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Two new species of *Distaplia* (Tunicata: Ascidiacea) from the SW Atlantic, Argentina

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Abstract

The ascidian fauna from the Southwestern Atlantic (Argentine Sea) have scarcely been studied and have rarely been sampled. The existing scanty ascidian records are from specimens collected by dredging many decades ago. During samplings in the San Matias Gulf (Río Negro, Patagonia), two new *Distaplia* species were found. *Distaplia naufragii* **sp. nov.** was collected in the subtidal zone attached to a shipwreck, while the other species, *Distaplia fortuita* **sp. nov.** was found released by the tides in the sandy intertidal zone. These two new species differ deeply from each other in the size and morphology of their zooids. They represent one third of the known species belonging to the family Holozoidae in the SW Atlantic. These results reinforce the importance of new studies in this extensive but little explored area that is, in addition, susceptible to invasion by non-native species.

Key words: ascidians, Holozoidae, *Distaplia naufragii* **sp. nov.**, *Distaplia fortuita* **sp. nov.,** San Matías Gulf, Patagonia

Resumen

La ascidiofauna del Atlántico sudoccidental (Mar Argentino) ha sido poco estudiada y rara vez muestreada. Los escasos registros de ascidias existentes provienen de ejemplares colectados con redes de fondo muchas décadas atrás. Durante muestreos realizados en el Golfo San Matías (Río Negro, Patagonia) se encontraron dos nuevas especies del género *Distaplia*. Una de ellas, *Distaplia naufragii* **sp. nov.** fue colectada en el sublitoral adherida al casco de un naufragio, mientras que *Distaplia fortuita* **sp. nov.** se encontró en el intermareal arenoso, depositada por las corrientes de marea. Ambas especies poseen profundas diferencias entre si en cuanto al tamaño y la morfología de sus zooides. Estas dos nuevas especies representan un tercio de las especies pertenecientes a la familia Holozoidae conocidas en el Atlántico sudoccidental. Estos resultados refuerzan la necesidad de nuevos estudios en esta área extensa, poco explorada y asimismo, susceptible a invasiones por especies exóticas.

Introduction

Southwestern Atlantic is an extensive region in which there has been very little sampling, resulting in a relatively low number of described ascidian species and a general lack of data. Significant taxonomic investigations have not been pursued since Van Name (1945). However, in the last ten years, many new species of ascidians have been described from the Brazilian Atlantic coast (Rocha 2002; Dias & Rodrigues 2004; Rocha & Costa 2005; Lotufo & Dias 2007; Rocha & Bonnet 2009; Bonnet & Rocha 2011; Kremer *et al.* 2011). Along the long Pacific Chilean coast, Sanamyan & Schories (2003) and Sanamyan *et al.* (2010) described four new aplousobranch species. In contrast, information on the SW Atlantic (Argentine Sea) is fragmented and the scarce published taxonomic studies

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are out of date (Amor 1964; Monniot 1970; Bastida 1971; Pisanó *et al.* 1971; Diehl 1977). In the last 40 years, only two new ascidian species have been reported for this extensive area: *Cnemidocarpa calypso* (Monniot 1970) and the deep-water ascidian *Culeolus likae* (Sanamyan & Sanamyan 2002), both of which were collected along the coastline of Buenos Aires Province.

To date, only four Holozoidae species has been reported along the Argentine Sea: *Sycozoa gaimardi*, *S. sigillinoides*, *S. umbellata* and *Distaplia cylindrica*, that latter species from the southern Patagonian shelf. The two new species described in the current paper are the most northern records of the genus *Distaplia* from the Argentine Sea.

Many efforts currently focus on compiling all information about marine exotic and cryptogenic species of the SW Atlantic, mainly in ports and harbors (Orensanz *et al.* 2002, Schwindt 2007, Boltovskoy *et al.* 2011). However, there is still a lack of information on marine natural areas, presumed to be "pristine", such as the San Matías Gulf. This study describes two new ascidian species of the genus *Distaplia* from shallow waters in a little explored gulf of Argentina.

Material and methods

The San Matias Gulf, northern Patagonia (Figure 1), is a semi-enclosed basin situated between 40° 47´ and 42° 13´ S on the Atlantic coast of South America and it is the second largest gulf in Argentina, with a maximum depth of 180 m. It has a semi-diurnal tidal regime and the average tidal amplitude is 7.62 m (maximum 9.2 m). The water temperature ranges, on average, from 10ºC in August (winter) to 18.2ºC in January (summer) at 20 m depth. The bottom sediment is dominated by sand near the coastline and gradually mixed with shell fragments, gravel and mud at depth (Morsan *et al.* 2010). The salinity is usually not lower than 34 ups (Guerrero & Piola 1997).

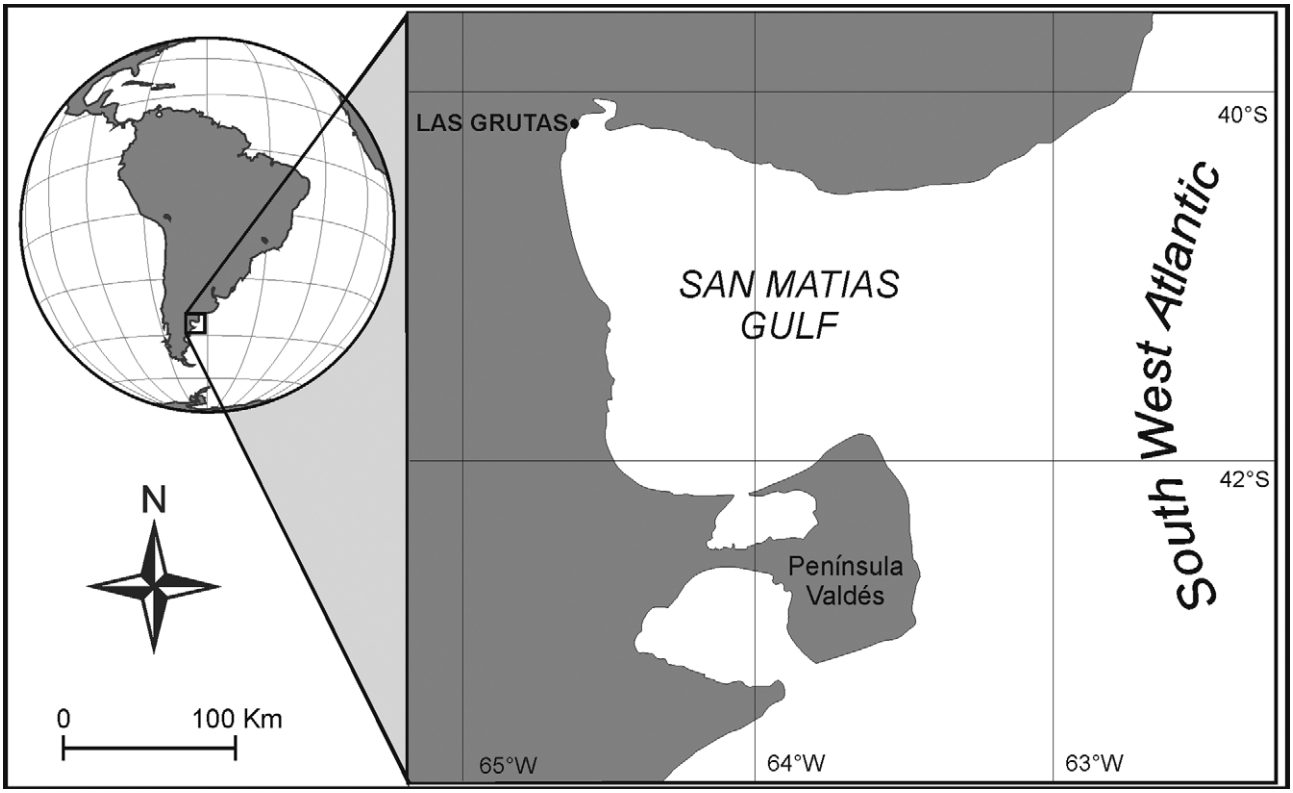

FIGURE 1. Map of the San Matias Gulf (SW Atlantic), showing Las Grutas town, the sampling site where the samples were collected.

In November 2005, two specimens of colonial ascidians released by tides in an area of sand beach off Las Grutas town were found, photographed *in vivo* to document colour, form and structure and fixed in 4% formalin. This material was quickly identified as *Distaplia* sp. (unpublished data) and stored in the collections of the Museo

de Zoología, Universidad Nacional de Córdoba, where it was overlooked until recently. In October 2011, new ascidians were collected by SCUBA diving in the shipwreck *"Don Félix"* (sunken in November 2007), near the coast of Las Grutas town at maximal depth of 25 m. The specimens were photographed, anesthetized with menthol crystals for two hours and fixed in 4% formalin diluted in seawater. The holotypes and paratypes were deposited in the collection of the Museo de Zoología, Universidad Nacional de Córdoba.

Descriptions

***Distaplia naufragii* sp. nov.**
(Figures 2A, B, C; 3A, B)

Material examined. Las Grutas (Río Negro), Argentina. Holotype: MZUC T00005, one colony **(**Fig. 2B**)** sampled on 30/X/2011 at 20 m depth from the shipwreck *"Don Félix"*(40° 51.784 S; 65° 03.802 W). Paratypes: MZUC T00006, six colonies: 40° 51.784 S; 65° 03.802 W; 30/X/2011, 20 m.

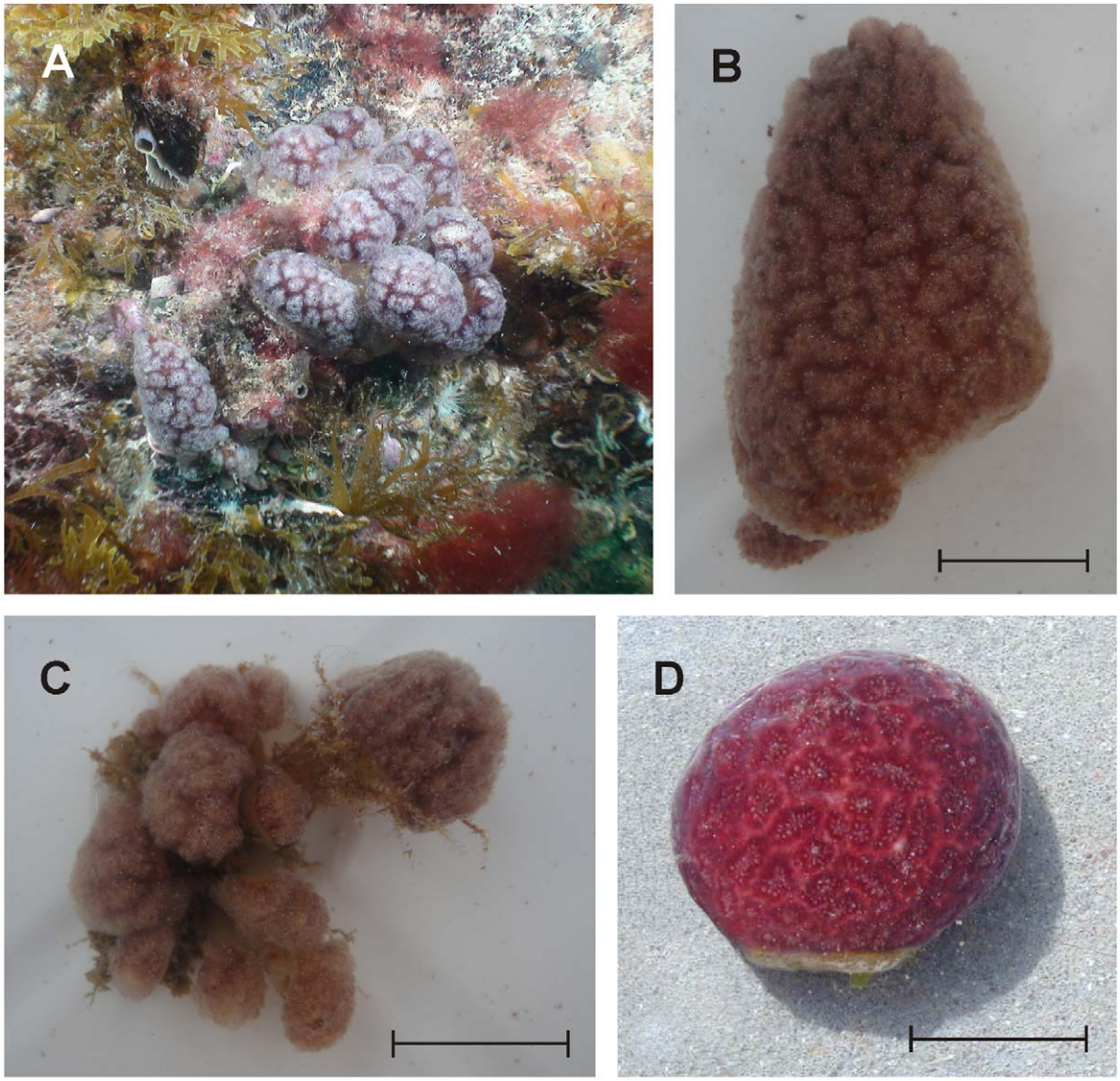

FIGURE 2. A, *Distaplia naufragii* **sp. nov.** at the shipwreck "Don Félix" (photo by R. Sahade). **B,** Large colony. **C,** Small colonies. **D**, Colony of *Distaplia fortuita* **sp. nov.** Scales: 2 cm.

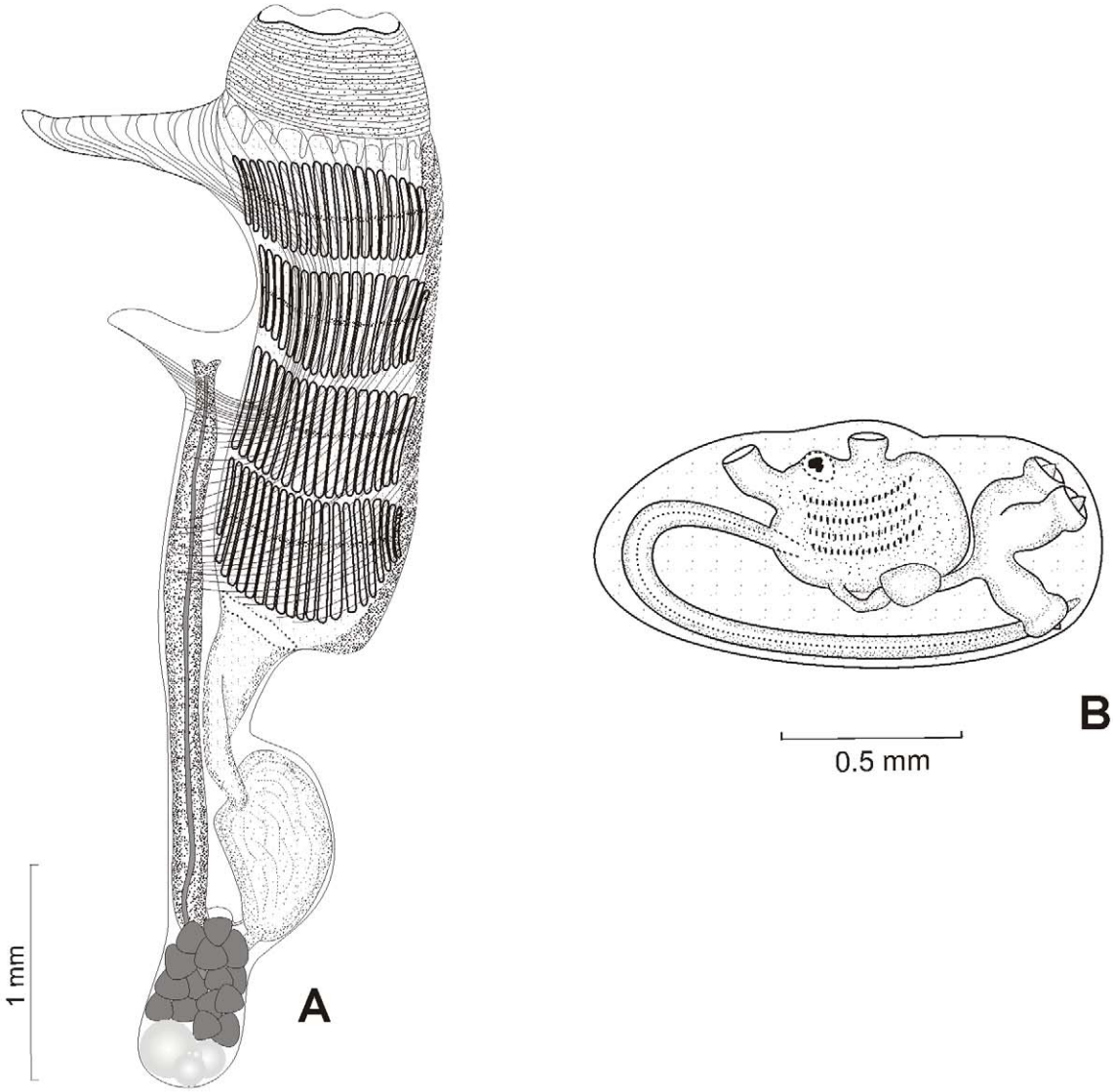

FIGURE 3. *Distaplia naufragii* **sp. nov. A**, zooid; **B**, larva.

Etymology. From *naufragii*, the genitive of the latin noun naufragium (= shipwreck).

Description. Small colonies consist of several mushroom-shaped lobes joined by short yellowish peduncles. Large colonies are composed of a cushion shaped lobe, which may reach 6.5 cm long by 4.5 cm high, attached to the substratum by a pale yellow small area. The basal portions of the colonies are burgundy colour while the upper layer of the tunic is semi-transparent allowing the observation of white pigment granules on the oral siphons of each zooid. The test is soft and free of epibionts. The zooids are perpendicular to the colony surface and organized in circular or elongated systems containing up to 25 zooids which converge into common cloacal aperture. The thoraces of the zooids are prominent and located in the uppermost layer of the tunic. Living colonies are easily recognizable underwater by the contrast between of burgundy basal area of the tunic and the white prominent zooids (Fig. 2A).

The zooids are large, reaching 5 mm or more in length when well expanded. The oral siphon, surrounded by abundant circular muscles, is smooth-edged or forming six sinuate lobes. The atrial aperture is wide, exposing much of the pharynx, and has a dorsal languet with plain edge.

The thorax bears about 40 thin oblique muscles (many of these are branched) on each side, most of which are concentrated around the opening of the atrial aperture. There are 15 to 21 thread-like oral tentacles of two sizes, arranged regularly in a single circle. The dorsal tubercle is small and circular, with an aperture of similar form. The four rows of long stigmata are crossed by well-developed parastigmatic vessels. There are about 18 to 21 stigmata in each half row. Three dorsal languets are displaced to the left side of the thoracic midline.

The slightly curved oesophagus connects to the stomach laterally. The oval stomach has conspicuous internal longitudinal ridges. Ridges are often curved, somewhat irregular or interrupted, giving a stretch-marked external appearance to the stomach wall. A pyloric gland extends between the stomach and the intestine. The intestine has a constriction situated at a short distance beyond the stomach. The bi-lobed anus opens at the level of the third row of stigmata.

The zooids are hermaphroditic. Gonads occupy the right side of the gut loop and consist of 8 to 15 male follicles and an ovary containing 7 to 9 oocytes of different sizes (one or two of them are large), situated mainly below the testis. The fine vas deferens ends at the same level as the anus.

The brood pouch projecting from the thorax, at the level of the fourth stigmata row, contains up to 6 developing larvae. The oval larval trunk may be up to 1.4 mm long. The larvae possess three adhesive papillae triangularly arranged; ampullae and epidermal vesicles were not observed. The tail is on the same level of the adhesive papillae and involves half or less of the larva. Each sensory vesicle contains both an otolith and an ocellus.

***Distaplia fortuita* sp. nov.**
(Figures 2D; 4A, B)

Material examined. Las Grutas (Río Negro), Argentina. Holotype: MZUC T00003, one colony **(**Fig. 2D**)**; washed ashore on sand beach off Las Grutas; 18/XI/2005. Paratypes from type locality: MZUC T00004, one colony; 18/XI/2005.

Etymology. From *fortuita* latin adjective meaning casual, by chance.

Description. Both colonies are almost spherical in shape. The holotype, the larger of the two available colonies, is up to 4 cm in length, with a small flat basal area. The cherry red colour of fresh colonies quickly faded when fixed in formalin. The tunic is soft and smooth without any incrustations. The pale zooids that may be seen through the tunic are organized in circular or elongated systems of up to 15 individuals.

The zooids reach a maximum of 2.8 mm but most are approximately 2 mm long. The elongated oral siphon is narrow but gets wider closer to the aperture, without lobes (smooth-edged) and with very few circular muscle bands. The atrial siphon exhibits two possible shapes: the most frequent is a tube-shaped siphon that projects upwards, with a slightly elongated upper margin. The other shape is a short siphon with smooth edge and situated close together with the oral siphon at the upper part of the thorax.

Each side of the thorax bears about 25 thin oblique muscles that extend from the ventral region to the dorsal line. There are about five to seven very small oral tentacles arranged in a circle. The pharynx has four rows of 15-17 stigmata per side and are crossed by parastigmatic vessels.

The aperture of the oesophagus is broad and the oesophagus enters the stomach laterally. The obliquely oriented stomach is oval and has an almost smooth wall, with some longitudinal fine stretch marks on its internal wall (not always noticeable). There is a constriction between the duodenum and the mid-intestine, but it is not possible to see unless gonads are removed. A pyloric ampulla, clearly visible above the gonads, extends between the stomach and the intestine. The bi-lobed anus opens at the level of the third row of stigmata.

All zooids of the two colonies are males. Testis consist of a cluster of 13 to 22 circular or oval-shaped follicles that lie beside the intestinal loop. The straight sperm duct originates from the centre of the testis and it opens near the anus. Some zooids possess a very small curved brood pouch (it was never developed).

The mature larvae lie in the upper layer of the colony; they are of a rounded shape and may be up to 1.4 mm long but are generally shorter (about 1.2 mm). The larval tail wraps in a half circle around the trunk, ending at the level of the adhesive papillae. Larvae have three adhesive organs arranged in triangle and their sensory vesicles each contain an otolith and an ocellus.

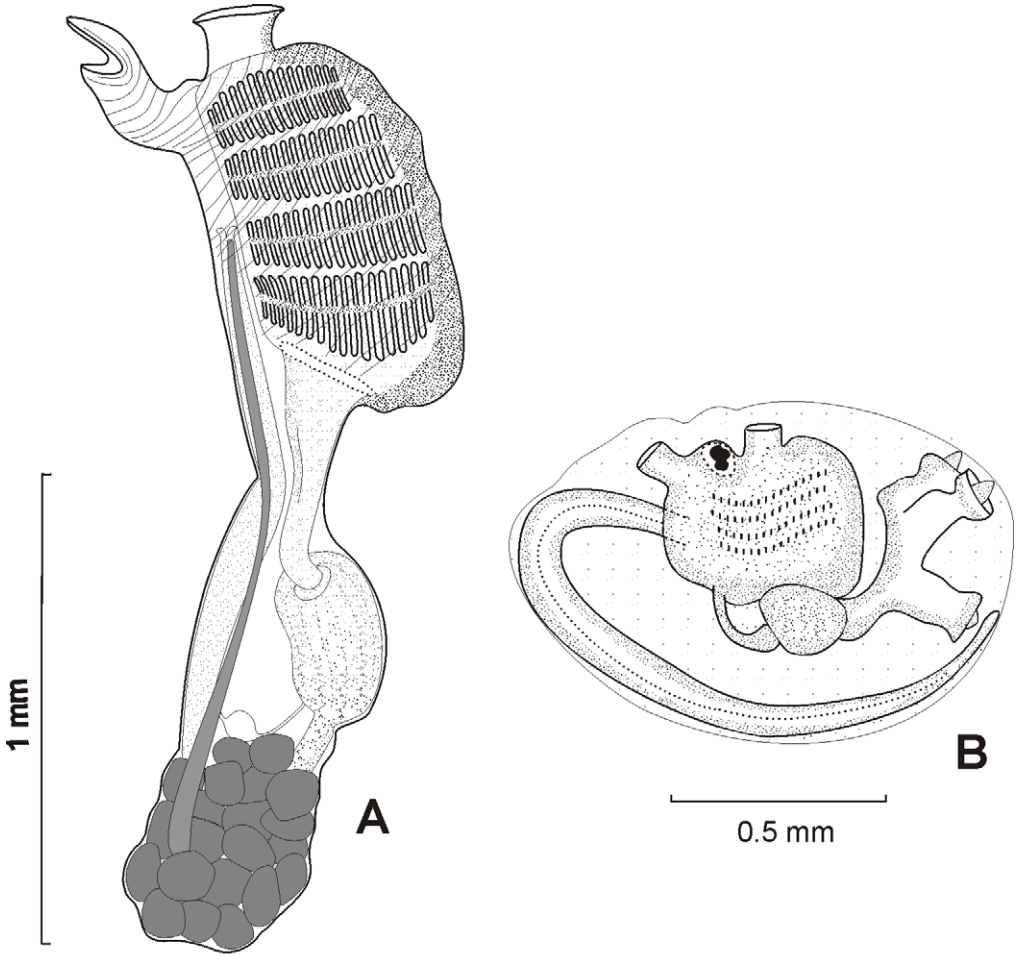

FIGURE 4. *Distaplia fortuita* **sp. nov. A**, zooid; **B**, larva.

Discussion

Both *Distaplia fortuita* and *D. naufragii* have similar cushion-shapes, although the smaller colonies of *D. naufragii* can also be mushroom-shaped. Despite the external similarity, the appearance of both species differs in colouration, size of the colonies and number of zooids surrounding the common cloacal opening. The zooids of *Distaplia fortuita* and *D. naufragii* are easily distinguished. The measurement of zooids revealed that those of *D. fortuita* are two or three times smaller than *D. naufragii.* In the former species, there are very few circular muscle bands around the never-lobed oral siphon, while in the latter the zooids show a strong circular musculature in the usually six-lobed oral siphon. The atrial siphon in *D. fortuita* is smaller and more closed than *D. naufragii.* Furthermore, *Distaplia fortuita* has fewer oral tentacles, thoracic muscles and stigmata than *D. naufragii***.** Finally, *D. fortuita* contains more testis follicles than *D. naufragii*. Both species have internal longitudinal ridges, but these are more evident or marked in *D. naufragii***.** While zooids of *D. fortuita* do not possess fully developed brood pouches, zooids of *D. naufragii* possess elongated brood pouches containing numerous developing larvae. The two colonies of *D. fortuita* were exclusively males. However, the presence of an undeveloped brood pouch suggest that there is a female phase at some time, probably after regression of the male phase. Although the larvae of both species are of similar size, they differ somewhat in the shape of their trunk, in that the larval thorax of *D. naufragii* is more elongated than that of *Distaplia fortuita***.**

A total of ten species belonging to the genus *Distaplia* have been reported in the Atlantic shallow waters (Rocha *et al*. 2012). *Distaplia bermudensis* Van Name, 1902 commonly forms an incrusting sheet with a thickness that varies between 2–7 mm. *D. bermudensis* has a smooth stomach, few testis follicles and oocytes, all located inside the intestinal loop, and a smaller larval trunk (0.8–1 mm). The species, common and widely distributed in the Caribbean (Van Name 1945), was also found in South eastern and Southern Brazil (Rocha & Costa 2005) and was also considered a relatively recent immigrant in the Mediterranean Sea (Mastrototaro & Brunetti 2006).

Distaplia stylifera (Kowalewsky 1874) is distinguished from the other West Atlantic *Distaplia* species by its characteristic pear-shaped or oval sac-like postabdomen (containing the reproductive organs) which connects to the abdomen by a very narrow and elongated neck. The species, widespread in the Caribbean, was also found on artificial substrata in São Paulo State, Brazil (Rocha *et al.* 2011).

Other two *Distaplia* species were recorded in the Caribbean: *Distaplia corolla* Monniot, 1974 and *Distaplia crassa* Monniot, 1983. Both species have a smooth walled stomach and neither have internal longitudinal ridges or fine marks, a clear diagnostic characteristic of the two new species presented in this paper. Furthermore, colonies of *D. corolla* are bright yellow or orange in colour and form cylindrical isolated lobes with zooids arranged in a ring, like a rosette. *D. crassa* have intestines without any morphological differentiation along their length and their ovaries are situated in the centre of the rosette of male follicles; thus, the gonads are entirely contained in the intestinal loop (Monniot 1983), differing from *Distaplia fortuita* and *D. naufragii***.**

Two *Distaplia* species have been reported from the North Atlantic: *Distaplia clavata* (Sars, 1851) and *Distaplia rosea* Della Valle, 1881. *D. clavata* consist of clavate or narrowly capitate colonies, sometimes with more than one head arising from a common expanded base, or occurring as flattened incrusting forms (Van Name 1945). According to Van Name (1945), Huntsman (1912) described living colonies as light yellow. The zooids of *D. clavata* share some characters in common with *D. naufragii*, including the size of zooids, the number of stigmata and the presence of ridges on the stomach wall (that are not very conspicuous, in contrast to *D. naufragii*). However, the figure drawn by Sars (1851) depicts a smaller cloacal siphon opening, fewer muscle fibres in the oral siphon and the thorax, a very robust vas deferens and indicates that all gonads lie over the intestinal loop, between the stomach and the mid-intestine (for comparison see Fig. 69 in Van Name, 1945). Inconspicuous colonies of *D. rosea* may be recognized not only by their characteristic rose-pink colour, but also by the peculiar soft sticky consistency of their tunic (Berrill 1950) *D. rosea* has fewer stigmata per row than our colonies. These two species recorded from the North Atlantic have external morphological characteristics completely different from the current samples.

Within the South East Atlantic, the slightly stalked colonies of *Distaplia capensis* Michaelsen, 1934 have zooids with only 7–9 stigmata per half row in the branchial sac. In *Distaplia skoogi* Michaelsen, 1924, like *D. stylifera*, the gonads are in a pedunculate pouch behind the abdomen, with the testis vesicles clustered around a central ovary (Monniot *et al*. 2001). The presence of two round ampullae at the base of each papilla is also a diagnostic feature in the small larvae of this species and is absent in the larvae of our species.

Only two *Distaplia* species have been recorded in the SW Atlantic to date. *Distaplia cylindrica* (Lesson 1830) is a circumpolar Antarctic species common not only in the Magellan area and the Patagonian shelf (Millar 1960; Sanamyan & Schories 2003), but also present up to 49º latitude south in the Pacific Ocean (Tatián & Lagger 2009). The externally observable cylindrical rods and the soft consistency of this species make it distinctive such that its identification could not be confused. The other *Distaplia* species recorded in the tip of South America (Strait of Magellan) and South Georgia Island is *Distaplia colligans* Sluiter, 1932. Its low and flattened encrusted colonies form thin sheets and its tunic is glassy gray or yellow (Sanamyan & Schories 2003).

Distaplia arnbackae Sanamyan *et al.* 2010 is a new species from the Pacific Ocean (Central Chile). Colonies of *D. arnbackae* are red in colour, but different from the present colonies: younger colonies consist of several small button shaped cormidiums containing mostly a single zooid system, while larger colonies are always encrusting sheets or flat cushions that rarely reach more than 5 mm thickness.

There are no other *Distaplia* species similar to the material here described. The morphology of the colonies and of the zooids justifies the proposal that they represent two new species.

As was stated, the SW Atlantic (Argentine Sea) is among the poorly known marine regions in terms of biodiversity (Orensanz *et al.* 2002). In the last ten years, several reports on the emergence of marine invasive, exotic and cryptogenic species in this vast region were published (Casas *et al.* 2004; Hidalgo *et al.* 2005; Penchaszadeh *et al*. 2005; Spivak *et al.* 2006; Calvo-Marcilese & Langer 2010; Tatián *et al*. 2010; Boltovskoy *et*

al. 2011; Fiori *et al*. 2012). Today, non-native ascidians are increasingly being documented throughout world (Shenkar & Swalla 2011). However, even knowing that ascidians have been recently involved in many cases of bioinvasion (Lambert 2007, 2009), little is known about the diversity and distributions of ascidians in the Argentine Sea.

The current results emphasize the need to increase the sampling effort for this group, and reinforce the importance of new studies in this area, considered pristine, but actually susceptible to biological invasions. Ascidian richness within the San Matias Gulf, examined by sampling for the first time using SCUBA diving, will be presented in a future publication.

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