New species of excavating sponges (Porifera: Demospongiae) on coral reefs from the Mexican Pacific Ocean

JOSÉ ANTONIO CRUZ-BARRAZA^{1,2}, JOSÉ LUIS CARBALLO¹, ERIC BAUTISTA-GUERRERO¹ AND HÉCTOR NAVA¹

¹Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México (Unidad Académica Mazatlán), Avenida Joel Montes Camarena s/n, Mazatlán, Sinaloa, México 82000, PO Box 811, ²Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Departamento de Oceanografía Biológica, km 107 Carretera Tijuana–Ensenada, 22860, Ensenada, Baja California, México

Three new species of coral reef boring sponges were found in remote coral reefs from Revillagigedo Island, an archipelago that is 386 km from the continent. Cliona medinae sp. nov. is a sponge with orange-yellow papillae characterized by short almost straight spirasters. Cliona tropicalis sp. nov., is a yellow papillate sponge with a spicule complement similar to the species included in the Cliona viridis complex. However, the new species differs from the rest of the species mainly in its external morphology and by differences in the size and shape of spicules. Thoosa purpurea sp. nov. is characterized by its purple colour, and the spicular complement formed by tylostyles, two amphiaster categories, bi- tri- and tetra-radiate oxyasters and smooth or microspined centrotylote oxeas. In addition, Cliothosa tylostrongylata sp. nov. is also described from coral reefs from the southern Mexican Pacific Ocean. This is a light red species were found exclusively excavating skeletons of live or dead corals of the genus Pocillopora. This study increases the number of boring sponges known from the Mexican Pacific Ocean to 22 species and it is the first study on marine sponge fauna from the Revillagigedo archipelago.

Keywords: taxonomy, Clionaidae, excavating sponges, East Pacific, coral reefs, Revillagigedo archipelago

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INTRODUCTION

Sponges belonging to the family Clionaidae D'Orbigny, 1851, constitute a diverse and important group of marine sponges, with the ability to excavate calcium carbonate substrates creating galleries connected by tunnels within the substrate in which they live (Rützler, 1975; Calcinai *et al.*, 2004).

Originally, all boring species were assigned to the family Clionaidae, on the basis of their common excavating ability (Laubenfels, 1936), but subsequently it was shown that this ability is not restricted to this family, and species were relocated in four different families, Clionaidae and Alectonidae (Hadromerida), Phloeodictyidae (Haplosclerida) and Acarnidae (Poecilosclerida) (Rützler, 2002). Currently, the family Clionaidae is defined by their spicular complement composed of tylostyles, and microscleres that include a large variability of spirasters, amphiasters, microxeas, microrabds and raphides (Rützler, 2002).

In the last decades, boring sponges have acquired a growing interest due to the impact that they cause on the destruction of calcareous structures such as corals (Risk *et al.*, 1995; Weil, 2002; Carballo *et al.*, 2008a, b), constituting the principal

Corresponding author: J.A. Cruz-Barraza Email: joseantonio@ola.icmyl.unam.mx endolithic bioeroders of coral framework (Hein & Risk, 1975; Risk *et al.*, 1995).

In order to be able to monitor bioerosion processes, the identification of species becomes more important. In most cases, boring sponges are easy to identify by the microsclere morphology (Carballo *et al.*, 2004), but there are species devoid of them, and cases where the spicule complement is quite similar between species, which cause confusion in taxonomic determination (Schönberg, 2002; Zea & Weil, 2003).

In an attempt to facilitate the identification of these problematic species, details of skeletal structures and morphological characteristics have been also included (Rosell & Uriz, 1991; Schönberg, 2002; Zea & Weil, 2003), as well as ecological and genetic information (Bavestrello *et al.*, 1996; Zea & Weil, 2003; Barucca *et al.*, 2007).

Coral reef boring sponges have received special attention in several taxonomic and ecological studies, principally in the Caribbean (Pang, 1973; Rützler, 1974, 1975; Hofman & Kielman, 1992; Zea & Weil, 2003) and the Indo-Pacific (Annandale, 1915; Thomas, 1972, 1975, 1985; Vacelet *et al.*, 1976; Calcinai *et al.*, 2000; Schönberg, 2000). In recent years, studies of boring sponges from the Mexican Pacific have provided a general vision of their diversity, distribution and ecology (Carballo *et al.*, 2004, 2007, 2008a, b; Bautista-Guerrero *et al.*, 2006; Nava & Carballo, 2008), but there still are a few undescribed species which undoubtedly play an important role in the bioerosion of East Pacific coral reefs (see Carballo *et al.*, 2008a; Nava & Carballo, 2008). In this paper, we describe four new species of the family Clionaidae which were found invading corals of the genus *Pocillopora*, which is the most common hermatypic coral of the East Pacific Ocean. *Cliona tropicalis* sp. nov. is one of the most important bioeroding sponges from Mexican Pacific coral reefs (identified as *Cliona* sp. in Carballo *et al.*, 2008a; Nava & Carballo, 2008). We also include the first records of boring sponges from the Revillagigedo archipelago, an important tropical area for corals, and completely unknown for its sponge fauna.

MATERIALS AND METHODS

The specimens were collected by SCUBA diving and snorkelling at sixteen localities of coral ecosystems from the Mexican Pacific Ocean (including Marías and Revillagigedo Islands) (Figure 1). The specimens were fixed in 4% formaldehyde for 24 hours and later transferred to 70% ethanol for storage. External morphology and skeletal elements were recorded for each species. The preparation of dissociated spicules, both for light microscopy (LM) 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. Tylostyle measurements are given in length \times shaft width; head width. The number in parentheses is the average.

Material has been deposited in the Colección de Esponjas del Pacífico Mexicano (LEB-ICML-UNAM), of the Instituto de Ciencias del Mar y Limnología, UNAM, in Mazatlán (México). The type material has been deposited in the Museo Nacional de Ciencias Naturales in Madrid (Spain) (MNCN), and in the British Museum of Natural History (BMNH) (London). Specific terms are used according to Boury-Esnault & Rützler (1997).

RESULTS

SYSTEMATICS Order HADROMERIDA Topsent, 1894 Family CLIONAIDAE d'Orbigny, 1851 Genus *Cliona* Grant, 1826 *Cliona medinae* sp. nov. (Figures 2 & 3)

TYPE MATERIAL

Holotype: MNCN 1.01/633, Isla Clarión, Roca Norte (Revillagigedo), $18^{\circ}47'14''N$ $110^{\circ}55'42''W$, 4 m depth, 12 March 2005.

Paratypes: BMNH 2010.11.01.4, Isla Clarión, Roca Norte (Revillagigedo), $18^{\circ}47'14''N 110^{\circ}55'42''W$, 4 m depth, 12 March 2005. LEB-ICML-UNAM-1238, Isla Clarión, Roca Norte (Revillagigedo), $18^{\circ}47'14''N 110^{\circ}55'42''W$, 4 m depth, 12 March 2005. LEB-ICML-UNAM-1252, Pináculo Norte (Revillagigedo), $18^{\circ}51'4''N 110^{\circ}59'53''W$, 4 m depth, 12 March 2005. LEB-ICML-UNAM-1260, Isla Clarión, Pináculo 2 (Revillagigedo), $18^{\circ}48'17''N 110^{\circ}56'21''W$, 4 m depth, 13 March 2005. LEB-ICML-UNAM-1661, Playa Blanca, Isla Socorro (Revillagigedo), $18^{\circ}48'56''N 111^{\circ}02'42''W$, 3 m depth, 5 May 2008. LEB-ICML-UNAM-1668, Bahía Braulia, Isla Socorro (Revillagigedo), $18^{\circ}43'44''N 110^{\circ}56'08''W$, 8 m depth, 7 May 2008.

DESCRIPTION

Papillate species found excavating live coral branches of the genus *Pocillopora*. The papillae extend by an area up to



Fig. 1. Reef location and distribution of the species. Numbers refer to the different species. (1) Cliona medinae sp. nov.; (2) C. tropicalis sp. nov.; (3) Cliothosa tylostrongylata sp. nov.; and (4) Thoosa purpurea sp. nov.

12 cm long. These are very small, circular or oval-shaped, from 0.3 to 0.8 mm in diameter (Figure 2A). They are relatively abundant and regularly distributed over the coral surface, usually spaced 0.5 to 1.7 mm from each other. The papillae are at surface level in preserved specimens. Oscules have not been observed and no distinction between ostial and oscular papillae was made because of the difficulty of recognizing each type after fixation. The papillae have firm consistency; the choanosome is soft and fleshy, and abundant inside the coral structure. Both papillae and choanosome are yellow-orange in living specimens; after fixation the colour turns to light brown. The diaphragms are easy to distinguish from the choanosomal tissue by having a dark yellow colour and oval shape (0.3-0.7 mm in diameter) (Figure 2B, C).

Erosion patterns: the species produces a regular network or reticulate galleries with spherical–ovoid (from 1.2 to 1.5 mm) to rectangular chambers with rounded borders (from 1.7 to 2.5 mm), which are densely distributed along the coral structure (Figure 2B–D). The sponge occupies the natural pores of the coral, boring only the walls that separate coral septae, producing large chambers as the result of fusion between two or more chambers. They measure from 2 to 8 mm in length and are 0.5 to 1 mm thick. The chambers are separated from each other by substrate walls from 0.2 to 0.6 mm thick, and the are connected by ducts from 180 to 330 μ m. The walls of the

chambers and tunnels present a pitted surface where chips have been removed (Figure 2D). They are polygonal from 20 to $50 \ \mu\text{m}$ in diameter (Figure 2E).

Skeletal structure: in the ectosomal papillae, the tylostyles are arranged in a palisade pattern, with tips toward the exterior. In the choanosome the tylostyles are irregularly scattered, single or in vague tracts. The spirasters are common in the choanosome tissue.

Spicules: tylostyles are mostly thin and lightly curved toward the upper third; the shafts are gradually tapering to the end, finishing in a hastate point. They have well-formed globular heads, sometimes with a small terminal knob (Figure 3A, B). Tylostyle measurements: $152-(176)-195 \times$ $2.5-(6)-7.5 \mu$ m. Head diameter: $7-(10)-13 \mu$ m. The spirasters (Figure 3C) are small and short, with a robust shaft, lightly curved toward the centre in a 'C' form, with the spines toward the convex side of the shaft. Spines are large and robust sometimes bi- or trifurcated, with sharp points. There are a few straight, amphiaster-like forms with rounded knobs along the shaft. Spiraster length: $10-(13)-15 \mu$ m.

ETYMOLOGY

The species is named after Mr Pedro Medina, who found the species for the first time in the Isla Clarión (Revillagigedo archipelago).



Fig. 2. Cliona medinae sp. nov. (A) Superficial view of the papillae; (B) cross-section view of a network of chambers; (C) detail of the joining of excavating chambers. Arrows show diaphragms; (D) scanning electron microscopy images of erosion pattern scars on the oval chamber wall; (E) detail of scars where chips were removed by the sponge tissue.



Fig. 3. (A) Scanning electron microscopy images of spicules of Cliona medinae sp. nov.; (B) tylostyles; (C) spirasters.

DISTRIBUTION

The species was found boring corals in different places from Clarión and Socorro Islands (Revillagigedo archipelago, Mexican Pacific Ocean) (Figure 1), between 3 m and 8 m depth.

REMARKS

There are only a few clionaid species with spirasters like *Cliona medinae* sp. nov. The closest species appear to be *C. dichotoma* Calcinai *et al.*, 2000 and *C. favus* Calcinai *et al.*, 2005, both from the Indo Pacific Ocean. *Cliona dichotoma* has spirasters similar in size to that of *C. medinae* sp. nov. However, in *C. dichotoma* they are exclusively amphiasters, with straight shaft and spines in the ends which are large and usually branched near the extremes. In *C. medinae* sp. nov., the spirasters are slightly curved toward the centre with spines toward the convex side of the shaft. Spines are large and robust sometimes bi- or trifurcated near the base of the shaft. *Cliona favus* also differs from *Cliona medinae* sp. nov. by having spirasters quite variable in shape, in spine number, and in their arrangement along the shaft, whereas *C. medinae* sp. nov. has spirasters with spines arranged

toward the convex side of the shaft. In addition, the presence of robust ensiform tylostyles and the very different geographical distribution clearly separates these two species from *C. dichotoma*. Tylostyles in *C. dichotoma* are also larger and thicker (from $200-350 \times 15-37 \mu$ m) than those in *Cliona medinae* sp. nov.

The species *C. euryphylle* Topsent, 1887 and *C. dioryssa* (Laubenfels, 1950) also have short almost straight spirasters. However, they have a thick shaft and they are densely spined by large and strong spines. The spirasters in *C. dioryssa* (from 11 to 41 μ m) and in *C. euryphylle* (from 4 to 30 μ m) are longer than those in *C. medinae* sp. nov. (from 10 to 15 μ m). Additionally, *C. euryphylle* and *C. dioryssa* possess a variety of spiraster forms, including large and slender spirasters, which are absent in *C. medinae* sp. nov.

Cliona tropicalis sp. nov. (Figures 4 & 5; Table 1)

TYPE MATERIAL

Holotype: MNCN 1.01/634 Bahía Tiburones (Isla Isabel, Nayarit), $21^{\circ}52'30''N$ 105 $^{\circ}54'54''W$, 2 m depth, 21 July 2005.

Paratypes: BMNH 2010.11.01.5, Bahía Tiburones (Isla Isabel, Navarit), 21°52′30″N 105°54′54″W, 2 m depth, 21 July 2005. LEB-ICML-UNAM-1165, Isla Lobos (Sinaloa), 23°13'49"N 106°27′43″W, 1 m depth, 19 August 2005. LEB-ICML-UNAM-1208, La Entrega (Oaxaca), 15°44′34″N 96°07′35″W, 6 m depth, 5 April 2005. LEB-ICML-UNAM-1273, Faro de Bucerías (Michoacán), $18^\circ 20' 49'' N \ 103^\circ 30' 33'' W, \ 8 \ m$ depth, 24 July 2005. LEB-ICML-UNAM-1309, San Agustín (Oaxaca), 15°41'09"N 96°13'46"W, 6 m depth, 9 April 2005. LEB-ICML-UNAM-1326, Bahía Tiburones (Isla Isabel, Nayarit), 21°52′30″N 105°54′54″W, 2 m depth, 21 July 2005. LEB-ICML-UNAM-1327, Punta Mita (Nayarit), 20°47'13"N 105°71'13"W, 2 m depth, 9 June 2005. LEB-ICML-UNAM-1337, Isla Cacaluta (Oaxaca), 15°43′08″N 96°09′43″W, 5 m depth, 5 June 2005. LEB-ICML-UNAM-1455, Bahía San Gabriel, Isla Espíritu Santo (Baja California Sur), 24°25'48"N 110°21′53″W, 5 m depth, 12 March 2007. LEB-ICML-UNAM-1463, Canal de San Lorenzo (Baja California Sur), 24°23′43″N 110°19′21″W, 5 m depth, 12 March 2007. LEB-ICML-UNAM-1639, Caleta de Bines, Isla Socorro (Revillagigedo), 18°44'10"N 110°57'37"W, 6 m depth, 6 May 2008. LEB-ICML-UNAM-1667, Playa Blanca, Isla Socorro (Revillagigedo), $18^{\circ}48'56''N 111^{\circ}02'42''W$, 3 m depth, 5 May 2008. LEB-ICML-UNAM-1690, Bahía Braulia, Isla Socorro (Revillagigedo), $18^{\circ}43'44''N 110^{\circ}56'08''W$, 6 m depth, 7 May 2008. LEB-ICML-UNAM-1767, Isla San Juanito, (Islas Marías), $21^{\circ}43'39''N 106^{\circ}40'25''W$, 3 m depth, 22 June 2008. LEB-ICML-UNAM-1769, Isla María Cleofas (Islas Marías), $21^{\circ}17'59''N 106^{\circ}16'24''W$, 3 m depth, 21 June 2008.

DESCRIPTION

Boring sponge with papillae and choanosome of a bright yellow colour (Figure 4A, B); after fixation colour turns to pale yellow or brown (Figure 4C, D). The papillae are very small, circular or oval-shaped, from 0.5 to 2 mm in diameter. They are numerous (4–8 papillae per cm²), and regularly scattered on the surface (from 0.5 to 1.2 mm apart) protruding 0.2-1.8 mm above the surface when the sponge is alive. After preservation they were contracting to the surface. The ostial papillae bear many sieve-like ostia, whereas oscular papillae have an oscule about 0.6 mm in diameter (Figure 4B). The papillae consistency is firm, while the choanosome is soft and fleshy, lightly



Fig. 4. *Cliona tropicalis* sp. nov. (A) Fragment of coral infested by the sponge; (B) detail of ostial and oscular papillae; (C) excavating chambers inside of coral; (D) detail of a chamber; arrow shows a diaphragm; (E) scanning electron microscopy (SEM) images of erosion pattern scars on the chamber wall; (F) SEM image detail of scars wall.

compressible. The diaphragms are dark yellow and oval-shaped, from 0.3 to 0.7 mm in diameter.

Excavation patterns: the species produced circular – elliptic chambers although some of them may be slightly irregular to polygonal-shaped ($_{3.8}$ mm × $_{2.4}$ mm in average). They are joined by ducts from 0.2 to 0.7 mm in diameter that have diaphragms at the ends ($_{0.35}$ mm in diameter on average). The main axis of the chambers is often parallel and near the coral surface (from 0.2 to 2 mm depth). The chambers are separated from each other by substrate walls from 0.1 to 1.7 mm thick. Sometimes the chambers may be fused and form larger galleries that occupy much of the coral skeleton inside. Erosion scars on chamber walls are smooth and polygonal-shaped from 60 to 95 μ m in diameter (Figure 4E, F).

Skeletal structure: in the ectosome, the tylostyles form a densely packed palisade with pointed ends sticking out, typical of the genus *Cliona*. The choanosome has loose, single or bundles of tylostyles and scattered spirasters throughout the tissue.

Spicules: the species has tylostyles and spirasters (Table 1). The tylostyles are slender and straight or lightly curved. The heads are typically well-formed, with a spherical or oval shape, although some of them are malformed with rounded borders (Figure 5A-C). Tylostyle measurements: 175-(193) – 280 μ m × 2.5 – (5) – 10 μ m. The head measures from 2.5 to 12.5 µm in diameter (8.6 µm average). The spirasters vary in morphology; the most common have a large, almost straight and thin shaft, sometimes with elaborate ends. The spines are relatively short bi-trifurcated and sparsely located around the shaft (Figure 5D). There are also short and straight or lightly curved spirasters, with relatively large branched spines, irregularly arranged along the shaft and wellelaborated spine bouquets at the ends (Figure 5E). Sinuous spiral-shaped spirasters (from 2-5 turns) with a profusely spined shaft are also common. They present spines relatively short and branching two, three or even more times, although rarely conical (Figure 5F). The spirasters measure 10-(25)-43 μm in length.



Fig. 5. Scanning electron microscopy images of spicules of *Cliona tropicalis* sp. nov. (A, B) Tylostyles; (C) detail of head of a tylostyle; (D) common large spirasters; (E) short almost straight spirasters with large and branched spines; (F) sinuous spiral-shaped spirasters.

Cliona species	External characteristics	Tylostyles	Spirasters	References
Pacific Ocean				
C. tropicalis sp. nov. Holotype MNCN 1.01/634	Yellow $\boldsymbol{\alpha}$ stage, with regular rounded papillae	150-(241)-277.5 × 3.8-(7)-10; 5-(10)-12.5	10-(16.5)-27.5	Present study
<i>C. tropicalis</i> sp. nov. Paratype LEB-ICML-UNAM-1208	Yellow $\boldsymbol{\alpha}$ stage, with regular rounded papillae	$175 - (227) - 275 \times 2.5 - (4.7) - 6.3; 5 - (7.2) - 8.7$	12.5-(19.5)-30	Present study
<i>C. tropicalis</i> sp. nov. Paratype LEB-ICML-UNAM-1273	Yellow $\boldsymbol{\alpha}$ stage, with regular rounded papillae	200-(230.5)-257.5 × 2.5-(4.3)-5.5; 7.5-(8.3)-10	15-(23)-37	Present study
C. tropicalis sp. nov. Paratype LEB-ICML-UNAM-1309	Yellow $\boldsymbol{\alpha}$ stage, with regular rounded papillae	$225 - (252.3) - 275 \times 2.5 - (5) - 7; 5 - (8.3) - 10$	10-(14.6)-22.5	Present study
C. orientalis Thiele, 1900	Marbled beige-brown to mottled dark grayish brown bark brown, α (irregular papilliae) and β stage.	158-423.7 × 2-10; 3-12	10-48.8	Schönberg, 2000
C. albimarginata Calcinai et al., 2005	Dark olive-brown β stage, encrusting species	222-336.7-3.4-5.7	9.2-20.7	Calcinai <i>et al.</i> , 2005
Caribbean Sea				
C. aprica Pang, 1973	Dark brown to brown black α - β stage, with fuse papillae	$232-399 \times 3.8-19; 3.8-19.5$	12.6-38.5	Zea & Weil, 2003
C. caribbaea Carter, 1882	Amber brown to grey brown. Thick encrustation covering completely the substratum	271-465 × 4.7-15.2; 7.1-18.1	14.9-47	Zea & Weil, 2003
C. tenuis Zea & Weil, 2003	Brown tissue with yellowish, greenish, reddish or orange tones. Thin entirely encrusting	199–380 × 33–14.3; 5.7–16.6	11-39	Zea & Weil, 2003
Mediterranean Sea				
C: viridis (Schmidt, 1862)	Dark brown or brownish green colour α - β - γ stage	$233.4 - 505.6 \times 9.8 - 14.8$	17.3-34.6	Rosell & Uriz, 2002
<i>C. parenzani</i> Corriero & Scalera-Liaci, 1997	Yellow, light olive or brownish. Encrusting to massive cushion shaped	300-500 × 7.5-15; 14-20	25-50	Corriero & Scalera-Liaci, 1997

Table 1. Comparative data for the external characteristics and dimension of spicules (µm) of *Cliona viridis*-complex, from Pacific, Caribbean and Mediterranean area. Dimensions of spicules are given as length by width of the shaft. Values in parentheses are means.

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Fig. 6. *Cliothosa tylostrongylata* sp. nov. (A, B) Transversal (A) and superficial (B) view of papillae; (C) cross-section of coral branches showing the chambers; (D) erosion pattern scars on the chamber wall; (F) detail of scars wall.

ETYMOLOGY

Tropicalis is derived from Latin and refers to the wide distribution of the species in tropical waters from the East Pacific Ocean.

DISTRIBUTION AND ECOLOGY

This species is common along the coast of the Mexican Pacific Ocean (Baja California Sur, Sinaloa, Nayarit, Michoacán and Oaxaca States). It is also found in distant islands of the archipelagos Marías and Revillagigedo (Figure 1). It is very common in coral reefs, from 2 to 8 m depth excavating live and dead corals of the genus *Pocillopora*. The new species *Cliona tropicalis* sp. nov. (identified as *Cliona* sp. in Carballo *et al.*, 2008a; Nava & Carballo 2008) is together with *C. vermifera* Hancock, 1867, one of the most important bioeroding sponges from coral reef ecosystems.

REMARKS

Based on the spicular morphology, *Cliona tropicalis* sp. nov. belongs to the *C. viridis*-complex group, which harbour the species *C. orientalis* Thiele, 1900 and *C. albimarginata* Calcinai *et al.*, 2005 from the Indo-Pacific Ocean; *C. caribbaea* Carter, 1882, *C. aprica* Pang, 1973 and *C. tenuis* Zea & Weil, 2003 from the Caribbean; and *C. viridis* (Schmidt, 1862) and *C. parenzani* Corriero & Scalera-Liaci, 1997 from the Mediterranean.

All species of the *Cliona viridis*-complex present a similar external morphology; they are usually encrusting-shaped sponges with a typical brown to dark olive-brown or olive green colour caused by symbiotic zooxantellae (Carballo

et al., 1994; Schönberg, 2000; Zea & Weil 2003; Calcinai *et al.*, 2005). This general pattern has never been observed in *C. tropicalis* sp. nov., which was always found in alpha stage with well-rounded yellow papillae. In the *C. viridis*-complex specimens in alpha stage have also been found, but these characteristically have irregular-shaped papillae and coloration similar to the beta and gamma forms (see Carballo *et al.*, 1994; Schönberg, 2000).

The spicular complement is also similar in all species of the *viridis*-complex, but light differences in size and morphology have been found between the species of the complex and *Cliona tropicalis* sp. nov. Tylostyles are from 320 to 500 μ m in the complex (maximum length), while in *C. tropicalis* sp. nov. tylostyles are smaller than 280 μ m long (see Table 1).

The geographically closest Pacific species to *Cliona tropicalis* sp. nov. is *C. orientalis*, which has spirasters with spines usually arranged at the convex side of the spicule parts (Thomas, 1979a; Calcinai *et al.*, 2000), forming little bouquets along the shaft (Schönberg, 2000) similar to our specimens. But, additionally, *C. tropicalis* sp. nov. has large and short almost straight spirasters, with spines arranged around the shaft.

The Caribbean species *Cliona aprica* differs from *C. tropicalis* sp. nov. by having spirasters with wide spires and profusely branched spines which form bouquets (Zea & Weil, 2003). The species *C. caribbaea* has long spirasters, narrowly turning (from 5 to 10 turns), sparsely to profusely spined, with short and branched spines in bouquets (Zea & Weil, 2003) and *C. tropicalis* sp. nov. has spirasters with fewer turns (2-5). Other spirasters are almost straight and large



Fig. 7. Scanning electron microscopy images of spicules of *Cliothosa tylostrongylata* sp. nov. (A) Tylostyles; (B) tylostrongyles (light microscope image); (C) smooth and nodulose amphiaster; (D) long branched amphiaster.

with short spines, or can be short with large branched spines. *Cliona tenuis* has a variety of spiraster shapes, widely turning spires (1-4 up to 7 turns), almost straight or u-shaped, profusely spined, with spines relatively short and branched in bouquets (Zea & Weil, 2003), which are very different from the typical spirasters of *C. tropicalis* sp. nov.

In the Mexican Pacific, two more species, *Cliona raromicrosclera* (Dickinson, 1945), and *C. vallartense* Carballo *et al.*, 2004 show similar characteristics to *C. viridis*-complex species (Carballo *et al.*, 2004). *Cliona vallartense*, differs from *C. tropicalis* sp. nov. by having thick and slightly bent spirasters with small conical or bifurcate spines, narrowly spaced along the shaft, while *C. tropicalis* sp. nov. has almost straight or strongly undulated spirasters and commonly branched spines. *Cliona vallartense* has tylostyles with a typical malformed head, while in *C. tropicalis* sp. nov., they have a commonly well-formed head. *Cliona raromicrosclera* has sinuous or straight spirasters, but also possesses typical small anthosigmas with spines arranged along the convex side, absent in our species. In addition, *C. raromicrosclera* has tylostrongyles, also absent in our species.

The record of *Cliona viridis* from the north-eastern Pacific area (Sim & Bakus, 1986) was considered invalid due to the lack of a morphological description (Carballo *et al.*, 2004).

Genus *Cliothosa* Topsent, 1905 *Cliothosa tylostrongylata* sp. nov. (Figures 6 & 7)

TYPE MATERIAL

Holotype: MNCN 1.01/636. San Agustín (Oaxaca), $15^{\circ}41'09''N 96^{\circ}13'46''W$, 6 m depth, 7 May 2010.

Paratypes: BMNH 2010.11.01.3 San Agustín (Oaxaca), 15°41′09″N 96°13′46″W, 6 m depth, 7 January 2010. LEB-ICML-UNAM-1193, San Agustín (Oaxaca), 15°41′09″N 96°13′46″W, 4 m depth, 18 July 2005. LEB-ICML-UNAM-1333, San Agustín (Oaxaca), 15°41′09″N 96°13′46″W, 6 m depth, 18 July 2005. LEB-ICML-UNAM-1881, San Agustín (Oaxaca), 15°41′09″N 96°13′46″W, 6 m depth, 7 January 2010. LEB-ICML-UNAM-2020, San Agustín (Oaxaca), 15°41′09″N 96°13′46″W, 6 m depth, 7 May 2010.

DESCRIPTION

Sponge with papillae very difficult to observe *in situ*, and distributed irregularly over the substratum surface. These are quite small, circular or slightly oval-shaped, from 0.4 to 1.5 mm in diameter, light red in life, and dark brown after preservation (Figure 6A, B). The choanosome is also light brown after preservation (Figure 6C). No distinction between inhalant and exhalant papillae was made. The papillae are firm in consistency but the choanosome is fragile.

Erosion patterns: chambers are elongated, slightly ovoid and often with the longer axis (from $3 \text{ mm} \times 1 \text{ mm}$ in diameter) parallel to the substrate surface. The chambers are connected to the papillae through narrow conical-shaped channels from 0.9 to 1.3 mm long (Figure E). The chambers and channel walls present a pitted polygonal shaped surface (10 to 60 µm in length) caused by bioerosion (Figure 6F).

Skeletal structure: in the periphery of the papillae there is a dense palisade of tylostyles, with their heads anchored in the tissue and pointed ends piercing the surface. In the choanosome, both megascleres and microscleres are irregularly dispersed.

Cliothosa species	Tylostyles	Tylostrongyles	Nodose amphiasters	Ramose amphiasters	Distribution
C. tylostrongylata sp. nov. MNCN 1.01/636	$132.5 - (175.3) - 250 \times 2.5 - (6.8) - 10;$ 5-(9)-12.5	$112.5 - (136.6) - 150 \times 9 - (9.5) - 10;$ 12.5 - (13.8) - 15	10-(12.2)-15	7.5-(15.5)-25	Oaxaca, Mexico
C. tylostrongylata sp. nov. LEB-ICML-UNAM-1333	$165 - (216) - 282 \times 5 - (5.5) - 6.5;$ 8.7 - (9.5) - 10	$137 - (153) - 167.5 \times 10 - (10.7) - 15;$ 12.5 - (12.8) - 15	12.5-(13.1)-15	12.5-(14.3)-15	Oaxaca, Mexico
C. tylostrongylata sp. nov. LEB-ICML-UNAM-1193	$103 - (198.6) - 286 \times 5 - (7.2) - 7.5;$ 3.3 - (7.8) - 11.3	$127.5 - (179.1) - 250 \times 8.8 - (9.6) - 11.3;$ 12.5 - (13) - 15	7.3 N = 1	8.5 - (9.3) - 10 N = 2	Oaxaca, Mexico
C. tylostrongylata sp. nov. LEB-ICML-UNAM-2020	$123 - (221.6) - 259.6 \times 1.7 - (5.8) - 12.5;$ 4-(8.2)-13	$145 - (181) - 251.17 \times 5 - (8.6) - 10.6;$ 8-(11.2)-14.5	7.8-(9.5)-11	8.8-(10.2)-14	Oaxaca, Mexico
Records of Cliothosa hancocki (Tops	sent, 1888) around the world				
C. hancocki sensu Rützler, 2002 Holotype measures	300-410 8-12		20×15	26 × 21	No further location given
C. hancocki sensu Rützler, 1973	$280 - (423.3) - 490 \times 7.5 - (10.8) - 13.8;$ 13.8 - (16.0) - 20		15.0-(21.3)-25.0	20.0-(25.8)-32.5	Tunisia Mediterranean
C. hancocki sensu Vacelet et al., 1976	275-450 × 10-15		20		Indian
C. hancocki sensu Schönberg, 2000	$166 - (380) - 526.7 \times 2 - (12) - 24;$ 6 - (15) - 24		10-(23)-30		Australia
C. hancocki sensu Calcinai et al., 2006	$192 - (316.4) - 460 \times 3 - (13.8) - 22; 8 - (16.7) - 25$		12-(15)-18	25-(29)-30	Vietnam
C. hancocki sensu Thomas, 1979b	$189 - (336) - 357 \times 4 - (10) - 16;$ 6 - 21		12	28	Mozambique
C. hancocki sensu Lévi, 1965	$275-450 \times 9-13$		20-23	10-12	Red Sea
C. hancocki sensu Pulitzer-Finali, 1993	230-410 × 5.5-11.5		Not found	22-33	East Africa

Table 2. Comparative data for dimension of spicules (µm), of *Clothosa tylostrongylata* sp. nov., from Mexican Pacific, and different records of *C. hancocki* (Topsent, 1888). Dimensions of spicules are given as length by width of the shaft. Values in parentheses are means.



Fig. 8. Thoosa purpurea sp. nov. (A) Cross-section of coral branches showing the chambers formed by sponge tissue; (B) scanning electron microscopy images of erosion on the walls of excavated chambers; (C) detail of ornamented pit.

Spicules: the megascleres are tylostyles and tylostrongyles (Table 2). The tylostyles are mostly straight, with the shaft wider in the middle part than at the ends. Their heads are well differentiated with a globular or oval form (Figure 7A). Tylostyles measure $103 - (202) - 286 \times 1.7 - (6.3) - 12.5 \mu m$; head 3.3-(7)-13 µm diameter. Tylostrongyles are less frequent than tylostyles. They are usually straight with a wellformed globular head (Figure 7B). Measurements: 112- $(162)-251 \ \mu\text{m} \times 5-(9.6)-15 \ \mu\text{m}$, and head diameter is $7.3-(2.7)-15 \mu m$. Microscleres are amphiasters in two categories (Table 2): (1) smooth and nodulose amphiasters, with short and thick shaft and rounded actins (Figure 7C); they measure $7.3-(11)-15 \mu m$ in length; and (2) long branched amphiasters with robust shaft and elongated actines (8 actines) which branch at the end in two or more spines (Figure 7D); they measure $7.5 - (12) - 25 \mu m$ in length.

DISTRIBUTION AND ECOLOGY

The species has been found excavating the coral *Pocillopora damicornis* at San Agustín (Oaxaca) (Figure 1), between 4 and 6 m depth. Despite extensive surveys undertaken in different coral reefs, the species was found only in this locality. This is one of the less common clionaid species from the Mexican Pacific coast.

ETYMOLOGY

The name 'tylostrongylata' alludes to presence of tylostrongyles as a distinctive characteristic of this species.

REMARKS

Cliothosa tylostrongylata sp. nov. is characterized mainly by having tylostrongyles, which are present in all specimens examined. This character has never been reported for any *Cliothosa* species.

The closest species to *Cliothosa tylostrongylata* sp. nov. is C. hancocki Topsent, 1888, with which it shares similar spicular complement and spicules morphology. The main difference between the two species is the presence of tylostrongyles in the new species. In addition, size of tylostyles is also different. In C. tylostrongylata sp. nov. tylostyles measure from 103 to 286 µm. Instead, the different records of C. hancocki show longer tylostyles: from 280 to 490 µm in the Mediterranean Sea (Rützler, 1973), from 275 to 450 µm in the Indian Ocean (Vacelet et al., 1976), from 166 to 443 µm in Australia (Schönberg, 2000) and from 192 to 490 µm in Vietnam (Calcinai et al., 2006) (see Table 2). Cliona quadrata (Hancock, 1849) is another species of this genus, but its validity has been questioned due to its similarity with C. hancocki (Calcinai et al., 2005). Nevertheless, C. quadrata does not have tylostrongyles or nodular amphiasters.

> Genus *Thoosa* Hancock, 1849 *Thoosa purpurea* sp. nov. (Figures 8 & 9)

TYPE MATERIAL

Holotype: MNCN 1.01/635, Caleta de Bines, Isla Socorro (Revillagigedo), $18^{\circ}44'10''N$ $110^{\circ}57'37''W$, 5 m depth, 6 May 2008.

Paratypes: BMNH 2010.11.01.6 Bahía Braulia, Isla Socorro (Revillagigedo), $18^{\circ}43'44''N$ $110^{\circ}56'08''W$, 8 depth, 5 November 2009. LEB-ICML-UNAM-1674, Caleta de Bines, Isla Socorro (Revillagigedo), $18^{\circ}44'10''N$ $110^{\circ}57'37''W$, 5 m depth, 6 May 2008. LEB-ICML-UNAM-1879, Caleta de Bines, Isla Socorro (Revillagigedo), $18^{\circ}44'10''N$ $110^{\circ}57'37''W$, 6 m depth, 5 November 2009. LEB-ICML-UNAM-1880, Bahía Braulia, Isla Socorro (Revillagigedo), $18^{\circ}43'44''N$ $110^{\circ}56'08''W$, 8 m depth, 5 November 2009.



Fig. 9. Scanning electron microscopy images of spicules of *Thoosa purpurea* sp. nov. (A) Amphiasters category with a few strong hook-like curved spines at the end; (B) amphiasters with abundantly microspined ends; (C) amphiasters both shaft and rays slender, ending in sharp point with large conical spines at side; (D) oxyasters bi- tri- tetra-radiate; (E) diverse spicular complement shaped of amphiasters, arrow show a centrotylote oxea.

DESCRIPTION

Sponge has very small and scarce papillae (Figure 8A). If the coral is fragmented, the purple tissue of the sponge is visible to the naked eye. Papillae are circular-shaped, from 0.7 to 1.2 mm in diameter. No distinction between inhalant and exhalant papillae was made. The choanosomal tissue is jelly-like and has a fleshy consistency. The colour in life, of both papillae and choanosome is bright purple; after fixation they turn dark brown or ochre.

Erosion patterns: the species produces spherical-ovoid (from 3 to 5 mm) to elongated chambers $(1.1 \times 0.3 \text{ cm} \text{ on average})$ generally with longer axis parallel to the substratum surface. The chambers are separated from each other by substrate walls (from 1.2 to 3.5 mm), and usually lie near of the surface (between 1 to 2 mm). They are connected to papillae by slender channels from 0.4 to 0.8 mm in diameter. Erosion marks (Figure 8B) circular to oval shaped from 31 to 62 μ m in diameter are evident in chambers (Figure 8C).

Spicules: the species has tylostyles, two amphiaster categories and oxyasters bi- tri- and tetra-radiate (Figure 9). Tylostyles not found in all specimens. Measurements: $105 - (186) - 222 \times 2.5 - (3) - 3.8 \mu m$; and $3.8 - (4.8) - 6.3 \mu m$ of

head diameter. Common amphiaster category (1) has 6 smooth rays at each side of the shaft, with extremes spinated and ending in a sharp point. Two types of amphiasters can be separated: (i) amphiasters have a stout or thin shaft and relatively large rays, with a few strong hook-like spines at the end (Figure 9A). They are very variable in size, $10-(30)-46 \ \mu m \times$ $5-(22)-38 \mu m$; and (ii) amphiasters guite similar to type 1, but rays are relatively shorter and have abundantly microspined ends (Figure 9B). Measurements: $18-(21)-25 \mu m \times$ $12-(14)-19 \mu m$. Less common amphiaster categories (2) have 7 rays on each side of the shaft, both shaft and rays are slender, ending in a sharp point with large conical spines (Figure 9C). Measurements: $18 - (20) - 39 \mu m \times 12 - (16.5) -$ 27 µm. Smooth oxyasters with an elongated or irregular centre at the junction, which can be biradiate, triradiate, or tetraradiate (Figure 9D). Measurements: $22-62 \mu m$. Centrotylote smooth or microspined oxeas are also present (see Figure 9E). Measurements: $105 - (186) - 222 \times 2.5 - (3) - ($ $3.7 \,\mu\text{m}; 3.75 - (4.8) - 6.2 \,\mu\text{m}$ in the centre.

ETYMOLOGY

The specific name *purpurea* alludes to the typical colour of the sponge.

DISTRIBUTION

The species was found excavating pocilloporid dead branches from Isla Socorro (Revillagigedo archipelago) (Figure 1), between 5 to 8 m deep.

REMARKS

Thoosa purpurea sp. nov. constitutes the third species of the genus *Thoosa* from the Eastern Pacific Ocean.

The only known species from the same area are *Thoosa* mismalolli Carballo et al., 2004 and *T. calpulli* Carballo et al., 2004, which are clearly different from *T. purpurea* sp. nov., in tissue colour and spicular complement. *Thoosa mismalolli* is a light brown sponge with bulbose amphiasters with reduced actines and very short spines. *Thoosa calpulli* is of a pale beige colour, with symmetrical oxyasters with six rays, biradiate oxyasters like bird wings, and smooth or spined centrotylote oxeas, which are missing in *T. purpurea* sp. nov.

A similar species to *Thoosa purpurea* sp. nov is *T. armata* Topsent, 1888 from Gabon (Atlantic Coast of Africa). The species has amphiasters with strong hook-like curved spines similar to those of *T. purpurea* sp. nov., but in addition, Topsent (1888) showed a spicular complement formed by pseudosterrasters and biradiate oxyasters resembling bird wings, which are absent in *Thoosa purpurea* sp. nov. In subsequent descriptions by the author from Seychelles (Topsent, 1904), and Azores (Topsent, 1918), he also mentioned the presence of pseudosterrasters.

Thoosa armata has been also recorded from the Red Sea and Indian Ocean (Lévi, 1965; Thomas, 1973). The specimens of Lévi (1965) have amorphous amphiasters and do not have tylostyles, and the specimens of Thomas (1973), possess globose nodular microspined amphiasters similar to *T. mismalolli* amphiasters.

DISCUSSION

The taxonomy of the sponges has been traditionally based on the skeletal elements and their arrangement in the body (see Hooper & Van Soest, 2002). In particular, species of the family Clionaidae are relatively easy to identify based on skeletal characteristics, shape and size of tylostyles and spirasters (Schönberg, 2000; Carballo *et al.*, 2004). However, some groups of species display an almost identical spicular complement (e.g. *Cliona viridis*-complex) that made their identification very difficult. In these cases, other characteristics like burrowing patterns, papillae shape, size and colour, and ecological characteristics, prove useful to discriminate between species (see Hartman, 1957; Rosell & Uriz, 1991, 1997, 2002).

The species of the *Cliona viridis*-complex are characterized by having a typical brown to dark brown, olive-brown, or olive-green colour, usually caused by symbiotic zooxanthellae (Calcinai *et al.*, 2005; Vacelet *et al.*, 2008). These species are highly destructive, both excavating and encrusting the substrate, and can cover surfaces from a few centimetres to 1 m in diameter (Schönberg, 2000; Rützler, 2002; Zea & Weil, 2003; Vacelet *et al.*, 2008). Most of the specimens reported in the alpha stage have irregular-shaped papillae and a typical dark colour (see Carballo *et al.*, 1994; Schönberg, 2000). *Cliona tropicalis* sp. nov. was always found growing with papillae, never encrusting the substrata, but the typical external characteristics of the *viridis* complex have never been seen in our specimens. In the last years molecular tools have provided alternative approaches to morphological taxonomy, principally in discrimination of species-complex (Sole-Cava & Boury-Esnault, 1999; Nichols & Barnes, 2005; Blanquer & Uriz, 2007). However, they are poorly implemented in boring sponges (Barucca *et al.*, 2007).

The new species Cliothosa tylostrongylata sp. nov. and Thoosa purpurea sp. nov. are also part of a species-complex represented by the species C. hancocki (Topsent, 1888) described from the Mediterranean Sea and T. armata Topsent, 1888, from Gabon (Atlantic Coast of Africa), respectively, which have a widespread distribution. At present, we could differentiate the new species based on the characteristics of spicular complements; T. purpurea sp. nov. lacks pseudosterrasters and biradiate oxyasters (like bird wings), which are present in T. armata; while C. tylostrongylata sp. nov. differs from C. hancocki, by having tylostrongyles as complementary megascleres, and smaller tylostyles than those recorded for C. hancocki. The subtle but consistent morpho-spicular differences, in addition to geographical distance, are sufficient argument to consider our specimens as separate species.

Our knowledge of the sponge fauna on Mexican Pacific coral reefs has increased in the last few years. Undoubtedly, boring sponges with 22 known species so far, constitute the group that has been more thoroughly studied, with the Mexican Pacific Ocean as one of the best known areas for boring sponges of the world (Carballo et al., 2004, 2007, 2008b; Bautista-Guerrero et al., 2006). However, this is the first contribution to the sponge fauna of the Revillagigedo archipelago. These islands are recognized by their high degree of endemism, because in the area organisms from the Indo-Pacific, Gulf of California and the Mexican Pacific converge. Although the archipelago has been the subject of numerous investigations on terrestrial and marine fauna and flora (Strong & Hanna, 1930; Durham & Barnard, 1952; Squires, 1959; Herrera, 1960; Rioja, 1960; Villalobos, 1960; Caso 1962; Chan, 1974; Holguin, 1994; Ketchum & Reves-Bonilla, 1997, 2001; Ochoa et al., 1998), there are no studies on Porifera.

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Correspondence should be addressed to:

J.A. Cruz-Barraza Instituto de Ciencias del Mar y Limnología Universidad Nacional Autónoma de México (Unidad Académica Mazatlán) Avenida Joel Montes Camarena s/n, Mazatlán Sinaloa, México 82000, PO Box 811 email: joseantonio@ola.icmyl.unam.mx