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Revalidation of *Leucetta floridana* (Haeckel, 1872) (Porifera, Calcarea): a widespread species in the tropical western Atlantic

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There is no consensus as to the level of intraspecific morphological plasticity in calcareous sponges, and so many species described in the nineteenth century were lumped into a few supposedly very polymorphic species during the 20th century. *Leucetta floridana* was originally described from Florida, but was subsequently considered as a junior synonym of the Pacific species *Leucetta microraphis*, in spite of the presence, in *L. floridana* only, of two size classes of tetractine spicules. We have compared, through DNA sequencing (ribosomal internal transcribed spacers, ITS1 and ITS2) and detailed morphological analyses, samples of *Leucetta* cf. *floridana* from the Atlantic (three sites in the Caribbean, and five along the Brazilian coast), with *L. microraphis* from the Pacific (Australia and New Caledonia). Not only did the genetic and morphological analyses confirm the taxonomic validity of *L. floridana*, but they also detected the presence of a new species of *Leucetta*, morphologically similar to and living in sympatry with *L. floridana* in the Brazilian coast.

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ADDITIONAL KEYWORDS: cosmopolitanism – internal transcribed spacers – molecular systematics – sponge – taxonomy.

INTRODUCTION

The systematics and taxonomy of many sponge genera are based on very few morphological characteristics (i.e. colour, texture, consistency, spicule shape and size, skeletal organization), which are often difficult to interpret as they are frequently variable or plastic (Maldonado *et al.*, 1999; Klautau *et al.*, 1999). During the 19th and the beginning of the 20th centuries, when the first large taxonomic monographs on Porifera were produced, many new species were described (e.g. Schmidt, 1862; Gray, 1867; Haeckel, 1872; Poléjaeff, 1883; von Lendenfeld, 1885; Carter 1886; Dendy, 1891, 1892). However, since then, many of those species have entered into synonymy, as their diagnostic morphological differences were reinterpreted as a result of intraspecific variability (e.g. Brøndsted, 1914; Tanita, 1942; Sarà, 1953; Burton, 1963). Consequently, widespread (even cosmopolitan) distributions became commonly postulated for Porifera species. Recently, morphological and molecular analyses have demonstrated that the putative enormous morphological variability of sponges was not the rule and that highly variable and widely distributed sponge species were not as common as previously thought (e.g. Solé-Cava *et al.*, 1991; Boury-Esnault, Solé-Cava & Thorpe, 1992; Hajdu & van Soest, 1992; Klautau, Solé-Cava & Borojevic, 1994; Muricy *et al.*, 1996; Klautau *et al.*, 1999; Klautau & Valentine, 2003).

Sponges of the genus *Leucetta* Haeckel, 1872 are amongst the most frequent calcareous sponges in shallow tropical regions (Borojevic *et al.*, 2002). The

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original description of the genus included leuconoid Calcarea with a skeleton exclusively composed of triactines. After several reinterpretations of the genus (Poléjaeff, 1883; von Lendenfeld, 1885, 1891; Dendy, 1892), *Leucetta* was finally established by Dendy & Row (1913). *Leucetta microraphis* (Haeckel, 1872) was originally described as a variety of *Leucetta primigenia*, and elevated to species level by von Lendenfeld (1885) based on specimens from Australia. In 1892, Dendy identified some specimens of *Leucetta* from Australia as *Leucandra microraphis*. Differently from von Lendenfeld, Dendy (1892) mentioned the presence of tetractines: 'Some specimens have a few quadriradiate spicules, while in others I cannot find any'. Later in the same article, Dendy described the skeleton of *L. microraphis* as 'dense and very irregular, consisting of scattered triradiates of two very different sizes, rather small and enormously large, the former being most abundant', without mentioning anymore the tetractines, perhaps suggesting that those few spicules with a fourth actine, an apical actine, were just rare complements (Dendy & Row, 1913). All prior descriptions (Haeckel, 1872; Ridley, 1884; von Lendenfeld, 1885) never mentioned the presence of tetractines.

In the Atlantic, two species were attributed to *Leucetta*: *Leucetta imberbis* (Duchassaing & Michelotti, 1864), described as *Medon imberbis*, and *Leucetta floridana* (Haeckel, 1872), described as *Leucaltis floridana* to include sponges from Florida with triactines of two size categories ('small' and 'big to giant'), and less abundant tetractines also of two size categories. *Leucaltis floridana* was reallocated to *Leucetta* by Dendy & Row (1913), when cortical tetractines became accepted within the scope of *Leucetta*. Burton (1963) postulated that the morphological differences between *L. floridana* and *L. microraphis* were not large enough to warrant their distinction at the specific level, and should, rather, be interpreted as the result of intraspecific variation of *L. microraphis*. That conclusion was followed by most authors, resulting in the acceptance that *L. microraphis* should be considered a cosmopolitan species.

Borojevic & Peixinho (1976) reported a high variability in the abundance of tetractines (both 'small' and 'big to giant') in specimens from Brazil. In that material, tetractines, although always present, were either very rare or very abundant. As the presence/absence of tetractines in *Leucetta* was regarded as intraspecific morphological variability with no taxonomic value, those authors accepted the synonymy proposed by Burton (1963) and named the Brazilian specimens *L. microraphis*. Later, Lehnert & van Soest (1998) questioned that synonymy, identifying specimens from Jamaica as *L.* aff. *floridana*. Given the widespread occurrence of cryptic species in sponges (reviewed in e.g. Boury-Esnault & Solé-Cava, 2004), the alleged cosmopolitanism of *L. microraphis* could be simply the result of taxonomical lumping of different species from the Atlantic and Pacific Oceans (Borojevic & Klautau, 2000).

The conspecificity of sponge populations from the Caribbean and tropical Brazil is also disputed. The first marine zoogeographers recognized a rich Caribbean fauna that contrasted with an impoverished Brazilian one (Ekman, 1953; Briggs, 1974). That dissimilarity was explained by coastal and oceanic barriers to gene flow between the two areas, like the high freshwater and sediment discharge from the Amazonas and Orinoco rivers; hemispheric separation of Atlantic north/south surface current system since the Tethys closure). However, the efficiency of those barriers may be limited to shallow depths and may have been less important during periods of sea level rise (Rocha, 2003; Wörheide, Solé-Cava & Hooper, 2005). Recent species inventories reported a high faunal similarity between the Caribbean and Brazil (Hechtel, 1976; Collette & Rützler, 1977; Rocha 2003). Nevertheless, for some groups, such as Porifera, a mixed scenario has been suggested, with some sponge species able to cross the Amazon barrier whereas others are not able to do so (Klautau *et al.*, 1999; Lazoski *et al.*, 2001; Wörheide, Solé-Cava & Hooper, 2005).

We have used molecular and morphological characters to confirm the taxonomic validity of *L. floridana*, and test if its distribution is restricted to the Caribbean or if it extends to Brazil. Internal transcribed spacers (ITS1 and ITS2) of rDNA were chosen for the molecular analyses because they have levels of molecular evolution compatible with taxonomic studies of sponges at the infrafamilial level (Wörheide, Nichols & Goldberg, 2004), including species of Leucettidae (Wörheide, Hooper & Degnan, 2002; Wörheide *et al.*, 2004).

MATERIAL AND METHODS

Sample sites and collection

Specimens of *L.* cf. *floridana* were collected by scuba diving, trawling, or dredging at seven localities throughout the tropical western Atlantic (Table 1, Fig. 1). For the morphological analyses, specimens of *L. microraphis* from New Caledonia, previously analysed by Borojevic & Klautau (2000), were also included. DNA sequences of *L. microraphis* and *Leucetta chagosensis* from Australia (Great Barrier Reef) were retrieved from GenBank (accession numbers AJ 633871, AJ 633872, and AF 458855, AF 458860, AF 458861, respectively).

Table 1. Specimens used in this study with collection sites, register numbers, and GenBank accession numbers of DNA sequences

Species	Collection site	Register number	GenBank accession no.	Type of analysis
Leucetta chagosensis	Australia (GBR)	QMG 313944	AF458855	Molec.
Leucetta chagosensis	Australia (GBR)	QMG 313946	AF458860	Molec.
Leucetta chagosensis	Australia (GBR)	QMG 313774	AF458861	Molec.
Leucetta microraphis	Australia (GBR)	QMG 313659	AJ633872	Molec.
Leucetta microraphis	Australia (GBR)	QMG 315140	AJ633871	Molec.
Leucetta microraphis	New Caledonia (NCA)	UFRJPOR 5139	–	Morph.
Leucetta microraphis	New Caledonia (NCA)	UFRJPOR 5140	–	Morph.
Leucetta microraphis	New Caledonia (NCA)	UFRJPOR 5142	–	Morph.
Leucetta microraphis	New Caledonia (NCA)	UFRJPOR 5355	–	Morph.
Leucetta cf. *floridana*	San Andrés, Caribbean (SAN)	UFRJPOR 5363	EU781971	Molec., Morph.
Leucetta cf. *floridana*	San Andrés, Caribbean (SAN)	UFRJPOR 5364	EU781972	Molec., Morph.
Leucetta cf. *floridana*	San Andrés, Caribbean (SAN)	UFRJPOR 5365	–	Morph.
Leucetta cf. *floridana*	San Andrés, Caribbean (SAN)	UFRJPOR 5366	EU781973	Molec., Morph.
Leucetta cf. *floridana*	San Andrés, Caribbean (SAN)	UFRJPOR 5367	EU781974	Molec., Morph.
Leucetta cf. *floridana*	Urabá, Caribbean (URA)	UFRJPOR 5356	-	Morph.
Leucetta cf. *floridana*	Urabá, Caribbean (URA)	UFRJPOR 5357	EU781970	Molec., Morph.
Leucetta cf. *floridana*	Urabá, Caribbean (URA)	UFRJPOR 5358	–	Morph.
Leucetta cf. *floridana*	Urabá, Caribbean (URA)	UFRJPOR 5359	EU781969	Molec., Morph.
Leucetta cf. *floridana*	Urabá, Caribbean (URA)	UFRJPOR 5360	EU781968	Morph.
Leucetta cf. *floridana*	Urabá, Caribbean (URA)	UFRJPOR 5361 INV-POR 542	–	Morph.
Leucetta cf. *floridana*	Urabá, Caribbean (URA)	UFRJPOR 5362 INV-POR 583	–	Molec., Morph.
Leucetta cf. *floridana*	Bocas del Toro, Panama (BDT)	PC BT 12	EU781989	Molec., Morph.
Leucetta cf. *floridana*	Bocas del Toro, Panama (BDT)	PC BT 22	EU781990	Molec., Morph.
Leucetta cf. *floridana*	Bocas del Toro, Panama (BDT)	PC BT 23	EU781991	Molec., Morph.
Leucetta cf. *floridana*	Ceará, Brazil (CEA)	MNRJ 8440	EU781983	Molec., Morph.
Leucetta cf. *floridana*	Ceará, Brazil (CEA)	MNRJ 8445	EU781984	Molec., Morph.
Leucetta cf. *floridana*	Ceará, Brazil (CEA)	MNRJ 8488	EU781980	Molec., Morph.
Leucetta cf. *floridana*	Ceará, Brazil (CEA)	MNRJ 8465	EU781982	Molec., Morph.
Leucetta cf. *floridana*	Ceará, Brazil (CEA)	MNRJ 8474	EU781981	Molec., Morph.
Leucetta cf. *floridana*	Abrolhos, Brazil (ABR)	UFRJPOR 4703	EU781979	Molec., Morph.
Leucetta cf. *floridana*	Rio Grande do Norte, Brazil (RGN)	BPOTPOR 202	EU781985	Molec., Morph.
Leucetta cf. *floridana*	Rio Grande do Norte, Brazil (RGN)	BPOTPOR 540	–	Morph.
Leucetta cf. *floridana*	Rio Grande do Norte, Brazil (RGN)	BPOTPOR 547	EU781986	Molec., Morph.
Leucetta cf. *floridana*	Rio Grande do Norte, Brazil (RGN)	BPOTPOR 569	EU781987	Molec., Morph.
Leucetta cf. *floridana*	Rio Grande do Norte, Brazil (RGN)	BPOTPOR 588	EU781988	Molec., Morph.
Leucetta cf. *floridana*	Rio Grande do Norte, Brazil (RGN)	BPOTPOR 591	–	Morph.
Leucetta cf. *floridana*	Rio Grande do Norte, Brazil (RGN)	BPOTPOR 610	EU781978	Molec., Morph.
Leucetta cf. *floridana*	Fernando de Noronha, Brazil (FNO)	MNRJ 8602	EU781977	Molec., Morph.
Leucetta cf. *floridana*	Fernando de Noronha, Brazil (FNO)	MNRJ 8609	EU781976	Molec., Morph.
Leucetta cf. *floridana*	Rocas Atoll, Brazil (RAT)	MNRJ 7630	–	Morph.
Leucetta cf. *floridana*	Rocas Atoll, Brazil (RAT)	MNRJ 7648	–	Morph.
Leucetta cf. *floridana*	Rocas Atoll, Brazil (RAT)	MNRJ 7725	EU781975	Molec., Morph.

It is also indicated if the specimen was used for morphological (Morph.) and molecular (Molec.) analyses. Specimens deposited at: Instituto de Investigaciones Marinas y Costeras, Museo Nacional de Historia Natural Marina (Santa Marta, Colombia) (INV-POR); Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil) (UFRJPOR); Collection number of specimens from Bocas del Toro, Panama (PC BT); Museu Nacional do Rio de Janeiro, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil) (MNRJ); Departamento de Zoologia, Universidade Federal de Pernambuco (Pernambuco, Brazil) (BPOTPOR).

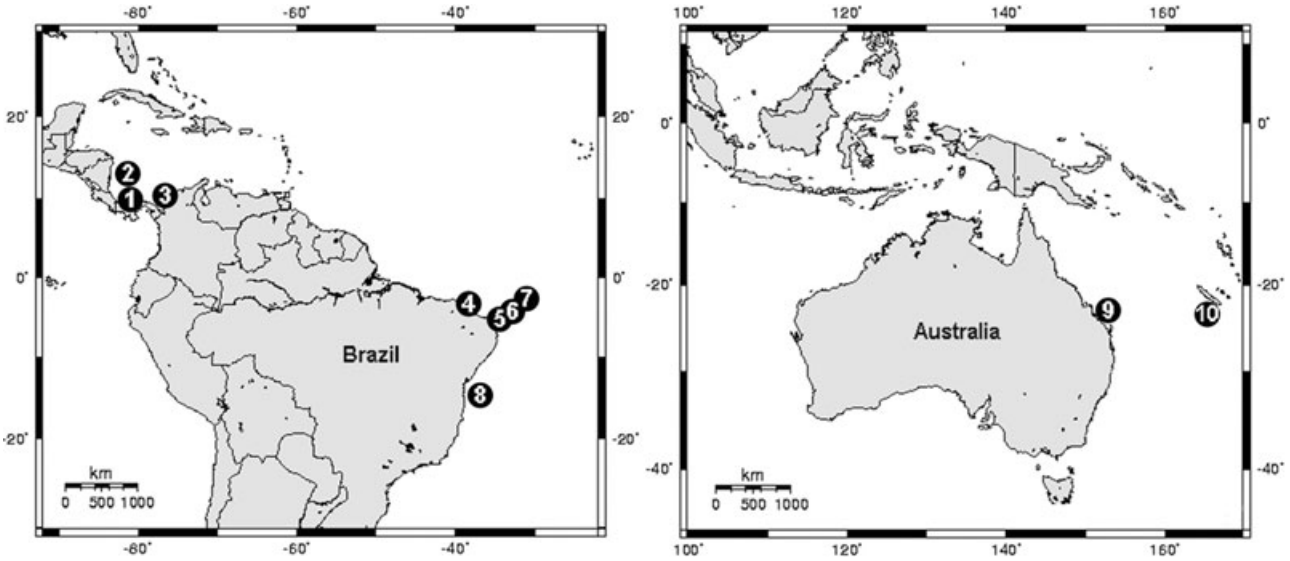

Figure 1. Sampling sites. Caribbean: 1 – Bocas del Toro (BDT), 2 – San Andrés Island (SAN), 3 – Urabá (URA); Brazil: 4 – Ceará (CEA), 5 – Rio Grande do Norte (RGN), 6 – Rocas Atoll (RAT), 7 – Fernando de Noronha Archipelago (FNO), 8 – Abrolhos Archipelago (ABR); Pacific: 9 – Australia (GBR), 10 – New Caledonia (NCA).

Specimens collected were fixed and preserved in alcohol 70% or 93% and deposited in the Porifera collections of several scientific institutions (Table 1).

Molecular data

Internal transcribed spacer (ITS) sequences of the rDNA were analysed from specimens of *L.* cf. *floridana* (western Atlantic specimens), *L. microraphis*, and *L. chagosensis* as outgroup. Genomic DNA was extracted from ethanol-preserved specimens by the guanidine/phenol chloroform protocol developed by Lôbo-Hajdu *et al.* (2004). The entire region comprising the two spacers (ITS1 and ITS2), and the 5.8S ribosomal DNA (approximately 900 bp) was amplified by PCR with the primers 18S (5′–TCA TTT AGA GGA AGT AAA AGT CG–3′) and 28S (5′–GTT AGT TTC TTT TCC TCC GCT T–3′) (Lôbo-Hajdu *et al.*, 2004). PCR mixes contained: buffer [Tris-HCl pH 8.8 (75 mM), $(NH_4)_2SO_4$ (20 mM), Tween 20 (0.01%)], bovine serum albumin (1 mg/mL), deoxynucleotide triphosphates (0.4 mM), 0.5 pmol/μL of each primer, $MgCl_2$ (2.5 mM), and one unit of Taq-DNA-polymerase. PCR steps consisted of 4 min/94 °C, 35 cycles (1 min/92 °C, 1 min/55 °C, 1 min/72 °C), and 6 min/72 °C.

Both DNA strands of each sample were sequenced directly in automatic sequencers by Macrogen. Sequences were edited using the program CHROMAS LITE 2.0 (http://www.technelysium.com.au) and BLAST searches (http://www.ncbi.nlm.nih.gov/blast/) confirmed their origin.

Sequence alignment and phylogenetic analyses

All sequences were aligned using the ClustalW algorithm in the program MEGA 4.0 (Tamura *et al.*, 2007) and the alignment produced was manually adjusted. All newly obtained sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/Genbank; Table 1).

Maximum likelihood (ML) analysis was performed in PHYML (Tamura *et al.*, 2007) using a heuristic search initiated with a starting tree estimated by neighbor-joining (NJ). Gaps were considered as missing data. The Tamura–Nei substitution model (Tamura & Nei, 1993) with a gamma distribution (TrN+G) was chosen with MODELTEST 3.7 using the Akaike information criterion (Posada & Crandall, 1998). Nodal support for ML was estimated using 1000 bootstrap pseudoreplicates (Felsenstein, 1985).

Maximum parsimony (MP) analysis was performed using PAUP 4.0b 10 (Swofford, 2002). A heuristic search was performed with 100 random additions, a maximum of ten trees retained at each step, and an overall maximum of 1000 trees. The stepwise addition and tree bisection reconnection algorithms were implemented with default settings. For node support, 1000 bootstrap replicates were performed. In MP and ML all characters were equally weighted.

A NJ tree (Saitou & Nei, 1987) was produced by MEGA 4.0 with the Jukes–Cantor model of nucleotide substitution (Jukes & Cantor, 1969). The choice of a

simpler model for the NJ tree was necessary because distance-based analyses are more sensitive to the increase in variance observed when larger number of parameters are used (Nei & Kumar, 2000). Complete deletion was used so that indel regions were not considered. Bootstrap of 1000 replicates was implemented. The overall topography of all trees was identical, so their results were condensed in a single tree (Fig. 2).

Morphological data

External morphology of specimens was observed in the field or from underwater photos and also under a stereoscopic microscope. Spicules and skeleton preparations followed standard procedures (Wörheide & Hooper, 1999; Klautau & Valentine, 2003). Spicule measurements of the width at the base of each actine and its length from tip to base were made using an ocular micrometer.

A Student *t*-test was used to compare spicule measurements between *L.* cf. *floridana* and *L. microraphis*, and between *L.* cf. *floridana* from the Caribbean and Brazil. Whenever data did not conform to assumptions of normality and homogeneity of variances, a Mann–Whitney test was used.

Photomicrographs were taken with a digital camera assembled on a Zeiss Axioscop microscope at the Laboratório de Tecnologia e Processamento de Imagens (PROIN), Instituto de Biologia (UFRJ).

RESULTS

Molecular data

Aligned sequences had a length of 890 bp, with 118 variable characters, of which 22 were parsimony informative. Three clades were formed, two of *L.* cf. *floridana* (clades A and B) and one of *L. microraphis* (clade C), all with high bootstrap support (Fig. 2). Clade A grouped Caribbean and Brazilian specimens, whereas clade B included only specimens from north-east Brazil (Ceará and Rio Grande do Norte). Sequence divergence between specimens of clades A and B was 2.4–3.1%, whereas specimens of those clades diverged 5.3–6.3% from *L. microraphis* (clade C) and 7.2–8.5% from *L. chagosensis* (clade D, the outgroup). Sequence divergence of specimens grouped within each clade varied from 0 to a maximum of 0.9 %.

Morphological data

Specimens of *L. microraphis* and *L.* cf. *floridana* could be clearly distinguished based on the presence of a second category of tetractines in the latter (Figs 3 and 4). Another difference found between *L. microraphis* and *L.* cf. *floridana* (clade A) was the significantly larger width of tetractines I in the latter (Tables 2 and 3). Also, tetractines I of Caribbean specimens of *L.* cf. *floridana* (clade A) were significantly shorter than those of the same clade from Brazil (Tables 2 and 3).

Specimens of clade A of *L.* cf. *floridana* had significantly longer triactines II than those of clade B (*Leucetta* sp., Tables 2 and 3). Specimens of the two clades could also be easily distinguished by the presence of atrium only in the specimens of clade A. Another morphological difference observed was the presence of cortical ridges in specimens of clade A (Fig. 4), which were absent in specimens of clade B (Fig. 6) and in *L. microraphis* (Fig. 3). The length of triactines I was shorter in specimens of *L.* cf. *floridana* (clade B) than in *L. microraphis* (Tables 2 and 3).

DISCUSSION

The morphological and molecular results show that *L. floridana* is a valid species, and that its distribution ranges from the Caribbean (type locality) to Brazil (clade A). Consequently, we reject the synonymy of *L. microraphis* and *L. floridana* proposed by Burton (1963). The high values of molecular divergence found in the ITS1–5.8S–ITS2 rDNA sequences between *L. floridana* and *L. microraphis* (5.3 to 6.3%) are six times higher than the intraspecific variation found in *L. floridana* (clade A; 0.0 to 0.9%), strongly supporting the validity of this species. The levels of inter- and intraspecific divergence observed in *Leucetta* are similar to those found using the same molecular segment in demosponge species. For example, for the same ITS1–ITS2 region, interspecific divergence observed in *Chondrilla* spp. varied between 7.1 and 14% (Usher *et al.*, 2004), and intraspecific divergence of *Crambe crambe* (Schmidt, 1862) from the Mediterranean and eastern Atlantic varied between 0.5 and 1.7% (Duran, Giriet & Turon, 2004). For the calcareous sponge *L. chagosensis* from the western Pacific, the intraspecific divergence in the same ribosomal region varied between 0.1 and 2.2% (Wörheide *et al.*, 2002; Wörheide, Epp & Macis, 2008).

The molecular divergence found between Caribbean and Brazilian populations of *L. floridana* (0.0 to 0.8%) was very low. In fact, the molecular divergence amongst individuals from the Caribbean was sometimes higher than between individuals from the Caribbean and Brazil. These results strongly support the conspecificity of Caribbean and Brazilian populations. In contrast, the molecular divergence between *L. floridana* (clade A) and the Brazilian specimens of clade B was too high for them to be considered conspecific individuals (2.0 to 3.1%), especially because some of those specimens were in sympatry. This result supports the presence of a second species of

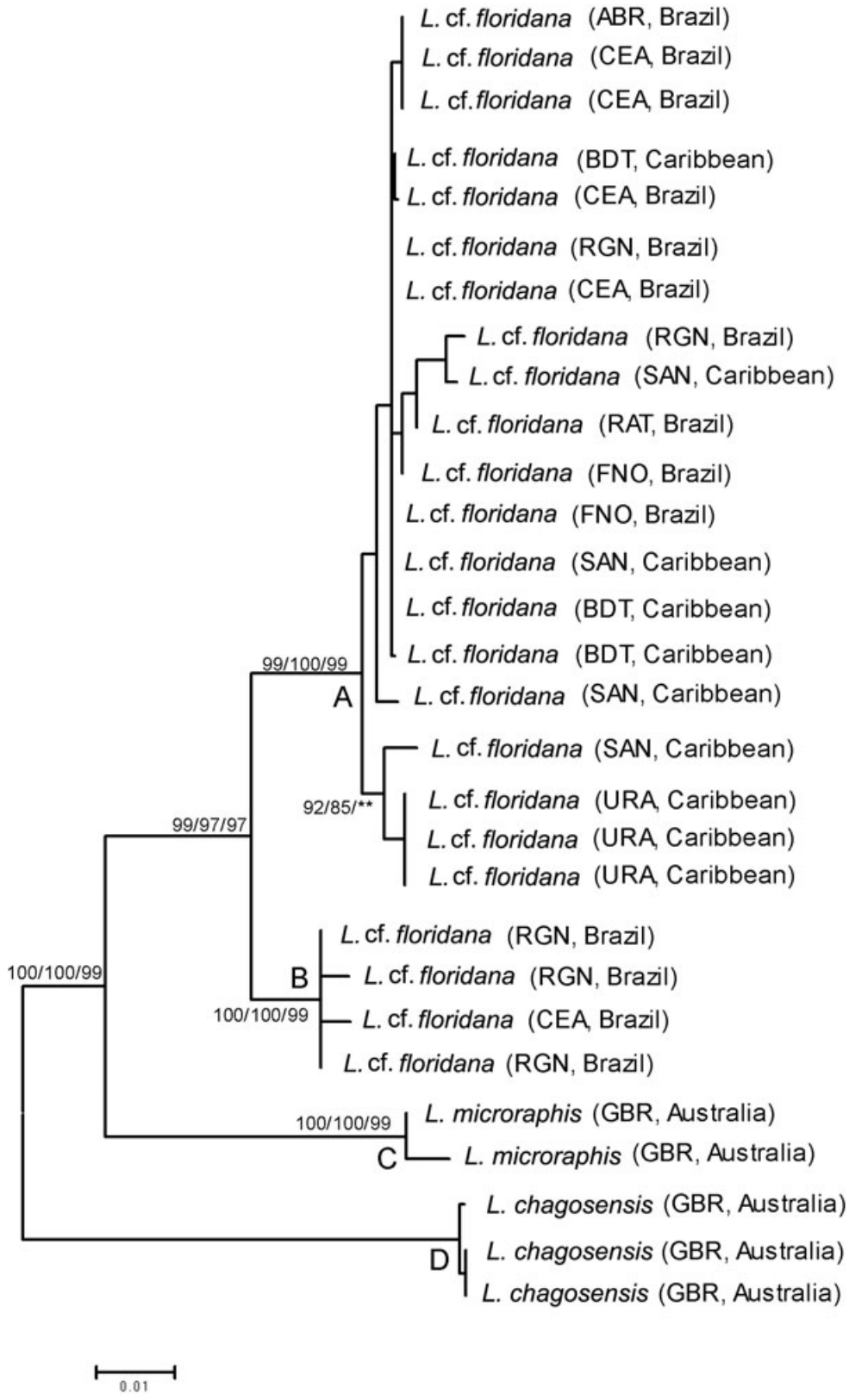

Figure 2. Maximum likelihood (ML) tree based on 890 bp of the internal transcribed spacer rDNA. Values on branches are bootstrap supports from ML, maximum parsimony, and neighbor-joining. Asterisks indicate bootstrap values below 50.

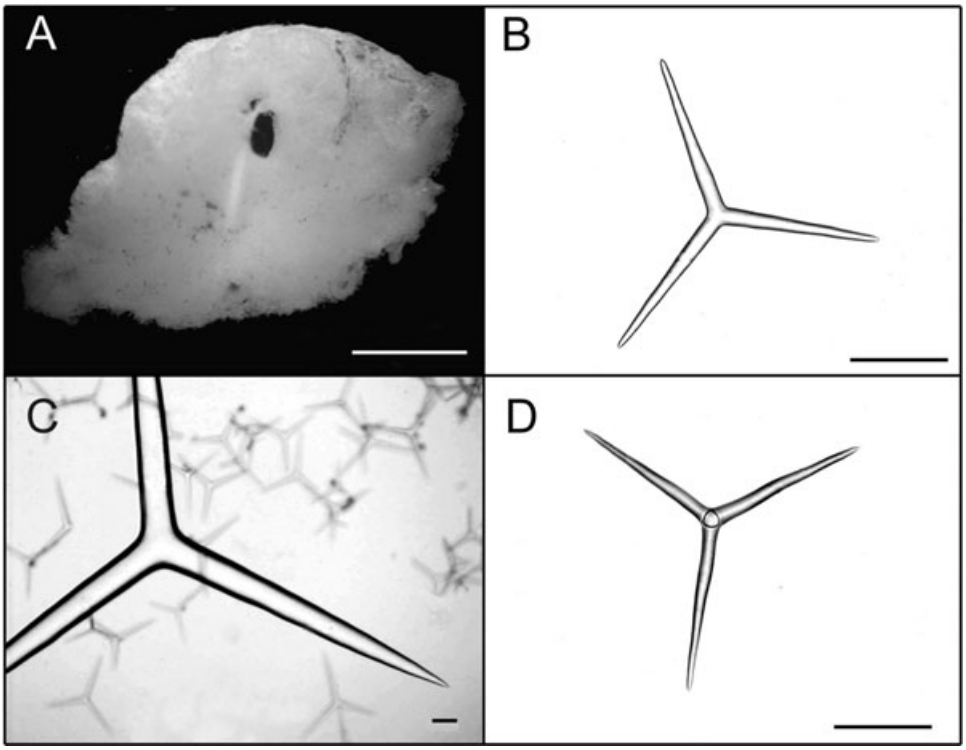

Figure 3. *Leucetta microraphis* from New Caledonia. A, preserved specimen; B, triactine I; C, triactine II and several triactines I and tetractines I; D, tetractine I. Scale bars: A = 1 cm; B–D = 100 μm.

Leucetta in Brazil. There is another described species of *Leucetta* in the Caribbean, *Leucetta imberbis*, but that is different from *Leucetta* sp. from Brazil because in the original description of *L. imberbis*, Duchassaing & Michelotti (1864) only reported the presence of triactines. Although Burton (1963) mentioned the presence of tetractines in *L. imberbis*, according to his measurements only tetractines I are present (triactine I: 910 μm × 13 μm; triactine II: 110 μm × 11 μm; tetractine I: 150 μm × 12 μm). The new species will be described in another article about calcareous sponges from north-east Brazil.

The presence of a second size category of tetractines in *L. floridana* is also strong evidence for the recognition of this species as valid and for its presence in the Caribbean and Brazil. The presence of two size categories of tetractines was consistently found, although sometimes tetractines II were rare or, in very few specimens, absent. Lehnert & van Soest (1998) reported those two categories of tetractines in high abundance in specimens from Jamaica. In specimens from Brazil, identified by Borojevic & Peixinho (1976) as *L. microraphis*, these two categories of tetractines were also reported. Thus, the presence of a second category of tetractines, independent of its abundance, seems to represent a good morphological character to distinguish *L. floridana* (or *Leucetta* sp.) from *L. microraphis*, as in the original sense of Haeckel (1872). However, as not all specimens of *L. floridana* had two categories of tetractines, spicule size (see Results), presence of cortical ridges, and geographical distribution (Atlantic Ocean) should also be considered for taxonomic purposes.

Both *L. floridana* and *Leucetta* sp. have two categories of tetractines, but they can be differentiated based on spicule size (length of triactines II), the presence of an atrium in *L. floridana*, (specimens of *Leucetta* sp. did not show a conspicuous atrium, except for MNRJ 8474), and texture of the surface, which was ridged in *L. floridana* and smooth in *Leucetta* sp.

Specimens of *Leucetta* without tetractines II were observed by Poléjaeff (1883), von Lendenfeld (1885) [both as *Leuconia dura* (= *L. microraphis sensu* Dendy, 1892)], de Laubenfels (1950) (as *L. floridana*) and Ridley (1884) (as *L. microraphis*) from Bermuda and Abrolhos (Brazil). As the descriptions given by those authors are very poor, a re-evaluation of those specimens must be carried out to confirm their taxonomic status. Similarly, the specimens reported by Jenkin (1908) as *Leucilla floridana* from Wasin (Eastern Atlantic) need to be re-analysed, but there

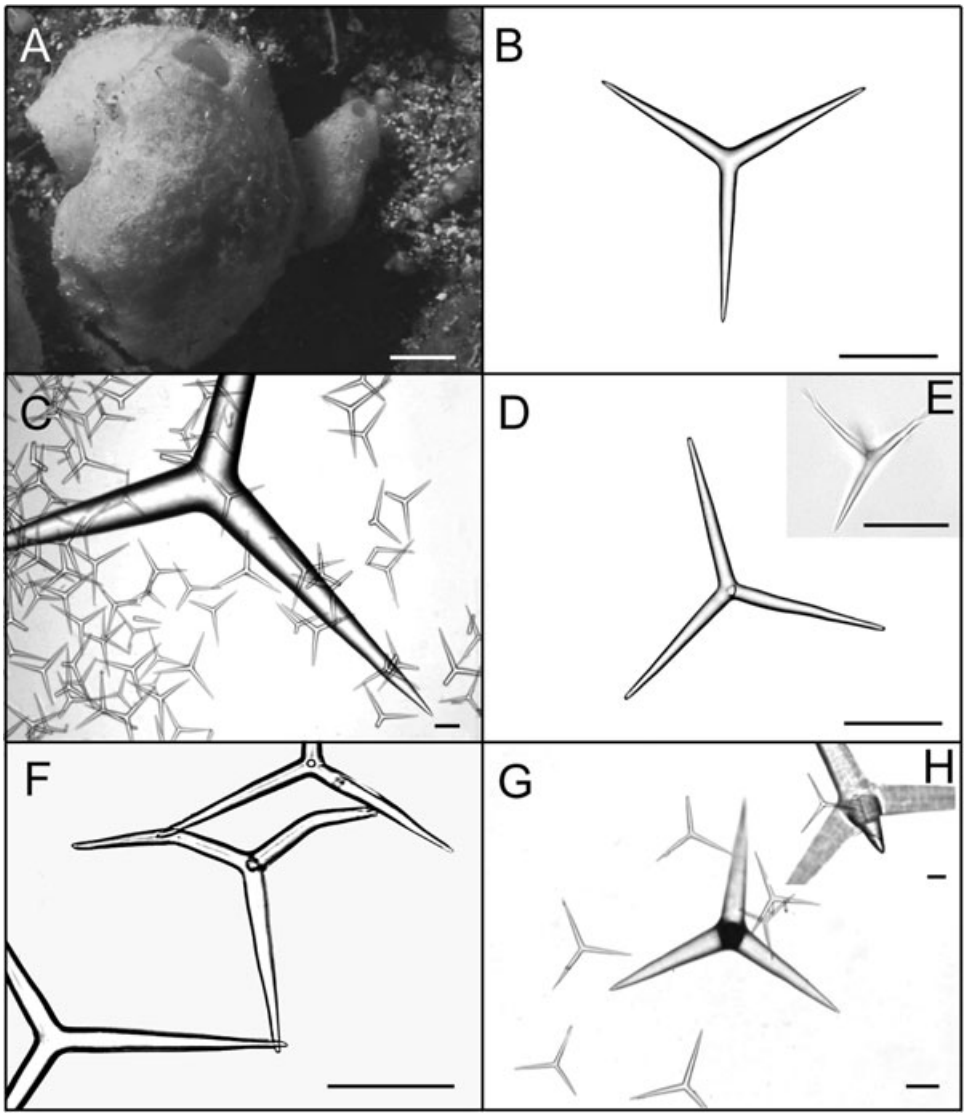

Figure 4. *Leucetta* cf. *floridana* from Brazil. A, live specimen (photo: F. Moraes); B, triactine I; C, triactine II and several triactines I; D, tetractine I; E, detail of the apical actine of tetractine I; F, sagittal tetractine I; G, tetractine II and several triactines I; H, detail of the apical actine of tetractine II. Scale bars: A = 1 cm; B–H = 100 μm.

is a good possibility that they are *L. floridana* or *Leucetta* sp.

The colours reported by Haeckel (1872) for *L. primigenia* were white, rarely red, or brown. However, he did not specify what colour corresponded to its varieties (*microraphis*, *isoraphis*, and *megaraphis*). The colour *in vivo* of *L. floridana* was also not reported by Haeckel. The colour found here in *L. floridana* specimens (light blue) differed from those reported by Dendy (1892) and Wörheide & Hooper (1999) for *L. microraphis* (green and greenish yellow, respectively). The colour *in vivo* of *Leucetta* sp. is pink.

RE-DESCRIPTION OF *LEUCETTA FLORIDANA* (HAECKEL, 1872)

PHYLUM PORIFERA GRANT, 1836

CLASS CALCAREA BOWERBANK, 1864

SUBCLASS CALCINEA BIDDER, 1898

ORDER CLATHRINIDA HARTMAN, 1958

FAMILY LEUCETTIDAE DE LAUBENFELS, 1936

GENUS *LEUCETTA* HAECKEL, 1872

Type species: Leucetta primigenia Haeckel, 1872 (by original designation).

Table 2. Spicule measurements (μm) of *Leucetta* spp.

	Length (μm)				Width (μm)					
Spicules	Min.	Mean	SD	Max.	Min.	Mean	SD	Max.	*N*	N
Clade A										
Leucetta floridana										
Triactine I	98.9	145.7	20.6	224.4	7.8	15.3	2.9	33.0	718	24
Triactine II	178.5	732.1	369.2	1864.8	21.0	94.8	52.8	233.1	636	24
Tetractine I	90.0	142.0	23.5	227.7	6.0	14.4	3.0	30.6	723	24
Tetractine II	237.7	761.2	355.6	2097.9	34.8	124.3	52.5	270.0	182	19
Clade B										
Leucetta sp.										
Triactine I	72.8	139.8	18.9	204.0	7.8	13.7	2.1	18.2	178	06
Triactine II	145.6	485.0	216.7	1102.5	20.8	64.1	30.5	148.2	135	06
Tetractine I	84.0	141.8	25.7	214.5	6.0	13.6	2.9	23.1	179	06
Tetractine II	236.3	562.4	208.9	938.6	41.7	87.7	34.3	148.2	22	05
Clade C										
Leucetta microraphis										
Triactine I	50.2	152.5	31.2	228.0	8.3	14.0	3.2	28.1	240	06
Triactine II	167.9	618.9	363.6	1631.7	14.6	71.0	47.3	264.1	172	06
Tetractine I	68.4	132.1	22.5	225.0	4.6	11.2	2.3	24.0	241	06

Min., minimum; Max., maximum; SD, standard deviation; *N*, number of spicules measured; **N**, number of specimens analysed.

Table 3. Pairwise spicule size comparisons

Spicule type	*L. floridana* × *L. microraphis*	*Leucetta* sp. × *L. microraphis*	*L. floridana* × *Leucetta* sp.	*L. floridana* Caribbean × Brazil
Triactine I				
Length	12.00	**2.453***	1.846	56.50
Width	0.877	1.018	12.00	58.50
Triactine II				
Length	1.059	1.618	**2.244***	58.00
Width	1.420	0.305	1.777	54.00
Tetractine I				
Length	1.660	0.745	0.392	**2.390***
Width	**3.710***	1.939	0.727	0.708
Tetractine II				
Length	–	–	0.658	1.787
Width	–	–	0.990	1.185

L., *Leucetta*; *t*-test value or Mann-Whitney *U*; *$P < 0.05$. Underlined text indicates Mann–Whitney *U* test results.

Diagnosis: Leucettidae with a homogeneous organization 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 or may be a large cavity (modified from Borojevic *et al.*, 2002).

LEUCETTA FLORIDANA (HAECKEL, 1872) (FIG. 5)

Synonymies: Leucaltis floridana Haeckel, 1872: 144, pl. xxvi, figures 1–17, pl. xxvii, figure 1 (original description).

Leucetta floridana; Dendy & Row, 1913: 734 (generic reallocation); Burton, 1963: 46, 252–253, figure 118 (proposed as junior synonym of *L. microraphis* Haeckel, 1872.

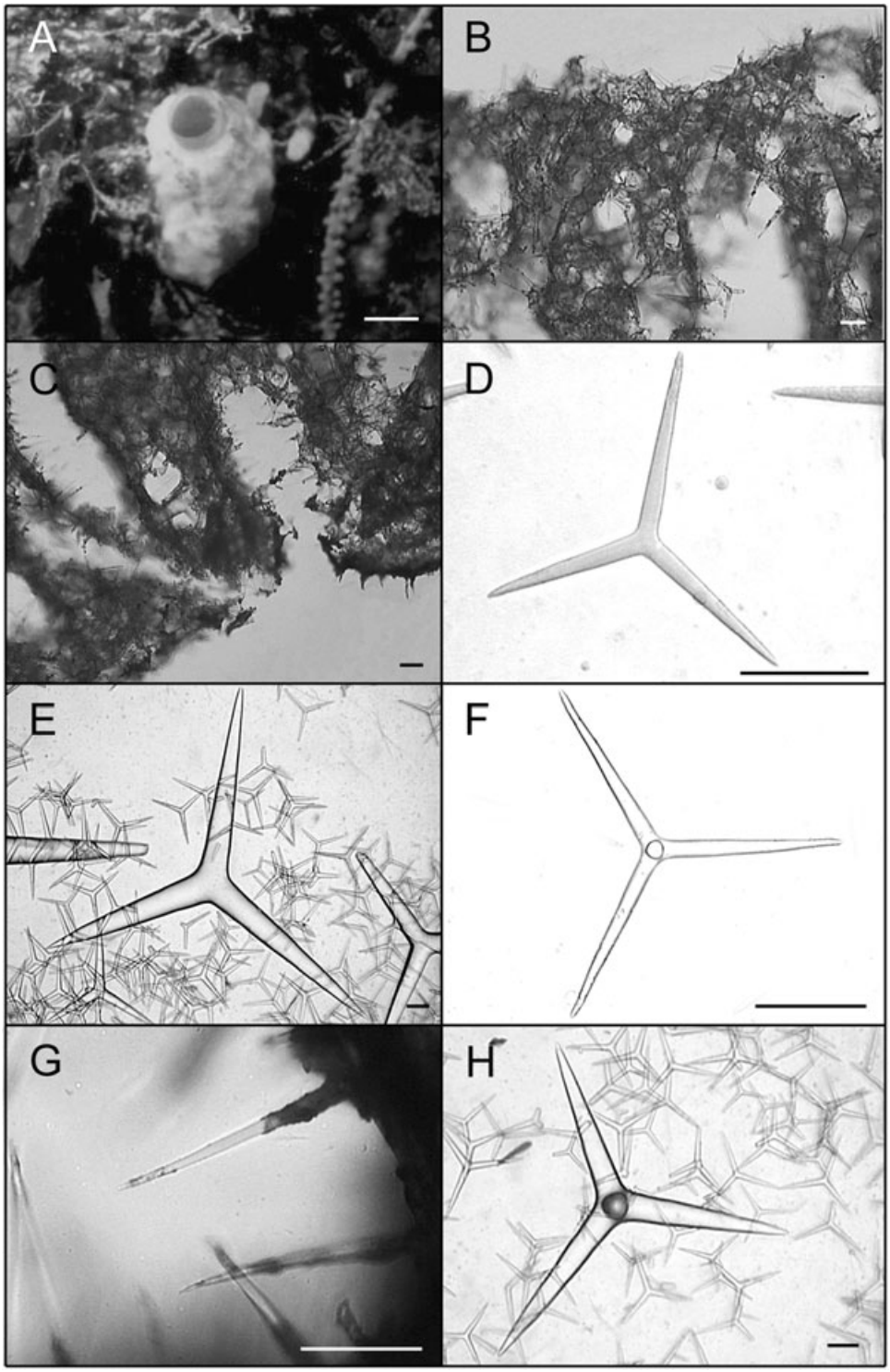

Figure 5. *Leucetta floridana* from the Caribbean (UFRJPOR 5360). A, *L. floridana in situ* (photo: S. Zea). B, transversal section of the cortex and choanosome; C, transversal section of the choanosome and atrium; D, triactine I; E, triactine II and the small triactines I; F, tetractine I; G, detail of the apical actine of tetractines I protruding into the atrium; H, tetractine II and several triactines I and tetractines I. Scale bars: A = 1 cm; B–H = 100 μm.

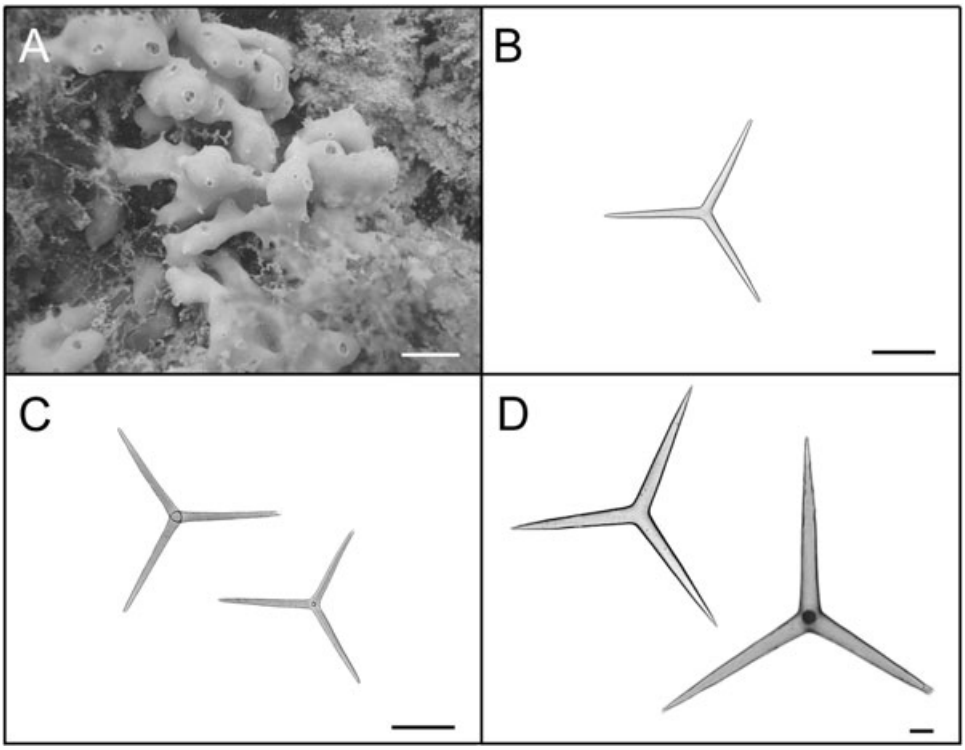

Figure 6. *Leucetta* sp. from Brazil. A, live specimen (photo: G. Muricy); B, triactine I; C, tetractines I; D, triactine II and tetractine II. Scale bars: A = 1 cm, B–D = 100 μm.

Leucetta microraphis; Borojevic & Peixinho, 1976: 1003–1005, figure 9 (*L. floridana* after Borojevic & Klautau, 2000).

Leucetta aff. *floridana*; Lehnert & van Soest, 1998: 99, figure 24.

Leucilla floridana; Jenkin, 1908: 453 (to be verified *sensu* Borojevic & Klautau, 2000; the description of that material does not allow its identification).

Leucetta floridana; de Laubenfels, 1950: 146, figure 64, pl. II (fig. 8) (*L. microraphis* after Borojevic, 1967).

Type material: Haeckel's specimens are lost *fide* Burton (1963).

Type locality: Coast of Florida. Collector A. Agassiz.

Reported distribution: Florida (Haeckel, 1872), Bermuda (de Laubenfels, 1950), Jamaica (Lehner & van Soest, 1998), Brazil: Ceará, Rio Grande do Norte, Rocas Atoll (Borojevic & Peixinho, 1976), Wasin (eastern Africa; Jenkin, 1908, to be confirmed).

Analysed material: – Bocas del Toro (Panama), PC BT 12, 22, 23 – San Andrés Island (Colombia), UFRJPOR 5363: Leeward-reef, 'West View', fossil wave-cut notch, 5 m of depth, coll. D. Valderrama, xi.2000; UFRJPOR 5364, 5365, 5366, 5367: 'La Piscinita', fossil wave-cut notch, 2–5 m of depth, coll. D. Valderrama, xi.2000. – Urabá (Colombia), UFRJPOR 5356: Sapzurro, 'Bajo El Palmar', inclined reef slope, 15 m of depth, coll. D. Valderrama, ii.2004; UFRJPOR 5357, 5358, 5359, 5360: 'Bajo Agua Viva', reef terrace, 15 m of depth, coll. D. Valderrama, ii.2004; INV-POR 583 (a fragment also in UFRJPOR 5362): reef base, 16 m of depth, coll. S. Zea, ix.1995; INV-POR 542 (a fragment also in UFRJPOR 5361): Cabo Tiburón, reef terrace, 9 m of depth, coll. S. Zea, ix.1995. – Ceará (Brazil), MNRJ 8440, 8445, 8465, 8481: Trawling, Station 30. – Rio Grande do Norte (Brazil), BPOTPOR 201, 202: Trawling 4, Station 4, xi.2003; BPOTPOR 540, 610: Risca das Bicudas, 10 m of depth, coll. F. Moraes and G. Muricy, iii.2007; BPOTPOR 634: Urca do Tubarão, 8 m of depth, coll. G. Muricy, iii.2007. – Rocas Atoll (Brazil), MNRJ 7630, 7648, 7725: Barretinha, 12 m of depth, coll. E. Hajdu, F. Moraes and M. Oliveira, xi.2003. – Fernando de Noronha Archipelago (Brazil), MNRJ 8602: Ressurreta, 4 m of depth, coll. F. Moraes, viii.2004; MNRJ 8609: Ilha Sela Gineta, 7 m of depth, coll. F. Moraes, viii.2004. – Abrolhos (Brazil), UFRJPOR 4703: Parcel das Paredes, 8 m of depth, coll. G. Muricy, x.1997.

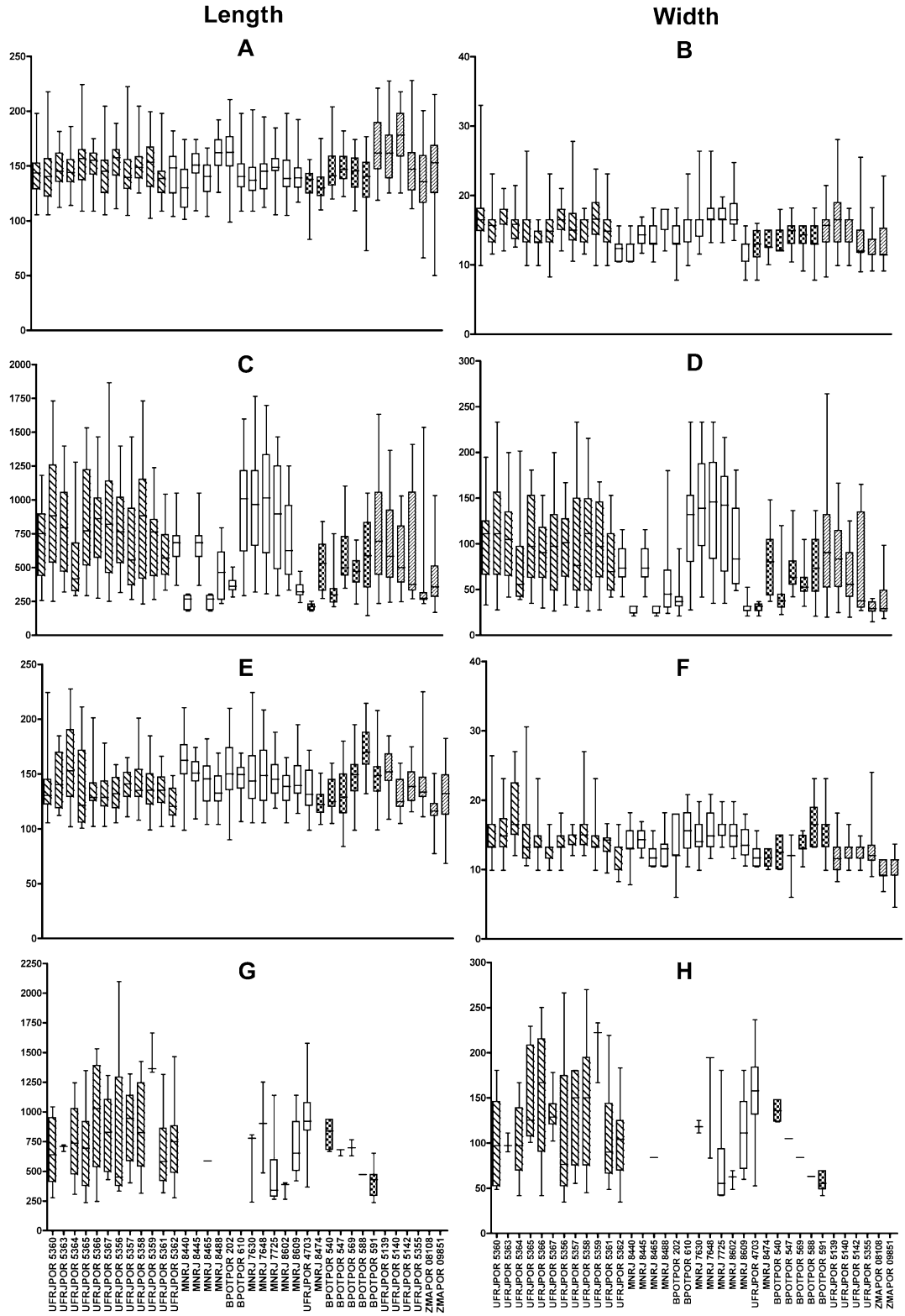

Figure 7. Box-and-whisker plot (maximum and minimum size; upper and lower quartiles and median) of spicule size of *Leucetta* spp. A, triactine I length; B, triactine I width; C, triactine II length; D, triactine II width; E, tetractine I length; F, tetractine I width; G, tetractine II length; H, tetractine II width. ▨ *Leucetta floridana* (Caribbean), □ *Leucetta floridana* (Brazil), ▩ *Leucetta* sp., ▨ *Leucetta microraphis*.

Suggested distribution: Florida (Haeckel, 1872), Jamaica (Lehnert & van Soest 1998), Brazil: Pará, Ceará, Rio Grande do Norte, Rocas Atoll, Paraíba, Pernambuco, Alagoas, Sergipe, Bahia, Espírito Santo (Borojevic & Peixinho, 1976). In addition: Colombia (Urabá and San Andrés Island), Brazil (Fernando de Noronha Archipelago).

Description

Solitary or grouped globular to pyriform tubes (Fig. 4A, 5A). Individuals may be highly deformed when encrusting small crevices. In photophylous environments, its colour is light blue. After preservation in ethanol colour becomes beige to dark brown.

Surface is rough and, in high wave energy environments, tends to be hispid because of the high spicule content. Consistence is always firm, varying from friable to hard. The osculum is localized at the top of the body. In deformed individuals, one to several oscula are localized at the top of apical projections. Below each osculum there is a wide atrial cavity, always hispid because of the apical actine of tetractines I. Numerous exhalant canals are dispersed in the atrium. In individuals with two or more oscula, wide canals may interconnect different atrial cavities.

The aquiferous system is leuconoid and the skeleton is disorganized, as typical of the genus. The cortex and the atrial wall are thin, whereas the choanosome is thick. Triactines II and tetractines II are concentrated in the cortex and lie tangentially to the surface, with the apical actine of tetractines penetrating the choanosome. Those spicules give a smooth appearance to the sponge. Subcortical holes may be present in abundance, and inhalant and exhalant canals are always profuse. Triactines I and tetractines I form the walls of subcortical holes and choanosomal canals, being tangentially aligned and densely packed around them. The apical actine of such tetractines conspicuously protrudes into exhalant canals. Triactines I also form an irregular meshwork along the entire body wall. The atrial wall is formed by triactines I and tetractines I tangentially aligned and densely packed around the atrium, projecting their apical actines into the atrial cavity and giving it a hispid appearance (Fig. 5B, C).

Spicules: Triactines I. These spicules are the most abundant. They are similar in shape to triactines II, although sagittal spicules may also be found. They are abundant in the choanosome, but sagittal spicules are mainly found tangentially aligned and densely packed around subcortical holes, choanosomal canals and the atrium [105.6–143.3 (±28.7) – 217.8/9.9–17.1 (±4.9) – 33.0 μm] ($N = 30$) (Figs. 4B, 5D, 7A, B).

Triactines II. They are regular, equiradiate, and equiangular. Actines are conical, with slightly sharp tips. Most lay tangentially to the surface and their size is very variable. Few can be found scattered in the choanosome, laying perpendicular to the atrium [257.4–696.2 (±279.7) – 1181.5/33.0–102.1 (±46.2) – 194.6 μm] ($N = 30$) (Figs. 4C, 5E, 7C, D).

Tetractines I. The basal system of these spicules is similar to that of triactines I. Apical actines are conical and smooth, with slightly sharp tips. They are straight or often undulated, with a single bend near the tip. Most tetractines I are tangentially aligned and densely packed around subcortical holes, choanosomal canals and the atrium. Nevertheless, apical actines only protrude conspicuously into exhalant canals and into the atrium. Very rarely, they are scattered in the choanosome, always in proximity to the canals, laying perpendicularly to the atrium. Sagittal tetractines may also be found [105.6–137.4 (±24.1) – 224.4/9.9–15.4 (±3.6) – 26.4 μm] ($N = 30$) (Figs. 4D–F, 5F, G, 7E, F).

Tetractines II. The basal system of these spicules and their distribution are similar to those of triactines II. These spicules can be abundant, rare or even be absent. Apical actines are conical, straight and smooth, and penetrate the choanosome [278.0–665.5 (±301.0) – 1042.5/48.7–102.5 (±51.3) – 180.7 μm] ($N = 8$) (Figs 4G, H, 5H, 7G, H).

Ecology and biogeography: Leucetta floridana can be found in semishadowed environments in reef terraces and slopes, where it can be encrusting in small crevices or erect under overhangs and on vertical slopes. This species seems to have a patchy distribution within a reef and is in general rare. It does not show any sign of predation or fouling. Nonetheless, if hard substrate is limited, it enters into direct contact with other organisms (i.e. corals and other sponges), when it shows external morphological alterations but no sign of tissue injury.

The presence of *L. floridana* in the Caribbean and Brazil provides new support for the existence of a sole zoogeographical province in the western tropical Atlantic. The Amazon River outflow penetrates 500 km offshore and 30 m deep (Rocha, 2003). Such discharge of freshwater could represent a significant barrier to gene flow between Caribbean and Brazilian populations. However, as observed for some other marine species (Lazoski *et al.*, 2001; Rocha, 2003; Wörheide *et al.*, 2005), *L. floridana* seems capable of maintaining gene flow between the two areas. There are no studies on the reproduction of *Leucetta* species, consequently we do not know if larvae of this genus have a long duration or not. Studies on reproduction of calcareous sponges that measured time to larval settlement showed that it takes from a few hours to a maximum of three days (Minchin, 1896; Amano & Hori, 2001; Leys & Eerkes-Medrano, 2005). Hence, it

is unlikely that larvae of *L. floridana* can cross the Amazon barrier between the Caribbean and Brazil. It seems more likely that the species has a continuous distribution including populations below the Amazon plume, in the sponge corridor found by Collette & Rützler (1977). To be sure about this, studies on the reproduction of *L. floridana* and collections under the Amazon plume should be conducted.

CONCLUSION

Based on morphological and genetic analyses, we conclude that *L. floridana* and *L. microraphis* are valid species. *Leucetta floridana* has two size categories of tetractines, whereas *L. microraphis* has only one. Also, *L. floridana* has thicker tetractines I, a larger atrial cavity, and a ridged surface. The two species are also genetically highly divergent in their internal ribosomal spacers. A second species of *Leucetta*, probably new to science, was also found in Brazil. This species can be differentiated from *L. floridana* based on spicule size (length of triactine II), by molecular data, by the absence of an atrium, and by its smooth surface. Our results reveal a widespread distribution of *L. floridana* in the western Atlantic and provide new support for the existence of a sole zoogeographical province in the western tropical Atlantic, joining the Caribbean and Brazilian faunas.

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