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Mesophotic sponges of the genus *Callyspongia* (Demospongiae, Haplosclerida) from Cuba, with the description of two new species

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Abstract

This study presents the description of two *Callyspongia* species new to science, and the distribution of all *Callyspongia* species recorded during the first joint Cuba–U.S. expedition to characterize Cuban mesophotic coral ecosystems (May–June 2017). Additionally, we propose a key to identify thin branching species of the genus in the Greater Caribbean. The observations here presented are the result of underwater explorations with the *Mohawk* Remotely Operated Vehicle dives at 35 sites around the island, at depths of 25–188 m, recording images and videos, and collecting specimens. The depth range of five species of *Callyspongia*, reported before in Cuba, has been extended to deeper waters. Two specimens of branching *Callyspongia* were collected and described: *Callyspongia (Callyspongia) pedroi* **sp. nov.** and *Callyspongia (Cladochalina) alcoladoi* **sp. nov.** Morphometric comparisons of external and skeletal traits show clear differences with the other *Callyspongia* species from the Central West Atlantic with a similar thin branching habit. *Callyspongia (C.) pedroi* **sp. nov.** consists of pinkish delicate cylindrical microconulose branches (3–6 mm in diameter, 10–14 cm long) that rarely anastomose, with relatively large oval oscules (1–3 mm in diameter), and a skeleton dominated by primary and secondary fiber reticulations, discretely cored by fusiform oxeas. *Callyspongia (C.) alcoladoi* **sp. nov.** consists of grayish, smooth thin branches (3–8 mm in diameter, 20–30 cm long), profusely dividing and occasionally anastomosing, with few spiny projections (2–4 mm wide, 2–4 cm long). Its skeleton includes a highly developed ectosomal tertiary reticulation within the secondary and primary reticulation, and fibers discretely cored by predominant mucronate oxeas. Clear morphometric differences of spicules and their skeletal reticulation distinguish these species from the other six thinly branching *Callyspongia* species known from the Caribbean.

Key words: taxonomy, Porifera, Callyspongiidae, *Callyspongia pedroi* **sp. nov.**, *Callyspongia alcoladoi* **sp. nov.**, Caribbean

Resumen

Este estudio presenta la descripción de dos especies de *Callyspongia* nuevas para la ciencia, y la distribución de todas las especies de *Callyspongia* registradas durante la primera expedición conjunta Cuba–EE.UU. para caracterizar a los ecosistemas coralinos mesofóticos cubanos (mayo–junio de 2017). Además, se propone una clave para identificar a las especies ramosas del género en el Gran Caribe. Las observaciones aquí presentadas son el resultado de las exploraciones submarinas realizadas con el *Mohawk Remotely Operated Vehicle* en 35 sitios alrededor de la isla, a profundidades de 25–188 m, grabando imágenes y videos, y colectando especímenes. El rango de profundidad de cinco especies de *Callyspongia*, reportadas anteriormente para Cuba, se extendió a aguas más profundas. Se colectaron y describieron dos especímenes de *Callyspongia* ramosas: *Callyspongia (Callyspongia) pedroi* **sp. nov.** y *Callyspongia (Cladochalina) alcoladoi* **sp. nov.** Las comparaciones morfométricas de los rasgos externos y esqueléticos muestran claras diferencias con las otras especies de *Callyspongia* del Atlántico Centro-Occidental con similar hábito ramoso–fino. *Callyspongia (C.) pedroi* sp. nov consiste en ramas rosáceas delicadas, cilíndricas y microconulosas (3–6 mm de diámetro, 10–14 cm de largo) que raramente se anastomosan, con óculos ovales relativamente grandes (1–3 mm de ancho), y el esqueleto dominado por una reticulación de fibras primarias y secundarias, conteniendo discretas oxeas fusiformes. *Callyspongia (C.) alcoladoi* **sp. nov.** consiste en ramas grisáceas lisas y delgadas (3–8 mm de diámetro, 20–30 cm de largo), que se dividen profusamente y ocasionalmente se anastomosan, con algunas proyecciones de aspecto espinoso (2–4 mm de ancho, 2–4 cm de largo).

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Su esqueleto presenta una reticulación ectosomal terciaria bien desarrollada, dentro de la reticulación secundaria y primaria, y fibras que contienen predominantemente discretas oxeas mucronadas. Las claras diferencias morfométricas de sus espículas y de su reticulación esquelética, distinguen a estas especies de las otras seis especies de *Callyspongia* ramosas-finas del Caribe.

Palabras clave: Taxonomía, Porifera, Callyspongiidae, *Callyspongia pedroi* **sp. nov.**, *Callyspongia alcoladoi* **sp. nov.**, Caribe

Introduction

Mesophotic reefs represent a continuum from shallow reefs occurring in tropical and subtropical regions extending from 30–45 m to 150 m (Baker *et al.* 2016; Turner *et al.* 2017). They are known to harbor high benthic diversity, some shared with shallow reefs and some exclusive to these ecosystems (Baker *et al.* 2016; Pomponi *et al.* 2018; Reed *et al*. 2018). Sponges constitute a dominant component of mesophotic reefs because of their species richness and their number of individuals (Slattery & Lesser 2012; Van Soest *et al.* 2014; Reed *et al.* 2018). In the Caribbean, it has been shown that sponge growth is higher on mesophotic reefs than on shallow reefs, and that diversity and abundance increase with depth (Liddell *et al.* 1997; Pawlik *et al.* 2013; Slattery & Lesser, 2015; Baker *et al.* 2016). Our knowledge about the composition and role of sponges on mesophotic reefs is incomplete, despite their suspected value for predicting threats to and environmental condition of these ecosystems (Lesser *et al*. 2009; Baker *et al*. 2016; Pomponi *et al.* 2018).

Pomponi *et al.* (2018) list literature reports of 241 sponge species from mesophotic reefs of the greater Caribbean, with 141 reported for the Greater Antilles (Cuba, Jamaica, Puerto Rico and Cayman Islands). However, preliminary results from the exploration of mesophotic reefs at 35 localities around Cuba showed 296 sponge taxa (Reed *et al.* 2018), indicating a gross current underestimate of Porifera biodiversity and the high probability to find new species. It is important to note that 25% of those morphospecies distinguished could only be assigned to a single class (i.e., Demospongiae) and a single order (i.e., Haplosclerida) or a single family (i.e., Petrosiidae.). This probably indicates that most species on mesophotic reefs are little known or new to science. Alcolado (2002; 2007), who described 263–280 sponge species in Cuba, indicates that there are probably many species to be described, particularly on reefs and in deep waters.

The genus *Callyspongia* Duchassing & Michelotti 1864 (Demospongiae, Haplosclerida, Callyspongiidae) is distributed throughout most tropical, temperate and cold waters; its species are frequently observed in the Eastern Pacific, Eastern South America, West Indies, West and South Africa, the Mediterranean Atlantic region, Indo–Malaysia, from Japan to China, and in the South and North-East and West Australian and New Zealand regions (Desqueyroux–Faúndez & Valentine 2002). In all, 261 species have been described in this genus, most inhabiting shallow waters (Desqueyroux–Faúndez & Valentine 2002; Van Soest *et al.* 2018), and ~180 of these have been accepted after critical review by taxonomists. Pomponi *et al.* (2018) report three species from mesophotic reefs in the Caribbean, *Callyspongia (C.) densasclera* Lehnert & Van Soest, 1999, *Callyspongia* cf. *pallida* Hechtel 1965, and *Callyspongia (C.) plicifera* (Lamarck 1814), and one species from Guyana, *Callyspongia (C.) scutica* Van Soest 2017. Eight species of *Callyspongia* have been reported from Cuban waters, making it a genus that is among the top five in species richness among all Cuban Porifera (Alcolado 2002; 2007).

During an expedition to Cuban mesophotic reefs, conducted in 2017 as a binational collaboration between Cuban and U.S. institutions, two very thin, rod shaped sponges, later identified as *Callyspongia* spp., were collected (Reed *et al.* 2018). There are currently six *Callyspongia* species with thin ramose habits described in the Central West Atlantic. These species are rare in abundance on shallow reefs, and their descriptions are published in a series of monographs that range from De Laubenfels (1936) to Van Soest (2017).

Our goal is to provide an inventory of the *Callyspongia* species found on Cuban mesophotic reefs, and to describe two species new to science: *Callyspongia (Callyspongia) pedroi* **sp. nov.** and *Callyspongia (Cladochalina) alcoladoi* **sp. nov.** We have created a simplified key to distinguish the rare ropey-branching *Callyspongia* spp. that inhabit Greater Caribbean coral reefs.

Materials and methods

Area of study. A joint Cuba–U.S. expedition was conducted May 14–June 12, 2017 to characterize for the first time the mesophotic coral ecosystems (MCEs) along the entire coastline of Cuba. *Mohawk* Remotely Operated Vehicle (ROV) dives at 35 sites (total transit around the island ~2778 km, depths 25–188 m) confirmed the presence of MCEs on all coasts of Cuba (Table 1). Topographically, the most conspicuous features of most dive sites were the deep island slope (125–>150 m), vertical wall (50–125 m), and deep fringing reef (30–50 m). In addition, dives were conducted on the slopes of two seamounts, Banco de San Antonio and Banco Silvertown (more details and map of the expedition track in Reed *et al.* 2018).

Collection methods. The *Mohawk* ROV was equipped with an Insite Pacific Mini Zeus high–definition CMOS color zoom camera, and a Kongsberg Maritime OE14–408 camera, both with 10 cm parallel lasers for scale (green still, red video). The ROV had a collection skid, consisting of a small 5–function manipulator, five suction buckets, and a thermally insulated biobox. ROV dives transected and documented habitat and species with 103 hours of high–definition video and 21146 digital images, and collected approximately 475 specimens of benthic macro–invertebrates and macro–algae, of which 117 were sponges. Sponges collected include two branching specimens of *Callyspongia* that were photographed both *in situ* and in the ship's lab, and then preserved in 70% ethanol. Both specimens have been deposited in the collections of Acuario Nacional de Cuba (ANC, Havana, Cuba).

Data processing & systematic descriptions. Metadata and habitat dive notes, including taxonomic identifications of specimens, were recorded during the dive into a Microsoft Access® database, which links to the ROV navigation data. From the images and Microsoft Access® database, species of *Callyspongia* were recorded by sites, including their depths. Systematic criteria for *Callyspongia* species identifications were adopted from Caribbean monographs, such as De Laubenfels (1936), Wiedenmayer (1977), and Van Soest (1980), and supplemented by data given in the World Porifera Database (Van Soest *et al.* 2018).

Depths registered for each *Callyspongia* species found during the expedition were compared with previously obtained information about Cuban sponges (unpublished data of Dr. P. M. Alcolado, and Porifera Microsoft Access® database, collections of Acuario Nacional de Cuba), and with references to the Central West Atlantic region (De Laubenfels 1936; Lehner & Van Soest 1998; 1999; Pulitzer–Finali 1986; Rützler *et al.* 2009; Van Soest 1980; 2017; Wiedenmayer 1977).

For describing the external morphology of specimens, photographic images were reviewed to analyze habit, structure, and color. Tissue fragments of each specimen were prepared for spicule and skeletal architecture studies (Díaz, 2007). Measurements of the spicules (length x width in µm, minimum–*average*–maximum) were limited to 25 for each sample, and only fully developed spicules were measured. Some fragments of both specimens were frozen, and thick sections were made by hand; typically, sections tangential and perpendicular to the surface were made. Skeletal structure was described, including measurements of fiber thickness (in µm) and mesh diameter (in µm), 10–20 each. Numbers of spicules coring the fibers were noted from each fiber type. All measurements and descriptions were made using light microscopy.

In order to compare our new species with other thin, stringy, and branching described *Callyspongia* spp., comparative morphometric tables were prepared (Tables 3, 4) and a key to the taxonomic identification of all similarly shaped *Callyspongia* species from the Central West Atlantic Atlantic is proposed.

Results

Mesophotic sponges of the genus *Callyspongia* from Cuba. During the Cuban mesophotic coral reef exploration of 2017, 296 sponge species were distinguished by their external morphology. Initially nine of these species were recognized as *Callyspongia* spp. (Reed *et al.* 2018), but after a more detailed subsequent analysis, seven species of *Callyspongia* were identified, which five as previously described species. Among 118 samples of sponges collected during this expedition, two *Callyspongia* species studied were undescribed species. These two are described below and named *Callyspongia (Callyspongia) pedroi* **sp. nov.** and *Callyspongia (Cladochalina) alcoladoi* **sp. nov.** The distribution of all seven species of *Callyspongia* reported from Cuba is summarized in Table 2.

TABLE 1. ROV dive sites on Cuba's mesophotic reefs during the R/V F.G. Walton Smith cruise, May 14 to June 12, 2017. Dive 10 (C–12) was aborted; MPA= Marine Protected Area.

Site	Dive No.	Date	Location	Latitude	Longitude	Depth Range (m)
C–04	1	18/05/2017	NW coast, Cayo Levisa MPA	22°53.4830'N	83°34.9699'W	25–188
C–04A	2,3	18/05/2017	NW coast, 2 nmi north of Cayo Arenas, Outside Cayo Levisa MPA	22°52.6388'N	83°39.0630'W	49–172
C–06	4,5	19/05/2017	NW coast, Los Pretiles MPA	22°30.4870'N	84°25.9078'W	43–170
C–07	6,7	20/05/2017	NW coast, Archipiélago de Los Colorados	22°13.4054'N	84°44.8370'W	45–178
C–10	8	21/05/2017	W coast, Banco de San Antonio MPA, east wall	22°00.9014'N	84°59.9478'W	25–183
C–10A	9	21/05/2017	W coast, Banco de San Antonio MPA, south wall	22°00.0175'N	85°00.9774'W	30–150
C–12A	11	22/05/2017	W coast, Faro Roncali, off lighthouse, Guanahacabibes National Park	21°52.1115'N	84°57.8245'W	45–156
C–12B	12	22/05/2017	W coast, Las Tumbas, Guanahacabibes National Park	21°55.4775'N	84°57.4944'W	38–148
C–15	13	23/05/2017	W coast, El Almirante, Punto de Buceo, Cabo Corrientes, Guanahacabibes National Park	21°48.2246'N	84°31.1370'W	25–145
C–16A	14	23/05/2017	W coast, Cabo Corrientes, Guanahacabibes National Park	21°45.5120'N	84°31.8741'W	28–150
C–21	15	25/05/2017	S coast, SW tip of Isla de la Juventud, Punta Pedernales MPA	21°35.2631'N	83°10.3254'W	25–158
C–21A	16	25/05/2017	S coast, SW Isla de la Juventud, bay on west side of point, Punta Francés MPA	21°36.0100'N	83°10.9436'W	28–150
C–23	17	26/05/2017	S coast, SE Isla de la Juventud, Punta Brava MPA	21°29.4195'N	82°39.2284'W	37–150
C–24	18	26/05/2017	S coast, E of Isla de la Juventud, Archipiélago de Los Canarreos, Cayo Matías	21°31.9245'N	82°27.4803'W	40–150
C–28	19	27/05/2017	S coast, Archipiélago de Los Canarreos, Cayo Rosario MPA	21°33.4690'N	81°44.4012'W	29–150
C–29	20	27/05/2017	S coast, Archipiélago de Los Canarreos, Outside Cayo Largo del Sur MPA	21°34.8540'N	81°33.1300'W	26–157
C–31A	21	28/05/2017	S coast, Golfo de Cazones, Punta Brava, Sur Ciénaga de Zapata	21°57.6502'N	81°10.3554'W	18–150
C–33A	22	28/05/2017	S coast, Bahía de Cochinos, east wall, Zapata National Park	22°07.4442'N	81°07.5186'W	30–150
C–38	23,24	31/05/2017	S coast, south of Cienfuegos, Banco Silvertown, west wall, proposed MPA	21°25.1806'N	79°56.3770'W	24–150
C–38A	25	31/05/2017	S coast, south of Cienfuegos, Banco Silvertown, south wall, proposed MPA	21°24.4700'N	79°54.9420'W	40–153
C–41	26	01/06/2017	S coast, Cayo Caballones, south slope, Jardines de La Reina MPA	20°47.4950'N	78°57.3230'W	30–150
C–48	27,28	02/06/2017	S coast, Cabo Cruz, Outside Desembarco del Gramma National Park	19°49.6000'N	77°44.5510'W	40–154
C–50	29,30	03/06/2017	S coast, Sierra Maestra, Chivirico Proposed MPA	19°56.5515'N	76°23.9372'W	27–155
C–52	31	04/06/2017	S coast, Sierra Purial, Punta Imías	20°02.6010'N	74°41.4980'W	60–161
C–52A	32	04/06/2017	S coast, Sierra Purial, east tip of Punta Imías, 1 nmi east of Dive 31	20°02.5130'N	74°40.3450'W	43–156
C–53A	33	05/06/2017	Eastern tip of NW Punta Maisí, Punta Silencio	20°19.0076'N	74°16.0018'W	54–155

……continued on the next page

TABLE 1. (Continued)

Site	Dive No.	Date	Location	Latitude	Longitude	Depth Range (m)
C–53B	34	05/06/2017	Eastern tip of NW Punta Maisí, Punta del Fraile	20°19.4825'N	74°13.9406'W	50–142
C–54	35	06/06/2017	NE coast, Outside Cabo Lucrecia MPA	21°05.5088'N	75°39.8211'W	45–151
C–54B	36	06/06/2017	NE coast, Cabo Lucrecia, proposed MPA	21°04.8171'N	75°38.5301'W	65–156
C–56	37,38	07/06/2017	NE coast, Archipiélago Sabana– Camagüey, Cayo Sabinal MPA	21°41.0960'N	77°10.2410'W	24–150
C–58	39	08/06/2017	NE coast, Archipiélago Sabana– Camagüey, Cayo Coco, proposed MPA	22°34.1920'N	78°22.3610'W	42–140
C–58A	40	08/06/2017	NE coast, Archipiélago Sabana– Camagüey, Cayo Coco, proposed MPA (just north of NW edge of MPA)	22°35.2620'N	78°27.7060'W	46–144
C–59A	41	09/06/2017	NE coast, Archipiélago Sabana– Camagüey, Cayo La Vela MPA	22°54.8586'N	79°41.8540'W	22–143
C–59C	42	09/06/2017	NE coast, Archipiélago Sabana– Camagüey, Outside Cayo Jutía MPA	22°59.4460'N	79°48.1880'W	30–147
C–60A	43	10/06/2017	NE coast, Archipiélago Sabana– Camagüey, Cayo Cruz del Padre, inside and outside MPA	23°17.7928'N	80°53.8093'W	40–161

TABLE 2. Species of *Callyspongia* found on Cuban mesophotic reefs, with distribution by sites.

Species/Sites	C–04A	C–07	C–10A	C–12A	C–12B	C–16A	C–28	C–29	C–33A	C–38	C–38A	C–41	C–48	C–50	C–53A	C–53B	C–54	C–54B	C–56	C–58	C–59A	C–59C	C–60A
Callyspongia (C.) alcoladoi sp. nov.									X							X							
Callyspongia (C.) armigera		X		X	X													X				X	
Callyspongia (C.) fallax			X	X	X		X																
Callyspongia (C.) pallida																		X					
Callyspongia (C.) pedroi		X		X			X	X				X		X	X		X			X	X	X	X
Callyspongia (C.) plicifera	X	X	X	X			X	X			X	X	X	X	X			X	X	X	X	X	
Callyspongia (C.) vaginalis						X			X	X			X			X					X		

TABLE 3. Morphometric comparison of external characteristics of the collected specimens of *Callyspongia pedroi* sp. nov. and *C. alcoladoi* sp. nov. with other *Callyspongia* species from the Central West Atlantic with a similar branching habit. Ref= References.

Species	Shape	Branch, max lenght x width (cm)	Surface	Consistency	Oscule size (mm)	Oscule distribution	Color	Ref
Callyspongia arcesiosa	Ramose form with two thin digitate branches	12 x 0.5	Almost smooth, a tendency for ascending fibers of the body to protrude from the surface	Delicately spongy, easily torn and cut	2–3		Greenish blue	1,2
Callyspongia (C.) armigera	Cylindrical branches, ramose, erect	40–42 x 0.5–3	Smooth or rugose, occasional spiky projections	Compressible, elastic	3–4	Aligned, one face of branches or in rows on opposite narrow sides	Gray, greenish, or light violet	1,3,6, 9
Callyspongia (C.) densasclera	Thin, finger shaped	5–8 x 0.3–1	Smooth, microhispid, soft thorns distributed irregularly	Firm and elastic	1–2	Flush with the surface	Pale, dirty–yellow	5
Callyspongia (C.) scutica	Erect, arborescent with long, sparingly dividing, often somewhat flattened branches (tendency in one plane) and ending swollen, but terminally pointed	25 x 0.4–1	Shiny smooth, unbroken, but with subdermal reticulation faintly visible beneath the skin	Firm, resilient	1–2	Flush, predominantly on branches side, on top of the branches undulations	Golden red–brown in alcohol	8
Callyspongia (C.) strongylophora	A tangled mass of irregularly anastomosing and dividing branches, no distinct basal mass	23 x 0.2–0.4	Rough, with sand and foreign material	Compressible, resilient, easily torn	1–3, irregular outline	Rows on branches	Yellow drab in spirit	4,7
Callyspongia (C.) tenerrima	Erect, not tangled, elongated many cylindrical branches depart from basal mass	25 x 0.5–3 23 x 2	Finely conulose	Tough and resilient	2–4	In rows, flush with the surface	Lavender, olive, pinkish purple	3,6,9
Callyspongia (C.) pedroi sp. nov.	Delicate cylindrical branches that rarely anastomose, no distinct base, the diameter at the tips sharply decreases, ending always in a pointed bent end	10–14 x 0.3–0.6	Smooth, rough to the touch, microconulose under the scope, subdermal round cavities (0.5–1 mm), no sand or foreign material	Soft, flimsy in consistency	1–3	Oval and flush to the surface, in rows on one side of the branch, with separation distances of 3–4 mm	Light reddish to pink	
Callyspongia (C.) alcoladoi sp. nov.	Delicate cylindrical branches with few spiny looking projections, profusely branching rarely anatomosing, no distinct base, the diameter at the tips sharply decreases, ending always in a pointed bent end	20–30 x 0.3–0.8 2–4 x 0.2–0.4 (spiny projections)	Very smooth, subdermal round cavities (0.25–0.8 mm), no sand or foreign material	Firm compressible	1–2	Round, slightly sinking from the surface, in rows on one side of the branch, keeping variable positions (0.2–2 cm apart)	Gray pinkish, light yellow	

1. Alcolado (unpublished data); 2. De Laubenfels (1936); 3. Duchassaing & Michelotti (1864); 4. Hartman (1955); 5. Lehnert & Van Soest (1999); 6. Pulitzer–Finali (1986); 7. Van Soest (1980); 8. Van Soest (2017); 9. Wiedenmayer (1977).

TABLE 4. Morphometric comparison of spicule–skeletal characteristics of collected specimens of *Callyspongia pedroi* sp. nov. and *C. alcoladoi* sp. nov. with other *Callyspongia* species from the Central West Atlantic with a similar branching habit. Ec.= Ectosome, Ch.= Choanosome, 1ries = primaries, 2ries = secondaries, 3ries = tertiaries, F=Fibers, M=Meshes, ss=Spicules, Ref=References.

Species	Spicules, length x width (μm)	Ec. 1ries–F (μm)	Ec. 2ries–F (μm)	Ec. 3ries–F (μm)	Ec. 1ries–M (μm)	Ec. 2ries–M (μm)	Ec. 3ries–M (μm)	Ch. 1ries–F (μm)	Ch. 2ries–F (μm)	Ch. 1ries–M (μm)	Ch. 2ries–M (μm)	Ref
Callyspongia arcesiosa	Oxeas 35–95 x 0.5–1.5 65–95 x 1–3	15 11–25	6 3–11		70–80 85–175	30–40 55–100		15 (1–7ss)		100		2 1
Callyspongia (C.) armigera	Oxeas fusiform 60–130 x 2–6 70–80 x 1.5–4	19–47	9–18		380–550	50–90						3 1 6
Callyspongia (C.) densasclera	Oxeas hastate, occasionally styles 184–232 x 6.5–8	25–80			400–500	100–200						5
Callyspongia (C.) scutica	Oxeas fusiform, sharply pointed, slightly curved 81–*104*–114 x 3.5–*5.1*–7	15–30 (1–3ss)	4–10		250–350	80–120		30–125 (5ss)	20–40 (3–4ss)	250–750		8
Callyspongia (C.) strongylophora	Strongylote 83–*88*–93 x 0.5–*1.27*–2.5	25–30	6–12		500–700	80–200		30–90 (4–11ss)	18–35 (1–2ss)			4
Callyspongia (C.) tenerrima	Strongylote 70–*74.0*–78 x 0.5 60–75 x 1.5–2 65–78 x 1–2	60–80 30–60 100	10–40 10–20 5–30		700 500–1000	150–200 50–140 50–400		100–250 (0–8ss) 100–200	8–90 (0–2ss) 30–100			7,6, 3,9
Callyspongia (C.) pedroi sp. nov.	Oxeas fusiform 60–*82.4*–100 x 1.25–*2.5*–5	20–40 (1–6ss)	10–15 (1–3ss)		120–425	50–350		20–40 (4–6ss)	10–15 (1–3ss)	200–500	40–330	
Callyspongia (C.) alcoladoi sp. nov.	Oxeas mostly mucronate, few fusiform 67.5–*73.3*–85 x 1.5–*2.3*–3 um	30–150 (1–6ss)	15–30 (1–2ss)	8–16	80–250	70–210	30–70	30–110 (1–6ss)	8–25 (1–2ss)	60–500	40–200	

1. Alcolado (unpublished data); 2. De Laubenfels (1936); 3. Duchassaing & Michelotti (1864); 4. Hartman (1955); 5. Lehnert & Van Soest (1999); 6. Pulitzer–Finali (1986); 7. Van Soest (1980); 8. Van Soest (2017); 9. Wiedenmayer (1977).

To differentiate the two new species collected in Cuba, a morphometric comparison of external characteristics (Table 3) and spicules and skeletal characteristics, respectively, (Table 4) of the collected specimens and all other *Callyspongia* species from the CW Atlantic with a similar thin branching habit was conducted.

Order Haplosclerida Topsent, 1928

Family Callyspongiidae de Laubenfels, 1936

Genus *Callyspongia* Duchassaing & Michelotti, 1864

Type species: *Callyspongia fallax* Duchassaing & Michelotti, 1864 (by subsequent designation; Burton 1934).

Diagnosis: Growth form varying greatly, from massive to ramose, lamellate, foliaceous to infundibuliform, tubular or lobate, repent or erect. Ectosomal skeleton a tangential network formed by secondary and, in places, finer tertiary fibers (triple mesh ectosomal layer), or less ramified and with regular size of mesh (single mesh ectosomal layer). Choanosomal skeleton, a well–developed network of primary longitudinal fibers, fasciculated or non–fasciculated, spongin sheath always present. Primary fibers ramified to form secondary and tertiary fine fibers and a tertiary choanosomal network, or non–ramified, connected by short, parallel, non–ramified, secondary fibers. There may be a peripheral condensation of the choanosomal skeleton (Desqueyroux–Faúndez & Valentine 2002).

Callyspongia (Callyspongia) pedroi sp. nov.

(Figs. 1a–e, 2a–d)

Material examined. Holotype: ANC 02.034, Cuba, Joint Cuba–U.S. Expedition R/V F.G. Walton Smith, site C–12A, Faro Roncali (W coast, Cuba), 21.868604° N 84.962657° W, depth 62.5 m, mesophotic coral reef, 22 May 2017, Collectors: M. C. Díaz & L. Busutil.

Description. External morphology: Delicate cylindrical branches (3–6 mm in diameter, 10–14 cm long) that rarely anastomose, smooth surface to the naked eye, microconulose when observed microscopically (Fig. 1a–e). No distinct base, but specimens attach to the substrate at a few points. Oscules 1–3 mm in diameter (Fig. 1d–e), oval and flush with the surface, separated by 3–4 mm, arranged in rows on one side of the branch. The diameter at the tips sharply decreases, ending always in a pointed, bent end. Color light reddish to pink externally, tan internally. Tan in alcohol. Smooth surface, but rough to the touch. Soft, flimsy in consistency. No sand or foreign material on the surface. Subdermal round cavities (0.5–1 mm), abundant throughout the body, are evident to the naked eye (Fig. 1d).

Spicules: Fusiform oxeas (Fig. 2d), 60–*82.4*–100 x 1.25–*2.5*–5 µm.

Skeleton: Ectosome and choanosome have well–developed primary and secondary tangential fibers (Fig. 2a–c); no tertiaries can be distinguished. Primary fibers (20–40 µm in diameter); secondary fibers (10–15 µm in diameter). In the ectosome, large meshes (120–425 µm in diameter) and smaller meshes (50–350 µm). In the choanosome, a longitudinal section of the branch shows a peripheral condensation of the skeleton, with regular and condensed meshes at the surface that become more variable towards the interior of the branch. Meshes are angular, with various shapes ranging from triangular, to square or polygonal. Large primary meshes range from 200–500 µm diameter, while smaller secondary meshes are 40–330 µm. All fibers are cored, primaries with 1–6 spicules, and secondaries with 1–3 spicules (Fig. 2c). The presence of a peripheral condensation of the reticulation, and overall skeletal morphology indicates the closeness of this species with the subgenus *Callyspongia* Duchassaing & Michelotti, 1864.

Distribution and ecology. Northwest, west, southwest, southeast and northeast coasts of Cuba (Table 2), mesophotic coral reef, depth range 44.4–102.4 m. At site C–50 (Table 1, depth 91.1 m) another specimen of similar size (12 cm long) and pink in color, similar to the holotype (Fig. 1b), was observed but not collected.

Remarks. Based on external morphology, primarily the delicate nature of the branches, *Callyspongia (C.) pedroi* **sp. nov.** is similar to *Callyspongia arcesiosa* De Laubenfels, 1936 and *Callyspongia (C.) densasclera* Lehnert & Van Soest, 1999. However, *Callyspongia (C.) pedroi* **sp. nov.** differs from *Callyspongia arcesiosa* by

the larger size of its fibers, and the diameter of the meshes that are at least double in size. The spicules of *Callyspongia (C.) pedroi* **sp. nov.** are 30% of the size of the spicules of *Callyspongia (C.) densasclera*, which is also described as having an abnormally dense conglomeration of spicules (measuring 184–232 x 6.5–8 μm) in the fibers, a fact not observed for *Callyspongia (C.) pedroi* **sp. nov.** *Callyspongia (C.) densasclera* has hastate oxeas, occasionally styles, while in *Callyspongia (C.) pedroi* **sp. nov.**, oxeas are always fusiform (Tables 3, 4).

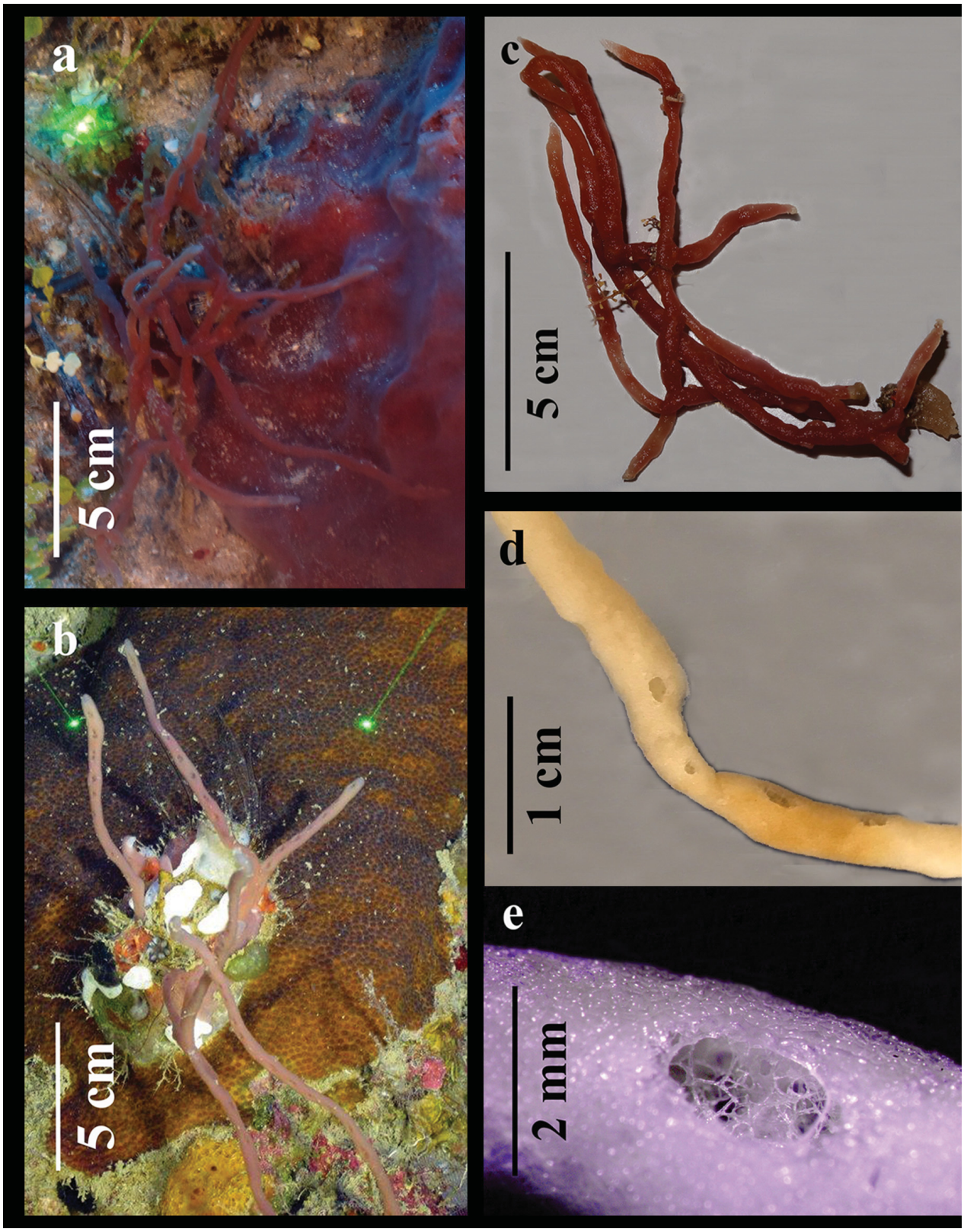

FIGURE 1. *Callyspongia (C.) pedroi* **sp. nov.** a) Holotype in live habit b) Other specimen in live habit, but not collected c-d) Holotype on deck e) Microconulose surface, and oval flushed osculum.

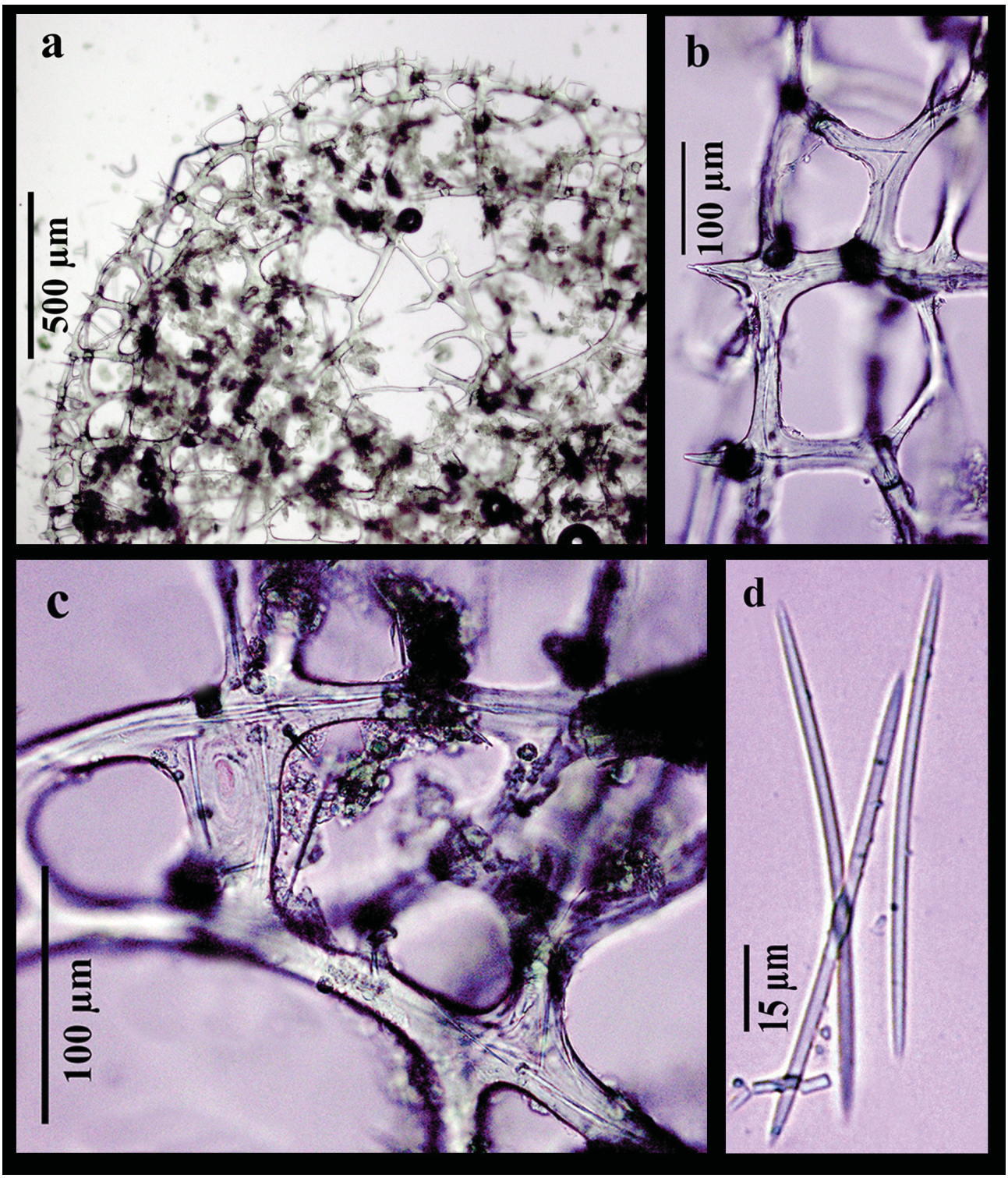

FIGURE 2. *Callyspongia (C.) pedroi* **sp. nov.** a) Perpendicular section of skeleton showing peripheral condensation of reticle (4X) b) Ectosome showing large meshes and spiny termination of the skeleton on the surface (20X) c) Fibers discretely cored (20X) d) Fusiform oxeas (400X).

***Callyspongia (Cladochalina) alcoladoi* sp. nov.**
(Figs. 3a–e, 4a–d)

Material examined. Holotype: ANC 02.035, Cuba, Joint Cuba–U.S. Expedition R/V F.G. Walton Smith, site C–53B, Punta del Fraile (NW Punta Maisí, Eastern tip of Cuba), 20.323166º N 74.232412º W, depth 51.5 m, mesophotic coral reef, 5 June 2017, Collectors: M.C. Díaz & L. Busutil.

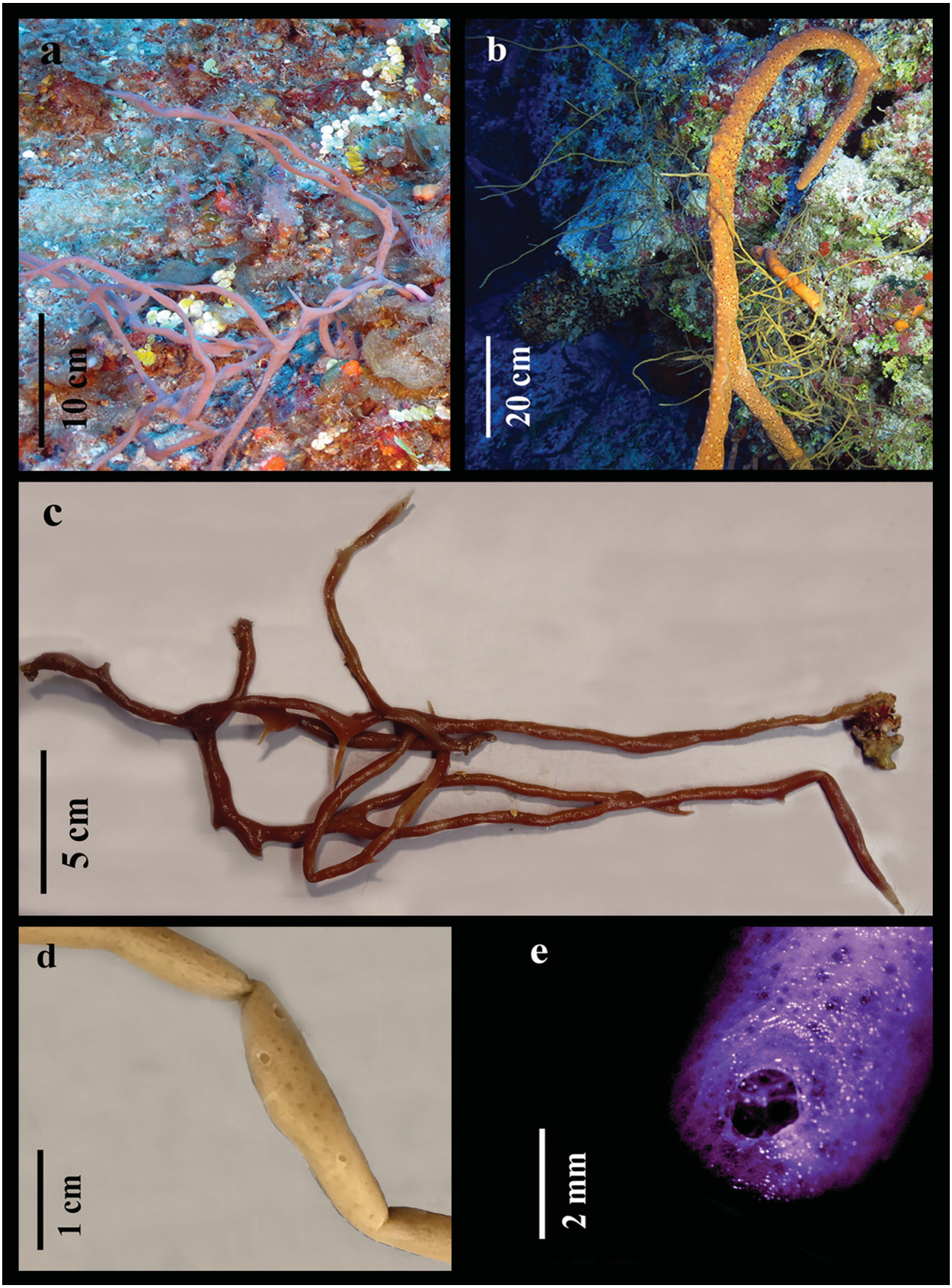

FIGURE 3. *Callyspongia (C.) alcoladoi* **sp. nov.** a) Holotype in live habit b) Other specimen in live habit, but not collected c-d) Holotype on deck e) Very smooth surface and round osculum, slightly sunken.

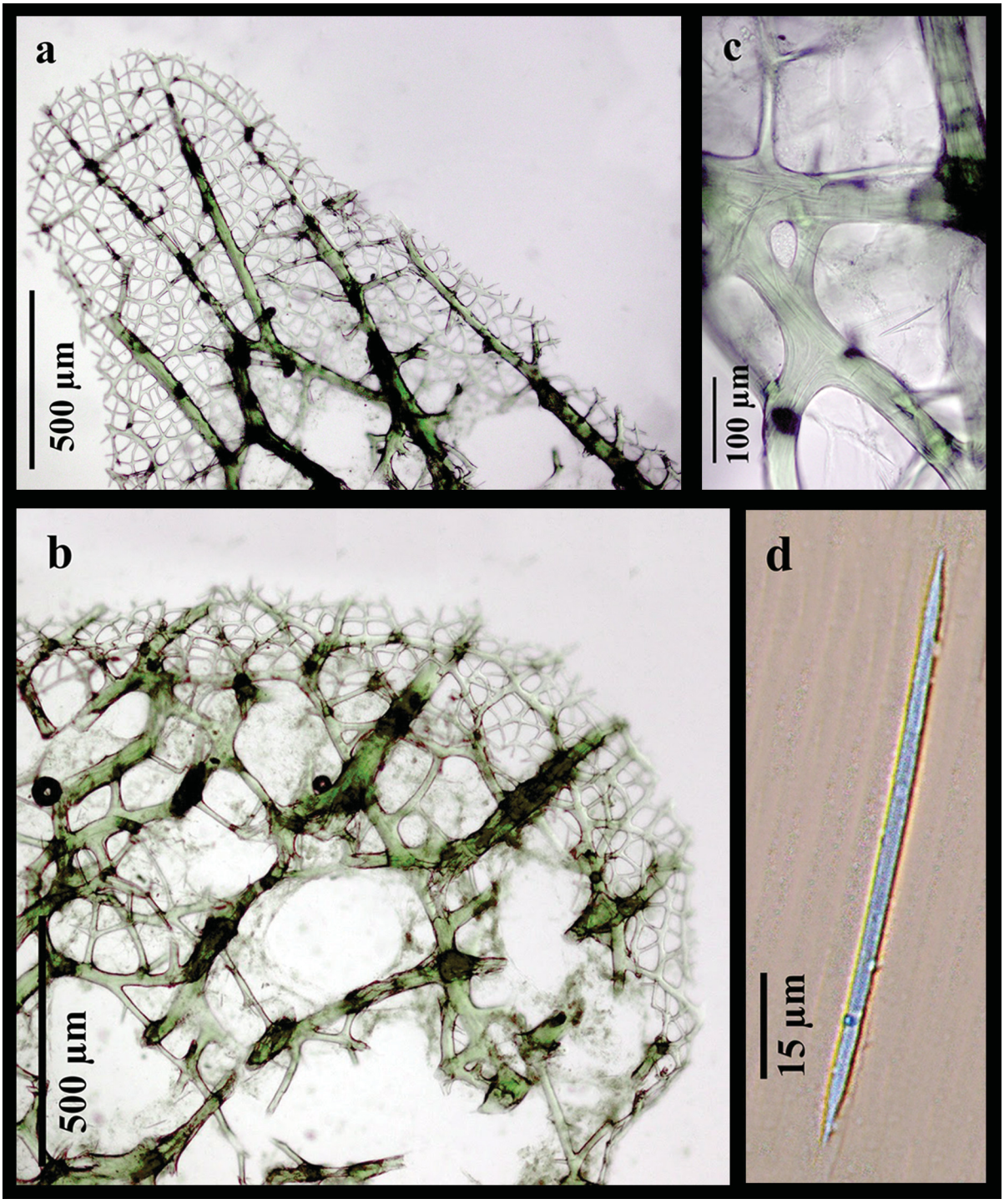

FIGURE 4. *Callyspongia (C.) alcoladoi* **sp. nov.** a) Tangential section of ectosome (4X) b) Longitudinal section shows primary, secondary, and tertiary fibers (4X) c) Fibers discretely cored (20X) d) Mucronate oxea (400X).

Description. External morphology: Delicate, smooth ropey and cylindrical branches (3–8 mm in diameter, 20–30 cm long), profusely branching and anastomosing, with a few spiny projections (2–4 mm wide, 2–4 cm long) that occasionally arise from the branches. The diameter is variable along the length of the branches, sharply decreasing at the tips, ending always in pointed ends (Fig. 3a–e). No distinct base, but specimens fixed to the substrate in two or three points. Oscules (1–2 mm in diameter) round and slightly sunken in relation to the surface (Fig. 3d–e), located in rows on one side of the branch, at variable positions (0.2–2 cm apart). Color gray-pinkish externally, tan internally. Tan in alcohol. Very smooth surface, visually and to the touch. Firm but compressible in consistency. No

sand or foreign material on the surface. Abundant subdermal round cavities (0.25–0.8 mm) throughout the sponge are visible to the naked eye (Fig. 3d).

Spicules: Oxeas (Fig. 4d), mostly mucronate, few fusiform, 67.6–*73.3*–85 x 1.5–*2.3*–3 μm.

Skeleton: Ectosome well–developed primary, secondary and tertiary tangential reticulation (Fig. 4 a–c). Primary fibers (30–150 μm in diameter), secondary fibers (15–30 μm) and tertiary fibers (8–16 μm), with primary meshes (80–250 μm), secondary meshes (70–210 μm) and tertiary meshes (30–70 μm). In the choanosome, a longitudinal section of the branch shows primary fibers (30–110 μm in diameter) that run longitudinally along the branches and are connected by secondary fibers (8–25 μm). Primaries occasionally anastomose forming wide, large meshes (60–500 μm), while secondary or smaller, more abundant meshes (40–200 μm) occur within them. Meshes are angular with various shapes ranging from triangular, to square or polygonal. Only primary and secondary fibers are cored, primaries with 1–6 spicules, and secondaries with 1–2 spicules (Fig. 4c). The pronounced development of a tertiary reticulation in the ectosome suggests the inclusion of this species in the subgenus *Cladochalina* Carter, 1885, despite the smooth surface of this species.

Distribution and ecology. East wall of Bahía de Cochinos (S coast of Cuba) and Punta del Fraile (NW Punta Maisí, Eastern tip of Cuba)(Table 2), mesophotic coral reef, depth range: 51.5–73.4 m. A larger specimen (approx. 1 m long) highly ramified and yellowish in color (Fig. 3b) was observed but not collected.

Remarks. *Callyspongia (C.) alcoladoi* **sp. nov.** does not represent the typical *Cladochalina*, which is characterized, by having the primaries forming bundles or fibrofascicles, and usually have species with spinose surface projections (i.e. mention here a couple of common *Cladochalina* species with spiny projections). However, the highly developed tertiary reticulation in the ectosome (typical among *Cladochalina* species), and the lack of a peripheral condensation in the reticle typical of the subgenus *Callyspongia* support the closer similarity of *Callyspongia (C.) alcoladoi* **sp. nov.** to this subgenus. The occasional spinose projections observed in *Callyspongia (C.) alcoladoi* **sp. nov.** may represent certain similarity to the spinose projections found in *Cladochalina* species. A comparative molecular study is necessary to validate the phylogenetic value of the current subgenera classification and the evolutionary relationships within the *Callyspongia* genus.

Callyspongia (C.) pedroi **sp. nov.**, *Callyspongia (C.) alcoladoi* **sp. nov.** and *Callyspongia (C.) strongylophora* Hartman, 1955 have similar characteristics, such as lack of a distinct base, and branches with oscules arranged in rows. However, *Callyspongia (C.) strongylophora* forms a tangled mass of irregularly anastomosing and dividing branches, with strongylote spicules, while the other two species are branching, rarely anastomosing, and have oxeas as spicules (Tables 3, 4).

Callyspongia (C.) alcoladoi **sp. nov.** and *Callyspongia (C.) scutica* Van Soest, 2017 are close in morphology, due to their smooth surface and pointed ends of the branches, but *Callyspongia (C.) scutica* branches are often somewhat flattened, with a tendency to have the branches in one plane, while *Callyspongia (C.) alcoladoi* **sp. nov.** has cylindrical branches, and branches in multiple planes. Furthermore, the primaries of *Callyspongia (C.) alcoladoi* **sp. nov.** in the ectosome are much thicker than those ones of *Callyspongia (C.) scutica*, and the fusiform oxeas are much larger in *Callyspongia (C.) scutica* (Tables 3, 4).

Etymology. Both species are dedicated to Dr. Pedro M. Alcolado, who dedicated his life to the study of marine sponges and made important contributions to the knowledge of Caribbean sponge taxonomy and ecology.

Key to branching *Callyspongia* species

(1)	Surface thorny to spiky	2
-	Surface smooth to microconulose or microhispid, never thorny	3
(2)	Short branches with various diameters (3–10 mm). Spicules hastate oxeas, occasionally styles (184–232 x 6.5–8 μm)	*C. (Callyspongia) densasclera*
-	Long, single or multiple branches, mostly erect (5–30 mm in diameter). Spicules oxeas (60–130 x 2–6 μm)	*C. (Cladochalina) armigera*
(3)	Branches depart from basal mass or a tangled mass of branches. Spicules strongylote	4
-	Branches dividing or rarely anastomosing. Spicules oxeas	5
(4)	Branches depart from basal mass, not tangled, 6–15 mm in diameter	*C. (Cladochalina) tenerrima*
-	Tangled mass of branches, 2–4 mm in diameter	*C. (Callyspongia) strongylophora*
(5)	Greenish blue, very thin digitate branches, 5 mm in diameter, 12 cm long	*C. arcesiosa*
-	Branches with pointed ends, 3–10 mm in diameter, primaries >25 μm wide, secondaries >1μm wide	6
(6)	Long branches, often somewhat flattened, with a tendency for the branches to be in one plane	*C. (Callyspongia) scutica*

- Delicate cylindrical branches without distinct base, but fixed to the substrate at a few points 7
(7) Color light reddish to pink. Skeleton without ectosomal tertiary reticulation . . . . . *C. (Callyspongia) pedroi* **sp. nov.**
- Color gray pinkish or light yellow. Branches with few spiny looking projections (2–4 mm width, 2–4 cm long). Ectosome with well–developed tertiary reticulation . . . . . *C. (Cladochalina) alcoladoi* **sp. nov.**

Discussion

The genus *Callyspongia* has an extensive distribution along the Cuban coast, with its seven species found in 23 of the 35 studied sites (65.7%) (Table 2). From 10 species listed in Cuba (Alcolado 2002; 2007; present work): two occur exclusively in shallow waters, six in both shallow and mesophotic depths, and two are exclusively mesophotic (Table 5). *Callyspongia (C.) pedroi* **sp. nov.** is the *Callyspongia* species with second widest distribution (12 of 35 sites studied, 34.3%), and the second deepest *Callyspongia* species. *Callyspongia (C.) plicifera* is the most widely distributed species (16 of 35 sites, 45.7%), and occurring at greater depths.

The present work characterizes two new *Callyspongia* species, *Callyspongia (C.) pedroi* **sp. nov.** and *Callyspongia (C.) alcoladoi* **sp. nov.**, thus increasing from 8 to 10 the number of *Callyspongia* species recognized for Cuba (Table 5).

The occurrence of thin branching *Callyspongia* specimens, rare but widely distributed along the mesophotic Cuban reefs, was the stimulus for closely examining the taxonomic features that distinguish these species that are seldom described in regional sponge studies. The goal of the expedition during which these samples were collected was to characterize mesophotic reefs around the coast of Cuba. Sample collections were a lower priority; sponges that appeared to be different from those already known were collected using the ROV. The two specimens collected were initially believed to belong to a single species of *Callyspongia*. Further characterization and close comparison of a complete set of morphological features showed clear differences between the two specimens, indicating as well that they do not conform to any of the previously described thin ropey *Callyspongia* species from the Caribbean (Tables 3, 4). Furthermore, the skeletal architecture shows affinities with different *Callyspongia* subgenera, *Callyspongia* and *Cladochalina*.

The depth distribution records of five species were extended in the present survey. The depth range of *Callyspongia (C.) armigera* (Duchassaing & Michelotti, 1864) is extended from 45 m to 81.3 m deep, *Callyspongia (C.) fallax* from 20 m to 64.4 m, *Callyspongia (C.) pallida* from 3 to 64 m, *Callyspongia (C.) plicifera* from 40 m to 172.2 m and *Callyspongia (C.) vaginalis* (Lamarck, 1814) from 70 to 80.4 m (Table 5).

Future collection of these *Callyspongia* species will allow further studies that may include histology, ultrastructural, and genetic barcoding. These studies will reinforce a clear delimitation of these unique and rare *Callyspongia* species common in deeper mesophotic reefs.

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TABLE 5. Species of the genus *Callyspongia* described for Cuba, with its distribution in shallow waters (S = 0–30 m) and mesophotic (M =>30–150 m); and the depth ranges (DR) reported for Cuba previously and in the present study; and for the Central West Atlantic region according to the literature. * = Species for which records of depth range increased. The depths in **bold** are records for the region. Ref = References.

Species	Cuba (1)			Cuba (Present)			CW Atlantic			
	S	M	DR (m)	S	M	DR (m)	S	M	DR (m)	Ref
Callyspongia (C.) alcoladoi sp. nov.					X	51.5–73.4				
Callyspongia arcesiosa	X		0.5–7				UK			2
*Callyspongia (C.) armigera**	X	X	2–45		X	31.1–**81.3**	X	X	4–48	6
Callyspongia (C.) debilis	-						X		0–20	9
*Callyspongia (C.) fallax**	X		1–20		X	33–**64.4**	X		2–6 3–18 10–15	7 6 5,3
*Callyspongia (C.) pallida**	X		3		X	**64.3**	X	X	1–2,5 25–62	7 8
*Callyspongia (C.) plicifera**	X	X	6–40		X	34.3–**172.2**	X	X	5–18 15 10–25 70–107	6 3 7,5 4
Callyspongia (C.) tenerrima	X	X	15–70				X		2–4	5
*Callyspongia (C.) vaginalis**	X	X	1–70	X	X	23.4–**80.4**	X	X	2–4 8–35 2–70	5 7 6
Callyspongia (C.) pedroi sp. nov.					X	44.4–102.4				

1. Alcolado (2002; 2007; unpublished data) &Porifera Microsoft Access® database, collections of Acuario Nacional de Cuba; 2. De Laubenfels (1936); 3. Lehnert & Van Soest (1998); 4. Lehnert & Van Soest (1999); 5. Pulitzer–Finali (1986); 6. Rützler *et al.* (2009); 7. Van Soest (1980); 8. Van Soest (2017); 9. Wiedenmayer (1977)

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