UNIVERSIDADE FEDERAL DO PARANÁ

ISABELA MONTEIRO NEVES

ASCÍDIAS DIDEMNIDAE (TUNICATA, ASCIDIACEA) DA REGIÃO DE BOCAS DEL TORO – PANAMÁ

Didemnidae ascidians (Tunicata, Ascidiacea) from Bocas del Toro – Panamá

CURITIBA

2015

ISABELA MONTEIRO NEVES

ASCÍDIAS DIDEMNIDAE (TUNICATA, ASCIDIACEA) DA REGIÃO DE BOCAS DEL TORO – PANAMÁ

Didemnidae ascidians (Tunicata, Ascidiacea) from Bocas del Toro – Panamá

Dissertação apresentada como requisito parcial à obtenção do grau de Mestre em Zoologia, no Curso de Pós-Graduação em Zoologia, Setor de Ciências Biológicas, da Universidade Federal do Paraná.

Orientadora: Profa. Dra. Rosana Moreira da Rocha

CURITIBA

2015

Ministério da Educação
UNIVERSIDADE FEDERAL DO PARANÁ
Setor de Ciências Biológicas
Programa de Pós-Graduacão Zoologia

TERMO DE APROVAÇÃO

Isabela Monteiro Neves

"*Ascídias Didemnidae (Tunicata, Ascidiacea) da região de Bocas del Toro – Panamá*"

Dissertação aprovada como requisito parcial para obtenção do grau de Mestre em Zoologia, do Setor de Ciências Biológicas da Universidade Federal do Paraná, pela seguinte Comissão Examinadora:

Professora Dra. Rosana Moreira da Rocha
Orientadora

Professor Dr. Tito Monteiro da Cruz
Membro Externo

Professora Dra. Tatiana Regina Moreno
Membro Externo

Curitiba, 06 de Fevereiro de 2015.

Programa de Pós-Graduação em Ciências Biológicas - Zoologia/UFPR
Setor de Ciências Biológicas - Departamento de Zoologia
Caixa Postal 19020 - CEP 81531-980 - Curitiba - Paraná
Telefone/FAX +55 (041) 3361-1641**

Agradecimentos

Meu agradecimento especial à minha família (Kuka, mãe, Flavinha e Paulinho) pela força, pela paciência, pelo amor e por estarem do meu lado nos piores e melhores momentos. Por serem meus exemplos de determinação, compreensão e coragem.

À Rosana Rocha, pela paciência, pelo incentivo, pelos ensinamentos, pelas lições e pelo exemplo de profissional.

Ao James Roper pelo auxílio nas traduções para o inglês.

Aos meus novos amigos (principalmente à Maíra, ao Jume, ao Garcia, ao Mado, ao Alemão e ao Roni) pelos momentos de pura alegria e companheirismo que me resgataram.

Aos velhos amigos (principalmente à Lu e ao Pedro) pela compreensão pelas mudanças que o mestrado trouxe para minha vida.

Aos colegas de laboratório (principalmente à Janaína Bumbeer) pela convivência recheada de cafés, de discussões científico-filosóficas e pelos resgates nos momentos de baixo rendimento.

Ao Smithsonian Tropical Research Institute e os professores e funcionários envolvidos pela oportunidade de fazer o curso Taxonomy and Biology of Tunicates e poder conhecer e realizar coletas em Bocas del Toro.

Ao Centro de Microscopia da Universidade Federal do Paraná pelas imagens em Microscopia Eletrônica de Varredura.

Á Capes pelo auxílio financeiro.

Resumo

Didemnidae é a família mais diversa e mais derivada da ordem Aplousobranchia (classe Ascidiacea). Compreende cerca de 600 espécies, representando 20% de todas as ascídias conhecidas até o momento. As águas tropicais do Caribe são conhecidas pela riqueza e abundância de invertebrados marinhos e a costa atlântica do Panamá é caracterizada pela presença de um mosaico de hábitats variados que proporcionam importantes substratos naturais para a comunidade incrustante. O presente trabalho apresenta descrições detalhadas de didemnídeos coletados entre os anos de 2003 e 2014 em 12 pontos do arquipélago de Bocas del Toro – Panamá. Foram identificadas 19 espécies, das quais cinco haviam sido registradas na região (*Diplosoma listerianum, Lissoclinum verrilli, Didemnum granulatum, D. psammatodes* e *Trididemnum orbiculatum*), sete são novos registros (*Lissoclinum abdominale, Didemnum calliginosum, D. cineraceum, D. perlucidum, D. vanderhorsti, Trididemnum palmae* e *T. thetidis*) e sete são espécies novas (*Diplosoma* sp. nov. 1, *Diplosoma* sp. nov. 2, *Lissoclinum* sp. nov. 1, *Lissoclinum* sp. nov. 2, *Didemnum* sp. nov., *Trididemnum* sp. nov. 1 e *Trididemnum* sp. nov. 2). Com estes resultados, o Panamá está entre as regiões do Caribe com maior número de didemnídeos registrados, mostrando a importância de se considerar a região objeto de esforço no monitoramento e na conservação dos diferentes hábitats e dos invertebrados marinhos que ali habitam.

Palavras-chave: *Aplousobranchia, ascídias coloniais, características morfológicas, Caribe, Diplosoma, Didemnum, Lissoclinum, taxonomia, Trididemnum, Tunicata.*

Abstract

Didemnidae is the most diverse and the more derived family from Aplousobranchia order (Ascidiacea class). Comprising ca. 600 species, that represents 20% of all known ascidians. The Caribbean tropical waters are known for the richness and abundance of marine invertebrates and the Panama Atlantic coast is characterized by the presence of different habitats that provide important natural substrates for sessile communities. This paper reports detailed descriptions of didemnids collected between 2003 and 2014 in 12 points in the archipelago of Bocas del Toro - Panama. Nineteen species were identified, of which five have been previously registered in the region (*Diplosoma listerianum*, *Lissoclinum verrilli*, *Didemnum granulatum*, *D. psammatodes* and *Trididemnum orbiculatum*), seven are new records (*Lissoclinum abdominale*, *Didemnum calliginosum*, *D. cineraceum*, *D. perlucidum*, *D. vanderhorsti*, *Trididemnum palmae* and *T. thetidis*) and seven are new species (*Diplosoma* sp. nov. 1, *Diplosoma* sp. nov. 2, *Lissoclinum* sp. nov. 1, *Lissoclinum* sp. nov. 2, *Didemnum* sp. nov., *Trididemnum* sp. nov. 1 and *Trididemnum* sp nov. 2). With these results, Panama is among the regions of the Caribbean Sea with the highest number of didemnids, showing the importance of considering the monitoring effort and conservation of different habitats and marine invertebrates that live in this region.

Keywords: *Aplousobranchia, colonial ascidians, Caribbean Sea, Diplosoma, Didemnum, Lissoclinum, morphology, taxonomy, Trididemnum, Tunicata.*

Index

Introduction

The class Ascidiacea, comprising ca. 3,000 species, is in the phylum Chordata and includes the majority of species in the subphylum Tunicata (Burighel & Cloney 1997). As with all tunicates, ascidians lack vertebrae, have a tunic (live tissue covering the body), a perforated pharynx, through which water is filtered to obtain food, a digestive tract that forms a loop with an internal anus, and bidirectional blood circulation (Brusca & Brusca 2007). Ascidians are strictly marine, sessile and tend to occur on consolidated substrates, and may be found at great depths (Lambert 2005). Species may be solitary or colonial and are divided among three orders: Stolidobranchia, Phlebobranchia and Aplousobranchia (Kott 2005).

Half of all known ascidian species are in the order Aplousobranchia (Shenkar & Swalla 2011), which comprises colonial species with simplified anatomy as a consequence of the reduced size of the zooids. They have a simplified pharynx, without longitudinal folds or internal blood vessels. The body of the zooid is divided in thorax and abdomen, with gonads associated with the digestive tract or rarely they are posterior to the intestinal loop, which is then called the post-abdomen (Monniot et al. 1991). Colony organization can be quite complex, in which zooids share channels and cloacae for water elimination as well as liberation of gametes, larvae and feces. When cloacae are absent from colonies, each zooid has two apertures (oral and atrial) on the colony surface.

The Didemnidae (subject of this study) is among the several families in the order Aplousobranchia. Didemnidae comprise 20% of all known species of Ascidiaceae, with ca. 600 described species. Not only it is this the most species rich family, but is also the most evolutionarily derived in the order (Moreno & Rocha 2008). The most species rich

genus is *Didemnum*, with around 230 species (Shenkar et al. 2014).

Didemnidae has nine genera with colonial species whose zooids are less than three mm long, with a body divided into thorax and abdomen. Colonies have cloacal apertures through which filtered water, feces and gametes pass. The tunic is extremely variable among species in its consistency due to the presence (or absence) of calcareous spicules that can vary in density (Monniot et al. 1991). Genera are differentiated based on the number of rows of pharynx in adults zooids and larvae, the number of follicles in the testis of the adults, form of the sperm duct (linear or spiral) and of the atrial aperture (aperture only, or tubular) and presence of calcarious spicules (Kott 2005). The genera *Didemnum, Diplosoma, Leptoclinides, Lissoclinum, Polysyncraton* and *Tridemnum* are found in the Atlantic Ocean (Rocha et al. 2012).

Based in phylogenetic analysis using morphology, the genus *Leptoclinides* is close to *Tridemnum*, *Didemnum* to *Polysyncraton* and *Diplosoma* to *Lissoclinum* (Moreno & Rocha 2008). Zooids in the genus *Leptoclinides* have four rows of pharyngeal stigmata (as do the larvae), tubular atrial aperture, two or more testicular lobes, spiral sperm duct, with relatively few coils. The genus *Tridemnum* is the only one whose zooids and larvae have three rows of pharyngeal stigmata. Zooids usually have a tubular atrial aperture and one testicular lobe and spiral sperm duct. The *Didemnum* have larvae with three rows of pharyngeal stigmata while zooids have four, a large, non-tubular atrial aperture, spherical testicular follicles and spiral sperm duct. Zooids (and larvae) in the *Polysyncraton* have four pharyngeal stigmata, large atrial aperture, more than two testicular follicles and spiral sperm duct with few turns. *Diplosoma* is the only genus without spicules in the tunic. Zooids have a large atrial aperture, one to three (usually two) testicular follicles and a straight sperm duct (Rocha et al. 2012). Zooids and larvae have four pharyngeal stigmata. *Lissoclinum* zooids and

larvae also have four pharyngeal stigmata, straight sperm duct, one or more testicular follicles and large atrial aperture (Rocha et al. 2012).

Tropical waters of the Caribbean have a wide diversity of Ascidians (Collin 2005; Rocha et al. 2005). In Panama, the province of Bocas del Toro has many different marine habitats, including rocky shores, coral reefs, mangroves and artificial substrates. Some species of ascidians are only found in particular habitats and because of the wide variety of habitats, many species are found there (Rocha et al. 2005). Bocas del Toro archipelago has a typical Caribbean Ascidian fauna, but with greater abundance, reflecting greater local productivity (Collin et al. 2009; Moreno, Faria & Rocha 2014). Many as yet undescribed species have been found in the region, which is considered the second most diverse in the Caribbean, after Guadeloupe (Rocha et al. 2005). Some areas of the Bocas del Toro archipelago are known to be biodiversity hotspots for other groups, including corals, gorgonians and sponges (Guzmán & Guevara 1999). Thus, Bocas del Toro should be considered as an important region for monitoring and conservation due to the wide diversity of habitats and species.

Despite the increase in research in the region in Bocas del Toro and the Caribbean, there is still much to be studied with respect to the Ascidiacea (Rocha et al. 2005), especially in the family Didemnidae, with many as yet unidentified species. Eleven species are already known from Panama, including *Didemnum conchyliatum* (Sluiter, 1898), *D. granulatum* Tokioka, 1954, *D. ligulum* Monniot, 1983, *D. psammatodes* (Sluiter, 1895), *D. speciosum* (Herdman, 1886), *Tridemnum orbiculatum* (Van Name, 1902), *T. savignii* (Herdman, 1886), *T. maragogi* Rocha, 2002, *Lissoclinum verrilli* (Van Name, 1902), *Diplosoma listerianum* (Milne-Edwards, 1841) and *Diplosoma simile* (Sluiter, 1909). Here we describe a total of 19 species in the Didemnidae found in the Bocas del Toro archipelago of Panama.

Materials and methods

Study area

The Republic of Panama is a country located in Central America (between latitudes 7°13'35 "N and 9°13'35" N and longitudes 82°56'20"E and 77°09'36" W). The archipelago of Bocas del Toro comprises nine islands located between the Almirante Bay and Laguna Chiriquí, on the Caribbean coast, northwest of the country (Figure 1).

The Almirante Bay is characterized by the presence of banks of marine phanerogams, reefs of well-developed coral and mangrove islands (Collin 2005). The Laguna de Chiriquí receives large flow from rivers in the region and the predominant ecosystem is characterized by the red mangrove - *Rhizophora mangle* (Collin 2005).

The animals were collected in the years 2003, 2006, 2008, 2009, 2011 and 2014, in 12 different locations that will be briefly described in the following lines (Figures 1, 2).

Bocas del Drago (9°25'00"N, 82°19'46"W) – is a channel in the east side of Colon Island. Sampling was performed at Colon Island margin, where a reef of dead corals, specially *Acropora palmata* (Lamarck, 1816) is the main substrate available. Most samples were found either underneath the coral still in the site or underneath dead coral pieces laying on the bottom, at 5 m of depth.

Bocas Marina (9º20'08"N, 82º14'48"W) – marina formed by three main floating docks in an entrance of a bay margined by mangrove. The bay also receives over flooding water from the sewage plant, thus eutrofication is high and ascidians cover the structures of the marina in high abundance. Maximum depth is around 6 m but most collections were made under 3 m.

Buoy 14 (9°25'05"N, 82°20'32"W) – this site is on the other margin of Bocas del Drago channel, called Punta Cauro. Buoy 14 is one of the buoys that mark the channel entrance. There is a coral reef formed mainly by *Agaricia tenuifolia* Dana, 1848 and collections were made mostly between 1 – 4 m deep, either underneath dead coral on the bottom or among the *Agaricia* sheets.

Cayo Agua Island (9°09'00"N, 82°01'00"W) and Crawl Key (9º15'05"N, 82º07'56"W) were also formed by coral reefs, with both hard coral and gorgonian patches. Crawl Key is a very large region, spread in the east most side of Bastimento Island, very shallow along the mangrove border. Different coral species form reefs and the site is very heterogeneous and diverse. Sampling was performed under 4 m usually underneath dead plates *Millepora* spp. accumulated on the bottom.

Cristobal (9°17'34"N, 82°15'24"W) and Solarte (9°17'30"N, 82°10'20"W) are sites covered by the red mangrove and the animals were collected from the prop roots in first meter of water. Patches of shallow coral reefs also can be found close to the bordering line of roots and they were also surveyed.

Garden (9°32'11"N, 82°22'00"W) and Hospital Point (9º20'04"N, 82º13'10"W) are diving sites surrounding Solarte Island. Garden has patches of coral reefs and sponge reefs mixed with patches of sand bottom that extend along a large region. Hospital Point has a vertical relief with many gorgonians projecting from the walls. Horizontal crevices furnish lots of rocky ceilings where ascidians can be found.

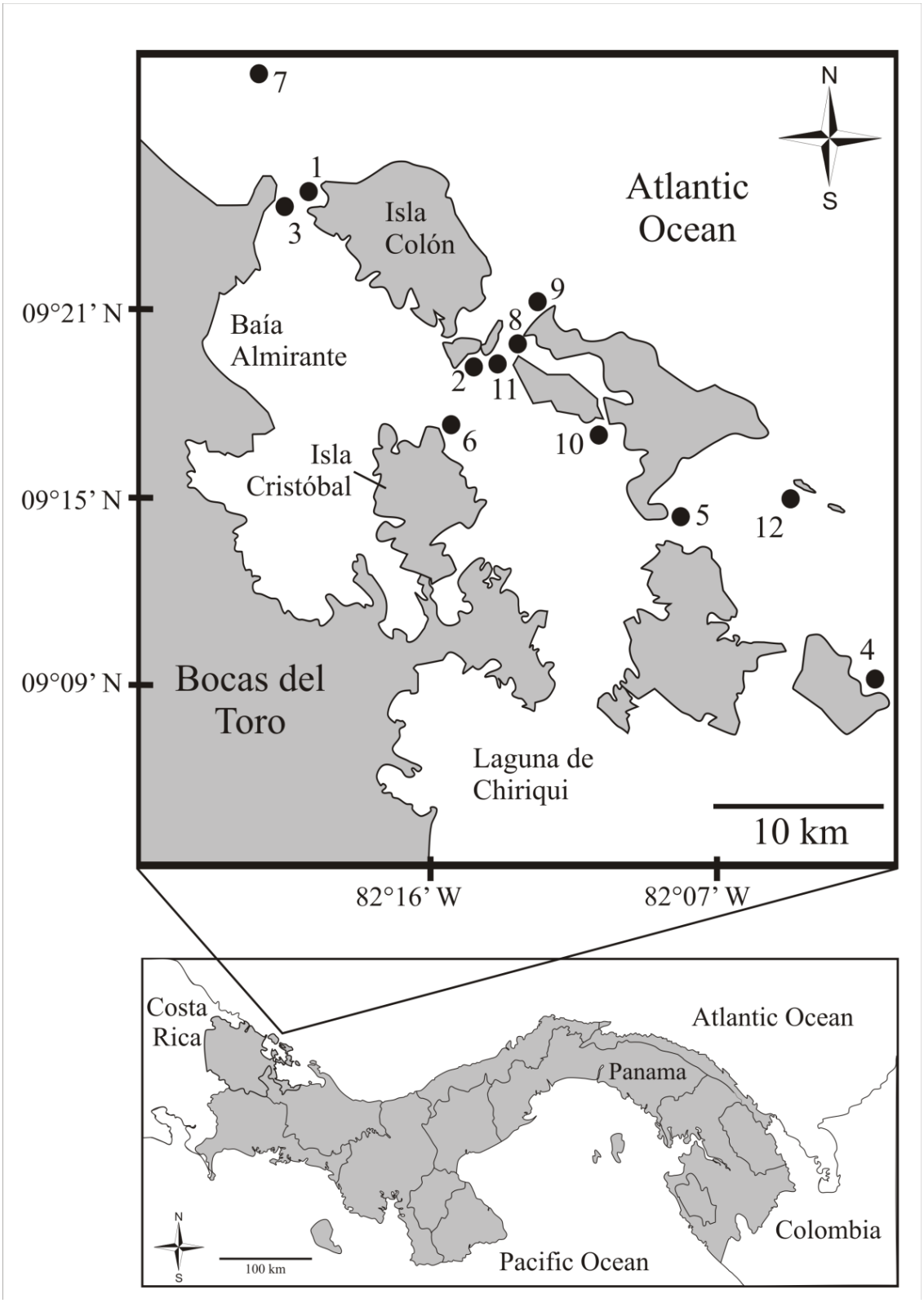

Figure 1. Study area and location of sampling sites. 1, Bocas del Drago; 2, Bocas Marina; 3, Buoy 14; 4, Cayo Agua Island; 5, Crawl Key; 6, Cristobal; 7, Garden; 8, Hospital Point; 9, Sachen; 10, Solarte; 11, Town Docks; 12, Zapatillas Island.

Sachen (9º21'11"N, 82º12'50"W) is a dive site located between Carenero and Bastimento Islands. Many years ago, a ferry sunk at this spot and its coral-coated walls

are forming a spectacular reef now. Many sponge species also cover the substrate and ascidians are usually attached to the sponge surfaces. Maximum depth is 12 m.

Town docks (9º20'07"N, 82º14'32"W) – front water construction in Bocas del Toro is made on stilts and most houses and restaurants have small docks with concrete or PVC-covered concrete. All these structures are completely fouled by sessile organisms and sampling occurred under 3 m deep.

Zapatillas Island (9°16'01"N, 82°03'30"W) – collection was performed underneath some large dead corals in the fringe of the subtidal zone.

Each collection site was sampled with different duration, but at no point was the effort under one hour. In most places the samples were taken in while snorkeling, sampling shallow depths, and only in Sachen, Hospital Point, Garden and Cayo Agua Island we used SCUBA diving, with sampling depths reaching 12 m. We did not consider the abundances of species and not all spots were sampled in each year. We tried to sample environments of different characteristics including meadows of the marine phanerogam *Thalassia testudinum*, mangrove roots, shallow coral reefs, coral formations on sandy substrate, and artificial substrates.

In the field, the samples were photographed before collection with or removed from the substrate with the aid of knives and spatulas. They were then anesthetized in menthol oil diluted in sea water and then, after two or three hours, were immersed in 4% formaldehyde (Monniot & Monniot 1972).

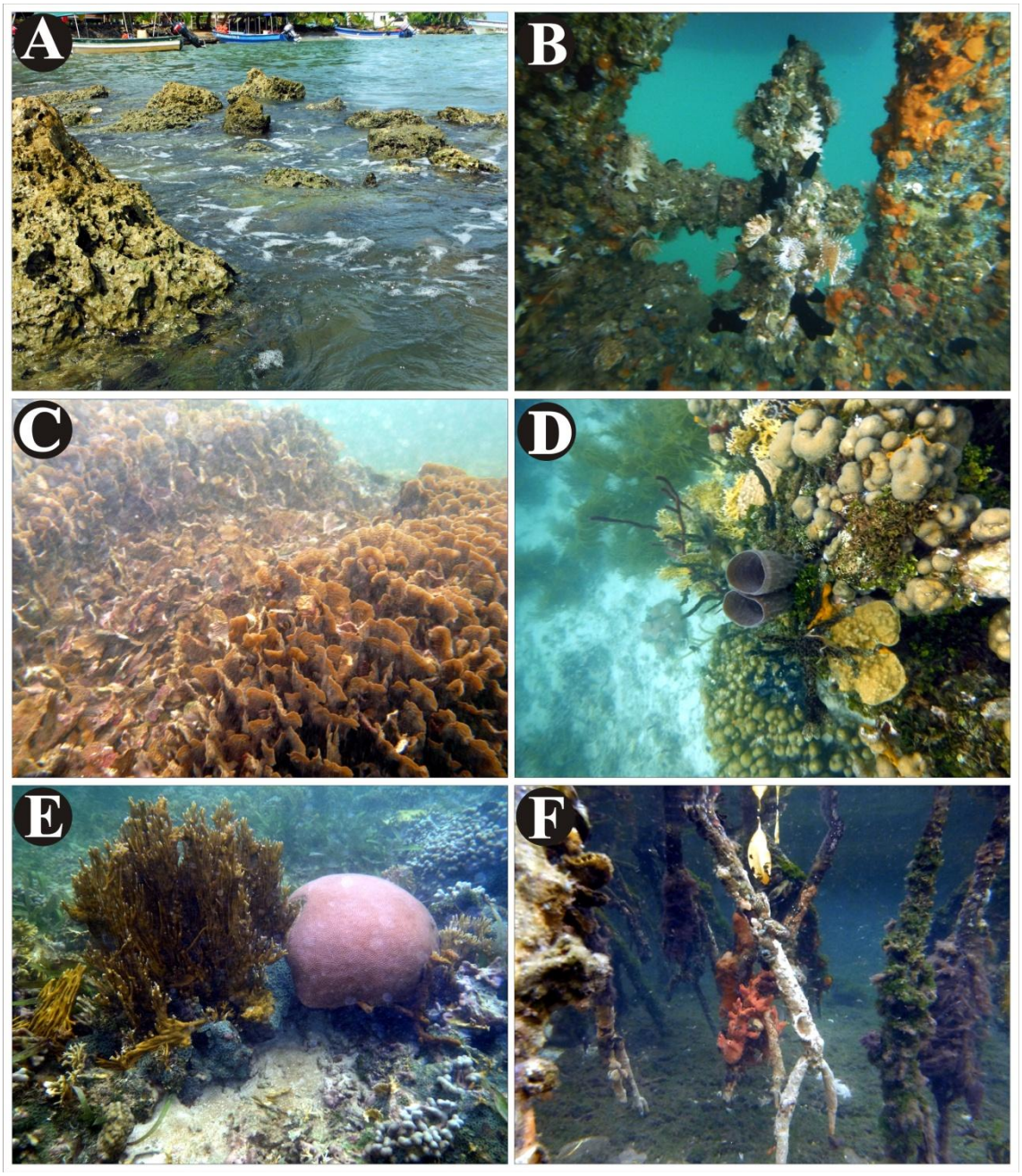

Figure 2. Sampling sites. A, Bocas del Drago; B, Bocas Marina; C, Buoy 14; D, Crawl Key; E, Cristobal; F, Solarte.

Analysis

The morphological analysis of the 69 samples began with the observation of external aspects of the colony, using the stereoscopic microscope. Subsequently, the colonies were cross sectioned and observation of the internal aspects of the colonies was achieved. A portion of approximately 1 cm^2 of the colonies of *Didemnum*, *Lissoclinum*

and *Trididemnum*, was submerged in 3-4% hydrochloric acid to dissolve the spicules and facilitate the removal of zooids and localization of larvae. Some zooids and larvae were separated from the tunic with the help of tweezers and entomological pins and stained with Harris hematoxylin for better visualization of structures.

Permanent slides were prepared with Durcuplan® (Sigma) resin and zooids and larvae were photographed and drawn using the Corel DrawX3 program (2005 version 13.0.0.576).

Sessions of scanning electron microscopy on Center of Microscopy, Universidade Federal do Paraná, were performed for better visualization and description of the morphology of spicules. The spicules were separated from the tunic by incineration (with an alcohol lamp) of a small piece of the colony followed by washing in bleach (sodium hypochlorite) to dissolve what remained of the tunic. The spicules were then stored in 70% alcohol. One drop of the homogenate of alcohol and spicules was put on a piece of double-sided adhesive copper tape attached to the appropriate stub for the JEOL JSM 6360-LV model of Scanning Electron Microscope. Spicules were observed and photographed after metalization with gold.

Finally, after the study of each material morphology, they were compared with published descriptions for the identification and knowledge of the geographical distribution of the species. Species new to science were described in detail.

Results

Among the 69 samples analyzed, 19 species were identified (Table I), five of which had already been recorded for the region of Bocas del Toro (*Diplosoma listerianum*, *Lissoclinum verrilli*, *Didemnum granulatum*, *D. psammatodes* e *Tridemnum orbiculatum*), seven are new records for the location (*Lissoclinum abdominale*, *Didemnum calliginosum*, *D. cineraceum*, *D. perlucidum*, *D. vanderhorsti*, *Tridemnum palmae* e *T. thetidis*) and seven are new species (*Diplosoma* sp. nov. 1, *Diplosoma* sp. nov. 2, *Lissoclinum* sp. nov. 1, *Lissoclinum* sp. nov. 2, *Didemnum* sp. nov., *Tridemnum* sp. nov. 1 e *Tridemnum* sp. nov. 2).

Table I. Didemnidae ascidians found in Bocas del Toro, Panama.

SPECIES	Locations*											
	1	2	3	4	5	6	7	8	9	10	11	12
Diplosoma listerianum		X			X						X	
Diplosoma sp. nov. 1		X										
Diplosoma sp. nov. 2		X										
Lissocinum abdominale		X			X			X	X	X	X	
Lissocinum verrilli									X			
Lissoclinum sp. nov. 1			X									
Lissoclinum sp. nov. 2					X				X			
Didemnum calliginosum		X			X			X			X	
Didemnum cineraceum	X	X									X	
Didemnum granulatum	X			X	X		X		X	X	X	X
Didemnum perlucidum		X			X						X	
Didemnum psammatodes					X					X	X	
Didemnum vanderhorsti								X				
Didemnum sp. nov.										X		
Tridemnum cf. *orbiculatum*						X						
Tridemnum palmae			X									
Tridemnum cf. *thetidis*						X						
Tridemnum sp. nov. 1								X				
Tridemnum sp. nov. 2	X											
Total	3	7	2	1	7	2	1	4	4	4	7	1

1, Bocas del Drago; 2, Bocas Marina; 3, Buoy 14; 4, Cayo Agua Island; 5, Crawl Key; 6, Cristobal; 7, Garden; 8, Hospital Point; 9, Sachen; 10, Solarte; 11, Town Docks; 12, Zapatillas Island.
X: Samples analyzed in this work. X: Samples analyzed before this work.

Genus *Diplosoma* Macdonald, 1859

Three species are described here followed by an identification key for Bocas del Toro species (Table II).

Table II. Tabular key for the identification of specimens in the genus *Diplosoma* in Bocas del Toro. Characters based on the present study and/or literature.

1	2	3	4	5	6	7	8	9	Species
U*,Y	0	9-10	L	0	2	0.5	2	2	*D. listerianum*
Gr	P	5-6	S	0	2	0.8	3	3	*D. simile*
U	0	6-9	0	0	1	0.5	3	4	*Diplosoma* sp. nov. 1
Be	0	5-10	M	P	2	0.5	1	2	*Diplosoma* sp. nov. 2

1. Living colony color: Be—beige, Gr—green, U—uncolored, U*—uncolored with some black spots, Y—yellow.
2. Symbiotic algae in the tunic: P—present, 0—absent.
3. Number of pharyngeal stigmata in half rows.
4. Length of the muscular process: L—longer than the abdomen, M—as long as the abdomen, S—shorter than the abdomen, 0—absent.
5. Glandular tissue on abdomen: P—present, 0—absent.
6. Number of testis follicles.
7. Maximum larval trunk size (μm).
8. Number of larvae embryos.
9. Number of pairs of ectodermal ampullae in larvae.

***Diplosoma listerianum* Milne-Edwards, 1841**

(Plate I A, B)

For synonymy see: Kott, 2001: 339.

Examined material: One colony, Crawl Key, Bocas del Toro, 9º15'05"N, 82º07'56"W, coll. Rosana Moreira da Rocha, 14 June 2009.

Description

Colony is encrusting, 5 cm long, very thin and delicate (1 mm thick). Living colony is transparent with light brown zooids. Zooids are arranged randomly without forming cloacal systems.

Zooids are about 1.2 mm long. The oral siphon is tubular and short, with six rounded small lobes. The atrial opening is wide exposing the four rows of stigmata. The muscular process projects from the base of the thorax and it is longer than the abdomen. In the pharynx, the first row contains about 10 stigmata, while the second, third and fourth have 9 stigmata in each side (based on one zooid).

The abdomen is larger than the thorax and light brown. The esophageal-rectal peduncle is short and curved, making the abdomen horizontal in relation to the vertical thorax. The stomach has an invagination in the region where it is penetrated by the esophagus, assuming a cordiform shape. The intestine is thick, with one constriction. It forms a second loop overlapping the stomach. The anus has a smooth margin at the level between the third and fourth row of stigmata. There are 2 or 3 stolonic vessels. Gonads are underneath the intestinal loop. The testis has 2 follicles that are bean-shaped. There is a small oocyte. Budding was observed in many zooids.

Larvae 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 close to each other, with 2 pairs of short, finger-like ampullae.

Remarks and Distribution

The species has a wide geographical distribution. However, from the sequencing of the COI gene of 234 colonies collected in 14 different localities, Pérez-Portela et al. (2013) found four monophyletic clades. They concluded that it is a species complex. In the region of Bocas del Toro they found clades A, C and D in Bastimentos Island and A was also found in Bocas Marina. The site where the colony described above was collected was not sampled by Pérez-Portela et al. (2013) and it is not possible to conclude to which clade it corresponds. Incongruence among descriptions of specimens from different regions might indicate different clades in these regions. For instance, in the description of Monniot (1983), *D. listerianum* from Guadeloupe has only about 8 stigmata in the first and second rows, 7 stigmata in the third and 6 in the fourth. However, in the description of Lafargue (1968), the species in France has 9-10 stigmata in the first row, 8-9 stigmata in the second and third and the 7-8 in the fourth, more similar to the specimen from Bocas. In Atlantic there are a few other species with 2 pairs of ampullae in the larvae. *Diplosoma glandulosum* Monniot, 1983 has glandular tissue surrounding the gut and testis (Monniot 1983); *D. tritestis* Monniot, 1984 has 3 follicles in the testis (Monniot 1984); *D. lafargueae* Vazquez 1993 has no muscle process, zooids are significantly larger and have less stigmata in the pharynx (Vazquez 1993) and *D. singulare* Larfargue 1968 has a single testis (Larfargue & Wahl 1987).

***Diplosoma* sp. nov. 1**

(Figure 3, Plate II)

Examined material: *Holotype:* One colony, Bocas Marina, Bocas del Toro, 9º20'8"N, 82º14'48"W, coll. Rosana Moreira da Rocha, 09 June 2009.

Description

Colony is encrusting, 10 cm long, very thin and delicate (1 mm thick). It crumbles easily when handled. Both living and preserved, it is transparent with colorless zooids.

Zooids are about 1 mm long and are surrounded by a thin tunic. The oral siphon is short, with six rounded small lobes. The atrial opening exposes the second and third rows of stigmata and part of the fourth. There is no muscular process. In the pharynx, the first row contains 9 stigmata, while the second contains 8, the third has 7 and the fourth has 6 stigmata in each side (based on one zooid). In some zooids the thorax is larger than the abdomen.

The esophageal-rectal peduncle is long and curved toward the ventral side. The stomach is yellow with an invagination in the region where it is penetrated by the esophagus, assuming an elongate cordiform shape. The intestine forms a second loop that overlaps the stomach. Gonads are underneath the intestinal loop. The testis has one spherical follicle. The sperm duct is enlarged along the testis, where it positioned inside a groove. There is one oocyte. Budding was observed in many zooids.

Larvae lie in the superficial layer of the colony and are gemmiparous (one oozooid and two blastozooids) with the trunk 0.5 mm long; the tail winds a little more than 1/3 around the trunk. Three adhesive papillae are present with thin long stalks, with 4 pairs of short, finger-like ampullae.

Remarks

Among the species of *Diplosoma* found in the Atlantic, the only species with one follicle in the testis is *D. singulare*. However, this species has the abdomen in vertical position with gonads on the right side and a non gemmiparous larva (Lafargue 1968). In other oceans, *D. ata* Monniot & Monniot, 1987 has also one follicle in the testis and the abdomen is perpendicular to the thorax, however the oral siphon has six very long leaf-like lobes, the pharynx has at least 10 stigmata in each half row, there are pigmented cells around the oral siphon, endostyle and gut, and the larva has a larger number of ampullae (Monniot & Monniot 1987). *Diplosoma pannosum* Monniot & Monniot, 2001 differs from the above species by the larger number of ampullae in the larvae, *D. unitestis* Monniot & Monniot, 2001 has a single ovary isolated in a pouch in the body wall and *D. redika* Monniot, 1994 has a protruding testicle outside the abdomen, 4 pairs of ampullae very distant from each other and larvae are incubated in the basal layer of the colony.

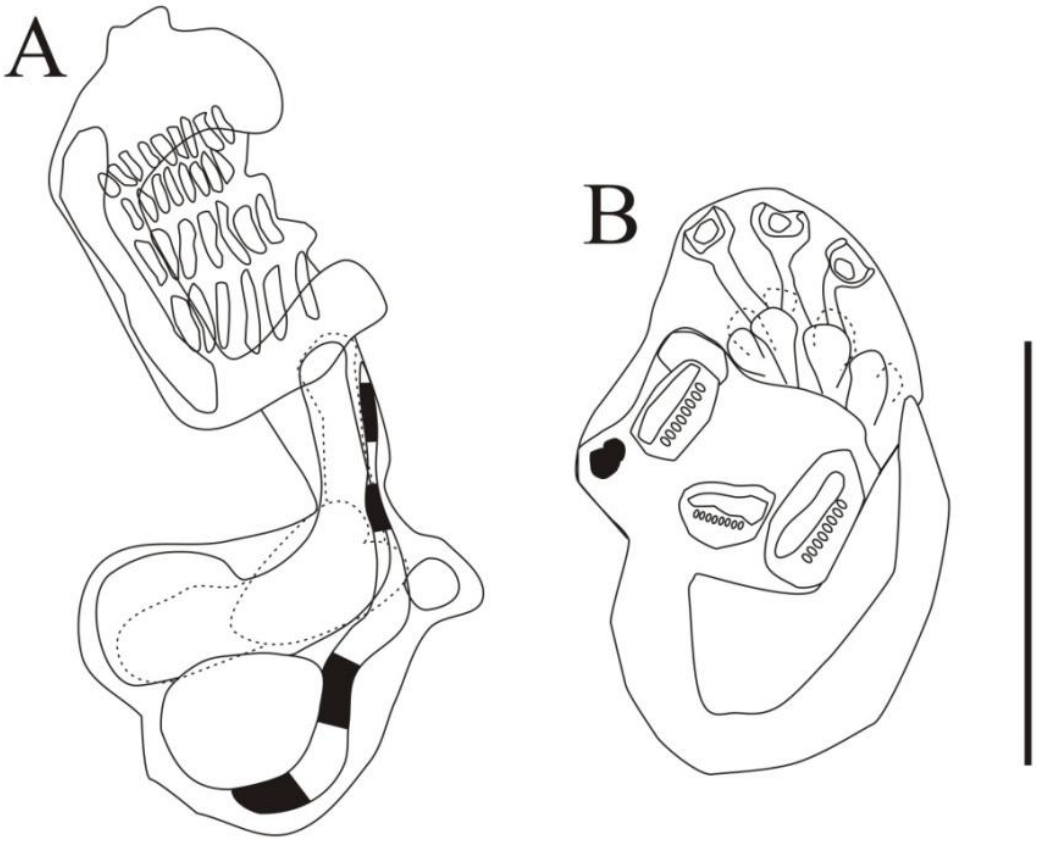

Figure 3. *Diplosoma* sp. nov. 1. A, zooid; B, larva. Scale bar, 0.5 mm.

***Diplosoma* sp. nov. 2**

(Figure 4, Plate III)

Examined material: *Holotype:* One colony, Bocas Marina, Bocas del Toro, 9º20'8" N, 82º14'48" W, coll. Rosana Moreira da Rocha, 12 June 2011.

Description

Colony is encrusting, 20 cm long, and very thin and delicate (1 mm thick). The living colony is beige with large and protruding cloacal cavities.

Zooids are about 1 mm long. The oral siphon is short, with six triangular small lobes. The atrial opening exposes all rows of stigmata. The muscular process projects from the base of the thorax, close to the endostyle, and it is the same size of the abdomen. In the pharynx, the first and second rows contains 10 stigmata, while the third have 6 to 8 and the fourth has 5 to 6 stigmata in each side.

The abdomen is light brown on posterior region. The stomach is large with an invagination in the region where it is penetrated by the esophagus, assuming a cordiform shape. The anus has a smooth margin at the level of the fourth row of stigmata. There are no stolonic vessels. Gonads are underneath the intestinal loop. The testis has two follicles which separation is difficult to see. It has a glandular tissue that binds it to the intestine. There are two oocytes. Budding was observed in many zooids.

Larvae are incubated in the lower layer of the. They are gemmiparous with the trunk 0.5 mm long; the tail winds a little less than halfway around the trunk. Three adhesive papillae are present with thin stalks, positioned close to each other, with 2 pairs of short, finger-like ampullae with pigmented ends. Oozooids are located in the anterior portion of the larvae

Remarks

Diplosoma glandulosum has a glandular tissue around the testis, but it only covers part of the intestinal loop, the species is smaller and have fewer stigmata on pharynx zooids (Monniot 1983). Another species that contains glandular tissue near the testis is *Diplosoma carnosum* Von Drasche, 1883, but the tissue also covers only part of the testicle and intestinal loop, and it can have more that 5 testicular follicles and has 4 pairs of ampullae inside the larvae (Lafargue & Wahl 1987).

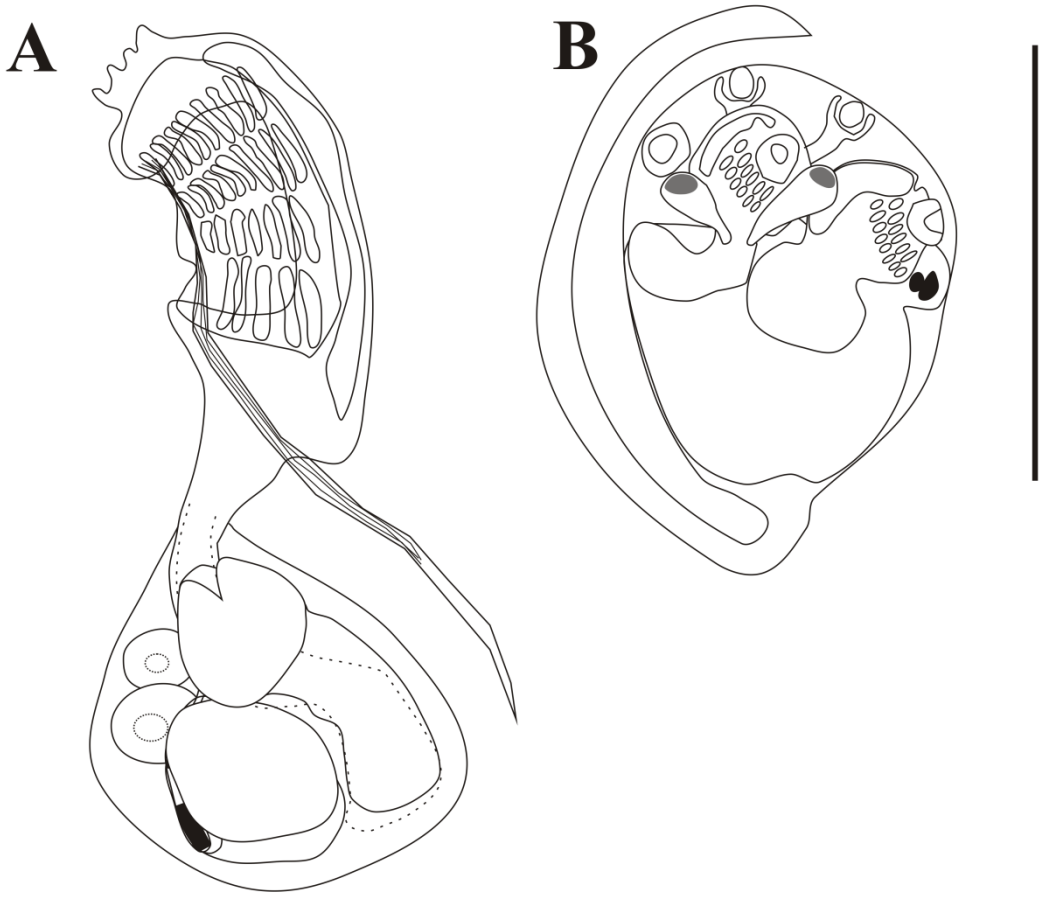

Figure 4. *Diplosoma* sp. nov. 2. A, zooid; B, larva. Scale bar, 0.5 mm.

Genus *Lissoclinum* Verrill, 1871

Four species are described here followed by an identification key for Bocas del Toro species (Table III).

Table III. Tabular key for the identification of specimens in the genus *Lissoclinum* in Bocas del Toro. Characters based on the present study and/or literature.

1	2	3	4	5	6	7	Species
many	60	8-10	0	G	0.4	4	*L. abdominale*
4	60	7-9	C, I	G	1.0	4	*L. verrilli*
4	210	9-10	C	?	?	?	*Lissoclinum* sp. nov. 2
4 or 3 plus many in central region	170	8-12	C	N	0.9	5-6	*Lissoclinum* sp. nov. 1

1. Number of spicule rays in optical transverse section.
2. Maximum spicule size (μm).
3. Number of pharyngeal stigmata in half rows.
4. Shape of the lateral organ: C—circular, I—irregular, 0—absent.
5. Larvae: G—gemmiparous, N—non-gemmiparous,
6. Maximum larval trunk size (μm).
7. Number of pairs of ectodermal ampullae in larvae.

***Lissoclinum abdominale* Monniot, 1983**

(Figure 5, Plate IV)

Examined material: One colony, Solarte, Bocas del Toro, 9º16’36"N, 82º10'15" W, coll. Rosana Moreira da Rocha, 9°17'30"N, 82°10'20"W, 04 July 2011.

Description

The encrusting colony is approximately 11 cm long and 3 mm thick. Tunic is transparent with some white spots where spicules are. Spicules are scarce and are around the zooids abdomen and larvae. Spicules are rounded and irregular, not exceeding 60 µm in diameter and have many needle-like rays.

The zooids are about 1.2 mm long. Thorax is larger than abdomen. Oral siphon has six small rounded lobes. The atrial aperture is wide open, exposing most of the pharynx and an atrial languet is absent. Thoracic lateral organs were not observed. In the pharynx, the rows contains from 8 to 10 stigmata on each side.

The location and shape of the stomach are typical; the intestinal loop has 1 constriction and forms a secondary loop that passes besides the stomach without overlaying it. The testis has a unique and spherical follicle. Ovary is dorsal to the testis and has two oocytes of approximately 0.20 mm and 0.05 mm. Budding in the esophageal region was observed in some zooids.

The larvae are gemmiparous (with two embryos) and more elongate than wide; trunk is approximately 0.4 mm in length, around which the tail winds a half of the way. The larvae have 3 adhesive papillae and 4 pairs of ectodermal ampullae with flattened and dark distal region (5 pairs was perceived on one side in one larva).

Remarks

Despite the similarities between zooids, *Lissoclinum abdominale* and *L. verrilli* have many differences in the spicules (which have only four rays in *L. verrilli*) and the size of the larvae (Rocha et al. 2005). *Lissoclinum perforatum* (Giard, 1872) has large thoracic lateral organ, pigmented larvae and spicules with rounded rays.

Distribution

Papua New Guinea (Monniot & Monniot 2001), New Caledonia (Monniot 1992), Belize (Goodbody 2000), Jamaica (Goodbody 2003), Guadeloupe (Monniot 1983) and Brazil (Rocha & Faria 2005).

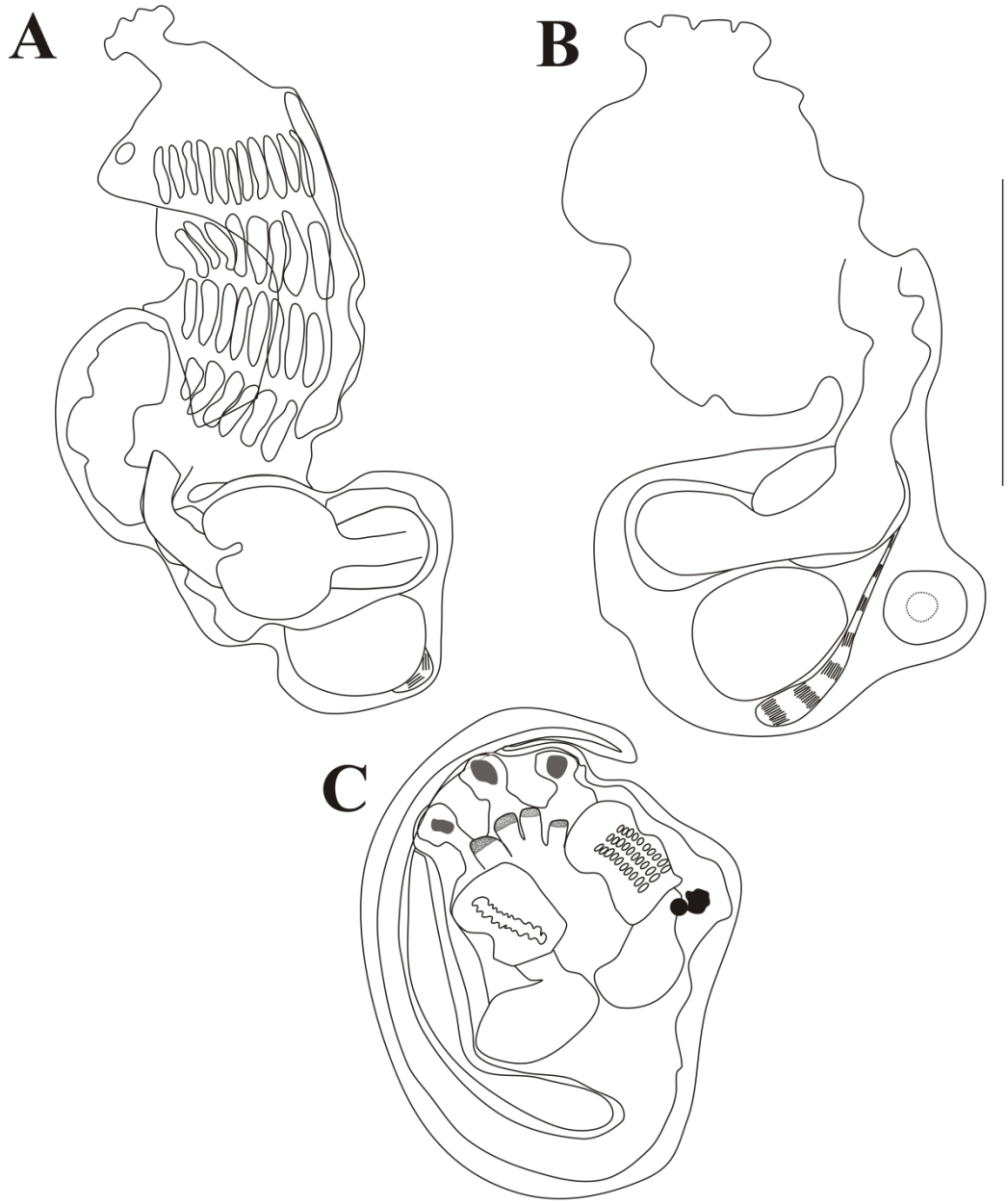

Figure 5. *Lissoclinum abdominale*. A, zooid (left side); B, zooid (right side); C, larva. Scale bar, 0.5 mm.

***Lissoclinum verrilli* (Van Name, 1902)**

(Plate V)

Echinoclinum verrilli Van Name 1902: Van Name, 1921: 341 figs 33-35; Van Name, 1945: 115, fig. 55; Millar, 1953: 300, fig. 12; Tokioka, 1958: 316 fig. 2; Kott, 1962: 312.

Examined Material: One colony, Sachen, Bocas del Toro, 9º21'11" N, 82º12'50"W, coll. Rosana Moreira da Rocha, 13 July 2011.

Description

The encrusting colony is about 1.5 cm long and 1.5 mm thick. Tunic is transparent with some white spots where spicules are. Spicules are scarce and are more common on the region of oral siphons. The most superficial layer of the colony has no spicules. The spicules are tetrahedral, with 45 to 60 µm in diameter.

Zooids are about 1 mm long. Thorax measuring twice the length of the abdomen. Oral siphon is tubular and have 6 small triangular lobes. The atrial aperture is small and has 2 muscular fibers on edge. Thoracic lateral organs were not observed. In the pharynx, the rows contains 7 and 9 stigmata in each side.

The stomach is yellowish a long time after fixation. The intestinal loop has 3 constrictions and doesn't form a secondary loop. The testis was not developed. Ovary is on the intestinal loop, has one oocyte of approximately 0.4 mm. No budding in the esophageal region was observed.

No larvae were found.

Remarks

The identification of this sample was based mainly on the morphology of spicules. The other important characteristics in identifying *Lissoclinum* spp. (larvae and developed testis) were absent in the sample and therefore the description remained incomplete. Original description of *L. verrilli* do not mentioned the musculature of the atrial opening, but in Monniot (1983) they are described in detail.

Distribution

Florida (Van Name 1945), Ghana (Millar 1953), Japan (Tokioka 1958) and Australia (Kott 1962).

***Lissoclinum* sp. nov. 1**

(Figure 6, Plate VI)

Examined material: *Holotype:* One colony, Buoy 14, Bocas del Toro, 9º2'07.8" N, 82º20'35.4" W, coll. Rosana Moreira da Rocha, 30 June 2009.

Description

The encrusting colony is about 4 cm long and 2 mm thick. Tunic is transparent with some white spots where spicules are. Spicules are scarce and more common in the region of oral siphons. The spicules have two distinct shapes. Some contains 3 large needle-like rays and numerous short, thin rays in the central region, measuring around 71 µm and others contain 3 conical rays and 14 short and truncated rays in central region, measuring 95 to 170 µm.

Zooids are about 1.4 mm long. Thorax and abdomen have the same size. Oral siphons are tubular with 6 small triangular lobes. Thoracic lateral organs are circular, protruding, at the level between second and third rows of stigmata. In the pharynx, the first row contains 12 stigmata, the second row contains 11, the third contains 10 and the fourth contains 8.

The esophageal-rectal peduncle is curved to dorsal side. Stomach is yellow long time after preserved. The intestinal loop has 3 constrictions. Testis is within the intestinal loop. Ovary is outside the intestinal wall, dorsal to the testis, close to stomach and has 2 oocytes of approximately 0.20 mm. Budding in the esophageal region was observed in some zooids.

The larvae are more elongate than wide, trunk is approximately 0.9 mm length, around which the tail winds a half of the way. Larvae have 3 adhesive papillae with long

stalks and 5 pairs of ectodermal ampullae (some larvae have 6 pairs). The sensory vesicle (with otolith and ocellus) is in the mid-dorsal region of the trunk of the larvae.

Remarks

Spicules of this species are quite unique. *Lissoclinum verrilli* differs from the species described above by its gemiparous larvae and spicules with 4 rays. *Lissoclinum triangulum* Millar, 1975 also has varied spicules, similar to the species above, but the larva is smaller and has only 4 pair of ampullae (Millar 1975).

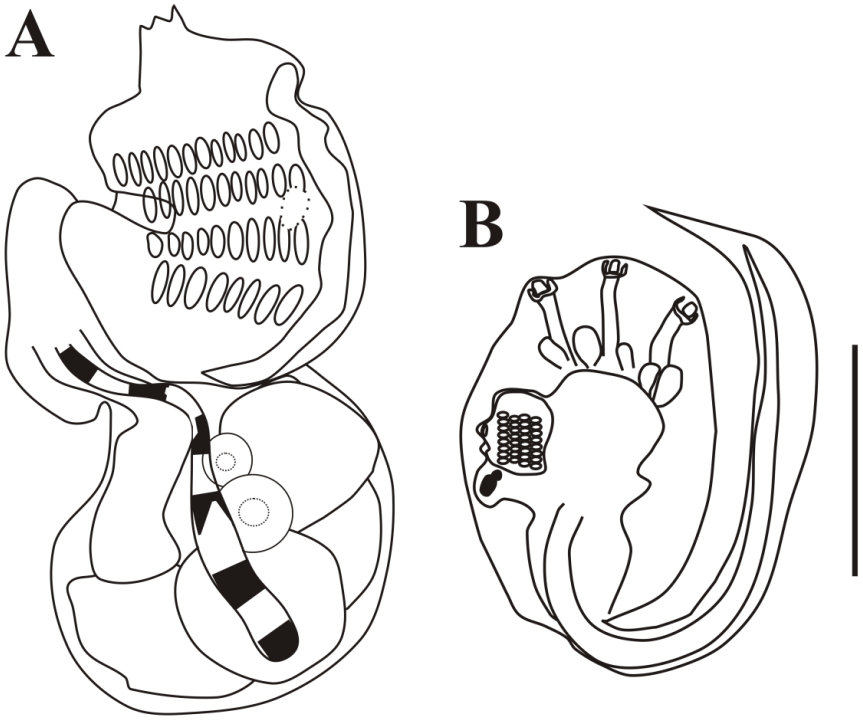

Figure 6. *Lissoclinum* sp nov. 1. A, zooid; B, larva. Scale bar, 0.5 mm.

***Lissoclinum* sp. nov. 2**

(Figure 7, Plate VII)

Examined Material: *Holotype:* One colony, Crawl Key, Bocas del Toro, 9º15'16" N, 82º08'10" W, coll. Rosana Moreira da Rocha, 22 June 2011. *Paratypes:* Three colonies, Crawl Key, Bocas del Toro, 9º15'16" N, 82º08'10" W, coll. Rosana Moreira da Rocha, 22 June 2011. One colony, Crawl Key, Bocas del Toro, 9º15'16" N, 82º08'10" W, coll. Rosana Moreira da Rocha, 25 August 2003. One colony, Sachen, Bocas del Toro, 9º15'16" N, 82º08'10" W, coll. Rosana Moreira da Rocha, 15 July 2011.

Description

The encrusting colonies are about 2 mm thick, not exceeding 2.5 cm in length. Tunic is transparent with some white spots where spicules are. Spicules are scarce and more common around zooids. Zooids are disposed on systems with approximately 12. Spicules are tetrahedral with conical rays, measuring 50 to 210 µm.

Zooids are about 0.8 mm long. Oral siphon is tubular with 6 small triangular lobes. The atrial aperture is strait and has muscular fibers on border. Thoracic lateral organs are circular, protruding, at the level between third and fourth rows of stigmata. In the pharynx, the rows contain 9 to 10 stigmata in each side.

The esophageal-rectal peduncle is long and curved to dorsal side. The stomach is yellow long time after preserved. The intestinal loop has 2 constrictions and second loop that partially covers the stomach. The testis is within the intestinal loop. Ovary dorsal to the testis, close to stomach and has 2 oocytes. Budding in the esophageal region was observed in some zooids.

No larvae were found.

Remarks

The spicules of this species are quite unique. Morphologically resemble with the spicules of *L. verrilli*, but are considerably larger. *Lissoclinum taratara* Monniot & Monniot, 1987 also has tetrahedral spicules, but they are small like those of *L. verrilli* (Monniot & Monniot 1987). *Lissoclinum tuheiavae* Monniot C. & Monniot F., 1987 have tetrahedral spicules larger than the *L. verrilli*, but the morphology differs substantially by having rays forming a rosette at the center (Monniot & Monniot 1987). *Lissoclinum sente* Kott, 2001 spicules may have from 4 to 8 rays (Kott 2001).

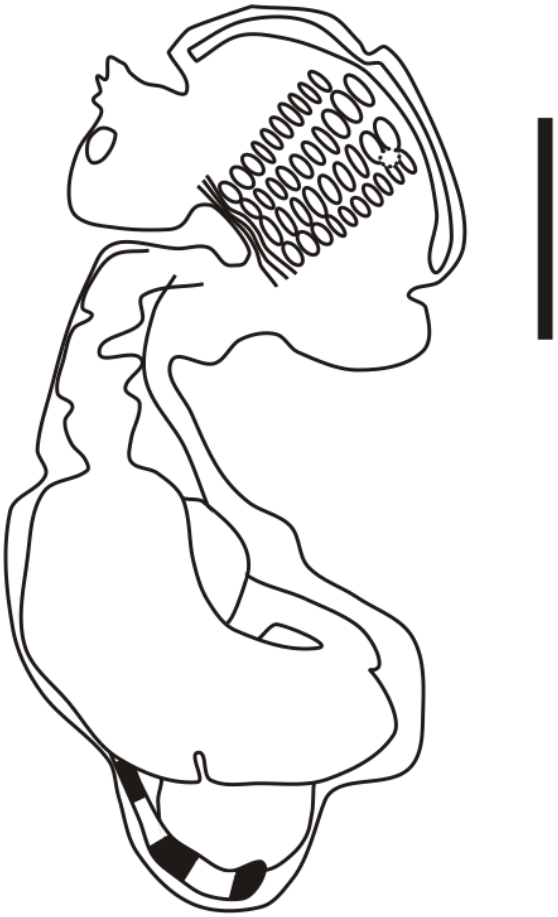

Figure 7. *Lissoclinum* sp nov. 2. A, zooid. Scale bar, 0.2 mm.

Genus *Didemnum* Savigny, 1816

Seven species are described here followed by an identification key for Bocas del Toro species (Table IV).

Table IV. Tabular key for the identification of specimens in the genus *Didemnum* in Bocas del Toro. Characters based on the present study and/or literature.

1	2	3	4	5	6	7	8	9	10	11	12	13	Species
W	S	A	40	6	0	5	C, O, P	S	6-7	0.4	4		*D. speciosum*
W	S, P	A, S	40	6	0	5-8	O, E	L	8	0.5	4	CC	*D. perlucidum*
W, P, Pi	S	A	40	4-7	0	5-7	C	M	7	0.4	4	SA	*D. conchyliatum*
Br, Be, O	S	L	20	12-14	0	8-10	C	S	6-7	0.4	4	GG	*D. calliginosum*
Br, B, Dp	S	L	20	12	0	5-8	C	L	8-9	0.5	8-10		*D. cineraceum*
Br, G	S	L	30	11-13	0	8	I	L	6-8	0.5	4	FP	*D. psammatodes*
Br, B, Dp	S	L	35	12	0	6-7	O	L	9	0.5	4		*D. vanderhorsti*
Bc	P	A	35	7-9	0	4-6	O, E	M, L	6-7	0.4	4		*D. granulatum*
O	S	A	40	4-8	0	4-6	C, E	S	8-9	0.4	4		*Didemnum* sp. nov.
O	S	A	50	10	P	6-10	C	S	6-9	0.75	10	DL	*D. ligulum*

1. Living colony color: B—black, Be—beige, Br—brown, Bc—brick, Dp—dark-purple, G— gray, O—orange, P—purple, Pi—pink, W—white.
2. Colony surface: P—with papillae, 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 rows.
8. Shape of the lateral organ: C—circular, E—elongate, I—irregular, O—oval, P—protruding.
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 coils of the sperm duct.
11. Maximum larval trunk size (μm).
12. Number of pairs of ectodermal ampullae in larvae.
13. Peculiar characteristics: CC—spicules less abundant along cloacal canals, SA—presence of symbiotic algae, GG—presence of c-shape gland inside intestinal loop, FP—presence of fecal pellets in the tunic, DL—presence of a small dorsal languet.

***Didemnum calliginosum* Monniot, 1984**

(Figure 8, Plate VIII)

Examined material: Two colonies, Bocas Marina, Bocas del Toro, 9º20'8" N, 82º14'48" W, coll. Rosana Moreira da Rocha, 21 June 2014. One colony, Crawl Key, Bocas del Toro, 9º15’05” N, 82º07’56” W, coll. Rosana Moreira da Rocha, 23 June 2014.

Description

Colonies are very thin (about 1 mm) and 4.5 to 6 cm long. Colony surface is beige, orange or brown. When preserved in formalin, the colonies are transparent with white zooids. Spicules are scarce, making the colony very delicate. Spicules are stellate, 15 to 20 µm, with many truncated rays.

Zooids are about 1 mm long. The oral siphon has six or more small rounded lobes. The atrial aperture is wide open, exposing most of the pharynx (except in contracted zooids) and an atrial languet is absent. The muscular process projects from the beginning of the esophageal-rectal peduncle and is shorter than the abdomen. Thoracic lateral organs are protruding and horizontally elongated, at the level of the fourth row of pharyngeal stigmata. In the pharynx, the first and the second rows contain 8 to 10 stigmata, while the third 8 to 9 and the fourth 8 to 10 stigmata in each side.

The abdomen is larger than the thorax. One, two or three stolonic vessels were observed in some zooids. The stomach is elongated (in zooids from the brown colony, it is rounded). The intestinal loop has two constrictions and does not form a secondary loop and passes besides the stomach without overlaying it (in zooids from the brown colony, the intestinal loop forms a secondary loop that covers the stomach). There is a c-shape gland inside the intestinal loop. In zooids from the brown colony, the gland is not

visible but it is possible to realize a depression where the gland would be. The testis has a unique and spherical follicle surrounded by 6 to 7 turns of the sperm duct. The ovarium has a small oocyte. No budding in the esophageal region was observed.

Larvae are oval, trunk is approximately 0.4 mm in length, around which the tail winds 3/4 of the way. Larvae are yellow when preserved, have three adhesive papillae with thick stalks, plus 4 pairs of ectodermal ampullae which are straight in the terminal portion. The sensory vesicle (including ocellus and otolith) is in the mid-dorsal region of the larval trunk.

Remarks

Didemnum calliginosum is similar to *D. cineraceum* (Sluiter 1898) in respect to the characteristics of colony, spicules and zooids, but they differ from each other in the characteristics of the larva (larvae of *D. cineraceum* are 1 mm long, have 8 pairs of ampullae on each side and 2 blastozooids) (Monniot 1983). *Didemnum vanderhorsti* Van Name, 1924 also presents many resemblances with *Didemnum calliginosum*, but they differ in the number of turns of spermiduct and in the presence of glands in the intestinal wall (*D. vanderhorsti* has 9 turns of the spermiduct and lacks the gland) (Rodrigues et al. 1998). The presence of a depression in the body wall of zooids, marking the location of the gland, may indicate that the zooids of the brown colony are on a physiological state in which the gland is atrophied or is not developed.

Distribution

Guadeloupe (Monniot 1984).

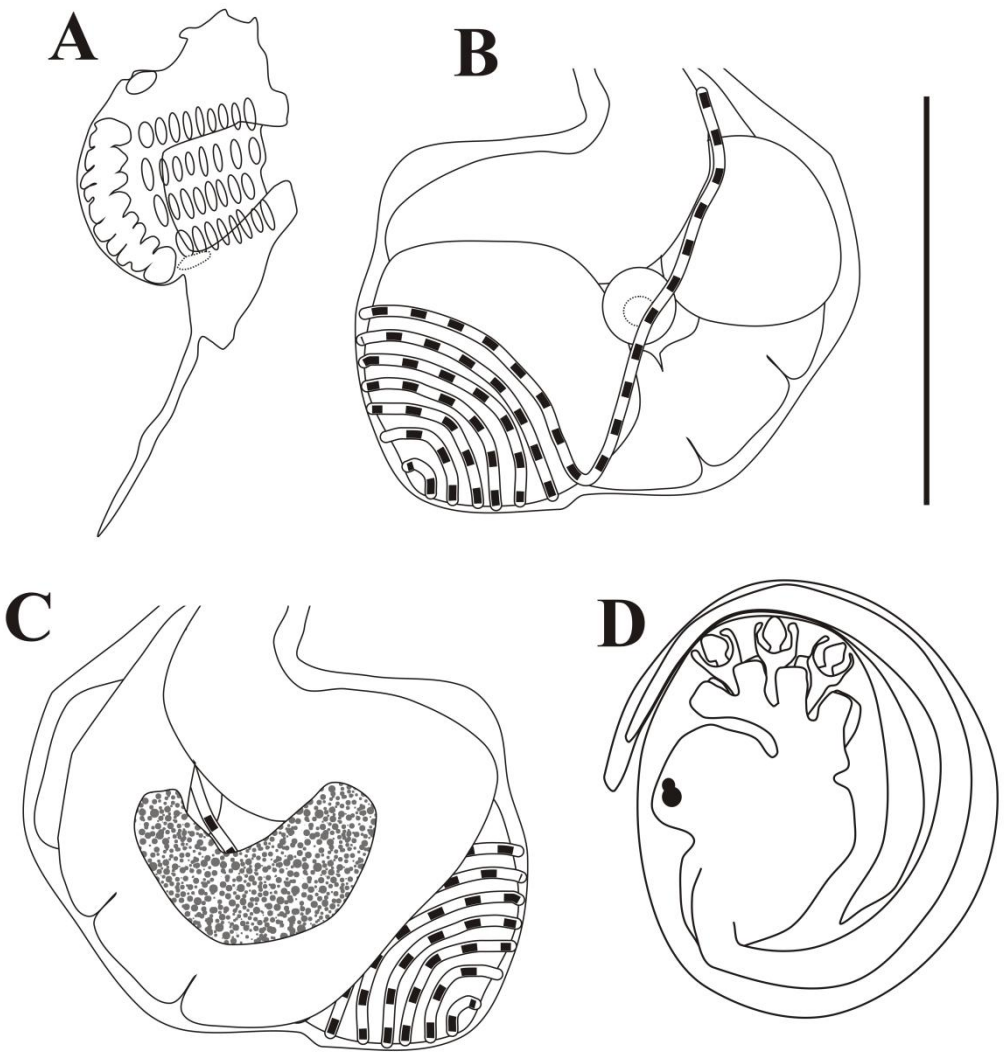

Figure 8. *Didemnum calliginosum*. A, thorax; B, abdomen; C, abdomen (with glandular tissue); D, larva. Scale bar, 0.5 mm

***Didemnum cineraceum* (Sluiter, 1898)**

(Figure 9, Plate IX)

Leptoclinum cineraceum Sluiter, 1898: 30, Pl. II fig. 41 e 41a, Pl. III fig. 48.

Examined material: Two colonies, Town Docks, Bocas del Toro, 9°20'13" N, 82°14'25" W, coll. Rosana Moreira da Rocha, 06 August 2008. One colony, Town Docks, Bocas del Toro, 9º20'07" N, 82º14'32" W, coll. Rosana Moreira da Rocha, 06 August 2008.

Description

The encrusting colonies are 5 to 8,5 cm long and 1 mm thick. Colonies surfaces are black or brown. Tunic is delicate, because spicules are not abundant. Spicules are stellate, 15 to 20 µm, with many truncated rays.

The zooids are about 1.5 mm long. Oral siphon has many small rounded lobes. The atrial aperture is wide open, exposing the second and third rows of stigmata and an atrial languet is absent. The muscular process projects from the base of the thorax and is longer than the abdomen (in some zooids, two times the size of abdomen). Thoracic lateral organs are circular, at the level of the fourth row of pharyngeal stigmata. In the pharynx, the first row contains 8 stigmata, the second and third contains 7, while the fourth contains 5.

The abdomen is larger than the thorax. The stomach is rounded; the intestinal loop has 2 constrictions and forms a secondary loop, partially overlaying the stomach. The gonads are within the intestinal loop, one beside other. The testis has a unique and spherical follicle surrounded by 8 to 9 turns of the sperm duct. In some zooids, the

ovarium is located above the testicle, where the spermiduct turns ends. No budding in the esophageal region was observed.

Larvae are gemmiparous, oval; trunk is approximately 0.5 mm in length, around which the tail winds more than half of the way. The larvae have three adhesive papillae with long stalks, plus 8 to 10 pairs of ectodermal ampullae. The embryos thoraxes have 8 stigmata on the first row. The sensory vesicle (including otolith and ocellus) is in the mid-dorsal region of the larval trunk.

Remarks

Didemnum cineraceum is similar to *D. calliginosum* in the characteristics of colony, spicules and zooids, but they differ of each other in the characteristics of the larva (larvae of *D. calliginosum* are smaller with fewer number of ampullae and are not gemmiparous) (Monniot 1983).

Distribution

New Caledonia (Monniot 1995), Sierra Leone (Monniot & Monniot 1994), Belize (Goodbody 2000), Jamaica (Goodbody 2003), Guadeloupe (Monniot 1983) and Brazil (Rocha et al. 2011).

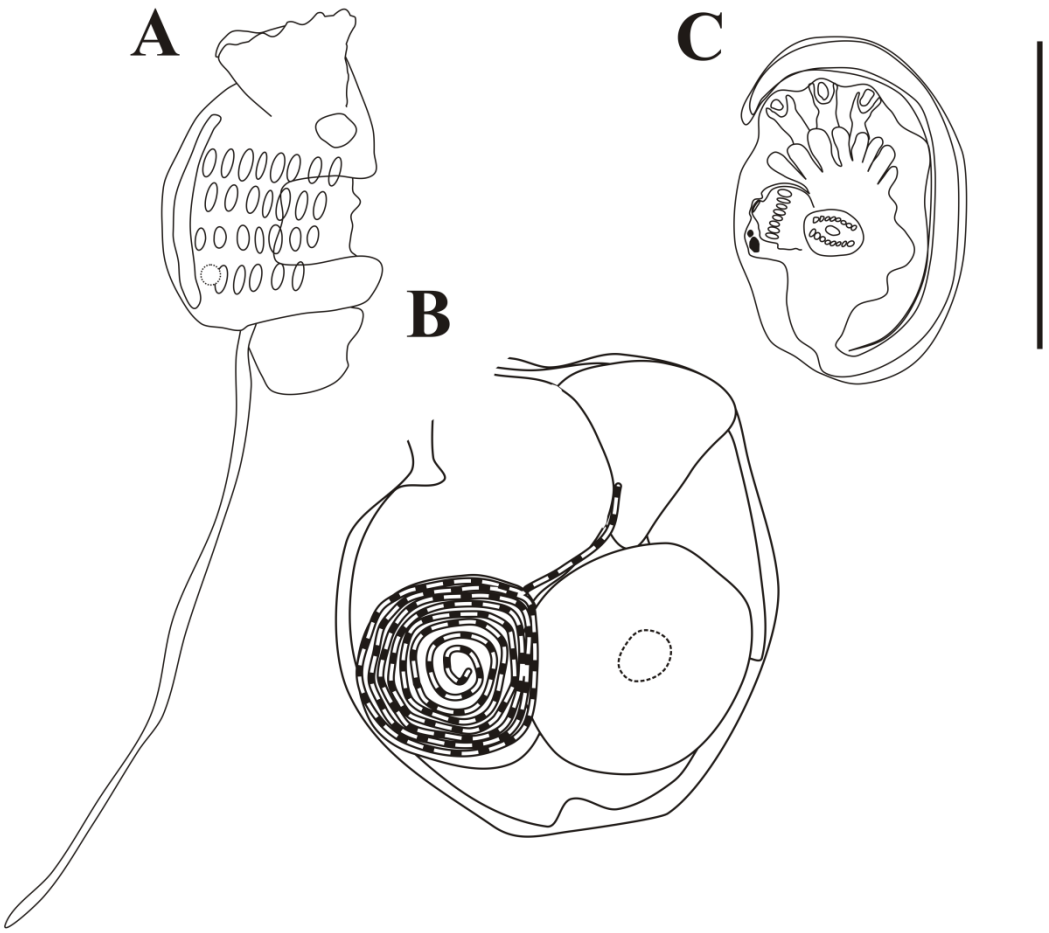

Figure 9. *Didemnum cineraceum*. A, thorax; B, abdomen; C, larva. Scale bar, 0.5 mm.

***Didemnum granulatum* Tokioka, 1954**

(Plate X)

Didemnum (Didemnum) moseleyi f. granulatum Tokioka, 1954: 244, pr XXI, fig. 1-4;

Didemnum moseley: Eldredge, 1967: 210;

Didemnum pele: Eldredge, 1967: 197;

Examined material: Two colonies, Crawl Key, Bocas del Toro, 9º15'16" N, 82º08'10" W, coll. Rosana Moreira da Rocha, 05 August 2003. Two colonies, Bocas del Drago, Bocas del Toro, 9°25'00" N, 82°19'46" W, coll. Rosana Moreira da Rocha, 07 August 2003. One colony, Cayo Agua Island, Bocas del Toro, 9°08'70" N, 82°00'97" W, coll. Rosana Moreira da Rocha, 08 August 2003. Three colonies, Garden, Bocas del Toro, 9º32'11" N, 82º22'00" W, coll. Rosana Moreira da Rocha, 16 June 2011.

Description

The encrusting colonies are approximately 0.5 to 6 cm long and about 1 mm thick. Colonies can be light pink, light orange, orange, light red, red with white borders. Some colonies have orange pigment in tunic and zooids. Spicules are dense on the surface of the firm colony and cluster in some spots, forming small papillae (easier to observe in magnification). Spicules are stellate, 10 to 35 µm in diameter, with 7 conical rays in optical optical transverse section.

Zooids are 0.9 mm long, abdomen and thorax are equal in size. The oral siphon has six triangular lobes. The atrial aperture is wide, exposing the rows of pharyngeal stigmata. The atrial languet is absent. The muscular process projects from the esophageal-rectal peduncle and is longer than the abdomen. Elongated lateral organs are

between the third and fourth rows of pharyngeal stigmata. Between 5 and 6 stigmata per row are on each side of the pharynx.

The esophagus is short; the stomach is rounded; the intestinal loop (with 3 constrictions) forms a secondary loop, covering part of the stomach. There are four small stolonic vessels. Gonads are located in the intestinal loop. The testis is single, spherical and surrounded by 7 or 8 coils of the sperm duct. The ovarium is above the testis and has 1 oocyte. No budding was observed.Larval trunk is oval, about 0.4 mm long, around which the tail winds full way.

Larvae have three linearly arranged adhesive papillae with short stalks, plus four pairs of ectodermal ampullae that are larger distally. The sensory vesicle (including ocellus and otolith) is in the mid-dorsal region of the larval trunk.

Remarks

This specie was originally classified as a subspecies of *Didemnum moseleyi* (Herdman 1886), but colonies of *D. moseleyi* have larger stellate spicules with more rays (11 in optical optical transverse section), and globular spicules. Zooids of *D. moseleyi* have more stigmata in the pharynx and nine sperm duct coils (Herdman 1886).

Distribution

Japan (Tokioka 1954), China (Kott & Goodbody 1980), Fiji (Kott 1981), New Caledonia (Monniot F. 1995), Polynesia (Monniot & Monniot 1987), Australia (Kott 2001, 2007), Sierra Leone (Monniot & Monniot 1994), Brazil (Rocha & Monniot 1993, 1995) and South Africa (Monniot et al. 2001).

***Didemnum perlucidum* (Sluiter, 1898)**

(Plate I C)

Examined material: One colony, Town Docks, Bocas del Toro, 9º20'07" N, 82º14'32" W, coll. Rosana Moreira da Rocha, 29 March 2009.

Description

Colony is white with gray canals, thin (about 1.5 mm) and has 5 cm in length. Spicules are abundant in the surface layer of the colony, but less abundant along cloacal canals. Spicules are stellate, not exceeding 30 µm in diameter and have 7 to 9 conic rays in optical transverse section.

The zooids are about 1.2 mm long. Thorax is larger than abdomen. Oral siphons have six triangular lobes. The atrial aperture is wide open, completely exposing the second and third rows and partly of the first and fourth rows. The muscular process projects from the base of the thorax and is longer than the abdomen. Thoracic lateral organs are elongated and located on the level of the the fourth row of stigmata. In the pharynx, the first and the second rows contain 8 stigmata and the third and the fourth contain 5 to 6 in each side.

The intestinal loop has 2 constrictions and forms a secondary loop that partially covers the stomach. Gonads are within the intestinal loop. The testis has a unique and spherical follicle surrounded by 6 turns of the sperm duct. The ovarium is located above the testicle. Budding in the esophageal region was observed in some zooids.

The larvae are more elongate than wide (0.4 mm trunk length) and the tail winds 3/4 around the trunk. Larvae have 3 adhesive papillae with large stalks and 4 pairs of ectodermal ampullae. The embryos thoraxes have 6 stigmata on the first and second

rows and 4 to 5 stigmata on the third. The sensory vesicle (with otolith and ocellus) is in the posterior region of the larval trunk.

Remarks

Colony aspect of *Didemnum perlucidum* is similar to *Didemnum ahu* Monniot & Monniot, 1987, but they differ from each other by the spicules morphology (*D. ahu* spicules are bigger and with smaller rays) (Monniot & Monniot 1987).

Distribution

Philippines (Monniot & Monniot 2001), Guam (Paulay et al. 2002; Lambert 2003), Indonesia (Monniot & Monniot 1996), Australia (Smale & Childs 2012), Tahiti (Monniot et al. 1985), Polynesia (Monniot & Monniot 1987), New Caledonia (Monniot F. 1995), Guadeloupe (Monniot 1983), Senegal (Monniot & Monniot 1994), Panama (Rocha et al. 2005), Venezuela (Rocha et al. 2010) and Brazil (Rocha 1995; Rocha & Monniot 1995, Kremer et al. 2010).

***Didemnum psammatodes* (Sluiter, 1895)**

(Plate I D)

Didemnum psammatode (Sluiter, 1895): Kott, 2001: 229.

To more synonymy see: Kott, 2001: 229 and Cascon & Lotufo 2005: 225.

Examined material: One colony, Crawl Key, Bocas del Toro, 9º15'16" N, 82º08'10" W, coll. Rosana Moreira da Rocha, 22 June 2011.

Description

Encrusting colony is 2.5 cm long and about 2 mm thick. Colony is light brown due the presence of fecal pellets in the tunic. Spicules are less abundant on the surface of the colony and are located around the oral siphons. Spicules are small and rounded, 5 to 30 µm in diameter, with many conical rays.

Zooids are 1.2 mm long, abdomen is larger than the thorax. Oral siphon is tubular and has six small triangular lobes. Atrial aperture is wide exposing all the rows of pharyngeal stigmata. Muscular process projects from the esophageal-rectal peduncle and it is shorter than the abdomen. No lateral organs were observed. There are 4 to 5 stigmata per row are on each side of the pharynx.

The esophagus is short; the stomach is rounded; the intestinal loop (with 2 constrictions) forms a secondary loop, covering part of the stomach. There are two small stolonic vessels. Gonads are located in the intestinal loop. The testis is single, spherical and surrounded by 8 coils of the sperm duct. The ovarium is above the testis and has 2 oocytes. No budding was observed.

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 four pairs of ectodermal ampullae. The sensory vesicle (including ocellus and otolith) is in the mid-dorsal region of the larval trunk.

Remarks

This species is easy to identify by the abundance of fecal pellets in the colony. The specimen described above is compatible with the descriptions of other parts of the world.

Distribution

The species has a pantropical distribution. For detail see Kott, 2001: 229.

***Didemnum vanderhorsti* Van Name, 1924**

Examined material: One colony, Hospital Point, Bocas del Toro, 9º20'04" N, 82º13'10"W, coll. Rosana Moreira da Rocha, 20 August 2006.

Description

Encrusting colony is 4 cm long and about 2 mm thick. Colony is light brown with some white regions due to the spicules. Spicules are scarce and are concentrated around the zooids. Spicules are small and rounded, 15 to 25 µm in diameter, with 9 short rays in optical optical transverse section.

Zooids are 1.0 mm long, thorax and abdomen are equal in size. Oral siphon has six small rounded lobes. Atrial aperture is wide exposing all the rows of pharyngeal stigmata. Atrial languet is absent. Muscular process projects from the esophageal-rectal peduncle and is shorter than the abdomen. Elongated lateral organs are at the level of the fourth row of pharyngeal stigmata. There are 8 to 10 stigmata per row are on each side of the pharynx.

The esophagus is long; the stomach is rounded, the duodenum is shorter than the stomach; the intestinal loop (with 1 constriction) forms a secondary loop, covering part of the stomach. Gonads are located in the intestinal loop. The testis is single, spherical and surrounded by 9 coils of the sperm duct. The ovarium is above the testis and has 3 oocytes. No budding was observed.

Larval trunk is oval, about 0.5 mm long, around which the tail winds half way. Larvae have three linearly arranged adhesive papillae, plus four pairs of ectodermal ampullae. The sensory vesicle (including ocellus and otolith) is in the mid-dorsal region of the larval trunk.

Remarks

Didemnum calliginosum has many resemblances with *Didemnum vanderhorsti*, but it differs in the smaller number of sperm duct coils and in the presence of a gland on the intestinal wall (Monniot, 1983).

Distribution

United States (Van Name 1924), Curaçao (Van Name 1924; Goodbody 1984) and Brazil (Cascon & Lotufo 2005; Millar 1958; Rocha & Monniot 1995; Rodrigues et al. 1998).

***Didemnum* sp. nov.**

(Figure 10, Plate XI)

Examined material: *Holotype*: One colony, Solarte, Bocas del Toro, 9°17'30" N, 82°10'20" W, coll. Rosana Moreira da Rocha, 04 August 2003. *Paratypes:* Five colonies, Solarte, Bocas del Toro, 9°17'30" N, 82°10'20" W, coll. Rosana Moreira da Rocha, 04 August 2003.

Description

Encrusting colonies are 3.0 to 7.0 cm long and about 0.7 mm thick. Living colonies are orange. In formalin colonies are white. Spicules are abundant on surface, and completely cover the wide cloacal canals. Spicules are stellate, 20 to 40 µm in diameter with 4 to 8 conical rays in optical transverse section, some rays with more pointed and others with rounded tips.

Zooids are 1 mm long, thorax is larger than the abdomen. Oral siphon has six large triangular lobes. Atrial aperture is wide exposing all the rows of pharyngeal stigmata. Muscular process projects from the esophageal-rectal peduncle and it is shorter than the abdomen. Elongated or circular lateral organs are small and at the level of the fourth row of pharyngeal stigmata. In the pharynx, the first and the second rows contain 6 to 5 stigmata, the third row contains 5 to 4 and the fourth contains 4.

The abdomen is elongated. The esophagus is longer than the stomach; the intestinal loop (with 2 constrictions) forms a secondary loop, covering the stomach. There are 3 small stolonic vessels. Gonads are located beneath the intestinal loop. The testis is single, spherical and surrounded by 8 to 9 coils of the sperm duct, that are thin and close

together. The ovarium is anterior to the testis and has 1 oocyte. Budding was observed in many zooids.

Larval trunk is oval, about 0.4 mm long, around which the tail winds 3/4 of the way. Larvae have three linearly arranged adhesive papillae with long stalks, plus four pairs of ectodermal ampullae which are long and straight. The sensory vesicle (including ocellus and otolith) is in the mid-dorsal region of the larval trunk.

Remarks

Many *Didemnum* species have orange colonies, but all descriptions available are different from *Didemnum* sp. nov. *Didemnum ahu* forms small colonies and the zooids have 10 coils of sperm duct (Monniot & Monniot 1987). *Didemnum inauratum* Monniot, 1983 also forms small colonies, has smaller spicules and smaller zooids (Monniot 1983). *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 *Didemnum* sp. nov. (Lafargue 1975; Rocha & Monniot, 1995). *Didemnum granulatum* differs by the presence of papillae filled with spicules on the tunic surface, by the smaller spicules and the number of coils of the sperm duct (Tokioka 1954). *Didemnum pseudofulgens* Médioni, 1970 has larvae with 8 – 10 pairs of ampullae (Médioni 1970). *Didemnum rodriguesi* Rocha & Monniot 1993 has small colonies with a reticulate pattern on the surface of the tunic, zooids with greater number of stigmata on pharynx and larvae with 8 pairs of ampullae and *Didemnum madeleinae* Monniot & Monniot, 2001 have longer larvae with 12 or more pairs of ampullae (Monniot & Monniot 2001).

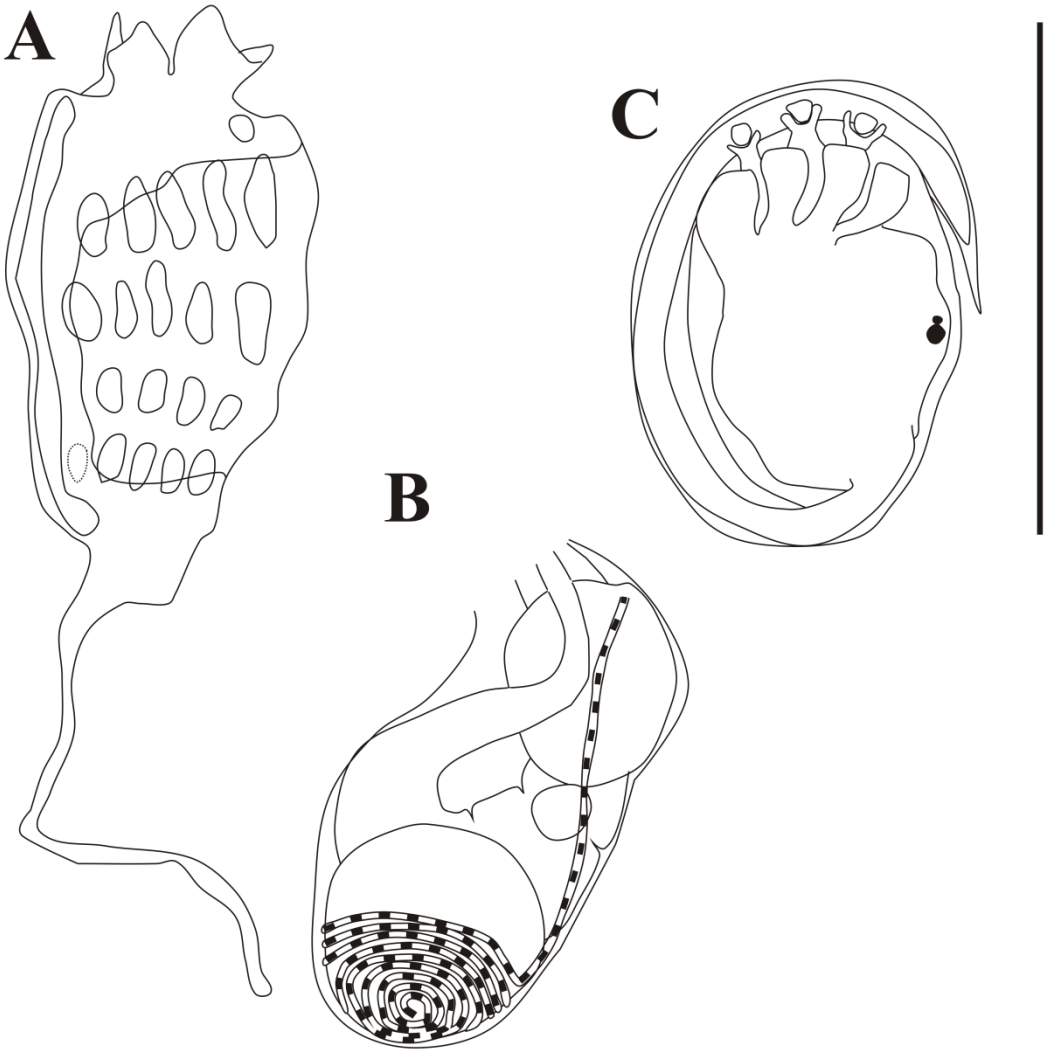

Figure 10. *Didemnum* sp. nov. A, thorax; B, abdomen; C, larva. Scale bar, 0.5 mm.

Genus *Trididemnum* Della Valle, 1881

Five species are described here followed by an identification key for Bocas del Toro species (Table V).

Table V. Tabular key for the identification of specimens in the genus *Trididemnum* in Bocas del Toro. Characters based on the present study and/or literature.

1	2	3	4	5	6	7	8	9	10	11	12	Species
W	0	S	50	7	S	5-7	S	7	0.5	4	1	*T.* cf. *orbiculatum*
W, B	0	S, B	80	9-13	A, S	12	M	10-12	?	?	?	*T. savignii*
Y	0	S	50	7-9	E	8-10	S	8	0.5	4	1	*T.* cf. *thetidis*
T	0	L	100	11	0	15-16	S	8-10	1.2	8	3	*Trididemnum* sp. nov. 1
Br-O	P	L	70	9-many	0	5-7	S	5	1.3	12-14	1	*T. palmae*
D-Br	P	S	60	8	0	?	L	8-9	0.8	8	1	*T.* sp. nov. 2

1. Living colony color: B—black, Br-O—brown-orange, D-Br—dark-brown, T—transparent, W—white, Y—yellowish.
2. Symbiotic algae: 0—absent, P—present.
3. Spicule density: S—abundant only on the surface, B— abundant only in the base, L—low abundance.
4. Maximum spicule size (μm).
5. Number of spicule rays in optical transverse section.
6. Thorax pigmentation: 0—absent, A— located on anterior region of the thorax, E—located on anterior end of endostyle, S— located on around the oral siphon.
7. Number of pharyngeal stigmata in half rows.
8. Length of the muscular process: L—longer than the abdomen, M—as long as the abdomen, S—shorter than the abdomen.
9. Number of coils of the sperm duct.
10. Maximum larval trunk size (μm).
11. Number of pairs of ectodermal ampullae in larvae.
12. Number of larvae embryos.

***Tridemnum* cf. *orbiculatum* (Van Name, 1902)**

(Plate I E)

Didemnum orbiculatum Van Name 1902: 361 Pl. 51 figs 32, 38, Pl. 61 figs. 127a, 128.

Examined material: Eight colonies, Cristobal, Bocas del Toro, 9°17'34" N, 82°15'24" W, coll. Rosana Moreira da Rocha, 13 August 2003.

Description

Colonies are very thin (about 1 mm) and are 3 to 8 cm in length. Its color is white (spicules) with brown (zooids). The spicules are very abundant at the surface layer of the colony. Spicules are stellate, 7 to 50 μm in diameter with 7 conical rays in optical transverse section.

The zooids are about 1 mm long. Oral siphons have six rounded lobes and are brown on the edges. The atrial aperture is tubular, located in the midline of the thorax, but oriented posteriorly. The muscular process projects from the base of the thorax and is shorter than the esophagus. Thoracic lateral organs are circular, at the level of the fourth row of stigmata. In the pharynx, the first rows contains 7 stigmata, the second contains 6, while the third 5.

The abdomen is the same size of thorax and has 1 or 2 stolonic vessels. The stomach is rounded; the intestinal loop forms a deep secondary loop, covering the stomach, and has 2 constrictions. The gonads are within the intestinal loop, one beside the other. The testis has a unique and spherical follicle surrounded by 7 turns of the sperm duct. The ovarium has two oocytes. No budding in the esophageal region was observed.

The larvae are more elongate than wide, with 0.5 mm trunk length, around which the tail winds half of the way. The larvae have 3 adhesive papillae with long stalks and 4 pairs of ectodermal ampullae. The sensory vesicle (with otolith and ocellus) is in the posterior region of the larvae trunk.

Remarks

There is great debate regarding the morphological characteristics that distinguish *Tridemnum orbiculatum* and *T. thetidis* (Van Name, 1945). According to the original description of *T. thetidis*, the species has yellowish colonies with lower density of spicules and smaller zooids (Van Name 1945). Many of the problems in identifying these species are due to the lack of details of gonads and larvae in the original descriptions. Monniot (1983) proposed synonymy between these species due to lack of morphological characters that differentiate. However, Dias et al. (2009) conducted an analysis comparing the allozyme alleles from colonies collected at different locations of the state of São Paulo - Brazil. They concluded that the colonies were of two different species (white morphotypes as *T. orbiculatum* and green and yellow morphotypes as *T. thetidis*). However, the descriptions of Monniot (1983) and Dias et al. (2009) do not agree with the number of turns of spermiduct of *T. thetidis*. The material described above was named as *T. orbiculatum* due to the three-dimensional growth pattern of colonies and greater abundance of spicules.

Distribution

Bermuda (Van Name 1921), Belize (Goodbody 2000), Brazil (Rodrigues et al. 1998; Rocha 2002), Guadeloupe (Monniot 1983) and Panama (Collin et al. 2005).

***Tridemnum palmae* Monniot, 1984**

(Figure 11, Plate XII)

Examined Material: Four colonies, Boia 14, Bocas del Toro, 9°25'05" N, 82°20'32" W, coll. Rosana Moreira da Rocha, 19 June 2011.

Description

The encrusting colonies are between 3 and 7 cm long and 2 mm thick. Colony surface are brown-orange, unevenly due to the presence of symbiotic algae, and cloacal cavities have a white border. Spicules are scarce and more common on the region of oral siphons. When preserved in formaldehyde, the colonies become greenish-brown coloration. Spicules are in three different shapes. Some are stellate not exceeding 70 µm in diameter, with 10 or 11 shorter and conical rays in optical transverse section. Others are spherical, 20 to 40 µm, with many truncated short rays. The third type has mixed characteristics between the others, with 9 conical rays and small truncated rays, not exceeding 60 µm. The most superficial layer of the colony has no spicules. In the middle and lower layers of the colonies, the spicules are abundant.

The zooids are approximately 0.9 mm long. Abdomen and thorax are the same size. Oral siphon has 6 small triangular lobes. Thoracic organs have circular shape, at the level of the second row of stigmata. In the pharynx, the first and third rows have 5 to 7 stigmata on each side, while the second row has 6 to 7.

Muscular process protrudes from the esophagus and is smaller than the abdomen. In some zooids, the muscular process was not observed. Stomach is located in the anterior portion of the abdomen and has smooth wall and cordiforme shape. The duodenum is shorter than the stomach. The intestinal loop has no second loop and no

constrictions. The intestine is thinner in the posterior portion. The testis is single, spherical and surrounded by 5 turns of sperm duct. Ovary has an oocyte of around 0.15 mm. Budding in the esophageal region was observed in some zooids.

The larvae are incubated in the tunic, in regions with a higher concentration of symbiotic algae. They are generally ovoid, trunk is approximately 1.3 mm in length, around which the tail winds a half of the way. The larvae have 3 fingerlike adhesive papillae distant among themselves with short and thin stalks, 12 to 14 pairs of short ampullae. It has a lot of symbiotic algae divided into two portions, one on each side of the trunk. Algae, after fixation, preventing visualization of the pharynx and the sensory vesicle.

Remarks

In the original description of *Tridemnum palmae*, color is described as white to greenish-brown (Monniot 1984). Since symbiotic algae are responsible for the color of these colonies, it is possible to change the color, due to the change in the relative amount of algae and spicules. Although *T. palmae* described by Monniot (1984) does not have muscular process, the characteristics of larvae and spicules resemble considerably with the material described above.

Tridemnum palmae has similarities in size and color of the colonies with *Tridemnum solidum* (Van Name 1902), but they differ significantly in the number of turns of spermiduct, the number of stigmata in the pharynx and the size of the larvae.

Distribution

Guadeloupe (Monniot 1984).

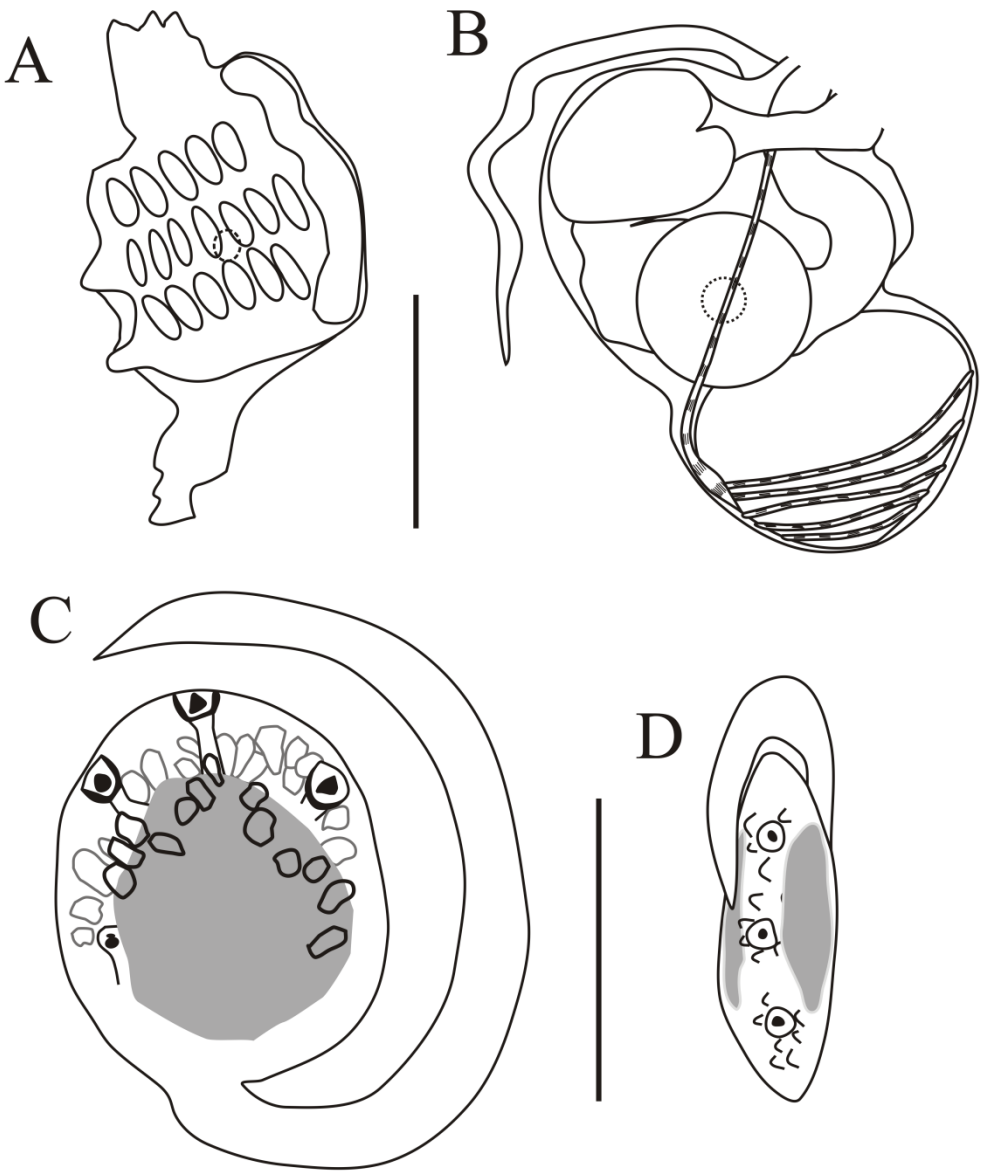

Figure 11. *Tridemnum palmae*. A, thorax; B, abdomen; C, larvae (algae in gray); D, anterior view of larvae, with two regions with algae (gray). Scale bars, A and B, 0.2 mm; C and D, 1 mm.

***Tridemnum* cf. *thetidis* (Van Name, 1945)**

(Figure 12, Prancha XIII)

Examined Material: One colony, Cristobal, Bocas del Toro, 9°17'34" N, 82°15'24" W, coll. Rosana Moreira da Rocha, 05 June 2009.

Description

The encrusting colony is approximately 9 cm long and 3 mm thick. Colony surface is yellowish unevenly. The spicules are more abundant on the surface of the colony being in lower density in the middle and basal layers. On the surface they are distributed according to the original description of the species: where is each zooid, there is a circular region without spicules but the central area has spicules, where is the atrial aperture. When preserved in formalin, the colony is white. The spicules are in three different shapes: some are stellate, not exceeding 50 µm in diameter, with 7 to 8 short and conical rays in optical transverse section. Others are also stellate, 35 to 40 µm, with many short and conical rays. The third type has both conical rays (9 in cross section) and truncated rays (many), not exceeding the 60 to 70 µm.

Zooids are approximately 1.2 mm long. The abdomen is larger than the thorax. The anterior end of the endostyle is pigmented, characteristic of the species. The oral siphon has 6 small and rounded lobes (triangular in some zooids). The atrial siphon is short, located at the second row of stigmata and its opening is narrow. The thoracic organs are circular and are positioned at the level of the third row of stigmata. In the pharynx, the first row has 10 stigmata on each side, while the second row has 8 and third has 9 (based on one zooid).

Muscular process protrudes of the esophageal-rectal peduncle and is smaller than the esophagus. Stomach has the usual size and location and the duodenum is shorter than the stomach, the intestinal loop has two constrictions and the second loop covers the stomach. The gonads are located in the intestinal loop, one beside the other. The testis is single, spherical and surrounded by 8 coils of sperm duct, which are thin and very close. The ovary has a small oocyte. Budding in the esophageal region was observed in some zooids.

Larvae are oval, trunk is approximately 0.5 mm in length, around which the tail winds full of the way. They have 3 adhesive papillae close together, with short, thin stalks, and 4 pairs of short and rounded ampullae. The sensory vesicle (with otolith and ocellus) is located in the mid-dorsal region of the larval trunk.

Remarks

Although there are no significant morphological differences between *Tridemnum orbiculatum* and *T. thetidis* (Van Name 1945), molecular analyzes showed differences between morphotypes white and yellow/green of these species. The material described above was named as *T. thetidis* due to the thinner colonies, lower abundance of spicules and yellowish color.

Distribution

Florida (Van Name 1945) and Brazil (Dias et. al 2009).

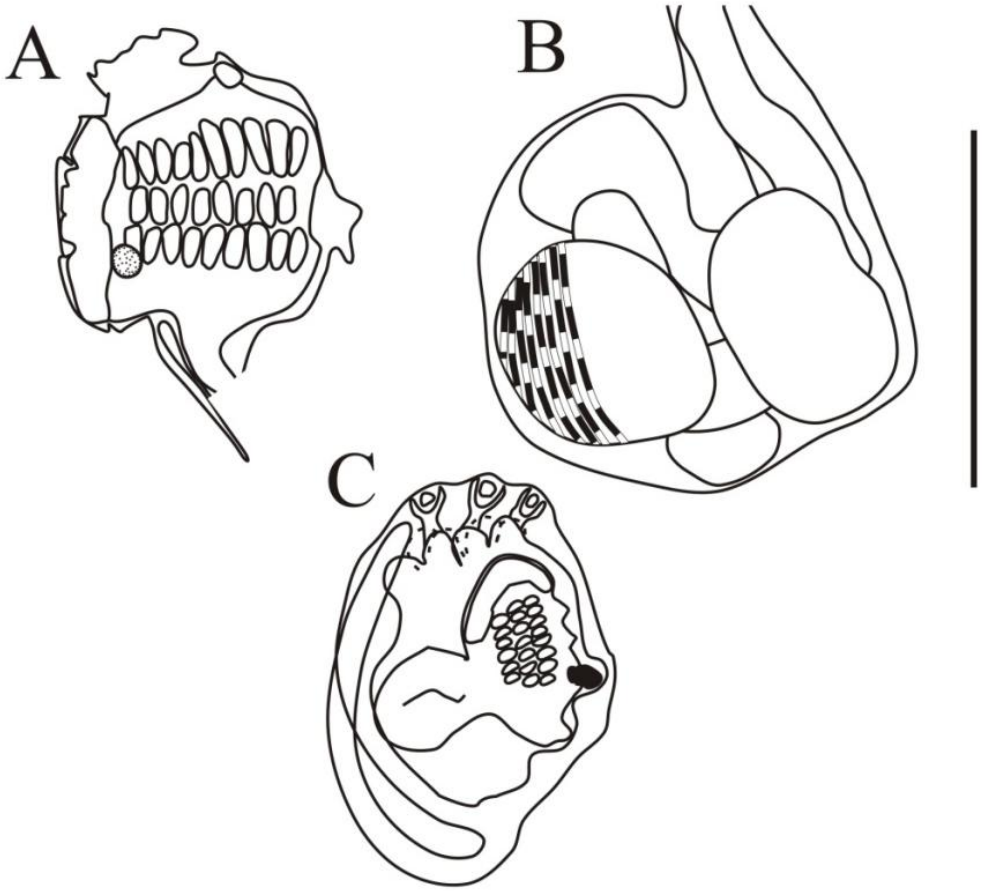

Figure 12. *Tridemnum* cf. *thetidis*. A, thorax; B, abdomen; C, larvae. Scale bar: 0.5 mm.

***Tridemnum* sp. nov. 1**

(Figure 13, Plate XIV)

Examined Material: *Holotype:* One colony, Hospital Point, Bocas del Toro, 9º20'04''N, 82º13'10''W, coll. Rosana Moreira da Rocha, 20 June 2006.

Description

The encrusting colony is approximately 8 cm long and 2 mm thick. When preserved in formalin, the colony is transparent with white spots where the spicules are. The spicules are located around the systems, with lower density in the first layer of the colony. The spicules are rounded, 45 to 100 µm in diameter, with 11 short and conical rays in optical transverse section.

Zooids are approximately 1 mm long. The abdomen and thorax have the same size. Oral and the atrial siphon are tubular, and the oral has 6 small rounded lobes. Thoracic lateral organs are small, have a circular shape, at the level of the third row of stigmata. The muscular process is shorter than the abdomen and protruding from the base of the endostyle. In pharynx the rows have 15 to 16 stigmata on each side.

The esophageal-rectal peduncle is short and curved to the ventral region; the stomach is round and yellowish even after fixation. Duodenum is shorter than the stomach. Intestinal loop has two constrictions and second loop that partially covers the stomach. The intestine is wider in the posterior portion. The testis is single, spherical and surrounded by 8 to 10 turns of sperm duct.

Larvae are oval, trunk is approximately 1.2 mm in length, around which the tail winds full of the way. The larvae have 3 adhesive papillae with long stalks and 8 pairs of long and thin ampullae with enlarged ends. They are gemmiparous and have 3

embryos in the final stages of development. The embryos have 9 stigmata in the first row, 7 stigmata in the second and 8 in the third row.

Remarks

Gemmiparous larvae are rare in *Tridemnum* species. The only species described with gemmiparous larvae is *Tridemnum pseudodiplosoma* (Kott, 1962), but it has four embryos and a larger number of ampullae (Kott 1962).

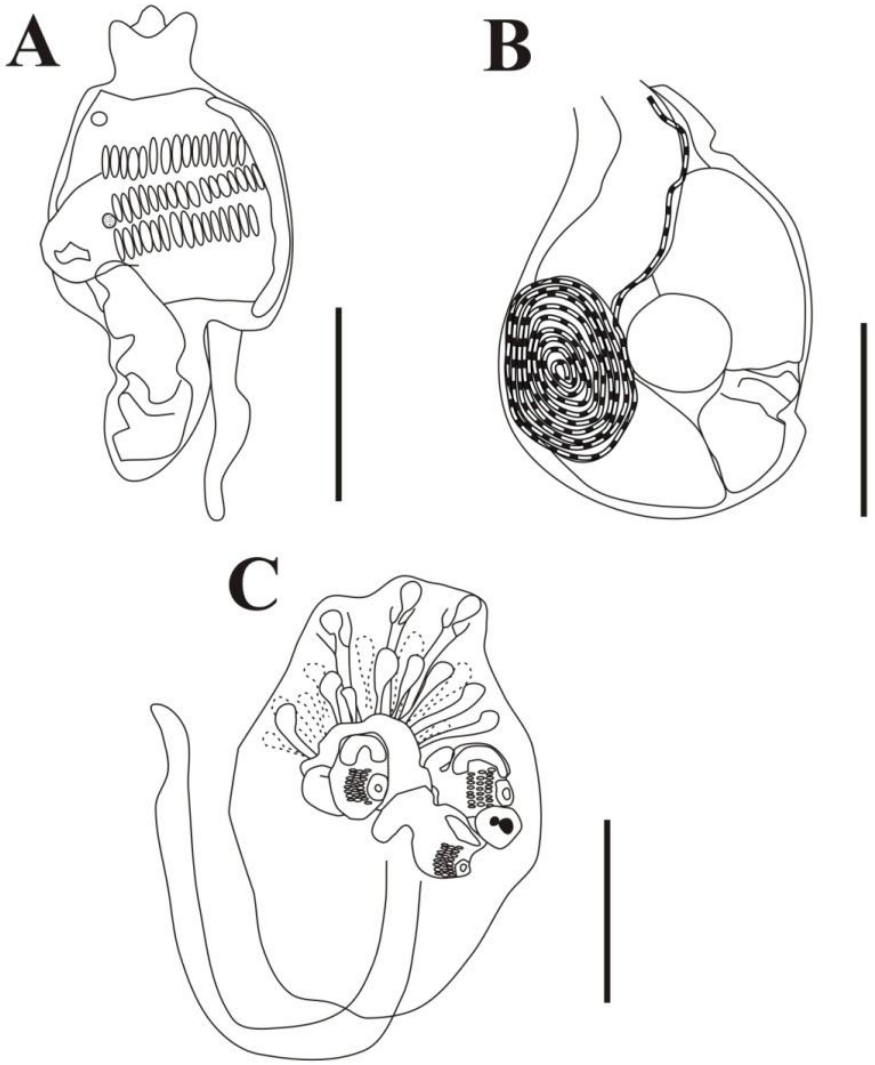

Figure 13. *Tridemnum* sp. nov. 1. A, thorax; B, abdomen; C, larvae. Scale bars, A, 0,2 mm; B, 0.2 mm; C, 0.5 mm.

***Tridemnum* sp. nov. 2**

(Figure 14, Plate XV)

Examined Material: *Holotype:* One colony, Bocas del Drago, Bocas del Toro, 9°25'00" N, 82°19'46" W, coll. Rosana Moreira da Rocha, 03 May 2009. *Paratypes:* Five colonies, Bocas del Drago, Bocas del Toro, 9°25'00" N, 82°19'46" W, coll. Rosana Moreira da Rocha, 03 May 2009.

Description

The encrusting colonies are approximately 3 to 7 cm long and 3 mm thick. Colony surfaces are dark brown with white spots where the spicules are more abundant (the regions between cloacas and border of colony). When preserved, colonies become greenish. Dark pigment was found in the colonies. The most superficial layer of the colony has no spicules, but in the middle and lower layers the spicules are abundant. The spicules measure 40 to 60 µm in diameter and have 8 short and conical rays in optical transverse section.

Zooids are about 1 mm long. The abdomen is larger than the thorax. The oral siphon has six long triangular lobes; the atrial siphon is large and cup shape with wide opening. Thoracic organs have circular shape, are very prominent and positioned at the first row of stigmata. It was not possible to count the number of stigmata in each row because the zooids were very contracted.

Muscular process protrudes from the esophageal-rectal peduncle and is longer than the abdomen. Intestinal loop has two constrictions and a second loop that do not cover the stomach. Gonads are located in the intestinal loop; the ovary is closest to the stomach. The testis is single, large and spherical, surrounded by 8 or 9 turns of sperm

duct. Ovary has a small oocyte. Budding in the esophageal region was observed in some zooids.

Larvae are oval, trunk is approximately 0.8 mm in length, around which the tail winds a half of the way. Larvae have 3 adhesive papillae and close to each other with long stalks and 8 pairs of short, finger-like ampullae. Many symbiotic algae are covering the entire trunk of the larva, except the region of the adhesive papillae and sensory vesicle. The algae, greenish after fixation, prevent visualization of the pharynx.

Remarks

Other brown species of *Tridemnum* with symbiotic algae that occur in the Atlantic differ from that described above. *Tridemnum maragogi* has 6 to 7 turns of spermiduct (Rocha 2002), while *T. cyanophorum* Larfargue & Duclaux, 1979 differs by the number of ampullae in the larvae (12 to 15 pairs), and the external appearance of colonies (Larfargue & Duclaux 1979). *Tridemnum solidum* has 10 turns of spermiduct and is also distinguished by the external appearance of the colony. *Tridemnum savignii* has smaller spicules (11 µm) and more turns of spermiduct (10-12) (Van Name 1945).

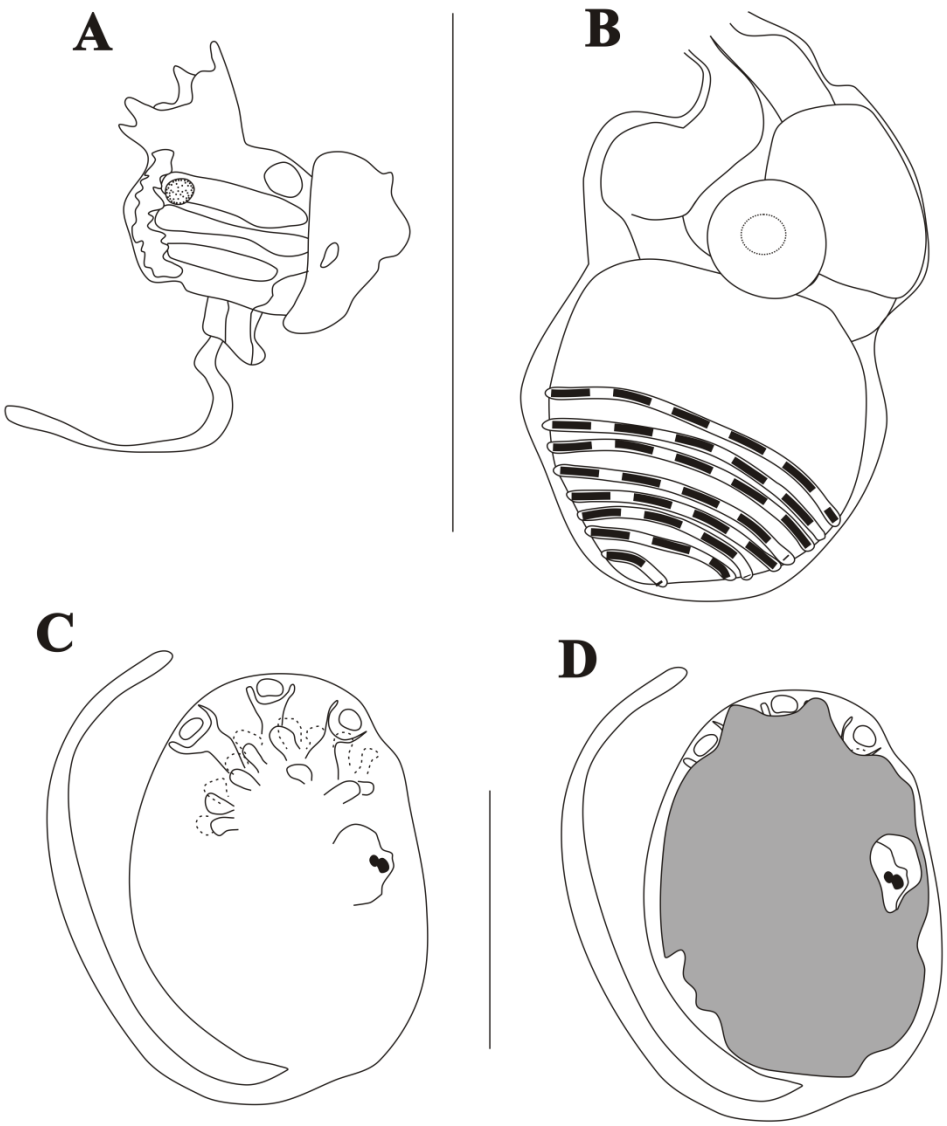

Figure 14. *Tridemnum* sp. nov. 2. A, thorax; B, abdomen; C, larvae without symbiotic algae; D, larvae with symbiotic algae. Scale bars, A and B, 0.5 mm; C and D, 0.5 mm.

Final Remarks

Altogether 19 species were identified Didemnidae to Bocas del Toro, and 14 of them had not been previously reported for the region. Adding these new records, the number of species of Didemnidae for the region amount to 24. The increase of 56% of the known didemnids in Panama is an important indicator that this family has been poorly studied in the region and deserves further attention, especially due to the large number of new species - which represent approximately 30% of all species.

The species were usually found in environments with coral reefs (eight sites), followed by artificial substrate habitats and mangroves (two sites each). Seven species were found only in coral reef habitats (*Lissoclinum verrilli*, *Lissoclinum* sp. nov. 1, *Lissoclinum* sp. nov. 2, *Didemnum vanderhorsti*, *Tridemnum palmae*, *Tridemnum* sp. nov. 1 and *Tridemnum* sp. nov. 2), three species were found only in mangroves (*Didemnum* sp. nov., *Tridemnum orbiculatum* and *T. thetidis*) and two species were found only in artificial substrate locations (*Diplosoma* sp. nov. 1 and *Diplosoma* sp. nov. 2). *Diplosoma listerianum*, *Didemnum calliginosum*, *D. cineraceum* and *D. perlucidum* were found in coral reefs and artificial substrate locations. *Lissoclinum abdominale*, *Didemnum granulatum* and *D. psammatodes* were less restricted, being found in the three different habitats sampled.

It is expected that many other species of didemnids are identified in the region, since this work did not include all the samples of *Didemnum* collected between 2003 and 2014. The samples belonging to the other three genera were all analyzed, but not all of them could be identified. Many colonies were not in reproductive period at the time of the sampling and therefore did not have important structures for the identification, such as developed larvae and gonads. Therefore, it is necessary to do more collections at

different times of the year, increasing the chance of sampling during the reproductive period of the different species. Thus, it is expected that the number of didemnids in the region will grow further, increasing the ecological and taxonomic importance of the Atlantic coast of Panama different habitats.

Many species have important similarities in their morphological characteristics and should be studied with care for their identification. The new species in this work could be confused with widely known species if not observed in detail. *Lissoclinum verrilli* and the two new species of the genus are distinguished only by the size and aspect of the spicules. The general characteristics of colonies and zooids are very similar. *Diplosoma* sp. nov. 2 could be mistakenly identified as *Diplosoma glandulosum* if the location and extent of the gland surrounding the intestine and testis had been disregarded. *Didemnum calliginosum* is similar to *D. cineraceum* in characteristics of colony, spicules and zooids, but they differ from each other in the morphology of the larvae. The only difference between *Didemnum calliginosum* and *Didemnum vanderhorsti* is the presence of a gland on the intestinal wall. *Tridemnum orbiculatum* and *T. thetidis* are only differentiated by the characteristics of colonies (color and growth pattern) and differences in size of zooids and larvae. The different clades of *Diplosoma listerianum* – probably different species (Pérez-Portela et al. 2013) are also morphologically cryptic and current identifications could change when differences are established.

Table VI lists all the species of Didemnidae registered for the Caribbean Sea, Florida and Bermuda (these two regions have been included because they are adjacent to the Caribbean and have very similar Ascidiacea fauna). Some localities already sampled in the region were excluded from the table because they do not contain didemnids. This can be attributed to the fact that many colonies are difficult to

recognize - either because they resemble sponges or because they attach to sheltered cryptic substrates - or because of the difficulty in identifying specimens of this family, which are not reported although presented in the collections.

Among the most common species (*Didemnum conchyliatum*, *D. psammatodes*, *Tridemnum orbiculatum*, *T. savignii*, *Lissoclinum fragile* and *Diplosoma listerianum*), only *L. fragile* was not previously registered in Bocas del Toro. There are records of this species on the Pacific coast of Panama, but not for the Atlantic coast. This is an interesting result, given that the species has a wide distribution in the Caribbean Sea and it has been considered introduced in other regions (Abdul Jaffar Ali et al. 2009; Carman et al. 2011; Rico et al. 2012). It is necessary to review the identification of this species both within and outside the Caribbean Sea, because its external appearance is very similar to other species of *Lissoclinum* not included in this paper.

Bocas del Toro has a high diversity of didemnids with only one species less than Guadeloupe (25 species recorded). This comparison reaffirms the importance of considering the Atlantic coast of Panama as an important region for monitoring and conservation of species, not only for Didemnidae, since all the Ascidiacea class and other groups of encrusting marine invertebrates are very diverse in the region.

Table VI. Didemnidae species in the Caribbean, Bermuda and Florida, based on Rocha et al. (2005); Rocha et al. (2010); and Cole (2012).

	Total	Aruba	Bermuda	Belize	Bocas del Toro	Bonaire	Cuba	Curaçao	Florida	Grenada	Guadeloupe	Guianas	Jamaica	Margarita	Puerto Rico	St. Lucia	St. Vincent	Tobago
Didemnum calliginosum Monniot, 1984	2				X						X							
Didemnum cineraceum (Sluiter, 1898)	6			X	X						X		X	X				X
Didemnum conchyliatum (Sluiter, 1898)	9			X	X			X		X	X		X	X		X		X
Didemnum duplicatum Monniot, 1983	6			X						X	X		X	X				X
Didemnum granulatum Tokioka, 1954	1				X													
Didemnum halimedae Monniot, 1983	2										X		X					
Didemnum inauratum Monniot, 1983	2			X							X							
Didemnum ligulum Monniot, 1983	2				X						X							
Didemnum perlucidum Monniot, 1983	3			X	X						X			X				
Didemnum psammathodes (Sluiter, 1895)	8	X		X	X					X	X		X			X	X	
Didemnum speciosum (Herdman, 1886)	1				X													
Didemnum vanderhorsti Van Name, 1924	4				X			X						X				X
Didemnum sp. nov.	1				X													
Polysyncraton amethysteum (Van Name, 1902)	3		X	X					X									
Polysyncraton louminae Monniot, 1984	1										X							
Trididemnum cyanophorum Lafargue & Duclaux, 1979	2			X							X							
Trididemnum hians Monniot, 1983	4		X	X							X		X					
Trididemnum maragogi Rocha, 2002	1				X													
Trididemnum orbiculatum (Van Name, 1902)	7		X	X	X			X			X			X				X
Trididemnum palmae (Monniot, 1984)	2				X						X							
Trididemnum savignii Herdman, 1886	7		X		X		X		X		X		X		X			
Trididemnum solidum (Van Name, 1902)	6		X	X			X	X			X				X			
Trididemnum sp. nov. 1	1				X													
Trididemnum sp. nov. 2	1				X													
Lissoclinum abdominale Monniot, 1983	3			X	X						X							
Lissoclinum fragile (Van Name, 1902)	8		X	X				X			X		X	X			X	X
Lissoclinum perforatum (Giard, 1872)	1										X							
Lissoclinum verrilli (Van Name, 1902)	5		X	X	X				X		X							
Lissoclinum sp. nov. 1	1				X													
Lissoclinum sp. nov. 2	1				X													
Diplosoma glandulosum Monniot, 1983	2			X							X							
Diplosoma listerianum (Milne-Edwards, 1841)	12	X	X	X	X	X		X	X		X	X	X	X				X
Diplosoma simile (Sluiter, 1909)	1				X													
Diplosoma tritestis Monniot, 1984	1										X							
Diplosoma virens (Hartmeyer, 1909)	1			X														
Diplosoma sp. nov. 1	1				X													
Diplosoma sp. nov. 2	1				X													
Leptoclinides latus Monniot, 1983	1										X							
Leptoclinides torosus Monniot, 1983	1										X							
Total		2	9	17	24	1	2	7	5	3	25	1	10	8	3	2	2	7

Plates

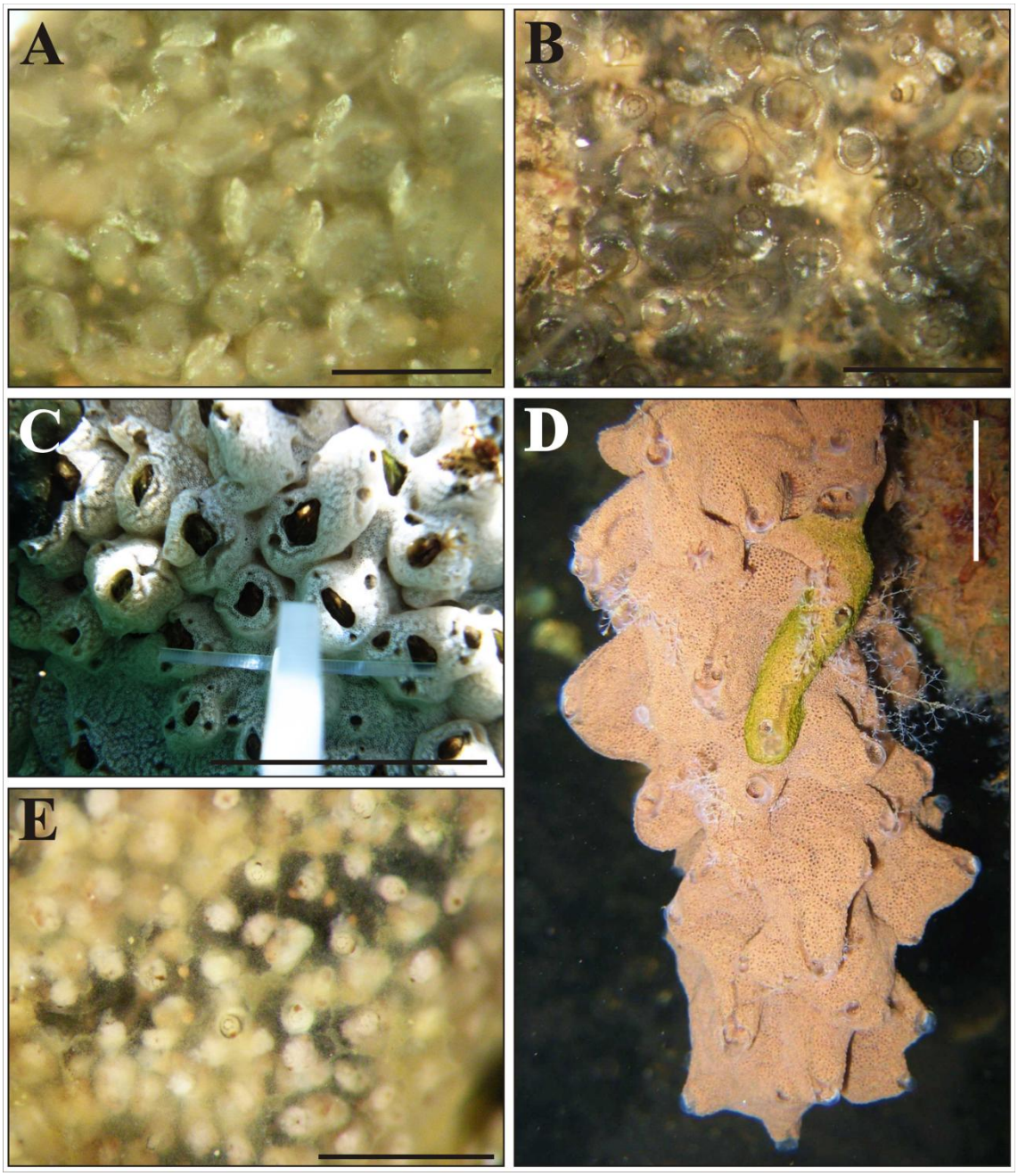

Plate I. A and B, *Diplosoma listerianum* (colonies detail); C, *Didemnum perlucidum*; D, *Didemnum psammatodes*; E, *Tridemnum* cf. *orbiculatum* (colony detail). Scale bars: A and B, 2 mm; C, 5 cm; D, 4 cm; E, 3 mm.

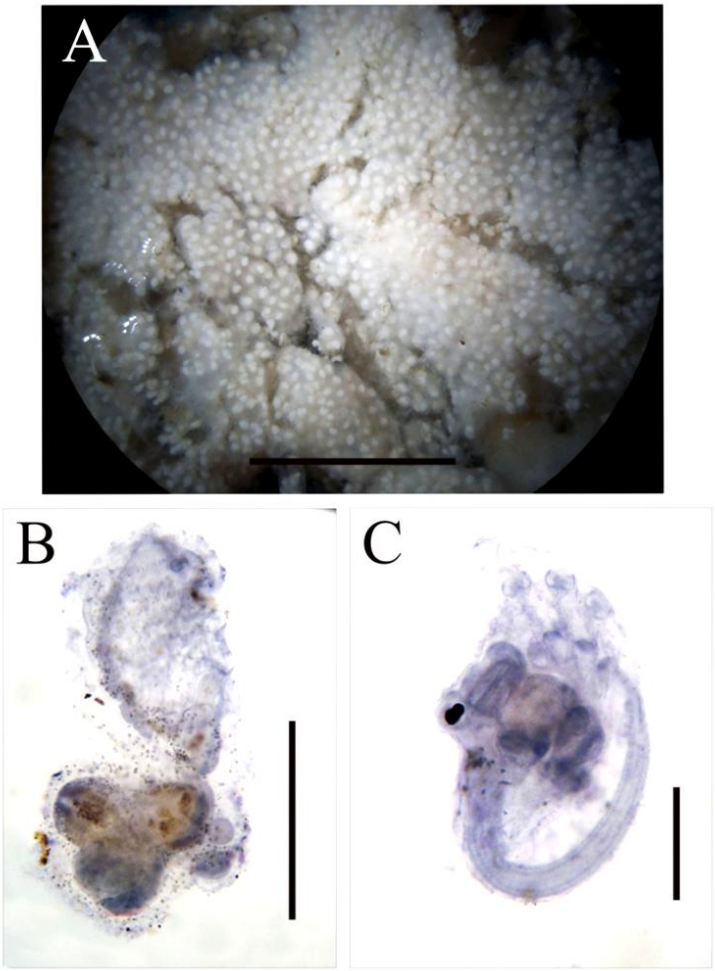

Plate II. *Diplosoma* sp. nov. 1. A, preserved colony; B, zooid; C, larva. Scale bars: A, 10 mm; B, 0.5 mm; C, 0.2 mm.

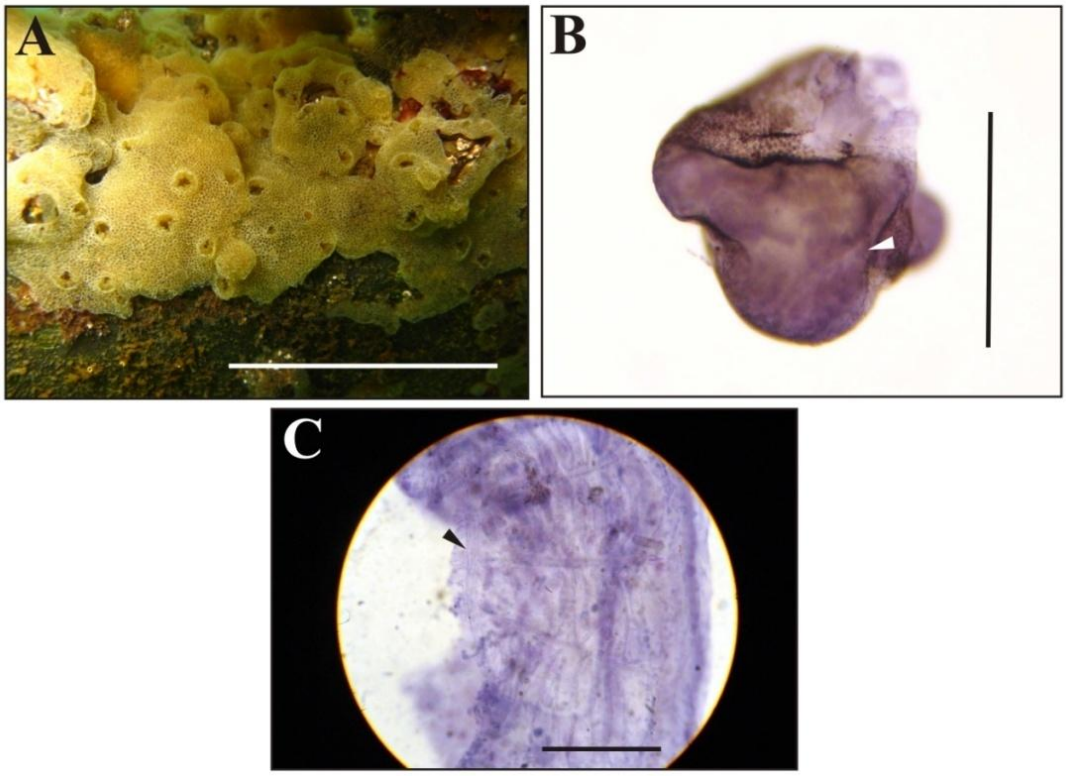

Plate III. *Diplosoma* sp. nov. 2. A, living colony; B, abdomen with a thin layer of epithelium that binds covers all structures (white arrow head); C, thorax with muscular fibers (black arrow head). Scale bars: A, 5 cm; B, 0.5 mm; C, 0.3 mm.

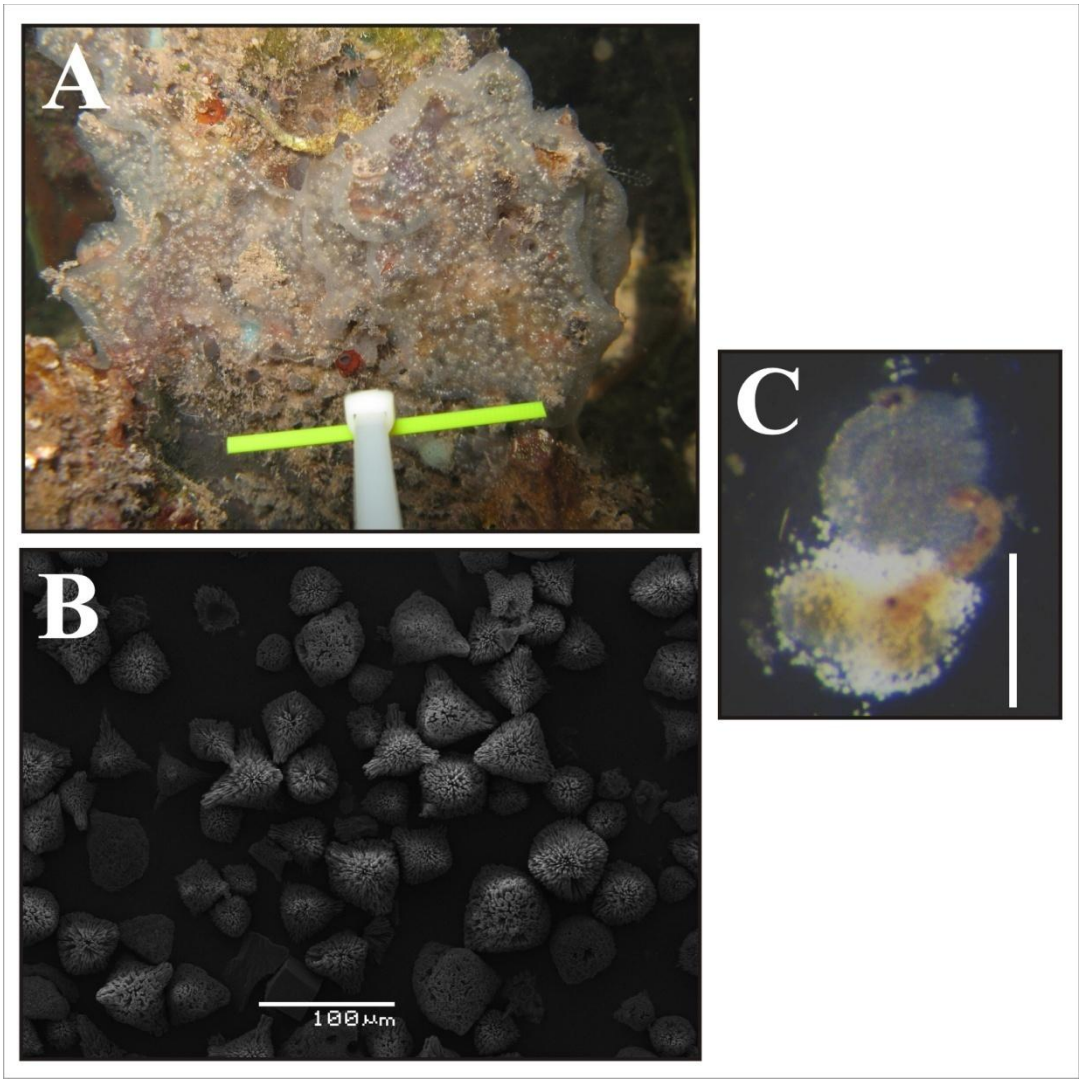

Plate IV. *Lissoclinum abdominale*. A, living colony; B, spicules; C, zooid. Scale bar, C, 0.5 mm.

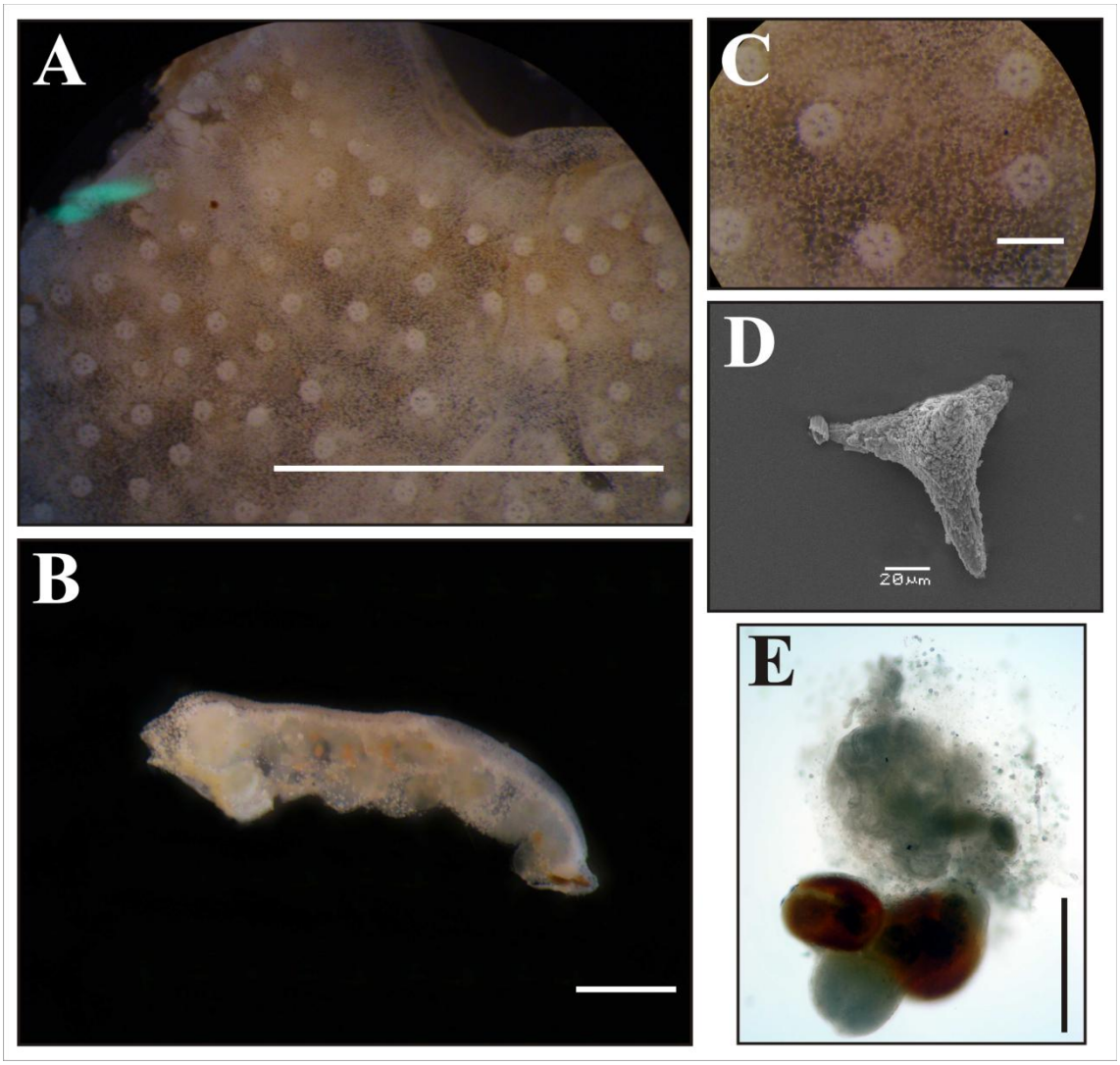

Plate V. *Lissoclinum verrilli*. A, preserved colony; B, cross section of preserved colony; C, detail of spicules distribution; D, spicule; E, zooid. Scale bars: A and B, 1.5 cm; C, 0.1 cm; E, 0.5 mm.

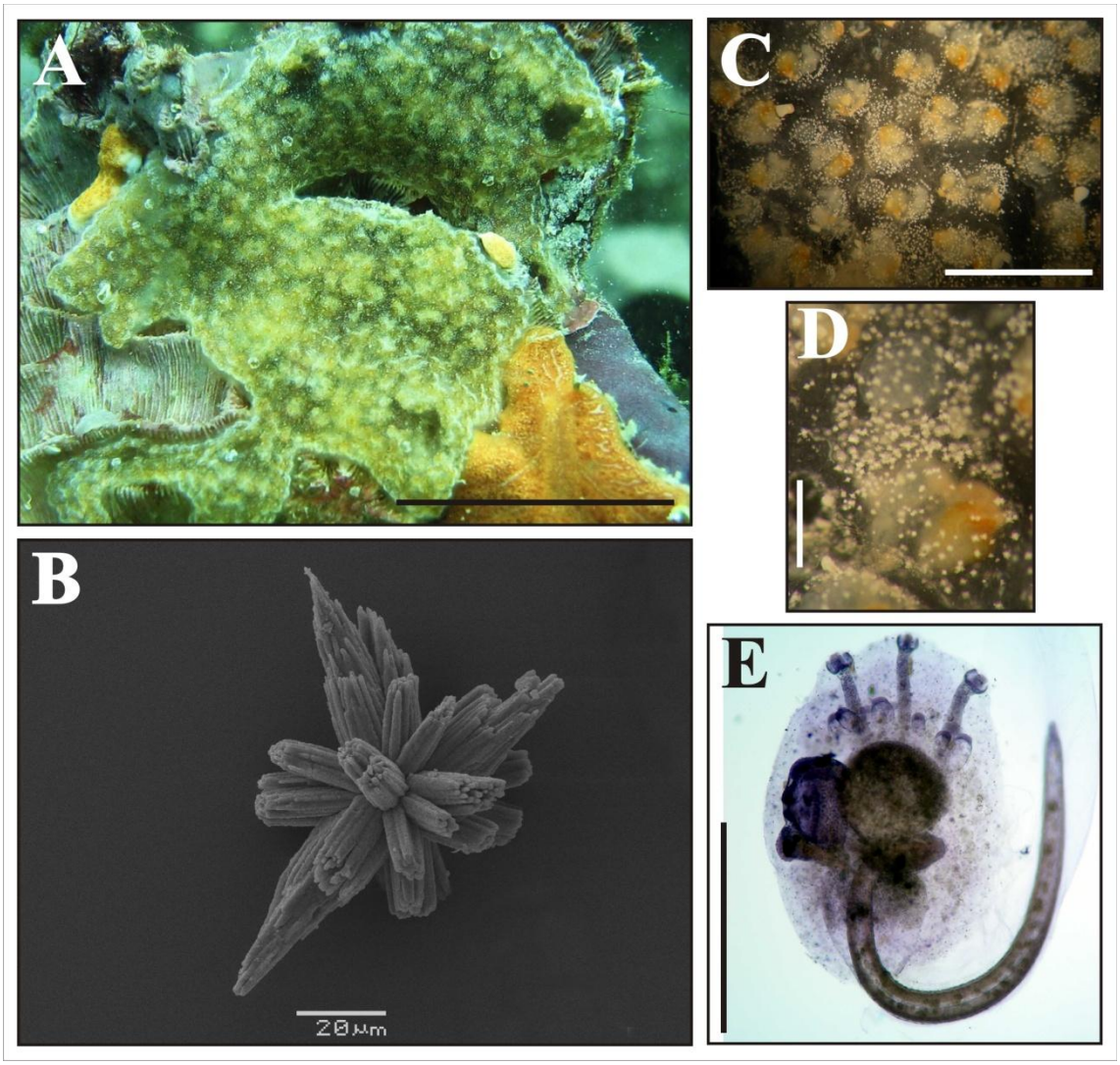

Plate VI. *Lissoclinum* sp nov. 1. A, living colony; B, spicule; C, colony detail; D, spicules around zooid; E, larva. Scale bars, A, 2 cm; C, 3 mm; D and E, 0.5 mm.

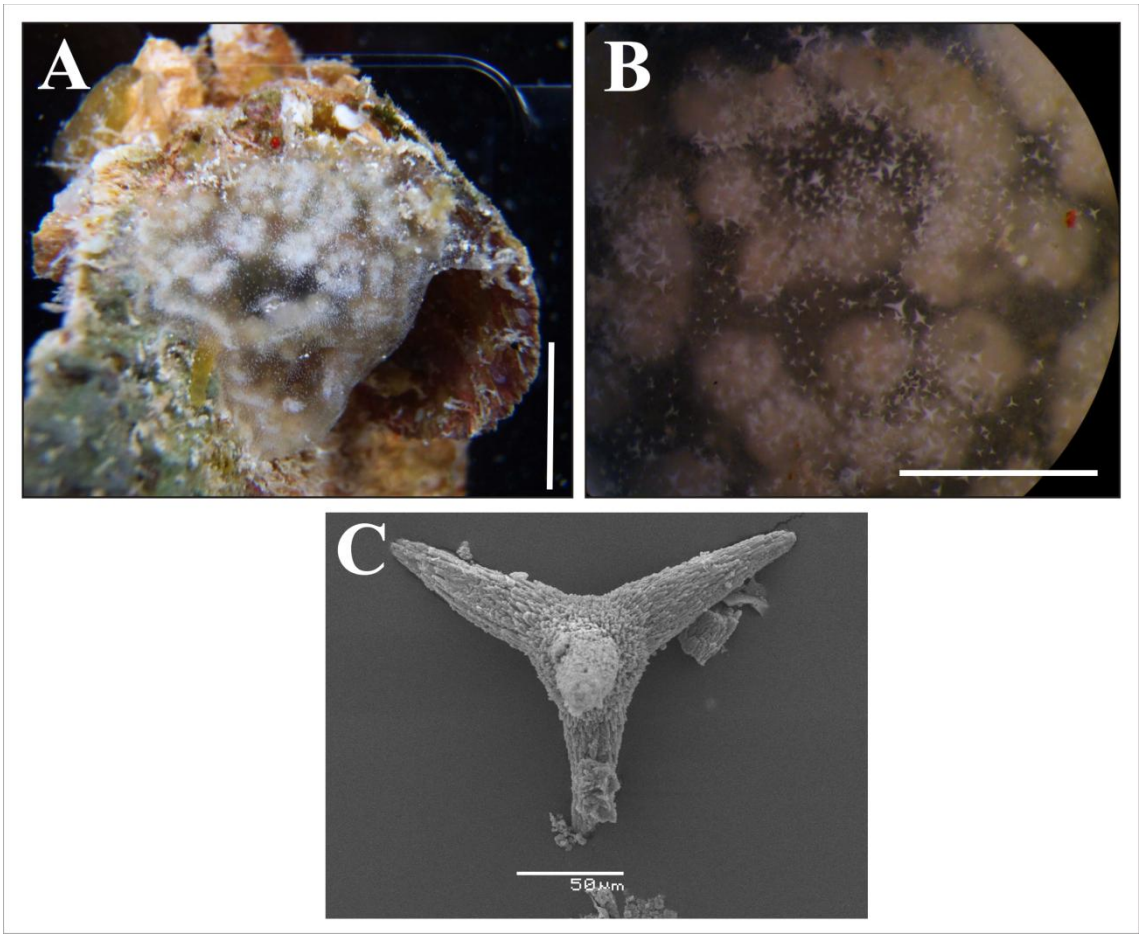

Plate VII. *Lissoclinum* sp nov. 2. A, living colony; B, spicules around zooids; C, spicule. Scale bars, A, 1 cm; B, 1 mm.

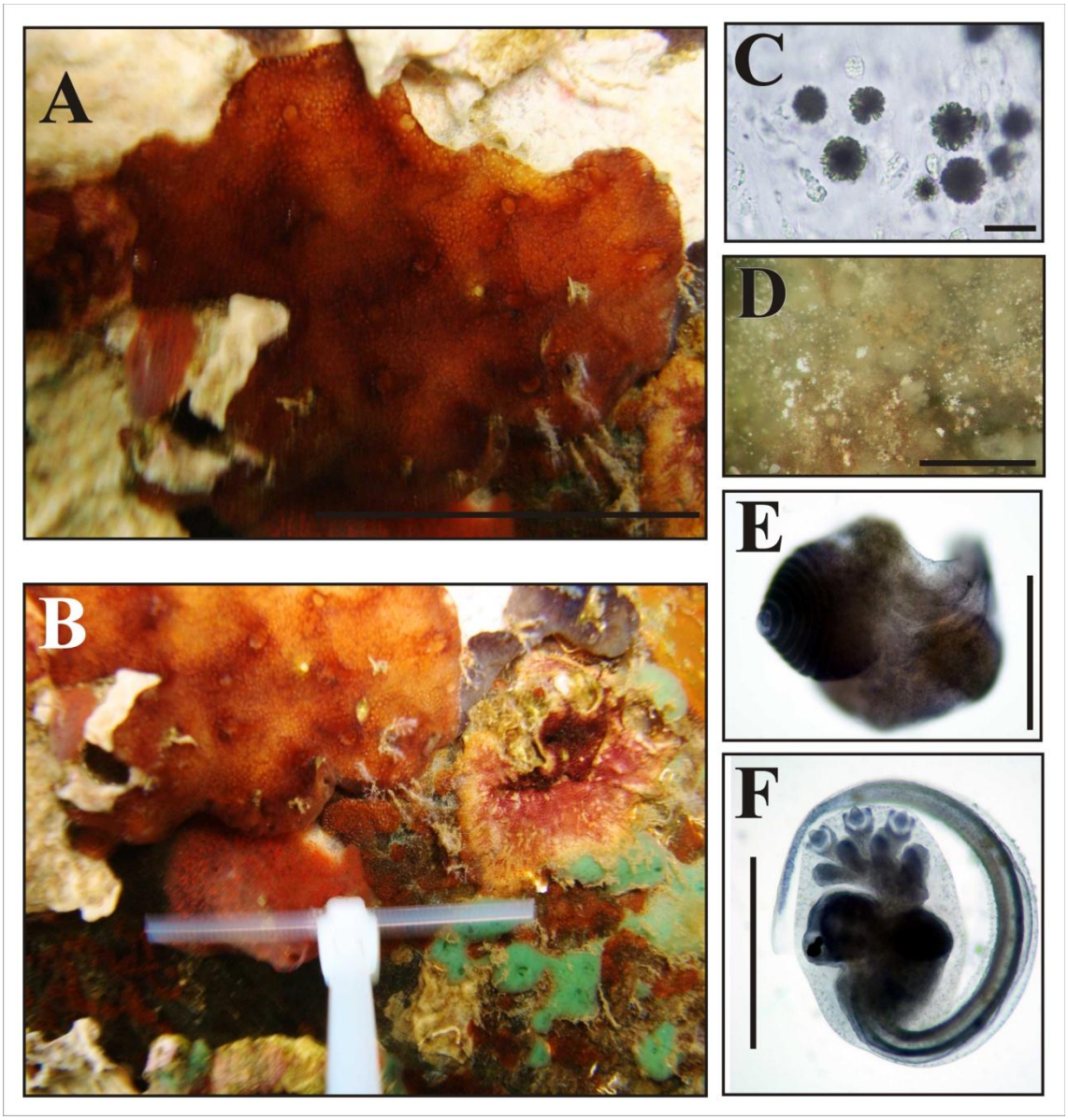

Plate VIII. *Didemnum calliginosum*. A and B, living colonies; C, spicules; D, colony detail; E: abdomen; F, larva. Scale bars: A and B, 5 cm; C: 15 µm; D, 1 cm; E, 0,5 mm; F, 0,3 mm.

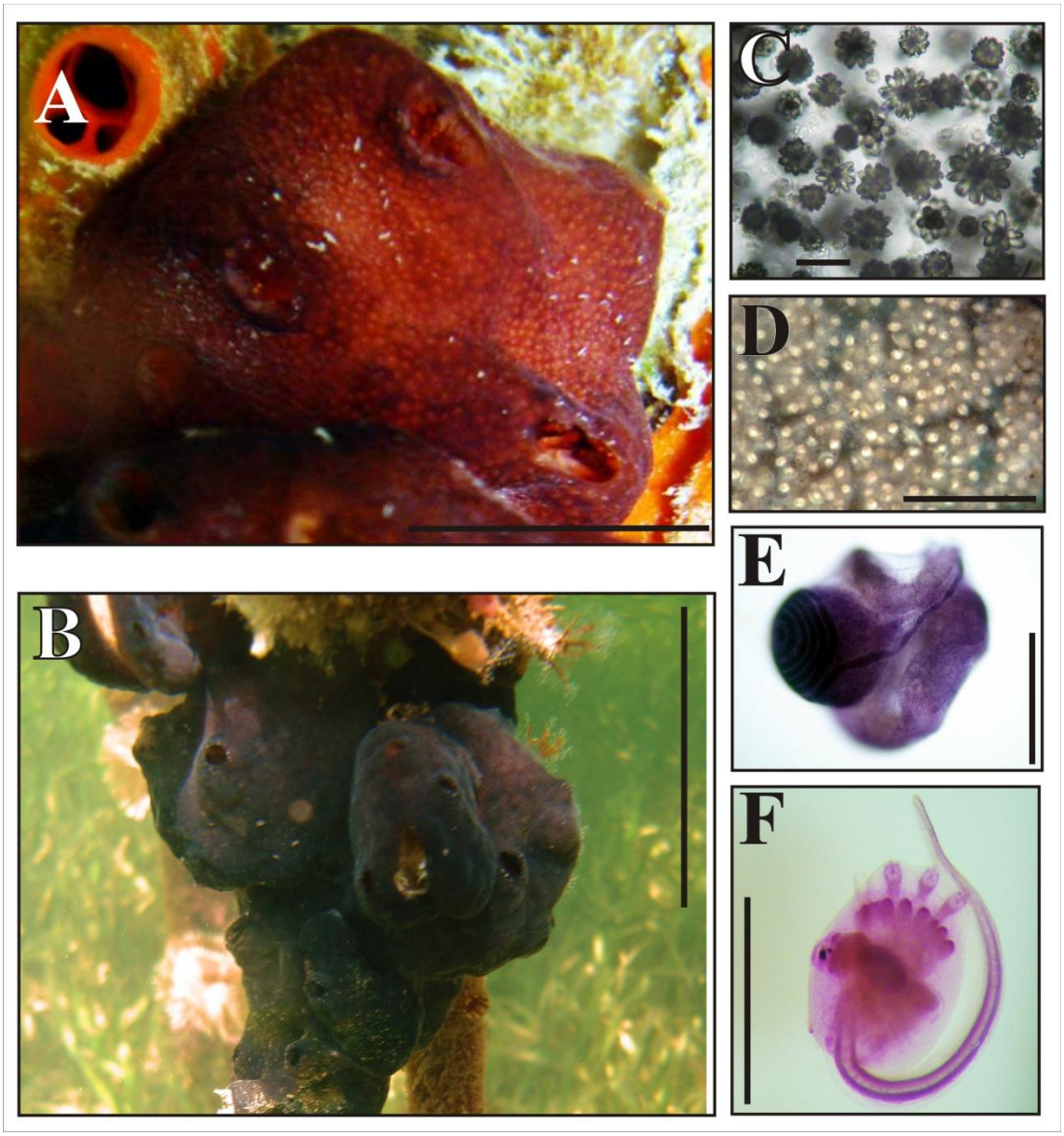

Plate IX. *Didemnum cineraceum*. A and B, living colonies; C, spicules; D, colony detail; E, abdomen; F, larva. Scale bars, A, 3 cm; B, 5 cm; C, 20 µm; D, 2 cm; E and F, 0.5 mm.

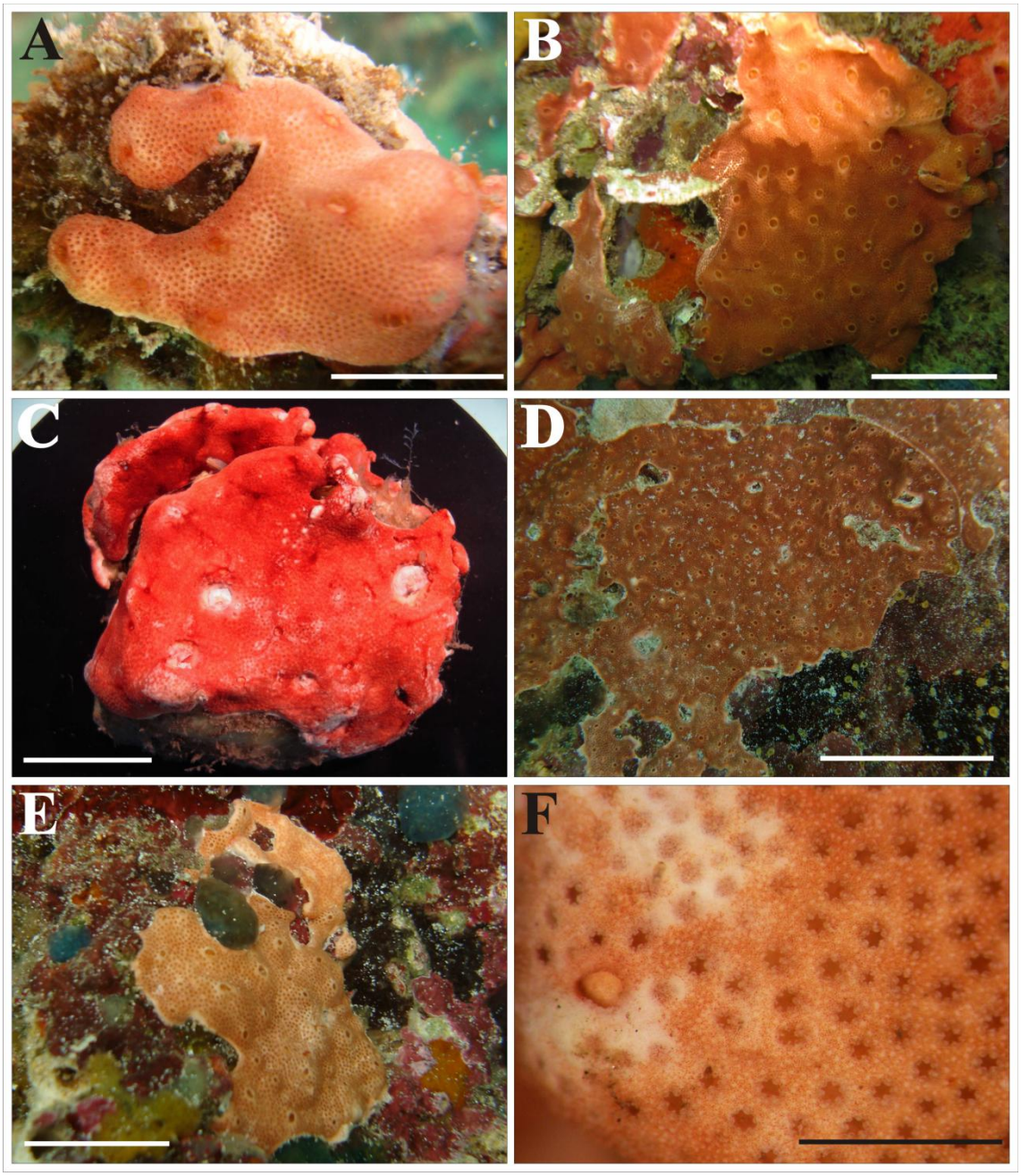

Plate X. *Didemnum granulatum.* A, B, C, D and E, living colonies; F, colony detail (papillae). Scale bars: A, B, C, D and E, 2 cm; F, 2 mm.

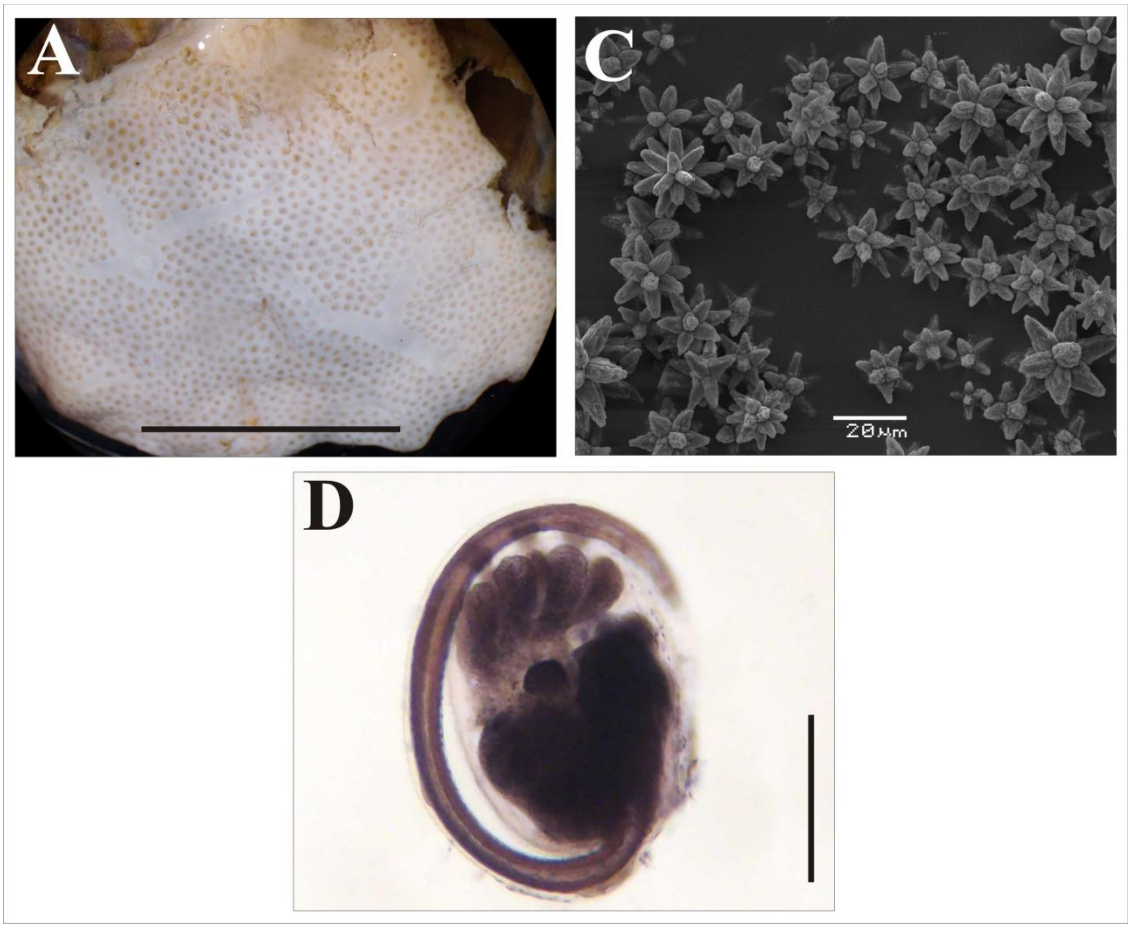

Plate XI. *Didemnum* sp. nov. A: preserved colony, B: spicules, C: preserved colony, D: larva. Scale bars: A, B: 2 cm; C: 0.2 mm

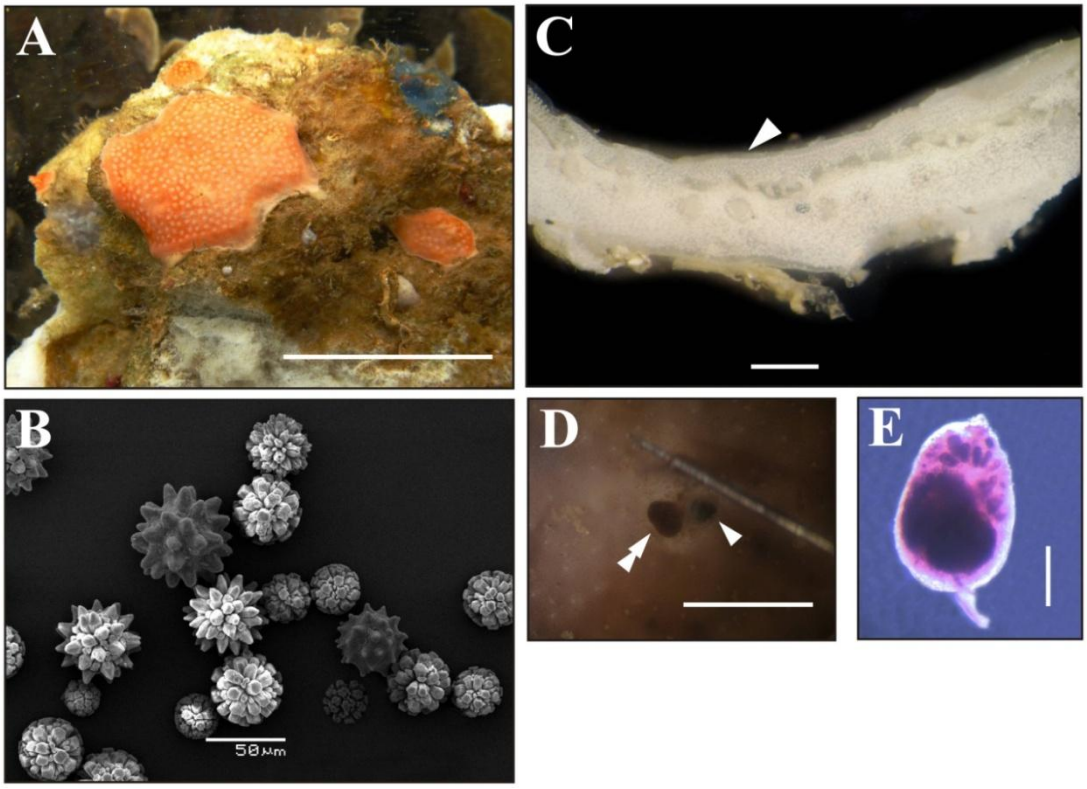

Plate XII. *Tridemnum palmae*. A, living colony; B, spicules; C, cross section of colony with superficial layer without spicules (arrow head); D, detail of colony showing were larvae are incubated (arrow head shows larvae and double arrow head shows algae); E, larvae. Scale bars, A, 5 cm; C, 0.5 cm; D, 0.5 cm; E, 0.2 mm.

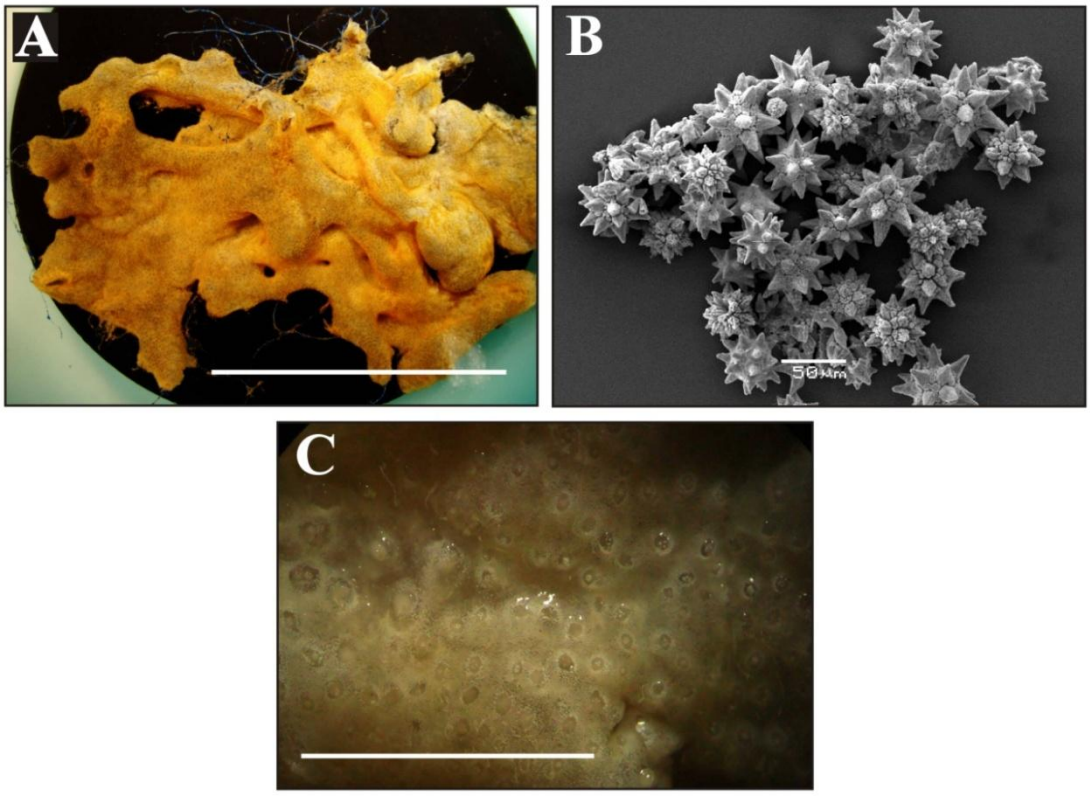

Plate XIII. *Tridemnum* cf. *thetidis*. A, freshly preserved colony; B, spicules; C, detail of spicules distribution; Scale bars, A, 5 cm; C, 0.5 cm.

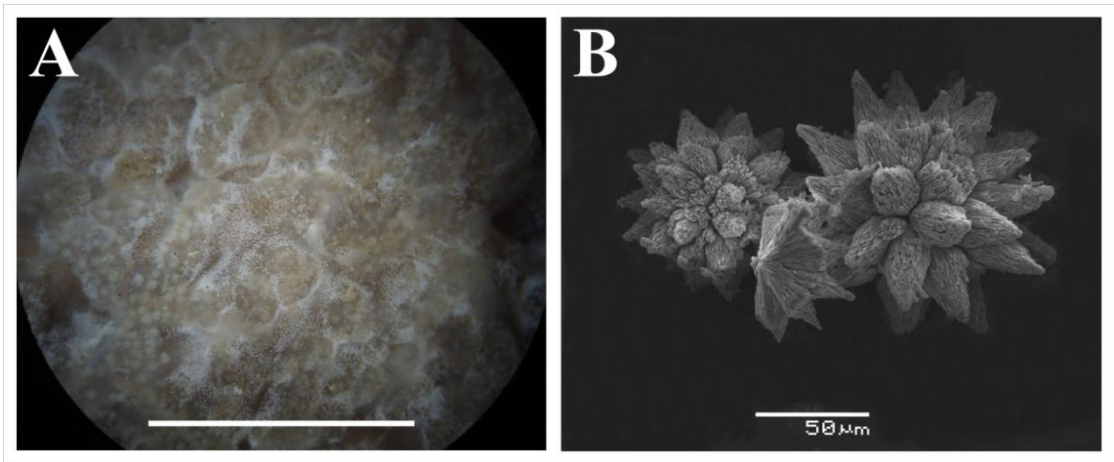

Plate XIV. *Tridemnum* sp. nov. 1. A, preserved colony; B, spicules. Scale bar, A, 0.5 mm.

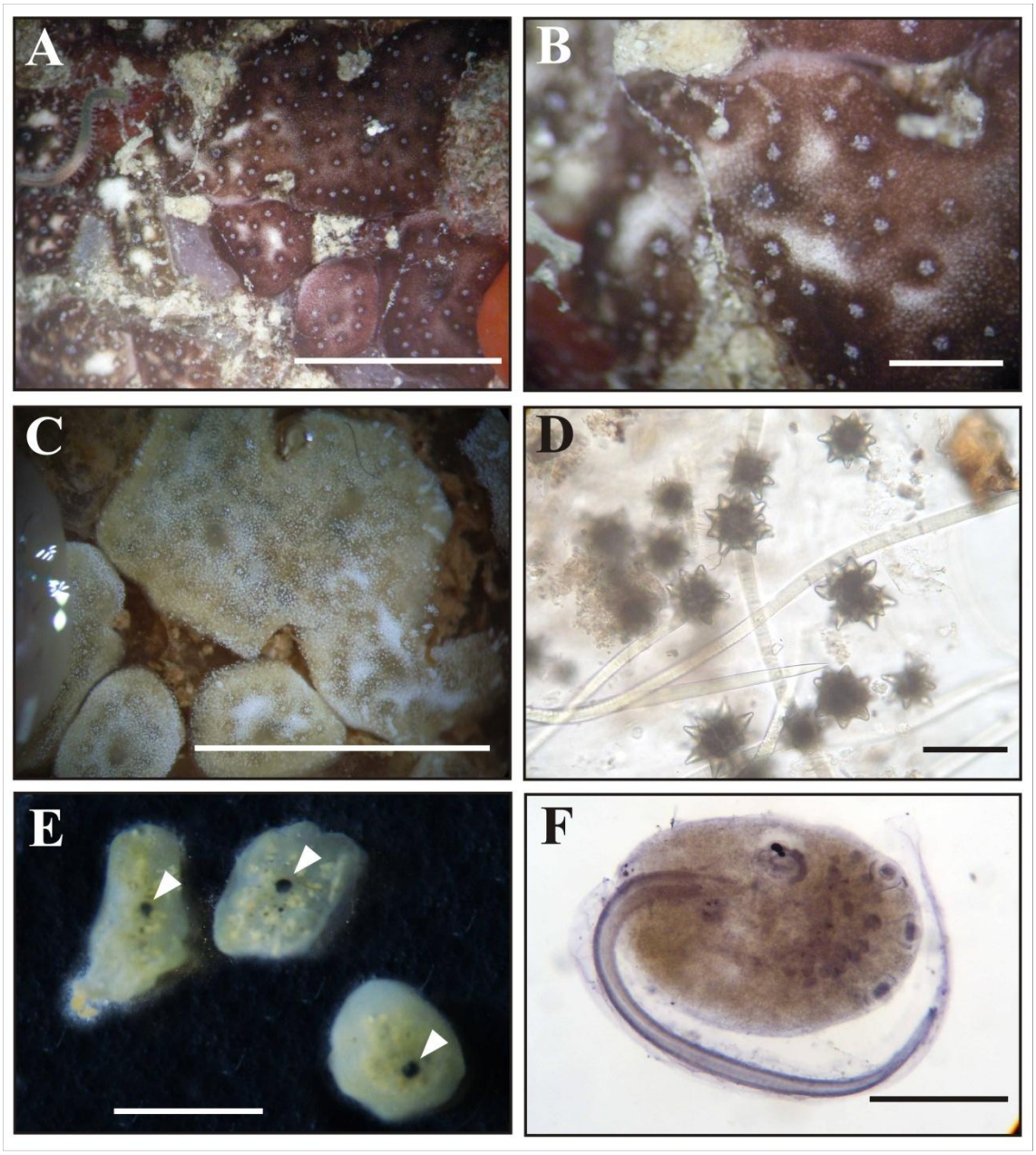

Plate XV. *Trididemnum* sp. nov. 2. A, living colonies; B, spicules distribution; C, preserved colonies; D, spicules; E, pigment spot in decalcified colonies (white arrow heads); F, larvae. Scale bar, A, 5 cm; B, 3 cm; C, 5 cm; D, 60 μm; E, 0.5 cm; F, 0.5 cm.

References

Abdul Jaffar Ali H, Sivakumar V, Tamilselvi M. 2009. World Journal of Fish and Marine Sciences 1(4):305–312.

Brusca RC, Brusca GJ. 2007. Invertebrados. Sinauer Associates, Sunderland: Massachusetts. 966 pages.

Brément E. 1913. Sur deux nouveaux Didemnidés (Synascidies) do Golfe do Lion. Bulletin de L'Institut Océanographique 257:1–7.

Burighel P, Cloney RA. 1997. Urochordata: Ascidiacea. Microscopic Anatomy of Invertebrates 15:221–347.

Carman MR, Bullard SG, Rocha RM, Lambert G, Dijkstra JA, Roper JJ, Goodwin A, Carman MM, Vail EM. 2011. Ascidians at the Pacific and Atlantic entrances to the Panama Canal. Aquatic Invasions 6 (4):371–380.

Cascon HM, Lotufo TMC. 2005. Biota Marinha da Costa Oeste do Ceará. Fortaleza. 268 pages.

Cole LL. 2012. Diversity and Distribution of Tunicata (Urochordata) in Tobago. Journal of Life Sciences 6:221–232.

Collin R. 2005. Ecological Monitoring and Biodiversity Surveys at the Smithsonian Tropical Research Institute's Bocas del Toro Research Station. Caribbean Journal of Science 41(3):367–373.

Collin R, D'Croz L, Gondola P, Del Rosario JB. 2009. Climate and Hydrological Factors Affecting Variation in Chlorophyll Concentration and Water Clarity in the Bahia Almirante, Panama. Smithsonian Contributions to the Marine Sciences 38:323–334.

Collin R, Díaz MC, Norenburg J, Rocha RM, Sánchez JA, Schulze A, Schwartz M, Valdés A. 2005. Photographic Identification Guide to Some Common Marine Invertebrates of Bocas Del Toro, Panama. Caribbean Journal of Science (41)3:638–707.

Della Valle A. 1881. Nouve contribuzioni alla storia naturale della ascidie composte del Golfo di Napoli. Atto della Reale Accademia del Lincei 3(10):431–498.

Dias GM, Abreu AG, Silva FOM, Solferini VN. 2009. Microgeographical differentiation between morphotypes of *Trididemnum orbiculatum* (Tunicata: Ascidiacea) in southeastern Brazil. Aquatic Biology 4:243–252.

Eldredge LG. 1967. A taxonomic review of the Indo-Pacific didemnid ascidians and descriptions of twenty three central Pacific species. Micronesica 2:162–261.

Giard AM. 1872. Recherches sur les ascidies composées ou synascidies. Archives de Zoologie Expérimentale et Générale 1:613–662

Goodbody I. 1984. The Ascidian Fauna of Two Contrasting Lagoons in the Netherlands Antilles: Piscadera Baai, Curaçao, and The Lac of Bonaire. Studies on the Fauna of Curaçao and other Caribbean Islands 202:21–61.

Goodbody I. 2000. Diversity and distribution of ascidians (Tunicata) at the Pelican Cays, Belize. Atoll Research Bulletin 480:301–333.

Goodbody I. 2003. The ascidian fauna of Port Royal, Jamaica I. harbor and mangrove dwelling species. Bulletin of Marine Science 73(2):457–476.

Guzmán HM, Guevara C. 1999. Arrecifes coralinos de Bocas del Toro, Panamá: III. Distribución, estructura y estado de conservación de los arrecifes de las islas Pastores, Cristobal, Popa y Cayo Agua. Revista de Biologia Tropical 47:659–675.

Hartmeyer R. 1909. Zur Terminologie der Didemnidae. Sitzungs-Ber. Ges. naturforsch. Freunde Ber. 1909: 575–581

Herdman WA. 1886. Report on the Tunicata collected during the years 1873-1876. Part 2. Ascidiae Compositae. Zoological Challenger Expedition 14(38):1–425.

Kott P. 1981.The Ascidians of the Reef Flats of Fiji. Proceedings of the Linnean Society of New South Wales 105(3):147–212.

Kott P. 1962. The ascidians of Australia. III. Aplousobranchiata Lahille: Didemnidae Giard. Australian Journal of Marine and Freshwater Research 13:265–334.

Kott P. 2001. The Australian Ascidiacea part 4, Aplousobranchia (3), Didemnidae Memoirs of the Queensland Museum 47:1–410.

Kott P. 2005. Catalogue of Tunicata in Australian Waters. Brisbane: Queensland Museum. 301 pages.

Kott P. 2007. New and little-known species of Didemnidae (Ascidiacea, Tunicata) from Australia (part 4). Journal of Natural History 41:17, 1163–1211.

Kott P, Goodbody I. 1980. Ascidians of Hong Kong. Proceedings of the First International Marine Biological Workshop: 503–554.

Kremer LP, Rocha RM, Roper JJ. 2010. An experimental test of colonization ability in the potentially invasive *Didemnum perlucidum* (Tunicata, Ascidiacea). Biological Invasions 12:1581–1590.

Lafargue F. 1968. Les peuplements sessiles de l'archipel de Glénan. II. Les Didemnidae - Sistématique - Écologie. Vie et Milieu 19(2A):353–446.

Lafargue F. 1975. Révision Taxonomique dês Didemnidae dês Côtes de France (Ascidies Composées). Les espéces de Banyils-sur-mer. Genre *Didemnum*. Première partie: Larves a trois ventouses. Annales de l'Institut Océanographique 51(2):173–194.

Lafargue F, Ducleaux G. 1979. Premier exemple, en atlantique tropical, d'une association symbiotique entre une ascidie Didemnidae et une Cyanophycéee Chroococcale: *Trididemnum cyanophorum* nov. sp. et *Synechocystis trididemni* nov. sp. Annales de I'Institut Océanographique 55(2):101–184.

Lafargue F, Wahl M. 1987. The didemnid ascidian fauna of France. Annales de l'Institut Océanographique 63(1):1–46.

Lambert G. 2003. Marine biodiversity of Guam: the Ascidiacea. Micronesica (35–36):588–597.

Lambert G. 2005. Ecology and natural history of the protochordates. Canadian Journal of Zoology 83:34–50.

Macdonald JD. 1859. On the anatomical characters of a remarkable form of compound Tunicata. Transactions of the Linnean Society of London (Zoology) 22:373–375.

Médioni A. 1970. Ascidies du benthus rocheux de Banyuls-Sur-Mer Didemnidae (Ascidies Composées). Vie et Milieu A21:25–48.

Millar RH. 1953. On a collection of ascidians from the Gold Coast. Proceedings of the Zoological Society of London 123(II):277–325.

Millar RH. 1958. Ascidians from Brazil. Annals and Magazine of Natural History 13(i):497–512.

Milne-Edwards H. 1841. Observations sur les ascidies composées des côtes de la Manche. Mémoires de l'Académie des Sciences de l'Institute de France 18:217–326.

Monniot C, Monniot F. 1972. Clé mondiale des genres d'ascidies. Archives de Zoologie Experimentale et Génerale 113:311–367.

Monniot C, Monniot F. 1987. Les ascidies de Polynésie Française. Mémoires du Muséum National d'Histoire Naturelle. Zoologie 136:1–155.

Monniot C, Monniot F. 1994. Additions to the inventory of eastern tropical Atlantic ascidians; arrival of Cosmopolitan species. Bulletin of Marine Science, 54(1):71–93.

Monniot C, Monniot F, Griffiths CL, Schleyer M. 2001. South African Ascidians. Annals of the South African Museum 108(1):1–141.

Monniot C, Monniot F, Laboute P. 1985. Ascidies du port de Papeete (Polynésie Française): relations avec le milieu naturel et d'autres ports. Bulletin du Muséum National d'Histoire Naturelle 4, 7(3):481–495.

Monniot C, Monniot F, Laboute P. 1991. Coral Reef Ascidians of New Caledonia. Paris. 247 pages.

Monniot F. 1983. Ascidies littorales de Guadeloupe I, Didemnidae. Bulletin du Muséum National d'Histoire Naturelle 5(1):5–49.

Monniot F. 1984. Ascidies littorales de Guadeloupe VIII. Questions de systématique évolutive posées par les Didemnidae. Mémoires du Muséum National D'Histoire Naturelle 4:885–905.

Monniot F. 1992. Ascidies de Nouvelle-Calédonie XII. Le genre *Lissoclinum* (Didemnidae) dans le lagon sud. Bulletin du Muséum National d'Histoire Naturelle 4:565−589.

Monniot F. 1994. Ascidies de Nouvelle-Calédonie. XV Le genre *Diplosoma*. Bulletin du Muséum National d'Histoire Naturelle 16A(1):3–11.

Monniot F. 1995. Ascidies de Nouvelle-Calédonie XV. Le genre *Didemnum*. Bulletin du Muséum National d'Histoire Naturelle 4(2-4):299−344.

Monniot F, Monniot C. 1996. New Collections of Ascidians from the Western Pacific and Southern Asia. Micronesica 29(2):133−279.

Monniot F, Monniot C. 2001. Ascidians from the tropical western Pacific. Zoosystema 23(2):201−383.

Moreno TR, Faria SB, Rocha RM. 2014. Biogeography of Atlantic and Mediterranean ascidians. Marine Biology 161:2023−2033.

Moreno TR, Rocha RM. 2008. Phylogeny of the Aplousobranchia (Tunicata: Ascidiacea). Revista Brasileira de Zoologia 25(2):269–298.

Paulay G, Kirkendale L, Lambert G, Meyer C. 2002. Anthropogenic Biotic Interchange in a Coral Reef Ecosystem: A Case Study from Guam. Pacific Science 56(4):403–422.

Pérez-Portela R, Arranz V, Rius M, Turon X. 2013. Cryptic speciation or global spread? The case of a cosmopolitan marine invertebrate with limited dispersal capabilities. Scientific Reports 3:1–10.

Rico A, Peralta R, Gappa JL. 2012. Succession in subtidal macrofouling assemblages of a Patagonian harbour (Argentina, SW Atlantic). Helgoland Marine Research 66:577–584.

Rocha RM. 1995. Abundance and distribution of sessile invertebrates under intertidal boulders (São Paulo, Brazil). Boletim do Instituto Oceanográfico 43(1):71−88.

Rocha RM. 2002. *Tridemnum maragogi* sp. nov. (Ascidiacea, Didemnidae) from Alagoas, Northeastern Brazil. Revista Brasileira de Zoologia 19(4):1105–1110.

Rocha RM, Guerra-Castro E, Lira C, Paul SM, Hernández I, Pérez A, Sardi A, Pérez J, Herrera C, Carbonini AK, Caraballo V, Salazar D, Diaz MC, Cruz-Motta JJ. 2010. Inventory of ascidians (Tunicata, Ascidiacea) from the National Park La Restinga, Isla Margarita, Venezuela. Biota Neotropica 10(1):1−10.

Rocha RM, Monniot F. 1993. *Didemnum rodriguesi* sp.nov. - a new didemnid ascidian common to Southeastem Brazil and New Caledonia. Annales de l'Institut océanographique 69: 261-265

Rocha RM, Monniot F. 1995. Taxonomic and Ecological Notes on Some *Didemnum* species (Ascidiacea, Didemnidae) from São Sebastião Channel, South-East Brazil. Revista Brasileira de Biologia 55(4):639−649.

Rocha, RM, Faria SR. 2005 Ascidians at Currais Islands, Paraná, Brazil: Taxonomy and Distribution. Biota Neotropica 5(2):2–20.

Rocha RM, Faria SB, Moreno TR. 2005. Ascidians from Bocas del Toro, Panama. I. Biodiversity. Caribbean Journal of Science 41(3):600–612.

Rocha RM, Dias GM, Lotufo TMC. 2011. Checklist das ascídias (Tunicata, Ascidiacea) do Estado de São Paulo, Brasil. Biota Neotropica. 11(1a):1–11.

Rocha RM, Zanata TB, Moreno TR. 2012. Keys for the identification of families and genera of Atlantic shallow water ascidians. Biota Neotropica 12(1):1–35.

Rodrigues SA, Rocha RM, Lotufo TMC. 1998. Guia Ilustrado para Identificação das Ascídias do Estado de São Paulo. São Paulo: Fapesp. 190 pages.

Savigny JC. 1816. Mémoires sur lês animaux sans verbèbres. Paris. 239 pages.

Shenkar N, Gittenberger A, Lambert G, Rius M, Moreira da Rocha R, Swalla BJ, Turon X. 2014. Ascidiacea World Database. http://www.marinespecies.org/ascidiacea (accessed 1 September 2014).

Shenkar N, Swalla BJ. 2011. Global Diversity of Ascidiacea. Plos One 6(6):2–12.

Sluiter CP. 1895. Tunicaten. In Semon R. Zoologische Fors-chungsreisen in Australien und den Malagischen Archipel. Denkschriften der Medizinisch-Naturwissenschaftlichen Gesellschaft zu Jena 8:163–186.

Sluiter CP. 1898. Beiträge zur Kenntniss der Fauna von Süd-Arrika. Mémoires de la Société zoologique de France 11:5–34

Sluiter CP. 1909. Die Tunicaten der Siboga Expedition. Pt II. Die merosomen Ascidien. Siboga Expedition 56B:1–112.

Smale DA, Childs S. 2012. The occurrence of a widespread marine invader, Didemnum perlucidum (Tunicata, Ascidiacea) in Western Australia. Biological Invasions 14(7):1325–1330.

Tokioka T. 1954. Invertebrate fauna of the intertidal zone of the Tokara Islands VII. Ascidians. Seto Marine Biological Laboratory III(3):239–264.

Tokioka T. 1958. Contributions to Japanese Ascidian Fauna. Publications of the Seto Marine Biological Laboratory 6 (3):73–85.

Van Name WG. 1902. The ascidians of the Bermuda Islands. Transactions of the Connecticut Academy of Arts and Sciences 11:325–412.

Van Name WG. 1921. Ascidians of the West Indian Region and Southeastern United States. Bulletin American Museum of Natural History 44:283–494.

Van Name WG. 1924. Ascidians from Curaçao. Bijdragen tot de Dierkunde, Amsterdam 23:23–32.

Van Name, W. G. 1945. The North and South American ascidians. Bulletin of the American Museum of Natural History 84:1–476.

Vazquez E. 1993. *Diplosoma lafargueae* n. sp., nouvelle espece de Didemnidae (ascidies composées) des côtes atlantiques ibériques. Vie Milieu 43(2-3):173–175.

Verrill AE. 1871. Descriptions of some imperfectly known and new ascidians from New England. American Journal of Science (3)1:54–446.

Von Drasche R. 1883. Die Synaseidien der Bueht von Rovigno (Adria). Wien:1–41.