

## New records of aplousobranch ascidians from Central Chile

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### Abstract

Seven shallow-water colonial ascidians of the order Aplousobranchia, including three new species, are identified in the material collected by SCUBA divers off the coast of Central Chile. Three species including *Aplidium falclandicum*, *A. fuegiense* and *Didemnum studeri* were known previously from Subantarctic and South Chile. The range of one species, *Distaplia arnbackae* n. sp., extends to south to Guaitecas Islands, and the rest three species (*Aplidium peruvianum*, *Aplidiopsis chilensis* n. sp. and *Eudistoma clivosum* n. sp.) are not known from more southern localities and may represent a northern component.

**Key words:** *Aplidiopsis*, *Eudistoma*, *Distaplia*, SE Pacific

### Introduction

Chilean ascidians are mainly known from the work of Ärnäck (1929), Van Name (1945, 1954), Monniot & Andrade (1983), Millar (1988), Clarke & Castilla (2000), Sanamyan & Schories (2003, 2004, 2007) and Lager *et al.* (2009). Species lists and geographical distribution information (Van Name, 1954; Lancellotti & Vasquez, 2000; Lee *et al.*, 2008; Lager *et al.*, 2009) indicate a higher species presence in Southern Chile compared to Central or Northern Chile. The biogeographical distribution of the Southern Chilean fauna is mainly influenced by the Antarctic circumpolar current and the South Pacific whereas the North Chilean fauna is more influenced by Peruvian coastal countercurrent (Lancellotti & Vasquez, 1999). This fact was used to explain differences in species numbers between Southern and Central Chile. However a closer look to the published data indicate that observed differences (Van Name, 1954; Lancellotti & Vasquez, 2000; Lee *et al.*, 2008) are mainly based on sampling effort during the last decades and do not depend on geographical latitude. Van Name (1954) as well as Lancellotti & Vasquez (2000) did not report any aplousobranch ascidian from Central Chile, whereas the species numbers reported by them were too high for Southern Chilean aplousobranch ascidians because several synonyms of the same species were reported as independent valid species. This error was repeated by Lee *et al.* (2008). Only Millar, (1988) mentioned the presence of the aplousobranch species *Aplidium* sp. between 33°S and 34°S.

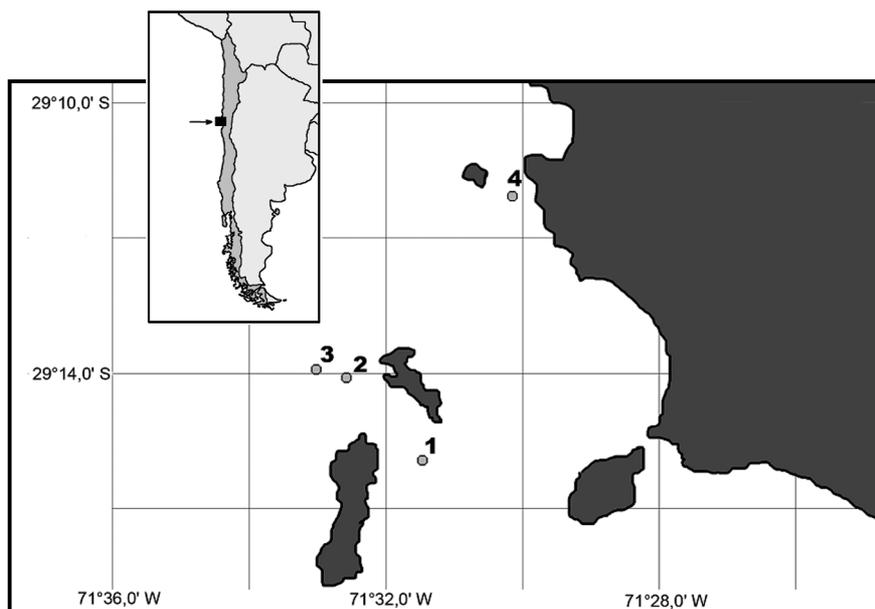
Actually a total number of 19 species must be considered as valid species of the suborder Aplousobranchia in Chile including *Aplidium irregulare* Herdmann, 1886, and *A. recumbens* Herdmann, 1886, whose status is problematic, as well as the status of most listed Didemnidae. Here we describe three new species, one of them was reported before as *Distaplia occidentalis* (Bancroft, 1899) and two species unknown from Chilean waters before. However, we were not able to identify didemnid ascidians to species level apart from *Didemnum studeri* Hartmeyer, 1911 on the basis of existing descriptions. At least seven aplousobranch species are present in Central Chile, demonstrating a significantly higher presence of ascidians in this region than reported before.

## Material and methods

**The study area.** Punta de Choros is situated 115 km northwest of Coquimbo (IV region). Air and water temperature are moderate and similar to each other. Summer average for Coquimbo is 18.9°C and winter average 11.2°C. Mean annual rainfall is about 127mm (Di Castri & Hajek, 1976). Mean water temperature varies between 13° and 18° C during the year (Montecino *et al.*, 2006). However large diurnal temperature fluctuations are in the nearshore water column are known from several sites along the coast of central Chile (Kaplan *et al.*, 2003).

In May 2008 we collected for the first time several specimen of *Aplidium peruvianum* near Las Tacas, 25km south of Coquimbo (30°05.61' S, 71°23.11' W). In November 2009 we performed a more detailed bioinventory of ascidians at 4 stations around the peninsula Punta de Choros. The sampling sites were: **1:** bajo tiburón, 29°15.31'S, 71°31.59'W, **2:** la gruta, 29°14.08'S, 71°32.59'W, **3:** Rhodymenia, 29°13.95'S, 71°33.02'W, **4:** wreck Lynch, 29°11.38'S, 71°30.15'W (Figure 1). Additional samples were collected between 2007 and 2009 in Southern Chile near Caleta la Arena (41°40.37'S, 72°39.48'W) and near the small village Yaldad, Chiloe (43°06.40'S, 73° 43.34'W).

**Sampling.** The samples were collected by SCUBA divers in individual flasks down to 40 m depth. Nearly all collected material was photographed *in vivo* to document colony form and substratum type. Time synchronization between the moment in which the images were taken and information received from the dive computer profile, allowed later correct allocation of water depth in which each sample was taken. Samples were fixed in 4 % formalin-seawater for taxonomic analysis. The specimens are deposited in the collection of Kamchatka Branch of the Pacific Institute of Geography (KBPIG).



**FIGURE 1.** Sampling sites near Punta de Choros (4. region, Chile). 1: Bajo Tiburon, 2: La gruta, 3: Rhodymenia, 4: wreck Lynch.

## Descriptions

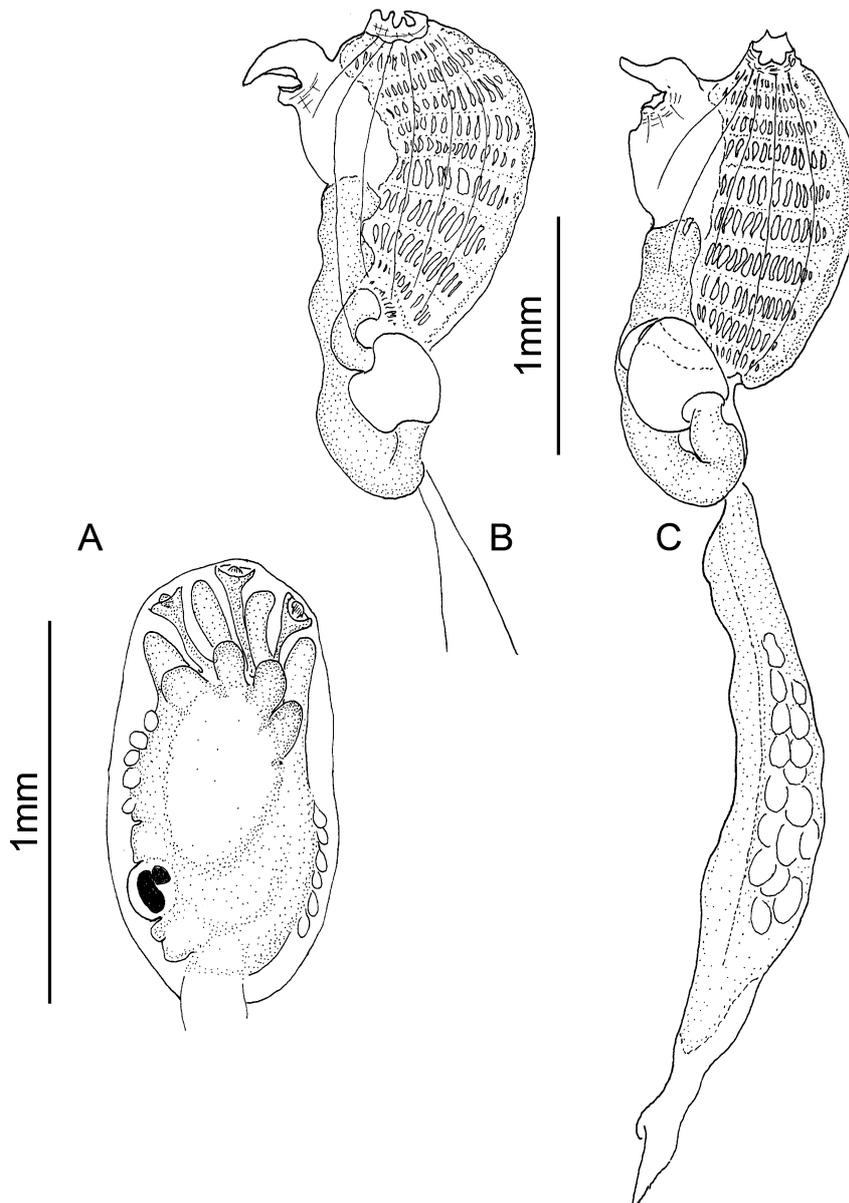
### *Aplidium falklandicum* Millar, 1960

(Figures 2A, 5A,B)

*Aplidium falklandicum* Millar, 1960: 33; 1970: 100; Monniot & Monniot, 1983: 15 (synonymy).

**Material examined:** Chile, 4. region: Punta de Choros, Rhodymenia, 10m, two colonies; La gruta, 25m, one colony.

**Remarks.** Examined specimens have solid oval colonies with a rather small area of attachment. Zooids are in small circular or oval systems (Figure 5A). The test is soft, gelatinous, transparent and colourless; the living specimens are whitish or colourless. Thread-like zooids are up to 10 mm long. Stomach wall has five longitudinal folds. According to Millar (1960) the number of rows of stigmata may vary from 12 to 23, a much wider range than occur in most *Aplidium* species. In the present specimens zooids have 12–14 rows of stigmata. Monniot & Monniot (1983) reported that the number of rows of stigmata is variable but rarely attain 16. Unusual white "thoracic triangles" described for this species by Millar (1960: 35) were not found in the present material. Other features of zooid agree well with the original description, and larva (Figure 2A) is identical to those illustrated by Millar (1960).



**FIGURE 2.** A, *Aplidium falklandicum*, larva. B, C, *Aplidiopsis chilensis* n. sp., zooids.

***Aplidium fuegiense* Cunningham, 1871**

(Figures 5C,D)

*Aplidium fuegiense* Cunningham, 1871: 66. Millar, 1960: 28; 1970: 100. Monniot, Monniot, 1983: 16. Sanamyan, Schories, 2003: 90.

Not *Aplidium fuegiense*: Van Name, 1954: 6.

**Material examined:** Chile, 10. region: Caleta Yervas Buenas, 18m, one colony; 4. region: Punta de Choros, Bajo tiburon, 15m, one colony; La gruta, 30m, one colony.

**Remarks.** Zooids of the present specimens agree with previous descriptions and colonies have small crowded oval bodies in the superficial layer of the tunic, a very characteristic feature allowing easy identification. In previous accounts, based on preserved material, the shape of colonies was described as extremely variable, and generally no obvious system of zooids could be recognized (Millar, 1960). Indeed, all examined preserved colonies are thick irregular masses without any recognizable systems. However, underwater photographs of the same specimens show that the shape of living colonies is rather characteristic: the surface is raised into several to numerous large conical lobes with a single cloacal siphon on the top of each lobe. Zooids open on the sides of these lobes and arranged along rather wide anastomosing cloacal canals converging to the top of the lobe (Figures 5C,D).

The material described by Van Name (1954) from Chile is certainly wrongly identified and consists of several species: reported number of stomach folds (12–24) is too high for *A. fuegiense* and range is too wide for one species.

#### ***Aplidium peruvianum* Sanamyan & Schories, 2004**

(Figure 5E,F)

*Aplidium peruvianum* Sanamyan, Schories, 2004: 193.

**Material examined:** Chile, 4. region: Punta de Choros, Rhodymenia, 10m, two colonies; La gruta, 10m, one colony; Bajo tiburon, 17m, one colony; Las Tacas (Coquimbo), several colonies.

**Remarks.** Originally described from the coasts of Peru the species is now recorded from Central Chile. It is characterized by hard massive colonies with distinctive circular, oval, or somewhat irregular systems of zooids separated by ridges of the test. The colonies and zooids resemble Atlantic species *A. stellatum* (Verrill, 1871), see Sanamyan & Schories (2004) and Sanamyan & Gleason (2009) for details.

#### ***Aplidiopsis chilensis* n. sp.**

(Figures 2B,C, 6A,B)

**Material examined:** Chile, 4. region: Punta de Choros, wreck Lynch, 20m, 20 November 2009, one colony. Holotype KBPIG 1397/1.

**Description.** Preserved material is represented by several fragments, largest of which is 2.5 cm wide and about 1.5 cm high, separated from a larger colony. Colony consists of numerous small crowded heads attached to a common base by short stalks rapidly narrowing basally. In the preserved specimen heads are almost flat topped; on underwater photograph of the living specimen they are more inflated and hemispherical (Figure 6B). Each head has one or a few circular systems, each with one large central cloacal opening on a short siphon visible clearly only in live. Sparse sand grains are attached to surface of the test but completely absent inside, otherwise the test is clear and transparent, especially in the upper expanded part of heads. Living specimen appears to be colourless, formalin preserved material is dull gray-brownish due to colour of zooids. Zooids are up to 4.5 mm long with wide thorax and narrower abdomen together about 2 mm. They are almost parallel to each other and open on the top of the head. Almost sessile branchial aperture has usual six small pointed lobes. The atrial aperture sometimes drawn into a wide short siphon with the upper border extending into a wide, rather short, simple atrial lip. Fine muscles encircle both apertures to form weak sphincters, and about 6 thin longitudinal muscles run from the branchial aperture to the bottom of the thorax where they bunch together into a weak band continuing to the end of abdomen. In general, thoracic muscles appear to be rather weak and in many zooids (preserved without anesthesia) thoraces are not contracted. The branchial sac has usually 10, rarely 11, rows of 11 to 13 stigmata.

The abdomen is two or three times shorter than the thorax. The gut loop is not twisted, but smooth walled asymmetrical stomach obliquely oriented and a short oesophagus curves at a right angle to enter to it (Figure 2B). Post pyloric part of the gut loop is short and the intestine has no clear division into regions. The rectum opens with a slit-like anus on the level of the middle of the thorax. No gastric reservoir was detected.

The postabdomen is not particularly long, being only slightly longer than the thorax and abdomen together, attached by a narrow stalk to the left side of the abdomen. Typically it is spindle-shaped, being wider in its middle part, and in most zooids is filled by parenchymatous tissue. Male follicles are in the middle of postabdomen, arranged in more than two rows. No ovary seen and no larva present in the examined material.

**Remarks.** The genus *Aplidiopsis* currently comprises 17 species no one of which has been recorded from the Pacific coasts of South America. The present species is characterized by a combination of characters including the shape of the colony, small number of rows of stigmata, position of the atrial languet on the upper rim of the atrial opening. *Aplidiopsis discoveryi* Millar, 1960 from New Zealand has 14 rows of stigmata and colony of irregular shape. Colony of *A. amoyense* Tokioka, 1967 from China is composed of similar lobes and has small number of rows of stigmata (12), but the systems are stellate rather than circular, and unlike the present species, the atrial languet is inserted far anterior to atrial aperture. *Aplidiopsis tokaraensis* Tokioka, 1954 also has 12 rows of stigmata but the number of stigmata in each row is much larger, 18–20. Other Pacific species also differ significantly from the present one: Australian *A. mammillata* Kott, 1992 and *A. sabulosa* Kott, 1992 have very long thoraxes (18 and 20 rows of stigmata) and *A. confluata* Kott, 1992 has different colony and zooids. Indonesian *A. ocellatus* Monniot & Monniot, 1996 has 17 rows of stigmata and different colony. New Caledonian *A. gelidus* has 11 rows of stigmata but different shape of postabdomen and has a different colony. Common North Pacific *A. pannosum* has very different massive colonies.

*Aplidiopsis chilensis* n. sp. so far is known from the single colony and most probably is a rare species in the studied region, we never saw similar colonies on hundreds of underwater photographs taken on diver accessible depths along the coasts of central and southern Chile.

### ***Eudistoma clivosum* n. sp.**

(Figures 3, 6C–E)

**Material examined:** Chile, 4. region, Punta de Choros: Bajo tiburon, 17m, several large fragments of one colony (Holotype KBPIG 1398/1); wreck Lynch, 23m, one colony; 25m, one colony. All specimens collected at November 2009.

**Description.** Colony is a wide thick mass attached by whole lower side. The edges of the colony are rounded and the surface produced into low lobes. The largest preserved fragment is about 8 cm wide and 2 cm thick. The test is tough and opaque, of spongy consistency (in preservative), without attached or embedded sand grains or other foreign matter. In live zooids are not visible through the test; in preserved colonies the test become slightly clearer (but still opaque) and contracted zooids may appear as white blotches visible through the superficial layer of the tunic. The colour in live varies from lilac to dirty purple, in formaline all specimens are dark purplish to dark brown. Zooids are not pigmented and are opaque whitish. They are arranged into very indistinct rudimentary systems consisting of two, three or four zooids with atrial apertures opening close to each other. Even on living specimens these systems are not always apparent, and in some parts of a colony zooids may appear as randomly distributed. No traces of any sort of depressions or shallow cloacal cavities are associated with systems.

Zooids are strongly contracted, no more than 4.5 mm long, and withdrawn to the base of the colony. Both siphons are six lobed, terminal, close to each other, of equal length or, often, the atrial siphon slightly displaced dorsally and longer than the branchial. The branchial sac has three rows of stigmata with 9 or 10 stigmata in the second and third rows (counted in several less contracted zooids); the first row has a few more stigmata but these cannot be counted precisely. The first row of stigmata slightly bents dorsally. Thin well spaced longitudinal muscles originate at the base of the branchial siphon, run on the sides of the thorax and continue as a wide band to the end of the abdomen. The wall of the abdomen is thick and opaque. Digestive

tract is typical for the genus with long oesophagus and smooth walled stomach in the end of abdomen. Post pyloric subdivision of the intestine into regions is obscured by contraction of zooids. Neither gastric vesicle nor pyloric gland were detected. Testis follicles are large and numerous, spread beside the gut loop in posterior end of the abdomen. The ovary is at the level of posterior end of the stomach and may contain a few rather large (0.5 mm) eggs. Up to two large eggs or developing embryos are in atrial cavity of many zooids in colonies collected in November, but tailed larva are few. The trunk of the larva is 0.9 mm long. Three adhesive organs are in the antero-median line. They have typical for *Eudistoma* structure with thick short stalks and wide platforms of numerous closely packed columnar cells in epidermal cups. Adhesive organs alternate with three smaller median ampullae.

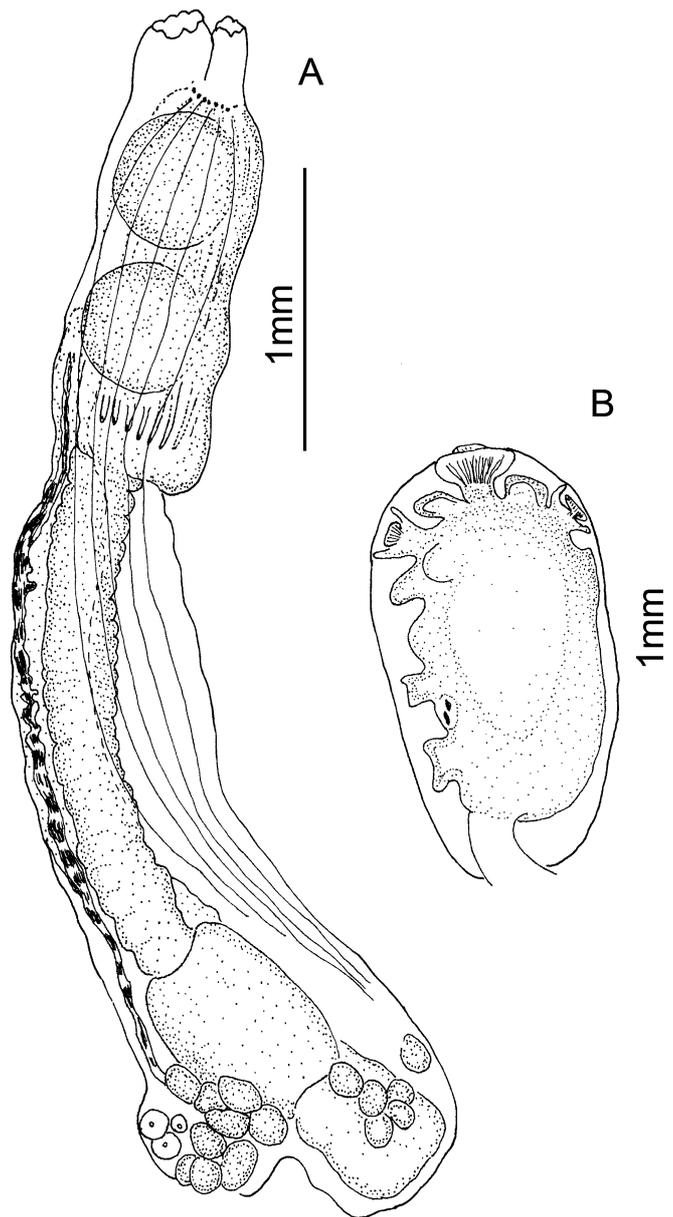


FIGURE 3. *Eudistoma clivosum* n. sp. A, zooid; B, larva.

**Remarks.** The genus *Eudistoma*, comprising 120 valid species, is especially diverse in tropics and rather few species occur in colder waters.

The main distinguishing characters of the present species are the shape and colour of the colony, hard tunic of spongy consistency without attached or embedded sand, and presence of rudimental circular systems without cloacal cavities. Zooids are similar to those of many other species of the genus, although a small

number of stigmata per row is noticeable. The species could be easily distinguished from both *Eudistoma* species known previously from the Pacific coasts of South America: *E. magalhaensis* (Michaelsen, 1907) originally described from Strait of Magellan and recently redescribed from Guaitecas Islands (Sanamyan & Schories, 2007), has a very different colony consisting of separate clavate lobes. And the second species, *E. pachecae* Van Name, 1945, based on a single small specimen from Bay of Panama, has thin transparent gelatinous colony which differ clearly from the present species. *Eudistoma clivosum* n. sp. is not similar to species known from Pacific coasts of North America and not appear to be closely related to any tropical or temperate species, most of which, with few exceptions, have more stigmata per row or differ in the structure of colony or larva.

The shape and colour of the colony, as appear on underwater photographs, allow distinguishing this species from other colonial ascidians occurring in the studied region. And as in the case of *Aplidiopsis chilensis* n. sp., we never saw similar colonies on photographs taken in more southern locations.

### ***Distaplia arnbackae* n. sp.**

(Figures 4, 6F–H)

*Distaplia* sp. Ärnäck, 1929: 14.

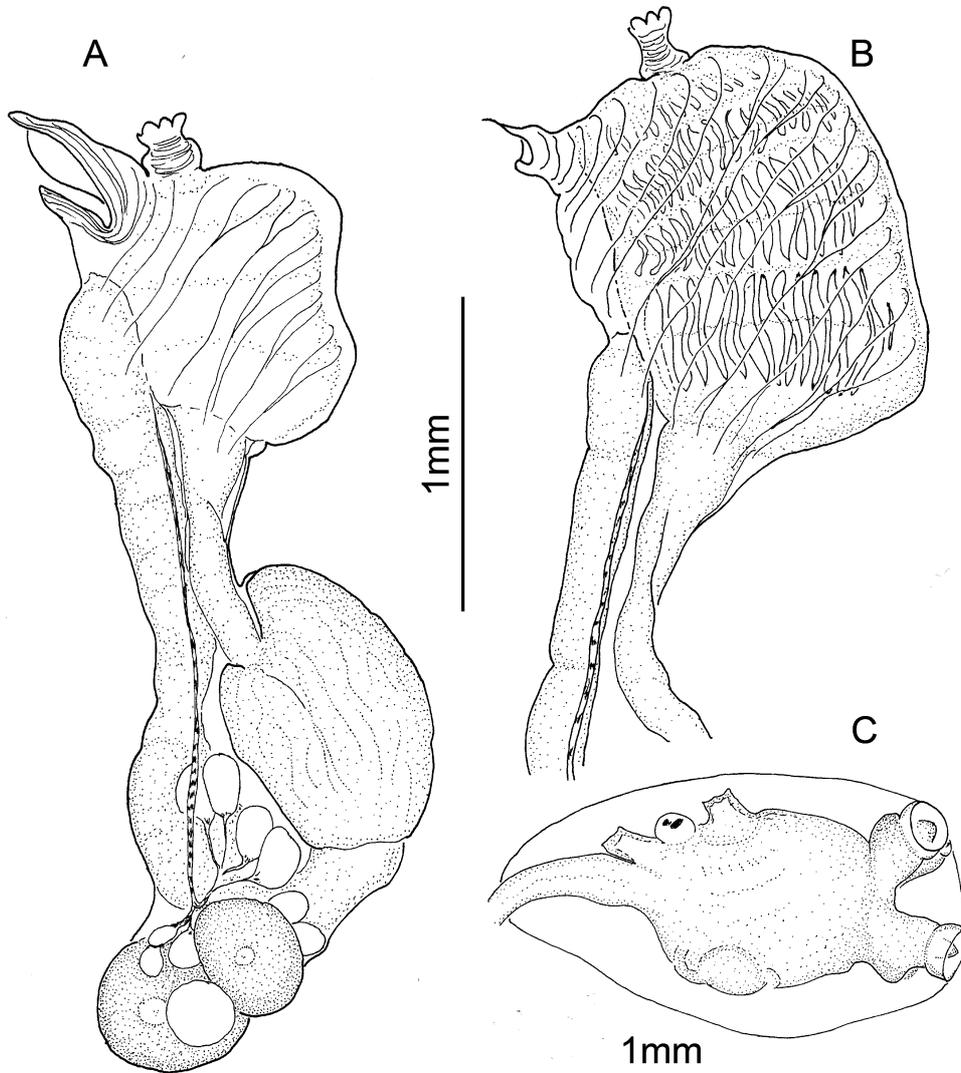
*Distaplia occidentalis*: Van Name, 1954: 8 (Not *D. occidentalis* Bancroft, 1899).

**Material examined:** Chile, 4. region, Punta de Choros: Bajo tiburon, 15m, 1 colony; 17m, several fragments; Chile, 10. region, Caleta la Arena, 04 April 2009, several colonies (Holotype KBPIG 1399/1). Additional specimens: 10. region: Caleta la Arena, several colonies, Yaldad (Chiloe), 25 February 2007, mussel culture, several colonies.

**Description.** Younger colonies consist of several small button shaped cormidium some of which are well separated and some fused together (Figure 6F); larger colonies are mostly solid encrusting sheets or cushions attached to substratum by the whole lower surface. In preservative they are rarely more than 5 mm thick and the largest collected colony is about 5 cm in extent. The test is free from encrusting or embedded sand and has soft spongy consistence characteristic for many *Distaplia* species. Zooids open on the upper surface only and are arranged into crowded circular or oval systems, each containing about 10 to 20 zooids with a cloacal opening in its centre. In younger colonies the systems are less crowded and are separated from each other by a shallow groove; smaller, button shaped colonies contain mostly a single system. The colour is very constant, all collected colonies as numerous seen on underwater photographs are always brilliant scarlet red, sometimes with sparse minute granules of white pigment especially between the systems, and often with white dot or a short white band on intersiphonal area of each zooid sometimes extending to the atrial languet. These markings may appear on photographs as small white splashes (Figure 6G,H). The colour is not retained in preservative (formaline) for a long time, the preservative extracts red pigment, become purple-violet, and colonies fade to gray in about one year.

Contracted zooids are no more than 3 mm long. Elongated branchial siphon has 6 small rounded lobes. Atrial aperture is a narrow transverse slit with short plain edged anterior languet; sometimes short atrial siphon is present (Figure 4B). Crowded circular muscles form a sphincter around the branchial siphon and similar, but more spaced muscles are around atrial aperture. About 15 fine spaced muscles are on the thorax. Some of them originate at the base of the branchial siphon, but most run obliquely from ventral side to the base of the thorax. Branchial sac has four rows of 15 or 16 long stigmata; each row is crossed by a parastigmatic vessel. Oesophageal neck is not especially long, being typically of the same length as the thorax. Obliquely oriented asymmetrical stomach has almost smooth wall, with faint and not always obvious internal plications. There are no apparent subdivisions of the intestine. A conspicuous elongated gastric reservoir lies in the gut loop and sometimes fills whole free space there. Gonads are on the right and below the pole of the gut loop. Male follicles form a loose rosette or a bunch with their ducts converging to the proximal end of the vas deferens in the centre of the bunch. One to three large ova are just below the gut loop.

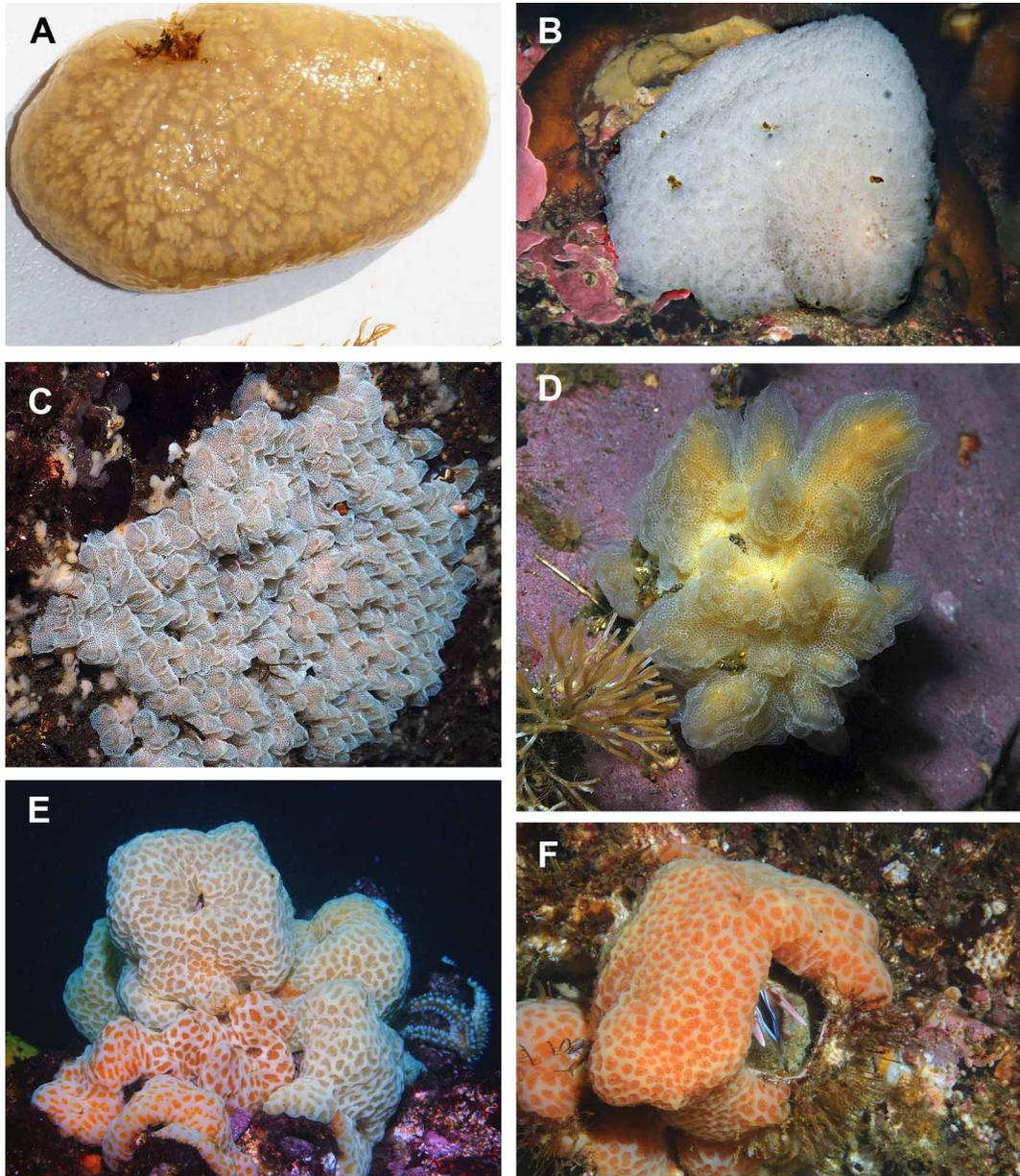
Several colonies contain numerous brood pouches with developing embryos and larvae which are mostly detached from zooids and freely lie in the tunic. Up to five larvae may present in each brood pouch. Larvae are elongated, with a trunk up to 1.1 mm long and a tail reaching a little more than halfway around it. An ocellus and an otolith are present. Adhesive organs are arranged triradially, two ventral ones have a single bulb at ventral side of the bases of their stalk, and a dorsal adhesive organ has two bulbs on the dorsal side of its stalk.



**FIGURE 4.** *Distaplia arnbackae* n. sp. A, B, zooids; C, larva.

**Remarks.** This species was described by Ärnback (1929) from Guaitecas Island, a location where it is rather common. A small colony examined by Ärnback's (1929) contained only immature zooids. Basing on the structure of zooid she pointed a close similarity of her material with Californian *D. occidentalis* but hesitated to identify Chilean specimens with that species referring to a large geographical distance between the records of these species. Van Name (1945) included Ärnback's record under the question to synonymy of *D. occidentalis*, and latter identified specimens from Chile with this species. He wrote: "In the present report I venture to assign the Chilean specimens to the North American species, as I am not able to mention any distinguishing characters." (Van Name, 1954: 9). Nevertheless, although zooids of Chilean species are similar to those of N. American *D. occidentalis*, the North and South American species appear to be distinct. Indeed, although several *Distaplia* species have distinctive zooids, in many others the zooids are virtually identical and in many cases features of a colony may be more important to delimitate species. Colonies of *D.*

*occidentalis* may be "light green, variable, dark brown, cadmium-yellow, brick-red, dirty white" (Ritter & Forsyth, 1917: 465) while in *D. arnbackae* **n. sp.** colour is very constant and hardly shows even a slight tint variation, being always bright red. Chilean specimens examined by Van Name (1954: 9) were of the same colour ("red, redbrown or vine red"). Further, unlike *D. arnbackae* **n. sp.**, which is always flat (with the exception of very small young colonies containing a single system), in *D. occidentalis* colonies are more or less mushroom shaped, with smaller area of attachment and expanded zooid-bearing top.



**FIGURE 5.** A, B, *Aplidium falklandicum*. C, D, *Aplidium fuegiense*. E, F, *Aplidium peruvianum*.

Colonies of *D. arnbackae* **n. sp.** are distinctive and could not be confused with other colonial ascidians occurring in the region. The species is recorded in central Chile from Coquimbo and its range extends to the south to Guaitecas Islands.

No other species of this genus are known from Chilean coasts north off Strait of Magellan. The colonies identified as *D. colligans* by Lager *et al.* (2009) from Lilihuapi Island (42°S) are certainly misidentified, they have much smaller larva than usually found in *Distaplia* and the colour photo (Lager *et al.*, 2009: Figure 2F) shows colonies very similar to colonies of *Diplosoma* species which is abundant in neighboring waters (e.g. Chiloe Island, our unpublished data).

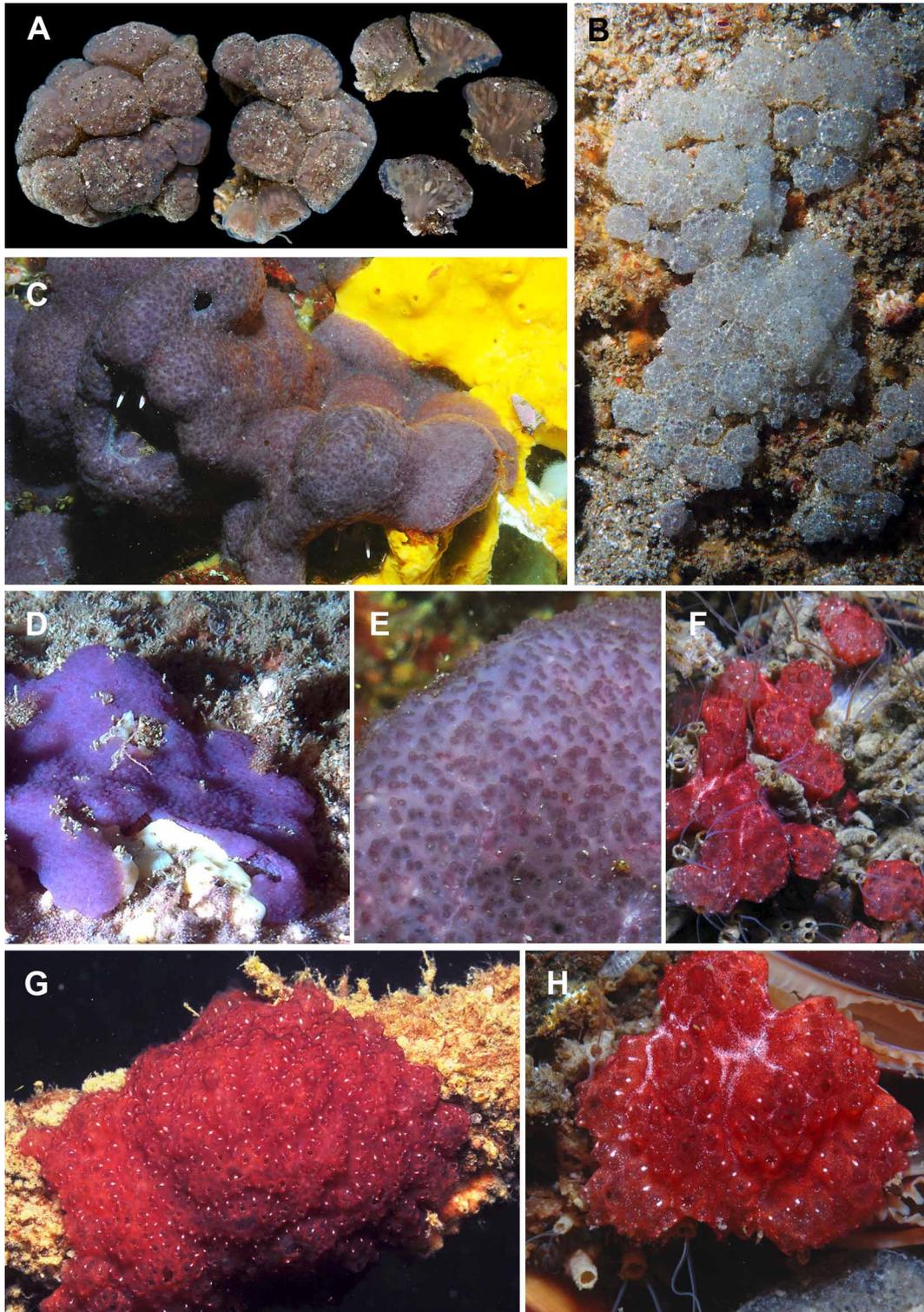


FIGURE 6. A, B, *Aplidiopsis chilensis* n. sp. C, D, E, *Eudistoma clivosum* n. sp. F, G, H, *Distaplia arnbackae* n. sp.

***Didemnum studeri* Hartmeyer, 1911**

*Didemnum studeri* Hartmeyer, 1911: 538. Van Name, 1945: 90. Monniot & Monniot, 1983: 43. Sanamyan & Schories, 2003: 94.

**Material examined:** Chile, 4. region: Punta de Choros, Isla Danmas, 12m, two colonies.

**Remarks.** Two colonies tentatively identified here as *D. studeri* have all characters of this species (including narrow branchial sac with only 5 or 6 stigmata per row and 6 coils of vas deferens) but spicules are distributed mainly in the superficial layer of the test. The specimens are similar to those from Magellan Strait (Sanamyan & Schories, 2003).

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