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Richness and zoogeography of ascidians (Tunicata: Ascidiacea) in eastern Canada

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ABSTRACT

Managers and policymakers in eastern Canada embrace science-based management of non-indigenous species and may benefit from having comprehensive regional species checklists at sub-national jurisdictional levels. In this paper, regional checklists provide an account of the richness of ascidians in eastern Canada. Records of 58 ascidians resulted from reviewing 108 published sources, accessing data from two online databases, and collecting some common indigenous ascidian specimens. Analysis comparing the similarity of species among nine regions indicates that there is greater similarity in species composition between contiguous regions than between non-contiguous regions and suggests that there are four zoogeographic clusters in eastern Canada. Our checklists can inform managers and policymakers of the diversity of the ascidian taxa and can minimise taxonomic uncertainties of established non-indigenous and prospective invading species, for example, by identifying indigenous species that are congeners of non-indigenous species. The maintenance of checklists can be a valuable tool for the management of non-indigenous species as baselines to estimate changes in richness and to document the invasion status of non-indigenous species over time. For example, more importance can be placed on the spread of non-indigenous ascidians from one zoogeographic cluster to another than spread within the same cluster.

Keywords: ascidians, indigenous, non-indigenous, cryptogenic, species richness, zoogeography, eastern Canada

INTRODUCTION

The understanding of the biological diversity of the ascidian fauna in Canada and the United States of America (USA) is relatively coarse in geographic terms. This coarse resolution is a result of collecting ascidian faunal and distributional data (mostly indigenous species) at relatively large spatial scales, often without any information on species abundance. At the continental scale, 82 species of ascidians inhabit the waters of western Canada and western USA (Pellegrin et al. 2007), and 88 are reported from eastern USA (Plough 1978). At smaller spatial scales in north-eastern North America, 37 ascidian species occur in the Gulf of St. Lawrence (GoSL; Brunel et al. 1998), 26 species in coastal Massachusetts (Shenkar and Swalla 2011), and 12 species in the Bay of Fundy (BoF; Shenkar and Swalla 2011). The rather low number of ascidian species reported for the BoF compared to neighbouring regions suggests either a low ascidian diversity, or that the ascidian faunal composition is poorly studied and the species richness underestimated. There may be a knowledge gap in the systematics of ascidians due to a low number of studies and a lack of taxonomic expertise in a given region (Schander and Willassen 2005; Kim and Byrne 2006).

Marine ecosystems are presently threatened by the invasion of non-indigenous ascidian (NIA) species, which have become both a Canadian (LeGresley et al. 2008; Ramsay et al. 2008a; Sephton et al. 2011; Deibel et al. 2014; McKenzie et al. 2016) and global problem, due to the displacement of native and commercially important species (Lambert 2007, 2009; Locke and Carman 2009; Zhan et al. 2015; Carman et al. 2016). In response, a growing number of investigators have aimed to understand the process, detection, and prevention of NIA introductions, to minimise their detrimental ecological and economic impacts and to control their potential spread. In southern California, as many as 14 NIA species have been identified (Lambert and Lambert 1998, 2003). In contrast, only five NIA species (*Botrylloides violaceus* Oka, 1927, *Botryllus schlosseri* (Pallas, 1766), *Ciona savignyi* Herdman, 1882, *Didemnum vexillum* Kott, 2002, and *Styela clava* Herdman, 1881) have been reported from British Columbia (Lambert 2003; Carver et al. 2006a, 2006b; Clarke and Therriault 2007; Epelbaum et al. 2009; Lambert 2009), and six NIA species (*Ascidrella aspersa* Müller, 1776, *B. violaceus*, *B. schlosseri*, *D.*

vexillum, *Diplosoma listerianum* (Milne-Edwards, 1841), and *S. clava*) and one cryptogenic ascidian species (*Ciona intestinalis* (Linnaeus, 1767), formerly *C. intestinalis* type B) have been reported in eastern Canada (Carver et al. 2006a, 2006b; Clarke and Therriault 2007; Locke et al. 2007; Sephton et al. 2011; Deibel et al. 2014; Moore et al. 2014; McKenzie et al. 2016). Presently, eastern Canada is susceptible to the potential introduction of other NIA species, such as those identified by Locke (2009), via shipping and climate-change processes. This susceptibility underscores the value of up-to-date knowledge on the distribution of NIA species at smaller spatial scales and for under-represented regions (e.g., the Arctic Ocean).

While the identification, invasion history, and geographic distribution of NIA species is useful information to industry, management, and policymakers, it is also valuable to have an understanding of indigenous ascidian (IA) species. Unfortunately, a good account of the local diversity and distribution of IA and NIA species can be inaccessible, incomplete, or missing. For instance, the IA fauna of coastal and offshore waters of Newfoundland might not be fully known because many specimens held at the provincial museum in St. John's have not been identified to species (K.C.K. Ma, personal observation) and might not be verifiable because older specimens described in the literature may have since been lost.

The main purpose of this investigation was to compile checklists of IA and NIA ascidian species of eastern Canada and to compare the species richness of the ascidian taxa among the coasts of the Arctic, Labrador, insular Newfoundland, the GoSL, and the Canadian Maritimes. We tested the hypothesis that there are significant differences in the composition of the ascidian fauna among regions, such that the faunal similarities between contiguous regions (i.e., having water bodies that share a boundary) are greater than between non-contiguous regions.

MATERIALS AND METHODS

Occurrence records

Ascidian records from 1852 to the present were collected from 108 published sources and two online databases: the Ocean Biogeographic Information System (OBIS; OBIS 2015); and the Global Biodiversity Information Facility (GBIF; GBIF 2015), accessed on 30 September, 2015, at <http://www.iobis.org/> and <http://www.gbif.org/>, respectively. See Appendix 1 for a list of sources organised by species and by region. We searched all ascidian records that were geographically referenced to Canada and excluded records from western Canada *post hoc*. Records that were not identified to the species level were excluded with the exception of *Heterostigma* sp. *Heterostigma* sp. was included because this genus is not represented by any species found in eastern Canada (see Goldsmit et al. [2014]). OBIS and GBIF contained ascidian occurrence records in 11 and 9 datasets, respectively (Appendix 1).

All scientific names were updated to be consistent with the World Register of Marine Species, which was accessed on 12 December, 2015, at <http://www.marinespecies.org/> (WoRMS Editorial Board 2015). All records from eastern Canada were categorised by region as follows: (1) the Arctic (from the Beaufort Sea to Baffin Bay; including Hudson Bay), (2) Labrador, (3) insular Newfoundland, (4) the Atlantic waters of Nova Scotia, (5) the Gulf of St. Lawrence (GoSL) waters of Quebec, (6) Prince Edward Island (PEI), (7) the GoSL waters of New Brunswick, (8) the GoSL waters of Nova Scotia, (9) the Bay of Fundy (BoF) waters of New Brunswick, and (10) the BoF waters of Nova Scotia (Figure 1).

Indigenous species with two or fewer records were evaluated for inclusion in the checklist. *Didemnum roseum* Sars, 1851 is an arctic species that is restricted to the Old World and is a close ally of the relatively common *Didemnum albidum* (Verrill, 1871) in the New World (Van Name 1945). *Halocynthia aurantium* (Pallas, 1787) is restricted to the Pacific coast and is very similar to the widely distributed *Halocynthia pyriformis* (Rathke, 1806) on the Atlantic coast (Van Name 1945; Millar 1966; Haydar 2010). Thus, to conform to the conventional treatment of their taxonomy based on their distributions, we considered records of *D. roseum* in eastern Canada to be *D. albidum* and records of *H.*

aurantium in eastern Canada to be *H. pyriformis*. We are uncertain about the identification of *Aplidium translucidum* (Ritter, 1901) (record from Kongulaksiarvik, Labrador), *Araneum sigma* Monniot C. & Monniot F., 1973 (offshore waters of Labrador), *Eugyra pedunculata* Traustedt, 1886 (Cambridge Bay, Nunavut), *Minipera papillosa* Monniot C. & Monniot F., 1974 (offshore waters of Labrador), *Minipera pedunculata* Monniot C. & Monniot F., 1974 (offshore waters of Labrador), *Molgula pugetiensis* Herdman, 1898 (Port de Grave, Newfoundland), *Polycarpa spongiabilis* Traustedt, 1883 (Halifax, Nova Scotia), *Pseudodiazona abyssa* Monniot C. & Monniot F., 1974 (offshore waters of Labrador), and *Styela squamosa* Herdman, 1881 (reported as *Styela milleri* Ritter, 1907; between Halifax and La Have Bank, Nova Scotia), listed in OBIS and GBIF. These nine species were not included in the checklist because of a lack of corroborating published literature to support their inclusion. Although *Clavelina conrescens* Hartmeyer, 1924, *Lissoclinum wandeli* Hartmeyer, 1924, and *Styela gelatinosa* (Traustedt, 1886) have been reported from the Davis Strait, off the west coast of Greenland, these species were not included because there are no other records from the coasts of Canada, and we believe these are primarily Old World species (Van Name 1945; Millar 1966). *Molgula occidentalis* Traustedt, 1883 (record from northern GoSL waters of Quebec) was excluded because it is distributed from North Carolina to the West Indies (Van Name 1945). The checklist included *Ascidia dijmphniana* (Traustedt, 1886) (record from Labrador) because it is a rare arctic species that has been documented in Baffin Bay (Van Name 1945), and *Hartmeyeria arctica* Korchynski, 1989 because it was originally described in Mason Bay, Northwest Territories (Korchynski 1989). As mentioned above, *Heterostigma* sp. is included in this checklist because there are no known species in the Arctic and in eastern Canada that belongs to this genus (Goldsmit et al. 2014). The checklist included *Molgula arenata* Stimpson, 1852 (a questionable record from the BoF waters of New Brunswick) because it is distributed from Massachusetts to New Jersey (Haydar 2010). As a result, a total of 13 ascidian species found in the sources we consulted were excluded from the checklist (Table 1).

Indigenous, non-indigenous, and cryptogenic criteria

Each ascidian species in this study was classified as indigenous, non-indigenous, or cryptogenic based on their historical, biogeographic range. *Ascidella aspersa*, *B. schlosseri*, *B. violaceus*, *D. listerianum*, *D. vexillum*, and *S. clava* are considered non-indigenous in eastern Canada (Carver et al. 2006a, 2006b; Clarke and Therriault 2007; Locke 2009; Mackenzie 2011; Moore et al. 2014). At a continental scale, Yund et al. (2015) suggest that *B. schlosseri* can be considered cryptogenic to north-eastern North America based on molecular evidence. However, in this study, *B. schlosseri* is not classified as cryptogenic in eastern Canada because Yund et al.'s (2015) findings were determined from specimens from only Nova Scotia (12 sites) and PEI (1 site), which may not be representative of all of eastern Canada. At a national scale, *C. intestinalis* (formerly *C. intestinalis* type B) is considered cryptogenic to eastern Canada; however, at a sub-national scale, this species is considered indigenous in the Arctic and non-indigenous in Îles-de-la-Madeleine (Quebec), PEI, and insular Newfoundland (see below). We assumed all other ascidian species to be indigenous.

Ascidella aspersa is indigenous to the waters throughout Europe (including the Mediterranean Sea and the British Isles; Stachowicz et al. 2002; Carlton 2009; Moore et al. 2014). *Botrylloides violaceus* and *S. clava* are indigenous to the north-western Pacific Ocean (Carlton 1979; Carver et al. 2006b; Clarke and Therriault 2007; Goldstien et al. 2011; Lejeusne et al. 2011). *Botryllus schlosseri* is widely considered indigenous to the Mediterranean Sea (Carver et al. 2006b; Lejeusne et al. 2011). Molecular evidence suggests that a clade of *B. schlosseri* (sub-clade Bs2) may be indigenous to north-eastern North America (Yund et al. 2015). Yund et al. (2015) recommend that *B. schlosseri* be considered cryptogenic in north-eastern North America because of the admixture of indigenous and non-indigenous haplotypes in some populations sampled in north-eastern North America. *Didemnum vexillum* is indigenous to Japan (Stefaniak et al. 2012). *Diplosoma listerianum* may be indigenous to the North Sea and the English Channel, where it was first described, but this species should be considered cryptogenic in European waters until more evidence is available (Haydar 2010; Mackenzie 2011). *B. violaceus* was likely introduced to eastern North America via transport of aquaculture material (Dijkstra et al. 2007), while *A. aspersa*, *B. schlosseri*, *D. listerianum*, *D. vexillum*, and *S. clava* may have been introduced via

fouled ship hulls (Van Name 1945; Bullard et al. 2007; Dijkstra et al. 2007; Therriault and Herborg 2008a; Haydar 2010; Mackenzie 2011).

Molecular and morphological evidence (Brunetti et al. 2015) and a cross-breeding study (Caputi et al. 2007) suggest that *C. intestinalis* type A and type B are two distinct species. They have been renamed *Ciona robusta* Hoshino & Tokioka, 1967 (formerly *Ciona intestinalis* type A) and *Ciona intestinalis* (Linnaeus, 1767) (formerly *Ciona intestinalis* type B). In the present study, we assume that *C. intestinalis* is the only *Ciona* sp. in eastern Canada based on the report of *C. intestinalis* type B from Newfoundland (Sargent et al. 2013) and the lack of reports of *C. intestinalis* type A from eastern Canada.

Ciona intestinalis is generally believed to be indigenous in European waters (Carver et al. 2006a; Therriault and Herborg 2008a; Zhan et al. 2010) and in arctic and subarctic waters (Van Name 1945; Berrill 1950; Millar 1966). Previously, there are several varieties of *C. intestinalis* described in the literature, including formae *typica*, *gelatinosa*, *longissima*, and *tenella* (Van Name 1945; Millar 1966; Therriault and Herborg 2008b). Formae *gelatinosa* and *longissima* were reported from arctic and subarctic waters of northern Europe (Van Name 1945; Millar 1966). Therefore, we considered *C. intestinalis* from the arctic waters of northern Canada (records from Foxe Basin and north-eastern Baffin Island; Atkinson and Wacasey 1989; Aitken and Fournier 1993) to be an indigenous species. Forma *tenella* is now no longer used, but was reported from northern New England and the BoF (Verrill 1871; Van Name 1945; Millar 1966). For the present study, *C. intestinalis* from the Atlantic waters of Canada, the GoSL, and the BoF can be considered as a cryptogenic species, except in Îles-de-la-Madeleine (Quebec), PEI, and insular Newfoundland. On these islands, the absence of historical records suggests that *C. intestinalis* can be considered as a non-indigenous species.

As a result of empirical and genetic evidence (Haydar 2010), *Molgula manhattensis* (De Kay, 1843), previously considered cryptogenic in eastern Canada, is believed to be indigenous. In the present study, we treat *M. manhattensis* as indigenous in eastern Canada despite its controversial origins.

Analysis

The pair-wise similarity between regions was evaluated with the Sørensen similarity index (Sørensen 1948), by calculating the quotient of similarity (QS),

$$QS = \frac{2C}{A + B}$$

where A and B represent the number of ascidian species in regions A and B , respectively, and C the number of species shared between these two regions. Accordingly, QS values range from 0 (dissimilar) to 1 (similar). We chose the Sørensen similarity index because the regions are distinct areas and the data consist of the presence and absence of ascidian species.

Non-metric, multi-dimensional scaling (nMDS) of ascidian species from nine regions of eastern Canada was generated using PRIMER (version 6). The GoSL waters of New Brunswick was not included in the nMDS because of the low richness of ascidian species ($n = 2$) in this region. Zoogeographic clusters were identified by applying a Simprof test with a 5% significance level.

Indigenous ascidian specimens from dive surveys

SCUBA divers from Memorial University of Newfoundland collected specimens of IA species from coastal harbours around Newfoundland between September 2009 and February 2010. Indigenous specimens were transported to the laboratory, where they were maintained alive in flowing unfiltered seawater. In the laboratory, living ascidian specimens were photographed within a few days of collection with a 7.1 megapixel, Canon PowerShot SD750 digital camera. A measuring tape was positioned under the glass beaker in each photograph to provide scale to the nearest 1 mm. The

specimen was left undisturbed for ≥ 1 min prior to photography.

RESULTS

Checklist

A total of 58 ascidian species (including *Heterostigma* sp.) from 10 families and 31 genera has been reported from eastern Canada (Table 2). We determined that 50 of these species are indigenous (photographs of common IA species collected from dive surveys are shown in Figure 2), six are non-indigenous, and two are cryptogenic (Appendix 2). Thirty-five species (60%) belong to the order Stolidobranchia, 15 (26%) to Aplousobranchia, and eight species (14%) to Phlebobranchia. Forty-one species were geographically referenced to the Arctic, 27 to Labrador, 36 to insular Newfoundland, and 36 to the Atlantic waters of Nova Scotia (Table 2). In the GoSL, 35 species were referenced to Quebec, 17 to PEI, two to New Brunswick, and 11 to Nova Scotia (Table 2). In the BoF, 34 species were referenced to New Brunswick, and 16 to Nova Scotia (Table 2). Notably, few species were reported from the BoF waters of Nova Scotia and the GoSL waters of PEI, New Brunswick, and Nova Scotia. Also, there are no reports of NIA species in the Arctic and Labrador regions. Furthermore, the distribution of all the ascidian species in this study (except that of *Heterostigma* sp., which is not known) were reviewed and categorised (Appendix 2). Based on the categories established by Haydar (2010), 26 ascidian species (46%) were limited to arctic and/or subarctic waters, 16 species (28%) exhibited a continuous amphi-Atlantic distribution, 11 (19%) exhibited a disjunct amphi-Atlantic distribution, and four (7%) were restricted to the north-eastern coast of North America.

There are three NIA species (*B. violaceus*, *D. vexillum*, and *S. clava*) in eastern Canada, which have IA species that are congeners. *Botrylloides aureum* (Sars, 1851) is an indigenous botryllid species reported in the Arctic, mainland Labrador, insular Newfoundland, and the GoSL waters of Quebec (Table 2). The indigenous didemnids *D. albidum* (Figure 2 E) and *Didemnum candidum* Savigny, 1816 are reported in

eastern Canada. *D. albidum* is more widely distributed (reported from nine out of ten regions investigated in this study) than is the patchily distributed *D. candidum* (reported from the GoSL waters of Quebec and the BoF waters of New Brunswick; Table 2). Three indigenous styelid species were identified in eastern Canada: *Styela canopus* (Savigny, 1816), *Styela coriacea* (Alder & Hancock, 1848), and *Styela rustica* Linnaeus, 1767 (Table 2). Geographically, all three of these styelids are found in the Atlantic waters of Nova Scotia (Table 2). In addition, *S. coriacea* and *S. rustica* are found in the Arctic, Labrador, insular Newfoundland, and the GoSL, and *S. canopus* and *S. coriacea* in the BoF waters of New Brunswick (Table 2).

Similarity of ascidian fauna among regions

Among the most similar regions in terms of ascidian fauna were insular Newfoundland and the GoSL waters of Quebec ($QS = 0.79$), the GoSL waters of Quebec and the Atlantic waters of Nova Scotia ($QS = 0.79$), and PEI and the GoSL waters of Nova Scotia ($QS = 0.79$; Table 3). The GoSL waters of New Brunswick region was the least similar region to the other regions of this study (QS ranged from 0.00-0.29; Table 3). The regions with highest mean values of QS were insular Newfoundland (mean $QS = 0.61 \pm 0.22$) and GoSL waters of Quebec (mean $QS = 0.61 \pm 0.21$; Table 3). The region with the lowest mean value of QS was the GoSL waters of New Brunswick (mean $QS = 0.13 \pm 0.10$; Table 3).

An nMDS analysis of the matrix of Bray-Curtis similarities in species composition revealed significant structure among the nine regions (the GoSL waters of New Brunswick region was excluded from the analysis due to the low number of species; Figure 3). This analysis indicates that neighbouring (contiguous) regions are more similar in species composition than are distantly separated (non-contiguous) regions. In addition, superimposed results from a cluster analysis revealed four significant clusters at greater than 60% and less than 70% similarity (clusters: Arctic-Labrador, Atlantic-northern GoSL, southern GoSL, and BoF waters of Nova Scotia). The Arctic-Labrador cluster is comprised of the Arctic and Labrador regions. Insular Newfoundland, the Atlantic waters of Nova Scotia, the GoSL waters

of Quebec, and the BoF waters of New Brunswick regions are grouped into the Atlantic-northern GoSL cluster. PEI and the GoSL waters of Nova Scotia regions form the southern GoSL cluster. Lastly, the BoF waters of Nova Scotia region forms its own cluster. This pattern is robust, with a 2D stress level of 0.04 (Figure 3).

DISCUSSION

The checklists presented in this study provide distributional information, species-by-species, and an account of the richness of ascidian species, region-by-region, of eastern Canada. Previously, a comprehensive description of ascidian species indigenous to different regions of eastern Canada was difficult to access in the literature. For instance, distributional information compiled by Van Name (1945), one of the most comprehensive and authoritative monographs of the ascidian fauna of North and South America, and by Brunel et al. (1998), for the GoSL, was organised by species rather than by region.

The geographic sizes (*e.g.*, the length of coastline) of the ten regions defined in this study are unequal (the Arctic region being the largest and, perhaps, the most complex). Therefore, larger regions could be divided into smaller regions. For example, future investigators might consider separating the GoSL waters of Quebec into possibly three smaller regions; for example, the northern coast of the GoSL (including Anticosti Island), the southern coast of the GoSL (*i.e.*, the Gaspé Peninsula), and the Îles-de-la-Madeleine. The division of larger regions into smaller ones may help to identify potential areas of knowledge gap (*e.g.*, the southern GoSL in the present study).

In the southern GoSL and the BoF waters of Nova Scotia, the low number of species suggests a possible knowledge gap in the systematics of ascidians in this area, *e.g.*, the GoSL waters of Nova Scotia (11 species; 7 sources), and the GoSL waters of New Brunswick (2 species; 3 sources). The low species richness in these regions may be due to a paucity of ascidian records from published sources of

literature and online databases, or lack of experts to morphologically identify ascidian specimens. Taxonomic expertise in a given region may be increasingly important to the management of NIA because some ascidian species are difficult to visually distinguish from closely related species. Therefore, regional checklists provide an account of IA species useful to managers and policymakers at relevant sub-national jurisdictional levels, because IA species that are congeners of established and prospective NIA species can be rapidly identified. The ability to positively identify known indigenous species that are congeners of possible non-indigenous species can minimise taxonomic uncertainties, especially if there are gaps in knowledge due to lack of taxonomic expertise.

In this study, the total number of sources of ascidian records (from published literature, online databases, and collection of specimens) was unevenly distributed among the regions. Also, the number of ascidian records varied among the sources. One possible explanation for this uneven distribution of data may be that the number and frequency of local ascidian faunal surveys is related to the proximity to historical and contemporary centres of biological research activities (government and academia). Yet, our findings indicated that neighbouring (contiguous) regions are more similar than distantly separated (non-contiguous) regions.

The identification of four zoogeographic clusters for the ascidian taxa in eastern Canada might be seen as useful areas for the management of NIA species. For example, the spread of NIA species from one cluster to another could represent a relatively more important management and policy issue than the spread of NIA species within the same cluster. However, the clusters and regions defined in this study are convenient geographic units and should not be viewed as distinct boundaries for the management of NIA species. As more NIA species invade in the future, the composition of the ascidian fauna among clusters and among regions may become increasingly more similar. Hence, the compilation and maintenance of species checklists of multiple neighbouring regions can be a valuable tool for the management of non-indigenous species because it serves as a baseline to estimate changes in species richness and to monitor the spread of non-indigenous species over time. Unfortunately, the

species checklist presented in this study includes many historical ascidian records and does not take into account possible extirpation of species from a given region.

The zoogeographic clusters identified in this study broadly correspond to the marine ecological provinces identified by Longhurst (2007) and Spalding et al. (2007). The partitioning of the ocean into provinces by Longhurst (2007) takes into consideration physical (*e.g.*, regional circulation and stratification) and biological (*e.g.*, surface chlorophyll) factors. According to Longhurst (2007), eastern Canada is comprised of two provinces: the Boreal Polar Province (BPLR), and the Northwest Atlantic Shelves Province (NWCS). The Arctic and Labrador regions belong to BPLR and the other regions of this study are part of NWCS. Longhurst (2007) further partition NWCS into smaller provinces: Newfoundland shelf (including the Grand Banks and Flemish Cap), the GoSL, Northeast shelf and Gulf of Maine (from Cabot Strait to Cape Hatteras), and South Atlantic Bight (from Cape Hatteras to the Florida Keys). For eastern Canada, the partitioning of provinces in Spalding et al. (2007) is consistent with Longhurst (2007), *i.e.* the Arctic province corresponds to BPLR and the Cold Temperate Northwest Atlantic province to NWCS.

Previously, at a larger spatial scale, Naranjo et al. (1998) used ascidian diversity to delineate marine boundaries along the Atlantic-Mediterranean shores, where eastern Canada was represented by two out of 17 biogeographical areas: Arctic, and north-western Atlantic (very similar to the provinces described by Longhurst [2007] and Spalding et al. [2007]). At this inter-continental scale, Naranjo et al. (1998) found that these two areas are grouped into one zoogeographic cluster (Bray-Curtis similarity) based on presence-absence of genera and species. For the case of eastern Canada, our study shows that this coarse understanding of ascidian biodiversity in terms of geographic resolution can be refined, and thus reveal meaningful structure in faunal composition at finer spatial scales.

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Figure 1 Map of eastern Canada showing the ten regions under consideration in this study: (1) the Arctic, (2) Labrador, (3) insular Newfoundland, (4) the Atlantic waters of Nova Scotia, (5) the Gulf of St. Lawrence waters of Quebec, (6) Prince Edward Island, (7) the Gulf of St. Lawrence waters of New Brunswick, (8) the Gulf of St. Lawrence waters of Nova Scotia, (9) the Bay of Fundy waters of New Brunswick, and (10) the Bay of Fundy waters of Nova Scotia. NB = New Brunswick; NL = Newfoundland and Labrador; NS = Nova Scotia; PEI = Prince Edward Island.

Figure 2 Common indigenous ascidian species found in eastern Canada. (A) *Dendrodoa carnea*; (B) *Aplidium glabrum*; (C) *Ascidia callosa*; (D) *Boltenia echinata*; (E) *Didemnum albidum* encrusting *Molgula* sp.; (F) *Halocynthia pyriformis*. Scale bars = 1 cm.

Figure 3 Non-metric, multidimensional scaling of the fauna of ascidian species from nine regions of eastern Canada showing four zoogeographic clusters. BoF = Bay of Fundy; GoSL = Gulf of St. Lawrence.

Map of eastern Canada showing the ten regions under consideration in this study: (1) the Arctic, (2) Labrador, (3) insular Newfoundland, (4) the Atlantic waters of Nova Scotia, (5) the Gulf of St. Lawrence waters of Quebec, (6) Prince Edward Island, (7) the Gulf of St. Lawrence waters of New Brunswick, (8) the Gulf of St. Lawrence waters of Nova Scotia, (9) the Bay of Fundy waters of New Brunswick, and (10) the Bay of Fundy waters of Nova Scotia. NB = New Brunswick; NL = Newfoundland and Labrador; NS = Nova Scotia; PEI = Prince Edward Island.

177x177mm (300 x 300 DPI)

Common indigenous ascidian species found in eastern Canada. (A) *Dendrodoa carnea*; (B) *Aplidium glabrum*; (C) *Ascidia callosa*; (D) *Boltenia echinata*; (E) *Didemnum albidum* encrusting *Molgula* sp.; (F) *Halocynthia pyriformis*. Scale bars = 1 cm.

154x133mm (300 x 300 DPI)

Non-metric, multidimensional scaling of the fauna of ascidian species from nine regions of eastern Canada showing four zoogeographic clusters. BoF = Bay of Fundy; GoSL = Gulf of St. Lawrence.

363x244mm (300 x 300 DPI)

Table 1 List of ascidian species, with fewer than two records in eastern Canada, that were excluded from the checklist.

Species	Region	Source(s) of record
<i>Aplidium translucidum</i> (Ritter, 1901)	Labrador	GBIF (2015), OBIS (2015)
<i>Araneum sigma</i> Monniot C. & Monniot F., 1973	Labrador	GBIF (2015)
<i>Clavelina conrescens</i> Hartmeyer, 1924	Arctic	Van Name (1945)
<i>Eugyra pedunculata</i> Traustedt, 1886	Arctic	OBIS (2015)
<i>Lissoclinum wandeli</i> Hartmeyer, 1924	Arctic	Van Name (1945)
<i>Minipera papillosa</i> Monniot C. & Monniot F., 1974	Labrador	OBIS (2015)
<i>Minipera pedunculata</i> Monniot C. & Monniot F., 1974	Labrador	OBIS (2015)
<i>Molgula occidentalis</i> Traustedt, 1883	Gulf of St. Lawrence waters of Quebec	Brunel et al. (1998)
<i>Molgula pugetiensis</i> Herdman, 1898	Insular Newfoundland	OBIS (2015)
<i>Polycarpa spongiabilis</i> Traustedt, 1883	Atlantic waters of Nova Scotia	GBIF (2015)
<i>Pseudodiazona abyssa</i> Monniot C. & Monniot F., 1974	Labrador	GBIF (2015)
<i>Styela gelatinosa</i> (Traustedt, 1886)	Arctic	Van Name (1945), Millar (1966)
<i>Styela squamosa</i> Herdman, 1881	Atlantic waters of Nova Scotia	GBIF (2015), OBIS (2015)

Table 2 Checklist of 58 extant ascidian species of eastern Canada. BoF = Bay of Fundy; GoSL = Gulf of St. Lawrence; NB = New Brunswick; NS = Nova Scotia. References are given in Appendix 1. The authority name and year, distribution, and status for each species are listed in Appendix 2.

Species	Region									
	Arctic	Labrador	Insular Newfoundland	Atlantic waters of NS	GoSL waters of Quebec	Prince Edward Island	GoSL waters of NB	GoSL waters of NS	BoF waters of NB	BoF waters of NS
<i>Aplidium glabrum</i>	x	x	x	x	x				x	x
<i>Aplidium mutabile</i>	x									
<i>Aplidium pallidum</i>	x		x	x	x				x	
<i>Aplidium spitzbergense</i>									x	
<i>Aplidium stellatum</i>									x	
<i>Ascidia callosa</i>	x	x	x	x	x				x	x
<i>Ascidia dijmphniana</i>	x	x								
<i>Ascidia obliqua</i>	x	x	x	x	x				x	
<i>Ascidia prunum</i>	x	x	x	x	x	x		x	x	
<i>Ascidiella aspersa</i>				x						
<i>Boltenia echinata</i>	x	x	x	x	x				x	x
<i>Boltenia ovifera</i>	x	x	x	x	x				x	x
<i>Bostrichobranchus pilularis</i>		x	x	x	x	x			x	x
<i>Botrylloides aureum</i>	x	x	x		x					
<i>Botrylloides violaceus</i>			x	x	x	x	x	x	x	x
<i>Botryllus schlosseri</i>			x	x	x	x	x	x	x	x
<i>Chelyosoma macleayanum</i>	x	x		x	x				x	
<i>Ciona intestinalis</i>	x		x	x	x	x		x	x	x
<i>Cnemidocarpa finmarkiensis</i>	x		x							
<i>Cnemidocarpa mollis</i>	x			x	x				x	
<i>Cnemidocarpa mortenseni</i>	x			x						
<i>Cnemidocarpa rhizopus</i>	x				x					
<i>Corella borealis</i>	x									
<i>Dendrodoa aggregata</i>	x	x	x	x	x					

<i>Dendrodoa carnea</i>			x	x	x	x			x	x
<i>Dendrodoa grossularia</i>	x	x	x	x	x				x	
<i>Dendrodoa pulchella</i>	x	x	x	x	x					
<i>Didemnum albidum</i>	x	x	x	x	x	x		x	x	x
<i>Didemnum candidum</i>					x				x	
<i>Didemnum vexillum</i>										x
<i>Diplosoma listerianum</i>				x	x					
<i>Distaplia clavata</i>	x		x	x	x				x	x
<i>Eudistoma vitreum</i>	x	x	x							
<i>Eugyra glutinans</i>		x	x		x					
<i>Halocynthia pyriformis</i>	x	x	x	x	x	x		x	x	x
<i>Hartmeyeria arctica</i>	x									
<i>Heterostigma</i> sp.	x				x					
<i>Kukenthalia borealis</i>	x	x								
<i>Leptoclinides faeroensis</i>	x		x	x						
<i>Lissoclinum aureum</i>	x		x						x	
<i>Microcosmus glacialis</i>	x		x	x						
<i>Molgula arenata</i>									x	
<i>Molgula citrina</i>	x	x	x	x	x	x		x	x	x
<i>Molgula complanata</i>	x	x	x		x	x			x	x
<i>Molgula griffithsii</i>	x	x	x	x	x	x			x	
<i>Molgula manhattensis</i>				x	x	x			x	x
<i>Molgula provisionalis</i>									x	
<i>Molgula retortiformis</i>	x	x	x	x					x	
<i>Molgula siphonalis</i>	x		x		x				x	
<i>Pelonaia corrugata</i>	x	x	x	x	x	x		x		
<i>Polycarpa fibrosa</i>	x		x	x	x	x		x	x	
<i>Rhizomolgula globularis</i>	x	x								
<i>Styela canopus</i>				x					x	
<i>Styela clava</i>				x		x				
<i>Styela coriacea</i>	x	x	x	x	x	x		x	x	
<i>Styela rustica</i>	x	x	x	x	x	x		x		
<i>Synoicum pulmonaria</i>	x	x	x							
<i>Trididemnum tenerum</i>			x	x					x	
Number of species	41	27	36	36	35	17	2	11	34	16
Number of published sources	15	10	25	28	19	55	3	7	32	13

Table 3 Quotient of similarity (*QS*) matrix and mean values of *QS* per region ($\pm$ one standard deviation; SD). BoF = Bay of Fundy; GoSL = Gulf of St. Lawrence; NB = New Brunswick; NS = Nova Scotia.

	Region										Mean <i>QS</i> ($\pm$ 1 SD)
	Arctic	Labrador	Insular Newfoundland	Atlantic waters of NS	GoSL waters of Quebec	Prince Edward Island	GoSL waters of NB	GoSL waters of NS	BoF waters of NB	BoF waters of NS	
Arctic	1.00	0.74	0.78	0.68	0.71	0.38	0.00	0.35	0.59	0.35	0.51 $\pm$ 0.26
Labrador		1.00	0.73	0.60	0.68	0.45	0.00	0.37	0.52	0.42	0.50 $\pm$ 0.23
Insular Newfoundland			1.00	0.78	0.79	0.57	0.11	0.47	0.71	0.54	0.61 $\pm$ 0.22
Atlantic waters of NS				1.00	0.79	0.60	0.11	0.47	0.74	0.54	0.59 $\pm$ 0.21
GoSL waters of Quebec					1.00	0.62	0.11	0.48	0.75	0.59	0.61 $\pm$ 0.21
Prince Edward Island						1.00	0.21	0.79	0.55	0.61	0.53 $\pm$ 0.16
GoSL waters of NB							1.00	0.29	0.11	0.21	0.13 $\pm$ 0.10
GoSL waters of NS								1.00	0.40	0.44	0.45 $\pm$ 0.14
BoF waters of NB									1.00	0.60	0.55 $\pm$ 0.20
BoF waters of NS										1.00	0.48 $\pm$ 0.13

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