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New Poecilosclerida from mesophotic coral reefs and the deep-sea escarpment in the Pulley Ridge region, eastern Gulf of Mexico: *Discorhabdella ruetzleri* n.sp. (Crambeidae) and *Hymedesmia (Hymedesmia) vaceleti* n.sp. (Hymedesmiidae)

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Abstract

Pulley Ridge, a limestone ridge that extends nearly 300 km along the southwestern Florida shelf in the eastern Gulf of Mexico, supports a mesophotic coral ecosystem (59 to 94 m deep), surrounded by deeper waters. An ongoing evaluation of Porifera biodiversity observed and collected during expeditions by Harbor Branch Oceanographic Institute (2003–2015) have shown the existence of approximately 102 sponge species, with at least 20 species new to science. The present paper describes two novel Poecilosclerida from mesophotic reefs and deep escarpments in the Pulley Ridge Region, Eastern Gulf of Mexico, namely *Discorhabdella ruetzleri* **n.sp.** (Crambeidae, Poecilosclerida) and *Hymedesmia (Hymedesmia) vaceleti* **n. sp.** (Hymedesmiidae, Poecilosclerida). This is the first record of *Discorhabdella* for the greater Caribbean and the Central West Atlantic. The skeleton of *D. ruetzleri* **n.sp.** includes the unique pseudoastrose acanthostyles of the genus, and it is distinct from congeners in the size ranges of the megascleres and in the occurrence of predominantly smooth instead of tuberose choanosomal and ectosomal subtylostyles. The intense blue color and the spicule combination of *Hymesdesmia (H.) vaceleti* **n.sp.** makes this species unique among other *Hymedesmia* spp. from the western Atlantic. The discovery represents a considerable expansion in the known biogeographical distribution of the genus *Discorhabdella* which is represented now by six species with a discrete geographic distribution (New Zealand, Azores, Western Mediterranean Sea, Eastern Pacific in Panama). This work is the first contribution to an ongoing effort to discover and document the importance of sponge biodiversity on mesophotic reefs and associated deep-water habitats in the Gulf of Mexico and the Caribbean.

Key words: new species, Porifera, crustose sponges, Crambeidae, Hymedesmiidae, Greater Caribbean

Introduction

Sponges are dominant components of mesophotic coral ecosystems and deeper communities surrounding them (Pomponi *et al.* 2018; Reed & Pomponi 1997; Slattery & Lesser 2012; Van Soest & Stentoft 1988;Van Soest *et al.* 2014; Van Soest 2017). Mesophotic coral ecosystems are distributed between 30–45 m and 150 m in depth and are of considerable importance due to their unique biodiversity and their potential to serve as a refuge for coral reef associated species (Reed 2016).

Recent taxonomic sponge surveys at mesophotic and associated deep water habitats (200–1000 m) using technical diving or TRIMIX, HOVs (human operated vehicles) and ROVs (remotely operated vehicles) in the Tropical Western Atlantic (Lehnert & Van Soest 1996, 1998, 1999; Reed & Pomponi 1997; Van Soest *et al.* 2014) have revealed a surprising number of previously unknown taxa demonstrating the importance of studying these ecosystems as sources of ecological, physiological, and biochemical novelty.

A recent review of the species associated with western Atlantic mesophotic coral reef ecosystems shows that from a total of 241 species recorded in the literature, 102 species (42%) also occur in shallow water, 40 species (17%) also occur in deeper water (> 150 m), 88 species (37%) occur exclusively at mesophotic depths, and 11 species (5%) occur at all three depth levels (Pomponi *et al.* 2018). Therefore, a significant percentage of the sponge biodiversity associated with mesophotic coral reefs is only found on particular biotopes at those depths, and usually represents fauna previously unknown to science.

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Pulley Ridge is a limestone ridge that extends nearly 300 km along the southwestern Florida shelf in the eastern Gulf of Mexico (Fig. 1). The southern terminus of Pulley Ridge supports a mesophotic coral ecosystem (MCE) at depths of 59 to 94 m and is the deepest known photosynthetic coral reef off the continental United States. Coral and associated fauna growth is supported by the Loop Current, the prevailing western boundary current in the Gulf of Mexico, which provides warm, clear waters to the Pulley Ridge Region (bottom temperatures range from 18.47° C to 28.48° C at 69 m) (Reed *et al.* 2016). A recent review summarizing decades of exploration (2003–2015) revealed that Pulley Ridge biodiversity consists of at least 95 species of macroalgae, 92 demosponges, 18 octocorals, 16 scleractinian corals, 9 antipatharian corals, and 86 fishes (Reed *et al.* 2017).

An ongoing taxonomic evaluation of sponge fauna collected from Pulley Ridge has shown that among the more than 102 species already identified, there is a group of unique morphotypes with a high possibility of several species new to science. Among those that are likely undescribed species are representatives of common shallow water genera such as *Aplysina, Callyspongia, Petrosia, Neopetrosia, Clathria, Auletta,* and *Ircinia*, and representatives of other genera that are rare in shallow waters, such as *Characella, Forcepia, Topsentia, Desmanthus, Dendrilla, Discorhabdella* and *Hymedesmia.* The morphological description of a novel sponge species requires the comparison of the external and internal morphology of the species in question with previously described species. In several cases, a further set of characters, such as genetic or chemical data, are required to distinguish species that might not present clear differences at the morphologic level.

The present study describes two species of thinly encrusting Poecilosclerida from the Pulley Ridge region, found growing on rubble (dead coral) or on other organisms (snails and solitary corals). Here we describe a species of the genus *Discorhabdella* Dendy 1924 (Crambeidae, Poecilosclerida) and a species of the genus *Hymedesmia* Bowerbank 1864 (Hymedesmiidae, Poecilosclerida).

For the genus *Discorhabdella*, a relatively rare genus with only five extant species described from sublittoral and bathyal depths worldwide (Van Soest *et al*. 2018), this represents the first report for the western Atlantic. The genus *Hymedesmia* is much more diverse worldwide and contains eight species described for the western Atlantic (Van Soest *et al*. 2018) distributed from shallow to deep waters of various Caribbean and south Atlantic regions.

Our goal is to promote the study of fauna from mesophotic coral reefs and deeper habitats associated with them and to demonstrate the importance to describe the often overlooked thinly encrusting fauna. Although these thin crusts may seem of little ecological importance due to their apparent low biomass, they may occupy unique niches, with unique evolutionary pathways and unknown functional roles in these ecosystems.

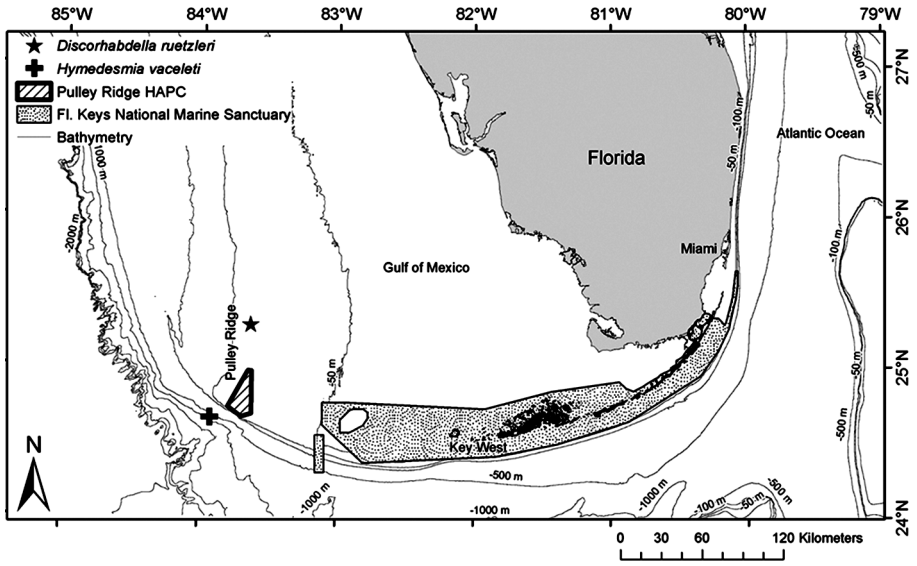

FIGURE 1. Map of the northeastern Gulf of Mexico showing the area of Pulley Ridge and the localities where the two new Poecilosclerida species were collected.

Materials and methods

Area of Study. Pulley Ridge is located in the Gulf of Mexico about 250 km west of the Florida coast and extends from north to south along the southwestern Florida platform at depths of 60–90 m for nearly 300 km (Reed 2016). To the west and south of Pulley Ridge, the Florida Escarpment drops off to >1000 m depths (Reed *et al.* 2017).

The material described herein was collected during the Florida Shelf-Edge Expedition II (2011), on board the NOAA R/V *Nancy Foster*, and surveys conducted during 2015, on board the University of Miami R/V *F. G. Walton Smith*. The expeditions were conducted by the National Oceanic and Atmospheric Agency (NOAA) Cooperative Institute for Ocean Exploration, Research, and Technology (CIOERT) at Harbor Branch Oceanographic Institute - Florida Atlantic University (HBOI-FAU), with support from the NOAA Office of Ocean Exploration and Research and the NOAA National Centers for Coastal Ocean Science. Sponges were collected in 2011 using the *Kraken* ROV (University of Connecticut) while diving on the south Pulley Ridge escarpment between 500–850 m, and in 2015 using the *Mohawk* ROV (University of North Carolina, Wilmington) while diving on north Pulley Ridge between 69–75 m.

Preparation of Samples. After collection, sponges were preserved in 70% ethanol. To study the skeleton, representative subsamples of the sponges were boiled in concentrated nitric acid, rinsed in distilled water, and dehydrated in a series of increasingly concentrated ethanol solutions (70-95%). Spicules were permanently mounted onto glass slides using Permount, and later observed and measured (25 spicule per each spicule type) using a light microscope (Olympus CX40) at HBOI-FAU. For scanning electron microscopy, a drop of dissociated spicules in ethanol was placed on a stub, dried overnight in an oven (60° C), and sputter-coated with gold-palladium. Observations were made with a Hitachi S-4800 at the electron microscopy facility at the U.S. Department of Agriculture, Fort Pierce. Type material was deposited in the Harbor Branch Oceanographic Museum (HBOM) at HBOI-FAU and stored at room temperature.

TABLE 1. Morphometric comparison of skeleton and distribution of *Discorhabdella* species worldwide. All spicule measurements in mm.

Species	Locality	Depth (m)	Principal tylostyles	Ectosomal subtylostyles	Pseudoastrose acanthostyles	Isochelae	Spined microrhabds	Sigmas
D. hindei Boury-Esnault *et al.* 1992	Alboran Sea, Mediterranean	534–604	1086 x 43	367 x 5.2	48 x 9 tyle 38	23 isancres	No	no
D. incrustans Dendy 1924	Three Kings, North Cape, New Zealand	180	1700 x 50	540 x 12	52/40	48	Yes oxy-discorhabds	no
D. littoralis Maldonado *et al.* 2001	Gulf of Chiriqui, Pacific Panama	10–30	117–300 x 5–10	130–180 x 2.5–4	2–40 tyle 10–18.5	no	No	13–15 x 1
D. tuberosocapitatum (Topsent 1890)	Azores	550–736	650 x 28	330	130	25	No	no
D. urizae Maldonado *et al.* 2001	Gulf of Chiriqui, Pacific Panama & Gulf of California, Mexico	55–73 & 340	380–750 x 19–42	180–220 x 5–7	23–27 tyle 15–26 subterminal spines	26–29	19–26 x 2–3	13–16 x 1
D. ruetzleri Present study	Pulley Ridge, Gulf of Mexico	60–80	470–810 x 5–13	260–340 x 3–7.5	17–40 x 2.5–7.5 tyle 7.5 – 20	20–25 x 1.5	15–18 x < 1	12–18 x <1
Fossil species: Oligocene deposits Hinde & Holmes 1892	Oamaru, South Island of New Zealand	bathyal	Tuberose tyles, broken spicules	na	43 x 8 tyle 35	na	na	na

Results

Two thinly encrusting sponges collected during the Pulley Ridge campaigns belong to two Poecilosclerida genera, *Discorhabdella* (family Crambeidae) and *Hymedesmia* (family Hymedesmiidae). *Discorhabdella* is referred to in the literature as a Tethyan "relict" (Boury-Esnault *et al.* 1992; Maldonado *et al.* 2001; Łukowiak 2016) and currently includes five extant species and one fossil species (Van Soest *et al.* 2018). Table 1 compares major skeletal characteristics and habit of the species of *Discorhabdella* currently described and of the species described herein. The second species with a similar growth form and with a hymedesmoid arrangement of the skeleton is here described as a novel species of *Hymedesmia.* Table 2 compares major skeletal components and habit of this species with eight species currently described for the western Atlantic (Van Soest *et al*. 2018).

TABLE 2. Morphometric comparison of skeleton and distribution of *Hymedesmia* species of the subgenera *Stylopus* or *Hymedesmia* in the Central West Atlantic. All spicule measurements in mm.

Species	Locality Depth (m)	Color	Acantho–tylostyle I	Acantho–tylostyle II	Tornotes to tylotes	Isochelae	Sigma
H. (S.) alcoladoi Van Soest 2017	off Suriname coast 64	brown in spirit	135–213 x 9–17	62–121 x 6.5–9	151–222 x 2–4	no	no
H. (H.) agaricicola Van Soest 1984	Curaçao 10	orange	91–194 x 4–5.5	48–57 x 2.5–3.5	111–142 x 1	I.18–21 II.8–12 arcuate	no
H. (H.) bonairensis Van Soest 2009	Bonaire 3.5–5	rich yellow	144–207 x 4.5–6.5	48–75 x 3–4.5	179–213 x 1.5–2.5	I. 17–24 II.9–11 arcuate	yes
H. (H.) caribica Lehnert and Van Soest 1996	Jamaica 76	light brown	200–250 x 5–10	70–105 x 4–7	220–255 x 3–5	20–27 arcuate	no
H. (H.) curacaoensis Van Soest 1984	Curaçao 10-33	bright red	205–229 x 8–10	57–114 x 3.5–6	182–230 x 1.5–2.5	28–33 arcuate	no
H. (H.) jamaicaensis Van Soest 1984	off Jamaica deep	transparent	133–276 x 4.5–9.5	53–106 x 3.4–7.5	160–228 x 0.5– 1	12–18 palmate	no
H. (H.) nummota De Laubenfels 1936	Florida Straits 1047	pale gray	970–1280 x 18–21	620 x 10	470–565 x 6–8	44–63 arcuate	no
H. (H.) palmatichelifera Van Soest 1984	Curacao 33	yellow reddish	293–361 x 6–8	66–91 x 4–7.5	167–232 x 1.5–2.5	palmate, 15–19	no
H. (H.) vaceleti Present study	Pulley Ridge 773	blue gray	280–400 x 10–12.5	100–150 x 7.5–10	370—540 x 7.5–10	45–60 x 5–10 arcuate	no

Taxonomic descriptions

Family Crambeidae Lévi, 1963 , sensu Van Soest 2002a

Genus *Discorhabdella* Dendy, 1924

***Discorhabdella ruetzleri* n. sp.**
(Figure 2)

Material collected: Holotype HBOM 003:02021 (sample 10–V–15–2–021).

Type locality: Gulf of Mexico, north Pulley Ridge, 80 m deep, on a ledge. *Mohawk* ROV dive 149. Collected by John Reed. Coordinates: latitude 25^{0}16.8990 N, longitude 83^{0}37.7850 W.

Etymology: the species is named in honor of Dr. Klaus Rützler, our mentor and an important contributor to the knowledge of sponges from the Caribbean and world-wide.

External morphology: thinly encrusting (1–3 mm) with amorphous, bulgy protuberances (Fig. 2A). Surface prominently hispid microscopically. Orange reddish color alive; white in spirit.

Spicules: long principal tylostyles to subtylostyles with predominantly smooth tyles, and less abundant slightly tuberose tyles in a ratio of 7:1 (Fig. 2B); principal subtylostyles measure 470–**598**–810 x 5–10.5–13 µm in length and width; small ectosomal substylostyles with smooth elongated tyles (260–**300**–340 x 3–4–7.55 µm), sigmas (12–18 x <1 µm), pseudoastrose acanthostyles, with elongated terminal or apical spine, measuring 17–**29.6**–40 x 2.5–**5**–7.5 µm, with spiny heads 7.5–**15**–20 µm wide (Fig. 2C, D); some pseudoastrose acanthostyles with a smoothly elongated apical spine, others with spiny areas distal from the terminal end of the apical spine (Fig. 2C, E); several smaller smooth forms of the acanthostyles are considered early growth forms of this spicule category. Extremely thin microspined oxea (15–18 µm < 1 µm) (Fig. 2F). Rare anchorate isochela (20–25 x1.5 µm), not seen in the spicule preparations, but observed on a section of the skeleton.

Skeleton: The skeletal arrangement consists of a hymedesmoid arrangement of a single layer of large principal tylostyles to subtylostyles, which pierce the sponge surface. The tyles of the principal megascleres are embedded in a spongin layer that is also echinated by densely packed pseudoastrose acanthostyles; ectosomal smooth subtylostyles are around and between the principal choanosomal subtylostyles. Sigmas and microxea are abundant in the ectosome. Two isochelae observed in the choanosme in one transversal section.

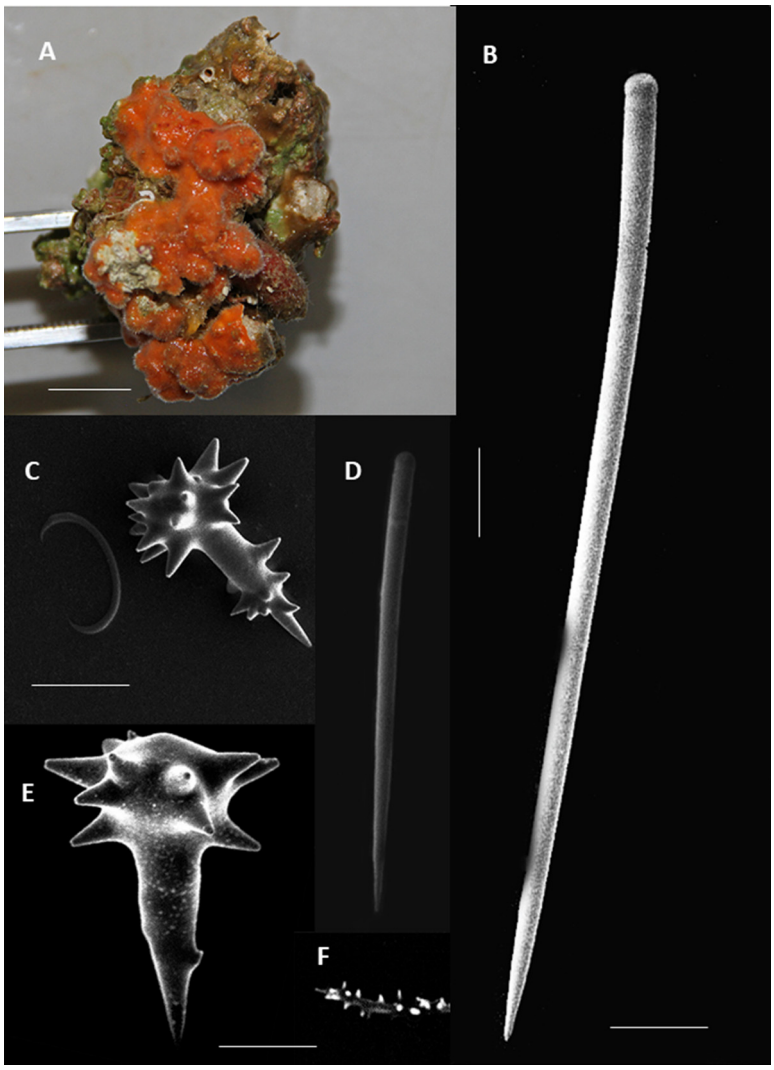

FIGURE 2. *Discorhabdella ruetzleri* **n.sp.** A. habitus (scale 1 cm), B. principal choanosomal subtylostyle with smooth tyle (scale 70 µm), C. pseudoastrose echinating acanthostyles with sub-terminal supination on apical spine, and sigma (scale 15µm), D. shorter choanosomal subtylostyles with smooth tyle (scale 100µm), E. pseudoastrose echinating acanthostyles with smooth apical spine (scale 10 µm), F. spined microrhabd (scale 10µm).

Remarks: Pseudoastrose acanthostyles typical of genus, and with the apical spine described for *D. hindei* Boury-Esnault, Pansini & Uriz, 1992 and *D. urizae* Maldonado, Carmona, Van Soest, & Pomponi 2001 are abundant in this species (Fig. 2C). The species presents skeletal similarities to *D. urizae* and *D. littoralis*, the two species described from the eastern Pacific side of Panama. *D. ruetzleri* **n. sp.** is very similar in skeletal composition to *D. urizae* Maldonado *et al.* 2001, a dark red thin crust originally collected on a boulder between 53–75 m deep in the Gulf of Chiriqui, off the Pacific coast of Panama, and later collected from the Gulf of California at 344 m (Aguilar-Camacho *et al.* 2012). The main difference between *D. ruetzleri* **n. sp.** and *D. urizae* is the predominance of smooth tylostyles (both ectosomal and choanosomal) in *D. ruetzleri* **n. sp.** versus the predominant and strongly tuberose tyles of *D. urizae* principal choanosomal tylostyles. The species are futher distinguished by the thickness of the principal tylostyles and the considerably smaller microoxea in *D. ruetzleri* **n. sp.** (Table 1). The discretely spined microxea of *D. urizae* and the shape and size of the pseudoastrose acanthostyles, with the spinose bulgy tyle and shortened spined base are very similar to *D. ruetzleri* **n. sp.**, suggesting a potential common origin of these two species. *D. littoralis* Maldonado *et al.* 2001 from the eastern Pacific lacks isochela, while in *D. ruetzleri* **n. sp.** they are very rare but observed in one skeletal section. The smooth subtylostyles of *D. littoralis* share similarity with the subtylostyles of *D. ruetzleri* **n. sp.** The color in *D. urizae* is described as dark red, and *D. littoralis* is bright red orange, while for *D. ruetzleri* **n. sp.**the color is orange reddish that in spirit becomes completely white.

Family Hymedesmiidae Topsent, 1928, sensu Van Soest 2002b

Hymedesmia Bowerbank, 1864

Hymedesmia (Hymedesmia) vaceleti n. sp.

(Figure 3)

Material collected: Holotype HBOM 003**:**02020 (sample 19–IX–11–1–007).

Type locality: Gulf of Mexico, south of Pulley Ridge, 773 m deep, escarpment, on sediment. Collected by John Reed and Shirley Pomponi. Coordinates: latitude 24°39.600 N, longitude 83°55.0420 W.

Etymology: The species is named in honor of Dr. Jean Vacelet, our mentor and an important contributor to the knowledge of sponge systematics and ecology from the Mediterranean, the Indian Ocean and the Caribbean.

External morphology: Blue, with irregular whitish spots, thin crust (1 mm thick), forming small patches (1–4 cm^2), growing over snails, sides of solitary corals, and calcareous tubes (Fig. 3A). Surface smooth and shiny. Discretely microhispid microscopically.

Spicules: Large acanthostyles (280—**345** –400 x 10–**10.3**–12.5 µm), with inflated head and large tuberculated spines (30–35 µm diameter) (Fig. 3C); smaller acanthostyles (100–**137**–150 x 7.5–**9**–10 µm) with spiny heads (12.5–15 µm in diameter) microspined all along (Fig. 3D); tornotes with stongyloid to styloid morphology (370–**461**–540 x 7.5–**8**–10 µm) (Fig. 3B); abundant arcuate isochela 45–60 µm long, and a thick flattened shaft 5–10 µm thick (Fig. 3E–F).

Skeleton: An hymedesmioid arrangement of large and small acanthostyles, that stand singly erect on a strong spongin basal layer, and tornotes that are strewn in the choanosome and in the ectosome (Fig. 3C).

Remarks: The combination of an intense blue color, large tornotes (> 400 µm in length), and the size of the acanthostyles differentiate *H. (H.) vaceleti* **n.sp.** from other *Hymedesmia* spp. from the western Atlantic (Table 2). The closest species among the previously described *Hymedesmia* (Table 2) is *H. (H.) nummota* De Laubenfels 1936, a thin pale gray crust (0.5–2 mm thick), with an irregularly lumpy surface growing on a piece of dead coral, collected in deep water (1047 m) off the coast of Florida (between Dry Tortugas and Cuba). The other seven species of *Hymedesmia* currently described from the western Atlantic have considerably smaller and thinner tornotes (< 255 µm in length, and < 4 µm in width). The three categories of megascleres in *H.* (*H*). *nummota* differ in size ranges from those in *H. (H.) vaceleti* **n. sp.** The size of the two types of acanthotylostyles of *H. (H.) nummota* are triple the length of those in *H. (H.) vaceleti* **n. sp.**, while the tornote strongyloids of *H. (H.)vaceleti* **n. sp.** can be 100 µm smaller than those in *H. (H.) nummota.* The size range of tornotes is very conserved and a significant character that distinguishes the different species of *Hymedesmia* from the western Atlantic (Van Soest, 2009). Therefore, taking into account the tremendous disparity in the size of acanthotylostyles and the distinct blue

color of *H. (H.) vaceleti*, we consider these differences sufficient evidence to classify the Pulley Ridge specimen as a different species from *H.(H.) nummota*.

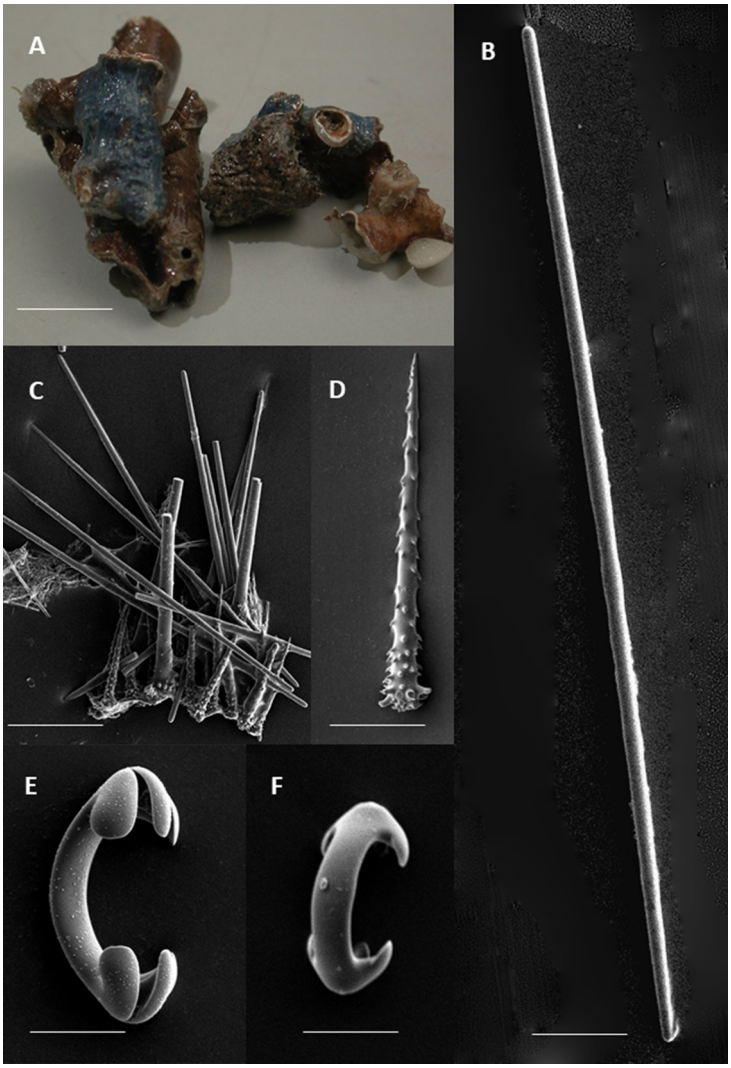

FIGURE 3. *Hymedesmia (Hymedesmia) vaceleti* **n.sp.** A. habitus, thinly encrusting on *Lophelia* rubble and snails (scale 1 cm), B. ectosomal smooth tornote (scale 40 µm), C. hymedesmoid arrangement of megascleres observed in fragment that had been previously treated with nitric acid for spicule dissociation (scale 100 µm), D. smaller category of acanthostyles (scale 37.5 µm), E. arcuate isochela, side view (scale 22 µm), F. arcuate isochela, back view showing flattened shaft (scale 27.5µm).

Discussion

Previous studies of the sponge fauna in Tropical Western Atlantic mesophotic coral reef ecosystems have shown that there is a considerable component of the sponge fauna (30–40%) that is unique to these habitats (see Pomponi *et al.* 2018). Certain genera such as *Auletta* and *Phakellia* (Axinellida), *Acanthella* (Bubaridae), *Halicnemia* (Stelligeridae), *Aulospongus* (Raspailiidae), *Penares* (Geodiidae), *Pachastrella* and *Characella* (Pachastrellidae), *Thrombus* (Thrombidae), *Siphonidium*, *Gastrophanella* and *Leiodermatium* (Siphonidiidae), *Scleritoderma* and *Aciculites* (Scleritodermidae), *Pseudotrachya* (Polymastiidae), *Coelosphaera* and *Forcepia* (Coelosphaeridae), *Desmanthus* and *Petromica* (Desmanthidae), *Antho* and *Echinochalina* (Microcionidae), *Phlyctaenopora* (Mycalidae), *Hymedesmia* (Hymedesmiidae), *Julavis* and *Parahigginsia* (Heteroxyidae), and *Topsentia* and *Spongosorites* (Halichondriidae) are among those that predominate at mesophotic depths and are absent or rare on shallow reefs. Pomponi *et al.* (2018) also reinforce the importance to study thin crusts that, although hard to collect, represent one of the least studied Porifera faunal components on mesophotic coral reef ecosystems.

During the evaluation of sponge biodiversity recorded on videos of Pulley Ridge mesophotic coral reef

ecosystems, 14 thinly encrusting sponge species were visually identified as representatives of the families Microcionidae and Spirastrellidae, or were only assigned to the class Demospongiae. After the taxonomic evaluation of those 14 collected samples, 12 species from 10 genera were distributed among nine families of seven Demospongiae orders (Agelasida, Axinellida, Bubarida, Clionaida, Poecilosclerida, Trachycladida, and Scopalinida). This underscores the importance of crustose sponges as a reservoir of biodiversity in mesophotic coral reef ecosystems and reinforces the need for taxonomic reference samples (versus identification from photos alone).

This study has added one Poecilosclerida genus, *Discorhabdella*, to the genera composing mesophotic coral ecosystem sponge fauna in the Tropical Western Atlantic. The finding of *D.ruetzleri* **n. sp.** is the first report of the genus for this important marine biogeographic region. The addition of the Gulf of Mexico to the previously known distribution for the genus places an important piece of the puzzle with respect to its biogeographic distribution, supporting the interpretation that the genus has a pantropical Tethyan origin and distribution (Azores, Gibraltar-Mediterranean, Panama-Eastern Pacific, New Zealand) (Boury-Esnault *et al.* 1992; Maldonado *et al.* 2001; Lukowiak 2016).

The correct classification and identification of sponges is not only pivotal for systematics, biodiversity, and biogeography but is highly relevant for the interpretation of ecological changes in mesophotic coral ecosystems and the ecological services that they provide, as well as for downstream applications of sponges, such as in biotechnology research.

Acknowledgements

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