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# First records of the order Polycladida (Platyhelminthes, Rhabditophora) from reef ecosystems of Alagoas State, north-eastern Brazil, with the description of *Thysanozoon alagoensis* sp. nov.

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The 230 km long coast of Alagoas State, in north-eastern Brazil, has diverse reef ecosystems, made from corals and of sandstone, that harbour a wide range of marine invertebrate fauna. Little is known about turbellarians of the order Polycladida in most parts of the Brazilian coast, with no record from Alagoas up to date. To fill this gap expeditions were conducted on the reefs from the central coast of Alagoas, where 11 Polycladida species were found: Pericelis cata, Enchiridium evelinae, Pseudoceros bicolor and a possible new colour variation of this species, Pseudoceros rawlinsonae, Pseudobiceros pardalis, Thysanozoon brocchii, Thysanozoon alagoensis sp. nov., Armatoplana leptalea, Adenoplana evelinae, Latocestus brasiliensis, Phaenocelis medvedica. The species are described in detail through photos of live specimens and histological sections. The present work adds six species to the north-eastern Brazilian coast, one of them a new species, and all 11 species are for the first time reported from Alagoas State. Also, it is the first time that Phaenocelis medvedica, Adenoplana evelinae, Latocestus brasiliensis and Armatoplana leptalea are illustrated by full colour photos of live specimens and histological sections.

Keywords: marine flatworms, Brazilian biodiversity, taxonomy

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# INTRODUCTION

Animals belonging to the order Polycladida are free-living platyhelminthes, with most species known from tropical seas. About 900 species have been described around the world (Newman & Cannon, 2003). Among them, about 125 were reported from the Tropical Western Atlantic, 70 occur in Brazil, 46 being endemic (Quiroga *et al.*, 2004a; Bahia *et al.*, 2014). From the Brazilian coast, the existing knowledge is mostly the result of works conducted between 1950 and 1970, based on specimens collected at São Paulo region (Marcus, 1947, 1948, 1949, 1950, 1952). Recent contributions, including new local and country records were made by Bahia & Padula (2009), Bahia *et al.* (2012, 2014) and Queiroz *et al.* (2013).

On the north-eastern Brazilian coast, such as in the state of Alagoas, reef ecosystems are abundant and have a rich biological diversity (Correia & Sovierzoski, 2009). These reefs include a benthic fauna, which provide various natural substrates for the Polycladida, composed mainly of sponges (Cedro *et al.*, 2007, 2011, 2013; Bispo *et al.*, 2014), corals (Correia, 2011) and bryozoans (Vieira *et al.*, 2007, 2008, 2010). A variety of algae also provides habitats for numerous

**Corresponding author:** J. Bahia Email: ju.bahia@yahoo.com associated organisms (Santos & Correia, 1994, 1995, 2001), which are important environments for flatworms and other small invertebrates such as opisthobranch molluscs (Padula *et al.*, 2012) and echinoderm brittle stars (Lima *et al.*, 2011, 2013). However, there is a significant lack of information, particularly about marine platyhelminthes in this region, with no record of the order Polycladida from Alagoas reef ecosystems. Herein we aim to present the first records of Polycladida from Alagoas coast, including the description of a new species.

# MATERIALS AND METHODS

Alagoas coast is approximately 230 km long, and it is limited by the Persinunga River on the north and by the São Francisco River on the south  $(8^{\circ}54'S-35^{\circ}9'W \text{ and } 10^{\circ}30'S-36^{\circ}23'W)$ , The main ecosystems that can be found are coral and sandstone reefs, lagoons, rivers and mangroves. The coral reefs were formed on calcareous sedimentary rock, composed of an aggregation of dead organisms, including skeletons of corals and hydrocorals combined with crusts of calcareous algae and other invertebrates (Correia & Sovierzoski, 2009). Many of these fringing reefs are located near the beach line, where the top of the reef platform is exposed during low tides. The sandstone reefs were formed by old sandbanks solidified through sedimentation, starting from chemical reactions with calcium carbonate from the Quaternary Period, and are generally arranged in rows parallel to the coastline and near the outlets of rivers and estuaries (Correia & Sovierzoski, 2009; Correia, 2011).

All specimens were manually collected under rocks at the intertidal zone or in the sublittoral by snorkelling or scuba diving along the edge of the reef platforms during low tide. Collections were conducted in January 2008 and January 2012, and were carried out in reef ecosystems along the central coast of Alagoas State, Brazil (Figure 1). Seven reef ecosystems were sampled: coral reefs of Ponta Verde (9°39'57"S- $35^{\circ}41'32"W$ ), Jatiúca (9°39'12"S- $35^{\circ}41'46"W$ ), Piscina dos Amores (9°40'39"S- $35^{\circ}42'10"W$ ), Pajuçara (9°41'06"S- $35^{\circ}43'22"W$ ) and Riacho Doce (9°34'55"S- $35^{\circ}39'25"$ ), and sandstone reefs of Francês (9°46'03"S- $35^{\circ}50'13"W$ ) and Saco da Pedra (9°44'26"S- $35^{\circ}48'59"W$ ) (Figure 1).

In the laboratory, specimens were photographed alive with a digital camera. Afterwards, they were fixed in 10% frozen formalin, following a modified methodology (Newman & Cannon, 2003), and transferred to 70% ethanol for histological preparation. Specimens were measured after fixation (length mm × width mm). The identification was based on morphological characteristics, colouration pattern, ocelli position and slides of the reproductive structures stained by haematoxylin-eosin method (Bolaños *et al.*, 2007). Specimens were compared with original descriptions and previous publications (Marcus, 1949, 1950, 1952; Marcus & Marcus, 1968; Bolaños *et al.*, 2007). Collected material was deposited on the Platyhelminthes collection in the Museu Nacional/ Universidade Federal do Rio de Janeiro, Brazil (MNRJ-PLAT).

# RESULTS

There were 35 specimens found belonging to 11 species of the order Polycladida. *Pericelis cata, Enchiridium evelinae, Pseudoceros bicolor, Pseudoceros rawlinsonae, Pseudobiceros pardalis, Thysanozoon brocchii, Thysanozoon alagoensis* sp. nov., *Armatoplana leptalea, Adenoplana evelinae, Latocestus brasiliensis* and *Phaenocelis medvedica*. The species *Enchiridium evelinae* was the most common, with 15 specimens collected, and found in all sampled reefs. Greater species richness was observed at Saco da Pedra reef. The lowest richness was found at Piscina dos Amores coral reef. All occurrences reported here are the first records of Polycladida from Alagoas ecosystems (Table 1). As some of the species found in this study were treated on previous papers by the authors, just the material examined, with measurements, distribution and remarks are included.

> SYSTEMATICS Order POLYCLADIDA Lang, 1884 Suborder COTYLEA Lang, 1884 Family PERICELIDAE Laidlaw, 1902 Genus Pericelis Laidlaw, 1902 Pericelis cata Marcus & Marcus, 1968 (Figure 2B)



Fig. 1. Map of reef environments studied at Alagoas State, north-eastern Brazil.

SPECIES	Ponta Verde	Francês	Riacho Doce	Saco da Pedra	Piscina dos Amores	Pajuçara	Number of specimens
Enchiridium evelinae	1	2	2	6	2	2	15
Pseudoceros bicolor				2			2
Pseudoceros rawlinsonae				1			1
Pseudoceros cf. bicolor		2					2
Pseudobiceros pardalis				1			1
Thysanozoon brocchii		1		2			3
Thysanozoon alagoensis sp. nov.				1			1
Pericelis cata				1			1
Phaenocelis medvedica	1						1
Armatoplana leptalea			3	1			4
Adenoplana evelinae			-	1		1	2
Latocestus brasiliensis	1					1	2
No. of species	3	3	2	9	1	3	Total:11/35

Table 1. Species and number of specimens found at each reef ecosystem on the Alagoas coast.

## EXAMINED MATERIAL

One mature specimen  $(18 \times 16 \text{ mm})$  as sagittal sections of reproductive structures (MNRJ-PLAT 96, 13 slides). Collected 26 January 2012 at Saco da Pedra sandstone reef, Marechal Deodoro, Alagoas, Brazil.

# GEOGRAPHIC DISTRIBUTION

This species was recorded from Curaçao (type locality; Marcus & Marcus, 1968), Colombian Caribbean (Quiroga *et al.*, 2004b), Cabo Frio, south-eastern Brazil (Bahia & Padula, 2009) and Salvador, north-eastern Brazil (Queiroz *et al.*, 2013). This is the first record of this species from Alagoas State.

## REMARKS

The specimen herein studied was smaller than those found at Cabo Frio, south-eastern Brazil (Bahia & Padula, 2009) and those from Bahia State, north-eastern Brazil (Queiroz *et al.*, 2013). During the collections two specimens of *P. cata* were placed in the same container as some specimens of the opisthobranch mollusc *Micromelo undatus*. When freed to be photographed the *Pericelis* were much damaged and one was not useful for study. This could have happened because



**Fig. 2.** (A) Enchiridium evelinae (MNRJ-PLAT 80); (B) Pericelis cata (MNRJ-PLAT 96); (C) Pseudoceros bicolor (MNRJ-PLAT 85); (D) Pseudoceros rawlinsonae (MNRJ-PLAT 87); (E) Pseudobiceros pardalis (MNRJ-PLAT 92).

of some fighting with the molluscs or some toxic chemical compound they might present.

Family PROSTHIOSTOMIDAE Lang, 1884 Genus Enchiridium Bock, 1913 Enchiridium evelinae Marcus, 1949 (Figure 2A)

# EXAMINED MATERIAL AND LOCALITY

One mature specimen (MNRJ-PLAT 77, 32 × 9 mm) collected 6 January 2008 at Brazil, Alagoas, Maceió, Ponta Verde coral reef. One mature specimen (MNRJ-PLAT 78,  $29 \times 8$  mm) collected 7 January 2008 at Pajuçara coral reef, Maceió, Alagoas, Brazil. Two specimens (MNRJ-PLAT 79,  $26 \times 6 \text{ mm}$  and  $30 \times 8 \text{ mm}$ ) collected 9 January 2008 at Francês sandstone reef, Maceió, Alagoas, Brazil. Two specimens (MNRJ-PLAT 80,  $21 \times 7$  mm and  $32 \times 10$  mm). One as sagittal sections of reproductive structures (21 slides). Collected 10 January 2008 at Riacho Doce coral reef, Maceió, Alagoas, Brazil. Five specimens (MNRJ-PLAT 81,  $6 \times 3$  mm,  $12 \times 5$  mm,  $16 \times 6$  mm,  $21 \times 7$  mm and  $23 \times 10^{-10}$ 8 mm) collected 11 January 2008 at Saco da Pedra sandstone reef, Marechal Deodoro, Alagoas, Brazil. Two specimens (MNRJ-PLAT 82,  $21 \times 7$  mm and  $22 \times 7$  mm) collected 13 January 2008 at Piscina dos Amores coral reef, Maceió, Alagoas, Brazil. One specimen (MNRJ-PLAT 83,  $18 \times$ 7 mm) collected 7 February 2008 at Pajuçara coral reef, Maceió, Alagoas, Brazil. One specimen (MNRJ-PLAT 84, 26 × 11 mm) collected 27 January 2012 at Saco da Pedra sandstone reef, Maceió, Alagoas, Brazil. All specimens preserved in 70% ethanol.

## GEOGRAPHIC DISTRIBUTION

Originally described from São Paulo State, south-eastern Brazil (Marcus, 1949) reported to Rio Grande do Norte State (Bahia *et al.*, 2012), Rio de Janeiro State (Bahia *et al.*, 2014) and now Alagoas State, north-eastern Brazil. It is also known from Curaçao (Marcus & Marcus, 1968). This species is considered by Rawlinson (2008) as representative of seagrass habitat. We found our specimens both in sandstone and coral reefs and *E. evelinae* was the most common species in our samplings. This is the first record of this species from Alagoas State.

# REMARKS

This species was observed copulating (video in Supplementary material). It presented reciprocal insemination and a somewhat violent penis-fencing behaviour. Unfortunately the following eggmass laying could not be observed. Also one specimen of *Enchiridium evelinae* ate a specimen of *Adenoplana evelinae* during a fortnight in the same Petri dish. We noticed differences in colouration between the specimens found in Alagoas and those from Rio de Janeiro, the latter sometimes has a different distribution of the dorsal brown spots, with them more densely disposed in the median line (personal observation).

Family PSEUDOCEROTIDAE Lang, 1884 Genus Pseudoceros Lang, 1884 Pseudoceros bicolor Verrill, 1901 (Figures 2C & 3)

# EXAMINED MATERIAL

One mature specimen  $(12 \times 8 \text{ mm})$  as sagittal sections of reproductive structures (MNRJ-PLAT 85, 9 slides). One mature specimen (MNRJ-PLAT 86, 10 × 8 mm) collected 26 January 2012. Both collected at Saco da Pedra sandstone reef, Marechal Deodoro, Alagoas, Brazil and preserved in 70% ethanol. One mature specimen (11 × 10 mm) as sagittal section of reproductive structures (MNRJ-PLAT 90, 16 slides). One mature specimen (15 × 10 mm) as sagittal section of reproductive structures (MNRJ-PLAT 91, 15 slides). Both collected 25 January 2012 at Francês sandstone reef, Marechal Deodoro, Alagoas, Brazil.

## GEOGRAPHIC DISTRIBUTION

Described from Bermudas (type locality; Verrill, 1901), reported from Curacao (Marcus & Marcus, 1968), Caribbean coast of Colombia (Quiroga *et al.*, 2004b), Florida, Virgin Islands, Jamaica, Belize, Honduras, Caribbean coast of Panama (Rawlinson, 2008) and south-eastern Brazil (Bahia & Padula, 2009). This is the first record of this species from north-eastern Brazil.



**Fig. 3.** *Pseudoceros* cf. *bicolor* (A) *in vivo*; (B) and (C) detail of the anterior region; (D) ventral view; (E) sagittal section of male reproductive structures; (F) sagittal section of male and female reproductive structures. ce, cerebral eyespots; cg, cement glands; cp, cement pouch; fg, female gonopore; ma, male atrium; mg, male gonopore; pe, penis; ph, pharynx; pv, prostatic vesicle; su, sucker; sv, seminal vesicle; te, tentacular eyespots; ut, uteri; va, vagina.

## REMARKS

The specimens MNRJ-PLAT 85 and 86 are in accordance with the description and posterior amendment. Specimens MNRI-PLAT 90 and MNRI-PLAT 91 (at Table 1 counted separately as Pseudoceros cf. bicolor) were somewhat different from the former specimens. Their background colour was vellowish orange with scattered white and dark spots (Figure 3A); whitish translucent marginal band with a thin light yellow outermost line, only seen in live specimens. Seminal vesicle muscularized and elongated. Prostatic vesicle rounded and small, located above the penial papillae (Figure 3E). They resemble Pseudoceros bicolor in its background coloration pattern, but it lacks the white marginal band with black languettes characteristic of this species. Also the colour has hints of orange that are absent in specimens from the same locality and from other regions of the Brazilian coast (Bahia & Padula, 2009), and the seminal vesicle of these specimens is more elongated than rounded as usually found in P. bicolor. The material differs from the recently described P. juani in body proportion length x width, this species has a more elongated body (Bahia et al., 2014) than the Pseudoceros specimens found in Alagoas. Also, the seminal vesicle of P. juani is proportionally much larger than in P. bicolor and in the two specimens studied. However both P. juani and the two studied specimens have both scattered white and dark spots and there is a hint of orange in Alagoas' specimens, which is the background colour of P. juani, therefore we cannot rule out that those specimens can be some morphotype between P. bicolor and P. juani, despite the latter having no record in Alagoas so far. These two specimens are here, for now, identified as P. bicolor; this should be confirmed through a future molecular analysis.

Pseudoceros rawlinsonae Bolaños, Quiroga & Litvaitsi, 2007 (Figure 2D)

# EXAMINED MATERIAL

One mature specimen  $(10 \times 6 \text{ mm})$  as sagittal sections of reproductive structures (MNRJ-PLAT 87, 18 slides). Collected 11 January 2008 at Saco da Pedra sandstone reef, Marechal Deodoro, Alagoas, Brazil.

## GEOGRAPHIC DISTRIBUTION

Originally described from the American Virgin Islands and Bonaire (Bolaños *et al.*, 2007). After the revision of *Pseudoceros bicolor* complex, *P. rawlinsonae* was also reported from Florida, Honduras, Jamaica, Bahamas, Curaçao (Litvaitis *et al.*, 2010) and south-eastern Brazil (Bahia & Padula, 2009, as *P. bicolor*; Bahia *et al.*, 2014). This is the first record of this species from north-eastern Brazil.

> Genus Pseudobiceros Faubel, 1984 Pseudobiceros pardalis (Verrill, 1900) (Figure 2E)

## EXAMINED MATERIAL

One specimen ( $45 \times 37$  mm) as sagittal sections of reproductive structures (MNRJ-PLAT 92, 35 slides). Collected 27 January 2012 at Saco da Pedra sandstone reef, Marechal Deodoro, Alagoas, Brazil. *Pseudobiceros pardalis* was described from Bermudas (Verrill, 1900) and reported from Bahamas, south Florida and Panama (Bolaños *et al.*, 2007). It was recently reported for the first time from Brazil, but south from Alagoas (Bahia *et al.*, 2014). This is the first record of this species from north-eastern Brazil.

#### REMARKS

Our specimens have lighter colouration than the ones in the original description (Verrill, 1900) and re-description (Bolaños *et al.*, 2007), but darker than reported from south-eastern Brazil (Bahia *et al.*, 2014), probably due to differences in size and the nutritional conditions of the animals. The specimens found in the Caribbean (Bolaños *et al.*, 2007) have a concentration of white dots near the margin that is not so clear in Brazilian specimens (Figure 2E), both from Alagoas and from Rio de Janeiro (Bahia *et al.*, 2014).

> Genus *Thysanozoon* Grube, 1840 *Thysanozoon brocchii* (Risso, 1818) (Figure 4)

## EXAMINED MATERIAL

Two specimens ( $22 \times 18$  mm and  $6 \times 4$  mm), the mature as sagittal sections of reproductive structures (MNRJ-PLAT 93, 29 slides). Collected 11 January 2008 at Saco da Pedra sandstone reef, Marechal Deodoro, Alagoas, Brazil. One specimen ( $17 \times 20$  mm) (MNRJ-PLAT 94) collected 25 January 2012 at Francês sandstone reef, Marechal Deodoro, Alagoas, Brazil.

#### GEOGRAPHIC DISTRIBUTION

Cosmopolitan species described from Naples, Italy (type locality; Risso, 1818) and other parts of the Mediterranean Sea, UK, south and west from Africa, Florida, Colombian Caribbean,



**Fig. 4.** *Thysanozoon brocchii* (A) *in vivo*; (B) detail of the ventral surface; (C) sagittal section of the male reproductive structures; (D) sagittal section of the female reproductive structures. cg, cement glands; fg, female gonopore; it, intestine; ma, male atrium; mg, male gonopore; mo, mouth; pe, penis; ph, pharynx; pv, prostatic vesicle; st, stylet; su, sucker; ut, uteri; va, vagina.

Brazil, Japan and New Zealand (Prudhoe, 1985; Quiroga *et al.*, 2004b). It was also reported from Canary Islands (Vera *et al.*, 2008), Argentina (Brusa *et al.*, 2009), from northeastern (Bahia *et al.*, 2012) and south-eastern Brazil (Bahia *et al.*, 2014). This is the first record from Alagoas State.

## REMARKS

The specimens found at Alagoas all have rounded papillae and brownish colouration (Figure 4A). This is not the case in some south-eastern Brazil and in the Mediterranean Sea specimens (Bahia *et al.*, 2014). Specimens from those areas can have slender papillae and black to greyish colouration, and European specimens present a red marginal band (Bahia pers. obs.).

Thysanozoon alagoensis sp. nov. (Figures 5 & 6)

# TYPE MATERIAL

Holotype: one specimen ( $19 \times 16$  mm), as sagittal sections of reproductive structures (MNRJ-PLAT 95, 26 slides). Collected 27 January 2012 at Saco da Pedra sandstone reef, Marechal Deodoro, Alagoas, Brazil, 4 m deep.

# GEOGRAPHIC DISTRIBUTION

The species is only known from the type locality.

# ETYMOLOGY

The specific name *alagoensis* makes reference to the geopolitical division of Brazil, Alagoas State, where the holotype specimen was found.

## DIAGNOSIS

Greyish background colour with reddish brown papillae, reddish brown margin and dark almost black tentacles with a hint of yellowish pigmentation on the border. Unpigmented line present at the median line of the body. Tentacular eyespots arranged in a line at the border and a group of eyespots at each tentacular tip. Pharynx ruffled with seven simple folds. Seminal vesicle elongated and huge. Its extension reaches the front of the penial papillae and the seminal duct come in a curve to join the penis. Prostatic vesicle small and comma shaped. Male atrium simple. Greatly developed spermiducal vesicles.

#### DESCRIPTION

*Colour*: Greyish background colour with reddish brown papillae, reddish brown margin and dark almost black tentacles (Figure 5A). Some papillae are more lightly coloured, more like the background colour. Unpigmented line present at the median line of the body from the tentacles until the last 1/6 of the body length (Figure 5A).

*Form*: Elongated with papillated dorsal surface (Figure 5B) with slender whitish tipped papillae.

*Tentacles*: Dark almost black tentacles with a hint of yellowish pigmentation on the border. More rounded than pointed, reaches 1 mm.

*Eyes*: Cerebral eyes numerous and arranged in a horseshoe shaped group (Figure 6B). Tentacular eyespots arranged in a line at the border (Figure 6A) and a group of eyespots at each tentacular tip (about 20). Frontally, in between psedotentacles, there are also clusters of eyespots, it is not clear if as an extension of the border eyespots groups or distinctive cluster



Fig. 5. *Thysanozoon alagoensis* sp. nov. (A) *in vivo*; (B) ventral view; (C) detail of the anterior margin, showing pseudotentacles; (D) detail of cerebral and tentacular eyespots; (E) sagittal section of female reproductive structures; (F) and (G) sagittal sections of male reproductive structures. ce, cerebral eyespots; cg, cement glands; fg, female gonopore; ma, male atrium; mg, male gonopore; pa, penis papilla; pe, pseudotentacular eyespots; ph, pharynx; pt, pseudotentacles; pv, prostatic vesicle; su, sucker; sv, seminal vesicle; ut, uteri; va, vagina.

(Figure 6A). Ventral eyespots groups (Figure 6C) with about 25 eyespots.

*Digestive system*: Pharynx ruffled with seven simple folds (Figure 5B). Reaches 6 mm. Mouth opens at 6 mm from the anterior margin.

*Epidermis and body wall*: Thin epidermis (Figure 5D) and body wall (0.04 mm), even thinner ventrally (0.01). Sucker with 0.7 mm diameter is 2 mm behind the female pore.

*Gonopores*: Two male and one female gonopores present, 1.5 mm apart (Figure 5B). Male pores at 7 mm from the anterior margin, both measure 0.5 mm and the female pore 1 mm.

*Male reproductive system*: Seminal vesicle elongated and huge (Figure 5E). Its extension reaches the front of the penial papillae and the seminal duct comes in a curve to join the penis. Penis papillae (Figure 5D) 0.18 mm. Prostatic vesicle small (0.09 mm) and comma shaped (Figure 5E). Its duct joins the ejaculatory duct and enters the penis papillae

(Figure 6D). Seminal vesicle elongated and spermiducal vesicles greatly developed and reaching the region right above the male gonopore (Figure 5E), maybe dislodging the seminal vesicle. Male atrium simple and 0.2 mm deep (Figure 5E).

*Female reproductive system*: Uteri well developed and full of eggs (0.089 mm diameter). Cement glands also well developed (Figure 5C).

## TAXONOMIC REMARKS

Our specimen has a simple male atrium, different from the folded atrium found in *Thysanozoon brocchii*. Also the *T. alagoensis* has a different colouration pattern, and the dorsal papillae of this species are slender and *Thysanozoon brocchii* have rounder ones (Table 2). Other similar species such as *Thysanozoon californicum* (Hyman, 1953a) also has slender papillae and elongated pharynx with seven simple folds, but the colouration pattern differs from the Brazilian species



Fig. 6. *Thysanozoon alagoensis* sp. nov. (A) detail of pseudotentacular eyespots; (B) cerebral eyespots; (C) ventral eyespots; (D) tentative reconstruction of serial sections. be, pseudotentacle border eyespots; ce, cerebral eyespots; cg, cement glands; ed, ejaculatory duct; fg, female gonopore; ma, male atrium; mg, male gonopore; pa, papilla; pt, eyespots between pseudotentacles; pv, prostatic vesicle; sb, spermiducal bulbs; su, sucker; sv, seminal vesicle; te, pseudotentacles tips eyespots; va, vagina; ve, ventral eyespots.

(Table 2). The most similar species found in the literature is Thysanozoon mirtae, recently described from Argentina (Bulnes et al., 2011). However, there are marked differences between them, T. mirtae has a smaller pharynx (5.5 mm) and with fewer folds. This is especially distinctive as the specimen from Argentina is much larger, almost double the size of ours, and it would be expected that a larger specimen would have a larger pharynx. Also, the pseudotentacles of the Argentinian species are colourless and the ones in T. alagoensis are dark with yellowish tips. The Argentinian species also present rounded black spots in the dorsal surface that are absent in our specimen (Bulnes et al., 2011). Both T. alagoensis and T. mirtae have slender, elongated papillae, which are also found in Thysanozoon skottsbergi (Bock, 1913) and T. distinctum (Stummer-Traunfels, 1895), but the former has darker pigmentation on the papillae and the latter has a golden yellow margin which is markedly different from the reddish brown margin of T. alagoensis sp. nov. and T. mirtae. Both also have apparent spermiducal vesicles, but in T. alagoensis sp. nov. they are greatly developed. Yet another difference between these two South American species is the parenchymatic musculature, developed in T. alagoensis sp. nov. and not in T. mirtae. Other valid species of the genus are compared in Table 2. It is repeatedly stated in the literature that the female system is very uniform throughout the genus, therefore it was not included in the comparative table.

> Suborder ACOTYLEA Lang, 1884 Family CRYPTOCELIDAE Laidlaw, 1902 Genus *Phaenocelis* von Stummer-Traunfels, 1933 *Phaenocelis medvedica* Marcus, 1952 (Figure 7)

## EXAMINED MATERIAL

One specimen ( $19 \times 5$  mm) as sagittal sections of reproductive structures (MNRJ-PLAT 97, 9 slides). Collected 6 January 2008 at Ponta Verde coral reef, Maceió, Alagoas, Brazil.

# GEOGRAPHIC DISTRIBUTION

This species was described from São Paulo State, Brazil (Marcus, 1952), and is also known from the Caribbean coast of Colombia (Quiroga *et al.*, 2004b). Here it is reported for the first time from north-eastern Brazil.

# DIAGNOSIS

Rosaceous colour with brown pigment spots in two rows longitudinal to the body, parallel to the body axis (Figure 7A). Marginal eyespots small and present all over the body margin, disposed in a line (Figure 7D). Cerebral eyespots scarce and disposed right in front of the brain; tentacular eyespots scarce in two small groups (Figure 7C, D). Pharynx 1/3 of the body size and centrally disposed. Muscular layers disposed in the following order: longitudinal, circular, diagonal and longitudinal (Figure 7B).

## REMARKS

Our specimen was immature and wounded at the level of reproductive structures, therefore on sagittal section it is possible only to identify the Lang's vesicle (Figure 6C, D).

Family STYLOCHOPLANIDAE Faubel, 1983 Genus Armatoplana Faubel, 1983 Armatoplana leptalea (Marcus, 1947) (Figure 8)

# EXAMINED MATERIAL

Three specimens ( $16 \times 5$  mm,  $10 \times 3$  mm and  $10 \times 3$  mm), one as sagittal sections of reproductive structures (MNRJ-PLAT 98). Collected 10 January 2008 at Riacho Doce coral reef, Maceió, Alagoas, Brazil. One specimen ( $11 \times$ 4 mm) (MNRJ-PLAT 99, 5 slides). Collected 11 January 2008 at Saco da Pedra sandstone reef, Marechal Deodoro, Alagoas, Brazil.

Thysanozoon species	Colour pattern	Papillae	Pharynx	Male system	Distribution	
T. brocchii (Risso, 1818)	Dark brown to yellowish brown, cream cross sometimes present at the dorsal surface	Slightly slender to rounded	Five simple folds	Seminal vesicle elongated and located diagonally to the body. Folded male atrium. Spermiducal vesicles absent	Naples, Italy, Mediterranean Sea, UK, south and west of Africa, Florida, Caribbean coast of Colombia, Brazil, Japan, New Zealand and Canagy Islands	
T. californicum Hyman, 1953a	Reddish grey with mauve margin, papillae yellowish cream in the median line, reddish grey in the rest of the body and mauve in the margins	Slender and elongated	Seven simple folds	Not sectioned	California	
<i>T. cruciatum</i> Schmmarda, 1859a	Light brown with reddish touch, cream cross can be present at the dorsal surface. Greyish brown papillae	Conic	Three simple folds	Not sectioned	New Zealand, Australia	
T. discoideum Schmmarda, 1859a	Yellowish orange to blood red, with dark reddish brown median line. Papillae brownish black to black. Dark brown tentacles	Slender, almost cylindrical	Five simple folds	Not sectioned	Sri Lanka and Eastern Africa	
T. distinctum Stummer-Traunfels, 1895	Light yellow, whitish median line and golden yellow margin. Blackish and whitish (more numerous) papillae. Tentacles blackish at base and yellow at time	Slender, small and elongated	Five simple folds	Oval seminal vesicle. Spermiducal vesicles absent	Edam and Java, Indonesia	
<i>T. flavotuberculatu</i> m Hyman, 1939d	Greyish with irregular small black flecks. Scarce yellow	Few oval papillae	?	Immature sectioned	Bermudas	
<i>T. hawaiiensis</i> Hyman,	Light ochre with dull green	Cylindrical	Five simple folds	Not sectioned	Hawaii	
<i>T. langi</i> Stummer-Traunfels, 1895	Bright dirty violet, with darker violet mottling over papillae	Short and rounded	Five simple folds	Not sectioned	Ambon, Indonesia	
T. minutum Stummer-Traunfels, 1895	Yellowish grey with live red median line, whitish margin and tentacle margin. Yellowish brown papillae with reddish brownish yellow and bright round spots	Short and conic	Five simple folds	Not sectioned. Vas deferens well developed	Java, Indonesia	
T. mirtae Bulnes et al. 2011	Greenish yellow covered with rounded black spots, reddish brown papillae, tentacles unpigmented	Slender and elongated	Three simple folds	Vas deferens form spermiducal vesicles. Seminal vesicle with well-developed muscular wall and is arranged dorsally to the male prostatic vesicle and stylet. Rounded prostatic vesicle	Argentina	
T. nigropapillosum (Hyman, 1959a)	Black with pale yellowish margin. Black papillae with white tips	Short and rounded	Five simple folds	Not sectioned	Ifaluk, Micronesia	
T. nigrum Girard, 1851	Black with grey patches and fine specks of white. Blackish papillae tinged with greenish yellow	Slender and pointed	?	Oval prostatic vesicle. Wide, long and coiled seminal duct	Florida and Bermudas	
					Continued	

 Table 2. Thysanozoon species characteristics comparison.

Thysanozoon species	Colour pattern	Papillae	Pharynx	Male system	Distribution
T. raphaeli Bolaños et al., 2007	Brown-blackish with yellowish orange papillae. Small white slash-like marks, hardly visible, in the margin. Black tentacles sometimes outlined by white marks	Short and rounded	?	Seminal vesicle elongated and prostatic vesicle rounded. Spermiducal vesicles absent	Belize and Panama
T. skottsbergi Bock, 1923c	Yellowish with touch of greenish grey, blackish median line, lighter papillae tipped with black. Black tentacles	Slender	Five simple folds	Not sectioned	Juan Fernandez Islands
T. alagoensis sp. nov.	Greyish background colour with reddish brown papillae, reddish brown margin and dark almost black tentacles with a hint of yellowish pigmentation on the border. Unpigmented line present at the median line of the body	Slender and elongated	Pharynx ruffled with seven simple folds	Seminal vesicle elongated and huge, reaches the front of penial papillae. Ejaculatory duct comes in a curve to join the penis. Prostatic vesicle small and comma shaped. Male atrium simple. Greatly developed spermiducal vesicles	Brazil

# Table 2. Continued

## GEOGRAPHIC DISTRIBUTION

This species was described from São Paulo State, Brazil (Marcus, 1947), and also known from Bahia State and localities in the Caribbean, such as Antigua, Barbuda, Curaçao and Florida (Marcus & Marcus, 1968). This is the first record of this species from Alagoas State.

## DIAGNOSIS

Light brown colouration, mostly transparent (Figure 8A). Few eyespots posteriorly to tentacular region; tentacular eyespots in a densely disposed group. Cerebral and pre-cerebral eyespots disposed in long parallel groups. Pharynx in the anterior half of the body. Vagina wall ciliated and muscular (Figure 8C, D). Long penis with stylet, seminal vesicle highly muscularized (Figure 8B), Lang's vesicle present, granular vesicle elongated and directed backwards.

## REMARKS

Our specimens slightly differ in the fact that the seminal vesicle is located under the granular vesicle and not behind it as illustrated in Marcus (1947: Figure 32), which can be due to the size of the specimens or fixation contraction. It is the first time that this species is illustrated with coloured photos of internal structures and live specimens.

# Family LATOCESTIDAE Laidlaw, 1902 Genus Latocestus Plehn, 1896 Latocestus brasiliensis Hyman, 1955 (Figure 9)

#### EXAMINED MATERIAL

One specimen ( $7 \times 1.5$  mm) as sagittal sections of reproductive structures (MNRJ-PLAT 101, 6 slides). Collected 7 February 2008 at Pajuçara coral reef, Maceió, Alagoas,



Fig. 7. Phaenocelis medvedica (A) in vivo; (B) sagittal section showing muscular layers; (C) fixed worm; (D) anterior region detail showing eyespots. ce, cerebral eyespots; cl, circular layer; dl, diagonal layer; es, eyespots; ll, longitudinal layer; lv, Lang's vesicle; me, marginal eyespots; te, tentacular eyespots.



Fig. 8. Armatoplana leptalea (A) in vivo; (B) sagittal section of the male reproductive structures; (C) and (D) sagittal sections of the female reproductive structures. cud, common uterine duct; fg, female gonopore; gv, granular vesicle; lv, Lang's vesicle; lvd, Lang's vesicle duct; mg, male gonopore; pe, penis; sv, seminal vesicle; va, vagina.

Brazil. One specimen  $(10 \times 2 \text{ mm})$  as sagittal sections of reproductive structures (MNRJ-PLAT 107, 8 slides). Collected 28 January 2012 in algae at Ponta Verde coral reef, Maceió, Alagoas, Brazil.

# GEOGRAPHIC DISTRIBUTION

In the original description Hyman (1955) did not mention the exact type locality and only writes the unspecific term 'off São Francisco'. However, in a later paper (Schmitt, 1926, p. 89), she states that the collector (Waldo L. Schmitt) travelled in

southern Brazil in 1925, including Santa Catarina State. Off the coast of this region there is an island called São Francisco do Sul, and, most probably, Hyman referred to it in the original description. It is the first time it is reported after almost 60 years after the original description. This is the first record of this species in north-eastern Brazil.

# DIAGNOSIS

Colour greyish beige (Figure 9A). Body form elongated, anterior part pointed, tentacles absent, marginal eyespots around



**Fig. 9.** Latocestus brasiliensis (A) in vivo; (B) detail of anterior region showing eyespots; (C) detail of the ventral posterior region showing mouth and gonopores; (D), (E) and (F) sagittal sections of reproductive structures. asv, accessory seminal vesicle; cud, common uterine duct; fg, female gonopore; gp, gonopores; gv, granular vesicle; lv, Lang's vesicle; lvd, Lang's vesicle duct; ma, male atrium; me, marginal eyespots; mg, male gonopore; mo, mouth; pce, precerebral eyespots; va, vagina.

all body, precerebral eyespots in a fan-like arrangement (Figure 9B). Pharynx at the last third of the body. Mouth opens at posterior part of the body right in front of reproductive structures. Male aparat directed forward (Figure 9D). Male and female gonopores separated (Figure 9D, F). Female atrium and vagina ciliated, vagina turned backwards and connects to Lang's vesicle by a repeatedly expanded and constricted duct (Figure 9F). Granular vesicle and accessory seminal vesicle very muscularized (Figure 9E), elongated and directed forward.

# REMARKS

Our specimens fit the original description and the redefinition of the genus by Faubel (1983). Other species of the genus, like *Latocestos callizona* Marcus, 1947 have different body form, eyespots arrangement and differences in reproductive structures both masculine and feminine. The species *Latocestus atlanticus* Plehn, 1896 also has a different eyespots arrangement. The species *Prolatocestus ocellatus* (Marcus, 1947) is mostly similar, but it has a common genital aperture and therefore is located in another genus. The material here studied fits the original description both internally and in eyespots arrangement.

> Family DISCOCELIDAE Laidlaw, 1902 Genus Adenoplana Stummer-Traunfels, 1933 Adenoplana evelinae Marcus, 1950 (Figure 10)

#### EXAMINED MATERIAL

One specimen (14 × 10 mm) as sagittal sections of reproductive structures (MNRJ-PLAT 102, 8 slides). Collected 18 January 2007 at Pajuçara, Maceió, Alagoas, Brazil. One specimen (12 × 6 mm), collected 9 January 2008 at Brazil, Alagoas, Maceió, Recife do Francês (eaten by a *Enchiridium evelinae*). One specimen (21 × 12 mm) (MNRJ-PLAT 103, 6 slides) collected 26 January 2012 at Saco da Pedra sandstone reef, Marechal Deodoro, Alagoas, Brazil, under rocks.

## GEOGRAPHIC DISTRIBUTION

This species was described from São Paulo State, Brazil (Marcus, 1950) and is endemic from Brazil. This is the first record of this species from north-eastern Brazil and it is the second and northernmost record in the Brazilian coast.

# DIAGNOSIS

Transparent body makes it possible to see gut contents (Figure 10A, B). Marginal eyespots (Figure 10C) surround all body, are arranged in a scattered broad line and become scarcer after the first third of the body. Tentacular eyespots located before the brain level (Figure 10C). Cerebral eyespots groups extend towards the margin and begin before the brain level (Figure 10C). Male and female gonopores separated. Prostatoid organs present in the penis tissue (Figure 10F) and granular vesicle; male atrium spacious, vagina, common uterine duct and Lang's vesicle duct ciliated (Figure 10E).



Fig. 10. Adenoplana evelinae (A) in vivo; (B) in vivo, ventral view; (C) detail of the anterior margin; (D) ventral view; (E) and (F) sagittal sections of reproductive structures. br, brain; ce, cerebral eyespots; fg, female gonopore; lvd, Lang's vesicle duct; me, marginal eyespots; mfg, male and female gonopores; mg, male gonopore; mo, mouth; ph, pharynx; po, prostatoids; spv, spermiductal vesicle; te, tentacular eyespots; va, vagina; vd, vas deferens.

External vagina directed forward and female ducts directed backwards, towards the Lang's vesicle.

### REMARKS

The other Tropical Western Atlantic *Adenoplana* species, *A. obovata* (Schmmarda, 1859a), does not have marginal eyespots surrounding the entire body margin (Hyman, 1955), as *Adenoplana evelinae* has. The South American species *A. platae* also has marginal eyespots all over the body, however it presents a rather elongated cerebral eyespots group and it begins at the brain level (Hyman, 1955).

# DISCUSSION

The north-eastern Brazilian coast is mostly unexplored concerning polyclad biodiversity and most works about Brazilian Polycladida have been made on the south-eastern coast (Marcus, 1949, 1950, 1952). Until recently, only two species were reported from this region: *Stylochoplana walser-gia* and *Armatoplana leptalea*, both from Bahia State, south of Alagoas State (Marcus & Marcus, 1968). Bahia *et al.* (2012), based on material from Rio Grande do Norte State, reported six other species: *Enchiridium evelinae, Phrikoceros mopsus, Pseudobiceros evelinae, Thysanozoon brocchii, Hoploplana divae.* And recently, Queiroz *et al.* (2013) recorded *Pericelis cata* from Bahia State.

The present work adds six species to the north-eastern Brazilian coast: *Pseudoceros bicolor, Pseudoceros rawlinsonae, Thysanozoon alagoensis* sp. nov., *Phaenocelis medvedica, Latocestus brasiliensis* and *Adenoplana evelinae*. One of them is a new species and all 11 species are for the first time reported from Alagoas State. It is also the first time that *Phaenocelis medvedica, Adenoplana evelinae, Latocestus brasiliensis* and *Armatoplana leptalea* are illustrated by full colour photos of live specimens and histological sections. Our results emphasize that the Order Polycladida is not well known on the Brazilian coast and that the biodiversity of reef areas in Alagoas State is underestimated. More studies focused on polyclads are necessary to access their biodiversity throughout the Brazilian coast.

The different areas sampled in this study are subject to different levels of human impact (Correia & Sovierzoski, 2010). The area with highest species richness, Saco da Pedra reef, has a good conservation status as it is within a Biological Reserve (Correia & Sovierzoski, 2009). In contrast areas such as Piscina dos Amores coral reef, Pajuçara and Jatiúca reefs are more impacted reef areas, inside the urban perimeter of the city of Maceió and the city harbour. Apparently, polyclads can be used as bioindicators of environmental quality as they are more abundant and diverse in more pristine areas (personal observation). Since the 1980s the urban occupation in Maceió, and in the Brazilian coast in general, is growing without much planning and care, which threatens the biodiversity of reef areas. It is alarming that we are losing biodiversity before we even know its identity, and studies such as this one are relevant efforts to prevent or call attention to it.

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# Supplementary material and methods

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0025315415000922.

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