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High genetic diversity and low differentiation in North American *Margaritifera margaritifera* (Bivalvia: Unionida: Margaritiferidae)

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Information on the genetic diversity and population genetic structure of threatened species is important for guiding management decisions. *Margaritifera margaritifera* (freshwater pearl mussel) occurs across western Russia, north and central Europe, and Atlantic drainages of north-eastern North America (NA). European populations of *M. margaritifera* are considered endangered, whereas NA populations are thought to be relatively secure. As such, the population genetics of *M. margaritifera* occurring in European rivers is relatively well studied while that of NA populations is not known. In this study, we investigated the genetic diversity and differentiation of *M. margaritifera* in Canada and the USA. Genetic diversity indices calculated from nine microsatellite loci were relatively high in the NA population. Analyses of genetic structure indicated that a single panmictic population exists for *M. margaritifera* in NA. However, there was evidence of substructure in some tributaries of the St. Lawrence River in Québec, Canada. The NA population of *M. margaritifera* has low genetic differentiation and high diversity, possibly resulting from large population size and high gene flow. Consequently, conservation of this species should focus primarily on maintaining favourable habitat conditions and connectivity for host fish.

ADDITIONAL KEYWORDS: conservation genetics – eastern pearlshell – freshwater pearl mussel – microsatellites – North Atlantic coast – population genetics.

INTRODUCTION

Unionid bivalves (order Unionida) are a diverse group of freshwater molluscs with a worldwide distribution (Graf & Cummings 2007). They play an important role in lotic and lentic ecosystems and their presence or absence in a lake or stream has important implications for aquatic ecosystem health (Vaughn & Hakenkamp, 2001; Bauer & Wächtler, 2001; Allen & Vaughn, 2011; Vaughn, 2018) and associated ecosystem functioning and services (Geist, 2011; Lummer, Auerswald & Geist, 2016; Richter *et al.*, 2016). Most unionids have a complex life history involving an obligate parasitic stage usually requiring an intermediate host fish for their larvae (termed glochidia in the families Margaritiferidae and Unionidae). While adult

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unionids are mostly sessile, glochidia can be dispersed long distances by movements of their host fish. Many species now suffer from severe population declines, and freshwater bivalve biodiversity is diminishing at a nearly unprecedented pace (e.g. Ricciardi & Rasmussen, 1999; Lydeard *et al.*, 2004; Haag, 2012; Lopes-Lima *et al.*, 2016).

The freshwater pearl mussel (*Margaritifera margaritifera* L.; common name eastern pearlshell in North America) is a unionid species that typically occurs in undisturbed headwater regions and small streams (Geist, 2010). It is known for its longevity, with lifespans exceeding 150 years in some parts of its distribution (Bauer, 1992). Their natural hosts include Atlantic salmon (*Salmo salar* L.), brown trout (*S. trutta* L.), and possibly brook charr [*Salvelinus fontinalis* (Mitchill, 1814)] (Geist, Porkka & Kuehn, 2006; Taeubert & Geist, 2017). Due to the vagility of its hosts (namely *S. salar* and *Salmo trutta fario*), *M. margaritifera* has a circumboreal distribution in northern Europe, eastern North America and Eurasia. Its distribution includes the arctic and temperate regions of western Russia, westwards through Europe to the north-eastern seaboard of North America and southwards to the Iberian Peninsula and central Europe (Young, Cosgrove & Hastie, 2001). The North American distribution of *M. margaritifera* extends from Pennsylvania north to Newfoundland and Labrador in Canada (Walker, 1910), comprising an extent of occurrence of approximately 1.05 million km^2. In Europe, *M. margaritifera* was historically found in high densities until the middle of the 19th century, often covering river bottoms in one or more layers. It has since declined substantially throughout its European range and is now endangered; few populations still have a significant number of juveniles present (Geist, 2010). Paradoxically, North American populations of *M. margaritifera* are considered relatively stable and secure (Williams *et al.*, 1993; NatureServe, 2017) with large and apparently healthy populations still found in many Atlantic coastal drainages of Canada and, to a lesser extent, north-eastern USA.

Due to the endangered status of *M. margaritifera* in Europe, and the publication of microsatellite markers for the species (Geist *et al.*, 2003), several studies on the molecular ecology, phylogeography and population structure of European populations have been published over the last two decades (Machordom *et al.*, 2003; Geist & Kuehn, 2005, 2008; Bouza *et al.*, 2007; Geist *et al.*, 2010; Karlsson, Larsen & Hindar, 2013; Stoeckle *et al.*, 2017). European studies generally show moderate to high levels of genetic differentiation that are partly explained by the accumulation of genetic drift due to small, declining populations. In northern and central Europe, the genetic structure was often independent of watershed with the highest levels of genetic diversity (Geist & Kuehn, 2005; Geist *et al.*, 2010), but in the southernmost populations on the Iberian Peninsula strong differentiation among watersheds and a much lower degree of genetic variability was found (Bouza *et al.*, 2007; Stoeckle *et al.*, 2017). Many populations in Europe show evidence of recent genetic bottlenecks possibly as a result of anthropogenically induced population declines (e.g. habitat alteration, pollution, overharvest; Geist & Kuehn, 2005; Geist *et al.*, 2010; Stoeckle *et al.*, 2017). Some populations showed genetic structure that may have resulted from differential host use (sea run trout and salmon vs. landlocked populations; Geist & Kuehn, 2008; Karlsson *et al.*, 2013).

Increased knowledge on the population genetics of *M. margaritifera* may additionally deliver important contributions to our understanding of the historical, phylogenetic and phylogeographical processes of post-glacial colonization patterns (Geist, 2010). While the endangered European populations of *M. margaritifera* are very well studied, relatively little is known about the apparently secure North American populations (Williams *et al.*, 1993; NatureServe, 2017). For this study, specimens were collected from across the North American distribution of *M. margaritifera* from Newfoundland in eastern Canada to Pennsylvania in the USA. The objectives of this study were to: (1) determine the genetic structure of North American *M. margaritifera*; (2) compare the genetic diversity among sampling locations; (3) explain the observed genetic structure in relation to colonization, historical geography and connectivity of populations; and (4) make recommendations for conservation and management of the species.

MATERIAL AND METHODS

Collection locations were selected at 23 sites across the North America distribution of the species from Newfoundland in the north-east, Québec in the west and Pennsylvania in the south (Table 1, Fig. 1). Mussels were collected by hand by snorkelling or wading. Small tissue samples for genetic analysis were obtained by taking a non-lethal biopsy from the mantle tissue of each mussel (Berg *et al.*, 1995). Mussels were opened gently along the ventral margin taking particular care to avoid tearing the adductor muscles, and small (~0.25-cm^2) clips of mantle tissue were taken from each mussel. Tissue biopsies were individually preserved in 95% ethanol in 2-mL cryovials labelled with a unique identifier and date collected. All mussels collected were placed back in their river habitat and area of capture in the correct infaunal orientation after biopsies were completed.

Table 1. Collection locations (waterbody, state/province, site code, location and watershed) and population genetics summary statistics for North American *Margaritifera margaritifera*

Waterbody, State/Province	Code	Location/watershed	N	N_A	A_r	A_p	H_O	H_E	F_{IS}	F	Bottleneck
Daniel's Brook, NL	DAN	Avalon Peninsula, Newfoundland	50	8.33	3.44	0.15	0.484	0.557	0.104	0.046	–/–/+
Bear Brook Pond, NL	BBP	South-western Newfoundland	50	7.78	3.27	0.11	0.441	0.515	0.157	0.046	–/–/+
Bethune's Brook, NL	BET	Avalon Peninsula, Newfoundland	50	8.78	3.51	0.09	0.495	0.579	0.103	0.025	–/+/+
Come-By-Chance River, NL	CBC	Southern Newfoundland	35	6.89	3.29	0.10	0.444	0.555	0.157	0.048	–/+/+
Indian River, NL	IND	Northern Newfoundland	20	5.22	3.12	0.07	0.403	0.537	0.224	0.045	–/–/–
Kennebecasis River, NB	KEN	St. John River/Bay of Fundy drainage	20	7.22	3.39	0.12	0.483	0.549	0.072	0.047	–/–/+
Musquodoboit River, NS	MUS	Atlantic Coast drainage	20	6.89	3.67	0.09	0.509	0.590	0.119	0.017	–/–/+
Miramichi River, NB	MIR	Gulf of St. Lawrence drainage	20	6.89	3.68	0.23	0.467	0.594	0.222	0.033	–/–/–
Stewiacke River, NS	STEW	Bay of Fundy drainage	20	6.11	3.55	0.11	0.477	0.601	0.234	0.046	–/–/+
Godbout River, QC	GOD	St. Lawrence Estuary	20	6.67	3.54	0.13	0.445	0.557	0.142	0.022	–/–/+
Morell River, PE	MOR	Gulf of St. Lawrence drainage	20	6.22	3.55	0.11	0.535	0.591	0.115	0.052	–/–/–
Fish Creek, NY	FIS	Oswego River/Lake Ontario drainage	20	5.44	3.31	0.09	0.428	0.533	0.266	0.075	–/–/+
Kayaderosseras Creek, NY	KAY	Upper Hudson River	20	5.00	3.01	0.09	0.398	0.474	0.161	0.163	–/–/+
Matane River, QC	MAT	Northern Gaspé Peninsula	42	9.56	3.59	0.15	0.553	0.586	0.047	0.018	–/+/+
Doncaster River, QC	DON	Southern Québec/Ottawa River drainage	30	7.67	3.50	0.18	0.589	0.580	-0.024	0.037	–/–/–
McLeod River, QC	MCL	Eastern Townships/upper St. Lawrence drainage	30	8.56	3.71	0.24	0.573	0.598	0.029	0.013	–/+/+
Berthier River, QC	BER	Southern Québec/upper St. Lawrence drainage	30	5.11	2.87	0.16	0.401	0.452	0.092	0.172	–/–/–
Etchemin River, QC	ETC	Central Québec/lower St. Lawrence drainage	30	7.67	3.60	0.31	0.547	0.564	-0.001	0.045	–/–/+
Malbaie River, QC	MAL	Central Québec/lower St. Lawrence drainage	30	6.00	3.22	0.18	0.519	0.538	0.049	0.079	–/–/+
Rivière aux Saumons, QC	ASA	Lac Saint Jean/Saguenay region, lower St. Lawrence drainage	30	7.56	3.56	0.16	0.507	0.577	0.109	0.046	–/–/+
Grasse River, NY	UAS	Upper St. Lawrence drainage	8	3.56	*	*	0.579	0.524	-0.147	0.168	–/–/–
Locust Creek, PA	UAD	Delaware River drainage	6	3.67	*	*	0.500	0.520	0.038	0.086	–/–/–
Swift River, MA	USW	Connecticut River drainage	44	6.89	3.38	0.13	0.507	0.552	0.171	0.056	–/+/+
Mean			*28*	*6.68*	*3.42*	*0.14*	*0.491*	*0.567*	*0.106*	*0.060*	

Number genotyped (N), mean number of alleles (N_A), mean rarefied allelic richness (A_r), mean rarefied private allele richness (A_p), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), F-value based on the 2MOD program (F), and results of tests for bottlenecks (significance at $\alpha = 0.05$ is indicated as '+' under the infinite allele, two-phase and step-wise mutation models, respectively).
*Not calculated due to small sample size.

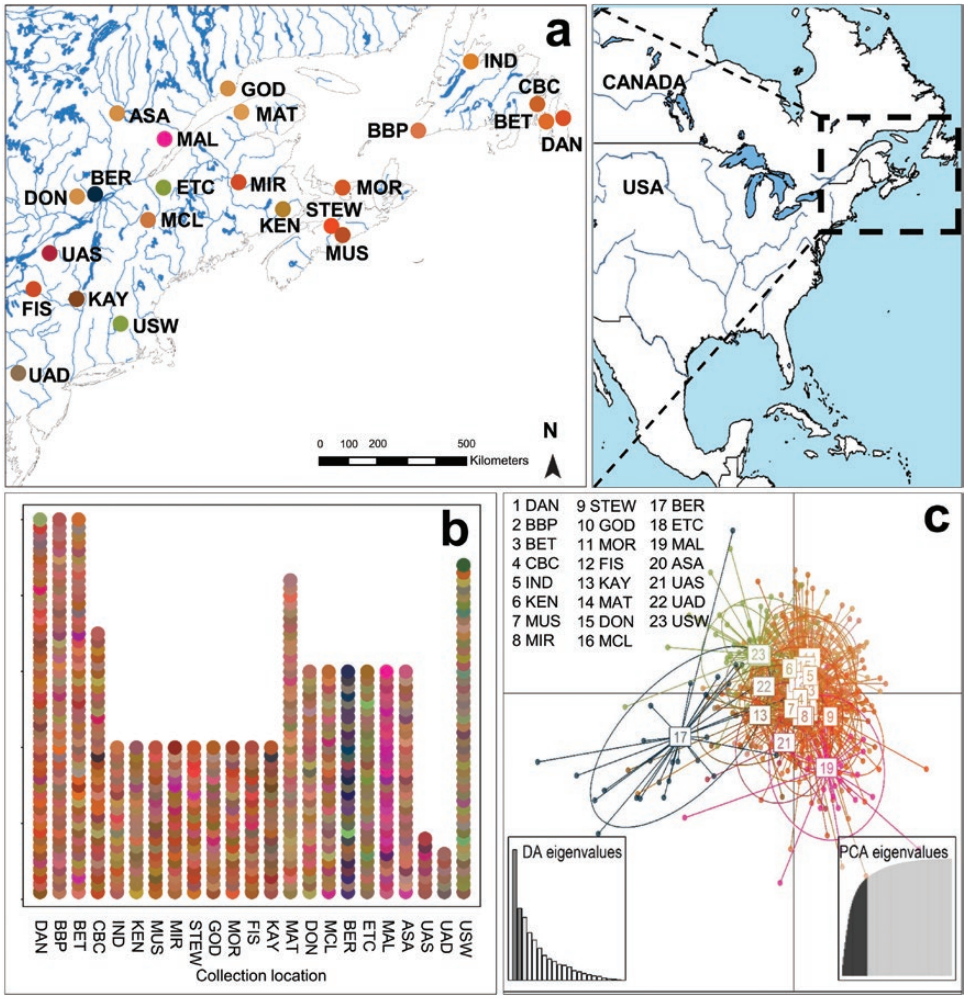

Figure 1. (A) Geographical location with genetic constitution (colour) for the analysed North American *Margaritifera margaritifera* populations. (B) Individual genetic characterization by population. The colour of the dots corresponds to the result of the DAPC (Jombart *et al.*, 2010). The similarity in colour of the dots indicates the genetic similarity of populations. (C) Clustering of individuals from each population (all depicted using the mean population colour according to the DAPC).

GENETIC METHODS

Genomic DNA was extracted from tissue samples using the standard phenol/chloroform method (Sambrook, Fristch & Maniatis, 1989), with slight modifications. Tissue lysis was performed in 600 µL of lysis buffer (20 mM Tris pH 8.0, 5 mM EDTA pH 8.0, 400 mM NaCl and 1% SDS) and 25 µL of proteinase K (10 mg/mL in 50 mM Tris pH 8.0, 1.5 mM calcium acetate). Digestion was performed at 55 °C overnight. In a first step, 600 µL of phenol was added to the sample. After centrifugation, the supernatant was washed with 300 µL phenol and 300 µL chloroform/isoamyl alcohol (24:1). The final wash was performed using 600 µL chloroform, following a precipitation step using cold isopropanol and 70% ethanol. The extracted DNA was preserved in Tris buffer (5 mM Tris pH 8.5) and incubated at 55 °C overnight to ensure optimal resuspension. These samples were then stored at –20 °C for subsequent analyses.

To allow comparability of results with other genetic studies on *M. margaritifera*, all genetic analyses were conducted based on genotyping of nine species-specific microsatellite markers as described by Geist et al. (2003, 2010), and Geist & Kuehn (2005, 2008). PCRs were performed in a total volume of 12.5 µL with the following components: 25–50 ng of genomic DNA, 200 nM of each primer, 0.2 mM of each dNTP, 3 mM $MgCl_2$ (2 mM $MgCl_2$ for locus MarMa5280), 1× PCR buffer (Solis Biodyne, Tartu, Estonia) and 0.25 U Taq DNA Polymerase (Solis Biodyne). PCR products were separated on 5% denaturing 19:1 acrylamide/bisacrylamide gels on an ALFexpressII DNA analyser and scored with ALLELELINKS 1.02 software (Amersham Pharmacia Biotech, Amersham,

UK). Electrophoresis was carried out with two internal standards (70 and 300 bp) in each lane. Additionally, an external standard (50–500 bp ladder) and a previously genotyped reference sample were included on each gel to standardize allele scoring and to facilitate cross-referencing among gels.

Statistical analyses

Each microsatellite locus was assessed for the presence of null alleles and genotyping errors using MICROCHECKER v.2.2.3 (van Oosterhout *et al.*, 2004) as a single dataset. If allele size difference frequencies deviated from the 95% confidence interval of simulated expectations, the test was deemed positive for null alleles. We included loci with null allele frequencies below 0.2, as this level has been shown to have very little impact on population delineation and divergence estimates (Dakin & Avise, 2004; Carlsson, 2008). Genetic diversity indices were calculated for each collection location and the dataset as a whole. GENALEX v.6.502 (Peakall & Smouse, 2006) was used to calculate the number of alleles (N_A), inbreeding coefficient (F_{IS}), observed and expected heterozygosities (H_O and H_E), and significance test of deviations from Hardy–Weinberg equilibrium (HWE). HP RARE (Kalinowski, 2005) was used to calculate mean allelic richness (corrected for sample sizes, A_r) and mean private allele richness (A_p). Relatedness between individuals was estimated based on the F value from the 2mod program (Ciofi *et al.*, 1999), which refers to the probability that two genes share a common ancestor within a population and correlates with effective population sizes. GENEPOP v.4.2 (Rousset, 2008) was used to test for linkage disequilibrium, using the log-likelihood ratio statistic with a dememorization number of 1000 with 100 batches and 1000 iterations per batch as a single dataset.

To identify any recent (i.e. within $2N_e$–$4N_e$ generations) genetic bottlenecks at any of the collection locations, sign tests were carried out using three models of evolution: the infinite alleles model (IAM), two-phase model (TPM) and stepwise mutation model (SMM). Variance and SMM proportions for the TPM model were set at 30 and 70%, respectively. Each model was iterated 1000 times to test significance ($\alpha = 0.05$). All bottleneck tests were conducted using BOTTLENECK v.1.2.02 (Piry, Luikart & Cornuet, 1999).

Genetic structure within and among collection locations was assessed using individual-based Bayesian assignment tests. STRUCTURE v.2.3.4 (Pritchard, Stephens & Donnelly, 2000) was used to determine the number of hypothesized groups, by evaluating the individual membership coefficients given a range of potential units of separation (K). Values of K from 1 to 25 (number of collection sites + 2) were analysed with ten iterations each to assess their consistency, likelihood and robustness (Pritchard *et al.*, 2000). The parameters given to STRUCTURE were 200 000 burn-in iterations and 1.5 million Markov chain Monte Carlo repeats, with assumptions of potential admixture (gene flow) between population and correlated allele frequencies. The analyses were run without a priori population information. STRUCTURE HARVESTER Web v.0.6.94 was then used to evaluate the most likely K value, using two methods of analysis (Earl & von Holdt, 2012). One was the Evanno method, which looks for the highest ΔK between sequential K values (Evanno, Regnaut & Goudet, 2005). The second method was to look for the highest mean estimate of the natural log of the probability of the data (Earl & von Holdt, 2012).

ARLEQUIN 3.0 software (Excoffier, Laval & Schneider, 2005) was used to quantify genetic population structure by analysis of molecular variance (AMOVA; Excoffier, Smouse & Quattro, 1992), and to incorporate molecular information based on allelic frequencies. Pairwise analyses of genetic divergence (F_{ST} and Jost's D_{est}; Wright, 1965; Jost, 2008) among sampling locations were made using GENALEX.

An analysis of genetic isolation by geographical distance between all 23 sampling sites was conducted using Mantel tests implemented in the Isolation By Distance Web Service (IBDWS) v.3.23 (http://ibdws.sdsu.edu) with 10 000 permutations (Jensen, Bohonak & Kelley, 2005). Geographical distance between sites was measured using ArcMap 10 (Environmental Systems Research Institute) and was considered to be the shortest possible water distance between collection sites (river kilometres in tributaries and straight lines across open water, avoiding land masses). Pairwise genetic distance was calculated using Nei's standard genetic distance (Nei, 1972) calculated in GENALEX. Mantel tests were also conducted in the same manner using linearized F_{ST} and D_{est} values.

Since Bayesian clustering techniques may produce biased results when faced with unequal sample sizes (Puechmaille, 2016), the results of STRUCTURE were verified with the multivariate Discriminant Analysis of Principal Components approach (DAPC; Jombart, Devillard & Balloux, 2010), which is less sensitive if sampling is uneven (Puechmaille, 2016). To combine genetic and geographical data, a synthesis map was generated with the first three scores of the DAPC implemented in the R-package adegenet (Jombart, 2008; Jombart *et al.*, 2010) for R v.2.12 (R Development Core Team 2016). DAPC, which uses no a priori geographical assumptions regarding the origin of samples, first extracts information by applying a principal component analysis (PCA). In a second calculation step, a discriminant analysis (DA) maximizes the between-group component of genetic variation.

RESULTS

GENETIC DIVERSITY

A total of 645 *M. margaritifera* specimens were collected from 23 collection locations in eastern North America (Table 1) and genotyped at nine microsatellite loci (Geist *et al.*, 2003). Microsatellite diversity varied among the loci, with 27 alleles at locus 5023 and three alleles at locus 2671. Allelic richness (adjusted for sample size) ranged from 2.87 alleles per locus in the Berthier River to 3.71 alleles per locus in the McLeod River. There were few private alleles found at any of the collection locations (A_p mean = 0.14, range 0.07–0.31) and only five collection locations (Malbaie, Etchemin, Berthier, McLeod and Miramichi rivers) had A_p values > 5% of the A_r values. Mean levels of observed heterozygosity ranged from 0.398 in Kayaderosseras Creek to 0.589 in the Doncaster River. Significant deviations from HWE after Bonferroni correction were found at 26 of 218 (11.9%) locus–collection site combinations (α = 0.0002427) (Supporting Information Table S1,). Of these, five were the result of heterozygote excess and 21 of heterozygote deficiency. Eight of the locus–collection site combinations showing heterozygote deficiency were at locus 5167. This locus also showed the highest estimated null allele frequencies (see below). Other deviations from HWE were not consistent across loci or collection locations, and thus locus 5167 was kept in all subsequent analyses. For the 2mod analysis, North American *M. margaritifera* populations yielded low *F*-value modes (0.013–0.172; mean 0.060; Table 1), which indicate that the probability of alleles being identical by descent is low and population structure is probably influenced by gene flow.

Potential null alleles were detected at two loci (4726 and 5167) with predicted null allele frequencies at these two loci of 0.071 and 0.170, respectively. These relatively low predicted null allele frequencies are below thresholds that would affect the results or interpretations of population-level analyses (Dakin & Avise, 2004; Carlsson, 2008); thus, all loci were included in the analyses. Only three of 730 locus–collection site combinations showed significant linkage after Bonferroni correction for multiple comparisons. Linkage was found between loci 3621 and 5023 in Bear Brook Pond (Newfoundland and Labrador; BBP), 3621 and 5167 in Come By Chance River (Newfoundland and Labrador; CDC), and 4726 and 5023 in the Swift River (Maine; USW). As linkage was not consistently found between any two loci across all sites, no further action was deemed necessary in subsequent analyses. Sign tests showed evidence for recent genetic bottlenecks at 16 of 23 collection locations using either the SMM or the TPM (Table 1). The IAM (not typically appropriate for microsatellite markers) did not indicate the presence of a recent bottleneck for any of the collection locations.

GENETIC STRUCTURE

Analysis using STRUCTURE indicated that there is probably only a single, panmictic genetic population for North American *M. margaritifera* when using the criteria of Evanno *et al.* (2005). However, when analysing the natural log of the probability of the STRUCTURE dataset, K = 6 was also supported (Supporting Information, Fig. S1,). The Evanno ΔK method also shows a peak at K = 6. At K = 6, samples from the Berthier River and the Malbaie River in the St. Lawrence River drainage both showed some genetic distinctiveness from all other collection locations (Fig. 2).

AMOVA revealed that 96.6% of the genetic variation was found among individuals within populations and only 3.4% was found among populations. The overall fixation index F_{ST} was 0.034. Pairwise F_{ST} and D_{est} were also generally low and mostly not significant among collection locations (Table 2), with more moderate levels of differentiation occurring with the Berthier River, the Malbaie River, the Grasse River, the Etchemin River (St. Lawrence River drainage in Québec), Kayaderosseras Creek (upper Hudson River drainage in New York) and the Swift River (Connecticut River drainage in Massachusetts). A Mantel test for isolation-by-distance did not find any significance (Nei's D: Mantel's r = 0.021, P = 0.375; F_{ST}: Mantel's r = 0.114, P = 0.143; D_{est}: Mantel's r < 0.001, P = 0.462).

The result of DAPC revealed high genetic similarity among individuals and among collection sites (Fig. 1). Only samples from the Berthier (BER) and Malbaie (MAL) rivers showed some genetic distinctiveness from

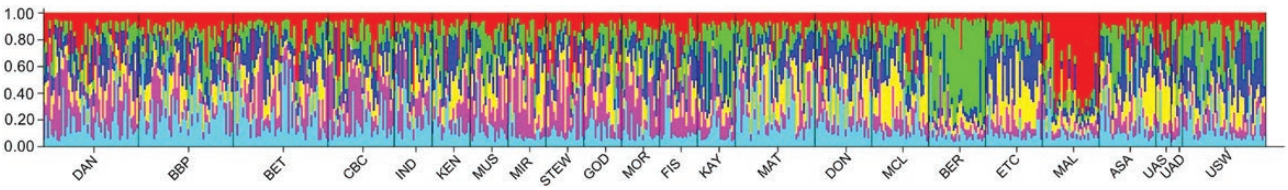

Figure 2. STRUCTURE bar plot output for 23 *Margaritifera margaritifera* collection sites in North America, without a priori populations assigned (admixture and correlated alleles assumed) for K = 6 genetic groupings. Site codes for collection locations are listed in Table 1.

Table 2. Pairwise population differentiation (D_{est} below diagonal and F_{ST} above diagonal) with each collection location represented by a cell

Collection location	DAN	BBP	BET	CBC	IND	KEN	MUS	MIR	STEW	GOD	MOR	FIS	KAY	MAT	DON	MCL	BER	ETC	MAL	ASA	UAS	UAD	USW
DAN	–	0.020	0.010	0.018	0.020	0.020	0.018	0.028	0.015	0.020	0.021	0.026	0.042	0.023	0.020	0.016	0.062	0.037	0.029	0.029	0.059	0.050	0.023
BBP	0.034	–	0.009	0.020	0.018	0.022	0.026	0.027	0.025	0.018	0.030	0.025	0.041	0.011	0.017	0.018	0.054	0.038	0.025	0.023	0.066	0.050	0.023
BET	0.010	0.008	–	0.013	0.013	0.015	0.015	0.019	0.014	0.013	0.020	0.019	0.038	0.010	0.011	0.011	0.057	0.028	0.023	0.019	0.053	0.041	0.022
CBC	0.027	0.029	0.016	–	0.014	0.015	0.016	0.022	0.015	0.011	0.024	0.020	0.033	0.017	0.016	0.015	0.051	0.030	0.034	0.024	0.074	0.042	0.017
IND	0.024	0.017	0.006	0.003	–	0.018	0.023	0.025	0.021	0.015	0.027	0.027	0.042	0.015	0.012	0.015	0.066	0.036	0.034	0.023	0.073	0.048	0.021
KEN	0.025	0.029	0.012	0.008	0.007	–	0.015	0.029	0.024	0.013	0.027	0.029	0.033	0.016	0.012	0.011	0.041	0.025	0.035	0.020	0.059	0.028	0.011
MUS	0.021	0.042	0.015	0.011	0.021	0.000	–	0.017	0.015	0.011	0.014	0.024	0.024	0.024	0.024	0.013	0.040	0.025	0.037	0.027	0.053	0.033	0.021
MIR	0.049	0.042	0.026	0.027	0.025	0.041	0.006	–	0.019	0.019	0.020	0.022	0.043	0.022	0.027	0.023	0.058	0.039	0.041	0.024	0.070	0.048	0.032
STEW	0.014	0.039	0.009	0.007	0.015	0.027	−0.002	0.010	–	0.017	0.012	0.024	0.043	0.022	0.023	0.018	0.064	0.039	0.030	0.025	0.060	0.046	0.028
GOD	0.023	0.017	0.005	−0.004	−0.004	−0.006	−0.012	0.007	0.004	–	0.021	0.018	0.021	0.018	0.019	0.012	0.042	0.021	0.028	0.020	0.065	0.029	0.014
MOR	0.031	0.054	0.031	0.034	0.033	0.037	−0.002	0.017	−0.011	0.015	–	0.031	0.036	0.027	0.027	0.020	0.053	0.037	0.038	0.026	0.054	0.041	0.027
FIS	0.039	0.033	0.023	0.019	0.026	0.036	0.023	0.017	0.024	0.006	0.044	–	0.027	0.030	0.025	0.025	0.053	0.034	0.041	0.023	0.065	0.042	0.035
KAY	0.072	0.063	0.067	0.048	0.059	0.042	0.022	0.069	0.071	0.011	0.054	0.025	–	0.046	0.045	0.031	0.033	0.025	0.054	0.043	0.077	0.038	0.032
MAT	0.047	0.012	0.010	0.026	0.011	0.017	0.041	0.034	0.036	0.019	0.051	0.053	0.087	–	0.009	0.012	0.059	0.035	0.027	0.016	0.062	0.044	0.021
DON	0.036	0.026	0.010	0.019	−0.001	0.002	0.038	0.047	0.033	0.020	0.049	0.037	0.082	0.006	–	0.013	0.058	0.029	0.031	0.015	0.059	0.043	0.019
MCL	0.025	0.028	0.010	0.016	0.007	−0.003	0.005	0.033	0.020	−0.001	0.026	0.035	0.047	0.013	0.011	–	0.051	0.026	0.020	0.015	0.043	0.033	0.015
BER	0.121	0.094	0.114	0.091	0.116	0.064	0.067	0.110	0.127	0.064	0.099	0.085	0.039	0.120	0.113	0.100	–	0.045	0.078	0.055	0.095	0.044	0.039
ETC	0.081	0.076	0.059	0.056	0.064	0.037	0.038	0.080	0.083	0.024	0.074	0.058	0.031	0.078	0.059	0.051	0.079	–	0.049	0.039	0.074	0.041	0.027
MAL	0.054	0.041	0.042	0.064	0.054	0.061	0.070	0.081	0.049	0.041	0.074	0.072	0.095	0.052	0.060	0.032	0.151	0.106	–	0.030	0.059	0.056	0.035
ASA	0.060	0.039	0.032	0.041	0.026	0.023	0.046	0.036	0.037	0.022	0.043	0.028	0.075	0.024	0.017	0.018	0.106	0.086	0.055	–	0.058	0.037	0.023
UAS	0.104	0.113	0.092	0.140	0.126	0.092	0.081	0.133	0.103	0.110	0.087	0.103	0.123	0.120	0.107	0.063	0.164	0.148	0.099	0.101	–	0.069	0.071
UAD	0.060	0.056	0.037	0.032	0.037	−0.011	0.001	0.043	0.038	−0.011	0.028	0.024	0.012	0.050	0.045	0.013	0.033	0.034	0.073	0.023	0.078	–	0.034
USW	0.043	0.042	0.042	0.025	0.026	0.003	0.030	0.062	0.050	0.009	0.048	0.062	0.049	0.041	0.032	0.019	0.067	0.053	0.070	0.041	0.138	0.017	–

Significance was calculated by permuting the dataset 10 000 times using a Bonferroni-corrected $\alpha = 0.0001976$. Collection location codes are as in Table 1. Dark grey cells were not significantly different from zero or had very low genetic differentiation (D_{est} or $F_{ST} < 0.01$); light grey cells had low genetic differentiation (D_{est} or $F_{ST} = 0.01$–0.05); green cells had moderate genetic differentiation (D_{est} or $F_{ST} = 0.05$–0.15); yellow cells had high genetic differentiation (D_{est} or $F_{ST} = 0.15$–0.25).

all other locations. These results are congruent with the STRUCTURE results and the F_{ST} calculations.

DISCUSSION

Despite the large North American distribution of *M. margaritifera*, little genetic differentiation among sampling locations across north-eastern North America was revealed using microsatellite markers, with a single genetic population being the most probable result. Genetic diversity (e.g. allelic richness and heterozygocity) in the North American population is considerably higher than in many threatened European populations (Geist & Kuehn, 2005; Bouza *et al.*, 2007; Geist *et al.*, 2010; Karlsson *et al.*, 2013; Stoeckle *et al.*, 2017). Differences in genetic diversity among North American and European populations of *M. margaritifera* may indicate that North American populations have not experienced recent declines and remain relatively healthy (e.g. a panmictic population, large population size and apparently high levels of gene flow across the distribution), in contrast to the situation in most European populations.

The low levels of genetic differentiation across the North American distribution of *M. margaritifera* are in stark contrast to what is found in Europe. Central and southern European populations show strong population genetic structure even at small spatial scales (i.e. among watersheds and among distances in a range of tens to hundreds of kilometres; Geist & Kuehn, 2005; Bouza *et al.*, 2007; Stoeckle *et al.*, 2017). Structure is even evident in northern European populations where genetic variability within populations was highest in all of Europe (Geist *et al.*, 2010; Karlsson *et al.*, 2013). Understanding the reasons why the North American population generally lacks genetic structure in contrast to European populations is important to consider and has implications for conservation of the species. Hypothesized reasons for the lack of structure in the North American population are: slow rate of molecular evolution, long life spans and generation times, the patterns of post-glacial colonization and possible ongoing gene flow.

The unionid family Margaritiferidae, to which *M. margaritifera* belongs, is an ancient group that probably arose during the Triassic or Jurassic with the current crown group of extant taxa arising in the Cretaceous (Huff *et al.*, 2004; Bolotov *et al.*, 2016). Margaritiferids are known for having slow rates of molecular evolution and a high degree of morphological conservatism, which apparently resulted in low species diversity in the family as a whole relative to most other unionid families (e.g. Unionidae and Hyriidae; Graf & Cummings, 2007). The slow rate of molecular evolution may also explain the low levels of genetic divergence among North American *M. margaritifera*. Given that only *c.* 10 000–15 000 years have passed since all North American *M. margaritifera* were concentrated into one or a few glacial refuges (see discussion below), the rate of molecular evolution (even in fast mutating microsatellite regions) may be too slow to show differentiation.

The life history of *M. margaritifera* may help to explain some of the lack of genetic structure across its North American range. The long life spans (> 100 years; Bauer, 1992) and generation times (at least 20 years; Bauer, 1987) of *M. margaritifera* mean that rates of evolutionary change (i.e. genetic drift and natural selection) will be very slow and there will be a long lag period before detectable changes in genetic diversity and structure can be observed even if populations become isolated (Hoffman *et al.*, 2017). It is possible that many of the collection locations sampled in this study are isolated, but do not yet show genetic isolation because the amount of time they have been isolated (*c.* < 10 000 years since post-glacial colonization) is not sufficient for genetic effects (Hoffman *et al.*, 2017) to be detected using microsatellite markers.

The general lack of genetic differentiation and structure in North American *M. margaritifera* may reflect the patterns of its post-glacial colonization in North America, in combination with the restriction of the species and its host fishes to cold waters. *Margaritifera margaritifera* probably occupied refugia in one or more hypothesized exposed and unglaciated continental shelf regions off the east coast of North America: Georges Bank off Massachusetts, the Sable Island bank off Nova Scotia and the Grand Banks off Newfoundland (Nedeau, McCollough & Swartz, 2000; Schmidt, 1986). The pattern of genetic structure revealed by the microsatellites supports a single glacial refugium, but it could be that these unglaciated regions were a single genetic population during the Pleistocene as well. River drainage patterns in New England and along the entire Atlantic coast of North America prior to the last glaciation were apparently in a somewhat different configuration from that at present and may have been quite variable as the Wisconsin glaciation ended (Caldwell, Hanson & Thompson, 1985) and allowed for gene flow among previously isolated populations. There may have also been freshwater connections between drainage basins in the form of both headwater proglacial lakes and deltaic areas near river mouths (reviewed by Nedeau *et al.*, 2000). Documented changes in drainage patterns are known to have occurred as a result of dams and impoundments in northern New England as recently as the mid-1800s (Nedeau *et al.*, 2000). Furthermore, a short-lived connection between the St. Lawrence River basin and the Hudson River may have existed via Lake Champlain *c.* 11 500 years ago (Underhill, 1986; Schmidt, 1986) that could have allowed for genetic exchange.

In addition to the relative youth of the modern North American *M. margaritifera* population, the lack of genetic structure and differentiation could also be the result of ongoing and frequent genetic exchange across the North American distribution. Contemporary gene flow in unionid mussels is mostly due to transport of encysted glochidia by fish. Intuitively, it would seem that *M. margaritifera* populations would be isolated because hosts would be unlikely to easily disperse among drainages that are separated by stretches of saline ocean environments. However, Atlantic salmon only imperfectly return to natal habitats after smolting to the ocean, thus potentially keeping the number of migrants per generation (N_m) greater than $N_m = 1$ necessary for maintaining gene flow and preventing genetic differentiation. It is estimated that between 1% and 6% of Atlantic Salmon found in any particular river were originally spawned in different rivers (termed 'strayers') (Petersson, 2016). Thus, based on large censuses and suspected large effective population sizes typically found in *M. margaritifera* (a single female can produce > 100 million glochidia in her lifetime; Bauer, 1987), the number of migrants per generation probably far exceeds $N_m = 1$ between any two drainages in the mussel's North American distribution. It is therefore likely that gene flow has occurred recently enough to have limited genetic differentiation across the North American distribution. It is unknown if glochidia encysted on the gills of salmon hosts would survive exposure to oceanic salinity levels, but encysted glochidia have been reported to be hardly influenced by ambient water conditions except for temperature (Taeubert, Gum & Geist, 2013) and it seems that this survival is probable given that gene flow has recently occurred among drainages separated by stretches of ocean.

While all of the analyses support a general lack of genetic structure across the North American distribution of *M. margaritifera*, there is some evidence of structure in at least two of the collection sites in tributaries of the St. Lawrence River in Québec: the Berthier and Malbaie rivers. The two locations do not appear to be unique in terms of diversity metrics, and the limited structure observed is probably a statistical artefact with little biological meaning. The Berthier River has the lowest allelic richness and heterozygosity values of any of the collection locations; however, the diversity metrics in the Malbaie River are near mean values. The Malbaie shows some evidence of a recent genetic bottleneck (as many collection locations do), but none of the tests for bottlenecks was positive for the Berthier River. Speculating on possible biological and biogeographical reasons why these collection locations were more distinct is difficult. Neither river has any impoundments that could genetically isolate or lead to land-locked mussel and host populations (e.g. King *et al.*, 2001; Karlsson *et al.*, 2013). It is possible that mussels at these collection locations may be using a different host, leading to genetic isolation (i.e. introduced brown trout or brook charr) with potential effects on genetic constitution, but this is simple speculation. Note that while the sampling coverage of *M. margaritifera* was considerable, it was not exhaustive. There may be landlocked and more inland populations in the North American distribution of *M. margaritifera* that do show greater genetic distinctiveness, as found in some European populations (e.g. Karlsson *et al.* 2013) and some landlocked North American Atlantic salmon populations (King *et al.*, 2001). Future studies on North American *M. margaritifera* may target potentially landlocked populations for genetic analyses.

The lack of genetic structure for North American *M. margaritifera* is quite different from the pattern observed for most other unionids and fish found along the Atlantic coast of North America. In the Atlantic coastal drainages, each river draining to the Atlantic Ocean appears to act as a biogeographical island in terms of species composition and endemism (Sepkoski & Rex, 1974) and genetic structure (King *et al.*, 1999; Kelly & Rhymer, 2005; Hasselman, Bradford & Bentzen, 2010), with limited opportunities for inter-basin dispersal by species or alleles. The general pattern of genetic structure among populations of North American unionids is somewhat more variable than those found in drainages along the Atlantic coast, but genetic structure among river drainages is a common finding (e.g. Zanatta & Wilson, 2011; Hewitt *et al.*, 2018; Mathias *et al.*, 2018). An important exception to the general pattern of genetic structure across major river drainages in North American unionids is provided by Pfeiffer *et al.* (2018) on *Megalonaias nervosa*. *Megalonaias nervosa* has a large distribution in eastern North America in the rivers draining into the Gulf of Mexico. Similar to *M. margaritifera*, *M. nervosa* showed very little genetic structure across its large distribution (from the upper Mississippi River to Mexico). Pfeiffer *et al.* (2018) used mitochondrial (COI) and nuclear (ITS1) DNA sequence data to assess the phylogeography of *M. nervosa*. These markers typically show lower diversity than do microsatellites and thus are not as likely to reveal genetic structure among populations. While studies using DNA sequence data are not necessarily directly comparable to studies using microsatellites to delineate genetic structure among populations, studies using a combination of both sequence data and microsatellites have shown that these datasets are typically congruent (e.g. Zanatta & Murphy, 2008; Hewitt *et al.*, 2018; Mathias *et al.*, 2018). It appears that the degree of genetic structure across the distribution of North American unionid species is largely dependent on the life history (e.g. generation time, life span, host attraction strategy, host specificity, host dispersal ability) of the mussel (Haag, 2012).

Other margaritiferids in North America show varying degrees of genetic diversity and structure across their distributions. *Margaritifera falcata* Gould, 1850, the western pearlshell, is distributed in streams across the Pacific Northwest, from northern California to northern British Columbia and Alaska. *Margaritifera falcata* showed shallow regional, but pronounced population-level structure using microsatellite markers (Mock *et al.*, 2013). This finding was comparable to those for European *M. margaritifera* (Geist & Kuehn, 2005; Bouza *et al.*, 2007; Geist *et al.*, 2010; Karlsson *et al.*, 2013; Stoeckle *et al.*, 2017), but contrasted the findings of this study for North American *M. margaritifera* in that North American populations showed shallow divergences at both regional and population levels. Like *M. margaritifera* populations, *M. falcata* was found to have low levels of genetic diversity across its distribution, possibly a result of low rates of molecular evolution (e.g. Bolotov *et al.*, 2016). *Margaritifera monodonta* (Say, 1829), the spectaclecase, was historically a widely distributed species in the highly dendritic Mississippi River drainage, but has experienced severe reductions in its distribution and is now considered to be threatened (Inoue *et al.*, 2014). Similar to *M. margaritifera*, based on a suite of microsatellite markers, *M. monodonta* showed little genetic structure and high levels of gene flow across most of its distribution in the Mississippi River drainage (with the exception of a distinct population in the Ouachita River in the southernmost part of its distribution). However, *M. monodonta* had much higher allelic richness and genetic diversity than North American or European *M. margaritifera*. Finally, *Margaritifera hembeli* Conrad, 1838, the Lousiana pearlshell, is a critically endangered and narrowly distributed species endemic to the Red River drainage along the coast of the Gulf of Mexico (Curole, Foltz & Brown, 2004). *Margaritifera hembeli* was found to have very low genetic diversity (using allozyme markers) and is again consistent with low rates of molecular evolution found in margaritiferids.

Conservation implications and conclusions

It seems that the North American population of *M. margaritifera* is apparently secure with a wide distribution, large population sizes, and evidence of recent recruitment at many of the locations visited for this study (Sollows, McAlpine & Munkittrick, 2013; D. T. Zanatta, personal observations). However, some populations (especially in the southern part of the North American distribution) are showing signs of declines (Strayer & Jirka, 1997; Nedeau *et al.*, 2000; Gouvernement du Québec, 2010; Connecticut DEP, 2013). Due to the longevity of the species, long lag-times between habitat degradation such as loss of connectivity and visible consequences on a population genetic level are to be expected (Hoffman *et al.*, 2017).

The results of this study provide valuable insight for the conservation and management of *M. margaritifera* in North America. The overall lack of genetic structure and similarity in genetic diversity among all of the locations sampled in North America for *M. margaritifera* indicate that maintaining population connectivity (i.e. avoiding barrier construction and encouraging barrier removals) and optimal habitat conditions may be most important for maintenance of the North American population of *M. margaritifera*. It is much easier and less costly to maintain currently secure populations than it is to restore depleted or extinct populations through artificial propagation (e.g. Gum, Lange & Geist, 2011), stocking or habitat restoration (e.g. Geist, 2015; Geist & Hawkins, 2016; McMurray & Roe, 2017). Only as a secondary conservation measure should North American *M. margaritifera* be propagated and/or relocated to other drainage basins to augment severely depleted or restore extinct populations. Relocating propagated juveniles could be done in North American *M. margaritifera* without any great worry of introducing new alleles or changing the genetic structure of the recipient population.

The most likely explanation for the overall lack of genetic structure in North American *M. margaritifera* is that these mussels have maintained gene flow across the region using a combination of movement by their salmonid hosts and the recent reconfigurations of drainages along the Atlantic coast of North American following deglaciation. Furthermore, there has probably not been sufficient time to show genetic differentiation across the region due a combination of low rates of molecular evolution found in margaritiferids, the long life spans and generation times of the mussels, and suspected large effective population sizes. Due to the parasitic nature of juvenile *M. margaritifera* on host fish, information on their genetic structure is also relevant to management of their hosts and thus more generally of other freshwater organisms in the region.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. STRUTURE HARVESTER results to determine the most likely K value. The Evanno method compares ΔK between sequential K values (a), and comparison of the mean estimated natural log of the probability of the data amongst K values, with the circle centred over the mean and the bar indicating the standard error (b).
Table S1. For each locus–collection site combination, the total number of *Margaritifera margaritifera* successfully genotyped, number of alleles observed, observed heterozygosity (H_O) and expected heterozygosity (H_E) are listed. Observed heterozygosity values significantly out of HWE after a table-wide Bonferroni correction ($\alpha = 0.0002427$) are shown in bold italics. Locus–collection site combinations with only one allele (H_O value in italics) or with no amplified alleles were not included in the Bonferroni correction calculation.