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## Research article

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# *Leptoclinides* (Ascidiacea, Didemnidae) from Brazil: new records and two new species

Livia M. OLIVEIRA<sup>1,\*</sup>, João Paulo CARVALHO<sup>2</sup> & Rosana M. ROCHA<sup>3</sup>

<sup>1,2,3</sup>Systematics and Ecology of Marine Invertebrates Laboratory, Zoology Department,  
Universidade Federal do Paraná, CP 19020, 81531-980 Curitiba, Brazil.

\*Corresponding author: [liviamoura87@gmail.com](mailto:liviamoura87@gmail.com)

<sup>2</sup>Email: [joaopcarvalho@ufpr.br](mailto:joaopcarvalho@ufpr.br)

<sup>3</sup>Email: [rmrocha@ufpr.br](mailto:rmrocha@ufpr.br)

<sup>1</sup>[urn:lsid:zoobank.org:author:2058206A-50D4-4956-8888-358701793D7F](https://zoobank.org/author:2058206A-50D4-4956-8888-358701793D7F)

<sup>2</sup>[urn:lsid:zoobank.org:author:D5916EB9-A22E-4821-824C-B17B7434026D](https://zoobank.org/author:D5916EB9-A22E-4821-824C-B17B7434026D)

<sup>3</sup>[urn:lsid:zoobank.org:author:E170DE7A-DB70-4E5B-8488-45FA680812DA](https://zoobank.org/author:E170DE7A-DB70-4E5B-8488-45FA680812DA)

**Abstract.** Twenty-two samples of *Leptoclinides* Bjerkan, 1905 collected along the Brazilian coast between 1998 and 2017 were examined. Herein we describe two new species (*Leptoclinides coronatus* sp. nov. and *Leptoclinides lotufoi* sp. nov.). We also extend the distribution of *L. latus* F. Monniot, 1983 and report that, for the first time, *L. torosus* F. Monniot, 1983 was found outside its type locality.

**Keywords.** Biodiversity, faunal survey, taxonomy, Tunicata, West Atlantic.

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## Introduction

Didemnidae Giard, 1872 has nine valid genera, distributed in all the oceans and depths, of which just six have been reported in the Atlantic waters. *Leptoclinides* is one of the most diversified genera in this family. It was proposed by Bjerkan (1905) when he described *L. faeroensis* Bjerkan, 1905, which is a boreal, Atlantic Ocean species (Carlisle & Carlisle 1954). Today, the genus is characterized by thick colonies with a wide variety of colors that can change even within the same species (Monniot 1989). Cloacal cavities are wide, with smooth or lobed common cloacal apertures (e.g., *L. latus* F. Monniot, 1983 and *L. cavernosus* Kott, 2001), or may be elevated and form a funnel or conical protuberance (e.g., *L. albamaculatus* Kott, 2001 and *L. coelenteratus* (Kott, 1962)). Typically, spicules are stellate, large (diameter > 50 µm), with conical, sometimes truncated, rays. Zooids have a tubular atrial siphon with a small opening (*L. latus* has an atrial languet in addition to the tube), a thorax with conspicuous muscle fibers that do not form a muscular process and a pharynx with four rows of stigmata. The testis is usually divided into several follicles, although it is undivided in the type species (*L. faeroensis*). The sperm duct is spiral, usually with five or fewer loose turns. Larvae, which are not gemmiparous, tend to be > 0.5 mm in length, and the oozoid in the larva have four rows of stigmata (Monniot *et al.* 1991; Kott 2001).

The genus *Leptoclinides* comprises 77 valid species (Shenkar *et al.* 2019), of which five are known from the Atlantic Ocean (Rocha *et al.* 2012; Paiva *et al.* 2015): *L. brasiliensis* Michaelsen, 1923, *L. crocotulus* Paiva *et al.*, 2015, *L. faeroensis* Bjerkan, 1905, *L. latus* F. Monniot, 1983 and *L. torosus* F. Monniot, 1983. Only two of these have been reported from coastal Brazil: *L. crocotulus*, currently only known from the Rocas Atoll (Paiva *et al.* 2015), and *L. latus*, described from Guadeloupe but also reported from the Arvoredo Biological Reserve in southern Brazil (Rocha *et al.* 2005).

Here, with this report, the distribution of two species previously known only from the western tropical Atlantic has been expanded to include Brazil. We also describe two new species. A third, previously undescribed species is presented, but we prefer not to name it due to the lack of larvae, whose diagnostics characteristics are necessary to complete the description of the species and compare it to known valid species.

## Material and methods

Samples were collected from 1998 to 2017 in the states of Bahia, Espírito Santo, Rio de Janeiro, Paraná and Santa Catarina. When collected, colonies were anesthetized with menthol, and then fixed and preserved in 4% formaldehyde neutralized with sodium hydroxide. Specimens are deposited in the Zoology Department Collection at the Federal University of Paraná, Curitiba, Brazil (DZUP) and in the Prof. Edmundo E. Nonato Biological Collection of the Oceanographic Institute of the University of São Paulo (ColBio).

We identified species using the colony, zooid, larval and spicule characteristics listed in Table 1. Specimens were dissected with transverse slices (2 to 4 mm thick) of the colonies, from which we removed zooids and larvae. Following dissection, zooids and larvae were stained with Harris haematoxylin and then mounted on slides using Durcupan® resin for microscopic examination.

Spicules were separated from the tunic and examined using scanning electronic microscopy (SEM) under a JEOL JSM-6360-LV model at the Universidade Federal do Paraná following routine procedures described in Oliveira *et al.* (2019).

## Results

Class Ascidiacea Blainville, 1824  
Order Aplousobranchia Lahille, 1886  
Family Didemnidae Giard, 1872  
Genus *Leptoclinides* Bjerkan, 1905

*Leptoclinides coronatus* sp. nov.

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Fig. 1

## Etymology

The name of this species refers to the crown-like arrangement of the lobes on the surface of the colony around the common cloacae.

## Material examined

### Holotype

BRAZIL • 1 colony; Espírito Santo, Guarapari, Escalvada Island; 20°41'58.77" S, 40°24'82" W; 8 m depth; 28 Mar. 2017; R.M. Rocha leg.; DZUP LEP-018, slide DZUP DID3.84.

**Table 1.** Key for the identification of the Atlantic species in *Leptoclinides* Berjan, 1905. Characters and abbreviations: **1.** Colour of living colony: Be = beige; Bg = brownish grey; Bl = black; G = grey; O = orange; S = salmon; V = violet; Y = yellowish. **2.** Colony shape: G = globular; L = laminar. **3.** Spicule distribution: R = restricted to the upper layer of the colony; W = whole colony. **4.** Spicules: D = high density; L = low density. **5.** Shape of the spicules: G = globular; S = stellate. **6.** Common cloacal aperture: L = lobed; S = smooth; P = with large projections. **7.** Size of the spicules ( $\mu\text{m}$ ). **8.** Number of muscular fibers on each side of the thorax. **9.** Atrial languet: 0 = absent; P = present. **10.** Number of coils of the sperm duct. **11.** Lateral thoracic organ position in relation to stigmata row. **12.** Number of testis follicles. **13.** Number of coils of the sperm duct. **14.** Size of larval trunk (mm). **15.** Number of pairs of ectodermal ampullae in larvae (unpaired ampullae).

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Species
O	L	R	L	S	S	30	12	0	10/12-10-10-9/10	3	3	3	0.8	9-10	<i>L. lotufol</i> sp. nov.
Be, G, Y	L	R	L, D	S	S	35-50	?	0	11/12-11-11-9	3-4	1	4-5	1.2-1.5	8-12	<i>L. faeroensis</i>
O, S	L	R	D	G	S	15-25	8-14	P	12/14-12/13-12/13-12/13	2-3	3-4	3	0.7-0.8	8	<i>L. latus</i>
O	L	W	D	G	L	12	7	0	10-9-9-8	3	3	2-3	?	?	<i>L. crocotilus</i> <sup>1</sup>
Bl, G, V	L	W	L, D	S	S	30-70	19-20	0	13-12-12-10/12	3-4	3-6	4-7	0.65-0.75	4	<i>L. torosus</i>
O, G	L	W	L	S	P	20-30	8	0	10-10-9-8	4	4-6	4-7	0.7	2(2)	<i>L. coronatus</i> sp. nov.
Bg, G, Y	L	W	L	S	?	<75	6-7	0	13	2-3	2-3	6	?	?	<i>L. brasiliensis</i> <sup>2</sup>
Be	G	W	L	S	S	30-55	5-6	0	8/10-8/10-8/10-7/9	3	3	4-6	?	?	<i>Leptoclinides</i> sp.

<sup>1</sup> Characteristics based on Paiva *et al.* (2015). <sup>2</sup> Characteristics based on Michaelsen (1923). ? no information available.

### Paratypes

BRAZIL • 1 colony; Espírito Santo, Guarapari, Ilha Rasa de Terra; 20°40'32" S, 40°22'1" W; 12–15 m depth; 27 Mar. 2017; R.M. Rocha leg.; DZUP LEP-016, slide DZUP DID3.82 • 1 colony; same collecting data as for holotype; DZUP LEP-020 • 1 colony; same collecting data as for holotype but 29 Mar. 2017; DZUP LEP-019.

### Description

Colonies were found exposed on granitic substrate between 8 and 15 m. They have an orange and white marbled appearance, and colony shape follows that of the substrate (Fig. 1A). Colonies can be 2–5 cm in diameter and 1–2 mm thick. Color in fixed specimens becomes grayish. Common cloacal apertures are wide, white with conical projections with many spicules along the margin (Fig. 1B). The tunic is cartilaginous with a smooth surface. Spicules are 20–30  $\mu\text{m}$  in diameter, uniformly, but not densely, distributed throughout the tunic. Spicules are star-like with 6 conical rays in cross section and, at the base, the rays are separated from one another by a matrix covered by small, rounded projections (Fig. 1C).

Zooids are about 1.6 mm long, with the thorax twice the length of the abdomen. The oral siphon is tubular, 0.44 mm long, with six short and rounded lobes, sometimes not easily visible. On each side of the thorax there are eight muscular fibers that extend from the oral siphon to just anterior to the rectal esophageal peduncle. The atrial siphon is long and oriented downward (Fig. 1D). Occasionally the atrial opening appears short, probably due to the position of the zooid within the colony. Both siphons have conspicuous circular musculature.

The pharynx has 10 stigmata per row on each side, except the last row, which has fewer stigmata (about 8–9). Stigmata are narrowly fusiform and slightly wider at the center. The thoracic organ is round, not raised, normally smaller than the size of the fold and located at the level of the fourth row of stigmata, almost in the middle of the thorax. The thoracic organ is often full of spicules.

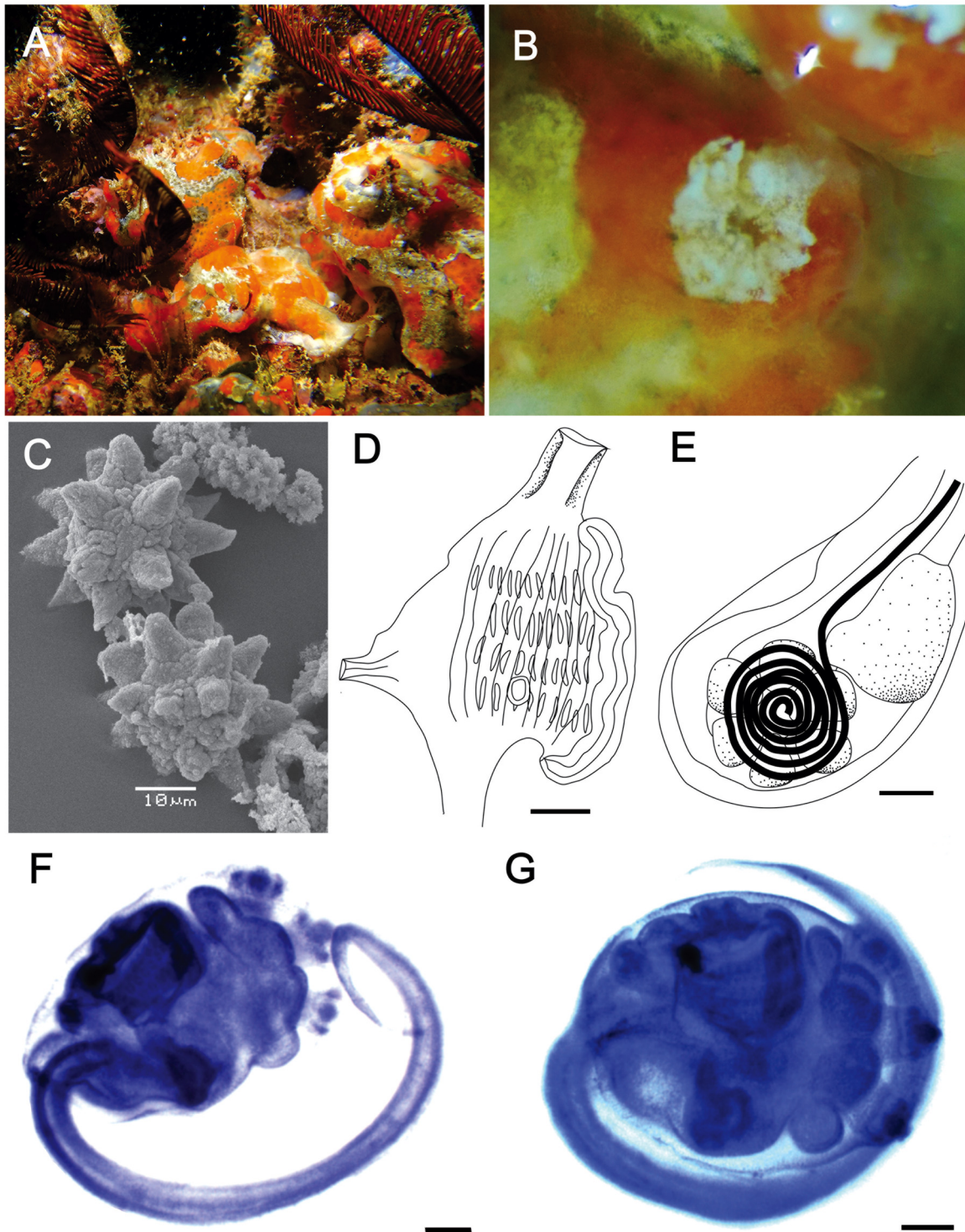
The abdomen is vertical, with a short and narrow esophagus. The stomach is large, elongate, and it is not overlapped by the intestinal loop (Fig. 1E). The testis has six rounded follicles. The sperm duct is spiral, with 4–6 counter-clockwise turns.

The larval trunk is ovoid and  $\sim 0.7$  mm in length. The tail curves  $\frac{3}{4}$  of the way around the trunk. Three adhesive papillae are equally spaced with short, wide peduncles. Two pairs of large ectodermal ampullae are centrally located, and there is also one single dorsal and ventral ampullae, both smaller, for a total of six ampullae. The sensorial vesicle is posterior and the statocyte either anterior or ventral to the ocellus. Mature larvae have four rows of stigmata in the pharynx, with 10 stigmata in the first three rows and nine stigmata in the last. No pigment or ectodermal vesicles are present.

### Remarks

*Leptoclinides coronatus* sp. nov. is very distinctive in comparison with the other congeners found in coastal Brazil. *Leptoclinides crocotulus* and *L. latus* are orange, but uniformly so and without swaths of white. *Leptoclinides crocotulus* is also different by the different shape and smaller (12  $\mu\text{m}$ ) size of spicules, the different size and shape of the siphons, smaller lateral thoracic organ and three testicular follicles with two or three turns of the sperm duct (Paiva *et al.* 2015). It is also different from *L. latus*, which has a globular-type spicule, an atrial languet and fewer turns of the sperm duct.

The Australian *L. imperfectus* (Kott, 1962) is marbled with orange and gray on a white background, but differs more clearly from *L. coronatus* sp. nov. in having papillae on the surface of the colony, a very long esophago-rectal peduncle, 6–8 testicular follicles and seven turns of the sperm duct and slightly



**Fig. 1.** *Leptoclinides coronatus* sp. nov., holotype (DZUP LEP-018). **A.** Colony *in situ*. **B.** Detail of the cloaca with lobes. **C.** Spicules. **D.** Thorax. **E.** Abdomen. **F–G.** Larvae in different stages in the same colony. Scale bars: D–G = 0.1 mm.

smaller larvae (trunk ~ 0.6 mm long) with four pairs of ectodermal ampullae (Kott 2001). Also marbled, *L. marmoreus* Brewin, 1956 differs in having a smooth-margin cloaca and nine turns of the sperm duct (Brewin 1956), while *L. marmoratum* (Sluiter, 1909) is not orange, but rather gray or dark blue, has longer (3 mm) zooids and spicules in a narrow layer under the bladder cells, in addition to small groups of spicules scattered around the tunic (Sluiter 1909; Millar 1975).

### Distribution

Type locality: Brazil: Espírito Santo.

### *Leptoclinides latus* F. Monniot, 1983

Fig. 2

*Leptoclinides latus* F. Monniot, 1983: 15, fig. 4, pl. 1B.

*Leptoclinides latus* – Rocha *et al.* 2005: 468, figs 6–8.

### Material examined

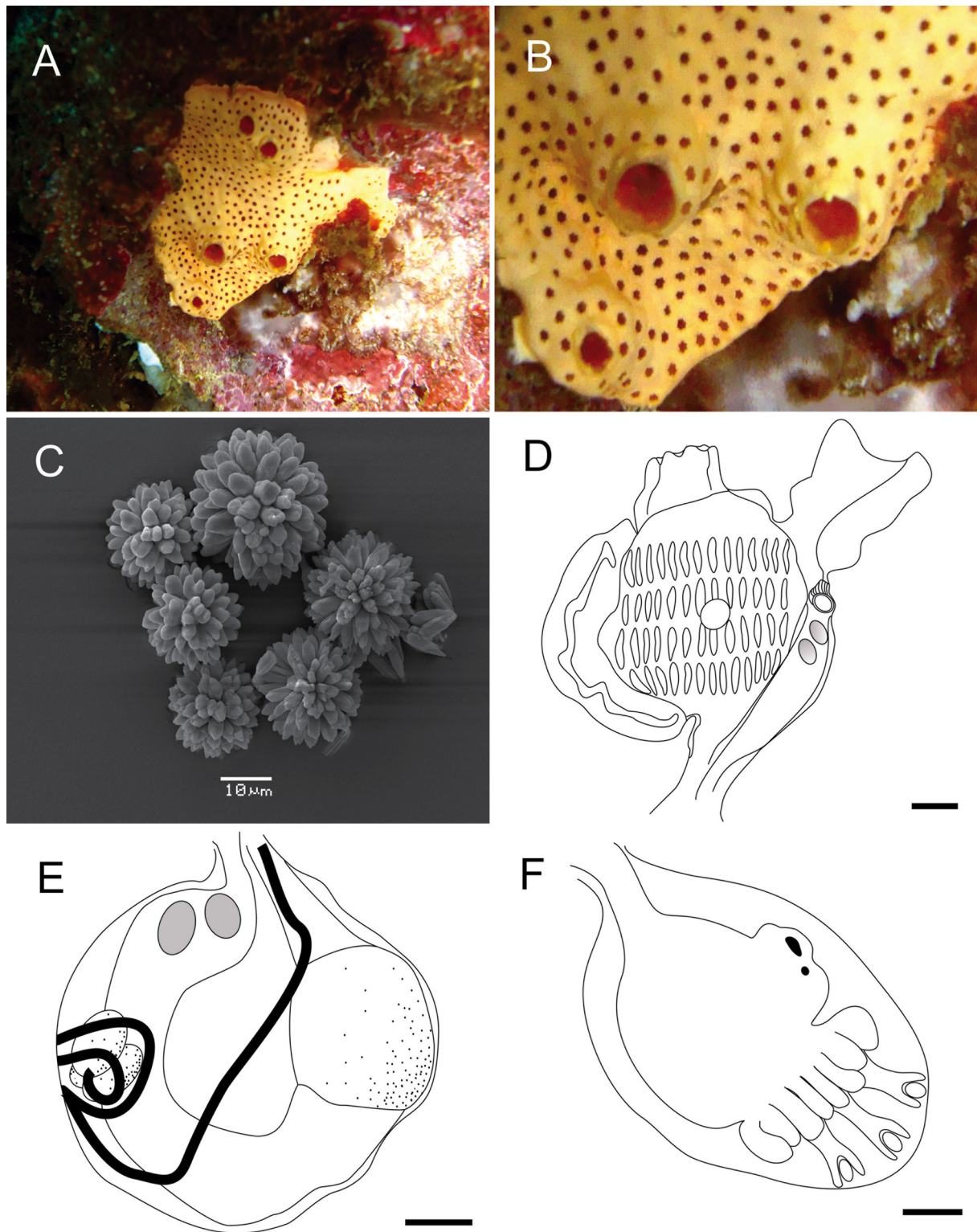
BRAZIL • 1 colony; Bahia, Salvador, São Marcelo Fort; 12°58'6" S, 38°31'5" W; 1 Aug. 1999; R.M. Rocha leg.; DZUP DID-185, slides DZUP DID3.32, DZUP DID3.33 • 1 colony; Espírito Santo, Guarapari, Escalvada Island; 20°41'59" S, 40°24'82" W; 13 m depth; 12 Feb. 2011; R.M. Rocha leg.; under rocks; DZUP LEP-008, slide DZUP DID3.79 • 2 colonies; same locality as preceding but 20°42'00" S, 40°24'30" W; 10 m depth; 26 Jan. 2012; G.A. Gamba leg.; DZUP LEP-010, DZUP LEP-021, slide DZUP DID3.80 • 2 colonies; same locality as preceding but 20°41'58.77" S, 40°24'27.82" W; 27 Jan. 2012; 11 m depth; G.A. Gamba leg.; DZUP LEP-013, DZUP LEP-015 • 1 colony; Guarapari, Beluccia shipwreck; 20°40'41" S, 40°21'41" W; 20 m depth; 28 Mar. 2017; R.M. Rocha leg.; DZUP LEP-014 • 1 colony; Rio de Janeiro, Arraial do Cabo, Saco do Cordeiro; 22°57'54" S, 41°59'57" W; 7–9 m depth; 10 Apr. 2001; R.M. Rocha leg.; under rock; DZUP DID-145 • 1 colony; Cabo Frio, Comprida Island; 22°53'23" S, 41°57'17" W; 29 Mar. 2011; R.M. Rocha leg.; DZUP LEP-012 • 1 colony; same locality but 31 Mar. 2011; DZUP LEP-022 • 1 colony; Papagaio Island; 22°53'55" S, 41°58'42" W; 9 May 2014; L.F. Skinner leg.; DZUP LEP-011 • 1 colony; São Paulo, São Sebastião, Alcatrazes Island; 24°06'33" S, 42°12'28" W; 8 m depth; 29 Jun. 2012; G.A. Gamba leg.; DZUP LEP-023 • 1 colony; Paraná, Pontal do Paraná, Currais Island; 25°44'12" S, 48°21'58" W; 14 May 2005; R.M. Rocha leg.; DZUP LEP-006 • 1 colony; Santa Catarina, São Francisco Sul, Graças Archipelago; 26°10'51" S, 48°28'55" W; 2 Apr. 2008; R.M. Rocha leg.; DZUP LEP-004 • 1 colony; Tamboretas Archipelago; 26°23'03" S, 48°31'25" W; 3 Mar. 2008; R.M. Rocha leg.; DZUP LEP-003 • 1 colony; Bombinhas, Lagoinha Beach; 27°08'43" S, 48°28'50" W; 14 Feb 1996; R.M. Rocha leg.; intertidal zone; DZUP LEP-007.

### Description

Colonies *in situ* are orangish or salmon in color, with a variation of darker or lighter forms (Fig. 2A–B), becoming white after fixation. Colonies are encrusting, with an irregular or somewhat oval outline, 3–5 cm wide and 2–4 mm thick and are more often found on the underside of rocks. Common cloacal apertures are large and abundant, round with a smooth border that when not well relaxed is somewhat wavy. The tunic is firm and easily broken due to abundant spicules that tend to be absent in the middle and base of the colony. In fixed colonies, the surface appears wrinkled and the oral siphons tend to become more salient. Spicules are globular, 15–30 µm in diameter, with cylindrical, relatively short, blunt, irregularly pointed rays (numbering about 14 in cross section, Fig. 2C).

Zooids, about 0.8–1.2 mm in length, have equally long thorax and abdomen. The oral siphon is short, tubular, with six rounded lobes around the margin. The atrial siphon is small, circular and smooth, in the





**Fig. 2.** *Leptoclinides latus* F. Monniot, 1983 (DZUP LEP-008). **A.** Colony *in situ*. **B.** Surface detail indicating lobed oral siphons and smooth cloacae. **C.** Spicules. **D.** Thorax. **E.** Abdomen. **F.** Larva. Scale bars: D–F = 0.1 mm.

middle of the thorax, between the second and third rows of stigmata. Some zooids have a bifurcate, wide, muscular, dorsal languet. The pharynx has 14 stigmata on each side of the first row and 13 stigmata in the remaining rows. There are six to eight muscle fibers in each side of the thorax. The thoracic organ is small, circular and between the second and third rows of the stigmata (Fig. 2D). The abdomen is vertical. The esophagus is short and narrow. The stomach is globular and is not overlapped by the intestinal loop. The testis has 3–4 small, oval follicles surrounded by two and a half or three, widely separated, turns of the sperm duct (Fig. 2E). Oocytes, present in the zooids of specimens DZUP DID-185 (Bahia) and DZUP LEP-014 (Espírito Santo), are 0.20–0.33 mm in diameter.

Larvae are oval-shaped, with a trunk 0.70–0.85 mm in length. Larvae have three adhesive papillae in a row, with long, slender, equally spaced peduncles, and eight pairs of elongated, ectodermal ampullae (Fig. 2F). The tail curves around  $\frac{3}{4}$  of the trunk. Larvae were found in specimens collected in November in Santa Catarina and in January to March (in immature state) in Espírito Santo.

### Remarks

*Leptoclinides latus* was first reported in the Caribbean in Guadalupe (Monniot 1983). In Brazil, it was first found at the island Arvoredo, in the state of Santa Catarina (Rocha *et al.* 2005). Today, the species is known to have a much wider distribution in Brazil, from Bahia to Santa Catarina, and is the most common Brazilian species in the genus *Leptoclinides*.

A few specimens had singular variations in characters when compared to most others. For example, the specimen from Bahia (DZUP DID-185) had a more open aperture and a longer rather than globular stomach, than is usually seen in most zooids. The colony collected in Cabo Frio (DZUP LEP-011) had five testicular follicles and the sperm duct with two turns only. Despite the variability described above, the remaining characters, including colony color, spicule size and shape, bifurcated atrial languet (where observed), and testis and larval shape and size, together clearly identify this species.

### Distribution

Type locality: Guadeloupe (Monniot 1983). Other localities: Brazil: Bahia, Espírito Santo, Rio de Janeiro, São Paulo and Paraná (this study), Santa Catarina (Rocha *et al.* 2005).

### *Leptoclinides lotufoi* sp. nov.

[urn:lsid:zoobank.org:act:31D0C00F-2263-4826-8CEC-9782A1704D49](https://doi.org/10.3896/urn:lsid:zoobank.org:act:31D0C00F-2263-4826-8CEC-9782A1704D49)

Fig. 3

### Etymology

The name of this species is in homage to Dr Tito Lotufo for his studies in ascidian diversity in northeastern Brazil. He collected this material and lent it to us for this study.

### Material examined

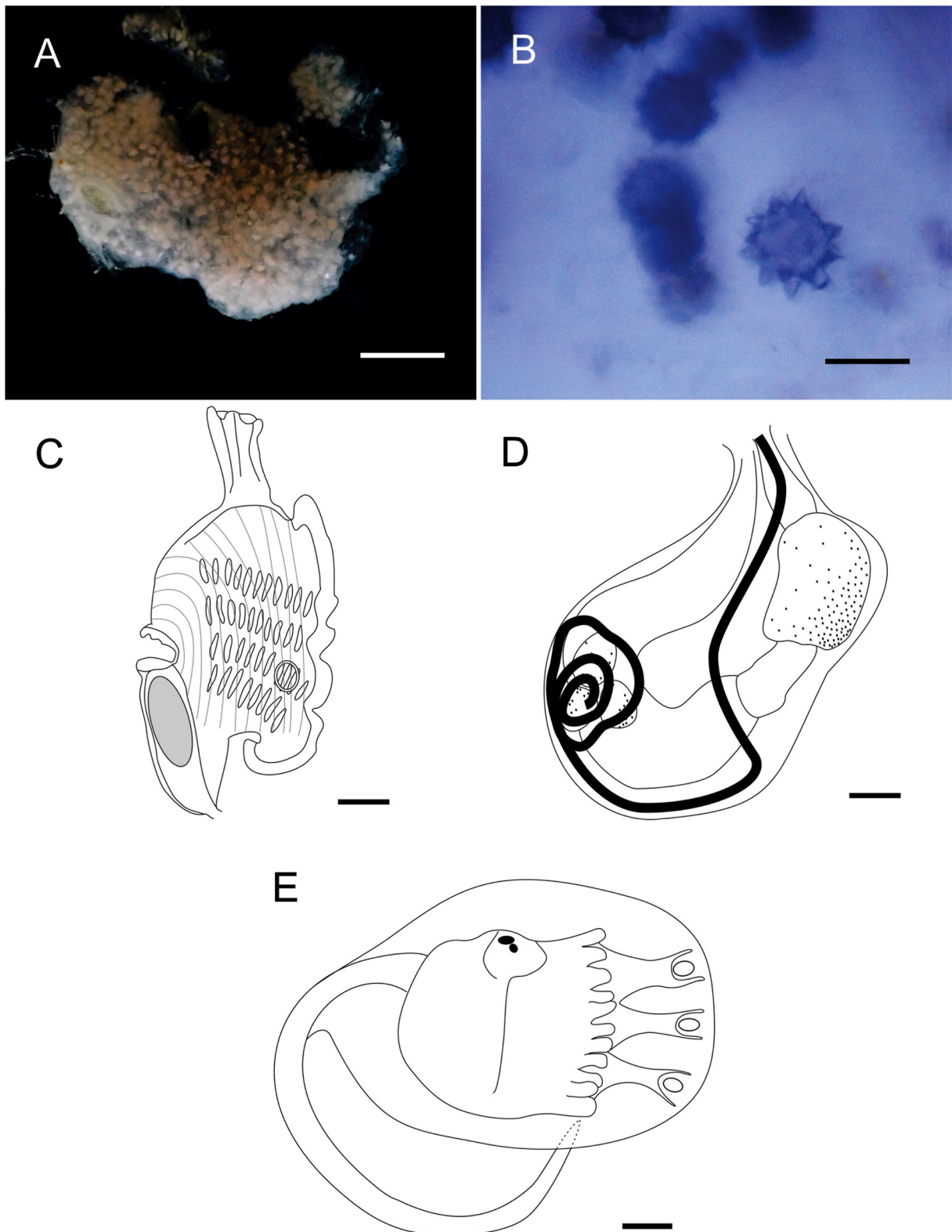
#### Holotype

BRAZIL • 1 colony; Rio de Janeiro, Angra Reis, Ilhota dos Porcos; 22°58'46" S, 44°19'47" W; 10 m depth; 10 Oct. 1998; T.M.C. Lotufo leg.; ColBio Tun 1245.

### Description

The only colony found was attached to a exposed granitic substrate around 10 m deep. This colony was encrusting and small (2 cm wide, 1 mm thick), orange, but lost most of its color when fixed in formalin (Fig. 3A). The tunic is malleable and delicate, with a wide cloacal cavity that extends from the thorax to the region of the esophago-rectal peduncle. Only one common cloacal aperture was seen in this





**Fig. 3.** *Leptoclinides lotufoi* sp. nov., holotype (ColBio Tun 1245). **A.** Preserved colony. **B.** Spicules under light microscope. **C.** Thorax. **D.** Abdomen. **E.** Larva. Scale bars: A = 0.5 cm; B = 30  $\mu$ m; C–E = 0.1 mm.

specimen; it is broad with a smooth margin. Spicules, in low density, almost unnoticeable, are mainly restricted to the surface layer of the tunic. Spicules are 20 to 30  $\mu\text{m}$  in diameter, with 10–12 (in cross section) conical, pointed rays (Fig. 3B). SEM images of the spicules could not be made because of their scarcity and the small size of the colony.

Zooids are orangish, about 1.5 mm long. Twelve muscle fibers run longitudinally along each side of the thorax. The oral siphon is 0.15–0.33 mm long with six rounded lobes. The atrial aperture is very short and opens between the third and fourth rows of the pharyngeal stigmata. The pharyngeal stigmata formula is 10–12, 10, 10, 9–10 per half row. The lateral thoracic organ is round, not salient, usually at about the third row of stigmata, occasionally at the second row, close to the endostyle (Fig. 3C). The stomach is slightly elongated and is not overlapped by the intestinal loop. The testis has three oval follicles, overlain by three open turns of the sperm duct (Fig. 3D).

Most of the larvae we found were immature. Larvae are oval, yellowish, with about 0.8 mm in trunk length, without vesicles. The tail curves about halfway around the trunk. There are three anterior adhesive papillae with long peduncles. Nine to eleven short, digitiform ampullae are on each side of the larva. The sensorial vesicle is mid-dorsal, with the statocyte somewhat anterior and ventral to the ocellus (Fig. 3E).

### Remarks

Although this species is being described from one single colony, its characters were different enough from those of other known species. *Leptoclinides lotufoi* sp. nov. is different from the other Atlantic congeners by having the following characteristics: few spicules restricted to the surface of the colony, few testicular follicles and turns of the sperm duct, and many ectodermal ampullae in the larvae. A few other species of *Leptoclinides* can have many ectodermal ampullae. The Australian *L. brandi* Kott, 2001 has a granular colony surface with a high density of spicules and smaller larvae with eight pairs of ampullae. *Leptoclinides durus* Kott, 2001 has many spicules and larger larvae with eight pairs of ampullae. *Leptoclinides echinus* Kott, 2001 has more testicular follicles (about 15) and larger larvae with eight pairs of ampullae. From New Caledonia, *L. multipapillatus* Monniot, 1989 has more testicular follicles, more turns of the sperm duct and more than 20 ectodermal ampullae (Monniot 1989; Kott 2001).

### Distribution

Type locality: Brazil: Rio de Janeiro.

### *Leptoclinides torosus* F. Monniot, 1983

Fig. 4

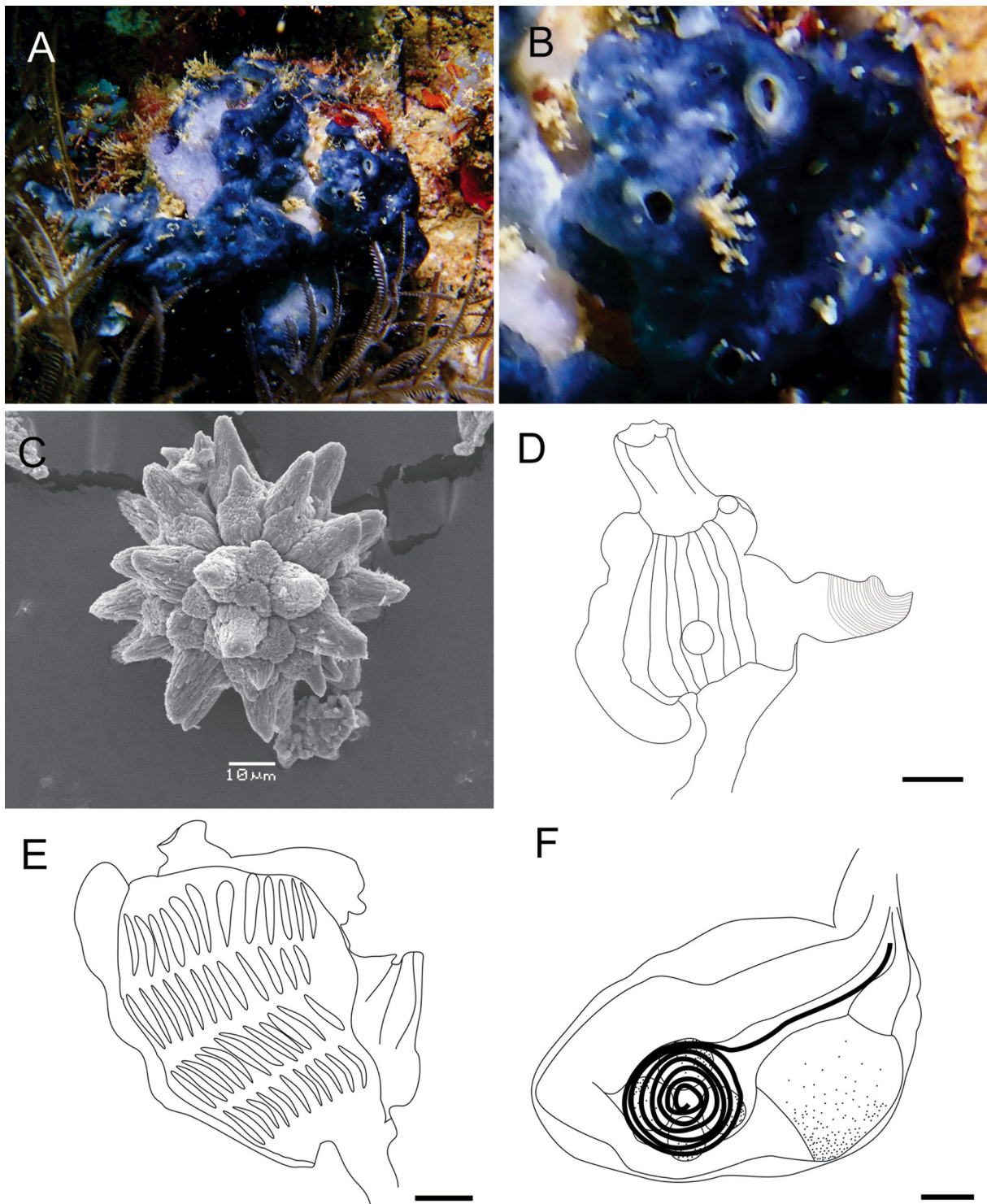
*Leptoclinides torosus* F. Monniot, 1983: 17, fig. 5; pl. 1D.

### Material examined

BRAZIL • 2 colonies; Bahia, Boa Viagem shipwreck; 12°55'56" S, 38°30'50" W; 7–8 m depth; 3 Aug. 1999; R.M. Rocha leg.; DZUP LEP-001, slides DZUP DID3.29 to DZUP DID3.31, DZUP DID-171 • 1 colony; Espírito Santo, Escalvada Island; 20°41'59" S, 40°24'82" W; 8 m depth; 28 Mar. 2017; R.M. Rocha leg.; DZUP LEP-017, slide DZUP DID3.83.

### Description

Colonies were found on exposed granitic substrate around 8 m deep. Brazilian specimens form black colonies *in situ* (Fig. 4A–B) that when fixed turn whitish brown. Colonies are 2.5–6.0 cm wide and 2–4 mm thick. The tunic is firm, smooth and has no encrustations. Spicules are star-shaped, usually



**Fig. 4.** *Leptoclinides torosus* F. Monniot, 1983. **A.** Colony *in situ*. **B.** Detail of the surface of the colony (DZUP LEP-017). **C.** Spicule. **D–E.** Thorax. Atrial siphon inadvertently removed during dissection in E. **F.** Abdomen (DZUP DID-171). Scale bars: D–F = 0.1 mm.

35  $\mu\text{m}$  in diameter; however, the larger ones can reach 70  $\mu\text{m}$  in diameter. The spicules are uniformly distributed in low density in the tunic, with 8–10, usually conical rays in cross section. Between the conical rays there are granulated areas that resemble very short truncated rays (Fig. 4C).

Zooids are  $\sim 1.2$  mm long, with a 0.5 mm long thorax and 0.7 mm long abdomen. The thorax has 7–8 longitudinal muscle fibers on each side (Fig. 4D). The oral siphon is 0.25 mm long, tubular and with six short, rounded lobes along the margin. In some zooids the oral siphon is difficult to see, appearing as though it has no lobes. The atrial siphon is tubular and arises from the base of the thorax, with many circular muscle fibers (19–20) along its length. The siphon opens at the level of the fourth row of stigmata, usually downward oriented. In some zooids, the atrial and oral siphons are about the same length. The thoracic organ is circular, not salient, usually less than or the same size as the stigmata and is at the level of the third (sometimes between the third and fourth) row of stigmata. The pharynx has 12–13 stigmata on each side, in the first three rows, and 10–12 in the last row (Fig. 4E). The abdomen is horizontal, the esophagus short and narrow. The stomach is globular and is not overlapped by the intestinal loop. The testis has four, rarely five, follicles, overlain by six tightly coiled turns of the sperm duct. One specimen (DZUP DID-171, from Bahia) had a testis with four follicles and only four turns of the sperm duct.

Larvae were only found in specimens from Bahia in August. They were immature and so some structures were not visible. The larval trunk was 0.65–0.75 mm long and the pharynx had 9–10 stigmata per half row.

### Remarks

The type location for *L. torosus* is Guadalupe (Monniot 1983), and here we report it for the first time in the southern Atlantic. In our samples, the colonies were thinner, and spicules were not distributed in the same way as in Guadalupe (restricted to the level of the abdomens), nor did they have papillae of spicules on the surface as in Monniot (1983). Otherwise, the color of the colony, spicule shape, number of testicular follicles, turns of the sperm duct and larval size all agree with the original description of the species.

### Distribution

Type locality: Guadeloupe (Monniot 1983). Additional records: Brazil: Bahia and Esp rito Santo (this study).

### *Leptoclinides* sp.

Fig. 5

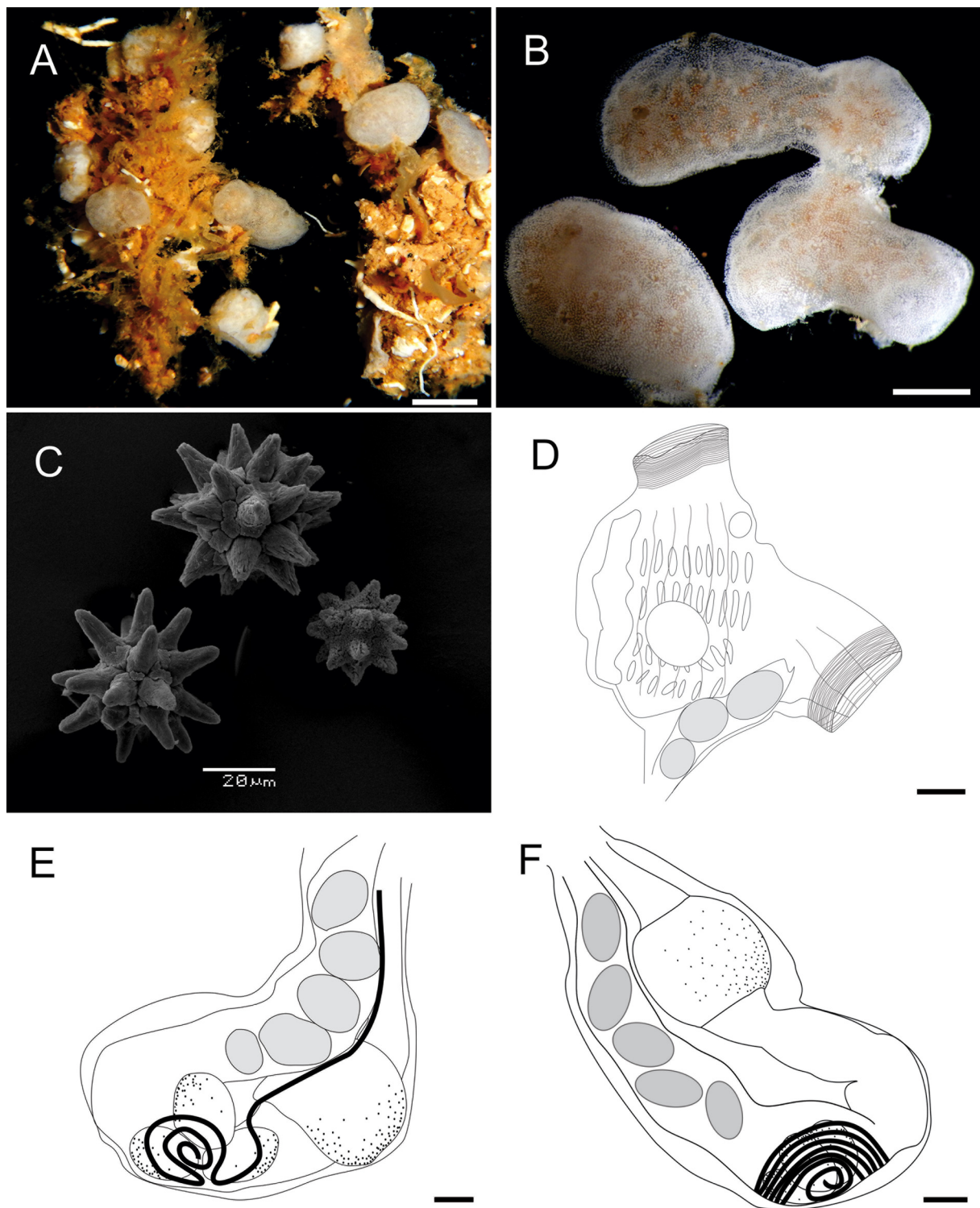
### Material examined

BRAZIL • several colonies; Esp rito Santo, Vila Velha, Pacotes Island; 20°21'04" S, 40°15'12" W; 12 m depth; 13 Feb. 2011; R.M. Rocha leg.; DZUP LEP-009, slide DZUP DID3.81.

### Description

Colonies are globular, small (0.5–1.0 cm wide), beige and translucent when fixed in formalin. Many colonies were found adhering to a branching alga, attached to a horizontal surface of granitic outcrop on the bottom. Many colonies had only one common cloacal aperture, while most colonies had two (Fig. 5A–B). The tunic is smooth and soft. Spicules (30–55  $\mu\text{m}$  in diameter) are low in density and uniformly distributed throughout the colony, mostly next to the surface layer. Spicules are stellate, with six to eight rays in cross-section, and rays are long and conical, with separate bases and granular patches between them (Fig. 5C).





**Fig. 5.** *Leptoclinides* sp. (DZUPLEP-009). **A.** Preserved colony on algae. **B.** Detail of preserved colonies. **C.** Spicules. **D.** Thorax. **E–F.** Abdomen. Scale bars: A = 1 cm; B = 0.5 cm; D–F = 0.1 mm.



Zooids (1–2 mm long) with thorax and abdomen of about the same length. The thorax has 5–6 longitudinal muscle fibers on each side. The oral siphon is short, wide and tubular, with evident circular muscle fibers. Its margin is slightly undulate, without clear lobes. The atrial siphon is downward oriented, tubular, but with a large diameter and without lobes in the aperture. Circular muscle fibers (19–20) are conspicuous. The first three rows in the pharynx have 8–10 stigmata, while the fourth has 7–9. The thoracic organ is circular, larger than the stigmata and not salient. It is at the level of the third row of stigmata in the middle of the thorax (Fig. 5D). In some zooids the organ is full of spicules.

The esophago-rectal peduncle is usually short and narrow, but some zooids have a long esophagus. The abdomen is vertical. The globular stomach may be slightly overlapped by the intestinal loop. There are three stoloniferous vessels of different sizes, with the largest being half the length of the abdomen. The testis has three follicles, surrounded by 4–6 counter-clockwise (few zooids with clockwise direction) turns of the sperm duct (Fig. 5E–F). Larvae were absent.

### Remarks

This lot was different from all others from Brazil or the Atlantic Ocean. A unique feature of this species was the globular, aggregated, small colonies. Despite their color, stellate spicule shape and male reproductive system, all of which are similar to those of *L. brasiliensis*, this species comprises a thin encrusting colony, with larger (2 ×) spicules, and an atrial siphon with a smaller diameter. While we suspect that this may be an undescribed species, we prefer not to name it here, due to the lack of diagnostic larvae and the few colonies we examined. It is possible that all the colonies we examined are part of the same clone and arose by fission, which is suggested by its aggregation, the small colony size and the usually single common cloaca.

### Distribution

Brazil (Espírito Santo).

### Discussion

*Leptoclinides* is the third most species-rich genus in the Didemnidae (Shenkar *et al.* 2019), yet only five species were previously known from the Atlantic Ocean (Rocha *et al.* 2012). The greatest number of species are known from the Indo-Pacific region and Australia (Kott 2001, 2004). The three species found in Brazil increase by almost 50% the species richness in the Atlantic Ocean and *L. latus* is the most common species of the genus in Brazil. Of the species described herein, only *L. latus* was found farther south than 20° S, and the rest are restricted to tropical waters. Given that didemnids are usually neglected in general surveys because of identification difficulties, we expect to find more new species in Atlantic tropical waters with further collections and study by specialists.

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Autor(en)/Author(s): Oliveira Livia M., Carvalho Joao Paulo, Rocha Rosana M.

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