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Research Article

Iceland: a laboratory for non-indigenous ascidians

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Abstract

Non-indigenous species (NIS) represent a serious problem worldwide, where ascidians are one of the most important taxa. However, little has been done to document the non-indigenous ascidians in Iceland, and over the past decade only two species had been recorded prior to the present study, *Ciona intestinalis* in 2007 and *Botryllus schlosseri* in 2011. To increase the knowledge of this taxon, extensive sampling was carried out in shallow waters around Iceland, during the summer 2018, in ports and on ropes of a long-line mussel aquaculture. In total, eleven species were identified, four native and seven NIS, of which *Diplosoma listerianum, Ascidiella aspersa, Botrylloides violaceus*, *Molgula manhattensis* and *Ciona* cf. *robusta*, are now reported for the first time in Iceland. The highest abundance of non-indigenous ascidians appeared among the ports in southwestern Iceland (Sandgerði, Hafnarfjörður). As pointed out for other regions, the most likely vector is maritime traffic (hull fouling and ballast water), although other vectors cannot be ruled out. The future expansion of these non-indigenous ascidians around Iceland must be monitored, where local maritime traffic could play an important role. Furthermore, global warming may facilitate the access and establishment of these species in colder areas with arctic influence (north and east of Iceland), which are likely still free of these species.

Key words: biofouling, global warming, maritime traffic, NE Atlantic

Introduction

Ascidians (Chordata: Tunicata) represent an important taxon of the non-indigenous species (NIS) worldwide (Lambert 2007) and have been suggested as good models for invasion success (Zhan et al. 2015). The adults are sessile and efficient non-selective filter feeders (Monniot 1979), reproducing by releasing short-lived lecithotrophic swimming larvae which involves a dispersion of short distances (Millar 1971; Svane and Young 1989). The appearance of some species at great distance from their native distribution range can only be explained by the intervention of man, through maritime traffic (hull fouling, ballast-water) and bivalve translocations for aquaculture (Monniot and Monniot 1983, 1994; Carlton and Geller 1993; Lambert 2001, 2002). In man-made environments, such as ports, marinas, as well as shell-fish aquaculture facilities, the non-indigenous

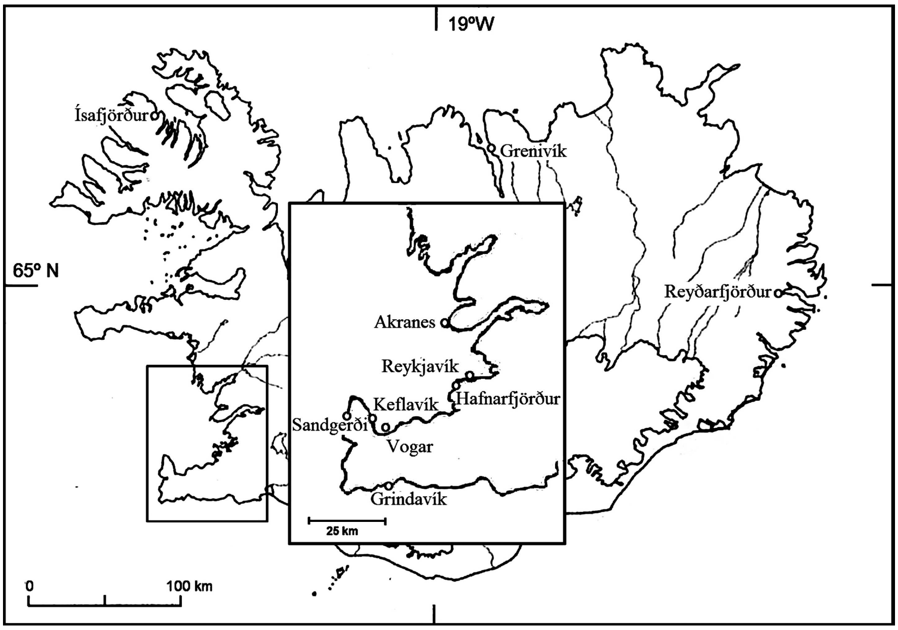

Figure 1. Location of the stations.

ascidians find adequate conditions to survive and resist in. From there they can expand locally through maritime traffic. Global warming, that particularly affects cold regions in altering biological interactions, may also increase the likelihood of invasions by NIS (Dijkstra et al. 2011).

Iceland is located between boreal and arctic waters. With an intermediate position between North America and Europe, it represents an interesting area for the study of recent NIS and their possible spread in cold waters. Ascidiofauna of Iceland has previously been reported to comprise 41 spp. (Hartmeyer 1923, 1924; Van Name 1945; Huus and Knudsen 1950; Lützen 1959; Millar 1966, 1974). The more recent BIOICE (Benthic Invertebrates in Icelandic Waters) project, with extensive sampling carried out in the years 1991 to 2004 at depths between 18 and 3000 m, has contributed 27 new ascidian records for Iceland (Ramos-Esplá 2016). In this regard, Thorarinsdottir et al. (2014) and Gunnarsson et al. (2015) reported 15 NIS of invertebrates and algae in Icelandic waters, one of which was *Ciona intestinalis*, first observed in 2007. Ramos-Esplá (2016) then added the colonial ascidian *Botryllus schlosseri* to the list of new NIS for Iceland.

Given the great expansion of NIS worldwide in recent decades, particularly in the North Atlantic (Carman et al. 2010; Martin et al. 2011; Bishop et al. 2015; Tsiamis et al. 2018), it was necessary to address the lack of knowledge of ascidians associated with ports and marine aquaculture facilities in Iceland.

Materials and methods

Study areas

During the summer 2018, between 7th of June and 18th of September, ten localities were sampled (Figure 1, Supplementary material Table S1): i) ports

(Akranes, Reykjavík, Hafnarfjörður, Sandgerði, Keflavík, Grindavík, Ísafjörður, Grenivík and Reyðarfjörður); and ii) *Mytilus edulis* long-line installation (in front of Vogar). For sampling in ports and mussel long-lines we followed the protocol of the rapid assessment survey (RAS; Pederson et al. 2005) where pontoons, buoys, tires, fences, ropes and kelp were observed during 1–2 hours/locality. It is important to note than there no official regulations regarding dock cleaning at the sampling sites (harbor managers, personal communication). The dominant fouling species at sampling sites were mussels (*Mytilus edulis*), barnacles (*Balanus balanus, B. crenatus, Semibalanus balanoides*) and macroalgae (*Alaria esculenta, Laminaria digitata, Ulva* spp.).

Treatment of samples and data

Ascidians were transported in seawater to the Southwest Iceland Nature Research Centre (SINRC) where they were anesthetized with menthol crystals, fixed in 10% seawater formalin, and 48 hours later preserved in 70% ethanol. For morphological taxonomy purposes, solitary and colonial (zooids) ascidians were dissected, some of them stained with Masson's haemalum, and mounted on permanent slides in Canada' balsam. The specimens are all stored at SINRC with the identification code: Asc-IF-001 to 057. We used the categories of the SACFOR scale (modified from Connor and Hiscock 1996) for the estimation of relative abundance in number of individuals/colonies observed in the different structures/locality. The range of values in the categories are based on exponentials of 3: (S) superabundant (> 81 individuals/colonies); (A) abundant (28–81); (C) common (10–27); (F) frequent (4–9); (O) occasional (2–3); and (R) rare (1).

Literature sources

A comprehensive literature search was conducted, including available scientific publications, "grey literature" (scientific congresses, technical reports, student theses), and web databases (Biodiversity Heritage Library, Google Scholar, Scopus, GBIF, NIS web pages). For the classification of the species we mainly followed Millar (1966) and other authors (Monniot 1969; Lafargue 1975; Saito et al. 1981; Hoshino and Nishikawa 1985; Brunetti et al. 2015) and updated nomenclature by Ascidiacea World Database (www.marinespecies.org/ascidiacea/). Data from personal observations were also included.

Results

A total of 238 ascidian specimens were collected during the 2018 summer survey, belonging to five families and 10 species (Table S2), and one to be confirmed (*Ciona robusta*). The solitary strategy dominates in the number of species (72.7%) and in the abundance (87.4%) over the colonial one. Seven of these species are considered as introduced to Icelandic waters:

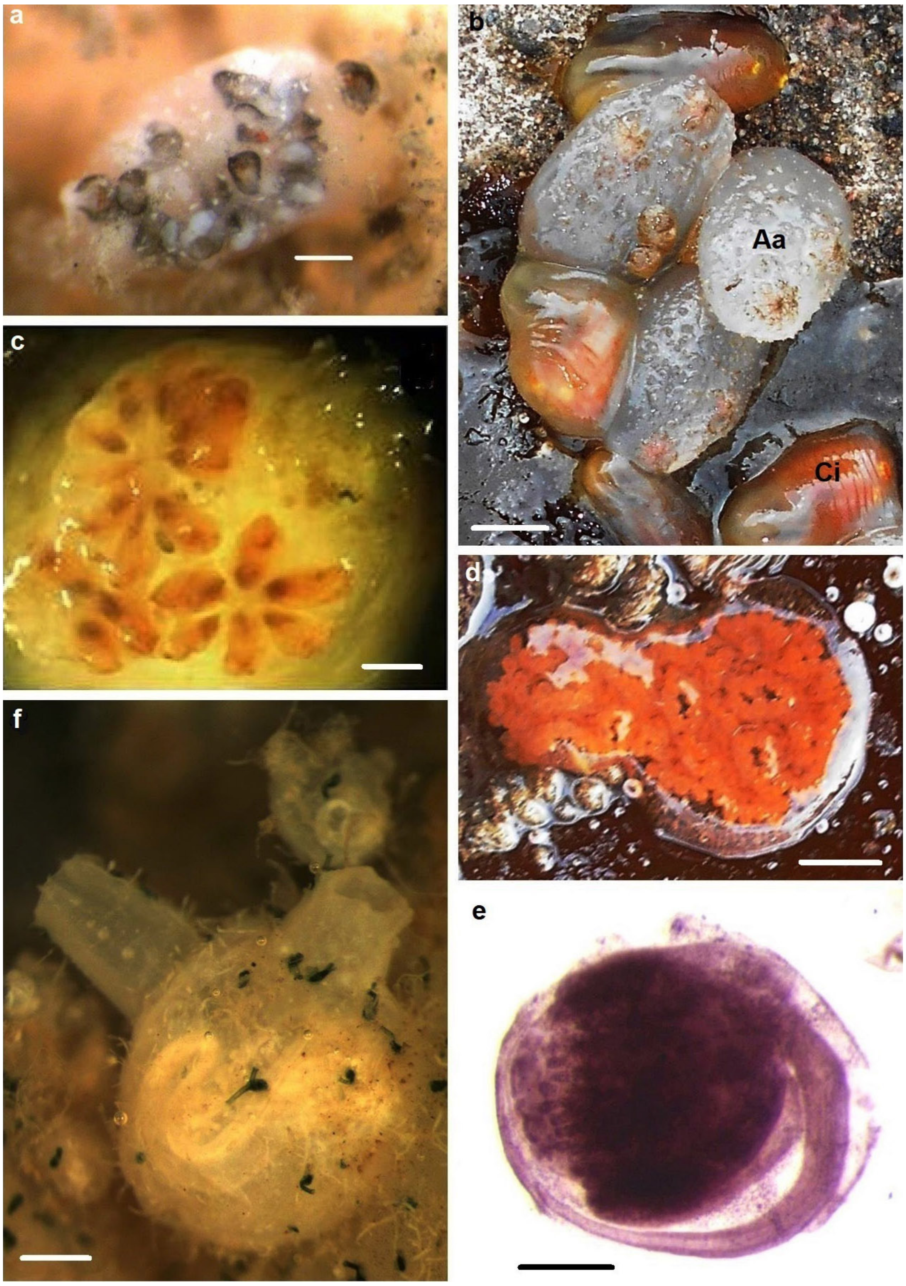

Figure 2. NIS ascidians: (a) *Diplosoma listerianum* (station Sa1); (b) *Ascidiella aspersa* (Aa) and *Ciona intestinalis* (Ci) on kelp blade (st. Sa3); (c) *Botryllus schlosseri* (st. Sa1); (d) colony of *Botrylloides violaceus* on kelp blade; (st. Ha2); (e) larvae of *Botrylloides violaceus*; (st. Ha2); (f) juvenile of *Molgula manhattensis* (st. Sa2). Scale bars: 0.5 mm (a, c, e); 10 mm (b); 5 mm (d); 1 mm (f).

Diplosoma listerianum, Ciona intestinalis, Ascidiella aspersa, Botryllus schlosseri, Botrylloides violaceus, Molgula manhattensis and *Ciona* cf. *robusta*. These species do not appear in the Millar' study (1966) or the comprehensive list of ascidians identified in the BIOICE project (Ramos-Esplá 2016). The four remaining species found in the present study are considered native in Iceland: *Ascidia callosa, Styela rustica, Halocynthia pyriformis* and *Molgula citrina*.

Diplosoma listerianum (Milne Edwards, 1841), species complex (Pérez-Portela et al. 2013): Only a small and immature colony (3 × 2 × 1 mm) was observed on *Ascidia callosa* test (Figure 2a). The colony and the zooids respond to the description of Millar (1966): gelatinous and transparent test, without spicules, and zooids with 4 rows of stigmata, without languet. Although they are immature zooids (spermiduct has not been observed), that colony corresponds to the genus *Diplosoma*; and the unique species widely distributed in northeastern Atlantic is *D. listerianum* (Millar 1966, 1970; Lafargue 1975). It has been introduced in the NW Atlantic and in other parts of the world (AquaNIS 2015; Fofonoff et al. 2018). Population status in Iceland: casual (only one immature colony has been observed). First record for Iceland.

Ciona intestinalis (Linnaeus, 1767): The specimens present smooth tunic around the siphons, without tubercles (*Ciona* type B, Figure 3a, b; Brunetti et al. 2015). Previously recorded in Iceland by Svavarsson and Dungal (2008) in 2007 at the commercial port of Straumsvík. Subsequently, it was found in 2010 by Björnsson (2011) at the ports of Reykjavík, Grindavík and Sandgerði. At present, *C. intestinalis* has only been recorded in SW Iceland, where it seems to be the non-indigenous ascidian dominating the artificial surfaces of the ports (Micael et al. 2020). It was not found in north and eastern Iceland (Ïsafjörður, Grenivík, Reyðarfjörður). Population status in Iceland: established.

Ciona cf. robusta Hoshino & Tokioka, 1967. The only specimen examined was 54 mm in height, presenting a cartilaginous and thick test (1.5 mm), like Styelinae, and tubercular prominences around the siphons (*Ciona* type A, Figure 3c, d). Due the conservation of the specimen, it has not been possible to observe the coloration of the gonoduct tips. Although the tunic and prominences around the siphons make it similar to *C. robusta*, (Hoshino and Nishikawa 1985; Brunetti et al. 2015), the species confirmation should be pending or not registered in Icelandic waters without additional samples. The species, possibly originally from NW Pacific, has been widely introduced in warm and cold regions (AquaNIS 2015; Fofonoff et al. 2018), although it has not yet been observed in waters near Iceland (Canada, Norway). Population status in Iceland: to be confirmed (although mature, only one specimen has been observed).

Ascidiella aspersa (Müller, 1776): The only specimen sampled in Sandgerði (Sa1, June 2018) measured 23 mm and was immature. Subsequently, specimens were sampled in July and August 2018 in the same locality (Sa2; Figure 2b) and Hafnarfjörður (Ha2), respectively. The five sampled specimens measured between 47–82 mm and were all mature. An important aspect of this species is the facilitation of substrate it provides for other species to settle on its test (macroalgae, sponges, hydrozoans, bryozoans and ascidians). This is particularly important in the summer period, when NIS begin to develop, so adequate surface is available for them. Thus, we have observed other non-indigenous ascidians on *A. aspersa* as *C. intestinalis*, *M. manhattensis* and *B. schlosseri*. The species is of NE Atlantic origin, currently, presenting a worldwide distribution (AquaNIS 2015; Fofonoff et al. 2018). Traustedt (1880) cited *Ascidia patula* (synonym of

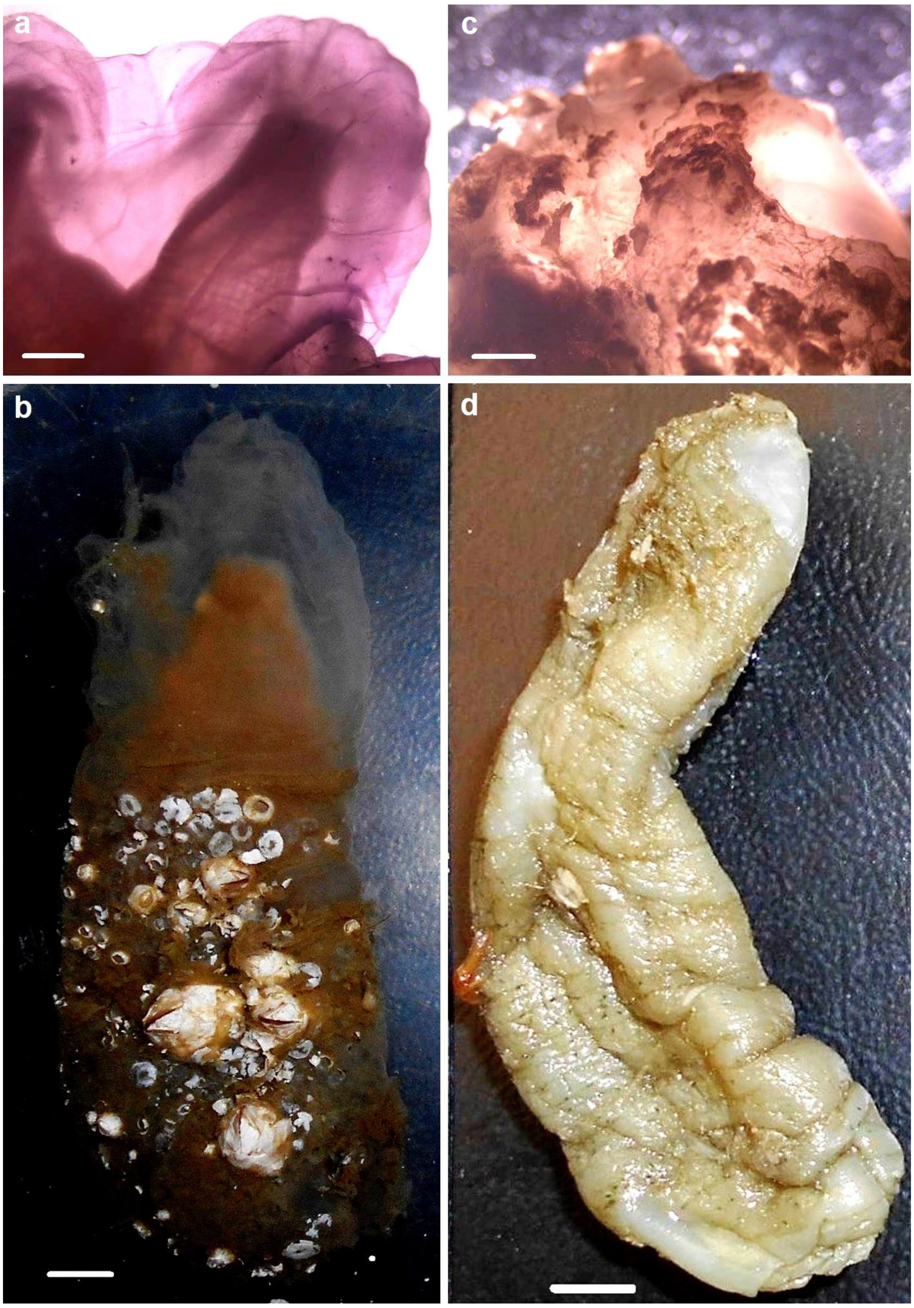

Figure 3. *Ciona intestinalis*: detail of the siphonal area (a), external appearance (b). *Ciona* cf. *robusta*: detail of the siphonal area (c); external appearance (d). Scale bars: 2 mm (a, c), 5 mm (b, d).

A. aspersa) from Iceland, but after Hartmeyer's (1923: 57) review of the boreal-arctic ascidiofauna, it was identified as *Ascidia obliqua*. Population status in Iceland: established (mature specimens in Sandgerði and Hafnarfjörður). First record for Iceland.

Botryllus schlosseri (Pallas, 1766), species complex (Bock et al. 2012): It was first observed on 11th of July in 2011 in front of Vogar on *Mytilus edulis* in a long-lines aquaculture facility, at approximately 3 m depth (Ramos-Esplá 2016). Subsequently, its presence has been detected in Sandgerði and Grindavík in high abundance. The colonies in June were immature (Figure 2c), while in July and August, the testicles were developed; larvae have not been observed. Together with a specimen of *C. intestinalis*, they are the only species that have till now been observed on mussel culture long-lines and buoys. The species is likely of NE Atlantic origin, and presents a worldwide distribution (AquaNIS 2015; Fofonoff et al. 2018). Population status in Iceland: established (mature colonies in Sandgerði and Grindavík).

Botrylloides violaceus Oka, 1927: Only sampled in Hafnarfjörður (Ha2), with four encrusting colonies (24–59 mm) with homogeneous orange and garnet color (Figure 2d); mature zooids and large larvae (2 mm) with 26–28 lateral ampullae (Figure 2e). Originally from NW Pacific, and has extended its distribution worldwide (AquaNIS 2015; Fofonoff et al. 2018). Population status in Iceland: casual (although colonies with larvae have been observed, it has only been found in Hafnarfjörður). First record for Iceland.

Molgula manhattensis (De Kay, 1843): The specimens sampled were between 3 and 29 mm, some of them fixed on *Ascidiella aspersa* (Figure 2f). It presents six branchial folds on each side, right rectilinear gonads, and the male genital papillae scattered over the gonad (Monniot 1969). The specimens sampled in June were immature, while in July and August the gonads were developed. The probable origin is the NW Atlantic, where it is considered native based on mtDNA analysis (Haydar et al. 2011). The species is currently distributed in NE (including the Mediterranean and Black Seas) where it is considered cryptogenic (Haydar et al. 2011), as well as in the SW Atlantic, and NW and NE Pacific (AquaNIS 2015; Fofonoff et al. 2018). Population status in Iceland: established (mature specimens in Sandgerði and Reykjavík). First record for Iceland.

Discussion

The non-indigenous ascidians mentioned above are considered introduced for Iceland, not cryptogenic (Carlton 1996), on the basis that they have been recently recorded (last two decades); for this we rely on Millar's review (1966), the BIOICE project (Ramos-Esplá 2016) and on local environmental assessment (grey literature), where these species do not appear in the inventories. However, it cannot be ruled out that some of these species may have arrived in the past by natural dispersal, e.g. on driftwood or algae (Millar 1971) though unproven, and in recent years increased their abundance due to global warming. Only *Ascidia callosa* could be considered cryptogenic since it was first cited for Iceland in 1950 (Huus and Knudsen 1950). However, Traustedt (1880) cited *Phallusia olrikii* in Iceland, currently a synonym for *A. callosa* (Hartmeyer 1923; Sanamyan 2007). At the present time, regarding the possible classification of the reported ascidians as invasive species (except for *C. intestinalis*), we cannot state or confirm anything, since they have only been observed in man-made localities and do not seem to extend outside of ports (Williamson and Fitter 1996). However, artificial structures do not only present an opportunity for NIS, since the tunics of other pioneer non-indigenous ascidians (as *Ascidiella aspersa*) also facilitate the settlement of larvae, permitting their survival and growth

(Figure 2a, c, f) from June to September with the maturation of specimens in this warmer period. Regarding the facility of bivalve aquaculture (Vogar), the non-indigenous ascidians *C. intestinalis* and *B. schlosseri* were observed and in low abundance (only one specimen of each species), while the native species *Styela rustica* and *Halocynthia pyriformis* were observed in greater abundance. This does not imply that in the coming years and, particularly, in the summer season, the previous NIS cannot become dominant, like in other mussel growing areas, e.g. off/on the east coast of North America (Carman et al. 2010).

The port of Sandgerði contains the highest number of non-indigenous ascidians reported in Iceland (4 spp.), and Micael et al. (2020) recently point to a higher abundance of *Ciona intestinalis* in in Sandgerði compared to the ports in Reykjavík and Grindavík. The reason for this is not known but facilitation of new space for settlement, such as the test of solitary non-indigenous ascidians (e.g. *Ascidiella aspersa*), together with favourable local conditions within Sandgerði harbour (a more stable range of salinity) could at least partly explain this difference. On the contrary, it is interesting to note that in the port of Akranes about 20 km north of Reykjavík, only *C. intestinalis* has been recorded; and in the northern and eastern ports (Ísafjörður, Grenivík, and Reyðarfjörður) no introduced species have been observed.

The isolation of non-indigenous ascidians in southwestern Iceland may be due to its recent introduction, and/or possible winter thermal barriers. Regarding temperature, the North Atlantic circulation reaches the south and the southwest coasts of Iceland, with annual temperatures ranging between 4–11 °C; while polar water reaches the north and east coasts, with temperatures between 2–9 °C (Storto and Masina 2016: C-GLORSv5 data). However, as elsewhere (Bishop et al. 2015), recently arrived species with a limited distribution are expected to expand due to global warming. In this regard, Stachowicz et al. (2002) observed a correlation between introduced species recruitment and interannual temperature variation, suggesting that ocean warming will facilitate the establishment and spread of introduced species, particularly in the mid and high latitudes of the Northern Hemisphere. Other temperate affinity non-indigenous ascidians (Locke 2009) are candidates to appear in Iceland in the coming years, such as *Styela clava* Herdman, 1881 and *Didemnum vexillum* Kott, 2002, common in Europe and North America (Lützen 1999; Ramsay et al. 2008; Nunn and Minchin 2009; Carman et al. 2010; Martin et al. 2011; Bishop et al. 2015).

Iceland Shelf is one of the most threatened areas, with a net temperature increase between 0.67 and 0.89 °C (records from 1982 to 2006; Belkin 2009). This means that the possible thermal barrier (mainly, winter water temperature) will become weaker and that NIS can extend their spread to the coldest areas westward, and later the northern and eastern Iceland, through shipping and/or by natural spread with the coastal current that runs in a clockwise direction around the island (Valdimarsson and Malmberg 1999). Iceland can probably be considered as an excellent "laboratory" to study the expansion of NIS towards very cold areas (Chan et al. 2019).

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Supplementary material

The following supplementary material is available for this article:

Table S1. Characteristics of the stations.

Table S2. Observed ascidians in ports and an in a mussel farm in Iceland (2018) with the relative abundance, reproduction stage, first year of record, native and population status.

Appendix 1. List of references for Table S2.

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