

# Integrative taxonomy of calcareous sponges (Porifera: Calcarea) from Réunion Island, Indian Ocean

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The Western Indian Ocean Province is reckoned for its rich marine diversity; however, sponges of the Mascarene Islands ecoregion are still poorly known. In La Réunion, only three species of class Calcarea have been registered. Hence, calcareous sponges were searched in seven sites representing various habitats of the Western coast of La Réunion, but found in only three of them. A total of 23 sponge samples was identified using morphological and molecular taxonomy. This sampling represents 11 species, all new records for the region, and seven of them are new to science: *Ascandra mascarenica* sp. nov., *A. oceanusvitae* sp. nov., *Janusya indica* gen. et sp. nov., *Leucascus tenuispinae* sp. nov., *Lelapiella tertia* sp. nov., *Soleneiscus intermedius* sp. nov. and *Leucandra ornata* sp. nov.; and a new genus, *Janusya* gen. nov. Based on results from this and from previous studies, we propose the synonymization of the order Murrayonida with Clathrinida. A very low sampling effort has thus increased the number of calcareous sponge species from the Mascarenes Islands ecoregion by 69 % and from La Réunion by 367%.

ADDITIONAL KEYWORDS: biodiversity – Indian Ocean – molecular systematics – morphological systematics – phylogenetic systematics – Porifera.

## INTRODUCTION

Although the Western Indian Ocean Province presents a rich sponge fauna, the Mascarene Islands ecoregion is still poorly known (Van Soest *et al.*, 2012; Van Soest & De Voogd, 2018). The Mascarene Islands ecoregion includes the islands of La Réunion, Mauritius and Rodrigues (Spalding *et al.*, 2007) and, to date, 66 species of sponges have been recorded from this ecoregion (Schuffner, 1877; Vacelet, 1977; Lévi, 1986; Bourmaud, 2003; Van Soest & De Voogd, 2018; Van Soest *et al.*, 2020), 16 belonging to the class Calcarea (Schuffner, 1877; Vacelet, 1977; Bourmaud, 2003; Van Soest & De Voogd, 2018).

Among these three islands, the sponge biodiversity of La Réunion is by far the least known. To our knowledge, the only studies that listed sponges from La Réunion were those of Vacelet (1977), a taxonomic work on sponges from French Polynesia, Madagascar and La Réunion; Bourmaud (2003), an inventory of the reef marine biodiversity of the western coast of La Réunion and Schleyer *et al.* (2016), a work on the benthos growing on submerged lava beds in the eastern coast of La Réunion. Only the first and the second studies recorded calcareous sponges, a total of three species: Vacelet (1977) described two hypercalcified calcareous sponges from La Réunion: the calcaroneans *Tulearinia stylifera* Vacelet, 1977 and *Plectroninia hindei* Kirkpatrick, 1900, and Bourmaud (2003) added the calcinean *Leucetta microraphis* Haeckel, 1872 to the list.

Calcarea represents the only sponge class whose skeleton contains calcite spicules (Borojević *et al.*,

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<http://zoobank.org/urn:lsid:zoobank.org:pub:3E370CBF-7DD1-4E73-BC37-81BF12EDFEED>

2002). These sponges are exclusively marine, viviparous and widely distributed at all latitudes and longitudes (Manuel *et al.*, 2002), from intertidal zones down to more than 4000 m deep (Rapp *et al.*, 2011). They are commonly neglected in inventories, possibly because of their small size and preference for cryptic habitats (e.g. Wörheide & Hooper, 1999; Klautau & Borojević, 2001; Van Soest *et al.*, 2012). It is not surprising that only three species were described from Réunion, considering that no specific sampling efforts have been made searching for *Calcarea* in the waters around this island.

To import skills that did not exist and improve knowledge on the sponge biodiversity, the Vie Océane association and the Marine Natural Reserve of Réunion (RNMR) organized a sponge training course in 2017. Designed on the same model as a previous project in Martinique (Pérez *et al.*, 2017), this course in sponge biology and taxonomy also aimed to provide a first sponge inventory together with first biological explorations of submarine caves. The focus on calcareous sponges resulted in taxonomic identifications and several descriptions presented here, and in a discussion on affinities of the calcareous sponge fauna across the Mascarene Islands, and between the Mascarene ecoregion and other ecoregions of the Western Indian Ocean.

## MATERIAL AND METHODS

### SAMPLE COLLECTION

Seven sites in the western part of La Réunion were investigated, three of them being shallow-water lagoons explored by free diving, the other four localities were reefs or caves explored by scuba diving down to 30 m deep: (1) Cap La Houssaye Reef (CAH2 – 21°1'3.2"S, 55°14'13.23"E), cliff and reef bench were explored; (2) Passe de l'Hermitage Reef (PAE2 – 21°5'11.50"S, 55°13'12.33"E), a fringing reef of the Saint Gilles Ermitage complex; (3) L'Hermitage Lagoon (PAE1 – 21°5'0.83"S, 55°13'24.48"E), a reef complex of La Saline-Ermitage, including the Closerie and the south part of the l'Hermitage Pass; (4) La Saline Lagoon (TRO1 – 21°6'0.73"S, 55°14'26.30"E), a reef complex of La Saline-Hermitage, a hole with water that replaces that of Saint Leu (La Varangue) in heavy swells; (5) Le Sec Jaune Caves (SJM2 – 21°12'25.63"S, 55°16'37.16"E), south area of Pointe au Sel, a cliff and reef banks with numerous overhangs and caves; (6) L'Étang Salé Lagoon (ESB1 – 21°16'5.22"S, 55°19'57.43"E), the southern part of the channel was investigated; (7) Portail Caves (ESB2 – 21°14'31.07"S, 55°17'41.24"E), old lava tunnels.

Each site was visited only once. While the shallow-water sites were explored by four to ten people, most sampling in caves was performed by only one scuba diver. Whenever possible, the studied sponges were photographed *in situ* before collection, and then fixed and preserved in ethanol 93%. A total of 23 calcareous sponges were collected and they were deposited in the Porifera Collection of the Universidade Federal do Rio de Janeiro (UFRJPOR).

### INSTITUTIONAL ABBREVIATIONS

GW: Gert Wörheide collection, Munich, Germany; MNRJ: Museu Nacional da Universidade Federal do Rio de Janeiro, Brazil; NHMUK: Natural History Museum, London, United Kingdom; PMR: Prirodoslovni Muzej Rijeka, Croatia; QM: Queensland Museum, Brisbane, Australia; RMNH: Naturalis Biodiversity Center, Leiden, The Netherlands; SAM: South Australian Museum, Adelaide, Australia; SMF: Senckenberg Museum, Frankfurt, Germany; SNSB-BSPG: Bayerische Staatssammlung für Paläontologie und historische Geologie, Staatlichen Naturwissenschaftlichen Sammlungen Bayern, Munich, Germany; UFRJPOR: Porifera collection of the Biology Institute of Federal University of Rio de Janeiro, Brazil; WAMZ: Western Australian Museum, Perth, Australia; ZMAPOR: Zoologisch Museum, Instituut voor Systematiek en Populatiebiologie, Amsterdam, The Netherlands

### MOLECULAR ANALYSES

Total DNA was extracted using the guanidine/phenol-chloroform protocol (Hillis *et al.*, 1996; modified from Sambrook *et al.*, 1989). To test the taxonomic status of the specimens and their phylogenetic relationships, two molecular markers were used: C-region of the 28S (referred here as C-LSU) for both subclasses Calcinea and Calcaronea, and the region comprising the spacers ITS1 and ITS2 and the 5.8S ribosomal DNA (referred as ITS) for Calcinea only. Polymerase chain reaction (PCR) sequencing conditions and sequence editing were the same used by our group in previous works (e.g. Córdor-Luján *et al.*, 2018).

In addition to the DNA sequences generated in this work, we also used sequences from GenBank (<https://www.ncbi.nlm.nih.gov/genbank>). DNA sequences were aligned through the online version of the program MAFFT v.7 (Katoh & Standley, 2013) using the strategy Q-INS-i (Katoh & Toh, 2008), taking the secondary structure of ribosomal DNA into consideration. The final alignment of 49 operational taxonomic units (OTU ITS sequences' of Calcinea was 1208 bp length, including gaps. A total of 357 conserved sites, 616 variable

sites and 136 singletons were retrieved (Supporting Information, Fig. S1). For the C-LSU, the final alignment comprised 71 OTU and 381 bp length, including gaps and a total of 139 conserved sites, 218 variable sites and 36 singletons (Supporting Information, Fig. S2). For Calcaronea, the C-LSU alignment had 45 OTU and a total length of 430 bp (including gaps), 273 conservative sites, 12 variable sites and 25 singletons (Supporting Information, Fig. S3). The substitution model chosen for the phylogenetic analyses was GTR+G+I for Calcinea (ITS and C-LSU) and TN93+G for Calcaronea (C-LSU only), according to the Model Selection in MEGA 7.0, using an initial automatic (neighbour-joining) tree and considering all sites of the alignment based on the Bayesian information criterion.

A maximum likelihood (ML) tree was generated using the software IQ-Tree 2 (Minh *et al.*, 2020) for each marker. Bootstrap supports were obtained with the ultrafast bootstrap (Hoang *et al.*, 2017) using 1000 pseudo-replicates (Felsenstein, 1985; Russo, 1997). Bayesian inferences (BI) were performed in MrBayes 3.1.2. (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), using the same evolutionary model parameters as selected for the ML analysis and two runs and four independent chains for  $10^6$  generations and a 25% burn-in, resulting in a majority consensus tree (of the sampled trees). Phylogenetic analyses were conducted using the CIPRES Science Gateway (Portal 2) online server (Miller *et al.*, 2012).

Both phylogenies of Calcinea (ITS and C-LSU) and Calcaronea (C-LSU) were midpoint-rooted, as the only appropriate outgroup would be the opposing subclass, and using such distant groups as outgroups would result in loss of information within closely related clades (Hess & Russo, 2007). However, we also built the trees with the opposing subclass as outgroups to confirm that there would be no important change in the topology of the midpoint-rooted trees (Supporting Information, Figs S4, S5).

The uncorrected  $p$ -distance was calculated in MEGA 7.0 to estimate the intraspecific and interspecific variability, considering the morphological identifications. Two criteria were used for the molecular identification of the specimens: the monophyly of morphologically conspecific specimens, and the genetic dissimilarity ( $p$ -distance) to the most closely related species with available DNA data.

Species names, location, voucher numbers and GenBank accession numbers of the DNA sequences generated in this work and those obtained from the GenBank database (<https://www.ncbi.nlm.nih.gov/genbank>) used in the phylogenetic tree are listed in Table 1.

## MORPHOLOGICAL ANALYSES

Slides of spicules and tangential ('in toto') were made according to standard protocols (Wörheide & Hooper, 1999; Klautau & Valentine, 2003). Skeleton sections were made, including fragments of the sponges, previously stained with a 5% fuchsin alcoholic solution, in paraffin and making thick sections (*c.* 500  $\mu$ m) with a microtome. The mounting medium used was Entellan (Merck). Spicule measurements (length and width at the base of the actines) were taken from 20 spicules, when possible, for every category in light microscopy using an ocular micrometer. The biggest and the smallest spicules were searched for, while the other 18 were randomly measured. The results are presented in tabular form presenting minimum (min), mean, standard deviation (SD) and maximum (max) values of both measurements, and number of analysed spicules ( $N$ ).

Photographs of preserved specimens were taken with a digital camera AxioCam ERc5s coupled to a Zeiss stereomicroscope. Slides photographs were taken with a Canon G12 camera coupled to a Zeiss Axioskop light microscope. Scanning electron microscopy (SEM) was performed in a JSM-6510 microscope at the Instituto de Biologia of the Universidade Federal do Rio de Janeiro. For this purpose, isolated spicules in ethanol 100% were transferred to stubs with double-sided carbon conductive tape and coverslip, dried and sputter coated with gold in a Balzers apparatus at the Instituto de Ciências Biomédicas of the Universidade Federal do Rio de Janeiro.

## RESULTS

Among the 23 calcareous sponges collected, we identified 11 species using both morphology and DNA sequences. All of them are new records for La Réunion. The subclass Calcinea was more diverse than Calcaronea, with eight and three species, respectively. Seven species are new to science, and include a new genus.

The species were distributed among only three sites: Passe de l'Ermitage, Reef and Cave (PAE2), Étang Salé, Portail Caves (ESB2) and Cap Lahoussaye Reef (CAH2; Fig. 1), all of them being caves, PAE2 appearing by far the richest. One single scuba-dive was not enough to cover the large networks of caves found in Le Sec Jaune Caves, what may explain the lack of calcareous specimens after the exploration of this cryptic habitat. On the other hand, whereas a much higher sampling effort was deployed in shallow waters (lagoons), no calcareous sponges could be found in this type of habitat.

**Table 1.** Species names, voucher numbers and GenBank accession numbers used in this study. \*Sequences generated in this study

Species	Locality	Voucher number	GenBank accession number	
			ITS	C-LSU
<b>Calcinea</b>				
<i>Arturia alcatraziensis</i>	Cape Verde, Africa	ZMAPOR 07061	KC843431	-
<i>Arturia spirallata</i>	Peru	MNRJ 11414	KC985142	-
<i>Arturia sueziana</i>	Red Sea	SNSB-BSPG GW3120	KY366410	KY366370
<i>Arturia sueziana</i>	Maldives	RMNH10112	-	MF872755
<i>Ascaletis reticulum</i>	Mediterranean Sea	UFRJPOR 6258	HQ588973	HQ589014
<i>Ascandra ('Ernstia') chrysops</i>	Indonesia	RMNH 1773	-	MF686061
<i>Ascandra contorta</i>	Mediterranean Sea	UFRJPOR 6327	HQ588970	HQ589011
<i>Ascandra corallicola</i>	Norway	UFRJPOR 6329	HQ588994	HQ589012
<i>Ascandra cf. crewsi</i>	French Polynesia	BMOO16290	KC843446	-
<i>Ascandra falcata</i>	Mediterranean Sea	UFRJPOR 5856	HQ588962	-
<i>Ascandra kakaban</i>	Indonesia	RMNH1696	-	MF686077
<i>Ascandra mascarenica</i> sp. nov.*	Réunion, Western Indian	UFRJPOR 8936	-	MW350111
<i>Ascandra oceanusvitae</i> sp. nov.*	Réunion, Western Indian	UFRJPOR 8944	-	MW350112
<i>Ascandra oceanusvitae</i> sp. nov.*	Réunion, Western Indian	UFRJPOR 8947	MW350104	-
<i>Ascandra spatulensis</i>	Adriatic, Croatia	PMR 17806	KP740024	-
<i>Bidderia bicolora</i>	Caribbean	UFRJPOR 8228	MG017978	MG017978
<i>Borojevia brasiliensis</i>	Brazil	UFRJPOR 5406	KX548909	HQ589015
<i>Borojevia cerebrum</i>	Mediterranean Sea	UFRJPOR 6322	HQ588964	HQ589008
<i>Brattegardia nansenii</i>	Greenland	UFRJPOR 6332	HQ588982	HQ589013
<i>Burtonulla sibogae</i>	Indonesia	RMNH9195	-	MF686091
<i>Clathrina aurea</i>	Brazil	MNRJ 8998	HQ588968	HQ589005
<i>Clathrina clathrus</i>	Mediterranean Sea	UFRJPOR 6315	HQ588965	HQ589009
<i>Clathrina conifera</i>	Brazil	UFRJPOR 8991	HQ588959	HQ589010
<i>Clathrina coriacea</i>	Norway	UFRJPOR 6330	HQ588986	HQ589001
<i>Clathrina curacaoensis</i>	Curaçao	UFRJPOR 6734	MF472607	MF472612
<i>Clathrina cylindractina</i>	Brazil	UFRJPOR 5206	HQ588979	HQ589007
<i>Clathrina lacunosa</i>	Norway	UFRJPOR 6334	HQ588991	HQ589020
<i>Clathrina lutea</i>	Brazil	UFRJPOR 5172	HQ588961	HQ589004
<i>Clathrina luteoculcitella</i>	Great Barrier Reef (GBR), Australia	QM G313684	HQ588989	JQ272283
<i>Clathrina ramosa</i>	Chile	MNRJ 10313	HQ588990	HQ589002
<i>Clathrina rotundata</i>	Red Sea	SMF 11636	KY711435	KY366399
<i>Clathrina rubra</i>	Adriatic Sea	PMR-14306	KC479088	KC479082
<i>Clathrina wistariensis</i>	Australia	QM G313663	HQ588987	JQ272303
<i>Ernstia klautauae</i>	Indonesia	ZMAPOR 8390	KC843451	-
<i>Ernstia klautauae</i>	Indonesia	RMNH9341	-	MF686062
<i>Ernstia rocasensis</i>	Brazil	UFRJPOR 6664	KX548928	-
<i>Ernstia tetractina</i>	Brazil	UFRJPOR 5183	HQ589000	HQ589021
<i>Janusya ('Arturia') adusta</i> comb. nov.	Seychelles, Western Indian	ZMAPOR 10612	-	MF872756
<i>Janusya ('Arturia') angusta</i> comb. nov.	Indonesia	ZMAPOR 8221a	-	MF686060
<i>Janusya ('Arturia') tubuloreticulosa</i> comb. nov.	Indonesia	RMNH5547	-	MF686059
<i>Janusya indica</i> sp. nov.*	Réunion, Western Indian	UFRJPOR 8932	MW350105	-
<i>Janusya indica</i> sp. nov.*	Réunion, Western Indian	UFRJPOR 8945	-	MW350113
<i>Lelapiella incrustans</i>	Vanuatu	QM G313914	-	JQ272306
<i>Lelapiella tertia</i> sp. nov.*	Réunion, Western Indian	UFRJPOR 8937	MW350106	MW350114
<i>Leucaltis clathria</i>	Panama, Caribbean Sea	UFRJPOR 6942	KC843452	KC869524

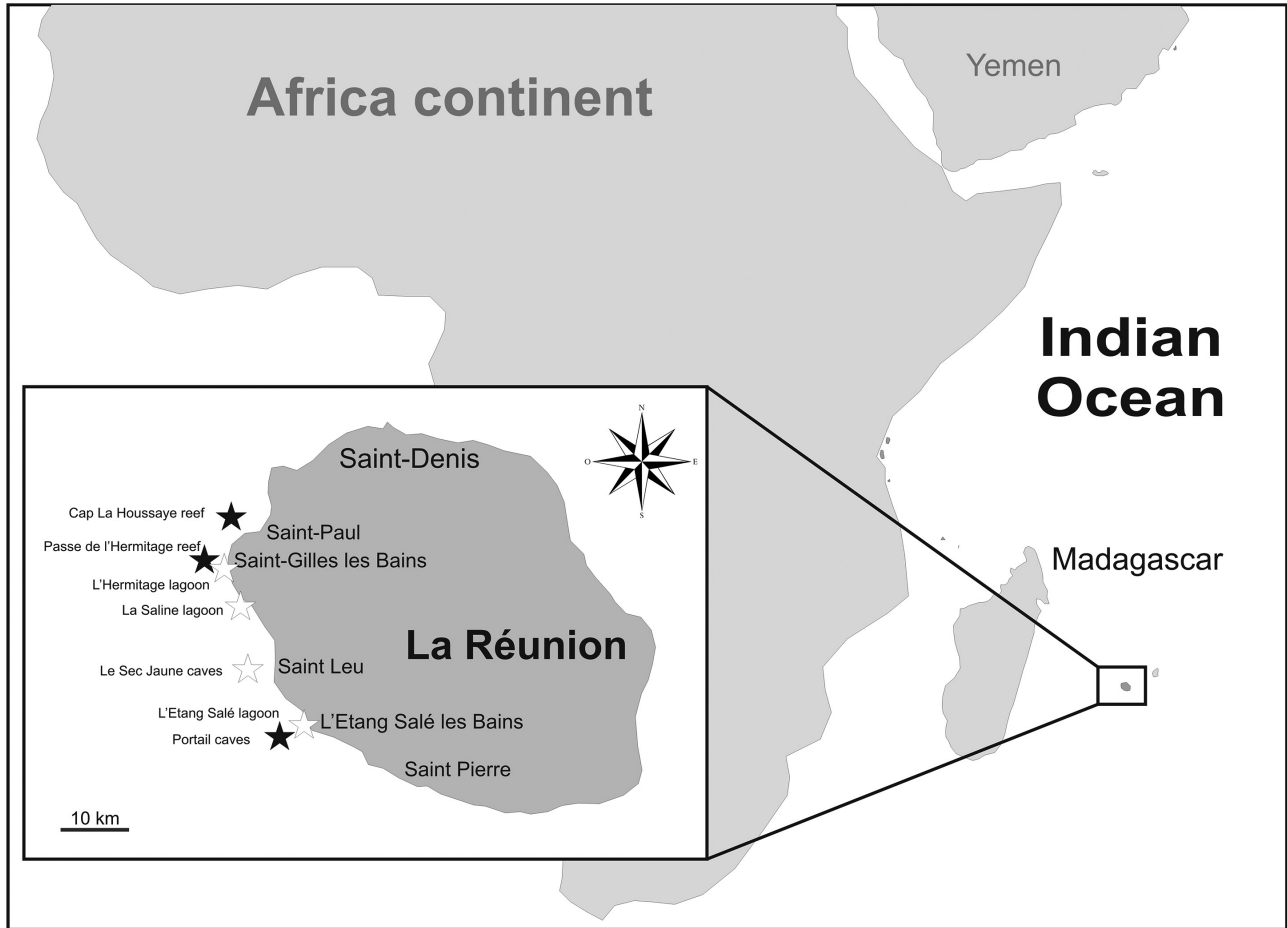


Table 1. Continued

Species	Locality	Voucher number	GenBank accession number	
			ITS	C-LSU
<i>Leucaltis clathria</i>	Panama, Caribbean Sea	UFRJPOR 6944	KU568493	-
<i>Leucaltis nodusgordii</i>	Australia	QM G316022	AJ633861	JQ272302
<i>Leucaltis nodusgordii</i> *	Réunion, Western Indian	UFRPORA 8930	MW350107	MW350115
<i>Leucaltis nodusgordii</i>	Seychelles, Western Indian	ZMAPOR 12436	-	MF872787
<i>Leucaltis nodusgordii</i>	Seychelles, Western Indian	ZMAPOR 12443	-	MF872788
<i>Leucaltis nodusgordii</i>	Indonesia	RMNH1772	-	MF686078
<i>Leucaltis nodusgordii</i>	Australia	H10	-	AY563542
<i>Leucascus flavus</i>	Indonesia	RMNH 2279	-	MF686080
<i>Leucascus simplex</i>	French Polynesia	BMOO16283	KC843454	-
<i>Leucascus simplex</i>	French Polynesia	UFRJPOR 6451	-	MN422254
<i>Leucascus</i> sp.	Australia	QM G316051	-	JQ272305
<i>Leucascus tenuispinae</i> sp. nov.*	Réunion, Western Indian	UFRJPOR 8927	-	MW350117
<i>Leucetta antarctica</i>	Antarctic	MNRJ 13798	KC849700	-
<i>Leucetta avocado</i>	Mauritius, Western Indian	NCI 108	-	KC869542
<i>Leucetta chagosensis</i> *	Réunion	UFRJPOR 8939	MW350108	MW350118
<i>Leucetta chagosensis</i>	Australia	-	-	AY563543
<i>Leucetta chagosensis</i>	Indonesia	ZMAPOR 13283	-	MF686085
<i>Leucetta chagosensis</i>	Rodrigues, Western Indian	RMNH11657 1	-	MF872790
<i>Leucetta chagosensis</i>	Rodrigues, Western Indian	RMNH11658	-	MF872791
<i>Leucetta chagosensis</i>	French Polynesia	UFRJPOR 6455	-	MN422257
<i>Leucetta chagosensis</i>	-	-	-	AM180994
<i>Leucetta chagosensis</i>	Australia	H10	AF458861	-
<i>Leucetta chagosensis</i>	French Polynesia	BMOO 16210	KC843455	-
<i>Leucetta chagosensis</i>	Saudi Arabia, Red Sea	SNSB-BSPG GW3052	KY366408	-
<i>Leucetta delicata</i>	Antarctic	SMF 11868	KC874654	-
<i>Leucetta floridana</i>	Brazil	UFRJPOR 5630	EU781968	-
<i>Leucetta floridana</i>	Panama, Caribbean Sea	P10x2	-	KC869538
<i>Leucetta foliata</i>	Western Australia	UFRJPOR 7149	-	KX499451
<i>Leucetta giribeti</i>	Antarctic	NHMUK:2017.1.19.1	KY670632	KY670632
<i>Leucetta microraphis</i>	GBR, Australia	QM G313659	AF479061	JQ272297
<i>Leucetta potiguar</i>	Brazil	MNRJ 8474	EU781981	-
<i>Leucetta purpurea</i>	Western Australia	UFRJPOR 7255	-	KX499450
<i>Leucetta</i> cf. <i>pyriformis</i>	Antarctic	MNRJ 13843	KC843457	-
<i>Leucetta sulcata</i>	Rodrigues	RMNH 11639_1	-	MF872798
<i>Leucetta villosa</i>	Maldives	QM G313662	-	JQ272295
<i>Leuclathrina translucida</i>	Maldives	SNSB-BSPG GW9394	-	LT899664
<i>Levinella prolifera</i>	GBR, Australia	QM G313818	-	AM180984
<i>Levinella thalassae</i>	Rockall Bank, Ireland	ZMAPOR 18150	-	MF686093
<i>Murrayona phanolepis</i>	Australia	QM G313992	-	JQ272304
<i>Murrayona phanolepis</i>	Australia	QM G316290	-	AM180998
<i>Nicola tetela</i>	Curaçao	UFRJPOR 6723	KU568492	-
<i>Soleneiscus intermedius</i> sp. nov.*	Réunion, Western Indian	UFRJPOR 8933	-	MW350119
<i>Soleneiscus hamatus</i>	Egypt, Red Sea	SNSB-BSPG GW2975	-	KY366365
<i>Soleneiscus radovani</i>	GBR, Australia	QM G313661	-	JQ272289
<i>Soleneiscus stolonifer</i>	GBR, Australia	QM G313668	-	JQ272290
<b>Calcaronea</b>				
<i>Aphroceras</i> sp. 1	Tasmania, Australia	SAM-PS0349	-	JQ272273
<i>Aphroceras</i> sp. 2	Coral Sea, Australia	QM G316285	-	AM181001
<i>Aphroceras</i> sp. nov.	St. Helena, South Atlantic	SA 87	-	MH385226

Table 1. Continued

Species	Locality	Voucher number	GenBank accession number	
			ITS	C-LSU
<i>Aphroceras</i> sp. nov.	St. Helena, South Atlantic	SA 89	-	MH385227
<i>Aphroceras seychellensis</i> comb. nov.*	Réunion, Western Indian	UFRJPOR 8926	-	MW350109
<i>Aphroceras seychellensis</i> comb. nov.*	Réunion, Western Indian	UFRJPOR 8935	-	MW350110
<i>Breitfussia schulzei</i>	West Greenland	FB 61	-	MH385228
<i>Grantia compressa</i>	Roscoff, France	SA 80	-	MH385242
<i>Grantiopsis cylindrica</i>	GBR, Australia	SNSB-BSPG GW973	-	JQ272266
<i>Grantiopsis heroni</i>	-	-	-	AY563539
<i>Leucandra ananas</i>	Trondheimsfjord, Norway	SA 136	-	MH385249
<i>Leucandra aspera</i>	-	-	-	AY563535
<i>Leucandra falakra</i>	Croatia, Adriatic Sea	PMR 13748	-	KT447560
<i>Leucandra</i> cf. <i>gausapata</i>	Antarctic	HT 15	-	MH385252
<i>Leucandra mozambiquensis</i>	Mozambique, West Africa	ZMA 22408B	-	MF872766
<i>Leucandra nicolae</i>	GBR, Australia	QM G313672	-	JQ272278
<i>Leucandra ornata</i> sp. nov.*	Réunion, Western Indian	UFRJPOR 8928	-	MW350116
<i>Leucandra penicillata</i>	West Greenland	FB 35	-	MH385256
<i>Leucandra pilula</i>	Seychelles, Western Indian	ZMA 10528	-	MF872767
<i>Leucandra spinifera</i>	Croatia, Adriatic Sea	IRB SG3	-	KT447561
<i>Leucandra tahuatae</i>	Tahuata, French Polynesia	UFRJPOR 6454	-	MN422253
<i>Leucandrilla</i> sp.	Galapagos	RMNH 11520	-	MF872768
<i>Leucilla antillana</i>	Curaçao, Caribbean Sea	UFRJPOR 6768	-	MF472615
<i>Leucosolenia botryoides</i>	Norway	SA 60	-	MH385257
<i>Leucosolenia complicata</i>	Norway	AA 201	-	MH385258
<i>Leucosolenia</i> cf. <i>corallorrhiza</i>	Greenland	FB 14	-	MH385270
<i>Paragranta waguensis</i>	Japan	RMNH 9317	-	KT277668
<i>Paraleucilla</i> sp. 1	St. Helena, South Atlantic	SA 83	-	MH385283
<i>Paraleucilla erpenbecki</i>	Mozambique, West Africa	ZMAPOR 22409C	-	MF872728
<i>Paraleucilla magna</i>	Algarve, Portugal	PT 06	-	MH385279
<i>Sycon caminatum</i>	Madeira Island	PT 02	-	MH385301
<i>Sycon villosum</i>	Møøyastaken, Norway	SA 24	-	MH385314
<i>Synute pulchella</i>	West Australia	WAMZ 1404	-	JQ272274
<i>Teichonopsis labyrinthica</i>	South Australia	SAM-PS 0228	-	JQ272264
<i>Ute ampullacea</i>	GBR, Australia	QM G313669	-	AM181002
<i>Ute glabra</i>	Portugal	PT 16	-	MH385315
<i>Ute gladiata</i>	Korsfjord, Norway	HT 47	-	MH385316
<i>Ute gladiata</i>	Trondheimsfjord, Norway	SA 134	-	MH385317
<i>Ute insulagemmae</i>	Seychelles, Western Indian	ZMAPOR 11562	-	MF872772
<i>Ute insulagemmae</i> *	Réunion, Western Indian	UFRJPOR 8925	-	MW350120
<i>Ute insulagemmae</i> *	Réunion, Western Indian	UFRJPOR 8929	-	MW350121
<i>Ute insulagemmae</i> *	Réunion, Western Indian	UFRJPOR 8938	-	MW350122
<i>Ute insulagemmae</i> *	Réunion, Western Indian	UFRJPOR 8942A	-	MW350123
<i>Ute insulagemmae</i> *	Réunion, Western Indian	UFRJPOR 8943A	-	MW350124
<i>Ute insulagemmae</i> *	Réunion, Western Indian	UFRJPOR 8948	-	MW350125
<i>Ute</i> aff. <i>syconoides</i>	GBR, Australia	GW975	-	JQ272270
<i>Ute</i> aff. <i>syconoides</i>	GBR, Australia	QM G313694	-	JQ272271
<i>Ute</i> aff. <i>syconoides</i>	Tasmania, Australia	QM G323233	-	JQ272269



**Figure 1.** Study area. Sampling localities in La Réunion, Indian Ocean. The black stars indicate the locations where calcareous sponges were found.

#### MOLECULAR RESULTS

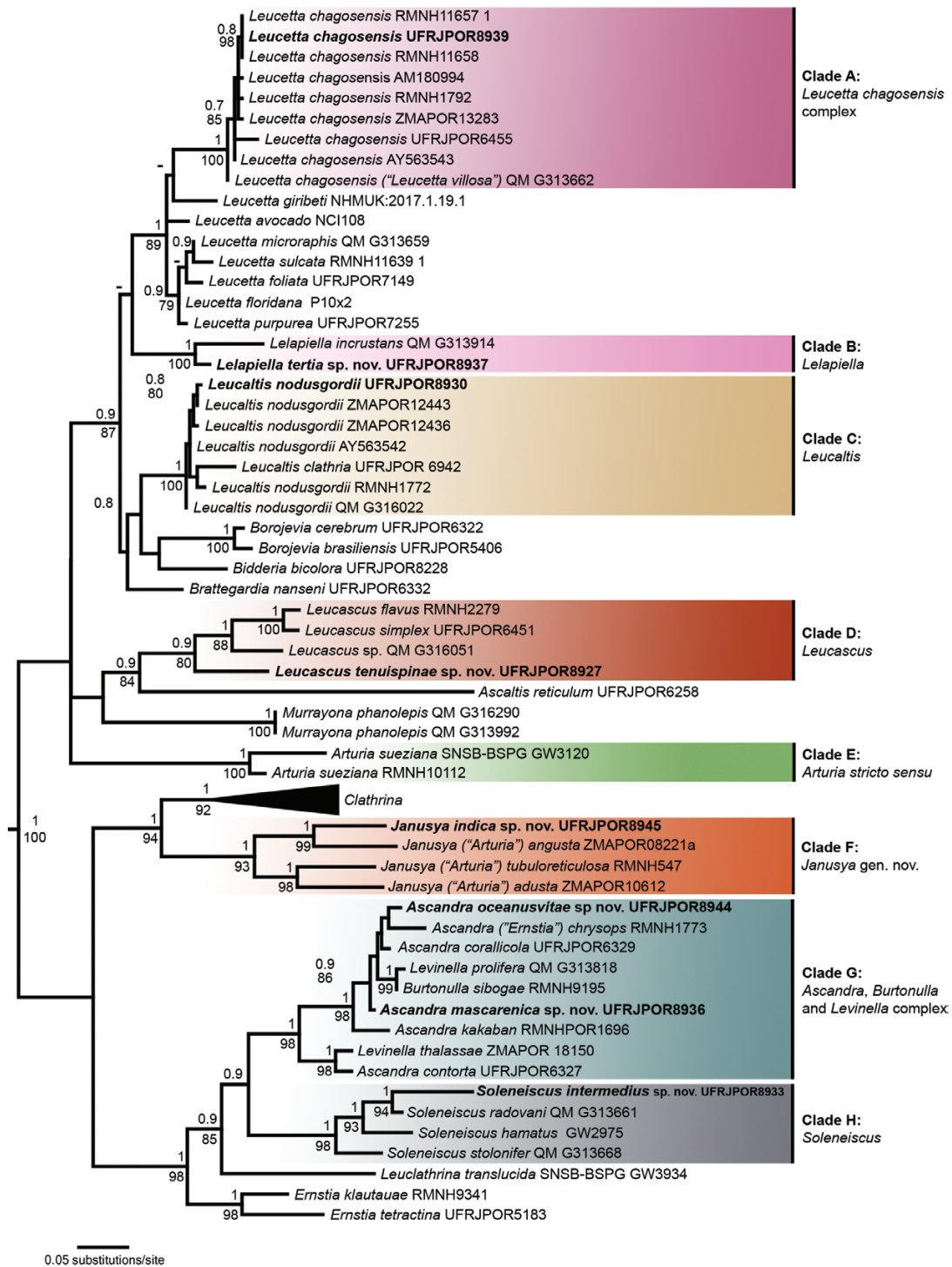
Besides the midpoint-rooted trees (C-LSU of *Calcinea* and *Calcaronea* and ITS of *Calcinea*), we also built C-LSU ML trees rooted with the opposing class (Supporting Information, Figs S4, S5). These trees showed almost the same topology of the midpoint-rooted trees but the midpoint-rooted trees showed better internal resolution. Consequently, we present our molecular results based on the midpoint-rooted phylogenies. Bootstrap and posterior probability values are shown in each ML tree.

#### CALCINEA PHYLOGENY (ITS AND C-LSU)

The recovered phylogenetic trees of C-LSU and ITS are shown in Figures 2 and 3, respectively. In both trees, we alphabetically named the clades with specimens from La Réunion (and *Arturia* Azevedo *et al.*, 2017 s.s.) from A to H. In both C-LSU and ITS trees, the polyphyletic genus *Leucetta* appears monophyletic (bootstrap/posterior

probability: 89%/1 and 73%/0.9, respectively), but only because we did not include *Pericharax* Poléjaeff, 1883 nor *Leucettusa* Haeckel, 1872 in the analyses (e.g. Klautau *et al.*, 2013; Lopes *et al.*, 2018). In clade A, the sequence of *Leucetta chagosensis* Dendy, 1913 from La Réunion grouped with other sequences of that species from the Indo-Pacific and with *L. villosa* Wörheide & Hooper, 1999 (Table 1; Fig. 2) with a bootstrap/posterior probability of 100%/1 in both trees. The intraspecific *p*-distance among them was 0.0–2.3% for the C-LSU and 1.5% for the ITS. This relatively high *p*-distance and wide-spread scenario suggest that *L. chagosensis* may be a complex of cryptic species, as already proposed by Wörheide *et al.* (2002, 2008), Klautau *et al.* (2020) and Pasnin *et al.* (2020). Our results indicate that *L. villosa* is part of this complex (see the ‘Taxonomic remarks’ of *L. chagosensis*).

Clade B contains the new species *Lelapiella tertia* (described below), although for the ITS tree we did not have a clade because this species was the first *Lelapiella* Vacelet, 1977 sequence generated. Because

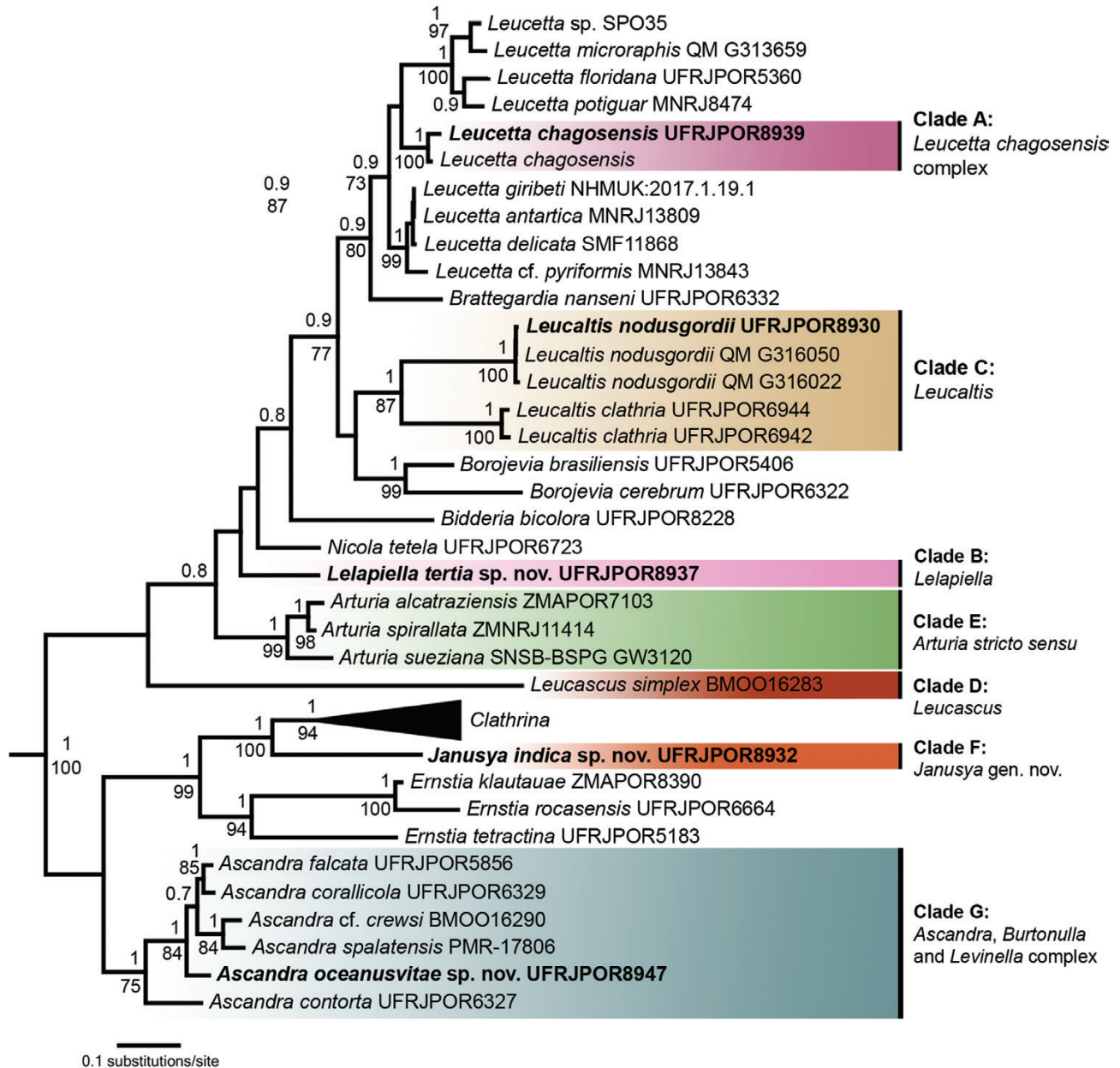


**Figure 2.** Maximum likelihood phylogenetic tree based on C-LSU sequences of the subclass Calcinea. Midpoint-rooted tree. Species sequences generated in this work are in bold. Clades containing species found in this work are highlighted. Support values are shown at the nodes. Support values are shown at the nodes (bootstrap > 70%; posterior probability > 0.7).

we included *Lelapiella incrustans* Vacelet, 1977 in our C-LSU dataset, both species grouped in a clade with a high bootstrap/posterior probability (100%/1)

and *p*-distance of 5.5%. *Lelapiella* did not group with sequences of *Murrayona phanolepis* Kirkpatrick, 1910, another species from the order Murrayonida.





**Figure 3.** Maximum likelihood phylogenetic tree based on ITS sequences of the subclass Calcinea. Midpoint-rooted tree. Species sequences generated in this work are in bold. Clades containing species found in this work are highlighted. Support values are shown at the nodes (bootstrap > 70%; posterior probability > 0.7).

Clade C contains sequences of *Leucaltis clathria* Haeckel, 1872 and *L. nodusgordii* (Poléjaeff, 1883). In the ITS tree, *L. nodusgordii* from La Réunion clustered in a highly supported clade with sequences from Australia (bootstrap/posterior probability: 100%/1) and with low *p*-distance (0.0–0.4%). This clade is closely related to the *L. clathria* clade (bootstrap/posterior probability: 87%/1), but with high genetic divergence of 11.1–11.9% (*p*-distance), confirming that it actually includes two different species and that

the genus is monophyletic. The C-LSU resolution for this clade was not satisfactory, because no lineage differentiation was recovered between *L. clathria* and *L. nodusgordii*. The sequences form a polytomic branch with low resolution and, except for the genus bootstrap/posterior probability (100%/1), all the bootstraps were low. The *p*-distances range from 0.3 to 3.6%.

For the new species *Leucascus tenuispinae* (described below; clade D), we got only a C-LSU sequence. The genus *Leucascus* Dendy, 1892 was found monophyletic

in our tree (bootstrap/posterior probability: 80%/0.9), *L. tenuispinae* grouping more distantly with *Leucascus* sp. (*p*-distance: 9.3%), *L. flavus* Cavalcanti *et al.*, 2013 (*p*-distance: 10.2%) and *L. simplex* Dendy, 1892 (*p*-distance: 9.9%).

In clade E, both molecular markers recovered the genus *Arturia stricto sensu*. In the ITS tree, it grouped *A. alcatraziensis* (Lanna *et al.*, 2007), *A. spirallata* Azevedo *et al.*, 2015 and *A. sueziana* (Klautau & Valentine, 2003) (bootstrap/posterior probability: 99%/1). Other sequences of *Arturia* clustered together with the independent lineage of the new genus *Janusya* (clade F; bootstrap/posterior probability: 93%/1 in the C-LSU tree). The new species *Janusya indica* (described below) was the only sequence available of this genus for the ITS marker. Therefore, no intra- or interspecific comments can be made. On the other hand, three other species of *Arturia lato sensu* were closely related to the new genus *Janusya* in the C-LSU tree and, consequently, should be transferred. They are *Janusya adusta* (Wörheide & Hooper, 1999) **comb. nov.**, *J. angusta* (Van Soest & De Voogd, 2015) **comb. nov.** and *J. tubuloreticulosa* (Van Soest & De Voogd, 2015) **comb. nov.** *Janusya angusta* grouped with *J. indica* (bootstrap/posterior probability: 99%/1; *p*-distance: 9.5%) and *Janusya adusta* grouped with *J. tubuloreticulosa* (bootstrap/posterior probability: 98%/1; *p*-distance: 10.1%). The variability among these branches ranged from 13.3% (between *Janusya indica* and *J. tubuloreticulosa*) to 14.7% (between *J. angusta* and *J. tubuloreticulosa*).

Clade G comprises the genera *Ascandra* Haeckel, 1872, *Burtonnulla* Borojević & Boury-Esnault, 1986 and *Levinella* Borojević & Boury-Esnault, 1986. Because no *Burtonnulla* nor *Levinella* representatives were available in the ITS tree, the genus *Ascandra* appeared monophyletic (bootstrap/posterior probability: 75%/1), but in the C-LSU, it was found non-monophyletic, with *Levinella thalassae* Borojević & Boury-Esnault, 1987, *L. prolifera* (Dendy, 1913) and *Burtonnulla sibogae* Borojević & Boury-Esnault, 1986 being related to *Ascandra* species (bootstrap/posterior probability: 98%/1). Interestingly, one sequence identified as *Ernstia chrysops* Van Soest & De Voogd, 2018 also grouped with the *Ascandra* clade. Re-analysing the description of this species, we confirm that it is an *Ascandra*: *A. chrysops* (Van Soest & De Voogd, 2018) **comb. nov.**

The *p*-distances for clade G range from 1.9% [between *A. corallicola* (Rapp, 2006) and *A. falcata* Haeckel, 1872] to 11.2% [between *A. contorta* (Bowerbank, 1866) and the new species *A. oceanusvitae*] for the ITS, and from 0.6% (between *L. prolifera* and *B. sibogae*) to 10.5% (between *A. chrysops* and *A. contorta*) for the C-LSU. Our results clearly indicate that *Levinella* and *Burtonnulla* should be synonymized.

Among clade H, the genus *Soleneiscus* Borojević *et al.* 2002 was monophyletic in the C-LSU tree (bootstrap/posterior probability: 98%/1). Our new species, *S. intermedius*, grouped with *S. radovani* Wörheide & Hooper, 1999 in a clade with elevated support (bootstrap/posterior probability: 94%/1) and *p*-distance (5.4%). The other two species are sister to this clade. The *p*-distances of the genus ranged from 5.4 to 10.9% [between the new species *S. intermedius* and *S. stolonifer* (Dendy, 1891)].

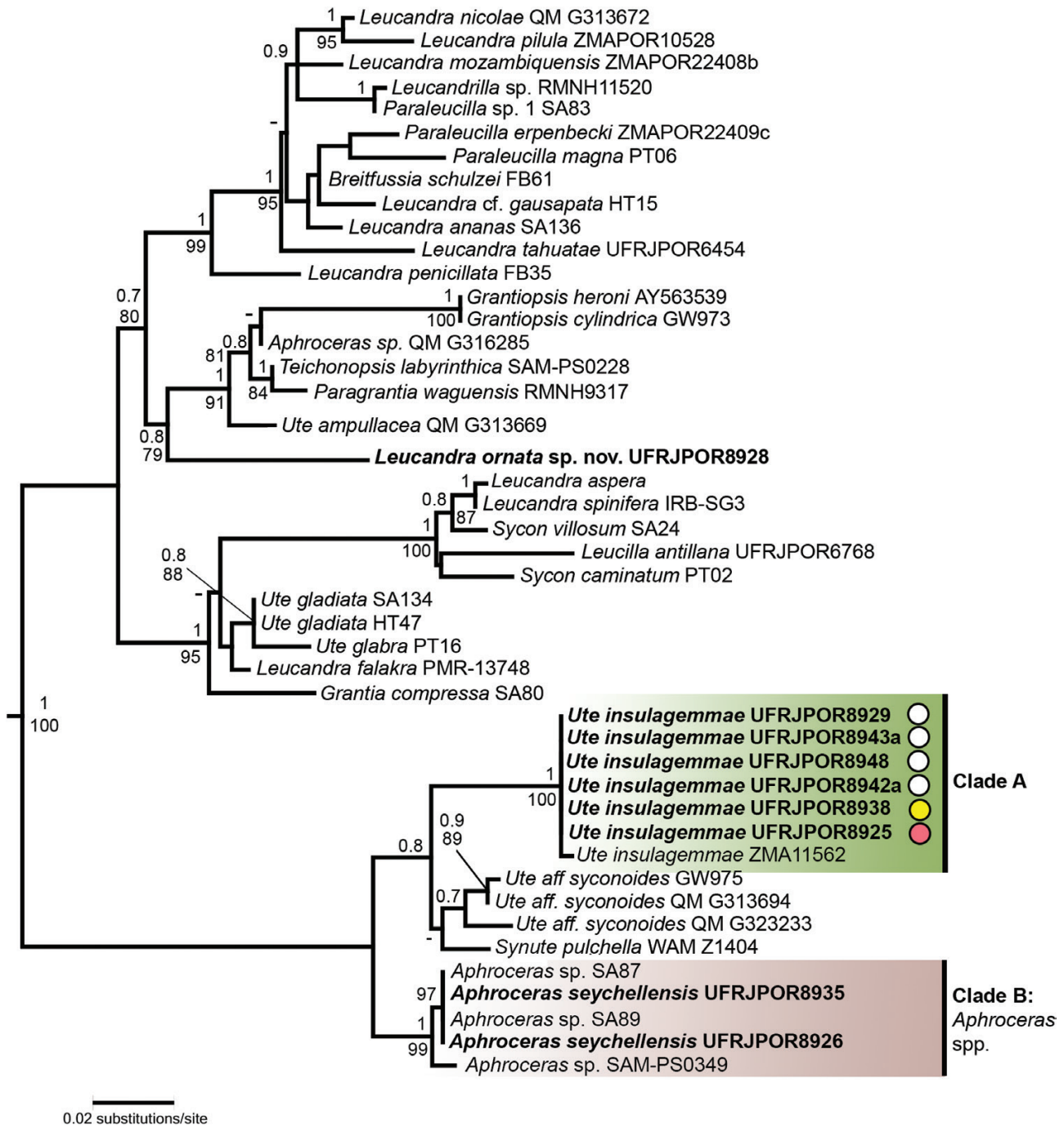
#### CALCARONEA PHYLOGENY (C-LSU)

In this study, we provided sequences for the new species *Leucandra ornata* (described below) and for the species *Aphroceras seychellensis* (Hôzawa, 1940) **comb. nov.** and *Ute insulagemmae* Van Soest & De Voogd, 2018. The obtained ML tree is shown in Figure 4. Corroborating previous results, the family Grantiidae Dendy, 1892 and the three genera were retrieved as non monophyletic. Two separated clades were defined for the cluster of species containing *A. seychellensis* (and other *Aphroceras* spp.) and one for *Ute insulagemmae*.

As a polyphyletic genus, *Leucandra* Haeckel, 1872 species were distributed in different clades within the subclass Calcaronea (e.g. Alvizu *et al.*, 2018). Our sequence of *Leucandra ornata* appears as a distinct lineage that groups with a clade formed by *Grantiopsis heroni* Wörheide & Hooper, 2003, *G. cylindrica* Dendy, 1892, *Ute ampullacea* Wörheide & Hooper, 2003, *Aphroceras* sp., *Teichonopsis labyrinthica* (Carter, 1878) and *Paragrantiopsis waguensis* Hôzawa, 1940 (bootstrap/posterior probability: 79%/0.8). These are members of Grantiidae, but also of the families Lelapiidae Dendy & Row, 1913, Jenkinidae Borojević *et al.*, 2000 and Amphoriscidae Dendy, 1892. Therefore, the genus assignment of this species was mostly made according to morphology.

The other sequences of specimens from La Réunion form two well-defined clades. Clade A reunited specimens from La Réunion, morphologically identified by us as *Ute insulagemmae*, with the holotype of this species from the Seychelles (Van Soest & De Voogd, 2018). The clade was supported by high bootstrap and posterior probability values (100%/1) and the genetic *p*-distance did not exceed 0.3%, confirming the morphological identifications. Besides, the specimens included in the phylogenetic tree represented different chromotypes (white, yellow and pink) and these grouped together, showing that for this species, different colours represent intraspecific polymorphism.

Clade B contains the specimens of *A. seychellensis* from La Réunion and other *Aphroceras* sequences available from GenBank. *Aphroceras seychellensis* groups conspecifically with two specimens from



**Figure 4.** Maximum likelihood phylogenetic tree based on C-LSU sequences of the subclass Calcaronea. Midpoint-rooted tree. Species sequences generated in this work are in bold. Clades containing species found in this work are highlighted. Coloured circles represent different chromotypes (yellow, pink or white) of *Ute insulagemmae*. Support values are shown at the nodes (Bootstrap > 70%; posterior probability > 0.7).

St. Helena (South Atlantic; bootstrap/posterior probability: 97%/< 0.7). This clade groups with another specimen of *Aphroceras* from Tasmania (Australia; bootstrap/posterior probability: 99%/1).

The four specimens of *A. seychellensis* presented  $p$ -distance of 0, and the comparison between this clade and the specimen from Tasmania presented  $p$ -distance of 0.8%.



## MORPHOLOGICAL RESULTS

PHYLUM PORIFERA GRANT, 1836

CLASS CALCAREA BOWERBANK, 1862

SUBCLASS CALCINEA BIDDER, 1898

ORDER CLATHRINIDA HARTMAN, 1958

FAMILY CLATHRINIDAE MINCHIN, 1900

GENUS *JANUSYA* GEN. NOV.

*Zoobank registration:* urn:lsid:zoobank.org:act:7F1DD58A-C896-4DF1-9DDC-817B4D30E400.

*Type species:* *Janusya indica* sp. nov.

*Etymology:* The new genus is dedicated to Jean Vacelet, who is affectionately known as Janus, in recognition for all his work on Porifera, including calcareous sponges.

*Diagnosis:* Calcinea in which the cormus comprises a typical clathroid body. A stalk may be present. The skeleton contains regular (equiangular and equiradiate) or subregular triactines and tetractines. Tetractines are less abundant than triactines, sometimes even rare, but they always have needle-like apical actines. Diactines may be added. The aquiferous system is asconoid.

***JANUSYA INDICA* SP. NOV.**

(FIGS 5, 6; TABLE 2)

*Zoobank registration:* urn:lsid:zoobank.org:act:79DA23AC-B3EE-42B0-8BB3-1DA5F592AF9A.

*Etymology:* Named in reference to the type locality located in the Indian Ocean.

*Diagnosis:* Yellowish to beige *Janusya* in which the cormus is incrusting to globulose, formed by regular and tightly anastomosed tubes with long, apical, water-collecting tube. The skeleton contains fusiform diactines, trichoxeas, triactines and rare tetractines.

*Type locality:* Passe de l'Hermitage, La Réunion, Indian Ocean.

*Type material:* Holotype – UFRJPOR 8945 (= 171109-PAE2-TP20). Paratype – UFRJPOR 8932 (= 171109-PAE2-TP6). Passe de l'Hermitage Reef, La Réunion, Indian Ocean, coll. T. Pérez, 9 November 2017, 18–20 m depth.

*Additional material examined:* UFRJPOR 8931 (= 171109-PAE2-TP5), UFRJPOR 8940 (= 171109-PAE2-TP16). Passe de l'Hermitage Reef,

La Réunion, Indian Ocean, coll. T. Pérez, 9 November 2017, 18–20 m depth.

*Colour:* Sponge yellowish beige in life (Fig. 5A, B) and light brown in ethanol (Fig. 5C, D).

*Morphology and anatomy:* Sponge incrusting to globulose (Fig. 5). Cormus clathroid, formed by regular and tightly anastomosed tubes (Fig. 5). One or few apical oscula, surrounded by membrane (Fig. 5A). The osculum is a long water-collecting tube, i.e. a larger tube with osculum that receives the water from several other thinner tubes. Underneath the osculum of globulose specimens, there is a cavity that is not lined by a continuous membrane. Cells with yellow granules are present and distributed homogeneously in the mesohyl of the specimens UFRJPOR 8945 and UFRJPOR 8932 (Fig. 6A, B, respectively). The aquiferous system is asconoid and the skeleton is composed of diactines, trichoxeas, triactines and rare tetractines (Fig. 6C–F).

*Spicules* (Table 2)

*Diactines:* Thin, fusiform, but with different tips. They are undulated and slightly curved (Fig. 6C).

*Triactines:* Equiangular and equiradiate (Fig. 6D), but often the unpaired actine is shorter than the paired ones (Fig. 6E). Actines are conical with blunt to sharp tips.

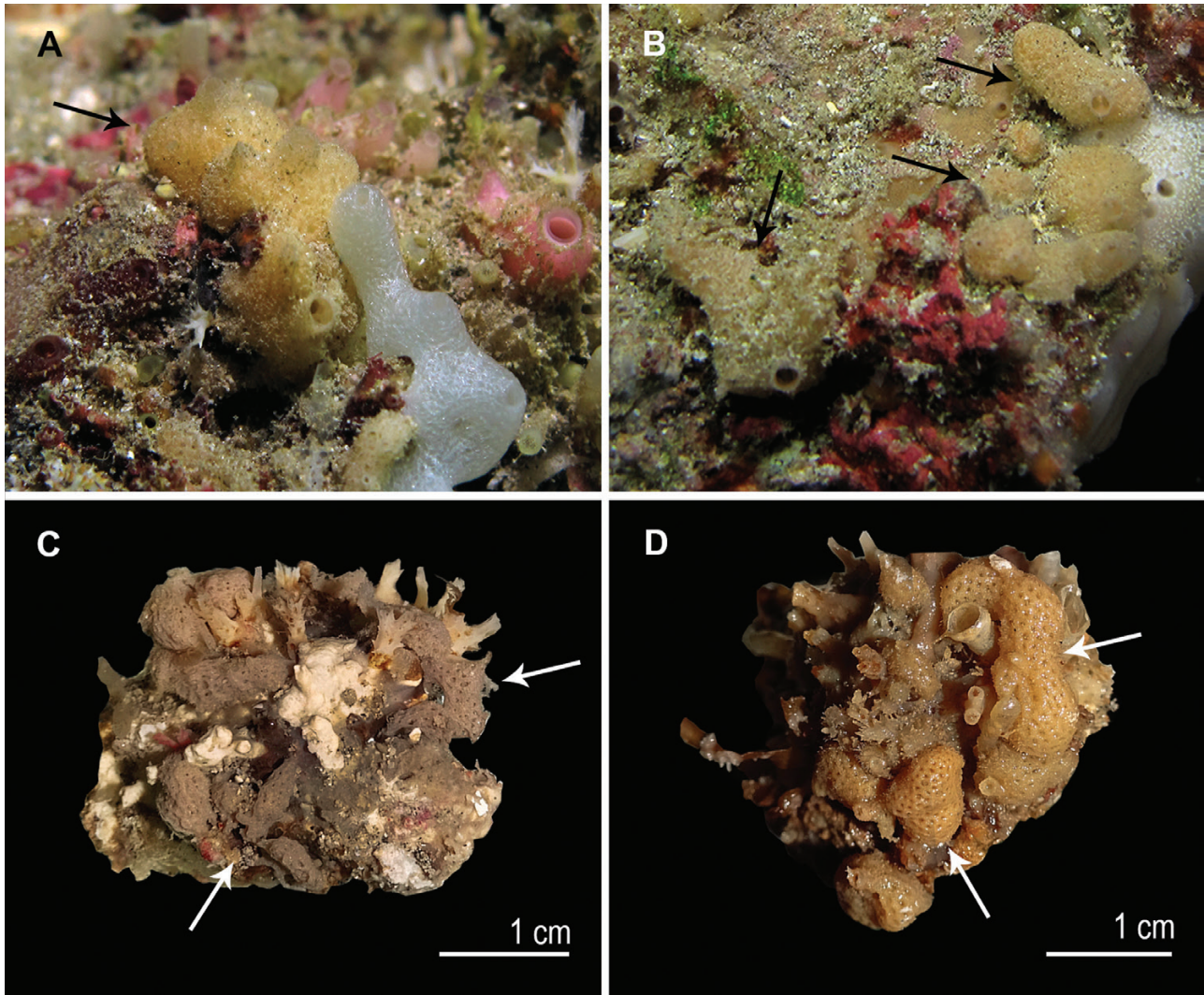
*Tetractines:* Equiangular and equiradiate, but often the unpaired actine is shorter than the paired ones in the triactines. Actines are conical with blunt to sharp tips (Fig. 6F). The apical actine is thin (needle-like), cylindrical, short, smooth and sometimes curved.

*Ecology:* Some individuals were associated with *Leucaltis nodusgordii* (UFRJPOR 8930) and *Ute insulagemmae* (Fig. 5A).

*Geographic distribution:* Mascarene Islands Ecoregion – La Réunion (this work).

*Taxonomic remarks:* The new genus *Janusya* is being proposed to reunite the new species described here and other sponges previously assigned to *Arturia*. The first diagnosis of *Arturia* was: 'Calcinea in which the 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). But it was modified later: 'Calcinea in which the cormus





**Figure 5.** *Janusya indica* A, specimen *in vivo* (UFRJPOR 8931), associated with *Leucaltis nodusgordii* (white sponge) and near *Ute insulagemmae* (pink sponge). B, several specimens *in vivo* (paratype; UFRJPOR 8932). C, fixed specimen (holotype; UFRJPOR 8945). D, fixed specimen (paratype; UFRJPOR 8932). Arrows point to specimens of *J. indica* gen. et sp. nov.

comprises a typical clathroid body. A stalk may be present. The skeleton contains regular (equiangular and equiradiate) triactines and few tetractines. Diactines may be added. Asconoid aquiferous system' (Fontana *et al.*, 2018).

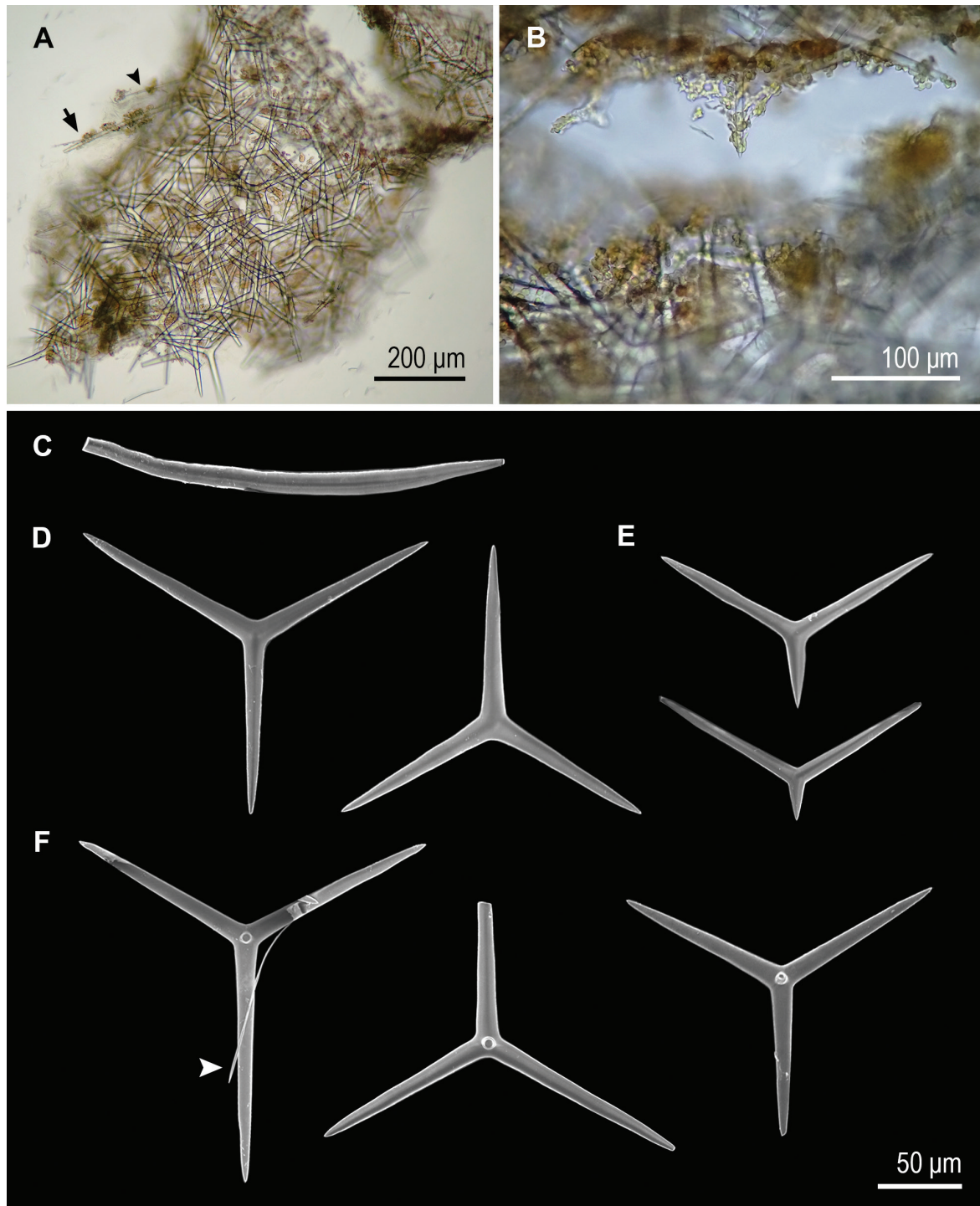
To clarify the difference between *Arturia* and *Janusya* we suggest a new modification in the diagnosis of *Arturia*: Calcinea in which the cormus comprises a typical clathroid body. A stalk may be present. The skeleton contains regular (equiangular and equiradiate) triactines and few tetractines. The apical actine of the tetractines is thinner than the basal ones, but it is conical, never needle-like. Diactines may be added. Asconoid aquiferous system (emend.).

Currently *Arturia* is composed of 15 species: *Arturia adusta*; *A. africana* (Klautau & Valentine, 2003);

*A. alcatraziensis*; *A. canariensis* (Miklucho-Maclay, 1868); *A. compacta* (Schuffner, 1877); *A. darwinii* (Haeckel, 1870); *A. dubia* (Dendy, 1891); *A. hirsuta* (Klautau & Valentine, 2003) – type species of the genus; *A. passionensis* (Van Soest *et al.*, 2011); *A. spirallata*; *A. sueziana*; *A. tenuipilosa* (Dendy, 1905); *A. trinidadensis* Azevedo *et al.*, 2017; *A. tubuloreticulosa*; and *A. vansoesti* Córdor-Luján *et al.*, 2018.

Voigt & Wörheide (2016) proposed to include '*Clathrina*' *adusta*, previously transferred to *Ernstia* by Klautau *et al.* (2013) in the genus *Arturia*. However, Voigt *et al.* (2017) showed in a molecular phylogeny that this species was actually not an *Arturia* and suggested that it could be a new genus.

Analysing morphologically all species of '*Arturia*' presented in our tree, we found that *A. alcatraziensis*,



**Figure 6.** *Janusya indica* A, cross-section of the specimen UFRJPOR 8945. B, cross-section of the specimen UFRJPOR 8932 with an apical actine of a tetractine pointing towards the lumen of a tube. C, diactine. D, triactines. E, triactines with shorter unpaired actine. F, tetractines (arrowhead points to a trichoxea).

*A. spirallata* and *A. sueziana* have the apical actine of their tetractines only a little thinner than the basal ones, whereas *A. adusta*, *A. angusta*, *A. tubuloreticulosa* and *Janusya indica* have thin

(needle-like) apical actines. Because the type species of *Arturia* (*A. hirsuta*) has apical actines only a little thinner than the basal ones, we consider the clade of *A. alcatraziensis*, *A. spirallata* and *A. sueziana* as the



**Table 2.** Spicule measurements of *Janusya indica*. H = holotype, P = paratype

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	Mean	SD	Max	Min	Mean	SD	Max	
UFRJPOR 8945 (H)	Diactine		270.3	396.4	68.2	497.3	10.8	14.8	3.2	21.6	15
	Triactine		105.3	122.3	12.7	145.8	10.8	13.7	1.3	16.2	20
	Tetractine	Basal	97.2	122.3	14.6	143.1	10.8	13.6	1.3	16.2	20
		Apical	78.3	82.4	4.1	89.1	4.1	5.2	0.6	5.4	06
UFRJPOR 8932 (P)	Diactine		194.6	281.1	69.2	378.4	3.2	8.8	2.7	10.8	15
	Triactine		121.5	145.3	16.7	180.9	13.5	17.0	1.7	20.3	20
	Tetractine	Basal	121.5	145.1	19.3	202.5	13.5	17.3	1.8	18.9	20
		Apical	51.3	67.5	10.6	81.0	4.1	5.1	0.6	5.4	05

true clade of *Arturia*. Therefore, our new species can be confirmed as a new genus named here *Janusya*. Moreover, reassessing the descriptions of each species currently assigned to *Arturia*, we concluded that the new genus *Janusya* should include *J. indica* (type species) and five new combinations: *J. adusta*, *J. angusta*, *J. trinidadensis*, *J. tubuloreticulosa* and, possibly, *J. darwinii*. The last is doubtful, because we did not find the type specimen of this species. Therefore, we used only the drawing published by [Haeckel \(1872\)](#). It is interesting to notice that three of them have a pseudoatrium: *J. adusta*, *J. angusta* and *J. indica*. Finally, *Janusya indica* can be differentiated from the other species of the genus, because it is the only species with diactines. Besides, the unpaired actine of its triactines and tetractines are usually shorter than the paired ones.

Sponges remaining assigned to *Arturia* include *A. hirsuta* (type species), *A. africana*, *A. alcatraziensis*, *A. canariensis*, *A. compacta*, *A. dubia*, *A. spirallata*, *A. sueziana*, *A. tenuipilosa* and *A. vansoesti*.

#### FAMILY DENDYIDAE DE LAUBENFELS, 1936

##### GENUS *SOLENEISCUS* BOROJEVIĆ ET AL., 2002

*Type species: Leucosolenia stolonifer* Dendy, 1891.

**Diagnosis:** Dendyidae that grow in the form of an individual ascon tube, with several ascon tubes growing upright from basal, stolon-like tubes, or in the form of creeping, ramified tubes (modified from [Borojević et al., 2002](#)).

##### *SOLENEISCUS INTERMEDIUS* SP. NOV.

(FIGS 7, 8; TABLE 3)

*Zoobank registration:* urn:lsid:zoobank.org:act:C9AE024F-7E3F-4B46-AD5F-A9E6704B59B8.

**Diagnosis:** *Soleneiscus* that grows in the form of ramified tubes at the base and whose skeleton is composed of diactines, rare triactines located only at the oscular region and two types of tetractines. The diactines are thin, maximum thickness of 7.5 µm.

**Etymology:** From Latin *intermedium*, meaning intermediate, in between, for the close morphological similarity with other species.

**Type locality:** Portail Caves, La Réunion, Indian Ocean.

**Type material:** Holotype – UFRJPOR 8933 (= 171110ESB2TP6). Portail Caves, La Réunion, Indian Ocean, coll. T. Pérez, 10 November 2017, 18–20 m depth.

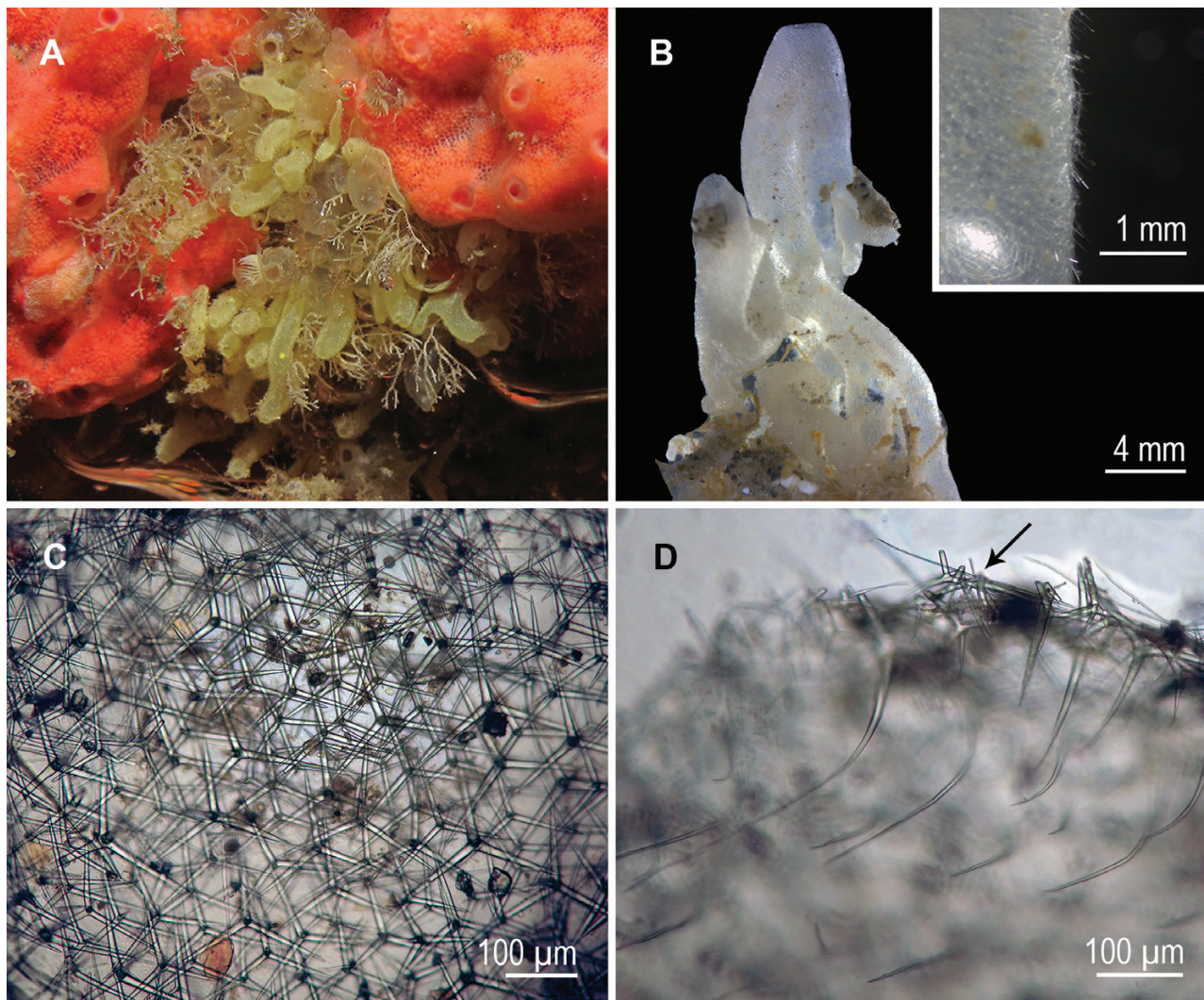
**Colour:** Sponge light yellow in life and white in ethanol (Fig. 7A, B).

**Morphology and anatomy:** Cormus formed by ramified and erect tubes, ending in a single osculum without a crown (Fig. 7A, B). Surface hispid due to the presence of diactines lying perpendicular to the surface (Fig. 7B). The lumen of the tubes is also hispid due to the long apical actines of the tetractines (Fig. 7C, D). Aquiferous system asconoid. Skeleton composed of diactines, two size categories of tetractines, and rare triactines (Fig. 8).

##### *Spicules* (Table 3)

**Diactines:** Thin, slightly curved and undulated. The tip that penetrates the cormus is thicker, but both of them are sharp (Fig. 8A).

**Triactines:** Regular to subregular, with variable size. They are rare. Actines are conical and sharp. They are present near the oscula (Fig. 8B).



**Figure 7.** *Soleneiscus intermedius* (holotype; UFRJPOR 8933). A, specimen *in vivo* living with a red Demospongiae, hydrozoans and ascidians. B, fixed specimen (external hispid surface of a tube in the inset). C, tangential section of a tube. D, lumen of a tube with the apical actine of tetractines. Arrow points to a diactine of the surface.

*Tetractines I:* Regular or sagittal, large. Actines are conical, sharp and curved (Fig. 8C).

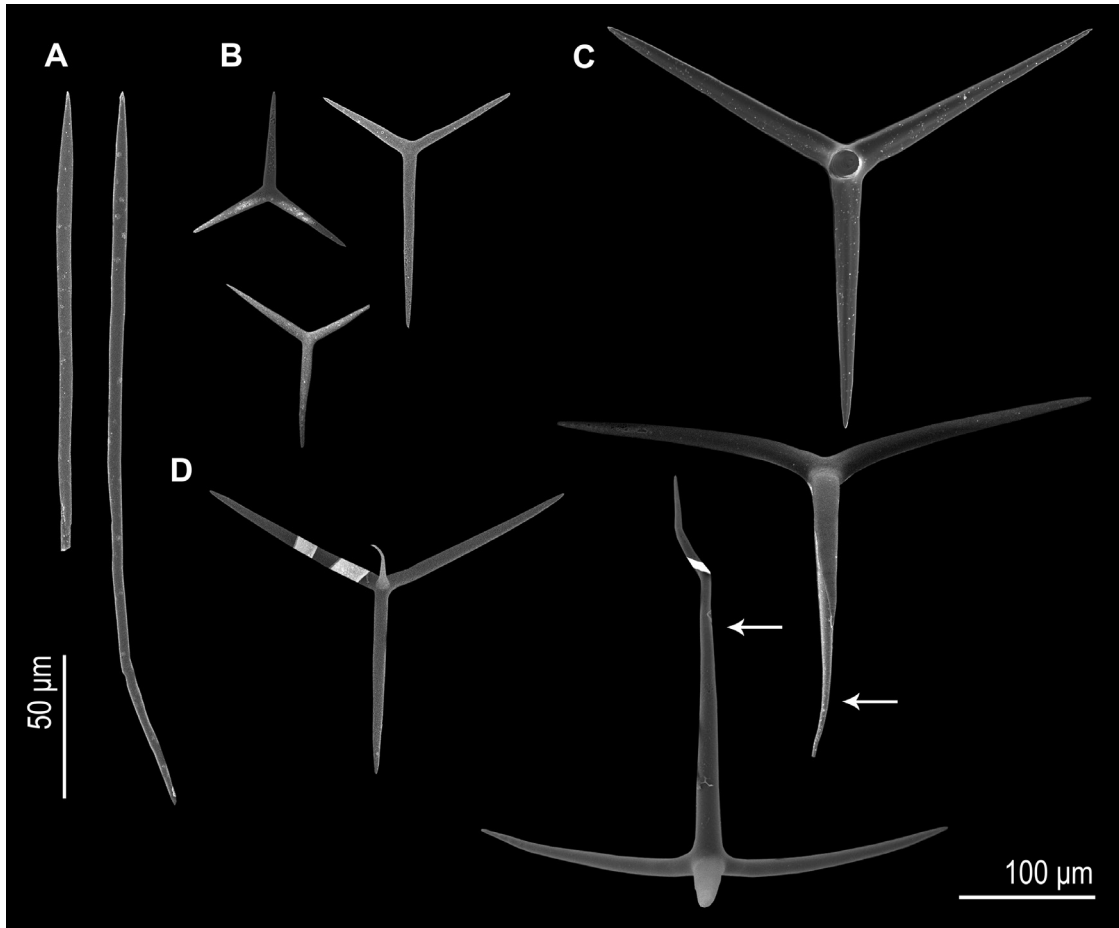
*Tetractines II:* Regular or sagittal, a little smaller than tetractine I. Actines are slightly conical and sharp. The apical actines are only a little thinner than the basal ones. They are thin, sharp and curved. The apical actines are huge. They are only a little thinner than the basal ones at the base but become thin (Fig. 8D).

*Ecology:* The specimen was growing among a red undetermined sponge, colonies of hydrozoans and ascidians (Fig. 7A).

*Geographic distribution:* Mascarene Islands Ecoregion – La Réunion (this work).

*Taxonomic remarks:* There are nine accepted species of *Soleneiscus*: *S. apicalis* (Brøndsted, 1931) from the Antarctic, *S. hamatus* Voigt *et al.*, 2017 from the Red Sea, *S. hispidus* (Brøndsted, 1931) from the Antarctic, *S. irregularis* (Jenkin, 1908) from East Africa, *S. japonicus* (Haeckel, 1872) from Japan, *S. olynthus* (Borojević & Boury-Esnault, 1987) from the Bay of Biscay, Atlantic, *S. pedicellatus* Azevedo *et al.*, 2015 from Peru, *S. radovani* from the Great Barrier Reef, Australia and *S. stolonifer* from South Australia. The new species *Soleneiscus intermedius* is morphologically similar to *S. hamatus*, *S. irregularis* and *S. stolonifer*, because the four species present diactines and two types of tetractines, one with large apical actine and another with thin apical actine. However, the new species has also triactines, but as they are rare and





**Figure 8.** *Soleneiscus intermedius* (holotype; UFRJPOR 8933). A, diactines. B, triactines. C, tetractines I (arrows point to apical actines). D, tetractines II.

present only near the osculum, they could have been overlooked by the other authors. Nonetheless, even ignoring the presence or absence of triactines, our new species can be differentiated from the others by the size of some spicules (Table 3).

Comparing the new species with *S. irregularis* and *S. stolonifer*, the diactines of *S. intermedius* are much smaller and thinner (*S. intermedius*: 250–445/5–7.5 µm; *S. irregularis*: 300–800/16–28 µm; *S. stolonifer*: 700/30 µm). The diactines of *S. intermedius* are most similar to those of *S. hamatus* (136–274/5–8 µm). However, these species are genetically distant (Fig. 4), showing that a similar morphology in this group does not, necessarily, represent conspecificity.

This observation also makes us question the identification of some specimens from the Great Barrier Reef (Australia), such as *S. stolonifer* (Wörheide & Hooper, 1999). In the original description of *S. stolonifer*, Dendy (1891) did not separate the tetractines in two different size categories. He mentioned variable

apical actines length of the tetractines (one developed and another short), but he did not separate them in different categories. Besides, the size of the diactines and the external morphology of the specimens from the Great Barrier Reef (Wörheide & Hooper, 1999) were different from those given by Dendy (1891) (see Table 3). These suggest that they are not conspecific, and that the specimens from the Great Barrier Reef probably represent a new species.

#### FAMILY LEUCALTIDAE DENDY & ROW, 1913

##### GENUS ASCANDRA HAECKEL, 1872

*Type species: Ascandra falcata* Haeckel, 1872.

**Diagnosis:** ‘Calcinea with loosely anastomosed tubes. Tubes are free, at least in the apical region. The skeleton contains regular (equiangular and equiradiate) or sagittal triactines and tetractines. The apical actine is thin (needle-like) or thick at the base.

**Table 3.** Spicule measurements of *Solenaiscus intermedius* and other *Solenaiscus* species. H = holotype

Species	Spicule	Actine	Length (µm)			Width (µm)			N			
			Min	Mean	SD	Max	Min	Mean		SD	Max	
<i>S. intermedius</i> sp. nov. UFRJ/POR 8933 (H)	Diactine		250.0	336.0	44.8	445.0	5.0	5.9	1.2	7.5	20	
	Triactine		105.0	116.3	14.6	145.0	10.0	11.9	2.5	15.0	06	
	Tetractine I	Paired		190.0	227.8	21.2	260.0	20.0	23.3	2.9	30.0	20
		Unpaired		135.0	221.8	39.7	285.0	15.0	23.0	4.1	30.0	20
	Tetractine II	Apical		75.0	230.5	175.8	>625.0	15.0	19.8	4.4	30.0	20
		Paired		135.0	158.5	11.0	175.0	10.0	10.0	0	10.0	20
<i>S. irregularis</i> after Jenkin 1908	Unpaired		115.0	155.3	20.4	195.0	15.0	23.0	4.1	30.0	20	
	Apical		25.0	50.0	18.4	>70.0	5.0	5.0	0	5.0	04	
	Diactine		300.0	-	-	800.0	16.0	21–24	-	-	28	-
	Tetractine I		150.0	-	-	220.0	-	20.0	-	-	-	-
	Tetractine II	Apical		210.0	-	-	260.0	16.0	-	-	20	-
		Apical		100.0	-	-	200.0	10.0	-	-	16	-
<i>S. stolonifer</i> after Dendy 1891	Diactine		120.0	-	-	150.0	-	7.0	-	-	-	
	Tetractine		-	700.0	-	-	-	5.7	-	-	-	
<i>S. stolonifer</i> after Wörheide & Hooper 1999	Tetractine		-	200.0	-	-	-	15.0	-	-	-	
	Diactine		150.0	207.8	-	260.0	4.0	5.7	-	8.0	25	
	Tetractine I		80.0	129.9	-	170.0	8.0	12.2	-	21.0	25	
	Tetractine II	Apical		-	-	-	500.0	-	-	-	-	-
		Paired		40.0	81.6	-	110.0	8.0	9.7	-	12.0	25
	<i>S. hamatus</i> after Voigt et al. 2017	Unpaired		80.0	124.4	-	160.0	8.0	9.7	-	12.0	25
Diactine			136.0	207.6	42.6	274.0	5.0	6.2	0.8	8.0	10	
Tetractine I		Basal (Regular)		84.0	179.5	49.1	291.0	9.0	13.0	90.7	19.0	20
		Paired		84.2	156.6	32.3	272.0	10	13.5	2.8	19.0	16
Tetractine II		Unpaired		199.0	251.1	24.1	291.0	9.0	13.5	3.1	19.0	16
		Apical		120.0	147.3	16.1	167.0	13.0	18.1	4.0	27.0	11
Tetractine II	Basal (Regular)		68.0	134.2	37.0	235.0	5.0	8.7	2.4	15.0	20	
	Paired		80.0	117.7	19.0	152.0	5.0	8.9	2.4	14.0	13	
	Unpaired		115.0	179.6	31.9	235.0	5.0	9.0	2.3	15.0	13	
	Apical		35.0	41.8	3.2	49.0	3.0	3.5	0.6	5.0	20	

Diactines may be added. Asconoid aquiferous system' (Klautau *et al.*, 2016).

**ASCANDRA MASCARENICA SP. NOV.**

(FIGS 9, 10; TABLE 4)

*Zoobank registration:* urn:lsid:zoobank.org:act:774A6B27-B4E3-4A85-830F-4BCFB2869574.

*Diagnosis:* *Ascandra* whose cormus is loosely anastomosed and has apical water-collecting tubes. Skeleton composed of diactines with one lanceolated tip, triactines and two size categories of tetractines. Apical actines are conical.

*Etymology:* The species name refers to ecoregion of the type locality, the Mascarene Islands.

*Type locality:* Passe de l'Hermitage, La Réunion, Indian Ocean.

*Type material:* Holotype – UFRJPOR 8936 (= 171109-PAE2-TP12), Passe de l'Hermitage Reef, La Réunion, Indian Ocean, coll. T. Pérez, 9 November 2017, 18–20 m depth.

*Colour:* White in life and light brown to white in ethanol (Fig. 9A, B).

*Morphology and anatomy:* Sponge formed by loosely anastomosed tubes (Fig. 9A, B). Large water-collecting tubes are present at the apical region (Fig. 9B). Surface hispid (Fig. 9C). Aquiferous system asconoid. Skeleton composed of tetractines of two sizes, diactines and few triactines (Fig. 9D). The diactines are perpendicular to the tubes.

*Spicules (Table 4)*

*Diactines:* One tip sharp and the other lanceolated. They are slightly curved and undulated (Fig. 10A).

*Triactines:* Equiangular and equiradiate. They are similar to the tetractines II, but they are less abundant. Actines are slightly conical and sharp (Fig. 10B).

*Tetractines I:* Equiangular and equiradiate. Actines are conical and sharp. The apical actine is conical, sharp, shorter than the basal ones and smooth (Fig. 10C).

*Tetractines II:* Equiangular and equiradiate. They are smaller than the tetractines I. Actines are slightly conical and sharp (Fig. 10D). The apical actine is conical but much thinner than in the tetractines I, sharp and smooth.

*Ecology:* This species was found protected from the sunlight and associated with a bryozoan, a hydrozoan and other sponges (Fig. 9A).

*Geographic distribution:* Mascarene Islands Ecoregion – La Réunion (this work).

*Taxonomic remarks:* Up to date, seven species of *Ascandra* with diactines have been described: *A. atlantica* (Thacker, 1908) from Cape Verde, *A. contorta* from the Mediterranean Sea, *A. densa* Haeckel, 1872 from Australia, *A. falcata* from the Adriatic Sea, *A. izuensis* (Tanita, 1942) from Japan, *A. minchini* Borojević, 1966 from the Mediterranean Sea and *A. sertularia* Haeckel, 1872 from the Indian Ocean (Java).

Considering only the shape of the diactines, the new species can be differentiated from six out of the seven species with diactines. While the diactine of *A. mascarenica* has one lanceolated tip, *A. atlantica*, *A. contorta* and *A. izuensis* have fusiform diactines with sharp tips, *A. densa* also has fusiform diactines with one end thicker than the other, *A. falcata* has sickle-shaped diactines with one rounded tip and *A. minchini* only has microdiactines with a thickening in the middle.

The remaining species, *A. sertularia*, is probably not an *Ascandra*. According to the drawings provided by Haeckel (1872), this species has only sagittal spicules, suggesting that it most probably belongs to the genus *Leucosolenia*. Dendy & Row (1913) already transferred this species to *Leucosolenia*, but they made this transference because of the asconoid aquiferous system of this species.

**ASCANDRA OCEANUSVITAE SP. NOV.**

(FIGS 11, 12; TABLE 5)

*ZooBank registration:* urn:lsid:zoobank.org:act:05205855-E843-4E38-B3EF-3DA6548EA5B2.

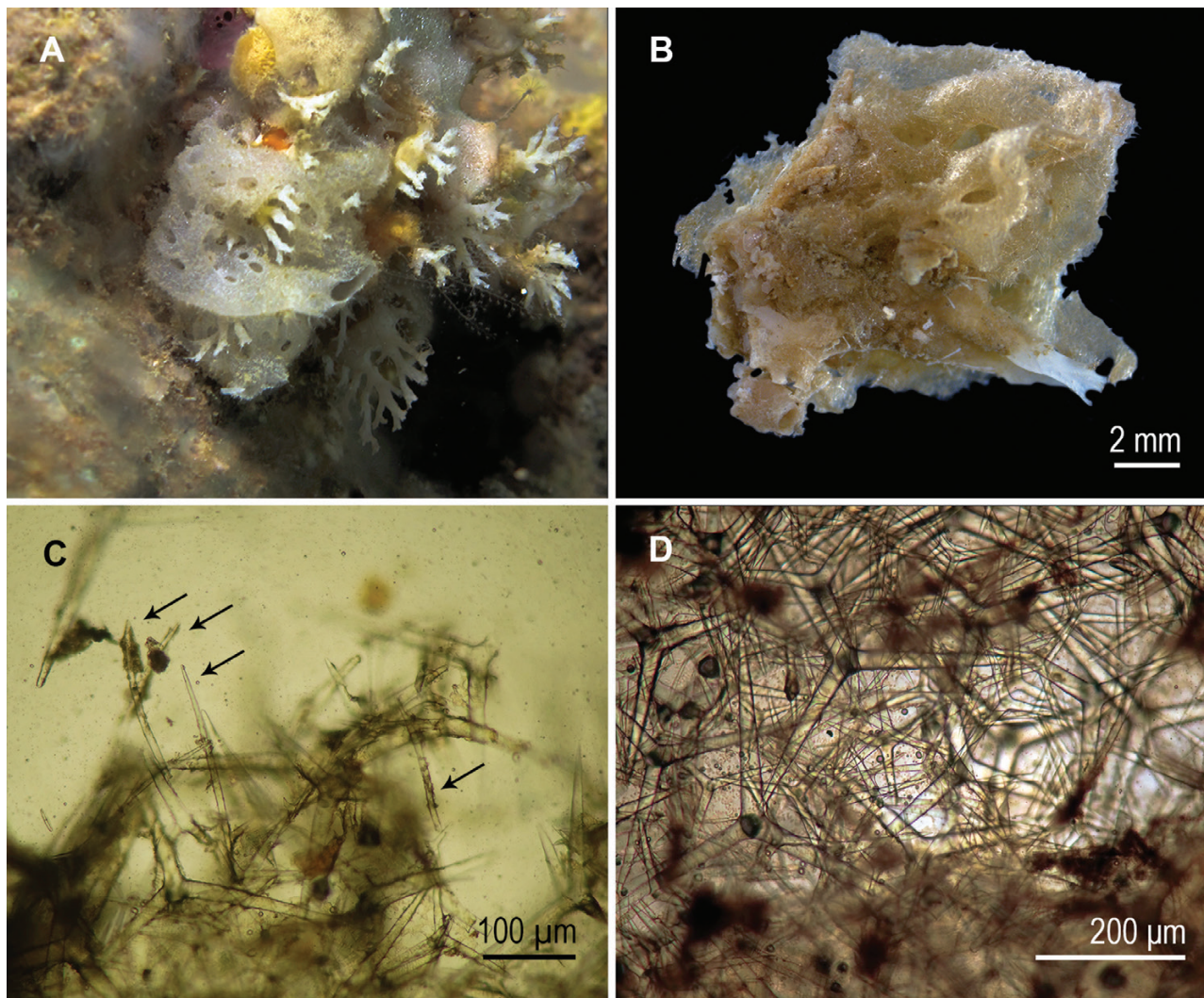
*Diagnosis:* *Ascandra* whose cormus is ramified, having only few anastomoses. Water-collecting tubes are not present. Skeleton composed of fusiform diactines, frequently with one tip larger, two size categories of triactines and of tetractines.

*Etymology:* For the Vie Océane Association, one of the institutions responsible for organizing the taxonomy course that allowed the collection of the sponges published here. The name is Latinized to *oceanus* (=ocean) and *vita* (=life).

*Type locality:* Passe de l'Hermitage, La Réunion, Indian Ocean.

*Type material:* Holotype – UFRJPOR 8944 (= 171109-PAE2-TP19). Paratype – UFRJPOR 8946





**Figure 9.** *Ascandra mascarenica* (holotype; UFRJPOR 8936). A, specimen *in vivo*, living among other sponges, bryozoans, and hydrozoans. B, fixed specimen. C, cross-section of a tube (arrows point to diactines). D, tangential section of the surface.

(= 171109-PAE2-TP21). Other material – UFRJPOR 8947 (= 171109-PAE2-TP22). Passe de l’Hermitage Reef, La Réunion, Indian Ocean, coll. T. Pérez, 9 November 2017, 18–20 m depth.

**Colour:** White in life and light brown or grey in ethanol (Fig. 11A, B).

**Morphology and anatomy:** Sponge formed by ramified tubes with few anastomoses and no water-collecting tubes (Fig. 11A, B). The surface of the tubes is hispid because of the presence of perpendicular diactines (Fig. 11C). Aquiferous system asconoid. The skeleton is composed of diactines, two size categories of triactines and tetractines. The larger triactines are rare. Diactines lie perpendicular to the tubes. The

small triactines are the most abundant spicules (Fig. 11D).

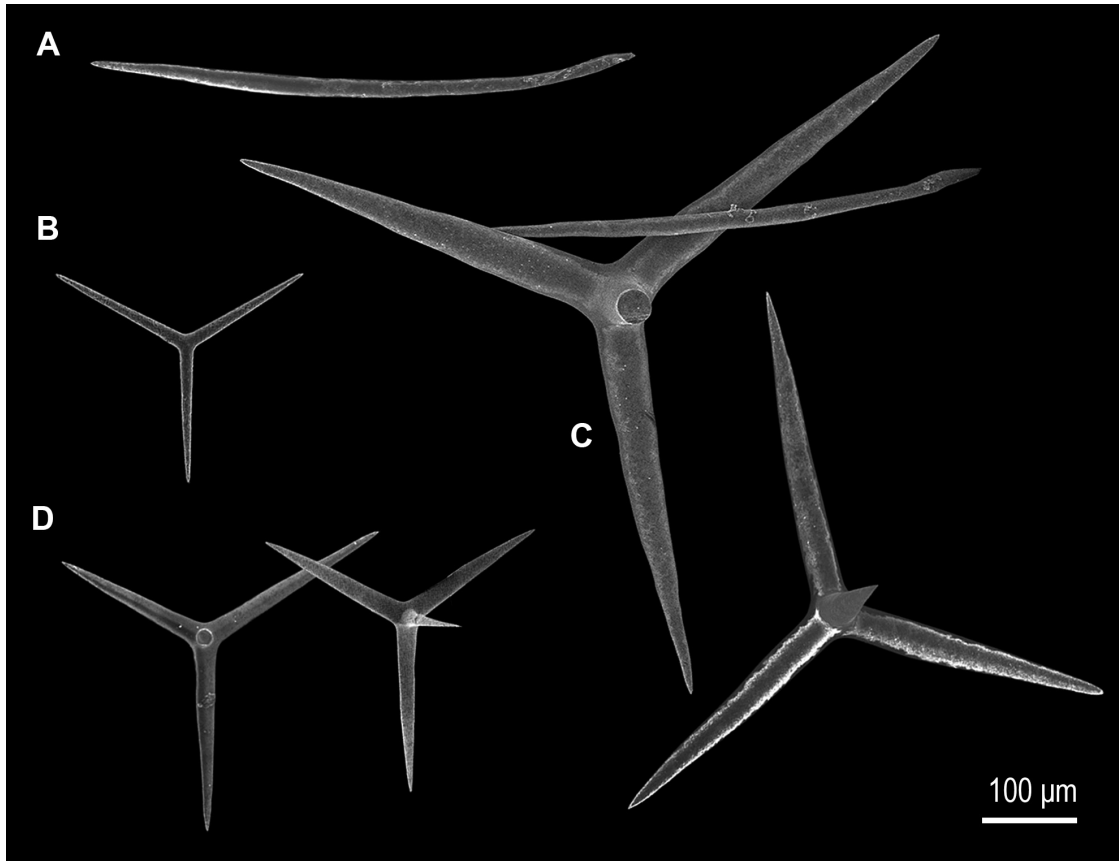
#### *Spicules* (Table 5)

**Diactines:** Fusiform but with one tip thicker than the other and frequently curved. The thicker tip penetrates the sponge body (Fig. 12A).

**Triactines I:** Equiangular and equiradiate to subregular. It is the largest category of triactine. They are similar to tetractine II. Actines are conical and sharp. They are rare.

**Triactines II:** Equiangular and equiradiate. Actines are slightly conical and sharp. They are the most abundant spicules (Fig. 12B).





**Figure 10.** *Ascandra mascarenica* (holotype; UFRJPOR 8936). A, lanceolated diactines. B, triactine. C, tetractines I. D, tetractines II.

**Table 4.** Spicule measurements of *Ascandra mascarenica*. H = holotype

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	Mean	SD	Max	Min	Mean	SD	Max	
UFRJPOR 8936 (H)	Diactine		200.0	328.0	91.8	530.0	10.0	13.5	3.3	20.0	20
	Triactine		125.0	160.3	18.1	190.0	10.0	14.3	2.4	20.0	20
	Tetractine I	Basal	270.0	356.5	79.8	500.0	30.0	35.5	6.0	50.0	20
		Apical	90.0	138.4	39.6	210.0	30.0	32.6	5.6	50.0	19
	Tetractine II	Basal	140.0	168.3	22.3	220.0	15.0	17.1	1.9	20.0	20
		Apical	42.5	109.9	75.0	350.0	5.0	6.1	1.7	10.0	20

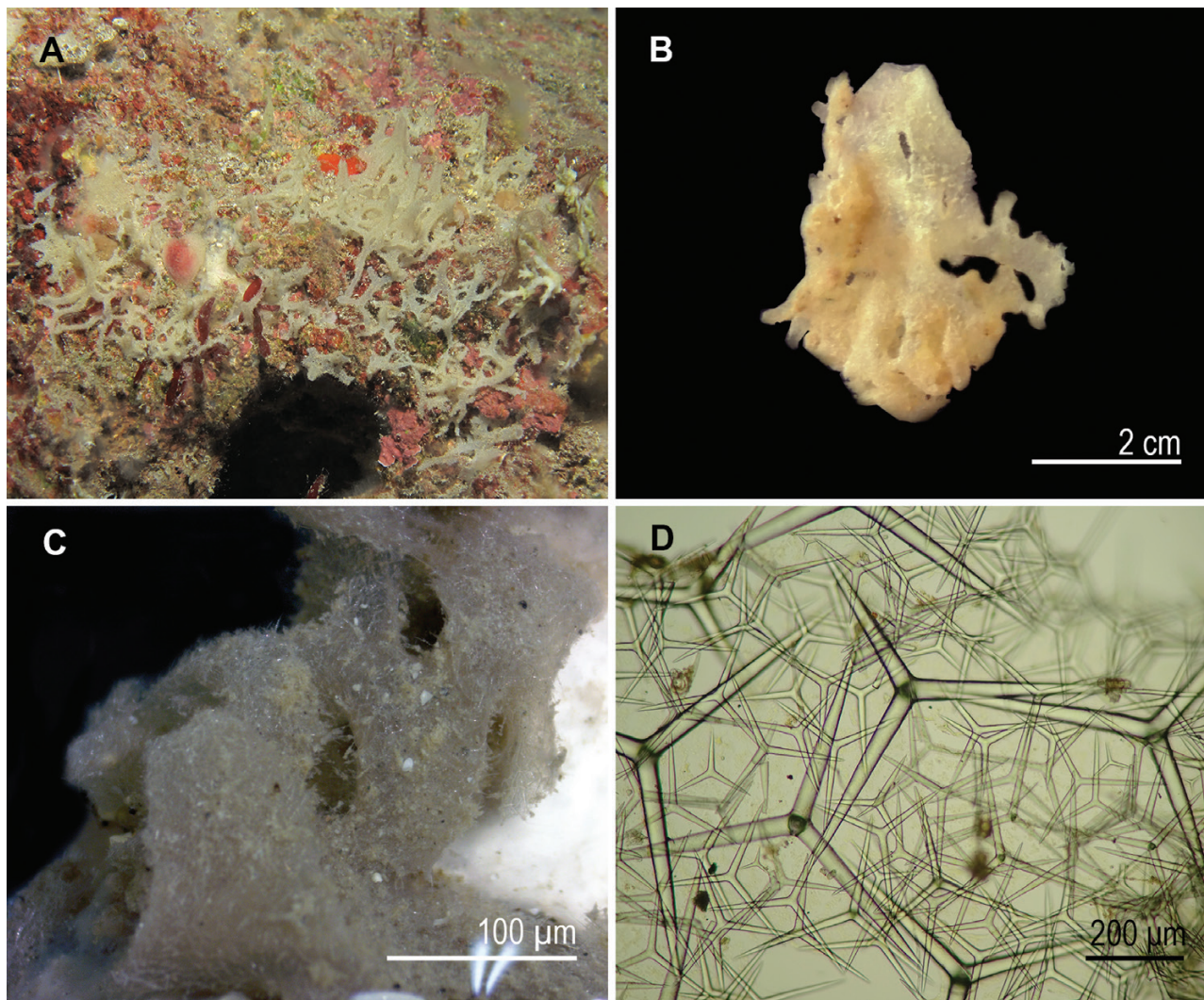
*Tetractines I*: Equiangular and equiradiate to subregular. This is the largest category of tetractine. Basal actines are conical and sharp. The apical actine is short, conical, sharp and smooth (Fig. 12C).

*Tetractines II*: Equiangular and equiradiate. Actines are slightly conical and sharp (Fig. 12D). The apical actine is short, conical, sharp and smooth.

*Ecology*: Specimens found in a submarine cave.

*Geographic distribution*: Mascarene Islands Ecoregion – La Réunion (this work).

*Taxonomic remarks*: Already considering that *A. sertularia* is in fact *Leucosolenia sertularia*, *Ascandra oceanusvitae* is the eighth species of



**Figure 11.** *Ascandra oceanusvitae* (holotype; UFRJPOR 8944). A, specimen *in vivo*. B, fixed specimen. C, hispid surface. D, tangential section of a tube.

*Ascandra* with diactines. As its diactines are fusiform, it can be differentiated from *A. falcata*, *A. mascarenica* and *A. minchini*, which do not have fusiform diactines. The fusiform diactines of *A. oceanusvitae* are similar to those of *A. atlantica*, *A. contorta*, *A. densa* and *A. izuensis*.

The new species can be differentiated from *A. contorta* and *A. densa* by, for example, the cormus, which is more tightly anastomosed in *A. contorta* and *A. densa*. Besides, it can also be differentiated by the presence of only one category of triactines and tetractines in both species, while *A. oceanusvitae* has two categories of triactines and of tetractines.

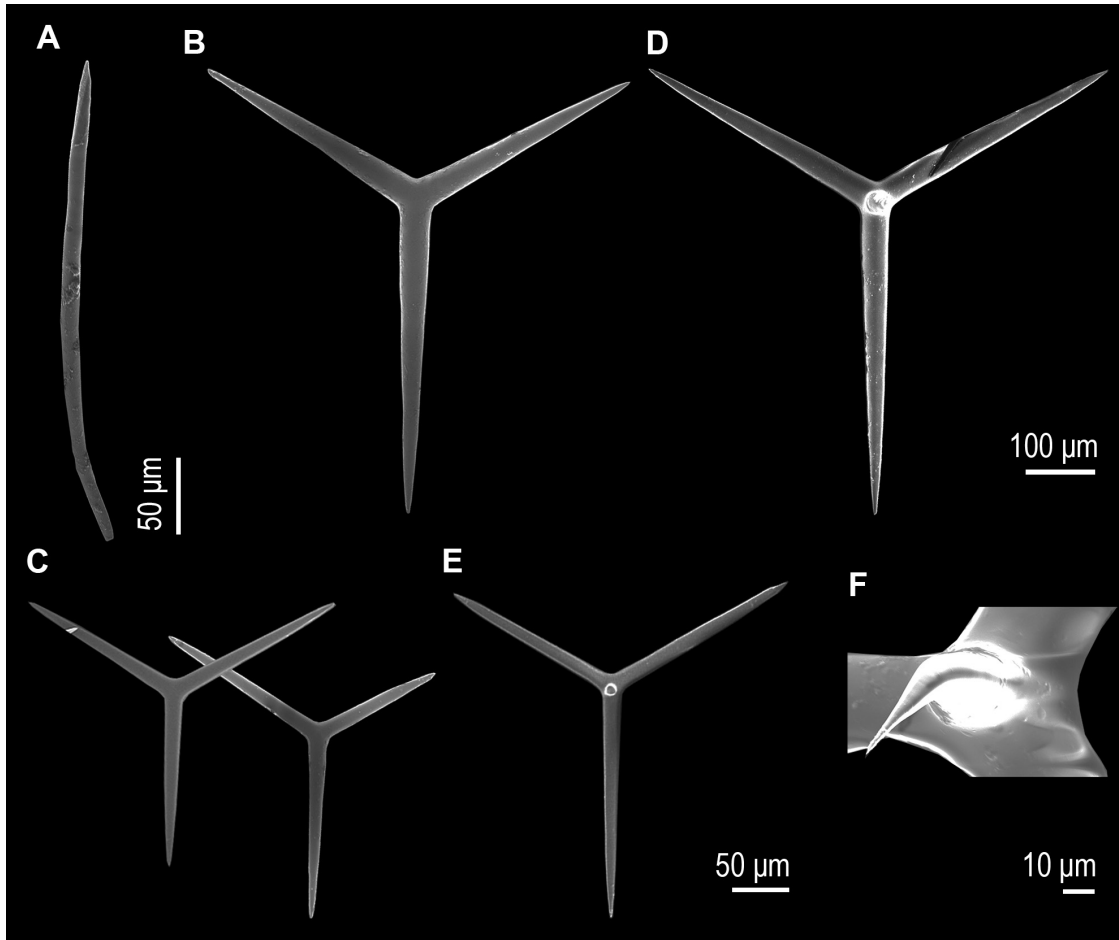
Consequently, the most similar species to the new one are *A. atlantica* from Cape Verde and *A. izuensis* from Japan. Although similar, they can be differentiated. *Ascandra oceanusvitae* has two size categories of

triactines, while *A. atlantica* and *A. izuensis* have only one category (the equivalent to the small category). Even though the largest category of triactine is rare in *A. oceanusvitae*, this new species can also be differentiated from the other two species by the size of the other spicules, which are much larger in the new species (Table 5).

#### GENUS *LEUCALTIS* HAECKEL, 1872

*Type species: Leucaltis clathria* Haeckel, 1872.

*Diagnosis:* Leucaltidae with a body composed of large, ramified and anastomosed tubes. Each tube has a distinct cortex, a choanoderm composed of elongated and ramified choanocyte chambers, and a central



**Figure 12.** *Ascandra oceanusvitae* (holotype; UFRJPOR 8944). A, diactine. B, triactine I. C, triactines II. D, tetractine I. E, tetractine II. F, apical actine of a tetractine II.

atrium. The choanoderm and the atrial wall have a secondary skeleton composed of small triactines and tetractines' (Borojević *et al.*, 2002).

*LEUCALTIS NODUSGORDII* (POLÉJAEFF, 1883)

(FIGS 13, 14; TABLE 6)

*Synonyms:* *Heteropegma nodusgordii* Poléjaeff, 1883: 45 (only the Torres Strait specimens); von Lendenfeld, 1885: 1107 (only the Australian specimens); Dendy, 1892: 113; Dendy, 1905: 230; Jenkin, 1908: 453. *Leucaltis bathybia* var. *mascarenica* Ridley, 1884: 628 (to be confirmed). *Clathrina latitubulata* Carter, 1886: 515 (to be confirmed). *Heteropegma latitubulata* Dendy, 1892: 114 (to be confirmed). *Leucaltis clathria* Dendy, 1913: 16; Dendy & Frederick, 1924: 483; Hôzawa, 1940: 136; Tanita, 1943: 393; Lévi, 1998: 75; Wörheide & Hooper, 1999: 876; Borojević & Klautau, 2000: 190. *Leuconia paloensis* (non Tanita, 1943), Colin & Arneson, 1995: 61; Gosliner *et al.*, 1996: 17; Erhardt

& Baensch, 1998: 21. *Leucaltis nodusgordii* Van Soest & De Voogd, 2015: 39, 2018: 66.

*Type locality:* Torres Strait, Australia.

*Material examined:* UFRJPOR 8930 (= 171109-PAE2-TP4). Photos: TP6029–6039. Passe de l'Hermitage Reef, La Réunion, Indian Ocean, coll. T. Pérez, 9 November 2017, 18–20 m depth.

*Colour:* White alive and light beige after fixation (Fig. 13A, B).

*Morphology and anatomy:* Sponge formed by large, individualized, anastomosed tubes (Fig. 13A, B). Each tube has its own cortex. Oscula located at the top of elevated tubes (Fig. 13A, B). The specimen was full of reproductive elements (Fig. 13C, D). The aquiferous system is composed of irregular, elongated and ramified choanocyte chambers



**Table 5.** Spicule measurements of *Ascandra oceanusvitae*, *Ascandra atlantica* (after Klautau & Valentine, 2003) and *Ascandra izuensis*. H = holotype, P = paratype

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N	
			Min	Mean	SD	Max	Min	Mean	SD	Max		
UFRJPOR 8944 (H)	Diactine		216.2	374.6	81.4	562.1	8.1	11.8	1.9	16.2	20	
	Triactine I		113.6	245.7	128.9	432.4	27.0	36.8	6.1	43.2	03	
	Triactine II		151.2	185.1	16.1	216.0	13.5	17.6	1.9	21.6	20	
	Tetractine I	Basal		345.6	462.2	62.3	583.2	32.4	47.0	6.3	54.0	20
		Apical		108.1	141.7	19.9	162.2	27.0	45.8	24.1	110.3	11
	Tetractine II	Basal		140.4	189.1	22.2	232.2	13.5	18.2	2.5	21.6	20
Apical			10.8	14.2	3.4	18.9	1.4	2.0	0.8	2.7	04	
UFRJPOR 8946 (P)	Diactine		275.0	375.7	45.9	435.0	10.0	11.7	2.4	15.0	15	
	Triactine I		-	-	-	-	-	-	-	-	-	
	Triactine II		157.5	176.3	17.7	230.0	12.5	13.9	2.4	22.5	20	
	Tetractine I	Basal		202.7	314.9	53.5	418.9	24.3	32.7	4.9	40.5	20
		Apical		-	-	-	-	-	-	-	-	-
	Tetractine II	Basal		160.0	184.5	14.1	205.0	12.5	14.5	1.5	17.5	20
Apical			15.0	16.3	1.8	17.5	5.0	5.0	0.0	5.0	2	
<i>Ascandra atlantica</i> (H)	Diactine		125.0	201.8	37.5	250.0	-	7.5	0.8	-	19	
	Triactine		75.0	108.3	16.5	140.0	-	10.0	1.5	-	30	
	Tetractine I	Basal		87.5	165.0	30.5	225.0	-	23.0	4.0	-	30
		Apical		40.8	69.1	23.8	102.0	-	18.9	7.0	-	13
	Tetractine II	Basal		85.0	106.6	13.5	145.0	-	9.8	1.5	-	30
		Apical		10.2	35.0	13.4	51.0	-	7.8	2.8	-	14
<i>Ascandra izuensis</i> (H)	Diactine		270.0	-	-	550.0	17.0	-	-	30.0	-	
	Triactine		85.0	-	-	120.0	8.0	-	-	14.0	-	
	Tetractine I	Basal		120.0	-	-	165.0	14.0	-	-	18.0	-
		Apical		140.0	-	-	190.0	10.0	-	-	14.0	-
	Tetractine II	Basal		60.0	-	-	95.0	8.0	-	-	10.0	-
		Apical		70.0	-	-	100.0	6.0	-	-	8.0	-

(Fig. 13D). There is a thick cortex composed of large triactines and tetractines, tangential to the surface (Fig. 13D, E). The choanosome has few spicules, tiny triactines and tetractines (Fig. 13D). The apical actine of the cortical tetractines penetrates the choanosome and, sometimes, the atrium. The atrial skeleton is composed of tiny triactines and tetractines (Fig. 13F).

#### Spicules (Table 6)

*Cortical triactines:* Large, equiangular and equiradiate or sagittal, with a curve between the paired actines. Actines are conical and sharp (Fig. 14A).

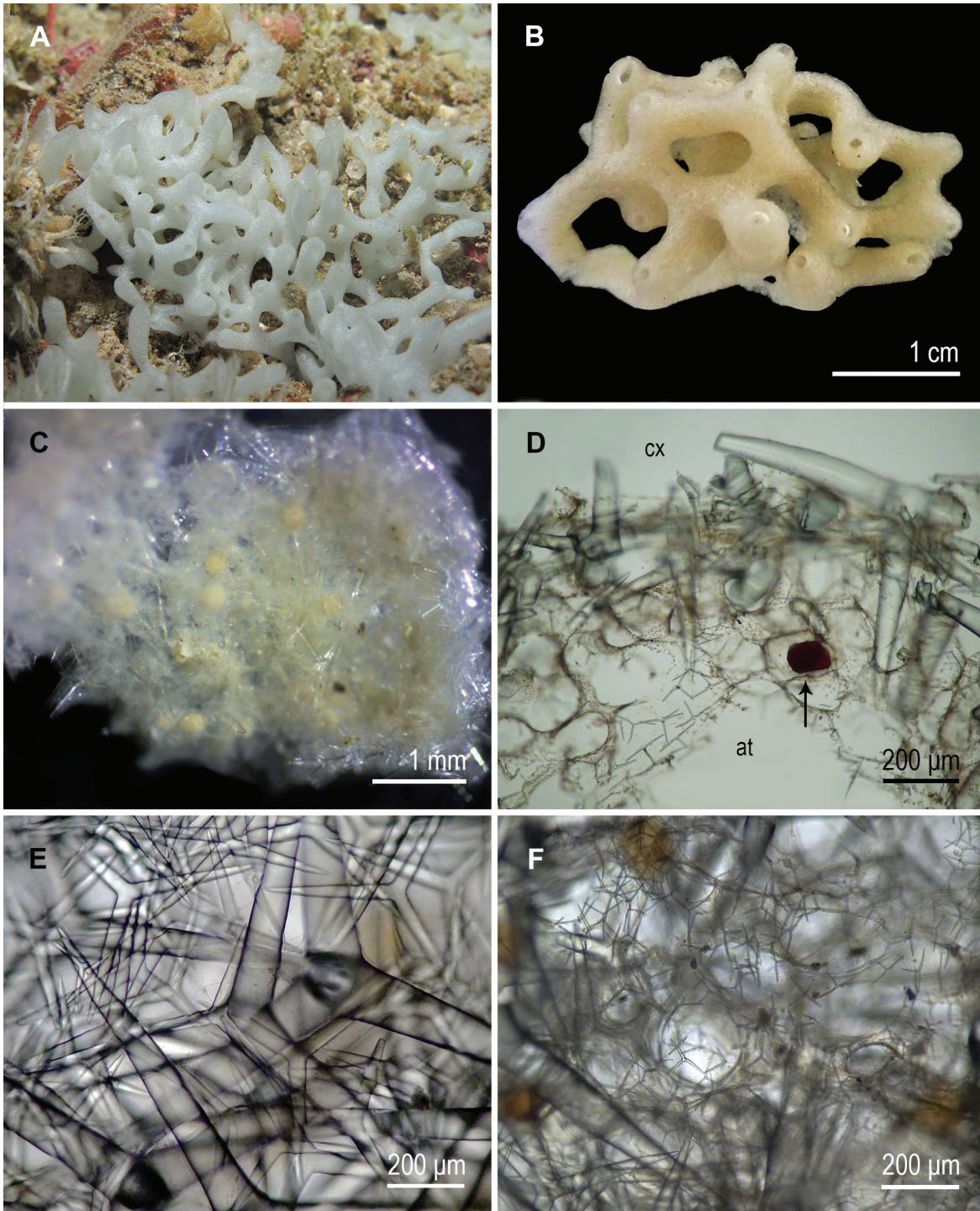
*Cortical tetractines:* Large, equiangular and equiradiate or sagittal. Actines are conical and sharp. The apical actine is longer than the basal ones (Fig. 14B).

*Choanosomal and atrial triactines:* Tiny, equiangular and equiradiate (Fig. 14C) or sagittal (Fig. 14D). Actines are cylindrical with rounded tips.

*Choanosomal and atrial tetractines:* Tiny, equiangular and equiradiate (Fig. 14E) or sagittal (Fig. 14F). Actines are cylindrical with rounded tips. The apical actine of the tetractines is conical and sharp and can be long.

*Ecology:* The specimen from Réunion was associated with two white nudibranchs and specimens of *J. indica* (UFRJPOR 8931) and *Ute insulagemmae* (Fig. 5A). *Leucaltis nodusgordii* has been found from 0 to 88 m depth in the Seychelles (Van Soest & De Voogd, 2018). Its colour alive varies from white to pink and lavender.

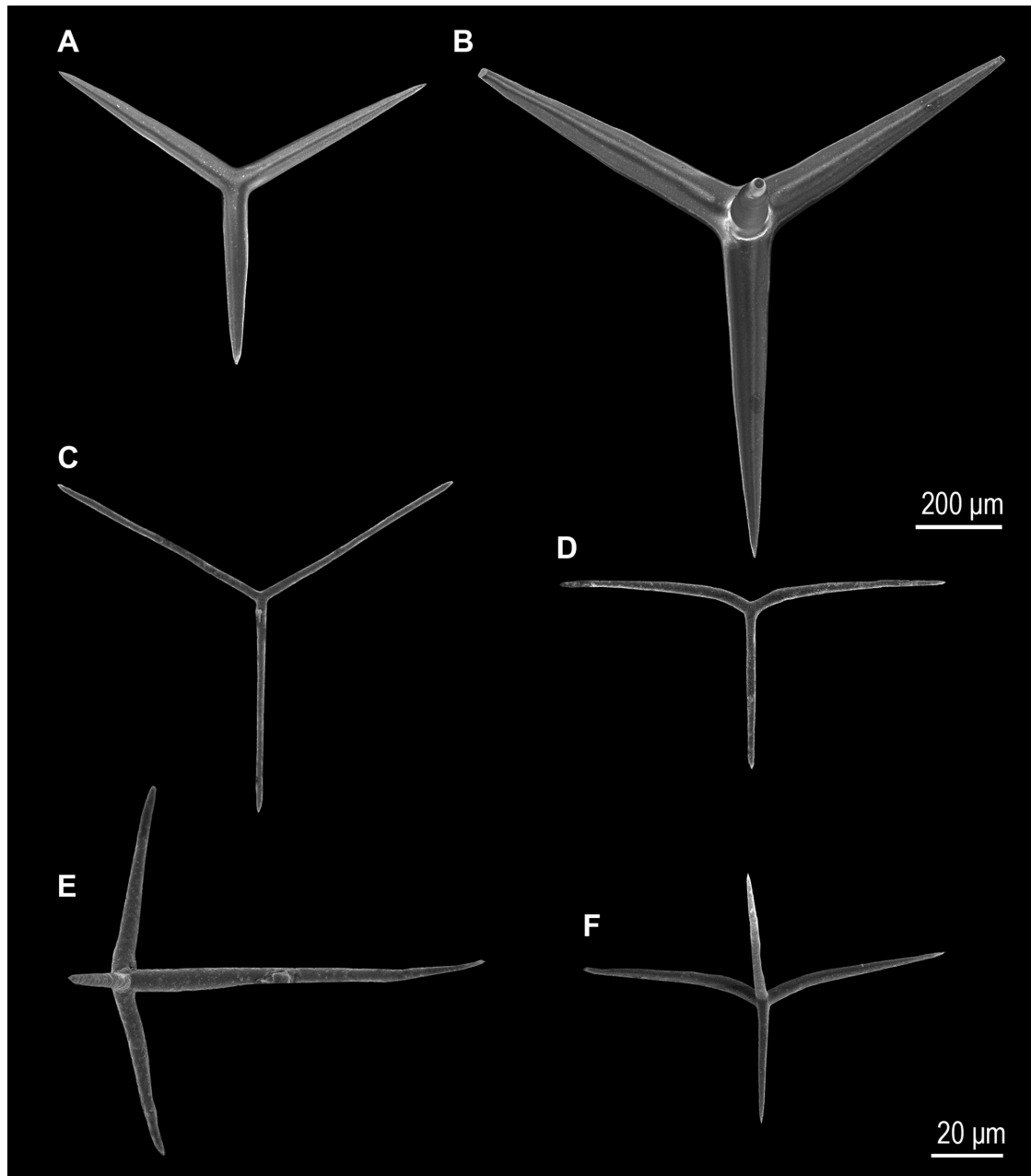
*Geographic distribution:* Torres Strait Northern Great Barrier Reef Ecoregion – Torres Strait (Poléjaeff,



**Figure 13.** *Leucaltis nodusgordii*. (UFRJPOR 8930). A, specimen *in vivo*. B, fixed specimen. C, choanosome full of yellow reproductive elements. D, cross-section (arrow points to a reproductive element). E, tangential section of the cortex. F, tangential section of the atrial wall. cx = cortex, at = atrium.

1883; von Lendenfeld, 1885; Dendy, 1892). Houtman Ecoregion – Houtman Abrolhos (Dendy & Frederick, 1924). Bassian Ecoregion – near Port Phillip Heads (Carter, 1886; Dendy, 1892); Bass Strait, Tasmania;

Westernport Bay, Victoria (Wörheide & Hooper, 1999). Central and Southern Great Barrier Reef Ecoregion – Egmont Reef (Dendy, 1913); Heron Island, GBR (Wörheide & Hooper, 1999). South India and Sri



**Figure 14.** *Leucaltis nodusgordii*. (UFRJPOR 8930). A, cortical triactine. B, cortical tetractine. C, regular choanosomal triactine. D, sagittal choanosomal or atrial triactine. E, regular choanosomal tetractine. F, sagittal choanosomal or atrial tetractine.

Lanka Ecoregion – Sri Lanka (Dendy, 1905); East African Coral Coast – Wasin (Jenkin, 1908); Seychelles Ecoregion – Amirantes group (Ridley, 1884; Dendy, 1913; Hôzawa, 1940; Van Soest & De Voogd, 2018); Mahé (Van Soest & De Voogd, 2018). Cargados Carajos/Tromelin Island Ecoregion – Cargados Carajos Atoll (Dendy, 1913). South Kuroshio – Naha, Okinawa Prefecture, Japan (Tanita, 1943). New Caledonia Ecoregion – Canal Woodin; Le Banc Gail (Borojević

& Klautau, 2000). Sulawesi Sea/Makassar Strait Ecoregion–North Sulawesi; Solomon Sea Ecoregion – Papua New Guinea (Van Soest & De Voogd, 2015). East African Coral Coast Ecoregion – Zanzibar (Van Soest & De Voogd, 2015). Mascarene Islands Ecoregion – La Réunion (this work).

*Taxonomic remarks:* Since Dendy (1913), *Heteropegma nodusgordii* was considered a synonym of *L. clathria*.



**Table 6.** Spicule measurements of *Leucaltis nodusgordii* (UFRJPOR 8930)

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	Mean	SD	Max	Min	Mean	SD	Max	
UFRJPOR 8930	Triactine I	Paired	210.0	368.3	72.1	475.0	30.0	46.8	7.8	60.0	20
		Unpaired	225.0	374.5	81.9	500.0	25.0	48.3	9.6	60.0	20
	Tetractine I	Basal	320.0	685.0	161.5	950.0	60.0	93.0	20.0	120.0	20
		Apical	160.0	265.0	73.3	320.0	70.0	72.5	5.0	80.0	04
	Triactine II	Paired	50.0	61.5	4.9	70.0	2.5	2.6	0.6	5.0	20
		Unpaired	25.0	60.1	10.5	72.5	2.5	2.6	0.6	5.0	20
	Tetractine II	Paired	40.0	63.2	7.0	72.5	2.5	3.6	1.3	5.0	20
		Unpaired	42.5	60.8	10.5	87.5	2.5	3.6	1.3	5.0	20
		Apical	15.0	22.5	9.4	37.5	2.5	4.0	1.3	5.0	10

Nonetheless, Klautau *et al.* (2013) showed that specimens from Australia identified as *L. clathria* were molecularly not conspecific with those from the Caribbean, type locality of this species. Later, considering this molecular analysis and their observation of large (cortical) sagittal triactines only in Caribbean specimens of *L. clathria*, Van Soest & De Voogd (2015) also concluded that specimens from the Indo-Pacific and the Atlantic were not conspecific. Consequently, they decided to name the Indo-Pacific specimens *L. nodusgordii*. In another paper, Van Soest & De Voogd (2018) maintained their proposition, although they could not find well-resolved clades separating these two species in their phylogenetic tree. This may be related to the use of C-LSU marker, which is too conserved for separating closely related species such as *L. clathria* and *L. nodusgordii*, which has been confirmed by our molecular analyses (Fig. 2), whereas a more variable marker such as ITS allowed to clearly separate clades (Fig. 3).

However, morphologically differentiating *L. nodusgordii* from *L. clathria* remains a challenge. Van Soest & De Voogd (2015) stated that these species could be differentiated by the presence of large (cortical) sagittal triactines, which were present only in *L. clathria*. Still, these large (cortical) sagittal triactines and tetractines were found in the specimen from Réunion (*L. nodusgordii*). On the other hand, atrial tetractines with long apical actines were also found in our specimen, and on a picture from a specimen analysed by Van Soest & De Voogd (2018: fig. 37E). And so far, such an unusual trait could not be observed by us in specimens of *L. clathria* in previous studies.

The record of *L. nodusgordii* in La Réunion is particularly interesting because as until recently (Van Soest & De Voogd, 2015), this species was considered a synonym of *L. clathria*, we did not know

if the distribution of *L. nodusgordii* was limited to the Eastern part of the Indian Ocean or if this species occurred also further West. With our study, we confirmed that *L. nodusgordii* is widely distributed along the Indian and Pacific Oceans, while *L. clathria* seems restricted to the Atlantic.

#### FAMILY LEUCASCIDAE DENDY, 1892

##### GENUS *LEUCASCUS* DENDY, 1892

*Type species: Leucascus simplex* Dendy, 1892.

*Diagnosis:* ‘Copiously branched and anastomosed choanocyte tubes covered by a continuous membrane. The exhalant aquiferous system is represented by a well-developed atrium delimited by a membrane with no choanoderm, supported by a specific skeleton of triactines and/or tetractines. The size of the spicules of the choanocyte tubes and of the cortical and atrial membranes is similar. Apical actine of the tetractines ornamented with spines. Solenoid aquiferous system’ (Cavalcanti *et al.*, 2013).

#### *LEUCASCUS TENUISPINAE* SP. NOV.

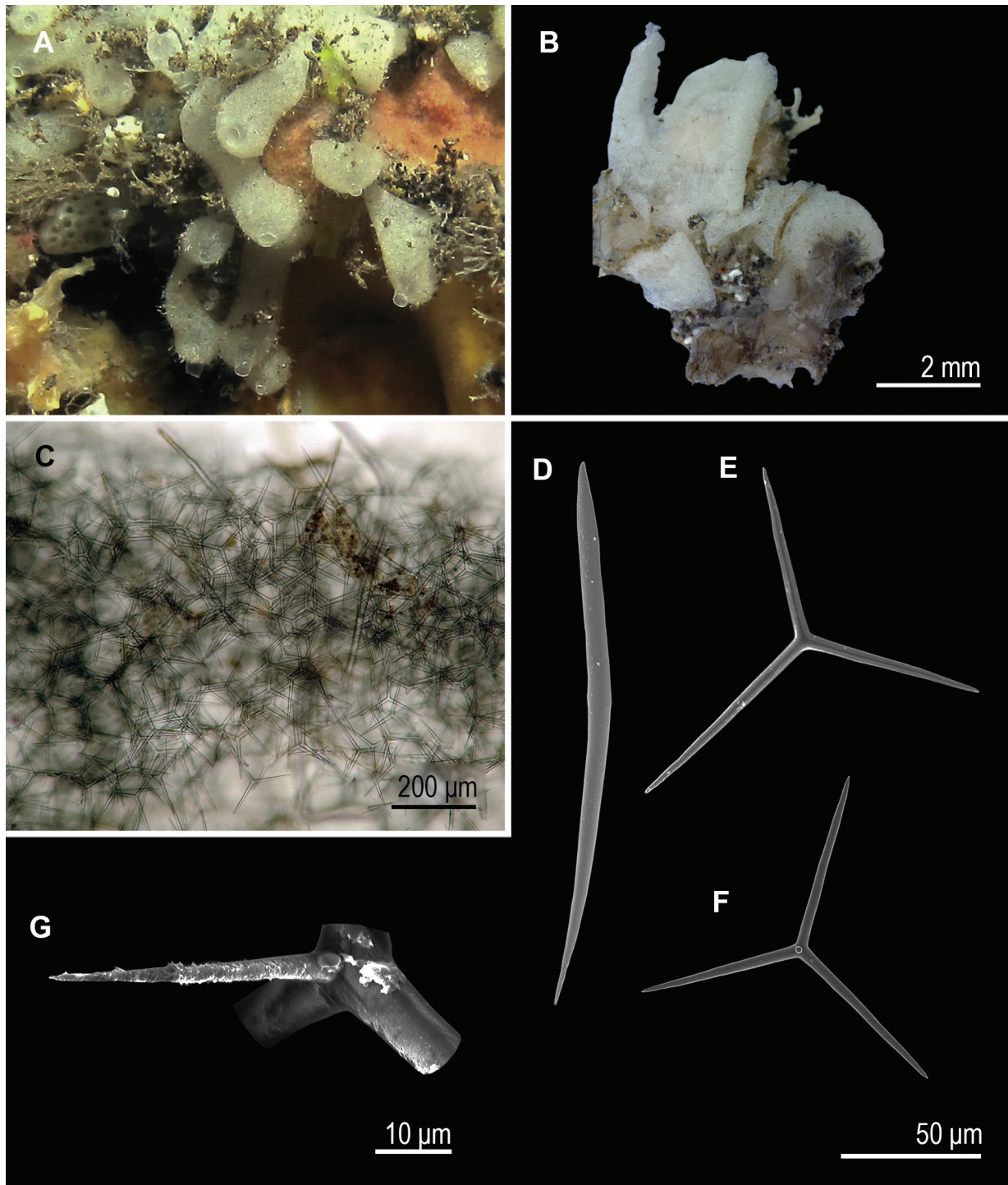
(FIG. 15; TABLE 7)

*Zoobank registration:* urn:lsid:zoobank.org:act:4EB114CF-5870-4338-95FE-09BE9C398196.

*Diagnosis:* Subglobular *Leucascus*. S skeleton composed of fusiform diactines, triactines and rare tetractines with short spines.

*Etymology:* For the microspines on the apical actine of the tetractines (*tenuis* = thin; *spinæ* = spines).

*Type locality:* Portail Caves, La Réunion, Indian Ocean.



**Figure 15.** *Leucascus tenuispinae* (holotype; UFRJPOR 8927). A, specimen *in vivo*. B, fixed specimen. C, tangential section of the surface. D, diactine. E, triactine. F, tetractine. G, apical actine of a tetractine with short spines.

**Table 7.** Spicule measurements of *Leucascus tenuispinae*. H = holotype

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	Mean	SD	Max	Min	Mean	SD	Max	
UFRJPOR 8927 (H)	Diactine		324.3	433.2	97.7	648.6	13.5	16.8	2.6	21.6	14
	Triactine		94.5	105.0	7.3	121.5	6.8	8.2	0.7	10.8	20
	Tetractine	Basal	94.5	105.2	7.1	116.1	8.1	8.1	0.0	8.1	20
		Apical	51.3	72.3	11.8	97.2	2.7	2.8	0.3	4.1	17

*Type material:* Holotype – UFRJPOR 8927 (= 171110-ESB2-TP2). Photos: TP6114–6116. Portail Caves, La Réunion, Indian Ocean, coll. T. Pérez, 10 November 2017, 25 m depth.

*Colour:* White alive and after fixation (Fig. 15A).

*Morphology and anatomy:* Sponge subglobular with a single apical osculum with membrane (Fig. 15A). A cortical membrane covers the anastomosed tubes. It was not possible to clearly observe the anastomosis because the specimen was compressed (Fig. 15B). The atrium is surrounded by a thin membrane. Aquiferous system solenoid. The sponge surface is hispid by the presence of diactines crossing it (Fig. 15A). There are also few trichoxeas, many triactines and rare tetractines (Fig. 15C).

#### Spicules (Table 7)

*Diactines:* Fusiform but with one end thicker than the other. They are slightly curved and undulated (Fig. 15D).

*Triactines:* Equiangular and equiradiate, sometimes subregular. Actines are slightly conical with sharp to blunt tips (Fig. 15E).

*Tetractines:* Equiangular and equiradiate. Actines are slightly conical with sharp to blunt tips (Fig. 15F). The apical actine is needle-like and covered by delicate short spines (Fig. 15G).

*Ecology:* Specimens were associated with colonial ascidians.

*Geographic distribution:* Mascarene Islands Ecoregion – La Réunion (this work).

*Taxonomic remarks:* Currently there are 11 accepted species of *Leucascus* in the world: *L. albus* Cavalcanti *et al.*, 2013; *L. clavatus* Dendy, 1892; *L. digitiformis* Klautau, Lopes *et al.*, 2020; *L. flavus*; *L. leptoraphis*

(Jenkin, 1908); *L. lobatus* Rapp, 2004; *L. neocaledonicus* Borojević & Klautau, 2000; *L. protogenes* (Haeckel, 1872 *sensu* Dendy, 1891); *L. roseus* Lanna *et al.*, 2007; *L. schleyeri* Van Soest & De Voogd, 2018; and *L. simplex* Dendy, 1892 (type species of the genus). From these, only *L. clavatus*, a species from Australia, and *L. schleyeri*, from South Africa, have diactines. The new species can be easily differentiated from them mainly by the fusiform shape of the diactines, which are club-shaped in *L. clavatus* and banana-shaped in *L. schleyeri*.

#### FAMILY LEUCETTIDAE DENDY & ROW, 1913

##### GENUS *LEUCETTA* HAECKEL, 1872

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

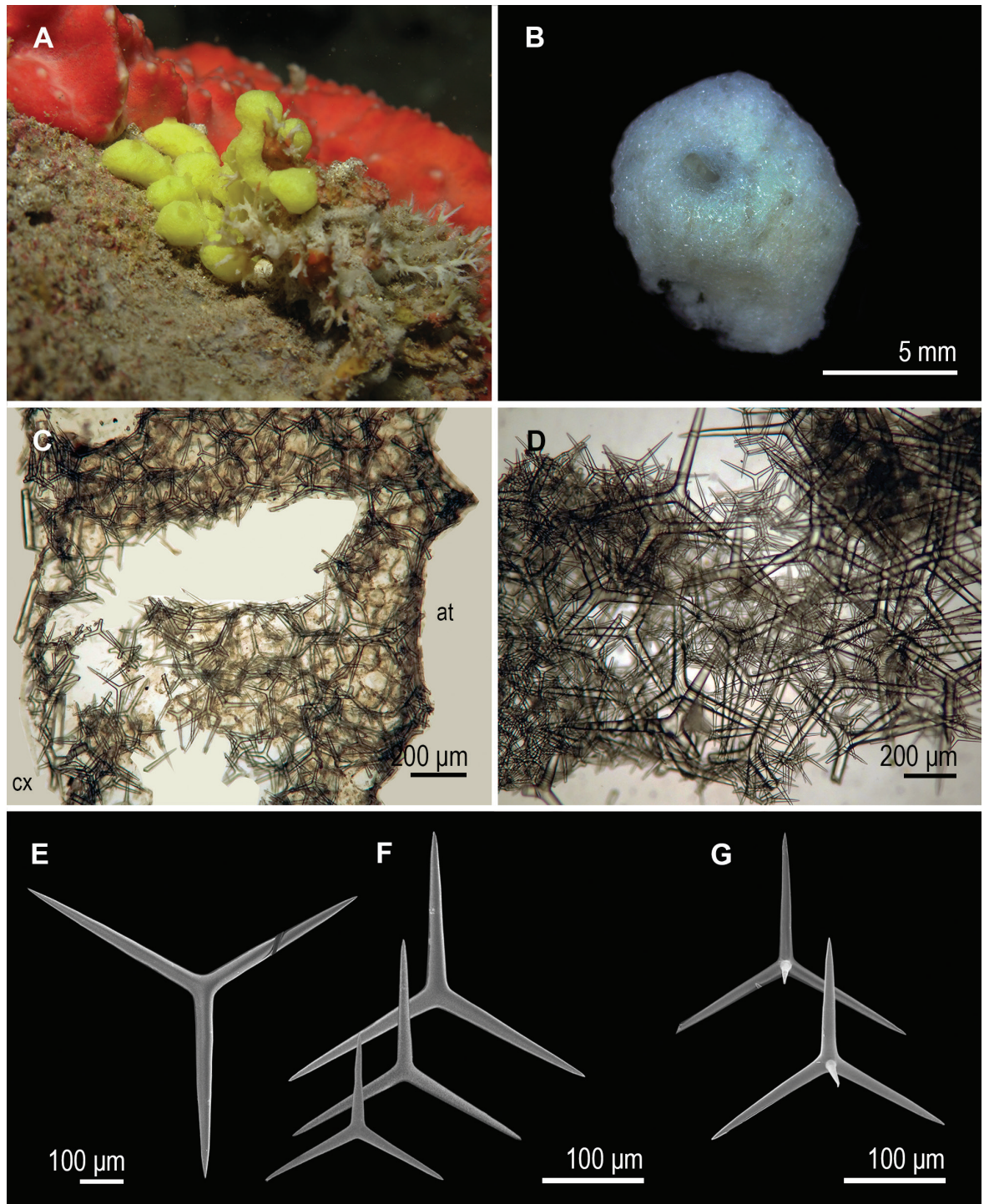
*Diagnosis:* ‘Leucettidae with a homogeneous organisation of the wall and a typical leuconoid aquiferous system. There is neither a clear distinction between the cortex and the choanoskeleton, nor the presence of a distinct layer of subcortical inhalant cavities. The atrium is frequently reduced to a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

##### *LEUCETTA CHAGOSSENSIS* DENDY, 1913

(FIG. 16; TABLE 8)

*Synonyms:* *Leucetta chagosensis* Dendy, 1913: 10; Dendy & Row, 1913: 733; Dendy & Frederick, 1924: 482; Burton, 1963: 241; Borojević, 1967: 2; Pulitzer-Finali, 1982: 89; Gosliner *et al.*, 1996: 16; Lévi, 1998: 77; Wörheide & Hooper, 1999: 882; Borojević & Klautau, 2000: 194; Wörheide *et al.*, 2002: 1753, 2005: 379; Baine & Harasti, 2007: 15; Wörheide *et al.*, 2008: 1; Voigt *et al.*, 2012: 101; Van Soest & De Voogd, 2015: 51, 2018: 76; George *et al.*, 2020: 285; Klautau *et al.*, 2020: 279; Pasnin *et al.*, 2020: 6. *Leucetta* sp. Colin & Arneson, 1995: 60 (photo 230). *Ascoleucetta sagittata* Cavalcanti *et al.*, 2013: 308; Van Soest & De Voogd, 2015: 49; Lopes *et al.*, 2018: 59; Klautau *et al.*, 2020: 265.





**Figure 16.** *Leucetta chagosensis*. (UFRJPOR 8939). A, specimen *in vivo*. B, fixed specimen. C, cross-section. D, tangential section of the cortex. E, triactine I. F, triactines II. G, tetractines. cx = cortex, at = atrium.

**Table 8.** Spicule measurements of *Leucetta chagosensis* (UFRJPOR 8939)

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	Mean	SD	Max	Min	Mean	SD	Max	
UFRJPOR 8939	Triactine I		315.0	410.0	57.9	500.0	25.0	38.3	7.9	55.0	20
	Triactine II		130.0	164.8	14.6	195.0	10.0	14.3	2.5	20.0	20
	Tetractine	Basal	77.5	124.1	22.5	155.0	10.0	13.1	2.9	17.5	20
		Apical	25.0	42.1	9.0	55.0	5.0	6.2	1.3	7.5	13

*Type locality:* Salomon, Chagos Archipelago, Indian Ocean.

*Material examined:* UFRJPOR 8939 (= 171109-PAE2-TP15). Photos: TP6059–6060. Passe de l'Hermitage Reef, La Réunion, Indian Ocean, coll. T. Pérez, 9 November 2017, 18–20 m depth.

*Colour:* Lemon-yellow alive and white after fixation (Fig. 16A, B).

*Morphology and anatomy:* Sponge formed by several globose parts, each one with a single apical osculum surrounded by membrane (Fig. 16A, B). Surface smooth and transparent. Canals can be seen below the surface. Although the atrium is reduced, it obviously receives a large number of exhalant canals. The aquiferous system is leuconoid (Fig. 16C). The skeleton is composed of two size categories of cortical triactines (Fig. 16D). The small triactines form the main part of the choanosomal skeleton, which has also rare small tetractines surrounding the canals. The atrial skeleton is composed of the small triactines and the rare tetractines.

#### Spicules (Table 8)

*Triactines I:* Large, equiangular and equiradiate. Actines are conical and sharp (Fig. 16E).

*Triactines II:* Small, equiangular and equiradiate. Actines are conical and blunt to sharp tips (Fig. 16F).

*Tetractines:* Small, equiangular and equiradiate. Actines are conical and blunt to sharp (Fig. 16G). The apical actine is short, conical and sharp.

*Ecology:* *Leucetta chagosensis* was found down to 25 m (Van Soest & De Voogd, 2018).

*Geographic distribution:* INDIAN OCEAN: Chagos Ecoregion – Salomon, Chagos Archipelago (Dendy, 1913); Houtman Ecoregion – Houtman Abrolhos (Dendy & Frederick, 1924); Maldives Ecoregion

– Maldives (Wörheide *et al.*, 2008; Van Soest & De Voogd, 2018; Pasnin *et al.*, 2020); Arabian (Persian) Gulf Ecoregion – Red Sea (Wörheide *et al.*, 2008; Voigt *et al.*, 2012; Van Soest & De Voogd, 2018; Pasnin *et al.*, 2020); India (Van Soest & De Voogd, 2018; George *et al.*, 2020). Seychelles Ecoregion – Seychelles (Van Soest & De Voogd, 2018); Western and Northern Madagascar Ecoregion – Madagascar (Van Soest & De Voogd, 2018); Mascarene Islands Ecoregion – Rodrigues (Van Soest & De Voogd, 2018; Pasnin *et al.*, 2020); La Réunion (Trentin & Massé, 2018; Pasnin *et al.*, 2020). PACIFIC OCEAN: Banda Sea Ecoregion – Indonesia; Sulawesi Sea Ecoregion – Sulawesi; Lesser Sunda Ecoregion – Bali (Wörheide *et al.*, 2002, 2008; Pasnin *et al.*, 2020); Eastern Philippines Ecoregion – Philippines (Wörheide *et al.*, 2008; Voigt *et al.*, 2012; Van Soest & De Voogd, 2015, 2018; Pasnin *et al.*, 2020); Great Barrier Reef, Australia; Tweed Moreton Ecoregion – Queensland, Australia (Wörheide & Hooper, 1999; Wörheide *et al.*, 2002, 2004, 2005, 2008; Voigt *et al.*, 2012; Pasnin *et al.*, 2020); Mariana Islands Ecoregion – Guam, South Kuroshio Ecoregion – Taiwan; Central Kuroshio Current Ecoregion – Japan (Wörheide *et al.*, 2002, 2008; Pasnin *et al.*, 2020); Bismarck Sea Ecoregion and Solomon Sea Ecoregion – Papua New Guinea, West Caroline Islands Ecoregion – Palau Islands (Wörheide *et al.*, 2002, 2005, 2008; Voigt *et al.*, 2012; Van Soest & De Voogd, 2015; Pasnin *et al.*, 2020); Vanuatu Ecoregion – Vanuatu; Fiji Islands Ecoregion – Fiji; Samoa Islands Ecoregion – Samoa (Wörheide *et al.*, 2002, 2004, 2008; Pasnin *et al.*, 2020); Society Islands Ecoregion – Society Island, French Polynesia; Tuamotus Ecoregion – Tuamotu Islands, French Polynesia (Wörheide *et al.*, 2002, 2005, 2008; Klautau *et al.*, 2020; Pasnin *et al.*, 2020); New Caledonia Ecoregion – New Caledonia (Borojević, 1967; Lévi, 1998; Borojević & Klautau, 2000); Coral Sea Ecoregion – Coral Sea (Wörheide *et al.*, 2008).

*Taxonomic remarks:* *Leucetta chagosensis* is a widespread species in the Indo-Pacific Ocean, which likely represents a species complex (Wörheide *et al.*, 2002, 2005, 2008; Voigt *et al.*, 2012; Pasnin *et al.*, 2020).

Descriptions of this species show variable morphologies, including variation in the external shape (globular to pyriform to massive lobate), colour after fixation (white or brown) and, perhaps, the spicules' size (Table 8; e.g. Dendy, 1913; Wörheide & Hooper, 1999; Borojević & Klautau, 2000; Van Soest & De Voogd, 2015, 2018; Klautau *et al.*, 2020). However, some characters seem to be constant, such as the bright yellow colour when alive, the large osculum and the conspicuous subsuperficial inhalant canals, running almost parallel.

Comparing measurements retrieved from the literature (Dendy, 1913; Wörheide & Hooper, 1999; Borojević & Klautau, 2000; Van Soest & De Voogd, 2015, 2018; Klautau *et al.*, 2020) and this work, including new measurements of the holotype given by Alencar (2012), we observed that only the size of the spicules given by Van Soest & De Voogd (2018) was different, being much larger than all the others. These authors did not mention if their measurements were based on one specimen or several but, curiously, in their description of specimens from the Red Sea and the Indian Ocean, we also found other morphological differences. They mentioned the presence of numerous tetractines in the atrium, whereas in other descriptions of *L. chagosensis* these spicules were referred as rare. Moreover, they mentioned the presence of a reduced atrium, while other authors mentioned a wide atrial cavity in this species. In that work, specimens from Indonesia, the Red Sea, the Maldives and Rodrigues grouped in the same clade, together with *L. villosa* from Australia and *Ascoleucetta sagittata* Cavalcanti *et al.*, 2013 from Indonesia (Van Soest & De Voogd, 2018). In this work, we also obtained a well-supported clade unifying specimens from Australia, French Polynesia, Indonesia, the Red Sea, Rodrigues and La Réunion, including *A. sagittata*, synonymized to *L. chagosensis* by Van Soest & De Voogd (2018) and *L. villosa* (Figs 2, 3).

Although Van Soest & De Voogd (2018) suggested that *A. sagittata* is a synonym of *L. chagosensis*, they considered *L. villosa* a valid species because of morphological evidence that might separate them. These characters are: colour alive (bright yellow in *L. chagosensis* and yellow-brownish in *L. villosa*), absence of sagittal spicules surrounding the osculum of *L. villosa* and its 'characteristic hairy surface' (Wörheide & Hooper, 1999). As colour in sponges can be variable, we should be careful about this character and, about the absence of sagittal spicules, we see some variation among *L. chagosensis* specimens. Regarding the 'hairy surface' that gave the name to the species, we analysed the holotype of *L. villosa* and found this to be colonial hydrozoans (Alencar, 2012). The same slight differences can be

seen between *L. chagosensis* and *A. sagittata*, as they can be differentiated only by the presence of sagittal spicules in the cortex and in the atrium of the latter and by the presence of large triactines only in the cortex.

Moreover, in a phylogeographic analysis performed with *L. 'chagosensis'* across the Indo-Pacific, *L. villosa* was considered to be possibly a hybrid with *L. chagosensis* (Wörheide *et al.*, 2008). Based on the mentioned morphological characters, *L. 'chagosensis'* seems to be variable and based on genetic studies, there is not enough differentiation to distinguish *L. villosa* from *L. chagosensis*. Therefore, we agree with Van Soest & De Voogd (2018) that *L. sagittata* is synonymous of *L. chagosensis* and we propose the same for *L. villosa*. Of course, it is possible that *L. chagosensis* is a species complex. A recent work using more variable markers (Pasnin *et al.*, 2020) found five lineages restricted to different biogeographic regions. The authors said they did not find morphological differences, but no morphological analyses were presented and they did not propose to split *L. chagosensis*. Hence, for the moment we consider *A. sagittata* and *L. villosa* synonyms of *L. chagosensis*.

ORDER MURRAYONIDA VACELET, 1981

FAMILY LELAPIELLIDAE BOROJEVIĆ *ET AL.*, 1990

GENUS *LELAPIELLA* VACELET, 1977

*Type species: Lelapiella incrustans* Vacelet, 1977 (by monotypy).

*Diagnosis:* Lelapiellidae encrusting and without fused spicules. Radial growing. Cortical skeleton composed chiefly of a layer of large tripods, diactines may be present. Choanosome with triactines and tetractines. Tracts of diactines may be present (modified from Borojević *et al.*, 2002).

***LELAPIELLA TERTIA* SP. NOV.**

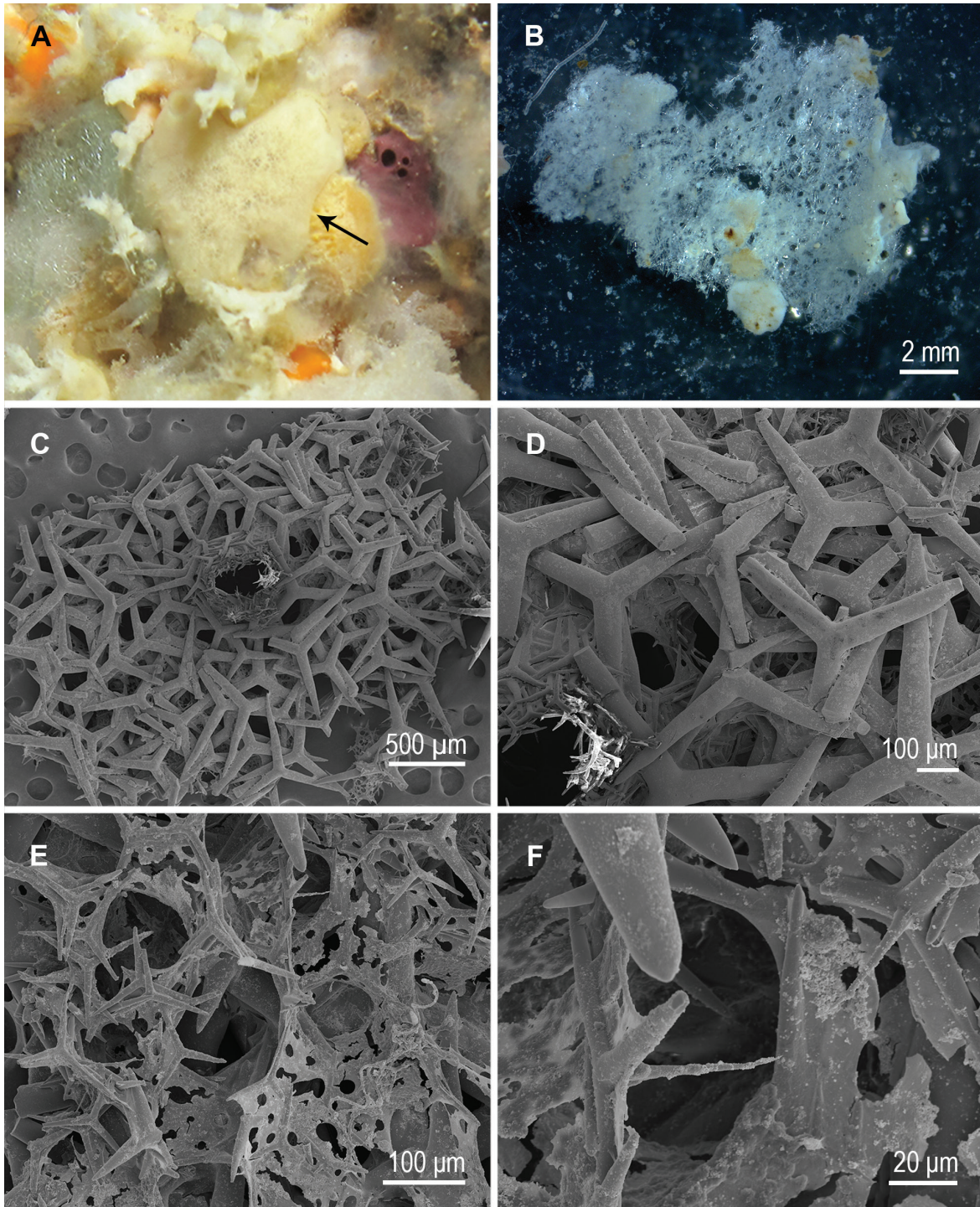
(FIGS 17, 18; TABLE 9)

*Zoobank registration:* urn:lsid:zoobank.org:act:AF5A1C7E-AA51-4D4B-A330-CAD0D653719E.

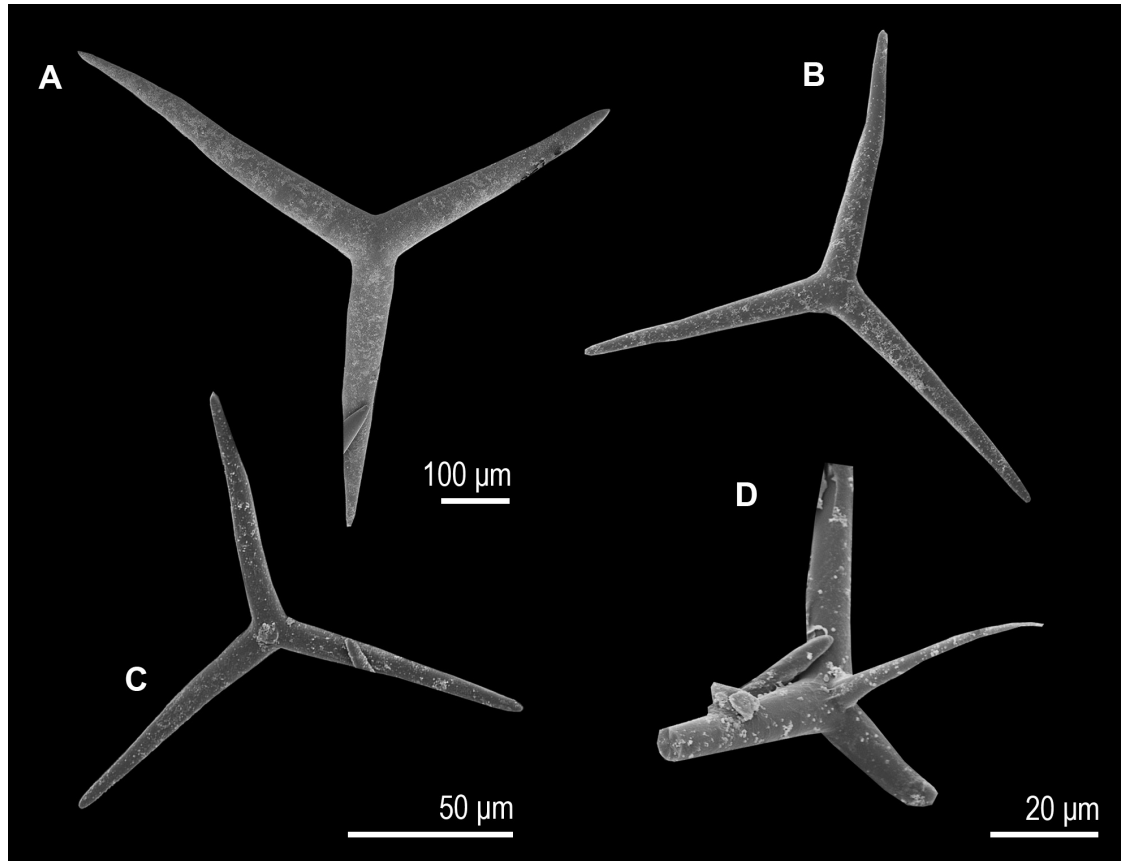
*Diagnosis:* *Lelapiella* with cortical tripods and choanosomal triactines and tetractines. Diactines and spicules tracts absent.

*Etymology:* From the Latin word *tertius*, which means third, because this is the third recognized species of the genus.





**Figure 17.** *Lelapiella tertia* (holotype; UFRJPOR 8937). A, specimen *in vivo* (arrow points to the sponge). B, fixed specimen. SEM: C, fragment of the specimen. D, tangential section of the surface. E, choanosome. F, tetractines in the choanosome.



**Figure 18.** *Lelapiella tertia* (holotype; UFRJPOR 8937). A, tripod. B, triactine. C, tetractine. D, apical actine of a tetractine.

*Type locality:* Passe de l'Hermitage, La Réunion, Indian Ocean.

*Type material:* Holotype – UFRJPOR 8937 (= 171109-PAE2-TP13). Passe de l'Hermitage Reef, La Réunion, Indian Ocean, coll. T. Pérez, 9 November 2017, 18–20 m depth.

*Colour:* Sponge white to beige in life and white in ethanol (although we have not observed this species during the collection, it corresponds probably to the sponge in Fig. 17A).

*Morphology and anatomy:* Sponge encrusting, with radial growing and oscula on the top of short cones (Fig. 17A–C). Aquiferous system leuconoid. Surface smooth. The skeleton is composed of large cortical tripods (Fig. 17D) and choanosomal triactines (Fig. 17E) and few tetractines (Fig. 17F). Sagittal triactines and tetractines similar to those of the choanosome support the oscula. Trichoxeas are also present.

#### *Spicules* (Table 9)

*Tripods:* Equiangular and equiradiate to sagittal. Huge. Actines are conical, stout with rounded to blunt tips (Fig. 18A).

*Triactines:* Equiangular and equiradiate to sagittal. Actines are conical and blunt (Fig. 18B).

*Tetractines:* Equiangular and equiradiate to sagittal. Actines are conical and blunt (Fig. 18C). The apical actine is conical, slightly curved and undulated, sharp and smooth (Fig. 18D).

*Ecology:* This sponge was growing close to *Ascandra mascarenica* sp. nov. (UFRJPOR 8936), also protected from sunlight and associated with a bryozoan and a hydrozoan.

*Geographic distribution:* Mascarene Islands Ecoregion – La Réunion (this work).

*Taxonomic remarks:* *Lelapiella inscrustans*, a species from Madagascar (Western Indian Ocean), is the



**Table 9.** Spicule measurements of *Lelapiella tertia*, *L. incrustans* and *L. sphaerulifera*. Abbreviations: c = curved; H = holotype; o = from the osculum; r = reniform; st = straight, from the tracts

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	Mean	SD	Max	Min	Mean	SD	Max	
<i>L. tertia</i> sp. nov. (H)	Tripod		118.9	490.8	150.3	713.5	32.4	75.7	16.0	97.3	20
	Triactine		67.5	82.1	7.5	94.5	6.8	9.9	1.6	13.5	20
	Tetractine	Basal	75.6	82.9	6.1	99.9	8.1	10.0	1.1	10.8	20
		Apical	27.0	41.3	8.6	54.0	4.1	5.4	0.7	8.1	20
<i>L. incrustans</i> (H)	Diactine (st)		80.0	-	-	200.0	6.0	-	-	20.0	-
	Diactine (c)		50.0	-	-	160.0	10.0	-	-	15.0	-
	Diactine (r)		-	35.0	-	-	-	12.0	-	-	-
	Tripods		65.0	-	-	400.0	32.0	-	-	60.0	-
	Triactine (o)	Paired	40.0	-	-	60.0	7.5	-	-	20.0	-
		Unpaired	37.0	-	-	55.0	10.0	-	-	15.0	-
	Tetractine	Basal	75.0	-	-	80.0	10.0	-	-	12.0	-
		Apical	50.0	-	-	80.0	10.0	-	-	12.0	-
<i>L. sphaerulifera</i>	Diactine (st)		126.0	193.2	-	297.0	13.0	17.2	-	27.0	-
	Diactine (c)		84.0	95.8	-	106.0	18.0	19.1	-	21.0	-
	Diactine (r)		26.0	30.2	-	36.0	17.0	18.2	-	20.0	-
	Tripods		171.0	226.2	-	285.0	36.0	46.0	-	57.0	-
	Tetractine	Basal	78.0	-	-	100.0	-	9.0	-	-	-
		Apical	10.0	-	-	20.0	-	-	-	-	-

type species of *Lelapiella* (Vacelet, 1977). In the same paper, a subspecies, *Lelapiella incrustans sphaerulifera*, from Moorea (French Polynesia) was also described and later elevated to the species rank (Van Soest & De Voogd, 2015). Both species are morphologically similar, being encrusting, circular and having large cortical tripods, choanosomal tetractines, triactines, straight diactines forming tracts, curved diactines and reniform diactines. According to Vacelet (1977), the creation of a subspecies was justified by differences in the size and shape of the spicules. For example, *L. incrustans* harbours tripods with ‘nipples’ and few reniform diactines, whereas *L. incrustans sphaerulifera* presents abundant smooth tripods and reniform diactines (Vacelet, 1977). Studying specimens from Indonesia, Van Soest & De Voogd (2015) identified them as *L. sphaerulifera*, elevating the subspecies to species status, although not explaining why. We are not certain that the specimens from Indonesia are *L. sphaerulifera*. Vacelet (1977) mentioned that his subspecies presented ‘nipples’ in some tripods, although some of them were smooth. Besides, he found triactines in his specimens from French Polynesia. Van Soest & De Voogd (2015) did not

mention the presence of ‘nipples’ in the tripods from the Indonesian specimens and showed images of a smooth tripod. Moreover, they did not mention the presence of triactines in their specimens.

Because we consider that *L. sphaerulifera* is a distinct species, the sponge we are describing from La Réunion has to be the third of the genus. Indeed, *Lelapiella tertia* can be differentiated from *L. incrustans* and *L. sphaerulifera* by skeleton composition and also by spicules size (Table 9). The new species does not have diactines, except for some trichoxeas. It is interesting that, similarly to *L. incrustans* and *L. sphaerulifera*, the new species is encrusting, grows radially, its oscula are on the top of short cones and its skeleton has tripods. However, the new species does not have the characteristic tract of diactines of *L. incrustans* and *L. sphaerulifera*, which convinced us to propose a new diagnosis for the genus.

Because our analyses corroborated that *Lelapiella* is a Calcinea (Borojević *et al.* 1990; Dohrmann *et al.*, 2006; Voigt *et al.*, 2012) and that it is not a Murrayonida (Dohrmann *et al.*, 2006; Voigt *et al.*, 2012; Fig. 5), we propose to synonymize Murrayonida with Clathrinida.



SUBCLASS CALCARONEA BIDDER, 1898

ORDER LEUCOSOLENIIDA HARTMAN, 1958

FAMILY GRANTIIDAE DENDY, 1892

GENUS *APHROCERAS* GRAY, 1858

*Type species: Aphroceras alcicornis* Gray, 1858.

*Diagnosis:* ‘Grantiidae with leuconoid organisation. The cortex is supported, at least in part, by giant longitudinal diactines’ (Borojević *et al.*, 2002).

***APHROCERAS SEYCHELLENSIS*** (HÓZAWA,  
1940) COMB. NOV.

(FIGS 19–21; TABLE 10)

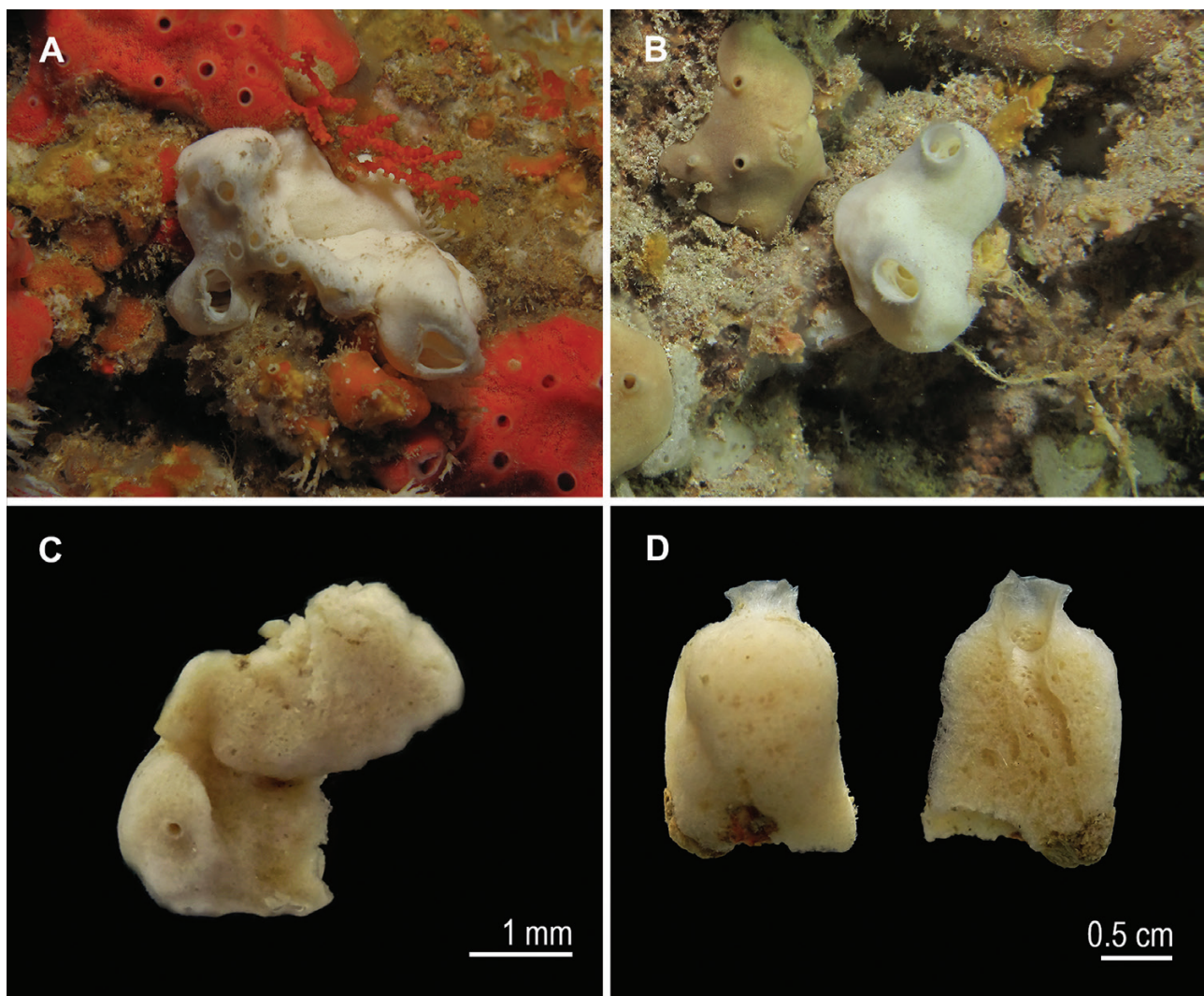
*Basionym:* *Leucandra seychellensis* Hózáwa, 1940:  
158; Burton, 1963: 519.

*Type locality:* Seychelles, Indian Ocean.

*Material examined:* UFRJPOR 8926 (= 171109-PAE2-TP2). UFRJPOR 8934 (= 171109-PAE2-TP9). UFRJPOR 8935 (= 171109-PAE2-TP10). Passe de l’Hermitage Reef, La Réunion, Indian Ocean, coll. T. Pérez, 9 November 2017, 18–20 m depth.

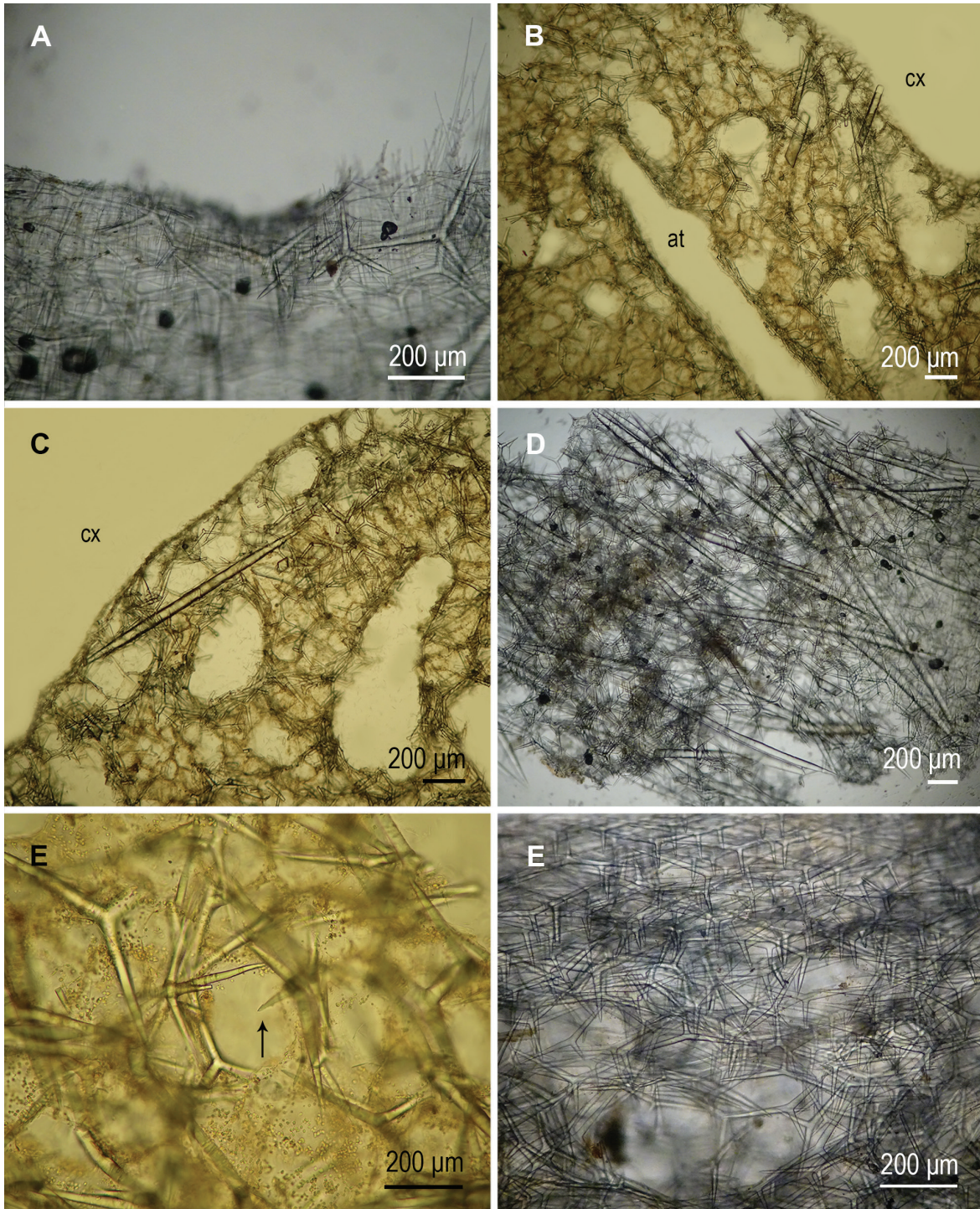
*Colour:* White alive but with a grey region around the oscula (Fig. 19A, B). White after fixation (Fig. 19C, D).

*Morphology and anatomy:* Sponge amorphous, massive, with several large and small apical oscula (Fig. 19A–D) surrounded by membrane (Fig. 19D) and



**Figure 19.** *Aphroceras seychellensis*. A, specimen *in vivo* (UFRJPOR 8926). B, specimen *in vivo* (UFRJPOR 8934). C, fixed specimen (UFRJPOR 8926). D, fixed specimen (UFRJPOR 8934).

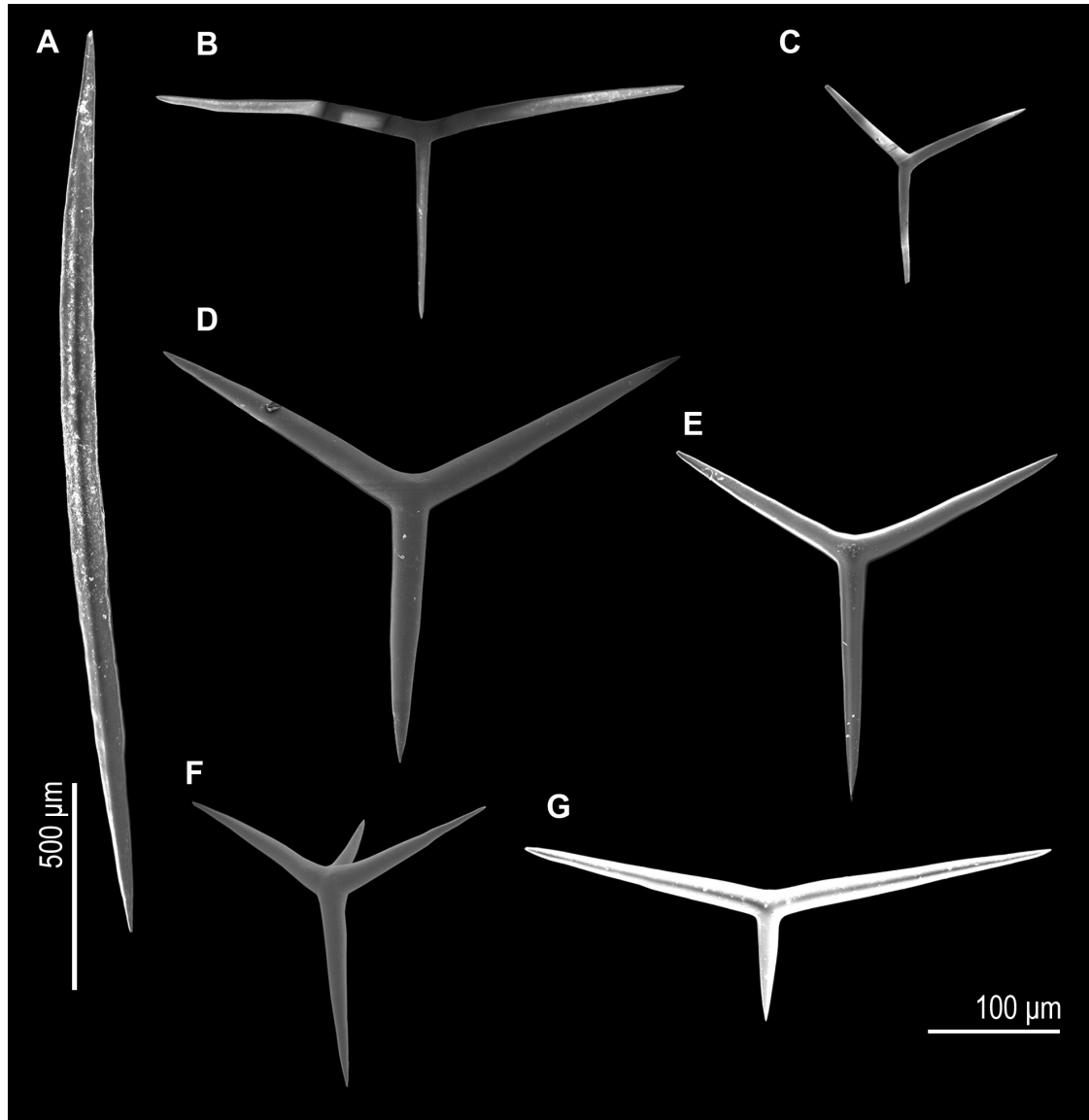




**Figure 20.** *Aphroceras seychellensis* (UFRJPOR 8926). A, oscular margin. B, cross-section. C, cross-section evidencing subcortical lacunae. D, tangential section of the cortex. E, choanosomal canal surrounded by a triactine and a tetractine (arrow points to the apical actine of the tetractine). F, tangential section of the atrial surface. cx = cortex, at = atrium.

a short crown of trichoxeas (Fig. 20A). The crown is supported by T-shape triactines with the unpaired actine thinner than the paired ones (Figs 20A, 21B). The huge cortical diactines protrude near the oscula

but lie tangentially under the surface in most of the body. As a result, the surface is smooth. The atrium can be reduced or large (Fig. 19D). It is possible to see the lacunae and canals below the cortex (Fig. 20B). The



**Figure 21.** *Aphroceras seychellensis* (UFRJPOR 8926). A, cortical diactine. B, oscular triactine. C, cortical triactine. D, choanosomal triactine. E, choanosomal triactine. F, tetractine of the canals. G, atrial triactine.

aquiferous system is leuconoid. The cortical skeleton is composed of longitudinal giant diactines and small triactines (Fig. 20B–D). The choanosome has dispersed triactines, and triactines and tetractines surrounding the canals (Fig. 20C, E). The triactines tend to reduce the angle between the paired actines when they are not surrounding the canals (i.e. less sagittal). The atrial skeleton has only triactines and they are larger than the cortical ones (Fig. 20F).

#### Spicules (Table 10)

**Diactines:** Giant and fusiform, with both tips sharp (Fig. 21A).

**Cortical triactines:** Small and sagittal. Actines are cylindrical and sharp (Fig. 21C).

**Choanosomal triactines:** Variable size, subregular to sagittal. Actines are conical or slightly conical and sharp. (Fig. 21D, E).

**Choanosomal tetractines (canals):** Sagittal, with curved paired actines. Actines are slightly conical to conical and sharp. The apical actine is conical, sharp and short (Fig. 21F).

**Atrial triactines:** Strongly sagittal. The paired actines are straight and much longer than the unpaired one. They are conical and sharp (Fig. 21G).



**Table 10.** Spicule measurements of *Aphroceras seychellensis* from La Réunion and from Seychelles (Hôzawa, 1940). H = holotype

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	Mean	SD	Max	Min	Mean	SD	Max	
UFRJPOR 8926	Cortical diactine		729.7	1695.0	406.1	2243.2	45.9	67.1	15.1	94.5	14
	Cortical triactine	Paired	59.4	93.4	19.6	121.5	5.4	7.3	1.5	10.8	20
		Unpaired	51.3	77.9	20.2	113.4	5.4	7.2	1.6	10.8	20
	Choanosomal triactine	Paired	94.5	169.4	45.6	243.0	13.5	21.5	4.9	32.4	20
		Unpaired	81.0	142.7	31.9	221.4	13.5	21.5	4.1	29.7	20
	Canal tetractine	Paired	124.2	153.3	18.4	183.6	16.2	18.9	2.7	24.3	09
		Unpaired	105.3	123.9	19.3	167.4	16.2	19.2	1.6	21.6	09
		Apical	51.3	58.8	5.7	70.2	10.8	13.5	1.1	16.2	13
	Atrial triactine	Paired	129.6	175.1	26.0	243.0	13.5	17.7	2.6	24.3	20
		Unpaired	45.9	75.3	17.2	113.4	13.5	15.5	1.9	18.9	20
UFRJPOR 8934	Cortical diactine		918.9	1621.6	422.7	2432.4	54.0	86.4	16.2	113.4	20
	Cortical triactine	Paired	59.4	83.4	16.5	113.4	5.4	7.1	1.4	9.5	20
		Unpaired	40.5	75.9	17.8	105.3	5.4	6.9	1.4	8.1	20
	Choanosomal triactine	Paired	86.4	172.9	34.9	240.3	16.2	26.1	5.3	40.5	20
		Unpaired	91.8	141.9	28.3	216.0	16.2	25.4	3.9	32.4	20
	Canal tetractine	Paired	124.2	153.9	21.1	183.6	16.2	19.4	3.5	24.3	05
		Unpaired	89.1	112.3	23.1	151.2	16.2	19.4	2.3	21.6	05
		Apical	54.0	60.9	6.4	70.2	10.8	13.1	1.0	13.5	07
	Atrial triactine	Paired	143.1	199.4	28.0	245.7	16.2	22.5	4.0	29.7	20
		Unpaired	40.5	66.2	12.1	83.7	16.2	21.0	3.0	27.0	20
Hôzawa 1940 (H)*	Cortical diactine		800.0	-	-	1050.0	45.0	-	-	65.0	-
	Cortical triactine		-	70.0	-	-	-	6.0	-	-	-
	Choanosomal triactine	Paired	140.0	-	-	240.0	20.0	-	-	30.0	-
		Unpaired	130.0	-	-	230.0	20.0	-	-	30.0	-
	Canal tetractine	Apical	-	50.0	-	-	-	14.0	-	-	-
	Atrial triactine	Paired	130.0	-	-	180.0	8.0	-	-	10.0	-
Unpaired		50.0	-	-	90.0	6.0	-	-	8.0	-	

\*Hôzawa (1940) also gave, for the cortical triactines, the following measurements: 260.0 µm length/ 20 µm width. We consider that this measurement belongs to the large choanosomal triactines.

**Ecology:** The specimen was attached to a coral and an undetermined red sponge (Fig. 19A).

**Geographic distribution:** Seychelles Ecoregion – Seychelles (Hôzawa, 1940); Mascarene Islands Ecoregion – La Réunion (this study).

**Taxonomic remarks:** Our specimens from La Réunion mostly match the description given by Hôzawa (1940) for *Leucandra seychellensis*, a species from the Seychelles. The only differences were the size of the diactines, larger and thicker in our specimens, and the atrial triactines, thicker in the specimens from La Réunion (Table 10). As we also observed some differences in spicule measurements among our specimens, we did not consider these differences strong enough to not consider the specimens from La Réunion conspecific with Hôzawa's specimen. The other difference we found concerns the

presence of large triactines in the cortex. Hôzawa (1940) mentioned the presence of small and large triactines in the cortex and in the choanosome. We observed that, although these large triactines are sometimes near the cortex in our specimens, they are mainly part of the choanosome. Therefore, we described the cortex as having only small triactines (and the diactines) and the choanosome as having the large triactines, small triactines (mainly surrounding canals) and tetractines only surrounding canals. Therefore, we identified our specimens as conspecific with those of Hôzawa.

The presence of diactines running parallel to the cortical surface, and not protruding through it, indicates that *L. seychellensis* is not *Leucandra* but *Aphroceras*. Therefore, we propose here to transfer this species to the genus *Aphroceras*. Moreover, Hôzawa (1940) pointed out that the Mediterranean *L. crambessa* Haekel, 1872 is also similar to *A. seychellensis* because of large parallel

diactines in the cortical skeleton. Both species, however, differ mainly in the composition of the atrial skeleton: tetractines are present in *L. crambessa*, whereas *A. seychellensis* only has triactines. Considering the positioning of the diactines of *L. cambressa*, this species also has to be transferred to *Aphroceras*.

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’ (Borojević *et al.* 2002).

*LEUCANDRA ORNATA* SP. NOV.

(FIGS 22–25; TABLE 11)

*Zoobank registration:* urn:lsid:zoobank.org:act:2F2F2C38-A216-4B1F-8A04-7CAA93B8F0EC.

*Diagnosis:* *Leucandra* with spined microdiactines in the cortical and in the atrial skeletons. The cortical microdiactines are frequently organized forming bouquets. Large cortical diactines are also present, along with cortical triactines. The choanosome contains regular triactines and sagittal triactines and tetractines. The atrial skeleton has microdiactines with spines and tetractines.

*Etymology:* From the Latin *ornatum*, ornate or decorated, for the presence of many diactines on the surface of this species.

*Type locality:* Cap La Houssaye Reef, La Réunion, Indian Ocean.

*Type material:* Holotype – UFRJPOR 8928 (= 171108-CAH2-TP2, Photos: TP5973–5975). Cap La Houssaye Reef, La Réunion, Indian Ocean, coll. T. Pérez, 8 November 2017, 10 m depth.

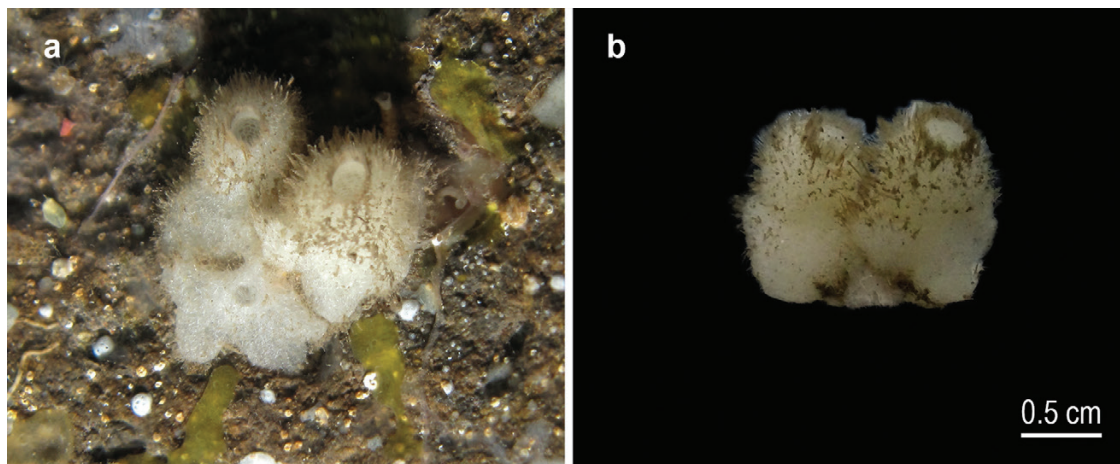
*Colour:* White alive (Fig. 22A) and after fixation (Fig. 22B).

*Morphology and anatomy:* Sponge vase-shaped with apical osculum surrounded by a crown of trichoxeas (Figs 22A, B, 24A) supported by sagittal triactines and tetractines (Fig. 23A). Surface hispid because of several large diactines protruding through the surface (Figs 22A, B, 23C, 24B). The aquiferous system is leuconoid. The cortical skeleton is composed of tangential triactines, large diactines and microdiactines protruding through the surface (Figs 22A, B, 23C, D, 24C). The cortical microdiactines form bouquets or lie tangentially to the cortex (Figs 23D, 24C). The choanosome contains large and small triactines. These small triactines are found spread in the choanosome or surrounding the canals, although tetractines are the main spicules in the canals (Fig. 23E). The atrial skeleton is composed of tetractines and microdiactines tangential to the atrium (Figs 23F, 24D).

*Spicules* (Table 11)

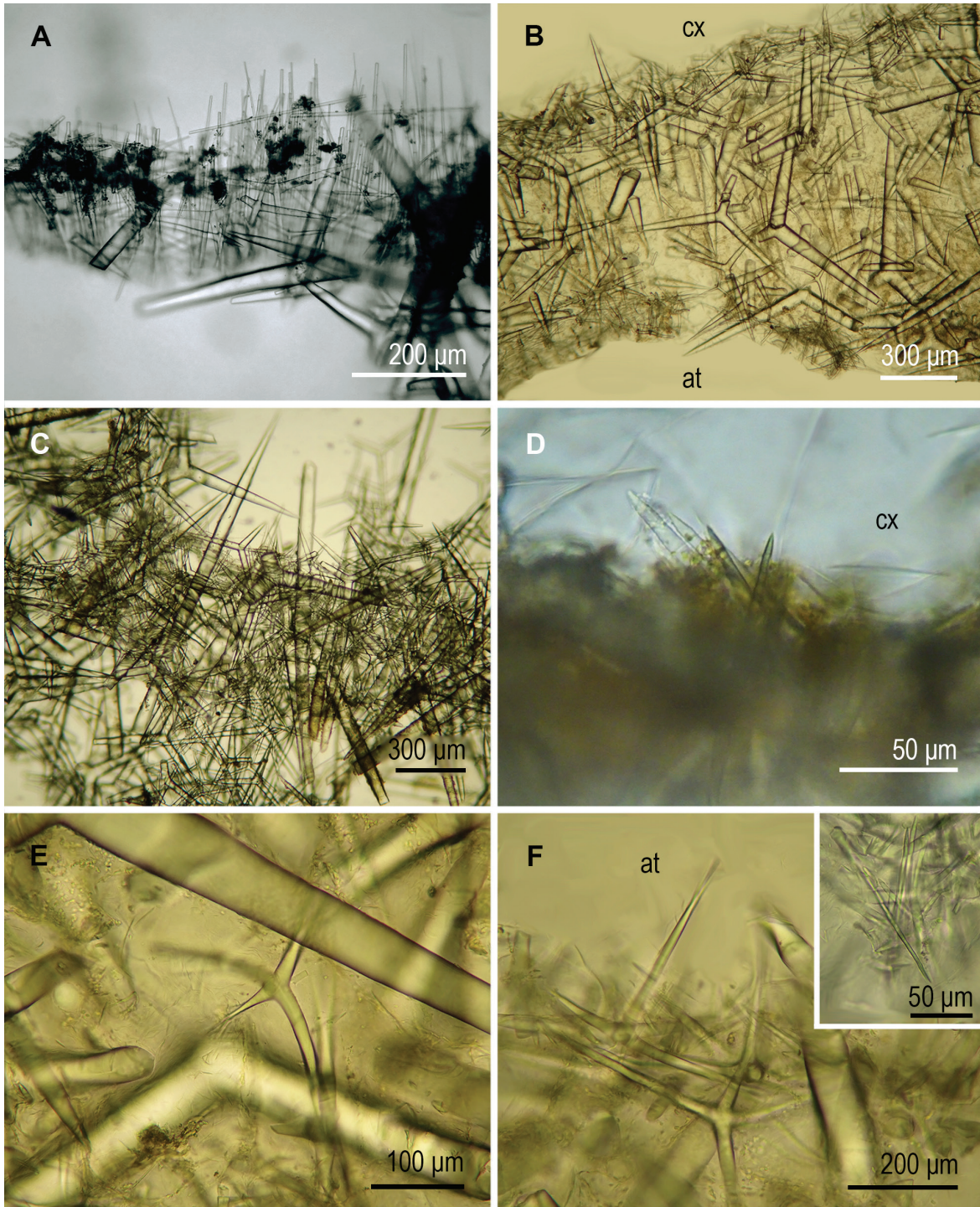
*Cortical diactines:* Huge and fusiform, with both tips sharp (Fig. 25A).

*Cortical microdiactines:* They are fusiform and slightly bent with sharp tips. The tip that lay outside



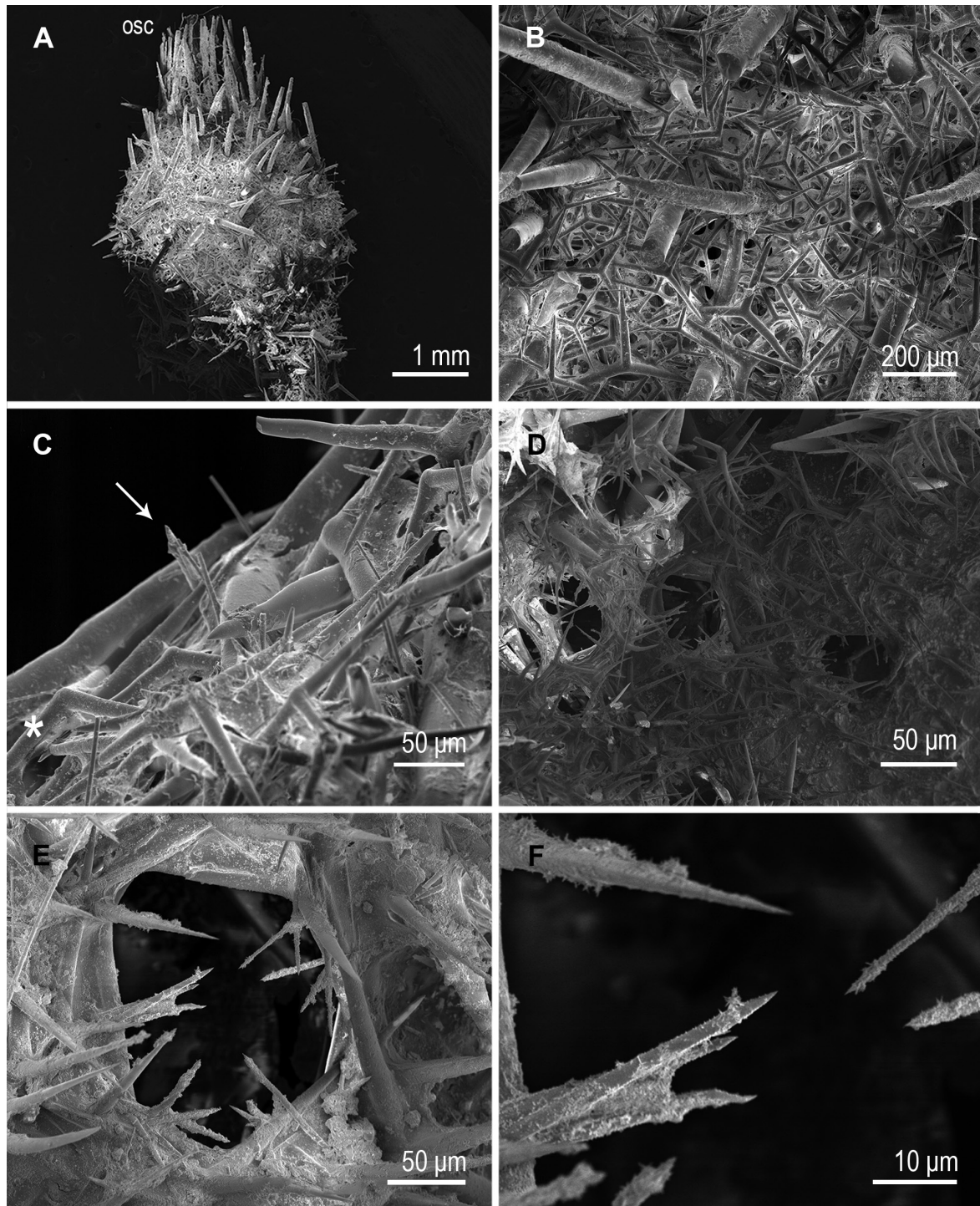
**Figure 22.** *Leucandra ornata* (holotype; UFRJPOR 8928). A, specimen *in vivo*. B, fixed specimen.



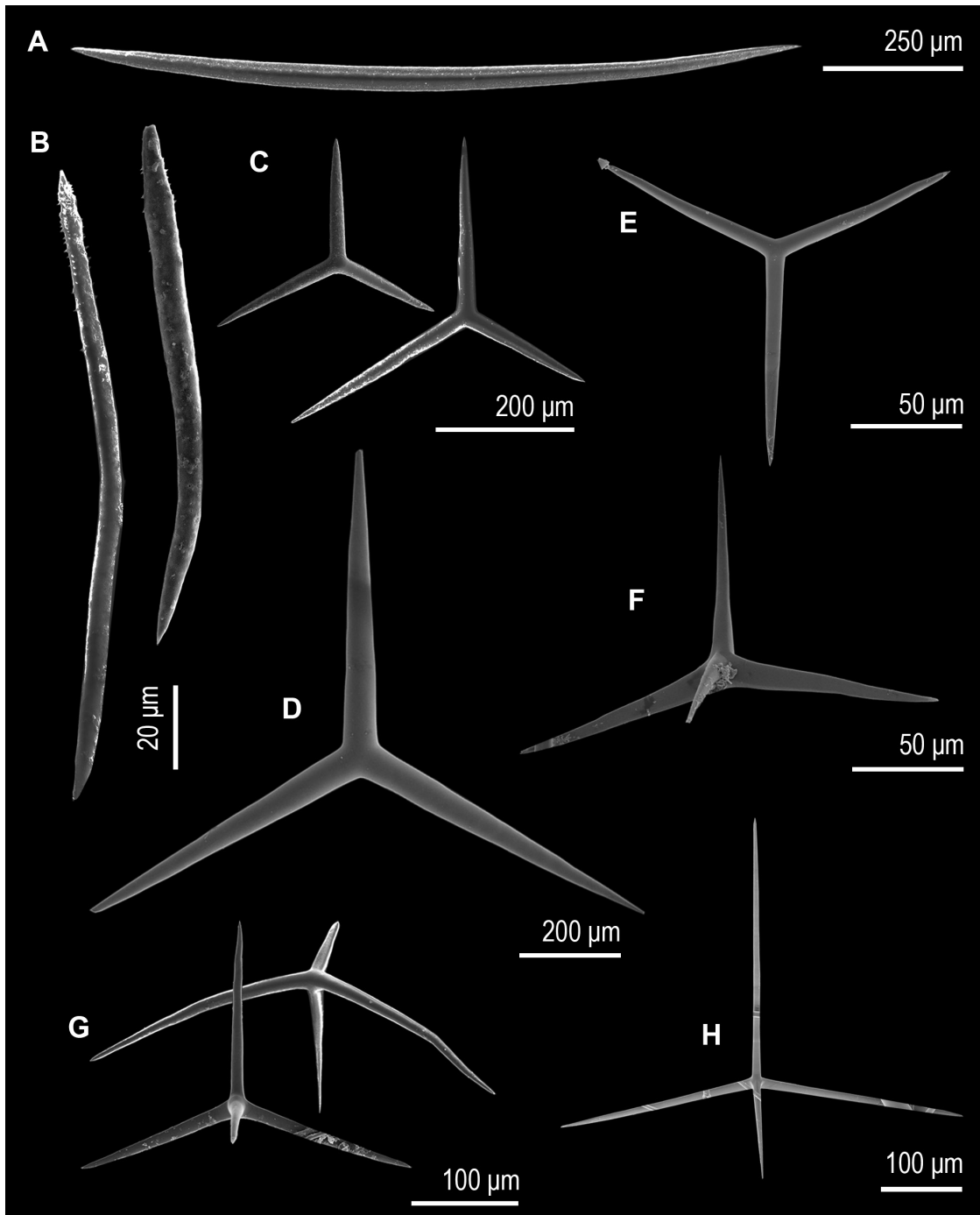


**Figure 23.** *Leucandra ornata* (holotype; UFRJPOR 8928). A, oscular margin. B, cross-section. C, tangential section of the cortex. D, detail of the cortex showing diactines II organised in bouquet. E, detail of the choanosome showing a tetractine of the canals. F, cross-section of the atrium (microdiactines in the inset). cx = cortex, at = atrium.





**Figure 24.** *Leucandra ornata* (holotype; UFRJPOR 8928). SEM: A, entire specimen. B, tangential section of the cortex. C, detail of the cortex showing a microdiactine (arrow) and a cortical triactine with elevated centre (asterisk). D, tangential section of the atrium. E, detail of an exhalant canal with microdiactines. F, detail of the microdiactines with spines. osc = osculum.



**Figure 25.** *Leucandra ornata* (holotype; UFRJPOR 8928). A, diactines. B, microdiactines. C, cortical triactines. D, choanosomal large triactine. E, canal triactine. F, tetractine of the canals. G, atrial tetractines. H, tetractine of the osculum.

**Table 11.** Spicule measurements of *Leucandra ornata* and the original measurements of *L. riojai*. H = holotype

Specimen	Spicule	Actine	Length (µm)			Width (µm)			N		
			Min	Mean	SD	Max	Min	Mean		SD	Max
UFRJPOR 8928 (H)	Cortical diactine		1162.3	1389.3	193.6	1621.8	54.0	62.1	5.4	67.5	05
	Cortical microdiactine		81.0	171.4	127.9	399.9	5.4	9.0	4.5	18.9	09
	Cortical triactine	Paired	118.9	264.0	99.7	486.5	16.2	25.4	8.9	43.2	20
		Unpaired	97.3	233.2	99.1	464.8	13.5	26.2	10.1	54.0	20
	Choanosomal triactine		540.5	671.2	58.6	783.8	37.8	61.9	8.7	75.6	30
	Canal triactine	Paired	162.2	194.6	28.6	216.2	16.2	18.0	1.6	18.9	03
	Canal tetractine	Unpaired	205.4	234.2	25.0	248.6	16.2	18.9	2.7	21.6	03
		Paired	162.2	227.6	23.4	259.4	16.2	20.5	2.2	24.3	20
	Atrial tetractine	Unpaired	173.0	231.3	26.3	270.3	16.2	20.5	2.2	24.3	20
		Apical	54.0	76.8	10.7	91.8	10.8	17.1	2.9	21.6	20
<i>L. riojai</i>	Atrial tetractine	Paired	129.7	230.3	41.4	324.3	13.5	16.6	2.8	21.6	20
		Unpaired	97.3	202.7	41.5	270.3	13.5	16.3	2.2	21.6	20
	Atrial microdiactine	Apical	108.0	143.1	20.6	197.1	13.5	15.8	1.6	18.9	20
			97.2	114.1	11.7	143.1	2.7	2.9	0.7	5.4	20
Cortical diactine		1600.0	-	-	2500.0	90.0	-	-	100.0	-	
Cortical microdiactine		100.0	-	-	240.0	-	-	-	-	-	
Cortical triactine		160.0	-	-	210.0	12.0	-	-	14.0	-	
Choanosomal triactine		180.0	-	-	240.0	20.0	-	-	30.0	-	
Atrial tetractine		-	-	-	-	-	-	-	-	-	
Atrial microdiactine		100.0	-	-	240.0	-	-	-	-	-	



the sponge has delicate spines. These spicules are distributed in bouquets or lay tangentially to the cortex (Fig. 25B).

*Cortical triactines*: Equiangular and equiradiate. Actines are conical and sharp (Fig. 25C).

*Choanosomal triactines*: Huge. Equiangular and equiradiate. Actines are conical and sharp (Fig. 25D).

*Triactines of the canals*: Small, rare. Sagittal. Actines are conical and sharp (Fig. 25E).

*Tetractines of the canals*: Small. Sagittal. Actines are conical and sharp (Fig. 25F). The apical actine is conical, sharp and smooth.

*Atrial tetractines*: Small. Sagittal. Actines are conical and sharp. The apical actine is conical, sharp, smooth and curved. It is thicker than the basal actines (Fig. 25G).

*Atrial microdiactines*: Thin, one tip is arrow-shaped and the other is sharp (Figs 23F, 24E). Small spines are present (Fig. 24F).

*Oscular tetractines*: Sagittal. Actines are cylindrical and sharp. The unpaired actine is longer than the paired ones. The apical actine is cylindrical, sharp, smooth and strongly curved. Consequently, it is similar to a chiacrine (Fig. 25H).

*Ecology*: This sponge was found in a submarine cave, among a sponge community mostly dominated by encrusting growth forms.

*Geographic distribution*: Mascarene Islands Ecoregion – La Réunion (this work).

*Taxonomic remarks*: Many species of *Leucandra* have microdiactines with spines in the atrial skeleton but, considering the whole skeleton composition of *L. ornata*, the most similar species is *L. riojai* Ferrer-Hernandez, 1918. This globulose species occurs in the Mediterranean Sea (Spain) and, although it has the same skeleton composition, we consider that some of the differences are enough to separate it from our new species. For example, Ferrer-Hernandez (1918) did not mention the presence of tetractines in the exhalant canals. Besides, our choanosomal triactines seem to be more regular than those of *L. riojai*. The size of the spicules is also different, being much larger in the new species, except for the large diactines, which are bigger in *L. riojai* (Table 11). Besides, in our species the cortical microdiactines are frequently organized in bouquets, while in *L. riojai* this kind of organization was not mentioned.

## GENUS *UTE* SCHMIDT, 1862

*Type species*: *Ute glabra* Schmidt, 1864.

*Diagnosis*: ‘Grantiidae with syconoid organization. The cortex is supported by giant longitudinal diactines, and the choanoskeleton is articulated, composed of several rows of triactines with occasional tetractines. There are no radial fascicles of diactines’ (Borojević *et al.*, 2002).

*UTE INSULAGEMMAE* VAN SOEST & DE VOOGD, 2018  
(FIGS 26–28; TABLE 12)

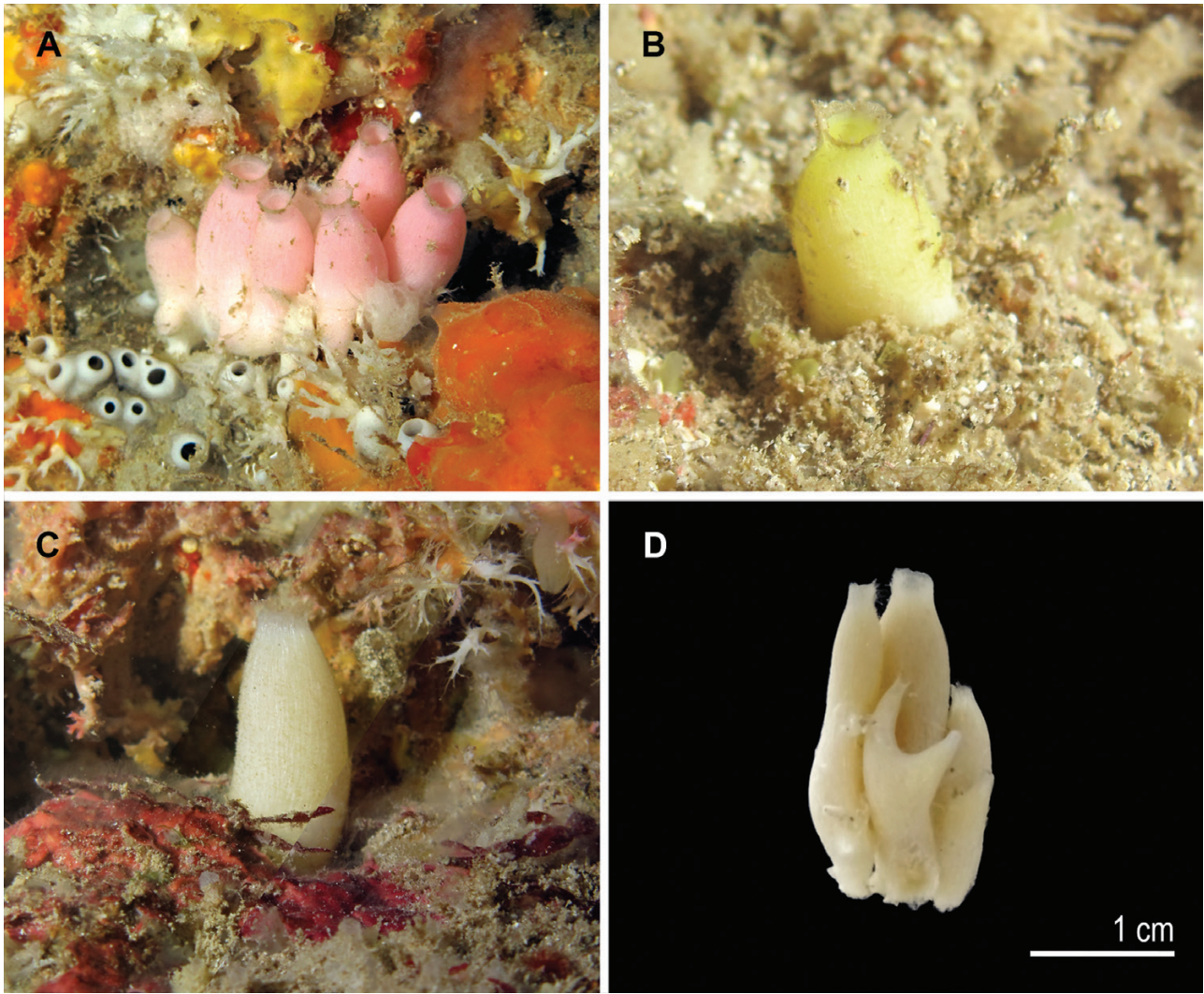
*Synonym*: *Ute insulagemmae*, Van Soest & De Voogd, 2018: 92.

*Material examined*: UFRJPOR 8925 (= 171109-PAE2-TP1, Photos: TP6017–6018, TP6051). UFRJPOR 8938 (= 171109-PAE2-TP14, Photo: TP6052). UFRJPOR 8943 (= 171109-PAE2-TP18, Photo: TP6050 or TP6057). UFRJPOR 8948 (= 171109-PAE2-TP23, Photo: TP6061). Passe de l’Hermitage Reef, La Réunion, Indian Ocean, coll. T. Pérez, 9 November 2017, 18–20 m depth. UFRJPOR 8929 (= 171110-ESB2-TP4, Photos: TP6129–6130). UFRJPOR 8942 (= 171110-ESB2-TP5, Photo: TP6131). Portail Caves, La Réunion, Indian Ocean, coll. T. Pérez, 9 November 2017, 25 m depth.

*Type locality*: Bijoutier Island, Amirantes, Seychelles, Indian Ocean.

*Colour*: This species can have several colours (Fig. 26). Some were mostly pink but white at the base (UFRJPOR 8925; Fig. 26A) or white with a pink sponge budding (UFRJPOR 8929) or even lemon-yellow (UFRJPOR 8938; Fig. 26B), white but pink near the osculum (UFRJPOR 8942) or completely white (UFRJPOR 8943, 8948; Fig. 26C). They all become beige in ethanol (Fig. 26D).

*Morphology and anatomy*: Sponge with a shape of vase with one single apical osculum surrounded by a crown of trichoxeas and with a delicate sphincter (Fig. 26A–D). The crown is supported by T-shape triactines (Fig. 27A). Frequently there are sponges budding from another and they keep connected to each other (Fig. 26D). Surface smooth, covered by huge longitudinal diactines (Fig. 27B–D). These diactines protrude through the surface near the osculum (Fig. 27A). The surface is perforated by ostia surrounded by tiny curved triactines, giving a honeycomb appearance to it (Fig. 27C, D). The aquiferous system is syconoid (Fig. 27B). The cortical



**Figure 26.** *Ute insulagemmae*. A, pink chromotype (UFRJPOR 8925). B, yellow chromotype (UFRJPOR 8938). C, white chromotype (UFRJPOR 8948). D, fixed specimen.

skeleton is composed of the giant longitudinal diactines and by the tiny triactines of the ostia (Fig. 27C). Below, the articulated tubar skeleton is composed of triactines pointing their unpaired actine to the surface (Fig. 27D, E). The subatrial skeleton has triactines and few tetractines and the atrial skeleton has only tetractines (Fig. 27E). The atrium is hispid (Fig. 27E, F).

#### *Spicules* (Table 12)

*Diactine*: Giant, fusiform with both tips sharp (Fig. 28A).

*Cortical triactine*: Tiny and sagittal. These spicules surround the ostia, consequently they have curved actines. Actines are conical and sharp and the unpaired actine is shorter than the paired ones (Fig. 28B).

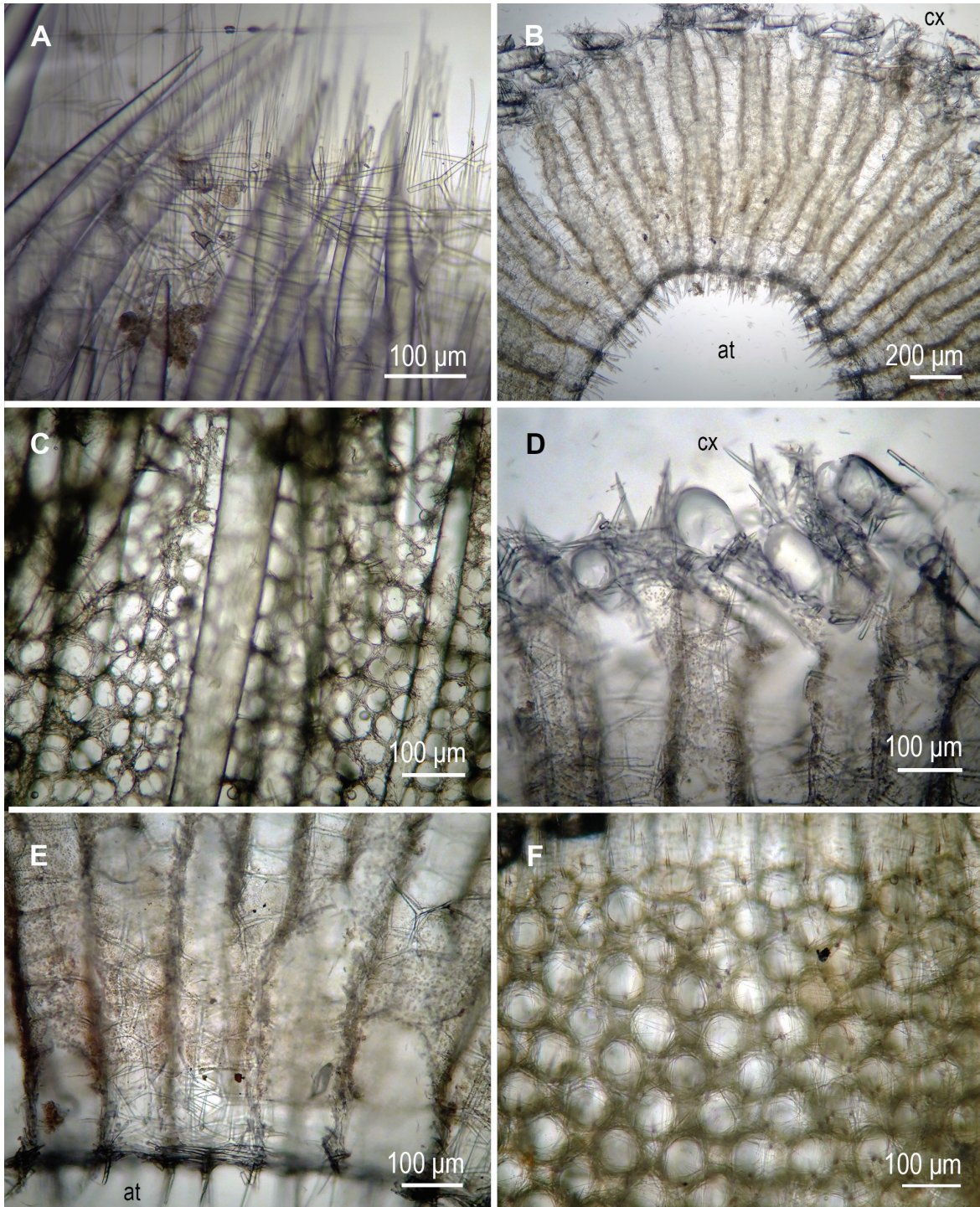
*Tubar triactines*: Sagittal. The unpaired actine is longer than the paired ones and each paired actine has a different length. They are conical and sharp (Fig. 28C).

*Subatrial triactine and tetractine*: Sagittal. The unpaired actine is longer than the paired ones. They are conical and sharp. The apical actine of the tetractines is short, conical, sharp and curved, pointing into the atrium (Fig. 28D, E).

*Atrial tetractine*: Sagittal. The basal actines are shorter and thinner than the apical one. They are conical and sharp. The apical actine is long, straight, conical and sharp (Fig. 28F).

*Ecology*: Observed in caves, some specimens being associated with ophiuroids. It was found in the Seychelles from 6 to 18 m deep.



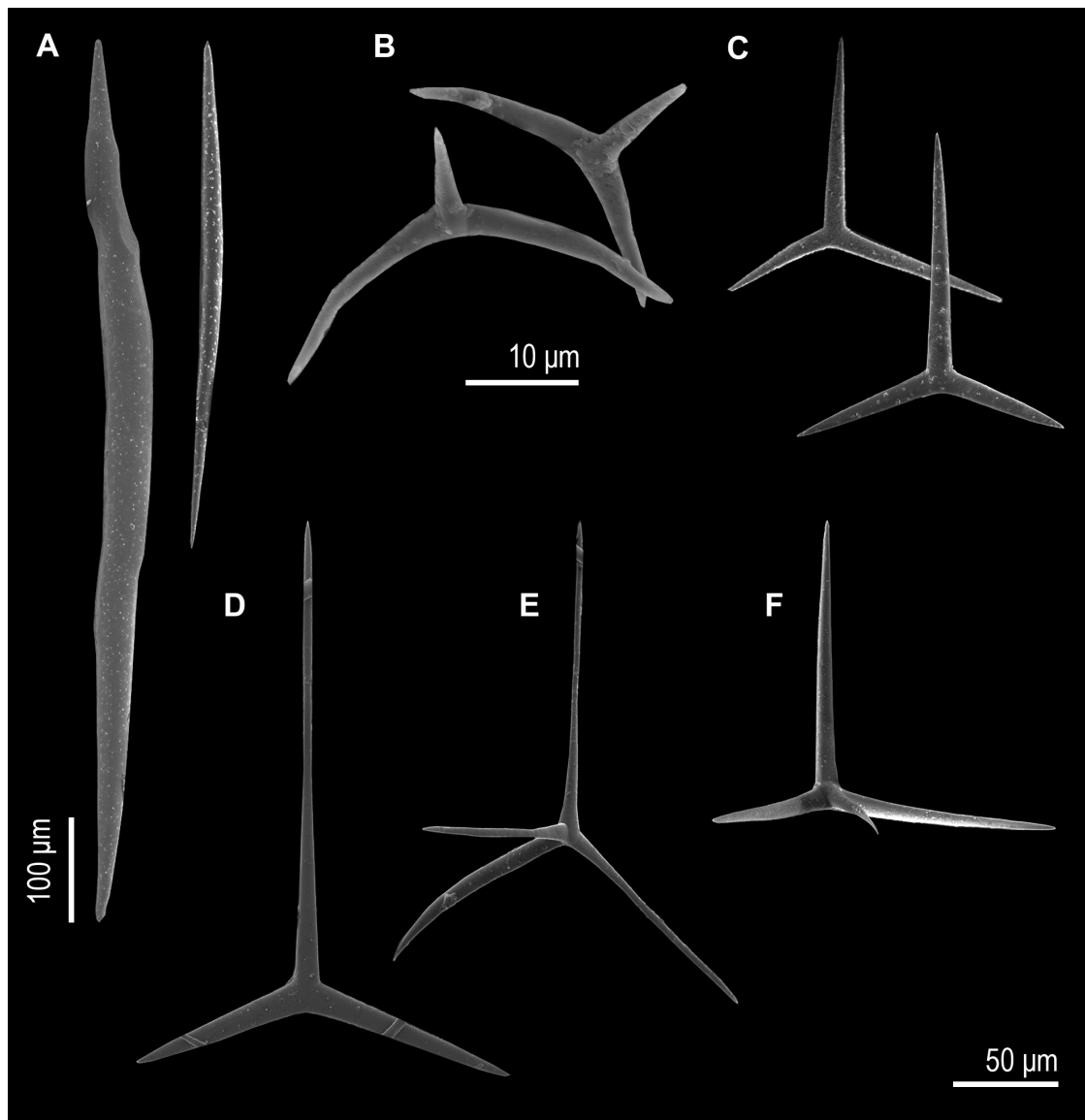


**Figure 27.** *Ute insulagemmae* (UFRJPOR 8928). A, oscular margin. B, cross-section. C, tangential section of the cortex. D, detail of the cortex. E, detail of the tubar, subatrial and atrial skeletons. F, tangential section of the atrial surface. cx = cortex.

*Geographic distribution:* Seychelles Ecoregion – Seychelles (Van Soest & De Voogd 2018); Mascarene Islands Ecoregion – La Réunion (this study).

*Taxonomic remarks:* *Ute insulagemmae* was described by Van Soest & De Voogd (2018) based on a ‘pale orange’ specimen collected in the Seychelles.





**Figure 28.** *Ute insulagemmae* (UFRJPOR 8928). A, diactines. B, cortical triactines. C, tubar triactines. D, subatrial triactine. E, subatrial tetractine. F, atrial tetractine.

Morphologically, except for the colour, our specimens match the description of the specimen from the Seychelles, including mostly the size of the spicules (Table 12). Considering all the analysed specimens, the diactines showed the largest size variation among the specimens, indicating that in this species spicule size can be variable. Colour is also variable, as shown by our molecular results.

## DISCUSSION

With this inventory, the number of known calcareous sponge species from the Mascarenes ecoregion increases

from 16 to 27, while that of La Réunion went from three to 14 (Table 13). Considering the two subclasses, Calcinea and Calcaronea, calcinean sponges continue to be the most diverse, with 17 species out of 27. Curiously, we did not find any of the species previously found in La Réunion (*T. stylifera*, *P. hindei* and *L. microraphis*) (Bourmaud, 2003) and of the 11 species found, seven are new to science, one of them belonging to a new genus (*Janusya*). The high number of species is remarkable, considering that our collections resulted from only seven short dives, most of them with only one sponge specialist, and in a limited area of La Réunion.

The knowledge about the occurrence of Calcarea in La Réunion seems to be fragmented. Among the

**Table 12.** Spicule measurements of *Ute insulagemmae* (UFRJPOR 8925). H = holotype

Specimen	Spicule	Actine	Length (µm)			Width (µm)			N		
			Min	Mean	SD	Min	Mean	SD			
			Max	Mean	SD	Max	Mean	SD			
UFRJPOR 8925 (pink and white at the base)	Diacpine		1540.5	2354.1	686.5	3783.8	54.1	83.8	14.9	108.1	20
	Cortical triactine	Paired	17.5	24.6	4.5	35.0	2.5	2.5	0	2.5	20
		Unpaired	7.5	15.4	5.4	25.0	2.5	2.5	0	2.5	20
	Tubar triactine	Paired	62.5	75.1	7.0	90.0	5.0	8.0	1.3	10.0	20
		Unpaired	85.0	97.3	7.9	115.0	7.5	8.4	1.2	10.0	20
	Subatrial triactine	Paired	62.5	95.5	17.4	142.5	5.0	8.6	2.9	15.0	20
		Unpaired	115.0	163.4	37.6	230.0	5.0	7.3	2.4	12.5	20
	Subatrial tetractine	Paired	80.0	113.1	16.4	137.5	7.5	7.6	0.6	10.0	20
		Unpaired	125.0	182.0	38.0	240.0	5.0	5.3	0.8	7.5	20
	Atrial tetractine	Apical	30.0	39.9	9.7	67.5	5.0	5.5	1.0	7.5	20
	Paired	55.0	91.0	20.1	100.0	5.0	9.0	2.9	12.5	05	
	Unpaired	37.5	63.0	23.3	100.0	5.0	9.0	2.2	10.0	05	
	Apical	87.5	106.9	12.1	130.0	5.0	7.8	1.4	10.0	20	
UFRJPOR 8938 (lemon yellow)	Diacpine		345.0	1428.3	1145.2	4500.0	15.0	66.5	41.6	125.0	20
	Cortical triactine	Paired	15.0	30.0	7.9	45.0	2.5	3.0	1.03	5.0	20
		Unpaired	10.0	15.6	4.2	25.0	2.5	2.5	0.0	2.5	20
	Tubar triactine	Paired	67.5	88.0	14.3	112.5	7.5	10.1	1.5	12.5	20
		Unpaired	55.0	84.9	14.0	102.5	7.5	9.9	1.7	12.5	20
	Subatrial triactine	Paired	55.0	82.3	17.8	117.5	5.0	8.8	1.9	12.5	20
		Unpaired	112.5	153.9	38.6	250.0	5.0	8.4	2.0	12.5	20
	Subatrial tetractine	Paired	62.5	87.5	16.5	107.5	7.5	8.3	1.7	12.5	10
		Unpaired	92.5	160.0	34.7	212.5	7.5	8.3	1.2	10.0	10
	Atrial tetractine	Apical	25.0	34.5	7.5	50.0	7.5	8.0	1.1	10.0	10
	Paired	47.5	86.2	16.7	107.5	5.0	8.2	1.5	10.0	15	
	Unpaired	50.0	83.5	14.7	100.0	7.5	8.3	1.2	10.0	15	
	Apical	17.5	59.0	29.0	100.0	5.0	7.5	1.5	10.0	12	

Table 12. Continued

Specimen	Spicule	Actine	Length (µm)			Width (µm)			N		
			Min	Mean	SD	Max	Min	Mean		SD	
			Max	SD	Max	SD	Max				
UFRJPOR 8943 (white)	Diacetine		200.0	1360.0	727.3	2500.0	15.0	66.5	33.1	125.0	20
	Cortical triactine	Paired	20.0	32.1	8.2	47.5	2.5	3.3	1.2	5.0	20
		Unpaired	7.5	19.9	8.9	35.0	2.5	2.6	0.6	5.0	20
	Tubar triactine	Paired	57.5	78.0	14.0	107.5	7.5	10.8	2.8	17.5	20
		Unpaired	57.5	89.8	17.7	125.0	7.5	10.6	2.0	15.0	20
	Subatrial triactine	Paired	50.0	84.6	17.7	112.5	5.0	10.1	3.8	15.0	20
		Unpaired	102.5	163.3	48.4	275.0	7.5	9.9	3.0	15.0	20
	Subatrial tetractine	Paired	85.0	95.8	16.6	115.0	7.5	9.2	1.4	10.0	03
		Unpaired	130.0	145.8	17.7	165.0	7.5	8.3	1.4	10.0	03
		Apical	25.0	49.2	25.0	75.0	7.5	9.2	1.4	10.0	03
ZMA Por 11562 (H)	Atrial tetractine	Paired	75.0	93.8	26.5	112.5	7.5	7.5	0.0	7.5	02
		Unpaired	62.5	68.8	8.8	75.0	7.5	7.5	0.0	7.5	02
		Apical	20.0	82.7	37.6	140.0	7.5	7.7	0.7	10.0	13
	Diacetine		256.0	1509.0	-	2520.0	13.0	68.1	-	116.0	-
	Cortical triactine	Paired	21.0	35.0	-	51.0	2.5	3.6	-	4.0	-
		Unpaired	5.0	9.4	-	18.0	2.0	4.1	-	6.0	-
	Tubar triactine	Paired	69.0	85.1	-	117.0	7.0	8.8	-	13.0	-
		Unpaired	52.0	79.5	-	112.0	8.0	10.2	-	13.0	-
	Subatrial triactine	Paired	78.0	97.0	-	112.0	9.0	12.2	-	14.0	-
		Unpaired	91.0	168.0	-	203.0	9.0	12.4	-	15.0	-
Subatrial tetractine	Paired	76.0	109.0	-	126.0	10.0	11.3	-	12.0	-	
	Unpaired	171.0	202.0	-	241.0	8.0	10.2	-	11.0	-	
	Apical	49.0	60.4	-	71.0	6.0	7.1	-	8.0	-	
Atrial tetractine	Paired	68.0	104.0	-	126.0	6.0	8.3	-	10.0	-	
	Unpaired	61.0	107.6	-	165.0	7.0	8.6	-	11.0	-	
	Apical	40.0	115.0	-	201.0	6.0	8.4	-	11.0	-	



**Table 13.** Species with reported occurrences in the Mascarenes Island ecoregion

Species	Locality	Reference
subclass Calcinea		
<i>Arturia compacta</i> (Schuffner, 1877)	Mauritius	Schuffner, 1877; Van Soest & De Voogd, 2018
<i>Ascandra mascarenica</i> sp. nov.	La Réunion	This study
<i>Ascandra oceanusvitae</i> sp. nov.	La Réunion	This study
<i>Borojevia pirella</i> Van Soest & De Voogd, 2018	Rodrigues	Van Soest & De Voogd, 2018
<i>Clathrina luteoculcitella</i> Wörheide & Hooper, 1999	Rodrigues	Van Soest & De Voogd, 2018
<i>Clathrina rodriguesensis</i> Van Soest & De Voogd, 2018	Rodrigues	Van Soest & De Voogd, 2018
<i>Ernstia naturalis</i> Van Soest & De Voogd, 2015	Rodrigues	Van Soest & De Voogd, 2018
<i>Janusya indica</i> gen. nov. sp. nov.	La Réunion	This study
<i>Lelapiella tertia</i> sp. nov.	La Réunion	This study
<i>Leucaltis mauritiana</i> Schuffner, 1877	Mauritius	Schuffner, 1877 – it is considered a <i>taxon inquirendum</i>
<i>Leucaltis nodusgordii</i> (Poléjaeff, 1883)	La Réunion	This study
<i>Leucascus tenuispinae</i> sp. nov.	La Réunion	This study
<i>Leucetta chagosensis</i> Dendy, 1913	La Réunion	This study
<i>Leucetta microraphis</i> Haeckel, 1872	La Réunion	Bourmaud, 2003
<i>Leucetta sulcata</i> Van Soest & De Voogd, 2018	Rodrigues	Van Soest & De Voogd, 2018
<i>Pericharax orientalis</i> Van Soest & De Voogd, 2015	Mauritius	Van Soest & De Voogd, 2018
<i>Soleneiscus intermedius</i> sp. nov.	La Réunion	This study
<b>Subclass Calcaronea</b>		
<i>Aphroceras seychellensis</i> (Hôzawa, 1940) comb. nov.	La Réunion	This study
<i>Leucandra claviformis</i> Schuffner, 1877	Mauritius	Schuffner, 1877; Van Soest & De Voogd, 2018
<i>Leucandra echinata</i> Schuffner, 1877	Mauritius	Schuffner, 1877; Van Soest & De Voogd, 2018
<i>Leucandra falcigera</i> Schuffner, 1877	Mauritius	Schuffner, 1877; Van Soest & De Voogd, 2018
<i>Leucandra ornata</i> sp. nov.	La Réunion	This study
<i>Plectroninia hindei</i> Kirk, 1900	La Réunion	Vacelet, 1977; Van Soest & De Voogd, 2018
<i>Sycettusa sycilloides</i> (Schuffner, 1877)	Mauritius	Schuffner, 1877; Van Soest & De Voogd, 2018
<i>Sycon oscari</i> Van Soest & De Voogd, 2018 (as <i>Sycandra tabulata</i> Schuffner, 1877)	Mauritius	Schuffner, 1877; Van Soest & De Voogd, 2018
<i>Tularinia stylifera</i> Vacelet, 1977	La Réunion	Vacelet, 1977
<i>Ute insulagemmae</i> Van Soest & De Voogd, 2018	La Réunion	This study

seven investigated localities, calcareous sponges were found in only three of them: Cap La Houssaye Reef, with one species (*Leucandra ornata* sp. nov.); Passe de l'Hermitage Reef, with eight species (*Aphroceras seychellensis*, *Ascandra mascarenica* sp. nov., *A. oceanusvitae* sp. nov., *Janusya indica* sp. nov., *Leucaltis nodusgordii*, *Leucetta chagosensis*, *Lelapiella tertia* sp. nov. and *Ute insulagemmae*); and Portail Caves, with three species (*Leucascus tenuispinae* sp. nov., *Soleneiscus intermedius* sp. nov. and *Ute insulagemmae*). One single site (Passe de l'Hermitage) presents most of the collected samples, confirming

that calcareous sponges require specific conditions to thrive. Further studies in other sites of the island could help understand the environmental requirements of calcareous sponges that may contribute to such distribution.

The species found in this work represent important findings for the systematics of Calcarea. Some of the new additions highlight inconsistencies in the traditional taxonomy, such as the paraphyly of orders and families in both Calcinea and Calcaronea (e.g. Voigt *et al.*, 2012; Klautau *et al.*, 2013; Lopes *et al.*, 2018). Considering the calcinean groups,

with the inclusion of *Lelapiella tertia* sp. nov. in the analysis, we once more recovered the paraphyly of Murrayonida (*Murrayona* and *Lelapiella*) and its placement among representatives of Clathrinida. For that reason, we opted to consider Murrayonida a junior synonym of Clathrinida, leaving Calcinea with a single monophyletic order. We were able to separate the problematic *Arturia* l.s. in two groups: the ‘true’ *Arturia* clade and the new genus *Janusya*, both with morphological and molecular support. However, some issues with other non monophyletic taxonomic groups, e.g. Leucaltidae (here represented by *Leucaltis* and *Ascandra*) and Clathrinidae, could still not be solved.

The two DNA markers currently available (C-LSU and ITS) for phylogenetic studies on Calcarea proved helpful for the integrative taxonomy. However, in some cases C-LSU provided inconclusive results because of the low evolutionary rate, being unable to separate close related species. For the subclass Calcaronea, the scenario is even harder, because only the C-LSU can be used, and it results in phylogenies that strongly conflict with the current morphological taxonomy. New markers are extremely important to provide a robust phylogenetic backbone and thus allow reorganization of taxa whose current markers render taxonomic decisions inconclusive.

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#### REFERENCES

**Alencar AR. 2012.** *Revisão taxonômica e filogenia da família Leucettidae de Laubenfels, 1936 (Porifera, Calcarea,*

*Calcinea)*. Unpublished D. Phil. Thesis, Universidade Federal do Rio de Janeiro.

- Alvizu A, Eilertsen MH, Xavier JR, Rapp HT. 2018.** Increased taxon sampling provides new insights into the phylogeny and evolution of the subclass Calcaronea (Porifera, Calcarea). *Organisms Diversity & Evolution* **18**: 279–290.
- Azevedo F, Córdor-Luján B, Willenz P, Hajdu E, Hooker Y, Klautau M. 2015.** Integrative taxonomy of calcareous sponges (subclass Calcinea) from the Peruvian coast: morphology, molecules, and biogeography. *Zoological Journal of the Linnean Society* **173**: 787–817.
- Azevedo F, Padua A, Moraes F, Rossi A, Muricy G, Klautau M. 2017.** Taxonomy and phylogeny of calcareous sponges (Porifera: Calcarea: Calcinea) from Brazilian mid-shelf and oceanic islands. *Zootaxa* **4311**: 301–344.
- Baine M, Harasti D. 2007.** *The marine life of Bootless Bay, Papua New Guinea*. Port Moresby: Motupore Island Research Centre.
- Bidder GP. 1898.** The skeleton and the classification of calcareous sponges. *Proceedings of the Royal Society of London* **64**: 61–76.
- Borojević R. 1966.** Éponges calcaires des côtes de France. II. Le genre *Ascandra* Haeckel emend. *Archives de Zoologie Expérimentale et Générale* **107**: 357–368.
- Borojević R. 1967.** Éponges calcaires recueillies en Nouvelle-Calédonie par la Mission Singer-Polignac. *Éditions de la Fondation Singer-Polignac, Paris* **2**: 1–12.
- Borojević R, Klautau M. 2000.** Calcareous sponges from New Caledonia. *Zoosystema* **22**: 187–201.
- Borojević R, Boury-Esnault N. 1986.** Une nouvelle voie d'évolution chez les éponges Calcinea: description des genres *Burtonella* n.g. et *Levinella* n.g. *Bulletin du Muséum National d'Histoire Naturelle, Paris* **8**: 443–455.
- Borojević R, Boury-Esnault N. 1987.** Calcareous sponges collected by N.O. Thalassa on the continental margin of the Bay of Biscaye: I. – Calcinea. In: Vacelet J, Boury-Esnault N, eds. *Taxonomy of Porifera from the NE Atlantic and Mediterranean Sea. NATO ASI Series, G13*. Berlin, Heidelberg: Springer-Verlag, 1–27.
- Borojević R, Boury-Esnault N, Vacelet J. 1990.** A revision of the supraspecific classification of the subclass Calcinea (Porifera, Class Calcarea). *Bulletin du Muséum National d'Histoire Naturelle (Paris)* **2**: 243–246.
- Borojević R, Boury-Esnault N, Vacelet J. 2000.** A revision of the supraspecific classification of the subclass Calcaronea (Porifera, class Calcarea). *Zoosystema* **22**: 203–263.
- Borojević R, Boury-Esnault N, Manuel M, Vacelet J. 2002.** Order Clathrinida Hartman, 1958. In: Hooper JNA, Van Soest RWM, eds. *Systema Porifera: a guide to the classification of sponges*. New York: Kluwer Academic/Plenum Publishers, 1141–1152.
- Bourmaud C. 2003.** *Inventaire de la biodiversité marine récifale à la Réunion*. Rapport de l'Association Parc Marin et du Laboratoire d'Ecologie Marine, ECOMAR, de la Réunion.
- Bowerbank JS. 1862.** On the anatomy and physiology of the Spongiadae. Part II. *Philosophical Transactions of the Royal Society of London*. London: The Royal Society, **152**: 747–836, pls XXVII–XXXV.

- Bowerbank JS. 1866.** *A monograph of the British Spongiadae, II.* London: Ray Society.
- Brøndsted HV. 1931.** Die Kalkschwämme. *Deutschen Südpolar Expedition 1901–3* **20**: 1–47.
- Burton M. 1963.** *A revision of the classification of the calcareous sponges.* London: British Museum (Natural History).
- Carter HJ. 1878.** On Teichonia, a new Family of calcareous sponges with description of two species. *Annals and Magazine of Natural History* **5**: 35–40.
- Carter HJ. 1886.** Descriptions of the sponges from the neighbourhood of Port Phillip Heads, South Australia, continued. *Annals and Magazine of Natural History* **15–18**: 431–441.
- Cavalcanti FF, Rapp HT, Klautau M. 2013.** Taxonomic revision of *Leucascus* Dendy, 1892 (Porifera: Calcarea) with revalidation of *Ascoleucetta* Dendy & Frederick, 1924 and description of three new species. *Zootaxa* **3619**: 275–314.
- Colin PL, Arneson C. 1995.** *Tropical Pacific invertebrates. A field guide to the marine invertebrates occurring on tropical pacific coral reefs, seagrass beds and mangroves.* Beverly Hills: Coral Reef Research Foundation.
- Cóndor-Luján B, Louzada TS, Hajdu E, Klautau M. 2018.** Morphological and molecular taxonomy of calcareous sponges (Porifera: Calcarea) from Curaçao, Caribbean Sea. *Zoological Journal of the Linnean Society* **182**: 1–67.
- Dendy A. 1891.** A monograph of the Victorian sponges. I. – The organisation and classification of the Calcarea Homocoela, with description of the Victorian species. *Transactions of the Royal Society of Victoria* **3**: 1–81.
- Dendy A. 1892.** Synopsis of the Australian Calcarea Heterocoela, with a proposed classification of the group and descriptions of some new genera and species. *Proceedings of the Royal Society of Victoria* **5**: 69–116.
- Dendy A. 1905.** Report on the sponges collected by Professor Herdman at Ceylon in 1902. *Reports on the Pearl Oyster Fisheries of the Gulf of Manaar* **3**: 59–246.
- Dendy A. 1913.** Report on the calcareous sponges collected by H. M. S. ‘Sealark’ in the Indian Ocean. *Transactions of the Linnean Society of London, Zoology* **16**: 1–29.
- Dendy A, Frederick LM. 1924.** On a collection of sponges from the Abrolhos Islands, Western Australia. *Journal of the Linnean Society of London, Zoology* **16**: 1–29.
- Dendy A, Row H. 1913.** The classification and phylogeny of the calcareous sponges, with a reference list of all the described species, systematically arranged. *Proceedings of the Zoological Society of London* **1913**: 704–813.
- Dohrmann M, Voigt O, Erpenbeck D, Wörheide G. 2006.** Nonmonophyly of most supraspecific taxa of calcareous sponges (Porifera, Calcarea) revealed by increased taxon sampling and partitioned Bayesian analysis of ribosomal DNA. *Molecular Phylogenetics and Evolution* **40**: 830–843.
- Erhardt H, Baensch HA. 1998.** *Meerwasser atlas 4. Wirbellose.* Melle: Mergus Verlag, 1214.
- Felsenstein J. 1985.** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.
- Ferrer-Hernandez F. 1918.** Esponjas del litoral de Asturias. *Trabajos del Museo Nacional de Ciencias Naturales* **36**: 1–39.
- Fontana T, Córdor-Luján B, Azevedo F, Pérez T, Klautau M. 2018.** Diversity and distribution of Calcareous sponges (subclass Calcinea) from Martinique. *Zootaxa* **4410**: 331–369.
- George AM, Van Soest RWM, Sluka RD, Lazarus S. 2020.** A checklist of marine sponges (Porifera) of peninsula India. *Zootaxa* **4885**: 277–300.
- Gosliner TM, Behrens DW, Williams GC. 1996.** *Coral reef animals of the Indo-Pacific: animal life from Africa to Hawaii exclusive of the vertebrates.* Monterey: Sea Challengers.
- Grant RE. 1836.** Animal kingdom. In: Todd RB, ed. *The encyclopedia of anatomy and physiology, Vol. 1.* London: Sherwood, Gilbert and Piper, 107–118.
- Gray JE. 1858.** Description of *Aphroceras*, a new genus of calcareous Spongiadae brought from Hong-Kong by Dr. Harland. *Proceedings of the Zoological Society of London* **26**: 113–114.
- Haeckel E. 1870.** Prodrum eines Systems der Kalkschwämme. *Jenaische Zeitschrift* **5**: 176–191.
- Haeckel E. 1872.** *Die Kalkschwämme. Eine monographie, Vols 1–3.* Berlin: Reimer.
- Hartman W. 1958.** A re-examination of Bidder’s classification of the Calcarea. *Systematic Zoology* **7**: 97–110.
- Hess PN, Russo CAM. 2007.** An empirical test of the midpoint rooting method. *Biological Journal of the Linnean Society* **92**: 669–674.
- Hillis D, Morritz C, Mable B. 1996.** *Molecular systematics, 2nd edn.* Sunderland: Sinauer Associates.
- Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. 2017.** UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* **35**: 518–522.
- Hôzawa S. 1940.** Reports on the calcareous sponges obtained by the zoological institute and museum of Hamburg. *Science Reports of the Tohoku Imperial University* **15**: 131–163.
- Huelsbeck JP, Ronquist F. 2001.** MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.
- Jenkin CF. 1908.** The Calcarea of the National Antarctic Expedition. *Natural History Reports* **4**: 182–311.
- Katoh K, Standley DM. 2013.** MAFFT multiple sequence alignment software v.7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.
- Katoh K, Toh H. 2008.** Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. *BioMedCentral Bioinformatics* **9**: 212.
- Kirkpatrick R. 1900.** XLIII. Description of sponges from Funafuti. *Journal of Natural History* **6**: 345–362.
- Kirkpatrick R. 1910.** On a remarkable pharetronid sponge from Christmas Island. *Proceedings of the Royal Society Series B* **83**: 124–133.
- Klautau M, Borojević R. 2001.** Calcareous sponges from Arraial do Cabo–Brazil (I: the genus *Clathrina*). *Zoosystema* **23**: 395–410.
- Klautau M, Valentine C. 2003.** Revision of the genus *Clathrina* (Porifera, Calcarea). *Zoological Journal of the Linnean Society* **139**: 1–62.



- Klautau M, Azevedo F, Córdor-Luján B, Rapp H, Collins A, Russo C. 2013. A molecular phylogeny for the order Clathrinida rekindles and refines Haeckel's taxonomic proposal for calcareous sponges. *Integrative and Comparative Biology* **53**: 447–461.
- Klautau M, Imešek M, Azevedo F, Pleše B, Nikolić V, Cetković H. 2016. Adriatic calcarean sponges (Porifera, Calcarea), with the description of six new species and a richness analysis. *European Journal of Taxonomy* **178**: 1–52.
- Klautau M, Lopes MV, Guarabyra B, Folcher E, Ekims M, Debitus C. 2020. Calcareous sponges from the French Polynesia (Porifera: Calcarea). *Zootaxa* **4748**: 261–295.
- Lanna E, Rossi AL, Cavalcanti FF, Hajdu E, Klautau M. 2007. Calcareous sponges from São Paulo State, Brazil (Porifera: Calcarea: Calcinea) with the description of two new species. *Journal of the Marine Biological Association of the United Kingdom* **87**: 1553–1561.
- de Laubenfels MW. 1936. A discussion of the sponge fauna of the Dry Tortugas in particular and the West Indies in general, with material for a revision of the families and orders of the Porifera. *Tortugas Laboratory Occasional Papers* **467**: 1–225.
- von Lendenfeld R. 1885. A monograph of the Australian sponges. III. The Calcispongiae. *Proceedings of the Linnean Society of New South Wales* **9**: 1083–1150.
- Lévi C. 1986. *Laocaetis perion* nov. sp., spongiaire hexactinellide Craticulariidae de l'océan Indien. *Bulletin du Muséum National d'Histoire Naturelle* **8**: 437–442.
- Lévi C. 1998. *Sponges of the New Caledonian lagoon*. Paris: Éditions ORSTOM.
- Lopes MV, Córdor-Luján B, Azevedo F, Pérez T, Klautau M. 2018. A new genus of calcareous sponge discovered in the Caribbean Sea: *Bidderia* gen. nov. (Porifera, Calcarea, Calcinea). *Zootaxa* **4526**: 56–70.
- Manuel M, Borojević R, Boury-Esnault N, Vacelet J. 2002. Class Calcarea Bowerbank, 1864. In: Hooper JNA, Van Soest RWM, Willenz P, eds. *Systema Porifera*. Boston: Springer.
- Miklucho-Maclay N. 1868. Beiträge zur kenntniss der spongien. I. Über *Guancha blanca*, einen neuen Kalkschwämm. *Jenaische Zeitschrift* **4**: 221–240.
- Miller MA, Pfeiffer W, Schwartz T. 2012. The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. *Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the extreme to the campus and beyond*. Chicago: ACM Press.
- Minchin EA. 1900. The Porifera. In: Ray Lankester E, ed. *A treatise on zoology, Vol. 2*. London: Adam & Charles Black.
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* **37**: 1530–1534.
- Pasnin O, Voigt O, Wörheide G, Murillo Rincón AP, Von der Heyden S. 2020. Indo-Pacific phylogeography of the lemon sponge *Leucetta chagosensis*. *Diversity* **12**: 466.
- Pérez T, Díaz MC, Ruiz C, Córdor-Luján B, Klautau M, Hajdu E, Lôbo-Hajdu G, Zea S, Pomponi SA, Thacker RW, Carteron S, Tollu G, Pouget-Culevier A, Thélamon P, Marechal JP, Thomas OP, Ereskovsky AV, Vacelet J, Boury-Esnault N. 2017. How a collaborative integrated taxonomic effort has trained new spongiologists and improved knowledge of Martinique Island (French Antilles, eastern Caribbean Sea) marine biodiversity. *PLoS One* **12**: e0173859.
- Poléjaeff N. 1883. Report on the Calcarea dredged by the HMS Challenger during the years 1873–1876. *Report on the Scientific Results of the Voyage Challenger (Zoology)* **8**: 1–76.
- Pulitzer-Finali G. 1982. Some new or little-known sponges from the Great Barrier Reef of Australia. *Bollettino dei Musei e degli Istituti Biologici dell'Università di Genova* **48**: 87–141.
- Rapp HT. 2004. The first record of the genus *Leucascus* Dendy, 1892 from the Atlantic Ocean, with description of *Leucascus lobatus* sp. nov. (Porifera, Calcarea, Calcinea) from Greenland. *Steenstrupia* **28**: 1–9.
- Rapp HT. 2006. Calcareous sponges of the genera *Clathrina* and *Guancha* (Calcinea, Calcarea, Porifera) of Norway (NE Atlantic) with the description of five new species. *Zoological Journal of the Linnean Society* **147**: 331–65.
- Rapp HT, Janussen D, Tendal OS. 2011. Calcareous sponges from abyssal and bathyal depths in the Weddell Sea, Antarctica. *Deep Sea Research Part II: Topical Studies in Oceanography* **58**: 58–67.
- Ridley SO. 1884. Spongiida. In: *Report on the zoological collections made in the Indo-Pacific Ocean during the voyage of HMS Alert, 1881–2*. London: British Museum (Natural History), 366–482.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- Russo CAM. 1997. Efficiencies of different statistical tests in supporting a known vertebrate phylogeny. *Molecular Biology and Evolution* **14**: 1078–1080.
- Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular cloning: a laboratory manual*. New York: Cold Spring Harbor Laboratory Press.
- Schleyer MH, Benayahu Y, Parker-Nance S, Van Soest RWM, Quod JP. 2016. Benthos on submerged lava beds of varying age off the coast of Reunion, western Indian Ocean: sponges, octocorals and ascidians. *Biodiversity* **17**: 93–100.
- Schmidt O. 1862. *Die Spongien des Adriatischen Meeres*. Leipzig: Wilhelm Engelmann.
- Schmidt O. 1864. *Supplement der Spongien des Adriatischen Meeres. Enthaltend die Histologie und systematische Ergänzungen*. Leipzig: Wilhelm Engelmann.
- Schmidt O. 1870. *Grundzüge einer Spongien-Fauna des atlantischen Gebietes*. Leipzig: Wilhelm Engelmann.
- Schuffner O. 1877. Beschreibung einiger neuer Kalkschwämme. *Jenaische Zeitschrift* **4**: 403–433.
- Spalding MD, Foz HE, Allen GR, Davidson N, Ferdaña ZA, Finlayson M, Halpern BS, Jorge MA, Lombana A, Lourie SA, Martin KD, McManus E, Molnar J, Recchia CA, Robertson J. 2007. Marine ecoregions of

- the world: a bioregionalization of coastal and shelf areas. *Bioscience* **57**: 573–583.
- Tanita S. 1942.** Calcareous sponges collected in the Kanto District, Japan. *Science Reports of the Tôhoku Imperial University* **17**: 17–69.
- Tanita S. 1943.** Studies on the Calcarea of Japan. *Science Reports of the Tôhoku Imperial University* **17**: 353–490.
- Thacker AG. 1908.** On collections of the Cape Verde Islands fauna made by Cyril Crossland, M.A. The calcareous sponges. *Proceedings of the Zoological Society of London* **49**: 757–782.
- Trentin F, Massé L. 2018.** Première approche de la biodiversité des éponges à la Réunion. *Rapport Vie Océane*. La Réunion: University of La Réunion.
- Vacelet J. 1977.** Éponges pharétronides actuelles et sclérosponges de Polynésie française, de Madagascar et de la Réunion. *Bulletin du Muséum National d'Histoire Naturelle de Paris* **444**: 345–368.
- Vacelet J. 1981.** Éponges hypercalcifiées ('Pharétronides' 'Sclérosponges') des cavités des récifs coralliens de Nouvelle-Calédonie. *Bulletin du Muséum National d'Histoire Naturelle* **3**: 313–351.
- Van Soest RWM, De Voogd NJ. 2015.** Calcareous sponges of Indonesia. *Zootaxa* **3951**: 1–105.
- Van Soest RWM, De Voogd NJ. 2018.** Calcareous sponges of the western Indian Ocean and Red Sea. *Zootaxa* **4426**: 1–160.
- Van Soest RWM, Kaiser K, Van Syoc R. 2011.** Sponges from Clipperton Island, East Pacific. *Zootaxa* **2839**: 1–46.
- Van Soest RWM, Boury-Esnault N, Vacelet J, Dohrmann M, Erpenbeck D, De Voogd NJ, Santodomingo N, Vanhoorne B, Kelly M, Hooper JNA. 2012.** Global diversity of sponges (Porifera). *PLoS One* **7**: e35105.
- Van Soest RWM, Hooper JN, Butler PJ. 2020.** Every sponge its own name: removing Porifera homonyms. *Zootaxa* **4745**: 1–93.
- Voigt O, Wörheide G. 2016.** A short LSU rRNA fragment as a standard marker for integrative taxonomy in calcareous sponges (Porifera: Calcarea). *Organisms, Diversity & Evolution* **16**: 53–64.
- Voigt O, Wülfig E, Wörheide G. 2012.** Molecular phylogenetic evaluation of classification and scenarios of character evolution in calcareous sponges (Porifera, Class Calcarea). *PLoS One* **7**: 1–16.
- Voigt O, Erpenbeck D, Gonzalez-Pech RA, Al-Aidaros AM, Berumen ML, Wörheide G. 2017.** Calcinea of the Red Sea: providing a DNA barcode inventory with description of four new species. *Marine Biodiversity* **47**: 1009–1034.
- Wörheide G, Hooper JNA. 1999.** Calcarea from the Great Barrier Reef. I: cryptic Calcinea from Heron Island and Wistari Reef (Capricorn-Bunker group). *Memoirs of the Queensland Museum* **43**: 859–891.
- Wörheide G, Hooper JNA. 2003.** New species of Calcaronea (Porifera: Calcarea) from cryptic habitats of the southern Great Barrier Reef (Heron Island and Wistari Reef, Capricorn-Bunker Group, Australia). *Journal of Natural History* **37**: 1–47.
- Wörheide G, Hooper JNA, Degnan BM. 2002.** Phylogeography of western Pacific *Leucetta* 'chagosensis' (Porifera: Calcarea) from ribosomal DNA sequences: implications for population history and conservation of the Great Barrier Reef World Heritage Area (Australia). *Molecular Ecology* **11**: 1753–1768.
- Wörheide G, Nichols S, Goldberg J. 2004.** Intragenomic variation of the rDNA internal transcribed spacers in sponges (phylum Porifera): implications for phylogenetic studies. *Molecular Phylogenetics and Evolution* **33**: 816–830.
- Wörheide G, Solé-Cava AM, Hooper JNA. 2005.** Biodiversity, molecular ecology and phylogeography of marine sponges: patterns, implications and outlooks. *Integrative and Comparative Biology* **45**: 377–385.
- Wörheide G, Epp SL, Macis L. 2008.** Deep genetic divergences among Indo-Pacific populations of the coral reef sponge *Leucetta chagosensis* (Leucettidae): founder effects, vicariance, or both? *BMC Evolutionary Biology* **8**: 24.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

**Figure S1.** ITS sequences aligned through the online version of the program MAFFT v.7 using the strategy Q-INS-i.

**Figure S2.** C-LSU sequences of Calcinea aligned through the online version of the program MAFFT v.7 using the strategy Q-INS-i.

**Figure S3.** C-LSU sequences of Calcaronea aligned through the online version of the program MAFFT v.7 using the strategy Q-INS-i.

**Figure S4.** ML tree based on the C-LSU sequences of the subclass Calcinea rooted with the subclass Calcaronea.

**Figure S5.** ML tree based on the C-LSU sequences of the subclass Calcaronea rooted with the subclass Calcinea.