm high.

GENERAL ORGANIZATION

Ectosome with a well defined cortex 200–350 μm thick. Subectosomal cavities well developed from 150 to 400 μm in diameter (Figure 12B). Aquiferous system leuconoid, with ovoid choanocytes chambers. Consistency firm but flexible, cartilaginous in alcohol.

Table 1. Comparison of spicule abundance and characteristics in *Plakina jamaicensis* and Mediterranean *Plakina* species. A, abundant; C, common; R, rare.

Spicules characters (sizes in μm)	*P. jamaicensis*	*P. endoumensis*	*P. trilopha*	*P. jani*	*P. weinbergi*
Diods (length)	A, 61 – 91	A, 60 – 75	A, 40 – 88	A, 43 – 100	R, 29 – 51
Triods (actine length)	A, 22 – 36	A, 18 – 30	A, 12 – 33	A, 16 – 37	R, 17 – 40
Calthrops (actine length)	A, 6 – 33	C, 20 – 32	C, 10 – 35	C, 16 – 38	R, 16 – 35
Monolophose calthrops (actine length)	R, 20 – 32	R, 25 – 35	R, 20 – 30	R, 22 – 38	C, 41 – 49
Dilophose calthrops (actine length)	R, 25 – 36	A, 27 – 35	R, 20 – 35	R, 30 – 35	C, 41 – 46
Trilophose calthrops (actine length)	R, 22 – 29	C, 15 – 20	A, 16 – 27	C, 19 – 32	A, 22 – 46
Tetralophose calthrops (actine length)	C, 17 – 26	R, 12 – 20	C, 10 – 25	C, 10 – 25	A, 19 – 35
Lophose calthrops (ramification pattern)	1m, 2d, ts	1p, ts	1 md, ts	1m, 2d, ts.	1 m/1 d

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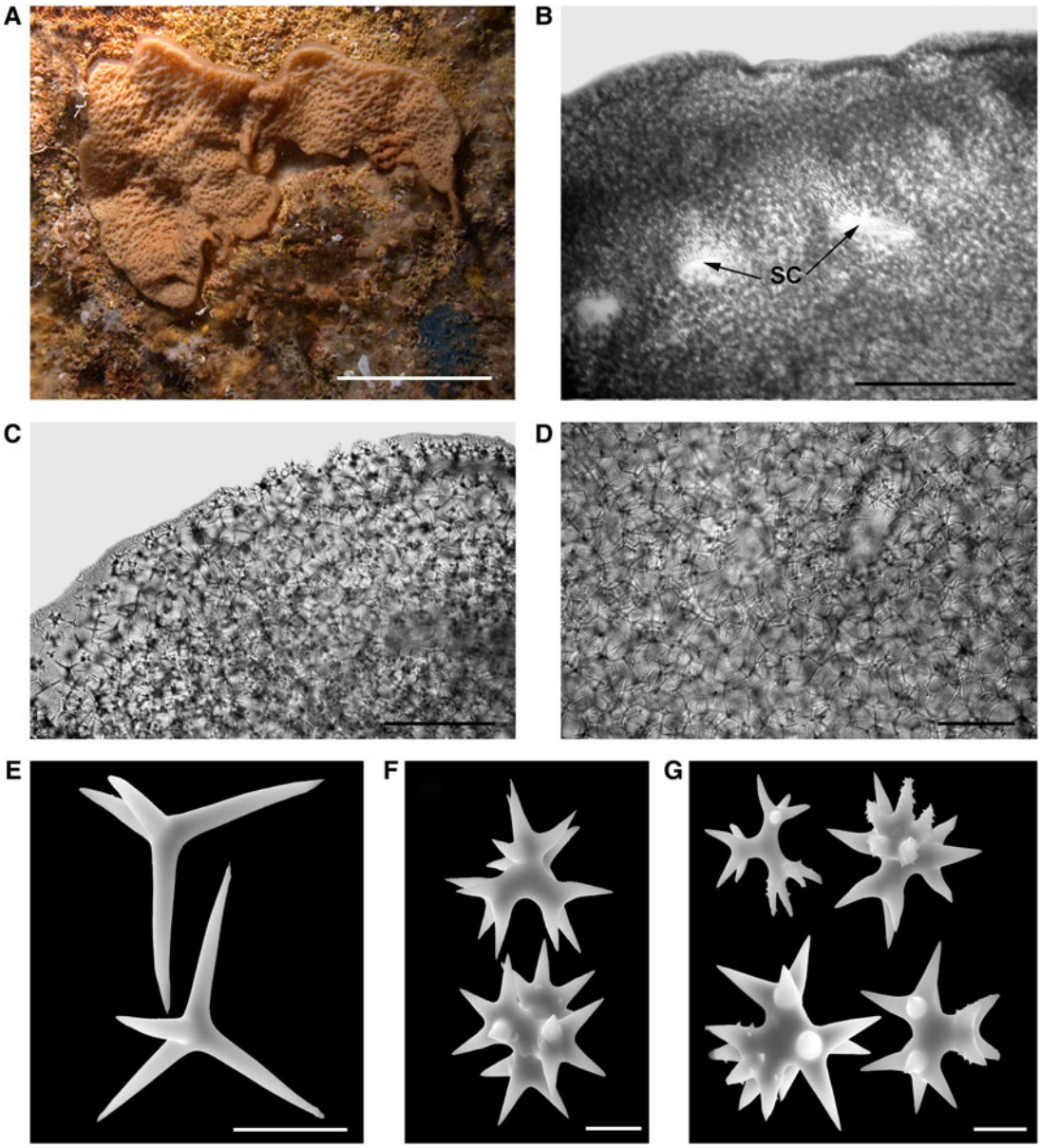

Fig. 12. *Corticium diamantense* sp. nov. (A) *In situ* close-up; (B) transverse section through ectosome and choanosome (LM); (C) transverse section through the ectosomal skeleton (LM); (D) transverse section through the choanosomal skeleton (LM); (E) calthrops; (F) tetralophose calthrops; (G) candelabra. SC, subdermal inhalant cavities. Scale bars: A, 5 cm; B, 200 μm; C, D, 100 μm; E, 20 μm; F, 10 μm; G, 10 μm.

SKELETON (FIGURE 12B–D)

Confused, with spicules scattered between choanocyte chambers. Candelabra and tetralophose calthrops concentrated at the surface and bordering canals.

SPICULES (FIGURE 12E–G)

Calthrops regular, non-lophose, in one size-class. Actines: 15–*31.5*–33/3–5.2 μm (N = 20; Figure 12E). Tetralophose calthrops very rare with a ramification pattern in which lophose actines have only a single proximal ramification point which gives rise to 3–4 conical, smooth rays (ramification pattern 1p, conical). Total length: 34.7–*36.8*–41.3 μm (N = 6; Figure 12F). Candelabra with equally ramified actines bearing conical ramification pattern in 2(rare)–4 rays. Fourth actine ramifies basally in 4–5 microspined rays. Total length: 28.4–*30.6*–41.1 μm long (N = 15; Figure 12G).

HABITAT

Sciaphilous, occurring on vertical walls of reef caves between 22 and 28 m depth.

REPRODUCTION

Sponges, collected in June, have well developed typical hollow cinctoblastula larvae about 320–340 × 200–220 μm.

DISTRIBUTION

Caribbean: Martinique (present study).

ETYMOLOGY

The specific name refers to the collecting locality, Le Diamant, Martinique.

TAXONOMIC REMARKS

So far, only one species of the genus *Corticium* was described from both the Atlantic Ocean and the Caribbean basin:

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C. quadripartitum Topsent, 1923. *Corticium diamantense* sp. nov. mainly differs from *C. quadripartitum* by the presence of abundant calthrops and by ramified candelabra with 2(rare)–4 rays, whereas *C. quadripartitum* possesses candelabra with 7–10 rays. *Corticium diamantense* sp. nov. differs from the type species *C. candelabrum* Schmidt, 1862 by the presence of tetralophose calthrops and absence of monolophose calthrops. *Corticium diamantense* sp. nov. also differs from all other known *Corticium*. *Corticium acanthastrum* Thomas, 1968 from India has a unique blood red colour, with presence of triods, absence of non-lophose calthrops and lophose calthrops of small size (21–33 μm). *Corticium bargibanti* Lévi & Lévi, 1983 from New Caledonia has a yellowish-grey colour, with absence of non-lophose calthrops, large lophose calthrops (73–75 μm) and candelabra (62–68 μm). *Corticium niger* Pulitzer-Finali, 1996 from New Guinea from *C. diamantense* sp. nov. is black, and characterized by large non-lophose calthrops (37–160 μm), and absence of lophose calthrops. Finally, *Corticium simplex* Lendenfeld, 1907 from N Australia differs from the new species by the absence of non-lophose and lophose calthrops.

Molecular analysis of the new and revised species

In addition to the morphological description, we determined partial Cytochrome b gene (*cob*) sequences for three new and the revised species of Homoscleromorpha described in this study: *Plakortis edwardsi* sp. nov. and *P dariae* sp. nov., *Plakina jamaicensis* Lehnert & van Soest, 1998 (two individuals) and *Corticium diamantense* sp. nov. (three individuals). In addition, *Plakortis angulospiculatus* collected at the Smithsonian marine station Bocas del Toro (S107-108) was also sequenced for comparison. Phylogenetic analysis of these sequences confirmed the genetic distinctiveness of the newly described species and revealed its affinities to other species of Homoscleromorpha (Figure 13). *Plakortis edwardsi* sp. nov. was closely related to *Plakortis halichondrioides* (96% sequence identity) while *Plakortis dariae* sp. nov. was most closely related to *P. simplex* (95% sequence identity, Figure 13). Interestingly, the sequence of *P. angulospiculatus* was 100% identical to that of *P. halichondrioides* reported in our previous study (Gazave *et al.*, 2010). The latter result was confirmed by comparison of two barcoding sequences from *P. angulospiculatus* collected in Belize (Porifera barcoding database ## 142, 137) which were also 100% identical to *cox1* sequence from *P. halichondrioides* (Gazave *et al.*, 2010), suggesting the conspecificity of both species. *Plakina jamaicensis* was most closely related to the *Plakina trilopha/P. jani* clade (97% average sequence identity, Figure 13). *Corticium diamantense* sp. nov. was most closely related to *C. candelabrum* from NW of Mediteranean Sea (98% sequence identity, Figure 13).

DISCUSSION

The five new species reported here belong to worldwide distributed genera previously known, or at least mentioned as far as *Oscarella nathaliae* sp. nov. is concerned, from the Caribbean basin (Muricy & Diaz, 2002).

The genus *Oscarella* includes 16 valid species known from different oceans around the world. Three additional indeterminate *Oscarella* sp. have been registered on the Brazilian coasts (Muricy & Moraes, 1998; Muricy & Hajdu, 2006). Several authors mentioned the occurrence of *Oscarella* in the Caribbean without further details (Díaz & van Soest, 1994; Rützler *et al.*, 2000; Díaz *et al.*, 2004; Díaz, 2005; Díaz & Rützler, 2009). *Oscarella nathaliae* sp. nov. occurring in southern Martinique, northern Jamaica and Guadeloupe was already illustrated from the Bahamas but remained non-described (Zea *et al.*, 2009). This species remarkably differs from other *Oscarella* species by its leaf-like thinly encrusting, flat body, loosely attached to the substrate and a perforated, not lobate surface. *Oscarella nathaliae* sp. nov. contains also two bacterial morphotypes and is characterized by two particular mesohylar cell types with inclusions.

Identification of *Oscarella* at the species level has always been tricky as this genus has no skeleton and as histological characters are generally unvaried (Boury-Esnault *et al.*, 1992; Diaz & van Soest, 1994; Muricy *et al.*, 1996; Muricy & Diaz, 2002; Muricy & Pearse, 2004; Ereskovsky, 2006; Ereskovsky *et al.*, 2009a, b; Pérez *et al.*, 2011). Several new species could recently be described thanks to ultrastructural and genetic methods. However, complementary tools appear necessary to further investigate *Oscarella* species diversity and phylogeny as a combination of molecular markers, biochemical fingerprints, symbiotic microbes investigation, and new morphological characters.

Nineteen species of the genus *Plakortis* are known throughout the world, four of which occur in the Caribbean Sea and more precisely in the Jamaican region: *P. angulospiculatus, P. zyggompha, P, halichondrioides* and *P. simplex* (Carter, 1879; Schulze, 1880; de Laubenfels, 1934; Hechtel, 1965; Boury-Esnault, 1973; Wiedenmayer, 1977; Pulitzer-Finali, 1986; Zea, 1987; Mothes & Bastian, 1993; Diaz & van Soest, 1994; Lehnert & van Soest, 1998; Moraes & Muricy, 2003).

In his recent work on the genus *Plakortis,* Muricy (2011) distinguished three groups of species based on spicule types and size: (1) *P. simplex* species group, including the nominal species and all other species with only diods and triods, with diods smaller than 190 μm long; (2) *P. angulospiculatus* species group, also with a nominal species complex and other species with only diods and triods, but with the largest diods varying between 190 μm and 300 μm long; and (3) *P. lita* species group, including species with microrhabds. *Plakortis myrae* sp. nov. is part of the *P. lita* species group because it is characterized by the presence of microrhabds. *Plakortis edwardsi* sp. nov. and *P. dariae* sp. nov. should be placed in the *P. simplex* species group, however, such placement is not supported by molecular data. Instead, *P. edwardsi* sp. nov. is grouped with *P. halichondroides/P. angulospiculatus* while *P. dariae* sp. nov. is grouped with *P. simplex* (Figure 13). Spicule size appears then to be a dubious character for species classification in the *Plakortis* genus and other characters are to be defined. As a side note, we originally identified *P. dariae* sp. nov. as *P. angulospiculatus* and its genetic distinctiveness lead to the 'posterior' recognition of morphological uniqueness (see also Blanquer & Uriz, 2007). Finally, our observation that *P. halichondrioides* and *P. angulospiculatus* share identical *cob* sequences supports the idea that at least some sponges assigned to *P. angulospiculatus* and *P. halichondrioides* are in fact conspecific (Diaz & van Soest, 1994).

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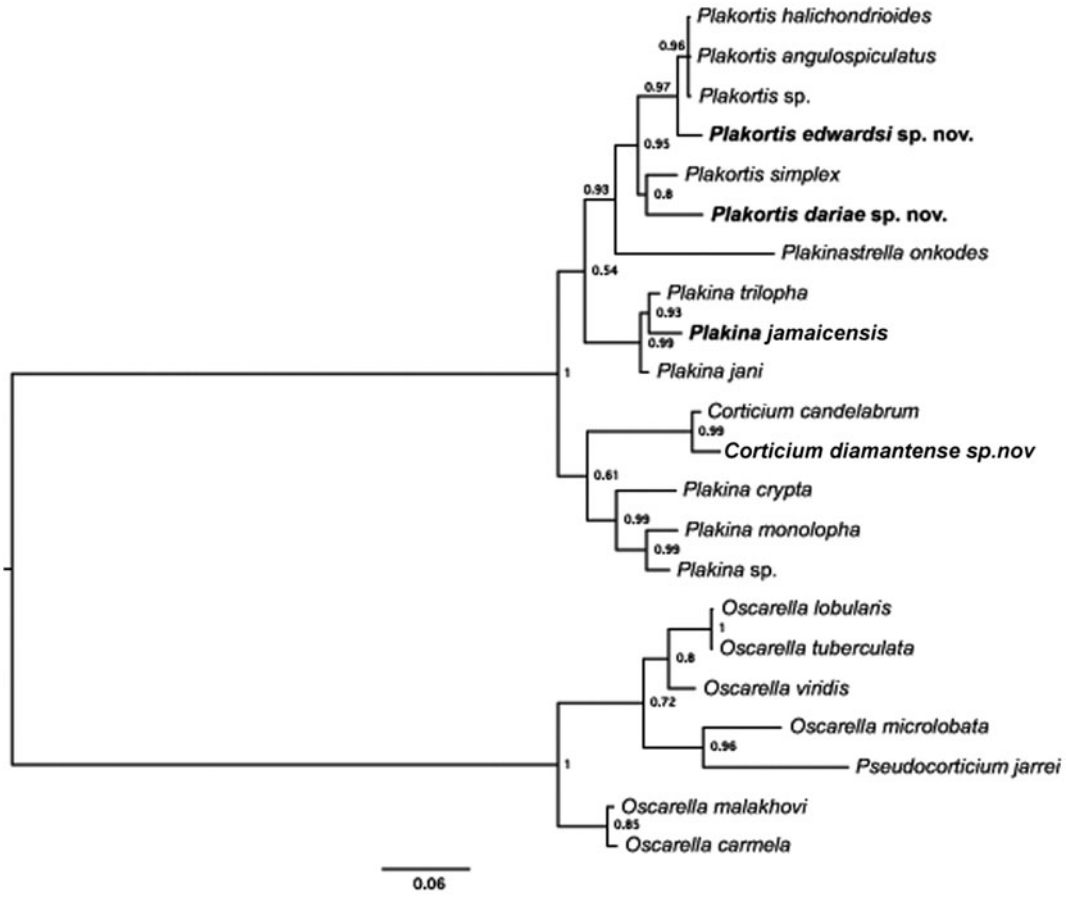

Fig. 13. Phylogenetic analysis of homoscleromorph relationships using mitochondrial *cob* sequences. Posterior majority-rule consensus tree obtained from the analysis under the HKY model in the MrBayes 3.2 program is shown. Two runs each with four independent chains were run for 5.0 million generations. The first 20% of these trees were discarded as burn-in. Convergence among the chains was monitored by comparison of maximum standard deviation of split frequencies for tree samples. The number at each node represents the Bayesian posterior probability. New species are indicated in bold.

In summary, the characteristics of the three new species found in Jamaica are consistent, and differ enough from the previously known *Plakortis* species to consider them as three different new species. A molecular phylogenetic analysis encompassing more *Plakortis* species will be necessary to investigate phylogenetic relationships inside of this large and complex genus.

Recent evidences based on nuclear ribosomal (18S and 28S) and mitochondrial coding sequences strongly support *Plakina* as a paraphyletic genus composed from at least two clades: B3 (including *Plakina jani* and *P. trilopha*) and B4 (including *Plakina monolopha* and *P. crypta*) (Gazave *et al.*, 2010). The clade B3 is characterized by its convoluted brain-like surface, the presence of a well developed mesohyl, well-differentiated ectosome, large subectosomal cavities and a tetralophose calthrops, whereas all these characters are absent in the clade B4. The two clades were also recovered in phylogenetic analysis conducted for this study based on partial mitochondrial *cob* sequences. Furthermore, our molecular and morphological data show that *Plakina jamaicensis* is most closely related to the *Plakina trilopha/P. jani* clade.

The genus *Corticium* Schmidt, 1862 includes six species with a wide-range distribution, from NW Mediterranean to Indian Ocean, Western Pacific (van Soest *et al.*, 2013). Only one species was described from both the Atlantic Ocean and the Caribbean basin: *C. quadripartitum* Topsent, 1923. *Corticium diamantense* sp. nov. differs from *C. quadripartitum* and from other *Corticium* species by the presence of regular non-lophose calthrops of one size-class, very rare tetralophose calthrops and candelabra with the fourth actine ramified basally in 4–5 microspined rays.

Our results based only on a few exploratory dives show that the sponge biodiversity in cryptic habitats of the Caribbean Sea is still poorly known, particularly for Homoscleromorpha that deserve more investigations.

The low diversity of this group in the Caribbean might only be a consequence of the lack of exploration, among other in cryptic habitats, like underwater caves and tunnels. This is confirmed by a more complete study of the Caribbean sponges that we have in preparation.

IDENTIFICATION KEY FOR THE WESTERN ATLANTIC SPECIES OF HOMOSCLEROMORPHA

1. Inorganic (spicular) skeletal complement present 2
— Inorganic (spicular) skeletal complement absent ..... ***Oscarella nathaliae* sp. nov.**

2. Skeleton mainly composed of diods, triods, and/or calthrops in one size-class 3
— Skeleton mainly composed of diods, triods and/or calthrops with a large size variation 9

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3. Lophose diods, triods, or calthrops complement the main skeleton of non-lophose spicules 4
— Lophose spicules absent, microscleres (microrhabs) present in some species 11

4. Heterolophose calthrops (candelabra) complement the main skeleton of non-lophose spicules present 5
— Lophocalthrops with one to four homogeneously ramified actines complement the main skeleton of non-lophose spicules present; candelabra absent 6

5. Non-lophose calthrops present ..... ***Corticium diamantense* sp. nov.**
— Non-lophose calthrops absent ..... *Corticium quadripartitum*

6. Diods absent 7
— Diods present 8

7. Mono- and dilophose calthrops absent, tertalophose calthrops present ..... *Plakina tetralopha*
— Monolophose calthrops present, tertalophose calthrops absent ..... *Plakina versatilis*

8. Di-, tri- and tetralophose calthrops absent .*Plakina elisa*
— Di-, tri- and tetralophose calthrops present ..... *Plakina jamaicensis*

9. Skeleton composed of non-lophose diods, triods and/or calthrops in three size-classes 10

10. Massive or thick encrusting, light brown, with large diods (22–230 μm) and large calthrops (33–152 μm)..... *Plakinastrella onkodes*
— Encrusting, black or grey externally, with small diods (15–130 μm) and small calthrops (10–50 μm) ..... *Plakinastrella microspiculifera*

11. Microscleres vermiform microrhabds present 12
— Microscleres absent 13

12. Triods present .....***Plakortis myrae* sp. nov.**
— Triods absent .....*Plakortis microrhabdifera*

13. Diods of two size-classes 14
— Diods of one size-class 15

14. The ectosomal skeleton has a loose confused arrangement .....***Plakortis edwardsi* sp. nov.**
— The ectosomal skeleton is distinctly reticulate ..... ***P. dariae* sp. nov.**

15. Sponge thinly encrusting (15 mm thick or less), diods less than 150 μm long 16
— Sponge massively encrusting (more than 15 mm thick), diods large (up to 220 μm long) 17

16. Diods up to 150 μm long, consistency compressible but resistant ..... *Plakortis zyggompha*
— Diods smaller than 100 μm, consistency very soft..... *Plakortis insularis*

17. Colour black or dark brown externally and internally, releases a dark exudate in alcohol ..... *Plakortis halichondrioides*
— Colour variable, light or dark brown, often with dark patches or with greenish tinges, but never black; no dark exudate released in alcohol ..... *Plakortis angulospiculatus*

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Correspondence should be addressed to:
A.V. Ereskovsky
Mediterranean Institute of Biodiversity and Ecology (IMBE), UMR 7263, Aix-Marseille Université, CNRS, IRD, Station Marine d'Endoume, Marseille, France
email: alexander.ereskovsky@imbe.fr

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