

Rapid Communication

Second record of *Diplosoma listerianum* (Milne-Edwards, 1841) five years after and 280 kilometres from the site of the first record in Nova Scotia

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Abstract

Five samples of *Diplosoma listerianum*, a non-indigenous colonial ascidian species complex, were collected via SCUBA between August 27 and September 28, 2017, from four sites in the coastal waters (depth ranging between 3.1 and 18.0 metres) around Brier Island, Nova Scotia, Canada. These samples constitute the second record of *D. listerianum* in Nova Scotia—five years after the first discovery and 280 kilometres (by sea) from the site of the first record. Semi-quantitative abundance of *D. listerianum* from Brier Island was determined and was found to be frequent or common in three of these four sites that were surveyed via SCUBA. The occurrence at several sites, coupled by the presence of eggs, ovaries, and larvae in some tissue samples suggest that there is presently an established population of *D. listerianum* off of Brier Island.

Key words: invasive species, eastern Canada, Didemnidae, Ascidiacea, Tunicata

Introduction

Diplosoma listerianum (Milne-Edwards, 1841) is a colonial ascidian species complex (Tunicata: Ascidiacea) that has a cosmopolitan distribution in marine waters and is non-indigenous to the coastal waters of northeast North America (Mackenzie 2011; Pérez-Portela et al 2013). The first record of this species from northeast North America was from the Isles of Shoals, Maine, in 1993 followed by an additional record from Portsmouth Harbour, New Hampshire in 1999 (Dijkstra et al. 2007). In eastern Canada the species was detected for the first time at Îles de la Madeleine, Quebec, in 2008, followed by records from Nine Mile Creek, Prince Edward Island (2011), and Lunenburg, Nova Scotia (2012; Mackenzie 2011; Simard et al. 2013; Moore et al. 2014; Ma et al. 2016). Additionally, suspected colonies of *D. listerianum* have been found in 2016 in southwest New Brunswick (Sephton et al. 2017). *D. listerianum* has been detected by multiple methods, including *in situ* (SCUBA), molecular probes, and settlement plates.

In Nova Scotia, despite targeted searches for *D. listerianum* after its initial detection (*i.e.*, first record; Moore et al. 2014), the absence of further evidence of this species suggests that its introduction has failed in Lunenburg (Sephton et al. 2017). In this report, the second record of *D. listerianum* is documented five years after the discovery of and 280 kilometres (by sea) from the site of the first record in Nova Scotia.

Material and methods

Five colonial ascidian samples were collected via SCUBA in August and September 2017 at four sites from the coastal waters around Brier Island, Nova Scotia, Canada (Table 1; Figure 1). Samples were collected during a baseline semi-quantitative habitat survey of Brier Island, following a modified Marine Nature Conservation Review protocol (Connor and Hiscock 1996). Most species were identified *in situ* but for more difficult groups, samples were collected to confirm identification. These samples are retained

Table 1. Date, location, and habitat description of samples of *Diplosoma listerianum* from Brier Island, Nova Scotia, Canada. Abundance of *D. listerianum* at each surveyed site was classified according to the semi-quantitative SACFOR scale, which classifies abundance in six categories from rare to super-abundant (Connor and Hiscock 1996).

ARC museum specimen no.	Date (dive no.)	Site name	Latitude, longitude	Depth (m)	Temperature (°C)	Salinity (ppt)	Abundance	Habitat description
ARC 81413	August 27, 2017 (170827-01)	Peter's Island	44.2586, -66.3367	3.1–6.1	11.58	32.41	Rare	Kelp forest (<i>Saccharina latissima</i> and <i>Laminaria digitata</i>) on a steep slope of cobbles and large boulders.
ARC 81414 and ARC 81415	August 28, 2017 (170828-03)	East side of Whipple Point	44.2337, -66.3894	4.8–7.8	11.93	32.41	Frequent	Large bedrock steps dominated by coralline and foliose red algae.
ARC 81416	August 30, 2017 (170830-01)	Gull Rock	44.2095, -66.3855	3.5–15.5	12.67	32.17	Frequent	Vertical bedrock wall with <i>Laminaria digitata</i> kelp forest on upper parts.
ARC 81417	September 28, 2017 (170928-01)	South side of Brier Island	44.2446, -66.3531	13.5–18.0	13.21	32.59	Common	Bedrock and boulder wall. Cover of kelp and foliose red algae. Large areas of <i>Diplosoma listerianum</i> encrusting over other fauna and flora.

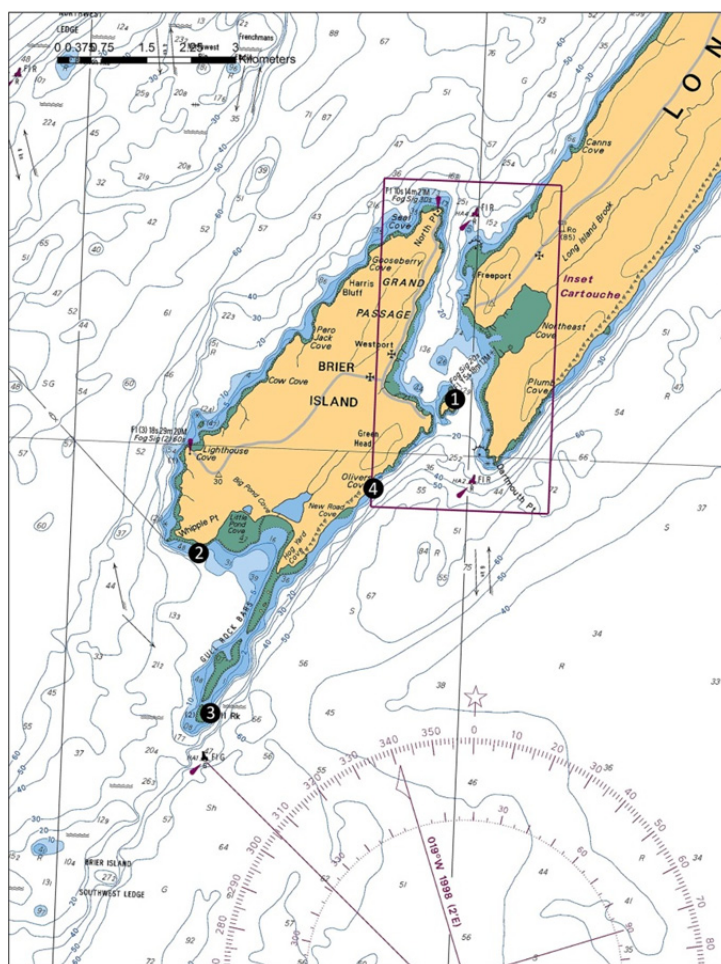


Figure 1. Locations of samples of *Diplosoma listerianum* collected from the coastal waters around Brier Island, Nova Scotia, Canada. 1 = Peter's Island; 2 = east side of Whipple Point; 3 = Gull Rock; and 4 = south side of Brier Island. Chart reproduced with permission from the Canadian Hydrographic Service.

in the collections of the Atlantic Reference Centre (ARC), Huntsman Marine Science Centre, New Brunswick, Canada. Samples were relaxed in seawater with the addition of menthol crystals for 10 to 14 hours (usually overnight) and then preserved in 10% seawater formalin buffered with marble chips (and a sub-

sample was preserved in 95% ethanol). After fixation in formalin for several days the samples were transferred into 50% isopropyl for long-term storage.

In October 2017, samples were examined for identification and imaging. The taxonomic keys to ascidian identification by Rocha et al. (2012) and by

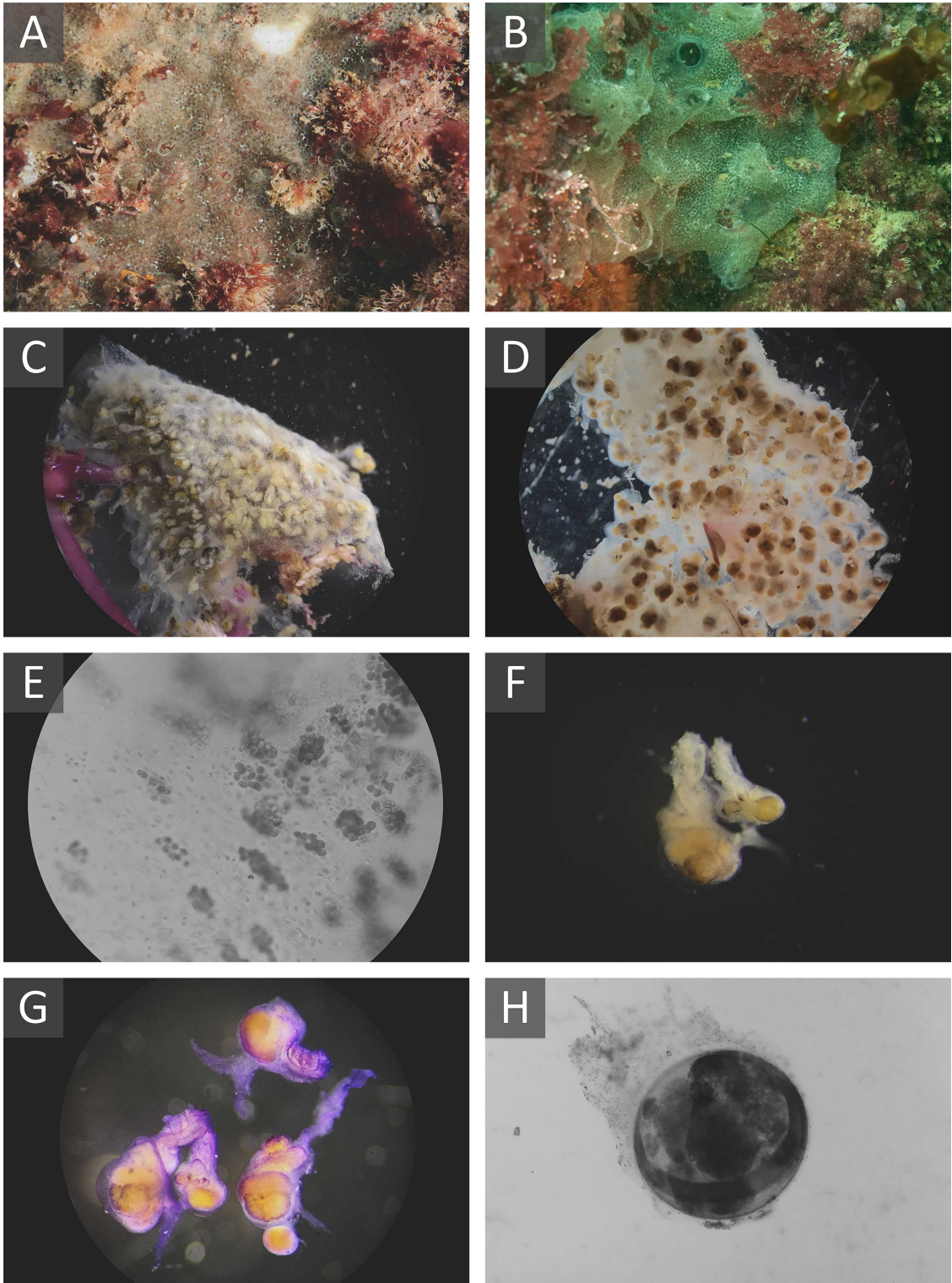


Figure 2. *Diplosoma listerianum* from coastal waters around Brier Island, Nova Scotia, Canada. (A, B) Live colonies photographed in the field; (C-D) preserved colonies illuminated under a stereomicroscope; (E) fragment of the tunic with granulations; (F) zooids; (G) zooids stained with haematoxylin; and (H) larva. The colour of the colony in panel (B) is not accurate due to camera lighting malfunction. Scale bar not available. Length of zooids ranged from 1 to 2 mm.

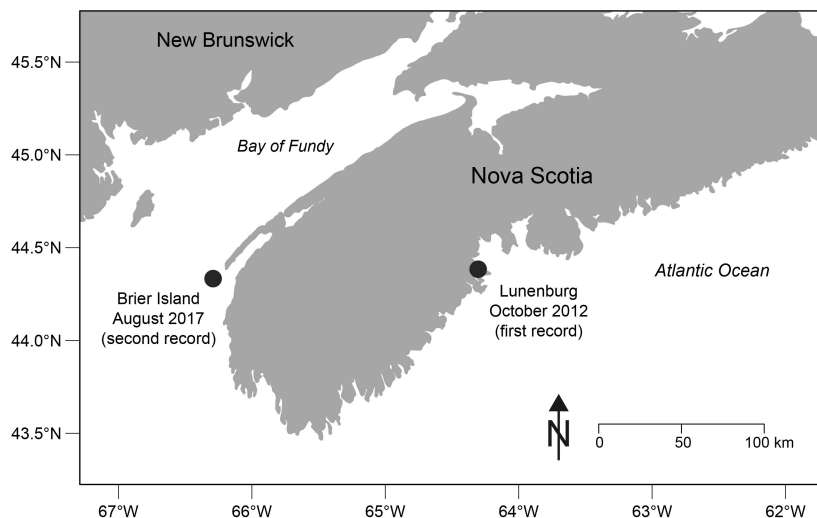


Figure 3. Locations of the first and second records of *Diplosoma listerianum* in Nova Scotia, Canada. The distance between the two locations is about 280 kilometres by sea.

Brunetti and Mastrototaro (2017) were used to identify specimens based on morphological features to genus and to species, respectively. Taxonomic descriptions in Van Name (1945) were also consulted. Additionally, Joyce A. Teixeira of Universidade Federal do Paraná (Brazil) examined digital photographs of the specimens for visual confirmation of species identity.

Results and discussion

A colonial mode of life, zooids completely embedded in the tunic, vertical arrangement of zooids, cloacal cavities, division of the body into thorax and abdomen, posterior position of the alimentary canal to the pharynx, four pharyngeal rows of stigmata, embryos and larvae in the tunic, and larvae with three adhesive papillae arranged in a line attribute these specimens to the family Didemnidae (Figure 2). Thin encrusting colonies with a soft tunic, lack of spicules, and white granulations (approximately 0.02 mm in diameter) on the tunic attribute these specimens to the genus *Diplosoma* (Figure 2). Translucent tunic, zooids ranging from 1 to 2 mm in length, thorax-abdomen ratio of 1:0.7, brown colouration around the siphons and on the abdomen, yellow colouration of organs, a two-lobed testis, and a straight sperm duct are characteristics that indicate they belong to the *D. listerianum* species complex (Figure 2).

The samples collected from Brier Island and described in this report constitute the second record of *D. listerianum* observed in Nova Scotia. This non-indigenous species was initially documented in 2012 in Lunenburg (Moore et al. 2014). This species has not been detected in Lunenburg since 2013 (Sephton

et al. 2017), which suggests that the introduction event in 2012 had failed and a population did not establish. The second record documented in this report being five years after and 280 kilometres from the site of the first record contribute to the understanding of the invasion history of *D. listerianum* in Nova Scotia (Figure 3).

The identification of possible vectors and pathways requires further investigation. Due to the limited natural dispersal ability of *D. listerianum*, spread of this species is likely to be anthropogenic (e.g., the movement of fouled boats, aquaculture products and gear, etc.; Mackenzie 2011; Pérez-Portela et al. 2013).

The abundance of *D. listerianum* was categorised as frequent or common in three of the four sites (Table 1). The presence of eggs, ovaries, and larvae in some of the samples from Brier Island indicates that the population is able to reach sexual maturity. One of the samples that was examined for this report was found encrusting the tunic of a native solitary tunicate, *Boltenia echinata* (Linnaeus, 1767). Other samples were found on either encrusting directly on bedrock and boulders or overgrowing turf algae and fauna. The occurrence at several sites, coupled by the presence of eggs, ovaries, and larvae in tissue samples suggest that there is an established population of *D. listerianum* in the coastal waters around Brier Island.

Globally, the *D. listerianum* species complex consists of at least four monophyletic clades (Pérez-Portela et al. 2013). Investigations into the species complex can help clarify the worldwide invasion pattern of *D. listerianum*. In eastern Canada, the clade(s) that the Canadian samples may belong to is presently unknown. To determine the clade(s) of the

eastern Canadian samples, an analysis of the mitochondrial sequences (*e.g.*, the cytochrome *c* oxidase subunit I) is required (Pérez-Portela et al. 2013). Furthermore, a more comprehensive genetic analysis of eastern Canada samples could provide important insights into the patterns of *D. listerianum* introductions; for instance, are all the introductions throughout eastern Canada coming from the same point of origin? The determination of donor site(s), vector(s), and pathway(s) associated with *D. listerianum* introductions in Nova Scotia can be informative in preventing future introductions before populations of this species become established and undergo secondary spread throughout the region. Additionally, long-term (multi-year) and large-scale (large geographic extent) monitoring of coastal waters for *D. listerianum* and other non-indigenous ascidian species in eastern Canada can be an effective approach to detect these species at early stages of the invasion process.

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