The LINNEAN SOCIETY of London ZOOLOGICAL Journal of the Linnean Society

Zoological Journal of the Linnean Society, 2014, **171**, 254–276. With 8 figures

Taxonomy of family Plakinidae (Porifera: Homoscleromorpha) from eastern Pacific coral reefs, through morphology and *cox1* and *cob* mtDNA data

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Received 2 August 2013; revised 6 January 2014; accepted for publication 10 January 2014

Sponges belonging to the class Homoscleromorpha have become a pivotal group to help understand early metazoan evolution. However, their complex systematics and cryptic habitat (e.g. dead coral, or under/amongst coral rubble or rock), hinders the recognition and classification of species. An extensive study carried out in coral reefs from the Mexican Pacific coast, Revillagigedo and Clipperton Islands, yielded 21 specimens and five species of Plakinidae: *Plakortis albicans*, *Plakinastrella clippertonensis*, ***Plakina muricyae* sp. nov.**, ***Plakina paradilopha* sp. nov.**, and ***Plakortis clarionensis* sp. nov.** Fragments of *cytochrome c oxidase subunit I* (*cox1*) and *cytochrome b* (*cob*) mtDNA were sequenced to generate DNA-barcoding of some species and to determine their phylogenetic relationships with other homosclermorphs. Molecular and morphological data placed *Plakina muricyae* sp. nov. together with the morphologically related species *Plakina monolopha* (*Plakina monolopha*-complex), whereas *Plakina paradilopha* sp. nov. seems to belong to the *Plakina* ***dilopha*** complex. According to spicule morphology and size, *Plakortis albicans* had been considered to be in the *Plakortis simplex*-complex. However, this was not supported by our molecular data and *Plakortis albicans* clustered with *Plakinastrella* sp. here. This is the first time that the standard *cox1* 'barcoding fragment' has been analysed for the systematics of Plakinidae, which showed to *Plakina* as a monophyletic group, which is congruent with the morphological hypothesis. The genus *Plakina* is here recorded for first time from Mexican Pacific waters. A taxonomic key for Homoscleromorpha species from the eastern Pacific region is also included.

doi: 10.1111/zoj.12137

ADDITIONAL KEYWORDS: DNA-barcoding – integrative taxonomy – Mexican Pacific – phylogeny.

INTRODUCTION

Sponges are the earliest metazoan group, and they are currently very abundant and diverse in benthic ecosystems worldwide, with about 8425 valid species so far (van Soest *et al*., 2013). Amongst poriferans, the small group class Homoscleromorpha is of great interest in evolutionary biology studies, owing to its pivotal position in helping to understand early metazoan evolution (Nichols & Wörheide, 2005; Gazave *et al*., 2012). Sponges in this class have unique characteristics, such as the presence of a basal membrane of type IV collagen and specialized cell junctions, which form a true epithelium (Ereskovsky *et al*., 2009). Several species are also known for their production of biologically active substances, many of which have potential value in the pharmacological field (Berrué *et al*., 2005; Holzwarth *et al*., 2005). As a result of this, the number of new species (Muricy, 2011; Ereskovsky, Lavrov & Willenz, 2014; Gazave *et al*., 2013) and studies on the phylogenetic relationships of the group have substantially increased in the last few years (see Borchiellini *et al*., 2004; Peterson *et al*., 2008; Sperling, Pisani & Peterson, 2007; Philippe *et al*., 2009; Sperling, Peterson & Pisani, 2009; Gazave *et al*., 2010, 2013; Ivanisêvić *et al*., 2011; Boury-Esnault *et al*., 2013).

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In the Systema Porifera (Hooper & van Soest, 2002), the order Homosclerophorida only included the family Plakinidae and seven valid genera (Muricy & Díaz, 2002). However, the incorporation of molecular data restored the pre-1995 systematics, when homosclerophorids remained subdivided in the families Plakinidae and Oscarellidae, which were allocated in the class Homoscleromorpha (Gazave *et al*., 2012). Currently, class Homoscleromorpha contains the order Homosclerophorida and two families, Plakinidae and Oscarellidae (Gazave *et al*., 2012). The first one harbours five genera: *Plakina* (with 25 spp.), *Plakortis* (with 22 spp.), *Plakinastrella* (with 12 spp.), *Placinolopha* (with six spp.) and *Corticium* (with seven spp.); whereas the second family has two genera: *Oscarella* (with 19 spp.), and *Pseudocorticium* (with one sp.) (van Soest *et al*., 2013).

Despite advances in molecular systematics, the taxonomy of Homoscleromorpha species is still difficult as a result of the taxon's morphological simplicity (Moraes & Muricy, 2003; Muricy, 2011). In fact, several species are involved in taxonomically controversial species complexes [e.g. *Oscarella lobularis* (Schmidt, 1862), *Plakortis simplex* Schulze, 1880 and *Plakina monolopha*, *Plakina dilopha*, and *Plakina trilopha* Schulze, 1880 (Boury-Esnault, Solé-Cava & Thorpe, 1992; Muricy *et al*., 1998; Ereskovsky *et al*., 2009; Boury-Esnault *et al*., 2013)].

Molecular markers have been implemented for sponge taxonomy and detection of cryptic species (e.g. Blanquer & Uriz, 2007; Xavier *et al*., 2010), and although they were not always successful in these matters, these markers provided interesting support to complement traditional taxonomy data. So, integrative taxonomy that combines different data sets is the best way for sponge identification, particularly in species complex groups (see Cruz-Barraza *et al*., 2012; Reveillaud *et al*., 2012; Gazave *et al*., 2013).

The highest species diversity of Homoscleromorpha is present in the Caribbean, Mediterranean, and West Pacific areas (Muricy & Díaz, 2002; Ereskovsky *et al*., 2009; Muricy, 2011; Ereskovsky *et al*., 2014). This is probably because of a larger tradition of sponge systematic studies (Ereskovsky *et al*., 2009, 2014; Gazave *et al*., 2010, 2013), whereas other areas remain poorly known. In the eastern Pacific region, in the 1980s there was no record of Homoscleromorpha, but in the last few years, the number of species has significantly increased (see below) (Green & Bakus, 1994; Desqueyroux-Faúndez & van Soest, 1997; Muricy & Pearse, 2004; Cruz-Barraza & Carballo, 2005; Lehnert, Stone & Heimler, 2005; van Soest, Kaiser & Syoc, 2011).

In this paper, we describe five species of Plakinidae associated with coral reef ecosystems from the eastern Pacific area, thus increasing the number of homosclerophorid species to 13. Three species described here are new to science, two *Plakina* and one *Plakortis*. This is also one of the few studies that includes Porifera records from the Revillagigedo and Clipperton Islands (Cruz-Barraza *et al*., 2011, 2012; van Soest *et al*., 2011).

We also sequenced *cytochrome c oxidase subunit I* (*cox1*) and *cytochrome b* (*cob*) gene fragments to generate DNA-barcoding of some species and determine their phylogenetic relationships with other Plakinidae. This is the first time that the standard mtDNA *cox1* barcoding fragment has been used for a phylogenetic analysis of the group. Finally, we present an identification key for homosclerophorid species from the eastern Pacific region.

MATERIAL AND METHODS

Specimen collection

Collections were carried out by the authors in ten coral reef habitats around the Nayarit and Oaxaca coasts, and Isabel, Revillagigedo, and Clipperton Islands (Fig. 1). Samples from Clipperton Island were obtained by donation from the marine biotechnology company Pharmamar SA. Specimens were collected by scuba diving and snorkelling and were immediately fixed in 4% formaldehyde for 24 h and later transferred to 70% ethanol for storage. A small fragment of some samples was taken and preserved in 100% ethanol for molecular analysis. The type material has been deposited in the Museo Nacional de Ciencias Naturales, Madrid (Spain) (MNCN) and in the Colección de Esponjas del Pacífico Mexicano (LEB-ICML-UNAM) of the Instituto de Ciencias del Mar y Limnología, UNAM, Mazatlán (México). Specific terms are used according to Boury-Esnault & Rützler (1997).

Morphology

External morphology, skeletal elements, and their arrangement were recorded for each individual. Dissociated spicules, both for light microscopy and scanning electron microscopy (SEM), were prepared following the techniques described by Rützler (1974). Spicule measurements were obtained from a minimum of 25 spicules chosen randomly from each specimen with the purpose of finding different size classes. The numbers within brackets are the means.

DNA purification, amplification and sequencing

Total genomic DNA was extracted using standard proteinase K digestion in cetyl trimethyl ammonium bromide extraction buffer and purified with a LiCl salting-out protocol, followed by organic extraction using chloroform–isoamyl alcohol, and subsequent ethanol precipitation (Aljanabi & Martinez, 1997). Specimens for which this protocol failed were subjected to a second

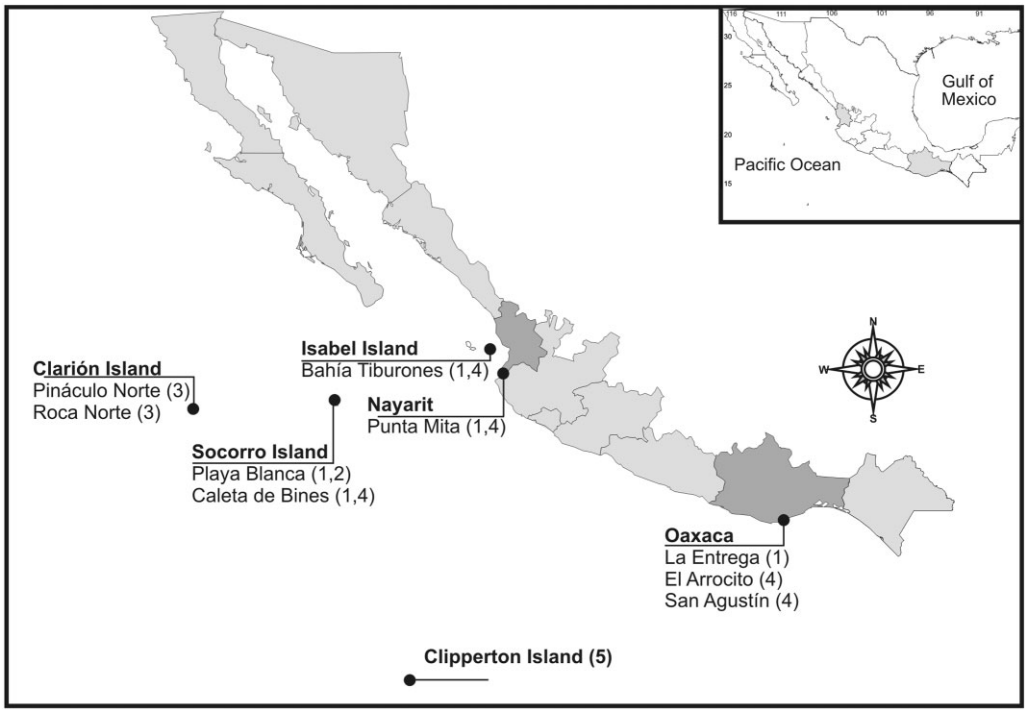

Figure 1. Distribution and sample localities of *Plakinidae* species from the Mexican Pacific coast. Numbers refer to different species as follows: 1, ***Plakina muricyae* sp. nov.**; 2, ***Plakina paradilopha* sp. nov.**; 3, ***Plakortis clarionensis* sp. nov.**; 4, *Plakortis albicans* Cruz-Barraza & Carballo, 2005; 5, *Plakinastrella clippertonensis* van Soest *et al*., 2011.

extraction using a SV Promega kit (Promega) following the manufacturer's instructions.

Fragments of *cob* and *cox1* mtDNA were PCR-amplified and sequenced from seven specimens of two different species (Table 1). For *cob* fragments we used the primers diplo-cob-f1m 5′-ATG TNT TNC CTT GRG GWC AAA TGT C-3′ and diplo-cob-r1m 5′-ATT WGG WAT WGA NCG YAA WAT NGC-3′ (Lavrov, Wang & Kelly, 2008). For the *cox1* Folmer fragment we used PLAKLCOdegF (modified from LCO1490; Folmer *et al*., 1994): 5′-TCW ACD AAY CAT AAA GAY ATW GG-3′; and C1J2165-R (from Erpenbeck *et al*., 2002 modified as reverse): 5′-CCN GGT AAA ATT AAA ATA TAA ACT TC-3′. PCR reactions were carried out in a volume of 12.5 µl and consisted of 6.0 µl distilled H_2O (sterile MilliQ), 0.75 µl dexoyribonucleotide triphosphates (0.2 mM), 0.75 µl $MgCl_2$ (8 mM), 0.70 µl of each primer (10 µM), 2.50 µl 5× PCR buffer (Promega), 0.2 µl *Taq* DNA polymerase, and 1 µl genomic DNA (*c*. 50–100 ng). Thermal cycling conditions were: initial denaturation at 94 °C for 2 min, and 35 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min, and a final extension of 72 °C for 5 min. PCR-products were run in a 1.5% agarose gel to corroborate the positive amplification. Products were purified using the Wizard purification kit (Promega) and sequenced in both directions using Applied Biosystems 3730xl DNA analysers by Macrogen, Korea.

Sequence analysis

Sequences were verified and edited with CODON CODE ALIGNER 2.0.1 (CodonCode Corporation). BLAST (National Center for Biotechnology Information/Blast) searches were used to verify the identity of sequences and check for possible contamination. Phylogenetic analysis included previously published *cox1* and *cob* mtDNA sequence fragments from Plakinidae and Oscarellidae species available in GenBank (Table 1). Sequences of *Amphimedon queenslandica* Hooper & van Soest, 2006 (DQ915601) were used as the outgroup. Two sequences of *Plakortis angulospiculatus* from Colombia and Panama were kindly supplied by Dr Dennis Lavrov. A Bayesian inference analysis was performed with MrBayes 3.2.1 (Ronquist & Huelsenbeck, 2003) using the Hasegawa-Kishino-Yano plus Invariant sites plus Gamma distributed model of sequence evolution, which was obtained through the JModelTest 2.0.1 program (Posada, 2008). The program was run with four Markov chains, each 10 000 000 generations long. These were sampled every 100 trees with a burn-in of 2500.

Table 1. Accession numbers and locality of the species/specimens used in this study

Homoscleromorpha-species	GenBank accession numbers: Mitochondrial genomes	GenBank accession numbers: *cox1*	GenBank accession numbers: *cob*	Collection sites
Corticium Schmidt, 1862				
C. diamantensis Ereskovsky *et al.*, 2014			KF915295	La Martinique, Le Diamant,
C. candelabrum Schmidt, 1862	HQ269363			Marseilles, France
Plakina Schulze, 1880				
***P. muricyae* sp. nov.**		KJ162930	KJ162928	Nayarit, Revillagigedo, and Oaxaca, México
P. jani Muricy *et al.*, 1998	HQ269360			Marseilles or La Ciotat, France
P. crypta Muricy *et al.*, 1998	HQ269352			La Ciotat, France
P. jamaicensis Lehnert & Soest, 1998			KF915293	Jamaica
P. trilopha Schulze, 1880	HQ269356			Marseilles, France
P. monolopha Schulze, 1880	HQ269351			Sete, France
Plakina sp.	HQ269354			Marseilles, France
Plakinastrella Schulze, 1880				
Plakinastrella sp.	EU237487			Florida, USA (Looe Keys)
Plakortis Schulze, 1880				
P. albicans Cruz-Barraza & Carballo 2005		KJ162931	KJ162929	Nayarit, Revillagigedo, and Oaxaca, México
P. simplex Schulze, 1880	HQ269362			La Ciotat, France
P. halichondrioides (Wilson, 1902)	HQ269359			Bocas del Toro, Panamá
P. angulospiculatus (Carter, 1882)		EF519536;		Belize
		EF519537		Belize
P. dariae Ereskovsky *et al.*, 2014			KF915292	Jamaica
P. edwardsi Ereskovsky *et al.*, 2014			KF915294	Jamaica
Oscarella Vosmaer, 1877				
O. lobularis (Schmidt, 1862)	HQ269361			Marseilles, France
O. carmela Muricy & Pearse, 2004	NC_009090			California, USA
O. malakhovi Ereskovsky, 2006	HQ269364			Japan Sea, Russia
O. microlobata (Schmidt, 1868)	HQ269355			Marseilles, France
O. tuberculata (Schmidt, 1868)	HQ269353			Marseilles, France
	JX963639			
O. viridis Muricy *et al.*, 1996	HQ269358			Marseilles, France
Oscarella sp.	JX963640			Marseilles, France
Pseudocorticium Boury-Esnault *et al.*, 1995				
P. jarrei Boury-Esnault *et al.*, 1995	HQ269357			Marseilles, France

cob; *cytochrome b*; *cox1*, *cytochrome c oxidase subunit I*.

RESULTS

SYSTEMATICS

PHYLUM PORIFERA GRANT, 1836

CLASS HOMOSCLEROMORPHA BERGQUIST, 1978

ORDER HOMOSCLEROPHORIDA DENDY, 1905

FAMILY PLAKINIDAE SCHULZE, 1880

GENUS *PLAKINA* SCHULZE, 1880

Type species
Plakina monolopha Schulze, 1880

Diagnosis
Encrusting to massive Plakinidae with a skeleton formed by diods, triods, calthrops, and lophocalthrops, homogeneously ramified with one, two, three, or four rays. Candelabra spicules (heterolophose calthrops) missing. Lophocalthrops are generally concentrated at the sponge surface and bordering canals. Development of the ectosome is variable, and subectosomal cavities may be present (e.g. *Plakina trilopha*). Choanocyte chambers are eurypylous or aphodal, usually with a radial arrangement around incurrent and excurrent canals (Muricy & Díaz, 2002).

PLAKINA MURICYAE SP. NOV.

(FIGS 2A–C, 3)

Material examined
Holotype: MNCN-1.01/694, Antiguo Corral del Risco (Nayarit), 20°46′20″N, 105°32′49″W, 3 m depth, 29.iii.2012. Paratypes: LEB-ICML-UNAM-1108, Punta Mita (Nayarit), 20°46′20″N, 105°32′49″W, 3 m depth, 19.ii.2005. LEB-ICML-UNAM-1213, La Entrega (Oaxaca) 15°42′50″N, 96°05′20″W, 4 m depth, 4.v.2005. LEB-ICML-UNAM-1523, Bahía Tiburones, Isabel Island (Nayarit), 21°50′38″N, 105°52′14″W, 2 m depth, 21.vii.2005. LEB-ICML-UNAM-1651, Playa Blanca, Socorro Island (Revillagigedo), 18°48′59″N, 111°02′42″W, 0.5 m depth, 6.v.2008. LEB-ICML-UNAM-2011, Caleta de Bines, Socorro Island (Revillagigedo), 18°44′10″N, 110°57′37″W, 6 m depth, 7.xi.2009. LEB-ICML-UNAM-2068, Antiguo Corral del Risco (Nayarit), 20°46′20″N, 105°32′49″W, 3 m depth, 29.iii.2012, LEB-ICML-UNAM-2069, Punta Mita (Nayarit), 20°46′20″N, 105°32′49″W, 3 m depth, 29.iii.2012.

Specimens examined for comparison: Plakina monolopha from the collection of Laboratorio de Biología Marina (LBM) of Sevilla University (Spain): LBM-379 bahía de Algeciras, Spain, 9.vi.1992; LBM-511 Bahía de Algeciras, Spain, 28.ix.1992.

ZooBank LSID: urn:lsid:zoobank.org:act:5BE60630-02A9-4A54-91E5-FE93D60795AB.

Etymology
The species is named after Dr Guilherme Muricy for his contribution to the study of the homosclerophorid taxonomy.

Diagnosis
Whitish to light green yellowish, thinly encrusting sponge, with smooth surface and rounded borders, characteristically with abundant subectosomal spaces regularly distributed. Spicules are diods, triods, and simple calthrops, which constitute a dense and sometimes confuse choanosomal alveolar structure, whereas the monolophose calthrops are arranged in a thin ectosomal layer.

Description
Thinly encrusting sponge from 1 to 4 mm thick, under coral rubbles and fragments of death corals, where it covers areas from 1 to 5 cm in diameter (Fig. 2A, B). The surface varies from smooth to irregular, with lightly elevated, rounded borders, which are visible to the naked eye. Some small lobules, from 140–170 μm in diameter and 120–200 μm high, are observed under the microscope. Subectosomal spaces are abundant and regularly distributed on the surface, giving it a punctate appearance; they measure from 100 to 315 μm in diameter. Oscula are scarce, circular in shape, of about 1 mm in diameter, slightly elevated, and distributed irregularly over surface. Consistency is fleshy and firm. Ectosome is a translucent membrane not easily detachable. Choanosome with circular or oval canals, about 100 μm in diameter. Colour is whitish to light green yellowish in life; preserved is ochre.

Spicules: diods, triods, simple calthrops and monolophose calthrops (Table 2). Diods are large, irregularly curved, some of them with a swelling slightly marked in the middle shaft (Fig. 3A). Diods always present, but they are less abundant than triods. They measure 35–92.5 μm long and 0.5–5 μm wide. Triods are the most abundant spicules. They possess equidistant rays, although can be irregular with 'T' or 'Y' shape (Fig. 3B). Rays measure from 6.25 to 37.5 μm long (spicules' total length 12.5–72.5 μm). Simple calthrops are scarce; they may possess rays with sharp point or with small spines and commonly one of the rays is slightly larger than the others (Fig. 3C). Calthrop rays measure from 6.25 to 30 μm long (total length 12.5–50 μm). Monolophose calthrops are common but not abundant; lophose rays have a simple ramification pattern: they ramify once at a medial position into two to four smaller rays with spined tips, sometimes smaller rays have a second bifurcation. Simple rays can end in a sharp or spiny point (Fig. 3D). Rays measure 7.5–25 μm long (total length 15–50 μm).

Skeleton: ectosomal structure is armed by a thin layer of monolophose calthrops with the ramified ray directed toward the surface. Choanosomal skeleton is formed by diods, triods, and calthrops, forming an alveolar, dense, and sometimes confuse reticulation, with meshes of 7.5–15 μm in diameter (Fig. 3E).

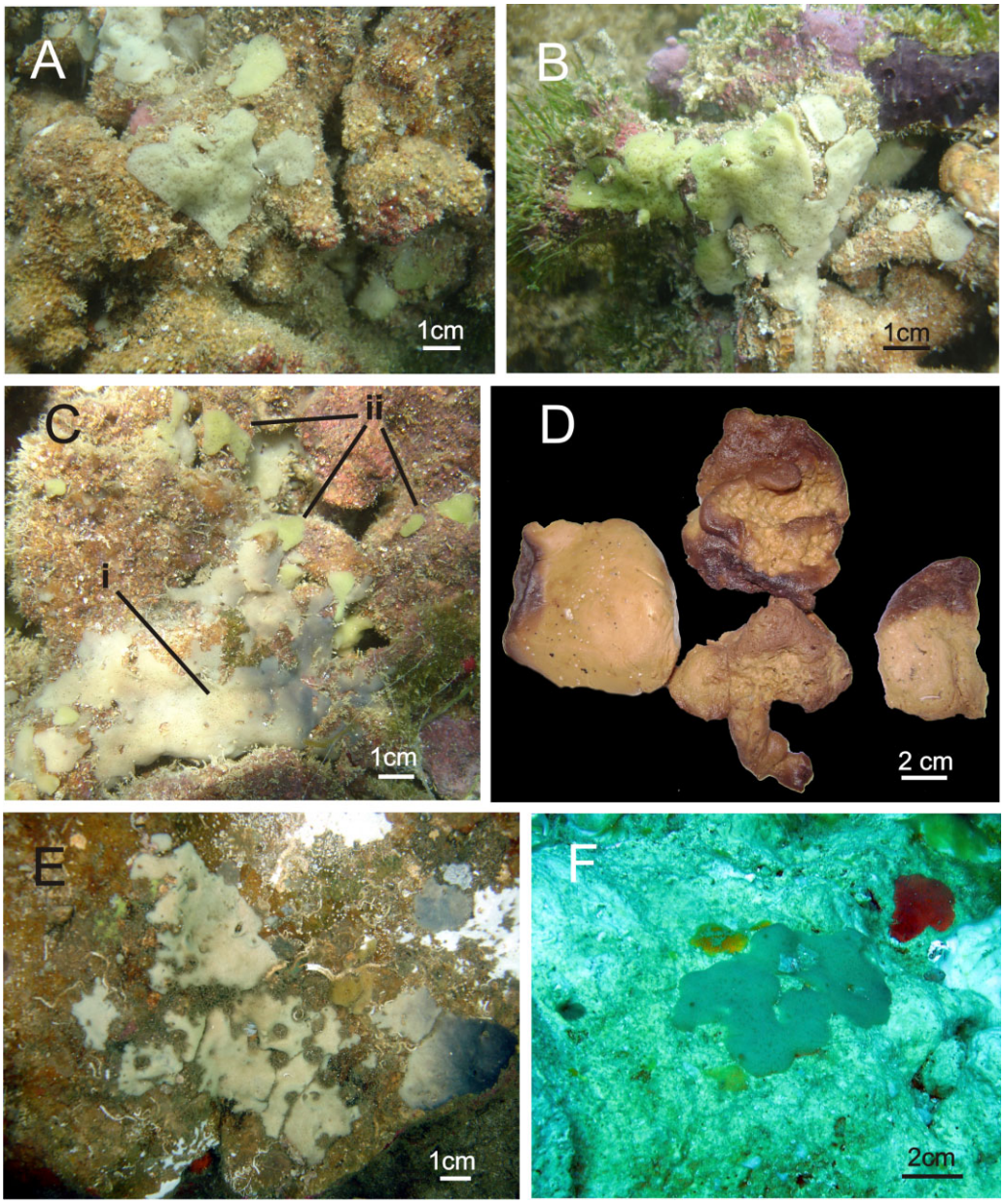

Figure 2. External morphologies of plakinid species from the eastern Pacific. A, B, ***Plakina muricyae* sp. nov.** C, i, *Plakina* ***paradilopha* sp. nov.**; ii, ***Plakina muricyae* sp. nov.** D, ***Plakortis clarionensis* sp. nov.** E, *Plakortis albicans* Cruz-Barraza & Carballo, 2005. F, *Plakinastrella clippertonensis* van Soest *et al*., 2011.

DNA barcode sequence data

We obtained sequences of three individuals from Isabel Island, (Nayarit, Mexico), which were designated here as the holotype and two paratypes. The three have an identical sequence for *cox1* and *cob*. Therefore, these results, together with the morphological similarity between the specimens, confirm that they belong to the same species. Comparison of our *cox1* 'barcode fragment' with similar sequences available in GenBank showed that *Plakina muricyae* sp. nov. differs by 4% from the nearest sequence, which corresponds to *Plakina monolopha* (HQ269351) from Sète, France. *Cob* comparison shows a similar result but differing by only 2% from *Plakina monolopha*.

Sequences of *Plakina muricyae* sp. nov. have been deposited in GenBank with accession number: KJ162928 (*cob*) and KJ162930 (*cox1*).

Ecology and distribution

The specimens were collected in coral reefs from the Mexican Pacific coast; Isabel Island (Nayarit), Oaxaca,

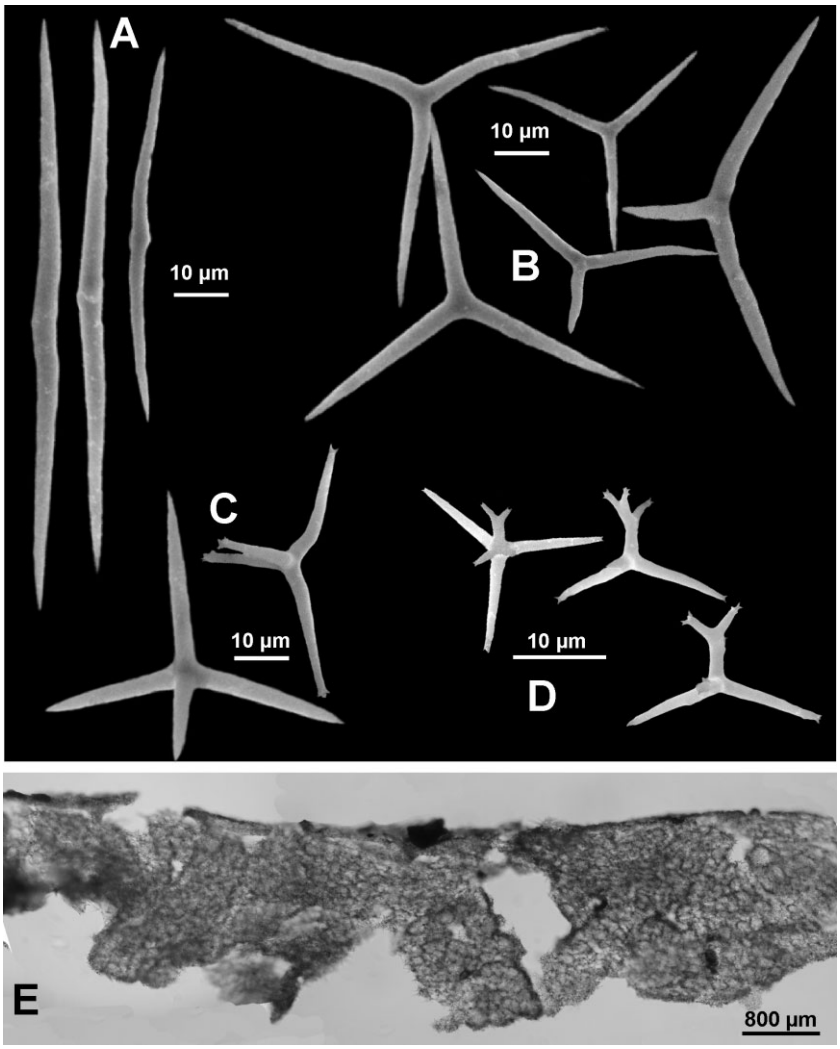

Figure 3. Scanning electron microscopy and light microscopy images of spicules and skeletal structure of ***Plakina muricyae* sp. nov.** A, diods. B, triods. C, calthrops. D, monolophose calthrops. E, transversal view of the choanosome.

and Socorro Island (Revillagigedo) (Fig. 1), from 0.5 to 6 m depth. All specimens were found in cryptic habitats, mainly under coral bases of the genus *Pocillopora*. Specimens from Punta Mita show a colour variation from white to light green yellowish, whereas at Revillagigedo only light green yellowish specimens were found.

Remarks

Plakina muricyae sp. nov. displays similar characteristics to the *Plakina monolopha* species complex, shares monolophose calthrops as only lophate spicules. *Plakina monolopha* was described from the Mediterranean Sea (Schulze, 1880), and it was then considered as a cosmopolitan species (see Muricy *et al*., 1998; Table 2). However, some authors mentioned that the non-Mediterranean specimens could be misidentified (e.g. Muricy *et al*., 1998). Here, we compared specimens of *Plakina muricyae* sp. nov. with specimens of *Plakina monolopha* from the Mediterranean Sea (Carballo, 1994; *Plakina monolopha*: LBM-379 Algeciras bay 9.vi.1992; LBM-511 Algeciras bay, 28.ix.1995). One of the main differences found between the two species is the presence of non-lophose calthrops with rays ending in three or four tiny spines in our specimens, which were not found in *Plakina monolopha*. Additionally, there are differences in spicule size between the species; *Plakina monolopha* possesses diods 50–67 µm long and 2–3 µm wide; triods with rays 44–55 µm long and 2–2.5 µm wide; calthrops with rays 20–30 µm long. Colour also varies between the species; *Plakina monolopha* is white or rose, whereas *Plakina muricyae* sp. nov. is whitish to light green yellowish. DNA sequence comparison of *cox1* and *cob* fragments between *Plakina muricyae* sp. nov.

Table 2. Comparative data for the dimensions of the spicules (in μm), locality, and other characteristics of ***Plakina muricyae* sp. nov.** and other species of the *Plakina monolopha* species complex. Values in parentheses are means

Plakina species	Diods (shaft length × width)	Triods (ray length – total length)	Calthrops (ray length – total length)	Shape/colour	Original locality/ references
***P. muricyae* sp. nov.** Holotype MNCN-1.01/694	39(74.3)92.5 × 1.25(2.53)3.75	7.5(22.5)35–15(24.6)32.5	S: 7.5(12.8)17.5–15(24.6)32.5 M: 11.3(13.8)20–22 (26.3)35	Encrusting/light green yellowish	Punta Mita, Nayarit/ Present study
***P. muricyae* sp. nov.** LEB-ICML-UNAM-1108	37.5(63.3)77.5 × 1.25(2.53)3.75	7.5(18.57)25–12.5(34.6)47.5	S: 9.6(14.12)17.16–15(29)45 M: 7.5(10.65)15–15(21.4)30	Encrusting/whitish	Punta Mita, Nayarit/ Present study
***P. muricyae* sp. nov.** LEB-ICML-UNAM-1213	52.5(74.25)87.5 × 1.25(2.43)3	6.25(19.72)25–12.5(38.6)50	S: 10(12.4)17.5–17.5(24.75)35 M: 8.75(12.4)25–17.5(24)50	Encrusting/whitish	La Entrega, Oaxaca/ Present study
***P. muricyae* sp. nov.** LEB-ICML-UNAM-1523	47.5(62.05)72.5 × 2.5(2.6)3.75	18.75(22.38)27.5–30(40.25)47.5	S: 10(14.4)20–20(29.4)40 M: 8.75(10.75)12.5–17.5(20.75)25	Encrusting/whitish	Bahía Tiburones, Isabel Island/ Present study
***P. muricyae* sp. nov.** LEB-ICML-UNAM-1651	35(71.05)92.5 × 0.5(2.93)5	7.5(22.67)35–15(42.24)72.5	S: 7.5(13.35)18.75–15(25.42)32.5 M: 11.25(13.15)20–17.5(23.9)35	Encrusting/whitish	Playa Blanca, Socorro Island (Revillagigedo)/ Present study
***P. muricyae* sp. nov.** LEB-ICML-UNAM-2011	55(76.5)90 × 1.25(3.05)5	6.25(24.9)37.5–12.5(47.5)72.5	S: 6.25(13.4)30–17.5(25)50 M: 7.5(12.76)20–15(22.43)37.5	Encrusting/whitish	Caleta de Bines, Socorro Island (Revillagigedo)/ Present study
***P. muricyae* sp. nov.** LEB-ICML-UNAM-2068	65(73)80 × 2.5(2.6)3.75	12.5(20.75)27.5–25(43.25)70	S: 12.5(18)25–22.5(35.3)47.5 M: 10(11.25)12.5–17.5(21.5)25	Encrusting/cream	Punta Mita, Nayarit/ Present study
***P. muricyae* sp. nov**. LEB-ICML-UNAM-2069	57.5(74.25)92.5 × 2.5(3.37)5	17.5(21.75)25–37.5(42)47.5	S: 6.25(11.9)15–12.5(24.24)30 M: 10(11.25)15–17.5(22.5)27.5	Encrusting/light green yellowish	Punta Mita, Nayarit/ Present study
Comparative species					
Plakina monolopha sensu Schulze, 1880	52.5(71.3)93.0 × 1.5–4.1	11.2(23.5)34.4 × 1.2–3.5	S: 15.5(18.9)31.0 × 1.2–3.2 M: 8.0(24.5)–31.8/1.3–2.5 cladome	Thinly encrusting/white or cream	Naples, Italy/ Muricy *et al*., 1998
P. monolopha sensu Thiele, 1898	80	20	M: 10–15 (rays)	Encrusting/whitish	Japan/ Thiele, 1898
P. monolopha sensu Arndt, 1927	75–90 × 3	33 × 3 (rays)	M: 16 (rays)	Encrusting/ brown-yellowish	Curaçao/ Arndt, 1927
P. monolopha sensu de Laubenfels, 1951	36 × 4	20–24 × 3–4 (rays)	S: 20–24 × 3–4 (rays) M: 12 × 3	–	Hawaii/ de Laubanfels, 1951
P. monolopha sensu Koltun, 1964	70–140 × 3–5	20–52 (rays)	S: 20–52 (rays) M: 10–20	–	Antarctica/ Koltun 1964
P. monolopha sensu Bergquist, 1968	72–96 × 4	20–28 × 4 (rays)	S: 20–28 × 4 (rays) M: 11–13 × 2	Encrusting/orange to yellowish	New Zealand/ Bergquist, 1968
P. monolopha sensu Thomas, 1970	63–109 × 2–6	21–42 × 2–5 (rays)	S: 21–42 × 2–5 (rays) M: 16	Encrusting/pale chocolate (dry)	India/ Thomas, 1970
P. monolopha sensu Pulitzer-Finali, 1983	94	32 (rays)	S: 32 (rays) M: 27–40 (total length)	–	Mediterranean/ Pulitzer-Finali, 1983
P. monolopha sensu Tanita & Hoshino, 1989	85–120 × 4–6	20–24 × 3–4 (rays)	S: 20–24 × 3–4 (rays) M: 20 (total length)	–	Japan/ Tanita & Hoshino, 1989
P. monolopha sensu Díaz & van Soest, 1994	80–90 × 3–5	30 × 3 (rays)	S: 21 × 3 (rays) M: 23 (total length)	–	Ireland/ Díaz & van Soest, 1994
P. monolopha sensu Carballo, 1994	50–67 × 2–3	44–54 × 2–2.5	S: 44–54 × 2–2.5 M: 20–30 (rays)	Encrusting/whitish	Iberian Peninsula/ Carballo, 1994
Plakina pacifica Desqueyroux-Faúndez & van Soest, 1997	67–184 × 4–12	47–77 × 4–9–47–77 × 5.6	S: 30–82 × 4.13 M: 13–19 × 1–3 and 19–60 × 4–12	Thin crust/beige	Galapagos Islands/ Desqueyroux-Faúndez & van Soest, 1997

M, monolophose; S, simple.

and *Plakina monolopha* (from France) showed that the species are closely related but with a significant genetic distance (Fig. 8). We considered that these morphological and molecular differences, together with the ample geographical distance, are sufficient to consider *Plakina muricyae* sp. nov. and *Plakina monolopha* to be different species. Morphological differences from other specimens cited as *Plakina monolopha* around the world can be seen in Table 2.

The only species from the eastern Pacific region with monolophose calthrops as seen in *Plakina muricyae* sp. nov. is *Plakina pacifica* Desqueyroux-Faúndez & van Soest, 1997. Both species possess triods, but they are relatively scarce in *Plakina pacifica*, and very abundant in *Plakina muricyae* sp. nov., in which they constitute the main skeleton. In addition, our specimens of *Plakina muricyae* sp. nov. possess monolophose calthrops in a single size category with rays measuring from 7.5 to 25 μm, whereas *P. pacifica* has monolophose calthrops in two size categories: rays measuring 13–15 μm and 19–45 μm long (Table 1).

PLAKINA PARADILOPHA SP. NOV.

(FIGS 2C, 4)

Material examined

Holotype: MNCN-1.01/695, Playa Blanca, Socorro Island (Revillagigedo), 18°48′59″N, 111°02′42″W, 0.5 m depth, 6.v.2008. Paratype: LEB-ICML-UNAM-1652, same collection data as holotype.

ZooBank LSID: urn:lsid:zoobank.org:act:5CAD744E-C33C-4D30-AD72-318DEA5B8A98.

Etymology

The species name is derived from the similarity of this species to the Mediterranean *Plakina dilopha*, mainly because of the composition of the dilophose spicules.

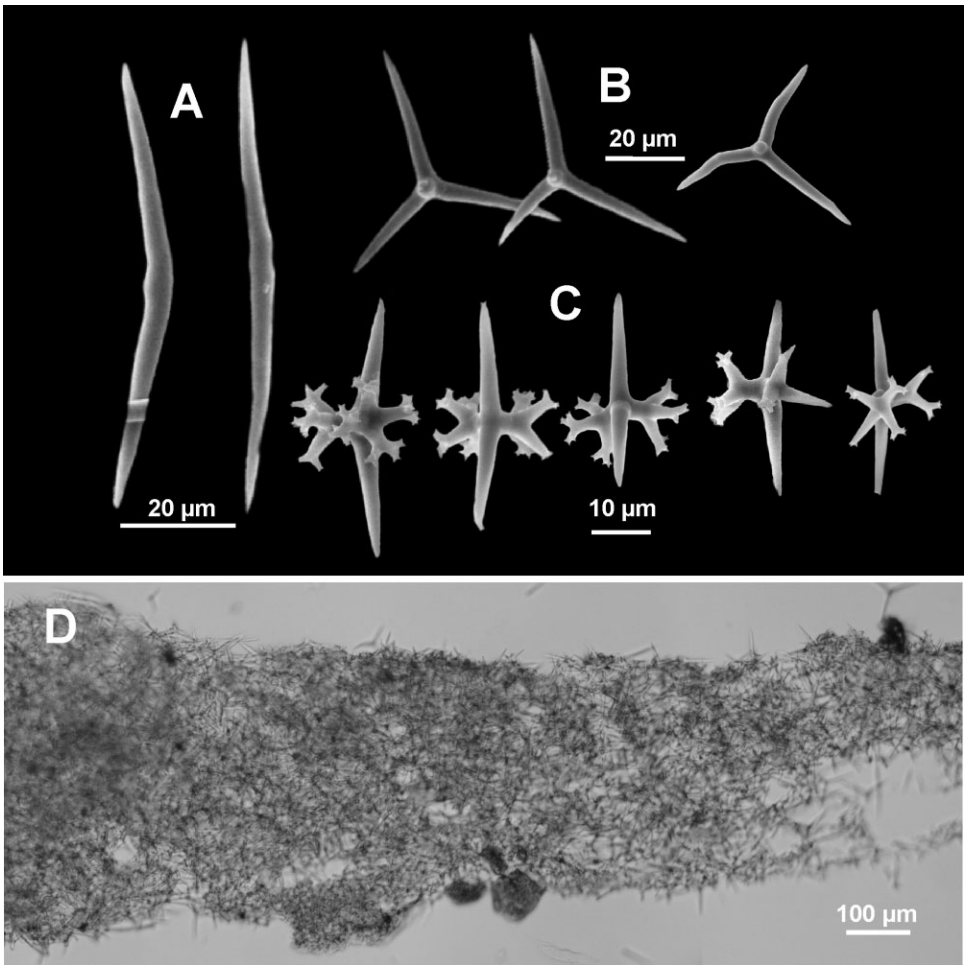

Figure 4. Scanning electron microscopy and light microscopy images of spicules and skeletal structure of ***Plakina paradilopha* sp. nov.** A, diods. B, triods. C, dilophose calthrops. D, transversal view of the choanosome.

Diagnosis

Whitish to bluish, thinly encrusting sponge with rounded edges, surface smooth but rough to the touch, with abundant subectosomal spaces and slightly elevated oscula irregularly distributed over the surface. Spicules are diods and simple calthrops, which constitute a dense and sometimes confuse choanosomal alveolar structure, whereas dilophose calthrops are arranged in a thin ectosomal layer.

Description

Encrusting sponge from, 0.2 to 0.4 mm thick, covering a small area of 10 × 3 cm on dead coral fragments of the genus *Pocillopora*. The surface is smooth with rounded edges, and rough to the touch. It is also characterized by abundant subectosomal spaces homogeneously distributed, which are visible to the naked eye (Fig. 2C). They are rounded to oval-shaped, from 100 to 250 μm in diameter. Oscules are circular to oval-shaped, of 1 to 2 mm in diameter, which are irregularly distributed and slightly elevated. The consistency is firm but easy to tear. Choanosomal aquiferous canals are common. They measure from 75 to 125 μm in diameter. Colour in life is cream to greyish-blue and greyish beige in ethanol preserved.

Spicules: diods, calthrops, and dilophose calthrops (Table 3). Diods are robust, curved, or straight, with an irregular, slight thickening in the middle of the shaft (Fig. 4A). Diods are very abundant. They are 62–100 μm long and 2.5–7.5 μm wide. Simple calthrops possess rays with a sharp point. These spicules are characterized by having one or two short rays as a knob, and the other one slightly larger than the others. The rays are commonly slightly curved at the end (Fig. 4B). Calthrops are also abundant; the rays are 15–35 μm long (total length 30–67.5 μm). Dilophose calthrops are less abundant, and they are characterized by having two lophose rays ramify medially into four or five small rays, which in turn ramify distally into two or three rays with spined tips (Fig. 4C). The simple rays measure 12.5–22.5 μm long and the lophose rays 5–12.5 μm long (total spicule length 22–42.5 μm).

Skeleton: ectosomal structure is formed mainly by dilophose calthrops with rays toward the surface. The choanosome is formed mainly by diods and simple calthrops, forming a dense structure, but sometimes with confuse alveolar reticulation with meshes measuring from 20 to 37.5 μm in diameter (Fig. 4D).

Ecology and distribution

The species was collected at Playa Blanca (Socorro Island, Revillagigedo archipelago; Fig. 1), at 0.5 m depth. Individuals were found between branches and bases of dead coral.

Remarks

Plakina paradilopha sp. nov. displays similar characteristics to the *Plakina dilopha* species complex. However, the species differs in details of the surface; *Plakina paradilopha* sp. nov. presents a regular smooth surface characterized by subectosomal spaces homogenously distributed and oscules slightly elevated, whereas *Plakina dilopha* possesses rounded holes of various sizes and oscules that do not protrude at the borders (*sensu* Schulze, 1880; see Muricy *et al*., 1998). Calthrops and diods are very scarce in *Plakina dilopha* (see Schulze, 1880; Topsent, 1895), but they are relatively abundant in *Plakina paradilopha* sp. nov. In addition, *Plakina dilopha* possesses triods (Desqueyroux-Faúndez & van Soest, 1997), which are absent in *Plakina paradilopha* sp. nov. Specimens of *Plakina* cf. *dilopha* with triods have also been cited from Ascension Island in the Atlantic (Díaz & van Soest, 1994).

Plakina microlobata is the only species of the *Plakina dilopha* species-complex found in the eastern Pacific region. This species is characterized by a smooth but strongly undulating, microlobate surface, which gives it the specific name (Desqueyroux-Faúndez & van Soest, 1997), whereas *Plakina paradilopha* sp. nov. has a smooth surface, even under the stereoscope. Skeletal differences are also important in both species; diods are larger in *Plakina paradilopha* sp. nov. (62.5–100 μm long and 2.5–7.5 μm wide) than in *Plakina microlobata* (45–60.5–72 × 1.5–2.75–4 μm). Additionally, *Plakina microlobata* possesses triods, which are absent in *Plakina paradilopha* sp. nov.

Plakina elisa (de Laubenfels, 1936) from the Caribbean possesses dilophose calthrops but also monolophose calthrops and diods with several sharp spines (de Laubenfels, 1936), which are absent in the diods of *Plakina paradilopha* sp. nov. (see Table 2).

Genus *Plakortis* Schulze, 1880

Type species

Plakortis simplex Schulze, 1880

Diagnosis

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 confuse, dense, without ectosomal specialization or differential location of spicules (Muricy & Díaz, 2002).

Table 3. Comparative data for the dimensions of the spicules (in μm), locality and other characteristics of *Plakina paradilopha* sp. nov. and other species of the *Plakina* ***dilopha*** species complex. Values in parentheses are means

Plakina species	Diods (shaft length × width)	Triods (ray length – total length)	Calthrops (ray length – total length)	Shape/colour	Original locality/ references
***P. paradilopha* sp. nov.** Holotype MNCN-1.01/ 695	62–(78.1)–100 × 2.5–(3.8)–7.5	None	S: 15(25.6)35–30(48)76.5 D: 12.5(18)20–22.5(35)40	Encrusting/white to greyish-blue	Playa Blanca, Socorro Island (Revillagigedo)/ Present study
***P. paradilopha* sp. nov.** LEB-ICML-UNAM-1652	62.5–(77.59)–100 × 2.5–(3.69)–7.5	None	S: 15(26)35–30(49)67.5 D: 5(9.6)12.5–22.5(35.2)42.5	Encrusting/white	Playa Blanca, Socorro Island (Revillagigedo)/ Present study
Comparative species					
Plakina dilopha Schulze, 1880	70–90	24–30 (rays)	S: 25–30 (rays) D: 25–30 (rays)	Encrusting/white	Mediterranean/ Schulze, 1880
P. cf. *dilopha* Díaz & van Soest, 1994	80–90 × 3	15–26 (rays)	S: absent? D: 13 × 2 (rays)		Ascension/ Díaz & van Soest, 1994
P. dilopha in Muricy *et al.*, 1998	65–75	20–30 (rays)	S: 20–30 (rays) D: 25–40	Crust/white	Trieste, Italy (Mediterranean)/ Muricy *et al.*, 1998
P. elisa (de Laubenfels, 1936)	Present	25 × 2 (rays)	S. absent? D: 12 × 1 (rays)		Panama (Caribbean)/ de de Laubenfels, 1936
P. elisa in Díaz & van Soest, 1994	80 × 4	18–23 × 4 (rays)	S: 18–24 × 4 (rays) D: 16 × 3 (rays)	None/blue	Curaçao / Díaz & van Soest, 1994
P. microlobata Desqueyroux-Faúndez & van Soest, 1997	60.5 × 2	24 × 2.2–38.6	S: 20.3 × 2–24–40 D: 11 × 1.7–16–27	Encrusting/beige	Galápagos Islands/ Desqueyroux-Faúndez & van Soest 1997
P. crypta Muricy *et al.*, 1995	35.3(64.6)80.8 × 1.5–3.2	15.0(23.4)35.5 × 1.5–3	S: 11(19.2)30.3 × 1.6–3.2 M: 19.3–(23.1)–30.5 × 1.5–2.5 cladome D: 19-(23.2)-26 × 1.5–2.0 cladome	White	La Ciotat, France/ Muricy *et al.*, 1998
P. atka Lehnert *et al.*, 2005	70–108 × 3–6; 80–95 × 8–10	28–33; 23–40 × 3–6	S: present but no data Te: 11–14 × 3–5	Encrusting/pink to reddish-brown	Atka Island, Alaska/ Lehnert *et al.*, 2005
Other *Plakina* species from East Pacific					
P. bioxea Green & Bakus, 1994	78–179 × 3–8	?	S: 29–39 × 4.5 13–19 × 4–5		California/ Green & Bakus, 1994
P. fragilis Desqueyroux-Faúndez & van Soest, 1997	66–96 × 5–7	29.2 × 3.25–42–61	S: 25.7 × 3.1–29–56 Tr: 13.5 × 3–18–25	Encrusting/none	Galápagos Islands/ Desqueyroux-Faúndez & van Soest, 1997
P. tanaga Lehnert *et al.*, 2005	85–97 × 2–4	24–50 × 2–5; 22–38 × 7–9	Rare, S: 24–50 × 2–5; 22–38 × 7–9	Encrusting/beige or light brown	Little Tanaga Strait/ Lehnert *et al.*, 2005

D, dilophose; M, monolophose; S, simple; Te, tetralophose; Tr, trilophose.

PLAKORTIS CLARIONENSIS SP. NOV.

(FIGS 2D, 5)

Material examined

Holotype: MNCN 1.01/696 Roca Norte, Clarión Island (Revillagigedo), 18°47′14″N, 110°55′42″W, 4 m depth, 12.iii.2005. Paratypes: LEB-ICML-UNAM-1240, Roca Norte, Clarion Island (Revillagigedo), 18°47′14″N, 110°55′42″W, 4 m depth, 12.iii.2005. LEB-ICML-UNAM-1253, Pináculo Norte (Revillagigedo), 18°51′4″N, 110°59′53″W, 4 m depth, 12.iii.2005.

ZooBank LSID: urn:lsid:zoobank.org:act:734EED7F-8AA6-4899-B3C3-1DE7346178A3.

Etymology

The epithet refers to the type locality Clarion Island (Revillagigedo archipelago) where the specimens of this species were collected.

Diagnosis

Dark brown (in preserved condition), encrusting to cushion-shaped sponge, with smooth surface. Consistency is cartilaginous-like, but inside is fleshy. Spicules are diods and triods, which are included in a dense collagen layer, without alveolar skeletal specialization.

Description

Encrusting to cushion-shaped sponge from 0.2 to 1.5 cm in thickness, spreading between coral branches and over rocks (Fig. 2D). The specimens cover areas up to 10 × 8 cm. Surface is uneven and rugose, with rounded bumps, which gives a granular appearance. Sponge borders in contact with the coral structures become irregular, taking the substrate's form. Between the

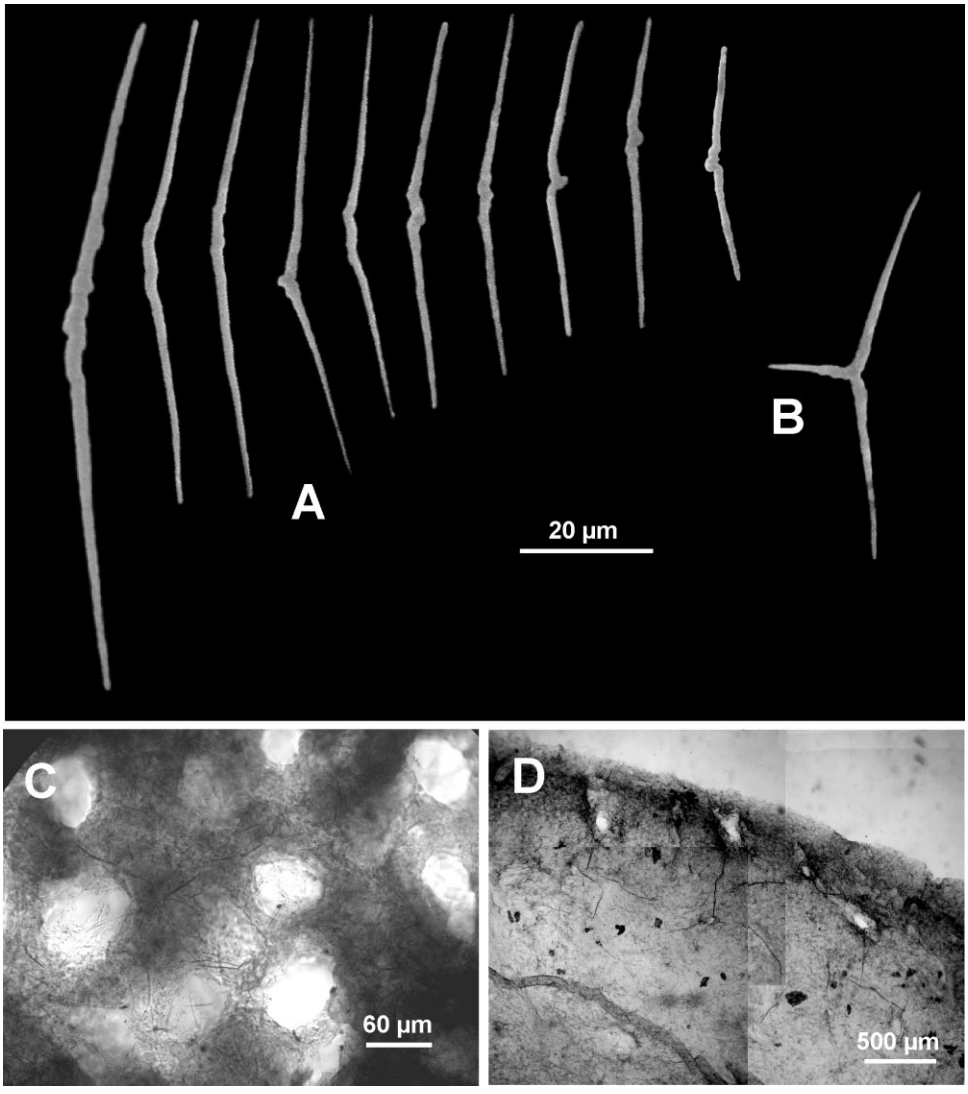

Figure 5. Scanning electron microscopy and light microscopy images of spicules and skeletal structure **of *Plakortis clarionensis* sp. nov.** A, diods. B, triods. C, tangential view of the ectosome. D, transversal view of the choanosome.

bumps there are small ostial pores of 330–670 μm in diameter. The surface also presents very small subectosomic canals 150–250 μm in diameter. Only one circular-shaped oscule was found, of 1 mm in diameter. The consistency of the surface is slightly cartilaginous, but the interior is fleshy. Colour in life was not observed; in spirit it is dark and light brown on the surface and the choanosome is pale yellow.

Spicules: diods and triods (Table 4). Diods are thin and elongated, typically curved in the centre, with one, two or more well-marked bent. The ends are straight with sharp or slightly rounded tips (Fig. 5A). Diods are abundant; they measure 17.5–77.5 μm long and 1.25–3 μm wide. Triods usually present equal angles, but can be uneven with 'T' or 'Y' shape (Fig. 5B). Sizes of the rays are variable, 10–30 μm long (total spicule length 20–62 μm).

Skeleton: the skeletal structure is formed by diods in abundance and some triods, which are included in a dense collagen layer, without alveolar or skeletal specialization and no apparent organization. The ectosome is a nondetachable pigmented layer (up to 1 mm in thickness), with dispersed diods (Fig. 5C), and very well differentiated from the choanosome (Fig. 5D). In the subectosomal area an agglomeration of spicules and pigments is present; this layer is also characterized by the presence of several canals, rather than the deep choanosome.

Ecology and distribution

Specimens were collected in two localities from Revillagigedo archipelago: Clarion Island and Pináculo Norte (Fig. 1). Specimens were found on pocilloporid coral branches and over rocks at 4 m depth.

Remarks

Currently, there are three species of *Plakortis* from the East Pacific coast: *Plakortis albicans* (see below for remarks on this species) and *Plakortis simplex* (Austin, 1996) from Mexico, and *Plakortis galapagensis* from the Galapagos Islands.

The species *Plakortis simplex* was originally described in the Mediterranean Sea (Schulze, 1880) and later cited around the world. However, recent authors have discussed the conspecificity of different populations, suggesting a species complex (sibling species). Therefore, the Mexican record by Austin (1996) is probably a different species. However, a more detailed description of this material would be necessary to confirm its taxonomic status.

The geographically closest species to *Plakortis clarionensis* sp. nov. is *Plakortis galapagensis*, which is different to the new species in external and internal characteristics. *Plakortis galapagensis* is an encrusting (5 mm thick), beige-coloured species whereas *Plakortis clarionensis* sp. nov. is more cushion-shaped (up to 1.5 cm thickness), dark brown sponge. *Plakortis galapagensis* presents an alveolar skeletal structure whereas *Plakortis clarionensis* sp. nov. has a dense collagen layer, without skeletal specialization. The spicules also vary in size; diods are larger and in two size categories in *Plakortis galapagensis* (126–165 by 4–8 μm and 27–92 by 1.5–4 μm) than in *Plakortis clarionensis* sp. nov., which only presents one, smaller size category (17.5–77.5 by 1.3–3 μm).

The species *Plakortis nigra* Lévi, 1953, from the Red Sea and *Plakortis insularis* Moraes & Muricy, 2003, from north-east Brazil are the only two species possessing small diods. The rest of the known species have diods bigger than 100 μm long (see Table 4). *Plakortis nigra* differs from *Plakortis clarionensis* sp. nov. in the black colour, porous surface, and because its skeletal structure is formed only by diods. *Plakortis insularis* is encrusting, massive, rounded or flat in shape, and dark to light brown in colour. The skeleton is very similar to *P. clarionensis* sp. nov., with diods and triods in low density forming a confuse structure. However, *P. insularis* has larger diods, which always end in a sharp tip, whereas the diods in *Plakortis clarionensis* sp. nov. are smaller and end in a sharp or rounded tip. *Plakortis insularis* produces a dark brown substance when preserved, which was not observed in our specimens of *Plakortis clarionensis* sp. nov. The consistency of *P. insularis* is soft, compressible, and fragile, whereas in *Plakortis clarionensis* sp. nov. it is elastic and resistant at the surface, although the interior is fleshy.

PLAKORTIS ALBICANS CRUZ-BARRAZA & CARBALLO, 2005 (FIGS 2E, 6)

Synonymy

Plakortis albicans Cruz-Barraza & Carballo, 2005: 4.

Material examined

LEB-ICML-UNAM-1106, Punta Mita (Nayarit), 20°46′20″N, 105°32′49″W, 3 m depth, 19.ii.2005. LEB-ICML-UNAM-1283, El Arrocito (Oaxaca) 15°44′25″N, 96°51′31″W, 4 m depth, 11.iv.2005. LEB-ICML-UNAM-1408, San Agustín (Oaxaca) 15°41′9″N, 96°13′46″W, 6 m depth, 9.iv.2005. LEB-ICML-UNAM-1718, Bahía Tiburones, Isabel Island (Nayarit), 21°50′38″N, 105°52′14″W, 2 m depth, 29.iii.2011. LEB-ICML-UNAM-2009, Caleta de Bines, Socorro Island (Revillagigedo), 18°44′10″N, 110°57′37″W, 6 m depth, 7.xi.2009. LEB-ICML-UNAM-2070, Punta Mita (Nayarit), 20°46′20″N, 105°32′49″W, 3 m depth, 29.iii.2012. LEB-ICML-UNAM-2071, Bahía Tiburones, Isabel Island (Nayarit), 21°50′38″N, 105°52′14″W, 2 m depth, 26.iii.2012. LEB-ICML-UNAM-2356, Bahía Tiburones, Isabel Island (Nayarit), 21°50′38″N, 105°52′14″W, 2 m depth, 29.xi.2012. LEB-ICML-UNAM-2357, Bahía Tiburones,

Table 4. Comparative data for the dimensions of the spicules (in μm), locality and other characteristics of ***Plakortis clarionensis* sp. nov.** and *Plakortis albicans*, and other similar (in morphology and geography) *Plakortis* species for comparison. Values in parentheses are means

Plakortis species	Diods (shaft length × width)	Triods (ray length – total length)	Shape/colour	Original locality/ references
***P. clarionensis* sp. nov.** MNCN-1.01/696	17.5(46)77.5 × 1.3(2.4)3	10(19.7)27.5–22(42)56	Cushion/dark brown	Roca Norte, Clarión Island (Revillagigedo)/ Present study
***P. clarionensis* sp. nov.** LEB-ICML-UNAM-1253	17.5(47)77.5 × 1.25(2)2.5	10(21)30–20(46)62	Cushion/dark brown	Pináculo Norte (Revillagigedo)/ Present study
Plakortis albicans				
P. albicans LEB-ICML-UNAM-1106	20(56.75)125 × 1.25(2.3)3.75	10(17.3)25–17.5(33)42.5	Encrusting/whitish	Punta Mita, Nayarit/ Present study
P. albicans LEB-ICML-UNAM-1283	25(84.2)125 × 1.5(3.2)5	15(25.4)37.5–27.5(51)37.5	Encrusting/beige	El Arrocito, Oaxaca/ Present study
P. albicans LEB-ICML-UNAM-1408	15(58.9)105 × 1.25(2.7)5	5(1.75)2.5–10(20.7)45	Encrusting/beige	San Agustín, Oaxaca/ Present study
P. albicans LEB-ICML-UNAM-1718	12.5(50.75)95 × 1.25(2.45)3.75	10(23)37.5–17.5(42.25)70	Encrusting/whitish	Bahía Tiburones, Isabel Island/ Present study
P. albicans LEB-ICML-UNAM-2009	17.5(54)120 × 1.25(2.6)5	7.5(24.7)37.5–15(46.25)67.5	Encrusting/beige	Caleta de Bines, Socorro Island (Revillagigedo)/ Present study
P. albicans LEB-ICML-UNAM-2070	15(64.8)110 × 0.5(2.18)2.5	6.25(20.10)35–12.5(39.8)62.5	Encrusting/whitish	Punta Mita, Nayarit/ Present study
P. albicans LEB-ICML-UNAM-2071	17.5(58.9)112.5 × 1.25(1.8)2.5	17.5(21.14)30–35(42)60	Encrusting/whitish	Bahía Tiburones, Isabel Island/ Present study
P. albicans LEB-ICML-UNAM-2356	22.5(57.7)102.5 × 0.5(2)3.75	6.25(18.6)25–12.5(37.3)50	Encrusting/whitish	Bahía Tiburones, Isabel Island/ Present study
P. albicans LEB-ICML-UNAM-2357	15(71)162.5 × 1.25(31.25)5	6.25(16.9)30–12.5(33.3)60	Encrusting/whitish	Bahía Tiburones, Isabel Island/ Present study
P. albicans LEB-ICML-UNAM-2358	15(74.7)150 × 0.25(2.3)5	5(19)35–10(37.3)72.5	Encrusting/whitish	Bahía Tiburones, Isabel Island/ Present study
Comparative individuals and species				
P. simplex Schulze, 1880	(Centrotylote) 60–150 × 3 × 6	25–50 × 3–6	Brown/lighted	Mediterranean/ Schulze, 1880
P. albicans Cruz-Barraza & Carballo, 2005	16.3–(70.1)–135 × 1.5–(3.5)–7	7.5–(23)–32.5	Encrusting/white	Lobos Island, Sinaloa (México)/ Cruz-Barraza & Carballo 2005
P. galapagensis Desqueyroux-Faúndez & van Soest, 1997	126–165 × 4–8 and 27–92 × 1.5–4	17–36 × 1.5–4 (rays)	Massive encrustation/ beige	Galápagos Islands/ Desqueyroux-Faúndez & van Soest, 1997

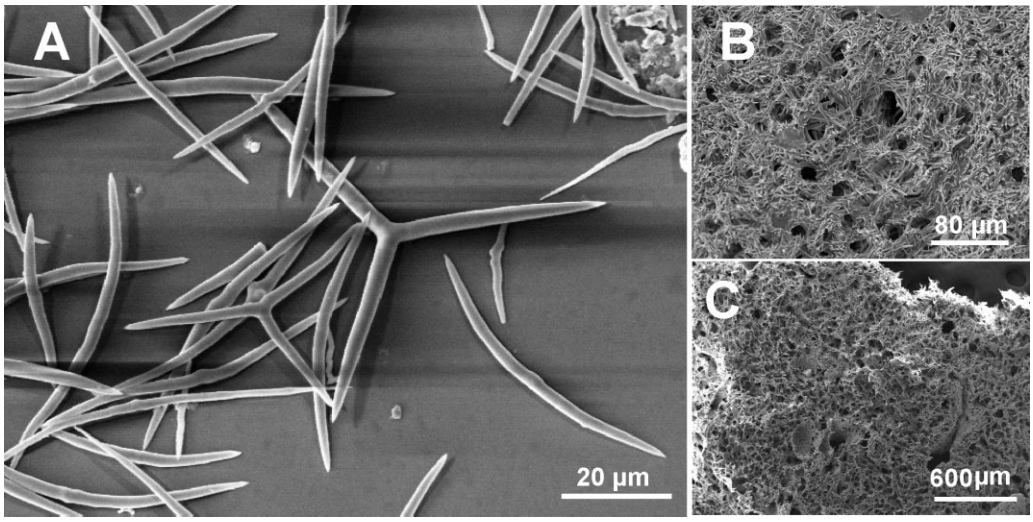

Figure 6. Scanning electron microscopy images of spicules and skeletal structure of *Plakortis albicans* Cruz-Barraza & Carballo, 2005. A, diods and triods. B, tangential view of ectosomal alveolar skeleton. C, transversal view of the choanosome.

Isabel Island (Nayarit), 21°50′38″N, 105°52′14″W, 2 m depth, 5.ix.2011. LEB-ICML-UNAM-2358, Bahía Tiburones, Isabel Island (Nayarit), 21°50′38″N, 105°52′14″W, 2 m depth, 5.ix.2011.

Description

Thickly encrusting species (from 1 to 2 mm in thickness), from 1 to 4 cm diameter, meandering between dead coral base and rocks (Fig. 2E). The surface is smooth, characteristically sculpted by subectosomal canals (50–150 µm in diameter). It also has subectosomal spaces, which are regularly distributed over the surface (from 33.3 to 83 µm in diameter). Oscula are circular-shaped and slightly elevated from the surface, measuring 0.5 mm in diameter. Consistency is slightly compressible but firm, easy to tear. Colour in life is white to ivory and dark greyish-blue, preserved it turns paler.

Spicules: diods and triods (Table 4). Diods are irregularly curved along the shaft, sometimes bent one or two times near the centre (Fig. 6A). They are abundant and variable in size, from 12.5 to 162.5 µm long by 0.3 to 5 µm width. The triods are less abundant than diods, with an irregular or equal-angled shaped. The rays are mostly straight or slightly bent. The size of the rays is very variable, 5–37.5 µm long, (total spicule length 10–72.5 µm).

Skeleton: ectosome possesses a tangential alveolar skeletal structure of spicule tracts (5 to 15 µm in diameter), with meshes from 15 to 50 µm in diameter (Fig. 6B). The choanosomal skeleton is a dense and relatively disorganized reticulation of spicule tracts, with meshes from 20 to 50 µm in diameter (Fig. 6C). Choanosomal canals from 70 to 350 µm in diameter.

DNA barcode sequence data

Two specimens from Isabel Island, (Nayarit) and two from the type locality, Mazatlan Bay (Cruz-Barraza & Carballo, 2005), were sequenced and 584 bp of *cox1* and 388 bp *cob* mtDNA were used for comparison. Individuals showed an identical sequence for both fragments, confirming together with morphology that they correspond to the same species. Comparison of our sequences with sequences available in GenBank showed that the nearest species to *Plakortis albicans* is *Plakinastrella* sp. from Florida, USA (EU237487.1 named as *Plakinastrella* cf. *onkodes*) with 99% similarity for *cox1* and 98% for *cob*. Although the specimen of this sequence appears in GenBank as *Plakinastrella* cf. *onkodes,* it was originally identified as *Plakortis angulospiculatus* (Carter, 1882) (by Lavrov *et al*., 2008), and in later revisions it was named as *Plakinastrella* sp. (by Gazave *et al*., 2010) and *Plakinastrella onkodes* (by Ereskovsky *et al*., 2014).

Sequences of *cox1* and *cob* of *Plakortis albicans* have been deposited in GenBank with accession numbers: *cox1 pending*; *cob*: *pending*.

Ecology and distribution

The species has been described from Lobos Island in Mazatlan Bay (Sinaloa, Mexico, East Pacific Ocean). In the present study, specimens were collected in shallow water (3 to 6 m depth) over dead corals from Bahía Tiburones (Isabel Island, Nayarit), Punta Mita (Nayarit), Caleta de Bines (Socorro Island, Revillagigedo), El Arrocito and San Agustín (Oaxaca). This is the first record for *Plakortis albicans* associated with coral reefs from the Mexican Pacific coast.

Remarks
Specimens described here as *Plakortis albicans* agree very well with the morphological description of the species (Cruz-Barraza & Carballo, 2005), and sequences also show a nucleotide composition identical to the specimens collected from the type locality, Mazatlán Bay.

Genus *Plakinastrella* Schulze, 1880

Type species
Plakinastrella copiosa Schulze, 1880

Diagnosis
Thinly to massively encrusting, sometimes lobate or tubular Plakinidae, usually tough in consistency and with surface smooth to the eye but rough to the touch. Subectosomal inhalant cavities present in some species; choanocyte chambers eurypylous or diplodal. Skeleton composed of non-lophose diods, triods, and/or calthrops with wide size variation, usually in three size classes. The small diactines are accumulated on the surface, either forming a palisade or disposed tangentially to the surface (Muricy & Díaz, 2002).

Plakinastrella clippertonensis van Soest, Kaiser & van Syoc, 2011 (Figs 2F, 7)

Material examined
LEB-ICML-UNAM-2034, Clipperton Island, 10°17′30″N, 109°14′59″W, 14 m depth, 28.ii.2005.

Description
Encrusting to cushion-shaped sponge (0.5 mm to up to 1 cm thick), covering an area of 8.4 × 3.5 cm. The surface is smooth to the naked eye, but rugose under the stereoscope. It also possesses homogeneously distributed subectosomal spaces (Fig. 2F). Oscula are circular-shaped and slightly elevated from the surface, measuring 0.2 to 0.5 mm in diameter. The consistency

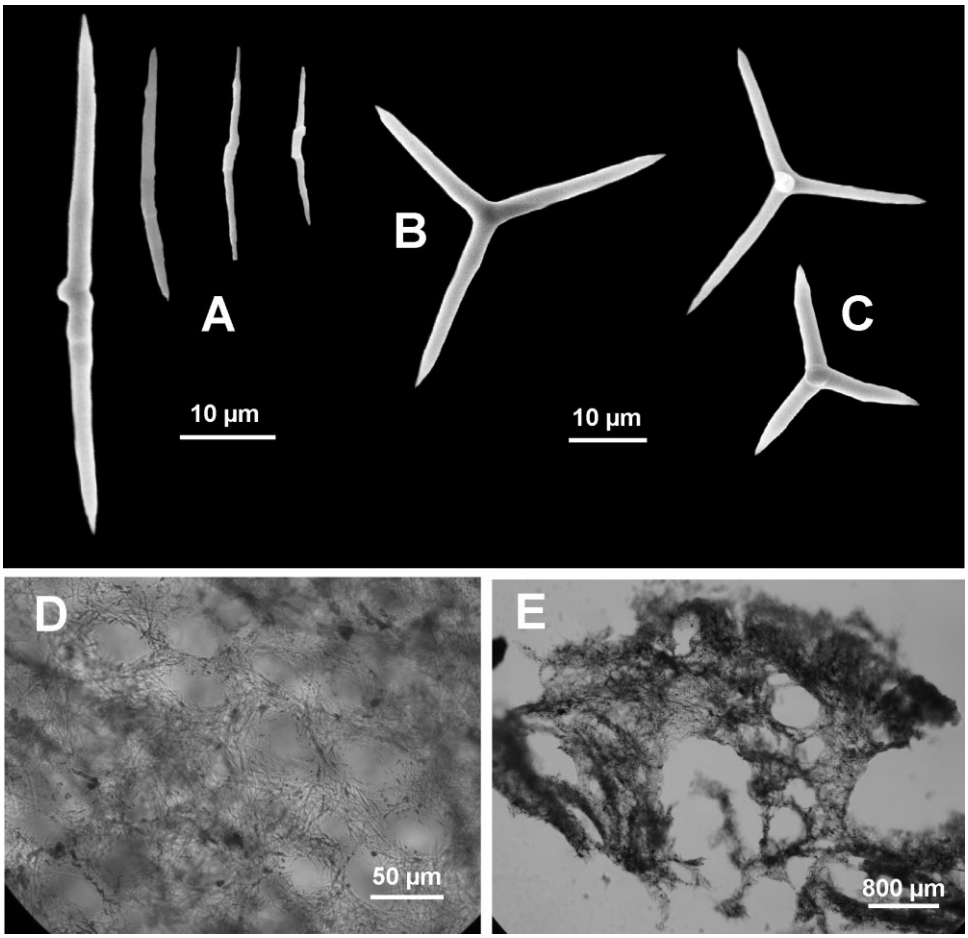

Figure 7. Scanning electron microscopy images of spicules and skeletal structure of *Plakinastrella clippertonensis* van Soest *et al*., 2011. A, diods. B, triods. C, simple calthrops. D, tangential view of the ectosome. E, transversal view of the choanosome.

is compressible but fragile. The colour is beige in preserved specimens.

Spicules: diods, triods, and calthrops. Diods are thin, widening to the middle shaft where there are one or two short bends (Fig. 7A). Diods are in a wide size range; they measure 10–(44.8)–70 μm long and 0.25–(1.35)–2.5 μm wide. Triods rays are mostly equidistant, thin but irregularly straight, sometimes with one ray smaller than the other two (Fig. 7B). The rays measure 3.75–25 μm long; [total spicule length 7.25–(13.43)–42.5 μm]. Calthrops can be equidistant or with one longer ray (Fig. 7C). The rays measure 3.75–22.5 μm long [total spicule length 7.5–(25.19)–42.5 μm].

Skeleton: the ectosomal skeleton consists of a tangential alveolar layer (20–30 μm in thickness) of spicule tracts (mainly diods), forming rounded meshes of 20–90 μm in diameter (Fig. 7D). The choanosomal skeleton is a dense structure formed mainly by diods, triods, and rare small calthrops. which form an irregular to alveolar pattern, with meshes of 30 to 55 μm in diameter (Fig. 7E). Choanosomal spaces are very abundant and vary from 70 to 500 μm in diameter.

Ecology and distribution

The species was described from Clipperton Island on dead corals in deep reef habitat (55 m) (van Soest *et al*., 2011). In the present study it was found on the same island under rock and corals at 14 m depth.

Remarks

The species was recently described by van Soest *et al*. (2011). Our specimen agrees in general aspects with the original description, although subtle differences are present.

The specimen described by van Soest *et al*., 2011, is cake-shaped, 1 cm thick; although we only analysed a small fragment, our specimen was cushion-shaped and about 1 cm thick (Fig. 2F). The original description also mentioned the skeleton as dense mass of diods and triods arranged around the aquiferous system, which is similar to our specimen (Fig. 7E), although we also found a not well-defined alveolar pattern in some sections of the sponge body. The ectosomal alveolar skeleton is similar too, although with rounded meshes slightly smaller in our specimens (50–100 μm vs. 20–90 μm, respectively). Diods and triods present a very similar size (diods: 4–75 μm in original description vs. 10–70 μm in our specimen; triod ray: 3.75–25 μm vs. 5–33 μm, respectively). However, simple calthrops are smaller than in our specimen (9–15 μm vs. 3.75–22.5 μm, respectively). In general, calthrops are very rare and only 12 were measured in the original description; we found a large number of spicules and a substantial range of measurements. To date, only two specimens of this species have been described. When more specimens are described, it will probably be possible to establish clearly the limits and ranges of spicule sizes.

Molecular analysis

The origin of our sequences was confirmed by BLAST search, thereby discarding the possibility of contamination. Comparison of all our sequences with others from GenBank showed high similarity with Plakinidae species (see DNA barcode in species description). Following previous molecular Homoscleromorpha analysis, we chose to use a fragment of the mitochondrial *cob* gene (388 bp) (Ereskovsky *et al*., 2014). Additionally, we also used 584 bp of the *cox1* mtDNA 'Barcoding Folmer fragment' to analyse the species differentiation and phylogenetic relationships of our Plakinidae; therefore sequences were aligned together with homosclerophorid sequences downloaded from GenBank (Table 1).

From the alignment, no insertion/deletion was detected. The topologies obtained by Bayesian inference are presented. The number at each node represents the Bayesian posterior probability (PP). Results from both gene fragments were mostly congruent with the phylogenetic hypothesis (Fig. 8). The two families, Plakinidae and Oscarellidae, were also clearly separated in our analysis. The relationships within Oscarellidae agreed between the two gene fragments, except in poorly supported clades of *Oscarella microlobata* and *Pseudocorticium jarrei* in the *cob* tree (65% PP), which were not resolved in the *cox1* topology (Fig. 8). Within Plakinidae, the phylogenetic relationships were also fairly similar, although the *cox1* topology was more congruent with the morphological hypothesis, restoring the monophyly of *Plakina* (70% PP), which was not supported in the *cob* reconstruction (Fig. 8).

Our results also show the affinities amongst our species and other Plakinidae. *Plakina muricyae* sp. nov. was grouped together with *Plakina monolopha* and *Plakina* sp. in both gene trees. The similarity between *Plakina muricyae* sp. nov. and *Plakina monolopha* is congruent with morphology, as both share the presence of monolophose calthrops. However, surprisingly *Plakortis albicans* showed major similarities with the specimen sequence identified as *Plakinastrella* sp. (EU237487) instead of with other *Plakortis* species. However, in both approximations this clade is closely related to other *Plakortis* species.

DISCUSSION

Diversity and distribution of the class Homoscleromorpha from the eastern Pacific region

For a long time, the eastern Pacific sponge fauna was considered amongst the least known in the world

KEY FOR SPECIES OF HOMOSCLEROMORPHA FROM THE EASTERN PACIFIC REGION

1. Mineral skeleton present……2
1′. Mineral skeleton absent……*Oscarella carmela*
2. Calthrops absent……3 (*Plakortis*)
2′. Calthrops present……5
3. Choanosomal skeleton with structure alveolar reticulation……4
3′. Choanosomal skeleton without a defined structure……***Plakortis clarionensis* sp. nov.**
4. Diods in a single large size category (12–162.5 μm)……*Plakortis albicans*
4′. Diods in two size categories (126–165 and 27–92 μm)……*Plakortis galapagensis*
5. Lophose calthrops absent……*Plakinastrella clippertonensis*
5′. Lophose calthrops present together with simple calthrops……6 (*Plakina*)
6. Only one type of lophose calthrops……7
6′. Two type of lophose calthrops (mono- and tetralophose)……*Plakina bioxea*
7. Monolophose calthrops……8
7′. No monolophose calthrops……9
8. Monolophose calthrops in a single category……***Plakina muricyae* sp. nov.**
8′. Monolophose calthrops in two categories……*Plakina pacifica*
9. Dilophose calthrops……10
9′. Trilophose or tetralophose calthrops……11
10. Smooth surface; dilophose calthrops in a single category……***Plakina paradilopha* sp. nov.**
10′. Microlobate surface; dilophose calthrops in two size categories……*Plakina microlobata*
11. Trilophose calthrops……12
11′. Tetralophose calthrops, conspicuously spined category of triods……*Plakina atka*
12. Large encrusting (1–1.5 cm) sponge with a convoluted surface pattern, rare triods (24–36 μm/ray)……*Plakina fragilis*
12′. Small, thin encrusting (0.3 mm) sponge without convoluted surface, triods in two shape categories: (1) smooth and thinner (24–50 μm/ray); (2) thicker, often with one short, spine near the base of each ray (22–38 μm/ray)……*Plakina tanaga*

(Gómez *et al*., 2002; van Soest *et al*., 2012). These sponges are mainly encrusting or endolithic species living in cryptic habitats such as dead coral and coral bases, or under/amongst coral rubble or rock (Carballo *et al*., 2008; present study).

However, in the last two decades, knowledge has increased significantly and has shown that there is a higher diversity of sponges than previously thought (Desqueyroux-Faúndez & van Soest, 1997; Lehnert, Stone & Heimler, 2005; Carballo *et al*., 2008, 2013; Cruz-Barraza *et al*., 2011, 2012), which suggests a potentially high unexplored diversity of cryptic sponges in the eastern Pacific.

Particularly, Homoscleromorpha, with about 91 valid described species, is not as diverse a group as other Porifera (e.g. Demospongiae). The Mediterranean and West Pacific with 23 and 21 species, respectively, are the richest areas, followed by the Caribbean with 17 species (van Soest *et al*., 2013); however, two decades ago, the group was completely unknown in the eastern Pacific region. Before the present work, ten species of four genera of Homoscleromorpha (*Plakina*, *Plakinastrella*, *Plakortis*, and *Oscarella*) were known (Desqueyroux-Faúndez & van Soest, 1997; Muricy & Pearse, 2004; Cruz-Barraza & Carballo, 2005; Lehnert *et al*., 2005; van Soest *et al*., 2011). This work has increased the number to 13 species, which are easy to distinguish from each other by differences in spicule composition and skeletal arrangement (see Key).

The genus *Plakina* is represented by eight species: *Plakina bioxea* Green & Bakus, 1994, from California (USA); *Plakina fragilis*, *Plakina microlobata*, and *Plakina pacifica* Desqueyroux-Faúndez & van Soest, 1997, from the Galapagos Islands; *Plakina atka* and *Plakina tanaga* Lehnert *et al*., 2005 from Atka Island (Alaska); and *Plakina muricyae* sp. nov. and *Plakina paradilopha* sp. nov. (present study), which constitute the first record of the genus *Plakina* from the Mexican Pacific.

The genus *Plakortis* harbours *Plakortis galapagensis* from the Galapagos Islands and *Plakortis albicans* from the Mexican Pacific. With the description of *Plakortis clarionensis* sp. nov., the number increases to three species. The brief description of the specimen described as *Plakortis* cf. *simplex* from Rocas Alijos (Mexican Pacific) does not allow us to determine its taxonomic status. It could be an undescribed species (Austin, 1996). The genera *Plakinastrella* and *Oscarella* are represented by only one species each, *Oscarella carmela*, from California (USA) and *Plakinastrella clippertonensis* from Clipperton Island. The latter species was also recorded from the type locality in the present study.

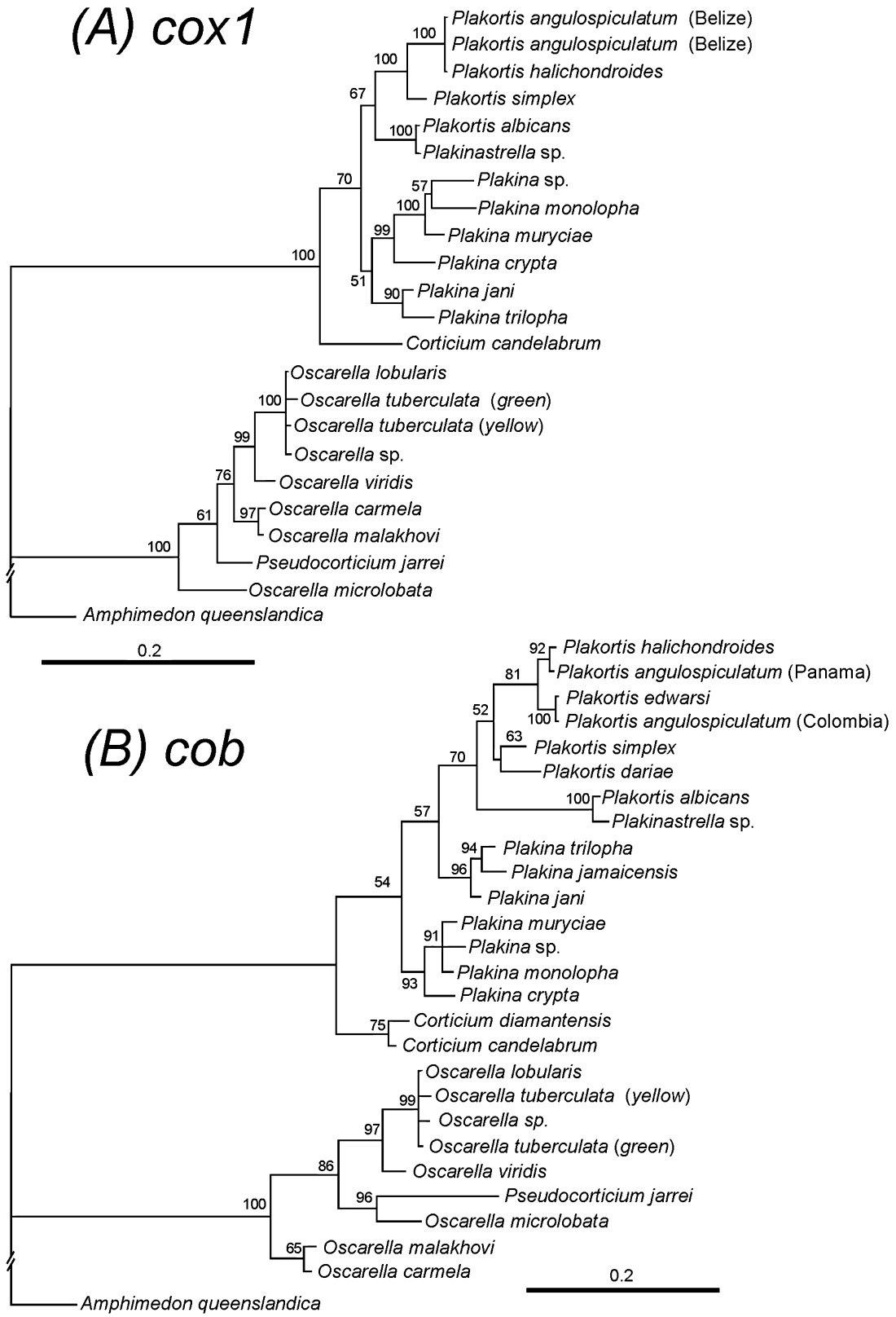

Figure 8. Phylogenetic reconstruction of *cytochrome c oxidase subunit I* (*cox1*; A) and *cytochrome b* (*cob*; B) mitochondrial markers. The topologies were obtained by Bayesian inference analysis with MrBayes. The number at each node represents the Bayesian posterior probability (%).

Molecular phylogeny and integrative approximation

Owing to the relevance of Homoscleromorpha for evolutionary studies, several approaches have emerged in order to clarify its internal and external phylogenetic relationships (Borchiellini *et al*., 2004; Sperling, Peterson & Pisani, 2009; Gazave *et al*., 2010, 2012, 2013; Ivanisêvić *et al*., 2011). In our analyses, internal relationships (Fig. 8) are mostly congruent with previous molecular hypotheses, which restored the two previously known families, Plakinidae and Oscarellidae (Gazave *et al*., 2010, 2012; Ivanisêvić *et al*., 2011; Ereskovsky *et al*., 2014).

In addition, except for the poorly supported clades of *Oscarella microlobata* and *Pseudocorticium jarrei* in the *cob* tree, which were not resolved in the *cox1* topology (Fig. 8), the relationships within Oscarellidae agreed with the two gene fragments that we used. These results are congruent with the current molecular hypothesis, which suggests the potential synonymy of *Oscarella* and *Pseudocorticium*; consequently Oscarellidae would be a monogeneric family (Gazave *et al*., 2013).

The family Plakinidae has shown distinct interactions using different molecular markers. Metabolic fingerprinting shows *Plakortis* as a sister clade of *Plakina* + *Corticium*, whereas *cox1* (I3M11) encompassed *Plakortis* in the *Plakina* clade, separated from *Corticium* (Ivanisêvić *et al*., 2011). Mitochondrial genomes and 28S rDNA sequences have presented *Plakina* as a nonmonophyletic group, with a clade formed by *Corticium*, *Plakina monolopha*, and *Plakina crypta*, and another clade formed by the other *Plakina* species (*Plakina jani* and *Plakina trilopha*). In contrast, 18S and *cob* data clustered together some *Plakina* (*Plakina jani* and *Plakina trilopha*) with the *Plakortis* + *Plakinastrella* clade (Gazave *et al*., 2010; Ereskovsky *et al*., 2014). In our analysis, the *cob* tree topologies are mostly similar to previous *cob* analyses, except for the genus *Corticium*, which is separated from all Plakinidae. This result was also supported by our *cox1* tree, placing *Corticium* as a valid genus. Moreover, *cox1* also shows a better resolved topology, congruent with the morphological hypothesis that shows *Plakina* as a monophyletic group (although with little support, 70% PP; Fig. 8).

From our *cox1* analysis, the two groups formed in the *Plakina* clade are consistent with two separated *Plakina* groups suggested previously in mitochondrial genomes, 28S rDNA sequences analyses (see above), and spicule morphology (lophate-calthrops) (Gazave *et al*., 2010). The two groups are represented by: *Plakina* with three- and/or tetralophose calthrops (*Plakina jani* and *Plakina trilopha*), and *Plakina* with monolophose or mono- and dilophose calthrops only (*Plakina monolopha*, *Plakina crypta*, *Plakina muricyae* sp. nov.). In this sense, Gazave *et al*. (2010) pointed out the importance of redefining the genus *Plakina* and subdivided it into two potential new genera corresponding to the abovementioned groups on the bases of morphology and genetic differences.

As we expected, in both of our analyses, *cob* and *cox1*, *Plakina muricyae* sp. nov. was grouped near *P. monolopha,* which is congruent with their morphological similarity (both with monolophose calthrops). The analyses also showed that these species are most closely related to *P. crypta*, which is also characterized by the presence of monolophose calthrops, but in contrast, this species also possesses scarce dilophose calthrops (Muricy *et al*., 1998). *Plakina paradilopha* sp. nov. could be part of this group because it has dilophose calthrops (like *P. crypta*). However, it was impossible to obtain DNA of this species although we tried to do so by various methods. In fact, it was impossible to obtain DNA of some species studied here because of the poor condition of some of the old specimens, and the difficulty in accessing the remote locations from where these specimens were collected.

Surprisingly, in both of our analyses *Plakortis albicans* showed a great affinity with *Plakinastrella* sp.; thus both are grouped as a sister clade with all the other *Plakortis* species (Fig. 8). According to spicule morphology and size, *Plakortis albicans* is currently considered in the *Plakortis simplex* species group (Muricy, 2011), together with species that have a spiculation composed of diods smaller than 190 μm long and triods. However, despite its morphological similarity, this is not supported by our molecular data (Fig. 8). A similar case is presented by *Plakortis dariae*, which by its spicular characteristics also would correspond to the *Plakortis simplex* group (see Ereskovsky *et al*., 2014), but molecular data did not support this placement, and it was grouped with *Plakortis angulospiculatus*. Our results support the previous idea that spicule size could be not a diagnostic character in *Plakortis* species identification (see Ereskovsky *et al*., 2014).

In order to determine if *Plakortis albicans* could be an *Plakinastrella*, we also carried out a careful review of new and old material (paratypes) of this species in order to find any sign of morphological characteristics that could suggest that this species is a *Plakinastrella*. We only found one thing in one specimen (LEB-1283), just one simple calthrop-like spicule, so we do not consider this important enough to suggest a new combination of *Plakortis albicans* with *Plakinastrella*. However, it is very interesting to consider the relationships between *Plakortis* and *Plakinastrella*, which have been discussed on several occasions owing to the difficulty of determining the boundaries between the two genera (Moraes & Muricy, 2003; Gazave *et al*., 2010; van Soest *et al*., 2011).

The traditional differences between *Plakortis* and *Plakinastrella* have been the presence of diods and triods in a single category for *Plakortis*; and diods, triods, and/or simple calthrops (not lophate) in several size classes in *Plakinastrella* (Muricy & Díaz, 2002). However, the emphasis in the distinction between the two genera has currently changed from the number of size categories, to the presence /absence of calthrops (e.g. Moraes & Muricy, 2003; Muricy, 2011; van Soest *et al*., 2011). In fact, several *Plakortis* species have been described that possess distinct categories of spicules (e.g. *Plakortis myrae*, *Plakortis dariae*, *Plakortis bergquistae*, and *Plakortis galapagensis*).

Molecular markers have suggested a possible common origin for the two genera, which is also supported by morphology because of the absence of lophose spicules. This could be an important synapomorphy, in contrast to all other genera with lophate spicules as *Plakina*, *Corticium*, and *Placinolopha* (Gazave *et al*., 2010).

The monophyly of *Plakortis* and its relationship to *Plakinastrella* seen in both of our analyses (Fig. 8) have also been suggested in previous studies (Gazave *et al*., 2010; Ereskovsky *et al*., 2014). In all previous cases the sequence EU237487 of *Plakinastrella* sp. was used, and this seems very interesting because when it was accompanied by other sequences of *Plakinastrella*, the phylogenetic reconstructions show different topologies. The 18S topology shows *Plakortis* as paraphyletic, with more affinity amongst *Plakinastrella* species and *Plakortis simplex* than amongst other congeneric species such as *Plakortis halichondrioides*. However, 28S showed a reciprocal monophyly of both genera when *Plakinastrella* sp. was not included (see Gazave *et al*., 2010). Unfortunately, the specimen corresponding to this sequence was not described in a publication and it appears named as *Plakinastrella* cf. *onkodes* in GenBank, but was first identified as *Plakortis angulospiculatus* (see Lavrov *et al*., 2008) and then as *Plakinastrella* sp. (in Gazave *et al*., 2010); currently it is identified as *Plakinastrella onkodes* (from Ereskovsky *et al*., 2014) but the taxonomic status may be considered uncertain. Therefore, it is important to increase the amount of sequences of *Plakinastrella* and in general of Plakinidae species from different localities across the world, which would give us better criteria to make more accurate systematic decisions.

ACKNOWLEDGEMENTS

The authors thank Clara Ramirez for help with the literature and Arturo Núñez, María de Los Angeles Herrera (ICML-Mazatlán), and Julio Cesar Moran and Manuel Salas (students) for their assistance in the sampling and in the laboratory. We also thank Ing. Israel Gradilla Martínez (Centro de Nanociencias y Nanoestructuras, UNAM) for the SEM photographs, and German Ramírez Reséndiz and Carlos Suárez (ICML-Mazatlán) for their computer assistance. We are grateful to the following sources of funding: Iniciativa de Apoyo complementario a la realización de Obras Determinadas-IB200711; Comisión Nacional Para el Conocimiento y uso de la Biodiversidad-FB1621JF19312, and Secretaria de Educación Pública-Consejo Nacional de Ciencia y Tecnología-102239. Collections in the Revillagigedo archipelago were carried out in May 2008 and November 2009 on board the El PUMA oceanographic ship, whereas the collection from Clipperton Island was donated by the marine biotechnology company Pharma-mar SA.

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