NOTA CIENTÍFICA

Polyzoa opuntia (Ascidiacea) in the Magellan Region, Chile: Colony shape changing with size and substrate

Polyzoa opuntia (Ascidiacea) en la región de Magallanes, Chile: Forma colonial cambia con el tamaño y tipo de sustrato

Juan I. Cañete[1]

The discovery of large colonial ascidians remain as unusual or striking fact (Ramos-Esplá & Ocaña, 2017). Morphotypes of elongated colonies has been cited in some ascidians families such as Didemnidae and Holozoidae (Millar, 1971; Monniot *et al.* 1991), with most singular case in *Distaplia cylindrica* from Antarctic waters which can reach to 7 m of length (Kott, 1969). Other reports shown colonies of *Eudistoma elongatum* (Herdman, 1886), *Distaplia cylindrica* (Lesson, 1830) and *Aplidium proliferum* (Milne Edwards, 1841) attaining to 2,5 m lenght (Page & Kelly, 2013; Ramos-Esplá & Ocaña, 2017). These species shown colonies elongated to cylindrical shape with form of drop or tears (Biosecurity New Zealand, 2007).

In *E. elongatum* there is a seasonal pattern in the colonial growth where the colonies regress and over-winter as small (c. 10 mm) cream buds and re-growing the following spring to larger colonies (Page & Kelly, 2013). Fast growth produce didemnid colonies of great size, extending over more than a square meter when the substrate is large enough. However, this colonies are encrusting (Monniot *et al*. 1991).

Some patagonian specimens of *Polyzoa opuntia* has been sized as large as 15 cm of length in some Chilean fjords (Tatián & Lagger, 2009). Others Chilean populations of *Polyzoa opuntia* form colonies of 10 cm or more in extent, comprising numerous zooids averaging about 10 mm height and 4 mm diameter. Zooids are typically cylindrical and connected by a basal test only (Turon *et al.* 2016b). However, these colonies are usually found on thin elongated objects, such as polychaete tubes, shells of bivalves or branches. The body wall of zooids removed from their test is muscular and thick.

The present study have as goals to report the presence of giant colonies in relation to previous studies of the ascidians *P. opuntia* in some locations of the Magellan Region, to show changes of colonial shape with size as with substrate as well as to describe the weight-length relationship in this colonial species. The large abundance and large size of colonies could produce problemas of biosecurity and fouling in aquaculture, in coastal building and in equipments disposed in Antarctic and Magellan Subantarctic areas (Biosecurity New Zealand, 2007).

Some colonies were collected during September, 2013 (Turon *et al*. 2016a, 2016b), which were used mainly to analyse the change of colonial shape with size around Punta Arenas (Cabo Negro Cape and Santa Ana Point), Magellan Strait, Chile. Other samples were collected between September to December of 2016 in Porvenir Bay, Punta Arenas and Puerto Williams, Beagle Channel, respectively (Table 1) and were surveyed by SCUBA diving or by pulling up ropes and collecting directly from pier or docks. Other colonies were collected during November, 2018, in an intertidal fringe fixed of a stranded ship (MV *Magallanes III*)

[1] Facultad Ciencias, Universidad de Magallanes,
Punta Arenas, Chile
✉ ivan.canete@umag.cl

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located in the sandy beach off Catalina Bay, Punta Arenas (Table 1). At each site, the colonies were randomly collected from artificial (docks, pilings, piers, aquaculture facilities) and natural substratum (shell of *Mytilus* spp; four species are reported in Magellan waters; Oyarzún *et al.* 2016). Sampling was exhaustive (i.e., typically, surveys lasted ca. 1 hr of diving and the depths studied ranged from 2 to 4 m). Surface temperature of sea water was recorded with a digital thermometer (± 0,1 °C) in both locations; salinity and dissolved oxygen were previosuly recorded in Porvenir Bay, showing estuarine and oxygenated condition along the year (Cañete *et al*. 2014).

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 1 or 2 ml of saturated menthol solution) and a cold treatment (keeping the trays in a freezer until ice formed on the surface).

Colonies were classified in three morphological patterns, which was related to the size: small were rounded, mid size with drop shape and the largest colonies were cylindrical and longest colonies. Colonies were counted according to shape pattern to estímate the frequency and each measured in the largest axe.

Total weight and length measurements were based on relaxed colonies collected in 2016 and 2018 (N = 104), considering all morphological pattern. All were dryed to the air during 10 minutes to eliminate remanent of water, weighted and recording the complete colony diameter (small colonies) or the total length (large colonies) using a common ruler, respectively. Samples were then split for preservation: most of the material was fixed in formaldehyde 4% for morphological observation. Ascidians were identified to species level using relevant literature (Sanamyan *et al.* 2003; Turon *et al.* 2016a, b). Taxonomy followed the guidelines of the World Register of Marine Species (Shenkar *et al*. 2017).

Specimens preserved in formaldehyde were deposited in the Systematic Room, Instituto de la Patagonia, Universidad de Magallanes, Punta Arenas, Chile (SRIP-UMAG, Codes 2927-900178).

This study considered the analysis of 104 colonies for morphological and quatitative analysis. Three morphological patterns were observed, according to the size: a) small colonies, with rounded shape, with 1 to 15 cm of diameter (55%) (Fig. 1a; Fig. 2), b) medium size colonies collected under a stranded, old boat from Puerto Williams, Navarino Island, Chile, which showing shaped drops colonies. This shape was observed in colonies vertically attached from subtrata (31%) (Fig. 1b; Fig. 2); similar shape was observed in colonies collected in a stranded ship from Catalina Bay, and c) elongated colonies, shaped cylindrical (between 80 to 240 cm) were uncommon (14%) (Fig. 1c-f) (Table 1; Fig. 2).

Cylindrical colonies were collected during September, 2016 in Chilota dock, Porvenir Bay, showing a total length of 240 cm and a weight of 440 g (Table 1). These colonies were observed floating in the water column in paralell position respect to the sea bed (Fig. 1f). The holdfast of colonies are smallest in relation to the rest of the colony; in the largest colony, the holdfast and peduncle have a length of 8 cm (Fig. 1d), while the rest of colony showing 232 cm of length. Other colonies of similar shape measuring between 100 to 180 cm of length (Fig. 2).

The vertically disposed colonies ranged from 15 to 100 cm in length, with a drop shape. In the specimens collected in Catalina Bay (Fig. 1g-i), the peduncle have similar length that the colony and small, gregarious buds were observed along of distal end.

The globular colonies were collected during September, 2013, showing gregariousness behaviour and settled on living *Mytilus* spp. shells suspended in the water column (Fig. 1 a).

There is a significant, positive, linear relationship between total length and weight in the colonies of *P. opuntia*. Colonies of 300 cm of length could attain a total weight of 500 g (Fig. 2). We collect some colonies smallest than 1 cm of diameter with globular shape. After 15 cm, the colonies attain a shaped drops (Fig. 1). The width of cylindrical colonies varied from 0.5 to 5 cm of diameter.

The main results of this study demonstrate a wide polymorphism in the *P. opuntia* colonies, the shape of colonies changing with size and the

Table 1. Locations of Magellan Region, Chile, where colonies of the ascidian *Polyzoa opuntia* where collected to study the relation between size and shape. Total length and weight are expressed as range (n.d.: no data).

	Locations					
Parameters	Chilota dock, Porvenir Bay, Tierra del Fuego	Arturo Prat dock, Punta Arenas	Cabo Negro dock, Pelicano Bay	Chilota dock, Porvenir Bay, Tierra del Fuego Island	Micalvi Pontoon, Puerto Williams, Navarino Island	MN Magallanes III Catalina Bay Punta Arenas
Type of subtrate	Living mytilid shells	Concrete dock	¿?? Dock	Steel dock	Hull of stranded ship	Hull of stranded ship
Date	September, 2013	September, 2013	November,2013	September, 2016	December, 2016	November, 2018
Latitude	53°18'13.20"S	53°10'14.02 S	52°55'47.84" S	53°18'13.20"S	54°56'06.10"S	53°06'23" S
Longitude	70°26'05.14" W	70°54'21.16" W	70148'08.07 W	70°26'05.14" W	67°37'05.20"W	70°52'42" W
Depth range (m)	2 -4	3-5	4	2	3-4	Intertidal
Length (cm)	2-6	50	40-60	30 – 240	1-60	15-80
Weight (g)	2-6	n.d.	n.d.	188-440	1-128	20-100
Number of colonies (N)	18	n.d.	n.d.	8	7	91
Shape	Globular	Shape of drop	Shape of drop	Shape of drop and cylindrical	Globular, drop and cylindrical shape	Mainly shape of drop
	6.5	6.9	8.3	6.5	9.6	7.1

shape could be affected by type (artificial or natural) and orientation of the substrate. Additionally, there is a positive, significant, linear relationship between size and weight of colonies, aspect not previously described in colonial ascidians (Table 1; Fig. 1 & 2).

The discovery of giant colonial ascidians remain as unusual or striking fact (Ramos-Esplá & Ocaña, 2017). Previous reports showing colonies of *Eudistoma elongatum* (Herdman, 1886), *Distaplia cylindrica* (Lesson, 1830) and *Aplidium proliferum* (Milne Edwards, 1841) attaining to 2.5 m lenght (Page & Kelly, 2013; Ramos-Esplá & Ocaña, 2017). Antarctic colonies of *Distaplia cylindrica* sized up 7 m (Kott, 1969). To our best knowledge, this finding in *P. opuntia* is one of the largest colonial ascidians reported in subantarctic waters.

In this study we found three morphological patterns in shallow waters (Fig. 1). The globular colonies living mainly on man-made and biological substrata such bivalves shells; the second and third type of colonies are fixed on most stable artificial substrata such as docks and hull of stranded ships. Thus, it is postulated that the colonial shape change with size (or age), stability and orientation of the substrate. To hypothesis level, is postulated that the shape of colonies could change seasonally.

In the present study, cylindrical colonies were collected in late winter and early spring in some Magellan locations. During this period low temperature prevails (Table 1). It is postulated that along winter the abundance of other colonial and solitary ascidians or predators could be reduced, helping to the growth in *P. opuntia*. High density of seawater under low temperature regime could favor the floatation of giant colonies in the water column (Barnes & Clarke, 2011); Porvenir Bay is a shallow embayment (< 12 m depth) and during winter sea surface temperature can attain to 1 to 2 °C (Cañete *et al.* 2014). Other reason could be that during winter and spring (July to November), the king crab boats remain in the fishing ground. No presence of boats in this period could reduce the opportunities of damage to the floating colonies of *P. opuntia*, allowing to attain largest size (Fig. 1e-f).

The weight-length relationship offer an opportunity to compare the differences in the fitting functions between solitary and colonial ascidians. For example, in *Ciona intestinalis* the fitting is an exponential function ($W=A\times e^{-Br}$; Du Clos *et al.* 2017), while in *P. opuntia* show a linear fitting (Fig.

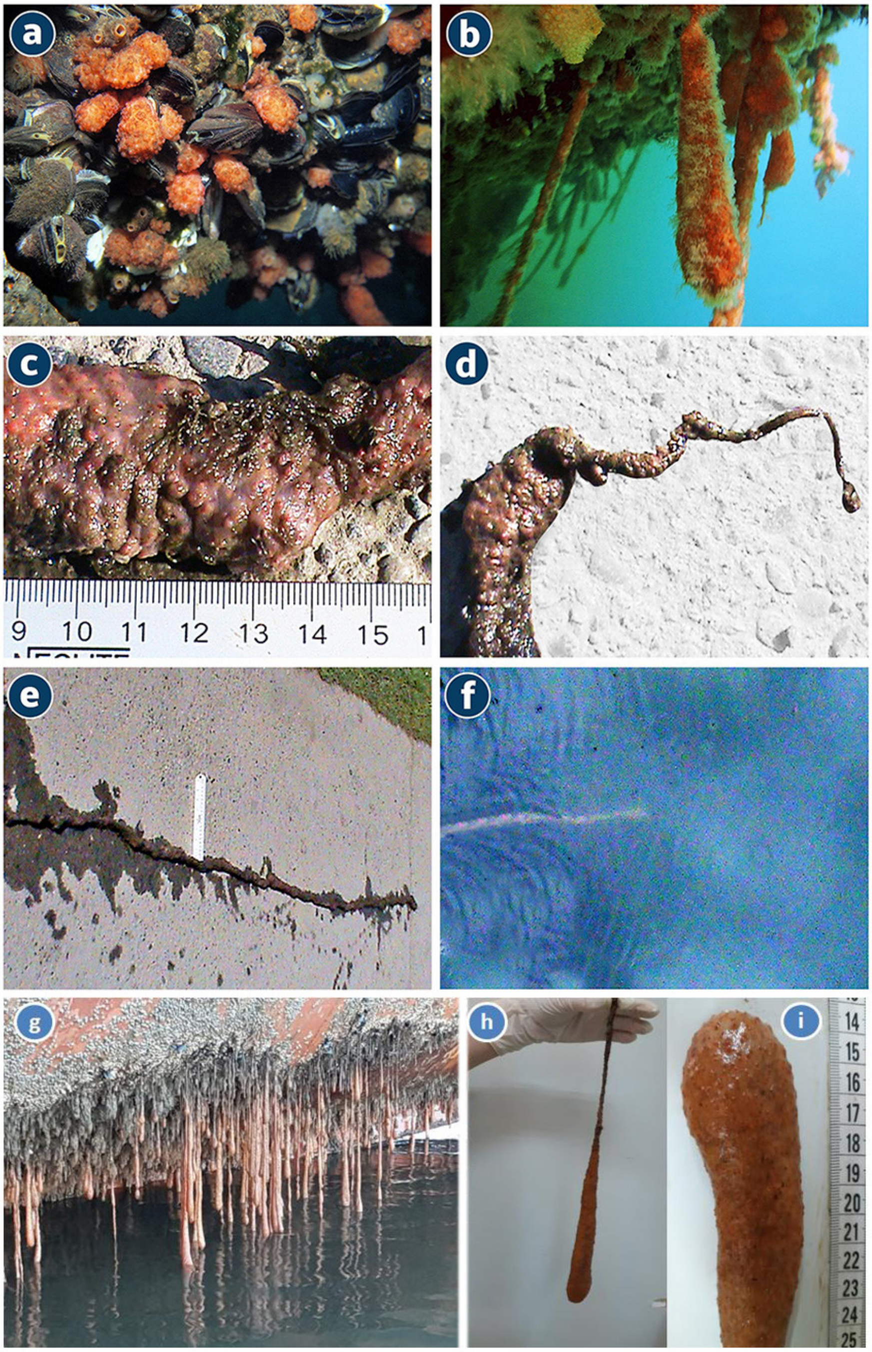

Fig. 1. *Polyzoa opuntia*: a) Fixation of globular colonies on the *Mytilus chilensis* shells suspended in the water column under Chilota dock, Porvenir Bay, Chile (September, 2013), Chile, b) Colonies with shape of drop under an old vessel hull off Puerto Williams, Navarino Island, Beagle Channel, Chile (December, 2016; 1.5 m depth), c) Detail of zooids in a section of the largest colony, d) shape of the colonial holdfast of the largest colony collected in this study (the length is nearly to 8 cm), e) largest colony stretched on the floor to denote its extension (ruler have a length of 20 cm; showed by black arrow), f) cylindrical colony suspended in the seawater, under Chilota dock, Porvenir Bay, Chile (September, 2016), g) Colonies with shape of drop fixed on stranded ship off Catalina Bay, Punta Arenas, Magellan Strait, Chile (November, 2018; intertidal), h) complete colony with detail of the length of peduncule and i) detail of the anterior end of a colony with drop shape, showing position of siphons (dark red spots).

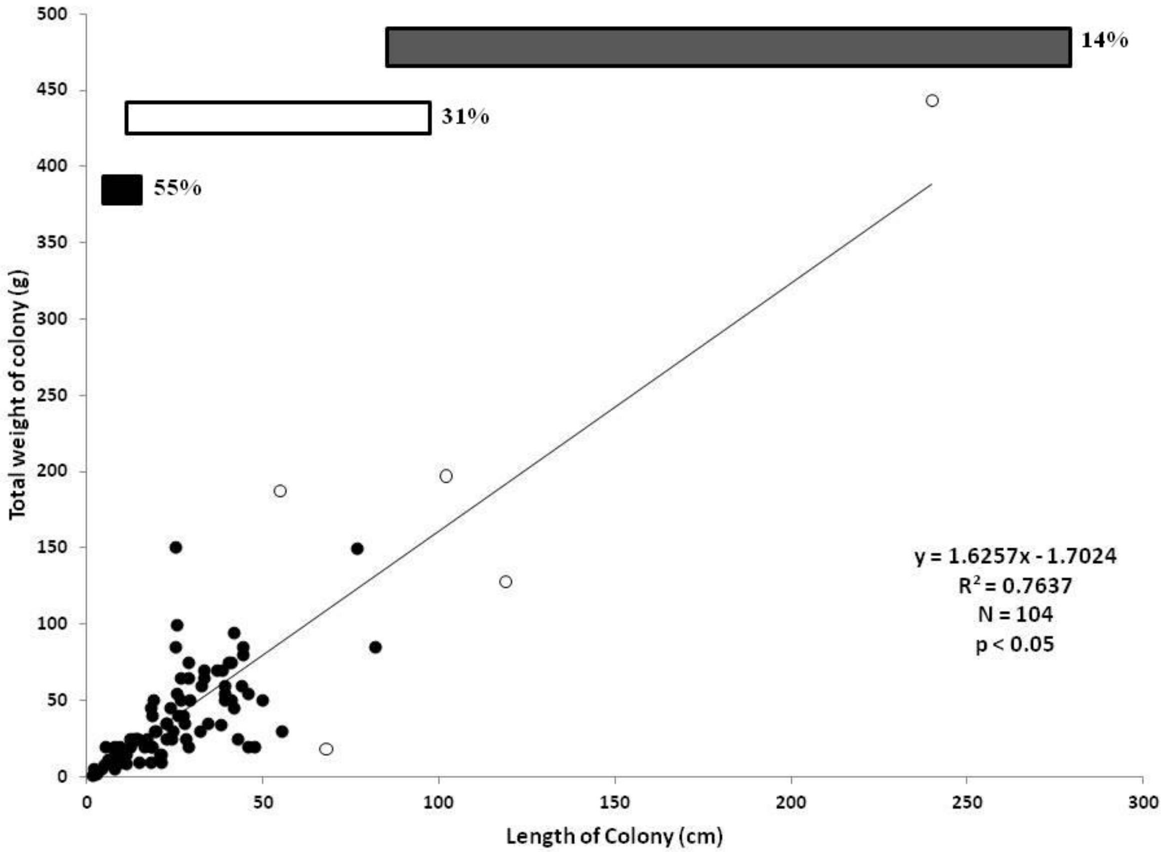

Fig. 2. *Polyzoa opuntia*. Length-weight relationship of colonies collected around Magellan Strait, Punta Arenas, Chile (○, 2016 & ●, 2018). Black rectangle indicate the range of size in globular colonies; white rectangle, the range of colonies with shape of drop; and gray rectangle, the range of length of cylindrical colonies. Percentages indicate the proportion of colonies in each morphological pattern.

2). No references to this length-weight relationship in colonial ascidians has been previously cited.

The fact of *P. opuntia* was found on mussel shells (Fig. 1 a) suggest a potential negative impact on bivalve aquaculture in southern Chile. However, low temperatures and salinities in shallow waters could limit the development of this colonial species (Turon *et al*. 2016a; Nagar & Shenkar, 2016; De Castro *et al*. 2018) in estuarine zones available to aquaculture in Magellan waters.

The artificial substrates observed in this study shows that colonies of *P. opuntia* coexist with other colonial fouling species such *Bugula*-like bryozoans, colonial ascidians as *Aplidium* and hydrozoans, shaping a community based in a guild of clonal taxa. Large biomass of this guild could to produce a fast organic enrichment of the bottoms with tunicates of dead colonies. In the present study, the accumulated weight of colonies produced near to 4.5 kg m^{-2} (N=104).

The colonial size/shape in ascidians seem be regulated by exogenous (hydrodynamic, interespecific competition, primary production, and anthropogenic environmental disturbances), showing typical aspects associated to the clonal life cycle (Ramos-Esplá & Ocaña, 2017). The large abundance, wide geographic distribution and the presence of giant colonies in *P. opuntia* suggest their potential use as biomodel to study the consequences of clonal life on the populational traits in a cold, estuarine system, where variable and extreme oceanographic conditions prevails (Jackson, 1977; Nakauchi, 1982; Caswell, 1985; Brown & Swalla, 2012; Stolfi & Brown, 2015).

P. opuntia is the only member of Stolidobranch colonial ascidian in Magellan waters.

The rest belong mainly to Aplausobranch colonial ascidians (12 species: *Distaplia colligans*, *D. cylindrica*, *Didemnum studeri*, *Trididdemnum auriculatum*, *Polysincraton trivolutum*, *Aplidium fuegiense*, *A. magellanicum*, *A. variabile*, *A. falklandicum*, *Synoicum georgianum, Eudistoma magalhaensis* and *Sycozoa gaimardi*) (Sanamyan & Schories, 2003; Schories *et al*. 2015, Turon *et al.* 2016a & b). This situation offer an interesting opportunity to compare the pattern of growth in colonial ascidians of cold and estuarine waters under a taxonomic perspective.

Additionally, the presence of *P. opuntia* in Subantarctic (Sanamyan & Schories 2003, Sanamyan & Schories, 2007; Lagger *et al*. 2009; Schories *et al*. 2015, Turon *et al.* 2016a & b) and Antarctic waters (Kott, 1969; Monniot & Monniot, 1983; Ramos-Esplá *et al.* 2005; Primo & Vázquez, 2007) offer opportunities to study the comparative growth of colonies under two cold regimes.

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