

Short Communication

***Ascidia ceratodes* (Huntsman, 1912) (Tunicata: Ascidiidae) off the northern Chilean coast: new record**

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ABSTRACT. The ascidian fauna of northern Chile (18° to 25°S) is poorly known. A member of the family Ascidiidae, *Ascidia ceratodes* (Huntsman, 1912), is reported in this study. We collected samples of *A. ceratodes* under intertidal boulders off the northern Chilean coast between Arica (18°S) and Iquique (20°S) (17 to 20°C; intertidal pool; <0.5 m depth; August, 2016). This finding verified a questionable record established by Van Name (1945) from Tocopilla (22°S), northern Chile. This record extends the confirmed geographical distribution of *A. ceratodes* along of the eastern Pacific coast from British Columbia, Canada, to northern Chile.

Keywords: *Ascidia*; warm temperate benthos; intertidal rocky shore; biodiversity; Southeastern Pacific

The ascidian fauna of the Chilean coast comprises around 72 species. Over the last decade, our knowledge about this taxon and its distribution in the Chilean coast has improved (Clarke & Castilla, 2000; Sanamyan & Schories, 2003; Lagger *et al.*, 2009; Sanamyan *et al.*, 2010; Tatián & Lagger, 2010; Madariaga *et al.*, 2014; Schories *et al.*, 2015; Turon *et al.*, 2016a,b); however, in order to accurately assess ascidians biodiversity, including the presence and distribution of non-indigenous species, new areas along the Chilean coast need to be studied (Turon *et al.*, 2016a,b). For example, Rocha *et al.* (2012) reported 26 species of the family Ascidiidae from the Atlantic coast of South America, while only nine species were reported on the Pacific coast (Van Name, 1945; Lambert & Lambert, 1998; Nova-Bustos *et al.*, 2010; Carman *et al.*, 2011; Monniot, 2013). This trend of greater diversity on the southwest Atlantic compared to Pacific coast has also been highlighted by Miloslavich *et al.* (2011) and by Clarke & Castilla (2000), Sanamyan & Schories (2003), Lagger *et al.* (2009), Sanamyan *et al.* (2010), Shenkar & Swalla (2011), Tatián & Lagger (2010), Madariaga *et al.* (2014), Schories *et al.* (2015) and Turon *et al.* (2016a,b).

Recent surveys of ascidians biodiversity on the northern Chilean coast (Clarke & Castilla, 2000; Schories *et al.*, 2015; Turon *et al.*, 2016a, 2016b) did not make any reference to members of the genus *Ascidia*. Only one previous study reported the species *A. meridionalis* Herdman, 1880 and *A. tenera* Herdman, 1880 from Magellan Strait, southern Chile (Schories *et al.*, 2015).

Van Name (1945) described the geographical distribution of *A. ceratodes* as ranging along the northern American Pacific coast from British Columbia south to California; however, he also referred to several questionable specimens collected from Ecuador to the northern Chilean coast (Tocopilla; 22°05'S, 70°11'W). Van Name (1954) only cited the presence of *A. ceratodes* in the northern Chilean coast to spite to collect intertidal ascidians between Iquique and Antofagasta (Stations 129, 131, 133 and 159 sampled during Lund University Chile Expedition 1948-1949). More recently, Carman *et al.* (2011) and Bonnet *et al.* (2013) reported the presence of *A. ceratodes* in the Pacific waters off Panama and replay the comment of Van Name (1945) on the possible presence of this species in the northern Chilean coast.

The study of the marine benthic communities of the northern Chilean coast is of great importance because the intense maritime traffic makes this region more susceptible to the introduction and establishment of tropical and subtropical, exotic species of ascidians (Turon *et al.*, 2016a, 2016b). Additionally, this area is under the influence of El Niño-Southern Oscillation (ENSO) events, putting it at risk of colonization of the northern Chilean coast by ascidians or other organisms from tropical latitudes (Lord *et al.*, 2015).

The most recent assessment of the northern Chilean ascidians only reached as far south as 25°S (Schories *et al.*, 2015). We aim to establish a new, extended record based mainly on a recent collection of intertidal ascidians sampled between Arica (18°S) and Iquique (20°S), including a report of *A. ceratodes*. Here, we confirm their presence in the northern Chilean intertidal rocky shore.

Sampling was performed in August, 2016. Localities around Arica and Iquique (Table 1) were surveyed by hooka diving, by pulling up ropes and raft cages as well as searching under intertidal boulders in rocky shore pools. Additionally, at each site, we sampled artificial substrates (docks, pilings, break-waves), shellfish markets (including the analysis of bivalves shells such as *Choromytilus chorus* and *Aulacomya ater*) and some intertidal natural communities. However, specimens of *A. ceratodes* were only collected under intertidal boulders settled on sandy beaches and in tide pools. Sampling was exhaustive, *i.e.*, it was continued until no further species were detected; typically, surveys lasted around 3 h and ranged from 0 to 1 m of depth. There is an important link between the temperature and the proportion of non-indigenous ascidians (Madariaga *et al.*, 2014; Lord *et al.*, 2015); temperature of the seawater found in tidal pools was therefore recorded using a digital thermometer ($\pm 0.1^\circ\text{C}$).

Ascidians were photographed *in vivo*, carefully collected in Ziploc bags, and taken to the laboratory within 2 h of collection. There, they were placed in trays and relaxed using a combination of menthol and cold treatment, keeping the trays in a freezer until ice formed on the surface (Turon *et al.*, 2016a,b). Samples were then split for preservation: most of the material was fixed in 4% formaldehyde for morphological observation, while some parts (usually muscular tissue) were preserved in absolute ethanol for molecular analyses. The observation of morphological features was routinely enhanced by staining with Bengal Rose or Methyl Blue stain. Specimens were identified to species level using the relevant literature (Rocha *et al.*, 2012) and in particular the available studies of the genus *Ascidia* in Central America (Van Name, 1945;

Tokioka, 1972; Carman *et al.*, 2011; Bonnet *et al.*, 2013). Taxonomy followed the guidelines of the World Register of Marine Species (Rocha *et al.*, 2013) and our comparative analysis was based mainly in the description of Bonnet *et al.* (2013). Additionally, five specimens of *A. ceratodes* were sent to Nadia Bonnet (Laboratório de Sistemática e Ecologia de Invertebrados Marinhos, Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil) to corroborate the identification. Specimens preserved in formaldehyde were deposited in the Systematic Room, Ascidian Collection, Department of Zoology, Universidade Federal do Paraná, Brazil (N. Bonnet and R.A. Rocha, *pers. comm.*) (5 specimens; DZUPASC 239) and in the Invertebrate Taxonomic Room, Universidad Católica del Norte, Coquimbo, Chile (J. Sellanes, *pers. comm.*) (3 specimens).

Ascidia ceratodes (Huntsman, 1912)

(Figs. 1-2)

Synonymy

Phallusia ceratodes Huntsman, 1912, p. 114, p. 121; 1912a, p. 117, pl. 10, Fig. 9, pl. 15, Figs. 3, 4, 7. *Ascidia californica* Ritter & Forysth, 1917, p. 454, pl. 38, Fig. 6, pl. 41, Figs. 24-27; Johnson & Snook, 1927, p. 593, fig. 692; Pratt, 1935, p. 745; MacGinitie, 1939, pp. 443, 446. *Ascidia eiseni* Michaelsen, 1923, p. 38, fig. 8. *A. ceratodes* Van Name, 1945, pp. 196-197, Fig. 109; Van Name, 1954, p. 16 (only cited); Bonnet *et al.* 2013, pp. 354-355, Fig. 2 (references to the presence of this species in the northern Chilean coast based on comments of Van Name, 1945).

Material examined

St 1: Arica, Laucho Beach (18°29'12.26"S, 70°19'36.00"W; intertidal pool, August, 2016; two specimens; sea water temperature 19.6°C). St. 2: Arica, Lisera Beach (18°29'34.52"S, 70°19'37.46"W; intertidal pool, 2 August 2016; five specimens; sea water temperature 18.6°C). St. 3: Iquique, Huaiquique (20°16'16.58"S, 70°07'52.90"W; intertidal pool; 4 August 2016; two specimens; sea water temperature 17.3°C). St. 4: Iquique, off Regional Government Building (20°13'03.63"S, 70°09'21.45"W; intertidal pool, 5 August 2016; four specimens; sea water temperature 18.2°C). All sites have <1 m depth, with the bottoms dominated by small boulders (30 to 40 cm of diameter), where the specimens were collected.

Description

Specimens are small (up to 2.5 cm total length) and are fixed to the substrate by the left side of the body, as is typical for other members of the family Ascidiidae (Monniot *et al.*, 1991). The living animal is yellowish

Table 1. Locations of specimens of *Ascidia ceratodes* collected in northern Chile. All sites were intertidal pools (<1 m depth) with a bottom dominated by small boulders (30 to 40 cm of size). Specimens were collected under these boulders. Numbers of specimens/boulder are shown in brackets.

Location	Date	Latitude	Longitude	T (°C)
Arica, Laucho Beach, (2 specimens)	2-8-2016	18°29'12.26"S	70°19'36.00"W	19.6
Arica, Lisera Beach, (5 specimens)	2-8-2016	18°29'34.52"S	70°19'37.46"W	18.6
Iquique, Huaiquique, (2 specimens)	4-8-2016	20°16'16.58"S	70°07'52.90"W	17.3
Iquique, off Regional, Government Building, (4 specimens)	5-8-2016	20°13'03.63"S	70°09'21.45"W	18.2

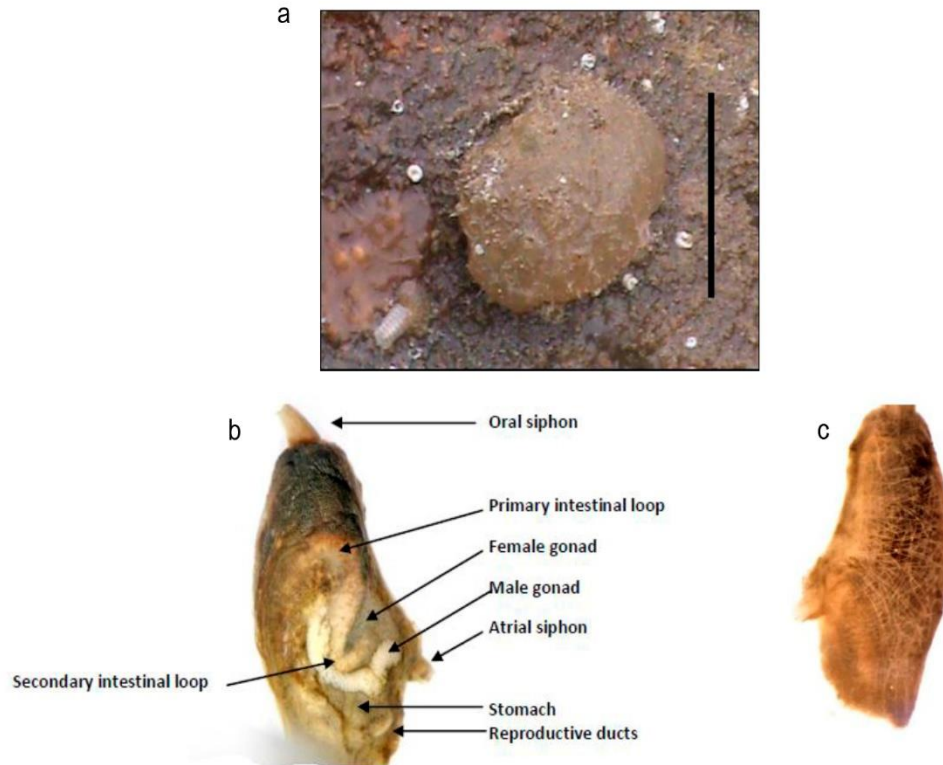


Figure 1. a) Living specimen of *Ascidia ceratodes* collected under intertidal boulder in the Lisera Beach, Arica, Chile (August, 2016). Bar scale = 20 mm length, b) same specimen without tunic; left view; c) same specimen, right view; white, fine lines are a reticulated of muscle fibers; oral siphon not showed.

with dark orange siphons, although these colors disappear after fixation. The tunic is translucent, depressed, with cartilaginous consistency (Fig. 1a). The body is oval, 1.0-2.0 cm long (including the oral siphon) and 0.5-2.0 cm wide, without the tunic. The color of the right side is different from that of the left side (Figs. 1b-1c). Usually, both siphons are small, but the oral siphon is the longer of the two. It has eight to nine lobes and six to eight red eye spots, while the atrial siphon has six lobes and six red eye spots (Figs. 2a-2b); both siphons present orange dots between the lobes, and neither has any projections on the margin. The atrial siphon is directed towards the posterior region of the body, at a right angle (Figs. 1b-1c). The V-shaped

neural ganglion is closer to the oral siphon. On the right side, the musculature is formed by a net of thick fibers (Fig. 1c). On the left side, the longitudinal muscles extending from the oral siphon are short. A band of short parallel transverse fibers is found in the dorsal region (Fig. 1c). The longitudinal muscle fibers in the siphons are not organized in bands (Figs. 2a-2b). An unknown typical brown net associated with the stomach and external wall of the posterior intestine was observed (Fig. 2c). According to Monniot *et al.* (1991; p. 22), this net could be tubules of the “pyloric gland” with possible excretory and digestive function.

Renal vesicles were not observed between the stomach and the ascendant loop of the intestine as

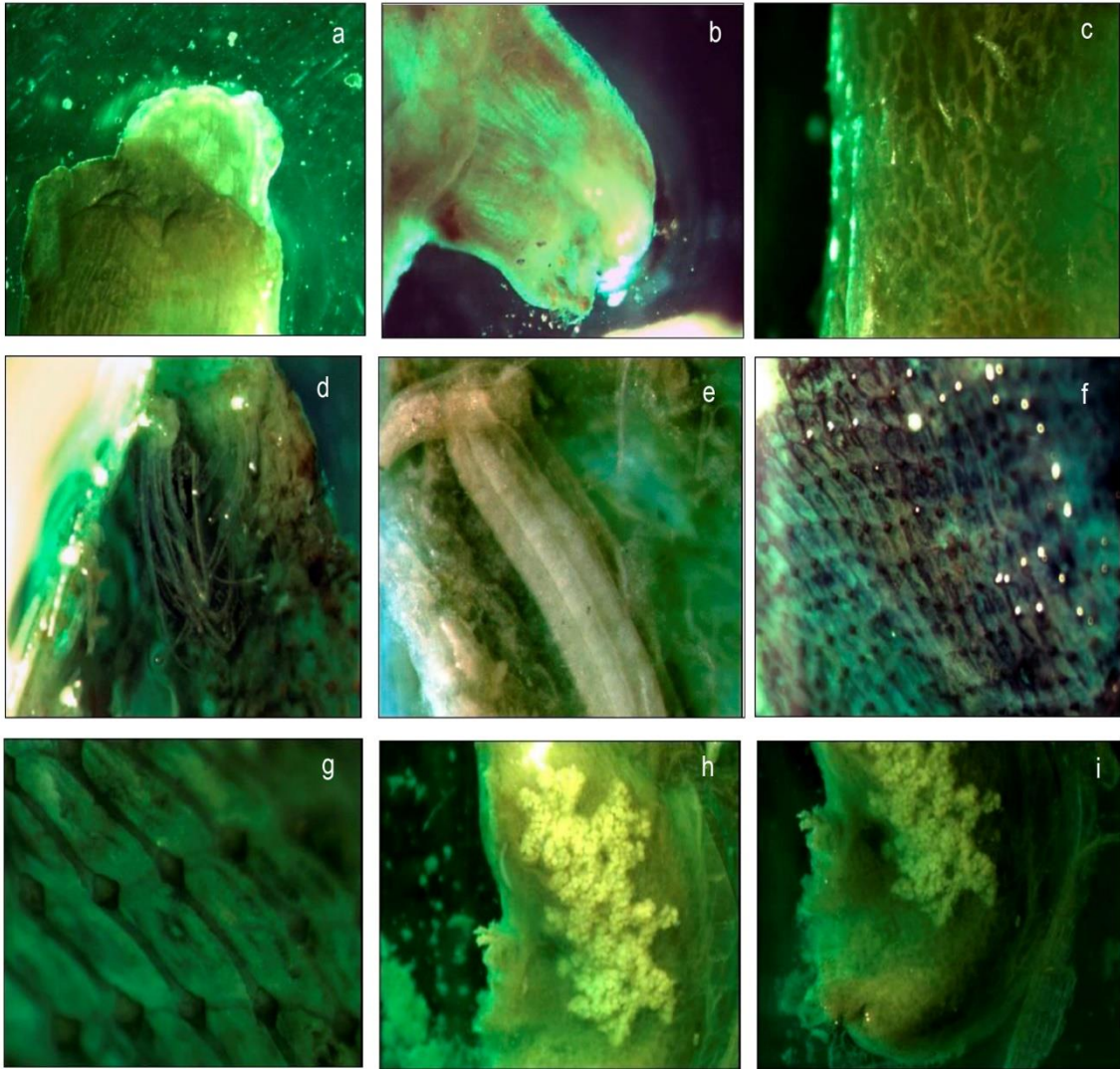


Figure 2. Internal anatomy of *Ascidia ceratodes*: a) oral siphon in contraction, viewed from the left side, b) atrial siphon, lateral view, c) unknown net associated to stomach and external wall of the posterior intestine; according to Monniot *et al.* (1991; page 22), could be tubules of the “pyloric gland”, d) oral tentacles, e) reproductive ducts and rectum in connection to atrial cavity, f) section of the pharynx, showing the branchial sac and arrangement of transverse and longitudinal vessels, g) primary papillae located at the posterior end of each longitudinal vessel, h) detail of the male gonad, viewed from the left side, i) portion of the male gonad and the first intestinal loop.

suggested by Bonett *et al.* (2013). In the basis of the oral siphon, it was possible to detect an area between 80 and 127 oral tentacles, of three sizes are present; the largest is 1.5-2.0 mm long and 90 μ m wide (Fig. 2d). The line of tentacles and the parapharyngeal groove is closed, with or without papillae. The peri-tubercular area is short, and the dorsal tubercle aperture is U-shaped, with or without enroled ends. Anteriorly, the dorsal lamina is double and passes by the left of the esophageal aperture to the end of the pharynx, close to the stomach; no papillae are present on the right side of the dorsal lamina at the level of the esophagus. There is

a narrow lamina on the right of the esophageal aperture. The pharynx has 24-38 longitudinal vessels on the right side, 23-35 longitudinal vessels on the left, and 47-81 transverse vessels; it is pleated, with five to seven stigmata per mesh (Fig. 2f). Distance between transversal vessels is 1.0 mm, that between longitudinal vessels, 0.6 mm spaces (Fig. 2g). The primary papillae in the pharynx are simple, bi or trilobed, this last form being the most common (Fig. 2g). There are secondary papillae in some parts of the pharynx. The alimentary canal occupies half or more of the left side of the body (Fig. 1b). The stomach is oval and large, with 9-11

internal longitudinal folds. The intestine forms a primary and a secondary loop (Fig. 1b). The anus is located very near the oral tentacles; it has a smooth or bilobed rim (Fig. 2e). The ovary is compact and localized inside the primary intestinal loop, where it is visible both from the outside and from the atrial cavity (Fig. 1b). The testicular follicles are elongated and overlies part of the stomach and intestine (Figs. 2h, 2i). Gonoducts open posterior to the anus aperture (Fig. 2e).

Taxonomic remarks

Van Name (1945) described *A. ceratodes* as similar to juveniles of *A. interrupta* Heller, 1978. Bonnet *et al.* (2013) agree that the absence of projections in the surface of the tunic, the larger number of oral tentacles, low number of longitudinal vessels in the pharynx, isodiametric intestine and conspicuous renal vesicles are characteristics, which distinguish *A. ceratodes* from *A. interrupta*. Northern Chilean specimens show a smaller number of oral tentacles and both siphons are shorter than Panamanian specimens (Bonnet *et al.*, 2013).

Geographic distribution

According to Van Name (1945), the geographical distribution of *A. ceratodes* ranges from British Columbia to California. His report makes reference to several specimens from Ecuador and northern Chile, which were catalogued as questionable due to the fact that the mantle musculature is coarser and heavier than in specimens from California. Later, Tokioka (1972) reported the species in Costa Rica, and Carman *et al.* (2011) extended its range to Panama. It is uncertain whether these tropical populations are in their native region and whether the species is hence expected to be found all along the Central America Pacific coast, or whether the northern Chilean populations were introduced through anthropogenic activities, such as maritime transport or dispersed by currents associated with previous or recent ENSO events. Sanamyan & Schories (2004) report four ascidians species from two locations in southern Peru, without reference to *A. ceratodes*. A possible reason for this may be that his sampling including only diving in subtidal rocky communities.

Ecological comments

The northern Chilean coast has been under persistent and sporadic influence of El Niño event of different magnitude in the last seven decades (1945-2015). However, *A. ceratodes* population persist living in the intertidal zone. This finding could be an example of littoral marine taxa that can resist the negative impacts of climate warming and a native species along the temperate coast from British Columbia, Canada to

Tocopilla, Chile (Carman *et al.*, 2011), including the northern Chilean coast. This wide geographical distribution is surprising since it covers approximately 71° latitude along the eastern Pacific coast.

A. ceratodes is a native species of the west coast of USA and have a thermal niche ranged between 16.2 and 26.7°C (Lord *et al.*, 2015; see Table 4). Thus, in physiological terms *A. ceratodes* could be classified as native, eurythermal species and could be a good bioindicator species for monitoring climate warming of mid-latitudes of the Chilean exposed coast.

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