Ascidian fauna (Tunicata, Ascidiacea) of subantarctic and temperate regions of Chile

Running title: Ascidian fauna of Chile

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ABSTRACT

We studied the ascidian fauna from two zones located in subantarctic (Punta Arenas, latitude 53º) and temperate Chile (Coquimbo, latitude 29º). The different oceanographic features of the two zones, with influence of the Humboldt Current in the north and the Cape Horn Current System and freshwater inputs in the south, led to markedly different ascidian faunas. A total of 22 species were recorded, with no shared species across the two areas (11 species each). The new species *Polyzoa iosune* is described, *Lissoclinum perforatum* is found for the first time in the Pacific Ocean, and *Synoicum georgianum* and *Polyzoa minor* are new to the Chilean fauna. The populations of *Ciona* in the Coquimbo area (formerly attributed to *Ciona intestinalis*) correspond to the species *Ciona robusta*. A total of 35 Cytochrome oxidase (COI) sequences of the standard barcode region have been obtained for 17 of the 22 species reported.

INTRODUCTION

The Chilean coasts provide a unique scenario for biodiversity studies, as it spans a wide range of latitude (ca. 39º) and includes climatic regimes from subtropical to Antarctic. The Chilean littoral is usually divided into three biogeographical units (Knox 1960, Camus 2001): (1) the northern littoral, which is part of the Peruvian Province and has warm-temperate biota (2-30ºS); (2) an Intermediate Area (30-42ºS), and (3) a Magellanic Province (42-56º) of cold-temperate and subantarctic conditions.

The ascidian fauna from the Chilean coasts was reviewed by Van Name (1954), who listed 51 species and noted that there was an important gap of knowledge of the northern coast. Later works (e.g., Monniot & Andrade 1983, Millar 1988, Clarke & Castilla 2000) raised the number of described species to 55. In the last decade, further additions (Sanamyan & Schories 2003, 2007, Lagger et al 2009, Tatián & Lagger 2010, Sanamyan et al. 2010) increased our knowledge of the ascidians from Chile, and the review by Schories et al. (2015) included 78 species. Notwithstanding these studies, and considering the wide geographic span and climatic variability of the Chilean coasts, many so far unreported ascidian species may exist in the area.

In this study we analysed the ascidian fauna of two contrasting areas: one located in the Magellanic Province (Punta Arenas in the Strait of Magellan, 52-53ºS), with subantarctic conditions, and the other right in the transition between the Peruvian Province and the Intermediate Area (Coquimbo zone, 29-30ºS) and thus of temperate nature. The oceanographic features of the two zones are markedly different, with influence of the Humboldt Current in the north, and the Cape Horn Current System, together with freshwater influxes, in the Magellanic zone (Fig. 1). The relative distribution of native and introduced species, as well as the implications of a likely increase of introductions associated with global warming and human activities, were discussed elsewhere (Turon et al., in press).

MATERIAL AND METHODS

The sampling was done in September and November-December of 2013. Localities around Punta Arenas and Coquimbo (Fig. 1, Table 1) were surveyed by SCUBA diving or by pulling up ropes and aquaculture cages. At each site, we sampled artificial substrates (docks, pilings, aquaculture facilities) and natural communities. Sampling was exhaustive, i.e., it was continued until no further species were detected; typically, surveys lasted ca. 1 hr and the depths studied ranged from 1 to 12 m. Two samples collected in 2012 by M.

Valdebenito from deeper waters and available at the Biological Collections Room of the Universidad Católica del Norte (SCBUCN) at Coquimbo were also included (Table 1).

Ascidians were photographed *in vivo*, carefully collected in Ziploc bags, and brought to the laboratory as soon as possible (within 2 hrs after collection). Ascidians were placed in trays and relaxed with a combination of menthol (adding some drops of saturated menthol solution) and a cold treatment (keeping the trays in a freezer until ice formed on the surface). Samples were then split for preservation: most of the material was fixed in formaldehyde 4% for morphological observation, while some parts (usually colony fragments or muscular tissue samples from solitary ascidians) were preserved in absolute ethanol for genetic analyses. To obtain scanning electron microscope (SEM) images of some features of the tunic, samples were critical-point-dried. Observation of calcareous tunic spicules was achieved by dissolving tunic fragments in sodium hypochlorite at high temperature. Spicules were then dehydrated in a graded alcohol series. Samples for SEM were sputter-coated with gold and observed in a Hitachi TM3000 microscope at the Microscopy Unit of the Center for Advanced Studies of Blanes.

The observation of morphological features was routinely enhanced by staining with Masson's haemalum, and ascidians were identified to species level using relevant literature. Taxonomy followed the guidelines of the World Register of Marine Species (Shenkar et al. 2015). Specimens preserved in formaldehyde were deposited in the Systematic Room of the Instituto de la Patagonia, Universidad de Magallanes, Punta Arenas, Chile (SRIP-UMAG, Codes 900174 to 900183) and the Biological Collections Room of the Universidad Católica del Norte (SCBUCN, codes SBUCN3942-3966).

Ascidian barcoding

DNA extractions from groups of zooids (colonial forms) or muscle tissue (solitary species) were used as templates for PCR amplification of a 520 to 632 bp fragment of the 5' region of the mitochondrial gene cytochrome oxidase subunit I (COI), corresponding to the standard barcoding partition (Hebert et al. 2003, Bucklin et al. 2011). DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen). Two sets of primer pairs were used for COI amplification depending on the species, the "universal" primers LCO1490 and HCO2198 (Folmer et al. 1994) and the ascidian-specific primers Tun_forward and

Tun_reverse2 (Stefaniak et al. 2009). Amplification was performed with 1 µL of each primer (10 µM), 12 µL (0.5 units) of MyTaqTM HS Red Mix DNA polymerase (Bioline), 10 to 20 µg/mL DNA, and PCR water to a total-reaction volume of 25 µL. The PCR program included initial denaturing at 95ºC for 1 min, followed by 35 amplification cycles of 95ºC for 15 sec; annealing at 45ºC (primers LCO1490 and HCO2198) or 42ºC (primers Tun_forward and Tun_Reverse2) for 15 sec; and extension at 72ºC for 10 sec. After the 35 amplification cycles, a final extension was carried out at 72ºC for 1 min. Sequencing reactions were carried out with the BigDyeTM terminator v. 3.1 and the same primer set used during the amplification step, and analyzed on an ABI Prism 3137xl automated sequencer (Applied Biosystems) available at UNCW Center for Marine Science. Other amplicons were generated at the Molecular Biology Laboratory of the Center of Advanced Studies of Blanes and sequenced (both strands) by Macrogen Inc. (Seoul, Korea). Pairwise consensus sequences for each sample were created using Geneious v. 8 (Kearse et al. 2012). A final alignment for all consensus sequences obtained in this study was built using ClustalW (Larkin et al. 2007) with a gap-opening penalty of 24 and a gap extension penalty of 4 (Erwin & Thacker 2007). Quality-checked sequences were archived in GenBank under accession numbers KU299754 to KU299781.

Descriptions

The species found in each region, substrate type and accession number of the corresponding COI sequence are listed in Table 2. We provide below descriptions (except for well-known species) and taxonomic and distributional remarks of the species found.

Order Aplousobranchia Lahille, 1886

Distaplia colligans Sluiter, 1932

Fig. 2

References and synonymy: *Distaplia colligans* Millar (1960) p. 77; Kott (1969) p. 32; Sanamyan & Schories (2003) p. 94.

Localities: 1S,2S,3S,6S,9S.

The species forms crusts ca. 5 mm in thickness of a bright yellow colour. Cloacal apertures are slightly raised over the colony surface, but no conspicuous zooid systems are visible. The tunic is soft and free of sand.

The zooids measure up to 3 mm. The atrial aperture is large with an anterior lip. The branchial sac has 4 stigmata rows with about 20 stigmata per half-row. A parastigmatic vessel cuts each row. At least 8 fine longitudinal muscles are counted at each side of the thorax. The abdomen is small, comprising the digestive loop with an elongated stomach whose surface is slightly wrinkled. The gonads lie in the centre of the intestinal loop, comprising several male follicles and a central mass of a few oocytes.

There were larvae in the colonies, apparently free in the tunic. They measure 1-1.5 mm of trunk length and bear an oozooid and three adhesive papillae. The stalks of the papillae had two lateral outgrowths in the ventral papilla and one each in the two dorsal papillae.

Remarks: this species is widely distributed in Antarctic and subantarctic waters (Monniot & Monniot 1983, Schories et al. 2015)

Didemnum studeri Hartmeyer, 1911

Fig. 3A,B,E; Fig. 12D

References and synonymy: *Didemnum studeri* Millar (1960) p. 56; Kott (1969) p. 75; Monniot (1970) p. 335.

Localities: 1S, 2S, 3S, 6S, 9S.

Whitish colonies forming extensive crusts, up to tens of cm in diameter and 3-4 mm in thickness. The tunic is filled with spicules 10-30 µm in size, stellate with about 10 short rays in optical section. Often, rays are blunt and irregular, giving an almost spherical appearance to the spicule. The general cavity is extensive at the thoracic level, often with accumulated faecal pellets.

The zooids are small and highly contracted. The branchial sac has a large aperture and 4 rows of stigmata. The thoracic organs lie at the posterior part of the branchial sac. There is a developed retractor muscle. The abdomina are embedded in the basal test and do not have gonads in the observed specimens.

Some colonies incubate larvae, 0.6 mm in trunk length, with three adhesive papillae and four pairs of ectodermal ampullae.

Remarks: this species is common in Antarctic and subantarctic waters (Monniot & Monniot 1983). Although we couldn't observe gonads in our material, the spicules, larvae and general shape of colonies and zooids allows the assignment of our specimens to this species.

Diplosoma listerianum (Milne Edwards, 1841)

References and synonymy: *Diplosoma macdonaldi* Van Name (1945) p. 109; *Diplosoma listerianum* (Berrill 1950) p. 125.

Localities: 1N, 2N, 3N, 6N, 8N.

Remarks: this well-known species is common in artificial structures and bivalve culture gear in the Coquimbo area, where it also occurs occasionally on natural substrates. *Diplosoma listerianum* is a widely distributed taxon, described in the NE Atlantic but nowadays present in all warm-temperate seas (Locke 2009). It has recently been shown to comprise five main mitochondrial clades, likely cryptic species (Pérez-Portela et al. 2013). The specimens from Coquimbo belong to Clade A of that work, the most invasive one. It was first reported in the SE Pacific by Pérez-Portela et al. (2013) in the Coquimbo and Antofagasta areas, and it was later reported also from the Patagonian Fjords zone (Schories et al. 2015).

Lissoclinum perforatum (Giard, 1872)

Fig. 3F

References and synonymy: *Lissoclinum argyllense* Lafargue (1968) p. 422; *Lissoclinum perforatum* (Lafargue 1975) p. 292.

Localities: 2N, 3N.

The colonies are encrusting, of whitish colour. The tunic has a moderate abundance of spicules. These are of ca. 40 µm and have a characteristic shape, with flabellate rays.

The common cavity of the colony is wide, with zooids included in strands of tunic. The branchial sacs are widely open to the common cavity, with wing-shaped thoracic organs in the middle part. The digestive system is small and not twisted (i.e., it has the stomach in ventral position). There were no gonads or larvae.

Remarks: this species has appeared on artificial structures and bivalve cultures in the Coquimbo area. *Lissoclinum perforatum* is an Atlanto-mediterranean

species that has been introduced in the western Atlantic (Monniot 1983, Rocha et al. 2005, Rocha and Bonnet 2009, Dias et al. 2013). This is the first finding of this species in the Pacific Ocean, representing a remarkable range expansion.

Polysyncraton trivolutum (Millar, 1960)

Fig. 3C,D,G

References and synonymy: *Polysyncraton trivolutum* Millar (1960) p. 58; Monniot & Monniot (1983) p. 43; Monniot et al. (2011) p. 10.

Localities: 5S.

This species forms crusts several cm across and 4-5 mm thick. The colour is white with a brownish tinge. The spicules are dense, in general less than 25 μm in diameter (occasionally reaching 40). They are stellate with short rays, up to ten in optical section. The general cavity of the colony is at the thoracic level, but it extends between groups of abdomina.

The thoraces reach 2 mm in length. They have an atrial languet that ranges from very small and simple to long and with several points. Cup-like thoracic organs are prominent between the second and third row of stigmata. No retractor muscle is present. The abdomina are smaller than the thoraces, and are in general in male phase. They have three testicular lobes surrounded by a sperm duct describing four coils. Some zooids also have a few developing oocytes adjacent to the testes.

Remarks: the presence of atrial languet, absence of retractor muscle, structure of thoracic organs and gonads allows the assignment of our specimens to this species. However, *P. trivolutum* is highly variable and may comprise in fact a group of closely related species (Monniot & Monniot 1983). This taxon is common in Antarctic and subantarctic regions (Monniot & Monniot 1983, Monniot et al. 2011, Schories et al. 2015).

Aplidium fuegiense (Cunningham, 1871)

Fig. 4A-C

References and synonymy: *Amaroucium fuegiense* Van Name (1945) p. 43; *Aplidium fuegiense* Monniot (1970) p. 325; Monniot & Monniot (1983) p. 16.

Localities: 2S, 7S, 8S.

Colonies of this species are cushion-like, measuring up to 10 cm in diameter and 4 cm in height. The colour in our specimens is brownish. The zooids form wide, meandering fields separated by narrow tunic stretches without zooids. The tunic is soft but consistent, particularly the outermost layer. The relaxed zooids measure over 20 mm in length (the thorax reaches ca. 5 mm, and the abdomen ca. 4 mm).

The oral siphon is six-lobed, and the atrial aperture is relatively wide, reaching from the second to the fifth row of stigmata. It is surmounted by a short languet that can be simple or, more often, have two, three, or more points. There are between 12 and 15 rows of stigmata (mostly 13-14), with over 20 stigmata per half-row, and 15-16 fine longitudinal muscles run longitudinally through each side of the thorax. The abdomen presents a folded stomach, usually with five folds plus the typhlosole. There is a posterior stomach and an elongated mid-intestine in vertical position in well relaxed zooids. The posterior intestine ends in a bi-lobed anus just at the posterior rim of the atrial aperture. Most zooids have well-developed gonads consisting of several reddish oocytes and white male testes occupying most of the post-abdomen. The space between the posterior intestinal loop and the ovary is filled by a folded and twisted spermduct. In a few zooids, undifferentiated embryos (up to four), of reddish colour, were seen in the posterior part of the peribranchial cavity.

Remarks: this species is widely distributed in Antarctic and subantartic waters. Most descriptions of the species report wide morphological variation, perhaps encompassing a group of ill-defined species. We did not observe the small orange-reddish bodies in the surface layer of test reported in other descriptions (Millar 1960, 1970, Sanamyan & Schories 2003), which may be regressing bodies. The convoluted spermduct between the ovary and the digestive system (Millar 1960, Monniot 1970, Monniot & Monniot 1983) may be a good diagnostic character.

Aplidium peruvianum Sanamyan & Schories, 2004

Figs. 5, 10A

References and synonymy: *Aplidium peruvianum* Sanamyan & Schories (2004) p. 193.

Localities: 1N, 6N.

The colonies form thick encrusting sheets, reaching tens of cm in the largest dimension and up to 2 cm in thickness. Several colours have been found, the commonest being beige-brownish. However, whitish, yellowish and reddish colonies are also observed. In all cases the colony organisation consists of circular zooid systems with one common cloacal aperture placed in depressions

of the colony surfaces and separated by elevated ridges of tunic. Sometimes the systems are elongated with more than one aperture. The tunic is strong and consistent. In reddish colonies the colour is intense in the zooid systems (with pigment concentrated in the thoraces) and fades in the ridges in-between.

The zooids reach 11 mm in length; the thorax measures up to 2.5 mm and the abdomen up to 2 mm. The thorax has a six-lobed oral siphon, and a narrow atrial opening facing the first two stigmata rows. There is an undivided atrial languet in the anterior rim of the aperture. When the zooids are contracted, minute denticulations are visible in the posterior part of the atrial aperture. There are from 11 to 14 stigmata rows (most often 12-13), with 15-20 slits per half-row. At each side of the branchial sac there are 14-16 fine longitudinal muscular bands.

The abdomen has a stomach with many ridges (up to 18-20). They are difficult to count as they are often interrupted, sometimes even substituted by irregular protuberances more or less aligned (particularly so in the side facing the intestine). There is a post-stomach and a mid-intestine in oblique or horizontal position at the lower end of the gut loop. The posterior intestine opens in a bi-lobed anus at the level of the fifth or sixth posterior-most stigmata row.

Some zooids have well-developed gonads, with oocytes adjacent to the digestive loop and male follicles filling the post-abdomen. There are also embryos in incubation in the atrial cavity, up to 6 of them more or less in a row. In red colonies, the oocytes and immature embryos are red in colour. Fully developed larvae are elongated, up to 1 mm in trunk length, and bear three adhesive papillae. There are four clusters of small vesicles. Two between the papillae, one dorsal and one ventral. The vesicles of each cluster seem to arise from a common stem.

Remarks. the species was described from Peru (hence its name) in 2004, and was later reported from the Coquimbo area in Chile (Sanamyan et al. 2010). This is the third finding of this species, which is well characterised by the colony aspect, stomach and larval morphology.

Aplidium variabile (Herdman, 1886)

Fig. 4D-F

References and synonymy: *Aplidium variabile* Millar (1960) p. 32; Kott (1969) p. 51; Monniot (1970) p. 331.

Localities: 7S.

Colonies were thick encrusting forms (up to 10 cm in diameter and 2.5 cm in thickness) of orange colour. The tunic is soft and free of sand. Circular or meandering systems of zooids can be seen in live material.

The zooids measure up to 16 mm, of which the (partially contracted) thoraces are up to 3 mm and the abdomina 1.5 mm. The oral siphon has six lobes and the atrial aperture is at the level of the third stigmata row. There is a short and simple atrial languet. There were 13 stigmata rows (occasionally 14-15) with 15-18 stigmata per half row. The digestive system comprises a stomach with ca. 14 longitudinal folds (some of those facing the intestine may not be complete). There is a post-stomach, an oblique or horizontal mid-intestine marking the lower part of the digestive loop, and a long posterior intestine with a couple of proximal caeca. The intestine ends near the atrial aperture.

There are well developed gonads in most zooids, with an ovary at some distance of the intestinal loop and male follicles loosely arranged in two or more rows. Some zooids had embryos in incubation in the peribranchial cavity, to the right of the intestine. Up to 9 embryos aligned in a row were observed (the alignment can be lost in the posterior part). The more developed embryos were fully formed larvae, with 0.75 mm of trunk length, three adhesive papillae, and numerous small vesicles in the anterior part of the larva.

Remarks: the colonies superficially resemble those of *A. fuegiense*, but the tunic is softer and some characteristics, such as the number of stomach folds, are widely diverging. The larval characteristics (not observed here for *A. fuegiense*) are also distinctive between these species (Millar 1960). It is a circum-subantarctic species extending from the littoral zone down to 500 m (Monniot & Monniot 1983).

Synoicum georgianum Sluiter, 1932

Fig. 6

References and synonymy: *Synoicum georgianum* Millar (1960) p. 47; Monniot (1970) p. 22; *Aplidiopsis georgianum* Kott (1969) p. 73.

Localities: 1S, 4S.

Colonies of this species form aggregates of small capitate heads (5-10 mm in diameter, 15 mm in height). These heads are united basally and often coalesce forming bigger units. From one to several systems of zooids form each head. The colonies from Muelle Loreto (locality 4S) are externally coated with sand, which does not penetrate in the inner tunic, while those from Bahía Porvenir (locality S1) are devoid of sand and whitish in colour. The internal tunic is soft and transparent.

The zooids measure up to 10 mm in length. The oral siphon has 6 lobes (sometimes subdivided). The atrial opening is narrow, forming a tube in front of the first row of stigmata, and is surmounted by a trifid languet. A couple of minute denticulations is sometimes visible in the posterior rim of the atrial aperture. The branchial sac has 10 to 12 stigmata rows with approx. 10 slits per half row. There are 7-9 longitudinal muscular bands at each side of the thorax. The abdomen has a quadrangular stomach, in which the oesophagus enters a bit sub-terminally. The stomach appears smooth-walled externally, except for the typhlosole, although a pentagonal shape is apparent in transverse sections. There is a post-stomach with a markedly thickened region, which connects to a slender mid-intestine in vertical position. The sharp transition between mid-intestine and posterior intestine lies at the bottom of the gut loop, and the posterior intestine ends about the level of the fourth posterior-most row of stigmata.

Some zooids have ovaries and testes (forming one or two rows) in the postabdomen, and only a few have brooding embryos (up to 4) posterolaterally (right side of the intestine) in the atrial cavity. No developed larvae could be seen.

Remarks: the fact that the incrustation with sand is a variable character had already been noted by Monniot & Gaill (1978). Although the number of stigmata rows is lower than reported by other authors (e.g., Millar 1960: 13-15 rows), the structure of the colonies, shape of the atrial aperture and digestive system is in agreement with descriptions of this species, which has a circum-antarctic distribution but had not been previously reported from Chile.

Order Phlebobranchia Lahille, 1886

Ciona robusta Hoshino & Tokioka, 1967

Fig. 7A

References and synonymy: *Ciona robusta* Hoshino & Tokioka (1967) p. 276; Brunetti et al. (2015) p. 186; *Ciona intestinalis* type A (Sato et al. 2012) p. 1611.

Localities: 2N, 3N, 6N.

Remarks: the cosmopolitan taxon *Ciona intestinalis* has been recently shown to lump together several cryptic species, of which the most widespread is the so-called *Ciona intestinalis* type A (Caputi et al. 2007, Zahn et al. 2010). Recent work (Brunetti et al. 2015, Pennati et al. 2015) has partially clarified the taxonomy of this species complex and has shown that *Ciona intestinalis* type A

is in fact *C. robusta*, a species described by Hoshino and Tokioka (1967) in Japan and later synonymized under *C. intestinalis*. The specimens observed had the diagnostic features of *C. robusta* (Sato et al. 2012, Brunetti et al. 2015), such as protuberances in the tunic near the siphons, and red pigment in the papillae at the distal end of the spermduct. The mitochondrial sequences obtained also confirm the identity of the Chilean specimens. *Ciona robusta* is an invasive species now established in northern-central Chile and is expanding its distributional range (Madariaga et al. 2014).

Corella eumyota Traustedt, 1882

Fig. 7B-D

References and synonymy: *Corella eumyota* Traustedt (1882) p. 273; Monniot (2013) p. 138.

Localities: 2N, 3N, 6N, 8N.

The specimens measure up to 3 cm in total length and are fixed ventrally or slightly tilted over the right side. The tunic is translucent and the mantle can be from whitish to orange. There are 7-8 red pigmented spots in the oral siphon and 4-6 in the atrial siphon.

The musculature of the mantle is well developed on the right hand side, where the fibres form a crisscrossed network dorsally and have a more transverse orientation ventrally. There are many simple tentacles (up to 100 visible). The neural gland has a "U" shaped aperture. The branchial sac is typical, with over 30 longitudinally arranged vessels and coiled stigmata below them. Slender dorsal languets mark the dorsal midline of the branchial sac.

The digestive, as is characteristic of this genus, lies on the right hand side. The stomach is folded (folds are better seen from the external side), and the intestine forms a wide loop occupying up to 3/4 of the right side of the mantle. The final part of the intestine runs adjacent to the dorsal languets and ends in an anus with minute lobes. The interior part of the digestive loop is occupied by the gonads, forming a compact mass of tissue. The genital apertures are inconspicuous (usually staining is required to observe them), on the inner side of the gonad mass, and consist of a narrow slit for the male papilla, and a wider aperture for the female gonad.

Remarks: Lambert (2004) was the first to note the particular shape and position of the genital ducts in *Corella eumyota*, and this character clearly separates this species from other congeners (Alurralde et al. 2013). *C. eumyota* was considered until recently the only species of the genus in the southern hemisphere, but Alurralde et al. (2013) and Monniot (2013) showed that there

are at least three species. Previous reports of *C. eumyota* should therefore be taken with caution. The Chilean populations should retain the original name of *C. eumyota*, described from Chile (Valparaiso), and recently introduced into the northern hemisphere (Lambert 2004).

Order Stolidobranchia Lahille, 1886

Asterocarpa humilis (Heller, 1878)

Figs 7A, 8

References and synonymy: *Cnemidocarpa robinsoni* Van Name (1945) p. 271; *Asterocarpa humilis* Kott (1985) p. 141; *Cnemidocarpa humilis* (Monniot et al. 2001) p. 98.

Localities: 2N, 3N.

The individuals of this species reach 4 cm in length, and are typically fixed by the ventral side, with both siphons close together in the flat upper surface. The species often forms clumps on top of artificial substrates or other ascidians. The tunic is hard and vivid orange in colour. In live specimens, the siphons are quadrangular, with four broad white-yellow stripes on a reddish background. In-between the wider stripes there are other fine and incomplete whitish bands. The internal tunic of the siphons has wide and blunt siphonal spines.

The mantle is muscular and strong. The inner part of the atrial siphon bears a velum with small papillae. The internal organization is typical of a stylelid, with simple tentacles, simple dorsal lamina, and four branchial folds. The number of longitudinal vessels on the folds is between 8 and 11, with 2-4 vessels between folds (and none between the dorsal-most fold and the dorsal lamina). The digestive has an ovoid stomach and an intestine forming a rather closed primary loop and a marked secondary loop. There are endocarps in the primary and secondary loops.

The gonads are characteristic of the species. On the right hand side they form an anastomosed mass of tubular gonads along the ventral midline. A few gonoduct apertures can be seen, facing different directions. On the left the gonad also forms a mass, but much smaller, anterior to the upper part of the intestinal loop.

Remarks: the species has been reported from the Juan Fernandez Islands as *Cnemidocarpa robinsoni* (Van Name 1945). Clarke & Castilla (2000) found it for the first time in continental Chile (Antofagasta Bay). This southern hemisphere species has been introduced into European waters (Bishop et al. 2013).

Botryllus schlosseri (Pallas, 1766)

References and synonymy: *Botryllus schlosseri* (Van Name 1945) p. 220; (Berrill 1950) p. 216.

Localities: 2N, 8N.

Remarks: the golden star tunicate is a model species for evo-devo studies and is distributed worldwide (Locke 2009). It has a marked polymorphism in chromatic patterns and colony shapes, and the long list of synonymies reported (e.g., Van Name 1945, Berrill 1950, Kott 1985) bears testimony of the convoluted taxonomical history of *B. schlosseri*. As in other widely distributed and polymorphic species, it has been recently shown that the taxon *B. schlosseri* is a complex of genetically differentiated species (Bock et al. 2012). The two mtDNA sequences obtained in this study correspond to Clade A in Bock et al. (2012), the most widespread and common but, in turn, subject to ongoing speciation processes (Griggio et al. 2014). *B. schlosseri* is abundant in artificial structures in the northern study zone. It has been reported in Chile by Van Name (1954) and Ben-Shlomo et al (2010).

Cnemidocarpa verrucosa (Lesson, 1830)

Fig 9

References and synonymy: *Cnemidorcarpa verrucosa* Lesson (1830a) p. 151; (Kott 1969) p. 106; Monniot & Monniot (1983) p 68; Monniot et al. (2011) p. 34.

Localities: 7S

Individuals are big (reaching 15 cm in height), cylindrical, posteriorly fixed and with anterior siphons. The colour is yellowish and the tunic is relatively thin but consistent, with plenty of tubercles and warts that give the species its name. The mantle has circular and longitudinal muscular bands. The siphons are featureless but for a thin reddish rim. Internally there are >30 simple oral tentacles, the aperture of the neural gland is "U" shaped with both horns rolled inwards. The dorsal lamina is smooth and simple, and there are four branchial folds with 1-3 longitudinal vessels between folds (none between the dorsal-most fold and the dorsal lamina) and 9-12 on the folds. The wide spacing between longitudinal vessels between folds results in large numbers of stigmata (up to 50 or more) in a given branchial mesh.

The digestive system comprises a voluminous stomach and an intestine describing a closed primary loop and a marked secondary loop ending in a scalloped anus. There are big endocarps near the digestive and between the

gonads at both sides. There are two big, elongated gonads at each side, with a terminal female opening surmounted by a small male papilla.

Remarks: this species is very abundant in Antarctic zones and, in the subantarctic area, it is known from the Magellanic region, South Georgia, and Kerguelen Islands (Monniot & Monniot 1983, Tatián & Lagger 2010).

Polyzoa iosune Turon & López-Legentil

Fig. 10

Localities: 6N.

Holotype: a colony has been designated as the holotype and deposited in the Museo Nacional de Historia Natural de Chile (code MNHNCL TUN-15001). Two other colonies (paratypes) are kept at the Biological Collections Room of the Universidad Católica del Norte (SCBUCN3962).

Several colonies of this new species have been found in the lower intertidal in the Coquimbo area. They form thick carpets by the union side by side of elongated zooids, reaching 17 mm in height. A single colony may cover hundreds of square centimetres. The tunic is of a clear brownish colour, and the siphons are pigmented in red. Small zooids appear in between larger ones, attached to their tunics. Thick stolons are found at the basis of the colony.

The tunic is thin but firm. The zooids have a weekly muscular body wall that allows the observation of some internal features. There is an inner oral velum and about 16 tentacles (big and medium size). The aperture of the neural gland is oval-vertical. There are 7 longitudinal vessels to the right and 4 (sometimes 5) to the left. Parastigmatic vessels cut the stigmata (up to 10 per mesh). Sometimes there are 2 and even 3 parastigmatic vessels between two consecutive transverse vessels. The digestive system forms an open primary loop, with a long and straight intestine reaching anteriorly towards the atrial siphon and ending in a smooth anus. The stomach has 12-16 folds and a pyloric caecum.

There are several endocarps (6-8) attached to the body wall at each side, round or elongated. Up to 18 gonads can be found on the right hand side, forming a row adjacent to the endostyle. The gonads are hermaphrodite and elongated. The male portion is basal and forms most of the gonad, and the female portion lies distally and apically. There is a short and wide oviduct opening distally with a scalloped aperture, and a long and thin subterminal spermduct. To the left there are only 5-7 hermaphrodite gonads between the endostyle and the ascending intestine. Some zooids brood larvae in the peribranchial cavity. The larval trunk length is up to 0.65 mm. When mature, the larvae feature a ring of

tubular anterior ampullae (6-7 pairs), three conical adhesive papillae, and a single pigmented spot.

Remarks: Monniot (1970) established a tabular key for the known species of Polyzoa. None matches the characters observed in the Chilean specimens. In particular, the number of longitudinal vessels falls within the variability of *P. minor* alone (Monniot 1973), a different species in terms of size, colony form, number of gonads and larval morphology. The only species featuring zooids joined together and of the size found here is *P. opuntia*, but it has consistently 8 longitudinal vessels per side, among other differences. On the other hand, and based on the COI sequences obtained here, *P. iosune* forms a well-suported clade (Neighbour Joining tree, results not shown), sister to the clade of *P. opuntia* sequences, with which it features 8-9% sequence differentiation. Species described more recently such as *P. exigua* and *P. nodosa* (Kott 1990), or *P. atlantica* (Sanamyan et al. 2009) are all clearly different in colony and zooid size and shape, as well as in internal features.

Derivatio nominis: this species is dedicated to María Jesús Uriz, colleague, friend, and great sponge specialist. Iosune (María Jesús in basc language) is here used as substantive in apposition.

Polyzoa minor Monniot, 1970

Fig. 11

References and synonymy: *Polyzoa minor* (Monniot 1970) p. 346; Monniot & Monniot (1973) p 721.

Localities: 6N.

We have found one colony of this colonial Styelidae, in the underside of a shallow boulder. The zooids (up to 3 mm in height) are united by stolons. There are small zooids and buds along the stolons. The tunic is whitish-brownish. Internally, there are about a dozen well-developed tentacles (and smaller ones in-between) and the neural gland opens in a vertical slit. The branchial sac has 6 longitudinal vessels on the right and 4 on the left. There are 7-9 stigmata, with parastigmatic vessels, in the wider meshes.

The digestive system forms an open loop and ends in a slightly bilobed anus. The stomach has ca. 12 folds and a long and curved pyloric caecum. There are three elongated endocarps attached to the body wall at each side.

There are up to 12 hermaphrodite gonads on the right, alongside the endostyle. They are sac-like or elongated, with a wide female opening at one end, and a male opening forming a slender tube in sub-terminal position. In some cases

the posterior gonads have only a ball-like male follicle. On the left there are from 2 to 6 gonads. When there are only two they are exclusively male. When they are more numerous, the posterior ones are hermaphrodite and the anterior ones male only. There are numerous larvae in incubation in the peribranchial cavity of some zooids. The larvae have a trunk length of up to 0.7 mm, three conical adhesive papillae and a single pigment spot.

Remarks: Monniot (1970) stressed that the species of the genus *Polyzoa* can be distinguished first by the number of branchial vessels, then by the structure of the stomach folds and, finally, by the form and shape of the colony. Our specimens, with 6 and 4 longitudinal vessels at each side, agree with the description of *P. minor* from the Kerguelen Islands (Monniot 1970). In a second report (Monniot & Monniot 1973) gives a more detailed description, highlighting some variability in the number of branchial vessels and the number of gonads. Our specimens have in general more gonads than described (up to 8 on the right and 4 on the left), but the general characters are in good agreement with the descriptions. The larvae are here mentioned for the first time in this species.

The fact that the species has been so far reported only from the Kerguelen Islands (S Indian Ocean) casts some doubt about its identification. There may be several *Polyzoa* with small zooids and more observations and genetic analyses are called for to clarify their taxonomic status. For the time being, in absence of clear discriminating characters, we assign this specimen to *P. minor* and expand therefore its distribution range to temperate E Pacific.

Polyzoa opuntia Lesson, 1830

Fig. 12

References and synonymy: *Polyzoa opuntia* Lesson (1830b) p. 437; Van Name (1945) p. 236; Kott (1969) p. 100; Monniot (1970) p. 344; *Polyzoa reticulata* Van Name (1945) p. 237; Kott (1969) p. 102.

Localities: 1S, 2S, 4S, 7S, 8S, 9S.

This colonial species is very abundant on the artificial substrates surveyed in the Punta Arenas zone. Typically, it forms stalked colonies up to 20 cm in length and of different shapes, the larger ones being normally elongated and flattened, the smaller cylindrical or simply conical. The colour in general is reddish, although a yellow form has also been found in Cabo Negro docks (locality 7S). In the colonies the zooids are united forming a compact mass where the zooids are encased. Convex protuberances with siphons mark the different zooids from the outside. However, the species can also form investing colonies with zooids clustered closely together, or with zooids completely separated linked only by stolons.

Each cylindrical zooid can measure up to 10 mm in length, with the siphons at the apical end and, in the case of compact colonies, one or two stolons at the basal end entering the common tunic. Internally there are ca. 25 well-developed simple tentacles with intervening smaller ones. The aperture of the neural gland is an oblique slit. The branchial sac has 8 longitudinal vessels on each side and up to ten stigmata per mesh. The digestive forms a simple loop without secondary curvature. The stomach is folded, with a curved pyloric caecum. At the level of the caecum a strand of tissue connects the stomach with the intestine, bridging the primary loop. There is a row of hermaphrodite gonads at both sides of the endostyle (10-12 on the right, 6-8 on the left). The gonads are elongated, with a basal testis and some oocytes on top. There is a terminal female opening and a subterminal male papilla forming a slender tube. The right row of gonads curves anteriorly and dorsally in the posterior part of the body. Small endocarps are scattered at both sides of the mantle.

Remarks: the variability of colonial shapes, from clearly separated zooids to compact stalked colonies, is a characteristic of this species. The stolonial form with separate zooids has been known as *Polyzoa reticulata* (Herdman, 1886), but it was later revealed to be the same species (Monniot & Monniot 1978, 1983). It is abundant in the Magellan region and the South Georgia Islands (Monniot & Monniot 1983).

Styela changa Monniot & Andrade, 1983

Fig. 13A-C

References and synonymy: *Styela changa* Monniot & Andrade (1983) p. 134; Millar (1988) p. 233.

Localities: 4N, 5N.

The body is globular or dorso-ventrally flattened, up to 12 mm in maximal dimension, fixed by the ventral side. The tunic is tough, brownish, and with tubercles and wrinkles. The mantle wall is very thin, particularly in the ventral region. It is very hard to extract an unbroken individual from the tunic.

Oral tentacles are simple, up to 30. The aperture of the neural gland is in the shape of a vertical slit and the dorsal lamina is smooth and low. There is an atrial velum with a circle of filiform tentacles extending anteriorly in a double row. The branchial sac has 4 folds on each side, with up to 8 stigmata per mesh, often crossed by parastigmatic vessels.

The digestive system is distinctive. It is formed by a cylindrical stomach with folds, and a prominent hepatic gland formed by branched diverticula that originate from two main stems in the anterior part of the stomach and smaller

ones located more posteriorly. The pyloric end of the stomach has a well-developed and coiled caecum. The isodiametric intestine forms a primary loop that can be more or less closed depending on the individual. The intestine is not flat against the mantle wall. Rather, it is quite unattached and is twisted in three dimensions. The secondary loop is little marked, except for a final bend of the rectum.

There are two gonads per side, each formed by an elongated and convoluted ovary and, ventrally, ramified male follicles. The male and female openings are close together at the distal end. The gonads, and particularly the male follicles, are unattached and become easily separated from the mantle. The posterior left gonad is partially under the intestine. Small endocarps are present at both sides.

Remarks: this species was described from Chile, off Coquimbo at 450 m depth (Monniot & Andrade 1983), and was later reported from the northern coast of Peru (05° S) between 200 and 300 m (Millar 1988). We have found it at ca. 100 m depth, thus enlarging its known bathymetric range. This is the third finding of this species, well characterized by its hepatic gland, of unique morphology among the Styelidae.

Styela paessleri (Michaelsen, 1898)

Fig. 13D-F

References and synonymy: *Styela paessleri* Millar (1960) p. 115; Kott (1969) p. 121.

Localities: 1S, 4S, 8S.

The individuals of this species tend to be cylindrical, up to 35 mm in height, fixed posteriorly and with the siphons close together in the distal part. The tunic is firm, brownish, with wrinkles and tubercles. The internal morphology is typical of the genus. There are over 40 simple oral tentacles in large individuals. The aperture of the neural gland is variable ("U", inverted "U", or other shapes). The branchial sac has a smooth dorsal lamina and 4 folds. The second and fourth on each side (6-8 vessels on each) are less developed than the first and third (10-14 vessels). There are up to 10 stigmata per mesh between folds, crossed by parastigmatic vessels. The atrial area has a field of filiform papillae.

The digestive system has an elongated stomach with folds (over 15). The primary loop is closed (the descending intestine is adjacent to the stomach) and the secondary loop is very open. The final part of the intestine has a characteristic twist. It is bent towards the atrial opening, and at this level there is a narrowing of the intestine at which end lies the anus with a fringed margin.

The gonads are characteristic, two at each side. They consist of a branched ovary and male follicles at the ends of the branches. The genital apertures (both male and female close together) are at the extreme of the gonads, and they generally point anteriorly, not towards the atrial siphon. Even in immature specimens, incipient branching gonads and their apertures are visible. In large individuals, accessory apertures exist at other parts of the branching gonad. There are plenty of endocarps at both sides of the mantle. In some specimens, embryos and larvae have been observed in the peribranchial cavity. The larvae have ca. 150 μm of trunk length and bear a single pigmented spot.

Remarks: this species, characterized by its gonad morphology, is limited to Falkland Islands and mainland Patagonia, down to 121 m (Kott 1969). The fact that it is able to brood larvae had not been previously described and is extraordinary for a species of *Styela*. Brooding has been described only in a few solitary styelids, belonging to the genera *Dendrodoa* and *Polycarpa* (Pérez-Portela et al. 2009).

Pyura chilensis Molina, 1782

Figs 8A; 14A,B

References and synonymy: *Pyura chilensis* Van Name (1945) p. 333.

Localities: 2N, 3N, 6N, 7N

The individuals have in general an ovoid shape, up to 5 cm in diameter. They form often aggregated clumps. The tunic is tough, its surface has wrinkles, protuberances and papillae. The colour is generally orange, but diverse tones of brown are also encountered, to almost black. The siphons are relatively close together, quadrangular and red-coloured. The internal tunic of the siphons has pointed spinules.

The mantle has a strong musculature and is of a vivid orange turning red in the siphonal area. The oral siphon has over 18 branched tentacles (with second and third order ramifications). The aperture of the neural gland is in the shape of an "U" with the horns rolled inwards, but it becomes extremely convoluted in large individuals. From the pre-pharyngeal area to the entrance of the oesophagus (which is relatively short as the siphons are close) a series of dorsal languets extends. The atrial siphon has a large bi-lobed velum. The branchial sac has 6 folds on each side, the ventral-most being the less developed. Except for these, there are 19 to 25 longitudinal vessels in each fold and 3-6 between folds. Up to 9 stigmata can be found in a single mesh in the central part of the branchial sac. In large individuals the posterior end of the longitudinal vessels on the folds protrudes freely near the oesophagus entrance (particularly those of the more ventral folds).

The digestive system forms an open primary loop and a slight secondary loop. There is a voluminous hepatic gland branching off from a common stem united to the stomach. Endocarps are present on the descending part of the intestine and the rectum, and they connect with the branches of the hepatic gland, thus the hepatic diverticula form a "bridge" over the primary loop. There is a well-developed gonad at each side. The left one enclosed in the primary loop. The right one strongly bent in the posterior part, reaching forward until the aperture of the atrial siphon. Each gonad is composed of alternating lobes at both sides of a central axis. The female portion occupies the proximal and basal part of these lobes and the male part lies distally. Endocarps appear over the gonad lobes. The ends of the two gonads and the anus lie close together posterior to the atrial siphon. Sometimes the distal parts of the two gonads coalesce and form a mass of tissue, to which the distal part of the rectum can also be fused. There are usually several apertures at the distal end of each gonad, and it is difficult to tell which corresponds to male or female ducts. One or several of the apertures point towards the atrial siphon, while the distal parts of other gonad ducts are bent 180º and point opposite it.

Remarks: surprisingly few descriptions exist for this species, which is very abundant on natural and artificial substrates in the Coquimbo area. The species is found in the low intertidal along the Chilean and Peruvian coast, and it sustains an important fishery in Chile (Davis 1995). Haye & Muñoz-Herrera (2013) highlighted the genetic diversity of this species in populations spanning 1800 Km of Chilean coastline, and the two sequences obtained here belong one each to their haplogroups 1and 2.

Pyura legumen (Lesson, 1830)

Fig. 14 C,D

References and synonymy: *Boltenia legumen* Lesson (1830a) p. 149; Millar (1960) p. 119; *Pyura legumen* Van Name (1945) p. 326; Kott (1969) p. 133; Monniot & Monniot (1983) p. 88.

Localities: 1S, 2S

The individuals are ovoid, up to 6 cm in maximal dimension. The oral siphon is terminal and the atrial siphon is widely separated from it. The individuals are borne on a stalk that originates ventrally. The tunic is thin but firm, of brownish-pinkish tones, and is covered by branched spines (to 0.5 mm in length). The tunic of the inner side of the siphons is carpeted by minute (70-100 μm) pointed spines. There are ca. 12 well-developed branched tentacles with smaller ones in-between. The aperture of the neural gland is "U" shaped with horns rolled inward. There are seven folds on each side of the branchial sac. The stomach is long, and the intestine describes a narrow primary loop all along the ventral side, with almost no secondary curvature. There is one gonad on each side, the

left one within the intestinal loop. The most prominent internal feature of this species is the presence of two big endocarp-like elongated masses at each side of the body (dorsal to the gonads and digestive tube), and three rounded, smaller ones surrounding the aperture of the atrial siphon.

Remarks:this is a well-known species present in the Patagonian shelf and the Falkland Islands. Sanamyan & Schories (2003) report its presence in the vicinity of Punta Arenas. Monniot & Monniot (1983) discuss the morphological features of the stalked *Pyura* species from the Antarctic and subantarctic waters.

Paramolgula gigantea (Cunningham, 1871)

Fig. 15

References and synonymy: *Molgula gigantea* Herdman (1882) p. 69; *Paramolgula gigantea* f *typica* Ärnbäck-Christie-Linde (1938) p. 15.

Localities: 1S, 2S, 7S.

Individuals up to 4 cm in diameter, body globular with whitish to brownish coloration. Tunic smooth or with small protuberances. There were no well-developed rhizoids, but the ventral part of the tunic often agglutinates some sediment and, occasionally, epibionts cover the tunic surface. The quadrangular siphons are short and relatively close together.

The mantle has abundant muscular fibres. The buccal siphon has ca. 15 heavily branched (up to 4 orders of branching) tentacles. The aperture of the neural gland forms an "U" tilted towards the right side and with the horns rolled inward. There is a smooth dorsal lamina. The branchial sac has 7 false folds, each formed by an elevated longitudinal vessel, on each side. Five conspicuous transverse vessels cross the branchial sac. In the meshes formed by the longitudinal and transverse vessels there is a network of smaller vessels underneath which lie the irregular stigmata, often arranged in spiral shapes.

The digestive has a stomach with a hepatic gland forming papillae (not arranged in rows) and a thin-walled intestine describing a double loop, the secondary loop completely closed. The renal sac on the right hand side is curved. The left gonad is adjacent to the distal part of the primary intestinal loop, in contact with the intestine and clearly outside the secondary loop. The right gonad is in contact with the renal sac. The gonads are elongated or globular, with a basal female part and testicular lobes occupying the sides and posterior part of the gonad. The female apertures are single, and there can be from one to four male papillae in each gonad.

Remarks: We have assigned the specimens of *Paramolgula* found in abundance in the Magellanic region to *P. gigantea*, a species previously synonymized with *P. gregaria* by Van Name (1945). This author noted the great variability of this taxon and decided to "lump" all species with similar appearance under *P. gregaria*. Later authors (e.g. Millar 1960, 1970, Kott 1969) have adopted that same list of synonyms, although some (e.g., Monniot & Monniot 1983) pointed out that the taxonomic status of this species needs revision.

Several authors have remarked the position of the left gonad in *Paramolgula gregaria* as being dorsal to, and partly in, the secondary loop of the digestive system (Van Name 1945, Kott 1969, Monniot & Monniot 1983). *P. gigantea* was described (as *Cynthia gigantea*) by Cunningham (1871) with little morphological information. Later on, Herdman (1882) described it in more detail (as *Molgula gigantea*) and defined the left gonad as lying "anteriourly to the intestine" (and so it has to be outside the secondary loop). In the same work, this author redescribed *P. gregaria* (as *Molgula gregaria*), a species described by Lesson (1830a), again with only superficial external description. According to Herdman, in *P. gregaria* the left gonad lies "in front of the intestine on the ventral side of the rectum" (thus in the aperture of the secondary loop). There are, therefore, two forms differing in the position of the left gonad. This difference is likely to be of taxonomic value, all other characters being in general similar (or at least with overlapping variability).

Ärnbäck-Christie-Linde (1938) reported *Paramolgula gigantea* from Tierra del Fuego and Falkland Islands, with the left gonad "above both primary and secondary intestinal loops". She also described a new form, *P. gigantea* f. *capax*, with the left gonad "in the concavity between the intestinal loop and the rectum, partly above the loop". Apparently she didn't realize that her *P. gigantea* f. *capax* was identical with *P. gregaria*, and indeed her drawings were used by Van Name (1945) to illustrate *P. gregaria*. For some reason, the distinction between species with the gonad outside the secondary loop and those with the gonads (partly) inside the secondary loop was not maintained and everything was considered as *P. gregaria* since Van Name (1945).

We have found only specimens with the left gonad totally outside the secondary loop, anterior to its aperture. We placed them in *P. gigantea*, which we consider a valid species. We have not found any "true" *P. gregaria* according to this character. Recent papers on the Chilean coasts report *P. gregaria* (Lagger et al. 2009, Tatián & Lagger 2010) and both refer that the left gonad is included in the secondary loop. This may be, however, a misinterpretation, as the figure in p. 904 of Tatián & Lagger (2010) clearly shows a gonad outside and anterior to the secondary loop. Clearly, all citations of *P. gregaria* need to be revised. *P. chilensis* Hartmeyer, 1914, a species with the left gonad outside the secondary loop may be the same as *P. gigantea*. Another described species, *P. canioi*

Monniot & Monniot, 1983, also has the left gonad outside the secondary loop, but it seems to be consistently of smaller size (up to 1.5 cm). It is likely that there are several species of *Paramolgula* with the left gonad in either position. The taxonomy of this genus still requires clarification, but this task is outside the scope of the present work. We note that our specimens have the left gonad outside the secondary loop, name them as *P. gigantea*, and call for caution when considering previous citations of the genus in the subantarctic area.

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Table 1. Localities sampled, with coordinates and depth range

Magellanic Region	Code	Depth range (m)	Latitude	Longitude
Bahía Porvenir 1	1S	0-3	-53.2991	-70.3813
Bahía Porvenir 2	2S	0-3	-53.3111	-70.435
Punta Carrera	3S	0-10	-53.5891	-70.92
Muelle Loreto	4S	0-2	-53.1652	-70.9008
Bahía Mansa	5S	0-10	-53.6086	-70.93
Miraflores	6S	0-5	-53.1786	-70.918
Cabo Negro	7S	0-4	-52.93	-70.8027
Muelle Enrique Cabello	8S	0-2	-53.1444	-70.8786
Muelle Prat	9S	0-4	-53.1705	-70.9058
Temperate Region				
La Herradura	1N	0-12	-29.9663	-71.3544
Muelles UCN	2N	0-2	-29.9663	-71.3536
Bahía Tongoy	3N	0-4	-30.2586	-71.5047
Off-shore N of Coquimbo	4N	101	-29.7188	-71.6086
Off-shore Huasco	5N	104	-28.5041	-71.3266
La Pampilla	6N	0-2	-29.9533	-71.3627
Punta Choros	7N	0-3	-29.2505	-71.4627
UCN aquaculture facilities	8N	0-1	-29.9655	-71.3544

Table 2. List of species found, with indication of the type of substrate (A: artificial, N: natural), region of collection, and accession numbers of the sequences generated (numbers in parenthesis indicate number of specimens for which the same sequence was retrieved).

	Species	Substrate	Region	Acc. Number
Aplousobranchia Lahille, 1886				
Holozoidae Berrill, 1950	*Distaplia colligans* Sluiter, 1932	N	Magellanic	KU299767
Didemnidae Giard, 1872	*Didemnum studeri* Hartmeyer, 1911	A,N	Magellanic	
	Diplosoma listerianum (Milne Edwards, 1841)	A,N	Temperate	KU299766 (2)
	Lissoclinum perforatum (Giard, 1872)	A	Temperate	KU299768
	Polysyncraton trivolutum (Millar, 1960)	N	Magellanic	
Polyclinidae Milne Edwards, 1841	*Aplidium fuegiense* (Cunningham, 1871)	A	Magellanic	
	Aplidium peruvianum Sanamyan & Schories, 200	N	Temperate	KU299754-56
	Aplidium variabile (Herdman, 1866)	A	Magellanic	KU299757
	Synoicum georgianum Sluiter, 1932	A	Magellanic	KU299781
Phlebobranchia Lahille, 1886				
Cionidae Lahille, 1887	*Ciona robusta* Hoshino & Tokioka, 1967	A,N	Temperate	KU299762 (2), KU299763
Corellidae Lahille, 1888	*Corella eumyota* Traustedt, 1882	A,N	Temperate	KU299765 (3)
Stolidobranchia Lahille, 1887				
Styelidae Sluiter, 1895	*Asterocarpa humilis* (Heller, 1878)	A	Temperate	KU299758 (2), KU299759
	Botryllus schlosseri (Pallas, 1766)	A	Temperate	KU299760-61
	Cnemidocarpa verrucosa (Lesson, 1830)	A	Magellanic	KU299764
	Polyzoa opuntia Lesson, 1830	A	Magellanic	KU299770-73
	Polyzoa minor Monniot, 1970	N	Temperate	
	Polyzoa iosune sp nov	N	Temperate	KU299774-75
	Styela changa Monniot & Andrade, 1983	N	Temperate	
	Styela paessleri (Michaelsen, 1898)	A	Magellanic	KU299779-80
Pyuridae Hartmeyer, 1908	*Pyura chilensis* Molina, 1872	A,N	Temperate	KU299776
	Pyura legumen (Lesson, 1830)	A	Magellanic	KU299777-78
Molgulidae Lacaze-Duthiers, 1877	*Paramolgula gigantea* (Cunningham, 1871)	A	Magellanic	KU299769 (3)

Figure captions

Figure 1. Map of the coast of Chile with indication of the sampling localities at the two areas surveyed. Locality codes as in Table 1. The main oceanic currents: west wind drift (wwd), Cape Horn Current (CHC) and Humbold Current (HC) are indicated.

Figure 2. *Distaplia colligans*. A, image of a colony; B, zooid as seen from the left hand side; C, Thoracic region as seen from the right hand side; D, larva. Scale bars: A, 2 cm; B,C,D, 0.5 mm.

Figure 3. Didemnid species. *Didemnum studeri*: A, colonies, B, anterior region of a larva; *Polysyncraton trivolutum*: C, image of a colony, D, thorax in dorsal (left) and lateral (right) view; E, spicules of *Didemnum studeri*; F, spicule of *Lissoclinum perforatum*; G, spicule of *Polysyncraton trivolutum*. Scale bars: A, 5 cm; B, 0.1 mm; C, 1 cm; D, 0.25 mm; E, 10 μm; F, 5 μm; G, 10 μm.

Figure 4. *Aplidium fuegiense*. A, image of a colony, B, thorax with a commensal crustacean inside; C, abdomen and postabdomen. *Aplidium variabilis*. D, colony; E, anterior part of a zooid; F, larva. Scale bars: A, 2 cm, B, 1 mm, C, 2 mm, D, 2 cm, E, 1 mm, F, 0.25 mm.

Figure 5. *Aplidium peruvianum*. A, colony; B, thorax; C, stomach; D, larva. Scale bars: A, 2 cm, B, 0.5 mm, C, 0.2 mm, D, 0.2 mm;

Figure 6. *Synoicum georgianum*. A, colony; B, zooid; C, abdomen (stained material); D. transverse section at the stomach level. Scale bars: A, 1 cm, B, 1 mm, C, D, 0.5 mm.

Figure 7. A, *Ciona robusta* on a buoy, note also some *Asterocarpa humilis* (orange-coloured). *Corella eumyota*. B, whole individual; C, dissected individual; D, right side of the mantle, branchial sac eliminated. Inset shows magnification

of the zone of the genital openings. The specimen in C and D has been stained. Scale bars: A, 10 cm, B, 2 mm, C, D, 1 mm.

Fig. 8. *Asterocarpa humilis*. A, some specimens on *Pyura chilensis* (orange papillae belong to *P. chilensis* tunic); B, close up of the siphons (a colony of *Diplosoma listerianum* grows on the tunic); C, dissected individual; D, close up of the digestive loop, branchial sac removed; E, gonads of the right-hand side, branchial sac removed. The specimens in C-E have been stained. Scale bars: A, 2 cm, B, 1 cm, C, 1 cm, D, E, 1 cm.

Fig. 9. *Cnemidocarpa verrucosa*, A, natural aspect; B, dissected body. Scale bar: A, B, 2 cm.

Figure 10. *Polyzoa iosune*. A, image of a colony carpeting the substrate and overgrowing *Aplidium peruvianum* (white masses) exposed at low tide; B, close-up of the apical part of the zooids; C, mantle of two zooids, vision of the right side (left of the image) and ventral vision (right of the image); D, dissected zooid; E, same zooid without branchial sac; F, G, details of the branchial sac; H, close-up of hermaphrodite gonads from the right side of the zooid (endostyle is towards the upper-left of the image); I, larva. Images D-H are of stained material. Scale bars: A, 5 cm, B, C, 5mm, D, E, 2 mm, F, 1 mm, G, 0.2 mm, H, 1 mm, I, 0.2 mm.

Figure 11. *Polyzoa minor*. A, colony; B, zooid dissected; C, left side of the body without branchial sac; D, close-up of hermaphrodite gonads from the right side of the body; E, larva. Images B-D are of stained material. Scale bars: A, 5 mm, B, C, 1 mm, D, 0.15 mm, E, 0.2 mm.

Figure 12. *Polyzoa opuntia*. A, colonies; B, zooid dissected without branchial sac (stained), inset shows magnification of gonads and endocarps on the left side. Scale bars: A, 2 cm, B, 1 cm.

Figure 13. *Styela changa*. A, whole individual; B, C, dissected digestive system as seen from the branchial side (B) and from the mantle side (C); D, Specimens of *Styela paessleri* partially overgrown by *Didemnum studeri*; E, dissected mantle of an immature individual, branchial sac removed (stained material); F,

anterior part of a larva. Scale bars: A, 2 mm, B, C, 0.5 mm, D,E, 1 cm, F, 50 μm.

Figure 14. A, *Pyura chilensis*, group of individuals (with some *Asterocarpa humilis*); B, dissected individual, branchial sac taken out; C, *Pyura legumen*, whole individual; D, close up of an oral siphon; D, siphonal spines. Scale bars: A, 5 cm, B, 1 cm, C, 2 cm, D, 0.5 mm, E, 20 μm.

Figure 15. *Paramolgula gigantea*. A, underwater image of an specimen from Porvenir Bay; B, dissected individual; C, same individual without branchial sac; D, close-up of the aperture of the left gonad (stained), showing one female (♀) and three male openings (short tubes around ♀); E, image of the branchial sac (stained). Scale bars: A, B, C, 2 cm, D, 2 mm, E, 0.2 mm.
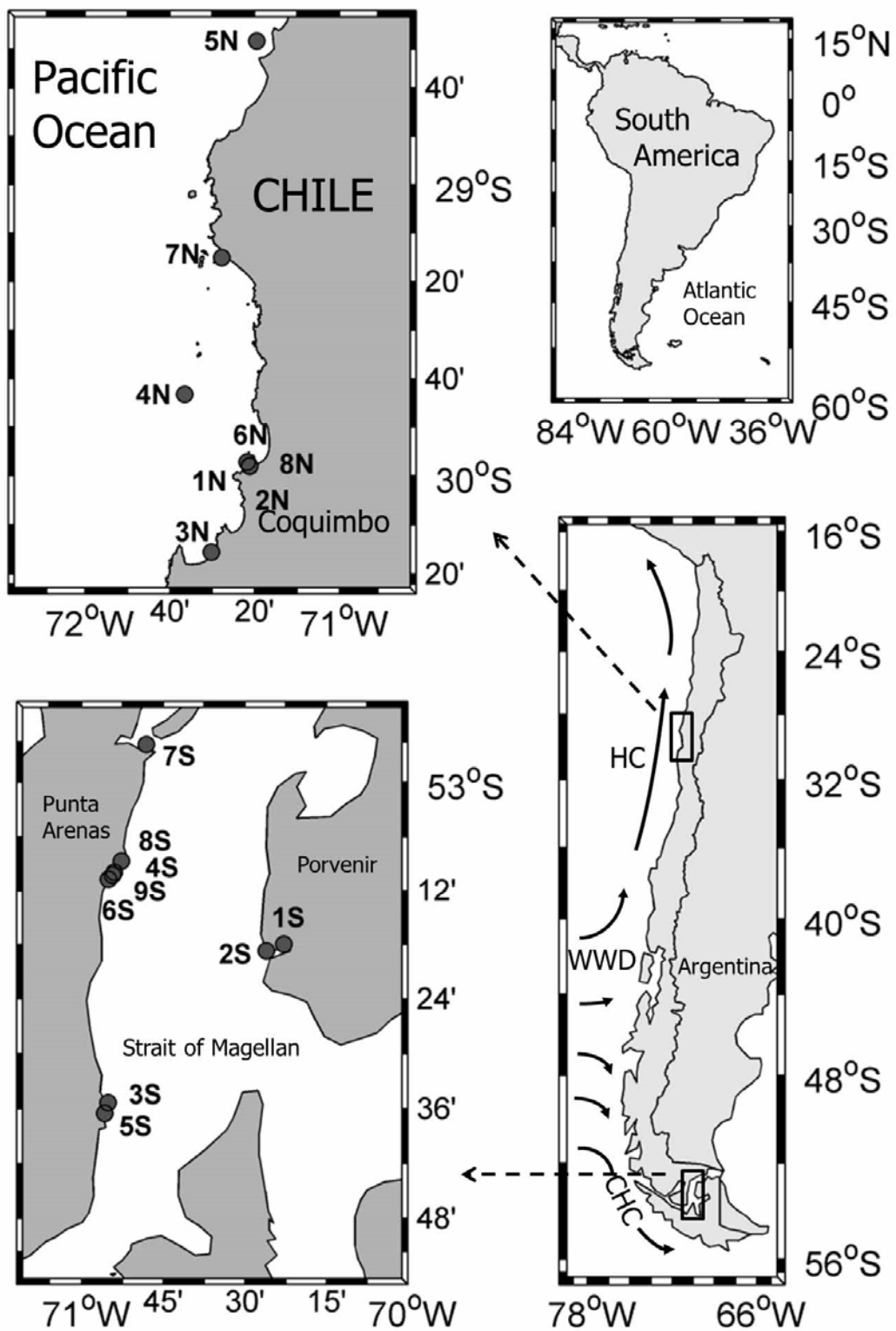
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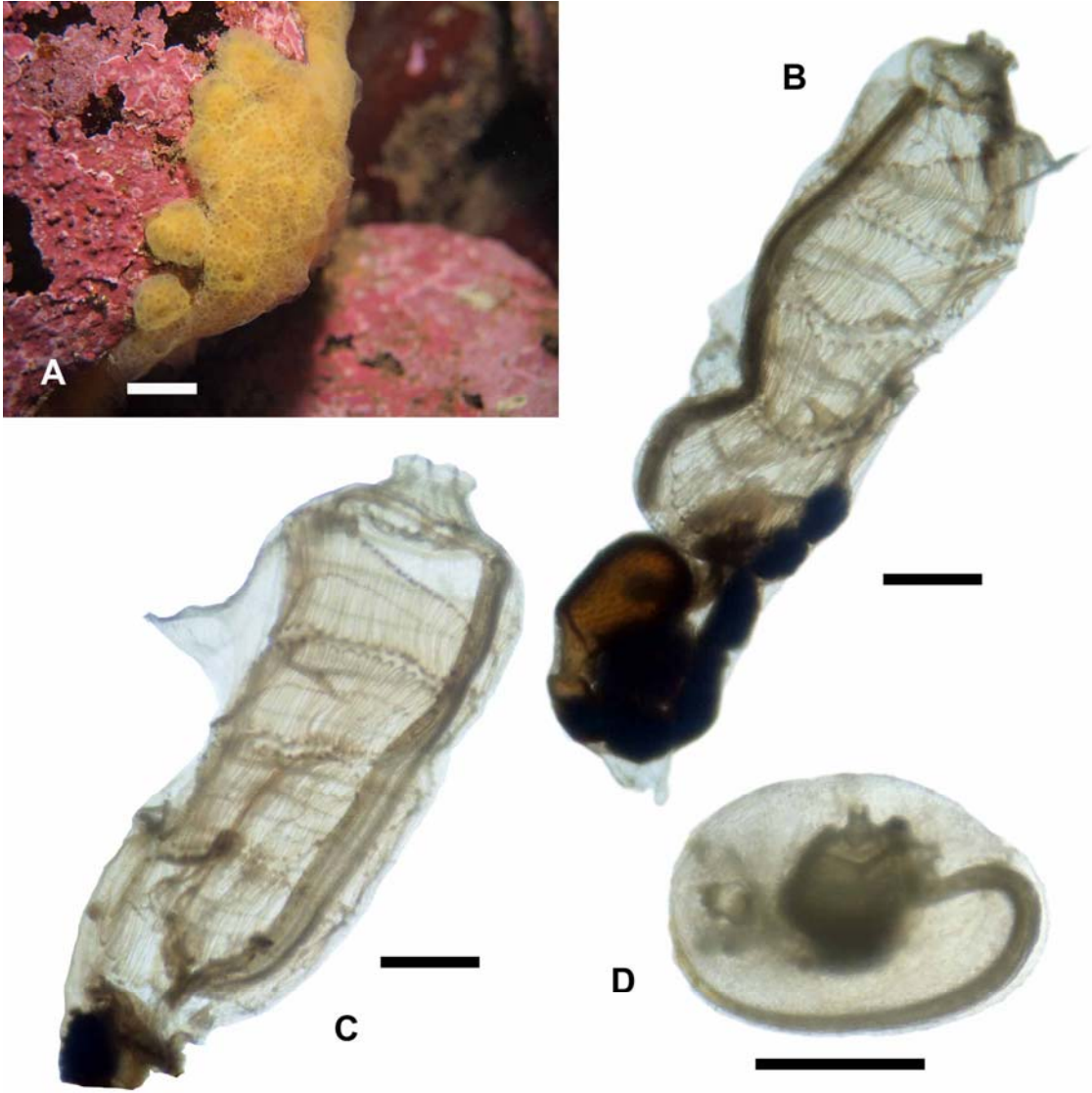
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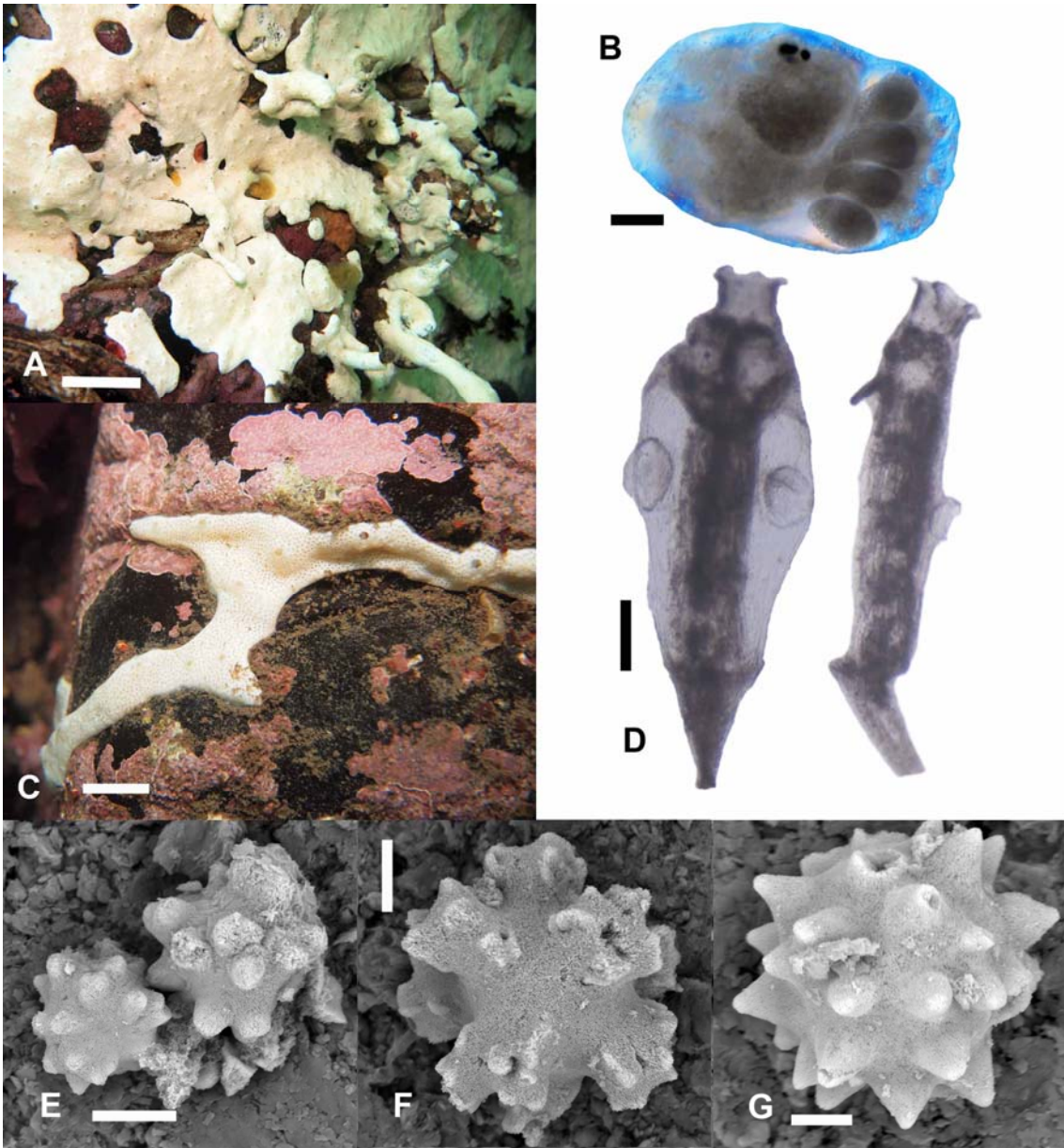
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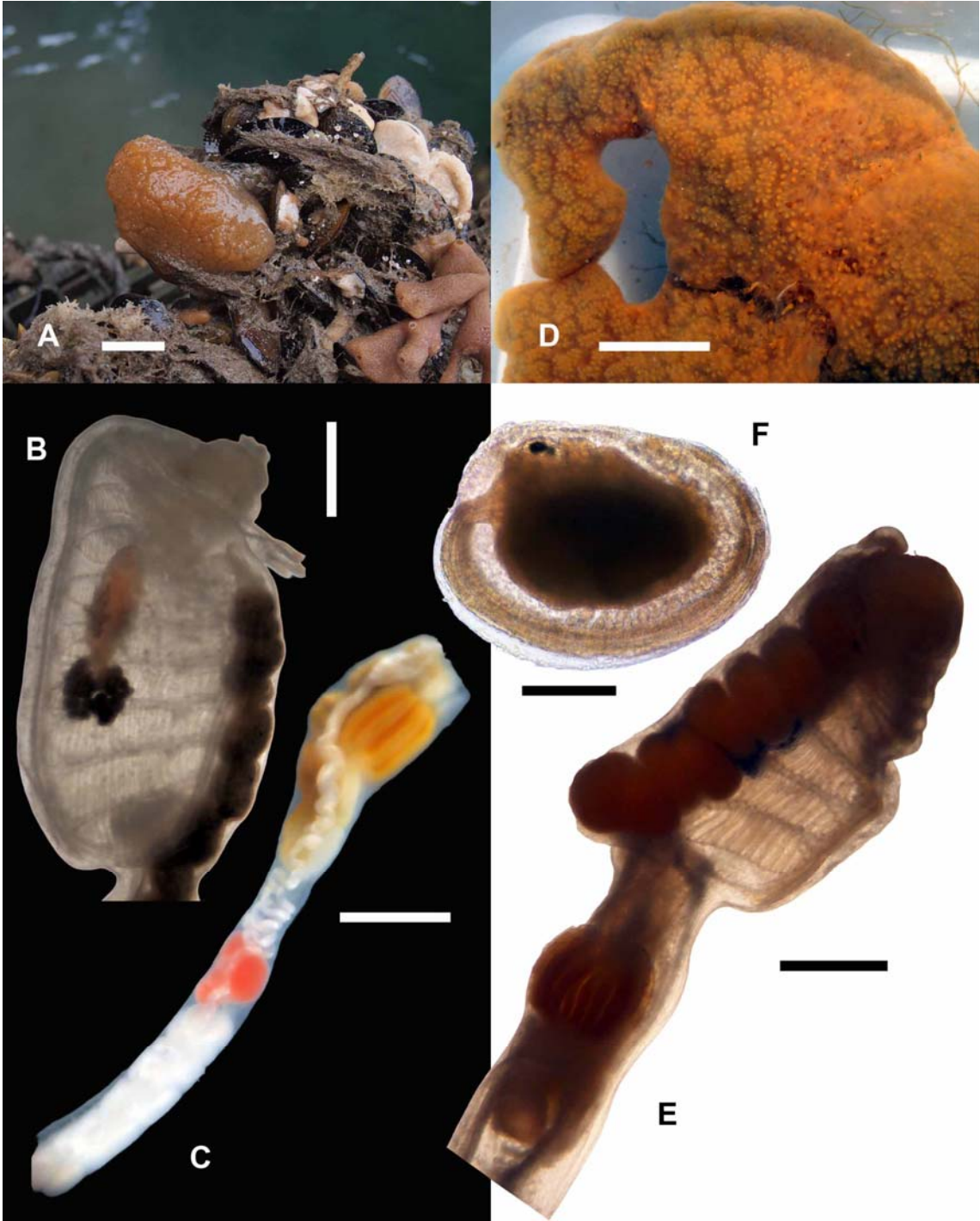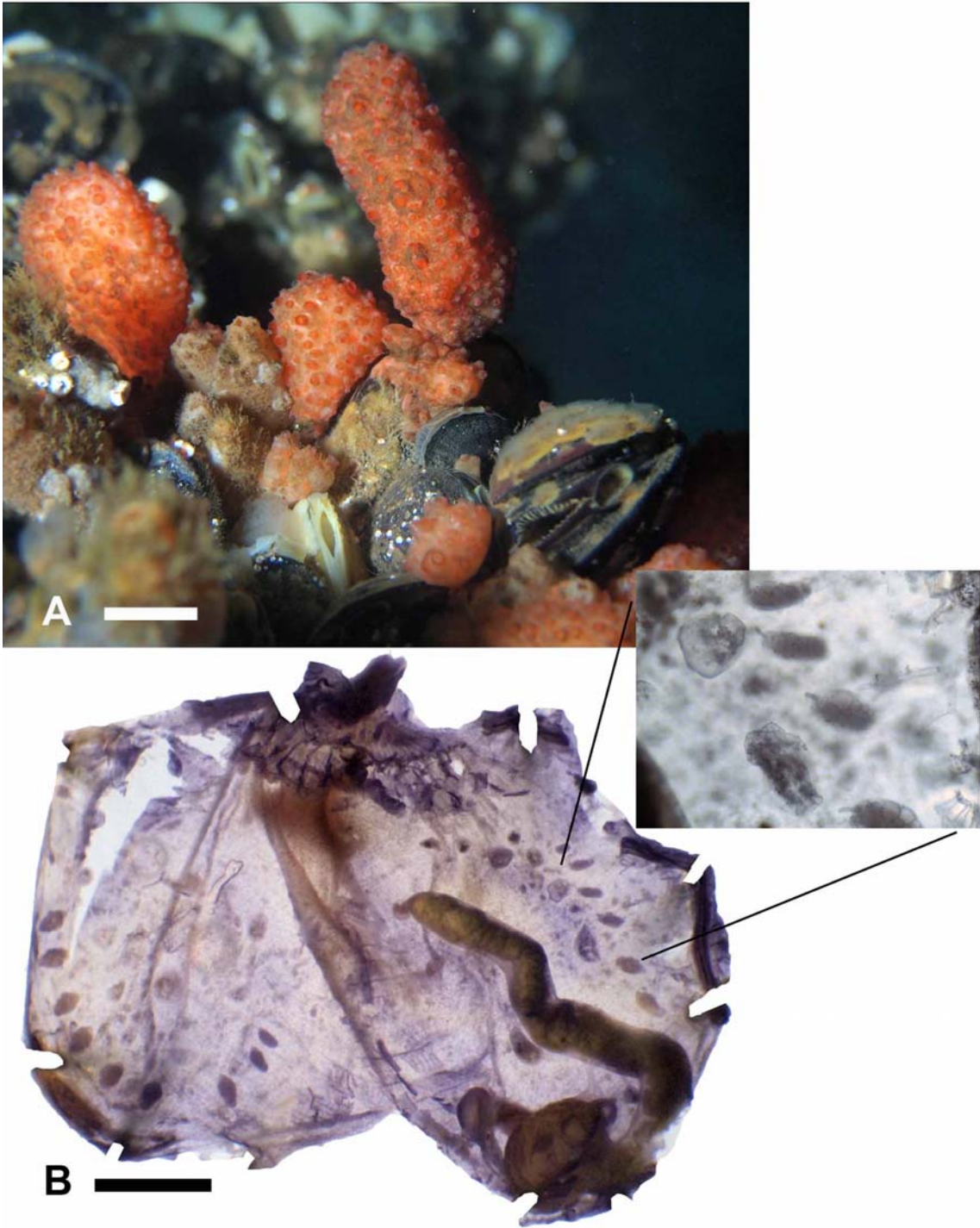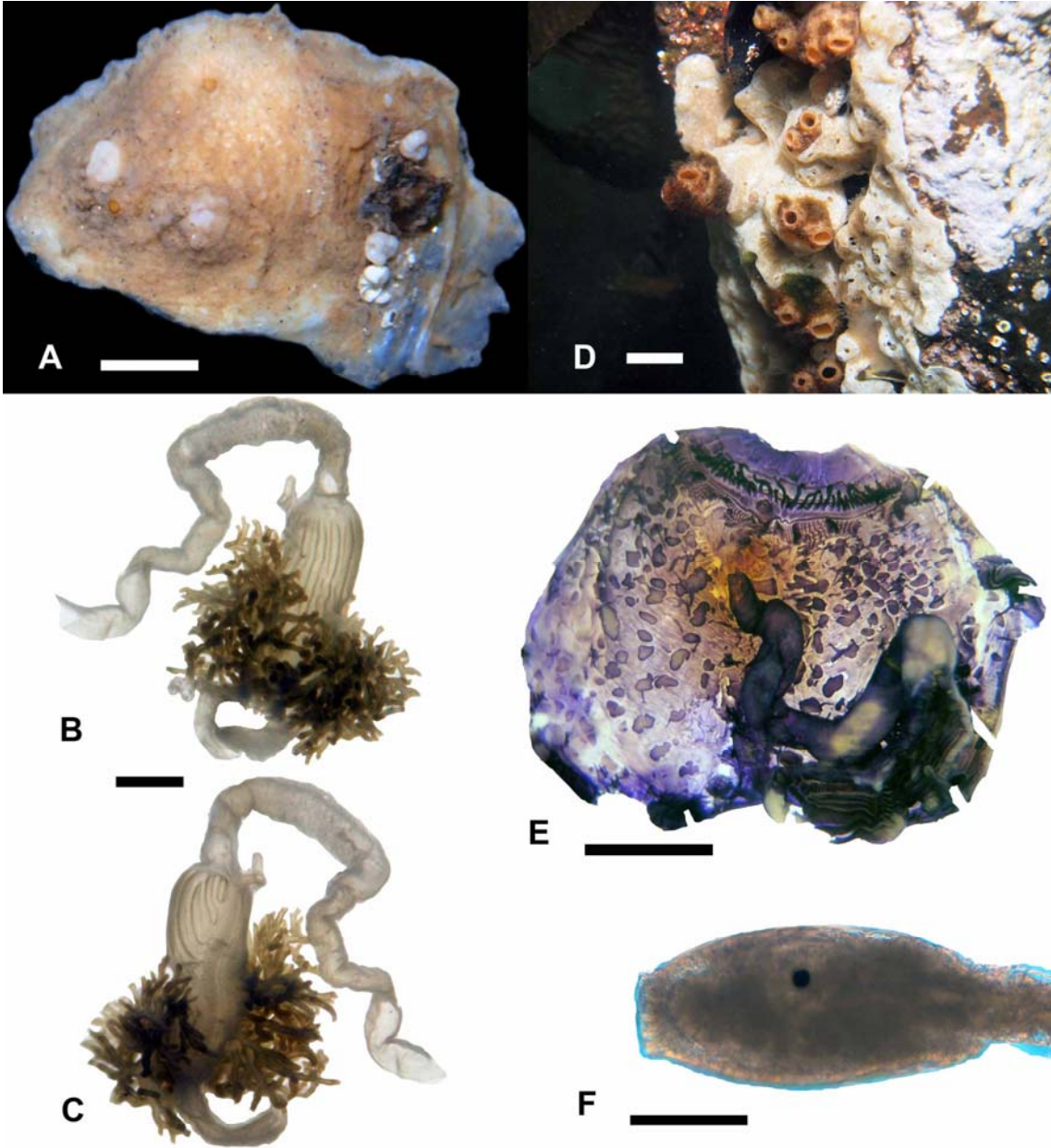
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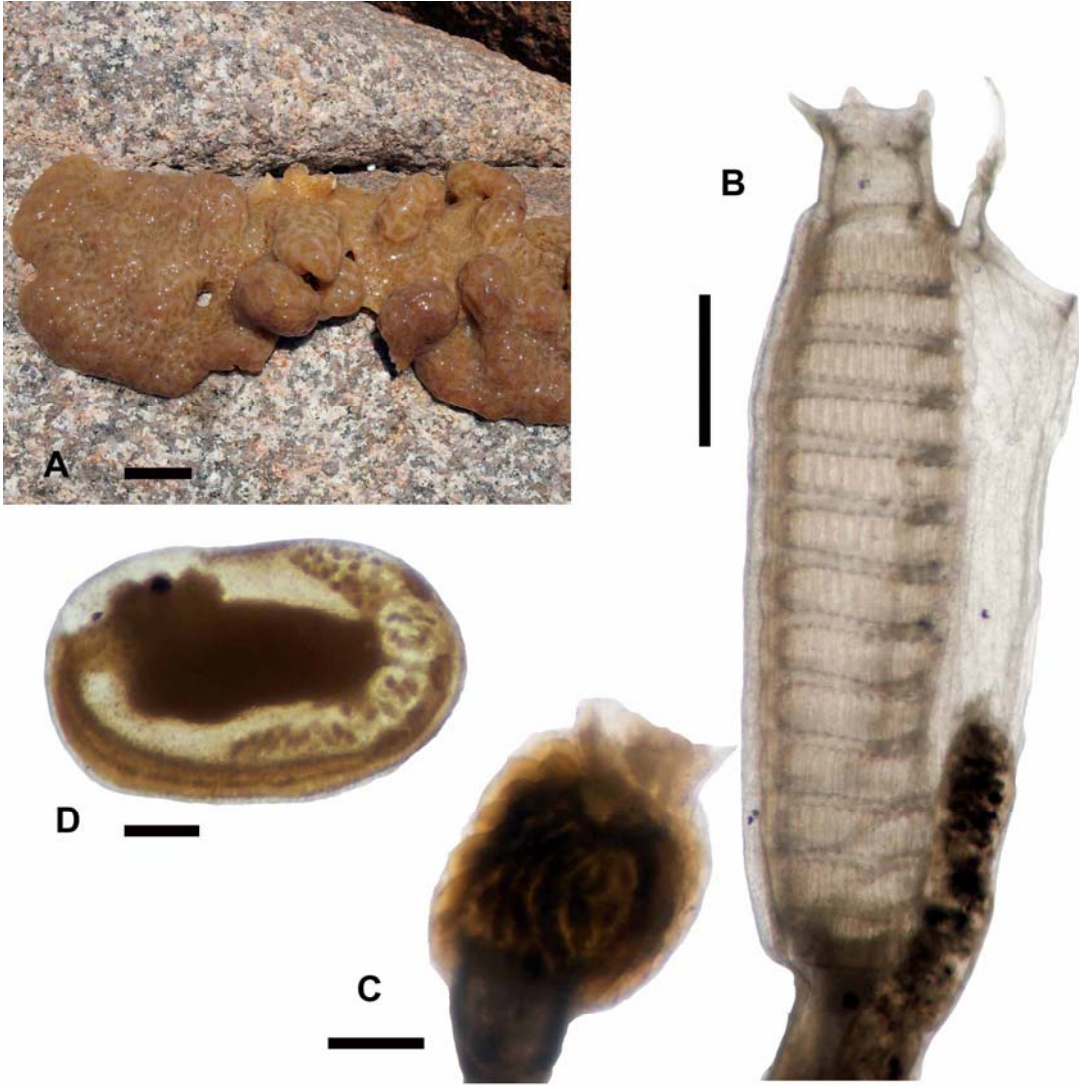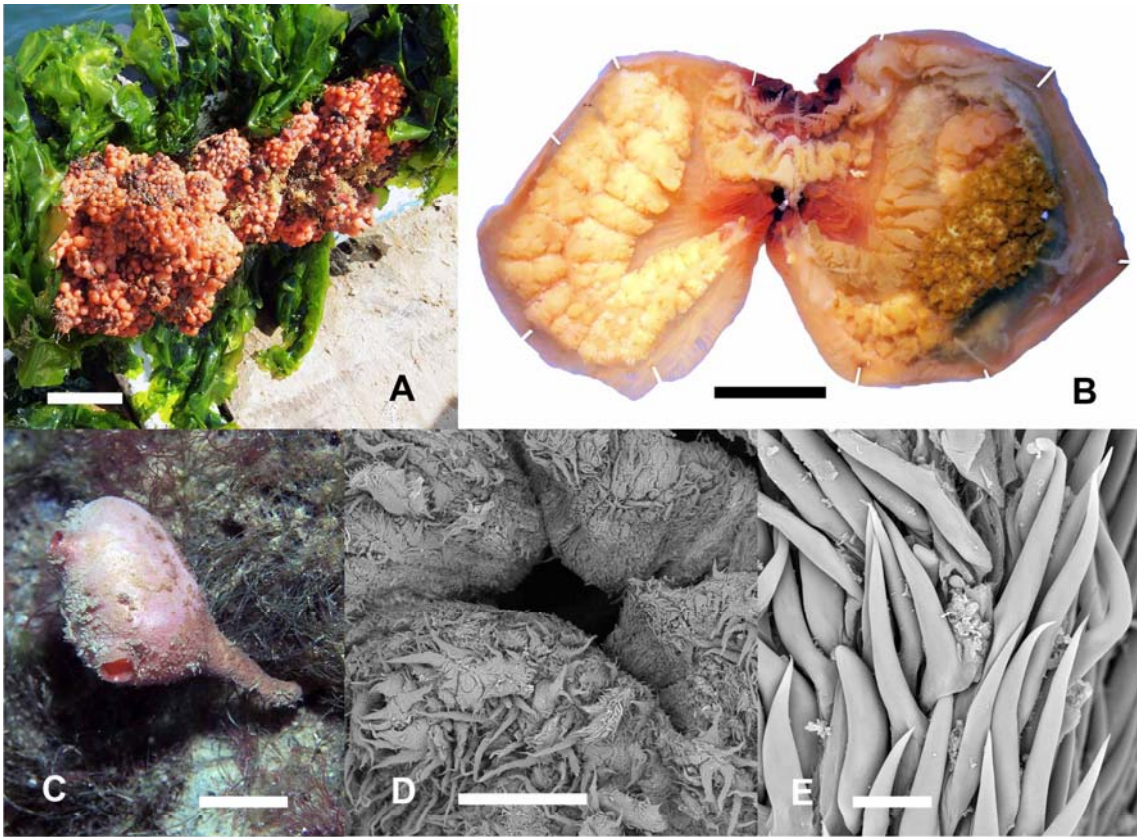
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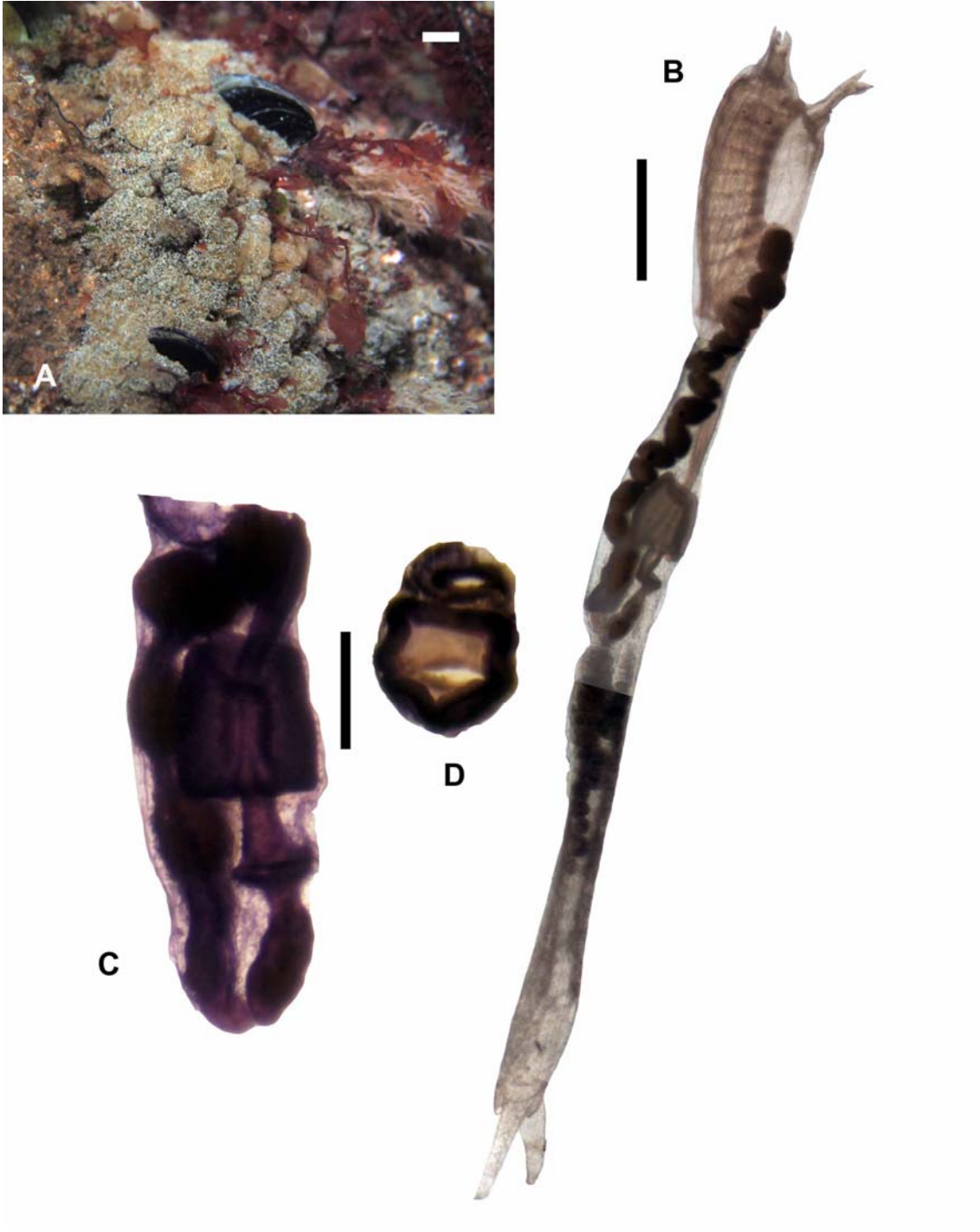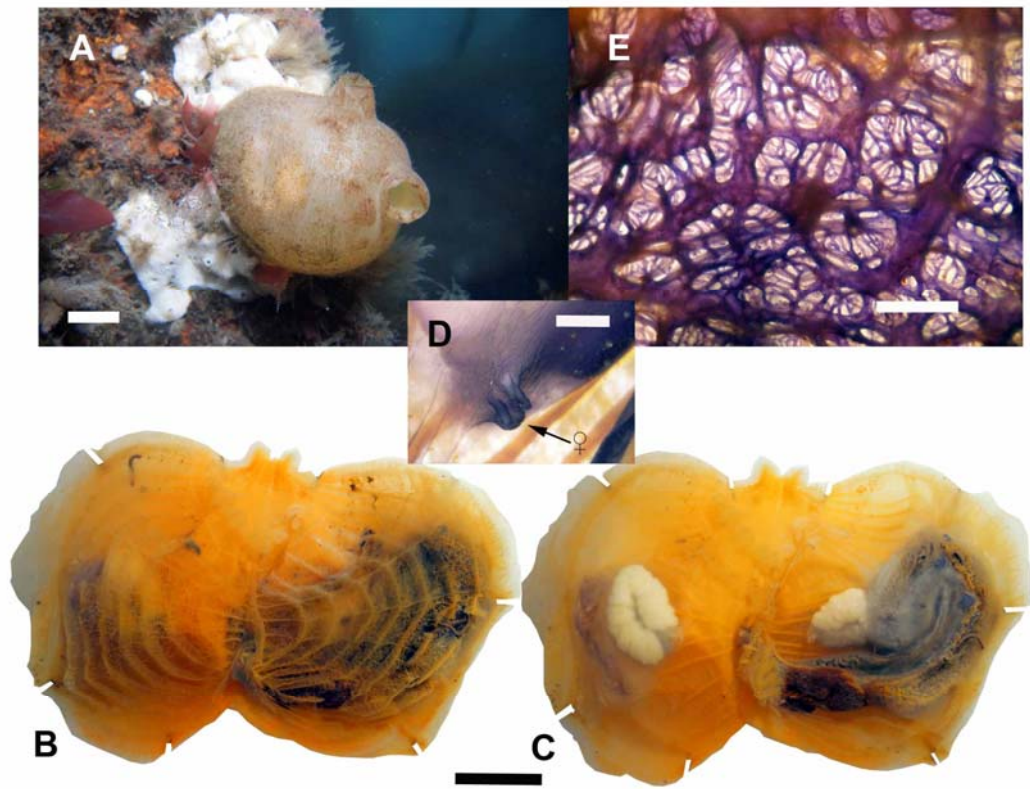
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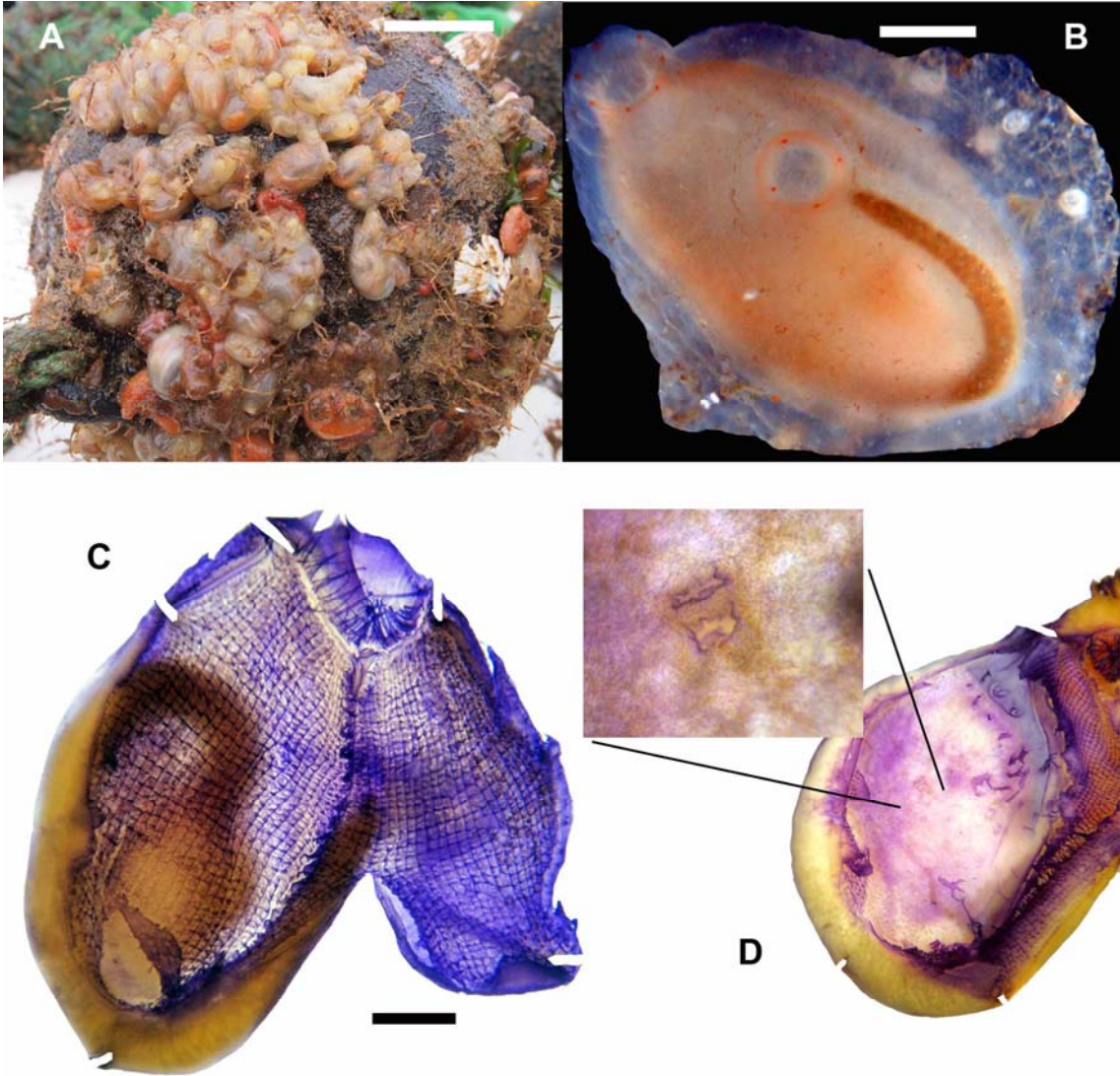
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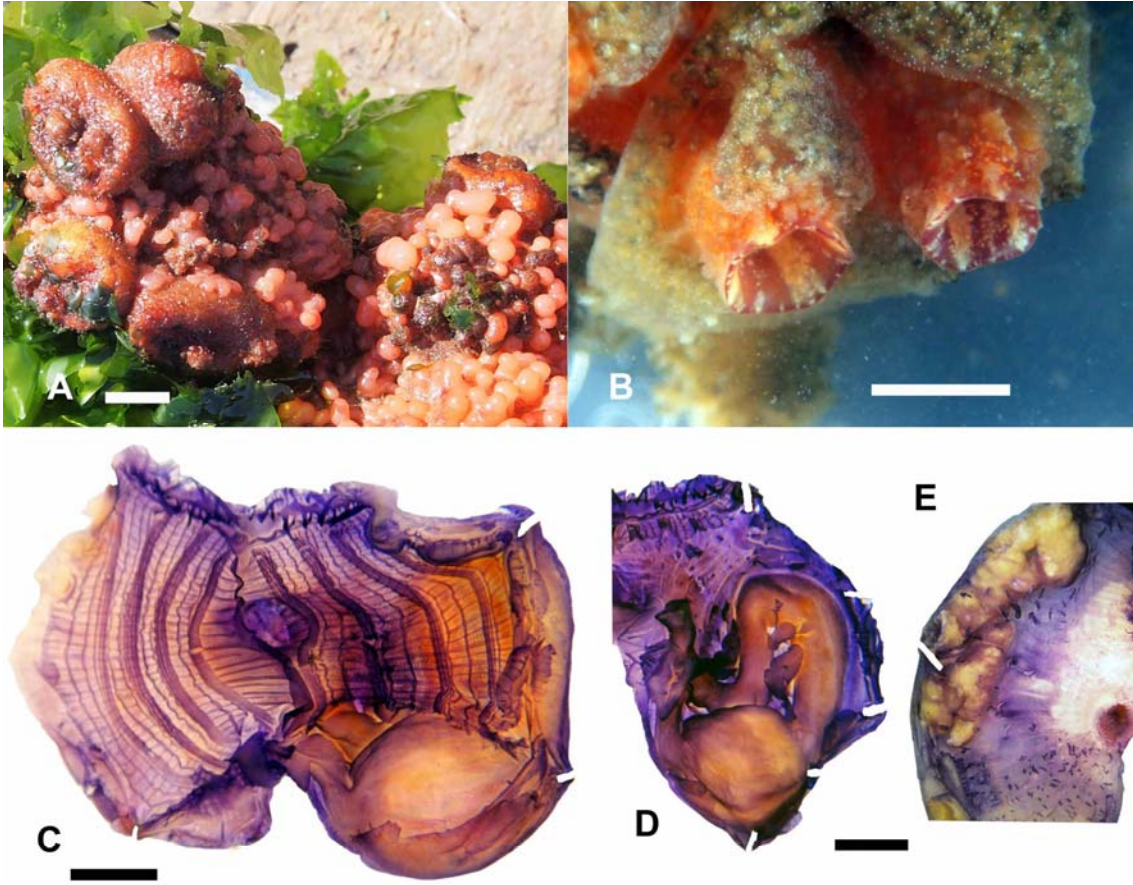
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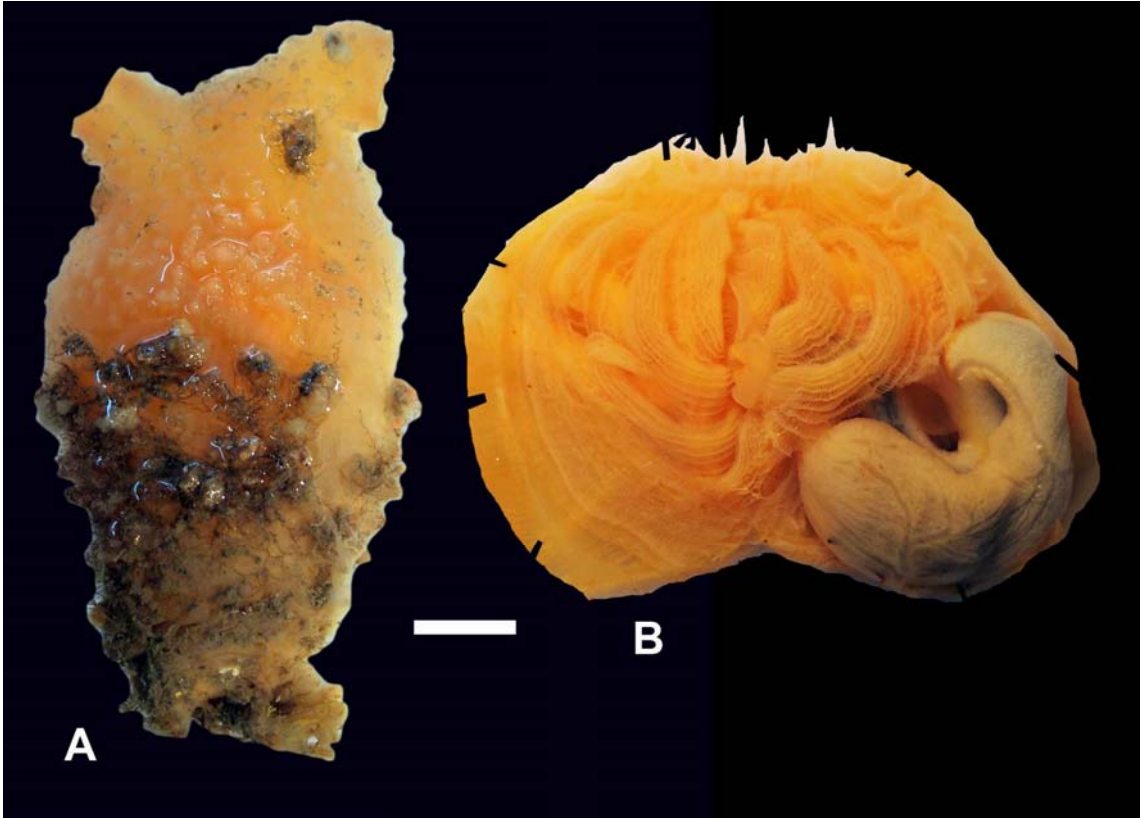
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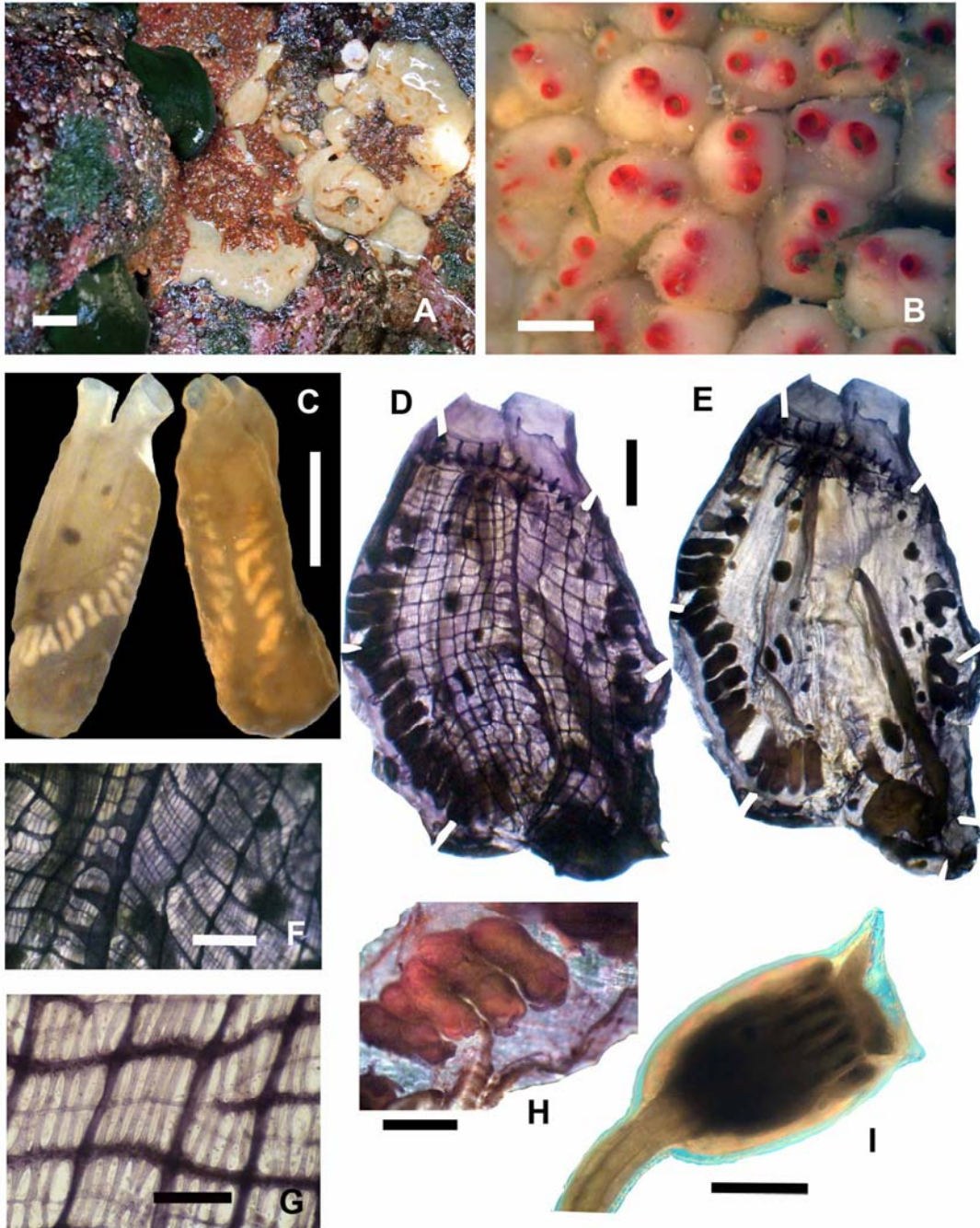
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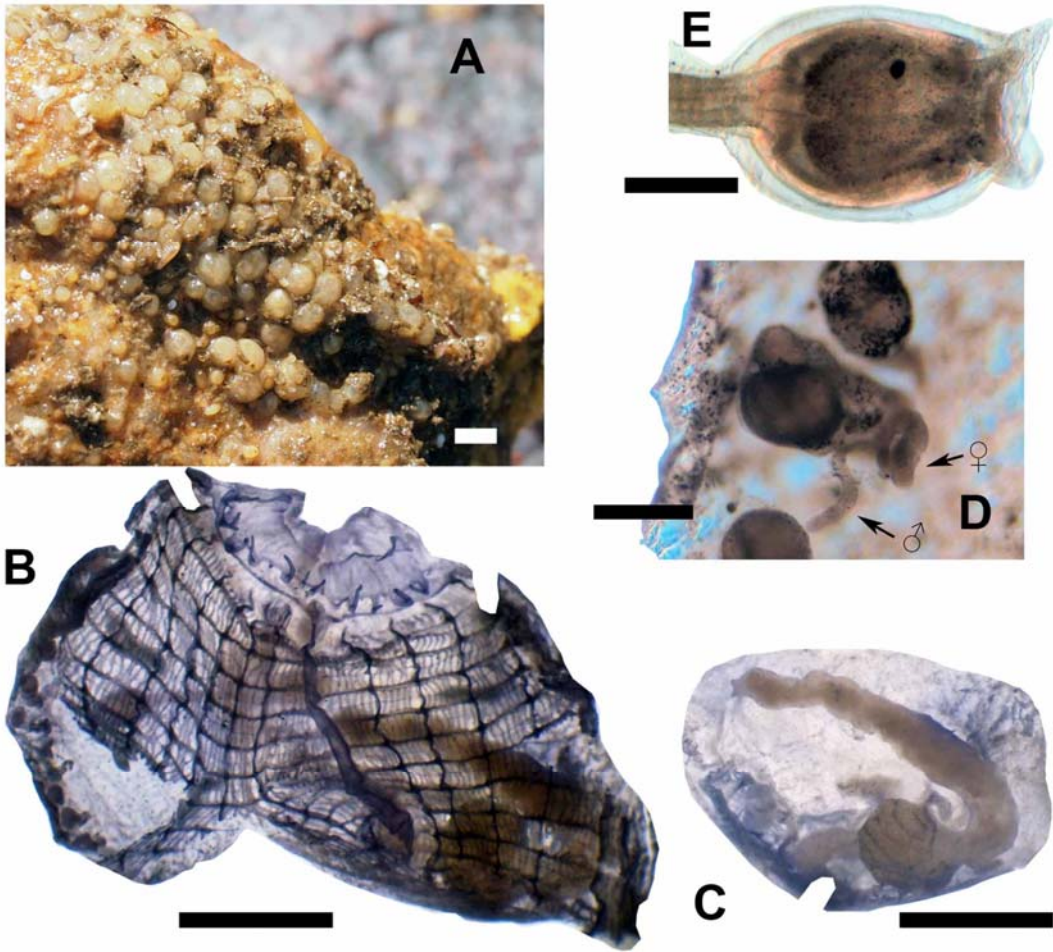
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