

# Morphological and molecular taxonomy of calcareous sponges (Porifera: Calcarea) from Curaçao, Caribbean Sea

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Despite the acknowledged high diversity of sponges in the Caribbean Sea, calcareous sponges from this region have been poorly studied. In order to start filling this gap, in this study we describe the calcareous sponges from Curaçao, Southern Caribbean. The specimens were collected by SCUBA in eight localities along the island of Curaçao and analysed by morphological and molecular (ITS and C-LSU) approaches. A total of 16 species were found and are described here. Ten species are new to science and are provisionally endemic to Curaçao: *Arturia vansoesti* sp. nov., *Clathrina curacaoensis* sp. nov., *Clathrina globulosa* sp. nov., *Grantessa tumida* sp. nov., *Leucandra caribea* sp. nov., *Leucilla antillana* sp. nov., *Leucilla micropilosa* sp. nov., *Leucandrilla quadriradiata* sp. nov., *Sycon conulosum* sp. nov. and *Sycon magnapicale* sp. nov. The formerly Brazilian endemic species *Borojevia tenuispinata*, *C. lutea*, *C. insularis* and *C. mutabilis* have their distribution widened to the Caribbean Sea. *Clathrina hondurensis* and *Leucetta floridana* are new records for Curaçaoan waters. With these new records, the diversity of calcareous sponges from the Caribbean Sea reaches 33 species. Some issues on the phylogeny of Calcarea are also discussed.

ADDITIONAL KEYWORDS: biodiversity – internal transcribed spacer – molecular phylogeny – Caribbean – alpha taxonomy – new species.

## INTRODUCTION

The Caribbean Sea is considered a global-scale hot-spot of marine biodiversity (Roberts *et al.*, 2002). In its 2 754 000 km<sup>2</sup> of area and over 13 500 km of coastline, it encompasses a high diversity of flora and fauna distributed in different ecosystems including coral reefs, mangroves and seagrasses (Miloslavich *et al.*, 2010). It is a semi-enclosed basin bounded by Central America in the west, Greater Antilles in the north, Lesser Antilles in the east and the northern coast of South America in

the south. It comprises five ecoregions of the Tropical Northwestern Atlantic Province (TNA, Spalding *et al.*, 2007): Western Caribbean, Southwestern Caribbean, Southern Caribbean, Greater Antilles and Eastern Caribbean.

The sponges (phylum Porifera) are considered one of the most diverse benthic faunal groups in Caribbean coral reefs and mangroves (Díaz & Rützler, 2011). Reef sponges outnumber almost three times the diversity of corals (Díaz & Rützler, 2001) and mangrove sponges can comprise up to 70% of the total root epiphytic diversity in several Caribbean localities (Díaz & Rützler, 2009). In oligotrophic ecosystems such as coral reefs, sponges play an important ecological role as they contribute to the energy recycling through the ‘sponge loop’ (de Goeij *et al.*, 2013).

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Among the four extant classes of Porifera, the class Calcarea Bowerbank, 1862 includes species which have a skeleton composed of calcium carbonate spicules. As these sponges are usually small, devoid of colour (Wörheide & Hooper, 1999; Rapp, 2006) and inhabit light-protected or cryptic environments (e.g. overhangs, caves, crevices; Pérez *et al.*, 2017), they are easily neglected in sponge fauna inventories. Furthermore, the plasticity of some morphological characters makes the identification of calcareous sponges difficult, sometimes requiring complementary approaches such as molecular analyses (e.g. Imešek *et al.*, 2014; Azevedo *et al.*, 2015, 2017; Klautau *et al.*, 2016; Voigt *et al.*, 2017). As a result, it is easily observed that the knowledge on the diversity of calcareous sponges is restricted to geographical areas to which specialized taxonomists had access.

The class Calcarea is divided in two monophyletic subclasses, Calcinea and Calcaronea Bidder, 1898; however, the phylogenetic relationships within each subclass are still not well understood. Several orders, families and even genera, which were diagnosed using morphological characters, turned out to be polyphyletic or paraphyletic when analysed with molecular approaches (Manuel *et al.*, 2003, 2004; Voigt, Wülfing & Wörheide, 2012; Voigt & Wörheide, 2016). Only within some Calcinea, skeletal traits match phylogenetic signal, allowing the description of monophyletic genera (Rossi *et al.*, 2011; Klautau *et al.*, 2013; Córdor-Luján & Klautau, 2016).

To date, only 19 species belonging to Calcarea have been reported from the Caribbean Sea (Van Soest *et al.*, 2017) including species of both subclasses, while in the Mediterranean Sea, a similar geographic area with a basin comprising 2 969 000 km<sup>2</sup> (Coll *et al.*, 2010), the number of recorded calcareous species is more than triple, *c.* 65 species (Van Soest *et al.*, 2017). This probably reflects the lack of taxonomic effort in the Caribbean Sea, a region considered a diversity hotspot for many other taxa. Recently, a study showed how knowledge of sponge diversity from another Caribbean island (La Martinique, Eastern Caribbean) was improved after sponge taxonomists were included in a biodiversity assessment (Pérez *et al.*, 2017).

Curaçao is an island localized in the Southern Caribbean ecoregion, 60 km north off Venezuela. This island is surrounded by fringing reefs with a total area of 7.85 km<sup>2</sup> situated at 20–250 m from the coast (Vermeij, 2012). Most of these reefs occur along the leeward coast and harbour higher coral coverage compared with other sites in the Caribbean (Bruckner & Bruckner, 2003; Vermeij, 2012; Jackson *et al.*, 2014). Despite being affected by natural and anthropogenic forces, the reefs of Curaçao are among the healthiest coral ecosystems remaining in the Caribbean region (Jackson *et al.*, 2014) and host a great diversity of organisms including endemic sponges.

The sponge diversity of Curaçao has been intensively assessed for many years and currently almost 156 species are known (Van Soest *et al.*, 2017) including only two calcareous sponges. The pioneering study of Arndt (1927) included the first record of Calcarea, *Leucilla amphora* Haeckel, 1872. Further studies on shallow-water sponges (Van Soest, 1978, 1980, 1981, 1984; Hajdu & Van Soest, 1992; Alvarez, Van Soest & Rützler, 1998; De Weerd, 2000; Van Soest & De Weerd, 2001), sponges from cryptic habitats (Van Soest, 2009) and deep-water sponges (Van Soest, Meesters & Becking, 2014) did not report any other Calcarea, although one of those works mentioned the presence of this class (Van Soest, 1981). More recently, a former Brazilian endemic species, *Nicola tetela* (Borojevic & Peixinho, 1976) was recorded for this island (Córdor-Luján & Klautau, 2016).

The only two species of Calcarea reported from Curaçao until recently motivated the onset of a detailed faunistic inventory and taxonomic study of these sponges. Integrating morphological and molecular information, here we report ten new species and six new records of Calcarea for this southern Caribbean island, thus corroborating the hypothesis that lack of records is totally unrelated to real low biodiversity.

## MATERIAL AND METHODS

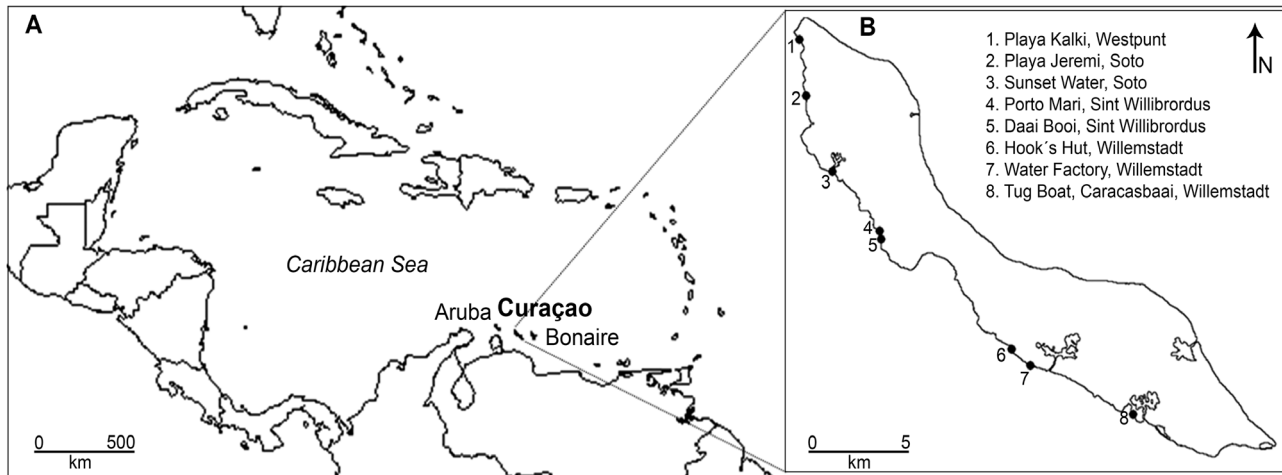
### STUDY AREA

Curaçao lies between 12°02'80" and 12°23'30"N and between 69°10'00" and 68°44'30"W. It is located off the NW coast of Venezuela, between Aruba (76 km NW) and Bonaire (41 km E) (Fig. 1A). The islands of Aruba, Bonaire and Curaçao are often referred to as the 'ABC islands'. Among these islands, Curaçao is the largest one with 59 km long, 14 km wide, with a total area of 443 km<sup>2</sup> (Pors & Nagelkerken, 1998).

Curaçao is a volcanic island located in the Leeward Antilles Ridge, a boundary zone between the Caribbean and the South American Tectonic Plates. This island was originated in the Cretaceous Period (88 Myr) and during later phases, it received different Miocene sediment depositions in certain areas (Beets, 1972). Its current geology has been shaped during the Quaternary (Hyppolyte & Mann, 2011).

### ANALYSED MATERIAL

In this study, eight localities along the western Curaçaoan coast were surveyed (Fig. 1B). The collections were performed by SCUBA down to 20 m depth in 2011. Before removing specimens from substrate, they were photographed in situ. At the Caribbean Research and Management of Biodiversity (CARMABI) Laboratory, specimens were fixed in 96%



**Figure 1.** Study area. A, Curaçao in the Caribbean Sea. B, sampled localities in Curaçao.

ethanol. All the samples are preserved in 96% ethanol and they are deposited in the Porifera Collection of the Biology Institute of the Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil.

#### MORPHOLOGICAL ANALYSES

The external morphology was examined through the observation of macroscopic characters on the preserved specimens and complemented with information from the in situ photographs and field notes. The anatomy was assessed through the analysis of the skeletal composition obtained from microscopy slides.

The preparation of microscopic slides of anatomical sections and dissociated spicules, as well as the spicule measurements followed standard procedures (Wörheide & Hooper, 1999; Klautau & Valentine, 2003). Spicule measurements of the species used for comparative purposes were obtained from the original descriptions or, otherwise, from more detailed recent descriptions of the type material. All spicule measurements are presented in tabular form, featuring length and width [minimum (min), mean, standard deviation (SD) and maximum (max)].

To illustrate the species descriptions, skeleton and spicule photographs were taken with a digital Canon camera coupled to a Zeiss Axioscop microscope. Scanning electron microscopy (SEM) micrographs of selected spicules with ornamentations were undertaken at the Biology Institute of the UFRJ on a JSM-6510 SEM equipment. The spicule preparations for SEM images followed Azevedo *et al.* (2015).

Identifications followed the Systema Porifera (Hooper & Van Soest, 2002) and additional appropriate literature (Klautau & Valentine, 2003; Klautau *et al.*, 2013; Azevedo *et al.*, 2017).

#### MOLECULAR ANALYSES

Total genomic DNA was extracted using the guanidine/phenol-chloroform protocol (Sambrook, Fritsch & Maniatis, 1989) or with a QIAamp DNA MiniKit (Qiagen) and stored at  $-20^{\circ}\text{C}$  until amplification. Two ribosomal DNA regions were amplified: the region containing the partial genes 18S and 28S, the spacers ITS1 and ITS2 and the 5.8S rDNA (named herein as ITS) and the C-region of 28S (named herein as C-LSU). ITS was amplified with the primers: fwd: 5'-TCATTTAGAGGAAG TAAAAGTCG-3' and rv: 5'-GTTAGTTTCTTTT CCTCCGCTT-3' (Lôbo-Hajdu *et al.*, 2004) for Calcinean species and C-LSU was amplified using the primers fwd: 5'-GAAAAGCACTTTGAAAAGAGA-3' (Voigt & Wörheide, 2016) and rv: 5'-TCCGTGTTTCAAGACGGG-3' (Chombard, Boury-Esnault & Tillier, 1998) for Calcinean and Calcaronean species.

The PCR mixture included 1× buffer (5× GoTaq Green Reaction Buffer Flexi, PROMEGA), 0.2 mM dNTP, 2.5 mM  $\text{MgCl}_2$ , 0.5  $\mu\text{g}/\mu\text{L}$  bovine serum albumin (BSA), 0.33  $\mu\text{M}$  of each primer, one unit of Taq DNA polymerase (Fermentas or PROMEGA) and 1  $\mu\text{L}$  of DNA in a final volume of 15  $\mu\text{L}$ . The PCR amplification comprised one first cycle of 4 min at  $94^{\circ}\text{C}$ , 35 cycles of 1 min at  $92^{\circ}\text{C}$ , 1 min at  $48^{\circ}\text{C}$  or  $50^{\circ}\text{C}$  and 1 min at  $72^{\circ}\text{C}$ , and a final cycle of 6 min at  $72^{\circ}\text{C}$ . Forward and reverse strands were automatically sequenced in an ABI 3500 (Applied Biosystems) at the Biology Institute of the Universidade Federal do Rio de Janeiro (UFRJ).

Sequences used in recent phylogenies (Klautau *et al.*, 2013, 2016; Azevedo *et al.*, 2015, 2017; Voigt & Wörheide, 2016; Voigt *et al.*, 2017) were retrieved from the GenBank database and are listed in Table 1 as well as those generated in this study. Sequences were aligned through the MAFFT v.7 online platform (Katoh

**Table 1.** Names, localities, voucher numbers and GenBank accession numbers of the specimens used in this study

Species	Locality	Voucher number	GenBank accession number	
			C-LSU	ITS
<b>CALCINEA</b>				
<i>Ascandra corallicola</i>	Norway	UFRJPOR 6329	HQ589012	HQ588994
<i>Borojevia</i> aff. <i>aspina</i>	Red Sea	SMF 11637	KY366400	KY366409
<i>Borojevia brasiliensis</i>	Brazil	UFRJPOR 5214	HQ589015	HQ588978
<i>Borojevia cerebrum</i>	Mediterranean Sea	UFRJPOR 6322	HQ589008	HQ588964
<i>Borojevia croatica</i>	Adriatic Sea	IRB-CLB6	KP740002	KP740023
<i>Borojevia</i> sp.	Pitcairn Islands	QMG 313824	JQ272287	-
<i>Borojevia tenuispinata</i>	Brazil	UFRJPOR 6484 (H)	-	KX548916
<i>Borojevia tenuispinata</i>	Brazil	UFRJPOR 6492	-	KX548917
<i>Borojevia tenuispinata</i> *	Curaçao	UFRJPOR 6700	MF472611*	MF472608*
<i>Borojevia trispinata</i>	Brazil	UFRJPOR 6487 (P)	-	KX548919
<i>Borojevia trispinata</i>	Brazil	UFRJPOR 5495	HQ589017	-
<i>Clathrina antofagastensis</i>	Chile	MNRJ 9289	HQ588985	HQ589003
<i>Clathrina aphrodita</i>	Peru	MNRJ 12994	-	KC985138
<i>Clathrina aurea</i>	Brazil	MNRJ 8998 (H)	HQ589005	HQ588968
<i>Clathrina blanca</i>	Adriatic Sea	PMR-14307	KC479085	KC479087
<i>Clathrina clathrus</i>	Mediterranean Sea	UFRJPOR 6315	HQ589009	HQ588974
<i>Clathrina conifera</i>	Brazil	UFRJPOR 8991	HQ589010	HQ588959
<i>Clathrina coriacea</i>	Norway	UFRJPOR 6330	HQ589001	HQ588986
<b><i>Clathrina curacaoensis</i> sp. nov.*</b>	Curaçao	UFRJPOR 6734 (H)	MF472612*	MF472607*
<i>Clathrina cylindractina</i>	Brazil	UFRJPOR 5206	HQ589007	HQ588979
<i>Clathrina fjordica</i>	Chile	MNRJ 8143	-	HQ588984
<i>Clathrina fjordica</i>	Chile	MNRJ 9964	HQ589016	-
<i>Clathrina insularis</i>	Brazil	UFRJPOR 6527	-	KX548920
<i>Clathrina insularis</i> *	Brazil	UFRJPOR 6530	-	MF803979*
<i>Clathrina insularis</i>	Brazil	UFRJPOR 6532 (H)	-	KX548921
<i>Clathrina insularis</i> *	Brazil	UFRJPOR 6533	-	MF803980*
<i>Clathrina insularis</i>	Brazil	UFRJPOR 6536	-	KX548922
<i>Clathrina insularis</i> *	Brazil	UFRJPOR 6537	-	MF803981*
<i>Clathrina insularis</i> *	Curaçao	UFRJPOR 6737	MF472614*	KC843435
<i>Clathrina lacunosa</i>	Norway	UFRJPOR 6334	HQ589020	HQ588991
<i>Clathrina lutea</i>	Brazil	UFRJPOR 5172	HQ589004	HQ588961
<i>Clathrina lutea</i>	Brazil	UFRJPOR 5173	-	HQ588976
<i>Clathrina lutea</i>	Brazil	UFRJPOR 6543	-	KX548923
<i>Clathrina lutea</i>	Brazil	UFRJPOR 6545	-	KC843442
<i>Clathrina lutea</i>	Curaçao	UFRJPOR 6761	-	KC843445
<i>Clathrina lutea</i>	Virgin Islands	ZMAPOR 08344	-	KC843444
<i>Clathrina lutea</i>	Florida, USA	UFRJPOR 5818	-	KC843443
<i>Clathrina luteoculcitella</i>	Great Barrier Reef	QMG 313684	JQ272283	HQ588989
<i>Clathrina mutabilis</i>	Brazil	UFRJPOR 6526 (H)	-	KX548925
<i>Clathrina mutabilis</i>	Brazil	UFRJPOR 6528 (P)	-	KX548926
<i>Clathrina mutabilis</i> *	Curaçao	UFRJPOR 6704	-	MF472600*
<i>Clathrina mutabilis</i> *	Curaçao	UFRJPOR 6719	-	MF472601*
<i>Clathrina mutabilis</i>	Curaçao	UFRJPOR 6733	-	KC843436
<i>Clathrina mutabilis</i> *	Curaçao	UFRJPOR 6735	-	MF472602*
<i>Clathrina mutabilis</i> *	Curaçao	UFRJPOR 6740	-	MF472603*
<i>Clathrina mutabilis</i> *	Curaçao	UFRJPOR 6741	MF472613*	KC843437
<i>Clathrina mutabilis</i> *	Curaçao	UFRJPOR 6743	-	MF472604*
<i>Clathrina mutabilis</i> *	Curaçao	UFRJPOR 6744	-	MF472605*
<i>Clathrina mutabilis</i> *	Curaçao	UFRJPOR 6747	-	MF472606*



Table 1. Continued

Species	Locality	Voucher number	GenBank accession number	
			C-LSU	ITS
<i>Clathrina nuroensis</i>	Peru	MNRJ 13032 (H)	-	KC985136
<i>Clathrina peruana</i>	Peru	MNRJ 12839 (H)	-	KC985135
<i>Clathrina primordialis</i>	Adriatic Sea	IRB-CLB3 = UFRJPOR 6863	KP739996	KP740016
<i>Clathrina ramosa</i>	Chile	MNRJ 10313 (P)	HQ589002	HQ588990
<i>Clathrina rotundata</i>	Red Sea	SMF 11636 (H)	KY366399	KY711435
<i>Clathrina rowi</i>	Red Sea	SMF 11632 (H)	KY366394	KY366401
<i>Clathrina rubra</i>	Adriatic	PMR-14306	KC479082	KC479088
<i>Clathrina sinusarabica</i>	Red Sea	GW3143	KY701522	-
<i>Clathrina sinusarabica</i>	Red Sea	SMF 11631	-	KY366407
<i>Clathrina wistariensis</i>	Australia	QMG 313663 (H)	JQ272303	HQ588987
<i>Ernstia tetractina</i>	Brazil	UFRJPOR 5183	HQ589021	HQ589000
<i>Leucetta antarctica</i>	Antarctic	MNRJ 13798	-	KC849700
<i>Leucetta chagosensis</i>	French Polynesia	BMOO 16210	-	KC843455
<i>Leucetta floridana</i>	Panama	PTL09-P100	-	KC843456
<i>Leucetta floridana</i>	Panama	P10X2	KC869538	-
<i>Leucetta floridana*</i>	Curaçao	UFRJPOR 6726	-	MF472609*
<i>Leucetta floridana*</i>	Curaçao	UFRJPOR 6765	-	MF472610*
<i>Leucetta floridana*</i>	Brazil	UFRJPOR 6480	-	MF803982*
<i>Leucetta microraphis</i>	Australia	QMG 313659	-	AJ633874
<i>Leucetta potiguar</i>	Brazil	UFPEPOR 569	-	EU781987
<i>Leucetta pyriformis</i>	Antarctic	MNRJ 13843	-	KC843457
CALCARONEA				
<i>Anamixilla torresi</i>	?	?	AY563636	-
<i>Aphroceras</i> sp.	Tasmania	SAM-PS0349	JQ272273	-
<i>Eilhardia schulzei</i>	Great Barrier Reef	QMG 316071	JQ272256	-
<b><i>Grantessa tumida</i> sp. nov.*</b>	Curaçao	UFRJPOR 6701 (H)	MF472616*	-
<b><i>Grantessa tumida</i> sp. nov.*</b>	Curaçao	UFRJPOR 6695 (P)	MF472617*	-
<i>Grantessa</i> aff. <i>intusarticulata</i>	Great Barrier Reef	GW979	JQ272278	-
<i>Grantia compressa</i>	?	?	AY563538	-
<i>Grantiopsis heroni</i>	Great Barrier Reef	QMG 313670 (H)	JQ272261	-
<i>Grantiopsis cylindrica</i>	Great Barrier Reef	GW973	JQ272263	-
<i>Leucandra aspera</i>	?	?	AY563535	-
<i>Leucandra falakra</i>	Adriatic Sea	UFRJPOR 8349 (H)	KT447560	-
<i>Leucandra nicolae</i>	Great Barrier Reef	QMG 313672 (H)	JQ272268	-
<i>Leucandra</i> sp.	Coral Sea	QMG 316285	JQ272265	-
<i>Leucandra spinifera</i>	Adriatic Sea	IRB-SG3 = UFRJPOR 8348 (H)	KT447561	-
<b><i>Leucandrilla quadriradiata</i> sp. nov.*</b>	Curaçao	UFRJPOR 6696 (P)	MF472619*	-
<b><i>Leucandrilla quadriradiata</i> sp. nov.*</b>	Curaçao	UFRJPOR 6705 (H)	MF472618*	-
<i>Leucascandra caveolata</i>	Great Barrier Reef	QMG 316057	JQ272259	-
<b><i>Leucilla micropilosa</i> sp. nov.*</b>	Curaçao	UFRJPOR 6755	MF472621*	-
<b><i>Leucilla antillana</i> sp. nov.*</b>	Curaçao	UFRJPOR 6768	MF472615*	-
<i>Leuconia nivea</i>	?	?	AY563534	-
<i>Leucosolenia</i> sp.	?	?	AY026372	-
<i>Paraleucilla dalmatica</i>	Adriatic Sea	UFRJPOR 8346 (H)	KT447566	-
<i>Paraleucilla magna</i>	Brazil	GW824	JQ272267	-
<i>Plectroninia novaecaledoniense</i>	Coral Sea	QMG 316300	AM181011	-
<i>Petrobiona massiliana</i>	Mediterranean Sea	GW1729	JQ272307	-

Table 1. Continued

Species	Locality	Voucher number	GenBank accession number	
			C-LSU	ITS
<i>Sycettusa</i> aff. <i>hastifera</i>	Red Sea	GW893	JQ272267	-
<i>Sycettusa</i> cf. <i>simplex</i>	Western Indian Ocean	ZMAPOR11566	JQ272279	-
<i>Sycettusa</i> sp.	?	?	AY563530	-
<i>Sycettusa tenuis</i>	Great Barrier Reef	QMG 313685	JQ272281	-
<i>Sycon ancora</i>	Adriatic Sea	UFRJPOR 8347 (P)	KT447568	-
<i>Sycon capricorn</i>	Great Barrier Reef	QMG 316187	JQ272272	-
<i>Sycon carteri</i>	Australia	SAM-PS0143	JQ272260	-
<i>Sycon ciliatum</i>	?	?	AY563532	-
<b><i>Sycon conulosum</i> sp. nov.*</b>	Curaçao	UFRJPOR 6707 (H)	MF472620*	-
<i>Sycon</i> cf. <i>villosum</i>	?	GW51115	KR052809	-
<b><i>Sycon magnapicale</i> sp. nov.*</b>	Curaçao	UFRJPOR 6748 (H)	MF472622*	-
<b><i>Sycon magnapicale</i> sp. nov.*</b>	Curaçao	UFRJPOR 6763 (P)	MF472623*	-
<i>Sycon raphanus</i>	?	?	AY563537	-
<i>Syconessa panicula</i>	Great Barrier Reef	QMG 313671 (H)	AM181007	-
<i>Synute pulchella</i>	West Australia	WAMZ 1404	JQ272274	-
<i>Teichonopsis labyrinthica</i>	South Australia	SAM-PS0228	JQ272264	-
<i>Ute</i> aff. <i>syconoides</i>	Tasmania	QMG 323233	JQ272269	-
<i>Ute</i> aff. <i>syconoides</i>	Great Barrier Reef	QMG 313694	JQ272271	-
<i>Ute ampullacea</i>	Great Barrier Reef	QMG 313669 (H)	JQ272266	-
<i>Vosmaeropsis</i> sp.	?	?	AY026372	-

H, holotype; P, paratype. (?) Information not provided. (\*) Sequences generated in the present study.

& Standley, 2013) using the strategy Q-INS-i (Kato & Toh, 2008). The length of the alignment (including gaps) of the ITS sequences was 1129 bp and that of the C-LSU sequences was 402 and 455 bp for *Calcinea* and *Calcaronea*, respectively. The nucleotide substitution model that best fit each alignment was indicated by the Bayesian Information Criterion in MEGA 6 (Nei & Kumar, 2000; Tamura *et al.*, 2013): GTR+G+I for both *Calcinea* ITS and *Calcaronea* C-LSU, and GTR+G for *Calcinea* C-LSU.

Phylogenetic reconstructions were performed under Maximum likelihood (ML) and Bayesian inference (BI) approaches, considering three sets of sequences: ITS *Calcinea*, C-LSU *Calcinea* and C-LSU *Calcaronea*. The ML analyses were conducted on MEGA 6 using an initial NJ tree (BIONJ) and a 1000 pseudo-replicates bootstrap. The BI reconstructions were obtained with MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) under  $10^6$  generations and a burn-in of 25% of the sampled trees, yielding a consensus tree of majority. The ITS phylogenetic tree was midpoint-rooted, whereas the C-LSU trees were rooted using species of the other subclass. In order to estimate the genetic intraspecific and interspecific variability, we calculated the uncorrected *p* distance in MEGA 6.

#### INSTITUTIONAL ABBREVIATIONS AND ACRONYMS

BMNH = The Natural History Museum, London, UK; GW = Gert Wörheide; IRB = Institut Ruđer Bošković, Zagreb, Croatia; MNRJ = Museu Nacional da Universidade Federal do Rio de Janeiro, Brazil; PMJ = Phyletisches Museum Jena, Germany; PMR = Prirodoslovni Muzej Rijeka, Croatia; QM = Queensland Museum, Australia; SAM = South Australian Museum, Australia; SMF = Senckenberg Museum, Frankfurt, Germany; UFRJPOR = Porifera collection of the Biology Institute of the Universidade Federal do Rio de Janeiro, Brazil; WAMZ = Zoological collection of the Western Australian Museum, Perth, Australia; ZMAPOR = Zoologisch Museum, Instituut voor Systematiek en Populatiebiologie, Amsterdam, The Netherlands.

#### RESULTS

Integrating morphological examination with molecular analyses, we identified 16 species among the 42 specimens analysed, including species of subclasses *Calcinea* and *Calcaronea*. A total of 25 sequences are provided herein: 16 sequences (including ITS and C-LSU) for five *Calcinea* species, and nine C-LSU sequences for six *Calcaronea* species. Both phylogenetic reconstruction

methods (ML and BI) yielded trees with similar topologies for both subclasses. The results of the molecular analysis are embedded within each species description.

#### DESCRIPTION OF TAXA

PHYLUM PORIFERA GRANT, 1836  
 CLASS CALCAREA BOWERBANK, 1862  
 SUBCLASS CALCINEA BIDDER, 1898  
 ORDER CLATHRINIDA HARTMAN, 1958  
 FAMILY CLATHRINIDAE MINCHIN, 1990  
 GENUS *ARTURIA* AZEVEDO, PADUA, MORAES, ROSSI,  
 MURICY & KLAUTAU, 2017

*Type species:* *Clathrina hirsuta* Klautau & Valentine, 2003.

*Diagnosis:* ‘Calcinea whose cormus comprises a typical clathroid body. A stalk may be present. The skeleton contains regular (equiangular and equiradiate) triactines and tetractines. However, tetractines are more rare. Diactines may be added. Asconoid aquiferous system’ (Klautau *et al.*, 2013).

#### *ARTURIA VANSOESTI* SP. NOV.

(FIG. 2; TABLE 2)

*Etymology:* Named after Rob Van Soest in recognition of his dedicated work on the taxonomy of sponges, including those from Curaçao.

*Type locality:* Daai Booi, St. Willibrordus, Curaçao.

*Material examined:* *Holotype.* UFRJPOR 6720, Daai Booi, St. Willibrordus, Curaçao (12°12'43.12"N, 69°05'8.42"W) 5.2 m depth, coll. B. Córdor-Luján, 19 August 2011. *Paratype.* UFRJPOR 6731, Sunset Waters, Soto (12°16'01.58"N, 69°07'44.85"W), 3–10 m depth, coll. B. Córdor-Luján, 20 August 2011.

*Diagnosis:* *Arturia* with cormus composed of loosely anastomosed tubes and water-collecting tubes. The skeleton is mainly composed of spicules with cylindrical and distally undulated actines with rounded tips. Yellow in life.

*Colour:* Yellow in life (Fig. 2A) and beige in ethanol (Fig. 2B).

*Morphology and anatomy:* This species has a massive smooth cormus composed of irregular and loosely anastomosed tubes. The holotype measures 0.7 × 0.6 × 0.2 cm (Fig. 2A, B). A water-collecting tube (2 × 1 mm) was present in the holotype (arrow in Fig. 2A). The aquiferous system is asconoid. No granular cells were observed.

*Skeleton:* The skeleton has no special organization and is composed of abundant triactines and rare tetractines (Fig. 2C).

*Spicules:* *Triactines.* Regular (equiangular and equiradiate). Abundant. Actines are cylindrical,

undulated at the distal part and with rounded tips (Fig. 2D). Size: 72.5–90.0/3.8–5.0 µm. *Tetractines.* Regular (equiangular and equiradiate). Rare. Basal actines are cylindrical, distally undulated and with rounded to blunt tips (Fig. 2E). The apical actine is the shortest actine (Fig. 2F). It is straight and smooth; however, some curved actines were also found. It has sharp or blunt tips. Size: 60.0–90.0/3.8–5.0 µm (basal actine) and 25.0/3.8–5.0 µm (apical actine).

*Ecology:* This sponge was found in a cryptic habitat, underneath coral boulders, and down to 10 m depth. No associated organisms were found.

*Geographical distribution:* Southern Caribbean ecoregion (provisionally endemic to Curaçao, present study).

*Taxonomic remarks:* The genus *Arturia* now comprises 12 valid species: *A. adusta* (Wörheide & Hooper, 1999), *A. africana* (Klautau & Valentine, 2003), *A. alcatraziensis* (Lanna, Rossi, Cavalcanti, Hajdu & Klautau, 2007), *A. canariensis* (Miklucho-Maclay, 1868), *A. darwinii* (Haeckel, 1870), *A. dubia* (Dendy, 1891), *A. hirsuta* (Klautau & Valentine, 2003), *A. spirallata* Azevedo, Córdor-Luján, Willenz, Hajdu, Hooker & Klautau, 2015, *A. tenuipilosa* (Dendy, 1905), *A. trinidadensis* Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017, *A. tubuloreticulosa* Van Soest & De Voogd, 2015 and *A. vansoesti* sp. nov. The species most resembling *A. vansoesti* sp. nov. in skeletal composition are *A. canariensis* from Canary Islands and *A. tubuloreticulata* from Indonesia, as the skeletons of these three species are mainly composed of triactines with cylindrical actines. Nonetheless, they have important differences.

The colour in vivo of *A. canariensis* is white, whereas in *A. vansoesti* sp. nov., it is yellow. Although spicule dimensions of these two species are very similar (Table 2), the spicules of *A. canariensis* are less cylindrical and less undulated than those of *A. vansoesti* sp. nov. In *A. vansoesti* sp. nov., the spicules bear rounded tips, while those in *A. canariensis* are blunt.

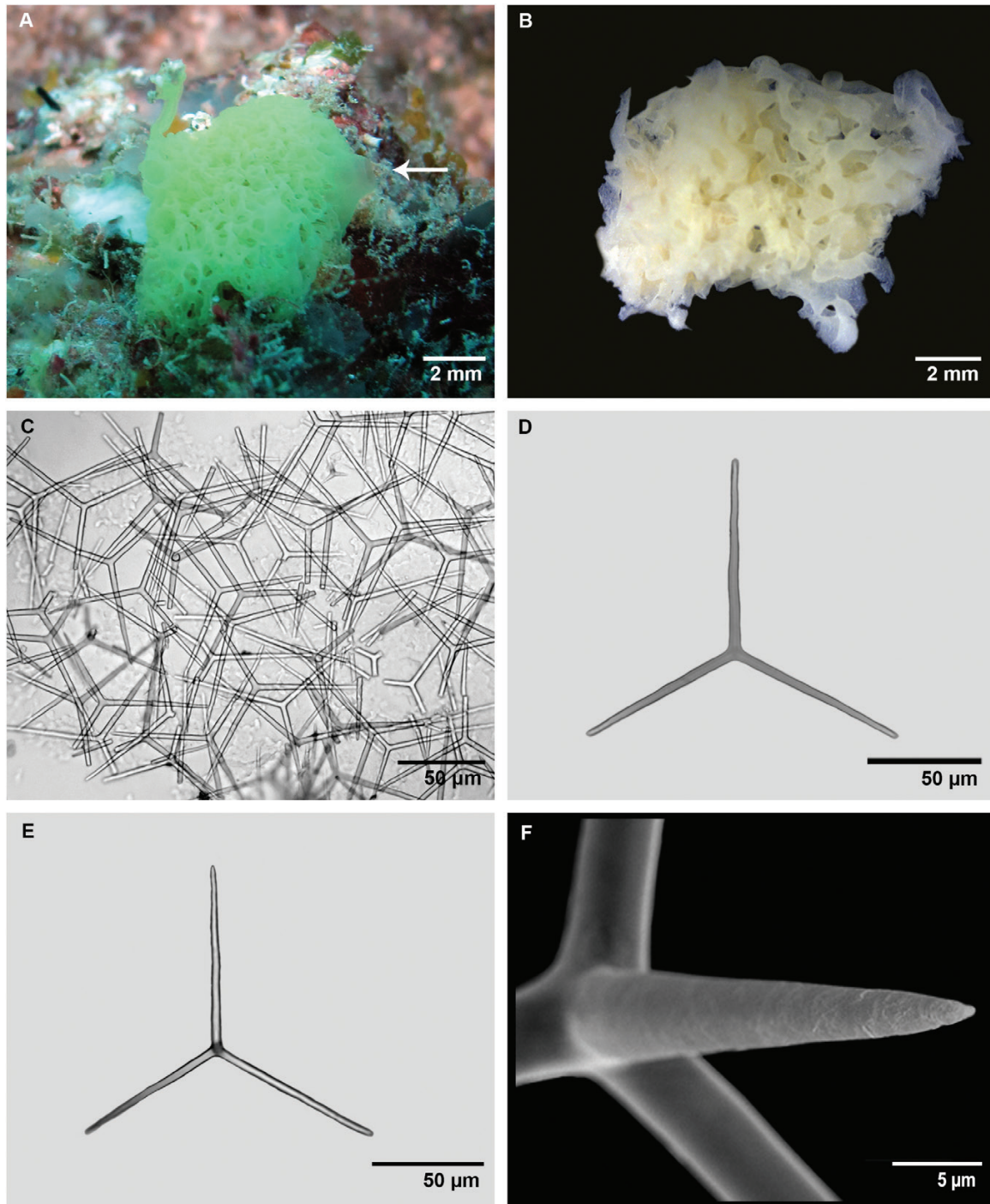
*Arturia tubuloreticulosa* is orange in life and its cormus has several oscula, whereas the yellow *A. vansoesti* sp. nov. has water-collecting tubes. Furthermore, the spicules of the Curaçaoan species have rounded tips, while in the Indonesian species, they are sharp or blunt.

*Arturia vansoesti* sp. nov. is the second species in the genus to be described from the Caribbean Sea, as Pérez *et al.* (2017) recently listed *A. hirsuta* for the Eastern Caribbean (La Martinique).

GENUS *BOROJEVIA* KLAUTAU, AZEVEDO,  
 CÓNDOR-LUJÁN, RAPP, COLLINS & RUSSO, 2013

*Type species:* *Asclatis cerebrum* Haeckel, 1872.





**Figure 2.** *Arturia vansoesti* sp. nov. (UFRJPOR 6720). A, specimen in vivo (arrow: water-collecting tube). B, specimen after fixation. C, tangential section of the skeleton. D, triactine. E, tetractine. F, apical actine of a tetractine.

**Diagnosis:** Calcinea in which the cormus comprises tightly anastomosed tubes. The skeleton contains regular (equiangular and equiradiate) triactines, tetractines, tripods and tetrapods. The apical actine of the tetractines has spines. Aquiferous system asconoid (Klautau *et al.*, 2013, emend.).

*BOROJEVIA TENUISPINATA* AZEVEDO, PADUA, MORAES, ROSSI, MURICY & KLAUTAU, 2017 (FIG. 3; TABLE 3)

**Synonymy:** *Borojevia tenuispinata* Azevedo *et al.*, 2017: 311–313.



**Table 2.** Spicule measurements of *Arturia vansoesti* sp. nov. (holotype = UFRJPOR 6720 and paratype = UFRJPOR 6731), *A. canariensis* (holotype = PMJ-Inv. Nr.Porif.103) and *A. tubuloreticulosa* (holotype = RMNH Por.5547)

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	Mean	SD	Max	Min	Mean	SD	Max	
UFRJPOR 6720	Triactine		72.5	79.8	4.9	87.5	3.8	4.6	0.6	5.0	30
	Tetractine	Basal	60.0	75.3	8.1	87.5	3.8	4.8	0.5	5.0	15
UFRJPOR 6731		Apical	25.0	25.0	0.0	25.0	3.8	4.4	0.7	5.0	6
	Triactine		77.5	81.8	3.7	90.0	3.8	4.9	0.3	5.0	14
PMJ-Inv. Nr.Porif.103*	Tetractine	Basal	65.0	81.7	6.9	90.0	3.8	4.9	0.3	5.0	17
	Triactine	Basal	62.5	74.3	5.8	87.5	-	5.0	0.0	-	30
RMNH Por.5547	Tetractine	Apical	35.0	43.8	5.6	55.0	-	5.0	0.0	-	15
	Triactine		63.0	112.1	-	138.0	4.0	5.3	-	6.5	-
	Tetractine	Basal	62.0	119.5	-	156.0	4.0	5.4	-	6.0	-
		Apical	39.0	-	-	132.0	3.5	-	-	6.5	-

\*Taken from Klautau & Valentine (2003).

**Material examined:** UFRJPOR 6700 and UFRJPOR 6708, Daai Booi, St. Willibrordus, Curaçao (12°12'43.12"N, 69°05'8.42"W), 3–5 m depth, coll. B. Córdor-Luján, 18 August 2011.

**Comparative material examined:** Holotype of *B. tenuispinata*. UFRJPOR 6484, Cabeço da Tartaruga, São Pedro e São Paulo Archipelago, Brazil (0°54'57"N, 29°20'46"W), 8–12 m depth, coll. G. Rodríguez and F. Azevedo, 16 June 2011.

**Colour:** White in life and in ethanol (Fig. 3A, B).

**Morphology and anatomy:** The biggest specimen (UFRJPOR 6700) is fragmented and the largest piece measures 0.7 × 0.4 × 0.3 mm (Fig. 3A). The cormus is massive, rough and compressible, composed of irregular and tightly anastomosed tubes (Fig. 3B). The tubes in contact with the substrate are less tightly anastomosed than the most superficial ones. No water-collecting tubes were observed. The aquiferous system is asconoid. No granular cells were observed.

**Skeleton:** The skeleton comprises tetractines, tetrapods, triactines and tripods. Tetrapods and tripods are located only in the external tubes (Fig. 3C), whereas tetractines and triactines can be present in the whole cormus (Fig. 3D).

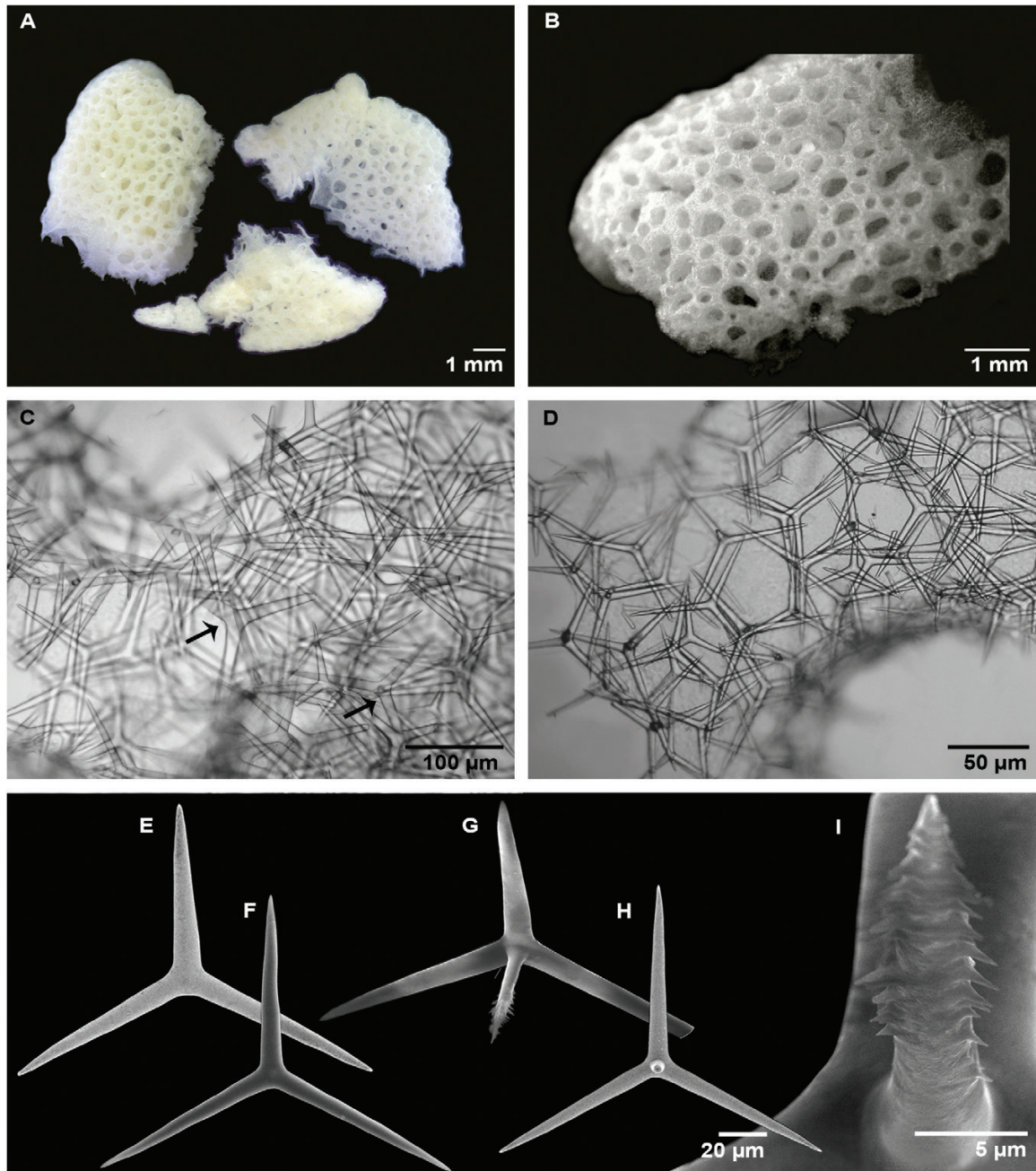
**Spicules:** *Tripods.* Regular (equiangular and equiradiate). Actines are straight, conical, with blunt tips. They do not have the raised centre of typical tripods, instead they look like stout conical triactines (Fig. 3E). Size: 65.0–162.5/10.0–22.5 µm. *Triactines.* Regular (equiangular and equiradiate). Abundant. Actines are straight, conical, with blunt tips (Fig. 3F).

Size: 62.5–87.5/6.2–8.8 µm. *Tetrapods.* Regular (equiangular and equiradiate). The basal actines are conical and stout, with blunt to sharp tips. The apical actine has spines similar to those of the tetractines. These tetrapods do not have the centre raised as common tetrapods. They have thicker actines than the tetractines and they are more conical (Fig. 3G). Size: 70.0–95.0/10.0–12.5 µm (basal actine) and 40/5 µm (apical actine). *Tetractines.* Regular (equiangular and equiradiate). The basal actines are straight, conical, with blunt tips (Fig. 3H). Few tetractines with curved paired actines were also found. The apical actine is shorter and thinner than the basal actines and has spines. The most common pattern of spine distribution observed consisted of spines arranged in rows spreading from about two-thirds of the actine up to the tip (Fig. 3I). Size: 62.5–92.5/7.5–8.8 µm (basal actine), 25.0–40.0/5.0–7.5 µm (apical actine).

**Ecology:** This species was found in a cryptic habitat, underneath boulders, down to 5 m depth. No organisms were found associated with this species.

**Geographical distribution:** São Pedro and São Paulo Islands, ecoregion of the Tropical Southwestern Atlantic (Azevedo *et al.*, 2017) and Southern Caribbean (Curaçao, this study).

**Molecular analysis:** *Borojevia* was recovered as a monophyletic clade with high support (pp = posterior probability, b = bootstrap) in the ITS (pp = 0.92, b = 72) and C-LSU (pp = 0.90, b = 86) phylogenetic trees (Figs 4 and 5, respectively). This clade included *Borojevia* aff. *aspina* (Klautau, Solé-Cava & Borojevic, 1994), *B. brasiliensis* (Solé-Cava, Boury-Esnault, Borojevic & Thorpe, 1991), *B. cerebrum* (Haeckel, 1872),



**Figure 3.** *Borojevia tenuispinata* (UFRJPOR 6700). A, fragmented specimen after fixation. B, detail of the cormus. C, tangential section of the skeleton of an external tube (left arrow: tripod; right arrow: tetrapod). D, tangential section of the cormus. E, tripod. F, triactine. G, tetrapod. H, tetractine. I, detail of the spined apical actine of the tetractine.

*B. croatica* Klautau, Imešek, Azevedo, Pleše, Nikolić & Četković, 2016, *Borojevia* sp., *B. tenuispinata* and *B. trispinata* Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017.

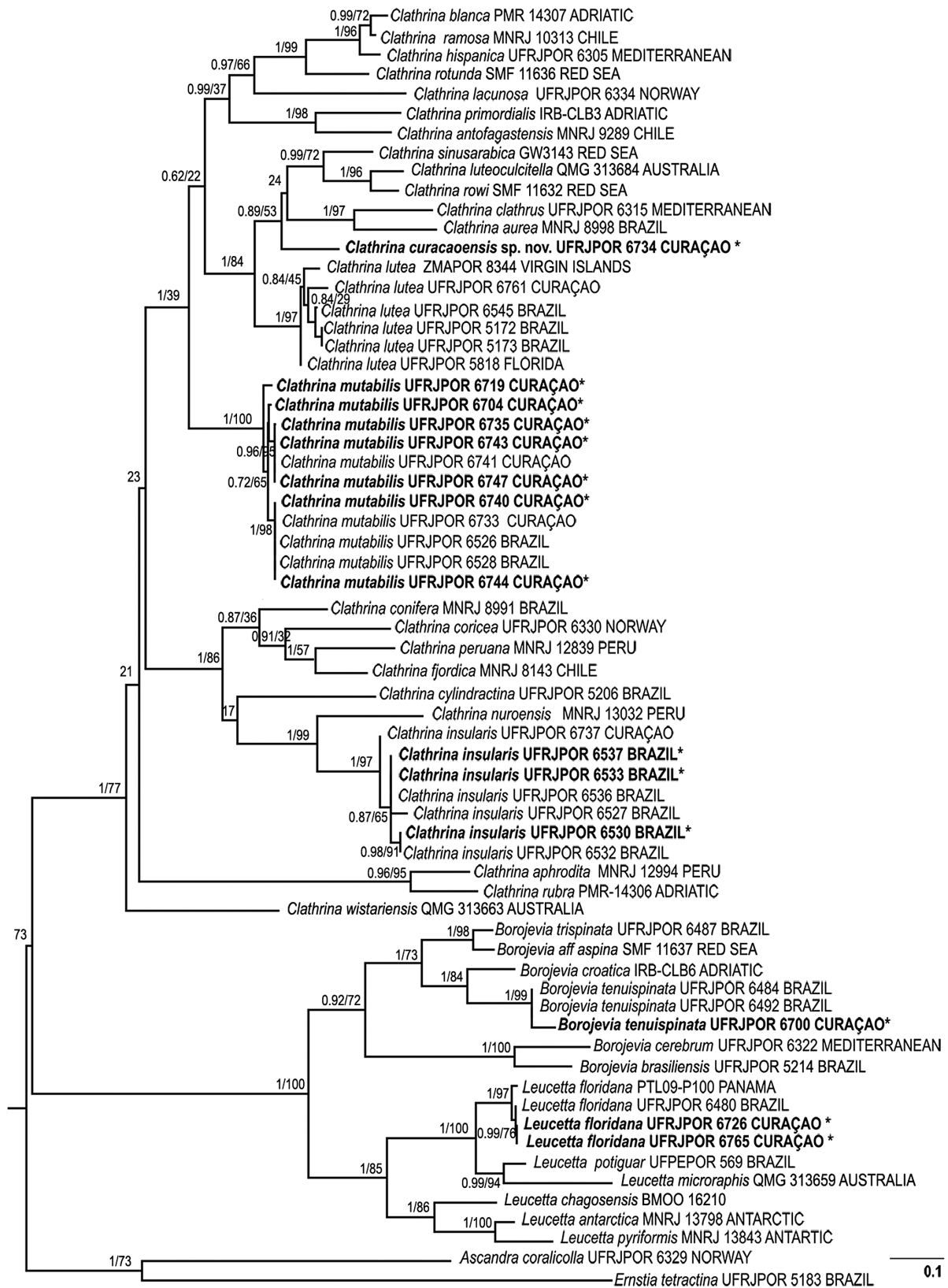
Within this genus, the ITS interspecific divergence (estimated by the uncorrected *p* distance) ranged from 1.1 (*B. aff. aspina* vs. *B. trispinata*) to 6.3% (*B. brasiliensis* vs. *B. tenuispinata*), whereas the C-LSU values

ranged from 1.0 (*B. aff. aspina* and *B. trispinata*) to 8.6% (*B. cerebrum* vs. *B. tenuispinata*).

In the ITS tree, the sequence of the specimen from Curaçao UFRJPOR 6700 clustered with the two sequences of *B. tenuispinata*, UFRJPOR 6484 (holotype) and UFRJPOR 6492 (paratype), forming a monophyletic clade with high support (*pp* = 1, *b* = 99). The genetic variation (*p* distance) among the three

**Table 3.** Spicule measurements of *Borojevia tenuispinata* from Curaçao (UFRJJPOR 6700 and UFRJJPOR 6708) and from the holotype of this species (UFRJJPOR 6484)

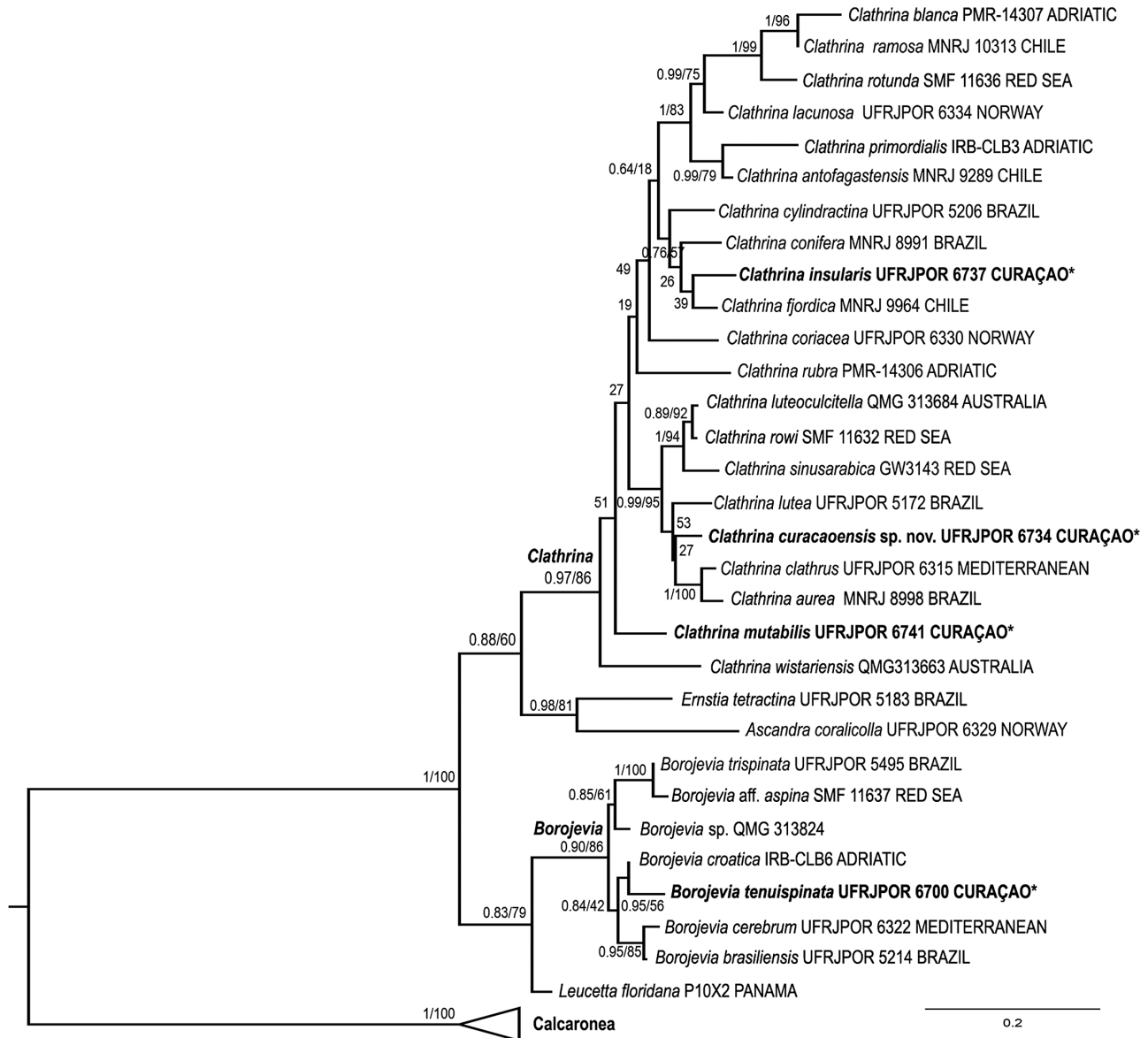
Specimen	Spicule	Actine	Length (µm)			Width (µm)			N		
			Min	Mean	SD	Max	Min	Mean		SD	Max
UFRJJPOR 6700	Triactine		62.5	75.3	7.9	87.5	6.2	7.8	0.8	8.8	23
	Tripod		75.0	103.4	17.4	162.5	10.0	12.8	2.3	22.5	38
	Tetractine	Basal	67.5	76.7	6.1	92.5	7.5	8.0	0.6	8.8	22
		Apical	25.0	33.6	4.5	40.0	5.0	7.2	0.8	7.5	9
UFRJJPOR 6708	Tetrapod	Basal	70.0	86.0	6.5	95.0	10.0	10.8	1.2	12.5	12
		Apical	-	40.0	-	-	-	5.0	-	-	1
	Triactine		62.5	71.4	6.0	82.5	7.5	8.5	0.6	8.6	9
	Tripod		65.0	91.2	14.9	125.0	10.0	12.3	1.7	15.0	48
UFRJJPOR 6484	Tetractine	Basal	62.5	70.2	6.0	77.5	7.5	7.9	0.6	8.8	12
		Apical	-	25.0	-	-	-	7.5	-	-	1
	Tetrapod	Basal	70.0	79.3	6.9	95.0	10.0	10.1	0.4	11.3	18
	Triactine	Basal	59.4	65.3	4.9	75.6	5.4	7.2	0.7	8.1	30
UFRJJPOR 6484	Tripod		56.7	80.9	11.9	102.6	8.1	10.1	1.4	12.2	30
	Tetractine	Basal	51.3	65.3	5.9	78.3	5.4	7.7	0.8	8.1	24
		Apical	27.0	40.0	8.6	56.7	4.1	4.9	0.8	6.8	20
	Tetrapod	Basal	64.8	72.9	5.9	81.0	9.5	10.4	0.7	10.8	6
	Apical	21.6	28.4	9.5	35.1	4.1	4.7	1.0	5.4	2	



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**Figure 4.** Bayesian phylogenetic tree inferred from the ITS sequences of the Calcinean species. Posterior probabilities and bootstrap values are given on the branches (pp/bootstrap). \*Sequences generated in this study.





**Figure 5.** Maximum likelihood phylogenetic tree inferred from the C-LSU sequences of the Calcinean species. Posterior probabilities and bootstrap values are given on the branches (pp/bootstrap). \*Sequences generated in this study.

specimens of *B. tenuispinata* ranged between 0 and 0.4%. The *p* distance between the holotype of *B. tenuispinata* and UFRJPOR 6700 was 0.4%.

In the C-LSU tree, the sequence of UFRJPOR 6700 appeared as a new lineage because no previous sequence of this species was obtained.

In both phylogenies, *B. tenuispinata* and *B. croatica* appeared as sister species with moderate to high support (ITS: pp = 1, b = 84 and C-LSU: pp = 0.95, b = 56). The divergence between these species was 1.9–2.4% and 3.3% in ITS and C-LSU phylogenies, respectively.

**Taxonomic remarks:** The genus *Borojevia* comprises eight species (Azevedo *et al.*, 2017). Among them, only *B. tenuispinata* from Brazil and *B. tetrapodifera* Klautau & Valentine, 2003 from New Zealand have their skeletons composed of tripods, tetrapods, triactines and tetractines. Differently from *B. tetrapodifera*, *B. tenuispinata* has tetrapods with spined apical actines.

The analysed specimens from Curaçao fit *B. tenuispinata* as their tetrapods also bear apical actines with spines. The only morphological difference observed is that tripods can attain larger sizes in the

specimens from Curaçao (65.0–162.5/10.0–22.5  $\mu\text{m}$ ) compared to the holotype (56.7–102.6/8.1–12.2  $\mu\text{m}$ ); however, this might be attributed to plasticity or to polymorphism. The molecular results reinforce the con-specificity of the Curaçaoan specimens with *B. tenuispinata*.

GENUS *CLATHRINA* GRAY, 1867

*Type species: Grantia clathrus* Schmidt, 1864.

*Diagnosis:* ‘Calcinea in which the cormus comprises anastomosed tubes. A stalk may be present. The skeleton contains regular (equiangular and equiradiate) and/or parasagittal triactines, to which diactines and tripods may be added. Asconoid aquiferous system’ (Klautau *et al.*, 2013).

*CLATHRINA CURACAOENSIS* SP. NOV.  
(FIG. 6; TABLE 4)

*Etymology:* Named after its type locality.

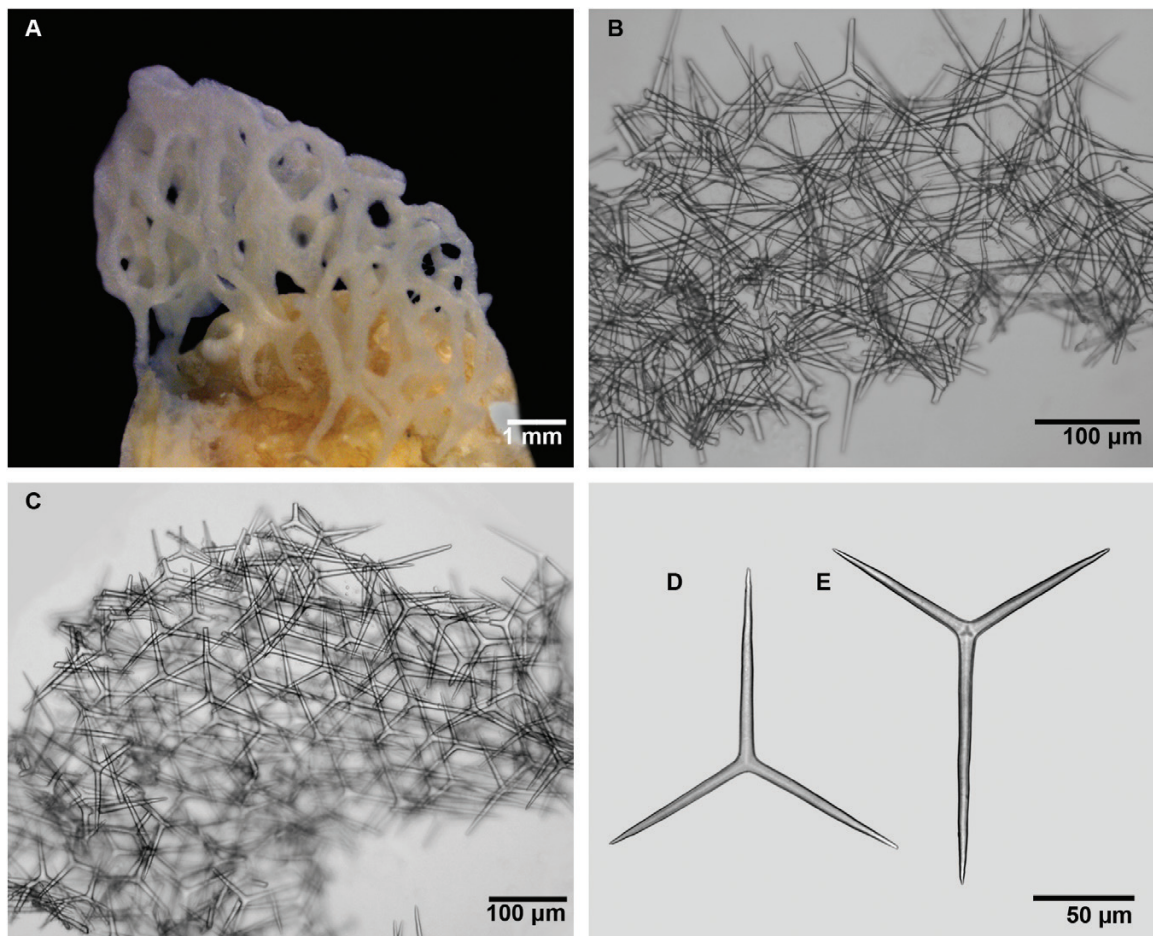
*Type locality:* Sunset Waters, Soto, Curaçao.

*Material examined:* *Holotype.* UFRJPOR 6734, Sunset Waters, Soto, Curaçao (12°16′01.58″N, 69°07′44.85″W), 3–10 m depth, coll. B. Cóndor-Luján, 20 August 2011.

*Diagnosis:* *Clathrina* with cormus formed by loosely anastomosed tubes. The skeleton is composed of regular and parasagittal triactines. Parasagittal triactines are only present in the tubes used for attachment to the substrate. Yellow in life.

*Colour:* Yellow in life and light beige in ethanol (Fig. 6A).

*Morphology and anatomy:* The analysed specimen has a smooth massive cormus (0.7 × 0.4 × 0.3 mm) composed of irregular and loosely anastomosed tubes (Fig. 6A). No water-collecting tubes were observed. The



**Figure 6.** *Clathrina curacaoensis* sp. nov. (UFRJPOR 6734). A, specimen after fixation. B, tangential section of the body (cormus). C, tangential section of an attachment tube. D, triactine I. E, triactine II.

**Table 4.** Spicule measurements of *Clathrina curacaoensis* sp. nov. (holotype = UFRJPOR 6734)

Spicule	Actine	Length (µm)				Width (µm)				N
		Min	Mean	SD	Max	Min	Mean	SD	Max	
Triactine I		87.5	102.8	13.6	130.0	7.5	8.5	1.0	10.0	20
Triactine II	Unpaired	99.9	119.9	13.1	137.7	8.1	8.2	0.3	8.8	10
	Paired	57.5	68.5	6.3	80.0	7.5	7.5	0.0	7.5	10

aquiferous system is asconoid. No granular cells were observed.

**Skeleton:** The skeleton has no special organization and it is exclusively composed of triactines (Fig. 6B). The tubes that attach the sponge to the substrate are composed of parasagittal triactines (Fig. 6C).

**Spicules:** *Triactines I.* Regular (equiangular and equiradial). Actines are slightly conical with blunt to sharp tips (Fig. 6D). Size: 87.5–130.0/7.5–10.0 µm. *Triactines II.* Parasagittal (equiangular). Actines are slightly conical with blunt to sharp tips (Fig. 6E). The unpaired actine is longer than the paired ones. Size: 57.5–80.0/7.5 µm (paired actine) and 99.9–137.7/8.1–8.8 µm (unpaired actine).

**Ecology:** The specimen was found underneath boulders and down to 10 m depth. No organisms associated with this species were observed.

**Geographical distribution:** Southern Caribbean (provisionally endemic to Curaçao, present study).

**Molecular analysis:** *Clathrina* was recovered as a monophyletic clade with high support in the ITS (pp = 1, b = 77) and C-LSU (pp = 0.90, b = 86) phylogenetic trees (Figs 4, 5). The ITS interspecific divergence ranged from 0.6 [*C. ramosa* (Azevedo, Hajdu, Willenz & Klautau, 2009) vs. *C. blanca* (Miklucho-Maclay, 1868)] to 11.2% (*C. rubra* Sarà, 1958 vs. *C. mutabilis* Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017), whereas the C-LSU values varied from 0.5 (*C. ramosa* vs. *C. blanca*) to 12.9% (*C. cylindractina* Klautau, Solé-Cava & Borojevic, 1994 vs. *C. ramosa*).

Within the large clade of *Clathrina*, *C. curacaoensis* sp. nov. appeared as a different lineage and grouped with other four yellow clathrinids (*C. aurea* Solé-Cava, Klautau, Boury-Esnault, Borojevic & Thorpe, 1991, *C. clathrus*, *C. lutea* Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017 and *C. luteoculcitella* Wörheide & Hooper, 1999) and two white clathrinids (*C. sinusarabica* Klautau & Valentine, 2003 and

*C. rowi* Voigt, Erpenbeck & Wörheide, 2017) forming a well-supported cluster (ITS: pp = 1, b = 84 and C-LSU: pp = 0.99, b = 95).

**Taxonomic remarks:** Among the seven species of the genus *Clathrina* that are yellow in vivo, namely, *C. aurea*, *C. chrysea* Borojevic & Klautau, 2000, *C. clathrus*, *C. insularis* Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017, *C. lutea* Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017, *C. luteoculcitella* and *C. mutabilis*, the latter is the species most resembling *C. curacaoensis* sp. nov. based on morphology.

*Clathrina curacaoensis* sp. nov. and *C. mutabilis* have a cormus composed of loosely anastomosed tubes and have water-collecting tubes (see below the description of *C. mutabilis*). Their skeletons bear triactines with actines of different lengths (even including parasagittal triactines). However, in *C. mutabilis* these spicules (triactines II of Azevedo *et al.*, 2017, size: 94.5–153.9/6.8–10.8 µm) occur in the whole cormus, whereas in *C. curacaoensis* sp. nov. (triactines II, size of the longest actine: 99.9–137.7/8.1–8.8 µm) they are restricted to the tubes used for attachment. Although being morphologically very similar, *C. curacaoensis* sp. nov. and *C. mutabilis* are molecularly distant. *Clathrina curacaoensis* sp. nov. clustered with six other *Clathrina* species, but not *C. mutabilis*.

Among the non-yellow clathrinids with stalk (former ‘ganchas’), *C. arnesenae* (Rapp, 2006) is most similar morphologically to *C. curacaoensis* sp. nov. Nonetheless, the skeleton of *C. arnesenae* does not comprise regular triactines as is the case of *C. curacaoensis* sp. nov.; instead, it is only composed of parasagittal triactines with cylindrical actines.

More recently, Voigt *et al.* (2017) described a white *Clathrina* from the Red Sea, *C. rotundata* Voigt, Erpenbeck & Wörheide, 2017. This species is very similar to *C. curacaoensis* sp. nov. as it also has a cormus composed of loosely anastomosed tubes, is devoid of a stalk and its skeleton is composed of parasagittal and regular triactines. However, in *C. rotundata*, the parasagittal triactines have cylindrical actines with



rounded tips and are evenly distributed in the tube wall, whereas in *C. curacaoensis* sp. nov., those spicules have slightly conical actines with blunt to sharp tips and are restricted to the attachment tubes. Regarding spicule dimensions, in *C. rotundata* parasagittal triactines are thinner (5.0–7.0 µm) and regular triactines are smaller (15.0–73.0/4.0–8.0 µm) than those in *C. curacaoensis* sp. nov. (7.5–8.8 and 87.5–130.0/7.5–10.0 µm, respectively). Besides these morphological differences, *C. rotundata* and *C. curacaoensis* sp. nov. appeared as very distant lineages in the phylogenetic tree.

***CLATHRINA GLOBULOSA* SP. NOV.**

(FIG. 7; TABLE 5)

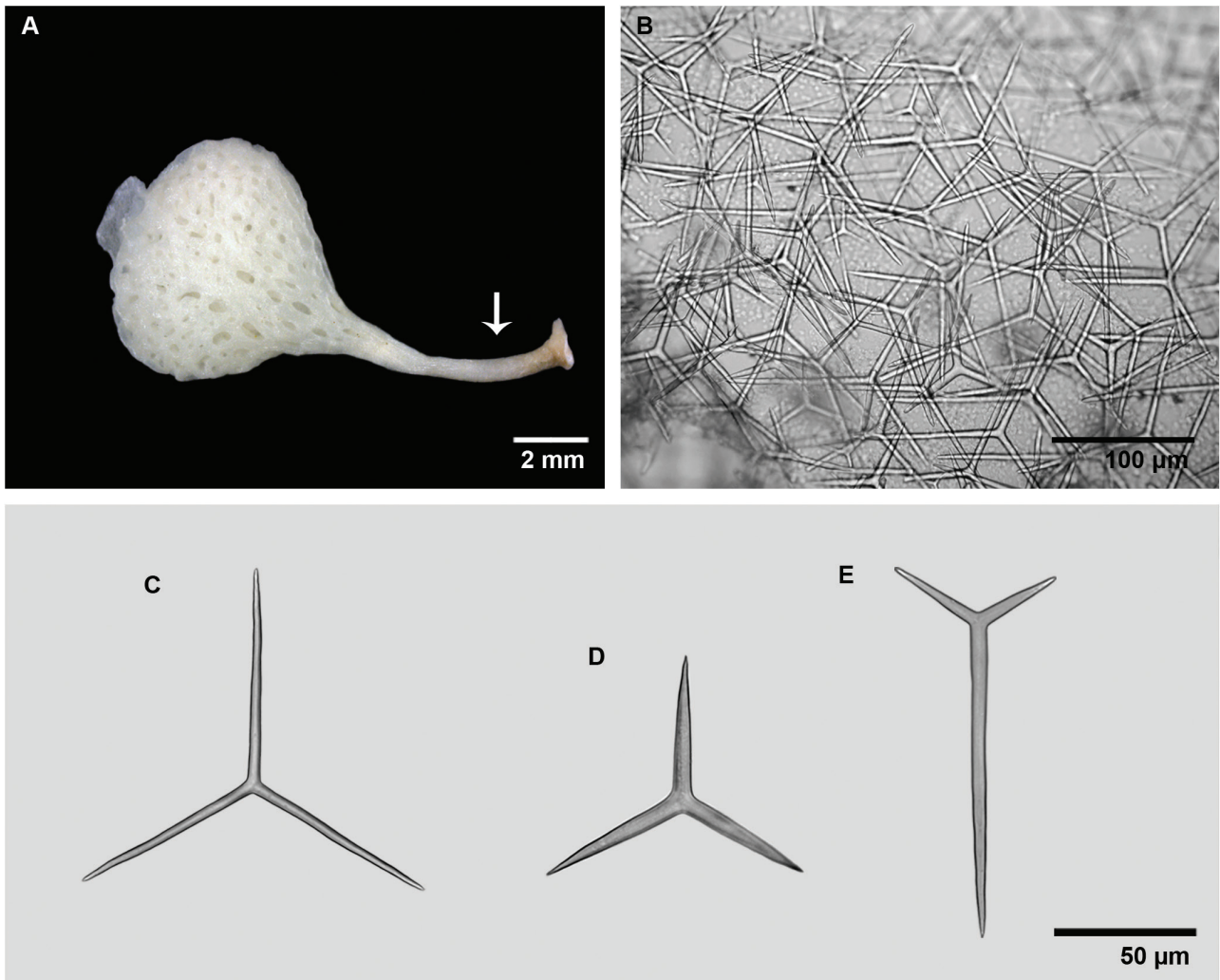
*Etymology:* From the Latin *globus* (=sphere), for its external morphology.

*Type locality:* Tug Boat, Caracasbaai, Willemstadt, Curaçao.

*Material examined:* *Holotype.* UFRJPOR 6759, Tug Boat, Caracasbaai, Willemstadt, Curaçao (12°04'08.20"N, 68°51'44.40"W) 10 m depth, coll. B. Cóndor-Luján, 23 August 2011. *Paratype.* UFRJPOR 6753, same as the holotype.

*Diagnosis:* *Clathrina* with cormus formed by a globular clathroid body with an apical water-collecting tube and a solid stalk. The skeleton of the clathroid body is composed of two size categories of triactines. The skeleton of the stalk is exclusively formed by parasagittal tractines. White in life.

*Colour:* White in life and in ethanol (Fig. 7A).



**Figure 7.** *Clathrina globulosa* sp. nov. (UFRJPOR 6759). A, specimen after fixation (arrow: stalk). B, tangential section of the clathroid body. C, triactine I. D, triactine II. E, triactine of the stalk.



**Table 5.** Spicule measurements of *Clathrina globulosa* sp. nov. (holotype = UFRJPOR 6759), *C. pellucida* (holotype = TMU-179) and *C. stipitata* (original description)

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	Mean	SD	Max	Min	Mean	SD	Max	
UFRJPOR 6759	Triactine I of the body	-	67.5	87.2	11.6	108.0	4.1	5.3	0.4	5.4	30
	Triactine II of the body	-	45.9	54.8	4.6	62.1	5.4	6.4	0.9	8.1	30
	Triactine of the stalk	Unpaired	94.5	119.8	21.6	151.2	5.4	7.9	1.8	9.4	7
TMU-179		Paired	40.5	51.8	5.7	62.1	8.1	8.1	0.0	8.1	10
	Triactine of the body	-	115.0	143.0	12.0	175.0	-	8.1	1.2	-	30
	Triactine of the stalk	Unpaired	120.0	173.0	18.0	195.0	-	8.5	1.6	-	30
<i>C. stipitata</i>		Paired	40.0	131.0	30.0	185.0	-	8.2	1.1	-	30
	Triactine	Unpaired	-	-	-	100.0	-	-	-	10.0	-
		Paired	-	70.0	-	-	-	8.5	-	-	-

**Morphology and anatomy:** This species has a globular clathroid body with an apical osculum and a stalk (Fig. 7A). The surface is smooth and the texture is soft. The consistency is compressible, even the stalk. In the holotype (UFRJPOR 6759), the clathroid body measures  $0.5 \times 0.5 \times 0.1$  cm and the stalk is  $0.5 \times 0.1 \times 0.1$  cm. The cormus is formed by irregular and tightly anastomosed tubes, which converge at the centre of the sponge forming a single apical osculum. The stalk is solid, formed by tubes without choanoderm (arrow in Fig. 7A). The aquiferous system is asconoid. No granular cells were observed.

**Skeleton:** The skeleton of the clathroid body has no special organization and is composed of two categories of regular triactines (Fig. 7B). The skeleton of the stalk is composed exclusively of parasagittal triactines, the unpaired actine of which is basipetally oriented. These spicules are more numerous and more closely placed in the median part of the stalk.

**Spicules:** *Triactines I of the clathroid body.* Regular (equiangular and equiradiate) or subregular. Frequent. Actines are cylindrical with blunt tips (Fig. 7C). Some of them are slightly undulated at the distal part. Size: 67.5–108.0/4.1–5.4 µm. *Triactines II of the clathroid body.* Regular (equiangular and equiradiate). Actines are conical and straight with sharp tips. Shorter and thicker than triactine I (Fig. 7D). Size: 45.9–62.1/5.4–8.1 µm. *Triactines of the stalk.* Parasagittal (equiangular). The paired actines are slightly conical and very short (sometimes they seem to be rudimentary). The unpaired actine is straight and cylindrical to slightly conical with sharp tips (Fig. 7E). Size: 40.5–67.5/5.4–8.1 µm (paired actine) and 94.5–199.8/5.4–8.1 µm (unpaired actine).

**Ecology:** This species was collected in a light-protected environment, underneath coral boulder, at 10 m depth. No associated organisms were found.

**Geographical distribution:** Southern Caribbean (provisionally endemic to Curaçao, present study).

**Taxonomic remarks:** Species of Clathrinidae with a clathroid body and a stalk were formerly included in *Guancha*, but recently those without tetractines were transferred to *Clathrina* (Klautau *et al.*, 2013). These species are: *C. arnesenae*, *C. blanca*, *C. camura* (Rapp, 2006), *C. challengerii* (Poléjaeff, 1883), *C. lacunosa* (Johnston, 1842), *C. macleayi* (Lendenfeld, 1885), *C. pellucida* (Rapp, 2006), *C. pulcherrima* (Dendy, 1891), *C. ramosa*, *C. sagittaria* (Haeckel, 1872) and *C. stipitata* (Dendy, 1891). Among them, *C. pellucida* from Norway and *C. stipitata* from Australia are the species resembling *C. globulosa* sp. nov. the most, as these three species have a cormus composed of tightly anastomosed tubes which converging in an apical osculum, and a skeleton composed of regular and sagittal triactines. However, they can be distinguished when additional features are considered.

*Clathrina pellucida* has a short stalk (less than 1/3 of the body) formed by true tubes with choanoderm and a skeleton exclusively composed of triactines with undulated actines, whereas *C. globulosa* sp. nov. has a solid stalk half the length of its whole body, and a skeleton which comprises triactines with straight actines.

The skeleton of the clathroid body of *C. stipitata* comprises parasagittal triactines with unpaired actines regularly arranged, pointing towards the stalk, whereas in *C. globulosa* sp. nov., it does not include parasagittal triactines, and is formed instead by regular and subregular disorganized triactines. Moreover, Dendy (1891) divides these spicules into dermal and deeper spicules. Dermal triactines are slightly curved with the centre uplifted, resembling a tripod, and deeper spicules are more nearly regular and more slender than the dermal ones. In *C. globulosa* sp. nov., spicules do not have this external and internal spicule distribution.

In neither of the above referred species, *C. pellucida* and *C. stipitata*, were spicules with comparable dimensions to those observed in the clathroid body of *C. globulosa* sp. nov. mentioned or illustrated in the original descriptions. Triactines from *C. pellucida* (115.0–175.0/8.1 ± 1.2 µm) and *C. stipitata* (unpaired actine: 100.0/10.0 µm and paired actine: 70.0/8.5 µm) are larger compared to *C. globulosa* sp. nov., even considering its two spicule categories (triactines I: 67.5–108.0/4.1–5.4 µm and triactines II: 45.9–62.1/5.4–8.1 µm).

*CLATHRINA HONDURENSIS* KLAUTAU & VALENTINE, 2003  
(FIG. 8; TABLE 6)

*Synonymy:* *Clathrina hondurensis* Klautau & Valentine, 2003: 46.

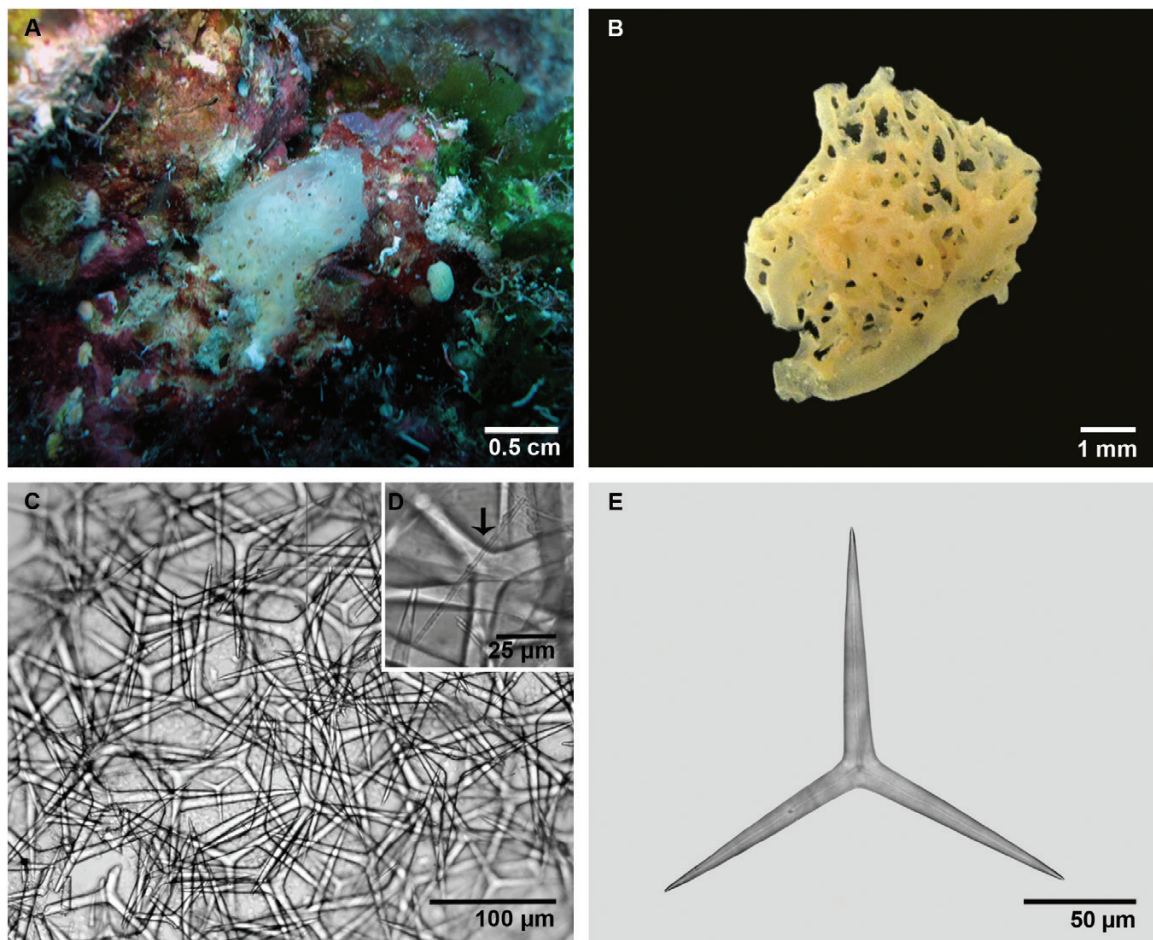
*Material examined:* UFRJPOR 6732, Porto Mari, St. Willibrordus, Curaçao (12°13'6.62"N, 69°05'13.26"W), 7.9 m depth, coll. B. Cóndor-Luján, 20 August 2011.

*Comparative material examined:* Holotype of *C. hondurensis*. BMNH 1938.3.28.4, Turneffe, Belize, Caribbean Sea, coll. J. H. Borley, 20–22 March 1935.

*Colour:* White in life (Fig. 8A) and yellow to light brown in ethanol (Fig. 8B).

*Morphology and anatomy:* The specimen is massive and it measures 1.4 × 1.0 × 0.2 cm (Fig. 8A). The surface is smooth and the consistency is compressible. The cormus is composed of irregular and tightly anastomosed tubes (Fig. 8B). No water-collecting tubes were observed. The aquiferous system is asconoid. No granular cells were observed.

*Skeleton:* The skeleton has no special organization (Fig. 8C) and is composed of triactines and rare trichoxeas (Fig. 8D, arrow).



**Figure 8.** *Clathrina hondurensis* (UFRJPOR 6732). A, specimen in vivo. B, specimen after fixation. C, tangential section of the body (cormus) skeleton. D, detail of trichoxea indicated by an arrow. E, triactine.

**Table 6.** Spicule measurements of *Clathrina hondurensis* from Curaçao (UFRJPOR 6732) and from the holotype of this species (BMNH 1938.3.28.4) and of *C. primordialis* (original description)

Specimen	Spicule	Length (µm)				Width (µm)				N
		Min	Mean	SD	Max	Min	Mean	SD	Max	
UFRJPOR 6732	Triactine	100.0	149.6	30.0	212.5	13.6	19.1	3.1	25	30
	Trichoxea	-	325.0	-	-	-	2.5	-	-	1
BMNH 1938.3.28.4*	Triactine	120.0	142.9	15.5	175.0	12.5	16.8	2.4	20	20
BMNH 1938.3.28.4**	Triactine	105.6	133.4	17.0	156.0	12.0	15.6	1.7	19.2	20
<i>C. primordialis</i>	Triactine	100.0	-	-	150.0	8.0	-	-	12.0	-

\*Present study.

\*\*Taken from Klautau &amp; Valentine (2003).

**Spicules:** *Trichoxeas*. Straight and very slender (Fig. 8D). Most of them are broken. The size of the unique entire trichoxea found is 325.0/2.5 µm. *Triactines*. Regular (equiangular and equiradiate). Actines are conical with sharp tips (Fig. 8E). Size: 100.0–212.5/13.6–25.0 µm.

**Ecology:** This specimen was collected underneath coral boulders at 7.9 m depth. No associated organisms were found.

**Geographical distribution:** Southwestern Caribbean (Belize, Klautau & Valentine, 2003) and Southern Caribbean (Curaçao, present study) ecoregions.

**Taxonomic remarks:** The external morphology and the shape of the triactines observed in the specimen from Curaçao are similar to *C. hondurensis* from Turneffe. However, in the original description of that species, trichoxeas were not reported and after re-examination of the slides of the holotype, we confirmed the absence of these spicules. As seen in other species, the presence of trichoxeas may not constitute a diagnostic character, as they seem to be plastic characters in *Clathrina* (Azevedo *et al.*, 2017).

Regarding spicule dimensions, although the triactines of the specimen from Curaçao can attain slightly larger sizes (100.0–212.5/13.6–25.0 µm) compared to the holotype of *C. hondurensis* (105.6–156.0/12.0–19.2 µm, taken from Klautau & Valentine, 2003), they are in the same size range.

It is important to point out that *C. hondurensis* was originally described based on a single specimen. Considering this, the presence of trichoxeas and of slightly larger triactines in the specimen from Curaçao could be attributable to intraspecific variation.

Recently, Klautau *et al.* (2016) considered the possible synonymy of *C. hondurensis* and *C. primordialis* (Haeckel, 1872). In fact, both species have similar

morphology and spicule size range. Nonetheless, it is possible to recognize thicker spicules in *C. hondurensis* (holotype: 12.0–19.2 µm) than in *C. primordialis* (holotype: 8.0–12.0 µm, Table 6). Based on this subtle but consistent difference, we decided to maintain *C. hondurensis* as a valid species and identify the Curaçaoan specimen as *C. hondurensis*.

Rützler *et al.* (2014) recorded *C. hondurensis* from another Belizean locality (Belize barrier reef near Carrie Bow); however, the skeleton of that specimen was composed of shorter and thinner triactines (85.0–100.0/8.0–12.0 µm) and thus, it does not seem to correspond to *C. hondurensis*.

*CLATHRINA INSULARIS* AZEVEDO, PADUA, MORAES, ROSSI, MURICY & KLAUTAU, 2017 (FIG. 9; TABLE 7)

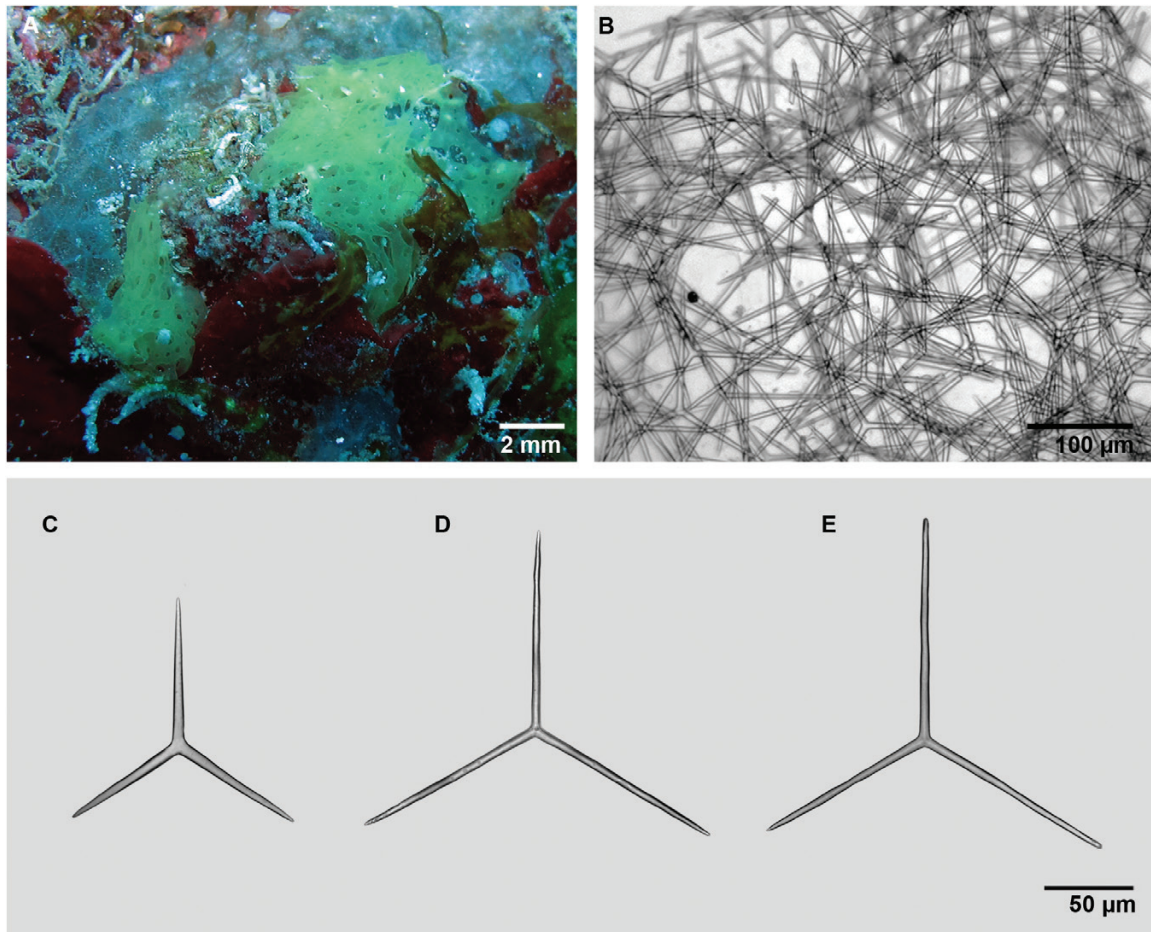
**Synonymy:** *Clathrina insularis* Azevedo *et al.*, 2017: 317–318.

**Material examined:** UFRJPOR 6737, Playa Jeremi, Soto, Curaçao (12°19'43.73"N, 69°09'07.80"W), 14.9 m depth, coll. B. Córdor-Luján, 22 August 2011.

**Additional material re-analysed:** UFRJPOR 6533, Cagarras, Fernando de Noronha Archipelago, Pernambuco, Brazil (03°48'34.59"S, 32°23'27.91"W), 15 m depth, coll. F. Azevedo and G. Rodríguez, 27 June 2011. UFRJPOR 6530 and UFRJPOR 6537, Ilha do Meio, Fernando de Noronha Archipelago, Pernambuco, Brazil (03°49'5.88"S, 32°23'36.6"W), 15 m depth, coll. F. Azevedo and G. Rodríguez, 27 June 2011.

**Comparative material examined:** Holotype of *C. insularis*. UFRJPOR 6532, Cagarras, Fernando de Noronha, Pernambuco, Brazil (03°48'34.59"S, 32°23'27.91"W), 15 m depth, coll. F. Azevedo and G. Rodríguez, 27 June 2011.





**Figure 9.** *Clathrina insularis* (UFRJPOR 6737). A, specimen in vivo. B, tangential section of the body (cormus). C, triactine I. D–E, triactines II.

**Table 7.** Spicule measurements of *Clathrina insularis* from Curaçao (UFRJPOR 6737) and from the holotype of this species (UFRJPOR 6532)

Specimen	Spicule	Length (µm)				Width (µm)				N
		Min	Mean	SD	Max	Min	Mean	SD	Max	
UFRJPOR 6737	Triactine I	52.5	62.6	6.7	77.5	3.8	5.2	0.5	6.3	30
	Triactine II	102.7	119.0	8.6	145.0	4.1	5.4	0.7	6.8	50
UFRJPOR 6532	Triactine I	47.5	76.4	12.5	97.5	5.0	6.1	0.8	7.5	30
	Triactine II	100.0	121.7	10.3	140.0	6.3	6.8	0.7	8.8	30

**Colour:** Pale yellow in life (Fig. 9A) and beige in ethanol.

**Morphology and anatomy:** The specimen is finely encrusting ( $0.4 \times 0.4 \times 0.1$  mm, Fig. 9A). The cormus is smooth, composed of irregular and loosely anastomosed tubes. Water-collecting tubes are not present. The aquiferous system is asconoid. No granular cells were observed.

**Skeleton:** The skeleton has no organization (Fig. 9B) and it is composed of two categories of triactines.

**Spicules:** *Triactines I.* Regular (equiangular and equiradiate). Actines are conical with sharp tips (Fig. 9C). Size: 52.5–77.5/3.8–6.3 µm. *Triactines II.* Regular (equiangular and equiradiate) or subregular (equiangular). Most frequent. Actines are cylindrical



to slightly conical, distally undulated and with sharp tips (Fig. 9D, E). Size: 102.7–145.0/3.7–6.6 µm.

**Ecology:** This sponge was found underneath coral boulders at 15 m depth. No associated organisms were observed.

**Geographical distribution:** Fernando de Noronha and Atol das Rocas ecoregion of the Tropical Southwestern Atlantic Province (Fernando de Noronha Archipelago, NE Brazil, Azevedo *et al.*, 2017) and Southern Caribbean (Curaçao, present study).

**Molecular analysis:** The Brazilian specimens of *C. insularis* UFRJPOR 6530, UFRJPOR 6533 and UFRJPOR 6537 were previously analysed in Azevedo *et al.* (2017), and herein, we provide their ITS sequences.

In the ITS phylogenetic tree, the sequence of the Curaçaoan specimen UFRJPOR 6737 clustered with the Brazilian specimens of *C. insularis* (UFRJPOR 6532 – holotype, UFRJPOR 6532 – paratype, UFRJPOR 6527, UFRJPOR 6530, UFRJPOR 6533 and UFRJPOR 6537) in a well-supported clade (pp = 1, b = 97, Fig. 4). The *p* distance among those specimens was very low, ranging from 0 to 0.2%. In the C-LSU phylogenetic tree, *C. insularis* (UFRJPOR 6737) appeared as a new lineage as no sequence of this species was previously obtained (Fig. 5).

*Clathrina nuroensis* Azevedo, Córdor-Luján, Willenz, Hajdu, Hooker & Klautau, 2015 appeared as the sister species of *C. insularis* (pp = 1, b = 99) in the ITS phylogeny (as previously found by Azevedo *et al.*, 2017). However, as no C-LSU sequence of *C. nuroensis* was available, it was not possible to determine its affinity to *C. insularis* in a C-LSU phylogeny. Nonetheless, using the set of C-LSU sequences available, *C. fjordica* Azevedo, Hajdu, Willenz & Klautau, 2009 appeared as the sister species of *C. insularis* with very low support (b = 39) in the ML analysis only (data not shown).

**Taxonomic remarks:** The external morphology and the skeletal composition of the Curaçaoan specimen match the original description of *C. insularis*, a species originally described from a Brazilian Oceanic Island (Fernando de Noronha) by Azevedo *et al.* (2017). This result is congruent with the molecular affinities found in the ITS and C-LSU phylogenies.

Previously, the only valid record of a yellow *Clathrina* in the Caribbean Sea corresponded to *C. aurea* from La Martinique (Pérez *et al.*, 2017). With this Curaçaoan record, *C. insularis* is the second *Clathrina* known to occur in both the Caribbean and Brazil.

*CLATHRINA LUTEA* AZEVEDO, PADUA, MORAES, ROSSI, MURICY & KLAUTAU, 2017  
(FIG. 10; TABLE 8)

**Synonymy:** *Clathrina primordialis* Lehnert & Van Soest, 1998: 99; *Clathrina lutea* Azevedo *et al.*, 2017: 318–320.

**Material examined:** UFRJPOR 6761, Porto Mari, St. Willibrordus, Curaçao, (12°13'6.62"N, 69°05'13.26"W), 10.6 m depth, coll. G. Lôbo-Hajdu, 20 August 2011.

**Comparative material examined:** Holotype of *C. lutea*. UFRJPOR 5173, Pedra Lixa, Abrolhos Archipelago, Caravelas, Bahia, Brazil (17°41'S, 38°59'W), 7 m depth, coll. C. Zilberberg & L. Monteiro, 21 March 2005.

**Colour:** Yellow in life (Fig. 10A) and beige in ethanol (Fig. 10B).

**Morphology and anatomy:** This specimen has a massive cormus (1.0 × 0.4 × 0.1 cm, Fig. 10A) composed of regular and tightly anastomosed tubes (Fig. 10B). Water-collecting tubes are present. The aquiferous system is asconoid. No granular cells were observed.

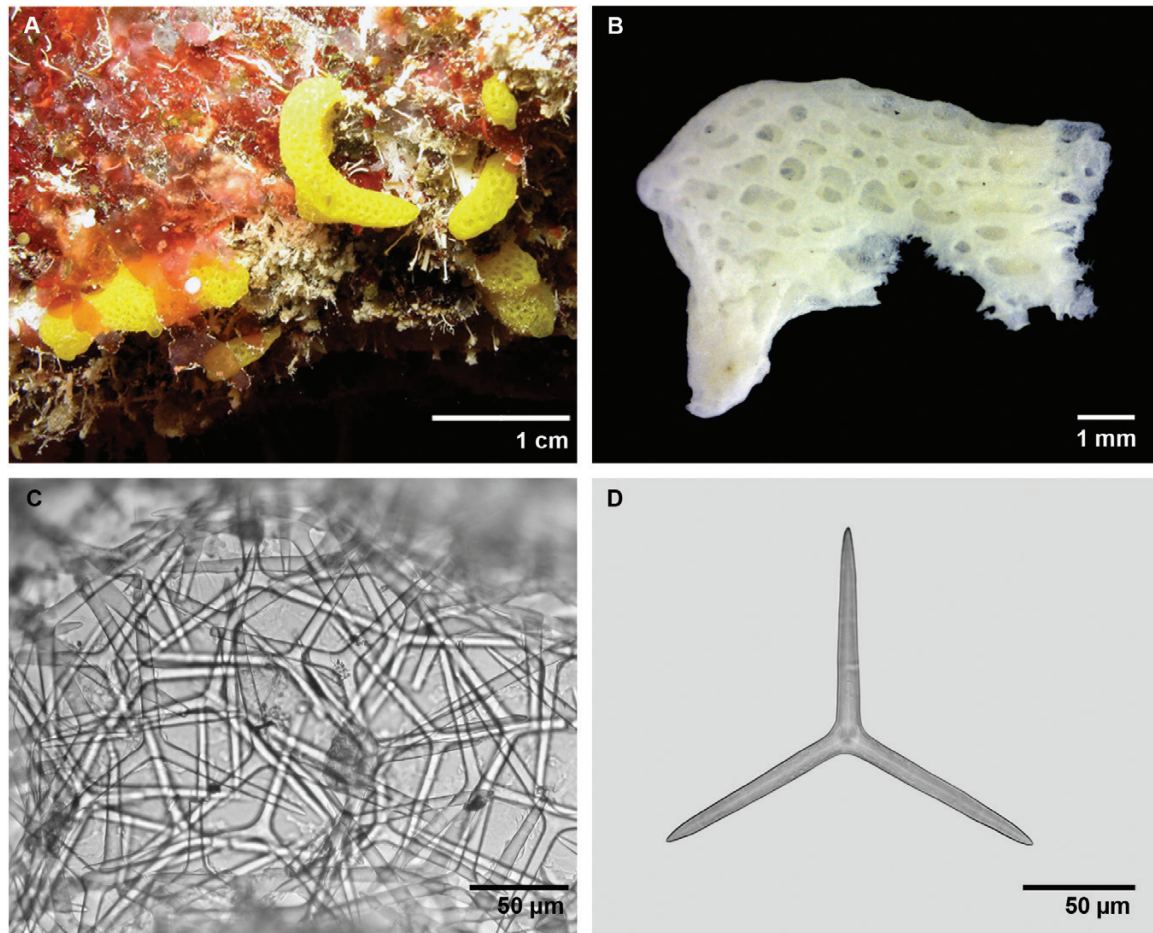
**Skeleton:** The skeleton has no organization and it is composed of one category of triactines (Fig. 10C). Some broken trichoxeas were found in the spicule slide.

**Spicules:** *Triactines*. Regular. Actines are cylindrical to slightly conical, slightly undulated and with blunt tips (Fig. 10D). Size: 75.0–97.5/7.5–10.0 µm.

**Ecology:** The specimen from Curaçao was collected in a light-protected environment, inside a small crevice, at 10.6 m depth. No associated organisms were observed.

**Geographical distribution:** This species has a widespread distribution. Tropical Northwestern Atlantic Province – Floridian ecoregion (Florida, USA, Klautau *et al.*, 2013), Eastern Caribbean (Virgin Islands, Klautau *et al.*, 2013) and Southern Caribbean (Curaçao, present study). Tropical Southwestern Atlantic Province – Eastern Brazil ecoregion (Abrolhos Archipelago, Azevedo *et al.*, 2017) and Fernando de Noronha and Atol das Rocas ecoregion (Rocas Atoll, Azevedo *et al.*, 2017).

**Molecular analysis:** In the ITS tree, the Curaçaoan specimen UFRJPOR 6761 grouped in the clade of *C. lutea* with high support (pp = 1, b = 97, Fig. 4). This clade included specimens from Brazil (holotype: UFRJPOR 5172, paratype: UFRJPOR 5173, and UFRJPOR 6545) and the Caribbean Sea (ZMAPOR8344 from Virgin Islands and UFRJPOR



**Figure 10.** *Clathrina lutea* (UFRJPOR 6761). A, specimen in vivo (photo taken by G. Lôbo-Hajdu). B, specimen after fixation. C, tangential section of the body (cormus). D, triactine.

**Table 8.** Spicule measurements of *Clathrina lutea* from Curaçao (UFRJPOR 6761) and from the holotype of this species (UFRJPOR 5173)

Specimen	Spicule	Length (µm)				Width (µm)				N
		Min	Mean	SD	Max	Min	Mean	SD	Max	
UFRJPOR 6761	Triactine	75.0	88.8	5.6	97.5	7.5	9.4	0.7	10.0	30
UFRJPOR 5173	Triactine	69.3	78.5	3.8	84.0	6.5	7.5	0.4	8.3	30

5818 from Florida). The  $p$  distance among these specimens ranged from 0 to 1.1%. The lowest value (0%) was found between UFRJPOR 5172 and UFRJPOR 5173, whereas the highest value (1.1%) was between ZMAPOR8344 and other specimens (UFRJPOR 5172, UFRJPOR 5173 and UFRJPOR 6761). The  $p$  distance between the Curaçaoan specimen (UFRJPOR 6761) and the holotype of *C. lutea* was 0.4%, considered within the intraspecific genetic variation of *Clathrina*.

**Taxonomic remarks:** Although the analysed specimen from Curaçao (UFRJPOR 6761) matches the morphological description of the Brazilian *C. lutea* (UFRJPOR 5173) and grouped in the same clade in the molecular phylogeny, they have some morphological differences. The skeleton of the Curaçaoan specimen has slightly larger triactines (75.0–97.5/7.5–10.0 µm) compared to those of the holotype of *C. lutea* (69.3–84.0/6.5–8.3 µm, Table 8). Moreover, some broken trichoxeas were found in the Curaçaoan specimen, whereas in the Brazilian specimens they were

not observed. These subtle differences can be attributed to plasticity as other specimens of *C. lutea* from several localities within the Caribbean have triactines with similar size as that of UFRJPOR 6761 and varied only by the presence or not of trichoxeas in their skeletons (B. Córdor-Luján, personal observation).

In this study, the high intraspecific variability observed among specimens morphologically identified as *C. lutea* (0–1.1%) overlapped with interspecific values of *C. blanca* and *C. ramosa* (0.6–1.1%, see Molecular analysis section of *C. curacaoensis* sp. nov. for value references). This suggests the presence of a species complex within *C. lutea*, or the synonymy of *C. blanca* and *C. ramosa*. A future phylogeographic study of *C. lutea* will certainly help to understand this result.

In 1998, Lehnert & Van Soest reported *C. primordialialis* from Jamaica. After revising the description of that record, including the in vivo figure (fig. 23, p. 97), we rather consider it as *C. lutea* based on its external morphology and spicule composition.

*CLATHRINA MUTABILIS* AZEVEDO, PADUA, MORAES,  
ROSSI, MURICY & KLAUTAU, 2017  
(FIG. 11; TABLE 9)

*Synonymy:* *Clathrina mutabilis* Azevedo et al., 2017: 320–321.

*Material examined:* UFRJPOR 6699, Hook's Hut, Willemstadt, Curaçao (12°07'18.94"N, 68°58'11.46"W), <10 m depth, coll. B. Córdor-Luján and G. Lôbo-Hajdu, 17 August 2011. UFRJPOR 6704 and UFRJPOR 6719, Playa Kalki, Westpunt, Curaçao (12°22'29.86"N, 69°09'30.63"W), 6.7 m depth, coll. B. Córdor-Luján and E. Hajdu, 21 August 2011. UFRJPOR 6712, Daai Booi, St. Willibrordus, Curaçao (12°12'43.12"N, 69°05'8.42"W), 4.9 m depth, coll. B. Córdor-Luján, 18 August 2011. UFRJPOR 6717, Water Factory, Willemstadt, Curaçao (12°06'30.88"N, 68°57'13.53"W), coll. B. Córdor-Luján, 19 August 2011. UFRJPOR 6733 and UFRJPOR 6735, Porto Mari, St. Willibrordus, Curaçao (12°13'6.62"N, 69°05'13.26"W), 7.9 m depth, coll. B. Córdor-Luján, 20 August 2011. UFRJPOR 6736, Playa Jeremi, Soto, Curaçao (12°19'43.73"N, 69°09'07.80"W), 4.9 m depth, coll. B. Córdor-Luján, 22 August 2011. UFRJPOR 6740, Sunset Waters, Soto, Curaçao (12°16'01.58"N, 69°07'44.85"W), 9–12 m depth, coll. B. Córdor-Luján, 22 August 2011. UFRJPOR 6741, Sunset Waters, Soto, Curaçao (12°16'01.58"N, 69°07'44.85"W), 8.9 m depth, coll. B. Córdor-Luján, 22 August 2011. UFRJPOR 6743, Sunset Waters, Soto, Curaçao (12°16'01.58"N, 69°07'44.85"W), 9.8 m depth, coll. B. Córdor-Luján, 22 August 2011. UFRJPOR 6744, Sunset Waters, Soto, Curaçao (12°16'01.58"N, 69°07'44.85"W), 7.2 m depth, coll. B. Córdor-Luján, 22 August 2011. UFRJPOR

6747, Sunset Waters, Soto, Curaçao (12°16'01.58"N, 69°07'44.85"W), 4.9 m depth, coll. B. Córdor-Luján, 22 August 2011. UFRJPOR 6750, Tug boat, Caracasbaai, Willemstadt, Curaçao (12°04'08.20"N, 68°51'44.40"W), coll. B. Córdor-Luján, 23 August 2011.

*Comparative material examined:* Holotype of *C. mutabilis*. UFRJPOR 6526, Cagarras, Fernando de Noronha, Pernambuco, Brazil (3°48'34.59"S, 32°23'27.91"W), 15 m depth, coll. F. Azevedo and G. Rodríguez, 27 June 2011.

*Colour:* Yellow in life (Fig. 11A, B) and white to beige in ethanol (Fig. 11C).

*Morphology and anatomy:* The specimens have a massive cormus composed of irregular and loosely anastomosed tubes (Fig. 11A). Water-collecting tubes are present. When tubes are contracted, this sponge has a more encrusting growth form (UFRJPOR 6743, Fig. 11B). The largest fragment deposited in the UFRJPOR collection belongs to the specimen UFRJPOR 6735 (Fig. 11C), which measures 0.8 × 0.6 × 0.2 cm. The aquiferous system is asconoid. No granular cells were observed.

*Skeleton:* The skeleton has no organization (Fig. 11D) and it is composed of two categories of triactines.

*Spicules:* *Triactines I.* Regular (equiangular and equiradiate) but some subregular spicules (equiangular but not equiradiate) were also found. Actines are conical, straight, with sharp tips (Fig. 11E). Smaller than triactines II. Size: 60.0–112.5/7.5–10.0 µm. *Triactines II.* Regular (equiangular and equiradiate), subregular (equiangular but not equiradiate) (Fig. 11F) or parasagittal (Fig. 11G). Frequent. Actines are slightly conical to cylindrical, slightly undulated, with blunt tips. Size: 100.0–195.0/5.0–11.3 µm.

*Ecology:* The specimens of *C. mutabilis* collected in Curaçao were found underneath broken corals at depths between 4 and 10 m but this species can occur down to 15 m depth (Azevedo et al., 2017). No associated organisms were found.

*Geographical distribution:* Fernando de Noronha and Atol das Rocas (Fernando de Noronha Archipelago; Azevedo et al., 2017) and Southern Caribbean (Curaçao, present study) ecoregions. *Clathrina mutabilis* was found in all sampled localities in Curaçao.

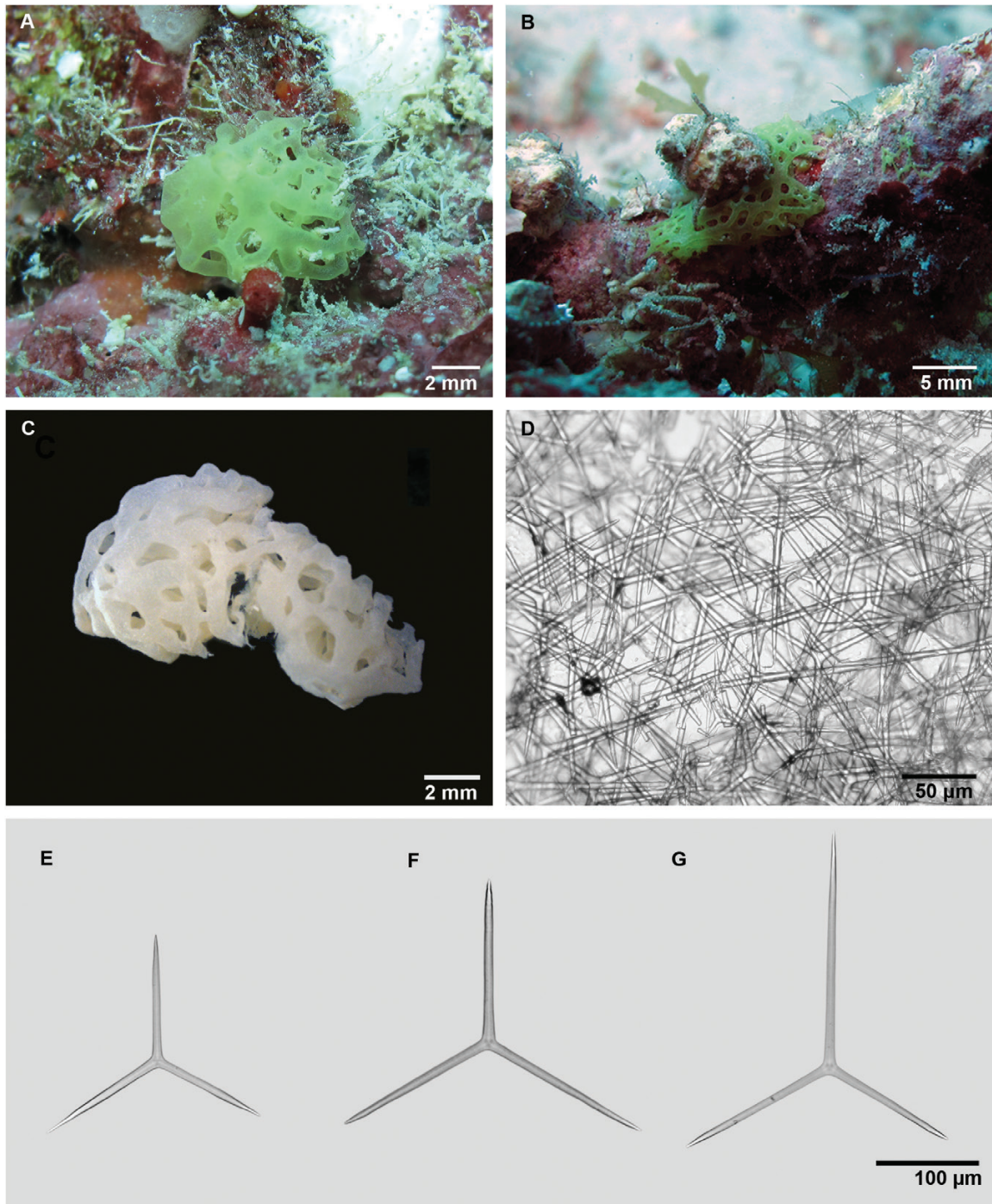
*Molecular analysis:* DNA sequences of *C. mutabilis* from Curaçao (UFRJOR 6733 and UFRJPOR 6741),



identified as *Clathrina* sp. nov. 4, were previously published by Klautau *et al.* (2013). Herein, we generated new sequences of this species.

In the ITS phylogenetic tree, the nine specimens from Curaçao clustered with the holotype

and paratype of *C. mutabilis* (UFRJPOR 6526 and UFRJPOR 6528, respectively), forming a monophyletic clade with high support ( $pp = 1$ ,  $b = 100$ , Fig. 4). The  $p$  distance among these specimens ranged from 0 to 0.6%. The lowest value (0%) was found between



**Figure 11.** *Clathrina mutabilis*. A–B, UFRJPOR 6704 and UFRJPOR 6743 in vivo. C, UFRJPOR 6735 after fixation. D, tangential section of the body (cormus). E, triactine I. F, triactine II. G, parasagittal triactine II. Skeleton photos were taken from UFRJPOR 6741.

**Table 9.** Spicule measurements of *Clathrina mutabilis* from Curaçao (UFRJPOR 6741, UFRJOR 6704, UFRJPOR 6745) and from the holotype (UFRJPOR 6526) and paratype (UFRJPOR 6528) of this species

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	Mean	SD	Max	Min	Mean	SD	Max	
UFRJPOR 6741	Triactine I		67.5	82.4	8.5	95.0	6.3	8.0	0.8	8.8	30
	Triactine II		100.0	123.8	10.4	145.0	7.5	8.9	0.9	10.0	30
	Parasagittal	Unpaired	125.0	166.0	17.3	195.0	5.0	8.2	1.1	10.0	30
UFRJPOR 6704	Triactine II	Paired	100.0	116.1	11.2	132.5	5.0	7.5	1.1	10.0	30
	Triactine I		60.0	95.5	12.6	112.5	7.5	8.8	1.2	10.0	20
	Triactine II		107.5	135.5	15.9	162.5	7.5	10.3	1.0	11.3	20
	Parasagittal	Unpaired	122.5	142.4	15.6	177.5	7.5	8.3	1.0	10.0	30
UFRJPOR 6745	Triactine II	Paired	75.0	98.7	12.8	127.5	6.3	7.6	0.5	8.8	30
	Triactine I		60.0	81.6	13.4	102.5	7.5	8.6	1.1	10.0	20
	Triactine II		100.0	126.0	18.1	160.0	7.5	9.0	1.1	10.0	20
UFRJPOR 6526	Parasagittal	Unpaired	150.0	169.5	13.1	195.0	7.5	9.1	1.0	10.0	30
	Triactine II	Paired	97.5	121.0	12.9	140.0	7.5	8.3	0.9	10.0	30
	Triactine I		56.7	69.8	7.9	91.8	8.1	8.4	0.6	9.5	20
UFRJPOR 6528	Triactine II		94.5	124.7	14.4	148.5	6.8	7.9	0.6	9.5	40
	Triactine I		54.0	78.3	24.1	159.3	8.1	9.5	0.9	10.8	20
	Triactine II		94.5	124.7	15.2	153.9	8.1	9.2	0.8	10.8	40

Brazilian specimens (UFRJPOR 6526 vs. UFRJPOR 6528) as well as between Curaçaoan specimens (e.g. UFRJPOR 6704 vs. UFRJPOR 6743, UFRJPOR 6733 vs. UFRJPOR 6740). The highest value (0.6%) was observed between UFRJPOR 6719 and other six specimens from Curaçao (UFRJPOR 6526, UFRJPOR 6528, UFRJPOR 6733, UFRJPOR 6735, UFRJPOR 6740 and UFRJPOR 6745). In the C-LSU phylogenetic tree, *C. mutabilis* (UFRJPOR 6741) appeared as an independent new lineage as no other sequences of this species was obtained previously (Fig. 5).

**Taxonomic remarks:** In the original description of *C. mutabilis*, water-collecting tubes were not observed as ‘the holotype was very small and the paratype was fragmented’ (Azevedo *et al.*, 2017). Now, water-collecting tubes were observed in the Curaçaoan specimens, providing evidence for this structure in *C. mutabilis*.

It is important to mention that the skeleton of the specimens from Curaçao has parasagittal triactines (referred as parasagittal triactines II in Table 9, Fig. 11G); however, the greater length (100.0–195.0 µm) of the unpaired actine matches the size range indicated for triactines II of *C. mutabilis* (94.5–153.9 µm, considering holotype and paratype) in the original description (Azevedo *et al.*, 2017). Therefore, they should not be regarded as a different spicule category, but as part of the plasticity observed in this species. Furthermore, the holotype of *C. mutabilis* (UFRJPOR 6526) formed a clade with the Curaçaoan specimens in the ITS phylogenetic tree.

FAMILY LEUCETTIDAE DE LAUBENFELS, 1936  
GENUS *LEUCETTA* HAECKEL, 1872

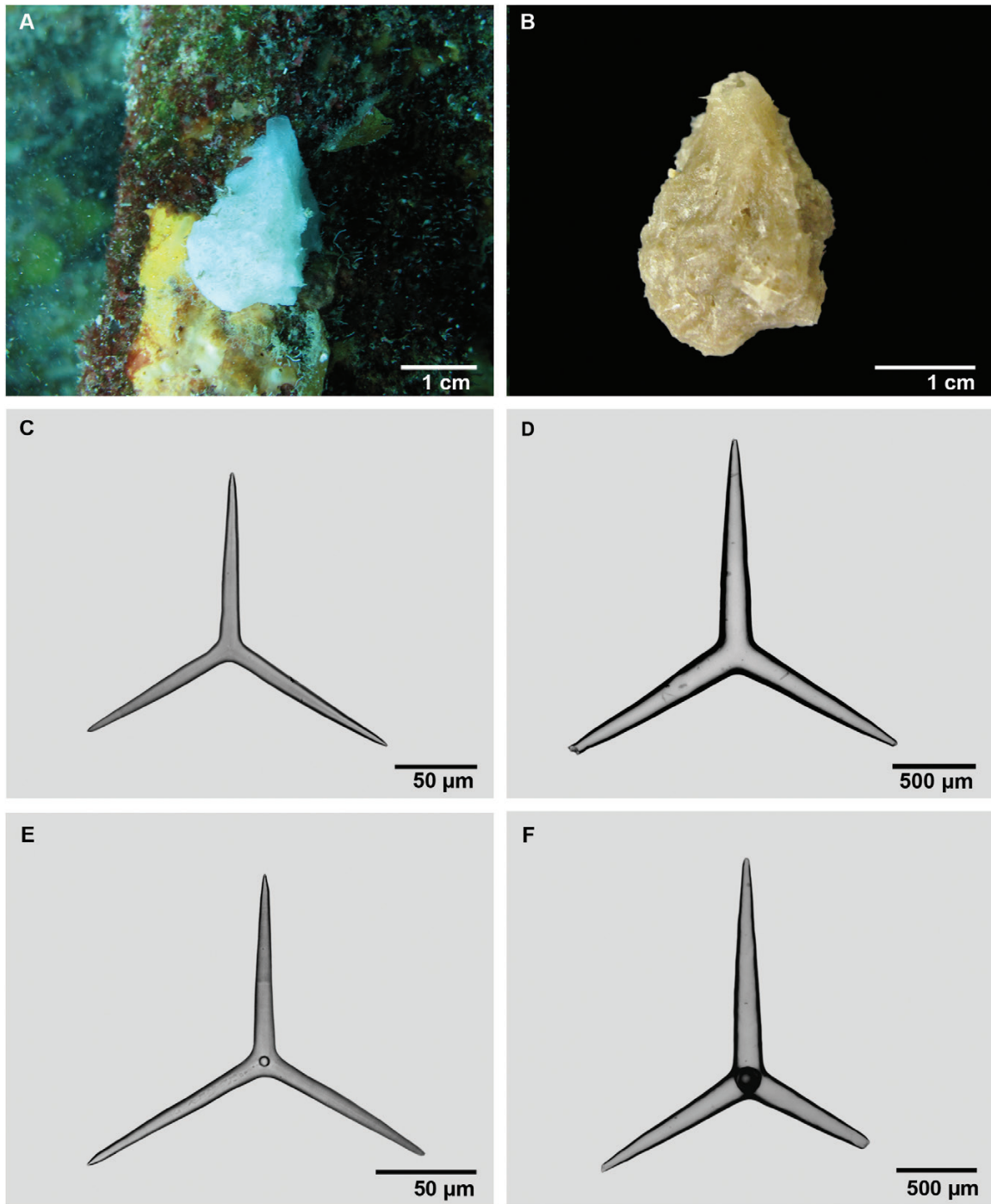
*Type species:* *Leucetta primigenia* Haeckel, 1872.

**Diagnosis:** ‘Leucettidae with a homogeneous organisation of the wall and a typical leuconoid aquiferous system. There is neither a clear distinction between the cortex and the choanoskeleton, nor the presence of a distinct layer of subcortical inhalant cavities. The atrium is frequently reduced to a system of exhalant canals that open directly into the osculum’ (Borojevic *et al.*, 2002).

*LEUCETTA FLORIDANA* HAECKEL, 1872  
(FIG. 12; TABLE 10)

**Synonymy:** *Amphoriscus floridanus*, *Dyssycus floridanus*, *Leucaltis floridana*, *Leucaltis impura*, *Leucaltis pura*, *Lipostomella floridana* Haeckel, 1872: 144; *Leucilla floridana* Jenkin, 1908: 453; *Leucetta floridana* de Laubenfels, 1950: 146; *Leucetta microraphis* Borojevic & Peixinho, 1976: 1003–1005; *Leucetta* aff. *floridana* Lehnert & Van Soest, 1998: 99; *Leucetta floridana* Valderrama *et al.*, 2009: 9–14; Lanna *et al.*, 2009: 7–9; Muricy *et al.*, 2011: 36–37; Rützler *et al.*, 2014: 102; Pérez *et al.*, 2017: 13; Azevedo *et al.*, 2017: 333–334, 336.

**Type specimen:** Haeckel’s type material of *L. floridana* is considered lost (Burton, 1963).



**Figure 12.** *Leucetta floridana* (UFRJPOR 6757). A, specimen in vivo. B, specimen after fixation. C, triactine I. D, triactine II. E, tetractine I. F, tetractine II.

*Type locality:* Coast of Florida, USA.

*Material examined:* UFRJPOR 6726, Water Factory, Willemstadt, Curaçao (12°06'30.88"N, 68°57'13.53"W), 17.8 m depth, coll. E. Hajdu, 19 August 2011. UFRJPOR 6757, Tug Boat, Caracasbaai, Curaçao (12°04'08.20"N, 68°51'44.40"W), 6.2 m depth, coll. B. Córdor-Luján,

23 August 2011. UFRJPOR 6765, Hook's Hut, Caracasbaai, Curaçao (12°07'18.94"N, 68°58'11.46"W), 13.3 m depth, coll. E. Hajdu, 18 August 2011.

*Colour:* White to light blue in life (Fig. 12A) and greyish white to brown in ethanol (Fig. 12B).



**Table 10.** Spicule measurements of *Leucetta floridana* from Curaçao (UFRJPOR 6726 and UFRJPOR 6757) and from the redescription of this species (UFRJPOR 5360)

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	Mean	SD	Max	Min	Mean	SD	Max	
UFRJPOR 6726	Triactine I		121.5	143.6	14.6	172.8	10.8	13.9	1.6	16.2	20
	Triactine II		972.0	1431.3	354.9	2378.4	129.6	214.5	52.5	345.6	21
	Tetractine I	Basal	105.0	139.1	23.3	200.0	12.5	15.2	2.2	20.0	20
		Apical	25.0	33.4	8.4	45.0	7.5	8.1	1.2	10.0	8
	Tetractine II	Basal	675.7	1228.2	293.6	1621.6	108.1	171.8	49.7	216.2	9
UFRJPOR 6757	Triactine I		87.5	126.7	22.2	175.0	10.0	15.5	3.2	22.5	30
	Triactine II		378.4	1383.3	673.9	2378.4	54.05	207.7	98.3	389.2	24
	Tetractine I	Basal	102.5	138.3	19.6	200.0	11.2	15.3	2.8	20.0	30
		Apical	40.0	44.4	4.3	50.0	10.0	10.0	0.0	10.0	4
	Tetractine II	Basal	464.9	1294.9	568.7	2162.2	108.1	172.0	59.1	270.3	9
UFRJPOR 5360*	Triactine I		105.6	143.3	28.7	217.8	9.9	17.1	4.9	33.0	30
	Triactine II		257.4	696.2	279.7	1181.5	33.0	102.1	46.2	194.6	30
	Tetractine I		105.6	137.4	24.1	224.4	9.9	15.4	3.6	26.4	30
	Tetractine II		278.0	665.5	301.0	1042.5	48.7	102.5	51.3	180.7	8

\*Valderrama *et al.* (2009).

**Morphology and anatomy:** This species has a massive growth form (Fig. 12A). The consistency is very rough and incompressible. The largest specimen (UFRJPOR 6726) measures 2.6 × 1.6 × 0.8 cm (Fig. 12B). The surface is ridged and hispid. The three analysed specimens had a single apical osculum (largest diameter = 0.5 cm). In the specimen UFRJPOR 6757, the osculum is particularly elongated and bears a very delicate margin. The atrial cavity is wide and hispid. The aquiferous system is leuconoid.

**Skeleton:** The skeleton is typical of the genus. It does not have special organization and it is composed of two size categories of triactines (I and II) and tetractines (I and II). The cortex and atrial wall are thin, whereas the choanosome is thick. Triactines II and tetractines II, which are the largest spicules, are found in the cortex and in the choanosome, tangentially positioned. Tetractines II are rare. Triactines I and tetractines I are spread in the choanosome and in the atrium. The apical actine of tetractines I penetrates the exhalant canals and the atrial cavity. Near the atrium, triactines I and tetractines I become sagittal.

**Spicules:** *Triactines I.* Regular. Actines are conical, straight, with blunt tips (Fig. 12C). Frequent. Sagittal triactines I were also observed. Size: 87.5–175.0/10.0–22.5 µm. *Triactines II.* Regular. Actines are conical, straight, with blunt tips (Fig. 12D). Highly variable size: 378.4–2378.4/54.1–389.2 µm. *Tetractines I.* Regular. Actines are slightly conical, straight, with blunt to sharp tips (Fig. 12E). The apical actine is smooth, thinner than the basal actines and has a sharp tip.

Sagittal tetractines I were also observed. Size: 102.5–200.0/11.2–20.0 µm (basal actine) and 25.0–50.0/7.5–10 µm (apical actine). *Tetractines II.* Regular. Rare. Actines are conical, straight, with blunt tips (Fig. 12F). Highly variable size: 464.9–2162.2/108.1–270.3 µm.

**Ecology:** This species was found underneath boulders close to some incrusting and massive demosponges (cf. *Clathria*). No associated organisms were found on the surface of the analysed specimens. The analysed specimens from Curaçao were collected between 6 and 18 m depth; however, *L. floridana* has been reported down to 100 m depth in NE Brazil (Lanna *et al.*, 2009).

**Geographical distribution:** This species has a widespread distribution. Tropical Northwestern Atlantic Province – Floridian (Florida: Haeckel, 1872), Bermuda (Bermudas: de Laubenfels, 1950), Greater Antilles (Jamaica: Lehnert & Van Soest, 1998), Eastern Caribbean (La Martinique: Pérez *et al.*, 2017) and Southern Caribbean (Urabá and San Andrés: Valderrama *et al.*, 2009 and Curaçao: present study) ecoregions, North Brazil Shelf Province (Pará, Brazil: Borojevic & Peixinho, 1976) and Tropical Northwestern Atlantic Province – Northeastern Brazil, Eastern Brazil, Fernando de Noronha and Atol das Rocas ecoregions (Borojevic & Peixinho, 1976; Lanna *et al.*, 2009; Valderrama *et al.*, 2009).

**Molecular analysis:** The ITS sequences of the specimens from Curaçao (UFRJPOR 6726 and UFRJPOR 6765) clustered within a monophyletic clade also composed of Brazilian (UFRJPOR 6480) and

Panamenian (PTL09-P100) specimens of *L. floridana* ( $pp = 1$ ,  $b = 97$ , Fig. 4). The Curaçaoan specimens showed a higher molecular affinity with the Brazilian specimen ( $p$  distance = 0%) than with the Panamenian one, despite their geographic distance ( $p$  distance = 0.4%).

**Taxonomic remarks:** Our specimens match the original description of *L. floridana* provided by Haeckel (1872) as well as the redescription of Valderrama *et al.* (2009). Haeckel's measurements are: triactines and tetractines I: 150.0–250.0/10.0–15.0  $\mu\text{m}$  and triactines and tetractines II: 700.0–1500.0/100.0–150.0  $\mu\text{m}$ . Those of Valderrama *et al.* (2009) are presented in Table 10. *Leucetta floridana* is not only one of the few species of Calcarea already reported from the Caribbean Sea, but it is also one of the most widespread species along the Western Tropical Atlantic.

SUBCLASS CALCARONEA BIDDER, 1898  
ORDER LEUCOLENIDA HARTMAN, 1958  
FAMILY AMPHORISCIDAE DENDY, 1893  
GENUS *LEUCILLA* HAECKEL, 1872

**Type species:** *Leucilla amphora* Haeckel, 1872.

**Diagnosis:** Amphoriscidae with sylleibid or leuconoid organization. The choanoskeleton is formed primarily by the apical actines of giant cortical tetractines and the unpaired actine of subatrial triactines or tetractines. It may contain dispersed spicules, but a typical articulated choanoskeleton is always absent (Borojevic *et al.*, 2002, emend.).

***LEUCILLA ANTILLANA* SP. NOV.**

(FIGS 13, 14; TABLE 11)

**Etymology:** From its presence in Curaçao, which is an island of the Leeward Antilles.

**Type locality:** Water Factory, Willemstadt, Curaçao.

**Material examined:** *Holotype.* UFRJPOR 6768, Water Factory, Willemstadt, Curaçao (12°06'30.88"N, 68°57'13.53"W), 9.9 m depth, coll. B. Cóndor-Luján, 23 August 2011.

**Diagnosis:** *Leucilla* with a skeleton composed of cortical tetractines, subatrial triactines and atrial tetractines, and leuconoid aquiferous system.

**Colour:** White to light blue in life (Fig. 13A) and white in ethanol (Fig. 13B).

**Morphology and anatomy:** This species has an irregular tubular shape being wider at the base (Fig. 13A). It measures 1.0 × 0.8 × 0.2 cm. The surface

is slightly hispid due to some protruding spicules. The osculum is apical and has a delicate crown of trichoxeas (arrow in Fig. 13B). The aquiferous system is leuconoid with subspherical to elongated choanocyte chambers ranging from 97.2 to 162.0  $\mu\text{m}$  of diameter.

**Skeleton:** The skeleton is characteristic of the genus (Fig. 13C). The cortical skeleton is exclusively formed by tetractines (Fig. 13D) with the basal actines tangentially positioned on the surface. The choanosomal skeleton is inarticulated, composed of the apical actine of the cortical tetractines (Fig. 13E, arrow), which occasionally crosses the atrial skeleton, and of the unpaired actine of the subatrial triactines (Fig. 13F, white arrow). The subatrial triactines do not form a continuous layer, instead, they are irregularly scattered in this region. The atrial skeleton is composed of tetractines with the apical actine projected into the atrium (Fig. 13F, black arrow).

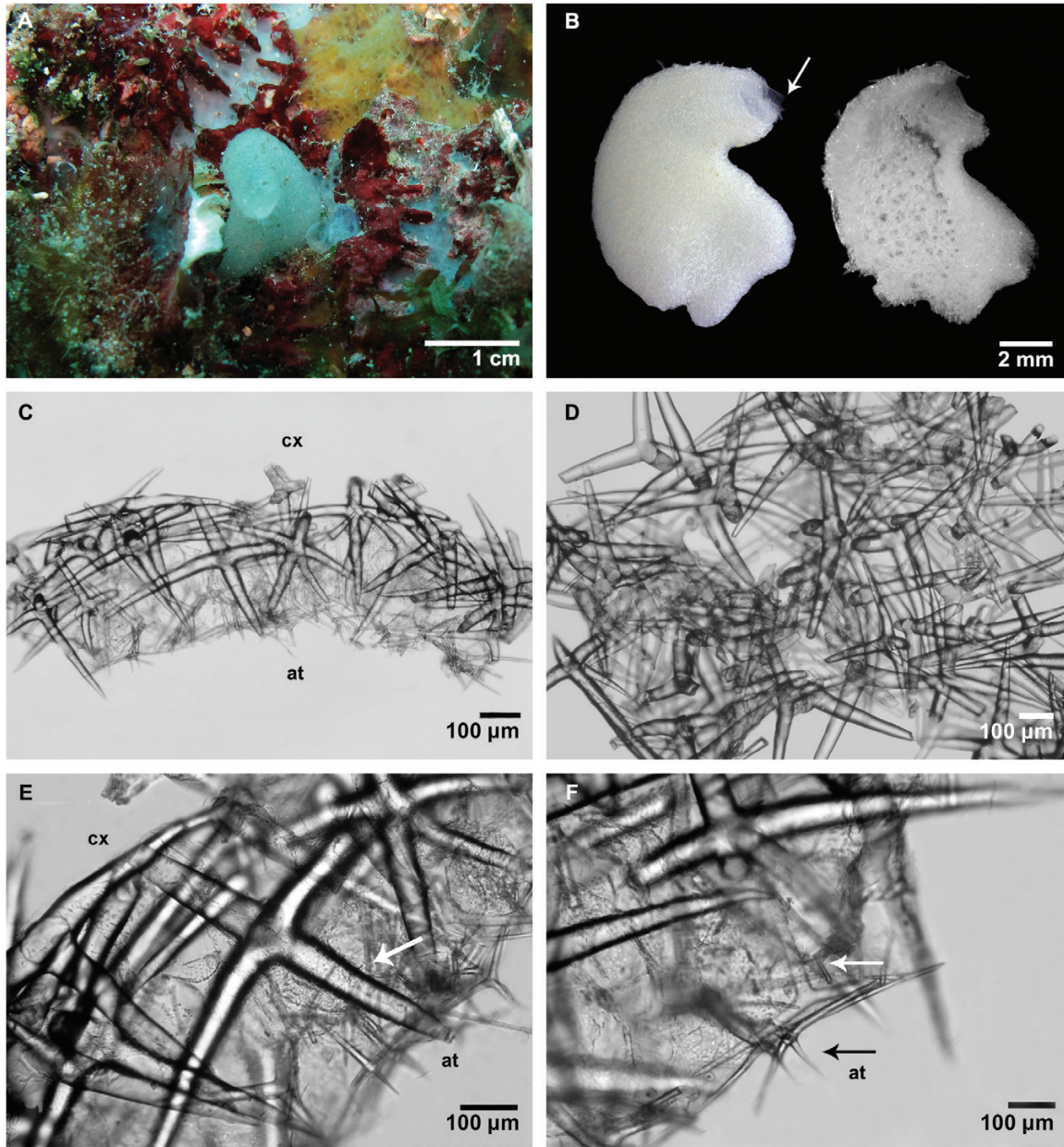
**Spicules:** *Cortical tetractines.* Sagittal. Actines are conical with sharp tips. The paired actines are frequently curved. The apical actine is straight and it is the longest actine (Fig. 14A). Some undulated apical actines were also observed. Size: 350.0–485.0/35.0–60.0  $\mu\text{m}$  (paired actine), 75.0–200.0/35.0–60.0  $\mu\text{m}$  (unpaired actine) and 285.0–550.0/35.0–55.0  $\mu\text{m}$  (apical actine). *Subatrial triactines.* Sagittal. Actines are conical with sharp tips. The paired actines are straight and smaller than the unpaired one (Fig. 14B, C). Some slightly curved paired actines were also observed. Highly variable size: 175.0–460.0/15.0–60.0  $\mu\text{m}$  (paired actine) and 225.0–490.0/15.0–55.0  $\mu\text{m}$  (unpaired actine). *Atrial tetractines.* Sagittal. Actines are conical with very sharp tips. The unpaired actine is slightly longer than the paired ones (Fig. 14D). The apical actine is the thinnest and shortest actine. Size: 120.0–270.0/10.0–12.5  $\mu\text{m}$  (paired actine), 185.0–355.0/10.0–12.5  $\mu\text{m}$  (unpaired actine) and 15.0–50.0/5.0–10.0  $\mu\text{m}$  (apical actine).

**Ecology:** This species was found underneath coral boulders at 9.9 m depth. No organisms were found associated with this species.

**Molecular analysis:** We provide the first C-LSU sequence of a *Leucilla* species, *L. antillana* sp. nov. In both phylogenetic reconstructions (ML and BI), *Leucilla* was not recovered as a monophyletic genus as the two *Leucilla* species used in this study, *L. antillana* sp. nov. and *L. micropilosa* sp. nov. (see below), appeared in different clades (Fig. 15).

**Geographical distribution:** Southern Caribbean (provisionally endemic to Curaçao, present study).





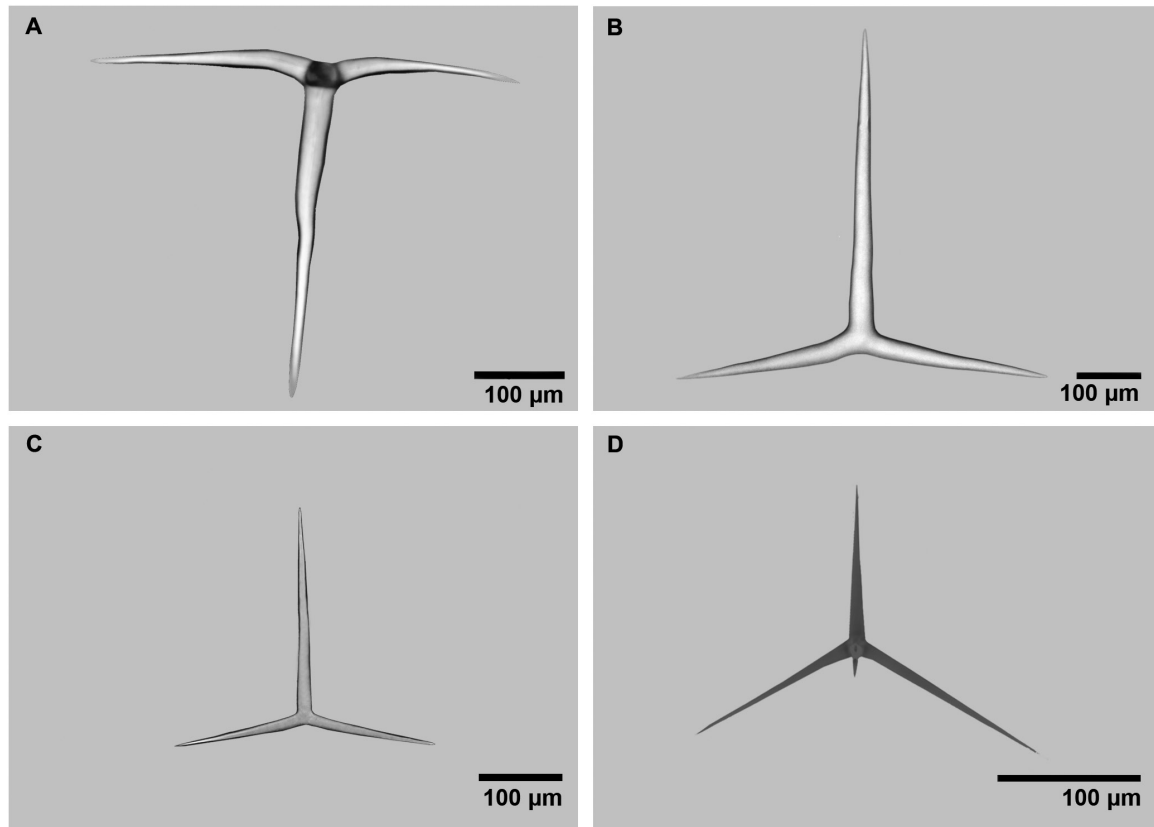
**Figure 13.** *Leucilla antillana* sp. nov. (UFRJPOR 6768). A, specimen in vivo. B, specimen after fixation with the crown of trichoxea (arrow). C, cross section of the skeleton. D, tangential section of the cortex. E, choanoskeleton with the apical actine of a tetractine (arrow). F, atrial skeleton with a subatrial triactine (white arrow) and an apical actine of an atrial tetractine (black arrow). cx, cortex; at, atrium.

*Taxonomic remarks:* The species that most resemble *L. antillana* sp. nov. are *L. amphora* (type locality: Puerto Rico or Barbados, Haeckel did not specify this), *L. capsula* Haeckel, 1872 (type locality: Agulhas Bank, South Africa), *L. uter* Poléjaeff, 1883 (type locality: Bermudas or Philippines, Poléjaeff did not specify this) and *L. sacculata* Carter, 1890 (type locality: Fernando de Noronha Archipelago, Brazil).

*Leucilla antillana* sp. nov. can be easily differentiated from *L. amphora* and *L. capsula* because the skeleton of the two latter species (*L. amphora* and *L. capsula*) is exclusively composed of tetractines (as described and illustrated by Haeckel, 1872), whereas the new species has subatrial triactines.

Different to *L. sacculata* and *L. uter*, the skeletons of which comprise cortical microdiactines and subatrial





**Figure 14.** Scicules of *Leucilla antillana* sp. nov. (UFRJPOR 6768). A, cortical tetractine. B, large subatrial triactine. C, small subatrial triactine. D, atrial tetractine.

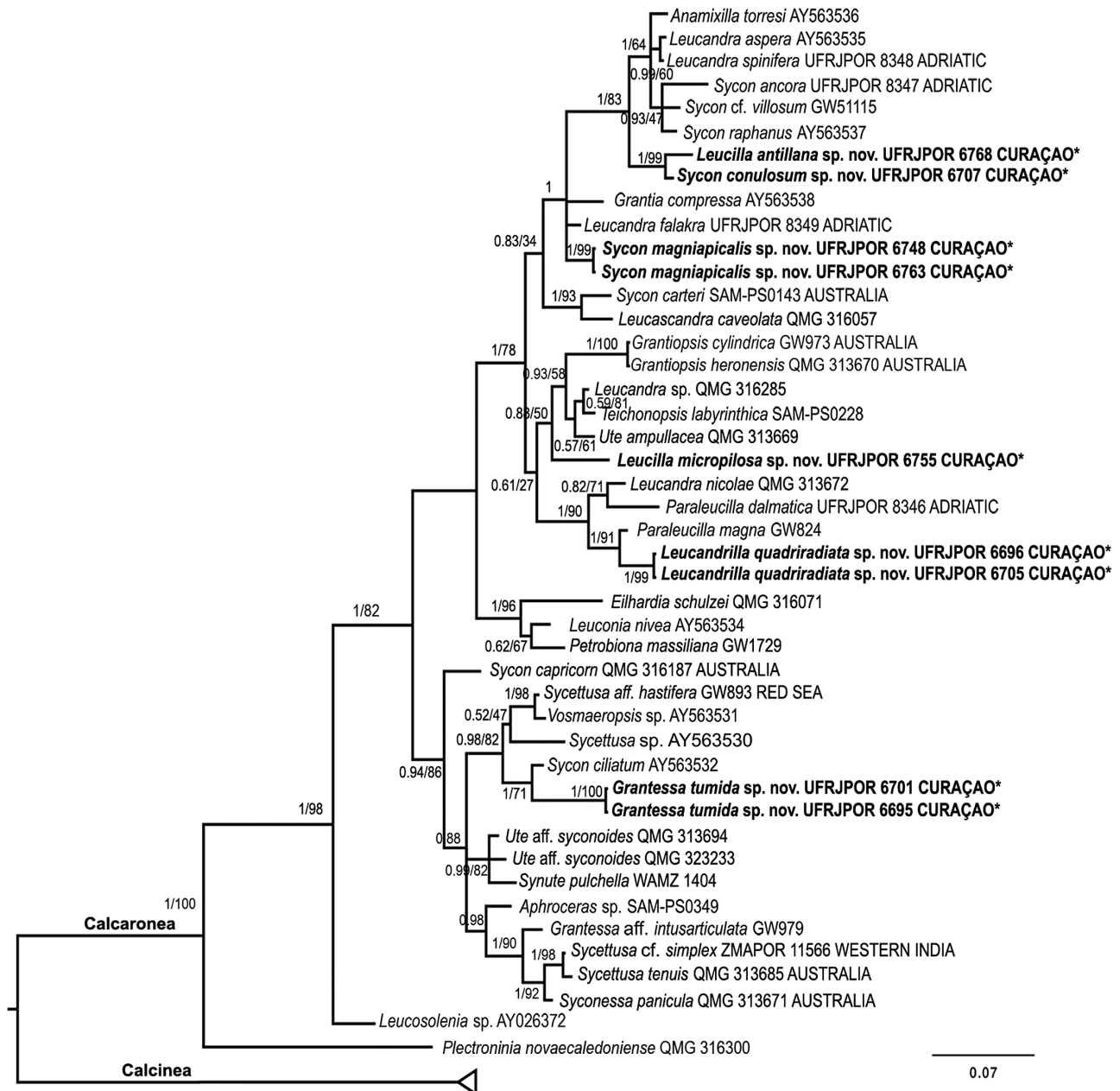
**Table 11.** Spicule measurements of *Leucilla antillana* sp. nov. (holotype = UFRJPOR 6768)

Spicule	Actine	Length (µm)				Width (µm)				N
		Min	Mean	SD	Max	Min	Mean	SD	Max	
Cortical tetractine	Paired	305.0	404.4	62.0	485.0	35.0	50.1	6.4	60.0	17
	Unpaired	75.0	131.0	45.3	200.0	35.0	48.8	7.4	60.0	10
	Apical	285.0	405.0	64.6	550.0	35.0	49.2	7.1	55.0	19
Subatrial triactine	Paired	175.0	276.0	91.3	460.0	15.0	32.8	13.1	60.0	20
	Unpaired	225.0	389.4	71.2	490.0	15.0	34.4	13.4	55.0	18
Atrial tetractine	Paired	120.0	224.0	34.8	270.0	10.0	10.3	0.8	12.5	20
	Unpaired	185.0	265.0	41.9	355.0	10.0	10.5	1.0	12.5	20
	Apical	15.0	30.0	8.9	50.0	5.0	8.0	1.7	10.0	20

tetractines (these latter can be found scattered in the choanosome of those species), *L. antillana* sp. nov. does not have any microdiactines nor subatrial tetractines in its skeleton. Considering the spicule dimensions of the other spicule categories of *L. sacculata* and *L. uter* provided in their original descriptions, the size ranges do not match the new species. *Leucilla antillana* sp. nov. has thinner spicules (15.0–60.0 µm) compared to *L. sacculata* (84.7 µm). The apical actine of

the cortical tetractines of *L. uter* is almost twice as long (400.0–1200.0 µm) as that of *L. antillana* sp. nov. (285.0–550.0 µm) and the atrial tetractines are thicker (20.0 µm vs. 10.0–12.5 µm).

*Leucilla antillana* sp. nov. is the second species of the genus *Leucilla* recorded from Curaçao. *Leucilla amphora* was also reported from that island by Arndt (1927). That author mentioned that Breitfuss was responsible for the identification and did not give a



**Figure 15.** Bayesian phylogenetic tree inferred from the C-LSU sequences of the Calcaronean species. Posterior probabilities and bootstrap values are given on the branches (pp/bootstrap). \*Sequences generated in this study.

description of the samples. Therefore, as we did not analyse those specimens, we can neither confirm nor invalidate that record.

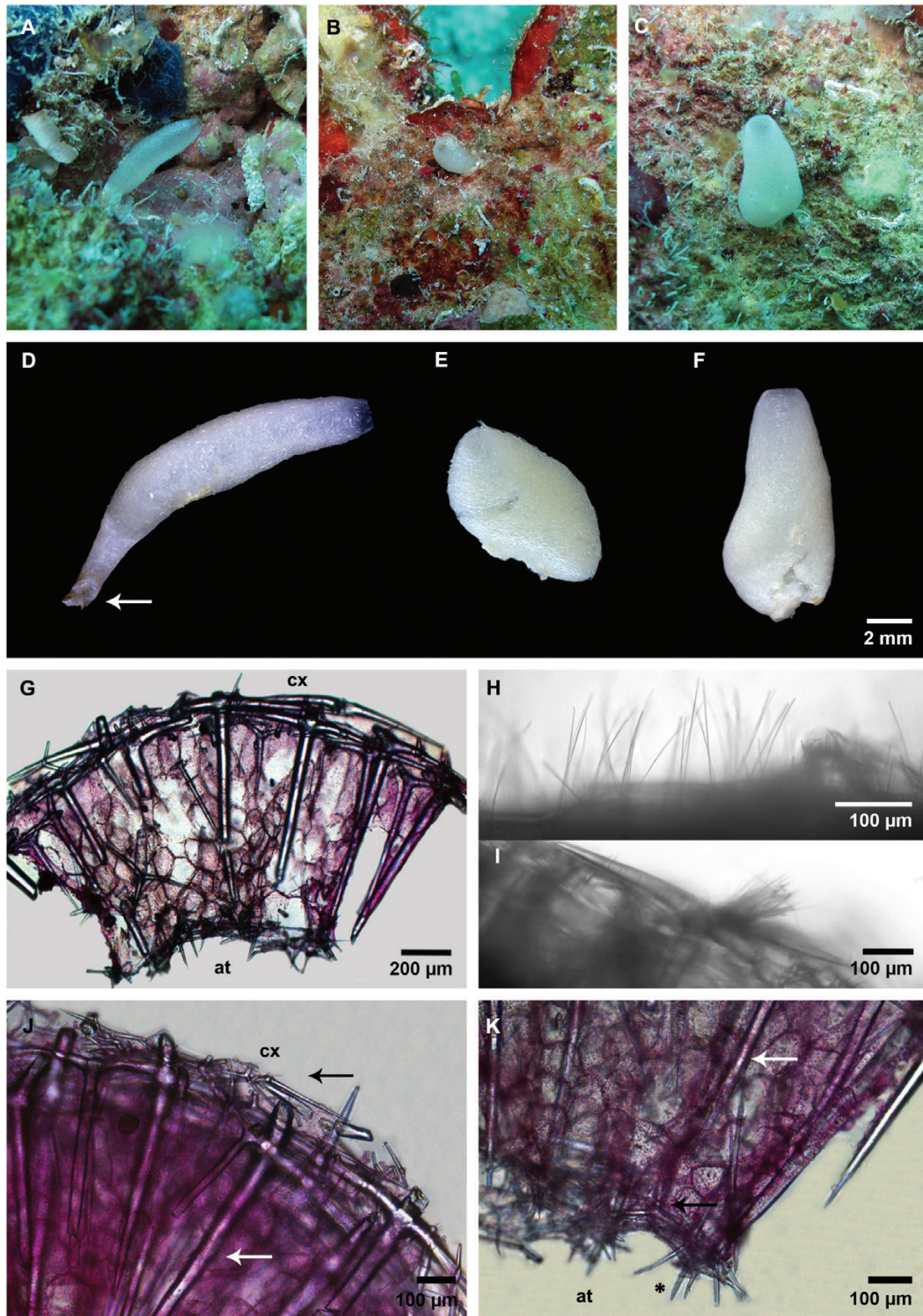
**LEUCILLA MICROPILOSA SP. NOV.**

(FIGS 16, 17; TABLE 12)

*Etymology:* From the Latin *pilosus* (=hairy), for the presence of microdiactines crossing the cortex.

*Type locality:* Tug Boat, Caracasbaai, Willemstadt, Curaçao.

*Material examined:* *Holotype.* UFRJPOR 6755, Tug Boat, Caracasbaai, Willemstadt, Curaçao (12°04'08.20"N, 68°51'44.40"W), 8.6 m depth, coll. B. Córdor-Luján, 23 August 2011. *Paratypes.* UFRJPOR 6739, Sunset Waters, Soto, Curaçao (12°16'01.58"N, 69°07'44.85"W), 13.1 m depth, coll. B. Córdor-Luján, 22 August 2011. UFRJPOR



**Figure 16.** *Leucilla micropilosa* sp. nov. A–C, UFRJPOR 6756, UFRJPOR 6739 and UFRJPOR 6755 in vivo. D–F, UFRJPOR 6756, UFRJPOR 6739 and UFRJPOR 6755 after fixation. G, cross section of the skeleton. H, microdiactines along the surface. I, tufts of microdiactines in UFRJPOR 6739. J, cortical skeleton showing triactines (black arrow) and apical actine of a subcortical tetractine (white arrow). K, choanosomal and atrial skeletons showing the apical actine of a subcortical tetractine (white arrow), unpaired actine of a subatrial triactine (black arrow) and apical actine of an atrial tetractine (\*). Skeleton photos were taken from UFRJPOR 6755, except when indicated. cx, cortex; at, atrium.



6756, Tug Boat, Caracasbaai, Willemstadt, Curaçao (12°04'08.20"N, 68°51'44.40"W), 8.6 m depth, coll. B. Córdor-Luján, 23 August 2011.

**Diagnosis:** *Leucilla* with a skeleton composed of cortical microdiactines, triactines and tetractines, subatrial triactines and atrial tetractines. The mean width of the cortical triactines varies from 12.0 to 14.4 µm. The mean length of the apical actine of the atrial tetractines varies from 49.7 to 78.8 µm. The aquiferous system is sylleibid.

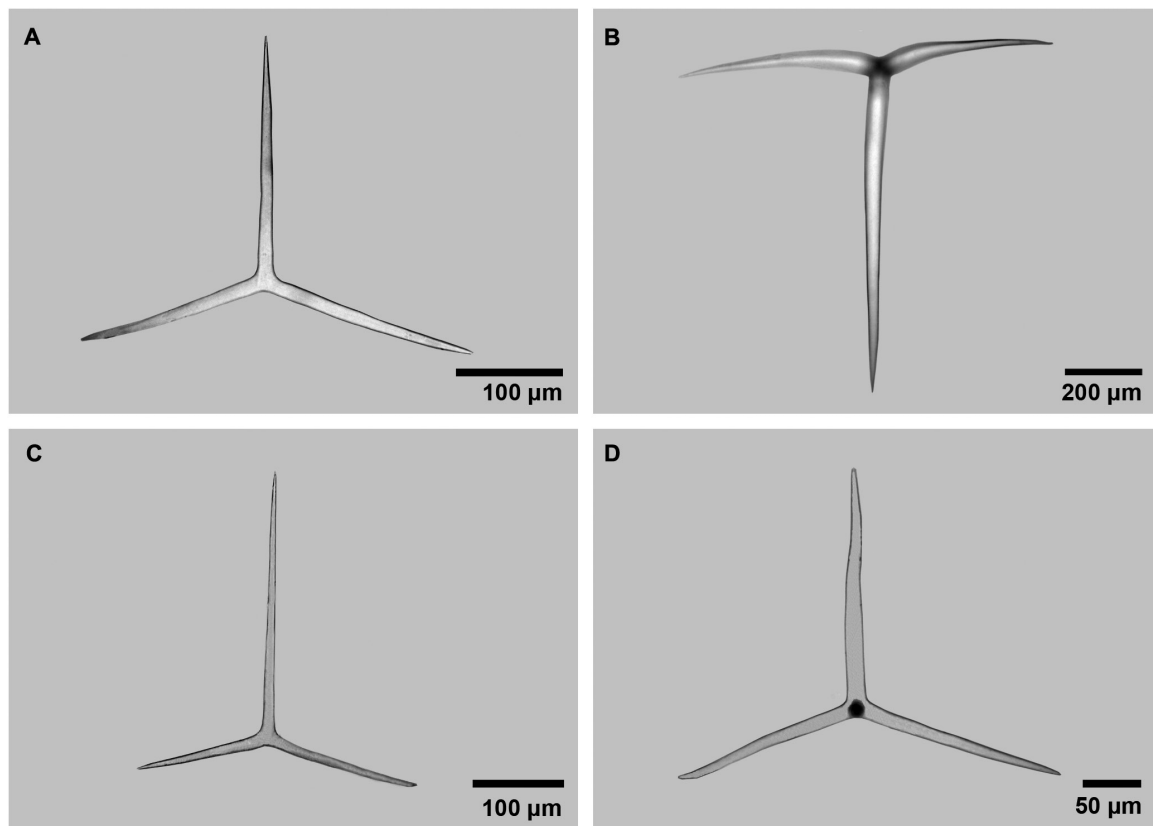
**Colour:** White to light blue in life (Fig. 16A–C) and white in ethanol (Fig. 16D–F). Transparent and bright.

**Morphology and anatomy:** This sponge has a variable external morphology (Fig. 16A–F), which can be tubular (Fig. 16A, D) to flattened sac-shaped (Fig. 16C, F) but always with an apical osculum. The surface is smooth, although (thin) microdiactines protrude through the surface. The consistency is rough. The holotype (UFRJPOR 6755) measures 1.2 × 0.5 × 0.2 cm. The osculum has a margin sustained by T-shaped triactines and it is surrounded by short

trichoxeas. The aquiferous system is sylleibid (Fig. 16G) with subspherical to elongated choanocyte chambers ranging from 62.2/54.1 to 135.1/81.1 µm. The aquiferous system is sylleibid.

**Skeleton:** The skeleton is typical of the genus (Fig. 16G). The cortical skeleton is composed of microdiactines perpendicularly positioned on the surface (Fig. 16H) or organized in tufts (only in UFRJPOR 6739, Fig. 16I), triactines and the basal actines of the tetractines. The triactines are distributed tangentially to the surface (Fig. 16J, arrow). The choanosomal skeleton is inarticulated, composed of the apical actines of the cortical tetractines and by rare subatrial triactines. The apical actine of the tetractines crosses the choanosome and occasionally reaches the atrium (Fig. 16J, K, white arrows). The unpaired actine of the subatrial triactines points toward the cortex (Fig. 16K, black arrow). The atrial skeleton is exclusively composed of tetractines with their apical actine protruding into the atrial cavity (Fig. 16K, asterisk).

**Spicules:** *Microdiactines*. Straight with sharp tips. Size: 27.0–94.5/1.1–1.4 µm. *Cortical triactines*.



**Figure 17.** Spicules of *Leucilla micropilosa* sp. nov. (UFRJPOR 6755). A, cortical triactine. B, subcortical tetractine. C, subatrial triactine. D, atrial tetractine.

**Table 12.** Spicule measurements of *Leucilla micropilosa* sp. nov. from Curaçao (holotype = UFRJPOR 6755 and paratypes = UFRJPOR 6755 and UFRJPOR 6756)

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	Mean	SD	Max	Min	Mean	SD	Max	
UFRJPOR 6739	Microdiactine		51.3	75.6	16.8	94.5	1.1	1.3	0.1	1.4	7
	Cortical triactine	Paired	102.6	204.0	51.7	310.5	8.1	14.4	2.8	18.9	14
		Unpaired	110.7	229.5	54.5	283.5	6.7	14.1	3.0	18.9	15
	Subcortical tetractine	Paired	356.4	475.2	71.1	572.4	32.4	52.9	9.2	64.8	20
		Unpaired	216.0	392.0	127.2	540.0	48.6	54.5	4.7	64.8	10
		Apical	162.0	449.6	158.8	658.8	21.6	49.7	12.4	64.8	19
	Subatrial triactine	Paired	81.0	177.5	46.7	240.3	8.1	13.7	3.7	21.6	20
		Unpaired	91.8	186.3	50.5	243.0	8.1	12.4	2.6	16.2	20
	Atrial tetractine	Paired	167.4	205.2	29.8	264.6	12.2	17.1	3.6	24.3	9
Unpaired		140.4	228.6	45.2	297.0	12.2	15.6	3.0	21.6	9	
Apical		51.3	65.1	11.4	81.0	8.1	11.5	1.9	13.5	8	
UFRJPOR 6755	Microdiactine		27.0	51.3	22.7	81.0	1.1	1.2	0.2	1.4	4
	Cortical triactine	Paired	113.4	174.2	32.3	240.3	8.1	14.0	2.6	18.9	20
		Unpaired	89.1	221.1	64.9	310.5	8.1	14.4	3.2	18.9	20
	Subcortical tetractine	Paired	172.8	223.2	62.6	367.2	21.6	30.5	7.5	43.2	14
		Unpaired	183.6	229.0	54.8	324.0	32.4	40.0	9.0	54.0	5
		Apical	291.6	491.8	217.8	1036.8	21.6	35.7	11.4	54.0	13
	Subatrial triactine	Paired	86.4	165.4	41.8	232.2	8.1	14.0	2.5	18.9	20
		Unpaired	135.0	242.2	61.6	351.0	9.5	13.7	2.4	18.9	20
	Atrial tetractine	Paired	113.4	173.3	34.1	229.5	10.8	14.0	1.7	16.2	20
Unpaired		83.7	200.0	56.6	283.5	10.8	15.6	2.5	21.6	19	
Apical		27.0	49.7	16.2	78.3	8.1	11.1	1.8	13.5	19	
UFRJPOR 6756	Microdiactine		45.0	61.7	16.7	87.5	1.0	1.2	0.1	1.2	6
	Cortical triactine	Paired	107.5	157.3	23.6	212.5	10.0	12.0	1.5	17.5	27
		Unpaired	157.5	195.4	23.0	230.0	10.0	12.2	0.8	12.5	26
	Subcortical tetractine	Paired	250.0	363.9	67.9	510.0	25.0	39.3	5.9	50.0	23
		Unpaired	250.0	279.0	23.3	315.0	30.0	37.0	4.5	40.0	5
		Apical	325.0	555.0	110.4	740.0	30.0	42.3	7.0	55.0	30
	Subatrial triactine	Paired	125.0	160.8	23.5	207.5	12.5	12.9	0.9	15.0	9
		Unpaired	255.0	346.3	110.2	610.0	10.0	13.8	2.4	18.8	15
	Atrial tetractine	Paired	100.0	142.3	25.4	212.5	10.0	11.9	1.7	15.0	27
Unpaired		125.0	184.7	43.4	250.0	10.0	12.4	1.8	17.5	22	
		Apical	55.0	78.8	17.8	115.0	10.0	10.3	0.8	12.5	10

Sagittal. Actines are smooth, conical, with sharp tips (Fig. 17A). Sometimes the paired actines are curved. Size: 102.6–310.5/8.1–18.9 µm (paired actine), 89.1–310.5/6.7–18.9 µm (unpaired actine). *Subcortical tetractines*. Sagittal. Actines are straight, smooth, conical, with sharp tips (Fig. 17B). The apical actine is very large. These are the largest spicules in this species. Size: 172.8–572.4/21.6–64.8 µm (paired actine), 183.6–540.0/30.0–64.8 µm (unpaired actine), 162.0–1036.8/21.6–64.8 µm (apical actine). *Subatrial triactines*. Sagittal. Actines are conical, smooth, straight, with sharp tips (Fig. 17C). The paired actines are shorter than the unpaired one and some are slightly curved. Sometimes, one paired actine is longer than the other.

Size: 81.0–240.3/8.1–21.6 µm (paired actine), 91.8–610.8/8.1–18.9 µm (unpaired actine). *Atrial tetractines*. Sagittal. Actines are conical, straight, smooth and have sharp tips. The unpaired actine is slightly longer than the paired ones (Fig. 17D). The apical actine is the shortest actine. Size: 100.0–264.6/10.0–24.3 µm (paired actine), 83.7–297.0/10–21.6 µm (unpaired actine), 27.0–115.0/8.1–13.5 µm (apical).

*Ecology*: Specimens from Curaçao were found underneath broken coral boulders between 8 and 13 m depth. No associated organisms were found. Some balls of sediment were found inside the atrial cavity of one of the specimens (UFRJPOR 6739).

*Geographical distribution:* Southern Caribbean (provisionally endemic to Curaçao, present study).

*Molecular analysis:* We provide the second DNA sequence for *Leucilla*, *L. micropilosa* sp. nov. In the C-LSU tree of Calcaronea (Fig. 15), none of the new *Leucilla* species, *L. antillana* sp. nov. or *L. micropilosa* sp. nov., clustered together with the other sequences of the Amphoriscidae family, namely, *Paraleucilla dalmatica* Klautau, Imešek, Azevedo, Pleše, Nikolić & Četković, 2016 and *P. magna* Klautau, Monteiro & Borojevic, 2004, supporting the non-monophyly of this family.

*Taxonomic remarks:* Although the sequence of *L. micropilosa* sp. nov. did not group with *L. antillana* sp. nov., we rather maintain this species as *Leucilla* following the current classification of the subclass Calcaronea (Borojevic, Boury-Esnault & Vacelet, 2000), which is based on morphological characters. However, a revision of the calcaronean genera, including *Leucilla*, is urgent. The descriptions and sequences of *L. antillana* sp. nov. and *L. micropilosa* sp. nov. provided herein will certainly contribute to an integrated revision of this genus.

*Leucilla* now comprises 15 valid species and among them, the species that resemble the most *Leucilla micropilosa* sp. nov. are *L. antillana* sp. nov. (described in the previous section) and *L. amphora sensu* Borojevic & Boury-Esnault (1987).

*Leucilla micropilosa* sp. nov. can be easily distinguished from *L. antillana* sp. nov. because the skeleton of the former has cortical triactines and microdiactines, whereas in the latter, those spicule categories are absent.

*Leucilla amphora* was originally described by Haeckel (1872), who analysed specimens from Puerto Rico and Barbados. Haeckel (1872) characterized the skeleton of *L. amphora* as being exclusively composed of tetractines. However, in the redescription of this species (Borojevic & Boury-Esnault, 1987), based on the examination of several specimens from Dakar (Senegal) and on Haeckel's slides, they mentioned the presence of cortical and subatrial triactines as well as some microdiactines ('microxeas').

We compared *L. micropilosa* sp. nov. with *L. amphora sensu* Borojevic & Boury-Esnault (1987) as they have similar external morphology and skeletal composition but they have slightly different spicule size and aquiferous system. Regarding spicule dimensions, *L. micropilosa* sp. nov. has thinner cortical triactines (holotype: paired actine =  $14.4 \pm 2.8 \mu\text{m}$  and unpaired actine =  $14.1 \pm 3.0 \mu\text{m}$ ) and atrial tetractines with longer apical actines (holotype:  $65.1 \pm 11.4 \mu\text{m}$ )

compared to *L. amphora sensu* Borojevic & Boury-Esnault (1987) ( $16.0\text{--}25.0$  and  $23.0\text{--}32.0 \mu\text{m}$ , respectively). In *L. amphora sensu* Borojevic & Boury-Esnault (1987), choanocyte chambers are positioned between the inhalant and exhalant system, whereas in *L. micropilosa* sp. nov. it is not possible to observe this arrangement.

FAMILY GRANTIIDAE DENDY, 1893  
GENUS *LEUCANDRA* HAECKEL, 1872

*Type species:* *Sycinula egedii* Schmidt, 1870.

*Diagnosis:* 'Grantiidae with sylleibid or leuconoid organization. Longitudinal large diactines, if present, are not restricted to the cortex, but lie obliquely across the external part of the sponge wall and protrude from the surface of the sponge' (Borojevic *et al.*, 2002).

***LEUCANDRA CARIBEA* SP. NOV.**  
(FIGS 18–20; TABLE 13)

*Etymology:* Named after its distribution in the Caribbean Sea.

*Type locality:* Tug Boat, Caracasbaai, Willemstadt, Curaçao.

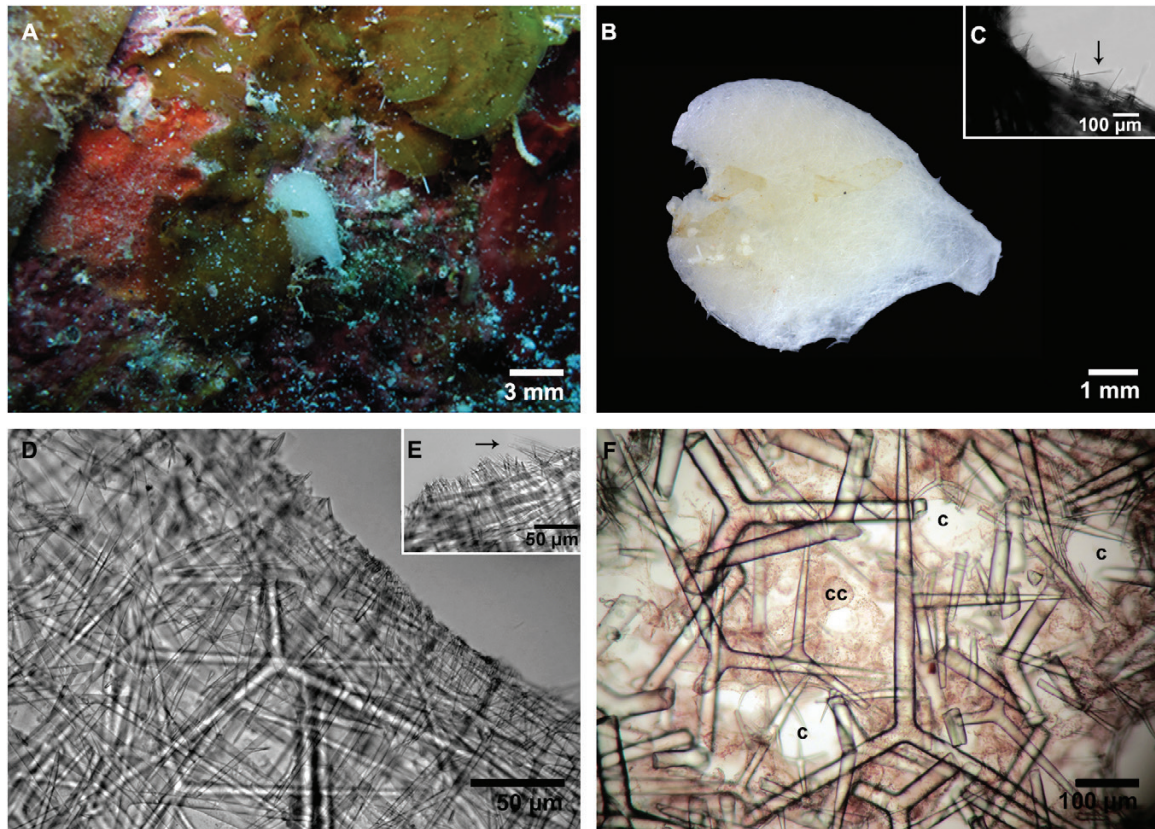
*Material examined:* *Holotype.* UFRJPOR 6754, Tug Boat, Caracasbaai, Willemstadt, Curaçao ( $12^{\circ}04'08.20''\text{N}$ ,  $68^{\circ}51'44.40''\text{W}$ ), 13.9 m depth, coll. B. Córdor-Luján, 23 August 2011.

*Diagnosis:* *Leucandra* with a sac-shaped body and leuconoid aquiferous system. The skeleton is mostly formed by triactines with some tetractines lining the choanosomal canals and atrial cavity. The choanosomal skeleton is composed of one category of triactines. Cortical trichoxeas are also present.

*Colour:* White in life (Fig. 18A) and beige in ethanol (Fig. 18B).

*Morphology and anatomy:* This species has a sac-shaped external morphology: it is wide at the base and becomes narrower near the osculum (Fig. 18A). It measures  $0.7 \times 0.3 \times 0.1 \text{ cm}$  (Fig. 18B). This sponge is quite smooth and compressible. Near the suboscular region there are short trichoxeas protruding through the surface (Fig. 18C). The osculum is apical. It is supported by triactines, tetractines and has a discrete crown of trichoxeas (Fig. 18D, E). The aquiferous





**Figure 18.** *Leucandra caribea* sp. nov. (UFRJPOR 6754). A, specimen in vivo. B, specimen after fixation. C, detail of trichoxeas (arrow) along the surface. D, tangential section of the oscular region. E, detail of the oscular trichoxeas (arrow). F, cross section of the skeleton. c, choanosomal canals; cc, choanocyte chambers.

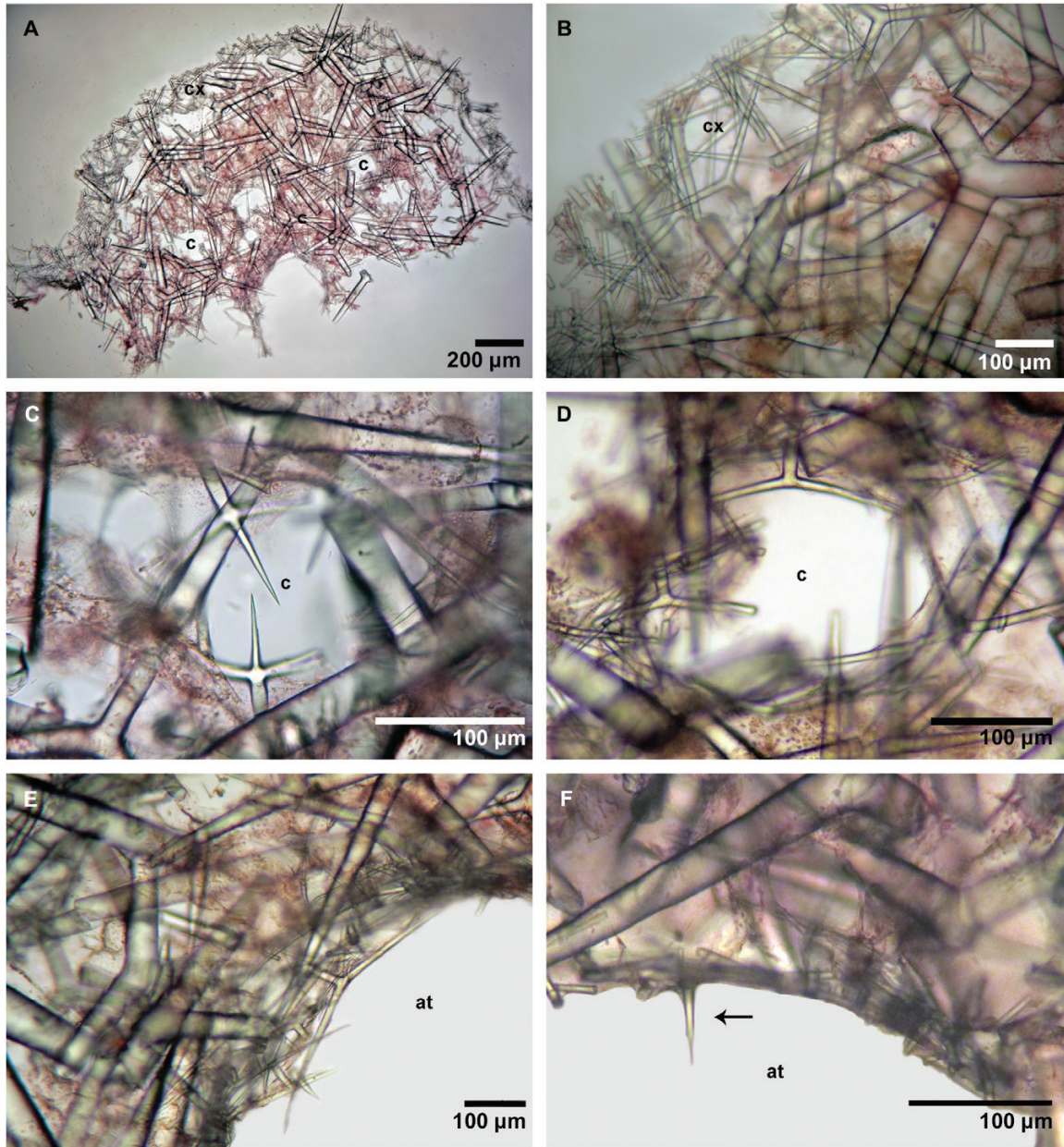
system is leuconoid with subspherical choanocyte chambers ranging from 28.0 to 34.0 µm in diameter (Fig. 18F). The diameter of the exhalant canals varies from 140.0 to 300.0 µm.

**Skeleton:** The skeleton is typical of the genus (Fig. 19A). As mentioned before, the oscular margin has a differentiated skeleton. It is composed of T-shaped triactines and tetractines tangentially positioned and short trichoxeas perpendicular to the surface. The cortical skeleton is composed of tangential triactines (Fig. 19B). The choanosomal skeleton does not have any special organization and it is formed by triactines of variable sizes (as shown in Figs 18F, 19A). Several exhalant choanosomal canals were observed within this region. They are surrounded by tetractines with the apical actine projected inside them (Fig. 19C). Some triactines lining the canals were also found (Fig. 19D). No subatrial skeleton was observed. The atrial skeleton

is formed by triactines (Fig. 19E) and rare tetractines (Fig. 19F). The apical actine of the tetractines protrudes into the atrial cavity (arrow in Fig. 19F).

**Spicules:** *Cortical triactines.* Subregular or parasagittal. Actines are slightly conical with sharp tips. The paired actines are frequently curved and slightly longer than the unpaired one, which is always straight (Fig. 20A). Size: 110.0–340.0/7.5–16.3 µm (paired actine) and 105.0–325.0/7.5–16.3 µm (unpaired actine). *Choanosomal triactines.* Regular or subregular. Actines are conical with sharp tips (Fig. 20B). They are the largest spicules in *L. caribea* sp. nov. Highly variable size: 370–960/25–75 µm. *Triactines and tetractines of the canals.* Sagittal. Actines are conical with sharp tips. The paired actines are curved, following the shape of the canals (Fig. 20C, D). The apical actine of the tetractines is smooth and it is thinner than the basal actines (as shown in Fig. 19C). The size of the triactines is similar to that

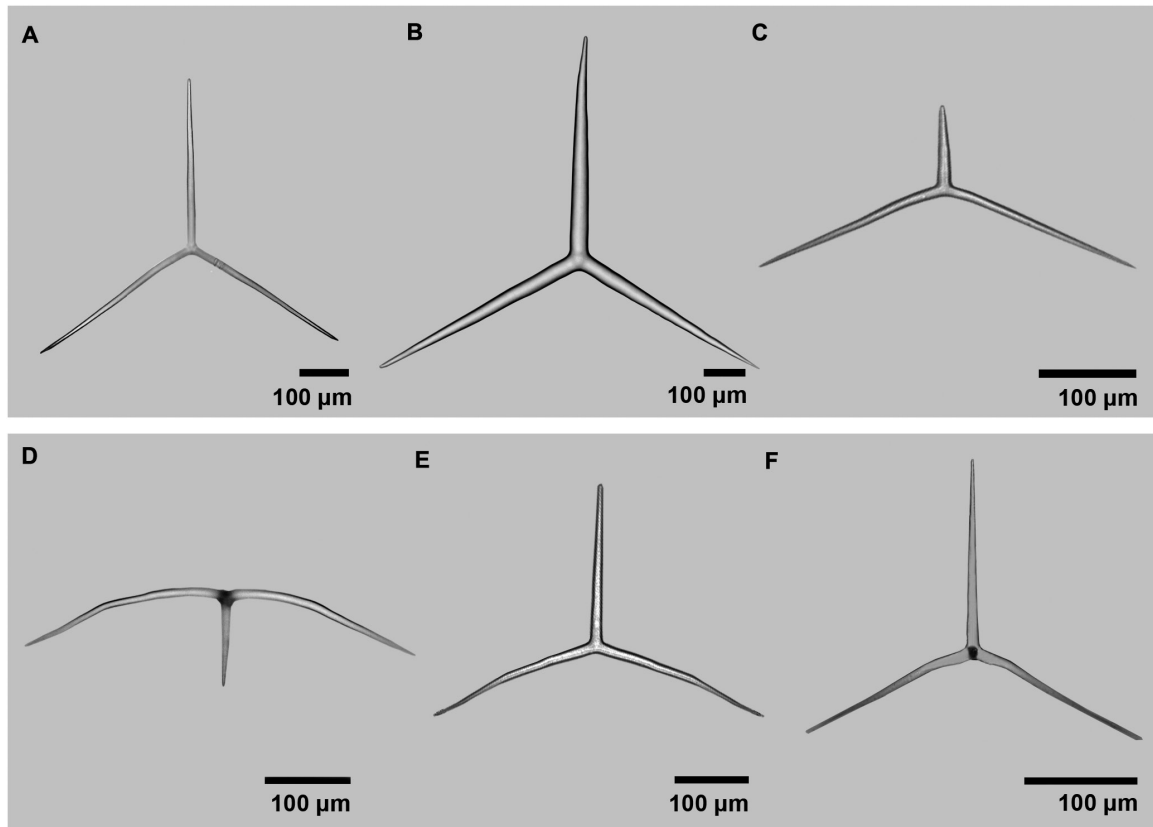




**Figure 19.** Skeleton of *Leucandra caribea* sp. nov. (UFRJPOR 6754). A, cross section of the skeleton. B, cortex. C, chaenosomal canal showing tetractines. D, chaenosomal canal showing triactines. E, atrial skeleton showing triactine. F, tetractine with the apical actine protruding into the atrial cavity (arrow). cx, cortex; c, chaenosomal canal; at, atrium.

of the tetractines. Size of tetractines: 112.5–220/7.5–12.5 µm (paired actine), 62.5–210.0/7.5–12.5 µm (unpaired actine) and 45.0–110.0/5.0–10 µm (apical actine). *Atrial triactines* (shown in Fig. 19E): Sagittal. Actines are conical with sharp tips. Compared to the cortical triactines, the atrial triactines are thinner and the angle formed by the paired actines is more

open. Size: 132.3–253.8/8.1–13.5 µm (paired actine) and 164.7–253.8/5.4–13.5 µm (unpaired actine). *Atrial tetractines*. Sagittal. Actines are conical with sharp tips (Fig. 20D). The apical actine is smooth. Size: 150.0–262.5/7.5–15.0 µm (paired actine), 162.0–297.0/8.1–16.2 µm (unpaired actine) and 35.0–132.5/7.5–12.5 µm (apical actine).



**Figure 20.** Spicules of *Leucandra caribea* sp. nov. (UFRJPOR 6754). A, cortical triactine. B, choanosomal triactine. C, triactine of choanosomal canals. D, tetractine of choanosomal canals. E, atrial triactine. F, atrial tetractine.

**Ecology:** This species was found underneath a coral boulder at 13.9 m depth. The basal region was attached to an algae (as shown in Fig. 18A, B).

**Geographical distribution:** Southern Caribbean (provisionally endemic to Curaçao, present study).

**Taxonomic remarks:** Among the species of *Leucandra* reported from the Caribbean Sea, namely *L. crustacea* (Haeckel, 1872), *L. barbata* (Duchassaing & Michelotti, 1864), *L. curva* (Schuffner, 1877), *L. multiformis* Poléjaeff, 1883, *L. rudifera* Poléjaeff, 1883, and *L. typica* (Poléjaeff, 1883) (Van Soest et al., 2017), only *L. typica* possesses a similar skeletal composition as that of *L. caribea* sp. nov. The skeletons of the other Caribbean species include cortical tetractines (*L. crustacea* and *L. curva*), diactines (*L. barbata*, *L. multiformis* and *L. rudifera*) and atrial grapple spicules (also in *L. rudifera*), which are spicule categories absent in *L. caribea* sp. nov.

*Leucandra caribea* sp. nov. can be differentiated from *L. typica* as the former species possesses an atrial skeleton mainly composed of triactines and few tetractines, whereas in the latter species, triactines and tetractines

are in the same proportion (or at least, Poléjaeff did not indicate the opposite). Besides, in the new species, the choanosomal canals are lined by tetractines and triactines and in *L. typica* they are only lined by tetractines. In *L. typica*, trichoxeas (<300.0/1.0 µm) are scattered in the choanosome and spindle-shaped microdiactines (100.0/4.0 µm) are concentrated in the subsclerular region, while in *L. caribea* sp. nov., trichoxeas (>100.0/1.2 µm) were found only in the subsclerular region.

Among the other species of *Leucandra* with skeletal composition and external morphology comparable to that of *L. caribea* sp. nov., *L. falakra* Klautau, Imešek, Azevedo, Pleše, Nikolić & Četković, 2016 from the Adriatic Sea is the one that most resembles the new species. Nonetheless, they have some important differences. The choanosomal skeleton of the Curaçaoan species is composed of one single type of triactine (370.0–960.0/25.0–75.0 µm) while in the Adriatic species, it is composed of small (paired actine: 94.5–180.9/8.1–13.5 µm and unpaired actine: 70.0–143.1/8.1–16.2 µm) and giant triactines (342.0–1047.6/48.6–118.8 µm). Additionally, although almost all the spicule categories have similar dimensions (Table 13), the unpaired actine of the atrial tetractines



**Table 13.** Spicule measurements of *Leucandra caribea* sp. nov. (holotype = UFRJPOR 6754) and of *L. falakra* (holotype = UFRJPOR 8349)

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	Mean	SD	Max	Min	Mean	SD	Max	
UFRJPOR 6754	Trichoxea (crown)					>105	1.1	1.2	0.2	1.6	15
	Trichoxea (cortex)					>108	1.1	1.2	0.2	1.6	10
	Cortical triactine	Paired	110.0	218.4	65.2	340.0	7.5	11.6	2.4	16.3	25
		Unpaired	105.0	219.3	63.7	325.0	7.5	12.6	2.4	16.3	25
	Choanosomal triactine		370.0	576.3	188.8	960.0	25.0	41.3	10.8	75.0	24
	Canal tetractine	Paired	112.5	179.3	32.0	220.0	7.5	9.8	1.8	12.5	10
		Unpaired	62.5	140.6	60.4	210.0	7.5	10.0	2.0	12.5	4
		Apical	45.0	70.9	19.0	110.0	5.0	7.9	1.3	10.0	14
	Atrial triactine	Paired	132.3	209.4	41.8	253.8	8.1	11.2	1.9	13.5	12
		Unpaired	164.7	210.6	35.0	253.8	5.4	10.1	3.4	13.5	6
	Atrial tetractine	Paired	150.0	206.9	28.7	262.5	7.5	11.6	1.9	15.0	20
		Unpaired	162.0	237.6	35.1	297.0	8.1	12.9	2.5	16.2	20
Apical		35.0	69.0	28.3	132.5	7.5	9.6	1.2	12.5	20	
UFRJPOR 8349	Cortical triactine	Paired	94.5	136.4	24.0	180.9	8.1	11.1	1.9	13.5	20
		Unpaired	70.2	106.0	18.8	143.1	8.1	11.4	2.4	16.2	20
	Cortical and choanosomal triactine		342.0	624.5	192.3	1047.6	48.6	81.5	20.6	118.8	23
	Choanosomal triactine	Paired	162.0	214.2	39.8	288.9	13.5	18.3	4.0	27.0	20
		Unpaired	108.0	189.7	58.9	351.0	13.5	19.8	4.1	29.7	20
	Canal tetractine	Paired	99.9	154.0	26.4	199.8	8.1	12.4	2.4	16.2	19
		Unpaired	45.9	143.0	56.5	288.9	9.5	12.4	1.9	16.2	19
		Apical	50.0	80.6	24.4	137.5	7.5	9.6	1.5	12.5	20
	Atrial triactine	Paired	140.4	222.7	33.7	294.3	9.5	15.1	2.5	20.3	30
		Unpaired	78.3	111.2	24.4	159.3	8.1	12.3	1.7	16.2	30
	Atrial tetractine	Paired	145.8	191.4	26.0	256.5	10.8	14.9	2.6	18.9	30
		Unpaired	59.4	92.0	22.1	126.9	10.8	13.1	1.7	16.2	16
Apical		67.5	110.3	30.3	162.0	8.1	11.9	2.8	16.2	15	

is shorter in *L. falakra* (59.4–126.9 µm) than in the new species (162.0–297.0 µm).

GENUS *LEUCANDRILLA* BOROJEVIC, BOURY-ESNAULT & VACELET, 2000

*Type species: Leucilla wasinensis* Jenkin, 1908.

*Diagnosis:* ‘Grantiidae with leuconoid organization. In addition to triactines the cortex contains tetractines, with the apical actines turned into the choanoderm. The articulated choanoskeleton is supported by subatrial triactine spicules, and numerous rows of choanosomal triactines and/or tetractines, with apical actines of cortical tetractines in the distal region’ (Borojevic *et al.*, 2000).

***LEUCANDRILLA QUADRIRADIATA* SP. NOV.**  
(FIGS 21–23; TABLE 14)

*Etymology:* Derived from the predominance of tetractines in the skeleton of the species.

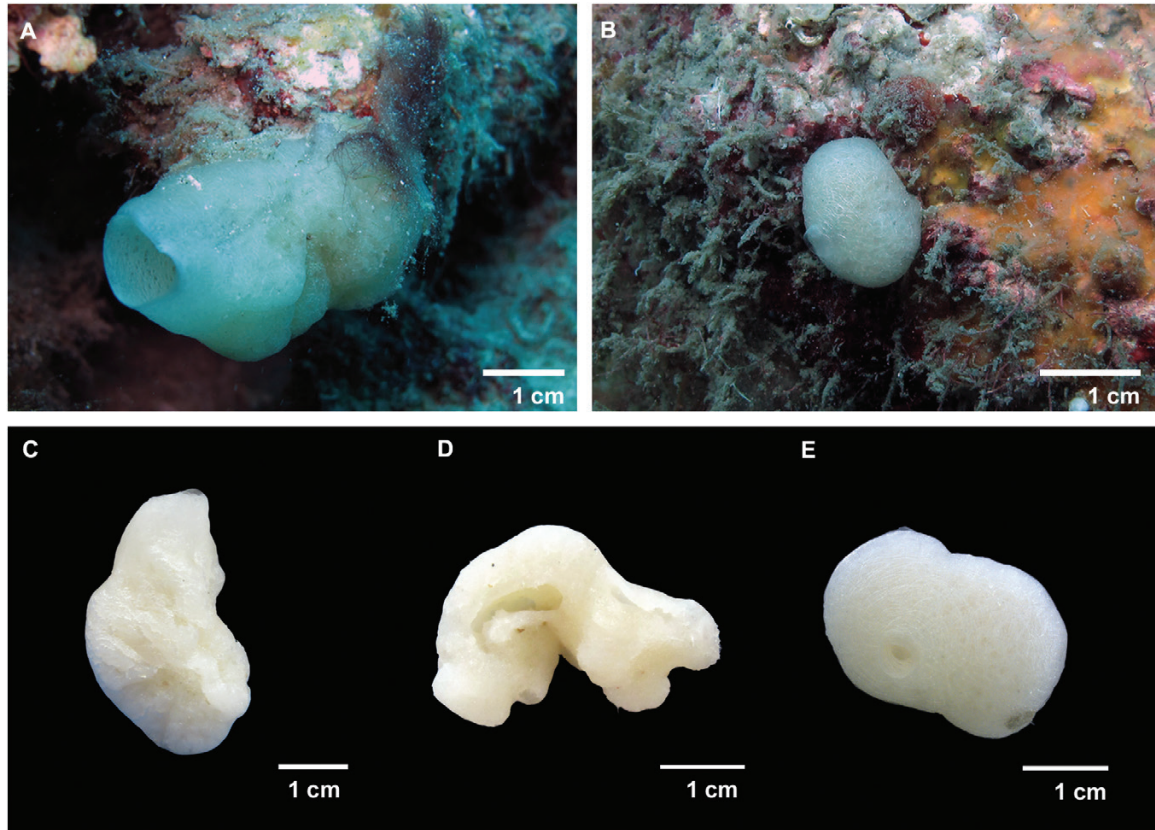
*Type Locality:* Water Factory, Willemstadt, Curaçao.

*Material examined:* *Holotype.* UFRJPOR 6705, Water Factory, Willemstadt, Curaçao (12°12′43.12″N, 69°05′8.42″W), 3–5 m depth, coll. B. Córdor-Luján, 18 August 2011. *Paratypes.* UFRJPOR 6696, Sunset Waters, Soto, Curaçao (12°07′18.94″N, 68°58′11.46″W), <10 m depth, coll. B. Córdor-Luján and G. Lôbo-Hajdu, 17 August 2011. UFRJPOR 6752, Tug Boat, Caracasbaai, Willemstadt, Curaçao (12°04′08.20″N, 68°51′44.40″W), 15.2 m depth, coll. E. Hajdu, 23 August 2011.

*Diagnosis:* *Leucandrilla* with a cylindrical body. The choanosomal and atrial skeletons are exclusively composed of tetractines.

*Colour:* White to light blue in life (Fig. 21A, B) and white to beige in ethanol (Fig. 21C–E).

*Morphology and anatomy:* This species has a cylindrical massive body (Fig. 21A–E). The holotype



**Figure 21.** *Leucandrilla quadriradiata* sp. nov. A–B, UFRJPOR 6732 and UFRJPOR 6696 in vivo. C–E, UFRJPOR 6732, UFRJPOR 6705 and UFRJPOR 6696 after fixation.

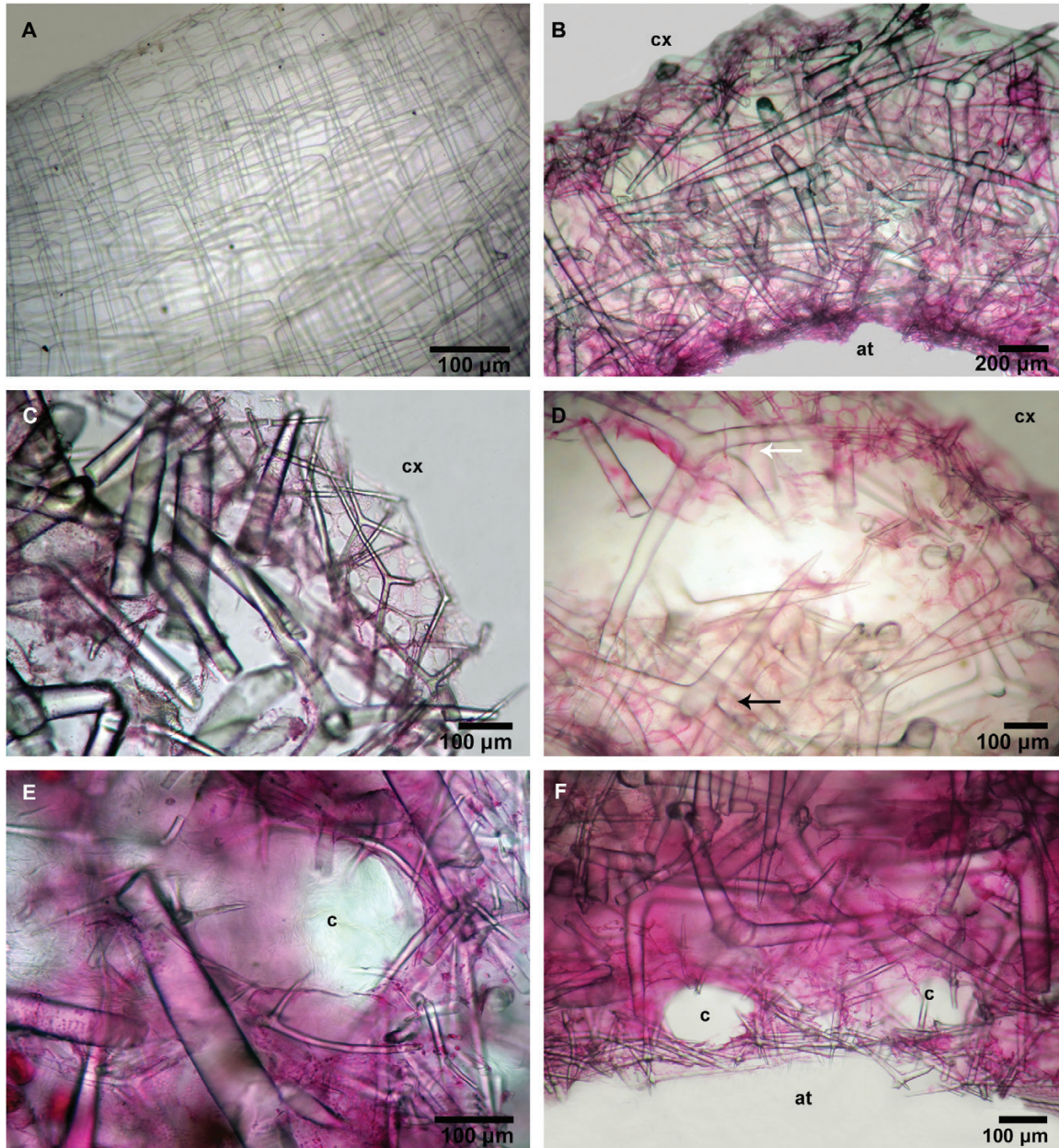
(UFRJPOR 6705) is fragmented ( $1.3 \times 1.0 \times 0.5$  cm). The largest specimen (paratype UFRJPOR 6752) measures  $3.8 \times 2.0 \times 0.8$  cm (Fig. 21C). The surface is slightly hispid and the consistency is hard. The osculum is apical and its diameter is 1.2 mm. The atrial cavity is not hispid. The aquiferous system is leuconoid with subspherical choanocyte chambers ranging from 75.6 to 162.0  $\mu\text{m}$  in diameter. The diameter of canals varied from 108.0 to 238.0  $\mu\text{m}$ .

**Skeleton:** In the three analysed specimens, the oscular margin is composed of T-shaped spicules including triactines and rare tetractines (Fig. 22A). The skeleton is not typical of the genus as it has rare pseudosagittal tetractines (Fig. 22B). The cortical skeleton is composed of triactines tangentially positioned on the surface (Fig. 22B, C) and of the basal actines of large sagittal tetractines. Some of them are pseudosagittal (Fig. 22D, white arrow). The apical actines of the cortical tetractines cross the choanosome and sometimes reach the atrial skeleton. The choanosomal skeleton is disorganized, formed by the same large tetractines of the cortex. The choanosomal canals are surrounded by small tetractines which have an apical actine projected

into them (Fig. 22E). The poorly developed subatrial skeleton is formed by rare tetractines (Fig. 22D, black arrow). The atrial skeleton is exclusively composed of tetractines with an apical actine which is projected into the atrial cavity (Fig. 22F).

**Spicules:** *Cortical triactines.* Sagittal. Actines are conical with blunt to sharp tips. Some paired actines are slightly curved (Fig. 23A). Highly variable size: 97.2–421.2/8.1–21.6  $\mu\text{m}$  (paired actine), 75.6–421.2/5.4–21.6  $\mu\text{m}$  (unpaired actine). *Cortical and choanosomal tetractines.* Sagittal. Actines are conical with sharp tips. The paired actines are slightly curved (Fig. 23B). Rare pseudosagittal-like tetractines were also observed (Fig. 23C). Size: 248.4–1188.0/27.0–75.6  $\mu\text{m}$  (paired actine), 162.0–564.0/27.0–64.8  $\mu\text{m}$  (unpaired actine) and 345.6–1122.0/21.6–75.6  $\mu\text{m}$  (apical actine). *Canal tetractines.* Sagittal. Actines are conical with sharp tips. The paired actines are curved following the shape of the canals and they are longer than the other actines (Fig. 23D). Size: 118.8–264.6/8.1–17.6  $\mu\text{m}$  (paired actine), 70.2–213.3/8.1–17.6  $\mu\text{m}$  (unpaired actine) and 40.5–132.3/8.1–13.5  $\mu\text{m}$  (apical actine). *Subatrial tetractines.* Sagittal. Actines are conical





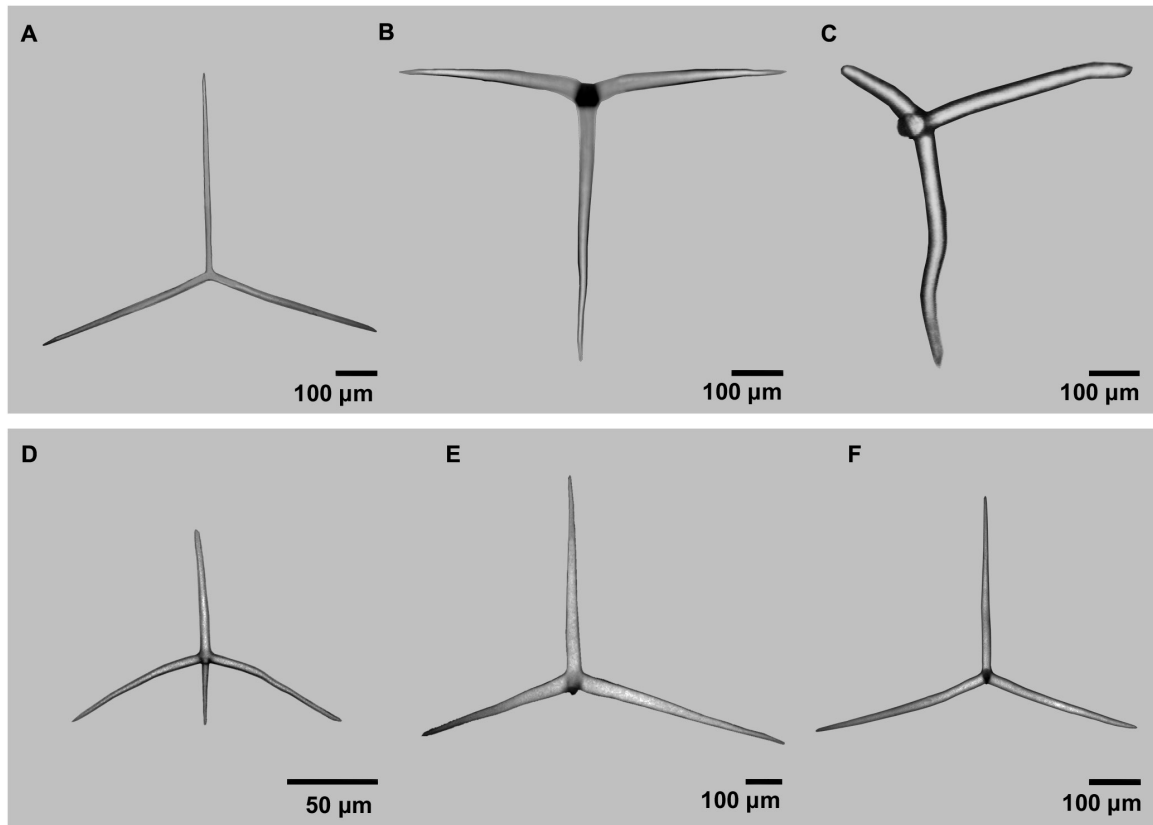
**Figure 22.** Skeleton of *Leucandrilla quadriradiata* sp. nov. (UFRJPOR 6705). A, osculum. B, cross section of the skeleton. C, cortex. D, choanoskeleton indicating the cortical tetractine (white arrow) and the subatrial tetractine (black arrow). E, choanosomal canal showing apical actines of tetractines. F, atrial skeleton. cx, cortex; at, atrium; c, choanosomal canal.

with sharp tips (Fig. 23E). The apical actine is shorter than the basal ones. Size: 205.2–993.6/21.6–75.6 µm (paired actine), 205.2–766.8/21.6–70.2 µm (unpaired actine) and 129.6–432.0/16.2–54.0 µm (apical actine). *Atrial tetractines.* Sagittal. Actines are conical with blunt to sharp tips (Fig. 23F). Sometimes, the paired actines are slightly curved. The apical actine is conical, shorter than the basal ones, smooth and has sharp tip. Size: 67.5–391.5/8.1–21.6 µm (paired actine),

59.4–270.0/8.1–21.6 µm (unpaired actine) and 21.6–108.0/6.4–13.5 µm (apical actine).

*Ecology:* The three specimens were collected in light-protected habitats at depths varying from 5 to 15 m. The holotype (UFRJPOR 6705) and one of the paratypes (UFRJPOR 6696) were collected underneath boulders. A polychaete was found inside one of the paratypes (UFRJPOR 6752).





**Figure 23.** Spicules of *Leucandrilla quadriradiata* sp. nov. (UFRJPOR 6705). A, cortical triactine. B, cortical tetractine. C, pseudosagittal cortical tetractine. D, tetractine of a canal. E, subatrial tetractine. F, atrial tetractine.

**Geographical distribution:** Southern Caribbean (provisionally endemic to Curaçao, present study).

**Molecular analysis:** The C-LSU sequences of the specimens of *L. quadriradiata* sp. nov., UFRJPOR 6696 (paratype) and UFRJPOR 6705 (holotype), clustered together in a monophyletic clade with high support (pp = 1, b = 99, Fig. 15). The genetic variability between them was 0%.

*Leucandrilla quadriradiata* sp. nov. appeared as a new lineage in a large clade recovered with high support values (pp = 1, b = 90). This clade was composed of one Grantiidae, *Leucandra nicolae* Wörheide & Hooper, 2003 and two Amphoriscidae *P. dalmatica* and *P. magna*. Within this clade, *P. magna* appeared as the sister species of *L. quadriradiata* sp. nov. (pp = 1, b = 91) with 2.2% of p distance.

**Taxonomic remarks:** The identification of this new species as *Leucandrilla* is not very clear. Pseudosagittal spicules are expected to be present only in the family Heteropiidae, and in that case, our new species would be closer to *Vosmaeropsis* Dendy, 1893. However, large cortical tetractines are not expected to be found

in that genus, instead they are characteristic of the family Amphoriscidae. Within that family, we would expect to allocate the new species to *Leucilla*, as it has leuconoid aquiferous system and the subatrial skeleton is adjacent to the atrial one. Nonetheless, the new species does not have an inarticulated skeleton, which is a diagnostic characteristic of *Leucilla*.

In the absence of phylogenetic studies indicating which morphological characters are good for the systematics of Calcaronea, we decided to consider the current morphological systematics and to place the new species in *Leucandrilla*, taking into account [Borojevic et al. \(2000\)](#): ‘In any calcaronean sponge with a strong cortex, some subcortical spicules may be in the position and have the shape of pseudosagittal spicules, due to the restriction of their growth by the rigidity of the cortical skeleton. They should not be interpreted as an indication that the sponge belongs to the family Heteropiidae’.

According to [Borojevic et al. \(2002\)](#), three species belong to the genus *Leucandrilla*: *L. intermedia* (Row, 1909) from the Red Sea, *L. lanceolata* (Row & Hôzawa, 1931) from Southwestern Australia and *L. wasinensis* from East Africa. Herein, we propose to transfer some

**Table 14.** Spicule measurements of *Leucandrilla quadriradiata* sp. nov. (holotype = UFRJPOR 6705 and paratypes = UFRJPOR 6696 and UFRJPOR 6752)

Specimen	Spicule	Actine	Length				Width				N
			Min	Mean	SD	Max	Min	Mean	SD	Max	
UFRJPOR 6696	Cortical triactine	Paired	97.2	273.2	77.4	399.6	8.1	14.9	3.6	21.6	20
		Unpaired	97.2	207.4	70.3	345.6	5.4	14.1	4.3	21.6	20
	Cortical tetractine	Paired	248.4	543.8	161.5	810.0	27.0	43.5	10.3	64.8	20
		Unpaired	162.0	271.1	90.0	399.6	32.4	37.8	5.7	43.2	10
		Apical	367.2	541.8	104.2	702.0	21.6	38.4	10.9	64.8	17
	Canal tetractine	Paired	118.8	200.7	39.9	256.5	8.1	12.6	2.8	17.6	20
		Unpaired	97.2	135.8	25.4	189.0	8.1	12.4	2.6	17.6	20
		Apical	54.0	89.4	23.1	124.2	8.1	10.2	1.8	13.5	20
	Subatrial tetractine	Paired	205.2	522.7	172.1	993.6	21.6	44.8	12.3	75.6	20
		Unpaired	205.2	388.3	113.8	637.2	21.6	35.4	7.9	48.6	20
		Apical	129.6	276.9	87.8	432.0	16.2	33.2	8.2	43.2	14
	Atrial tetractine	Paired	67.5	201.1	57.5	297.0	8.1	13.1	3.2	18.9	19
		Unpaired	59.4	187.5	54.3	240.3	8.1	12.6	3.4	18.9	11
		Apical	32.4	62.9	23.0	105.3	6.4	9.3	2.1	14.9	11
	UFRJPOR 6705	Cortical triactine	Paired	129.6	307.3	85.0	410.4	9.5	13.8	3.2	21.6
Unpaired			129.6	261.4	65.3	367.2	9.5	14.0	3.4	21.6	12
Cortical tetractine		Paired	259.2	639.4	211.0	972.0	27.0	54.3	13.5	70.0	20
		Unpaired	162.0	350.1	103.3	464.4	27.0	39.6	11.4	64.8	12
		Apical	345.6	511.2	147.7	864.0	37.8	51.3	8.7	70.2	20
Canal tetractine		Paired	143.1	210.7	32.1	264.6	8.1	12.5	2.5	17.6	20
		Unpaired	97.2	143.3	29.1	205.2	9.5	13.1	2.3	17.6	18
		Apical	62.1	86.4	16.7	124.2	8.1	9.6	1.5	13.5	20
Subatrial tetractine		Paired	259.2	489.8	207.9	864.0	32.4	48.3	13.2	70.2	20
		Unpaired	216.0	405.0	131.6	669.6	32.4	48.3	11.8	70.2	20
		Apical	129.6	279.0	99.5	432.0	21.6	35.1	9.4	54.0	12
Atrial tetractine		Paired	135.0	230.0	69.4	391.5	8.1	13.8	3.5	21.6	20
		Unpaired	94.5	164.4	43.8	264.6	9.5	13.4	3.1	21.6	20
		Apical	27.0	46.4	14.0	64.8	8.1	9.5	2.3	13.5	19
UFRJPOR 6752		Cortical triactine	Paired	118.8	276.5	89.3	421.2	10.8	15.0	2.7	20.3
	Unpaired		75.6	242.5	84.9	421.2	12.2	15.1	2.3	18.9	20
	Cortical tetractine	Paired	313.2	689.6	222.4	1188.0	32.4	52.9	11.0	75.6	20
		Unpaired	194.4	360.7	134.3	594.0	32.4	36.7	7.1	54.0	10
		Apical	367.2	693.6	232.2	1122.0	32.4	51.3	11.0	75.6	20
	Canal tetractine	Paired	118.8	186.4	46.5	245.7	8.1	12.0	2.4	16.2	20
		Unpaired	70.2	128.5	41.2	213.3	8.1	10.9	2.6	16.2	20
		Apical	40.5	81.7	23.5	132.3	8.1	10.2	1.7	13.5	20
	Subatrial tetractine	Paired	280.8	518.4	191.1	853.2	32.4	46.5	10.7	59.4	13
		Unpaired	302.4	502.7	167.1	766.8	32.4	44.7	9.4	54.0	11
		Apical	216.0	332.6	66.1	432.0	27.0	40.0	10.6	54.0	10
	Atrial tetractine	Paired	153.9	205.9	48.0	332.1	9.5	13.8	3.0	21.6	29
		Unpaired	126.9	202.8	40.9	270.0	9.5	13.8	3.1	21.6	24
		Apical	21.6	60.5	23.0	108.0	6.7	9.8	1.9	14.9	30

species of the genus *Leucandra* to *Leucandrilla* as their original descriptions match the current accepted diagnosis of *Leucandrilla*. Those species are: *L. connectens* Brønsted, 1927 from New Zealand, *L. fernandensis* (Breitfuss, 1898) from Juan Fernández Islands (Chile), *L. ovata* (Poléjaeff, 1883) from Kerguelen Islands, *L. pallida* Row & Hôzawa, 1931 and *L. thulakomorpha*

Row & Hôzawa, 1931 from Southwestern Australia and *L. tropica* Tanita, 1943 from Japan. Among these nine species, none has the same skeletal composition as *L. quadriradiata* sp. nov. (Table 15). The new species can be easily differentiated from the others as it is the only *Leucandrilla* with chaonosomal and atrial skeletons exclusively composed of tetractines.

**Table 15.** Skeletal composition of *Leucandra* species proposed to be transferred to the genus *Leucandrilla*

Species	Cortex	Choanosome	Atrium
<i>Leucandra connectens</i>	Triactines Tetractines	Triactines Diactines Tetractines	Triactines Diactines Tetractines
<i>Leucandra fernandensis</i>	Tetractines Diactines Microdiactines Trichoxeas	Triactines	Triactines (rare) Tetractines
<i>Leucandra ovata</i>	Triactines Tetractines (rare) Microdiactines	Triactines	Triactines Microdiactines
<i>Leucandra pallida</i>	Triactines Tetractines Diactines Microdiactines	Triactines Tetractines	Tetractines Microdiactines
<i>Leucandra thulakomorpha</i>	Triactines Tetractines Diactines Trichoxeas	Triactines	Tetractine (subatrial, few) Tetractines
<i>Leucandra tropica</i>	Triactines Tetractines Diactines	Triactines	Triactines (subatrial) Triactines Tetractines

FAMILY HETEROPIIDAE DENDY, 1893  
GENUS *GRANTESSA* LENDENFELD, 1885

*Type species: Grantessa sacca* Lendenfeld, 1885.

*Diagnosis:* ‘Heteropiidae with syconoid organization and an articulated choanoskeleton. A thin cortex is formed by triactines but lacks longitudinal large diactines. The distal part of the radial tubes is frequently decorated by tufts of radially arranged diactines, indicating a close relationship to the genus *Syconessa*’ (Borojevic *et al.*, 2002).

***GRANTESSA TUMIDA* SP. NOV.**  
(FIGS 24, 25; TABLE 16)

*Etymology:* From the latin *tumidus* (=swollen), for the presence of subatrial and atrial spicules with distally swollen unpaired actines.

*Type locality:* Daai Booi, St. Willibrordus, Curaçao.

*Material examined:* *Holotype.* UFRJPOR 6701, Daai Booi, St. Willibrordus, Curaçao (12°12'43.12"N, 69°05'8.42"W), 3–5 m depth, coll. B. Cóndor-Luján, 18 August 2011. *Paratypes.* UFRJPOR 6695, Hook's Hut, Willemstadt, Curaçao (12°07'18.94"N, 68°58'11.46"W), 6.3 m depth, coll. B. Cóndor-Luján and G. Lôbo-Hajdu, 17 August 2011. UFRJPOR 6766, Sunset Waters,

Soto, Curaçao (12°16'01.58"N, 69°07'44.85"W), 13.1 m depth, coll. B. Cóndor-Luján, 23 August 2011.

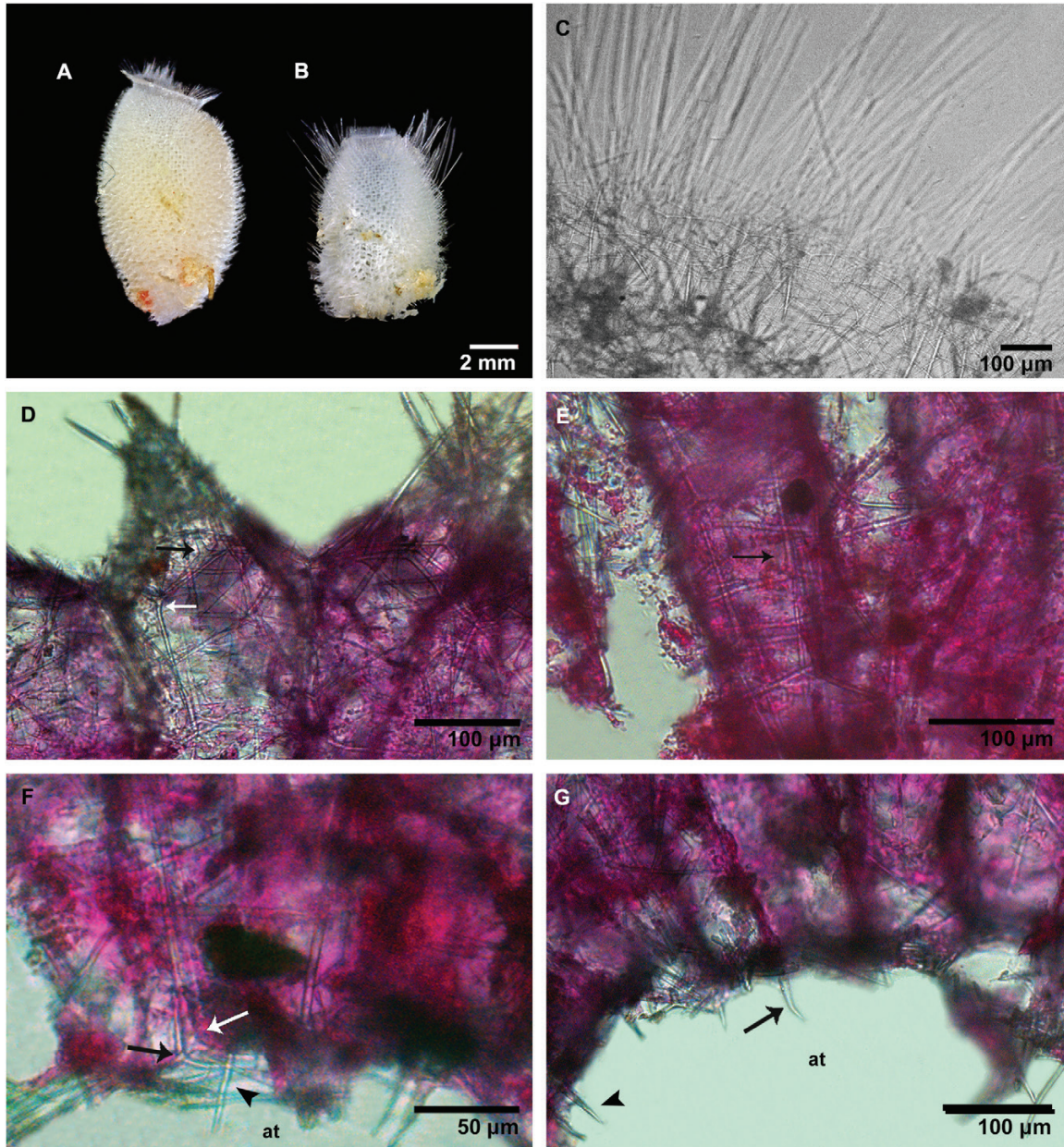
*Diagnosis:* *Grantessa* with a solitary cylindrical body and a hispid surface. Distal cones decorated by tufts of diactines are evident. The tubar skeleton is exclusively composed of triactines. The atrial skeleton is composed of one category of triactines and two categories of tetractines (I and II). The unpaired actines of the atrial triactines and of atrial tetractines I are distally swollen. The apical actine of tetractines I is curved and of tetractines II is straight.

*Colour:* Beige in life and beige to white in ethanol (Fig. 24A, B).

*Morphology and anatomy:* This species has a tubular to sac-shaped body with an apical osculum surrounded by a crown of trichoxeas. The holotype (UFRJPOR 6701) is the largest specimen and measures 0.5 × 0.2 × 0.1 cm (Fig. 24A). The surface is smooth to the touch although diactines protrude through the surface. The consistency is compressible. The aquiferous system is syconoid with elongated choanocyte chambers ranging from 75.0/13.0 to 87.0/20.0 µm.

*Skeleton:* The osculum is surrounded by a crown of trichoxeas supported by T-shaped triactines and rare

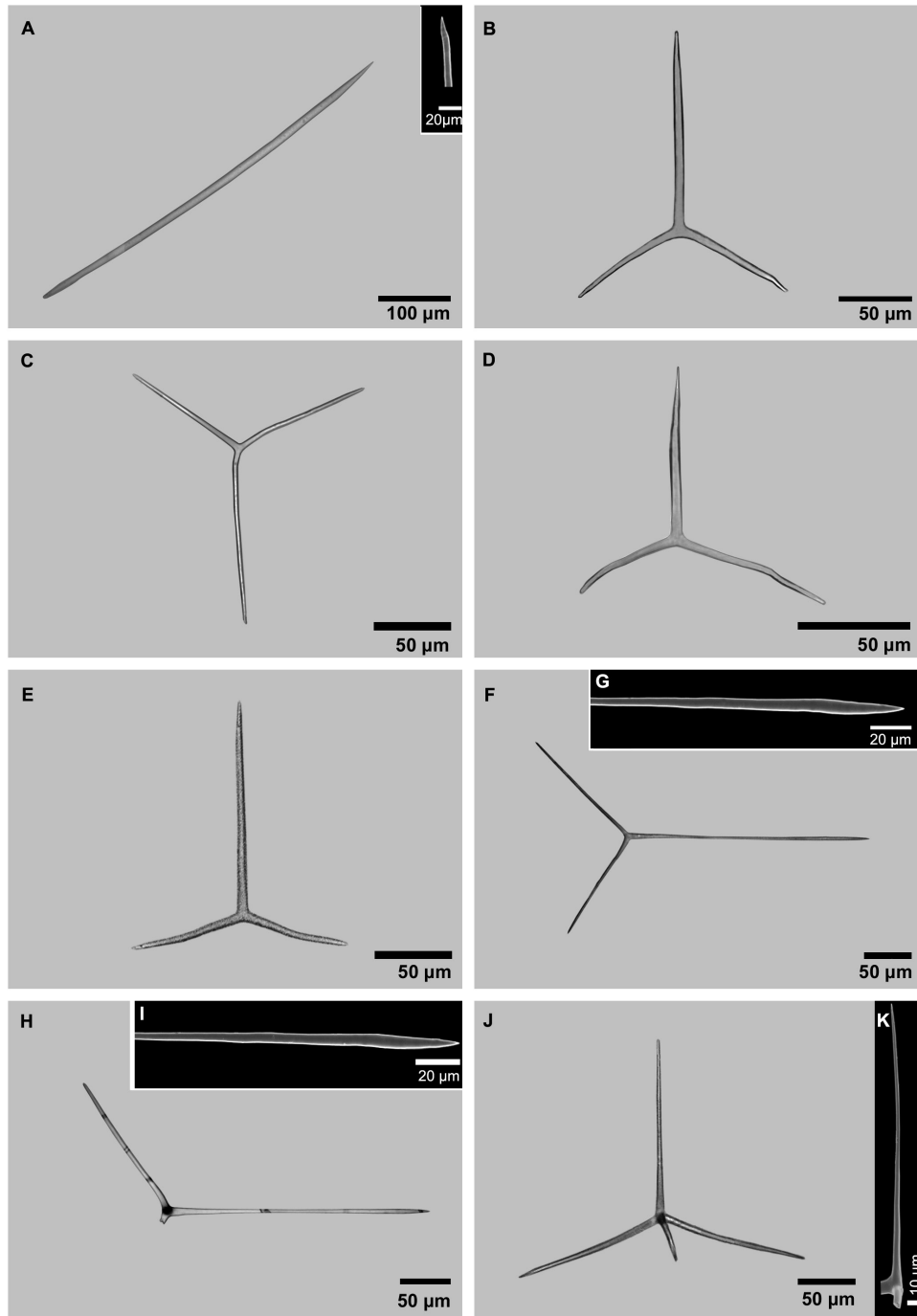




**Figure 24.** *Grantessa tumida* sp. nov. A–B, UFRJPOR 6701 and UFRJPOR 6766 after fixation. C, osculum. D, distal part of the skeleton indicating a cortical triactine (black arrow) and a pseudosagittal subcortical triactine (white arrow). E, tubar skeleton indicating a tubar triactine (arrow). F, subatrial skeleton showing subatrial triactine (white arrow), atrial tetractine I (black arrow) and atrial tetractine II (arrowhead). G, atrial skeleton showing apical actines of tetractine I (arrow) and tetractine II (arrowhead). All skeleton photos were taken from the specimen UFRJPOR 6701. at, atrium.

tetractines (Fig. 24C). The skeleton is typical of the genus. The cortical skeleton is composed of diactines and triactines (Fig. 24D). The diactines are arranged in tufts, to which trichoxeas can be added. Rare diactines penetrate obliquely the choanosome but do not reach the atrium. The triactines are tangentially positioned with the paired actines laying tangentially to the subcortical region (Fig. 24D, black arrow). The

subcortical skeleton is composed of pseudosagittal triactines (Fig. 24D, white arrow) with the longest paired actine (actine 1) penetrating the choanosome. The tubar skeleton is articulated, composed of several rows of triactines (Fig. 24E, arrow) with the unpaired actine pointing to the cortex. The subatrial skeleton is composed of triactines (Fig. 24F, white arrow). The atrial skeleton is composed of one category of triactines



**Figure 25.** Scicules of *Grantessa tumida* sp. nov. (UFRJPOR 6701). A, diactine. B, cortical triactine. C, subcortical triactine. D, tubar triactine. E, subatrial triactine. F, atrial triactine. G, detail of the swollen tip of the atrial triactine. H, atrial tetractine I. I, detail of the swollen tip of the unpaired actine of the atrial tetractine I. J, atrial tetractine II. K, detail of the unpaired actine of the atrial tetractine II.

and two categories of tetractines (I and II, black arrow and arrowhead, respectively, in Fig. 24F). Atrial triactines and tetractines I have the unpaired actine

tangential to the atrium, supporting it. Tetractines I and II have their apical actine penetrating the atrial cavity (Fig. 24F, arrow and arrowhead, respectively).

**Table 16.** Spicule measurements of *Grantessa tumida* sp. nov. (holotype = UFRJPOR 6766 and paratypes = UFRJPOR 6695 and UFRJPOR 6701)

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	Mean	S	Max	Min	Mean	S	Max	
UFRJPOR 6695	Diactine		108.0	250.6	99.6	432.0	5.4	9.7	2.8	16.2	20
	Cortical triactine	Paired	48.6	62.1	9.8	81.0	5.4	5.4	0.0	5.4	20
		Unpaired	51.3	93.9	21.7	121.5	5.4	5.6	0.5	6.8	20
	Pseudosagittal triactine	Paired (1)	67.5	106.7	27.3	140.4	4.1	5.1	0.6	5.4	15
		Paired (2)	51.3	73.7	17.9	108.0	4.1	5.1	0.6	5.4	13
		Unpaired	54.0	93.4	24.5	124.2	4.1	5.1	0.6	5.4	15
	Tubular triactine	Paired	51.3	91.8	20.8	121.5	5.4	6.4	1.1	8.1	20
		Unpaired	99.9	153.4	35.3	207.9	5.4	6.0	0.8	8.1	20
	Subatrial triactine	Paired	43.2	83.5	21.8	108.0	4.1	5.2	0.9	6.8	15
		Unpaired	70.2	156.2	60.8	229.5	4.1	5.8	0.9	6.8	14
	Atrial triactine	Paired	94.5	119.8	17.4	148.5	4.1	5.1	0.6	5.4	14
		Unpaired	159.3	223.3	32.9	283.5	5.4	6.1	1.0	8.1	20
	Atrial tetractine I	Paired	94.5	119.2	14.0	148.5	4.1	5.3	0.6	6.8	14
		Unpaired	162.0	222.3	29.5	270.0	5.1	5.6	0.5	6.8	18
	Atrial tetractine II	Apical	24.3	30.2	5.2	37.8	4.1	5.0	0.7	5.4	6
		Paired	78.3	117.5	22.7	148.5	4.1	5.1	0.6	5.4	12
		Unpaired	121.5	196.9	53.9	270.0	5.4	6.4	1.2	8.1	12
		Apical	24.3	56.2	17.8	86.4	4.1	4.9	0.7	5.4	10
UFRJPOR 6701	Diactine		108.0	360.7	110.0	615.6	5.4	10.8	3.5	16.2	20
	Cortical triactine	Paired	56.7	78.4	10.7	102.6	5.4	5.5	0.6	8.1	20
		Unpaired	86.4	117.5	15.8	140.4	5.4	6.0	0.8	8.1	20
	Pseudosagittal triactine	Paired (1)	78.3	125.1	17.5	145.8	5.4	5.4	0.0	5.4	20
		Paired (2)	56.7	92.2	15.6	108.0	4.1	5.2	0.5	5.4	20
		Unpaired	78.3	105.3	13.9	129.6	4.1	5.3	0.8	8.1	20
	Tubular triactine	Paired	78.3	88.0	7.9	105.3	5.4	6.1	1.1	8.1	20
		Unpaired	72.9	129.2	27.7	189.0	5.4	6.2	1.2	8.1	20
	Subatrial triactine	Paired	48.6	74.9	11.8	89.1	4.1	5.1	0.6	5.4	16
		Unpaired	35.1	151.6	59.2	243.0	4.1	5.6	1.0	8.1	16
	Atrial triactine	Paired	89.1	120.4	19.8	162.0	4.1	5.2	0.5	5.4	20
		Unpaired	162.0	223.4	27.2	270.0	5.4	6.3	1.2	8.1	20
	Atrial tetractine I	Paired	94.5	125.3	18.6	151.2	4.1	5.1	0.6	5.4	10
		Unpaired	189.0	230.6	28.3	270.0	5.4	6.3	1.3	8.1	12
		Apical	13.5	29.2	11.7	43.2	4.1	5.1	0.6	5.4	5
	Atrial tetractine II	Paired	83.7	115.0	25.9	145.8	4.1	5.1	0.6	5.4	10
		Unpaired	129.6	179.6	49.3	280.8	5.4	5.9	1.0	8.1	8
		Apical	21.6	46.5	24.4	86.4	4.1	5.1	0.6	5.4	9
UFRJPOR 6766	Diactine		194.4	381.2	138.2	637.2	5.4	9.7	2.8	16.2	20
	Cortical triactine	Paired	51.3	79.4	12.7	102.6	4.1	5.3	0.4	5.4	20
		Unpaired	81.0	128.9	22.3	175.5	4.1	5.5	0.8	8.1	20
	Pseudosagittal triactine	Paired (1)	78.3	110.7	18.1	137.7	4.1	5.3	0.3	5.4	15
		Paired (2)	62.1	84.6	14.2	113.4	2.7	5.0	0.8	5.4	15
		Unpaired	56.7	101.8	26.9	126.9	4.1	5.3	0.4	5.4	10
	Tubular triactine	Paired	48.6	95.6	13.3	118.8	5.4	6.0	1.0	8.1	20
		Unpaired	83.7	145.9	27.9	205.2	5.4	5.9	0.8	8.1	20
	Subatrial triactine	Paired	40.5	80.3	17.3	99.9	4.1	5.3	0.6	6.8	16
		Unpaired	67.5	162.0	47.6	224.1	4.1	5.7	1.0	6.8	16
	Atrial triactine	Paired	97.2	123.1	13.2	148.5	4.1	5.7	1.1	8.1	20
		Unpaired	189.0	233.1	26.7	283.5	5.4	6.1	1.0	8.1	20
	Atrial tetractine I	Paired	94.5	121.5	16.9	148.5	5.4	6.2	1.1	8.1	10
		Unpaired	189.0	240.5	28.7	283.5	5.4	6.1	1.1	8.1	11
		Apical	13.5	21.2	8.4	35.1	5.4	5.4	0.0	5.4	6
	Atrial tetractine II	Paired	97.2	118.8	17.5	137.7	4.1	5.1	0.7	5.4	4
		Unpaired	108.0	160.7	53.4	234.9	5.4	6.4	1.3	8.1	4
		Apical	27.0	79.0	23.2	97.2	5.4	5.6	0.4	6.8	8



**Spicules:** *Diactines*. Fusiform with tips usually sharp (Fig. 25A). Size: 108.0–637.2/5.4–16.2 µm. *Cortical triactines*. Sagittal. Actines are conical with sharp tips. The paired actines are less straight than the unpaired one (Fig. 25B). Size: 48.6–102.6/4.1–8.1 µm (paired actine), 51.3–175.5/4.1–8.1 µm (unpaired actine). *Subcortical triactines*. Pseudosagittal. Actines are conical with sharp tips. One of the paired actines is shorter and more curved than the other. The unpaired actine is straight (Fig. 25C). Size: 67.5–145.8/4.1–5.4 µm (paired actine 1), 51.3–113.4/2.7–5.4 µm (paired actine 2), 54.0–129.6/4.1–8.1 µm (unpaired actine). *Tubar triactines*. Sagittal. Actines are conical, slightly curved with sharp tips. The paired actines have almost the same size or one of them is shorter than the other one. The unpaired actine is straight and usually longer than the paired ones (Fig. 25D). Size: 48.6–121.5/5.4–8.1 µm (paired actine), 72.9–205.5/5.4–8.1 µm (unpaired actine). *Subatrial triactines*. Sagittal. Actines are slightly conical with sharp tips. The unpaired actine is straight and longer than the paired ones. The paired actines are inwardly curved (Fig. 25E). Some paired actines with different lengths in the same spicule were also found. Size: 40.5–108.8/4.1–6.8 µm (paired actine), 35.1–243.0/4.1–8.1 µm (unpaired actine). *Atrial triactines*. Sagittal. Actines are conical with sharp tips. The unpaired actine is elongated and swollen at the distal part (Fig. 25F, G). Size: 89.1–162.0/4.1–8.1 µm (paired actine), 159.3–283.5/5.4–8.1 µm (unpaired actine). *Atrial tetractine I*. Sagittal. Actines are conical with sharp tips. The unpaired actine is elongated and it is swollen at the distal part (Fig. 25H, I). The apical actine is curved, with the base being much thicker than the tip, which is blunt. Size: 94.5–151.2/4.1–8.1 µm (paired actine), 162.0–283.5/5.1–8.1 µm (unpaired actine), 13.5–43.2/4.1–5.4 µm (apical actine). *Atrial tetractines II*: Sagittal. Actines are conical with sharp tips. The unpaired actine is longer than the paired ones (Fig. 25J, K). The apical actine is straight. Size: 78.3–148.5/4.1–5.4 µm (paired actine), 108.0–280.8/5.4–8.1 µm (unpaired actine), 21.6–97.2/4.1–6.8 µm (apical actine).

**Ecology:** The specimens were found underneath coral boulders at depths varying from 5 to 13 m. The paratype UFRJPOR 6695 was covered with sediment when collected. No associated organisms were observed.

**Geographical distribution:** Southern Caribbean (provisionally endemic to Curaçao, present study).

**Molecular analysis:** The sequences of the holotype (UFRJPOR 6701) and of the paratype UFRJPOR 6695 of *Grantessa tumida* sp. nov. formed a monophyletic clade with high support (pp = 1, b = 100, Fig. 15) and 100% of genetic similarity (0% of *p* distance).

*Grantessa tumida* sp. nov. did not group with the other *Grantessa* species included in the phylogenetic tree, *G. aff. intusarticulata* (Carter, 1886), which may indicate the non-monophyly of this genus. On the other hand, *G. tumida* sp. nov. evidenced a closer affinity to *Sycon ciliatum* as they clustered together (pp = 1, b = 71). The *p* distance between these two species was 5.5%.

**Taxonomic remarks:** Among the 28 valid species of the genus *Grantessa*, only *G. ramosa* (Haeckel, 1872) from South Africa and *G. tenhoveni* Van Soest & de Voogd, 2015 from Indonesia have atrial spicules with swollen unpaired actine as also observed in *G. tumida* sp. nov. Nonetheless, the skeleton of our new species differs from *G. tenhoveni*, as it bears neither subatrial tetractines nor atrial tetractines with long apical actines (660.0–960.0 µm) as described for the Indonesian species. Therefore, *G. ramosa* is the most similar species to *G. tumida* sp. nov.

*Grantessa ramosa* was originally described by Haeckel (1872) and redescribed by Borojevic (1967). Although the specimens described in both studies are very similar, it is possible that they belong to different species as they differ mainly in the composition of the cortical skeleton and spicule dimensions (Tables 16 and 17). Consequently, we compared the specimens from Curaçao with each description separately, i.e. *G. ramosa sensu* Haeckel (1872) and *G. ramosa sensu* Borojevic (1967).

*Grantessa ramosa sensu* Haeckel (1872) is a ramified sponge with a smooth surface, whereas *G. tumida* sp. nov. forms a single tube with hispid surface. According to Haeckel's description, the cortical diactines of *G. ramosa* are distally swollen (see Plate 54, fig. 1s), located perpendicularly to the surface (close to each other) and are not supported by cortical triactines. In contrast, in *G. tumida* sp. nov. cortical diactines are fusiform, arranged in tufts and supported by cortical triactines. Moreover, Haeckel (1872) mentioned that the apical actines of the atrial tetractines were 'Nagethier-Schneidezahns' (thick and sharp-pointed as shown in Plate 54, fig. 1a), whereas in *G. tumida* sp. nov., the apical actine of the atrial tetractines can be curved (tetractines II) or straight (tetractines I). Regarding spicule dimensions, although almost all the spicule categories of *G. ramosa sensu* Haeckel (1872) match those of *G. tumida* sp. nov. (Tables 16 and 17), diactines are shorter in the former (60.0–80.0 µm) than in the latter (108.0–637.2 µm).

Differing from *G. tumida* sp. nov., *G. ramosa sensu* Borojevic (1967) is a sponge with variable external morphology, being ramified or solitary. Although very similar in skeletal composition, the spicules of these two species are not identical. Borojevic (1967) described one category of atrial tetractines; however, in figure 14 (p. 206) it is possible to distinguish two categories of

**Table 17.** Spicule measurements of *Grantessa ramosa* from the original description (Haeckel, 1872) and from the redescription (Borojevic, 1967)

Specimen	Spicule	Actine	Length (µm)				Width (µm)			
			Min	Mean	S	Max	Min	Mean	S	Max
<i>Grantessa ramosa</i> (original description)	Diactine		60.0	-	-	80.0	-	-	-	-
	Tubar triactine	Paired	40.0	-	-	80.0	6.0	-	-	8
		Unpaired	80.0	-	-	120.0	6.0	-	-	8
	Subatrial triactine	Paired	40.0	-	-	80.0	6.0	-	-	8
		Unpaired	160.0	-	-	200.0	6.0	-	-	8
	Atrial tetractine	Paired	50.0	-	-	80.0	6.0	-	-	8
		Unpaired	100.0	-	-	120.0	6.0	-	-	8
		Apical	20.0	-	-	30.0	-	8.0	-	-
	<i>Grantessa ramosa sensu</i> Borojevic (1967)	Diactine	-	150.0	-	-	380.0	6.0	-	-
Cortical triactine*		Unpaired	70.0	-	-	150.0	8.0	-	-	12
Subcortical triactine		Cortical	80.0	-	-	180.0	15.0	-	-	18
		paired								
		Internal	150.0	-	-	240.0	15.0	-	-	18
Tubar triactine		Unpaired	100.0	-	-	200.0	14.0	-	-	16
		Unpaired	150.0	-	-	230.0	14.0	-	-	18
Subatrial triactine		Paired	80.0	-	-	120.0	9.0	-	-	11
		Unpaired	180.0	-	-	250.0	12.0	-	-	14
Atrial triactine		Paired	60.0	-	-	180.0	8.0	-	-	10
		Unpaired	200.0	-	-	400.0	8.0	-	-	10
Atrial tetractine		Paired	60.0	-	-	180.0	-	25.0	-	-
		Unpaired	<200	-	-	<400	-	25.0	-	-
	Apical	-	-	-	140.0	-	25.0	-	-	

\*Paired actines slightly longer than the unpaired actine.

atrial tetractines: one with very long unpaired actine (left, fig. 14g) and another with short unpaired actine (right, fig. 14g). In *G. tumida* sp. nov., both categories of atrial tetractines (I and II) have long unpaired actine (see Spicules section). Furthermore, compared to *G. ramosa sensu* Borojevic (1967), almost all the spicule categories have thinner actines in *G. tumida* sp. nov.: cortical triactines (8.0–12.0 µm vs. 4.1–8.1 µm), subcortical triactines (14.0–18.0 µm vs. 2.7–8.1 µm), tubar triactines (14.0–18.0 µm vs. 5.4–8.1 µm), subatrial triactines (9–14 µm vs. 4.1–8.1 µm), atrial triactines (8.0–10.0 µm vs. 4.1–8.1 µm) and atrial tetractines (4.1–8.1 µm vs. 25.0 µm).

In both descriptions of *G. ramosa*, Haeckel and Borojevic agreed that the distal cones are not visible macroscopically, whereas, in *G. tumida* sp. nov., short distal cones are evident probably due to the longer diactines of the new species.

Although the analysed specimens from Curaçao did not cluster with the other *Grantessa* species in the C-LSU phylogeny (*Grantessa* aff. *intusarticulata*), we identified these specimens as *Grantessa* following the morphological classification of Systema Porifera (Borojevic *et al.*, 2002). *Grantessa* aff. *intusarticulata*

and *G. tumida* sp. nov. have many similarities (syconoid aquiferous system, articulated choanoskeleton and pseudosagittal subcortical triactines); however, only *G. tumida* sp. nov. has radial tubes decorated with tufts of diactines resembling distal cones. Whether this difference has phylogenetic signal in *Grantessa* or not should be tested in further molecular studies. The addition of the sequence from the type species of the genus, *G. sacca*, in future phylogenetic analyses would help to understand the phylogenetic affinities within *Grantessa*.

FAMILY SYCETTIDAE DENDY, 1893  
GENUS *SYCON* RISSO, 1827

*Type species:* *Sycon humboldti* Risso, 1827.

*Diagnosis:* ‘Sycettidae with radial tubes partially or fully coalescent; distal cones are decorated by tufts of diactines. The inhalant canals are generally well defined between the radial tubes and are often closed at the distal end by a membrane that is perforated by an ostium, devoid of a skeleton. There is no continuous cortex covering the distal ends of the radial tubes.



Skeleton of the atrium and of the tubes composed of triactines and/or tetractines' (Borojevic *et al.*, 2002).

***SYCON CONULOSUM* SP. NOV.**

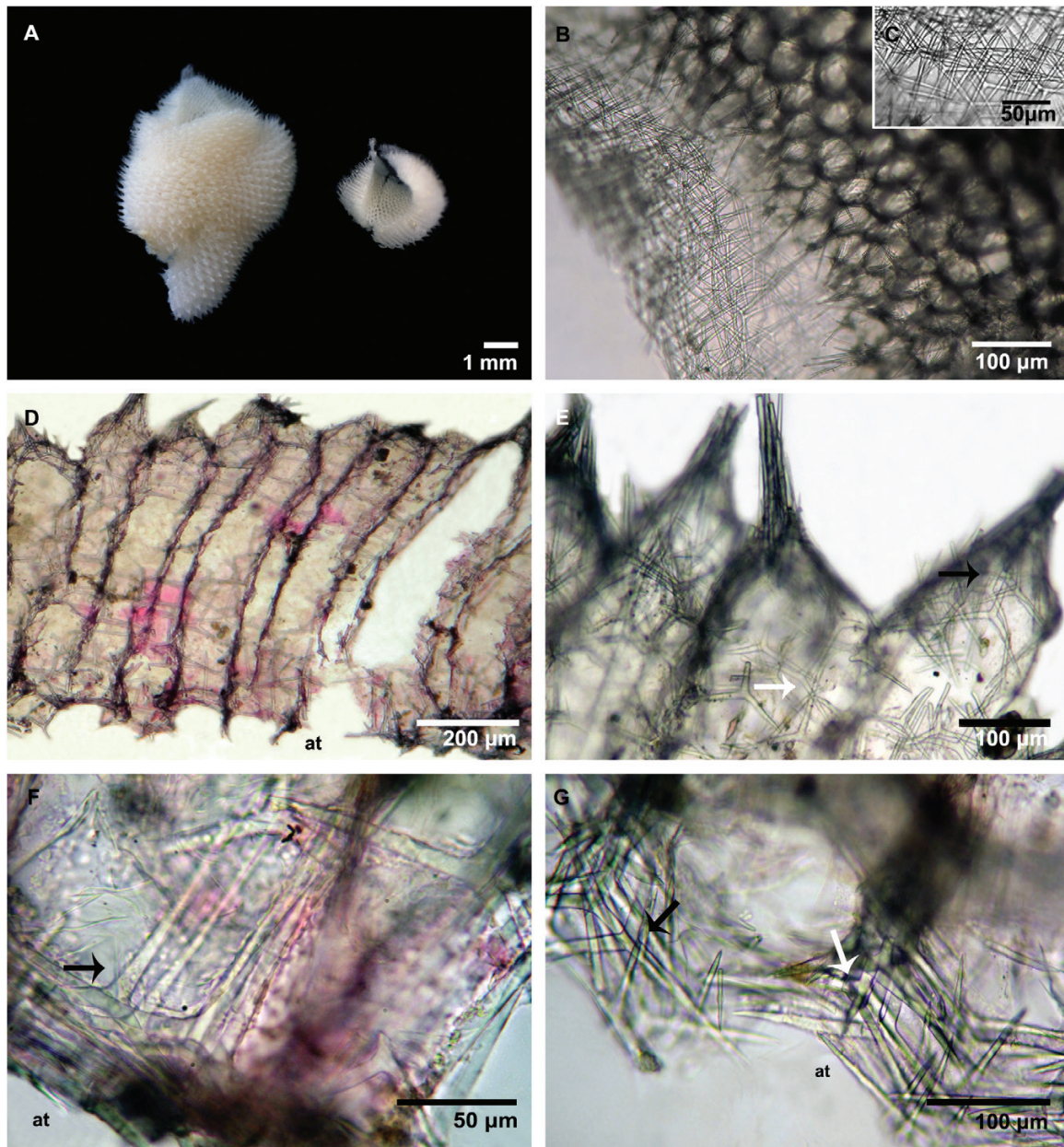
(FIGS 26, 27; TABLE 18)

*Etymology:* Derived from the conulose appearance of the surface.

*Type Locality:* Daai Booi, St. Willibrordus, Curaçao.

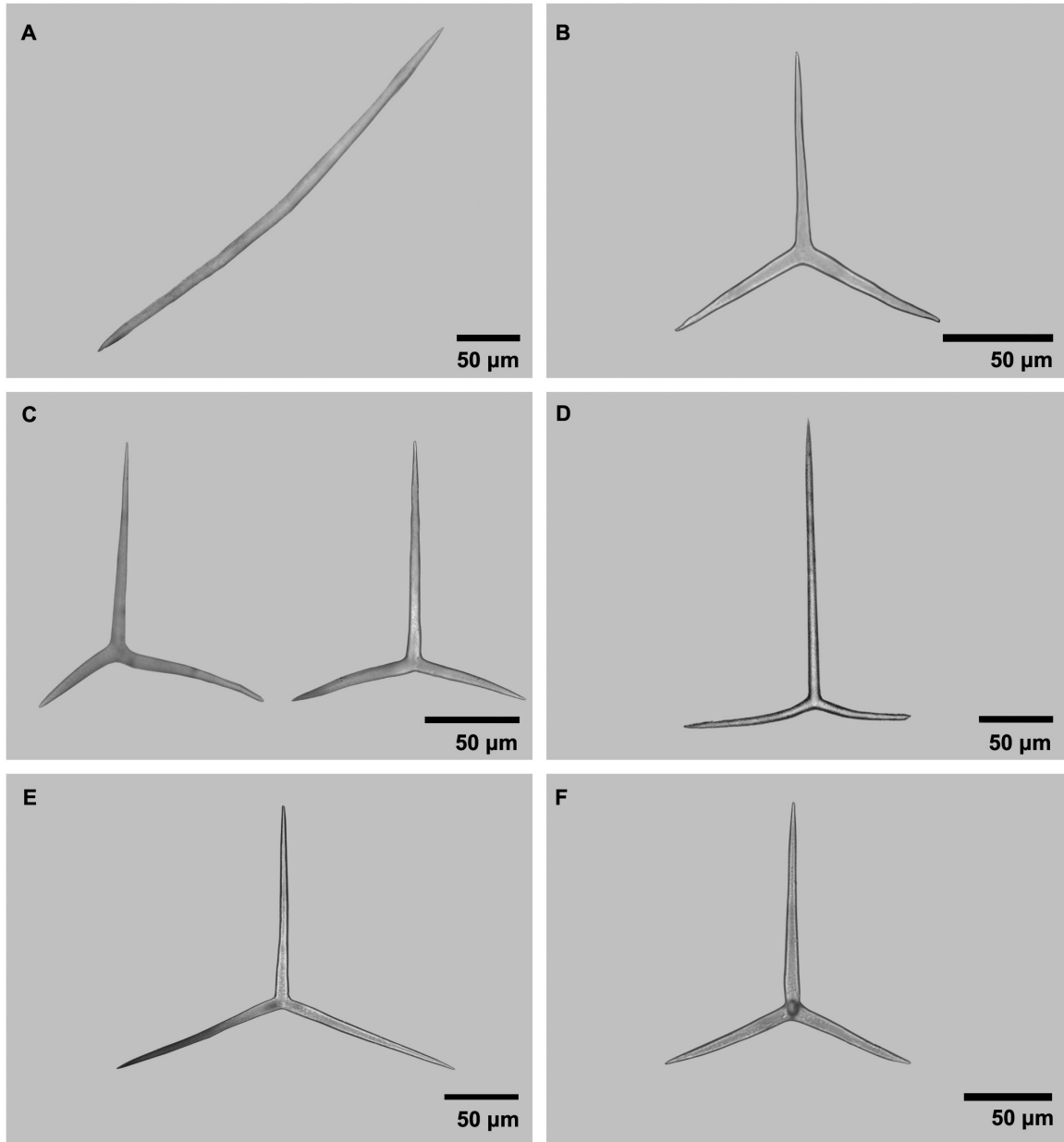
*Material examined:* *Holotype.* UFRJPOR 6707, Daai Booi, St. Willibrordus, Curaçao (12°12'43.12"N, 69°05'8.42"W), 3–5 m depth, coll. B. Cóndor-Luján, 18 August 2011.

*Diagnosis:* *Sycon* with a conulose appearance due to its separated distal cones. Skeleton mainly composed of triactines. Tetractines are only present in the atrial skeleton and they are less abundant than the atrial triactines.



**Figure 26.** *Sycon conulosum* sp. nov. (UFRJPOR 6707). A, specimen after fixation. B, osculum. C, detail of the oscular T-shaped triactines. D, cross section of the skeleton. E, part of the skeleton with a tuft of diactines, a triactine of the distal cone (black arrow) and a tubar triactine (white arrow). F, subatrial skeleton indicating a subatrial triactine (arrow). G, atrial skeleton indicating atrial triactine (black arrow) and atrial tetractine (white arrow). at, atrium.





**Figure 27.** Spicules of *Sycon conulosum* sp. nov. (UFRJPOR 6707). A, diactine of the distal cone. B, triactine of the distal cone. C, tubar triactines. D, subatrial triactine. E, atrial triactine. F, atrial tetractine.

**Colour:** White in life and beige in ethanol (Fig. 26A).

**Morphology and anatomy:** This species has a vase shape being larger in the central part of the body (Fig. 26A). It measures  $0.9 \times 0.7 \times 0.1$  cm. It has a hispid conulose appearance because of its separated distal cones which are supported by diactines protruding the surface. The apical osculum (diameter = 0.9 mm) has no crown and it is supported by sagittal triactines (Fig. 26B, C). The atrium is not hispid. The radial

tubes are fully coalescent (Fig. 26D). The aquiferous system is syconoid with elongated choanocyte chambers that ranged in size from  $810.0/140.4$  µm to  $939.6/216.0$  µm.

**Skeleton:** The skeleton of the distal cones is composed of short diactines, rare trichoxeas and triactines (Fig. 26E, black arrow). The tubar skeleton is articulated, composed of several rows of triactines (Fig. 26E, white arrow). The subatrial skeleton is

**Table 18.** Spicule measurements of *Sycon conulosum* sp. nov. (holotype = UFRJPOR 6707).

Spicule	Actine	Length (µm)				Width (µm)				N
		Min	Mean	SD	Max	Min	Mean	SD	Max	
Diaactine		100.0	216.5	48.0	275.0	6.3	8.4	1.3	11.3	20
Triactine (distal cone)	Paired	50.0	77.4	16.4	112.5	5.0	7.2	1.6	10.0	20
	Unpaired	65.0	124.1	28.9	175.0	5.0	6.4	1.3	8.8	20
Tubar triactine	Paired	40.0	63.5	10.5	80.0	3.8	7.4	1.7	10.0	20
	Unpaired	62.5	106.4	21.2	155.0	3.8	7.3	1.7	10.0	20
Subatrial triactine	Paired	42.5	65.1	9.9	80.0	5.0	5.8	1.2	8.8	20
	Unpaired	82.5	135.1	20.2	172.5	5.0	7.1	1.6	10.0	20
Atrial triactine	Paired	80.0	103.0	11.8	130.0	7.1	10.0	2.2	14.3	14
	Unpaired	80.0	126.6	23.7	172.5	5.0	7.9	1.9	10.0	14
Atrial tetractine	Paired	62.5	77.5	11.4	90.0	7.5	9.1	1.9	11.3	4
	Unpaired	92.5	111.3	20.3	130.0	7.5	8.8	1.4	10.0	4
	Apical	15.0	27.9	9.6	40.0	5.0	7.3	1.7	10.0	12

exclusively composed of triactines (Fig. 26F, arrow). The atrial skeleton is composed of triactines (Fig. 26G, black arrow) and few tetractines (Fig. 26G, white arrow). The apical actine of the atrial tetractines penetrates the atrial cavity.

**Spicules:** *Diaactines*. Fusiform with tips usually sharp (Fig. 27A). Size: 100.0–275.0/6.3–11.3 µm. *Triactines of the distal cones*. Sagittal. Actines are conical with sharp tips. The paired actines are frequently curved (Fig. 27B). In the distal part of the cone, the unpaired actine is very long. Size: 50.0–102.5/5.0–10.0 µm (paired actine) and 65.0–175.0/5.0–8.8 µm (unpaired actine). *Tubar triactines*. Sagittal. Actines are conical with sharp tips. The paired actines are slightly curved. One of the paired actines can be shorter and more curved than the other. The unpaired actine is straight and it is generally slightly longer than the paired ones (Fig. 27C). Size: 40.0–80.0/3.8–10.0 µm (paired actine) and 62.5–155.0/3.8–10.0 µm (unpaired actine). *Subatrial triactines*. Sagittal. Actines are slightly conical with sharp tips. The paired actines are inwardly curved. Some paired actines have different lengths. The unpaired actine is straight and longer than the paired ones (Fig. 27D). Size: 42.5–80.0/5–8.8 µm (paired actine) and 88.5–172.5/5.0–10.0 µm (unpaired actine). *Atrial triactines*. Sagittal. Actines are conical, straight and have sharp tips. The unpaired actine is usually longer than the paired ones (Fig. 27E). They are the largest and the less sagittal spicules of this species. Size: 80.0–130.0/7.1–14.3 µm (paired actine) and 80.0–172.5/5.0–10.0 µm (unpaired actine). *Atrial tetractines*. Sagittal. Actines are conical, straight with sharp tips (Fig. 27F). The apical actine is the shortest actine of this species. Size: 62.5–90.0/7.5–11.3 µm (paired actine),

92.5–130.0/7.5–10.0 µm (unpaired actine) and 15.0–40.0/5.0–10.0 µm (apical actine).

**Ecology:** This species was found underneath boulders down to 5 m depth. No associated organisms were observed.

**Molecular analysis:** *Sycon* was recovered as a polyphyletic genus in both ML and BI phylogenies (Fig. 15). Only *S. ancora* Klautau, Imešek, Azevedo, Pleše, Nikolić & Četković, 2016, *S. cf. villosum* (Haeckel, 1870) and *S. raphanus* Schmidt, 1862 formed a monophyletic group (pp = 0.93, b = 47). The other species (*S. carteri* Dendy, 1893, *S. conulosum* sp. nov. and *S. magnapicale* sp. nov.) appeared as different lineages and clustered with species of other families.

*Sycon conulosum* sp. nov. nested in a cluster (pp = 1, b = 83) that included species of Amphoriscidae (*L. antillana* sp. nov.), Grantiidae [*Leucandra aspera* (Schmidt, 1862) and *L. spinifera* Klautau, Imešek, Azevedo, Pleše, Nikolić & Četković, 2016], Jenkinidae (*Anamixilla torresi* Poléjaeff, 1883) and Sycettidae (*Sycon ancora*, *S. cf. villosum* and *S. raphanus*). Within this cluster, *S. conulosum* sp. nov. showed a closer molecular affinity to *L. antillana* sp. nov. as these species appeared in the same clade with high support (pp = 1, b = 99). The *p* distance between *S. conulosum* sp. nov. and *L. antillana* sp. nov. was 2.2%.

**Geographical distribution:** Southern Caribbean (provisionally endemic to Curaçao, present study).

**Taxonomic remarks:** Within the 88 accepted species of *Sycon* (Van Soest et al., 2017), 13 have a skeletal composition similar to the specimen analysed from

Curaçao: *S. ampulla* (Haeckel, 1870) from the Atlantic coast of South America, *S. barbadense* (Schuffner, 1877) from Barbados, *S. brasiliense* Borojevic, 1971 from Brazil, *S. boreale* (Schuffner, 1877) from Norway, *S. dunstervillea* (Haeckel, 1872) and *S. elegans* (Bowerbank, 1845) from South Africa, *S. formosum* (Haeckel, 1870) from the Antilles, *S. humboldti* from the Western Mediterranean, *S. raphanus* Schmidt, 1862 from the Adriatic Sea, *S. scaldiense* (Van Koolwijk, 1982) from the Netherlands, *S. setosum* Schmidt, 1862, *S. schmidti* (Haeckel, 1872) and *S. tuba* Lendenfeld, 1891 also from the Adriatic Sea and *S. villosum* (Haeckel, 1870) from the Antilles. However, they differ in external morphology or spicule dimensions (Tables 18 and 19).

*Sycon ampulla* has a highly variable external morphology (solitary, ramified, with or without stalk and with or without oscular crown); however, it always has an oscular neck. This feature is not present in *S. conulosum* sp. nov. Haeckel (1872) differentiated two varieties within *S. ampulla*, *S. ampulla* var. *alopecurus* (from Venezuela), devoid of a stalk and with diactines longer than the actines of the triactines of the distal cones, and *S. ampulla* var. *petiolata* (from Brazil), characterized by the presence of a stalk composed of parasagittal triactines and of shorter diactines in the distal cones. *Sycon conulosum* sp. nov. most resembles *S. ampulla* var. *alopecurus* but differs in spicule dimensions (Tables 17 and 18). *Sycon ampulla* (var. *alopecurus* and var. *petiolata*) has atrial spicules (triactines and tetractines) with smaller unpaired actines (60.0–85.0 µm), longer and thinner apical actines (40.0–60.0/5.0 µm, rarely 100.0 µm) and longer diactines (200.0–500.0 µm) compared with *S. conulosum* sp. nov., the corresponding measurements of which are: 80.0–172.5 µm (unpaired actines), 15.0–40.0/5.0–10.0 µm (apical actines) and 100.0–275.0 µm (diactines).

The skeletons of *S. barbadense* and *S. conulosum* sp. nov. are quite similar in spicule shape and dimensions. However, in the former species, diactines are longer (400.0 µm), and the apical actine of the tetractines is longer and thicker (80.0/13.0 µm) than in the latter species. Different to *S. conulosum* sp. nov., the atrium of *S. barbadense* is hispid. As Schuffner (1877) did not give more details about the external morphology, no further comparisons regarding the conulose appearance can be made.

Although *S. brasiliense* and *S. conulosum* sp. nov. have similar external morphology, their skeletons are not identical. In *S. brasiliense*, diactines have lanceolate tips and they rarely occur in the distal cones, while in *S. conulosum* sp. nov. they are fusiform and very common. Regarding spicule dimensions, the apical actines of the atrial tetractines are slightly longer ( $\leq 100.0$  µm) in *S. brasiliense* than in the new species (15.0–40.0 µm) and there is also a small difference in the width of the actines of the subatrial triactines,

which are thicker in *S. brasiliense* (10.0–12.0 µm) than in *S. conulosum* sp. nov. (5.0–10.0 µm)

*Sycon boreale* can be easily distinguished from *S. conulosum* sp. nov. because the tubar skeleton of the former species is mainly composed of equiangular triactines and sagittal triactines rarely occur, whereas in the latter species, the tubar skeleton is exclusively composed of sagittal triactines and equiangular triactines are restricted to the skeleton of the distal cones. In addition, in *S. conulosum* sp. nov., the diactines (100.0–275.0 µm) and the apical actines of the atrial tetractines (15.0–40.0 µm) are shorter compared to *S. boreale* (500.0 and 50.0 µm, respectively).

*Sycon elegans* was originally described by Bowerbank (1845) and its spicule measurements were provided by Haeckel (1872). *Sycon elegans* differs from *S. conulosum* sp. nov. in the skeleton arrangement of the distal cones. In the former, the tufts of diactines are more dense and more continuously distributed (see Bowerbank, 1864: Plate XVII, fig. 6) than in the latter. In *S. elegans*, the triactines of the distal cones have a particular shape (see Haeckel, 1872: Plate 54, fig. 3). The unpaired actine is curved, longer and thicker (200.0–400.0/25.0–35.0 µm), and the paired actines are thicker (50.0–90.0/25.0–35.0 µm) compared to those of *S. conulosum* sp. nov. (unpaired actine: 65.0–175.0/5.0–8.8 µm and paired actine: 50.0–102.5/5.0–10.0 µm). Furthermore, *S. elegans* has an osculum with both vertical and horizontal ornamentation – mentioned by Bowerbank (1864) and illustrated by Haeckel (1872: Plate 58, fig. 3), whereas *S. conulosum* sp. nov. is devoid of any particular ornamentation except for its slender oscular membrane.

Both *S. dunstervillea* and *S. formosum* were originally described as varieties of *S. elegans* (*Sycandra elegans*) by Haeckel (1872) based only on the shape of the apical actines of the atrial tetractines, being straight and with sharp tip in *S. dunstervillea* and distally swollen in *S. formosum*. Therefore, the differences previously pointed out between *S. elegans* and *S. conulosum* sp. nov. are also valid to distinguish *S. dunstervillea* and *S. formosum* from *S. conulosum* sp. nov. In addition, *S. conulosum* sp. nov. can be differentiated from *S. formosum* by the shape of the apical actine of the atrial tetractines which is not distally swollen.

Unlike *S. conulosum* sp. nov., *S. humboldti* has a hairy body with an osculum surrounded by a crown of diactines. As no description of the skeletal composition of *S. humboldti* was provided by Risso (1827), we retrieved spicule sizes from Haeckel's description (Table 18). *Sycon humboldti* has two size categories of diactines (I: 500.0–2000.0/20.0–40.0 µm and II: 200.0–400.0/5.0–20.0 µm), which do not exactly match the diactines of *S. conulosum* sp. nov. (100.0–275.0/6.3–11.3 µm), and it also has longer apical actines (100.0–120.0 µm).



**Table 19.** Original measurements ( $\mu\text{m}$ ) of *Sycon ampulla*, *S. barbadense*, *S. boreale*, *S. humboldti*, *S. raphanus*, *S. scaldiense*, *S. setosum*, *S. schmidti*, *S. tuba* and *S. villosum*

Species	Distal cone	Tubar triactine	Subatrial triactine	Atrial triactine	Atrial tetractine
<i>Sycon ampulla</i>	Diactine: 100–150/5 (var. <i>petiolata</i> ) 200–500/5 (var. <i>alopecurus</i> ) Diactine: 400/9	Paired: 50–80/5 Unpaired: 100–150/5	-	60–80/5	Unpaired: 60–80/5 Apical: 40–60/5 (rarely 100)
<i>Sycon barbadense</i>	Diactine: 400/9	Paired: 80 Unpaired: $\leq 150$	-	$\leq 200/9$	Paired: $\leq 150/9$ Unpaired: $\leq 200/9$ Apical: 80/13 Apical: 50/13
<i>Sycon boreale</i>	Diactine: 500/9	Paired: 50 Unpaired: 180	-	Paired: shorter. Unpaired: 180/6.8	Paired: 120/8 Unpaired: 80/8 Apical: 120–160/12–16
<i>Sycon elegans</i> *	Diactine: 200–250/2–20 Paired (triactine): 50–90/25–35 Unpaired (triactine): 200–400/25–35	Paired: 50–90/25–35? Unpaired: 200–400/25–35?	Paired: 80–100/10 Unpaired: 100–120/10	Paired: 120/8 Unpaired: 80/8	Paired: 120/8 Unpaired: 80/8 Apical: 120–160/12–16
<i>Sycon humboldti</i> *	Diactine I: 500–2000/20–40 Diactine II: 200–400/5–20 Paired (triactine): 30–60/10–15 Unpaired (triactine): 150–250/20–30 Diactine: 1000–3000/20–24	80–120/8–12	-	Paired: 50–120/8 Unpaired: 50–200/5–8	Paired: 50–120/8 Unpaired: 50–200/5–8 Apical: 100–120
<i>Sycon raphanus</i> *	Diactine: 500–700/5–10 Triactine: 30–60/5–10	Paired: 100–180/10–12 Unpaired: 150–250/10–12	Paired: 100–80/5–8 Unpaired: 150–250/5–8 Paired: 80–200/5–10 Unpaired: 180–350/5–10	(Subregular to rarely sagittal) 150–250/8–10 Paired: 100–280/5–10 Unpaired: 200–300/5–10	(Subregular to sagittal) Basal: 150–250/8–10 Apical: 60–120 Paired: 100–280/5–10 Unpaired: 200–300/5–10 Apical: 180–350/5–10
<i>Sycon scaldiense</i>	Diactine: 1000–3000/20 Diactine: 100–200/1 Diactine I: 100–300/10 Diactine II: 100–500/20–30 Triactines: Paired: very short. Unpaired: 200/10 Diactine: 300/10	Paired: 80–160/5–8 Unpaired: 120–200/5–8 Paired: 100–150/10 Unpaired: 200–300/10	-	Subregular 100–115/5 Unpaired: 300/10	Subregular Apical: 300–600/5 Basal: 200–400/10–15 Apical: 40–50/12–16
<i>Sycon tuba</i>	Diactine: 300/10	Paired: 280/7 Unpaired: 220/7	-	Paired: 280/7 Unpaired: 320/7	Paired: 280/7 Unpaired: 320/7 Apical: 200–260/7 Paired: 100–150/5–8 Unpaired: 300–400/5–8 Apical: 500–800/ $\leq 10$
<i>Sycon villosum</i>	Diactine: 1000–3000/10–40 Triactine: 100–200/5–8	100–300/30	Sagittal Paired: 100/30 Unpaired: 300/10–30	Sagittal Paired: 100/30 Unpaired: 300/10–30	Paired: 100–150/5–8 Unpaired: 300–400/5–8 Apical: 500–800/ $\leq 10$

\*Taken from Haeckel (1872).

*Sycon raphanus* has a variable external morphology (Haeckel, 1872; Klautau *et al.*, 2016). However, it can be differentiated from *S. conulosum* sp. nov. by the presence of triactines with curved paired actines in the skeleton of the distal cones and of the tubar skeleton, both of which are absent in our new species. In *S. raphanus*, diactines are larger (1000.0–3000.0/20.0–24.0 µm) and apical actines are longer (60.0–120.0 µm) than in *S. conulosum* sp. nov.

Different to *S. conulosum* sp. nov., *S. scaldiense* is not conulose; instead, it is very hispid due to diactines protruding the surface and it also has a crown of trichoxeas. The known colour of *S. scaldiense* is grey or brown, whereas in *S. conulosum* sp. nov., it is white when alive and beige in ethanol. Regarding spicule dimensions, diactines, subatrial triactines, and atrial triactines and tetractines are longer and the triactines of the distal cones are shorter in *S. scaldiense* than in the new species (Tables 18 and 19).

*Sycon conulosum* sp. nov. can be recognizable from *S. schmidtii* by the lack of an oscular neck. Besides, *S. schmidtii* possesses atrial tetractines with apical actines bearing a constriction in their middle part and triactines of the distal cones with curved unpaired actines. These two spicule categories are absent in the *S. conulosum* sp. nov. Similar to *S. humboldtii*, *S. schmidtii* has two types of diactines, diactines I (100.0–300.0/10.0 µm) which match those of *S. conulosum* sp. nov., and diactines II (100.0–500.0/20.0–30.0 µm) which are not present in the new species. In general, the spicules of *S. conulosum* sp. nov. are smaller than those of *S. schmidtii* (Tables 18 and 19).

It is easy to differentiate *S. conulosum* sp. nov. from *S. setosum* as the latter species has a long crown (almost the same length of the sponge body, Schmidt, 1862: Plate 1, fig. 3) which is absent in the former species. In *S. setosum*, diactines are substantially larger (1000.0–3000.0/20.0 µm) and the apical actines of the atrial tetractines are much longer (300.0–600.0 µm) than in *S. conulosum* sp. nov., the spicules of which only attain 275.0/11.3 µm (diactines) and 40.0/10.0 µm (apical actines of tetractines).

*Sycon tuba* and *S. conulosum* sp. nov. have similar external morphology and skeletons; however, their aquiferous systems are different. While in the new species the choanocyte chambers are coalescent, in *S. tuba* they are free and are only connected at the atrial cavity (Lendenfeld, 1891: Plate XI, fig. 81). In fact, this anatomic characteristic would place *S. tuba* within *Sycetta*, if we followed Systema Porifera (Borojevic *et al.*, 2002).

*Sycon villosum* has a very hispid atrium because of the presence of tetractines with very long apical actines (500.0–800.0 µm) which are longer than those of *S. conulosum* sp. nov. In *S. villosum*, diactines are also larger (1000.0–3000.0/10.0–40.0 µm) than in the

new species. The ornamentation of the distal cone of *S. villosum* is very different to that of *S. conulosum* as in the former species it is composed of dense tufts of diactines and triactines (as shown in Haeckel, 1872: Plate 58, fig. 1) and in the latter species, diactines do not form similar vertical tufts.

Of the cited species where the original descriptions or posterior redescription provided information on the skeleton of the oscular margin, *S. conulosum* sp. nov. is the only species where the osculum is exclusively supported by triactines. In some species, the osculum is supported by triactines and tetractines (*S. brasiliense*) to which trichoxea (*S. barbadense*), diactines (*S. ampulla*) or diactines and microdiactines (*S. raphanus*) can be added, whereas in the other species, it is supported by tetractines and trichoxeas (*S. boreale* and *S. schmidtii*).

In all the descriptions of the species used for comparison herein, the authors did not differentiate the proportion of triactines and tetractines present in the atrium. This may indicate that these spicules were in the same proportion or that the authors did not consider the spicule proportion to be an important character. Nonetheless, in *S. conulosum* sp. nov., triactines are more frequent than tetractines in the atrial skeleton and we consider this a diagnostic character for this species.

**SYCON MAGNAPICALE SP. NOV.**  
(FIGS 28, 29; TABLE 20)

*Etymology:* From the Latin *magna* (=large), for the long apical actine of the atrial tetractine.

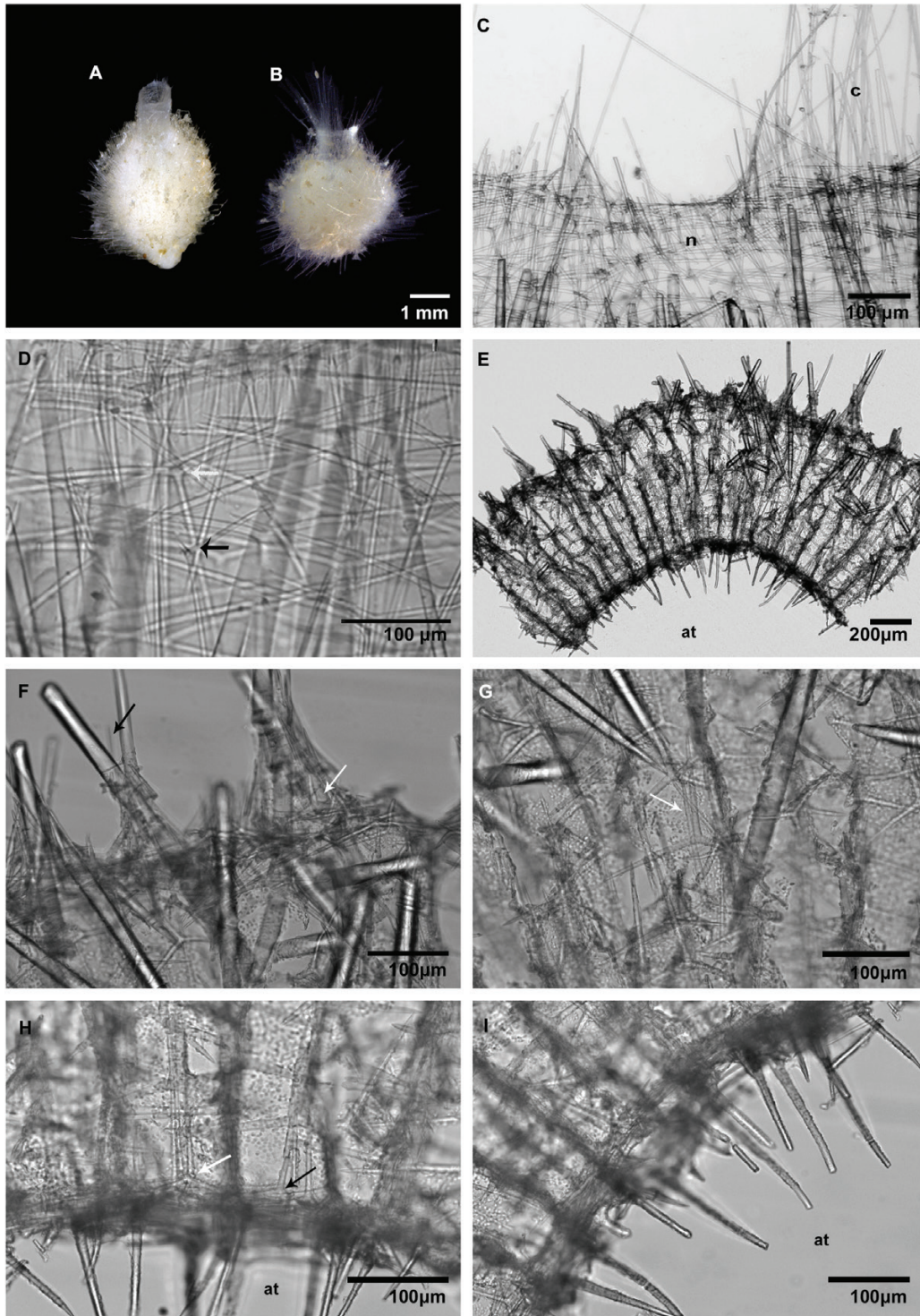
*Type locality:* Tug Boat, Caracasbaai, Willemstadt, Curaçao.

*Material examined:* *Holotype.* UFRJPOR 6748, Tug Boat, Caracasbaai, Willemstadt, Curaçao (12°04'08.20"N, 68°51'44.40"W), 14.9 m depth, coll. B. Córdor-Luján, 23 August 2011. *Paratype.* UFRJPOR 6763, Water Factory, Willemstadt, Curaçao (12°06'30.88"N, 68°57'13.53"W), 8.4 m depth, coll. E. Hajdu, 23 August 2011.

*Diagnosis:* *Sycon* with globular body composed of an oscular neck and a crown. Skeleton composed of diactines and triactines in the distal cones, tubar triactines, subatrial tetractines and triactines, and atrial tetractines. Tetractines with long apical actines projected into the atrium.

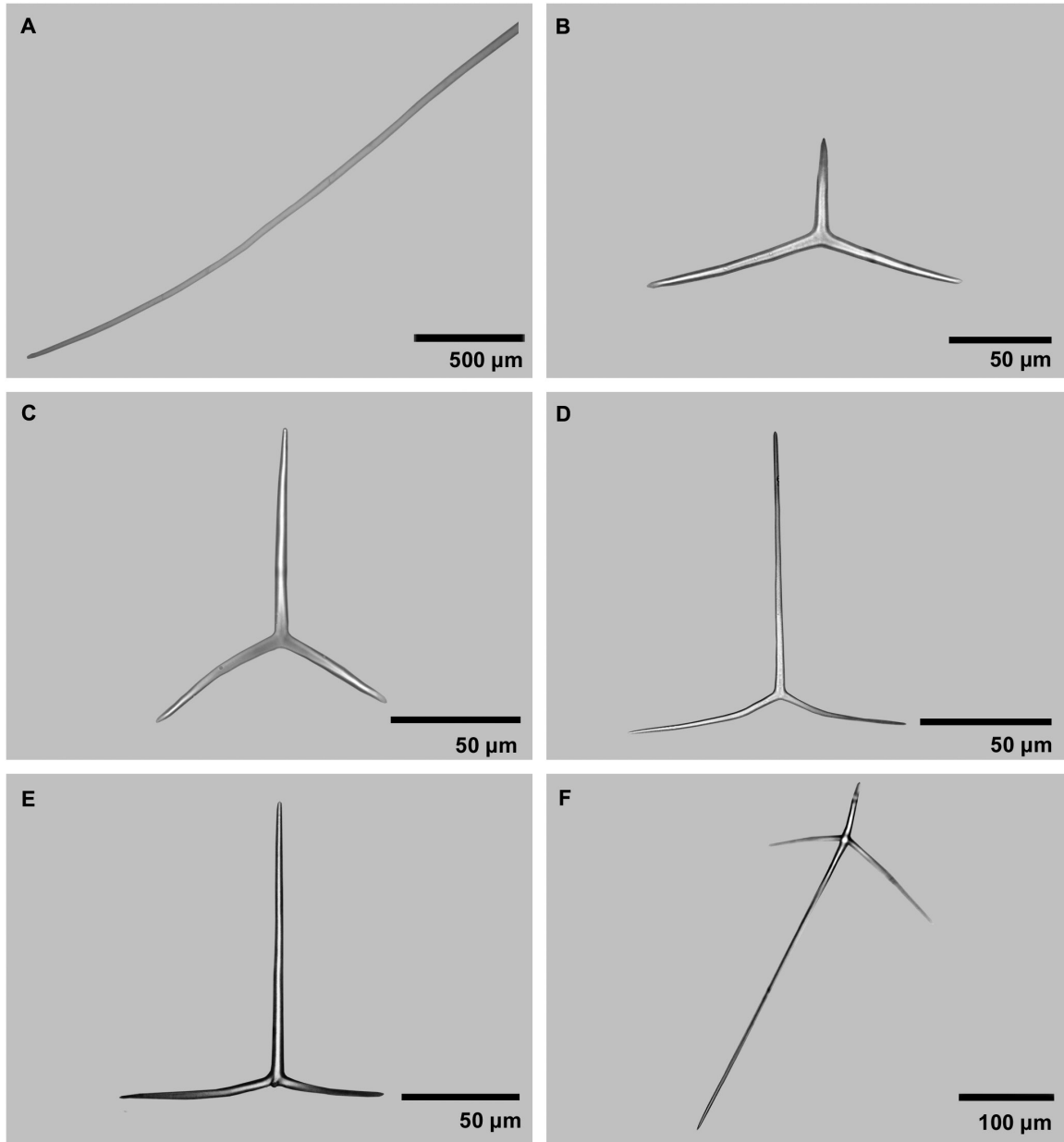
*Colour:* White to beige in life and yellowish in ethanol (Fig. 28A, B).

*Morphology and anatomy:* This species has a globular body with an apical osculum (Fig. 28A, B). The holotype



**Figure 28.** *Scycon magnapicale* sp. nov. A–B, UFRJPOR 6748 and UFRJPOR 6763 after fixation. C, skeleton of the osculum indicating the neck (n) and the crown (c). D, detail of the oscular T-shaped triactine (white arrow) and tetractine (black arrow). E, cross section of the skeleton. F, distal cones with diactines (black arrow) and triactines (white arrow). G, tubar skeleton indicating a tubar triactine (arrow). I, subatrial skeleton with tetractine (white arrow) and triactine (black arrow). J, atrial skeleton with tetractines. All skeleton photos were taken from UFRJPOR 6748. at, atrium.





**Figure 29.** Spicules of *Sycon magnapicale* sp. nov. (UFRJPOR 6748). A, diactine. B, triactine of the distal cone. C, tubar triactine. D, subatrial triactine. E, subatrial tetractine. F, atrial tetractine.

measures  $6.5 \times 2.5 \times 0.1$  cm (Fig. 28A). The consistency is hard, although compressible. The surface is very hispid with long diactines and trichoxeas protruding through the surface. The osculum has a neck with a delicate crown of trichoxeas. In the paratype, the crown is more conspicuous (Fig. 28B). The radial tubes are fully coalescent. The atrial cavity is hispid and the aquiferous system is syconoid.

**Skeleton:** The osculum has a neck composed of T-shaped triactines and tetractines and a crown of

trichoxeas (Fig. 28C). The triactines (white arrow) and tetractines (black arrow) are arranged parallel to each other (Fig. 28D). The skeleton of the body is typical of the genus (Fig. 28E). The distal cones have diactines (Fig. 28F, black arrow), triactines (Fig. 28F, white arrow) and rare trichoxeas tangentially positioned. Some longer diactines cross the choanosome and occasionally reach the atrium. The tubar skeleton is articulated, exclusively formed by rows of triactines with the unpaired actine pointing to the distal cones (Fig. 28G). The subatrial skeleton is composed of

**Table 20.** Spicule measurements of *Sycon magnapicale* sp. nov. (holotype = UFRJPOR 6748 and paratype = UFRJPOR 6763)

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N	
			Min	Mean	SD	Max	Min	Mean	SD	Max		
UFRJPOR 6748	Trichoxea		864.0	-	-	-	1.3	3.6	1.2	5.4	20	
	Diactine		>2835	-	-	-	23.0	26.4	1.3	27.0	12	
	Triactine (distal cone)	Paired		64.8	110.6	17.9	145.8	5.4	9.7	1.6	10.8	20
		Unpaired		40.5	97.7	34.5	175.5	5.4	8.8	1.8	10.8	20
	Tubar triactine	Paired		54.0	94.0	26.9	135.0	5.4	7.2	1.6	10.8	19
		Unpaired		35.1	65.5	25.2	118.8	5.4	6.3	1.2	8.1	20
	Subatrial triactine	Paired		67.5	88.2	16.6	126.9	4.1	5.5	0.7	6.8	20
		Unpaired		121.5	184.4	29.6	245.7	5.4	6.1	0.9	8.1	20
	Subatrial tetractine	Paired		75.6	106.0	20.2	143.1	4.1	5.5	1.0	8.1	20
		Unpaired		143.1	194.5	23.5	237.6	5.4	7.4	1.1	8.1	20
		Apical		16.2	28.9	15.1	75.6	2.7	4.7	0.9	6.8	19
	Atrial tetractine	Paired		116.1	157.0	19.4	183.6	5.4	8.3	1.3	10.8	15
		Unpaired		29.7	67.5	34.3	151.2	5.4	7.5	1.5	10.8	13
Apical			221.4	313.6	84.4	496.0	5.4	8.8	1.7	10.8	11	
UFRJPOR 6763	Trichoxea		216.0	671.7	386.0	1690.2	1.4	3.2	2.0	5.4	20	
	Diactine		>2970	-	-	-	24.3	27.4	1.7	32.4	17	
	Triactine (distal cone)	Paired		54.0	89.1	24.3	153.9	5.4	6.8	1.2	8.1	20
		Unpaired		40.5	64.4	21.5	121.5	5.4	5.9	1.0	8.1	20
	Tubar triactine	Paired		54.0	104.1	23.2	129.6	5.4	8.5	1.3	10.8	20
		Unpaired		94.5	156.6	25.7	189.0	5.4	8.8	1.5	10.8	20
	Subatrial triactine	Paired		72.9	91.0	13.0	113.4	2.7	4.9	1.0	6.8	20
		Unpaired		67.5	169.0	37.6	234.9	4.1	6.1	1.3	8.1	20
	Subatrial tetractine	Paired		75.6	103.8	14.6	132.3	5.4	5.7	0.8	8.1	20
		Unpaired		148.5	191.3	24.9	232.2	5.4	7.1	1.5	10.8	20
		Apical		10.8	20.9	9.6	51.3	2.7	4.2	1.2	5.4	20
	Atrial tetractine	Paired		110.7	148.4	25.4	237.6	6.8	9.7	1.8	13.5	20
		Unpaired		32.4	63.5	25.9	113.4	5.4	9.3	2.6	16.2	20
		Apical		124.2	227.0	56.5	345.0	8.1	10.8	2.5	16.2	19

tetractines (Fig. 28H, white arrow) and rare triactines (Fig. 28H, black arrow) with the unpaired actine pointing to the surface. The atrial skeleton is formed by tetractines. The basal actines lie tangentially and the very long apical actine is projected into the atrium (Fig. 28I).

*Spicules: Trichoxeas.* Straight with tips always broken. Thicker than usual. Size: >1690.2/1.4–5.4 µm. *Diactines.* Fusiform and straight. The proximal tip is sharp and the distal tip was always found broken (Fig. 29A). Size: >2970.0/23.0–32.4 µm. *Triactines of the distal cones.* Sagittal. Actines are conical with sharp tips. Some paired actines can be slightly longer than the unpaired one (Fig. 29B). Size: 54.0–153.9/5.4–10.8 µm (paired actines) and 40.5–175.5/5.4–10.8 µm (unpaired actine). *Tubar triactines.* Sagittal. Actines are conical with sharp tips (Fig. 29C). Some paired actines are curved. Size: 54.0–135.0/5.4–10.8 µm

(paired actine) and 35.1–189.0/5.4–10.8 µm (unpaired actine). *Subatrial triactines.* Sagittal. Actines are conical with sharp tips. The unpaired actine is straight and longer than the paired ones (Fig. 29D). The paired actines are inwardly curved. In some triactines, one paired actine can be longer than the other. Size: 67.5–126.9/2.7–6.8 µm (paired actine) and 67.5–245.7/4.1–8.1 µm (unpaired actine). *Subatrial tetractines.* Sagittal. Actines are conical with sharp tips. The unpaired actine is straight and is longer than the basal actines. It can be slightly conical. The paired actines are inwardly curved (Fig. 29E). The apical actine is the smallest actine present in this species. Size: 40.0–143.1/4.1–8.1 µm (paired actines), 143.1–237.6/5.4–10.8 µm (unpaired actine) and 10.8–75.6/2.7–6.8 µm (apical actine). *Atrial tetractines.* Sagittal. The basal actines are conical with sharp tips (Fig. 29F). The apical actine is slightly conical to cylindrical, straight, smooth and very long. It can

be straight or curved. Size: 110.7–237.6/5.4–13.5 µm (paired actines), 29.7–151.2/5.4–16.2 µm (unpaired actine) and 124.2–496.0/5.4–16.2 µm (apical actine).

**Ecology:** The specimens of this species were collected underneath coral boulders at 8 and 15 m depth. They were found partially covered with sediment. No associated organisms were observed.

**Geographical distribution:** Southern Caribbean (provisionally endemic to Curaçao, present study).

**Molecular analysis:** The two sequences of *S. magnapicale* sp. nov. (holotype UFRJPOR 6748 and paratype UFRJPOR 6763) grouped together with high support (pp = 1, b = 99, Fig. 15) and 100% genetic similarity. The phylogenetic affinities between *S. magnapicale* sp. nov. and the other species were not conclusive. In the ML phylogenetic tree only (data not shown), *Leucandra falakra* (Grantiidae) appeared as the sister species of *S. magnapicale* sp. nov., but with low support (b = 48).

**Taxonomic remarks:** The difference between *Grantia* and *Sycon* is the presence of a 'cortex composed of tangential triactines and/or tetractines, occasionally with small perpendicular diactines' (Borojevic *et al.*, 2000) in *Grantia*, whereas in *Sycon* there is no such continuous cortex and the distal ends of the radial tubes form cones which are decorated by tufts of diactines (Borojevic *et al.*, 2002). The specimens from Curaçao do not perfectly match the diagnosis of either *Grantia* or *Sycon* as it was possible to distinguish some radial tubes covered by tangential triactines and perpendicular diactines and other radial tubes with distal cones decorated by tufts of diactines. Nonetheless, we decided to allocate the new species to *Sycon* due to the presence of distal cones. However, we would not be surprised if this species is reallocated to *Grantia* or to another genus in future studies.

The most remarkable characteristic of *S. magnapicale* sp. nov. is the presence of tetractines with long apical actines projected into the atrium. Other species of *Sycon* that also have this skeletal feature are *S. defendens* Borojevic, 1967 from South Africa, *S. minutum* Jenkin, 1908 from Zanzibar, *S. plumosum* Tanita, 1943 from Paulau (Carolinas Islands), *S. setosum* and *S. villosum*. Among them, the species that most resembles *S. magnapicale* sp. nov. is *S. plumosum*, as both species have the same skeletal composition. *Sycon setosum* and *S. villosum* differ from the new species by not having subatrial tetractines. *Sycon defendens* and *S. minutum* bear tubar tetractines which are absent in *S. magnapicale* sp. nov.

Although *S. magnapicale* sp. nov. and *S. plumosum* have similar skeletal composition, they greatly differ in spicule size. Spicule measurements of *S. plumosum* from its original description are provided here. Diactines: 800–3000/30–35 µm, triactines of the distal cone: 200–240/16 µm (paired actine) and 140–200/16 µm (unpaired actine), tubar triactines: 170–240/15–18 µm (paired actine) and 270–360/15–18 µm (unpaired actine), subatrial triactines: 120–180/8–10 µm (paired actine) and 250–360/8–10 µm (unpaired actine), subatrial tetractines: 120–180/8–10 µm (paired actine) and 250–360/8–10 µm (unpaired actine) and 70–100/8–10 µm (apical actine) and atrial tetractines: 170–200/12–16 µm (paired actine), 200–280/12–16 µm (unpaired actine) and 130–350/12–16 µm (apical actine). Compared to *S. plumosum*, all the spicule categories but the diactines and atrial tetractines are smaller in *S. magnapicale* sp. nov. Additionally, *S. plumosum* lacks the oscular neck observed in both Curaçaoan specimens of *S. magnapicale* sp. nov.

In the phylogenetic tree, *S. magnapicale* sp. nov. did not cluster with any other *Sycon* with well-defined distal cones (namely *S. ancora*, *S. carteri*, *Sycon* cf. *villosum*, *S. raphanus* and *S. conulosum* sp. nov.). Maybe a future detailed analysis of the presence or absence of well-defined distal cones in *Sycon* would guide us to an understanding of the polyphyletic position of this genus in molecular phylogenies (Voigt *et al.*, 2012; Voigt & Wörheide, 2016).

## DISCUSSION

### BIODIVERSITY AND BIOGEOGRAPHY

The known diversity of calcareous sponges from Curaçao has increased from two to 18 species, ten species in Calcinea and eight in Calcaronea, which is somewhat at odds with the considerably larger worldwide diversity of the latter subclass (531 calcaroneans vs. 209 calcineans). Within the subclass Calcinea, the richest genus was *Clathrina* with six species: *C. curacaoensis* sp. nov., *C. globulosa* sp. nov., *C. hondurensis*, *C. insularis*, *C. lutea* and *C. mutabilis*. This is not surprising as *Clathrina* is one of the most speciose genera in Calcinea (54 species beside those describe here; Klautau *et al.*, 2013; Van Soest *et al.*, 2017) and is always well represented in studies of calcareous sponges (e.g. Klautau & Borojevic, 2001; Rapp, Klautau & Valentine, 2001; Imšek *et al.*, 2014; Azevedo *et al.*, 2015, 2017; Klautau *et al.*, 2016; Voigt *et al.*, 2017). In Calcaronea, *Leucilla* was the richest genus with three species, *L. amphora*, *L. antillana* sp. nov. and *L. micropilosa* sp. nov., and was followed by *Sycon* with two species, *S. conulosum* sp. nov. and *S. magnapicale* sp. nov. The calcinean genus *Borojevia* as well as the



**Table 21.** List of species from the Caribbean Sea including their distribution according to Spalding *et al.* (2007) and references

Species	Distribution	Ecoregion, province, realm	References
<b>Subclass Calcaronea</b>			
<i>Amphoriscus testiparus</i> (Haeckel, 1872)	Cuba (TL)	Greater Antilles, TNA, TA	<a href="#">Haeckel (1872)</a>
<i>Amphoriscus urna</i> Haeckel, 1872	Venezuela (TL)	S Caribbean, TNA, TA	<a href="#">Haeckel (1872)</a>
<b><i>Grantessa tumida</i> sp. nov.</b>	Curaçao (TL)	S Caribbean, TNA, TA	This study
<i>Leucandra barbata</i> (Duchassaing & Michelotti, 1864)	Dry Tortugas Virgin Islands (TL) Colombia NE Brazil NE Brazil and SE Brazil	Floridian, TNA, TA E Caribbean, TNA, TA SW Caribbean, TNA, TA NE Brazil, TSA, TA E Brazil, TSA, TA	<a href="#">de Laubenfels (1936)</a> <a href="#">Duchassaing &amp; Michelotti (1864)</a> <a href="#">Rozemeijer &amp; Dulfer (1987)</a> <a href="#">Borojevic &amp; Peixinho (1976)</a> <a href="#">Borojevic &amp; Peixinho (1976)</a>
<b><i>Leucandra caribea</i> sp. nov.</b>	Curaçao (TL)	S Caribbean, TNA, TA	This study
<i>Leucandra crustacea</i> (Haeckel, 1872)	Bermuda Venezuela (TL)	Bermuda, TNA, TA S Caribbean, TNA, TA	<a href="#">de Laubenfels (1950)</a> <a href="#">Haeckel (1872)</a>
<i>Leucandra curva</i> (Schuffner, 1877)	Barbados	E Caribbean, TNA, TA	<a href="#">Schuffner (1877)</a>
<b><i>Leucandrilla quadriradiata</i> sp. nov.</b>	Curaçao (TL)	S Caribbean, TNA, TA	This study
<i>Leucilla amphora</i> Haeckel, 1872	Puerto Rico (TL?) Barbados (TL?) Curaçao Senegal, West Africa	Greater Antilles, TNA, TA E Caribbean, TNA, TA S Caribbean, TNA, TA Sahelian Upwelling, WAT, TA	<a href="#">Haeckel (1872)</a> <a href="#">Haeckel (1872)</a> <a href="#">Arndt (1927)</a> <a href="#">Borojevic &amp; Boury-Esnault (1987)</a>
<b><i>Leucilla antillana</i> sp. nov.</b>	Curaçao (TL)	S Caribbean, TNA, TA	This study
<b><i>Leucilla micropilosa</i> sp. nov.</b>	Curaçao (TL)	S Caribbean, TNA, TA	This study
<i>Sycon ampulla</i> (Haeckel, 1872)	Venezuela (TL?) SE Brazil and S Brazil (TL?) Azores, Portugal	S Caribbean, TNA, TA SE Brazil, WTSA, TeSA Azores Canaries Madeira, Lusitanian, TeNA	<a href="#">Haeckel (1872)</a> <a href="#">Haeckel (1872)</a> <a href="#">Topsent (1892)</a>
<i>Sycon barbadense</i> (Schuffner, 1877)	Barbados (TL)	E Caribbean, TNA, TA	<a href="#">Schuffner (1877)</a>
<b><i>Sycon conulosum</i> sp. nov.</b>	Curaçao (TL)	S Caribbean, TNA, TA	This study
<i>Sycon formosum</i> (Haeckel, 1870)	Cuba (TL)	Greater Antilles, TNA, TA	<a href="#">Haeckel (1870, 1872)</a>
<b><i>Sycon magnapicale</i> sp. nov.</b>	Curaçao (TL)	S Caribbean, TNA, TA	This study
<i>Sycon villosum</i> (Haeckel, 1870)	Norway Ireland North coast of France  British Isles Florida Puerto Rico (TL?) Barbados (TL?) Venezuela	S Norway, NES, TeNA Celtic Seas, NES, TeNA Celtic Seas, NES, TeNA  Celtic Seas, NES, TeNA Floridian, TNA, TA Greater Antilles, TNA, TA E Caribbean, TNA, TA S Caribbean, TNA, TA	<a href="#">Burton (1930)</a> <a href="#">Haeckel (1872)</a> <a href="#">Haeckel (1872); Borojevic, Cabioch &amp; Lévi (1968)</a> <a href="#">Haeckel (1872)</a> <a href="#">Haeckel (1872)</a> <a href="#">Haeckel (1870, 1872)</a> <a href="#">Haeckel (1870, 1872)</a> <a href="#">Haeckel (1872)</a>

Table 21. Continued

Species	Distribution	Ecoregion, province, realm	References
<b>Subclass Calcinea</b>			
<i>Arturia hirsuta</i> Klautau & Valentine, 2003	Stil Bay (TL), Cape Town, South Africa	Agulhas Bank, Agulhas, TeSAf	Klautau & Valentine (2003)
	Cape Verde	Cape Verde, WAT, TA	Klautau <i>et al.</i> (2013)
	La Martinique	E Caribbean, TNA, TA	Pérez <i>et al.</i> (2017)
<b><i>Arturia vansoesti</i> sp. nov.</b>	Curaçao (TL)	S Caribbean, TNA, TA	This study
<i>Ascaltis panis</i> (Haeckel, 1870)	Florida, USA	Floridian, TNA, TA	Haeckel (1870)
	Carrier Bow Cay, Belize	W Caribbean, TNA, TA	Rützler <i>et al.</i> (2014)
	Cape Verde, West Africa	Cape Verde, WAT, TA	Thacker (1908)
<i>Borojevia tenuispinata</i> Azevedo <i>et al.</i> , 2017	Curaçao	S Caribbean, TNA, TA	This study
	São Pedro e São Paulo Archipelago (TL), NE Brazil	São Pedro e São Paulo Islands, TSA, TA	Azevedo <i>et al.</i> (2017)
<i>Clathrina aurea</i> (Solé-Cava <i>et al.</i> , 1991)	La Martinique	E Caribbean, TNA, TA	Pérez <i>et al.</i> (2017)
	FN, NE Brazil	FN Archipelago and Atoll das Rocas, TSA, TA	Muricy <i>et al.</i> (2011)
	Potiguar Basin, NE Brazil	NE Brazil, TSA, TA	Lanna <i>et al.</i> (2009)
	Abrolhos Archipelago, NE Brazil	E Brazil, TSA, TA	Azevedo <i>et al.</i> (2017)
	SE Brazil (TL: Rio de Janeiro)	SE Brazil, WTSA, TA	Solé-Cava <i>et al.</i> (1991)
	S Peru	Humboldtian, WTSEP, TeSAf	Azevedo <i>et al.</i> (2015)
<b><i>Clathrina curacaoensis</i> sp. nov.</b>	Curaçao (TL)	S Caribbean, TNA, TA	This study
<b><i>Clathrina globulosa</i> sp. nov.</b>	Curaçao (TL)	S Caribbean, TNA, TA	This study
<i>Clathrina hondurensis</i> Klautau & Valentine, 2003	Belize (TP)	W Caribbean, TNA, TA	Klautau & Valentine (2003)
	Carrie Bow Cay, Belize	W Caribbean, TNA, TA	Rützler <i>et al.</i> (2014)
	Curaçao	S Caribbean, TNA, TA	This study
<i>Clathrina insularis</i> Azevedo <i>et al.</i> , 2017	Curaçao	S Caribbean, TNA, TA	This study
	FN (TL), NE Brazil	FN and Atoll das Rocas, TSA, TA	Azevedo <i>et al.</i> (2017)
<i>Clathrina lutea</i> Azevedo <i>et al.</i> , 2017	Florida, USA	Floridian, TNA, TA	Klautau <i>et al.</i> (2013)
	Jamaica	Greater Antilles, TNA, TA	Lehnert & Van Soest (1998); this study
	Curaçao	S Caribbean, TNA, TA	This study
	Virgin Islands	E Caribbean, TNA, TA	Klautau <i>et al.</i> (2013)
	Abrolhos Archipelago (TL), NE Brazil	E Brazil, TSA, TA	Azevedo <i>et al.</i> (2017)
<i>Clathrina mutabilis</i> Azevedo <i>et al.</i> , 2017	Curaçao	S Caribbean, TNA, TA	This study
	FN Archipelago (TL), NE Brazil	FN and Atoll das Rocas, TSA, TA	Azevedo <i>et al.</i> (2017)
<i>Leucaltis clathria</i> Haeckel, 1872	Florida (TL), USA	Floridian, TNA, TA	Haeckel (1872)
	Bocas del Toro, Panama	SW Caribbean, TNA, TA	Klautau <i>et al.</i> (2013)
	Guyana Shelf	Guianian, NBS, TA	Van Soest (2017)
	N Brazil	Amazonia, NBS, TA	Borojevic & Peixinho (1976)
	NE Brazil	NE Brazil, TSA, TA	Borojevic & Peixinho (1976); Lanna <i>et al.</i> (2009)
	SE Brazil	E Brazil, TSA, TA	Borojevic & Peixinho (1976)

**Table 21.** *Continued*

Species	Distribution	Ecoregion, province, realm	References
<i>Leucetta floridana</i> (Haeckel, 1872)	Bermuda	Bermuda, TNA, TA	de Laubenfels (1950)
	Florida (TL), USA	Floridian, TNA, TA	Haeckel (1872)
	Jamaica and Cayman Islands	Greater Antilles, TNA, TA	Lehnert & Van Soest (1998); Miloslavich <i>et al.</i> (2010)
	Panama and Colombia	SW Caribbean, TNA, TA	Valderrama <i>et al.</i> (2009); Klautau <i>et al.</i> (2013)
	Curaçao	S Caribbean, TNA, TA	This study
	La Martinique	E Caribbean, TNA, TA	Pérez <i>et al.</i> (2017)
	N Brazil	Amazonia, NBS, TA	Borojevic & Peixinho (1976)
	NE Brazil	NE Brazil, TSA, TA	Borojevic & Peixinho (1976); Lanna <i>et al.</i> (2009); Valderrama <i>et al.</i> (2009)
	FN Archipelago and Rocas Atoll	FN and Atoll das Rocas, TSA, TA	Borojevic & Peixinho (1976); Valderrama <i>et al.</i> (2009)
	NE Brazil and SE Brazil	E Brazil, TSA, TA	Borojevic & Peixinho (1976); Valderrama <i>et al.</i> (2009)
<i>Leucetta imberbis</i> (Duchassaing & Michelotti, 1864)	Virgin Islands (TL)	E Caribbean, TNA, TA	Duchassaing & Michelotti (1864)
	Jamaica	Greater Antilles, TNA, TA	Lehnert & Van Soest (1998)
<i>Leucettusa corticata</i> (Haeckel, 1872)	Cuba	Greater Antilles, TNA, TA	Haeckel (1872)
	New Zealand?	?, S New Zealand?, TAA	Kelly <i>et al.</i> (2009)
<i>Nicola tetela</i> (Borojevic & Peixinho, 1976)	Curaçao	S Caribbean, TNA, TA	Cóndor-Luján & Klautau (2016)
	NE Brazil (TL: Bahia State)	E Brazil, TSA, TA	Borojevic & Peixinho (1976)

TL, type locality; ?, doubtful record. Provinces in the Tropical Atlantic (TA) realm – NBS: North Brazil Shelf, TNA: Tropical Northwestern Atlantic, TSA: Tropical Southwestern Atlantic, WAT: West African Transition. Other provinces – NES: Northern European Seas, WTSA: Warm Temperate Southwestern Atlantic, WTSEP: Warm Temperate Southeastern Pacific. Other realms – TAA: Temperate Australasia, TeNA: Temperate Northern Atlantic, TeSAf: Temperate Southern Africa, TeSAm: Temperate South America. E: Eastern, N: Northern, NE: Northeastern, S: Southern, SE: Southeastern, SW: Southwestern, W: Western.

calcareous genera *Grantessa* and *Leucandrilla* constitute not only new records of genera for Curaçao, but also for the entire Caribbean Sea.

The finding of ten species provisionally endemic to Curaçao (55.6%, 10/18), *A. vansoesti* sp. nov., *C. curacaoensis* sp. nov., *C. globulosa* sp. nov., *G. tumida* sp. nov., *L. caribea* sp. nov., *L. quadriradiata* sp. nov., *L. antillana* sp. nov., *L. micropilosa* sp. nov., *S. conulosum* sp. nov. and *S. magnapicale* sp. nov., might indicate a high endemism of Calcarea in this island; however, it is also possible that this is merely mirroring how understudied this class is in the whole Caribbean region.

With the addition of these Curaçaoan records, the valid number of calcareous species reported from the Caribbean Sea rose from 19 to 33 (see Table 21), an increase of 73.7%. Within the Caribbean Sea, the richest ecoregion is the Southern Caribbean, with 22 valid species (Haeckel, 1870, 1872; Arndt, 1927; this study). The Eastern Caribbean has ten species (Duchassaing & Michelotti, 1864; Haeckel, 1870, 1872; Schuffner, 1877; Pérez *et al.*, 2017), and it is followed by the Greater Antilles with eight species (Haeckel, 1870, 1872; Lehnert & Van Soest, 1998).

The Southwestern Caribbean and the Western Caribbean possess three and two species records, respectively (Rozemeijer & Dulfer, 1987; Klautau & Valentine, 2003; Valderrama *et al.*, 2009; Klautau *et al.*, 2013; Rützler *et al.*, 2014).

Previously, only six species were known to be shared by the Caribbean Sea and Brazil, namely, *Clathrina aurea*, *Leucaltis clathria*, *Leucandra barbata*, *L. floridana*, *N. tetela* and *S. ampulla* (Muricy *et al.*, 2011; Cóndor-Luján & Klautau, 2016; Pérez *et al.*, 2017). In this study, we expanded the geographic distribution of four species formerly considered as endemic to the Brazilian oceanic islands (*B. tenuispinata*, *C. insularis*, *C. lutea* and *C. mutabilis*; Azevedo *et al.*, 2017) to the Caribbean (Curaçao). Therefore, ten species (31.3%, 10/32) are currently shared between these two regions. This result suggests a Caribbean-Brazilian affinity for Calcarea and questions the role of the Amazon and Orinoco Rivers as effective barriers to the dispersal of calcareous sponges.

Although the Caribbean-Brazilian affinity in Calcarea is being reported for the first time in this study, it was previously observed for several



Demospongiae (Hechtel, 1976; Collette & Rützler, 1977; Van Soest & Hajdu, 1997; Klautau *et al.*, 1999; Lazoski *et al.*, 2001; Moraes, 2011; Muricy *et al.*, 2011; Moura *et al.*, 2016). Hechtel (1976) reported numerous Demospongiae from both the West Indies and Brazil, and suggested that larvae of those species could cross the Orinoco and Amazon barriers, assuming the occurrence of hard substrate availability close to both river's discharges. One year later, Collette & Rützler (1977) not only reported 'Caribbean' sponges underneath the mouth of the Amazon River, but also mentioned that there was no apparent effect of freshwater on the sponges and that there was abundant hard substrata on the bottom of the Amazon mouth. Recently, Moura *et al.* (2016) reported the existence of a calcium carbonate system underneath the Amazon river plume, which would act as a connectivity corridor for wide depth-ranging reef-associated species, such as sponges. Considering this, it is also expected to find these ten shared species in intermediate localities (e.g. in the Venezuelan Southeastern Coast, Guyanas or in the Northern Brazilian Coast). In fact, *L. clathrina* and *L. floridana* have already been recorded in such intermediate localities (Borojevic & Peixinho, 1976; Van Soest, 2017).

#### MOLECULAR TAXONOMY AND PHYLOGENY OF CALCAREA

##### *Subclass Calcinea*

The phylogenetic affinities inferred from our ITS and C-LSU trees were congruent with the classification proposed by Klautau *et al.* (2013) using ITS, and by Voigt & Wörheide (2016) using C-LSU. With the addition of new sequences (*C. curacaoensis* sp. nov. and *B. tenuispinata*), we supported the monophyly of the genera *Clathrina* and *Borojevia* as obtained in other recent studies (Azevedo *et al.*, 2017; Voigt *et al.*, 2017).

According to Klautau *et al.* (2016), 'the development of a peduncle and of parasagittal spicules probably appeared only once in the evolution of *Clathrina*' as former guanchas (*C. blanca*, *C. hispanica*, *C. ramosa*) clustered in a monophyletic clade in their phylogenetic tree (fig. 16, p. 38). In this study, *C. curacaoensis* sp. nov., a species with parasagittal spicules (but without peduncle), did not group with the referred species (former guanchas), indicating that at least parasagittal spicules appeared independently more than once in *Clathrina*.

In Calcinea, the molecular information obtained from both DNA markers (ITS and C-LSU) certainly contributed to the identification of species. DNA similarity (*p* distance) and tree topology (well-supported monophyletic clades) were appropriate approaches for delimiting species and genera, as pointed out by Azevedo *et al.* (2017) using ITS sequences. It was

also possible to test whether or not the morphological variation observed among some conspecific specimens corresponded to true interspecific polymorphism/plasticity. Azevedo *et al.* (2017) suggested that trichoxeas do not seem to be reliable characters to differentiate species in *Clathrina* as specimens of *C. mutabilis* with and without trichoxeas clustered together in their ITS tree (Fig. 18, p. 338). We confirmed this result adding sequences of Curaçaoan specimens of *C. mutabilis* in the phylogenetic tree, and we also found the same pattern in another species, *C. lutea*.

##### *Subclass Calcaronea*

As in previous Calcaronean phylogenies where different DNA markers were analysed (18S and 28S: Dohrmann *et al.*, 2006; 28S: Voigt *et al.*, 2012; Klautau *et al.*, 2016 and C-LSU: Voigt & Wörheide, 2016), our C-LSU phylogenetic reconstruction recovered several taxa as non-monophyletic.

Voigt *et al.* (2012) found the family Amphoriscidae not to be a monophyletic group; however, they could not elucidate the affinities within each Amphoriscidae genus as enough sequences were not available. Klautau *et al.* (2016) recovered a well-supported clade (*pp* = 0.95, *b* = 0.64) formed only by *Paraleucilla* species (*P. magna* and *P. dalmatica*), which suggested the possible monophyly of that genus. In the present study, using a larger set of sequences, we confirmed that Amphoriscidae is not monophyletic and refuted the monophyly of *Paraleucilla* and *Leucilla*.

With a reduced number of Heteropiidae sequences (*n* = 2: *Sycettusa* sp. and *Vosmaeropsis* sp.), Manuel *et al.* (2003, 2004) supported the monophyly of that family. In subsequent studies using more sequences (Dohrmann *et al.*, 2006; Voigt *et al.*, 2012), Heteropiidae was not recovered as a monophyletic taxon and nor were the Heteropiidae genera *Sycettusa* and *Ute*. In this work, we found Grantessa, another Heteropiidae genus, as not monophyletic.

*Sycon* is one of the largest genera within Calcaronea, currently including 90 species. Nonetheless, our knowledge of the molecular phylogenetic affinities within this genus is based only on a few species. As in previous phylogenies (Manuel *et al.*, 2003, 2004; Dohrmann *et al.*, 2006; Voigt *et al.*, 2012; Klautau *et al.*, 2016; Voigt & Wörheide, 2016), *Sycon* was recovered as a polyphyletic genus in this study.

In Calcaronea, our results corroborated that the C-LSU marker is suitable for species identification as proposed by Voigt & Wörheide (2016); nonetheless, the correct allocation of these species to genera was not possible when only considering molecular information. An integrative revision including morphology and new molecular markers is necessary to improve the systematics of Calcaronea.

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