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Geographic patterns of genetic diversity in two species complexes of Canadian marine bivalves

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ABSTRACT

Larval development has strong impacts on dispersal potential and gene flow among populations of marine invertebrates. However, Pleistocene glaciations have also played an important role in shaping population structure in benthic taxa in the Northern Hemisphere, even those with planktotrophic larvae. Each glacial advance tended to fragment species distributions, often separating populations for long periods and setting the stage for their differentiation. This study examines patterns of sequence divergence of the mitochondrial cytochrome *c* oxidase subunit I gene in North American populations of two bivalve species complexes, *Hiatella arctica s. l.* and *Macoma balthica s. l.*, with complementary data from the nuclear internal transcribed spacer-2 (ITS2) gene for the latter. Our results confirm the presence of two known species from the *M. balthica* complex in Canada, but also provide evidence for a third clade in Atlantic Canada. Our study confirms that the *H. arctica* complex in Canada contains at least four species, with support for a novel clade (*Hiatella* N) in the northeastern Pacific. Our results extend the range of a previously identified *Hiatella* clade (K) to include the northwestern Atlantic. Both *M. balthica s. l.* and *H. arctica s. l.* have broad Holarctic distributions and planktotrophic larvae, but this work reveals differences in phylogeographic structure and genetic diversity.

INTRODUCTION

Life-history attributes, vicariance events and environmental tolerances all play a role in determining where species occur (Reid, 1990; Hewitt, 2000). However, contemporary patterns of population structure in the Northern Hemisphere can only be understood by considering dispersal capacity and past population subdivision in response to glacial events and how these factors impacted population isolation and differentiation (e.g. Wares & Cunningham, 2001). Patterns of population structure in marine invertebrates with a sessile adult stage are interesting because dispersal potential in these taxa is largely dictated by the presence or absence of a planktonic larval stage (Meehan, 1985; Lee & Boulding, 2009). Some larvae spend weeks in the plankton and passive dispersal by oceanic currents can cause enough gene flow to ensure near panmixis across the species' range (Jablonski, 1986; Arndt & Smith, 1998; Lee & Boulding, 2009). Although planktotrophic species may demonstrate less regional genetic divergence than those lacking a planktonic stage, substantial population structure occurs in some species with planktonic larvae. For instance, *Macoma balthica* shows clear genetic divergence between populations in the northwestern and northeastern Atlantic (Väinölä, 2003; Nikula, Strelkov & Väinölä, 2007).

Glacial cycles had a dramatic impact on Canadian marine environments. During glacial maxima, sea levels declined by up to 170 m and the Arctic Ocean was covered by ice. The northwestern Atlantic was heavily impacted by the last glacial maximum (LGM), but the Cordilleran ice sheet on the west coast produced lesser impacts (Warner, Mathewes & Clague, 1982; Bernatchez & Wilson, 1998; Rohling *et al.*, 1998; Hewitt, 2000; Mandryk *et al.*, 2001; Marko, 2004). In fact, Marko *et al.* (2010) suggested that many rocky-shore species in the northeastern Pacific were able to persist during the LGM. In addition, the recurrent opening and closing of the Bering Strait linked to glacial cycles promoted the intermittent isolation and exchange of species between the Pacific and Atlantic Oceans (Briggs, 1970; Vermeij, 1991; Taylor & Dodson, 1994; Dodson *et al.*, 2007). During each glacial advance, species retreated to refugia and then expanded their range during the subsequent interglacial (Hewitt, 2000). For example, studies of mtDNA diversity in two species with planktonic larvae—the capelin, *Mallotus villosus*, and the green sea urchin, *Strongylocentrotus droebachiensis*—demonstrated their persistence in glacial refugia (Addison & Hart, 2005; Dodson *et al.*, 2007). Because each glacial advance tended to fragment species distributions in a

consistent way, populations were repeatedly separated for prolonged periods, setting the stage for their differentiation (Hewitt, 1996; Maggs *et al*., 2008; Dapporto, 2009). As species expanded their range during interglacials, divergent lineages re-established contact at sites along Canada's coasts (Harper & Hart, 2007). Species with similar life history attributes, but differing ecological preferences, have undoubtedly responded differently to glacial cycles (Bernatchez & Wilson, 1998).

Holarctic species are ideal candidates for determining how glacial cycles have shaped contemporary patterns of genetic variation. This study targets two wide-ranging bivalves, *Hiatella arctica* and *Macoma balthica*, both with a planktonic larval phase. There is evidence that each of these taxa is best viewed as a species complex. Coan (1971) recognized *M. petalum* as a sibling species of *M. balthica* from the northwestern Atlantic, and genetic analyses later revealed divergent lineages of *M. balthica* in the eastern and western North Atlantic (Meehan, 1985; Meehan, Carlton & Wenne, 1989). Based on DNA sequence analysis, Väinölä (2003) confirmed the status of *M. petalum* as a separate species and uncovered four lineages in the *M. balthica* complex. Nikula *et al*. (2007) corroborated these results using additional molecular data, and further discovered that *M. b. balthica* likely consists of two lineages (C and D). Both studies highlighted the role of Pleistocene glacial cycles in shaping genetic structure in this complex. Lastly, a recent study by Layton, Martel & Hebert (2014) explored DNA barcode variation in marine molluscs and highlighted three *M. balthica* clades in Canada.

Hiatella arctica also has a complex taxonomic history with two species names (*H. pholadis* and *H. striata*) synonymized with it (Marshall & Gofas, 2015). Taxonomic decisions are complicated because adults of *Hiatella* display considerable phenotypic plasticity, shell shape varying with the substrate and microhabitat type (Alison & Marincovich, 1982). Recent work examining variation of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene has highlighted the occurrence of multiple *Hiatella* clades across North America (Layton *et al*., 2014) with at least 11 taxa in the Northern Hemisphere as a whole (Laakkonen, Strelkov & Väinölä, 2015). As in the case of *M. balthica*, Layton *et al*. (2014) highlighted cryptic diversity in the *H. arctica* complex, but did not examine the patterning of genetic diversity in these groups. The present study addresses this gap by using DNA barcodes (COI sequences) to compare patterns of phylogeographic structure in Canadian populations of *H. arctica s. l.* and *M. balthica s. l.*, with a secondary goal of advancing understanding of the taxonomic status of their component lineages. We expand on prior work by both incorporating additional localities in Canada and sequences of the nuclear internal transcribed spacer-2 (ITS2) gene for the *M. balthica* complex.

METHODS

Sample collection, vouchers and data deposition

From 4 to 60 specimens of the *Macoma balthica* and *Hiatella arctica* complexes were collected at 33 sites in Alaska, British Columbia, Labrador, Manitoba, New Brunswick, Newfoundland, Nova Scotia and Prince Edward Island between 2007 and 2014 (Fig. 1). Specimen details, sequences and trace files are available from the Dataset dx.doi.org/10.5883/DS-COIPOP on BOLD (Ratnasingham & Hebert, 2007) and specimens are held at the Biodiversity Institute of Ontario. Sequences have also been deposited in GenBank (accession numbers: KP977574-KP977969, KP977970-KP978014). Specimens were obtained from rock crevices, algal mats and holdfasts in the intertidal zone and also from subtidal habitats using otter trawls, dredges and SCUBA diving. A lateral incision was made along each shell to ensure proper preservation of internal tissues when transferred to 90% ethanol. Specimens were then stored at −20 °C until ready for tissue sampling. All specimens were examined at the Canadian Museum of Nature to ensure that they matched the morphological description of *H. arctica* and *M. balthica*. Subspecies designations for *M. balthica* follow Väinölä (2003) and Nikula *et al*. (2007), while *Hiatella* lineage assignments follow Laakkonen *et al*. (2015). For simplicity, we employ species names in the broad sense throughout the rest of this section.

DNA amplification and sequencing

Tissue from the adductor muscle was removed from each specimen and placed in cetyltrimethylammonium bromide (CTAB) lysis buffer solution with proteinase K. The samples were then incubated for 12 h at 56 °C before DNA extraction was carried out using a manual glass-fibre plate method (Ivanova, Fazekas

Figure 1. Collection sites for *Hiatella arctica s. l.* and *Macoma balthica s. l.* Sample sizes for each species at each site are shown in pie diagrams.

& Hebert, 2008). Following incubation, the DNA was eluted with 40 µl of ddH_2O. After re-suspension, 2 µl of each DNA extract was placed into a well in a separate plate with 18 µl ddH_2O to ensure the dilution of salts or mucopolysaccharides that could inhibit PCR. Both COI and ITS2 were amplified from *M. balthica*, while only COI was amplified from *H. arctica*. Species-specific primer sets were used for COI: HiaF1/HiaR1: AAGTTGTAATCATCGAGATATTGG and TAGACTTCT GGGTGCCCGAAAAACCA for *H. arctica* and MMacF1/LepR1: CTTTTATTAGCTGCACCTGATAT and TAAACTTCTGGA TGTCCAAAAAATCA for *M. balthica* (S. Prosser, unpublished). Universal ITS2 animal primers were also used for *M. balthica*: CAS5p8sFc: TGAACATCGACATTTYGAACGCACAT and CAS28sB1d: TTCTTTTCCTCCSCTTAYTRATATGCTTAA (Ji, Zhang & He, 2003). Each well was filled with 2 µl of diluted DNA and the following reagents to generate a 12.5 µl PCR reaction mix: 6.25 µl 10% trehalose, 2 µl ddH_2O, 1.25 µl 10× PCR buffer, 0.625 µl $MgCl_2$ (50 mM), 0.125 µl of each forward and reverse primer (10 µM), 0.0625 µl dNTP (10 mM) and 0.06 µl Platinum Taq polymerase. The thermocycling regime consisted of one cycle of 1 min at 94 °C, 40 cycles of 40 s at 94 °C, 40 s at 52 °C and 1 min at 72 °C, and finally 5 min at 72 °C. An E-GelH 96 (Invitrogen) was used to check 3 µl of each PCR product and reactions that generated an amplicon were bidirectionally sequenced using PCR primers and BigDye v. 3.1 on an ABI 3730xl DNA Analyzer (Applied Biosystems). Sequences were manually edited using CodonCode (CodonCode Corporation) and an amino acid alignment for COI was generated by eye in MEGA v. 6 (Tamura *et al.*, 2013). All ITS2 sequences were aligned with Kalign2 (Lassmann, Frings & Sonnhammer, 2009).

Data analysis

Neighbor-joining (NJ) trees for COI (both species) and ITS2 (*M. balthica*) were constructed in MEGA v. 6 using a Tamura-3-parameter substitution model and 1,000 bootstrap replicates (Tamura, 1992; Tamura *et al.*, 2013). A model test in MEGA v. 6 returned the lowest Bayesian Information Criterion value for the Tamura-3-parameter substitution model for both the *Macoma* and *Hiatella* datasets. The *M. balthica* and *H. arctica* NJ trees were rooted with outgroups (*Macoma inquinata* and *Panopea generosa*, respectively) and each COI cluster showing more than 2% divergence was treated as a distinct clade. These NJ trees also included 13 and 11 COI sequences from previously identified lineages from Väinölä (2003) and Laakkonen *et al.* (2015), respectively. The COI sequences generated by Laakkonen *et al.* (2015) only partially overlap (105 bp) with the barcode region. Clustering patterns in each NJ tree were compared with those in the corresponding median-joining haplotype network that was constructed in Network v. 4.6.1 (fluxus-engineering.com; Bandelt, Forster & Röhl, 1999). Haplotype networks were recreated in TCS v. 1.21 to identify ancestral haplotypes (Clement, Posada & Crandall, 2000).

Arlequin v. 3.5 (Excoffier & Lischer, 2010) was employed to examine patterns of genetic structure in each species using COI sequences. Haplotype and nucleotide diversity for each population was calculated with pairwise differences, and Tajima's *D* test of neutrality with 1,000 simulated samples was used to test for evidence of nonneutral evolution and population size fluctuation (Nei, 1987; Tajima, 1989). The proportion of unique haplotypes was also quantified to ascertain the geographic location(s) with the highest genetic exclusivity. An analysis of molecular variance (AMOVA) was conducted with pairwise differences and significance was tested with 1,000 permutations (Kimura, 1980). The AMOVA results were used to determine whether the majority of genetic variation in each species existed within or between populations, as a measure of regional differentiation (Excoffier & Lischer, 2010). Fixation indices (F_{ST}) were estimated with 1,000 permutations to determine the partitioning of variance (Weir & Cockerham, 1984). Slatkin's linearized F_{ST} (Slatkin, 1993) was plotted against geographic distance to test for isolation-by-distance (IBD) and ultimately to infer whether genetic variation among populations reflects long-term historical divergence or geographic distance (Slatkin, 1993; Kyle & Boulding, 2000; Marko, 2004; Keever *et al.*, 2009). In order to test the significance of IBD, a Mantel test with 1,000 permutations was conducted in Arlequin v. 3.5 (P-values <0.05 were treated as significant) after geographical distance between sites was calculated using Google Earth.

RESULTS

Sequence and haplotype diversity

The 197 COI and 45 ITS2 sequences from the *Macoma balthica* complex ranged in length from 377 to 655 bp (mean = 429 bp) and 210 to 349 bp (mean = 326 bp), respectively. The 199 COI sequences of *Hiatella arctica* ranged in length from 550 to 655 bp (mean = 648 bp). All sequences of variable length were included in the construction of NJ trees and haplotype networks, but *M. balthica s. l.* and *H. arctica s. l.* sequences were trimmed to 378 and 588 bp for the Arlequin analysis. All sequences contained less than 1% ambiguous bases and lacked stop codons and double peaks. The considerable length variation in sequences for the *M. balthica* complex reflected the need to employ internal primers to recover sequences from some specimens with degraded DNA. Maximum and mean intraspecific divergences in the *H. arctica* complex were 23.5 and 6.9%, respectively, while maximum and mean intraspecific divergences in the *M. balthica* complex were 15.4 and 8.8%, respectively (Fig. 2).

A NJ tree indicated that four lineages were present within *H. arctica s. l.* (Fig. 3). Three corresponded to haplotypes I, K and L of Laakkonen *et al.* (2015), while the fourth from British Columbia and southeastern Alaska was new and was designated as *Hiatella* lineage N. *Hiatella* K was previously reported from multiple locations in Europe (Laakkonen *et al.*, 2015), but this work revealed it to be present in the northwestern Atlantic also. None of the sequences in this study clustered with the other eight lineages found by Laakkonen *et al.* (2015) (i.e. European lineages C, D, E and F, northeastern Pacific lineages G and H, Bering Sea lineage M, and northwestern Pacific lineage J) based on a 105-bp overlapping segment of the barcode region of COI. Haplotypes A and B of Laakkonen *et al.* (2015) are from the Southern Hemisphere (Chile and New Zealand, respectively) and were excluded from the NJ tree because they did not cluster with any Northern Hemisphere lineages.

The COI NJ tree revealed three clades in the *M. balthica* complex from Canada, representing *M. b. balthica*, *M. balthica* NFLD and *M. petalum* (Fig. 4). No Canadian sequences clustered with *M. b. rubra* (Fig. 4). The ITS2 NJ tree provided support for two clades; one containing *M. b. balthica* and *M. balthica* NFLD lineages and the second a clade of *M. petalum*. One specimen (POPMA047-14) collected from New Brunswick was assigned to *M. petalum* in the COI tree, but to *M. b. balthica*/*M. balthica* NFLD in the ITS2 tree (Fig. 5).

Most sequences (163 of 199) and haplotypes (66 of 83) of the *Hiatella* complex fell into a dominant lineage (L) that was found at all sites. All 66 haplotypes of this dominant clade showed low divergence, differing from one another by just 1 to 3 mutational steps (Fig. 6A). The remaining 17 haplotypes included representatives of three clades, two from the northeastern Pacific (*Hiatella* I and *Hiatella* N) and a third from New Brunswick (*Hiatella* K) (Fig. 6A, B). TCS suggested that a northwestern Atlantic haplotype was ancestral to *Hiatella* L (Fig. 6A). Subsequent analysis of intraspecific variation in *H. arctica* only

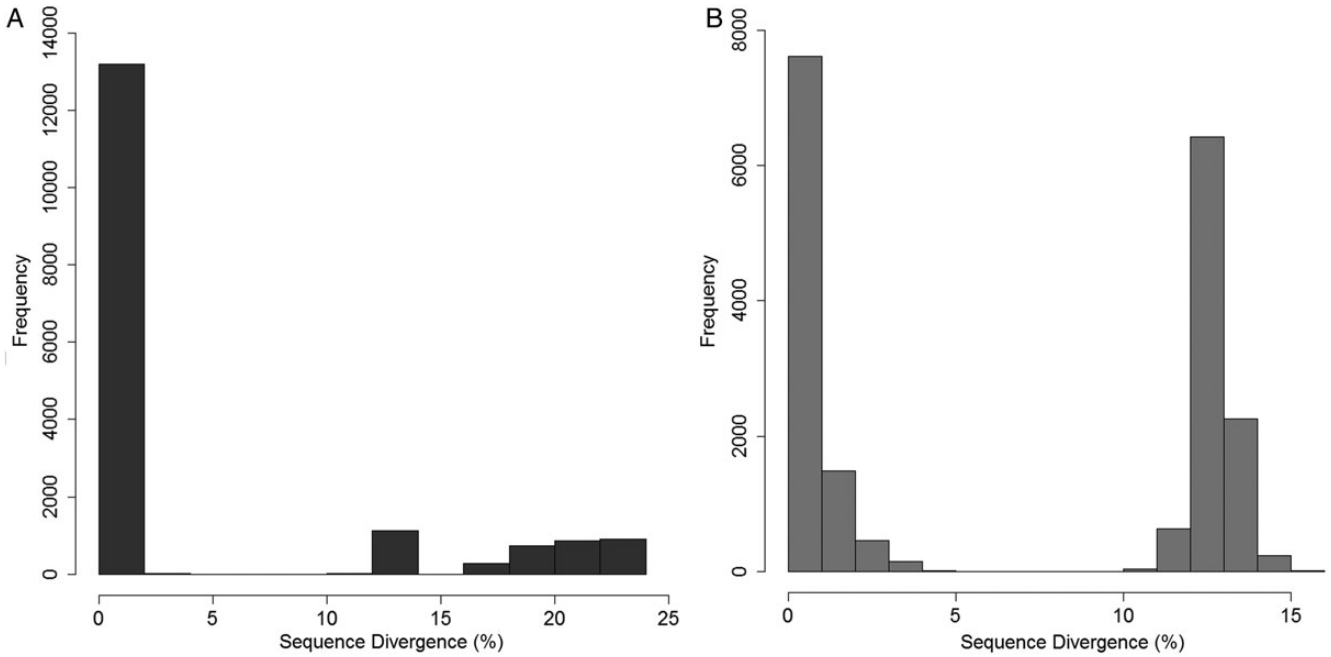

Figure 2. Intraspecific COI sequence divergence (% K2P) for *Hiatella arctica s. l.* (**A**) and *Macoma balthica s. l.* (**B**).

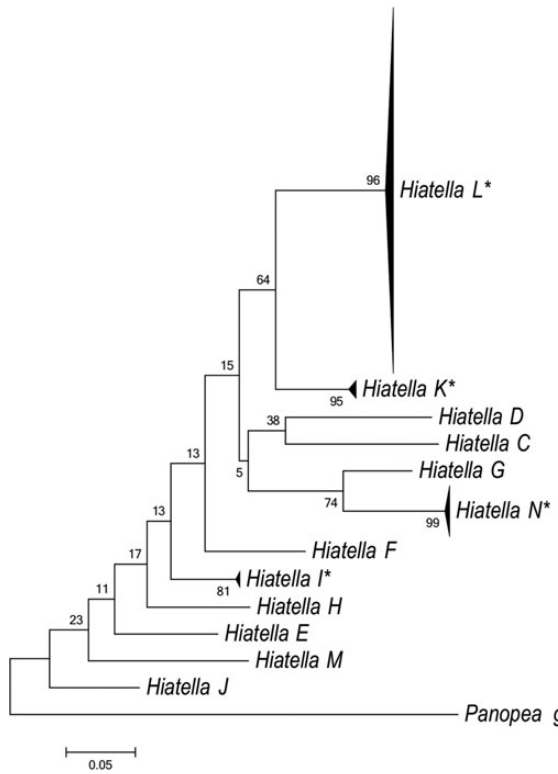

Figure 3. Neighbour-joining tree (COI) based on a Tamura-3-parameter substitution model with gamma distribution for *Hiatella arctica s. l.* NJ tree includes sequences from this study and from Laakkonen *et al.* (2015). Lineage names follow Laakkonen *et al.* (2015) and clades discovered in this study are indicated with asterisks.

examined specimens belonging to *Hiatella* L. The other lineages are likely to represent sibling species unrecognized in current taxonomy.

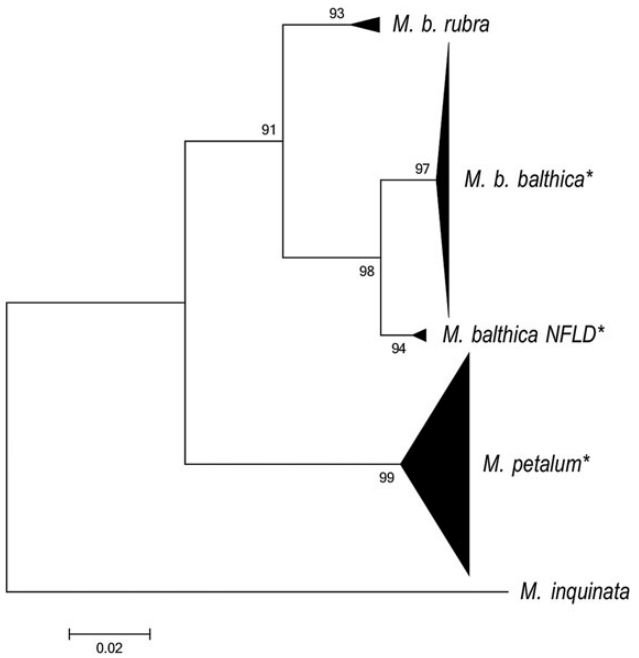

Figure 4. Neighbour-joining tree (COI) based on a Tamura-3-parameter substitution model for *Macoma balthica s. l.* NJ tree includes sequences from this study and European sequences obtained from GenBank. Subspecies names suggested by Väinölä (2003) and Nikula *et al.* (2007) are provided and clades discovered in this work are indicated by asterisks.

The three lineages in the *M. balthica* complex showed a maximum intraspecific divergence of 15.4% (Fig. 4). *Macoma b. balthica* was more broadly distributed than *M. petalum* and *M. balthica* NFLD, occurring in Alaska, British Columbia, Labrador and Manitoba (Fig. 7A, B). *Macoma petalum* had a narrower distribution, being found in New Brunswick, Newfoundland and Prince Edward Island, while *M. balthica* NFLD was only detected in Newfoundland (Fig. 7A, B). A deep

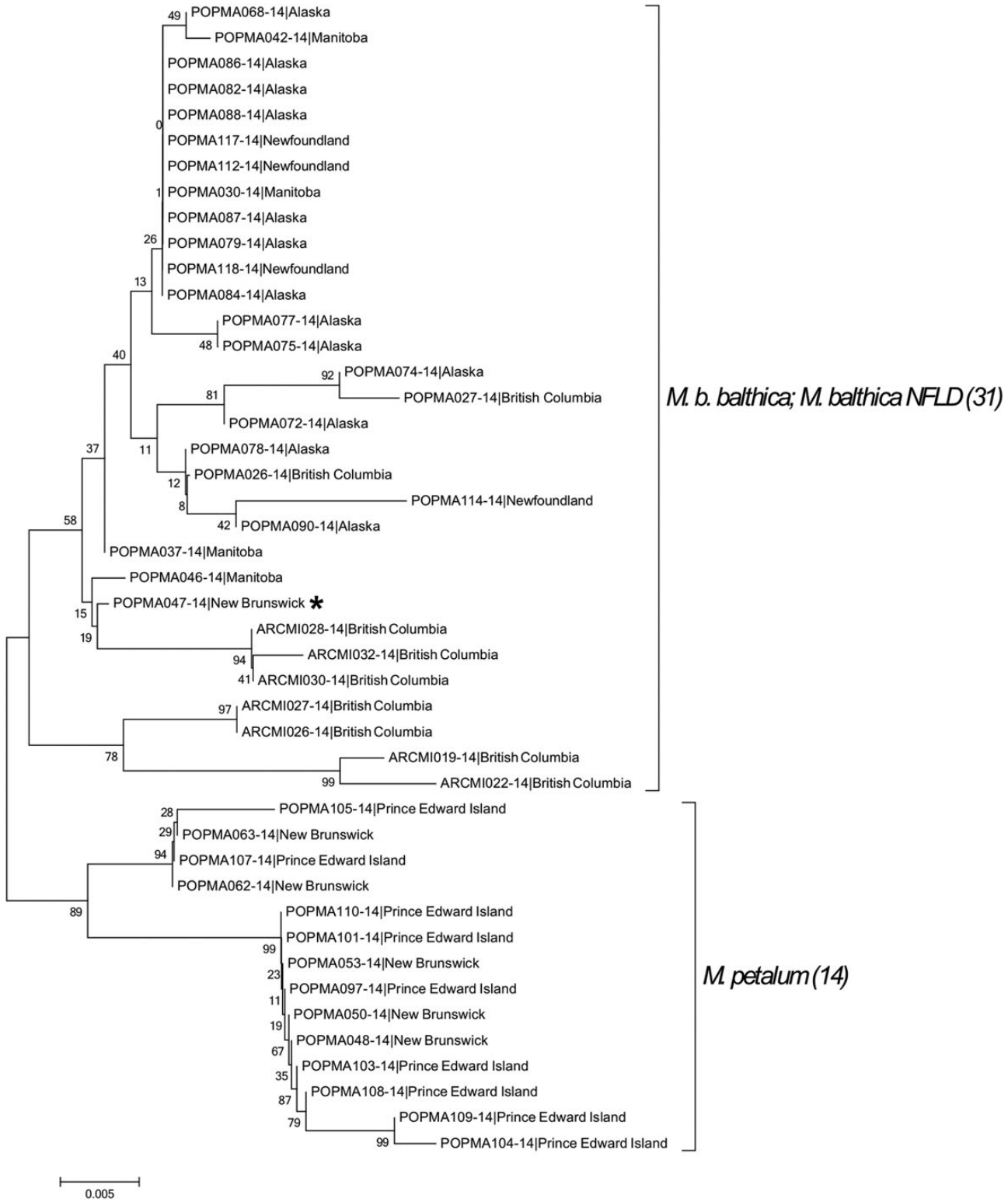

Figure 5. Neighbour-joining tree (ITS2) based on a Tamura-3-parameter substitution model for *Macoma balthica s. l.* Subspecies names suggested by Väinölä (2003) and Nikula *et al.* (2007) are provided and bootstrap probabilities are shown. The specimen highlighted with an asterisk clusters with *M. b. balthica*/*M. balthica* NFLD in the ITS2 NJ tree and with *M. petalum* in the COI NJ tree.

divergence between *M. b. balthica* and *M. petalum* was corroborated by the high number of mutational steps that separated the two clusters (Fig. 7A). Conversely, only four mutational steps separated *M. b. balthica* and *M. balthica* NFLD in the haplotype network (Fig. 7A).

Patterns of genetic diversity

Table 1 provides population genetic parameters for populations of each species, excluding *Hiatella* lineages I, K and N. Populations in Churchill, Manitoba showed the lowest variation for *Hiatella* L,

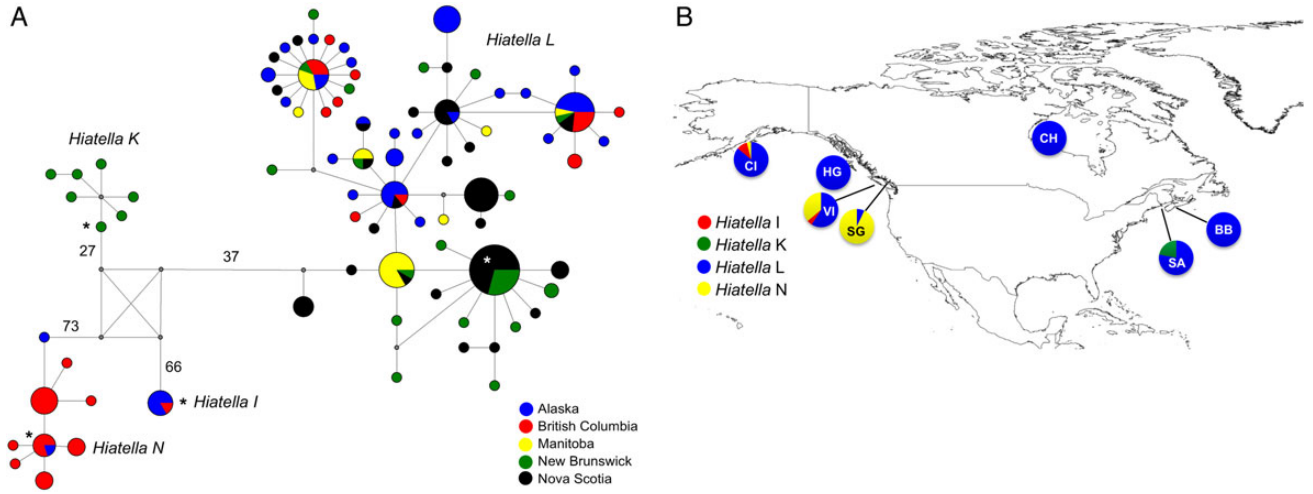

Figure 6. A. Median-joining network of COI haplotypes for *Hiatella arctica s. l.* constructed using Network v. 4.6.1. All mutational steps are three or less unless indicated by a numeral. Presumptive ancestral haplotypes are marked with an asterisk. **B.** Proportions of four *Hiatella* COI lineages at each sampling location in this study (CI, Cook Inlet, Alaska; HG, Haidi Gwaii, British Columbia; VI, Vancouver Island, British Columbia; SG, Strait of Georgia, British Columbia; CH, Churchill, Manitoba; BB, Bonne Bay, Newfoundland; SA, St Andrews, New Brunswick).

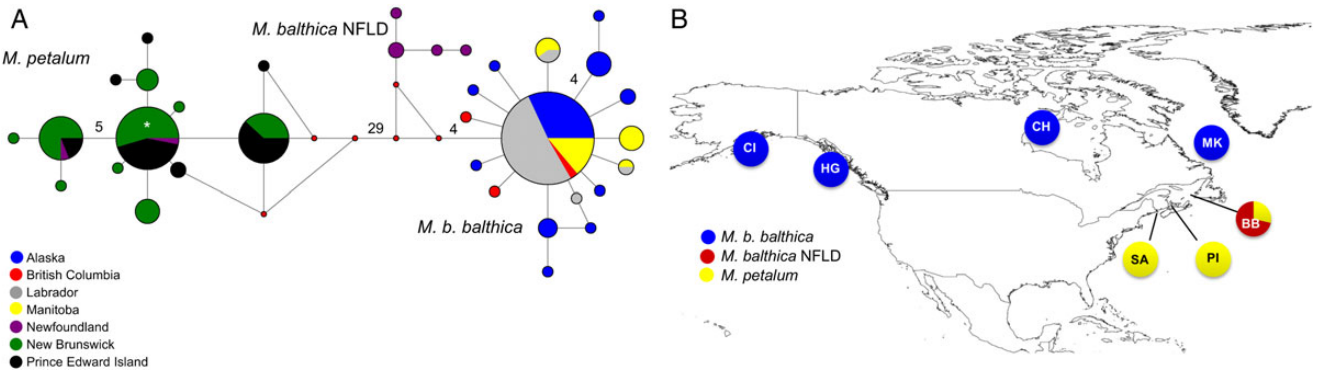

Figure 7. A. Median-joining network of COI haplotypes for *Macoma balthica s. l.* constructed using Network v. 4.6.1. All mutational steps are three or less unless indicated by a numeral. Presumptive ancestral haplotypes are marked with an asterisk. **B.** Proportions of *M. b. balthica*, *M. balthica* NFLD and *M. petalum* at each sampling location, based on COI data (CI, Cook Inlet, Alaska; HG, Haidi Gwaii, British Columbia; CH, Churchill, Manitoba; MK, Makkovik, Labrador; BB, Bonne Bay, Newfoundland; PI, Pinette, Prince Edward Island; SA, St Andrews, New Brunswick).

while populations in Labrador and Prince Edward Island had the lowest genetic diversity for *M. b. balthica* and *M. petalum*, respectively. Haplotype diversity was highest in populations of *Hiatella* L from Alaska and in populations of *M. b. balthica* and *M. petalum* from Newfoundland. However, nucleotide diversity was highest in *Hiatella* L populations from New Brunswick and in populations of *M. b. balthica* and *M. petalum* from Alaska and Newfoundland. Nucleotide and haplotype diversity did not differ significantly between species. Tajima's D values were significantly different from zero for populations of *Hiatella* L in New Brunswick and populations of *M. b. balthica* in Alaska and Labrador.

Population structure

A comparison of genetic structure revealed more phylogeographic structure in *Hiatella* L ($F_{ST} = 0.16$) than *M. b. balthica* ($F_{ST} = 0.09$) and *M. petalum* ($F_{ST} = 0.06$). Most of the genetic variation in *Hiatella* L and *M. b. balthica* was partitioned within individual populations (Table 2). Fixation indices provided further insight into the partitioning of variation in *Hiatella* L and *M. b. balthica*. In *Hiatella* L, populations from Nova Scotia and New Brunswick showed little divergence ($F_{ST} = 0.02$), while those from British Columbia and New Brunswick were the most divergent ($F_{ST} = 0.30$). Populations of *M. b. balthica* from Alaska and British Columbia were the most similar ($F_{ST} = 0.02$), while those from British Columbia and Labrador were the least similar ($F_{ST} = 0.34$). *Macoma petalum* also showed a high F_{ST} (0.22) between populations in Newfoundland and Prince Edward Island, although these sites are only separated by the 40-km wide Northumberland Strait. When Slatkin's linearized F_{ST} values were plotted against geographic distance, only *Hiatella* L showed evidence of IBD (Fig. 8A) with a strong, positive correlation ($R^2 = 0.86$) and a Mantel test confirmed its significance ($P = 0.004$). IBD results for *M. petalum* and *M. balthica* NFLD were excluded due to insufficient data.

DISCUSSION

Systematics and cryptic diversity

Incorporating sequences for recognized subspecies provided insight into the taxonomic status of both bivalve complexes. Our

results from both a mitochondrial (COI) and nuclear (ITS2) marker support findings that *Macoma b. balthica* occurs in the northeastern Pacific and the Arctic (e.g. Nikula *et al.*, 2007), but our work has also shown that this clade occurs in the northwest Atlantic (Labrador). Moreover, a maximum intraspecific divergence of 15.4% between *M. balthica* NFLD and *M. petalum* in their zone of sympatry in eastern Canada supports their recognition as sibling species. Our results also provide evidence for a third clade in Newfoundland which is 3% divergent from *M. b. balthica*, although this result was only supported by COI data. Conversely, these sequences clustered into a single clade in the ITS2 NJ tree, likely due to the slower evolutionary rate of this nuclear gene. Nonetheless, these results corroborate previous work that revealed three unique clades in the *M. balthica* complex in Canada (Layton *et al.*, 2014). The discovery of a separate cluster in Newfoundland (*M. balthica* NFLD) is also concordant with the results of Nikula *et al.* (2007), who found a unique subclade of *M. b. balthica* (C) in the northwestern Atlantic using the COIII gene. Future work should aim to determine if *M. b. balthica* and *M. balthica* NFLD show incipient or complete reproductive isolation. The discordance between COI and ITS2 for a single *Macoma* specimen, but without heteroplasmy, may reflect an advanced generation hybrid, so the potential for hybridization between these taxa should be investigated.

Table 1. Genetic diversity in populations of *Hiatella* L, *Macoma b. balthica*, *M. balthica* NFLD and *M. petalum* as measured by number of sequences (n), number of haplotypes (H), haplotype diversity (h), nucleotide diversity (Π) and Tajima's D for COI data.

Species	Population	n	H	h	Π	D
Hiatella L	Alaska	43	23	0.94	0.0072	−1.40
	British Columbia	16	10	0.92	0.0052	0.09
	Manitoba	19	7	0.71	0.0058	−0.55
	New Brunswick	25	18	0.93	0.0084	−1.58*
	Nova Scotia	60	25	0.88	0.0077	−1.18
Macoma b. balthica	Alaska	38	12	0.69	0.0045	−1.48*
	British Columbia	4	3	0.83	0.0035	−0.75
	Labrador	39	4	0.20	0.0005	−1.57*
	Manitoba	19	4	0.66	0.0019	−0.20
Macoma balthica NFLD	Newfoundland	5	4	0.90	0.0056	0
Macoma petalum	New Brunswick	51	12	0.83	0.0090	0.01
	Newfoundland	2	2	1	0.016	0
	Prince Edward Island	33	9	0.74	0.0053	−1.04

Tajima's D values significantly different from zero are marked with an asterisk.

Table 2. Overall genetic structure measured by AMOVA for the widespread lineages *Hiatella* L and *Macoma b. balthica* using COI data.

Species	Variation	Df	Sum of squares	Variance	% Variation	P-value
Hiatella L	Among	4	59	0.41	16.3	0
	Within	158	335	2.12	83.7	
Macoma b. balthica	Among	3	5	0.05	8.8	0
	Within	96	49	0.51	91.2	

P values <0.05 were treated as significant.

Similarly, incorporating *Hiatella* COI sequences from Laakkonen *et al.* (2015) confirmed the presence of multiple lineages in North America, including the widespread lineage L and additional lineages in the NE Pacific (I) and the NW Atlantic (K). Four unique *Hiatella* clades were detected by previous work in Canada (Layton *et al.*, 2014), but the extensive molecular dataset generated by Laakkonen *et al.* (2015) allowed us to assign clade names to them. Sequence divergences (COI) between the four *Hiatella* clades ranged from 11.8 to 23.5%, suggesting that species status is warranted. Furthermore, our COI dataset provides the first evidence for *Hiatella* K in the northwestern Atlantic and for a novel lineage (*Hiatella* N) in the northeastern Pacific. These results add to earlier findings of genetic diversity in this complex (Layton *et al.*, 2014; Laakkonen *et al.*, 2015) and emphasize the need for a taxonomic revision of *Hiatella*.

Comparison of genetic diversity and structure

Although a widespread species was present in both complexes, *Hiatella* L showed more regional sequence variation in Canadian

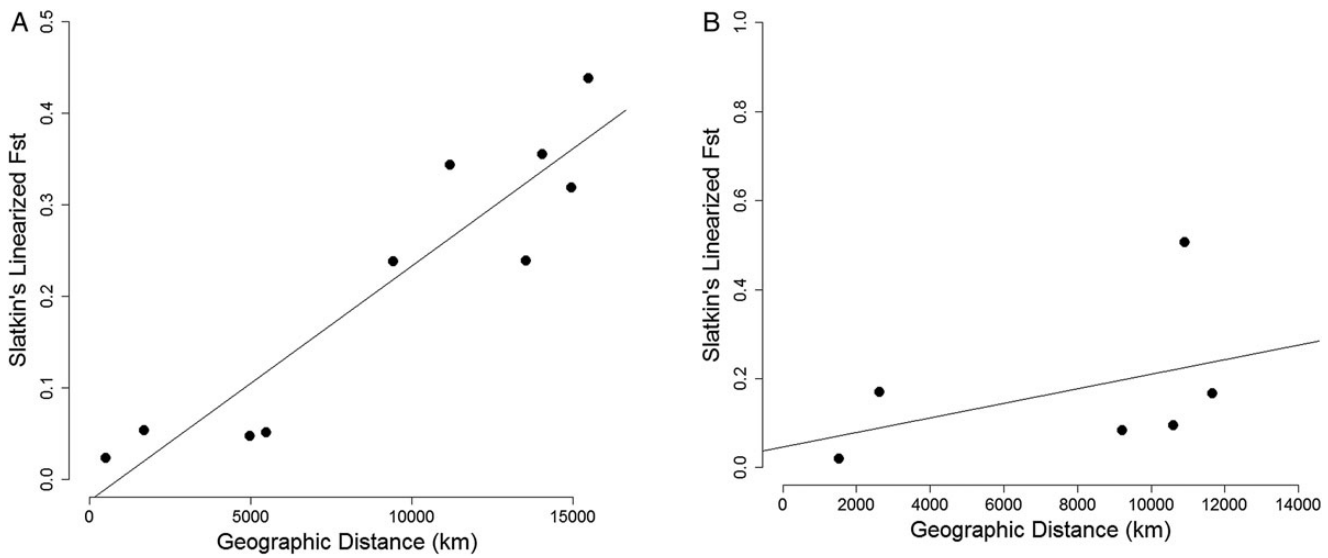

Figure 8. F_{ST} (Slatkin's linearized) values plotted against geographic distance for populations of *Hiatella* L (**A**) and *Macoma b. balthica* (**B**) to examine the extent of isolation by distance using COI data. Results of Mantel test for significance are: **A**: $R^2 = 0.86$, $P = 0.004$; **B**: $R^2 = 0.18$, $P = 0.15$.

waters than *M. b. balthica*. Contrasts in genetic variation between species with similar life-history strategies have been observed in other molluscs, including the direct-developing gastropods *Nucella lamellosa* and *N. ostrina* (Marko, 2004). *Hiatella* L and *M. b. balthica* also differ in their habitat and feeding behaviour (Newell, 1965; Ali, 1970; Hines & Comtois, 1985), which may influence population structure. In fact, Marko (2004) suggested that ecological factors may often be more important than dispersal ability in determining population structure. Nevertheless, the presence of the widespread lineages *Hiatella* L and *M. b. balthica* suggests that these species have considerable gene flow among their populations. Particularly for *Hiatella* L, low regional divergence may reflect the use of secondary dispersal mechanisms, including transportation of juveniles in ballast water and adults in kelp holdfasts (Helmuth, Veit & Holberton, 1994). In any case, patterns of genetic structure in marine invertebrate populations of North America are largely influenced by past glacial cycles.

Patterns of population fragmentation during glacial periods are often reflected in measures of genetic diversity. Reduced genetic diversity, due to glaciation and subsequent population expansion, has been demonstrated in populations of marine fishes and in *Mya arenaria* (Bernatchez & Wilson, 1998; Lasota, Hummel & Wolowicz, 2004; Strasser & Barber, 2009). However, some taxa exposed to repeated glaciations exhibit high genetic diversity. For instance, the high allelic diversity of *Nucella lapillus* has been linked to rapid re-expansion following a severe population bottleneck (Colson & Hughes, 2004). A similar process could explain the high diversity in populations of both *Hiatella* L and *M. petalum* from New Brunswick, despite severe glacial conditions in the region (Briggs, 1970; Wares & Cunningham, 2001). Northeastern Pacific populations of the widespread lineages *Hiatella* L and *M. b. balthica* were also diverse, which may reflect their foundation through postglacial admixture (Kelly *et al.*, 2006; Sakaguchi *et al.*, 2011). By contrast, the low diversity of populations at Churchill (Manitoba) may be a consequence of the relatively recent (8,000 year BP) formation of Hudson Bay (Ashworth, 1996).

Glacial refugia and secondary contact

High haplotype diversity in some northern populations can be invoked as evidence for persistence in glacial refugia (Marko, 2004). Therefore, high haplotype diversity in populations from the northeastern Pacific, Newfoundland and New Brunswick suggests the possible existence of multiple refugia in these areas. In the Pacific Ocean alone, recent work has shown that populations of Atlantic Cod (*Gadus morhua*) survived in two refugia in the northeastern Pacific and two refugia in the northwestern Pacific (Bigg, 2014). Moreover, Nikula *et al.* (2007) suggested that multiple refugia existed for *M. balthica*, likely explaining the deep divergences in this complex.

In addition to refugial sites, genetic diversity can be elevated in zones of admixture as well (Petit *et al.*, 2003; Kelly *et al.*, 2006; Sakaguchi *et al.*, 2011). The mixing of phylogenetic lineages in admixture zones combines variants derived from two or more refugia (Provan & Bennett, 2008). Secondary contact and postglacial admixture of allopatric populations previously isolated in different marine refugia may explain the pattern of haplotype distribution in *Hiatella* L. A section of the haplotype network for *Hiatella* L consists of haplotypes from only the northwestern Atlantic and Manitoba, while the remaining section of the network contains haplotypes from all localities, suggesting secondary contact and subsequent admixture of divergent lineages. The opening of the Bering Strait allowed for interchange between faunas in Canada's three oceans, with the majority of migrations occurring from the Pacific to the Atlantic (Briggs, 1970; Vermeij, 1991). This pattern of haplotype partitioning may reflect multiple trans-Arctic migrations and subsequent mixing of divergent lineages in the Atlantic, a pattern observed in both *M. balthica* and *Mytilus edulis* (Väinölä, 2003; Nikula *et al.*, 2007). It is clear that the genetic structure of these planktotrophic bivalves has not only been influenced by their dispersal potential, but shaped by Canada's glacial history.

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