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## New species of Didemnidae (Tunicata: Ascidiacea) from the tropical coast of Brazil

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### Abstract

The family Didemnidae includes 20% of all known ascidians with six genera in the Atlantic. Here, we describe five new species in the Didemnidae from central coastal Brazil. Four species are in the genus *Didemnum* and were found in Bahia (*D. aurantium*, *D. flammacolor*, *D. lambertae*, *D. longigaster*), while one *Diplosoma* (*D. citrinum*) was found both in Bahia and Espírito Santo.

**Key words:** Brazil, South Atlantic, taxonomy, biodiversity, *Didemnum*, *Diplosoma*

### Introduction

The family Didemnidae, the most species-rich in the Ascidiacea (Shenkar & Swalla 2011), currently comprises almost 600 species (20% of the total species of ascidians) and the most speciose genus, *Didemnum*, has approximately 230 valid species (Shenkar et al. 2014). The Didemnidae includes nine genera of colonial species whose zooids are less than 3 mm in length and the body is divided into thorax and abdomen. Zooids eliminate water, feces and gametes through an extensive system of cloacal channels that converge and discharge through a few openings on the colony surface. Tunic consistency varies due to the calcareous spicules that may or may not be present and when present, may vary in density (Monniot et al. 1991).

Didemnid genera are recognized primarily by the number of rows of pharyngeal slits in adult zooids and larvae, number of testis follicles in adult zooids, shape of the spermiduct (straight or spiral), shape of the atrial opening (wide, small or tubular) and the presence of calcareous spicules in the colony (Kott 2001). Genera found in the Atlantic are *Didemnum*, *Trididemnum*, *Polysyncraton*, *Leptoclinides*, *Lissoclinum* and *Diplosoma* (Rocha et al. 2012).

Eleven species are currently known from Bahia: *Didemnum ahu* Monniot & Monniot, 1987, *D. apersum* Tokioka, 1953, *D. candidum* Savigny, 1816 (probable mis-identification), *D. granulatum* Tokioka, 1954, *D. ligulum* Monniot, 1983, *D. perlucidum* Monniot, 1983, *D. psammatodes* (Sluiter, 1895), *D. speciosum* Herdman, 1886, *D. vanderhorsti* Van Name, 1924, *Diplosoma listerianum* (Milne-Edwards, 1841) (registered as *Diplosoma macdonaldi* Herdman, 1886) and *Trididemnum orbiculatum* Van Name, 1902 (Van Name 1945; Lotufo 2002). Eleven species are also known from Espírito Santo: *Didemnum galacteum* Lotufo & Dias, 2007, *D. granulatum*, *D. ligulum*, *D. perlucidum*, *D. psammatodes*, *D. rodriguesi* Rocha & Monniot, 1993, *D. vanderhorsti*, *Diplosoma glandulosum* Monniot, 1983, *Leptoclinides brasiliensis* Michaelsen, 1923, *Leptoclinides latus* Monniot, 1983, and *Trididemnum orbiculatum* (Lotufo 2002; Lotufo & Dias 2007).

Here, we describe five new didemnid species from Bahia, one of which was also found in Espírito Santo.

## Material and methods

Animals were collected in Bahia in 2004 and 2007 and in Espírito Santo in 2012 (Fig. 1), by SCUBA diving at depths to 15 m. Artificial and natural substrates were sampled. In Bahia, sampling locations included Boião da Barra, Canal Madre Deus, Yatch Club, Germânia wreck, Ondina, Porto da Barra, Quebramar Norte and Quebramar Sul (Fig. 1A). In Espírito Santo, samples were taken at Escalvada Island (Fig. 1B).

Animals were photographed *in situ* and then collected with substrate or by removal from the substrate. Samples were placed in menthol diluted in sea water for two hours for relaxation and then fixed in 4% formaldehyde. Anesthesia, fixation and dissection procedures followed Monniot & Monniot (1972).



**FIGURE 1.** Atlantic coast of Brazil, highlighting the states of Bahia (A) and Espírito Santo (B) with sampling localities: Boião da Barra (1), Canal Madre de Deus (2), Yatch Club (3), Germânia wreck (4), Ondina (5), Porto da Barra (6), Quebramar Norte (7), Quebramar Sul (8), Ilha Escalvada (9).

Holotypes were deposited in the Museum of Zoology, University of São Paulo (MZUSP) and paratypes in the collection of Ascidiacea, Department of Zoology, Federal University of Paraná (DZUP), Brazil.

## Results

Five new species are described here followed by an identification key for Brazilian species in the genus *Didemnum* (Tab. 1).

**TABLE 1.** Tabular key for the identification of specimen of the genus *Didemnum* in the Brazilian coast. Characters based on present study and/or literature.

1. Living colony color: B—black, Be—beige, Br—brown, Bc—brick, Dp—dark-purple, G—gray, O—orange, R—red, W—white.
2. Colony surface: P—with papillae, R—reticulated, S—smooth.
3. Spicule density: A—abundant in the whole colony, S—abundant only on the surface, L—low abundance.
4. Maximum spicule size (µm).
5. Number of spicule rays in optical transverse section.
6. Atrial languet: P—present, 0—absent.
7. Number of pharyngeal stigmata in half row.
8. Shape of the lateral organ: C—circular, E—elongate, I—irregular, O—oval, P—protruding, V—variable, Z—z-shape.
9. Length of the muscular process: L—longer than the abdomen, M—as long as the abdomen, S—shorter than the abdomen.
10. Number of testis follicles.
11. Number of coils of the sperm duct.
12. Maximum larval trunk size (µm).
13. Number of pairs of ectodermal ampullae in larvae.

1	2	3	4	5	6	7	8	9	10	11	12	13	Species
Be, Br, Bc	P	A	35	7–9	0	6, 6, 5, 4	O, E	M, L	1	6–7	0.4	4	<i>D. granulatum</i>
W, G	P	S	33	10–12	0	?	E, P	S	1	6–8	0.6	4	<i>D. apersum</i>
W	S, P	A, S	40	6	0	7–8, 7, 6–7, 5–6	O, E	L	1	8	0.5	4	<i>D. perlucidum</i>
W	S	A	30	9	0	9, 8, 7, 6	O	S	2	7–8	0.8	4	<i>D. galacteum</i>
W	S	A	40	6	0	5	C, O, P	S	1	6–7	0.4	4	<i>D. speciosum</i>
W, O	S	A	30	4*	0	5, 4, 4, 3	O	S	1	7–9	0.4	4	<i>D. tetrahedrum</i>
B, Br, Dp	S	S, L	50	8–10	0	10, 9, 9, 8	C	S	1	6–9	1.5	6–1 0	<i>D. cineraceum</i>
Br, G	S	L	30	11–13	0	8, ?	I	L	1	6–8	0.5	4	<i>D. psammatores</i>
B, Br, Dp	S	L	35	12	0	6, 7, 7, 6	O	L	1	9	0.5	4	<i>D. vanderhorsti</i>
W, Be	S	L	60	8–10	0	6, 6, 5, 4	O, P	L	1	6–8	0.4	4	<i>D. ahu</i>
Be	S	A	100	10	0	5–6	C	L	1	8	0.4	6	<i>D. longigaster</i>
O	S	A	50	10	P	10, 9, 6, 6	C	S	1	6–9	0.75	10	<i>D. ligulum</i>
O	S	A	30	8	0	5–7	C, I, O, P	S	1	8–10	0.5	4	<i>D. aurantium</i>
R	S	A	25	6	0	6–9	I, P	S	1	7	0.4	5	<i>D. lambertae</i>
R	S	A	20	7–10	0	6–7	C, P	S	1	9	0.4	4	<i>D. flammacolor</i>
R, O	R	A	40	6	0	7, 7, 7, 5	E	M	1	6–9	0.6	8	<i>D. rodriguesi</i>

\* total number of rays

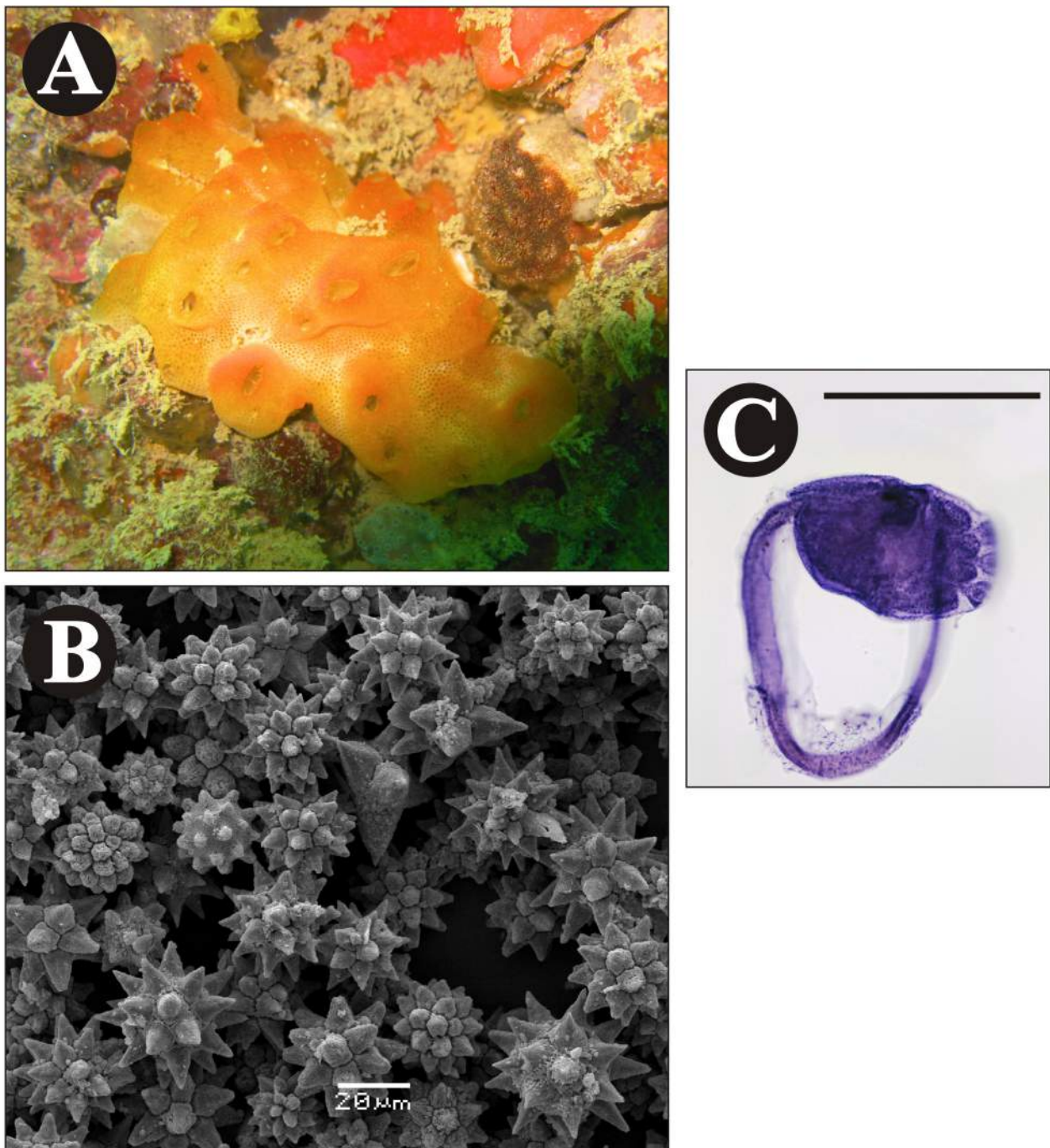
### *Didemnum aurantium* sp. nov. Rocha & Neves

(Figs 2, 3)

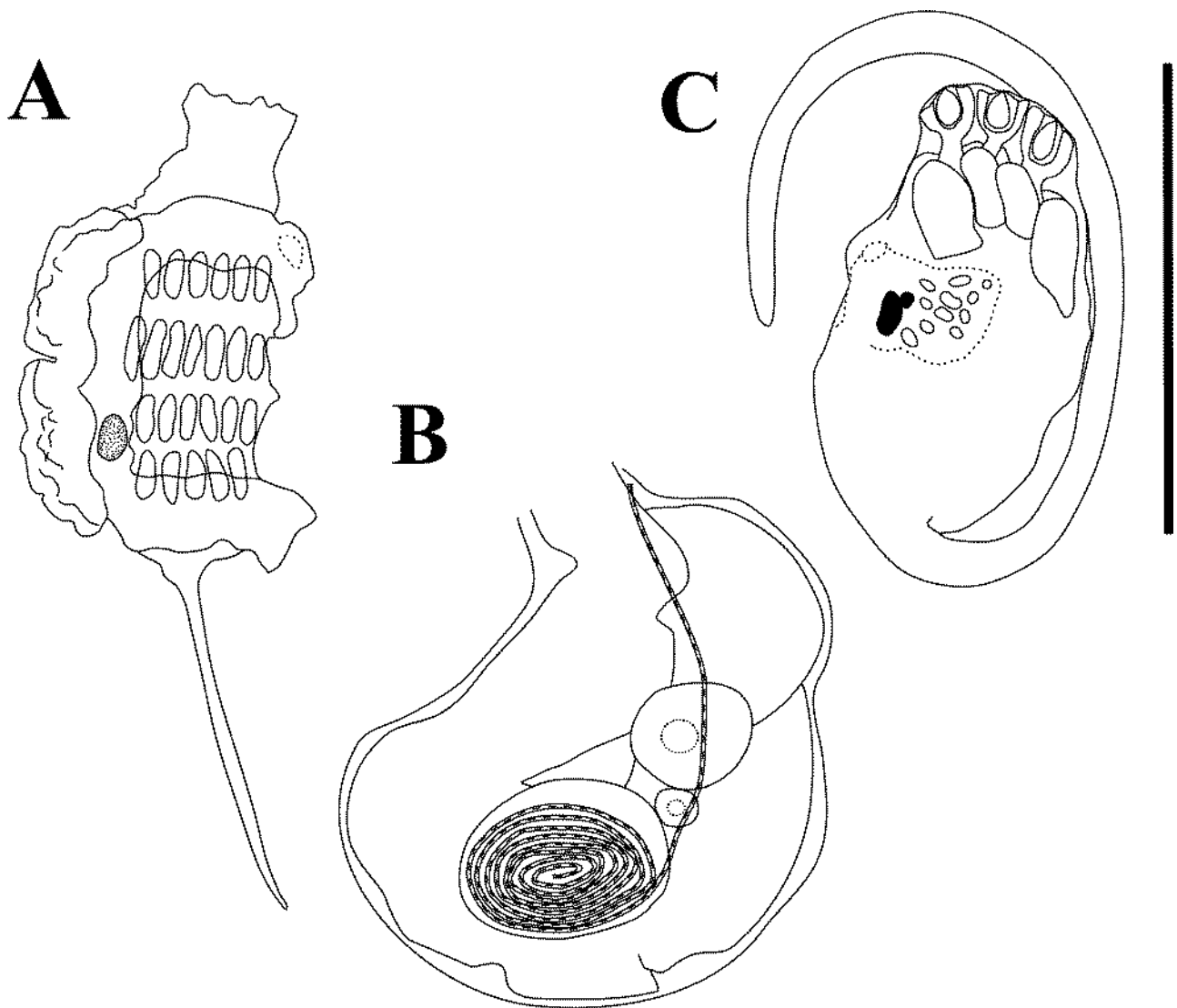
**Examined material:** Holotype: MZUSP 00050 one colony, Quebramar Norte, Salvador, Bahia, 12°57'52" S,

38°30'57" W, 5 m, Col. R. M. Rocha, 11.12.2007. Paratypes: DZUP DID-354 one colony, Canal Madre Deus, Salvador, Bahia, 12°45'48" S, 38°39'17" W, Col. R. M. Rocha, 07.06.2004; DZUP DID-355 one colony, Quebramar Norte, Salvador, Bahia, 12°57'52" S, 38°30'57" W, Col. R. M. Rocha, 08.06.2004; DZUP DID-356 one colony, Quebramar Sul, Salvador, Bahia, 12°58'22" S, 38°31'09" W, Col. R. M. Rocha, 10.06.2004; DZUP DID-357 one colony, Naufrágio Germânia, Salvador, Bahia, 13°00'34" S, 38°31'59" W, 6.5 m, Col. R. M. Rocha, 10.12.2007; DZUP DID-358 one colony, Iate Clube, Salvador, Bahia, 12°59'57" S, 38°31'52" W, 3 a 4 m, Col. R. M. Rocha, 13.12.2007; DZUP DID-384 7 colonies, Quebramar Norte, Salvador, Bahia, 12°57'52" S, 38°30'57" W, 5 m, Col. R. M. Rocha, 11.12.2007.

**Etymology.** The name refers to the bright orange color of the colony under water.



**FIGURE 2.** *Didemnum aurantium* sp. nov. A, living colony. B, spicules. C, larva. Scale bar: C = 0.5 mm.



**FIGURE 3.** *Didemnum aurantium* sp. nov. A, thorax. B, abdomen. C, larva. Scale bar: 0.5 mm.

The colonies are bright orange (ranging between darker and lighter oranges) with slightly darker cloacal cavities (Fig. 2A) and variable in size, ranging from 1.5 to 5.0 cm in length. When filled with water the large cloacal cavities give the colony surface a hilly appearance. When fixed in formalin, colonies are very thin (about 1 mm) and become white with some rose-colored spots. Clumps of orange pigment were found in the tunic during the dissection of some colonies. Spicules are abundant in the surface layer of the colony, giving them a firm, brittle consistency. Spicules are less abundant in the middle and lower layers of the tunic. Spicules which are smaller (30 mm) are stellate and have eight long or short conical rays in optical transverse section. Larger spicules (40 to 50 mm) tend to have longer and fewer rays (Fig. 2B). Zooids are about 1 mm long and are difficult to remove from the tunic. Oral siphons have six small lobes. The atrial aperture is wide and exposes most of the pharynx and the atrial languet is absent. The muscular process projects from the base of the thorax and is variable in length, but is usually shorter than the abdomen. Thoracic lateral organs protrude, with variable shape, 35 to 45  $\mu$ m in maximum length, and they are located between the third and fourth row of pharyngeal stigmata. The pharynx has four rows of stigmata and 5 to 7 slits per row on each side (Fig. 3A).

The abdomen is slightly larger than the thorax and stolonial vessels were not observed. The stomach is rounded. The duodenum is shorter than the stomach. The intestinal loop forms a shallow secondary loop, that can pass beside the stomach or slightly overlay it. Gonads are within the intestinal loop. The testis has a unique and spherical follicle surrounded by 8 to 10 narrow and tightly packed turns of the sperm duct (Fig. 3B). The ovarium has a large (0.2 mm) and smaller oocyte. Budding in the esophageal region was observed in a few zooids.



Mature larvae were found in only one colony that was collected during the winter (June 2004) and immature larvae were found in a single colony during the summer (December 2007). Larvae are more elongate than wide (0.47 mm trunk length) and the tail winds three-quarters of the way around the trunk. Larvae are not pigmented, have three linearly arranged adhesive papillae on thin stalks, plus four pairs of finger-like ectodermal ampullae. The sensory vesicle (with otolith and ocellus) is in the mid-dorsal region of the larval trunk (Figs. 2C, 3C).

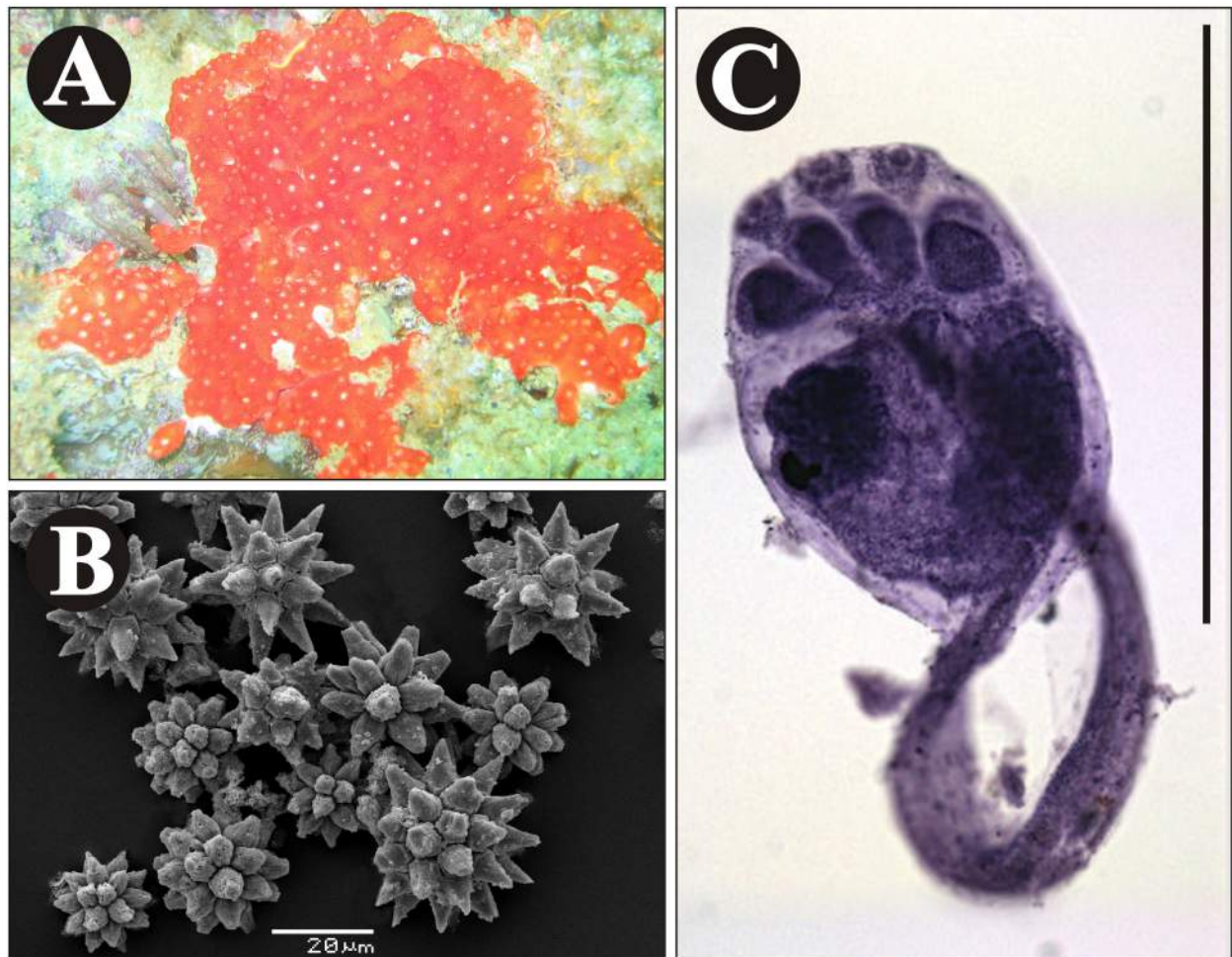
**Remarks.** Many *Didemnum* species have orange colonies, but all descriptions available are different from *Didemnum aurantium* n. sp. *Didemnum ahu* and *D. inauratum* Monniot, 1983 have spicules with rounded ray tips, which are pointed in *D. aurantium* (Monniot 1983; Rocha et al. 2005). *Didemnum drachi* Lafargue, 1975, *D. peyrefittense* Brément, 1913, *D. coriaceum* Von Drasche, 1883 and *D. ligulum* have a greater number of ectodermal ampullae in the larvae than *D. aurantium* (Lafargue 1975; Rocha & Monniot 1995). *Didemnum granulatum* differs by the presence of papillae filled with spicules on the tunic surface (Tokioka, 1954).

***Didemnum flammacolor* sp. nov. Rocha & Neves**

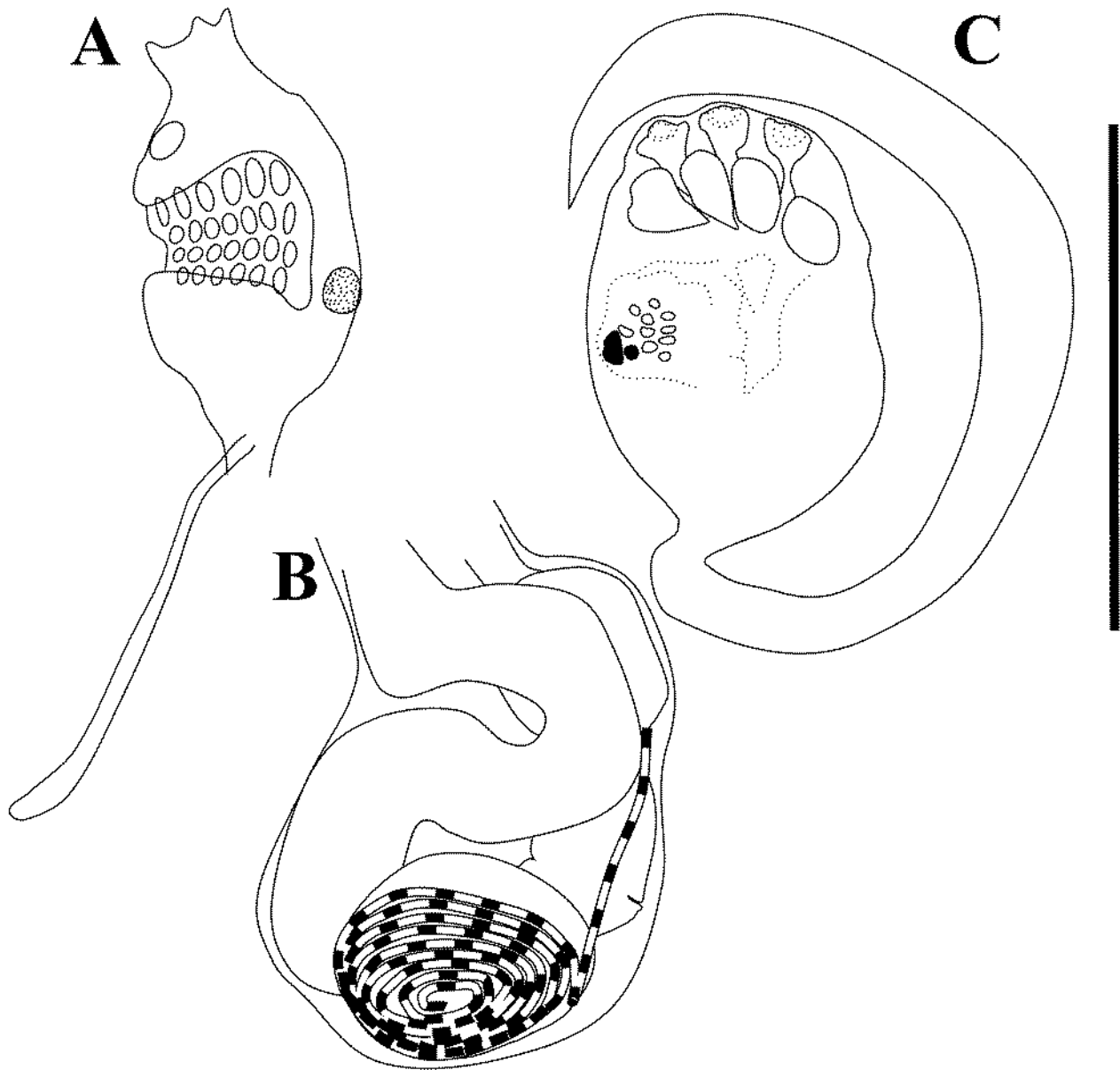
(Figs 4, 5)

**Examined material:** Holotype: MZUSP 00090 one colony, Ondina, Salvador, Bahia, 13°00'33" S, 38°31'41" W, Col. R. M. Rocha, 06.06.2004.

**Etymology.** The name refers to the red color of the living colony.



**FIGURE 4.** *Didemnum flammacolor* sp. nov. A, living colony. B, spicules. C, larva. Scale bar: C = 0.5 mm.



**FIGURE 5.** *Didemnum flammacolor* sp. nov. A, thorax. B, abdomen. C, larva. Scale bar: 0.5 mm.

The encrusting colony is approximately 3 cm long and 1 mm thick. Colony surface is red and the rim of cloacal cavities is white due to the presence of spicules (Fig. 4A); when fixed in formaldehyde, the color fades to pinkish. Spicules are abundant throughout the surface, giving the colony a firm and brittle consistency; spicules are less abundant in the middle and lower layers of the tunic. Spicules are stellate, approximately 30  $\mu\text{m}$  in diameter with eight tapered rays in cross section; some spicules are approximately 20  $\mu\text{m}$  in diameter and have 7–10 conical and truncated rays in optical transverse section (Fig. 4B).

Zooids are about 1 mm long. The oral siphon has six small triangular lobes. The atrial aperture is wide open, exposing most of the pharynx and an atrial languet is absent. The muscular process projects from the esophageal-rectal peduncle and is variable in length and may be absent in some zooids, and tends to be shorter than the abdomen. Thoracic organs are circular, 80  $\mu\text{m}$  in length, protruding, at the level of the fourth row of pharyngeal slits. There are between 6 and 7 stigmata per row on each side of the pharynx (Fig. 5A).

The abdomen is larger than the thorax. One small stolonial vessel was observed in some zooids. The esophagus is short. The location and shape of the stomach are typical; the duodenum is shorter than the stomach; the intestinal loop forms a deep secondary loop, covering the stomach. The single testis is spherical and surrounded by nine coils of the sperm duct (Fig. 5B). No oocytes were found.

Larvae are oval, trunk is approximately 0.4 mm in length, around which the tail winds three-quarters of the way. Larvae are non-pigmented, have three linearly arranged adhesive papillae that are close together on short stalks, plus four pairs of rounded ectodermal ampullae. The sensory vesicle (including ocellus and otolith) is in the mid-dorsal region of the larval trunk (Figs. 4C, 5C).

**Remarks.** The known *Didemnum* species with reddish colonies differ from *D. flammacolor* in many ways. *Didemnum moseleyi* (Herdman, 1886) and *D. pseudofulgens* Médioni, 1970 have spicules with a greater number of short, conical rays (Kott 2001; Médioni 1970). *Didemnum rodriguessi* colonies have a reticulate pattern on the surface of the tunic and a different number of larval ampullae (Rocha & Monniot 1993; Monniot 2010). *Didemnum membranaceum* Sluiter, 1909 has larger spicules (0.1 mm) with 4–6 conical rays in transverse optical section (Monniot & Monniot 2008). *Didemnum madeleinae* Monniot & Monniot, 2001 have larvae with 12 or more pairs of ampullae and the trunk is longer (Monniot & Monniot 2001). *Didemnum coccineum* Von Drasche, 1883 and *D. amourouxi* Lafargue, 1976 also have a greater number of ampullae but only two adhesive papillae (Lafargue & Wahl 1987). Some other species of *Didemnum* have 9 turns of sperm duct and larvae with 3 adhesive papillae and 4 pairs of ampullae. *Didemnum crescente* Kott, 2001 has larger spicules (70 µm), longer retractor muscles and 0.6 mm long larval trunk (Kott 2001). *Didemnum cuculiferum* (Sluiter, 1909) has longer retractor muscle and larger larvae (0.57 mm). *Didemnum fragilis* Sluiter 1909, has a fragile colony with circular spicules and larger larvae (0.78 mm) (Monniot & Monniot 1987). *Didemnum hiopaa* Monniot & Monniot, 1987 form small and transparent colonies and has spicules with larger number of rays. *Didemnum incantum* (Herdman, 1899) has longer retractor muscle, fewer pharyngeal stigmata in each row (4–6), and the spicules rays have a tetrahedral basis. *Didemnum vulgare* Kott, 2001 has larger spicules (60 µm) with more rays and longer retractor muscle (Kott, 2001).

### ***Didemnum lambertae* sp. nov. Rocha & Neves**

(Figs 6, 7)

**Examined material:** Holotype: MZUSP 00091 one colony, Porto da Barra, Salvador, Bahia, 13°00'14" S, 38°32'01" W, 3 m, Col. R. M. Rocha, 09.12.2007. Paratypes: DZUP DID-351 one colony, Germânia wreck, Salvador, Bahia, 13°00'34" S, 38°31'59" W, 6 m, Col. R. M. Rocha, 10.12.2007; DZUP DID-352 one colony, Quebramar Norte, Salvador, Bahia, 12°57'52" S, 38°30'57" W, 5 m, Col. R. M. Rocha, 11.12.2007; DZUP DID-353 one colony, Boiã da Barra, Salvador, Bahia, 13°00'32" S, 38°32'19" W, 15 m, Col. R. M. Rocha, 12.12.2007; DZUP DID-366 one colony, Quebramar Sul, Salvador, Bahia, 12°58'22" S, 38°31'09" W, Col. R. M. Rocha, 07.08.1999.

**Etymology.** Name given to honor Gretchen Lambert because of her many contributions to the knowledge of ascidian biodiversity.

Encrusting colonies are 5 to 20 cm long and about 3 mm thick. Colonies are red, some having cloacal apertures with a white rim due to the presence of spicules but others uniformly colored (Fig. 6A). After preservation in formalin they turn white. Spicules are dense on the surface of the firm and brittle colony, and are less dense in the middle and basal layers of the tunic. Spicules are small and stellate, less than 25 µm in diameter, with long and cylindrical rays, rounded at the tip. There are six rays in optical transverse section (Fig. 6B).

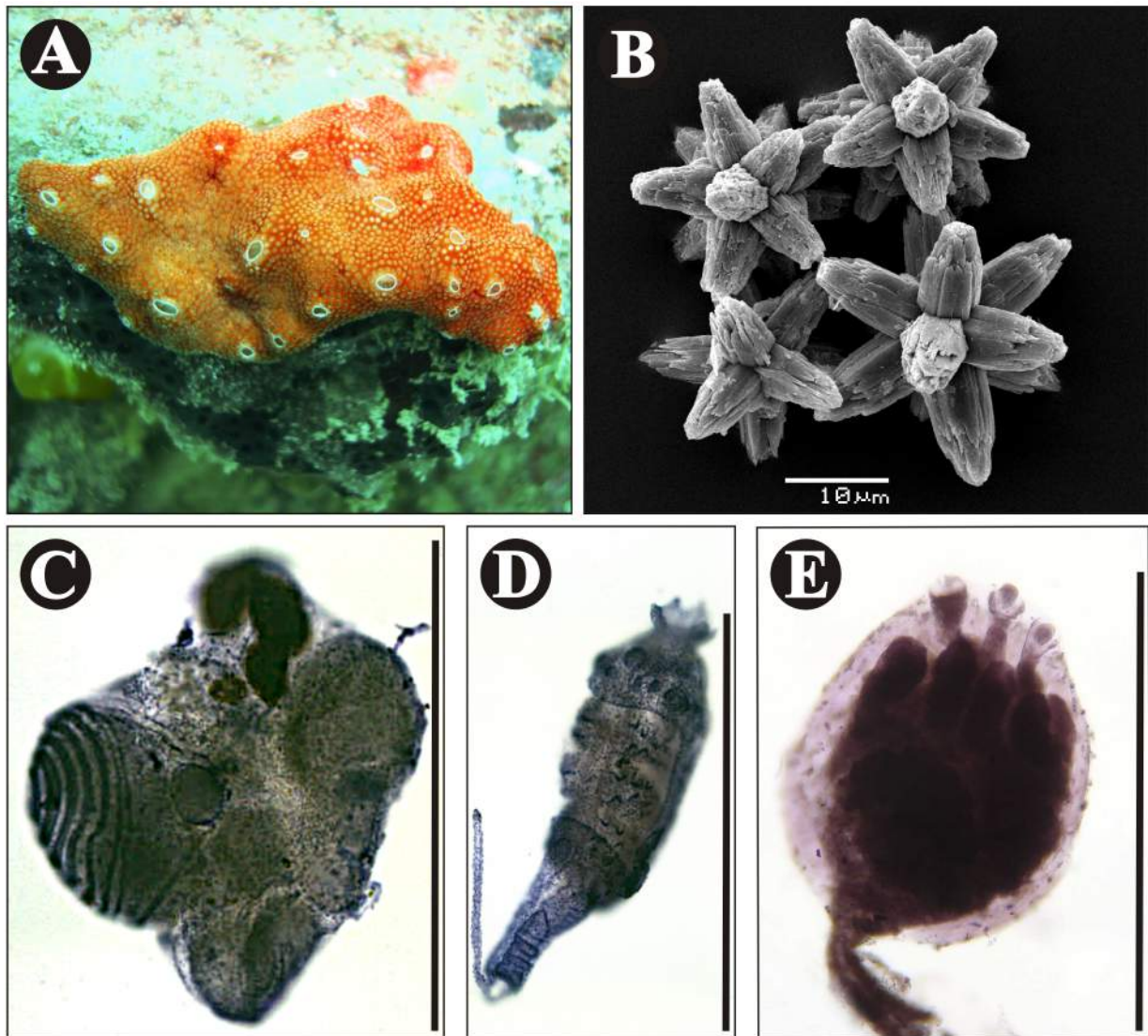
Zooids are between 1.0 and 1.8 mm long, thorax and abdomen are equal in size. The oral siphon has six small triangular lobes. The atrial aperture is wide exposing the second and third rows of pharyngeal slits. The atrial languet is absent. The muscular process projects from the esophageal-rectal peduncle and it is shorter than the abdomen. Lateral organs are protruding between the third and fourth rows of stigmata, but shape varies among zooids: some are oval and other are z-shaped. Between 6 and 9 stigmata per row are on each side of the pharynx (Fig. 6D, 7A).

The esophagus is short; the stomach location and shape are typical, the duodenum is shorter than the stomach; the intestinal loop (with 2–3 constrictions) forms a deep secondary loop, covering the stomach. There are two small stolonial vessels. Gonads are located in the intestinal loop, one beside the other. The testis is single, spherical and surrounded by 7 coils of the sperm duct (Fig. 6C, 7B). The ovary has one oocyte (0.3 mm). A few zooids were budding.

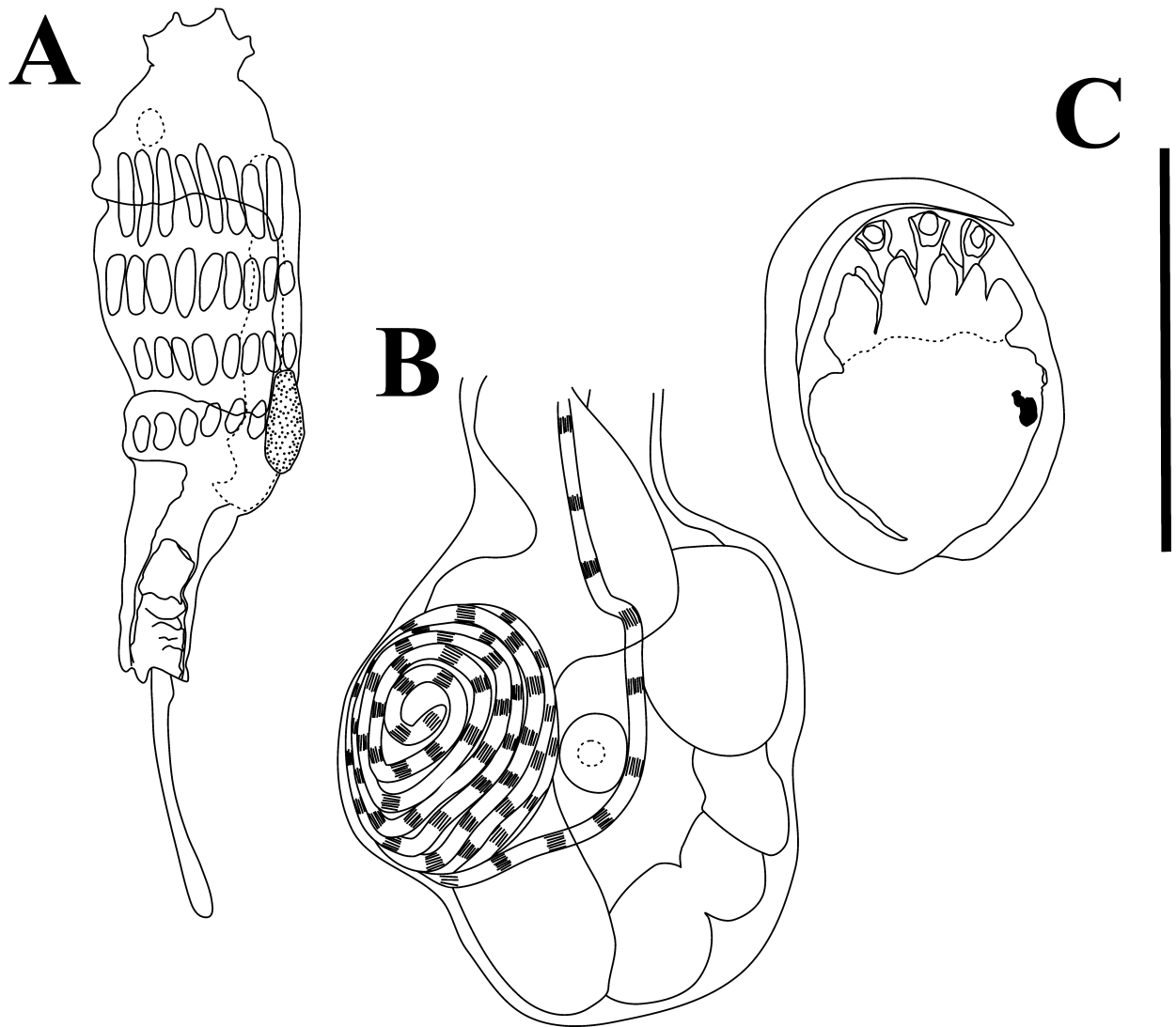
Larval trunk is oval, about 0.4 mm long, around which the tail winds half way. Larvae have three linearly arranged adhesive papillae with long stalks, plus five pairs of ectodermal ampullae. The sensory vesicle (including ocellus and otolith) is in the mid-dorsal region of the larval trunk (Fig. 6E, 7C).



**Remarks.** Colonies of *Didemnum lambertae* and *D. flammacolor* are similar in external appearance, but notable differences are in spicules (number and shape of rays), zooids (number of pharyngeal stigmata, size of lateral organs, shape of intestine and number of sperm duct coils), and larvae (number and shape of ampullae). Few species of *Didemnum* have larvae with 5 pairs of ampullae. *Didemnum nekozita* Tokioka, 1967 is found at greater depths (20–30 m), has posterior stomach and fewer stigmata in each half-row of the pharynx (five). *Didemnum mutabile* Monniot Monniot, 1987 has smaller larvae (0.28 mm) and larger spicules with short rays (35–40  $\mu\text{m}$ ) (Monniot & Monniot 1987). *Didemnum fuscum* Sluiter, 1909 has larger spicules (60–90  $\mu\text{m}$ ), fewer stigmata in the pharynx (4–6 per half row) and 9 turns of the sperm duct. *Didemnum pecten* Kott, 2001 has larger spicules (56  $\mu\text{m}$ ) and 9 turns of the sperm duct. *Didemnum poecilomorpha* Monniot & Monniot, 1996 has larger spicules (60  $\mu\text{m}$ ), 8 turns of the sperm duct and larvae with symbiont algae. *Didemnum sordidum* Kott, 2001 has larger spicules (65  $\mu\text{m}$ ), smaller zooids (0.6 mm), and many granule brown cells in zooids and larvae (Kott 2001). *Didemnum domesticum* Kott, 2004 has only six turns of the sperm duct (Kott 2004).



**FIGURE 6.** *Didemnum lambertae* sp. nov. A, living colony. B, spicules. C, abdomen. D, thorax. E, larva. Scale bars: C–E = 0.5 mm.



**FIGURE 7.** *Didemnum lambertae* sp. nov. A, thorax. B, abdomen. C, larva. Scale bar: 0.5 mm.

***Didemnum longigaster* sp. nov. Rocha & Neves**

(Figs 8, 9)

**Examined material:** Holotype: MZUSP 00092 one colony, Quebramar Norte, Salvador, Bahia, 12°57'52" S, 38°30'57" W, 4 m, col. R. M. Rocha, 11.12.2007.

**Etymology.** The name refers to the elongated stomach that is uncommon in didemnids.

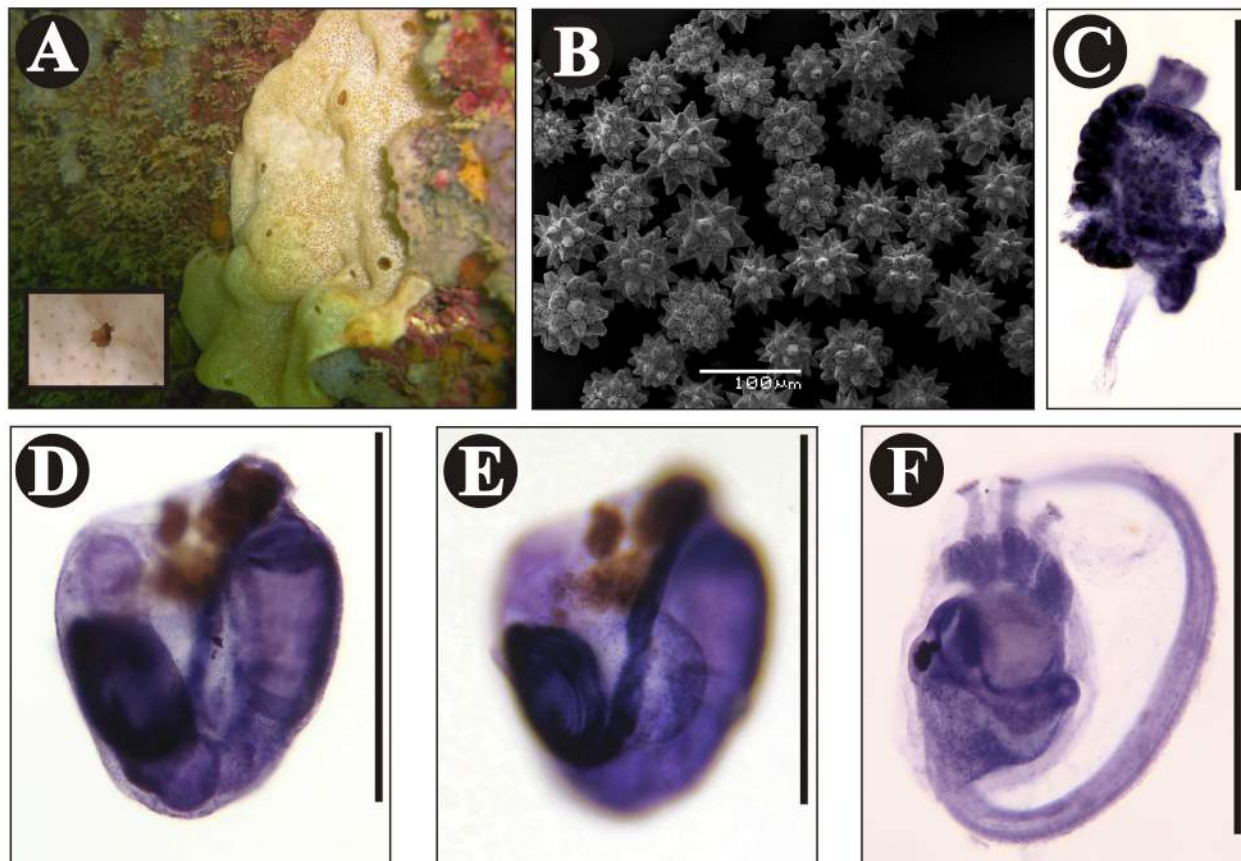
The colony is encrusting, around 10 cm long and 2 mm thick. Beige with orange zooids, the colony has large circular cloacae with lobed rim (Fig. 8A). When preserved in formalin the colony turns white. Spicules are very dense in the surface of the colony, but less so in the middle and basal tunic layers. Spicules are large and stellate, between 60 and 100  $\mu$ m with 9–10 short conic rays in optical transverse section (Fig. 8B).

Zooids are 1.5 mm long. When relaxed, the thorax is larger than the abdomen. The oral siphon is tubular with shallow rounded lobes. The atrial aperture is wide exposing the pharynx, atrial languet is absent. The muscular process projects from the base of the thorax and is longer than the abdomen. Thoracic organs are elongated, protruding, located between the third and fourth rows of stigmata close to the endostyle. Each row has 5–6 stigmata on each side (Figs. 8C, 9A).

No stolonial vessels were observed in the abdomen. The esophagus is short. In general, all zooids have elongated stomach, but not all of them as long as in Figs. 8D and 9B. The intestine does not form a secondary loop

and the ascending portion passes beside, without overlaying, the stomach. The testis has a single and spherical follicle, surrounded by 8 coils of the sperm duct (Figs. 8E, 9B). Larvae are oval, 0.4 mm trunk length, around which the tail winds half way. Three adhesive papillae are present, with long and wide stalks plus six pairs of ectodermal ampullae. The sensorial vesicle (including ocellus and otolith) is in the mid-dorsal region of the larval trunk (Figs. 8F, 9C).

**Remarks.** No other *Didemnum* species has an elongated stomach as in *D. longigaster*. The combination of this elongated stomach, large spicules and larvae with six pairs of ectodermal ampullae is also unique. In the Atlantic, two other species are of the same colony color. *Didemnum granulatum* have a beige color morph, but the colony surface has small projections filled with spicules, that are much smaller than in *D. longigaster* (Rocha et al. 2005). *Didemnum vexillum* Kott, 2002 does not occur in Brazilian coastal waters, and it has smaller spicules and larger larvae (Kott 2002).

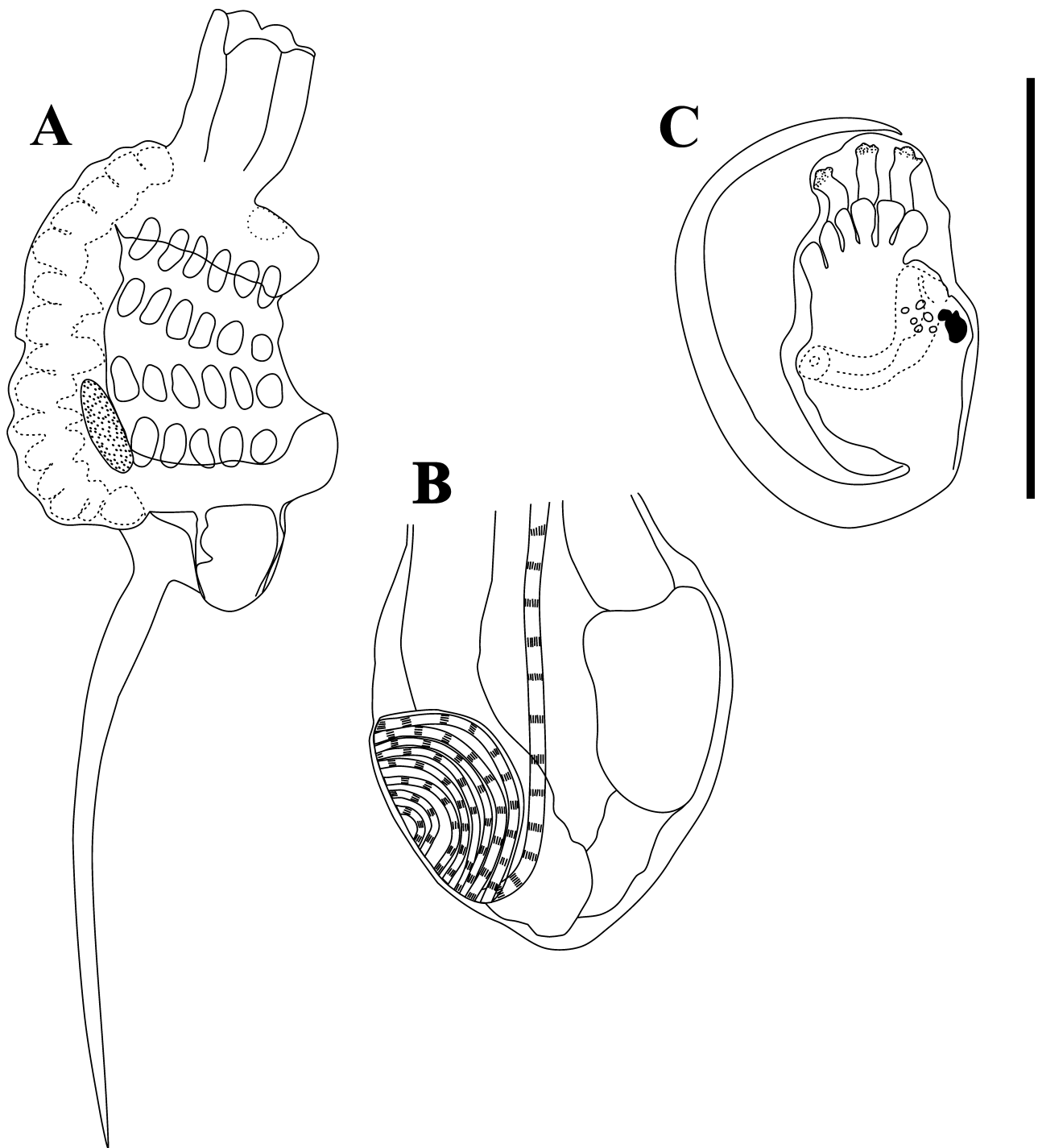


**FIGURE 8.** *Didemnum longigaster* sp. nov. A, living colony (cloacal aperture in detail). B, spicules. C, thorax. D, abdomen showing the long stomach on the right. E, abdomen showing the sperm duct. F, larva. Scale bars: C–F = 0.5 mm.

***Diplosoma citrinum* sp. nov. Rocha & Gamba**  
(Figs 10, 11)

**Examined material:** Holotype: MZUSP 00093 one colony, Ilha Escalvada, Guarapari, Espírito Santo, 20°42'00"S, 40°24'30"W, 1 m, col. R. M. Rocha, 12.02.2011. Paratypes: DZUP DIPL-37 one colony, Ilha Escalvada, Guarapari, Espírito Santo, 20°42'00"S, 40°24'30"W, 12 m, Col. R. M. Rocha, 26.01.2012; DZUP DIPL-38 one colony, Ilha Escalvada, Guarapari, Espírito Santo, 20°42'00"S, 40°24'30"W, 10 m, Col. R. M. Rocha, 27.01.2012; DZUP DIPL-39 3 colonies, Boião da Barra, Salvador, Bahia, 13°00'32" S, 38°32'19" W, 10–13.5 m, Col. R. M. Rocha, 12.12.2007; DZUP DIPL-40 one colony, Ondina, Salvador, Bahia, 13°00'33" S, 38°31'41" W, Col. R. M. Rocha, 06.06.2004.

**Etymology.** The name of the species refers to the yellow color of the living colony.



**FIGURE 9.** *Didemnum longigaster* sp. nov. A, thorax. B, abdomen. C, larva. Scale bar: 0.5 mm.

Colonies are encrusting 2–6 cm long, and very thin and delicate (1 mm thick). Living colonies have extensive cloacal cavities filled with water, which makes them appear thicker than they really are. The tunic is transparent and the yellow zooids are responsible for the colony color. When preserved in formalin zooids turn light brown. Zooids are arranged randomly without forming systems. Cloacal openings are circular, large and randomly distributed on the surface of the colony (Fig. 10A).

Zooids are embedded in a thin test and connected in pairs by thin projections of the test that extend to the basal layer of the colony, where the larvae are. Zooids are between 0.6 and 1.2 mm long. The oral siphon is tubular and short, with six rounded small lobes. The atrial opening exposes the second and third rows of stigmata. The muscular process projects from the base of the thorax and it is shorter than the abdomen. In the pharynx, the first

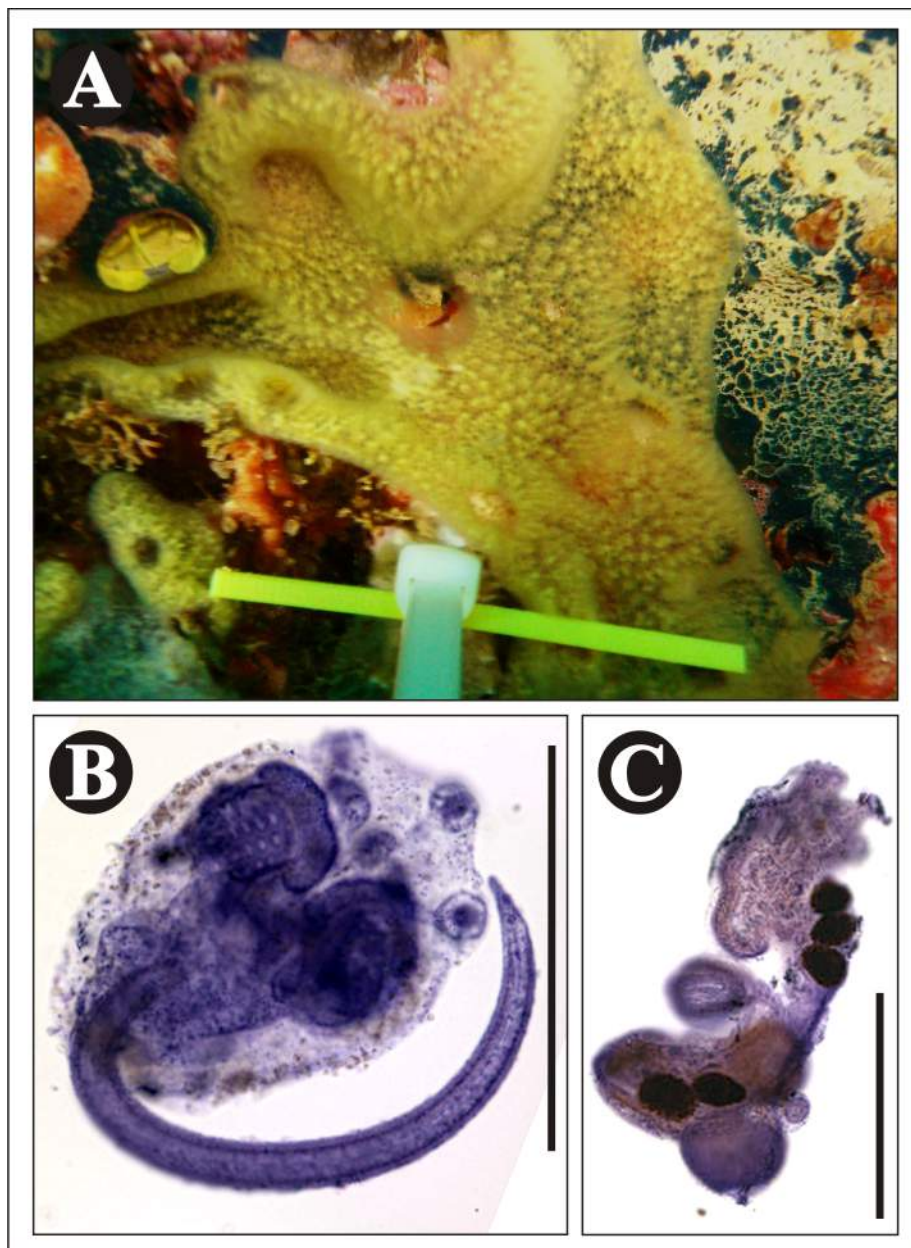


row contains 8–10 stigmata, while the second and third have 7–9 and the fourth has 6–9 slits in each side (Figs. 10C, 11A).

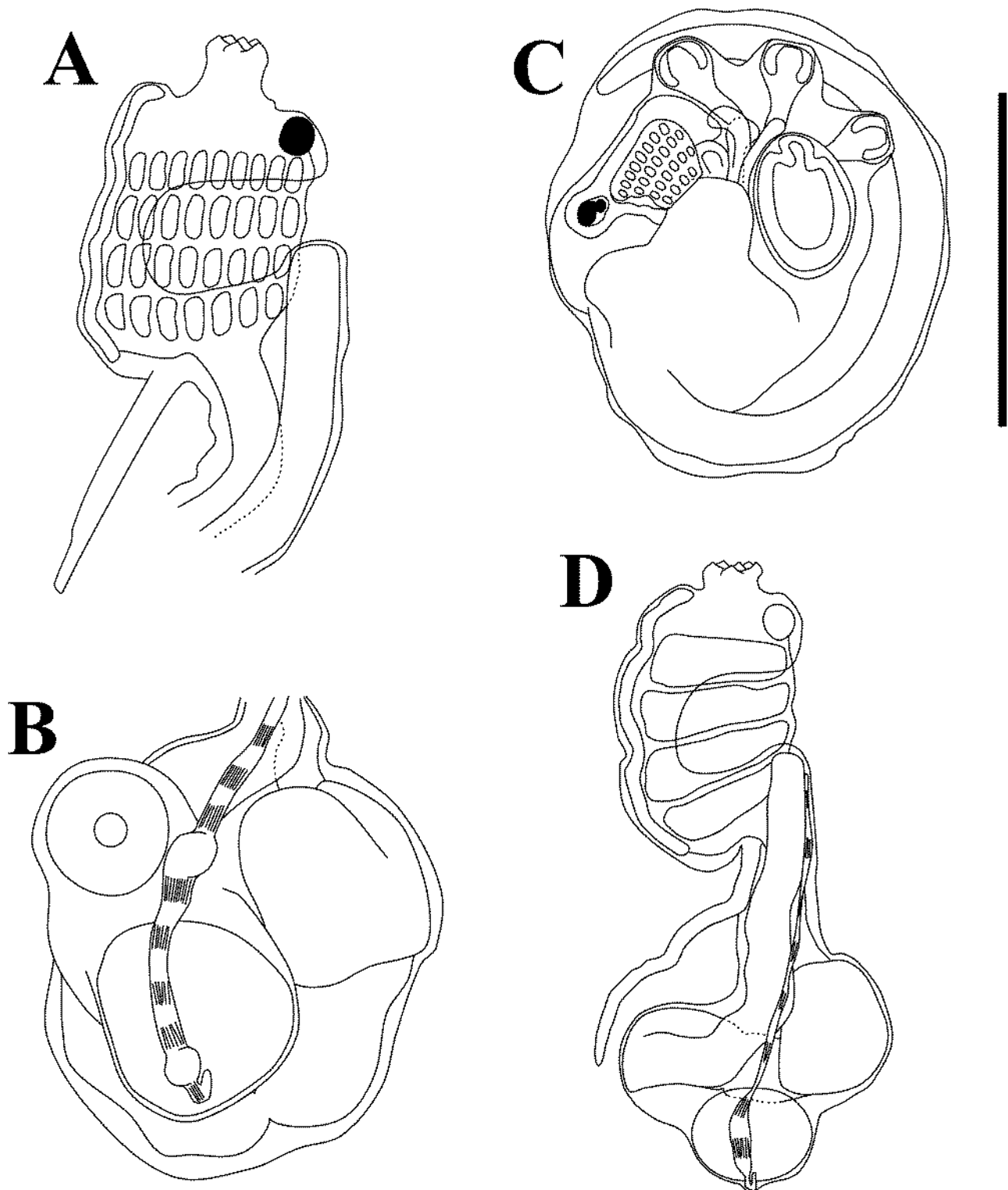
The abdomen is larger than and perpendicular to the thorax. The esophageal-rectal peduncle is long. The stomach is large with an invagination in the region where it is penetrated by the esophagus, assuming a cordiform shape. The duodenum is shorter than the stomach and ends in a strong constriction. The anus has a smooth margin at the level between the third and fourth rows of stigmata (Figs 10C, 11D). There are two or three stolon vessels.

Gonads are inside the intestinal loop, which means they appear underneath the abdomen, because the intestine is horizontally oriented. The testis has one spherical follicle. There is a large (0.3 mm) and another smaller oocyte. Budding was observed in many zooids (Fig. 10C).

Larvae were found only in colonies collected in Espírito Santo. They are gemmiparous with the trunk 0.6 mm long; the tail winds a little more than halfway around the trunk. Three adhesive papillae are present with long stalks, positioned a distance from each other, with three pairs of short, finger-like ampullae. The oozoid pharynx has four rows of stigmata with 7–8 slits in the first and second rows and 4–6 in the third and fourth rows (Figs. 10B, 11C).



**FIGURE 10.** *Diplosoma citrinum* sp. nov. A, living colony (yellow bar = 5 cm). B, larva. C, zooid without tunic. Scale bars: B, C = 0.5 mm.



**FIGURE 11.** *Diplosoma citrinum* sp. nov. A, thorax. B, abdomen (posterior view). C, larva. D, zooid. Scale bar: 0.5 mm.

**Remarks.** Of the species of *Diplosoma* found in the Atlantic, only *D. singulare* Lafargue, 1968 has one follicle in the testis. However, in that species, the abdomen is vertical with gonads on the right side, with non gemmiparous larva having four pairs of ampullae (Lafargue 1968). In other waters, among species with one follicle in the testis, *D. ata* Monniot & Monniot, 1987 has the oral siphon with six very long leaf-like lobes and pigmented cells, the pharynx has at least 10 slits in each half row, and the larva has a larger number of ampullae (Monniot & Monniot 1987). *Diplosoma pannosum* Monniot & Monniot, 2001 differs from *D. citrina* by the color of the colony and number of ectodermal ampullae in the larva. *Diplosoma unitestis* Monniot & Monniot, 2001 has a unique ovary



isolated within a pouch and *D. redika* Monniot, 1994 has uncolored colonies and larvae with four pairs of ectodermal ampullae and three embryos (Monniot & Monniot 2001).

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