



Research Article

Too cold for invasions? Contrasting patterns of native and introduced ascidians in subantarctic and temperate Chile

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Abstract

We analysed the biodiversity of ascidians in two areas located in southern and northern Chile: Punta Arenas in the Strait of Magellan (53° latitude, subantarctic) and Coquimbo (29° latitude, temperate). 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. Both regions were surveyed twice during 2013 by SCUBA diving and pulling ropes and aquaculture cages. Both artificial structures and natural communities were sampled. A total of 22 species were identified, three of them reported for the first time in Chilean waters: *Lissoclinum perforatum*, *Synoicum georgianum*, and *Polyzoa minor*. The first is an introduced species found here for the first time in the Pacific. No species occurred in both regions, highlighting the very different environmental conditions of subantarctic vs. temperate waters. In spite of exhaustive searches in aquaculture facilities and on artificial structures such as harbour docks and piers, no introduced species were found in the Punta Arenas area. Conversely, 5 out of 11 (45%) species found in northern Chile were introduced. The Coquimbo area has a history of ship traffic dating back at least 150 years, and cultures of native (e.g. scallop) as well as exotic species (e.g. abalone) have been deployed for ca. 35 years. Some of the introduced species, such as *Ciona robusta* (formerly *C. intestinalis* sp. A), constitute pests for scallop culture facilities in the area, causing serious losses to local farmers. It is surprising that the Punta Arenas zone, with a history of ship traffic dating back ca. 500 years and over 25 years of sustained mussel and salmon aquaculture activity, is apparently free from introduced species. The ascidian cover on artificial structures is high, but it is made up of native species such as *Paramolgula* sp., *Cnemidocarpa verrucosa*, or *Polyzoa opuntia*. It is hypothesized that cold waters (5 to 11°C) are the determining factor hindering the development of introduced ascidians, which tend to be temperate-warm water species. The ongoing warming in the Southern Cone may change this picture and continued monitoring is strongly advised.

Key words: tunicates, ship traffic, exotic species, bivalve cultures, Strait of Magellan, Coquimbo

Introduction

The Chilean coasts provide an ideal setting for the study of marine introductions, as the country spans a very wide range of latitude (ca. 36°), climatic regimes from subtropical to Antarctic, and markedly different current patterns (Thiel et al. 2007). Spalding et al. (2007) recognized two provinces in the littoral areas of Chile, the Warm

Temperate Southeastern Pacific and the Magellanic provinces, separated at around 42° S. Other authors have advocated for a biogeographical division of the Southeastern Pacific into three units (Knox 1960; Camus 2001), including a Peruvian Province (2–30°S) of warm-temperate biota, an Intermediate Area (30–42°S), and a Magellanic Province (42–56°) of temperate-subantarctic biota. The break between the first two zones is marked by temperature, hydrology and geographical features (reviewed

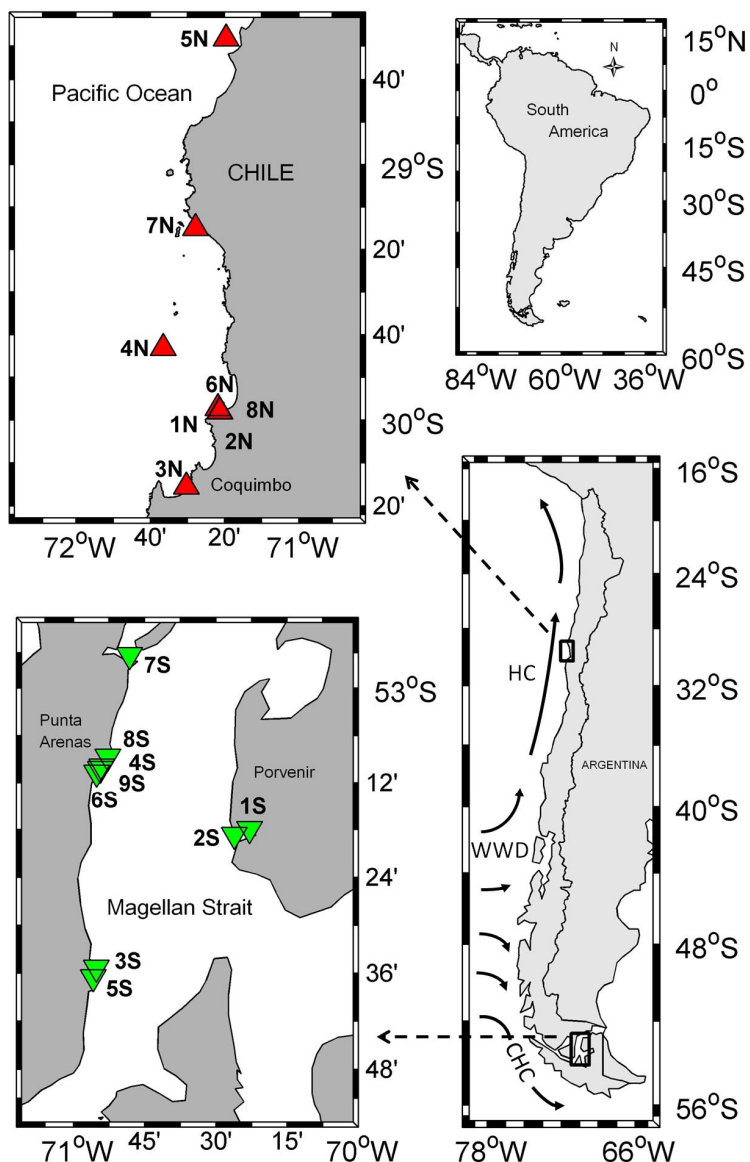


Figure 1. Map of the coast of Chile with indication of the sampling localities at the two areas surveyed. Locality codes as in supplementary Table S1. The main oceanic features: west wind drift (WWD), Cape Horn Current (CHC) and Humboldt Current (HC) are indicated.

in Camus 2001). The break between the last two regions, at around 42°S, is widely recognized as the steepest transition in the area (Lancellotti and Vasquez 2000; Camus 2001; Thiel et al. 2007; Palma et al. 2014) and coincides with the presence of fjords, massive freshwater inputs and, particularly, the west wind divergence (WWD) area, in which the main current splits into two branches running north and south due to the approach to the continent. In very general terms, this generates two main ocean currents, to the north the Humboldt Current (HC) and flowing

southwards the Cape Horn Current (CHC) (Figure 1). For a detailed review of the current systems in the Chilean shores see Shaffer et al. (1999) and Thiel et al. (2007). In addition, the Strait of Magellan region has historically been the connection hub between the Atlantic and the Pacific oceans (particularly before the opening of the Panama canal). The Magellanic area has thus supported intense ship traffic for over 500 years (Martinic 1977). Aquaculture activities, on the other hand, are well developed all along the Chilean coast (Thiel et al. 2007).

Biological invasions in marine systems are usually associated with shipping, artificial canals, or aquaculture activities. The first includes translocation of organisms in ballast water or as ship fouling, and the artificial canals opened for navigation created new direct connections between seas and oceans (Carlton and Geller 1993; Gollasch et al 2000; Lambert 2002; Zenetos et al. 2012). Introductions related to aquaculture include translocation of both target species and accompanying epibiota that hitchhike the stocks (Naylor et al. 2001; Minchin 2007). It is therefore expected that Chilean waters should have a heavy payload of introduced species, potentially impacting natural communities and interfering with economic activities. A recent review lists 32 introduced marine species in Chile (Castilla et al. 2005).

Ascidians constitute a prominent group among marine introduced taxa (Lambert 2007; Locke and Hanson 2011). Introduced ascidians often thrive on artificial substrates (e.g. López-Legentil et al. 2015; Airoidi et al. 2015) and are a cause of concern in shellfish aquaculture industry around the world (e.g. Bourque et al. 2007; Gittenberger 2009; Adams et al. 2011; Arens et al. 2011, Fitridge et al. 2012). Ascidians are an ideal group for the study of introduction processes (Zhan et al. 2015), because they have a limited natural dispersal capability (Svane and Young 1989; Rius et al. 2010), making them reliant on human transport for long-distance dispersal. In addition, recurrent introductions seem common in ascidians and increase the probability of long-term establishment in a new area (e.g. Dupont et al. 2010; Pineda et al. 2011; Rius et al. 2012, 2015).

In this study we surveyed the biodiversity of ascidians in two areas located in southern and northern Chile: Punta Arenas in the Strait of Magellan (52–53° latitude) and Coquimbo (29–30° latitude, at the southern limit of the Peruvian Province). Our study therefore includes warm-temperate and cold subantarctic zones, with a large latitudinal gap (over 22°) between the two study areas. The record of human activities in these areas is also different: the Magellanic region has a history of ship traffic dating back ca. 500 years, and over 25 years of sustained mussel/salmon aquaculture activity. These aquaculture activities, however, are concentrated in less saline waters (<25 psu) in the Patagonian fjords area, at some distance from Punta Arenas, which hosts important ports. The Coquimbo area has a record of ship traffic dating back at least 150 years, but with shipping levels about one third those of Punta

Arenas and adjacent ports (Keller et al. 2010). On the other hand, in the Coquimbo area cultures of local (e.g. scallop) as well as exotic (e.g. abalone, Pacific oyster) species have been deployed for ca. 35 years (Di Salvo et al. 1984; Thiel et al. 2007).

We concentrated our sampling effort in artificial structures and aquaculture settings to specifically look for introduced ascidians, but also included natural communities for comparison. Previous ascidian surveys in Chile have mostly focused on natural assemblages (e.g., Sanamyan and Schories 2003; Lagger et al. 2009; Sanamyan et al. 2010; Tatián and Lagger 2010). We foresaw a highly divergent composition of the natural ascidian fauna, given the latitudinal (and hence climatic) differences. We also expected a heavy load of introduced species in both areas, probably more related to shipping in the Magellanic region, and to bivalve aquaculture in the Coquimbo area.

Methods

Most of the sampling was done in September and November–December of 2013. Localities around Punta Arenas and Coquimbo (Figure 1, supplementary Table S1) were surveyed. At each site, we sampled artificial substrates (docks, pilings, aquaculture facilities) by SCUBA-diving or by pulling ropes and aquaculture cages. For comparison, we also sampled natural communities on rocky bottom habitats down to 12 m by SCUBA-diving. Sampling was exhaustive, i.e., it was continued until no further species could be detected; typically surveys lasted ca. 1 hr. Two samples collected in 2012 by M. Valdebenito and available at the collection of the Universidad Católica del Norte at Coquimbo were also included from deeper waters (ca. 100 m, Table S1).

Ascidians were photographed *in vivo*, collected, and brought to the laboratory as soon as possible (within 2 hrs after collection). They were relaxed with menthol and cold treatment (placing them in a freezer until ice started forming in the surface of the containers). Ascidians were identified to species level using the relevant literature. Taxonomy followed the Ascidiacea World Database (Shenkar et al. 2015). We assigned each species a native, introduced or cryptogenic status (Carlton 1996, 2009) following the distributional and historical information available in the literature.

The specimens preserved were deposited in the Systematic Room of Instituto de la Patagonia, Universidad de Magallanes, Punta Arenas, Chile (SRIP-UMAG, Codes 900174 to 900183).



Figure 2. Some of the species identified at the Magellanic zone (left), and at the temperate zone (right). A, *Distaplia colligans*; B, *Polyzoa opuntia* (stalked form); C, *Ciona robusta* (formerly *C. intestinalis* sp A) (on scallop culture gear); D, *Asterocarpa humilis* (on top of *Pyura chilensis*).

Results

We identified a total of 22 species belonging to 8 families. It is noteworthy that no species was found in both regions surveyed, each having exactly one half (11) of the species identified. A detailed list of species found at each locality is given in supplementary Table S1. *Lissoclinum perforatum* (Giard, 1872) is recorded for the first time in the Pacific, and *Polyzoa minor* Monniot, 1970 is found for the first time outside its type locality in S. Indian Ocean. *Synoicum georgianum* Sluiter, 1932 had been previously found only in localities of the South Indian and Southwestern Atlantic oceans. An unidentified *Polyzoa* sp. (probably a new species) was found in the Coquimbo area. The species that we identified as *Paramolgula* sp. has been referred to in previous works as *P. gregaria* (Lesson, 1830) (e.g., Tatián and Lager 2010), a taxon that likely lumps several species (Van Name 1945) and requires clarification. Full taxonomic details and discussion will be published elsewhere. Some representative species from both areas are illustrated in Figure 2.

In the Magellanic region, most species (8) were found exclusively on artificial substrates (including cultured bivalves), and two were observed only on natural substrates (Table 1). *Didemnum studeri* Hartmeyer, 1911 was the only

species present in both types of communities. In the Coquimbo area, the distribution was more even, with four species exclusive to natural substrate, three on artificial substrate, and four species [*Diplosoma listerianum* (Milne Edwards, 1841), *Ciona robusta* Hoshino and Tokioka, 1967, *Corella eumyota* Traustedt, 1882, and *Pyura chilensis* Molina, 1872] present on both (Table 1).

Most species could be assigned either a native or introduced status based on available distributional data. Only *Polyzoa minor*, with two widely separated geographical records, and *Polyzoa* sp. were left as cryptogenic. As for the introduced ascidians, *Asterocarpa humilis* (Heller, 1878), a southern Hemisphere species recently expanded into the northern Hemisphere (Bishop et al. 2013), is here regarded as introduced in Chile following Clarke and Castilla (2000). *Botryllus schlosseri* (Pallas, 1776) is widespread in warm and temperate areas in both hemispheres (Locke 2009) and is likely of Northwest Atlantic origin (Yund et al. 2015, but see López-Legentil et al. 2006 and Lejeune et al. 2011). Our specimens of the genus *Ciona* were genetically and morphologically (following Sato et al. 2012) attributable to the so-called *Ciona intestinalis* (Linnaeus, 1767) Clade A or sp. A (Caputi et al. 2007; Zhan et al. 2010), the most invasive clade of this species complex, whose correct name has been recently

Table 1. List of the species identified in this study, with information on their status (native, introduced, cryptogenic), type of substrate (A, artificial; N, natural), and region where it has been found.

	Species	Status	Substrate	Region
Aplousobranchia Lahille, 1886				
Didemnidae Giard, 1872	<i>Didemnum studeri</i> Hartmeyer, 1911	Native	A,N	Magellanic
	<i>Diplosoma listerianum</i> (Milne Edwards, 1841)	Introduced	A,N	Temperate
	<i>Lissoclinum perforatum</i> (Giard, 1872)	Introduced	A	Temperate
	<i>Polysyncraton trivolutum</i> (Millar, 1960)	Native	N	Magellanic
Holozoidae Berrill, 1950	<i>Distaplia colligans</i> Sluiter, 1932	Native	N	Magellanic
Polyclinidae Milne Edwards, 1841	<i>Aplidium fuegiense</i> (Cunningham, 1871)	Native	A	Magellanic
	<i>Aplidium peruvianum</i> Sanamyan and Schories, 2004	Native	N	Temperate
	<i>Aplidium variable</i> (Herdman, 1866)	Native	A	Magellanic
	<i>Synoicum georgianum</i> Sluiter, 1932	Native	A	Magellanic
Phlebobranchia Lahille, 1886				
Cionidae Lahille, 1887	<i>Ciona robusta</i> Hoshino and Tokioka, 1967	Introduced	A,N	Temperate
Corellidae Lahille, 1888	<i>Corella eumyota</i> Traustedt, 1882	Native	A,N	Temperate
Stolidobranchia Lahille, 1887				
Styelidae Sluiter, 1895	<i>Asterocarpa humilis</i> (Heller, 1878)	Introduced	A	Temperate
	<i>Botryllus schlosseri</i> (Pallas, 1766)	Introduced	A	Temperate
	<i>Cnemidocarpa verrucosa</i> (Lesson, 1830)	Native	A	Magellanic
	<i>Polyzoa opuntia</i> Lesson, 1830	Native	A	Magellanic
	<i>Polyzoa minor</i> Monniot, 1970	Cryptogenic	N	Temperate
	<i>Polyzoa</i> sp.	Cryptogenic	N	Temperate
	<i>Styela changa</i> Monniot and Andrade, 1983	Native	N	Temperate
	<i>Styela paessleri</i> (Michaelson, 1898)	Native	A	Magellanic
Pyuridae Hartmeyer, 1908	<i>Pyura chilensis</i> Molina, 1872	Native	A,N	Temperate
	<i>Pyura legumen</i> (Lesson, 1830)	Native	A	Magellanic
Molgulidae Lacaze-Duthiers, 1877	<i>Paramolgula</i> sp.	Native	A	Magellanic

established to be *Ciona robusta* (Brunetti et al. 2015; Pennati et al. 2015). Likewise, *Diplosoma listerianum* comprises different species, present in all seas except Antarctica (Locke 2009) and our specimens were genetically attributed to Clade A described by Pérez-Portela et al. (2013), the most widespread by human activities. Pérez-Portela et al. (2013) were the first to report the species in Chile in the Coquimbo area, and it was later found in the Patagonian Fjords zone (Schories et al. 2015). *Lissoclinum perforatum* is a European species that has expanded its distribution into the Azores (Monniot 1974), the Caribbean (Monniot 1983), and the Brazilian coast (Rocha et al. 2005; Rocha and Bonnet 2009; Dias et al. 2013). It is here signalled for the first time in the Pacific Ocean. The remaining species were classified as native because they have been described on natural substrates in the general areas studied and/or have a known non-disjunct distribution that includes the zones here analysed. In the case of *Corella eumyota*, recent revisions of this taxon, widespread in antarctic and sub-antarctic regions (and now introduced in European waters, Lambert 2004), have revealed the existence of several species (Alurralde et al. 2013; Monniot 2013). The Chilean populations, however, retain

the original name of *Corella eumyota*, a species described from Chile (Valparaíso) and thus considered here as native.

The distribution of native and introduced species in the two areas studied showed markedly contrasting trends. No introduced species were identified in the Magellanic localities, while 5 out of 11 species were introduced in the area surveyed in northern Chile.

Discussion

Two main results stem from our study: first, the differentiation between the ascidian faunas of the two regions surveyed; second, an apparent absence of introduced species in the Punta Arenas zone, as opposed to almost 50% of introduced species in northern Chile (Coquimbo). A very recent review of antarctic and southeastern Pacific ascidians (Schories et al. 2015) places the number of species reported from Chilean waters (including Juan Fernández and Desventuradas Islands) up to 78. In our study we identified 22 species in a spatially restricted sampling (2 zones). We increase the known ascidian fauna of Chile by three species: *Lissoclinum perforatum*, *Synoicum georgianum*, and *Polyzoa minor*, the former

being introduced and found for the first time in the Pacific Ocean.

In their comprehensive review, Monniot and Monniot (1983) already noted that the ascidian faunas of the antarctic and subantarctic regions were completely different from those found northwards in all continents. In their review of the distribution of ascidians from southeastern Pacific, Schories et al. (2015) also distinguished on the Chilean coasts a northern zone with southern limit at 30° (equivalent to the Peruvian Province), a transition zone (30–40°) and a southern (Magellanic) zone southwards. Sixty-seven species were reported for the southern zone, 19 for the transition zone, and 19 from the northern zone. However, the number of species found only in a given area was 50 for the southern zone, 3 for the transition zone, and 5 for the northern zone, highlighting the isolation of the southern ascidian fauna. In a study on natural substrates near Punta Arenas, Sanamyan and Schories (2003) reported 9 species, all with antarctic and subantarctic distributional range. Five of these species have been sampled in our work [*Aplidium fuegiense* (Cunningham, 1871), *Aplidium variabile* (Herdman, 1866), *Didemnum studeri*, *Distaplia colligans* Sluiter, 1932, and *Pyura legumen* (Lesson, 1830)]. The first four species are also included in the list of 12 species reported recently in the northern limit of the Magellanic region (Chiloé area, Lagger et al. 2009), while two species found by these authors, *Corella eumyota* and *Pyura chilensis*, were present in our samples from Coquimbo. The Chiloé area, at about 42°S, has therefore some transitional characteristics between the ascidian fauna of the two regions here analysed. Three of the species found in Punta Arenas (*Didemnum studeri*, *Aplidium fuegiense*, *Polysia opuntia* Lesson, 1830) extended northwards up to the northern Chilean coast (Sanamyan et al. 2010, Schories et al. 2015). Conversely, there is only one report in the Punta Arenas area of one species found here in Coquimbo, an old report by Traustedt (1885) of *Ciona intestinalis* (now *C. robusta*) in the Strait of Magellan. However, this report is highly dubious (Monniot and Monniot 1983), and the species is likely to be *Ciona antarctica* Hartmeyer, 1911. No specimen of *C. robusta* was found in spite of intense sampling of harbors and piers and there is no basis in our view to sustain the presence of this species in the Magellanic region at present.

Temperature, a major driver of ascidian distribution (Lambert 2005), is markedly different between the two regions here surveyed, with a

yearly mean of ca. 6.6°C (range 5° to 11°C) in the Punta Arenas zone (Antezana 1999), and ca. 15.5°C (range 12° to 22°C) in the Coquimbo area (Thiel et al. 2007). This is probably the main factor determining differences in faunal components. In addition, the fjord areas of the northern Magellanic region, with influxes from freshwater and associated salinity changes (Antezana 1999; Iriarte et al. 2010), are also likely to act as barriers and prevent the spread of species. In general, ascidians are not able to withstand salinities below 20–25‰ (reviewed in Lambert 2005). Upwelling events, particularly if they bring to the surface layer waters depleted in oxygen (Thiel et al. 2007), can further impede the stepping-stone exchange of species among areas.

Castilla et al. (2005) noted that the number of non-indigenous species reported for Chilean waters (32 species) is noticeably lower than that of other geographic areas such as North America and New Zealand. One potential explanation lies in the scarcity of studies of artificial substrates such as those occurring in ports and harbours where introduced species are more abundant. Four ascidian species are listed in Castilla's report: *Pyura praeputialis* (Heller, 1878), *Ciona intestinalis* (now *C. robusta*), *Asterocarpa humilis* and *Molgula ficus* (Macdonald, 1859), while *Botryllus schlosseri* is listed as likely introduced. *Diplosoma listerianum* (Pérez-Portela et al. 2013; Schories et al. 2015) and *Lissoclinum perforatum* (present work) should be added to this list, totalling 7 introduced ascidian species reported for Chile. Of these, *Pyura praeputialis* and *Ciona robusta* are worth noting. The first one forms dense carpets in the lower intertidal and is able to completely modify the landscapes, thus acting as a bioengineer species (Castilla et al. 2004) fostering increased biodiversity. However, it is only found in Antofagasta Bay and adjacent waters, some 700 km north of Coquimbo (Castilla et al. 2002). *Ciona robusta*, on the other hand, causes serious damage and important economic losses to cultures of the scallop *Argopecten purpuratus* (Lamarck, 1819) (Uribe and Etchepare 2002) and is expanding its range in northern-central Chile (Madariaga et al. 2014). Almanza et al. (2012) reported recruitment of *Ciona robusta* on top of the giant kelp *Macrocystis pyrifera* (Linnaeus) C. Agardh, 1820, and we have found some of the introduced ascidians (*Ciona robusta* and *Diplosoma listerianum*) inhabiting natural substrates, which points to a worrisome ability for dispersal outside man-made, artificial environments and highlights the need for continued monitoring efforts. A

genetic assessment of introduced species is also necessary to elucidate the fitness of these populations, the propagule pressure, the existence of recurrent introductions, and other parameters of interest for their management (Rius et al. 2015).

The absence of introduced species in a zone with heavy maritime traffic such as Punta Arenas is remarkable. Active harbours in the area, such as Muelle Prat or Cabo Negro docks (Keller et al. 2010), sustain a dense fouling of ascidians, much as other harbours worldwide, but in this case this fauna is not made up of the usual introduced species, but instead is composed of native forms [mainly *Polyzoa opuntia*, *Didemnum studeri*, *Paramolgula* sp., and *Cnemidocarpa verrucosa* (Lesson, 1830)]. The cause for such absence can only be speculated upon, but low temperatures may impede the proliferation of non-indigenous ascidians in the Magellanic region. Introduced ascidians are usually tropical or temperate. Shenkar and Swalla (2011) list 64 documented global non-indigenous species of ascidians, of which 14 are restricted to tropical environments and 50 are from temperate or temperate-cold regions. Rius et al. (2014) showed how the expansion of some introduced ascidians correlated with their developmental thermal tolerances in another system with a marked temperature gradient due to currents (South African shores). Colder waters in the Chilean Patagonia can explain why introduced species that have been able to proliferate in Patagonian waters of the Atlantic shelf (Orensanz et al. 2002) are not found in Chile, with differences in sea water temperature for the same latitude range of 3–6°C between the Pacific and the Atlantic, due to currents and upwellings (Castilla et al. 2005). Some introduced ascidians, such as *Asciidiella aspersa* (Müller, 1776) and *Styela clava* Herdman, 1881, are already present in Argentinian Patagonia (Tatián et al. 2010; Pereyra et al. 2015) but have not been found in Chile. It is probable that extreme temperature events, rather than mean thermal values over the year, are limiting the success of introduced ascidians in the Magellanic region. A possible exception is *Asterocarpa humilis*, which we could not detect in our samples from the Magellanic zone, but that has been reported from Ushuaia (Schwindt et al. 2014, under the name *Cnemidocarpa robinsoni* Hartmeyer, 1916) and deserves close monitoring at the Magellan strait. Of course, other parameters besides temperature (salinity, oxygen levels, phytoplankton abundances) may be relevant, and further experimental work is required to

assess the factor or combination of factors that so far has hampered the establishment of introduced ascidians in the southern Magellanic area.

The apparent scarcity of introduced species in the Chilean southernmost areas is likely to be a labile one, as a rise in temperature can make extensive areas habitable by species that nowadays cannot withstand low temperatures. Rius et al. (2014) have reported ongoing range expansions of non-indigenous ascidians in South Africa linked to climate change. El Niño (ENSO) events have been reported to foster southward expansion of some non-indigenous species in Chile (Castilla et al. 2005). Chilean Patagonia encompasses one of the major fjord systems in the world and five large Ice Fields, providing one of the world's largest freshwater reservoirs. This region is sustaining in recent years intense anthropogenic pressures linked to commercial projects in hydroelectricity, tourism, and salmon and bivalve culture (Pantoja et al. 2011). The downside of increased anthropogenic presence and economic activities lies in the threats these developments pose to the natural communities (Iriarte et al. 2010). The southwest Atlantic is already impacted north of central Patagonia (Orensanz et al. 2002), and populations of important introduced species seem to be expanding at a fast rate. The situation in southwest Atlantic calls for caution when considering the future of the Chilean southern shores. A sustained monitoring effort in different latitudinal zones of Chile is necessary for early detection of new arrivals or significant range expansions of introduced species and for the implementation of contingency plans.

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Supplementary material

The following supplementary material is available for this article:

Table S1. The sampling localities, with species present, dates, depth, and coordinates.

This material is available as part of online article from:

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