Research Article

Morphological identification of two invading ascidians: new records of *Ascidiella aspersa* (Müller, 1776) from Nova Scotia and *Diplosoma listerianum* (Milne-Edwards, 1841) from New Brunswick and Quebec

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Citation: Ma KCK, Hawk HL, Goodwin C, Simard N (2019) Morphological identification of two invading ascidians: new records of *Ascidiella aspersa* (Müller, 1776) from Nova Scotia and *Diplosoma listerianum* (Milne-Edwards, 1841) from New Brunswick and Quebec. *BioInvasions Records* 8(1): 50–64, https://doi.org/10.3391/bir.2019.8.1.06

Received: 5 July 2018
Accepted: 9 October 2018
Published: 6 November 2018

Handling editor: Mary Carman

Abstract

We report new observations of two invasive ascidian species, the solitary *Ascidiella aspersa* and the colonial *Diplosoma listerianum*, from Eastern Canada, which include some records from new sites. Because these species share many superficial characteristics with native and non-native species in the region, identifications based on detailed taxonomic studies of internal structures—and not only on external features—are essential and can complement molecular methods. In this report, specimens of *A. aspersa* collected from Nova Scotia in 2013 and *D. listerianum* from New Brunswick and Quebec in 2017 were described morphologically to confirm species identity. Specimens of both were collected one year after initial discoveries in Nova Scotia and New Brunswick, respectively. Specimens of *D. listerianum* from Quebec were collected after a six-year hiatus. This report contributes a new record of *A. aspersa* 30 km (by sea) north of the site of the first record in Canada. It also confirms the first record of *D. listerianum* and documents its rapid spread in New Brunswick. This report is the first time that *A. aspersa* from Eastern Canada and *D. listerianum* from New Brunswick and the second time that *D. listerianum* from Quebec was explicitly identified based on an examination of internal features. Species confirmation of these easily overlooked or misidentified taxa contributes a time-stamp on current invasion fronts and suggests that the risk of further introductions and spread of these invasive species is present at un-invaded sites throughout Eastern Canada.

Key words: invasive species, monitoring, Canada, Ascidiidae, Didemnidae, Ascidiacea, Tunicata

Introduction

Aquatic invasive species can impact ecosystem functioning and harm local economies, for instance, by disrupting shellfish aquaculture operations (Carman et al. 2010; Adams et al. 2011). Various ascidian species are considered aquatic pests around the world, and they are commonly found fouling underwater structures in ports, marinas, and aquaculture sites. As of 2018, seven species of invasive ascidians have been reported in the

waters of Eastern Canada (Ma et al. 2017a): *Ascidiella aspersa* (Müller, 1776), *Botrylloides violaceus* Oka, 1927, *Botryllus schlosseri* (Pallas, 1766), *Ciona intestinalis* (Linnaeus, 1767), *Didemnum vexillum* Kott, 2002, *Diplosoma listerianum* (Milne-Edwards, 1841), and *Styela clava* Herdman, 1881 (*e.g.*, Moore et al. 2014, Sephton et al. 2017). In Eastern Canada, the current distributions of *D. listerianum* (first detected in 2008), *A. aspersa* (in 2012), and *D. vexillum* (in 2013) are geographically localised relative to the other five invasive ascidian species (Moore et al. 2014; Ma et al. 2016; Ma et al. 2018; Simard et al. 2013; Vercaemer et al. 2015; Sephton et al. 2017).

The native range of *D. listerianum* is unknown and the species is considered cryptogenic in parts of the world where it is already established (Rocha and Kremer 2005). In the northwest Atlantic Ocean (including Eastern Canada), *D. listerianum*—considered an invasive species—was first reported in Maine in 1993 and again in New Hampshire in 1999 (Dijkstra et al. 2007). In Eastern Canada, *D. listerianum* was initially reported in the Quebec province in 2008 and in Nova Scotia in 2012 (Simard et al. 2013; Moore et al. 2014). Molecular traces of *D. listerianum* (*i.e.*, larvae and eDNA) were detected in water samples collected in Quebec and in Prince Edward Island in 2010 and 2011 (Ma et al. 2016). As larval dispersal in colonial ascidians is limited (< 10 m; Grosberg 1987; Olson and McPherson 1987), detection therefore indicated that there were established populations nearby. The primary method of detection for this invasive species was by a species-specific 18S rDNA PCR-based assay (Willis et al. 2011; Moore et al. 2014; Ma et al. 2016) with only one record using morphology (Ma et al. 2018).

Ascidiella aspersa is native to European waters and it is considered invasive in other regions around the world, including Eastern Canada (Mackenzie 2011; Moore et al. 2014). On the eastern coast of North America, this species was first reported in New England in the 1980s and has since been found as far north as Casco Bay, Maine (Mackenzie 2011; Moore et al. 2014). The first recorded observation of *A. aspersa* in Eastern Canada was in 2012 in Lunenburg Harbour, Nova Scotia (Moore et al. 2014). Identification of these first specimens was performed by expert examination of external features in digital photographs. The methods of identification used for subsequent records of *A. aspersa* on the southern shore of Nova Scotia have not been published even though this species was detected through recent monitoring efforts (Sephton et al. 2017). To date, no confirmed records of *A. aspersa* have been reported from sites outside of the southern shore of Nova Scotia (Ma et al. 2017a; Sephton et al. 2017).

Our report describes specimens of *A. aspersa* collected in Nova Scotia and *D. listerianum* collected in New Brunswick one year after initial discoveries in their respective provinces. In addition, it presents new records of *D. listerianum* collected from Îles-de-la-Madeleine, Quebec, after

Table 1. Distribution and percent cover (semi-quantitative abundance opportunistically estimated from the underside of floating docks) of *Diplosoma listerianum* from harbours and marinas in southwest New Brunswick between September and October 2017.

Site	Latitude (ºN)	Longitude (ºW)	Percent cover
Saint John	45.2723	66.0657	Not detected
Lorneville	45.1923	66.1486	Not detected
Five Fatham Hole	45.1867	66.2575	Not detected
Chance Harbour	45.1226	66.3514	Not detected
Dipper Harbour	45.0943	66.4180	Not detected
Boynes Cove	45.1309	66.4979	Not detected
Blacks Harbour	45.0558	66.7943	Not detected
Back Bay	45.0559	66.8632	Not detected
L'Etete	45.0513	66.8954	Not detected
Stuart Town	45.0190	66.9377	Not detected
St. Andrews Biological Station	45.0825	67.0848	Not detected
St. Andrews Harbour	45.0714	67.0544	0–5%
Beaver Harbour	45.0688	66.7387	0–5%
Lords Cove	45.0060	66.9466	5–25%
Leonardville	44.9717	66.9527	5–25%
Fairhaven	44.9640	67.0080	25–50%
North Head	44.7630	66.7494	25–50%
Ingalls Head	44.6626	66.7563	25–50%
Seal Cove	44.6491	66.8385	25–50%

a six-year hiatus. The species identities of these specimens were determined by examining multiple internal structures in addition to external features. As the first confirmed record of *D. listerianum* in New Brunswick, this report also documents its rapid spread in the Bay of Fundy, and the presence of this invasive species fouling the hull of a recreational boat in the Passamaquoddy Bay.

Materials and methods

Collection and preparation of specimens

In September 2013, 18 individuals of *A. aspersa* were collected from the undersides of floating docks and submerged stabiliser chains at the Lunenburg Yacht Club, located in the far southwest corner of Mahone Bay, Nova Scotia (44.4108°N; 64.3217°W). Concurrently, two specimens were collected from wooden piers in Lunenburg Harbour (44.3750°N; 64.3083°W), near the initial 2012 collection site of Moore et al. (2014). Lunenburg Harbour and the Lunenburg Yacht Club are on opposite sides of a peninsula and are separated by approximately 30 km of coastline. Although tissue relaxation steps (*e.g.*, Stefaniak and Heupel 2016) are commonly used to prevent the distortion of muscles for viewing after preservation, specimens at these locations were large and numerous so that relaxation was not employed and fresh specimens were merely preserved in 95% ethanol.

Between September and October 2017, 19 harbours and marinas in southwest New Brunswick were opportunistically surveyed for the presence of *D. listerianum* (see Table 1 for a list of sites). The percent cover of *D. listerianum* as part of the fouling community growing on the underside of floating docks was determined semi-quantitatively. Submerged sections

Figure 1. Detection of colonies of *Diplosoma listerianum* fouling the underside of a recreational boat during the end-of-season haul-out at Bayside, New Brunswick, Canada, on 9 October 2017. (A) One of the recreational boats in the process of being hauled out of the water; (B) the underside of a boat fouled by *D. listerianum* and another invasive colonial ascidian *Botrylloides violaceus*; (C) a colony of *D. listerianum* that was detached from the boat hull. Photo credit: K.C.K. Ma.

of 20 different 1-m wide dock sections, as viewed from the surface, were each designated one of six percent cover levels (not detected, < 5%, 5 to 25%, 25 to 50%, 50 to 75%, and 75 to 100%). At sites where *D. listerianum* were present, entire colonies were collected from kelp blades (*Laminaria* spp.) that were growing on the undersides of floating docks. In addition, the undersides of 20 recreational boats were visually inspected while out of the water at Bayside, New Brunswick (45.0672°N; 67.0353°W), during the end of season haul-out of boats (Figure 1). Colonies of *D. listerianum* found fouling these boats were collected. Finally, a single colony of *D. listerianum* was collected via SCUBA on a herring weir from Sandy Island (44.9714°N; 66.9138°W) as part of a bio-blitz surveying the Passamaquoddy Bay region in May 2017. This site is a disused weir and the specimen was collected from the surface of the wooden weir piling. Immediately after collection, all specimens (from kelp blade, boat, and weir substrates) were relaxed in seawater with added menthol crystals before being preserved in 95% ethanol (Stefaniak and Heupel 2016).

In October 2017, several colonies of *D. listerianum* were collected from the permanent dock via SCUBA and from collector plates in the Havre-Aubert marina, Îles-de-la-Madeleine, Quebec (47.2360°N; 61.8343°W). At the site, ten collector arrays were deployed such that each array consisted

of one PVC plate (dimensions: 10 × 10 cm) and three Petri dishes (diameter: 24 cm). This sampling strategy to monitor for marine invasive species was adopted starting in 2016 (see Simard et al. 2013 for a description of the sampling strategy used between 2006 and 2015 in Îles-de-la-Madeleine). Similar to the protocol used in New Brunswick, all specimens were relaxed in a seawater bath with menthol crystal immediately after collection and then preserved in 10% solution of formaldehyde. Different methods of ascidian relaxation and preservation were used (*e.g.*, ethanol without relaxation, ethanol with relaxation, formaldehyde with relaxation) because collection events were part of different investigations; however, all specimens, regardless of method, were in suitable condition for dissection. The percent cover of *D. listerianum* was estimated from a video made of the permanent dock and floating docks on 28 November 2017. For the entire site, the percent cover on the permanent dock (n = 240 underwater wooden planks around the perimeter of the structure) and on the undersides of floating docks was estimated semi-quantitatively (see above) from the video sequence.

Taxonomic identification

Preserved specimens of *D. listerianum* and *A. aspersa* were photographed and an initial identification was made using external features. Next, specimens were dissected by removing the tunic. The body of the *A. aspersa* was cut ventrally from the posterior to the anterior. For *D. listerianum*, individual zooids and larvae were isolated from the tunic matrix. Haematoxylin was used to stain tissue samples to illuminate important morphological features (such as tentacles and vessels). Ascidian taxonomic references and species descriptions were used to target morphological features that distinguish these two species from similar taxa in Canada and around the world (Van Name 1945; Rocha et al. 2012; Brunetti and Mastrototaro 2017).

Results and discussion

Species identification

Solitary ascidians collected from Lunenburg, Nova Scotia were confirmed as *A. aspersa*. The specimens ranged from 0.5 to 3 cm (mean: 1.57 cm) in length (Figure 2A), and the tunics were firm, translucent, papillated, and (in life) greyish-blue in colour with pink spots around the siphons (Figure 2B). The oral and atrial siphons each had six to eight lobes (Figure 2B). These specimens presented an undivided ovate body (Figure 2C), a pharynx wall without any folds (Figure 2D), a continuous and finely toothed dorsal lamina (Figure 2E), 16 to 18 simple oral tentacles, and a single aperture at the C-shaped dorsal tubercle, which was turned towards the anterior (both horns rolled inwards; Figure 2F). The alimentary canal

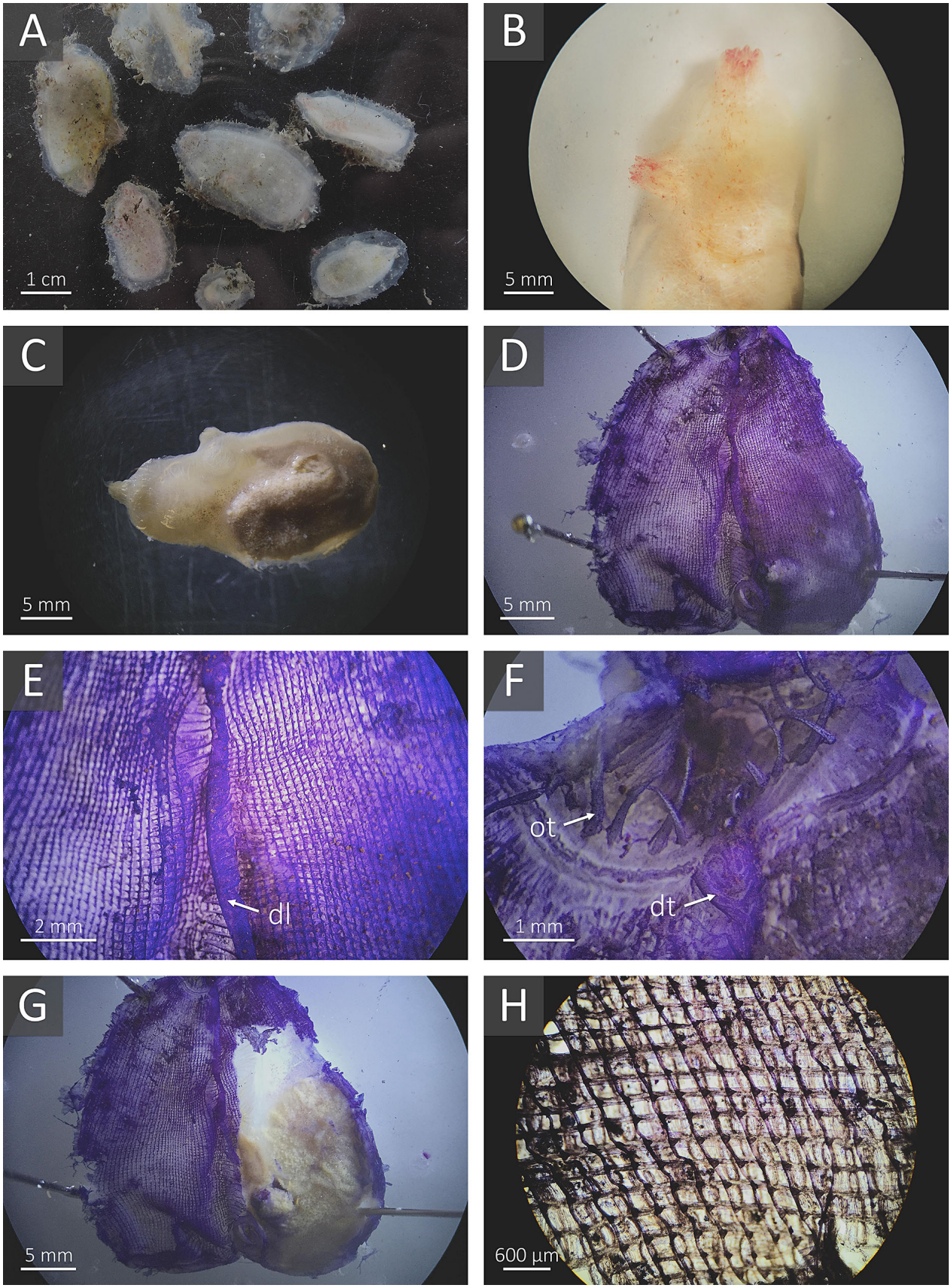

Figure 2. *Ascidiella aspersa* from Lunenburg, Nova Scotia. (A) Whole, live individuals immediately following collection; (B) right side of live individual with two pigmented siphons; (C) left side of the body of a preserved individual with the tunic removed; (D) dissected body stained with haematoxylin; (E) stained pharynx and dorsal lamina (dl); (F) stained oral tentacles (ot) and dorsal tubercle (dt); (G) view of the alimentary canal and gonads on the left side of the body after removing a section of the pharynx; and (H) stained section of the pharynx. Photo credit: K.C.K. Ma.

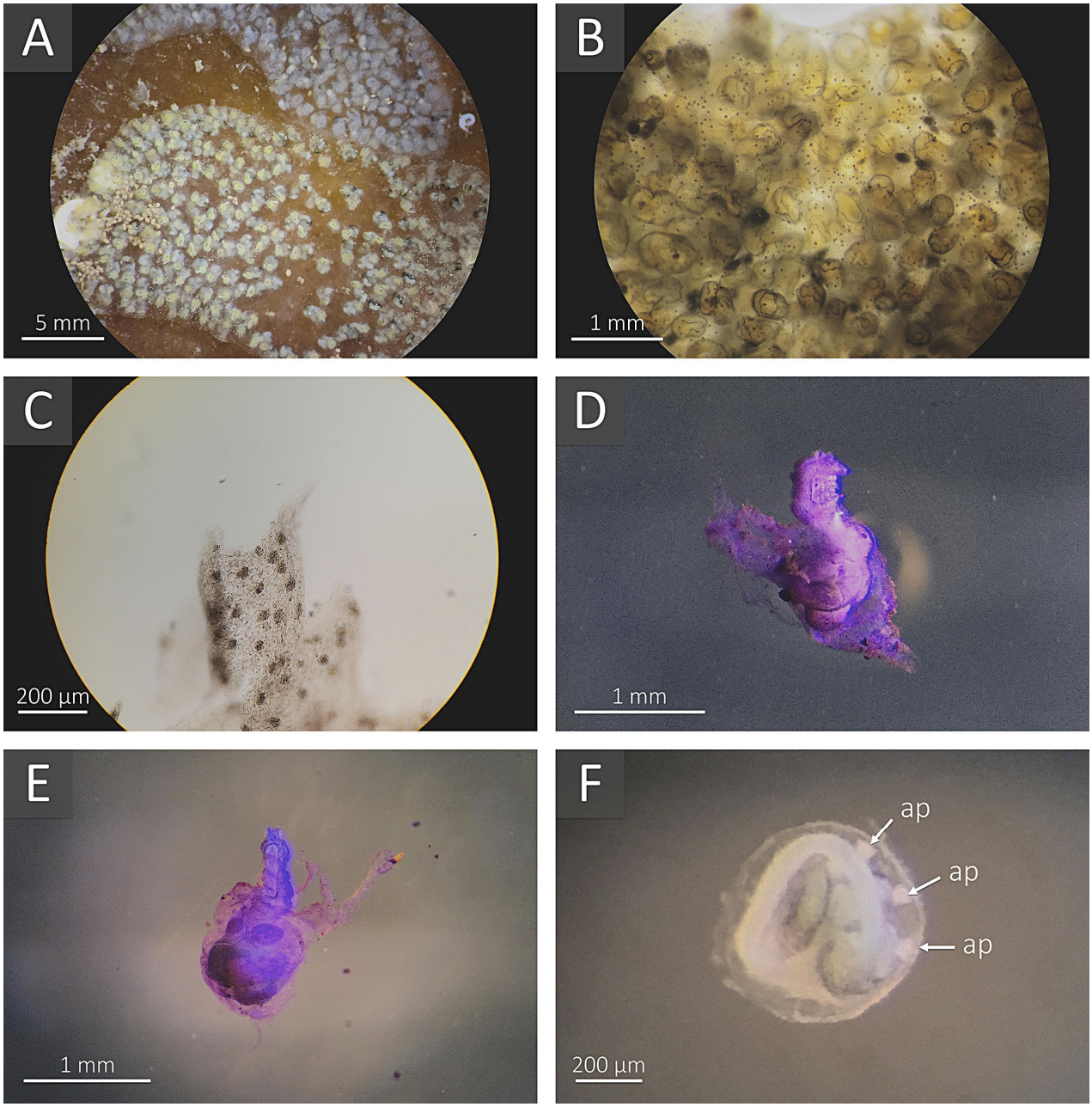

Figure 3. *Diplosoma listerianum* from coastal waters of southwest New Brunswick, Canada. (A) Live colonies on a kelp blade; (B) close-up of a live colony; (C) fragment of the tunic with granulations; (D, E) zooids stained with haematoxylin; and (F) unstained larva and adhesive papillae (ap). Photo credit: K.C.K. Ma.

was located on the left side of the body and gonads were attached to the intestinal wall (Figure 2G). Pharyngeal papillae supported 60 to 62 complete longitudinal vessels without projecting into the lumen (lack of intermediate branchial papillae and lack of secondary papillae). Pharyngeal stigmata were straight (Figure 2H), arranged in 50 to 110 pharyngeal rows. Finally, consistent with one of the most distinguishing characters of *A. aspersa,* there were more longitudinal vessels than tentacles.

Ascidian colonies collected from coastal waters of southwest New Brunswick (Figure 3) and from Havre-Aubert, Quebec (Figure 4), were confirmed as *D. listerianum*. These specimens presented a colonial mode of

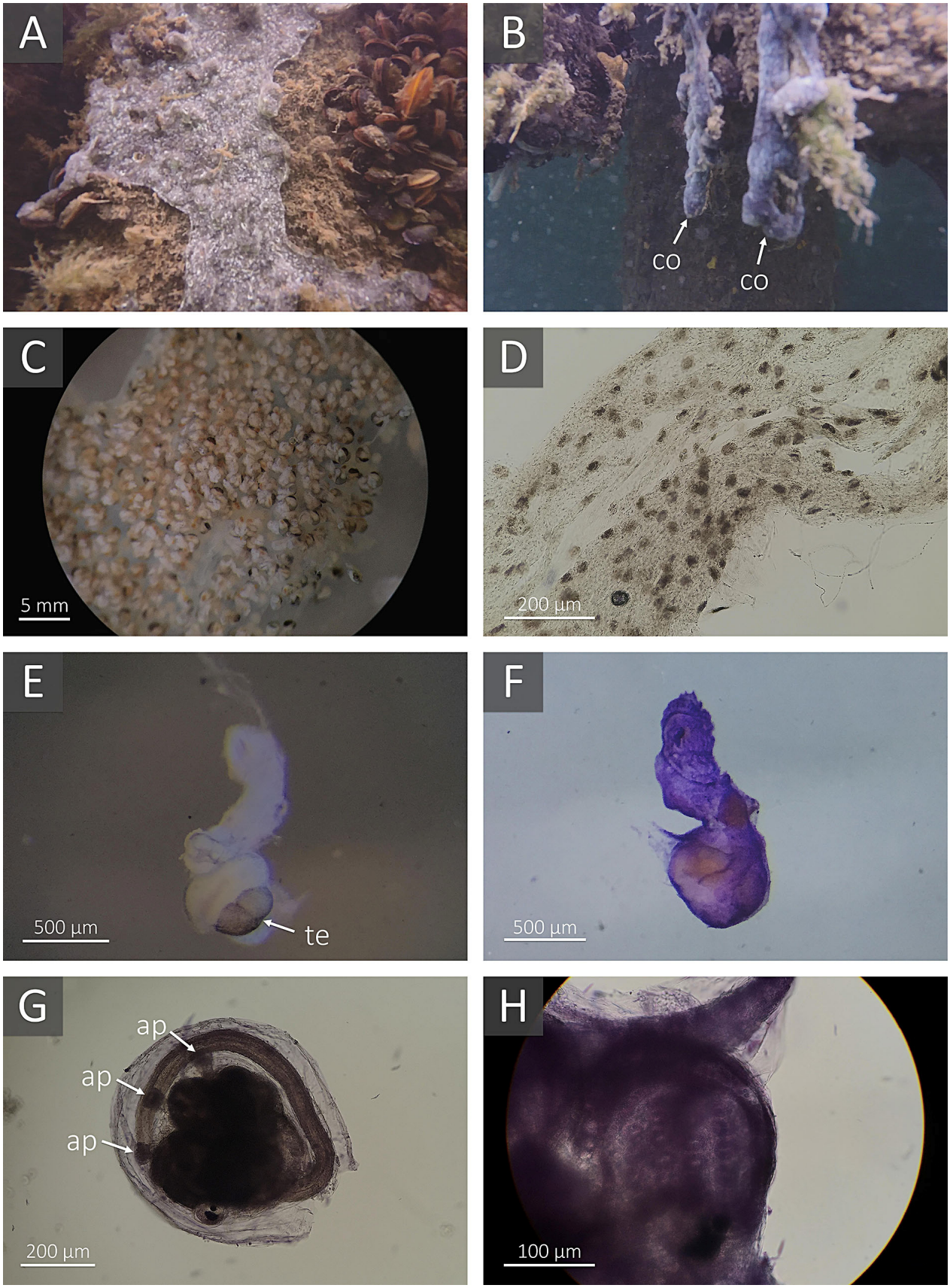

Figure 4. *Diplosoma listerianum* from Havre-Aubert, Îles-de-la-Madeleine, Quebec, Canada. (A) Live colony on dock structure; (B) live colonies (co) with lobate growth form; (C) preserved colony; (D) fragment of the tunic with granulation; (E) unstained zooid and its two-lobed testis (te); (F) zooid stained with haematoxylin (G) stained larva and adhesive papillae (ap); and (H) stained oozooid with four rows of stigmata. Images in panels A and B were extracted from video footage. Scale bars are not available for images in panels A and B. Photo credit: N. Simard (A, B), K.C.K. Ma (C–H).

life (sheet-like and lobate growth forms), zooids (1.2 to 1.4 mm in length) that were completely embedded in the tunic, atrial siphons that were opened into a cloaca system, and brown colouration of organs (Figures 3A, B; 4A–C). The greyish tunic was soft and translucent, no spicules were present in the tunic, and white granulations were observed in the tunic (Figures 3C and 4D). The zooid body was divided into two parts (thorax and abdomen; ratio of 1.0 to 0.7), the atrial aperture was wide (not tubular), the testis was two-lobed, and the sperm duct was straight (Figures 3D, E and 4E, F). There were four rows of straight pharyngeal stigmata. Brooding embryos and larvae were found in both the New Brunswick and Quebec specimens. The larva exhibited three adhesive papillae arranged in a line (Figures 3F and 4G) and an oozooid with four rows of stigmata (Figure 4H).

Invasion of the south shore of Nova Scotia by Ascidiella aspersa

Lunenburg Harbour was the site of the first record of *A. aspersa* in Nova Scotia in 2012. As part of the government's monitoring program, this species has been observed in waters in Lunenburg Harbour in subsequent years (2013 and 2014) but not in 2015 (Sephton et al. 2017). In 2013 and 2014, the species was also found on moorings and lines at an aquaculture lease in Corkum's Island, approximately 3.5 km south of Lunenburg Harbour. Although *A. aspersa* was not detected at the Lunenburg Yacht Club (approximately 30 km north of Lunenburg Harbour by sea) by the government's monitoring program in 2013 and in 2015 (Sephton et al. 2017), we detected this species in both Lunenburg Harbour and the Lunenburg Yacht Club in 2013. The detection of *A. aspersa* at these two sites suggests that recreational boats (largely pleasure sailing and recreational fishing) are the most likely vector responsible for its regional spread within the south shore of Nova Scotia (*e.g.*, Simard et al. 2017). In fact, the connectivity of the harbour and Mahone Bay regions by recreational boating activities appears to be extensive (Simard et al. 2017).

The lack of detection of *A. aspersa* in Lunenburg Harbour and vicinity in 2015 suggests that local populations may have failed (Sephton et al. 2017), but it is yet unknown whether local populations are reseeded by boats passing among connected regional sites. In the summer of 2016, suspected individuals of *A. aspersa* were reported for the first time in Shelburne, Nova Scotia, which is approximately 140 km (by sea) south of Lunenburg (Sephton et al. 2017). As of spring 2018, there have been no additional records of *A. aspersa* in other waters of Eastern Canada, and this report is the first confirmation of *A. aspersa* in Eastern Canada using detailed morphological identifications (Supplementary material Table S1).

Rapid invasion of southwest New Brunswick by Diplosoma listerianum

Although colonies of *D. listerianum* had previously been reported from nine of the 14 monitored sites (locations of these sites were not recorded) for

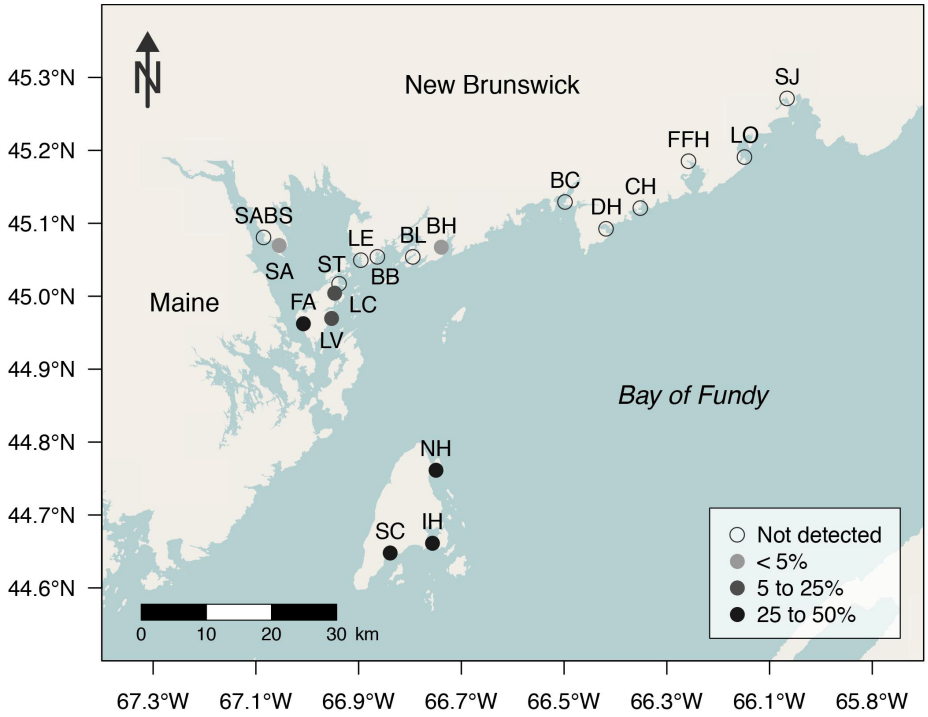

Figure 5. Distribution and percent cover of the fouling community on the underside of floating docks (semi-quantitative abundance) of *Diplosoma listerianum* in southwest New Brunswick in the autumn of 2017, one year after its initial discovery. BB = Back Bay; BC = Boynes Cove; BH = Beaver Harbour; BL = Blacks Harbour; CH = Chance Harbour; DH = Dipper Harbour; FA = Fairhaven; FFH = Five Fathom Hole; IH = Ingalls Head; LC = Lords Cove; LE = L'Etete; LO = Lorneville; LV = Leonardville; NH = North Head; SA = St. Andrew's Harbour; SABS = St. Andrew's Biological Station; SC = Seal Cove; SJ = Saint John; ST = Stuart Town. This map does not include records of *D. listerianum* collected from the underside of a boat in Bayside and from the herring weir in Sandy Island.

the first time in the New Brunswick coast of the Bay of Fundy, the identification of these specimens was not confirmed by morphological examination (Sephton et al. 2017). Consequently, colonies presented here in this report are the first confirmed records of *D. listerianum* in New Brunswick based on an examination of internal structures (Table S1).

The invasion of New Brunswick by this species was considerably rapid. Within a single year since its first detection, it increased its geographic extent by approximately 800 km^2 (estimated based on occurrence records). In 2017, this invasive colonial ascidian was present at 50% of sampled sites (Table 1). Abundance tended to be greater from sites on Deer Island and Grand Manan Island, which are more southerly (Figure 5). Surveys were not conducted on Campobello Island due to logistic constraints. Within our study area, no colonies were observed at sites north of Beaver Harbour (Figure 5). We hypothesise that a greater abundance of *D. listerianum* represents an older introduction event under the assumptions that (1) at a given site, population size is a function of time elapsed since initial introduction event, and (2) a greater abundance on recruitment plates is a proxy for a larger population size. Hence, the latitudinal pattern in *D. listerianum* abundance that was observed in our report is suggestive of

northward spread. These findings are consistent with the known distribution of *D. listerianum* in neighbouring waters: (1) established populations have been reported from coastal waters immediately south of New Brunswick in Maine, USA, and (2) geographically and temporally disjunct detection of this species early in the invasion process from other neighbouring Canadian provinces (Quebec, Nova Scotia, and Prince Edward Island). Notably, the detection of this species in other Canadian provinces can be interpreted as separate introduction events based on the disjunct distribution in space and time revealed through the relatively high spatial and temporal resolution of monitoring in Eastern Canada (Ma et al. 2016).

We found several colonies of *D. listerianum* on only one of the 20 inspected recreational boats (Figure 1). All surveyed boats were hauled out for the winter of 2016, placed in the water in May or June of 2017, moored in St. Andrews Harbour (New Brunswick), and hauled out in September or October for the winter of 2017. Anecdotally, these boats remained within the Passamaquoddy Bay for the entire 2017 season (KCK Ma, *pers. observ.*). Movement of fouled maritime vessels, aquaculture equipment, and aquaculture products are the main vectors for the introduction of invasive ascidians (Lambert and Lambert 1998; Carman et al. 2010). Ascidians foul boats by the attachment of settling larvae on hulls, rudders, sea chests, etc. (presumably when boats are berthed). Once fouling ascidians are attached to the hull, they can often survive the transit through water from the site of entrainment (donor location) to the site of introduction (recipient location). Introduction occurs at a previously uncontaminated site when individuals attached to hulls (1) reproduce and release gametes and or larvae at the recipient location and or (2) propagate by viable fragments dislodged from fouled hulls (Clarke Murray et al. 2012). The presence of *D. listerianum* fouling boat hulls provides evidence that the movement of recreational boats is a vector for the spread of *D. listerianum* in the Passamaquoddy Bay. Finally, our detection of *D. listerianum* attached to the Sandy Island weir suggests that the local spread of this species may be associated with recent boating activities and the availability of artificial substrate (*e.g.*, Locke et al. 2007) because, even though the weir is now disused for fishing, it remains a popular destination for recreational boaters. Leonardville and Lords Cove are less than five kilometres away and are the closest sites to the weir with confirmed detections of *D. listerianum* (see Table 1). This short distance, in theory, is well within the range of local boat traffic.

Invasion of Îles-de-la-Madeleine, Quebec, by Diplosoma listerianum

Îles-de-la-Madeleine (Quebec) is a group of islands located in the Gulf of St. Lawrence, geographically close to Prince Edward Island. Colonies of *D. listerianum* were initially discovered from Havre-Aubert on Îles-de-la-Madeleine in September and October 2008 (first records); a taxonomist

identified these samples by morphology (Simard et al. 2013; Ma et al. 2016). Prior to the present report, the last confirmed detection of *D. listerianum* from Havre-Aubert (and, overall, from Îles-de-la-Madeleine) occurred in 2011 (Ma et al. 2016). As a result of recent monitoring efforts described in the present report, *D. listerianum* was detected again from Havre-Aubert in 2017 after a 6-year hiatus (Table S1).

During the SCUBA video survey of Havre-Aubert in 2017, the permanent dock structures were estimated from video footage to have between 5 and 25% coverage by *D. listerianum* (primarily by large colonies), and at least 60% of the individual wooden planks were colonised. Colonies were also detected on the undersides of floating docks but with lower coverage (0 to 5%). This moderate yet homogeneous coverage is within the range that has been previously observed for another invasive ascidian species in Eastern Canada during its peak growth season (Ma et al. 2017b).

Although *D. listerianum* was not detected between 2011 and 2016, the population size of this species could have been so small that it was simply below the threshold of detection. The analysis of collector arrays used for monitoring during 2016 and 2017 is currently ongoing (N. Simard, *unpubl. data*). The re-appearance of *D. listerianum* in Îles-de-la-Madeleine in 2017 could be due to (1) a recent introduction event, (2) increased survival and growth as a response to inter-annual climate variation (for instance, Osman and Whitlatch [2007] has linked local disappearance to mean winter temperatures below approximately 4 °C), (3) increases in population size as a result of new artificial substrate available for rapid colonisation (*i.e.*, the wooden planks of the permanent dock in Havre-Aubert were replaced with new ones in 2016), or, theoretically, (4) improved sampling effort (however, this is unlikely because SCUBA surveys were done annually, except in 2016, in Havre-Aubert since its initial discovery). The anticipated results of annual monitoring using collector arrays might provide additional information to interpret long-term patterns of *D. listerianum* detection in Îles-de-la-Madeleine.

The detection of *D. listerianum* late in the season in October (by SCUBA divers) in Havre-Aubert is consistent with the tendency of initial discoveries of invasive ascidians in Eastern Canada to be during the autumn months (Ma et al. 2016). Also around the same time, small colonies were observed on a few collector arrays retrieved in October 2017 as part of the government's monitoring program from the same site (N. Simard, *unpublished data*). Once this species becomes firmly established in the Havre-Aubert marina, the population will likely exhibit seasonal variability in abundance.

Identification methods used for Canadian ascidian records

Three methods of species identification have been accepted by peer-reviewed journals in reporting records of invasive ascidians, each with benefits,

limitations, and sources of error. First, visual examination of external features, often from *in situ* photographs, is the method most likely to lead to misidentification (*e.g.*, being mistaken for other congeners) due to overlapping morphological features or distinguishing features hidden behind debris. Nevertheless, the first record of *A. aspersa* in Eastern Canada (Moore et al. 2014) was based solely on identification of specimens from field photographs. Second, PCR-based assays targeting species from environmental samples are rapid and sensitive detection tools (*e.g.*, Willis et al. 2011). In fact, PCR-based assay contributed the first record of *D. listerianum* from coastal waters of Prince Edward Island (Ma et al. 2016). However, detection from environmental samples, particularly when physical specimens have yet to be observed, is susceptible to false positives due to PCR contamination (Darling and Mahon 2011). The third method for invasive ascidian identification in Eastern Canada is the evaluation and description of key morphological features; however, the reporting of identifications using this method has not been standardised and sometimes absent (*e.g.*, Callahan et al. 2010; Sargent et al. 2013). Until now, few early records of invasive ascidians have provided detailed taxonomic descriptions of internal structures as conclusive evidence of detection (*e.g.*, Ma et al. 2018). In our report, we provided photographs and descriptions of our taxonomic examinations of key internal structures of two invasive ascidian species. Therefore, we advocate that morphological identification and reporting of invasive ascidian species should not be limited to external features, particularly since ascidians have wide variation in morphology and features are commonly veiled by sediment or epibionts. Furthermore, the best practice is to utilise multiple (if not all three) methods to provide evidence of species identification. The detection of *D. vexillum* in Nova Scotia (Moore et al. 2014; Vercaemer et al. 2015) is a prime example of the use of multiple methods (*i.e.*, examination of spicules and PCR-based assays of morphologically identified specimens) to confirm the identity of a potentially high-impact invasive species.

Conclusions

Historical records and the circumstances leading to the early detections of invasive species are invaluable for re-constructing biological invasions. Therefore, it is critical to confirm the identities of invasive species as early as possible to avoid the possibility of misidentification and mismanagement of invasive species. An invasive species should be properly identified to reliably distinguish it from similar species from anywhere in the world (*e.g.*, congeners). For instance, there are three species belonging to the *Ascidiella* genus and 43 species belonging to the *Diplosoma* genus (WoRMS Editorial Board 2018). Furthermore, species confirmations of early detections using the best available tools is a vital step to rapidly responding to biological invasions, *e.g.*, the discovery of *D. vexillum* in

Eastern Canada (Moore et al. 2014). We provide species confirmations based on the morphology of two invasive ascidians (*A. aspersa* and *D. listerianum*) that are in the process of invading Eastern Canada. In particular, a more rigorous approach of species identification is critically missing for *A. aspersa* from this region. Our report provides a snapshot of the rapid invasion of *D. listerianum* into the southwest region of the Bay of Fundy two years after its initial detection via government monitoring in 2016. We also document the re-appearance of *D. listerianum* in the coastal waters of Îles-de-la-Madeleine, Quebec, after a six-year hiatus. These early patterns of detection suggest that Eastern Canada is at risk of further regional spread and re-introduction of these two invasive ascidians unless appropriate eradication measures are taken (*e.g.*, Deibel et al. 2014).

Acknowledgements

Financial and logistical support for the fieldwork and laboratory component of this study was provided by the NSERC Canadian Aquatic Invasive Species Network II (CAISN), Fisheries and Oceans Canada (DFO), and the Huntsman Marine Science Centre (via the Atlantic Reference Centre and the Dr. R.C. Frazee and Dr. W.B. Scott Student Research Award). Training in ascidian taxonomy was financially supported by Québec-Océan and the Smithsonian Tropical Research Institute (STRI). Special thanks to Christopher McKindsey (DFO Quebec Region) and Ladd E. Johnson (Université Laval) for in-kind field support for sampling in 2013 and 2017. Specimens collected by CG, HLH, KCKM, and NS; identifications and photomicrography performed by KCKM; manuscript prepared by HLH and KCKM; additional contributions made by CG and NS. The authors are grateful to the handling editor and to two anonymous reviewers who have improved this manuscript with their comments and suggestions.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Confirmed records of *Ascidiella aspersa* and *Diplosoma listerianum* from eastern Canada by means of morphological methods.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2019/Supplements/BIR_2019_Ma_etal_Table_S1.xlsx